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Published in:
Environmental Science and Pollution Research

DOI:
[10.1007/s11356-008-0020-5](https://doi.org/10.1007/s11356-008-0020-5)

[Link to publication](#)

Citation for published version (APA):

Sáez, M., de Voogt, P., & Parsons, J. R. (2008). Persistence of perfluoroalkylated substances in closed bottle tests with municipal sewage sludge. *Environmental Science and Pollution Research*, 15(6), 472-477. <https://doi.org/10.1007/s11356-008-0020-5>

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Persistence of perfluoroalkylated substances in closed bottle tests with municipal sewage sludge

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Received: 14 May 2008 / Accepted: 27 May 2008 / Published online: 2 July 2008

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Abstract

Background, aim, and scope Perfluoroalkylated substances (PFAS) are chemicals with completely fluorinated alkyl chains. The specific properties of the F–C bond give PFAS a high stability and make them very useful in a wide range of applications. PFAS also pose a potential risk to the environment and humans because they have been recently characterized as persistent, bioaccumulative, and toxic. The objective of this work is to study the bacterial degradation of PFAS under aerobic and anaerobic conditions in municipal sewage sludge as a contribution toward understanding their environmental fate and behavior.

Materials and methods Bacterial communities from sewage sludge were exposed to a mixture of PFAS under aerobic or anaerobic conditions. Individual PFAS concentrations were determined in the experiment media at different exposure

times using liquid chromatography–mass spectrometry analysis after extraction with solid-phase extraction.

Results The PFAS analyses of samples of sludge showed repeatable replicate results, allowing a reliable quantification of the different groups of PFAS analyzed. No conclusive evidence for PFAS degradation was observed under the experimental conditions tested in this work. Reduction in concentrations, however, was observed for some PFAS in sludge under aerobic conditions.

Discussion The largest concentration decrease occurred for the fluorotelomer alcohols (FTOHs), especially for the 8:2 FTOH, which have been described as biodegradable in the literature. However, this concentration decrease could be due to different causes: sorption to glass, septa, or matrix components, as well as bacterial activity. Therefore, it is not certain that biodegradation occurred.

Conclusions PFAS are very recalcitrant chemicals, especially when fully fluorinated. Although some decreases in concentration have been observed for some PFAS, such as the FTOHs, there is no conclusive evidence for biodegradation. It can be concluded that the PFAS tested in these experiments are non-biodegradable under these experimental conditions.

Recommendations and perspectives Since the presence of PFAS is ubiquitous in the environment and they can be toxic, more research is needed in this field to elucidate which PFAS are susceptible to biodegradation, the conditions required for biodegradation, and the possible routes followed. A possible inhibitory effect of PFAS on bacteria, the threshold concentrations, and conditions of inhibition should also be investigated.

Responsible editor: Henner Hollert

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Keywords Aerobic biodegradation ·
Anaerobic biodegradation · Fluorotelomer alcohols ·
FTOHs · Perfluoroalkylated substances · PFAS · Sludge

1 Background, aim, and scope

Perfluoroalkylated substances (PFAS) are a group of chemicals, including oligomers and polymers, with unique properties due to their highly fluorinated structure. These properties make them suitable for a broad range of applications including surface treatment (oil-, grease-, and water-resistant coatings on paper and textile products) and performance chemicals (fire-fighting foams, industrial surfactants, acid mist suppression, insecticides, etc.; USEPA 2002; Hekster et al. 2003). These industrial and consumer uses have increased enormously over the last decades, and hence, the release of PFAS to the environment has also increased. A total of 3,200–7,300 tonnes of total PFAS have been estimated to have been globally discharged, both directly and indirectly (Prevedouros et al. 2006).

PFAS, mainly perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), have been detected in the abiotic environment (Berger et al. 2004; Yamashita et al. 2005; Skutlarek et al. 2006) and in biota (fish, birds, and mammals; Hansen et al. 2001) in a large part of the world, including such remote areas as the Arctic (Giesy and Kannan 2001; Tomy et al. 2004; Smithwick et al. 2005). Their transport to remote areas is unexpected because PFOS, PFOA, and related substances are much more water-soluble and less volatile than the typical persistent organic pollutants that undergo long-range transport. Recent studies suggest that fluorotelomer alcohols (FTOH), which are volatile and can be more easily transported to remote locations, can be degraded to PFOA in the environment and hence may explain the discovery of PFOA in remote locations (Ellis et al. 2004, 2005; Berti et al. 2005). Another hypothesis is that direct long-range transport of PFOS and PFOA may occur via ocean currents (Taniyasu et al. 2004).

