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Biological and Chemical Transformation of Per- and Polyfluoroalkyl Substances for
Contaminated Groundwater Site Management and Remediation

by

Emily Kathryn Cook

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Engineering - Civil and Environmental Engineering

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Lisa Alvarez-Cohen, Chair

Professor David Sedlak

Associate Professor Celine Pallud

Spring 2022

Biological and Chemical Transformation of Per- and Polyfluoroalkyl Substances for
Contaminated Groundwater Site Management and Remediation

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Emily Kathryn Cook

Abstract

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Emily Kathryn Cook

Doctor of Philosophy in Engineering - Civil and Environmental Engineering

University of California, Berkeley

Professor Lisa Alvarez-Cohen, Chair

This dissertation covers both biological and chemical transformation of per- and polyfluoroalkyl substances (PFAS) as well as their interactions with microorganisms and common PFAS co-contaminants. In Chapter 2, I investigate the biotransformation pathway of a PFAS commonly detected in aqueous film forming foam impacted (AFFF) groundwater. Sites impacted by AFFFs can contain co-contaminants that can stimulate biotransformation of polyfluoroalkyl substances. In this chapter, I compare how microbial enrichments from AFFF-impacted soil amended with diethyl glycol monobutyl ether (found in AFFF), aromatic hydrocarbons (present in co-released fuels), acetate and methane (substrates used or formed during bioremediation) impact the aerobic biotransformation of an AFFF-derived six-carbon electrochemical fluorination (ECF) precursor N-dimethyl ammonio propyl perfluorohexane sulfonamide (AmPr-FHxSA). I found that methane and acetate oxidizing cultures resulted in the highest yields of identifiable products (47% and 36%, respectively), including perfluorohexane sulfonamide (FHxSA) and perfluorohexane sulfonic acid (PFHxS). Using these data, I propose and detail a transformation pathway. Additionally, I examined chemical oxidation products of AmPr-FHxSA and FHxSA to provide insights on remediation strategies for AmPr-FHxSA. I demonstrate mineralization of these compounds using sulfate radical and test their transformation using the total oxidizable precursor (TOP) assay. While perfluorohexanoic acid accounted for over 95% of the products formed, I demonstrate here for the first time two ECF-based precursors that produce PFHxS during TOP assay. These findings have implications for monitoring PFAS during site remediation and application of the TOP assay at sites impacted by ECF-based precursors.

In Chapter 3, I test the interference of PFAS with bioremediation of co-contaminants. In source zones where PFAS from AFFF are commingled with co-contaminants such as trichloroethene (TCE) and fuel hydrocarbons benzene, toluene, ethylbenzene, and xylene (BTEX), high concentrations of PFAS could impact bioremediation efforts of these co-

contaminants. Bioremediation of chlorinated solvents and BTEX has been practiced for decades, usually incorporating the augmentation of *Dehalococcoides* for the former, or oxygen sparging to encourage in situ aerobic microbial communities for the latter. In this study, we exposed two cultures, an anaerobic co-culture containing *Dehalococcoides mccartyi* strain 195 with a sulfate reducer, as well as an aerobic AFFF-impacted enrichment culture to various PFAS to test the inhibition of specific PFAS to TCE and BTEX degradation. Specifically, we amended 1 or 10 μM of perfluorohexane sulfonic acid (PFHxS), perfluorohexane sulfonamide (FHxSA), n-dimethyl perfluorohexane sulfonamido amine (AmPr-FHxSA), an electrochemical fluorination produced AFFF, or non-fluorinated surfactant control sodium dodecyl sulfate (SDS) to the cultures. We tested the TCE and BTEX degradation, adenosine triphosphate (ATP) production, and metabolites to determine possible mechanisms of PFAS toxicity. We found that the anaerobic dechlorinating co-culture was resistant to individual PFAS inhibitory effects but was impacted by 1,000x diluted and 100x diluted 3M AFFF. The aerobic BTEX-degrading enrichment culture experienced substrate degradation inhibition when exposed to FHxSA and AmPr-FHxSA, but not to PFHxS or SDS. Based on ATP and amino acid data, I hypothesize that main mechanisms are FHxSA permeating the cell membrane, AmPr-FHxSA interfering with proteins, and the AFFF dilutions negatively impacting the cell's ability to produce ATP via the proton motive force.

Finally, in Chapter 4 I explore a possible in situ destruction technique for PFAS and co-contaminant remediation. Military bases and airports are often contaminated by PFAS due to the repeated use of AFFFs from decades of training exercises, equipment testing, and extinguishing of fuel and solvent-based fires. Pump-and-treat coupled to sorption processes is a common ex situ remediation strategy, but is often expensive and requires decades of operation due to long-term diffusion and dissolution of contaminants. Alternatively, in situ chemical oxidation is an inexpensive strategy during which oxidants (e.g., persulfate, hydrogen peroxide) are injected into an aquifer to react with contaminants in situ. Specifically, heat-activated persulfate oxidation (HAPO) creates highly reactive sulfate radicals that can mineralize perfluorocarboxylic acids (PFCAs) and some polyfluoroalkyl substances. Sulfate radicals, however, can be scavenged by groundwater constituents, reducing the efficiency of PFCA transformation. In this chapter, I conduct HAPO experiments under conditions realistic to groundwater aquifers; I test low-temperature HAPO, examine inhibitors of successful PFAS destruction at 40°C, and examine different persulfate dosing regimes. I find that weekly doses of persulfate (50-300 mM) are needed for >95% PFAS destruction in aquifer solid-groundwater slurries and that neutralization after treatment results in the removal of dissolved, mobile metals from the groundwater. This chapter provides practical insights to performing HAPO as an in situ PFAS source zone remediation pilot.

Dedications

To Henry and Nancy Cook. I'm honored to be the next Dr. Cook in the family.

To Suzy and John Roper for your strength and support.

To Max and Mila for lighting up this world.

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Finally, thank you to Eric for making the best quesadillas.

Chapter 1

Introduction

1.1 Motivation

Synthetic chemicals play an important role in modern society. There are over 86,000 non-confidential chemical compounds listed on the EPA's Toxic Substances Control Act Chemical Substance Inventory, and anywhere between 25,000 and 86,000 are in use in the United States.[1] The European Chemicals Agency, on its Classification and Labelling Inventory, lists over 205,000 chemical compounds manufactured or imported.[2] Of these, there are 286 perfluorinated compounds listed and over 20,000 fluorinated compounds total. With the sheer number and volume of chemicals in use globally today, managing the production, use, disposal, environmental fate, and potential health consequences of each compound is extremely challenging. Consequently, history is full of instances where chemicals have been used and later found to be toxic and/or harmful to the environment, including: chlorofluorocarbons depleting the ozone layer;[3] trichloroethene, now a known human carcinogen that was used in the food industry and as a dry cleaning solvent; and, more recently, per- and polyfluoroalkyl substances (PFAS), the “forever chemicals” that have been discovered to be extremely environmentally persistent as well as toxic.

Despite these past and inevitable future events, humans use, interact with, and are exposed to innumerable chemicals per week: PFAS in water- and oil-repellent coatings and products, phthalates in shampoos and conditioners, triclosan in antibacterial soaps, surfactants in laundry detergent, food preservatives, carbon monoxide and nitrogen oxides from car exhausts, volatile organic compounds (VOCs) from asphalt and tar off gassing, solvents and VOCs in paints, chlorine and disinfection byproducts in drinking water, brominated flame retardants in household items, and many more. PFAS are a class of chemicals unlike any other; being both hydrophobic and oleophobic, a unique combination, these compounds are used in hundreds of consumer and industrial products across almost all industry branches (e.g., aerospace, biotechnology, electronic industry, energy sector, food production, mining, oil & gas, etc.).[4] Understanding now that PFAS are extremely challenging to transform or remove from water and other point sources for human exposure, the extent of PFAS

contamination will be an important topic of study for decades, if not longer.

The motivation of this dissertation is to contribute to the growing understanding of PFAS, particularly in reference to groundwater contamination and treatment. With this work, I explain how bacteria may biotransform PFAS into different subclasses, show how some PFAS negatively impact co-contaminant-degrading microbial communities, and suggest groundwater remediation strategies for PFAS.

1.2 Properties and Uses of PFAS

Per- and polyfluoroalkyl substances (PFAS) are a chemical class containing thousands of individual compounds, many of which exist in drinking water and in household products that humans use every day.[4] For instance, PFAS are key ingredients in products that boast “stain resistance” and “durable water repellency”, including carpets, upholstery, non-stick cookware, food packaging, cosmetics, and waterproof outdoor wear.[5, 6]

Part of their consumer product ubiquity is because these fluorinated organics resist hydrolysis, thermolysis, and chemical degradation,[7] as well as impart both hydro- and oleophobicity. The stability and persistence of PFAS can be attributed to the fully fluorinated carbon alkyl chains.[8] Fluorine, the most electronegative element, draws covalently shared electrons from the carbon, making the C-F bond short and extremely strong; multiple fluorine atoms bonded to the same carbon atom intensifies this effect. Although most PFAS have this fluorinated alkyl chain, PFAS compounds vary based on carbon chain length, non-fluorinated carbon chain length, and non-fluorinated moieties, creating a vast diversity of PFAS structures and classes.

In addition to countless household products, PFAS have also been used extensively in aqueous film-forming foams (AFFFs), used on military bases and at municipal airports since the 1960s to suppress fuel- and solvent-based fires.[8] In addition, AFFFs have been used for training exercises and equipment checks (i.e., in non-emergency situations). PFAS are the key ingredients for optimal performance in AFFFs (which are composed also of glycol-based solvents and non-fluorinated hydrocarbon surfactants) in part because their surfactant properties, imparted from their hydrophobic and oleophobic tails and their hydrophilic head groups, are able to efficiently lower surface tension, spreading the AFFF into a thin layer between the fuel and oxygen, starving the fire and earning the name film-forming foam.[9] There have been several AFFF manufacturers (e.g., 3M Company, Ansul, National Foam, etc.) each with their own PFAS combinations in their formulae.[10, 11] The users of AFFFs could purchase a range of these products, resulting in groundwater contaminated with potentially dozens or hundreds of different PFAS over the course of decades.

Through manufacturing and industrial discharge, landfilling of PFAS-containing consumer products, and the use of AFFFs, plus their propensity to remain stable in the environment, PFAS have been found contaminating water sources across the globe, making them a top drinking water and groundwater remediation concern.

1.3 PFAS Classes, Terminology, Diversity

There are three major groups of AFFF-related PFAS that contaminate groundwater: perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkyl sulfonic acids (PFSAs), and polyfluoroalkyl substances. PFCAs and PFSAs are fully fluorinated carbon chains with a carboxylic or sulfonic acid head group, respectively, which are deprotonated at environmentally relevant pH ranges (Figure 1.1). PFCAs and PFSAs together are known as perfluoroalkyl acids (PFAAs). These compounds can have a range of fluorinated carbons, though the most commonly used PFAS contain between 3-9 carbon atoms. Especially historically common are the eight-carbon compounds including perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), sometimes referred to as “C8s”.

Polyfluoroalkyl substances, broadly, have a fully fluorinated carbon chain plus a non-fluorinated part of the molecule that is more susceptible to oxidation than the perfluoroalkyl chain; as a result, many can transform into PFAAs via biotic or abiotic processes and are often referred to as “precursors”. [12] More specifically, polyfluoroalkyl substances in AFFFs can be broken into two groups: those produced by electrochemical fluorination (ECF) and those produced by telomerization. ECF-derived precursors are found in 3M AFFFs and often have a sulfonyl group (e.g., sulfonamido) adjacent to the fully fluorinated carbon tail (Figure 1.1). Telomerization-derived precursors are known as fluorotelomers, and often have a non-fluorinated alkyl chain adjacent to the fluorinated carbon tail (Figure 1.1). These distinctions are important because they determine potential transformation products via oxidation processes (e.g., through hydroxyl or sulfate radical oxidation) or via biological processes. Due to the diversity in non-fluorinated head groups as well as the possible variations in fluorinated carbon tail length, there are thousands of PFAS used in industry, and often dozens of precursors and PFAAs can be contaminating AFFF-impacted groundwater. A list of PFAS focused upon in this dissertation are shown in Table 1.1.

Besides these thousands of known PFAS, there are still more being uncovered. Recently, an in-depth analysis of ECF- and fluorotelomer-based AFFFs revealed 40 new classes of PFAS that had yet to be adequately quantified. [13] The majority of the revealed PFAS classes were ECF-derived, meaning that the ECF process creates many unintended products, while the telomerization process produces much fewer. Some of the novel classes of PFAS were found only in tested groundwaters (not in the AFFF formulations), meaning that they were likely the products of biotic and/or abiotic transformation in situ. [13] The scope of possible PFAS present in the environment is staggering, underlining the importance of understanding the fate and transformation of these compounds naturally and via engineered solutions.

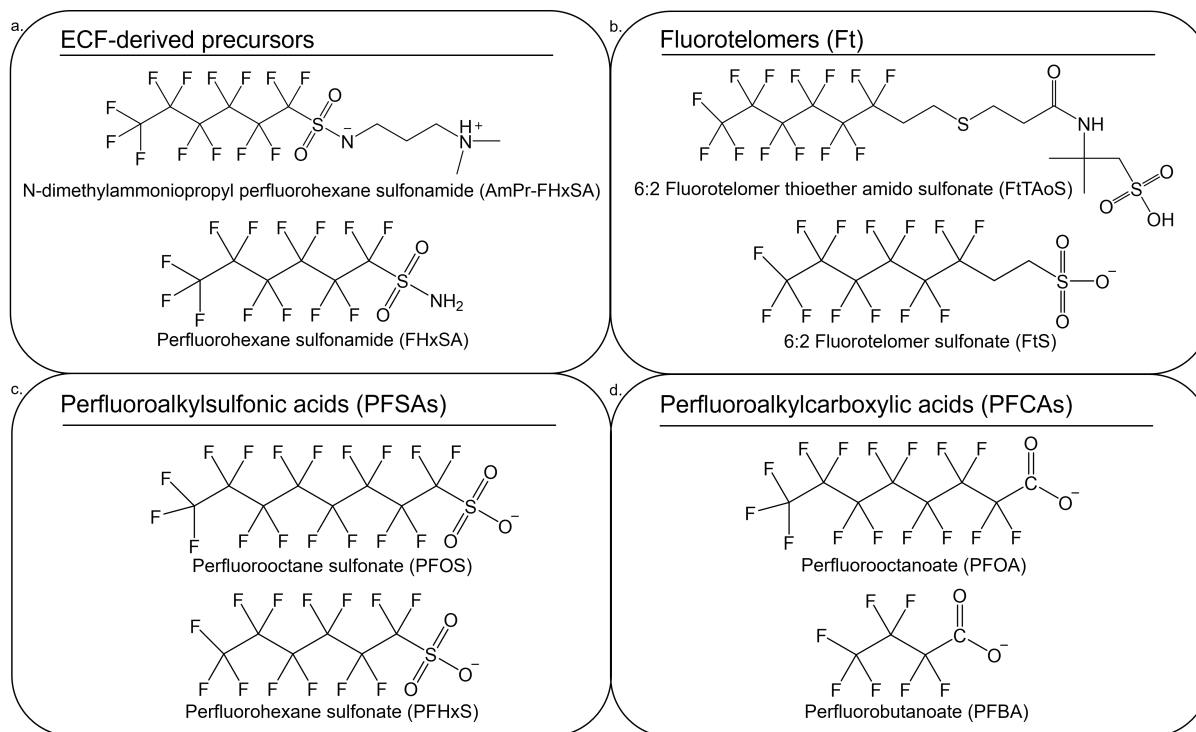


Figure 1.1: Representation of classes of PFAS. a) ECF-derived precursors are found in 3M AFFFs, and often have an SO_2 group adjacent to the fluorinated alkyl chain (see AmPr-FHxSA and FHxSA). b) Fluorotelomer precursors have non-fluorinated carbons adjacent to the fluorinated chain; fluorotelomer naming convention 6:2, for example, refers to the number of fluorinated carbons followed by the number of non-fluorinated carbons adjacent. c) PFSAs and d) PFCAs together are known as PFAAs; these are deprotonated at environmentally-relevant pH values, as shown here.

1.4 Contamination and Prevalence of PFAS

The following sections call out several key PFAS-contaminated matrices and sources, but these are not an exhaustive list. Beyond groundwater, treatment plants, and industrial sites, PFAS exist in oceans, seas, sediment, soils, glaciers, snowpack, rainwater, and even in the blood of polar bears.[14, 15, 16, 17] Their persistence, stability, and widespread use in consumer and industrial products have resulted in their ubiquity.[18, 19, 20, 21] In chemical hazard assessment, regulators often refer to persistence, bioaccumulation, and toxicity together when making decisions on chemicals to watch and regulate. However, a recent paper argued that persistence alone could be a hazard assessor, and should be weighted more heavily in managing a specific chemical.[22] In this paper, the authors list common, major issues surrounding persistent chemicals including: long-range transport,

delayed identification and unexpected effects, and technical and financial implications for remediation.[22] All of these ring true for PFAS contamination. Recent news reports have cited the estimation of \$2.1 billion that the US Department of Defense will have to pay to remediate PFAS from military bases.[23] This estimation does not include the costs of public utilities to monitor and remove PFAS from water and wastewater treatment plants, nor the monitoring of PFAS in landfill leachate, nor the costs for remediation and treatment in the rest of the world. Not to mention, this cost does not include the often invisible costs that PFAS have had, are having, and will continue to have for human health. In short, the properties that make PFAS such useful chemicals in industrial and consumer products, their resistance to degradation, have resulted in their detection in countless environments across the world.

Groundwater

AFFF is one major source of PFAS in groundwater, and is a main focus of this dissertation. Mentioned above, one use of AFFFs was during firefighter training exercises, which could involve igniting a fuel or solvent in an unlined pit or on a concrete slab, then using AFFF to smother the resulting fire.[24] As a result of these activities, as well as the use and historically improper disposal of solvents and other chemicals, AFFF-impacted groundwater often contains many co-contaminants. In addition to the diverse suite of accumulated PFAS, along with other AFFF ingredients such as glycol solvents (e.g., diethylene glycol monobutyl ether (DGBE)) and hydrocarbon surfactants, co-contaminants may include chlorinated solvents such as TCE (a degreasing agent used on aircrafts), 1,4-dioxane (a chlorinated solvent stabilizing agent), and fuel components such as benzene, toluene, ethylbenzene, and xylene (BTEX). The coexistence of these contaminants with PFAS are a major focus of this dissertation.

Landfills are also considered a major source of PFAS in groundwater. PFAS-containing products (e.g., carpets, upholstery, food wrappings, clothes, old cookware, etc.) are often landfilled and, over time, PFAS can leach from these products into what is known as landfill leachate. Similarly to AFFF-contaminated sites, leachates are complex mixtures of contaminants that can be challenging to treat. Unfortunately, landfill leachates have historically been collected and sent to wastewater treatment plants (WWTPs) for treatment. Indeed, a 2017 study analyzing PFAS loadings in 95 leachate samples estimated a yearly PFAS mass release of 600 kg/year into landfill leachate sent to WWTPs.[25] PFAS, however, are not broken down by WWTP processes and these plants become another ongoing, concentrating source of PFAS to the environment.[26]

Water Treatment Plants

As described below in the section “PFAS Recalcitrance to Treatment Technologies”, PFAS are not removed by conventional drinking and wastewater treatment processes. For example, coagulation, flocculation, sedimentation, disinfection (e.g., ozonation, chlorine dioxide,

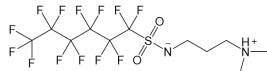
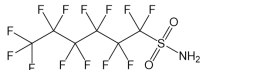
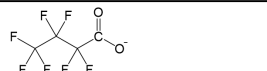
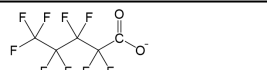
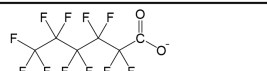
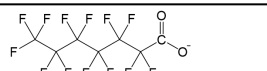
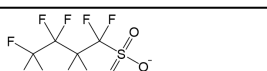
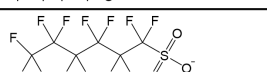
Acronym	Full name	Carbons	Fluorinated Carbons	Structure
Precursors				
AmPr-FH _x SA	N-dimethyl ammonio propyl perfluorohexane sulfonamide	11	6	
FH _x SA	Perfluorohexanesulfonamide	6	6	
PFCAs				
PFBA	Perfluorobutanoic acid	4	3	
PFPeA	Perfluoropentanoic acid	5	4	
PFHxA	Perfluorohexanoic acid	6	5	
PFHpA	Perfluoroheptanoic acid	7	6	
PFOA	Perfluorooctanoic acid	8	7	
PFSAs				
PFBS	Perfluorobutanesulfonic acid	4	4	
PFHxS	Perfluorohexanesulfonic acid	6	6	
PFOS	Perfluorooctanesulfonic acid	8	8	

Table 1.1: PFAS most relevant to this disseration.

chlorination, chloramination), micro- or ultrafiltration, advanced oxidation processes, anaerobic digestion, and activated sludge have all been shown to be ineffective at removing or transforming PFAAs.[7, 27, 28, 29] A common removal strategy is granular activated carbon (GAC) but, as is discussed in later section “Sorption and Removal Technologies”, GAC has variable efficiencies for removing PFAS; for example, shorter PFAS are not removed by GAC as well as longer chain PFAS. There have been some studies showing that precursors can transform into PFAAs during WWTP processes,[30, 31, 32] but because of the recalcitrance of PFAAs, WWTPs concentrate PFAS and have become point sources as wastewater effluent is discharged into environmental waters that then go through drinking water treatment plants.[26]

In 2016, research showed definitively for the first time that 194 public water supplies across the United States were contaminated with PFAS.[26] These suppliers served approximately 16.5 million Americans. This study, though extremely important, quite probably underestimated the number of Americans affected by PFAS exposure in drinking water. For one, this study only examined six PFAS compounds—PFBS, PFHxS, PFOS, PFHpA, PFOA, (see Table 1.1 for structures and full names) and perfluorononanoic acid (PFNA)—and, for another, this study only looked at public water suppliers serving over 10,000 people.[26] Because the researchers did not examine small water systems, groundwater wells, or the myriad more PFAS (e.g., PFBA, PFPeA, PFHxA, perfluoroalkyl ether acids, polyfluoroalkyl substances, etc.) that have since been detected in treatment plants, the number of Americans impacted by PFAS in drinking water is possibly much larger.

In addition to concentrating PFAS in water ways, WWTPs are point sources of PFAS in biosolids. Approximately half of biosolids produced at US WWTPs is land applied as agricultural fertilizer due to its richness in nitrogen and phosphorus; this occurs in all 50 states.[33, 34] Land-applying PFAS-laden biosolids has become yet another national research priority,[35] as PFAS can enter the roots, leaves, and fruits of many types of crops.[36] In addition to this 50% being land applied, about 22% of biosolids are landfilled,[34] potentially serving as another major PFAS source in landfill leachate.

Discharge From Industrial Users and Manufacturers

Industrial contamination is one of the first known sources of PFAS in surface water. For example, the lawsuit that opened the floodgates of PFAS scrutiny in March 2001—of PFAS toxicity and the extent of its use and contamination—surrounded DuPont and their PFAS discharge in Parkersburg, West Virginia.[37] DuPont was purchasing PFOA from its manufacturer, the 3M Company, and while both companies understood that it had toxic health impacts (both for laboratory animals and for their own workers), DuPont did not disclose this information to the public or to the EPA and continued discharging PFOA into the environment.[37] Since then, 3M has also been sued by the state of Michigan for PFAS contamination in the state.

Aqueous industrial sources of PFAS contribute to groundwater and surface water PFAS contamination,[26] but industries and manufacturers can also contribute to the PFAS pollu-

tion cycle via airborne emissions and subsequent deposition.[38] Airborne PFAS release and deposition can also arise from the attempt to destroy PFAS waste at industrial incinerators.

Human Exposure

Humans are exposed to PFAS every day via fast food packaging,[5] non-stick cookware, tap water,[26] and bottled water,[39] and even in dust.[40] Therefore, it may come as no surprise that nearly every human in the United States has PFAS in their bodies. A study in 2007 tested serum samples from a representative sample of over 2,000 American participants;[41] PFOS, PFOA, PFHxS, and PFNA were detected in over 98% of the samples, though concentrations decreased between the two sampling points (2003-2004 sample and 1999-2000 sample) due to industrial phase-out of PFOS between 2000-2002.[42] While some PFAS concentrations in humans decreased, a more recent study has shown an increase in total organofluorine in plasma samples, indicating that humans are still in contact with PFAS in their daily lives, though the PFAS are new and may be unidentified.[43] Some of the studied health impacts of PFAS are discussed in the next section, “Health Impacts of PFAS”.

The PFAS Cycle

From these sections, it is clear that PFAS have integrated themselves into our world in a type of (nearly) indestructible cycle. Figure 1.2 shows a representation of this cycle. Although environmental engineers have been doing what they can to manage PFAS in the environment, the fact remains that PFAS are robust chemicals that resist most forms of degradation: chemical, thermal, and biological.[7] Dubbed “the forever chemicals” by many news outlets, scientists across the world are questioning the full extent of the ubiquitous PFAS contamination that began seven decades ago and will continue for countless more.

1.5 Health Impacts of PFAS

Such ubiquity of PFAS in our lives and environment is startling when considering that several of the most common PFAS have been shown to be toxic and bioaccumulative. For example, the two most historically used and studied PFAS, PFOA and PFOS, have been positively correlated with kidney and testicular cancers, thyroid disruption, and liver damage.[44, 45]

Indeed, as early as the 1960s, companies 3M and DuPont understood that PFOA was toxic to lab animals including rats and rabbits in their own internal toxicity research.[37] Additionally, DuPont conducted toxicity studies on their own workers who interacted with PFOA. After 3M found that PFOA caused rat birth defects, DuPont discovered that out of seven pregnant women in their Teflon division (Teflon is also known as polytetrafluoroethylene (PTFE)), two had children with eye defects.[37] None of these results were shared with the workers or the public at the time.

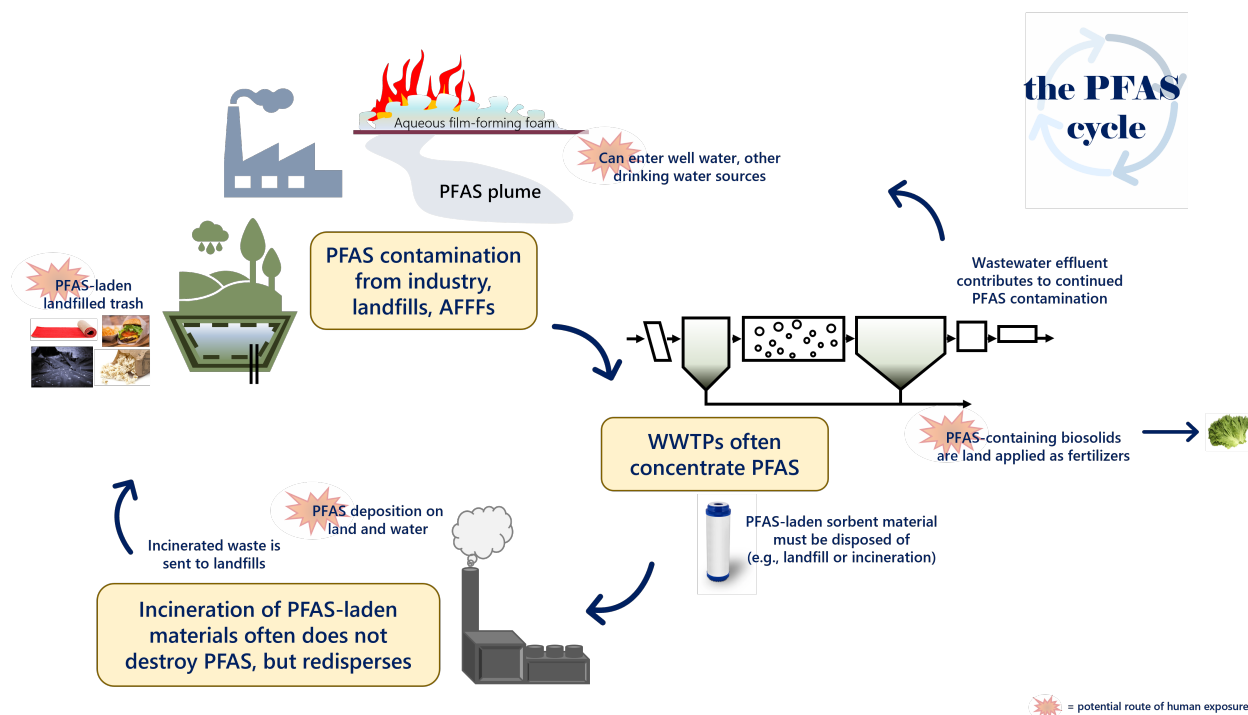


Figure 1.2: A simplistic representation of the global PFAS cycle. Red stars indicate where human exposure can occur.

It has been reported that DuPont had been polluting a West Virginian town, Parkersburg, with PFOA for years, causing illness in its residents and livestock.[37] When all of this information and more was uncovered and made public by the attorney suing DuPont, he—with the town’s permission—used the money won in settlement to conduct a research study on how the PFAS contamination affected the town’s population.[37] This decision was significant because human epidemiology studies for potentially toxic chemicals at this scale are nearly impossible to conduct. From the epidemiological study, the team established definitively that there was a “probable link” between PFOA and the following health impacts: kidney and testicular cancers, thyroid disease, high cholesterol, pre-eclampsia, and ulcerative colitis.[37]

Since then, countless toxicity studies have been conducted internationally on various laboratory fish and mammals as well as bacteria for individual and combined PFAS compounds beyond just PFOA.[46] These studies have confirmed and further linked PFAS with cancer, altered immune and thyroid function, kidney and liver disease, lipid and insulin dysregulation, and negative impacts to reproductive and developmental outcomes.[46]

1.6 PFAS Recalcitrance to Treatment Technologies

Destructive methods that are effective for organic contaminants have been shown to be ineffective in treating PFAS. For example, many chemical oxidants (e.g., ozone, permanganate) and microbial transformation processes such as reductive dehalogenation do not work with PFAS.[47] Additionally, as mentioned previously, PFAS are not susceptible to many conventional water treatment processes. Some removal processes such as GAC, however, are effective but success depends on the chain length and headgroup of the PFAS molecule, as described below. Indeed, a treatment strategy that removes one compound might not remove another. Because PFAS in contaminated matrices most often exist in mixtures with different classes, chain lengths, and head groups, finding one treatment strategy to remove or transform all present PFAS remains challenging.

Sorption and Removal Technologies

Sorption technologies take advantage of the hydrophobicity and headgroup charge of PFAS to adsorb the compounds, removing them from the aqueous phase. The shorter the C-F chain length, however, the less hydrophobic the PFAS (i.e., the more they stay in the aqueous phase, not on a sorbent). While some have suggested that hydrophobicity dominates sorption, electrostatic attraction/repulsion of the head functional groups also contributes.[48] Put together, PFASs usually adsorb better than PFCAs, and long chain PFAS adsorb better than short chain PFAS with the same type of head group. This has been shown to be the case with GAC, ion exchange (IX) resin, and sediments.[29, 48, 49] With PFAS existing in complex mixtures, competition for sorption sites will arise; specifically, long-chain PFAS can displace short-chain PFAS from the sorbent material,[49] resulting in incomplete removal.

Beyond these inconsistencies of adsorptive removal, there is the question of what to do with the PFAS-contaminated sorbents after treating the water. Often, the materials are either landfilled, incinerated, or attempted to be regenerated. GAC regeneration techniques include using solvents such as basic ethanol to desorb the PFAS,[50] which still leaves a PFAS waste stream to manage.

In contrast to adsorption strategies, removal strategies such as filtering are generally more effective for a range of PFAS. Reverse osmosis (RO), for example, effectively removes many PFAS (greater than 90% in most cases, but ranging 50-99% for PFAAs) but concentrates them in its reject stream, which requires further treatment.[27, 29] Nanofiltration performs similarly to RO but, with both technologies, neutral precursors may not be completely removed, as well as some of the shorter-chain PFAAs (e.g., PFBA).[29] Compared to RO, ultra filtration is less effective; one study showed less than 15% removal of PFOA and about 25% removal of PFOS.[27]

Destructive Technologies

Holistic, realistic, and cost-effective PFAS destructive treatment strategies remain elusive despite much research on PFAS transformation. Because PFAS are highly oxidized molecules, some groups have investigated advanced reduction processes, such as using hydrated electrons (also known as solvated electrons). The most common reductive systems studied thus far are UV/sulfite and UV/iodide; these processes work by using ultraviolet light to convert a chemical solute to produce hydrated electrons, which are strong reducing agents (-2.9 V).[51] Hydrated electrons can perform a direct electron transfer with organic compounds, such as PFAS, resulting in defluorination.[51] Some downsides are the high pH values often needed, the need for an anoxic water matrix, the inhibitory effect of co-occurring non-PFAS organics, and the high energy cost from UV irradiation. Additionally, one reaction mechanism of reductive defluorination is the exchanging of H atoms for F atoms on the PFAS molecule; while eventually that can lead to a completely defluorinated alkyl chain, transformation products can include only partially defluorinated PFAS.

Electrochemical treatment is another widely studied PFAS treatment technique. PFAS exposed to an anode (e.g., mixed metal oxide or boron-doped diamond anodes) can be mineralized; the anode extracts an electron from the headgroup of the PFAS molecule, thus beginning a stepwise unzipping process (described in the section “Heat-Activated Persulfate Oxidation (HAPO)”). This process has been shown effective under various conditions for both PFOS and PFOA, plus some mixtures of PFAAs, and researchers are exploring other PFAS matrices, including AFFF-impacted groundwater.[52, 53] While the unzipping mechanism is similar to persulfate oxidation, electrochemical oxidation works for both PFOA and PFOS, while HAPO works only for PFOA (and other PFCAs).

Advanced oxidation processes can also transform various PFAS. While there are many oxidants in use for in situ remediation of organic contaminants, to date, only persulfate is known to mineralize two major classes of PFAS. Heat-activated persulfate oxidation of PFAS is described in detail in the next section and is a major focus of this dissertation. Hydroxyl radicals are another oxidant that can transform PFAS, but results in the transformation of polyfluoroalkyl substances to PFAAs. This process is harnessed in the total oxidizable precursor assay, which is also described below.

1.7 Chemical Oxidation of PFAS

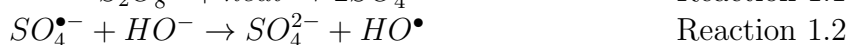
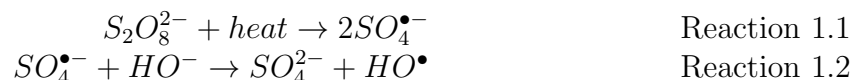
In situ chemical oxidation (ISCO) is a common groundwater remediation strategy that involves injecting an aqueous oxidant into an aquifer for contaminant transformation.[54] There are many types of oxidants (e.g., peroxide, permanganate, ozone, persulfate) that can transform a wide range of organic contaminants (e.g., chlorinated solvents, polycyclic aromatic hydrocarbons, pesticides, fuel hydrocarbons), though this dissertation will focus on the hydroxyl radical and the sulfate radical (via persulfate) for PFAS transformation. As mentioned in “PFAS Classes, Terminology, Diversity”, polyfluoroalkyl substances can be transformed

into PFAAs by chemical or biological oxidation. Using hydroxyl radicals, many precursors can be transformed to PFCAs,[12] but the hydroxyl radical cannot transform the PFAAs further. Sulfate radicals, in contrast, can mineralize precursors and PFCAs at an acidic pH, though are ineffective at transforming PFSAAs.[55] These processes will primarily be discussed in this dissertation in the context of the total oxidizable precursor (TOP) assay,[12] which utilizes hydroxyl radicals, and heat-activated persulfate oxidation (HAPO), which utilizes sulfate radicals.

TOP Assay

The TOP assay was developed as a method to quantify the mass of unknown precursor PFAS in a given aqueous matrix to forms that can be quantified.[12] Precursor PFAS are so diverse in structure—and new forms continue to be discovered—that many of the compounds cannot be quantified by current mass spectrometry methods. Most PFAAs, however, have commercially available mass labeled standards that ensure accurate quantification in many matrices. Therefore, the TOP assay works by converting precursor PFAS into PFAAs that can be measured by liquid chromatography mass spectrometry (LC-MS) (Figure 1.3). By measuring the PFAAs before and after the TOP assay reaction, one can estimate the mass of unmeasurable precursor PFAS in a given sample.

The TOP assay is useful and accessible for estimating the unknown PFAS. The reaction works by adding sodium hydroxide and persulfate (150 mM and 60 mM, respectively) to an aqueous or slurry sample and letting the mixture react for 6-8 hours at 85°C. The heat activates (splits) the persulfate, creating sulfate radicals that instantaneously react with the HO^- , creating hydroxyl radicals (Reactions 1.1 and 1.2).[12]



It has been shown that ECF-based precursors exposed to the TOP assay are transformed into the PFCA of equivalent carbon number (e.g., an eight carbon sulfonamide such as perfluorooctane sulfonamide (FOSA) transforms to the eight carbon PFOA), while fluorotelomers transform into a mixture of shorter chain PFCAs (e.g., 8:2 fluorotelomer sulfonate (FtS) transforms to PFOA, PFHpA, PFPeA, PFBA and a small percentage to PFNA).[12]

The TOP assay is particularly useful as a measurement tool, but some researchers have also experimented with the hydroxyl radical for in situ remediation projects. Precursors, with charged non-fluorinated head groups, are often more sorbtive to soil matrices than PFAAs. Therefore, some have proposed using hydroxyl radicals in situ to transform a mixed PFAS plume into PFAAs to be more easily pumped out of the aquifer for further ex situ treatment processes.[56, 57]

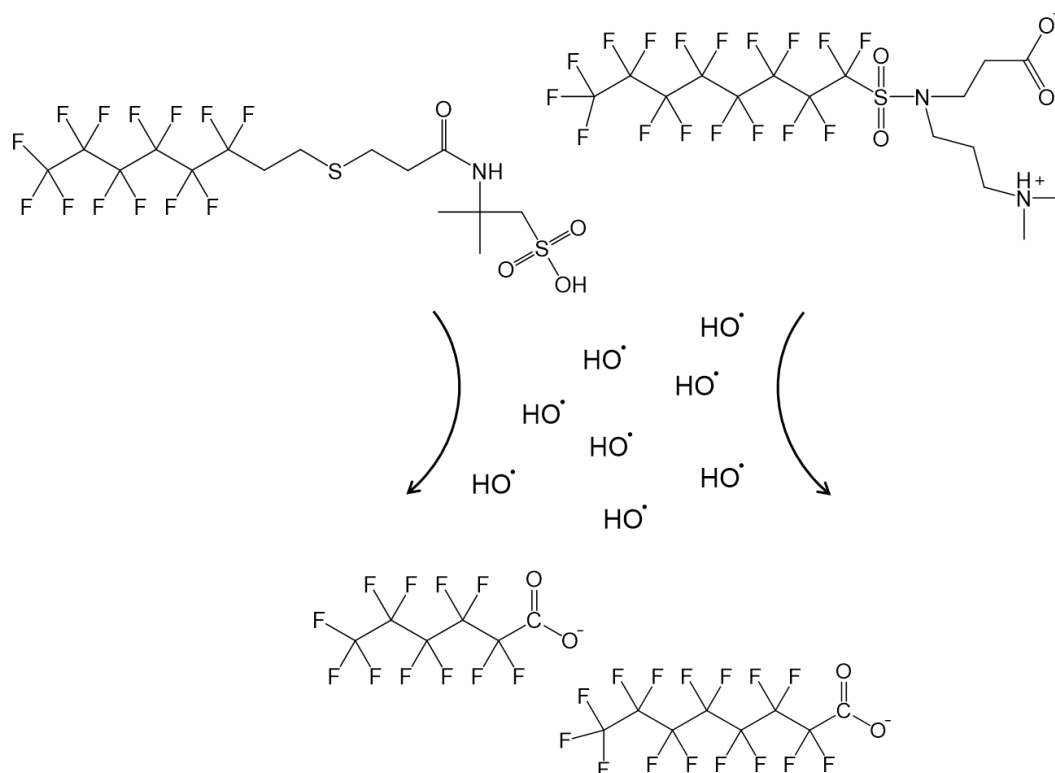


Figure 1.3: A fluorotelomer (left) and ECF (right) precursor can be oxidized by hydroxyl radicals to form PFCAs during the TOP assay. This oxidation method is effective at quantifying the approximate concentration of unknown PFAS precursors in a given PFAS matrix.

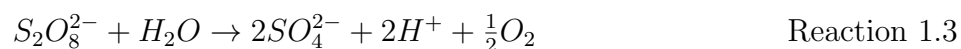
Heat-Activated Persulfate Oxidation (HAPO)

Activated persulfate is known to transform two PFAS classes—PFCAs and precursors—into shorter chain PFCAs and mineralized products.[55, 58, 59] Persulfate can be activated by several mechanisms (e.g., heat, high pH, transition metals) to create one or two sulfate radicals ($\text{SO}_4^{\bullet-}$), which are strong, non-selective oxidants that can transform organic pollutants (Reaction 1.1). Specifically, Bruton & Sedlak showed that HAPO can completely transform PFOA and some precursor compounds into shorter-chain PFCA products, ultimately leading to mineralization into F^- and CO_2 . [55, 58] Precursor PFAS are first transformed into PFCAs, then those produced PFCAs undergo further transformation (Figure 1.4).

A mechanism of PFCA transformation by sulfate radical proposed by Kutsuna and Hori [60] and summarized by Vecitis [7] describes a stepwise “unzipping” of PFOA to create shorter chain PFCAs, which begins with a direct electron transfer from the carboxylate headgroup to the sulfate radical. Interestingly, it has been shown that PFCA destruction by sulfate radical can only occur at a pH <3. To understand this pH dependence, a group

modeled different scenarios of sulfate radical reacting with PFCAs, and hypothesized and defended that deprotonated PFOA reacting with a protonated sulfate radical ($\text{HSO}_4^{\bullet-}$) was the most favorable reaction, which would happen at pH values less than about 3.[61] A more recent study disagreed with the protonated sulfate radical theory, showing that the radical does not protonate when accounting for water molecules in their calculations.[62] Rather, they suggested that the pH dependence of PFCA transformation was attributed to acid catalysis of heat-activated persulfate, coupled with the decrease in sulfate radical scavengers at these acidic pH values.[62]

Regardless of the mechanism, as persulfate degrades to sulfate radical and eventually to sulfate, two moles of protons are produced per mole of persulfate (Reaction 1.3), naturally driving down the solution pH to the required acidity for PFCA mineralization.



This pH requirement, however, proves to be a challenging requirement for successful transformation of PFCAs. For example, groundwater aquifers naturally have buffering capacity in the form of carbonate-based minerals. A buffer will prevent or delay the acidification of the PFAS solution matrix, negatively impacting PFCA destruction. A main focus of this dissertation (Chapter 4) is exploring inhibitors of in situ HAPO for PFAS treatment and suggesting methods to overcome these inhibitions, especially that of aquifer solids.

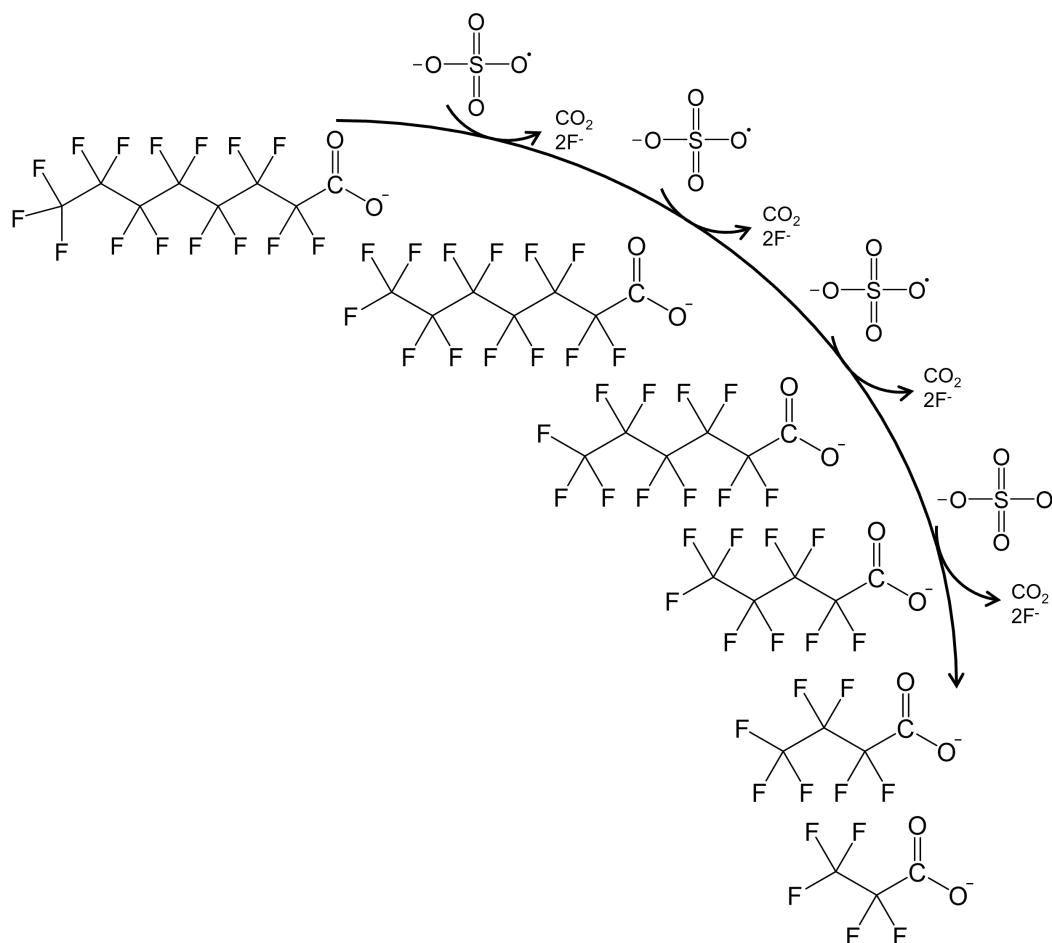


Figure 1.4: Representation of step-wise unzipping of PFCAs by the sulfate radical. These reactions produce CO_2 and F^- as well as the shorter chain PFCAs.

1.8 Biotransformation of PFAS

As recalcitrant as PFAS are, there has been very little success in finding either isolated microorganisms or microbial communities capable of transforming PFAAs. There are no naturally-occurring fluorinated alkyl compounds and, therefore, no microorganisms have been identified to date that have had the evolutionary opportunity to adapt to utilize PFAAs as a carbon or energy source. More research has been done on how microorganisms can transform polyfluoroalkyl substances, or PFAA precursors, under aerobic and anaerobic conditions in WWTPs and in groundwater matrices. This research is important to understand how precursors might transform in situ with existing microbial communities in AFFF-contaminated aquifers. These microbial transformations could result in unknown PFAS biotransformation products as well as increasing the concentrations of PFAAs over time, which are important factors to monitor for management and treatment of AFFF-impacted sites. While there have been many studies investigating precursor biotransformation by activated sludge in WWTPs, this section will focus on biotransformation of AFFF-based precursors under groundwater relevant conditions.

