Feammox process for biodegradation of PFAS

Note: This version does not include some material that was presented and is currently under review or about to be submitted

Peter R. Jaffé and Shan Huang

Department of Civil and Environmental Engineering

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PRINCETON UNIVERSITY

Oxidation of NH_4^+ under Fe reducing conditions

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

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

anaerobic autotrophic

Clement *et al*. (2005); Shrestha *et al.* (2009)

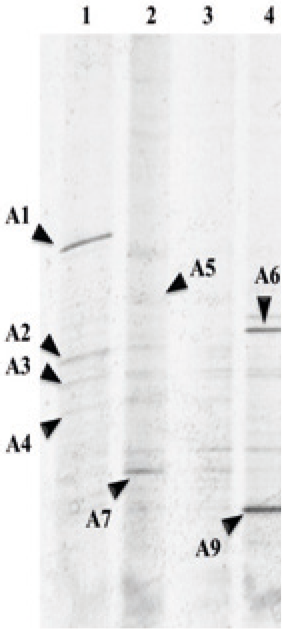
Sawayama* (2006); Yang *et al*. (2012)

***Feammox**

Many studies showing Feammox in different settings.
e.g. Ding et al. 2014; Yang et al. 2018;
Guan *et al.* 2018; Yi B*, et al.* , 2019 …

Results of long-term enrichment cultures
Identification of microorganism responsible for Feammox

An uncultured ***Acidimicrobiaceae* bacterium A6**, whose closest cultivated relative is *Ferrimicrobium acidiphilum* (with 92% identity) and *Acidimicrobium ferrooxidans* (with 90% identity)



