Microbial enhancement of selenium removal in chemically modified zeolite columns

Presenter: Ishani Kulasekara Advisors: Dr. Lambis Papelis, Dr. Yanyan Zhang Institution: NMSU

Background

Selenium (Se) is a naturally occurring metalloid and an essential nutrient for mammals. However, high selenium concentrations can lead to adverse health effects in humans and other animals.

Anthropogenic selenium sources include mines, agriculture, oil refineries, and coal-fired power plants and can result in water contamination.

Selenium speciation and redox chemistry is complex. Selenium compounds include selenides (Se^{-II}), elemental selenium (Se^{0}), selenites (Se^{+IV}), and selenates (Se^{+VI}).

Biological treatment methods have drawn attention because of efficiency, low cost, and less harm to the environment.

Research Objectives

1. To determine the sorption affinity of selenium oxyanions of different oxidation states for sodium-pretreated, iron-coated zeolite.
2. To study the effect of microbial activity on selenium oxidation state in the absence and presence of iron-coated zeolites.
3. To study the removal of selenium oxyanions in columns in the presence of microbes and iron-modified zeolites

Selenium Standards	US EPA	WHO
Drinking water	50 ppb	40 ppb
Surface water	5 ppb	

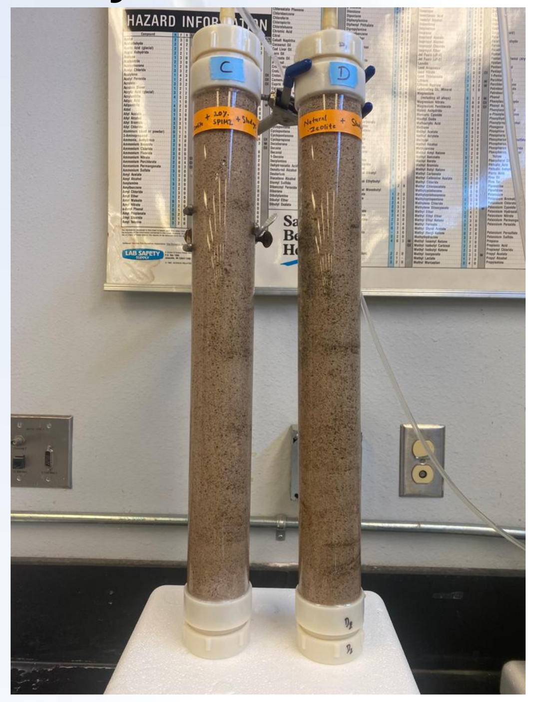
Materials and Methods

Sodium-Pretreated Iron-Modified Zeolite (SPIMZ) - The iron modification was completed by a precipitation process with ferric hydroxide. The sodium pre-treatment was completed by an exchange process with sodium chloride.

Column experiments-Dynamic sorption-reduction experiments were conducted by using two sets of columns separately for selenite and selenate oxyanions. In each case, one column was fully packed with natural zeolite while the other column was composed of 80% natural and 20% iron-coated zeolites, by mass. After packing, nutrients were injected into the columns; approximately 200 mL of sludge were added as microbial inoculum.

The initial selenium concentration (Se^{+IV} or Se^{+VI}) was 790 ppb, the pH was 7.5, and the flow rate was 3 mL/min.

Experiments were conducted under different operational conditions, including different initial selenium concentration and reduced nutrient input.



Results

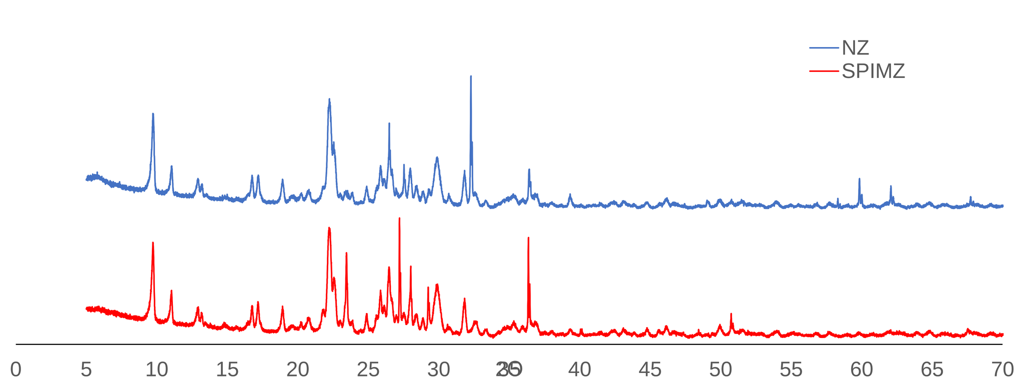


Figure 1 : XRD spectrum for natural zeolite and iron-modified zeolite

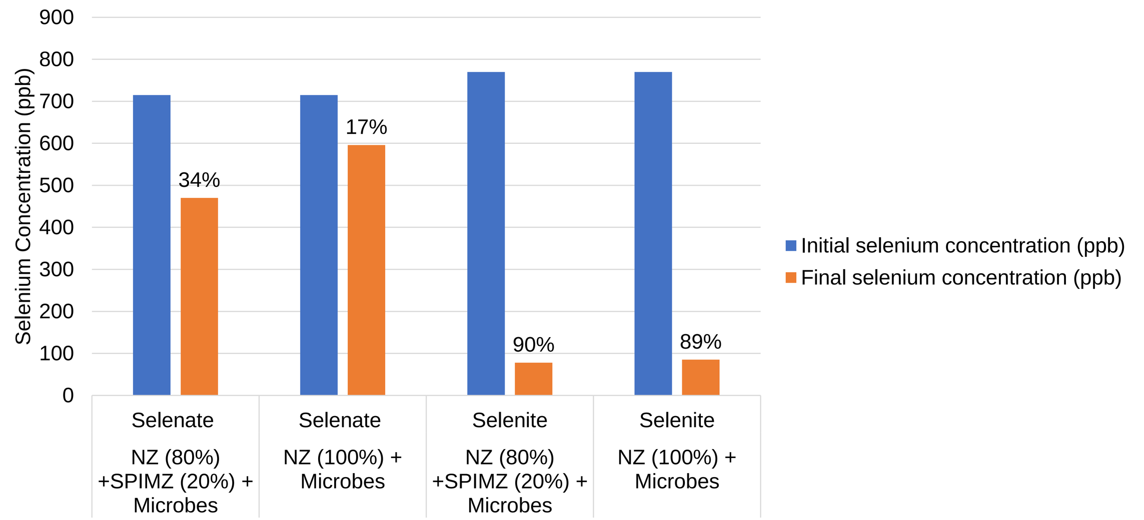


Figure 2: initial and final selenium concentration in different columns

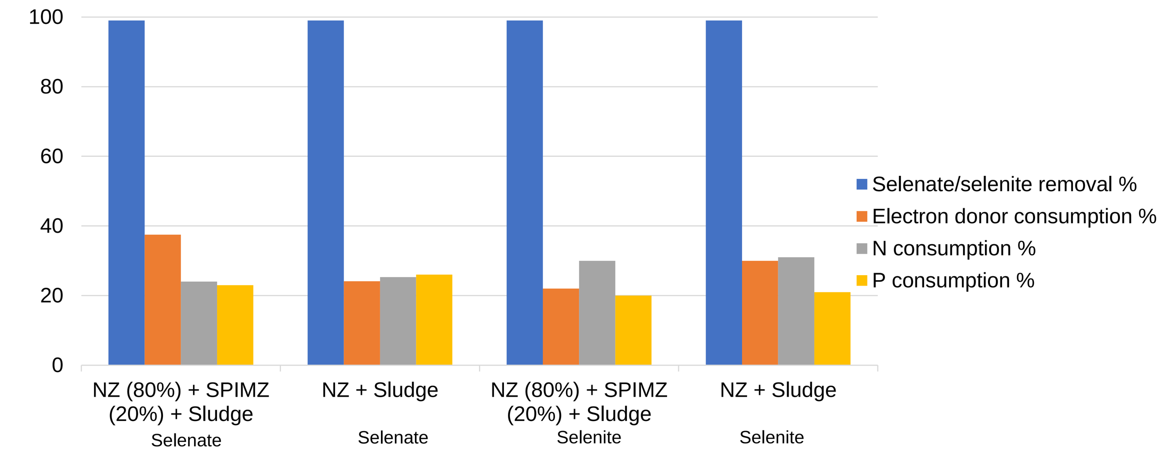


Figure 3 : Average data for different columns through 3 weeks continuous flow

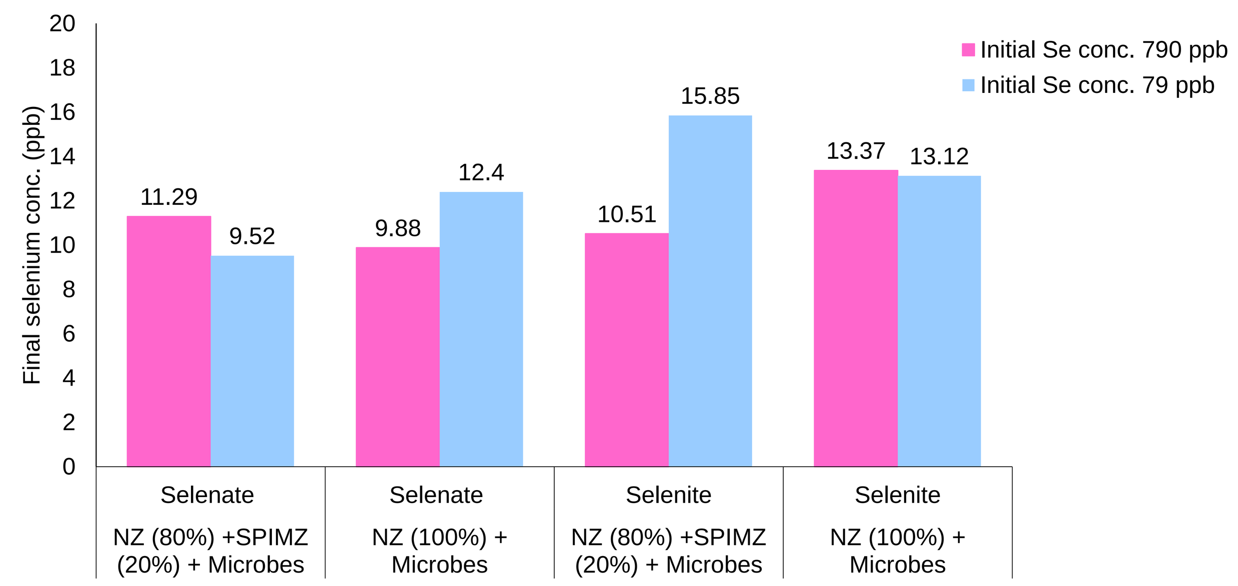


Figure 4: Effect of initial selenium concentration on selenium removal

Analysis and Conclusion

- Compared to the natural zeolite, SPIMZ XRD spectra include peak corresponding to iron oxide at 2θ values of 27°, 32.5° and 40°. The presence of iron oxide increases the sorption capacity of the zeolites for anions (Figure 1).
- At the beginning, maximum selenate removal (34%) was observed with coated zeolite, twice as high compared to the results with natural zeolite. Maximum selenite removal was 89% in the column with modified zeolite (Figure 2).
- With time, as the biofilm developed inside the columns, selenium reduction in all four columns reached approximately 99% (Figure 3).
- Regardless of the initial selenium concentration, all four columns have generated effluents which are well below the permissible level (40 ppb, WHO) (Figure 4).
- Adsorption combined with microbial reduction system is a promising method to treat selenium and generate high quality effluent.

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Acknowledgement
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Effect of Fe (III) and/or Sulfate on Microbiological Cr (VI) Reduction by Mixed Microbial Cultures

Researchers: Srivatsan Mohana Rangan
Advisors: Rosa Krajmalnik-Brown and Anca G. Delgado
Institution: Arizona State University

Thrust: Environmental Protection and Restoration
Use Case: Remediation of Soil and Groundwater
Project # 16

Introduction

- Hexavalent chromium (Cr (VI)) is a human carcinogen and mobile in the subsurface due to its high water-solubility.
- However, Cr (III) is ~1000 times less toxic and readily precipitates, making it immobile in the subsurface.
- *In situ* microbial Cr (VI) reduction and precipitation of Cr (III) compounds is a cost-effective and environmentally friendly way of soil and groundwater remediation.
- Further, *in situ* iron (Fe) and SO_4^{2-} can be bio-reduced to Fe (II) and HS^-, which as strong Cr (VI) reductants.

