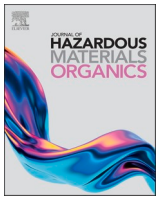
1 (2025) 100002

Contents lists available at ScienceDirect

Journal of Hazardous Materials: Organics

journal homepage: www.sciencedirect.com/journal/journal-of-hazardous-materials-organics



Biotransformation of perfluorooctanoic acid by a newly isolated *Stenotrophomonas acidaminiphila* OA1 strain from Quebec

Nihed Tellili [a], Saba Miri [a,b], Satinder K. Brar [c], Tarek Rouissi [a,*,1]

[a] Institut National de la Recherche Scientifique, Centre Eau Terre Environnement, Université du Québec, 490 Rue de la Couronne, Québec City, QC G1K 9A9, Canada
[b] Faculty of Health Sciences and wellness, Humber polytechnic, Toronto, Ontario M9W 5L7, Canada
[c] Department of Civil Engineering, Lassonde School of Engineering, York University, Toronto, Ontario M3J 1P3, Canada

ARTICLE INFO

Keywords:
PFOA
PFAS
Bioremediation
Biodegradation
Stenotrophomonas acidaminiphila

ABSTRACT

Perfluorooctanoic acid (PFOA), a persistent and bioaccumulative compound within the broader class of per- and polyfluoroalkyl substances (PFAS), poses a significant challenge to environmental remediation efforts due to its chemical stability and resistance to conventional treatments. This study explores the potential of bacterial strains isolated from a historically petroleum-contaminated site for PFOA biodegradation. Among the three isolates enriched and screened in mineral medium supplemented with 100 mg L^{-1} PFOA, strain OA1 showed the highest resistance and transformation potential. The selected isolate was identified as *Stenotrophomonas acidaminiphila* OA1. Batch and fed-batch experiments conducted in enriched medium containing 0.5 mg L^{-1} PFOA showed no apparent toxicity, with *S. acidaminiphila* OA1 maintaining stable growth over a 30-day period. PFOA degradation reached 45 % under batch conditions, while fed-batch showed lower transformation rates (35 %). ^{1}H NMR spectroscopy suggested the transformation mechanism of PFOA by *S. acidaminiphila* OA1. This work represents the first report of PFOA biotransformation by *Stenotrophomonas acidaminiphila* and contributes to the expanding body of knowledge on microbial interactions with perfluorinated compounds.

1. Introduction

The extensive use of fluorinated organic compounds in a variety of applications such as refrigerants, surfactants, lubricants, pharmaceuticals, pesticides, and aqueous film-forming foams has led to their widespread release into the environment [10,49]. Among these, perfluorooctanoic acid (PFOA; $C_7F_{15}COOH$) stands out as a synthetic long-chain perfluoroalkyl carboxylic acid belonging to the broader group of per- and polyfluoroalkyl substances (PFAS) [30]. PFOA's amphiphilic nature, along with its exceptional chemical and thermal stability, attributed to its fully fluorinated carbon chain and terminal carboxylate group, has underpinned its extensive industrial and commercial use since the 1950s. PFOA has been widely used in producing stain-resistant coatings, firefighting foams, and various industrial products, as well as in synthesizing fluoropolymers like PTFE for non-stick and waterproof materials [18,25].

PFOA has become a contaminant of global concern due to its widespread environmental presence, bioaccumulative nature, and harmful effects on both human and ecological health [31,44]. National monitoring of U.S. tap water (2016–2021) reported cumulative PFAS concentrations ranging from 0.35 to 346 ng L^{-1}, with PFOA detected in ~15 % of samples and ranking among the most frequently observed compounds [39]. In parallel, a nationwide multi-site study of communities with PFAS-contaminated drinking water (2019–2023) reported elevated serum PFOA concentrations compared with national averages from NHANES 2017–2020, underscoring ongoing human exposure risks [32].

In reaction to growing concerns, major manufacturers such as 3 M voluntarily began phasing out PFOA production between 2000 and 2002 [29]. More recently, 3 M announced its plan to cease all PFAS manufacturing by the end of 2025 [33]. In addition, the U.S. Environmental Protection Agency (EPA) recently established legally enforceable drinking water standards, setting Maximum Contaminant Levels (MCLs) at 4 ppt for PFOA and PFOS, and 10 ppt for PFNA, PFHxS, and GenX (HFPO-DA), with monitoring requirements beginning in 2027 and full compliance by 2029 [45].

Conventional PFAS remediation strategies have largely relied on physical and chemical techniques, including adsorption using granular

* Corresponding author.
E-mail address: Tarek.Rouissi@inrs.ca (T. Rouissi).
[1] ORICID ID: 0000–0001-9039–213X

https://doi.org/10.1016/j.hazmo.2025.100002
Received 25 July 2025; Received in revised form 6 September 2025; Accepted 29 September 2025
Available online 3 October 2025

activated carbon or ion exchange resins, membrane-based filtration methods such as reverse osmosis and nanofiltration, and destructive processes like incineration, sonolysis, and advanced oxidation [35,36]. While these methods are effective under controlled conditions, they are frequently associated with high operational costs, elevated energy demands, and complex maintenance requirements, limiting their scalability and accessibility in real-world environmental contexts [15,48]. Considering these limitations, biologically based remediation strategies are gaining attention as potentially low-cost, environmentally sustainable alternatives. However, their application is still limited by slow degradation rates in some cases, incomplete mineralization that may lead to the accumulation of more toxic intermediate products campared to the parental contaminant, and challenges in scaling under uncontrolled field conditions such as variable temperature, fluctuating pH, as well as presence of competing contaminants [28,37]. Bacterial species capable of transforming and/or defluorinating PFAS have been isolated from a wide range of environments, including rivers, lakes, marine sediments, contaminated soils near industrial facilities and landfills, as well as sewage and wastewater treatment plant effluents [3]. These strains have been studied under various redox conditions (aerobic, anaerobic, and anoxic) using specialized enrichment culture media to assess their degradation capabilities. For instance, *Pseudomonas aeruginosa* achieved a 27.9 % transformation of PFOA at an initial concentration of 10 mg L^{-1} under aerobic conditions (96 h, ~30 °C, pH 7.0), while *Pseudomonas putida* converted 19.0 % under the same conditions [9]. In another study, *Dietzia aurantiaca* completely degraded 6:2 fluorotelomer sulfonate (6:2 FTS) at ~70 mg L^{-1} under aerobic conditions (200 rpm at room temperature over 32 days) [26]. Additionally, *Dehalococcoides mccartyi* and *Moraxella* sp. strain B have also been identified for their potential in PFAS degradation, although specific degradation efficiencies and experimental conditions were not detailed in their respective studies [53].

Although several PFAS-degrading bacterial strains have been identified, the overall diversity and availability of effective PFOA-degrading microorganisms remain limited. Known strains often show low degradation efficiency and suffer growth inhibition when exposed to high concentrations of the compound as the sole carbon source [3,37,46]. The isolation of new strains therefore offers the opportunity to expand our understanding of microbial diversity involved in PFAS degradation and to uncover novel biodegradation capabilities and mechanisms. This study aimed to identify bacterial strains capable of degrading PFOA and to evaluate their degradation efficiency under comparative conditions. Further analysis sought to gain insights into the degradation kinetics and the biotransformation process of PFOA.

2. Materials and methods

2.1. Standard chemicals of PFOA and media

PFOA was purchased from Sigma-Aldrich (Oakville, ON, Canada) and used for microbial culture and standard solutions in LC-MS/MS analysis. Perfluoro-n-[1,2-$^{13}C_2$]octanoic acid was purchased from Wellington Laboratories (Guelph, ON, Canada) and used for internal standard in LC-MS/MS analysis. PFOA-stock solutions at 100 and 500 mg L^{-1} were prepared with ultrapure water for the isolation and the biodegradation assays. Mineral salts medium (MSM) was prepared with the following composition (per liter of ultrapure water): 5 g NH_4NO_3, 1 g KH_2PO_4, 2 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7\,H_2O$, 2 g NaCl, 0.05 g $CaCl_2{\cdot}2\,H_2O$, pH 7.0 ± 0.2 [52]. Luria-Bertani (LB) medium, used for both plating and liquid cultures, was prepared with 10 g tryptone, 5 g yeast extract, and 10 g NaCl per liter of ultrapure water, adjusted to pH 7.0 ± 0.2. For solid media, agar was added at a concentration of 15 g L^{-1} .

