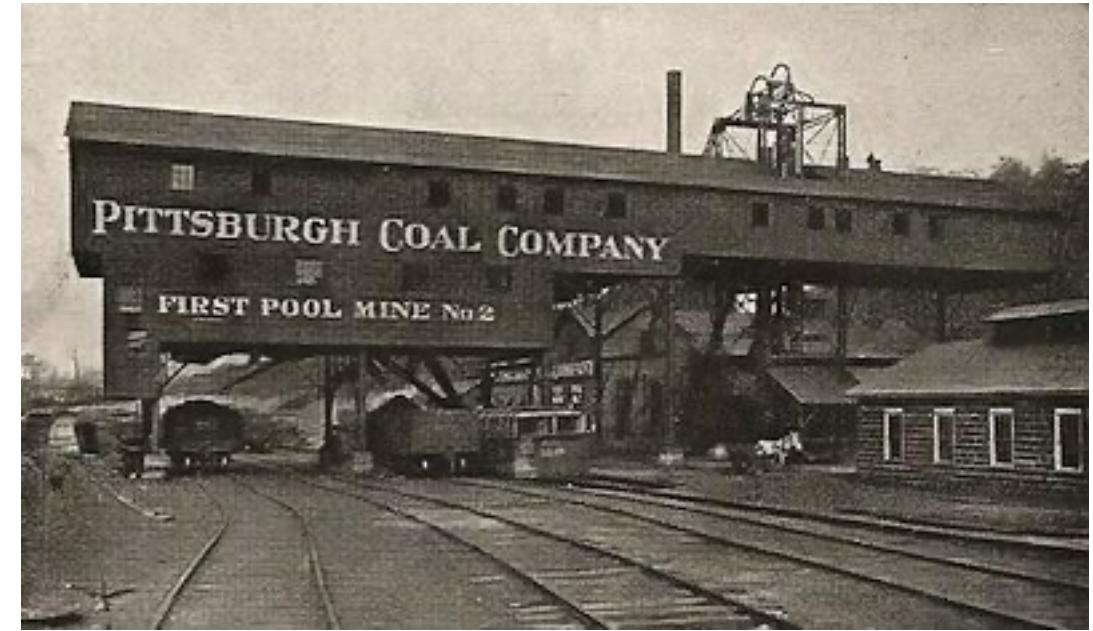


Bioremediation & Biocontamination of Abandon Coal-Mine Drainage Impacted Waters

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Duquesne Univ.
NASLR Fall 2021

Mining Pollution (Coal)

- Anthropogenic Environmental Pollution
- Pennsylvania
 - ~11K mines
 - ~5K km streams
- Until 1945 No regulations & No restoration = Abandoned Mine Drainage (AMD)
- Environmental health – water, wildlife
- Financial cost
 - Recreation (fishing, kayaking)
 - **\$15-50 Billion to Restore in PA**



Boyce Park
Passive Remediation
System



AMD Contaminants

AMD = Watershed Pollution

- Pyrite exposure to O₂ & H₂O
- Production of Sulfuric acid
- Solubilization of Heavy Metals & Metalloids

Water Quality Limits for Constituents Found in AMD			
	PME	CCC	SMCL
Iron (Fe)	7 PPM	1 PPM	0.3 PPM
Manganese	5 PPM	NA	0.05 PPM

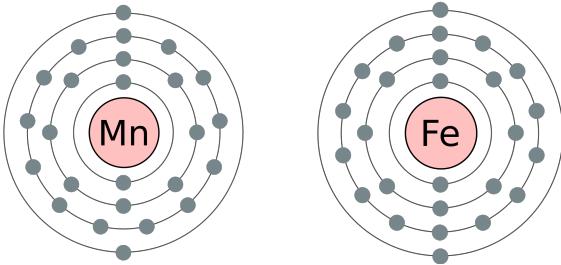
Iron

- Orange-Yellow
- Staining

Manganese

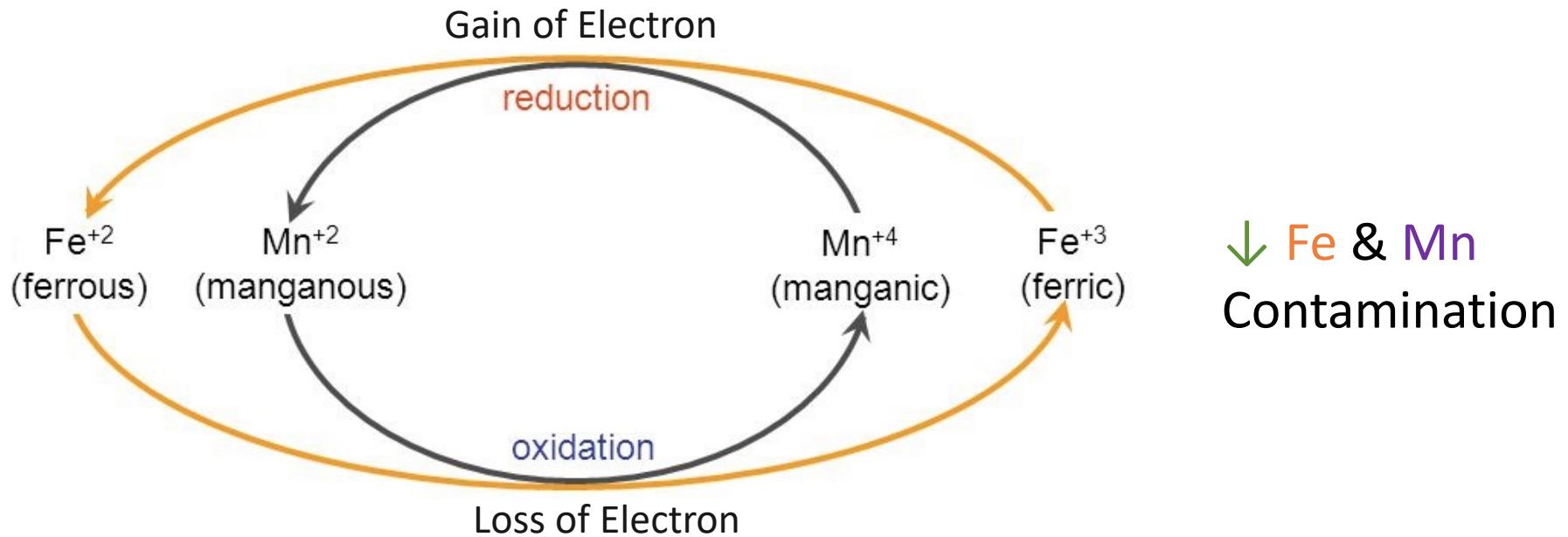
- Staining / Corrosion
- Impair DNA Replication/Repair
- Manganese toxicity
 - Cognitive Impairment & Parkinson-like Symptoms

Chemical Oxidation States & Cycling



Element	Oxidation States											
Manganese (Mn)		-3	-2	-1	0	+1	+2	+3	+4	+5	+6	+7
Iron (Fe)	-4		-2	-1	0	+1	+2	+3	+4	+5	+6	+7
Nitrogen (N)		-3	-2	-1	0	+1	+2	+3	+4	+5		