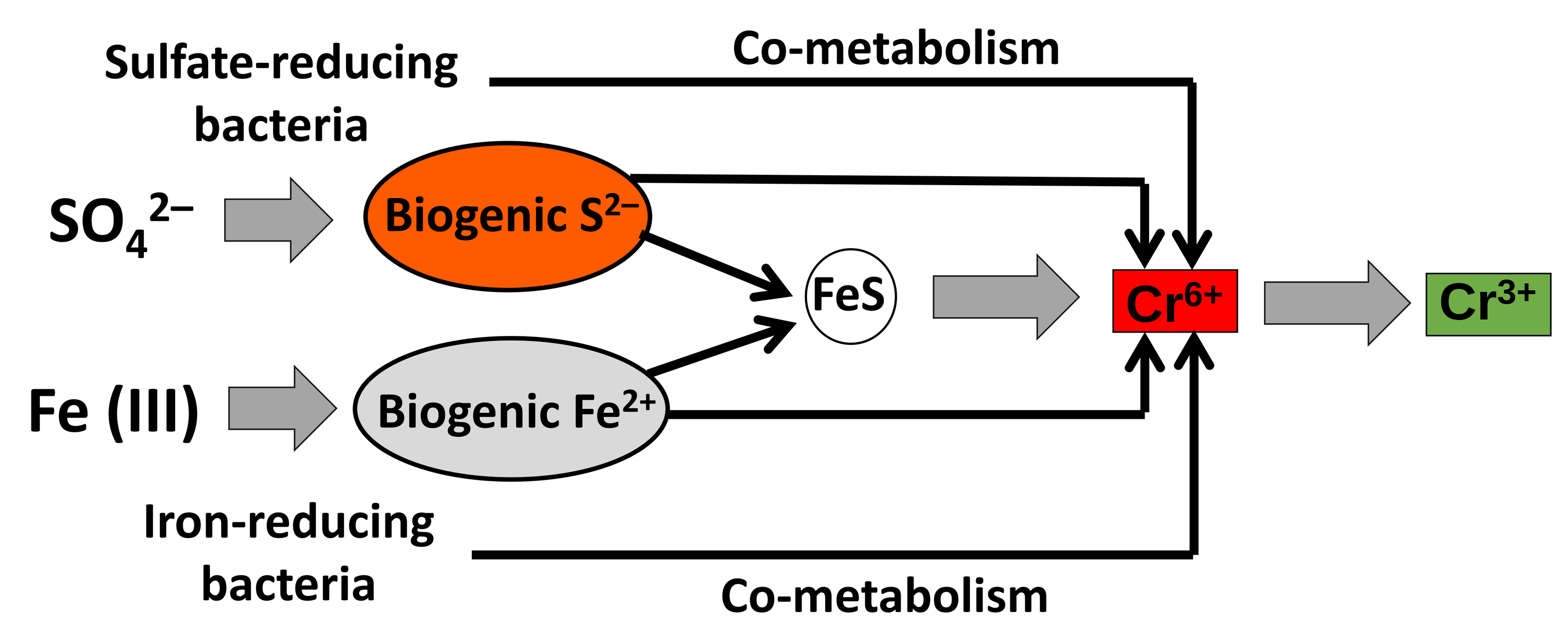
Cr (VI) co-occurrence with iron (Fe)

- Chromite ore ($FeCr_2O_4$)
- Ferrochrome alloy (FeCr)

Cr (VI) co-occurrence with sulfate (SO_4^{2-})

- Acid rock drainage
- Electroplating wastewater.

Background



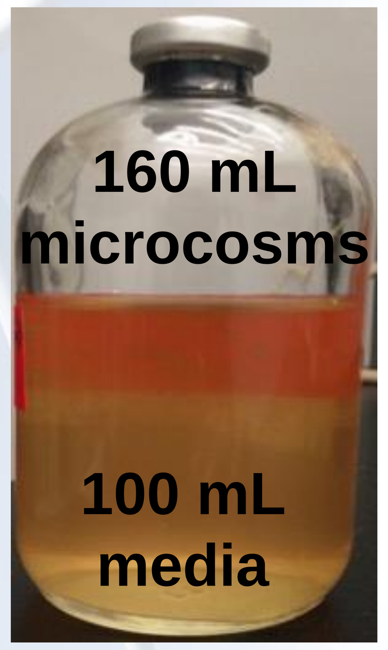
Research objectives

- To evaluate the effect of Fe (III) and/or SO_4^{2-} on microbiological Cr (VI) reduction by enriched Cr (VI)-reducing mixed cultures.

Materials and Methods

Experimental conditions in anaerobic mineral media

1. Cultures only
2. Culture + Fe (III)
3. Culture + SO_4^{2-}
4. Culture + Fe (III) + SO_4^{2-}

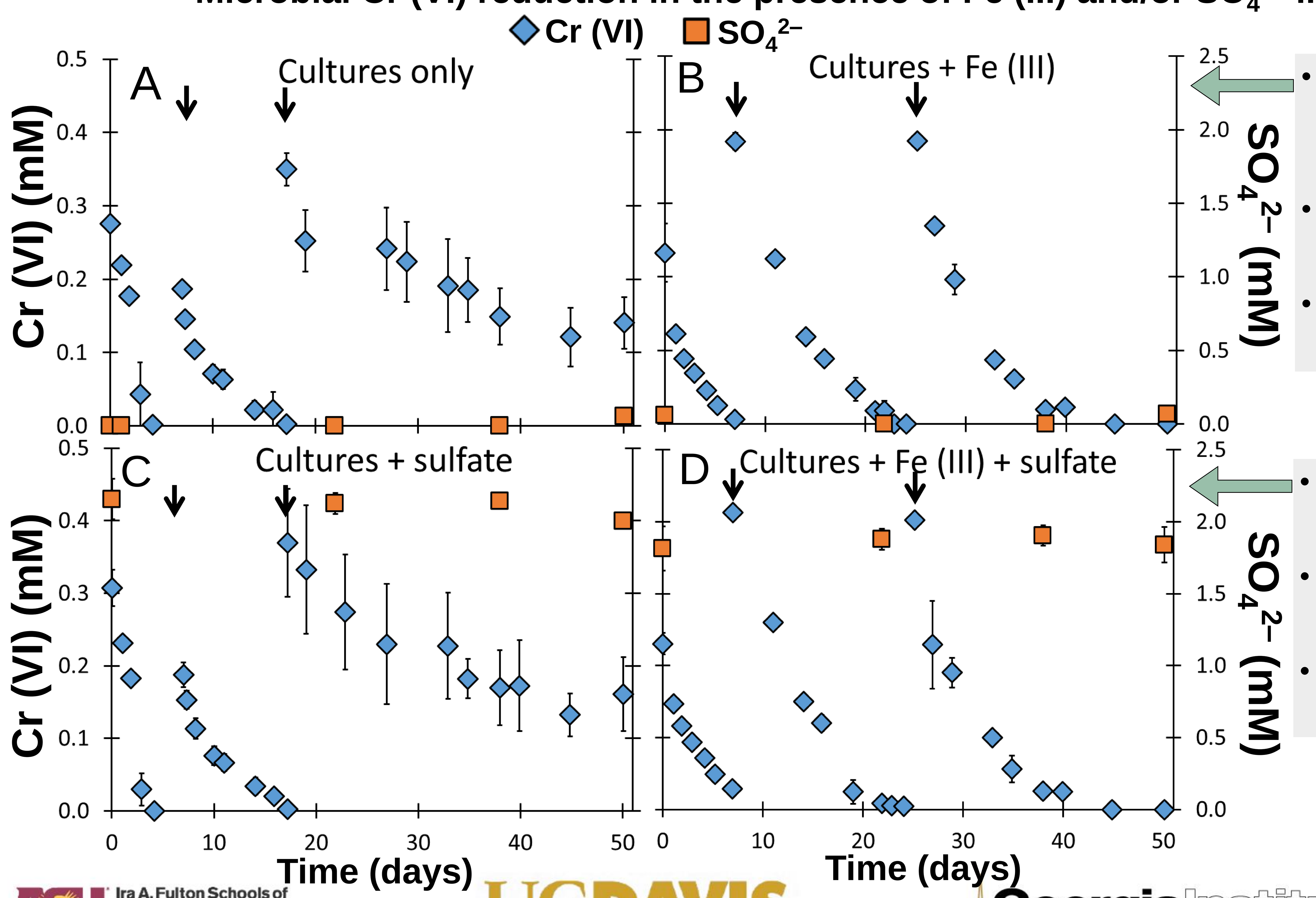


Notes

- Fe (III) (5 mM) was added as $FeCl_3.6H_2O$.
- SO_4^{2-} (2 mM) was added Na_2SO_4.
- 2 mL of each of Cr (VI)-reducing mixed cultures developed from two different sources was added to all conditions.
- Lactate (5 mM) was added as $C_3H_5NaO_3$.
- Yeast extract (500 mg/L) was added to all conditions.

Results & Discussion

Microbial Cr (VI) reduction in the presence of Fe (III) and/or SO_4^{2-} in anaerobic media



- Presence of Fe (III) reduced Cr (VI) reduction rate initially during the first 20 days.
- However, Fe (III) increased Cr (VI) reduction rate from days 25-50.
- Potentially due to generation of biogenic Fe (II) after day 25.

- Microbial SO_4^{2-} reduction was absent/negligible.
- SO_4^{2-} did not have any effect on microbial Cr (VI) reduction.
- Potentially due to lack/absence of active sulfate reducing bacteria.

Year 7 plans

Preparation for artificial soil experiments

- Iron-reducing bacteria and sulfate-reducing bacteria have been enriched separately.
- Prepared artificial soil and synthetic groundwater.

Artificial soil components

- Kaolinite
- Bentonite
- Sand
- Humic acid

Fe (III)-minerals

- Goethite (FeOOH)
- Hematite (Fe_2O_3).

Research Objective

- To study the effect of Fe (III)-minerals and SO_4^{2-} separately and combined on microbial Cr (VI) reduction by using artificial soil and synthetic groundwater.

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Field Demonstration of P and N Removal from Tile Drainage

Presenter: Michael Edgar

Advisors: Treavor Boyer, Nasser Hamdan

Institution: Arizona State University

Thrust: Environmental Protection and Restoration

Use Case: Soil and Groundwater Remediation

Project: PR42

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Background & Motivation

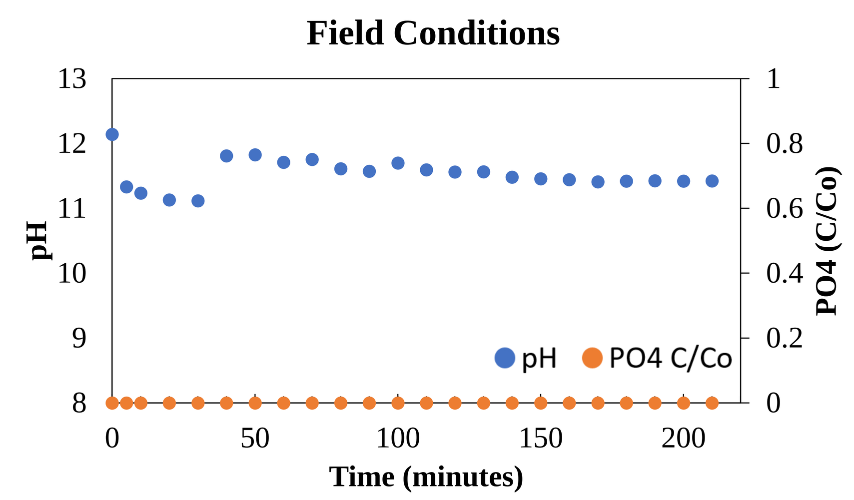
- Phosphorus (P) and Nitrogen (N) in water leads to the over-fertilization of aquatic plants, increasing the rate of eutrophication in surface waters.
- Fox Lake is receiving nutrient runoff from upstream agriculture and has been experiencing eutrophication.
- Steel slags have been shown to remove high levels of total and dissolved P in lab and field-scale demonstrations
- Slag drives P removal through a combination of adsorption and mineral precipitation, by leaching $Ca(OH)_2$, increasing pH, and forming hydroxyapetite
- Field conditions have inhibited P precipitation with sporadic flow and high alkalinity

Research Objectives

- Use a field-scale slag filter to reduce P levels in real agricultural runoff at flow rates of up to 2,000 gal/min
- Evaluate the impact of field conditions on P removal by steel slag, including high alkalinity, low/no-flow conditions, and varied slag grain size distributions.
- Evaluate N removal using constructed wetlands or biological ion exchange under the conditions produced by the steel slag filter

