Defluorination of Perfluorooctanoic Acid (PFOA), Perfluorooctanesulfonic Acid (PFOS), and other Perfluoroalkyl Acids (PFAAs) by *Acidimicrobium* sp. strain A6

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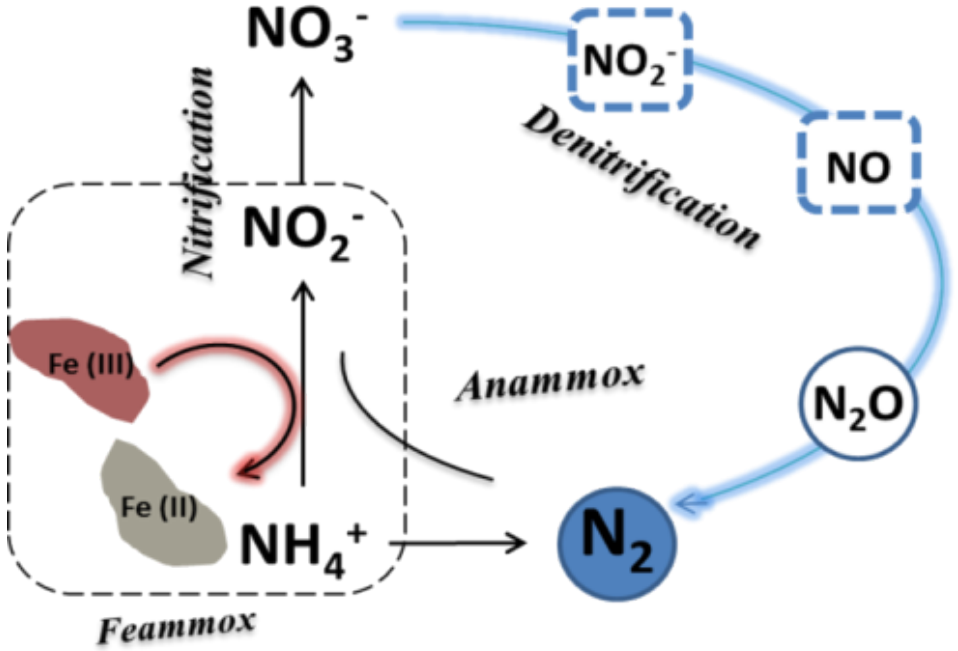
Oxidation of NH_4^+ under Fe-reducing conditions (Feammox)



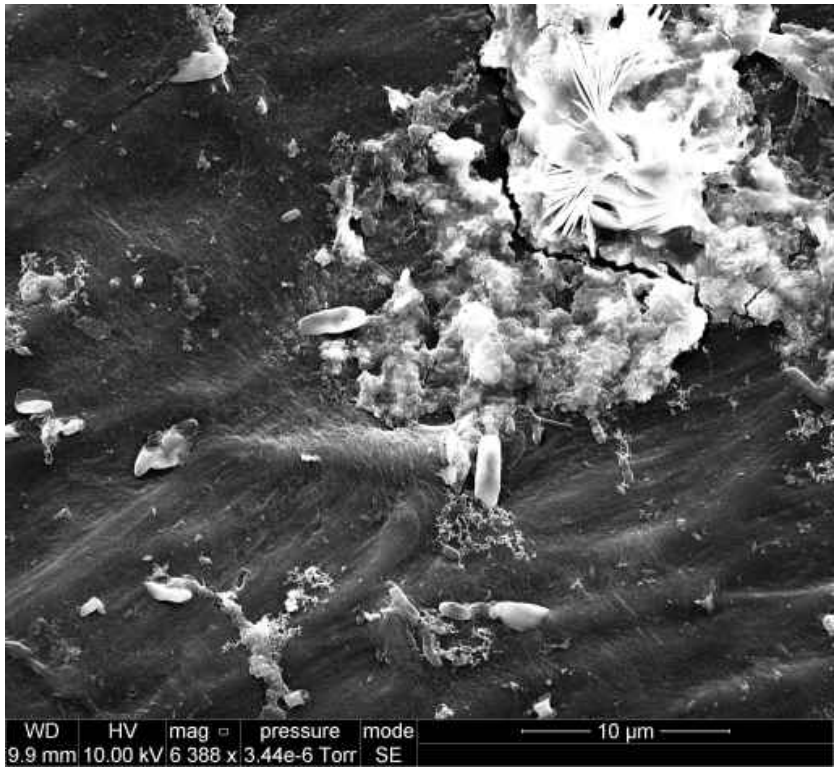
$$3Fe_2O_3 \cdot 0.5H_2O + 10H^+ + NH_4^+ \rightarrow 6Fe^{2+} + 8.5H_2O + NO_2^-$$

anaerobic
autotrophic

$$\Delta G_r \leq -145.08\, kJ\ mol^{-1}$$

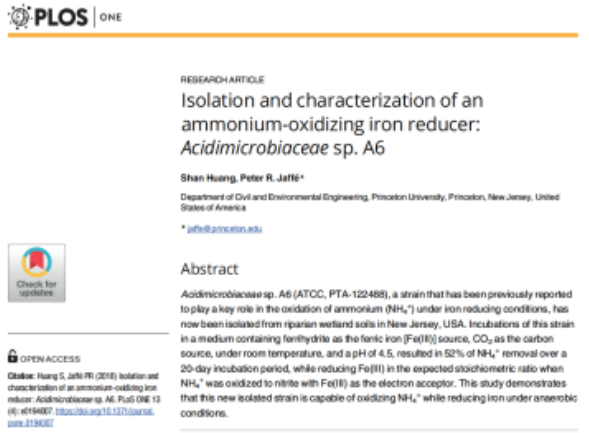
Clement *et al*. (2005); Shrestha *et al*. (2009), Sawayama (2006); Yang *et al*. (2012); Huang *et al* (2016), many recent papers on Feammox …

