npj | clean water

Review

Published in partnership with King Fahd University of Petroleum & Minerals

Article in Press

https://doi.org/10.1038/s41545-026-00646-8

Engineered biofilms as compartments regulating PFAS fate in water and wastewater treatment

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Rabia Zafar[1,2,4], Shahzeb Tariq[2,4], Mairaj Bibi[3], Keugtae Kim[2] ✉ & Jin Hur[1] ✉

Per- and polyfluoroalkyl substances (PFAS) are persistent contaminants in water and wastewater treatment, yet their behavior within engineered biofilms remains poorly resolved. This review develops a PFAS-specific, biofilm-centered framework linking PFAS molecular properties with extracellular polymeric substances, microbial surfaces, biofilm structure, and reactor conditions to explain partitioning, biological accessibility, transformation, retention, and release. A biofilm-centered mass-balance perspective is proposed to distinguish reversible retention, precursor transformation, remobilization, and verified loss from the tracked PFAS inventory. Biological transformation is emphasized as being most relevant to selected polyfluorinated precursors rather than terminal perfluoroalkyl acids. Key research needs include spatially and temporally resolved PFAS inventories and integrated measurements of biofilm and reactor states. Overall, apparent PFAS removal should not be equated with true elimination without accounting for retention, transformation, and remobilization.

Per- and polyfluoroalkyl substances (PFAS) have become a major challenge for water and wastewater treatment because of their exceptional persistence and continuous release from industrial, commercial, and domestic sources[1,2]. Their strong carbon-fluorine bonds make many PFAS resistant to conventional biological and physicochemical treatment, contributing to their widespread occurrence in surface waters, groundwater, wastewater, drinking-water sources, and biosolids[3–5]. Increasing evidence of ecological and human health risks has accelerated regulatory attention and shifted research priorities from occurrence toward understanding PFAS behavior in engineered treatment systems[6,7]. However, PFAS fate remains difficult to predict owing to differences in perfluoroalkyl chain length, functional groups, ionization state, and precursor chemistry, which govern their interactions with biological matrices, organic matter, mineral surfaces, and treatment environments[1,8,9]. Moreover, many treatment processes primarily redistribute or temporarily retain PFAS among aqueous, solid, and biological phases rather than achieve complete destruction[10,11].

PFAS encompass chemically diverse compounds that differ in perfluoroalkyl structure, functional groups, ionization behavior, and precursor characteristics. Although this review considers terminal perfluoroalkyl acids (PFAAs), including perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), the framework also acknowledges less extensively studied PFAS classes, including perfluoroalkane sulfonamides (FASAs) and fluorotelomer ethoxylates (FTEOs). FASAs exhibit compound- and pH-dependent ionization behavior and may occur as neutral, anionic, cationic, or zwitterionic species depending on their molecular structure and functional groups. These characteristics contribute to compound-specific sorption and microbial transformation behavior[12,13]. FTEOs are fluorinated ethoxylate surfactants that have been detected in indoor dust and industrial wastewater, indicating their environmental occurrence and relevance[13]. Experimental research has demonstrated that FTEOs can undergo biotransformation to fluorotelomer ethoxylate carboxylates, although the formation of PFAAs observed in one study was attributed to residual fluorotelomer alcohols in the commercial mixture[14,15]. These differences in charge state, molecular structure, and functional groups may influence PFAS sorption, electrostatic interactions, and biological accessibility in environmental matrices. Accordingly, PFAS interactions with EPS components, microbial surfaces, and aqueous microdomains cannot be inferred solely from the behavior of conventional anionic PFAAs. However, the extent to which these compound-specific properties regulate transport, partitioning, and transformation within intact engineered biofilms remains insufficiently resolved.

Within this context, water and wastewater treatment systems represent important control points for PFAS retention, transformation, and redistribution throughout the urban water cycle[16,17]. Biofilm-based systems are

[1]Department of Environment and Energy, Center for Earth and Environment Research, Sejong University, Seoul, South Korea. [2]Department of Convergent Environmental Science, Dongguk University-Seoul, Seoul, South Korea. [3]Department of Biochemistry, Cellular and Molecular Biology, University of Tennessee, Knoxville, TN, USA. [4]These authors contributed equally: Rabia Zafar, Shahzeb Tariq. ✉e-mail: kkt38@dongguk.edu; jinhur@sejong.ac.kr

particularly relevant owing to their high biomass retention and operational stability, creating spatially heterogeneous environments that regulate contaminant transport and microbial exposure[18–20]. Unlike suspended biomass, engineered biofilms consist of structured microbial communities embedded within an extracellular polymeric substance (EPS) matrix, where interactions among bulk water, EPS, microbial surfaces, and internal aqueous domains can influence contaminant accessibility and fate[21–23]. EPS contains proteins, polysaccharides, lipids, extracellular DNA, and humic-like substances that provide chemically diverse microenvironments within the biofilm matrix[20,24,25]. These matrix components may influence PFAS partitioning, diffusion, and accessibility, although the extent and direction of these interactions depend on PFAS molecular characteristics and biofilm conditions.

Previous studies of PFAS in treatment systems have examined aqueous attenuation, adsorption, precursor biotransformation, and accumulation in biomass or biosolids[26,27]. Recent reviews have similarly addressed PFAS occurrence and fate in wastewater treatment plants, precursor transformation, biological treatment, and sludge or biosolids management[28,29]. A comparison of representative reviews and their major research emphasis is provided in Table S1. However, these existing studies often investigate PFAS sorption, transformation, or biological effects separately, whereas the mechanisms linking PFAS molecular properties with the spatial organization of intact engineered biofilms, EPS-mediated partitioning, microbial accessibility, and reactor-level transport remain insufficiently established. This gap is particularly important because the mechanisms governing PFAS fate may differ according to molecular characteristics, including chain length, functional groups, and whether compounds are terminal PFAAs or transformable precursors. Biological transformation is primarily relevant to selected polyfluorinated precursors, whereas terminal PFAAs are highly resistant to microbial biotransformation[30,31]. In contrast, chain length can influence phase distribution and retention, with long-chain PFAS generally showing greater association with biomass and sludge, whereas short-chain PFAS tend to remain more mobile in the aqueous phase and are more frequently discharged with treated effluent. Thus, the central question is not whether biofilms universally remove PFAS but under which physicochemical and biological conditions they meaningfully alter PFAS distribution, precursor transformation, persistence, and subsequent release.

Accordingly, this review critically evaluates the conditions under which engineered biofilms influence PFAS fate and distinguishes among mechanisms governing temporary retention, aqueous transport, precursor transformation, redistribution, and remobilization. Rather than treating PFAS as a chemically uniform contaminant class or assuming that biofilms universally promote PFAS removal, the review links PFAS molecular properties with biofilm characteristics and reactor conditions to identify when biological systems meaningfully influence PFAS fate and when their influence is limited. The review therefore aims to: (1) integrate PFAS transport, compartment-specific partitioning, transformation, remobilization, and mass-balance behavior within engineered biofilms; (2) evaluate biofilm responses to PFAS exposure, including cellular stress, EPS-mediated adaptation, microbial community restructuring, and implications for process stability; and (3) identify major knowledge gaps and measurement needs required to establish a PFAS-specific, spatially and temporally resolved understanding of PFAS fate in biofilm-based water and wastewater treatment systems.

Review scope, literature search, and conceptual boundaries

This review focuses on PFAS fate and biological responses in engineered-biofilm-based water and wastewater treatment systems. These include biological activated-carbon (BAC) filters, moving bed biofilm reactors (MBBRs), membrane bioreactors (MBRs), membrane-aerated biofilm reactors (MABRs), biological aerated filters (BAFs), and related attached-growth processes. Studies involving activated sludge, biosolids, extracted EPS, microbial consortia, and other biological or abiotic matrices were included when they provided mechanistic evidence relevant to PFAS interactions that may occur in engineered biofilms. Evidence from these systems was used to inform broader mechanisms, such as PFAS–biomass and PFAS–EPS interactions, precursor transformation, and cellular responses, rather than as direct evidence of compartment-specific behavior in intact biofilms. In contrast, conclusions that depend specifically on attached-growth architecture were interpreted only where biofilm-specific evidence was available. These concern spatial transport, internal redox stratification, compartment-specific accessibility, and detachment or remobilization.

The applicability and transferability of these mechanisms across engineered-biofilm configurations are also expected to depend on the system. BAC filters, MBBRs, MBRs, MABRs, and BAFs differ in biofilm thickness, hydrodynamic shear, biomass retention, and oxygen transfer, which can influence diffusion pathways, EPS organization, and the development of spatial redox gradients. Therefore, the mechanistic relationships discussed in this review are not assumed to apply uniformly across reactor types. Instead, PFAS partitioning, accessibility, transformation, retention, and remobilization are interpreted in the context of the specific biofilm architecture and operating conditions. Where direct evidence for a particular reactor configuration is unavailable or limited, the corresponding mechanism is treated as indirect evidence or mechanistic inference rather than a universally applicable process. Studies focused primarily on destructive PFAS treatment or lacking relevance to biological or biofilm-mediated processes were excluded. To formalize this distinction, the evidence considered in this review was categorized as direct evidence, indirect evidence, or mechanistic inference, as summarized in Table S2. Accordingly, the conclusions of this review are intended to provide a PFAS-specific mechanistic framework rather than system-independent predictions across reactor configurations.

A structured Scopus search was conducted for peer-reviewed literature published from 2016 through July 2026. The search used the Scopus TITLE-ABS-KEY fields and combined PFAS-related terms (PFAS, “per- and polyfluoroalkyl substance*”, perfluoroalkyl*, polyfluoroalkyl*, PFOA, PFOS, PFAA*, PFCA*, PFSA*, and fluorotelomer*) with biofilm and biological-treatment terms (biofilm*, “extracellular polymeric substance*”, EPS, “attached growth”, “attached-growth”, “activated sludge”, biosolid*, “biological wastewater treatment”, “biological water treatment”, “biological activated carbon”, biofiltration, “biofilm reactor*”, MBBR, MABR, “membrane bioreactor*”, and “biological aerated filter*”). Retrieved records were retained when they addressed PFAS in a relevant biological or biofilm-treatment context and provided information on PFAS partitioning, transport, retention, precursor transformation, or biological responses. Studies focused primarily on destructive PFAS treatment, or lacking relevance to biological or biofilm-mediated processes, were excluded. After deduplication and application of the predefined screening criteria, 356 records were retained as candidates for bibliometric analysis. The retained studies were organized into seven themes: (1) transport, retention, and redistribution; (2) EPS-mediated partitioning; (3) microbial responses and adaptation; (4) biofilm reactors and operations; (5) precursor transformation and fate; (6) predictive management and modeling; and (7) PFAS molecular determinants.

Quantitative synthesis was evaluated by endpoint, but cross-study pooling was generally not appropriate because studies differed in biological matrix, EPS extraction and sample preparation, biofilm growth and operating conditions, PFAS exposure and water chemistry, analytical and normalization procedures, and reporting of replication and uncertainty. These differences produced non-equivalent endpoints, such as distribution coefficients, retardation factors, removal percentages, biofilm concentrations, biological responses, and precursor-product yields, often without compatible variance estimates. Accordingly, quantitative values were retained on their original measurement bases rather than combined into pooled effects or formal heterogeneity statistics. A claim-level summary of the supporting evidence, quantitative contrasts, and conflicting or unresolved findings is provided in Table S3.