Transformation of Fluorotelomer Polyfluoroalkyl Substances

Fluorotelomer precursors can be biotransformed into PFCAs under aerobic conditions.[63] Several types of fluorotelomers have been investigated under groundwater conditions, including fluorotelomer alcohols (FTOH) and fluorotelomer thioether amido sulfonates (FtTAoS) (Table 1.2). While they can ultimately transform to PFCAs, both 8:2 and 6:2 FTOH form intermediate products including the fluorotelomer with a carboxylic acid or ketone head-group replacing the alcohol, which can then transform to PFCAs of equal or fewer carbon number.[64] Incubation times range from 1-7 months and PFCA yield ranges from 1-40%.[64]

8:2, 6:2, and 4:2 FtTAoS were investigated for aerobic biotransformation using an enrichment culture seeded by soil from an AFFF-contaminated air force base.[65] Each of these three compounds disappeared from the cultures within 20 days of incubation, producing transformation products such as fluorotelomer sulfonates (FtS), fluorotelomer carboxylic acids (FTCA), fluorotelomer unsaturated carboxylic acids (FtUCA), and PFCAs (Table 1.2).[65]

6:2 FtTAoS was also investigated under anaerobic, sulfate-reducing conditions; this study resulted in about 75% of parent product transformation over the course of 270 days—much slower than the aerobic study.[66] Additionally, this study showed no similar transformation products compared to the aerobic study; no PFCAs were produced under sulfate-reducing conditions, emphasizing that PFAS precursors will produce different biotransformation products depending on the aquifer's redox conditions.

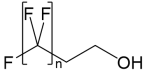
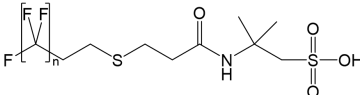
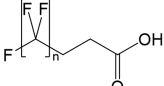
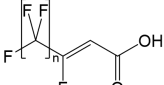
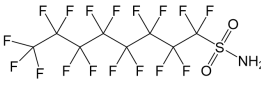
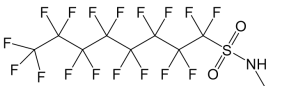
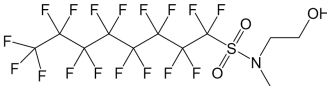
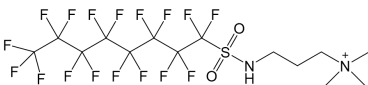
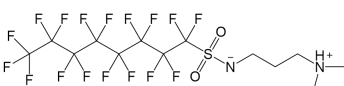
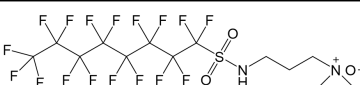
Acronym	Full name	Structure
Fluorotelomers		
FTOH	Fluorotelomer alcohol $n = 3, 5, 7$	
FtTAoS	Fluorotelomer thioether amido sulfonate $n = 3, 5, 7$	
FTCA	Fluorotelomer carboxylic acid	
FtUCA	Fluorotelomer unsaturated carboxylic acid	
ECF-based sulfonamide precursors		
FOSA	Perfluorooctane sulfonamide	
EtFOSA	N-ethyl perfluorooctane sulfonamide	
EtFOSE	N-ethyl perfluorooctane sulfonamido ethanol	
PFOSAmS	Perfluorooctane sulfonamide quaternary ammonium salt	
AmPr-FOSA	N-dimethyl ammonio propyl perfluorooctane sulfonamide	
PFOSNO	Perfluorooctane sulfonamido amine oxide	
PFOSi	Perfluorooctane sulfinate	

Table 1.2: PFAS precursors that have been studied for biotransformation pathways under groundwater relevant conditions.

Transformation of ECF-Based Polyfluoroalkyl Substances

Many ECF-based precursors are known as PFSA precursors in biotransformation studies due to the sulfonyl group adjacent to the fully fluorinated alkyl chain.[63] Of these sulfonamido precursors, perfluorooctane sulfonamide (FOSA), N-ethyl perfluorooctane sulfonamide (EtFOSA), N-ethyl perfluorooctane sulfonamido ethanol (EtFOSE), perfluorooctane sulfonamide quaternary ammonium salt (PFOSAmS), perfluorohexane sulfonamido amine also known as N-dimethyl ammonio propyl perfluorooctane sulfonamide (PFOSAm also known as AmPr-FOSA), and perfluorooctane sulfonamido amine oxide (PFOSNO) have been studied for biotransformation pathways (Table 1.2).[67, 68, 69]

Researchers hypothesize similar aerobic biotransformation pathways of these eight-carbon sulfonamido precursors. If the compound has a terminal carboxylic acid headgroup (e.g., PFOSB), this group may first be cleaved, leaving a secondary amine as the terminal group.[69] From here, methyl groups on the secondary amine may be cleaved, followed by the N-group itself, leaving a propanoic or acetic acid head group adjacent to the sulfonamide group.[67, 69] Irrespective of the exact pathway, these results suggest that the sulfonamide FOSA is a key transformation product, leading to PFOS sometimes with the eight-carbon sulfinate—perfluorooctane sulfinate (PFOSi)—as a proposed intermediate between FOSA and PFOS.[69]

These studies all focus on the eight carbon precursors, which eventually can form PFOS. Less studied, however, and a focus of this dissertation (Chapter 2), is the biotransformation of the six carbon ECF-based precursors such as N-dimethyl ammonio propyl perfluorohexane sulfonamide (AmPr-FHxSA) using a range of microbial communities by exposing an enrichment culture to different carbon sources.

Potential Biotransformation of PFAAs

There have been decades of research on microbial dehalogenation, specifically dechlorination, for the organic groundwater contaminants perchloroethene (PCE) and TCE. Dechlorinating microorganisms, such as species within *Dehalococcoides*, can utilize chlorinated solvents as their electron acceptor while using H_2 as an electron donor and acetate as a carbon source. When PFAS research became prominent, many environmental microbiologists attempted to use dechlorinating cultures to transform PFAAs, hoping that they may be shown to perform defluorination of these fluorinated alkyl chains. Unfortunately, however, there has been no published work of successful defluorination of PFAAs using these dechlorinating bacteria.

A recent study, however, reported the success of an ammonium-oxidizing, iron-reducing bacterium, *Acidimicrobium* sp. strain A6, that successfully transformed PFOS and PFOA.[70] While there was only about 50% removal of PFOA and about 23% removal of PFOS over 100 days, the study remains unique in reporting the defluorination of two PFAAs. They reported fluoride concentration increases as well as some shorter chain PFAAs as transformation products.[70] While promising, this study has not yet been confirmed by outside

researchers, no doubt partly due to the difficulties growing and maintaining the reported culture.

1.9 Impact of PFAS on Biotransformation of Co-Contaminants

Although microorganisms may be able to biotransform precursor PFAS, some PFAS, especially at higher concentrations, could be toxic and inhibitory to microorganisms. These negative impacts are especially important to consider when investigating bioremediation of common PFAS co-contaminants. When it comes to the impacts of PFAS on cells, there are many more studies on eukaryotic cells for insights on human and animal health impacts [71, 72, 73, 74] or on model membranes [75, 76] compared to bacterial studies, which are more important for bioremediation. Of the bacterial studies, groups have investigated the effects of PFAS on the phospholipid bilayer membranes as well as inhibitory effects of high concentrations of PFAS and AFFFs. It has been found that both long and short chain PFAS partition into phospholipid membranes of gram-negative and gram-positive bacteria.[77] and that PFOS and PFNA can increase membrane permeability of *Aliivibrio fischeri*. [78] Bacterial cells rely on a robust membrane to create a proton gradient (protons enriched on the outside of the membrane) for energy production via the electron transport chain. Therefore, disrupting cell membranes and increasing permeability can increase the movement of protons across the membrane, disrupting this gradient and reducing the efficacy of cellular energy production.

When exposing high concentrations of AFFFs to a TCE-dechlorinating enrichment culture, fluorotelomer-based AFFFs Ansul and National Foam inhibited TCE degradation, while 3M AFFF slowed dechlorination but did not completely inhibit as the other foams did.[79] The same study showed inhibition of TCE dechlorination in the presence of isolated 6:2 FtSaB, a key ingredient in the fluorotelomer-based AFFFs showing that this PFAS was particularly inhibitory, rather than simply the presence of surfactants in the AFFFs generally.[79] Another study showed inhibition of PFAAs (when amended on the order of mg/L) on both a dechlorinating enrichment culture as well as isolated *Dehalococcoides mccartyi* strain 195.[80] In the enrichment culture, the presence of PFAAs repressed the main dechlorinating bacterium and resulted in an increase in abundance of methanogenic archaea.[80] These studies were all performed under anaerobic conditions. Interestingly, aerobic toluene-degrading bacterium *Rhodococcus jostii* strain RHA1 exposed to PFAAs did not exhibit substrate degradation inhibition, but did express an increase in biofilm formation as well as stress-related genes.[81] These comparisons may point to aerobic bacteria being more resistant to the presence of PFAS compared to anaerobic cultures.

While these studies focused on higher concentrations of PFAS to observe when inhibition may occur, what is lacking is an investigation on the impacts of lower, environmentally-relevant concentrations of PFAS on microbial functions, especially on the functions and effi-

cacy of co-contaminant-degrading bacterial communities. Additionally, comparing the effects of individual PFAS compounds, especially the more common six-carbon precursor PFAS, on both aerobic and anaerobic microbial communities will be helpful to elucidate which PFAS may be more harmful to bioremediation processes at a particular AFFF-contaminated site.

1.10 Outline of Dissertation

The goal of this research was to investigate biological and chemical transformation of different PFAS in AFFF-contaminated groundwater. Biotransformation of AFFF-relevant PFAS precursors (Chapter 2) is still a developing field, and it is important to understand which precursors may biologically transform to produce PFAAs, at what yields, and under which conditions. Next, I wanted to investigate how high concentrations of PFAS near source zones may inhibit bioremediation processes of common co-contaminants TCE and BTEX (Chapter 3). Finally, with chemical transformation, HAPO has been shown to be effective at transforming PFCAs at high temperatures with low solids concentrations. My goal was to expand on that understanding—to lower the reaction temperature to one that is more field-feasible, investigate the reaction of PFAS with common co-contaminants, determine what range of persulfate doses would be required for an ISCO site using HAPO, and to test post-HAPO neutralization of the groundwater slurry solution (Chapter 4). These goals and my results are addressed in the following chapters:

Chapter 2: Biological and Chemical Transformation of the Six-Carbon Polyfluoroalkyl Substance N-dimethyl ammonio propyl perfluorohexane sulfonamide (AmPr-FHxSA)

AmPr-FHxSA was tested for biotransformation pathways and product yields using four different microbial communities enriched using different carbon sources. Biotransformation products were compared between the four communities and with chemical oxidation products for a thorough understanding of the behavior of AmPr-FHxSA and how to manage it at AFFF-contaminated sites. This chapter has been submitted for publication; co-authors are listed in Appendix A.

Chapter 3: PFAS Impacts on the Bioremediation of Co-Contaminants TCE and BTEX

An anaerobic co-culture that dechlorinates TCE and an aerobic enrichment culture that degrades BTEX were exposed to different PFAS at two concentrations to determine how PFAS may inhibit bioremediation at source zones. Bacterial cells were analyzed for adenosine triphosphate and amino acid production, as well as contaminant degradation. The conditions were compared for a better understanding of PFAS toxicity mechanisms on co-contaminant-degrading bacteria.

Chapter 4: Considerations for Applying In Situ Heat-Activated Persulfate Oxidation for PFAS Treatment

HAPO was tested at 40°C with co-contaminants and high concentrations of aquifer solids to inform persulfate dosing requirements for an in situ HAPO field project.

Chapter 5: Conclusions

The implications of this work are discussed in this chapter, as well as suggestions for future research needs.

Chapter 2

Biological and Chemical Transformation of the Six-Carbon Polyfluoroalkyl Substance AmPr-FHxSA

2.1 Introduction

Aqueous film-forming foam (AFFF), used on military bases and at municipal airports since the 1960s, is a major source of per- and polyfluoroalkyl substances (PFAS) in groundwater.[13] In the last two decades, AFFF manufacturers have transitioned from primarily long-chain perfluoroalkyl acids (PFAAs) and polyfluoroalkyl substances that can be PFAA precursors in favor of shorter chain (≤ 6 perfluorinated alkyl chain) PFAS.[42] However, historical AFFF formulations were not entirely based on the “C8” chemistries. For example, 3M Lightwater contained significant quantities of precursors to perfluorohexane sulfonate (PFHxS),[18] which persists in the environment, can bioaccumulate, and is toxic to humans and animals.[82, 83, 84, 85] To address this hazard, individual states have adopted strict drinking water standards, including 18 and 51 ng/L for PFHxS in New Hampshire and Michigan, respectively.[86, 87]

PFAAs can form in the environment through biotic and abiotic transformation of polyfluoroalkyl substances (i.e., precursors) used in AFFF formulations.[68, 69] For example, fluorotelomer polyfluorinated compounds are aerobically biotransformed to perfluoroalkyl carboxylic acids (PFCAs).[63, 65] Some perfluorooctyl-based polyfluorinated substances produced by electrochemical fluorination (ECF), such as perfluorooctane sulfonamide (FOSA), undergo aerobic biotransformation to produce perfluoroalkyl sulfonic acids (PFSAs), albeit at rates slower than those observed for fluorotelomers.[30, 67] Understanding precursor biotransformation and biotransformation intermediates are important in determining optimal remediation strategies and improving environmental fate and forensic analyses.

Remediation of AFFF-impacted sites is often complicated by the presence of multiple co-contaminants, creating many possible carbon sources for microbial communities.[88, 9] These potential carbon sources, likely present at higher concentrations than the PFAS, could drive precursor biotransformation through their effect on the microbial community composition and function.[89] Co-contaminants at these sites may include non-PFAS organics such as hydrocarbons and/or glycols in the AFFF formulations (often as diethyl glycol monobutyl ether (DGBE)),[79] as well as fuel components such as benzene, toluene, ethylbenzene, and xylene (BTEX).[11] Methane also may be present from methanogenic activity in microbial communities, and acetate is a common fermentation product from electron donors (e.g., lactate) added at bioremediation sites stimulated for detoxification of chlorinated solvents.[90] These latter two carbon sources could be especially important at transition zones between groundwater and surface waters, between anaerobic and aerobic zones.

Olivares et al found that PFAS enrichment cultures supplemented with BTEX as an additional carbon source resulted in a greater percentage of PFAA transformation products formed from the parent 6:2 fluorotelomer thioether amido sulfonate (FtTAoS), a major fluorotelomer found in Ansul AFFFs, compared to a treatment supplemented with DGBE as the carbon source.[91] These results indicate that at sites where biotransformation is occurring, the composition of precursors and PFAAs is likely dependent on the carbon sources present, which could influence the choice of remediation strategy. However, the impact of carbon source has yet to be investigated for ECF-based precursor transformation product composition. Several perfluorooctyl ECF-based precursors have proposed biotransformation pathways,[69] but their six-carbon analogues such as N-dimethyl ammonio propyl perfluorohexane sulfonamide (AmPr-FHxSA), a zwitterionic precursor often found at AFFF source zones[92, 93] have not been investigated and compared. Moreover, the divergence of pathways caused by different microbial communities has not been tested. Because PFHxS, potentially a biotransformation product of AmPr-FHxSA,[18, 69, 30, 67, 94, 95] is bioaccumulative and an immune suppressant,[85] it is important to understand the ongoing sources of PFHxS exposure and other potentially harmful perfluorohexyl-based transformation products that could be produced by microbial activities in the environment.

While biological transformation of ECF-based precursors can lead to the formation of PFSA, chemical transformation yields PFCAs that are more easily treated. For example, many ECF-based precursors including N-ethyl perfluorooctanesulfonamide (N-EtFOSA) and FOSA have been shown to produce PFCAs during chemical oxidation with hydroxyl radicals,[96, 12, 97] which can be mineralized by activated persulfate oxidation.[55, 58] PFSA are not reactive in these oxidative radical treatments. Understanding the conditions that promote biotic production of PFSA can inform remediation decisions, such as utilizing chemical oxidation before PFSA concentrations can increase from microbial transformation of precursors.

The objective of this study was to compare AmPr-FHxSA aerobic biotransformation products under different carbon sources to understand product yield and composition with a range of aerobic microbial communities and to demonstrate that chemical oxidation can result in AmPr-FHxSA mineralization, which could be used as a remediation strategy. We

hypothesized that biological transformation would likely end in PFHxS with perfluorohexane sulfonamide (FHxSA) as a key intermediate, while chemical transformation would produce perfluorohexanoic acid (PFHxA) that could be mineralized by acidic sulfate radical oxidation. We also hypothesized that the rates of biotransformation and product yields of AmPr-FHxSA would be influenced by the carbon source. We created enrichment cultures from an AFFF-contaminated site by adding four carbon sources (methane, acetate, DGBE, and BTEX) separately, evaluated the resulting divergent microbial communities, and compared biotransformation products with chemical oxidation products. We analyzed these pathways to better understand how to treat and manage this six-carbon ECF-based precursor, as well as other PFAS with similar structures.

2.2 Materials and Methods

Chemicals

PFAS analytical standards (C4—C10 PFCAs; 4:2, 6:2, and 8:2 fluorotelomer sulfonates; 5:3, 7:3, 6:2, 8:2 fluorotelomer carboxylic acids; 6:2, 8:2 fluorotelomer unsaturated carboxylic acids, AmPr-FHxSA, and FHxSA) and mass labeled standards were purchased from Wellington Laboratories (Guelph, ON, Canada). AmPr-FHxSA used for biotransformation experiments was purchased from Chemieliva Pharmaceutical Co., Ltd (China, 97%) and FHxSA from Synquest Laboratories Inc. (USA, 95%). DGBE was purchased from TCI chemicals (>99%). BTEX compounds (>98%), methane (99%), and sodium acetate, were purchased from Sigma Aldrich. All other chemicals used in media or analyses were purchased from Sigma Aldrich or Fisher Scientific at the highest purity available. The ECF-based AFFF used in the first microcosm test was a Cal Guardian 3M AFFF 3%, generously given to our lab by Jennifer Field. Concentrations of PFAS in this formulation are included in Table B.1.

Microcosms

Experiments were performed with either diluted AFFF containing AmPr-FHxSA or AmPr-FHxSA by itself. AFFF-based microcosm experiments are described in SI Text B.1. For the four carbon source experiment, AmPr-FHxSA was added to each condition. Each enrichment consisted of triplicate live treatments and autoclaved controls. One set of triplicate medium-only controls were conducted without any of the four carbon sources added. All controls were amended with 0.5 mL of an antibiotics mixture (1 mg/mL in MilliQ water of kanamycin, chloramphenicol, and streptomycin) to suppress biological activity.[98] All bottles contained 19.5 mL of ammonium mineral salts (AMS) media (Text B.3), 0.5 mL of phosphate buffer, and 5 mL of inoculum that contained trace amounts of soil (Text B.5) (5 mL water added to medium-only controls) in 160 mL glass serum bottles. Methane, acetate, DBGE, and BTEX were added to achieve a target concentration of about 16 mM carbon. AmPr-FHxSA was added from a 10 mM stock solution (in 1:1 isopropyl alcohol: methanol) to a final

concentration of approximately 0.2 μM . Background PFAS concentrations from the AFFF-impacted soil-derived inocula were 1-2 orders of magnitude lower than the concentration of AmPr-FHxSA; peak areas measured by high resolution mass spectrometry (HRMS) from Day 0 are listed in Table B.5, while quantitative data from a liquid chromatography triple quadrupole mass spectrometer (LC-MS/MS) are listed in Table B.6. Since only AmPr-FHxSA was added to this experiment, the remaining PFAS present were assumed to come from the inocula.

The microcosms were placed horizontally in a 30°C shaking incubator and sampled (500-700 μL) weekly for the first half of the experiment, then every 14 days. Aqueous samples were stored at -20°C and were measured within a week. Samples for PFAS analysis were immediately diluted in basic methanol (2% NH_4OH) (25% sample in final mixture) and stored at -20°C until LC-MS/MS analysis. Oxygen was monitored weekly and supplemented when below 5% of the headspace (Text B.4; Figure B.3). Carbon sources were measured weekly and amended when needed (weekly for DGBE, acetate, and BTEX, once for methane). DOC was measured as a proxy for DGBE and acetate (Figure B.4). BTEX and methane were measured directly with a gas chromatograph coupled to a flame-ionization detector (GC-FID) (Text B.4; Figures B.5 and B.6). Adenosine Triphosphate (ATP) was also monitored as a proxy for biological activity (Text B.6, Figure B.7).

Total Oxidizable Precursor Assay and Heat-Activated Persulfate oxidation

The total oxidizable precursor (TOP) assay[12] was performed on the final time point of the four carbon source experiments as well as pure AmPr-FHxSA and FHxSA. For the microcosms, 50 μL of slurry samples were diluted in 750 μL of MilliQ water with sodium hydroxide and sodium persulfate (67 mM and 20 mM). These mixtures were left overnight in an 85°C water bath, quenched with methanol, and neutralized with HCl. For the pure compounds, concentrated stocks of AmPr-FHxSA and FHxSA were diluted (200-400 nM) into MilliQ water with 120 mM sodium hydroxide and 60 mM sodium persulfate (3 mL total volume). After 12 hours of reaction time, 3 mL of methanol was added to the solutions, and these were stored at 4°C until LC-MS/MS analysis.

To simulate conversion of ECF-based precursors during in situ chemical oxidation with heat-activated persulfate, AmPr-FHxSA and FHxSA (200-400 nM) were separately added to a 20 mL solution of MilliQ water and 50 mM sodium persulfate. These were placed in an 85°C water bath for 24 hours; the heat-activated persulfate caused the solution pH to drop and remain below 3 for the experiment. Unheated controls were kept at 4°C to prevent heat-activation. Samples were immediately diluted (10x) in basic methanol (2% NH_4OH) and stored at -20°C.

LC-MS/MS

Samples stored in methanol were vortexed, centrifuged (10 minutes at 15,000g), and transferred into LC-MS/MS vials with mass-labeled internal standard mixture (2 $\mu\text{g/L}$ [^{13}C]-PFAS) (Table B.2). Samples were analyzed with an Agilent 1260 Infinity Binary HPLC System coupled to an Agilent 6460 triple quadrupole mass spectrometer. Chromatographic separation was achieved using a Zinc-Diol (12.5 $\times$ 4.6 mm, 6 μm) and Zorbax C18 XDB guard column (12.5 $\times$ 4.6 mm, 5 μm) before a Zorbax C18 XDB analytical column (50 $\times$ 4.6 mm, 1.8 μm). The LC-MS/MS configuration was modified to decrease PFAS contamination from the system as reported before.[12] Samples were run in ESI +/- “pole-switching mode” within the same injection. The mobile phase was a water/methanol gradient, each with 10 mM ammonium acetate at 0.4 mL/min (Table B.3). HRMS analysis is described in the SI (Text B.7).

Microbial Community Analyses

Genomic DNA was isolated for 16S rRNA gene amplicon sequencing at the end of the carbon source experiment. Biomass slurry samples (2 mL) were taken from each live treatment on day 70 and centrifuged at 10,000g for 10 minutes. After decanting the supernatant, DNA was extracted from pellets using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. DNA quantity and quality was assessed using a NanoDrop Spectrometer (Thermo Fisher Scientific, Wilmington, DE, USA). The extracted DNA was stored in sterile, parafilm 1.5 mL centrifuge tubes at -80°C and sent to Novogene Corporation Inc. (Sacramento, CA, USA) for bacterial 16S rRNA amplification and sequencing (Text B.8).

2.3 Results and Discussion

Targeted PFAS Analysis

Biotransformation of AFFF containing AmPr-FHxSA was first attempted but results were confounded by suspected inhibition by high concentrations of PFOS, the presence of multiple parent compounds, and abiotic loss of parent compounds (Text B.9, Figures B.1 and B.2). To isolate possible aerobic biotransformation pathways of AmPr-FHxSA in the absence of PFOS or other inhibitors in AFFF, we performed biotransformation experiments by adding only AmPr-FHxSA with either methane, acetate, DGBE, or BTEX as a carbon source. We hypothesized that enrichments developed from these four conditions would result in functionally divergent microbial communities with different transformation products and yields. After 70 days of incubation, the concentration of AmPr-FHxSA decreased under all conditions but had the most significant disappearance (83% decrease) in the acetate-oxidizing cultures, followed by the methane-oxidizing cultures (Figure 2.1). The live methane cultures resulted in the highest concentrations of generated targeted transformation products (Figure

2.1a-c)—about 70 nM and 9 nM of FHxSA and PFHxS, respectively, or approximately 40% and 6.7% of the loss of the parent.

Similar to the AFFF biotransformation experiment (Text B.9), AmPr-FHxSA decreased in the medium-only (Figure B.8) and autoclaved controls (Figure 2.1) but without detectable FHxSA or PFHxS. Some AmPr-FHxSA loss could be explained by trace amounts of soil or biofilms not captured in sampling, as the autoclaved controls had less AmPr-FHxSA loss compared to the medium-only controls (Table B.8, Figure B.8). However, because of the medium-only controls, we believe that the majority of the AmPr-FHxSA decrease is primarily due to sorption to the container. Low recovery could also have occurred from sorption to the needle syringe used for sampling or from aggregation or accumulation at interfaces, including air-water.[99] Studies have shown that AmPr-FHxSA has a propensity to sorb to surfaces,[93] can be unstable from two oxidizable nitrogens and a lone pair of electrons,[69] and perhaps has temperature-dependent instability like other sulfonamides.[100] Despite the decrease in AmPr-FHxSA, autoclaved and medium-only controls did not form the stable intermediate FHxSA or terminal product PFHxS (Figure 2.1). Because of the challenges with AmPr-FHxSA, we prioritized analyzing the differences in transformation products between the biotic cultures and abiotic controls.

In the methane, acetate, and DGBE cultures, approximately 12-15 nM FHxSA was present at the start of the experiment due to the AFFF-impacted inoculum source (Table B.6); this background was not in the BTEX culture, presumably because its inoculum source was older and had been amended with fresh media several times, diluting FHxSA below the limit of detection. Although the DGBE and BTEX cultures did not produce FHxSA, low concentrations (2.6 and 2.1 nM, respectively) of PFHxS were produced. This lack of FHxSA suggests that AmPr-FHxSA might be able to transform to PFHxS without accumulation of FHxSA, or that other intermediates not targeted in the LC-MS/MS method can be hydrolyzed directly to PFHxS. Given the stability of FHxSA in the environment,[95] it is unlikely that this compound was formed as an intermediate that was quickly transformed to PFHxS.

We compared the percentage of FHxSA and PFHxS production (based on initial parent concentration) in the live controls to the perfluorooctyl-based analogue in literature. In this study, we found FHxSA production to be approximately 40% and 36% in the methane and acetate cultures, respectively, after 70 days compared to Liu et al[69] who reported approximately 7.5% FOSA production after 90 days. PFHxS production was 5%, 2%, 2%, and 1% for methane, acetate, DGBE, and BTEX cultures over 70 days compared to 2.6% PFOS production after 90 days. Although it is challenging to compare based on the different microbial communities and conditions, our results suggest that some aerobic microbial communities can result in much faster rates of FHxSA production compared to FOSA and slightly faster or similar rates, depending on the community, of PFHxS production compared to PFOS.

Additionally, because some literature suggests a difference in biotransformation rates of branched versus linear isomers of ECF-based precursors,[98] we examined the ratios of branched versus total (linear + branched) peak areas of AmPr-FHxSA and PFHxS on day 70 (Table B.9). Linear AmPr-FHxSA precursors may be more readily transformed into PFHxS

by soil microbial communities (discussed in Text B.10).

To evaluate the mass balance, we performed the TOP assay on day 70 samples for the four carbon source treatments. FOSA and similar eight carbon ECF-based precursors have been shown to produce PFOA during the TOP assay.[12, 97] Therefore, we expected the TOP assay to produce PFHxA in concentrations similar to the initial concentration of AmPr-FHxSA, minus the PFHxS produced biotically. Indeed, the only detected PFAAs after the TOP assay were PFHxA and PFHxS (Figure B.9) but resulted in incomplete balances likely due to sorption of AmPr-FHxSA as mentioned. An appreciable fraction of the PFHxA produced likely came from the oxidation of FHxSA, which was a biotransformation product during the experiment. For example, 70 nM of FHxSA was produced by day 70 in the methane enrichments, which, if completely transformed to PFHxA during the TOP assay, would account for about 77% of the PFHxA produced during the TOP assay. For the acetate, DGBE, and BTEX cultures, this ratio was 45, 20, and 3.5%, respectively. The remaining PFHxA produced during the assay presumably came from other biotransformation products and/or from the remaining AmPr-FHxSA. Interestingly, the PFHxS concentrations after the assay were greater than the concentrations measured on day 70 of the biotransformation experiment. As will be discussed, this could point to some transformation of perfluoroalkyl sulfonamides to PFHxS during the TOP assay.

Non-targeted PFAS Analysis

We performed HRMS on samples from days 0 and 70 of the live treatments, and on day 70 of the autoclaved sets to explore non-targeted transformation products (Tables B.5, B.7). Combining the results from HRMS and LC-MS/MS, in addition to some proposed transformation products in recent literature and in *enviPath* (Figures B.12 and B.13), a transformation pathway is proposed in Figure 2.2. Overall, the methane and acetate cultures had the greatest number of transformation products identified, both containing perfluorohexanesulfonamido propanoic acid (FHxSA-PrA), perfluorohexane sulfonamido acetic acid (FHxSAA), FHxSA, perfluorohexane sulfinate (PFHxSi), and PFHxS, and the acetate cultures additionally containing N-oxidedimethylammoniopropyl-perfluorohexanesulfonamide (OAmPr-FHxSA). In the DGBE cultures FHxSAA, PFHxSi, and PFHxS were measured, and in the BTEX cultures only PFHxSi and PFHxS. Perfluoroalkyl sulfonamido acids such as FHxSA-PrA and FHxSAA have been detected in the environment, including in AFFF-impacted groundwater and landfill leachate.[13, 101, 102]

We detected FHxSA-PrA in the methane and acetate live cultures, as well as one DGBE replicate, though at about an order of magnitude lower peak area than the acetate and methane cultures (Table B.7). On the proposed pathway between AmPr-FHxSA and FHxSA-PrA, we include potential demethylated intermediates based on *enviPath* predictions (Figures B.12 and B.13) and Liu et al's[69] investigation of perfluorooctyl-based ECF-based precursor biotransformation. Also proposed on *enviPath* and in literature was an aldehyde intermediate (Figure 2.2).[103] FHxSA-PrA was additionally measured in one of the DGBE

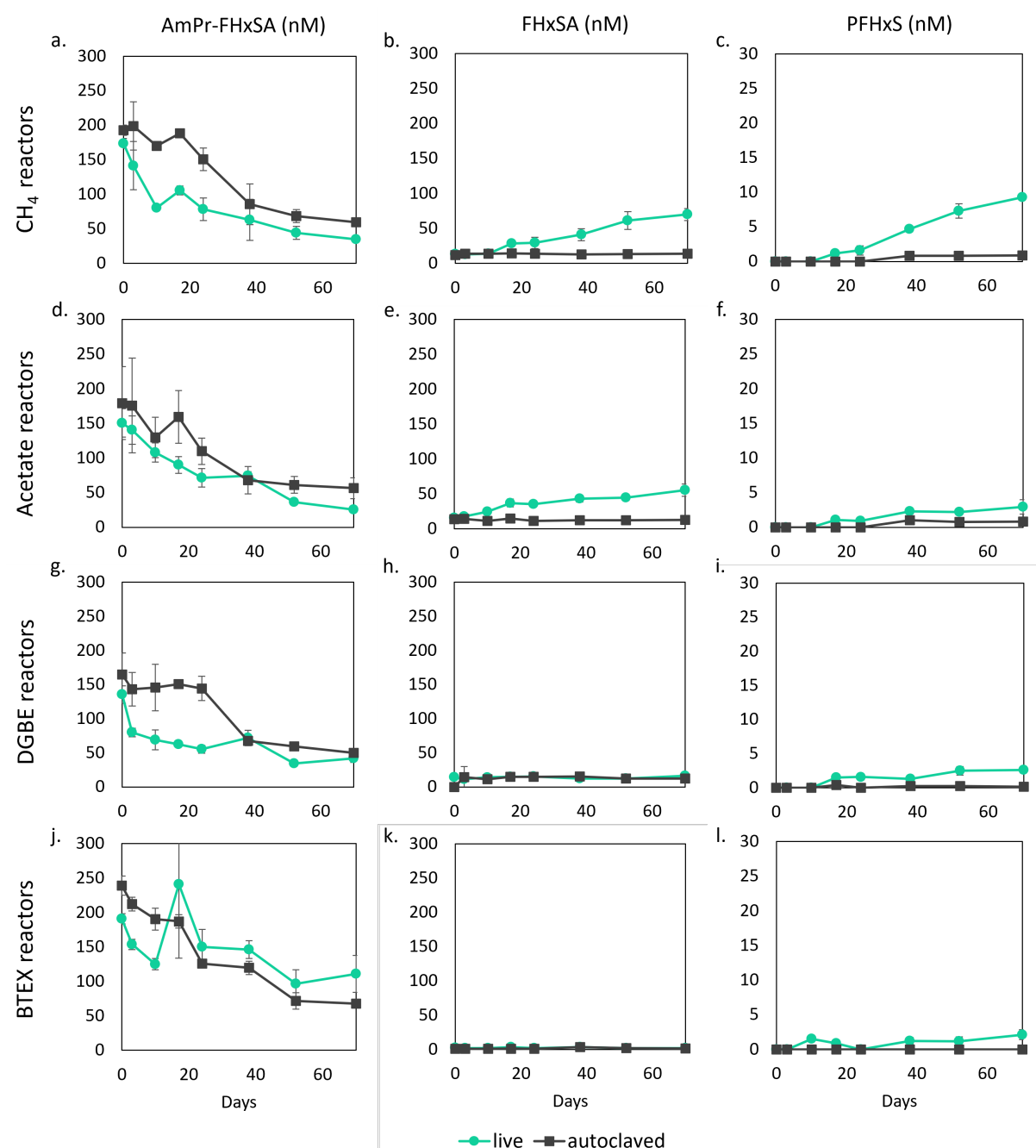


Figure 2.1: From left to right, AmPr-FHxSA depletion and FHxSA and PFHxS production (all in nM) over 70 days under aerobic conditions with four carbon sources: a-c) methane (CH₄), d-f) acetate, g-i) DGBE, and j-l) BTEX. Error bars represent standard deviation of triplicate reactors. Green circles represent the live cultures, while gray squares represent autoclaved controls.

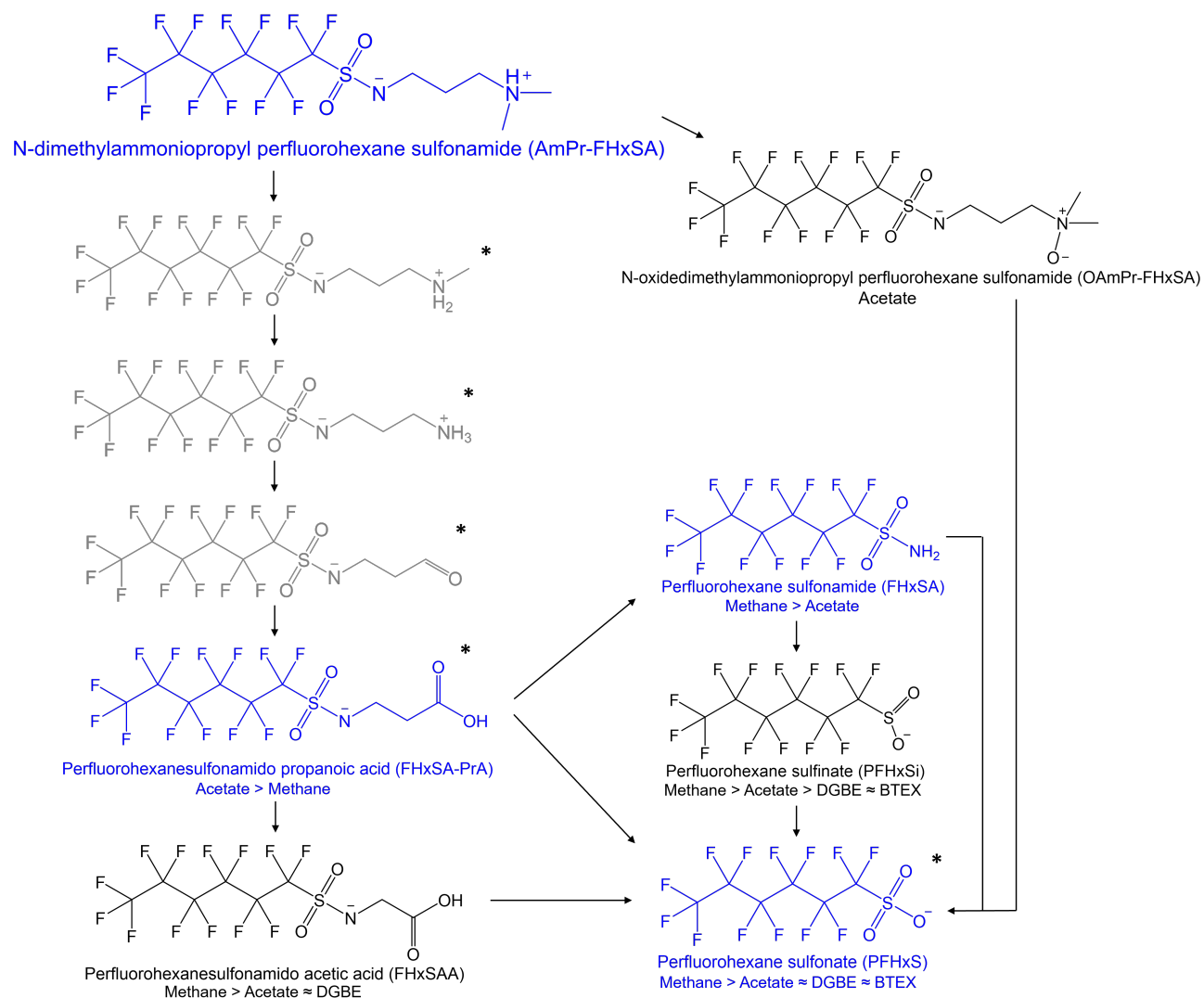


Figure 2.2: Proposed transformation pathway of AmPr-FHxSA under various carbon source treatments. The faded structures in gray were not measured for in this study but proposed in literature [69] for the eight-carbon analogues and/or on envPath. Asterisks denote products proposed via envPath. The blue structures were measured in higher abundances via HRMS.

autoclaved controls with a similar peak area as the live replicate, opening the possibility of abiotic transformation to FHxSA-PrA in the DGBE condition.

FHxSAA was likely produced after FHxSA-PrA and was detected in the methane, acetate, and DGBE live cultures, though at an order of magnitude less than FHxSA-PrA. This acetic acid could then lead to FHxSA, which increased in the methane and acetate cultures as shown in Figure 2.1. We also suggest that FHxSA-PrA can lead directly to FHxSA, as

proposed by Liu et al. for the eight-carbon homologs.[69] While FHxSAA could be an intermediate between FHxSA-PrA and FHxSA, we believe it would be a minor pathway based on *enviPath*'s predictions and literature. For example, Rhoads et al[30] observed FOSA before detecting FOSAA in activated sludge biotransformation of Et-FOSE and thus suggested that FOSAA to FOSA was a minor pathway. Similarly, Yin et al[66] proposed that direct dealkylation of Et-FOSA to FOSA was faster than biotransformation of FOSAA to FOSA, corroborating these hypotheses. Nevertheless, it is also possible that FHxSAA is a transient intermediate.

FHxSA could then be hydrolyzed to either PFHxSi or directly to PFHxS. Rhoads et al[30] proposed perfluorooctane sulfinate (PFOSi) as a biotransformation intermediate between FOSA and PFOS, and PFOSi has been found to be highly correlated with FOSA in a marine mammal study.[104] The methane cultures had the highest peak area of PFHxSi, which correlates with high concentrations of FHxSA and PFHxS in this treatment. Conversely, FHxSA concentrations did not increase in the DGBE or BTEX cultures despite increasing concentrations of PFHxS (Figure 2.1), indicating that there was rapid transformation to PFHxSi or PFHxS, resulting in no accumulation of FHxSA. Another possibility is that AmPr-FHxSA, FHxSA-PrA, and/or FHxSAA could directly produce PFHxS via biotic hydrolysis.[69] To inform this possibility, we can consider the perfluorooctyl equivalent, FOSA. FOSA has been shown to be quite stable biotically and is readily measured at AFFF-impacted sites and urban surface waters, as is FHxSA.[88, 95, 105] The half-life of FOSA in aerobic soils has been predicted to be >700 days.[98] Many ECF-based biotransformation studies that measured FOSA and PFOS as key transformation products did not observe sequential peak times over the course of their experiments that would have suggested FOSA transforming to PFOS.[69, 67, 98, 103, 106] In a recent follow up FHxSA biotransformation experiment with acetate as a carbon source, we measured less than 2% PFHxS after 90 days of incubation (data not shown), compared to 2.4% PFHxS in the acetate cultures observed in this study after 70 days. Therefore, while FHxSA may be a key biotransformation product of ECF-based precursors, it could be a rate limiting bottleneck in the pathway shown in Figure 2.2. This hypothesis is supported by high frequency detection in surface waters impacted by AFFF.[95] The recalcitrance of FHxSA underscores the significance of perfluoroalkyl sulfonamides and the need to include short-chain homologs in monitoring lists. More research should be done on their prevalence in the environment, potential toxicity, and human exposure.

While PFHxSi peak areas increased between days 0 and 70 for the methane, acetate, DGBE, and BTEX cultures, it was also detected in the methane, DGBE, and BTEX autoclaved controls, indicating that this compound could be produced abiotically (Table B.7). Finally, PFHxS was found in each of the four carbon source live cultures (and in none of the autoclaved controls) indicating that the production of PFHxS necessitates a biological step. Interestingly, when comparing the pathway step probabilities in *enviPath*, transformation of any of the precursors (i.e., AmPr-FHxSA, FHxSA-PrA, and the grayed-out theoretical intermediates) directly to PFHxS was more probable than the stepwise transformation shown on the left side of Figure 2.2. Additionally, Chen et al[68] suggests that the eight-carbon

equivalent to OAmPr-FHxSA could biologically transform directly to PFOS, meaning that OAmPr-FHxSA similarly could be an immediate precursor to PFHxS. Because there are potentially several direct biotransformation precursors to PFHxS (e.g., FHxSA-PrA to PFHxS, FHxSA to PFHxS, etc.), it is possible that several of these pathways can occur simultaneously. If FHxSA biotransformation to PFHxS is as slow as FOSA to PFOS, FHxSA could prove to be a long-term biotic source of PFHxS at AFFF-contaminated sites, even long after AmPr-FHxSA or other ECF-based precursors have transformed. Based on our results, this could be especially important where methane and acetate are present carbon sources.

Microbial Community Analysis

We extracted the DNA for each of the live treatments on day 70 to compare the phyla, genera, and diversity of each of the four carbon source enrichment cultures (Figure 2.3, Figure B.10). The BTEX, methane, and DBGE cultures were predominantly composed of Proteobacteria (57%, 77%, and 77% respectively), whereas the acetate culture was primarily made up of Actinobacteria (56%) with a smaller proportion of Proteobacteria (31%) (Figure B.10). Actinobacteria made up an appreciable, though lesser proportion of the three Proteobacteria-dominant cultures (5-27%). These two phyla were similarly found to dominate a PFAS aerobic soil microcosm experiment when not provided an external source of organic carbon,[106] suggesting that Proteobacteria and Actinobacteria are tolerant to PFAS contamination.

The methane culture, which contained the highest concentrations of transformation products, was also composed of an appreciable relative abundance of *Acidobacteria* (8%) compared to the other three treatments ($\leq 2\%$), and a modest relative abundance of *Gemmatimonadetes* ($> 1\%$), which was not seen in any of the other three enrichments. These two phyla are less frequently discussed in the literature but may also be tolerant to PFAS.[107, 108, 109] The methane cultures were dominated by *Ramlibacter* and *Cupriavidus* genera (additional discussion in Text B.11) and had a much larger fraction of low abundance taxa compared to the other three cultures (Figure 2.3a), which likely contributed to the significantly ($p < 0.05$) greater alpha diversity in this culture (Figure 2.3b). Greater biodiversity entails a broader suite of soluble enzymes that could react with precursors, leading to the higher yield of FHxSA and PFHxS.

The acetate cultures were dominated by *Mycobacterium* (52%), which is known to transform alkanes, and has been shown to transform 6:2 fluorotelomer alcohol.[110] Given this and its high relative abundance, we hypothesize that *Mycobacterium* was in part responsible for biotransformation of PFAS in the acetate culture. However, we were unable to find experimental evidence in literature confirming this genus's ability to transform ECF-based precursors like AmPr-FHxSA.

Cupriavidus, in addition to having a high abundance in the methane cultures, was also found in high abundance in the DGBE cultures, which had much fewer transformation products. Their shared high abundance of *Cupriavidus* likely contributed to the close clustering of these two enrichments when the relative abundances were plotted in principal component

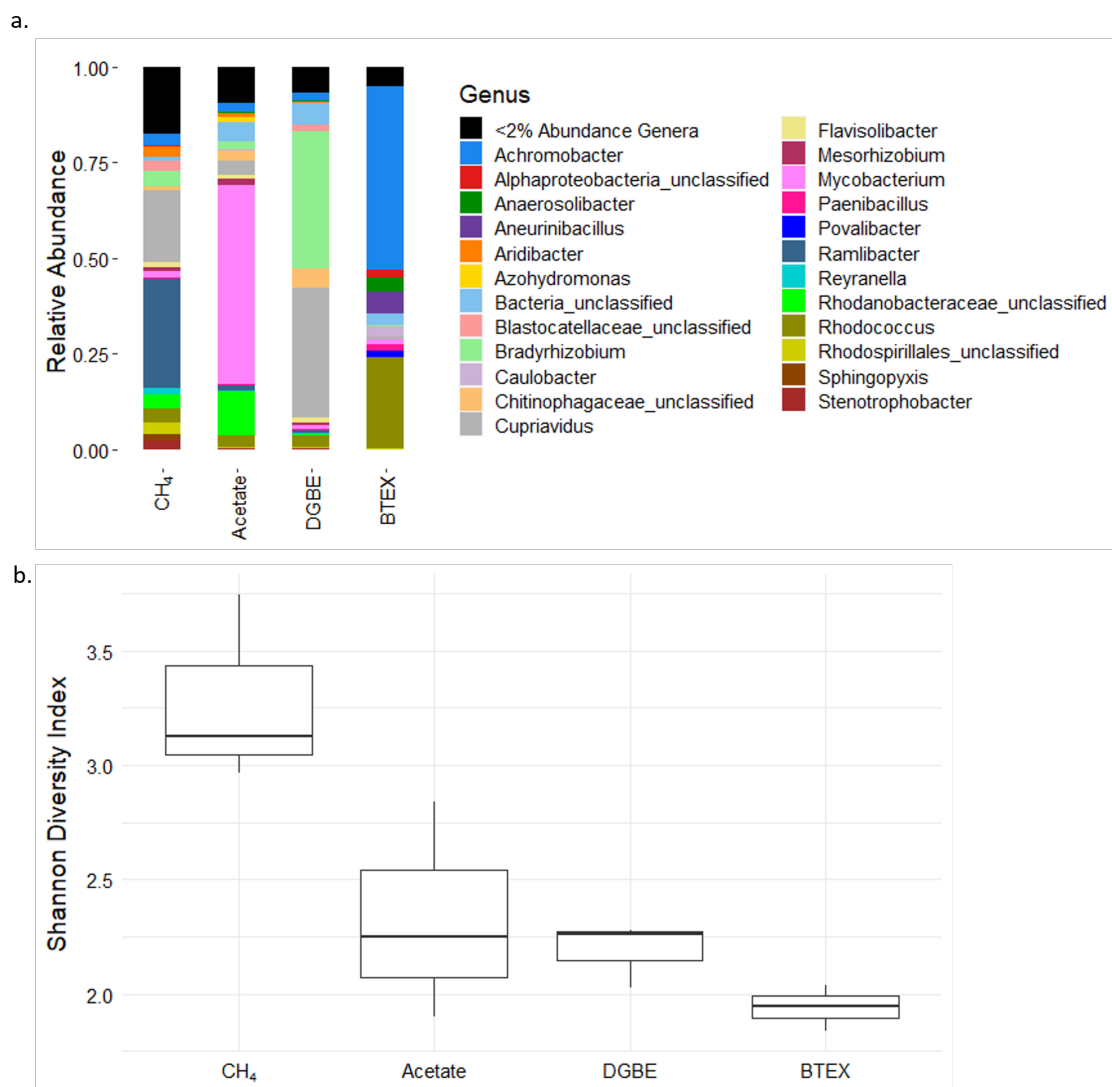


Figure 2.3: The a) genera and b) Shannon Diversity Index from each of the four carbon source conditions. Phyla are shown in Figure B.10. DNA was extracted from day 70 aqueous samples. Phyla and genera were averaged between the triplicate live samples.

analysis space (Figure B.11), though did not correlate with biotransformation product yield between these two carbon sources.