Acidimicrobium sp. Strain A6
(ATCC, PTA-122488)



A6 can also use NH_4^+ and H_2 as electron donor
A6 is an autotroph
A6's doubling time ~ 10 – 14 days
A6 is electrogenic

Not a "domesticated" organism. Difficult to grow. Does not take off in many incubations…


PLOS ONE

RESEARCH ARTICLE

Isolation and characterization of an ammonium-oxidizing iron reducer: *Acidimicrobiaceae* sp. A6

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OPEN ACCESS

Abstract

Acidimicrobiaceae sp. A6 (ATCC, PTA-122488), a strain that has been previously reported to play a key role in the oxidation of ammonium (NH_4^+) under iron reducing conditions, has now been isolated from riparian wetland soils in New Jersey, USA. Incubations of this strain in a medium containing ferrihydrite as the ferric iron [Fe(III)] source, CO_2 as the carbon source, under room temperature, and a pH of 4.5, resulted in 52% of NH_4^+ removal over a 20-day incubation period, while reducing Fe(III) in the expected stoichiometric ratio when NH_4^+ was oxidized to nitrite with Fe(III) as the electron acceptor. This study demonstrates that this new isolated strain is capable of oxidizing NH_4^+ while reducing iron under anaerobic conditions.

Cells are rod-shaped, 1.5–3 mm long by 0.5 mm wide. Gram-positive.

Current data suggests A6 only uses solid Fe(III) phases as an electron acceptor.

The pure strain needs to be re-cultured ~ every 2 to 3 months.

After growing on H_2, A6 no longer oxidizes NH_4^+.

Putative dehalogenase genes in the A6 genome

Acidimicrobiaceae sp. A6's genome contains multiple dehalogenase genes (GenBank accession numbers MK358459-MK358462), including a gene for a:

Reductive dehalogenase homolog (*rdhA*),

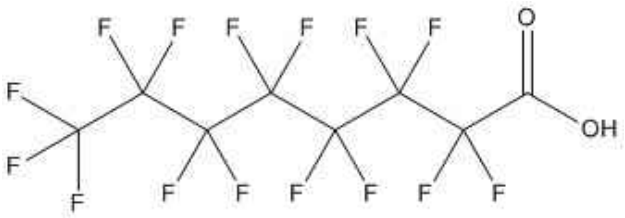
Fluoroacetate dehalogenase homolog (*FceA*),

Two putative haloacid dehalogenase genes (*dhl_1* and *dhl_2*).

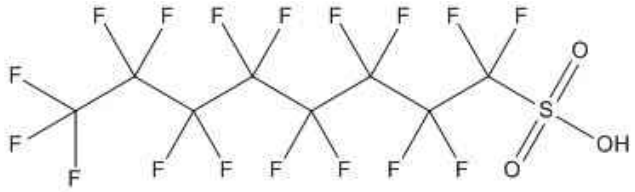
A similar *rdhA is* present in *Acidimicrobiaceae* bacterium TMED77, discovered in Mediterranean Sea sediments, 2017.

Defluorination of PFAS by *Acidimicrobium* sp. Strain A6

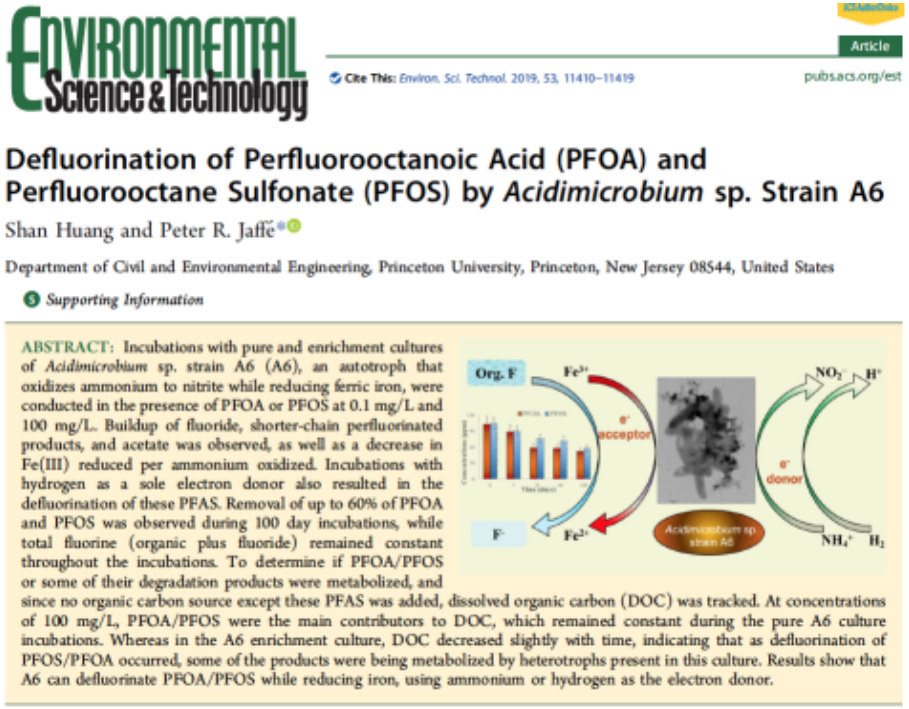
- Incubation with PFOA and/or PFOS at 0.1, 1.0, 10, and 100 mg/l
- NH_4^+ or H_2 as electron donor. Ferrihydrite as the Fe(III) electron acceptor
- About 40- 50% of PFOA and PFOS removal in 100 days by A6 enrichment culture.
- Production of shorter carbon chain perfluoroalkyl acids (PFAAs) was observed



