Microbial biotransformation of aqueous film-forming foam derived polyfluoroalkyl substances

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Highlights;

- Microbial biotransformation of AFFF-derived PFASs was reviewed.
- ECF-derived and fluorotelomer-derived PFASs share head-group transformation pathways.
- Dealkylation at N- and S-head groups are dominant biotransformation mechanisms.
- FASAs are semi-recalcitrant transformation products, and not all microcosm transformation products are found in the field.
- Further research is needed for transformation mechanisms on secondary amide and sulfonamides adjacent to fluorinated tails.

ABSTRACT (181/300)

Per- and polyfluoroalkyl substances (PFASs) used in aqueous film-forming foam (AFFF) comprise some perfluoroalkyl substances but a larger variety of polyfluoroalkyl substances. Despite their abundance in AFFF, information is lacking on the potential transformation of these polyfluoroalkyl substances. Due to the biological and chemical stability of the repeating perfluoroalkyl -$(CF_2)_n$- moiety common to all known AFFF-derived PFASs, it is not immediately evident whether the microbial biotransformation mechanisms observed for other organic contaminants also govern the microbial biotransformation of polyfluoroalkyl substances. This manuscript aims to: 1) review the literature on the aerobic or anaerobic microbial biotransformation of AFFF-derived polyfluoroalkyl substances in environmental media; 2) compile and summarize proposed microbial biotransformation pathways for major classes of polyfluoroalkyl substances; 3) identify the dominant biotransformation intermediates and terminal biotransformation products; and 4) discuss these findings in the context of environmental monitoring and source allocation. This analysis revealed that much more is currently known about aerobic microbial biotransformation of polyfluoroalkyl substances, as compared to anaerobic biotransformation. Further, there are some similarities in microbial biotransformations of fluorotelomer and electrochemical fluorination-derived polyfluoroalkyl substances, but that differences may be largely due to head group composition. Dealkylation, oxidation, and hydrolytic reactions appear to be particularly important for microbial biotransformation of AFFF-derived polyfluoroalkyl substances, and these biotransformations may lead to formation of some semi-stable intermediates. Finally, this review discusses key knowledge gaps and opportunities for further research.

1. Historical usage of AFFF

Per- and polyfluoroalkyl substances (PFASs) are a large group of synthetic organic compounds that are highly stable and persistent in the environment. The unique properties of PFASs stem from the extent of their highly fluorinated chains and various non-fluorinated functional groups. PFASs have been used in industrial and consumer applications since the 1940s (ITRC, 2020), most commonly when oil/water repellency and low surface tension are needed, including firefighting foams. Aqueous film-forming foams (AFFFs) are complex proprietary formulations that contain percent levels of PFASs as well as solvents and hydrocarbon surfactants which, when combined, afford AFFF the functionality required for its purpose (Korzeniowski et al., 2018). AFFF has been used as a very effective agent for fighting Class B fires, but its extensive use in training and accident responses has led to significant contamination of water resources (Barzen-Hanson et al., 2017b; De Solla et al., 2012; Hatton et al., 2018). Broadly speaking, AFFF can be categorized as containing electrochemical fluorination (ECF)-derived PFASs (such as those developed and sold by 3M) and fluorotelomer (FT)-based PFASs (such as those developed and sold by Ansul, Chemguard, Angus, National Foam, Buckeye, etc.). With the exception of 3M's Lightwater, which historically has contained significant quantities of PFOS as well as polyfluoroalkyl substances (3M, 1997; Baduel et al., 2017; Fitzgerald et al., 2019), most AFFF formulations examined (to date) appear to primarily contain polyfluoroalkyl substances. The reasons for the complexity of PFAS composition include synthesis impurities (Arsenault et al., 2008; Lehmler, 2005; Norman and C.Regina, 1993) and their use as mixtures, because products were often formulated by mixing one or more families of PFASs (Bertocchio et al., 1991; Boardman, 2004; Martin, 2012). As the polyfluoroalkyl substances can transform to the more persistent perfluoroalkyl acids (PFAAs), elucidating the PFAS compositional changes upon release of AFFF to the environment has important environmental and public health implications. For the scope of this review, here we define AFFF-derived PFASs as any PFASs observed in AFFF formulations or at AFFF-impacted sites and their transformation products. Some of these PFASs may also be present in the environment as a result of their use in other (i.e., non-AFFF) products. A list of AFFF-derived PFASs developed from the existing literature is provided in Table S1.

Polyfluorinated compounds present in AFFF can result in the formation of specific PFAAs, but also semi-stable polyfluorinated intermediates (Chen et al., 2020; Liu et al., 2021; Mejia-Avendaño et al., 2016). As documented to date, the PFAS composition at AFFF-impacted sites tends to be dominated by the perfluoroalkyl sulfonates (PFSAs, namely PFOS)(Bräunig et al., 2017; Nickerson et al., 2021), though a few recent studies have reported precursors of perfluoroalkyl carboxylates (PFCAs) (D'Agostino and Mabury, 2014; Mejia-Avendaño et al., 2017) being dominant, likely due to the use of FT-based AFFF.

However, FT-based AFFFs have been in use for some time (including C6-based AFFFs, Korzeniowski et al., 2018, Mejia-Avendaño et al., 2017), and fluorotelomer compounds, primarily those with X:2 polyfluoroalkyl structures, have been widely detected at AFFF-impacted sites (Martin et al., 2019; Mejia-Avendaño et al., 2017; Nickerson et al., 2020). While fluorotelomer compounds with X:3 and X:1:2 (X = 5, 7, 9, 11 and 13) polyfluoroalkyl structures have been recently detected in AFFF-impacted sites, at present, these detections are limited (Dauchy et al., 2019 Chemosphere; Mejia-Avendaño et al., 2017 EST). More broadly, over the last decade, the use of the total oxidizable precursor (TOP) assay (Houtz and Sedlak, 2012) and total fluorine measurements (Trojanowicz et al., 2011), when coupled with degradation studies (Chen et al., 2020; Dinglasan et al., 2004; Lange, 2018), have suggested a potentially significant role of PFAA precursors, particularly at AFFF-impacted sites, serving as sources of PFAAs. Thus, the presence of PFAA precursors at AFFF-impacted sites could result in slow transformation and release of PFAAs to downgradient receptors (Adamson et al., 2020; De Solla et al., 2012; Munoz et al., 2017; Schultz et al., 2004).

Transformations of polyfluoroalkyl substances to PFAAs can proceed both chemically and biologically (Chen et al., 2020; D'Agostino and Mabury, 2017a; Liu et al., 2021). Unfortunately, existing microbial transformation pathway prediction models (i.e., Eawag-PPS, Envipath, etc) are primarily derived from knowledge of microbial biocatalytic reactions and biodegradation pathways for other organic (non-fluorinated) chemicals. Although such knowledge has been used by some investigators when analyzing PFAS transformations in the laboratory, the prediction and estimation of PFAS transformations may be different from other chemicals of environmental concern due to the stability of the carbon-fluorine structure, the strong electron-withdrawing effect in the hydrophobic perfluoroalkyl tail (Dimitrov et al., 2004), and the hydrophilic head-group chemistries. Predictive microbial biotransformation tools would be beneficial for AFFF-impacted sites, as nearly 100 classes of ECF-derived and/or FT–based compounds have been detected in neat AFFF and/or AFFF-impacted soil, sediment, groundwater or surface water (Barzen-Hanson et al., 2017b; D'Agostino and Mabury, 2014; Maizel et al., 2021; Mejia-Avendaño et al., 2017; Moe et al., 2012; Place and Field, 2012; Schultz et al., 2004). In particular, such tools could be particularly helpful when trying to ascertain the source of specific PFASs: although unique AFFF-derived PFASs are likely to present near AFFF source zones, due of transformation but also differences in transport, the PFAS mixture composition is likely significantly simplified with distance (and time) from the point of release (Charbonnet et al., 2021). Thus, PFAS source apportionment tools may need to account for these processes if an AFFF source is suspected.

Herein, we have conducted a detailed review of the microbial biotransformation pathways that have been elucidated for AFFF-derived polyfluoroalkyl substances. Specifically, this manuscript aims to: 1) review the literature on the aerobic or anaerobic microbial biotransformation of AFFF-derived polyfluoroalkyl substances in environmental media; 2) compile and summarize proposed microbial biotransformation pathways for major classes of polyfluoroalkyl substances; 3) identify the dominant biotransformation intermediates and terminal products; and 4) discuss these findings in the context of environmental monitoring and environmental source allocation. Finally, we also discuss critical knowledge gaps and opportunities for further research.

2. Microbial Biotransformations of AFFF-Derived Polyfluoroalkyl Substances

Studies on the biotransformation of polyfluoroalkyl substances have almost always observed alterations of the non-fluorinated head groups of the various substances, whereas for FT-based substances, partial degradation of perfluoroalkyl chains may also follow. To date, most studies on polyfluoroalkyl substance microbial biotransformation have focused either on the 6-carbon (e.g., 6:2 fluorotelomer compounds) or 8-carbon perfluoroalkyl chain length (e.g., PFOS precursors; Table 1). Several studies have shown that, in general, one may expect the microbial biotransformation pathways for otherwise identical C6 vs C8 chemicals to be the same (other than the length of the perfluoroalkyl component in the intermediates)(Harding-Marjanovic et al., 2015). Thus, for the purposes of this review, if a particular pathway has been observed for the “C8” member of a PFAS subclass, we assumed the “C6” pathway can also occur. However, the kinetics of those microbial biotransformations are likely dependent on the length of the perfluorocarbon chain due to enzyme specificity and changes in physical-chemical properties.

