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Review

Recent Progress and Challenges in Microbial Defluorination and Degradation for Sustainable Remediation of Fluorinated Xenobiotics

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Abstract

Fluorinated xenobiotics, such as per- and polyfluoroalkyl substances (PFAS), fluorinated pesticides, and pharmaceuticals, are extensively used across industries, but their extreme persistence, driven by the high carbon–fluorine (C–F) bond dissociation energy (~485 kJ/mol), poses serious environmental and health risks. These compounds have been detected in water, soil, and biota at concentrations from ng/L to µg/L, leading to widespread contamination and bioaccumulation. Traditional remediation approaches are often costly (e.g., EUR >100/m^3 for advanced oxidation), energy-intensive, and rarely achieve complete degradation. In contrast, microbial defluorination offers a low-energy, sustainable alternative that functions under mild conditions. Microorganisms cleave C–F bonds through reductive, hydrolytic, and oxidative pathways, mediated by enzymatic and non-enzymatic mechanisms. Factors including electron donor availability and oxygen levels critically influence microbial defluorination efficiency. Microbial taxa, including bacteria, fungi, algae, and syntrophic consortia, exhibit varying defluorination capabilities. Metagenomic and microbial ecology studies continue to reveal novel defluorinating organisms and metabolic pathways. Key enzymes, such as fluoroacetate dehalogenases, cytochrome P450 monooxygenases, reductive dehalogenases, peroxidases, and laccases, have been characterised, with structural and mechanistic insights enhancing the understanding of their catalytic functions. Enzyme engineering and synthetic biology tools now enable the optimisation of these enzymes, and the design of microbial systems tailored for fluorinated compound degradation. Despite these advances, challenges remain in improving enzyme efficiency, broadening substrate specificity, and overcoming physiological constraints. This review emphasises the emerging promise of microbial defluorination as a transformative and green solution, uniquely integrating recent multidisciplinary findings to accelerate the development of sustainable microbial defluorination strategies for effective remediation of fluorinated xenobiotics.

Keywords: microorganisms; enzymes; dehalogenase; defluorination; C–F bond; biodegradation; xenobiotics; pollutants; pharmaceuticals; per- and polyfluoroalkyl substances

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1. Introduction

Fluorinated organic compounds have experienced a substantial rise in use across industrial and consumer sectors due to their distinctive physicochemical properties, such as water and oil repellence, thermal stability, and exceptional chemical inertness [1]. These

characteristics make them essential in a wide array of applications, including pharmaceuticals, agrochemicals, textiles, cosmetics, refrigeration fluids, electronics, and medical devices [2–5]. Among them, per- and polyfluoroalkyl substances (PFAS), often referred to as "forever chemicals", represent a particularly prominent and diverse class. They are defined by the presence of strong carbon–fluorine (C–F) bonds, which are among the most stable in organic chemistry [5]. While this bond strength provides functional benefits, it also leads to extreme environmental persistence, making many fluorinated xenobiotics highly resistant to natural degradation processes [6].

The widespread production, usage, and disposal of fluorinated xenobiotics have resulted in their global distribution across environmental matrices, including soil, surface- and groundwater, sediments, and living organisms [7]. Many of these compounds are sufficiently volatile or soluble to undergo long-range atmospheric and aquatic transport. Once released into the environment, they resist both abiotic and biotic degradation. Some exhibit soil half-lives that extend well beyond a year [8]. Their persistence, combined with the potential to bioaccumulate and biomagnify through food webs, raises significant concerns for both ecological and human health [9]. Biomonitoring studies have consistently detected these chemicals in human blood, including in vulnerable populations such as pregnant women and newborns [10]. Reported health effects include endocrine disruption, immune suppression, metabolic disorders, developmental and reproductive toxicity, and potential carcinogenicity [8–10]. Several fluorinated compounds function as endocrine-disrupting chemicals by mimicking fatty acids or interfering with thyroid hormone pathways [11].

From an ecological perspective, exposure to fluorinated xenobiotics has been associated with harmful effects in aquatic invertebrates, amphibians, and fish. This contributes to biodiversity loss and disruptions in ecosystem function [9,12]. Environmental contamination originates from various sources, including direct industrial discharges, leaching from treated consumer products, and improper waste handling. Their high mobility and chemical resilience make them particularly difficult to manage in groundwater and drinking water supplies, where traditional treatment systems often prove inadequate [13].

Conventional remediation technologies, such as activated carbon adsorption, ion exchange resins, advanced oxidation processes, and high-temperature incineration, are often expensive, energy-intensive, and ineffective in achieving complete degradation of persistent pollutants such as fluorinated xenobiotics, including PFAS [14]. In some cases, these methods generate toxic by-products or merely transfer the contaminants to another environmental medium. As a result, there is an urgent need to develop more sustainable, efficient, and cost-effective approaches for the remediation and management of fluorinated xenobiotics and PFAS [15]. Addressing these challenges has become a key priority for environmental researchers and policymakers worldwide.

Microbial defluorination, the biological cleavage of carbon–fluorine (C–F) bonds by microbes or their enzymes, is a promising strategy for degrading persistent fluorinated xenobiotics. It leverages specialised consortia or enzymes to break down xenobiotics under mild, eco-friendly conditions [16]. Compared to conventional methods, microbial approaches are not only less energy- and resource-intensive but also offer the potential for complete mineralisation without generating hazardous by-products. Unlike conventional methods, it operates under ambient conditions, often using renewable electron donors and producing minimal by-products. Though still developing, advances in microbial ecology, metagenomics, and enzyme engineering have deepened our understanding of fluorine-degrading microbes and defluorinases [17,18]. These advances have revealed novel microbial taxa and biocatalysts capable of C–F bond cleavage, even in complex compounds. This biological strategy represents a novel and scalable route towards green remediation, with significant implications for environmental sustainability. Microbial defluorination is

emerging as a viable, low-impact complement to existing remediation methods, especially for cost-effective, in situ applications [19]. Aligned with green chemistry and circular bioeconomy principles, this low-impact solution addresses fluorinated pollution, though research is needed to improve efficiency and real-world applicability.

This review provides a comprehensive synthesis of current knowledge on microbial defluorination as a sustainable strategy to remediate fluorinated xenobiotics. It covers the chemistry, environmental presence, and toxicity of fluorinated compounds, followed by C–F bond cleavage via reductive, hydrolytic, and oxidative pathways. It also explores microbial taxa, key enzymes, and insights from metagenomics, molecular studies, and synthetic biology while identifying challenges and gaps to guide future innovation.

2. Fluorinated Xenobiotics: Chemistry and Environmental Impact

The diverse applications of fluorinated organic compounds span multiple industries, with each sector benefiting from the unique properties imparted by the presence of fluorine atoms. These properties, such as water and oil repellence, heat resistance, and chemical stability, enhance the performance of various products [1]. Figure 1 provides an overview of these applications, categorised into sectors, such as pharmaceuticals, agrochemicals, refrigeration, textiles, cosmetics, electronics, and medical devices.

Fluorinated compounds are widely utilised across multiple industries due to the unique physicochemical properties conferred by fluorine atoms, such as high electronegativity, strong C–F bonds, and lipophilicity. In the pharmaceutical sector, the incorporation of fluorine significantly enhances drug efficacy by improving lipophilicity, membrane permeability, metabolic stability, and target binding affinity. This has led to the development of widely used fluorinated drugs, such as ciprofloxacin, fluoxetine, and efavirenz [20,21]. In agriculture, fluorinated agrochemicals, like fluometuron, fludioxonil, and triflumuron, exhibit enhanced herbicidal, fungicidal, and insecticidal activities, contributing to improved crop protection and yield [3,22]. The refrigeration and air conditioning industries rely heavily on hydrofluorocarbons (HFCs) and hydrofluoroolefins (HFOs) as replacements for ozone-depleting substances, offering favourable thermodynamic properties and lower ozone depletion potential [23].

In the firefighting and manufacturing sectors, per- and polyfluoroalkyl substances (PFAS) are employed due to their exceptional thermal stability, surfactant properties, and resistance to water and oil, although increasing regulatory attention is focused on their environmental persistence and bioaccumulation potential [1,5]. In consumer products, including textiles and paper, fluorinated compounds provide effective water, oil, and grease resistance, yet their long-term environmental impact is substantial [4]. Fluorinated solvents such as perfluoromethylcyclohexane are used in cosmetics to enhance texture and spreadability [24], while fluorinated gases like sulfur hexafluoride and octafluorocyclobutane are essential in the electronics and semiconductor industries for plasma etching and insulation [25]. In biomedical applications, fluorinated compounds such as hexafluorobenzene and perfluorodecalin are utilised in medical imaging and as synthetic oxygen carriers due to their high gas solubility and biocompatibility [26].

Collectively, the versatility and functional benefits of fluorinated compounds have driven their widespread adoption. However, this extensive usage has also led to growing environmental and health concerns, particularly surrounding the persistence, mobility, and toxicity of certain fluorinated pollutants such as PFAS, highlighting the urgent need for safer, sustainable, and degradable alternatives.

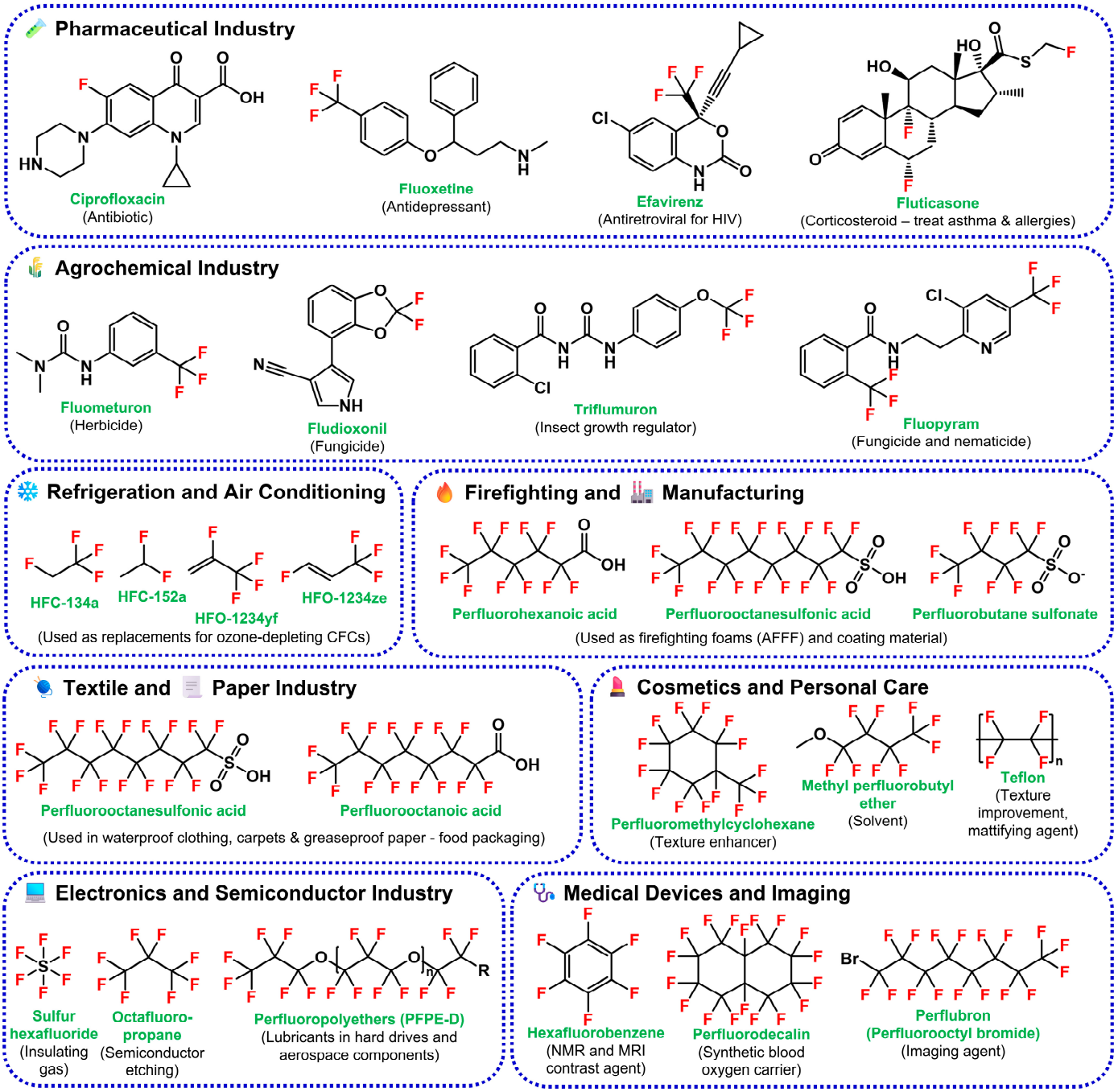


Figure 1. Overview of industrial applications of fluorinated organic compounds across diverse sectors. Key uses include pharmaceuticals, agrochemicals, refrigeration, firefighting, textiles, paper, cosmetics, electronics, and advanced medical devices and imaging.

2.1. Properties of the Carbon–Fluorine (C–F) Bond

The unique physicochemical properties of fluorinated organic compounds are widely used in pharmaceuticals, agrochemicals, and as environmental contaminants, which are largely due to the distinctive characteristics of the carbon–fluorine (C–F) bond. Figure 2 provides a schematic overview of the defining features of the C–F bond, illustrating their mechanistic basis and implications for the chemical reactivity, environmental stability, and biodegradation resistance of these compounds. Compared to carbon–hydrogen or other carbon–halogen bonds, the C–F bond is chemically unique and significantly more robust,

which contributes to the persistence and inertness of fluorinated compounds in natural and engineered systems [27].

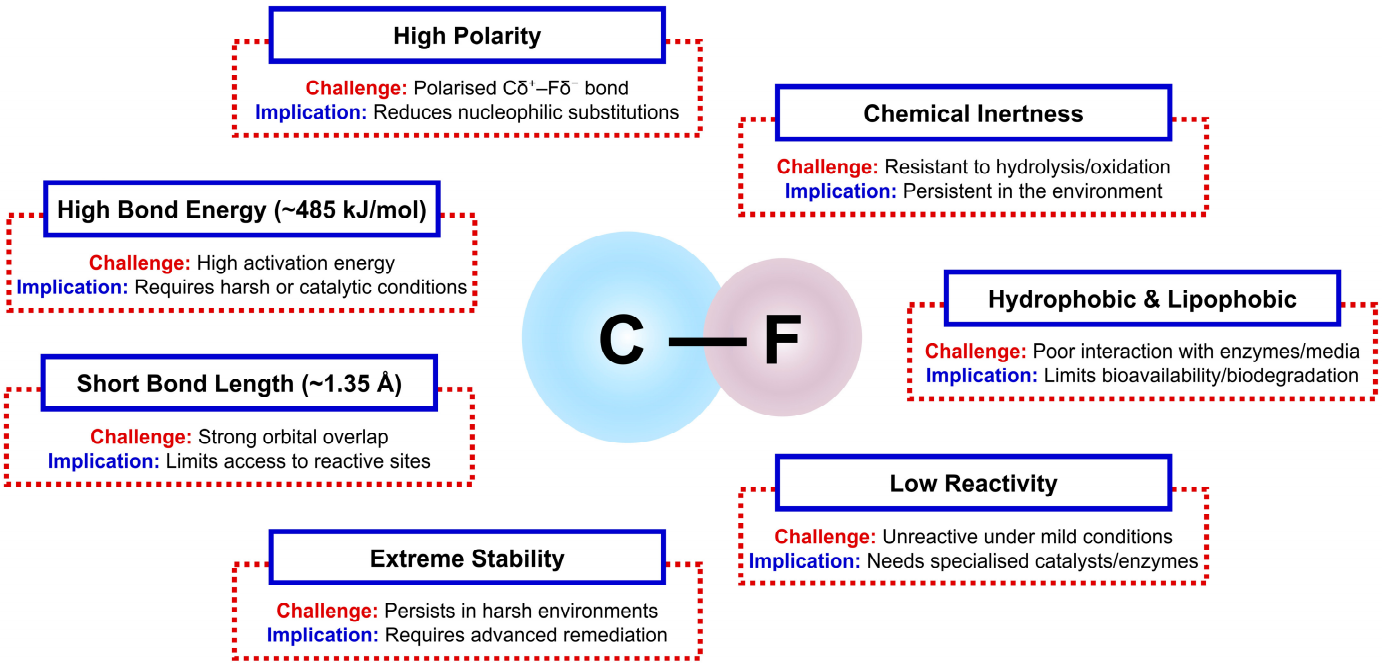


Figure 2. Key properties of the carbon–fluorine (C–F) bond and their industrial implications, highlighting how its high polarity, strength, and stability lead to challenges in reactivity, biodegradability, and the need for specialised processing and remediation.