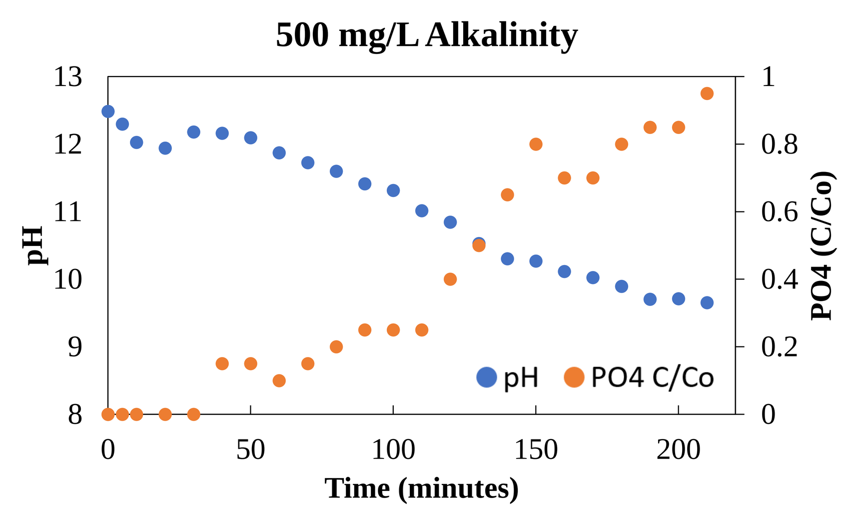
Lab-Scale Studies

Original Field Conditions

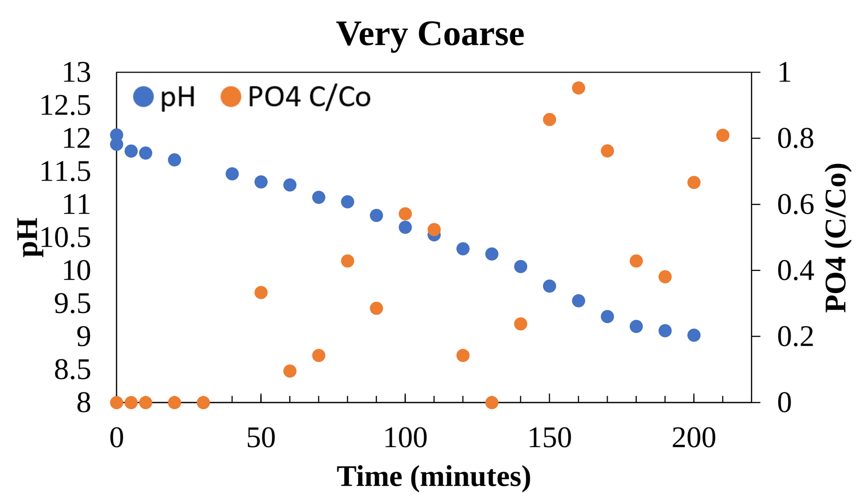


Original test conditions included
- 250 mg/L $CaCO_3$ alkalinity
- 0.20 mg/L influent PO_4^{3-}
- coarse slag gradation 9-12 mm
- 75/25 basic oxygen furnace to blast furnace slag ratio

Increased Alkalinity



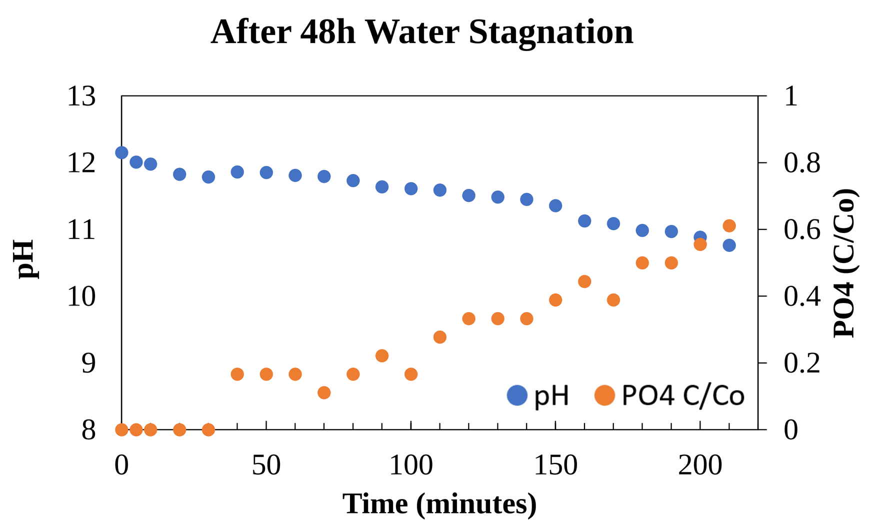
Larger Grain Size



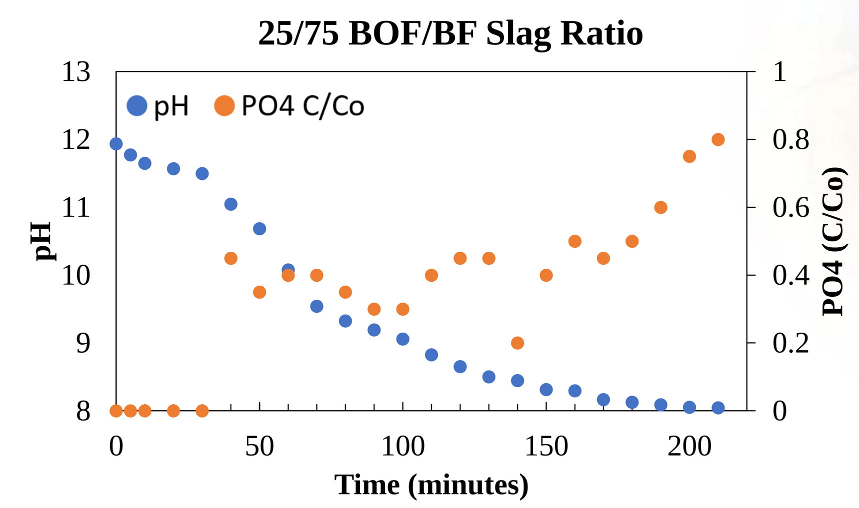
Key Results

- High alkalinity results in increased $CaCO_3$ precipitation and inhibits phosphate mineral precipitation
- Higher grain sizes resulted in preferential flow paths that made adsorption and precipitation unpredictable
- 48 h of water stagnation had little impact on pH, but did cause P removal to decrease over time
- Lower BOF/BF ratios resulted in rapid decreases in pH, indicating BOF slag is essential for P mineral precipitation

Sporadic Flow



BOF/BF Ratio



Industry Collaboration and Stakeholders

- Phoenix Services LLC has providedin-kind contribution of slag for this project (~ 25 tons)
- Geo-Logics Associates is providing engineering services including design, construction, and sampling via local firm Kunkel Engineering
- Local farm owners and agronomist providing land and services for the project
- Other stakeholders include Fox Lake Protection & Rehabilitation District and the WI Dept. of Natural Resources

Timeline and YR-7 Work

- Incorporate a constructed wetland or bioreactor system amenable to achieving simultaneous denitrification
- Test regeneration strategies for the filter media
- Continue lab – scale parallel experiments and publish

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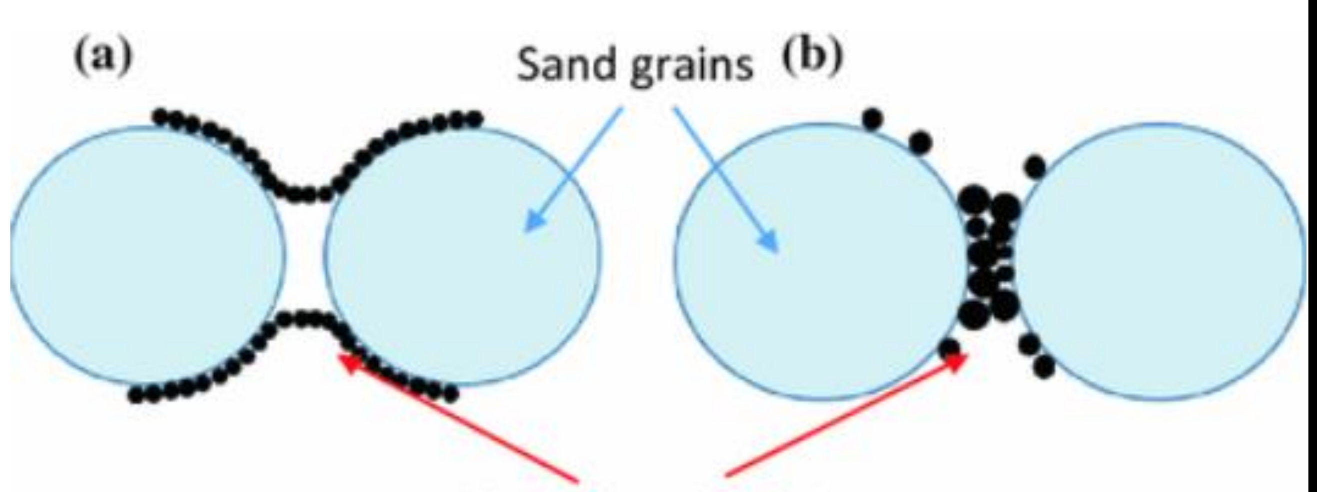
Electrokinetic Sub-surface Transport for mineral Precipitation and Soil Remediation

Presenter: Benjamin Agbo Advisors: Cesar I Torres, Leon Van Paassen Institution: ASU

Background

A study was conducted to evaluate the various alternatives in which electro – kinetic (EK) transport can aide in calcium carbonate precipitation approaches for ground improvement. We demonstrated ion dispersal and the removal of high concentrations of ammonium chloride from the reactor, a known undesired by-product of EICP.

Electrokinetic is the study of the dynamics of charged fluids. The dynamic is influenced by electrical potential difference, pressure difference, concentration gradient and even gravity. Electrokinetics is used to aide EICP because of the chemistry of sand.

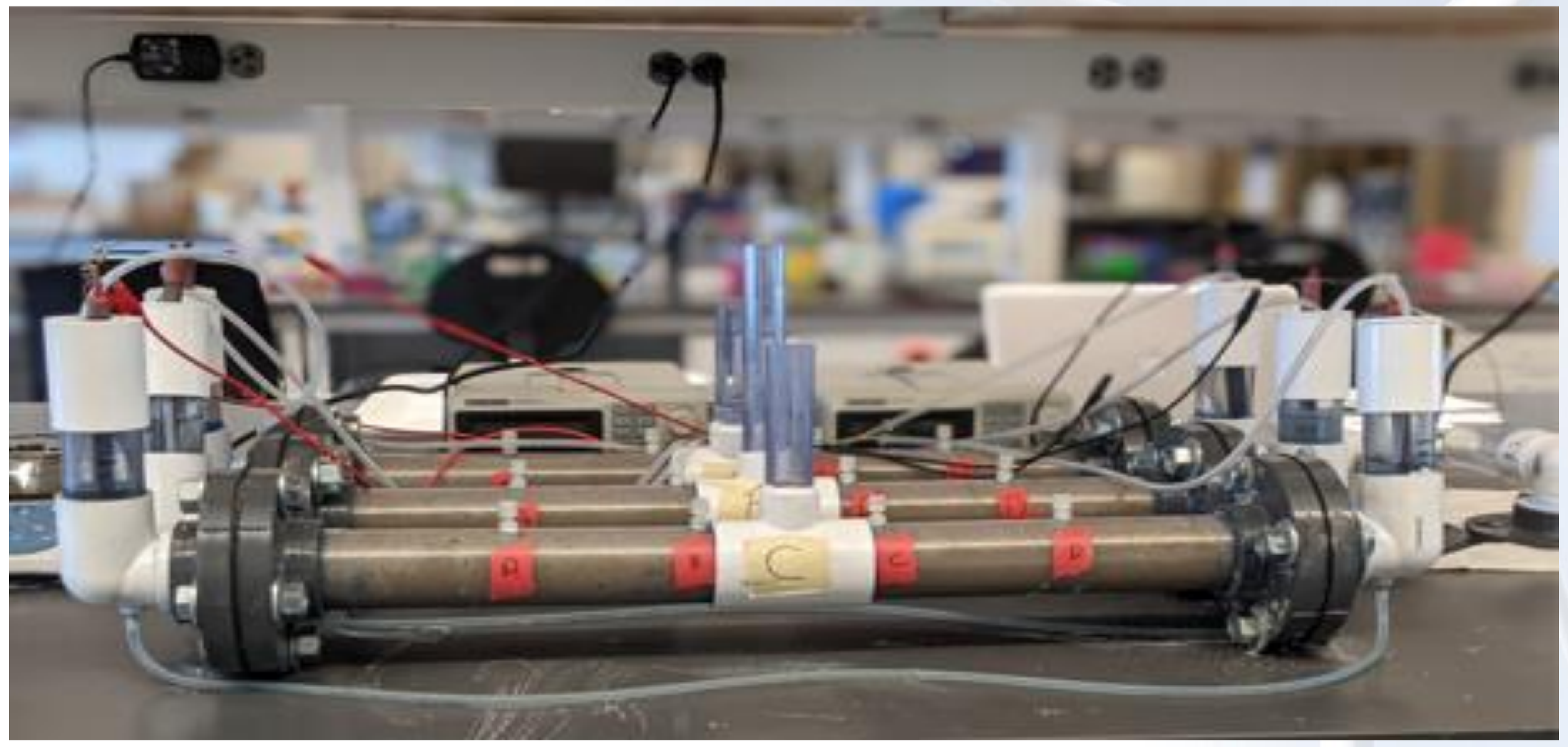


Under high ionic concentrations, like those found in EICP applications, transport of ions occurs mainly through electromigration and directly correlated to the current applied. However, electroosmosis, the transport of water under an electric field, is an important phenomenon to consider under the high ionic movement applications considered.