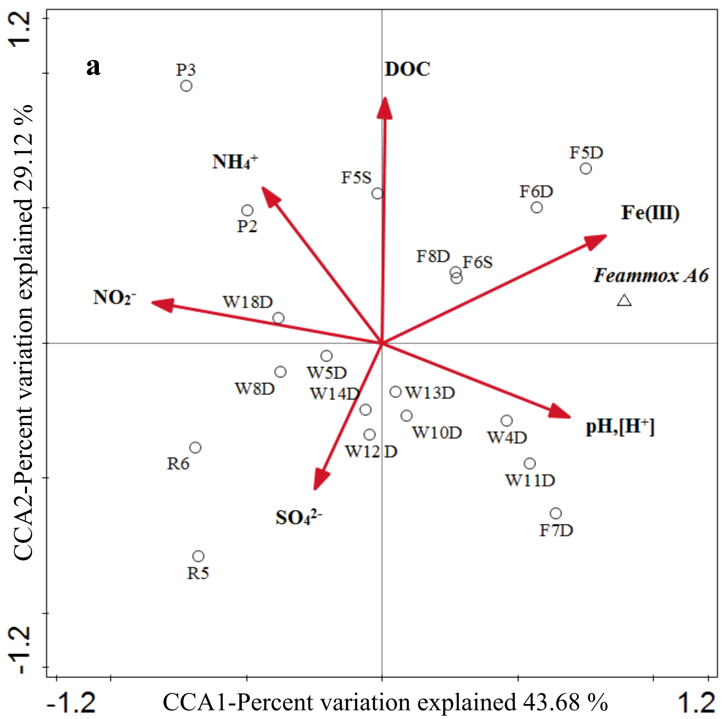
DGGE Bands

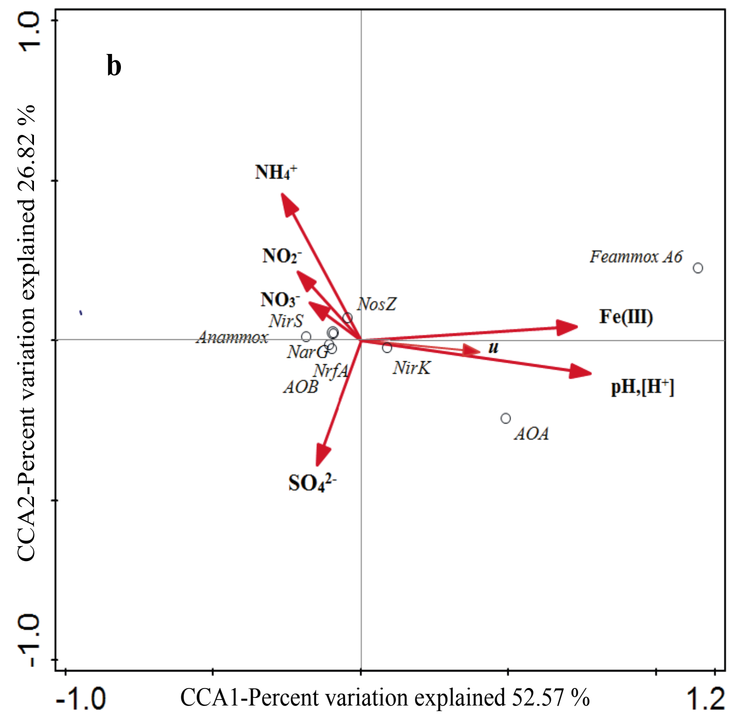
Incubation with ferrihydrite + NH_4Cl + $NaHCO_3$ (lane 1-4)

0, 30, 90 and 160 days

Huang and Jaffé, 2015, *Biogeosciences*, 12, 769-779.

Correlation between *Acidimicrobiaceae* bacterium A6 and soil Fe(III) content, pH, and other NH_4^+ oxidizers

