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Bacterial Biodegradation of Perfluorooctanoic Acid (PFOA) and Perfluorosulfonic Acid (PFOS) Using Pure *Pseudomonas* Strains

Florentina Laura Chiriac *,†, Catalina Stoica †, Cristina Iftode, Florinela Pirvu, Valentina Andreea Petre, Iuliana Paun, Luoana Florentina Pascu, Gabriela Geanina Vasile and Mihai Nita-Lazar

National Research and Development Institute for Industrial Ecology—ECOIND, Drumul Podu Dambovitei Street 57–73, 060652 Bucharest, Romania; catalina.stoica@incdecoind.ro (C.S.); cristina.iftode@ecoind.ro (C.I.); florinela_pirvu@yahoo.com (F.P.); petrevalentinaandreea@gmail.com (V.A.P.); iuliana.paun@incdecoind.ro (I.P.); ecoind@incdecoind.ro (L.F.P.); gabriela.vasile@incdecoind.ro (G.G.V.); mihai.nita@incdecoind.ro (M.N.-L.)

* Correspondence: laura.chiriac@incdecoind.ro

† These authors contributed equally to this work.

Abstract: The principal objective of the present research involved the achievement of high biodegradation degrees of perfluorooctanoic acid (PFOA) and perfluorosulfonic acid (PFOS) using pure individual bacterial strains. The use of such microorganisms can contribute to the improvement of the wastewater treatment process in sewage treatment plants through bioaugmentation or other bioremediation processes. Thus, in this study, we investigated the biodegradation potential of PFOA and PFOS. Bacterial strains tested in this study were from the *Pseudomonas* genus, namely: *Pseudomonas aeruginosa* and *Pseudomonas putida*, due to their known capacity to degrade xenobiotic compounds. The results indicated that *Pseudomonas aeruginosa* was able to transform 27.9% of PFOA and 47.3% of PFOS in 96 h, while *Pseudomonas putida* managed to transform 19.0% of PFOA and 46.9% of PFOS in the same time frame. During the biodegradation tests, PFHxA was recognized as the principal biotransformation product of PFOA in the presence of *Pseudomonas aeruginosa*, and PFPeA, PFPxA and PFHpA were recognized as the biotransformation products in the presence of *Pseudomonas putida*. For PFOS, only two biotransformation products (PHHxA and PFHpA) were observed as a consequence of biodegradation by both bacterial strains.

Keywords: PFOA; PFOS; *pseudomonas* strains; biodegradation; kinetics; biotransformation products

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1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are recognized being highly difficult organic contaminants to treat in aquatic ecosystems [1]. PFAS are used widely in a variety of customer products. For this reason, these substances are considered to be omnipresent in the environment and are highly persistent. Perfluorooctanoic acid (PFOA) and perfluorosulfonic acid (PFOS) are two of the most common PFAS; they have been extensively produced and can also be obtained by degradation processes of various precursors. PFOS and PFOA were found in many environmental compartments and represent a great concern for both biota and human health [2,3]. The negative effects of PFOA and PFOS on the wildlife and humans could be summarized in the wide distribution, huge persistence, bioaccumulation predisposition and potential toxicological repercussions [4–7]. Therefore, finding new biodegradation methods for destruction of these organic pollutants represents a matter of increasing necessity, as does considering economically and eco-friendly methods.

In spite of the initial discouraging experimental data regarding the biodegradability of PFAS under environmental conditions, because of their high resistance, scientists continued to analyze different microorganisms for their ability to degrade PFAS. In the last decade, several studies discovered that different single or mixed microbial types were able to degrade some PFAS compounds, such as perfluorinated alkyl acids (PFAA), PFOA and PFOS [8–12].

The cases where biodegradation has been successful have created opportunities for biological treatment with PFAS, although the transformation depends on the systemic intricacy of the PFAS species, the microscopic organism types and the environmental circumstances.

It is well known that the carbon–fluoride covalent bond is one of the strongest in organic chemistry. For this reason, the direct enzymatic action and microbial degradation of such substances are hard to accomplish [13,14]. Even so, some recent studies have showed that naturally existing microorganisms' own enzymes could break the carbon–fluoride bonds in normal conditions (25 °C and neutral pH) [15–17]. Moreover, some bacteria strains can catalyze the splitting of the C-F bond through reductive [17–19], oxygenolytic [20], hydration [21] and hydrolytic [22] mechanisms.

Studies on this topic have reported that bacterial species degrade PFAS in the presence or absence of oxygen (Table 1). Almost all of the accessible studies present the biodegradation of PFAS compounds using different cultures isolated from solid environmental compartments (soil, sludge or sediments) [23–25]. Biodegradability studies of PFAS and fluorotelomer species using bacterial strains were predominantly communicated in the preceding literature. Following the biodegradability studies of some fluorotelomers, using activated sludge in the presence of free oxygen, it was observed that some similar major products were obtained during the process, among them perfluorohexanoic acid (PFHxA) and perfluoropentanoic acid (PFPeA) [23]. While limited fluorinated PFAS compounds are rapidly transformable under different microorganisms' activities, completely fluorinated species, such as PFOA and PFOS, have higher stability and were assumed to be non-biodegradable [24,26,27].

