CRITICAL REVIEW

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Biodegradation of per- and polyfluoroalkyl substances: mechanisms, challenges, and emerging strategies for sustainable remediation

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Per- and polyfluoroalkyl substances (PFAS) are highly persistent synthetic chemicals that pose serious environmental and public health risks due to their resistance to degradation, bioaccumulative nature, and toxicity. Their widespread occurrence in water, soil, and biota underscores the urgent need for effective remediation strategies. Conventional methods such as adsorption, filtration, and chemical oxidation, often fail to achieve complete mineralization and may generate harmful by-products. Biodegradation, driven by microbial and enzymatic processes, has emerged as a promising sustainable alternative. This review evaluates recent advances in PFAS biodegradation, focusing on the role of bacteria, fungi, and enzymatic mechanisms, as well as the influence of environmental factors on degradation efficiency. Innovative strategies including enzyme immobilization, phytoremediation, hybrid chemical–biological systems, and machine learning-based predictive modeling are evaluated for their potential to enhance treatment efficiency. Remaining challenges include incomplete understanding of metabolic pathways and limited scalability. A future research roadmap is proposed to integrate metabolic engineering, system optimization, and field-scale validation toward effective, sustainable PFAS biodegradation. This review provides a comprehensive synthesis of current knowledge and outlines strategic directions to advance PFAS biodegradation research and its practical implementation.

Water impact

PFAS persistence poses a major threat to water quality, as conventional treatments often fail to achieve complete degradation or avoid secondary waste. This review synthesizes advances in microbial, fungal, and enzymatic PFAS biodegradation, highlighting mechanistic insights, current limitations, and emerging hybrid approaches. It outlines research priorities needed to develop scalable, sustainable strategies for effective PFAS destruction in water systems.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic fluorinated chemicals developed in the 1940s, comprising over 7 million compounds meeting broad PFAS definitions according to PubChem (September 2023).[1] They feature highly stable carbon–fluorine (C–F) bonds along a 4–16-carbon hydrophobic chain and a polar functional group. This exceptional bond strength confers remarkable chemical and thermal stability, earning PFAS the designation of "forever chemicals".[2] Their amphiphilic nature underpins widespread industrial use in firefighting foams, repellents, coatings, packaging, and surfactants.[3] Recently, certain

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fluorinated pharmaceuticals and xenobiotics, such as fluoxetine and other polyfluorinated drugs, have also been discussed under the broad PFAS category due to their environmental persistence and resistance to biodegradation.[4] However, decades of extensive use have resulted in global environmental contamination, with firefighting foams identified as a dominant source in soil and groundwater near training sites, where persistent compounds such as PFOS, 6:2 FTSA, and 6:2 FTAB are frequently detected.[3] These occurrences underscore gaps in international regulation and disposal standards, particularly in military and industrial operations.

PFAS bioaccumulate due to chain-length-dependent persistence, with long-chain species such as PFOS and PFOA exhibiting slow degradation and efficient gastrointestinal absorption. Human exposure occurs mainly through contaminated water and food, and measured concentrations

in wastewater treatment plant (WWTP) effluents often exceed regulatory thresholds, reaching more than 100 ng L^{-1} compared with the United States Environmental Protection Agency (U.S. EPA) drinking-water limit of 70 ng L^{-1}.[3] Exposure has been linked to endocrine disruption, immune suppression, liver toxicity, and cardiovascular effects, and emerging evidence connects PFAS burden to aggravated COVID-19 outcomes.[5] In animals, PFAS induce immunotoxic and carcinogenic responses, while incomplete degradation releases fluoride ions that further stress microbial and ecological systems.[2] Despite growing toxicological evidence, global enforcement remains fragmented, underscoring the urgent need for harmonized regulation and effective destruction technologies.

Various technological approaches have been explored for PFAS remediation, broadly classified into physical, chemical, and biological methods. Physical removal techniques, such as anion exchange and carbon-based adsorption, can efficiently capture PFAS but do not achieve degradation, merely transferring the contaminants between media. Membrane filtration systems, including nanofiltration and reverse osmosis, also separate PFAS effectively but produce concentrated brines requiring costly disposal. Thermal destruction can mineralize PFAS at very high temperatures but remains energy-intensive and economically impractical for large-scale operations.[6] Chemical degradation methods, including electrochemical, photochemical, and plasma-based oxidation, have shown promise for breaking C–F bonds under controlled laboratory conditions. However, their performance strongly depends on solution chemistry and operational parameters, and scale-up remains a major challenge.[7] Moreover, their efficiency depends strongly on pH and matrix composition, and they may yield intermediate products of uncertain toxicity.[6] Consequently, these conventional approaches offer partial solutions rather than sustainable remediation.

In contrast, biological treatments using PFAS-degrading bacteria and fungi have emerged as promising, cost-effective, and environmentally compatible alternatives. Recent studies demonstrate limited but measurable defluorination, and coupling biological with chemical or electrochemical processes may enhance degradation while lowering energy costs.[6] However, most investigations remain restricted to laboratory settings, focusing on a narrow set of legacy compounds, leaving the degradation pathways and efficacy against short-chain and emerging PFAS largely unresolved.

Given the persistence, bioaccumulative nature, and toxicity of PFAS, developing technologies that achieve complete mineralization to non-toxic end products is a global priority (Nasrollahpour *et al.*, 2024).[8] Existing physical and chemical methods provide temporary containment, whereas integrated, mechanistically informed bioremediation frameworks hold greater potential for scalable, field-ready applications. This review therefore focuses on recent advances, mechanistic insights, and remaining research challenges in PFAS biodegradation and hybrid treatment systems, aiming to guide the development of next-generation destruction strategies supported by regulatory and environmental monitoring frameworks.

Recent progress in PFAS remediation has increasingly focused on biodegradation strategies that offer sustainable and energy-efficient alternatives to conventional treatments. Among these, enzymatic degradation has attracted significant attention for its ability to catalyze specific PFAS bond cleavage under mild conditions. Natural and engineered enzymes enable targeted degradation, yet low catalytic efficiency, long reaction times, and limited substrate compatibility continue to hinder practical deployment.[9] Enzyme immobilization, a technique adapted from biofuel catalysis, has emerged as a promising approach to enhance enzyme stability and reusability, thereby improving overall degradation efficiency. Nevertheless, the substrate specificity of most PFAS-active enzymes remains poorly understood, restricting their application across structurally diverse PFAS compounds. Plant-based remediation represents another innovative but underexplored approach. A recent study that examined 28 PFAS compounds reported that hemp absorbed 10 of them, showing a marked preference for those containing carboxylic head groups and shorter carbon chains. Furthermore, hydrothermal liquefaction of the contaminated biomass achieved almost complete degradation, with efficiencies approaching 100 percent.[10] Although these results highlight the potential of phytoremediation, uncertainties regarding PFAS re-release from plant tissues and the lack of long-term field validation continue to limit large-scale application.

Despite encouraging laboratory results, microbial and enzymatic pathways involved in PFAS biodegradation are still not fully characterized, and the field remains in an early stage of development. Establishing reliable and scalable bioprocesses could transform PFAS remediation by combining low environmental impact with high selectivity and cost-effectiveness. Accordingly, this review synthesizes recent advances in PFAS biodegradation and hybrid degradation systems, identifies the remaining mechanistic and scalability challenges, and outlines future research priorities to bridge current knowledge gaps. Unlike earlier reviews that examine single aspects of PFAS degradation, this paper presents an integrated analysis that links microbial, enzymatic, and hybrid treatment mechanisms. Its novelty lies in evaluating both conventional and emerging remediation approaches for their real-world applicability, degradation efficiency, and environmental sustainability. Furthermore, it extends beyond legacy compounds such as PFOS and PFOA to address transformation pathways of newer industrial alternatives, including GenX and PFBS, which remain underrepresented in existing research. The review specifically aims to (i) clarify the dominant microbial and enzymatic mechanisms driving PFAS defluorination, (ii) assess the potential of hybrid treatment systems for achieving complete mineralization, and (iii) identify current technological and regulatory barriers hindering large-scale implementation. By

398 | *Environ. Sci.: Water Res. Technol.*, 2026, **12**, 397–420

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connecting degradation mechanisms with regulatory and environmental perspectives, this review provides a forward-looking framework for the development of next-generation PFAS destruction technologies. An overview summarizing contamination pathways, treatment limitations, and emerging biological strategies is illustrated conceptually in Fig. 1, establishing the foundation for the discussions that follow.

2. Detection and analytical challenges in PFAS monitoring

Accurate detection and quantification of PFAS are fundamental to evaluating biodegradation, since the apparent disappearance of parent compounds or formation of transformation products depends entirely on analytical precision and sensitivity. Analytical bias can mislead biodegradation interpretation: for instance, adsorption losses or ion-suppression effects may be mistaken for degradation, whereas background contamination may produce false persistence. PFAS analysis is intrinsically difficult because of their wide structural diversity, ultra-trace environmental concentrations, and the widespread presence of fluorinated materials in laboratories. Polytetrafluoroethylene (PTFE)

components in tubing, vial caps, or SPE frits release fluorinated residues that elevate blanks and obscure true signals. To mitigate this, EPA 1633 and ISO 21675 require replacement of PTFE with polyether ether ketone (PEEK) or polyethylene parts, single-use SPE cartridges, and delay columns that trap system-derived PFAS before injection. Such control measures are indispensable when tracking low-mass intermediates in biodegradation assays.

Matrix interferences further complicate interpretation. Dissolved organic carbon, surfactants such as cetyltrimethylammonium bromide, and proteins reduce electrospray efficiency, yielding matrix-suppression factors (SF) of roughly 0.6–0.9 compared with reagent water. This suppression can artificially lower apparent PFAS concentrations in complex samples such as sludge, biomass, or enzyme cultures, giving the illusion of degradation. ^{13}C-PFAS surrogates are used as internal standards to correct for such variability, while spike-and-recovery tests (70–130% acceptance, ISO 21675 Section 12.1) verify extraction efficiency. Field blanks and ultrapure reagents also help confirm that concentration changes reflect biochemical rather than analytical processes. To contextualize these challenges, Table 1 summarizes benchmark analytical methods (EPA 533, 537.1, 1633, ISO 21675, and ISO 25101),
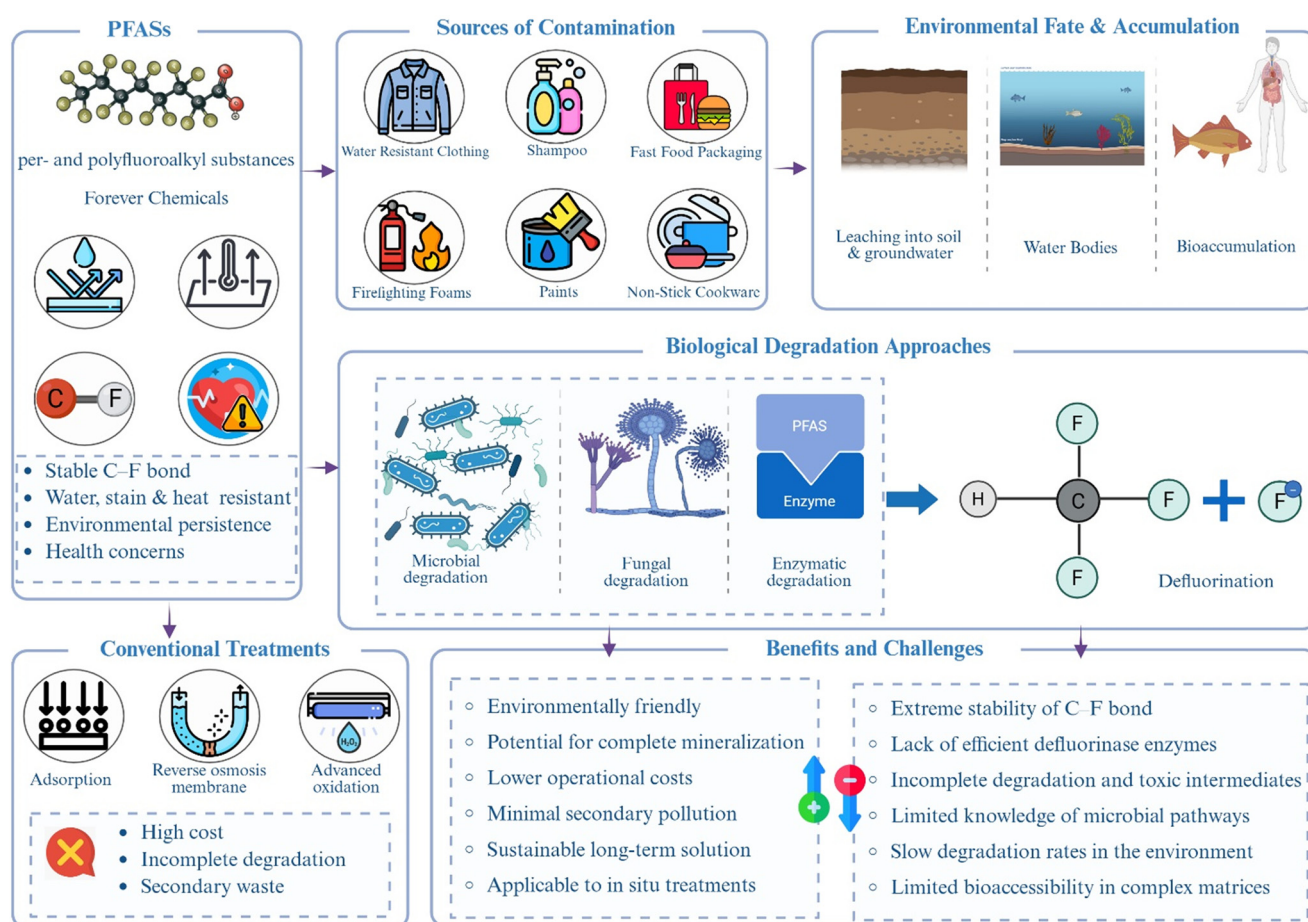