A key structural feature of the C–F bond is its exceptionally high bond dissociation energy, approximately 485 kJ/mol, which surpasses that of most other carbon–element bonds. This high bond energy results in a substantial activation energy barrier for bond cleavage, often necessitating the use of extreme thermal conditions, strong reductants, or highly specialised catalytic or enzymatic systems. The bond length is also relatively short, around 1.35 Å, indicating a strong orbital overlap between carbon and fluorine atoms. This structural compactness limits the accessibility of reactive sites within the molecule, thereby reducing overall reactivity. Furthermore, the high electronegativity of fluorine induces a significant dipole moment in the C–F bond, generating a partial positive charge on the carbon atom and a partial negative charge on the fluorine atom. This polarity reduces the carbon's susceptibility to nucleophilic substitution, further enhancing the stability of fluorinated molecules under both chemical and biological conditions [27,28].

In addition to its chemical inertness, the presence of multiple C–F bonds can confer both hydrophobic and lipophobic characteristics to the parent molecule. Such dual-phase repellency limits the compound's solubility in aqueous and lipid environments, thereby reducing its interactions with enzymes, transporters, and microbial systems [29]. This diminished bioavailability poses challenges for the uptake, metabolism, and eventual degradation of fluorinated compounds, contributing to their recalcitrance and potential for bioaccumulation. While the intrinsic properties of the C–F bond are advantageous for the development of stable pharmaceuticals and industrial materials, they also present significant challenges for environmental remediation and sustainable chemical design [30]. A mechanistic understanding of the C–F bond is crucial for advancing the application and remediation of fluorinated xenobiotics in natural and engineered environments.

2.2. Environmental Occurrence and Pathways of Contamination

Fluorinated xenobiotics, particularly PFAS, comprise a diverse group of synthetic organofluorine compounds used extensively in industrial applications, firefighting, and consumer products [5]. Their distinctive physicochemical attributes, including strong carbon–fluorine bonds, thermal stability, hydrophobicity, and resistance to degradation, make them environmentally persistent and globally pervasive. These substances are routinely detected in air, water, soil, sediments, and biological tissues. Contamination occurs through both localised point sources (e.g., industrial effluents and firefighting training sites) and widespread diffuse sources (e.g., landfill leachate, wastewater discharge, and consumer product residues), with further dissemination facilitated by atmospheric and hydrological transport [8–10].

2.2.1. Sources of Fluorinated Xenobiotics

Fluorinated xenobiotics, particularly PFAS, persist in the environment due to their extensive industrial use, high mobility, resistance to degradation, and bioaccumulation [12]. Major sources of PFAS contamination include industrial manufacturing facilities producing fluoropolymers and surfactants, emitting legacy compounds like PFOA and PFOS via air and wastewater; aqueous film-forming foams (AFFFs) used in firefighting, which introduce long-chain PFAS into soils and groundwater; consumer products such as food packaging and textiles, which release PFAS into indoor environments and wastewater; and landfills and wastewater treatment plants, which act as diffuse reservoirs releasing PFAS-rich leachates and biosolids into ecosystems [31]. The complex, multifaceted emission pathways highlight the urgent need for integrated monitoring, robust regulation, and advanced remediation strategies. Figure 3 illustrates the major sources of fluorinated xenobiotics, particularly PFAS and their environmental release pathways into soil and groundwater. These include industrial discharges, landfill leachates, wastewater effluents, and the use of PFAS-containing products, which contribute to widespread subsurface contamination.

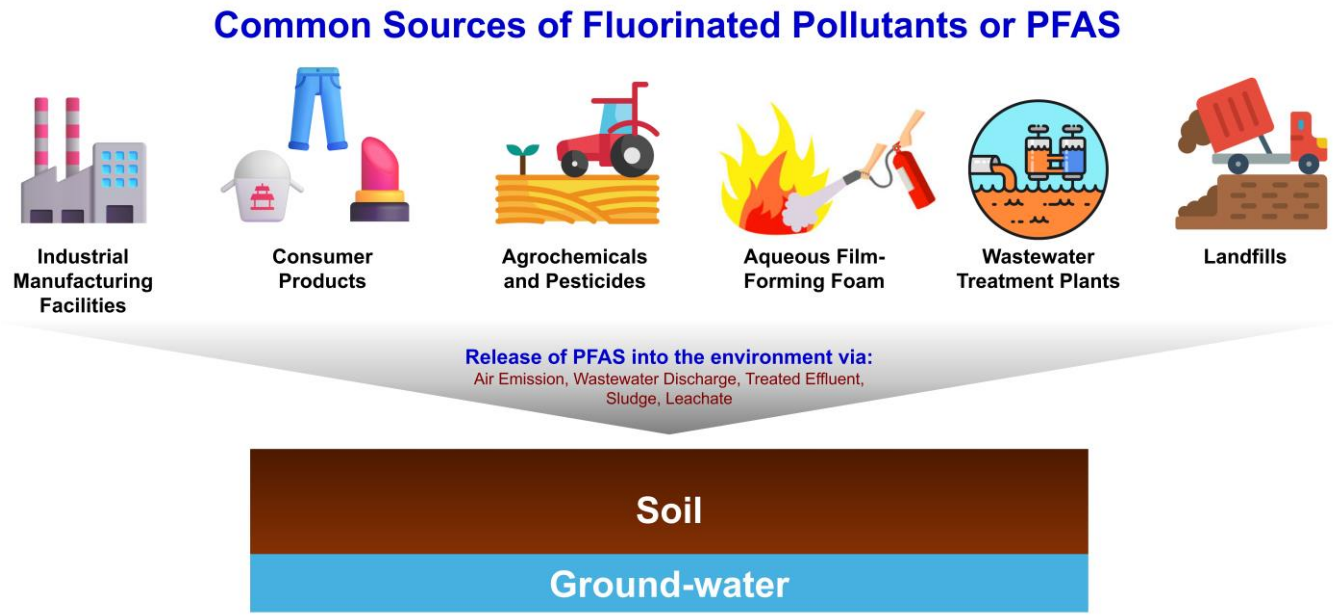


Figure 3. Major sources and environmental pathways of fluorinated pollutants (PFAS) into soil and groundwater.

2.2.2. Environmental Pathways and Fate of Fluorinated Xenobiotics

Upon release, fluorinated xenobiotics undergo diverse transport and transformation processes governed by their molecular properties and environmental interactions, resulting

in widespread persistence across aquatic, terrestrial, atmospheric, and marine systems [32]. Globally, the annual production and use of fluorinated compounds exceed approximately 230,000 tonnes/year, with industrial and consumer applications contributing substantially to environmental fluorinated waste generation [33]. For instance, in the United States alone, over 50,000 tonnes/year of PFAS-containing waste are produced annually from manufacturing, product disposal, and wastewater treatment facilities.

These compounds contaminate surface waters and groundwater via industrial effluents, firefighting runoff, landfill leachate, and wastewater discharge, with mobility influenced by molecular structure and environmental conditions [34]. Concentrations of PFAS in affected surface- and groundwater typically range from nanograms per litre (ng/L) to several micrograms per litre (μg/L), though near industrial hotspot concentrations it can reach milligrams per litre (mg/L) [35]. In soils, retention and movement depend on chain length, functional groups, and soil properties, affecting pollutant bioavailability and leaching potential. Volatile fluorinated precursors released during manufacturing and usage can undergo long-range atmospheric transport and photochemical transformation, resulting in their global distribution and deposition, even in remote regions [36]. Concentrations of these volatile precursors in air and snow in remote areas are commonly in the picograms per cubic meter (pg/m^3) range [37]. Marine environments receive fluorinated xenobiotics through riverine input, wastewater discharge, and atmospheric deposition, with ocean currents facilitating their widespread distribution into sediments and biota [38]. Sediment contamination near the affected sites can range from nanograms per gram (ng/g) to micrograms per gram (μg/g) dry weight [20].

Among these, per- and polyfluoroalkyl substances (PFAS) are particularly concerning due to their high water solubility, resistance to degradation, protein-binding bioaccumulation, and biomagnification through food webs, resulting in significant exposure risks to apex predators and humans via contaminated food, water, and dust [9,38]. Human serum concentrations of PFAS such as PFOA and PFOS are typically at low ng/mL values but can be significantly elevated in populations with high exposure [39]. This complex environmental behaviour underscores the need for comprehensive monitoring, regulation, and remediation strategies.

2.3. Health and Ecological Risks Associated with Fluorinated Xenobiotics

Fluorinated xenobiotics, including pesticides, agrochemicals, industrial chemicals, pharmaceuticals, and dyes, are characterised by strong carbon–fluorine bonds that impart exceptional stability and environmental persistence. Among them, per- and polyfluoroalkyl substances (PFAS) are particularly concerning due to their extensive use, persistence, bioaccumulation, and toxicity [9]. PFAS resist degradation, leading to chronic exposure through contaminated water, food, air, and soil, posing significant risks to human health and ecosystems [8,9].

2.3.1. Human Health Risks

Long-chain PFAS, such as perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), and perfluorohexane sulfonate (PFHxS), bind strongly to serum proteins and bioaccumulate in organs (liver and kidneys), exhibiting biological half-lives spanning years to decades [39]. Epidemiological and toxicological studies link PFAS exposure to endocrine disruption (thyroid, reproductive, and adrenal), developmental toxicity (low birth weight and immune suppression), hepatotoxicity, immunotoxicity (reduced vaccine response), metabolic disorders (dyslipidaemia, hypertension, and insulin resistance), and possible carcinogenicity (kidney and testicular cancers) [40]. Recent clinical studies have reported associations between serum PFAS concentrations and altered liver enzyme levels, impaired

immune response to vaccination, increased cholesterol levels, and risk of preeclampsia in pregnant women. These findings reinforce the relevance of PFAS exposure as a public health concern. Vulnerable groups—children, pregnant women, and populations near contamination sources—are at heightened risk due to increased susceptibility and cumulative exposures [10]. Differences in toxicity exist between long- and short-chain PFAS, but the persistence and bioaccumulative nature of all PFAS necessitate ongoing research into their chronic health effects [9].

2.3.2. Ecological Risks

PFAS bioaccumulate in wildlife by binding to proteins (e.g., albumin), accumulating primarily in the liver, blood, and kidneys across aquatic and terrestrial species. Aquatic organisms absorb PFAS from water and sediments, while terrestrial predators accumulate PFAS via contaminated prey [31–35]. Documented effects include reproductive toxicity (reduced hatching and altered sex ratios), endocrine disruption (steroid and thyroid hormone interference), immunotoxicity, and neurological/behavioural impairments, threatening ecosystem stability, especially among keystone species. PFAS biomagnify through food webs, with soil and sediment microbial communities adversely affected, showing reduced diversity, enzymatic activity, and nutrient cycling, impacting soil health and productivity [41]. Emerging short-chain and replacement PFAS (e.g., GenX and PFBS) exhibit high environmental mobility and uncertain toxicity, posing additional ecological risks [42]. Complex mixture toxicities and interactions with co-stressors such as climate change complicate risk assessments. Despite regulatory limits, bans, and remediation efforts, PFAS persistence requires sustained monitoring, safer chemical alternatives, and advanced remediation technologies [34].

3. Microbial Defluorination: Mechanisms of C–F Bond Cleavage

The carbon–fluorine (C–F) bond, with a bond dissociation energy of ~485 kJ/mol, is the strongest in organic chemistry. This exceptional thermodynamic stability confers chemical inertness, thermal resilience, and environmental persistence to fluorinated xenobiotics, particularly per- and polyfluoroalkyl substances (PFAS) [28]. The recalcitrance of these compounds has led to their pervasive accumulation in environmental matrices and biota, necessitating effective remediation strategies. Among them, microbial defluorination, as the enzymatic cleavage of C–F bonds by microorganisms, emerges as a critical process for the environmental detoxification and mineralisation of fluorinated contaminants [29,30].

To contextualise microbial defluorination within existing remediation technologies, Table 1 presents a comparative analysis of microbial and physicochemical methods. This overview delineates mechanistic pathways, efficacy, scalability, cost-effectiveness, environmental impact, and substrate specificity. Additionally, it summarises key microbial taxa, enzymatic systems, and corresponding substrates, providing a comprehensive reference for evaluating the advantages and limitations of microbial defluorination relative to alternative approaches.

Microorganisms have evolved diverse enzymatic strategies for defluorination, facilitated by selective pressures and adaptive metabolic evolution. These strategies are mechanistically classified into three principal categories—reductive, hydrolytic, and oxidative defluorination—each governed by distinct enzyme systems, redox conditions, and chemical structures of the fluorinated compounds [43]. The fundamental biochemical transformations underlying microbial defluorination are illustrated in Figure 4, highlighting the mechanistic distinctions among reductive, oxidative, and hydrolytic C–F bond cleavage pathways.

Table 1. Comparative overview of microbial and physicochemical defluorination approaches.

Parameter	Microbial Defluorination	Physicochemical Treatments
Mechanism	Enzymatic C–F cleavage occurs via hydrolytic (haloacid dehalogenases from *Burkholderia* sp. and *Streptomyces*; fluoroacetate), reductive (reductive dehalogenases from *Cloacibacillus* and *Dehalococcoides*; PFOA), oxidative (cytochrome P450s from *Thamnidium* and *Cunninghamella*; fluorobenzene), and radical-mediated (peroxidases and laccases from *Phanerochaete* and *Pleurotus*; fluoxetine) pathways	Advanced oxidation (UV, ozone, and persulfate), pyrolysis, UV photolysis, and plasma treatment
Target Specificity	High; enzyme-substrate specificity allows for targeted degradation (e.g., laccases in *Pleurotus ostreatus* active on fluorinated phenols)	Moderate; broad-spectrum but non-specific
Scalability	Moderate; requires controlled bioreactor conditions, strain enrichment, and biofilm optimisation	High; widely used in industrial-scale wastewater treatment and soil remediation
Cost-Effectiveness	Generally low-cost; uses renewable inputs, but may incur time and optimisation costs for strain development and process integration	High cost; requires significant energy and chemical inputs
Environmental Impact	Low; biologically mediated, minimal secondary pollution, and manageable fluoride release	Risk of forming toxic by-products and greenhouse gases
Degradation Rate	Slower; it often ranges from days to weeks, depending on the organism and conditions	Rapid (minutes to hours), though sometimes incomplete
By-Product Toxicity	Low; fluoride ion is the main by-product, which can be managed biologically	Variable; potentially toxic intermediates may form
Adaptability to Mixed Pollutants	High; microbial consortia (e.g., enriched sludge with alkane sulfonate monooxygenase and cytochrome P450—PFOS degradation) allow for flexibility and syntrophic interactions	Limited; often requires sequential or combined treatments
Limitations	Requires microbial adaptation, pathway elucidation, and system engineering; uncultivated species and enzymes remain poorly characterised	High energy demand, incomplete mineralisation, and limited selectivity
Example Applications	Bioreactors, constructed wetlands, and engineered consortia for PFAS/pharmaceutical removal	Industrial oxidation units, incineration plants, and chemical reactors
Reference	[16–19]	[13–15]

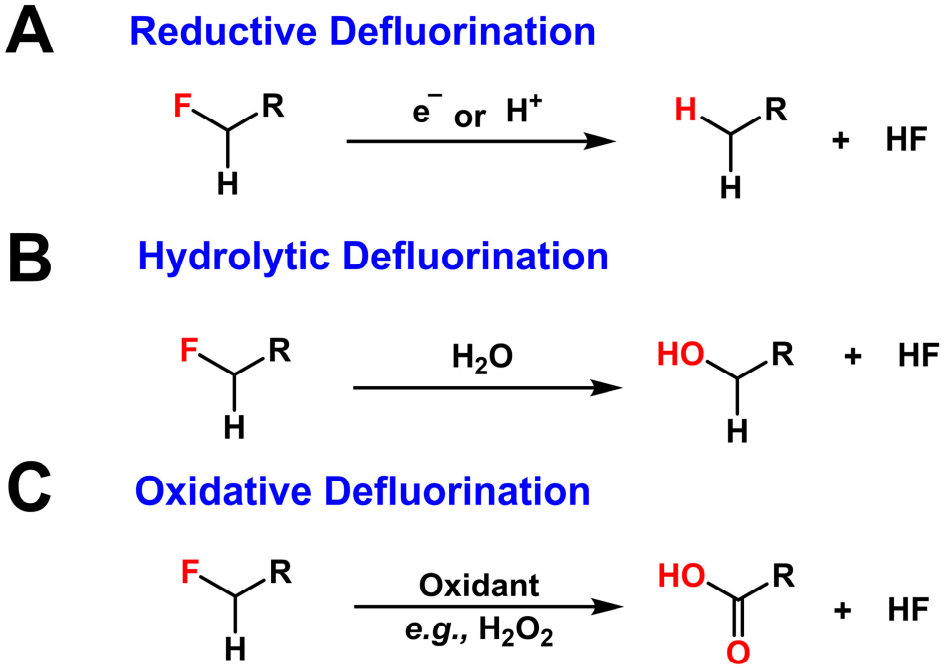


Figure 4. Representative microbial mechanisms of C–F bond cleavage: (**A**) reductive defluorination via electron/proton-mediated substitution; (**B**) hydrolytic defluorination via nucleophilic attack by water; (**C**) oxidative defluorination through oxygen incorporation (e.g., H_2O_2). The figure is sourced from References [17,29,30,43].