Table 1. PFOA and PFOS biotransformation by different bacterial strains.

Compound (Conc, mg/L)	Bacterial Strains	Conditions	Biotransformation Products	Biodegradation Efficiency	Test Time (Days)	References
PFOA (0.1/100)	*Acidimicrobium* sp. strain A6	Anaerobe	PFBA; PFPeA; PFHxA; PFHpA	63% (0.1 mg/L) 50% (100 mg/L)	100	[8]
PFOS (0.1/100)	*Acidimicrobium* sp. strain A6	Anaerobe	PFBA; PFBS	60% (0.1 mg/L) 47% (100 mg/L)	100	[8]
PFOA/PFOS (5 each)	Mixed culture	Aerobe/anaerobe sludge	Not reported	0% (aerobe) 100% (anaerobe)	30	[28]
PFOA (500)	*Pseudomonas Parafulva* strain YAB1	Aerobe	Not reported	48%	5	[10]
PFOS (1.8)	*Pseudomonas aeruginosa* strain HJ4	Aerobe	PFBS; PFHxS	67%	2	[9]
PFOS (1000)	*Pseudomonas plecoglossicida* 2.4-D	Aerobe soil	Not reported	75%	90	[29]
PFOS (1000)	*Pseudomonas plecoglossicida* 2.4-D	Aerobic mineral medium	PFHpA	100%	6	[29]
PFHxS (0.2)	*Pseudomonas* sp. strain PS27	Aerobe	Not reported	32%	10	[30]
PFHxS (0.2)	*Pseudomonas* sp. strain PDMF10	Aerobe	Not reported	28%	10	[30]

Based on the low levels of PFAS substances in wastewater treatment plants' effluents, both aerobic and anaerobic degradation of PFOA and PFOS were tested [28]. The data revealed that none of the PFOA or PFOS were transformed under aerobic conditions; in comparison, the anaerobic condition achieved almost maximum degradation of PFOA and PFOS.

Pseudomonas sp. looks to be an appropriate candidate for the biodegradation treatment of PFOA and PFOS, as well as for other similar compounds. The achievement of PFOS biotransformation was communicated for the first time in 2014, with up to 67% removal efficiency [9]. The major biotransformation products of PFOS under *Pseudomonas aeruginosa* strain HJ4 activity were perfluorobutanesulfonic acid (PFBS) and perfluorohexanesulfonic acid (PFHxS) [9]. *Pseudomonas plecoglossicida* was later revealed to biotransform PFOS with higher removal efficiency (up to 75% in soil and up to 100% in laboratory mineral medium) [29]. In 2016, the bacterial strain *Pseudomonas parafulva* YAB1 was isolated from soil contaminated with perfluorinated compounds, and it was shown to reduce PFOA 13–48% under laboratory conditions [10]. Recently, in 2019, the biological removal of PFAS species using Acidimicrobium sp. Strain A6 proved to be an alternative biodegradation technique of PFA compounds, using them as electron acceptors in the Feammox bacterial procedure [8].

Recent research on PFAS biodegradation has demonstrated that the *Gordonia sp*. aerobic strain NB4-1Y could remove fluorotelomer compounds with great efficiency (up to 88%) within 7 days [31]. In addition, *Pseudomonas plecoglossicida* 2.4-D was able to degrade PFOS, obtaining perfluoroheptanoic acid (PFHpA) as major biotransformation product, in less than 6 days [29].

An important and well-known benefit of biodegradation procedures is cost efficiency, with minimum investments in resources and energy. Anyway, compared to physical–chemical approaches, the most important disadvantage of removing organic compounds based on microorganisms' activity is that it is a time-consuming process. The biodegradation process of PFAS can take from a few days to a few weeks for equilibrium to be established (Table 1), compared to physical–chemical treatments, which can take a much shorter period of time. Therefore, a significant direction of study was to find new effective bacterial strains that can quickly remove PFAS from various environmental matrices. While different suppositions for biodegradation are confused, preliminary data suggest the probability of fast biodegradation for PFAS compounds, encouraging future investigations for the development of PFAS biodegradation technology using bacterial strains.

The purpose of the study was to investigate the ability and efficiency of two *Pseudomonas* bacterial strains, namely *Pseudomonas aeruginosa* and *Pseudomonas putida*, to degrade PFOA and PFOS. The use of such microorganisms can contribute to the improvement of the wastewater treatment process in wastewater treatment plants (WWTPs) through bioaugmentation or other bioremediation processes. Bacteria are economical alternatives compared to other processes, such as the catalytical ones, which can constitute an additional step in WWTPs. The study followed three principal objectives: (a) to evaluate the biodegradation degree of PFOA and PFOS by *P. aeruginosa* and *P. putida* during 96 h of incubation; (b) to evaluate the pollution stress caused by PFOA and PFOS to the *Pseudomonas* bacteria strains; (c) to establish a possible (bio)degradation pathway for PFOA and PFOS.

