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Defluorination of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) by *Acidimicrobium* sp. Strain A6

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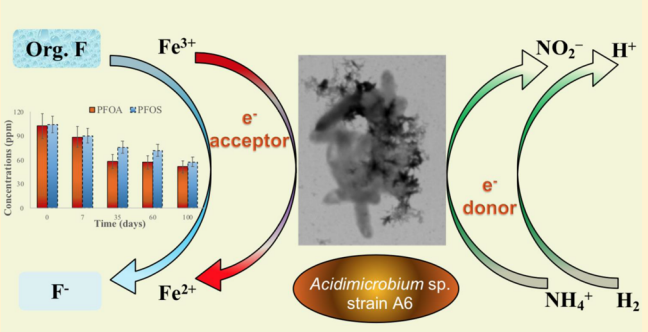
Supporting Information

ABSTRACT: Incubations with pure and enrichment cultures of *Acidimicrobium* sp. strain A6 (A6), an autotroph that oxidizes ammonium to nitrite while reducing ferric iron, were conducted in the presence of PFOA or PFOS at 0.1 mg/L and 100 mg/L. Buildup of fluoride, shorter-chain perfluorinated products, and acetate was observed, as well as a decrease in Fe(III) reduced per ammonium oxidized. Incubations with hydrogen as a sole electron donor also resulted in the defluorination of these PFAS. Removal of up to 60% of PFOA and PFOS was observed during 100 day incubations, while total fluorine (organic plus fluoride) remained constant throughout the incubations. To determine if PFOA/PFOS or some of their degradation products were metabolized, and since no organic carbon source except these PFAS was added, dissolved organic carbon (DOC) was tracked. At concentrations of 100 mg/L, PFOA/PFOS were the main contributors to DOC, which remained constant during the pure A6 culture incubations. Whereas in the A6 enrichment culture, DOC decreased slightly with time, indicating that as defluorination of PFOS/PFOA occurred, some of the products were being metabolized by heterotrophs present in this culture. Results show that A6 can defluorinate PFOA/PFOS while reducing iron, using ammonium or hydrogen as the electron donor.



INTRODUCTION

PFAS (per- and polyfluoroalkyl substances) are known to be among the most challenging organic pollutants to remediate in aquatic and terrestrial environments.[1] PFAS are found in a wide range of consumer products, they are ubiquitous in the environment, and are extremely recalcitrant. Among them, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), which have been manufactured widely and can also be formed via degradation of precursors, are the most common PFAS present in many environmental settings and are of increasing concern.[2,3] Studies indicate that PFOA and PFOS pose considerable threats to the natural environment and human health because of their wide distribution, extraordinary persistence, bioaccumulation tendencies, and potential toxicological effects.[4−7] Therefore, identifying methods to mineralize these PFAS is a topic of growing urgency.

The carbon−fluorine (C−F) bond is the strongest covalent bond in organic chemistry,[8] hence biodegradation of perfluoroalkyl chemicals is difficult and usually limited to molecules, or regions of molecules, that are not fully fluorinated[3] such as fluorobenzene,[9] fluoroacetate,[10] perfluorohexylethanol,[11,12] and perfluorohexylsulfonate.[13] Fluorotelomer compounds, which have an ethyl group between the perfluoroalkyl chain and the rest of the nonfluorinated functional groups, can undergo extensive transformations, resulting in a partial defluorination or shortening of the perfluoroalkyl chain.[14] These findings on fluorotelomer transformation are consistent with those on degradations of difluoromethane sulfonate (DFMS) and 2,2,2-trifluoroethane sulfonate (TES), all of which contain C−H bonds,[15] indicating that the lack of C−H bonds in perfluorinated compounds like PFOA and PFOS makes them more diffcult to degrade.

Studies of successful PFOA and PFOS biodegradation, especially mineralization, are limited. It has been reported that PFOS and PFOA can be eliminated using activated sludge as a seed and incubated under anaerobic conditions, although the buildup of fluoride ion (F^-) was not observed.[16,17] In studies by single cultures, a decrease of about 32% of PFOA was observed after a 96 h incubation with *Pseudomonas parafulva*,[18] and 67% of PFOS was biologically decomposed by *Pseudomonas aeruginosa* over 48 h.[19] The degradation of PFOS by *Pseudomonas plecoglossicida* was studied, which showed that this strain can use PFOS as a carbon source, transforming it to perfluoroheptanoic acid and releasing F^-.[20] Although reductive defluorination has been shown to be energetically favorable,[1] up to date, no bioreductive defluorination of perfluorinated compounds such as PFOA and PFOS has been reported in the literature.

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Anaerobic oxidation of ammonium (NH_4^+) under iron-reducing conditions, referred to as Feammox, is a relatively novel pathway in the nitrogen cycle. Here, NH_4^+ is oxidized to nitrite (NO_2^-) coupled to the reduction of ferric iron [Fe(III)] to ferrous iron [Fe(II)].[21] An autotrophic microorganism responsible for this process, *Acidimicrobium* sp. strain A6 (ATCC, PTA-122488; referred to as A6 from here on), has been identified[21] and isolated.[22] The environmental conditions at sites where the Feammox process occurs and A6 is present have been described and show that the organism is common at sites where the pH is <7 and soils are iron rich.[23] The Feammox process has been reported to occur by many investigators in many different environmental settings.[24−28] The Feammox stoichiometry when ferrihydrite is the Fe(III) source can be written as:[21]

$$3\ Fe_2O_3{\cdot}0.5\ H_2O + 10\ H^+ + NH_4^+ \rightarrow 6\ Fe^{2+} + 8.5\ H_2O + NO_2^- \quad (1)$$

For this reaction and environmental/incubation conditions described previously,[21] the change in Gibbs free energy (ΔG_r) is 145.08 kJ/mol NH_4^+. Although oxidation of NH_4^+ to N_2 has been reported for the Feammox process,[25] incubations with A6 have shown that NO_2^- is the product of the NH_4^+ oxidation by this organism.[21,22] It was also shown that in addition to NH_4^+, A6 is capable of using H_2 as an electron donor while reducing Fe(III).[22] Furthermore, it was shown that A6 is unable to utilize common organic electron donors as its carbon source, but that it does incorporate CO_2 into its biomass.[22] Given that it has been shown that A6 can cometabolically degrade TCE and PCE,[29] the objective of this research was to determine if PFAS, specifically PFOA and PFOS, can be biodegraded/defluorinated by either the pure A6 and/or an A6 enrichment culture while oxidizing NH_4^+ or H_2 under iron-reducing conditions.