3.1. Reductive Defluorination

Reductive defluorination predominates under anaerobic or highly reducing conditions, involving the stepwise substitution of fluorine atoms with hydrogen via electron transfer (Figure 4A). This process is catalysed by reductive dehalogenases, a class of vitamin-B12- or flavin-dependent enzymes that utilise electron donors such as H_2 or reduced ferredoxins [44]. The mechanism involves a nucleophilic attack on the carbon centre, resulting in the elimination of fluoride ions (F^-) or hydrogen fluoride (HF).

Recent studies have demonstrated that specific anaerobic consortia, such as those enriched in *Desulfovibrio* and *Sporomusa*, as well as hybrid systems integrating electroactive materials, can significantly enhance PFAS defluorination, broadening the applicability of this pathway beyond model compounds [45,46]. This pathway is particularly effective for aliphatic fluorinated compounds bearing terminal fluorines or other electron-withdrawing groups. *Dehalococcoides* spp., for instance, have demonstrated defluorination of compounds such as fluoroacetate [47]. However, the process is kinetically slow, requires strict anaerobiosis, and is limited by the narrow substrate specificity of native enzymes, thus constraining its environmental scalability without bioaugmentation or engineered bioreactors.

3.2. Hydrolytic Defluorination

Hydrolytic defluorination proceeds via nucleophilic substitution, where hydroxide ions or water attacks electrophilic carbon centres bearing fluorine substituents, displacing the fluorine with a hydroxyl group [48]. The resulting transformation yields alcohols, acids, or other oxygenated derivatives, accompanied by the release of free fluoride (Figure 4B).

Hydrolytic dehalogenases (e.g., defluorinases), identified in select bacterial genera, mediate this reaction. The pathway is generally limited to monofluorinated compounds or activated fluorinated aromatics, where the C–F bond is sufficiently polarised for nucleophilic attack [17]. A classic example includes the defluorination of fluoroacetate via fluoroacetate dehalogenase [43]. Though less applicable to highly fluorinated PFAS, this mechanism offers promise for enzyme engineering, particularly under ambient, aerobic conditions conducive to scalable biocatalysis.

3.3. Oxidative Defluorination

Oxidative defluorination is among the most versatile and environmentally relevant pathways, occurring predominantly under aerobic conditions (Figure 4C). It involves the incorporation of molecular oxygen (or oxygen-derived radicals) into fluorinated substrates via enzymes, such as monooxygenases, dioxygenases, peroxidases, and laccase [49]. This oxidation generates labile intermediates (e.g., fluorohydrins, gem-diols, or α-hydroxy acids) that spontaneously eliminate fluoride due to instability.

Enzymes, such as laccases, lignin peroxidases, and manganese peroxidases, have demonstrated partial defluorination of perfluoroalkyl acids and aromatic PFAS [50]. Notably, oxidative biocatalysis may be enhanced in consort with advanced oxidation processes (AOPs), where reactive oxygen species (ROS), generated via UV/H_2O_2, ozonation, or photocatalysis, mimic enzymatic oxidative mechanisms [51]. However, oxidative defluorination can result in toxic or persistent intermediates, necessitating integrated pathways or post-treatment for complete mineralisation.

A comparative overview of the key characteristics of reductive, hydrolytic, and oxidative defluorination mechanisms is presented in Table 2.

Table 2. Comparison of C–F bond cleavage mechanisms.

Feature	Reductive Defluorination	Hydrolytic Defluorination	Oxidative Defluorination
Basic mechanism	Electron- and proton-mediated replacement of F with H	Nucleophilic substitution of F by OH^- or H_2O	Attack by reactive oxygen species (ROS) or oxidants, leading to cleavage of C–F
Type of reaction	Redox (reduction)	Hydrolysis (nucleophilic substitution)	Redox (oxidation)
Electron flow	Electron gain at carbon (reduction)	No net redox change	Electron loss from carbon (oxidation)
Typical conditions	Anaerobic/reducing (e.g., presence of H_2 or electron donors)	Mild aqueous or basic conditions, sometimes enzymatic	Aerobic/oxidative (e.g., presence of H_2O_2, ROS, or photolysis)
Catalysts/enzymes	Reductive dehalogenases, metal catalysts (e.g., Pd/C and Ni), and microbes like *Dehalococcoides*	Defluorinases, hydrolytic enzymes, and strong bases	Peroxidases (e.g., lignin peroxidase and Mn peroxidase), Fenton reagent, laccases, and photocatalysts
By-products	HF (gas or aqueous), alkane, or partially defluorinated hydrocarbons	F^- (aqueous), alcohols, or carboxylic acids	F^- and oxidised intermediates (e.g., aldehydes, ketones, and acids)
Environmental relevance	Anaerobic biodegradation (e.g., groundwater and sediments)	Biodegradation by specific microbes in aerobic/neutral pH conditions	Abiotic or enzymatic degradation under aerobic/oxidative conditions
Rate and relative efficiency	Typically slow; depends on electron donor availability and anaerobic conditions; moderate to low efficiency	Moderate; enzyme-dependent with limited substrate scope and availability	Broad substrate range with fast degradation rates, but potentially toxic by-products
Substrate preference	Perfluoroalkyls with terminal halogens or activated groups	Fluorinated aromatics and short-chain fluorinated carboxylates	Perfluorinated carboxylic/sulfonic acids and fluorinated aromatics
Limitations	Slow reaction rates; requires strict anaerobic conditions; limited knowledge of many reductive enzymes	Narrow substrate specificity; enzyme stability issues; less effective for highly fluorinated compounds	Possible formation of toxic by-products; high energy input; not always fully mineralising
Typical applications	Anaerobic bioreactors for halogenated organics and PFAS remediation	Enzymatic treatment of mild fluorinated pollutants; biocatalysis in wastewater treatment	Advanced oxidation processes (AOPs) and photolytic degradation in water treatment and soil remediation
Examples	- *Dehalococcoides* reducing C–F in fluoroacetate	- Enzymatic hydrolysis of fluoroacetate	- Laccase-mediator oxidation of fluorophenols
	- Ni-based catalysts in PFAS	- Base-catalysed cleavage of aryl-F	- H_2O_2/UV treatment of PFOA
References	[43–47,51]	[17,43,48]	[49–51]

Microbial defluorination encompasses a suite of specialised and diverse enzymatic mechanisms that challenge the formidable stability of C–F bonds [47,48]. While current approaches are often substrate-specific and limited in efficiency, advances in metagenomics, enzyme engineering, and synthetic biology hold promise for developing robust, scalable systems for the microbial remediation of fluorinated xenobiotics.

4. Microbial Degradation of Fluorinated Xenobiotics

4.1. Bacterial Defluorination and Degradation

Bacteria are highly promising candidates for the biodegradation and biotransformation of fluorinated xenobiotics due to their exceptional metabolic diversity, enzymatic flexibility, and ecological adaptability [18–21]. Their genomes encode a broad spectrum of enzymes, such as dehalogenases, monooxygenases, dioxygenases, and reductases, which facilitate the cleavage of the carbon–fluorine (C–F) bond [43]. This bond is among the strongest in organic molecules and contributes significantly to the environmental persistence of fluorinated compounds [30]. Bacteria can rapidly adapt to new environmental pressures, including anthropogenic pollutants, by acquiring or modifying metabolic pathways. This allows them to utilise fluorinated xenobiotics either directly as carbon and energy sources or indirectly through co-metabolism [52]. Additionally, bacteria often function within multispecies communities, where metabolic intermediates are exchanged and sequentially transformed by different members. This cooperative degradation strategy is particularly effective in complex environments such as soil and groundwater. The widespread occurrence of bacteria in contaminated habitats further supports their ecological importance as natural agents in the remediation of persistent organic pollutants, including fluorinated substances [18,21].

Several bacterial species and consortia have been identified that are capable of degrading various fluorinated compounds, particularly per- and polyfluoroalkyl substances

(PFAS). For example, *Labrys portucalensis* F11, isolated from industrially contaminated soil, has been shown to metabolise over 90 percent of perfluorooctane sulfonic acid (PFOS) within 100 days. This strain can also degrade 6:2 fluorotelomer sulfonate and 5:3 fluorotelomer carboxylic acid, with evidence of defluorination [53]. Anaerobic bacteria such as *Acetobacterium* species have demonstrated the ability to reductively defluorinate unsaturated PFAS, including PFMeUPA, PFUPA, and 6:2 FTUCA. These bacteria utilise specialised enzymes and fluoride efflux systems to counteract the intracellular toxicity associated with fluoride release [54]. *Acidimicrobium* sp. strain A6 has also shown potential to degrade both PFOS and perfluorooctanoic acid (PFOA) [55]. Members of the genus *Gordonia* are capable of cleaving long-chain fluorotelomers such as 6:2 FTSA and related sulfonamides, although they often leave behind persistent perfluorinated carboxylic acids [56]. Species like *Pseudomonas plecoglossicida* and *Pseudomonas aeruginosa* have demonstrated high efficiency in PFAS transformation, highlighting the central role of this genus in microbial biodegradation of fluorinated pollutants [57].

Figure 5 provides a comprehensive visual summary of microbial degradation pathways for the following two major fluorinated xenobiotics: perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). It highlights the sequential biotransformation steps, the resulting intermediate products, and the specific bacterial species and consortia responsible for these transformations. PFOA, a fully fluorinated carboxylic acid with an eight-carbon backbone, undergoes stepwise degradation to progressively shorter-chain perfluorinated carboxylic acids (PFCAs), including PFHpA (C7), PFHxA (C6), PFPeA (C5), PFBA (C4), and PFPrA (C3). This degradation ultimately yields trifluoroacetic acid (TFA) and inorganic fluoride ions, indicating successful defluorination. Several bacterial species have been identified as key players in the degradation of PFOA and its transformation products. Notably, *Pseudomonas plecoglossicida* strain DD4 [58], *Delftia acidovorans* [59], *Pseudomonas mosselii* [60], *Ensifer adhaerens* M1 [61], and *Acidimicrobium* sp. strain A6 [62] have all demonstrated the capacity to degrade PFOA through various metabolic pathways. Additionally, mixed anaerobic consortia composed of multiple microbial taxa have also shown the ability to break down PFOA and its intermediates [63]. These transformations typically proceed via oxidative, hydrolytic, or reductive defluorination mechanisms, depending on the microbial species and environmental conditions. The degradation process is often a result of metabolic cooperation among different microorganisms within microbial communities, where one species may initiate the breakdown, and others further degrade the intermediates (Figure 5A).

Similarly, PFOS is a perfluorinated compound characterised by a sulfonic acid functional group instead of a carboxyl group, which also undergoes microbial degradation, although often at slower rates due to the stronger stability of the sulfonic moiety. Microbial degradation of PFOS involves a series of transformation steps that generate intermediate compounds, including fluorinated alcohols, like perfluorooctanol, shorter-chain perfluorinated sulfonic acids (PFHpS, PFHxS, and PFBS), and PFCAs (PFHpA, PFHxA, PFPeA, and PFBA), likely formed via oxidative cleavage of the sulfonic group. The appearance of trifluoropropanoic acid (TFPrA) and fluoride ions indicates defluorination, suggesting partial or complete PFOS breakdown. A wide range of microbial taxa are known to be involved in the degradation of PFOS. These include *Ensifer adhaerens* M1 [61], *Ensifer moralensis* H16 [64], *Acidimicrobium* sp. strain A6 [62], and multiple species of *Pseudomonas*, such as *Pseudomonas aeruginosa* [57], *Pseudomonas plecoglossicida* 2.4-D [65], and *Pseudomonas putida* [57]. In addition to these isolates, complex microbial consortia have also been implicated in PFOS degradation. These consortia often include a combination of *Paracoccus*, *Hyphomicrobium*, *Roseobacter*, *Flavobacteria*, members of the family *Micromonosporaceae*, and various *Gammaproteobacteria* [66]. The cooperation among these diverse microorganisms

likely facilitates multi-step PFOS degradation, with different species contributing distinct enzymes and metabolic functions (Figure 5B).

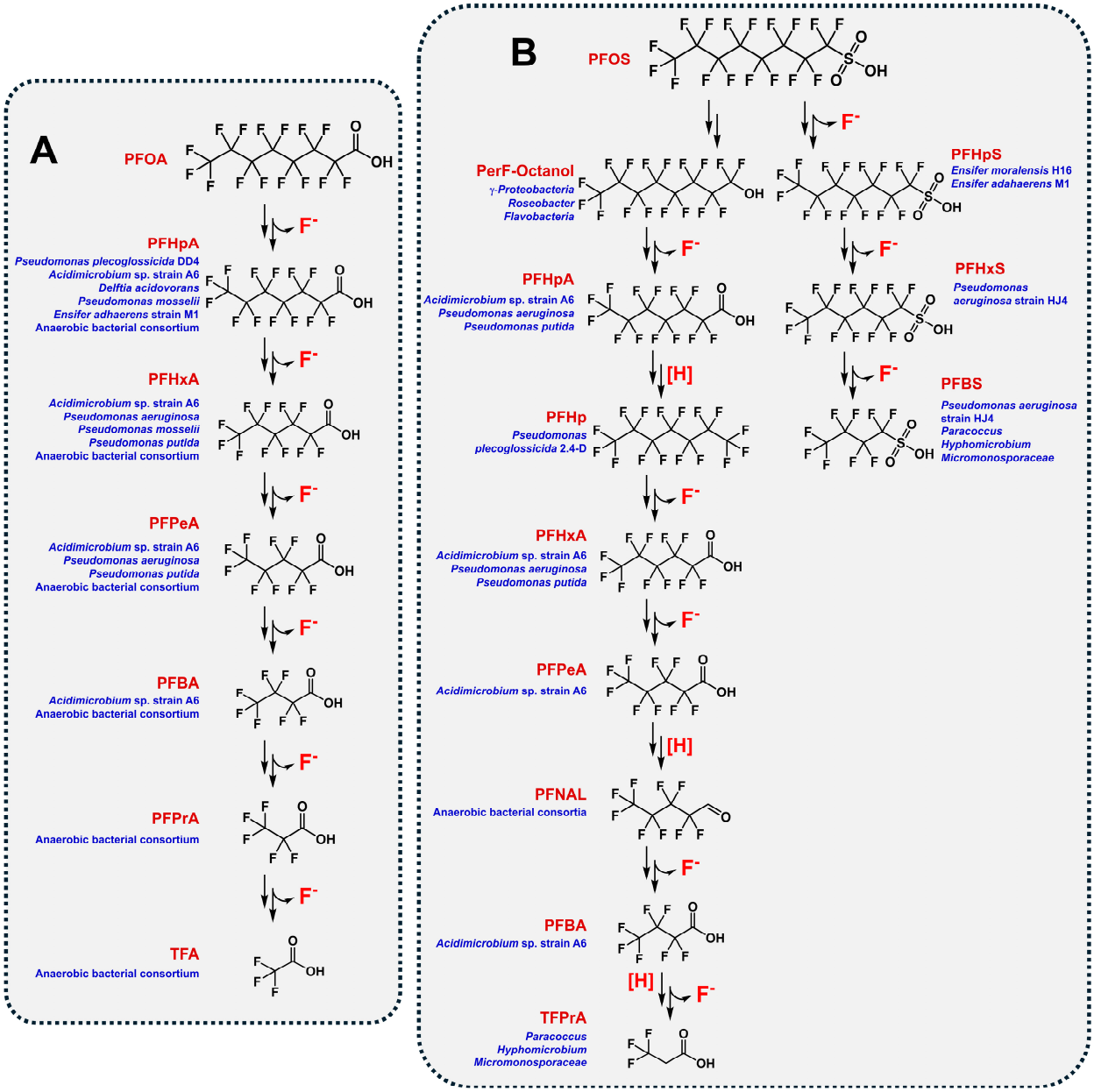


Figure 5. Bacterial degradation intermediate metabolites of PFOA (**A**) and PFOS (**B**). Abbreviations of metabolites are highlighted in red, and the bacterial species responsible for each transformation are indicated in blue. PFOA (perfluorooctanoic acid), PFHpA (perfluoroheptanoic acid), PFHxA (perfluorohexanoic acid), PFPeA (perfluoropentanoic acid), PFBA (perfluorobutanoic acid), PFPrA (perfluoropropanoic acid), TFA (trifluoroacetic acid), fluoride ion (F^-), PFOS (perfluorooctane sulfonic acid), PerF-Octanol (perfluorooctanol), PFHpS (perfluoroheptane sulfonic acid), PFHp (perfluoroheptane), PFHxS (perfluorohexane sulfonic acid), PFNAL (perfluorononanal), PFBS (perfluorobutane sulfonic acid), and TFPrA (trifluoropropanoic acid). The figure is sourced from References [54–63].

