

Biotransforming the “Forever Chemicals”: Trends and Insights from Microbiological Studies on PFAS

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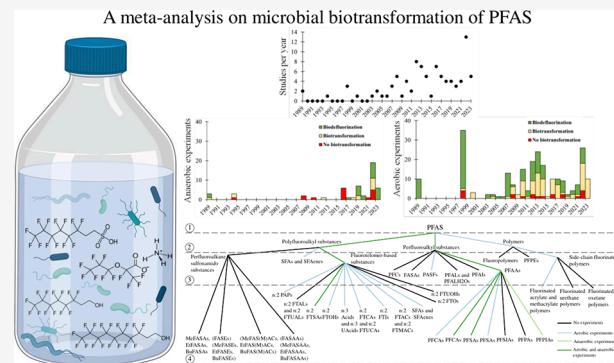
Supporting Information

ABSTRACT: Per- and polyfluoroalkyl substances (PFAS) are recalcitrant contaminants of emerging concern. Research efforts have been dedicated to PFAS microbial biotransformation in the hopes of developing treatment technologies using microorganisms as catalysts. Here, we performed a meta-analysis by extracting and standardizing quantitative data from 97 microbial PFAS biotransformation studies and comparing outcomes *via* statistical tests. This meta-analysis indicated that the likelihood of PFAS biotransformation was higher under aerobic conditions, in experiments with defined or axenic cultures, when high concentrations of PFAS were used, and when PFAS contained fewer fluorine atoms in the molecule. This meta-analysis also documented that PFAS biotransformation depends on chain length, chain branching geometries, and headgroup chemistry. We found that the literature is scarce or lacking in (i) anaerobic PFAS biotransformation experiments with well-defined electron acceptors, electron donors, carbon sources, and oxidation-reduction potentials, (ii) analyses of PFAS biotransformation products, and (iii) analyses to identify microorganisms and enzymes responsible for PFAS biotransformation. To date, most biotransformation research emphasis has been on 8:2 fluorotelomer alcohol (8:2 FTOH), 6:2 fluorotelomer alcohol (6:2 FTOH), perfluorooctanesulfonic acid (PFOS), and perfluorooctanoic acid (PFOA). A wide array of PFAS remains to be tested for their potential to biotransform.

KEYWORDS: PFAS, biotransformation, biodegradation, microbial, bacteria, bioremediation, perfluoroalkyl, polyfluoroalkyl, defluorination, meta-analysis

INTRODUCTION: PFAS, A BRIEF OVERVIEW AND THEIR ENVIRONMENTAL RELEVANCE

Per- and polyfluoroalkyl substances (PFAS) are contaminants of concern for human and environmental health because of their wide distribution, degradation recalcitrance, bioaccumulation tendencies, and toxicological effects.^{1–9} The Organisation for Economic Co-operation and Development (OECD) defines PFAS as fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon (C) atom (without any hydrogen, chlorine (Cl), bromine, or iodine atoms attached to it).¹⁰ The possibility of PFAS biotransformation or biodegradation is often dismissed out of hand: PFAS are the “forever chemicals”.^{11–13} This review revisits this “conventional wisdom” by synthesizing research outcomes from studies of the microbial biotransformation of diverse PFAS molecules. Here, we examine microbiological PFAS transformation studies, regardless of outcome, in order to synthesize PFAS biotransformation knowledge. Although reviews of PFAS biotransformation processes and pathways exist,^{14–33} to the authors’ knowledge, meta-analyses of microbial PFAS biotransformation have not been performed on the existing literature. Our overarching goal was to identify



the conditions under which PFAS biotransformation is known to or may occur. Overall, we document that PFAS biotransformation or lack thereof is dependent on a variety of factors including chain length, chain branching geometries, whether the molecule is fully saturated with fluorine (F) atoms, headgroup chemistry, among others. In general, PFAS molecules that are fully saturated and have longer C chains are less likely to be susceptible to microbial biotransformation.

PFAS are human-made substances used for surface coatings due to their surfactant properties.³⁴ The C–F bond is the strongest covalent bond and is considered the main factor limiting PFAS biotransformation.^{14,15,35–37} The three electron pairs that surround each F atom and the shielding of C atoms by F atoms render PFAS resistant to reactions with acids, bases, reductants, and oxidants.³⁴ A perspective on C–F

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		Chemical structure	Name	Culture used in biotransformation experiments
RCRA-regulated PFAS most researched PFAS	perfluoroalkyl ether carboxylic acid		2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (GenX)	wastewater aerobic activated sludge ^{106 (δ)} anaerobic defluorinating enrichment ^{106 (δ)}
			perfluorobutane sulfonic acid (PFBS)	wastewater sludges and sediments ^{80 (δ)} wastewater activated sludge ^{93 (δ)}
	perfluoroalkyl acid		perfluoroctanoic acid (PFOA)	<i>Acidimicrobium</i> sp. strain A6 or A6 enrichment ^{82 (γ), 88, 89} <i>Pseudomonas parafulva</i> strain YAB ⁶²
			perfluorooctane sulfonic acid (PFOS)	<i>Acidimicrobium</i> sp. strain A6 and A6 enrichment ^{82 (γ)} <i>Pseudomonas plecoglossicida</i> strain 2.4-D ⁸¹ <i>Pseudomonas aeruginosa</i> strain HJ4 ^{79 (γ)}
	fluorotelomer alcohol		8:2 fluorotelomer alcohol (8:2 FTOH)	<i>Pseudomonas butanovora</i> ⁶⁵ <i>Pseudomonas oleovorans</i> ⁶⁵ <i>Pseudomonas</i> sp. OCY4 and sp. OCW4 ⁶⁰
			6:2 fluorotelomer alcohol (6:2 FTOH)	<i>Pseudomonas butanovora</i> ^{65, 75} <i>Pseudomonas oleovorans</i> ^{65, 75} <i>Pseudomonas fluorescens</i> DSM 8341 ⁷⁵ <i>Mycobacterium austroafricanum</i> JOB-5 ⁷⁵ <i>Rhodococcus jostii</i> RHA1 ⁷⁷
	fluorotelomer sulfonic acid		6:2 fluorotelomer sulfonic acid (6:2 FTSA)	<i>Rhodococcus jostii</i> RHA1 ⁷⁷ <i>Gordonia</i> sp. NB4-1Y ⁹¹

Figure 1. Seminal PFAS and selected microbial cultures used in PFAS biotransformation or biodegradation experiments. (δ) GenX or PFBS has not yet been reported to biodegrade or biotransform. (γ) indicates that the claim of PFAS biotransformation has been disputed and not convincingly demonstrated and/or biotransformation results have not been reproduced by others.

chemistry depicted greater complexity of C–F bond cleavage due to variability in bond strength, location relative to other (non C–F) bonds, the relative hydrophobicity of perfluorocarbons, and the toxicity of the free fluoride ion (F^-), among other factors.²⁶