Perfluorooctanoic acid (PFOA)



Perfluorooctane sulfonic acid (PFOS)



ENVIRONMENTAL Science & Technology

Article

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

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Supporting Information

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

Further questions addressed here

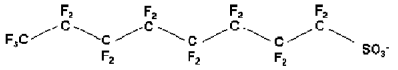
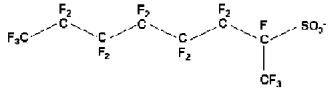
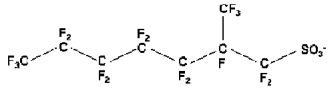
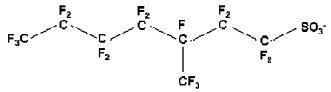
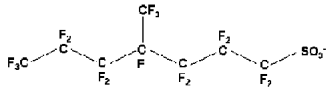
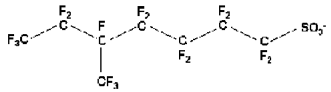
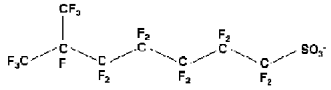
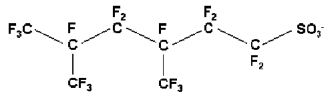
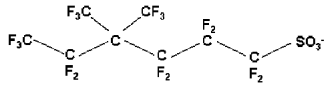
Legend
Complete: ✓
Ongoing: O

			Status
1	Branched vs. linear isomer defluorination		✓
2	NH_4^+ vs. H_2 as electron donor for defluorination		✓
3	Defluorination of intermediate products (smaller PFAAs)		✓
4	Can defluorination be enhanced via frequent addition of amendment and spent solution withdrawal?		✓
5	Identify putative functional gene		O

Defluorination of branched vs. linear PFOA and PFOS

Biodefluorination of PFOA and PFOS isomers that are: linear, have a single branch, and two branches.

- Fe(III)+NH_4+ 0.1 ppm isomers.
- Fe(III)+H_2 + 0.1 ppm isomers.

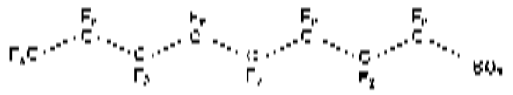
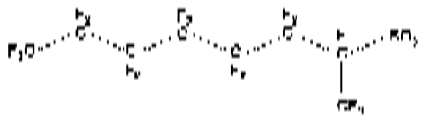
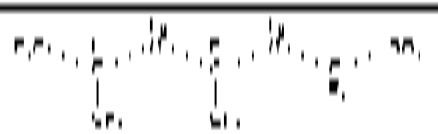
Chemical Name	Abbreviation	PFOS Isomer Chemical Structure
n-perfluoro-1-octanesulfonate	L-PFOS	
perfluoro-1-methyl-heptanesulfonate	P1MHpS	
perfluoro-2-methyl-heptanesulfonate	P2MHpS	
perfluoro-3-methyl-heptanesulfonate	P3MHpS	
perfluoro-4-methyl-heptanesulfonate	P4MHpS	
perfluoro-5-methyl-heptanesulfonate	P5MHpS	
perfluor-6-methyl-heptanesulfonate	P6MHpS	
perfluoro-3,5-dimethyl-hexanesulfonate	P35DMHxS	
perfluoro-4,4-dimethyl-hexanesulfonate	P44DMHxS	

Isomer source; Wellington Laboratories, LLC

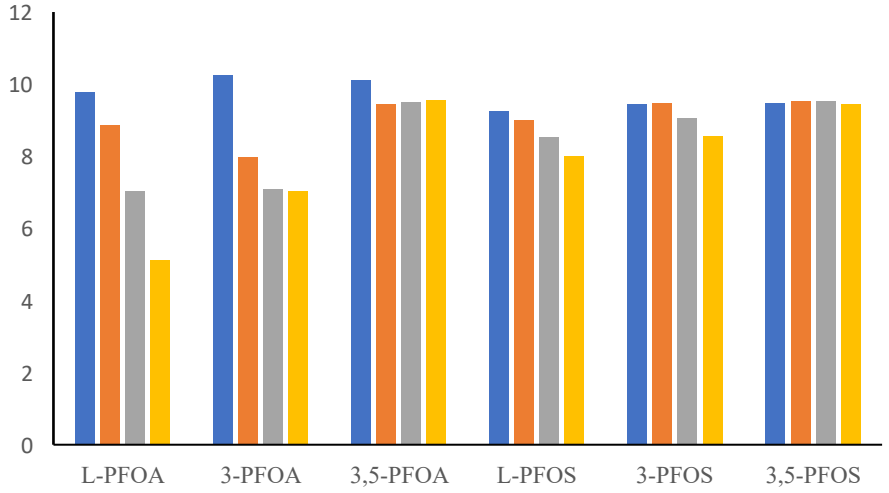
Defluorination of branched vs. linear PFOA and PFOS with NH_4^+ as electron donor

Goal: Assess occurrence of defluorination for branched vs. linear PFOA and PFOS.