2.2. Screening and selection of PFOA-degrading strain

A soil sample, considered a potential source of PFOA-degrading microorganisms, was collected from a historically petroleum-contaminated industrial site near the Port of Montreal, Canada. 5 % (w/v) soil suspension was prepared in sterile MSM supplemented with 0.1 % yeast extract and 0.1 mg L^{-1} of PFOA. The slurry was incubated at 30 ± 1 °C under continuous shaking (150 rpm) for 7 days. To enrich PFOA-degrading microbes, sequential subculturing was conducted by transferring 5 % (v/v) of the pre-enriched culture into fresh MSM containing 0.1 % yeast extract and incrementally increasing PFOA concentrations (1, 10, and 100 mg L^{-1}). Each subculture cycle lasted 7 days under the same incubation conditions. In the final enrichment step, the bacterial community was subcultured in MSM amended solely with 100 mg L^{-1} PFOA [52]. This concentration is considered high and exerts toxic effects on microbial communities; thus, strains capable of growing at this level were considered as PFOA-resistant [20]. After four enrichment cycles, acclimated cultures were serially diluted and plated onto both PFOA-supplemented LB and MSM agar plates to isolate individual colonies.

Following isolation, the strains were screened for PFOA resistance by culturing individually in a 100 mg L^{-1} PFOA-MSM at 30 ± 1 °C for 165 h. Individual colonies of the isolated strains were pre-cultured in LB medium at 30 ± 1 °C under orbital shaking (150 rpm) overnight. Bacterial cells were harvested by centrifugation (10,000 × g, 10 min) and rinsed 3 times with a sterile phosphate buffer solution (pH 7.4) [PBS; composed (per liter) of 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4,]. Each of the obtained cells were resuspended in 50 mL of fresh MSM to achieve a target inoculum density of 10^7 CFU mL^{-1} . Experimental flasks were then amended with 100 mg L^{-1} PFOA and incubated under identical conditions. Cultures were sampled at 0, 22, 45, 69, 93, and 165 h to monitor bacterial growth via optical density at 600 nm ($OD_{600\,nm}$) [34]. Experiments were performed in duplicate.

2.3. PFOA-biodegradation tests using monoculture and consortium

The isolated strains were assessed for PFOA degradation potential by culturing individually, and as a consortium, in LB liquid medium supplemented with 0.5 mg L^{-1} PFOA. Pre-culture cell densities were quantified by 10-fold serial dilution and plate counting (triplicate). Based on these counts, the required inoculum volume was added to obtain a starting density of 10^7 CFU mL^{-1} , and the total working volume of each assay was adjusted to exactly 50 mL with LB medium. For the consortium, the inoculum volume for each strain was adjusted based on its measured CFU mL^{-1} to ensure equal contributions of viable cells, resulting in a combined starting density of 10^7 CFU mL^{-1} , consistent with the monocultures. All cultures were incubated at 30 ± 1 °C with continuous shaking (150 rpm) [9]. Samples were collected at 0, 7, 15, and 20 days, with corrections applied for evaporation losses based on pre- and post-sampling flask weights at each sampling interval [56]. Residual PFOA concentrations were quantified via LC-MS/MS (described below). Experiments were performed in duplicate, with abiotic control (no inoculum), and results are expressed as mean ± standard deviation. Statistical significance was assessed using two-way ANOVA (Analysis of Variance) followed by the Siegel–Tukey post hoc test, with $p < 0.05$ considered significant. Analyses were performed with SigmaPlot version 11. The strain demonstrating the highest degradation efficiency, designated strain OA1, was selected for further experiments.

2.4. Identification of selected isolate

Identification of the bacterial isolate was performed through 16S ribosomal RNA (rRNA) gene sequencing, following procedures described in a previous study [27]. Briefly, genomic DNA was extracted from freshly cultured isolates using the NucleoSpin® Microbial DNA kit (Macherey–Nagel, Germany). DNA concentration and purity were assessed using a Tecan NanoQuant Plate™ (Austria). The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using a thermal cycler (Eppendorf, Germany) and universal primers 27 F

(5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492 R (5′-TACGGYTACCTTG TTACGACTT-3′). PCR products were purified using the QIAquick® PCR Purification Kit (Qiagen, Germany), then sequenced at the DNA sequencing facility of the Ottawa Hospital Research Institute (Ottawa, Canada). The resulting sequences were compared against the NCBI GenBank 16S rRNA database using the BLAST algorithm. A sequence identity of greater than 99 % was required for species-level identification. Phylogenetic analysis was conducted using MEGA7 software, employing the neighbor-joining method [27]. The purified strain was preserved in 25 % (v/v) glycerol and stored at −80 °C for long-term viability.

2.5. PFOA-biodegradation tests using OA1 strain

PFOA degradation was assessed under two operational modes [24]. In the batch mode, the system was closed, with no additional nutrients provided throughout the experiment. In contrast, the fed-batch mode operated as a semi-open system, in which sterile LB medium (10 % v/v) was aseptically added every 7–10 days to maintain metabolic activity over time. Strain OA1 was first cultured in LB medium at 30 ± 1 °C with orbital shaking (150 rpm) for 32–48 h to reach the mid-exponential growth phase. The cell concentration was determined by standard plate counts, and experimental cultures were inoculated to achieve target initial cell densities of 10^7, 10^8, 10^9, and 10^{10} CFU mL^{-1}. No washing step was required before inoculation, as the composition of the pre-culture and experimental medium (LB) was identical. All tests were conducted in 125 mL sterilized Erlenmeyer flasks containing 50 mL of LB medium amended with 0.5 mg L^{-1} PFOA. Preliminary tests confirmed that shaking at 150 rpm for 125 mL Erlenmeyer flasks containing 50 mL working volume provided sufficient aeration, and no signs of oxygen limitation were observed under these conditions. Cultures were incubated at 30 ± 1 °C with continuous shaking for 30 days. Experiments were performed in triplicate, alongside abiotic controls (no inoculum) and cell growth controls (no PFOA), and results are presented as mean ± standard deviation. Differences among conditions were assessed using two-way ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$ (SigmaPlot v11). Culture purity and density were evaluated through regular plating on LB. Aliquots (1 mL) were collected at 0, 7, 15, 20, and 30 days, with corrections applied for evaporation losses based on pre- and post-sampling flask weights [56]. Residual PFOA concentrations were quantified via LC-MS/MS. The degradation efficiency (%) was calculated using the Equation (1):

$$\text{Degradation efficiency (\%)} = \frac{C_0 - C_t}{C_0} \times 100 \quad \text{Eq.(1)}$$

where C_0 is the initial concentration (mg L^{-1}), and C_t is the concentration at time t (mg L^{-1}). This equation expresses the percentage of PFOA removed from the aqueous phase over the course of the experiment.

The relationship between PFOA removal efficiency and cultivation time for strain OA1 was modeled using linear regression.

2.6. Quantification of PFOA

Samples from cultures were collected and centrifuged at 10,000 × g for 10 min to remove bacterial cells. The resulting supernatant was subjected to heat treatment at 80 °C for 10 min, then diluted fivefold with Milli-Q water. An aliquot of 25 µL of the isotopically labeled internal standard (M2PFOA, 1 ng mL^{-1}) was added to each sample prior to LC-MS/MS analysis. PFOA concentrations were analyzed using a high-performance liquid chromatography system coupled with a tandem mass spectrometer (LC-MS/MS), following the method described by [40]. Chromatographic separation was carried out on a HyperSil GOLD C18 column (Thermo Scientific; 2.1 × 100 mm, 3 µm particle size) maintained at 40 °C. The mobile phase consisted of (A) 1 mM ammonium acetate in water and (B) methanol, delivered at a flow rate of 0.3 mL min^{-1} under the following gradient: 20 % B (0–0.5 min), ramped to 95 % B (7–10 min), and re-equilibrated to 20 % B (10.5–14 min) (see Supplementary Table S3.1). A 10 µL injection volume was used via autosampler. Mass spectrometric detection was performed in negative electrospray ionization mode (ESI^-) with the following settings: spray voltage −4500 V, source temperature 300 °C, and nitrogen used as both nebulizing and collision gas. Quantification was conducted in multiple reaction monitoring (MRM) mode with a dwell time of 100 ms per transition. Specific MRM transitions and parameters for PFOA and M2PFOA are provided in Supplementary Table S3.2. PFOA concentrations were calculated by comparing the peak areas of the samples to a calibration curve generated from serial dilutions of reference standards. Results were corrected for analytical recovery using the internal standard (M2PFOA). Analyses were performed on biological triplicates, with one technical replicate per sample.

