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Biotransformation of AFFF Component 6:2 Fluorotelomer Thioether Amido Sulfonate Generates 6:2 Fluorotelomer Thioether Carboxylate under Sulfate-Reducing Conditions

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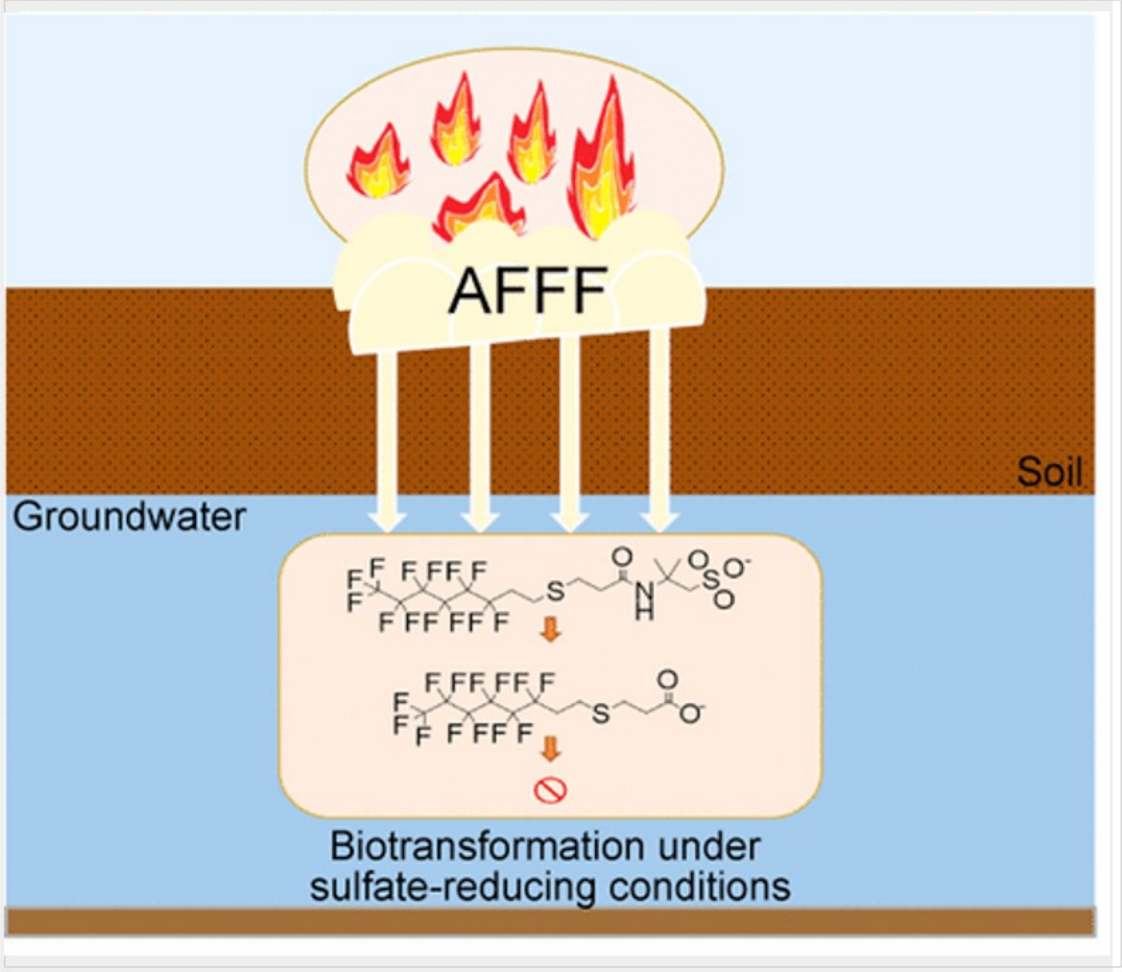
Abstract