2. Materials and Methods

2.1. Chemicals and Reagents

Analytical standards (purity > 99%): perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorosulfonic acid (PFOS), perfluorononanoic acid (PFNA) were obtained from Sigma Aldrich (Saint Louis, MO, USA). Acetonitrile (ACN, HPLC grade) and Methanol (MeOH, HPLC grade) were provided by Merck (Darmstadt, Germany) and ammonium acetate (≥98%) from Sigma-Aldrich (Darmstadt, Germany). Ultrapure water was obtained in-house using a Milli-Q water system purchased from Merck (Darmstadt, Germany).

Specific solid culture medium, namely trypticase soy agar, was obtained from VWR Chemicals (Leuven, Belgium). The liquid culture medium, namely acetamide broth, was acquired from Scharlau (Spain). *Pseudomonas aeruginosa* WDCM 00026 VT 000263 bacterial

strain was purchased from (Saint Louis, Missouri, US), while *Pseudomonas putida* (ATCC 17514) was obtained from American Type Culture Collection, Manassas, VA, USA.

2.2. Pseudomonas Strain Culture Growth

Evaluation of the biodegradation capacity of PFOA and PFOS by referenced bacterial strains was carried out using two bacterial strains from the genus *Pseudomonas*, namely *Pseudomonas aeruginosa* (B1) and *Pseudomonas putida* (B2). Initially, the inoculation of the bacterial strains was performed on solid nutrient medium for 24 h (35 °C incubation temperature) (Binder GmbH, Germany). After this period, a single colony from each of *P. aeruginosa* and *P. putida* was relocated to a particular liquid culture medium, liquid broth with acetamide (AN). Acetamide broth is a liquid culture medium recommended for the differentiation of non-fermentative Gram-negative bacteria, especially for the bacteria identification and confirmation of the genus *Pseudomonas*. The incubation period in the liquid medium was carried out for 24 h at 37 °C and 130 rpm (Incubator Innova 44acquired from New Brunswick Scientific,). Bacterial growth was measured using the UV-VIS spectrophotometer (VWR International, Radnor, PA, USA) at 600 nm absorbance. Bacterial strains with optical density (OD) equal to 0.2 were used in the biodegradation experiments of PFOA and PFOS.

2.3. Bacterial Growth Inhibition Evaluation

Bacterial strains with OD_{600nm} equal to 0.2 were incubated for 96 h in presence of PFOS and PFOA at three different concentrations: 10 mg/L, 1 mg/L, 0.1 mg/L. All the experimental determination of the absorbance value, which is closely correlated with the bacterial growth, was established according to a negative control represented by the unseeded bacterial growth medium.

Bacterial growth rate in presence of the three PFOS and PFOA concentration levels was quantified at OD_{600nm} by comparison with a positive control consisting of each bacterial strain (BM) in the specific growth medium (acetamide broth), without the chemical compounds. Furthermore, aliquots of 1 mL were extracted from the incubation at 0 h, 2 h, 4 h, 6 h, 24 h, 48 h, 72 h and 96 h after contamination and were kept at −20 °C until the LC-MS analysis. Acetamide broth with each individual compound (PFOA/PFOS-M) represented the control sample for the analytical experiments.

2.4. Chemical Analysis

The experimental analysis was performed through liquid chromatography–tandem mass spectrometry technique (LC/MS-MS) using an Agilent 1260 and an Agilent 6410B (Agilent Technologies, Santa Clara, CA, USA). The parent compounds and biotransformation products' chromatographic separation was achieved on a Zorbax Eclipse C18 chromatographic column (2.1 × 100 mm, 3.5 µm). The column temperature was set at 30 °C. Samples of 10 µL were injected using an autosampler. The compounds were eluted using a mobile phase consisting of aqueous 5mM ammonium acetate (A) and MeOH (B). The flow rate was 0.2 mL/min. The gradient elution program is shown in Table S1, having a stop time of 25 min. To increase the sensitivity of the determination, the acquisition of the triple quadrupole detector was set in MRM mode with 6 time segments (Table S2 and Figure S1). The optimized MS and ESI operational parameters are showed in Tables S3 and S4. The biodegradation efficiency was evaluated using the Equation (1)

$$\text{Biodegradation (\%)} = (C_0 - C)/C_0 \times 100 \quad (1)$$

where C—concentration of the organic compound (mg/L) at time t, C_0—initial concentration of the organic compound (mg/L).

2.5. Sample Preparation for Analytical Detection

The test and control samples were thawed at room temperature and vortexed for 1 min. The sample preparation method focused on the precipitation of proteins and other

interfering compounds existing in the growth medium, with an organic solvent such as acetonitrile to minimize the matrix effect that can affect the mass spectrometric detection. To a 200 µL sample (medium—M, *P. aeruginosa* or *P. Putida* in medium—B1/B2-M, PFOA or PFOS in medium—PFOA/PFOS-M and *P. aeruginosa* or *P. Putida* incubated with PFOA or PFOS at different concentration—B1/B2-PFOA/PFOS-C), 400 µL ACN was added, vortexed (1 min) and centrifuged (at 14,000 RPM for 5 min). After the precipitate was decantated, 400 µL of the supernatant was moved to a 2 mL vial.

