Author/s:
Harris, BA;Zhou, J;Clarke, BO;Leung, IKH

Title:
Enzymatic Degradation of PFAS: Current Status and Ongoing Challenges

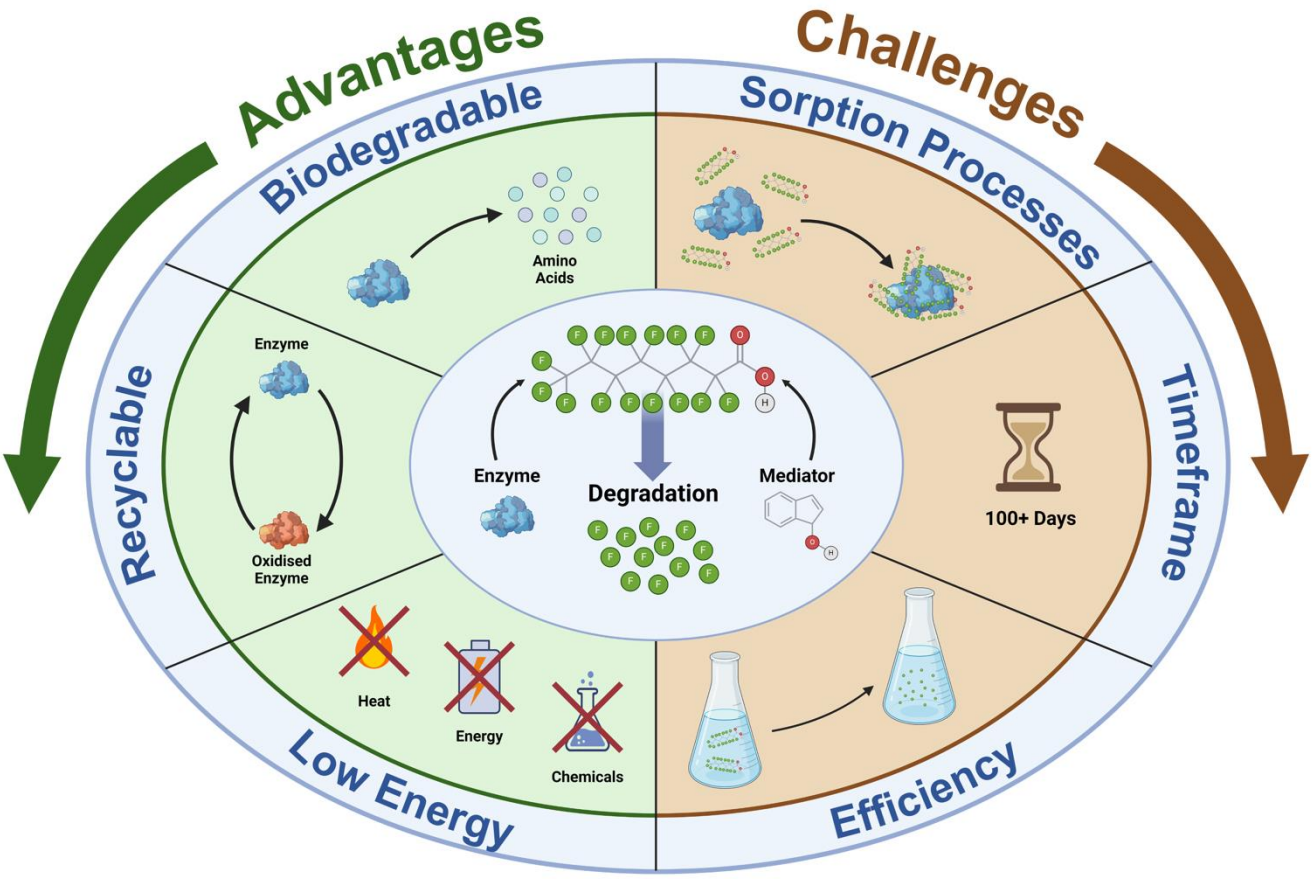
Date:
2025-01-14

Citation:
Harris, B. A., Zhou, J., Clarke, B. O. & Leung, I. K. H. (2025). Enzymatic Degradation of PFAS: Current Status and Ongoing Challenges. ChemSusChem, 18 (2), https://doi.org/10.1002/cssc.202401122.

Persistent Link:
https://hdl.handle.net/11343/355447

Enzymatic Degradation of PFAS: Current Status and Ongoing Challenges.

Benjamin A. Harris,[a,b] Jinpeng Zhou,[a,b] Bradley O. Clarke,*[b] and Ivanhoe K. H. Leung*[a]



[a] Mr. B. A. Harris, Mr. J. Zhou, Dr. I. K. H. Leung
School of Chemistry and Bio21 Molecular Science & Biotechnology Institute
The University of Melbourne
Parkville, VIC 3010, Australia.
ivanhoe.leung@unimelb.edu.au

[b] Mr. B. A. Harris, Mr. J. Zhou, Dr. B. O. Clarke
Australian Laboratory for Emerging Contaminants, School of Chemistry
The University of Melbourne
Parkville, VIC 3010, Australia.
brad.clarke@unimelb.edu.au

Abstract: Per- and polyfluoroalkyl substances (PFAS) are often considered the quintessential example of industrial chemical pollution – they are toxic and ubiquitous environmental contaminants that are extremely difficult to degrade. There has been a large research focus on the development of effective and renewable degradation technologies. In comparison to traditional pollutant degradation techniques, such as advanced oxidation processes and electrochemistry, degradation of PFAS using extracellular enzymes offers an eco-friendly solution as enzymes are biodegradable, recyclable and have low energy and chemical requirements. This review outlines the current understanding of extracellular enzymatic degradation of PFAS with a focus on reported results and proposed degradation mechanisms. More importantly, this review highlights limitations that hinder the application of enzymes for PFAS degradation and proposes critical future research that is needed to improve the applicability of this promising remediation strategy.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic organic substances that are characterized by a carbon chain that is either partially (poly-) or fully (per-) fluorinated.[1] The strength of the carbon-fluorine (C-F) bond, which is recognized as the shortest and strongest covalent bond, gives PFAS unique chemical characteristics.[2] The C-F bond is responsible for the high stability, and high lipo- and hydrophobicity of PFAS, rendering them repellant to water and oil.[3] As a result, PFAS have been extensively used in a variety of manufacturing processes and consumer products, notably non-stick cookware (e.g., Teflon™), fire-fighting foams, food packaging, clothing, and paints.[3] The two most studied PFAS are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). However, their use is being phased out, and novel replacement compounds, such as hexafluoropropylene oxide dimer acid (GenX) and perfluoroethylcyclohexane sulfonate (PFECHS), are now being found within the environment.[4]

Due to their prolific use and high stability, PFAS have become ubiquitous and persistent environmental contaminants,[5] detected in water systems (including drinking water),[6] sediment,[7] soil,[8] and air.[9] In addition, many PFAS are highly mobile in both air and water, meaning they can be dispersed far from their original source and can contaminate even remote areas.[10] The mobility and degradation resistance of PFAS results in environmental concentrations increasing over time.[11] From environmental and industrial sources human exposure to PFAS is nearly unavoidable: PFAS has been measured in the blood of nearly entire populations of developed countries.[12] Furthermore, PFAS can bind to blood lipoproteins, notably human serum albumin,[13] resulting in extended presence of many PFAS in human blood for several years.[14] This also makes many PFAS bio-accumulative, and enables them to biomagnify, meaning they increase in concentration from lower to higher trophic levels.[15] Human and wildlife exposure to PFAS is alarming - even low-level concentrations have been associated with cancers,[16] increased risk of obesity,[17] and altered hormone function leading to birth defects and reduced reproductive capacity.[18]

1.1 PFAS Classification

The PFAS family is broad, representing over 4,000 compounds.[19] PFAS classification can be split into polymers and non-polymers.[1] Of the non-polymer PFAS, perfluoroalkyl substances occupy a large presence in current literature. These compounds are characterized by a fully fluorinated carbon chain, except for carbons within functional groups. Perfluoroalkyl substances include but are not limited to perfluoroalkyl carboxylic acids (PFCAs) [e.g., PFOA], perfluoroalkyl ether carboxylic acids (PFECAs) [e.g., GenX] and perfluoroalkyl sulfonic acids (PFSAs) [e.g., PFOS, PFECHS]. The other category of non-polymer PFAS comprises polyfluoroalkyl substances, instead characterized by partial fluorination of non-functional group carbons. Polyfluoroalkyl substances include the fluorotelomer substances, fluorotelomer carboxylic acids (n:2 FTCAs), fluorotelomer sulfonic acids (n:2 FTSs) and fluorotelomer alcohols (n:2 FTOHs). Fluoropolymers are the main category of PFAS polymers, and includes polytetrafluoroethylene (PTFE) [e.g., Teflon™], and polyvinylidene fluoride (PVDF). This classification is summarized in Figure 1, and does not encompass all categories of PFAS, but only those referenced in the context of this review.