Beyond PFAS, several other classes of fluorinated xenobiotics have been reported to undergo microbial degradation. Fluoroacetate degradation has been observed in genera including *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Streptomyces*, and *Pseudomonas*, often via fluoroacetate dehalogenases that hydrolyse the C–F bond to produce glycolate and fluoride [67]. For instance, *Delftia acidovorans* strain D4B expresses haloacid dehalogenases (DeHa2 and

DeHa4) that are active against mono- and difluoroacetates [59]. *Rhodococcus* sp. NJF-7 has been reported to defluorinate monofluorinated medium-chain alkanes, likely initiated by monooxygenases [68]. *Thauera aromatica* K172 was anaerobically cultivated at 30 °C and pH 7.8 in mineral salt medium with benzoate/4-F-benzoate (2.7 mM) or toluene/4-F-toluene (2 mM) as sole carbon sources, using nitrate as the terminal electron acceptor; toluene cultures included 2% (*v*/*v*) paraffin. Fluorinated substrate depletion and fluoride release were quantified by reversed-phase HPLC and ion chromatography, respectively. Under denitrifying conditions, *T. aromatica* degrades 2- and 4-fluorobenzoate via promiscuous enzymes, including class I benzoyl-CoA reductase and enoyl-CoA hydratases/hydrolases, generating unstable intermediates that spontaneously defluorinate [69]. *Pseudomonas pseudoalcaligenes* KF707 can utilise fluorinated biphenyls as carbon and energy sources via dioxygenase-mediated pathways similar to those used in polychlorinated biphenyl (PCB) degradation [70]. Furthermore, strains such as *Sphingomonas* spp. SS3 and SS33 are capable of degrading 4-fluoro- and 4,4′-difluoro-diphenyl ethers through dioxygenase-catalysed oxidation, forming intermediates like fluorophenol and fluorocatechol, which are further mineralised [71]. Table 3 presents various bacterial strains capable of degrading or transforming other fluorinated xenobiotics, including PFAS, pharmaceuticals, pesticides, and industrial compounds, highlighting both successful biodegradation cases and compounds that remain highly recalcitrant.

Table 3. Other examples of bacterial biodegradation involve fluorinated xenobiotics.

Compound	Bacterial Strain(s)	Degradation Outcome	Reference
PFAS (per- and polyfluoroalkyl substances)			
Perfluorooctanoic acid (PFOA)	*Pseudomonas parafulva strain* YAB1	Partial defluorination (48%)	[72]
Perfluorooctane sulfonate (PFOS)	*Pseudomonas aeruginosa strain* HJ4	Minimal biodegradation	[73]
6:2 Fluorotelomersulfonic acid (6:2 FTSA)	*Gordonia sp. strain* NB4-1Y	Degradation up to ~88%	[74]
6:2 Fluorotelomer alcohol (6:2 FTOH)	*Pseudomonas fluorescens* DSM 83413, *Pseudomonas butanovora*, *Pseudomonas oleovorans*, *Mycobacterium vaccae* JOB5	Degradation up to ~80–100%	[75]
Fluorinated pharmaceuticals			
Fluoxetine (Prozac)	*Bacillus subtilis* DSM 3477, *Comamonas testosteroni* DSM 12678, *Pseudomonas knackmussii* B-13 DSM 6978	Produce trifluoroacetic acid and fluoride ion	[21]
Ciprofloxacin, ofloxacin, norfloxacin, and enrofloxacin	*Thermus thermophilus* strain C419	Attenuated antibacterial activity	[76]
Fluorouracil (5-FU)	*Vibrio fischeri*	Via uracil metabolism	[77]
Flurbiprofen	*Streptomyces griseus* DSM40236 and ATCC13273, and *Streptomyces subrutilis* DSM40445 *Bacillus subtilis* IM7, *Bacillus megaterium* NCIMB8291 and *B. megaterium* ATTC14581	Produce hydroxylated and amidated metabolites	[78]
Flutamide	*Rhodotorula mucilaginosa* ATCC 20129	Produce three metabolites via hydrolysis, nitroreduction, and N-acetylation	[79]
Fluorinated pesticides and agrochemicals			
Trifluralin	*Bacillus* sp. TF-1 *and Arthrobacter aurescens* CTFL7	Biodegradation up to 88%	[80]
β-cyfluthrin and λ-cyhalothrin	*Bacillus* sp. MFK14	Fluoride from β-cyfluthrin and TFA from λ-cyhalothrin are end-products.	[81]
Acifluorfen	*Bacillus* sp. Za	Aminoacifluorfen is produced as a non-toxic end-product	[82]
Fomesafen	*Lysinibacillus* sp. ZB-1	Degradation up to 81.32%	[83]
Oxyfluorfen	*Sphingomonas wittichii* RW1	Degradation up to 75%	[84]
Isopyrazam	*Xanthomonas axonopodis* and *Pseudomonas syringae*	Degradation up to 80% and 86%, respectively	[85]
Fluorinated industrial compounds and intermediates			
Fluorobenzene and 3-fluorocatechol	*Burkholderia fungorum* FLU100	Complete metabolism via 2-fluoromuconate pathway	[86]
4-Fluorobenzoate	*Pseudomonas knackmussii* B-13 DSM 6978	Conversion to 4-fluorocatechol	[87]
4-Fluorobenzaldehyde	*Arthrobacter* sp. strain G1 *and Ralstonia* sp. strain H1	Degradation via *ortho*-cleavage pathway	[88]

Despite these encouraging examples, several challenges continue to limit the efficiency of bacterial degradation of fluorinated xenobiotics. The high bond dissociation energy of the C–F bond results in slow degradation rates, particularly for fully fluorinated compounds [30]. The release of fluoride ions during biodegradation is also problematic due to its toxicity to microbial cells, necessitating specific fluoride detoxification or export systems [89]. Moreover, isolating highly efficient strains and elucidating the specific enzymes and metabolic pathways involved in defluorination remain significant scientific challenges. To address these barriers, research is turning to genomics, synthetic biology, and microbial ecology. Genetic engineering can enhance defluorination or introduce new pathways, while stable microbial consortia may improve the breakdown of complex mixtures [90]. Coupling biological treatment with physicochemical methods like oxidation or sorption also holds promise [51]. Overall, bacteria are central to the biodegradation of fluorinated xenobiotics and advancing our understanding of their genetic and enzymatic mechanisms, alongside innovative biotechnological approaches, is critical for reducing the environmental persistence of these pollutants. However, due to inherent limitations in bacterial degradation pathways, fungi also play a vital complementary role. Filamentous fungi, equipped with a diverse array of oxidative and hydrolytic enzymes, such as cytochrome P450 monooxygenases, laccases, peroxidases, and esterases, provide alternative biochemical routes to defluorinate and transform recalcitrant fluorinated compounds. This enzymatic versatility broadens the scope and enhances the overall efficiency of microbial remediation strategies.

4.2. Fungal Defluorination and Degradation

Fungal biodegradation and biotransformation represent crucial biological processes for the environmental breakdown of fluorinated xenobiotics, including pharmaceutical compounds [91]. These compounds, characterised by strong carbon–fluorine bonds, tend to persist in the environment and resist microbial degradation. However, filamentous fungi possess a wide repertoire of oxidative and hydrolytic enzymes, including cytochrome P450 monooxygenases, laccases, peroxidases, and esterases, enabling them to perform diverse biotransformations [20]. These include hydroxylation, N- and O-dealkylation, amide hydrolysis, and ring cleavage. Through these pathways, fungi can significantly alter the structure, bioactivity, and environmental behaviour of fluorinated drugs, making them valuable agents in bioremediation and green chemistry applications [92].

Figure 6 presents representative examples of fungal-mediated biotransformation of fluorinated pharmaceuticals, highlighting the metabolic versatility of various fungal species. Ciprofloxacin, a widely prescribed fluoroquinolone antibiotic, undergoes extensive fungal metabolism by *Irpex lacteus*, *Pleurotus tigrinus*, *Dichomitus squalens*, *Trametes versicolor*, *Pleurotus ostreatus*, *Ganoderma striatum*, and *Mycoacia ramanniana* [93,94]. Key transformations include N-dealkylation of the piperazine ring, hydroxylation, and oxidative cleavage, generating multiple demethylated and hydroxylated derivatives indicative of phase I- and II-like fungal activities (Figure 6A). Similarly, ofloxacin is metabolised by *I. lacteus*, *D. squalens*, *T. versicolor*, and *P. ostreatus* via N-dealkylation, oxidation, and N-oxide formation, producing metabolites with modifications at nitrogen and oxygen atoms of the piperazine ring, suggesting regioselective oxidative mechanisms [95] (Figure 6B). Pradofloxacin, a third-generation fluoroquinolone, undergoes hydroxylation at both aromatic and aliphatic sites by *G. striatum*, implicating monooxygenase activity and indicating possible alterations in pharmacokinetics and toxicity [95] (Figure 6C). Norfloxacin is transformed by a broad spectrum of fungi, including *I. lacteus*, *D. squalens*, *P. tigrinus*, and *T. versicolor*, through N-dealkylation, hydroxylation, and side-chain cleavage, producing diverse oxidative and ring-cleaved products that reflect the enzymatic plasticity of fungal systems [93,94] (Figure 6D). Danofloxacin is oxidised by *Xylaria longipes* at a nitrogen-containing moiety, forming a ketone derivative, likely through oxida-

tive deamination or secondary amine oxidation, representing a distinct metabolic route [96] (Figure 6E). Enrofloxacin, structurally analogous to ciprofloxacin, is biotransformed by *G. striatum* and *M. ramanniana* via similar pathways, with N-dealkylation and oxidation targeting the piperazine ring and adjacent carbons, yielding more hydrophilic and potentially less bioactive metabolites [97,98] (Figure 6F). Sarafloxacin is metabolised by white-rot fungi *M. ramanniana* and *Phanerochaete chrysosporium* via demethylation and oxidation, resulting in structural alterations to both aromatic and aliphatic regions, which may reduce environmental persistence and biological activity [99,100] (Figure 6G). The non-steroidal anti-inflammatory drug (NSAID) flurbiprofen is transformed by *Cunninghamella elegans* and *Aspergillus oryzae*, primarily via aromatic ring hydroxylation and side-chain oxidation to carboxylic acids, demonstrating fungal capacity to modify both hydrophobic and aliphatic moieties and enhance biodegradation in soil and aquatic matrices [101,102] (Figure 6H). Flutamide, an antiandrogen containing trifluoromethyl groups, is metabolised by *C. elegans*, *Rhodotorula mucilaginosa*, and *Beauveria bassiana* through hydroxylation and amide bond hydrolysis, generating polar metabolites with potentially reduced endocrine-disrupting potential [79,103]. These reactions showcase the fungal ability to cleave robust amide linkages and transform fluorinated aromatic structures (Figure 6I). Lastly, flumequine, a veterinary fluoroquinolone, is degraded by *C. elegans* through hydroxylation and oxidation reactions, forming ketone and hydroxylated products, likely mediated by fungal dehydrogenases and monooxygenases, contributing to enhanced solubility and reduced environmental persistence [104] (Figure 6J). Collectively, these examples illustrate the breadth of enzymatic strategies employed by fungi to transform structurally diverse and environmentally persistent fluorinated pharmaceuticals. The biotransformation pathways underscore the potential of fungal systems in bioremediation approaches aimed at mitigating pharmaceutical contamination in terrestrial and aquatic ecosystems.

Fungi have emerged as promising agents for the biodegradation of PFAS, owing to their unique and diverse enzymatic systems, particularly oxidative enzymes, such as cytochrome P450s, lignin peroxidases, and manganese peroxidases, although evidence for complete defluorination remains limited [20,49]. The white-rot fungus *Phanerochaete chrysosporium* has been extensively studied for its ability to aerobically transform fluorotelomer alcohols (FTOHs); for instance, Tseng et al. demonstrated that 6:2 FTOH was converted into metabolites, such as PFPeA, PFHxA, and PFBA, via intermediates like 6:2 FTUCA, while 8:2 FTOH yielded PFHpA, PFNA, and PFOA, with up to 56% of the products remaining unidentified, suggesting the presence of complex, multi-pathway metabolic networks [105]. Under ligninolytic conditions, Tseng also reported a 50% increase in fluoride ion release from PFOA after 10 days, implying potential enzymatic defluorination by ligninolytic enzymes [106]. Mass spectrometry (MS/MS) data further corroborated the degradation of PFAS via the loss of HF and CF_2 groups, consistent with sequential HF eliminations and carbon chain shortening, as described by Ayala-Cabrera et al. [107]. In parallel, *Cunninghamella elegans* has demonstrated highly efficient PFAS biotransformation, largely attributed to its potent cytochrome P450 (CYP)-mediated xenobiotic metabolism. Khan and Murphy reported complete biotransformation of 6:2 FTOH into 5:3 FTCA within 48 h, an activity fully suppressed by CYP inhibition, underscoring the critical role of these enzymes [108]. The fungi were cultured in Sabouraud dextrose broth and agar at 28 °C, with mycelia grown for 120 h, homogenised in sterile water, and inoculated (5 mL) into 45 mL SDB and then incubated at 28 °C and 150 rpm for 72 h. Organically extractable intermediates were identified using an Agilent 7890B GC–5977A MS with HP-5MS column after silylation (40 µL MSTFA, 60 °C, 2 h), injection (1 µL, 20:1 split), and analysis under a temperature program from 55 °C (4 min) to 300 °C at 10 °C/min in the scan mode. Moreover, when combined with the photocatalyst BiOI, *C. elegans* facilitated a synergistic effect, achieving 90% degradation and 60% defluorination of PFOA, with distinct fluorometabo-

lites arising from fungal biotransformation [109]. Further work revealed CYP5208A3 from *Cunninghamella elegans* is a highly promiscuous monooxygenase capable of metabolising a broad spectrum of xenobiotic substrates, including the perfluoroalkyl substance 6:2 fluorotelomer alcohol. Its activity towards PFAS is specifically enabled when co-expressed with the fungal cytochrome P450 reductase CPR_B, highlighting the adaptability and biotechnological potential of fungal CYP/CPR systems in PFAS biotransformation [110]. While current evidence underscores the capacity of fungi to generate shorter-chain acids and partially defluorinated products from PFAS precursors, the complete mineralisation of these persistent pollutants remains a key challenge. Continued research is, therefore, essential to optimise fungal enzyme systems, clarify degradation pathways, and conclusively link biotransformation to fluoride ion release, thereby paving the way for sustainable, fungi-based PFAS bioremediation strategies [49].

