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Opinion

A Synergistic Approach to Per- and Polyfluoroalkyl Substance Treatment That Includes Microbial Bioremediation and Considers Degradation Fluxes

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The aim of the author is to propose a change of the approach to the management of fluorinated pollutants in waste and water streams, in which the linear treatment of pollutants could be replaced by the integration of a synergistic system including biological treatments and a focus on the secondary streams produced by conventional and less conventional technological solutions in order to avoid the translation of the problem or, even worse, the production of equally harmful compounds.

Per- and polyfluoroalkyl substances (PFASs) have been widely used in industrial production and commercial products, notably textiles, food packaging, firefighting foams, refrigerant gases, nonstick coatings, cosmetics, medical devices, plant protection products, etc. [1,2]. This is a class of chemicals in which hydrogen atoms in the carbon skeleton or chain are partially or totally replaced by fluorine atoms, as well as by a terminal functional group where the head can be a sulfonate or carboxylate group [3]. Fluorine is characterized by a high electronegativity (one of the most electronegative elements in the periodic table), a high ionization potential, and a low polarizability, which is also responsible for the ionic character of PFASs and the higher bond strength compared to similar hydrocarbons. When bonded to carbon, fluorine forms one of the strongest and most inert single bonds found in organic compounds, with a bond dissociation energy up to 531.5 kJ·mol^{-1} [4]. The chemical structure of PFASs, characterized by an optimal overlap between the 2s and 2p orbitals of the fluorine with the C orbitals forming the C-F bonds, induces the simultaneous formation of multiple dipolar resonance structures along the chain. Furthermore, the strength of the C-F bonds increases further as the number of fluorine atoms bonded to the central carbon increases, coupled with a chemical inertia and kinetic stability mainly due to the shielding of the central carbon atom by the fluorine, thus making a nucleophilic attack on the central carbon atom difficult [5]. Due to the high electronegativity, the C-F bonds in the perfluorinated tail are highly polarized. When the hydrogen atom is replaced by the fluorine atom in the alkyl chain, the effect produced is a decrease in the pKa value. To the properties related to the chemical structure of PFASs, physical properties must also be added. Indeed, the larger van der Waals radius of fluorine compared to hydrogen (1.47 Å and 1.2 Å, respectively) leads to a drastic change in the conformation of the resulting molecule. On the other hand, PFASs show very weak intramolecular and intermolecular interactions due to the low polarizability of fluorine, which is characterized by a much higher volatility and lower boiling point than, for example, their hydrocarbon counterparts of similar molecular mass [5]. The low intermolecular forces also result in the exceptionally low surface tension of PFASs, which is responsible for their excellent surface wettability and amphiphilic and oleophobic

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character, which makes them excellent surfactants, allowing the surface tension of water to be reduced from 7.2 × 10^6 N/m to 1.5–2.0 × 10^6 N/m compared to 2.5–3.5 × 10^6 N/m for their hydrocarbon counterparts [5,6]. A great concern of PFASs in the environment arises from their bioaccumulative nature, which is governed by three important physicochemical parameters, namely, water solubility, vapour pressure, and critical micelle concentration (CMC), which not only affects their transport in the natural environment but can be utilized for PFAS removal from aqueous media [4]. Amongst all PFASs, perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have had the longest production history, but their use is being phased out to make way for new replacement compounds such as hexafluoropropylene dimer acid (GenX) and perfluoroethylcyclohexane sulphonate (PFECHS). Since many PFASs (precursors) can be easily degraded into other persistent forms, long-chain PFASs (C8–C14) and their sodium and ammonium salts have been added to the candidate list of controlled substances in the EU and to the list of new Persistent Organic Pollutants (POPs) under the Stockholm Convention [7,8]. With the ban on long-chain PFASs, short-chain PFAs (perfluoroalkyl carboxylic acid (PFCA) < C8, perfluoroalkane sulfonic acid (PFSA) < C7) have been produced and used as substitutes in large quantities. Apart from the US and some developed countries, regulations and enforcement in some less developed countries are relatively lax [9]. However, as PFAS contamination becomes a global problem, regulations in less developed areas of the world are expected to become more stringent. In the same direction, analytical techniques have also made a positive contribution. Detecting PFAS is a challenge because these compounds contain no chromophores or electroactive groups and are therefore not optically or electrochemically active. These properties make the application of low-cost analysis methods difficult. Different approaches that take advantage of the interaction of PFASs with redox-active molecules and the development of molecular receptors for PFASs have been reported in an attempt to achieve the selectivity and sensitivity needed for detection. Anyway, the low regulatory limits, e.g., 70 parts per trillion (70 ng/L or ppt) imposed by the US EPA, or 10 ppt in some states, pose additional challenges for detection. In addition, very recently, the US EPA (https://www.federalregister.gov/d/2023-05471, accessed on 1 January 2025) has released new guideline limits at levels as low as 0.004 ppt for PFOA in drinking water, posing significant challenges and increasing the need to develop ultrasensitive methods for detection. The increased regulation will involve a significant increase in testing; therefore, there is an urgent need to expand the arsenal of analytical methodologies with a significantly higher sensitivity and a lower cost than currently used methods [10]. In general, current techniques allow the measurement of a limited number of PFASs, i.e., 18 following the US EPA protocol and 21 using the ASTM protocol. Advances have indeed been observed in chromatography techniques [11]. A comprehensive review carried out by Zarębska and Bajkacz [12] considered the advancement of PFAS analytical methodologies over the last ten years. The paper outlined some of these, for instance, LC-ESI-MS/MS in the measurement mode for anionic PFASs and cationic and zwitterionic PFASs [12]. Most PFASs remain unidentified due to a lack of authentic standards. Key requirements remain to be defined to enable the development of field-usable methods for PFAS analysis, including the development of new materials and molecular receptors capable of selectively binding a single PFAS or a class of PFASs (e.g., short-chain or long), methods and mechanisms of optical and electrochemical signal amplification to achieve the low detection limits imposed by regulatory agencies (for example, very low ng/L or ppt), and validation and standardization criteria [10]. Currently, the interaction of PFASs with different types of materials is largely unknown, which limits the ability to innovate in this area. It is generally known that electrostatic and hydrophobic interactions contribute to the adsorption of PFASs [10]. Particular attention has historically been given to the treatment of municipal drinking