Though the kinetics of microbial biotransformation are critical to predicting the long-term fate of these substances, most studies have been conducted with mixed cultures or environmental media, which makes direct comparison of the limited data challenging. For this review, we are principally interested in the intermediates that can be formed and the pathways leading to the terminal PFAAs. Though more studies are now attempting to identify the potentially responsible microorganisms or enzymes for AFFF polyfluoroalkyl substances, there is mounting evidence of a complex interplay between environmental factors and the biotransformation (e.g. co-contaminant stimulation, nutrients, redox conditions) (Olivares et al., 2022; Shaw et al., 2019; Yang et al., 2022). Assessing the extent to which specific microbial strains, species, or consortia are responsible for microbial biotransformations of PFASs is beyond the scope of this review.

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148 **2.1. Microbial biotransformations of fluorotelomer precursors**

149 In general, degradation of AFFF-derived X:2 fluorotelomer surfactants has been well studied
150 (D'Agostino and Mabury, 2017a; Harding-Marjanovic et al., 2015; Liu and Mejia Avendaño, 2013; Weiner
151 et al., 2013; Yi et al., 2018). Degradation of X:2 fluorotelomer surfactants has been investigated for 6:2
152 FTSAPr-DiMeAn, 6:2 FTSAPr-B, and 6:2 FTTh-PrAd-DiMeEtS (figure 1, PFAS naming rule is in
153 Supplementary Information-2). These first two classes, X:2 FTSAPr-DiMeAn (also known as X:2 FTAA) and
154 X:2 FTSAPr-B (also known X:2 FTAB), have been detected in National Foam (Houtz et al., 2013; Place and
155 Field, 2012), Fireade (Place and Field, 2012), Hazard control tech (F-500) (D'Agostino and Mabury, 2017b)
156 and Angus (Tridol S, Niagara 1-3, Forexpan) (D'Agostino and Mabury, 2017b). X:2 FTSAPr-B is also known
157 by its trade name Capstone 1157 (Chemours/Dupont, earlier name Forafac 1157)(Moe et al., 2012) and
158 Chemguard FS-157 (Martin, 2012). Further, the X:2 FTTH-PrAd-DiMeEtS class was detected in Ansul
159 (D'Agostino and Mabury, 2017b; Place and Field, 2012), Chemguard (Place and Field, 2012), Hazard
160 control tech F-500 (D'Agostino and Mabury, 2017b), and Angus (Tridol S, Niagara 1-3, Forexpan)
161 (D'Agostino and Mabury, 2017b; Place and Field, 2012). These polyfluoroalkyl compounds identified in
162 AFFFs included varied perfluoroalkyl chain lengths (C4, C6, C8, or C10) and yet shared similar pathways
163 (Harding-Marjanovic et al., 2015); for simplicity, the discussion hereafter will focus on the C6 homologue.
164 A composite transformation pathway for these three key fluorotelomer precursors is illustrated in Figure
165 1 for the C6 homologue. Of particular note are the shared fluorotelomer sulfonate (FTS) intermediate
166 for many precursors, which may help explain the frequent detection of FTS at many AFFF-impacted sites
167 (Baduel et al., 2017; Schultz et al., 2004).

168 Microbial biotransformations of AFFF-derived fluorotelomers appear primarily in the head group
169 (as opposed to the fluorinated tail), with N-dealkylation and oxidation reactions often observed. Sulfur
170 atom oxidation (S-Oxidation) appears to readily occur for sulfide (or thia, -S-) groups and sulfoxides (-SO-
171). For example, X:2 FTTh-PrAd-DiMeEtS (X:2 FtTAoS (Harding-Marjanovic et al., 2015; Yi et al., 2018) or
172 X:2 FTSAS (Weiner et al., 2013) were readily converted, under aerobic and anaerobic conditions, to
173 FTSO-PrAd-DiMeEtS, $FTSO_2$PrAd-DiMeEtS and finally to fluorotelomer alcohols (FTOHs) or FTSs, all of
174 which can be further transformed to PFCAs. FTOHs and FTSs as parent compounds can show extensive
175 transformation and defluorination (Liu and Mejia Avendaño, 2013), but the defluorination of other
176 fluorotelomers with a bulkier head group was limited to the CF-CH moieties at the junction of the
177 perfluoroalkyl tail and the non-fluorinated head group. Although partial defluorination of FTSs is
178 explained with the presence of one or more hydrogen atoms at the α-carbon, allowing for ready access

to the carbon-sulfur bond (Key et al., 1998), the intermediates are rarely observed in the field where FTSs are detected. Carbon-sulfur bond cleavage is believed to be closely linked to microbial sulfur metabolism. Transformations of FTOHs and FTSs to FTCAs and further PFCAs have been well studied (Kim et al., 2014, 2012; Liu et al., 2010; Zhang et al., 2016), and are reviewed elsewhere (Liu and Mejia Avendaño, 2013). Though some have reported the transformation of 6:2 FTS to 6:2 FTOH (Marchington, 2008) , this has not always been observed. It is also likely that the lack of 6:2 FTOH detection in some studies may be due to its reactivity and high instrumental detection limits. Alternatively, Wang et al. (2011) reported alkanesulfonate oxidation of 6:2 FTS to an aldehyde, with subsequent transformation to PFCAs.

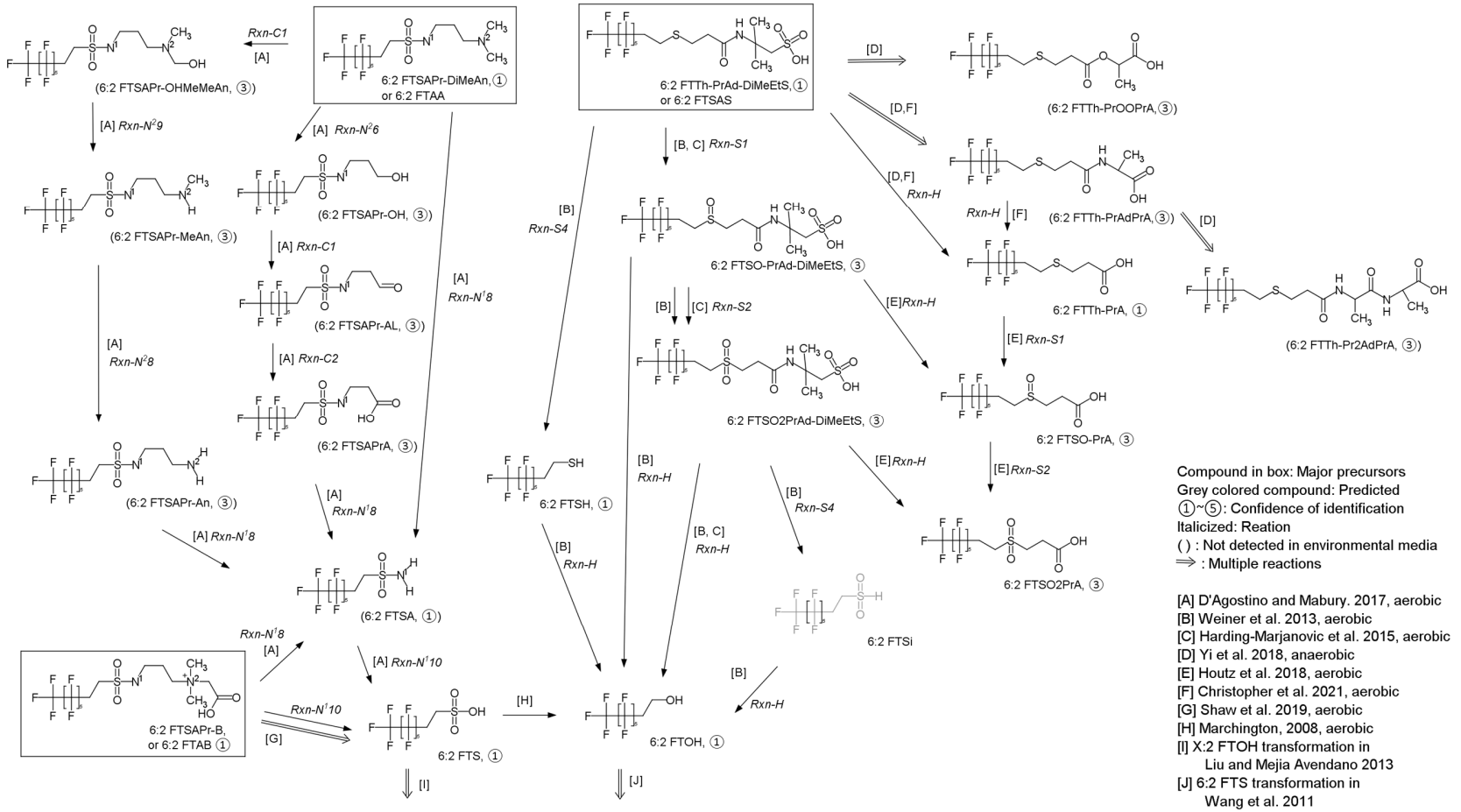

Figure 1. Microbial biotransformation of precursors to the 6:2 FTS intermediate in soil and sludge under aerobic and anaerobic conditions (except [G]). Major precursors are 6:2 FTSAPr-DiMeAn, 6:2 FTTh-PrAd-DiMeEts, and 6:2 FTSAPr-B. For structures having more than one nitrogen atom, each nitrogen has been numbered on the right upper side for easy differentiation of transformation mechanisms, e.g. N^2. 6:2 FTSAPr-B and 6:2 FTSAPr-DiMeAn can directly transform to 6:2 FTOH and 6:2 FTS (D'Agostino and Mabury, 2017a). Shaw et al. proposed direct and multiple transformation to 6:2 FTS in their pure culture experiment. Confidence of identification was assigned according to criteria defined by Schymanski (Schymanski et al., 2014), ①: highest confidence.