Figure S1 summarizes the resulting literature landscape. Keyword co-occurrence analysis showed that PFAS fate was most frequently linked with sorption, accumulation, biosolids, reactive oxygen species, and related biological processes (Fig. S1a). Thematic mapping further showed that transport/retention and EPS-mediated partitioning were the most extensively represented research areas, whereas studies integrating PFAS molecular properties, biofilm characteristics, biological responses, transformation, and predictive management were comparatively limited (Fig. S1b, d). Publication activity increased during the 2020-July 2026 period, shown in Fig. S1c; the underlying literature search and broader bibliometric analyses covered publications from 2016 through July 2026. This imbalance highlights the need for the biofilm-centered synthesis developed in the following sections. Aerobic granular sludge, wetland-substratum biofilms, and bioelectrochemical biofilms were not treated as core reactor categories in the present synthesis because the review was structured around the engineered water- and wastewater treatment configurations defined above. However, studies from these systems were retained where they provided mechanistic evidence relevant to the broader PFAS–biofilm framework.

Determinants of PFAS–biofilm interactions

Physicochemical properties governing PFAS interactions with engineered biofilms

PFAS interactions with engineered biofilms are governed jointly by molecular properties and the heterogeneous biofilm matrix, which comprises hydrated channels, EPS-rich domains, microbial surfaces, and redox-stratified microenvironments. Microsensor and imaging studies of mature biofilms have demonstrated spatial oxygen and redox gradients; however, these studies generally do not resolve PFAS concentrations or distributions across the corresponding biofilm regions[32,33]. Accordingly, the potential influence of redox stratification on PFAS accessibility, partitioning, and precursor transformation should be regarded as a mechanistic hypothesis rather than a directly established PFAS-specific process. Perfluoroalkyl chain length is a major determinant of PFAS partitioning, with long-chain PFAS generally exhibiting stronger hydrophobic and interfacial interactions and greater affinity for organic-rich matrices than short-chain homologs[34–36].

Evidence from sorption studies supports a greater association of long-chain PFAS with organic-rich and protein-containing matrices; however, direct evidence demonstrating preferential accumulation within hydrophobic EPS of intact engineered biofilms remains limited. Thus, their potentially greater retention within EPS-rich or cell-associated regions should be regarded as a mechanistic inference rather than an established biofilm-specific outcome. Short-chain PFAS are generally expected to remain more mobile because of their lower affinity for hydrophobic and interfacial domains. Functional head-group chemistry further influences hydration, charge interactions, and affinity for proteinaceous and mineral-associated domains[37,38]. The amphiphilic nature of PFAS also favors accumulation at aqueous-organic interfaces[1,39], potentially influencing exchange among EPS, microbial, and aqueous domains. For precursors, biotransformation can further alter hydrophobicity, charge, and mobility[15,40–42]. PFAS partitioning within biofilms should, therefore, be considered a condition-dependent process in which molecular structure, matrix composition, and biofilm architecture collectively influence retention and accessibility, while transformation and subsequent redistribution may occur for selected PFAS and precursors.

Structure and biochemical features of biofilms

The heterogeneous architecture of engineered biofilms creates distinct extracellular, cellular, and hydrated domains that regulate PFAS accessibility and partitioning. The EPS matrix represents the primary interaction interface and contains chemically diverse fractions rather than functioning as a uniform sorbent[43,44]. Protein-associated EPS domains may provide important PFAS interaction sites through hydrophobic and charge-dependent interactions (Fig. 1). Recent studies of activated sludge and extracted EPS implicate protein abundance, amide functionality, and protein conformation in PFOA/PFOS binding or redistribution[45,46]. In a direct model-component comparison, protein-loaded beads reduced free PFOA by 38.4% after 48 h, compared with 6.3% for cellulose-loaded beads at 1000 ng L^{-1}[47]. However, these studies use different experimental endpoints and do not establish a common protein-specific effect size in native intact engineered biofilms. Current evidence therefore supports protein involvement in PFAS–EPS interactions, but not a universal protein-over-polysaccharide preference.

These observations provide evidence for PFAS–protein interactions but should not be interpreted as depth-resolved evidence of PFOS/PFOA localization within intact reactor biofilms. In contrast, polysaccharides mainly maintain hydration and diffusion pathways, potentially facilitating the transport of more mobile short-chain PFAS through the biofilm matrix[48,49]. The possibility that polysaccharide-rich, hydrated domains facilitate transport of more mobile short-chain PFAS is therefore a mechanistic inference rather than a directly demonstrated depth-resolved transport pathway. Thus, EPS composition may influence PFAS distribution and accessibility within biofilms, although the magnitude and direction of these effects depend on PFAS molecular characteristics and the structural properties of the biofilm matrix.

Microbial cells form a second major interaction domain, where PFAS association can trigger biological responses or enable precursor transformation. Cell surfaces contain membrane lipids, transport proteins, and extracellular enzymes that determine accessibility of contaminants to metabolically active microorganisms[12,39]. Although terminal PFAAs such as PFOS and PFOA are highly resistant to biodegradation, cellular exposure can induce oxidative stress, membrane damage, and compensatory EPS production, with stronger responses often reported for PFOS than for PFOA[50,51]. By contrast, polyfluoroalkyl precursors may undergo enzymatic transformation after reaching metabolically active biofilm regions, where they can be converted sequentially to terminal PFAAs[52]. These observations indicate that microbial accessibility is relevant to biological responses and precursor transformation, but they do not establish that greater biofilm-associated PFAS accumulation necessarily results in greater transformation.

Interconnected mechanisms governing PFAS–biofilm interactions

PFAS fate within engineered biofilms involves interconnected processes rather than a universally validated sequence from the biofilm-water interface to EPS, microbial cells, and subsequent transformation or redistribution (Fig. 1). These processes occur across heterogeneous biofilm compartments and collectively determine PFAS retention and persistence. Initial interactions occur at the biofilm-water interface, where EPS provides the primary extracellular interaction domain. Whole-sludge measurements generally show stronger PFOS than PFOA partitioning, although the magnitude is matrix- and process-dependent. Recent primary studies reported higher PFOS K_d values across four sludge conditions from two WWTPs, six thermophilic anaerobic-digestion conditions, and EBPR sludge[53–55]. A recent synthesis across nine WWTPs similarly reported PFOS K_d values exceeding PFOA in 13 of 14 wastewater-solid/process conditions, with K_d ranges of 142–29,429 L kg^{-1} for PFOS and 19–949 L kg^{-1} for PFOA; the only reversed condition occurred in anaerobic sludge[56]. These observations support a recurrent, but not universal, tendency toward stronger PFOS partitioning in wastewater solids. Protein-associated EPS interactions are considered separately because isolated EPS represents a different measurement endpoint. The study-specific K_d values, matched PFOS-PFOA comparisons, and corresponding methodological limitations are summarized in Table S3.

Stronger sorption of sulfonates than comparable carboxylates further highlights the role of head-group chemistry[57,58]. However, these observations primarily provide evidence for PFAS partitioning in bulk treatment-associated solids or isolated EPS fractions and should not be interpreted as direct validation of a sequential PFAS pathway through intact biofilm compartments. Within biofilms, PFAS mobility can be influenced by diffusion, reversible partitioning, matrix porosity, and chemically

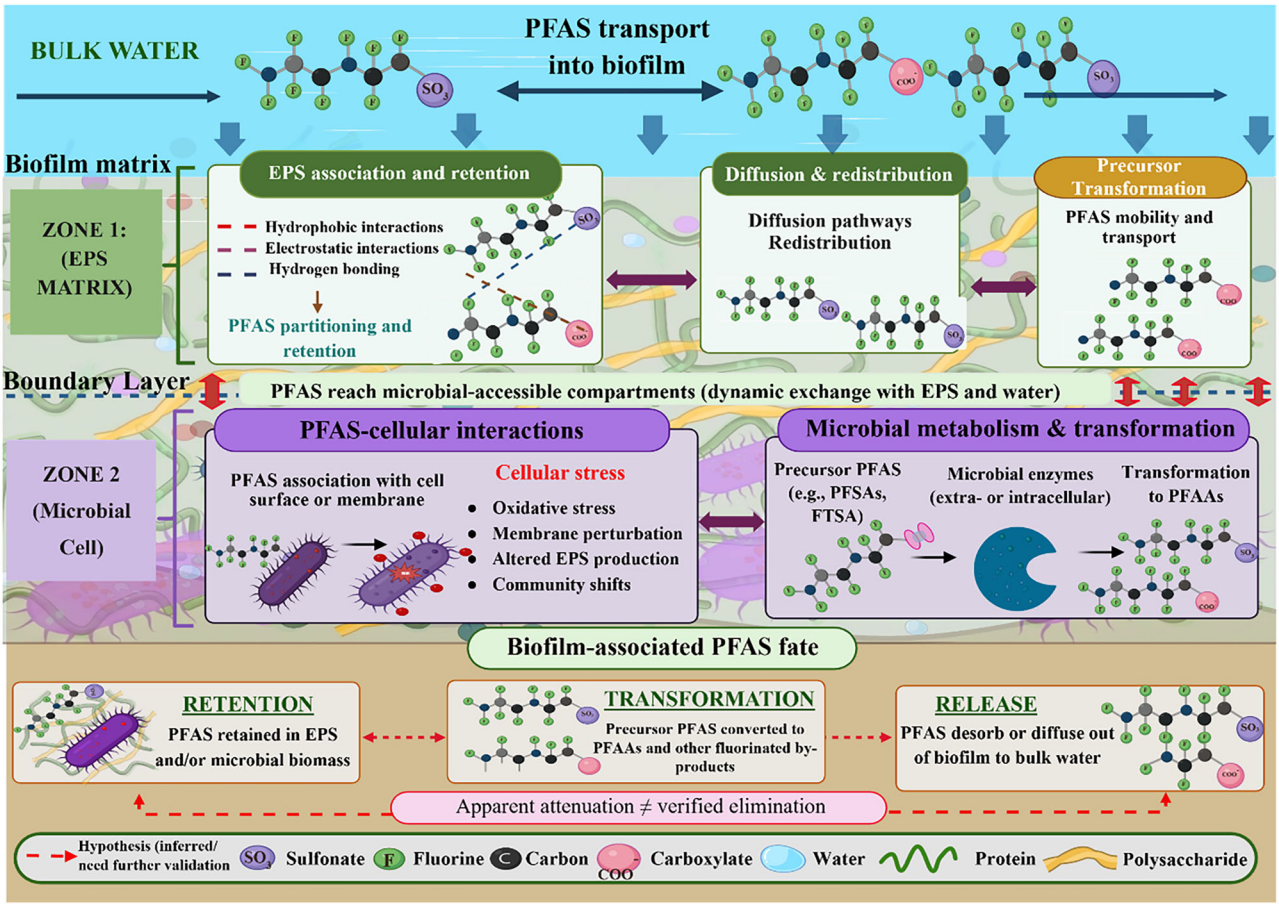


Fig. 1 | Sequential mechanisms governing PFAS fate within engineered biofilms. PFAS entering from the bulk water first encounter the biofilm-water interface and the extracellular polymeric substance (EPS) matrix (Zone 1), where protein-rich and polysaccharide-rich EPS regulate PFAS partitioning, retention, diffusion, and redistribution through hydrophobic, electrostatic, and hydrogen-bonding interactions, as well as through matrix porosity and hydration. PFAS that reach microbial-accessible compartments subsequently interact with microbial cells (Zone 2), potentially inducing cellular stress, oxidative stress, membrane perturbation, altered EPS production, and community-level responses. PFAS precursors may also undergo microbial transformation through extracellular or intracellular enzymatic processes, generating PFAAs and other fluorinated intermediates. These coupled processes ultimately determine three major biofilm-associated PFAS outcomes: retention, transformation, and release/remobilization to the bulk water.

heterogeneous EPS domains[21,59,60]. Short-chain PFAS may remain comparatively mobile because of their generally weaker association with organic-rich phases, whereas longer-chain compounds can exhibit greater association with biological or organic matrices[16,56,61]. The resulting exchange among aqueous and biofilm-associated domains is therefore expected to depend on PFAS chemistry and local biofilm conditions. PFAS interactions with microbial-accessible regions may influence cellular exposure and, for selected polyfluorinated precursors, subsequent transformation[50,62]. In contrast, polyfluoroalkyl precursors may undergo enzymatic transformation to shorter-chain PFAAs and other fluorinated intermediates when they become accessible to metabolically active microorganisms[63,64]. However, these processes are not necessarily sequential or mutually dependent: PFAS may remain associated with extracellular domains without reaching microbial cells. The framework presented here should therefore be interpreted as a conceptual map of potentially interacting PFAS–biofilm processes rather than a validated compartment-resolved sequence. Direct evidence linking PFAS localization across EPS, microbial, and aqueous biofilm compartments with subsequent transformation or redistribution remains limited. However, the simultaneous measurement of oxygen or redox conditions with depth-resolved PFAS distributions and transformation products remains necessary to establish whether these gradients directly regulate PFAS fate within intact biofilms.

