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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Oxidation of $\text{NH}_4^+$ under Fe reducing conditions

$$3\text{Fe}_2\text{O}_3 \cdot 0.5\text{H}_2\text{O} + 10\text{H}^+ + \text{NH}_4^+ \rightarrow 6\text{Fe}^{2+} + 8.5\text{H}_2\text{O} + \text{NO}_2^-$$

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

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$_4$Cl + NaHCO$_3$ (lane 1-4)
0, 30, 90 and 160 days
Correlation between *Acidimicrobiaceae* bacterium A6 and soil Fe(III) content, pH, and other NH₄⁺ oxidizers

Isolation of *Acidimicrobiaceae* bacterium A6

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

Gnome of A6 shows the presence of

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

![Graph showing the relationship between mRNA copies of FMO gene and NH$_4^+$ oxidation rate. The equation of the line is $y = 5.1229x - 0.428$ with $R^2 = 0.53051$.](image-url)
Potential applications of Feammox other than $\text{NH}_4^+$ oxidation:
Selected organics that are degraded by oxygenases

- $\text{CH}_4 \rightarrow \text{CH}_3\text{OH}$
- $\text{C}_6\text{H}_6 \rightarrow \text{C}_6\text{H}_5\text{OH}$
- $\text{HClC}≡\text{CCl}_2 \rightarrow \text{HClC-CCl}_2$
  - trichloroethylene $\rightarrow$ trichloroethylene epoxy
- $\text{C}_6\text{H}_6 \rightarrow \text{C}_6\text{H}_5\text{OH}$
  - benzene $\rightarrow$ 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


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**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Formula</th>
<th>MW</th>
<th>Change in concentration (mg/l)</th>
<th>F- produced (mg/l)</th>
<th>% defluorination of parent compound degraded</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFBA (Heptafluorobutyric acid)</td>
<td>CF₃CF₂CF₂COOH</td>
<td>214.0</td>
<td>41.3</td>
<td>24.9</td>
<td>97.0</td>
</tr>
<tr>
<td>PFOA (Perfluorooctanoic acid)</td>
<td>CF₃(CF₂)₆COOH</td>
<td>414.1</td>
<td>44.4</td>
<td>29.5</td>
<td>96.5</td>
</tr>
<tr>
<td>(2,2,2-Trifluoroethyl Nonafluorobutanesulfonate)</td>
<td>C₆H₂F₁₂O₃S</td>
<td>382.12</td>
<td>29.3</td>
<td>16.3</td>
<td>93.2</td>
</tr>
<tr>
<td>6:2 FTS (6:2 Fluorotelomer sulfonate) **</td>
<td>C₈H₅F₁₃O₃S</td>
<td>428.2</td>
<td>18.0</td>
<td>11.4</td>
<td>109.4</td>
</tr>
<tr>
<td>8:2 FTOH (8:2 Fluorotelomer Alcohol) **</td>
<td>C₁₀H₅F₁₇O</td>
<td>464.1</td>
<td>25.1</td>
<td>17.5</td>
<td>100.0</td>
</tr>
<tr>
<td>PFBS (Perfluorobutane sulfonic acid)</td>
<td>C₄HF₉O₃S</td>
<td>300.1</td>
<td>35.2</td>
<td>15.9</td>
<td>79.3</td>
</tr>
<tr>
<td>PFOS (Perfluorooctane sulfonic acid)</td>
<td>C₈HF₁₇O₃S</td>
<td>500.1</td>
<td>39.2</td>
<td>18.0</td>
<td>71.0</td>
</tr>
<tr>
<td>8:2 FTS (8:2 Fluorotelomer sulfonate)</td>
<td>C₁₀H₄F₁₇O₃S</td>
<td>527.2</td>
<td>23.5</td>
<td>6.9</td>
<td>47.7</td>
</tr>
<tr>
<td>6:2 FTOH (6:2 Fluorotelomer Alcohol)</td>
<td>C₈H₅F₁₃O</td>
<td>368.1</td>
<td>19.4</td>
<td>7.1</td>
<td>54.6</td>
</tr>
<tr>
<td>PFNS (Perfluorooctane sulfonamide)</td>
<td>C₈H₂F₁₇NO₂S</td>
<td>499.1</td>
<td>18.5</td>
<td>6.1</td>
<td>51.0</td>
</tr>
<tr>
<td>ADONA (Ammonium 4,8-dioxa-3H-perfluorononanoate)</td>
<td>C₇H₅F₁₂NO₄</td>
<td>393.1</td>
<td>12.3</td>
<td>1.0</td>
<td>14.6</td>
</tr>
<tr>
<td>8:2 diPAP (8:2 Fluorotelomer phosphate diester)</td>
<td>C₂₀H₉F₃₄O₄P</td>
<td>990.2</td>
<td>16.2</td>
<td>1.8</td>
<td>17.2</td>
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** in ethanol
**Feammox PFAS Incubation Screening Experiment**
initial PFAS concentration ~ 100 mg/l