1.2 PFOA & PFOS: Representative PFAS

A common criticism is the disproportionate research focus on the degradation of PFOA and PFOS with the assumption that findings can be extrapolated across all PFAS molecules.[20] This is a valid argument as PFAS are highly diverse and variations in hydrophobicity, bioavailability and degradation susceptibility within the class can significantly influence the efficacy of proposed degradation strategies between compounds.[21]

The use of PFOA and PFOS has been restricted over the past two decades, and thus are considered as ‘legacy’ PFAS compounds.[22] However, this does not render them environmentally irrelevant or inconsequential, in fact they tend to be detected at higher concentrations than their introduced replacement compounds in the environment.[23] A key reason is that a prevalent environmental fate of many PFAS precursors, particularly fluorotelomers, is their conversion to PFCAs and PFSAs through aerobic microbial metabolism.[24] Extensive studies have characterized the aerobic biodegradation pathway

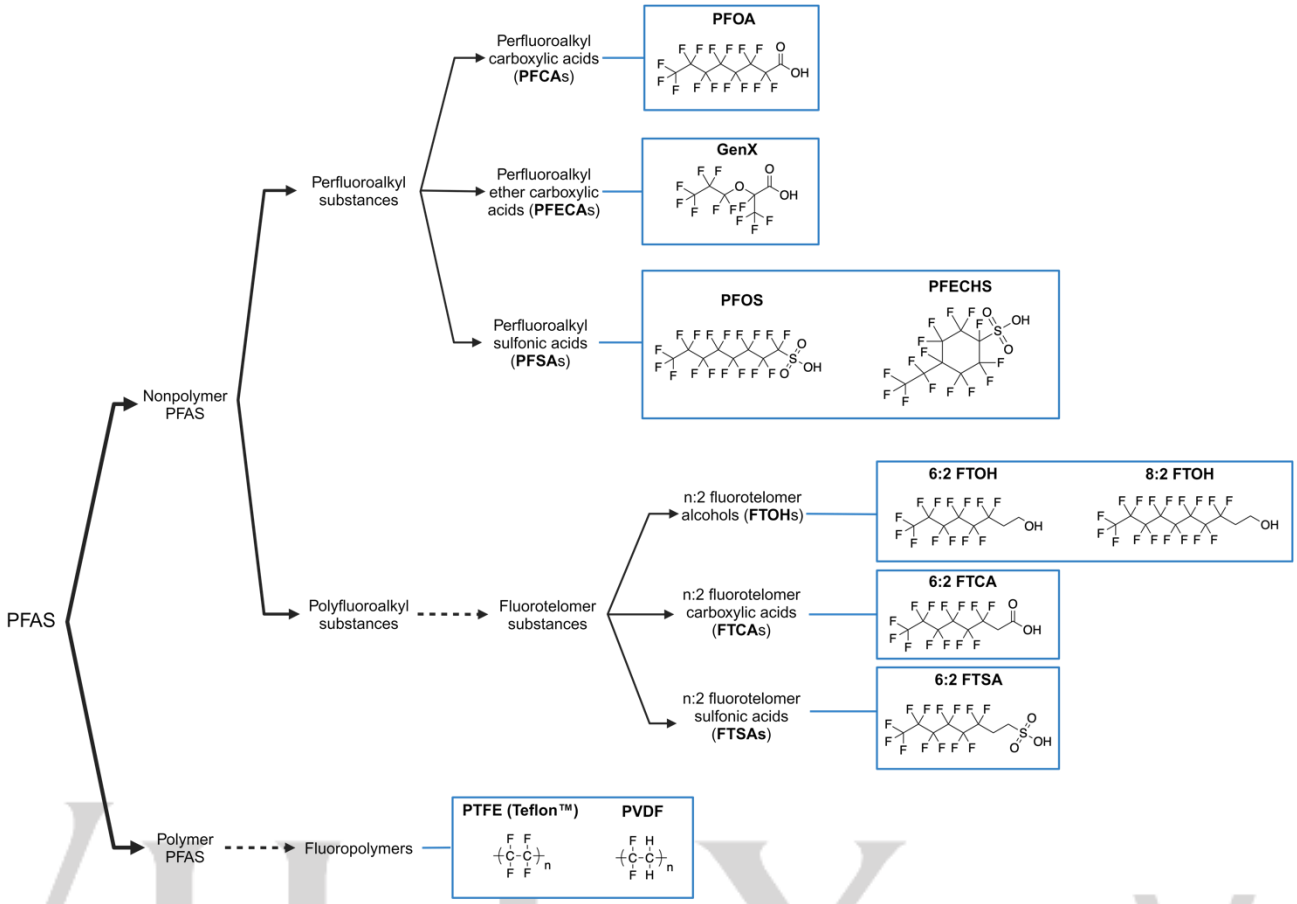


Figure 1. Classification of per- and polyfluoroalkyl substances (PFAS) compounds. This does not include all PFAS classifications, only those that are mentioned in this review.

of 6:2 FTOH, 8:2 FTOH, as well as 6:2 FTSA and 6:2 FTCA, which primarily yield fully fluorinated products like PFOA and PFOS.[25-27] Notably, biodegradation products can also yield PFAS with different functional groups compared to their precursors: n-Ethyl perfluorooctanesulfonamidoethanol (EtFOSE), a sulfonamide, transforms to PFOS, a sulfonic acid, as a terminal metabolite.[28] Furthermore, other natural abiotic processes, including photochemical degradation and atmospheric transformation, contribute to increases in PFCA formation from precursor transformation.[29-30] Consequently, OECD estimates about one thousand PFAS will naturally degrade to PFCAs in the environment.[31]

Another key reason contributing to the elevated environmental levels of PFCA and PFSA is their exceptional chemical stability compared to the fluorotelomer based PFAS: the C-F bond exhibits enhanced stability when multiple C-F bonds are adjacently present in fully fluorinated chains.[31-32] For this reason, PFCAs and PFSAs exhibit the most environmental persistence and degradation resistance. It is reasonable to rationalize that degradation techniques proven effective for these highly stable PFAS, are likely to be applicable for the remediation of other PFAS compounds with relatively lower thermodynamic stability, albeit with minor adjustments to treatment conditions.

2. PFAS Degradation: Non-Enzymatic Approaches

2.1. Non-biological PFAS Treatment Methods

The most established treatment options for PFAS is physical removal using granular activated carbon,[34] reverse osmosis,[35] and foam fractionation.[36] While removal treatments do not address target PFAS degradation, they provide an opportunity to concentrate PFAS prior to destructive technologies. Various wastewater treatment plants currently employ advanced oxidation processes (AOPs) for the treatment of other environmental contaminants.[37] AOPs generate highly reactive oxidizing radicals, such as hydroxyl (•OH) or superoxide radicals (O_2•-), to initiate pollutant degradation. Initial research into PFAS degradation AOPs was promising, however efficacy of these techniques specifically targeting PFAS is currently disputed.[38-39] Additionally, some waste-water treatment plants employing AOPs observed detectable increase of targeted PFAS, including PFOA and PFOS, from inflow to outflow, implying AOP induced transformation of PFAS precursors.[40] Other PFAS degradation pathways include incineration,[41] and electrochemistry;[42] however, they are limited by their intensive energy consumption, lack of specificity, high cost, and potential for producing toxic by-products.

2.2. Limitations of Microbial PFAS Degradation

Biological treatment strategies, utilizing microbes or enzymes to degrade PFAS, are emerging as promising and sustainable alternatives to traditional physicochemical methods. Enzymatic systems offer several key advantages: lower energy and chemical requirements,[43] recyclability of the enzymes, and natural degradation into harmless amino acids.[44] For these reasons, enzymatically catalyzed techniques are currently employed industrially to degrade various non-PFAS contaminants, including industrial organic dyes, heavy metals, plastics, and pesticides.[45] While microbial degradation may seem the optimal approach for efficient biodegradation, with optimized intracellular techniques and diverse array of enzymes, its application to PFAS degradation has been limited. Only a handful of instances of microbial degradation of PFAS have been documented, and the process is notably slow.[46-47] The challenge of microbial degradation largely arises from biology's historical avoidance of fluorine.[48] While fluorine is abundantly available in the Earth's crust, surpassing the prevalence of even nitrogen and phosphorus,[49] natural occurrences of fluorinated substances are rare. Only a dozen monofluorinated natural products have been documented, and fully fluorinated products appear to be non-existent in biological processes within nature.[50]

The paucity of biological fluorine can be attributed to the thermodynamic stability of the C-F bond, which provides no energetic benefit when reduced.[51] Instead, the high bond dissociation energy involved may result in net energy drain on cellular resources.[52] Further, C-F reduction would likely yield fluoride, and the intracellular toxicity of fluoride to bacteria is well documented.[53] Fluoride can inhibit several essential enzymes including those used in glycolysis, respiration, and energy production pathways.[54] Thus, through evolutionary selective pressure, bacteria have developed processes to avoid and resist fluoride exposure, rather than evolving degradative pathways for fluorinated compounds that could produce fluoride.[55-56] Another key factor restricting intracellular degradation of PFAS is their limited bioavailability and cellular uptake, stemming from the hydrophobic and surfactant like nature of these compounds.[57] For these reasons, using extracellular enzymes emerges as a promising approach – they tend to exhibit higher efficiency and could circumvent the bioavailability constraints that limit microbial degradation efforts.[58]

2.3. Direct Enzymatic C-F Bond Cleavage: Fluoroacetate Dehalogenase

Fluoroacetate dehalogenase (FAcD) is a bacterial enzyme capable of breaking the C-F bond in the monofluorinated compound fluoroacetate.[59] Fluoroacetate is a naturally occurring toxin produced by certain plants and gram-positive bacteria as a chemical defense mechanism against mammals.[60] The degradation of fluoroacetate by FAcD produces glycolic acid.[61] While the direct cleavage of the C-F bond is energetically taxing, the subsequent metabolism of glycolic acid provides sufficient metabolic energy to sustain the growth of FAcD-expressing microbes.[62] The defluorination reaction proceeds through a nucleophilic substitution (S_N2) mechanism, where the carboxylate anion of the aspartate group acting as a nucleophile (Figure 2).[63] Fluorine is recognized as a notoriously poor leaving group due to the strength of the C-F bond, however the 'halide pocket' triad containing tryptophan, tyrosine and histidine provide hydrogen bonding and electrostatic interactions to facilitate release of fluoride.[64] Substitution of any of these auxiliary amino acids results in loss of activity of FAcD towards fluoroacetate.[65] Following fluoride release, the ester-enzyme intermediate is

Benjamin A. Harris is a Master of Science (Chemistry) student at the University of Melbourne, where he also earned his bachelor's degree. His current research focusses on developing sustainable technologies to combat chemical pollution, particularly emerging contaminants. Benjamin aims to contribute innovative solutions and insights to address pressing environmental challenges in his work. He is keen to extend his research pursuits into a doctoral program.