2.2. Microbial biotransformations of ECF precursors

The microbial biotransformation of ECF substances used in consumer products has been noted since early 2000 (Benskin et al., 2013, 2012; Lange, 2000; Mejia Avendaño and Liu, 2015; Rhoads et al., 2008), while the biotransformation of AFFF-derived ECF polyfluorinated substances only began receiving attention in recent years (Chen et al., 2020; Liu et al., 2021; Mejia-Avendaño et al., 2016). Large numbers of these chemicals in multiple classes (Backe et al., 2013; Barzen-Hanson et al., 2017b), as well as limited access to compounds with meaningful purity (Barzen-Hanson et al., 2017b; Joudan et al., 2019), have hampered the research, while the complexity of biological intermediates analysis (Dubocq et al., 2019; McDonough et al., 2019) and possibly toxicity hindering microbial activity (Cai et al., 2019) pose additional challenges. Limited data are available on the transformation pathway of quaternary ammonium polyfluoroalkyl surfactants (TAmPr-FACA and TAmPr-FASA, Mejia-Avendaño et al., 2016), polyfluoroalkyl amine oxides (OAmPr-FACA and OAmPr-FASA, Chen et al., 2020), and polyfluoroalkyl amine betaines (CMeAmPr-FACA and CMeAmPr-FASA, Liu et al., 2021). These classes share degradation intermediates and pathways due to R_f-C(O)N(H)-C_3H_6-N(CH_3)$_2$- (or R_f-SO_2N-C_3H_6-N(CH_3)$_2$-) in their structure. The proposed pathways for the amide-containing TAmPr-FOAA + OAmPr-FOAA + CMeAmPr-FOAA and the sulfonamide-containing TAmPr-FOSA + OAmPr-FOSA + CMeAmPr-FOSA biodegradation in aerobic soil are illustrated in Figure 2 and Figure 3, respectively.

In prior laboratory studies for these classes, several intermediate structures along specific pathways were predicted but not always observed under the experimental conditions (Chen et al., 2020; Mejia-Avendaño et al., 2016). For example, aldehyde or alcohol functional groups in the head group (among the grey colored compounds in Figure 2 and Figure 3) were not detected in experiments, possibly due to their transient nature but also because aldehydes and alcohols typically exhibit higher limits of detection when analyzed by liquid chromatography high-resolution mass spectrometry (LC-HRMS), which is the primary instrumental approach used to elucidate transformation intermediates. With the exception of the initial differences in transformation for the amine groups via tertiary amine-N-Oxide reduction from the OAmPr-group, the N-dealkylation from the TAmPr-group, and N-deacetylation from the CMeAmPr-group, the transformation pathways appear to be shared for the amide-based and sulfonamide-based precursors. Dealkylation of the amide or sulfonamide in the head group, as well as oxidative removal and hydrolysis, were reported as the main transformation reactions (Chen et al., 2020; Liu et al., 2021; Mejia-Avendaño et al., 2016). Degradation of FOAA-PrA did not produce perfluorooctane amide, but directly transformed to PFOA: when adjacent to a perfluoroalkyl tail, amide hydrolysis may be a more likely reaction (R_f-C(O)-N(H)-R → R_f-COOH) than N-dealkylation. In OAmPr-

FASA, TAmPr-FASA, and CMeAmPr-FASA transformation (Figure 3), the observed major microbial biotransformation reaction was sulfonamide oxidative N-dealkylation (R_f-SO_2-N(H)-R → R_f-SO_2-NH_2), which appears to dominate as compared to the competing sulfone oxidation (R_f -SO_2-N(H)-R → R_f -SO_3). Differences in sulfonamide vs. carboxamide reactivities have been previously observed (Chataigner et al., 2007).

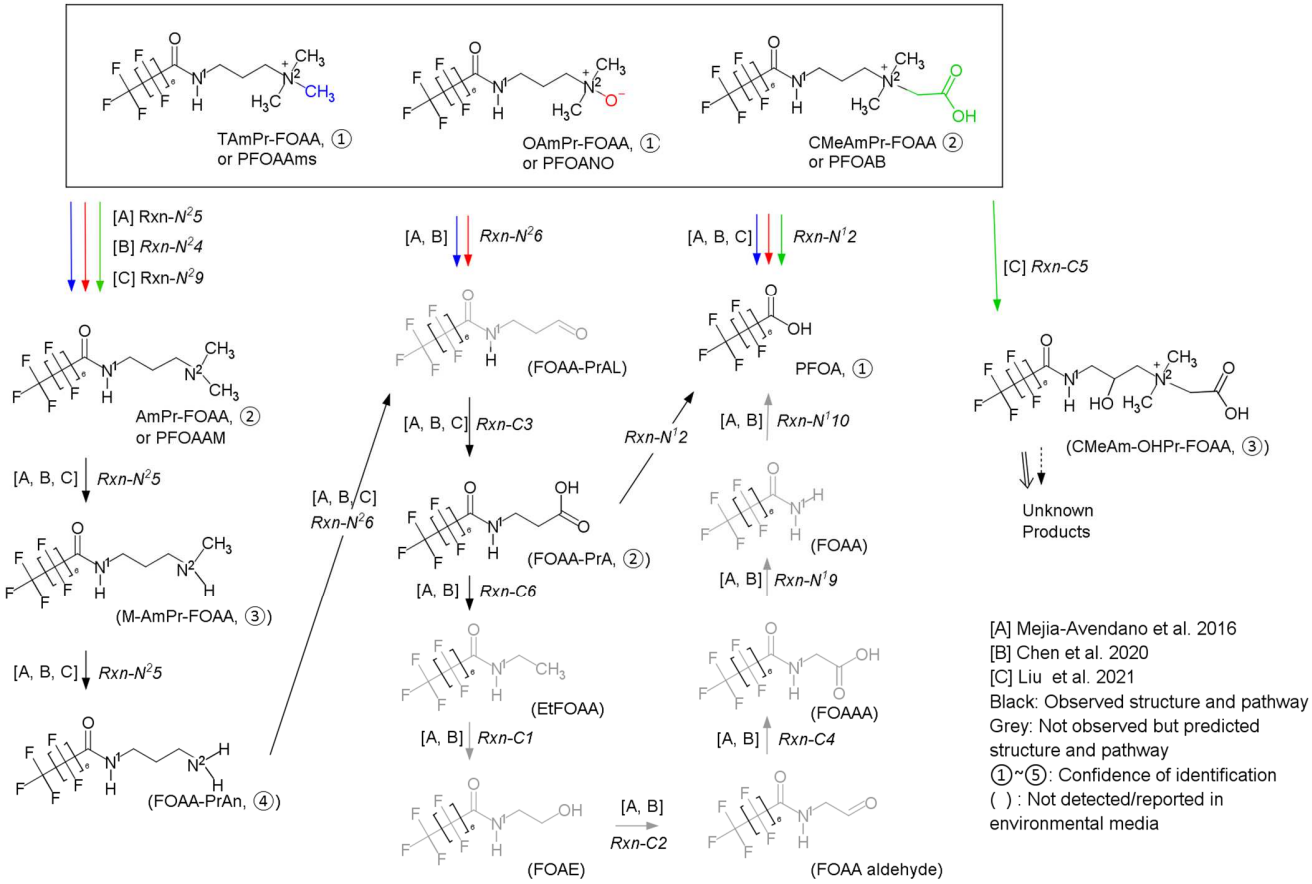

Figure 2. Microbial biotransformation of TAmPr-FOAA, OAmPr-FOAA, and CMePrAm-FOAA in aerobic soils. Except for the initial transformation via trimethylamine-N-Oxide reduction from OAmPr-FOAA and N-dealkylation from TAmPr-FOAA, all transformation mechanisms are shared for those compounds. Compounds in grey were predicted as intermediates but not observed. Compound names in bracket are proposed as intermediates in the cited papers but not (yet) reported in AFFF-impacted environmental media. For structures having more than one nitrogen atom, each nitrogen has been numbered on the right upper side for easy differentiation of transformation mechanisms, e.g. N^2. Color of arrow represents headgroup-specific transformation pathway. Confidence of identification was assigned according to criteria defined by Schymanski (Schymanski et al., 2014), ①: highest confidence.