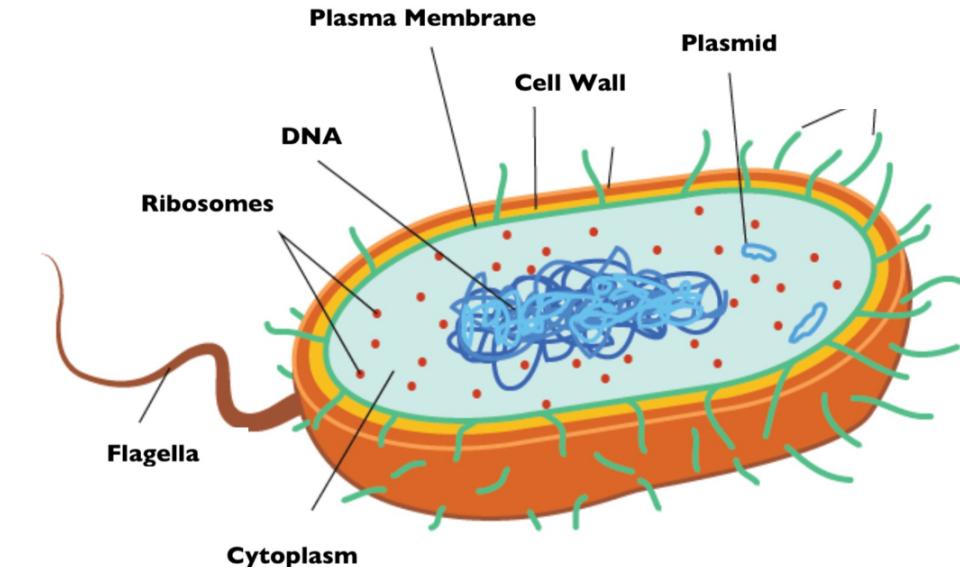
↑ Fe & Mn
Contamination



Remediation of Acidic AMD

Passive Remediation

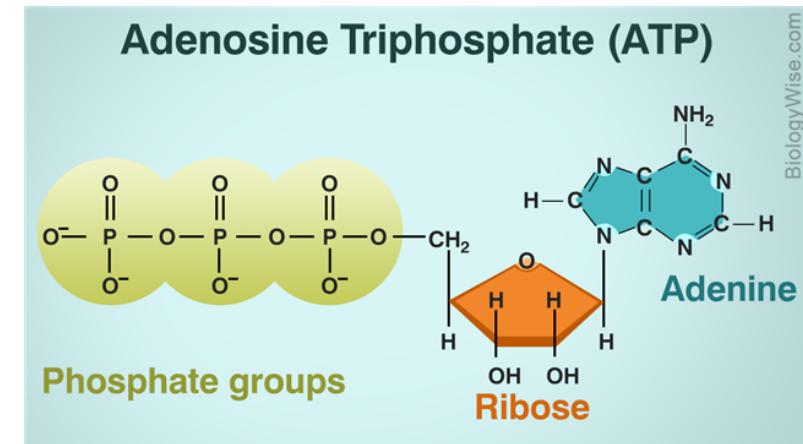
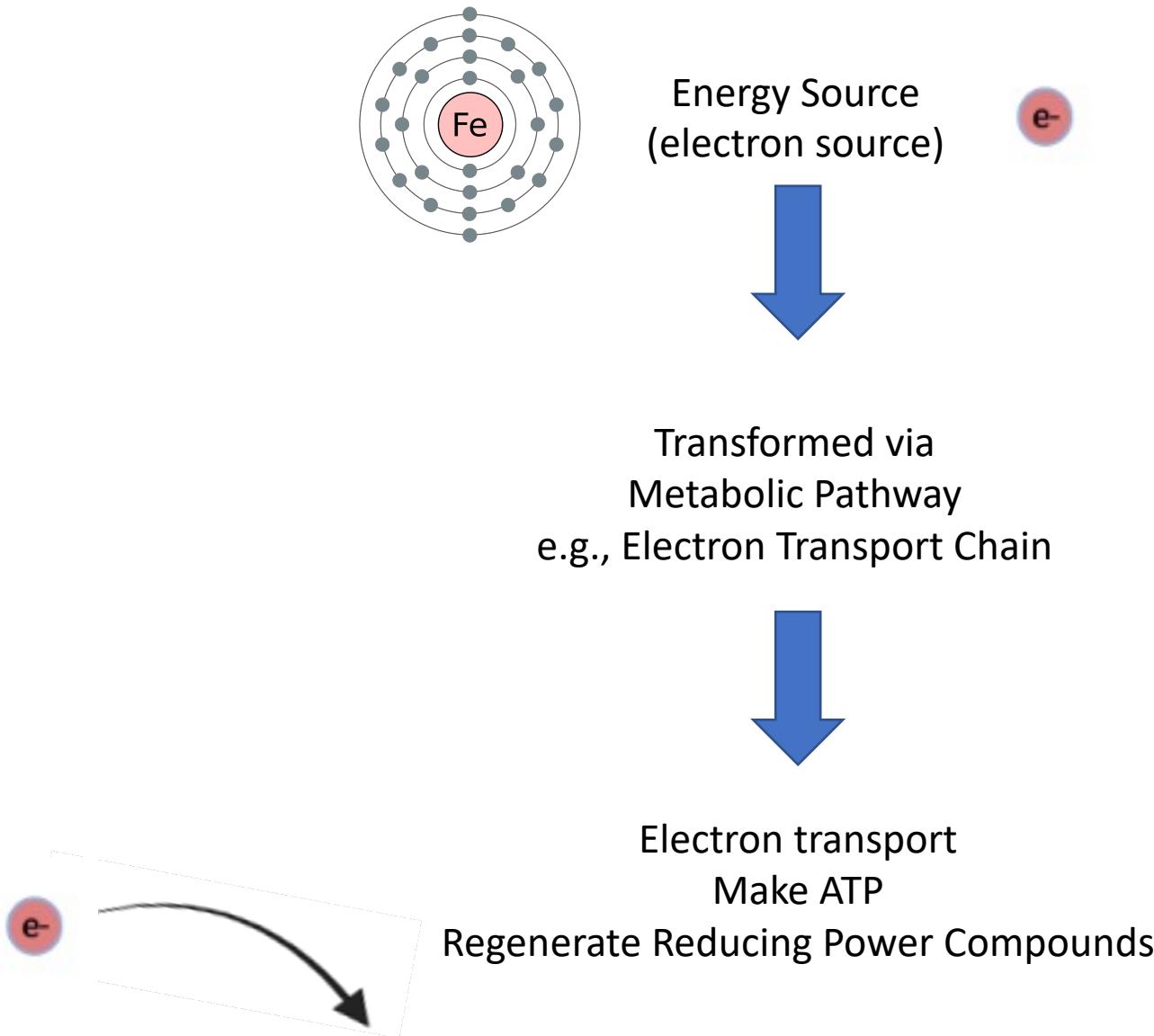
- Low \$ & maintenance long-term
- Settling ponds
- Vertical flow ponds
- Aeration fountain
- Limestone beds
- Wetlands
- Biogeochemical
 - Microbial influence