The fate of per- and polyfluoroalkyl substances (PFASs) in aqueous film-forming foams (AFFFs) under anaerobic conditions has not been well characterized, leaving major gaps in our understanding of PFAS fate and transformation at contaminated sites. In this study, the biotransformation of 6:2 fluorotelomer thioether amido sulfonate (6:2 FtTAoS), a component of several AFFF formulations, was investigated under sulfate-reducing conditions in microcosms inoculated with either pristine or AFFF-impacted solids. To identify the transformation products, we used high-resolution mass spectrometry and employed suspect-screening and nontargeted compound identification methods. These analyses demonstrated that 6:2 FtTAoS was transformed primarily to a stable polyfluoroalkyl compound, 6:2 fluorotelomer thioether propionate (6:2 FtTP). It did not undergo further reactions to produce the perfluoroalkyl carboxylates and fluorotelomer sulfonates and carboxylates that were observed during aerobic transformations. Here, the 6:2 FtTP was recalcitrant to biotransformation, indicating the stability of the thioether group under sulfate-reducing conditions. The total oxidizable precursor (TOP) assay was used to assess the presence of other PFASs. Although nearly all of the PFAS mass initially present was recovered from the pristine microcosms, only 67% of the initial

PFAS mass was recovered from the contaminated microcosms, suggesting the formation of volatile biotransformation products or those that could not be detected by the TOP assay.

Introduction

Aqueous film-forming foams (AFFFs) have been used since the 1960s to extinguish hydrocarbon and solvent-based fires at airports, refineries, and military sites.(1,2) As a result, per- and polyfluoroalkyl substances (PFASs) in AFFFs have been released into the environment during emergency responses and firefighting training exercises, where they pose potential threats to drinking water supplies.(3−15) Fluorotelomer thioether amido sulfonates (FtTAoSs), also known by the trade name Lodyne, make up a group of PFAS homologues present in AFFF formulations made by at least three manufacturers. They have been detected in groundwater and soil at firefighter training sites.(7,11,14) Although aerobic microcosm studies demonstrated that FtTAoSs are transformed into perfluoroalkyl carboxylates (PFCAs) and fluorotelomer sulfonates (FtSs) and carboxylates (FtCAs), the anaerobic biotransformation of FtTAoSs is still unknown.(16,17)

Previous studies have demonstrated that anaerobic microbial activities play important roles in the transformation of some classes of PFASs, but not all of them.(18−21) In cultures inoculated with anaerobic digester sludge, *n*:2 fluorotelomer alcohols (FtOHs) (*n* = 6 or 8) were mainly transformed into *n*:2 and (*n*–1):3 FtCAs and *n*:2 fluorotelomer unsaturated carboxylates (FtUCA) (*n* = 6 or 8).(21) Similar biotransformations have also been observed in laboratory anaerobic bioreactors treating municipal solid waste containing disubstituted polyfluorinated phosphate esters (DiPAPs).(18) Additionally, methanogenic cultures were demonstrated to convert *N*-methyl perfluorobutane sulfonamido ethanol (MeFBSE) and *N*-ethyl perfluorooctane sulfonamido ethanol (EtFOSE) into their respective perfluoroalkyl sulfonamide acetates and perfluoroalkyl sulfinates.(19) In contrast, perfluoroalkyl sulfonamide acetates were recalcitrant to biotransformation in methanogenic culture, and FtSs were persistent in both methanogenic or sulfate-reducing cultures.(18,20) The lack of information about the anaerobic biotransformation of fluorotelomer thioethers highlights the importance of further studies of PFAS biotransformation at AFFF-impacted groundwater sites.

This study was conducted to assess the biotransformation potential and products of 6:2 FtTAoS under sulfate-reducing conditions, a prevalent redox condition in groundwater environments.(22)Microcosms with both pristine and AFFF-impacted solids were employed along with high-resolution mass spectrometry (MS) to assess biotransformation.

Materials and Methods

Chemicals

Descriptions of the chemicals are provided in the Supporting Information.

Construction of Sulfate-Reducing Microcosms

The solid samples were sieved through sterile 1 mm mesh to remove coarse grains and plant debris and stored in an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) with a nitrogen and hydrogen (2–3%) atmosphere (<2 ppm O_2). Approximately four grams (dry weight) of either pristine or AFFF-contaminated solids were used as microbial inocula. Anaerobic live microcosms, medium-only controls (no solids), and autoclaved controls were constructed in triplicate with 50 mL of reduced defined mineral salts medium and an amendment of AFFF manufactured by Ansul in 2008 (Supplemental Materials and Methods, Table S1).(14,16,23) All live and control microcosms were prepared with a N_2/CO_2 headspace [90/10 (v/v)] in 160 mL glass serum bottles that were capped with butyl rubber septa and sealed with aluminum crimp caps. Resazurin was added as a redox indicator, and cysteine (0.6 mM) and sulfide (0.4 mM) were added as reducing reagents.(23) At the beginning of incubations, 50 mM sodium sulfate was amended as an electron acceptor along with 50 μL of neat AFFF and 1.5 mM diethylene glycol butyl ether (DGBE, the primary organic solvent in the AFFF formulation) as the electron donor and carbon source for the microcosms. The initial 6:2 FtTAoS concentration in the microcosms was approximately 20 μM. A set of live and autoclaved microcosms were also constructed with an amendment of Zonyl FSA, a commercial product known to mainly contain 6:2 and 8:2 fluorotelomer thioether propionate (FtTP), to investigate the biotransformation potential of 6:2 FtTP. Additional information about the construction of the microcosms is provided in the Supporting Information.