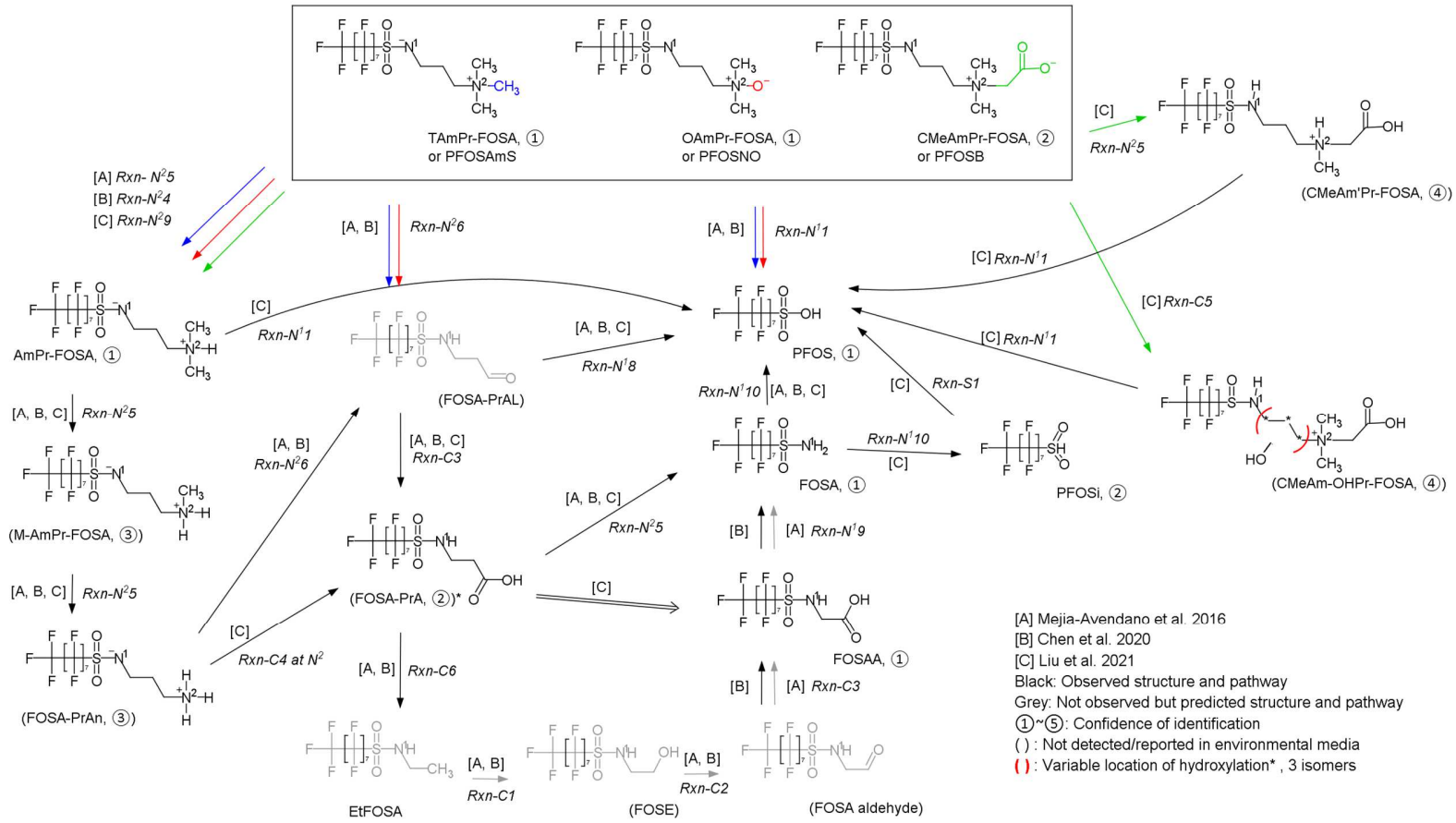

Figure 3. Microbial biotransformation of TAmPr-FOSA, OAmPr-FOSA, and CMeAmPr-FOSA. Except for the initial transformation via trimethylamine-N-Oxide reduction from OAmPr-FOSA and N-dealkylation from TAmPr-FOSA, and N-deacylation from CMeAmPr-FOSA all transformation mechanisms for those compounds are shared. Compounds in grey were predicted as intermediates but not observed. Compound names in bracket are proposed as intermediates in the cited papers but not (yet) reported in AFFF-impacted environmental media. CMeAm-OHPr-FOSA-1 and CMeAm-OHPr-FOSA-2 are isomers and pathway flow followed as it is described in the cited paper. For structures having more than one nitrogen atom, each nitrogen has been numbered on the right upper side for easy differentiation of transformation mechanisms, e.g. N^2. *FOSA-PrA is isomer of MeFOSAA. Color of arrow represents headgroup-specific transformation pathway. Confidence of identification was assigned according to criteria defined by Schymanski (Schymanski et al., 2014), ①: highest confidence.

2.3. Different precursors from different products sharing pathways and intermediates

Details of microbial biotransformation pathways would be constructive in elucidating sources of PFASs present in the environment. For example, FOSAA and FOSA (as well as PFOS) were all detected from the transformation of the sulfonamide-containing OAmPr-FOSA, TAmPr-FOSA, and CMeAmPr-FOSA (Figure 3). However, FOSAA and FOSA are also degradation intermediates of N-ethyl perfluorooctane sulfonamido acetic acid (EtFOSAA), which can be oxidatively transformed from N-ethyl perfluorooctane sulfonamidoethanol (EtFOSE, Figure 4). EtFOSE is a monomer that can be released from various PFAS-based commercial products, including fabrics and food packaging (Benskin et al., 2013; Lange, 2018; Mejia Avendaño and Liu, 2015; Rewerts et al., 2018; Rhoads et al., 2008; Zhang et al., 2017). Presumably, similar transformation processes occur for N-methyl perfluorooctane sulfonamidoethanol (MeFOSE), which also can be released from side-chain PFAS polymers used in food packaging and fabric protection (Benskin et al., 2012), leading to the formation of N-methyl perfluorooctane sulfonamido acetic acid (MeFOSAA), and eventually to PFOS. Therefore, FOSA detection alone may not always indicate an ECF AFFF source, as this sulfonamide oxidative N dealkylation (R_f-SO_2-N< → R_f-SO_2-N(H)-) reaction is likely common for many of these ECF polyfluoroalkyl sulfonamide-containing substances.

While the presence of FOSA may not always enable clear identification of the parent product released, MeFOSAA is a structural isomer of perfluorooctane sulfonamido propanoic acid (FOSA-PrA). Although both can form FOSA and eventually PFOS, MeFOSAA (nor EtFOSAA) is not likely to be produced from AmPr-FOSA degradation (Figure 1). Thus, while the detection of FOSA or FOSAA at a site could imply multiple sources of these important PFOS precursors, the presence of EtFOSAA and MeFOSAA (the latter importantly distinguished from its structural isomer FOSA-PrA) at a site could suggest significant impacts from non-AFFF PFAS sources. This is supported by limited field data: EtFOSA and EtFOSAA (nor other chain lengths, Mejia-Avendaño et al., 2017; Nickerson et al., 2020) have only rarely been detected at AFFF-impacted sites (when looked for), and another class (MeFASA) is also rarely detected (Favreau et al., 2017; Nickerson et al., 2020). Instead, detection of FOSA-PrAn and FOSA-PrA (which are proposed degradation intermediates of TAmPr-FOSA, OAmPr-FOSA and CMeAmPr-FOSA; Figure 3) or their homologs (i.e., FHxSA-PrA; (Nickerson et al., 2021a)) would support the notion that these PFASs are primarily AFFF-derived. Unfortunately, they do not appear to accumulate and may transform rapidly to other intermediates (Chen et al., 2020). The presence of these relatively unstable intermediates in environmental samples, therefore, may imply a significant source of their AFFF-derived precursors.

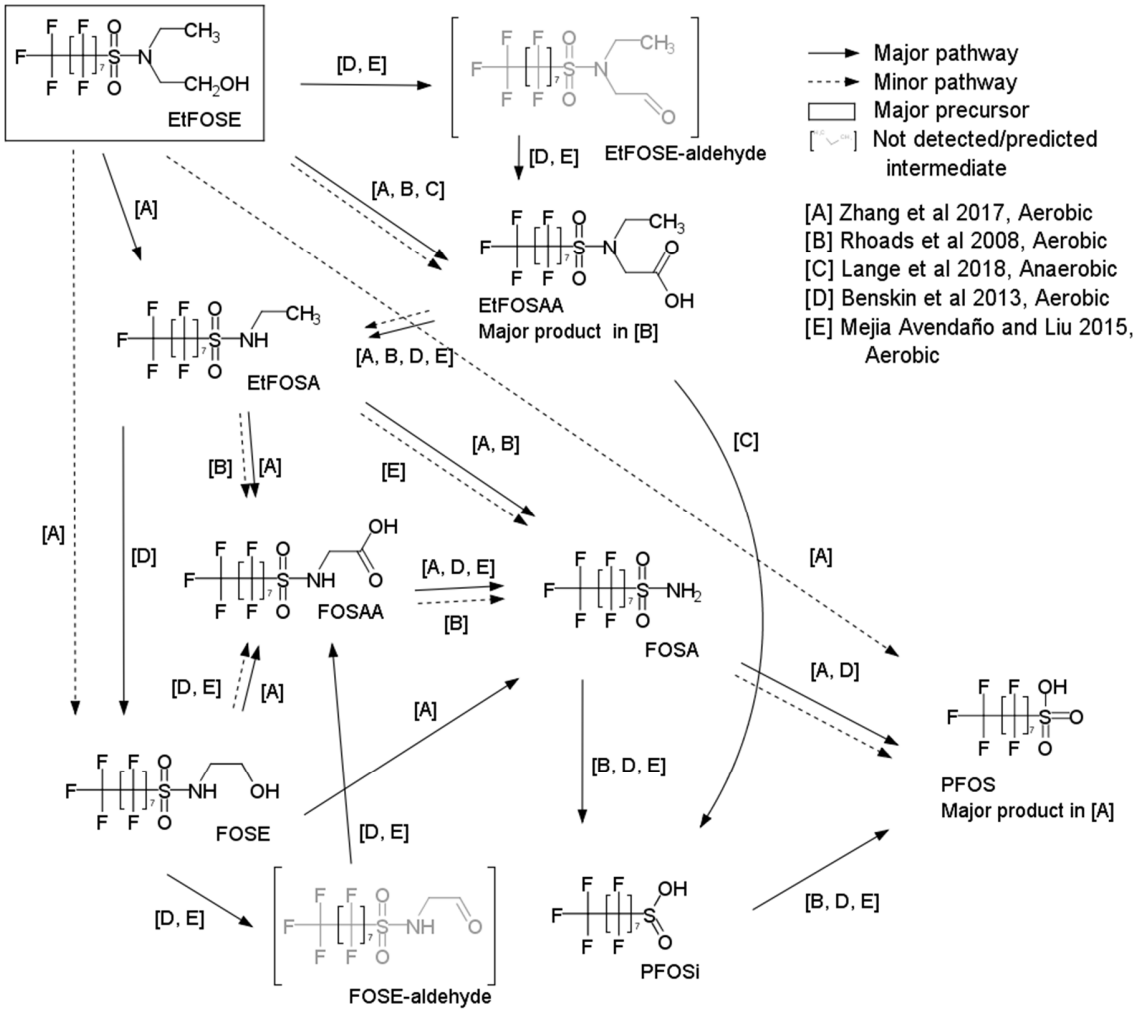

Figure 4. Microbial biotransformation of EtFOSE under aerobic and anaerobic conditions.