water, aqueous film-forming foam (AFFF), and industrial wastewater, but complex matrices such as sludge derived from water treatment and sludge derived from other fermentation bioprocesses (e.g., digestate used as soil improver) have been neglected. In this sense, the approaches followed remain removal or separation, destruction, and sequestration, or a combination of these. More specifically, treatment techniques for PFASs include adsorption, membrane separation, or more emerging methods such as foam fractionation, as well as incineration or less conventional methods such as supercritical or electrochemical oxidation, hydrothermal alkaline treatment, thermal plasma, and sonolysis [13]. The above technologies can remove PFASs to a certain extent, but their treatment effects, operating conditions, removal mechanisms, and applicability take into account energy-intensive consumption, high costs, and the potential for producing toxic by-products. For some of these techniques, the problem associated with treating fluxes containing these compounds shifts strategically to finding additional treatments capable of treating secondary fluxes with higher concentration factors. As an alternative to the approaches just described, there are also approaches involving the use of biological systems, which can be counted as biosorption, bioremediation, or biodegradation. Several studies have explained the application of bioremediation in the degradation of pesticides, petroleum hydrocarbons, and other chlorinated substances, but the capability of biological agents to degrade PFASs has been poorly understood [14]. One option involves microorganisms breaking the C-F bond under aerobic or anaerobic conditions either by oxidation (addition of an oxygen atom between the C-F bond) or reduction (addition of an electron between the C-F bond). In both cases, considerable energy is required to catalyze the reaction [15]. The microbial cleavage of fluorinated alkyl compounds presupposes the presence of at least one hydrogen atom in the alkyl chain for the primary attack. The additional difficulty of oxidatively replacing fluorine atoms lies in their ability to form a dense hydrophobic layer surrounding the carbon–carbon bonds, preventing oxidative degradation. This characteristic element of the fluorine-saturated carbon chain offers resistance to oxidation or utilization by microorganisms as a source of carbon and energy. An example of such an application is certain bacteria like *Pseudomonas* sp. or *Acidimicrobium* sp., which can bioaccumulate these compounds under alkanotropic conditions [16]. Specifically, *Pseudomonas mosselii* would make this degradation possible through the halogen dehalogenase gene (dhaA), the haloacetate dehalogenase H-1 gene (dehH1), the fluoride ion transporter (crcB), and the alkane sulphonate monooxygenase gene (ssuE). As for the *Acidimicrobium* sp. genome (strain A6), genes encoding a homologue of reductive dehalogenase (RdhA), a homologue of fluoroacetate dehalogenase (FceA), and two putative haloacid dehalogenases (dhl_1 and dhl_2) were identified [17]. Ding et al. [18] showed that there is a correlation between the RdhA gene and perfluorinated alkyl acid (PFAA) removal during the incubation period, whereas the expression of dhl_1 and dhl_2 was related to dehalogenation and did not change in the presence of PFAAs. It was also observed that the CrcB gene plays a crucial role in the removal of F from within the cell, thus attenuating the toxic F-building [17].