In addition to PFAS, fungi are also increasingly recognised for their role in the biodegradation of other diverse environmental pollutants, including pesticides, dyes, polycyclic aromatic hydrocarbons (PAHs), and halogenated compounds, owing to their metabolic versatility and robust extracellular enzymatic systems, particularly cytochrome P450 monooxygenases, laccases, peroxidases, and hydrolases, which enable transformation or partial mineralisation of recalcitrant xenobiotics under variable environmental conditions, though efficiency and completeness of degradation remain compound-specific and strain-dependent [20]. Filamentous fungi, such as *Cunninghamella elegans*, *Aspergillus sydowii*, *Penicillium decaturense*, and *Fusarium proliferatum*, have demonstrated the ability to metabolise pesticides, like cyhalothrin, transfluthrin, methyl parathion, and allethrin; however, degradation is not always complete, as shown by the persistence of trifluoromethyl moieties in λ-cyhalothrin after fungal treatment [22,111–113]. Dye degradation is commonly mediated by fungal laccases and peroxidases, with species such as *Fusarium oxysporum*, *Aspergillus tabacinus*, and *Cunninghamella elegans*, effectively decolourising dyes like aniline blue, crystal violet, and malachite green, particularly when biofilm-associated or supplemented with inducers [114–117]. *Cunninghamella elegans* biofilm simultaneously degrades malachite green and biosorbs Cr(VI) in a semi-continuous system, maintaining high efficiency and reusability across multiple treatment cycles even under high salt and metal ion conditions [117]. Similarly, Munck et al. demonstrated that muslin cloth effectively induced biofilm formation in *Coriolopsis* sp., significantly enhancing the biodegradation and removal efficiency of triphenylmethane dyes compared to free mycelium forms [118]. For PAHs, fungi preferentially degrade molecules with fewer aromatic rings through oxidative and conjugative pathways. *Dentipellis* sp. showed cytochrome P450 and dehydrogenase upregulation in response to PAH exposure [119]. Baldantoni et al. demonstrated that compost amendment enhances the early-stage degradation of anthracene and benzo(a)pyrene in contaminated soils, particularly when combined with a fungal consortium (*Pleurotus ostreatus*, *Pleurotus eryngii*, *Armillaria mellea*, and *Stropharia ferii*), likely by stimulating peroxidase activity and supporting its potential in bioremediation strategies [120]. Biofilm-forming fungi such as *C. elegans* demonstrated significantly higher fluoranthene degradation rates compared to planktonic forms, and mixed yeast biofilms rapidly catabolised PAHs and phenol in petrochemical-contaminated environments [121]. Fungi also engage in the transformation of halogenated compounds; for example, *P. ostreatus* metabolised > 99% of PCB mixtures, forming hydroxylated and chlorinated biphenyl derivatives [122], though in vivo degradation by laccase-producing fungi like *Trametes versicolor* is variable, indicating intracellular transformation rather than extracellular enzymatic breakdown [123]. Germain et al. isolated 12 fungal strains from PCB-contaminated soil and sediment, identifying four strains—*Penicillium chrysogenum*, *P. citreosulfuratum*, *P. canescens*, and *Aspergillus jensenii*—that degraded over 70% of PCBs. Among these, *P. canescens* uniquely reduced PCB-related

toxicity, with enzymatic activities such as laccases and peroxidases induced variably depending on the strain [124]. Indigenous fungi such as *Aspergillus* and *Penicillium* from contaminated sites show potential for PCB degradation, though comprehensive mechanistic studies are still needed [49]. Overall, fungi offer promising biocatalytic potential for environmental remediation, but achieving complete mineralisation, particularly of highly halogenated or fluorinated compounds, remains a major challenge, highlighting the need for further research into fungal metabolism, enzyme engineering, and biofilm-enhanced bioprocesses to optimise their application in biodegradation systems.

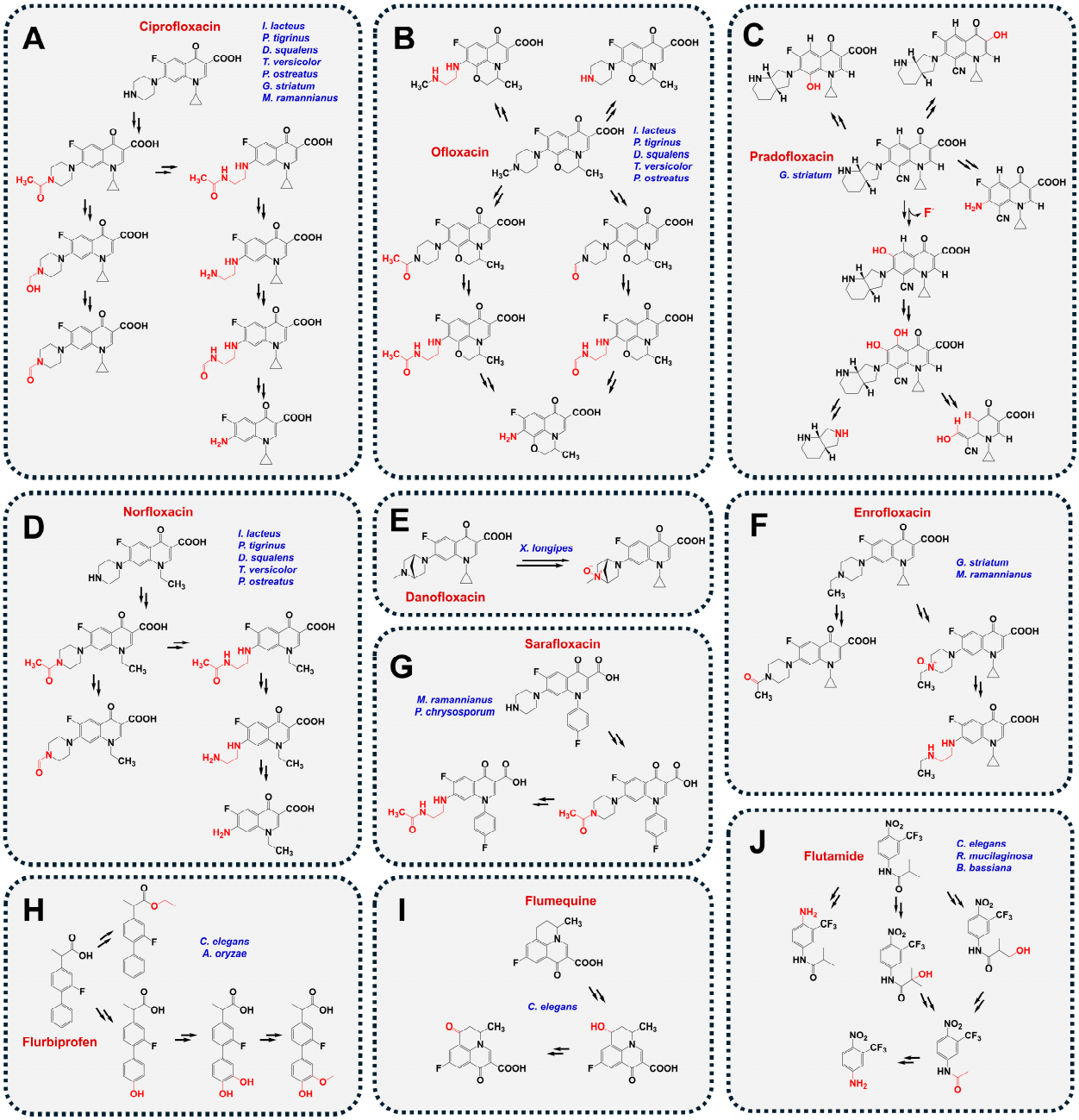


Figure 6. Fungal-mediated biodegradation and biotransformation pathways of selected fluorinated pharmaceuticals. Each panel (**A**–**J**) illustrates the metabolic conversions of individual compounds—ciprofloxacin (**A**), ofloxacin (**B**), pradofloxacin (**C**), norfloxacin (**D**), danofloxacin (**E**), enrofloxacin (**F**), sarafloxacin (**G**), flurbiprofen (**H**), flumequine (**I**), and flutamide (**J**)—by various fungal species. Red highlights indicate the structural modifications introduced by fungal enzymatic activity. Fungal species responsible for each transformation are indicated in blue. The figure is sourced from References [90–101].

In addition to fungi, algae represent another critical group of microorganisms with unique strategies for tackling fluorinated xenobiotics, expanding the biological toolkit available for environmental remediation.

4.3. Algal Defluorination and Degradation

While bacteria and fungi are widely recognised for their direct defluorination capabilities, algae, particularly microalgae, are increasingly acknowledged as important agents in the biodegradation and remediation of fluorinated xenobiotics. Algae employ a multifaceted strategy to mitigate these persistent pollutants [125]. Primarily, they function as effective biosorbents by leveraging abundant surface functional groups, such as hydroxyl, amino, and carboxyl moieties, to adsorb fluorinated compounds from aqueous environments [126]. This adsorption process significantly reduces the bioavailable concentration of these contaminants. Beyond surface binding, certain algal species, such as *Synechocystis* sp. PCC 6803, have demonstrated the ability to internalise PFAS, with studies showing that up to 88% of PFOS is sequestered intracellularly, while approximately 37% of PFOA is partially transformed via yet-unidentified metabolic pathways [127]. Although complete enzymatic defluorination by algae is less frequently observed than in specialised bacterial taxa, preliminary bioinformatic analyses of *Synechocystis* suggest the presence of putative transporters and enzymes that may enable the biotransformation of fluorinated compounds and contribute to the detoxification of released fluoride ions.

Li et al. demonstrated that *Chlorella pyrenoidosa* can tolerate up to 100 mg/L of 3-fluorophenol, with over 50% removal after 240 h, primarily via adsorption. In this study, *C. pyrenoidosa* was cultured in Blue–Green 11 medium at 25 ± 1 °C, 4000 lux (1:1 light–dark), and 150 rpm, with ~1 × 10^5 cells/mL in 250 mL flasks and exposed to 3-fluorophenol (10, 50, and 100 mg/L). Samples were collected every 48 h for 240 h to assess the biomass, 3-fluorophenol concentration (by GC-MS), and biochemical indicators. The algae exhibited stress responses, such as increased superoxide dismutase (SOD) and catalase (CAT) activities, along with elevated malondialdehyde (MDA) and reactive oxygen species (ROS) levels, while activating metabolic pathways including glycerol phospholipid metabolism, autophagy, glycosylphosphatidylinositol (GPI)-anchored protein biosynthesis, and phenylpropanoid biosynthesis. These findings highlight its potential as an effective biosorbent for the bioremediation of the fluorinated pollutant 3-fluorophenol [128].

In addition, algae often engage in synergistic interactions with bacterial consortia, forming integrated microalgae–bacteria systems that significantly outperform monocultures in the bioremediation of fluorinated xenobiotics. These consortia leverage complementary metabolic activities and ecological cooperation, enhancing pollutant degradation efficiency and overall system stability. For instance, studies have reported a 40–90.5% increase in chemical oxygen demand (COD) removal during wastewater treatment when using microalgae–bacteria consortia [129]. This integration not only accelerates contaminant breakdown but also enables concurrent benefits, such as biomass production, biohydrogen generation, and resource recovery, presenting a sustainable and eco-friendly strategy for addressing fluorinated xenobiotic contamination in aquatic ecosystems. Beyond algae-associated systems, more diverse and functionally specialised microbial consortia, particularly those structured around syntrophic interactions, are gaining attention for their enhanced defluorination capabilities and ecological robustness.

4.4. Microbial Consortia and Syntrophic Interactions

Microbial consortia, particularly those exhibiting syntrophic interactions, have emerged as promising biological tools for the biodegradation and defluorination of persistent environmental contaminants such as PFOS [63]. Due to the exceptional stability of the

C–F bond, one of the strongest in organic chemistry, the microbial cleavage of this bond presents a major biochemical challenge. However, recent studies have begun to reveal how diverse microbial communities, rather than individual species, may possess the enzymatic toolkit necessary to facilitate such transformations [130].

Despite the well-documented resistance of PFOS and its short-chain alternatives to microbial degradation under both aerobic and anaerobic conditions, a recent study reported a rare instance of partial PFOS biodegradation [131]. A microbial consortium enriched from PFOS-contaminated soils and supplemented with methanol achieved a 56.7% reduction in PFOS concentration over 20 days, excluding adsorption effects. The consortium was dominated by *Hyphomicrobium* species (~47%) alongside a substantial proportion of unclassified microbes (~53%), suggesting a synergistic mechanism underpinning C–F bond cleavage. Comparative KEGG-based pathway analysis revealed distinct differences between the consortium and *Hyphomicrobium* sp. MC1, highlighting the potential metabolic contributions of uncultured or poorly characterised taxa in facilitating PFOS degradation [132].

Microbial consortia represent a promising strategy for PFAS biodegradation, largely due to syntrophic interactions that enable cooperative metabolism among diverse taxa [133,134]. These interactions, involving the exchange of metabolic intermediates, are essential for overcoming the high energetic barriers associated with defluorination [135]. For example, one species may initiate PFOS degradation by releasing less fluorinated intermediates, which are subsequently metabolised by other community members—an important mechanism when no single organism possesses the full enzymatic machinery for complete breakdown [136]. The addition of co-metabolic substrates like methanol or acetate can further enhance microbial activity by supplying electron donors or auxiliary carbon sources, thereby improving both the rate and extent of defluorination. However, challenges, such as PFAS toxicity, microbial identification, and variability across environmental matrices, must be addressed to optimise these consortia for effective and scalable remediation [135].

An illustrative example of the effectiveness of microbial consortia is provided by Fernandes and colleagues, who investigated the biodegradation potential of consortia derived from estuarine sediment and activated sludge. These consortia were assembled for the removal of fluorinated pharmaceuticals, including paroxetine and bezafibrate [137]. Among the five consortia tested, three achieved removal efficiencies greater than 97%. Fluoride ion release, measured using a fluoride ion-selective electrode, confirmed active C–F bond cleavage. *Pseudomonas* species were dominant across all consortia, while *Acinetobacter* species were consistently detected in static culture conditions. This work highlights the biodegradation and defluorination potential of native microbial consortia from diverse environmental sources and underlines the taxonomic diversity involved in these processes. Such consortia may provide the foundation for future bioremediation tools aimed at environmental restoration.

Metagenomic analysis plays a crucial role in revealing how soil microbial communities respond to PFOS and PFOA stress. Exposure to these perfluorinated compounds increased bacterial richness and diversity but disrupted key functional pathways such as carbohydrate metabolism and membrane transport [138]. Sorn et al. further elucidated these mechanisms by applying metagenome-assembled genomes and metatranscriptome analyses to a bacterial consortium enriched from activated sludge, capable of reducing PFOS. Their study identified key genes encoding enzymes such as alkanesulfonate monooxygenase, (S)-2-haloacid dehalogenase, and putative cytochrome P450, which were highly expressed during PFOS biotransformation. These findings underscore the critical role of specific enzymes in biosorption and defluorination processes and reinforce the potential of such consortia in PFOS remediation [139].

Although PFOS remains a particularly recalcitrant compound, the combination of functional microbial enrichment, syntrophic community interactions, and the strategic use of co-substrates is beginning to reveal new defluorination mechanisms [134,135]. Synthetic ecology approaches that build upon naturally adapted consortia, as demonstrated in both PFOS and fluorinated pharmaceutical degradation studies, show considerable promise for bioremediation [135,136]. These strategies not only offer effective pathways for the detoxification of highly fluorinated compounds but also open opportunities to explore novel microbial metabolisms that remain poorly understood or uncultivated.

Building upon these metagenomic and ecological insights, the enzymatic underpinnings of defluorination have gained significant attention as a molecular framework for biodegradation.

5. Enzymatic Defluorination of Fluorinated Xenobiotics

Enzymatic systems offer promising molecular insights into the defluorination of organic compounds, a process critical for environmental remediation and detoxification. As depicted in Figure 7, various enzyme classes facilitate the cleavage of carbon–fluorine bonds through distinct mechanisms. These include hydrolytic defluorination by haloacid dehalogenases, reductive defluorination by reductive dehalogenases, oxidative defluorination catalysed by cytochrome P450 or monooxygenases, and radical-mediated defluorination by peroxidases and laccases. Each pathway highlights specific enzymatic strategies for overcoming the remarkable stability of the C–F bond, providing valuable blueprints for the design of efficient defluorination technologies.

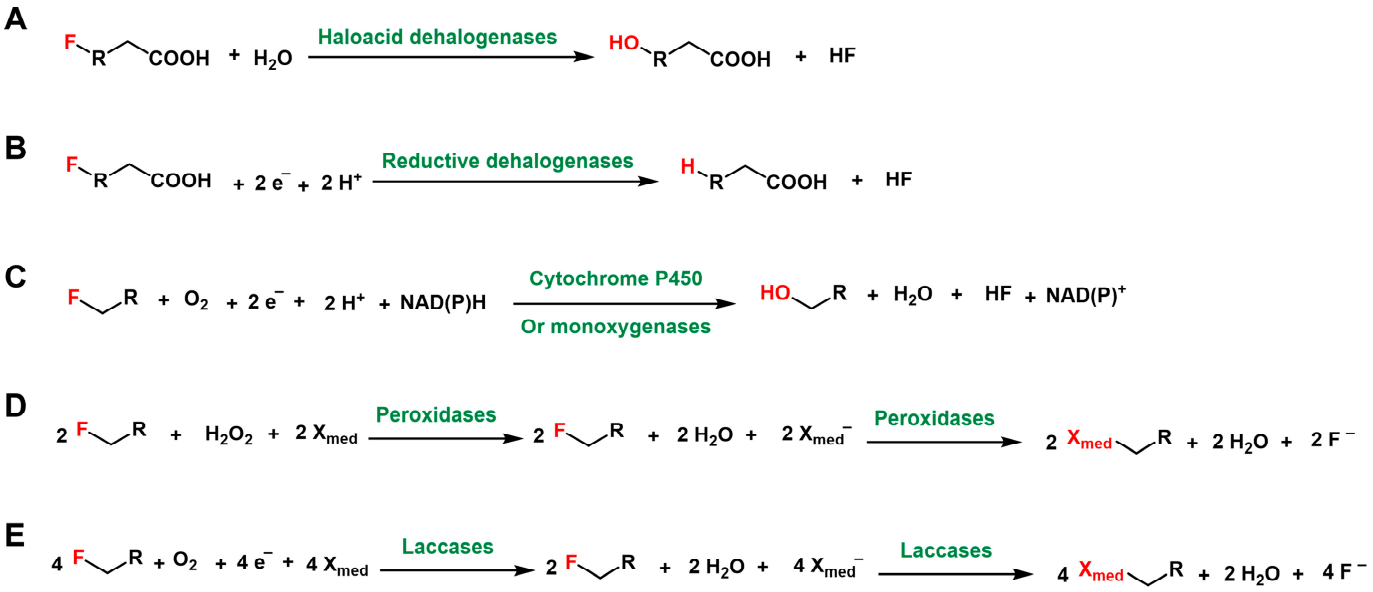


Figure 7. Enzymatic defluorination reactions of fluorinated compounds: (**A**) hydrolytic defluorination catalysed by haloacid dehalogenases; (**B**) reductive defluorination mediated by reductive dehalogenases; (**C**) oxidative defluorination involving cytochrome P450 enzymes or monooxygenases; (**D**) peroxidase-catalysed defluorination reactions; (**E**) laccase-mediated oxidative defluorination.