2.6. QA and QC Section

The experiments were performed in triplicate. For each sample sequence, two blank samples, containing ultra-pure water and methanol, and 2 standard solutions (1 and 75 µg/L) were analyzed. The calibration curves were fit in the range of 0.1 and 100 µg/L (R2 > 0.9996), and all samples were diluted to their suitable concentration. The method's sensibility was tested based on the analysis of blank samples (ultrapure water and MeOH). The detection (LOD) and quantitation (LOQ) limits were determined to be 0.3 and 1 µg/L, respectively, with signal-to-noise ratio of 10:1. Standard deviations were situated between 3 and 5%.

2.7. Statistical Analysis

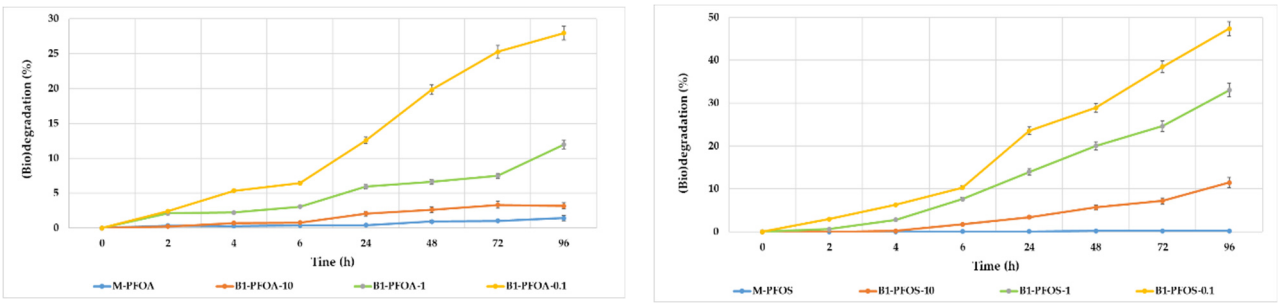
The statistical analysis was performed using Microsoft Office Excel 2021.

3. Results

3.1. Decrease of PFOA and PFOS in the Presence of Pseudomonas Strains

During the tested period (96 h), for the control experiments regarding the abiotic degradation of PFOA and PFOS, a decrease in concentrations of the two analytes in the growth medium was observed. The values of abiotic degradation ranged between 1.12 and 1.42% for PFOA and between 0.16 and 0.22% for PFOS.

Under the *P. aeruginosa* activity, a decrease in PFOA levels was observed over time (up to 96 h). The biodegradation degree rose as the tested concentration lowered (Figure 1a). Thus, at the end of the experiments, after 96 h, the degradation percentage of PFOA was 3.17% in the experiments in which 10 mg/L PFOA was used, 12.0% for the concentration of 1 mg/L and 27.9% for 0.1 mg/L PFOA. The reported percentages were corrected with those obtained in the control experiment. The increase in the percentage of biodegradation with the decrease in the concentration of the organic compound, in the presence of the *P. aeruginosa*, was also observed for the experiments regarding the PFOS biodegradation (Figure 1b). The values of the biodegradation percentages were obtained after subtracting the values obtained in the control experiment. Thus, in descending order of the tested concentrations, the biodegradation percentages were 11.5% for 10 mg/L PFOS, 33.3% for 1 mg/L PFOS and 47.4% for 0.1 mg/L PFOS, respectively.



(**a**) (**b**)

Figure 1. Biodegradation (percentage) of PFOA (**a**) and PFOS (**b**) during the incubation with *P. aeruginosa*.

The same pattern could be observed for the experiments performed in the presence of *P. putida*. The percentage of biodegradation increased with the decrease of the test

concentration (Figure 2). Thus, the biodegradation percentages, calculated after the values obtained for control experiment were subtracted, were 1.84% for the test concentration of 10 mg/L, 12.3% for 1 mg/L and 19.0% for 0.1 mg/L PFOA (Figure 2a), respectively. For the three PFOS concentrations tested, the biodegradation percentages obtained after 96 h from the start of the experiments were 6.35% for 10 mg/L, 26.6% for 1 mg/L and 46.9% for 0.1 mg/L (Figure 2b).

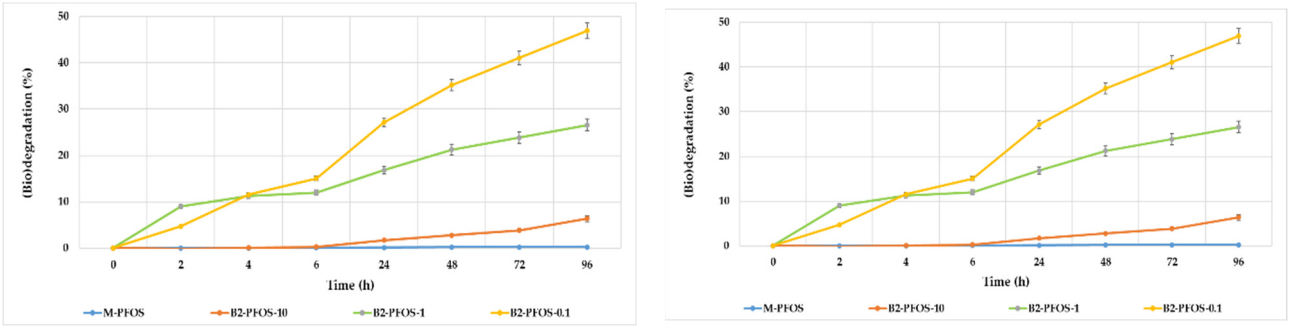


Figure 2. Biodegradation (percentage) of PFOA (**a**) and PFOS (**b**) during the incubation with *P. Putida.*