Year 6 Summary

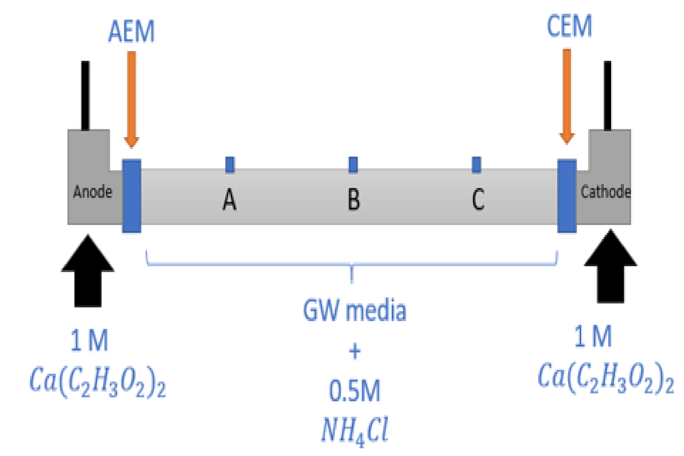
- Show the movement of calcium ion through a diluted media like sand using electrokinetics.
- Induce calcium carbonate precipitate throughout the sand and test for the confinement strength and efficiency of the precipitation.
- Removed high concentration of ammonium chloride concentrations residue from the soil using electrokinetics.
- Interned for Geosyntec over the summer of 2021

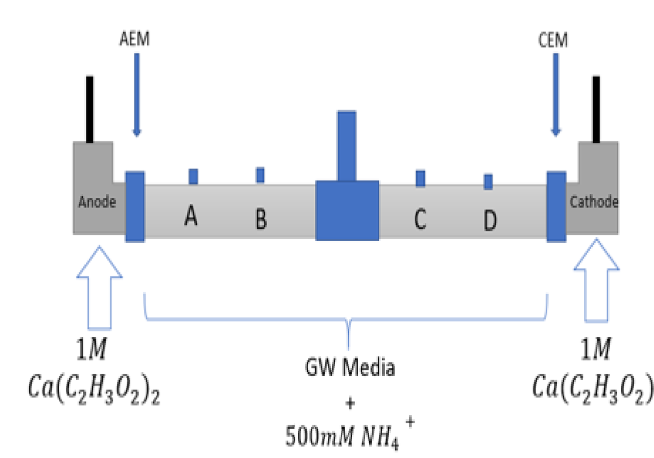
Year 7 Plans

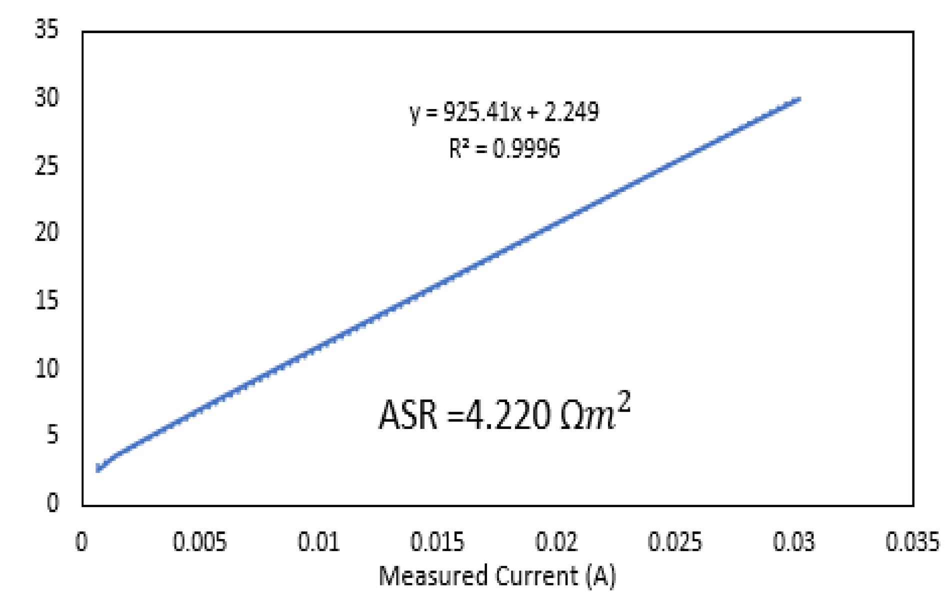


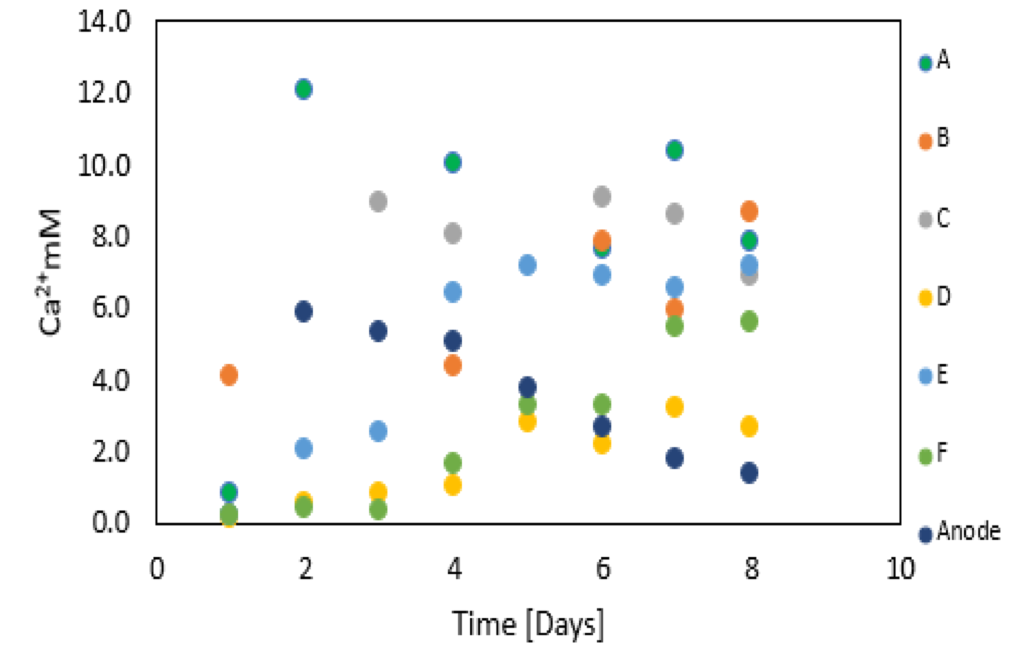
- ❑ Anode/Cathode recycle experiments during ammonium chloride removal
- ❑ EICP Post-treatment removal
- ❑ EK BIO follow up on transport and electrochemical degradation of TCE.

