



Effects of Coating Iron Phases with Polyacrylic Acids on the Ammonium Oxidation/PFAS Defluorination by *Acidimicrobium* sp. A6 (A6)

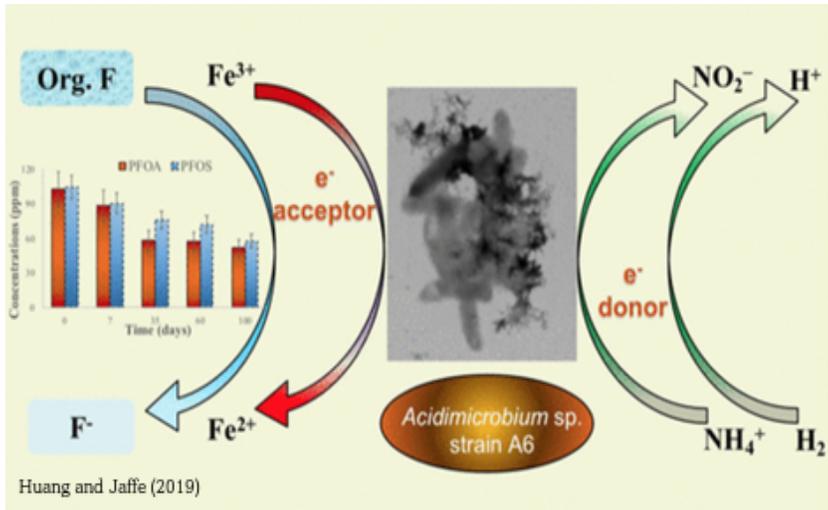
Princeton University

Jinhee Park, Shan Huang, Bruce E. Koel, and Peter R. Jaffe

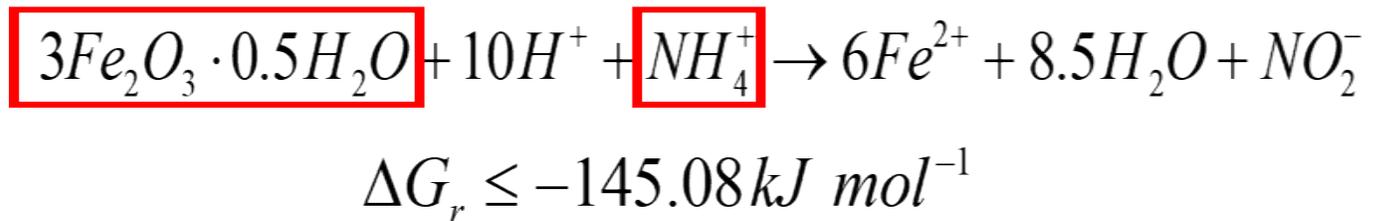
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PFAS Biodegradation

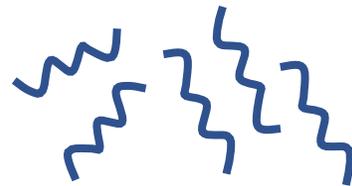
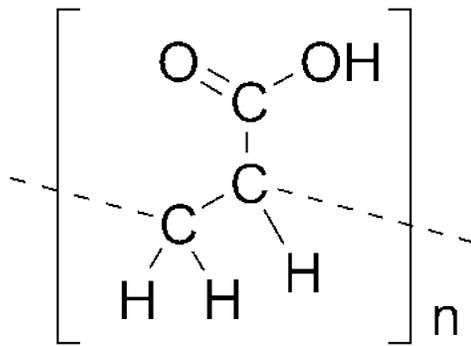


- Huang and Jaffe (2019) found that Feammox bacterium *Acidimicrobium* sp. Strain A6 (A6) defluorinate PFAS, including the more stable perfluoroalkyl acids (PFAAs).



- To implement active bioremediation using A6, NH_4^+ (or H_2) and Fe(III)) phases need to be supplied and spatially distributed.
- ❖ However, supplying and distributing solid iron oxide in sediments or aquifers is challenging as **positively charged iron oxides** are typically retained by negatively charged sub-surface components.

Use of the Polyacrylic acid (PAA) as the Surface Modifier for Ferrihydrite



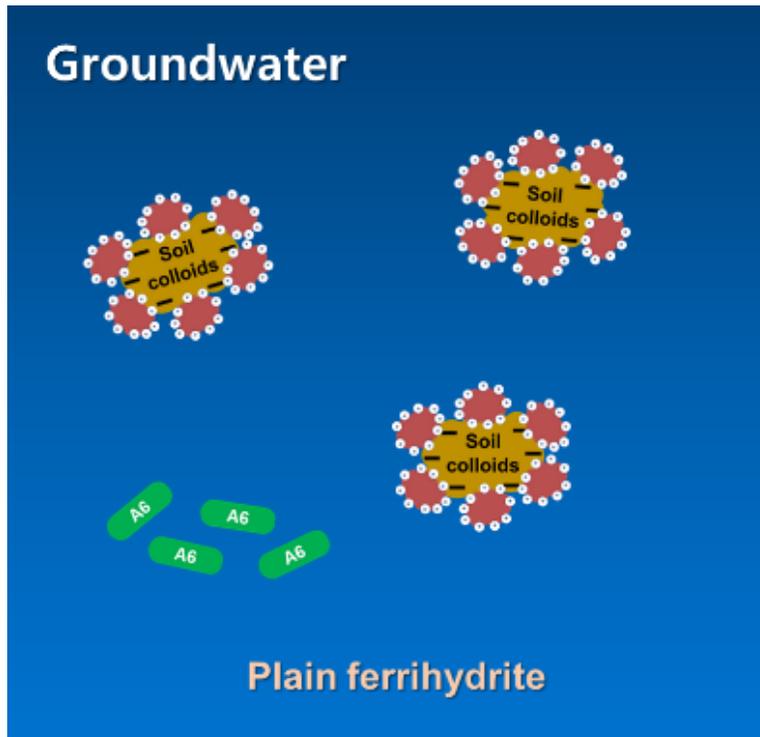
PAA chains

- Polyacrylic acids (PAAs) can be used as the stabilizer for ferrihydrite ($\text{Fe}_2\text{O}_3 \cdot 0.5\text{H}_2\text{O}$).
- PAAs are nontoxic, widely used in consumer products and biomedical applications, and available in various molecular weights.
- Our previous showed that PAA coating on ferrihydrite changed the zeta potential of the particle from **positive** to **negative**, which enhanced transport in soil columns.
- PAA-coated ferrihydrite retained iron reduction by *Goebacter sulfurreducens* compared to bare ferrihydrite.

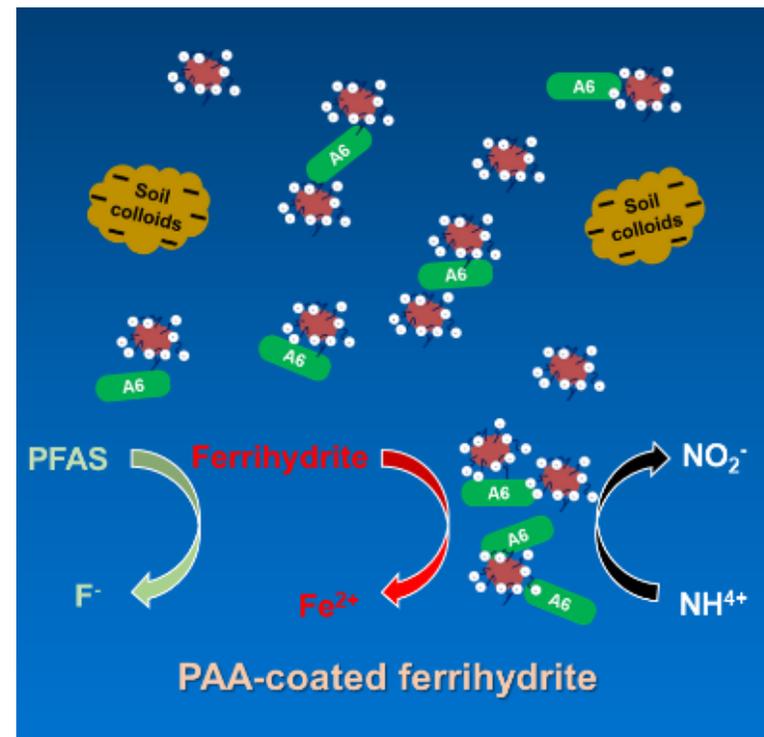
Q. Can A6 utilize PAA-coated ferrihydrite as an electron acceptor?

Primary Goals

- ❖ A schematic of A6-Mediated PFAS Degradation using PAA-Coated Ferrihydrite in Aquifers



VS.