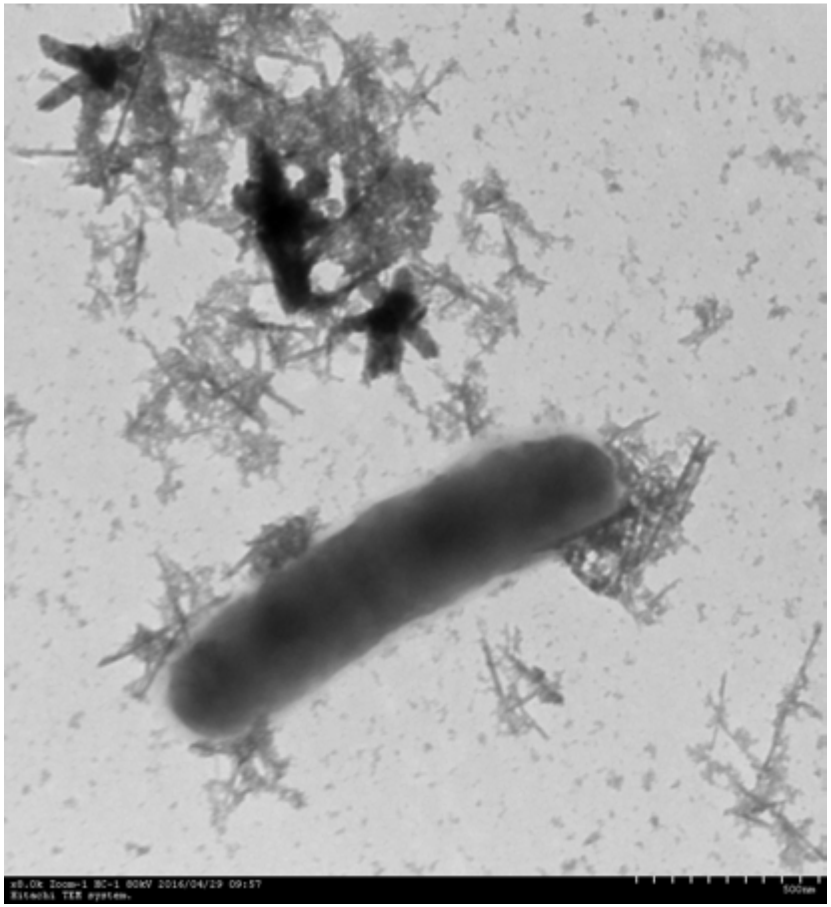


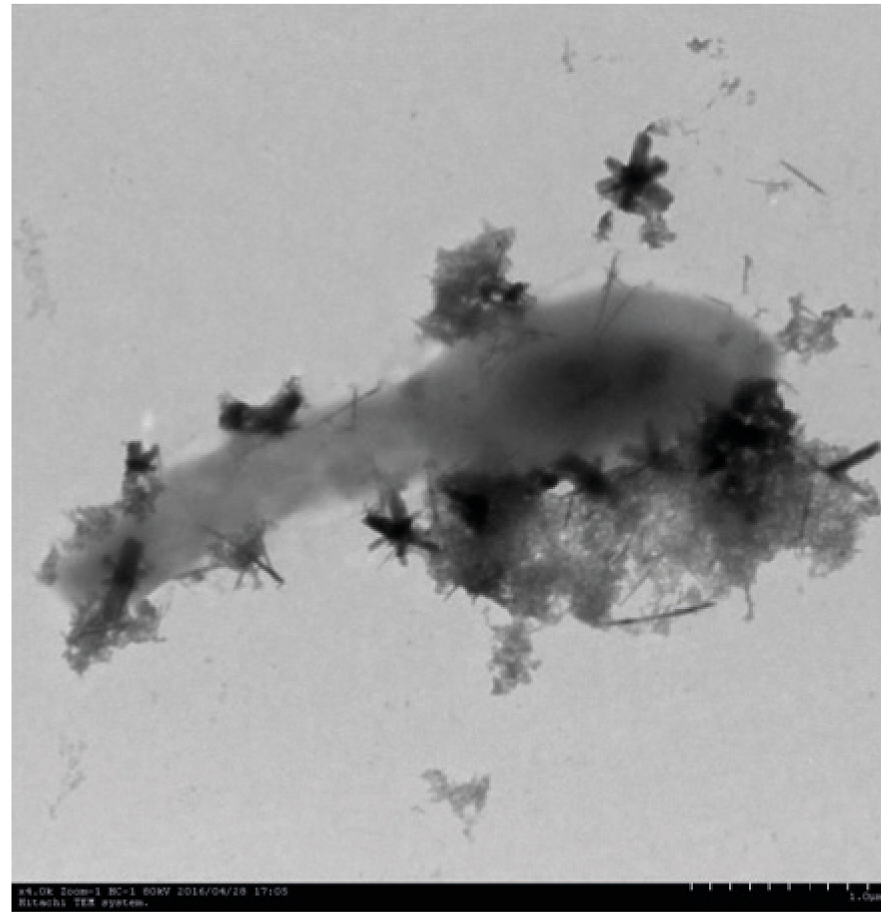


Huang et al., 2016, *Soil Biology and Biochemistry*, 2016, Vol. 98, pp. 148-158.

Isolation of *Acidimicrobiaceae* bacterium A6

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

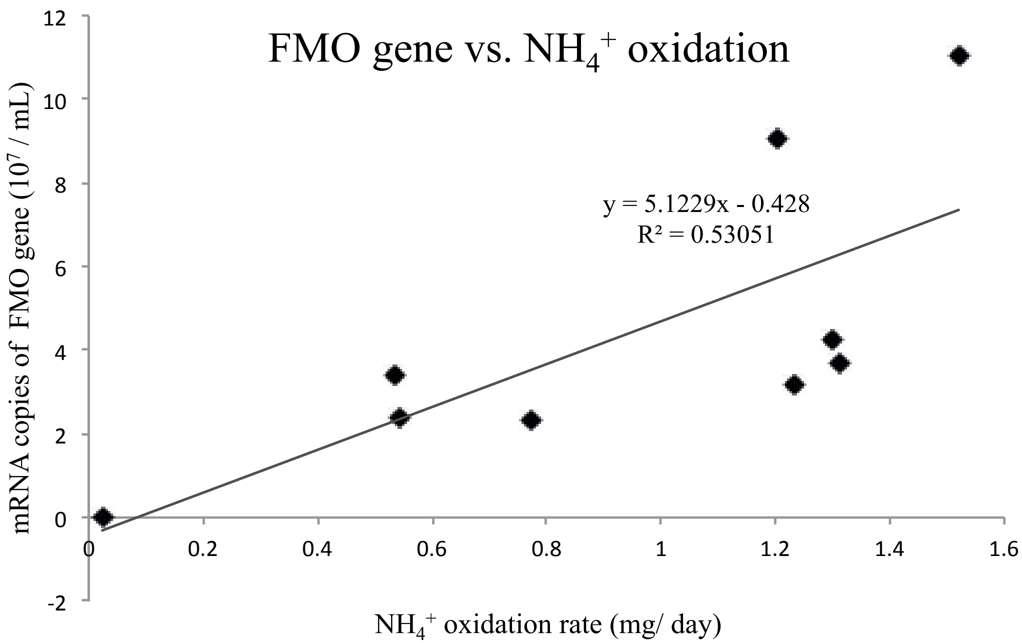




Huang, S., and P.R. Jaffé, *PLOS ONE*, Vol. 13, No. 4, 2018, e0194007

Gnome of A6 shows the presence of

- A group of novel oxygenase related genes (GenBank accession numbers: MG011983-MG012003)