5.1. Dehalogenases

Dehalogenases are enzymes that catalyse the cleavage of carbon–halogen bonds in organohalide compounds. They are key biocatalysts in microbial pathways that degrade toxic halogenated pollutants. Based on their mechanism, dehalogenases are broadly classified into hydrolytic (e.g., haloacid dehalogenases), oxidative, and reductive dehalogenases. These enzymes play crucial roles in detoxifying and transforming halogenated compounds

into less toxic or more bioavailable forms, thereby facilitating bioremediation of contaminated environments [17]. Among them, hydrolytic and reductive dehalogenases have received significant attention for their potential in defluorination of per- and polyfluoroalkyl substances (PFAS), due to their unique mechanisms of breaking strong C–F bonds [43].

5.1.1. Haloacid Dehalogenases

Haloacid dehalogenases are a class of hydrolases that catalyse the hydrolytic cleavage of carbon–halogen bonds, particularly within haloalkanoic acids. These enzymes are typically divided into L-, D-, and DL-2-haloacid dehalogenases based on substrate specificity [140]. Among them, L-2-haloacid dehalogenases are well-characterised for converting L-2-haloalkanoic acids to their corresponding D-2-hydroxyalkanoic acids [141]. Fluoroacetate dehalogenases, a subclass of 2-fluoroacid dehalogenases, exhibit the capacity to cleave the strong C–F bond in fluoroacetate, a toxic organofluoride naturally produced by microorganisms like *Streptomyces cattleya* and various plants [142].

Fluoroacetate dehalogenases are phylogenetically widespread and found in *Pseudomonas* [143], *Delftia acidovorans* [144], *Serratia liquefaciens* [18], *Burkholderia* [145], and *Rhodopseudomonas* [146]. Key enzymes, such as H-1 from *Delftia acidovorans* B (formerly *Moraxella* sp. B), FA1 from *Burkholderia* sp. FA1, and RPA1163 from *Rhodopseudomonas palustris* CGA009, have been biochemically and structurally characterised. These enzymes follow a two-step S_N2 mechanism involving an enzyme-ester intermediate and a nucleophilic water molecule [147]. Site-directed mutagenesis and quantum-mechanical/molecular-mechanical (QM/MM) modelling of FA1 and RPA1163 have elucidated the roles of conserved catalytic triads (Asp-His-Asp) and active site residues in stabilising the transition state and facilitating fluoride release [148,149]. Notably, residues like Trp150 and Tyr212 (in FA1) and Trp156 and Tyr219 (in RPA1163) form critical hydrogen-bond networks that lower the activation energy for C–F bond cleavage [148–150].

The kinetics and thermodynamics of enzymatic defluorination are critical in determining the degradation efficiency. In haloalkane dehalogenases, Schanstra and Janssen showed that halide release is rate-limited by a slow conformational change (k_{cat} ~3–3.5 s^{-1}) [151], while Krooshof et al. found that catalytic triad mutations reduced activity by over 200-fold [152]. For fluoroacetate dehalogenase, Li et al. reported a higher k_{cat} of 330 min^{-1} for 2-fluoropropionic acid, linked to efficient active site geometry [153]. In more complex systems, Sima et al. demonstrated that PFAS defluorination by *Acidimicrobium* sp. A6 was coupled to ammonium oxidation rates, reflecting thermodynamic dependencies [154]. These studies highlight that enzyme dynamics, activation energy, and substrate structure all influence defluorination performance.

Beyond fluoroacetate, these enzymes demonstrate substrate promiscuity. RPA1163, for instance, defluorinates 2-fluoropropionic acid, difluoroacetate, and even bulky substrates like 2-fluoro-2-phenylacetic acid [155]. Structural and functional studies on other haloacid dehalogenases, such as POL0530 from *Polaromonas* sp. and RJO0230 from *Rhodococcus jostii* strain RHA1, reveal compact conformations and conserved catalytic machinery that enhance fluorine coordination [156]. These enzymes also exhibit activity on environmentally relevant polyfluorinated substances (e.g., 6:2 FTOH and 6:2 PAPs) [157,158].

Furthermore, genetic engineering approaches have introduced haloacid dehalogenase genes into *E. coli*, enabling limited defluorination of persistent fluorochemicals like PFOA [159]. While enzymatic activity in such systems remains modest, these findings highlight the potential of haloacid dehalogenases in bioremediation [160]. Continued structural, mechanistic, and synthetic biology investigations will be essential for harnessing and enhancing their biodefluorination capabilities for environmental applications.

5.1.2. Reductive Dehalogenases

Reductive dehalogenases (RDases) are key enzymes used by anaerobic microorganisms to dehalogenate halogenated compounds via reductive mechanisms. These enzymes fall into the following two categories: respiratory RDases and catabolic RDases [43,161]. Respiratory RDases are oxygen-sensitive, membrane-associated proteins that utilise halogenated compounds as terminal electron acceptors in anaerobic respiration. They are exported to the periplasm via twin-arginine translocation and bind to membrane anchors [162]. Catabolic RDases, by contrast, are cytoplasmic and more oxygen-tolerant, participating in energy-yielding degradation pathways [43,163]. Both types of RDases contain cobalamin and [4Fe–4S] clusters at their active sites, enabling electron transfer and substrate reduction via cobalt–halide interactions [164].

While RDases are well known for the reductive dehalogenation of organochlorides, bromides, and iodides, evidence for enzymatic reductive defluorination remains limited [86]. The high redox potential required to reduce the C–F bond makes this reaction thermodynamically challenging under physiological conditions [165]. However, abiotic systems using cobalamin with Ti(III) citrate or zerovalent zinc have successfully defluorinated branched or unsaturated PFAS, suggesting that similar enzymatic mechanisms may be feasible under tailored microbial conditions [166,167].

Several microbiological studies have inferred reductive defluorination activity. In methanogenic systems, degradation of trifluoroacetate (TFA) into difluoroacetate and fluoroacetate has been observed, though reproducibility has been limited. More convincingly, *Cloacibacillus porcorum* strain MFA1 was shown to stoichiometrically defluorinate monofluoroacetate (MFA) into acetate, without detectable intermediates or known fluoroacetate dehalogenase genes [168]. A breakthrough study identified *Acidimicrobium* sp. strain A6 as capable of defluorinating PFOA and PFOS using hydrogen or ammonium as electron donors and iron(III) as an acceptor [169]. Genomic analysis revealed a putative RDase gene (A6RdhA), but the sequence was incomplete [170]. A highly similar full-length gene (T7RdhA) was found in marine microbial metagenomes, encoding an Fe-S-S protein with a norpseudo-B12 cofactor [171]. Structural modelling suggested PFOA-binding potential, although direct enzymatic evidence remains lacking [170]. Additionally, in the *Dehalococcoides*-containing consortium KB1, defluorination of C6–C8 unsaturated fluorinated compounds occurred in the presence of vitamin B12. Despite the detection of defluorination products, no RDase gene transcription was observed [44]. These studies imply the possibility of enzymatic defluorination but fall short of confirming a definitive RDase-mediated pathway [172].

Additional support for the feasibility of microbial reductive defluorination under tailored conditions comes from recent studies integrating both microbial and electrochemical approaches. Jin et al. revealed that an anaerobic community dominated by *Desulfovibrio* and *Sporomusa* species achieved significant defluorination of chlorinated PFCAs after hydrolytic dichlorination [45]. Furthermore, Che et al. reported enhanced PFAS degradation using a bioelectrochemical system, wherein defluorinating microbes interfaced with electroactive materials. This hybrid setup enabled deeper defluorination than achievable through biological or electrochemical methods alone, emphasising the role of material–microbe synergy in advancing PFAS remediation [46].

Haloacid and reductive dehalogenases represent two important enzymatic strategies for breaking down halogenated compounds. While haloacid dehalogenases have demonstrated clear activity against C–F bonds in specific substrates, reductive dehalogenases remain underexplored in this context. The evidence for their role in PFAS degradation is still emerging, often indirect, and requires further validation [43,172]. Continued research into enzyme discovery, structural analysis, and bioengineering will be crucial for advancing

our understanding of enzymatic defluorination. Harnessing these enzymes could play a key role in developing effective and eco-friendly solutions for PFAS bioremediation.

5.2. Cytochrome P450s and Monooxygenases

Cytochrome P450 enzymes (CYPs) are a large and diverse superfamily of haem-thiolate monooxygenases found across all domains of life—Archaea, Bacteria, and Eukaryotes [173]. These enzymes play crucial roles in numerous physiological and metabolic processes, including the biosynthesis of secondary metabolites, sterols, and the detoxification of xenobiotics [174]. Fungal CYPs are particularly important for their ability to metabolise a wide variety of endogenous and exogenous compounds, contributing to cellular metabolism, environmental adaptation, pathogenicity, and, notably, the biotransformation of hazardous substances [49]. Although they share several conserved structural motifs, the overall sequence similarity among fungal CYPs is low, with current phylogenetic analyses clustering them into approximately 15 clades [175]. Their catalytic activities extend beyond hydroxylation to include epoxidation, dehalogenation, decarboxylation, demethylation, denitrification, desulfurisation, and desaturation reactions, making them highly versatile in substrate transformation [176]. CYPs typically operate through a catalytic cycle that involves electron transfer from NADPH (or NADH) via cytochrome P450 reductase (CPR) and, sometimes, cytochrome b5 to the haem iron at the active site [49]. The cycle begins with the reduction of the haem iron, followed by the binding of molecular oxygen and the formation of a highly reactive ferryl-oxo intermediate known as Compound I. This species abstracts a hydrogen atom from the substrate, creating a substrate radical that recombines with the hydroxyl group on the haem, producing the monooxygenated product and returning the enzyme to its resting state [177]. Fungal CYPs may be present in different forms depending on their interaction with redox partners, as follows: single-component fusion enzymes, two-component CPR-CYP systems, or three-component systems involving CPR, cytochrome b5, and CYP [49,110,178].

The expanding availability of fungal genomic resources, such as those from the 1000 Fungal Genomes Project, has facilitated the functional characterisation of fungal CYPs through heterologous expression in model systems like *Saccharomyces cerevisiae* and *Pichia pastoris*. However, expressing functional CYPs remains challenging due to issues such as their membrane association, the incorporation of haem groups, and the need for efficient coupling with redox partners [179]. Various strategies, including host selection, vector optimisation, codon adjustment, and protein engineering, have been employed to overcome these limitations [180]. For example, a comprehensive screening of the CYPome of *Thamnidium elegans* in *S. cerevisiae* led to the identification of CYP5312A4, which catalysed an unusual 14α-hydroxylation of testosterone [181]. Similarly, co-expression studies involving CYP5208A3 from *Cunninghamella elegans* and different CPRs in *P. pastoris* highlighted the importance of specific CYP-CPR pairings for optimal monooxygenase activity, supporting the hypothesis that fungi may broaden their xenobiotic degradation capabilities through multiple redox partner interactions [110].

The catalytic versatility of CYPs also extends to the biodegradation of halogenated and fluorinated xenobiotics. For instance, mammalian CYPs such as P450 2E1 have been shown to perform oxidative defluorination on inhalation anaesthetics like sevoflurane, generating reactive metabolites [182]. Similarly, $P450_{BM3}$-F87G from *Bacillus megaterium* catalyses the oxidative defluorination of 4-fluorophenol, forming benzoquinone, which is subsequently reduced to hydroquinone via NADPH-dependent reactions [183]. In microbial systems, $P450_{CAM}$ from *Pseudomonas putida* has demonstrated reductive dehalogenation activity on perfluorinated and chlorinated substrates, such as 1,1,1-trichlorotrifluoroethane and

trichlorofluoromethane, in the presence of electron donors like titanium(III) citrate, with products including carbon monoxide and halogenated intermediates [184].

In terms of per- and polyfluoroalkyl substances (PFAS), studies have identified fungal and bacterial P450s as participants in the defluorination of 6:2 fluorotelomer alcohol (6:2 FTOH). For example, strain RHA1 exhibited significantly higher fluoride release when exposed to 6:2 FTOH in the absence of P450 inhibitors, suggesting the involvement of a P450 enzyme, later cloned and expressed in strain PD631, which confirmed its role in 6:2 FTOH degradation. Moreover, the same gene was found to be upregulated during the defluorination of 6:2 fluorotelomer sulfonic acid (6:2 FTSA) in RHA1 [185]. In fungi like *C. elegans* and *P. chrysosporium*, evidence of P450 involvement has been demonstrated through inhibition studies and the measurement of NADPH-CPR activity, respectively [108–110,186]. Additionally, heterologous expression of CYP5208A3 and its associated CPR in *P. pastoris* revealed their ability to convert 6:2 FTOH to 6:2 FTCA, further supporting the role of fungal CYPs in PFAS metabolism [108,110].

In addition to CYPs, monooxygenases such as alkane monooxygenases and butane monooxygenases have also been implicated in the degradation of fluorinated compounds [187]. The well-studied three-component butane monooxygenase system from *Pseudomonas butanovora* includes a diiron hydroxylase, a reductase that delivers electrons from NADH, and a regulatory component [188]. These enzymes, primarily oxidise alkanes (C2–C9) and alcohols (C2–C4), have also been associated with co-metabolic dehalogenation under aerobic conditions [189,190]. Likewise, alkane monooxygenase from *Pseudomonas putida* GPo1, an integral membrane-bound ω-hydroxylase, catalyses the terminal hydroxylation of C5–C12 alkanes into primary alcohols [191]. Given the structural similarity between linear alkanes and fluorotelomer alcohols (FTOHs), researchers hypothesised that these enzymes might also metabolise FTOHs [192]. Indeed, strains harbouring these monooxygenases have been reported to defluorinate 4:2, 6:2, and 8:2 FTOHs, as well as 6:2 polyfluoroalkyl phosphates (6:2 PAPs), resulting in the sequential loss of CF2 groups and the formation of shorter-chain perfluorocarboxylic acids (PFCAs) [157,158,192,193]. While further mechanistic studies are required to clarify the exact roles of butane monooxygenases in FTOH degradation, there is stronger evidence for the involvement of alkane monooxygenases, especially given that higher gene copy numbers of *alkB*, the gene encoding this enzyme, have been correlated with increased fluoride release during the biodegradation of both FTOHs and 6:2 PAPs [157,158].

Monooxygenases, especially cytochrome P450s (CYPs) and alkane/butane monooxygenases, are powerful biocatalysts capable of initiating the activation and transformation of persistent fluorinated xenobiotics [49]. Although significant advances have been made in identifying and engineering these enzymes for defluorination, several challenges remain, including difficulties with heterologous expression, coupling with appropriate redox partners, and achieving complete mineralisation of per- and polyfluoroalkyl substances (PFAS) [185]. Future research should focus on the structural and mechanistic understanding of CYP–substrate interactions, utilise functional metagenomics and directed evolution to improve enzyme specificity and catalytic efficiency, and integrate multi-omics approaches to uncover natural defluorinating pathways [138,194]. Effectively harnessing these enzymes could provide sustainable and effective solutions for PFAS bioremediation.