3. Importance of redox conditions and co-contaminants

Aerobic microbial biotransformations were studied for both AFFF-derived PFASs and other PFAS-based industrial and consumer products, but there are limited data for the microbial biotransformation of AFFF-derived PFASs under anaerobic conditions. In one of the few studies (to date), 6:2 FTTh-PrAd-DiMeEts degradation was assessed under anaerobic conditions (Field et al., 2017; Yi et al., 2018). Oxidation at the sulfide (thioether) was observed under aerobic conditions, while head group amide hydrolysis and conjugation were observed under sulfate-reducing conditions (Yi et al., 2018) (Figure 5). Degradation of 6:2 FTTh-PrAd-DiMeEts was mostly active in nitrate-reducing conditions, while 6:2 FTTh-PrA, which is a microbial biotransformation intermediate observed under more reducing anaerobic conditions, was not detected (Field et al., 2017; Yi et al., 2018). Another distinctive observation in anaerobic microbial biotransformations is the absence of common intermediates such as FTSs and other dealkylated products. This supports a concern that transformation intermediate product yields derived from well-controlled laboratory experiments may not sufficiently explain field observations under dynamic redox conditions. Further, these observations suggest that the spectrum of microbial biotransformation products that can be formed and/or observed is likely dependent on the local redox conditions, among many other factors.

In addition to redox conditions altering the observed PFAS composition at AFFF-impacted sites, microbial biotransformation of precursors could occur co-metabolically by microorganisms primarily growing on comingled substrates such as solvent and AFFF-derived hydrocarbon surfactants, particularly if they are stimulated through nutrient additions. For example, the microbial biotransformation of 6:2 FTSAB was observed during biopile treatment of a site impacted by aromatic hydrocarbons (Li et al., 2019). Moreover, increased release of PFAAs has been linked to sparging oxygen to remediate co-contaminants (McGuire et al., 2014). DGBE and hydrocarbon surfactants, which often exist at much higher concentrations than PFASs, are likely used as a sole carbon source for many microorganisms (Montagnolli et al., 2017). Enhanced release and spread of PFASs from treatment could influence microbial communities associated with PFAS transformations (Harding-Marjanovic et al., 2016; O'Carroll et al., 2020), with consequences for co-contaminant degradation (Fitzgerald et al., 2019). Oxidations (involving oxygenases and/or oxidases) are some of the major types of reactions for many FT-derived compounds and are favored under aerobic conditions across a wide array of microbial systems, with microbial biotransformation of the parent compound often occurring within days (Table 1). Increased expression of genes that encode for oxygenases was detected in 6:2 FTSAB biotransformation with Gordonia sp. (Bottos et al., 2020), and the involvement of Alkanesulfonate Monooxygenase in FTS

desulfonation has been observed (Yang et al., 2022). Co-contaminants inducing elevated expression of genes that encode aromatic hydrocarbon dioxygenases (which can catalyze reactions with a wide range of xenobiotics (Wackett, 2009)) could also stimulate precursor microbial biotransformations in AFFF-impacted sites co-impacted by fuel releases (Olivares et al., 2022). On the other hand, shifted or suppressed microbial biotransformation of co-contaminants was influenced by the type of AFFF (possibly due to the PFAS composition), type of co-contaminant or carbon source, and nutrient levels (Harding-Marjanovic et al., 2016; Li et al., 2019; Montagnolli et al., 2017). Unfortunately, the effects of co-contaminants on microorganisms transforming PFASs are not yet well understood.

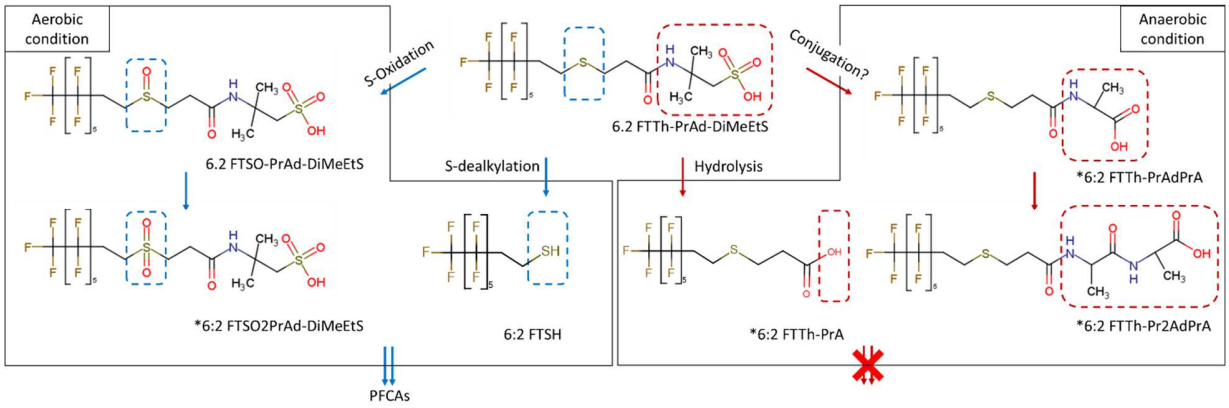

Figure 5. Comparison in 6:2 FTTh-PrAd-DiMeEtS microbial biotransformation under aerobic and anaerobic conditions. Under aerobic conditions, S-oxidation, S-dealkylation and desulfonation proceeded the downstream reactions to form PFCAs (Harding-Marjanovic et al., 2015; Weiner et al., 2013; Yang et al., 2022) while transformation on the secondary amine in the head group was dominant under anaerobic conditions, and no further degradation was observed (Yi et al., 2018).

4. Similarities and Differences for ECF and FT-derived Polyfluoroalkyl Substance Microbial biotransformations

Microbial biotransformations of FT-based polyfluoroalkyl substances have been observed at the headgroup and also at the hydrocarbon spacer immediately adjacent to the perfluorinated tail. This latter process of eventual biotransformation to PFAAs has been well documented (Liu and Mejia Avendaño, 2013). For the initial reactions occurring in the head group, FT-derived polyfluoroalkyl substance transformations appear to be similar to ECF-derived polyfluoroalkyl substance transformations. N-dealkylations appear to be key reactions on the backbone of sulfonamide- and amide–containing substances in both FT-based and ECF-based substance transformations, while C-oxidations are commonly observed on carboxylates, alcohols, and aldehydes such as 6:2 FTSAPr-AL > 6:2

FTSAPrA (fig 1) and FOAA-PrAL > FOAA-PrA (fig 2) (Table 2). S-oxidation was distinctively observed in FT-based substances having sulfide (-S-) and sulfoxide (-SO-) groups under aerobic conditions, including for 6:2 FTSO-PrA transformation to 6:2 $FTSO_2PrA$: to date, no ECF-derived substances with similar chemistries have been observed.

Though the focus here is on the pathways by which polyfluoroalkyl substances are transformed, the rates of some of these microbial biotransformations may be highly dependent not only on the structure of the non-fluorinated portion of the molecules, but also the tested environmental conditions (Table 1). Eight ECF-based PFASs with N-containing head groups have been well studied under aerobic soil conditions (Chen et al., 2020; Liu et al., 2021; Mejia-Avendaño et al., 2016). Though the time to 50% disappearance (DT_{50}) of TAmPr-FOSA was not measurable during the 180-d study, the DT_{50} values of the other compounds were in the following order: CMeAmPr-FOSA (675 d), AmPr-FOSA (47.5 d), and OAmPr-FOSA (15d). The order is slightly different for carboxamide precursors: CMeAmPr-FOAA (266-630 d), TAmPr-FOAA (127 d), AmPr-FOAA (14.2 d), and OAmPr-FOAA (3-7d). In comparison, as reported by Li et al. (Li et al., 2019), 6:2 FTSAPr-B, which has the same head group (R_f-N1-$(CH_2)_3$-$N(CH_3)_2$-CH_2COOH) as CMeAmPr-FOSA, displayed a half-life of 31 d in a petroleum and AFFF-impacted soil as compared to the 675 d for CMeAmPr-FOSA in a different aerobic soil (Liu et al., 2021). Differing test conditions complicate this comparison, as testing conditions likely play a major role in relative rates of microbial biotransformation; despite the relatively quick disappearance in soil, 68±13% of 6:2 FTSAPr-B remained after 109 d during an aerobic WWTP sludge test (D'Agostino and Mabury, 2017a), whereas the opposite was observed for the ECF-derived EtFOSAA, which exhibited a DT_{50} of ~10 d in a similar sludge test (Rhoads et al., 2008), but 245-335 d in aerobic soils (Mejia Avendaño and Liu, 2015; Zhang et al., 2017). Clearly, variable test conditions may lead to highly variable transformation rates, and it is not immediately evident whether generalizations can be made with respect to the transformation rates of ECF vs. FT chemistries with nearly identical headgroups.