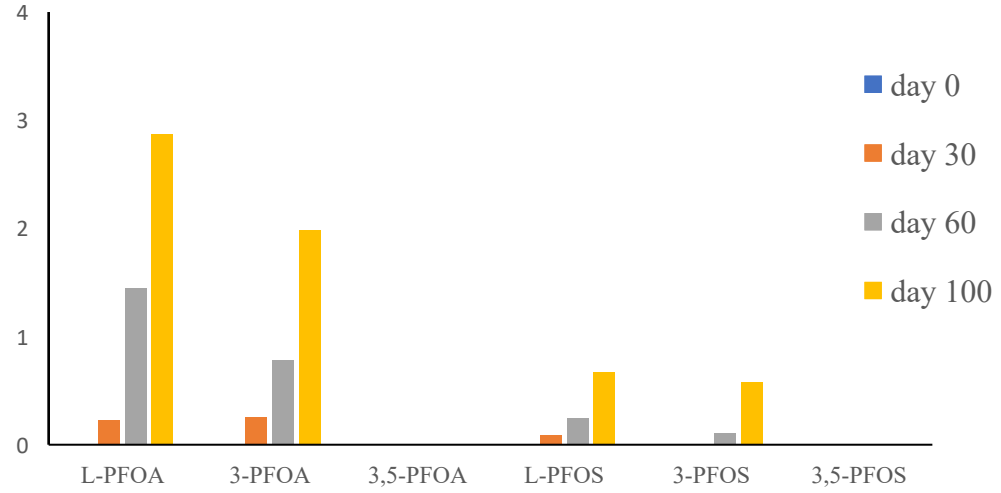
Result: Linear as well as single branched PFOA and PFOS isomers are more readily defluorinated.

Chemical Name	Abbreviation	PFOS Isomer Chemical Structure
n-perfluoro-1-octanesulfonate	L-PFOS	
perfluoro-1-methyl-heptanesulfonate	P1MHpS	
perfluoro-3,5-dimethyl-hexanesulfonate	P35DMHxS	

Concentrations of PFAS (mg/L)



Concentrations of F^- (mg/L)



- Fe(III)+NH_4+ 0.1 ppm isomers.
- Fe(III)+H_2 + 0.1 ppm isomers.

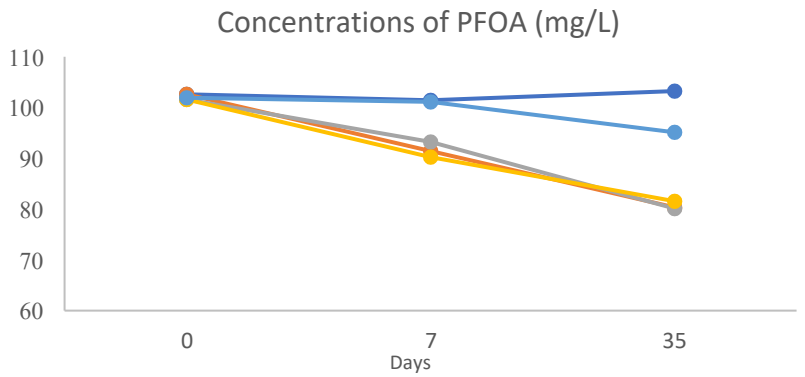
For H_2 as electron donor, incubations were only for 25 days, but results are similar.

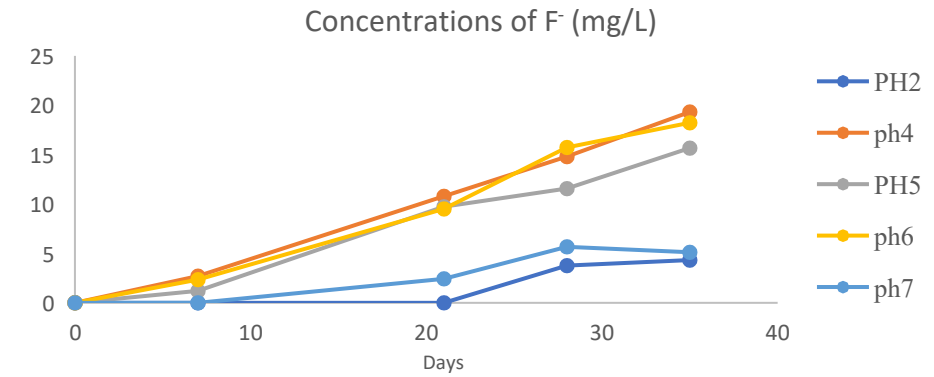
Task 2: H_2 as electron donor and effects of pH

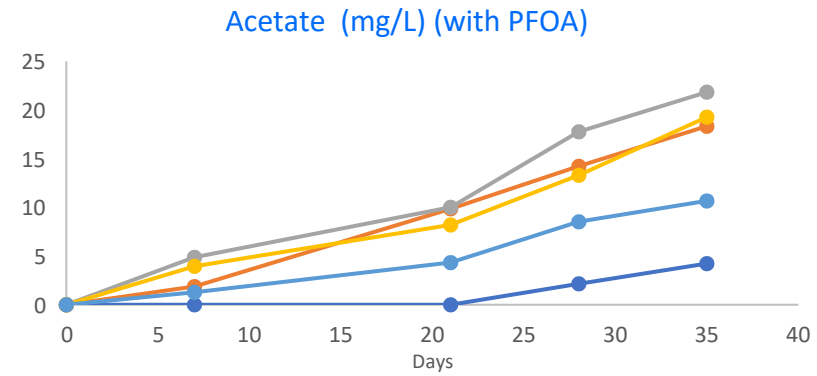
Goal: Assess occurrence of defluorination with alternative electron donors (H_2 vs. NH_4^+).