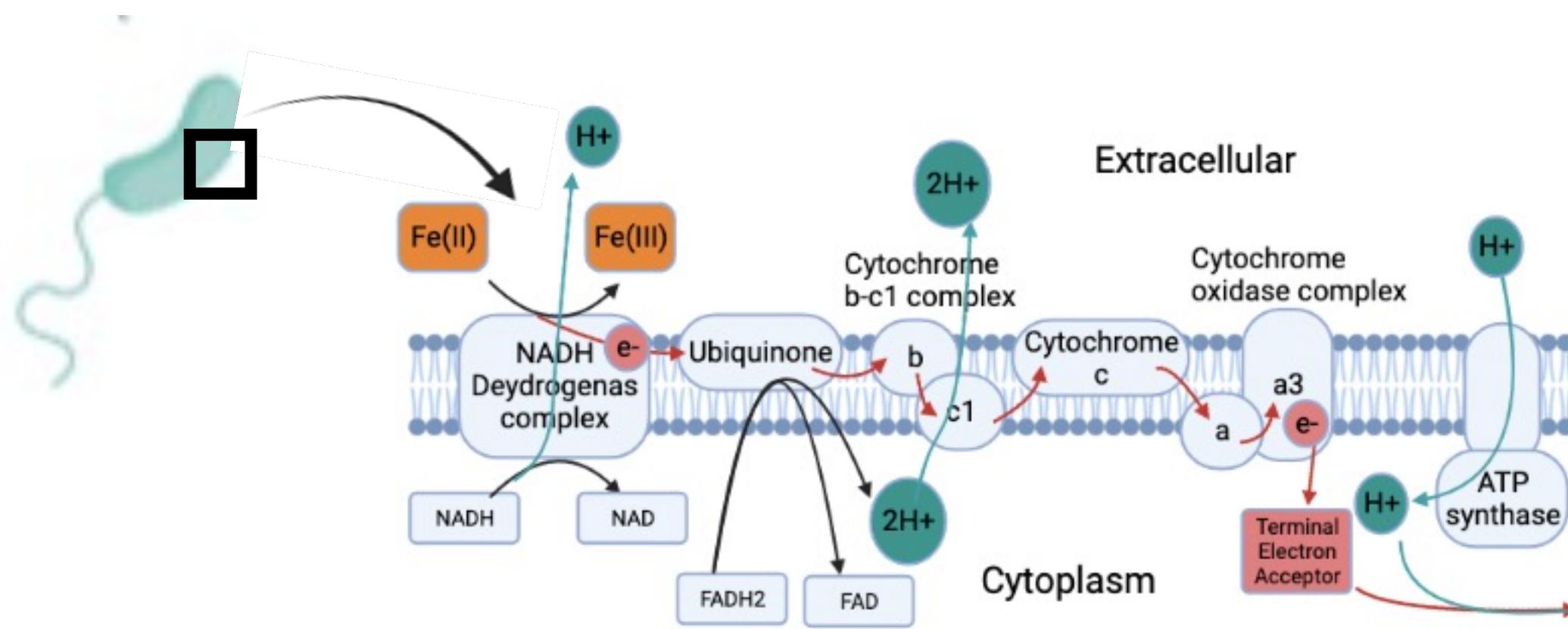
Microbes/Microorganisms

- Microscopic organism
 - Too small to be seen by the naked eye
- Bacteria, fungi, algae

Microbial Metabolism

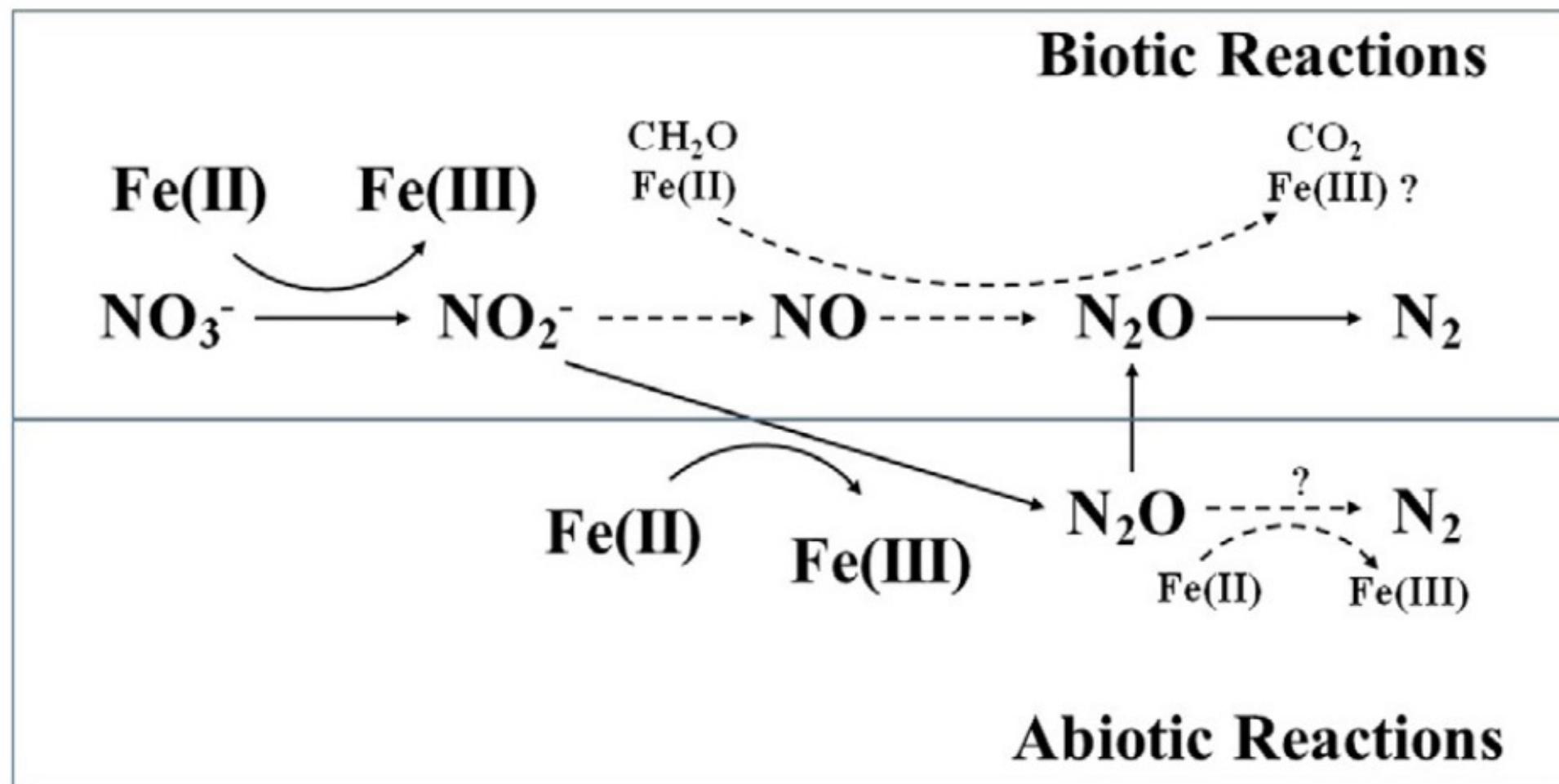


Bioremediation via Microbial Metabolism



- Electron transport chain – Oxidation (Loss of e^-)
- Microbes have many variations of cytochromes
- Cytochromes can be optimized to accept electrons from Fe & potentially