Slurry samples withdrawn from microcosms were mixed with methanol [1/1 (v/v)] and stored at −20 °C prior to PFAS quantification.(16) For the total oxidizable precursor (TOP) assay, 100 μL of mixed slurry/methanol samples were dried under a gentle N_2 gas flush before the addition of TOP assay reagents to prevent methanol interference (details in the Supplemental Materials and Methods).(16) To identify biotransformation products, pooled slurries from the triplicate microcosms were purified by solid phase extraction (SPE) as described previously.(16)

Quantitative Liquid Chromatography–Tandem MS (LC–MS/MS) Analysis

Quantification of PFASs, including 6:2 FtTAoS, 6:2 FtTP, 6:2FtS, and PFCAs (C4–C12) (ion structures shown in Table S2), was achieved via LC–MS/MS (6410, Agilent Technologies, Santa Clara, CA) as reported previously.(11,24) The newly identified transformation products were analyzed semiquantitatively using either Zonyl FSA or 6:2 FtTAoS as the standard reference (Table S3). Details of these semiquantitative methods are provided in the Supporting Information(Supplemental Materials and Methods). The TOP assay was used to quantify the total amount of PFASs in the microcosms as described previously.(24)

Identification of Biotransformation Products

Biotransformation products were identified using the MS analysis workflow shown in Figure S1. High-resolution electrospray mass spectrometry (HR-ESI-MS) and high-resolution nanoESI MS/MS (HR-nanoESI-MS/MS) analyses were performed in negative ion mode at the QB3/Chemistry Mass Spectrometry Facility at the University of California, Berkeley. Accurate masses from HR-ESI-MS analyses were analyzed using either suspect-screening or nontargeted analysis with the criterion that the mass error relative to the exact mass of a potential transformation product be no more than 5 ppm (Figure S1, Supplemental Materials and Methods). The proposed structures of potential biotransformation products were further analyzed using HR-nano-ESI-MS/MS. Fragment interpretation was performed using in silico fragment analysis software, ACD/MS fragmenter 2015 (Advanced Chemical Development, Toronto, ON). The proposed structures were annotated when mass errors were less than 5 mDa or 15 ppm between the observed accurate mass and the theoretical exact mass of proposed fragments.(9) The certainty of the structure identification was evaluated using a confidence level system described by Schymanski et al.(25) In brief, the highest confidence, level 1 (confirmed structure identification), was achieved when a compound standard or a standard reference containing the target compound was available to be analyzed together with the proposed product after in silico fragment prediction. Level 2 (probable structure identification) was achieved when previously reported MS/MS spectra and fragmentations were available for a compound of interest or when the structure could be determined unambiguously via fragmentation analysis. When fragmentations were informative for possible structure(s) but unable to determine one exact structure, level 3 (tentative identification) was established. Level 4 identification was achieved when a molecular formula could be unambiguously assigned using HR-ESI-MS data, but MS/MS fragmentation data were not available.(25) The names of transformation products and their previous names in the literature are listed in Table S2.

Results and Discussion

Biotransformation of 6:2 FtTAoS in Microcosms

Throughout the experiments, strictly anaerobic conditions were maintained, as indicated by the colorless appearance of the resazurin indicator. Active sulfate-reducing activities were confirmed in both sets of microcosms, and details are provided in the Supporting Information (Figure S2, Supplemental Materials and Methods).