Fig. 1 Conceptual overview of PFAS contamination, treatment challenges, and biodegradation strategies.

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Environ. Sci.: Water Res. Technol., 2026, **12**, 397–420 | **399**

Table 1 Performance characteristics of benchmark PFAS analytical methods

Method/standard	Matrix applicability	Sensitivity metric (units)	Typical recovery[a] (%)	Target PFAS scope	Relevance to biodegradation studies
EPA 537.1	Drinking water	LCMRL 0.53–6.3 ng L^{-1}	80–120	18 PFAS including PFOA, PFOS, PFNA, GenX	Reliable for legacy PFAS; limited retention for short-chain acids
EPA 533	Drinking water (short-chain focused)	LCMRL 1.4–16 ng L^{-1}	94–128% (at 10 ng L^{-1}), 96–111% (at 80 ng L^{-1})	25 PFAS (C$_4$–C$_{12}$ acids and sulfonates plus ethers)	Detects short-chain transformation products (PFBA, PFPeA); isotope-dilution and anion-exchange SPE reduce bias
EPA 1633	Water, soil, biosolids, tissue	MDL ≤ 0.8 ng L^{-1}; ML ≈ 1.6–16 ng L^{-1}	70–130	≈40 PFAS (acids, sulfonates, telomers, precursors)	Multi-matrix method; carbon cleanup plus isotope dilution; most suitable for complex biodegradation media
ISO 21675	Drinking, surface and waste water (<2 g L^{-1} SPM)	LOQ ≥ 0.2 ng L^{-1}	70–120	≈20 PFAS (C$_4$–C$_{14}$ acids and sulfonates)	Harmonized LC–MS/MS guideline; useful for cross-study QA/QC comparability
ISO 25101	Drinking, ground, surface water	LOQ 2 ng L^{-1} (PFOS), 10 ng L^{-1} (PFOA) (scope)	80–110	PFOS and PFOA (2 analytes)	Foundational PFOS/PFOA method; limited scope but valuable for validation

[a] Percent recoveries refer to fortified-matrix samples reported in the respective methods. When not explicitly listed, a general acceptance range of 70–130% is assumed. Matrix-suppression factors (SF ≈ 0.6–0.9) are matrix-specific and should be measured experimentally for each biodegradation setup.

highlighting their sensitivity, recovery performance, and applicability to biodegradation research.

These method comparisons illustrate how analytical uncertainty directly limits the interpretability of biodegradation results. When recovery variability approaches ±20%, a 15% concentration drop may fall within analytical error rather than true transformation. Therefore, any claim of PFAS degradation must be accompanied by recovery, suppression, and detection-limit data from the same analytical run. EPA 1633 and 533 currently provide the most comprehensive and transferable frameworks for multi-matrix biodegradation assays, while EPA 537.1 remains suitable for legacy PFAS in aqueous systems and ISO 21675 offers internationally harmonized QA/QC guidance. In summary, PFAS biodegradation studies must explicitly link observed concentration changes to analytical confidence. Rigorous contamination control, isotope-labeled internal standards, spike-recovery validation, and transparent reporting of detection limits ensure that apparent "biodegradation" reflects real molecular transformation rather than measurement uncertainty.[11–15]

While LC–MS/MS remains the benchmark for quantitative PFAS analysis because of its validated methods, high precision, and regulatory alignment, its scope is inherently limited to predefined analyte lists. In contrast, high-resolution mass spectrometry (HRMS) and non-targeted analysis (NTA) expand detection beyond these lists by providing sub-ppm mass accuracy, full-scan acquisition, and feature discovery workflows that can identify previously unrecognized PFAS, transformation products, and intermediates. Recent studies using HRMS-based suspect screening have reported hundreds of additional fluorinated features through accurate-mass filtering and homologue-series algorithms. However, HRMS and NTA currently have lower quantification robustness, less standardized data processing, and limited reproducibility compared with LC–MS/MS. For this reason, targeted LC–MS/MS continues to provide the most reliable quantitative data, whereas HRMS and NTA complement it by revealing new PFAS species and transformation pathways.[16] Integrating these approaches enhances the reliability and completeness of PFAS biodegradation studies.

3. Limitations of existing PFAS treatment methods

Understanding PFAS occurrence in wastewater and environmental matrices is critical for designing and evaluating biodegradation strategies. The types and concentrations of PFAS present, as well as the performance of conventional non-biological treatments, determine the baseline conditions against which biodegradation approaches must be developed. PFAS are extensively detected in WWTPs and contaminated soils across the globe, highlighting the persistent and mobile nature of these compounds. As shown in Table 2, legacy PFAS such as PFOA and PFOS, along with newer alternatives like GenX and ADONA, have been reported in WWTP effluents in North America, Europe, Asia, and Africa. This widespread occurrence illustrates that conventional wastewater treatment not only fails to eliminate PFAS effectively but can also transform precursor compounds into more stable and persistent forms.[3] In summary, Table 2 demonstrates that PFAS contamination is a global issue, with both legacy and emerging PFAS detected in diverse regions, underscoring the inability of conventional WWTPs to completely remove these compounds. However, the available data show large regional differences in monitoring scope and

Table 2 Dispersal of PFAS molecules in WWTPs worldwide

Regions	Number of WWTPs	PFAS detected	Ref.
Canada (Cambridge Bay, Victoria Island, Ontario)	42	PFBA, PFOA, PFOS, GenX	17
China (Beijing, Bohai Sea, Daling River Basin, Guangzhou, Guanting Reservoir, Jinan, Jiangsu, Jiangsu Hi-tech, Liaoning, Qingdao, Shanghai, Shandong, Shenyang, Taihu Lake, Tianjin, Wenzhou, Wuxi, Xihe River, Yanghe River, Yingcheng)	100	PFBA, PFHxA, PFOA, PFOS, PFNA, PFDA, PFBS, PFHxS, PFHpA, FTOHs, FTCAs, FOSA, Cl-PFAES	18
Finland (Turku, Espoo, Helsinki, Porvoo)	4	PFDA, PFHxA, PFOS, PFOA	19
France (Nancy)	1	PFOA, PFOS	20
France (North)	1	PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, 6:2 & 8:2 FTSA, 6:2, 8:2, 10:2 & 12:2 FTOH	21
Germany (Bavaria)	71	PFNA, PFHxS, PFHpA, PFOS, PFDA, PFOSA, PFBS, PFDS, PFHxA, PFOA	22
Germany (Halle)	—	PFNA, PFHxA, PFOS, PFBS, PFOA	23
Germany (Hesse)	5	PFNA, PFHxS, PFPeA, PFBS, PFDA, PFHpA, PFBA, PFOS, PFHxA, PFOA	24
Germany (River Alz)	—	ADONA	25
Greece (Athens, Mytilene)	2	PFHpS, PFHxS, PFPeA, PFNA, PFHpA, PFBS, PFDA, PFOS, PFHxA, PFOA	26
India (Chennai)	—	PFOA, PFOS	27
Japan (Osaka)	—	PFHxA	28
Kenya (Bungoma, Busia, Kakamega, Kisumu, Kisii, Mumias)	6	PFDS, PFHxA, PFDA, PFOSA, PFHxS, PFNA, PFOS, PFOA	29
Nigeria (Lagos, Oyo, Ogun)	10	PFHxS, PFDA, PFHpA, PFOS, PFHxA, PFBS, PFNA, PFOA	30
South Africa (Gauteng Province)	3	PFHxS, PFDA, PFBA, PFHxA, PFOS, PFPeA, PFOA	31
South Korea (Hyung-san River, Gyeongju, Pohang)	3	PFOS, PFHxA, PFBS	32
South Korea (Seoul, Incheon, Daejeon, Daegu, Busan, Gwangju)	81	PFDA, N-EtFOSA, PFHxS, PFHpA, PFOS, PFNA, PFHxA, PFOA	33
South Korea (other)	25	PFBS, PFHxS, PFBA, PFHxA	34
Spain (Albufera Natural Park, Valencia)	—	PFHxS, PFPeA, PFOS, PFOA, PFHxA	35
Spain (Alzira, Loja, Cuenca)	16	PFHpA, PFPeA, PFHxS, PFBA, PFHpS, PFDA, PFOS, PFHxA, PFBS, PFNA, PFOA	36
Thailand (Bangkok)	2	PFOS, PFNA, PFHpA, PFBS, PFHxA, PFBA, PFOA	37
Thailand (Central & Eastern)	2	PFDA, PFHxS, PFHpA, PFNA, PFOS, PFHxA, PFOA	38
Vietnam (Hanoi)	—	PFOA, PFOS	39
Vietnam (Red River)	—	PFDA, PFOS, PFNA, PFOA	39

Abbreviations: the complete names for each of the PFAS mentioned in the table are listed below: perfluoroalkyl carboxylic acids (PFCAs): perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroalkyl sulfonic acids (PFSAs): perfluorobutane sulfonic acid (PFBS), perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorodecane sulfonic acid (PFDS), fluorotelomer sulfonic acids (FTSAs): 6:2 fluorotelomer sulfonic acid (6:2 FTSA), 8:2 fluorotelomer sulfonic acid (8:2 FTSA), fluorotelomer alcohols (FTOHs): 6:2 fluorotelomer alcohol (6:2 FTOH), 8:2 fluorotelomer alcohol (8:2 FTOH), 10:2 fluorotelomer alcohol (10:2 FTOH).

target compounds, suggesting uneven analytical coverage and possible underestimation in areas with limited surveillance.