2.7. NMR Analysis of PFOA transformation products

^{19}F and ^1H NMR spectroscopy were employed to monitor changes in PFOA structure and detect potential transformation products. All experiments were performed on a Varian Inova 500 MHz NMR spectrometer, equipped with a QCI HFCN helium cryoprobe, SampleJet autosampler, and ATMA autotune system, controlled by MestReNova 9.0.1 software. ^{19}F spectra were acquired using the zgfhigqn.2 pulse sequence (1D ^{19}F with inverse-gated ^1H decoupling). A broad spectral window (-80 to −130 ppm) was used to capture potential signals from fluorinated degradation products beyond the parent PFOA (-CF_3 resonance ≈ −81.5 ppm). Key acquisition parameters included: acquisition time = 500 ms, relaxation delay = 3 s, calibrated 90° pulse ≈ 11 µs, and enough transients (typically 400–1096) to ensure detection sensitivity. The primary goal was to identify new ^{19}F resonances indicative of fluorinated metabolites. Complementary ^1H spectra were acquired on the same samples using the zgesgp pulse sequence. This aimed to detect signals from potential non-fluorinated organic degradation products or changes in background signals [5,21].

NMR analysis targeted degradation time points (0 h, 22 h, 45 h, 93 h, and 165 h) from cultures of OA1 (10^7 CFU mL^{-1}) grown in MSM containing 100 mg L^{-1} PFOA. Degradation samples were prepared in 10 % D_2O within 3 mm SampleJet NMR tubes (180–200 µL final volume). NMR spectra were recorded for biological duplicates, with one technical replicate per sample.

3. Results and discussion

3.1. Isolation and screening

Three bacterial strains (OA1, OA2, and OA3), exhibiting distinct colony morphologies, were isolated from enrichment cultures based on their ability to grow on LB and MSM agar plates supplemented with PFOA (Figs. 1A and 1B). Their growth in liquid MSM containing 100 mg L^{-1} PFOA was monitored over 165 h as shown in Fig. 1C. Among the tested isolates, the OA1 strain demonstrated the highest resistance to PFOA, as evidenced by a steady increase in optical density (OD_{600}) during the incubation period. The OD_{600} of OA1 peaked at ~0.4 after 45 h and remained stable through 165 h, indicating sustained growth under high PFOA levels. In contrast, OA2 and OA3 exhibited a decline in OD_{600} beyond 45 h, suggesting a lower tolerance to PFOA (Fig. 1C). Our results confirmed that only the OA1 strain was capable not only of surviving but also of actively growing under PFOA-induced stress, highlighting its potential for degradation even under strict nutrient conditions. These findings position OA1 as a strong candidate for further test for identification and bioremediation of PFOA.

Earlier studies have demonstrated that PFOA can negatively affect bacterial cells, even at low concentrations. It can damage cell membranes, cause oxidative stress, and interfere with DNA function, which impact cell growth and survival [6,16]. For instance, *Pseudomonas aeruginosa* exposed to just 0.1 mg L^{-1} PFOA showed a 16 % decrease in

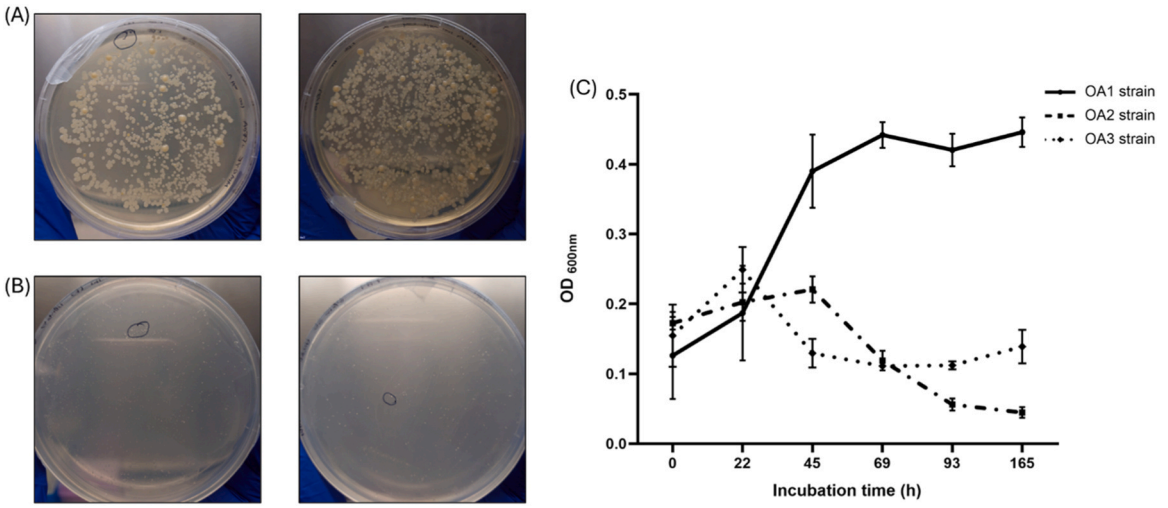


Fig. 1. Growth capacity of bacterial isolates OA1, OA2, and OA3 on minimal medium containing PFOA. (A) Colony formation on LB agar plates supplemented with PFOA; (B) Colony formation on MSM agar plates supplemented with PFOA; (C) Growth kinetics of OA1, OA2, and OA3 strains in liquid MSM medium containing 100 mg L^{-1} PFOA, monitored by OD_{600} over 165 h. Data are presented as mean ± SD from duplicate cultures.

optical density (OD_{600}) within the first two hours. Although this inhibition dropped to 5 % after 24 h, the initial response highlights the stress PFOA can cause before adaptation begins [9]. In contrast, strain OA1 demonstrated remarkable tolerance to PFOA exposure with minimal signs of initial inhibition even at a much higher PFOA concentration (100 mg L^{-1}). OA1 maintained steady growth over a 165-hour period, pointing to an unusually high tolerance level and suggesting that this strain possesses intrinsic resistance mechanisms. Similar trends were observed in a recent study by [47], where the presence of PFOA in drinking water systems was associated with increased growth of certain bacterial communities, such as *Hyphomicrobium*, *Bradyrhizobium*, and *Microbacterium*. Similarly, long-term PFAS exposure in soil has been shown to shift microbial community structure and enrich PFAS-tolerant taxa such as *Proteobacteria*, *Burkholderiales*, and *Rhodocyclales* [50]. These findings highlight that while PFOA is often considered broadly toxic, some bacteria can not only tolerate it but may even thrive in its presence. OA1 appears to belong to this emerging group of PFAS-tolerant strains.

3.2. Identification of selected strain

The 16S rRNA gene sequence analysis of strain OA1 revealed 99.77 % sequence identity to *Stenotrophomonas acidaminiphila*. Furthermore, BLAST analysis showed that OA1 shares 100 % sequence similarity with *Stenotrophomonas acidaminiphila* strain YFMCD4.4, indicating a potential close taxonomic relationship or possible identity with this strain. The 16S rRNA gene sequence of OA1 has been deposited in the NCBI GenBank database under the accession number PV916235.

The identification of strain OA1 as *S. acidaminiphila* represents a significant extension of the phylogenetic spectrum of known PFOA-degrading bacteria, which has been largely confined to *Pseudomonas* species [38,55]. Notably, *Pseudomonas parafulva* strain YAB1, isolated from soil proximal to a fluorochemical production site, demonstrated a PFOA degradation efficiency of 32.4 % under optimal laboratory conditions (30°C, pH 7, 2 % inoculum, 500 mg L^{-1} initial concentration) within 96 h [52]. Similarly, *Pseudomonas mosselii* strain 5, isolated from pesticide-contaminated soil, achieved complete degradation of 1 mg L^{-1} PFOA over 7 days under aerobic conditions at 28 °C [7]. Other species within the genus, including *Pseudomonas putida* and *Pseudomonas aeruginosa*, have displayed concentration-dependent PFOA removal efficiencies [9]. Beyond *Pseudomonas*, *Acidimicrobium* sp. strain A6 has been implicated in PFOA biodegradation (63 %) at 0.1 mg L^{-1} , in 100 days of incubation under anaerobic conditions [12,13,14].

In contrast to the best of our knowledge, no previous studies have reported the isolation of *Stenotrophomonas* strains capable of degrading PFOA. For instance, [11] identified *Stenotrophomonas* as the dominant bacterial genus in sediments downstream of a PFAS contamination source, representing 99.2 % relative abundance, with a strong positive correlation to PFAS concentration. These findings suggest that members of this genus are not only highly tolerant of PFAS but may also possess metabolic capabilities to thrive in such environments. However, no direct evidence has previously linked *Stenotrophomonas acidaminiphila* to active PFOA biotransformation. The identification of OA1 as a PFOA-degrading *S. acidaminiphila* strain thus provides the first empirical confirmation of its functional involvement in PFOA degradation. This discovery expands the currently narrow taxonomic scope of documented PFOA degraders and highlights *Stenotrophomonas* as a previously unrecognized genus with PFOA bioremediation potential. The phylogenetic distinction of *S. acidaminiphila* from established *Pseudomonas* degraders also suggests the presence of unique metabolic pathways, providing new perspectives on the microbial mechanisms underlying PFAS degradation.