MATERIALS AND METHODS

Feammox Incubation Experiments. Two hundred milliliters of either a pure A6 culture[22] or an A6 enrichment culture, obtained from a Feammox laboratory-scale continuous-flow membrane reactor,[21] which has been operated for 180 days, were mixed with 500 mL of an anoxic inorganic Fe(III)−NH_4^+ enrichment medium.[22] This medium consisted of 7.5 mM 6-line ferrihydrite ($Fe_2O_3{\cdot}0.5H_2O$, prepared according to Cornell and Schwertmann[30]), 2.81 mM NH_4Cl, 0.19 mM $(NH_4)_2SO_4$, 0.24 mM $NaHCO_3$, 0.71 mM $KHCO_3$, 0.07 mM KH_2PO_4, 0.41 mM $MgSO_4{\cdot}7H_2O$, and 0.40 mM $CaCl_2{\cdot}2H_2O$, in addition to 1 mL/L of a trace element solution[31] and 1 mL/L of a vitamin solution (ATCC MD-VS). The mixture of medium and culture was then shaken in an anaerobic chamber for 24 h to be homogenized prior to the incubations. An electron shuttling compound, 9,10-anthraquinone-2,7-disulphonic acid (AQDS), was added to the pure A6 culture at 25 μM, which was shown to be needed to grow the pure A6 culture.[22] These mixtures were evenly distributed into multiple 10 mL serum vials in an anaerobic chamber and were sealed with butyl rubber stoppers. The headspace (2 mL) of each vial was vacuumed and then flushed with a N_2/CO_2 (80:20) mixture to achieve anoxic conditions and provide additional CO_2 to the vials, which is required by A6 for its growth. All steps for the inoculation and isolation were carried out under sterilized conditions (autoclaved vials, and the media filtered through a 0.2 μm membrane). The E_h in the vials was −135 ± 17 mV, and the initial pH was 4.5 ± 0.2 and increased to 5.0 ± 0.2 during the incubations.

Effects of PFOA and PFOS on the growth of A6 and its Feammox activity were evaluated via incubations at initial concentrations of 0.24 μM, 2.4 μM, 0.024 mM, and 0.24 mM PFOA and 0.20 μM, 2.0 μM, 0.02 mM, and 0.20 mM PFOS (equivalent to 0.1 mg/L, 1.0 mg/L, 10 mg/L, 100 mg/L, and 200 mg/L PFOA/PFOS).

PFOA and PFOS Incubations with NH_4^+ as Electron Donor. Incubations as described above were conducted with initial concentrations of both 0.24 μM and 0.24 mM (0.1 mg/L and 100 mg/L) PFOA as well as 0.20 μM and 0.20 mM (0.1 mg/L and 100 mg/L) PFOS. The high concentration (0.24 mM PFOA and 0.20 mM PFOS), much higher than what is most often found in contaminated environments, was selected to allow for more accurate F^- and sulfate (SO_4^{2-}) analyses, fluorine and carbon balance, and to evaluate the effect of defluorination on the reduction of Fe(III). For each set of experiments, several controls were prepared, including a positive control that was identical as described above but without PFAS to compare the Feammox activity in their absence. One set was prepared as described above with the PFAS and was autoclaved. One set was prepared as above with the PFAS and no NH_4^+ to link the PFAS degradation to Feammox activity (or lack thereof in the absence of NH_4^+). One set was prepared as described above but without adding a Fe(III) source. Finally, one set was prepared with the PFAS, but instead of seeding the vials with A6, they were seeded with *Geobacter sulfurreducens* ATCC 51573 (with an initial concentration of 0.12×10^7 copies/mL) and augmented with 0.5 mM acetate to determine if the iron reduction and buildup of Fe(II) might be responsible for the degradation of these PFAS. All vials were placed on a rotary shaker at 150 rpm at 30 °C for the incubations. On days 0, 7, 35, 60, and 100, three vials were destructively sampled for Fe(II), NH_4^+, PFOA/PFOS, dissolved organic carbon (DOC), and microbial analyses. An additional three vials were sacrificed, filtered, and stored at 4 °C to be available if required for further analyses. Given the large time interval between analyzing the data collected on day 60 and day 100, and since data from day 100 are key to assess how much a chemical constituent has changed during the incubations, samples from three stored vials collected on day 60 were analyzed for all compounds measured at the same time as the 100 day samples to ascertain that the accuracy of the various analytical techniques had not changed over time. Hence, n = 6 for day 60.