The BTEX culture was the least diverse (Figure 2.3b), though not significantly less so than the acetate and DGBE cultures. It was dominated by *Achromobacter* and *Rhodococcus*, both of which are known BTEX degraders.[111, 112] Combined, these two organisms accounted for 72% of the relative abundance in this culture. It is possible that this extreme specialization is responsible for the slower rate of AmPr-FHxSA biotransformation. While the enzymes produced may have been efficient in degrading BTEX, they appear to be unable to transform AmPr-FHxSA in an appreciable amount. This result diverges from the findings on fluorotelomer biotransformation in our laboratory,[91] which showed that adding BTEX as an additional carbon source resulted in more PFAA product formation.

All cultures contained *Rhodococcus*, which can participate in sulfur oxidation in 6:2 fluorotelomer sulfonamido alkyl betaine.[113] It has been proposed in perfluorooctyl-based PFAS biotransformation studies that PFOSi is a biotic precursor to PFOS, this step microbially mediated via sulfur oxidation.[69] If PFHxSi was indeed biotically transformed to PFHxS in our experiments, *Rhodococcus* could be responsible for this step, being able to oxidize the sulfinate head group to a sulfonate.

Chemical Transformation of AmPr-FHxSA and FHxSA

While biotransformation of AmPr-FHxSA can lead to the production of PFHxS under a range of microbial communities, applying chemical oxidation such as sulfate radical oxidation as a remediation strategy could prevent the biotic production of PFSA from AFFF-impacted source zones. To explore different chemical oxidation processes, we first performed the TOP assay on AmPr-FHxSA and FHxSA individually (Figure 2.4). With both AmPr-FHxSA and FHxSA, most of the mass was converted to PFHxA after 12 hours. Unexpectedly, a small amount of PFHxS was produced, forming about 1% of the product in AmPr-FHxSA TOP assay and 4.3% in the FHxSA TOP assay. To quantify the PFHxS, we concentrated the samples for PFHxS to fall within the range of the calibration curve. No PFHxS was detected in the controls. Additionally, this experiment was repeated three times and each time produced 1-1.5% of PFHxS from AmPr-FHxSA and 4-5% PFHxS from FHxSA. To date, it has been widely accepted that PFSA are not produced during the TOP assay; while fluorotelomer-based precursors result in a suite of PFCA in the assay, ECF-based precursors are reported to be converted to the corresponding chain-length PFCA (e.g., FOSA to PFOA).[96, 12, 97] Robel et al[114] found PFOS production during reaction of textiles during the TOP assay but could not isolate which precursor might have been its oxidation precursor. Therefore, this could be the first example of specific PFAS producing a PFSA during the assay. Although the PFHxS yield is low, we believe that this result underscores the unique properties of sulfonamides and should be considered in TOP assay analyses with perfluoroalkyl sulfonamides as ignoring PFSA formation could cause underestimation of total PFAS at sites with high ECF-based precursor concentrations.

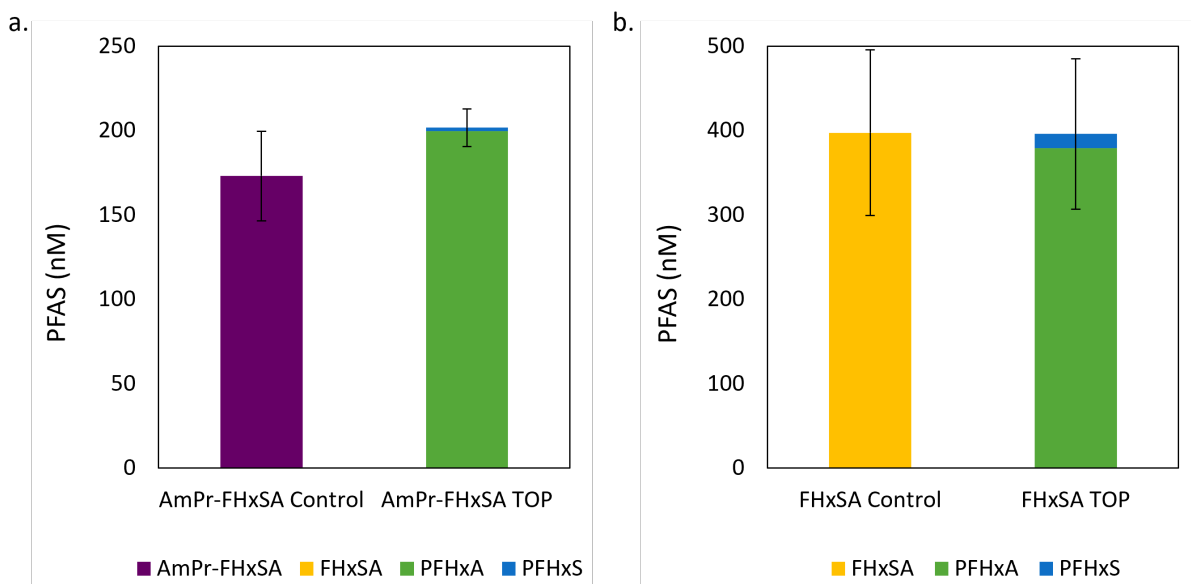


Figure 2.4: TOP assay results from reacting pure a) AmPr-FHxSA and b) FHxSA in Milli-Q water. Both precursors converted mostly to PFHxA via hydroxyl radical, but some PFHxS was detected. Error bars represent triplicates.

We then compared the TOP results with potential mineralization of AmPr-FHxSA and FHxSA using sulfate radicals during heat-activated persulfate oxidation, as activated persulfate oxidation of PFAS has been proposed as a potential in situ remediation technique.[55, 58, 62] Sulfate radicals from activated persulfate can transform precursor PFAS into PFCAs and at pH values below 3 these radicals can sequentially oxidize PFCAs, leading to mineralization.[55, 58, 59] Upon adding sodium persulfate and placing these reactors in an 85°C water bath, persulfate quickly converts to sulfate radicals and produces protons ($\text{S}_2\text{O}_8^{2-} + \text{H}_2\text{O} \rightarrow 2\text{SO}_4^{\cdot-} + 2\text{H}^+ + \frac{1}{2}\text{O}_2$), acidifying the solution.[55] AmPr-FHxSA and products were fully transformed in less than five hours, while FHxSA and products were fully transformed in about 24 hours (Figure 2.5), showing that these ECF-based precursors can be transformed under acidic conditions using activated persulfate. AmPr-FHxSA was first transformed to FHxSA and carboxylic acids, which underwent chain shortening. While FHxSA took longer to transform, it was no longer detected after five hours, suggesting that FHxSA is more recalcitrant than AmPr-FHxSA.

Similarly to the TOP assay, PFHxS was detected during heat-activated persulfate oxidation of FHxSA (Figure 2.5b). One of the triplicates contained PFHxS at the first sample point, which might indicate some rapid transformation of FHxSA to PFHxS since the controls did not contain PFHxS (Figure B.14). Across the triplicates, the concentration of PFHxS appears to increase between 0 and 5 hours (Table B.11), and levels off after 5 hours; this timing corresponds to when FHxSA was no longer detected (between 3 and 5 hours).

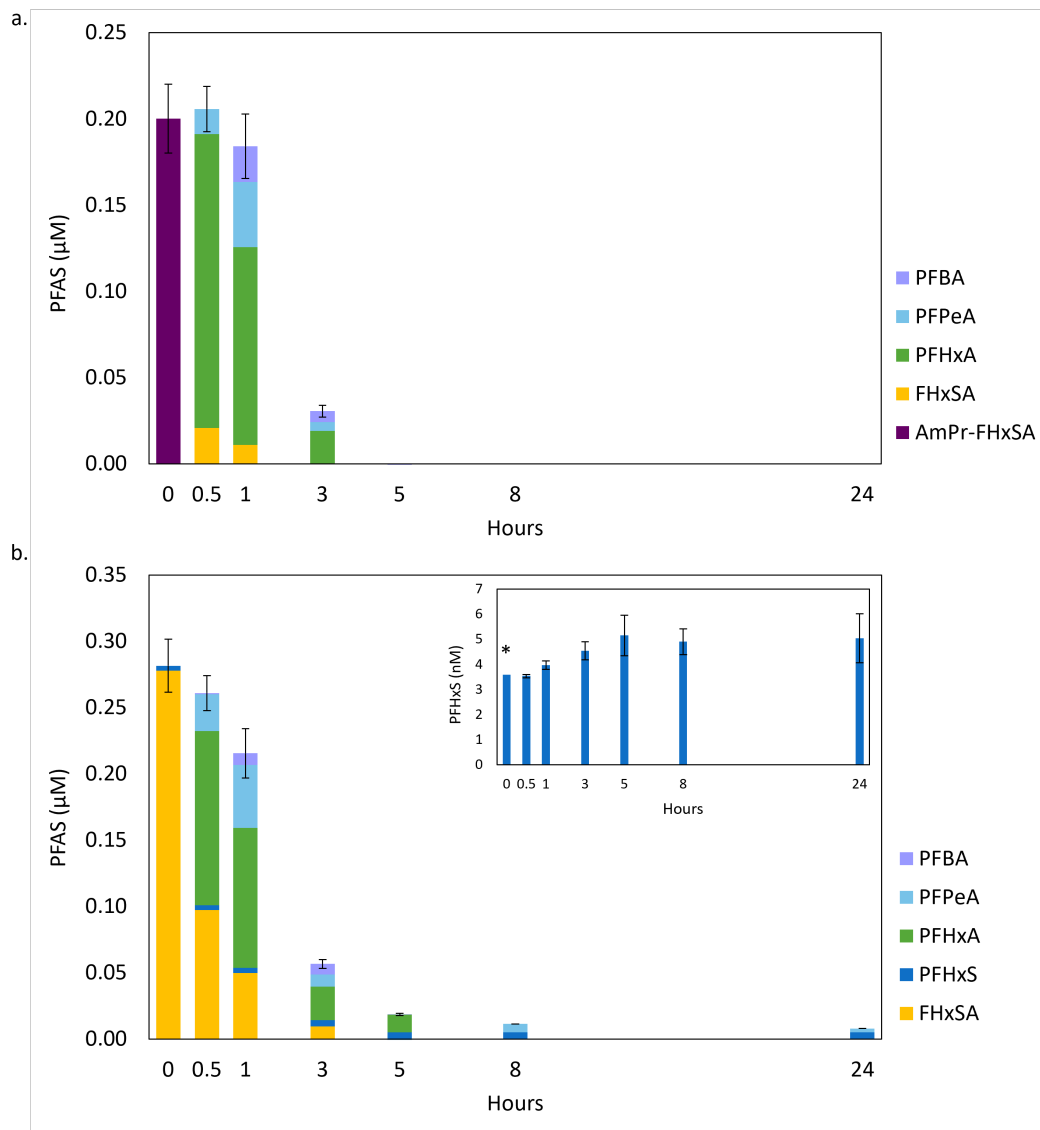


Figure 2.5: Heat-activated persulfate oxidation conducted on a) AmPr-FHxSA and b) FHxSA diluted in MilliQ water with 50 mM sodium persulfate. The pH quickly dropped to below 3 within the first hour and remained acidic. Inset in b) is the concentration of PFHxS in nM, increasing within the first 5 hours of reaction time, corresponding to the time it takes FHxSA to transform. The asterisk (*) indicates that PFHxS was only detected in one of the triplicate samples at hour 0. PFPeA = perfluoropentanoic acid; PFBA = perfluorobutanoic acid. Error bars represent triplicate reactors. Controls are shown in Figure B.14.

2.4 Implications

We found that different microbial communities impacted by available carbon sources will affect AmPr-FHxSA biotransformation yields and product composition. Specifically, with methane and acetate as carbon sources, the resulting microbial communities had higher yields of FHxSA and PFHxS compared to DGBE and BTEX, and additionally contained a greater diversity of intermediate products. The methane oxidizing community was the most diverse, which could point to a correlation between microbial diversity and increased biotransformation of ECF-based precursors. Lack of microbial diversity could especially be significant at sites where there has been a long history of BTEX contamination, or other commonly used solvents, that could result in a site's microbial community being more enriched or specialized for a particular substrate, resulting in less precursor biotransformation. Additionally, while FHxSA is a main biotransformation product with the microbial communities utilizing methane and acetate, this compound could be a bottleneck, resulting in slow, long-term PFHxS production. In our study, each of the microbial communities tested resulted in some PFHxS production; however, chemical oxidation of both AmPr-FHxSA and FHxSA primarily results in PFHxA under the TOP assay, which can be mineralized under heat-activated acidic persulfate oxidation. Therefore, persulfate oxidation could be used as a remediation tool for an AFFF-contaminated site to prevent long-term biotic production of FHxSA and PFHxS. However, a small fraction of FHxSA also transforms to PFHxS during chemical oxidation and appears to be more recalcitrant than AmPr-FHxSA during heat-activated persulfate oxidation. These latter two results point to the unique characteristics of perfluoroalkyl sulfonamides, which should be carefully monitored at AFFF-contaminated sites, especially FHxSA and other short-chain sulfonamides that are not usually included in monitoring lists.

Chapter 3

PFAS Impacts on the Bioremediation of Co-Contaminants TCE and BTEX

3.1 Introduction

Per- and polyfluoroalkyl substances (PFAS) are performance enhancing substances in aqueous film-forming foams (AFFFs), added for their ability to drastically lower the surface tension of a solution, increasing an AFFF's ability to quickly quench fuel and solvent fires.[11] Because some legacy PFAS such as perfluorooctane sulfonate (PFOS) have been shown to be bioaccumulative and toxic,[45, 46] shorter-chained PFAS have been introduced as regrettable substitutions for longer-chained PFAS, including perfluorohexane sulfonate (PFHxS) and n-demethyl perfluorohexane sulfonamido amine (AmPr-FHxSA), which are both electrochemical fluorination (ECF)-based PFAS in AFFFs produced by 3M. Recently, researchers have shown the widespread prevalence of shorter-chained PFAS in AFFF-impacted groundwater, including PFHxS and AmPr-FHxSA, as well as perfluorohexane sulfonamide (FHxSA), a biotransformation product of perfluorohexyl-based ECF precursors including AmPr-FHxSA.[69, 93, 95, 115] In one study, FHxSA was the dominant ECF-based sulfonamide present in groundwater samples.[116] Several groups have started reporting the biotransformation pathways and rates of ECF-based precursors, but no one has yet reported on the potential toxicity of these compounds on source zone relevant microbial communities.

Moreover, few studies have demonstrated PFAS toxicity potential or mechanisms on bacterial cells as opposed to eukaryotic cells. When aerobic toluene-degrading *Rhodococcus jostii* strain RHA1 was exposed to a combination of 11 perfluoroalkyl acids (PFAAs) (10 mg/L each for a total PFAS exposure of 110 mg/L), toluene degradation was not impacted by PFAS but the cells experienced enhanced aggregation as well as upregulation of stress-related genes.[81] The partitioning of PFAAs into bacterial cell membranes and model membranes has been found to increase with PFAS chain length.[77] The effect was greater for perfluoroalkyl sulfonic acids (PFSAs) compared to perfluoroalkyl carboxylic acids (PFCAs) and higher for gram-negative cells.[77] PFAS disruption of cell membranes is important

because an increase in membrane fluidity can disrupt the required cross-membrane proton gradient required for energy production via the electron transport chain. This same group also investigated the effect of PFAAs on the quorum sensing ability and bioluminescence of aerobic marine bacterium *Aliivibrio Fischeri*, [78], which is also impacted by membrane integrity. It was found that perfluorobutane sulfonic acid (PFBS) and, to a lesser extent, PFHxS increased metabolic rates of the bacterium possibly due to proton leakage from the compromised membrane, [78] while 50 mg/L of PFOS decreased its metabolic rate and 50 mg/L of PFHxS increased quorum sensing. [78]

An anaerobic trichloroethene (TCE)-degrading enrichment culture was exposed to three types of AFFF as well as key PFAAs and precursors contained in the AFFFs. [79] Because AFFFs contain an array of hydrocarbon surfactants, not just fluorinated surfactants, as well as glycol solvents, it was found that fermentable organics in AFFFs could stimulate anaerobic dehalogenation, while the PFAS themselves could inhibit it. [79] Specifically, fluorotelomer-based AFFFs inhibited TCE dechlorination, while 3M AFFF did not. [79] Additionally, high concentrations (i.e., 16 and 32 mg/L) of precursor fluorotelomer sulfonamido betaine (FtSaB) inhibited TCE degradation, while fluorotelomer thioether amido sulfonate (FtTAoS) (45 mg/L) did not; it was hypothesized that the positively charged betaine group in FtSaB was in part responsible for this difference [79] as cationic amines are known antibacterials. [117] In addition, AFFF might contain anticorrosives, which target specific microbial populations similar to the use of molybdate to deter sulfate-reducing bacteria. [118]

These studies provide crucial insights into how PFAS impact bacterial cells; some data gaps remain, including comparing various hexafluoroalkyl PFAS and determining the potentially different mechanisms of toxicity occurring under anaerobic and aerobic conditions. In addition, evaluating the robustness of a groundwater microbial community as well as a defined co-culture would improve our understanding of the benefits of microbial interactions in overcoming toxicity. Because ECF-based AFFFs were historically more commonly used compared to fluorotelomer-based AFFFs, [11] we wanted to understand the potential toxicity of hexafluoroalkyl ECF-based PFAS, specifically AmPr-FHxSA, its biotransformation product FHxSA, PFHxS, and a 3M AFFF containing high concentrations of AmPr-FHxSA and PFHxS. We sought to determine if hexafluoroalkyl PFAS negatively impact microorganisms capable of degrading PFAS source zone co-contaminants for bioremediation, specifically for co-contaminants benzene, toluene, ethylbenzene, and xylene (BTEX) as well as trichloroethene (TCE). We hypothesized an aerobic enrichment would be more robust compared to anaerobic enrichments, and that a mixture of PFAS (i.e., the 3M AFFF) would be more inhibitory to the cultures compared to the individual PFAS.

In this study, we exposed an aerobic BTEX-degrading enrichment culture (seeded from an AFFF-contaminated site) as well as an anaerobic TCE-degrading co-culture to various ECF-based PFAS and an ECF-based AFFF to determine possible inhibitory effects on these bacteria. We tested 1 μ M and 10 μ M (i.e., approximately 0.5 and 5 mg/L) of the various compounds, took biomass samples, and investigated the effect of electron donor/acceptor consumption, adenosine triphosphate (ATP) production, amino acid production, and community changes to determine how bioremediation processes may be affected by the presence

of PFAS.

3.2 Materials and Methods

Chemicals

PFAS analytical standards (including C4-C10 PFCAs, FHxSA, and AmPr-FHxSA) and mass labeled standards were purchased from Wellington Laboratories (Guelph, ON, Canada). BTEX compounds (>98%) were purchased from Sigma Aldrich, and TCE from Acros Organics (99.6%). All other chemicals used in media or analyses, including LC-grade water, methanol, acetonitrile, and isopropanol, were purchased from Sigma Aldrich or Fisher Scientific at the highest purity available. The ECF-based AFFF used, a Cal Guardian 3M AFFF 3%, was donated to our group by Prof. Jennifer Field at Oregon State University; PFAS concentrations for this AFFF are shown in Table C.1.

Microcosms

Anaerobic dechlorinating co-culture

An anaerobic inoculum stock of a co-culture constructed with *Dehalococcoides mccartyi* strain 195 and *Desulfovibrio vulgaris* Hildenborough (Dhc195 and DvH, henceforth) was maintained—fed TCE and lactate regularly—in a 30°C incubator until needed. For the inhibition tests, triplicates were exposed to PFHxS, FHxSA, AmPr-FHxSA, 3M AFFF (diluted 100x, 1,000x, or 10,000x), or a non-fluorinated surfactant control sodium dodecyl sulfate (SDS). A triplicate set with no surfactant was included as a control. This experiment was conducted thrice—once with approximately 1 μ M surfactant added, with 10 μ M, and with various AFFF dilutions. Added to 160 mL glass serum bottles was 5 mL of anaerobic inoculum, 94 mL of sterile BAV1 medium (Text B.2) and 0.5 mL of a vitamins mixture as previously described,[119, 120] 0.5 mL of 1 M sodium lactate, 10 μ L of neat TCE, and the respective surfactants with a headspace ratio of N₂/CO₂ 80/20. Where aqueous surfactant stock solutions were possible (SDS, PFHxS, diluted AFFF), the surfactant was added directly to reach the target concentration. For solvent-based surfactant stocks (FHxSA and AmPr-FHxSA), the required volume was first added to the empty, sterile bottles under sterile conditions, and dried with a slow stream of filtered (0.22 μ m) N₂ until the solvent evaporated. Then, the sterile media components were added via filtered needle syringe into the surfactant-containing bottles. The bottles with all microcosm components except for the inoculum were equilibrated for approximately a day before the addition of inoculum and the start of the experiment. These were kept in sealed glass serum bottles on their side in a 30°C incubator for the entirety of the experiment.

Aerobic BTEX enrichment culture

An aerobic enrichment culture seeded from an AFFF-contaminated site (as described in Chapter 2)[115] has been maintained in our laboratory since early 2020 with regular feedings of BTEX, fresh medium, and oxygen. For the inhibition tests, 2.5 mL of this inoculum culture was added to 22 mL of AMS medium (Text B.3), 0.5 mL of a phosphate buffer, 10 μ L of neat BTEX mixture (i.e., 2.5 μ L of each compound), and the respective PFAS, as described above. The purpose of a lower volume in these batches was to maintain a high ratio of headspace to aqueous solution for oxygen availability to this aerobic culture. These bottles were kept on their side in a 30°C shaking incubator (150 rpm) for the experiment. Like the anaerobic experiments, these microcosms were first equilibrated without inoculum for about a day for PFAS dissolution and BTEX equilibration.

In all experiments, headspace was sampled regularly (1-3 days for chlorinated solvents and every day for BTEX), and aqueous samples were taken for ATP and metabolomics analyses. The BTEX enrichment culture was additionally sampled for DNA extraction at the end of the 10 μ M PFAS experiment for community comparison.

Anaerobic toluene enrichment culture

A short test was conducted to compare the aerobic BTEX results with an anaerobic enrichment community. Similarly to the aerobic BTEX culture, the anaerobic culture was started by adding AFFF-contaminated soil (from an air force base in Michigan) to bottles of anaerobic BAV1 medium, vitamin mixtures including B12, and 1 mM lactate[120] with a N₂/CO₂ 80/20 headspace with either excess nitrate or sulfate added as electron acceptors. For the inhibition tests, dilutions of the 3M AFFF (diluted 100x, 1,000x, or 10,000x) or AmPr-FHxSA (1 or 10 μ M) were added to 160 mL sealed glass serum bottles with 5 mL of inoculum, 94 mL of sterile BAV1 medium,[119] 0.5 mL vitamins, [119, 120] 0.5 mL of 1 M sodium lactate, 10 μ L of neat toluene.

Metabolomics Extractions

Metabolomics extractions were performed based on Fiehn et al.[121] Aqueous unfiltered (0.5 mL) and filtered (2 mL of BTEX aerobic enrichment and 5 mL of TCE co-culture) were taken near the end of the first cycle of substrate/electron acceptor degradation and frozen at -20°C until extraction. Briefly, these aqueous samples were mixed with 1:1 isopropanol/acetonitrile at a 1:3 ratio (e.g., 0.5 mL aqueous sample, 1.5 mL solvent mixture), vortexed for 10 seconds, shaken for 5 minutes at 4°C, and centrifuged at 12,800g for 2 minutes at 4°C. About 90% of the supernatant was removed and the remaining sample was dried and resuspended in 250 μ L of 1:1 acetonitrile:water. This mixture was vortexed briefly and centrifuged for 2 minutes at 12,800g. About 150 μ L of this mixture was transferred into a vial for LC-MS/MS analysis, and the remaining 100 μ L was transferred to a 2 mL centrifuge tube for subsequent derivatization protocol.

For the derivatization, this 100 μ L mixture was dried, then supplemented with 10 μ L of a 28 mg/mL methoxyamine hydrochloride solution in pyridine, 20 μ L of pyridine, and 5 μ L d-myristic acid (0.75 mg/mL in hexane) and vortexed at 40°C for 90 minutes. Then, 70

μL of N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was added and samples were vortexed again at 40°C for 50 minutes. This mixture was transferred to a vial for full scan GC-MS analysis.

LC-MS/MS Analyses

PFAS were analyzed on LC-MS/MS as previously described (Chapter 2).[91, 115]

Targeted metabolites were analyzed on LC-MS/MS using established methods.[122]. The acetonitrile/water samples were run using a HILIC Plus column (4.6 x 100 mm, $3.5\ \mu\text{m}$). Organic mobile phase was 95% acetonitrile, 5% water, 0.1% formic acid, with 10 mM ammonium formate. Aqueous mobile phase contained 0.1% formic acid and 10 mM ammonium formate. These were run at the ratios shown in Table C.2 at 0.4 mL/minute. Analytes are shown in Table C.3.

GC-MS and GC-FID Analyses

Derivatized samples for metabolomics were run on a GC-MS (Agilent 5977B) with 1 μL as the injection volume. A DB5 capillary column (30 m x 250 μm i.d., 0.25 μm film thickness; J&W Scientific, Folson, CA) was used with a PTV injection (Gerstel CIS4 injector) in the splitless mode. Constant flow (1.7 mL/min) was used; the MS was used in the electron impact mode and we additionally ran full scans for each sample, using m/z ratios 15-800. The inlet temperature was set at 125°C and ion source and quadrupole were maintained at 250 and 200°C , respectively.

Headspace samples (100 μL) for BTEX and TCE concentrations were taken every day for BTEX and every several days for TCE and measured on GC-FID as previously described (Chapter 2).[91, 115, 123]

Additional Microbial Analyses

ATP was measured using an assay adapted from Hammes et al[124] as previously described in Kennedy et al[125] and Miller et al.[126] Briefly, filtered (0.22 μm) (for extracellular ATP concentrations) and unfiltered (for total ATP concentrations) microcosm samples were diluted 10-100x with Milli-Q water. 500 μL of this dilution as well as a 50 μL aliquot of ATP Reagent BacTiter-GloTM (G8231, Promega Corporation, Madison, WI) were each incubated for 3 minutes at 38°C ; then, the Reagent was added to the 500 μL sample, incubated for an additional 20 seconds, and measured using a Luminometer (GloMax® 20/20 Single Tube Luminometer, Model No. E6080, Promega Corporation, Madison, WI). Sample concentrations were compared with a curve made with rATP stocks (P1132; Promega Corporation, Madison WI).

At the end of the BTEX enrichment experiment with 10 μM PFAS, 4 mL slurry samples were taken, centrifuged at $10,000g$ for 10 minutes, decanted, and used for DNA extraction using a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) according to the manufacturer's

instructions and as previously described (Chapter 2).[115] Extracted DNA was stored at -80°C and sent to Novogene Corporation Inc. (Sacramento, CA, USA) for bacterial 16S rRNA amplification and sequencing. Data processing was performed as previously described (Chapter 2).[115]

3.3 Results and Discussion

Co-Contaminant Degradation

Dhc and Dvh exist in a syntrophic relationship that results in more rapid and robust dechlorination of TCE to ethene, as previously described.[127] Dhc uses chlorinated solvents as electron acceptors, transforming TCE to cis-dichloroethene (cis-DCE), vinyl chloride (VC), and finally to ethene with the support of sulfate-reducing Dvh. When we exposed this co-culture to 1 and 10 μM individual PFAS and SDS, there was no effect on the rate of dehalogenation of TCE to ethene compared to the no-surfactant controls (Figure C.1). The only condition that resulted in inhibition of TCE dechlorination was the addition of 1,000x diluted ECF-based AFFF (Figure C.1), which had approximately equivalent concentrations of AmPr-FHxSA (6.5 μM) compared to the AmPr-FHxSA-only triplicates. Additionally, this dilution of AFFF contained approximately 3.8 μM PFHxS and 20 μM of PFOS, which could have contributed to the inhibition of the AFFF (SI Table C.1).

To further investigate the impacts of the AFFF replicates, we repeated this experiment with three dilutions of the AFFF: 100x, 1,000x, and 10,000x (Figure 3.2). The 10,000x diluted replicates performed similarly to the control, while the 1,000x diluted set resulted in a delay in TCE degradation and subsequent cis-DCE, VC, and ethene production (Figure 3.2). In contrast, the microcosms that contained 100x diluted AFFF resulted in complete inhibition of TCE dechlorination, with no production of cis-DCE, VC, or ethene (Figure 3.2). Therefore, the combined effects of the PFAS in the 1,000x diluted AFFF delayed TCE degradation while the concentration range of 100x diluted AFFF—38 μM PFHxS, 65 μM AmPr-FHxSA, and 200 μM of PFOS—inhibited the culture entirely. Since PFOS was present in the AFFF at an order of magnitude higher than the next highest PFAS, the concentration of PFOS could have controlled whether or not the AFFF dilution was inhibitory, but this could have also been due to other ingredients in the AFFF.

The aerobic BTEX-degrading enrichment culture, which uses BTEX as its carbon source and electron donor, did not exhibit any inhibitory effects when exposed to 1 μM of PFAS and 10,000x diluted AFFF (Figure C.2). When exposed to 10 μM PFAS or 1,000x diluted AFFF, however, both FHxSA and AmPr-FHxSA completely inhibited BTEX consumption compared to every other triplicate condition, including the 1,000x AFFF dilution (Figure 3.3). When we repeated this experiment with increasing dilutions of AFFF (Figure C.3), the cultures exposed to 1,000x and 100x dilutions were more inhibited compared to the control and 10,000x AFFF dilutions, in contrast to the 1,000x dilution results in Figure 3.3 where little to no inhibition was observed at that dilution.

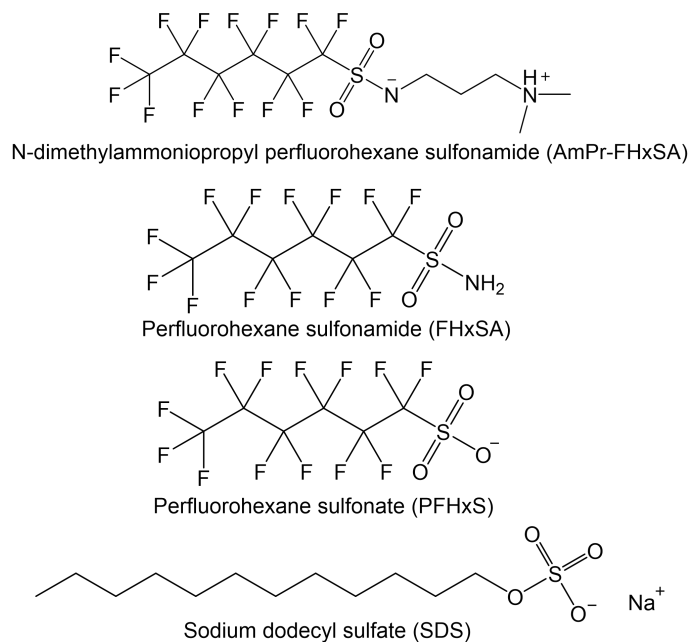


Figure 3.1: Surfactants tested in this study. All contained hexafluoroalkyl chains, except for the non-PFAS control, SDS.

The inconsistencies in AFFF inhibition could be attributed to variability of PFAS behavior in the bulk solution, especially in mixed surfactant solutions. PFAS, like non-fluorinated surfactants, have a concentration value called the critical micelle concentration (CMC), the concentration above which they can form aggregates known as micelles.[128] When PFAS are mixed with hydrocarbon surfactants, like those present in AFFFs, the CMC can be decreased by hydrocarbon surfactants of opposite headgroup charges, or increased by hydrocarbon surfactants with the same headgroup charge.[129, 130] Along with the decrease in CMC, the surface activity of the affected PFAS may increase, meaning that PFAS will concentrate more at interfaces compared to in the bulk solution.[129, 130] Additionally, it has been shown that salts can affect the surface activity and CMC of fluorinated surfactants,[99] which may play a role in these experiments due to the mineral salts medium used for the microcosms. With this complexity of factors impacting PFAS bulk solution behavior, the PFAS from the AFFF, including PFOS, AmPr-FHxSA, and PFHxS, might interact less with bacteria in bulk solution and instead exist in a micellar or hemi-micellar forms or at air-water or surface-water interfaces. In contrast, FHxSA and AmPr-FHxSA when added alone, may be better distributed in bulk solution thereby interacting more with the bacteria and inhibiting their activity, as shown in Figure 3.2.

It is interesting that FHxSA and AmPr-FHxSA were the only two compounds in isolation to inhibit the aerobic BTEX culture, as their headgroups are neutral and zwitterionic, re-

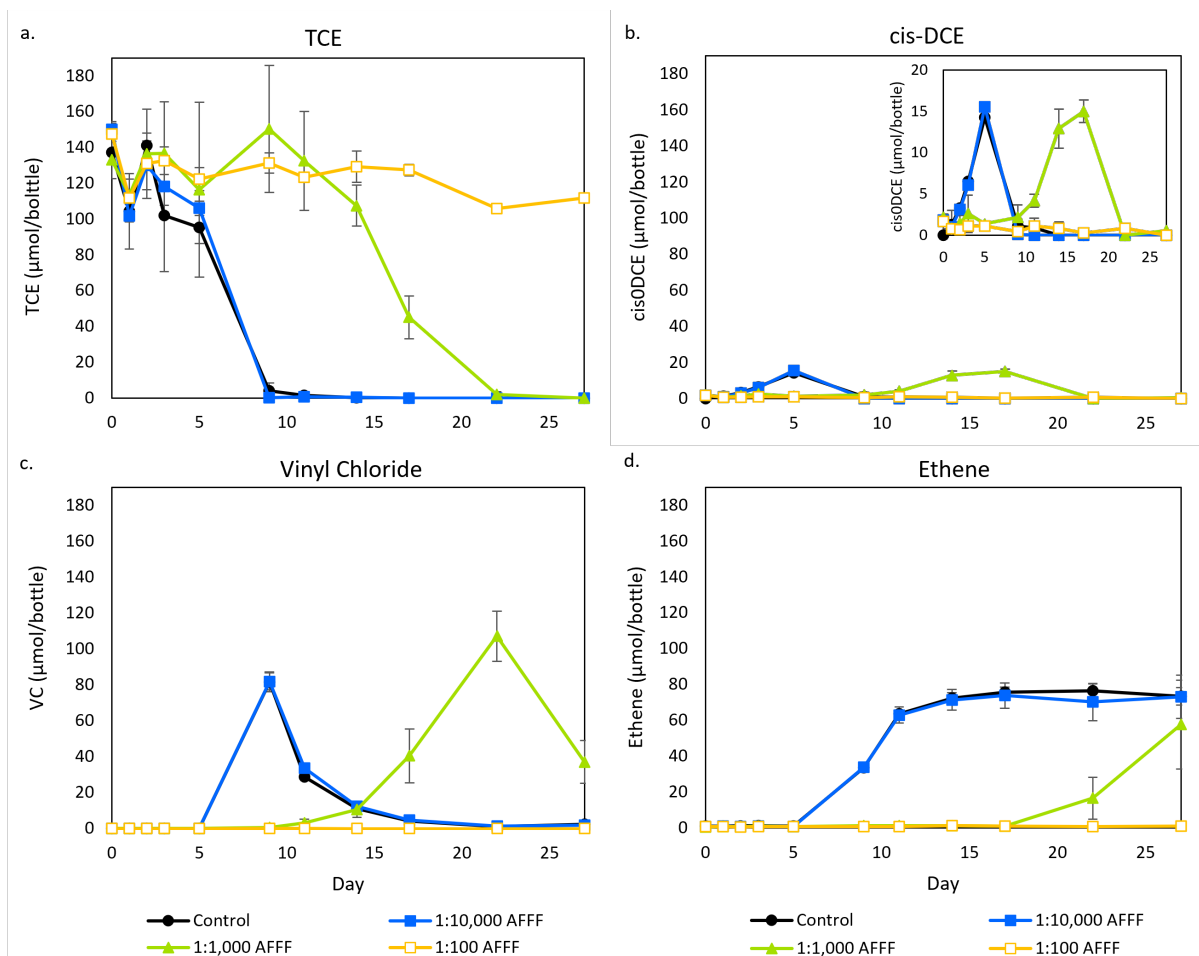


Figure 3.2: a) TCE dechlorination and subsequent production of b) cis-DCE, c) VC, and d) ethene by the anaerobic co-culture. This culture was exposed to increasing dilutions of an ECF-based AFFF: black circles are no-AFFF control, blue squares 10,000 diluted AFFF, green triangles 1,000x diluted, and yellow/white squares 100x diluted. Error bars represent triplicate bottles.

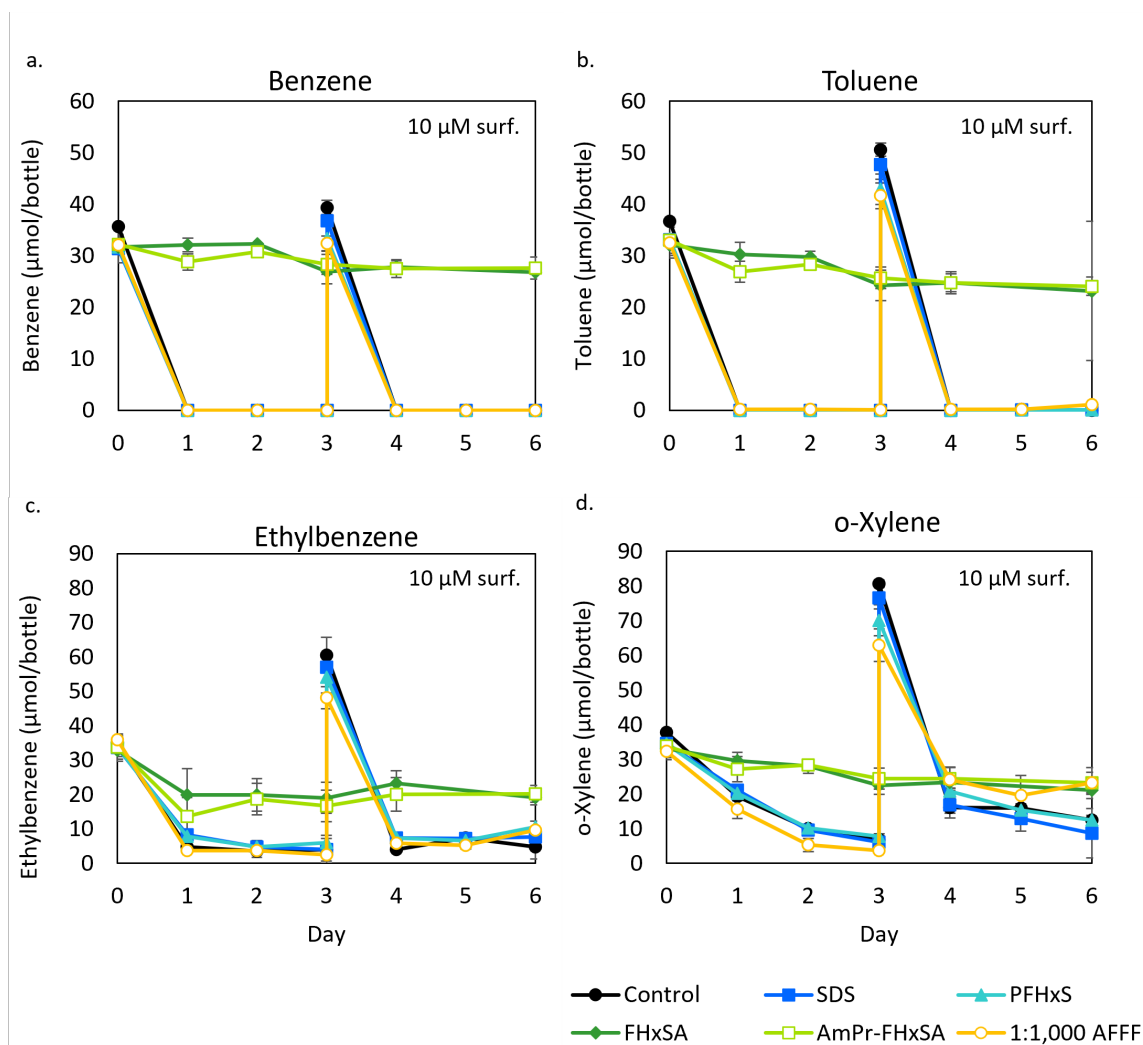


Figure 3.3: Aerobic degradation of a) benzene, b) toluene, c) ethylbenzene, and d) ortho-xylene by an AFFF-impacted enrichment culture. Black circles are no-PFAS control, blue squares SDS, turquoise triangle PFHxS, dark green diamond FHxSA, light green/white square AmPr-FHxSA, and yellow/white squares 1,000x diluted AFFF. Error bars represent triplicate bottles. Additional BTEX was added Day 3 to all reactors that had already consumed most of their substrate; additional BTEX was not added to the AmPr-FHxSA and FHxSA cultures because they had not demonstrated BTEX degradation.

spectively (Figure 3.1). Negatively charged PFAs have been shown to disrupt bacterial cell membranes,[77, 78] but the effects of neutral and zwitterionic PFAS on membrane integrity have not been reported to our knowledge. It is possible that the neutral FHxSA permeated cell membranes more effectively than negatively charged surfactants, and that the zwitterion AmPr-FHxSA could have interfered with the membrane and membrane-bound proteins such as ATP synthase, as described in the next section.

We then compared the aerobic BTEX enrichment culture with anaerobic cultures enriched under nitrate- and sulfate-reducing conditions using toluene as electron donor and carbon source. Neither added AmPr-FHxSA (1 or 10 μM), nor any of the three AFFF dilutions inhibited toluene degradation in these cultures (Figures C.4 and C.5).

Finally, it should be noted that the highest concentrations of PFAS and AFFF tested impacted the solubility of these volatile compounds, especially of the chlorinated solvents. This effect is evident in the higher error bars for the volatile compounds with higher PFAS concentrations shown in Figures 3.2 and C.1 for TCE, the variation of Day 0 TCE concentrations in Figure C.1e compared to the control, as well as the larger error bars in Figure C.3 for BTEX. Many surfactants, not just PFAS, can affect the solubility of co-contaminants,[131, 132] demonstrating that at sites with volatile organics coexisting with PFAS, and AFFF specifically, solubility and detection may be impacted.

ATP Production

ATP is present in all organisms as energy currency. ATP is produced during respiration via glycolysis, the Krebs cycle, and the electron transport chain, with aerobic respiration resulting in more ATP production than anaerobic respiration. With the electron transport chain, electrons are transferred from higher-energy compounds to lower ones, while protons are pumped from inside the cell to outside in periplasmic space. The proton pumping creates a gradient, with the inside of the cell becoming more negative, and the outside becoming more positive; this gradient results in potential energy known as the proton motive force. The protons that build up in the periplasmic space reenter the cell by means of the membrane bound protein ATP synthase (ATPase), which transports approximately three protons to produce one molecule of ATP. Contaminants, including surfactants such as some PFAS, interfere with the generation of ATP, which in turn can interrupt microbial metabolic processes and eventually lead to cell death. Surfactants could potentially affect ATP production in a few ways. One way could be by damaging membrane integrity, creating a leaky membrane that reduces the proton motive force, thus reducing the efficiency of ATPases. Another is by impacting the secondary structure of proteins,[133] potentially including membrane-bound proteins like the ATPase, or even inactivating and/or extracting proteins from the membrane.[134]

The anaerobic dehalogenation co-culture resulted in no observable ATP production impacts for either the 1 μM surfactant conditions or for the 10 μM surfactant conditions, but there was an impact for the AFFF exposed replicates (Figures C.6 and C.7). The cultures exposed to 1,000x diluted AFFF contained about 24% ATP compared to the control on day 16, while the 100x AFFF cultures had 18% of the ATP (Figure C.7). The genus *Dehalococ-*

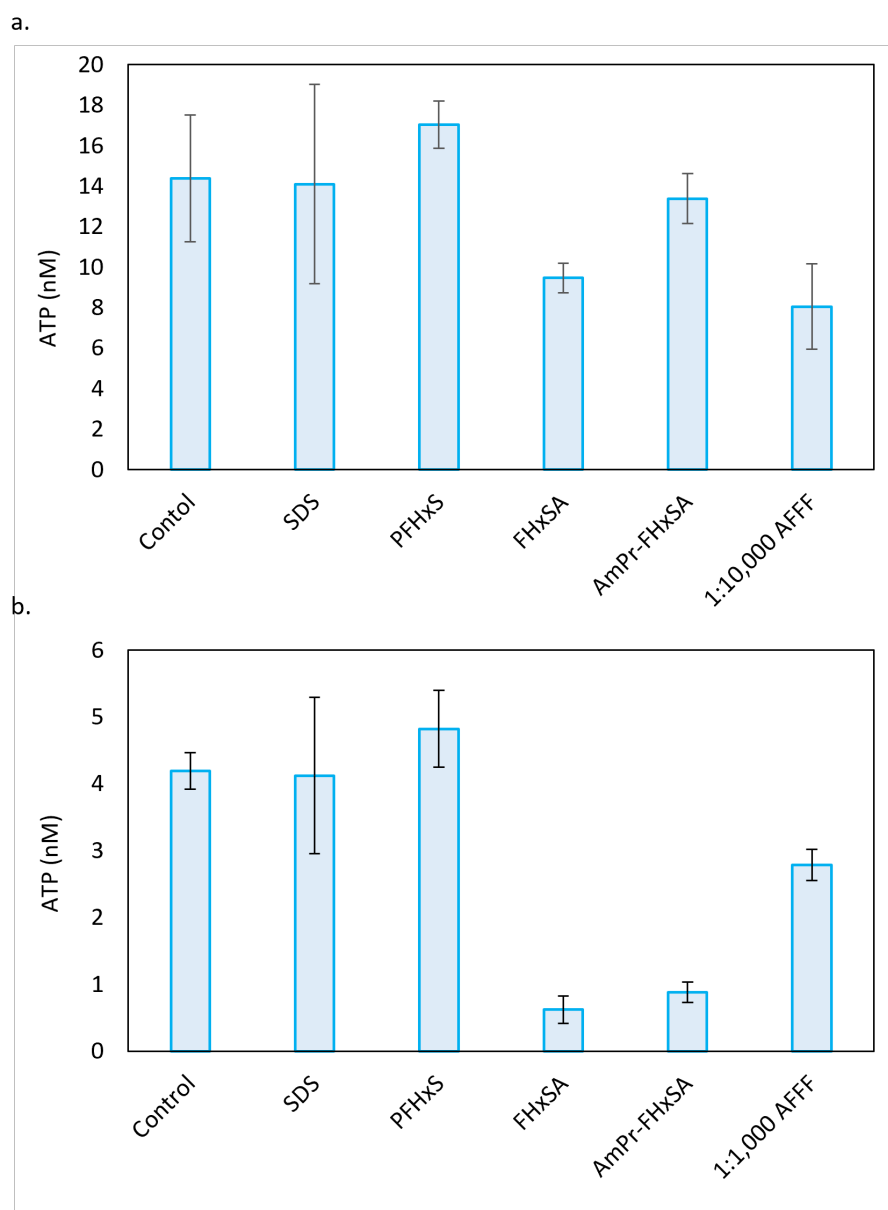


Figure 3.4: Total ATP concentrations from the BTEX enrichment culture on a) day 2 of the 1 μ M surfactant experiment (see Figure C.2) and b) day 2 of the 10 μ M surfactant experiment (see Figure 3.3).

coides has been shown to have a unique version of a cell wall; instead of peptidoglycan, the cell wall structure resembles the S-layer protein subunit walls that are typical of archaea.[135] These S-layer type proteins could be the main contributor to the co-culture's resistance to PFAS, especially when the compounds are added individually. Indeed, it has been shown that *Dehalococcoides* species can be resistant to peptidoglycan biosynthesis associated antibiotics.[136]

In the aerobic BTEX enrichment culture, replicates exposed to 1 μ M SDS, PFHxS, and AmPr-FHxSA resulted in similar ATP concentrations as the control (Figure 3.4). The FHxSA- and AFFF-exposed bottles, however, resulted in about 66% and 56% of the ATP of the control, respectively (Figure 3.4). The lower ATP concentration in the 10,000x diluted AFFF set corresponds to the delayed BTEX degradation shown in Figure C.2 for this test. The FHxSA condition, however, showed no inhibition in BTEX consumption even though the ATP concentrations were impacted. In the 10 μ M surfactant tests with the BTEX enrichment, the control, SDS, and PFHxS cultures had similar ATP concentrations of 4 nM ATP (Figure 3.4) while the FHxSA and AmPr-FHxSA cultures, which inhibited BTEX degradation (Figure 3.3), had about 15% and 21%, respectively, of the ATP concentration of the control (Figure 3.4). Although the 1,000x AFFF dilution in this experimental set (Figure 3.3) did not result in BTEX inhibition, the ATP concentrations in these cultures were about 66% of the control cultures (Figure 3.4). It is possible that since aerobic microorganisms produce more ATP than anaerobic ones, that the 1 μ M FHxSA set and the AFFF conditions simply reduced the cultures' aerobic respiration ATP-production efficiency rather than completely inhibiting the cell's ability to respire by inactivating proteins or destroying the cell membrane.