- (1) To determine the optimal PAA coating conditions for ferrihydrite in an A6 growth medium
- (2) To investigate the effect of PAA-coated ferrihydrite on A6 activities

Optimizing PAA Coating Conditions for Ferrihydrite in an A6 Growth Medium

(1) Ferrihydrite coating with different molecular weight of PAAs in an A6 growth medium

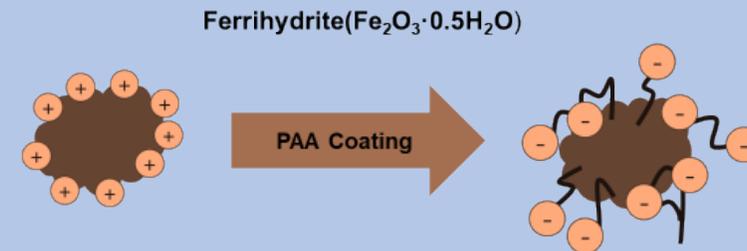
- ① Prepared 2.1K-PAA, 6K-PAA, 2540K-PAA, and 450K-PAA.
- ② Mixed a suspension of ferrihydrite and the PAA solution to give a proper solid ratio of PAA: ferrihydrite.



A6 growth medium	Conc. (mM)
NH_4Cl	3.82
$(\text{NH}_4)_2\text{SO}_4$	0.59
NaHCO_3	0.24
KHCO_3	0.71
KH_2PO_4	0.08
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.41
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.41

Zeta Potential (ZP)

- A key indicator of the stability of the dispersion, with a **higher absolute value** indicating greater repulsion between particles and greater stability.

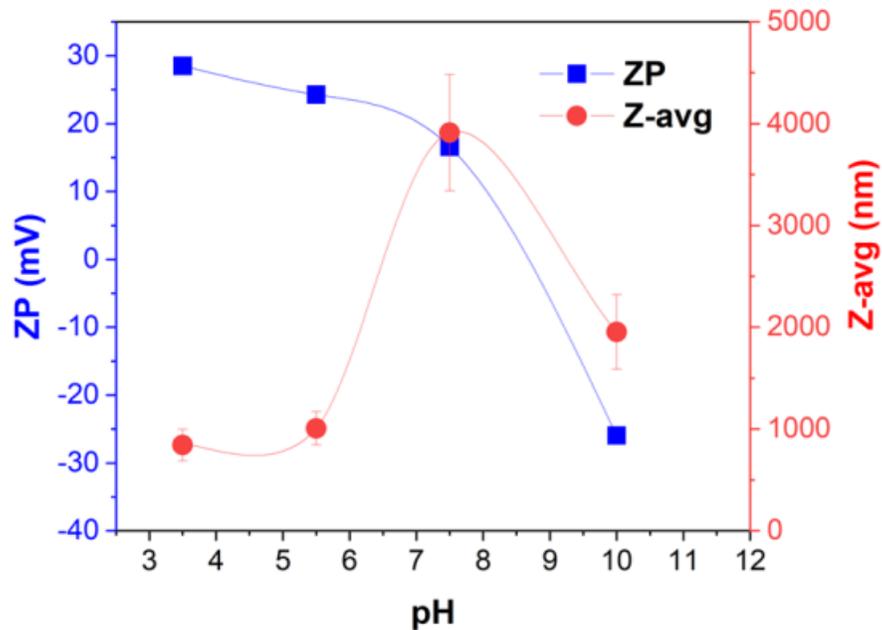


(2) Measurements

- ① PAA sorption (Total Organic Carbon; TOC and UV-vis spectrometry)
- ② Hydrodynamic Diameter (Dynamic Light Scanning; DLS)
- ③ Zeta Potential (Electrophoretic Lights Scanning; ELS)
- ④ Surface Morphology (Environmental Scanning Electron Microscopy; ESEM)

Effect of pH on Particle Stability

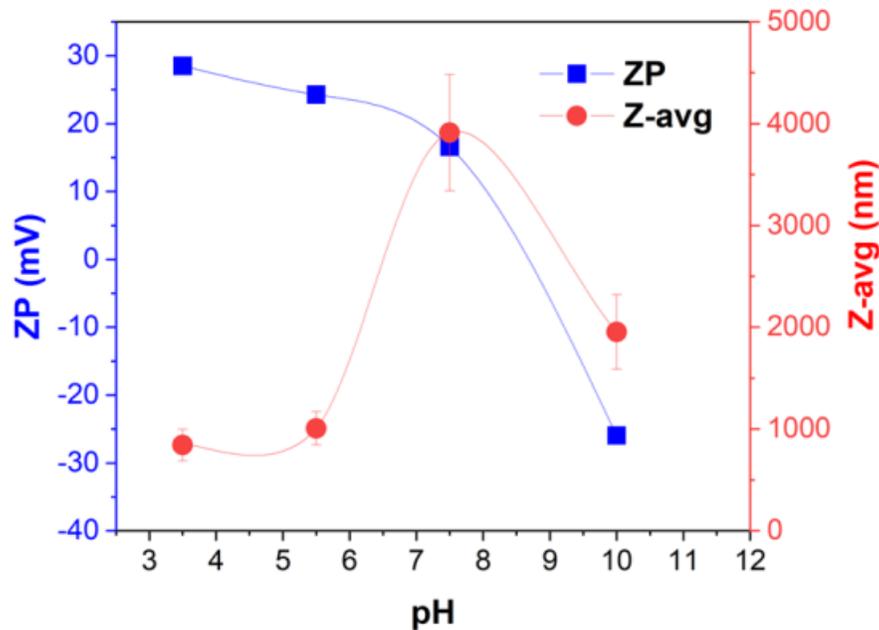
(1) Bare ferrihydrite



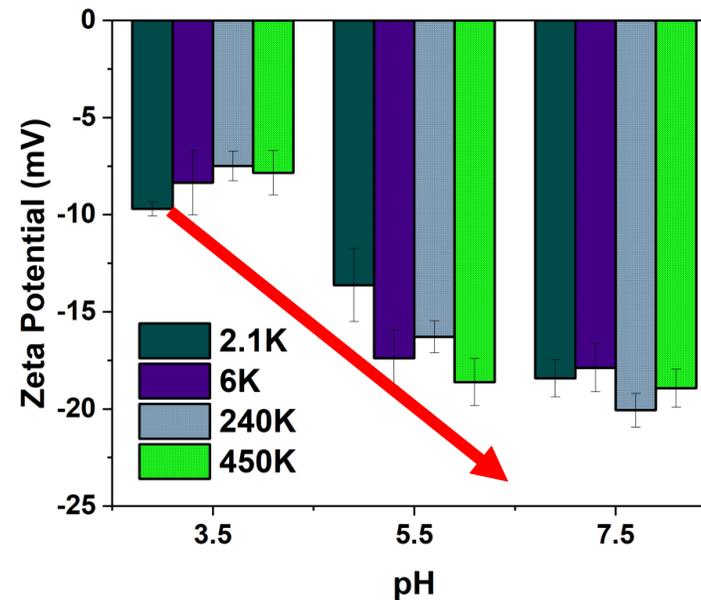
- **pH of A6 growth medium** affects zeta potential and particle diameter of bare ferrihydrite.
- Bare ferrihydrite particles show **positive zeta potentials** below pH_{iep} ($\text{pH} \sim 8$).
- Increasing pH leads to aggregation and decreased colloidal stability.

Effect of pH on Particle Stability

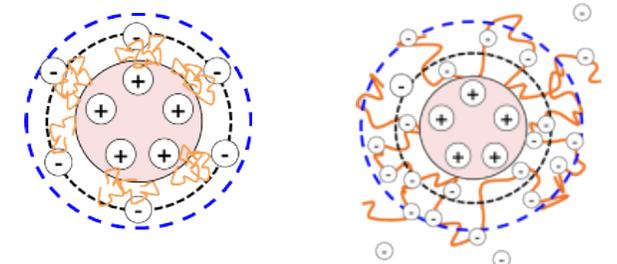
(1) Bare ferrihydrite



(2) PAA-coated ferrihydrite (1/10 wt%)