PFOA and PFOS Incubations with H_2 as Electron Donor. Hydrogen (H_2) was tested as an alternate electron donor to NH_4^+ for A6 to conduct PFAS degradation/defluorination. For this purpose, pure A6 and A6 enrichment cultures were incubated with the same medium plus PFAS, but without NH_4^+, and with H_2 in the headspace of the vials. A small amount of NH_4^+ (0.15−0.20 mM) was still present in the vials to which no NH_4^+ was added, which originated from the transfer of cells from the inoculum and was the nitrogen source required for bacterial growth in these incubations. Five incubation sets were conducted in parallel for both the pure A6 and the A6 enrichment cultures including (1) PFOA/PFOS + Fe(III) + NH_4^+, (2) PFOA/PFOS + Fe(III) + H_2, (3) PFOA/PFOS + H_2, (4) PFOA/PFOS + Fe(III) + NH_4^+ + H_2, and (5) autoclaved control of PFOA/PFOS + Fe(III) + H_2. With the exception of the samples to which no NH_4^+ was added, the concentrations of PFOA/PFOS, Fe(III), and NH_4^+

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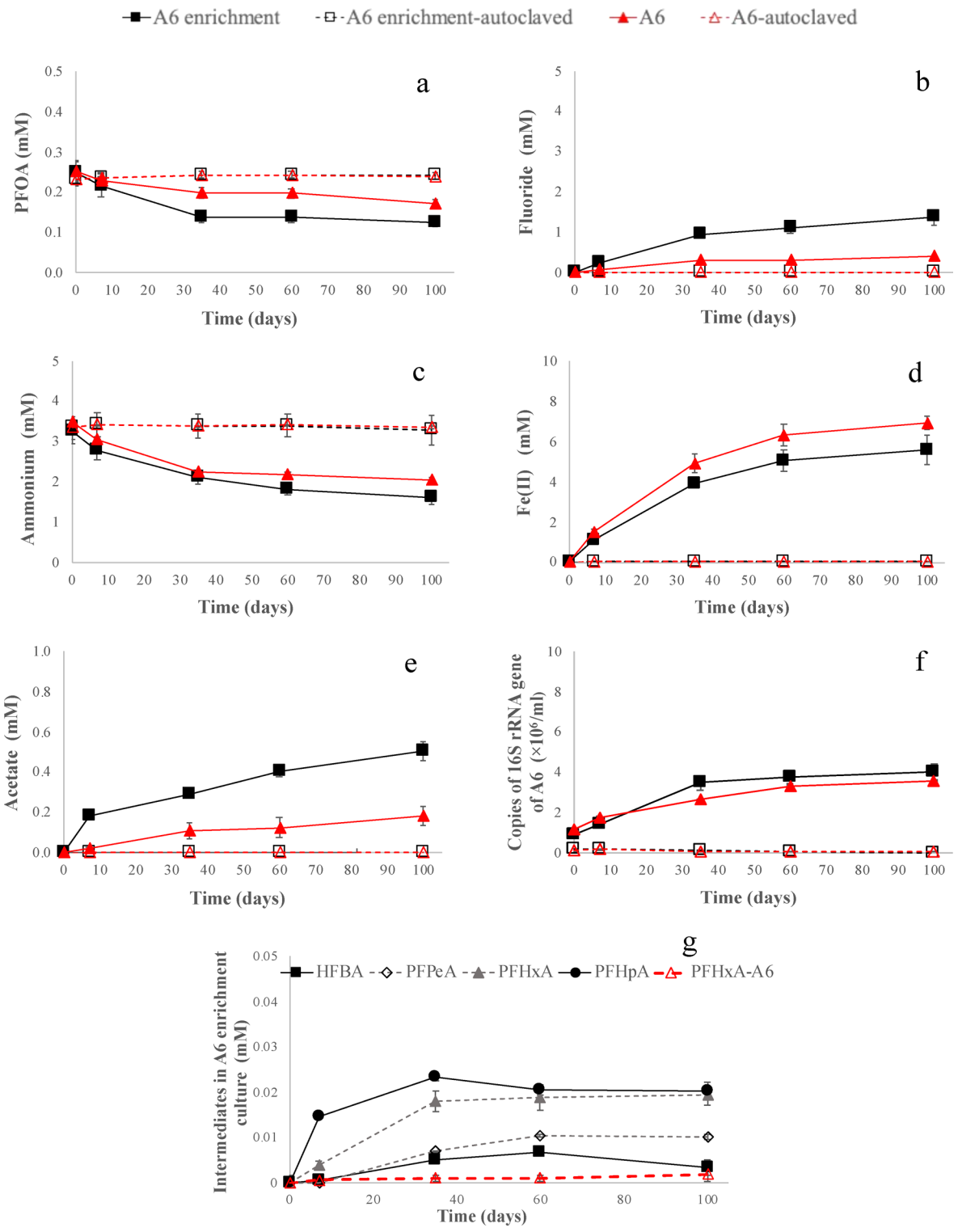

Figure 1. Results of PFOA 0.24 mM (100 mg/L) incubations with pure A6 and A6 enrichment cultures and NH_4^+ as electron donor [av. (SD), n = 3 for all samples, except for day 60 for which n = 6].

were the same as for the experiments with NH_4^+ as electron donor. The incubations with NH_4^+ instead of H_2 as electron donor were run in parallel to compare the effects of these two electron donors on the degradation of PFAS, under the exact same initial culture activity, bacterial numbers, and incubation conditions.

For each incubation, 2 mL of either a pure A6 or an A6 enrichment culture was mixed with 18 mL of the medium including 0.24 mM PFOA or 0.20 mM PFOS in a 50 mL vial. The headspace of each vial was vacuumed and then, for the incubations with H_2 as electron donor, was filled with a N_2/H_2 (80:20) mixture, while for those with NH_4^+ as electron donor vials were filled with a N_2/CO_2 mixture (80:20). Vials were then placed on a rotary shaker at 150 rpm at 30 °C for a 35 day incubation period. Three subsamples were collected destruc-

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tively on day 0, 7, 21, and 35 for Fe(II), NH_4^+, F^-, DOC, and PFOA/PFOS analyses.

