Bacterial Nitrate Reduction by *Paraburkholderia yinzercillus* Contributes to Iron Bioremediation in Abandoned Coal 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

<table>
<thead>
<tr>
<th>EPA Iron Limits (PPM)</th>
<th>Mine Effluent</th>
<th>Aquatic Life</th>
<th>Drinking Water</th>
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<tbody>
<tr>
<td></td>
<td>7.0</td>
<td>1.0</td>
<td>0.3</td>
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</tbody>
</table>

Acidic Conditions Fe(II) Oxidation Microbial Driven (Bird et. al 2011)
Microbial Iron Oxidation = Iron Bioremediation in AMD

Iron Oxidation (FeOx)

Iron Oxidation (FeOx) biotic

Iron Oxidase

Fe^{2+} \rightarrow Fe^{3+}

Nitrate Dependent Iron Oxidation (NDFO)

Nitrate Dependent Iron Oxidation (NDFO) biotic

Nitrate Reductase

Fe^{2+} + NO_3^- \rightarrow NO_2^- + Fe^{3+}

Microbial Bioremediation

↓ Contamination (+) Impact Precipitate Metals

Remediation

No Moderate High

Oxidation Loss of Electron

Reduction Gain of Electron
Iron Oxidation (FeOx) & Nitrate Dependent Iron Oxidation (NDFO) Frequency at Boyce Park

- Slurry from Boyce plated for single colonies
- Single colonies inoculated into a 96-well plate

Colorimetric ferrozine assay measures Fe$^{2+}$ + NaNO$_3$ = NDFO

- Purple = Fe$^{2+}$
- Clear = (+) FeOx/NDFO

1 well = 1 single screen
Each pond 570 screens FeOx & NDFO
Boyce System 4,560 screens FeOx & NDFO
9,120 total screens

2.1% - 11.4% FeOx in all ponds of the system
1.4% - 6.0% NDFO in all ponds of the system
**Paraburkholderia yinzercillus Nitrate Reduction Concurrent Iron Oxidation**

*Paraburkholderia* grown overnight in R2A

Inoculate into Sterile Synthetic AMD
10% R2A pH 4.0
5 mM NaNO$_3$

**Increased Fe Contamination**

**Decreased Fe Contamination**

From samples inoculated with *Paraburkholderia yinzercillus*, produce nitrite, decrease Fe(II). Bacterial growth is detected over this time period and the pH of the AMD remains acidic.

**NRBO**
Does not happen without NO$_3$ added
Not observed in HK *yinzercillus*
Not observed in -NR isolate

Chemical NaNO$_3$ added to Sterile AMD results in FeOx
*Paraburkholderia yinzercilllus* expresses *napA* during nitrate reduction

*Paraburkholderia* grown overnight in R2A

- Negative for NO₂
  - Addition of NaNO₃
  - Incubated until (+) for NO₂ byproduct
  - Extract RNA
  - Reverse Transcribe cDNA
  - PCR

novel *napA* and *rpoB* primer sets

Image
Nitrite Byproduct of *Paraburkholderia yinzercillus* Drives Iron Oxidation

*Paraburkholderia* grown overnight in R2A

- Negative for NO₂
- Addition of NaNO₃
- Incubated until (+) for NO₂ byproduct
- Filter sterilized with 0.2 μM filter

**50% Filtrate**

**90% Filtrate**

Measure soluble Fe²⁺

Ferrozine Assay

**Fe(II) % change** when bacterial byproduct NO₂ added

- From Day 0 to Day 7
  - S. B1 decreased by 2% ± 5%
  - 50% filtrate decreased 66% ± 8%
  - 90% filtrate decreased soluble iron by 80% ± 0%

\[ n = 3 \]

Two factor ANOVA with replication

- Significant difference between groups (Sterile v. 50% v. 90%), \( p < 0.00001 \)
- Sig. diff. between days, \( p < 0.00001 \)
- Sig. diff. between the samples over time, \( p < 0.00001 \)
Burkholderiales are Present in the acidic **Boyce Park** and acidic **Middle Branch** Passive Remediation System

**Classification**
- **Domain**: Bacteria
- **Phylum**: Pseudomonadota
- **Class**: Betaproteobacteria
- **Order**: Burkholderiales
- **Family**: Burkholderiaceae
- **Genus**: Paraburkholderia
- **Species**: yinzercillus

**ASV** = amplicon sequence variant

**RStudio**
- **Dada2 pipeline**
- **Silva database v138.1**

**Burkholderiales** in all ponds
- **Boyce**: 2.80% - 9.70%
- **Middle Branch**: 3.65% - 9.97%

System sampling & MiSeq 16S by MV
Iron Oxidation in AMD

**FeOx**
- **Iron Oxidase**: $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$
- **Metabolism**: Acidic, Aerobic, **Iron Oxidizers (cytochromes?)**

**NDFO**
- **Nitrate Reductase**: $\text{NO}_3^- \rightarrow \text{NO}_2^-$
  - **Biotic**: Neutral, Anaerobic, Undetermined
  - **Abiotic**: Geochemical
  - **Metabolism**: Acidic, Aerobic, **Nitrate Reducers (napAB)**
Conclusions

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

*Paraburkholderia yinzercilllus* is capable of remediating iron via bacterial nitrate reduction chemical iron oxidation metabolism.

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

Burkholderiales are present in all ponds at Boyce Park and at Middle Branch.

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

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I’m looking for a postdoc starting early 2025, let’s connect!!
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Funding

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