Iron & Nitrogen Coupling Redox Reactions

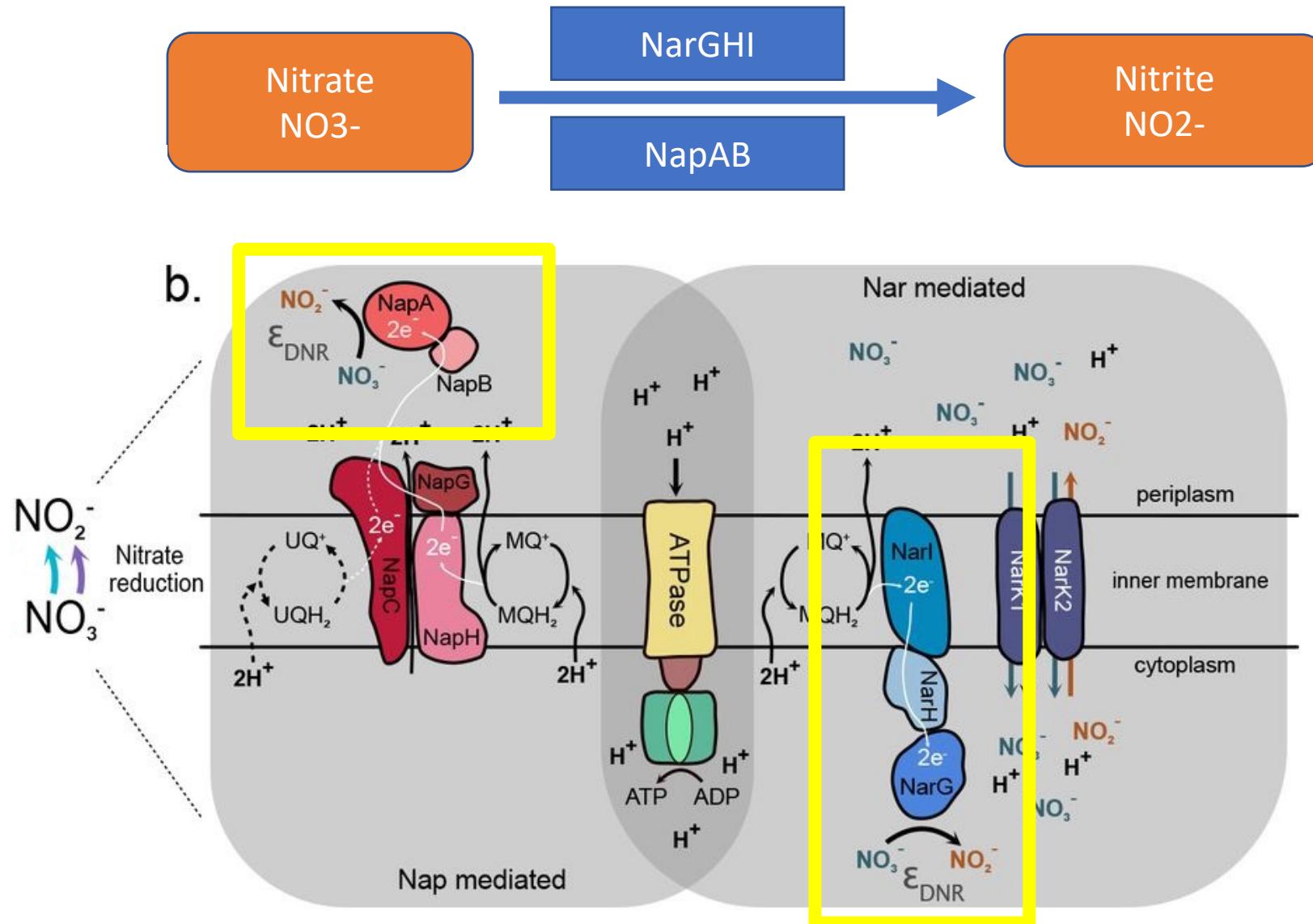


Redox reactions are coupled (Oxidation + Reduction)

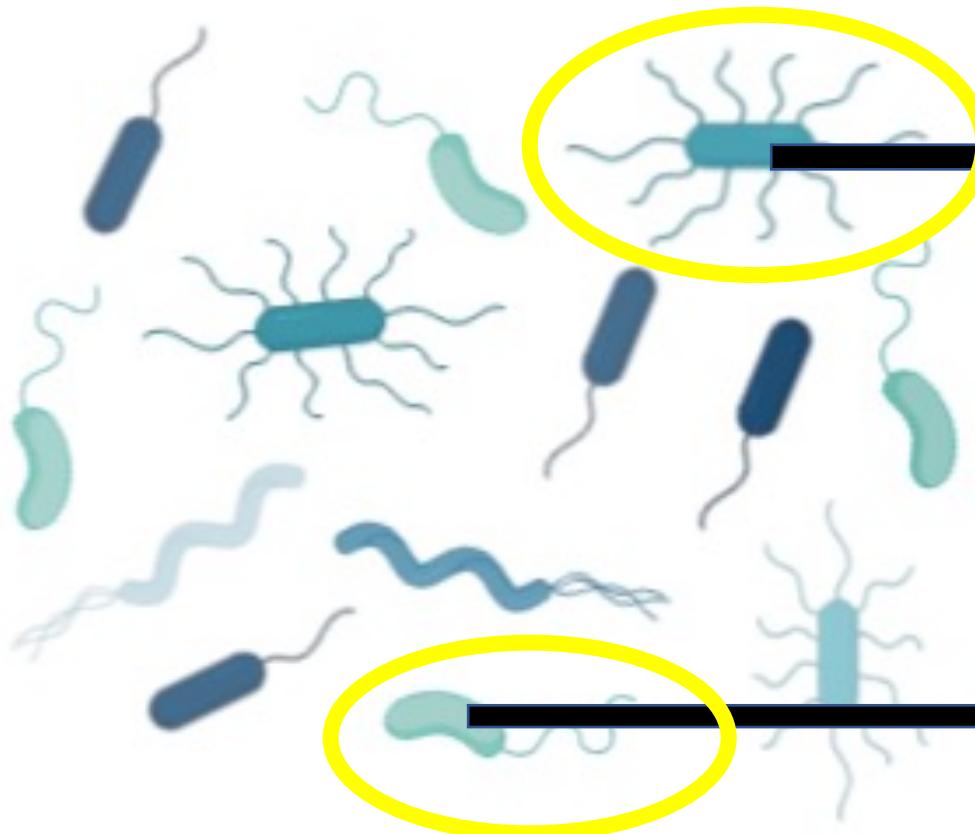
Smith et. al 2017

Iron Bioremediation & Nitrogen Metabolism

Dissimilatory Nitrate Reduction



Role of the Microbe



- **Bioremediation**
 - Remediate Toxic Sites
 - Positive Impact
 - ↓ Contamination

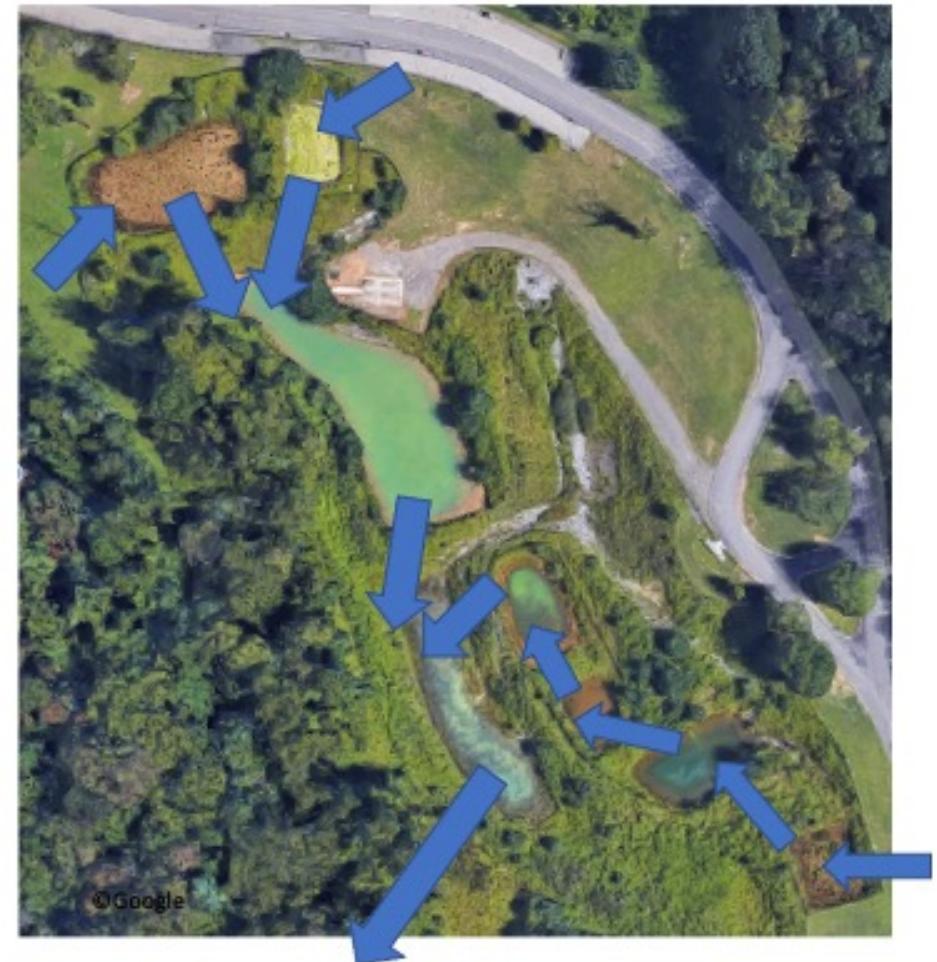
- **Biocontamination**
 - ↑ Toxicity of Polluted Sites
 - Negative impact
 - ↑ Contamination