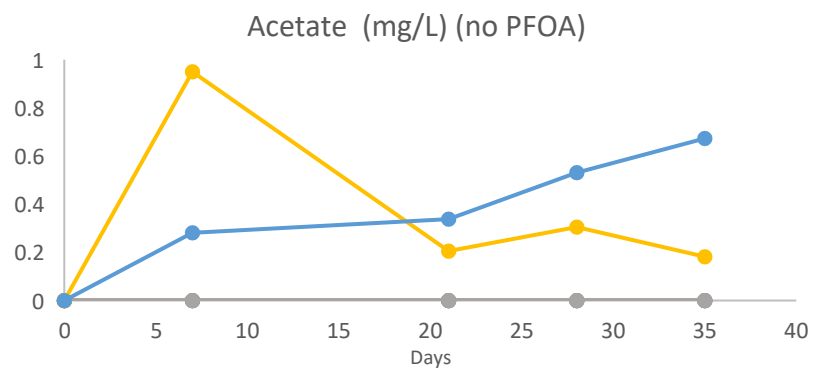
Result: H_2 can be used an alternative electron donor during defluorination of PFOA at various pH.

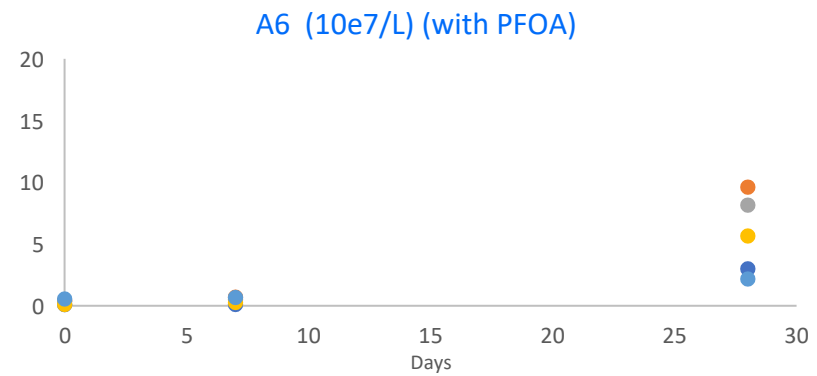
Effect of pH is similar for NH_4^+ and for H_2 but seems to occur at slightly higher pH values.

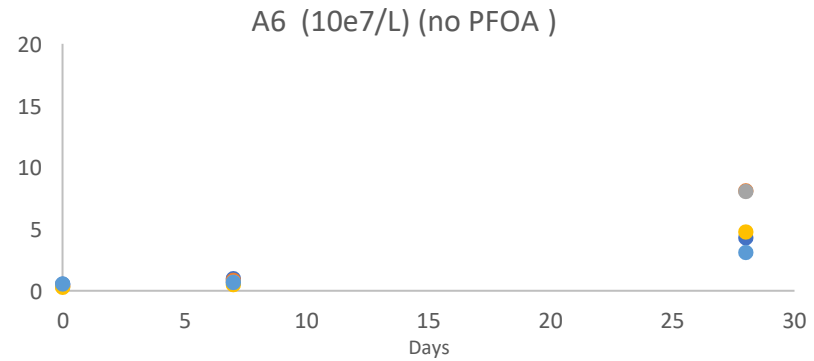












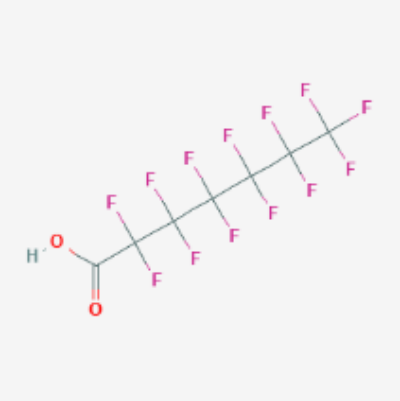
Task 3: Defluorination of intermediate degradation products (shorter C chain PFAAs)

PFBA	Perfluorobutanoic acid, C_3F_7COOH
PFBS	Perfluorobutane sulfonate, $C_4F_9SO_3^-$
FOSA	Perfluorooctane sulfonamide, $C_8F_{17}SO_2NH_2$
PFOA	Perfluorooctanoic acid, $C_7F_{15}CO_2H$
PFOS	Perfluorooctane sulfonate, $C_8F_{17}SO_3^-$
PFHpS	Perfluoroheptane sulfonate, $C_7F_{15}SO_3^-$
PFHxS	Perfluorohexane sulfonate, $C_6F_{13}SO_3^-$
PFPeA	Perfluoropentanoic acid, $CF_3(CF_2)_3COOH$
PFHpA	Perfluoroheptanoic acid, $C_6F_{13}CO_2H$

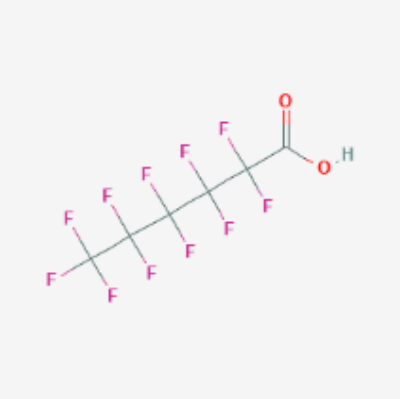
Defluorination of perfluoro -hepta-, -hexa, -penta, and butanoic acid was investigated with a series of batch incubations.