5.3. Peroxidases

Peroxidases are haeme-containing enzymes widely distributed among fungi, bacteria, plants, and animals, and they play a vital role in the oxidative degradation of a broad spectrum of environmental pollutants [195]. These enzymes utilise hydrogen peroxide (H_2O_2) or organic hydroperoxides as co-substrates to initiate a catalytic cycle that generates highly

reactive radical intermediates, which facilitate the breakdown of complex and recalcitrant organic molecules [196]. The catalytic mechanism typically involves the following three main oxidative states: The resting state enzyme reacts with H_2O_2 to form a Fe^{3+}-hydroperoxo complex, known as Compound 0, which is then converted to Compound I (Fe^{4+}=O with a porphyrin or protein radical). Compound I abstracts a hydrogen atom from an organic substrate (X-H), generating a substrate radical (X•) and forming Compound II (Fe^{4+}=O). Compound II is subsequently reduced back to the resting state, completing the cycle and releasing an additional radical species. This radical-based catalysis underlies the oxidative transformation and detoxification of phenols, polycyclic aromatic hydrocarbons (PAHs), pesticides, polychlorinated biphenyls (PCBs), dyes, endocrine-disrupting compounds (EDCs), and other xenobiotics [197].

Among the most prominent fungal peroxidases are lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP), all of which are secreted by white-rot fungi and are key players in lignin degradation and xenobiotic transformation [49,198,199]. LiP and MnP both rely on H_2O_2, but MnP additionally requires Mn^{2+}, which it oxidises to Mn^{3+}. The resulting Mn^{3+} ions act as diffusible oxidants capable of initiating ligninolytic and pollutant-degrading reactions [20,49]. VP combines the catalytic features of both LiP and MnP, enabling it to oxidise a broader range of phenolic and non-phenolic substrates. The versatility and broad substrate specificity of peroxidases make them attractive candidates for environmental bioremediation, including the transformation of fluorinated xenobiotics such as per- and polyfluoroalkyl substances (PFAS) [49].

Recent research has begun to explore the roles of peroxidases in PFAS biodegradation [200]. Notably, LiP and MnP have been implicated in the biotransformation of fluorinated compounds such as 5:3 fluorotelomer carboxylic acid (5:3 FTCA). When *Phanerochaete chrysosporium* was cultured in the presence of 5:3 FTCA, the genes encoding LiP (*lipD*) and MnP (*mnp1*) exhibited time-dependent upregulation compared to PFAS-free controls, with transcript levels peaking on day 28 [186]. In parallel, LiP activity progressively increased, while the MnP activity remained stable before a late-stage upsurge, suggesting that these enzymes contributed to the biotransformation of 5:3 FTCA or were upregulated in response to the compound or its metabolites.

Although the precise mechanisms of defluorination remain to be fully elucidated, product identification and theoretical modelling suggest that peroxidase-mediated reactions may facilitate stepwise cleavage of C–F bonds, leading to defluorinated intermediates and potentially fluoride release. However, mechanistic clarity is still lacking, particularly regarding the fate of fluorine atoms and the stability of intermediates under oxidative conditions [201]. Despite their promise, peroxidase-based bioremediation faces practical challenges, including enzyme instability under fluctuating pH and temperature conditions, sensitivity to inhibitors, and limitations in reuse. These hurdles are being addressed through strategies such as protein engineering and immobilisation. For instance, a LiPH8 triple mutant, developed by Son et al., exhibited improved thermostability and a ten-fold increase in half-life at pH 2.5 [202]. Additionally, immobilisation of MnP on magnetic nanoparticles or chitosan-based nanocomposites has enhanced thermal stability, broadened the operational pH range, and improved catalytic efficiency across multiple pollutant removal cycles [203]. Overall, while further research is needed to define the enzymatic mechanisms of PFAS defluorination, fungal peroxidases, particularly LiP, MnP, and VP, offer promising tools for the oxidative transformation of persistent fluorinated xenobiotics.

5.4. Laccases

Laccases are multicopper oxidases produced by a wide range of organisms, including bacteria, fungi, plants, and insects. These enzymes, particularly abundant in white-rot fungi,

play a key role in lignin degradation and the oxidative breakdown of various aromatic pollutants [204]. Structurally, fungal laccases such as those from *Trametes versicolor* contain four copper atoms organised into the following three distinct centres: the mononuclear type 1 (T1), mononuclear type 2 (T2), and binuclear type 3 (T3). These copper centres form a trinuclear cluster (T2/T3), which is essential for oxygen reduction and catalysis [205].

Laccases catalyse single-electron oxidations of phenols, polyphenols, aromatic amines, and other organic substrates. The T1 copper site initially accepts an electron from the substrate, which is subsequently relayed to the T2/T3 cluster. This cluster reduces molecular oxygen to water via a four-electron reduction, completing the catalytic cycle [206]. Importantly, laccases use molecular oxygen as the terminal electron acceptor, eliminating the need for externally supplied hydrogen peroxide, which can be detrimental to enzyme stability in peroxidase systems [207]. However, many substrates are not directly oxidisable by laccase due to redox potential limitations. In such cases, small-molecule redox mediators, such as 1-hydroxybenzotriazole (HBT) or 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), are used. These mediators form stable radical cations upon laccase oxidation and subsequently diffuse from the active site to oxidise target compounds via non-specific radical-based pathways [205–207].

Recent studies have demonstrated the potential of laccase-mediated enzyme-catalysed oxidative humification reactions (ECOHRs) for degrading persistent fluorinated xenobiotics, particularly PFOA and PFOS [201,202]. Luo et al. reported that laccase from *Pleurotus ostreatus*, combined with the mediator HBT, facilitated ~50% degradation of PFOA (0.5 μM) over 157 days [208]. High-resolution mass spectrometry identified partially fluorinated shorter-chain alcohols and aldehydes, and 28% of fluorine was released as fluoride ions, indicating partial defluorination. Interestingly, defluorination products did not include short-chain perfluorinated acids, suggesting that degradation involved radical-mediated decarboxylation, side-chain cleavage, and rearrangement processes [208–210].

The efficiency of laccase-HBT systems in PFAS degradation is influenced by the presence of metal ions. Cu^{2+} and Fe^{3+}, which can complex with PFOA or PFOS, enhanced degradation rates, likely by reducing electrostatic repulsion and improving proximity between negatively charged PFAS molecules and the laccase/HBT system [209,210]. In contrast, ions such as Mg^{2+} or Mn^{2+} had negligible effects. In PFOS degradation experiments, Cu^{2+} facilitated a 59% removal, while Mg^{2+} led to a 35% degradation over 162 days [209]. In both cases, partially defluorinated intermediates were detected, again supporting a free-radical-driven mechanism.

Beyond synthetic mediators, natural materials have also been explored. For instance, soybean meal was validated as an effective organic mediator for in-soil PFOA degradation via laccase-induced ECOHRs [211]. Furthermore, both purified (*Pleurotus ostreatus*) and crude laccase extracts (from *Pycnoporus* sp. SYBC-L3) showed comparable efficacy, indicating the robustness of the catalytic system and its potential for field application [43].

While promising, laccase applications in environmental remediation face challenges including enzyme instability at high temperatures or extreme pH, low yields from native hosts, and the high cost of mediators. Advances in enzyme engineering and immobilisation have addressed some of these limitations. For example, fusing a laccase gene with a carbohydrate-binding module and expressing it in *Saccharomyces cerevisiae* BJ1824 improved thermostability and pH tolerance [212]. Similarly, immobilising laccase from *T. versicolor* on chestnut biochar enhanced activity towards polycyclic aromatic hydrocarbons (PAHs), extended operational stability, and broadened the enzyme's effective pH and temperature ranges [213].

In summary, laccase-mediated systems, particularly when combined with appropriate redox mediators and metal cofactors, demonstrate significant potential for the oxidative

degradation and partial defluorination of persistent fluorinated pollutants like PFOA and PFOS [208–211]. The underlying mechanisms appear to involve free radical formation, decarboxylation, and side-chain oxidation rather than direct C–F bond cleavage [211]. Further mechanistic studies and optimisation are needed to enhance defluorination efficiency and expand applicability to a broader range of per- and polyfluoroalkyl substances.

Table 4 summarises the major enzyme classes involved in the microbial defluorination and degradation of fluorinated xenobiotics, including haloacid dehalogenases, reductive dehalogenases, cytochrome P450s, monooxygenases, and oxidative enzymes such as peroxidases and laccases. These enzymes play diverse roles in cleaving the chemically stable C–F bond through various catalytic mechanisms.

Table 4. Enzymes involved in defluorination and degradation of fluorinated xenobiotics.

Feature	Haloacid Dehalogenases	Reductive Dehalogenases	Cytochrome P450s and Monooxygenases	Oxidative Enzymes (Peroxidases and Laccases)
Examples	L-haloacid dehalogenases D-haloacid dehalogenases DL-2-haloacid dehalogenases Fluoroacetate dehalogenases (FA1, H-1, RPA1163, POL0530)	T7RdhA RDase A6RdhA RDase Other RDases	CYP5208A3 $P450_{BM3}$-F87G $P450_{CAM}$ Alkane monooxygenase Butane monooxygenase	Peroxidases (lignin peroxidase, manganese peroxidase, versatile peroxidase) Laccases (multi-copper oxidases)
Mechanism	SN2 hydrolysis via enzyme-ester intermediate	Reductive cleavage via cobalamin + [4Fe–4S] clusters	Monooxygenation via Compound I (Fe^{4+}=O), hydroxylation via electron transfer	Electron transfer oxidations, radical generation, substrate oxidation
Microbial sources	*Streptomyces cattleya* *Pseudomonas* spp. *Serratia liquefaciens* *Delftia acidovorans* *Burkholderia* sp. FA1 *Rhodopseudomonas palustris* *Polaromonas* sp. *Rhodococcus jostii* RHA1	*Cloacibacillus porcorum* *Acidimicrobium* sp. A6 *Dehalococcoides*-containing consortium KB1	*Thamnidium elegans* *Cunninghamella elegans* *Phanerochaete chrysosporium* *Pseudomonas putida* *Bacillus megaterium* *Pseudomonas butanovora*	*Phanerochaete chrysosporium* *Pleurotus ostreatus* *Pycnoporus* sp. SYBC-L3 *Trametes versicolor* Bacteria Plants
Substrate scope and examples	Fluoroacetate 2-FPA Difluoroacetate 6:2 FTOH 6:2 PAPs	MFA TFA PFOA PFOS C6–C8 unsaturated PFAS	6:2 FTOH 6:2 FTSA 4-Fluorophenol Alkanes (C2–C12) fluorotelomer alcohols	Aromatic compounds Phenolics PFOA PFOS Fluorinated phenols
PFAS relevance/key findings	Demonstrated defluorination of fluoroacetate and analogues Enzyme engineering (e.g., in *E. coli*) shows PFOA defluorination potential	Emerging evidence—that vitamin B12 stimulation enhances PFAS defluorination—indirect links to RDases Some stoichiometric MFA defluorination	Confirmed defluorination of 6:2 FTOH and FTSA Potential co-metabolic PFAS transformation due to substrate similarity	Some evidence of PFAS precursor oxidation Partial defluorination via radical attack Enhances biodegradation with mediators
References	[140–160]	[161–172]	[173–194]	[195–213]

5.5. Enzymatic and Synthetic Biology Approaches to Improve Microbial Defluorination

The strength and stability of the carbon–fluorine (C–F) bond make fluorinated organic compounds remarkably persistent in nature [29]. These properties have led to widespread environmental contamination from substances like per- and polyfluoroalkyl substances (PFAS), which resist conventional degradation [214]. However, recent advances in enzyme engineering are opening new pathways for tackling this environmental challenge through bio-based defluorination strategies.

One of the most promising developments is the engineering of enzymes to break down C–F bonds, a feat once considered highly improbable due to the bond's exceptionally high dissociation energy. A notable breakthrough was the modification of cysteine dioxygenase, a natural enzyme that typically contains a protein-derived cysteine–tyrosine cofactor [215]. By substituting the tyrosine at position 157 with 3,5-difluorotyrosine, researchers successfully enabled the enzyme to cleave the fluorine atoms during the cofactor formation process [216]. This marked one of the first demonstrations of engineered defluorination within the enzyme itself, highlighting the potential of synthetic biology to enhance or reprogram enzyme function.

5.5.1. Thermodynamic and Environmental Factors Influencing Microbial Defluorination

Microbial defluorination efficiency is heavily governed by thermodynamic and kinetic parameters, as well as environmental conditions. For example, Liu et al. demonstrated that moxifloxacin degradation via $CoFe_2O_4$-activated peroxymonosulphate follows pseudo-first-order kinetics (k_{obs} = 0.194 min^{-1}), with sulphate radicals ($SO_4\bullet^-$) serving as the primary reactive species driving defluorination and related degradation pathways [217]. This underscores the pivotal influence of reactive species generation and catalyst activation on the rates and outcomes of microbial defluorination processes.

The availability of electron donors, such as NH^{4+}, activated carbon, and sucrose, significantly enhances microbial Fe(III) reduction, which, in turn, improves the removal of PFAS like PFOA and PFOS in wetland sediments [218]. These electron shuttles promote the growth of iron-reducing bacteria and alter organic carbon composition, creating favourable conditions for microbial degradation. Furthermore, environmental redox conditions and electron donor availability critically influence microbial degradation pathways. Under anaerobic conditions with iron oxide, PFOA and PFOS act as electron acceptors, while electron shuttles facilitate electron transfer and enhance microbial degradation [219]. Since microbes generally lack the enzymatic machinery to use PFAS directly as energy sources, they rely on external electron donors to drive co-metabolic oxidation. Variations in the enhancement effects between PFOA and PFOS also reflect their differing chemical properties and microbial accessibility, underscoring the importance of electron donor availability and environmental conditions for efficient PFAS bioremediation.

Collectively, these studies underscore that thermodynamic and kinetic constraints, modulated by environmental factors like electron donor presence and oxygen levels, are pivotal in optimising microbial defluorination processes. Advances in enzyme engineering, metagenomics, and synthetic biology offer promising avenues to overcome these limitations and develop scalable bioremediation strategies.

5.5.2. Engineering Microbial Hosts and Discovery of Novel Defluorinases

Building upon advances in enzyme engineering, recent research has shifted towards identifying new defluorinase enzymes and engineering microbial hosts capable of withstanding the toxic fluoride ions released during defluorination [220]. Since the release of fluoride can impose significant cytotoxic stress—especially when degrading heavily fluorinated compounds like perfluorooctanoic acid (PFOA), which can liberate up to 15 fluoride ions per molecule—robust microbial systems are essential for effective biodegradation. This has driven efforts to enhance microbial fluoride tolerance by improving fluoride export mechanisms and optimising host physiology to sustain high defluorination activity under environmentally relevant conditions [221]. Two major export proteins, CLCFs (fluoride/proton antiporters from the CLC family) and Flucs (passive fluoride channels, often encoded by *crcB*), play key roles in maintaining low intracellular fluoride concentrations [222,223]. These transporters are widespread across bacterial taxa, with over 85% of strains encoding at least one [224]. Synthetic biology approaches now incorporate such transporters into chassis strains and apply adaptive evolution using intermediate fluorinated compounds to incrementally boost microbial resilience and degradation efficiency [220].

Another major avenue is the use of surrogate fluorinated substrates with features that facilitate growth or selection, which allows for adaptive laboratory evolution and enzyme discovery. Organisms such as *Pseudomonas putida*, known for their natural fluoride resistance, have been leveraged in such experiments, showing great potential when paired with rational strain engineering and evolution under selective pressure [225].

5.5.3. Enzymatic Mechanisms and Structure-Guided Enzyme Engineering

Fluoride elimination represents an energetically favourable biochemical pathway for defluorination compared with the direct hydrolysis or reduction of the robust carbon–fluorine bond. This process generates reactive intermediates, such as fluorinated olefins, which can undergo subsequent enzymatic transformations, including oxidation, reduction, or hydration, ultimately yielding more readily degradable products [29]. Notably, enzymes such as pyruvate decarboxylase have demonstrated the ability to catalyse defluorination reactions, exemplified by the turnover of 3-fluoropyruvate, highlighting the catalytic potential of natural enzymes in this context [226]. Leveraging detailed insights into enzyme structure–function relationships, structure-guided engineering enables rational modification of enzyme active sites to enhance specificity, catalytic efficiency, and stability for defluorination applications.

Fluorotelomers are widely used in industrial applications, presenting another opportunity for enzymatic defluorination. Their structure includes methylene groups adjacent to functional headgroups like sulfonates or carboxylates, providing enzymatic entry points [52]. Enzymes such as alkanesulfonate monooxygenase have been implicated in the degradation of sulfonated telomers, exploiting proton abstraction mechanisms that mirror those used in chemical defluorination with bases like NaOH [227,228].