Bradley O. Clarke is a Senior Lecturer in Environmental Science and Analytical Chemistry at the University of Melbourne (UoM) and lead researcher of the Australian Laboratory for Emerging Contaminants (ALEC). Prior he was Program Manager of Environmental Science at RMIT University (Melbourne, Australia) 2016–2019 and has held research positions at Imperial College (London) and the University of Arizona. Brad specializes in characterizing the fate, mobility, and impact of legacy and emerging persistent organic pollutants.

Jinpeng Zhou earned his Master of Science (Chemistry) degree in 2023 under the joint supervision of Dr. Ivanhoe Leung and Dr. Bradley Clarke at the University of Melbourne. He then began his Ph.D. in 2024 at Griffith University, supervised by Professor Frederic Leusch, Dr. Steven Melvin, and Dr. Peta Neale. Jinpeng's research interests revolve around using cutting-edge techniques like high-resolution chromatography, mass spectrometry, NMR metabolomics, and cell-based bioassays to evaluate the impacts of emerging contaminants on the environment, aquatic life, and human health across multiple biological levels.

Ivanhoe K.H. Leung is a Senior Lecturer in Biological Chemistry at the University of Melbourne. He earned his undergraduate and doctoral degrees in Chemistry and completed postdoctoral research at the University of Oxford. Prior to joining Melbourne, he spent seven years at the University of Auckland as a Lecturer/Senior Lecturer in Chemical Biology. His current research focuses on: (1) mechanistic enzymology, particularly the relationships between protein structure, function, and modulation; (2) enzyme technology, including the use of enzymes for bioremediation; and (3) inhibitor discovery.

readily hydrolyzed by water, resulting in the production of glycolic acid.[66] This step is similarly stabilized by auxiliary aspartate and histidine residues within the FAcD active site.

Unfortunately, applying FAcD to PFAS is restricted by inherent limitations of the S_N2 mechanism. The nucleophilic attack of the carboxylate anion is sterically hindered when there are multiple fluorine substitutions at the site of attack.[67] Further, the bond strength of C-F increases when adjacent C-F bonds are present.[33] As such, di- and trifluoroacetate were unreactive in microbial wastewater studies despite measurable decreases in fluoroacetate.[68] There is evidence that FAcD is capable of degrading other monofluorinated species as well but has not yet shown potential in interacting with secondary substituted carbons.[69]

Selective pressure to detoxify fluoroacetate likely drove the development of a specific enzyme, FAcD, capable of defluorination in affected organisms. However, this evolutionary pressure is relatively rare, as only around a dozen known fluorinated natural products have been discovered to date.[50] This likely limits the presence of naturally occurring enzymes capable of efficiently breaking one C-F bond, let alone those that can completely defluorinate fully fluorinated chemicals engineered for the purpose of being stable. Given these hurdles, it is unlikely we will discover natural enzymes that have potential to be used in degradation of PFAS. Overcoming these hurdles would likely require engineering enzymes specifically designed for this purpose.[63]

Several other enzymes have demonstrated the ability to catalyze the direct cleavage of C-F bonds in various fluorinated compounds. Evidence suggests that aromatic C-F cleavage has been achieved by 4-fluorobenzoate dehalogenase, 4-fluorophenol monooxygenase, and peroxygenase-like LmB2.[70] Further, recombinant *Pseudomonas putida* was engineered to express a defluorinase enzyme from *Deltia acidovorans,* which allowed the metabolism of several unnatural monofluorinated compounds.[71] However, in comparison to FAcD the mechanism of cleavage and characterization of these enzymes are poorly understood and requires further development.

3. Enzymatic Degradation of PFAS

Recent studies have reported promising findings that indicate that several extracellular enzymes may facilitate the degradation of PFOA or PFOS, with the results of these studies summarized in Table 1.[72-78] These enzymes, which fall under the broader category of ligninolytic enzymes, include laccases, and several peroxidases, such as horseradish peroxidase (HRP), lignin peroxidase (LiP) and manganese peroxidase (MnP).[79] Ligninolytic enzymes are produced by wood-rotting fungi and are excreted extracellularly to catalyze the breakdown of lignin, which is an extremely stable plant polymer used as a major structural component of cell walls in wood and bark.[80] Interestingly, lignin is extremely resistant to microbial degradation.[81] Ligninolytic enzymes catalyze the oxidation of lignin to produce carbon dioxide and small organic acids that are used as carbon sources in its metabolic cycle. The oxidative capacity of ligninolytic enzymes have been identified as a promising approach to bioremediation of various environmental contaminants beyond PFAS, including polyaromatic hydrocarbons,[82] endocrine disrupting chemicals,[83] polychlorinated compounds, and other polymers.[84] Given these results, it is unsurprising that the degradation of PFAS has been investigated. Colosi and co-authors first documented the degradation of PFAS using ligninolytic enzymes, specifically reporting 68% transformation of PFOA when in the presence of an organic substrate 4-methoxyphenol.[72] The results of this study will be discussed in detail later in this review.

Evidence suggests that ligninolytic enzymes cannot directly interact with PFOA or PFOS. Consequently, the reported degradation pathways proceed through *indirect* degradation of PFAS, via the generation of reactive free radicals through the interaction of the enzyme with an aromatic mediator substrate. To be clear, this is distinct from the mechanistic approach proposed in remediation efforts against aromatic pollutants. In such cases, ligninolytic enzyme induced detoxification involves direct oxidation of pollutants into free radicals, which can subsequently couple, polymerize, and precipitate from solution.[85]

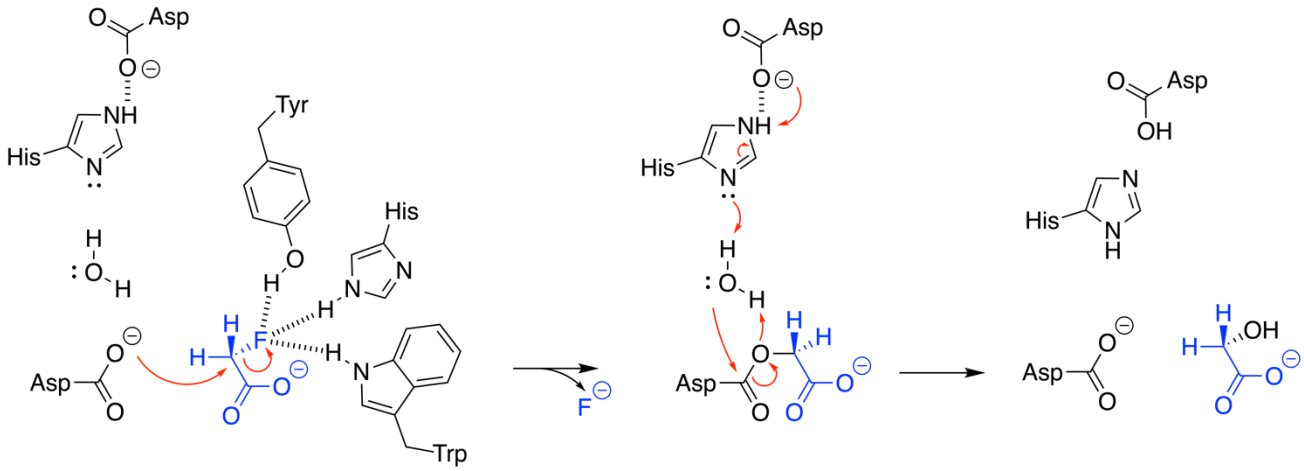


Figure 2. Proposed 2-step defluorination pathway of fluoroacetate catalyzed by fluoroacetate dehalogenase (FAcD).[62] In the first step, an SN2 reaction proceeds with an aspartate functioning as a nucleophile, resulting in the loss of the fluoride and formation of the enzyme-glycolyl intermediate. The loss of fluoride is stabilized by the presence of tyrosine, histidine and tryptophan auxiliary amino acids. In the second step, water hydrolyses the intermediate with stabilization from auxiliary histidine and aspartate groups, resulting in formation of glycolic acid.

3.1. Peroxidases

Peroxidases catalyze the oxidation of various substrates, such as aromatic compounds, by reducing hydrogen peroxide or other peroxides.[86] Peroxidases are widely distributed throughout nature, and are produced by a variety of sources, including microbes, plants and animals.[87] In terms of peroxidases studied for PFAS degradation, horseradish peroxidase (HRP) is derived from horseradish plants, while lignin peroxidase (LiP) and manganese peroxidase (MnP) are found in wood-rotting fungi. Peroxidases exhibit remarkable diversity, including heme-containing peroxidases (e.g., HRP and LiP), non-heme peroxidases (e.g., MnP), and thiol peroxidases. Microbes excrete peroxidases extracellularly for various purposes, including to decompose complex organic polymers, such as lignin, or to detoxify reactive oxygen species, such as H_2O_2, to less harmful molecules.[88]

The peroxidase family share a catalytic cycle (Figure 3).[89] Upon reduction of hydrogen peroxide, the enzyme is oxidized from its ground state and forms to a radical cation intermediate. Here, the radical will be transferred onto the aromatic substrate, generating the reactive radical species, and concurrently reducing the enzyme. In the final step, the enzyme returns to the ground state when oxidizing a second aromatic substrate, generating another reactive radical species. In the proposed mechanisms discussed, the generated radical species will directly interact with PFOA or PFOS to facilitate their degradation.