- Fe(III)+ NH_4^+ & 10 ppm PFAS
- Fe(III)+H_2 & 10 ppm PFAS
- under anaerobic condition.

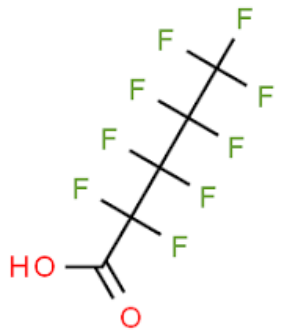
PFHpA



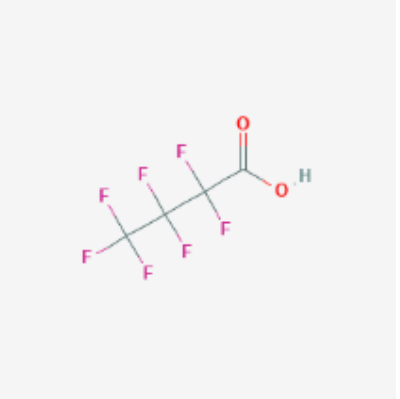
PFHxA



PFPeA

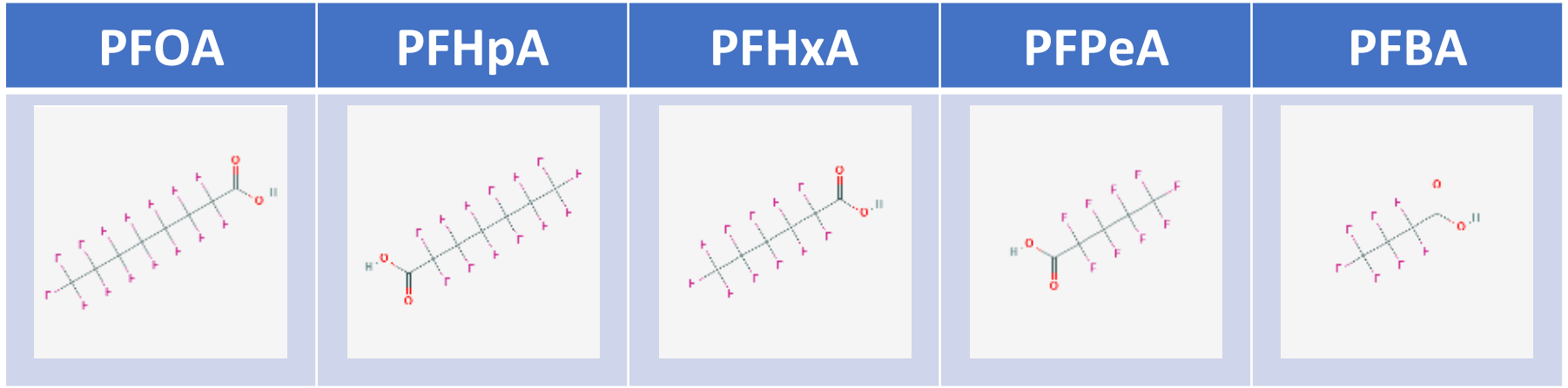


PFBA



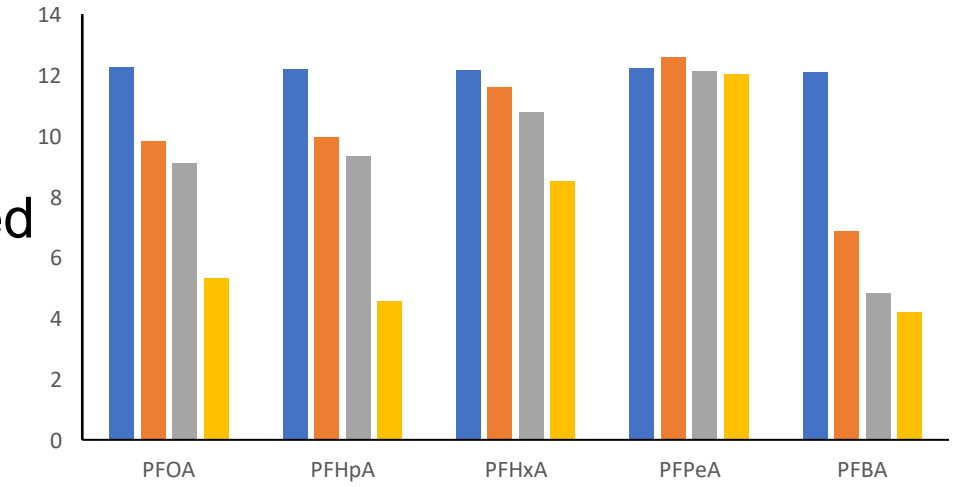
Defluorination of intermediate degradation products (NH_4^+ as electron donor)

Goal: Assess occurrence of defluorination of intermediate degradation products, such as PFHpA, PFHxA, PFPeA, and PFBA.