PFAS-relevant microbial traits and biofilm function

Microbial traits may influence whether PFAS exposure results primarily in cellular stress, adaptation, or precursor transformation. Oxidative-stress tolerance and associated cellular defense mechanisms may support the maintenance of metabolic activity under PFAS exposure, while membrane remodeling may alter cell-surface properties and interactions with amphiphilic PFAS[65,66]. At the biofilm scale, changes in EPS composition, including its protein content, may modify PFAS–matrix interactions and potentially influence PFAS partitioning and microbial accessibility[12]; however, the contribution of specific EPS components remains insufficiently resolved. For polyfluorinated precursors, the presence and activity of relevant transforming enzymes represent additional factors that may influence biological transformation and product formation[67,68]. These findings provide a potential mechanistic link between PFAS molecular properties, microbial accessibility, and biological responses. However, direct evidence connecting specific microbial traits with PFAS fate in intact engineered biofilms remains limited, and many proposed relationships are inferred from studies of isolated microorganisms, extracted EPS, suspended biomass, or related environmental systems.

Environmental and operational factors modulating PFAS–biofilm interactions

Water chemistry and coexisting solutes. Water chemistry modulates PFAS partitioning within engineered biofilms by altering EPS

conformation and the intermolecular interactions that govern contaminant accessibility. Because most environmentally relevant PFAAs remain predominantly anionic near circumneutral pH, pH effects are expected to arise mainly from changes in EPS charge and binding-site accessibility rather than PFAS speciation. Protonated functional groups, extracellular proteins, mineral-associated domains, and multivalent cations can create localized interaction sites that reduce electrostatic repulsion and promote PFAS association[69,70]. Increasing ionic strength and the presence of Ca^{2+} or Mg^{2+} can further enhance sorption through electrical double-layer compression and cation bridging[71,72]. However, these effects are compound-dependent and appear particularly relevant to long-chain PFAS, including sulfonates, for which hydrophobic and interfacial interactions can contribute substantially to association with organic-rich matrices[73]. They should therefore not be generalized as a dominant mechanism for more mobile short-chain compounds such as GenX or PFBS.

Beyond electrostatic screening, multivalent cations may participate in specific interactions between anionic PFAS headgroups and negatively charged functional groups in environmental matrices. For example, Ca^{2+} and Fe^{3+} have been reported to enhance the sorption of perfluoroalkyl carboxylic acids in soils, with the results attributed in part to cation-bridging interactions between PFAS and negatively charged sorption domains[74]. Metal cations can also bind to carboxyl, phosphoryl, hydroxyl, and other functional groups within EPS, thereby modifying its surface charge and chemical accessibility[75]. In principle, these interactions may influence PFAS partitioning within EPS-rich biofilm regions by altering local electrostatic conditions, interfacial association, and the availability of binding domains. However, the direct contribution of Fe(III)- or Cu(II)-mediated bridging to PFAS retention and intra-biofilm mass transfer has not been sufficiently established in intact engineered biofilms. Evidence from soils, mineral surfaces, and metal-EPS studies[76,77] should therefore be interpreted as mechanistic support rather than direct confirmation of the same process within engineered-biofilm matrices.

Importantly, the effects of metal-mediated interactions are not necessarily consistent across PFAS compounds or environmental conditions. They may vary with the PFAS headgroup and chain length, pH, metal identity and concentration, and the properties of the interacting surface. For example, a recent surface-complexation study reported that Cu(II), Cd(II), and Fe(II) produced PFAS-specific effects on the adsorption of PFOS, PFDA, PFOA, and GenX, indicating that metal-mediated interactions do not uniformly enhance PFAS sorption[76]. Accordingly, increased metal concentrations should not automatically be interpreted as promoting PFAS retention, as competition, complexation, surface charge, and organic-matter interactions may produce compound-specific outcomes. Whether these interactions subsequently influence PFAS diffusion, membrane permeation, intracellular exposure, or protein-binding processes within engineered biofilms remains unresolved and requires direct investigation.

Temperature may also affect diffusion, EPS dynamics, and microbial activity, although PFAS biotransformation appears to be controlled primarily by precursor structure, enzyme specificity, and microbial metabolism[78]. Coexisting organic constituents can further modify PFAS partitioning. NOM/DOM and soluble microbial products compete for EPS binding domains while altering matrix hydration and organization. Humic substances can reduce PFAS sorption through competitive site occupation, whereas DOM can shift PFAS distribution between aqueous and solid phases depending on chain length and functional chemistry[79,80]. Protein-rich microbial products may provide additional hydrophobic interaction domains for long-chain PFAS, while shorter-chain compounds such as GenX and PFBS remain more mobile because of their weaker affinity for organic matrices[81,82]. Overall, water chemistry should be viewed as a compound- and matrix-dependent modulator of PFAS–biofilm interactions rather than as a universal mechanism that produces the same response across PFAS classes.

Biofilm development as a modulator of PFAS–biofilm interaction

Biofilm properties evolve during reactor operation through biomass accumulation, EPS production, structural reorganization, and microbial succession. These changes are commonly described by biofilm thickness, biomass, EPS composition, structural heterogeneity, transport characteristics, and community composition[21,48,83]. Since these properties regulate diffusion and interaction domains, PFAS partitioning may change during biofilm development rather than remain fixed[22,25,84,85].

Increasing EPS accumulation may increase the opportunity for association of long-chain PFAS with protein-rich extracellular domains, whereas short-chain PFAS are expected to remain comparatively mobile[17,86]. Importantly, the relationship between total biofilm-associated PFAS and cellular exposure remains unresolved. Extracellular retention could reduce cellular accessibility if binding or diffusion limits transport, but paired measurements of total biofilm PFAS, cell-associated PFAS, and microbial responses are needed to test this mechanism. Continuous changes in biomass, EPS turnover, porosity, and detachment may therefore alter PFAS distribution among extracellular, cellular, and aqueous compartments[87,88]. Supporting this concept, a study reported that progressive biofilm development in a full-scale biological activated-carbon filter reduced PFAS adsorption by limiting access to internal sorption sites[56]. This plant-scale observation demonstrates that biofilm development can modify PFAS-associated sorption behavior, but it does not by itself establish a universal relationship between biofilm age and PFAS retention or transformation. Evidence directly linking longitudinal changes in biofilm structure to PFAS retention, transformation, or remobilization remains scarce. In particular, PFAS releases following biofilm detachment or other structural disturbances should be regarded as a potential pathway unless directly demonstrated through paired measurements of biofilm-associated and aqueous PFAS inventories.

Operational driver shaping PFAS behavior in engineered-biofilm systems

Reactor operation indirectly regulates PFAS behavior by controlling biofilm architecture, mass transfer, and contaminant-biomass contact (Table 1). Hydrodynamic conditions strongly affect biofilm attachment, growth, detachment, and transport processes[59,89]. Low shear generally favors thicker, EPS-rich biofilms, whereas higher shear promotes compaction and biomass turnover[90], potentially altering PFAS diffusion, partitioning, and accessibility[91]. Similar effects are expected in MBBRs, where carrier-associated biofilms develop heterogeneous physicochemical gradients[92], although their specific influence on PFAS fate remains poorly resolved.

Solids retention time provides another operational control by regulating biomass retention and contaminant contact time. Long-SRT systems such as MBRs may enhance the association of hydrophobic and long-chain PFAS with biological solids[93,94]. Likewise, BAC filters combine activated-carbon sorption with biofilm development, creating coupled physicochemical and biological domains that can influence PFAS retention and precursor transformation[95,96]. These systems should nevertheless be distinguished from conventional biofilm reactors because activated carbon provides an additional sorption phase that can independently influence PFAS distribution. Evidence from biological-treatment systems indicates that PFAS exposure can affect reactor-level treatment performance, although the magnitude and direction of these effects vary with PFAS identity, exposure concentration, and reactor configuration. In a sequencing batch biofilm reactor (SBBR), exposure to GenX and PFHxA at 100 µg/L was associated with decreases of approximately 28% in ammonium-nitrogen removal, 10% in COD removal, and 1% in nitrate-nitrogen removal[97]. The study also reported changes in predicted functional-gene abundances and PFAS-associated EPS secretion, indicating that microbial responses and nitrogen-conversion pathways may not be affected uniformly. Similarly, in a suspended-growth sequencing batch reactor, PFOS exposure at 100 mg/L reduced COD removal from 92% to 23% and ammonium-nitrogen removal from 98% to 35% after 20 days, accompanied by increased EPS and soluble microbial product production, sludge bulking,

Table 1 | Operational drivers shaping biofilm-mediated PFAS fate in engineered treatment systems

Operational driver	Biofilm response	Potential PFAS fate implication	PFAS-specific Evidence status	Representative references
Hydrodynamic shear/mixing	Biofilm thickness; density; EPS; detachment; mass transfer	PFAS transport and accessibility; potential redistribution with biofilm detachment	PFAS-specific outcome not directly established; implication primarily inferred from biofilm behavior.	24,89,90,195
Hydraulic retention time (HRT)	Contact time; substrate exposure; microbial activity	PFAS–biofilm interaction; potential opportunity for precursor transformation	Direct linkage between HRT and PFAS fate in intact biofilms remains insufficiently demonstrated.	2,93,94
Solids retention time (SRT)/ biomass age	Biomass retention; succession; EPS maturation; functional populations	PFAS retention; potential influence on precursor transformation	PFAS-specific relationship with biomass age or SRT requires further validation.	21,25,111
Reactor configuration	Support material; biofilm distribution; biomass accumulation; redox zones	PFAS partitioning; transport; retention; potential differences	PFAS-specific evidence is configuration-dependent and remains limited.	18,20,95,96
Backwashing/air scouring	EPS disruption; detachment; biomass loss; restructuring	PFAS redistribution; potential remobilization during detachment	PFAS release has been reported in specific treatment contexts; biofilm-specific remobilization requires direct validation.	99,100
Substrate/nutrient availability	Microbial activity; EPS production; community composition; redox gradients	PFAS accessibility; potential precursor transformation	PFAS-specific outcome not directly established; implication primarily mechanistic.	23,149,196

The table summarizes how reactor operating conditions influence biofilm structure and function and identifies their potential implications for PFAS transport, partitioning, retention, precursor transformation, and release. The PFAS-specific evidence status is reported separately to distinguish experimentally demonstrated outcomes from mechanistic inferences and research needs.

and impaired settling performance[98]. However, the high PFOS concentration and suspended activated-sludge configuration limit the direct extrapolation of these findings to environmentally relevant exposure conditions and intact biofilm systems. These findings suggest that PFAS exposure can produce measurable changes in organic-matter and nitrogen-removal performance, but such effects cannot be inferred solely from changes in EPS production, microbial community composition, oxidative-stress indicators, or predicted functional genes. Accordingly, assessments of PFAS impacts on biological treatment should integrate reactor-level measurements of COD or TOC removal, ammonium oxidation, nitrate/nitrite conversion, and treatment recovery following exposure or operational disturbance to determine whether biological changes translate into impaired process performance or resilience.