The biotransformation analysis of these microcosms focused primarily on 6:2 FtTAoS, the most abundant PFAS in the amended AFFF formulation.(16) The onset of 6:2 FtTAoS biotransformation was observed after 50 days in microcosms with pristine or AFFF-contaminated solids. By day 270, approximately 75% of the 6:2 FtTAoS had been transformed in all live microcosms (Figure 1). The biotransformation rates of 6:2 FtTAoS in the pristine and contaminated microcosms were approximately 41 and 47

nM/day, respectively, which were significantly slower than the aerobic biotransformation rate previously reported.(16) Transformation products observed in aerobic microcosms, including PFCAs and 6:2 FtS, were not detected in either set of anaerobic microcosms.(16) These results indicate that the anaerobic biotransformation of 6:2 FtTAoS under sulfate-reducing conditions proceeded through a different transformation pathway.

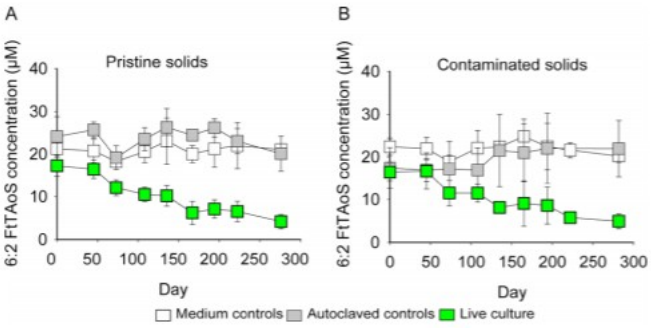


Figure 1. Microbial transformation of 6:2 FtTAoS in sulfate-reducing microcosms inoculated with (A) pristine or (B) contaminated solids. Error bars show the standard deviation of averages from triplicate microcosms.

Identification of Biotransformation Products

Nine potential biotransformation products were identified in the live microcosms in addition to the parent compound 6:2 FtTAoS (Table 1). Two transformation products were identified at confidence levels 1 and 2; four products were identified at confidence level 3, and one product was identified at confidence level 4 (Table 1). Two additional ions that could potentially be MS artifacts were also identified.

Table 1. Potential Biotransformation Products Identified from Automated Suspect-Screening and Manual Nontargeted Analysis of HR-ESI-MS Accurate Mass Measurements

			mass (*m*/*z*)			
	abbreviated name	molecular ion formula	exact	accurate[a]	mass error (ppm)	level of confidence[e]
A	6:2 FtTAoS	$C_{15}H_{17}F_{13}NO_4S_2^-$	586.0396	586.0390[b]	0.68	1
B	6:2 FtTP	$C_{11}H_8F_{13}O_2S^-$	451.0043	451.0040	0.66	1

		mass (*m*/*z*)			
abbreviated name	molecular ion formula	exact	accurate[a]	mass error (ppm)	level of confidence[e]
CNA[f]	$C_8H_4F_{13}S^-$	378.9828	378.9829	1.06	NA[f]
DNA[f]	$C_{22}H_{17}F_{26}O_4S_2^-$	903.0159	903.0153[c]	0.55	NA[f]
E 6:2 FtTPlA	$C_{14}H_{13}F_{13}NO_3S^-$	522.0414	522.0412	0.38	3
F 6:2 FtTPlAA	$C_{17}H_{18}F_{13}N_2O_4S^-$	593.0785	593.0780	0.84	3
G 6:2 FtTPoP	$C_{14}H_{12}F_{13}O_4S^-$	523.0252	523.0252	0.38	3
HNA[f]	$C_{15}H_{17}F_{13}NO_6S_2^-$	618.0295	618.0290[d]	0.81	4
I 6:2 FtSiAoS	$C_{15}H_{17}F_{13}NO_5S_2^-$	602.0346	602.0340	1.00	2
J 6:2 FtSiP	$C_{11}H_8F_{13}O_3S^-$	466.9992	466.9990	0.42	3

[a] Accurate mass measured by HR-ESI-MS.

[b] Parent compound.

[c] A dimer compound of *m*/*z* 451, no MS/MS data.

[d] Precursor ion abundance too low for MS/MS analysis.

[e] The level of confidence of each proposed transformation product was determined using a scheme described by Schymanski et al.(25)

[f] Not applicable.

