Biological and Chemical Transformation of a PFAS Precursor with Insights into PFAS Fate and Forensics

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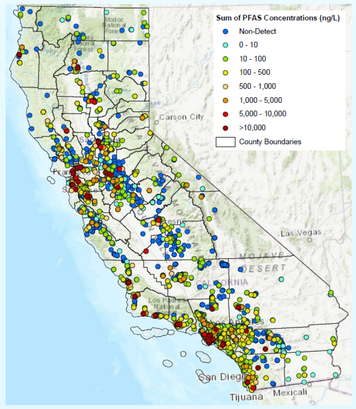
Poster Group 1

BACKGROUND/OBJECTIVES

Sites impacted by per- and polyfluoroalkyl substances (PFAS) from aqueous film forming foam (AFFF) contain co-contaminants that can stimulate biotransformation of polyfluoroalkyl substances. Here, we compare how microbial enrichments from AFFF-impacted soil amended with diethyl glycol monobutyl ether (DGBE; found in AFFF), aromatic hydrocarbons (present in co-released fuels), acetate and methane (substrates used or formed during bioremediation) impact the aerobic biotransformation of N-dimethyl ammonio propyl perfluorohexane sulfonamide (AmPr-FHxSA), a common AFFF ingredient designated as a precursor made by electrochemical fluorination, or an ECF-based precursor. Our objective was to compare biotransformation rates and products using different microbial communities to provide insights into the fate of AmPr-FHxSA and similar ECF-based PFAS precursors. Additionally, we examined chemical oxidation products of AmPr-FHxSA and its biotransformation product perfluorohexane sulfonamide (FHxSA) to compare biological versus chemical transformation products.

Understanding PFAS products that could be formed with different microbial communities can inform which PFAS to target for source attribution forensics analyses. When PFAS analytical methods are limited to a set of 30-40 compounds, most of them perfluoroalkyl acids, PFAS mass including biotransformation products may be missed, thus underestimating PFAS contamination in a given sample and missing out on key forensics data.

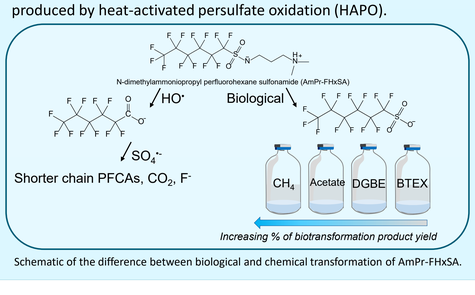


PFAS Prevalence in California (GeoTracker, 2023). Base map is ESRI's ArcGIS Online world topographic map, obtained April 2023.

EXPERIMENTAL APPROACH/ACTIVITIES

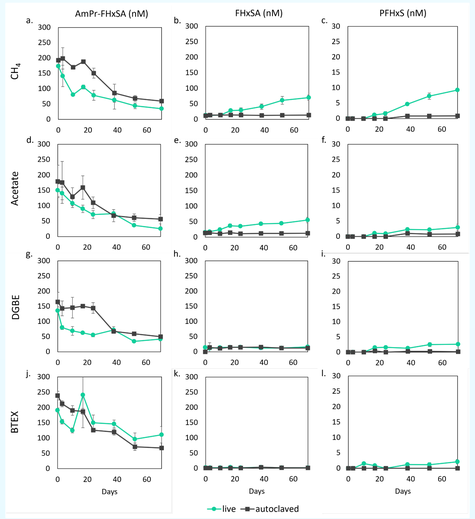
We performed aerobic biotransformation batch tests in triplicate over 70 days, with an enrichment culture seeded from an AFFF-impacted site, and four different carbon sources to impact the microbial community. Carbon sources used were DGBE, aromatic hydrocarbons (mixture of benzene, toluene, ethylbenzene, and xylene), acetate, and methane. AmPr-FHxSA was added to each triplicate, and PFAS were monitored weekly or biweekly using liquid chromatography tandem mass spectrometry. Additionally, high resolution mass spectrometry (HRMS) was performed at the start and end of the experiment to capture non-targeted products.