Potential applications of Feammox other than NH_4^+ oxidation: **Selected organics that are degraded by oxygenases**

MMO

$$CH_4 \rightarrow CH_3OH$$

MMO

$$HClC{=}CCl_2 \rightarrow HCl\overset{O}{C\text{-}C}Cl_2$$

trichloroethylene → trichloroethylene epoxy

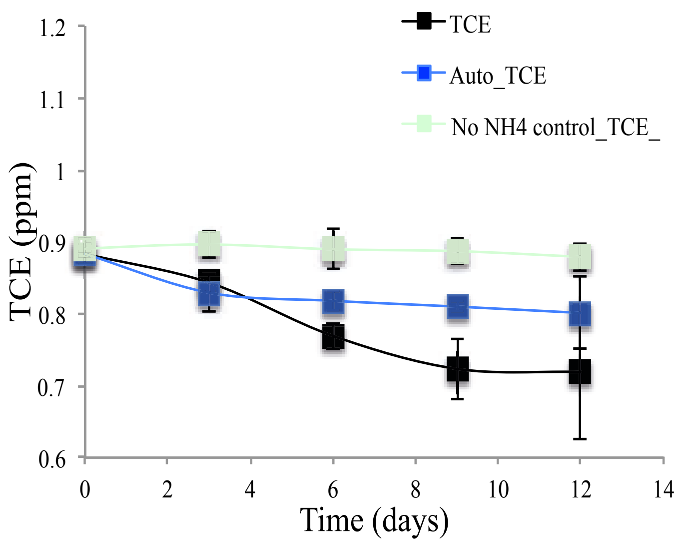
DIO

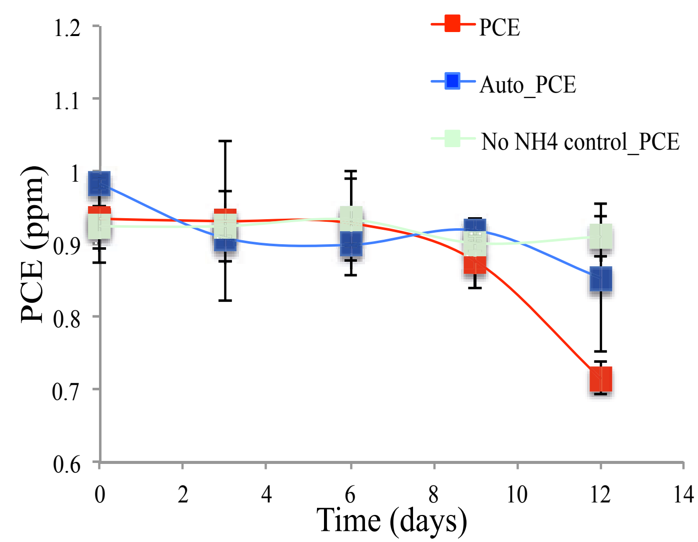
$$C_6H_6 \rightarrow C_6H_5OH$$

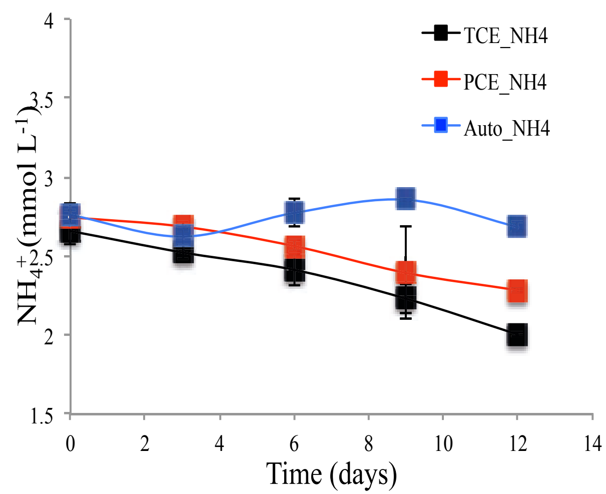
DIO

benzene → toluene

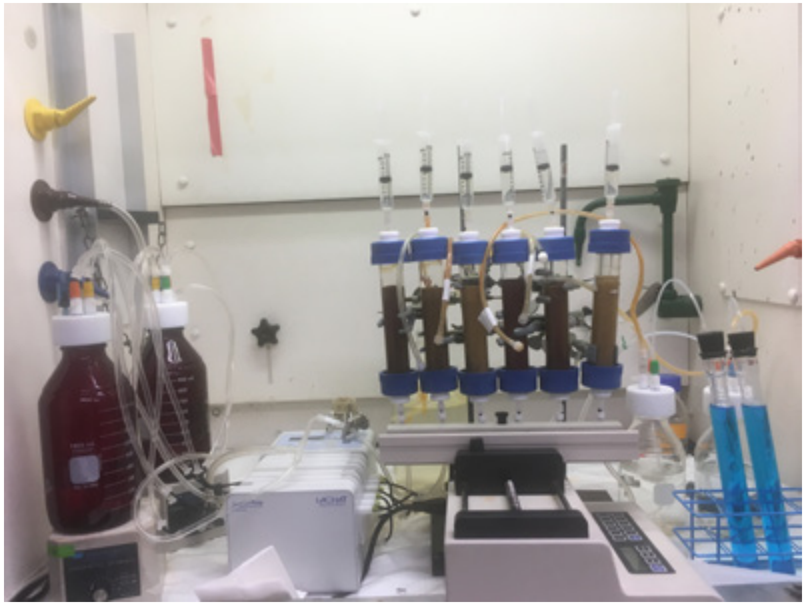
TCE, PCE, and NH_4^+ concentrations vs. time during incubation experiments with the pure culture of A6