Chemical and Microbiological Analyses. PFAS were analyzed commercially by the Guangdong Institute of Microbiology (China) and Eurofins (USA) via ultraperformance liquid chromatography−tandem mass spectrometry (UPLC-MS−MS: Agilent 1290-6430A) following well-established methods.[32,33] All compounds were quantified with a standard curve in the range of 0.5−100 μg/L, after making appropriate dilutions with methanol. A blank control was also included in each sample sequence to ascertain that the target compounds were not detected in their absence. A Zorbax SB-C18 (10 mm × 2.1 mm, 1.7 μm) column was used at 50 °C, with an isocratic mobile phase composed of (A) (32:0.15% (v/v) acetic acid in water) and (B) (68:0.15% (v/v) acetic acid in methanol) with a flow rate of 0.4 mL/min. The eluent gradient started with 25% (B) for 0.5 min, and then was lineally increased to 95% (B) in 6 mins and held at that gradient for 5 min, and eventually returned to the initial conditions within 1 min and held for 2 mins for equilibration during the injection interval. Detection was done by negative electrospray ionization (ESI) mass spectrometry in a multiple reaction monitoring mode (MRM) (more detailed information is provided in Section S1.1 and Table S1 of the Supporting Information).

Redox potential and pH in the incubation vials were measured using a Hach HQ40d multi-probe meter (Hach, Loveland, CO, USA). DOC and POC (particulate organic carbon) were analyzed using a Shimadzu TOC-5000A analyzer for samples filtered through or retained by 0.2 μm aperture filters.[21,34] Fe(II) was analyzed via the ferrozine assay method.[35] Different ions were analyzed for filtered samples (0.2 μm) via an ion chromatograph (IC) with a conductivity detector (ICS-3000, Dionex Co., USA), with an AS18 column (4 mm i.d. × 200 mm) for anions and a CS16 column (4 mm i.d. × 200 mm) for cations.[21,23]

Since precise F^- analyses are key in this research, and since they can be affected by various interferences, multiple QA precautions were taken. The analytical method for F^- was modified based on methodologies described in the literature.[36,37] Two separate IC analyses were conducted. The first one used an AS-18 column with a KOH solution (20 mM) as the eluent, flow rate = 0.80 mL min^{-1}, and the suppressor current was set at 85 mA. The other one used an AS12A column (4 mm i.d. × 200 mm), with 7.5 mM $NaHCO_3$/22.5 mM Na_2CO_3 as the eluent, flow rate = 1.00 mL min^{-1}, and the suppressor current was set at 50 mA. Dionex retention time standards were used as external calibration curves, which contained F^- at seven equal-increment dilutions up to a concentration of 5.2 mM. There was no significant difference ($p < 0.05$) in the measured F^- concentration between IC methods, hence the values reported here for all incubations with the higher PFAS concentrations, and used for the fluoride balances, are for the AS-18 column with KOH as the eluent. Retention times and the chromatograms of the analysis of F^- and other ions are shown in Table S2. As an additional confirmation, F^- was also analyzed using a perfectION combination electrode (Mettler-Toledo, USA). The concentrations measured with the F^--specific electrode were within 10% of those measured via IC and were not significantly different ($p < 0.05$). F^- measurements for the low-concentration PFAS incubations were somewhat higher using the electrode, and the difference between the IC and the electrode measurements was significant.

Total DNA was extracted from samples of the 100 day incubations with 0.24 mM PFOA and 0.20 mM PFOS (Section S1.2, Supporting Information). Sequencing of the 16S rRNA genes from the A6 enrichment cultures over the incubation period was performed on an Illumina MiSeq platform (Section S1.3, Supporting Information). The 16S rRNA genes of A6 were quantified through quantitative PCR (qPCR) analyses (Section S1.4, Supporting Information).[21,23]

Statistical Analysis. The normality and homoscedasticity of the data was examined. Significance for all tests was accepted at the 0.05 level. All the analyses were performed using SPSS v12.

RESULTS AND DISCUSSION

Degradation of PFOA with NH_4^+ as Electron Donor. Effects of PFOA on the Feammox activity (in terms of NH_4^+ removed and Fe(III) produced, as well as on A6 numbers) were found to be negligible within the concentration of interest over a 2 week incubation and are discussed in Section S2 and Figure S1 of the Supporting Information. Figure 1 shows results for incubations with an initial PFOA concentration of 0.24 mM (100 mg/L) for the pure A6 and the A6 enrichment cultures. Comparable NH_4^+ removal, Fe(II) and NO_2^- production, and A6-16S rRNA gene numbers during the incubations (Figure 1c, d, f and Figure S3a) show that both cultures had very similar Feammox activity. While all incubations were set up without adding organic carbon, buildup of acetate (Figure 1e) was detected in both cultures during incubations with PFOA, and to higher levels than in the positive control without PFOA (Figures S4c and S6c).

Over the 100 day incubations, a 0.12 mM (50%) decrease of PFOA was detected in the A6 enrichment culture and 0.08 mM (33%) in the pure A6 culture (Figure 1a). Four intermediate products, including perfluorobutanoic acid (HFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), and perfluoroheptanoic acid (PFHpA) (Figure 1g) were detected in the A6 enrichment culture incubations, neither of which was present initially nor in any control. Only perfluorohexanoic acid (PFHxA) was detected in the incubation with the pure A6 culture, and at much lower concentrations (0.12 ± 0.05 μM) than in the A6 enrichment culture. After the incubation, 1.36 mM of F^- was detected in the A6 enrichment culture, and 0.42 mM in the pure A6 culture incubations (Figure 1b).

During the same period, the 16S rRNA gene numbers of A6 increased from 1.15×10^6 to 3.57×10^6 copies/mL in the pure culture and from 0.87×10^6 to 4.02×10^6 copies/mL in the enrichment culture (Figure 1f).

There were no changes in concentration of any of the chemical species monitored in the autoclaved controls, and there was no PFOA removal or F^- generation in the cultures without Fe(III) or NH_4^+, nor in the incubations with *G. sulfurreducens* (Figures S4 and S6). Over the 100 day incubations, *Geobacter* increased from 0.12×10^7 to 2.12×10^7 copies/mL and produced 6.98 mM Fe(II) (Figure S6).