The widespread presence of PFAS in wildlife, their bioaccumulation, potential toxicity, and adverse health effects makes the study of their environmental fate a major concern. The extreme strength of the C–F bond that makes PFAS suitable for certain uses also makes them potentially significant environmental contaminants due to their persistence. Although they are not easily degraded, there are few studies showing that a primary biodegradation of some perfluorinated compounds (like N-EtFOSE) to PFOS and PFOA (3M 2000a) can be observed, but there is no evidence for further degradation (3M 2000b, c, d, 2001).

It appears that aerobic biodegradation of polyfluorinated chemicals occurs in the non-fluorinated part of the molecule, without loss of fluorine (Remde and Debus 1996), and only takes place for non-perfluorinated compounds. Defluorination, however, has been reported for some fluorinated compounds containing carbon–hydrogen bonds, such as trifluoroethane sulfonate, 6:2 FTOH (Key et

al. 1998), and recently, also for 8:2 FTOH (Dinglasan et al. 2004; Wang et al. 2005a, b). These last studies describe the aerobic degradation of 8:2 FTOH ($C_8F_{17}CH_2CH_2OH$, the most commercially important FTOH) in sludge (Dinglasan et al. 2004; Wang et al. 2005a) and sediment (Wang et al. 2005b), as well as the possible degradation routes. Recently, biodegradation of FTOH, N-EtFOSAA, and other PFAS was proposed to contribute to the levels of PFOA and PFOS in waste water treatment plant sludge, effluents, and sediment (Higgins et al. 2005; Sinclair and Kannan 2006).

The objective of this paper is to contribute to a better understanding of the persistence of PFAS in the environment. For that purpose, biodegradation experiments of PFAS under aerobic and anaerobic conditions have been performed using bacterial consortia from municipal sewage sludge. Experiments have been performed with mixtures of PFAS since microorganisms in the environment are always exposed to mixtures of these compounds.

2 Materials and methods

2.1 Chemicals

High-performance liquid chromatography (HPLC) grade methanol (MeOH) was purchased from Brunschwig Chemie, Amsterdam, The Netherlands. Deionized water was used throughout the experiments (16.2 M Ω /cm). All chemicals were at least reagent grade. The biodegradation experiments were performed using mixtures of PFAS, which are shown in Table 1 together with their suppliers. All PFAS had purity higher than 96%, and all concentrations reported in this paper were corrected for the purity. The C₁₈ Sep-Pak cartridges were supplied by Waters Chromatography BV, Etten-Leur, The Netherlands, and the Acrodisc Syringe Filters with 0.2 μ m GHP Membrane were purchased from VWR International BV, Amsterdam, The Netherlands. The HPLC separation was performed with an Eclipse XDB C18 column of 150 \times 2.1 mm internal diameter and 3.5 μ m particle size, purchased from Agilent Technologies. Mass-labeled ¹³C₂-PFOA was used as internal standard.

2.2 Experimental conditions

The biodegradation experiments were based on the OECD guideline 301D (closed bottle test, OECD 1992) with slight modifications. Several batches of experiments were performed (Table 2) depending on the conditions (aerobic or anaerobic) and the mixture of PFAS tested. In all the experiments, bacteria were exposed to a mixture of PFAS with an initial nominal concentration of about 4 and 2 mg/l for mixtures 1 and 2, respectively. Mixture 1 consists of