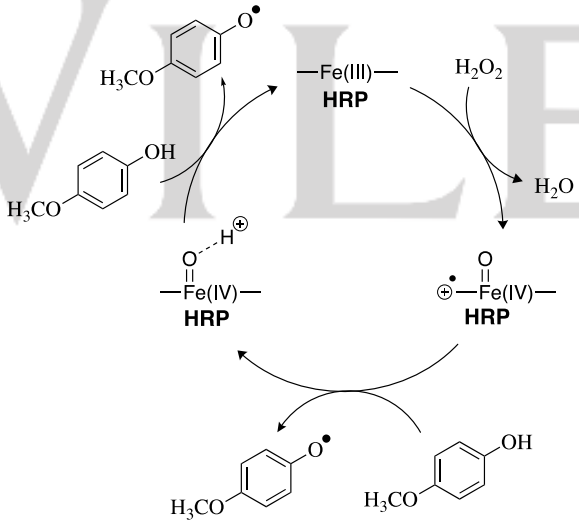


Figure 3. Catalytic cycle of heme containing peroxidases with mediator (e.g.4-methoxyphenol) that produces the reactive organic radical.[89] The catalytic cycle begins with peroxides acting as electron acceptors. This generates a radical cation enzyme intermediate. The radical cation then transfers the radical to the organic mediator and generates a reactive radical. The enzyme is then able to react with another organic mediator to generate a second reactive radical and the enzyme returns to the ground state.

Colosi et al. (2009) were the first to report the potential of free enzymes in degrading PFOA.[72] They used HRP, hydrogen peroxide and 4-methoxypehnol as an organic mediator, resulting in the 68% transformation of PFOA over a 6-hour period. To date, this is the largest reported depletion of parent PFAS achieved in a relatively short 6-hour time course. However, the fluoride ion concentration only increased to 2.0 mg/ml, representing less than 1% defluorination efficiency. Gas chromatography mass spectrometry (GC-MS) identified several fully fluorinated by-products, including alkenes and cyclic products, which are even more environmentally persistent than PFOA.[90] The enhanced stability of a fully fluorinated product is attributed to the absence of functional groups, like carboxylic acids or sulfonate groups, which typically provide a vulnerable point for degradation.[91]

Colosi et al. proposed that methoxy radical generated by the HRP catalyzed oxidation of 4-methoxyphenol initiates a decarboxylation of PFOA and subsequent formation of C_8F_{15}• (Figure 4). The decarboxylation and radical formation step are proposed in all subsequent papers, though do differ in how this radical proceeds.

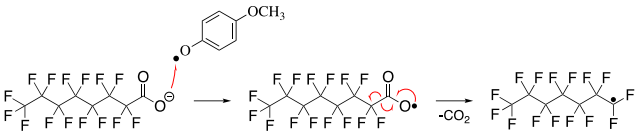


Figure 4. Proposed decarboxylation pathway to initiate PFOA degradation. The organic radical (e.g., 4-methoxyphenol) abstracts an electron from the PFOA carboxylate anion, generating an oxygen centered radical. This rapidly leads to loss of CO_2 and the formation of the C_8F_{15}• radical. This mechanism is commonly proposed for extracellular PFAS degradation, with variations in the organic radical species.

Based on the limited data available in literature, we suggest the use of peroxidase to degrade PFOA warrants further critical evaluation, and the results should be interpreted cautiously. Supplementary degradation routes beyond enzymatic processes may exist, or some of the observed reduction could be attributed to physical removal rather than true chemical degradation. For instance, high performance liquid chromatography equipped with an ultraviolet detector (UV-HPLC) is not an optimal choice for PFOA analysis. PFOA exhibits very low UV absorbance, resulting in poor sensitivity and making it challenging to accurately quantify PFOA concentrations.[92] Additionally, PFOA has demonstrated an ability to undergo photodegradation when exposed to UV light in presence of H_2O_2 and Fe(III), reagents that are present in Colosi et al.'s study.[93] Under these conditions 80% of PFOA was degraded within just 3 hours. This raises the concern that UV-induced degradation pathways could contribute to artificial loss of PFOA during analysis. A more appropriate analytical method would be liquid chromatography mass spectrometry (LC-MS), which is preferred for sensitive and accurate analysis of PFOA and other perfluorinated compounds.[94] Secondly, the potential of sorption processes contributing measurement artifacts in free enzyme degradation will be discussed later in this review but is relevant to consider when analyzing the results of this study.

Further, despite reporting the highest degradation efficiency amongst enzymatic approaches, this result has not been replicated. In fact, only subsequent study has used HRP for PFOA degradation, reporting 20% degradation over two hours.[74] The absence of additional research corroborating the high PFAS transformation efficiency of HRP suggests potential challenges in reliably reproducing these initial findings.

In addition to HRP, other peroxidases LiP and MnP have also been investigated for their potential to degrade PFOA.[73-74, 95] However, the results of these studies are less promising (Table 1). For example, while 54% increase in fluoride ions was measured when PFOA was treated with LiP and MnP over 10 days, this accounted for less than 1% defluorination efficiency and direct PFOA concentrations were not measured.[73]

Table 1: Summary of current results regarding extracellular enzymatic degradation. Abbreviations: PFAS, per- and polyfluoroalkyl substance; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonate; HRP, Horseradish Peroxidase; LiP, Lignin Peroxidase; MnP, Manganese Peroxidase; HBT, 1-hydroxybenzotriazole; NP, not provided in study; N/A, not applicable.

PFAS Compound			Enzyme			Mediator		Conditions				Results			
Identity	Concentration	Analytical technique	Identity	Activity	Source	Identity	Concentration	Reaction Time	pH & Temperature	Medium	Other	Parent Compound Disappearance Efficiency	Defluorination Efficiency	Notes	Reference
PFOA	850 µM	UV-HPLC	HRP	0.015 U/mL	*Armoracia rusticana*	4-methoxyphenol	200 µM	6 h	pH 7, room temperature	Aqueous	Phosphate buffer, 750 µM H_2O_2	68%	<1%		[72]
PFOA	500 mg/L	NP	LiP & MnP	NP	*Phanerochaete chrysosporium*	N/A	N/A	10 d	NP	Aqueous	N/A	N/A	<1%	PFOA not measured	[73]
PFOA	24 µM	LC-MS/MS	HRP	0.1 U/mL	*Armoracia rusticana*	4-methoxyphenol	210 µM	2 h	pH NP, Room temperature	Aqueous	150 µM H_2O_2	20%	N/A	Comparison study, only best results for each enzyme shown	
PFOA	1 µM	LC-MS/MS	Laccase	10 U/mL	NP	4-methoxyphenol	200 µM	120 h	pH NP, Room temperature	Aqueous	N/A	30%	N/A		[74]
PFOA	30 µM	LC-MS/MS	LiP	0.005 U/mL	NP	Veratryl alcohol	200 µM	1 h	pH NP, Room temperature	Aqueous	100 µM H_2O_2	23%	N/A		
PFOA	1 µM	LC-MS/MS	Laccase	1 U/mL	*Pleurotus ostreatus*	HBT	20 µM	137 d	pH 4.5, 22˚C	Aqueous	Mineral buffer	50%	28%		[75]
PFOA	100 µM	LC-MS/MS	Laccase	1 U/mL	*Pleurotus ostreatus*	HBT	20 µM	156 d	pH 4.5, 22˚C	Aqueous	Mineral buffer	47%	N/A	Laccase (1U/mL) & HBT (20 µM) redosed every 6 days	[76]
PFOS	1 µM	LC-MS/MS	Laccase	1 U/mL	*Pleurotus ostreatus*	HBT	20 µM	162 d	pH 4.9, 22˚C	Aqueous	10 mM Cu^{2+}	59%	31%		[77]
PFOA	1 µM	LC-MS/MS	Laccase	1 U/mL	*Pleurotus ostreatus*	HBT	20 µM	36 d	pH NP, 22˚C	Aqueous	20g soybean meal extract	29%	N/A		[78]
PFOA	0.5 µg/g	LC-MS/MS	Laccase	60 U	*Pycnoporus* sp. SYBC-L3	N/A	N/A	140 d	NP	Soil Slurry	50mg soybean meal	40%	N/A		

3.2. Laccase

Laccases are versatile multi-copper oxidase enzymes, capable of catalyzing the oxidation of a broad range of substrates, including phenolic and non-phenolic species.[96] Laccase are found in fungi, bacteria, plants, and insects, where they perform a variety of biological functions, including the degradation of lignin.[88] Unlike peroxidases that use peroxides as electron acceptors, laccase couple the oxidation of substrates with the four-electron reduction of O_2, producing water as the only by-product. This has made them attractive for various industrial environmental practices, including as biocatalysts for remediation of industrial dyes,[97] aromatic organic pollutants,[98] and herbicides.[99] Notably, fungal laccases tend to have a higher redox potential than plant or bacterial laccase, making them more popular in industrial and bioremediation applications.[100]

Laccase typically has four copper atoms at its active site, at a resting state of Cu(II).[96] The catalytic cycle of laccase involves one electron oxidations of four substrate molecules, producing four active radical species (Figure 5). These substrate derived radical species are proposed to interact with and initiate the degradation of PFOA and PFOS (Figure 4).[73, 74-78] Concurrently, the four-electron reduction of oxygen to water allows the regeneration of the oxidation state of laccase and continuation of the catalytic cycle.