Current physical and chemical treatment technologies primarily separate PFAS from water rather than destroy them. Adsorption techniques such as granular activated carbon and ion-exchange resins are often effective for long-chain PFAS but perform poorly for short-chain variants. Reported removal efficiencies vary from about 20% to 100%, depending on material and conditions. These methods therefore achieve removal but not destruction, transferring PFAS to solid waste that requires further treatment or disposal.[9,40] Membrane filtration processes, including reverse osmosis and nanofiltration, provide high separation efficiency but generate concentrated brine waste and are seldom cost-effective at full scale.[9] Their rejection rates typically range from 70% to 100%, but high energy and brine management costs limit full-scale use. Advanced oxidation processes like UV or electrochemical treatments show promise in laboratory conditions, achieving high (>90%) degradation at mg L^{-1} levels yet requiring substantial energy input, which restricts real-world applicability.[9,41]

In soil and groundwater, PFAS remediation is further complicated by their differential mobility and sorption behavior. Long-chain PFAS tend to adhere to soil particles and organic matter, while short-chain compounds are more mobile and infiltrate groundwater. Field studies have found 6:2 FTSA and 6:2 FTAB at high concentrations in soil and groundwater at former firefighting training sites, demonstrating PFAS persistence even years after use.[42] Furthermore, PFOS has been observed up to two meters below the surface, with modeling suggesting it can remain in unsaturated zones for decades.[43,44]

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Environ. Sci.: Water Res. Technol., 2026, **12**, 397–420 | **401**

Table 3 Varying PFAS removal and treatment methods

Treatment	Procedure to remove PFAS	Location	Pros	Cons	Tested PFAS	Efficiency (%)	Ref.
Physical	Using materials that are highly adsorbent (e.g., biochar, activated carbon, silica gel, resins)	In situ or ex situ	Cost-effective; utilizes purchasable materials	Requires large concentrations of adsorbent; only effective for long-chain PFAS; may associate with other pollutants	C3 to C11 PFCAs, C4, C6, C8 PFSAs, C8 FOSA, GenX, PFPeA, PFHxA, PFOA, PFOS	22–100% (depending on material and conditions)	46–48
Physical	Use biochar as an adsorbent with activated or inactivated biofilm	Pilot	Nonpolar interactions	Less availability due to biofilm blocking adsorption sites	C3 to C11 PFCAs, C4, C6, C8 PFSAs, C8 FOSA	58, 42, and 22	47
Physical	Use purolite A860 as an adsorbent along with anion exchange resin	Lab	Ion exchange	Organic ions diffuse out of pores, inorganic ions hinder access	GenX	100	48
Physical	Use activated carbon as an adsorbent	Pilot	Nonpolar interactions	—	PFPeA, PFHxA, PFOA, PFOS	61	40
Physical	Use Fe_3O_4 nanopowder and fluorinated vermiculite, pre-treating wastewater with centrifugation	Lab	Efficient and specific PFOS adsorption based on fluorophilicity	—	PFOA	98	49
Physical	Use anionic ion exchange resin (IX)	Lab	Ion exchange	Higher concentration of resin requires more NOM	PFOA and PFOS	70	48
Physical	Use anionic ion exchange resin (IX)	Lab	Ion exchange	Organic and inorganic ions compete for adsorption sites	PFOA, PFOS, PFBA, PFBS, and GenX	99	31
Physical	Adsorption onto activated sludge (pre-treated with 45-day aged sludge)	Full	—	—	PFOS	94	31
Physical	Adsorption onto activated sludge (pre-treatment of wastewater with SPE)	Full	—	—	All PFAS	82	31
Physical	Use DMAPAA-Q hydrogel polymers as adsorbent	Lab	Long tails of PFAS and PFSA hydrophobically interact	Anion concentrations decrease adsorption of short-chain PFAAs	All PFAS, GenX, PFBS, ADONA, PFBA, PFOA, F-53B, PFOS	100	50
Physical	Heat PFAS compounds to 1000 °C	Ex situ	Potential for mineralization of PFAS	Costly; environmental damage; high resource consumption	All PFAS (high temperature)	Low to moderate (depends on temperature and PFAS type)	51
Physical	Heat PFAS compounds to 1000 °C	Ex situ	Potential for mineralization of PFAS	Costly; environmental damage; high resource consumption	All PFAS (high temperature)	Low to moderate (depends on temperature and PFAS type)	51
Biological	Use living organisms (fungi, bacteria, plants) to decompose or aggregate PFAS	Ex situ	Easy to implement; sustainable; low cost	Slow process; limited degradation; lacks extensive research	All PFAS (varies by organism)	Low (varies significantly)	52
Biological	Use SPE (solid phase extraction) to pretreat wastewater before biological filtration	Full scale	Sustainable, reduces PFAS concentration	Limited efficiency for some PFAS types	All PFAS	41	31
Biological	Biological treatment to remove PFAS-related odours from raw water	Full scale	Efficient for raw water treatment	Not focused on PFAS removal, mainly for odour	All PFAS	70	53
Mechanical	Use stainless steel cathode and boron-doped diamond anode for PFAS oxidation	Full scale	High efficiency, near 100% degradation	Requires significant energy and equipment	All PFAS	~100% (organic carbon removal >90%)	53
Mechanical	Use filtration methods (e.g., nanofiltration, reverse osmosis)	Ex situ	Functional across broad pH ranges	Costly; does not degrade PFAS; mass-dependent efficiency	All PFAS (varies by filter type)	70–100% (depends on PFAS type and filter)	53

Table 3 (continued)

Treatment	Procedure to remove PFAS	Location	Pros	Cons	Tested PFAS	Efficiency (%)	Ref.
Mechanical	Wash soil with water to separate PFAS compounds	*Ex situ*	Potential for land rehabilitation; minimal tech input	Prolonged duration; can cause water pollution	All PFAS	Low to moderate (depends on soil and method)	53

A wide range of treatment techniques has been evaluated, as summarized in Table 3. These include physical, thermal, mechanical, and biological approaches. Despite extensive testing, most are limited by poor scalability, high operational cost, or low efficiency for short-chain PFAS. Many laboratory experiments also use concentrations (5–1000 mg L^{-1}) that are far above environmental levels, making translation to field conditions uncertain.[45]

Most current treatment technologies address PFAS removal rather than molecular destruction. Scale-up failures of adsorption systems arise from mass-transfer limitations, competitive sorption with natural organic matter, and difficulties regenerating exhausted media. Short-chain PFAS, which are more soluble and less hydrophobic, break through sorbents early, and media replacement generates secondary PFAS-laden waste requiring high-temperature disposal. Likewise, advanced oxidation and reduction processes often fail to fully defluorinate PFAS because the C–F bond energy (485 kJ mol^{-1}) exceeds that of most oxidants. Partial transformation yields short-chain acids that remain persistent, while high energy demand and electrode fouling constrain field deployment.[54]

Recognizing these shortcomings has encouraged development of biological and hybrid treatment frameworks. Integrating physicochemical separation with microbial or enzymatic defluorination can combine removal and destruction within the same treatment train. Hybrid bio-electrochemical and photo-biocatalytic systems exploit microbial electron transfer or enzyme activity to cleave weakened intermediates produced during pretreatment. These multi-stage, synergistic systems represent the most promising path toward complete PFAS destruction.[55] In summary, while adsorption, filtration, and oxidation methods improve removal efficiency, their inability to break the C–F bond highlights the importance of biologically assisted or hybrid approaches for achieving genuine molecular degradation.

4. Biodegradation mechanisms of PFAS

4.1. Microbial degradation pathways

Microbial degradation has been investigated as a potential strategy for PFAS removal, offering a sustainable and cost-effective alternative to physicochemical treatments. Various bacterial and fungal strains, as well as microbial consortia, have demonstrated the ability to degrade or transform PFAS under specific environmental conditions. Despite extensive research, complete PFAS mineralization remains a challenge due to the extreme stability of the C–F bond.[56] Microbial pathways primarily involve defluorination reactions, occurring through enzymatic breakdown or co-metabolic transformation in the presence of co-substrates. Both aerobic and anaerobic conditions have been studied, each presenting unique degradation mechanisms and efficiencies. The major microbial and enzymatic pathways of PFAS biodegradation, including aerobic, anaerobic, fungal, biochemical, and integrated strategies, are summarized in Fig. 2.

The susceptibility of PFAS to microbial attack depends strongly on molecular structure, particularly chain length, headgroup chemistry such as carboxylate and sulfonate, and the presence of ether linkages, which influence enzyme accessibility and reaction energetics.

Experimental studies have further demonstrated these pathways under a variety of laboratory conditions. However, most experiments are performed under controlled environments that may not reflect the complexity of real contaminated sites. Table 4 provides an overview of microbial degradation studies on PFAS, highlighting the microbial species involved, degradation conditions, by-products formed, and observed efficiencies. Yet, comparisons between studies remain difficult because of differences in PFAS types, concentrations, microbial strains, and experimental setups, making it challenging to determine which systems are most promising for real-world applications.

4.1.1. Bacterial systems and defluorination routes

4.1.1.1. Aerobic microbial defluorination. Certain bacteria degrade PFAS aerobically using oxygenase enzymes, such as monooxygenases and dioxygenases, which initiate the breakdown of fluorinated chains. The genus *Pseudomonas* has been widely studied for its ability to degrade PFAS. *Pseudomonas* YAB-1 exhibited 48.1% degradation efficiency for PFOA within four days under aerobic conditions with glucose as a co-substrate.[58] A genetically modified strain (*Pseudomonas* YAB1 mutant F3-52) demonstrated 58.6% degradation efficiency, indicating potential for enhanced microbial degradation through genetic modification.[58] However, the need for glucose or other co-substrates adds to operational costs and limits application in nutrient-poor environments.

Pseudomonas plecoglossicida strain DD4 showed complete degradation (100%) of PFOA (1000 mg L^{-1}) within four days,

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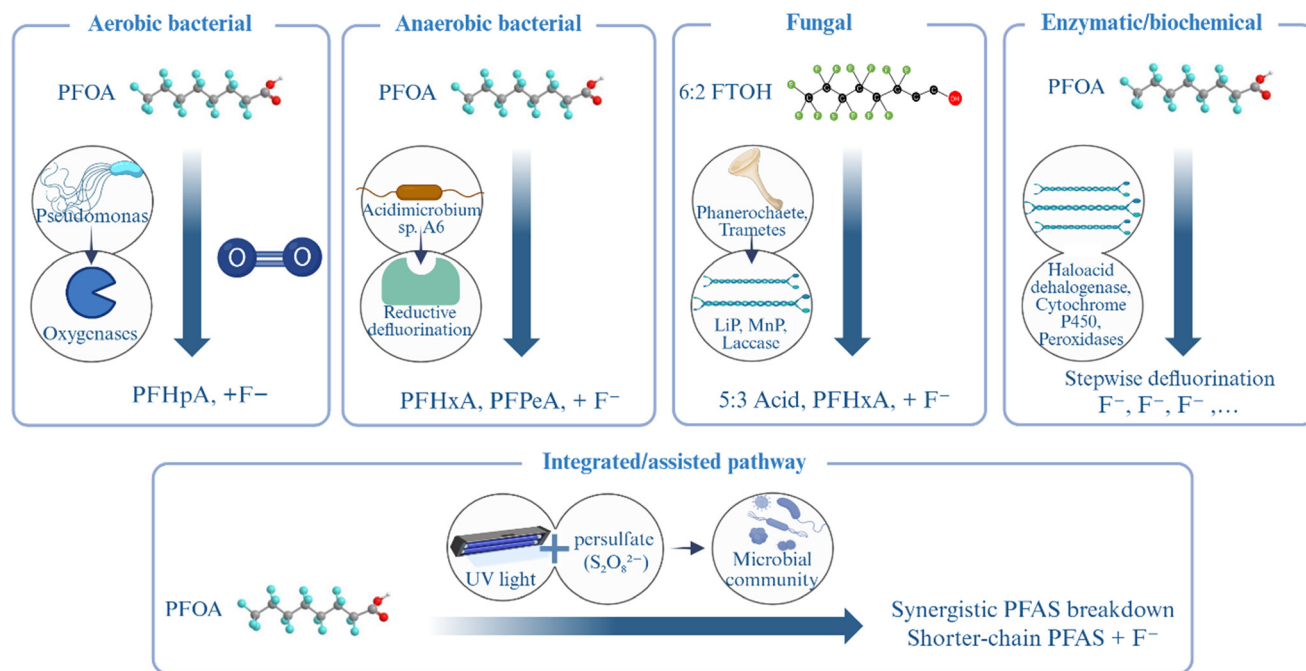*Environ. Sci.: Water Res. Technol.*, 2026, **12**, 397–420 | **403**

Fig. 2 Mechanisms of PFAS biodegradation.

producing fluoride and perfluoroheptanoic acid as degradation products.[59] Such degradation is attributed to oxygenase-mediated alpha oxidation supported by haloacid dehalogenases from *Delftia acidovorans*, which catalyze stepwise C–F substitution in carboxylated PFAS. Synergistic systems using *Stenotrophomonas*, *Bacillus*, *Pseudomonas*, and *Brevundimonas* in activated sludge achieved 46.6–49% PFOA removal, with enhanced degradation observed under photocatalysis-aided conditions.[59] However, despite these advances, the aerobic degradation route remains highly limited by its dependence on external carbon sources, high energy demand, and limited enzyme stability, which reduce its practicality for large-scale or nutrient-limited field applications.