Stenotrophomonas acidaminiphila is known for its ability to degrade a wide range of environmental pollutants, making it a relevant candidate for bioremediation applications [19]. Notably, it has demonstrated the ability to metabolize persistent polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene, pyrene [23]. It has also been identified as part of a bacterial consortium capable of breaking down benzopyrene, a particularly potent carcinogen [1,2].

In addition to hydrocarbons, this species can degrade pesticides like fipronil and chlorothalonil [43,54]. Furthermore, this bacterium has shown activity against biologically derived toxins. It degrades microcystins produced during cyanobacterial blooms [51], as well as aflatoxin B1, a potent mycotoxin contaminating food crops [41]. This metabolic versatility highlights the potential of *S. acidaminiphila* as a promising candidate for mitigating both chemically and biologically contaminated environments.

3.3. PFOA Biodegradation using monoculture and consortium

The PFOA degradation efficiency of a bacterial consortium composed of three individual strains (OA1, OA2, and OA3) and their respective monocultures over a 20-day incubation period is shown in Fig. 2**.** All strains were tested under identical conditions, with an initial PFOA concentration of 0.5 mg L^{-1} in LB medium and bacterial concentrations standardized at 10^7 CFU mL^{-1} . The results showed that strain

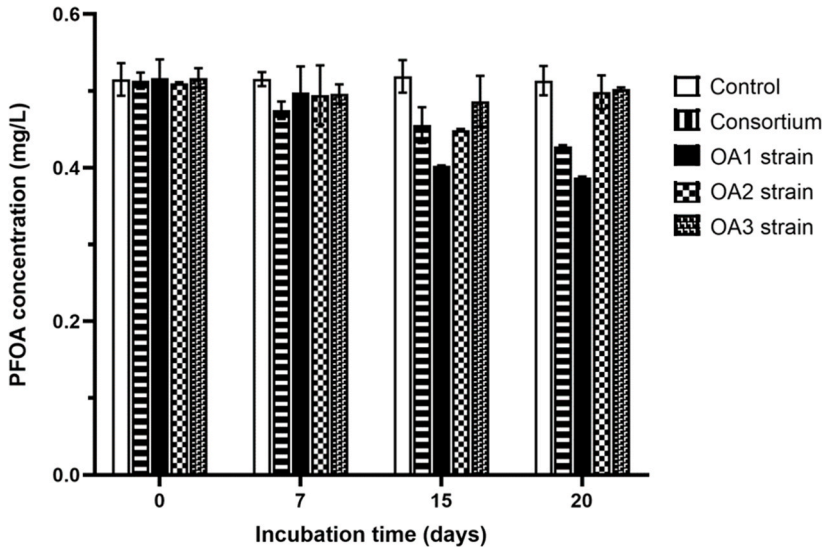


Fig. 2. PFOA degradation efficiency by a bacterial consortium composed of three strains (OA1, OA2, and OA3), compared to each strain in monoculture. Data are presented as mean ± SD from duplicate cultures.

S. acidaminiphila OA1 exhibited the greatest reduction in PFOA concentration by day 20, achieving a 20 % decrease (higher than that observed for OA2, OA3 and even the bacterial consortium). Statistical analysis indicated no significant difference between *S. acidaminiphila* OA1 and the consortium ($p = 0.187$). However, *S. acidaminiphila* OA1 was significantly more effective than OA2 and OA3 ($p < 0.001$). The difference in biodegradation capacity between OA2 and OA3 was minimal and not statistically significant ($p = 0.997$). Although the consortium demonstrated moderate performance, it did not surpass *S. acidaminiphila* OA1, suggesting that combining the strains did not enhance degradation efficiency (Fig. 2). These results indicate that *S. acidaminiphila* OA1 is the primary contributor to PFOA degradation, even within the consortium. The absence of inhibitory interactions during isolation initially led to the hypothesis of a potential synergistic effect; however, the data suggest that *S. acidaminiphila* OA1 alone accounts for most of the observed biodegradation activity. These findings align with and diverge from trends reported in previous research. Previous studies have highlighted the potential advantages of microbial consortia for the biodegradation of persistent pollutants, including perfluorinated compounds, due to their metabolic diversity and resilience to environmental fluctuations (Liang *et al.*, 2024; [46]). For example, Liang *et al.* (2024) showed that consortia predominantly comprised of *Hyphomicrobium* species (46.7 %) along with unclassified microorganisms (53.0 %) achieve higher removal rates of PFOS compared to single strains, with some studies reporting up to 56.7 % reduction over 20 days under optimized conditions with auxiliary substrates [22]. The rationale is that consortia can compensate for the metabolic limitations of individual strains, allowing for more complete or robust degradation pathways.

However, the present results indicate that, at least for the tested strains and conditions, the consortium did not enhance PFOA degradation beyond what was achieved by the most effective single strain (OA1). This outcome suggests that the additional strains in the consortium may not have contributed complementary metabolic functions necessary for further degradation, or that potential interspecies interactions did not facilitate a cooperative effect. Similar observations have been noted in recent studies, where the effectiveness of consortia can fluctuate depending on community composition, stability, and the presence of key degraders [46]. In some cases, the degradation activity of consortia is driven largely by a dominant strain, with other members providing limited or even inhibitory effects (Liang *et al.*, 2024).

These findings stand in contrast to the study by Tang et al. [42], where a native microbial consortium derived from PFAS-contaminated aquifer solids achieved a 13.52 % biological removal efficiency of PFOA after a 10-month incubation period under anoxic conditions. In their case, the microbial community was complex and enriched with low-abundance taxa such as *Flexilinea flocculi*, *Bacteriovorax stolpii*, and *Sphingomonas*, which were correlated with fluoride ion generation indicating active defluorination processes. Interestingly, although defluorination was confirmed, the overall removal efficiency was relatively low, possibly due to the slow metabolism and low growth rate of anaerobic communities [42].

3.4. Batch and Fed-Batch biodegradation of PFOA by S. acidaminiphila OA1

Batch and fed-batch experiments were conducted to assess the effect of initial cell density (10^7 to 10^{10} CFU mL^{-1}) and nutrient addition (10 % LB from day 7) on PFOA degradation by *S. acidaminiphila* OA1. For all degradation assays, actively growing OA1 cells harvested at mid-exponential phase were transferred into fresh LB medium supplemented with 0.5 $mg{\cdot}L^{-1}$ PFOA.

As shown in Fig. 3A, all tested cell densities exhibited progressive PFOA removal over the 30-day incubation period. Statistical analysis confirmed that while time was a highly significant factor ($p < 0.001$), the starting bacterial concentration did not significantly affect the final degradation efficiency across a 1,000-fold inoculum range ($p = 0.596$, two-way ANOVA; no significant pairwise differences by Tukey's test, $p > 0.05$). By day 30, PFOA removal efficiencies reached 42.0 % (10^7 CFU mL^{-1}), 43.7 % (10^8), 42.0 % (10^9), and 45.3 % (10^{10}) (Fig. 3A). Linear regression confirmed a consistent, time-dependent degradation pattern across all densities (Table 1), with the steepest slope ($a = 1.525$) observed at the highest tested concentration (10^{10} CFU mL^{-1}).

As illustrated in Fig. 3B, all fed-batch operations resulted in lower degradation efficiencies than batch operations by day 30 across all bacterial concentrations: 29.2 % vs. 42.0 % for 10^7, 32.1 % vs. 43.7 % for 10^8, 27.4 % vs. 42.0 % for 10^9, and 31.3 % vs. 45.3 % for 10^{10}. Linear regression further confirmed that the degradation rate was 28.5 % lower in fed-batch mode, with a steeper slope (1.080, $R^2 = 0.964$, $p = 0.018$) compared to batch (slope = 1.510, $R^2 = 0.992$, $p = 0.004$) at 10^8 CFU mL^{-1} (Table 1).