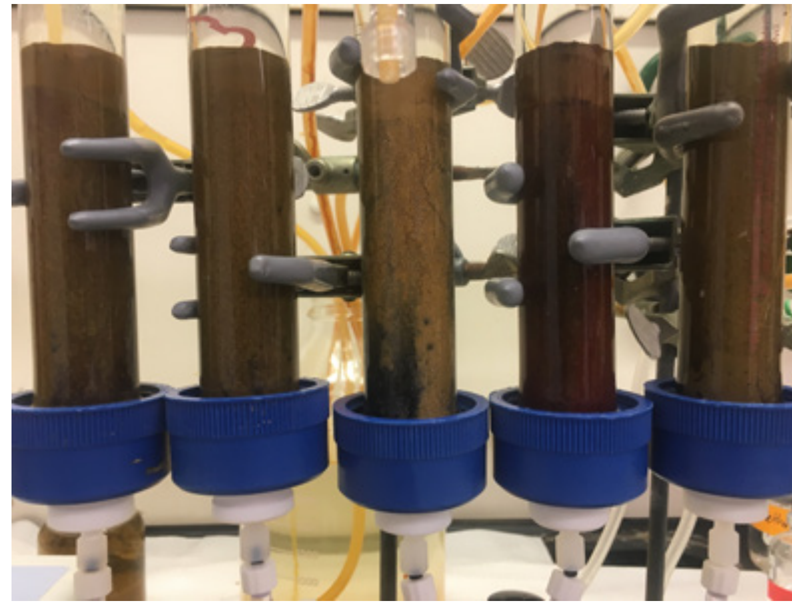






Bioaugmentation of soil columns with an A6 enrichment culture for enhanced TCE degradation





Columns operated for 10 days with 1 mg/l TCE in the influent

Velocity ~ 1 m/d (4 hour residence time)

TCE removal in seeded columns ~ 10%. No removal in non-seeded controls

Acidimicrobiaceae bacterium A6 was low in the seeded columns ~ $10^2 - 10^3$ cells/gr

Ge, J., S. Huang, I. Han, and P.R. Jaffé, 2019. *Environmental Pollution*, Vol. 247, pp. 248-255, https://doi.org/10.1016/j.envpol.2019.01.066.