PFAS have various industrial purposes that involve lowering surface tension, facilitating foam spreading, and fire smothering. PFAS are used in the manufacture of a wide variety of consumer goods and aqueous film forming foams (AFFFs) used to extinguish hydrocarbon and solvent-based fires.^{38,39} As longer chain PFAS are undergoing increased scrutiny and regulation, there is a transition to incorporate more shorter-chain and branched PFAS into the product design. Further toxicological research is necessary to understand the human health and environmental implications of this transition.^{40–43} Perfluoroctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) production in the United States has been phased out, although importation continues.⁴⁴ In 2024, the US Environmental Protection Agency (EPA) added nine PFAS, their salts, and their structural isomers to its list of hazardous constituents in the Resource Conservation and Recovery Act (RCRA)—PFOA, FOS, perfluorobutanesulfonic acid (PFBS), hexafluoropropylene oxide-dimer acid (HFPO–DA, commonly known as GenX chemicals), perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS), perfluorodecanoic acid, perfluorohexanoic acid, and perfluorobutanoic acid.^{7,9,45} Additionally, the EPA has added final National Primary Drinking Water Regulation (NPDWR) for six PFAS at low ppt levels: PFOA, PFOS, PFHxS, PFNA, HFPO–DA, and mixtures containing two or more of PFHxS, PFNA, HFPO–DA, and PFBS.⁴⁶

PFAS are structurally and compositionally diverse and have been categorized broadly into perfluoroalkyl substances, polyfluoroalkyl substances, and polymeric substances.⁴⁷ Figure 1 depicts this categorization. Polymers are multifluorinated repeating moieties of many subclasses.⁴⁷ Perfluoroalkyl substances are compounds where F atoms occupy all binding

sites on the C chain, except for Cs associated with a functional group (e.g., the C–S bond of the sulfonic group in PFOS). Nonpolymer fluoroalkyl substances include perfluoroalkyl and polyfluoroalkyl substances. Polyfluoroalkyl substances are compounds that have at least two F atoms but are not fully saturated with Fs (e.g., 6:2 fluorotelomer alcohol (6:2 FTOH) and 8:2 fluorotelomer alcohol (8:2 FTOH) in Figure 1; see also Buck et al.⁴⁷). PFAS are surfactants because they are comprised of an oleophobic and hydrophobic perfluorocarbon tail and a hydrophilic charged head.³⁴

PFAS manufacture began in the 1940s.⁶ In the 1960s, various chemical manufacturers created formulations of PFAS-containing AFFFs to extinguish flammable-liquid fires.⁶ After the initial manufacture of PFAS for firefighting, a proliferation occurred across the consumer marketplace with PFAS incorporated into products such as water-repellent outdoor gear, nonstick cookware, and stain-resistant carpeting and furniture. It was estimated that up to 6900 t of PFAS were released between 1951 and 2004.⁴⁸ An estimate from 2017 forecasted a release of up to 6420 t of PFAS in the environment by 2030.⁴⁹

PFAS emissions exist in air, water, and soil and migrate between these media. Here, we focus on water and soil, as they are the media where PFAS microbial biotransformation is most likely to occur. PFAS emissions to surface water and groundwater include releases from manufacturing sites, use and disposal of PFAS-containing consumer products, firefighting/crash training facilities, wastewater facilities, and use of contaminated biosolids.^{48,50,51} Due to their low pK_a values (0.5–3.8), a majority of perfluoroalkyls exist as dissociated conjugate bases at the environmentally relevant pH range of 5 to 9.^{52,53} As PFAS have weak van der Waals interactions, they do not form strong intermolecular relationships with their surrounding phase and, thus, tend to move into the atmosphere.⁵⁴

Marine currents and aerosols transport PFAS over long distances due to their surfactant properties and accumulation

in upper sea surface microlayers.^{48,55} While K_{oc} values do not exist for many perfluoroalkyls, PFOA's $\log K_{oc}$ ($2.06 \text{ L kg}^{-1}_{oc}$) and PFOS's $\log K_{oc}$ ($2.57\text{--}3.1 \text{ L kg}^{-1}_{oc}$) indicate that they will not adsorb strongly to soil or sediment but will be moderately mobile in groundwater.^{48,50,56,57} Perfluoroalkyls migrate in groundwater rather than remain at the initial source location. In water, PFOS and PFOA have been shown to be stable, with pH-independent hydrolysis reaction half-lives of 41 and 92 year, respectively.⁵⁸ Fluorotelomer alcohols and perfluoroalkyl sulfonamides may transform to PFOA and PFOS in water or soil and, therefore, can be precursors or sources of these substances.^{59,60} We discuss PFAS biotransformation at length in later sections. PFAS contamination is ubiquitous and persistent, necessitating remediation.⁶¹ The cost-prohibitive and *ex situ* nature of engineered treatment technologies provides an opportunity to explore PFAS biotransformation for *in situ* remediation. Using PFAS biotransformation for *in situ* treatment would be beneficial because of the large number of PFAS-contaminated sites, the low cleanup limits established, and public health implications. Future successes in the development of foundational knowledge for PFAS biotransformation could lead to comprehensive watershed and organismal protection *via* nature-based sustainable approaches.

This study provides a synopsis and a meta-analysis of the PFAS microbial biotransformation literature up to 2023. Trends and promising developments in PFAS biotransformation research include a growing number of studies being published each year as the impetus from public health concern, regulation, and scientific knowledge grow. In the field of microbial PFAS biotransformation, a preponderance of studies has been conducted under aerobic conditions, but a few studies also documented anaerobic biotransformation of some PFAS. While PFAS were originally thought to be recalcitrant compounds, microbes able to biotransform PFAS aerobically or anaerobically have been identified; this makes it possible to begin to establish PFAS structure- and condition-dependent PFAS biotransformation relationships. We can possibly gain insights into PFAS defluorination using microbial dechlorination as a guide. Furthermore, we identify needed conventions of PFAS microbial biotransformation studies to produce foundational knowledge that should hasten the development of PFAS bioremediation technologies.

METHODS

We reviewed 97 published studies from 1989 to 2023. The surveyed studies contained 288 experimental conditions and researched the ability of microorganisms to biotransform or biodegrade more than 100 fluorinated compounds, most of which were PFAS. For the purposes of this analysis, we define experimental conditions as a unique experimental setup with independent results. Therefore, publications typically have multiple experimental conditions. Examples of setup changes that were considered as unique experimental conditions within one publication include changes in fluorinated compound tested, changes in fluorinated compound concentration, addition of nonfluorinated substrates, or incubation with a different culture, among others.

Approximately 79% of the included studies used PFAS. The remaining studies used simple fluorinated substances such as fluorobenzene, sodium fluoroacetate, and 1-fluorodecane, among others. While PFAS are the focus of this meta-analysis, biotransformation studies of simple fluorinated compounds may also provide insights into conditions for PFAS microbial

biotransformation.⁶² To facilitate comparisons between studies, we converted aqueous PFAS concentrations to mM, converted dosages expressed as mass-to-mass ratio to mg kg⁻¹ of soil or sludge, and converted incubation time to days (d). The concentration of F⁻ released during incubation (mM) was used to calculate a total F⁻ released (%) relative to the original fluorinated substance.