Our findings notably deviate from the established understanding that biodegradation efficiency depends strongly on active biomass, where higher cell densities typically enhance pollutant removal. This principle

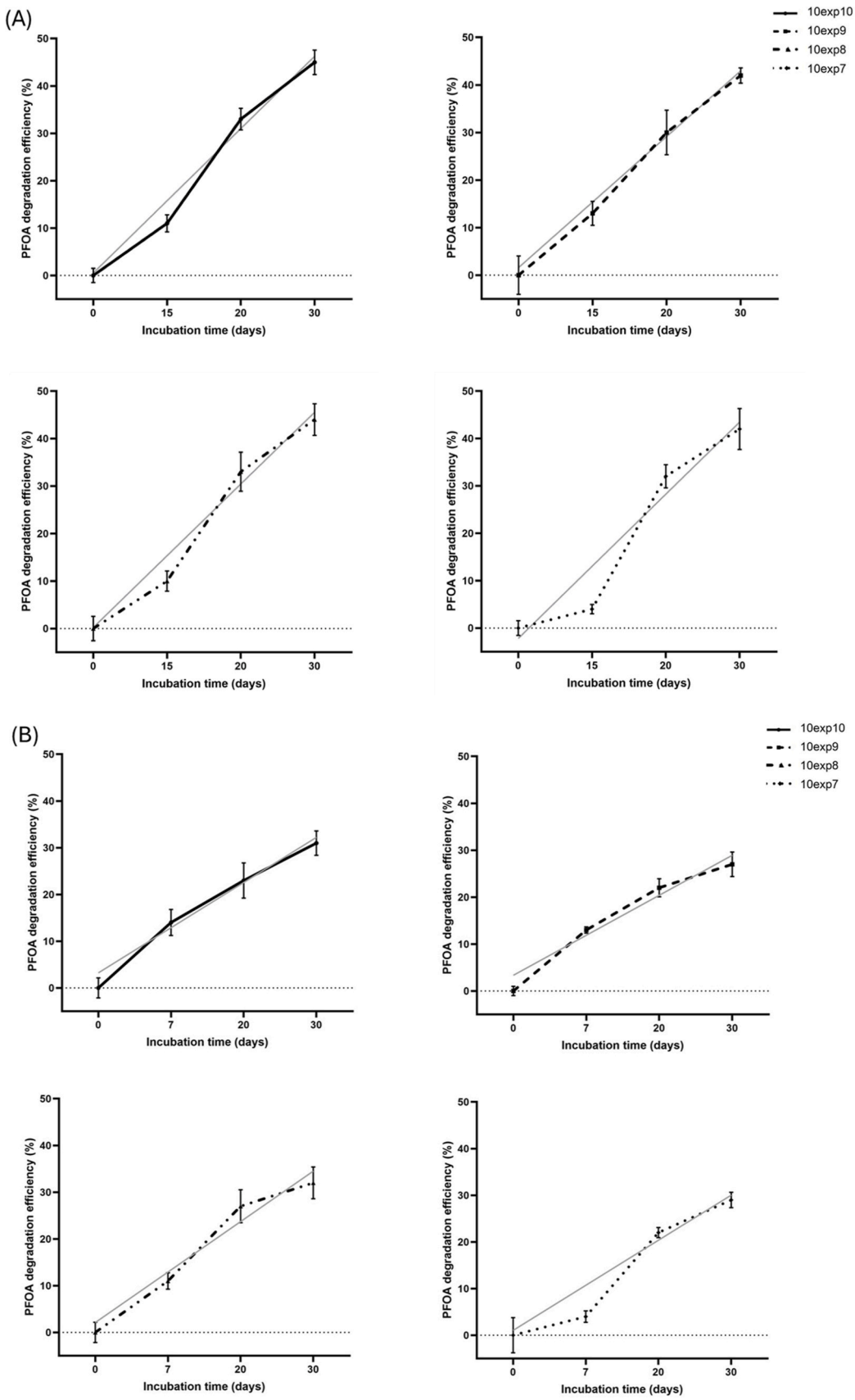


Fig. 3. PFOA degradation by *Stenotrophomonas acidaminiphila* OA1 under (A) batch and (B) fed-batch conditions. Data represent mean ± SD of triplicate cultures. Lines indicate zero-order kinetic fits with respective slopes (a) and R^2 values.

Table 1
PFOA biodegradation kinetic parameters obtained in batch and fed-batch modes.

Mode	Concentration	b	a	R^2	P value
Batch	10^{10}	0.513	1.525	0.995	0.002
	10^{9}	1.611	1.378	0.989	0.004
	10^{8}	0.239	1.510	0.988	0.004
	10^{7}	−2.177	1.521	0.968	0.016
Fed-batch	10^{10}	3.221	0.967	0.947	0.027
	10^{9}	3.354	0.852	0.926	0.038
	10^{8}	2.115	1.080	0.964	0.018
	10^{7}	−0.965	1.033	0.979	0.011

b = slope of the regression equation; a = intercept of the regression equation; R^2 = coefficient of determination; P-value = significance level of regression fit.

is evident in most microbial PFOA degradation studies, including the study on strain YAB1, where increasing the inoculum from 1 % to 2 % boosted PFOA degradation; however, higher inocula (>2 %) caused growth inhibition due to nutrient limitation [52]. Similarly, Chiriac et al. [9] reported that *P. putida* achieved a 19 % reduction in PFOA concentration over a 96-hour incubation period. Although the authors did not explicitly provide the cell count used, they did mention that bacterial strain with an optical density of 0.2 at 600 nm was used in the experiments, suggesting that degradation rates were linked to the number of active cells [9]. Unlike these strains, *S. acidaminiphila* OA1 showed a degradation pattern that was primarily time-dependent and unaffected by initial cell density, pointing to a distinct metabolic strategy.

In comparison with batch mode, fed-batch conditions resulted in lower final degradation efficiency and slower degradation kinetics. The addition of LB likely caused a metabolic shift away from PFOA metabolism, a dilution effect, and potentially disrupted the catabolic balance, reducing both viability and degradation efficiency. This interpretation is supported by viable cell counts at day 30, showing 15 % reduced viability in fed-batch (8.5×10^8 CFU mL^{-1}) compared to batch (10^9 CFU mL^{-1}) at equivalent starting concentrations of 10^9 CFU mL^{-1}. The reduced degradation efficiency under fed-batch conditions indicates that *S. acidaminiphila* OA1 preferentially metabolizes the simpler nutrients present in LB medium over PFOA. This preference aligns with observations in activated sludge systems where PFAS removal is often dominated by adsorption, with biodegradation being minor or inhibited [8], and where PFAS presence can severely disrupt nutrient removal performance and microbial ecology [17]. Therefore, while conventional bioreactor design emphasizes maintaining high active biomass through nutrient management, our findings with *S. acidaminiphila* OA1 suggest that such strategies could be counterproductive for PFOA degradation by this specific strain, as supplemental nutrients divert metabolic activity away from the target pollutant.

Table 2 provides an overview of reported PFOA biodegradation performances by different bacterial strains, summarizing tested concentrations, degradation efficiencies, experimental conditions, and literature sources. This comparison places the results obtained with *S. acidaminiphila* OA1 into a broader context, highlighting its relative efficiency under similar or varying conditions. Despite these promising performances, microbial approaches to PFAS degradation still present several limitations. The stability of the carbon–fluorine bond makes microbial breakdown inherently difficult, and only a few microorganisms have evolved the capacity to attack these compounds. As a result, degradation is often slow and incomplete, leading to the accumulation of intermediate products such as shorter-chain PFAS, and the release of inorganic fluoride can further inhibit microbial activity. Moreover, microbial performance is strongly influenced by environmental variables such as pH, temperature, and co-contaminants, and most published studies remain restricted to laboratory conditions, with limited evidence at pilot or field scale [28]. These limitations highlight the need for continued optimization and, potentially, integration of microbial strategies with complementary remediation technologies.

Table 2
Comparative Overview of PFOA Biodegradation by Various Bacterial Strains.

Strain	Conc. (mg L^{-1})	Degradation effenciency (%)	Conditions	References
Pseudomonas mosselii strain 5	1	100	Aerobic, 28 °C, 7 days	Chetverikov et al., [7]
Pseudomonas plecoglossicida DD4	1000	100	Aerobic, 28°C, 96 h	(Chetverikov *et al.*, 2022)
Pseudomonas parafulva strain YAB1	500	32.4(48.1)	30°C, pH 7, 2 % inoculum, 96 h, (with 1 g/L glucose as co-substrate)	Yi et al., [52]
Pseudomonas putida	0.1, 1 and 10	1.84; 12.3; 19.0	Aerobic, 96 h (higher efficiency at lower conc.)	Chiriac et al., [9]
Pseudomonas aeruginosa	0.1, 1 and 10	3.17; 12.0; 27.9	Aerobic, 96 h (higher efficiency at lower conc.)	Chiriac et al., [9]
Acidimicrobium sp. Strain A6	0.1 and 100	63; 50	Anaerobic, 30°C, 100 days	(Huang *et al.*, 2019)
Stenotrophomonas acidaminiphila OA1	0.5	45.3	Batch mode, aerobic, 30°C, pH 7, 30 days	This work

Conc. = initial PFOA concentration; Degradation efficiency (%) = percentage of PFOA removed relative to initial concentration. Values in parentheses indicate efficiency in the presence of a co-substrate.