Interestingly, it has been reported that at low concentrations SDS can stimulate ATPase activity (approximately ≤ 10 μ M) before drastically reducing it above 20 μ M.[134] This observation is reflected in our experiments; with 10 μ M surfactants in the TCE culture, the ATP production was similar in the SDS and PFHxS exposed cultures (Figure C.6) and slightly higher in the PFHxS exposed cultures in the aerobic BTEX enrichment (Figure 3.4).

It has been shown that PFAS interference with cell membranes as well as proteins is chain length and headgroup dependent. For example, PFAS binding to proteins can be impacted by the types of amino acids near open binding sites that can interact with the charged PFAS headgroups.[137] Shen et al[138] showed that PFAS can enter a cell's lipid bilayer by first switching orientation so that the fluorinated tail faces the membrane; this flip allows the PFAS to overcome the energy barrier associated with entry to the membrane. PFASs penetrate the membrane bilayer better than PFCAs [138] and the eight-carbon sulfonamide perfluorooctane sulfonamide (FOSA) incorporated more than PFOS in another study.[139] With increasing concentrations of PFAS, lipophobic PFAS in the lipid bilayer can result in lipid removal from the bilayer, which can result in a disordered membrane structure.[139] Based on our results and literature, we hypothesize that FHxSA was penetrating cell membranes in the aerobic BTEX culture, while zwitterionic AmPr-FHxSA was associating with charged amino acids from membrane-bound proteins, inhibiting proper function.

Metabolites

We measured the relative concentrations of metabolites in the anaerobic TCE co-cultures and aerobic BTEX enrichment cultures (Figures 3.5, C.9-C.11). Figure 3.5 shows a heatmap of the relative metabolites for the TCE dechlorinating co-culture and the BTEX enrichment culture exposed to increasing dilutions of AFFF. The TCE dechlorinating culture generally had clearer trends as shown on the heatmaps, potentially because there were only two species in the culture responding to the PFAS. The BTEX enrichment, however, had more varied results; as there were 3-5 key genera in these cultures (Figure 3.6), the culture likely has a network of materials exchange and co-dependence, leading to more varied metabolites concentrations as each species may react differently to the surfactant exposure.

For the TCE dechlorinating co-culture, the control sets clustered similarly to the 10,000x diluted AFFF, with the TCE culture containing sarcosine whereas the AFFF exposures did not. The 1,000x AFFF dilution resulted in higher relative concentrations of tyrosine and histidine compared to the control and 10,000x AFFF dilution. Tyrosine can be linked to polysaccharide production,[140] while histidine residues are common in phosphoproteins.[141] Interestingly, aspartic acid, serine, and threonine are also common in phosphoproteins; the phosphorylation of proteins is a key cellular mechanism to either activate or deactivate an enzyme based on it being phosphorylated or dephosphorylated or cellular signaling for a cell to respond to a change in its environment.[142] Therefore, aspartic acid, serine, and threonine having a much higher relative concentration in the 100x dilution compared to the other sets, and histidine much lower, could indicate the phosphorylation or dephosphorylation of these residues on particular proteins in response to the presence of the AFFF. Additionally, aspartic acid is used to synthesize both lysine and peptidoglycan. Since lysine decreases in concentration from the control to 10,000x and then 1,000x AFFF dilutions, this could point to negative interactions with peptidoglycan synthesis.

BTEX Community Composition

On day 4 of the 10 μ M surfactant test with the aerobic BTEX enrichment cultures, we sampled biomass for DNA extraction and sequencing to compare differences between the cultures exposed to different surfactants. Unfortunately, potentially because of the high concentrations of surfactants used, several of the samples failed the quality control measures due to amplification failure. It is possible that the surfactants interfered with the DNA extraction procedure; in the future, when analyzing PFAS-impacted microbial communities, a PFAS extraction method may need to be first employed before DNA extraction to avoid downstream amplification errors. Therefore, the results that passed quality control, the inoculum source, FHxSA, and AmPr-FHxSA exposed cultures, are described here.

The inoculum was dominated by *Rhodococcus* and *Achromobacter* (Figure 3.6a), two known BTEX degraders,[112, 111] as we have previously reported (Chapter 2).[115] Cultures exposed to FHxSA and AmPr-FHxSA, in addition to *Rhodococcus* and *Achromobacter*, also contained *Sediminibacterium* and unclassified *Alphaproteobacteria*. The inoculum and the

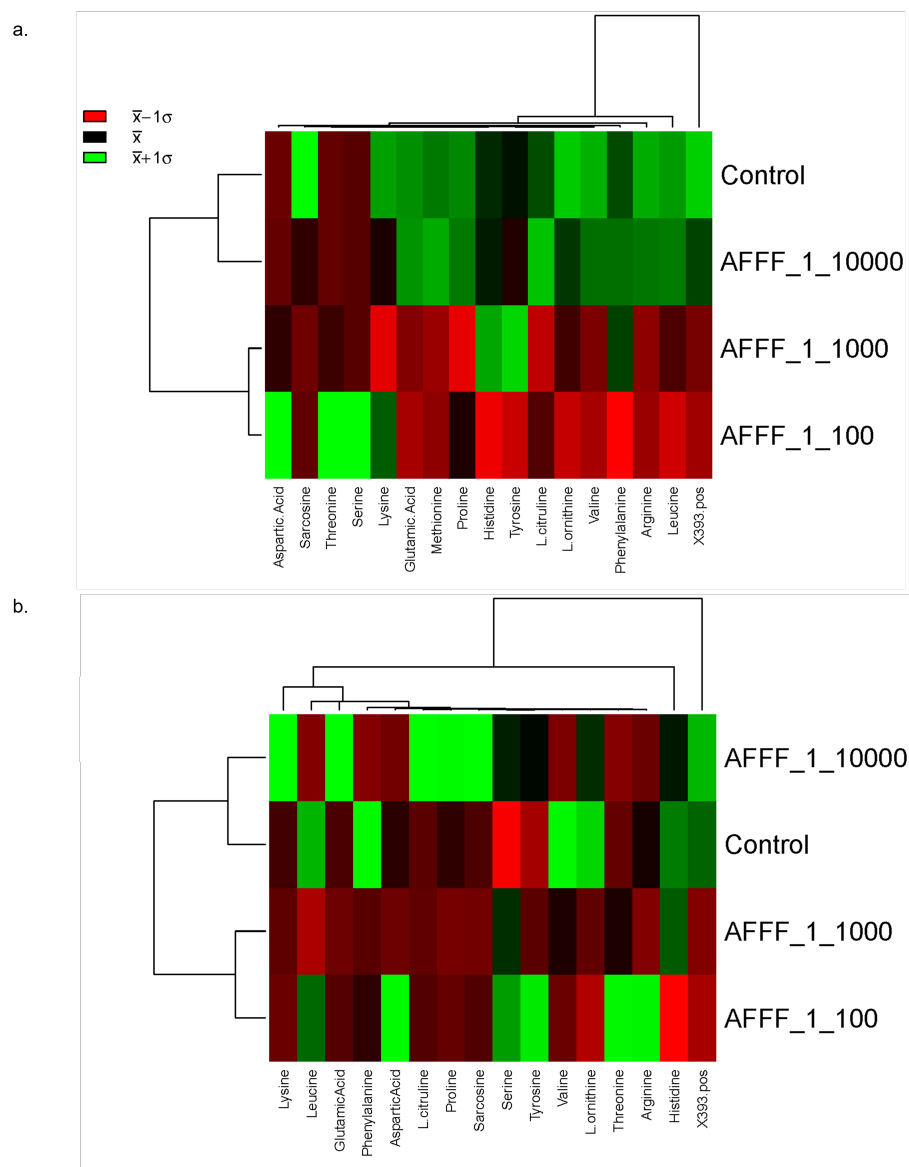


Figure 3.5: Metabolites measured in the a) anaerobic TCE co-culture and b) aerobic BTEX enrichment culture with the three dilutions of AFFF.

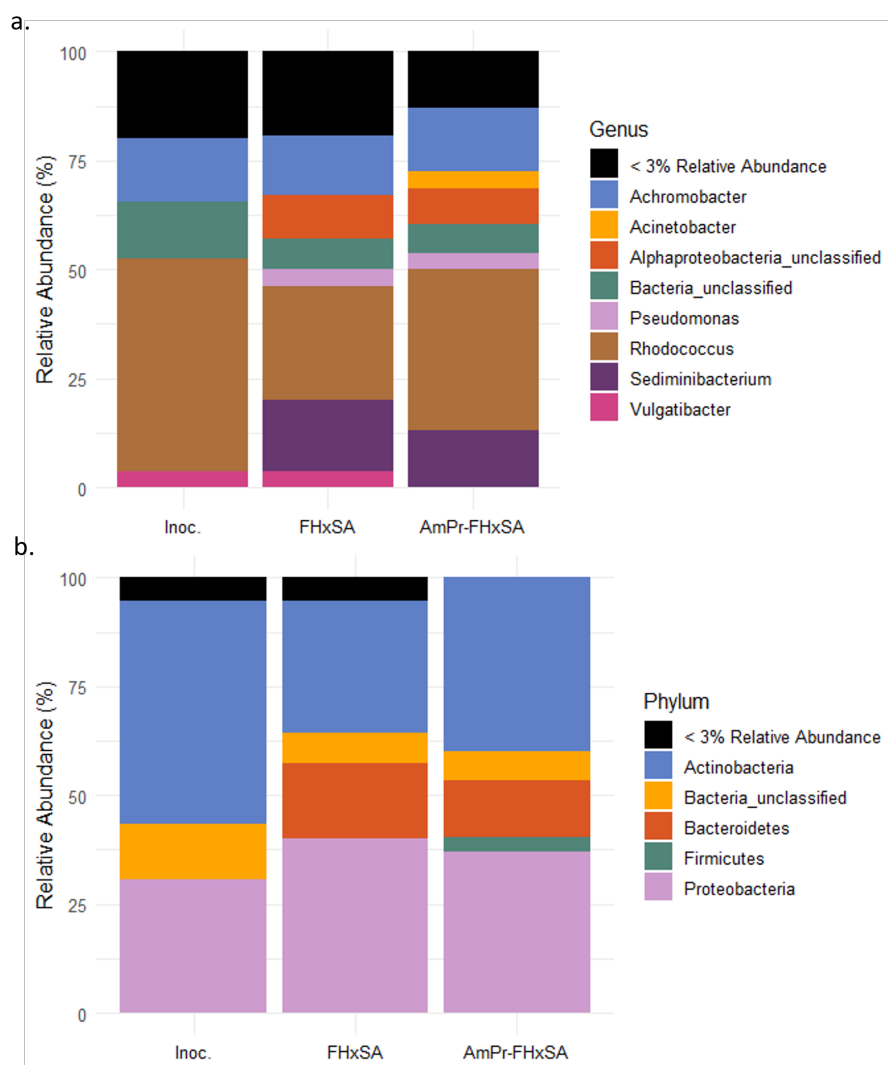


Figure 3.6: a) Genera and b) Phyla of the inoculum, 10 μ M FHxSA exposed cultures, and 10 μ M AmPr-FHxSA exposed cultures in the aerobic BTEX degrading enrichment culture.

FHxSA culture shared a small abundance of *Vulgatibacter*, while the AmPr-FHxSA cultures were unique in containing *Acinetobacter*.

Acinetobacter are gram-negative bacteria known for being antibiotic resistant bacteria, including to compounds such as penicillin and aminoglycoside (Doughari et al 2011).[143] In addition to being antibiotic resistant, *Acinetobacter* are generally robust organisms, being able to persist in a range of temperatures and moisture contents. In studies investigating bioremediation of petroleum hydrocarbons, *Acinetobacter* has been demonstrated to be able to use fuels as their carbon source and electron donor.[144, 145] In fact, one study showed an enrichment of *Acinetobacter* in contaminated soils compared to pristine, which further points to their robust and persistent nature.[144] It is interesting that, based on its persistence, *Acinetobacter* was not detected with significant abundance in the FHxSA cultures. Its absence could be attributed to the ability of FHxSA to passively diffuse into cell membranes compared to AmPr-FHxSA, leading to inhibition of this genus.

There is not much literature on the *Vulgatibacter* genus, which was found in the inoculum and in the FHxSA cultures. This genus contains gram negative obligate heterotrophic aerobes.[146] *Sediminibacterium*, present in both FHxSA and AmPr-FHxSA cultures, is similarly an understudied genus with only seven species,[147] which are also gram negative heterotrophic aerobes. Its higher abundance with the PFAS cultures compared to the control suggests that *Sediminibacterium* can withstand high PFAS concentrations. Interestingly, *Pseudomonas* was present in both the FHxSA and AmPr-FHxSA cultures, its higher abundance compared to the control suggesting that this genus could be more resistant to PFAS compared to *Rhodococcus*, which declined in abundance compared to the control (Figure 3.6). While *Rhodococcus* was sensitive to the presence of PFAS, *Achromobacter*, the other key BTEX-degrader, seems unaffected evidenced by its consistent relative abundance in all cultures.

3.4 Implications

At sites with multiple contaminants, high concentrations of AFFF may inhibit anaerobic chlorinated solvent bioremediation, while AFFF and potentially FHxSA and AmPr-FHxSA individually can negatively impact aerobic BTEX degradation. PFAS may act by interfering with cells' proton motive force, which drives ATP production thus reducing the cell's ability to create energy currency. Neutral PFAS such as FHxSA may be able to penetrate cell membranes more effectively than negatively charged PFAS, and zwitterionic PFAS may use electrostatic interactions to interfere with cell proteins. Additionally, changes in metabolite concentrations point to certain amino acids associated with cell signaling and protein activation being impacted by the presence of AFFF. In source zones where AFFF concentrations are the highest, transformation of co-contaminants by microbial communities may be inhibited, while zones with lower AFFF concentrations or individual PFAS species will be less likely to interfere with bioremediation processes.

Chapter 4

Considerations for Applying In Situ Heat-Activated Persulfate Oxidation for PFAS Treatment

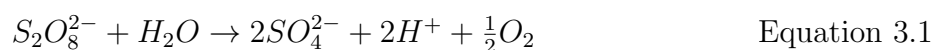
4.1 Introduction

With near-ubiquitous per- and polyfluoroalkyl substances (PFAS) water contamination,[18, 19, 20, 21] treatment technologies are needed that can mineralize PFAS, especially at point sources. PFAS are an active ingredient in aqueous film-forming foams (AFFFs), a firefighting foam used for decades on military bases and at airports.[9, 11, 10] Because of repeated contamination events, a diverse suite of PFAS have migrated into groundwater aquifers around the country, endangering drinking water for millions of people.[26] Remediation of PFAS has become a priority in much of the world, as several of the most common PFAS are toxic and bioaccumulative.[44, 45]

PFAS treatment is often discussed in terms of removal or destruction. A common strategy used for mitigating human exposure to PFAS is removal by granular activated carbon (GAC), either at source zones using ex situ pump-and-treat or at water treatment plants.[148, 149, 150, 151] Researchers have shown that long-chain PFAS are preferentially adsorbed onto GAC over short-chain PFAS, and perfluoroalkylsulfonic acids (PFSAs) over perfluoroalkylcarboxylic acids (PFCAs), resulting in variable removal efficiencies for individual PFAS compounds.[29, 48, 49] Further, spent GAC is disposed of via incineration, but research has shown that many PFAS remain in the ash after incineration and some volatilize, escaping destruction.[152] Therefore, rather than mineralizing PFAS, incinerators can become new sources by depositing PFAS in the surrounding area. Beyond this ongoing source of PFAS, sorption using pump-and-treat often takes decades and therefore is an expensive treatment option for source zones. Given these drawbacks, an in situ treatment technology that can mineralize PFAS at source zones would be a major step in reducing the mass of PFAS persisting in drinking water sources.

In situ chemical oxidation (ISCO) is a source zone treatment strategy that has been used for decades for organic groundwater contaminants, including chlorinated solvents and hydrocarbon fuel compounds.[54] Persulfate can be activated in several ways (e.g., heat, basic pH) to create even more reactive sulfate radicals.[153] Sulfate radicals can mineralize two major classes of PFAS: PFCAs (under acidic pH conditions) via an unzipping mechanism leading to CO_2 and F^- , as well as polyfluoroalkyl substances.[55, 58, 59] While neither persulfate nor the sulfate radical has been shown to transform perfluorooctane sulfonic acid (PFOS) [55, 58, 59] or other PFSAs, sulfate radicals can mineralize the polyfluoroalkyl substances, including those created via electrochemical fluorination (ECF), that might otherwise transform into PFSAs biologically as shown in literature and in Chapter 2,[69] thus preventing the formation of these dead-end transformation products. Additionally, persulfate has been shown to transform organic co-contaminants such as 1,4-dioxane; hydrocarbon fuel components benzene, toluene, ethylbenzene, and xylene (BTEX); and chlorinated solvents.[154, 155] Therefore, at point sources of PFCAs and polyfluoroalkyl substances where organic co-contaminants exist, activated persulfate oxidation could be an effective PFAS destructive treatment for mass reduction of mixed contaminants.

Although activated persulfate oxidation can mineralize polyfluoroalkyl substances, PFCA mineralization has been shown to be only possible at pH ranges below 3.[58] Researchers have hypothesized that this requirement is due to the sulfate radical needing to be protonated to interact with the anionic PFCAs [61] or because low pH decreases the likelihood of HO^- scavenging of sulfate radicals given similar rate constants observed for PFBA mineralization between pH 0.5-4[62], though some researchers show that HO^- scavenging is only significant at much higher pH values.[58] While the reason for an acidic pH is still hypothesized, the degradation of persulfate creates protons (Equation 3.1) that acidify the aquifer environment proportional to the persulfate dose, allowing PFCA transformation to occur.



Beyond pH, additional challenges include radical scavengers such as labile organic compounds and chloride that may compete with the target PFAS and co-contaminants for sulfate radicals,[55, 58] decreasing the treatment efficacy. Bruton and Sedlak showed the possibility of mineralizing PFCAs in a perfluorooctane carboxylic acid (PFOA)-only matrix, and in an AFFF mixture with the main precursor 6:2 fluorotelomer thioether amido sulfonate (FtTAoS) at 85°C without and with 20 g/L aquifer solids.[55, 58] Park et al demonstrated PFOA degradation at 50°C with 20 g/L of aquifer solids, and 6:2 fluorotelomer sulfonic acid (FTSA) degradation without solids present at 40 and 50°C.[59] Although these studies demonstrated the ability of heat-activated persulfate to mineralize PFCAs, they did not explore the efficiency and possible inhibitors of heat-activated persulfate oxidation (HAPO) at 40°C, more likely to be used under field conditions, or the effects of pH, buffering minerals, co-contaminants, and more realistic (e.g., 1,000 g/L) concentrations of aquifer solids at this low activation temperature. Using realistic concentrations of aquifer solids is especially

important for informing what range of persulfate doses are needed for in situ oxidation of PFAS.

The aim of this research was to evaluate low temperature in situ HAPO for PFAS source zones. We tested the transformation of PFAS and co-contaminants at low temperatures with potential in situ inhibitors including high solids concentrations, and suggest persulfate dose concentrations and frequency to overcome the challenges of in situ HAPO.

4.2 Materials and Methods

Chemicals

Sodium persulfate (>99.0%) and potassium persulfate (>99.99%) were purchased through Sigma Aldrich, calcium carbonate (>99.0%) and 1,4-dioxane were purchased through Fisher Scientific, trichloroethene (TCE) from Acros Organics (99.6%), and sodium hydroxide was purchased through VWR at >99.99% purity. All other chemicals were purchased at the highest purity available through either Fisher or Sigma. LC-MS-grade water and methanol were purchased through Fisher Scientific. All PFAS standards, including mass labelled standards, were purchased through Wellington Laboratories. Concentrated Ansul and 3M AFFFs were graciously donated by Prof. Jennifer Field at Oregon State University from the AFFF archive in the Field laboratory.

Soil Characterization

The two soil samples used in the temperature, calcium carbonate, pre-acidification, and co-contaminant experiments, from air force bases in Arizona (AZ) and Oklahoma (OK), were sent to the UC Davis Analytical Lab for measurement of total nitrogen, total carbon, total organic carbon, and some metals (zinc, manganese, iron, and copper) (UC Davis) (Table D.1). Samples were dried at 100°C, ground, and sieved at 600 µm before shipment.

HAPO Variable Temperature Batch Tests

HAPO experiments were conducted with 50 mM sodium persulfate ($\text{Na}_2\text{S}_2\text{O}_8$), in the presence or absence of AZ or OK aquifer solids (20 g/L) at 85, 65, and 40°C using either Ansulite (Ansul) or 3M AFFF formulations that were diluted 1:10,000 (1-3 µM PFAS in final concentrations). Experiments at 65 and 85°C were conducted in a temperature-controlled water bath, while 40°C experiments were in an incubator. All solids used were dried at 100°C, homogenized, and passed through a 600 µm sieve before use in experiments. These tests were done in duplicate.

Calcium Carbonate and Pre-acidification Batch Tests

Duplicate batch experiments were conducted by adding increasing masses of calcium carbonate to Milli-Q water for final concentrations 2, 5, 10, 25, and 50 mM. 3M and Ansil AFFFs were added for a total PFAS concentration of 1-4 μM . Then, aliquots of a 500 mM sodium persulfate stock solution were added for a final persulfate concentration of 50 mM. The sealed tubes were placed in an incubator set to 40°C and were sampled periodically.

The pre-acidified tests were performed similarly as to what is described above, in duplicate and with 20 g/L aquifer solids, but additionally were acidified to a target pH 2-3 using 1 N sulfuric acid.

Co-contaminants batch tests

TCE-contaminated HAPO experiments were conducted with either 1 mg/L or 10 mg/L of TCE in sealed glass 160 mL serum bottles with 20 g/L of AZ aquifer solids. They were first pre-acidified with 1 N sulfuric acid to pH 2-3, then persulfate was added for a final concentration of 50 mM. 1,4-Dioxane-contaminated HAPO experiments were done in triplicate without solids present; 1 ppm of 1,4-dioxane was added to the AFFF-diluted Milli-Q water. This test was conducted first in a 40°C water bath and aqueous samples were taken regularly for about 14 hours. After this period, the reactors were moved into a 40°C incubator for PFAS sampling on a daily basis.

High solid slurry batch tests

PFAS-contaminated groundwater and aquifer solids (5-35' below ground surface) were generously donated by John Kornuc of the US Navy, obtained from a US Navy site with known PFAS contamination in Florida (FL). The contaminated groundwater contained approximately 1.2 μM of PFCAs and 1.4 μM of precursors, the latter expressed by the difference between PFCA concentrations before and after the total oxidizable precursor (TOP) assay (Figure D.9). Additionally, the total PFSA concentration was about 4.5 μM . Although the PFSA concentrations exceed that of precursors and PFCAs, we found these materials to be a good fit for HAPO tests due to the low organic concentrations in the groundwater (44 mg/L) and low alkalinity 1.1 mM (110 mg/L as CaCO_3).

In addition, we tested dissolved metals in the groundwater and solids using acid digestion (sulfuric acid pH 3 and aqua regia) to quantify the maximum amount of metals that could be released upon acidification via HAPO (Figures D.10-D.12). Metals were measured on an inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7700 Series). FL aquifer solids were dried at 100°C, homogenized, and sieved at 600 μm before use in chemical oxidation experiments.

Experiments were conducted by adding a 1:1 ratio of dried solids with the contaminated groundwater (e.g., 100 g solids and 100 mL groundwater). Concentrated stock solutions (e.g., 500 mM or 2 M) of sodium persulfate were added to the target final solution concentration.

Initial samples were taken immediately, without any heat-activation. These bottles were kept in a 40°C incubator and manually mixed periodically. Slurry samples were taken and centrifuged to separate the solids; supernatant was then diluted 1:1 with methanol and stored (-20°C) for LC-MS/MS analysis or frozen as aqueous samples for other analyses (i.e., persulfate, dissolved organic carbon (DOC), etc.). At the end of this experiment, a solids extraction test was performed, as previously described.[18, 99]

Analytical methods

The TOP assay was conducted by adding diluted aqueous PFAS samples into a 7 mL vial with sodium hydroxide and sodium persulfate for final concentrations of 67 mM and 20 mM, respectively. These mixtures were left overnight (≤ 16 hours) in an 85°C water bath for heat-activation of the persulfate; 1 mL of methanol was added to quench the reaction. The vials were neutralized with HCl and stored in a 4°C fridge until LC-MS/MS analysis.

Persulfate was measured using a colorimetric assay adapted from Liang et al.[156] Briefly, a diluted sample was added to a potassium iodide (KI) reagent (0.462 g NaHCO_3 and 9.13 g KI in 100 mL Milli-Q water) and left to react in the dark for 30 minutes. Persulfate oxidizes I^- to I_2 , which turns the solution shades of yellow. The samples were transferred to quartz cuvettes, measured on a spectrophotometer (352 nm), and concentrations calculated with a standard curve.

Aqueous samples for DOC were stored in a -20°C freezer and measured within a week of sampling using a Total Organic Carbon Analyzer (Shimadzu TOC-VCSH).

TCE was measured by injecting 100 μL of headspace into a gas chromatograph coupled to flame-ionization detection (GC-FID) (Agilent 7890A) with a GS-GasPro column as previously described.[123]

Chloride and chlorate were measured using an ion chromatograph (Thermo Scientific Dionex AS-DV; AS23 4x250 mm analytical column) as previously described.[58]

1,4-dioxane was measured using a gas chromatograph coupled to a mass spectrometer (Waters Quattro microTM GC) using solid phase microextraction as described previously.[157] Samples were diluted within the calibration range and NaCl was added for a final composition of 30% NaCl. Concentrations were calculated against a standard curve.

Methanol-diluted samples for PFAS analysis were vortexed for 10 minutes and centrifuged for 10 minutes at 10,000 g . They were then further diluted into 2 mL amber LC vials with LC-grade water and methanol, with an internal standard mixture (2 $\mu\text{g/L}$ 13C-labeled PFAS). Samples were separated using a Zinc-Diol guard column coupled to Zorbax C18 XDB guard and analytical columns. Another C18 column served as a delay column at the outlet of the methanol pump to decrease the possibility of PFAS contamination from the instrument. The mobile phase was a water/methanol gradient, each with 10 mM ammonium acetate (Table D.2) and targeted PFAS are listed in Table B.2.

4.3 Results and Discussion

Temperature Dependence on Persulfate Activation and PFCA Transformation

Previous experiments in our lab demonstrated the efficacy of HAPO of some precursor compounds and PFCAs at 85°C in the presence of low concentrations (i.e., 20 g/L) of aquifer solids.[55, 58] Our first goal was to establish the efficacy of precursor and PFCA destruction at lower temperatures. Lowering the temperature could lower remediation cost and enhance potential for complementary bioremediation processes, thereby broadening applications of persulfate ISCO. We performed HAPO on diluted (10,000x) 3M and Ansul AFFFs (to test both an ECF-based AFFF and a fluorotelomer-based AFFF) in MilliQ water at 65°C and 40°C (Figure 4.1a-d).

In the absence of solids, at an activation temperature of 65°C, >95% of precursors and PFCAs were removed within 60 hours for both AFFFs (Figure 4.1a and c). The pH decreased to below 3 within 5 hours and the persulfate was nearly depleted after 56 hours (Figure D.1). The 40°C test resulted in >90% PFCA transformation for both AFFFs (Figures 4.1b and 4.1d) over 9 days. The pH decreased to below 3 within 5 days, and about half of the persulfate remained after 15 days (Figure D.1). The PFASs in the 3M AFFF were not oxidized by HAPO (Figure D.2). Previous work at 85°C with a 21,000x diluted Ansul AFFF resulted in 90% PFAS transformation within 8 hours.[55] Together, these results show that activation temperatures impact the transformation rate of precursors and PFCAs, but that even at the lowest activation temperature (40°C), PFAS mineralization of AFFF samples could be achieved. It is interesting to note that PFCAs were transformed at 40°C even with half of the persulfate remaining.

Introducing aquifer solids (20 g/L of AZ solids; 30 mM HCO_3^- measured by titration) still resulted in PFCA transformation (93% removal) at 65°C after 56 hours (Figure 4.1e), though no PFCA transformation was observed at 40°C (Figure 4.1f). Precursors transformed into PFCAs between days 0 and 5 at 40°C, but no subsequent degradation occurred (Figure 4.1f) due to a persistent circumneutral pH. After 27 days, 30 mM of persulfate remained in solution (data not shown), indicating that persulfate availability was not limiting PFCA transformation.

Inhibition of Buffering Capacity

To confirm that pH buffering inhibited complete mineralization of PFCAs and precursors in AFFF at 40°C, we tested HAPO at this temperature with 3M and Ansul AFFFs and increasing concentrations of calcite (no added aquifer solids) to mimic a range of buffering capacities (Figures 4.2, D.3, and D.4). Precursors transformed into PFCAs regardless of pH over the first day of the experiment. Once the buffering capacity was depleted and the pH dropped to below 3, PFCA transformation began and continued until the end of the experiment as expected; higher concentrations of calcite resulted in longer lag times before

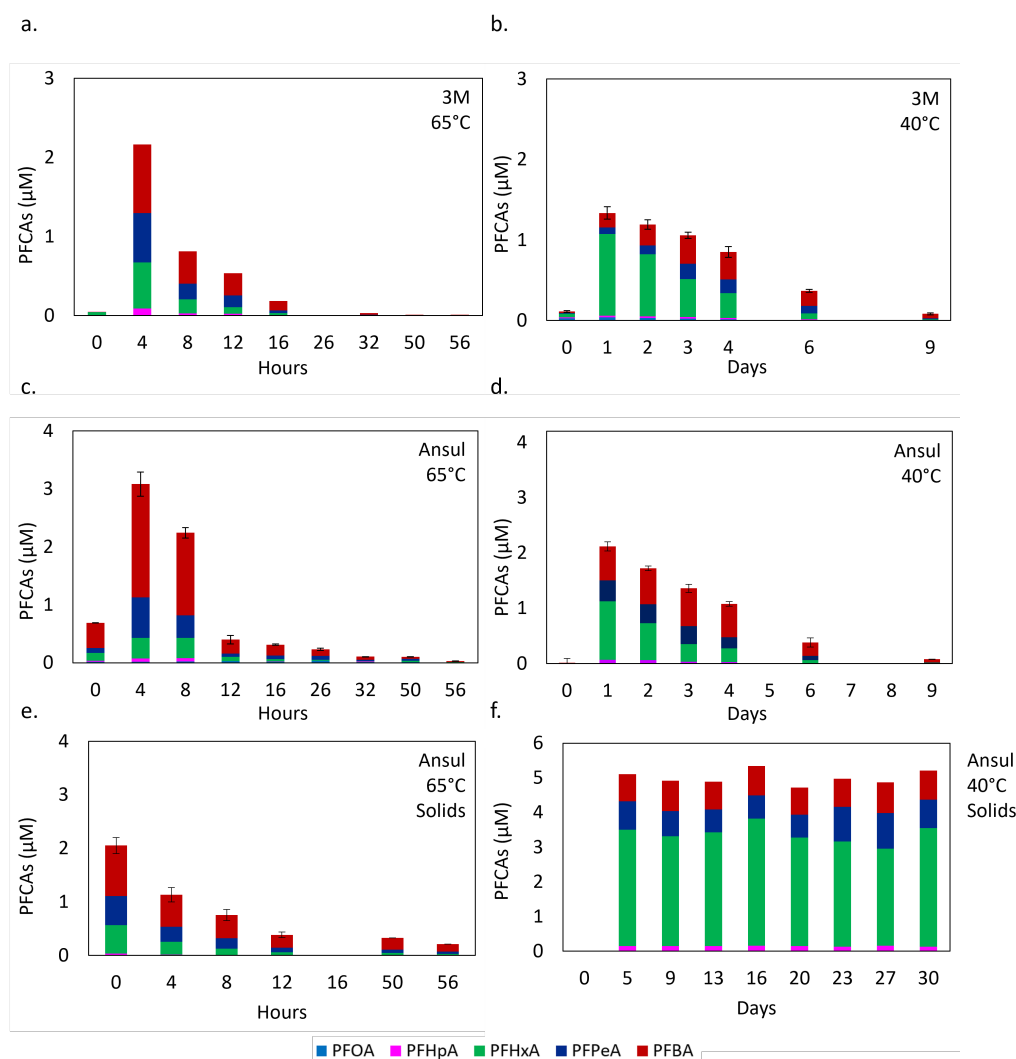


Figure 4.1: HAPO of AFFFs with 50 mM sodium persulfate. 3M AFFF shown in parts a and b and Ansul AFFF shown in c, d, e, and f. Parts a, b, c, and d show PFCAs transformation in a MilliQ water solution, and e and f show Ansul AFFF with 20 g/L of aquifer solids. The left graphs show the experiments conducted at 65°C (a, c, and e) while the right graphs show the results at 40°C (b, d, and f). At 40°C, PFCAs transformation was inhibited in the presence of solids even over an extended 30 day sampling period, though precursors transformed into PFCAs.

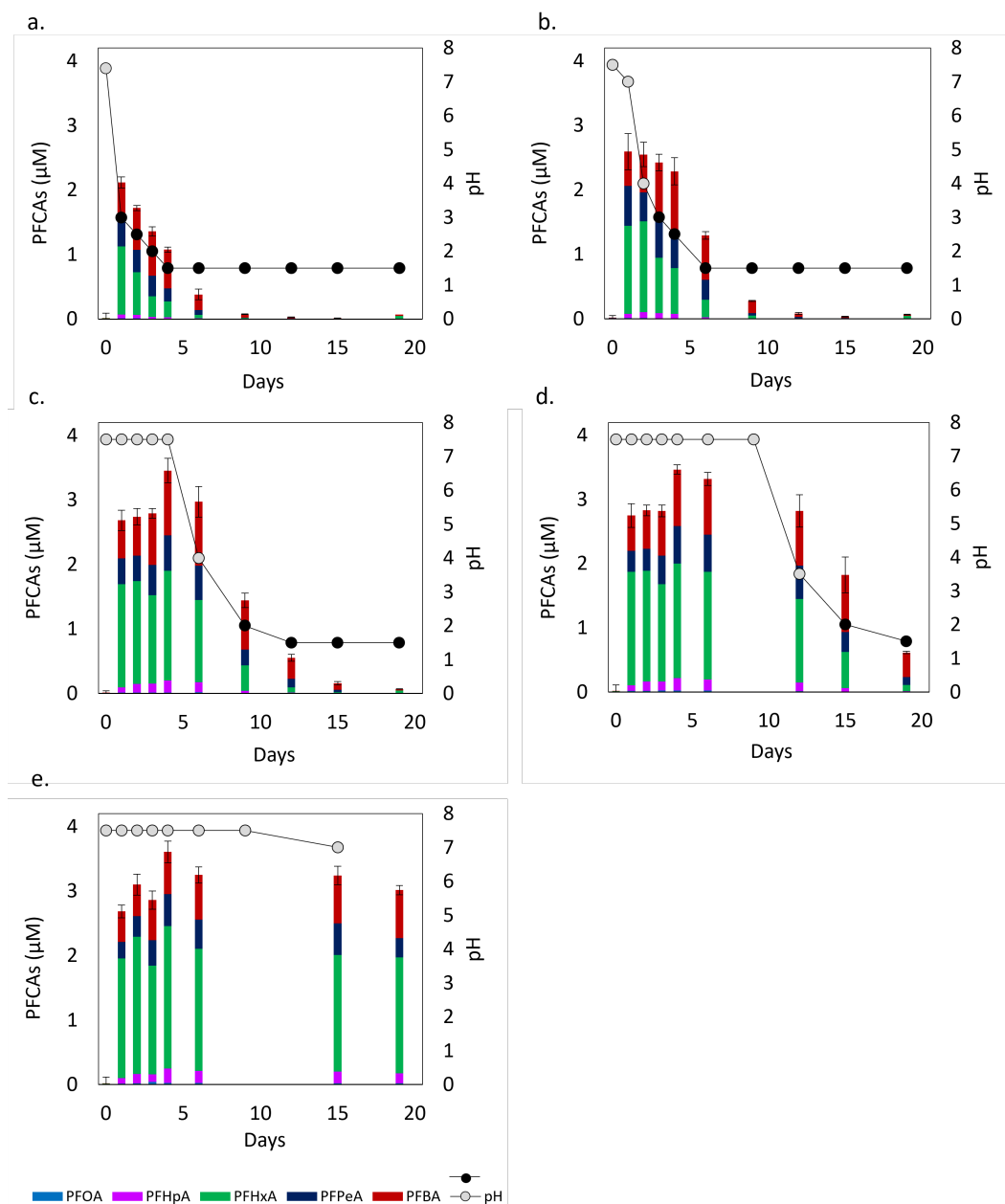


Figure 4.2: HAPO of Ansul AFFF diluted in MilliQ water at 40°C with 50 mM sodium persulfate and increasing concentrations of calcite (no aquifer solids present): a) 0 mM, b) 2 mM, c) 5 mM, d) 10 mM, and e) 25 mM calcite. The left y-axis shows PFCA concentrations, while the right y-axis shows pH measurements. The gray circles indicate a $\text{pH} > 3$, which is too high for PFCA transformation, and the black filled circles indicate $\text{pH} \leq 3$, which is amenable to PFCA transformation by sulfate radical.

PFCA transformation began (Figure 4.2). As mentioned above, two groups have recently published studies on the pH-dependence of activated persulfate oxidation of PFCAs.[61, 62] Although the mechanisms is still unclear, it is integral to have an acidic solution before PFCA mineralization can occur. If employing HAPO at field sites, engineers should expect to see PFCA concentrations increase at $\text{pH} > 3$, while precursors transform, and only see PFCA concentrations decrease when the pH falls below 3.

Acidification as HAPO Pre-treatment

Higher concentrations of buffering minerals in an aquifer would mean a higher concentration of persulfate needed to acidify the groundwater, increasing the amount of persulfate needed in the field. To overcome this buffering-induced delay, and to demonstrate a possible pretreatment step, we tested the acidification of water-soil slurries prior to HAPO. We conducted batch tests using both the AZ and OK aquifer solids (Table D.1) that were amended with either 3M or Ansul AFFF. We compared pre-acidified bottles (to pH 2-3 with 1 N sulfuric acid) to those without pre-acidification (Figures 4.3 and D.5).

Over the course of about 20 days, the slurries with sulfuric acid addition showed greater PFCA degradation than the slurries without the addition of acid (Figures 4.3 and D.5). Following pre-acidification, the AZ slurries showed quicker PFCA degradation than the OK slurries (Figure D.5), likely due to higher total carbon concentrations in the OK solids (Table D.1). After acidifying to pH 2-3, the pH of the OK slurry increased over the first two days, which postponed the onset of PFCA destruction until day 9 (Figure D.5). It is likely that additional acid or persulfate would have resulted in faster transformation and higher removal of PFCAs during the timeframe of the experiment. The AZ slurries achieved a greater degree of PFCA destruction after about 20 days because the pH remained below 3. These results indicate that the dissolution of acid neutralizing minerals in aquifer solids could halt PFCA transformation, even if the slurry is initially acidified, and that pH must be monitored throughout treatment for best PFAS removal.

The results from the calcite and pre-acidification experiments indicate that groundwater and aquifer solids buffering can be overcome by designing persulfate dose accordingly (activation of higher persulfate concentrations results in higher H^+ concentrations) or pre-acidifying the groundwater. Needing the groundwater to be acidic for PFCA transformation could result in greater hazard and requires caution if implementing this strategy as an in situ PFAS source zone treatment.

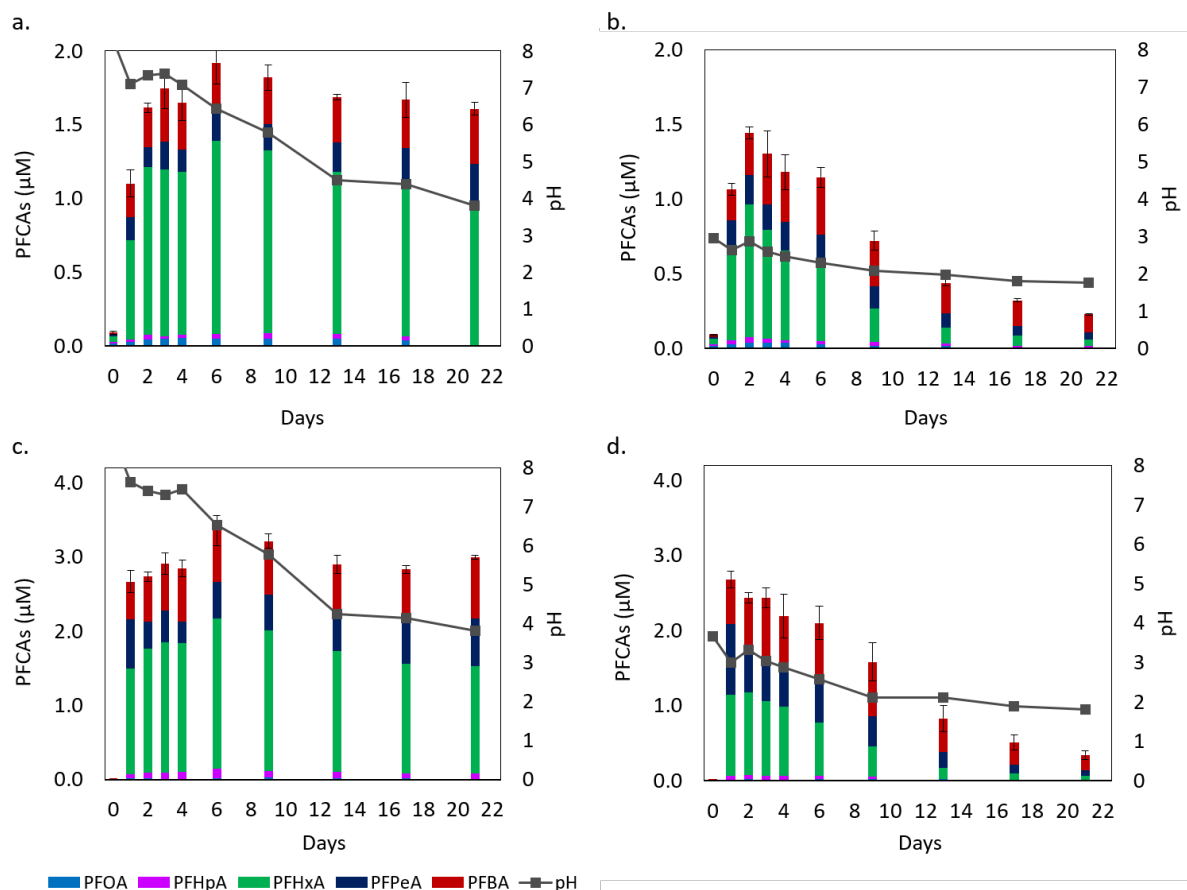


Figure 4.3: HAPO at 40°C with 50 mM sodium persulfate using 20 g/L AZ solids. a) 3M AFFF without a pre-acidification step, b) 3M AFFF with a pre-acidification step, c) Ansul AFFF without a pre-acidification step, and d) Ansul AFFF with a pre-acidification step. Acidification was conducted using 1 N sulfuric acid until the pH was 3.

Transformation of PFAS Uninhibited by Co-contaminants

Because common co-contaminants in AFFF-impacted sites include chlorinated solvents and 1,4-dioxane,[9, 158] we evaluated potential competition from these organics for sulfate radicals during HAPO of PFAS. Two concentrations of TCE (7.6 and 76 μM) were added to soil slurries pre-acidified with sulfuric acid before beginning HAPO at 40°C. Samples taken hourly at the start of the experiment showed rapid transformation (within 12 hours) of TCE in the presence of both types of AFFF (Figure 4.4a). Samples taken daily tracked PFAS and chloride concentrations from TCE. After about three weeks, PFCA transformation resulted in about 90-93% removal of precursors plus generated PFCAs in the co-contaminated slurry (Figure D.6). These results are comparable to those from Figure 4.3, indicating that the presence of TCE did not affect PFAS transformation. There appears to be mild inhibition of precursor transformation into PFCAs (taking 2 days compared to 1 day in previous experiments), potentially due to sulfate radical scavenging by chloride from TCE (Figure D.6).

After an initial increase of chloride concentrations from the degradation of TCE, the chloride was oxidized to chlorate (which occurs at $\text{pH} < 5$) (Figure D.7), yielding 70 μM of chlorate by day 3.[159] The US EPA lists a maximum drinking water concentration of chlorate at 56 $\mu\text{g/L}$, and therefore chlorate formation would need to be monitored carefully under HAPO conditions, especially if near any drinking water sources. However, after treating PFAS with HAPO, any remaining PFASs plus the salinity of the treated groundwater due to high concentrations of persulfate would prevent this groundwater from being a downstream source of drinking water.