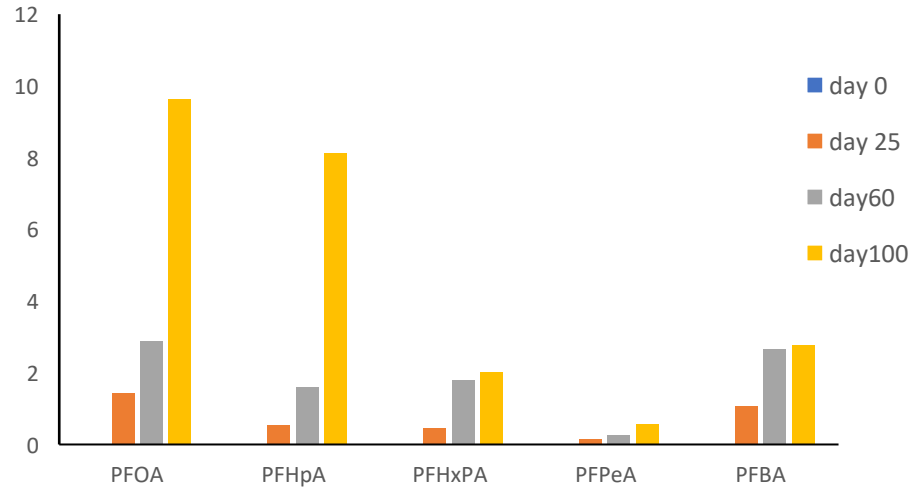


Results: All intermediates were defluorinated except PFPeA. PFPeA analyzed via NMR for isomer composition (linear vs. branched), but results did not suggest high presence of branched isomers.

Concentrations of PFAS (mg/L)



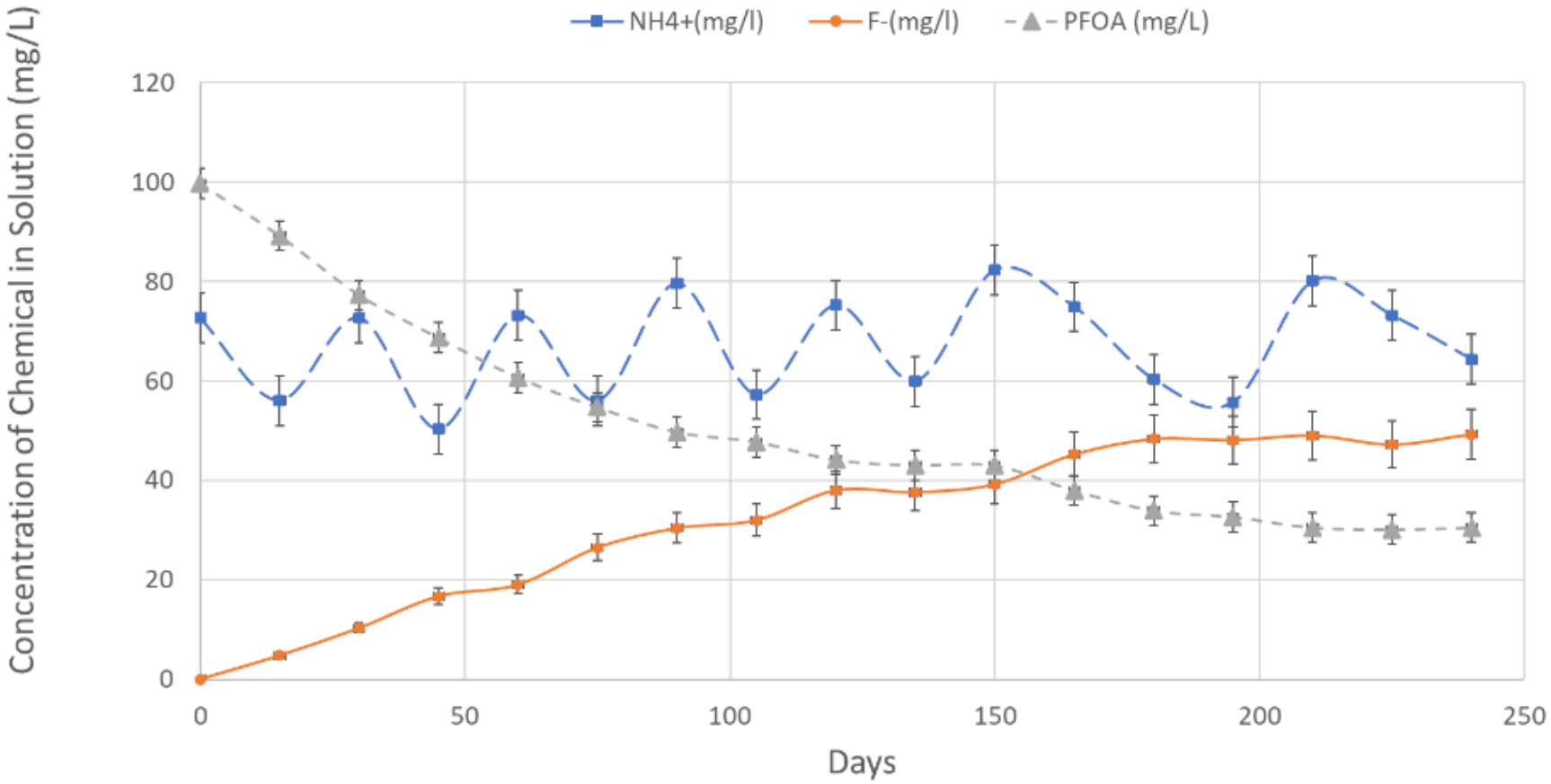
Concentrations of F^- (mg/L)



Task 4: Enhanced defluorination in batch incubations by rejuvenating th medium

Goal: Assess ability to enhance defluorination of PFAS. Prior studies resulted in diminishing returns at ~50% reduction in initial PFOS or PFOA concentration.