The transformation product that produced peaks of the highest intensity in the MS analyses was proposed to be 6:2 FtTP (*m*/*z* 451) by suspect-screening analysis. Fragmentation analysis using HR-nanoESI-MS/MS indicated the presence of characteristic moieties, including 6:2 thiolate (*m*/*z*379) and related ions with serial losses of −HF groups (20 Da) (Figure S3A). Although the mass error of the fragment from the functional head was greater than the 15 ppm criterion, the identification of the product as 6:2 FtTP was confirmed by comparison with the standard reference, Zonyl FSA. This product accumulated to similar concentrations in both sets of live microcosms at the end of the incubation but was absent in the medium and autoclaved controls (Figure S4). Although this compound was previously reported as a component in AFFF, our analysis represents the first reported detection of 6:2 FtTP as a major biotransformation product.(10)

Two ions related to 6:2 FtTP were observed in our MS analysis, at *m*/*z* 379 and 903 (Table 1 and Figure S5A–D). These ions showed accumulation in live microcosms along with 6:2 FtTP. However, they were also detected in the Zonyl FSA reference material, rendering them unlikely to be biotransformation products, but rather either artifacts of mass spectrometry or abiotic transformation products of 6:2 FtTP (Figure S5E,F).

Our MS analysis also led to the identification of three ions occurring at low abundance, namely, those at *m*/*z* 522, 523, and 593 (Table 1). Suspect screening suggested that the ion at *m*/*z* 522 was 6:2 fluorotelomer thioether propanoyl alaninate (6:2 FtTPlA). The nontargeted analysis implied that the ions at *m*/*z* 523 and 593 were fluorotelomer thioether propanoyl oxy propanoate (6:2 FtTPoP) and 6:2 fluorotelomer thioether propanoylalanylalaninate (6:2 FtTPlAA), respectively. Because the structure of 6:2 FtTP was confirmed by the standard reference, Zonyl FSA, the MS/MS spectrum of 6:2 FtTP was used as a reference to interpret the structures of the three ions (*m*/*z* 522, 523, and 593). The characteristic fragment at *m*/*z* 379 was observed in all three ions with an *m*/*z* value almost identical to that of the respective reference fragment in 6:2 FtTP (Figure S3B–D and Table S5), confirming the presence of a fluorotelomer thioether backbone structure (Figure S3B–D). However, the mass errors of the other mass fragments obtained at *m*/*z* 522 and 523 were greater than the 15 ppm criterion, hindering the structural determination of the functional head due to an inability to differentiate multiple potential isomers associated with the same molecular formula. Therefore, the identification of ions at *m*/*z* 522 and 523 as 6:2 FtTPlA and 6:2 FtTPoP, respectively, was only tentative, resulting in the confidence level 3 identification for both biotransformation products (Table 1). Interestingly, the observation of a CO_2 loss (44 Da) in the mass fragmentation spectra of both products confirms the presence of a carboxylate group in these two compounds (Figure S3B,C). Similarly, our

analysis indicates a confidence level 3 for *m*/*z*593 of which the functional head is likely a propionylalanylalaninate, which could produce acryloylalanylalaninate (*m*/*z* 213) as a result of heterolytic cleavage between the thioether and its adjacent carbon during MS/MS fragmentation (Figure S3D). The production of fragments at *m*/*z*196 and 124 was likely from acryloylalanylalaninate (*m*/*z* 213) as a result of a neutral loss of water (18 Da) and a breakage of an amide bond in the dipeptide moiety (89 Da). Although an increase in the level of 6:2 FtTPlAA (*m*/*z* 593) at the end of the incubation was observed in both sets of live microcosms, increases of *m*/*z* 522 and 523 were detected only in pristine and contaminated microcosms, respectively (Figure S6).

Suspect-screening analysis also suggested the presence of potential transformation products containing sulfinyl (*m*/*z* 602) or sulfonyl (*m*/*z* 618) moieties (Table 1). The HR-nanoESI-MS/MS spectrum of the ion at *m*/*z* 602 was similar to previously reported MS/MS spectra for 6:2 fluorotelomer sulfinyl amido sulfonate (6:2 FtSiAoS), indicating the probable structure of 6:2 FtSiAoS at confidence level 2 (Figure S3E).(16,17) Decreases in the 6:2 FtSiAoS concentration were observed only in the live microcosms between day 0 and the end of the anaerobic experiments. This indicates that 6:2 FtSiAoS, a minor component in the AFFF formulation, was biotransformed under anaerobic conditions (Figure S7). This is different from the previous observation that 6:2 FtSiAoS can be generated from both biotic and abiotic reactions in the aerobic microcosms.(16) An ion at *m*/*z* 618 was detected by HR-ESI-MS but was impossible to identify definitively because of its weak response. This molecule was also present in the AFFF formulation, and the extent of observed biotransformation was insignificant (Figure S7).