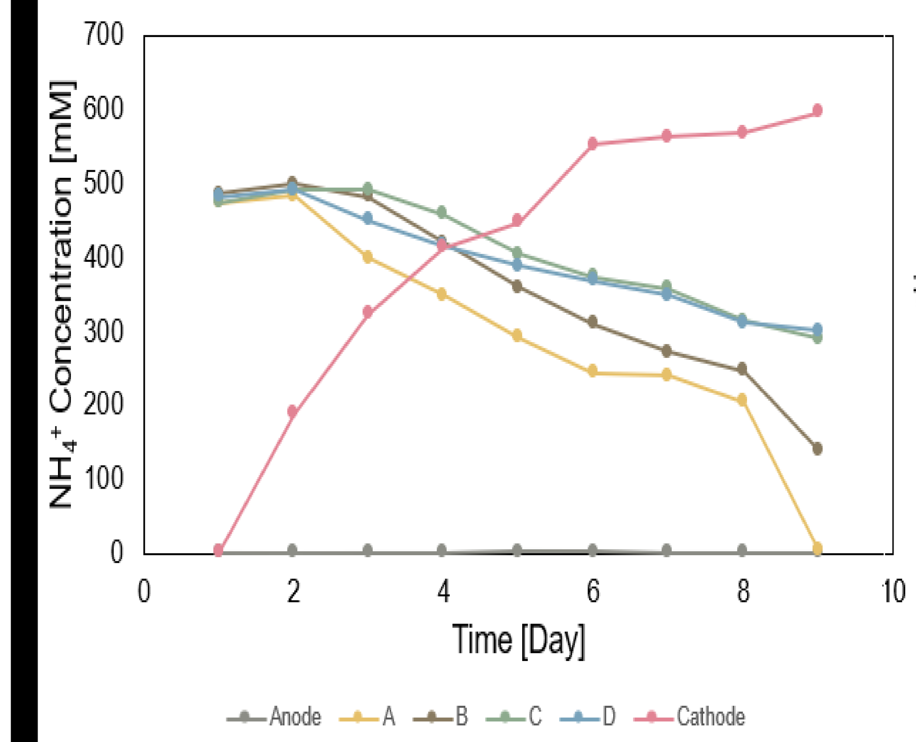
Year 6 Results

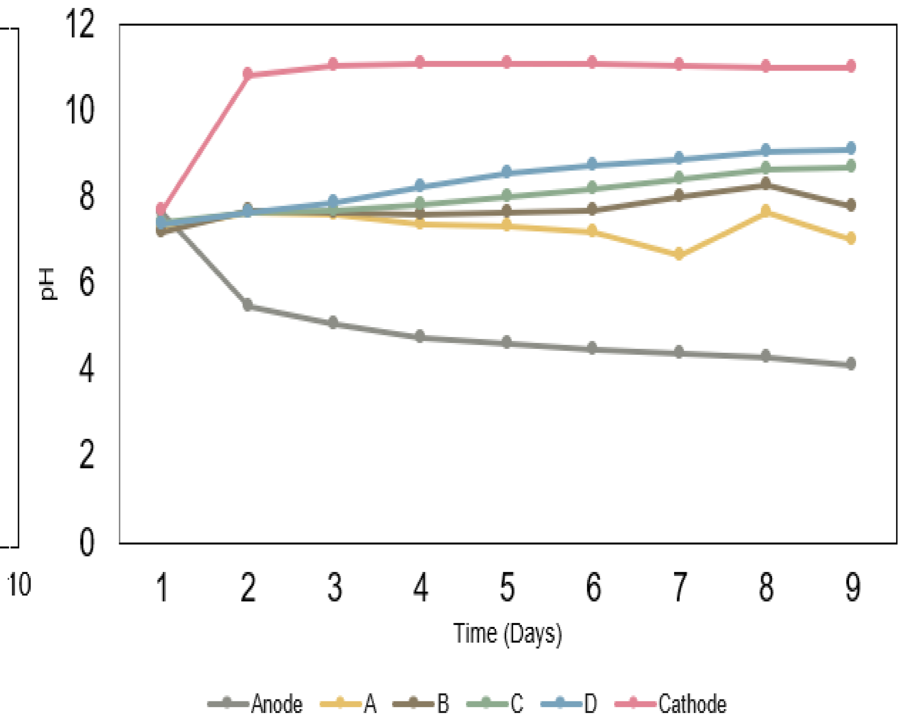


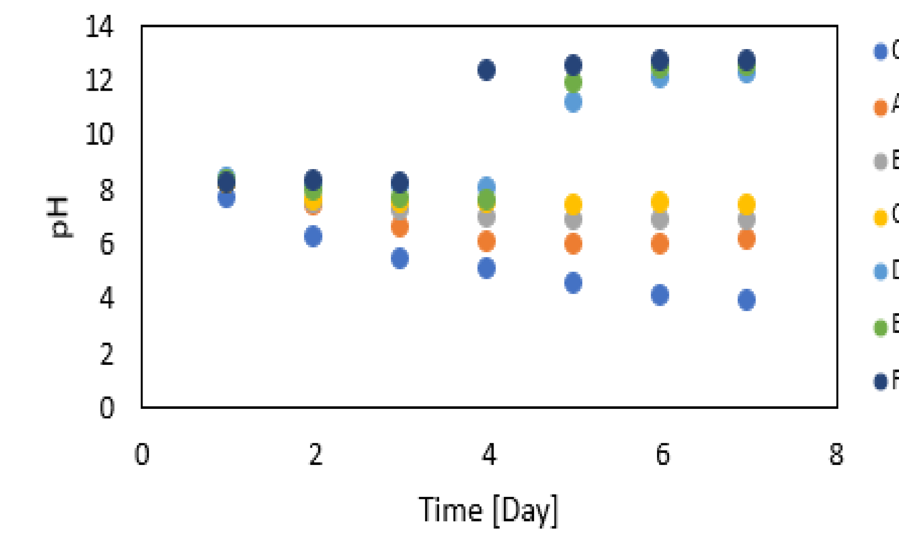


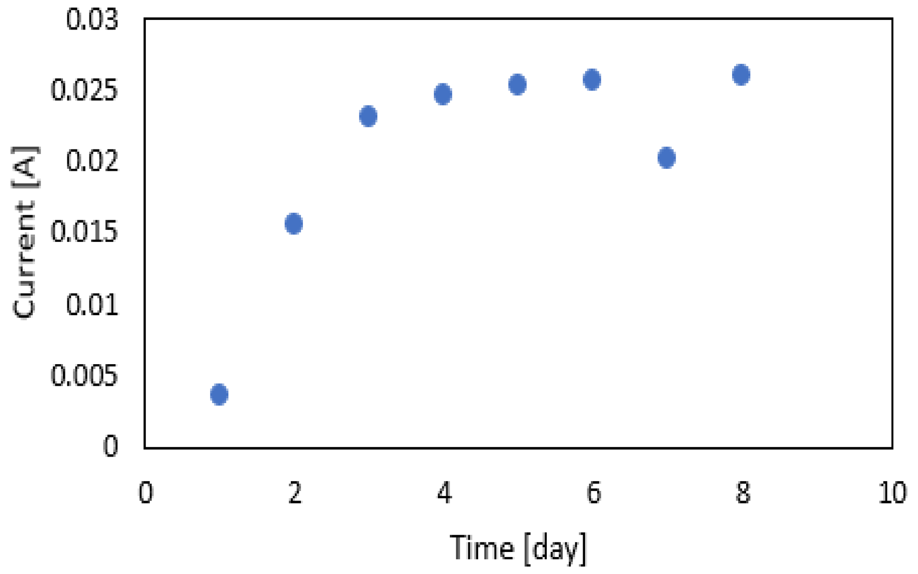












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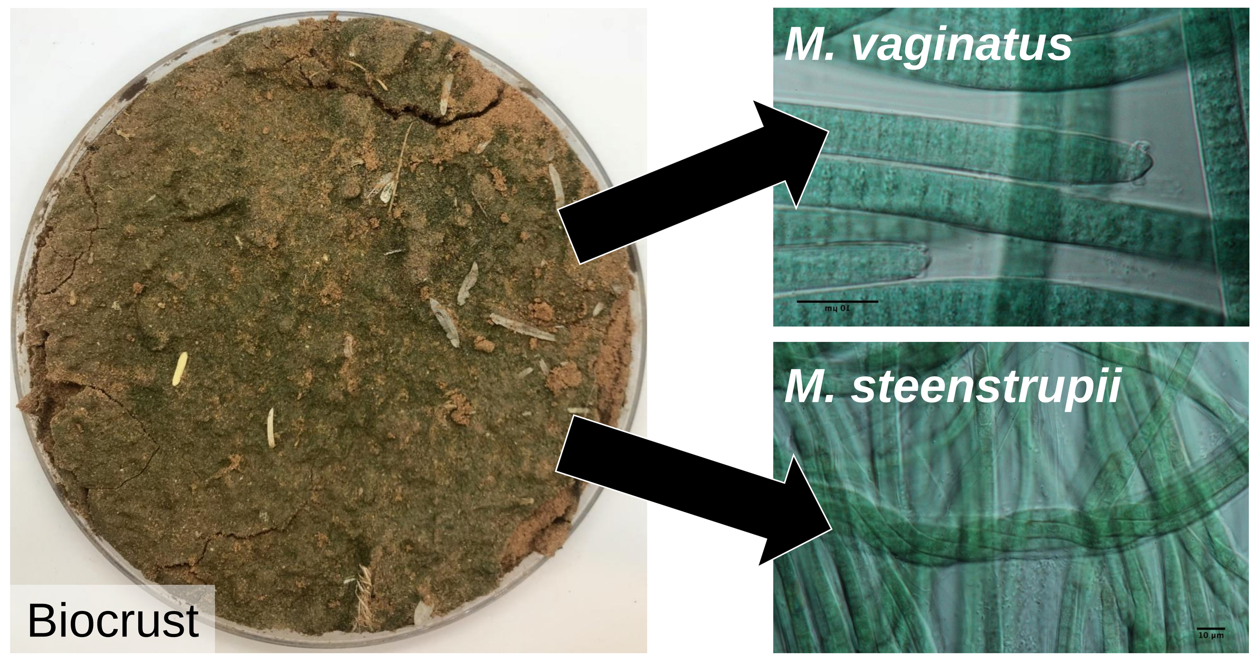
Restoration of soil crust cover (biocrust) for erosion and fugitive dust mitigation in degraded soils and solar farms

Presenter: Luis Gonzalez-de-Salceda   Advisor: Ferran Garcia-Pichel   Institution: ASU

Background

- Biological soil crusts (biocrusts) are microbial topsoil communities that naturally mitigate erosion and fugitive dust.
- Inoculation of cultivated biocrust inoculum can speed establishment of biocrust communities and mitigate wind/water erosion and fugitive dust generation in degraded areas.



Research Objectives

1. Demonstrate biocrust inoculum production technology [(1)] at field scale using mobile microbial nursery facility
2. Determine beneficial effect of different microbial compositions on biocrust establishment [(2)]
3. Evaluate biocrusts as a sustainable dust mitigation strategy for solar energy providers

Phase 1: Biocrust Inoculum Production (Completed)

A: Collection and Isolation

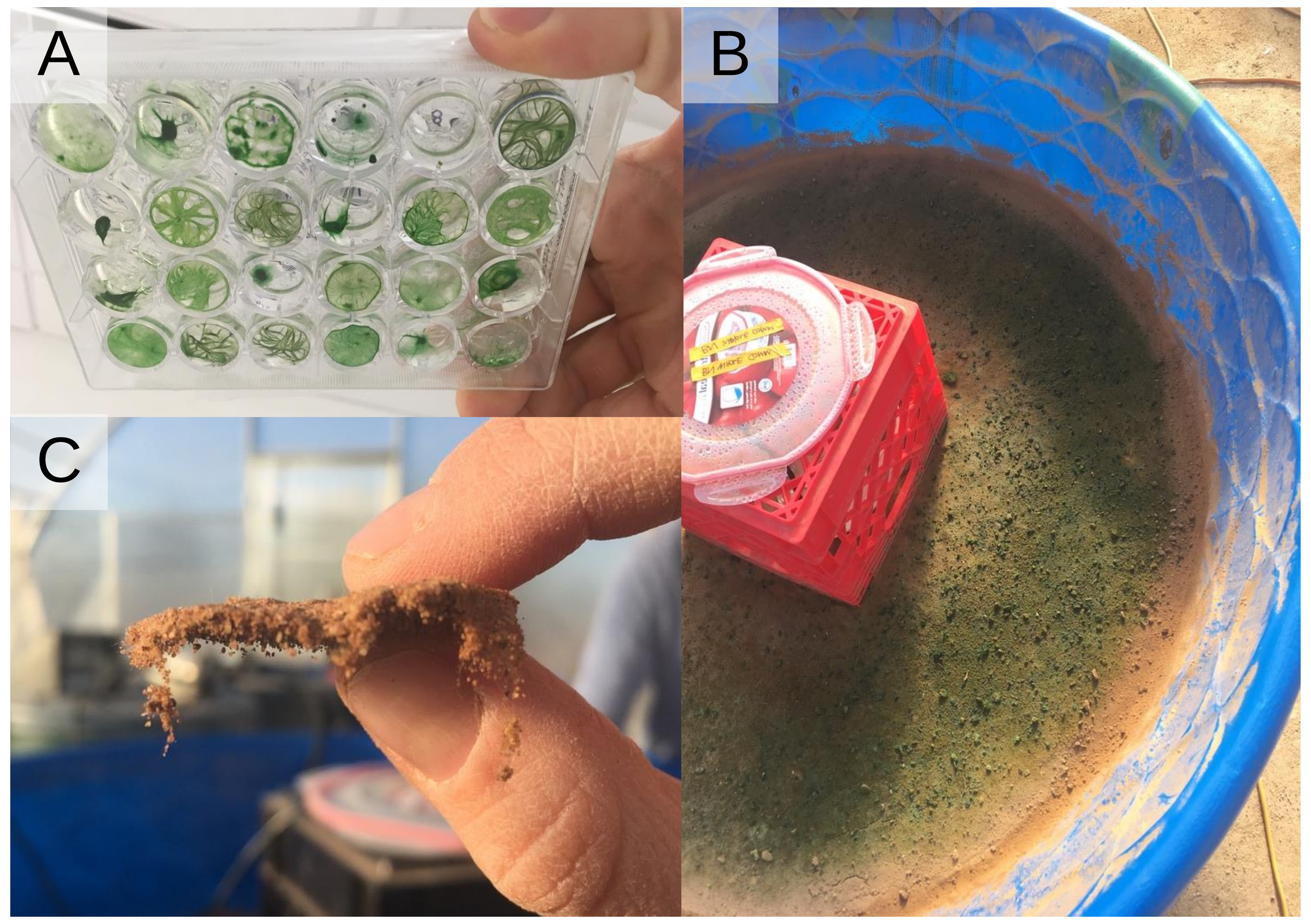
Bacterial components of biocrust isolated from locally-sourced biocrust remnants

B: Scale-up of biocrust biomass

Cyanobacterial isolates and whole biocrusts communities scaled-up using fog-irrigated soil substrate (FISS) technology.

C: Harvest biocrust inoculum

Harvested nursery biocrust dried and stored until field transplantation.

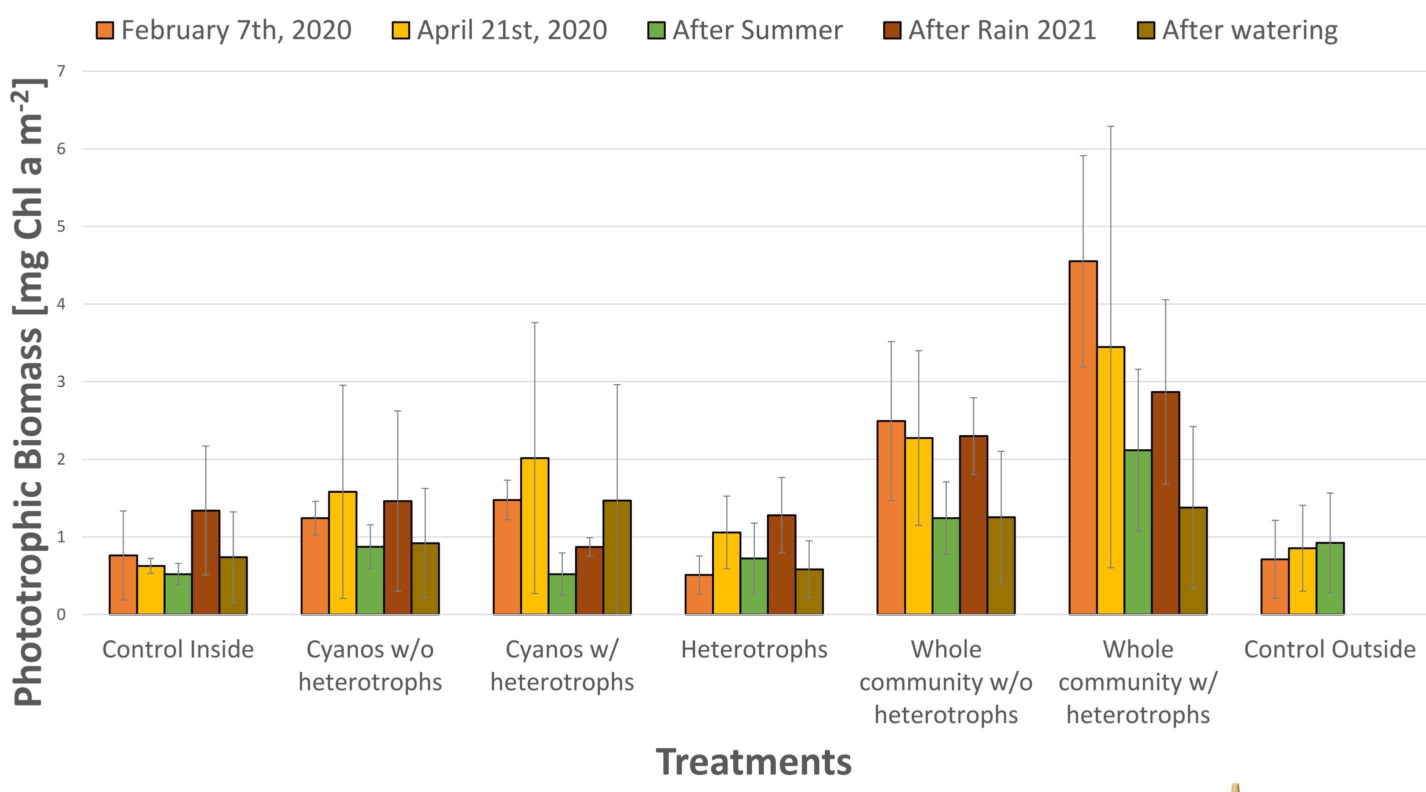


Phase 2: Site Inoculation and Monitoring (Ongoing)

Inoculation and initial assessments completed Spring 2020 with biannual (Winter/Summer) samplings scheduled over several years.