Results for the incubations with an initial concentration of 0.24 μM (0.1 mg/L) show a 0.15 μM (63%) PFOA removal for both the pure A6 and the A6 enrichment cultures (Figure S8). Due to the low concentrations, neither the intermediate nor end products were analyzed. The higher relative error of the F^- analysis at this concentration and lack of quantified

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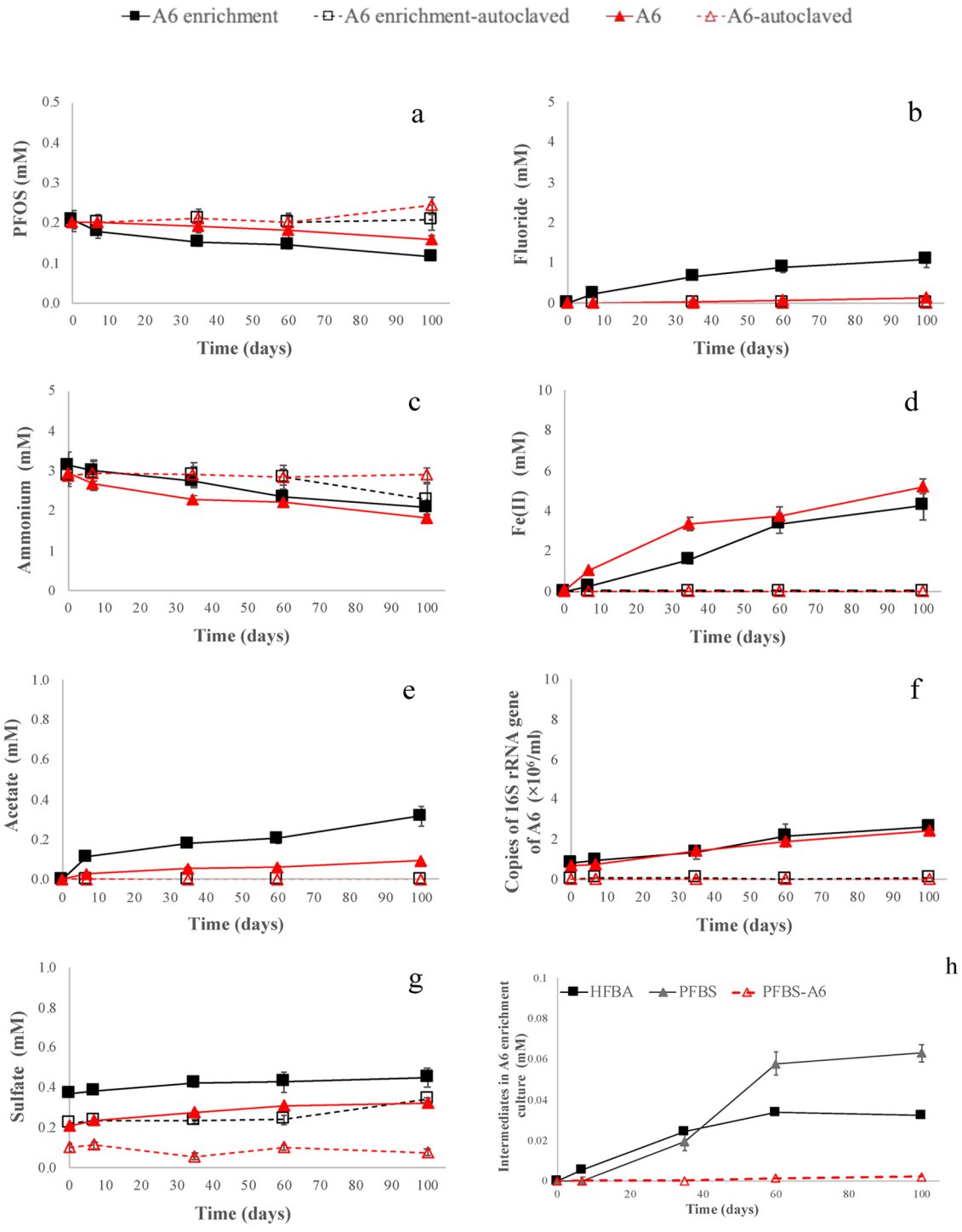

Figure 2. Results of PFOS 0.20 mM (100 mg/L) incubations with pure A6 and A6 enrichment cultures and NH_4^+ as electron donor [av. (SD), n = 3 for all samples, except for day 60 for which n = 6].

intermediates does not allow for an accurate fluorine balance. Acetate concentrations in the incubations with 0.24 μM PFOA (Figure S8e) are somewhat higher than those in the positive control (Figure S4c) but not significantly different.

Degradation of PFOS with NH_4^+ as Electron Donor. Similar to the results for PFOA, effects of PFOS on Feammox activity were negligible as discussed in Section S2 and Figure S2 in the Supporting Information. As shown in Figure 2, 0.045 mM (23%) of PFOS was removed by the pure A6 culture over 100 days of incubation, and 0.093 mM (47%) by the A6 enrichment culture. Intermediates detected in the A6 enrichment culture include HFBA and perfluorobutane sulfonate (PFBS) (Figure 2h), while the only intermediate detected in the pure A6 culture incubation, and at a much lower concentration, is PFBS.

