

FINAL REPORT

Biotransformation and Potential Mineralization of PFOS, PFHxS,
and PFOA by Acidimicrobiaceae sp. A6 under Iron
Reducing Conditions

SERDP Project ER20-1219

JULY 2021

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REPORT DOCUMENTATION PAGE					<i>Form Approved</i> OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) 29/07/2021		2. REPORT TYPE SERDP Final Report			3. DATES COVERED (From - To) 9/23/2019 - 9/23/2021	
4. TITLE AND SUBTITLE Biotransformation and Potential Mineralization of PFOS, PFHxS, and PFOA by Acidimicrobiaceae sp. A6 under Iron Reducing Conditions				5a. CONTRACT NUMBER 19-P-0108		
				5b. GRANT NUMBER		
				5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Peter R. Jaffé, Shan Huang, Matthew Sima Princeton University Ian Ross Arcadis Jinxia Liu McGill University				5d. PROJECT NUMBER ER20-1219		
				5e. TASK NUMBER		
				5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Princeton University Civil and Environmental Engineering Princeton, NJ 08544					8. PERFORMING ORGANIZATION REPORT NUMBER ER20-1219	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Strategic Environmental Research and Development Program (SERDP) 4800 Mark Center Drive, Suite 16F16 Alexandria, VA 22350-3605					10. SPONSOR/MONITOR'S ACRONYM(S) SERDP	
					11. SPONSOR/MONITOR'S REPORT NUMBER(S) ER20-1219	
12. DISTRIBUTION/AVAILABILITY STATEMENT DISTRIBUTION STATEMENT A. Approved for public release: distribution unlimited.						
13. SUPPLEMENTARY NOTES						
14. ABSTRACT The objective of this research was to verify these findings, determine if other compounds of interest at DoD sites, like PFHxS, could also be degraded, and gain further insights into the mechanism by which A6 defluorinates these PFAS, including the role of the two dehalogenase genes expressed during the defluorination of these PFAS.						
15. SUBJECT TERMS Biotransformation, Potential Mineralization, PFOS, PFHxS, PFOA, Acidimicrobiaceae sp. A6, Iron Reducing Conditions						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UNCLASS	18. NUMBER OF PAGES 88	19a. NAME OF RESPONSIBLE PERSON Peter R. Jaffé	
a. REPORT UNCLASS	b. ABSTRACT UNCLASS	c. THIS PAGE UNCLASS			19b. TELEPHONE NUMBER (Include area code) 609-258-4653	

FINAL REPORT

Project: ER20-1219

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¹ Additional funding for this effort was provided by the Princeton Catalysis Initiative

² Work conducted at NJIT was not funded by this project

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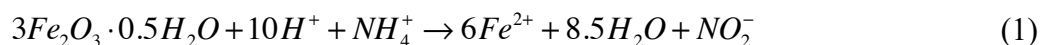
ACRONYMS AND ABBREVIATIONS

A6	<i>Acidimicrobium</i> sp. Strain A6
aa	amino acid
AJS ESI	Agilent Jet Stream Electrospray Ionization
AQDS	anthraquinone-2,6-disulfonate
BBMAP	A splice-aware global aligner for DNA and RNA sequencing reads
bp	base pairs
CD-HIT	Cluster Database at High Identity with Tolerance
CO ₂	Carbon dioxide
<i>dhl_1</i> and <i>dhl_2</i>	Haloacid dehalogenase genes
DCP	dichloropropane
DNA	Deoxyribonucleic acid
DoD	Department of Defense
F	Fluorine
F ⁻	Fluoride
<i>FceA</i>	Fluoroacetate dehalogenase homolog
Fe(II)	Iron(II)
Fe(III)	Iron(III)
GC	Gas chromatography
H-PFOA chemical formula is	it is PFOA with one F substituted for H I don't think it has a name. The
KOH	Potassium hydroxide
LCA	lowest common ancestor
m	meter
mg/l	milligrams per liter
min	minute
mL	milliliter
mL/min	milliliter per minute
mm	millimeter
MRM	multiple reaction monitoring mode
MS	Mass Spectrometry
N ₂	Dinitrogen
NCBI	National Center for Biotechnology Information
ng/mL	nanograms per milliliter
NH ₄ ⁺	Ammonium

nM	nanometer
nr	Non-redundant
nt	nucleotide
NTC	no template controls
ORF	Open Reading Frame
OTU	Operational taxonomic units
PCR	Polymerase chain reaction
PFAA	Perfluoroalkyl acid
PFAS	Perfluoroalkyl and polyfluoroalkyl substances
PFHxS	Perfluorohexanesulphonic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
pH	power of hydrogen
ppm	parts per million
qPCR	quantitative polymerase chain reaction
RDases	Reductive dehalogenases
<i>rdh</i>	Reductive dehalogenase homologous
<i>rdhA</i>	Reductive dehalogenase homolog
<i>rdhB</i>	Reductive dehalogenase anchoring protein
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
rpm	revolutions per minute
RT	Reverse Transcription
s	seconds
TCP	1,2,3-trichloropropane
μL	microliter
μm	micrometer
UPLC	High-Performance Liquid Chromatography
°C	degrees Celsius

Introduction and Objectives

Acidimicrobium sp. Strain A6 (A6 for brevity) is a novel bacterium, capable of oxidizing NH_4^+ while reducing ferric iron, as shown in equation 1 (Huang and Jaffé, 2015).



This process has been named Feammox (Sawayama, 2006). A6 has recently been isolated (Huang and Jaffé, 2018) and its genome has been sequenced. Through metagenomic analyses, it was revealed that the genome of A6 contains multiple dehalogenase genes, which were also detected in the A6 enrichment culture (GenBank accession numbers MK358459-MK358462). These include a gene for a reductive dehalogenase homolog (*rdhA*), a gene for a fluoroacetate dehalogenase homolog (*FceA*), and two putative haloacid dehalogenase genes (*dhl_1* and *dhl_2*).

Results of initial incubations with PFOA and PFOS as well as other PFAS, based on which our proposal was written, revealed that A6 could degrade these PFAS and produce F^- during the Feammox process as well as several shorter carbon chain PFAAs. Our initial results also revealed that both, the *rdhA* and *FceA* genes are expressed when PFAS are being defluorinated.

The objective of or proposed research was to verify these findings, determine if other compounds of interest at DoD sites, like PFHxS, could also be degraded, and gain further insights into the mechanism by which A6 defluorinates these PFAS, including the role of the two dehalogenase genes expressed during the defluorination of these PFAS.

Results presented in Huang and Jaffé (Huang and Jaffé, 2019), showed a good fluorine balance for PFOA/PFOS incubations with the A6 enrichment culture, but there was unaccounted fluorine in the pure A6 culture, which increased over the incubation time. We speculated that a partially

defluorinated PFOA/PFOS compound was being produced, which the pure culture was either unable to or not efficient in degrading. At that time, no partially defluorinated PFOA/PFOS was on the list of analytes that were being looked for in the analyses. Identifying such a compound would shed further insights into the defluorination process by A6, and a goal was to determine if a such compounds could be detected.

I. PFOA, PFOS, and PFHxS incubations with pure A6 and A6 enrichment cultures

Objectives

As mentioned above, the objective was to verify that PFOA/PFOS can be defluorinated by A6, and also test if PFHxS can be defluorinated. Furthermore, these incubations will allow for a careful monitoring of the expression of the *rdhA* and *FceA* genes during these incubations. It was decided to use initial concentrations of ~ 10 mg/l, which is substantially lower than the 100 mg/l used previously, but it still allows for tracking of F⁻ produced.

Methods

1. Incubation set up

200 mL of a pure or enrichment A6 culture, obtained from an anaerobic incubation amended with 10 ppm PFOA and PFOS for 90 days, were washed 3 times in the Feammox medium without Fe(III), centrifuged, and resuspended in 400 mL of anoxic inorganic Fe(III)-NH₄⁺ enrichment medium (Huang and Jaffé, 2018). These cultures were evenly distributed into multiple 30-mL serum vials in an anaerobic chamber and were sealed with butyl rubber stoppers. The headspace (5 mL) of each vial was vacuumed and then flushed with a N₂/CO₂ (80:20) mixture to achieve anoxic conditions, then incubated in a shaker at 25 °C. The initial pH of the cultures was 5.0 ± 0.2 .

The experiments were carried out with initial concentrations of PFOA (98%, Sigma-Aldrich), PFOS (SigmaAldrich 40% solution), or PFHxS (98%, Sigma-Aldrich) set to 10 mg/L. For each set of experiments, one autoclaved control and one medium control with the same PFAS and PFAS concentration was prepared. All vials were placed on a rotary shaker at 150 rpm at 25 °C for 60-

day incubations. On day 0, 7, 25, and 60, three vials were destructively sampled for Fe(II), NH_4^+ , PFAS, and microbial analyses.

2. Chemical analyses

The quantitative analysis of PFAS in the liquid phases (using 0.22 μm filters) was performed via High-Performance Liquid Chromatography coupled to Tandem Mass Spectrometry (UPLC-MS-MS: Agilent 1290-6430A), by SGS-CSTC Standards Technical Services Co., Ltd Guangzhou, Guangdong, China following well established methods.

All compounds were quantified with a multiple-point (7 to 9 points) calibration curve is executed in calibration levels of 1–40 ng/mL, 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. An Agilent ZORBAX SB-C18 (150 '2.1 mm, 3.5 μm) column was used at 30°C, with an isocratic mobile phase composed of (A) (85% ammonium acetate solution) and (B) (100% acetonitrile) with a flow rate of 0.3 mL/min. The eluent gradient started with (B) for 2 min, and then was lineally increased to 90% (B) in 3 min and held at that gradient for 5 min, and eventually returned to the initial conditions within 0.1 min and held for 4.9 min for equilibration during the injection interval. Detection was done by negative AJS ESI mass spectrometry in a multiple reaction monitoring mode (MRM). The SGS Quality Control Report and Equipment /Methods Information are shown in Appendix IV.

Redox potential and pH were measured using a Hach HQ40d multi-probe meter (Hach, Loveland, CO, USA). Fe(II) was analyzed via the ferrozine assay method as described in our previous study (Huang and Jaffé, 2018, 2019). Anions and cations were analyzed using a Dionex™ Ion Chromatograph (LC3000) with an AS-18 and CS-16 column, respectively. For the fluoride

analyses, 2 mL samples were filtered (0.2 μm) and then fluoride measured immediately via IC using an AS-18 column (flow rate = 1.00 mL min⁻¹, detection limit = 0.010 mg/l) with 25 mM KOH as the eluent. Dionex™ Retention Time Standards for all the anion analyses were used. As an additional confirmation, samples were also analyzed for fluoride spectrophotometrically, using a perfectION™ combination electrode (Mettler-Toledo, USA). The values reported here are for the AS-18 column with KOH as the eluent. The concentrations measured with the fluoride electrode were within 10% of those measured via IC and were only used as a separate confirmation.

3. Microbial analyses

Total DNA and RNA was extracted from each subsample, as described in the manual of the Fast DNA™ spin kit for soil (MP Biomedicals) or RNA PowerSoil® Total RNA Isolation Kit (Bio 101, Qiogene Inc. CA, USA). The concentrations and purity of the isolated DNA/ RNA were checked using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Marietta, OH, USA), and its integrity was evaluated by electrophoresis on ethidium bromide-stained agarose gels (Thermo Scientific 2X RNA Loading Dye).

To further study the changes in the microbial communities during incubation with different PFAS in the enrichment cultures, sequencing of 16S rRNA genes of each selected subsample (on day 0 and day 60) was performed on an Illumina MiSeq platform. The V4-V5 region of the 16S rRNA gene of bacteria was amplified using primer-set 515F-806R following methods suggested by Caporaso *et al* (Caporaso *et al.*, 2012). PCR reactions were carried out with a Phusion® High-Fidelity PCR Master Mix (New England Biolabs), and the PCR products were quantified and purified before sequencing. Sequencing libraries were generated using TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following the manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit® 2.0 Fluorometer (Thermo

Scientific) and Agilent Bioanalyzer 2100 system and was then sequenced on an Illumina MiSeq platform at Novogene Co. (Beijing, China), and 250bp paired-end reads were generated.

A total of 13275-17043 sequences were obtained for each sample. Operational taxonomic units (OTUs) were clustered with a 97% similarity cut-off using the Uparse software (Uparse v7.0.100, <http://drive5.com/uparse/>), which resulted in 817-1032 Operational Taxonomic Units (OTUs) for each sample. The taxonomy of each sequence was analyzed with Mother (version v.1.30.1) against the Silva SSUrRNA database using a confidence threshold of 0.8-1.

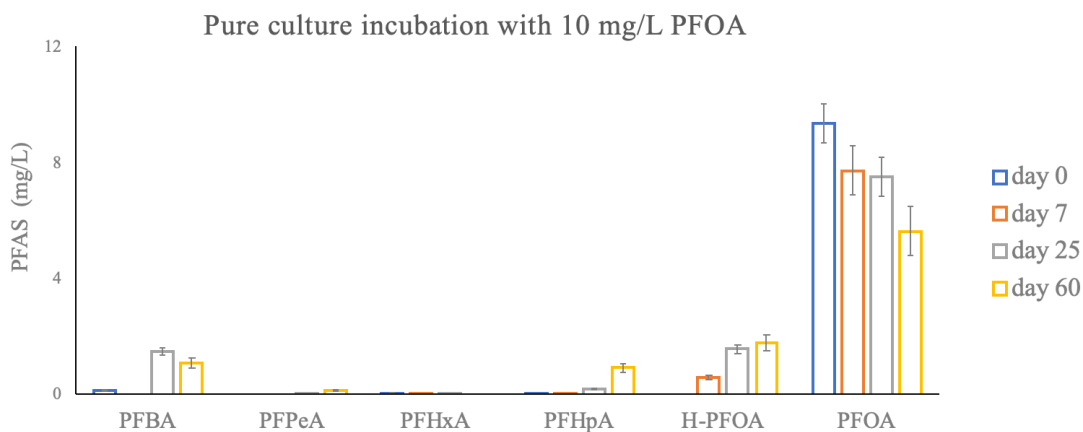
To quantified A6 numbers in both enrichments and pure cultures, 16S rRNA genes of A6 were targeted with primer set acm342f (5'-GCAATGGGGGAAACCCTGAC-3') - acm439r (5'-ACCGTCAATTTTCGTCCCTGC3'), and primer set 33F (5'-GGCGGCGTGCTTAACACAT-3') - 232R (5'-GAGCCC GTCCCAGAGTGATA-3') (Huang and Jaffé, 2018, Shuai and Jaffé, 2019). through qPCR analyses on a StepOnePlus qPCR system (Thermo Fisher Scientific, USA). Tenfold serial dilutions of a known copy number of the plasmid DNA were subjected to real-time PCR in triplicate to generate an external standard curve. Each assay contained a standard, independent triplicate template for each soil sample, and triplicate no template controls (NTC). Each PCR mixture (20 µL) was composed of 10 µL of SYBR Premix Ex Taq™ II (2×), 0.8 µL 10 nM of each forward and reverse primer, 0.4 µL ROX Reference Dye II, 6 µL ddH₂O, and 2.0 µL of template DNA (TaKaRa Biotechnology, Japan). The PCR program was initiated for 30 s at 94 °C, followed by 40 cycles of 5 s at 94 °C, 30 s at 55°C, and 30 s at 70°C. Melting curves were generated after each assay to check the specificity of amplification. PCR efficiencies were 90–103% and only the results with correlation coefficients above 0.98 were used.

Results

1. Degradation of PFAS during incubations with the pure A6 culture

Concentrations of PFOA, PFOS, and PFHxS, as well as that of intermediates produced over the 60-day incubations with the pure A6 culture are shown in Figure I.1. Consistent with our previously published results (Huang and Jaffé, 2019), there is more degradation of PFOA than of PFOS. A higher fraction of PFHxS seems to have been degraded than PFOS. We are uncertain why the degradation of PFHxS shows fewer intermediates than that of PFOS.

Interestingly, and not reported previously, we do see the production of H-PFOA during the degradation of PFOA. Controls, also shown in Figure I.1 show little if any change in the PFAS concentrations over the incubation time. Controls consisted of autoclaved vials with A6 and non-autoclaved medium controls without A6, both with PFAS added.



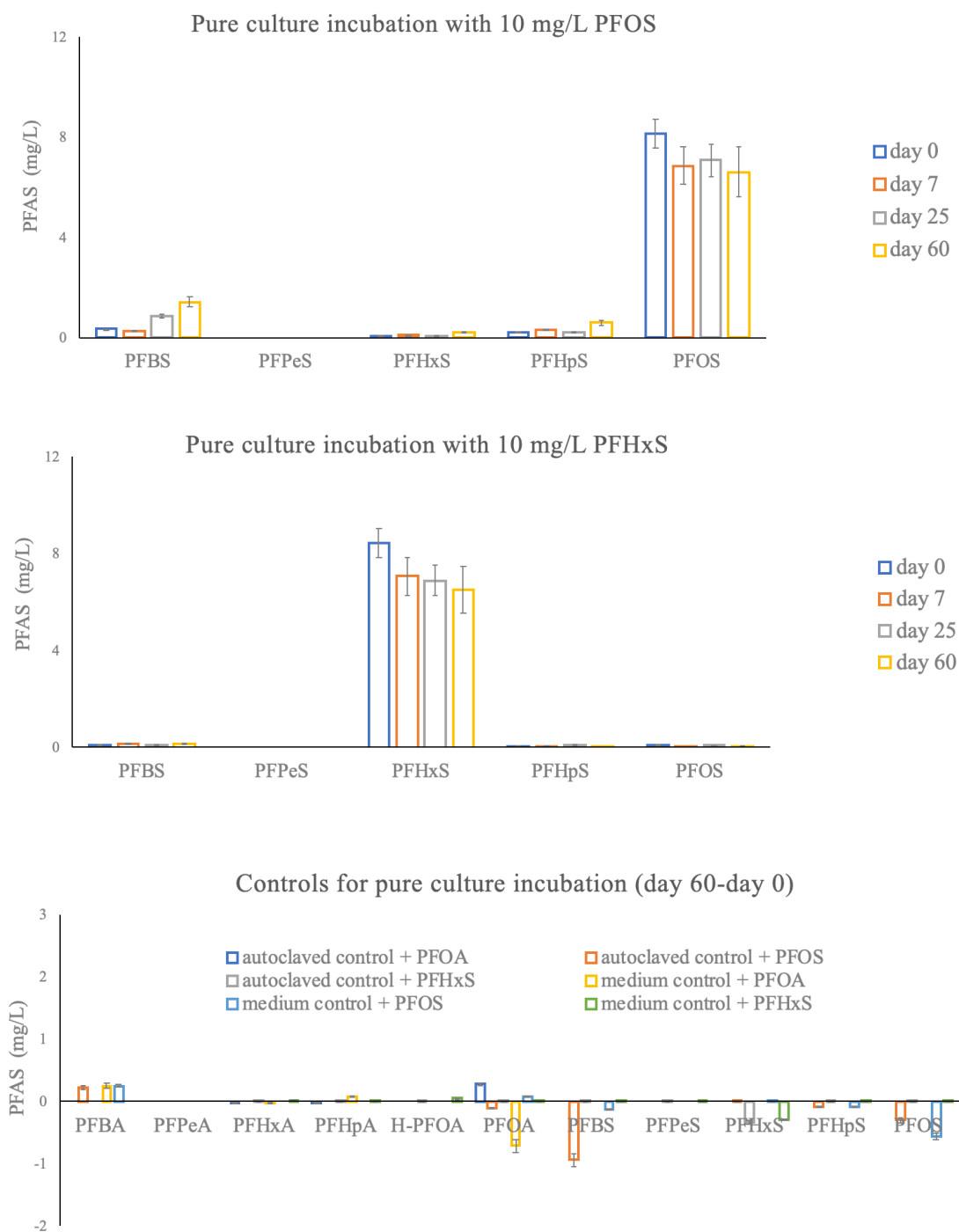


Fig. I.1 PFAS concentrations vs. time during incubations with the pure A6 culture

Statistical analysis: The decrease in PFOA and PFHxS between day 1 and day 60 is significant for the results shown above at the 5% level. The decrease in PFOS was due to the larger standard deviation in the measurement's not significant at the 5% level. In the degradation of PFOA the buildup of products was statistically significant for all intermediates except for PFHxA. The production of the all intermediates shown in the degradation of PFOS and PFHxS was statistically significant.

The production of fluoride during these incubations is shown in Figure I.2. A gradual increase in F^- is observed for the incubations of PFOA, PFOS, and PFHxS, which in combination with the decrease in their concentration and production of intermediates is a strong indication of PFAS biodegradation. In contrast, the change in F^- concentration in the controls was negligible.

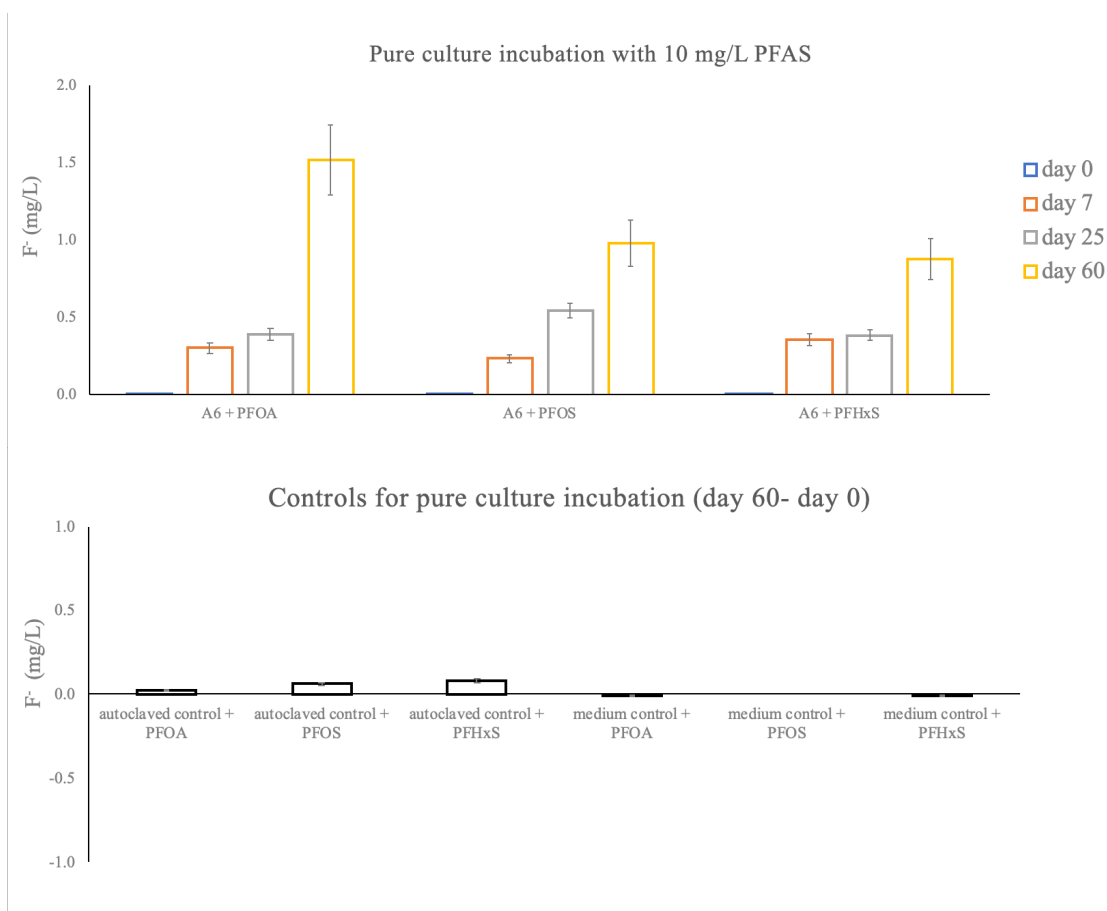


Fig. I.2 Production of F^- during the incubations with the pure A6 culture

Statistical analysis: The production of fluoride over the 60-day incubation was statistically significant at the 5% level for all incubations, and so was the increase in fluoride between day 25 and day 60, indicating that the reaction was still proceeding during this time interval.