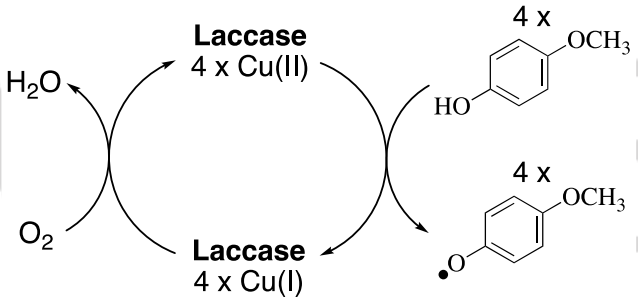


Figure 5. Catalytic cycle of laccase with mediator (e.g., 4-methoxyphenol) that produces the reactive organic radical.[96] The mediator oxidation is a one electron process that converts one laccase Cu(I) to Cu(II), four of these reactions are coupled with a four-electron reduction of molecular oxygen to water.

The potential of laccase to degrade both PFOA and PFOS has been reported.[73-78] Of these results, the most promising was the transformation of 59% of PFOS with a defluorination efficiency of 31% over a 162-day period, using 1-hydroxybenzotriazole as a mediator.[77] The closer alignment between transformation and defluorination efficiency in this case lends more credibility and applicability to the findings.

Importantly, findings indicated that degradation of PFOA and PFOS could be further optimized by incorporating cationic metals.[76-77] The proposed mechanism is that the positively charged metals can form ionic associations with both the negatively charged PFOA and laccase enzyme. This ionic 'bridge' shortens the distance between the species, thus increasing the likelihood of the HBT radical reacting with and degrading PFOA. Specifically, PFOA degradation was boosted by the inclusion of Cu^{2+} and Fe^{3+}, while PFOS degradation was improved by the presence of Mg^{2+} and Cu^{2+}.[76-77] Remarkably, Fe^{3+} does not form complexes with PFOS, and Mg^{2+} does not form complexes with PFOA, underscoring the importance of the specific metal cation involved.[76-77] It is important to note that the presence of Fe^{3+} ions inhibited the laccase-induced removal of bisphenol A (BPA), suggesting that metal ions should be avoided in polymerization-based removal of pollutants.[101]

Laccase offers several advantages over the use of peroxidases. Firstly, the use of oxygen as an electron acceptor allows these systems to occur naturally within the environment, without an external source of peroxides. Thus, laccase degradation reactions do not need to be replenished as frequently as those using peroxidases. Secondly, laccase can retain its catalytic activity for days, while inactivation of peroxidases occurs on the scale of hours.[101] Further, laccase exhibits high salt tolerance, maintaining stability at concentrations as high as 1.0 M NaCl, with the salt potentially even aiding in laccase solubility.[97] Considering seawater typically contains approximately 0.5 M NaCl, laccase degradation systems could prove effective in marine environments. Thirdly, laccase has demonstrated a capacity to degrade PFOA without the addition of an organic mediator, and can instead use phenolic organic matter naturally present in soils.[78] This means that laccase could potentially degrade PFAS in environmental samples without the need for chemical additives, and these degradative pathways may already be occurring naturally. For these reasons, it is not surprising that researchers have primarily chosen to study laccase rather than peroxidases for the remediation of PFAS-contaminated environments.

4. Challenges of Enzymatic Degradation of PFAS

Despite the potential of extracellular enzymatic degradation for PFAS remediation, there are several uncertainties within the existing literature that call for further investigation before practical implementation is considered. This section will offer insight into some of these uncertainties particularly concerns regarding potential PFAS sorption processes, proposed degradative mechanisms, identification of degradation by-products, and deactivation conditions of enzymes.

4.1. Sorption Processes

A recent study demonstrated that a proportion of observed decreases in PFAS concentrations during enzymatic degradation studies might be attributed to experimental artifacts caused by PFAS sorption onto the enzymes, rather than true degradation.[102] Steffens et al. examined a laccase-mediator degradation for both PFOA and PFOS over 96 hours. Initially, after 24 hours, they observed a reduction of 13% reduction in PFOA and a 20% reduction in PFOS. However, upon extracting the reactions with basic methanol after 96 hours, there was no significant decrease in PFOA and PFOS mass. The authors propose that the absence of significant degradation over time could be attributed to the PFAS-enzyme sorption. Notably, substantial sorption of PFOA only occurred in the presence of the mediator, HBT, suggesting mediators can alter the protein surface structure upon oxidation, thereby facilitating PFOA adsorption. This observation may elucidate why controls from prior degradation experiments, where PFAS reduction did not occur in the absence of both enzyme and mediator, did not detect this phenomenon. Further, under conditions where the system

effectively degraded a proxy compound carbamazepine (CBZ), no significant PFOA removal was observed. This indicates the system could generate the radical species proposed to be responsible for PFAS degradation.

Sorption of PFAS to proteins is a well-documented phenomenon. Serum albumin, including human serum albumin (HSA), contains multiple non-specific binding sites that can selectively bind PFAS compounds.[103] This suggests that large proteins, like laccase and peroxidases, likely hold potential sites for PFAS sorption. Additionally, hemp proteins demonstrated a remarkable capacity to remove 98% of PFOS from contaminated wastewater.[104] Given the strong binding affinity between PFAS and various proteins, PFAS-enzyme binding needs to be considered in future enzymatic degradation studies.

Likewise, it is essential to consider PFAS sorption when choosing experimental glassware. Both PFOA and PFOS have demonstrated the potential, up to 50% loss for PFOS, to adsorb to glassware made of polypropylene and polyethylene.[105] Further, fluorinated polymers also must also be avoided: polytetrafluoroetlyhene (PTFE) and polyvinylidene fluoride (PVDF) may contain residual PFAS.[24]

4.2. Unconfirmed Degradation Mechanism

Before implementing any PFAS treatment technology, a fully descriptive and complete degradation mechanism is essential. Without this knowledge there is a risk of forming unknown, potentially more stable and toxic fluorinated by-products. Further, by-products likely vary significantly across different PFAS compounds, requiring in-depth analysis beyond just PFOA and PFOS. Crucially, a complete mass balance, including identifying and quantifying all reaction products, is necessary to demonstrate true mineralization, rather than just partial transformation.[106] Additionally, without understanding precisely how these reactions function, the radical reactions involved cannot be properly controlled or optimized to achieve high PFAS degradation efficiency.

So far in enzymatic degradation studies, high resolution mass spectrometry (HRMS) has been used to identify a subset of reaction products.[72,75-78] However, their concentrations have never been quantified, preventing a complete fluorine mass balance. Consequently, there is a high likelihood not all transformation products are accounted for. Even so, these identified products include fully fluorinated alkenes (Figure 6a) and fully fluorinated cyclic compounds (Figure 6b), which are likely more stable than the original PFOA and PFOS precursors.[89] Notably, the cyclic by-products can be classified as fluorinated volatile organic compounds, which are highly problematic due to their high global warming potential.[107] Further, targeted LC-MS methods have failed to detect shorter-chain PFCAs, but they are still included in the proposed degradation mechanisms in Luo 2015, calling into question the accuracy and completeness of the reported pathways (Figure 6c).[75]

Another consideration with these studies is that degradative studies often focus solely on reporting the disappearance of the parent compound as the main statistic, frequently overlooking the defluorination efficiency. In fact, only four out of the ten studies have reported defluorination efficiency. However, the defluorination efficiency holds greater significance as it indicates the release of inorganic fluorine, which is the ultimate objective of degradation studies. To be realistically implemented and meet the U.S. EPA's standard for zero fluorinated pollution, PFAS degradation technologies must achieve near-complete mineralization, thus requiring defluorination efficiencies closer to 100%.[108] Thus far, enzymatic degradation studies reporting the defluorination efficiency have yielded a maximum of 31% after 162 days, significantly below the requisite threshold for environmental implementation (Table 1).[77]