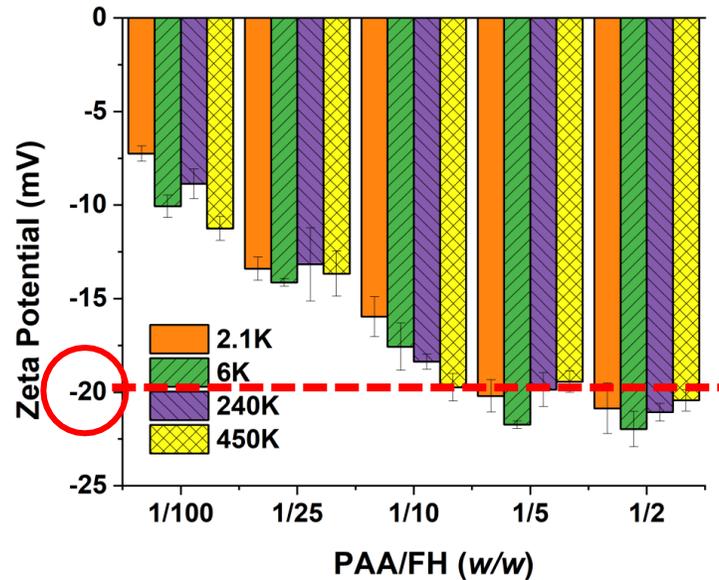
Dissociation of COOH groups



--- Stern plane
- - - Slipping plane

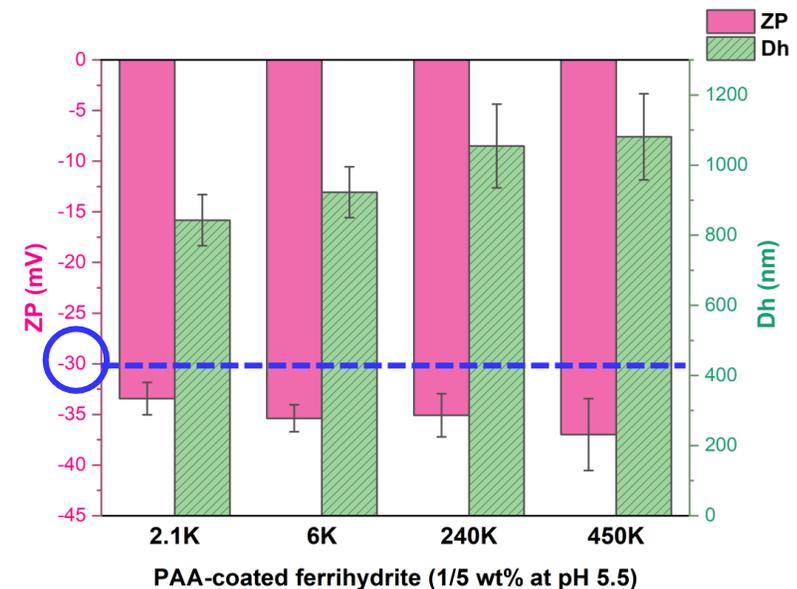
- Behavior of PAA-coated ferrihydrite was investigated at pH 3.5 to pH 7.5.
- At a ratio of 1:10, PAA-coated ferrihydrite particles exhibit negative zeta potential.
- Polymer adsorption and dissociation of COOH groups contribute to the change in zeta potential.

Effect of PAA loadings on Particle Stability

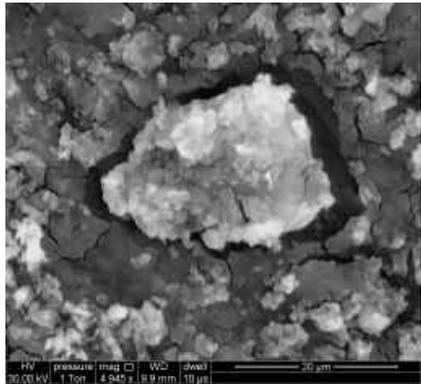


- A moderate colloidal stability is obtained at a PAA:ferrihydrite **mass ratio of 1:5** in the A6 growth medium (pH 5.5).
- In the A6-growth medium, adjusting the Ca^{2+} concentration to 0.1 mM resulted in **highly stable colloids** with negative zeta potentials lower than -30 mV for all PAA-coated particles..

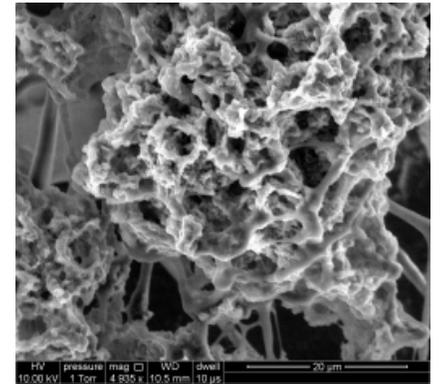
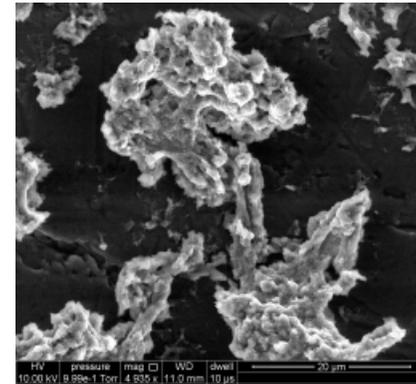
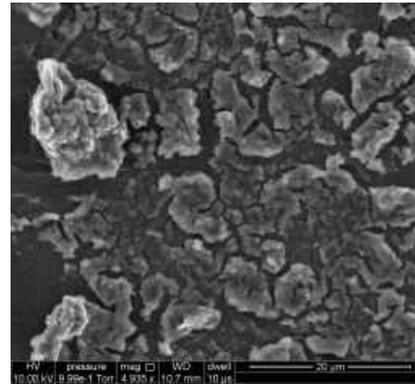
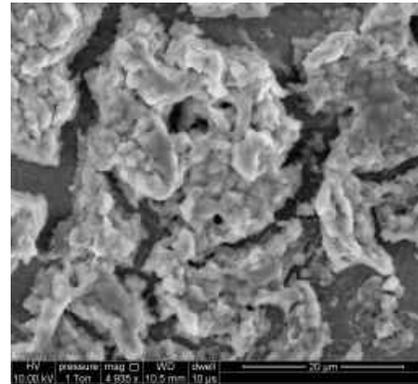
Zeta Potential (mV)	Stability Behavior
$\pm 0-10$	Rapid coagulation or flocculation
$\pm 10-20$	Relatively stable
$\pm 20-30$	Moderately stable
$> \pm 30$	Highly stable



ESEM Images of Bare vs. PAA-coated Ferrihydrite



vs.



Bare ferrihydrite

2.1K-ferrihydrite

6K-ferrihydrite

240K-ferrihydrite

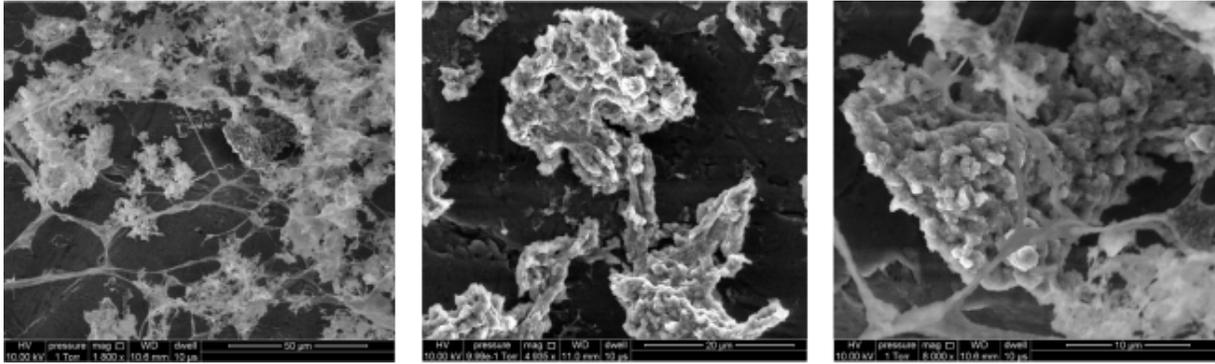
450K-ferrihydrite

Increasing the molecular weight of PAA

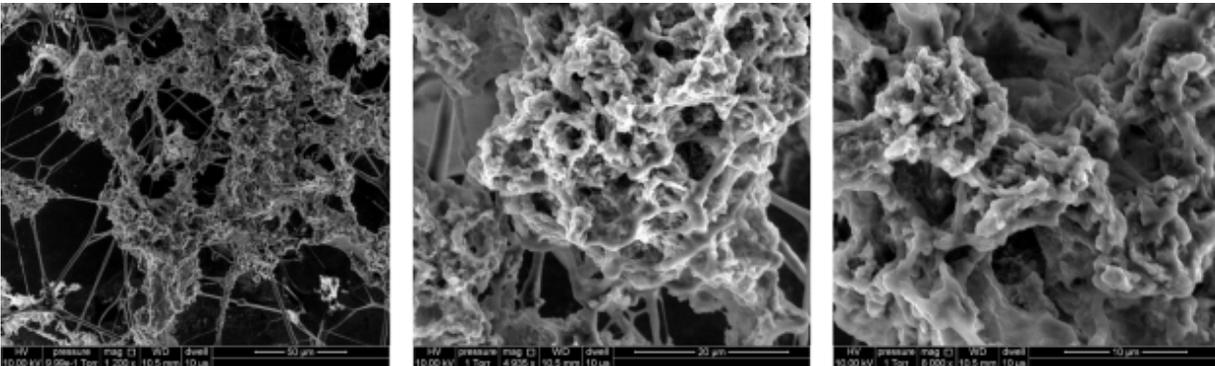
- The images of PAA-coated ferrihydrite demonstrate that PAA is incorporated into ferrihydrite, forming flocs that have porosity on the surface of the aggregates.
- The 240K and 450K-coated ferrihydrite show interconnected structures of polymer and ferrihydrite aggregates, forming a three-dimensional hydrogel network.

