Bacterial Nitrate Reduction Contributes to the Geochemical Oxidation of Iron and Subsequent Remediation from Acidic Abandoned Mine Drainage

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Coal-Mining in Pennsylvania

Began 1700s, unregulated until 1945

Abandoned legacy mines = Abandoned Mine Drainage (AMD)

- ~11,000 mines
- ~5,000 km of streams
- $29.1 billion recreation industry
- $15-50 billion to remediate

Soluble Metal Contamination

Iron

- Poisonous
- Associated with CA
- Increased infection risk
Boyce Park Passive Remediation of AMD

- Acidic Conditions Fe(II) Oxidation Microbial Driven (Bird et. al 2011)
  - Iron Oxidizing Bacteria (FeOB)
  - *Nitrate Dependent Iron Oxidizing (NDFO)*?

- Biogeochemical/Microbial Influence?

<table>
<thead>
<tr>
<th>Iron (PPM)</th>
<th>EPA Limits</th>
<th>Boyce</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mine Effluent</td>
<td>Aquatic Life</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Bioremediation
Remediation improved through microbes

Iron Remediation in Acidic AMD

Bioremediation
Remediation improved through microbes

Microbial Bioremediation
↓ Contamination
Positive (+) Impact
Precipitate Metals
Iron Oxidation in AMD

<table>
<thead>
<tr>
<th>Iron Oxidation (FeOx)</th>
<th>AMD pH</th>
<th>Oxygen</th>
<th>Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic</td>
<td>Aerobic</td>
<td>Iron Oxidizers (cytochromes?)</td>
<td></td>
</tr>
</tbody>
</table>

1. Fe²⁺ → Iron Oxidase → Fe³⁺

Neutral Nitrate Dependent Iron Oxidation (n-NDFO)

<table>
<thead>
<tr>
<th>Neutral Nitrate Dependent Iron Oxidation (n-NDFO)</th>
<th>pH</th>
<th>Anaerobic</th>
<th>Meteorism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral</td>
<td>Anaerobic</td>
<td>Undetermined (cytochromes? napAB?)</td>
<td></td>
</tr>
</tbody>
</table>

1. Fe²⁺ → Iron Oxidase → Fe³⁺

2. NO₃⁻ → Nitrate Reductase → NO₂⁻

Acidic NDFO (a-NDFO) / Nitrate Reduction indirect Iron Oxidation (NRiFO)

<table>
<thead>
<tr>
<th>Acidic NDFO / Nitrate Reduction indirect Iron Oxidation (NRiFO)</th>
<th>pH</th>
<th>Anaerobic</th>
<th>Meteorism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic</td>
<td>Aerobic</td>
<td>Nitrate Reducers (napAB)</td>
<td></td>
</tr>
</tbody>
</table>

1. NO₃⁻ → Nitrate Reductase → NO₂⁻

2. Fe²⁺ → Fe³⁺

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1. Fe²⁺

2. NO₃⁻
Frequency of **Iron Oxidation (FeOx)** & **acidic Nitrate Dependent Iron Oxidation (a-NDFO)** at Boyce Park

Single colonies inoculated into a 96-well plate

Ferrozine assay measure Fe$^{2+}$

Each pond screened

- 570 FeOx
- 570 NDFO

Boyce System 9,120 screens

- **193 FeOx positive** / 4,560 FeOx screens
- **125 a-NDFO positive** / 4,560 a-NDFO screens
### Identification of NRFO Bacterial Isolates as *Paraburkholderia sp.*

<table>
<thead>
<tr>
<th>NDFO Isolate</th>
<th>16S <em>rrn</em> % Identification</th>
<th>Iron Oxidation</th>
<th>Nitrate Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV18</td>
<td>100% <em>Paraburkholderia sp</em>¹</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>AV25</td>
<td>99% <em>Paraburkholderia sp</em>²</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>AV26</td>
<td>99% <em>Paraburkholderia sp</em>²</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

1. Whole genome sequencing of NRFO bacterial isolate AV18
2. Sanger sequencing ID NRFO bacterial isolates AV25 and AV26
Bacteria *Paraburkholderia sp. AV18* Nitrate Reduction
Concurrent Iron Oxidation

*Paraburkholderia sp. AV18*
Sterile AMD
Addition of **Nitrite** Causes **Iron Oxidation** in AMD

Spike NO₂

NO₂
Sterile AMD
Test Ability of Filtrate (+) NRFO from *Paraburkholderia sp. AV18* to Drive FeOx Abiotically

100% Sterile AMD
50% Filtrate
90% Filtrate

1st (+) NRFO Experiment

*Paraburkholderia sp. AV18*

\[ \text{NO}_2^- \rightarrow \text{Fe}^{2+} \]

DAY 0

DAY 4

Filter Sterilize (+) NRFO

Nitrite Byproduct of *Paraburkholderia sp. AV18* Drives Iron Oxidation
cDNA Confirmation that *napA* is Expressed During Nitrate Reduction

1. Grow *Paraburkholderia sp. AV18* (-) Nitrate (NaNO₃)

2. (+) 10 mM NaNO₃⁻

3. Measure NO₂⁻ Production
   - -1 m
   - 30 m
   - Negative (-) NO₃⁻ Reduction to NO₂⁻
   - Positive (+) NO₃⁻ Reduction to NO₂⁻

4. Concurrently Extract RNA over Time

5. Reverse Transcribe (RT) RNA into cDNA

6. RT-PCR

**Table:**

<table>
<thead>
<tr>
<th></th>
<th><strong>napA</strong>⁺</th>
<th></th>
<th><strong>rpoB</strong>⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Ladder</td>
<td>-1</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>Time (m)</td>
<td>-1</td>
<td>5</td>
<td>30</td>
</tr>
</tbody>
</table>

**Image:**

DNA ladder gel showing bands for each time point.
Conclusions

Microbes present within the acidic AMD at Boyce Park can aide in iron remediation.

*Paraburkholderia sp.* AV18 is capable of remediating iron via bacterial nitrate reduction *indirect iron oxidation (NRFO)* metabolism.

Novel *napA* primers indicated that the gene is expressed during nitrate reduction.

Working on paper for NRFO story
Second paper for Novel *Paraburkholderia sp.*
Thank You!!

PhD Advisor
Nancy Trun, PhD

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I’m looking for a postdoc starting Summer/Fall 2024, let’s connect!!

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