We also exposed AmPr-FHxSA and FHxSA to the total oxidizable precursor (TOP) assay, which is used to quantify the mass of unknown PFAS precursors in a mixed PFAS sample. For this reason, the TOP assay can be an extremely useful tool for PFAS forensics analyses to capture precursor mass and close mass balances. Finally, we tested mineralization of AmPr-FHxSA using sulfate radicals produced by heat-activated persulfate oxidation (HAPO).



Schematic of the difference between biological and chemical transformation of AmPr-FHxSA.

RESULTS

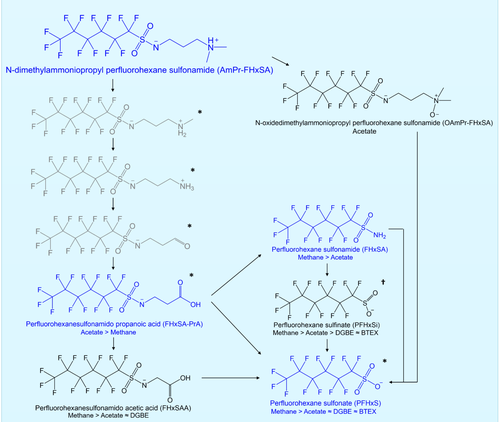


From left to right, AmPr-FHxSA depletion and FHxSA and PFHxS production (nM) over 70 days under aerobic conditions with four carbon sources: a-c) methane (CH_4), d-f) acetate, g-i) DGBE, and j-l) BTEX. Error bars represent standard deviation of triplicate reactors. Green circles represent the live cultures, while gray squares represent autoclaved controls.

We found FHxSA production to be approximately 33 and 28% in the methane and acetate cultures, respectively, after 70 days compared to Liu et al. (2021) who reported approximately 7.5% perfluorooctane sulfonamide (FOSA) production after 90 days. Perfluorohexane sulfonic acid (PFHxS) production was 5.4, 2, 1.9, and 1% for methane, acetate, DGBE, and BTEX cultures over 70 days compared to 2.6% perfluorooctane sulfonic acid (PFOS) production after 90 days. Although 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 those its eight-carbon analog FOSA and slightly faster or similar rates, depending on the community, of PFHxS production compared to those of PFOS.

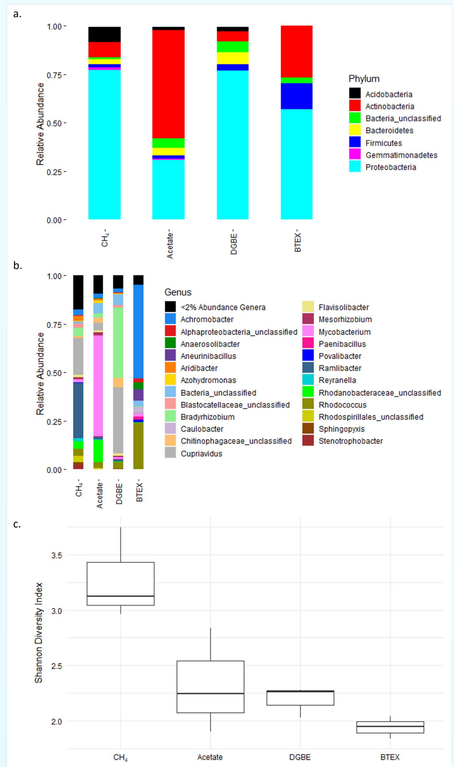
We also examined the ratios of branched versus total (linear + branched) peak areas of AmPr-FHxSA and PFHxS on day 70, and linear AmPr-FHxSA precursors appeared to be more readily transformed into PFHxS.

Based on HRMS results and targeted LC-MS/MS products, a biotransformation pathway for AmPr-FHxSA is shown below.



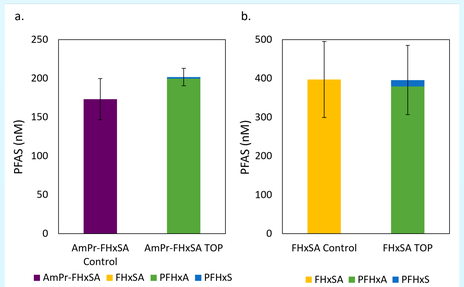
Proposed transformation pathway of AmPr-FHxSA. The faded structures in gray were not measured in this study but proposed in literature (Liu et al 2021) for the eight-carbon analogues and/or on enviPath. * denotes products proposed via enviPath. † Denotes PFHxSi had low peak areas. The blue structures were measured with larger peak areas (suspect screening) or concentration (targeted analysis). AmPr-FHxSA, FHxSA, and PFHxS were detected in LC-MS/MS and HRMS; remaining were detected using HRMS suspect screening.