Suspect screening indicated that the ion at *m*/*z* 467 was either 6:2 fluorotelomer sulfinyl propanoate (FtSiP) or 2-OH, 6:2 FtTP. Fragmentation analysis of the HR-nanoESI-MS/MS spectrum showed multiple fragments that can be generated from either proposed product. However, two high-intensity fragments, *m*/*z* 334 and 314, were likely produced from 6:2 FtSiP, containing a sulfinyl instead of a thiol moiety, which supports the presence of 6:2 FtSiP with a confidence level of 3 (Figure S3F). The concentration of 6:2 FtSiP increased in the live microcosms during the incubation period (Figure S7).

Semiquantitative analysis indicated that 6:2 FtTP generated at the end of the experiment accounted for approximately 36 and 30% (by mole) of the initial 6:2 FtTAoS added to the pristine and contaminated microcosms, respectively. Other identified fluorotelomer compounds at the end of the experiments accounted for <0.1% of the added 6:2 FtTAoS (Figures S6 and S7).

Biotransformation of 6:2 FtTP and Proposed Anaerobic Biotransformation Pathway of 6:2 FtTAoS

To elucidate the biotransformation potential of 6:2 FtTP, we constructed live and autoclaved microcosms with Zonyl FSA (approximately 8 μM 6:2 FtTP)

and observed minor changes in 6:2 FtTP concentrations over a 150 day incubation (Figure S8). The absence of 6:2 FtSiP, 6:2 FtTPlAA, 6:2 FtTPlA, and 6:2 FtTPoP formation in 6:2 FtTP-amended microcosms indicates that they were formed through a transformation pathway that did not include 6:2 FtTP as an intermediate.

The possible transformation reactions driving the production of 6:2 FtTPlAA (*m*/*z* 593) from 6:2 FtTAoS could have occurred via an alanine conjugation of an alanine-containing intermediate, possibly 6:2 FtTPlA (*m*/*z* 522), that was produced through anaerobic desulfonation and decarboxylation.(21,26) Conjugation of amino acids is a common cellular pathway in the metabolism of xenobiotic carboxylates and was previously reported during the biotransformation of 6:2 FtOH in a fungal culture and in mammalian liver cells.(27,28) The formation of 6:2 FtTPoP (*m*/*z* 523) was unexpected. Because of its low concentration (0.8 and 10.8 nM in pristine and contaminated microcosms, respectively) (Figure S6), it was unknown whether it was produced from 6:2 FtTAoS or from an unidentified minor component in the AFFF formulation. It is unlikely that 6:2 FtSiP was a transformation product of 6:2 FtTAoS. Rather, it may have been produced from 6:2 FtSiAoS through an amide hydrolysis reaction similar to the pathway that generated 6:2 FtTP from 6:2 FtTAoS (Figure S9).

On the basis of these analyses, an anaerobic biotransformation pathway for 6:2 FtTAoS was proposed (Figure 2). Under the tested conditions, 6:2 FtTAoS was predominantly transformed into 6:2 FtTP (*m*/*z* 451), likely catalyzed by a microbial amidase. Biotransformation of 6:2 FtTAoS also produced smaller amounts of 6:2 FtTPlA (*m*/*z* 522), 6:2 FtTPlAA (*m*/*z* 593), and possibly 6:2 FtTPoP (*m*/*z* 523).