Alternatively, it is possible to imagine an approach focused on the possibility of targeting the Cl-C bond, as in the case of one of the metabolic pathways of *Dehalobacter* sp. [19]. To grow, these microorganisms utilize organohalide respiration (OHR), which is the energy metabolism of anaerobic bacteria that are able to use halogenated organic compounds as terminal electron acceptors [20]. In the case of microalgae, the biosorption process of PFASs involves their binding to extracellular compounds (EPS) or the algal cell wall. In the case of bioaccumulation, PFASs are transferred through the cell wall to specific proteins on the intracellular surface.

PFASs are often low-pKa acids with a poor ability to cross cell membranes by passive diffusion. Therefore, many PFASs are candidates as substrates for a number of transport

proteins that facilitate their entry into and transport within cells. Important proteins for the distribution of PFASs include albumin, fatty acid binding proteins (FABP), and organic anion transporters (OATs). In addition, longer-chain PFASs with larger hydrophobic regions can associate with phosphoproteins and lipoproteins in biological systems.

Biotic and abiotic changes (e.g., salinity, temperature, reproductive stage, and health status) often lead to dynamic and reactive physiological changes that alter the prevalence and localisation of many proteins, including PFAS-related proteins [21].

The behaviour of species such as *Scenedesmus* sp. or *Chlorella* sp. is potentially suitable for PFAS adsorption or accumulation. Thus, during the bioaccumulation process, the production of ROS (reactive oxygen species) may contribute to cell dysfunction to the point of cellular death [22]. The most used fungi for the degradation of toxic contaminants are the brown-rot fungus *Aspergillus niger* and the white-rot fungus *Phanerochaete chrysosporium*. It has been shown that the remediation, treatment, and decomposition of PFASs are feasible using the ECOHR (enzyme-catalyzed oxidative humification reaction) [22]. ECOHRs commonly take place in the soil system and are characterized by a series of oxidative reactions during the humification process. ECOHRs are carried out by natural extracellular enzymes secreted by white and brown rotting fungi, namely laccases. In general, biodegradation is promoted by co-metabolism mechanisms of enzymes with oxidative or hydrolytic activity [23]. Enzymes cited in the literature with the ability to catalyze de-fluorination reactions include oxidoreductases, laccases, soy peroxidase, chlorine peroxidase, and dehalogenase. Laccases and peroxidases are most often used in remediation processes because of their ability to generate reactive free radicals through an interaction of the enzyme with an aromatic mediator substrate that breaks down the basic pollutant into smaller products that are more easily biologically degradable. In these specific cases, the detoxification induced by ligninolytic enzymes involves the direct oxidation of pollutants into free radicals, which can subsequently couple, polymerise, and precipitate in the solution. Laccases are multi-copper oxidase enzymes that are able to catalyze the oxidation of a wide variety of phenolic and non-phenolic substrates [24]. Colosi and coauthors first documented the degradation of PFASs using ligninolytic enzymes, specifically reporting a 68% transformation of PFOA when in the presence of the organic substrate 4-methoxyphenol [22]. Peroxidases catalyse the oxidation of various substrates, such as aromatic compounds, by reducing hydrogen peroxide or other peroxides. Peroxidases are widely distributed throughout nature and are produced by various sources, including microbes, plants, and animals. The enzyme is oxidized from its basal state and forms a cationic radical intermediate, which is transferred to the aromatic substrate, generating reactive radical species and simultaneously reducing the enzyme and detoxifying reactive oxygen species into less harmful molecules. In the final step, the enzyme returns to its basal state when it oxidizes a second aromatic substrate, generating another reactive radical species. In the proposed mechanisms, the radical species generated interacts directly with PFASs to facilitate its degradation. There are other types of enzymes such as peroxygenase (P450), which is able to replace 'F' in the F–C bond with a transition metal. The electronegative 'F' in the F–C bond has an attraction towards the transition metal cations. The enzyme P450 contains a cation that possesses activity and is modified by the 'heme' group. The biodegradation of environmentally hazardous fluoroaromatics was mainly associated with oxygenase-dependent defluorination reactions. For the bacterium *Thauera aromatica*, a new oxygen-independent defluorination pathway was identified for the complete degradation of fluoroaromatics concomitant with denitrification; thus, it takes place in anoxic conditions. The use of enzymes offers advantages such as operation at low or high concentrations of pollutants, less sludge production, and less energy required. However, at the same time, negative effects such as the change in conformation and the impossibility of reusing the en-

zyme except in the case of immobilization on a support or in the presence of insolubilisation cannot be denied [25]. In any case, one of the challenges in the field of PFAS degradation is related to the optimisation of the enzymatic catalysis process or its biosynthesis.

In conclusion, the need to target PFAS treatment in an alternative manner is crucial not only for the reason listed in the manuscript but also to anticipate the problems associated with the treatment of nontraditional matrices with a more complex structure such as the post-fermentation digestates, composts destined for spreading, or organic soil improvers; for the latter, a conventional approach involving the use of membrane separations, adsorption, or advanced oxidation methods would be unfeasible.

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