RESULTS

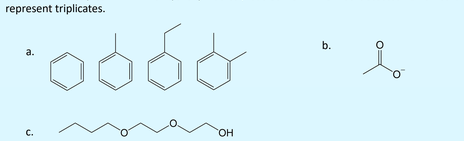


The a) phyla, b) genera, and c) Shannon Diversity Index from each of the four carbon source conditions. DNA was extracted from day 70; results are averaged between triplicate live samples.

While biotransformation of AmPr-FHxSA can lead to the production of PFHxS, applying chemical oxidation such as sulfate radical oxidation as a remediation strategy could prevent the biotic production of PFSAs from AFFF-impacted source zones. Performing the TOP assay on AmPr-FHxSA and FHxSA, most of the mass was converted to PFHxA after 12 hours. Unexpectedly, a small amount of PFHxS was produced, forming ~1% of the product in AmPr-FHxSA TOP assay and 4.3% in the FHxSA TOP assay.

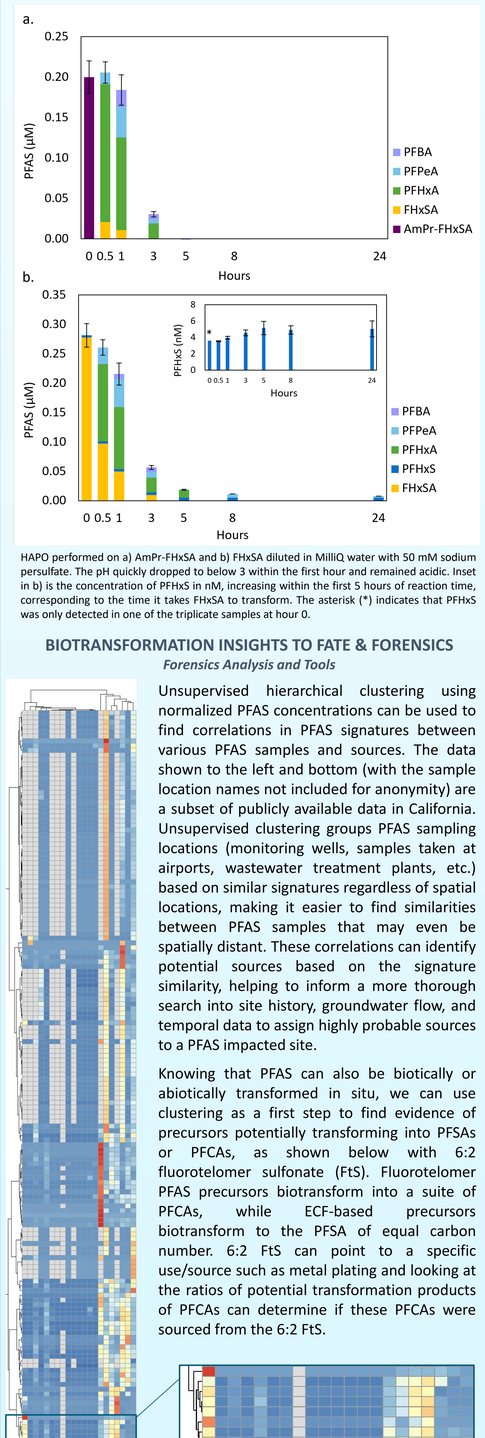


TOP assay results from reacting a) AmPr-FHxSA and b) FHxSA in Milli-Q water. Both precursors converted mostly to PFHxA via hydroxyl radical, but some PFHxS was detected. Error bars represent triplicates.



Carbon sources used in microcosm experiments. a) BTEX compounds in order from left to right benzene, toluene, ethylbenzene, and ortho xylene; b) acetate; and c) DGBE. Methane is also one of the carbon sources used, the structure simply CH_4.