Operational disturbances, including backwashing, membrane cleaning, air scouring, hydraulic fluctuations, and biomass wasting, can periodically restructure biofilms and alter the release or redistribution of retained PFAS[99,100]. Such disturbances provide plausible pathways for redistribution or release of biofilm-associated PFAS, but their contribution to PFAS remobilization should be considered evidence-dependent. Where backwashing, wasting, detachment, or PFAS concentration changes have been directly measured, these observations can support a remobilization pathway; otherwise, release should be treated as a testable hypothesis rather than an established consequence of biofilm disturbance. Reactor operation should therefore be considered a key regulator of PFAS accessibility and redistribution within biofilm systems rather than merely a characteristic of treatment configuration.

PFAS fate within biofilm-based water and wastewater treatment systems

Physicochemical regulation of PFAS partitioning, retention, and reservoir formation

PFAS retention within engineered biofilms is governed by dynamic partitioning among EPS, microbial biomass, and hydrated aqueous microdomains. The EPS matrix represents a major extracellular interaction domain because of its abundance and chemical heterogeneity, with variations in EPS composition influencing PFAS retention during biological treatment[88,101,102]. However, EPS-associated PFAS are not permanently immobilized. Reversible sorption, desorption, and diffusion continuously redistribute PFAS among extracellular polymers, microbial cells, and aqueous pores[103,104]. This partitioning evolves as biofilm structure and chemistry change during reactor operation. Coexisting organic matter can reduce PFAS retention by competing for hydrophobic interaction sites and modifying matrix physicochemistry[5,105]. Thus, PFAS retention reflects not only compound-specific affinity but also the changing accessibility of interaction domains within the biofilm.

Continuous redistribution can convert transient sequestration into a dynamic PFAS reservoir. Residence time within individual biofilm compartments determines whether retained PFAS remain extracellularly sequestered, become accessible to microorganisms, or are remobilized to the bulk water[106,107]. Biofilm development can further modify these processes by changing internal transport pathways and access to sorption domains. For example, biomass and EPS accumulation in biological activated carbon reduced PFAS access to internal adsorption sites, while biofilm growth altered contaminant transport within biologically active filtration media[56,108]. Accordingly, biofilm-associated PFAS should be considered a dynamic contaminant inventory rather than a permanently removed fraction[27,109]. Decreases in aqueous PFAS concentrations may therefore represent temporary retention rather than irreversible removal[4,110]. This reservoir perspective provides an important mechanistic link between physicochemical partitioning, microbial accessibility, precursor transformation, and subsequent remobilization under changing operational conditions.

Biofilm-mediated microbial transformation of PFAS

Transformation of polyfluorinated precursors is expected to depend on the fraction that reaches metabolically active microorganisms, but the

relationship between this accessible fraction and total biofilm-associated inventory has not been quantified. Although PFAS may accumulate within EPS-rich extracellular domains, only compounds reaching metabolically active microorganisms are available for enzymatic transformation[26,111–113]. Biofilm heterogeneity, therefore, creates a potential disconnect between contaminant retention and transformation because EPS sequestration and diffusion limitations can restrict cellular accessibility[114,115].

Transformation potential is strongly constrained by PFAS structure. Polyfluorinated precursors are the principal PFAS class for which microbial transformation is well documented. Fluorotelomer compounds, polyfluoroalkyl phosphate esters, and fluorinated amides contain functional groups that can undergo hydrolysis, oxidation, reduction, or other biotransformation reactions before complete removal of fluorine from the molecule[2,116,117]. These reactions can produce intermediate and terminal perfluoroalkyl products with different physicochemical properties and environmental behavior. This precursor transformation should be distinguished from defluorination of terminal PFAAs. Terminal PFAAs contain highly stable perfluoroalkyl structures, and their biological transformation generally requires cleavage of strong C–F bonds. Evidence for substantial microbial defluorination of terminal PFAAs under environmentally relevant conditions remains limited[60,118]. Engineered biofilms may nevertheless provide conditions conducive to precursor transformation through long biomass retention, microbial consortia, redox stratification, and retention of extracellular enzymes and co-metabolic substrates[119–121]. These features may support sequential and redox-dependent transformations, although direct evidence demonstrating that intact engineered biofilms enhance PFAS transformation relative to suspended-growth systems remains limited[111,112].

Importantly, enhanced precursor transformation should not be equated with defluorination or destruction. Transformation may simply redistribute fluorinated material among intermediate and terminal products without substantially reducing the total fluorine or PFAS-derived fluorinated inventory. Extensive defluorination remains uncommon because effective C–F cleavage requires the simultaneous availability of suitable substrates, enzymes, microorganisms, redox conditions, electron donors, and favorable thermodynamics[53,122]. Thus, longer residence time, higher biomass, or greater microbial diversity should not be interpreted as direct evidence of enhanced defluorination[123,124]. Sustained PFAS defluorination has been demonstrated mainly for selected compounds under specialized laboratory conditions, whereas substantial mineralization of terminal PFAS has not been established in full-scale biofilm systems[29,105]. Transformation can also alter subsequent PFAS fate by generating products with different partitioning and mobility characteristics[125,126]. PFAS transformation should therefore be evaluated according to the specific transformation step and product formed, with precursor biotransformation distinguished from verified defluorination and mineralization.

Remobilization and secondary release

Biofilm-associated PFAS reservoirs should not be interpreted as permanently removed contaminant pools. Engineered biofilms undergo continuous biomass turnover, EPS renewal, detachment, and structural reorganization during reactor operation[80,127,128]. PFAS retained within these matrices are therefore better viewed as dynamic inventories whose stability depends on both physicochemical partitioning and biofilm evolution. As PFAS retention is governed largely by hydrophobic, electrostatic, and interfacial interactions, changes in pH, ionic strength, dissolved organic matter, competing solutes, and redox conditions can shift partitioning between aqueous and biofilm-associated phases[79,80,103]. Biofilm turnover and operational disturbances, including backwashing, hydraulic flushing, air scouring, media cleaning, and biomass wasting, may further destabilize retained PFAS by altering EPS organization and internal transport pathways[114,129,130]. However, direct evidence linking these processes to PFAS remobilization remains limited. Most existing studies quantify PFAS in influent, effluent, sludge, or biosolids[2,111,120], whereas compartment-specific inventories, reservoir stability, and release kinetics within biofilms remain poorly resolved[29,131]. Future studies should therefore combine stable-isotope tracing, spatially resolved PFAS analysis, long-term reactor monitoring, and mechanistic mass balances to distinguish temporary retention from persistent sequestration and to determine when engineered biofilms act as stable sinks or sources of secondary PFAS release.

Proposed biofilm-centered PFAS mass balance

Conventional assessments of PFAS removal generally rely on influent–effluent concentration differences. Although these measurements provide information about net aqueous attenuation, they do not independently resolve PFAS redistribution among the aqueous phase, attached biomass, EPS, carrier material, suspended solids, and other downstream compartments[26,110]. Plant-scale mass-balance studies demonstrate the importance of considering these compartments. For example, following an aqueous film-forming foam (AFFF) release, effluent discharge and biosolids accounted for approximately 55% and 45%, respectively, of the estimated PFOS outputs, illustrating that apparent treatment-associated attenuation may reflect phase redistribution rather than chemical destruction[132].

This limitation is particularly relevant to engineered-biofilm systems, where EPS, microbial biomass, and biofilm architecture may regulate PFAS transport, partitioning, accessibility, transformation, and release[19,102,133]. Therefore, a biofilm-centered mass balance should be established with an explicitly defined system boundary and a clearly specified analytical target. The target may be an individual PFAS, a defined group of compounds, targeted PFAS together with measured precursors, extractable organic fluorine (EOF), or a broader fluorine-containing inventory. These targets are not interchangeable because a decrease in one PFAS species may result from transformation into another fluorinated compound rather than from the removal of fluorine from the system.

A mass balance for an individual PFAS species i within the defined biofilm-associated compartment can be expressed as:

$$\frac{dM_{BF,i}}{dt} = J_{in,i} - J_{out,i} + J_{Formation,i} - J_{consumption,i} \quad (1)$$

Here M_i represents the inventory of PFAS species iii within the defined biofilm-associated compartment; $J_{\mathrm{net,i}}$ the net physical flux of species iii across the compartment boundary, calculated as the difference between incoming and outgoing fluxes. The transformation terms, $J_{\mathrm{Formation,i}}$ and $J_{\mathrm{consumption}}$, represent the formation and depletion of species iii through precursor transformation or other chemical conversion processes. Accordingly, the net transformation contribution is defined as:

$$J_{TR,net,i} = J_{Formation,i} - J_{consumption,i} \quad (2)$$

In this equation, $J_{\mathrm{out,i}}$ includes physical release from the biofilm-associated compartment through desorption, diffusion, biofilm detachment, backwashing, biomass wasting, or other operational disturbances. Consequently, a separate remobilization term should not be added to Eq. (1) unless it is explicitly defined as a subset of $J_{\mathrm{out,i}}$. This formulation avoids double-counting physical release.

The mass-balance terms are influenced by PFAS molecular properties, biofilm structure and chemistry, microbial activity, water chemistry, and reactor operation. Equation (1) is intended as a conceptual and experimentally parameterizable framework rather than an equation that can be solved from aqueous concentration data alone. The measurement strategy must be adapted to the selected system boundary and analytical target.

Operational estimation of mass-balance terms

Gross aqueous input, system-level attenuation, and biofilm-specific transport

The gross aqueous mass input to the complete biofilm-containing system can be estimated from the influent flow rate and aqueous PFAS

concentration:

$$J_{in,system}(t) = Q(t)C_{influent}(t) \quad (3)$$

where $Q(t)$ is the influent flow rate (volume time^{-1}) and $C_{\text{influent}}(t)$ is the aqueous PFAS concentration in the influent (mass volume^{-1}). The corresponding aqueous output is:

$$J_{out,aqueous}(t) = Q(t)C_{effluent}(t) \quad (4)$$

For a system operating at a defined flow rate, the difference between the influent and effluent mass flow rates can be expressed as:

$$J_{net\ attenuation}(t) = Q(t)[C_{influent}(t) - C_{effluent}(t)] \quad (5)$$

Equation (5) provides a system-level net aqueous attenuation estimate. It should not be interpreted directly as the inward transport of PFAS into the biofilm because the observed influent–effluent difference may also include: (1) sorption to reactor walls, tubing, membranes, or carrier material; (2) association with attached and suspended solids; (3) accumulation in EPS or other non-biofilm compartments; (4) transformation of precursors into targeted or non-targeted PFAS; (5) sampling and analytical losses; and (6) unmeasured aqueous or solid-phase outputs[29,134]. Therefore:

$$J_{biofilm,in} \neq Q\left[C_{influent} - C_{effluent}\right] \quad (6)$$

Unless the contributions of other sinks, storage compartments, transformation processes, and outputs have been independently quantified or reasonably excluded, a biofilm-specific inward flux requires additional compartment-resolved measurements. These may include time-resolved quantification of PFAS in the bulk liquid, attached biofilm, EPS-associated fractions, carrier material, suspended solids, detached biomass, and other relevant compartments. The biofilm-associated inventory should be normalized to a defined basis, such as carrier mass, biofilm dry mass, reactor volume, surface area, or measured biofilm volume. For example, the concentration of PFAS associated with a harvested biofilm can be converted into an inventory using:

$$M_{BF,i} = C_{BF,i}m_{BF} \quad (7)$$

This distinction is important because PFAS mass-balance studies in wastewater systems have shown that target PFAS concentrations may increase across treatment even when bulk fluorine indicators remain relatively stable. For example, a study reported an increase in the summed concentration of 31 target PFAS from approximately 22–47 ng L^{-1} in influent to 140–213 ng L^{-1} in effluent, while EOF remained within a similar range of approximately 2.3–3.5 µg-F L^{-1}. The authors interpreted these results as consistent with the transformation of non-target PFAS precursors into measurable PFAS. This observation illustrates why influent–effluent target-PFAS differences should be interpreted together with precursor and fluorine measurements rather than as evidence of direct biofilm uptake or destruction[134].