3.5. Investigation on possible PFOA transformation products

As *S. acidaminiphila* OA1 strain exhibited a progressive reduction in PFOA concentration over time, a survey on possible metabolites was performed using ^{1}H and ^{19}F NMR spectroscopy. NMR analysis targeted selected degradation time points (0 h, 22 h, 45 h, 93 h, and 165 h) from *S. acidaminiphila* OA1 cultures grown under stress conditions, in MSM supplemented with 100 mg L^{-1} PFOA. Although this condition did not correspond to the most pronounced degradation observed across all trials, it was chosen to assess whether any detectable transformation could occur under extreme exposure. The goal was to capture potential early-stage metabolites or structural changes within a short timeframe, even before significant overall degradation had occurred.

^{1}H ^{1}D NMR spectra revealed multiple novel signals that were absent in the non-inoculated control sample (Fig. 4A and B). Given that native PFOA is fully fluorinated and contains no hydrogen atoms on its carbon backbone, these signals provide clear evidence of PFOA biotransformation by strain OA1. The most informative signals appeared in the downfield region between 3.0 and 3.8 ppm (Fig. 4A). This area contained a series of multiplets, particularly between 3.4 and 3.8 ppm, which are characteristic of protons on a carbon adjacent to carboxylic group or one or more strongly electronegative atoms, such as fluorine. Likely structures would include –CH_2–COOH, –CH_2–CF_2–. This observation strongly suggests the introduction of hydrogen onto the PFOA backbone via hydrodefluorination specifically at the α-carbon, a chemically reactive site. Hydrodefluorination is a plausible pathway that would yield a product like COOH−CH_2−$(CF_2)_6$−CF_3, whose α-methylene protons would resonate in the observed range. The absence of new signals in the typical aliphatic region (1.2–1.4 ppm) argues against extensive defluorination or the formation of long hydrocarbon chains. Further analysis of the ^{1}H spectrum revealed a sharp singlet at 8.35 ppm, a hallmark of a proton in a highly deshielded environment (Fig. 4B).

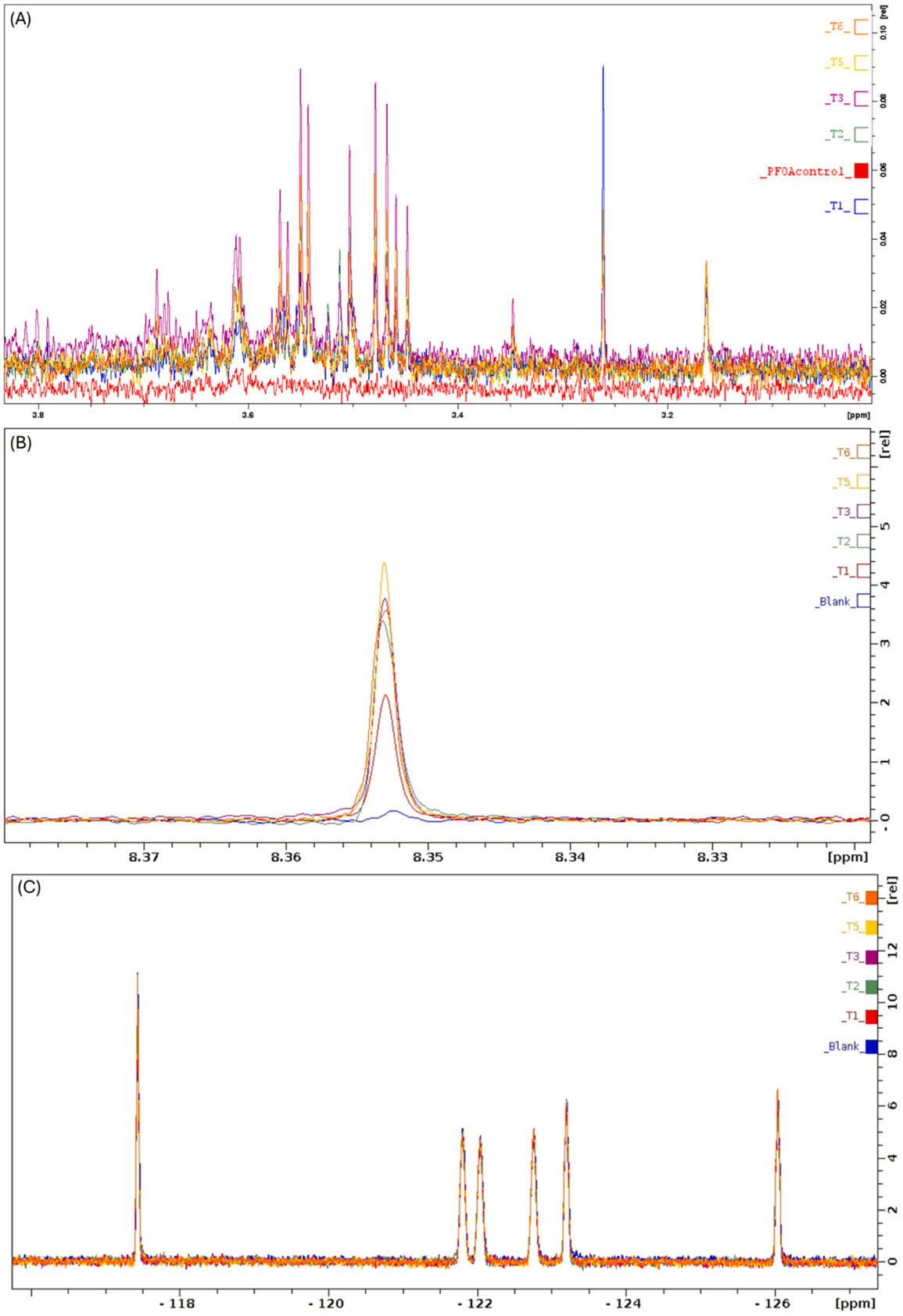


Fig. 4. NMR analysis of PFOA biotransformation by strain OA1. **(A)** ^{1}H NMR spectra 3.0–3.8 ppm; **(B)** ^{1}H NMR spectra 8.32–8.37 ppm; **(C)** ^{1} ^{9}F NMR spectra. Time points: T1 = 0 h, T2 = 22 h, T3 = 45 h, T5 = 93 h, T6 = 165 h.

Such downfield shift with singlet multiplicity is likely attributable to a small, oxidized formyl group (–CHO) metabolites such as formic acid (HCOOH), suggesting that oxidative chain scission is another key transformation pathway. Additionally, three distinct singlets between 3.1 and 3.4 ppm (Fig. 4A) point to the formation of small, non-fluorinated breakdown products. Their singlet multiplicity excludes coupling to adjacent fluorine, suggesting structures like methyl esters ($-O-CH_3$) or short-chain alcohols.

Complementary ^{1} ^{9}F NMR analysis showed no significant changes in the fluorine signals of PFOA over the incubation period (Fig. 4**C**). The absence of additional ^{1} ^{9}F signals does not exclude transformation but implies that only a limited number of fluorinated positions were affected, or that fluorinated intermediates remain below the detection threshold of the current NMR acquisition settings. This finding, when paired with the ^{1}H NMR data, supports a model of localized, rather than global, transformation. The perfluorinated carbon skeleton of the bulk PFOA remains largely intact, with modifications confined to specific, reactive sites.

Notably, the chemical shifts of all ^{1}H signals were consistently detected across all time points (22–165 h), indicating the steady accumulation of stable transformation products. This consistency implies that the observed metabolites result from selective, enzyme-driven mechanisms rather than random chemical degradation. Taken together, the NMR evidence points to a clear model of early-stage PFOA biotransformation by *S. acidaminiphila* OA1, involving targeted hydrodefluorination at reactive carbons and subsequent oxidative cleavage. However, complementary detection systems and more experiments are needed, to further validate the exact chemical identity of the metabolites and their toxicity.

To our knowledge, this is the first study to apply NMR spectroscopy for capturing early-stage structural changes in PFOA during bacterial transformation. Previous research has used ^{1} ^{9}F NMR to trace fluorophenol degradation by *Rhodococcus* ssp. Bondar et al., [4]. Both Chiriac et al. [9] and Huang *et al.* (2019) reported the sequential shortening of the perfluoroalkyl chain in PFOA, identifying intermediates such as PFHpA, PFHxA, and PFPeA using LC-MS analysis ([9]; Huang *et al.*, 2019). Our approach provides a complementary method that captures a more immediate picture of the transformation process. Although definitive intermediates were not resolved in this instance, our work establishes a methodological proof-of-concept for using NMR to gain direct structural insights into the initial steps of long-chain PFAS biodegradation, while also highlighting the current limitation of not assessing overall mineralization.