Depending on the environmental conditions, there may be a build-up of semi-stable intermediates for both FT-based and ECF-based chemistries. For example, in an abiotic study, Chen and colleagues reported a lack of PFOS formation from OAmPr-FOSA, suggesting FOSA as the final abiotic transformation product (Chen et al., 2020). In biological systems, this final step in the transformation (e.g., FOSA to PFOS) appears to be relatively slow as well: in studies of EtFOSA transformation (Liu et al., 2019; Rhoads et al., 2008; Zhang et al., 2017), the half-life for the formation of FOSA was 10.8–11.2 d, with the half-life for the formation of PFOS from FOSA up to 60 times longer (106–712 d) (Liu et al 2019, Zhang et al 2017). The difference in rates might be explained by hydrolysis models of perfluorinated

sulfonamides (Rayne and Forest, 2009), which suggest that compounds with N-alcohol and N-carboxylic substitutions were likely to undergo intramolecular attacks that could lead to the formation of sulfonates. Similarly, in abiotic and aerobic microbial biotransformation studies, 6:2 FTSAPr-DiMeAn and 6:2 FTSAPr-B transformed to 6:2 fluorotelomer sulfonamide (6:2 FTSA), which was suggested as the key transformation product with 6:2 FTS (D'Agostino and Mabury, 2017a). To date, the subsequent microbial biotransformation of X:2 FTSA has not been evaluated, though the eventual formation of PFCAs is certainly likely. Altogether, these data suggest that for both FT-based and ECF-derived polyfluorinated substances, a potential build-up of sulfonamides (FASAs and/or X:2 FTSAs) in soil and water is possible. Indeed, recent experimental evidence points to FASAs being important intermediates released from soil columns (Maizel et al., 2021; Nickerson et al., 2021b) and is supported by monitoring AFFF impacted surface waters (D'Agostino and Mabury, 2017b). Interestingly, the pathway prediction software platform EnviPath (Lorsbach et al., 2016) currently does not predict the formation of FASAs such as FHxSA in AmPr-FHxSA biotransformation, indicating a possible need to particularly understand the enzymes and kinetics possibly involved in this type of microbial biotransformation.

Importantly, both ECF-derived and FT-based chemistries can be sources of PFCAs to the environment. PFOA was directly generated from polyfluorinated amide precursors (Chen et al., 2020; Liu et al., 2021; Mejia-Avendaño et al., 2016), indicating that FT-based AFFFs may not be the only source of PFOA. In that study, the authors noted (but did not quantify) an apparent increase in the branching of the residual TAmPr-FOAA and OAmPr-FOAA, suggestive of preferential transformation of linear isomers, whereas the isomeric distribution of PFOA in the live soils fluctuated. Interestingly, the percent of branched PFOA (~12%) remained constant in the sterile soils (Chen et al., 2020). As purely linear (>95%) PFCAs have been linked to FT-based chemical sources, isomeric profiling may be an important tool for differentiating PFCA sources (Buck et al., 2011). Unfortunately, too little isomeric data (particularly isomer-specific transformation rates) are available to indicate specific polyfluorinated precursors, though the presence of branched PFCAs strongly indicates an ECF source.

5. Observations from field studies

As PFASs derived from AFFFs are complex and exist in neutral, anionic, cationic, and zwitterionic forms, their fate and transport upon release to the environment is complicated and depends on the type of AFFF and the environment into which the release occurs. PFASs observed in AFFF and environmental samples from AFFF-impacted sites are available in Table S1 in Supplementary Information. To fully delineate specific releases, spatial profiling of various transformation intermediates may be necessary.

For example, anionic PFASs showed further soil profile penetration and distribution than PFASs with positively charged functional groups (Nickerson et al., 2021b). Various head groups (with the same tail length) also display different desorption trends in soil column leaching experiments with field-collected soils (Maizel et al., 2021). Nickerson reported several N-substituted sulfo propyl perfluorohexane sulfonamides ($C_6F_{13}SO_2$-NR-$C_3H_6SO_3$, R varied, SPrAmPr-FHxSAPrS, DiOHPrAm-MeOHPr-FHxSAPrS, EtOH-AmPr-FHxSAPrS) at an AFFF-impacted site and their possible dealkylation to form to SPr-FHxSA ($C_6F_{13}SO_2$-N-$C_3H_6SO_3$, N-sulfo propyl perfluorohexane sulfonamide) followed by FHxSA ($C_6F_{13}SO_2$-NH_2). These data suggested that as the transformation proceeded, the transformed intermediate was transported further from the initial source zone.

Transformation mechanisms observed from laboratory studies can also help explain compounds observed at AFFF-impacted sites. EtOH-AmPr-FASA-EtOH (Figure 6: [1]), which has (to date) only been reported in neat AFFF products (Barzen-Hanson et al., 2017, C=2~8), could transform to CMeAmPr-FASAA via alcohol oxidation and then aldehyde oxidation under oxidative conditions. CMeAmPr-FASAA has been detected in groundwater (Barzen-Hanson et al., 2017b) and soils from AFFF impacted fields (Nickerson et al., 2020), but not (as of yet) in AFFF. CMeAmPr-FASAA can be further degraded to CMeAmPr-FASA via Sulfonamide N-dealkylation. Figure 6-[2] shows a transformation pathway proposed from a recent field PFAS evaluation (Nickerson et al., 2021b) starting from diOHPrAm-MeOHPr-FASAPrS. While all compounds have been previously detected in AFFF (Barzen-Hanson et al., 2017a), not all were detected in soil and groundwater (Nickerson et al., 2021b). Of particular note is that three different secondary sulfonamides may transform to SPr-FASA and further FASA via sulfonamide N-dealkylation.

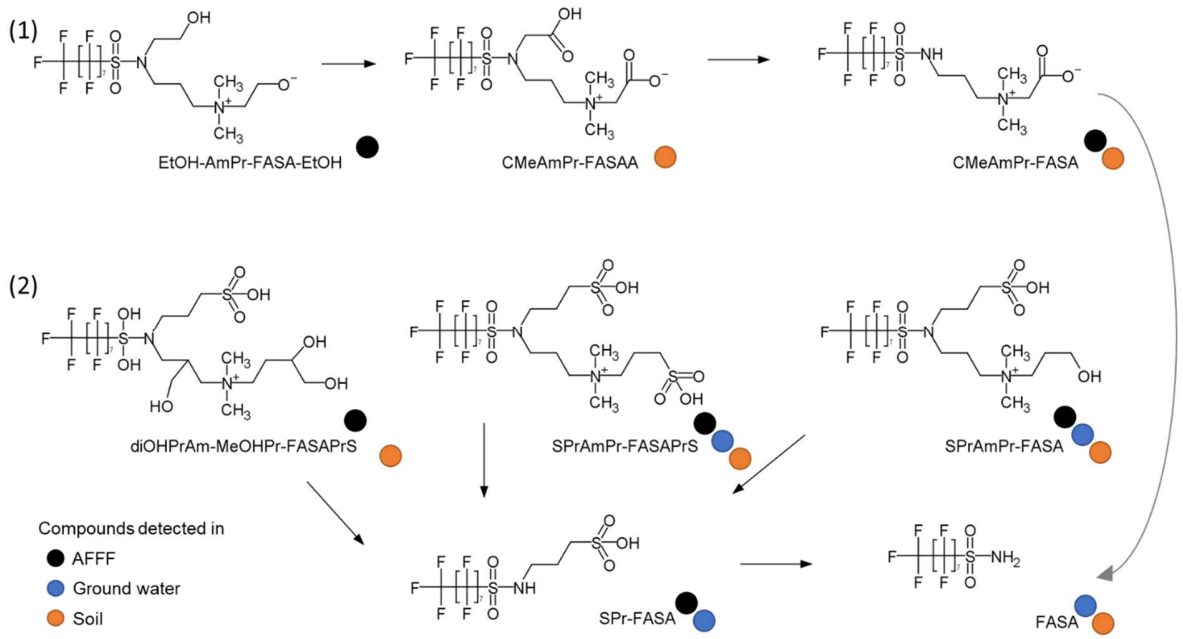

Figure 6. Possible microbial biotransformation between reported PFASs in AFFF and environmental media. All transformation mechanisms except EtOH-AmPr-FASA-EtOH → CMeAmPr-FASAA (alcohol oxidation and aldehyde oxidation under oxidative conditions) is sulfonamide N-dealkylation. Colored circles represent the type of samples in which these compounds have been detected. (1) possible transformation of chemicals detected soil, groundwater, and AFFF (Barzen-Hanson et al., 2017b; Mejia-Avendaño et al., 2017; Munoz et al., 2016; Nickerson et al., 2020);(2) proposed transformation of chemicals detected in the AFFF impacted site soil (Nickerson et al., 2021b). The chemicals were also observed in other soil, groundwater, and AFFF (Barzen-Hanson et al., 2017b; Mejia-Avendaño et al., 2017; Munoz et al., 2016; Xiao et al., 2017). More information is available in table S1 in Supplementary Information.