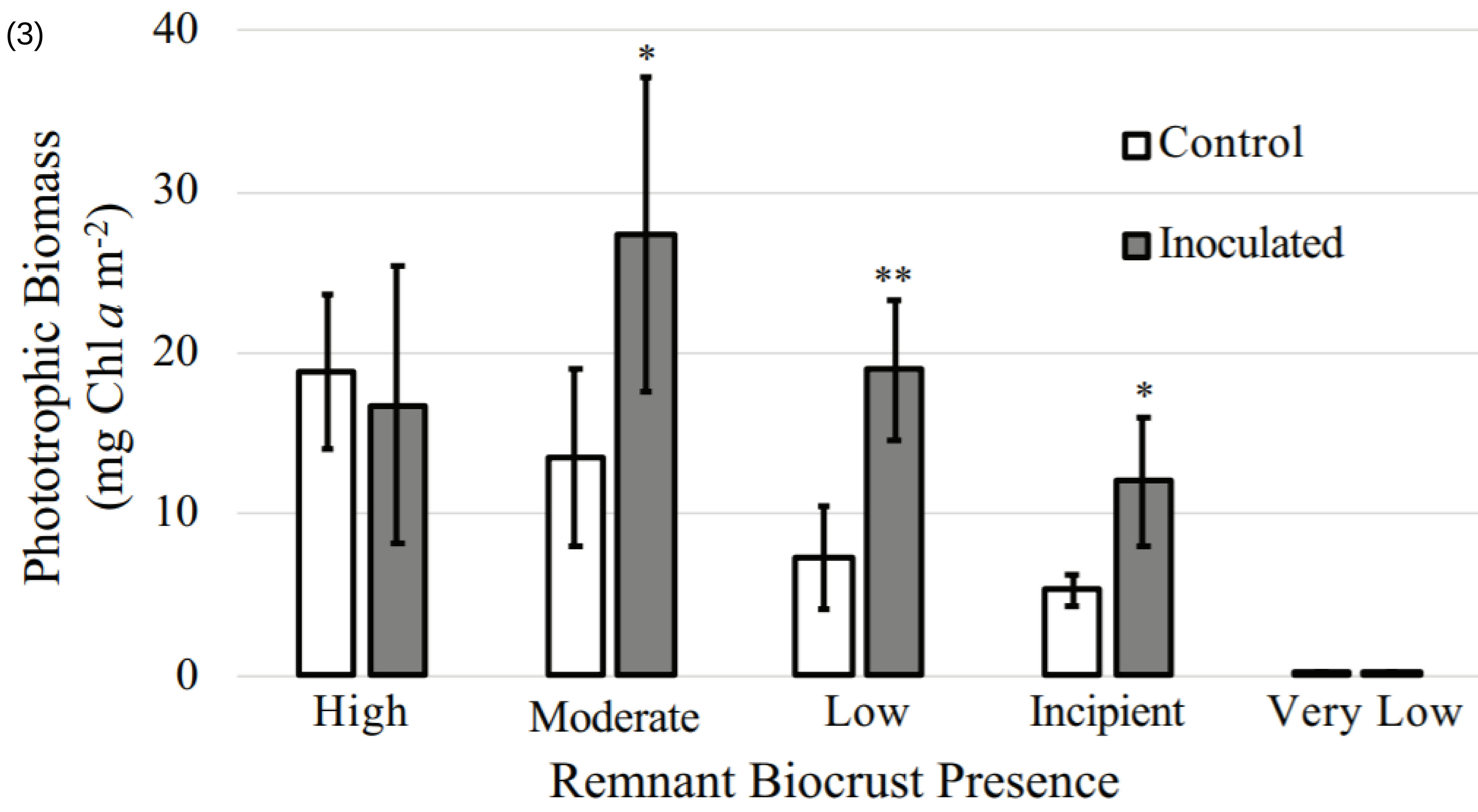
Long-term monitoring of experimental plots will include:

- Visual assessments
- Chlorophyll *a* determinations
- Microbial community composition analysis



Heterotrophs speed biocrust establishment

(3)



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(1) **Nelson, C.**, Giraldo-Silva, A., Garcia-Pichel, F. 2020. A symbiotic nutrient exchange within the cyanosphere microbiome of the biocrust cyanobacterium, *Microcoleus vaginatus. The ISME Journal.* DOI: 10.1038/s41396-020-00781-1

(2) **Nelson, C.**, Giraldo-Silva, A., Garcia-Pichel, F. 2020. A fog-irrigated soil substrate (FISS) system unifies and optimizes cyanobacterial biocrust inoculum production. *Appl. Environ. Microbiol.* DOI: 10.1128/AEM.00624-20

(3) **Nelson, C.**, & Garcia-Pichel, F. (2021). Beneficial cyanosphere heterotrophs accelerate establishment of cyanobacterial biocrust. Applied and environmental microbiology, AEM0123621 .

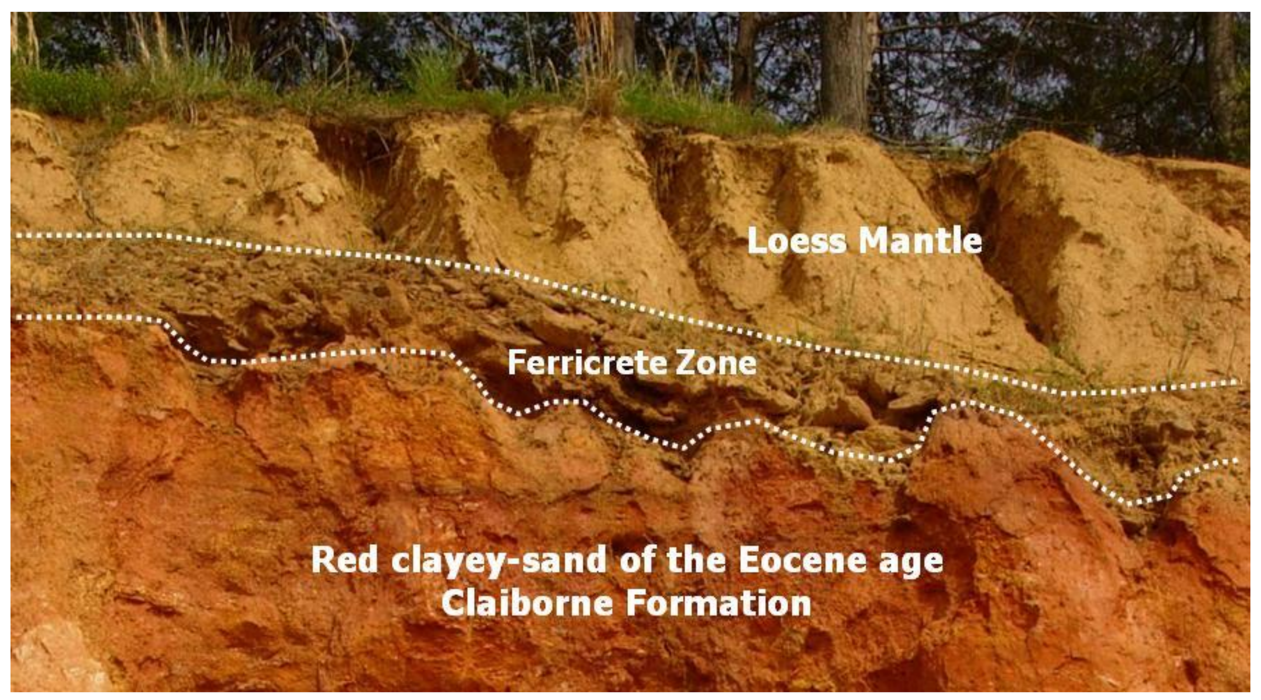
This work was supported in part by the Jornada Basin LTER Graduate Research Fellowship Program and Center for Bio-mediated and Bio-inspired Geotechnics.

Microbially Inspired Iron Precipitation for Permeability Reduction

Presenters: Sahil Kanawade, Zach Hubbard Advisors: Cesar Torres, Leon Van Paassen Institution: ASU

Background

Natural iron bands in the earth's crust result from the (a)biotic oxidation and precipitation of iron at the oxic-anoxic interface.



We aim to determine if this can be replicated and applied to various geotechnical problems like bio-cementation, permeability reduction, erosion control, and dust-mitigation while overcoming the limitations of other processes.

Broad Research Objective

Study both anaerobic and aerobic reactions, under both chemical and (imminently) biological regimes to determine how iron precipitation can be used to most optimally alter the property of soils.

Advantages of MIIP

1. Oxygen is readily available from the air and iron is widely distributed beneath the earth's surface
   - Deriving its reagents from the environment, MIIP is highly economical on a theoretical basis
2. Iron oxidizing bacteria are found naturally beneath the earth's surface
   - A wide variety of bacteria that do not require cultivation are at our disposal
3. The by-products of iron oxidation are protons and water
   - No hazardous materials will be released into the environment

Current Scope of MIIP

Oxide precipitation can be conducted using either ferrous "Fe(II)" or ferric "Fe(III)" iron products. We have initially chosen to oxidize iron using oxygen as an e^{-}acceptor.

$$4Fe^{2+} + O_2 + 8OH^{-} \rightarrow 4Fe(OH)_3{}^{o}$$
$$4Fe(OH)_3{}^{o} \rightarrow [Fe(OH)_3]_4$$

Because abiotic and biotic oxidation compete for iron in the feed, no bacteria were introduced at this stage so that a procedure could be established for later comparison against chemical oxidation.

While various applications exist, our current focus regards **permeability reduction using two methods**:

1. **Counter-diffusion** approach
2. **Periodic Flush** approach

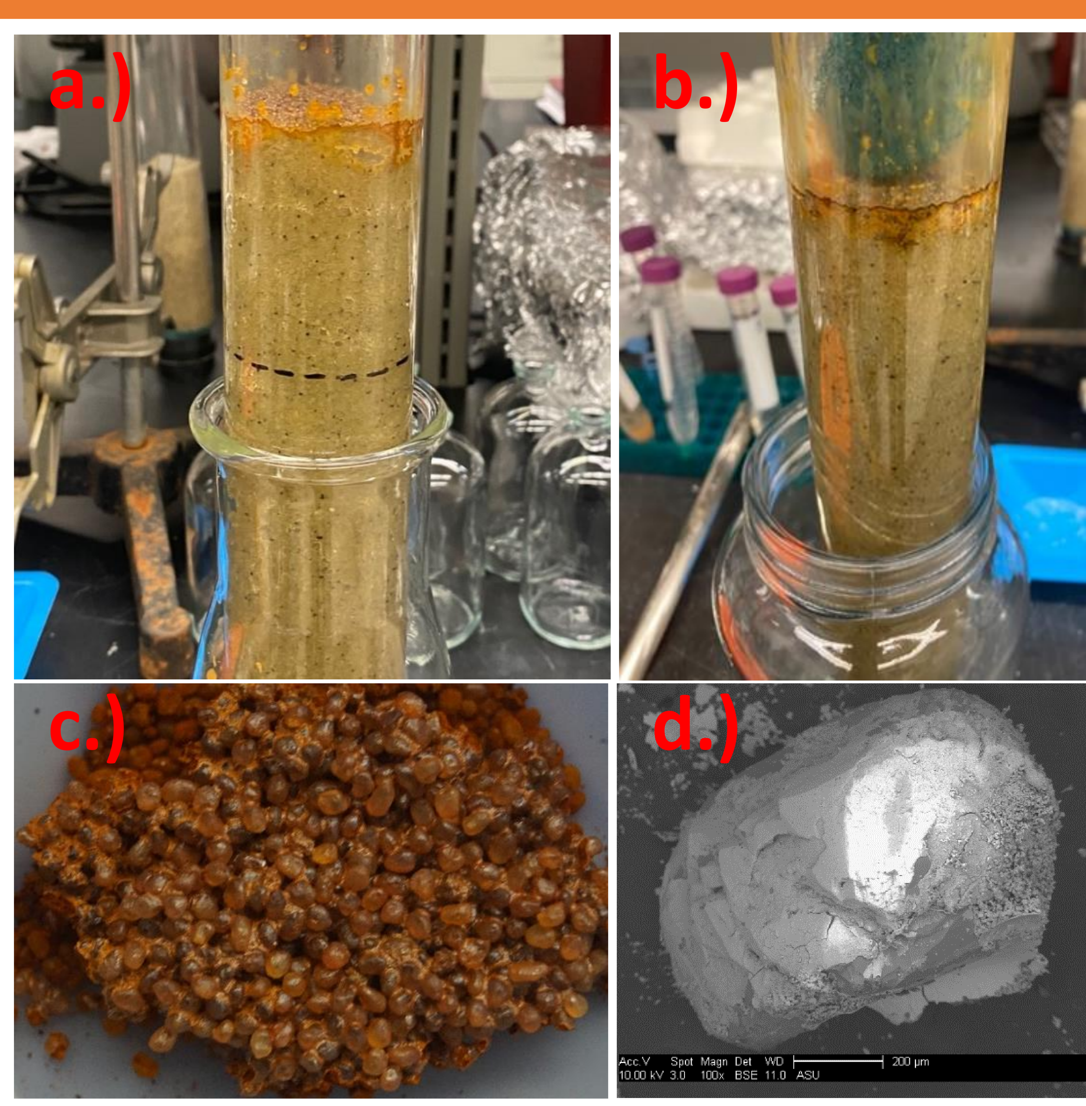
Experimental Approaches

Iron (II) chloride, various organic buffers (2:1 ratio), and DI/DO water are mixed with Ottawa 20-30 sand in a column of ~ 4cm diameter and 10cm length under aerobic conditions.