4. Conclusion

This study demonstrated the successful isolation and characterization of a high-performing PFOA-degrading bacterium, strain OA1, identified as *Stenotrophomonas acidaminiphila*, from a petroleum-contaminated soil environment. *S. acidaminiphila* OA1 demonstrated consistent and efficient PFOA degradation under batch conditions, reaching up to 45 % removal in 30 days, a performance superior to both other isolates and consortium. Interestingly, degradation efficiency was not significantly affected by inoculum size across a 1,000-fold range, challenging common assumptions that higher biomass enhances biodegradation. In contrast to many PFAS biodegradation strategies that rely on maximizing active cell densities, *S. acidaminiphila* OA1 maintained its activity regardless of cell concentration, suggesting an alternative degradation mechanism. Nutrient supplementation in fed-batch mode further revealed that additional of extra organic matter may disrupt degradation performance, likely due to metabolic prioritization or dilution effects. To deepen our understanding of this process, ^{1}H and ^{1} ^{9}F NMR spectroscopy was employed to investigate early-stage transformation products. To our knowledge, this is the first application of NMR to specifically track structural changes in PFOA during bacterial transformation. Overall, the findings highlight *S. acidaminiphila* OA1's potential as a robust candidate for potential scalable bioremediation applications targeting persistent contaminants like PFOA. Although promising, future evaluations of biodegradation performance in real water matrices such as groundwater, surface water, or industrial wastewater are still needed to fully assess the performance of this newly isolated strain.

Environmental Implication

This study demonstrates, for the first time, the ability of *Stenotrophomonas acidaminiphila* OA1 to biotransform perfluorooctanoic acid (PFOA), a persistent and toxic PFAS compound, under environmentally relevant conditions. The strain maintained growth at realistic contamination levels and achieved up to 45 % degradation over 30 days without nutrient supplementation. NMR analysis revealed selective backbone modifications, suggesting a targeted degradation mechanism. These findings highlight OA1's potential as a robust candidate for sustainable bioremediation of fluorinated contaminants, offering a low-cost and biologically viable alternative to current energy-intensive PFOA treatment technologies.

CRediT authorship contribution statement

Nihed Tellili: Writing – original draft, Methodology, Investigation, Data curation. **Saba Miri:** Writing – original draft, Formal analysis. **Kaur Brar Satinder:** Supervision, Funding acquisition. **Rouissi Tarek:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank the funding supported by the Natural Sciences and Engineering Research Council of Canada (Discovery Grant) and Inrs startup Grant.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.hazmo.2025.100002.

Data availability

Data will be made available on request.

References

[1] S.M. Davoodi, S. Miri, S.K. Brar, R. Martel, Formulation of synthetic bacteria consortia for enzymatic biodegradation of polyaromatic hydrocarbons contaminated soil: soil column study, Environ. Sci. Pollut. Res. 30 (28) (2023) 72793–72806.

[2] A. Aziz, P. Agamuthu, F.O. Alaribe, S.H. Fauziah, Biodegradation of benzo[a] pyrene by bacterial consortium isolated from mangrove sediment, Environ. Technol. 39 (4) (2018) 527–535.

[3] A. Berhanu, I. Mutanda, J. Taolin, M.A. Qaria, B. Yang, D. Zhu, A review of microbial degradation of per- and polyfluoroalkyl substances (PFAS): biotransformation routes and enzymes, Sci. Total Environ. 859 (Pt 1) (2023) 160010.

[4] V.S. Bondar, M.G. Boersma, E.L. Golovlev, J. Vervoort, W.J. Van Berkel, Z. I. Finkelstein, I.P. Solyanikova, L.A. Golovleva, I.M. Rietjens, ^{19}F NMR study on the biodegradation of fluorophenols by various rhodococcus species, Biodegradation 9 (6) (1998) 475–486.

[5] D. Camdzic, R.A. Dickman, D.S. Aga, Total and class-specific analysis of per- and polyfluoroalkyl substances in environmental samples using nuclear magnetic resonance spectroscopy, J. Hazard. Mater. Lett. 2 (2021) 100023.

[6] C. Chen, Y. Fang, X. Cui, D. Zhou, Effects of trace PFOA on microbial community and metabolisms: microbial selectivity, regulations and risks, Water Res 226 (2022) 119273.

[7] S. Chetverikov, G. Hkudaygulov, D. Sharipov, S. Starikov, D. Chetverikova, Biodegradation potential of C(7)-C(10) perfluorocarboxylic acids and data from the genome of a new strain of pseudomonas mosselii 5(3), Toxics 11 (12) (2023).

[8] Chiavola A., Di Marcantonio C., Boni M.R., Biagioli S., Frugis A., Cecchini G. (2020) PFOA and PFOS Removal Processes in Activated Sludge Reactor at Laboratory Scale. Frontiers in Water-Energy-Nexus—Nature-Based Solutions, Advanced Technologies and Best Practices for Environmental Sustainability. (Cham, 2020//), Naddeo V, Balakrishnan M,Choo K-H (Édit.) Springer International Publishing, p 375-377.

[9] F.L. Chiriac, C. Stoica, C. Iftode, F. Pirvu, V.A. Petre, I. Paun, L.F. Pascu, G. G. Vasile, M. Nita-Lazar, Bacterial biodegradation of perfluorooctanoic acid (PFOA) and perfluorosulfonic acid (PFOS) using pure pseudomonas strains, Sustainability 15 (18) (2023).

[10] L.G.T. Gaines, Historical and current usage of per- and polyfluoroalkyl substances (PFAS): a literature review, Am. J. Ind. Med 66 (5) (2023) 353–378.

[11] C. Guo, L. Ahrens, S. Bertilsson, M.J.L. Coolen, J. Tang, Riverine microbial communities impacted by per- and polyfluoroalkyl substances (PFAS) emissions from a fluoropolymer-manufacturing plant, J. Hazard Mater. 457 (2023) 131803.

[12] S. Huang, C. Chen, X. Peng, P.R. Jaffé, Environmental factors affecting the presence of acidimicrobiaceae and ammonium removal under iron-reducing conditions in soil environments, Soil Biol. Biochem. 98 (2016) 148–158.

[13] S. Huang, P.R. Jaffe, Defluorination of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) by acidimicrobium sp. Strain A6, Environ. Sci. Technol. 53 (19) (2019) 11410–11419.

[14] S. Huang, P.R. Jaffé, Characterization of incubation experiments and development of an enrichment culture capable of ammonium oxidation under iron-reducing conditions, Biogeosciences 12 (3) (2015) 769–779.

[15] ITRC (2020) PFAS — Per- and Polyfluoroalkyl Substances — Treatment Technologies. https://pfas-1.itrcweb.org/12-treatment-technologies.

[16] Y. Jeong, K. Vyas, J. Irudayaraj, Toxicity of per- and polyfluoroalkyl substances to microorganisms in confined hydrogel structures, J. Hazard Mater. 456 (2023) 131672.

[17] J. Ji, L. Peng, M.M. Redina, T. Gao, A. Khan, P. Liu, X. Li, Perfluorooctane sulfonate decreases the performance of a sequencing batch reactor system and changes the sludge microbial community, Chemosphere 279 (2021) 130596.

[18] S.M. Khumalo, B.F. Bakare, S. Rathilal, The occurrence and bioremediation of emerging polyfluorinated compounds in water bodies: a mini review, Appl. Sci. 12 (23) (2022).

[19] A. Kumar, L. Rithesh, V. Kumar, N. Raghuvanshi, K. Chaudhary, Abhineet, A. K. Pandey, Stenotrophomonas in diversified cropping systems: friend or foe? Front Microbiol 14 (2023) 1214680.

[20] J.A. LaFond, P.B. Hatzinger, J.L. Guelfo, K. Millerick, W.A. Jackson, Bacterial transformation of per- and poly-fluoroalkyl substances: a review for the field of bioremediation, Environmental Science Advances 2 (8) (2023) 1019–1041.

[21] S.T. Larda, Y. Ayotte, M.M. Denk, P. Coote, G. Heffron, D. Bendahan, F. Shahout, N. Girard, M. Iddir, P. Bouchard, F. Bilodeau, S. Woo, L.J. Farmer, S.R. LaPlante, Robust strategy for Hit-to-Lead discovery: NMR for Sar, J. Med. Chem. 66 (19) (2023) 13416–13427.

[22] Y. Liang, A. Ma, Investigating the degradation potential of microbial consortia for perfluorooctane sulfonate through a functional "top-down" screening approach, PLoS One 19 (5) (2024) e0303904.

[23] N. Mangwani, S.K. Shukla, S. Kumari, T.S. Rao, S. Das, Characterization of stenotrophomonas acidaminiphila NCW-702 biofilm for implication in the degradation of polycyclic aromatic hydrocarbons, J. Appl. Microbiol 117 (4) (2014) 1012–1024.

[24] F. Marchetto, M. Roverso, D. Righetti, S. Bogialli, F. Filippini, E. Bergantino, E. Sforza, Bioremediation of Per- and Poly-Fluoroalkyl substances (PFAS) by synechocystis sp. Pcc 6803: a chassis for a synthetic biology approach, Life (Basel) 11 (12) (2021).