PAA Cross-linking in the A6 Growth Medium (without A6)

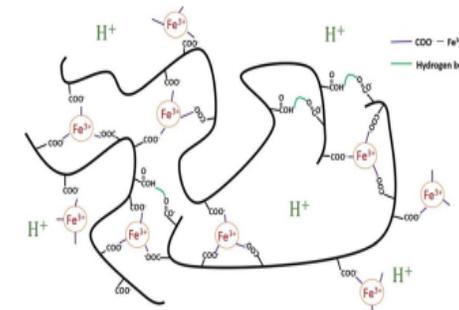
240K-ferrihydrate



450K-ferrihydrate

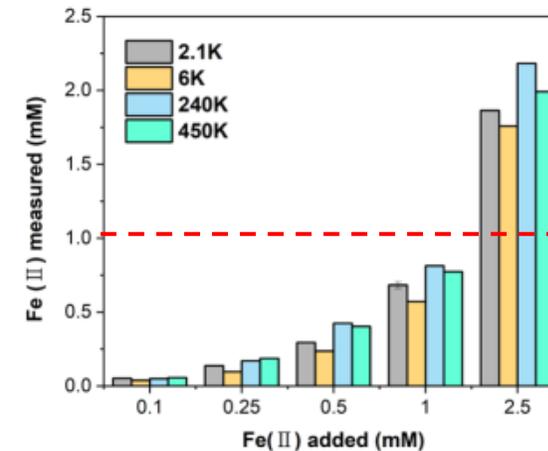


Ionic + chemical cross-linking generates PAA hydrogels



(<https://doi.org/10.1039/C6TA01441K>)

Fe(II)-PAA complex



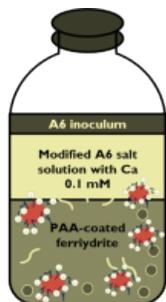
Impact of PAA-Coated Ferrihydrite on A6 Activity

(1) Feammox incubations in the presence of 1 mg/L of PFOA

- ① Prepared A6-enrichment cultures incubations using the modified A6 growth medium at pH 5.5.
- ② Added bare ferrihydrite (control) or PAA-coated ferrihydrite as the electron acceptor.



A6-enrichment
cultures inoculation



Incubations for
50 days

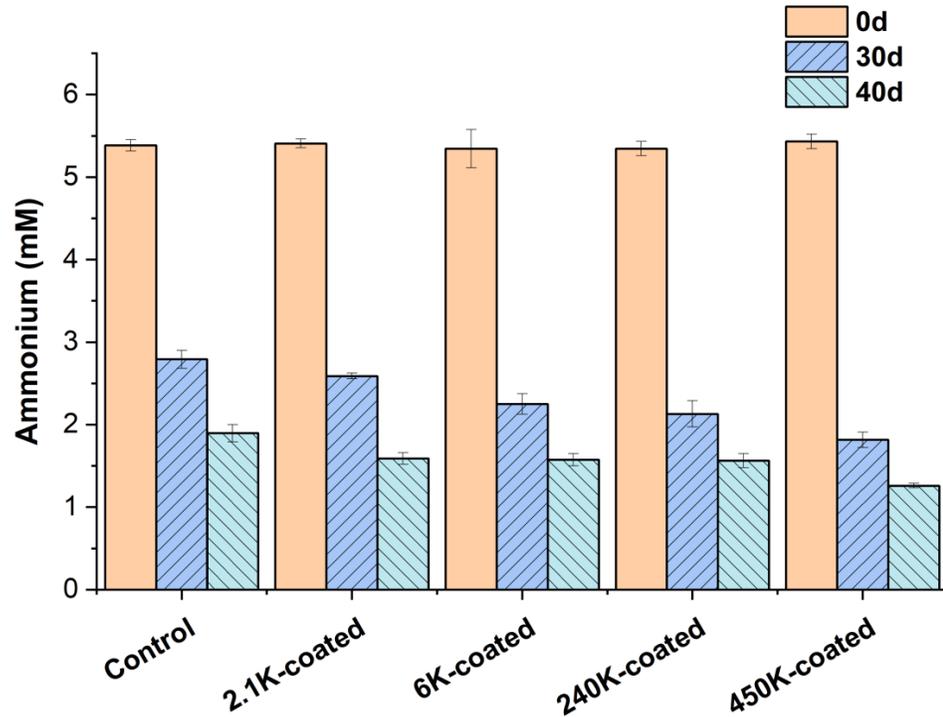
- *Ammonium removal*
- *Charge transfer resistance*
- *PFOA decrease*
- *Fluoride production*
- *Short-chain PFAS observation*
- *A6 copy numbers*
- *Microbial community structure*

A6 growth medium	Conc. (mM)
NH ₄ Cl	3.82
(NH ₄) ₂ SO ₄	0.59
NaHCO ₃	0.24
KHCO ₃	0.71
KH ₂ PO ₄	0.08
MgSO ₄	0.41
CaCl ₂	0.10

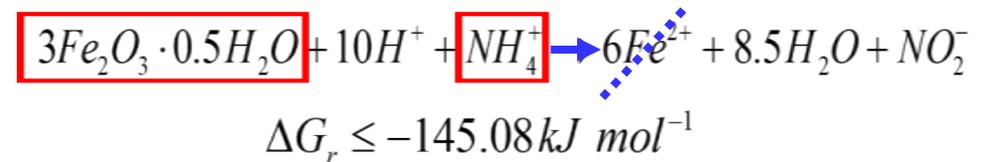
(2) Measurements

- ① Ion Chromatography (IC)
- ② Electrochemical impedance spectroscopy (EIS)
- ③ Ultraperformance Liquid Chromatography-tandem Mass Spectrometry (UPLC-MS-MS)
- ④ qPCR
- ⑤ 16S sequencing

Ammonium Oxidation during A6-enrichment Incubations



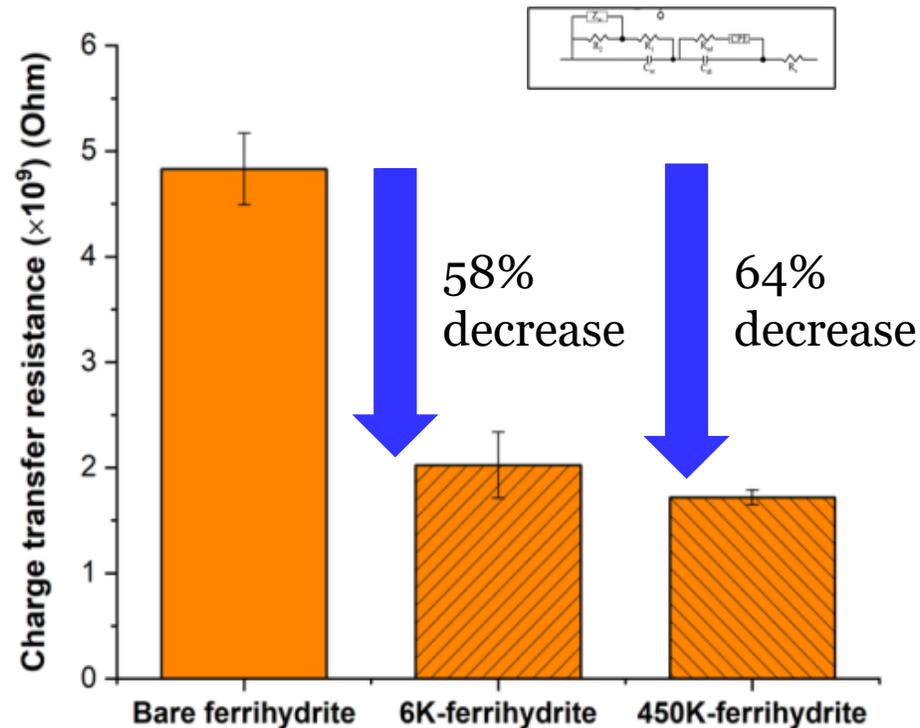
- By day 14, the concentration of NH_4^+ decreased by 44%.
- At the end of the incubation period, NH_4^+ reductions were significantly higher in all PAA-coated samples compared to the control.
- These observations indicate that A6 can utilize PAA-coated ferrihydrite as an electron acceptor.
- Furthermore, the presence of PAA may enhance the activity of A6.