"Methods and Compositions for Nitrogen Removal Using Feammox Microorganisms," Patent No. US 9,815,723, Nov. 14, 2017

Gnome of A6 shows the presence of

- A6's gnome and the A6 enrichment culture also revealed the presence of reductive dehalogenases (RDases) (GenBank accession numbers: MK358459-MK358462)
- Can we defluorinated PFAS via Feammox?

Feammox PFAS Incubation Screening Experiment

initial PFAS concentration ~ 100 mg/l

Similar results with 0.1 mg/l and 1 mg/l

Compound		MW	Change in concentration (mg/l)	F^- produced (mg/l)	% defluorination of parent compound degraded
HFBA (Heptafluorobutyric acid)	$CF_3CF_2CF_2COOH$	214.0	41.3	24.9	97.0
PFOA (Perfluorooctanoic acid)	$CF_3(CF_2)_6COOH$	414.1	44.4	29.5	96.5
(2,2,2-Trifluoroethyl Nonafluorobutanesulfonate)	$C_6H_2F_{12}O_3S$	382.12	29.3	16.3	93.2
6:2 FTS (6:2 Fluorotelomer sulfonate) **	$C_8H_5F_{13}O_3S$	428.2	18.0	11.4	109.4
8:2 FTOH (8:2 Fluorotelomer Alcohol)**	$C_{10}H_5F_{17}O$	464.1	25.1	17.5	100.0
PFBS (Perfluorobutane sulfonic acid)	$C_4HF_9O_3S$	300.1	35.2	15.9	79.3
PFOS (Perfluorooctane sulfonic acid)	$C_8HF_{17}O_3S$	500.1	39.2	18.0	71.0
8:2 FTS (8:2 Fluorotelomer sulfonate)	$C_{10}H_4F_{17}O_3S$	527.2	23.5	6.9	47.7
6:2 FTOH (6:2 Fluorotelomer Alcohol)	$C_8H_5F_{13}O$	368.1	19.4	7.1	54.6
PFNS (Perfluorooctane sulfonamide)	$C_8H_2F_{17}NO_2S$	499.1	18.5	6.1	51.0
ADONA (Ammonium 4,8-dioxa-3H-perfluorononanoate)	$C_7H_5F_{12}NO_4$	393.1	12.3	1.0	14.6
8:2 diPAP (8:2 Fluorotelomer phosphate diester)	$C_{20}H_9F_{34}O_4P$	990.2	16.2	1.8	17.2

** in ethanol

Feammox PFAS Incubation Screening Experiment

initial PFAS concentration ~ 100 mg/l

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HFBA (Heptafluorobutyric acid)	$CF_3CF_2CF_2COOH$	214.04	41.3	24.9	97.0
PFOA (Perfluorooctanoic acid)	$CF_3(CF_2)_6COOH$	414.07	44.4	29.5	96.5
(2,2,2-Trifluoroethyl Nonafluorobutanesulfonate)	$C_6H_2F_{12}O_3S$	382.12	29.3	16.3	93.2
6:2 FTS (6:2 Fluorotelomer sulfonate) **	$C_8H_5F_{13}O_3S$	428.17	18.0	11.4	109.4
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** in ethanol

Short-term incubation of PFAS contaminated sediments augmented with ferrihydrite

		Day 0	Day 14
		mg/kg	mg/kg
$CF_3CF_2CF_2COOH$	Heptafluorobutyric acid (HFBA)	0.003	0.017
$CF_3(CF_2)_6COOH$	Perfluorooctanoic acid (PFOA)	0.045	0.064
$C_4HF_9O_3S$	Perfluorobutane sulfonic acid (PFBS)	0.007	0.021
$C_8HF_{17}O_3S$	Perfluorooctane sulfonic acid (PFOS)	0.029	0.068
$C_8H_5F_{13}O$	6:2 Fluorotelomer Alcohol (6:2 FTOH)	0.085	0.019
$C_{10}H_5F_{17}O$	8:2 Fluorotelomer Alcohol (8:2 FTOH)	0.14	0.040
$C_8H_5F_{13}O_3S$	6:2 Fluorotelomer sulfonate (6:2 FTS)	0.066	0.014
$C_{10}H_4F_{17}O_3S$	8:2 Fluorotelomer sulfonate (8:2 FTS)	0.033	0.021