Liu and colleagues (Liu et al., 2021) examined the microbial biotransformation of N-substituted head groups. In their study, polyfluorinated quaternary amines and betaines had the highest stability, followed by tertiary amines, while an amine oxide was transformed as quickly as EtFOSAA. The various transformations primarily occurred at N^2 (see parent compound in Figures 1 and 2) and rarely at the amide or sulfonamide (N^1 in these figures). Among 83 PFAS classes observed in AFFF-impacted environmental media (Backe et al., 2013; Baduel et al., 2017; Barzen-Hanson et al., 2017b; Nickerson et al., 2020; Xiao et al., 2017), 57 compound classes have been reported with a sulfonamide/amide or quaternary ammonium in the head group, of which 41 classes are ECF-derived and 16 classes are FT-derived. As discussed above, research has primarily focused on secondary sulfonamides (e.g. EtFOSA) or secondary amides (e.i. AmPr-FOAA), with the exception being EtFOSE transformation (Table S2 in SI). However, 19 out of 41 classes of ECF-based AFFF-derived PFASs are tertiary sulfonamides (N,N-

sulfonamide, R-S(=O)$_2$N(R_1)R_2). Tertiary sulfonamides were apparently abundant in 3M products (TAmPr-FASA-PrA, CMeAmPr-FASA-PrA, EtOH-AmPr-FASA-PrA) (Barzen-Hanson et al., 2017b; Place and Field, 2012), and also have been observed as some of the highest concentration PFASs in AFFF-impacted soils (TAmPr-N-MeFASA, AmPr-FASA-PrA, and CMeAmPr-FASAA) (Nickerson et al., 2020). The detection frequency and levels in soils, in conjunction with their presence in AFFF, warrant the need to further study the transformation of tertiary sulfonamides and their potential transport.

From this review, it is clear that not all compounds observed in controlled bench-scale laboratory experiments (names in brackets in Figures 1, 2, and 3) have been reported in AFFF-impacted environmental media (i.e., soil, sediment, or water). While this suggests that a broader list of compounds should be monitored at AFFF-impacted sites, this may also be due to differences in biogeochemical conditions, the presence of co-contaminants, and remediation history. In addition, some computationally predicted intermediates were not always detected under experimental conditions (compounds colored in grey in Figures 1, 2, and 3). The opposite could be true as well; for instance, FHxSA is not currently predicted as a microbial biotransformation product of AmPr-FHxSA in EnviPath. Finally, though there are many intermediates in the 6:2FTSAPr-DiMeAn transformation pathway (Figure 1) observed in laboratory experiments, to date, only 6:2 FTS has been reported in field-collected samples.

Finally, in addition to the aforementioned isomers FOSA-PrA and MeFOSAA (and their shorter perfluoroalkyl chain length equivalents), the detection of isomers is likely common in field samples, especially for sites where multiple chemistries have been released (likely most AFFF-impacted sites). With more than one type of fluorochemistry released, this could convolute the delineation of specific precursors. For example, X:2 FTSA-Pr-MeAA (R_f-C_2H_4-SO_2-N(H)-C_3H_6N(CH_3)CH_2COOH) is an isomer of AmPr-FASA-PrA (R_f-SO_2-N(C_2H_4COOH)-C_3H_6N(CH_3)$_2$): the latter is ECF-derived, while the former is FT-derived and has been reported in AFFF (Moe et al., 2012) but not (as of yet) in environmental media. For the X:2 FTSA-Pr-MeAA class, one might expect biotransformation products and PFCA production. For the AmPr-FASA-PrA class, the final products likely present in groundwater and soil are primarily PFSAs. In these cases, the presence of linear vs. branched isomers and/or the detection of transformation intermediates could help differentiate which isomers are present (in the absence of pure analytical standards) and inform environmental source apportionment and site management activities.

6. Implications and Data Gaps

Elucidating the microbial biotransformation pathways for polyfluoroalkyl substances requires validation through the detection of PFASs in AFFF impacted samples in various media. To date, this pathway work has primarily focused on extant peer-reviewed literature. Further, only a dozen or so research papers have specifically reported on novel and newly identified AFFF-derived PFASs. An incomplete mapping and understanding of transformation products for polyfluoroalkyl substances have led some to refer to this uncharacterized PFAS mass as "dark matter" (Ruyle et al., 2021). Indeed, with the absence of laboratory and/or field data for many intermediate compounds, it may be difficult to ascertain from what specific precursors certain PFASs are derived. With improvements in transformation prediction models enabled by PFAS-relevant reaction mechanisms (i.e., Table 2.), it may be possible to develop more robust microbial biotransformation predictions. Further laboratory and field-based studies are needed to validate the data collected to date and fill in the gaps with respect to PFAA precursors and their microbial biotransformation.

One additional tool that could prove useful in filling in these polyfluorinated substance data gaps is the broader use of LC-HRMS data. The identity of various transformation intermediates is typically not clear without confirmation via HRMS, ideally with the help of an HRMS fragmentation spectral library (i.e., HRMS MS2 libraries). Such a library should be as comprehensive as possible, as an insufficiently developed library would limit the discussion to those detected and confirmed from previous studies. When coupled with a larger list of PFAS suspects for HRMS analysis, this could prove a valuable tool for PFAS pathway identification and/or source allocation. Developing an HRMS suspect list (for parent ion matching) is a recursive process that requires comparing what has actually been observed in the literature with what might be predicted based on updated transformation prediction tools. Importantly, the U.S. National Institute of Standards and Technology (NIST) has recently published and is now maintaining a list of PFASs for use in HRMS suspect analyses ("NIST PFAS Data Repository," 2020). The need to include compounds predicted based on known or suspected transformations is becoming increasingly important. For example, FOSA-PrA was detected from the degradation of AmPr-FOSA (Chen et al., 2020; Liu et al., 2021; Mejia-Avendaño et al., 2016): this intermediate has not yet been identified as the main ingredient in any commercial product. With the recognition that these intermediates can be formed, the prevalence of these compounds at AFFF-impacted sites can now be assessed. Analysis of specific precursors may prove particularly useful in identifying environmental PFAS sources and/or managing AFFF-impacted sites.

507 Table 1. Reported microbial biotransformation of PFASs discussed in this review and their test condition with mass balance and product yield.

Starting compound (name used in the cited manuscript)	Degradation rate*	mass balance and % yield of transformation products	Incubation condition	Test Duration	Citation
6:2 FTSAPr-B (6:2 FTAB)	70.4% reduction in 168 h	Mass balance 70.8–99.1%, 22% volatile, 49% water soluble	Pure *Gordonia sp.* strain NB4-1Y inoculum culture under aerobic and sulfur-limiting condition	7 d	Shaw et al. 2019
	68±13% remained in 109 d	96-99% mass balance from an intact sample, 3-6 mol % from products	WWTP sludge under aerobic	109 d	D'Agostino et al. 2017
	Original sample: Total 24% reduction in 60 d with high nutrient addition Spiked sample: $t_{1/2}$ 31d in 6:2 FTAB	no significant product mass relative to initial FTAB	soil impacted by petroleum oil spill-firefighting activities under aerobic condition	60 d	Li et al. 2019
6:2 FTSAPr-DiMeAn (6:2 FTAA)	53±6% remained in 109 d	84-91 % mass balance from intact sample, 12-16 mol % from products (>50% of 6:2FTSA)	WWTP sludge under aerobic	109 d	D'Agostino et al. 2017
6:2 FTTh-PrAd-DiMeEtS (6:2 FtTAoS or 6:2FTSAS)	~75 % reduction in 282 d	96 ± 8 % mass balance in clean solids at day 276, 67 ± 6% for contaminated soil at d 282	Pristine or AFFF-contaminated solids from groundwater site, under anaerobic-sulfate reducing condition	282 d	Yi et al. 2018
	Below LOQ after 42 d	32 % of metabolite yield for quantifiable PFAS	Aerobic wastewater treatment plant (WWTP) sludge	42 d	Weiner et al. 2013
X:2 FTTh-PrAd-DiMeEtS (X:2 FtTAoS	Complete disappearance and biotransformation in 45 d with two aliquots of AFFF addition	Mass balance ~ >10% at d 60. 80-100% from precursor oxidation assay at d 60	Contaminated soil collected from a firefighter training area	60 d	Harding-Marjanovic et al. 2015
	NA	58.3 % (6.3 kg total PFAS in effluent, 10.8 kg total PFAS in influent)	Sample grab from WWTP under aerobic condition	NA	Houtz et al. 2018
CMeAmPr-FOAA (PFOAB), AmPr-FOAA (PFOAAm) as impurity	DT_{50} 266-630 d for CMeAmPr-FOAA	Mass balance 81-113% including impurities 32.6 mol% PFOA	Aerobic soil	150 d	Liu et al., 2021
	DT_{50} 14 d for AmPr-FOAA			180 d	
CMeAmPr-FOSA (PFOSB), AmPr-FOSA (PFOSAm) as impurity	DT_{50} 675 for CMeAmPr-FOSA	Mass balance 67-103% including impurities 0.52 % FOSA, 0.064 % FOSAA, and 1.5 % PFOS for 90 d from CMeAmPr-FOSA	Aerobic soil	150 d	
	DT_{50} 47.5 for AmPr-FOSA	8 % FOSA, 0.01 % FOSAA, and 2.7 % PFOS from initial AmPr-FOSA impurity for 90 d		90 d	
TAmPr-FOAA (PFOAAmS)	DT_{50} 127 for TAmPr-FOAA	30.1 % of PFOA, 73.1 % mass balance	Aerobic soil	6 mo	Mejia-Avendaño et al. 2016
TAmPr-FOSA (PFOSAmS)	no $DT_{50}$from no significant TAmPr-FOSA level change	0.3% of PFOS, near 100% mass balance mostly from TAmPr-FOSA	Aerobic soil	6 mo	Mejia-Avendaño et al. 2016
OAmPr-FOAA (PFOANO)	DT_{50} 3–7 d (>99% removal in 90 d)	15-21 % PFOA by 90 d, 18-21% of mass balance	Aerobic soil	90 d	Chen et al. 2020
OAmPr-FOSA (PFOSNO)	DT_{50} ~15 d (97% removal in 90 d)	Mass balance 20-49%, 5-33% for products	Aerobic soil	90 d	Chen et al. 2020
EtFOSE¶	$t_{1/2}$ 1860 d	Mass balance 92%, 2 % loss in 180 d	WWTP Sludge under anaerobic condition (N2 atmosphere)	35 d	Lange et al. 2018
MeFBSE¶	$t_{1/2}$ 35.8 d	Mass balance 122%, MFBSE 25%, MeFBSAA 57%, PFBSi 40%			