Net transformation contribution ($J_{TR,\ net}$)

The transformation term represents the net formation or consumption of the tracked PFAS species through precursor transformation or other chemical transformations. Its sign depends on the PFAS species being tracked. For example, transformation of a precursor may decrease the precursor inventory while increasing the inventory of a terminal PFAA or another transformation product[26,116].

For an individual PFAS species:

$$J_{TR,net,i} = J_{Formation,i} - J_{consumption,i} \quad (8)$$

The formation and consumption terms cannot generally be estimated from targeted LC–MS/MS data alone because targeted methods cover only a predefined list of compounds. Transformation may generate shorter-chain PFAAs, intermediate products, and unidentified fluorinated compounds that are not included in the analytical method[11,54] For an individual PFAS species, transformation therefore represents a change in the inventory of that species and does not necessarily indicate loss of total fluorine from the system.

The estimation of $J_{\text{TR,net,i}}$ should therefore combine, where feasible, (1) targeted LC–MS/MS analysis of terminal PFAAs, known precursors, and transformation products; (2) assays for oxidizable precursors, such as total oxidizable precursor (TOP) analysis, where appropriate; (3) EOF analysis to quantify extractable organofluorine not represented by the targeted analyte list; (4) total fluorine or other fluorine mass measurements when analytically feasible; (5) time-resolved sampling of the aqueous phase and biofilm-associated compartments; and (6) abiotic and biological controls to distinguish transformation from sorption, desorption, and other non-biological processes[11,135].

EOF and total fluorine should not be treated as interchangeable measurements. EOF represents a defined extractable organofluorine fraction, whereas total fluorine measurements aim to capture a broader fluorine inventory depending on the analytical method and sample preparation. A wastewater-effluent study reported that a substantial fraction of measured EOF was not explained by the monitored PFAS analytes, illustrating the limitations of relying exclusively on targeted PFAS analysis[134].

A decrease in targeted PFAS concentrations accompanied by relatively stable EOF may be consistent with redistribution or transformation into non-target fluorinated pools. However, stable EOF alone cannot identify the transformation products, establish their location, or demonstrate that all fluorine remains within the defined system boundary. Evidence for net defluorination, therefore, requires complementary fluorine mass-balance measurements, adequate analytical recovery, and controls that account for unmeasured compartments and potential analytical losses[136]. For a total-fluorine inventory, internal transformation between fluorinated species should not be counted as fluorine loss because formation and consumption terms represent redistribution within the fluorinated inventory when the relevant fluorine-containing species and pools are captured within the defined system boundary. A total-fluorine balance for a clearly defined system can be expressed as:

$$\frac{dM_{F,total}}{dt} = J_{F,i} - J_{out,in} + J_{F,out} - J_{F,unaccounted} \quad (9)$$

where $M_{\text{F,total}}$ is the measured total-fluorine inventory within the defined system boundary, $J_{\text{F,in}}$ and $J_{\text{F,out}}$ are fluorine fluxes entering and leaving that boundary, and $J_{\text{F,unaccounted}}$ represents fluorine that is not accounted for within the measured inventory. Importantly, $J_{\text{F,unaccounted}}$ should not automatically be interpreted as verified defluorination. It may reflect analytical recovery limitations, unmeasured compartments, unidentified fluorinated compounds, or incomplete sampling. The term should only be assigned to chemical defluorination when supported by suitable evidence.

Physical release and remobilization

In Eq. (1), physical release from the biofilm-associated compartment is represented by $J_{\text{out,i}}$. For practical interpretation, this term may be separated into different release pathways:

$$J_{in,i} = J_{desorption,i} + J_{detachment,i} + J_{operational,i} - J_{other,i} \quad (10)$$

where the terms represent release through desorption or diffusion, biomass detachment, operational disturbances such as backwashing or wasting, and other measured or estimated pathways.

The release flux should not be assumed to be the thermodynamic reverse of the inward transport flux. PFAS interactions with EPS, biomass, carrier material, and other solid phases may involve kinetic limitations,

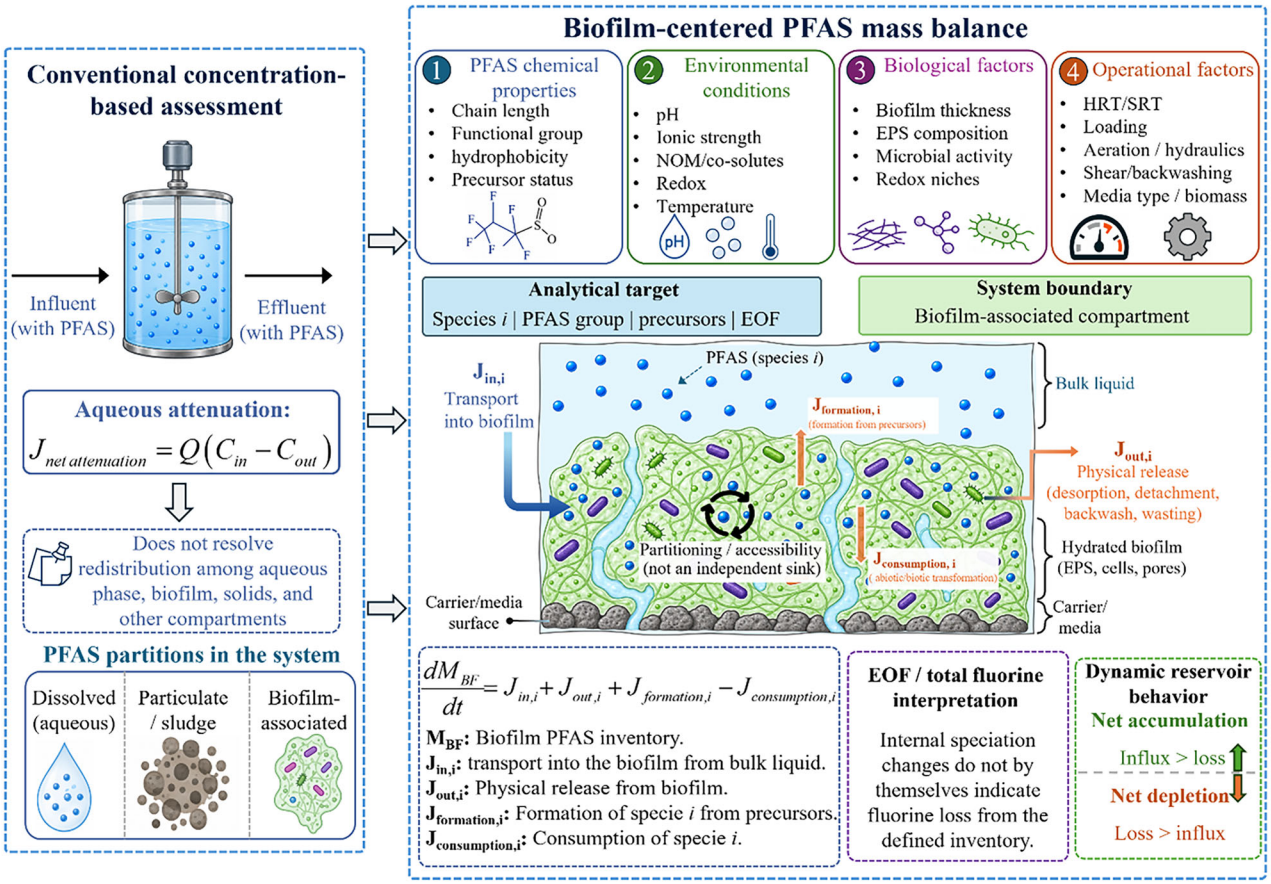


Fig. 2 | Conceptual comparison between conventional concentration-based PFAS assessment and a biofilm-centered PFAS mass-balance framework. Conventional assessment relies primarily on influent–effluent concentration differences to quantify apparent aqueous attenuation, without resolving PFAS redistribution among dissolved, particulate, and biofilm-associated pools. The proposed biofilm-centered framework integrates PFAS chemical properties, environmental conditions, biological factors, and reactor operating conditions to describe PFAS transport from the bulk liquid into the biofilm and subsequent partitioning, transformation/speciation shifts, and remobilization or release. The biofilm is conceptualized as a dynamic PFAS reservoir, in which PFAS inventory is governed by transport influx, net transformation, and remobilization/loss processes. Accordingly, apparent decreases in aqueous PFAS concentrations may reflect biofilm accumulation, partitioning, or transformation rather than complete PFAS removal, whereas remobilization, detachment, backwashing, or operational disturbances may contribute to secondary release.

heterogeneous binding environments, diffusion constraints, and adsorption–desorption hysteresis. PFAS release may also depend on biofilm age, EPS composition, matrix restructuring, contact time, concentration gradients, and operational conditions. The potential for PFAS release from loaded solid materials is influenced by water chemistry, ionic strength, pH, dissolved organic matter, and competitive interactions, although the magnitude and direction of these effects are compound- and matrix-dependent[29,137] (Fig. 2).

Practical estimation of the release terms may involve: (1) measuring PFAS concentrations in the liquid phase before, during, and after a controlled hydraulic or operational disturbance; (2) quantifying PFAS associated with detached biomass, wasted solids, and backwash material; (3) conducting time-resolved desorption experiments using intact biofilm material or representative carrier-associated biomass; (4) comparing PFAS release under different hydraulic conditions, ionic strengths, contact times, and water chemistries; and (5) fitting the resulting data using kinetic or non-equilibrium desorption models when sufficient observations are available.

A biphasic or multi-rate model may be required when an initially rapid release phase is followed by a slower release. However, fitted rate constants should be interpreted cautiously because they may represent combined effects of diffusion, site heterogeneity, matrix restructuring, and analytical extraction limitations rather than a single molecular desorption process.

Defining retention, transformation, and true loss

Temporary sequestration, precursor transformation, and true loss represent different outcomes and should be distinguished according to the tracked inventory and system boundary. Temporary sequestration refers to PFAS association with EPS, microbial biomass, carrier material, or other biofilm-associated compartments without evidence that the fluorinated structure has been destroyed. The compound may remain chemically intact and may subsequently be released through desorption, diffusion, detachment, or operational disturbance.

Precursor transformation refers to the conversion of one fluorinated compound into another. For example, a precursor may be converted into a terminal PFAA, a shorter-chain fluorinated product, or another intermediate[67,116]. This process changes the chemical identity and distribution of fluorinated compounds but does not necessarily decrease the total-fluorine inventory. If a precursor is converted into a terminal PFAA that remains within the biofilm-associated compartment, the process should be classified as precursor transformation accompanied by biofilm-associated retention, rather than true loss. Precursor concentration may decrease while the concentration of the terminal PFAA increases, and the fluorine remains within the defined system boundary.