1. Oxygen will saturate the sand/solution mixture at the sand's surface and diffuse into the bulk of the column. As ferrous iron is consumed, it will diffuse from the bulk toward the air interface → Thin layer of precipitate at the sand/air interface
2. Iron solution is periodically introduced and drained from the column. This will deposit a thin layer over each sand kernel – decreasing O_2 diffusional resistance, permitting permeability reduction over the entire column, and allowing for greater pH control

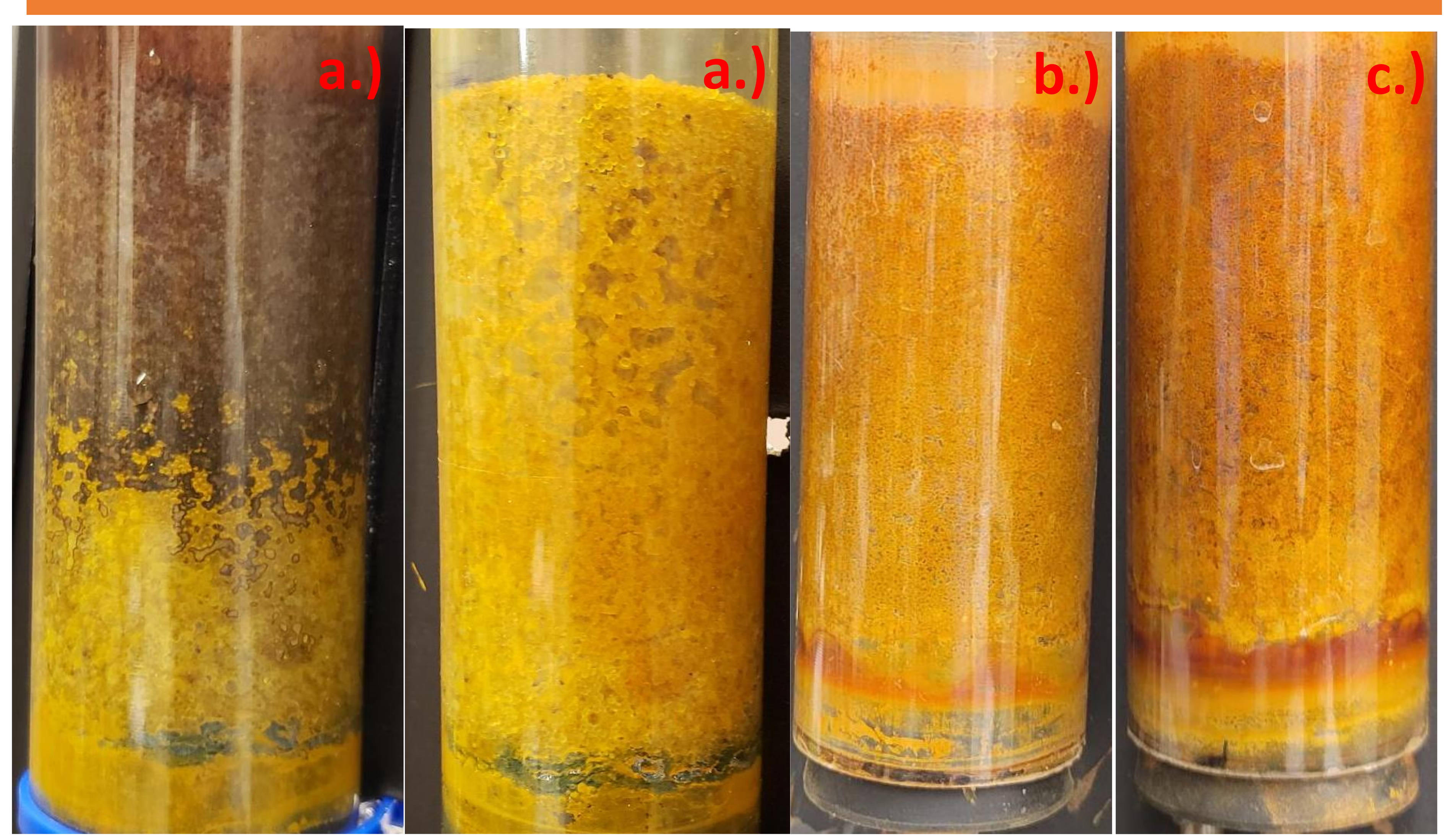
Yielding permeability reductions ranging from ~45 to 93%, using .1M to 1M Fe(II) and various organic buffers (primarily acetate/propionate)

Counter-Diffusion



Precipitation results after one week of treatment using a.) .5 Molar Fe(II) and b.) 1 Molar Fe(II), c.) top (~ 2mm) crust after 1M Fe(II) treatment, and d.) SEM image of sand after 1M Fe(II) treatment

Periodic Flush



Precipitation results following 3 days of treatment with 30-minute intervals between each flush using a.) 1 Molar Fe(II) (second image is after washing the column), b.) 0.5 Molar Fe(II) with propionate as a buffer, c.) .5 Molar Fe(II) using acetate as a buffer

Some Permeability Reduction Results

- pH reductions below ~6 restrict the kinetics, despite substantial Fe(II) and increasing O_2 concentrations after 4 days.
- High buffer concentrations may lead to the generation of soluble iron-organic complexes that congeal upon dehydration and are easily removed
- High iron supersaturation may result in small nucleates that are more-readily dissolved, form only in solution, and fail to adhere to the sand

Method	Buffer	Iron Concentration (M)	Buffer : Iron (M/M)	Permeability Reduction (%)
Flush	Acetate	1	2.3 to 1	57.0 ± 2.9
Flush	Acetate	1	2 to 1	47.0 ± 1.2
Flush	Acetate	0.5	2 to 1	66.3 ± 1.1
Flush	Propionate	0.5	2 to 1	91.5 ± .27
Flush	propionate	0.1	2 to 1	58.0 ± .65
Flush	propionate	0.25	2 to 1	69.7 ± 2.71
CD	Acetate	1	2 to 1	56.8 ± 3.6
CD	Acetate	0.5	2 to 1	49.3± 2.2
CD	Acetate	0.5	2 to 1	42.5 ± .66
CD	Propionate	0.5	2 to 1	80.4 ± 1.1

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Acknowledgement
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Changes in methane emissions and microbes in landfill cells manipulated for sustainable biogas

Presenter: Paul Brewer **Advisor: Hinsby Cadillo-Quiroz** **Institution: ASU**

Background

Goal: High resolution monitoring of changes in CH_4 release after water injection to an arid landfill bioreactor for sustainable practices

- Can microbial changes be predictors of improved methanogenesis and rate of ground change?
- Can spatial CH_4 flux mapping based on Eddy Covariance methods provide a remote tool for sustainable management of CH_4 yield or emissions?
- Landfills as engineered geotechnical systems

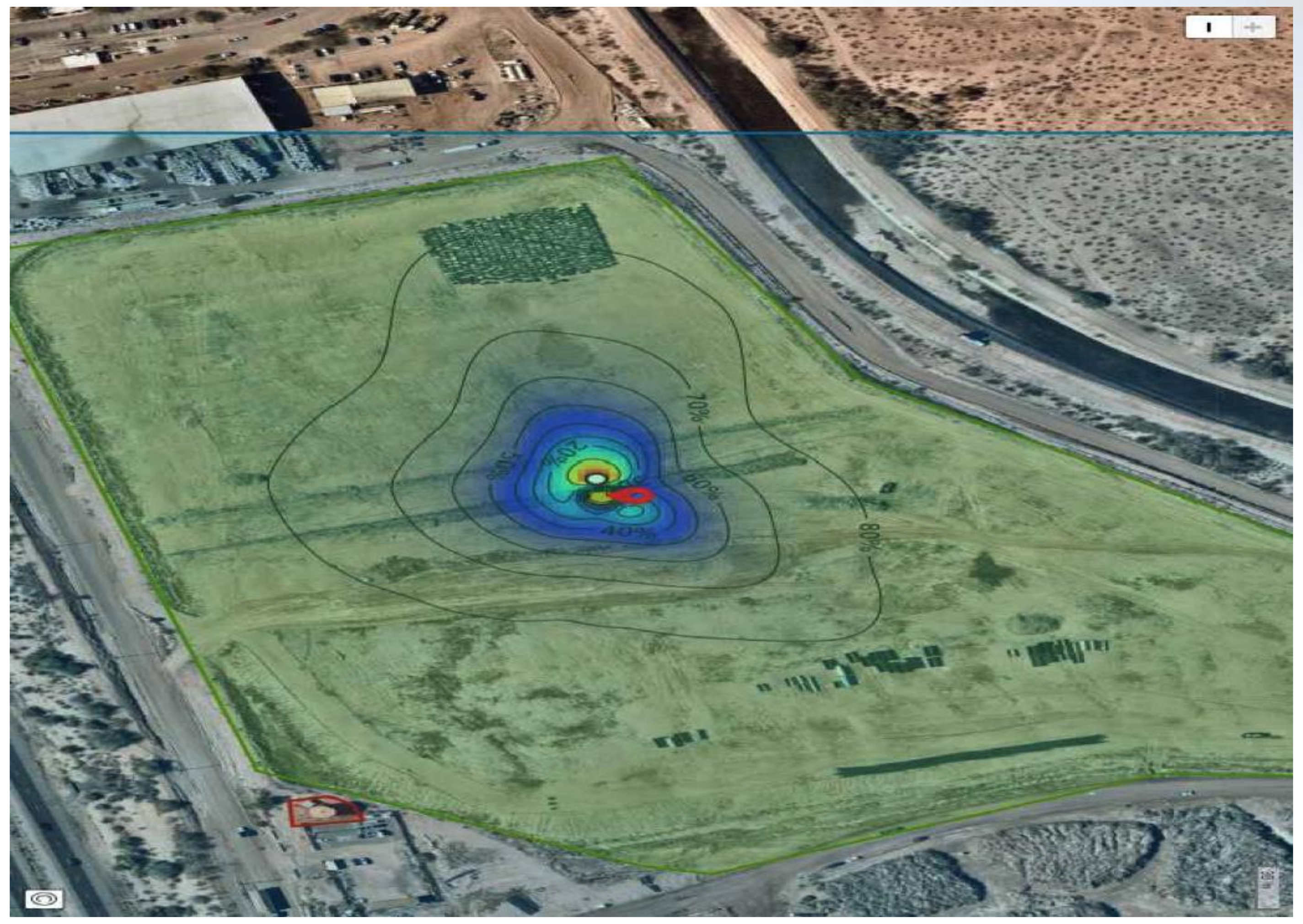
SALT RIVER LANDFILL
An Enterprise of the Salt River Pima-Maricopa Indian Community

Research Objective

1. Develop a new Discrete Area Source Eddy Covariance method to be evaluated by EPA regulators
2. Establish a CH_4 emission, microbial and soil surface baseline as well as catalog changes as water injections are underway in two landfill cells.
3. Testing a new method for surface mapping and location of high emission points in landfills

All work in continuous collaboration and funding with CBBG Industry Partner SRL.