As for the incubations with PFOA, comparable NH_4^+ oxidation, and Fe(III) and NO_2^- production were found in both cultures during these incubations (Figure 2c, d and Figure S3b). The number of A6-16S rRNA genes in the pure culture increased from 0.70 × 10^6 to 3.32 × 10^6 copies/mL, and from

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Table 1. Fluorine Balance for the PFOA Degradation Experiment with the A6 Enrichment Culture[a]

times (days)	F in PFOA (mM)	F in HFBA (mM)	F in PFPeA (mM)	F in PFH × A (mM)	F in PFHpA (mM)	F^- (mM)	total F (mM)
0	3.72 (0.49)	0 (<0.001)	0 (<0.001)	0 (<0.001)	0 (<0.001)	0 (<0.001)	3.72
7	3.21 (0.36)	0.0034 (<0.001)	0 (<0.001)	0.04 (0.001)	0.19 (0.01)	0.25 (0.01)	3.69
35	2.10 (0.20)	0.036 (0.002)	0.064 (0.001)	0.06 (0.025)	0.30 (0.01)	0.95 (0.08)	3.51
60	2.07 (0.19)	0.050 (0.004)	0.095 (0.002)	0.19 (0.031)	0.27 (0.01)	1.10 (0.12)	3.77
100	1.87 (0.14)	0.024 (0.011)	0.01 (0.004)	0.30 (0.022)	0.27 (0.02)	1.36 (0.19)	3.83

[a][av (SD), n = 3 for all samples, except for day 60 for which n = 6]

0.85×10^6 to 3.51×10^6 copies/mL in the enrichment culture (Figure 2f). The F^- measurements show that 1.07 mM of F^- was produced during the 100 day incubation with the A6 enrichment culture, which is almost 10 times higher than the amount of F^- produced in the pure A6 culture incubation (Figure 2b). An increase of 0.13 mM SO_4^{2-} was observed in the pure A6 culture during the PFOS incubations (Figure 2g), which was not observed in the incubations with PFOA. Autoclaving the medium in the presence of ferrihydrite resulted in a decrease in the dissolved SO_4^{2-} concentration as can be seen in Figure 2g. The noisier baseline of the autoclaved control makes it harder to state conclusively that there was a significant increase in the SO_4^{2-} concentration in the A6 enrichment culture. Furthermore, the presence of sulfate reducers (see microbial community discussions below), decrease in SO_4^{2-} over time in the positive control (Figure S7g), and coupled to the decrease in DOC vs time in the A6 enrichment culture (see dissolved carbon balance section below) indicate that sulfate reduction could have occurred in the A6 enrichment culture incubations, which makes it difficult to determine if sulfate was produced from the degradation of PFOS in these incubations.

There were no changes in concentration of any of the chemical species monitored in the autoclaved controls where there was no PFOS removal or F^- generation in the cultures without Fe(III) or NH_4^+ and neither was there PFOS removal in the incubations with *G. sulfurreducens* (Figures S5 and S7). Over the incubation period, *G. sulfurreducens* numbers increased from 0.12×10^7 to 1.98×10^7 copies/mL, producing 6.24 mM Fe(II) (Figure S7).

Results for the incubations with an initial concentration of 0.20 μM (100 μg/L) PFOS shown in Figure S9 show that the pure A6 culture removed 0.07 μM (35%) PFOS and the A6 enrichment culture removed 0.12 μM (60%) over the incubation period.

It is interesting to note that, in general, the fraction of PFOA/PFOS removed by each culture was similar for the incubations with the high and the low PFOA/PFOS concentration.

Degradation of PFOA and PFOS with H_2 as Electron Donor. A comparable amount of PFOA removal was observed during 35 days of incubation in both the pure A6 and the A6 enrichment cultures when either Fe(III) + NH_4^+ or Fe(III) + H_2 were present (Figure S10a). Similar results were observed for the PFOS incubations (Figure S11a). No PFAS removal was observed in the autoclaved controls (Figures S10a and S11a), nor in any of the other controls discussed above.

A noticeable increase over time of fluorinated degradation products (HBFA, PFPeA, PFHxA, and PFHxA for the PFOA incubations and HBFA and PFBS for the PFOS incubations) was detected in the A6 enrichment culture incubations (Figures S10g,h and S11g,h). A buildup of fluorinated degradation products was not observed in the pure A6 culture incubations.

Sulfate buildup in the PFOS incubations (Figure S11g) was significant for the pure A6 culture incubations when either NH_4^+ or H_2 was the electron acceptor, while for the enrichment culture the increase in sulfate was only significant when NH_4^+ was the electron donor.

Characteristics of the Microbial Community during Incubations with and without PFOA/PFOS. The microbial community analysis reveals the presence of denitrifiers (*Ralstonia* and *Bacillus*),[38,39] iron reducers (*Acidimicrobium* and *Aciditerrimonas*),[40] and sulfate reducers (*Desulfosporosinus*).[41,42] These organisms might have been using the NO_2^- generated from the Feammox process, Fe(III), and SO_4^{2-} present in the medium as electron acceptors to oxidize the defluorinated or partially defluorinated products from the PFOS/PFOA defluorination, resulting in the decreasing DOC vs time in the A6 enrichment culture.

Although the short term effects of PFOA and PFOS were negligible on the Feammox activity, the microbial community analysis from the A6 enrichment culture of samples collected on day 0 and day 60 shows that the composition of the community with PFOA diverged over time from that with PFOS, while the change of the composition in samples without PFAS was much smaller (Figure S12).

It is also interesting to note that *Acidimicrobium* was dominant at the onset of the incubations (Figure S12). Although copy numbers of A6 increased during the 60 day incubations (more in the presence of PFOA than PFOS) the relative population of the *Acidimicrobium* decreased in the positive control incubations, more so in the presence of PFAS, and most in the presence of PFOS. The larger relative *Acidimicrobium* population in the PFOA incubations (22.6%) vs the PFOS incubations (10.4%) is consistent with the higher 16S rRNA gene numbers of A6 in that incubation, and a higher Feammox activity in terms of NH_4^+ removal (39% higher than in the PFOS incubation) and Fe(II) production (47% higher than in the PFOS incubation) over the same time period. The higher Feammox activity in the PFOA A6 enrichment culture incubations also corresponds to a higher PFOA removal and F^- production vs PFOS removal and F^- production in the PFOS incubations. Hence, it appears that, at these rather high concentrations, PFOS has a more detrimental effect on A6 than PFOA. Further work is needed to explore the threshold concentrations where this effect becomes less marked, and if it may have an impact on long-term PFOS bioremediation schemes with A6.