Short-term incubation results of PFAS contaminated sediments augmented with ferrihydrite

Concentration (mg/l)	Day 0	Day 14
F^-	3.9	14.3
SO_4^{2-}	43.2	271.1
Acetate	20.9	37.2
NH_4^+	33.4	7.2
Fe(II)	24.3	168.9

Challenges for Applications of the Feammox Process for Organic Contaminant Removal

- Can we operate reactors with easily available Fe(III) sources?
- Can we produce significant A6 biomass for bioaugmentation without a high Iron(II,III) oxide content (e.g., magnetite, Fe_3O_4)?
- **The Challenge: Fe(III):NH_4^+ = 6:1**

A6 is Electrogenic

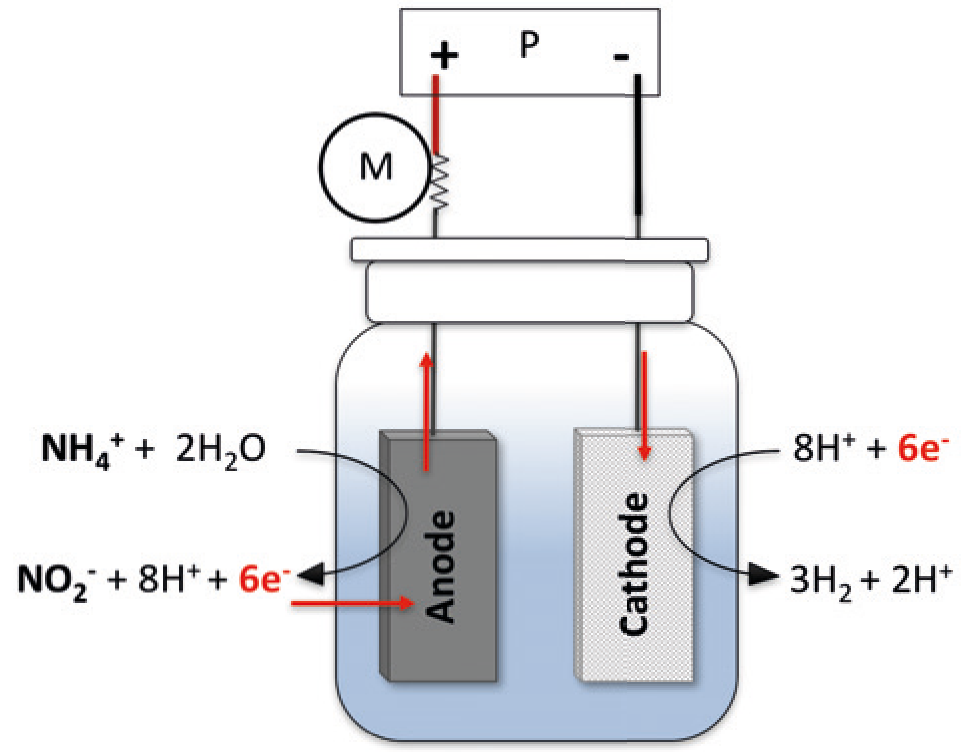
- A6 can colonize an anode, when two connected electrodes are submerged in soil or a solution with a natural or imposed redox potential difference between electrodes
- We could grow A6 in an Fe(III) free solution/reactor
- Ruiz, M., W. Shuai, and P.R. Jaffé, *Applied and Environmental Microbiology*, 2018
  DOI: 10.1128/AEM.02029-18