❖ Fe(II) sequestration by the PAA-containing solution may enhance the Feammox reaction.

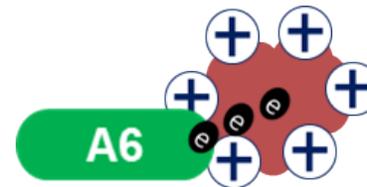
Reduced Charge Transfer Resistance with PAAs

❖ Charge transfer resistance without A6

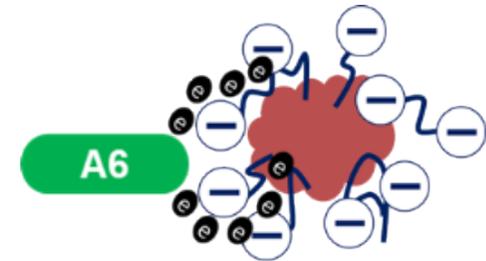


❖ A possible impact of PAA-coated ferrihydrite on the electron transfer by A6

Bare-ferrihydrite



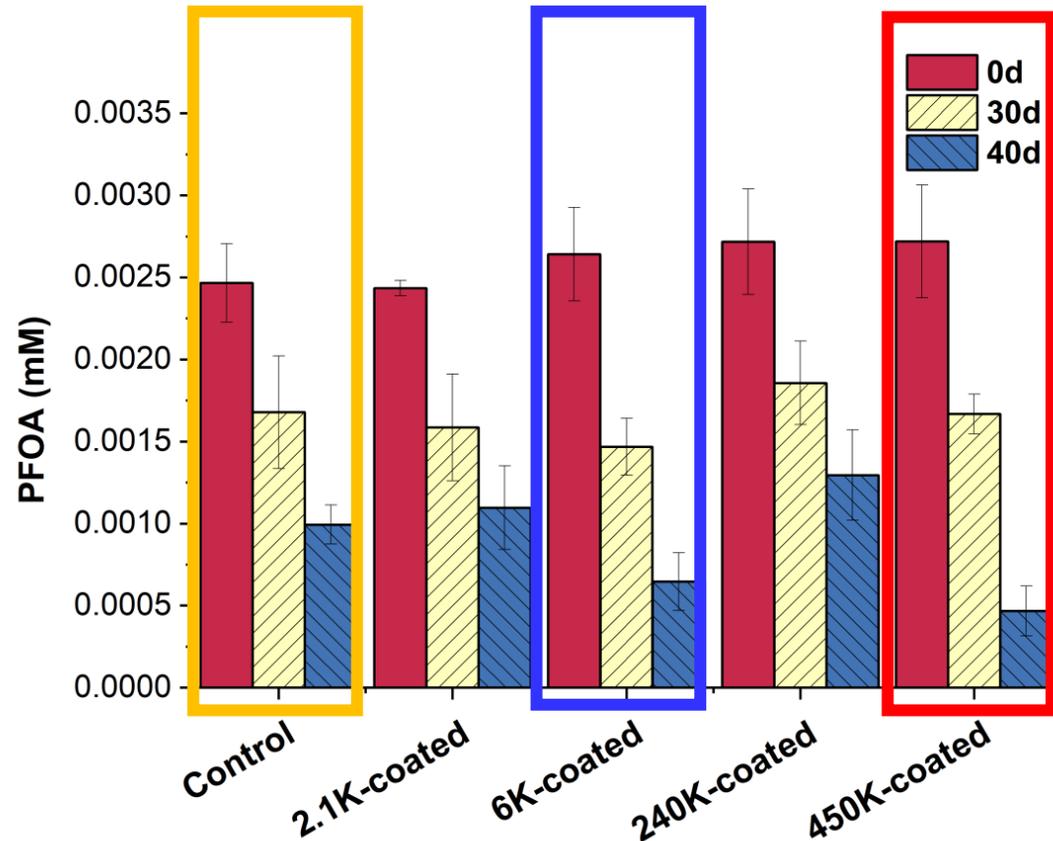
PAA-coated ferrihydrite



- Reduced charge transport resistance with 6K and 450K PAA coatings might be a potential contributing factor.

- The charge transport resistance of bare ferrihydrite was measured to be $(4.8 \pm 0.34) \times 10^{10}$ Ohm.
- In contrast, the 6K-coated and 450K-coated ferrihydrite exhibited significantly lower resistance values.

PFOA Degradation during A6-enrichment Incubations



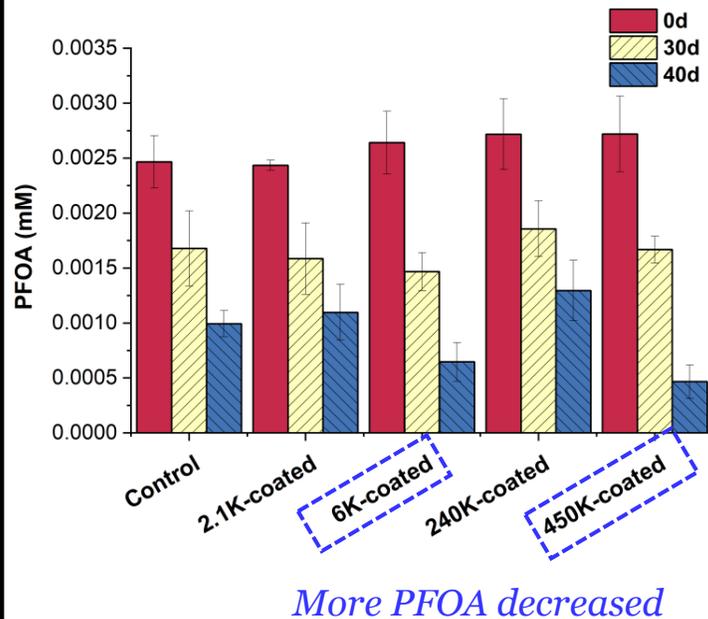
- Over the incubation period, PFOA concentration decreased in all samples.

60% vs. 76% vs. 83%

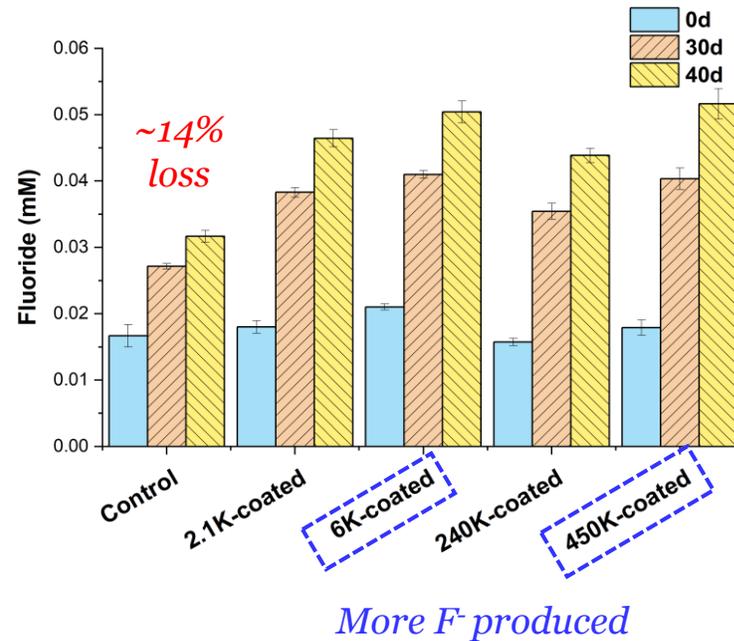
- Notably, after 40 days, the incubations with 6K and 450K-coated ferrihydrite exhibited significantly greater PFOA degradation compared to the other treatments and the control.