Fluorine Balance. The results for PFOA/PFOS degradation allow for a fluorine balance during each incubation, which for the A6 enrichment culture is shown in Table 1 for PFOA and in Table S3 for PFOS. Results show a good fluorine balance, which gives confidence in the accuracy of the analyses shown in Figures 1 and 2.

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The same fluorine balances for incubations with the pure A6 culture are shown in Table S4 for PFOA and in Table S5 for PFOS. The fluorine balance for the pure A6 culture incubations shows a slight decreasing trend in total quantified fluorine over time for both the incubation with PFOA and with PFOS, indicating that some fluorinated intermediates might have been present but were not detected. Standards for partially fluorinated intermediates were not available and, if present, such compounds were not detected.

Effect of Defluorination on Fe(III) Reduction. As shown by Equation 1, the expected molar ratio of Fe(II) produced to NH_4^+ oxidized is 6:1. If during the defluorination process, some electrons from the oxidation of NH_4^+ are transferred to the PFAS to free F^- instead of being transferred to Fe(III) to produce Fe(II), one should expect a commensurate decrease in Fe(III) reduction. Results of the Fe(II)/NH_4^+ ratio vs time during the A6 enrichment culture incubations with PFOA and PFOS are shown in Table 2 and in Table S6, respectively, and in Table S7 and in Table S8 for the pure A6 culture with PFOA and PFOS, respectively. The first column in each table shows the observed Fe(II)/NH_4^+ ratio for incubations with PFOA/PFOS, the second column shows the ratio of [Fe(II) + F^-]/NH_4^+ for incubations with PFOA/PFOS, while the third column shows the Fe(II)/NH_4^+ ratio of the positive controls that were incubated in parallel without PFOA/PFOS. The actual Fe(II)/NH_4^+ ratio observed during Feammox incubations is usually less than 6:1, which is attributed to (i) a less efficient Fe(II) extraction especially early on during the incubations, (ii) when AQDS is present, the reduction of AQDS and buildup of AH_2QDS, which accumulates early on during incubations,[43] and (iii) the possible oxidation of Fe(II) by NO_2^-, especially when the pH is >6.[44]

Table 2. Molar Ratio of Fe(II) Produced to NH_4^+ Removed for PFOA Incubations with the A6 Enrichment Culture[a]

time day	with PFOA Fe(II)/NH_4^+	with PFOA [Fe(II) + F-]/NH_4^+	control without PFOA Fe(II)/NH_4^+
7	2.37 (0.36)	2.90 (0.45)	3.21 (0.41)
35	3.40 (0.21)	4.22 (0.19)	4.28 (0.15)
60	3.49 (0.16)	4.25 (0.11)	4.49 (0.16)
100	3.41 (0.15)	4.24 (0.17)	4.62 (0.11)

[a][av (SD), n = 3 for all samples, except for day 60 for which n = 6]

By day 100, the Fe(II)/NH_4^+ ratio in the controls without PFOA and PFOS was between 4.6 and 4.8 in the A6 enrichment culture and between 4.8 and 5.1 in the pure A6 culture. In contrast, the respective Fe(II)/NH_4^+ ratio in the presence of these PFAS was lower (3.4 to 4.0 in the A6 enrichment culture and 4.6 and 4.8 in the pure A6 culture), while the [Fe(II) + F^-]/NH_4^+ was closer to the positive controls. These analyses suggest that these PFAS may be acting as alternative electron acceptors to Fe(III), and hence, that the F^- production could be due to reductive defluorination. Incubations without Fe(III) (Figures S6a and S7a) did not result in defluorination, indicating that these PFAS, at the experimental conditions described here, cannot be used as sole electron acceptors for the growth of A6 using NH_4^+ or H_2 as electron donors. Results also show that in the presence of these PFAS, even at the high concentrations used here, the large majority of electrons were transferred to Fe(III).

Dissolved Organic Carbon Balance. DOC analyses were performed immediately after sampling during the 35 day incubations, and DOC balances were performed on these 0.24 mM PFOA and 0.20 mM PFOS incubations with both NH_4^+ and H_2 as electron donors.

As A6 numbers increased during the incubations, with CO_2 as the carbon source (Figure 1f and Figure 2f), the particulate organic carbon (POC) increased from 1.42 mM to 2.05 mM and from 1.29 mM to 1.78 mM in the 100 day PFOA and PFOS pure A6 culture incubations, respectively. In contrast, DOC remained constant over the incubation time in the pure A6 culture (Table 3 and Table S9 as well as Figures S10f and S11f). Although no organic carbon (except the PFAS) was supplied to any vials, there was a background DOC in the positive control (without PFAS), ranging from 0.18 ± 0.02 to 0.36 ± 0.03 mM over the 35 day incubations for the pure A6 and the A6 enrichment culture, respectively, which is attributed to the long-term fixation of organic carbon by this autotroph. At 0.24 mM PFOA and 0.20 mM PFOS, their contribution to the total DOC is an order of magnitude higher than that of the background DOC. Given that the DOC fixed during the 35 day incubation period is negligible in comparison to the DOC from the PFAS added, a constant DOC vs time in the pure A6 culture incubations indicates that none of the PFAS that were being degraded nor their degradation products were oxidized to CO_2.