4.1.1.2. Anaerobic microbial defluorination. Anaerobic degradation relies on reductive defluorination mechanisms in which PFAS serve as electron acceptors. *Acidimicrobium* sp. strain A6 has demonstrated up to 63% PFOA degradation at 0.1 mg L^{-1} and 50% at 100 mg L^{-1} under anaerobic conditions, generating degradation products such as perfluorohexanoic acid (PFHxA) and perfluoroheptanoic acid (PFHpA).[57] The same strain degraded both PFOA and PFOS (60% each) under similar conditions.[52] This shows potential for anaerobic systems, but their slower degradation rates limit their use where fast results are needed.

In iron-reducing environments, corrinoid and metal-dependent dehalogenases coupled with extracellular electron transfer mediate reductive C–F cleavage. The A6 system exemplifies Feammox-linked PFAS defluorination, and its integration with microbial electrolysis cells enhanced PFOA removal to nearly 77 percent, lowering redox potential barriers.[61] These electro-assisted systems demonstrate that

external electron donors can overcome the low redox potential of PFOA and PFOS, which is otherwise thermodynamically unfavorable for microbial respiration.[75] While anaerobic degradation appears promising, its efficiency is slower, requiring 100+ days for substantial PFAS breakdown, compared to aerobic degradation, which occurs within days to weeks. Additionally, anaerobic pathways often lead to the accumulation of intermediate byproducts rather than complete mineralization. These byproducts may still be harmful and mobile, which means treatment is only partially effective unless followed by another process.

4.1.2. Fungal degradation pathways and hybrid bioprocesses. Although bacterial degradation of PFAS has been widely studied, fungal biodegradation remains an underexplored but promising approach. Many fungi, particularly white-rot fungi such as *Phanerochaete chrysosporium* and brown-rot fungi such as *Aspergillus niger*, produce extracellular enzymes including lignin peroxidases (LiP), manganese peroxidases (MnP), and laccase, which generate reactive radicals capable of attacking C–F and C–S bonds. Under aerobic co-metabolic conditions, *Phanerochaete chrysosporium* achieved about 50 percent transformation of 6 : 2 fluorotelomer alcohol (6 : 2 FTOH) and 70 percent of 8 : 2 FTOH within 28 days, while *Gloeophyllum trabeum* and *Trametes versicolor* reached 23 percent and 6 percent degradation efficiency, respectively, producing partially defluorinated intermediates such as 5 : 3 acid, 6 : 2 FTUCA, and PFHxA. In contrast, *Aspergillus niger* and *Phanerochaete chrysosporium* showed no measurable PFOA transformation after 35 days.[42] In addition, recent studies have shown that the zygomycete fungus *Cunninghamella elegans* can biotransform 6 : 2 fluorotelomer alcohol within 48 hours

Table 4 Summary of microbial degradation of PFAS under different experimental conditions

Type of PFAS compound	Initial concentration (mg L^{-1})	Microorganism involved	Environmental setup	By-products formed	Breakdown efficiency (%)	Testing period (days)	Experimental conditions	Ref.
PFOA	0.1 or 100	*Acidimicrobium* sp. strain A6	Anaerobic	PFHpA, HFBA, PFHxA, PFPeA	63% (0.1 mg L^{-1}) and 50% (100 mg L^{-1})	100	—	57
PFOA and PFOS	0.1 and 100	*Acidimicrobium* sp. A6	Anaerobic	PFHpA (PFOA), PFHxA, HFBA, PFPeA; PFBS and HFBA (PFOS)	60% (PFOA), 60% (PFOS)	100	pH = 4.5–5, 30 °C	52
PFOA	—	*Pseudomonas* YAB-1 (NR040859)	Aerobic	Not reported	48.10%	4	1 g L^{-1} glucose	58
PFOA	1200	*Pseudomonas* YAB1 mutant F3-52	Not reported	Not reported	58.60%	—	—	58
PFOA	500	*Pseudomonas parafulva* strain YAB1	Aerobic	Not reported	48%	5	—	58
PFOA	1000	*Pseudomonas plecoglossicida* strain DD4	Aerobic	Fluoride (132 mg L^{-1}), perfluoroheptanoic acid	100.00%	4	—	59
PFOA	0.5	Activated sludge bacteria: *Stenotrophomonas, Bacillus, Pseudomonas,* and *Brevundimonas*	Aerobic	PFHxA and PFHpA were observed only under synergistic conditions, with no detection in individual setups	46.6 ± 5.7% removal (microbial tests); 49.0 ± 7.2% removal (phytoremediation); 69.3 ± 5% removal (synergetic system over 700 min)	—	Combination of photocatalysis and aerobic biological remediation	60
PFOA	—	*Acidimicrobium* sp. strain A6, microbial electrolysis cells	Anaerobic A6 as electron acceptor NH$_4^+$ as electron donor	Not reported	77% decrease with A6 highly enriched culture; 48.1% decrease in A6 enrichment culture	—	—	61
PFOA	—	*Acidimicrobium* sp. strain A6, biosolids	Anaerobic	PFBA, PFHxA, fluoride, PFPeA, PFHpA	37–68% decrease	150	Augmentation with A6 and/or ferrihydrite, with sterilized controls incorporated	9
PFOA	100	Haloacid dehalogenase enzyme (DeHa I) from *Delftia acidovorans,* expressed in *E. coli*	Aerobic	~1.25 μM fluoride in *E. coli* with DeHa I	Not reported, fluoride produced after 4 h	—	—	62
PFOA	—	Bacterial consortium from anaerobic digestion	Anaerobic	Not reported	20.4–38%	—	Higher biodegradation observed with higher PFOA concentration, up to 3 mg L^{-1}	63
PFOA	1	*Acidimicrobium* sp. strain A6, with PAA-coated ferrihydrite	Anaerobic	PFCAs ranging from C7 to C10	Enhanced removal with PAA-coated ferrihydrite compared to bare ferrihydrite	40	Highest fluoride production for 450 K-coated ferrihydrite (~0.005 mM)	59
PFCAs (C7–C10)	0.1–1	*Pseudomonas mosselii* with potential haloalkane genes	Aerobic	—	Fluoride, PFHpA, PFHpA, PFHxA, PFPeA, PFBA detected	—	T = 25 °C; proposed mechanisms: decarboxylation, hydroxylation, hydrolysis, dehydrogenation, dehalogenation	

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Environ. Sci.: Water Res. Technol., 2026, **12**, 397–420 | **405**

Table 4 (*continued*)

Type of PFAS compound	Initial concentration (mg L⁻¹)	Microorganism involved	Environmental setup	By-products formed	Breakdown efficiency (%)	Testing period (days)	Experimental conditions	Ref.
PFOS	1400–1800	*Pseudomonas aeruginosa* strain HJ4	Aerobic	PFBS; PFHxS	67%	48	T = 30–37 °C, pH 7–9, no mention of abiotic controls	33
PFHxS	1000	*Pseudomonas* species (PS27 & PDMF10)	Aerobic	Not reported	24% (by PS27), 15% (by PDMF10)	5	Proposed mechanism: bioaccumulation, PFHxS was not utilized as a source of carbon or energy, maximum bioaccumulation was observed when ethanol and octane were combined	52
PFOS	1000	*Ensifer moralensis* H16	Aerobic	Fluoride, PFHpS	88%	2	Batch fermentation, T = 28 °C, pH 6.8–7.2, proposed mechanism: monooxygenase elimination of sulfonate group	59
PFOS	1000	*Ensifer adahaerens* M1	Aerobic	Fluoride, PFHpS	Complete removal	6	Incubations, proposed mechanism: sulfonate removal *via* monooxygenase activity	64
PFOS	1	Anaerobic microbial consortia	Anaerobic	2,2,3,4,4,5,5,5-nonafluoropentanal	24%	10	T = 35 °C, pH 6, cometabolic activity was detected when simulated sewage served as a co-substrate	65
PFOS	2	Bacterial consortium with *Paracoccus*, *Hyphomicrobium*, and *Micromonosporacea*	Aerobic	PFBS, 3,3,3-trifluoropropionic acid	70.00%	42	Microbial capsules enhanced biodegradation, activated sludge-derived bacterial consortia, initial PFAS concentration: 2 mg L⁻¹	46
PFOS	0.1 or 100	*Acidimicrobium* sp. strain A6	Anaerobic	HFBA; PFBS	60% (0.1 mg L⁻¹) and 47% (100 mg L⁻¹)	100		58
PFOA	500	*Pseudomonas parafulva* strain YAB1	Aerobic	Not reported	48%	5		59
PFOS	1000	*Pseudomonas plecoglossicida* 2.4-D	Aerobic soil	Not reported	75%	90		59
PFOS	1000	*Pseudomonas plecoglossicida* 2.4-D	Aerobic mineral medium	PFHpA	100%	6		59
PFOS	1000	*Pseudomonas plecoglossicida* 2.4-D	Aerobic	Fluoride (≤150 mg L⁻¹) and perfluoroheptane	75%	6	T = 26–30 °C, pH 6.8–7.2, NaCl concentration within 5%, proposed mechanism: desulfonation, PFOS served as the carbon source; no reduction observed in the medium control	59
6 : 2 FTAA	1.5	Mixed culture	Aerobic sludge	PFBA, PFHxA, 6 : 2 FTOH, 6 : 2 FTSAm, PFPeA, 5 : 3 FTCA	~12%	109		66
6 : 2 FTAB	1.5	Mixed culture	Aerobic sludge	PFHxA, 6 : 2 FTOH, 5 : 3 FTCA, PFPeA, 6 : 2 FTSAm	~3%	109		66

406 | *Environ. Sci.: Water Res. Technol.*, 2026, **12**, 397–420

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Table 4 (*continued*)