The amount of NH_4^+ removed during this incubation period is shown in Figure I.3 and shows a decreased NH_4^+ removal in the incubations with PFHxS, indicating a lower Feammox activity in these vials. The final pH on day 65 in the different vials of the incubations with the three PFAAs was between 5 and 5.5.

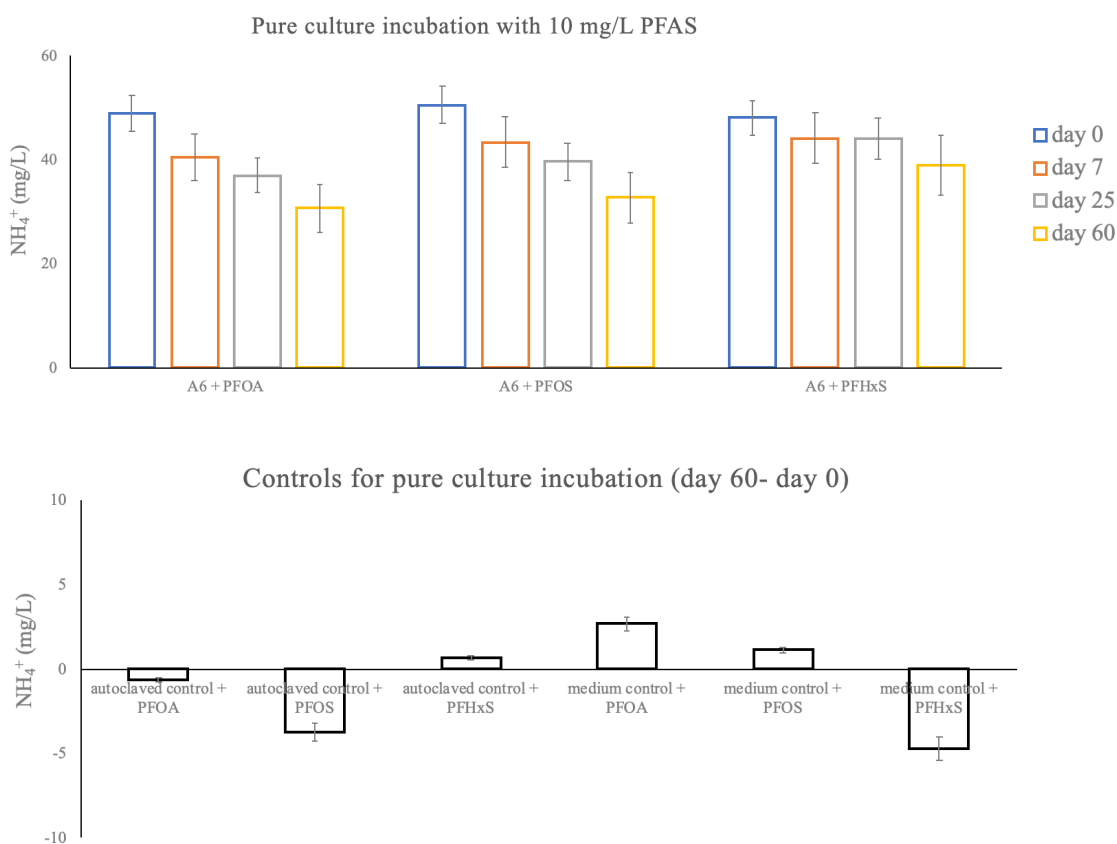
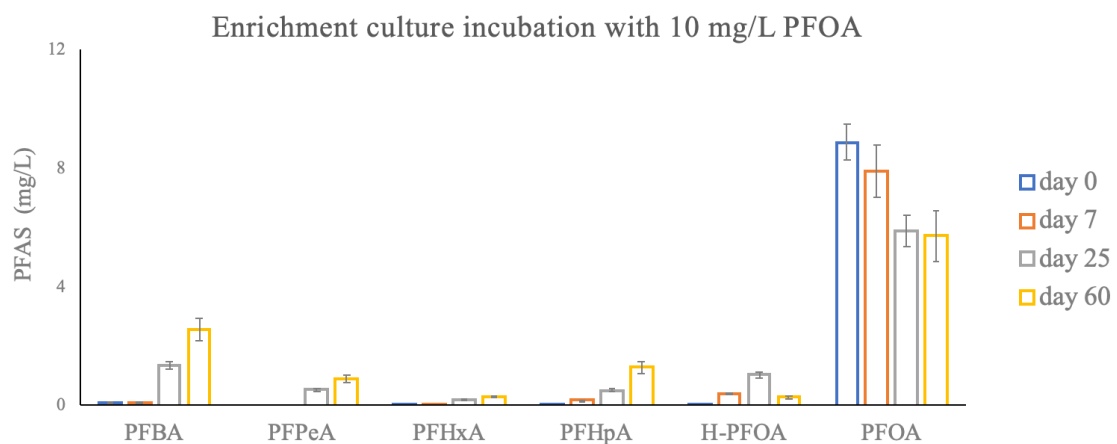


Fig. I.3 NH_4^+ concentrations during the incubations and controls with the pure A6 culture

Statistical analysis: The decrease in NH_4^+ concentration over the incubation time is significant at the 5% level for all incubations shown above.

2. Degradation of PFAS during incubations with the A6 enrichment culture

Concentrations of PFOA, PFOS, and PFHxS, as well as that of intermediates produced over the 60-day incubations with the A6 enrichment culture are shown in Figure I.4. Again, consistent with our previously reported findings, there is a more robust removal of PFOS in the enrichment culture incubations than in the pure culture incubations, and there is also an increase in PFHxS removal in the enrichment culture over that observed in the pure culture. The production of H-PFOA was observed again in the PFOA incubations, although concentrations were lower and they do not seem to build up with increasing incubation time, reaching the highest concentration on day 25 vs. at the end of the incubation as for the pure culture.



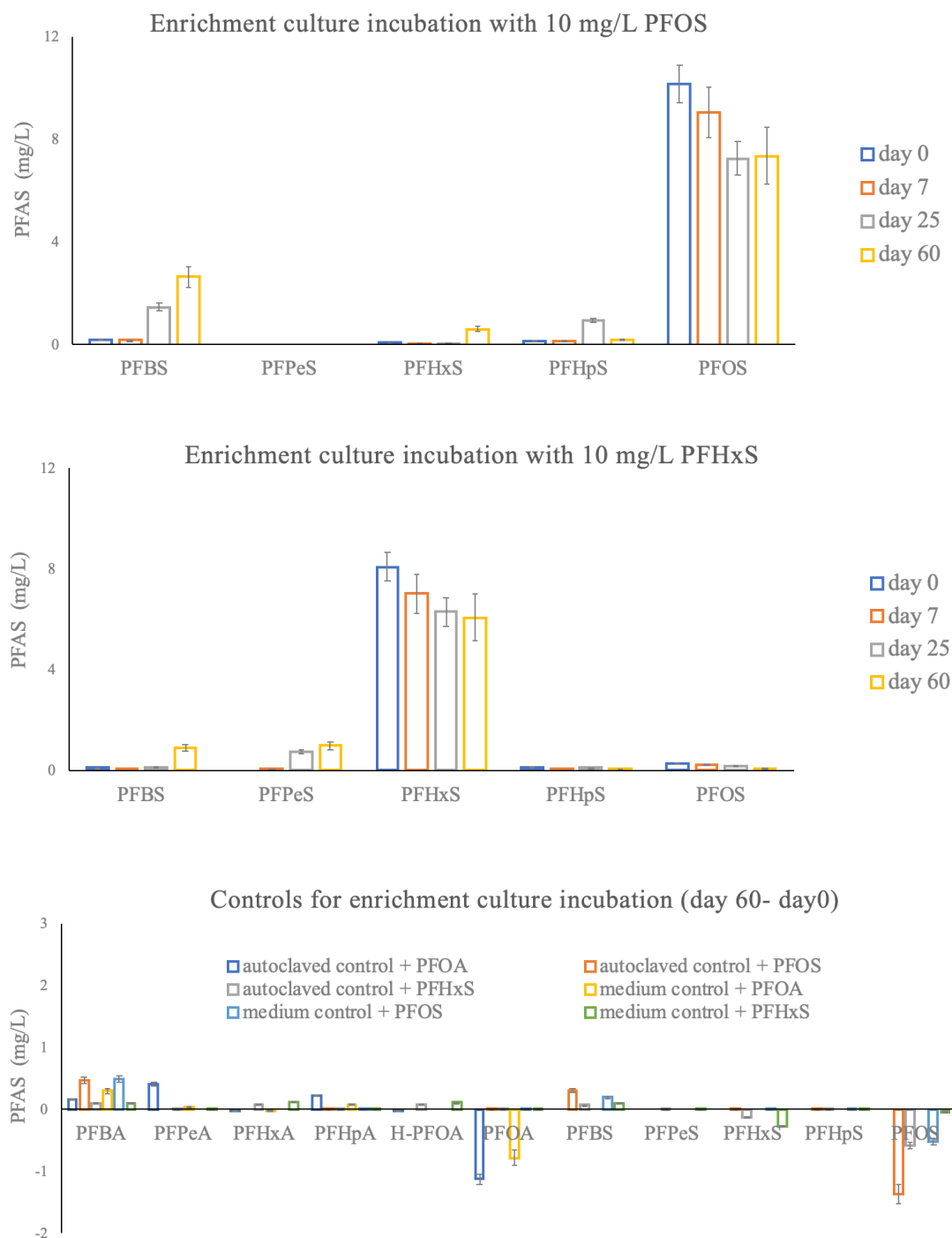


Fig. I.4 PFAS concentrations vs. time during incubations with the enrichment A6 culture

Statistical analysis: The decrease in PFOA, PFOS, and PFHxS concentration as well as the production of intermediates is statistically significant at the 5% level in all of these enrichment culture incubations. The decrease in PFOA and PFHxS concentration is still significant between days 25 and 60, while that for PFOS is not.

The production of F^- during these incubations is shown in Figure I.5, showing again a significant increase in F^- over time while F^- production in the controls is negligible.

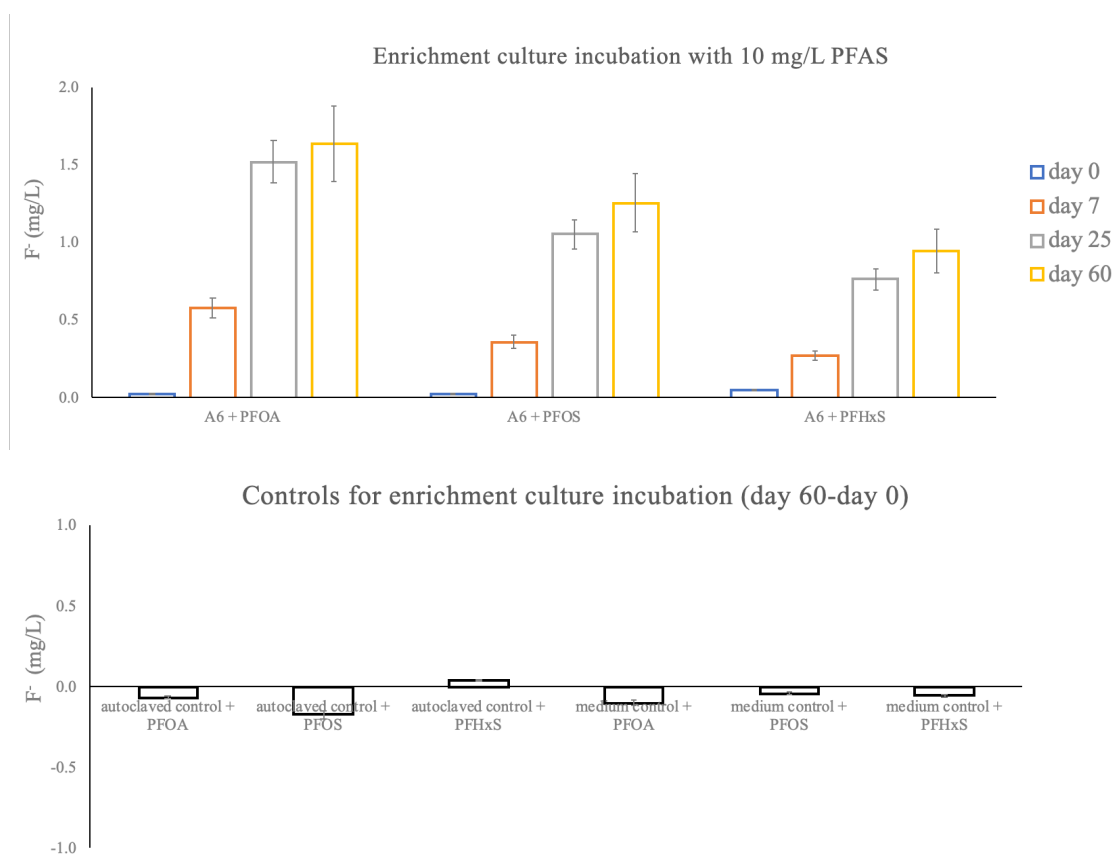


Fig. I.5 Production of F^- during the incubations with the enrichment A6 culture

Statistical analysis: The production of fluoride over during these incubations as well as that between days 25 and 60 is significant at the 5% level for all incubations.

The removal of NH_4^+ during the incubation time is shown in Figure I.6 and is consistent with our Feammox incubations in that there is usually more NH_4^+ removal in enrichment cultures than in pure cultures (Huang and Jaffé, 2018, 2019).

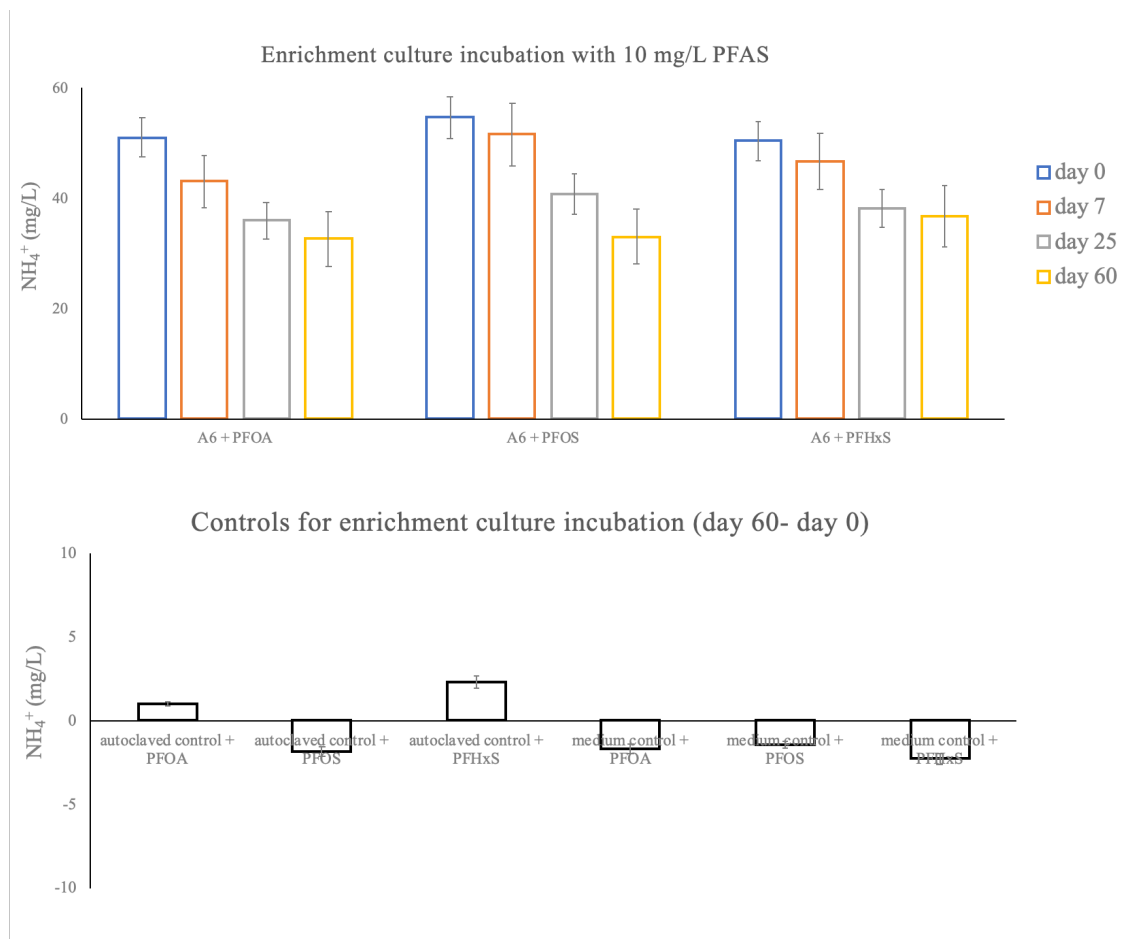


Fig. I.6 NH_4^+ concentrations during incubations with the enrichment A6 culture

Statistical analysis: The decrease in NH_4^+ concentration is significant at the 5% level over the incubation time for all incubations.

3. Fluorine balance

An accounting of fluorine in the incubations at each timepoint was performed considering the organic fluorine of each PFAS monomer plus fluoride, this includes impurities at = 0. For the pure

A6 incubations the ratio of total F at $t = 0$ to total F at $t = 60$ days was: 0.82, 0.86, and 1.07 for the incubations with PFOA, PFOS, and PFHxS respectively. These same ratios for the A6 enrichment culture incubations were: 0.69, 0.86, and 0.88 for the incubations with PFOA, PFOS, and PFHxS respectively. These balances are not as close as those we obtained in Huang and Jaffé, 2019, and a major reason might be that the concentrations used here were one tenth of those we did the F balance on previously, magnifying possible errors. Further work is needed to determine how to achieve the most accurate F balances and potential sources of error. Higher pH makes F- analyses challenging, as shown in Appendix I, but the pH here was sufficiently low that this should not have been a problem.

4. A6 numbers during pure and enrichment culture incubations

A6 numbers expressed in the pure and enrichment culture incubations vs. time, expressed as copies of A6-16S rRNA, are shown in Figure I.7. Overall A6 numbers were similar in the pure and enrichment culture incubations, showing growth over the incubation time in all cases. Lowest growth of A6 was in the pure culture with PFOS, which was also the one that resulted in the lowest degree of PFAS degradation, which might be linked to these low numbers of A6. Since A6 numbers were similar in the pure and enrichment cultures but PFAS removal in the enrichment culture, especially for PFOS was higher, this indicates that other organisms play an important role in the overall defluorination process initiated by A6. We have consistently observed changes in the enrichment culture composition with time (see next section), with concomitant changes in PFAS degradation. Further studies need to focus on the role of other organisms in the A6 enrichment culture and their effect on the overall PFAS degradation.

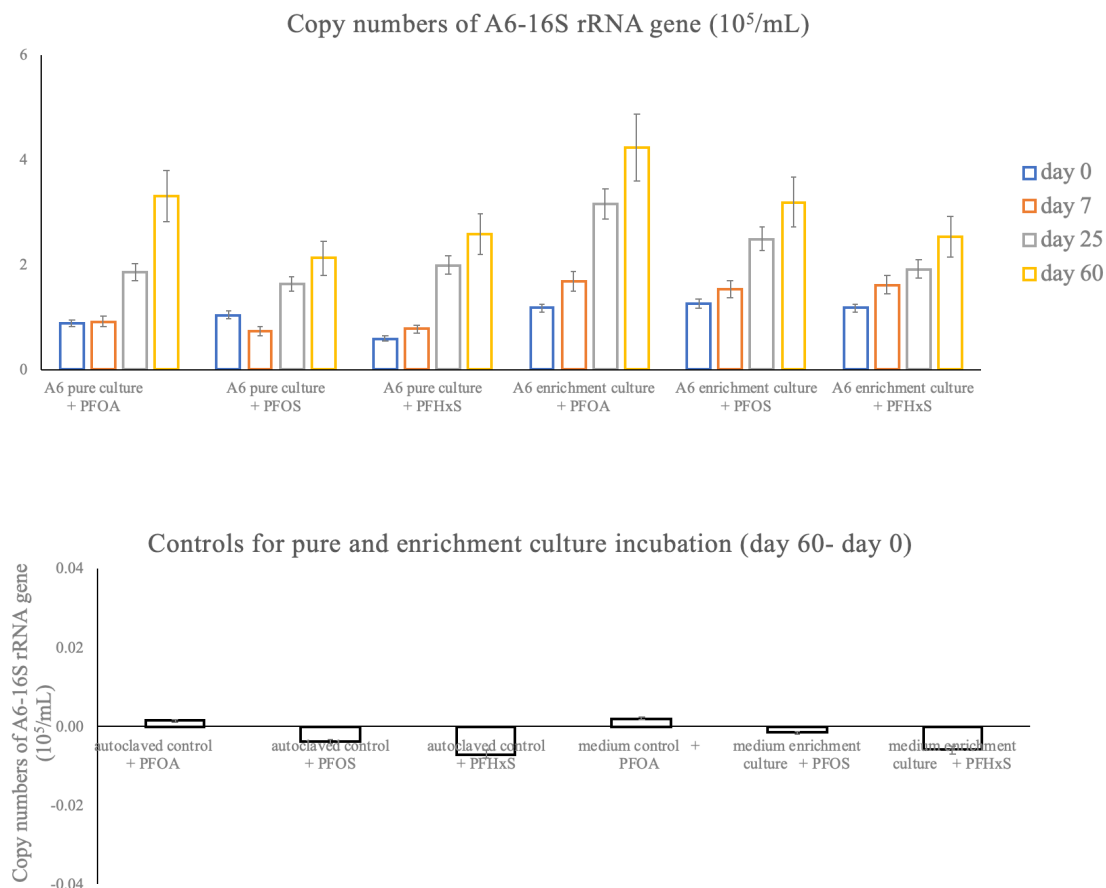


Fig. I.7 A6 numbers during pure and enrichment culture incubations.