Similarly, 1,4-dioxane was added to diluted 3M and Ansul AFFFs in MilliQ water (Figure 4.4b) and heat-activated at 40°C to determine if this added organic content decreased the PFCA transformation efficiency. 1,4-Dioxane was quickly transformed within 8 hours; because this reaction occurred so quickly, it is likely that persulfate as well as sulfate radicals were able to transform this compound. PFCA transformation remained unaffected (Figure D.8), though precursors took 2 days to transform into PFCAs similarly to the TCE tests. All DOC was depleted to below 5 mg/L (Figure D.9) by the end of the experiment.

PFCAs are much more recalcitrant than organic co-contaminants such as TCE and 1,4-dioxane. Even with a high ratio of oxidant to PFAS, it takes days for PFCAs to mineralize, versus only hours for the co-contaminants to mineralize at 40°C. These co-contaminants may be oxidized by the persulfate anion ($\text{S}_2\text{O}_8^{2-}$) without activation, while PFCAs can only be transformed by the sulfate radical at acidic pH values. Additionally, rate constants of sulfate radicals with longer-chain PFCAs are orders of magnitude lower than with natural organic matter (NOM) and chloride (Tables D.3 and D.4). At an AFFF remediation site, engineers may see slower initial transformation of precursors into PFCAs if there are organic co-contaminants present, and may require higher doses of persulfate.

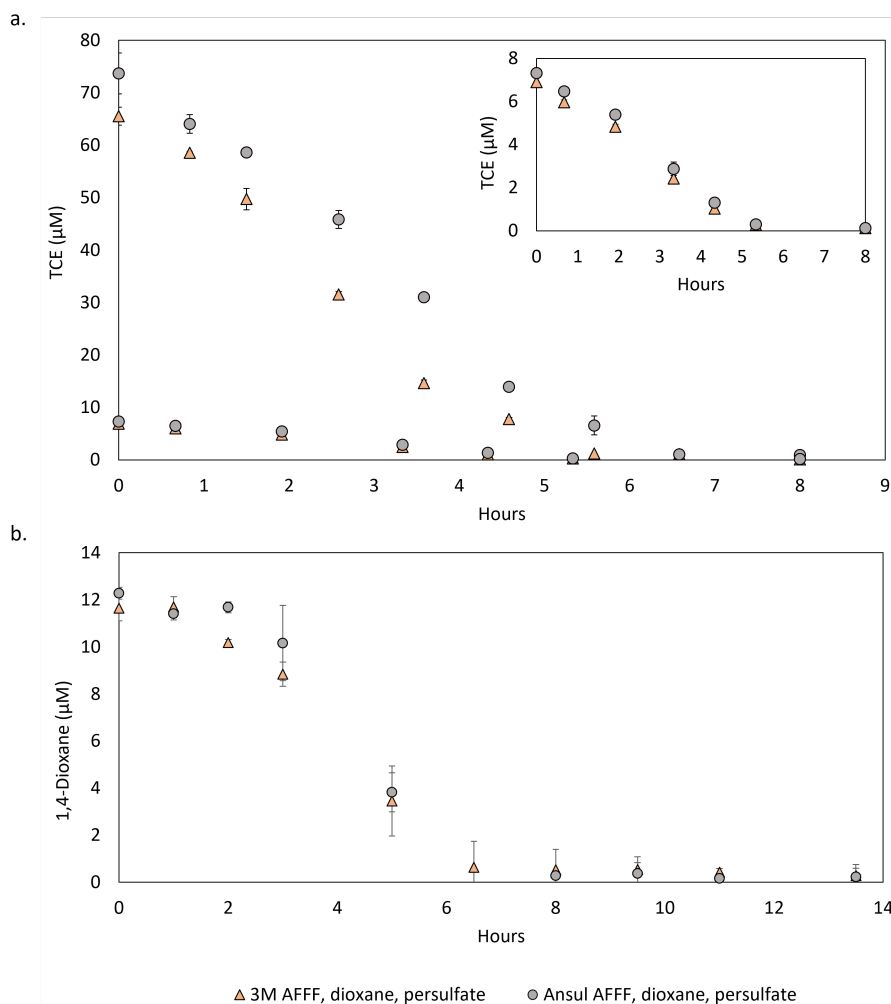


Figure 4.4: a) Two concentrations of TCE (7.6 and 76 μM , or 1 and 10 mg/L, respectively) were degraded by 50 mM persulfate within eight hours in a slurry solution of AFFFs diluted (1:10,000) in Milli-Q water with 20 g/L aquifer solids at 40°C. The slurries were acidified to 2 before the addition of persulfate on Day 0. Inlaid is the lower TCE concentration data. Error bars represent duplicate reactors. b) Degradation of 1,4-dioxane in under 8 hours with 50 mM sodium persulfate and diluted 3M and Ansul AFFFs, done in triplicate. 3M AFFF experiments are denoted with orange triangles, Ansul AFFF with gray circles.

Mimicking Aquifer Conditions and Persulfate Dosing

The aforementioned HAPO experiments were all conducted with 50 mM persulfate and, where solids were present, 20 g/L were added. To more closely simulate aquifer conditions, the following experiments consisted of a one-to-one ratio of groundwater to solids (i.e., 1,000 g/L). In addition, we simulated post-HAPO treatment steps including neutralization to monitor concentrations of dissolved metals. These experiments were performed using PFAS-contaminated groundwater and solids from an air force base in Florida (FL). The PFAS concentrations detected via the TOP assay are shown in Figure D.10, the metals concentrations are shown in Figures D.11-D.13, and the DOC concentration of the groundwater was 60 mg/L.

Preliminary HAPO tests using 50, 100, and 200 mM persulfate added on day 0 resulted in only precursor transformation into PFCAs, and no further PFCA degradation (data not shown). Another one-to-one test was conducted with a 500 mM persulfate dose added day 0, followed by a 100 mM persulfate addition after three weeks (Figure D.14). While it resulted in 50% PFCA transformation, this test, too, resulted in the stalling of PFCA transformation.

We then tested various iterations of a weekly dosing experiment, during which we sampled weekly and added persulfate doses each week after sampling. We performed these experiments initially with 200 mM and 500 mM sodium persulfate; both tests resulted in >95% PFAS removal after four or five doses of persulfate (Figure 4.5).

With the success of weekly persulfate doses, we then tested lower persulfate concentrations, 50 and 100 mM, compared to 200 mM and 300 mM (Figure 4.6). The pH of all reactors dropped to below 4 within one day, and below 3 by the third day (data not shown). Our results showed over 97% of PFAS destruction under both 50 and 100 mM weekly persulfate doses, though a longer reaction time was required. For all treatments, the DOC decreased by about 50% after 63 days, suggesting that some recalcitrant organics were resistant to persulfate oxidation and could have contributed to sulfate radical scavenging (Figure D.15).

The success of low doses of persulfate during 40°C HAPO opens up various possibilities for dosing for PFAS treatment. Adding more lower doses of persulfate would result in less cost for chemicals because less persulfate and neutralizing agent (e.g., carbonate or NaOH) would be required. However, the cost savings of many lower dose events would have to be considered with operations costs with having fewer, high concentration oxidant delivery events. In ISCO field studies, it is common to have 1-2 oxidant delivery events with a median duration of 4-6 days each.[160] HAPO for PFAS destruction, on the other hand, may require at least 4 delivery events depending on the chosen persulfate concentration.

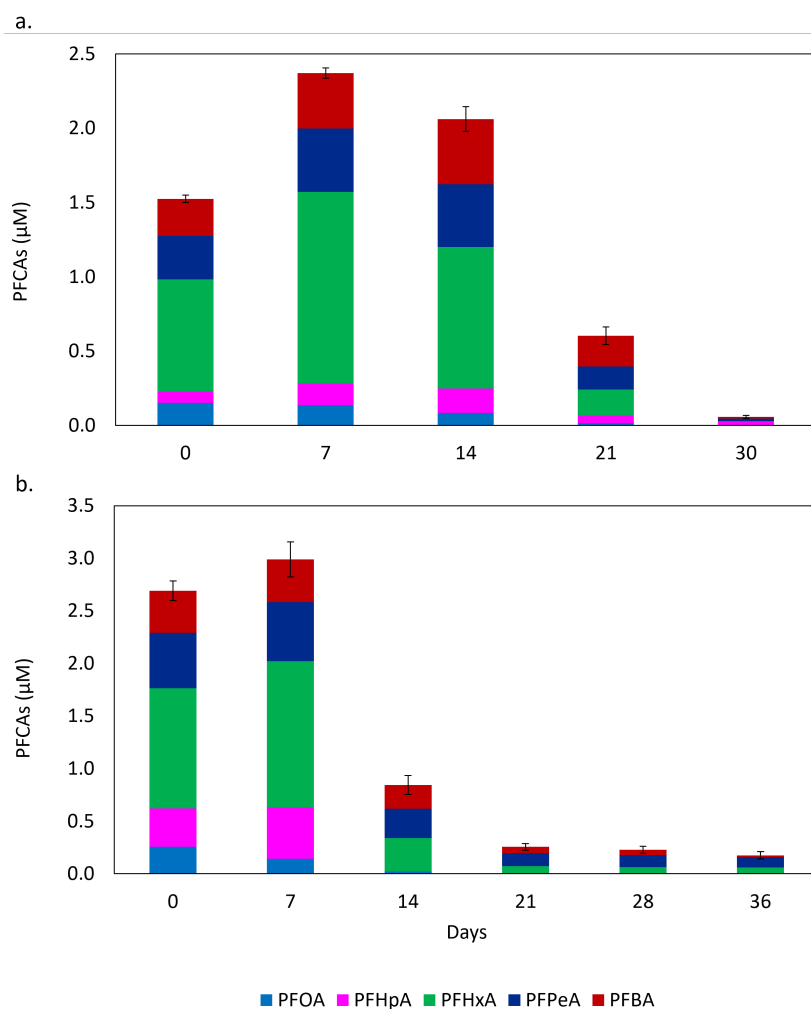


Figure 4.5: HAPO at 40°C of contaminated FL groundwater and aquifer solids (1,000 g/L). Doses of a) 200 mM persulfate were added on Days 0, 7, 14, and 21, and b) 500 mM persulfate were added Days 0, 7, 14, 21, and 28.

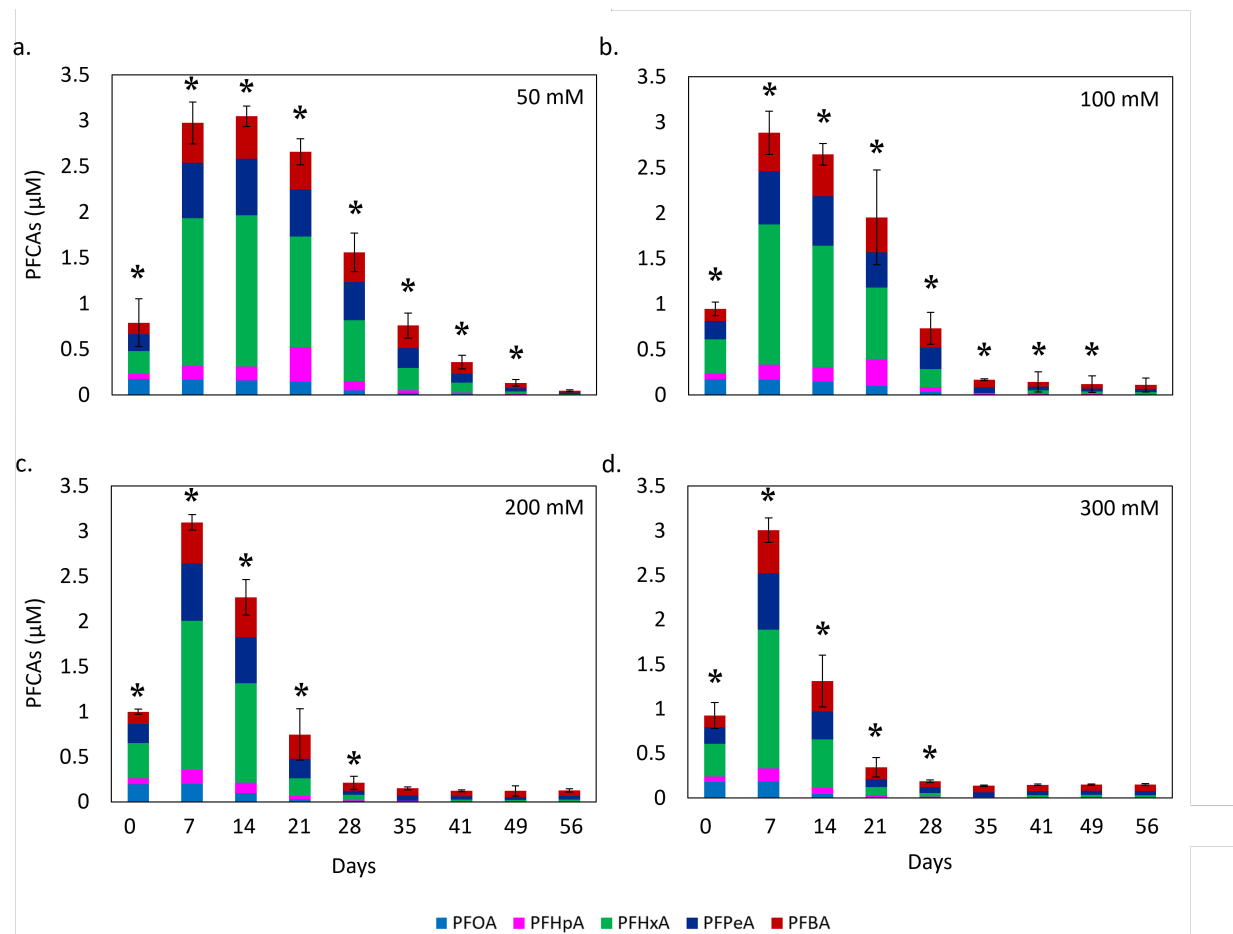


Figure 4.6: HAPO at 40°C of contaminated groundwater and aquifer solids (1,000 g/L), with a) 50 mM, b) 100 mM, c) 200 mM, and d) 300 mM sodium persulfate doses added weekly, shown by the asterisks. Error bars represent triplicate reactors.

Post-treatment neutralization

Once HAPO treatment of an AFFF-contaminated site is completed, the groundwater would have to be neutralized. We investigated adding calcite or sodium hydroxide to neutralize the slurry tests from the HAPO test shown in Figure 4.6, and monitored how metals were affected. For the final time point of the experiment, increasing concentrations of persulfate (the weekly doses of 50, 100, 200, and 300 mM persulfate) added resulted in higher concentrations of dissolved metals measured (Figure 4.7). After neutralizing the slurries with either calcite (55 g/L for the 50 mM persulfate dose condition, up to 135 g/L for the 300 mM persulfate dose condition) or sodium hydroxide (27 g/L for the 50 mM persulfate dose condition, up to 70 g/L for the 300 mM persulfate dose condition), metals concentrations decreased significantly; both neutralization methods resulted in precipitation and 99% removal of metals from the aqueous phase (Figure 4.7).

4.4 Implications

Low temperature (40°C) HAPO can be an effective PFAS remediation strategy at AFFF source zones, though with some key limitations. The aquifer should have low chloride concentrations, low buffering mineral content, low concentrations of toxic metals, and ideally be composed of precursor PFAS and/or PFCAs. Co-contaminants such as 1,4-dioxane and chlorinated solvents (and potentially many others) will be transformed under HAPO and much more quickly than the PFAS. Repeated injections of persulfate are likely needed for best transformation (50-300 mM, over several weeks) depending on the aquifer conditions. While some metals may be mobilized based on the acidic pH caused by HAPO, neutralization post-treatment can precipitate and immobilize the vast majority. Unintended byproducts such as chlorate and mobilized metals should be monitored post-HAPO and post-neutralization, as well as the pH of the aquifer.

ISCO projects have been steadily increasing since the 1990s for a range of organic contaminants, under a range of geological conditions, and to achieve different remediation goals (e.g., mass reduction, maximum concentration limits, alternative (risk-based) cleanup levels)[160]. Although in situ HAPO as an ISCO treatment for PFAS contaminants has its own set of challenges and inhibitions, it is one tool in the PFAS treatment toolbox that can mineralize PFAS in situ, an advantage over ex situ treatment options. Some industrial PFAS sites as well as landfill leachates with a higher concentration of PFCAs and precursors could be better suited for HAPO compared to some AFFF sites that have a high abundance of PFOS.

HAPO is a unique destruction method because it mineralizes two major classes of PFAS without creating a slew of unknown transformation products. Each site and PFAS-contaminated matrix is unique, and HAPO can, and should, be one of the many tools to help break the global, persistent PFAS cycle.

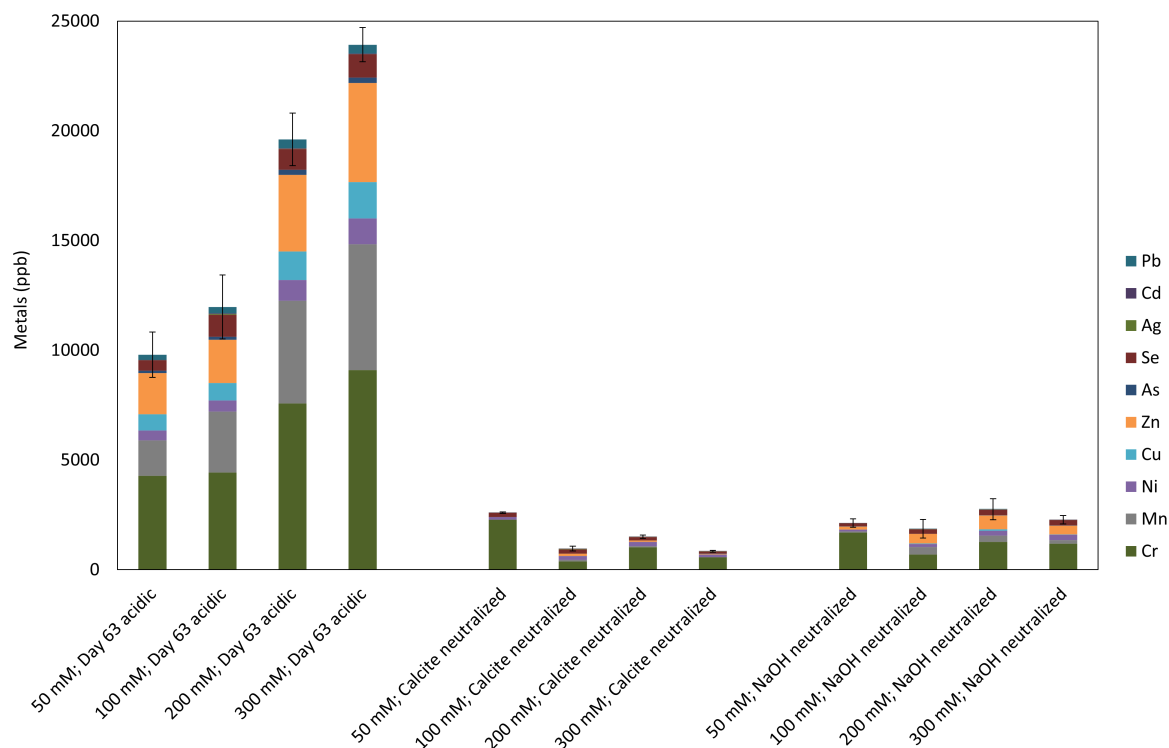


Figure 4.7: Dissolved metal concentrations ($\mu\text{g/L}$) resulting from the various persulfate concentrations added during 40°C HAPO experiment with 1,000 g/L aquifer solids. Results are shown immediately after the experiment (in the first four columns) and after calcite (second four columns) or NaOH neutralization (final four columns). Metals shown are chromium (Cr), manganese (Mn), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), silver (Ag), cadmium (Cd), and lead (Pb).

Chapter 5

Conclusions

This dissertation investigates PFAS in source zones: how they may biotransform, how they may impart toxicity on microbial communities, and how they might be treated for AFFF source zone remediation projects. Experimental designs included controlled microcosm experiments to transform PFAS, TCE, or BTEX in Chapters 2 and 3, and chemical oxidation studies using heat-activated persulfate in Chapter 4. Key data collection and analysis included PFAS concentrations and composition using LC-MS/MS and HRMS, microbial substrate degradation, microbial electron acceptor utilization, DNA extraction and sequencing, cellular ATP and metabolite production, and solution conditions such as pH, persulfate, buffer, anions, DOC, and metals.

ECF-based PFAS precursors such as AmPr-FHxSA can biotransform into PFHxS, with many potential intermediates, with varying microbial communities. The long-term production of PFHxS is troubling due to its mobility in the environment and its persistence, toxicity, and bioaccumulation capability. Being able to treat PFAS precursors such as AmPr-FHxSA before they can biotransform to PFASs should be a remediation priority for site engineers who are monitoring the PFAS concentrations and composition of AFFF-impacted sites. At a contaminated site where bioremediation processes are being considered for treatment of chlorinated solvents or BTEX, PFAS individually or 3M AFFFs could negatively impact remediation. PFAS may impact which microbial species are present and could impact cellular mechanisms such as their ability to generate energy and perform cell signalling. Considering a site with multiple contaminants, HAPO could be a feasible source zone treatment strategy for PFAS and co-contaminants. Although HAPO requires an acidic pH, once achieved, PFCAs are reliably mineralized. HAPO would especially be useful at sites with high concentrations of precursors (which could therefore prevent biotic production of PFASs) and high concentrations of PFCAs. HAPO could potentially have many other uses; if achieving an acidic pH in a well-buffered aquifer is not an option, HAPO could be used in ex situ scenarios where high concentrations of PFAS need to be mineralized. This could include PFAS from sorbent materials or even stockpiled fluorotelomer-based AFFFs.

5.1 Biotransformation of AmPr-FHxSA

With different carbon sources controlling microbial community composition and function, AmPr-FHxSA aerobically biotransforms to produce FHxSA, FHxSA-PrA, FHxSAA, PFHxSi, and PFHxS under methane and acetate oxidizing conditions, FHxSAA, PFHxSi, and PFHxS under DGBE oxidizing conditions, and PFHxSi and PFHxS with BTEX as the carbon source. Methane oxidizing conditions resulted in the highest yield of AmPr-FHxSA biotransformation products as well as the greatest alpha diversity out of the four enrichment cultures, dominated by *Cupriavidus* and *Ramlibacter* genera. This result in particular could point to a correlation between microbial diversity and PFAS precursors biotransformation yields; a site with many potential carbon sources and greater microbial diversity could result in a higher yield of precursors biotransformation while a site that is more specialized for one substrate could result in lower biotransformation rates and product yields.

Literature suggests that sulfonamides are key intermediates between ECF-based precursors and PFSA; while FHxSA is a main biotransformation product with methane and acetate carbon sources in this study, it could be a bottleneck rather than a main precursor to PFHxS based on its accumulation and the evidence that other intermediates can be biotic precursors to PFSA without the presence of the sulfonamide. With the accumulation of FHxSA in the environment, and its extremely similar structure to PFHxS, this compound in addition to perfluoroalkyl sulfonamides of other chain lengths should be added to monitoring lists and studied for potential toxicity.

Comparing biotransformation products with chemical oxidation processes, AmPr-FHxSA and FHxSA primarily produce PFHxA under the TOP assay, which can be mineralized under HAPO. However, FHxSA also produces small yields of PFHxS during chemical oxidation and appears to be more recalcitrant than AmPr-FHxSA during HAPO. The production of sulfonamides during the TOP assay was surprising and, though its yield was 1-2%, this result displays that there are some precursors that do not perform as predicted during the TOP assay. There could be more classes of precursors that produce PFSA during the assay, which would impact how we analyze TOP assay results and which compounds to monitor more carefully for their oxidation product yield. In particular though, this result points to the unique characteristics of perfluoroalkyl sulfonamides; these compounds and their short-chain homologs should be carefully monitored at AFFF-contaminated sites for their fate and long term biotransformation.

AmPr-FHxSA is just one ECF-based hexafluoroalkyl PFAS precursor found in 3M AFFFs. As referenced in Chapter 2, there have been many studies investigating the biotransformation rates and product yields of ECF-based octafluoroalkyl PFAS precursors that can produce PFOS. Additionally, new research in our laboratory shows that, when performing the TOP assay on various 3M AFFFs, short- and ultra-short chain PFCAs are produced, meaning that there are short- and ultra-short chain (perfluoroalkyl chains containing four carbons or fewer) precursors in these formulations. With the known eight- and six-carbon precursors in AFFFs, plus four-carbons and potentially shorter perfluoroalkyl chained precursors, there is a wide range of PFAS in AFFF-impacted aquifers that have the potential to biotransform to

a range of perfluoroalkylsulfonamides, PFASs, and intermediates similar to those shown in Chapter 2 of varying chain lengths. One way to avoid this biotic production of sulfonamides and PFASs from ECF-based AFFFs in impacted groundwater could be to perform in situ chemical oxidation on the source zone, as described in Chapter 4.

5.2 Toxicity of PFAS on Co-Contaminant Degrading Bacteria

Biotransformation of compounds like AmPr-FHxSA may be more likely to occur where the concentrations of individual PFAS are closer to those used in Chapter 2, on the order of 200 nM. Where PFAS are more concentrated, however, they could prove to be inhibitory to groundwater relevant bacteria, including communities that can be utilized in the bioremediation of PFAS co-contaminants TCE and BTEX.

An anaerobic dechlorinating co-culture of *Dehalococcoides mccartyi* strain 195 and *Desulfovibrio vulgaris* Hildenborough did not experience dehalogenation inhibition with individual PFAS at 1 and 10 μ M, but did at high dilutions of 3M AFFF. 100x and 1,000x diluted AFFF completely inhibited or stalled, respectively, the culture's utilization of chlorinated solvents. I hypothesized that components in the AFFF, potentially PFOS or an antimicrobial present in the formulation, was primarily responsible for this effect. Additionally, the ATP production of this culture was unaffected by AmPr-FHxSA, FHxSA, PFHxS, or SDS; only the AFFF dilutions decreased the ATP detected. Because ATP production can be impacted by disturbing the cell membrane and the proton motive force, I hypothesize that the unique structure of the cell wall of *Dehalococcoides mccartyi* strain 195 contributed to their resistance to these hexafluoroalkyl PFAS. Only when exposed to high concentrations of AFFF did this culture become overwhelmed. The relative concentrations of measured metabolites revealed that histidine and tyrosine decreased in the presence of 100x diluted AFFF, while aspartic acid, threonine, and serine increased. These results can indicate the activation/deactivation of enzymes via phosphorylation or cell signaling to external threats, such as the toxicity of AFFF components. I show in Chapter 3 that this co-culture proves to be relatively resistant to AmPr-FHxSA, FHxSA, PFHxS, and SDS individually, and succumbs to toxicity impacts with concentrated AFFF.

The aerobic BTEX enrichment culture, which was seeded from soil from an AFFF-contaminated site, displayed different impacts to the PFAS. At 10 μ M, AmPr-FHxSA and FHxSA inhibited the culture, resulting in no BTEX degradation. This substrate consumption inhibition also corresponded with a drastic decrease in ATP concentrations, which signifies the near-death of this culture. Interestingly, the triplicate set exposed to 1,000x diluted AFFF still consumed the BTEX comparatively to the controls, but had a 30% decrease in ATP concentration. This result could signify that the membrane and proton motive force was impacted so that ATP production was less efficient but not enough to inhibit BTEX consumption. Another possibility is that more ATP was being used up in this culture; since

ATP is energy currency in the cell, it could have been used for cell signalling and response to the AFFF. Additionally, slight changes in the BTEX enrichment culture community composition point to which genera of bacteria may be more resistant to PFAS. Compared to the inoculum source, the cultures exposed to FHxSA and AmPr-FHxSA contained lower abundances of *Rhodococcus*, higher relative abundances of *Sediminibacterium* and an unclassified *Alphaproteobacterium*, and of *Vulgatibacter* and *Acinetobacter* for FHxSA and AmPr-FHxSA cultures, respectively.

Overall, these results show that microbial communities can be resistant to PFAS. Generally, 1 μ M of PFAS did not impact any of the bacteria tested, and 10 μ M of FHxSA and AmPr-FHxSA only impacted the BTEX community. The FHxSA could have been particularly impactful due to its neutral headgroup, which could partition in cell membranes more effectively than negatively charged PFAS. The AmPr-FHxSA could have impacted the culture by its zwitterionic headgroup interacting with charged aspects of proteins, potentially deactivating them.

5.3 HAPO Treatment of PFAS and Co-Contaminants

If bioremediation is not an option for source zone treatment of PFAS co-contaminants, HAPO could be an effective in situ remediation technique for mineralizing common organic contaminants as well as precursor PFAS and PFCAs.

In Chapter 4, I demonstrate the efficacy of low temperature (40°C) HAPO for PFAS transformation. Although higher temperatures have been shown to be effective in transforming PFAS with activated persulfate, 40°C would be an easier temperature to achieve and maintain in the field. The lower temperature also means lower cost of energy for a HAPO project. For HAPO at 40°C, however, the buffering capacity of the aquifer materials plays a large role; because the pH must be below 3 for PFCA transformation to occur, the slower rate of persulfate activation and proton production delays the acidification of the buffered groundwater-solids slurry, effectively preventing PFCA transformation. This effect can be overcome by either pre-acidifying the solution or by using higher persulfate doses. In fact, when testing a groundwater-solids slurry of 1,000 g/L, much closer to mimicking a groundwater aquifer, high (>50 mM weekly doses) doses are required to transform PFCAs. I found that adding eight doses of 50 mM persulfate or five doses of 200 mM transformed $\leq 95\%$ of the PFAS in solution over approximately two months for the 50 mM doses and one month for the 200 mM. Compared to in situ chemical oxidation projects reported in literature, which on average utilized 1-2 injection events with a median duration of 4-6 days, adding 2-3 extra injection events is feasible but would of course increase the cost and duration of the project. At source zones with high concentrations of precursors, PFCAs, and organic co-contaminants, this cost could be entirely justified to mineralize PFAS.

5.4 Current and Future Needs for PFAS Research and Management

Although there are dozens of labs around the country studying treatment and toxicity of PFAS, and dozens of PhD students writing their dissertations on such topics, our understanding of PFAS classes, prevalence, toxicity, and treatment/disposal is still, unfortunately, limited. A recent paper summarized some of these shortcomings recently; in “Addressing Urgent Questions for PFAS in the 21st Century”, authors Ng et al discuss six key questions that must be addressed for the proper management of PFAS.[161] Their questions are:

1. What are the global production volumes of PFAS, and where are they used?
2. Where are the unknown PFAS hotspots in the environment?
3. How can we make measuring PFAS globally accessible?
4. How can we safely manage PFAS-containing waste?
5. How do we understand and describe the health effects of PFAS exposure?
6. Who pays the costs of PFAS contamination?

To these, I would also add:

1. What are the most effective and sustainable PFAS treatment/removal options for small drinking water systems and/or groundwater wells, and who pays for it?
2. What are the composition and yields of volatile of PFAS from incinerators; how do these compounds impact air quality and how do deposited PFAS impact nearby ecosystems?

Many of these questions require policy and regulation that is quite slow in being written and enforced. What we need is the regulatory push to implement most promising technologies to date to remove mass in PFAS source zones and to destroy stockpiled PFAS waste. Additionally, we need regulations to restrict who uses PFAS in what products, how to handle their manufacturing waste, and how to monitor the life cycle of PFAS-containing products. Overall, we need to start widespread implementation of PFAS destruction technologies as well as removal of PFAS from non-essential consumer products such as outdoor wear, cookware, food packaging, carpets, and upholstery.

The saturation of PFAS in consumer and industrial markets has proven extremely harmful to humans and the environment. While we created a class of non-reactive, useful compounds to improve products’ quality and usefulness, we have created a ubiquitous contaminant present in groundwater, surface water, air, soil, and organisms across the world.

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Appendices

A Author contributions

Chapter 2: Biological and chemical transformation of the six-carbon polyfluoroalkyl substance AmPr-FHxSA

This Chapter has been submitted for publication and is still currently under review.

- Emily K. Cook: Experimental design, sampling, analysis, manuscript writing and editing
- Christopher I. Olivares: Experimental design, sampling, analysis, manuscript editing
- Edmund (Ned) H. Antell: 16S data analysis and figure design, manuscript writing (microbial community analysis section) and editing
- Shan Yi: Experimental design and mentorship
- Anastasia Nickerson: HRMS running and analysis
- Youn Jeong Choi: HRMS running and analysis
- Christopher P. Higgins: Mentorship and manuscript editing
- David L. Sedlak: Mentorship, conceptualization, funding acquisition, manuscript editing
- Lisa Alvarez-Cohen: Mentorship, conceptualization, funding acquisition

Chapter 3: PFAS impacts on bioremediation success of co-contaminants TCE and BTEX

- Emily K. Cook: Experimental design, sampling, analysis, manuscript writing and editing
- Edmund (Ned) H. Antell: 16S data analysis and figure design
- Christopher I. Olivares: Experimental design, sampling, analysis, manuscript editing
- David L. Sedlak: Mentorship, funding acquisition, manuscript editing
- Lisa Alvarez-Cohen: Mentorship, conceptualization, funding acquisition

Chapter 4: Considerations for applying in situ heat-activated persulfate oxidation for PFAS treatment

- Emily K. Cook: Experimental design, sampling, analysis, manuscript writing and editing
- Christopher I. Olivares: Experimental design, analysis, manuscript editing
- Daniel Ocasio: Data collection for some SI figures D.11-D.13
- Shan Yi: Experimental design and mentorship
- Yilu Sun: Experimental design and sampling
- David L. Sedlak: Mentorship, conceptualization, funding acquisition, manuscript editing
- Lisa Alvarez-Cohen: Mentorship, conceptualization, funding acquisition

B Supplementary Information for Chapter 2

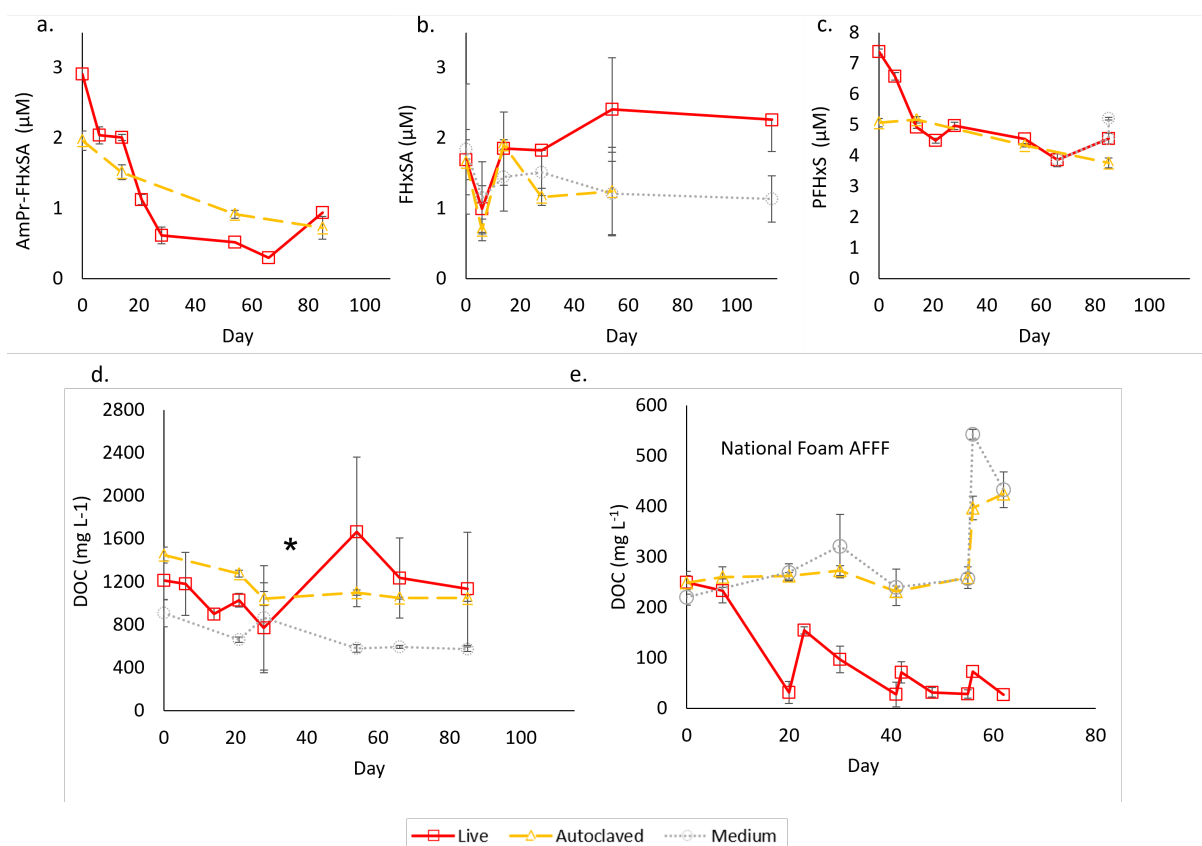


Figure B.1: ECF-based AFFF biotransformation study amended with DGBE. Concentrations of a) AmPr-FHxSA, b) FHxSA, c) PFHxS, and d) DOC for the ECF-based AFFF biotransformation test. Compared to the e) DOC degradation during a fluorotelomer-based AFFF biotransformation test, there was little to no DOC degradation in the ECF AFFF test, indicating recalcitrant non-fluorinated surfactants. Asterisk denotes respike with DGBE as additional carbon. 1:1,000 dilution AFFF.

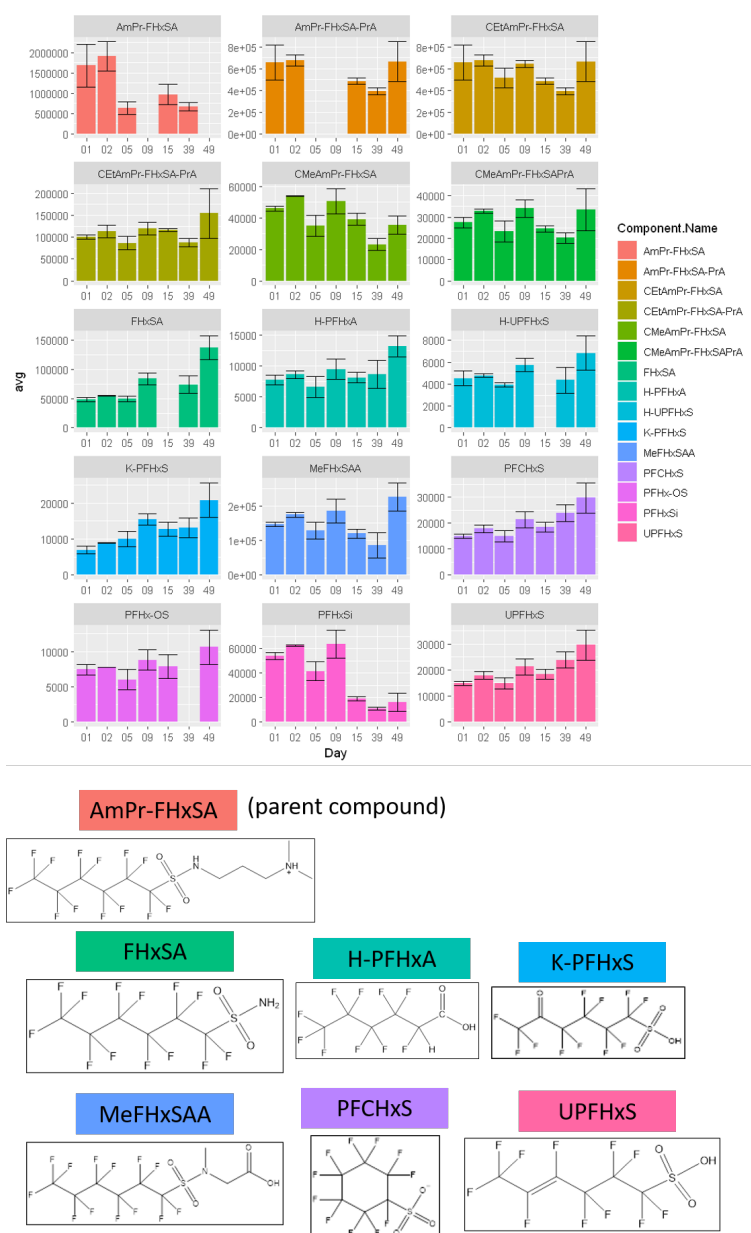


Figure B.2: HRMS results, and relevant structures, from the ECF-based AFFF biotransformation experiment.

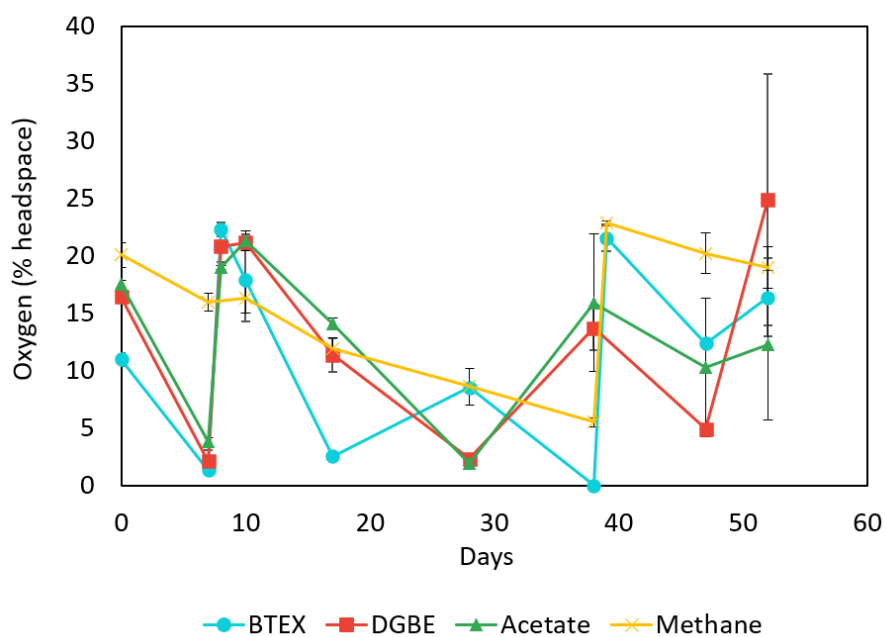


Figure B.3: Oxygen consumption and replenishment for the four carbon source experiment.

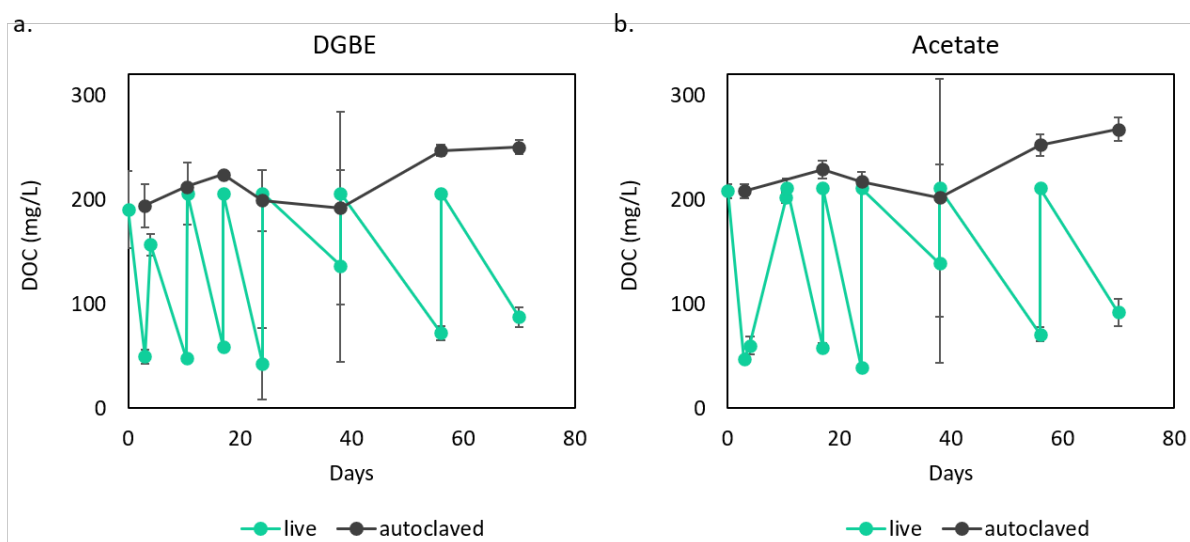


Figure B.4: DOC degradation for the DGBE and acetate cultures.

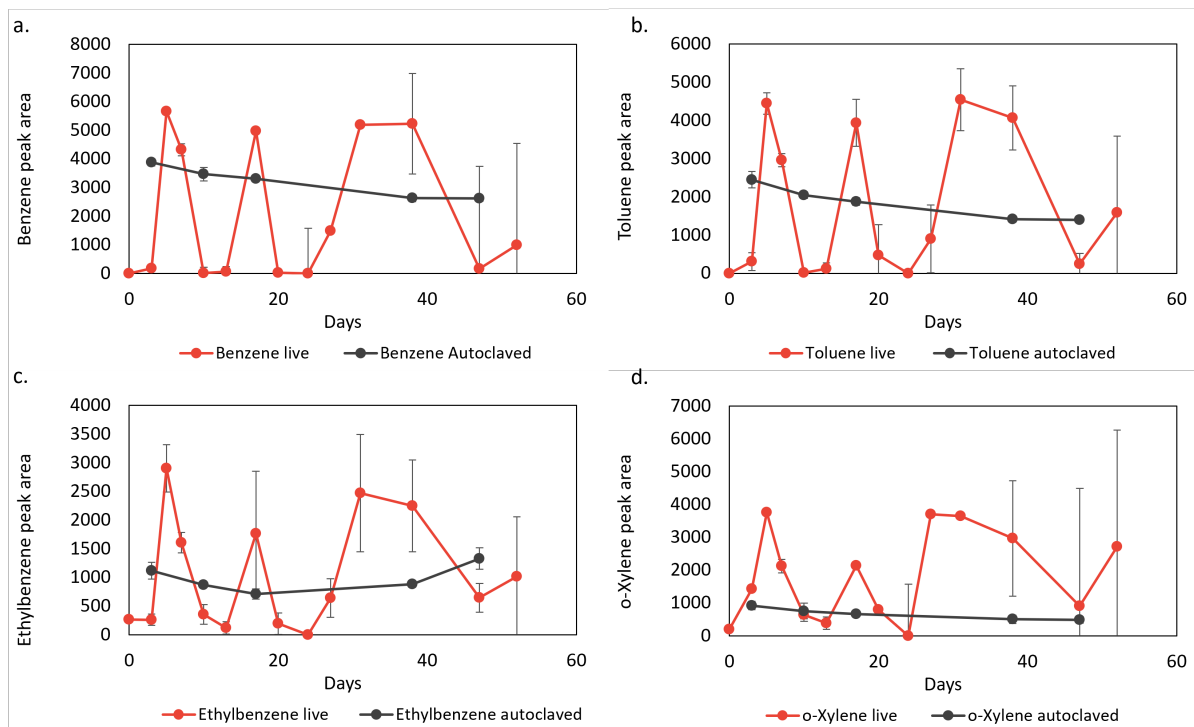


Figure B.5: BTEX measurements for the live and autoclaved triplicate cultures.