In microbial electrolysis cells (MECs), apply an external potential

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

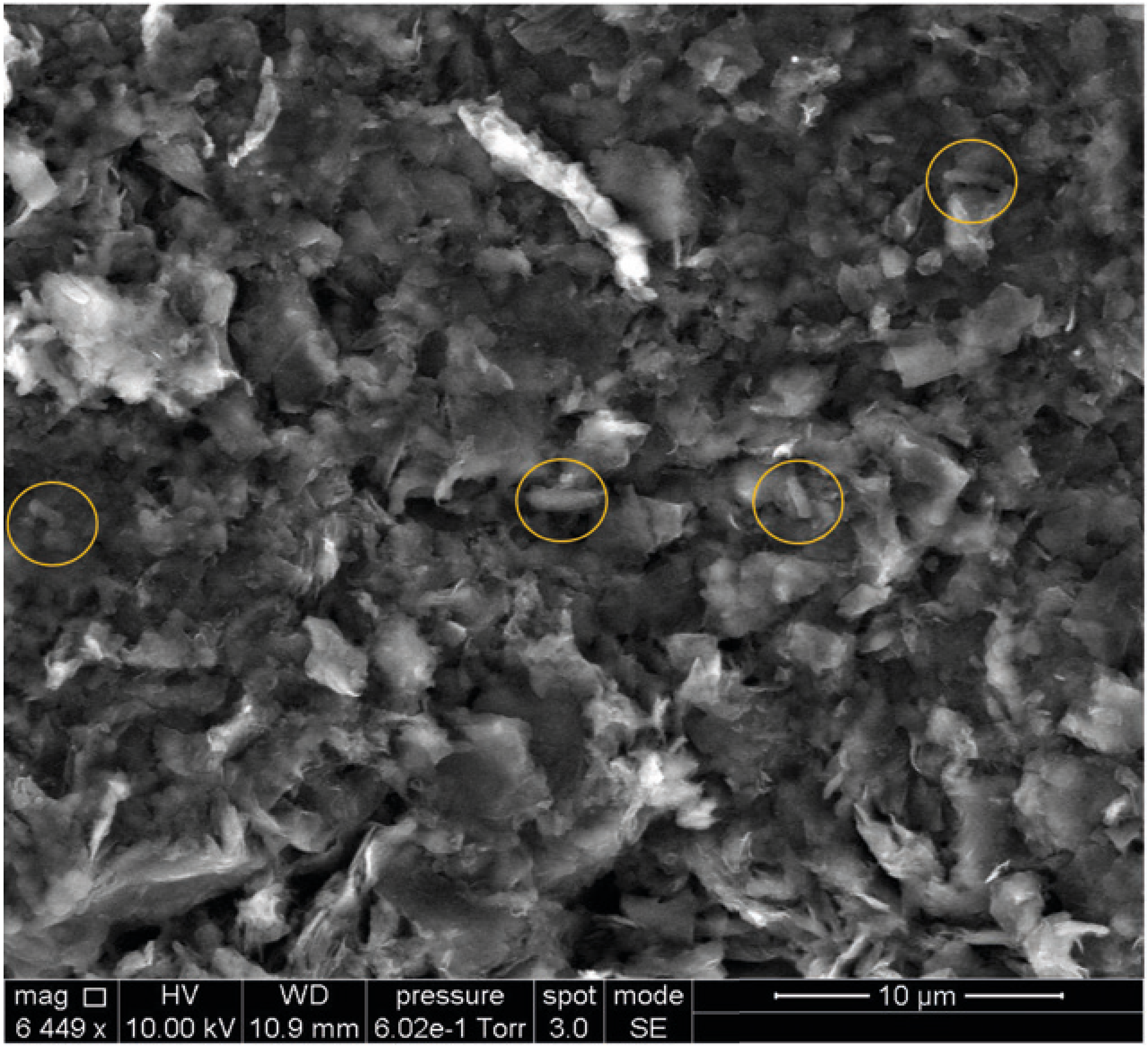
Reaction without Fe(III):

$NH_4^+ + 2H_2O \rightarrow NO_2^- + 3H_2 + 2H^+$



(Call and Logan, 2011)

E-SEM image of graphite anode of MEC operated with live A6



~2.8 10^4 cells/cm^2
vs.
2.11 x 10^9 cells/ml

Conclusions

- A6 has a novel oxygenase-related enzyme responsible for ammonium oxidation that can also oxidize co-metabolically various recalcitrant organic contaminants.
- A6 has reductive dehalogenaszes that can defluorinated PFAS, including PFOA and PFOS
- We can bioaugment aquifer and wetland soils with A6 to enhance Feammox activity (NH_4^+ oxidation, TCE, PFAS degradation).

Thank You!

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