Total F Balance during A6-enrichment Incubations

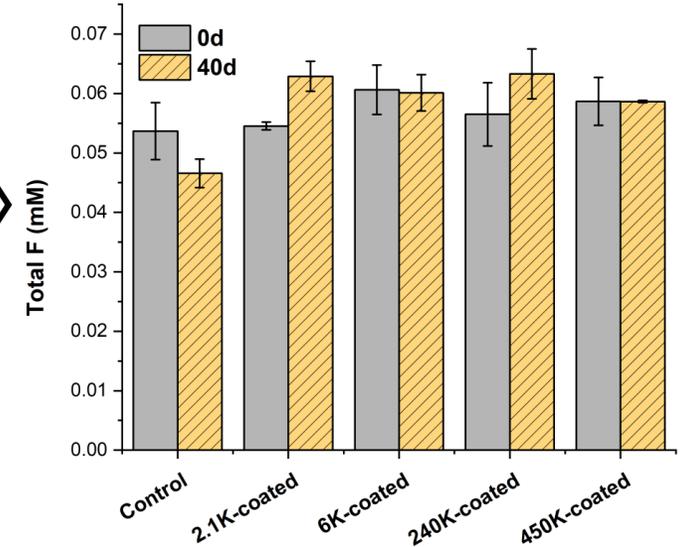
(1) PFOA decrease



(2) F⁻ production



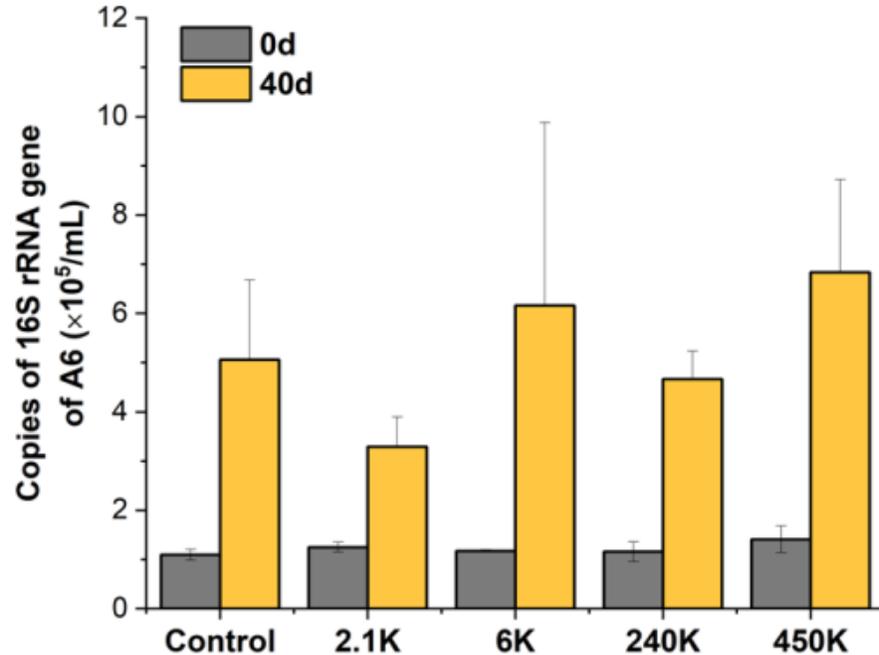
(3) Total F balance



❖ A relatively complete defluorination of PFOA during the Feammox reaction.

- PFOA >>>> PFHpA, PFHxA, PFPeA, and PFBA
- The occurrence of intermediates was sporadic and not specific to PAA coating.

A6 Growth



Sample	Periods	Total OTU	Relative abundance of A6 (%)
Original	0 d	2413	0.44
Control	40 d	4353	0.57
2.1K		7290	0.44
6K		7079	0.44
240K		6269	0.36
450K		8733	0.49

- A6 numbers increased in the presence of PAA-coated ferrihydrite despite large error bars.
- A6 remained the most abundant bacterium among these microbial communities over the 40-day incubation period.

TAKE-HOME MESSAGE

- 1) PAA-coated ferrihydrite particles serve as **electron acceptor** for Feammox and PFOA degradation by A6.
- 2) **6K and 450K PAA-coated ferrihydrite** show the most substantial positive effect on A6-based PFOA defluorination due to PAA-Fe(II) complex and reduced charge transfer resistance.
- 3) PAAs increase total bacterial numbers and do not negatively affect A6 growth after 40 days of incubation, leading to **increased A6 numbers**.
- 4) Further investigation is needed to optimize PAA-coated ferrihydrite for in situ bioremediation using commercially available iron oxides (e.g., goethite).

Acknowledgment



Prof. Peter R. Jaffé
Civil and Environmental Engineering
Princeton University



Prof. Bruce E. Koel
Chemical and Biological Engineering
Princeton University



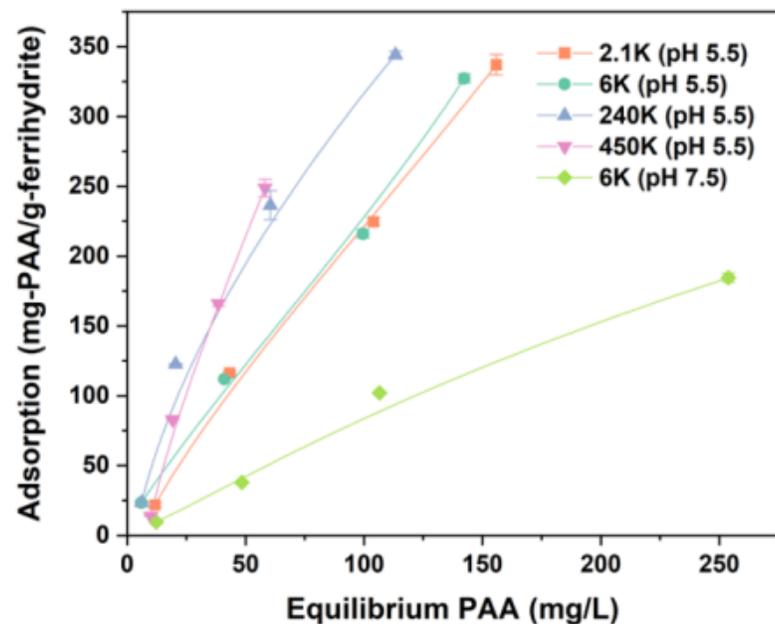
Dr. Shan Huang
Civil and Environmental Engineering
Princeton University

❖ *We gratefully acknowledge the support of the National Institutes of Health (NIH) for funding this research.*

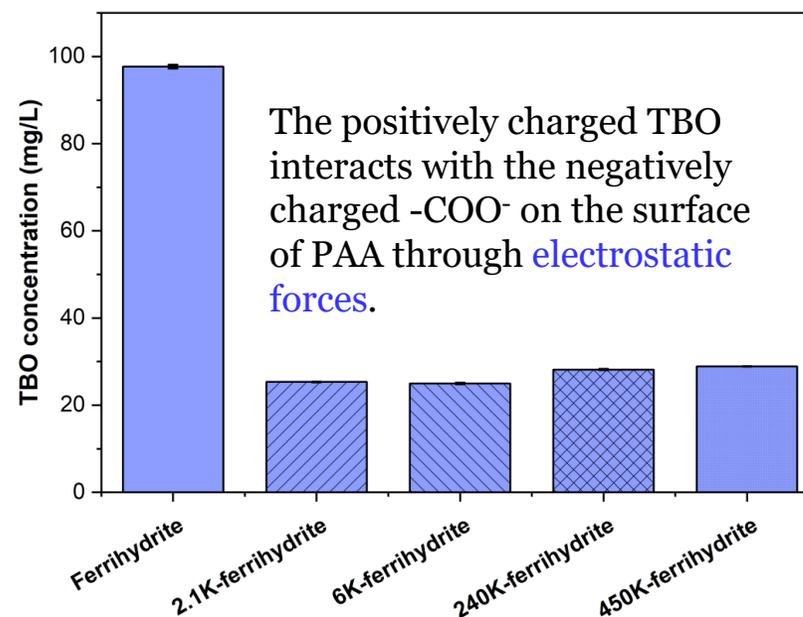


PAA Sorption

(1) PAA isotherm (pH 5.5 vs. pH 7.5)