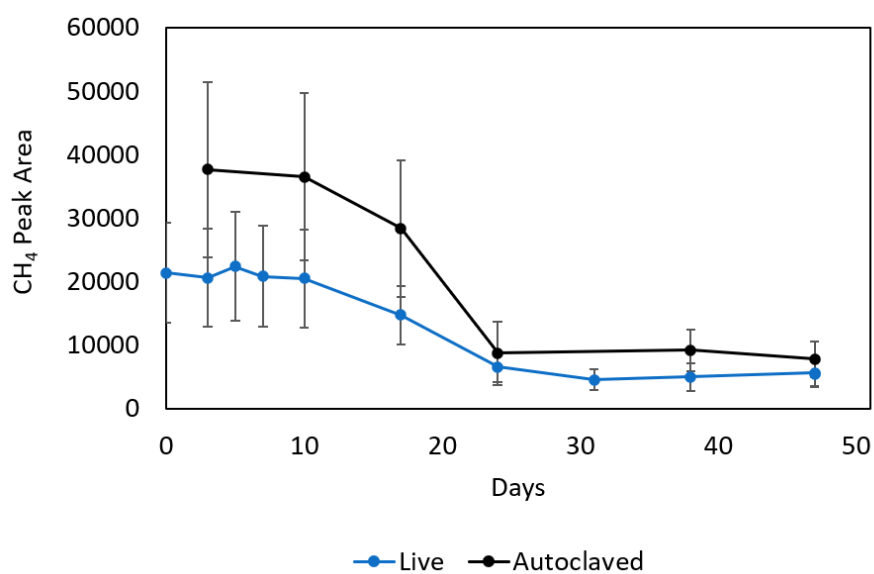


Figure B.6: Methane measurements for live and autoclaved triplicate cultures.

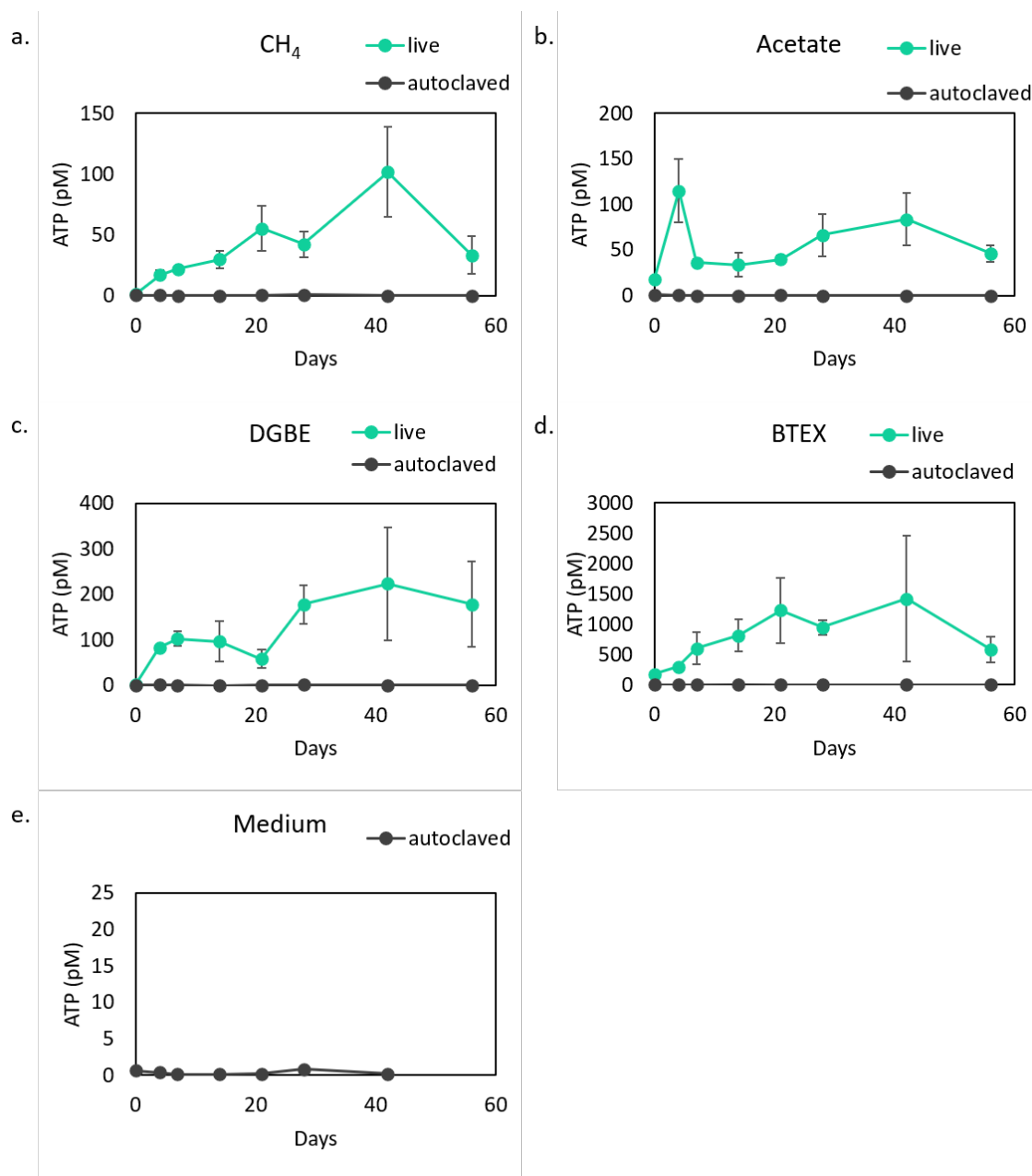


Figure B.7: ATP measurements for the live and autoclaved controls for each of the four carbon sources, as well as the medium controls.

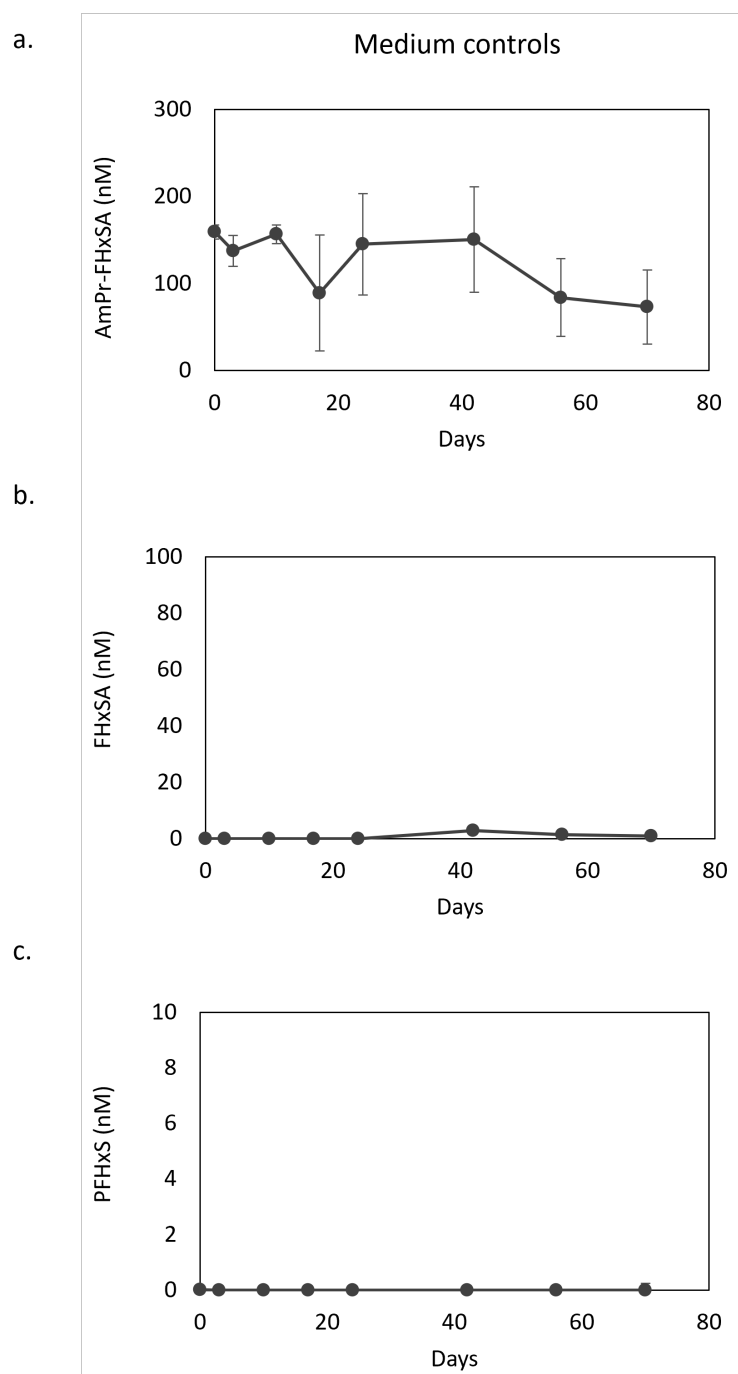


Figure B.8: PFAS measurements for the medium controls of the four carbon source experiment using LC-MS/MS.

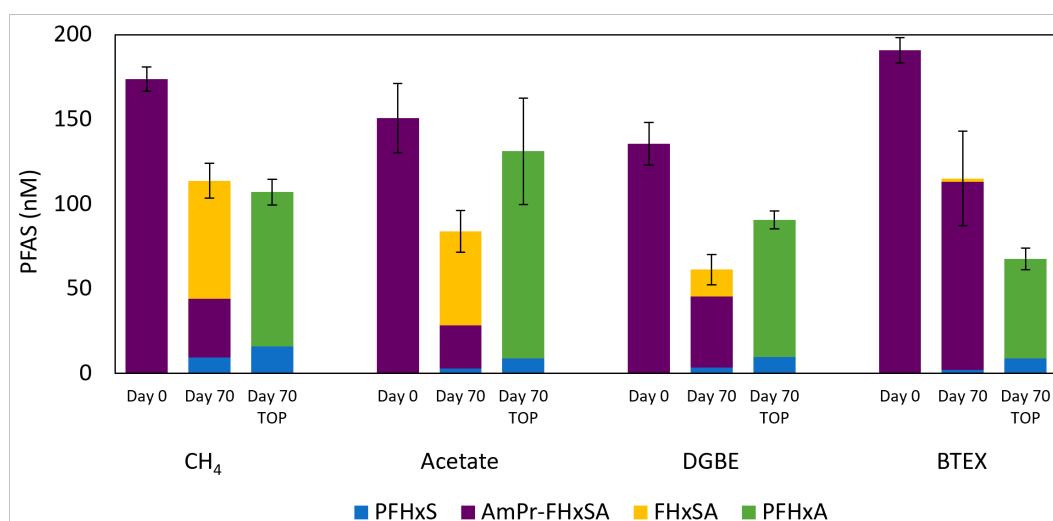


Figure B.9: The concentrations of parent on day 0, parent and biotransformation products on day 70, and the TOP assay results conducted on day 70 samples.

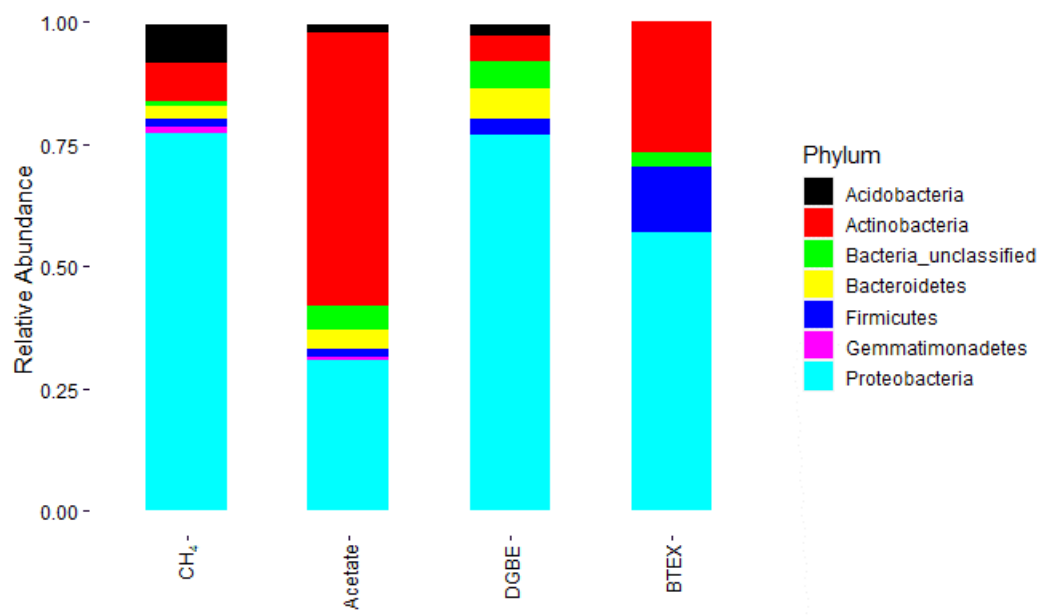


Figure B.10: Phyla from each of the four carbon source enrichments. The results were averaged between triplicate live samples, taken on day 70.

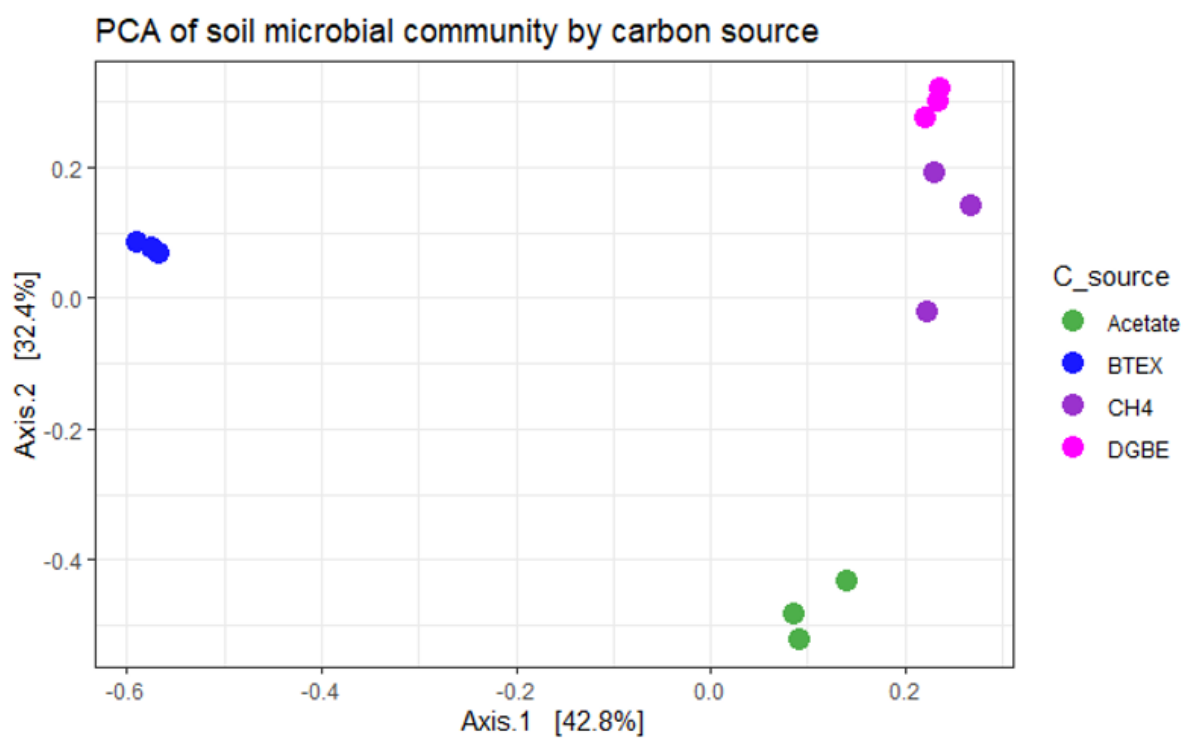


Figure B.11: Principal component analysis space of each of the four carbon sources according to their detected genera.

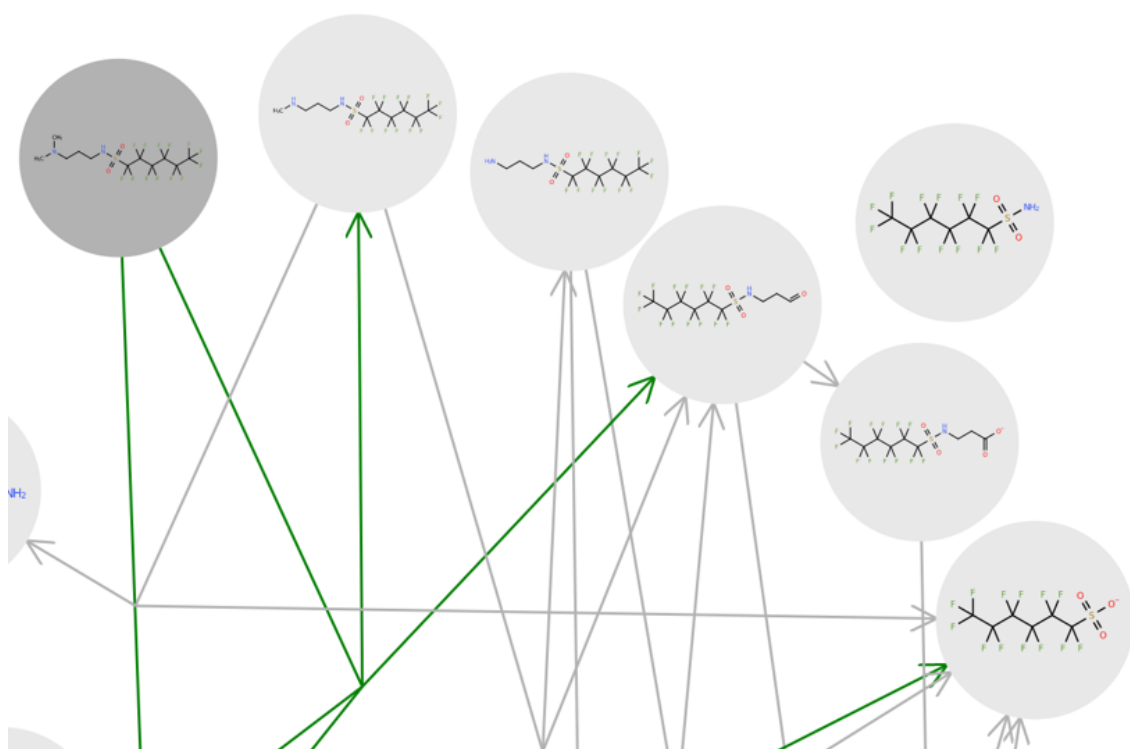


Figure B.12: envPath proposed pathway zoomed in on the fluorinated compound results. SMILES ID used:

FC(C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(S(=O)(NCCCN(C)C)=O)(F)F)(F)F.

FHxSA is shown here, but was manually added by us during analysis; it was not originally suggested as a transformation product of this parent.

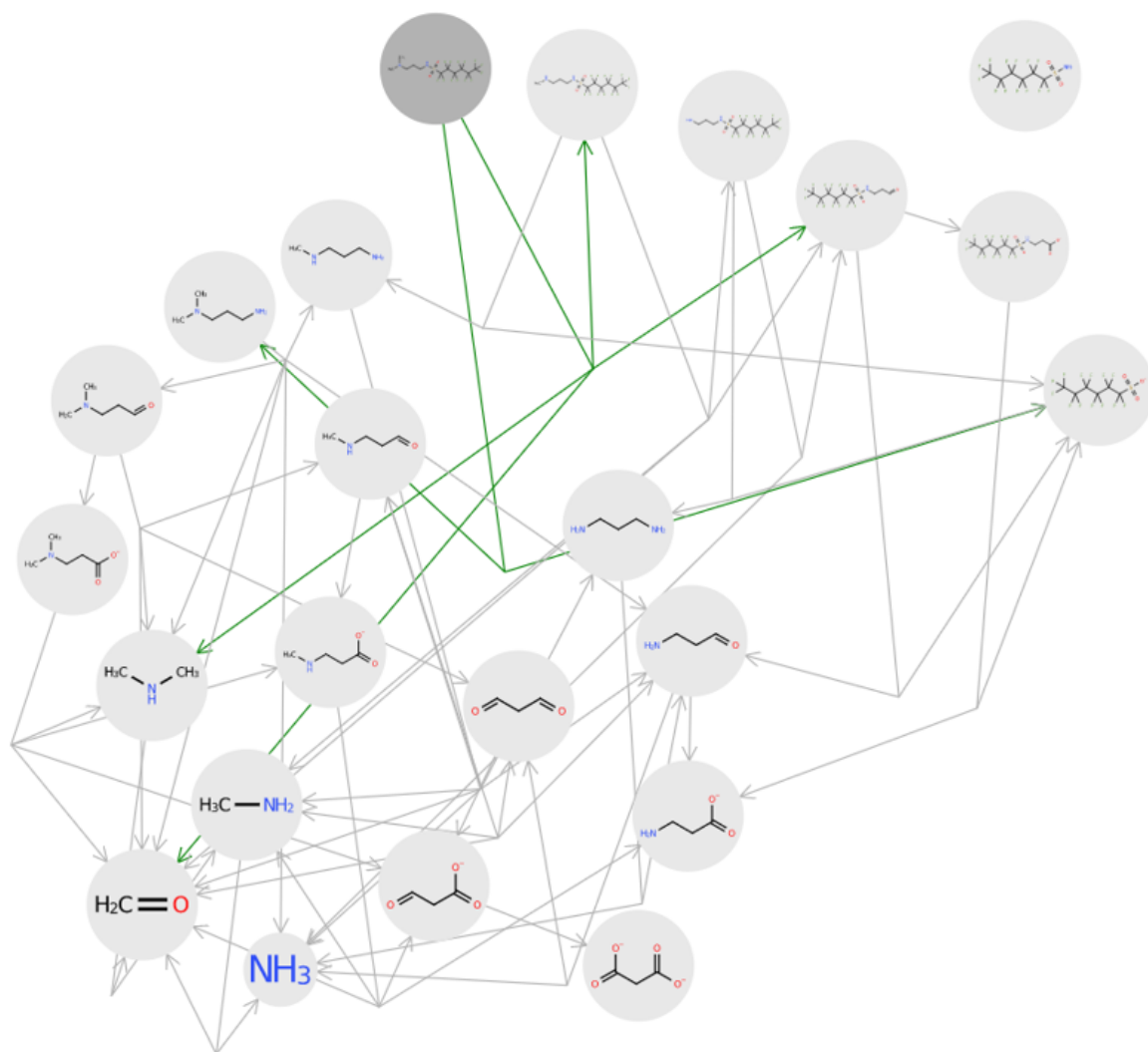


Figure B.13: envPath proposed pathway. SMILES ID used:

FC(C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(S(=O)(NCCCN(C)C)=O)(F)F)(F)F.

FHxSA is shown here, but was manually added by us during analysis; it was not originally suggested as a transformation product of this parent.

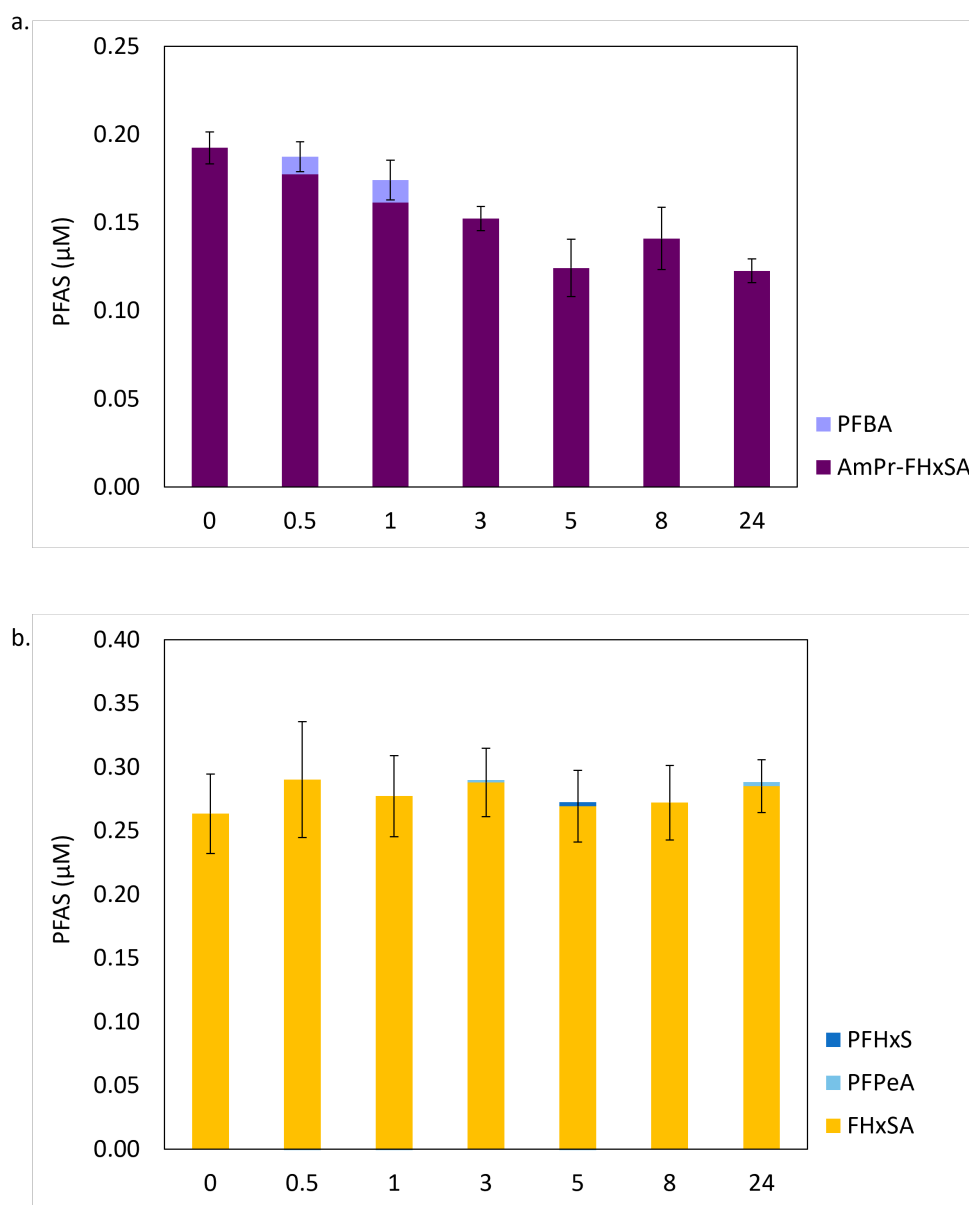


Figure B.14: Controls for heat-activated persulfate oxidation of a) AmPr-FHxSA and b) FHxSA. These controls were kept in a 4°C fridge to avoid heat-activation of the persulfate.

μM	PFPeA	PFHxA	PFHpA	PFOA	PFBS	PFHxS	PFOS	Ampr-FHxSA
ECF-based AFFF	453 ± 57	526 ± 50	167 ± 18	442 ± 57	742 ± 17	1841 ± 7	16761 ± 2191	4755 ± 613

Table B.1: PFAS concentrations in ECF-based AFFF. Duplicate samples run on the LC-MS/MS of the diluted AFFF stock solution. Error is simply the difference between the two replicates.

Compound	Internal Standard	Precursor Ion	Product Ion	Fragmentor Voltage (V)	Collision Energy (V)	Polarity
MPFBA	-	217	172	50	5	Neg
MPFPeA	-	266	222 221	60	2	Neg
MPFHxA	-	315	270	60	5	Neg
MPFOA	-	417	372	70	2	Neg
MPFNA	-	468	423	70	5	Neg
MPFHxS	-	403	103	150	40	Neg
MPFOS	-	503	80	190	60	Neg
PFBA	MPFBA	213	169	50	2	Neg
PFPeA	MPFPeA	263	219 68.9	60 92	2 8	Neg
PFHxA	MPFHxA	313	269 119	80	2 15	Neg
PFHpA	MPFOA	363	319 169	80	2	Neg
PFOA	MPFOA	413	369 169	80	3 14	Neg
PFNA	MPFNA	463	419 219	80	2 15	Neg
PFBS	MPFHxS	299	99 80	120	30 70	Neg
PFHxS	MPFHxS	399	99 80	125	50 80	Neg
PFOS	MPFOS	499	99 80	122	50 80	Neg
FHxSA	MPFOS	398	78	125	36	Neg
FOSA	MPFOS	498	78	125	36	Neg
6-2 FtS	MPFOS	427	407 80	140	25 35	Neg
AmPr-FHxSA	MPFOS	485	85	135	30	Pos

Table B.2: PFAS in Targeted LC-MS/MS Analyses.

Time	Water %	Methanol %
0	75	25
5	50	50
13	5	95
16	5	95
16.5	75	25
23	75	25

Table B.3: LC-MS/MS mobile phase gradient. LC-MS/MS was run with water and methanol mobile phase, each with 10 mM ammonium acetate.

Time	Water %	Methanol %
0	90	10
0.5	50	50
8	1	99
13	1	99
13.5	90	10
20	90	10

Table B.4: HRMS mobile phase gradient. HRMS was run with water and methanol mobile phase, each with 10 mM ammonium acetate.

Sample Name	FHxSA	PFOs	PfHxS	8:2 FTS	6:2 FTS	PFPes	PFBS	PFPPrS	PfHpS	AmPr-FHxSA	MeFHxSAA	PFEtA	FPeSA	PfHxSi	OAmPr-FHxSA	FHxSAA	O-U-PFOA	AmPr-FBSA	MeFPeSAA
Acetate Live Day 0 - dup1	27500	27104	N/A	N/A	920	N/A	681	324	156	104837	NA	NA	NA	1600	1992	NA	1199	NA	NA
Acetate Live Day 0 - dup2	28925	27756	N/A	N/A	1092	N/A	954	564	315	105161	NA	NA	2710	1535	NA	NA	NA	NA	NA
BTEX Live Day 0 - dup1	2963	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	118783	NA	NA	NA	997	NA	NA	NA	NA	NA
BTEX Live Day 0 - dup2	2758	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	113625	NA	NA	NA	NA	NA	NA	NA	NA	NA
DGBE Live Day 0 - dup1	22063	18294	N/A	N/A	1264	N/A	881	446	N/A	99020	NA	NA	3135	NA	NA	NA	NA	NA	NA
DGBE Live Day 0 - dup2	23759	18742	N/A	N/A	1390	N/A	1033	400	N/A	104277	NA	14741	829	NA	NA	NA	NA	NA	NA
Methane Live Day 0 - dup1	21933	14072	N/A	243	479	N/A	552	259	N/A	100040	NA	NA	1800	NA	NA	NA	NA	NA	NA
Methane Live Day 0 - dup2	21956	15672	N/A	194	594	N/A	624	244	N/A	98886	NA	NA	1872	NA	3109	NA	NA	NA	NA

Table B.5: Target areas detected on HRMS for day 0. Background PFAS from inoculum source, plus the added AmPr-FHxSA. MeFHxSAA: N-methylperfluorohexane sulfonamido acetic acid; PFEtA: perfluoro-n-ethanoic acid; FPeSA: perfluoropentane sulfonamide; PFHxSi: perfluorohexane sulfinate; OAmPr-FHxSA: N-oxidedimethylammonioethylperfluorohexanesulfonamide; FHxSAA: Perfluorohexane sulfonamido acetic acid; O-U-PFOA: Oxa-Unsaturated-PerFluoroOctanoic Acid; AmPr-FBSA: N-dimethyl ammonio propyl perfluorobutane sulfonamide; MeFPeSAA: N-methylperfluoropentane sulfonamido acetic acid

Sample	N-TAmP-FHxSA	FHxSA	AmPr-FHxSA	6-2 FtTAoS	6-2 FtS	6-2 FtSaB	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFBS	PFHxS	PFOS
Name	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L	ug/L
std00	0.00	0.00	0.01		0.23	0.18		0.04		0.04	0.01	0.02			0.00
std01	0.19	0.10	0.19	0.06	0.26	0.26	0.14	0.28	0.30	0.26	0.23	0.21	0.22		0.16
std02	0.45	0.40	0.43	0.54	0.37	0.34	0.59	0.44	0.45	0.50	0.49	0.47	0.49	0.57	0.30
std03	0.99	0.86	0.96	1.37	0.97	1.15	1.08	0.92	0.99	1.02	1.04	1.00	0.90	0.78	0.97
std04	2.24	2.16	2.19	2.52	2.35	2.36	2.21	2.15	2.23	2.41	2.33	2.32	1.98	2.03	2.23
std05	4.99	4.69	4.85	4.99	5.32	4.75	5.05	5.25	4.85	5.08	5.13	5.20	5.54	5.60	4.82
std06	7.55	7.47	7.67	5.75	8.10	7.69	7.15	7.51	7.89	7.27	7.35	7.51	7.33	7.33	7.45
std07	10.08	10.18	10.14	10.92	9.50	10.07	10.35	10.06	10.16	10.15	10.20	9.94	10.02	10.03	10.18
BTEX_d0 live_01	0.00	0.00	1.61	0.02	0.25	0.18	0.13	0.00	0.00	0.04	0.00	0.00	0.00	0.08	0.00
BTEX_d0 live_02	0.00	0.00	2.21	0.00	0.26	0.18	0.13	0.02	0.00	0.04	0.00	0.00	0.00	0.05	0.00
BTEX_d0 live_03	0.00	0.00	2.14	0.04	0.00	0.18	0.13	0.00	0.00	0.04	0.00	0.00	0.00	0.09	0.02
BTEX_d0 autoclaved_01	0.00	0.00	2.91	0.00	0.23	0.19	0.13	0.00	0.00	0.04	0.00	0.00	0.00	0.08	0.05
BTEX_d0 autoclaved_02	0.00	0.00	2.39	0.07	0.24	0.18	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.09	0.03
BTEX_d0 autoclaved_03	0.00	0.00	2.31	0.06	0.23	0.18	0.13	0.00	0.00	0.05	0.00	0.00	0.00	0.07	0.00
DGBE_d0 live_01	0.01	0.10	1.51	0.06	0.23	0.19	0.14	0.00	0.00	0.04	0.00	0.00	0.00	0.07	0.13
DGBE_d0 live_02	0.00	0.08	1.71	0.02	0.27	0.19	0.14	0.00	0.00	0.04	0.00	0.00	0.00	0.07	0.00
DGBE_d0 live_03	0.00	0.09	1.64	0.03	0.27	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00
DGBE_d0 autoclaved_01	0.00	0.48	1.46	0.02	0.24	0.19	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.09	0.06
DGBE_d0 autoclaved_02	0.00	0.43	2.08	0.07	0.00	0.18	0.13	0.03	0.00	0.04	0.00	0.00	0.00	0.09	0.05
DGBE_d0 autoclaved_03	0.00	0.73	2.27	0.00	0.24	0.18	0.16	0.00	0.00	0.05	0.00	0.00	0.00	0.09	0.04
Acetate_d0 live_01	0.00	0.14	1.43	0.02	0.24	0.18	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.09	0.03
Acetate_d0 live_02	0.00	0.14	1.31	0.00	0.25	0.00	0.13	0.00	0.00	0.04	0.00	0.00	0.00	0.09	0.01
Acetate_d0 live_03	0.00	0.11	1.59	0.08	0.24	0.00	0.13	0.00	0.00	0.04	0.00	0.00	0.00	0.06	0.04
Acetate_d0 autoclaved_01	0.00	0.06	1.15	0.00	0.26	0.00	0.13	0.00	0.00	0.04	0.00	0.00	0.00	0.09	0.05
Acetate_d0 autoclaved_02	0.00	0.13	1.41	0.00	0.23	0.18	0.00	0.01	0.00	0.04	0.00	0.00	0.00	0.08	0.06
Acetate_d0 autoclaved_03	0.00	0.06	2.01	0.03	0.24	0.18	0.13	0.00	0.00	0.04	0.00	0.00	0.00	0.10	0.07
CH4_d0 live_01	0.00	0.02	1.50	0.00	0.24	0.19	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.05
CH4_d0 live_02	0.00	0.09	1.65	0.00	0.28	0.18	0.13	0.00	0.00	0.04	0.00	0.00	0.00	0.07	0.04
CH4_d0 live_03	0.00	0.04	1.63	0.00	0.28	0.19	0.13	0.00	0.00	0.04	0.00	0.00	0.00	0.08	0.00
CH4_d0 autoclaved_01	0.00	0.08	1.26	0.03	0.27	0.18	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00
CH4_d0 autoclaved_02	0.00	0.07	1.76	0.00	0.26		0.14	0.00	0.00	0.04	0.00	0.00	0.00	0.07	0.00
CH4_d0 autoclaved_03	0.00	0.09	2.83	0.04	0.24		0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.08	0.00
Medium_d0 autoclaved_01	0.00	0.00	1.48	0.03	0.24	0.18	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.07	0.02
Medium_d0 autoclaved_02	0.00	0.00	1.63	0.00	0.23	0.18	0.13	0.00	0.00	0.04	0.00	0.00	0.00	0.08	0.05
Medium_d0 autoclaved_03	0.00	0.00	1.52	0.00	0.23	0.18	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.09	0.02

Table B.6: Background PFAS ($\mu\text{g/L}$; without dilution factor included) detected via LC-MS/MS on day 0.

Sample Name	FHxSA	pFOS	pFHxS	K2	FTS	FBSA	K2	FTS	pFPeS	pPBs	pPFRs	pFHPS	AmPr-FHxSA	MeFHxSAA	pPEIA	FPeSA	pFHxSi	QAmPr-FHxSA	FHxSAA	O-U-PFOA	AmPr-FBSA	MeFPeSAA
Acetate Live Day 70 - rep1	93171	32031	8559	1544	1453	5133	1017	N/A	287	N/A	33118	50994	NA	3178	NA	1352	NA	NA	2068	NA	NA	
Acetate Live Day 70 - rep2	72931	22086	N/A	4794	1209	1503	696	N/A	311	N/A	20706	44683	33523	2610	2107	7136	973	1136	NA	NA		
Acetate Live Day 70 - rep3	76876	94717	7894	2598	1222	957	774	N/A	326	1533	25143	49034	NA	3085	2750	7518	1149	NA	NA	NA		
Acetate Autoclaved Day 70 - rep1	18139	13590	N/A	501	1028	N/A	1011	956	390	N/A	40221	NA	NA	2622	NA	NA	NA	1075	NA	NA		
Acetate Autoclaved Day 70 - rep2	1575	11628	N/A	1216	1323	1116	761	N/A	456	N/A	38914	NA	NA	2597	NA	NA	NA	NA	NA	NA		
Acetate Autoclaved Day 70 - rep3	18636	13058	N/A	529	1248	N/A	889	652	341	N/A	59316	NA	NA	2840	NA	NA	1363	NA	NA	NA		
BTEX Live Day 70 - rep1	1844	3496	N/A	1493	N/A	1190	N/A	N/A	N/A	N/A	90615	NA	NA	1341	NA	NA	NA	NA	NA	NA		
BTEX Live Day 70 - rep2	2044	1715	N/A	993	N/A	1028	N/A	N/A	N/A	N/A	63163	NA	NA	NA	NA	NA	NA	NA	NA	NA		
BTEX Live Day 70 - rep3	1433	1513	N/A	393	N/A	N/A	N/A	N/A	N/A	N/A	64472	NA	NA	NA	NA	NA	NA	NA	NA	NA		
BTEX Autoclaved Day 70 - rep1	1144	3767	N/A	1001	N/A	815	N/A	1664	N/A	N/A	49980	NA	NA	1877	NA	NA	NA	NA	NA	NA		
BTEX Autoclaved Day 70 - rep2	967	1972	N/A	393	N/A	N/A	N/A	N/A	N/A	N/A	48971	NA	79317	NA	1605	NA	NA	NA	NA	NA		
BTEX Autoclaved Day 70 - rep3	1086	2565	N/A	1007	N/A	1081	N/A	N/A	N/A	N/A	47754	NA	NA	NA	NA	NA	NA	NA	NA	NA		
DGBE Live Day 70 - rep1	22850	19060	9161	885	1917	1039	905	N/A	299	279	24538	3651	NA	2137	2094	NA	NA	NA	NA	NA		
DGBE Live Day 70 - rep2	17391	30109	7545	14860	1982	8043	899	N/A	395	N/A	30663	NA	NA	2173	858	NA	1023	NA	NA	NA		
DGBE Live Day 70 - rep3	17612	12414	N/A	N/A	1853	N/A	1016	N/A	513	N/A	27836	NA	NA	NA	1430	NA	NA	NA	NA	NA		
DGBE Autoclaved Day 70 - rep1	69451	39052	N/A	565	3136	N/A	2105	2755	980	702	37381	3583	NA	8970	4025	NA	NA	NA	786	NA		
DGBE Autoclaved Day 70 - rep2	65599	31981	N/A	1252	3375	1068	1830	2558	1073	614	37500	NA	NA	8116	3615	NA	NA	NA	3052	NA		
DGBE Autoclaved Day 70 - rep3	60897	40704	N/A	815	3237	1302	1905	2488	940	545	35735	NA	NA	7595	3415	NA	NA	NA	2266	1014		
Methane Live Day 70 - rep1	90930	17126	37871	260	1507	N/A	646	N/A	163	N/A	26359	15274	NA	NA	3061	NA	3948	NA	NA	NA		
Methane Live Day 70 - rep2	110248	26286	33528	417	1315	N/A	619	N/A	169	185	28954	26598	NA	2538	4074	NA	5302	1042	NA	NA		
Methane Live Day 70 - rep3	90906	19112	27891	252	1180	N/A	616	N/A	194	N/A	24876	26813	NA	2436	5094	NA	NA	554	NA	NA		
Methane Autoclaved Day 70 - rep1	18888	13013	N/A	N/A	709	N/A	378	N/A	250	N/A	39833	NA	NA	1886	1756	NA	NA	722	NA	NA		
Methane Autoclaved Day 70 - rep2	20619	13547	N/A	254	714	N/A	743	N/A	N/A	170	42938	NA	NA	2048	2508	NA	NA	712	NA	NA		
Methane Autoclaved Day 70 - rep3	18608	10738	N/A	253	706	N/A	425	N/A	225	N/A	59454	NA	NA	1582	3532	NA	NA	NA	NA	NA		

Table B.7: Target areas detected on HRMS for day 70. MeFHxSAA: N-methylperfluorohexane sulfonamido acetic acid; PFEtA: perfluoro-n-ethanoic acid; FPeSA: perfluoropentane sulfonamide; PFHxSi: perfluorohexane sulfinate; OAmPr-FHxSA: N-oxidedimethylammonioethyl-perfluorohexanesulfonamide; FHxSAA: Perfluorohexane sulfonamido acetic acid; O-U-PFOA: Oxa-Unsaturated-PerFluoroOctanoic Acid; AmPr-FBSA: N-dimethyl ammonio propyl perfluorobutane sulfonamide; MeFPeSAA: N-methylperfluoropentane sulfonamido acetic acid

	% decrease of AmPr-FHxSA	Std Dev	T test
Methane live	80.1	0.6	0.0004
Methane autoclaved	69.2	1.0	
Acetate live	83.0	2.6	0.0035
Acetate autoclaved	68.4	1.4	
DGBE live	69.0	5.4	0.9387
DGBE autoclaved	68.7	6.8	
BTEX live	42.2	11.7	0.1433
BTEX autoclaved	71.3	0.9	
Medium control	54.1	2.7	--

Table B.8: Percentage of AmPr-FHxSA decrease between days 0 and 70, averaged between the triplicate reactors. The T test was performed in Excel to compare AmPr-FHxSA removal between the live and autoclaved reactors.

	Ampr Day 70				PFHxS Day 70		
	Average	Std Dev	T test		Average	Std Dev	T test
Methane Live	17.4	0.18	0.003		2.8	0.31	0.139
Methane Autoclaved	15.7	0.28			11.5	6.01	
Acetate Live	28.2	2.09	0.005		9.5	4.46	0.236
Acetate Autoclaved	15.4	0.69			22.3	9.73	
DGBE Live	15.2	1.39	0.642		8.6	6.48	0.471
DGBE Autoclaved	15.8	0.36			14.8	5.68	
BTEX Live	10.5	2.11	0.064		11.8	5.07	—
BTEX Autoclaved	14.5	0.27			—	—	
Medium Control	17.8	3.08	—		—	—	—

Table B.9: Percent branched AmPr-FHxSA and PFHxS on Day 70. The stock solution of AmPr-FHxSA contained $9.3 \pm 1.4\%$ branched AmPr-FHxSA as measured on the LC-MS/MS (Table B.9).

$\mu\text{g/L}$	Area	$\mu\text{g/L}$	Area	$\mu\text{g/L}$	Area	
Linear + Branched	Linear + Branched	Linear only	Linear only	Estimated branched	Estimated branched	% branched area
2.1182	8135	1.879	7170	0.2392	965	11.9
2.3826	8079	2.1806	7363	0.202	716	8.9
3.8943	11244	3.5108	10107	0.3835	1137	10.1
3.1713	42418	2.8017	37068	0.3696	5350	12.6
3.7806	48168	3.434	43453	0.3466	4715	9.8
3.4234	42826	3.1131	38651	0.3103	4175	9.7
9.2994	88439	8.5155	80787	0.7839	7652	8.7
8.1966	87744	7.51	80171	0.6866	7573	8.6
8.6443	89220	7.9596	81950	0.6847	7270	8.1
8.2864	85142	7.5954	77830	0.691	7312	8.6
8.7451	89792	8.0631	82591	0.682	7201	8.0
8.1137	85882	7.4892	79070	0.6245	6812	7.9
15.6915	151797	14.3283	138390	1.3632	13407	8.8
14.6414	140922	13.438	129133	1.2034	11789	8.4
11.574	113234	10.5942	103431	0.9798	9803	8.7
					Average	9.3
					Std dev	1.4

Table B.10: Analysis of AmPr-FHxSA stock. Stock was diluted in MilliQ water (over several runs on the LC-MS/MS) to calculate the branched percentage.

	Raw PFHxS Conc ug/L						
Hour	0	0.5	1	3	5	8	24
FHxSA HAPO rep 1	0	0.127	0.149	0.177	0.215	0.196	0.218
FHxSA HAPO rep 2	0.129	0.130	0.139	0.154	0.163	0.165	0.159
FHxSA HAPO rep 3	0	0.126	0.141	0.160	0.179	0.169	0.167
	PFHxS Conc nM						
Hour	0	0.5	1	3	5	8	24
FHxSA HAPO rep 1	0.00	3.52	4.14	4.91	5.97	5.45	6.06
FHxSA HAPO rep 2	3.59	3.60	3.86	4.27	4.53	4.59	4.42
FHxSA HAPO rep 3	0.00	3.49	3.91	4.45	4.96	4.69	4.63

Table B.11: Concentrations of PFHxS during persulfate oxidation of FHxSA in triplicate.

Text B.1: AmPr-FHxSA-Containing AFFF Microcosms

AFFF microcosms were performed in triplicate using a mineral salts medium (Text B.2), a 5% soil inoculum from a separate Air Force base in South Dakota, and a 1:1000 dilution of neat AFFF. The inoculum contained trace amounts of soil. Autoclaved and medium-only controls were also included in triplicate. 50 mL of liquid slurry was added to 250 mL bottles to allow for ample oxygen exchange into the liquid. The bottles were capped with Mininert valve caps, and incubated in the dark (130 rpm, 30°C). 500-700 μ L were taken about once a week (then less frequently the latter half of the experiment). About 400 μ L of the sample was frozen aqueous, and 100 μ L were diluted with 300 μ L of basic methanol (2% NH_4OH) for PFAS analysis; all were stored in -20°C until analysis. Oxygen and CO_2 were monitored in the headspace using Hewlett Packard 5890 Series II gas chromatograph coupled to a thermal conductivity detector (GC-TCD) held isocratically at 35°C (Text B.4) and dissolved organic carbon (DOC) was measured using a Total Organic Carbon Analyzer (Shimadzu TOC-VCSH) as an indication of substrate consumption in the AFFF. DGBE was amended once. To compare microbial consumption of non-fluorinated carbon between 3M AFFF and fluorotelomer-based AFFF, additional microcosms under similar conditions described above were amended with either 1:1000 diluted ECF-based AFFF or the fluorotelomer-based National Foam AFFF (Figure B.2). Upon depletion of DOC, we amended the microcosms with 1.5 mM of DGBE. Heat-killed and medium-only controls were also included for this experiment.