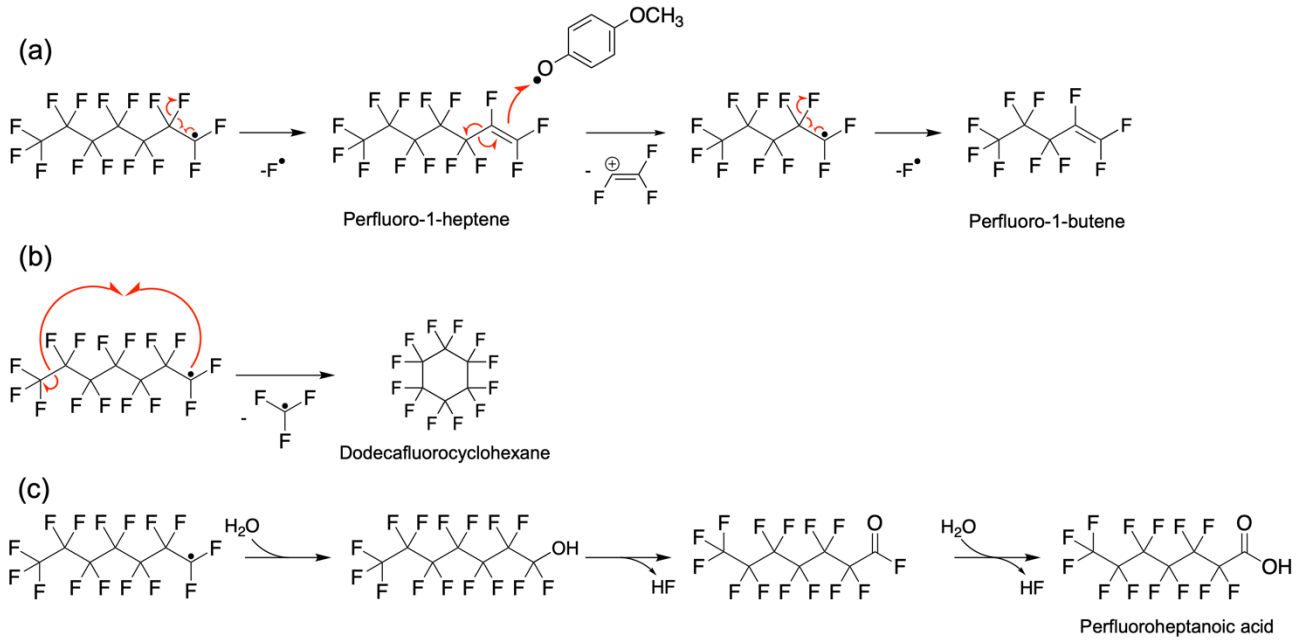


Figure 6. Proposed mechanisms of the reactive radical from Figure 4. **(**a) Mechanism proposed to generate fully fluorinated alkenes (perfluoro-1-heptene and perfluoro-1-butene), adapted from Colosi 2009.[72] (b) mechanism proposed to generate fully fluorinated cyclic compounds (dodecafluorocyclohexane), adapted from Colosi 2009.[72] (c) Mechanism proposed to generate shorter chain PFCAs (perfluoroheptanoic acid), despite the absence of shorter PFCAs identified in targeted LC-MS, adapted from Luo 2015.[75]

4.3. Use of Organic Mediators

Hydroxyl (•OH) and superoxide (O_2•-) have reported limited efficacy in degrading PFOA, despite their stronger oxidative potential compared to HBT and methoxy radicals.[38-39] It has been hypothesized, but not conclusively confirmed, that the extended lifetime of the mediator radicals may enable them to reach and react with PFAS before being quenched by solvent.[74] However, the underlying rationale for the superior degradative effectiveness of organic radicals remains unclear and requires further investigation to fully understand the mechanistic differences between various radical species.

Further, the fate of the mediators used must also not be ignored. The common mediators, HBT and 4-methoxyphenol, are themselves listed as harmful to aquatic life and potentially toxic to humans.[109-110] Notably, in degradation studies, the concentrations of these mediators are often at least 10-100 times higher than the target PFAS compounds. This raises the concern that simply replacing one set of toxic substances with another is not a complete environmental solution. While using less toxic mediators is possible, it may compromise the overall degradation efficiency.[111]

4.4. Inherit Challenges in Enzymatic Degradation of PFAS

Enzymatic degradation of PFAS faces several inherent challenges that are yet to be fully overcome. To date, all enzymatic degradation experiments conducted have been performed under optimized laboratory conditions, which do not reflect the complexities of real-world environmental samples. Even in these controlled settings, the degradation efficiency and timeframe remain suboptimal for practical implementation, with the best result reporting 59% degradation of PFOS after 162 days.[77] This lengthy timeframe is far from what would be required for effective real-world applications, where technologies capable of working within days would be needed. Further, numerous studies using laccase involved re-dosing of laccase and HBT every 6 days, and therefore attributing degradation solely to the initial concentrations of laccase and HBT would be inaccurate.[75-78]

Using laccase and peroxidases in PFAS degradation presents another inherent challenge as they can undergo deactivation through several common pathways. Counterintuitively, simply increasing mediator concentrations does not necessarily enhance degradation.[112] In fact, excessively high mediator levels can instead lead to enzyme deactivation through two mechanisms: 1) the organic radicals formed can recombine and precipitate, causing the enzymes to adsorb and be removed from the solution, and 2) the reactive radicals can oxidize and inactivate the Fe(III) centers in peroxidases.[113-114] Additionally, peroxidases are susceptible to inactivation by excessive H_2O_2 levels, which can promote the formation of inactive enzyme species.[115-116] Laccase and peroxidases can also be deactivated by their own products.[116] As such, careful optimization of reaction conditions and co-factor concentration is necessary to avoid enzyme deactivation and high catalytic activity, making these systems quite delicate.

Immobilizing laccase and peroxidase enzymes on solid interfaces may address the outlined inherent challenges of enzymatic PFAS degradation.[117] Immobilized enzymes can exhibit enhanced kinetic ability, higher activity, and improved stability compared to free enzymes, helping to overcome issues like enzyme deactivation and low degradation efficiency.[97,119] Additionally, the reusability of immobilized enzymes can improve the overall cost-effectiveness of the process.[120] However, deepening our mechanistic understanding of free enzymatic degradation should be the primary focus, before adding an additional layer of complexity with immobilization.

Given the vast array of PFAS compounds, degradation research must extend beyond PFOA and PFOS. While PFCA's and PFSA's are known for their high environmental persistence, there is large chemical diversity amongst PFAS and developing a technology effective in degrading some compounds may not be applicable to others. [31-32] Therefore, a broader focus on various PFAS is essential to develop adaptable and effective treatment technologies.

5. Summary and Outlook

The lack of naturally occurring highly fluorinated compounds in the environment has meant there has been little evolutionary pressure for microbes to develop the ability to degrade fluorinated compounds. A known exception is the enzyme fluoroacetate dehalogenase, which can break a single carbon-fluorine bond. However, FAcDs limited capability is far from sufficient for comprehensively degrading and mineralizing the complex, highly fluorinated PFAS compounds. This explains why finding biological systems capable of comprehensively degrading and mineralizing PFAS has been so difficult. This highlights the challenges encountered in identifying biological systems capable of comprehensively degrading and mineralizing PFAS.

Nonetheless, the enzymes laccase and peroxidase have shown potential for indirectly degrading PFAS compounds PFOA and PFOS. However, the evidence supporting their efficacy remains incomplete and requires further investigation. A key limitation is sorption processes may contribute to removal of PFAS, escaping analytical detection rather than true degradation.

Further, providing the fluoride ion concentration is crucial in PFAS degradation studies, to demonstrate mineralization rather than partial transformation to potentially more stable and toxic by-products. Importantly, this must be paired with comprehensive identification and quantification of all reaction products to ensure a complete fluorine mass balance and validate the proposed degradation pathways.

Beyond enzymatic degradation, the persistence, ubiquity, and inertness of PFAS clearly demonstrates the critical need for use of sustainable and, at the very least, degradable materials in the future. We should prioritize designing materials that prevent pollution, rather than relying on technologies to remediate it.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The graphical abstract, table of contents graphic and Figure 1 were created with BioRender.com. We acknowledge the financial support received from the University of Melbourne. IKHL

acknowledges the financial support received from the Selby Scientific Foundation. BAH acknowledges the financial support received from the Norma Hilda Schuster (nee Swift) Scholarship.

Author Contributions

Benjamin A. Harris: Conceptualization, Writing: original manuscript draft, subsequent drafts, tables & figures, front piece image, table of contents, formatting, reviewing & editing. **Jinpeng Zhou:** Conceptualization, Writing: early draft on mechanistic aspects. **Bradley O. Clarke:** Conceptualization, supervision, writing: review and editing. **Ivanhoe K. H. Leung:** Conceptualization, supervision, writing: review & editing.

Keywords: PFAS • enzymatic degradation • bioremediation • defluorination • sustainable chemistry • enzymes

[1] R. C. Buck, J. Franklin, U. Berger, J. M. Conder, I. T. Cousins, P. de Voogt, A. A. Jensen, K. Kannan, S. A. Mabury, S. P. J. van Leeuwen, *Integrated Environmental Assessment and Management* **2011**, *7*, 513-541.

[2] T. Hiyama, *Organofluorine compounds: chemistry and applications*, Springer Science & Business Media, **2013**.

[3] J. Glüge, M. Scheringer, I. T. Cousins, J. C. DeWitt, G. Goldenman, D. Herzke, R. Lohmann, C. A. Ng, X. Trier, Z. Wang, *Environmental Science: Processes & Impacts* **2020**, *22*, 2345-2373.

[4] H. Mahoney, Y. Xie, M. Brinkmann, J. P. Giesy, *Eco Environ Health* **2022**, *1*, 117-131.

[5] H. Brunn, G. Arnold, W. Körner, G. Rippen, K. G. Steinhäuser, I. Valentin, *Environmental Sciences Europe* **2023**, *35*, 20.

[6] C. A. McDonough, J. L. Guelfo, C. P. Higgins, *Curr Opin Environ Sci Health* **2019**, *7*, 13-18.

[7] S. Newton, R. McMahen, J. A. Stoeckel, M. Chislock, A. Lindstrom, M. Strynar, *Environ Sci Technol* **2017**, *51*, 1544-1552.

[8] A. L. Duchesne, J. K. Brown, D. J. Patch, D. Major, K. P. Weber, J. I. Gerhard, *Environ Sci Technol* **2020**, *54*, 12631-12640.

[9] N. Yu, H. Guo, J. Yang, L. Jin, X. Wang, W. Shi, X. Zhang, H. Yu, S. Wei, *Environmental Science & Technology* **2018**, *52*, 8205-8214.