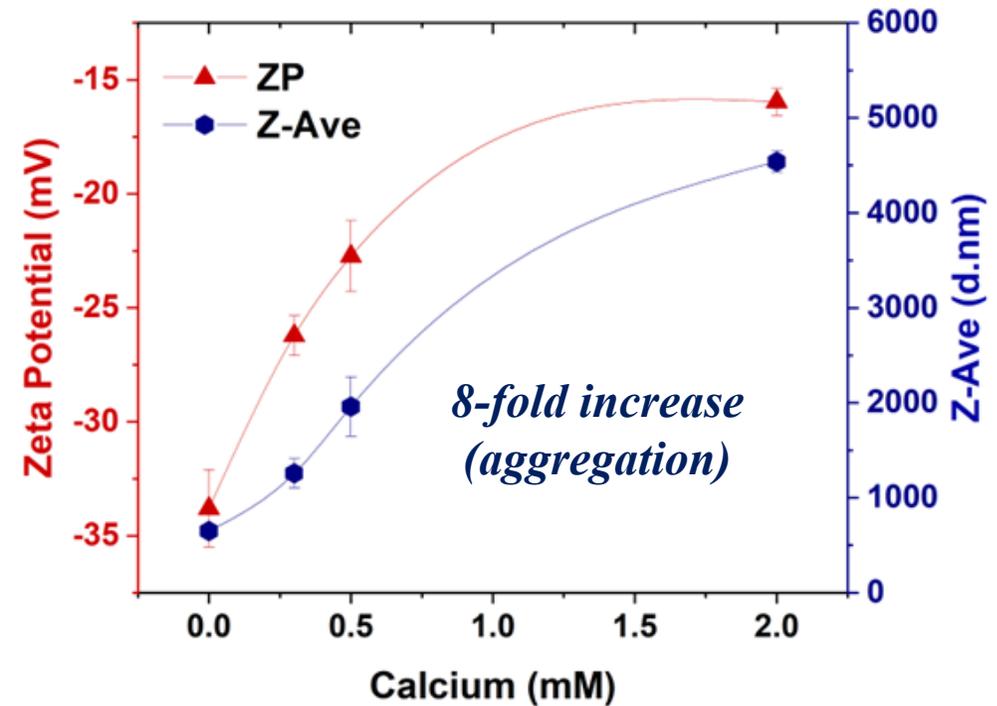
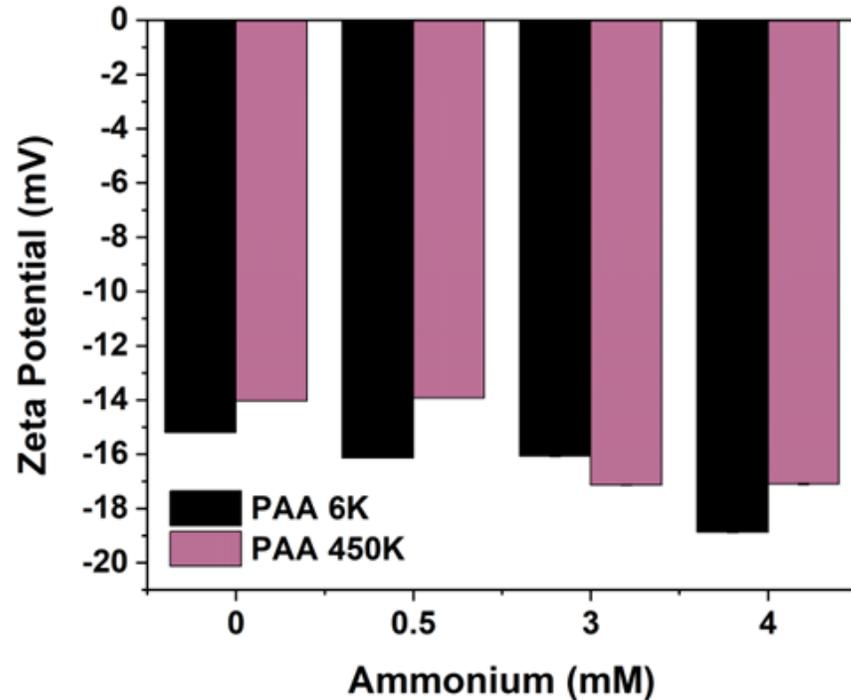


(2) PAA sorption with Toluidine Blue O (TBO)
(PAA/ferrihydrite = 1/5 wt% at pH 5.5)



- Higher molecular weight PAAs have stronger sorption than lower molecular weight PAAs, and sorption decreases with increasing pH, as seen for the 6K PAA.
- PAA coating on the ferrihydrite surface enhances TBO sorption by creating negatively charged sites, which is supported by the zeta potential measurements.

Effect of Selected Cations on Particle Stability



Short-chain PFAS Intermediates

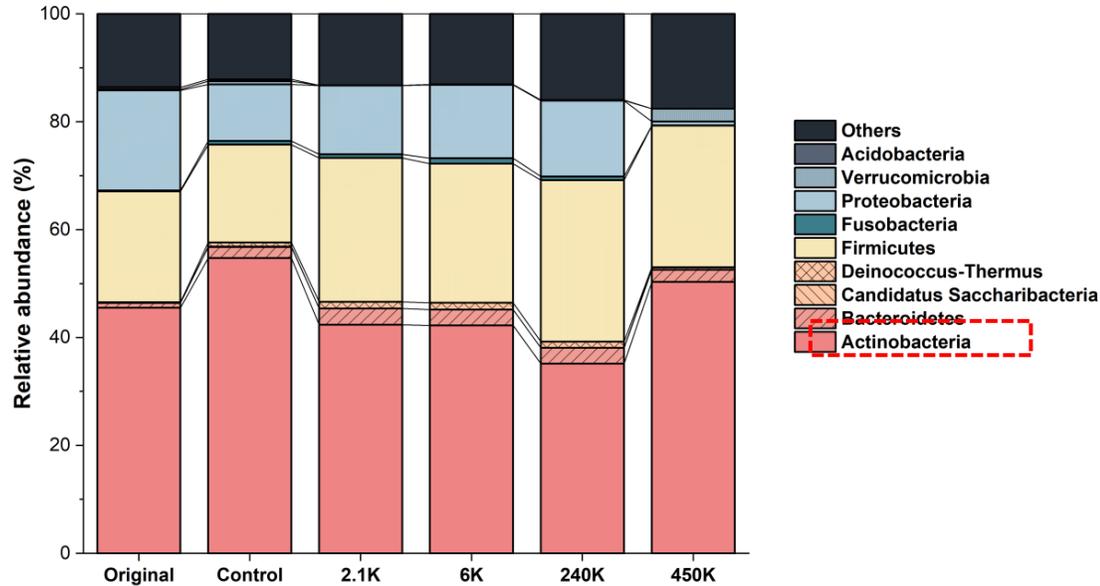
Ctrl	F in PFOA	F in PFHpA	F in PFHxA	F in PFPeA	F in PFBA	F ⁻	Total F
0d	$(3.70 \pm 0.36) \times 10^{-2}$		$(1.47 \pm 1.39) \times 10^{-7}$		$(9.27 \pm 5.78) \times 10^{-9}$	$(1.67 \pm 0.17) \times 10^{-2}$	$(5.37 \pm 0.48) \times 10^{-2}$
30d	$(2.52 \pm 0.52) \times 10^{-2}$		$(4.70 \pm 4.41) \times 10^{-7}$		$(4.06 \pm 5.66) \times 10^{-7}$	$(2.72 \pm 0.04) \times 10^{-2}$	$(5.23 \pm 0.51) \times 10^{-2}$
40d	$(1.49 \pm 0.18) \times 10^{-2}$	$(7.16 \pm 5.32) \times 10^{-8}$	$(6.53 \pm 5.06) \times 10^{-7}$		$(6.10 \pm 4.12) \times 10^{-7}$	$(3.17 \pm 0.09) \times 10^{-2}$	$(4.66 \pm 0.24) \times 10^{-2}$
2.1K	F in PFOA	F in PFHpA	F in PFHxA	F in PFPeA	F in PFBA	F ⁻	Total F
0d	$(3.65 \pm 0.07) \times 10^{-2}$				4.85×10^{-9}	$(1.8 \pm 0.09) \times 10^{-2}$	$(5.45 \pm 0.07) \times 10^{-2}$
30d	$(2.38 \pm 0.49) \times 10^{-2}$			6.36×10^{-7}	$(4.30 \pm 5.83) \times 10^{-7}$	$(3.83 \pm 0.07) \times 10^{-2}$	$(4.65 \pm 0.37) \times 10^{-2}$
40d	$(1.65 \pm 0.38) \times 10^{-2}$	1.53×10^{-7}	4.02×10^{-6}		$(1.17 \pm 0.49) \times 10^{-7}$	$(3.97 \pm 0.27) \times 10^{-2}$	$(6.29 \pm 0.25) \times 10^{-2}$
6K	F in PFOA	F in PFHpA	F in PFHxA	F in PFPeA	F in PFBA	F ⁻	Total F
0d	$(3.96 \pm 0.43) \times 10^{-2}$				1.09×10^{-8}	$(2.10 \pm 0.05) \times 10^{-2}$	$(6.06 \pm 0.42) \times 10^{-2}$
30d	$(2.20 \pm 0.26) \times 10^{-3}$			$(1.72 \pm 2.03) \times 10^{-7}$	$(1.16 \pm 0.22) \times 10^{-8}$	$(4.10 \pm 0.06) \times 10^{-2}$	$(6.30 \pm 0.24) \times 10^{-2}$
40d	$(9.69 \pm 2.65) \times 10^{-3}$		$(2.14 \pm 2.15) \times 10^{-7}$	1.83×10^{-7}	$(3.61 \pm 0.96) \times 10^{-7}$	$(5.04 \pm 0.17) \times 10^{-2}$	$(6.01 \pm 0.31) \times 10^{-2}$