Text B.2: Modified BAV1 Media Recipe

One liter of BAV1 media was prepared by adding 10 mL of salts solution (1 g NaCl ; 0.5 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$; 0.2 g KH_2PO_4 ; 0.3 g NH_4Cl ; 0.3 g KCl ; 15 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ per liter of Milli-Q water), 1 mL of trace elements solution (10 mL of 25% w/w HCl ; 1.5 g $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$; 0.19 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; 0.1 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 70 mg ZnCl_2 ; 6 mg H_3BO_3 ; 36 mg $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$; 24 mg $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$; 2 mg $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ per liter of Milli-Q water), 1 mL of Se/Wo solution (6 mg $\text{Na}_2\text{SeO}_3 \cdot \text{H}_2\text{O}$; 8 mg $\text{NaWO}_4 \cdot 2\text{H}_2\text{O}$; 0.5 g NaOH per liter of Milli-Q water), and 2.52 g of NaHCO_3 .

Text B.3: AMS Media Recipe

One liter of Ammonium Mineral Salts (AMS) media was prepared by adding salts (0.66 g $(\text{NH}_4)_2\text{SO}_4$; 1.0 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.015 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 1 mL AMS trace elements solution (0.5 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 0.4 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 0.02 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$; 0.015 g H_3BO_3 ; 0.01 g $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$; 0.25 g EDTA; 0.05 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; 0.005 g $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ per liter of Milli-Q water), 1 mL Stock A (5.0 g Fe-Na EDTA; 2.0 g $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ per liter of Milli-Q water), and 20 mL phosphate buffer (113 g K_2HPO_4 ; 47.0 g KH_2PO_4 per Liter of Milli-Q water). Specifically, the AMS recipe without the phosphate buffer was added to individual bottles, capped, and

sterilized in an autoclave. The phosphate buffer solution was filter-sterilized separately and added to the autoclaved bottles under sterile conditions.

Text B.4: GC Methods: GC-FID and GC-TCD

BTEX and methane were measured on a gas chromatograph coupled to flame-ionization detection (GC-FID) (Agilent 7890A) with a GS-GasPro column, injecting 100 μL of headspace air. For BTEX, the oven was set at 50°C for two minutes, then ramped 50°C/min until it reached 260°C. It was held at 260°C for two minutes. With this method, the retention times were 3.9, 4.8, 5.6, and 5.8 for benzene, toluene, ethylbenzene, and ortho-xylene. Methane was measured using a different method throughout which the oven remained at 70°C with the retention time being about 1.1 minutes. In both methods, the injector port and detector were kept at 250°C.

Oxygen and carbon dioxane were measured on a Hewlett Packard 5890 Series II gas chromatograph coupled to a thermal conductivity detector (GC-TCD) with an inner stainless steel column held at 35°C. The retention times for CO₂ and O₂ were 0.8 and 1.2 minutes.

Text B.5: Inoculum Preparation for Four Carbon Source Experiment

To prepare enrichments from selecting for each of the desired carbon sources, homogenized surficial soil (12.5 g/L) from the South Dakota AFFF-impacted site was combined with 200 mL of ammonium mineral salts (AMS) media (Text B.2) in 1 L bottles. The target carbon (BTEX, methane, acetate, or DGBE) concentration added was 1 mM, not including any soil-derived carbon. These enrichments were kept in a 37°C incubator and were periodically amended with oxygen and carbon until the start of the experiment. The BTEX inoculum was the oldest (maintained 8 months before use), while the methane, acetate, and DGBE inocula were started in November 2020 (one month before use).

Text B.6: Adenosine Triphosphate (ATP) Assay

To confirm microbial activity, ATP was measured each week for all reactors—live, autoclaved, and medium-only (Figure B.8) using a luminescence-based assay adapted from Hammes et al [124]. Briefly, a reagent was made using a BacTiter-Glo™ Buffer solution and a lyophilized BacTiter-Glo™ Substrate (Promega). Reagent and MilliQ water were separately heated in a heating block for three minutes at 38°C, then combined (50 μL Reagent, 450 μL diluted sample), vortexed, and incubated at 38°C for 20 seconds. The sample was then transferred to a Luminometer (GloMax® 20/20 Single Tube Luminometer Model No. E6080) to be measured as an integral in relative light units over 10 seconds. These units were converted to ATP concentrations using a standard curve using a rATP stock (Promega; Cat. No. P1132). All live reactors maintained ATP concentrations above 50 pM for most of the experiment (much more for the DGBE and BTEX enrichments), while autoclaved and medium controls

remained at 0 pM. Aqueous samples for ATP measurements were analyzed within 6 hours of sample collection.

Text B.7: HRMS Analysis

Samples from the AFFF-based biotransformation study, as well as day 0 and day 70 time-points from the four carbon source biotransformation study were sent to Colorado School of Mines for HRMS analysis. These samples were run on an ultra-high pressure liquid chromatography system (uHPLC, SCIEX Nexera LC) equipped with Sciex X500R Quadrupole Time-of-Flight Mass Spectrometer (QToF-MS) system. A delay column (Luna C18, 100Å, 30×3 mm, 5 μm) was installed for removal of potential contamination from the LC system. The analytical column was two Agilent Zorbax Diol guard columns (12.5×4.6 mm, 6 μm), a Phenomenex C18 guard column (2×4 mm), and Phenomenex Gemini C18 column (110Å, 100×3 mm, 5 μm), and column temperature was maintained at 40°C. The injection volume was 1000 μL. The mobile phase was a water/methanol gradient, each with 20 mM ammonium acetate at 0.6 mL/min (Table B.3). Data acquisition on SWATH® in Data-Independent Acquisition mode allowed full scan of the infused sample and the data set was processed using Sciex OS software for both target analysis using analytical standard chemicals and nontarget screening with a list of suspected PFAS as previously described.[93]

Text B.8: Microbial Community Analyses

At Novogene Corporation Inc. (Sacramento, CA, USA), DNA concentrations were normalized to 10 ng/μL and amplified targeting the V3-V4 region (470bp fragment length). The amplicons were then assessed for quality on an Agilent 5400 Fragment Analyzer (Agilent, Palo Alto, CA, USA) before library preparation and sequencing on an Illumina NovaSeq6000 PE250 platform (Illumina, San Diego, CA, USA). All downstream processing was done in-house using MOTHUR v.1.43.0 [162] following the MiSeq SOP. Briefly, raw reads were merged, trimmed, and filtered for quality. Unique sequences were aligned against the SILVA 16S rRNA reference database (v.132),[163] clustered into operational taxonomic units based on 97% similarity and assigned taxonomic identities to the genus level using the RDP v.18 reference training set.[164] Singletons were removed and the data was transferred to R for all downstream analysis. The *phyloseq* package in R was used to process output files from MOTHUR into relative abundance using the averaged triplicates and plotted using *ggplot2*. Diversity indices were calculated using the *microbiome* package. Statistical analysis was performed using base R using ANOVA and Tukey's HSD Test for post-hoc analysis.

Text B.9: AFFF Microcosm Experiments Results and Discussion

In the initial AFFF experiments, consumption of organic carbon was slower in the enrichments containing the ECF-based formulation than those containing the fluorotelomer-based formulation (Figure B.1) despite adequate oxygen in both systems. This suggests that some

component(s) in the ECF-based AFFF can inhibit the microbial community, such as PFOS, which has been shown to be toxic to microorganisms[165, 166, 80] and was present at concentrations three times that of AmPr-FHxSA (Table B.1). After 85 days, live and autoclaved controls showed a similar AmPr-FHxSA concentration decrease. Though not statistically significant compared to controls when averaged, two of the three replicates showed twice the concentration of FHxSA compared to the autoclaved controls (Figure B.1). However, the multiple possible precursors within the formulation confounded the analysis. While we performed HRMS on multiple timepoints throughout the experiment, many of the results were not statistically significant compared to the initial timepoint (Figure B.2), making it challenging to draw conclusions on the fate of AmPr-FHxSA. There was no indication of biological activity in the autoclaved controls based on ATP (data not shown), which suggests any decreases of AmPr-FHxSA in the autoclaved controls were because of abiotic processes.

Text B.10: Branched versus Linear AmPr-FHxSA Results and Discussion

All reactors had a higher percentage of branched compared to the AmPr-FHxSA stock (Table B.9), suggesting that linear AmPr-FHxSA sorbed more than the branched isomer. Moreover, the methane and acetate live controls had higher percentages of branched AmPr-FHxSA on day 70 compared to the autoclaved controls. While the AmPr-FHxSA stock solution contained an average branched percentage of $9.3 \pm 1.4\%$ (Table B.9), the acetate live cultures resulted in 28% branched AmPr-FHxSA remaining on day 70 compared with 15.4% in the autoclaved reactors. This result indicates that, especially in the acetate cultures, linear AmPr-FHxSA was more likely to be biotransformed compared to its branched isomer. Additionally, the percentage of branched PFHxS produced, particularly in the methane treatments (2.8% branched), was lower than the percentage branched of the parent compound, further suggesting that linear PFHxS precursors may be more readily transformed into PFHxS by soil microbial communities.

Text B.11: Microbial Community Additional Results and Discussion

Interestingly, none of the taxonomic assignments in our results corresponded to typical methanotrophs at the family, order, or genus level. The methanotrophic culture was composed of two dominant genera: *Ramlibacter* and *Cupriavidus*. Heterotrophic *Cupriavidus* has been shown to contribute growth cofactors to the autotrophic methanotrophs,[109] so it is possible that *Ramlibacter* were the methanotrophs, supported by *Cupriavidus*. A species of *Ramlibacter* has been demonstrated to oxidize butane [167] and, while there has been no research demonstrating its ability to oxidize methane, there is significant structural and sequence similarity between methane monooxygenase and butane monooxygenase.[168] Furthermore, facultative methanotrophs capable of oxidizing other carbon sources such as ac-

etate [169] and propane [170] have been discovered in recent years, making it possible that *Ramlibacter* is a facultative methanotroph. Given the high relative abundance of *Ramlibacter* and its ability to oxidize short-chain alkanes, this species is the strongest candidate organism that oxidized methane, and possibly transformed AmPr-FHxSA. However, metagenomic sequencing or growing the organism in isolation would be required to determine its true metabolic capabilities.

C Supplementary Information for Chapter 3

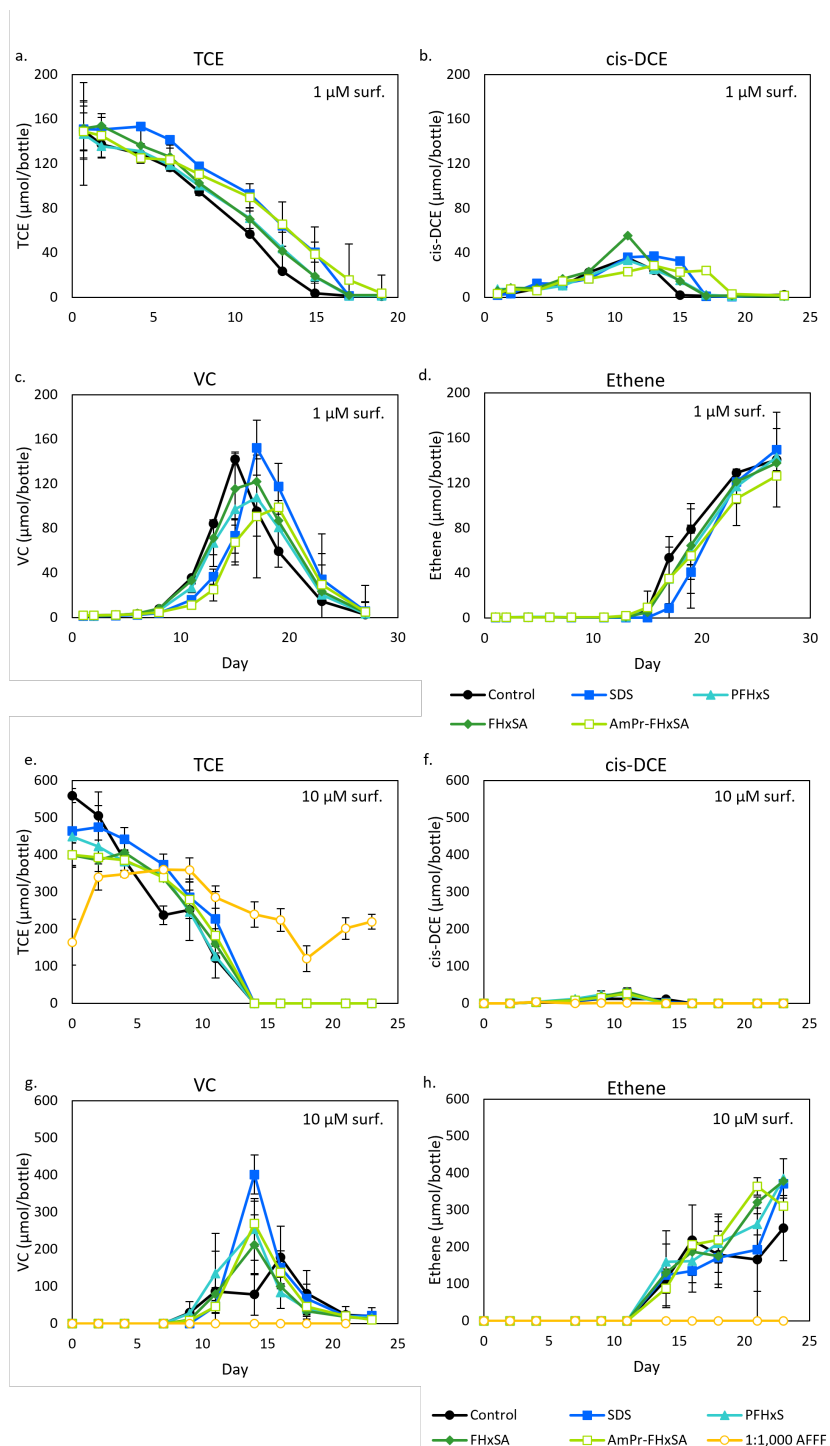


Figure C.1: TCE (a,e), DCE (b,f), and VC (c,g) dehalogenation and ethene production (d,h) with the anaerobic culture exposed to 1 μM of surfactants (a-d) or 10 μM surfactants (e-g). Black circles are control, blue squares are non-fluorinated control SDS, light blue triangles PFHxS, dark green diamond FHxSA, light green and white squares AmPr-FHxSA, and yellow white circles 1,000x diluted AFFF.

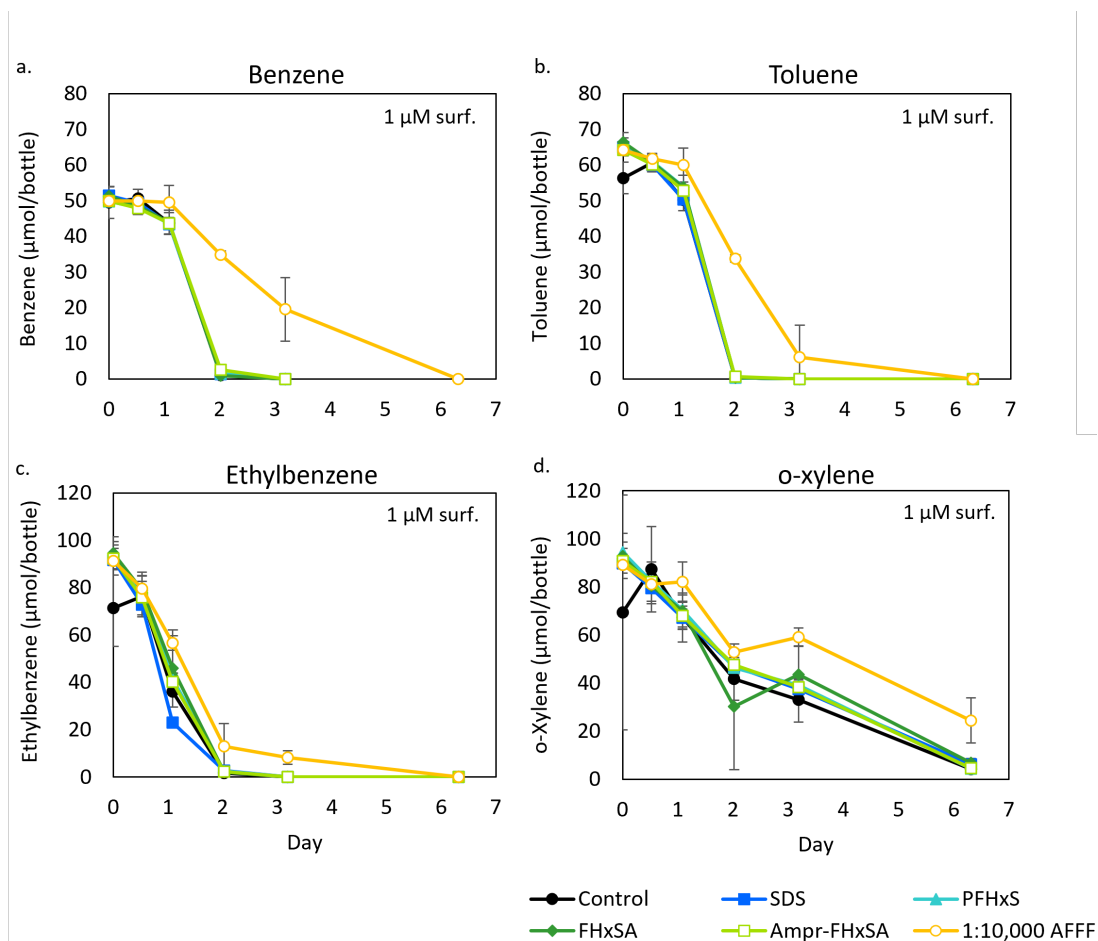


Figure C.2: Aerobic BTEX degrading culture consuming a) benzene, b) toluene, c) ethylbenzene, and d) ortho-xylene in the presence of $1 \mu\text{M}$ surfactants. Black circles are control, blue squares are non-fluorinated control SDS, light blue triangles PFHxS, dark green diamond FHxSA, light green and white squares AmPr-FHxSA, and yellow white circles 10,000x diluted AFFF.

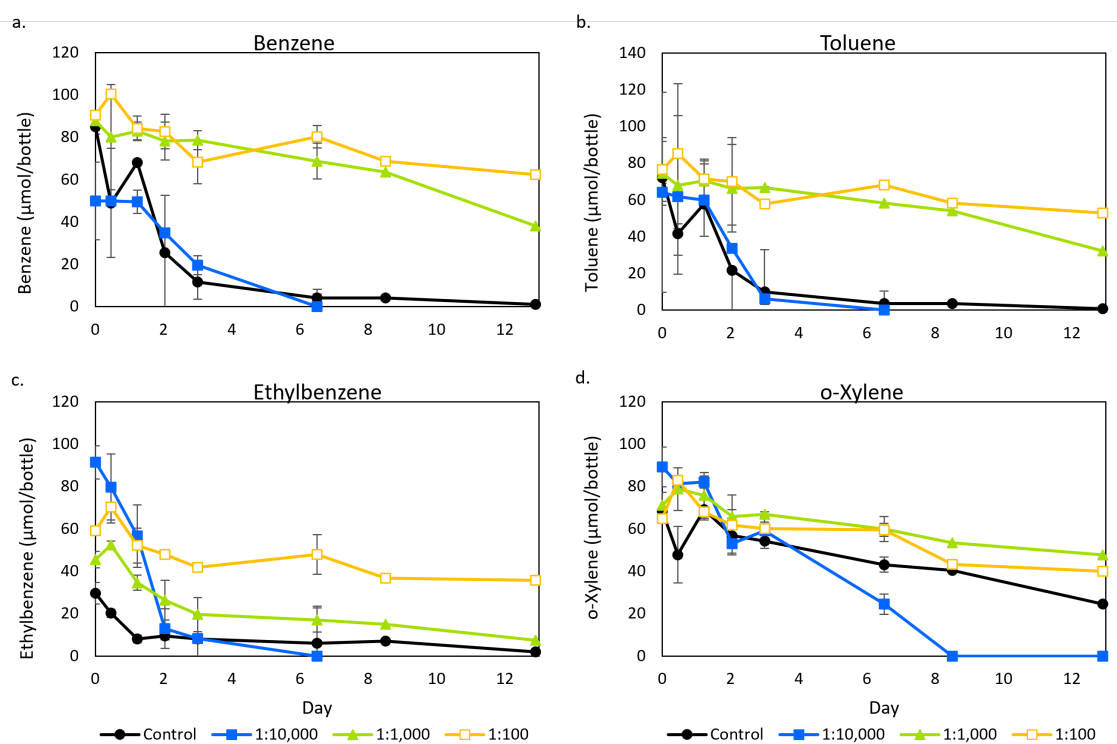


Figure C.3: Aerobic BTEX degrading culture consuming a) benzene, b) toluene, c) ethylbenzene, and d) ortho-xylene in the presence of dilutions of AFFF. Black circles are the control, blue squares are 10,000x diluted AFFF, light green triangles 1,000x diluted AFFF, and yellow white circles 100x diluted AFFF.

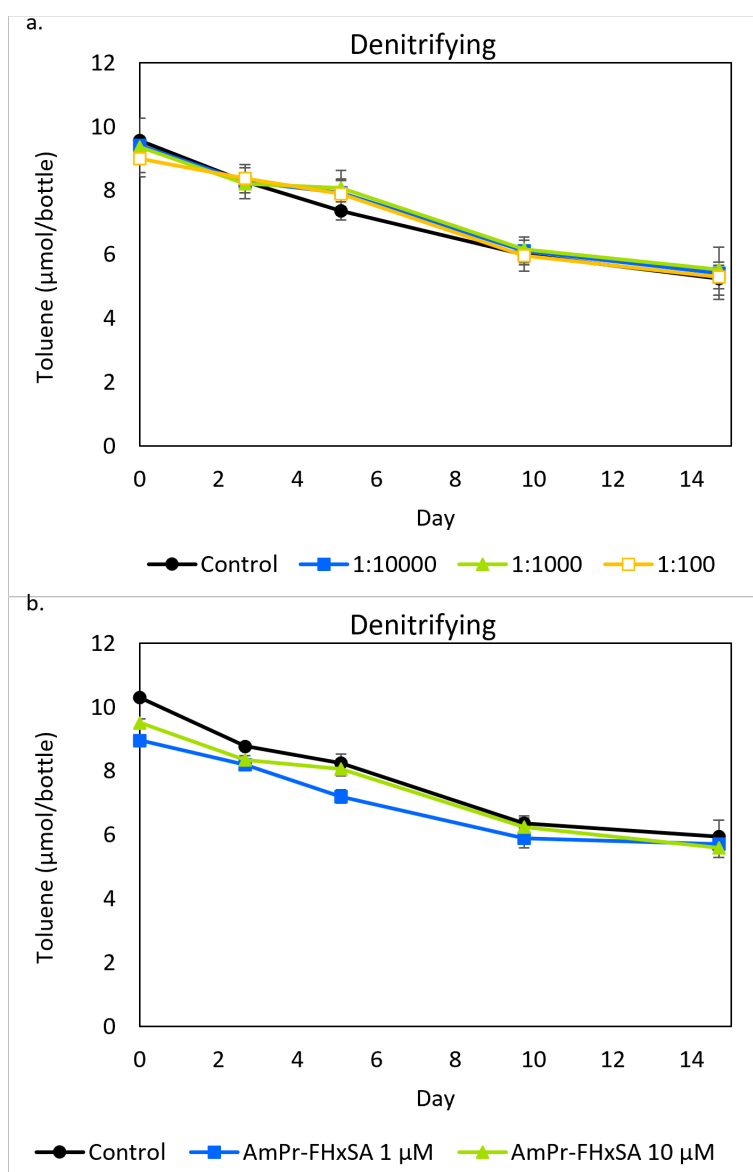


Figure C.4: Anaerobic toluene degradation with the anaerobic enrichment culture under nitrate reducing conditions, exposed to a) dilutions of AFFF or b) two concentrations of AmPr-FHxSA.

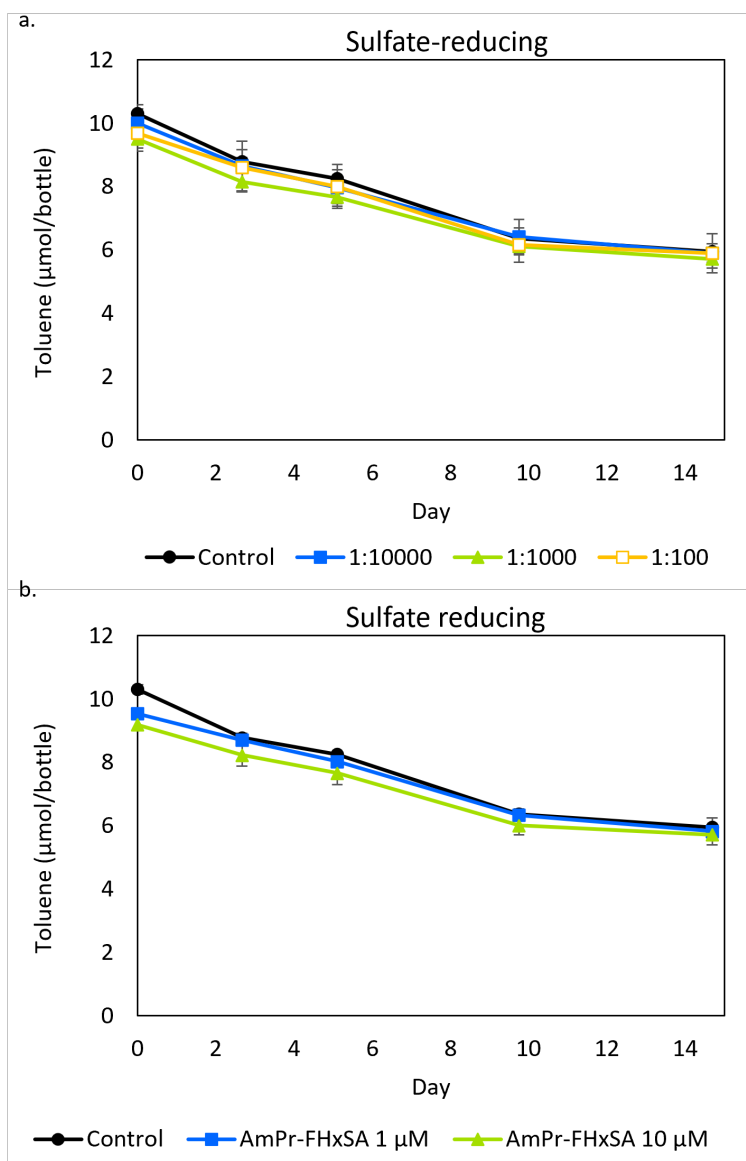


Figure C.5: Anaerobic toluene degradation with the anaerobic enrichment culture under sulfate reducing conditions, exposed to a) dilutions of AFFF or b) two concentrations of AmPr-FHxSA.

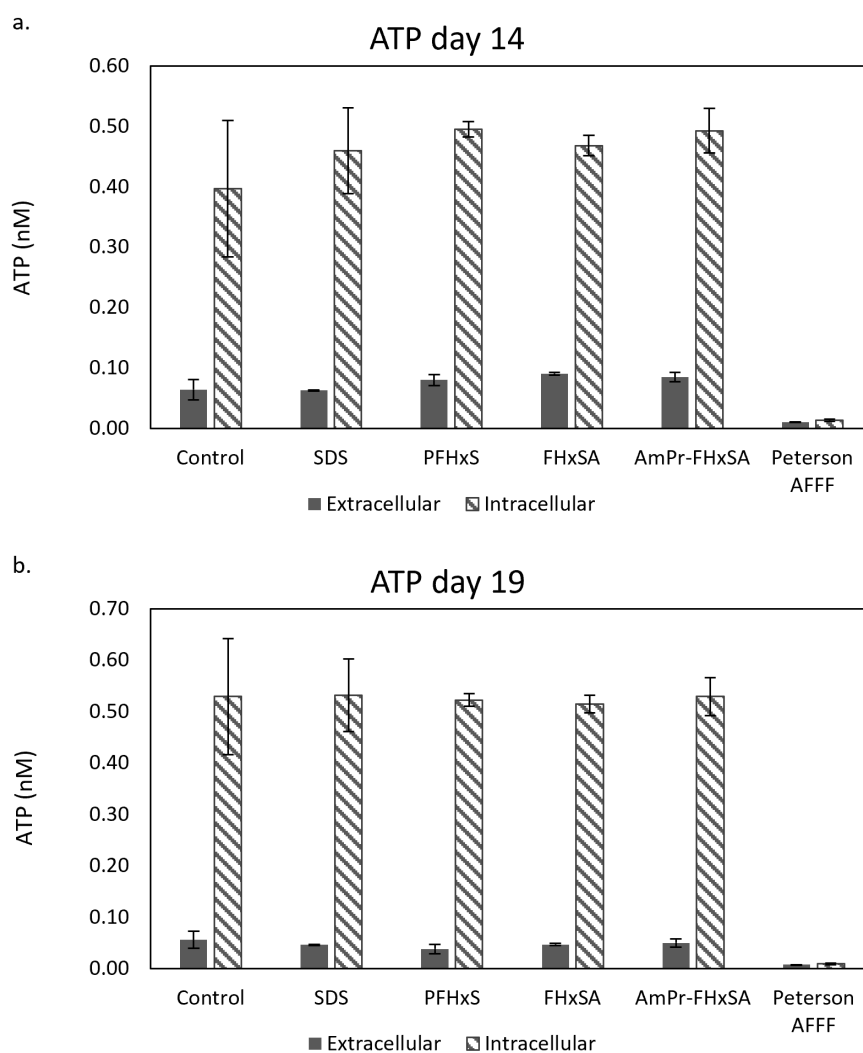


Figure C.6: ATP concentrations in nM from the TCE co-culture on day 14 of the 10 μM surfactant experiment, as shown in Figure S1e-h. Extracellular, shown in solid gray, represents ATP measured after filtering the biomass samples. Intracellular, shown in striped gray, represents ATP measured in total without filtering with the extracellular concentration subtracted. Error bars represent standard deviation of triplicate cultures.

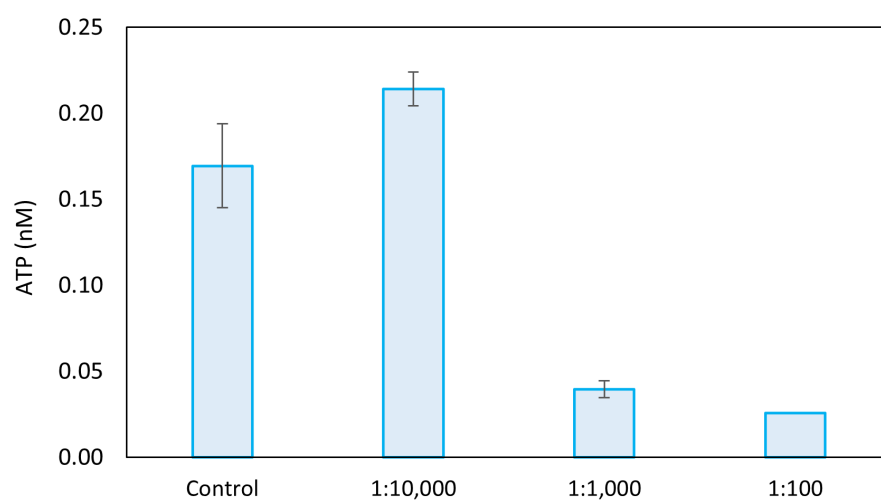


Figure C.7: ATP concentrations in nM from the TCE co-culture on day 16 of the AFFF dilution experiment as shown in Figure 3.2.

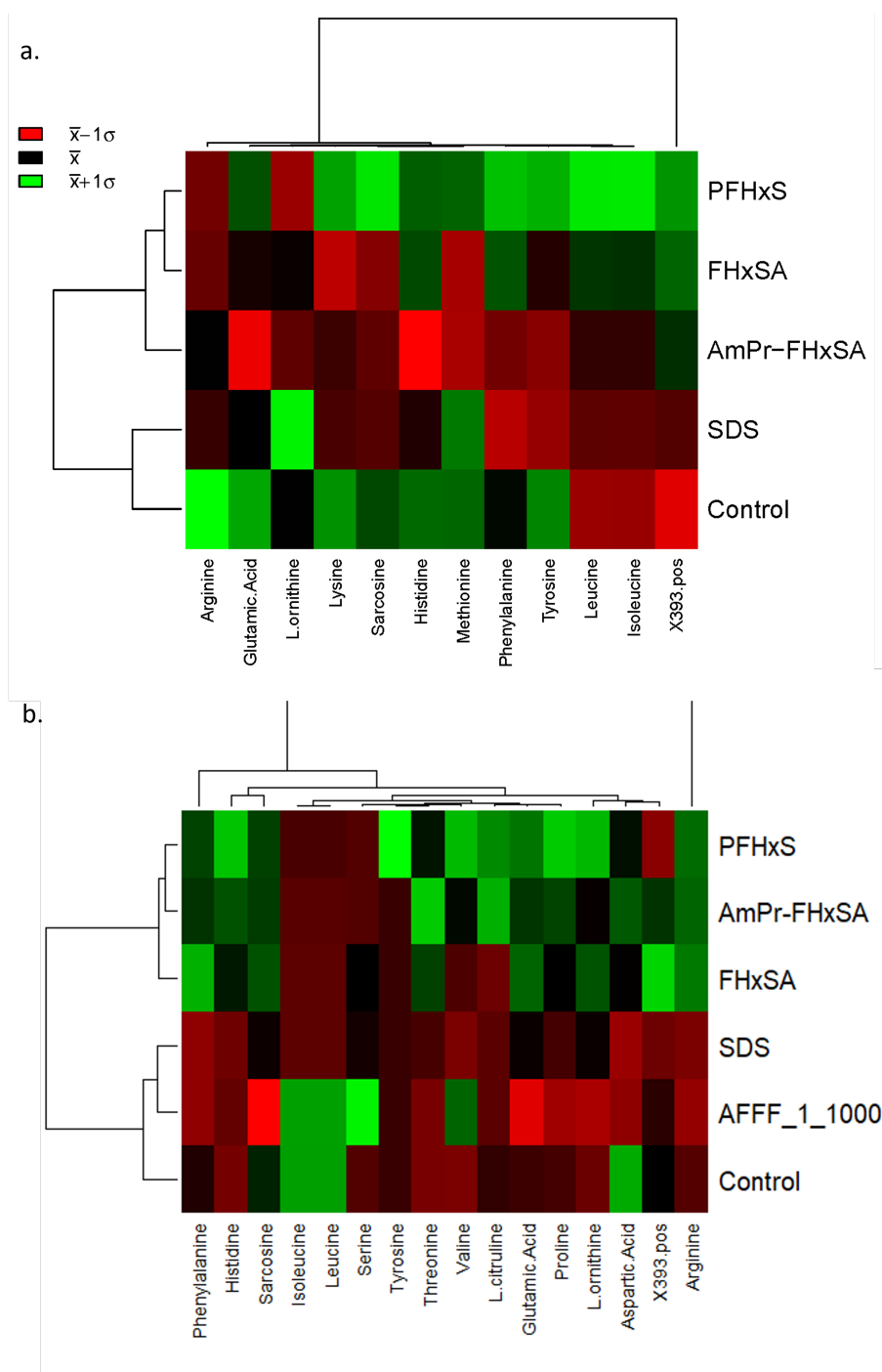


Figure C.8: Total (unfiltered) metabolites measured in anaerobic TCE co-culture experiments for a) 1 μM surfactant exposures and b) 10 μM surfactant exposures.

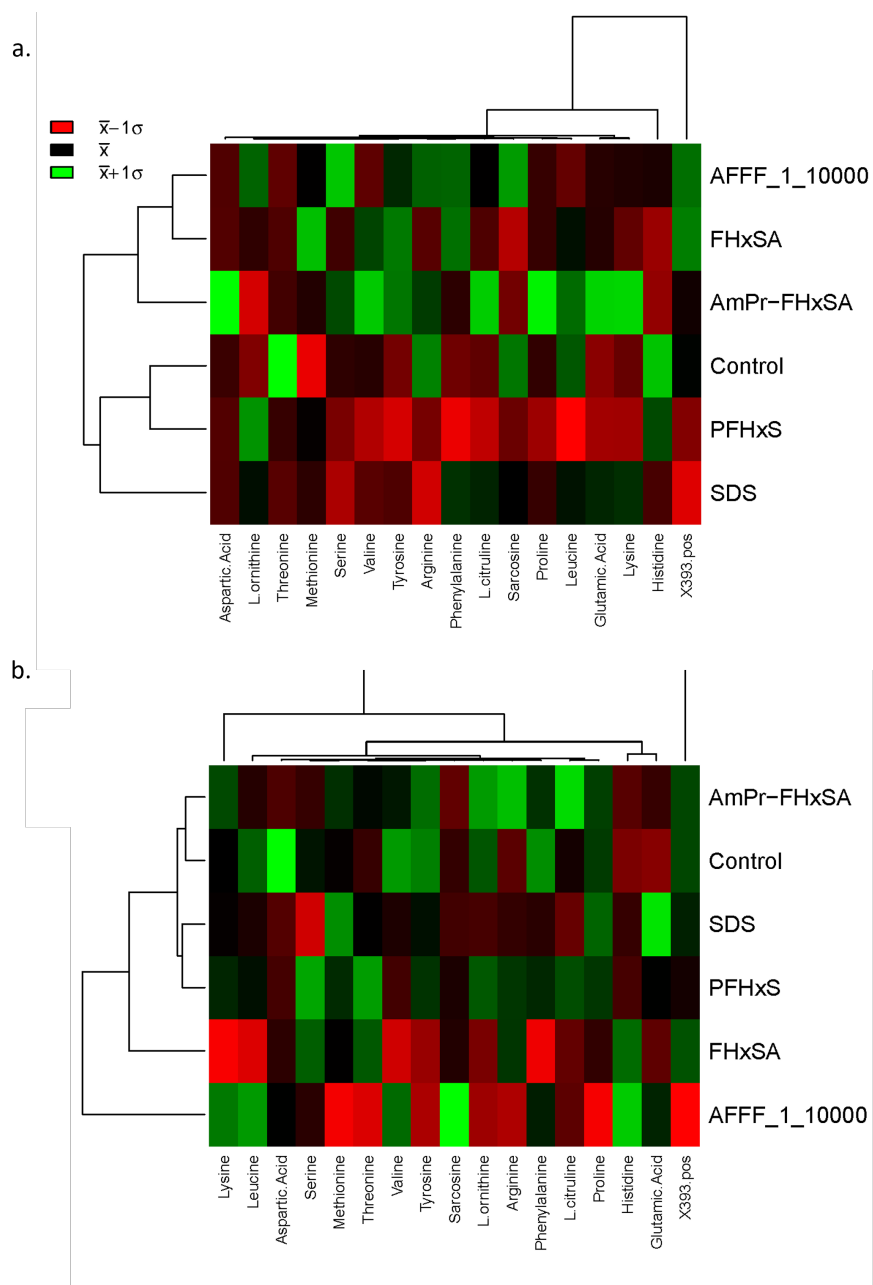


Figure C.9: Metabolites measured in aerobic BTEX degrading enrichment culture with 1 μ M surfactant exposures a) filtered, or extracellular metabolites, and b) unfiltered, or total metabolites measured.

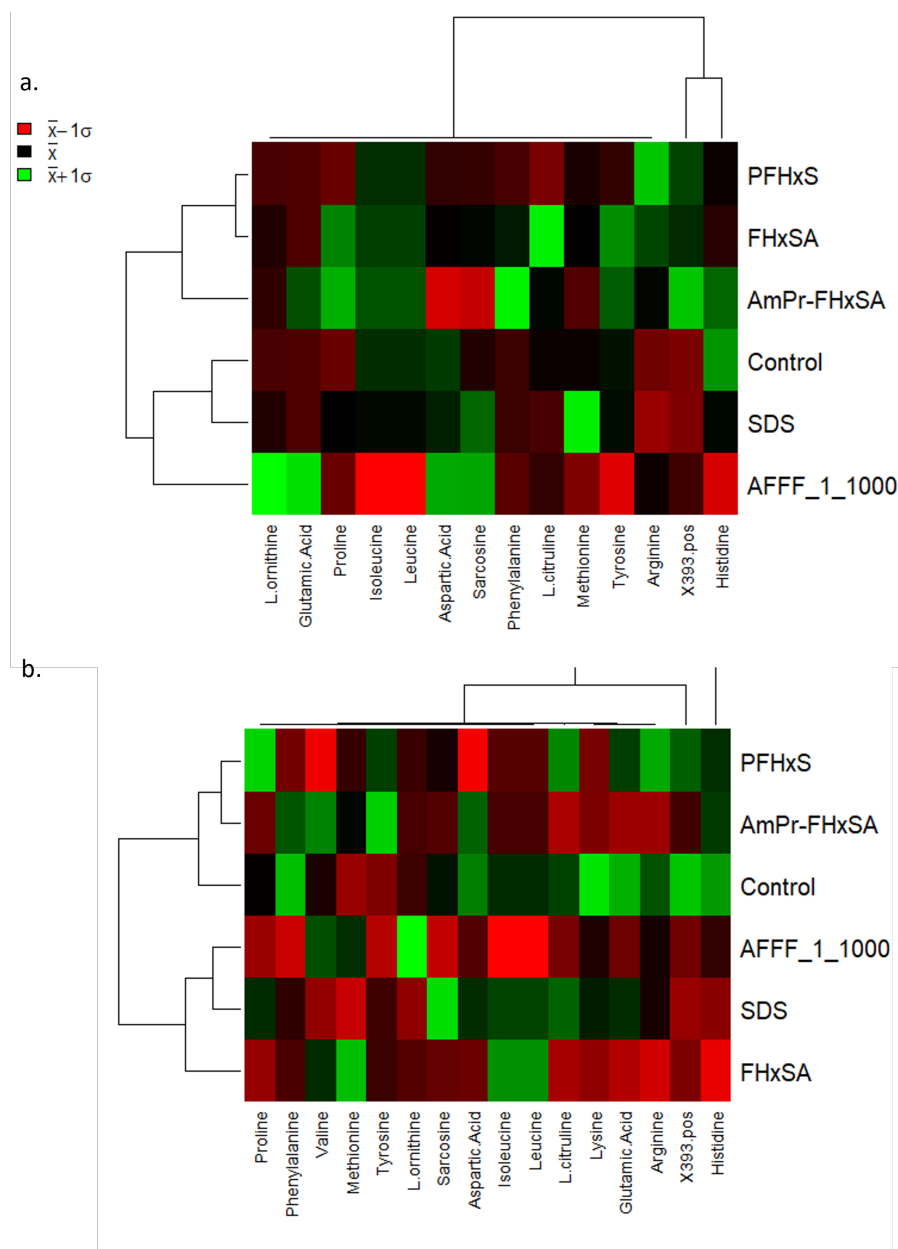


Figure C.10: Metabolites measured in aerobic BTEX degrading enrichment culture with 10 μ M surfactant exposures a) filtered, or extracellular metabolites and b) total metabolites, unfiltered.

μM	PFPeA	PFHxA	PFHpA	PFOA	PFBS	PFHxS	PFOS	Ampr-FHxSA
ECF-based AFFF	453 ± 57	526 ± 50	167 ± 18	442 ± 57	742 ± 17	1841 ± 7	16761 ± 2191	4755 ± 613

Table C.1: PFAS concentrations in ECF-based AFFF. Duplicate samples run on the LC-MS/MS of the diluted AFFF stock solution. Error is simply the difference between the two replicates.

Time	A %	B %
0	0	100
2	0	100
7	30	70
7.7	30	70
9	60	40
9.5	60	40
10.25	70	30
12.75	0	100
17	0	100

Table C.2: Eluent gradient for LC-MS/MS metabolites method.

Compound	Precursor Ion	Product Ion	Fragmentor	Collision Energy	Polarity
Glutathione-s2	613.2	355	100	5	+
393-pos	393.3	57.2	80	40	+
Glutathion	308	308	92	0	+
Oxaloacetic acid-con	271	157	92	8	-
Oxaloacetic acid	271	113	92	12	-
Tyrosine	182.1	136.1	25	12	+
L-citrulline	176	159.1	72	4	+
L-citrulline-conf	176	70.2	72	20	+
Arginine	175.1	70.1	75	28	+
Aconitic acid	169	125.1	68	4	-
Phenylalanine	166.1	120.1	25	5	+
Hilidine	156.1	110	25	12	+
Methionine	150	104	75	8	+
Glutamic acid	148.1	84.1	75	16	+
Lysine	147.1	84.1	50	16	+
Aspartic acid	134	74	50	15	+
L-ornithine	133	116	72	4	+
L-ornithine	133	70.2	72	16	+
Leucine	132.1	86.1	25	8	+
Isoleucine	132.1	86.1	25	8	+
Threonine	120	74.1	25	10	+
Valine	118.1	72.1	25	8	+
Proline	116.1	70.1	50	16	+
2-methyl oxobutanoate	116	74	180	10	-
Maleic acid	115	71.2	46	8	-
Serine	106.1	60.1	25	8	+
Sarcosine	90.1	44.2	46	12	+
Alanine	90.1	44.1	25	8	+
Glycine	76	30.1	25	5	+

Table C.3: Analytes for metabolite LC-MS/MS analysis.

D Supplementary Information for Chapter 4

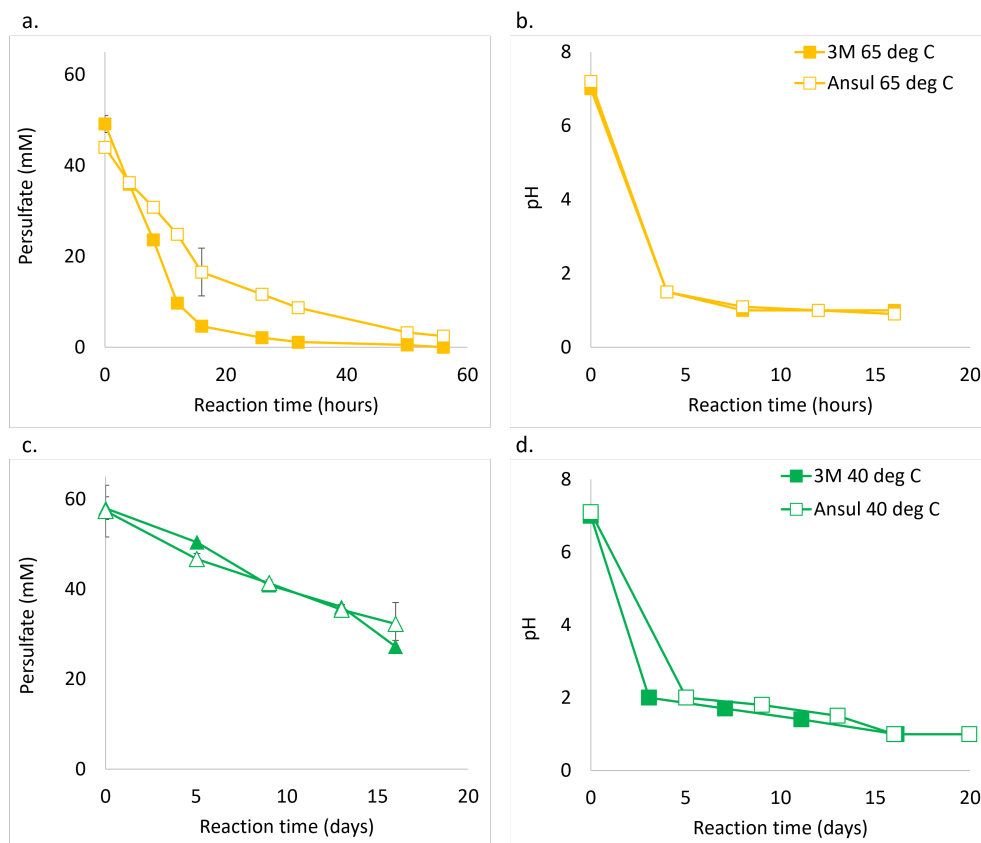


Figure D.1: HAPO on two AFFFs with 50 mM sodium persulfate added. a) Persulfate concentration and b) pH are shown for the 65°C tests, on the timescale of hours. c) Persulfate concentration and d) pH for the 40°C tests on the timescale of days. Open markers show results for Ansul AFFF tests; filled markers show results for 3M AFFF tests. Error bars represent duplicate reactors.