Boyce Passive Remediation System

Ponds	Fe (PPM)	Mn (PPM)
Pond 1	4.49	0.52
Pond 2	4.04	0.60
Pond 3	4.36	0.60
Pond 4	4.75	0.64
Pond 5	1.42	0.29
Pond 6	3.69	0.45
Pond 7	3.21	0.34
Pond 8	3.87	0.46
Pond 8 - Effluent	<u>1.03</u>	<u>0.45</u>

ABOVE CCC & SMCL

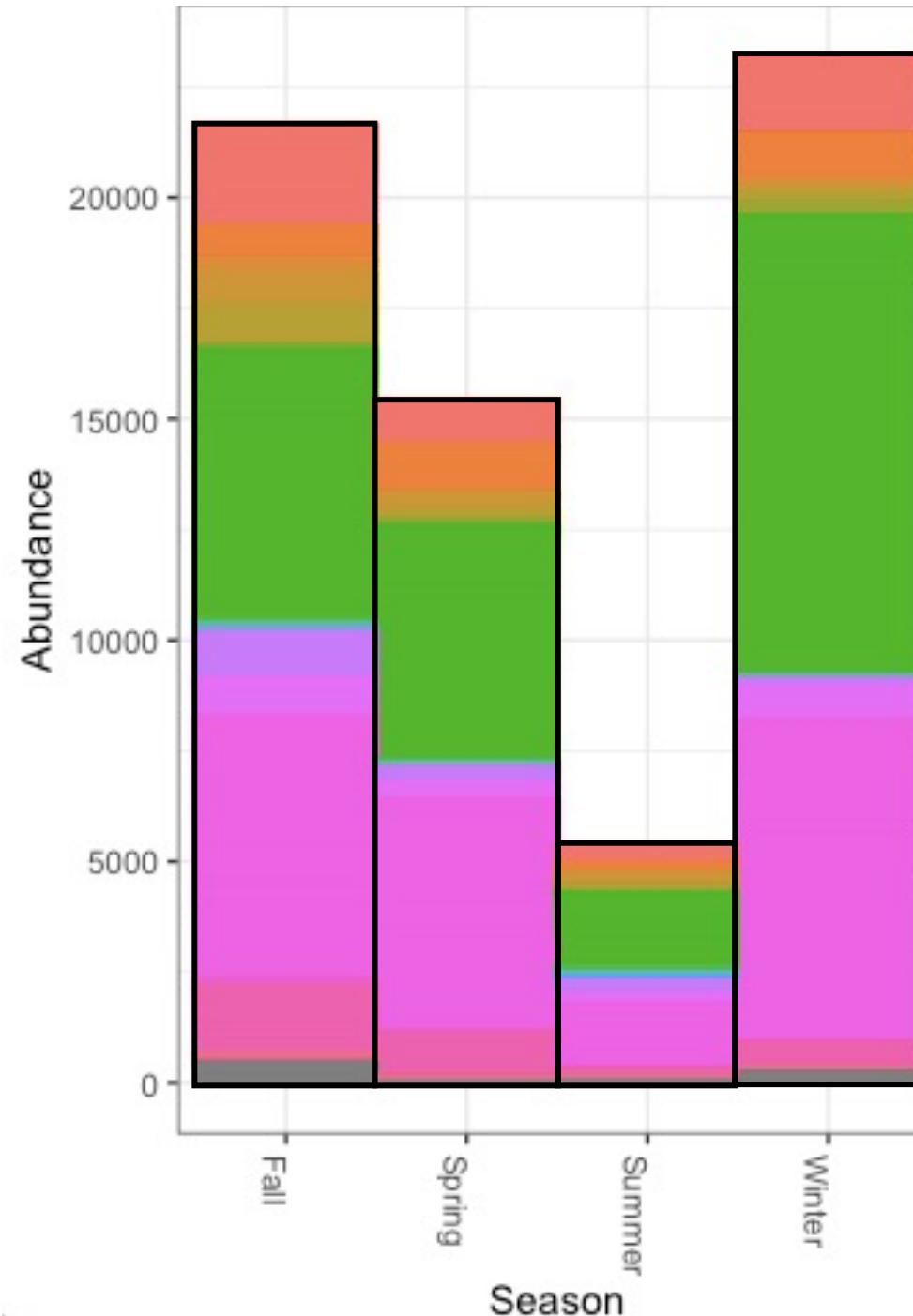
Constructed 2008 \$300K
Acidic System



Metals do not readily precipitate because of the low pH

Microbes are present in Boyce Passive Remediation System Pond 1

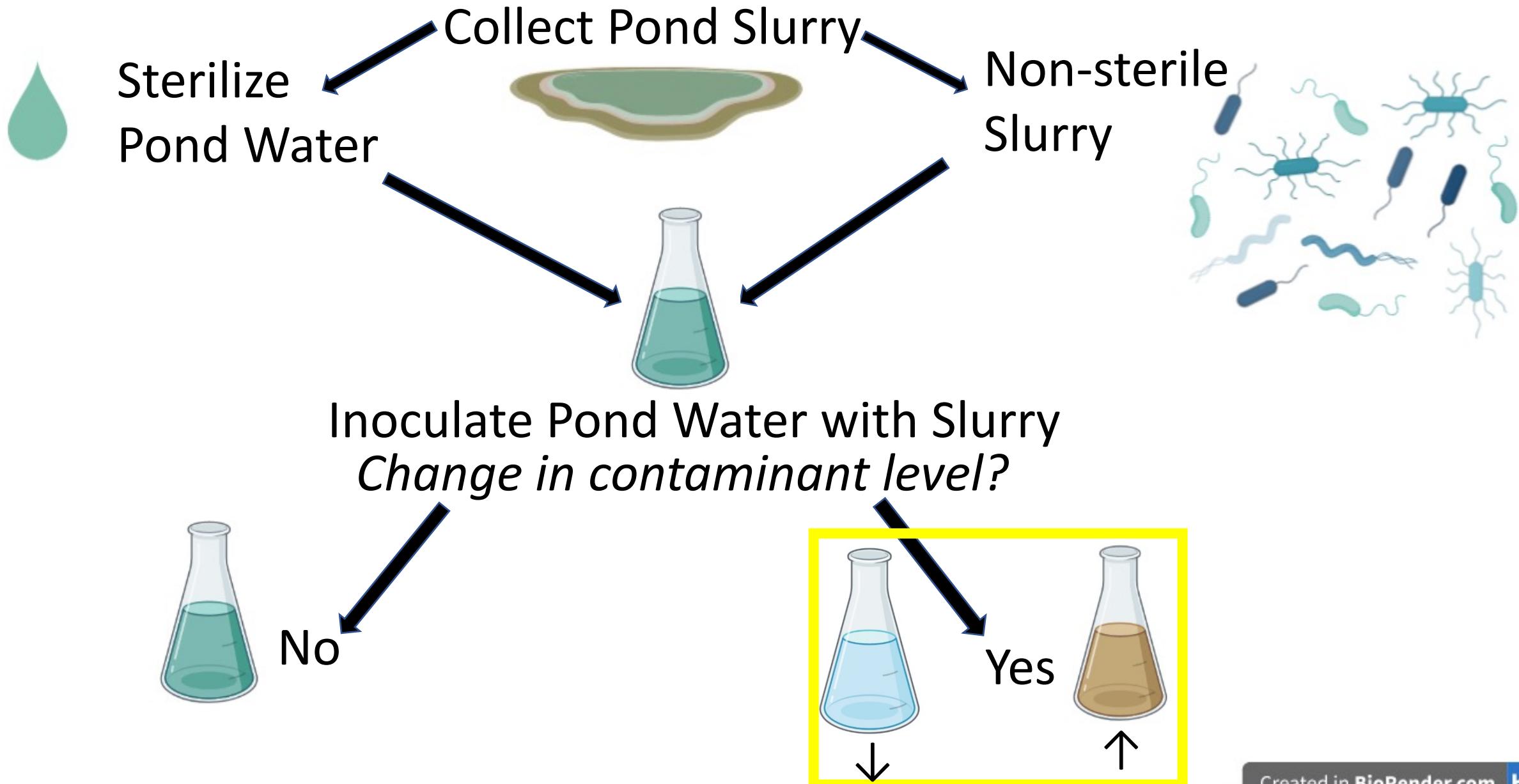
~1/3 of the microbes in the system are identified in the literature as capable of iron remediation