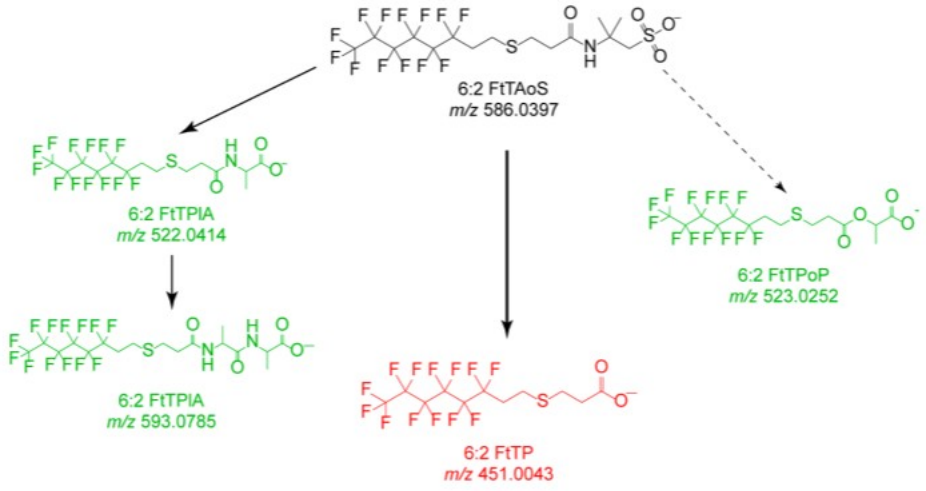


Figure 2. Proposed biotransformation pathway of 6:2 FtTAoS under strictly anaerobic conditions. Level of confidence in the structures identified using high-resolution mass spectrometry: red, confirmed structures (level 1); green, tentative candidates (level 3).(25) The dashed arrow indicates the less certain pathway.

Mass Balance in the Biotransformation of 6:2 FtTAoS

We used the ratio of PFCAs recovered from the TOP assay of live microcosms to their corresponding autoclaved controls at each time point to indicate the mass changes during biotransformation. Approximately 121 ± 15, 115 ± 7, and 96 ± 8% of PFASs were recovered on days 0, 194, and 276, respectively, from the live microcosms inoculated with pristine solids, and 114 ± 10, 98 ± 13, and 67 ± 6% of PFASs were recovered on days 0, 135, and 282, respectively, from live microcosms with contaminated solids (Figure S10) (the errors represent one standard deviation of biological triplicates). Statistical analyses indicated that the decrease in recovered PFAS mass between day 0 and the end of the experiment was insignificant in pristine microcosms but was significant in contaminated microcosms. This suggests that a PFAS mass balance was achieved in the pristine solid microcosms, with 6:2 FtTAoS and 6:2 FtTP accounting for ~60% of initial amended 6:2 FtTAoS at the end of the experiment, but was not achieved in the contaminated microcosms. The complete PFAS mass balance in the pristine microcosms indicates that the remaining transformation products (other than 6:2 FtTP) were converted into PFCAs by the TOP assay. The incomplete mass balance observed in the contaminated solid microcosms could be due to the generation of transformation products that were volatile or were not converted into the PFCAs measured by the TOP assay. The TOP assays were performed on slurry samples, ruling out the potential loss of mass by irreversible binding to solids.(29)

Environmental Implications

This study demonstrates that PFASs present in common AFFF formulations biotransformed much more slowly under sulfate-reducing conditions than under aerobic conditions. The PFCAs, which are thought to be the terminal products of fluorotelomer PFASs under aerobic conditions, were not formed in the anaerobic microcosms tested in this study. Anaerobic microbes transformed 6:2 FtTAoS into an apparent persistent product, 6:2 FtTP, and possibly other fluorotelomer thioethers. These results demonstrate the inert properties of the thioether group under sulfate-reducing conditions.(16,30) Interestingly, 6:2 FtTP was not observed during aerobic biotransformation of 6:2 FtTAoS, potentially because the oxidation of the thioether group was significantly faster than the hydrolysis reaction of the amido group.(16) Thus, if 6:2 FtTP is exposed to aerobic microbial activities, it would likely biotransform into 6:2 FtS and PFCAs similar to the parent compound, 6:2 FtTAoS, perhaps via sulfinyl and sulfonyl intermediates.(16) The detection of sulfonyl derivatives of FtTP in AFFF-impacted groundwater suggests a potential for similar oxidation of FtTPs under aerobic conditions.(9)

Despite the different AFFF-exposure histories of the two sets of solids used to inoculate the microcosms, the rate of 6:2 FtTP generation in the microcosms, which was produced through amide hydrolysis, was similar. This similarity suggests amide hydrolysis reactions acting on PFAS may be present in dissimilar microbial communities. Indeed, amide hydrolysis was also found to be the major aerobic reaction converting the perfluorooctane-amido

quaternary ammonium salt in 3 M AFFF to PFOA in soil microcosms.(31) Because amidases are a group of hydrolytic enzymes existing in all domains of organisms, understanding the ubiquity of amide hydrolysis would help better predict the fate of amido-bearing PFASs in the environment.(10,14,32)

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