Type of PFAS compound	Initial concentration (mg L⁻¹)	Microorganism involved	Environmental setup	By-products formed	Breakdown efficiency (%)	Testing period (days)	Experimental conditions	Ref.
6:2 FTSAS	2	Mixed culture	Aerobic sludge	PFHpA, 6:2 FTOH, 5:3 FTCA, PFHxA, PFPeA, 5:3 FTCA, 6:2 FTUCA	~32%	42		67
6:2 FTSA	1.8–2.6	Mixed culture	Aerobic sludge	PFHxA, 5:2 ketone, PFPeA, 5:2 sFTOH	~6%	90		68
6:2 FTSA	~1.3	Mixed culture	Aerobic or anaerobic sludge	PFPeA, 5:2 sFTOH, 6:2 FTOH, 5:3 acid, 6:2 FTCA	~80% (aerobic); 0% (anaerobic)	100		46
6:2 FTSA	25.6	*Gordonia* sp. strain NB4-1Y	Aerobic	5:2 sFTOH, 6:2 FTOH, 6:2 FTUA, 5:2 ketone, 6:2 FTCA	~88%	7		69
6:2 FTAB	~34.2	*Gordonia* sp. strain NB4-1Y	Aerobic	6:2 FTUA, 5:2 sFTOH, 6:2 FTOH, 5:2 ketone, 6:2 FTCA	~85%	7		69
4:2 FTOH	0.01	Mixed culture	Aerobic landfill soil	TFA, PFPeA, PFBA	~28%	32		70
6:2 FTOH	2.8	Mixed culture	Aerobic sludge	5:3 acid, PFHxA, 6:2 FTCA, 5:2 sFTOH, 6:2 FTUA	~60%	90		71
6:2 FTOH	2.9	Mixed culture	Aerobic soil	PFPeA, 5:3 acid, 5:2 sFTOH	~67%	90		71
6:2 FTOH	1.6	*Mycobacterium vaccae* JOB5	Anaerobic sludge	5:3 acid, 6:2 FTUCA, PFHxA, 6:2 FTCA	~70%	181		72
6:2 FTOH	4.125	*Pseudomonas fluorescens* DSM 8341	Aerobic	5:3 acid, 6:2 FTUCA, 5:2 sFTOH, 6:2 FTCA, 5:2 ketone	~110%	28		72
6:2 FTOH	4.125	*Pseudomonas oleovorans*	Aerobic	PFHxA, 5:2 ketone, 6:2 FTUCA, 5:3 acid, 5:2 sFTOH, 6:2 FTCA	58–81%	28		72
6:2 FTOH	4.125	*Pseudomonas butanovora*	Aerobic	5:2 sFTOH, 6:2 FTUCA, 5:3 acid, 5:2 ketone, 6:2 FTCA	87–113%	28		72
6:2 FTOH	3	*Gloeophyllum trabeum*	Aerobic	PFHxA, 5:3 acid, 6:2 FTUCA	~23%	28		73
6:2 FTOH	3	*Trametes versicolor*	Aerobic	6:2 FTUCA, PFHxA, 5:3 acid	~6%	28		73
6:2 FTOH	1.7	*Phanerochaete chrysosporium*	Aerobic	5:3 acid, PFHxA, 6:2 FTUCA	~40%	28		73
8:2 FTOH	~0.18	Mixed culture	Anaerobic sludge	8:2 FTUCA, 7:3 acid, 8:2 FTCA	~55%	181		46
Trifluoropentanoic acid	500	Mixed culture	Aerobic sludge	Not reported	~6%	224		46
MeFBSE	976	Mixed culture	Anaerobic sludge	MeFBSAA; PFBSI	97%	108		46
EtFOSE	1010	Mixed culture	Anaerobic sludge	EtFOSAA; PFOSI	2%	108		74
PFAA precursors	Not available	Mixed culture	Not available	PFBS, PFCAs (C5 to C7)	Not available	60		74
5:3 FTCA	2	*Pseudomonas oleovorans, P. fluorescens* DSM 8341	Aerobic	4:3 acid, PFPeA	100% biotransformation, 10% defluorination	90	pH = 7, T = 30 °C	72

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Environ. Sci.: Water Res. Technol., 2026, **12**, 397–420 | **407**

through cytochrome P450-mediated oxidation, forming 5 : 3 fluorotelomer carboxylic acid as the main product and confirming its enzymatic defluorination potential.[76] These differences highlight the enzyme-specific nature of PFAS oxidation, which depends on the redox potential and mediator activity of LiP, MnP, and laccase systems.[77] Unlike bacteria, fungi rely on co-metabolism because PFAS cannot serve as their sole carbon or energy source, and the addition of lignocellulosic materials such as wood chips or wheat straw can enhance enzyme expression and PFAS bioavailability.[78]

Recent studies have demonstrated that combining fungal enzymes with electrochemical or photocatalytic processes improves desulfonation and increases fluoride release. For instance, manganese peroxidase integrated with photocatalytic pretreatment enhanced fluoride recovery compared to enzyme-only systems. Laccase mediator systems also achieved partial PFAS removal up to 60 percent after long incubation periods, but confirmation of true defluorination requires fluoride mass balance and product-specific high-resolution mass spectrometry to distinguish degradation from adsorption or mediator oxidation.[77] Overall, while fungal systems show potential for PFAS biotransformation, major gaps remain regarding cross-comparative studies, enzyme substrate specificity, and the influence of co-metabolic substrates under environmentally relevant conditions. Further work is required to identify robust enzyme–mediator pairs and optimize hybrid fungal–catalytic systems that can sustain high activity and stability in complex environmental matrices.[79]

4.1.3. Mixed microbial communities and synergistic strategies. Microbial consortia, composed of diverse bacterial and fungal communities, have shown greater stability and efficiency in PFAS degradation than individual strains. These systems mimic natural microbial ecosystems, where different species cooperate in breaking down complex pollutants. A microbial consortium isolated from PFOS- and PFOA-contaminated river sediments in Japan demonstrated 16–36% degradation when using chemoheterotrophic bacteria alone, but efficiency increased to 46–69% when combined with fungi and yeast. Similarly, an anaerobic microbial consortium known as WBC-2, enriched with chlorinated volatile organic compounds, achieved 46.4% PFOS removal within 45 days, forming intermediates such as PFHxS, PFFeS, and PFBA, which indicate a desulfonation step followed by sequential defluorination.[42]

The cooperative metabolism in these consortia involves complementary redox functions, where aerobic bacteria such as *Pseudomonas* initiate headgroup oxidation and anaerobic *Acidimicrobium*-like organisms complete reductive defluorination. Fungal members contribute extracellular peroxidases and laccase activity, enhancing degradation of long-chain PFAS and promoting transformation of intermediates. The integration of bacterial and fungal metabolism increases overall defluorination and minimizes accumulation of partially oxidized by-products. When

Acidimicrobium sp. strain A6 was coupled with microbial electrolysis cells, degradation efficiency reached approximately 77%, demonstrating the potential of electro-bioremediation to overcome redox limitations.[61] These findings confirm that microbial cooperation and electrochemical enhancement can significantly increase PFAS removal and fluoride release compared with single-strain systems. Despite these advances, microbial consortia approaches still face challenges in reproducibility and optimization, as interspecies interactions vary with pH, temperature, and available electron donors. Future studies should focus on identifying the genetic and enzymatic mechanisms driving community-level PFAS transformation, employing isotope-labeled PFAS tracers to confirm defluorination pathways, and validating these results under environmentally relevant conditions.

To consider the points mentioned above, a comparative evaluation of microbial systems under realistic conditions shows that aerobic microbial systems generally achieve faster PFAS degradation within days to weeks because oxygenase enzymes promote oxidative defluorination. However, they often require organic co-substrates and rarely achieve complete mineralization. Anaerobic systems proceed more slowly over weeks or months but can break stronger carbon–fluorine bonds through reductive pathways, particularly in iron-reducing or electro-assisted environments. Mixed microbial communities combine both oxidative and reductive mechanisms, leading to higher overall defluorination and lower accumulation of intermediate products. Under environmentally relevant conditions with fluctuating pH, temperature, nutrient scarcity, and competing electron acceptors, the performance of all systems declines, underscoring the importance of biostimulation, genetic optimization, and pilot-scale testing to ensure field applicability.

4.1.4. Challenges in microbial PFAS degradation. Incomplete degradation and byproduct formation remain major obstacles to microbial PFAS biodegradation. Many microbial systems transform PFAS into shorter-chain intermediates rather than achieving complete defluorination, which may increase environmental mobility and toxicity. Defluorinated products such as PFBA, PFPeA, and PFHxA remain persistent and require further degradation.[60] This highlights a need for treatment systems that target full mineralization instead of partial conversion.

Anaerobic processes require weeks to months to achieve significant degradation, while aerobic degradation is often incomplete. Long incubation times limit real-world applicability for large-scale remediation. Many microbial species also depend on co-metabolism, requiring the presence of organic co-substrates (*e.g.*, glucose, organic acids) for cometabolic transformation, which limits scalability. These requirements can increase operational costs and reduce efficiency in field applications, making microbial PFAS degradation slow, strongly dependent on external co-substrates, and difficult to sustain under

408 | *Environ. Sci.: Water Res. Technol.*, 2026, **12**, 397–420

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variable field conditions, which limit its large-scale applicability.

Optimal degradation conditions (*e.g.*, pH 4.5–5, temperature 25–30 °C, electron donors) are difficult to maintain in real-world contaminated sites.[80] Performance in field conditions often differs from lab-scale results due to fluctuating environmental factors. Furthermore, while certain oxygenases, dehalogenases, and peroxidases have been implicated in PFAS degradation, specific gene clusters responsible for defluorination remain poorly characterized, necessitating further research into the genetic and enzymatic mechanisms that drive microbial PFAS breakdown.

Advancements in metabolic engineering, synthetic microbial consortia, and electro-assisted biodegradation present opportunities for enhancing microbial PFAS degradation. Further research should focus on field-scale validation, improved biostimulation strategies, and genetic modifications to enhance enzyme activity. Combining microbial approaches with physicochemical treatment technologies may provide a more comprehensive and efficient solution for PFAS-contaminated environments. Integrated systems may help overcome the limits of individual approaches and offer a more flexible strategy for different contamination scenarios.

4.2. Biochemical mechanisms of PFAS defluorination

Biotransformation of PFAS primarily occurs through reductive and oxidative defluorination, with pathways that may or may not involve direct cleavage of the C–F bond. The high stability of the C–F bond, with a dissociation energy of up to 130 kcal mol^{-1}, makes its cleavage challenging under normal environmental conditions[46] Microbial processes offer a potentially viable route for PFAS degradation, yet complete mineralization remains largely inefficient. The complexity of PFAS structures and their resistance to microbial metabolism necessitates integrated approaches that combine microbial degradation with advanced oxidation or reduction strategies.

Fluoride exposure presents numerous health and environmental concerns, yet microbial PFAS degradation remains limited due to thermodynamic constraints. The redox potential of PFOS and PFOA ($\sim$−450 mV) is too low to be coupled with bacterial respiratory chains for ATP production, making electron transfer from common microbial growth substrates thermodynamically unfavorable.[81] Consequently, only a few bacterial strains have demonstrated the ability to defluorinate PFAS, necessitating further research into microbial metabolic pathways and enzymatic mechanisms involved in PFAS degradation. These energetic barriers explain why so few microbes can perform defluorination effectively. Understanding the metabolic limits of microbes can help in designing better biostimulation or engineering strategies.

4.2.1. Reductive defluorination. The concept of reductive defluorination has its origins in the 1960s, when microbial degradation of halogenated pesticides was first investigated.

Like PFAS, chlorinated synthetic pesticides were recognized for their high persistence in the environment. Early studies demonstrated that metallo-enzymes and their associated cofactors facilitated reductive dehalogenation of chlorinated compounds.[82] However, despite progress in reductive dichlorination, these enzymes were found to be ineffective in catalyzing reductive defluorination of PFAS due to the high bond strength of C–F.[82] This highlights the need to discover or engineer new enzymes that are better suited for attacking the C–F bond, since current systems evolved for different types of pollutants. Despite these early efforts, the inefficiency of known reductive dehalogenases toward PFAS can be attributed to three interrelated limitations: (i) the exceptionally high bond-dissociation energy of the C–F bond, which exceeds the catalytic redox potential of most corrinoid- and Fe–S-cluster-based enzymes; (ii) restricted substrate accessibility caused by the hydrophobic and strongly electron-withdrawing surface of perfluoroalkyl chains that limits enzyme–substrate interactions; and (iii) the lack of evolutionary adaptation, since most known dehalogenases evolved to target chlorinated or brominated substrates rather than fluorinated carbon frameworks. Collectively, these biochemical and structural constraints explain why naturally occurring reductive systems rarely achieve measurable PFAS defluorination under environmental conditions.[83]

Complete defluorination has been observed under specific microbial conditions. For instance, bacterial inoculum derived from activated sludge successfully degraded perfluorododecanol, resulting in no detectable intermediate metabolites. Similarly, a bacterium isolated from fluoroacetate-producing plants was capable of completely defluorinating perfluorododecanol at a rate of 25.3 mg per 10^9 cells per hour.[73] However, these successes are rare and often tested at high PFAS concentrations, which may not represent typical environmental levels.