We used qualitative parameters (e.g., aerobic and anaerobic incubation conditions) to divide the experimental conditions into subclasses. For the purposes of this study, we defined biotransformation as the change in the bond structure or redox state of a molecule. We defined biodegradation as the breakdown of C bonds within a molecule. In our analyses, we categorized PFAS to be biotransformed when the original publication asserted that biotransformation occurred from evidence of (i) a decrease in the concentration of the tested PFAS or (ii) detection of transformation products. We categorized a PFAS to be biodeflourinated when criteria (i) and (ii) were met but also when there was (iii) an increase in F⁻ concentration and/or (iv) a mass balance demonstrating biodeflourination. The meta-analysis yielded 236 experimental conditions as aerobic and 52 experimental conditions as anaerobic from 97 studies. We used a student's *t* test to search out statistical differences between quantitative categorizations, where $p > 0.05$ = not significant (NS), $p < 0.05 > 0.01 = *$, $p < 0.01 > 0.001 = **$, and $p < 0.001 = ***$. The parameters extracted from all studies are listed in Table S1 in the Supporting Information.

RESULTS AND DISCUSSION

This meta-analysis of PFAS biotransformation was conducted with the objective of identifying trends in experimental conditions linked with promising biotransformation and biodeflourination outcomes. Of the 288 experimental conditions reviewed in 97 studies, 82% were set up under aerobic conditions. The most frequently tested PFAS were 8:2 FTOH (11 studies),^{59,60,63–70} 6:2 FTOH (9 studies),^{65,67,71–77} PFOS (7 studies),^{78–84} PFOA (10 studies),^{36,62,82–89} and 6:2 FTSA (4 studies).^{77,80,90,91} Only 7% of analyzed studies investigated biotransformation of mixtures of PFAS.^{92–97} The focus on PFAS biotransformation in aerobic conditions is opposite to the intense focus on dehalogenation of chlorinated compounds under anaerobic conditions.⁹⁸

Figure 2 summarizes the temporal trends, experimental conditions (aerobic or anaerobic), and outcomes in the PFAS microbial biotransformation literature. Most of the reviewed studies (82%) documented the biotransformation of fluorinated chemicals (Figure 2A-C), but a concentration of F⁻ released was reported in only 41% of the studies. Evidence for biotransformation in the studies without concentrations of F⁻ included decreases in PFAS concentrations, the detection of fluorinated transformation products, and indicators of microbial growth. As of June 2023, F⁻ released from polyfluorinated compounds, including PFAS, at $\geq 90\%$ was described in 4 out of 28 studies (Figure 2B–C).^{78,99–101} In contrast, 14 publications (22 experimental conditions) showed $> 90\%$ F⁻ release for monofluorinated compounds under aerobic conditions (Figure 2B–C).

Aerobic Studies with Some Documentation of Biotransformation. Of the 236 aerobic microbial biotransformation experimental conditions, 92% documented biotransformation of fluorinated molecules in live treatments and no biotransformation in sterile controls (Figure 2B). This

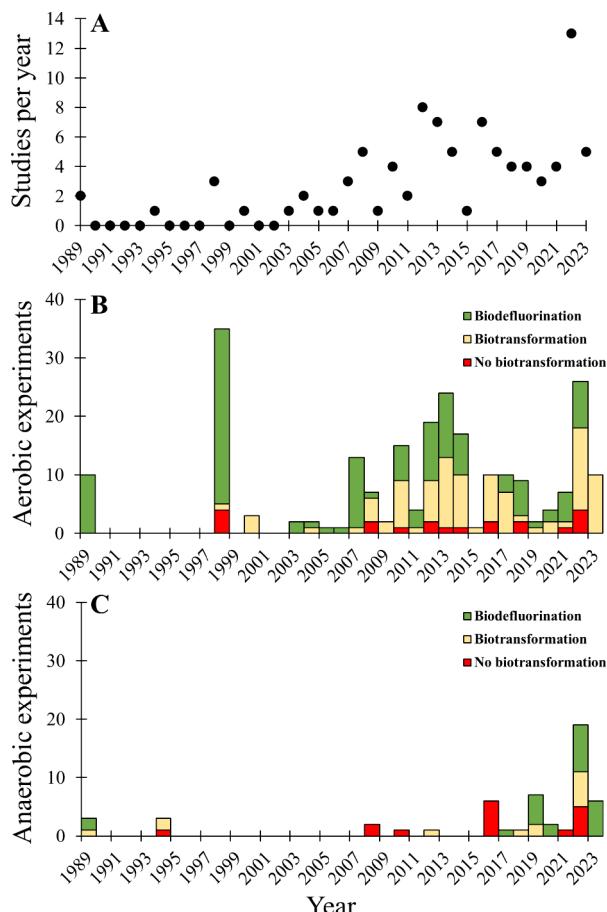


Figure 2. (A) Publications in which fluorinated compounds (PFAS and non-PFAS) were investigated for their potential to biotransform. Outcome of fluorinated compounds (mostly PFAS) biotransformation under (B) aerobic conditions (aerobic studies) and (C) anaerobic conditions (anaerobic studies).

preponderance of biotransformation points to a need to move away from viewing PFAS as “forever chemicals”. Of the studies that achieved F⁻ release with PFAS containing 3–17 F atoms, the average release was $45\% \pm 37\%$ of the total possible F⁻ concentration, and 29 experimental conditions documented F⁻ release of at least 90%. In studies with PFAS containing greater than 5 F atoms, only 27 experimental conditions documented F⁻ release (2-amino-5-(pentafluorosulfanyl)phenol, 4:2 fluorotelomer alcohol (4:2 FTOH), 6:2 FTOH, 6:2 fluorotelomer sulfonate (6:2 FTS), 6:2 fluorotelomer unsaturated carboxylic acid (6:2 FTUCA), 8:2 FTOH, 4,4,4-trifluoro-3-(trifluoromethyl)crotonic acid (MeUC 5d), PFOA, and PFOS).^{59,60,65,71,76–78,81,87,102–106} The highest F⁻ released from these experimental conditions was 80%, while the average was $17\% \pm 27\%$.^{59,60,65,71,76–78,81,87,102–106}

The first documentation of aerobic perfluoroalkyl acid biotransformation occurred with *Pseudomonas* sp. strain D2. Strain D2 was reported to biotransform 1H,1H,2H,2H-perfluoroctanesulfonate (H-PFOS) to six volatile transformation products containing oxygen and F but not sulfur.⁷⁸ However, PFOS was not biotransformed by strain D2. *Pseudomonas plecoglossicida* 2.4-D was later shown to use PFOS as a sole source of C with complete utilization in 6 d while transforming PFOS to perfluoroheptanoic (perfluoroenanthic) acid and releasing F⁻.⁸¹ Additionally, *Delftia acidovorans*, a

microorganism with several haloacid dehalogenases identified in its genome, can grow with PFOA as a sole C source.⁸¹