True loss should be reserved for cases in which fluorine is demonstrated to leave the defined tracked inventory through a supported mechanism, such as verified defluorination, or where transfer to an

explicitly monitored external compartment is quantified. A decrease in aqueous concentration or targeted PFAS concentration alone is not sufficient to establish true loss[136].

Integrating biological accessibility into mass balance

Compartment-specific partitioning should not be included as an independent loss term when it only redistributes PFAS among EPS, microbial biomass, hydrated biofilm domains, and carrier-associated compartments within the defined inventory. Instead, partitioning may regulate residence time, diffusion, microbial exposure, transformation, and subsequent release. Biological accessibility can therefore be represented as a state-dependent regulating factor rather than as a separate sink:

$$J_{F,i} = f_{acc} J_{TR,\,potential} \quad (11)$$

where f_{acc} represents the fraction of the relevant PFAS inventory that is accessible to the biological or chemical process under prevailing conditions. This factor is conceptual or model-estimated unless supported by an independent experimental proxy. It should not be equated directly with the total amount of PFAS associated with the biofilm. Potential proxies include concentration-dependent transformation or uptake rates; desorption fractions; depth-resolved PFAS concentration measurements; diffusion or mass-transfer experiments; comparisons between intact and disrupted biofilm matrices; and measurements of local microbial activity or redox conditions. These proxies do not measure accessibility identically and should not be treated as interchangeable. Greater biofilm-associated PFAS should not be assumed to indicate greater microbial exposure or transformation. Whether total inventory tracks the biologically accessible fraction remains unresolved and requires paired compartment-specific PFAS and biological-response measurements.

Kinetics of remobilization and biofilm aging

Remobilization should be considered a time-dependent process rather than an instantaneous reversal of PFAS transport. PFAS may associate with biofilm matrices through multiple interaction environments with different release rates. Early-stage adsorption, diffusion into internal matrix regions, EPS restructuring, biofilm maturation, and changes in ionic composition may produce different desorption behavior over time. For example, a study observed an elevated PFAS release during the initial filtration period following BAC backwashing, directly demonstrating operationally induced remobilization[99].

Biofilm aging may alter the accessibility of PFAS binding sites, matrix porosity, and diffusion pathways. Similarly, hydraulic disturbances may increase release by detaching outer biofilm layers, restructuring the EPS matrix, or exposing previously inaccessible material. The direction and magnitude of these effects are expected to depend on PFAS chemistry, biofilm composition, operational conditions, and the timescale of the disturbance. A practical assessment should therefore distinguish between rapidly desorbed PFAS, slowly released or diffusion-limited PFAS, PFAS released with detached biomass, and PFAS remaining in the biofilm-associated inventory after the disturbance. Time-resolved desorption and disturbance experiments, combined with measurements of both aqueous and solid-associated PFAS, are needed to estimate these fractions. Where adsorption–desorption hysteresis is observed, release parameters should be estimated independently rather than calculated by assuming that the adsorption rate is directly reversible[137].

Overall, a biofilm-associated PFAS inventory should not be interpreted as equivalent to irreversible removal. Physical retention, precursor transformation, microbial accessibility, and remobilization may occur simultaneously. A decrease in a precursor may represent transformation into a terminal PFAA, while release from the biofilm may transfer PFAS to another reactor or downstream compartment rather than eliminate it from the treatment system. Accordingly, quantitative models should integrate PFAS molecular properties, biofilm architecture, microbial activity, operational conditions, and compartment-specific fluxes to determine whether biofilm-associated PFAS is retained, transformed, or subsequently released under realistic treatment conditions.

Biofilm responses to PFAS exposure and implications for functional stability of engineered-biofilm systems

Cellular stress and metabolic responses

Following transport and compartment-specific partitioning, biologically accessible PFAS may interact with metabolically active microorganisms within engineered biofilms. However, direct evidence from intact engineered-biofilm systems remains limited. Much of the current evidence for PFAS-associated membrane perturbation, oxidative stress, and metabolic reallocation is derived from PFAS-exposed microorganisms, pure cultures, or non-biofilm systems, and therefore provides mechanistic insight rather than direct confirmation of responses within intact biofilms[50,110]. The microbial cell envelope represents an important interface for PFAS-cell interactions[52,130] (Fig. S2a). Owing to their amphiphilic nature, PFAS can associate with membrane lipids and proteins, altering membrane fluidity, permeability, and electrochemical properties[52,138]. Such changes may affect substrate transport, proton-motive-force maintenance, respiration, and cellular signaling. Whether these effects occur to a similar extent within engineered biofilms depends on the accessibility of PFAS to cells and on the physicochemical properties of the surrounding biofilm matrix.

Biofilm architecture introduces additional complexity because PFAS exposure is unlikely to be spatially uniform. EPS, biomass density, diffusion resistance, and local physicochemical gradients can influence the fraction of PFAS reaching metabolically active cells[139,140]. The EPS matrix may, therefore, provide a degree of protection by retaining PFAS away from cells, but it may also create localized PFAS-enriched domains or alter diffusion pathways, depending on PFAS properties and EPS chemistry. Consequently, membrane-level responses observed in planktonic or pure-culture systems cannot be assumed to occur uniformly throughout an engineered biofilm. Direct measurements linking local PFAS accessibility with cellular responses within intact biofilms are still needed.

Membrane perturbation can promote electron leakage from respiratory pathways and increase reactive oxygen species (ROS) production[26,50,110,141]. Excess ROS can damage lipids, proteins, nucleic acids, and enzyme cofactors, triggering antioxidant defenses, DNA repair, protein quality control, and membrane repair[142,143]. These protective responses can increase cellular maintenance requirements and potentially divert resources from growth and biosynthesis. Consistently, PFAS exposure has been associated with changes in ATP production, respiration, and carbon metabolism[144,145], suggesting that metabolic allocation may shift under PFAS stress. However, because these observations are derived largely from non-biofilm systems, their magnitude and ecological significance within engineered biofilms remain uncertain. In intact biofilms, oxygen, nutrient, redox, and PFAS concentration gradients may further generate spatially heterogeneous physiological responses[22,146,147].

PFAS exposure may also influence EPS production or composition, but the extent to which such changes are specifically induced by PFAS remains insufficiently resolved. Changes in protein-rich EPS could modify the abundance or accessibility of charged and hydrophobic domains capable of interacting with PFAS, whereas changes in polysaccharide-rich components may affect matrix and diffusion resistance[88,129,148]. These properties could, in turn, modify PFAS retention and accessibility. However, EPS quantity and composition are also strongly influenced by biofilm maturation, nutrient availability, hydrodynamic conditions, and microbial succession[149,150]. Therefore, changes in EPS observed during PFAS exposure cannot be attributed to PFAS-induced remodeling without appropriate controls and characterization of the initial biofilm state. In particular, evidence that PFAS bind to EPS does not by itself demonstrate that PFAS exposure causes active EPS production or compositional restructuring. The contribution of PFAS-specific EPS remodeling to PFAS retention, transformation, protection, or redistribution, therefore, remains an important but insufficiently tested hypothesis.

Microbial community restructuring and functional adaptation

Cellular responses to PFAS exposure may extend to changes in microbial community organization and biofilm function[22,129] (Fig. S2b). However, current evidence does not indicate a universal taxonomic response. Reported enrichment or suppression of specific microbial groups varies across studies, with PFAS identity, exposure concentration, exposure duration, reactor configuration, substrate availability, and operating conditions all potentially contributing to the observed response[44,151]. These factors should therefore be considered together rather than attributing community restructuring solely to PFAS identity.

PFAS identity may influence microbial selection through differences in chain length, functional groups, charge, molecular structure, and interactions with the biofilm matrix, which collectively affect PFAS accessibility to microorganisms[16,102]. Exposure concentration may determine the intensity of physiological stress, whereas exposure duration may distinguish transient physiological responses from sustained ecological selection or adaptation. Short-term exposure may primarily produce stress-related changes in activity, while prolonged exposure could favor populations with greater tolerance to membrane perturbation, oxidative stress, or altered resource allocation. However, concentration-response and time-resolved comparisons across PFAS classes remain limited, making it difficult to establish general thresholds for community-level effects.

Unlike biodegradable organic contaminants, PFAS generally do not serve as microbial carbon or energy sources[44,152]. Consequently, prolonged exposure is more likely to favor microorganisms with traits related to oxidative-stress resistance, membrane adaptation, and metabolic flexibility than specialized PFAS-utilizing populations[152–154]. Such adaptation can occur even when the overall fluorinated inventory remains persistent, because precursor transformation may change PFAS speciation without complete destruction or mineralization[155,156].

Biofilm architecture further shapes these ecological responses by creating spatial gradients in PFAS exposure, oxygen, nutrients, electron donors, and redox conditions[129,157]. Populations in PFAS-accessible regions may therefore experience stronger selection pressure than those in less exposed compartments[158,159]. Biofilm maturity, thickness, EPS composition, and hydrodynamics can therefore influence which microorganisms are exposed and the duration or intensity of exposure[160]. This spatial organization distinguishes engineered biofilms from homogeneous planktonic systems and provides a mechanistic basis for heterogeneous community responses.

PFAS exposure may also alter functional interactions within biofilm consortia, including cross-feeding, syntrophy, metabolite exchange, extracellular enzyme production, and carbon utilization[158,161]. Nevertheless, evidence directly linking PFAS exposure to changes in these interactions within intact engineered biofilms remains limited. Functional redundancy may preserve process performance despite taxonomic restructuring[162,163]; consequently, taxonomic changes alone should not be interpreted as evidence of impaired reactor function. Assessments of PFAS effects should instead integrate community composition with functional indicators such as metabolic activity, extracellular enzyme activity, precursor transformation, and reactor-level treatment performance.

Implications for biofilm function and treatment performance

PFAS-induced changes in microbial physiology, EPS properties, and community organization may ultimately affect biofilm-mediated treatment performance (Fig. S2c). Organic-matter removal depends on extracellular hydrolysis, substrate diffusion, microbial uptake, and intracellular metabolism[164,165]. PFAS-associated changes in EPS quantity and composition may alter matrix permeability and substrate transport, while EPS-mediated sequestration can simultaneously reduce microbial PFAS exposure and increase mass-transfer resistance[130,166]. Thus, effects on carbon removal are likely governed by the balance among cellular stress, EPS protection, and substrate accessibility rather than by direct toxicity alone[129]. This balance may differ spatially within a biofilm. PFAS retained in outer matrix regions may have limited immediate access to microorganisms, whereas PFAS that penetrate deeper compartments could affect cells responsible for key metabolic processes. Conversely, a strong association with EPS may prolong PFAS residence in the biofilm and influence subsequent redistribution when environmental conditions change. These possibilities highlight the need to distinguish PFAS concentration in the total biofilm from that in the fraction that is biologically accessible. Measurements of overall removal or accumulation, therefore, provide limited information on whether PFAS exposure is affecting microbial function.

Nutrient transformations may be similarly affected because nitrifying and denitrifying populations occupy spatially distinct biofilm niches defined by oxygen, substrate, and redox gradients[83,149]. PFAS-associated changes in microbial activity, community structure, or EPS organization could alter these niches and their functional activity. Previous studies have reported PFAS-associated community changes accompanied by altered nitrogen-transformation potential, although the direction and magnitude of these responses vary with PFAS properties, exposure conditions, and reactor configuration[130,167,168]. Accordingly, changes in nitrogen removal should be interpreted together with measurements of relevant functional populations and process rates rather than solely from taxonomic composition.