Table 1 The two mixtures of PFAS used in the biodegradation experiments

Mixture	PFAS	Structure	Abbreviation	Supplier
1	Decafluorohexanoic acid	C ₅ F ₁₁ COOH	PFHxA	ABCR
	Pentadecafluorooctanoic acid	C ₇ F ₁₅ COOH	PFOA	Acros
	Heptafluorooctanoic acid	C ₈ F ₁₇ COOH	PFNA	Aldrich
	Heptafluorooctanesulfonic acid (K salt)	C ₈ F ₁₇ SO ₃ K	PFOS	Fluka
2	Nonafluorobutanesulfonic acid	C ₄ F ₉ SO ₃ H	PFBS	Apollo Sc.
	Perfluoroundecanoic acid	C ₁₀ F ₂₁ COOH	PFUnA	Apollo Sc.
	Perfluorooctane sulfonamide	C ₈ F ₁₇ SO ₂ NH ₂	PFOSA	ABCR
	1,1,2,2 H-perfluorooctane sulfonic acid	C ₆ F ₁₃ C ₂ H ₄ SO ₃ H	6:2 FTS	Interchim
	1,1,2,2 H-perfluorooctanol	C ₆ F ₁₃ C ₂ H ₄ OH	6:2 FTOH	ABCR
	1,1,2,2 H-perfluorodecanol	C ₈ F ₁₅ C ₂ H ₄ OH	8:2 FTOH	DuPont
IS	Pentadecafluorooctanoic acid	C ₇ F ₁₅ COOH	¹³ C-PFOA	DuPont

PFHxA, PFOA, PFNA, and PFOS, whereas mixture 2 contains perfluorobutyl sulfonate (PFBS), perfluoroundecanoic acid (PFUnA), perfluorooctanesulfonamide (PFOSA), 6:2 fluorotelomer sulfonate (FTS), 6:2 FTOH, and 8:2 FTOH. All the PFAS tested are fully fluorinated, except the telomers 6:2 FTS, 6:2 FTOH, and 8:2 FTOH, which have two CH₂ units.

Experimental bottles were run as independent duplicates with parallel sterile controls (bottles treated identically to experimental ones except for the autoclaving after inoculating). Each bottle contained 40 ml mineral medium (12 mM KHPO₄, 9 mM Na₂HPO₄, 5.6 mM NH₄Cl, 5.1 mM NaCl, 0.75 mM CaCl₂, 0.49 mM MgCl₂, 9.5 mM NaHCO₃, and 2 mM Na₂S) and 10 ml of inoculum (10 ml sludge supernatant). The reductant (Na₂S) was obviously absent from aerobic bottles. The bottles were kept under constant agitation and natural light period, although protected from UV radiation. The anaerobic experiments, kept in darkness, were performed under a nitrogen atmosphere in viton-sealed bottles, with resazurine as redox indicator.

Vitamins, trace elements, and a solution of lactate, acetic, and pyruvic acids, as extra carbon source, were also added. When most of the experiments had been running for 3–4 weeks (depending on the series), ethanol was added, since it has been shown to enhance the aerobic biodegradation of fluorotelomer alcohols (Wang et al. 2005b).

The activated sludge was taken from RWZI Westpoort, a waste water treatment plant in the West of the city of Amsterdam (The Netherlands), and transported to the

laboratory where it was left for about 1–2 h to settle. Then, the supernatant was removed and kept with aeration until experiment bottles were filled (within a few hours).

2.3 Analytical conditions

2.3.1 Sample extraction

PFAS were extracted from supernatant of the sludge experiments (both aerobic and anaerobic) with C₁₈ SPE cartridges as described elsewhere (de Voogt et al. 2005). The cartridges were previously activated with 10 ml of MeOH followed by 10 ml of distilled water. The cartridges were eluted with 10 ml MeOH, after which the volume was reduced under a gentle stream of N₂ and filtered before analysis.

2.3.2 Detection and quantification of PFAS

PFAS analysis was performed using HPLC–electrospray ionization mass spectrometry (Thermoquest Navigator) under a flow of 0.25 ml/min of a mixture of 90% of 5 mM CH₃COONH₄ in water and 10% of MeOH. At 12 min, the MeOH was increased to 100%, kept there for 2 min, and returned to the initial condition in another 5 min. The probe was kept at 220°C and 4.6 kV, while the entrance cone voltage was –20 V (Sáez and de Voogt 2006). For identification and quantitative analysis, the masses (*m/z*) corresponding to the pseudo molecular ions [M–H][–] were selected (de Voogt and Sáez, 2006), except for the FTOHs,

Table 2 Experimental conditions of the different batches of the biodegradation experiments of PFAS