Aerobic fluorotelomer-based substance biotransformation has also achieved significant breakthroughs. 8:2 FTOH was found to be biologically defluorinated and mineralized under conditions that may occur in wastewater treatment plants forming shorter-chain metabolites.⁵⁹ Research with 8:2 FTOH showed that *Pseudomonas* species OCY4 and OCW4 transformed 8:2 FTOH using octanol as a C source, possibly via a cometabolic process.⁶⁰ A successive study on 6:2 FTOH showed a short half-life (<2 d) and indicated that alternate biotransformation pathways from those for 8:2 FTOH were likely preferential, removing multiple –CF₂– groups.⁷¹ The same research team then compared 6:2 FTS biotransformation to 6:2 FTOH.¹⁰² Results showed that 6:2 FTS was biotransformed by activated sludge from wastewater treatment plants at a lower rate (63.7% of 6:2 FTS concentration remaining at 90 days), possibly due to slow aerobic desulfonation. It was concluded that 6:2 FTS is an unlikely major source of perfluorinated transformation products in wastewater effluent.¹⁰²

Dietzia aurantiaca J3 was also shown to biotransform 6:2 FTS with alkanesulfonate monooxygenase and alkanesulfonate ABC transporters upregulated in the presence of 6:2 FTS.¹⁰⁴ A study with *Rhodococcus jostii* RHA1 demonstrated defluorination and desulfonation of 6:2 FTSA.⁷⁷ Desulfonation of 6:2 FTSA was a requisite prior to defluorination, and a higher expression of alkane monooxygenase was linked to concurrent biotransformation of 6:2 FTOH.⁷⁷ The understanding of fluorotelomer alcohol biotransformation was also enhanced by studies with *Pseudomonas butanovora* (butane oxidizer) and *Pseudomonas oleovorans* (octane oxidizer). These strains defluorinated 4:2 FTOH, 6:2 FTOH, and 8:2 FTOH with a greater defluorination for 4:2 FTOH.⁶⁵

Incubation periods in aerobic studies ranged from 3 h (0.125 d) to 730 d, with an average incubation period of 36 ± 80 d. Contaminant concentrations ranged over several orders of magnitude: 3 to 1000 μmol PFAS g⁻¹ soil and 3 nM to 20 mM PFAS in water. In studies employing a mixed culture or a microbiome, the microorganisms responsible for PFAS biotransformation were only identified in seven studies.^{70,76,107–111} Microbes from 19 genera have been identified to biotransform fluorinated compounds under aerobic conditions (Table S2). Based on the number of experimental conditions, the most studied genus was *Pseudomonas*, followed by *Rhodococcus* and *Labrys* (Table S2). Biotransformation of PFAS by mixed cultures has been observed, but the microbial community was not analyzed or reported. Therefore, those studies were not included in Table S2.^{59,60,63,64,66,67,73,74,90,92,95,96,105,112–123}

Aerobic Studies without Biotransformation. Compounds not biotransformed under aerobic conditions in surveyed studies included trifluoromethanesulfonic acid (TFMS), PFOS, PFOA, EtFOSE-based phosphate diester (SAmPAP Diester), perfluoropropionic acid (PFPrA), 3H-perfluoro-3-[(3-methoxy-propoxy)propanoic acid (3H-PFPrA), toluene-2,4-di(8:2 fluorotelomer urethane), perfluoropentanoic acid (PFPeA), Gen X, and three PFAS mixtures.^{36,75,78,80,85,93,95–97,105} In the aerobic subset of the database, the compound with the highest F content had 34 F atoms per molecule (8:2 fluorotelomer urethane), and the average F number was 18.7. Not counting the PFAS mixtures,^{93,96,97} the average molecular weight was 352 g⁻¹.

mol. Compared to the average molecular weight of 291 g mol⁻¹ of all the fluorinated compounds in this meta-analysis, these nonbiotransformed molecules had higher F content and molecular weight. Figures 3A and S1 illustrate that PFAS with a higher molecular weight are less likely to be defluorinated aerobically.

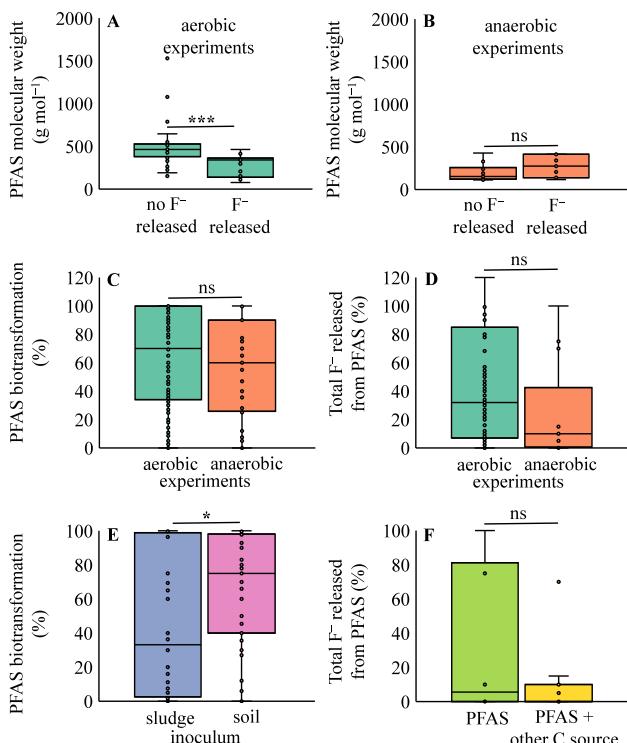


Figure 3. Experimental outcomes for biotransformation of PFAS and other fluorinated compounds as a function of (A–D) aerobic/anaerobic conditions, (E) source of microbial inoculum (either sludge or soil), and (F) absence/presence of an additional carbon (C) source in anaerobic experiments. $p > 0.05$ = not significant (ns), $p < 0.05 > 0.01$ = *, $p < 0.01 > 0.001$ = **, $p > 0.001$ = ***. The corresponding references for each panel from which the data were extracted for analyses are shown in Table S4.