[10] H. Hamid, L. Y. Li, J. R. Grace, *Environmental Pollution* **2018**, *235*, 74-84.

[11] Z. Habib, M. Song, S. Ikram, Z. Zahra, *Pollutants* **2024**, *4*, 136-152.

[12] K. Kato, L.-Y. Wong, L. T. Jia, Z. Kuklenyik, A. M. Calafat, *Environmental science & technology* **2011**, *45*, 8037-8045.

[13] M. Forsthuber, A. M. Kaiser, S. Granitzer, I. Hassl, M. Hengstschläger, H. Stangl, C. Gundacker, *Environment International* **2020**, *137*, 105324.

[14] M. Dourson, B. Gadagbui, *Regulatory Toxicology and Pharmacology* **2021**, *126*, 105025.

[15] J. M. Conder, R. A. Hoke, W. De Wolf, M. H. Russell, R. C. Buck, *Environ Sci Technol* **2008**, *42*, 995-1003.

[16] K. Steenland, A. Winquist, *Environ Res* **2021**, *194*, 110690.

[17] G. Liu, K. Dhana, J. D. Furtado, J. Rood, G. Zong, L. Liang, L. Qi, G. A. Bray, L. DeJonge, B. Coull, *PLoS medicine* **2018**, *15*, e1002502.

[18] B. P. Rickard, I. Rizvi, S. E. Fenton, *Toxicology* **2022**, *465*, 153031.

[19] A. J. Williams, L. G. Gaines, C. M. Grulke, C. N. Lowe, G. F. Sinclair, V. Samano, I. Thillainadarajah, B. Meyer, G. Patlewicz, A. M. Richard, *Frontiers in environmental science* **2022**, *10*, 850019.

[20] A. Berhanu, I. Mutanda, J. Taolin, M. A. Qaria, B. Yang, D. Zhu, *Science of The Total Environment* **2023**, *859*, 160010.

[21] Y. Deng, Z. Liang, X. Lu, D. Chen, Z. Li, F. Wang, *Chemosphere* **2021**, *283*, 131168.

[22] S. Y. Wee, A. Z. Aris, *npj Clean Water* **2023**, *6*, 57.

[23] R. A. Brase, E. J. Mullin, D. C. Spink, *Int J Mol Sci* **2021**, *22*.

[24] J. Liu, S. Mejia Avendaño, *Environment International* **2013**, *61*, 98-114.

[25] N. Wang, B. Szostek, R. C. Buck, P. W. Folsom, L. M. Sulecki, J. T. Gannon, *Chemosphere* **2009**, *75*, 1089-1096.

[26] L. Zhao, P. W. Folsom, B. W. Wolstenholme, H. Sun, N. Wang, R. C. Buck, *Chemosphere* **2013**, *90*, 203-209.

[27] V. Méndez, S. Holland, S. Bhardwaj, J. McDonald, S. Khan, D. O'Carroll, R. Pickford, S. Richards, C. O'Farrell, N. Coleman, M. Lee, M. J. Manefield, *Science of The Total Environment* **2022**, *829*, 154587.

[28] J. P. Benskin, M. G. Ikonomou, F. A. Gobas, T. H. Begley, M. B. Woudneh, J. R. Cosgrove, *Environmental science & technology* **2013**, *47*, 1381-1389.

[29] E. F. Houtz, D. L. Sedlak, *Environ Sci Technol* **2012**, *46*, 9342-9349;

[30] U. Schenker, M. Scheringer, M. MacLeod, J. W. Martin, I. T. Cousins, K. Hungerbühler, *Environmental Science & Technology* **2008**, *42*, 3710-3716.

[31] OECD, *OECD Papers* **2006**, *6*.

[32] D. M. Lemal, *The Journal of Organic Chemistry* **2004**, *69*, 1-11.

[33] W. R. Dolbier Jr, *Journal of Fluorine Chemistry* **2005**, *126*, 157-163.

[34] V. Franke, M. Ullberg, P. McCleaf, M. Wålinder, S. J. Köhler, L. Ahrens, *ACS ES&T Water* **2021**, *1*, 782-795.

[35] E. W. Tow, M. S. Ersan, S. Kum, T. Lee, T. F. Speth, C. Owen, C. Bellona, M. N. Nadagouda, A. M. Mikelonis, P. Westerhoff, C. Mysore, V. S. Frenkel, V. deSilva, W. S. Walker, A. K. Safulko, D. A. Ladner, *AWWA Water Sci* **2021**, *3*, 1-23.

[36] A. C. E. We, A. Zamyadi, A. D. Stickland, B. O. Clarke, S. Freguia, *Journal of Hazardous Materials* **2024**, *465*, 133182.

[37] M. A. Oturan, J.-J. Aaron, *Critical Reviews in Environmental Science and Technology* **2014**, *44*, 2577-2641.

[38] H. Javed, C. Lyu, R. Sun, D. Zhang, P. J. J. Alvarez, *Chemosphere* **2020**, *247*, 125883;

[39] H. Javed, J. Metz, T. C. Eraslan, J. Mathieu, B. Wang, G. Wu, A.-L. Tsai, M. S. Wong, P. J. J. Alvarez, *Environmental Science & Technology Letters* **2020**, *7*, 653-658.

[40] A. K. Venkatesan, C. S. Lee, C. J. Gobler, *Sci Total Environ* **2022**, *847*, 157577.

[41] L. J. Winchell, J. J. Ross, M. J. M. Wells, X. Fonoll, J. W. Norton, Jr., K. Y. Bell, *Water Environ Res* **2021**, *93*, 826-843.

[42] Y. Su, U. Rao, C. M. Khor, M. G. Jensen, L. M. Teesch, B. M. Wong, D. M. Cwiertny, D. Jassby, *ACS Applied Materials & Interfaces* **2019**, *11*, 33913-33922.

[43] E. Shahsavari, A. Schwarz, A. Aburto-Medina, A. S. Ball, *Current Pollution Reports* **2019**, *5*, 84-92.

[44] Roohi, K. Bano, M. Kuddus, M. R. Zaheer, Q. Zia, M. F. Khan, G. M. Ashraf, A. Gupta, G. Aliev, *Curr Pharm Biotechnol* **2017**, *18*, 429-440.

[45] A. Saravanan, P. S. Kumar, D. N. Vo, S. Jeevanantham, S. Karishma, P. R. Yaashikaa, *J Hazard Mater* **2021**, *419*, 126451.

[46] S. Huang, P. R. Jaffé, *Environmental Science & Technology* **2019**, *53*, 11410-11419;

[47] Y. Yu, K. Zhang, Z. Li, C. Ren, J. Chen, Y.-H. Lin, J. Liu, Y. Men, *Environmental science & technology* **2020**, *54*, 14393-14402.

[48] L. P. Wackett, in *Methods in Enzymology, Vol. 696* (Ed.: R. B. Stockbridge), Academic Press, **2024**, pp. 65-83.

[49] C. J. Allegre, J.-P. Poirier, E. Humler, A. W. Hofmann, *Earth and Planetary Science Letters* **1995**, *134*, 515-526.

[50] M. C. Walker, M. C. Chang, *Chemical Society Reviews* **2014**, *43*, 6527-6536.

[51] D. Harper, D. O'Hagan, C. Murphy*, Vol. 3*, **2005**, pp. 56-56.

[52] L. P. Wackett, *Microbial Biotechnology* **2022**, *15*, 773-792.

[53] N. R. Johnston, S. A. Strobel, *Arch Toxicol* **2020**, *94*, 1051-1069.

[54] A. Strunecka, O. Strunecky, *Applied Sciences* **2020**, *10*, 7100.

[55] E. R. Chellaiah, P. Ravi, R. Uthandakalaipandian, *Folia Microbiologica* **2021**, *66*, 569-578.

[56] Y. Liao, B. W. Brandt, J. Li, W. Crielaard, C. Van Loveren, D. M. Deng, *J Oral Microbiol* **2017**, *9*, 1344509.

[57] M. Hu, C. Scott, *Applied and Environmental Microbiology* **2024**, *0*, e00157-00124.

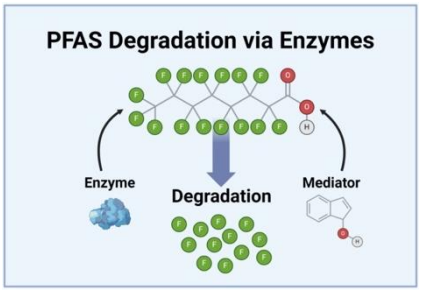
[58] S. Mahendra, L. H. Rome, V. A. Kickhoefer, M. Wang, **2016**.

[59] T. Kamachi, T. Nakayama, O. Shitamichi, K. Jitsumori, T. Kurihara, N. Esaki, K. Yoshizawa, *Chemistry (Weinheim an der Bergstrasse, Germany)* **2009**, *15*, 7394-7403.

[60] L. E. X. Leong, S. Khan, C. K. Davis, S. E. Denman, C. S. McSweeney, *Journal of Animal Science and Biotechnology* **2017**, *8*, 55.