PFAS exposure may also influence long-term process resilience. The stability of attached-growth systems depends on spatial microbial organization, EPS-mediated protection, and functional redundancy[140,162]. Changes in resource allocation, matrix properties, or microbial interactions could potentially alter the capacity of biofilms to maintain treatment functions during continued PFAS exposure or recover following disturbance. However, direct evidence linking PFAS exposure to resilience metrics such as recovery time, sustained process stability, functional redundancy, or resistance to secondary disturbances remains limited. In particular, it remains unclear whether PFAS-induced physiological stress produces measurable deterioration in reactor-level stability when biofilm protection and functional redundancy are retained.

Therefore, PFAS effects on engineered-biofilm performance should be evaluated across multiple levels of organization, from PFAS accessibility and cellular stress to EPS properties, microbial community structure, process rates, and reactor-level performance. A reduction in microbial activity or a shift in community composition does not necessarily translate into treatment failure if functional redundancy compensates for the affected populations. Conversely, apparently stable bulk treatment performance may mask changes in PFAS partitioning, microbial stress, or functional vulnerability that become important under prolonged exposure or changing operating conditions. Integrating molecular, physicochemical, and reactor-level measurements is consequently necessary to determine when PFAS exposure represents a reversible physiological perturbation versus a sustained threat to biofilm functional stability.

From mechanistic understanding to predictive management of PFAS–biofilm systems

The mechanisms synthesized in this review indicate that PFAS behavior in engineered biofilms cannot be predicted reliably from influent concentration or compound identity alone. Existing PFAS models across wastewater, soil, adsorption, and membrane systems indicate that predictions can benefit from integration of chemical, environmental, and operational descriptors[169–172]. For biofilm systems, a critical missing domain is the evolving biofilm state. For biofilm systems, a critical missing domain is the evolving biofilm state. However, frequent PFAS measurements and online biofilm-state data remain scarce. Therefore, predictive management should be developed in stages. In the near term, the framework is most realistically applied through periodic assessments informed by mass balance that combine PFAS measurements, biofilm characterization, and operating records. These observations can form the longitudinal evidence base required for subsequent predictive modeling. Accordingly, Fig. 3 presents a biofilm-centered framework that links multi-domain measurements with compartment-scale mechanisms and outlines a progression from present-day monitoring to mechanism-informed prediction.

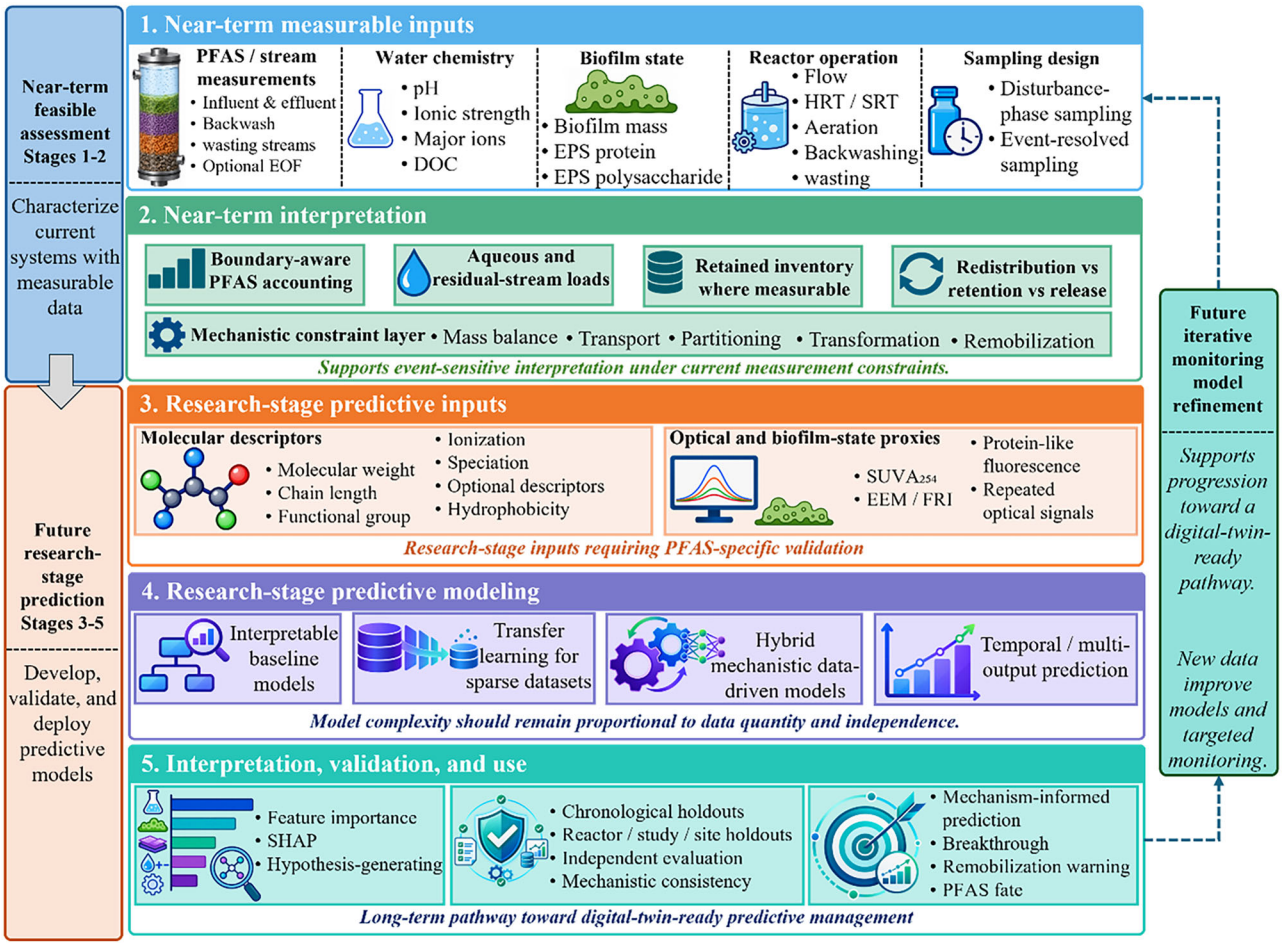


Fig. 3 | Staged framework for developing PFAS-specific predictive management in engineered-biofilm systems. The framework progresses from near-term feasibility and interpretation using currently measurable system information (Stages 1–2) toward research-stage predictive development, validation, and application (Stages 3–5). Near-term assessment integrates measurable PFAS inputs, aqueous and residual-stream loads, retained inventories, and redistribution versus release within a boundary-aware mass-balance framework, constrained by transport, partitioning, transformation, and remobilization processes. Research-stage development introduces PFAS-specific molecular descriptors and optical/biofilm-state proxies to support predictive modeling, including interpretable baseline models, transfer learning for sparse datasets, hybrid mechanistic-data-driven approaches, and temporal or multi-output prediction. The final stage emphasizes independent and temporally structured validation, including chronological, reactor/site, and other holdout strategies, together with feature-importance and mechanism-consistency analyses to evaluate model interpretability and robustness. The framework ultimately links mechanism-informed prediction with PFAS fate assessment, remobilization warnings, and targeted monitoring. The progression from near-term measurable inputs to research-stage predictive modeling represents a proposed research pathway rather than a currently validated predictive system; research-stage descriptors, proxies, models, and proposed applications require PFAS-specific experimental validation and quantitative performance assessment. Dashed/iterative pathways indicate that new measurements and validation can be used to refine model development and subsequent monitoring strategies.

A practical initial dataset should include paired influent and effluent target PFAS concentrations with corresponding flow rates and sampling times; measurements of PFAS in exported backwash or wasting streams with the associated water volumes or dry-solid masses; representative biofilm mass and EPS protein and polysaccharide measurements; and matched water chemistry and operating conditions[132]. If quantification of the retained PFAS inventory is an objective, PFAS concentrations in representative biofilm- or carrier-associated material, along with the corresponding material mass, are required. Samples collected before, during, and after at least one defined operational disturbance can provide information on short-term redistribution or release. Elevated PFAS concentrations during early filtration following BAC backwashing illustrate the value of event-resolved monitoring[99]. When precursor transformation or unresolved organofluorine is relevant, a bulk organofluorine measurement, such as extractable organic fluorine (EOF), can complement targeted PFAS analysis, provided that compatible sample phases and fluorine-equivalent quantities are compared[134]. Therefore, the minimum dataset should be defined by measurement domains rather than by a universal number or frequency of samples, because the necessary temporal resolution depends on hydraulic residence time, loading variability, and the process or disturbance under investigation.

The framework integrates four input domains: PFAS chemistry, water chemistry, biofilm state, and reactor operation. For near-term assessment, these domains should prioritize variables obtainable through periodic sampling and operating records. PFAS chemistry includes target identity, concentration, precursor status, and mixture composition; biofilm state is represented initially by biofilm mass and EPS protein/polysaccharide content; and water chemistry and reactor operation include relevant physicochemical conditions, hydraulic loading, residence time, and disturbance history. Because EPS measurements are sensitive to extraction and analytical procedures, consistent extraction and normalization are necessary for comparisons across sampling periods and systems[173]. Depth-resolved PFAS distributions, intracellular PFAS, detailed transport and redox profiles, and continuous optical proxies are not prerequisites for this initial assessment and remain research measurements

These measurements provide a practical basis for PFAS accounting within a defined treatment boundary, allow changes in aqueous and residual-stream loads to be identified, and permit assessment of whether an operational disturbance coincides with redistribution or release. Recent inter-stage mass-balance analyses show that PFAS can follow different liquid and solid pathways within treatment systems, while experiments with aged granular activated carbon demonstrate that changes in feedwater composition can induce desorption and earlier breakthrough of previously retained PFAS[174,175]. These observations support phase-resolved and event-sensitive monitoring when persistent retention must be distinguished from redistribution or release. Therefore, Fig. 3 presents a measurement-to-interpretation pathway: information from sampled water, residual, and biofilm, together with operational information, provides the basis for mass-balance and event interpretation, whereas advanced prediction remains a separate research extension. At the first predictive tier, routinely collected plant variables can support forecasts of PFAS occurrence and concentration in treatment outputs. For example, Dong et al. developed models across 200 wastewater treatment plants to predict total and individual PFAS in influent, effluent, and biosolids from treatment-process, wastewater-source, operational, and water-quality variables[169].

Within this future predictive research stage, mechanistic understanding should constrain both model formulation and interpretation. The biofilm-centered mass-balance framework provides a defined system boundary, inventory terms, and conservation relationships that can guide prediction targets and physical-consistency checks. Recent PFAS and biofilm modeling studies identify PFAS molecular properties, water chemistry, biofilm characteristics, and reactor-operation variables as relevant predictor domains[59,169,172]. For PFAS chemistry, candidate molecular descriptors include molecular weight, fluorinated chain length, functional groups, hydrophobicity, and condition-dependent ionization or speciation. A machine-learning analysis of 1,274 PFAS sorption records incorporated molecular weight, hydrophobicity, and pK_a, with molecular weight and hydrophobicity among the most influential PFAS-specific predictors[176]. Molecular weight and chain length have likewise been identified as important variables in machine-learning analyses of PFAS membrane removal[172]. Quantum-chemical descriptors may provide additional molecular-level information where their contribution is mechanistically relevant. A recent DFT-machine-learning study integrating 1961 PFAS adsorption observations identified the PFAS HOMO–LUMO gap among the influential predictors of biochar adsorption[177]. Therefore, such descriptors should be evaluated for incremental predictive value rather than included indiscriminately.