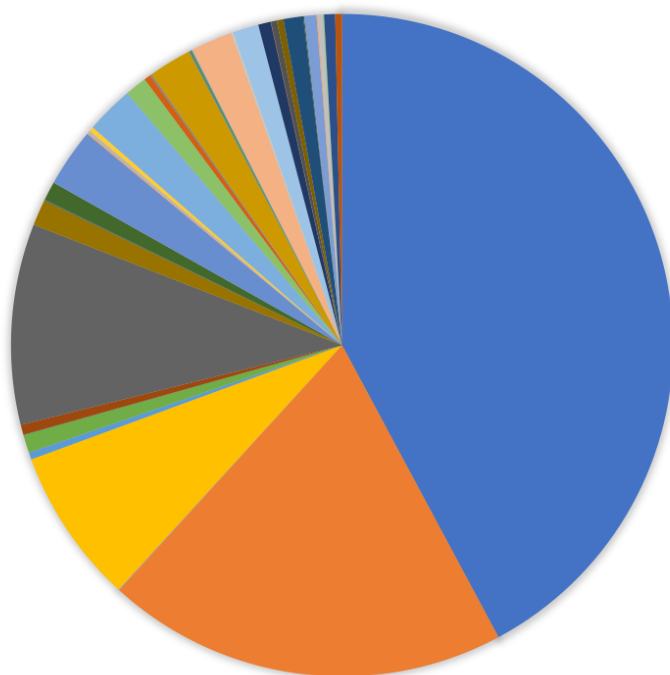
Statistical analysis: The increase of A6 numbers over the incubation time is statistically significant at the 5% level for all pure and enrichment culture incubations shown here.

5. Shift of the microbial community during incubations with the A6 enrichment culture

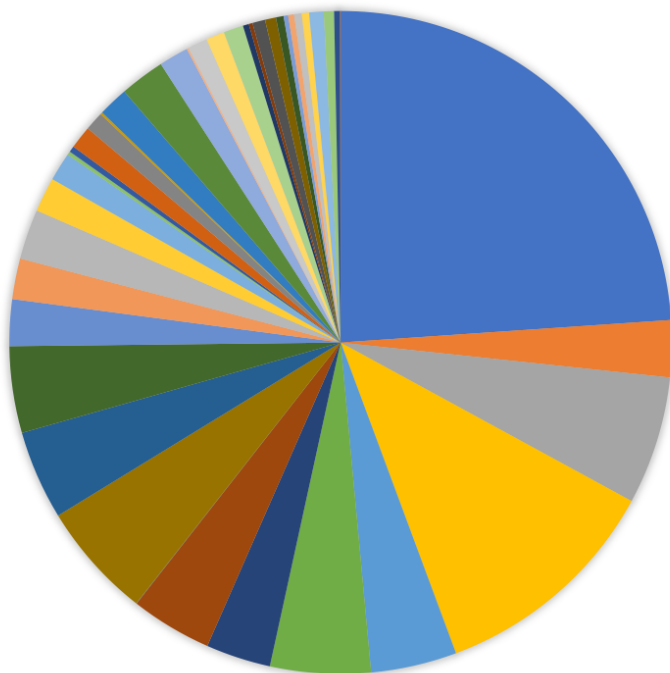
The changes in the microbial communities over the 60-day incubations with the A6 enrichment culture and with 10 mg/L PFOA, 10 mg/L PFOS, and 10 mg/L PFHxS were studied base on the 16S rRNA gene sequencing data (Fig. I.8). These results represent about 70-85% of the overall

microbial community. In the initial A6 enrichment culture (day 0), *Acidimicrobiaceae* was the major fraction (42.1%) of the total bacterial community, followed by an *Ralstonia* (20.2%) and *Sediminibacterium* (10.1%). After 60 days of incubation, the percentage of *Acidimicrobiaceae* changed to 24.7%, 27.2% and 18.6% in the enrichment cultures with PFOA, PFOS, and PFHxS, respectively. *Burkholderiaceae* became the dominant denitrifier after the incubation with these PFAS. *Desulfosporosinus*, which are known to have dehalogenation abilities, increased during the incubation process in samples amended with PFOS and PFHxS (Fig. I.8), although it is not known if they did play a role in the defluorination process.

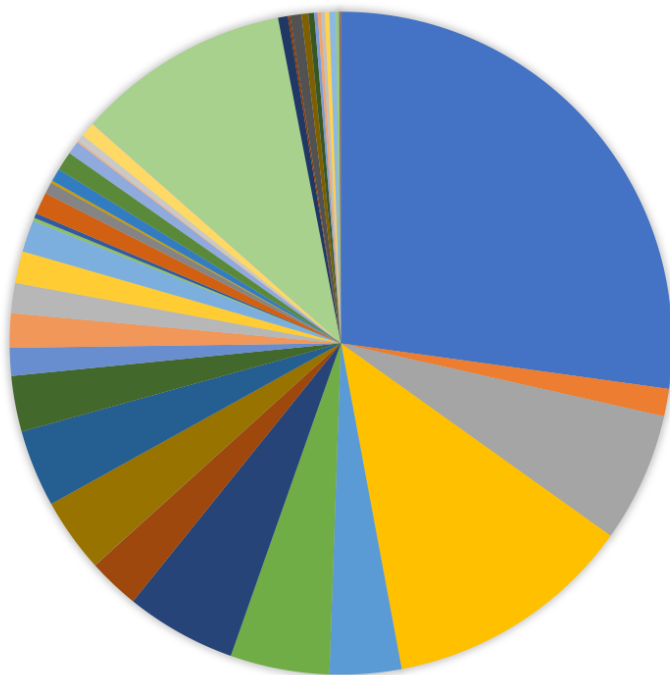
A6 enrichment culture (Day 0)



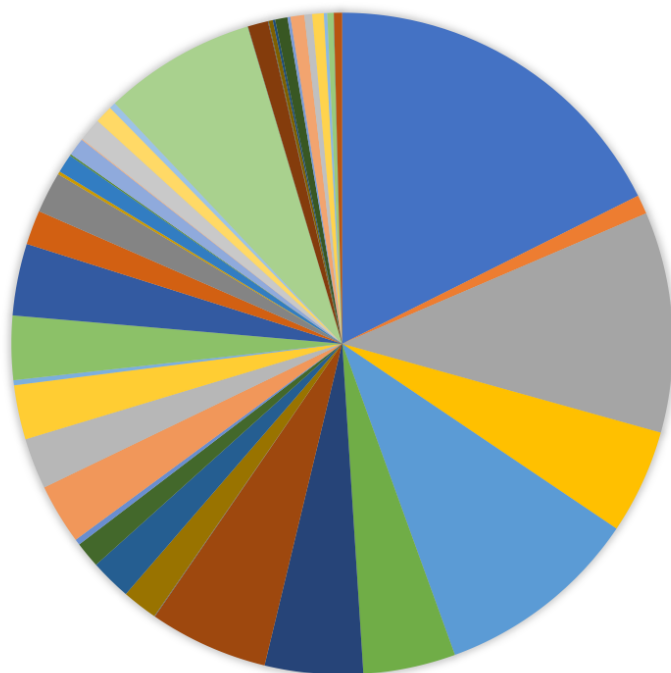
A6 with 10 mg/L PFOA (Day 60)



A6 with 10 mg/L PFOS (Day 60)



A6 with 10 mg/L PFHxS (Day 60)



■ Acidimicrobiaceae	■ Ralstonia	■ unidentified_Spirochaetaceae	■ unidentified_Burkholderiaceae
■ Novosphingobium	■ Hyphomicrobium	■ Caldisericum	■ Amphiplicatus
■ Sediminibacterium	■ Mycobacterium	■ Leptolinea	■ Mesorhizobium
■ unidentified_Clostridiales	■ Yersinia	■ Anaerolinea	■ unidentified_Christensenellaceae
■ Zoogloea	■ Azospira	■ Geobacter	■ unidentified_Rikenellaceae
■ Dechloromonas	■ Bradyrhizobium	■ Rhodobacter	■ Leucobacter
■ unidentified_Rhizobiaceae	■ Acinetobacter	■ unidentified_Hyphomonadaceae	■ Blastopirellula
■ Lactobacillus	■ Desulfosporosinus	■ Desulfovibrio	■ Brachymonas
■ Cellulomonas	■ unidentified_Alphaproteobacteria	■ Sulfurovum	■ Sphingopyxis
■ unidentified_Enterobacteriaceae	■ unidentified_Rhizobiales	■ Ferruginibacter	■ Petrimonas
■ Planctopirus	■ unidentified_Nitrospiraceae	■ Holophaga	■ Pseudomonas

Fig. I.8. Microbial Community over 60 days incubation with the A6 enrichment culture under different conditions

6. Conclusions

Results presented here confirm our previous findings that PFAS such as PFOA, PFOS and as shown here also PFHxS can be defluorinated by pure A6 and A6 enrichment cultures while oxidizing NH_4^+ and reducing Fe(III). This is based on the decreased in concentration of the initial

PFAS, production of F^- , and production of smaller carbon-chain PFAAs while these changes were negligible in the various controls.

A novel finding is the detection of H-PFOA, which built up to higher levels in the pure culture vs. the enrichment culture. The missing fluorine in the H-PFOA was on the alpha carbon, indicating that that is the carbon where defluorination of PFOA is initiated. There is clearly a need to study the formation of these partially defluorinated PFAA's in more detail and for a wider range of compounds.

The expression of the *rdhA* gene increased throughout the incubations with the three PFAAs.

A6 numbers increased throughout the incubations, although the relative numbers in the enrichment culture decreased, most likely because the defluorinated PFAS provided a carbon source for heterotrophs. No organic carbon was added to either the pure or enrichment culture, and organic carbon present would have been the result of carbon fixation by A6 and carbon turnover. Interestingly, the enrichment culture for incubations with PFOS and PFHxS were more similar to each other on day 60 than the culture of the incubations with PFOA. Clearly, at the PFAS concentrations used here, selection of the individual PFAS affects the culture's community composition. The effect might of course be less marked at more environmentally relevant PFAS concentrations.

II. Expression of reductive dehalogenase genes during incubations with different PFAS

Background

Organohalide compounds can serve as final electron acceptors in an energy-conserving process, wherein a halogen–carbon bond is broken, and the halogen atom is liberated as a halide, during organohalide respiration.

Reductive dehalogenases (RDases) are a group of enzymes catalyzing the cleavage of the carbon-halogen bond. These membrane-associated enzymes have attracted great interest for the detoxification of organohalide pollutants. Reductive dehalogenase homologous genes (*rdh*) typically comprise an operon containing *rdhA*, the gene for the catalytically active enzyme, and *rdhB*, a gene encoding a putative membrane-anchoring protein, sometimes as well as one or more members of *rdhTKZECD*, genes associated with reductive dehalogenase genes (Hug *et al.*, 2013).

Reductive dehalogenases are mostly attached to the periplasmic side of the cytoplasmic membrane, and related to the cobamide (or vitamin B12) family of enzymes. They contain a cobalamin at its catalytic active site, where actual reductive reaction occurs. Two conserved features exist in the *RdhA* proteins, including two iron – sulfur cluster-binding motifs and a twin-arginine signal motif for translocation to or across the cell membrane (Maillard *et al.*, 2003).

RDases share a specific function and do not necessarily share high sequence similarity and are known to catalyze the dehalogenation of a broad range of substrates. For example, *VcrA*, *BvcA*, *TceA*, *MbrA*, *PceA*, *PrdA* can catalyze chlorinated ethenes degradation, *CfrA*, *DcrA*, *DcaA* can catalyze chlorinated ethanes degradation, and *CbrA*, *CprA* can catalyze the degradation of chlorinated aromatics (Hug *et al.*, 2013). Reductive dehalogenase encoding genes have been identified in a wide variety of strictly anaerobic bacteria, including *Desulfitobacterium*,

Dehalobacter, *Dehalococcoides* *Dehalogenimonas*, and *Sulfurospirillum*, and in micro- aerophilic bacteria as in the case of *Anaeromyxobacter*, etc. (Jugder et al., 2016).

In this study, we focus on the pure A6 and A6 enrichment cultures that have shown to be capable of defluorinating PFAS, and query PFAS-degrading enzymes in these, establishing the relationship between defluorination and RDases.

Methods

1. DNA Extraction, Metagenome Sequencing, and Sequence Analysis

For genomic DNA extraction, cells from A6 were collected by centrifugation at 15,000 ×g for 15 minutes, and Genomic DNA was extracted as described in the manual of PureLink™ Microbiome DNA Purification Kit (Thermo Fisher Scientific, USA).

The metagenomics sequencing libraries were generated using NEB Next® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) following the manufacturer's recommendations. Then, the library was sequenced on an Illumina Hiseq X-ten platform and 150 bp paired-end reads were generated. Raw data were cleaned (Trimmomatic, v.0.36), assembled and removed short fragment (<500 bp) (MEGAHIT, v1.0.6), and predicted the ORF to filter the length information shorter than 90 nt (MetaGeneMark, Version 3.38). Then, the CD-HIT(Version: 4.) was adopted to remove redundancy and obtain the unique initial gene catalogue (the genes here refer to the nucleotide sequences coded by unique and continuous genes), which is clustered by identity 95%, coverage 90%, and chosen the longest of the representative sequences. The clean data of each sample was mapped to the initial gene catalogue using BBMAP software and to get the number of reads. The abundance information of each gene in each sample was based on the number of mapped reads and the length of the gene.

The DIAMOND software was applied to blast the Unigenes using the NR database of NCBI. The table containing the average depth of genes and the abundance information of each sample in each taxonomy hierarchy (kingdom, phylum, class, order, family, genus, species) were obtained based on the LCA annotation result. The abundance of a specie in one sample equals the sum of the gene abundance annotated for the specie; the gene average depth of a specie in a sample equals the sum of the gene average depths whose abundance are nonzero. Functional predictions based on KEGG database, eggNOG database, and CAZy database were applied using the DIAMOND software. All sequencings were generated at the Guangdong Magigene Biotechnology Co., Ltd. (Guangzhou, China).

2. *Quantitative PCR (qPCR), and Reverse Transcription- (RT-) qPCR*

DNA and RNA that was extracted from incubations with different PFAS on day 0, 7, 20, and 60 were used to monitor the abundance and activity of dehalogenase genes. qPCR, targeting *FceA* and *rdhA* genes was carried out using primer set *fceA*_5f (5'-GATCCGATTGGCCCTCAACT-3') - *fceA*_5r (5'-GAGCCACCTCGAAAGAGAGG-3'), and primer set *rdhA*_5f (5'-GACGTCTTCTGCCGATCACA-3') - *rdhA*_5r (5'-GGGCTGTCTCCAACAGTTCA-3'), and was conducted on a StepOnePlus qPCR system (Thermo Fisher Scientific, USA). mRNA copy numbers of transcribed *rdhAs* were examined by RT-qPCR using the One Step SYBR PrimeScript RT-PCR Kit II (TaKaRa, Otsu, Japan). The RNA recovery rates of the samples in this study varied with total RNA concentration and were in the range of 2–12%. The transcription rate of *rdhA* was calculated by dividing the mRNA copy numbers by the gene copy numbers determined in parallel via qPCR.

3. Identification of Ortholog Groups (OGs) of reductive dehalogenase

To determine the similarity of A6's *rdhA* to that of existing reductive dehalogenase, the sequence of A6's *rdhA* was compared the *rdh_all_aa_DATE.fasta* database using BLAST as described in Hug et al. (2013), and hits with an identity of $\geq 90\%$ were kept.

4. Mutant construction and assay of reductive dehalogenase activity

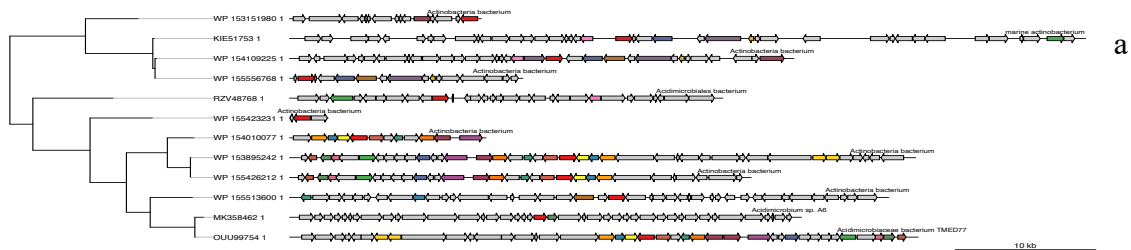
A protocol modified from Thongdee *et al.* (2008) was used to construct the FAM1-*rdhA* and the FAM1-*fceA* mutants. The upstream and downstream regions of *rdhA* and *FceA* were amplified and subsequently fused together with two rounds of PCR. The PCR fragments were ligated into the pEX18Gm plasmid vector for the *rdhA* and *FceA* knockout mutant.

Results

1. Reductive Dehalogenase and Associated Genes

As mentioned above, the genome of A6 contains genes with similarities to that of reductive dehalogenases, which were also detected in the A6 enrichment culture (GenBank accession numbers MK358459-MK358462) (Fig II.1). Which includes a gene for Reductive dehalogenase homolog (*RdhA*), a gene for Fluoroacetate dehalogenase homolog (*FceA*), and two putative haloacid dehalogenase gene (*dhl_1* and *dhl_2*).

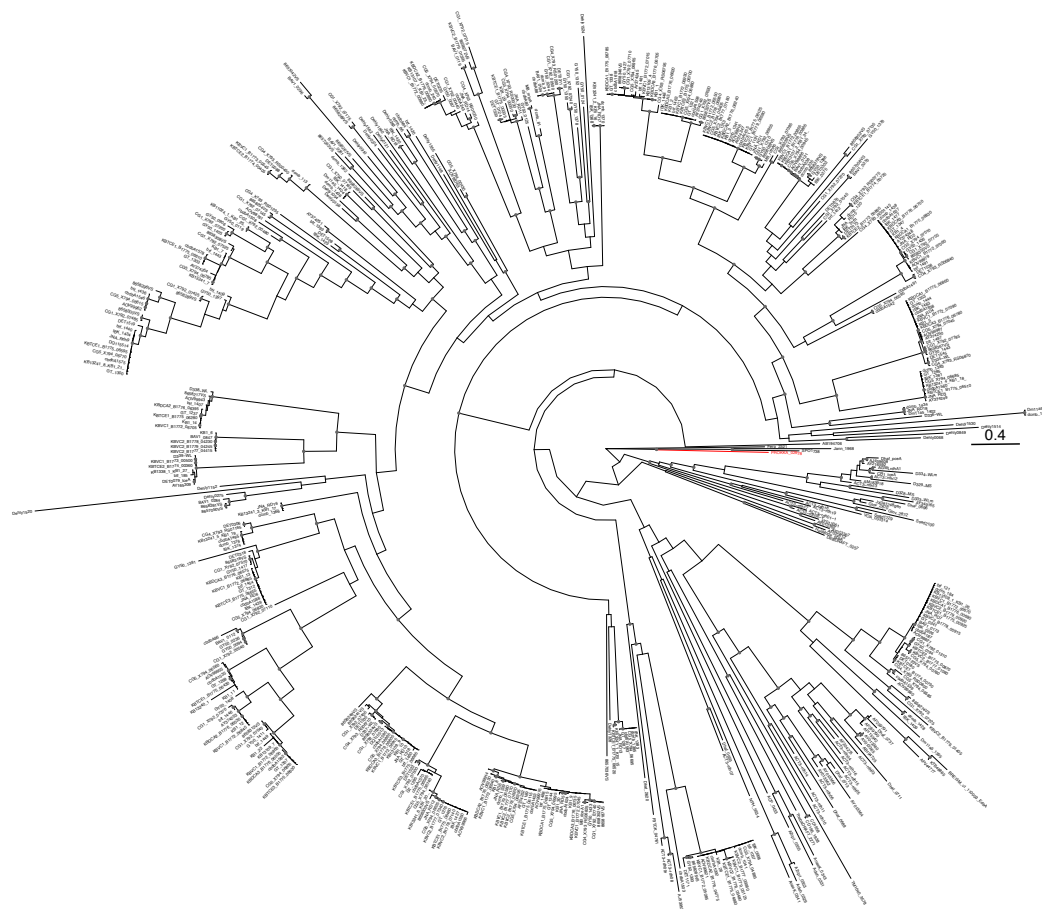
The Reductive dehalogenase homolog is 302 aa in length, and the top BLAST homologs are 4Fe-4S dicluster domain-containing proteins in Acidimicrobiaceae and Actinobacteria (Fig.II.2)



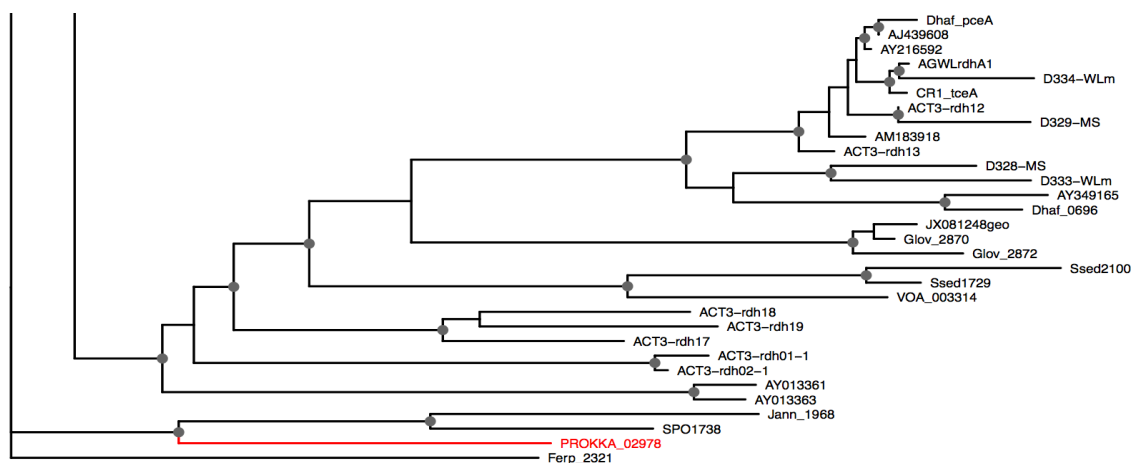
PROKKA_02970 ATP-dependent DNA helicase PcrA	
PROKKA_02971 GMP synthase [glutamine-hydrolyzing]	
PROKKA_02972 putative oxidoreductase/MSMEI_1564	
PROKKA_02973 putative oxidoreductase/MSMEI_1564	
PROKKA_02974 hypothetical protein	
PROKKA_02975 Putative fluoride ion transporter CrcB	fluoride ion transporter?
PROKKA_02976 hypothetical protein	closest hit in the database is a conserved domain of unknown function
PROKKA_02977 Putative fluoride ion transporter CrcB	fluoride ion transporter?
PROKKA_02978 Putative reductive dehalogenase	putative reductive dehalogenase
PROKKA_02979 putative Nudix hydrolase NudL	
PROKKA_02980 60 kDa chaperonin 2	might play a role in protein folding/expression?
PROKKA_02981 10 kDa chaperonin	
PROKKA_02982 tRNA N6-adenosine threonylcarbamoyltransferase	
PROKKA_02983 Mycothiol acetyltransferase	
PROKKA_02984 hypothetical protein	
PROKKA_02985 tRNA threonylcarbamoyladenosine biosynthesis protein TsaE	
PROKKA_02986 Uracil DNA glycosylase superfamily protein	
PROKKA_02987 Alanine racemase	
PROKKA_02988 ADP-dependent (S)-NAD(P)H-hydrate dehydratase	
PROKKA_02989 Bifunctional NAD(P)H-hydrate repair enzyme Nnr	
PROKKA_02990 Holo-[acyl-carrier-protein] synthase	
PROKKA_02991 HTH-type transcriptional activator CmpR	
PROKKA_02992 hypothetical protein	putative hydrolytic dehalogenase
PROKKA_02993 Glycosyl transferases group 1	
PROKKA_02994 Glutamine-fructose-6-phosphate aminotransferase [isomerizing]	
PROKKA_02995 Phosphoglucosamine mutase	

b

Fig. II.1 Closest (a) genome neighborhoods and (b) dehalogenase gene neighborhood



a



b

Fig. II.2 *rdhA* maximum-likelihood phylogeny

2. Transcription of *FceA* and *rdhA* in response to F^- production

To identify the *FceA* and *rdhA* genes involved in the PFAS degradation during incubations with the A6 enrichment or pure cultures, RT-qPCR targeting mRNA of both genes was conducted. Both *FceA* and *rdhA* showed increase in transcription during the incubations amended with PFAS as compared to incubations without PFAS, especially the *rdhA* gene expression was more than twice as high in the PFAS amended incubations than in the no PFAS controls (Fig. II.3). Based on the results of the first 7 days of incubation, the relationship between the expression of the reductive dehalogenase genes and PFAS defluorination (F^- production) were analyzed and are shown in Figure II.4. Results show a much higher correlation between the expression of the *rdhA* gene and F^- produced than between the expression of the *FceA* gene and F^- produced.