4.2.2. Oxidative defluorination. Unlike reductive defluorination, oxidative defluorination of PFAS occurs primarily in compounds that are not fully fluorinated, such as fluorotelomer alcohols and certain side-chain fluorinated structures. In these cases, the presence of residual hydrogen atoms or functional groups allows oxidative enzymes to attack the molecule and destabilize adjacent C–F bonds. Metallo-enzymes including oxygenases, oxidases, and peroxidases have been linked to such partial PFAS degradation. For instance, oxygenases in *Pseudomonas* strains and extracellular enzymes produced by white-rot fungi (*Phanerochaete*, *Trametes*) have been shown to catalyze oxidative transformations that yield shorter-chain acids and release fluoride ions. However, these enzymes are generally ineffective against fully fluorinated compounds such as PFOA or PFOS, where the absence of hydrogen atoms and the presence of resistant trifluoromethyl (-CF$_3$) groups hinder oxidative metabolism.[70] This highlights that while oxidative pathways play an important role in transforming certain PFAS precursors and intermediates, they are insufficient on their own to address the most recalcitrant perfluoroalkyl acids.

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Environ. Sci.: Water Res. Technol., 2026, **12**, 397–420 | **409**

Oxidative systems face comparable mechanistic barriers. Oxygenases and peroxidases typically initiate catalysis through hydrogen abstraction or electron-rich intermediates, yet fully fluorinated PFAS lack such reactive sites. In addition, fluorine's high electronegativity imposes a strong negative inductive effect that deactivates adjacent carbons and suppresses radical propagation. Consequently, even when oxidative pathways begin partial defluorination, they often stall at thermodynamically stable intermediates, yielding short-chain PFAS rather than complete mineralization.[77]

4.2.3. Integrated approaches for PFAS biodegradation. Given the limitations of microbial PFAS degradation, recent studies have explored integrated techniques that combine microbial degradation with advanced oxidation or reduction treatments. One such approach is photodegradation-assisted microbial defluorination, where oxidative UV–persulfate or reductive UV–sulfite treatments are used to enhance PFAS breakdown. In laboratory studies, these approaches have demonstrated high efficiency in degrading 6:2 flu and 6:2 FTS.[6] This suggests that using chemical pre-treatment can make PFAS more accessible to microbes, improving overall degradation.

When subjected to oxidative UV–persulfate treatment, 6:2 FTS was rapidly degraded into short-chain PFAS ($C_2–C_7$) within just 10 minutes[6] However, polyfluorinated PFAS compounds exhibit lower reactivity in aqueous environments than their perfluorinated counterparts. This suggests that a single approach is likely insufficient for complete PFAS degradation. Instead, a stepwise activation process, where polyfluorinated molecules are first converted into perfluorinated molecules, can increase their susceptibility to aqueous electron-mediated degradation. These findings support the need for multi-stage treatment systems rather than one-step solutions. Each step targets a different limitation in PFAS structure or reactivity. Studies on *Pseudomonas fluorescens* DSM 8341 have demonstrated the ability to cleave C–F bonds, providing some of the earliest evidence of microbial PFAS degradation[46] However, additional investigations are required to determine the efficiency and feasibility of microbial PFAS defluorination at environmentally relevant concentrations.

Integrated and hybrid systems attempt to overcome these enzymatic bottlenecks by chemically weakening the C–F bond before microbial attack. Pre-oxidation or UV–sulfite reduction introduces hydroxyl or carboxyl functionalities that enhance substrate polarity, electron transfer, and microbial accessibility. This synergy between physicochemical activation and enzymatic metabolism compensates for the intrinsic energetic and structural limitations of individual pathways, providing a more feasible route toward near-complete PFAS mineralization.[77]

4.2.4. Alternative strategies beyond direct C–F bond cleavage. In addition to direct C–F bond cleavage, alternative PFAS remediation strategies have been investigated. Soil washing, for instance, represents a low-technology, *ex situ* remediation technique that involves removing PFAS-contaminated soil and treating it with solvents or surfactants. Although effective, this method is often costly and carries a risk of secondary water contamination.[42]

Furthermore, plasma-based degradation techniques have demonstrated the ability to degrade PFOA within nanoseconds under high-energy conditions.[46] While effective, these treatments remain highly energy-intensive and expensive, making them impractical for large-scale applications. These methods may be better suited for small, high-risk sites rather than broad applications due to cost and energy demands.

4.3. Enzymatic PFAS degradation

Enzymatic degradation of PFAS has been explored as a potential mechanism for breaking down these persistent pollutants, with studies identifying key enzymes capable of catalyzing defluorination reactions. Despite limited efficiency in complete mineralization, enzymatic processes provide insight into microbial adaptations for PFAS metabolism. The role of specific enzyme families, including cytochrome P450 monooxygenases, peroxidases, and dehalogenases, has been studied to understand their contribution to PFAS degradation. However, reported enzymatic systems show considerable variation in kinetics, substrate specificity, and turnover efficiency, which strongly influence their applicability.

Comparative analyses indicate that cytochrome P450 monooxygenases exhibit broad substrate specificity but very low catalytic turnover toward fully fluorinated PFAS. Their heme–iron active center enables oxidation of mono- or partially fluorinated carbons, yet steric hindrance and fluorine's strong electronegativity restrict electron transfer, limiting defluorination to initial transformation steps. Apparent catalytic constants are generally below $1\ s^{-1}$, reflecting slow reaction kinetics. In contrast, oxidoreductases such as laccases, lignin peroxidase, and manganese peroxidase catalyze radical-mediated oxidation with moderate substrate affinity (typical K_m values between 0.1 and 1 mM), but the reactions proceed over long incubation periods and show low selectivity for PFAS chain length or headgroup type. Dehalogenases, including haloalkane dehalogenase (dhaA) and haloacetate dehalogenase (dehH1), exhibit narrower substrate ranges but higher mechanistic precision, targeting α-fluorinated carbons adjacent to electron-withdrawing groups. Their turnover rates are usually around $10^{-2}\ s^{-1}$, which remains insufficient for practical mineralization. Overall, dehalogenases offer higher specificity, while oxidoreductases provide broader but slower transformation capacity.[75,83]

Recent research has demonstrated that *Acidimicrobium* sp. strain A6 facilitates the degradation of PFOA and PFOS under anaerobic conditions, achieving up to 63% removal over 100 days.[59] The defluorination process resulted in the release of fluoride ions (F^-), suggesting incomplete decomposition of perfluorinated compounds. A linear correlation between

410 | *Environ. Sci.: Water Res. Technol.*, 2026, **12**, 397–420

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fluoride release and PFCA concentration was observed, indicating a stepwise degradation pathway. However, the accumulation of fluoride ions can cause downstream inhibition of microbial activity. The presence of the fluoride ion transporter gene (crcB) has been identified as a key factor in mitigating fluoride toxicity, allowing bacteria to expel excess fluoride ions, thereby enabling continued PFAS metabolism.[59]

Several enzymes have been linked to PFAS biodegradation, among which cytochrome P450 monooxygenases have shown the ability to initiate defluorination. These enzymes contain a heme prosthetic group with an iron cation, which interacts with fluorinated carbon chains due to fluorine's high electronegativity. This interaction facilitates oxidative cleavage of monofluorinated compounds, though the degradation efficiency against highly fluorinated PFAS molecules remains limited.[42] The strong electron-withdrawing properties of fluorine reduce the availability of reactive sites, thereby hindering the ability of P450 monooxygenases to catalyze defluorination in perfluorinated compounds. In contrast, dehalogenases achieve stronger binding with fluorinated substrates but require specific redox conditions and cofactors for activity. These comparative trends highlight that enzyme efficiency is controlled by both structural accessibility of the substrate and the reaction mechanism of the enzyme.[59]

Additional studies have demonstrated the role of oxidoreductases, including laccases, in PFAS transformation. Oxidative humification reactions mediated by laccases have been shown to degrade PFOA by 40% over 140 days in a soil slurry system, while 24% degradation was observed over 36 days.[6] Laccases, primarily extracted from soybean meal, generate highly reactive free radicals, which can attack the carbon backbone of PFAS molecules. These radicals initiate oxidation pathways that transform perfluorinated compounds into smaller fluorinated byproducts. In soil environments, laccase activity has been identified as a primary mechanism of PFOA degradation, facilitating its transformation under aerobic conditions. However, the long treatment times and low efficiency raise concerns about practical field use, especially in dynamic soil conditions where enzyme activity can fluctuate.

Apart from laccases, other peroxidases such as lignin peroxidase (LiP) and manganese peroxidase (MnP) have been investigated for their role in PFAS degradation. These enzymes, commonly involved in lignin degradation, are heme-containing oxidoreductases activated by hydrogen peroxide (H_2O_2). Their strong oxidative potential enables them to catalyze electron transfer reactions, facilitating the breakdown of fluorinated organic compounds (UCLA Electronic Theses Tseng, 2012). Unlike general fungal peroxidases, LiP and MnP exhibit strong electron-deficient properties, which enhance their ability to degrade persistent organic pollutants. This suggests that peroxidase-based oxidation systems could contribute to PFAS degradation, although their efficiency remains dependent on reaction conditions such as pH, redox potential, and enzyme–substrate affinity.

Genomic studies of *Pseudomonas mosselii* have identified key enzymatic pathways involved in PFCA biotransformation, with approximately 50% of bacterial isolates containing genes encoding laccases and dehalogenases (UCLA Electronic Theses Tseng, 2012) several key genes have been implicated in enzymatic PFAS degradation, including haloalkane dehalogenase (dhaA) and haloacetate dehalogenase H-gene (dehH1), which facilitate C–F bond cleavage at the α-carbon position.[59] Furthermore, alkanesulfonate monooxygenase (ssuE) has been found to catalyze the desulfonation of organosulfonate substrates, promoting PFCA degradation under sulfate-limited conditions. The role of fluoride ion transporter genes, such as crcB, has been confirmed in preventing fluoride ion accumulation during PFAS metabolism, thereby reducing cytotoxicity and enabling sustained microbial activity. Additionally, redox-active dehalogenase (rdhA) genes have been associated with fluoride release and PFAS removal, with gene knockout studies in *Acidimicrobium* sp. strain A6 confirming the essential role of rdhA in microbial PFAS defluorination.[84]

Despite the identification of these enzymatic pathways, PFAS degradation remains highly inefficient, primarily due to enzyme specificity and limited substrate affinity. Most naturally occurring microbial enzymes have evolved to degrade chlorinated or brominated organic pollutants, which exhibit lower bond dissociation energies compared to fluorinated compounds. The lack of evolutionary pressure for microbial adaptation to PFAS degradation further limits the efficiency of enzymatic pathways. Overall, current comparisons indicate that enzymatic PFAS degradation is constrained more by reaction kinetics than by thermodynamics. Oxidoreductases act on a wider range of PFAS types but at slow rates, whereas dehalogenases display higher catalytic precision but restricted substrate compatibility. These mechanistic contrasts underline the need for enzyme engineering to improve substrate binding, cofactor regeneration, and catalytic turnover for effective C–F bond cleavage.[42]

Further research is required to optimize enzyme reaction conditions, substrate binding interactions, and cofactor availability to improve PFAS degradation efficiency. Immobilization of enzymes onto solid supports or the use of enzyme–microbe hybrid systems could enhance the stability and catalytic efficiency of enzymatic PFAS transformation. While enzymatic degradation alone may not achieve complete PFAS mineralization, its integration into bioelectrochemical systems or advanced oxidation processes may provide a viable strategy for PFAS remediation in contaminated environments.

5. Factors influencing PFAS biodegradation

The biodegradation of PFAS is influenced by a complex interplay of factors, including microbial species, co-

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Environ. Sci.: Water Res. Technol., 2026, **12**, 397–420 | **411**

substrates, environmental conditions, and the inherent chemical structure of the PFAS compounds.

5.1. Microbial communities and their functional roles

The efficiency of PFAS biodegradation is closely linked to the specific microbial species present and their interactions within the community. For instance, a mixed microbial culture derived from aerobic sludge achieved a mere 6% degradation of 6:2 fluorotelomer sulfonic acid (6:2 FTSA), whereas a culture from aerobic sediment accomplished up to 80% degradation of the same compound. This disparity underscores the significance of microbial origin and community composition in PFAS degradation. These differences show that even small variations in microbial sources can lead to large changes in degradation potential, suggesting that microbial selection is a critical step in system design.