Repeated or continuous optical measurements may provide complementary inputs during the research stage for characterizing changes in water quality and biofilm state. Specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) is defined as ultraviolet absorbance at 254 nm (UVA_{254}) normalized to dissolved organic carbon (DOC), whereas fluorescence regional integration (FRI) divides an excitation-emission matrix (EEM) into predefined regions and integrates fluorescence intensity within each region[178]. In aged granular activated-carbon (GAC) columns, fluorescence-derived DOM fractions and $SUVA_{254}$ reflected changes in organic-matter composition that coincided with PFAS adsorption and desorption; higher feedwater aromaticity was associated with earlier ^{14}C-PFOA breakthrough[175]. Continuous protein-like fluorescence has also been demonstrated in wastewater treatment. Online sensor measurements agreed closely with benchtop fluorescence over nine days of continuous operation (R^2 of 0.99)[179]. In addition, three-dimensional EEM spectroscopy has been used experimentally to characterize PFOA interactions with sludge-derived EPS and protein-associated binding[180]. These findings support optical measurements as candidate contextual predictors, but they do not yet establish a direct relationship with PFAS saturation, cellular stress, or breakthrough in BAC biofilms. Therefore, PFAS-specific predictive use requires paired optical, PFAS, and EPS/biofilm measurements, controls for matrix interference, and prospective validation.

At a predictive tier with greater mechanistic resolution, molecular and biofilm-state descriptors can refine compound- and reactor-specific forecasts. Recent studies illustrate this input-to-target relationship: PFAS molecular and process variables have been used to predict membrane removal[172]; adsorbate, water-matrix, and carbon properties have supported early-breakthrough prediction for recalcitrant micropollutants in granular activated carbon[181]; and biofilm density, thickness, dissolved oxygen, and hydraulic residence time have supported contaminant-removal prediction in a biofilm reactor[182]. Together, these studies show how chemical, matrix, and biofilm-state information can progressively extend prediction from bulk treatment outputs toward process-specific behavior.

As longitudinal PFAS–biofilm datasets develop, model complexity should remain proportional to the number and independence of available observations. Interpretable models provide an appropriate baseline, while transfer learning and hybrid mechanistic-data-driven approaches may help when system-specific datasets remain limited. Transfer learning can adapt information learned from a data-rich source system to a target system with fewer observations; this approach has been demonstrated for effluent-quality prediction in data-limited wastewater treatment systems[183]. Therefore, transfer learning represents a potential strategy for making better use of limited target-system data, but it does not replace independent model evaluation.

Once candidate inputs are defined, hybrid wastewater treatment models provide a route to integrate mechanistic constraints with data-driven prediction. First-principles process knowledge can be embedded as physical constraints, while data-driven components represent unresolved nonlinear relationships[184]. Recent wastewater treatment studies show that hybrid architectures can preserve mechanistic process structure and improve prediction of treatment outcomes[185–187]. For example, a study developed an Activated Sludge Model No. 2d (ASM2d)-long short-term memory (LSTM) hybrid model. In this model, first-principles wastewater-process knowledge was embedded as physical constraints, and the data-driven component represented unresolved process dynamics. The model achieved test-set R^2 values of 0.92, 0.91, and 0.94 for COD, total phosphorus, and total nitrogen, respectively[184]. Therefore, data-driven components should complement, rather than replace, mechanistic understanding by representing relationships that remain insufficiently parameterized from process knowledge alone. They should not be used to infer unmeasured compartment-specific fluxes or to force closure of an incomplete PFAS mass balance. For PFAS–biofilm systems, hybrid modeling should remain a research-stage approach until sufficiently resolved longitudinal datasets and independent evaluation become available.

Model interpretation requires the same restraint. Feature-importance values and Shapley additive explanation (SHAP) values describe how a fitted model uses available predictors but do not establish causal or mechanistic relationships[188]. Their interpretation requires additional caution when predictors are correlated, as SHAP outputs can be sensitive to feature collinearity and dependencies among predictors[189,190]. Therefore, model-attribution patterns should be treated as a basis for hypothesis generation and evaluated against independent compartment measurements, controlled operational disturbances, or other mechanistically targeted observations before they are interpreted as evidence of a PFAS–biofilm mechanism. For this reason, SHAP-based interpretation and related model-diagnostic approaches require further research, independent validation, and mechanistically targeted measurements before operational interpretation.

Transferability is essential for future predictive applications. Validation should be matched to the intended prediction task and should preserve the temporal and hierarchical structure of the observations. Chronological holdouts provide a direct test of prediction for later operating periods, whereas withholding complete reactors, experimental campaigns, studies, or sites provides a stronger assessment of transfer to new systems when sufficient independent groups are available[191]. When too few independent reactors, campaigns, or sites are available for a credible external test, model performance should be described as internally validated or exploratory rather than as evidence of generalizability. Depth-resolved measurements,

advanced optical or molecular predictors, hybrid models, and more integrated predictive systems should remain research-stage components until sufficiently resolved longitudinal datasets and independent evaluation become available. Only after these requirements are met could a future PFAS–biofilm digital twin extend this predictive hierarchy through recurrent state updates. New PFAS, biofilm, water-quality, and operating measurements could update the virtual representation, while validated mechanistic and data-driven models could forecast effluent breakthrough, retained inventory, remobilization, and changes in biofilm or treatment state. Recent water-treatment digital-twin frameworks combine real-time process data with mechanistic or machine-learning models to support system-state forecasts and operational decisions[192,193].

Knowledge gaps and future directions for PFAS–biofilm engineered systems

Despite growing recognition of biofilms as regulators of PFAS fate, the mechanisms controlling their long-term retention, transformation, and release remain poorly resolved. Most available knowledge derives from suspended biomass, sludge, or abiotic sorption systems, whereas engineered biofilms introduce spatial heterogeneity, dynamic EPS chemistry, variable microbial accessibility, and continuous structural turnover. A key research priority is therefore to determine how these biofilm-specific features regulate PFAS distribution, biological exposure, transformation, and remobilization, and how such knowledge can support predictive management.

First, the spatial distribution of PFAS within intact biofilms remains largely unknown. Bulk measurements of water, sludge, biomass, or whole biofilms cannot distinguish PFAS retained within EPS from those present in hydrated domains or associated with microbial cells. Because both PFAS properties and biofilm structure govern compartment-specific partitioning[21,23], spatially resolved measurements are needed across biofilm depth and among extracellular, cellular, and aqueous compartments. Such information is essential for distinguishing total retention from biologically accessible PFAS. Second, PFAS inventories should be resolved temporally as biofilms develop and undergo disturbance. Biofilm growth and turnover continuously alter EPS composition, thickness, porosity, diffusion, and detachment[194], while biofilm development can modify PFAS accessibility to underlying sorptive domains[96]. Longitudinal studies spanning biofilm development, stable operation, and defined disturbances are needed to determine whether biofilms function as persistent sinks or temporary reservoirs capable of episodic secondary release[99]. Third, the relationship between PFAS retention and biological exposure requires direct validation. Whether total biofilm-associated PFAS reflects the fraction accessible to metabolically active cells remains unresolved. Future studies should therefore couple compartment-specific PFAS localization with microbial stress responses, metabolic activity, and precursor transformation. Particular attention should be given to aerobic, anoxic, and anaerobic niches in which precursor transformation may generate intermediate PFAAs or unresolved organofluorine species. Fourth, changes in the microbial community need to be linked to function rather than taxonomy alone. Reported taxonomic responses to PFAS vary widely with compound composition, exposure duration, reactor configuration, and operating conditions. Because PFAS generally do not support substrate-driven enrichment, future work should distinguish stress-induced community restructuring from functional adaptation and determine whether these changes affect EPS properties, precursor transformation, carbon and nitrogen removal, or resilience to disturbance.

Current quantitative evidence remains strongly endpoint-specific. Tables S3 summarizes sludge-water distribution coefficients, whereas Tables S4 compiles other quantitative PFAS–biofilm-related observations while retaining their original matrices, units, and reported uncertainty. In biofilm-coated sand, PFOA retardation factors ranged from 1.11 to 1.38 at 1 mM NaCl and from 1.15 to 1.29 at 20 mM NaCl across tested biofilm conditions, whereas natural periphytic biofilms contained 1.96–20.1 ng g^{-1} wet-weight Σ_{16} PFAS. Other studies report component-specific interactions, composite porous-media distribution coefficients, biological-response metrics, filter-removal outcomes, or precursor-product yields on different experimental bases (Table S4). These measurements cannot be combined into a single cross-study range without conflating distinct endpoints. Comparable EPS-only PFAS concentration ranges and intact engineered-biofilm-water distribution coefficients remain particularly limited.

At minimum, studies should report PFAS identity and concentration, water chemistry, operating conditions, biofilm biomass and structure, EPS composition, microbial activity, analytical detection limits, and treatment performance. Compartment-resolved PFAS measurements in water, EPS, biomass, carrier-associated material, and detached solids should be combined with precursor-product and organofluorine analyses when transformation is evaluated. Such standardized longitudinal datasets are essential for cross-study comparison, quantitative mass balances, and development of transferable predictive models for PFAS–biofilm systems.

Conclusions

This review advances a biofilm-centered and PFAS-specific perspective in which engineered biofilms are considered dynamic compartments that can alter PFAS distribution and persistence rather than universal barriers for PFAS removal. Biofilms may temporarily store PFAS through association with biomass and EPS, while selected polyfluorinated precursors may undergo biological transformation without necessarily resulting in destruction of the fluorinated mass. Consequently, reductions in aqueous-phase PFAS concentrations should not be interpreted as evidence of permanent removal, because PFAS can be redistributed into biofilm-associated pools and subsequently remobilized or released as transformation products or other fluorinated compounds. The extent and direction of these processes depend on PFAS-specific properties, biofilm structure and composition, microbial activity, mass-transfer conditions, and reactor operation. This perspective, therefore, emphasizes the need to distinguish reversible retention, precursor transformation, remobilization, and verified destructive loss when evaluating biological-treatment systems. Overall, the key contribution of this review is to move beyond the assumption that engineered biofilms universally remove PFAS. Instead, it provides a framework for identifying when, why, and for which PFAS-biofilm-mediated processes meaningfully influence distribution, precursor transformation, persistence, and subsequent release. This PFAS-specific and mechanism-based perspective provides a basis for evaluating biological-treatment systems beyond conventional influent–effluent removal metrics and for distinguishing apparent attenuation from verified PFAS elimination. Future research should move beyond conventional influent–effluent measurements toward spatially and temporally resolved PFAS inventories that track aqueous and biofilm-associated pools together with transformation products and subsequent release. Such measurements are necessary to determine when biofilms meaningfully influence the fate of individual PFAS and to establish whether observed concentration decreases represent temporary storage, redistribution, transformation, or verified elimination.

Data availability

No datasets were generated or analyzed during the current study.

Received: 3 September 2026; Accepted: 11 October 2026;
Published online: 28 October 2026

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Author contributions

Rabia Zafar: Conceptualization, writing – original draft. Shahzeb Tariq: Conceptualization, writing – original draft. Mairaj Bibic: Visualization. Keugtae Kim: Writing – review & editing. Jin Hur: Writing – review & editing, funding acquisition.

Funding
This work was supported by grants from the National Research Foundation of Korea (NRF), funded by the Korean government (RS- 2024-00340645) and from Korea Environment Industry & Technology Institute (KEITI) through research and development for high-salinity wastewater treatment in the rechargeable battery industry Project, funded by Korea Ministry of Climate, Energy and Environment (MCEE) (RS-2026-25500759).

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41545-026-00646-8.

Correspondence and requests for materials should be addressed to Keugtae Kim or Jin Hur.

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