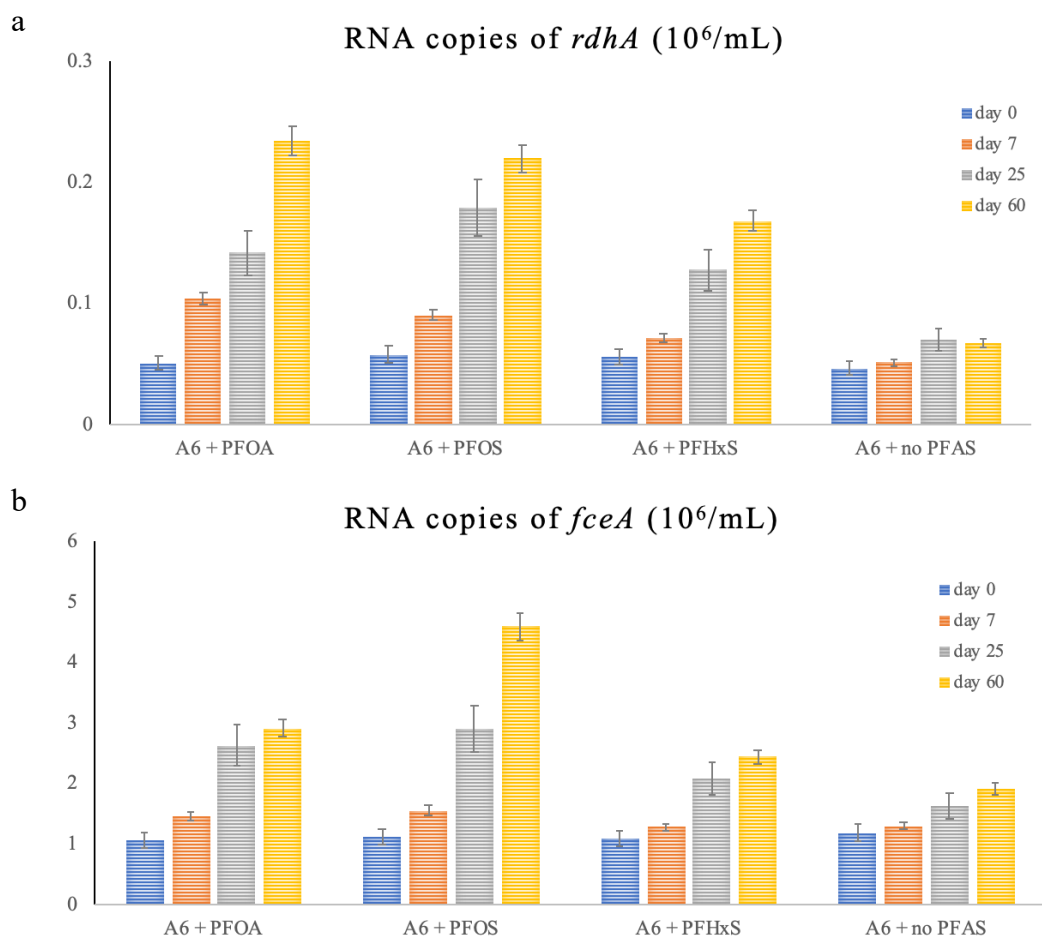


Fig. II.3 Expression of (a) *rdhA* genes and (b) *FceA* gene during incubations

Statistical analysis: In the presence of these PFAAs the increase in the expression of the *rdhA* gene is significant at the 5% level over the incubation time, and not in their absence, while the increase in expression of the *FceA* gene is significant at the 5% level in all incubations.

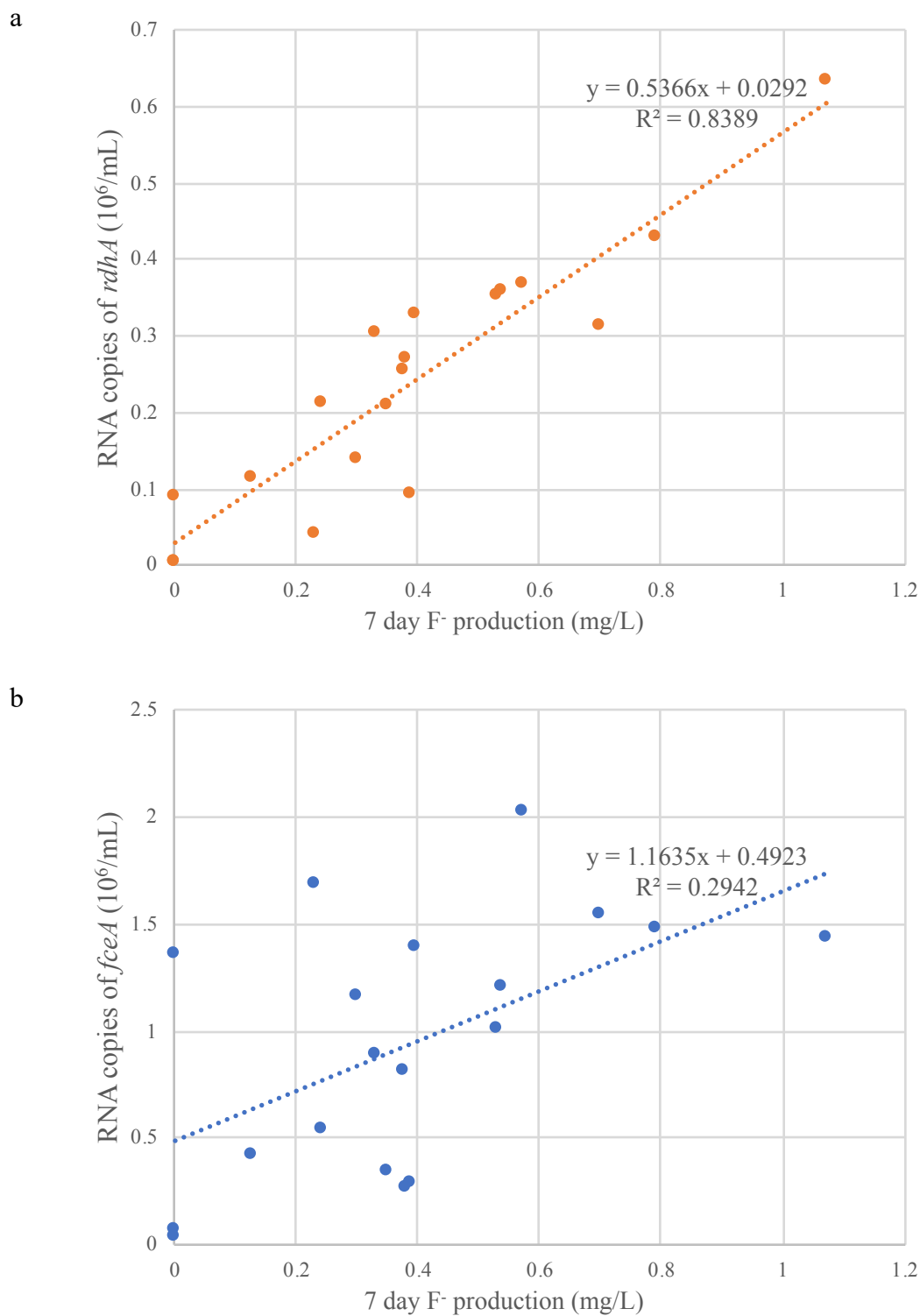


Fig. II.4 Expression of (a) *rdhA* genes and (b) *FceA* gene vs. F^- production over 7 days in the pure A6 culture for the PFOA, PFOS, and PFHxS incubations

Results of incubations with the A6 culture (wild type) and mutants where either the *rdhA* or *FceA* gene was knocked out are shown in Figure II.5.

As shown in Figure II.5, compared to the A6 wild type, most of the FAM1-*rdhA* mutants lost their ability of F^- production, while the FAM1-*fceA* mutants still showed some F^- production after 72-hour of incubations with 100 mg/L PFOA, as shown in Figure II.5.

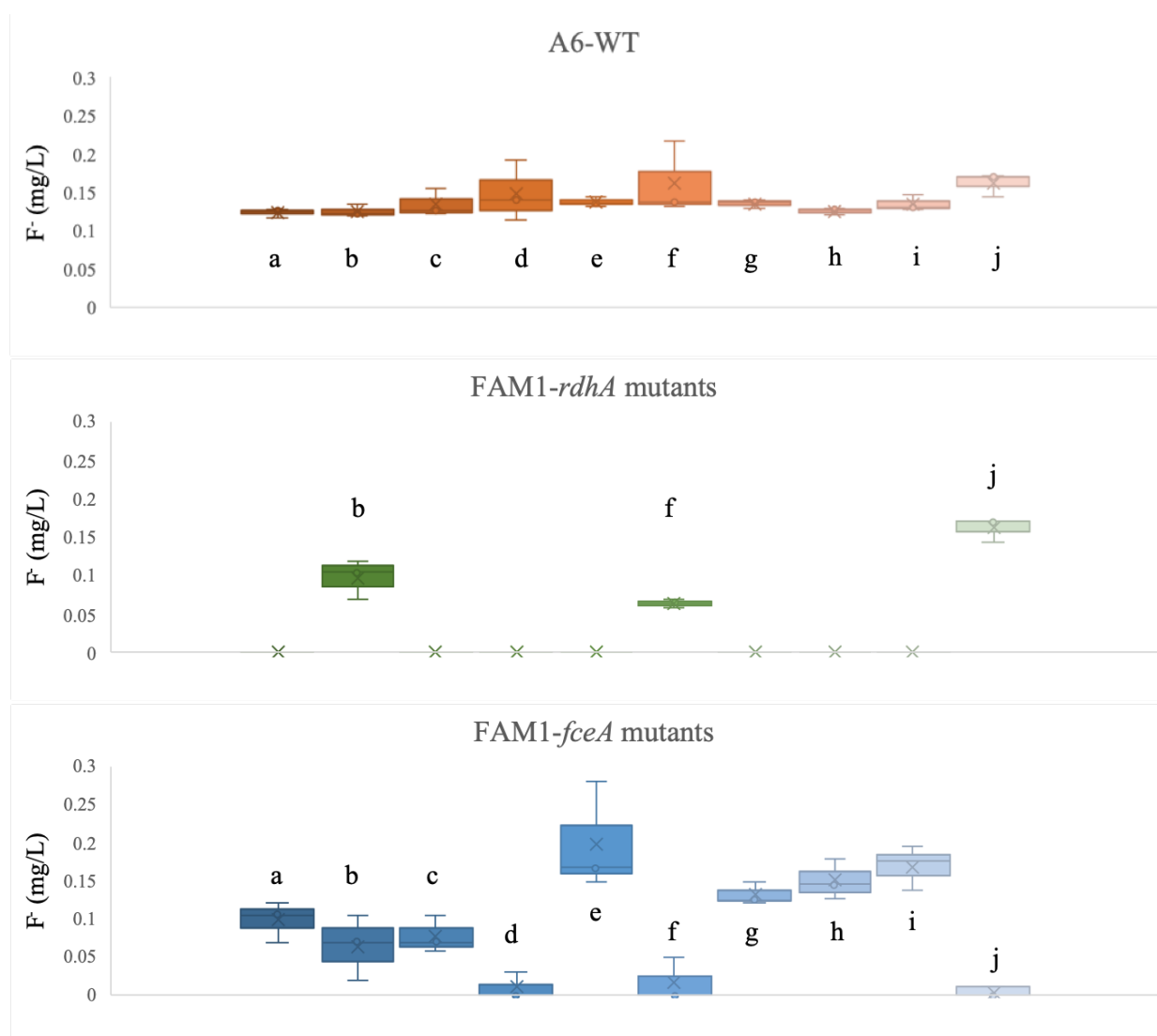


Fig. II.5 Fluoride production of A6 wild type, the FAM1-*rdhA* and FAM1-*fceA* mutants after 72-hour incubations with 100 mg/L PFOA

Results shown in Figure II.5 show 3 *rdhA* mutants that did produce F⁻ in the incubations and 3 *fceA* mutants that did not produce F⁻, showing that the construction of mutants, especially for anaerobes, is not always successful Thongdee *et al.* (2008).

The high correlation of the expression of the *rdhA* gene with F⁻ produced and the findings that most of the *rdhA* mutants did not produce F⁻ during these incubations, indicates that the reductive dehalogenase in A6 plays a key role in the defluorination of PFAS by A6.

III. Synergistic degradation of 1,2,3-Trichloropropane and PFOA during Feammox incubation.

The objective here was to determine if A6 is also able to dechlorinate a chlorinated compound, and if so, if dechlorination would be favored over defluorination.

Given the interest in 1,2,3 TCP we tested if A6 is capable of degrading TCP and if so, what the interactions are if we supply a mixture of 1,2,3 TCP and PFOA to incubations with A6. Although 1,2,3 TCP is rather recalcitrant, several studies have shown that it can be degraded aerobically and anaerobically (Tratnyek *et al.*, 2008).

Methods

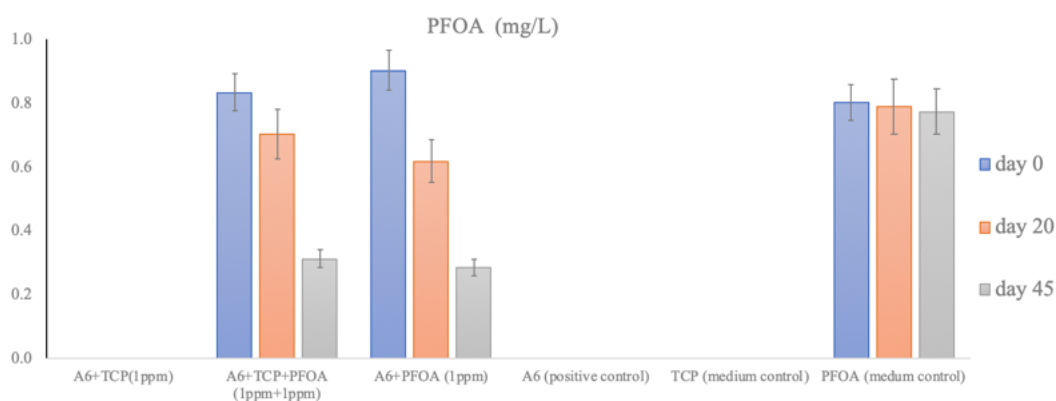
The incubation experiments were carried out in two groups: (1) experimental group, and (2) control group. Incubations in the experimental group were Feammox A6 enrichment cultures with 0.6 mg/L TCP, 1mg/L PFOA, and 0.6 mg/L TCP + 1mg/L PFOA, respectively. Here, the 5mL of cultures, which contained about 10^5 copies/mL of A6, were incubated in 50mL vials with 30 mL inorganic Fe(III)-NH₄⁺ medium. There were three control treatments which consisted of incubations with medium and 0.6 mg/L TCP, medium and 1mg/L PFOA, both without A6, as well as an A6 enrichment culture without TCP or PFOA, all controls were autoclaved. All the vials were sealed with butyl rubber stoppers and aluminum caps. After being vacuumed and then flushed with a N₂/CO₂ (80:20) mixture to achieve anoxic conditions and provide additional CO₂ for the growth of A6, the vials were shaken at 25°C for 45 days. Each test was repeated in triplicate and the results averaged. Subsamples were taken on day 0, 25, and 45, and their chemical and microbial characteristics were analyzed.

The TCP, and its degradation intermediates were assayed by SGS-CSTC Standards Technical Services Co., via chiral GC analysis using the chiral column β -dex 225 (30 m x 0.25 mm x 0.25 μ m, Supelco) under the following conditions: 100 °C for 6 min, 10 °C/min up to 220 °C, and 15 min at 220 °C; retention times were 18.1 min for TCP and 12.1 min for DCP, respectively. Other chemical and microbial characteristics analysis methods are consistent with those described above.

Results

1. Degradation of PFOA and TCP during incubations with the enrichment A6 culture

During the 45-day incubations, PFOA decreased from 0.902 to 0.283 mg/L, and from 0.832 to 0.311 mg/L in samples amended with both only PFOA, and PFOA+TCP, respectively. While, TCP concentration changed from 0.483 to 0.213 mg/L, and from 0.573 to 0.132 mg/L in samples amended with only PFOA and with PFOA+TCP, respectively (Fig. III.1). There was no significant difference in the PFOA and TCP degradation rate between these treatments. Dichloropropane (DCP) was found in samples with TCP degradation, and there was no significant PFOA or TCP removal in the control samples (Fig. III.1). In addition to DCP there was at least one other compound being produced, which at this point has not been identified.



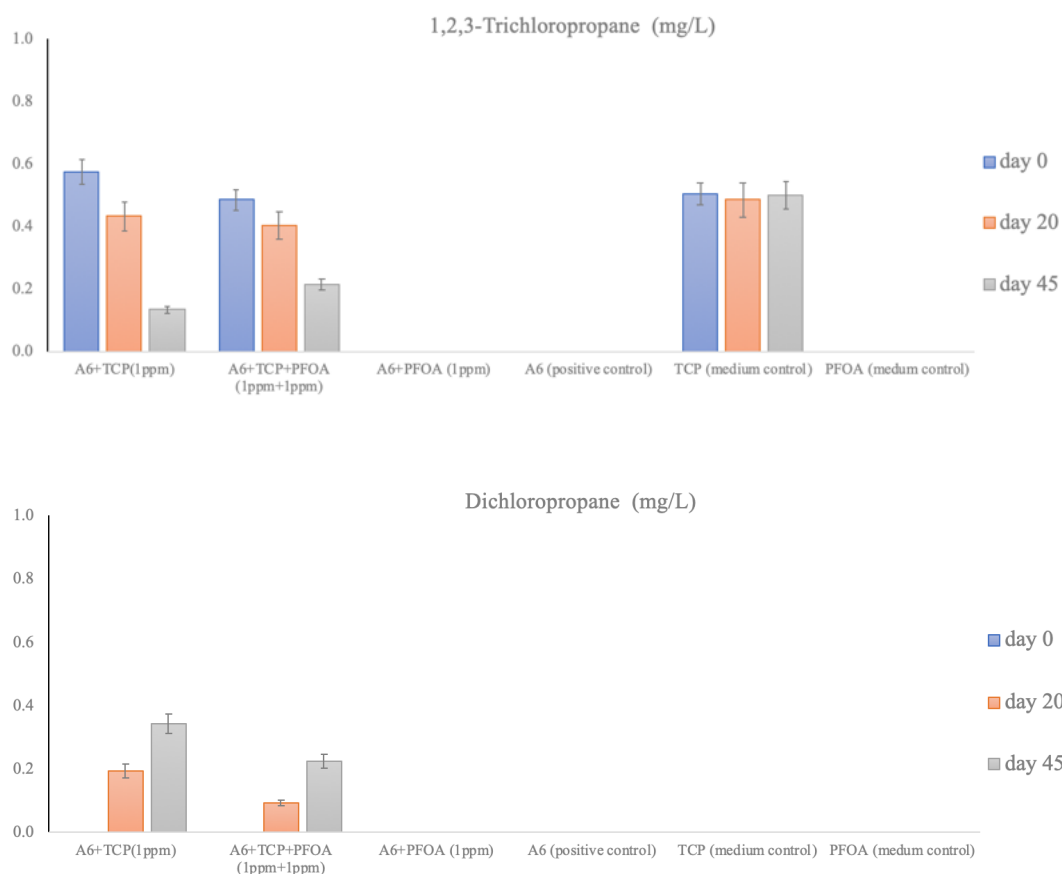


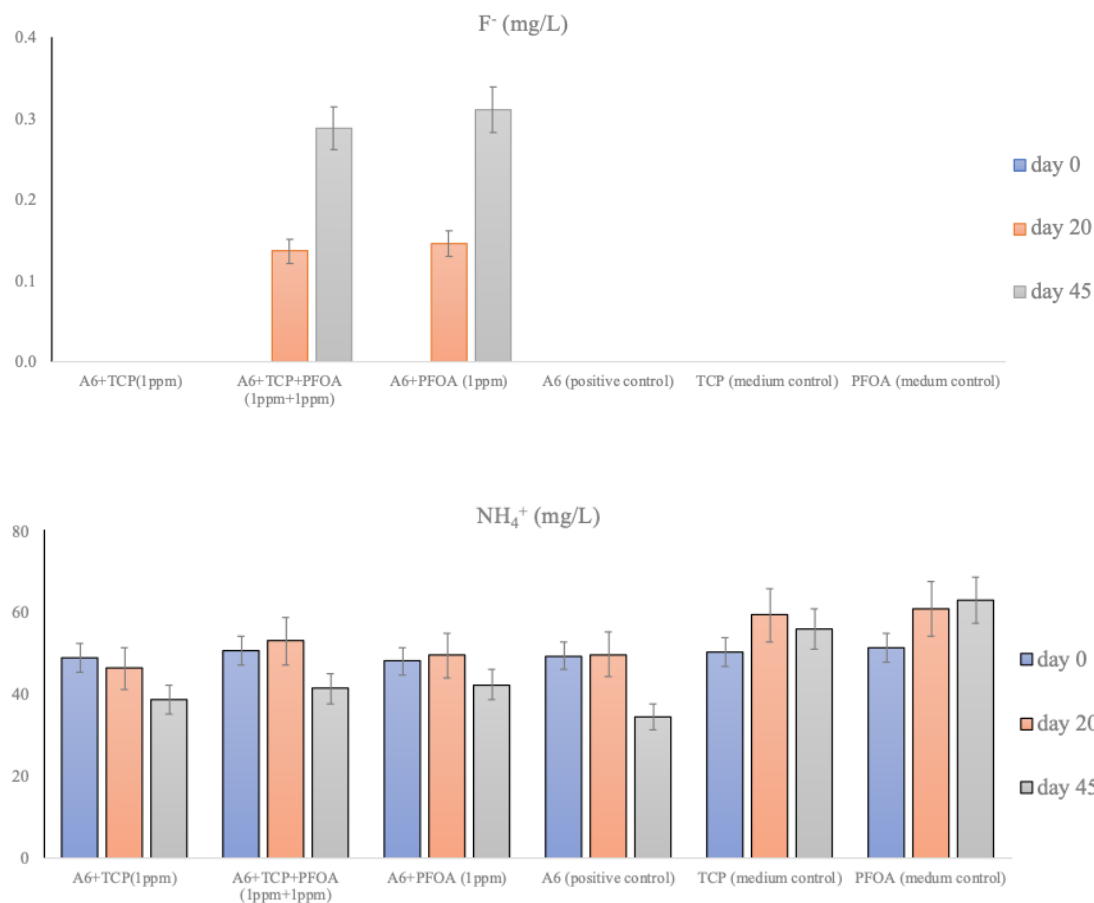
Fig. III.1 PFOA, TCP, and DCP concentration during incubations

Statistical analysis: The decrease in 1,2,3 TCP concentration was significant at the 5% level in the presence and absence of PFOA, while no significant change was observed in the controls. Similarly, the increase in DCP was significant in the active incubations with and without TCP.