EtFOSE¶	EtFOSE $t_{1/2}$ 25.2-30.8 d EtFOSA $t_{1/2}$ 19.5-29.5 d EtFOSAA $t_{1/2}$ 245-335 d	Mass balance 85-115%, ~<20% for EtFOSE, EtFOSAA ~>50%	Aerobic soil	180, 210 d	Zhang et al. 2017
EtFOSA¶	$t_{1/2}$ 13.9 ± 2.1 d	Mass balance 71% at 182 d (51% for sterile control), EtFOSA 2.21%, FOSA 30.3%, FOSAA 34.2 %, PFOS 4%	Aerobic soil	182 d	Mejia-Avendaño et al. 2015
EtFOSE¶	$t_{1/2}$ 44 d (25°C) and 160 d (4°C)	Mass balance 87 (25°C) and 107% (4°C)	Aerobic condition with Marine Sediment	120 d	Benskin et al. 2013
EtFOSE¶	T1/2 <1d for EtFOSE T1/2 ~10d for EtFOSAA	110% mass balance and 66% EtFOSAA, EtFOSE <0.3%	Sludge under aerobic	10 d	Rhoads et al. 2008

*Degradation rate : $t_{1/2}$ (half-life) or DT_{50} (time for 50% of a substance to disappear). If not mentioned in the manuscript, test period and reduction described.; ¶: not AFFF specific PFAS described in figure 4.

510 Table 2. Observed microbial biotransformation reactions of PFASs

Code	Reaction	Subreaction	Test Condition	Parent	Product	Parent structure	Product structure
Rxn-H	Hydrolysis	Hydrolysis	-	-	-	R1-R2	R1-OH, R2-H
Rxn-C1	C-oxidation	Monohydroxylation	Aerobic	Alkane	Alcohol	-C(R1)R2-H	-C(R1)-R2OH
Rxn-C2	C-oxidation	Alcohol oxidation	Aerobic	Primary Alcohol	Aldehyde	-CH2OH	-COH
Rxn-C3	C-oxidation	Aldehyde oxidation	Aerobic	Aldehyde	Carboxylic acid	-COH	-COOH
Rxn-C4	C-oxidation	Carboxylation	Aerobic	Alkane	Carboxylic acid	-RCH2	-RCOOH
Rxn-C5	C-oxidation	hydroxylation	Aerobic	Alkane	Alkane hydroxy	-C-R	-C(-OH)-R
Rxn-C6	Decarboxylation	Decarboxylation	Aerobic	Carboxylic acid	Alkane	-COOH	-H
Rxn-C7	C-oxidation	Alcohol dehydrogenation	Anaerobic	Ketone	Secondary alcohol	-C(=O)-R	-C(R)H-OH
Rxn-C8	C-oxidation	Alcohol dehydrogenation	Anaerobic	Aldehyde	Primary alcohol	-C(=O)H	-CH2OH
Rxn-S1	S-oxidation	Sulfide-oxidation	Aerobic	Sulfide	Sulfoxide	-S-R	-S(=O)-R
Rxn-S2	S-oxidation	Sulfoxide-oxidation	Aerobic	Sulfoxide	Sulfone	-S(=O)-R	-S(=O)2-R
Rxn-S3	S-oxidation	S-oxidation	Aerobic	Sulfinate	Sulfonate	-S(=O)2H	-S(=O)2-OH
Rxn-S4	S-dealkylation	S-dealkylation	Aerobic	Thioether	Thiol	-S-R	-S-H
Rxn-N1	N-oxidation	Sulfonamide hydrolysis	Aerobic	Sulfonamide	Sulfonate	-S(=O)2-NH2	-S(=O)2-OH
Rxn-N2	N-oxidation	Amide hydrolysis	Aerobic	Amide	Carboxylate	-CO-NH2	-COOH
Rxn-N3*	N-oxidation	Dehydrogenation	Aerobic	Amine	Imine	-NR2	=NR
Rxn-N4	N-reduction	Trisubstituted-N-oxide reduction	Aerobic	tri-substituted Amine N-oxide	Tertiary Amine	-N(R1)R2-OH	-N(R1)R2
Rxn-N5	N-dealkylation	N-dealkylation	Aerobic	Tertiary amine	Secondary amine	-N(R1)-R2	-NH-R2
Rxn-N5	N-dealkylation	N-dealkylation	Aerobic	Secondary Amine	Primary Amine	-NH-R	-NH2
Rxn-N6	N-dealkylation	Oxidative removal	Aerobic	Primary Amine	Aldehyde or Ketone	-NH2	-C(=O)-H
Rxn-N7	N-dealkylation	N-dealkylation	Aerobic	Methylammonium derivatives	Tertiary Amine	-NR3	-NR2
Rxn-N8	N-dealkylation	Sulfonamide N-dealkylation	Aerobic	Tertiary sulfonamide	Secondary sulfonamide	-S(=O)2-N(R1)-R2	-S(=O)2-NH-R2
Rxn-N8	N-dealkylation	Sulfonamide N-dealkylation	Aerobic	Secondary sulfonamide	Primary sulfonamide	-S(=O)2-NH-R	-S(=O)2-NH2
Rxn-N9	N-deacetylation	N-deacetylation	Aerobic	Tertiary amine, R1	Secondary amine	-N(R1)-R2	-N-R1
Rxn-N10	N-deamination	N-deamination	Aerobic	Sulfonamide	sulfonate	-NH2	-H
Rxn-F1*	F elimination	H substitution		CF moiety	H-substituteC3d CF	R1CF2-R2	R1-C(F)H-R2

511 *Not in the figures in this paper but described in Kim et al., 2014& 2012; Liu et al., 2010; Liu and Mejia Avendaño, 2013; and Zhang et al., 2016 of

512 FTOHs and FTSs transformations to FTCAs and further PFCAs.

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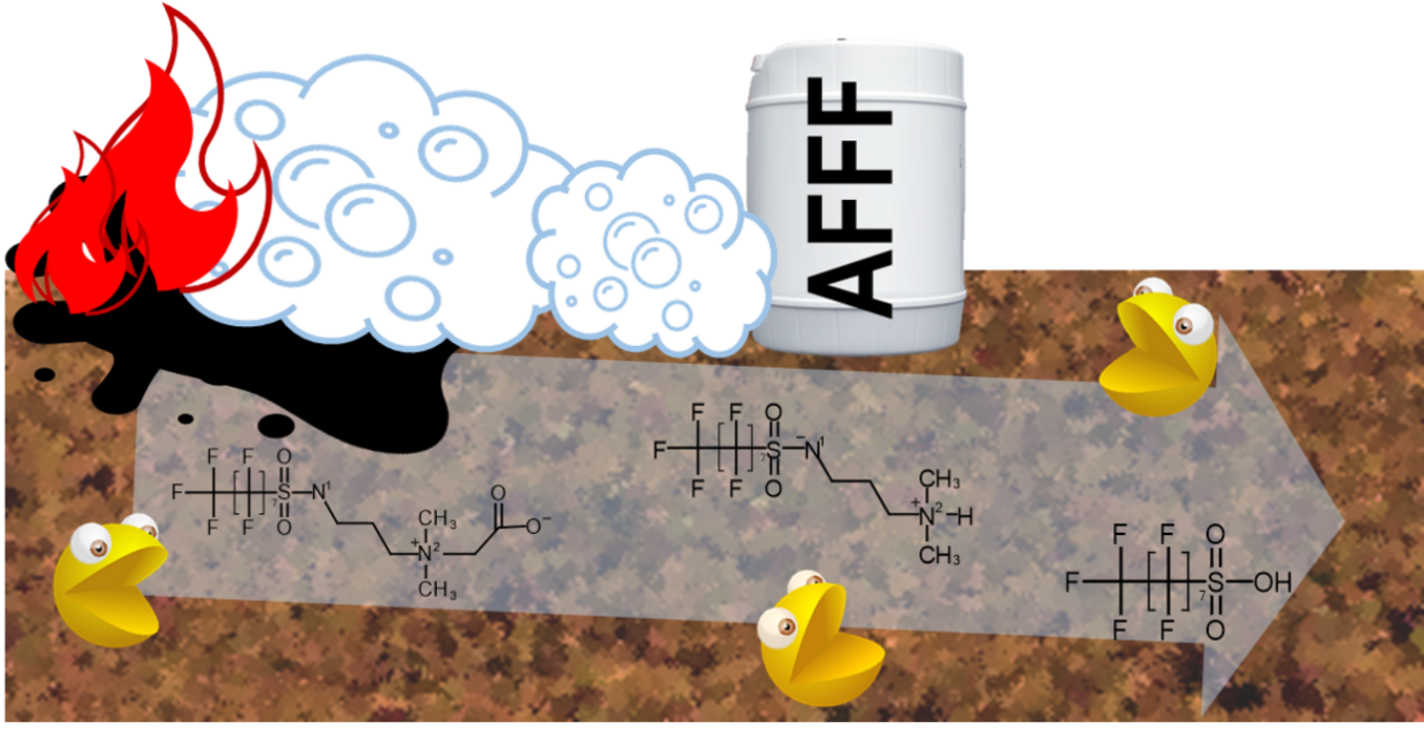
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