Comparing the results obtained in the presence of the two bacterial strains, it can be stated that *P. aeruginosa* shows a higher efficiency in terms of the PFOA biodegradation process, while, for PFOS, both bacterial strains affect biodegradation in a similar way for the concentration tested.

3.2. Bacterial Growth Inhibition in Presence of PFOA and PFOS

The growth inhibition degree of *P. aeruginosa* in the presence of PFOA increased with the increase in the tested substance concentration. The lowest percentage of inhibition of the growth of *P. aeruginosa* was observed in the case of the concentration of 0.1 mg/L PFOA, varying from 16% in the first 2 h of contact to 23% after 6 h, dropping to 5% after 24 h. Thus, an adaptation of the bacteria in the presence of PFOA after 24 h was noted both in the presence of the concentration of 0.1 mg/L and in the case of 1 mg/L, a fact corroborated with the increase in the percentage of degradation of the substance together with the decrease in the degree of inhibition of bacterial growth (Table S5 and Figure 3a). The experiments regarding the evaluation of the *P. aeruginosa* growth inhibition in the presence of PFOS presented the same characteristics compared to the inhibition test of the same bacteria incubated with PFOA, except that an inhibition of approximatively 3 times higher was observed for the lower concentrations in the first 2 h of contact and up to 24 h. An adaptation of *P. aeruginosa* in the presence of PFOS was noted after 24 h, when growth inhibition was considerably reduced (Table S7 and Figure 3b). This fact correlates with the increase in the biodegradation percentage of PFOS (Figure 1b) along with the decrease in the *P. aeruginosa* inhibition degree after 24 h and up to 96 h.

Regarding the growth inhibition degree of *P. putida*, a greater variation was observed compared to *P. aeruginosa* throughout the test in the presence of the same concentration levels of PFOA. The inhibition degree was 100% at the highest concentration tested, 10 mg/L PFOA, while, at lower values, the percentage of growth inhibition fluctuated up to 72 h (Tables S6 and S7). The growth inhibition degree of *P. putida* in the presence of PFOS was higher than in the presence of PFOA for all tested concentrations. Unlike *P. aeruginosa*, for *P. putida*, a greater variation was observed during the 96 h of the growth inhibition test in the presence of PFOS. The *P. putida* growth inhibition was proportionally lower for the lowest concentration (0.1 mg/L PFOS), indicating that approximately 80% of the viable bacterial population, after 48 h of contact, would have led to a compound degradation of 46.9%, compared to the PFOS concentration of 1 mg/L, at which the percentage of biodegradation was approximately 2 times lower. The biodegradation percentage of 26.6% in the case of

1 mg/L PFOS can also be deduced from the fact that more than half, 56%, of the *P. putida* bacterial population was inhibited after 48 h of contact (Table S8 and Figure 4b).

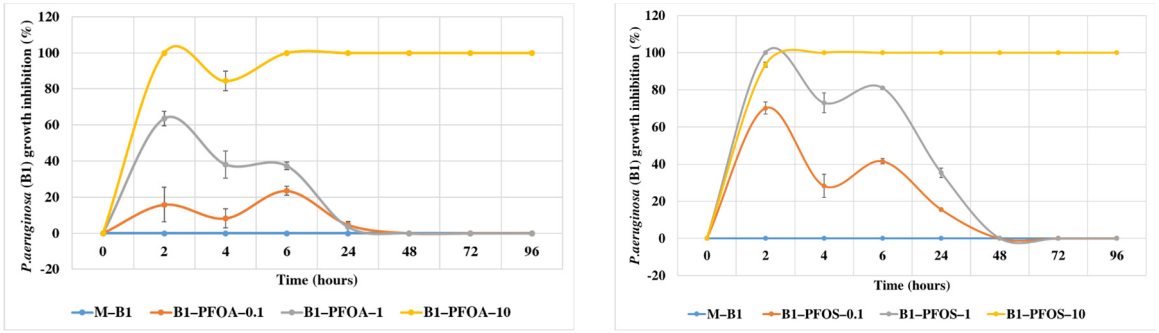


Figure 3. The growth inhibition degree of *P. aeruginosa* in the presence of PFOA (**a**) and PFOS (**b**).