2. F^- , NH_4^+ , and $Fe(II)$ concentrations during incubations with the A6 enrichment culture

F^- production was only detected in the incubations with PFOA, and in range of 0.29-0.31 mg/L after 45 days of incubation (Figure III.2). NH_4^+ removal and $Fe(II)$ production were observed in all the incubations with A6, indicating that the Feammox reaction was active, and there was no significant difference in the NH_4^+ removal efficiency between the incubations with TCP/ PFOA.

No obvious NH_4^+ removal and Fe(II) production were observed in the medium controls without A6 (Fig. III.2). Similarly, Fe(II) production was about the same for the incubations with PFOA and or TCP, with perhaps a slight increased production in the vials without these compounds, and negligible Fe(II) production in the controls without A6.



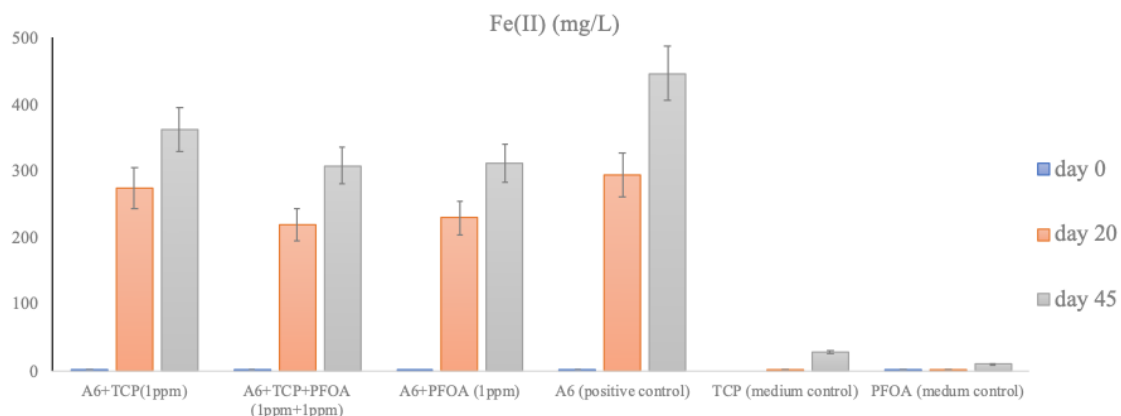


Fig. III.2 F^- , NH_4^+ , and $Fe(II)$ concentrations during incubations

Statistical analysis: The production of fluoride during the incubations was significant at the 5% level in the active incubations containing PFOA. The removal of ammonium was significant at the 5% level in all active incubations, except A6 + PFOA. Increase in $Fe(II)$ was significant at the 5% level in all incubations.

3. Transcription of dehalogenase genes during the incubations

Total RNA was extracted from cultures on day 45, and RT-qPCR was used to study the transcriptions of dehalogenase genes under different treatments. RT-PCR products of *rdhA* and *FceA* genes in samples amended with PFOA showed transcription levels at least one order of magnitude higher than those in samples without PFOA. However, *dhl_1* and *dhl_2* gene showed higher expression levels in samples with TCP (Fig. III.3).

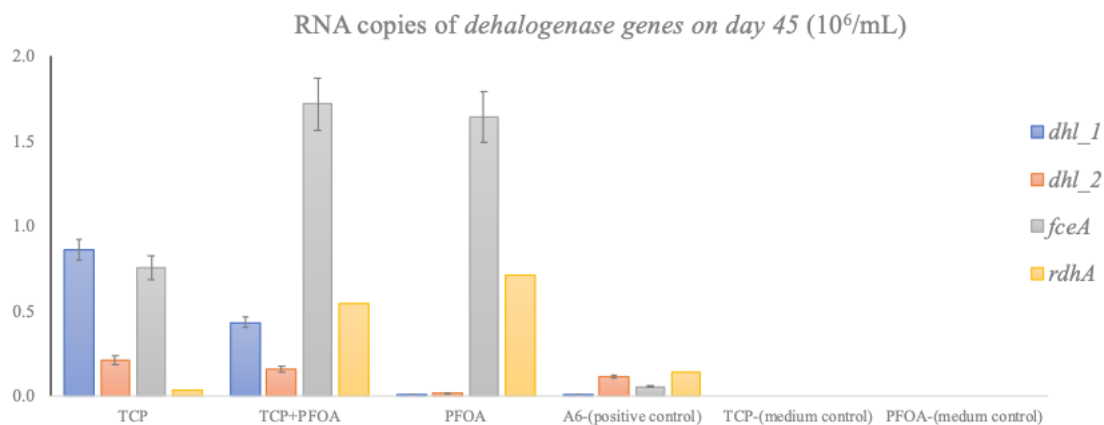


Fig. III.3 RNA copies of dehalogenase genes during incubations

Note: The TCP degradation experiments presented here are considered rather preliminary results and need to be repeated for both TCP and ideally other chlorinated compounds, but of interest to this research focusing on PFAS degradation is that there seems to be no direct and measurable interaction between the degradation of a chlorinated and a fluorinated compound by A6.

IV. PFOA defluorination as a function of the initial A6 biomass

So far, all incubations that have been performed were with comparable A6 biomass. The objective here was to conduct incubations with PFOA where the initial A6 biomass was varied over four orders of magnitude to determine, the initial A6 biomass will affect the defluorination kinetics. The methodology employed for these incubations is as described above, and the higher A6 concentrations were achieved via centrifugation. Results, using pure A6 cultures are shown in Figure IV.1

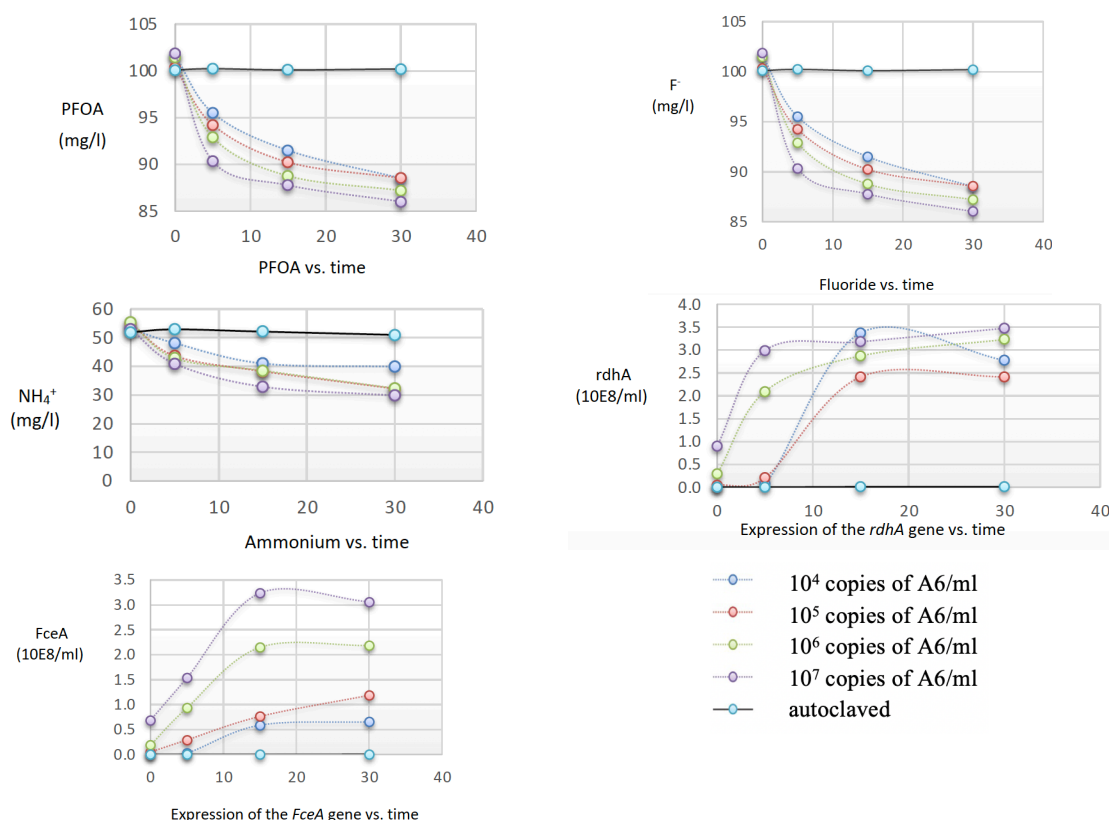


Fig. IV.1 Results of incubations with PFOA as a function of the A6 biomass

Whereas in most biodegradation experiments biodegradation one typically observes a linear relationship between biomass and biodegradation rates, results presented here clearly indicate a monotonic, but not linear, increase in all the variables measured. This non-linearity may be attributed to limitations of the electron transfer to the solid ferric iron phase. Future work should

examine the kinetics as a function of the Fe(III) surface and various concentrations of electron shuttling compounds, such as AQDS, both of which were maintained constant in these experiments. An initial modeling effort of these kinetics is outlined in the next section.

V. Kinetics of PFAS defluorination by A6

Here we use the rate of NH_4^+ oxidation, Fe(III) reduction, PFAS degradation, F^- production, and biomass growth to formulate a simple model to simulate the various PFOA and PFOS degradation experiments presented here, and to further constrain the modeling, we also include the experiments described in Huang and Jaffé, 2019. We assume that the Feammox reaction is first order with respect to NH_4^+ and with respect to Fe(III) and given the non-linear relationship between the A6 biomass and the Feammox process, as well as PFOA degradation as a function of the A6 biomass, shown in the previous section (Section IV), we explore the relation of biomass in these kinetics. For this purpose, the following equations have been formulated:

$$\frac{dC_{\text{NH}_4}}{dt} = -k_{\text{NH}_4} C_{\text{FeIII}} C_{16\text{S}}^{1/n} C_{\text{NH}_4} \quad (1)$$

$$\frac{dC_{\text{FeIII}}}{dt} = -k_{\text{FeIII}} C_{\text{NH}_4} C_{16\text{S}}^{1/n} C_{\text{FeIII}} \quad (2)$$

$$\frac{dC_{\text{PFAS}}}{dt} = -f k_{\text{NH}_4} C_{\text{FeIII}} C_{\text{NH}_4} C_{16\text{S}}^{1/n} C_{\text{PFAS}} \quad (3)$$

$$\frac{dC_{\text{F}^-}}{dt} = f_{\text{F}} \frac{dC_{\text{PFAS}}}{dt} \quad (4)$$

$$\frac{dC_{16\text{S}}}{dt} = f_{16\text{S}} \frac{dC_{\text{NH}_4}}{dt} - k_d C_{16\text{S}} \quad (5)$$

Where C_{NH_4} , C_{FeIII} , C_{PFAS} , and C_{F^-} are concentrations of NH_4^+ , Fe(III), PFAS, F^- , in solution ($\mu\text{g/ml}$), respectively. $C_{16\text{S}}$ is the 16S rRNA gene in solution (million copies per ml). k_{NH_4} and k_{FeIII} are oxidation rate constant of NH_4^+ and reduction rate constant of Fe(III), respectively (with a derived unit of $(\text{ml}^{(1+1/n)})/(10 \cdot \text{copies}^{(1/n)} \cdot \text{mg} \cdot \text{hr})$). f , f_{F^-} , and $f_{16\text{S}}$ are fractions (dimensionless) of NH_4^+ oxidized used for PFAS defluorination, F^- production, and A6 growth. k_d is A6 death rate and assumed to be negligible over the timeframe of the experiments simulated in this study.

$$\frac{dC_{PFAS}}{dt} = -fk_{NH4}C_{FeIII}C_{NH4}C_{rdhA}^{1/m}C_{PFAS} \quad (6)$$

Equation (6) is an alternate formulation for PFAS degradation, using the *rdhA* expression instead of the A6 biomass.

We found that the best fit, when using equation 1 through 5 was obtained when $n \geq 3$ for C_{16S} . Therefore, we used $n=3$.

Using equation 6 instead of equation 3 with $m = 3$ gave only a marginally decrease in the least square error of the overall fit.

Results

The above equations were fitted to the data, and the best fit parameters obtained for individual sets of incubations are shown in Table V1.

Table V.1. Fitted parameters for the 100 mg/L and 0 mg/L initial PFAS concentrations.

Treatment	k_{NH4}	k_{FeIII}	f	f_F	f_{16S}	k_{FeIII}/k_{NH4}	$Fe(II)/NH_4$
PFOA, 100 mg/L, A6	2.97×10^{-7}	5.46×10^{-6}	0.0128	0.3099	0.1165	18.38	5.93
PFOA, 100 mg/L, A6E	3.88×10^{-7}	4.25×10^{-6}	0.0245	0.4957	0.1097	10.95	3.52
PFOA, 0.0 mg/L, A6	3.18×10^{-7}	6.20×10^{-6}			0.1115	19.50	6.29
PFOA, 0.0 mg/L, A6E	2.54×10^{-7}	5.45×10^{-6}			0.1886	21.46	6.92
PFOS, 100 mg/L, A6	3.37×10^{-7}	3.89×10^{-6}	0.0560	0.0266	0.0445	11.54	3.72
PFOS, 100 mg/L, A6E	2.06×10^{-7}	2.59×10^{-6}	0.3795	0.1798	0.0826	12.57	4.06
PFOS, 0.0 mg/L, A6	2.74×10^{-7}	5.08×10^{-6}			0.1217	18.54	5.98
PFOS, 0.0 mg/L, A6E	2.74×10^{-7}	5.04×10^{-6}			0.1642	18.39	5.84

Simulation results for the incubations with PFOA and 10^7 copies/ml of A6 are shown in Figure V.1.

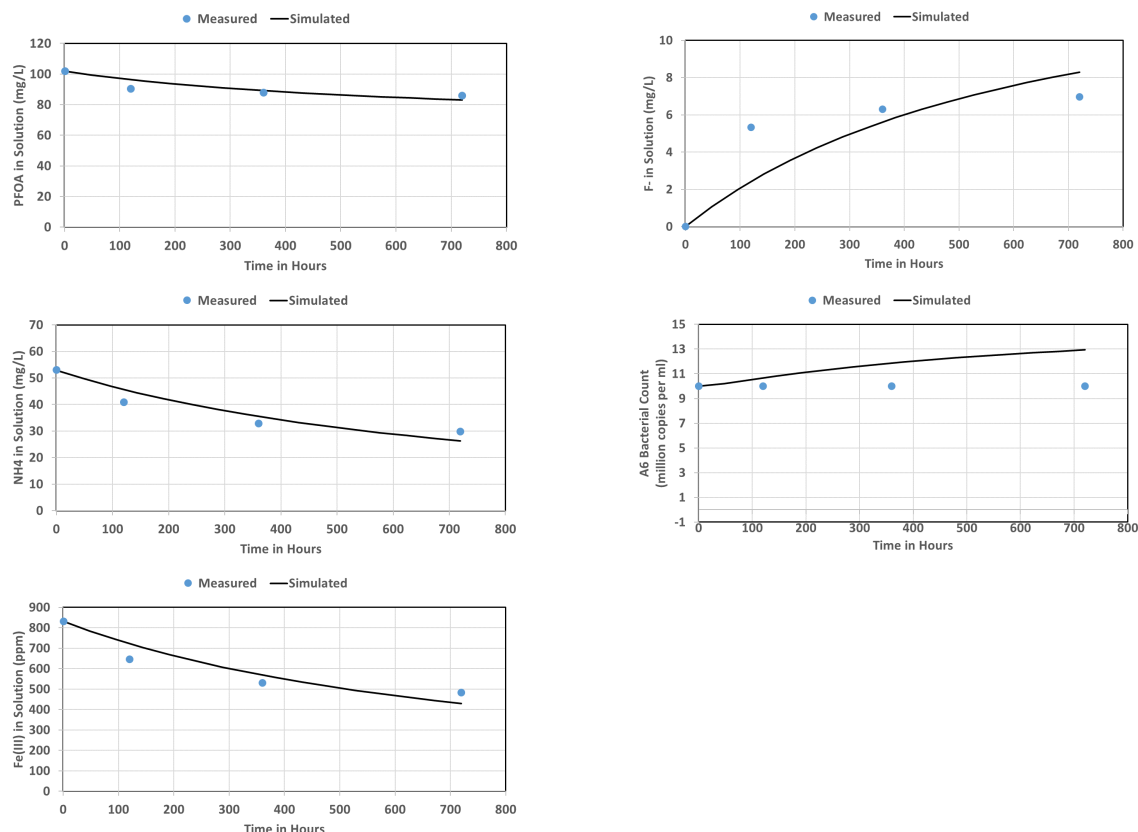


Fig. V.1 Measured and simulated PFOA, NH_4^+ , F^- , Fe(III) , and A6 concentrations for 10^7 copies of A6/ml

Results show that the PFAS defluorination kinetics are proportional to the rate of NH_4^+ oxidation, which is not unexpected given that NH_4^+ is the electron donor for the reductive defluorination of PFAS by A6. It is interesting, but perhaps not surprising for an iron reducing process that the kinetics of NH_4^+ oxidation and PFAS defluorination are not linearly related to the A6 biomass.

We conclude from this brief initial kinetic study that the best way to increase the PFAS defluorination rates is by increasing the rate of the Feammox process. Our previous studies have shown that the addition of electron shuttling compounds, such as AQDS, can, under certain circumstances, significantly increase the rate of NH_4^+ oxidation by A6. So far we have not explored the effect of electron shuttling compounds on the PFAS defluorination rate.

VI. Conclusions

The key conclusions from this research include:

1. PFOA, PFOS, and PFHxS can be defluorinated by the pure A6 and A6 enrichment cultures during the oxidation of NH_4^+ by A6 while reducing Fe(III). Consistent with our previous findings, PFOA is defluorinated at a higher rate than PFOS, specially by the pure A6 culture.
2. For all PFAS tested so far, a gene for a reductive dehalogenase homolog (*rdhA*) and a gene for a fluoroacetate dehalogenase homolog (*FceA*), are always expressed when PFAS are being defluorinated by A6.
3. There is a strong correlation between the expression of the *rdhA* gene and the production of F^- during the incubations. This correlation is much weaker for the *FceA* gene.
4. Consistent with the stronger correlations described above, gene knockout experiments indicate that the *rdhA* gene plays a key role in the defluorination of PFAS. Defluorination still proceeded when the *FceA* gene was knocked out.
5. The detection of H-PFOA during the incubations with PFOA, with the missing fluorine likely from the alpha carbon, indicates that the first step in the defluorination is the removal of the fluorine on that carbon. Since there is no detected defluorination in most of the incubations with the *rdhA* mutants, this first defluorination step is attributed to the *rdhA*.
6. The role of the *FceA* in the PFAS degradation process, if any, is not clear at this point.
7. So far, we only examined degradation by A6 of 1,2,3 TCP as a chlorinated compound of interest. The preliminary results indicate that A6 is capable of degrading TCP and produce

DCP in this process. While the *rdhA* gene was not expressed, or less than in the positive control, during the degradation of TCP, the two putative haloacid dehalogenase genes (*dhl_1* and *dhl_2*) were expressed, indicating that defluorination and dechlorination by A6 is carried out by different enzymes.

8. Incubations of mixtures of 1,2,3 TCP and PFOA show that all genes discussed above are expressed, and that the presence of either compound does not affect the degradation of the other compound, confirming the conclusion that defluorination and dechlorination by A6 are separate processes.
9. To avoid a long lag time, A6 requires acclimation to PFAS. Acclimation results in PFAS carryover, including their degradation products, even after multiple washing of the cultures, which needs to be accounted for in these experiments for their proper design and interpretation. The high carryover made it impossible to detect the production of intermediates in the incubations with a much lower Feammox activity that are described in Appendix II.
10. The defluorination kinetics of PFOA/PFOS are first order with respect to their concentration over the concentration range examined. The kinetics are proportional to the rate of NH_4^+ (electron donor) oxidation, and for the experimental conditions discussed here, are proportional to the A6 biomass to the 1/3 power.
11. Given the direct proportionality between the PFAS defluorination rate and the NH_4^+ oxidation rate, and the proportionality to the A6 biomass only to the 1/3 power, enhancing the Feammox process by optimizing the electron transfer to the ferric iron mineral surface via the use of electron shuttles or different Fe(III) phases/concentrations, might be required to significantly enhance the PFAS defluorination rate by A6, and should be a strategy examined in addition to increasing the A6 biomass.

Appendix I. TOC and F⁻ analyses in Feammox incubations with PFAS

I. a. Using Total Organic Carbon (TOC) in to measure Per- and polyfluoroalkyl substances (PFAS) in Solution in the absence of other organic compounds

In Huang and Jaffé, 2019, TOC was used for a carbon balance when the only organic compound added to the incubations with *Acidimicrobium* A6 (an autotroph) was PFOA or PFOS at initial concentrations in the 100 mg/l range. Use of TOC for such purposes has been reported previously by Shimadzu, 2015 and by Bonyadinejad et. al., 2016. The goal here was to verify the accuracy of such analyses over the concentration range we used previously.

The concentrations of multiple PFAS (including PFOA, PFHpA , PFPeA, PFBA, PFOS, PFHxS, PFBS) was evaluated on the basis of TOC content using the TOC-LCPH high-temperature (720 °C) catalytic combustion total organic carbon analyzers (Shimadzu TOC-5000(A)).

PFAS was dissolved in DI water, and aqueous solutions were prepared at 20 mg/L, 10 mg/L, 5 mg/L, 2 mg/L, and 1 mg/L. For PFOS and PFHxS, because these two solutions are prone to foaming, TOC measurement was conducted using the TC-IC method.

Results

The results are shown in Table A1 and Fig. A1 and confirm that using the high- temperature catalytic combustion TOC analyzer, allows for an accurate analysis of the TOC contributed by multiple PFAS in solution with high detection rates in the concentration range studied and in the absence of other organic compounds. Hence, tracking TOC can be used to conduct a carbon balance on PFAS in autotrophic incubations such as described by Huang and Jaffé, 2019, at least for the concentration range examined here.