Results: Periodic exchange of the growth medium enhanced defluorination – ~70% reduction in initial PFOA concentration.

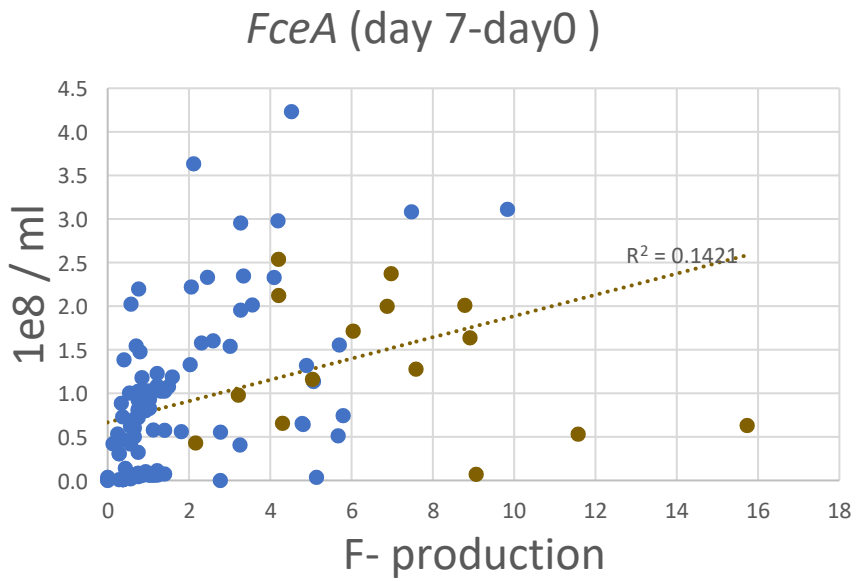


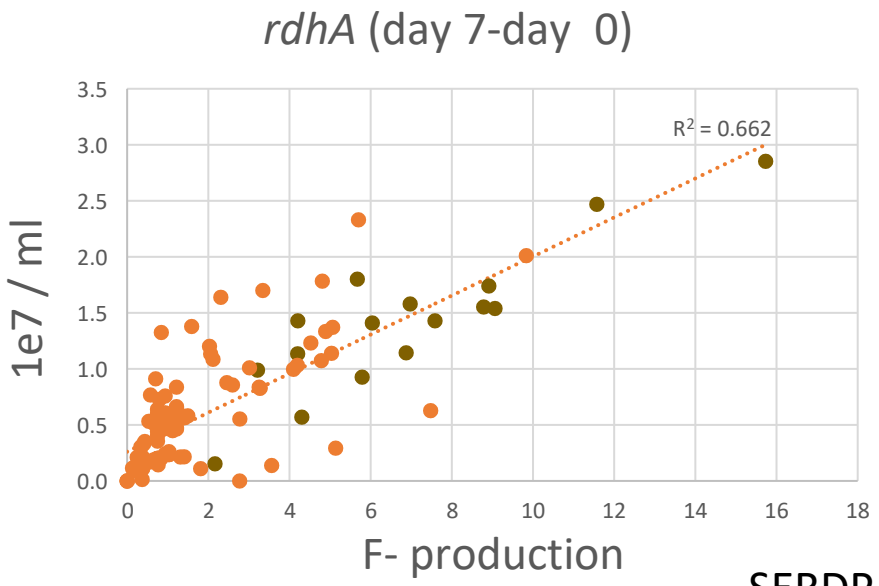
Task 5: Expression of putative functional genes during the incubations

Goal: Assess expression of reductive dehalogenase (*rdhA*) gene and fluoroacetate dehalognase (*fceA*) gene during defluorination to suggest functional gene identification for future mechanism study and biomarker development.

Results: Stronger correlation between the expression of *rdhA* and F^- produced. Findings were supplemented with collaboration from UMN.

The haloacid dehalogenase genes are not expressed during defluorination but are expressed during dichlorination (TCP)





Summary of Key Findings

- **Task 1:** Linear and single branched PFOA and PFOS monomers can be defluorinated. PFOA/PFOS with multiple branches are more resistant to defluorination within the timeframe and design of these incubations.
- **Task 2:** When H_2 is the electron donor as opposed to NH_4^+:
  - defluorination can be achieved at slightly higher pH values,
  - *rdhA* expression is increased,
  - and *rdhA* expression exhibits a stronger correlation with F^- produced.
- **Task 3:** Smaller carbon chain PFAAs are also degradable, except PFPeA, which is being further investigated.
- **Task 4:** Periodic replenishment of growth medium and headspace CO_2, which also results in adjustment of pH, and withdrawal of Feammox products [Fe(II) and NO_2^-] can enhance the defluorination of PFAS and Feammox activity.
- **Task 5:** *rdhA* is a key gene implicated in PFAS defluorination, given the strong correlation between its expression and F- produced.

Acknowledgements

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- ExxonMobil Environmental and Property Solutions Company
- SERDP