- Top Phylum:
- Acidobacteria
 - Actinobacteria
 - Cyanobacteria
 - Proteobacteria
 - Verrucomicrobia
 - NA

*Can we identify microbes that
impact iron and/or manganese
contamination remediation?*

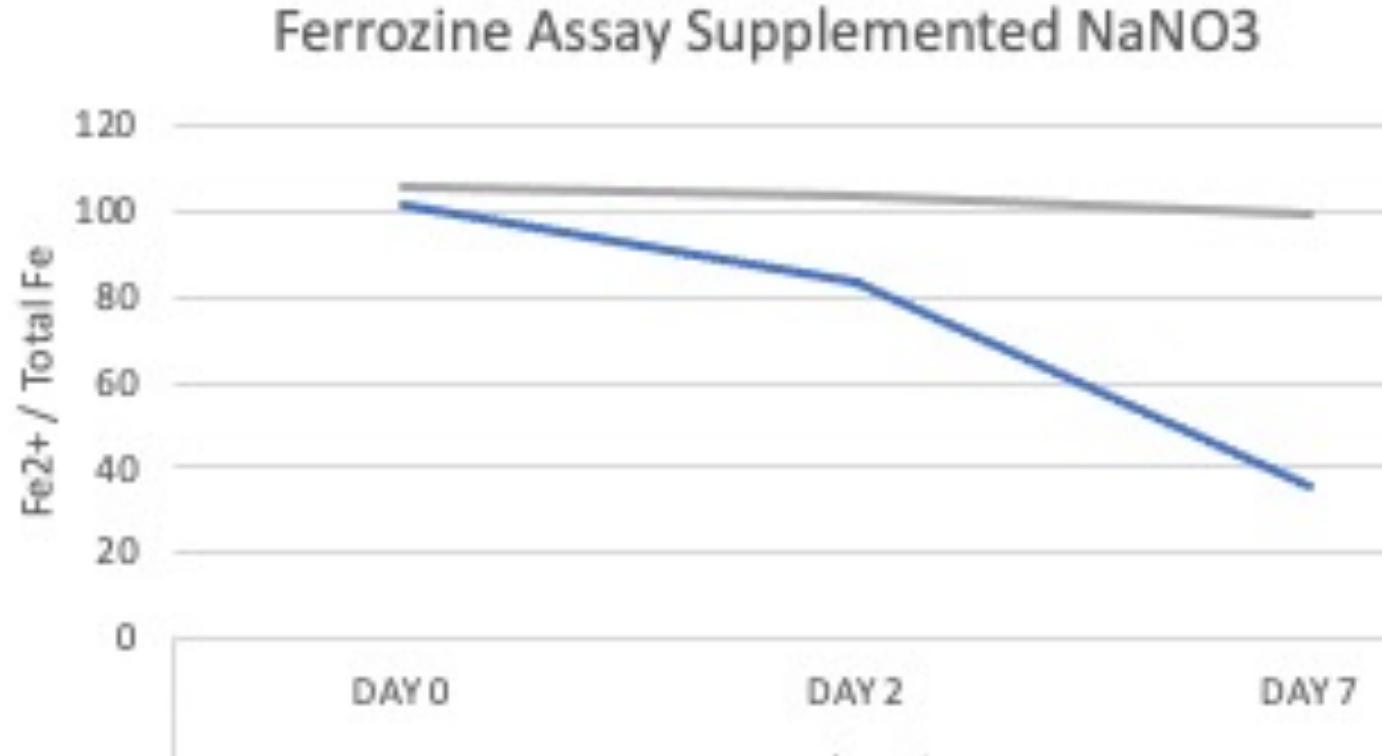
Boyce Passive Remediation System



Bacterial Isolate AV1 bioremedies iron

Increased Fe Contamination

AV1 identified as
Paraburkholderia
sp.