Table A1. PFAS theoretical vs. measured value as Total Organic Carbon (TOC)

	PFAS Theoretical Value (mg/L)	TOC Measurement Value (mg C/L)	PFAS Measurement Value (mg/L)	Error
PFOA	0	0.10	0.44	
	1	0.20	0.88	7.2%
	5	1.24	5.36	-8.5%
	10	2.12	9.15	-9.5%
	20	4.20	18.10	-21.9%
PFHpA	0	0.00	0.00	
	1	0.31	1.36	35.6%
	5	1.07	4.62	-7.6%
	10	2.14	9.28	-7.2%
	20	3.28	14.21	-28.9%
PFPeA	0	0.00	0.00	
	1	0.25	1.10	35.6%
	5	1.11	4.88	-7.6%
	10	2.36	10.38	-7.2%
	20	4.71	20.73	-28.9%
PFBA	0	0.00	0.00	
	1	0.19	0.85	-15.4%
	5	0.96	4.27	-14.6%
	10	1.67	7.42	-25.8%
	20	3.66	16.30	-18.5%
PFOS	0	0.10	0.54	
	1	0.21	1.10	10.4%
	5	0.98	5.13	2.5%
	10	1.82	9.46	-5.4%
	20	4.08	21.27	6.3%
PFHxS	0	0.00	0.00	
	1	0.17	0.93	-7.4%
	5	0.67	3.71	-25.8%
	10	1.59	8.81	-11.9%
	20	3.60	19.98	-0.1%
PFBS	0	0.00	0.00	
	1	0.18	1.13	-16.9%
	5	0.77	4.81	-16.9%
	10	1.71	10.71	-16.9%
	20	2.95	18.41	-16.9%

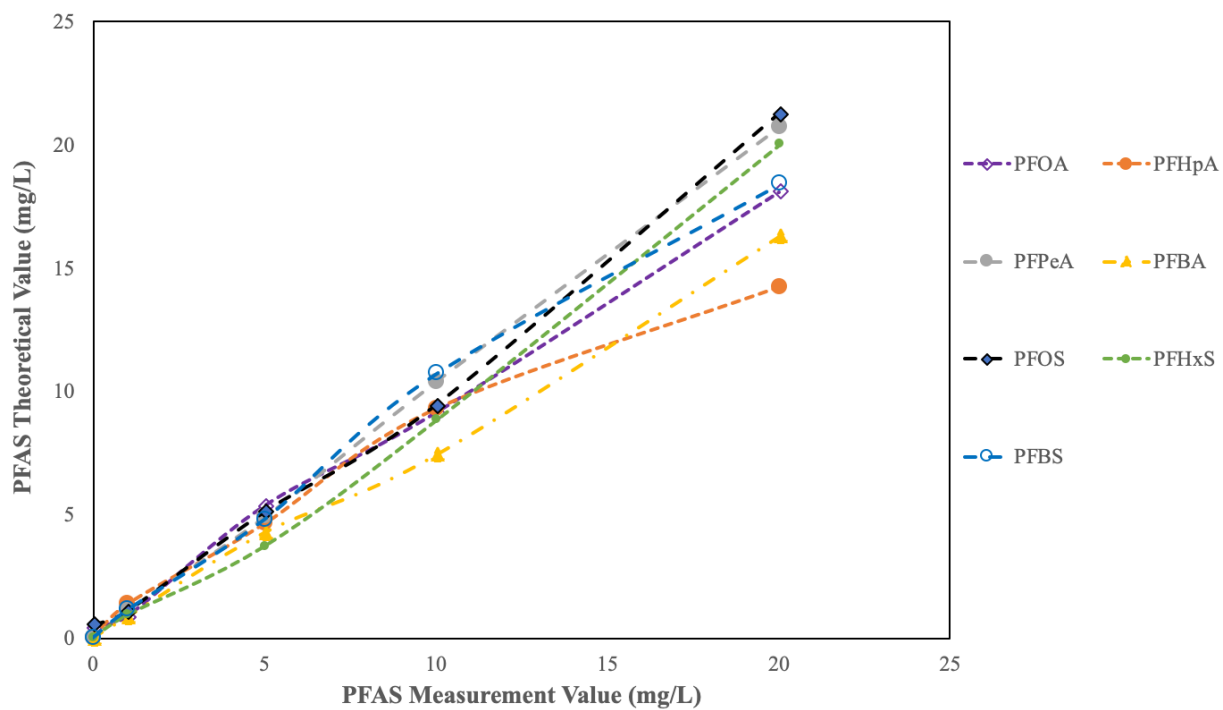


Figure A1. PFAS measured via UPLC-MS-MS vs. PFAS concentration estimated from TOC

Conclusions: For the concentration range tested using the TOC-LCPH high-temperature (720 °C) catalytic combustion total organic carbon analyzers (Shimadzu TOC-5000(A)) resulted in reliable quantification of PFAS expressed in terms of their TOC.

I. b. Measurement of Fluoride in Feammox medium under different pH conditions

In Huang and Jaffé, 2019, working with high PFOA/PFOS concentrations (100 mg/l) we obtained good fluorine balances (organic F in the parent compound and in the smaller PFAAs produced + F⁻ produced) during the defluorination of these compounds. As expected, at lower PFAS concentrations a fluorine balance was much more challenging. We have since then conducted incubations with high PFAS concentrations, some of which, have not yielded a good F balance. Usually with F missing at the end of the incubations. Hence, the objective here was to determine under what circumstances we might expect to obtain a good F balance and why in other incubations that is not achieved.

The production of hexafluoroferrates(III) has been reported by Chaudhuri and Islam, 1986, and might be contributing to the poor F⁻ recovery under certain pH values in Feammox incubations, which contain Fe(III) sources.

1. During Feammox reaction, Fe(III) is reduced to Fe(II), which will be partially or completely re-oxidized to Fe(III) under aerobic conditions when pH in solution is higher than 3. When the pH is higher, NO₂⁻ formed during the Feammox oxidation of NH₄⁺ can also react with Fe(II) to form Fe(III).

2. This free Fe(III) with F⁻ in solution, will then form several stable binary fluoro complexes of Fe(III), [FeF₆]³⁻, [FeF₅]²⁻, [FeF₄]⁻ and causing F⁻ to be undetectable.

3. [FeF₆]³⁻, is relatively stable under acidic and neutral conditions, but can generate Fe(OH)₃ releasing F⁻ in strong alkaline environments: $\text{Na}_3\text{FeF}_6 + 3\text{NaOH} = \text{Fe(OH)}_3\downarrow + 6\text{NaF}$

The Feammox process carried out by *Acidimicrobium* A6 requires acidic conditions (Huang and Jaffé, 2018). The process consumes protons as shown in Eq. 1, resulting in an increase in pH during the incubations.



Furthermore, since *Acidimicrobium* A6 is an autotroph, CO₂ is added to the incubation vials headspace. Consumption of that CO₂ without replacement will result in additional increases in the pH. The final pH in the incubations described by Huang and Jaffé, 2019, as well as the incubations described here, remained below 5, but in many incubations the final pH can yield values that increase to slightly below 7.

To study the effect of the pH on Fe(II) oxidation to Fe(III), which may then cause the complexation of F⁻ during the F⁻ analysis, experiments were conducted by spiking known concentration (1 mg/L, 3 mg/L, and 10 mg/L) of F⁻ to Feammox medium with 3mM ferrihydrite and 1mM FeCl₂. The pH of medium was then adjusted to a range of pH levels: pH 4, pH 5, pH 6, and pH 7. After 24 hours of oxic / anoxic incubation, the recovery of F⁻ in solution was determined.

For the fluoride analyses, 2 mL samples were filtered (0.2 µm) and then fluoride measured immediately via IC using an AS-18 column (flow rate = 1.00 mL min⁻¹, detection limit = 0.010 mg/l) with 25mM KOH. Dionex™ Retention Time Standards for all the anion analyses were used. As an additional confirmation, samples were also analyzed for fluoride spectrophotometrically, using a perfectION™ combination electrode (Mettler-Toledo, USA). The values reported here are for the AS-18 column with KOH as the eluent. The concentrations measured with the fluoride electrode were within 10% of those measured via IC and were only used as a separate confirmation.

Results

Table. A2 Dissolved F^- concentration and percentages of fluoride recovered after 24-hours of oxic incubation under different pH values spiked with 1 mg/L, 3 mg/L, and 10 mg/L of F^- .

		<i>F⁻ spike 1mg/L</i>	<i>F⁻ spike 3 mg/L</i>	<i>F⁻ spike 10mg/L</i>
F^- concentration (mg/L) in liquid phase	pH=4	0.9221	2.679	8.974
	pH=5	0.9257	2.637	8.699
	pH=6	0.8545	2.157	8.939
	pH=7	0.2577	1.984	7.218
% of F^- recovery	pH=4	92.2%	89.3%	89.7%
	pH=5	92.6%	87.9%	87.0%
	pH=6	85.4%	71.9%	89.4%
	pH=7	25.8%	66.1%	72.2%

Conclusions

1. pH in solution gradually increases during the Feammox incubation. When pH is greater than 3, increasing pH results in increasing oxidation of Fe(II) with NO_2^- during the incubations, and with O_2 when vials are opened for analyses. In the presence of F^- , when Fe(II) is oxidized to Fe(III), fluoro complexes of Fe(III) are formed, which will affect the F^- analysis. Results of F^- recovery in the liquid solution under different pH conditions (pH =4, 5, 6, and 7) show 7.4 % to 10.7% decrease in the F^- detection for a pH=5. As the pH increased, the % recovery of F^- decreased gradually (Table A2). These results indicate that when the final pH observed in the Feammox incubations is near 6 or higher, a full recovery of the F^- produced is not expected.

2. To avoid generating fluoro complexes of Fe(III), especially when the end pH of the incubations is >5 , it is necessary to suppress the conversion of Fe(II) to Fe(III). Feasible methods include acidified filtered samples (pretreatment of IC analysis), and conduct F^- detection under anoxic conditions. Neither of these solutions will prevent the oxidation of Fe(II) by the NO_2^- produced during the Feammox incubations.

Appendix II. Experimental validation of biodegradation of PFOS and PFOA by an A6 enrichment culture

This work was conducted by Prof. Jinxia Liu
Department Civil Engineering
McGill University
Under a subcontract to McGill University

1. Culture preparation and Feammox activity monitoring at Princeton

300 mL of A6 enrichment culture, obtained from a 180-day long anaerobic incubation amended with 10 ppm PFOA and PFOS, were washed 3 times by centrifuging and resuspending with 300 mL of anoxic inorganic Fe(III)-NH₄⁺ enrichment medium. These cultures were evenly distributed into multiple 30-ml serum vials in an anaerobic chamber and were sealed with butyl rubber stoppers. The headspace (5 mL) of each vial was vacuumed and then flushed with a N₂/CO₂ (80:20) mixture to achieve anoxic conditions. The initial pH of the cultures was 4.5 ± 0.2.

Cultures were then shipped to McGill. Subsamples were taken at Princeton every 7 days for Fe(II), NH₄⁺, F⁻ and microbial analyses. The exact timeline of handling the cultures is depicted in Figure A2. When cultures arrived at McGill they were stored in a refrigerator. Cultures that were retained at Princeton were handled in the same manner as those studied at McGill, except the shipping event, which could not be reproduced. On day 28, 10 ppm PFOS was amended to the cultures at both, McGill and Princeton, and incubated at a shaker at 25 °C.

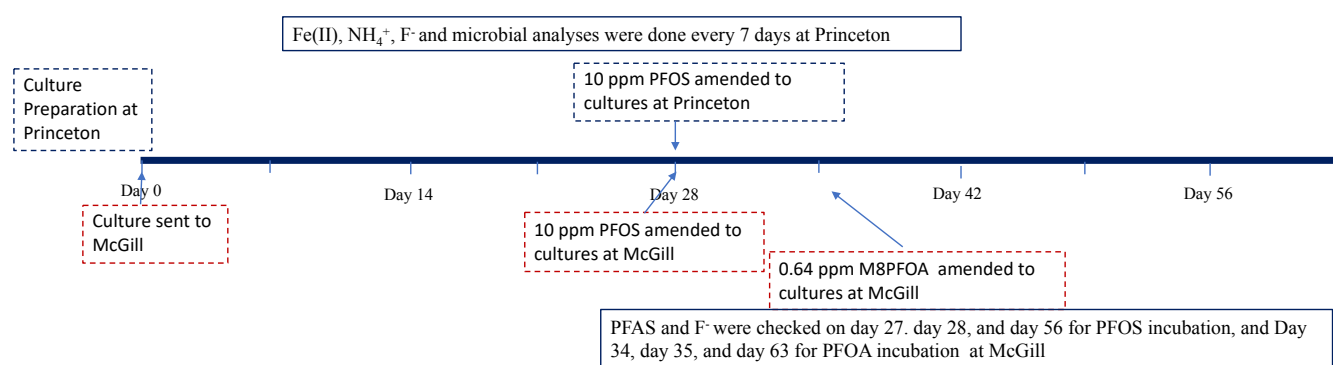


Figure A2. Timeline of culture handling at Princeton and McGill

Results

Incubations conducted at Princeton University

Removal of NH_4^+ , production of Fe(II) and F^- , as well as A6 numbers, monitored at Princeton are shown in Figures A3, A4, A5, and A6, respectively.

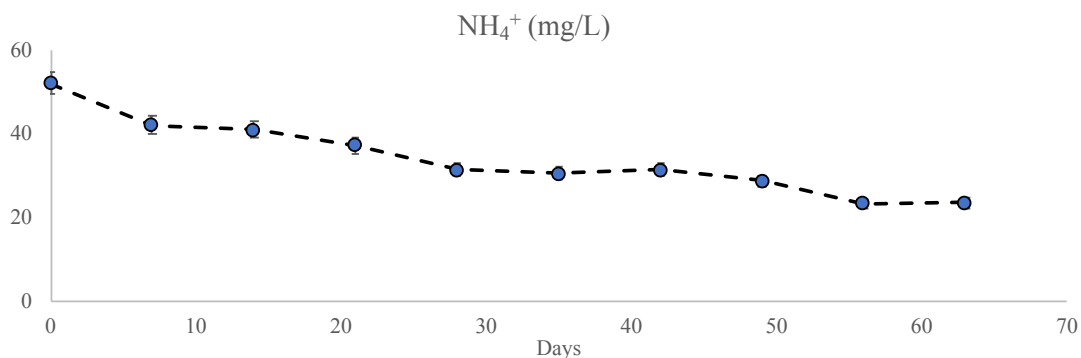


Figure A3. Ammonium removed vs. time during the culture storage and incubation

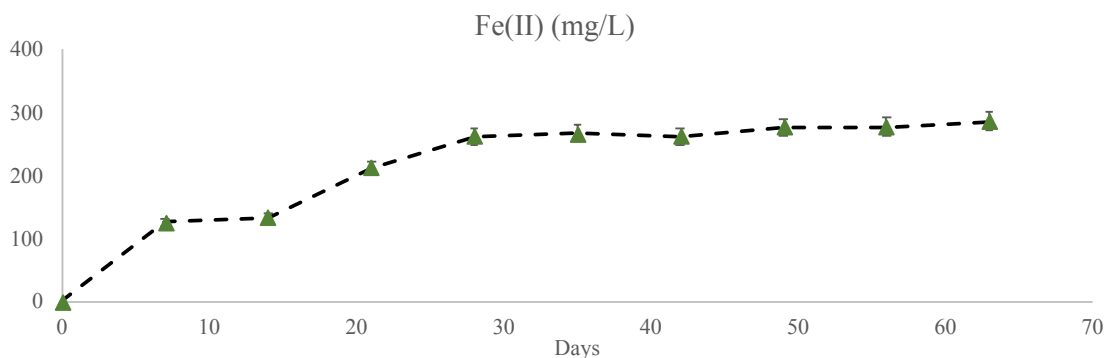


Figure A4. Ferrous iron produced vs. time during the culture storage and incubation

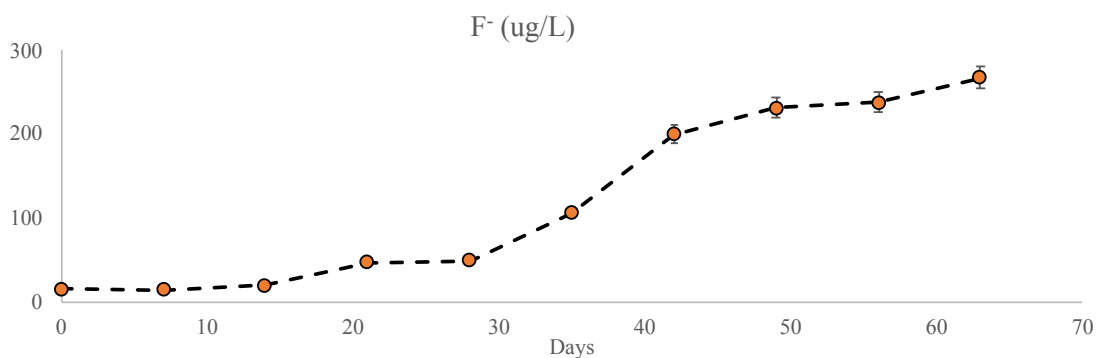


Figure A5. Fluoride produced vs. time during the culture storage and incubation

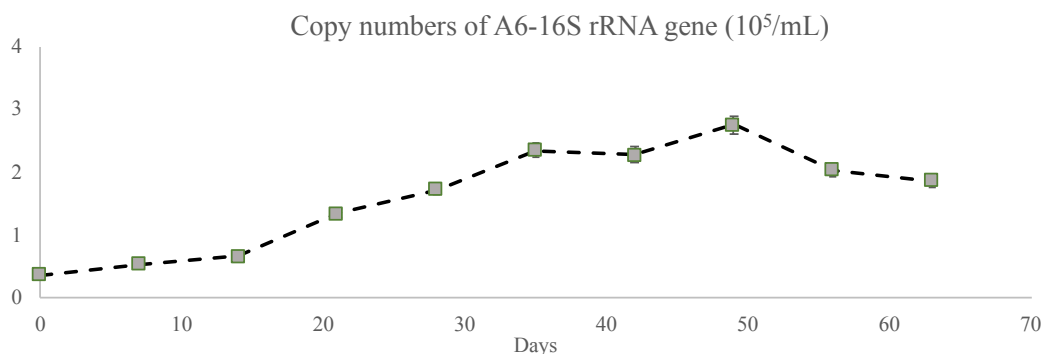


Figure A6. A6 copy numbers vs. time during the culture storage and incubation

Focusing on the amount of NH_4^+ removed (Fig. A3) and Fe(II) produced (Fig. A4), specially from day 28 onwards, when the culture was spiked with PFOA, shows that there was little if any Feammox activity in the cultures monitored at Princeton. We assume that the activity was similar in the cultures incubated at McGill, although the effect of shipping, and perhaps different incubation methods and handling, might have resulted in difference in their performance. Based on the results discussed in Section V, that the rate of defluorination is linked to the amount of NH_4^+ oxidized, these results indicate that defluorination was likely to be minimal. This is corroborated by the results shown in Fig. A5 that show that the amount of F^- produced, which was less than 300 $\mu\text{g/l}$ (Princeton incubations) vs. 1.5 mg/l in other incubations with the same amount of PFOA conducted for this project (Fig. I.2).

Incubations conducted at McGill University

On day 28 vials were spiked with PFOS (new SigmaAlrich 40% solution) and M8PFOA (^{13}C -labeled PFOA). For this purpose, vials were degassed and purged with N_2/CO_2 , and spiked with PFOS (21 μM , 10ppm) aqueous solution or M8PFOA (1.6 μM , 0.6ppm) in salt medium. Vials were then incubated for days in an incubator shaker at 25 °C. A 0.5 mL volume was removed from the

sealed vials on day 0 and 28 to quantify the PFAS and fluoride concentrations. Abiotic controls were prepared at McGill using concentrated Feammox medium and 2-line ferrihydrite solutions sent by Princeton. The methanol solvent in the M8PFOA solution was evaporated and M8PFOA was re-dissolved in the degassed salt medium.

PFAS analysis by LC-MS/MS

After collection, all samples were frozen at -20 °C until analyzed at the end of the 28-day incubation. Samples were collected and analyzed for days (-1), 0 and 28.

Fluoride analysis by IC

A 14-day spike-recovery test was performed to establish the proper sample preparation method (centrifugation only; centrifugation followed by acidification; acidification followed by centrifugation). “Centrifugation only” was chosen to prepare day(-1), 0 and 28 samples from the 28-day incubation.

Results

The measured concentrations of PFOA and PFOS after the vials had been spiked are shown in Fig. A7a and A6b, respectively, and show that the initial concentration of the parent compounds (PFOA and PFOS) had a large variability among replicates.

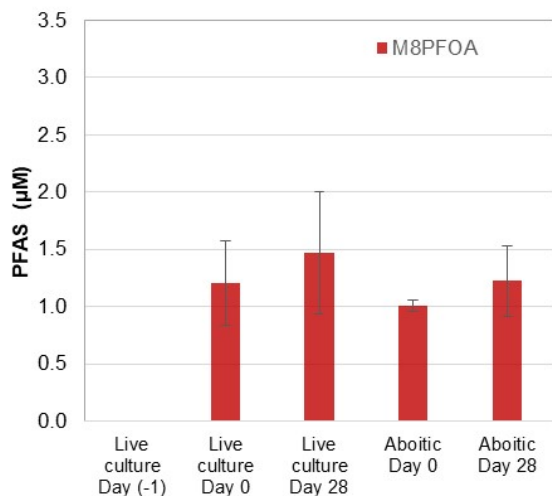


Fig. A6a. Spiked [M8PFOA] = 1.6 μ M

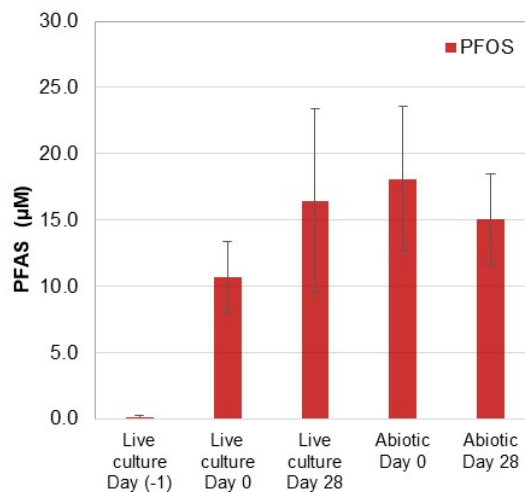


Fig. A6b. Spiked [PFOS] = 21 μ M

Results of the analyses also show that even though the acclimated A6 enrichment culture was triple washed, as described above, prior to shipping, it still contained significant background with PFBS as the major impurity in the background, followed by PFOA and PFOS (Figs. A7a and A7b). Other impurities detected and that are most likely the result of carryover are shown in Fig. A8.