RESULTS



HAPO performed 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.

BIOTRANSFORMATION INSIGHTS TO FATE & FORENSICS

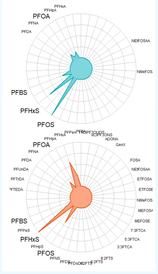
Forensics Analysis and Tools

Unsupervised hierarchical clustering using normalized PFAS concentrations can be used to find correlations in PFAS signatures between various PFAS samples and sources. The data shown to the left and bottom (with the sample location names not included for anonymity) are a subset of publicly available data in California. Unsupervised clustering groups PFAS sampling locations (monitoring wells, samples taken at airports, wastewater treatment plants, etc.) based on similar signatures regardless of spatial locations, making it easier to find similarities between PFAS samples that may even be spatially distant. These correlations can identify potential sources based on the signature similarity, helping to inform a more thorough search into site history, groundwater flow, and temporal data to assign highly probable sources to a PFAS impacted site.

Knowing that PFAS can also be biotically or abiotically transformed in situ, we can use clustering as a first step to find evidence of precursors potentially transforming into PFSAs or PFCAs, as shown below with 6:2 fluorotelomer sulfonate (FtS). Fluorotelomer PFAS precursors biotransform into a suite of PFCAs, while ECF-based precursors biotransform to the PFSA of equal carbon number. 6:2 FtS can point to a specific use/source such as metal plating and looking at the ratios of potential transformation products of PFCAs can determine if these PFCAs were sourced from the 6:2 FtS.

BIOTRANSFORMATION INSIGHTS TO FATE & FORENSICS

Similarly, compounds like FHxSA and FOSA can point to ECF-based precursors transforming to PFHxS and PFOS, respectively, with ratios of precursor to products over time informing the approximate quantity of products like PFHxS biotically produced from a precursor. Understanding these relationships and measuring more precursors like FOSA and FHxSA will provide insights into if increasing concentrations of PFHxS and PFOS in a sample is due to transport or due to the biotransformation of ECF-based precursors.



Data Gaps and Next Steps

PFAS Data Density → Unfortunately, not all states like California have as dense of a PFAS database. Still, forensics can help with source attribution by comparing signatures of well characterized sources to your unknown PFAS sample.

FHxSA and FOSA Analysis → These are key biotransformation products for ECF-based precursors that can point to specific AFFF formulations for source attribution and are not currently included in EPA approved analytical methods 533, 537, or 537.1.

Diverse Precursor Data → Any additional precursor data can help with the granularity of your PFAS dataset; if possible, conducting the TOP assay on a subset of your samples can improve your understanding of your total PFAS and PFAS precursor classes.

Temporal Data → Precursor-to-PFSA ratio over time could point to biotransformation of ECF-based precursors. Precursor-to-PFCA ratio over time could point to biotransformation of fluorotelomer precursors.

→ The Fifth Unregulated Contaminant Monitoring Rule (UCMR5) will help fill this data gap for public water systems with the sampling of 29 PFAS 2-4 times over 12 months.

LIST OF ACRONYMS

6:2 FtS	6:2 fluorotelomer sulfonate	ng/L	nanograms per liter
AFFF	aqueous film forming foam	nM	nanomolar
AmPr-FHxSA	N-dimethyl ammonio propyl perfluorohexane sulfonamide	PFAS	per- and polyfluoroalkyl substances
		PFBA	perfluorobutanoic acid
BTEX	benzene, toluene, ethylbenzene, xylene	PFCA	perfluoroalkyl carboxylic acid
CH_4	methane	PFHxA	perfluorohexanoic acid
DGBE	diethyl glycol monobutyl ether	PFHxS	perfluorohexane sulfonic acid
ECF	electrochemical fluorination	PFPeA	perfluoropentanoic acid
FHxSA	perfluorohexane sulfonamide	PFOS	perfluorooctane sulfonic acid
HAPO	heat-activated persulfate oxidation	PFSA	perfluoroalkyl sulfonic acid
HRMS	high resolution mass spectrometry	TOP	total oxidizable precursor assay
mL	milliliters	µL	microliters

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