[61] Y. Li, Y. Yue, H. Zhang, Z. Yang, H. Wang, S. Tian, J.-b. Wang, Q. Zhang, W. Wang, *Environment International* **2019**, *131*, 104999.
[62] L. P. Wackett, *Microorganisms* **2022**, *10*.
[63] M. Marciesky, D. S. Aga, I. M. Bradley, N. Aich, C. Ng, *Journal of Chemical Information and Modeling* **2023**, *63*, 7299-7319.
[64] P. W. Chan, A. F. Yakunin, E. A. Edwards, E. F. Pai, *Journal of the American Chemical Society* **2011**, *133*, 7461-7468.
[65] K. Jitsumori, R. Omi, T. Kurihara, A. Kurata, H. Mihara, I. Miyahara, K. Hirotsu, N. Esaki, *Journal of bacteriology* **2009**, *191*, 2630-2637.
[66] S. Miranda-Rojas, I. Fernández, J. Kästner, A. Toro-Labbé, F. Mendizábal, *ChemCatChem* **2018**, *10*, 1052-1063.
[68] Y. Yue, J. Fan, G. Xin, Q. Huang, J. B. Wang, Y. Li, Q. Zhang, W. Wang, *Environ Sci Technol* **2021**, *55*, 9817-9825.
[69] D. A. M. Alexandrino, I. Ribeiro, L. M. Pinto, R. Cambra, R. S. Oliveira, F. Pereira, M. F. Carvalho, *New Biotechnology* **2018**, *43*, 23-29.
[70] H. J. Seong, S. W. Kwon, D.-C. Seo, J.-H. Kim, Y.-S. Jang, *Applied Biological Chemistry* **2019**, *62*, 62.
[71] G. Dodge Anthony, J. Thoma Calvin, R. O'Connor Madeline, P. Wackett Lawrence, *mBio* **2023**, *15*, e02785-02723.
[72] L. M. Colosi, R. A. Pinto, Q. Huang, W. J. Weber, Jr., *Environmental Toxicology and Chemistry* **2009**, *28*, 264-271.
[73] N. S.-l. Tseng, UCLA **2012**.
[74] J. Qingguo, *SERDP Project* **2013**.
[75] Q. Luo, J. Lu, H. Zhang, Z. Wang, M. Feng, S.-Y. D. Chiang, D. Woodward, Q. Huang, *Environmental Science & Technology Letters* **2015**, *2*, 198-203.
[76] Q. Luo, Z. Wang, M. Feng, D. Chiang, D. Woodward, S. Liang, J. Lu, Q. Huang, *Environmental Pollution* **2017**, *224*, 649-657.
[77] Q. Luo, X. Yan, J. Lu, Q. Huang, *Environmental Science & Technology* **2018**, *52*, 10617-10626.
[78] Q. Luo, S. Liang, Q. Huang, *Journal of Hazardous Materials* **2018**, *359*, 241-247.
[79] A. Kumar, R. Chandra, *Heliyon* **2020**, *6*, e03170.
[80] L. D. Sánchez-Corzo, P. E. Álvarez-Gutiérrez, R. Meza-Gordillo, J. J. Villalobos-Maldonado, S. Enciso-Pinto, S. Enciso-Sáenz, *J Fungi (Basel)* **2021**, *7*.
[81] F. J. Ruiz-Dueñas, A. T. Martínez, *Microb Biotechnol* **2009**, *2*, 164-177.
[82] X. Wen, Y. Jia, J. Li, *Chemosphere* **2009**, *75*, 1003-1007.
[83] A. O. Falade, L. V. Mabinya, A. I. Okoh, U. U. Nwodo, *Microbiologyopen* **2018**, *7*, e00722.
[84] L. P. Wackett, S. L. Robinson, *Biochem J* **2020**, *477*, 2875-2891.
[85] F. K. de Oliveira, L. O. Santos, J. G. Buffon, *Food Research International* **2021**, *143*, 110266.
[86] R. Morsi, M. Bilal, H. M. Iqbal, S. S. Ashraf, *Science of the total environment* **2020**, *714*, 136572.
[87] N. Bansal, S. S. Kanwar, *The Scientific World Journal* **2013**, *2013*, 714639.
[88] G. Janusz, A. Pawlik, J. Sulej, U. Świderska-Burek, A. Jarosz-Wilkołazka, A. Paszczyński, *FEMS microbiology reviews* **2017**, *41*, 941-962.
[89] G. I. Berglund, G. H. Carlsson, A. T. Smith, H. Szöke, A. Henriksen, J. Hajdu, *Nature* **2002**, *417*, 463-468.
[90] E. Paulechka, A. Kazakov, *J Chem Eng Data* **2019**, *64*.
[91] A. Raza, S. Bardhan, L. Xu, S. S. R. K. C. Yamijala, C. Lian, H. Kwon, B. M. Wong, *Environmental Science & Technology Letters* **2019**, *6*, 624-629.
[92] K. L. Rodriguez, J. H. Hwang, A. R. Esfahani, A. Sadmani, W. H. Lee, *Micromachines (Basel)* **2020**, *11*.
[93] S. M. Mitchell, M. Ahmad, A. L. Teel, R. J. Watts, *Environmental Science & Technology Letters* **2014**, *1*, 117-121.
[94] A. Di Giorgi, N. La Maida, O. Taoussi, S. Pichini, F. P. Busardò, A. Tini, A. Di Trana, *Journal of Pharmaceutical and Biomedical Analysis Open* **2023**, *1*, 100002.
[95] L. Zhou, W. Li, J. Zhang, H. Mao, *Chemosphere* **2023**, *345*, 140427.
[96] L. Arregui, M. Ayala, X. Gómez-Gil, G. Gutiérrez-Soto, C. E. Hernández-Luna, M. Herrera De Los Santos, L. Levin, A. Rojo-Domínguez, D. Romero-Martínez, M. C. Saparrat, *Microbial cell factories* **2019**, *18*, 1-33.
[97] N. Sharma, I. K. H. Leung, *Int J Biol Macromol* **2021**, *190*, 574-584.
[98] N. Sharma, I. K. H. Leung, *Frontiers in Chemistry* **2021**, *9*.
[99] U. N. Dwivedi, P. Singh, V. P. Pandey, A. Kumar, *Journal of Molecular Catalysis B: Enzymatic* **2011**, *68*, 117-128.
[100] S. Li, Q. Liu, J. Liu, K. Sun, W. Yang, Y. Si, Y. Li, Y. Gao, *Chemosphere* **2022**, *307*, 135685.
[101] L. Mao, L. M. Colosi, S. Gao, Q. Huang, *Environmental Science & Technology* **2011**, *45*, 5966-5972.
[102] S. D. Steffens, E. H. Antell, E. K. Cook, G. Rao, R. D. Britt, D. L. Sedlak, L. Alvarez-Cohen, *Environmental Science & Technology Letters* **2023**, *10*, 337-342.
[103] M. S. Hannah, W. J. Thomas, D. R. Kylie, M. B. Scott, *bioRxiv* **2023**, 2023.2011.2010.566613.
[104] B. D. Turner, S. W. Sloan, G. R. Currell, *Chemosphere* **2019**, *229*, 22-31.
[105] J. E. Zenobio, O. A. Salawu, Z. Han, A. S. Adeleye, *Journal of Hazardous Materials Advances* **2022**, *7*, 100130.
[106] S. J. Smith, M. Lauria, C. P. Higgins, K. D. Pennell, J. Blotevogel, H. P. H. Arp, *Environmental Science & Technology* **2024**, *58*, 2587-2590.
[107] J. S. Casey, S. R. Jackson, J. Ryan, S. R. Newton, *Journal of Chromatography A* **2023**, *1693*, 463884.
[108] J.-M. Arana Juve, B. Wang, M. S. Wong, M. Ateia, Z. Wei, *Current Opinion in Chemical Engineering* **2023**, *41*, 100943.
[109] Sigma-Aldrich, Mar 2024 ed., **2024**.
[110] Sigma-Aldrich, Aug 2023 ed., **2024**.
[111] A. I. Cañas, S. Camarero, *Biotechnology Advances* **2010**, *28*, 694-705.
[112] L. Mao, S. Luo, Q. Huang, J. Lu, *Sci Rep* **2013**, *3*, 3126.
[113] S. Nakamoto, N. Machida, *Water Research* **1992**, *26*, 49-54.
[114] M. A. Ator, P. O. De Montellano, *Journal of Biological Chemistry* **1987**, *262*, 1542-1551.
[115] R. Nakajima, I. Yamazaki, *Journal of Biological Chemistry* **1987**, *262*, 2576-2581.
[116] M. Arnao, M. Acosta, J. Del Rio, R. Varon, F. Garcia-Canovas, *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology* **1990**, *1041*, 43-47.
[117] S. Dasgupta, K. E. Taylor, J. K. Bewtra, N. Biswas, *Water Environ Res* **2007**, *79*, 858-867.
[118] S. F. F. Zofair, S. Ahmad, M. A. Hashmi, S. H. Khan, M. A. Khan, H. Younus, *Journal of Environmental Management* **2022**, *309*, 114676.
[119] L. I. FitzGerald, J. F. Olorunyomi, R. Singh, C. M. Doherty, *ChemSusChem* **2022**, *15*, e202201136.
[120] M. K. Al-Sakkaf, I. Basfer, M. Iddrisu, S. A. Bahadi, M. S. Nasser, B. Abussaud, Q. A. Drmosh, S. A. Onaizi, *Nanomaterials (Basel)* **2023**, *13*.

Entry for the Table of Contents



The concept of degrading per- and polyfluoroalkyl substances (PFAS) using enzymes is exciting – it could be a sustainable and environmentally friendly way to destroy toxic chemicals contaminating our environment. However, due to the persistent nature of PFAS, the field faces many challenges, including low efficiencies, long timeframes, and inconsistencies with results. This review offers an insightful, and at times critical analysis, into the current state of enzymatic degradation of PFAS.