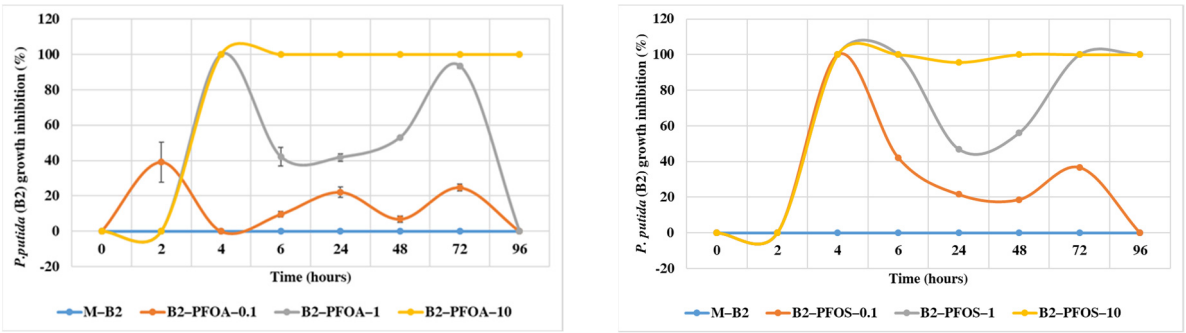


Figure 4. The growth inhibition degree of P. putida in the presence of PFOA (**a**) and PFOS (**b**).

3.3. Biotransformation Products Sustain the PFOA and PFOS Biodegradation by Pseudomonas Strains

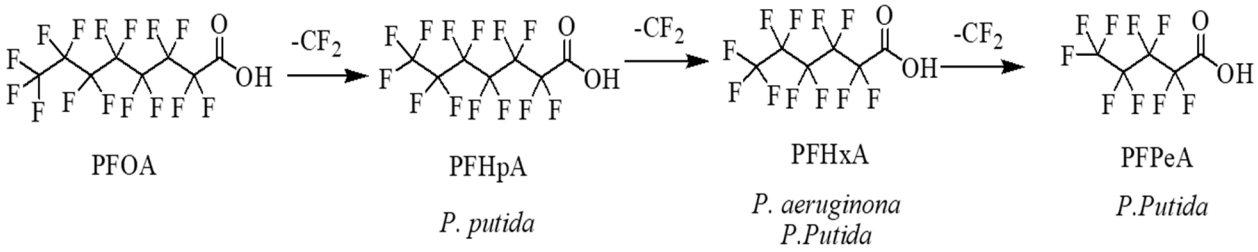
Since PFOA and PFOS quantification might be influenced by sample preparation and manipulation, identification and quantitation of biodegradation products with shorter aliphatic chains in the final samples of every experiment was used as supplementary key confirmation of PFOA and PFOS biodegradation. During the experiments that involved studies on the biodegradation of PFOA in the presence of the bacterial strain *P. aeruginosa*, the formation of a single biodegradation product was observed, namely PFHxA (Table 2). In the test where 0.1 mg/L PFOA was used, the formation of PFHxA was observed 4 h after the start of the experiment and increased from 0.12 µg/L to 0.57 µg/L. For the test in which 1 mg/L PFOA was used, the formation of PFHxA was also observed after 4 h, but the concentration increased from 0.56 µg/L to 0.60 µg/L at 24 h, after which it started to decrease, reaching 0.36 µg/L at the end of the experiment (96 h). This decrease can be based on the biodegradation of this compound under the influence of the bacterial strain.

In the presence of *P. putida*, PFOA biodegradation led to the formation of three biodegradation products: PFHxA, PFHpA and PFPeA, the latter being determined only in the case of the experiment in which the PFOA concentration was 0.1 mg/L (Table 2). The formation of biodegradation products followed a similar pattern, their presence being observed after 4 h from the start of the experiments. Their concentration increased over time until 48 h, after which they began to decrease, being biodegraded, in turn, by *P. putida*. Despite the fact that its formation was observed only in one test, PFPeA was detected with the higher values, the concentration domain being situated at 0.39 and 28 µg/L.

Table 2. Concentration values (μg/L) of PFOA biodegradation intermediates detected during the incubation experiments with *Pseudomonas* bacterial strains.

Time (h)	B1-FFOA-0.1	B1-FFOA-1	B2-PFOA-0.1			B2-PFOA-1		
	PFHxA	PFHxA	PFPeA	PFHxA	PFHpA	PFPeA	PFHxA	PFHpA
0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4	0.12 ± 0.004	0.56 ± 0.024	0.39 ± 0.014	0.10 ± 0.003	0.17 ± 0.007	<LOQ	0.12 ± 0.004	0.22 ± 0.009
24	0.19 ± 0.008	0.60 ± 0.025	1.47 ± 0.057	0.12 ± 0.004	0.19 ± 0.008	<LOQ	0.16 ± 0.006	0.31 ± 0.013
48	0.35 ± 0.013	0.52 ± 0.022	28.0 ± 1.092	0.12 ± 0.004	0.23 ± 0.010	<LOQ	0.19 ± 0.007	0.25 ± 0.011
72	0.42 ± 0.016	0.41 ± 0.017	23.5 ± 0.917	0.10 ± 0.003	0.19 ± 0.008	<LOQ	0.15 ± 0.006	0.21 ± 0.009
96	0.57 ± 0.021	0.36 ± 0.015	16.3 ± 0.636	0.05 ± 0.002	0.16 ± 0.007	<LOQ	0.13 ± 0.005	0.17 ± 0.007

Following the results obtained, it can be stated that the biodegradation mechanism of PFOA by *Pseudomonas* strains consisted in obtaining biodegradation products with shorter aliphatic chains by removing a -CF_2 group. The proposed biodegradation pathway is presented in Scheme 1.