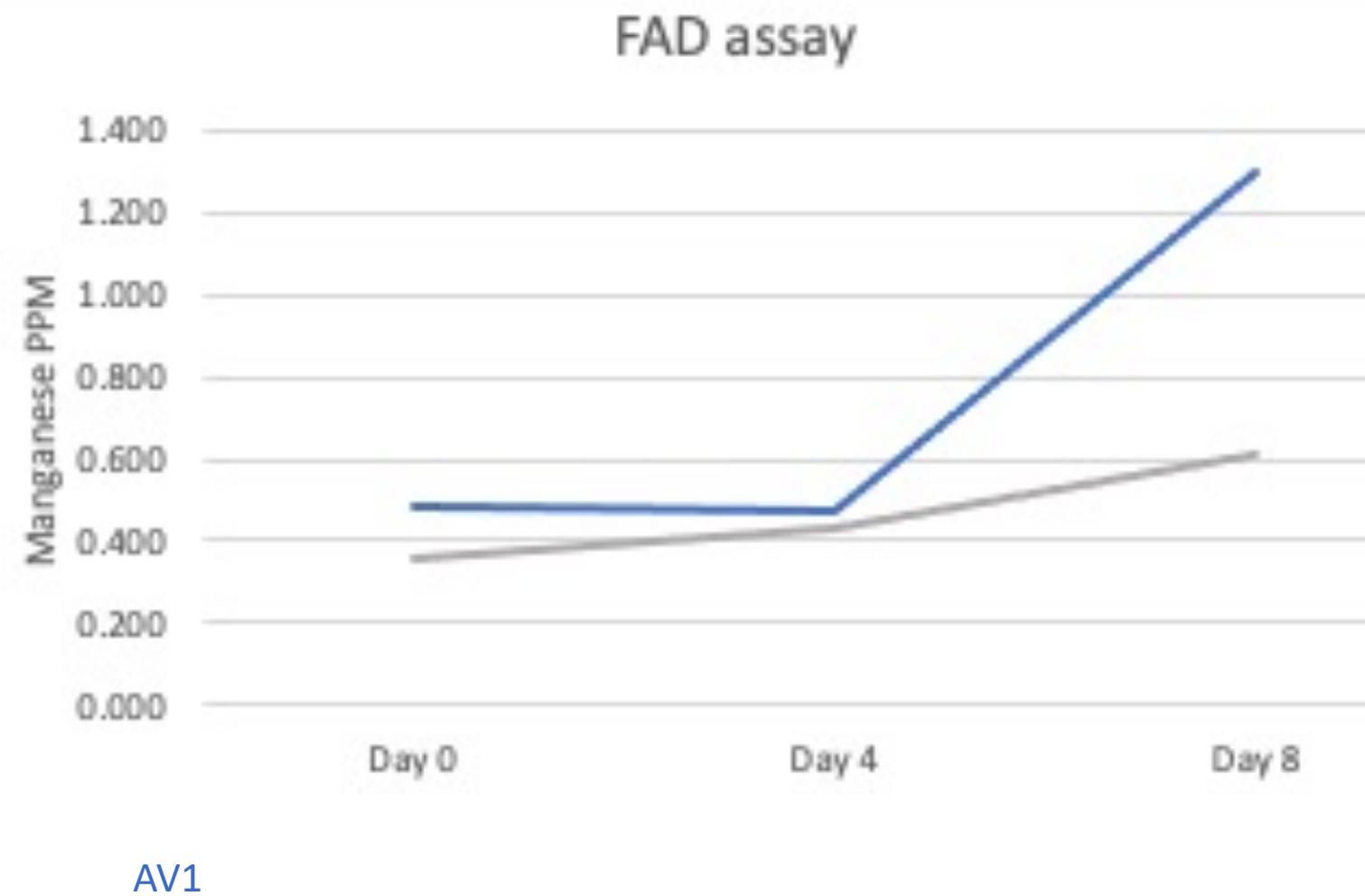


AV1

Sterile Boyce passive remediation system pond 1 water (Control)