Recent studies underscore the importance of understanding enzyme structure–function relationships to overcome the inherent limitations of natural enzymes in defluorination reactions. For example, Marciesky et al. emphasised the promise of in silico enzyme design to improve radical-generating enzymes such as laccases and peroxidases. This rational approach combines computational modelling and experimental screening to enhance enzyme specificity, catalytic efficiency, and structural stability for PFAS degradation [229].

Laccases, in particular, have attracted attention for PFAS biotransformation. However, their practical use requires improvements in thermostability, reusability, and activity [230]. Modern protein engineering tools like PROSS and FuncLib now enable structure-guided mutation of laccases by leveraging evolutionary conservation and stability predictions. This has led to the development of more robust bacterial laccases, which offer broader pH tolerance and thermal resistance than their fungal counterparts [231–233]. These optimised enzymes are more suitable for industrial-scale PFAS degradation.

Equally important are innovations in screening platforms to identify effective defluorinases. Traditional negative selection methods are hindered by fluoride toxicity; thus, fluoride-resistant microbial hosts like *Pseudomonas putida* and *Saccharomyces cerevisiae* are increasingly employed for functional screening of enzyme libraries [234]. Furthermore, the integration of microfluidic droplet sorting and fluoride-sensitive biosensors such as FluorMango riboswitches allows for high-throughput screening of defluorinating enzymes, even when catalytic efficiencies are low [235,236].

Machine learning and computational approaches are beginning to play a role in identifying and improving candidate enzymes [237]. Predictive tools have been used to model C–F bond dissociation energies and guide mutagenesis, improving the efficiency of defluorinase design. For instance, a modified connectivity-based hierarchy (CBH) scheme enables accurate and efficient computation of C–F bond dissociation energies in PFAS, guiding enzyme mutagenesis for improved defluorinase design [238]. Furthermore, integrating multi-enzyme systems for complete PFAS breakdown, using oxidoreductases and cofactor-dependent enzymes, may require formulated enzyme cocktails or even small bioreactor systems [239].

5.5.4. Synthetic Biology and Microbial Consortia for Scalable Bioremediation

Advances in synthetic biology provide powerful tools for constructing modular, multi-step defluorination pathways within single microbial hosts or consortia, enabling efficient breakdown of complex PFAS molecules. Cutting-edge genetic engineering techniques, such as CRISPR-based genome editing, Golden Gate cloning, and pathway refactoring, facilitate the assembly and precise control of synthetic metabolic circuits tailored to target specific fluorinated substrates [240]. Implementation of artificial operons, synthetic promoters, and tuneable expression systems enhances metabolic flux through these pathways while mitigating the accumulation of toxic intermediates. Such engineered microbial platforms, including defined consortia, offer promising, scalable solutions for in situ and ex situ bioremediation of persistent fluorinated environmental contaminants.

In practical applications, engineered microbes or consortia could be deployed in bioreactors, such as packed-bed, membrane, or fluidised-bed systems, where operational parameters can be optimised for PFAS removal from contaminated water sources [114,241,242]. Additionally, community-based ecological restoration efforts using native or augmented microbial consortia offer a promising avenue for in situ bioremediation of fluorinated compounds in soil or groundwater environments [15]. Such efforts may integrate environmental DNA (eDNA) monitoring, omics-based community optimisation, and habitat engineering to ensure microbial persistence and activity in natural systems.

Together, these recent advancements in structure-guided enzyme engineering, host strain development, and high-throughput screening technologies represent a transformative shift in the field. While the natural evolution of enzymes against fluorinated compounds has been limited, modern enzyme engineering, synthetic biology, and adaptive evolution offer powerful tools to bridge this gap. These strategies not only expand our enzymatic toolbox but also pave the way for effective bioremediation of persistent environmental pollutants.

6. Challenges and Limitations

The degradation of fluorinated xenobiotics, particularly per- and polyfluoroalkyl substances (PFAS), presents a multifaceted challenge due to their exceptional chemical stability and environmental persistence [243]. Traditional remediation methods, such as high-temperature incineration, advanced oxidation processes (AOPs), and activated carbon adsorption, often suffer from high operational costs, energy demands, incomplete mineralisation, and potential formation of toxic by-products. In contrast, microbial and enzymatic strategies offer a potentially sustainable, low-energy, and environmentally friendly alternative for the breakdown of C–F bonds under mild conditions. However, the application of biological defluorination is still in its early stages and faces numerous barriers that hinder efficiency, scalability, and practical deployment in real-world settings. These challenges can be broadly categorised into those associated with microbial degradation systems and those specific to isolated enzymatic processes.

6.1. Challenges in Microbial Defluorination

Bacteria possess immense metabolic diversity and adaptive capabilities, positioning them as potential agents for the biodegradation of fluorinated xenobiotics. However, this promise is hampered by several limitations. Chief among these is the high stability of the carbon–fluorine (C–F) bond, one of the strongest in organic chemistry, which severely limits degradation rates, particularly for fully fluorinated compounds such as many PFAS [27,28]. Additionally, the release of toxic fluoride ions during defluorination poses a threat to microbial viability, necessitating efficient detoxification or efflux systems that are often absent or suboptimal [89,244]. The identification and isolation of microbial strains with

robust, sustained defluorination capabilities is an ongoing scientific challenge, compounded by a limited understanding of the enzymes and metabolic pathways underpinning these processes [18,245].

Efforts to replicate microbial degradation in complex environmental matrices such as soil or groundwater often fall short due to competing substrates, microbial diversity, and fluctuating physicochemical conditions [246]. Even when degradation occurs, persistent and toxic intermediates, such as short-chain perfluorinated carboxylic acids (PFCAs), may accumulate, undermining remediation goals [60,61]. Highly fluorinated compounds, such as trifluoroacetic acid (TFA), remain notably recalcitrant [247]. Furthermore, the metabolic conditions required for degradation vary; while oxidative processes dominate in aerobic environments, some anaerobic bacteria perform reductive defluorination [172]. This necessitates finely tuned redox management in remediation contexts. In many cases, microbial defluorination relies on co-metabolism, where an additional growth substrate is required to trigger transformation of the target xenobiotic, adding further complexity to bioremediation strategies [52].

Fungal systems, while offering diverse oxidative and hydrolytic enzymes, face similar constraints. Fungi often achieve only partial mineralisation of fluorinated compounds, frequently generating intermediates that retain fluorinated moieties. Their degradation capabilities are highly strain- and compound-specific, with performance sensitive to environmental factors such as pH, temperature, and nutrient availability [138,140,194]. Though enzymes, such as cytochrome P450s, laccases, and peroxidases, hold promise, significant work is needed to optimise these systems through enzyme engineering [17,18]. The formation and maintenance of biofilms for consistent degradation performance under field conditions remain a research frontier, and in many instances, degradation occurs intracellularly rather than through secreted enzymes, limiting the direct accessibility of pollutants [248]. Mechanistic understanding of fungal-mediated defluorination remains limited, further constraining rational design of effective fungal bioremediation approaches [89].

Algae are an eco-friendly remediation option, but their direct enzymatic defluorination is poorly understood. They primarily act through biosorption or sequestration rather than true C–F bond cleavage, and disposal of fluorinated biomass raises environmental concerns [126]. Degradation is typically slow and reliant on bacterial symbiosis, and large-scale applications face resource and infrastructure challenges [129].

6.2. Challenges in Enzymatic Defluorination

Enzymatic degradation provides precise molecular tools to dissect and exploit defluorination pathways yet faces distinct and formidable challenges. The C–F bond's high bond dissociation energy poses a thermodynamic barrier that requires specialised active sites or significant energy input, conditions difficult to replicate under physiological or environmental settings. Although dehalogenases are diverse, those capable of cleaving C–F bonds efficiently and broadly are rare, with much of the existing evidence for enzymatic defluorination being indirect or inferred from metabolic studies [28–30].

Enzyme specificity further limits their application. While some exhibit broad substrate ranges, many act only on narrow classes of compounds [150,249]. The release of fluoride during catalysis can inhibit or denature enzymes, reducing their efficiency and lifespan [250]. Additionally, isolated enzymes often lack the stability and protective environment of the native cell, making their deployment in environmental settings (e.g., ex situ bioreactors) technically demanding [217].

Many defluorinating enzymes require cofactors, such as haem iron, NADPH, or cobalamin, whose supply and regeneration in engineered systems are challenging and often costly [49,166,176]. Reductive dehalogenases, in particular, are oxygen-sensitive, complicat-

ing their application outside of strictly anaerobic settings [161]. Cytochrome P450 enzymes (CYPs), while promising oxidative defluorinators, are notoriously difficult to express functionally in heterologous hosts due to challenges related to membrane integration, haem incorporation, and electron transport [110].

A further limitation is the lack of direct biochemical evidence for many proposed enzymatic pathways [18,245]. While genome and transcriptome data often suggest potential candidates, few enzymes have been isolated and characterised with confirmed defluorination activity, slowing the translation of molecular insights into applied technologies [138,139]. The high cost and complexity associated with producing purified enzymes at scale for environmental remediation also limit their practical deployment, with whole-cell microbial systems often proving more viable [251].

Various microbial and enzymatic challenges hinder efficient defluorination, including narrow substrate specificity, low catalytic efficiency, enzyme instability under environmental conditions, and host physiological limitations. Proposed solutions include protein engineering to enhance enzyme robustness, rational design of synthetic microbial consortia, and the integration of systems biology and machine learning for pathway optimisation, as summarised in Table 5.

Table 5. Microbial and enzymatic challenges and their solutions in defluorination.

Challenge	Description	Possible Solutions/Alternatives	Reference
Microbial defluorination			
High stability of the C–F bond	Exceptional bond strength makes microbial cleavage thermodynamically unfavourable	Use engineered or naturally evolved enzymes (e.g., dehalogenases and cytochrome P450s); apply redox mediators or co-substrates to drive reaction thermodynamics	[27,28]
Fluoride toxicity	Released fluoride ions inhibit microbial growth and metabolism	Engineer fluoride-exporting proteins or fluoride-resistant strains; integrate fluoride-trapping materials (e.g., calcium salts and biochar) in bioreactors	[89,232]
Isolation of efficient strains	Few strains degrade fluorinated compounds; often narrow substrate range	Metagenomic mining, directed evolution, or synthetic biology to discover or engineer strains with broader defluorination capacity	[18,233]
Environmental variability	Inconsistent pH, redox, nutrients, and microbial competition limit effectiveness	Use adaptive consortia, in situ conditioning (bioaugmentation and biostimulation), and microencapsulation for environmental stability	[235]
Incomplete mineralisation	Many pathways leave partially degraded products	Combine aerobic and anaerobic treatments; co-culture with complementary degraders; apply sequential bioreactor systems	[60,61]
Formation of persistent intermediates	Transformation may yield short-chain PFAS, still persistent and toxic	Apply oxidative post-treatment (e.g., UV, ozone, and AOPs); design enzymes targeting terminal groups of intermediates	[60,61,235]
Anaerobic vs. Aerobic conditions	Differing strategies complicate unified bioremediation approaches	Design modular treatment systems that switch between aerobic and anaerobic conditions; engineer facultative microbes	[179]
Co-metabolism dependency	Requires primary substrates to co-metabolise fluorinated compounds	Provide low-cost, renewable co-substrates (e.g., glycerol and acetate); develop strains capable of autonomous degradation	[55]
Strain & compound specificity	Activity is highly specific to the organism and compound	Develop multi-strain consortia; use machine learning to predict enzyme–substrate compatibility; engineer broad-spectrum enzymes	[138,140,194]
Reproducibility in complex environments	Lab-scale degradation is often not replicable in the field	Use pilot-scale microcosms to test real conditions; employ microbial encapsulation and bioaugmentation with native-strain compatibility screening	[242]
Algal biosorption, not degradation	Algae adsorb rather than degrade fluorinated compounds	Combine algae with bacteria for biosorption and biodegradation; functionalise algae to express defluorinating enzymes	[126]
Dependency on microbial consortia	Algal/fungal degradation depends on bacterial interactions	Engineer synthetic microbial consortia with defined roles; identify keystone strains for co-culture optimisation.	[133–139]
Limited mechanistic insight (fungi/algae)	Degradation pathways are poorly characterised	Apply omics (transcriptomics, proteomics, and metabolomics); use isotope tracing and pathway reconstruction	[89]
Challenges in biofilm engineering	Biofilm development for defluorination is underdeveloped	Use microfluidic systems to select effective biofilm formers; apply material science for tailored scaffolds; use quorum sensing modulators	[244]
Enzymatic defluorination			
Thermodynamic barrier of C–F bond	High bond dissociation energy limits cleavage under physiological conditions	Use oxidoreductases coupled to redox-active cofactors; apply artificial electron donors/acceptors; couple with energy-yielding pathways	[28–30]
Limited discovery of specific enzymes	Few enzymes have been identified for C–F cleavage	Metagenomics, functional screening of extreme environments, and AI-based enzyme prediction models can accelerate discovery	[245]

Table 5. *Cont.*

Challenge	Description	Possible Solutions/Alternatives	Reference
Substrate specificity vs. promiscuity	Overly narrow or broad specificities limit use	Engineer enzyme active sites via directed evolution or rational design for enhanced selectivity; develop modular domains for substrate targeting.	[150,250]
Fluoride-mediated inhibition	Accumulated fluoride ions inhibit or denature enzymes	Use fluoride scavengers or precipitation strategies (e.g., with Ca^{2+}); engineer fluoride-resistant enzymes or fluoride-export mechanisms	[251]
Cofactor dependency	Expensive/unstable cofactors like flavins and cobalamin are required	Engineer cofactor regeneration systems (e.g., NAD(P)H cycles); use cell-free lysates or co-expression of cofactor biosynthesis pathways	[49,166,176]
Limited stability of isolated enzymes	Enzymes often lose activity outside their native contexts	Immobilise enzymes on stable carriers; engineer thermostable and solvent-tolerant variants	[251]
Oxygen sensitivity (e.g., RDases)	Many enzymes require strict anaerobic conditions	Use anaerobic reactors; engineer oxygen-tolerant variants or apply encapsulation to shield enzymes from oxygen	[162]
Difficulties in heterologous expression	Functional expression is often challenging	Use optimised expression hosts (e.g., *S. cerevisiae* and *P. pastoris*); co-express chaperones and their redox partners, as well as cofactor assembly proteins; codon optimisation	[110]
Lack of direct biochemical evidence	Proposed pathways lack in vitro validation	Reconstitute systems using purified components; conduct enzyme kinetics and structural studies with fluorinated model substrates	[18,233]
High cost and low scalability	Industrial enzyme production is often too costly	Develop low-cost fermentation systems; use cell lysates or whole-cell biocatalysts; implement scalable immobilisation and reuse systems	[251]

7. Conclusions and Future Directions

The environmental persistence and bioaccumulative behaviour of fluorinated organic pollutants, owing to the exceptional stability of the carbon–fluorine bond, present critical challenges to ecosystem and human health. While conventional remediation techniques, such as adsorption, incineration, and AOPs, have dominated current strategies, they often fall short in terms of cost, environmental safety, and sustainability. In this context, microbial defluorination emerges as a novel and promising paradigm, leveraging biological catalysis to achieve selective C–F bond cleavage under ambient conditions. This review underscores the unique potential of microbial and enzymatic systems to overcome the limitations of traditional approaches, highlighting recent breakthroughs in understanding the underlying mechanisms of biological defluorination. The environmental persistence and bioaccumulative behaviour of fluorinated organic pollutants, owing to the exceptional stability of the carbon–fluorine bond, present critical challenges to ecosystem and human health. Microbial defluorination has gained prominence as a sustainable and biologically driven solution for the remediation of these recalcitrant compounds. Mechanistic insights into reductive, hydrolytic, and oxidative pathways, mediated by both enzymatic and non-enzymatic processes, have uncovered a broad range of defluorinating microbial taxa and catalytic systems. Key enzymes, such as fluoroacetate dehalogenases, cytochrome P450s, reductive dehalogenases, peroxidases, and laccases, have demonstrated significant potential for fluorine release, supported by recent advances in structural biology, metagenomics, proteomics, and synthetic biology.

Despite these developments, improving enzyme kinetics, expanding substrate scope, and ensuring functional activity in complex environmental matrices remain major obstacles. Future research should prioritise the discovery of novel defluorinating genes and organisms through high-throughput metagenomic screening, functional annotation, and directed evolution strategies. The rational design of engineered microbial consortia and synthetic microbiomes with an optimised division of metabolic labour can enhance system resilience and degradative efficiency. Integration of machine learning and systems biology will further enable predictive modelling and pathway optimisation. Bridging the gap between laboratory-scale findings and field applications requires interdisciplinary collaboration and

the development of supportive regulatory and policy frameworks to advance the practical deployment of microbial defluorination technologies.

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