Scheme 1. Biodegradation pathway proposed for PFOA during the incubation with *Pseudomonas* strains.

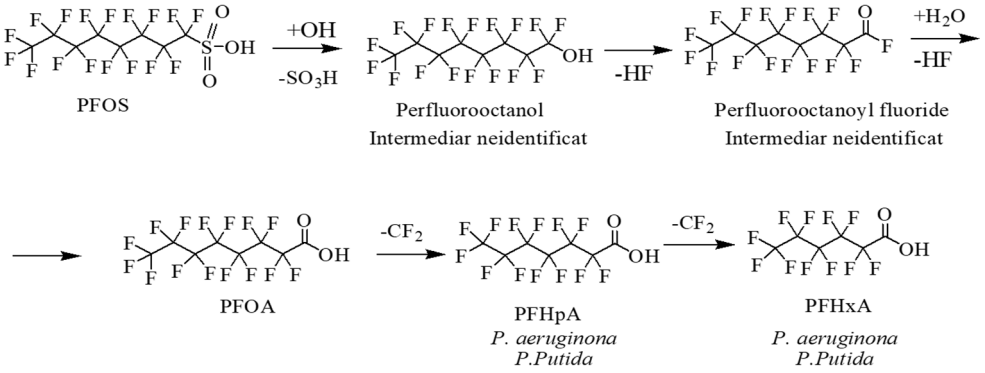
During the biodegradation experiments involving the incubation of *P. aeruginosa* with PFOS, at low concentrations (0.1 mg/L and 1 mg/L), two biodegradation products were identified (Table 3). The formation of the perfluoro alkylated derivative with six carbon atoms in the aliphatic chain, PFHxA, was observed early, after 2 h of the tests beginning. In the first experiment (0.1 mg/L PFOS), its concentration increased until 48 h, reaching a maximum of 0.45 μg/L, after which it decreased, reaching 0.38 μg/L. In the case of the higher concentration of PFOS, 1 mg/L, the values determined for PFHxA increased up to 0.64 μg/L at 24 h, after which they decreased, reaching a value of 0.16 μg/L at the end of the experiment (after 96 h). A similar behavior was observed for the counterpart with seven carbon atoms in the aliphatic chain, for both concentrations of PFOS (0.1 mg/L and 1 mg/L). Its formation was identified starting at 2 h and recorded an increase for 24 h, reaching values up to 0.26 μg/L. After this period, the concentrations begin to decrease over time.

The experiments carried out in the presence of *P. putida* also highlighted the formation of the 2 biodegradation products (PFHxA and PFHpA), when the concentrations of PFOS involved were 0.1 and 1 mg/L, respectively (Table 3). In the case of these experiments, the formation of these products was observed starting 6 h from the beginning of the incubation experiments. The maximum values were determined in the samples taken 72 h after the experiments started, and then the values decreased in the following 24 h. The decrease in concentrations of biodegradation products after a certain period of time can be due, also, to their degradation by *Pseudomonas* strains. For experiments that involved the highest concentration of PFOA and PFOS (10 mg/L), the formation of biodegradation products was not observed.

Table 3. Concentration values (μg/L) of PFOS biodegradation intermediates detected during the incubation experiments with *Pseudomonas* bacterial strains.

Time (h)	B1-FFOS-0.1		B1-FFOS-1		B1-FFOS-0.1		B1-FFOS-1	
	PFHxA	PFHpA	PFHxA	PFHpA	PFHxA	PFHpA	PFHxA	PFHpA
0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2	0.16 ± 0.006	0.11 ± 0.005	0.13 ± 0.005	0.10 ± 0.004	<LOQ	<LOQ	<LOQ	<LOQ
4	0.15 ± 0.006	0.22 ± 0.009	0.23 ± 0.009	0.14 ± 0.006	<LOQ	<LOQ	<LOQ	<LOQ
6	0.34 ± 0.013	0.24 ± 0.010	0.36 ± 0.014	0.22 ± 0.008	0.12 ± 0.005	<LOQ	0.12 ± 0.004	0.19 ± 0.008
24	0.44 ± 0.016	0.26 ± 0.011	0.64 ± 0.025	0.24 ± 0.009	0.13 ± 0.005	0.11 ± 0.005	0.14 ± 0.005	0.26 ± 0.011
48	0.45 ± 0.017	0.16 ± 0.007	0.35 ± 0.014	0.22 ± 0.008	0.18 ± 0.008	0.16 ± 0.007	0.23 ± 0.009	0.29 ± 0.012
72	0.44 ± 0.017	0.13 ± 0.005	0.16 ± 0.006	0.11 ± 0.004	0.41 ± 0.017	0.36 ± 0.015	0.64 ± 0.024	0.19 ± 0.008
96	0.38 ± 0.014	<LOQ	<LOQ	<LOQ	0.26 ± 0.009	0.21	0.46 ± 0.017	<LOQ

Although a relatively high percentage of biodegradation of PFOS was recorded, the concentrations tested were low, and, implicitly, the amount biodegraded was small, which made it impossible to identify the unknown intermediates through an analytical screening method. Thus, the proposed mechanism regarding the biodegradation of PFOS by the bacterial strains *P. aeruginosa* and *P. Putida* is presented in Scheme 2.