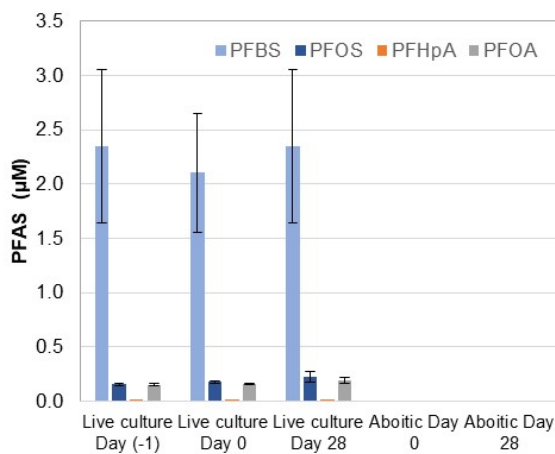


Fig. A7a. Background in sample spiked with $[M8PFOA] = 1.6 \mu M$

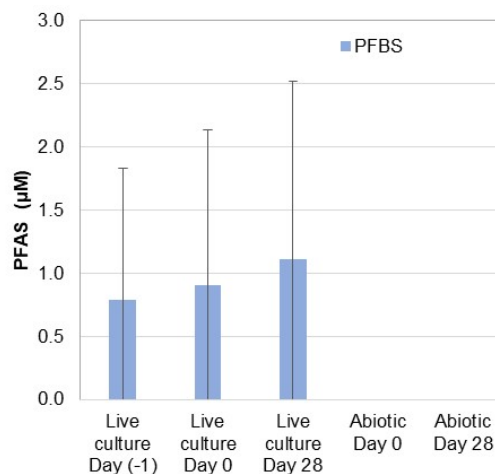


Fig. A7b. Background in sample spiked with $[PFOS] = 21 \mu M$

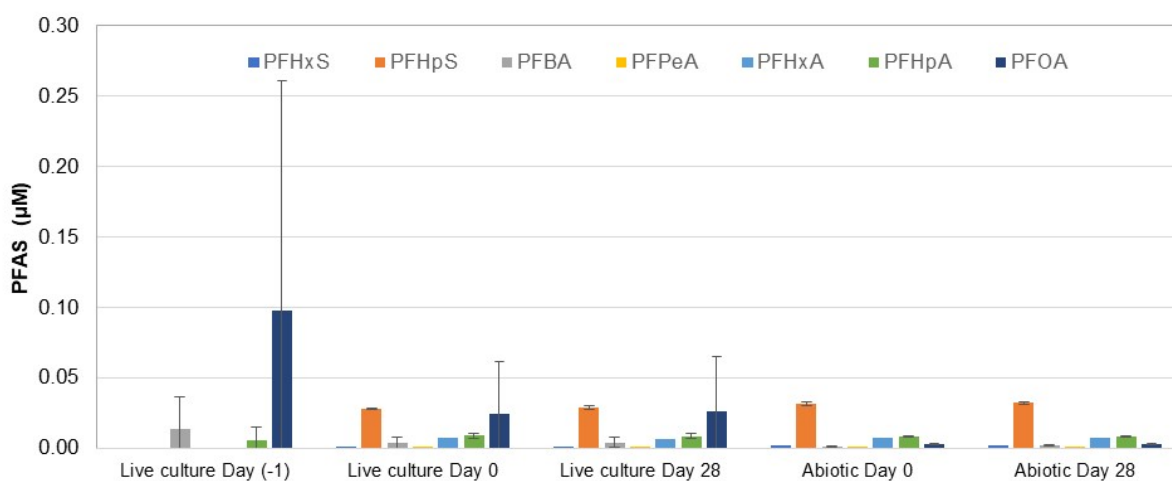


Fig. A8. Other background compounds in samples spiked with $PFOS = 21 \mu M$

Fluoride analyses, shown in Fig. A9 do not show a production of F^- over the incubation period for the incubations conducted at McGill.

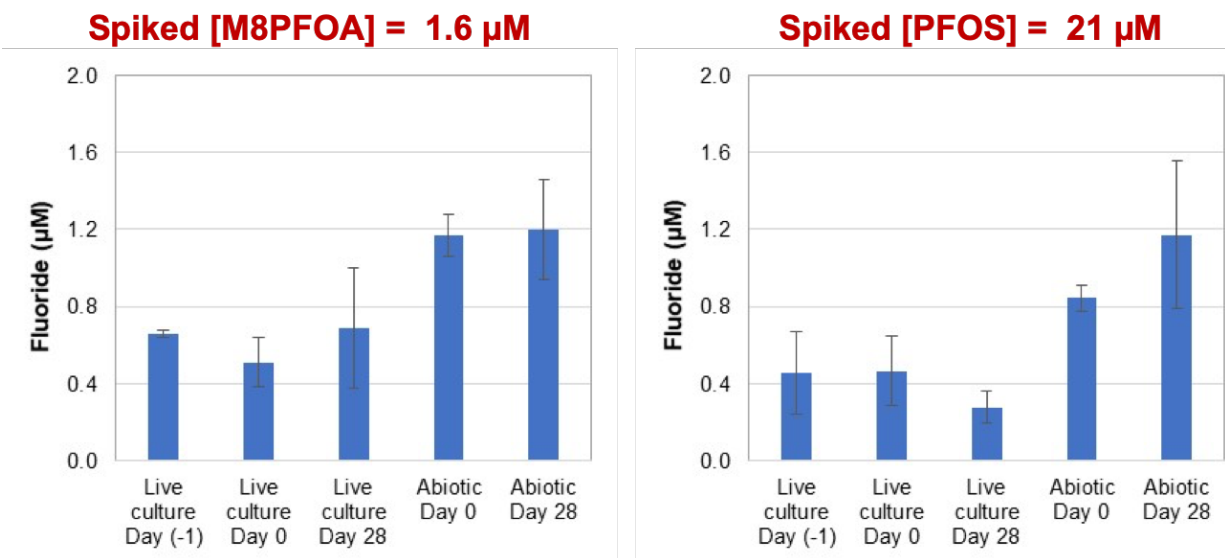


Fig. A9. Fluoride concentrations in the live culture and abiotic vials

Conclusions

Although a very small amount of F^- production was observed in the incubations conducted at Princeton, no F^- production was observed in the incubations conducted at McGill. The relatively large initial variability in the parent compound (PFOS or M8PFOA) makes it difficult to assess if small changes in their concentrations might have occurred during the incubations. This problem is exacerbated by the presence of potential intermediates that come from the acclimated culture. Hence, the results of these incubation are inconclusive and do not demonstrate PFOA or PFOS defluorination.

We are uncertain if the culture itself was less active than usual, or if the shipping process, followed by the storage in a refrigerator for 28 days inactivated the culture. Based on the NH_4^+ removal monitored at Princeton, we did not expect defluorination, although some F^- production was still observed, hence we that there was a chance that the incubations at McGill would show production of some degradation.

Unfortunately, due to the pandemic there was little time to repeat these experiments and funding for the analyses had been exhausted.

An important finding from these analyses is that even with multiple culture washing, traces of the PFSA that was used for the culture acclimation, as well as its shorter carbon chain degradation PFASs can still be detected. This makes it a challenge to track potential intermediates that appear at only very small concentrations.

Appendix III. Detection of H-PFOA as a PFOA defluorination metabolite by the A6 enrichment culture conducted at NJIT

This work was conducted by Prof. Mengyan Li and Chen Wu
Department of Chemistry and Environmental Science
New Jersey Institute of Technology

While growing and testing the cultures and analyzing their numbers, NH_4^+ removal and Fe(II) production was conducted at Princeton under this project, work conducted at NJIT was not funded by this project but is shown as additional relevant information for this project.

The objective here was to investigate the defluorination products independently by an outside laboratory. For this purpose, we teamed up with the laboratory of Prof. Mengyan Li at the New Jersey Institute of Technology (NJIT), since his lab has established an analytical approach to screen PFAS metabolites using high resolution mass spectrometry (HRMS). A further advantage of collaborating with NJIT is that the travel distance to NJIT is one hour, allowing us to prepare active cultures, that were picked up by a student of Prof. Li, taken to NJIT, spiked, and incubations started within a few hours.

Procedure

A separate PFOA-acclimated A6 enrichment culture was grown and its Feammox activity monitored at Princeton as described above. Two vials of this active enrichment culture and one vial with the Feammox medium were provided to NJIT for this purpose. The experimental design, shown in Table A3, consisted of two identical biologically active runs and a control containing no A6. These vials were picked up at Princeton and taken directly to NJIT where they were spiked upon arrival with 10 ppm of PFOA and placed into an incubator shaker at 25 °C. At NJIT, vials were sampled on day 14 for PFOA defluorination metabolite screening. A parallel set of incubations was conducted on day 7, 14 and 90 at Princeton to analyze for NH_4^+ , Fe(II), and A6 numbers. A microbial community analysis for this culture has not been performed to determine difference between these cultures.

Table A3. Experimental setup for the incubations conducted at NJIT

SETUP	A6 enrichment	liquid media
PFOA+A6_1	+	+
PFOA+A6_2	+	+
Abiotic control	-	+

Results

Utilization of NH_4^+ , production of Fe(II), A6-16S copy numbers are shown in tables A4 to A6, respectively. Results show Feammox activity in terms of NH_4^+ utilization and Fe(II) production.

Table A4. NH_4^+ concentrations

NH_4^+ (ppm)	Day 7	Day 14	Day 90
PFOA+A6_1	48.1	39.8	30.4
PFOA+A6_2	47.3	36.1	25.4
Abiotic control	51.2	50.1	50.6

Table A5. Fe (II) production

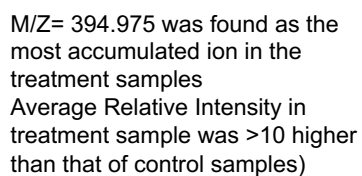
Fe (II) (ppm)	Day 7	Day 14	Day 90
PFOA+A6_1	25.4	67.8	131.3
PFOA+A6_2	30.4	81.3	142.8
Abiotic control	6.3	5.2	7.6

Table A6. A6-16S copy numbers

A6-16S ($10^5/\text{mL}$)	Day 7	Day 14	Day 90
PFOA+A6_1	0.58	1.16	4.03
PFOA+A6_2	0.59	1.21	5.63
Abiotic control	0	0	0

Table A8. H-PFOA detected in the incubations with PFOA with relative intensity compared to a 10 ppm internal standard on day 14

Based on the relative intensity with respect to the standard, which was 10 mg/L, these results indicate a H-PFOA concentration on day 14 on the order of 100 ppb.



Conclusion

Using HRMS, the presence of H-PFOA, with the missing F from the alpha carbon, was detected on day 14, while it was not detected in the abiotic control, and is an additional confirmation of the production of H-PFOA that was also observed in the incubations described in Section I.

These are very preliminary but encouraging results, and more incubations and analyses are under way by the NJIT group.

Appendix IV. SGS Quality Control Report and Equipment Information



ORGANIC ANALYSIS									
QC REPORT Laboratory Control Sample				Batch : 21-02688		Date of Extraction : 2021/4/14			
				Matrix : Water		Date of Analysis : 2021/4/16			
ITEMS	METHODS	MDL	UNIT	Blank Result	Laboratory Control Sample				
					Spike Conc.	Rec.%	Control Range		
							Low	High	
PFBA	EPA537-2008	20	µg/L	<20	10	81	70	130	
PFPeA	EPA537-2008	20	µg/L	<20	10	100	70	130	
PFHxA	EPA537-2008	20	µg/L	<20	10	113	70	130	
PFBS	EPA537-2008	20	µg/L	<20	10	96	70	130	
PFPHpA	EPA537-2008	20	µg/L	<20	10	101	70	130	
PFOA	EPA537-2008	20	µg/L	<20	10	104	70	130	
PFHxS	EPA537-2008	20	µg/L	<20	10	103	70	130	
PFNA	EPA537-2008	20	µg/L	<20	10	104	70	130	
PFHpS	EPA537-2008	20	µg/L	<20	10	97	70	130	
PFOS	EPA537-2008	20	µg/L	<20	10	106	70	130	
PFPeS	EPA537-2008	1000	µg/L	<20	500	86	70	130	



ORGANIC ANALYSIS				Date of Extraction : 2021/4/14 Batch : SHE21-02688 Date of Analysis : 2021/4/16 Matrix : Water Duplicate Sample : SHE21-02688-01			
QC REPORT Duplicate Sample				Duplicate Sample			Control Range RPD %
ITEMS	METHODS	MDL	UNIT	Sample Result	Check Sample Result	RPD %	
PFBA	EPA537-2008	20	µg/L	<20	<20	-	-
PFPeA	EPA537-2008	20	µg/L	<20	<20	-	-
PFHxA	EPA537-2008	20	µg/L	<20	<20	-	-
PFBS	EPA537-2008	20	µg/L	<20	<20	-	-
PFPHpA	EPA537-2008	20	µg/L	<20	<20	-	-
PFOA	EPA537-2008	20	µg/L	<20	<20	-	-
PFHxS	EPA537-2008	20	µg/L	<20	<20	-	-
PFNA	EPA537-2008	20	µg/L	<20	<20	-	-
PFHpS	EPA537-2008	20	µg/L	<20	<20	-	-
PFOS	EPA537-2008	20	µg/L	<20	<20	-	-
PFPeS	EPA537-2008	1000	µg/L	<1000	<1000	-	-



Equipment information

NO.	METHOD	Equipment Name	Inv No.	Model No
1	EPA537-2008	Agilent 1260/6460	1260/6460	SG16517306

Remark:

Test method is not in CMA and CNAS accredited scope,the test report shall only be used for client scientific research, teaching, internal quality control, product research and development, etc... and just for client internal reference.

End of report

Appendix V. Data for figures presented in the report

- *Measurements (M)*
- *Mean*
- *Standard deviation (SD)*

Fig. I.1 PFAS concentrations vs. time during incubations with the pure A6 culture

Pure culture incubation with 10mg/L PFOA

	Day0					Day7					Day25					Day60				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
PFBA	0.12	0.13	0.17	0.14	0.03	0.00	0.00	0.00	0.00	0.00	1.42	1.65	1.37	1.48	0.15	1.05	1.21	0.98	1.08	0.12
PFPeA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.02	0.01	0.01	0.12	0.14	0.11	0.13	0.02
PFHxA	0.04	0.02	0.08	0.05	0.03	0.00	0.00	0.00	0.00	0.00	0.03	0.02	0.01	0.02	0.01	0.00	0.00	0.00	0.00	0.00
PFHpA	0.00	0.01	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.19	0.23	0.15	0.19	0.04	0.86	0.77	1.08	0.90	0.16
H-PFOA	0.00	0.00	0.00	0.00	0.00	0.42	0.67	0.64	0.58	0.14	1.33	1.69	1.67	1.56	0.20	1.72	1.57	2.03	1.77	0.23
PFOA	9.07	10.02	8.99	9.36	0.57	7.24	7.33	8.63	7.73	0.78	7.05	7.60	7.86	7.50	0.41	5.65	6.44	4.79	5.63	0.83

Pure culture incubation with 10mg/L PFOS

	Day0					Day7					Day25					Day60				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
PFBS	0.44	0.31	0.27	0.34	0.09	0.22	0.18	0.33	0.24	0.08	0.83	0.89	0.86	0.86	0.03	1.03	1.50	1.77	1.43	0.37
PFPeS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFHxS	0.05	0.05	0.11	0.07	0.03	0.10	0.13	0.06	0.10	0.03	0.05	0.07	0.10	0.07	0.02	0.23	0.20	0.18	0.20	0.02
PFHpS	0.17	0.22	0.31	0.23	0.07	0.29	0.30	0.35	0.31	0.03	0.22	0.19	0.22	0.21	0.02	0.60	0.55	0.65	0.60	0.05
PFOS	7.55	8.17	8.69	8.14	0.57	7.57	6.82	6.23	6.87	0.67	7.50	7.20	6.56	7.09	0.48	7.22	6.90	5.73	6.62	0.79

Pure culture incubation with 10mg/L PFHxS

	Day0					Day7					Day25					Day60				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
PFBS	0.11	0.11	0.09	0.10	0.01	0.15	0.11	0.19	0.15	0.04	0.11	0.10	0.10	0.10	0.00	0.15	0.18	0.19	0.18	0.02
PFPeS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFHxS	8.05	8.53	8.74	8.44	0.35	7.04	7.59	6.58	7.07	0.50	6.64	6.69	7.35	6.89	0.40	6.52	7.21	5.84	6.52	0.69
PFHpS	0.05	0.06	0.11	0.07	0.03	0.05	0.06	0.04	0.05	0.01	0.10	0.10	0.12	0.11	0.01	0.00	0.00	0.01	0.00	0.00
PFOS	0.11	0.11	0.09	0.10	0.01	0.15	0.11	0.19	0.15	0.04	0.11	0.10	0.10	0.10	0.00	0.15	0.18	0.19	0.18	0.02

Controls for pure culture incubation (day 60-day 0)

	autoclaved control + PFOA		autoclaved control + PFOS		autoclaved control + <u>PFHxS</u>		medium control + PFOA		medium control + PFOS		medium control + <u>PFHxS</u>	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PFBA	0.0000	0.0000	0.2270	0.0184	0.0000	0.0000	0.2540	0.0179	0.2500	0.0175	0.0000	0.0000
PFPeA	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
<u>PFHxA</u>	-0.0057	0.0006	0.0000	0.0000	0.0039	0.0004	-0.0157	0.0025	0.0000	0.0000	0.0286	0.0012
PFHpA	-0.0099	0.0011	0.0000	0.0000	0.0011	0.0001	0.0873	0.0096	0.0000	0.0000	0.0008	0.0001
H-PFOA	0.0000	0.0000	0.0000	0.0000	0.0052	0.0004	0.0000	0.0000	0.0000	0.0000	0.0496	0.0031
PFOA	0.2797	0.0269	-0.1043	0.0111	0.0029	0.0000	-0.7125	0.0534	0.0770	0.0085	0.0107	0.0012
PFBS	0.0000	0.0000	-0.9400	0.0862	0.0146	0.0016	0.0000	0.0000	-0.1180	0.0117	0.0101	0.0011
<u>PFPeS</u>	0.0000	0.0000	0.0000	0.0000	0.0032	0.0004	0.0000	0.0000	0.0000	0.0000	0.0060	0.0001
<u>PFHxS</u>	0.0000	0.0000	0.0140	0.0009	-0.3349	0.0235	0.0000	0.0000	0.0155	0.0015	-0.2899	0.0309
<u>PFHpS</u>	0.0000	0.0000	-0.0917	0.0101	0.0017	0.0002	0.0000	0.0000	-0.0766	0.0078	0.0023	0.0002
PFOS	0.0000	0.0000	-0.3001	0.0204	0.0023	0.0002	0.0000	0.0000	-0.5613	0.0611	0.0043	0.0004

Fig. I.2 Production of F⁻ during the incubations with the pure A6 culture

Pure culture incubation with 10mg/L PFAS

	Day0					Day7					Day25					Day60				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6 + PFOA	0.001	0.001	0.001	0.001	0.000	0.321	0.287	0.294	0.301	0.018	0.402	0.400	0.367	0.390	0.020	1.258	1.566	1.729	1.518	0.239
A6 + PFOS	0.004	0.003	0.002	0.003	0.001	0.212	0.202	0.284	0.233	0.045	0.468	0.562	0.593	0.541	0.065	0.778	0.875	1.279	0.977	0.266
A6 + PFHxS	0.001	0.004	0.004	0.003	0.002	0.352	0.303	0.398	0.351	0.048	0.381	0.377	0.395	0.384	0.010	0.772	0.897	0.964	0.878	0.097

Controls for pure culture incubation (day 60-day 0)

	autoclaved control + PFOA		autoclaved control + PFOS		autoclaved control + PFHxS		medium control + PFOA		medium control + PFOS		medium control + PFHxS	
(mg/L)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
F ⁻	0.0270	0.0021	0.0624	0.0086	0.0821	0.0190	-0.0030	0.0003	0.0000	0.0000	-0.0043	0.0008

Fig. I.3 NH₄⁺ concentrations during incubations with the pure A6 culture

Pure culture incubation with 10mg/L PFAS

	Day0					Day7					Day25					Day60				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6 + PFOA	47.6	47.6	51.5	48.9	2.3	40.0	44.3	36.9	40.4	3.7	35.0	39.7	36.1	36.9	2.4	27.7	32.0	32.5	30.7	2.6
A6 + PFOS	52.1	52.6	46.9	50.5	3.1	47.3	44.0	39.0	43.4	4.2	36.3	40.3	42.3	39.6	3.1	29.9	33.7	34.6	32.7	2.5
A6 + PFHxS	46.9	46.7	50.7	48.1	2.2	49.1	43.2	39.9	44.1	4.7	42.3	43.2	46.5	44.0	2.2	35.4	40.9	40.6	39.0	3.1

Controls for pure culture incubation (day 60-day 0)

	autoclaved control + PFOA		autoclaved control + PFOS		autoclaved control + PFHxS		medium control + PFOA		medium control + PFOS		medium control + PFHxS	
(mg/L)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NH ₄ ⁺	-0.650	0.027	-3.736	0.062	0.679	0.082	2.688	-0.003	1.137	0.001	-4.707	0.003

Fig. I.4 PFAS concentrations vs. time during incubations with the enrichment A6 culture

Enrichment culture incubation with 10mg/L PFOA

	Day0					Day7					Day25					Day60				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
PFBA	0.11	0.10	0.09	0.10	0.01	0.09	0.08	0.07	0.08	0.01	1.42	1.24	1.31	1.32	0.09	2.33	2.51	2.75	2.53	0.21
PFPeA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.53	0.46	0.53	0.51	0.04	0.80	0.87	0.95	0.87	0.07
PFHxA	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.01	0.00	0.01	0.17	0.16	0.22	0.18	0.03	0.34	0.29	0.19	0.27	0.08
PFHpA	0.01	0.01	0.02	0.01	0.01	0.17	0.17	0.13	0.16	0.02	0.54	0.50	0.44	0.49	0.05	1.51	1.10	1.20	1.27	0.21
H-PFOA	0.01	0.00	0.01	0.01	0.00	0.37	0.43	0.34	0.38	0.05	1.13	0.93	1.01	1.02	0.10	0.25	0.27	0.25	0.26	0.01
PFOA	8.37	8.91	9.30	8.86	0.47	8.65	7.42	7.61	7.89	0.66	5.40	6.09	6.12	5.87	0.41	6.33	5.58	5.21	5.71	0.57

Enrichment culture incubation with 10mg/L PFOS

	Day0					Day7					Day25					Day60				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
PFBS	0.22	0.21	0.20	0.21	0.01	0.16	0.14	0.18	0.16	0.02	1.54	1.36	1.50	1.47	0.09	2.77	2.44	2.70	2.64	0.18
PFPeS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFHxS	0.06	0.06	0.06	0.06	0.00	0.04	0.05	0.04	0.04	0.00	0.03	0.04	0.03	0.03	0.00	0.63	0.63	0.54	0.60	0.05
PFHpS	0.15	0.14	0.17	0.15	0.01	0.01	0.11	0.25	0.13	0.12	0.96	0.95	0.86	0.92	0.06	0.18	0.17	0.18	0.18	0.01
PFOS	9.57	10.16	10.75	10.16	0.59	8.40	9.00	9.72	9.04	0.66	6.82	7.05	7.89	7.25	0.56	6.37	7.32	8.36	7.35	1.00