Anaerobic Studies with Documentation of Biotransformation. PFAS-biotransformation studies under anaerobic conditions are relatively few: 52 anaerobic experimental conditions were surveyed, with 72% having some documentation of PFAS biotransformation and 46% showing a concentration of F⁻ was released during incubation (Figure 2C).^{82,84,88,89,105,124–132} The most frequently studied compounds were PFOA (10 experimental conditions), PFOS (4 experimental conditions), and 6:2 FTSA (5 experimental conditions). The incubation periods ranged from 1.25 to 341 d, with an average incubation period of 90 ± 93 d. Of the 37 anaerobic studies documenting biotransformation, inocula included axenic bacterial cultures (10 studies), soils (7 studies), reductively dechlorinating mixed microbial cultures (11 studies), a Feammax culture (6 studies), and wastewater activated sludges (3 studies). Table S3 tabulates the microorganisms associated with the biotransformation of PFAS in anaerobic conditions. Studies utilizing pure or enrichment cultures included *Pseudomonas oleovorans* and *Pseudomonas butanovora*, *Acidimicrobium* sp. strain A6, the reductively dechlorinating culture KB-1, and a mixed culture derived

from an AFFF site. Only a few examples are available for anaerobic PFAS-degrading mixed cultures.^{69,82,105,129} Three anaerobic studies with mixed cultures included analyses of the microbial community composition. However, putative defluorinating microbes were not identified, and, thus, those studies were not included in Table S3.^{105,131,133}

In a study where culture KB-1, an enrichment culture containing *Dehalococcoides mccartyi*, was tested under anaerobic conditions, incubations achieved >90% removal for (E)-perfluoro(4-methylpent-2-enoic acid) (PFMeUPA) (14% biodeflourination) and 4,5,5,5-tetrafluoro-4-(trifluoromethyl)-2-pentenoic acid (FTMeUPA) (4–5% biodeflourination) in 150 and 70 d, respectively.¹³¹ *Geobacter* and *Dehalobacter* were assessed for their involvement in biodeflourination.¹³¹ *Geobacter* showed no growth in PFMeUPA-fed culture, but *Dehalobacter* exhibited growth in PFMeUPA/FTMeUPA-fed culture. When the defluorination capabilities of *Dehalobacter restrictus* strain PER-K23 were examined, F⁻ was not released from PFMeUPA and FTMeUPA.¹³¹ Culture KB-1 was also evaluated in a study on the importance of C double bonds in microbial defluorination of unsaturated per- and polyfluorinated carboxylic acids.¹⁰⁵ It was found that α,β -unsaturation was key for reductive defluorination and hydrogenation of fluorinated carboxylic acids. Additionally, reductive defluorination was less favorable than hydrogenation of the double bonds, and unsaturated fluorinated carboxylic acid structures with trifluoromethyl ($-\text{CF}_3$) branches at the α/β -carbon had greater degradability.¹⁰⁵ Finally, a culture derived from an AFFF-containing site was shown to transform 65–84% of the concentration of 6:2 fluorotelomer thioether amido sulfonate (6:2 FtTAoS at 20 μM) in 300 days under nitrate-reducing conditions, while 6:2 FtTAoS biotransformation was slower under sulfate-reducing conditions.¹³³ These recent studies, which are breakthroughs in documenting PFAS biotransformation and defluorination under anaerobic conditions (Figure 2C),^{69,82,84,88,89,131,133} may enable tailored design of degradable fluorinated compounds as alternatives to existing PFAS.

Anaerobic Studies without Biotransformation. Of all anaerobic experiments, 29% did not achieve biotransformation.^{80,85,90,93,105,125} The range of F atoms in these PFAS was between 3 and 17 per molecule (average 9.6 F per molecule). The molecular weight of PFAS compounds tested under anaerobic conditions where biotransformation was not recorded ranged from 113 to 500 g mol⁻¹ (avg. 349 g mol⁻¹), which is above the average molecular weight of 291 g mol⁻¹ (Figure 3B). The molecular weight is an important metric as molecules with a higher molecular weight may have longer C chains and therefore also more Fs. In contrast to aerobic studies, anaerobic conditions did not show a statistically significant relationship between molecular weight and extent of defluorination (Figure 3B).

PFAS Category-Specific Biotransformation Trends. PFAS chemical structure affects biotransformation, but only a few PFAS subtypes have been extensively studied. Analyses by PFAS subtype could identify structural factors relevant to enhancing or suppressing biotransformation or biodegradation. Figure 4, which illustrates whether PFAS subtypes have been studied for their biotransformation potential, highlights that microbial biotransformation experiments have not been conducted for about 50% of the categories (shown in black lines, Figure 4). This is consistent with the nascent nature of this field and underscores that thousands of PFAS have yet to be assessed for microbial biotransformation.