Experiment code	Conditions	Medium	PFASs tested	Duration (months)	Sampling schedule (weeks)
A 1	Aerobic	Sludge	Mixture 1	3.5	0, 1, 2, 3, 7, 15
A 2	Aerobic	Sludge	Mixture 2	2	0, 1, 2, 3, 4, 9
An 1	Anaerobic	Sludge	Mixture 1	3.5	0, 1, 2, 3, 7, 15
An 2	Anaerobic	Sludge	Mixture 2	2	0, 1, 2, 3, 4, 9

which were detected as the acetate adduct ions $[M-H + CH_3COO]^-$. The concentration of each PFAS was calculated using a five-point calibration curve determined with external standards. Concentrations were corrected for extraction and injection losses by the addition of $^{13}C_2$ -PFOA before extraction. The average recovery of $^{13}C_2$ -PFOA was $70.5 \pm 20.6\%$. As the mass-labeled $^{13}C_2$ -PFOA was not yet available during the first two experiments performed (A1, An1), 6:2 FTS was used as an internal standard in these experiments.

3 Results

The simultaneous analysis of different groups of PFAS presents several difficulties, such as extraction from complex matrices, lack of proper and well-characterized standards, and lack of robust analytical methods. The quality assurance in this study was realized through recovery tests and independent replicate analyses. Recoveries in general were satisfactory (greater than 60%), and the replicates showed good repeatability.

3.1 Aerobic experiments

The time trends of the PFAS during the biodegradation experiments performed with sludge under aerobic conditions is shown in Fig. 1. No significant decrease is observed for any of the PFAS tested (with the exception of PFHxA, 6:2 FTOH, and 8:2 FTOH) over a period of up to either 9 or

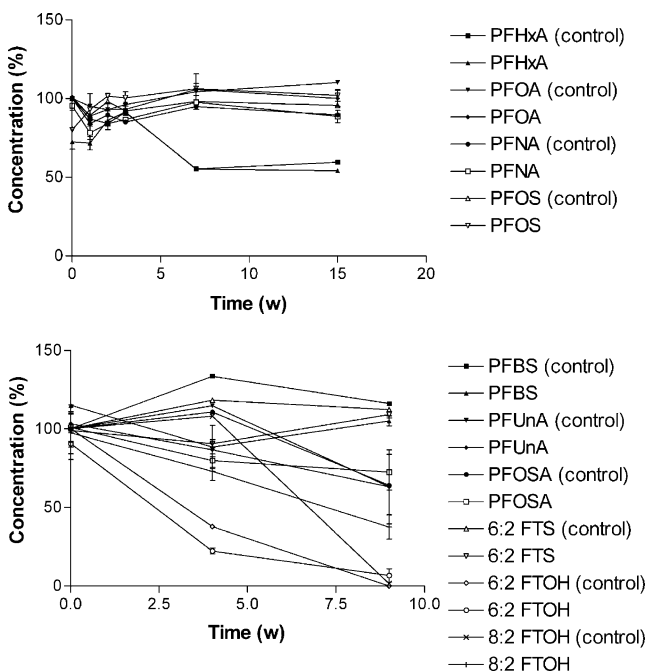


Fig. 1 Trends in the concentrations of PFAS (as percentage of the initial concentration) in sludge under aerobic conditions

15 weeks. The concentrations of PFHxA, 6:2 FTOH, and 8:2 FTOH also decreased in the controls during the experiment. The decrease is thus not conclusive evidence for biodegradation, since the decrease in concentration could be due to processes other than microbial metabolism. It is, however, also possible that incomplete sterilization of the controls could be responsible for the removal of these compounds in the controls.

3.2 Anaerobic experiments

Results of the experiments performed with sludge under anaerobic conditions are presented in Fig. 2, showing again a good reproducibility of the data. None of the tested compounds showed a significant decrease in the concentrations under these conditions during the course of the experiments (9 or 15 weeks, depending on the batch). Interestingly, there was no decrease in the concentrations of PFHxA, 6:2 FTOH, and 8:2 FTOH in these incubations, suggesting that the removal of these compounds in the aerobic experiments could indeed be due to biodegradation. The lack of biodegradation observed under anaerobic conditions is consistent with the fact that anaerobic biodegradation is a slower process than aerobic biodegradation.