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<td>HFBA (Heptafluorobutyric acid)</td>
<td>CF₃CF₂CF₂COOH</td>
<td>214.04</td>
<td>41.3</td>
<td>24.9</td>
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<tr>
<td><strong>PFOA (Perfluorooctanoic acid)</strong></td>
<td>CF₃(CF₂)₆COOH</td>
<td>414.07</td>
<td>44.4</td>
<td>29.5</td>
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<td>(2,2,2-Trifluoroethyl Nonafluorobutanesulfonate)</td>
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</tr>
<tr>
<td>PFNS (Perfluoroctane sulfonamide)</td>
<td>C₈H₂F₁₇NO₂S</td>
<td>499.14</td>
<td>18.5</td>
<td>6.1</td>
</tr>
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<td>ADONA (Ammonium 4,8-dioxa-3H-perfluorononanoate)</td>
<td>C₇H₅F₁₂NO₄</td>
<td>393.09</td>
<td>12.3</td>
<td>1.0</td>
</tr>
<tr>
<td>8:2 diPAP (8:2 Fluorotelomer phosphate diester)</td>
<td>C₂₀H₉F₃₄O₄P</td>
<td>990.21</td>
<td>16.2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

** in ethanol
### Short-term incubation of PFAS contaminated sediments augmented with ferrihydrite

<table>
<thead>
<tr>
<th>Compound</th>
<th>Day 0</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CF₃CF₂CF₂COOH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heptafluorobutyric acid (HFBA)</td>
<td>0.003</td>
<td>0.017</td>
</tr>
<tr>
<td><strong>CF₃(CF₂)₆COOH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfluorooctanoic acid (PFOA)</td>
<td>0.045</td>
<td>0.064</td>
</tr>
<tr>
<td><strong>C₄HF₉O₃S</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfluorobutane sulfonic acid (PFBS)</td>
<td>0.007</td>
<td>0.021</td>
</tr>
<tr>
<td><strong>C₈HF₁₇O₃S</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perfluorooctane sulfonic acid (PFOS)</td>
<td>0.029</td>
<td>0.068</td>
</tr>
<tr>
<td><strong>C₈H₅F₁₃O</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6:2 Fluorotelomer Alcohol (6:2 FTOH)</td>
<td>0.085</td>
<td>0.019</td>
</tr>
<tr>
<td><strong>C₁₀H₅F₁₇O</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:2 Fluorotelomer Alcohol (8:2 FTOH)</td>
<td>0.14</td>
<td>0.040</td>
</tr>
<tr>
<td><strong>C₈H₅F₁₃O₃S</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6:2 Fluorotelomer sulfonate (6:2 FTS)</td>
<td>0.066</td>
<td>0.014</td>
</tr>
<tr>
<td><strong>C₁₀H₄F₁₇O₃S</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:2 Fluorotelomer sulfonate (8:2 FTS)</td>
<td>0.033</td>
<td>0.021</td>
</tr>
</tbody>
</table>
Short-term incubation results of PFAS contaminated sediments augmented with ferrihydrite

<table>
<thead>
<tr>
<th>Concentration (mg/l)</th>
<th>Day 0</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>F^-</td>
<td>3.9</td>
<td>14.3</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>43.2</td>
<td>271.1</td>
</tr>
<tr>
<td>Acetate</td>
<td>20.9</td>
<td>37.2</td>
</tr>
<tr>
<td>NH$_4^+$</td>
<td>33.4</td>
<td>7.2</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>24.3</td>
<td>168.9</td>
</tr>
</tbody>
</table>
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, Fe₃O₄)?
- **The Challenge:** Fe(III):NH₄⁺ = 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
  DOI: 10.1128/AEM.02029-18
In microbial electrolysis cells (MECs), apply an external potential

\[
\text{NH}_4^+ + 3\text{Fe}_2\text{O}_3 \cdot 0.5\text{H}_2\text{O} + 10\text{H}^+ \rightarrow \text{NO}_2^- + 6\text{Fe}^{2+} + 8.5\text{H}_2\text{O}
\]

Reaction without Fe(III):

\[
\text{NH}_4^+ + 2\text{H}_2\text{O} \rightarrow \text{NO}_2^- + 3\text{H}_2 + 2\text{H}^+
\]

(Call and Logan, 2011)
E-SEM image of graphite anode of MEC operated with live A6

~2.8 \times 10^4 \text{ cells/cm}^2 vs. 2.11 \times 10^3 \text{ 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 dehalogenases that can defluorinated PFAS, including PFOA and PFOS.
- We can bioaugment aquifer and wetland soils with A6 to enhance Feammox activity ($\text{NH}_4^+$ oxidation, TCE, PFAS degradation).
Thank You!

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