[25] J.N. Meegoda, B. Bezerra de Souza, M.M. Casarini, J.A. Kewalramani, A review of PFAS destruction technologies, Int J. Environ. Res Public Health 19 (24) (2022).

[26] V. Mendez, S. Holland, S. Bhardwaj, J. McDonald, S. Khan, D. O'Carroll, R. Pickford, S. Richards, C. O'Farrell, N. Coleman, M. Lee, M.J. Manefield, Aerobic biotransformation of 6:2 fluorotelomer sulfonate by dietzia aurantiaca J3 under sulfur-limiting conditions, Sci. Total Environ. 829 (2022) 154587.

[27] S. Miri, A. Rasooli, S.K. Brar, T. Rouissi, R. Martel, Biodegradation of p-xylene-a comparison of three psychrophilic pseudomonas strains through the lens of gene expression, Environ. Sci. Pollut. Res Int 29 (15) (2022) 21465–21479.

[28] S. Miri, T. Robert, S.M. Davoodi, S.K. Brar, R. Martel, T. Rouissi, J.M. Lauzon, Evaluation of scale-up effect on cold-active enzyme production and biodegradation tests using pilot-scale bioreactors and a 3D soil tank, J. Hazard Mater. 450 (2023) 131078.

[29] Novak C., Parker J., Twaddell O. (2023) A SURVEY OF TRENDS IN PFAS REGULATION AND LITIGATION. *Brief* 52(4):16+. https://link.gale.com/apps/doc/A780930118/AONE?u=anon~89ce1178&sid=googleScholar&xid=5a836ae2.

[30] OECD (2021) *Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl Substances: Recommendations and Practical Guidance*. Parisp.

[31] OECD (2022) *Portal on Per and Poly Fluorinated Chemicals.*, ⟨https://www.oecd.org/en/topics/sub-issues/risk-management-risk-reduction-and-sustainable-chemistry.html⟩.

[32] M. Pavuk, J.L. Adgate, S.M. Bartell, E. Bell, L.M. Brown, R.J. Laumbach, L. A. Schaider, van T' Erve TJ, J.M. Bailey, J.C. Botelho, A.M. Calafat, C.R. Cutler, S. Forand, J.M. Graber, T. James-Todd, Z. Jeddy, K. Kato, N. Mowry, A.S. Nair, P. Ohman-Strickland, P. Rago, A.M. Schaefer, A.P. Starling, V.M. Vieira, M. M. Weems, K.F. Wiant, F.J. Bove, Multi-site study of communities with PFAS-contaminated drinking water: methods, demographics, and PFAS serum concentrations, Environ. Int 202 (2025) 109589.

[33] R. Prabhudesai, C. D'Costa, D. Denny, P. Awasthi, London. 3M in 2024: Tiding Over Colossal Challenges, SAGE Publications: SAGE Business Cases Originals, 2025.

[34] A. Presentato, S. Lampis, A. Vantini, F. Manea, F. Dapra, S. Zuccoli, G. Vallini, On the ability of perfluorohexane sulfonate (PFHxS) bioaccumulation by two pseudomonas sp. Strains isolated from PFAS-Contaminated environmental matrices, Microorganisms 8 (1) (2020).

[35] H. Vatankhah, R.H. Anderson, R. Ghosh, J. Willey, A. Leeson, A review of innovative approaches for onsite management of PFAS-impacted investigation derived waste, Water Res. 247 (2023) 120769.

[36] M. Riegel, S. Egner, F. Sacher, B. Beuthe, M. Bonte, M. Hjort, Rev. Water Treat. Syst. PFAS Remov. (2020).

[37] E. Shahsavari, D. Rouch, L.S. Khudur, D. Thomas, A. Aburto-Medina, A.S. Ball, Challenges and current status of the biological treatment of PFAS-Contaminated soils, Front Bioeng. Biotechnol. 8 (2020) 602040.

[38] J.P. Skinner, A. Raderstorf, B.E. Rittmann, A.G. Delgado, Biotransforming the "forever chemicals": trends and insights from microbiological studies on PFAS, Environ. Sci. Technol. 59 (11) (2025) 5417–5430.

[39] K.L. Smalling, K.M. Romanok, P.M. Bradley, M.C. Morriss, J.L. Gray, L.K. Kanagy, S.E. Gordon, B.M. Williams, S.E. Breitmeyer, D.K. Jones, L.A. DeCicco, C.A. Eagles-Smith, T. Wagner, Per- and polyfluoroalkyl substances (PFAS) in United States tapwater: comparison of underserved private-well and public-supply exposures and associated health implications, Environ. Int 178 (2023) 108033.

[40] S. Sorn, H. Hara-Yamamura, S. Vet, M. Xiao, E.M.V. Hoek, R. Honda, Biological treatment of perfluorooctanesulfonic acid (PFOS) using microbial capsules of a polysulfone membrane, Chemosphere 329 (2023) 138585.

[41] J. Suo, T. Liang, H. Zhang, K. Liu, X. Li, K. Xu, J. Guo, Q. Luo, S. Yang, Characteristics of aflatoxin B(1) degradation by stenotrophomonas acidaminiphila and it's combination with black soldier Fly larvae, Life (Basel) 13 (1) (2023).

[42] Z. Tang, T.M. Vogel, Q. Wang, C. Wei, M. Ali, X. Song, Microbial defluorination of TFA, PFOA, and HFPO-DA by a native microbial consortium under anoxic conditions, J. Hazard Mater. 465 (2024) 133217.

[43] S. Uniyal, R. Paliwal, R.K. Sharma, J.P.N. Rai, Degradation of fipronil by stenotrophomonas acidaminiphila isolated from rhizospheric soil of zea mays, 3 Biotech 6 (1) (2016) 48.

[44] US EPA (2022) *Our Current Understanding of the Human Health and Environmental Risks of PFAS.*, ⟨https://www.epa.gov/pfas/our-current-understanding-human-health-and-environmental-risks-pfas⟩.

[45] US EPA (2024) *Final PFAS National Primary Drinking Water Regulation.* ⟨https://www.epa.gov/sdwa/and-polyfluoroalkyl-substances-pfas⟩.

[46] L.P. Wackett, Why is the biodegradation of polyfluorinated compounds so rare? mSphere 6 (5) (2021) e0072121.

[47] H. Wang, X. Tao, H. Yin, X. Xing, B. Shi, The perfluorooctanoic acid accumulation and release from pipelines promoted growth of bacterial communities and opportunistic pathogens with different antibiotic resistance genes in drinking water, J. Hazard Mater. 478 (2024) 135600.

[48] D.M. Wanninayake, Comparison of currently available PFAS remediation technologies in water: a review, J. Environ. Manag. 283 (2021) 111977.

[49] Y. Xie, G. Chen, A.L. May, J. Yan, L.P. Brown, J.B. Powers, S.R. Campagna, F. E. Loffler, Pseudomonas sp. Strain 273 degrades fluorinated alkanes, Environ. Sci. Technol. 54 (23) (2020) 14994–15003.

[50] R. Xu, W. Tao, H. Lin, D. Huang, P. Su, P. Gao, X. Sun, Z. Yang, W. Sun, Effects of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) on soil microbial community, Micro Ecol. 83 (4) (2022) 929–941.

[51] F. Yang, Y. Zhou, L. Yin, G. Zhu, G. Liang, Y. Pu, Microcystin-degrading activity of an indigenous bacterial strain stenotrophomonas acidaminiphila MC-LTH2 isolated from lake taihu, PLoS One 9 (1) (2014) e86216.

[52] L.B. Yi, L.Y. Chai, Y. Xie, Q.J. Peng, Q.Z. Peng, Isolation, identification, and degradation performance of a PFOA-degrading strain, Genet Mol. Res 15 (2) (2016).

[53] Y. Yu, K. Zhang, Z. Li, C. Ren, J. Chen, Y.H. Lin, J. Liu, Y. Men, Microbial cleavage of C-F bonds in two C(6) Per- and polyfluorinated compounds via reductive defluorination, Environ. Sci. Technol. 54 (22) (2020) 14393–14402.

[54] Q. Zhang, H. Liu, M. Saleem, C. Wang, Biotransformation of chlorothalonil by strain stenotrophomonas acidaminiphila BJ1 isolated from farmland soil, R. Soc. Open Sci. 6 (11) (2019) 190562.

[55] Z. Zhang, D. Sarkar, J.K. Biswas, R. Datta, Biodegradation of per- and polyfluoroalkyl substances (PFAS): a review, Bioresour. Technol. 344 (Pt B) (2022) 126223.

[56] L. Zhou, W. Li, J. Zhang, H. Mao, Removal of perfluorooctanoic acid (PFOA) in the liquid culture of phanerochaete chrysosporium, Chemosphere 345 (2023) 140427.