Short-chain PFAS Intermediates

240K	F in PFOA	F in PFHpA	F in PFHxA	F in PFPeA	F in PFBA	F ⁻	Total F
0d	$(4.08 \pm 0.05) \times 10^{-2}$				$(4.15 \pm 5.61) \times 10^{-8}$	$(1.57 \pm 0.06) \times 10^{-2}$	$(5.65 \pm 0.53) \times 10^{-2}$
30d	$(2.79 \pm 0.38) \times 10^{-2}$			1.91×10^{-8}	$(3.86 \pm 4.00) \times 10^{-7}$	$(3.54 \pm 0.12) \times 10^{-3}$	$(6.33 \pm 0.42) \times 10^{-2}$
40d	$(1.94 \pm 0.41) \times 10^{-2}$	$(8.60 \pm 2.74) \times 10^{-8}$	$(1.08 \pm 0.79) \times 10^{-6}$		$(1.33 \pm 1.77) \times 10^{-6}$	$(4.39 \pm 0.11) \times 10^{-2}$	$(6.33 \pm 0.42) \times 10^{-2}$

450K	F in PFOA	F in PFHpA	F in PFHxA	F in PFPeA	F in PFBA	F ⁻	Total F
0d	$(4.08 \pm 0.52) \times 10^{-2}$					$(1.79 \pm 0.12) \times 10^{-2}$	$(5.87 \pm 0.40) \times 10^{-2}$
30d	$(2.50 \pm 0.18) \times 10^{-2}$	1.37×10^{-8}		1.46×10^{-8}	$(7.94 \pm 3.24) \times 10^{-9}$	$(4.04 \pm 0.16) \times 10^{-2}$	$(6.54 \pm 0.26) \times 10^{-2}$
40d	$(7.00 \pm 2.28) \times 10^{-3}$	$(0.97 \pm 1.06) \times 10^{-7}$	$(1.41 \pm 1.37) \times 10^{-6}$		$(1.96 \pm 1.08) \times 10^{-7}$	$(5.16 \pm 0.23) \times 10^{-2}$	$(5.86 \pm 0.02) \times 10^{-2}$

Microbial Community Structure



Sample	Periods	Total OTU	Relative abundance of A6 (%)
Original	0 d	2413	0.44
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- The main microbial structure did not shift a lot in the presence of PAA, and the abundance of the phylum Proteobacteria was prominent in all samples except for the 450K-coated sample.
- PAA-coated ferrihydrite did not inhibit A6 growth and the presence of PAA might be beneficial for the growth of heterotroph.

Microbial Community Structure

Genus (Original on 0d)	OTU	Relative abundance	Family	Class
Acidimicrobium	1063	0.41	Acidimicrobiaceae	Acidimicrobia
Staphylococcus	363	0.14	Staphylococcaceae	Bacilli
Cobetia	201	0.08	Halomonadaceae	Gammaproteobacteria
Exiguobacterium	74	0.03	Bacillaceae	Bacilli

Genus (Control on 40d)	OTU	Relative abundance	Family	Class
Acidimicrobium	2472	0.54	Acidimicrobiaceae	Acidimicrobia
Exiguobacterium	558	0.12	Bacillaceae	Bacilli
Pseudomonas	92	0.02	Pseudomonadaceae	Gammaproteobacteria
Unclassified Erysipelotrichaceae	73	0.02	Erysipelotrichaceae	Erysipelotrichia

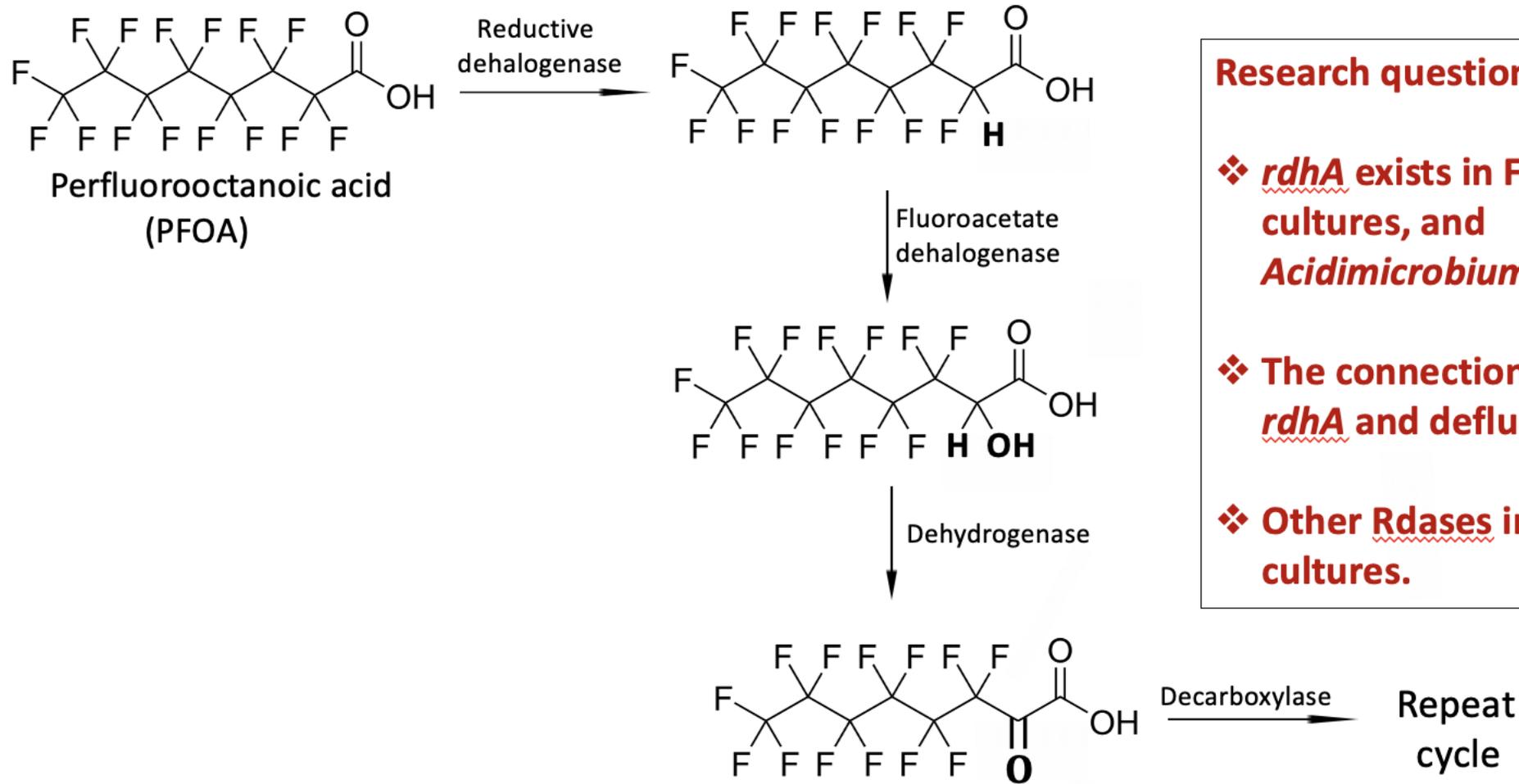
Genus (2.1K on 40d)	OTU	Relative abundance	Family	Class
Acidimicrobium	3176	0.42	Acidimicrobiaceae	Acidimicrobia
Exiguobacterium	1272	0.17	Bacillaceae	Bacilli
Unclassified Erysipelotrichaceae	398	0.05	Erysipelotrichaceae	Erysipelotrichia
Pseudomonas	287	0.04	Pseudomonadaceae	Gammaproteobacteria

Genus (6K on 40d)	OTU	Relative abundance	Family	Class
Acidimicrobium	3083	0.42	Acidimicrobiaceae	Acidimicrobia
Exiguobacterium	1396	0.19	Bacillaceae	Bacilli
Pseudomonas	191	0.03	Pseudomonadaceae	Gammaproteobacteria
Unclassified Erysipelotrichaceae	172	0.02	Erysipelotrichaceae	Erysipelotrichia

Genus (240K on 40d)	OTU	Relative abundance	Family	Class
Acidimicrobium	2275	0.34	Acidimicrobiaceae	Acidimicrobia
Exiguobacterium	1243	0.19	Bacillaceae	Bacilli
Unclassified Erysipelotrichaceae	405	0.06	Erysipelotrichaceae	Erysipelotrichia
Pseudomonas	267	0.04	Pseudomonadaceae	Gammaproteobacteria

Genus (450K on 40d)	OTU	Relative abundance	Family	Class
Acidimicrobium	4249	0.48	Acidimicrobiaceae	Acidimicrobia
Faecalibaculum	1155	0.13	Erysipelotrichaceae	Erysipelotrichia
Lactobacillus	640	0.07	Lactobacillaceae	Bacilli
Akkermansia	207	0.02	Akkermansiaceae	Verrucomicrobiae

One Plausible Reaction Pathways of PFAS Degradation by A6 (Huang et al.)



Research questions and aims

- ❖ *rdhA* exists in Feammox cultures, and *Acidimicrobium*?
- ❖ The connection between *rdhA* and defluorination?
- ❖ Other Rdases in Feammox cultures.

This invokes a role for both reductive and hydrolytic dehalogenases