Scheme 2. Biodegradation pathway proposed for PFOS during the incubation with *Pseudomonas* strains.

4. Discussion

The results obtained in the present study are very promising and can be compared with those reported in the specialized literature (Table 1). Studies carried out using the bacterium *Acidimicrobium sp.* Strain A6 to remove PFOA and PFOS in the aerobic environment reported values of the removal degree up to 63% for 0.1 mg/L PFOA and up to 50% for a test concentration of 100 mg/L PFOA in 100 days, while, for PFOS, the removal degree was 60% and 47% for test concentrations of 0.1 mg/L and 100 mg/L PFOS, respectively, in 100 days [30]. Also, PFOA could be removed up to 48% in an aerobic environment in 5 days by the bacteria *P. parafulva Strain YAB1* [10], while the initial concentration of 1.8 mg/L and 500 mg/L were removed up to 67% in 2 days by *P. aeruginosa strain HJ4* [9] and up to 75% in 90 days by the bacterium *P. plecoglossicida* [29], respectively.

The present work showed no abiotic PFOA and PFOS degradation during 96 h and, subsequently, no degradation by-products. In the presence of bacterial strains, there was observed a decrease in PFOA and PFOS concentrations, which was correlated to a raise of biodegradation by-products such as PFPeA, PFHxA and PFHpA. Singh and his collaborators proposed a PFOA and PFOS defluorination, causing a transformation into PFHxA and PFHpA [32]. This transformation fitted our experimental model only when bacterial strains were added, which meant that bacteria could trigger defluorination and C-C bond oxidation [8]. The exact bacterial enzymatic equipment involved in PFOA and PFOS biodegradation, and, subsequently, defluorination, is not yet characterized and will serve as the main theme of a further study.

Despite the fact that not many research papers focused their studies on the identification of biodegradation products, PFPeA, PFHxA and PFHpA were commonly biotrans-

formation products of PFOA reported in the literature, while, for PFOS, compounds such as PFBA, PFBS, PFHxS and PFHpA were communicated as the major biotransformation products [8,9,29].

The results of this research open new possibilities for the use of *P. aeruginosa* and *P. putida* in the bioremediation of PFOA and PFOS. Improving the bioremediation processes by bioaugmentation could represent a simple, efficient, fast, environmentally safe and cost-effective technology to treat PFAS-contaminated water.

5. Conclusions

The results obtained in this study demonstrate that pure *Pseudomonas* strains, namely *P. aeruginosa* and *P putida*, could successfully biodegrade two of the most used perfluoroalkyl substances, PFOA and PFOS. Under specific conditions, the PFOA concentration was biologically transformed up to 27.9% and 19%, respectively, after 96 h of incubation time with *P. aeruginosa* and *P. putida*, respectively. In the case of PFOS, the two bacteria were more effective compared to PFOA. The biodegradation percentages obtained were up to 47.3% in the presence of *P. aeruginosa* and up to 46.9% in the presence of *P. putida*.

During the biodegradation tests, biotransformation products of the two parent compounds could be identified. During the PFOA biodegradation by *P. aeruginosa*, only one biodegradation product was identified (PFHxA), while the biodegradation by *P. putida* led to the identification of three biodegradation products (PFPeA, PFPxA and PFHpA). The biodegradation of PFOS by both bacteria strains generated the same two biodegradation products in both cases (PFPxA and PFHpA). Although it was observed that PFOA and PFOS had a significant impact on the bacterial community, especially at high concentrations, the adaptation of *Pseudomonas* chains was observed in all experiments, throughout the 96 h incubation period. The promising results support the use of *Pseudomonas* strains and their metabolic pathways, validating the conceptualization of using *Pseudomonas*-based bacterial models for PFOA and PFOS removal.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su151814000/s1, Table S1: The gradient elution program used for the separation of PFOA, PFOS and their biotransformation products; Table S2: Time segment set to increase the method sensibility; Figure S1: MRM chromatogram recorded for 50 µg/L PFAS standard solution; Table S3: MS optimized operational parameters; Table S4: ESI operational parameters; Table S5: *P. aeruginosa* growth inhibition (%)/contact time (h); Table S6: *P. putida* growth inhibition (%)/contact time (h); Table S7: *P. aeruginosa* growth inhibition (%)/contact time (h); Table S8: *P. putida* growth inhibition (%)/contact time (h).

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