High concentrations of PFAS can exert toxic effects on microbial communities, inhibiting their growth and metabolic activities. Exposure to 20 mg L^{-1} of perfluorooctanoic acid (PFOA) impeded microbial growth and reduced the removal efficiency of dissolved organic carbon in sludge processes.[42] Prolonged exposure also led to shifts in microbial community structure, favoring PFOA-tolerant species such as *Bacteroidetes*, *Proteobacteria*, and *Acidobacteria*.

5.2. Role of co-substrates

The presence of co-substrates can significantly influence PFAS biodegradation rates. In studies examining the degradation of 6:2 fluorotelomer alcohol (6:2 FTOH), the addition of formate as an external substrate had varying effects depending on the microbial strain. For *Mycobacterium vaccae* JOB5, formate addition did not impact degradation products, whereas for *Pseudomonas fluorescens* DSM 8341, formate supplementation led to the production of metabolites with fewer carbon–fluorine bonds, indicating enhanced degradation.[85]

Co-substrates can also alter degradation pathways. For example, *Pseudomonas butanovora* degrades 6:2 FTOH into products such as 6:2 FTCA, 6:2 FTUCA, 5:2 ketone, 5:2 sFTOH, and PFHxA under aerobic conditions. When lactate was used as a co-substrate, additional products like 5:3 acid and 5:3 u acid were formed, suggesting the activation of alternative degradation pathways.[85]

5.3. Environmental conditions

Environmental factors, including media composition, organic material availability, and redox potential, are critical determinants of PFAS biodegradation.[85] Oxygen availability, for instance, can influence both the biodegradability and reaction kinetics of PFAS. Some studies suggest that oxygen-independent pathways may offer greater degradation efficiency compared to oxygen-dependent mechanisms.[86] This raises the possibility of designing low-oxygen or anaerobic systems for specific PFAS, especially when oxygen demand or control is impractical.

Temperature and pH also play pivotal roles. The biodegradation rate of EtFOSE in marine sediments varied with temperature, exhibiting a half-life of 44 days at 25 °C and extending to 160 days at 4 °C.[85] pH affects the sorption behavior of PFAS, thereby altering their bioavailability to microbes. For instance, the biodegradation rate of EtFOSE is approximately five times higher at a slightly alkaline pH of 7.8 compared to an acidic pH of 5.5.[85] Under anaerobic Fe(III)-reducing conditions, *Acidimicrobium* sp. A6 achieved 63% removal of PFOA and PFOS after 100 days, whereas *Labrys portucalensis* F11 metabolized over 90% PFOS within the same period under aerobic conditions. Fungal and mixed microbial systems maintain optimal activity at 25–30 °C and pH 6–8, with more than 70% loss in efficiency outside this range.[83]

5.4. Impact of chemical structure

The inherent chemical structure of PFAS compounds, including chain length and functional groups, significantly influences their biodegradation potential. Short-chain perfluoroalkyl carboxylic acids (PFCAs) exhibit greater mobility, water solubility, and resistance to degradation compared to their long-chain counterparts.[59] This increased stability poses challenges for remediation efforts. It also means that new-generation PFAS, often designed to be short-chain for reduced toxicity, may still be more difficult to remove through biological means.

Functional groups within PFAS molecules contribute to their stability through various interactions. Polar head groups, such as carboxyl and sulfonyl groups, engage in electrostatic interactions and hydrogen bonding, while the hydrophobic carbon backbone promotes the formation of micelles. Notably, short-chain PFAS are less prone to micelle formation, resulting in adsorption as single units at pore sites, which complicates their removal compared to aggregated long-chain PFAS.[86]

The strong carbon–fluorine bonds characteristic of PFAS, with dissociation energies around 450 kJ mol^{-1}, render these compounds highly resistant to degradation[42] This chemical robustness necessitates the exploration of innovative strategies to effectively degrade PFAS in environmental settings.

In summary, the biodegradation of PFAS is governed by a multifaceted array of factors encompassing microbial community composition, availability of co-substrates, environmental conditions, and the specific chemical structures of the compounds. A comprehensive understanding of these determinants is essential for developing effective bioremediation strategies for PFAS-contaminated environments.

6. Machine learning applications for predicting PFAS degradation pathways

The prediction of degradation pathways for PFAS has increasingly benefited from advanced machine learning (ML)

412 | *Environ. Sci.: Water Res. Technol.*, 2026, **12**, 397–420

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methodologies. Traditional mechanistic models often struggle with the complex, nonlinear interactions that govern PFAS transformation under varied treatment conditions. In response, ML models have demonstrated capabilities to predict degradation behavior across diverse PFAS structures and environmental settings with increasing accuracy and efficiency.[87]

Recent studies have demonstrated that ensemble models such as random forests and gradient-boosted decision trees, such as XGBoost, can predict PFAS degradation outcomes in electrochemical systems by correlating operational parameters, such as applied voltage and electrode material, with degradation efficiency. In a related development, Jeong et al. (2024)[88] utilized transformer-based deep learning models combined with molecular simulations to predict PFAS transport across polyamide membranes, revealing structure–activity relationships that govern PFAS retention, partitioning, and potential transformation at membrane interfaces. Their findings highlighted that electrostatic interactions and molecular polarity significantly influence PFAS–membrane interactions, offering insights into how molecular properties affect separation performance and potentially subsequent degradation behavior, insights that would be difficult to derive from experimental observations alone.

Another critical development was introduced by Raza et al. (2019),[89] who used ML models including random forest, LASSO regression, and feed-forward neural networks (FNN) to predict C–F bond dissociation energies without relying on computationally intensive quantum mechanical calculations. Their models achieved mean absolute deviations of less than 0.70 kcal mol^{-1}, sufficient to guide degradation pathway analysis and reactor design. Importantly, these predictions required only 2D molecular connectivity information, significantly reducing computational burdens and enabling high-throughput screening of PFAS candidates for degradation susceptibility.

Machine learning models have also been applied to predict PFAS degradation under dynamic and untested environmental conditions. Recent research shows that ML models can extrapolate degradation behaviors under varied pH, temperature, and salinity conditions, offering a practical tool for designing treatment systems adaptable to field realities. Moreover, unsupervised learning techniques such as t-distributed stochastic neighbor embedding (t-SNE) have been used to cluster PFAS molecules based on intrinsic C–F bond characteristics, providing automated chemical classification schemes that inform degradation strategy selection.[87]

Despite these advances, several limitations persist. A major challenge is the scarcity of degradation-specific datasets, particularly for short-chain and emerging PFAS like GenX and ADONA. This data limitation restricts the generalization capacity of ML models trained predominantly on legacy compounds such as PFOA and PFOS. Furthermore, while ML models can predict degradation rates and pathways with increasing accuracy, most existing studies do not account for the environmental toxicity of transformation products, an essential factor in comprehensive remediation planning. Even when intermediates are predicted, toxicity data for these byproducts are rarely available or integrated into model outputs, limiting their usefulness for environmental risk assessment. The absence of curated datasets linking structure, degradation rate, and ecotoxicity particularly constrains ML model validation for emerging PFAS. Additionally, many of the high-performing models, particularly deep neural networks, lack interpretability, complicating their acceptance in regulatory frameworks where mechanistic transparency is required.[89]

Looking forward, several strategies have been proposed to enhance the applicability of ML to PFAS degradation. Building extensive, curated, open-access databases that include degradation intermediates, reaction kinetics, and environmental toxicity data is a priority. The development of hybrid modeling approaches that integrate mechanistic chemical knowledge with data-driven learning is expected to provide models that are both predictive and interpretable. Furthermore, applying explainable AI (XAI) techniques to existing ML models can uncover the molecular drivers behind degradation trends, bridging the current gap between prediction and understanding. These future directions are crucial to transforming machine learning from a predictive tool into a comprehensive platform for rational PFAS degradation design.[87]

7. Challenges and limitations in PFAS biodegradation

Despite growing efforts to develop effective degradation strategies, several key obstacles continue to hinder progress. The persistence of PFAS in natural environments, the formation of toxic degradation by-products, and the limited understanding of microbial and enzymatic pathways all contribute to the complexity of their biodegradation. Addressing these challenges requires a deeper insight into the mechanisms governing PFAS degradation and the environmental factors that influence these processes.

7.1. Persistent barriers to biodegradation

Despite the urgent need for effective PFAS degradation, several persistent barriers hinder complete biodegradation. The primary challenge arises from the exceptional stability of the C–F bond, which is the strongest single bond in organic chemistry. This stability not only prevents environmental breakdown but also limits enzymatic and microbial defluorination mechanisms. Degradation occurs through stepwise processes such as decarboxylation, desulfonation, and defluorination, which proceed at a slow rate and are often incomplete under natural conditions[84] Furthermore, the structural diversity of PFAS molecules, characterized by various functional groups including sulfonates, carboxylates, and phosphates, complicates degradation pathways.[85]

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Environ. Sci.: Water Res. Technol., 2026, **12**, 397–420 | 413

Quantification of degradation products, such as fluoride ions released during defluorination, is hampered by analytical challenges. Detecting short-chain and ultra-short-chain PFAS remains particularly difficult because they are highly soluble, weakly retained on chromatographic columns, and often fall below LC–MS/MS detection thresholds. Incomplete fluoride mass balance further complicates assessment of defluorination efficiency in field samples, where matrix interferences and co-eluting species obscure true fluoride release. These analytical limitations create uncertainty in verifying complete mineralization under environmental conditions. Factors such as low detection limits, interference from environmental matrices, and the potential formation of fluoride complexes with surrounding molecules hinder accurate assessment of degradation efficiency.[84] Although some polyfluorinated compounds can undergo partial defluorination under controlled conditions, these methods often generate smaller, persistent perfluorinated by-products that remain resistant to further degradation.[85,90] The inability to detect or quantify by-products accurately creates uncertainty around the true effectiveness of any treatment process.

Microbial degradation is further limited by the structural features of PFAS molecules. Compounds with carbon–hydrogen (C–H) bonds in the alpha position are more susceptible to microbial enzymatic activity. This has been demonstrated in studies where 3,3,3-trifluoropropionic acid achieved an 85 percent defluorination rate, whereas 2,2-difluoropropionic acid and 2,2,3,3-tetrafluoropropionic acid remained unaffected after exposure to wastewater treatment plant sludge.[85] However, no biological or chemical method currently exists to cleave multiple C–F bonds simultaneously under environmental conditions, reinforcing the classification of PFAS as "forever chemicals".[90]

Environmental factors also impose constraints on PFAS biodegradation. Variability in oxygen availability, the concentration of PFAS, and the presence of co-substrates influence microbial activity. For instance, desulfonation of 6 : 2 fluorotelomer sulfonic acid (FTSA) by *Rhodococcus jostii* RHA1 was observed only under sulfur-poor conditions, while under sulfur-rich conditions, no degradation occurred.[85] This dependence on environmental conditions limits the development of a standardized degradation approach. Additional parameters affecting PFAS degradation include the composition of the surrounding medium, bioavailability, toxicity, and the presence of organic matter and metal ions, all of which influence microbial metabolism and enzymatic activity. These combined factors make scaling up from lab to field particularly challenging, as they can vary widely from one site to another.

7.2. Toxic by-products from degradation

A major limitation of PFAS biodegradation is the formation of toxic by-products, which persist in the environment and pose long-term health risks. These degradation intermediates include shorter-chain PFAS compounds and fluoride ions.

Incomplete degradation often results in the formation of perfluorobutanoic acid (PFBA) and perfluoropentanoic acid (PFPeA), which are more mobile and water-soluble than their longer-chain counterparts, making them more difficult to remove during wastewater treatment.[91] While longer-chain PFAS tend to adsorb to soil and sediments, limiting their mobility, shorter-chain PFAS can migrate between surface water and groundwater, increasing the risk of contamination.[71] This means that degradation does not always reduce environmental impact—it may increase mobility and spread.