Enrichment culture incubation with 10mg/L PFHxS

	Day0					Day7					Day25					Day60				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
PFBS	0.09	0.10	0.12	0.11	0.01	0.16	0.14	0.18	0.16	0.02	0.13	0.13	0.11	0.12	0.01	0.93	0.97	0.81	0.90	0.08
PFPeS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.73	0.77	0.72	0.74	0.03	0.95	0.97	1.03	0.98	0.04
PFHxS	8.50	8.19	7.56	8.08	0.48	0.04	0.05	0.04	0.04	0.00	6.03	6.08	6.81	6.31	0.43	5.37	6.07	6.77	6.07	0.70
PFHpS	0.12	0.10	0.12	0.11	0.01	0.01	0.11	0.25	0.13	0.12	0.10	0.11	0.10	0.10	0.00	0.06	0.06	0.05	0.06	0.00
PFOS	0.29	0.27	0.25	0.27	0.02	8.40	9.00	9.72	9.04	0.66	0.20	0.18	0.19	0.19	0.01	0.07	0.07	0.07	0.07	0.00

Controls for enrichment culture incubation (day 60-day 0)

	autoclaved control + PFOA		autoclaved control + PFOS		autoclaved control + PFHxS		medium control + PFOA		medium control + PFOS		medium control + PFHxS	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PFBA	0.158	0.015	0.481	0.028	0.104	0.026	0.297	0.026	0.486	0.039	0.107	0.013
PFPeA	0.404	0.011	0.000	0.000	0.009	0.005	0.040	0.005	0.000	0.000	0.009	0.001
PFHxA	-0.006	0.000	0.000	0.000	0.087	0.001	-0.005	0.001	0.000	0.000	0.123	0.006
PFHpA	0.222	0.027	0.005	0.001	0.006	0.009	0.074	0.009	0.005	0.001	0.004	0.001
H-PFOA	-0.013	0.002	0.000	0.000	0.078	0.000	0.000	0.000	0.000	0.000	0.115	0.014
PFOA	-1.121	0.076	0.002	0.000	0.001	0.054	-0.778	0.054	0.003	0.000	0.016	0.002
PFBS	0.000	0.000	0.309	0.027	0.071	0.000	0.000	0.000	0.197	0.039	0.103	0.025
PFPeS	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.006	0.001
PFHxS	0.000	0.000	0.006	0.001	-0.118	0.000	0.000	0.000	0.003	0.000	-0.275	0.023
PFHpS	0.000	0.000	0.016	0.002	0.007	0.000	0.000	0.000	0.015	0.002	0.001	0.000
PFOS	0.000	0.000	-1.365	0.154	-0.578	0.000	0.000	0.000	-0.519	0.043	-0.038	0.004

Fig. I.5 Production of F⁻ during the incubations with the enrichment A6 culture

Enrichment culture incubation with 10mg/L PFAS

	Day0					Day7					Day25					Day60				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6 + PFOA	0.028	0.026	0.022	0.025	0.003	0.571	0.585	0.587	0.581	0.009	1.515	1.527	1.506	1.516	0.010	1.819	1.321	1.766	1.635	0.274
A6 + PFOS	0.024	0.023	0.023	0.024	0.000	0.356	0.353	0.368	0.359	0.008	1.046	1.044	1.063	1.051	0.010	1.102	1.250	1.405	1.252	0.152
A6 + PFHxS	0.042	0.046	0.052	0.047	0.005	0.275	0.274	0.263	0.270	0.007	0.756	0.770	0.759	0.761	0.007	0.806	0.847	1.169	0.941	0.199

Controls for enrichment culture incubation (day 60-day 0)

	autoclaved control + PFOA		autoclaved control + PFOS		autoclaved control + PFHxS		medium control + PFOA		medium control + PFOS		medium control + PFHxS	
(mg/L)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
F ⁻	-0.0660	0.0063	-0.1716	0.0089	0.0412	0.0053	-0.1001	0.0110	-0.0413	0.0054	-0.0549	0.0060

Fig. I.6 NH₄⁺ concentrations during incubations with the enrichment A6 culture

Enrichment culture incubation with 10mg/L PFAS

	Day0					Day7					Day25					Day60				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6 + PFOA	54.6	50.9	47.7	51.1	3.4	39.3	42.1	47.8	43.1	4.4	37.1	38.8	31.9	35.9	3.6	36.9	32.4	28.6	32.6	4.2
A6 + PFOS	52.0	52.8	59.2	54.7	4.0	46.5	50.7	57.7	51.6	5.7	43.5	37.1	41.7	40.8	3.3	30.4	30.1	38.8	33.1	4.9
A6 + PFHxS	51.1	53.3	46.8	50.4	3.3	44.0	43.6	52.6	46.7	5.1	35.2	38.1	40.9	38.1	2.9	31.3	37.3	41.8	36.8	5.3

Controls for enrichment culture incubation (day 60-day 0)

	autoclaved control + PFOA		autoclaved control + PFOS		autoclaved control + PFHxS		medium control + PFOA		medium control + PFOS		medium control + PFHxS	
(mg/L)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NH ₄ ⁺	1.009	0.104	-1.875	0.094	2.308	0.139	-1.690	0.195	-1.449	0.115	-2.291	0.247

Fig. I.7 A6 numbers during pure and enrichment culture incubations.

Copy numbers of A6-16S rRNA gene (10⁵/mL)

	Day0					Day7					Day25					Day60				
(10 ⁵ /mL)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6 pure culture + PFOA	0.83	0.88	0.96	0.89	0.07	0.92	0.89	0.94	0.91	0.03	1.87	1.81	1.90	1.86	0.05	3.93	3.41	2.59	3.31	0.68
A6 pure culture + PFOS	1.02	1.08	0.99	1.03	0.04	0.69	0.65	0.85	0.73	0.10	1.49	1.73	1.67	1.63	0.12	2.37	2.11	1.88	2.12	0.25
A6 pure culture + PFHxS	0.63	0.60	0.53	0.59	0.05	0.72	0.65	0.93	0.77	0.15	2.06	2.12	1.80	1.99	0.17	2.26	2.34	3.14	2.58	0.49
A6 enrichment culture + PFOA	1.29	1.09	1.13	1.17	0.11	1.58	1.59	1.84	1.67	0.15	2.85	3.11	3.51	3.16	0.33	3.53	4.41	4.78	4.24	0.64
A6 enrichment culture + PFOS	1.22	1.40	1.14	1.25	0.13	1.35	1.65	1.61	1.54	0.16	2.32	2.81	2.34	2.49	0.28	3.57	3.11	2.87	3.18	0.36
A6 enrichment culture + PFHxS	1.22	1.40	1.14	1.25	0.13	1.72	1.65	1.48	1.62	0.12	1.95	1.76	2.02	1.91	0.14	2.26	2.34	3.00	2.53	0.41

Controls for pure and enrichment culture incubation (day 60-day 0)

	autoclaved control + PFOA		autoclaved control + PFOS		autoclaved control + PFHxS		medium control + PFOA		medium control + PFOS		medium control + PFHxS	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10 ⁵ /mL	0.001	0.001	-0.004	0.002	-0.007	0.004	0.002	0.002	-0.002	0.001	-0.006	0.003

Fig. II.3 Expression of (a) *rdhA* genes and (b) *FceA* gene during incubations

RNA copies of *rdhA* (10⁶/mL)

	Day0					Day7					Day25					Day60				
(10 ⁶ /mL)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6 + PFOA	0.059	0.032	0.060	0.050	0.016	0.136	0.093	0.083	0.104	0.028	0.137	0.139	0.149	0.142	0.006	0.194	0.203	0.305	0.234	0.062
A6 + PFOS	0.051	0.065	0.056	0.057	0.007	0.095	0.073	0.102	0.090	0.015	0.145	0.190	0.201	0.179	0.030	0.218	0.238	0.203	0.219	0.017
A6 + PFHxS	0.062	0.045	0.060	0.056	0.009	0.071	0.064	0.080	0.071	0.008	0.097	0.119	0.164	0.127	0.034	0.176	0.168	0.159	0.168	0.008
A6 + no PFAS	0.046	0.034	0.057	0.046	0.011	0.063	0.045	0.044	0.051	0.011	0.074	0.055	0.079	0.069	0.013	0.076	0.063	0.062	0.067	0.008

RNA copies of *fceA* (10⁶/mL)

	Day0					Day7					Day25					Day60				
(10 ⁶ /mL)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6 + PFOA	1.015	1.029	1.098	1.047	0.044	1.361	1.369	1.647	1.459	0.163	2.628	2.259	2.963	2.617	0.352	2.461	2.974	3.264	2.900	0.407
A6 + PFOS	1.137	1.027	1.148	1.104	0.067	1.261	1.520	1.842	1.541	0.291	2.718	2.658	3.323	2.900	0.368	4.234	4.128	5.386	4.583	0.698
A6 + PFHxS	1.054	1.108	1.054	1.072	0.031	1.332	1.249	1.229	1.270	0.054	2.099	2.263	1.886	2.083	0.189	2.191	2.219	2.874	2.428	0.386
A6 + no PFAS	1.142	1.184	1.191	1.172	0.026	1.192	1.270	1.413	1.292	0.112	1.800	1.601	1.483	1.628	0.160	1.703	1.797	2.231	1.911	0.282

Fig. II.5 Fluoride production of A6 wild type, the FAM1-*rdhA* and FAM1-*fceA* mutants after 72-hour incubations with 100 mg/L PFOA

A6-WT

F ⁻ (mg/L)	<i>a</i>					<i>b</i>					<i>c</i>					<i>d</i>				
	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
	0.117	0.127	0.128	0.124	0.006	0.120	0.123	0.135	0.126	0.008	0.122	0.126	0.156	0.135	0.019	0.193	0.114	0.140	0.149	0.040
	<i>e</i>					<i>f</i>					<i>g</i>					<i>h</i>				
	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
	0.132	0.145	0.137	0.138	0.007	0.217	0.137	0.132	0.162	0.048	0.129	0.137	0.140	0.135	0.005	0.120	0.129	0.128	0.126	0.005
	<i>i</i>					<i>j</i>														
	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD										
	0.131	0.127	0.148	0.135	0.011	0.145	0.170	0.171	0.162	0.015										

FAM1- *rdhA* mutants

F ⁻ (mg/L)	<i>a</i>					<i>b</i>					<i>c</i>					<i>d</i>				
	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
	0.000	0.000	0.000	0.000	0.000	0.120	0.123	0.135	0.126	0.008	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	<i>e</i>					<i>f</i>					<i>g</i>					<i>h</i>				
	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
	0.000	0.000	0.000	0.000	0.000	0.217	0.137	0.132	0.162	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	<i>i</i>					<i>j</i>														
	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD										
	0.000	0.000	0.000	0.000	0.000	0.147	0.169	0.170	0.162	0.013										

FAM1- *fceA* mutants

F ⁻ (mg/L)	<i>a</i>					<i>b</i>					<i>c</i>					<i>d</i>				
	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
	0.120	0.105	0.069	0.098	0.026	0.020	0.105	0.069	0.065	0.043	0.059	0.105	0.069	0.078	0.024	0.000	0.000	0.030	0.010	0.017
	<i>e</i>					<i>f</i>					<i>g</i>					<i>h</i>				
	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
	0.120	0.105	0.069	0.098	0.026	0.020	0.105	0.069	0.065	0.043	0.059	0.105	0.069	0.078	0.024	0.000	0.000	0.030	0.010	0.017
	<i>i</i>					<i>j</i>														
	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD										
	0.195	0.175	0.138	0.169	0.029	0.000	0.010	0.000	0.003	0.006										

Fig. III.1 PFOA, TCP, and DCP concentration during incubations

PFOA (mg/L)

	Day0					Day20					Day45				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6+TCP(1ppm)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A6+TCP+PFOA (1ppm+1ppm)	0.940	0.780	0.777	0.832	0.094	0.708	0.705	0.696	0.703	0.006	0.354	0.359	0.220	0.311	0.079
A6+PFOA (1ppm)	0.904	0.878	0.924	0.902	0.023	0.618	0.620	0.614	0.617	0.003	0.290	0.252	0.307	0.283	0.028
A6 (positive control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TCP (medium control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PFOA (medium control)	0.805	0.799	0.799	0.801	0.003	0.780	0.786	0.799	0.788	0.010	0.784	0.796	0.739	0.773	0.030

1,2,3-Trichloropropane (mg/L)

	Day0					Day20					Day45				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6+TCP(1ppm)	0.579	0.553	0.588	0.573	0.018	0.440	0.436	0.418	0.431	0.012	0.140	0.126	0.131	0.132	0.007
A6+TCP+PFOA (1ppm+1ppm)	0.474	0.498	0.477	0.483	0.013	0.409	0.408	0.390	0.402	0.011	0.252	0.218	0.169	0.213	0.041
A6+PFOA (1ppm)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A6 (positive control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TCP (medium control)	0.497	0.500	0.515	0.504	0.010	0.481	0.475	0.493	0.483	0.009	0.509	0.496	0.490	0.498	0.010
PFOA (medium control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Dichloropropane(mg/L)

	Day0					Day20					Day45				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6+TCP(1ppm)	0.000	0.000	0.000	0.000	0.000	0.190	0.195	0.191	0.192	0.003	0.381	0.345	0.303	0.343	0.039
A6+TCP+PFOA (1ppm+1ppm)	0.000	0.000	0.000	0.000	0.000	0.101	0.096	0.079	0.092	0.011	0.203	0.212	0.254	0.223	0.027
A6+PFOA (1ppm)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A6 (positive control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TCP (medium control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PFOA (medium control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Fig. III.2 F⁻, NH₄⁺, and Fe(II) concentrations during incubations

F⁻(mg/L)

	Day0					Day20					Day45				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6+TCP(1ppm)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
A6+TCP+PFOA (1ppm+1ppm)	0.000	0.000	0.000	0.000	0.000	0.139	0.110	0.159	0.136	0.025	0.269	0.262	0.332	0.287	0.038
A6+PFOA (1ppm)	0.000	0.000	0.000	0.000	0.000	0.138	0.129	0.171	0.146	0.022	0.359	0.307	0.265	0.310	0.047
A6 (positive control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
TCP (medium control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PFOA (medium control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

NH₄⁺(mg/L)

	Day0					Day20					Day45				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6+TCP(1ppm)	47.8	45.4	53.6	48.9	4.2	47.6	51.0	40.2	46.3	5.5	38.9	41.7	35.7	38.8	3.0
A6+TCP+PFOA (1ppm+1ppm)	50.7	52.6	48.3	50.5	2.2	48.0	53.4	57.6	53.0	4.8	45.9	42.5	35.5	41.3	5.3
A6+PFOA (1ppm)	48.7	43.7	51.9	48.1	4.2	48.3	47.0	53.2	49.5	3.3	41.2	47.0	38.8	42.3	4.2
A6 (positive control)	49.5	45.2	53.2	49.3	4.0	48.2	50.6	50.1	49.7	1.3	37.0	31.6	34.4	34.3	2.7
TCP (medium control)	50.6	52.7	47.3	50.2	2.7	63.9	59.7	54.5	59.4	4.7	54.7	56.3	56.8	55.9	1.1
PFOA (medium control)	54.2	47.6	51.8	51.2	3.3	65.8	60.3	56.7	60.9	4.6	66.1	63.5	58.9	62.8	3.7

Fe(II) (mg/L)

	Day0					Day20					Day45				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6+TCP(1ppm)	0.7	0.8	0.8	0.8	0.1	255.2	285.6	281.2	274.0	16.4	400.4	361.9	321.5	361.3	39.5
A6+TCP+PFOA (1ppm+1ppm)	1.5	1.2	1.7	1.5	0.2	212.5	216.8	226.2	218.5	7.0	348.4	301.9	273.0	307.8	38.1
A6+PFOA (1ppm)	0.6	0.5	0.5	0.5	0.0	203.8	245.2	239.5	229.5	22.5	279.0	303.0	351.3	311.1	36.8
A6 (positive control)	1.6	2.0	1.7	1.8	0.2	297.3	316.4	268.1	293.9	24.3	428.3	417.2	492.5	446.0	40.7
TCP (medium control)	0.0	0.0	0.0	0.0	0.0	1.6	1.6	2.7	2.0	0.6	22.5	24.6	35.6	27.6	7.0
PFOA (medium control)	0.7	0.9	0.9	0.8	0.1	0.6	0.5	1.3	0.8	0.4	9.8	10.8	10.2	10.3	0.5

Fig. III.3 RNA copies of dehalogenase genes during incubations

	<i>dhl_1</i>					<i>dhl_2</i>				
(10 ⁶ /mL)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6+TCP(1ppm)	0.878	0.812	0.878	0.856	0.038	0.232	0.201	0.197	0.210	0.019
A6+TCP+PFOA (1ppm+1ppm)	0.432	0.404	0.458	0.431	0.027	0.137	0.156	0.166	0.153	0.015
A6+PFOA (1ppm)	0.006	0.001	0.008	0.005	0.004	0.013	0.019	0.014	0.015	0.003
A6 (positive control)	0.001	0.004	0.004	0.003	0.002	0.102	0.112	0.125	0.113	0.012
TCP (medium control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PFOA (medium control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	<i>fceA</i>					<i>rdhA</i>				
(10 ⁶ /mL)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
A6+TCP(1ppm)	0.787	0.704	0.774	0.755	0.045	0.021	0.032	0.043	0.032	0.011
A6+TCP+PFOA (1ppm+1ppm)	1.873	1.632	1.639	1.715	0.137	0.589	0.437	0.597	0.541	0.090
A6+PFOA (1ppm)	1.583	1.777	1.553	1.638	0.122	0.793	0.772	0.577	0.714	0.119
A6 (positive control)	0.045	0.051	0.062	0.053	0.009	0.133	0.172	0.124	0.143	0.026
TCP (medium control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PFOA (medium control)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Fig. IV.1 Results of incubations with PFOA as a function of the A6 biomass

PFOA vs. time

	Day0					Day5					Day15					Day30				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
G-1-1	100.5	107.5	96.8	101.6	5.5	96.7	90.5	99.2	95.5	4.5	88.5	88.5	97.5	91.5	5.2	88.5	82.5	94.5	88.5	6.0
G-2-1	101.5	105.5	94.0	100.3	5.8	92.7	94.5	95.5	94.2	1.4	90.6	90.5	89.5	90.2	0.6	88.7	88.6	88.3	88.5	0.2
G-3-1	101.5	99.3	103.5	101.4	2.1	86.4	91.5	100.7	92.9	7.2	92.2	84.7	89.4	88.8	3.8	84.7	92.2	84.7	87.2	4.3
G-4-1	104.1	98.2	103.4	101.9	3.2	92.4	93.1	85.6	90.3	4.1	79.6	87.9	95.9	87.8	8.2	85.9	82.6	89.6	86.0	3.5
A6-autoclaved	102.8	98.2	99.3	100.1	2.4	105.2	100.8	94.7	100.2	5.3	102.9	101.4	96.0	100.1	3.6	101.6	102.9	96.1	100.2	3.6

F⁻ vs. time

	Day0					Day5					Day15					Day30				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
G-1-1	0.00	0.00	0.00	0.00	0.00	0.48	0.55	0.50	0.51	0.04	3.39	3.84	3.85	3.69	0.27	4.71	4.65	4.99	4.78	0.18
G-2-1	0.00	0.00	0.00	0.00	0.00	0.65	0.60	0.80	0.68	0.11	4.61	4.65	4.69	4.65	0.04	5.31	4.74	5.13	5.06	0.29
G-3-1	0.00	0.00	0.00	0.00	0.00	1.95	1.94	2.07	1.98	0.07	4.95	4.91	4.85	4.90	0.05	6.84	6.75	7.03	6.87	0.14
G-4-1	0.00	0.00	0.00	0.00	0.00	5.79	4.83	5.34	5.32	0.48	6.79	6.31	5.82	6.31	0.48	7.03	6.96	6.93	6.97	0.06
A6-autoclaved	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

NH₄⁺ vs. time

	Day0					Day5					Day15					Day30				
(mg/L)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
G-1-1	53.3	50.5	55.5	53.1	2.5	50.3	46.5	47.6	48.1	2.0	41.1	41.2	40.7	41.0	0.3	39.4	38.3	42.0	39.9	1.9
G-2-1	54.0	53.2	57.7	55.0	2.4	40.0	45.1	46.0	43.7	3.2	40.7	38.7	35.5	38.3	2.6	31.8	32.6	32.2	32.2	0.4
G-3-1	55.3	54.7	56.0	55.3	0.6	42.7	40.7	45.2	42.9	2.3	38.1	37.7	39.8	38.5	1.1	31.8	32.3	32.9	32.3	0.6
G-4-1	50.1	53.0	55.8	53.0	2.9	40.4	40.1	42.3	40.9	1.2	31.5	34.1	33.1	32.9	1.3	30.6	29.3	29.6	29.8	0.7
A6-autoclaved	51.8	55.5	48.5	51.9	3.5	51.8	51.8	55.1	52.9	1.9	54.3	52.0	50.1	52.1	2.1	53.4	51.3	48.3	51.0	2.6

rdhA vs. time

	Day0					Day5					Day15					Day30				
(10 ⁶ /mL)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
G-1-1	0.003	0.002	0.002	0.002	0.000	0.068	0.074	0.073	0.072	0.004	2.447	3.314	4.371	3.377	0.964	2.952	2.441	2.937	2.776	0.291
G-2-1	0.047	0.043	0.039	0.043	0.004	0.176	0.186	0.257	0.207	0.044	2.405	3.259	1.575	2.413	0.842	2.480	2.687	2.078	2.415	0.310
G-3-1	0.259	0.294	0.311	0.288	0.026	2.269	2.050	1.960	2.093	0.159	2.167	3.151	3.302	2.873	0.616	3.473	3.158	3.066	3.232	0.214
G-4-1	0.856	0.933	0.901	0.897	0.039	2.997	2.924	3.046	2.989	0.061	3.089	2.988	3.469	3.182	0.254	3.468	3.458	3.505	3.477	0.025
A6-autoclaved	0.004	0.004	0.004	0.004	0.000	0.002	0.002	0.002	0.002	0.000	0.008	0.009	0.011	0.009	0.001	0.010	0.008	0.012	0.010	0.002

fceA vs. time

	Day0					Day5					Day15					Day30				
(10 ⁶ /mL)	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD	M1	M2	M3	Mean	SD
G-1-1	0.001	0.002	0.001	0.001	0.000	0.035	0.037	0.026	0.033	0.006	0.583	0.572	0.610	0.588	0.019	0.692	0.641	0.632	0.655	0.032
G-2-1	0.063	0.040	0.047	0.050	0.011	0.275	0.297	0.303	0.292	0.015	0.726	0.826	0.737	0.763	0.055	1.188	1.155	1.218	1.187	0.031
G-3-1	0.163	0.183	0.216	0.187	0.027	0.916	0.931	0.949	0.932	0.017	2.130	2.363	1.949	2.147	0.208	2.169	2.182	2.209	2.187	0.020
G-4-1	0.660	0.694	0.709	0.688	0.025	1.661	1.487	1.455	1.534	0.111	3.424	3.160	3.122	3.235	0.164	3.074	3.034	3.077	3.062	0.024
A6-autoclaved	0.002	0.002	0.002	0.002	0.000	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.003	0.003	0.003	0.000

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