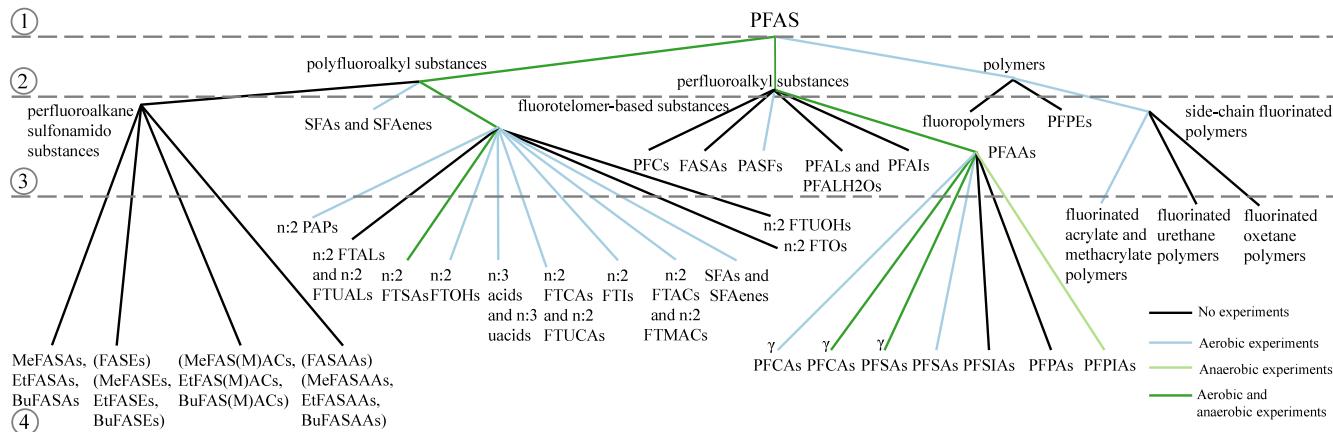


Figure 4. PFAS categorization (levels 1 through 4) and their state of microbial biotransformation evaluation. Biotransformation studies conducted under aerobic conditions (blue lines), anaerobic conditions (light green lines), or both (dark green lines) are shown. Black lines signify the absence of published attempts at microbial biotransformation. Categories (level 1 through 4) adhere to chemical nomenclature literature.⁴⁷ (γ) signals the inclusion of data from studies where the claim of PFAS biotransformation has been disputed or not convincingly demonstrated and/or biotransformation results have not been reproduced by others. Acronyms are defined as follows: BuFASA = *N*-butyl perfluoroalkane sulfonamide, BuFASAA = *N*-butyl perfluoroalkane sulfonamido acetic acid, BuFASE = *N*-butyl perfluoroalkane sulfonamido ethanol, BUFA(M)AC = *N*-butyl perfluoroalkane sulfonamidoethyl acrylate and methacrylate, EtFASA = *N*-ethyl perfluoroalkane sulfonamide, EtFASAA = *N*-ethyl perfluoroalkane sulfonamido acetic acid, EtFASE = *N*-ethyl perfluoroalkane sulfonamido ethanol, EtFAS(M)AC = *N*-ethyl perfluoroalkane sulfonamidoethyl acrylates and methacrylates, FASA = perfluoroalkane sulfonamide, FASAA = perfluoroalkane sulfonamido acetic acid, FASE = perfluoroalkane sulfonamido ethanol, MeFASA = *N*-methyl perfluoroalkane sulfonamide, MeFASAA = *N*-methyl perfluoroalkane sulfonamido acetic acid, MeFASE = *N*-methyl perfluoroalkane sulfonamido ethanol, n:2 FTAC = n:2 fluorotelomer acrylate, n:2 FTAL = n:2 fluorotelomer aldehyde, n:2 FTCA = n:2 fluorotelomer carboxylic acid, n:2 FTI = n:2 fluorotelomer iodide, n:2 FTMAC = n:2 fluorotelomer methacrylate, n:2 FTO = n:2 fluorotelomer olefin, n:2 FTOH = n:2 fluorotelomer alcohol, n:2 FTUAL = n:2 fluorotelomer unsaturated aldehyde, n:2 FTUCA = n:2 fluorotelomer unsaturated carboxylic acid, n:2 FTUOH = n:2 fluorotelomer unsaturated alcohol, n:2 PAP = n:2 fluorotelomer phosphate, n:3 acids = n:3 saturated acid, n:3 uacid = n:3 unsaturated acid, PASF = perfluoroalkane sulfonyl fluoride, PFAA = perfluoroalkyl acid, PFAI = perfluoroalkyl iodide, PFAL = perfluoroalkyl aldehyde, PFALH2O = perfluoroalkyl aldehyde hydrate, PFCA = perfluoroalkyl carboxylic acid and perfluoroalkyl carboxylate, PFPA = perfluoroalkyl phosphonic acid, PFPIA = perfluoroalkyl phosphinic acid, PFSA = perfluoroalkyl sulfonic acid and perfluoroalkanesulfonate, PFSIA = perfluoroalkyl sulfenic acid, SFA = semifluorinated *n*-alkane, SFAene = semifluorinated *n*-alkene.

Table 1. Comparison of Experimental Outcome for PFAS Categorized as n:2 FTOH

PFAS	Experimental conditions (#)	% experimental conditions with biotransformation	Avg. % biotransformation achieved	% experimental conditions with biodeflourination	Avg. % F ⁻ released	Reference
4:2 FTOH	4	100	Not available	100	9	75
6:2 FTOH	14	100	53	43	19	65, 67, 71–77, 134, 135
8:2 FTOH	26	100	49	17	17	59, 60, 63, 64, 66, 67, 134, 136

Figure 4 divides PFAS into secondary (polyfluoroalkyl substances, perfluoroalkyl substances, polymers), tertiary (i.e., fluorotelomer-based substances, side-chain fluorinated polymers), and quaternary (i.e., perfluoroalkyl carboxylic acids (PFCAs), n:2 fluorotelomer phosphates (n:2 PAPs)) categories. While all secondary categories have been explored to some degree, seven tertiary and 11 quaternary categories remain unexamined (Figure 4). Of the PFAS categorizations that have been explored, research has been focused on fluorotelomer substances and perfluoroalkyl acids (PFAAs). Table S5 presents the outcomes of fluorotelomer substances under experimental conditions. In the following sections, we describe microbial biotransformation trends first by category and then by specific substances nested within that category. Due to the complexity of PFAS categorizations and data limitation, we focused the analysis on categories and substances that appear more frequently in the literature.

Biotransformation of PFAS Categorized as n:2 Compounds. PFAS categorized as n:2 compounds have C chains of varying lengths (n) and differing head groups (e.g.,

alcohol, urethane). We compared studies with the same headgroup (FTOHs) at different C chain lengths to evaluate the effect of C chain length on biotransformation. PFAS as n:2 FTOHs tested for microbial biotransformation ranged in length from four C (4:2) to eight C (8:2). Our comparison showed that % F⁻ released decreases with increasing C chain length (Table 1). All 4:2 FTOH and 6:2 FTOH experiments were performed under aerobic conditions. Of the 26 experimental conditions with 8:2 FTOH, 23 were performed under aerobic conditions. The three 8:2 FTOH conditions carried out anaerobically were from a single study where 8:2 FTOH was shown to biotransform but the concentration of F⁻ was not measured.⁶⁹ 8:2 FTOH biotransformation under nitrate-, sulfate-, and iron-reducing conditions revealed differences in biotransformation products and extent of biotransformation of 8:2 FTOH. Nitrate-reducing conditions resulted in the greatest 8:2 FTOH biotransformation and more diverse biotransformation products.⁶⁹

We also compared biotransformation at a set chain length with different head groups to assess how head groups affect

biotransformation. When comparing 8:2 compounds that contain 17 F (8:2 FTCA, 8:2 FTSA, and 8:2 FTUCA) to 8:2 FTOH, the non-8:2 FTOH compounds achieved 68–100% biotransformation, while 8:2 FTOH was less biotransformed (59% avg.).^{113,114,117} 8:2 FTOH appears to be biotransformed to a lesser extent than other 8:2 compounds due to the alcohol moiety. These trends in PFAS categorized as n:2-compounds reinforce that recalcitrance to biotransformation increases with chain length and, thus, the number of F atoms. However, how the headgroup of PFAS influences biotransformation for a set C chain length is a topic just beginning to be explored.^{77,91,113,122,132} There is a pressing need for analyses of how similar PFAS are biodegraded depending on C chain length, F saturation, head groups, and bond placement.¹⁰⁵

Biotransformation of PFOS and PFOA. Comparing PFOS and PFOA biotransformation is important because these EPA-regulated PFAS are considered to be dead-end transformation products from the thousands of PFAA precursor compounds in commerce.¹³⁷ PFOS and PFOA may be rate-limiting for PFAS biotransformation. To date, multiple studies reported no biotransformation for PFOA and PFOS.^{36,78,80,85,86} Though considered “forever” dead-ends, some evidence from the literature suggests that PFOA and PFOS can biotransform, and, in some cases, they can defluorinate.^{62,81,83,84,87–89} Transformation products of PFOA and PFOS identified to date include PFBS, PFHxS, heptafluorobutyric acid (HFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), and perfluoroheptanoic acid (PFHpa).^{84,87–89} The literature shows that PFOA is biotransformed more frequently than PFOS (Table S7). However, the extent of biotransformation (% PFAS transformed) and microbial defluorination (% F[−] released) was not significantly different when comparing PFOA to PFOS (Table S7).