Decreased Fe Contamination

Bacterial Isolate AV1 increases biocontamination of manganese



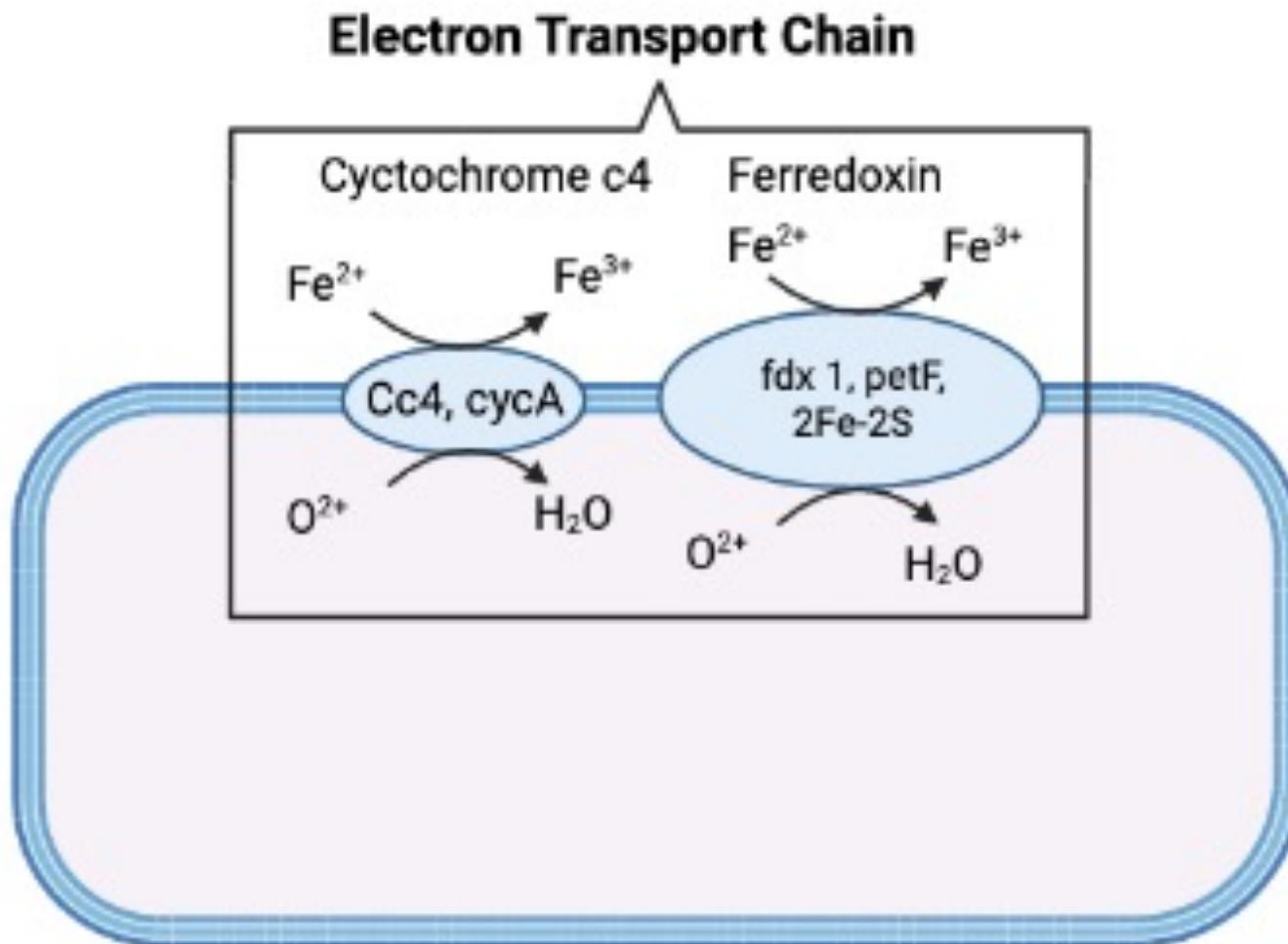
Increased Mn Contamination



Decreased Mn Contamination

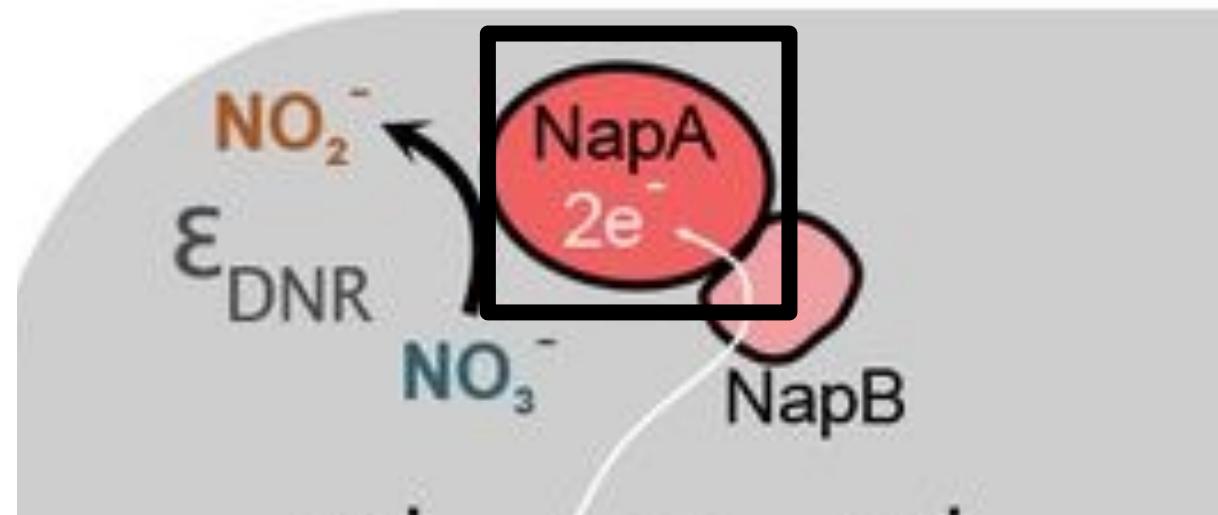


Predicted Iron Metabolism of Isolate AV1



Predicted Nitrogen Metabolism Genes

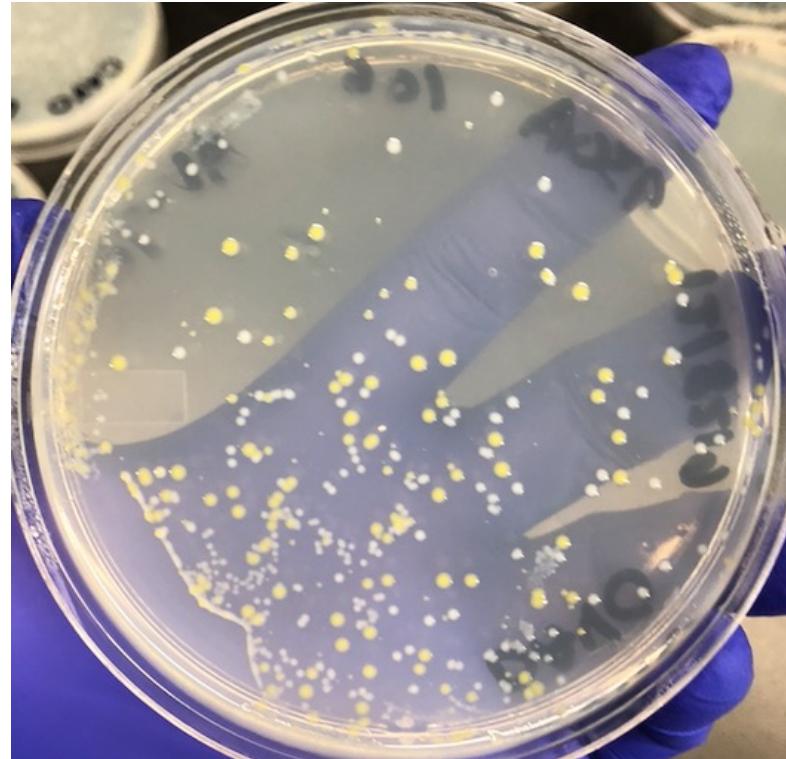
Dissimilatory Nitrate Reduction



Marietou et. al 2005
Nitrate reduction
possible lacking NapB

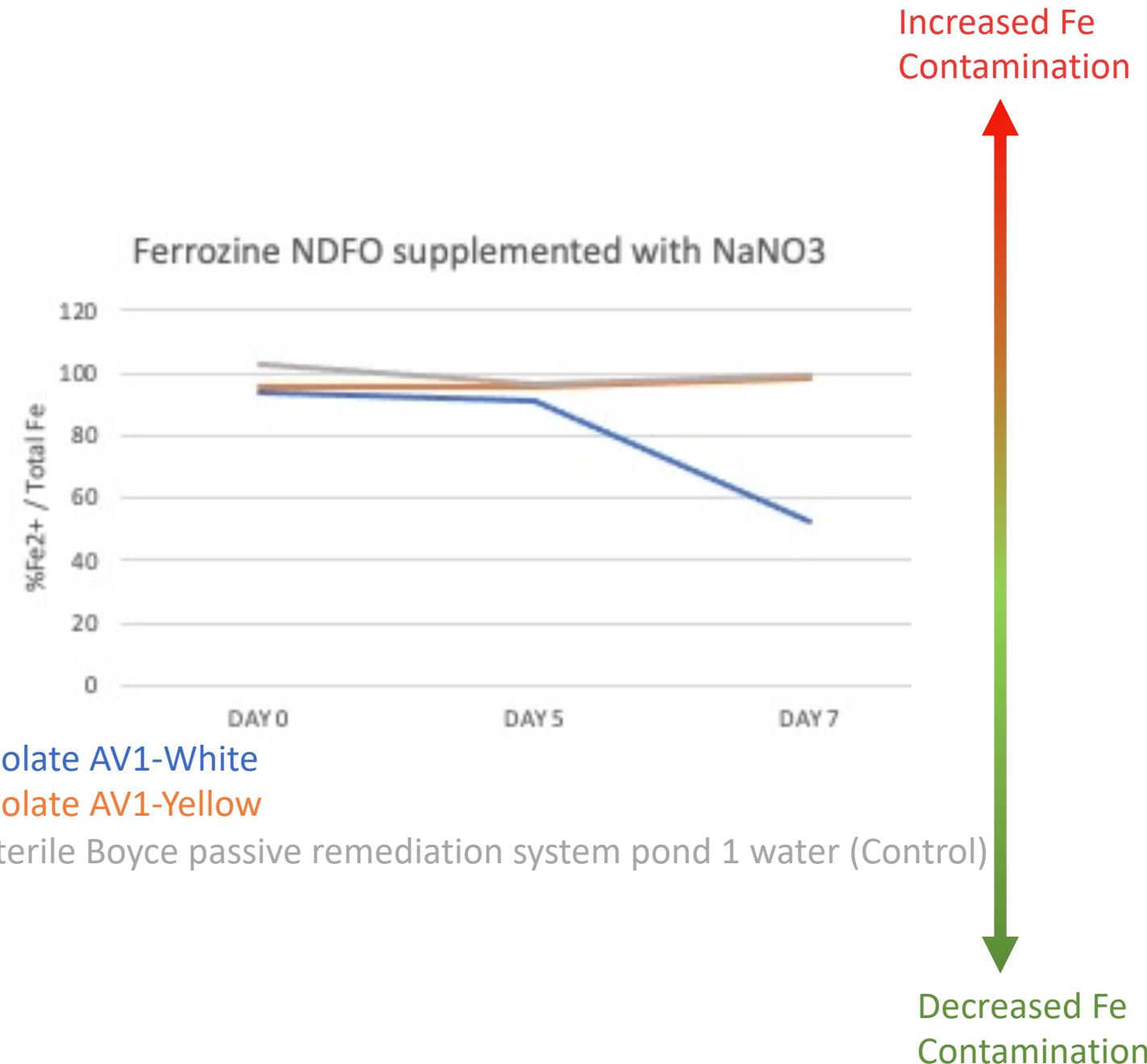
Characterization of *Paraburkholderia* sp. AV1

White & Yellow Colonies



AV1-White isolate
bioremedies iron

AV1-Yellow isolate
does not
bioremediate iron



Future Direction

- Determine identity of AV1 white & yellow colonies
- Further investigate microbial metabolism responsible for remediation & contamination
 - Study relationships between two bacteria in bioremediation
- Determine if dissimilatory nitrate reduction pathway is complete – NapB?
- Determine the abundance of these microbes in the passive remediation system – Bioindicator

Thank you!

Dr. Nancy Trun

Dr. John Stoltz

Dr. Jan Janecka

Dr. Johnathan Senko

Dr. Michelle Valkanas

Kayla Brennan

Pratham Patel

Remy Pastierik

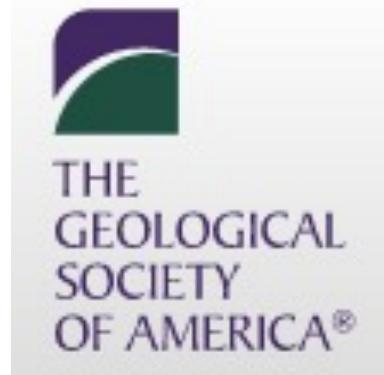
Evan Oblak

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Maria Strish

Stoltz Lab – Dr. Cantlay

Duquesne – Biology Department



National Science Foundation
WHERE DISCOVERIES BEGIN



Questions?