Ultra-short-chain PFAS, defined as compounds with one to three carbon atoms, present an additional challenge. These compounds, including trifluoroacetic acid (TFA) and pentafluoropropionic acid, have been detected in surface water and drinking water sources. Studies have reported that in Canadian rivers and rainwater, approximately 40 percent of PFAS contamination is attributable to ultra-short-chain compounds. Similarly, drinking water supplies in Germany contain these compounds at concentrations ranging from one to ten nanograms per liter.[90] These ultra-short-chain compounds often bypass conventional filtration systems, making them a hidden but critical concern in drinking water safety. Current wastewater treatment processes are unable to recover or degrade these ultra-short-chain PFAS, necessitating further research into effective remediation strategies.

The release of fluoride ions as a by-product of defluorination presents another environmental concern. Fluoride ions can persist in aquatic systems, form complexes with other elements, or remain mobile, posing risks to both human health and ecosystems. Given that degradation often results in the accumulation of smaller yet still persistent and toxic by-products, partial biodegradation does not eliminate the environmental and health risks associated with PFAS contamination.

7.3. Current gaps in understanding microbial and enzymatic pathways

One of the most important limitations in PFAS biodegradation research is the incomplete understanding of microbial and enzymatic pathways. While certain microbial strains have demonstrated the ability to partially degrade PFAS, the specific mechanisms and enzymes involved remain poorly characterized. For example, studies have reported the partial defluorination of polyfluorinated C6 compounds in mixed microbial cultures, but the exact microbial species and enzymatic cofactors responsible for the process have not been conclusively identified.[92] This lack of clarity makes it difficult to reproduce results or develop consistent treatment models. Researchers hypothesize co-metabolic interactions, rather than the activity of a single species, drive PFAS degradation, making pathway elucidation more complex.

414 | *Environ. Sci.: Water Res. Technol.*, 2026, **12**, 397–420

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Enzymatic degradation remains similarly unclear. Although enzymes such as haloacid dehalogenases (DeHa I and DeHa II) have shown the ability to catalyze defluorination in controlled environments, their activity is highly substrate-specific.[85] Reductive defluorination is believed to require cobalt corrinoid cofactors, but the specific cofactors necessary for efficient PFAS degradation have not been identified[92] Additionally, existing knowledge of dehalogenation enzymes, primarily derived from studies on chlorinated compounds, may not be directly applicable to PFAS due to differences in bond strength and molecular structure.

To address these knowledge gaps, researchers have proposed using intermediate product analysis in time-series sampling to infer microbial degradation pathways. By identifying degradation intermediates and linking them to known enzymatic processes using bioinformatics tools such as the KEGG database, researchers can determine potential enzymatic and genetic mechanisms involved in PFAS biodegradation.[42] However, this approach requires further validation and expansion to encompass the wide structural diversity of PFAS compounds.

A further gap exists in the translation of laboratory findings to real-world environmental conditions. Most biodegradation studies have been conducted under controlled laboratory settings, with limited research on PFAS degradation in contaminated environmental sites.[84] Expanding research to include field studies in PFAS-contaminated environments will provide critical insights into the applicability and limitations of bioremediation strategies (Fig. 3).

8. Future directions and perspectives

The persistence of PFAS in the environment makes clear that conventional approaches to biodegradation are not sufficient. The high strength of the C–F bond and the structural diversity of PFAS have consistently limited microbial and enzymatic efforts. Yet, advances in biotechnology, synthetic biology, and hybrid treatment technologies are opening new possibilities. Future research must combine molecular-level discoveries with system-level integration and field-scale validation, ensuring that laboratory insights can translate into workable solutions under real-world conditions.

8.1. Next-generation biotechnological innovations

Engineering of microbes and enzymes specifically tailored for PFAS is one of the most promising areas for future exploration. Laboratory work has already shown that microbial performance can be improved through genetic manipulation. For example, genome shuffling of *Pseudomonas parafulva* strain YAB-1 generated a mutant (strain F3-52) capable of increasing PFOA degradation from 48.1% to 58.6% over 96 hours in the presence of glucose.[84] This demonstrates that precise modifications can yield measurable improvements. Building on this, CRISPR-based

editing and adaptive laboratory evolution may create strains that perform reliably in complex environmental conditions.

Enzyme engineering offers similar opportunities. Existing dehalogenases such as fluoroacetate dehalogenase (FAcD) are limited to monofluorinated substrates, but homology modeling and molecular docking can help redesign active sites for PFAS backbones. Radical-based systems, such as glycyl radical enzymes (GREs), have already demonstrated activity on other highly fluorinated substrates, making them strong candidates for future development.[93] Integrating computational design with high-throughput experimental screening could accelerate discovery of robust PFAS-active enzymes.

Bio-electrochemical systems, including microbial electrolysis cells (MECs), provide another promising route. These systems can harness microbial metabolism to generate electrons for reductive defluorination. Notably, *Acidimicrobium* sp. strain A6 has been shown to degrade PFOA under electrochemical conditions, producing fluoride ions as end products.[94] Optimizing electrode materials, electron transfer mediators, and electroactive microbial consortia will be crucial for improving the efficiency and scalability of such systems.

8.2. Unconventional bioremediation routes

Fungi, algae, and synthetic consortia represent unconventional but promising biological systems that may complement bacterial degradation. White-rot fungi such as *Phanerochaete chrysosporium* and *Trametes versicolor* produce extracellular enzymes (LiP, MnP, laccases) that have been shown to act on PFAS precursors, though current efficiencies are low.[9] Optimizing enzyme–substrate interactions, enzyme immobilization, and co-substrate additions may significantly enhance their utility. Algal systems also offer potential. Certain algal species can uptake PFAS and degrade them *via* cometabolic pathways, while simultaneously producing biomass that can be further processed.[94] This dual functionality aligns with sustainability goals and warrants further development through photobioreactor designs and metabolic pathway analysis. Synthetic biology approaches may also enable the design of microbial consortia with complementary roles. In such consortia, one strain may initiate C–F bond cleavage while others consume the resulting intermediates, preventing accumulation of toxic byproducts. Advancing this concept will require integration of genomics, transcriptomics, and proteomics to guide rational community design and maintain stability in complex environments.[59]

8.3. Hybrid and integrated approaches for scale-up

Given the resilience of PFAS, hybrid approaches that combine biological and physicochemical treatments are likely to be most effective. Adsorption with granular activated carbon (GAC) remains a widely used technology, but its effectiveness decreases with short-chain PFAS.

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Environ. Sci.: Water Res. Technol., 2026, **12**, 397–420 | **415**

Pairing GAC with biodegradation could pre-concentrate PFAS, enhancing microbial access and breakdown.[91] Advanced oxidation processes (AOPs) such as UV–persulfate and sonochemistry can pre-treat PFAS, destabilizing the C–F bond and creating intermediates that are more biodegradable. Coupling AOPs with microbial consortia or engineered enzymes may therefore accelerate mineralization. Similarly, immobilizing peroxidases on nanoparticles has been shown to stabilize enzymatic activity and mitigate fluoride toxicity, though environmental risks of nanomaterials must be carefully assessed.[93] However, nanoparticle-based enzyme carriers can leach reactive metal ions or persist as secondary contaminants in soils and sediments, requiring long-term monitoring of ecotoxicity and mobility. Electrode-driven systems may also release corrosion products or generate reactive oxygen species that impact microbial viability and water chemistry, necessitating risk-managed scale-up designs. Hybrid electrochemical–biological systems are another promising direction, where electroactive microbes are paired with electrode-driven radical generation. Such systems could provide controlled and efficient PFAS breakdown and should be evaluated under pilot-scale conditions.

8.4. Translational gaps and field validation

Despite progress in laboratory studies, future work must validate promising microbes, enzymes, and consortia in real contaminated groundwater, soils, and sediments. Pilot-scale demonstrations in bioreactors, constructed wetlands, or MEC-based systems will be essential to assess feasibility, while life cycle and risk assessments should accompany these trials, particularly when hybrid technologies involve nanoparticles or aggressive oxidants. Future demonstrations

Fig. 3 Existing knowledge gaps in PFAS biodegradation.

should therefore quantify nanoparticle recovery, energy demand, and secondary emissions to determine the practical limits of hybrid bioreactors under field conditions. Regulatory frameworks must also evolve to ensure that PFAS breakdown products are non-toxic and do not accumulate. Developing standardized testing protocols for degradation pathways and byproducts will be crucial for enabling field-scale deployment.

9. Conclusions

This review has provided a comprehensive analysis of the current state of PFAS biodegradation, emphasizing its challenges, mechanisms, and the necessity of effective remediation strategies. The persistence of PFAS in the environment, due to their highly stable carbon–fluorine bonds, remains a major obstacle, necessitating innovative approaches for their degradation. It is clear that traditional biological systems alone are not sufficient, and interdisciplinary solutions are needed.

A key point is that while microbial and enzymatic degradation have shown promise, no universal degradation mechanism exists due to the chemical diversity of PFAS compounds. Some microbial strains, such as *Pseudomonas mosselii* and *Pseudomonas plecoglossicida*, have demonstrated potential in breaking down specific PFAS molecules. However, degradation often results in the accumulation of toxic intermediates, such as fluoride ions and short-chain PFAS, which can be more persistent and mobile in the environment. This underscores the need for holistic approaches that ensure complete mineralization rather than mere transformation of these pollutants. Partial degradation may reduce concentrations but not environmental risk. Future strategies must prioritize end-product safety and persistence.

Additionally, this review highlights the limitations of current biodegradation studies. Many investigations are conducted under controlled laboratory conditions that may not translate effectively to real-world environmental systems. There is a pressing need for field-scale validation of degradation pathways, as well as mass balance studies to confirm true PFAS mineralization rather than redistribution. Establishing standardized assessment protocols that include fluoride quantification, product identification, and isotope-based mass balance verification is required to distinguish genuine mineralization from partial transformation. These standardized methods would enable consistent comparison across studies and guide the development of scalable remediation strategies.

Another critical challenge is the absence of standardized assessment methods for PFAS biodegradation. The integration of advanced analytical techniques, such as high-resolution mass spectrometry and isotopic tracing, will be essential in verifying degradation efficiency and understanding transformation pathways. Moreover, research into synergetic microbial consortia and enzyme engineering
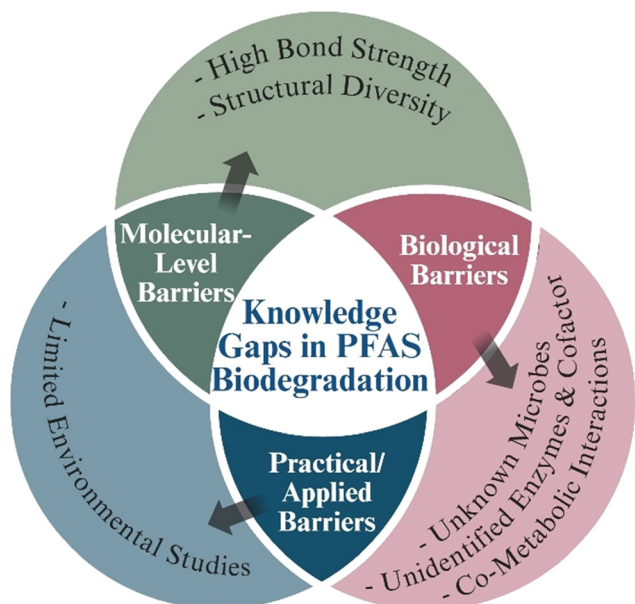
could pave the way for more efficient PFAS degradation strategies.

Ultimately, while promising advancements have been made in understanding and advancing PFAS biodegradation, many knowledge gaps remain. Addressing these gaps will require interdisciplinary collaboration across microbiology, chemistry, environmental science, and engineering. As research progresses, ensuring that biodegradation strategies are both effective and scalable will be key to mitigating the environmental and health risks posed by PFAS contamination.

Conflicts of interest

There are no conflicts of interest to declare.

Data availability

This review did not generate or analyze any new data, and no primary research results, software, or code are included.

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