While PFOA and PFOS biotransformation is the current frontier in this field, multiple seminal studies have received critiques about their methodologies and conclusions.^{79,82,138–141} For the advancement of PFAS microbial treatment, it is important that researchers incorporate rigorous variable-isolating experimental designs and appropriate analytical methodologies. A study reported that PFOS biotransformation occurred under aerobic conditions in experiments with *Pseudomonas aeruginosa* strain HJ4,⁷⁹ but these results were later contested^{138,139} and have yet to be reproduced by others. *Acidimicrobium* sp. strain A6 was reported to degrade PFOA and PFOS with a buildup of shorter-chain perfluorinated transformation products and release of F[−].⁸² *Acidimicrobium* sp. strain A6 is a Feammonox autotroph that anaerobically oxidizes ammonium to nitrite while reducing ferric iron.⁸² Strain A6 can use either ammonium or H₂ as the electron donor.⁸² The tested PFOA concentrations were degraded at 50% (0.12 mM PFOA initial) by the A6 enrichment culture and 33% (0.08 mM PFOA initial) by strain A6 pure culture in 100 d.⁸² The A6 enrichment culture was reported to degrade the concentration of PFOS by 47% (initial of 0.093 mM), while the pure A6 culture degraded 23% of the initial (0.045 mM) PFOS concentration in 100 d.⁸² *Acidimicrobium* sp. strain A6 was also tested in a microbial electrolysis cell and in biosolids; results showed 60% and 70% transformation of 100 mg L^{−1} PFOA in 18 and 150 d, respectively.^{88,89} Experimental studies with *Acidimicrobium* strain A6 were followed by modeling studies

comparing PFOA degradation kinetics for the following conditions: differing PFOA concentrations, varying abundance of strain A6, and semicontinuous growth conditions.⁸⁴ Coupling the experiments with modeling led to an interpretation that the rate of PFOA defluorination was proportional to the ammonium-oxidation rate. The rates of defluorination and ammonium oxidation increased monotonically in a nonlinear fashion with greater abundance or biomass of strain A6.⁸⁴ While promising, there is current contention over the veracity and replicability of PFOA and PFOS biotransformation by *Acidimicrobium* sp. strain A6. Concerns have been raised about possible complicating factors regarding abiotic removal via adsorption of PFAAs to ferrihydrite, the unvalidated use of a TOC analyzer to track C mass balance for PFAAs, and the implausibility of the proposed microbial degradation mechanisms.¹⁴⁰

Analyses of PFAS Structure-Dependent Biotransformation. To extract structure dependent relationships to biotransformation, we first compared the PFAS categories, PFAAs and fluorotelomer-based substances. Fluorotelomer-based substances exhibited significantly greater biotransformation (%) than PFAAs (Figure S3). Second, we compared subcategories within the PFAAs (PFCAs, PFPiAs, and PFSAs) and fluorotelomer-based substances (n:3 acids, n:2 FTCSAs, n:2 FTCAs, n:2 FTOH) (see Figure S4). We found that there are no statistically significant differences between PFAS biotransformation (%) and F[−] released (%) for the PFAA subcategories, PFCAs, PFPiAs, and PFSAs (Figure S4). Within the fluorotelomer-based substance subcategories, n:2 FTCSAs showed greater biotransformation (%) than n:3 acids, n:2 FTCAs exhibited greater biotransformation (%) than n:2 FTOHs, and n:2 FTOHs displayed a greater F[−] release (%) than any of the other tested fluorotelomer subcategories (Figure S4). The latter results may be, in part, a consequence of the disproportionate research efforts in n:2 FTOH biotransformation compared to the other fluorotelomer-based substance subcategories.

Fluorotelomer substances with a set C chain length and number of F have different functional groups, e.g., alcohol, iodides, sulfonate, and carboxylic acids (Figure S5). Only n:2 compounds could be investigated for a relationship between biotransformation and functional groups at a set C chain length and F atom number (Figure S5). Statistical analyses showed no significant differences in biotransformation (%) or F[−] released (%) for 6:2 compounds. 8:2 FTOH had greater transformation (%) than 8:2 FTS, but no significant differences in F[−] released (%) were noted for 8:2 substances as 8:2 FTOH (Figure S5). These are first glimpses into the complex structure-dependent biotransformation of PFAS and should not be viewed as prescriptive.

Overall Trends in PFAS Biotransformation. Our meta-analysis of the PFAS literature sought trends in successes and lack thereof for biotransformation of PFAS. The following are some overall trends, which may help elucidate experimental next steps. Of the experimental conditions reviewed, 82% were aerobic, with only 18% being anaerobic. PFAS was biotransformed and biodeflourinated in 92% and 51% of the aerobic studies, respectively. Anaerobic studies documented PFAS biotransformation and microbial defluorination in 71% and 46% of the studies, respectively. While the overall percentages are similar, F[−] release was documented much more frequently in aerobic studies (Figure 3D). Further research is necessary to substantiate that PFAS biotransforma-

tion and, especially, biodeflourination are faster and more complete under aerobic conditions.

Experiments using cultures isolated/enriched from soils rather than sludges had a higher PFAS biotransformation (Figure 3E), which may provide guidance for using soils as good sources of PFAS biotransforming microorganisms. According to the database used in our study, only a few anaerobic studies have shown F^- release when an organic electron donor was provided (Figure 3F).^{105,131} The only studies to demonstrate F^- release and PFAS reductive defluorination and hydrogenation under anaerobic conditions in the presence of an additional organic donor used KB-1 culture and either lactate or acetate.^{105,131} The comparison in Figure 3F includes data from different PFAS (e.g., different chemical structures) with and without an additional C source. A comparison of specific PFAS (e.g., same chemical structure) with and without an additional C source will provide critical insights into the role of non-PFAS C sources on PFAS fate and biotransformation. Such comparative analysis was not possible at the time of writing this manuscript due to the scarcity of data.

Conditions that correlated with a higher likelihood of or more extensive biotransformation were aerobic conditions, pure/defined cultures, higher concentrations of PFAS, and fewer F atoms in the fluorinated substance. Nonideal conditions for PFAS biotransformation—i.e., a lower likelihood of biotransformation or biodeflourination—included anaerobic conditions, mixed cultures, a heavily fluorinated substrate at a low concentration, and the presence of an additional nonfluorinated C source. PFAS with a higher number of F atoms exhibited significantly less transformation (%) and less total F^- released (%) (Figure S2). For metabolic PFAS biotransformation, the presence of a nonfluorinated C source may decrease the likelihood of PFAS utilization as a C source, as microbes may preferentially utilize the nonfluorinated, labile C sources. For cometabolic reactions where a non-PFAS C source or electron donor is required (one compound serving as both the C source and electron donor), the type of growth substrate needed to elicit the production of PFAS-relevant cometabolism enzymes is the subject of much current research.^{33,60,77,85,109,132}

We noted that significant differences exist between PFAS biotransformation (%) and total F^- released (%) for well-studied PFAS categories (n:2 FTOH, n:2 FTCA, n:2 FTSA, n:3 acid, PFCA, PPPIA, PFSA) (Figure S2). Fluorotelomer substances have significantly higher biotransformation (%) than do PFAAs (Figure S3). Within fluorotelomer substances, n:2 FTOH was the subcategory with the highest degree of biotransformation (Figure S4). We were not able to determine which functional group results in an increased likelihood of biotransformation for compounds of a set C chain length and number of Fs (Figure S5). Importantly, these trends are derived from the existing literature surveyed for the purpose of this study. Due to the limitations of performing a meta-analysis on diverse and complex multivariable research, we cannot, with certainty, attribute differences in biotransformation potential to a single factor. The trends highlighted in this study are meant to be informative of factors which appear to be important in determining PFAS biotransformation potential. Further experimentation that employs a wider range of redox conditions and more effectively explores the structure-dependent relationships of PFAS defluorination surely will lead to reassessment or revisions of these trends.