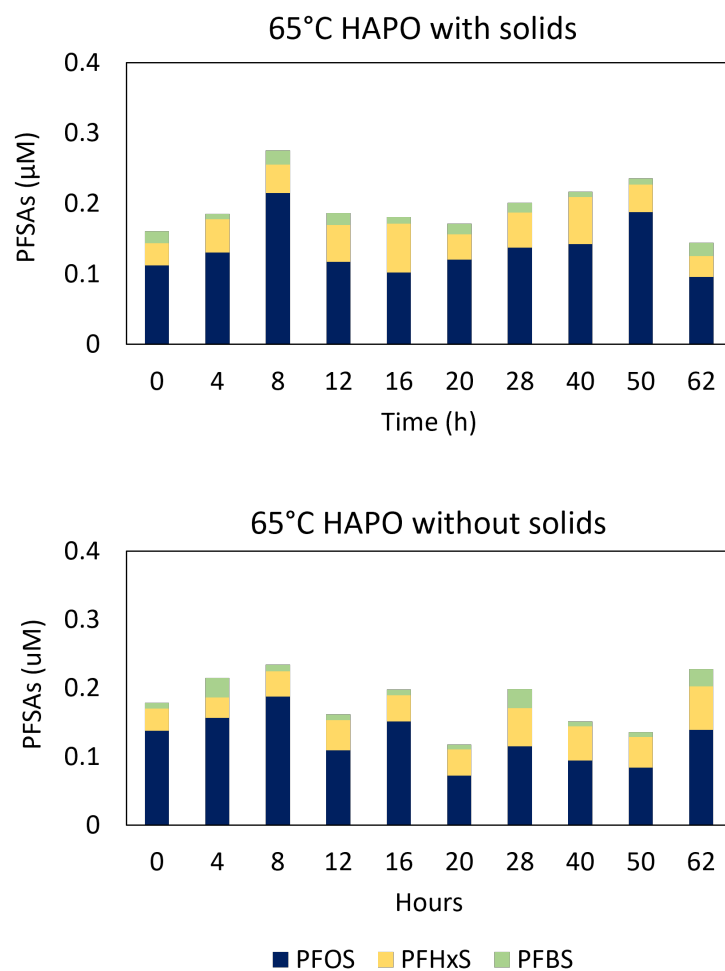


Figure D.2: PFSA concentrations, including PFOS, perfluorohexane sulfonic acid (PFHxS), and perfluorobutane sulfonic acid (PFBS), 65°C HAPO with and without aquifer solids present. PFSAs were not transformed or removed by HAPO.

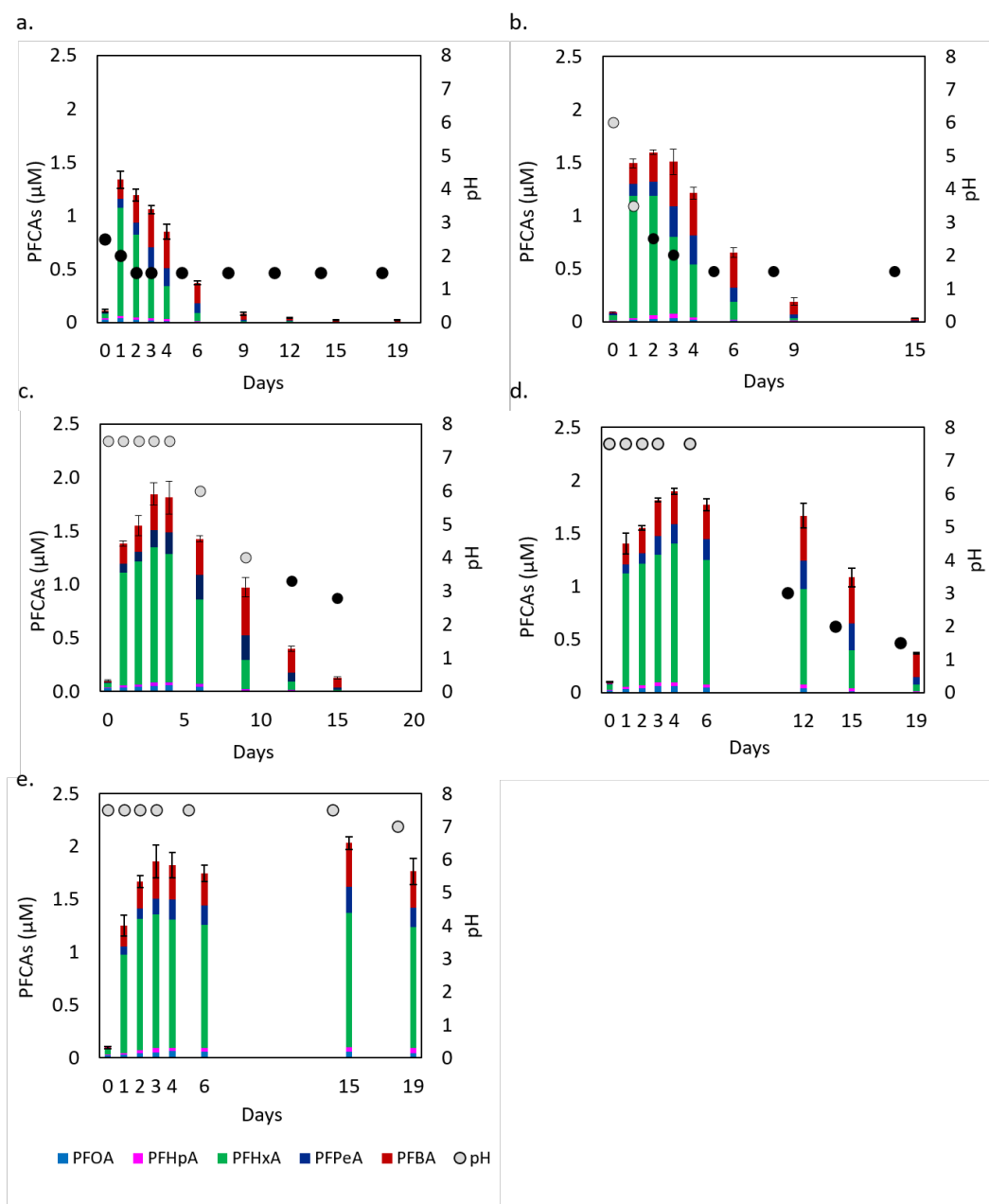


Figure D.3: HAPO at 40°C with 50 mM sodium persulfate and 3M AFFF diluted in Milli-Q water with increasing concentrations of calcite (no aquifer solids present): a) 0 mM, b) 2 mM, c) 5 mM, d) 10 mM, and e) 25 mM. The left y axis shows PFCA concentrations in μM , while the right y axis shows pH measurements; the gray circles denote that the pH is too high for PFCA transformation, and the black filled circles denote a pH amenable to PFCA transformation by sulfate radical.

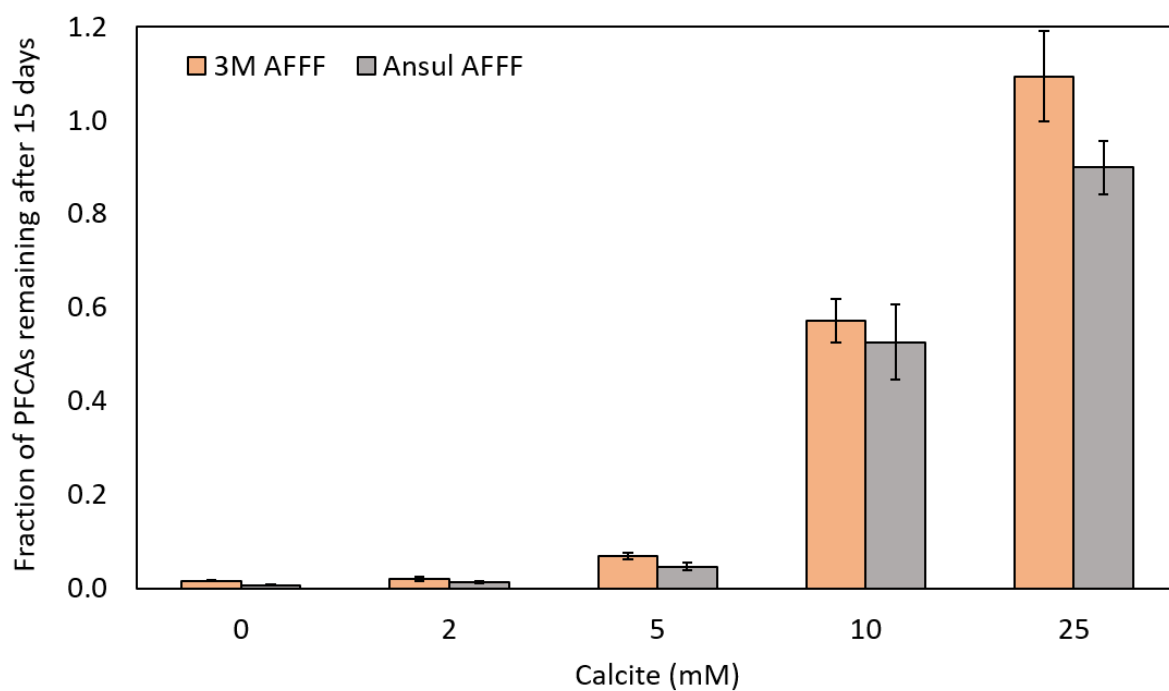


Figure D.4: Summary of the calcite results, comparing the fraction of PFCAs remaining from the 3M AFFF and Ansul AFFF after 15 days with increasing concentrations of calcite present.

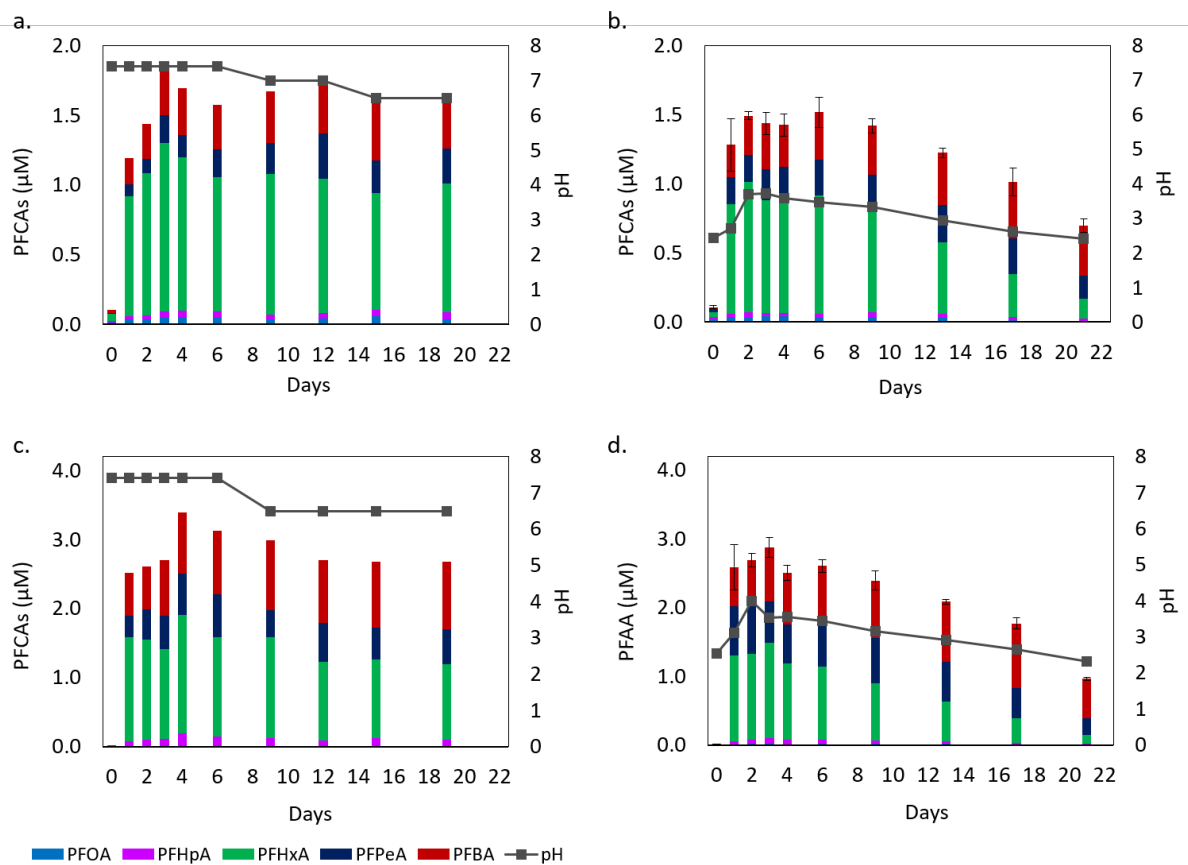


Figure D.5: HAPO at 40°C with 50 mM sodium persulfate using 20 g/L OK solids. a) 3M AFFF without a pre-acidification step, b) 3M AFFF with a pre-acidification step, c) Ansul AFFF without a pre-acidification step, and d) Ansul AFFF with a pre-acidification step. Acidification was conducted using 1 N sulfuric acid until the pH was 2-3.

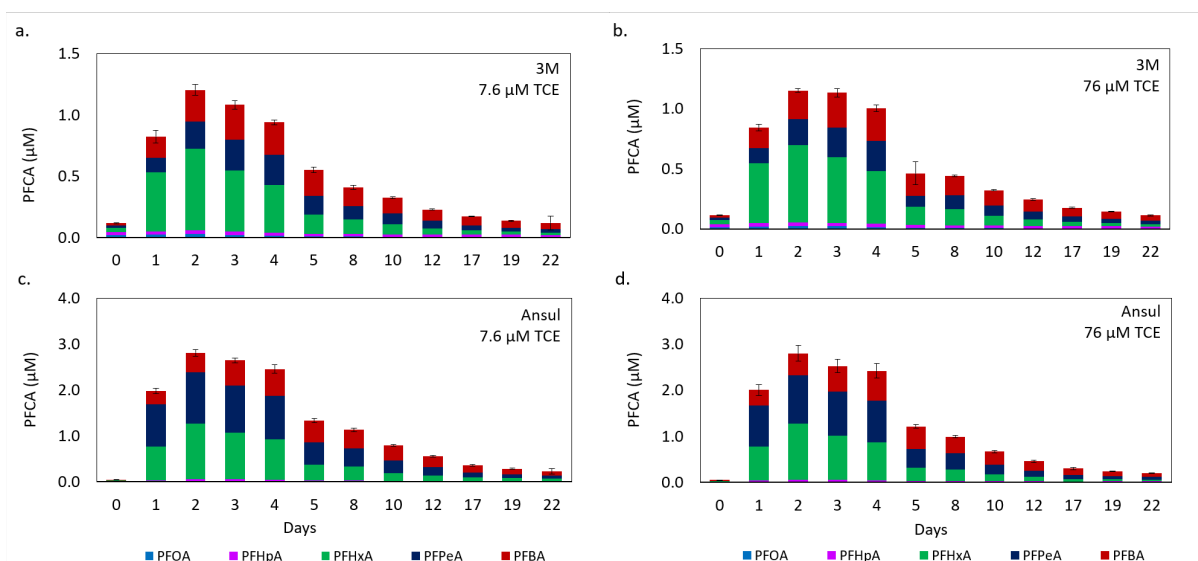


Figure D.6: PFCA transformation over three weeks at 40°C in a slurry co-contaminated with a) 3M AFFF with 7.6 μM TCE, b) 3M AFFF with 76 μM TCE, c) Ansul AFFF with 7.6 μM TCE, and d) Ansul AFFF with 76 μM TCE. No PFCA transformation inhibition is observed in comparing the two tests to previous experiments without TCE.

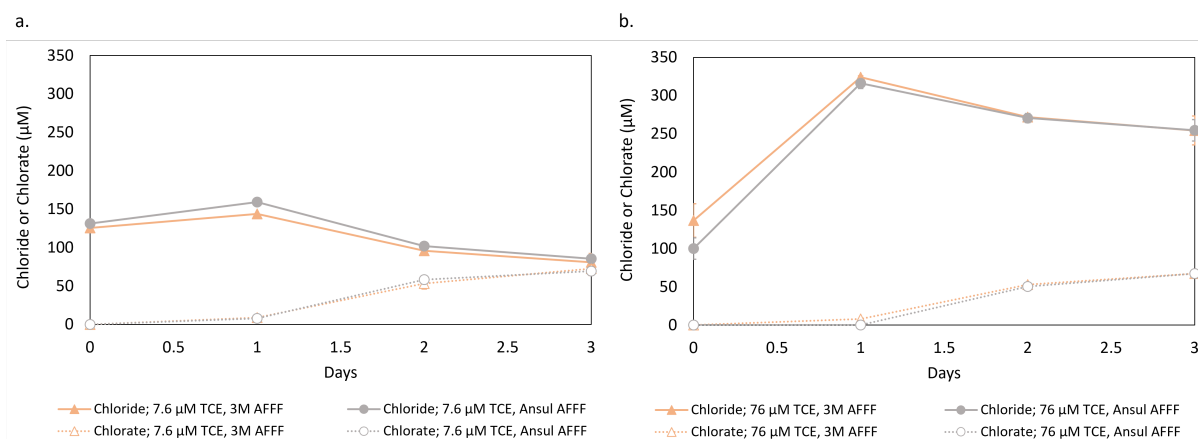


Figure D.7: Chloride and chlorate production from HAPO of TCE-AFFF mixtures for a) 7.6 μM (1 mg/L) TCE and b) 76 μM (10 mg/L) TCE. Although TCE is mineralized to nontoxic products (chloride and CO_2), the chloride can be further oxidized to create chlorate, a toxic byproduct. At relevant pH ranges (below 3 for PFCA HAPO treatment), chloride radical is oxidized to chlorate.

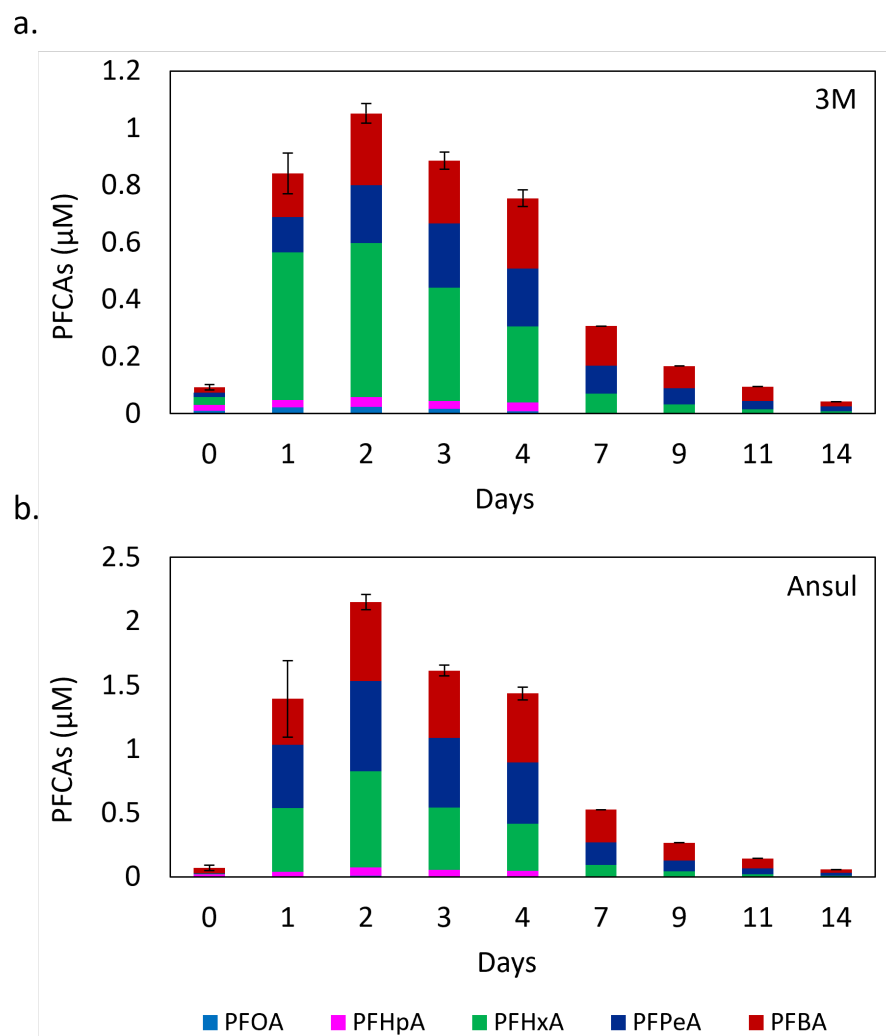


Figure D.8: PFCA transformation from 3M and Ansul AFFFs in the presence of 1 ppm dioxane.

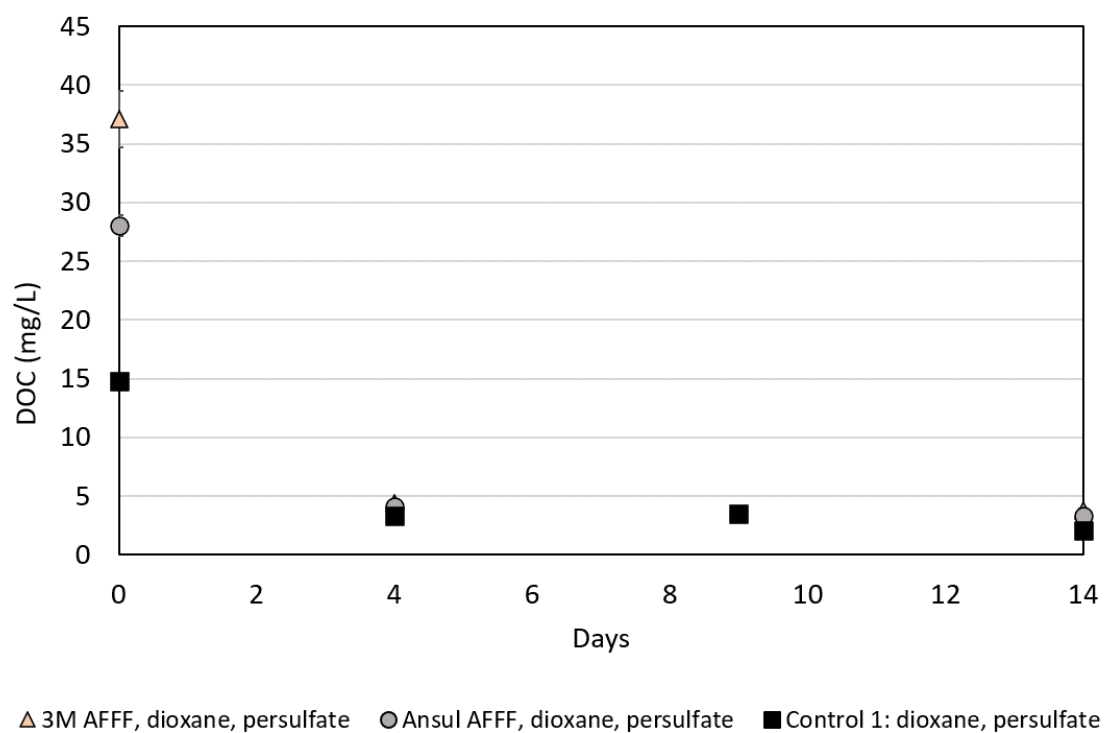


Figure D.9: DOC degradation during 50 mM sodium persulfate heat-activated experiment in the presence of AFFFs and 1,4-dioxane. Most DOC was mineralized by HAPO after the two week experiment.

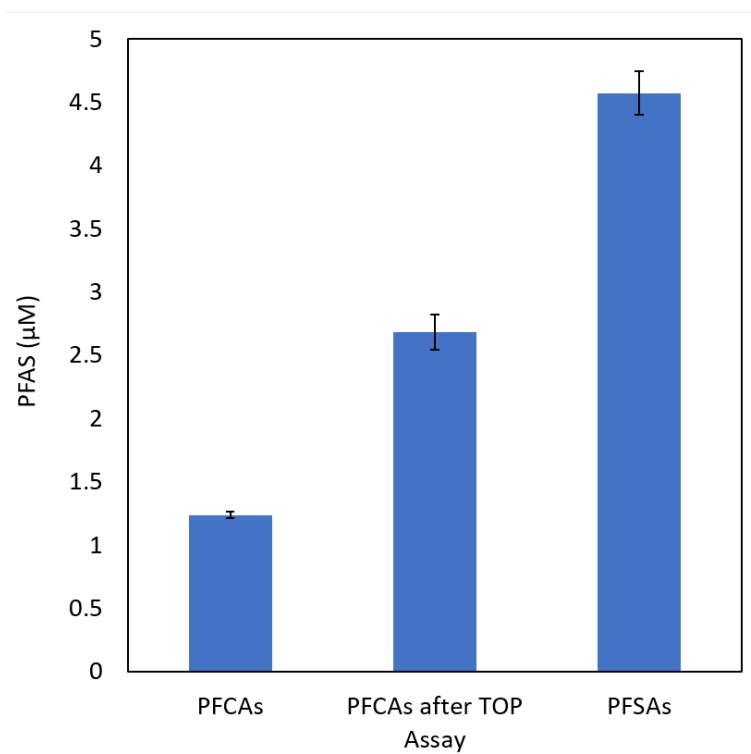


Figure D.10: PFAS concentrations in FL contaminated groundwater. The difference between the PFCAs and PFCAs after TOP assay is the estimated total precursor concentration.

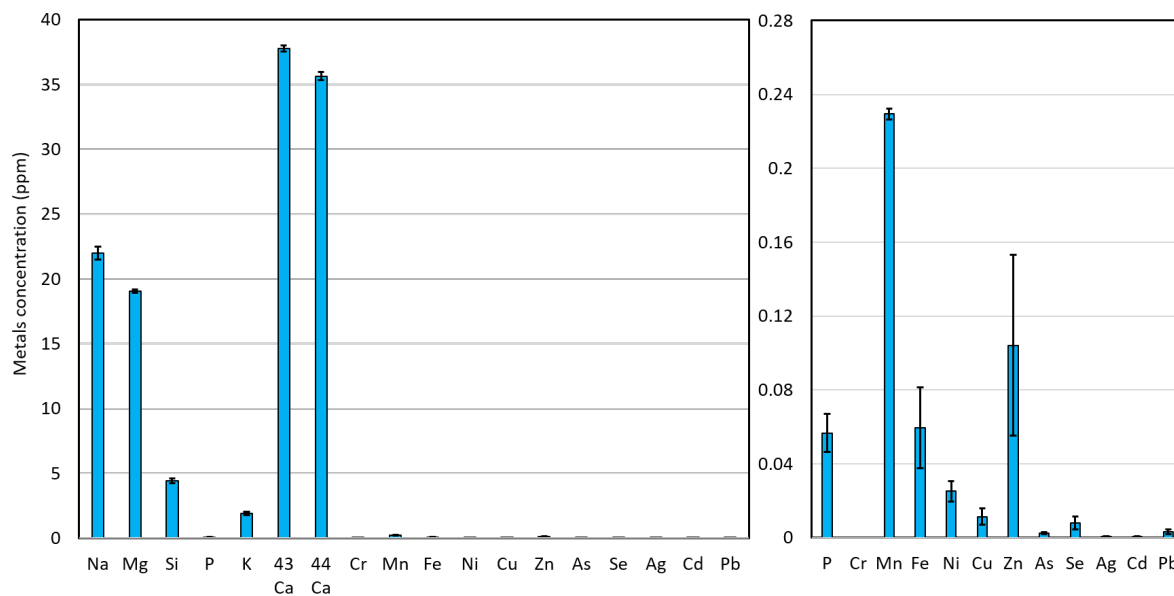


Figure D.11: Concentration of metals (ppm) in PFAS-contaminated FL groundwater.

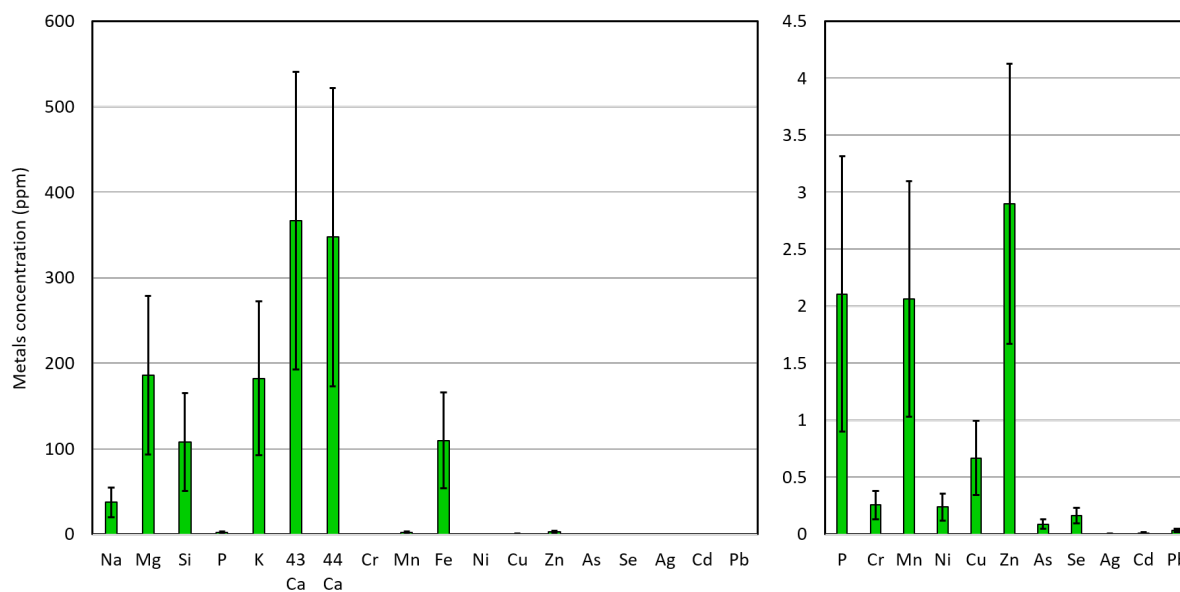


Figure D.12: Concentration of metals from sulfuric acid digested FL aquifer solids.

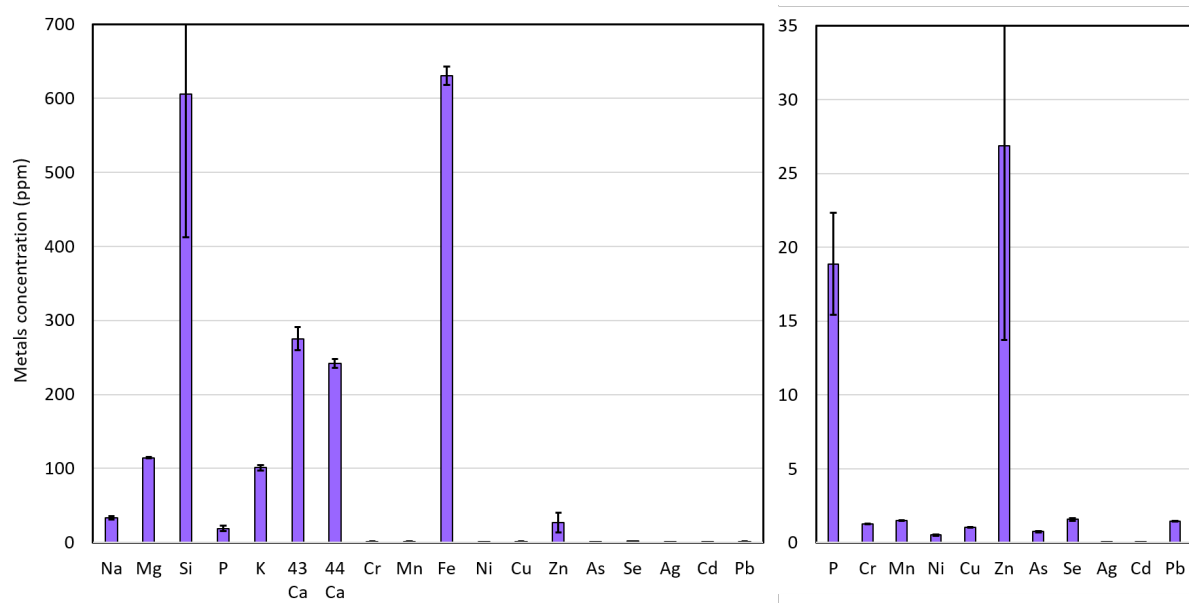


Figure D.13: Concentration of metals from Aqua Regia digested FL aquifer solids.

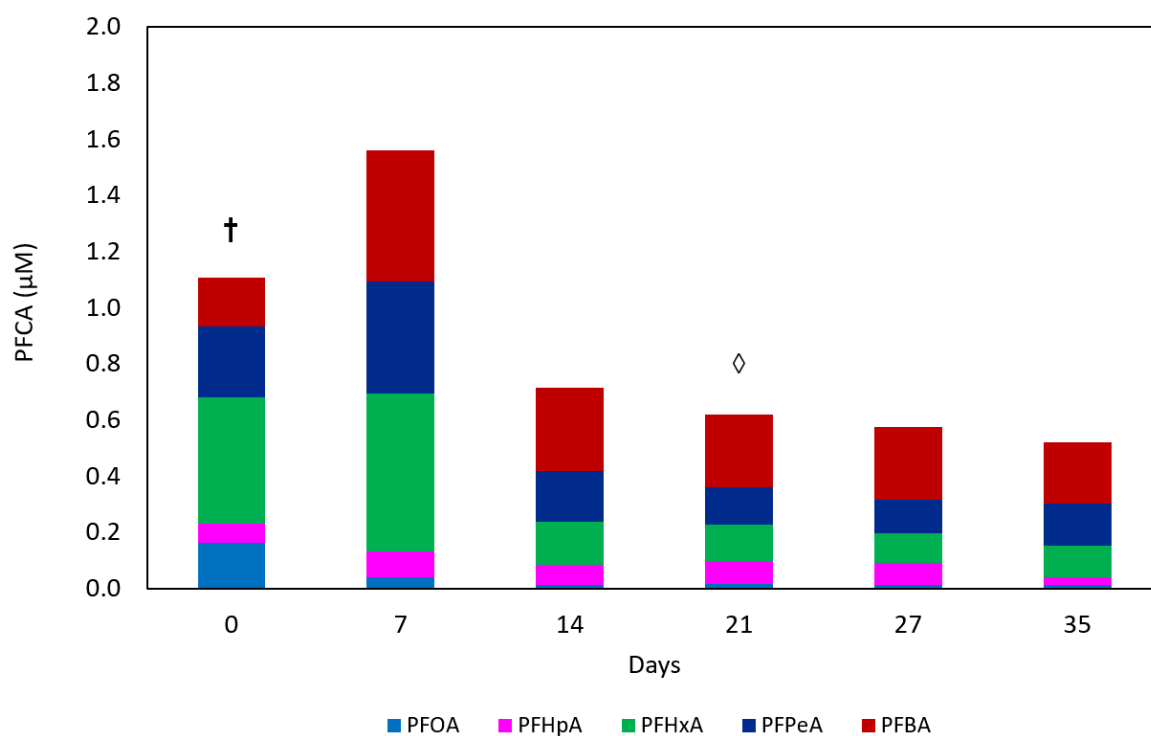


Figure D.14: High solids slurry test of HAPO at 40°C of contaminated groundwater and aquifer solids at a 1:1 ratio. 500 mM persulfate was added on Day 0 (cross), followed by an additional dose of 100 mM persulfate added Day 21 (diamond), which did little to promote further PFCA transformation.

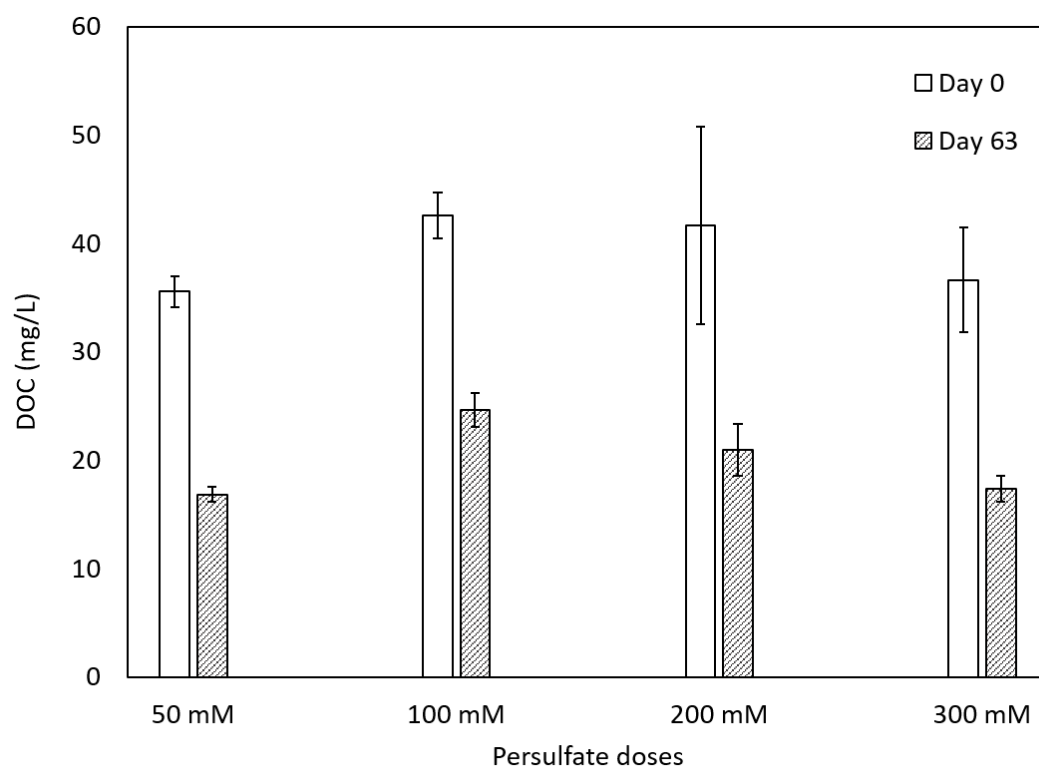


Figure D.15: DOC from the HAPO test in Figure 3.6. Although PFAS were mineralized, some recalcitrant organic matter remained at the end of the experiment.

Sample No.	Description	N (Total) %	C (Total) %	TOC %	Zn (Total) ppm	Mn (Total) ppm	Fe (Total) ppm	Cu (Total) ppm
1	Davis-Monthan,	<0.020	0.73	<0.02	57	338	16100	17
1 dup	AZ solids	<0.020	0.75	<0.02	55	342	15400	17
2	Tinker, OK AFB	<0.020	1.15	<0.02	37	840	15300	24
2 dup	solids	<0.020	1.18	<0.02	35	810	13300	22

Table D.1: Soil characteristics for HAPO tests measured at UC Davis. Nitrogen (N), Carbon (C), total organic carbon (TOC), zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu).

Time	A %	B %
0	90	100
2	90	100
8	5	70
12	5	70
12.50	90	40
19	90	40

Table D.2: LC-MC/MS mobile phase gradient for negative mode.

Reactants		Products		Rate constant (M ⁻¹ s ⁻¹)	Reference
Radical propagation					
SO ₄ ^{•-}	HO [•]	HSO ₅ ⁻		1.00E+10	Das, T.N. 2001
SO ₄ ^{•-}	SO ₅ ^{•-}	S ₂ O ₈ ²⁻	0.5O ₂	8.96E+09	Qian, Yajie, et al. 2015
SO ₄ ^{•-}	SO ₄ ^{•-}	S ₂ O ₈ ²⁻		8.20E+09	Criquet & Leitner 2009
HO [•]	HO ₂ [•]	H ₂ O	O ₂	6.60E+09	Sehested et al 1968
HO [•]	HO [•]	H ₂ O ₂		5.00E+09	Kamel et al 2009
SO ₄ ^{•-}	HO ₂ [•]	HSO ₄ ⁻	O ₂	3.50E+09	Jiang et al. 1992
SO ₅ ^{•-}	SO ₅ ^{•-}	S ₂ O ₈ ²⁻	O ₂	2.20E+08	Das, T.N. 2001
SO ₅ ^{•-}	SO ₅ ^{•-}	SO ₄ ^{•-}	SO ₄ ^{•-} O ₂	2.10E+08	Das, T.N. 2001
SO ₄ ^{•-}	OH ⁻	HO [•]	SO ₄ ²⁻	8.30E+07	Neta et al 1988
HO [•]	H ₂ O ₂	H ₂ O	HO ₂ [•]	2.70E+07	Buxton et al 1988
S ₂ O ₈ ²⁻	HO [•]	HSO ₄ ⁻	SO ₄ ^{•-} 0.5O ₂	1.20E+07	Peternel et al 2013
SO ₄ ^{•-}	H ₂ O ₂	HSO ₄ ⁻	HO ₂ [•]	1.20E+07	Jiang et al. 1992
SO ₄ ^{•-}	HSO ₅ ⁻	SO ₅ ^{•-}	HSO ₄ ⁻	1.00E+06	Das, T.N. 2001
SO ₄ ^{•-}	S ₂ O ₈ ^{•-}	S ₂ O ₈ ²⁻	SO ₂ O ₂	9.92E+05	Qian, Yajie, et al. 2015
HO ₂ [•]	HO ₂ [•]	H ₂ O ₂	O ₂	8.30E+05	Bielski 1985
HO [•]	HSO ₄ ⁻	SO ₄ ^{•-}	H ₂ O	3.74E+05	Yu et al 2004
SO ₄ ^{•-}	S ₂ O ₈ ²⁻	S ₂ O ₈ ^{•-}	SO ₄ ²⁻	1.80E+05	Yu et al 2004
SO ₄ ^{•-}	H ₂ O	HO [•]	HSO ₄ ⁻	1.82E+03	Herrmann et al 1995
S ₂ O ₈ ^{•-}	SO ₄ ²⁻	S ₂ O ₈ ²⁻	SO ₄ ^{•-}	9.50E-02	Yu et al 2004
S ₂ O ₈ ^{•-}	H ₂ O	HO [•]	H ⁺ S ₂ O ₈ ²⁻	8.30E-04	Qian, Yajie, et al. 2015
PFCAs					
SO ₄ ^{•-}	C ₂ F ₅ COOH	CF ₃ COOH	Byproducts	9.31E+07	Qian, Yajie, et al. 2015
SO ₄ ^{•-}	C ₃ F ₇ COOH	C ₂ F ₅ COOH	Byproducts	1.05E+07	Qian, Yajie, et al. 2015
SO ₄ ^{•-}	C ₄ F ₉ COOH	C ₃ F ₇ COOH	Byproducts	1.26E+06	Qian, Yajie, et al. 2015
SO ₄ ^{•-}	C ₅ F ₁₁ COOH	C ₄ F ₉ COOH	Byproducts	7.02E+05	Qian, Yajie, et al. 2015
SO ₄ ^{•-}	C ₆ F ₁₃ COOH	C ₅ F ₁₁ COOH	Byproducts	2.68E+05	Qian, Yajie, et al. 2015
SO ₄ ^{•-}	C ₇ F ₁₅ COOH	C ₆ F ₁₃ COOH	Byproducts	2.59E+05	Qian, Yajie, et al. 2015
Aquifer Constituents					
SO ₄ ^{•-}	Fe ²⁺	SO ₄ ²⁻	Fe ³⁺	9.90E+08	Neta et al 1988
SO ₄ ^{•-}	NOM	Byproducts		2.35E+07	Xie et al 2015
SO ₄ ^{•-}	Mn ²⁺	SO ₄ ²⁻	Mn ³⁺	2.00E+07	Neta et al 1988
SO ₄ ^{•-}	CO ₃ ²⁻	CO ₃ ^{•-}	SO ₄ ²⁻	6.50E+06	Zuo et al 1999
SO ₄ ^{•-}	HCO ₃ ⁻	CO ₃ [•]	H ⁺ SO ₄ ²⁻	3.60E+06	Chameides & Davis 1982
SO ₄ ^{•-}	HPO ₄ ²⁻	HPO ₄ ^{•-}		1.20E+06	Neta et al 1988
SO ₄ ^{•-}	NO ₃ ⁻	NO ₃ [•]	SO ₄ ²⁻	3.60E+05	Neta et al 1988

Table D.3: Rate constants with sulfate radicals and PFAS and aquifer constituents

<i>Chloride</i>						
$\text{SO}_4^{\bullet-}$	$\text{ClO}_2^{\bullet} + \text{H}_2\text{O}$	ClO_3^-	SO_4^{2-}	2H^+	8.23E+09	Qian, Yajie, et al. 2015
$\text{Cl}^{\bullet}$	Cl^-	$\text{Cl}_2^{\bullet-}$			8.00E+09	Yu et al 2004
$\text{Cl}_2^{\bullet-}$	$\text{Cl}_2^{\bullet-}$	Cl_2	2Cl^-		6.41E+09	Neta et al 1988
$\text{HO}^{\bullet}$	Cl^-	$\text{ClOH}^{\bullet-}$			4.30E+09	Jayson et al 1973
$\text{HO}^{\bullet}$	$\text{ClO}_2^{\bullet}$	ClO_3^-	H^+		4.00E+09	Neta et al 1988
$\text{Cl}^{\bullet}$	$\text{Cl}_2^{\bullet-}$	Cl_2	Cl^-		2.10E+09	Yu & Barker 2003
$\text{HO}^{\bullet}$	ClO_2^-	$\text{ClO}_2^{\bullet}$	OH^-		1.00E+09	Neta et al 1988
$\text{Cl}^{\bullet}$	CO_3^{2-}	Cl^-	$\text{CO}_3^{\bullet-}$		5.00E+08	Yang et al 2014
$\text{SO}_4^{\bullet-}$	Cl^-	SO_4^{2-}	$\text{Cl}^{\bullet}$		4.70E+08	Yuan et al 2014
$\text{Cl}^{\bullet}$	SO_4^{2-}	$\text{SO}_4^{\bullet-}$	Cl^-		2.50E+08	Yu et al 2004
$\text{Cl}^{\bullet}$	HCO_3^-	Cl^-	$\text{CO}_3^{\bullet-}$	H^+	2.20E+08	Yang et al 2014
$\text{Cl}^{\bullet}$	$\text{Cl}^{\bullet}$	Cl_2			8.80E+07	Wu et al 1980
$\text{Cl}^{\bullet}$	H_2O	$\text{ClOH}^{\bullet-}$	H^+		1.30E+03	Liang et al 2006
<i>pH</i>						
$\text{HSO}_4^{\bullet}$		$\text{SO}_4^{\bullet-}$	H^+		pKa < 1.9	
HSO_4^-		SO_4^{2-}	H^+		pKa = 1.9	
H_2CO_3		HCO_3^-	H^+		pKa = 6.3	
HCO_3^-		CO_3^{2-}	H^+		pKa = 10.3	

Table D.4: Rate constants with sulfate radicals and chloride species, and relevant pKa values.