Table 3. DOC Balance for Incubations with PFOA−A6 Pure Culture[a]

parameters	day 0	day 7	day 21	day 35
measured DOC, with NH_4^+ (mM)	1.96 (0.09)	1.98 (0.09)	1.94 (0.09)	1.93 (0.08)
estimated DOC with NH_4^+ (mM)	2.04	2.04	2.01	2.03
% difference	4.1%	3.1%	3.6%	5.2%
measured DOC, with H_2 (mM)	1.95 (0.08)	1.97 (0.08)	1.96 (0.08)	2.00 (0.08)
estimated DOC with H_2 (mM)	2.06	2.03	1.96	1.77
% difference	5.6%	3.1%	0%	11.5%

[a][av (SD), n = 3]

DOC was measured as described above as well as estimated. DOC as a function of incubation time was estimated based on the concentration and molecular formula of the initial PFAS, fluorinated intermediates, and acetate for each timepoint. The background DOC from the positive control (no PFAS) was then added to these estimated DOC values (for the pure A6 culture: 0.18 mM for PFOA and 0.17 mM for PFOS; for the A6 enrichment culture: 0.35 mM for PFOA and 0.38 mM for PFOS incubations). Hence, a close match between the measured and estimated DOC indicates that the major dissolved organic compounds, which includes the original PFAS and their degradation products (fluorinated and nonfluorinated organics), have been properly quantified.

Table 4 and Table S10 show the measured and estimated DOC vs time for the incubations with the A6 enrichment culture when either NH_4^+ or H_2 is used as electron donor, for incubations with PFOA and PFOS, respectively. Results show that the DOC decreased at a rate of ~5.7 μM day^{-1}, which is attributed to the presence of heterotrophic bacteria that can grow on PFOA/PFOS degradation products such as acetate while using electron acceptors such as NO_2^- (Figure S3), Fe(III), or SO_4^{2-}. The measured and estimated DOC values are in close agreement, indicating that the key degradation

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Table 4. DOC Balance for Incubations with PFOA−A6 Enrichment Culture[a]

parameters	day 0	day 7	day 21	day 35
measured DOC, with NH_4^+ (mM)	2.38 (0.09)	2.32 (0.09)	2.19 (0.08)	2.1 (0.07)
estimated DOC with NH_4^+ (mM)	2.33	2.25	2.20	2.23
% difference	2.1%	3.0%	0.5%	6.2%
measured DOC, with H_2 (mM)	2.40 (0.10)	2.28 (0.09)	2.18 (0.08)	2.09 (0.07)
estimated DOC with H_2 (mM)	2.35	2.25	2.18	2.05
% difference	2.1%	1.3%	0%	2.0%

[a][av. (SD), n = 3]

intermediates contributing to the DOC have been identified and properly quantified.

Table 3 and Table S9 show the measured and estimated DOC vs time for the incubations with the pure A6 culture with NH_4^+ or H_2 as an electron donor for incubations with PFOA and PFOS, respectively. In contrast to the A6 enrichment culture, here the DOC remains constant with time, indicating that, as expected, A6 does not utilize PFOA or PFOS nor any of their degradation products as carbon source. Results again show a good agreement between measured and estimated DOC.

Production of Shorter Chain Fluorinated Compounds. The production during incubations with the A6 enrichment culture of shorter-chain fluorinated compounds such as HFBA, PFPeA, PFHxA, and PFHpA during the degradation of PFOA, and the production of PFBS and HFBA during the degradation of PFOS show that in addition to defluorination, the C−C bond of these molecules was oxidized. Production of SO_4^{2-} in the PFOS incubations indicates that the C−S bond was oxidized.

Concentrations of shorter carbon chain−perfluorinated intermediates and of acetate in the incubations with the pure A6 culture were much lower than those in the A6 enrichment culture and not detected when H_2 was the electron donor. As mentioned above, the decrease over time in total fluorine quantified (0.56 mM for PFOA and 0.58 mM for PFOS over 100 days, Tables S4 and S5) indicates the possibility of the formation and accumulation of other fluorinated organics in the pure A6 culture that were not detected. Hence, further research is needed to determine what fluorinated intermediates that have not been identified here might be produced, and when and to what degree the pure A6 culture is capable of breaking the C−C bond.

Although the Feammox activity in the pure and enriched A6 culture was similar in terms of NH_4^+ removed and Fe(II) produced as well as A6 numbers, the A6 enrichment culture had a higher overall PFOA and PFOS removal and production of smaller perfluorinated compounds, acetate, and F^-. This indicates the possibility of a synergistic interaction between A6 and other organisms in the A6 enrichment culture during the degradation of these PFAS, where once a perfluorinated compound has been partially defluorinated by A6, heterotrophs in the A6 enrichment culture are capable of further degrading these intermediates. Since A6 is an autotroph and no organic carbon was added to the incubations, the increase in acetate above that in the positive control during the incubations with the PFAS, coupled with the carbon balance, indicates that complete defluorination of PFOA and PFOS may be achieved by these cultures. Incubations with ^{13}C-labeled PFAS will have to be conducted to confirm that the acetate is a product from the PFAS degradation.

Possible Environmental Settings for PFAS Remediation via Feammox. Given that the Feammox process has been reported in many environmental settings, as long as the pH is acidic and soils are iron rich, the results presented here indicate that under the right conditions, the Feammox process could be stimulated to achieve PFAS biodegradation in contaminated sediments and groundwater systems. The Feammox process has been stimulated successfully in constructed wetland mesocosms[45] and microbial electrolysis cells[46] for the removal of NH_4^+ and in soil columns for the removal of TCE.[29]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b04047.

More details on analytical methods, sample chromatograms for the PFAS, and F^- analyses; tables for fluorine balances, molar ratio of Fe(II) produced to NH_4^+ removed, and DOC balances, not shown in the main text; figures showing short term effects of PFOA and PFOS on Feammox activity, nitrate production during incubations, results of various controls of the PFOA/PFOS incubations, results of PFOA and PFOS incubations at the lower concentrations, results contrasting H_2 and NH_4^+ as electron donor for PFOA and PFOS incubations; and microbial community analysis of the enrichment culture (PDF)

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Notes

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