The observed trends and the bacteria known to biotransform PFAS (Tables S2 and S3) make it clear that our understanding of PFAS biotransformation is deficient. A systematic approach to studying PFAS biotransformation is needed to avoid experimental repetition while gleaning the most information from each experimental condition.

Comparison of Derived PFAS Trends to Microbial Dechlorination. As Cl and F are halogens, dechlorination trends may provide insights into which defluorination experiments might yield greater F^- release. Microbial dechlorination is a much more developed field than microbial defluorination due to chlorinated organics being long-standing regulated contaminants. Furthermore, the strength of C–Cl bonds is small compared to C–F bonds.^{20,24,98,142} Microbial dechlorination processes frequently applied for the bioremediation of chlorinated compounds, such as chlorinated ethenes, include reductive dechlorination in anaerobic conditions and cometabolism in aerobic conditions.

Reductive dechlorination rates and extent have been documented to diminish because of an insufficient electron-donor supply, competition for hydrogen (H_2) from the activity of other terminal electron-accepting microbial processes, a paucity of the specialized bacteria capable of dechlorinating some intermediates, and the presence of inhibitory compounds, among others.^{98,143–146} Complete reductive dechlorination occurs only under highly reducing conditions: sulfate-reducing or methanogenic.^{143,147} In aerobic conditions, the following factors can limit cometabolic dechlorination: increasing Cl saturation, too little dissolved oxygen, toxicity of transformation products, and competitive inhibition by co-contaminants or cometabolites.^{148,149}

Biotransformation of PFAS may depend on some of the same factors. For example, recent studies demonstrated PFAS defluorination by reductively dechlorinating cultures and by either stimulation or inhibition of parallel reductive dechlorination, depending on the microbial culture used and PFAS concentration.^{105,131,145,150–152} This uncertainty of PFAS's effect on reductive dechlorination complicates bioremediation implementations as many contaminated sites have chlorinated contaminants and PFAS present. The value of reductive dechlorination makes plain the importance of further PFAS biotransformation research under anaerobic conditions. The small literature on PFAS biotransformation under anaerobic conditions generally does not well define the redox conditions. As anaerobic microbial PFAS biotransformation may occur under a limited range of oxidation–reduction potential, defining, monitoring, and purposefully altering such conditions during experimentation may lead to breakthroughs and more easily reproducible results. (Homo)acetogenesis and methanogenesis are processes typically co-occurring and/or competing with reductive dechlorination for hydrogen.^{144,146,153} PFAS studies have not yet directly assessed the effects of PFAS on acetogenesis and methanogenesis.

While reductive dechlorination is the most prevalent process in the microbial dechlorination literature, studies employing aerobic conditions dominate the PFAS microbial biotransformation literature. Aerobic metabolic dechlorination is not widely deployed for bioremediation, but aerobic cometabolism-based dechlorination is widely researched. If aerobic microbial defluorination processes reflect aerobic microbial dechlorination processes, then aerobic PFAS cometabolism should be a significant research avenue. PFAS-cometabolism studies are increasing and cometabolism may be a promising

avenue for PFAS defluorination breakthroughs.^{33,60,77,85,109,132} A review argues for the importance of PFAS biotransforming cometabolic reactions by raising concerns about published metabolic defluorination pathways.³³ One nonmetabolic reaction is the desulfonation of sulfur-containing precursors under sulfur-limiting conditions, most likely through a hydrolysis reaction without energy yield.³³ As most sites have relatively low PFAS concentrations, it is likely that PFAS biotransforming metabolic reactions may be limited, increasing the importance of nongrowth supporting cometabolic reactions for PFAS biotransformation.

FUTURE NEEDS AND RECOMMENDATIONS

Much remains to be accomplished in achieving a predictable microbial PFAS biotransformation. The literature to date, although lacking in quantity, standardization, and anaerobic studies, provides a direction on high-priority steps for potentially fruitful future research. Understanding the limits of biotransformation is constantly evolving. The identities of microbial PFAS biotransformers and PFAS-transforming enzymes may be found through metagenomic studies of contaminated environments or proteomic studies of PFAS degraders whose entire genomes have already been sequenced.¹⁵⁴ While enzymes for PFAS biotransformation are not currently known, P-450-type or similar enzymes may be able to cleave C–F bonds.^{23,155} In organic chemistry, F in C–F bonds can be replaced with transition metals. Therefore, transition-metal-dependent enzymes may release F from C–F bonds.^{20,24} Additionally, mixed-function oxidases and horseradish peroxidases have been documented to be responsible for monofluorinated compound defluorination.^{156,157} Identifying PFAS-biotransformation genes and enzymes will enable the design of applicable biotechnologies.

To achieve these needs, we recommend the following:

- Anaerobic biotransformation experiments should be carried out with well-defined electron acceptors and electron donors at a range of oxidation–reduction potentials.
- Further experimentation with aerobic PFAS cometabolism is essential to assess the contribution of cometabolism to PFAS biotransformation.
- PFAS biotransformation products should be measured, and this endeavor may require developing new or more-sensitive analytical capabilities.
- Mass and electron balances should be performed on PFAS experiments to understand fates of F, C, and electrons.
- A greater variety of the commercially produced PFAS should be tested for biotransformation potential to clarify structure-dependent biotransformation relationships.
- Microbial community structure and function should be analyzed to identify PFAS transformers, their key biotransformation genes, and microorganisms with supporting roles in PFAS biotransformation.
- Experiments should evaluate hypotheses on why bacteria may perform PFAS biotransformation reactions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.4c04557>.

Variables extracted from PFAS biotransformation experiments (Table S1), aerobic and anaerobic PFAS biotransforming microbes (Tables S2–S3), publications used to generate Figures 3 and S1 (Table S4), outcome of biotransformation experiments with PFAS as fluorotelomer-based substances (Table S5), 8:2 compounds (Table S6), and PFOS and PFOA (Table S7), effect of number of F atoms per molecule (Figure S2, Table S8) and tertiary PFAS categorizations on biotransformation (Figure S3, Table S9), comparison of biotransformation outcomes between PFAAs and fluorotelomer substances (Table S10, Figure S4), comparison of biotransformation outcomes between subcategories within PFAAs and fluorotelomer substances (Table S11, Figure S5) ([PDF](#))

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Notes

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