4 Discussion

The simultaneous analysis of different groups of PFAS is complicated by the different properties of the FTOHs

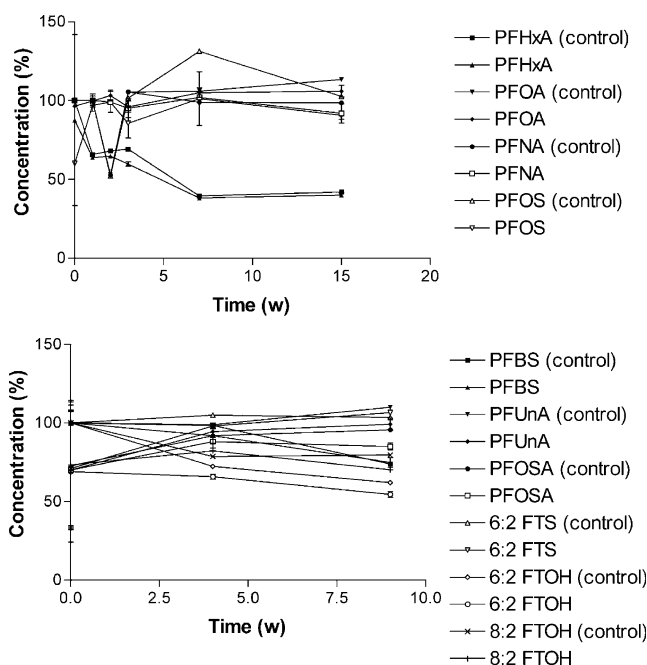


Fig. 2 Trends of the concentrations of PFAS (as percentage of the initial concentration) under anaerobic conditions

(lower water solubility and higher volatility) compared with the perfluorocarboxylic acids and sulfonates, resulting in lower recoveries and higher detection limits, and therefore hindering the performance of the biodegradation experiments. The correction of the concentration of the tested PFAS with ^{13}C -PFOA (internal standard) may lead to higher errors for those PFAS whose properties differ from those of PFOA. When the experiments were performed, this was the only labeled standard available, but this problem is nowadays being solved by the increasing number of mass-labeled standards available. However, quality assurance measures gave satisfactory results.

There is no conclusive evidence for biodegradation of PFAS in sludge under aerobic conditions. Although decreases in PFHxA, 6:2 FTOH, and 8:2 FTOH concentrations were observed, their concentrations in the control bottles also decreased, and it is therefore not possible to confirm that they were indeed due to biodegradation. The concentration decrease could also be due to a non-biological degradation process, losses not due to degradation or even to incomplete sterilization of the control bottles. Aerobic biodegradation of 8:2 FTOH has been reported in sludge by Dinglasan et al. (2004) and by Wang et al. (2005a, b), but this could not be confirmed in the present experiments. No evidence for degradation of any of the PFAS was observed under anaerobic conditions. This result is consistent with the lack of anaerobic biodegradation of PFAS reported elsewhere in the literature.

Based on the results presented in this paper, it can therefore be concluded that the PFAS tested in these experiments are non-biodegradable under the experimental conditions used in this study, despite using municipal sewage sludge, which presumably has a history of exposure to PFAS. Similar experiments with sediment contaminated with PFAS also showed no evidence for biodegradation of any of the PFAS tested (data not shown).

5 Conclusions and recommendations

It is reasonable to assume that the lack of degradation of PFAS observed in these experiments is probably caused by the stability of the C–F bond, although there are examples of microbially catalyzed defluorination reactions (Parsons et al. 2008). As is the case with reductive dechlorination or debromination, reductive defluorination is energetically favorable and, under anaerobic conditions, releases more energy than that available as a respiratory process from sulfate reduction or methanogenesis. Several species of anaerobic microorganisms are known to utilize reductive dechlorination as a respiratory process. We should therefore consider the possibility that microorganisms will eventually adapt to utilize defluorination as a source of energy. Hence,

the situation for PFAS may be comparable to that of chlorinated organic compounds several decades ago. For many years, organochlorine compounds were considered to be recalcitrant, whereas today reductive chlorination reactions of many organochlorines, including polychlorinated biphenyls and dioxins, are regularly observed in anaerobic environments. Further studies should, however, be performed to investigate whether longer exposure times or higher exposure concentrations could lead to the development of degradation capability in microbial populations in sludges and sediments.

Acknowledgment This research work was financially supported by the European Union, Project PERFORCE (NEST-508967); by the Ministerio de Educación y Ciencia (Spanish Ministry for Education and Science); and DuPont Chemical Solutions Enterprise, Wilmington, Delaware, USA. The authors would like to thank the staff of the RWZI Westpoort for their assistance in the sludge sampling.

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