Proposed manipulation and monitoring



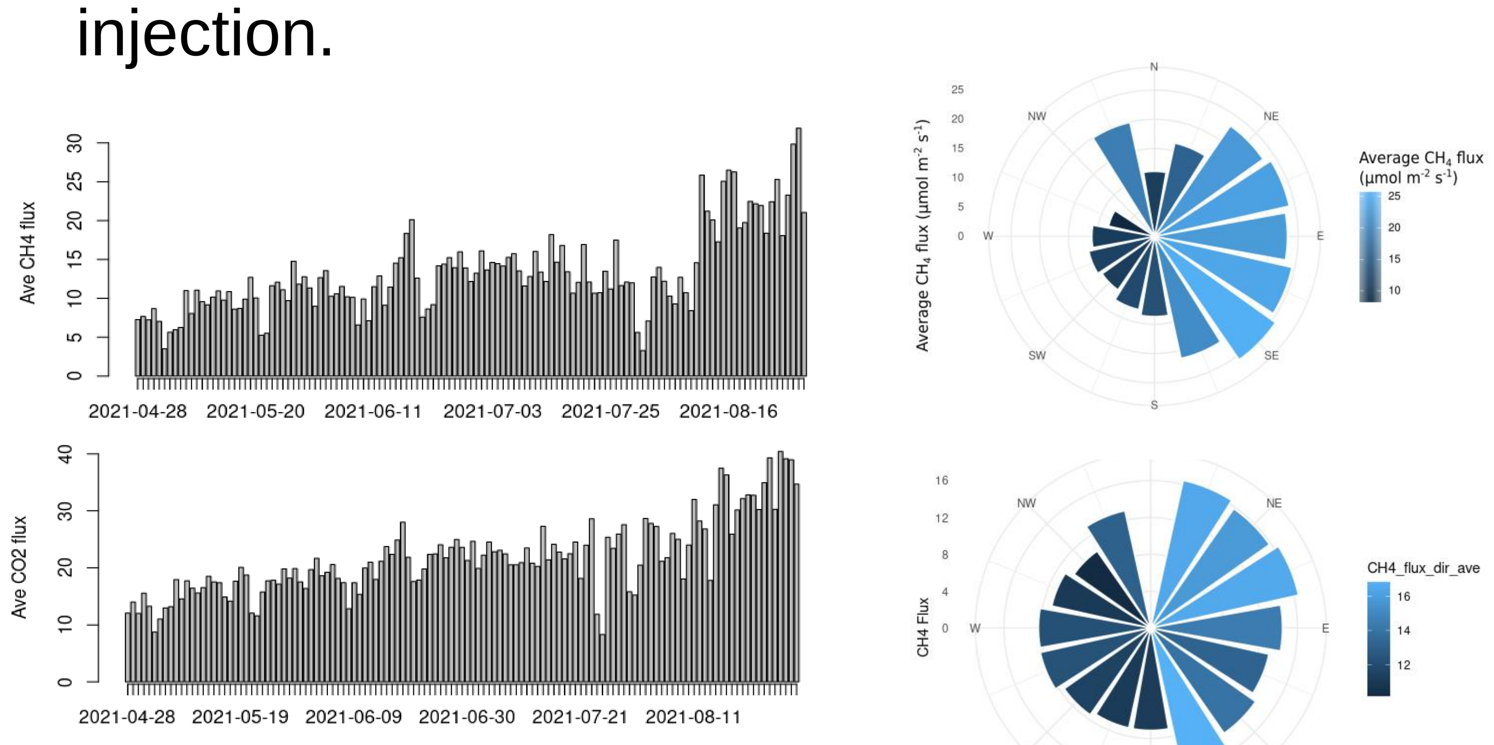
- Located at Salt River Landfill (SRL) in Scottsdale, AZ.
- DASEC envelope is >50% of the 33 acre landfill cell
- Leachate and freshwater liquid injections following baseline emissions assessment

Phase 1: Baseline monitoring: CH_4 with DASEC, microbial from leachate pumps, and soil surface mapping (externally done). 9 months

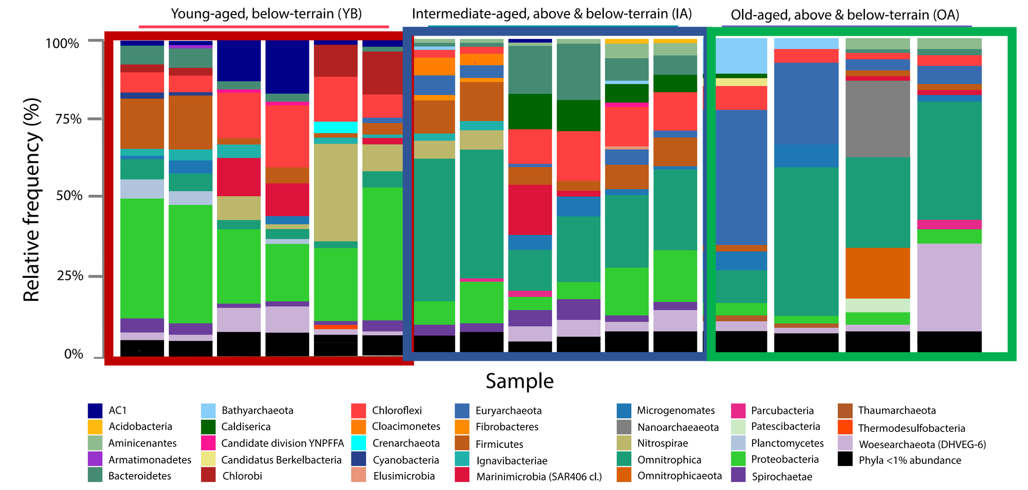
Phase 2: Water injection for several months, begun in February 2021 and ongoing; monitor associated emission and microbial changes

Phase 2: monitoring after baseline and water injection period

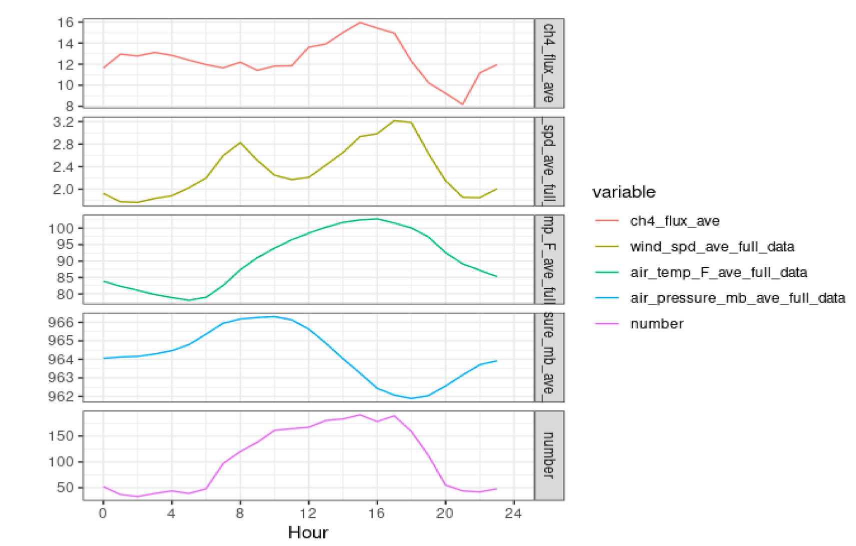
Since liquids injection (Feb 2021) surface CH_4 emissions remained at or below pre-injection levels until Aug 2021 when emissions increased 60%. Spatial patterns of emissions (East higher, West lower) have been maintained since injection.



Distribution of temporal and spatial GHG as evaluated by DAEC



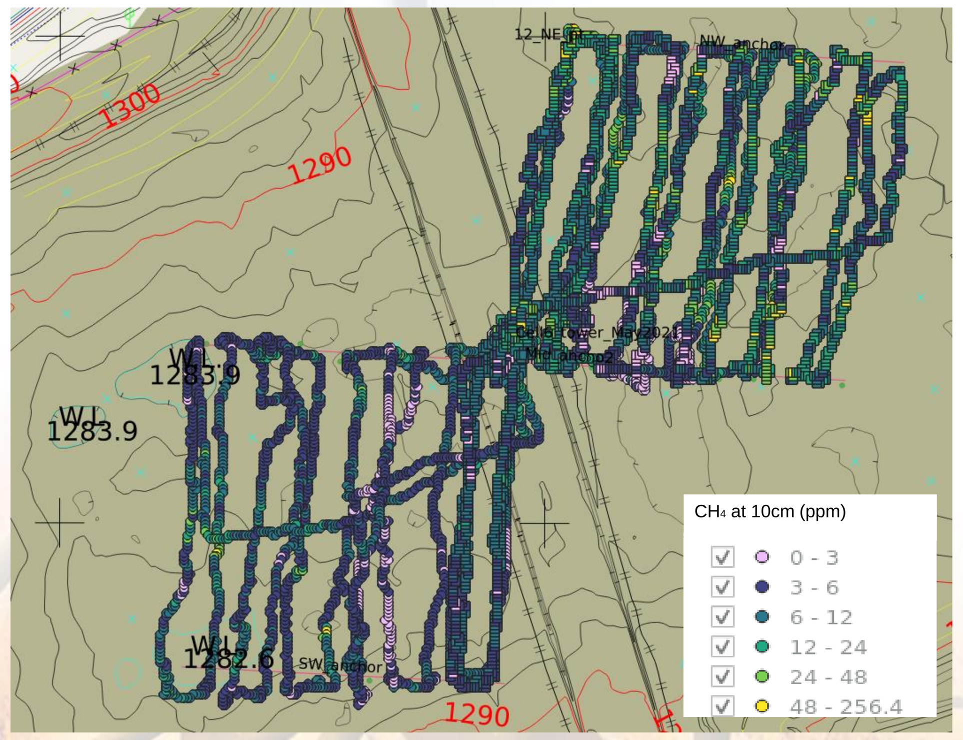
16S rRNA based microbial community baseline assessment : Cell VI is OA (green) and cell II-IV is YB (red)



Averaged daily environmental condition associated to EC tower

Analysis and directions for progress

- Need to differentiate seasonal drivers vs water pumping induced effects in recent change in surface CH_4 emissions
- Developing a rapid, high resolution method for identifying emission hotspots
- Microbial baseline suggests three main niches and states of microbial community in relationship to cell type but association with flux needs to be modeled
- Output from water addition on CH_4 yield is continuously monitored
- LCA analysis is needed and is new collaborative avenue
- Landfill response is expected in multiyear term.



Rapid walking assessment provides fine resolution CH_4 flux mapping: This is an EC tower independent method to map emission cold and hotspot and management change

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UC DAVIS UNIVERSITY OF CALIFORNIA

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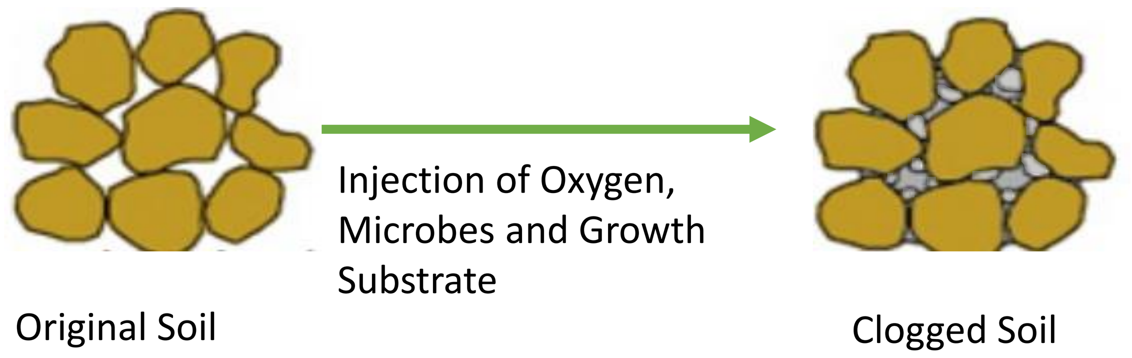
Enhanced Control Of Microbial Activity And Substrate Delivery Via Inhibitors For In-situ Contaminant Treatment

Presenter: Justin Skinner **Team: Anca Delgado, Nasser Hamdan, Jacob Chu** HALEY ALDRICH **Institution: ASU**

Background

- Bioremediation of large diffuse contaminant plumes often exhibits excessive microbial growth near the substrate injection points leading to bioclogging.
- Bioclogging decreases permeability and effectiveness of in-situ bioremediation for many contaminant types.
- To minimize bioclogging, microbial inhibitors may be used to temporarily deter microbial growth allowing for better operational control.
- Bioremediation of TCE via aerobic cometabolism is a feasible approach for large dilute plumes where low TCE concentration limits metabolic microbial growth.

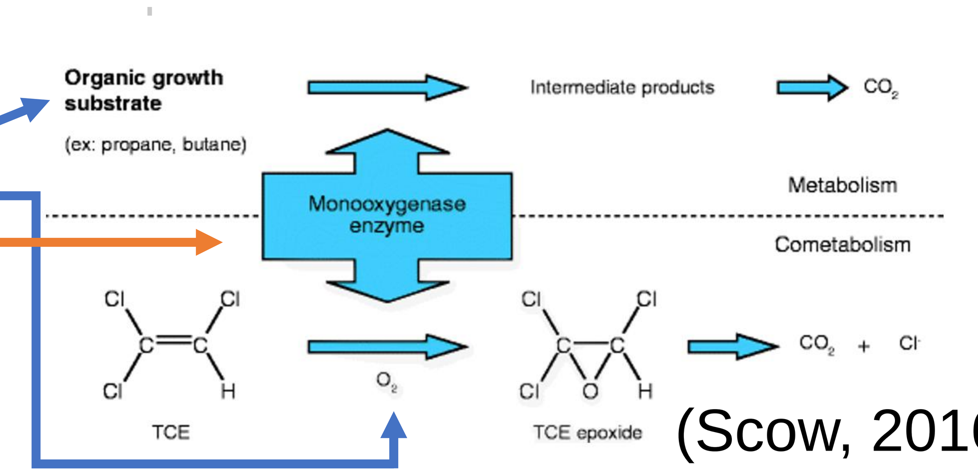


Research Objective

- Determine the effect of acetylene, a microbial inhibitor, on co-metabolic biodegradation of trichloroethene (TCE).
- Acetylene inhibits growth by binding to oxygenase enzymes which both metabolically degrade hydrocarbons and cometabollically degrade TCE.
- Growth substrate and oxygen must be injected into the subsurface, therefore increasing the likelihood of bioclogging.

Must be injected increasing likelihood of bioclogging.

Acetylene inhibits this enzyme.

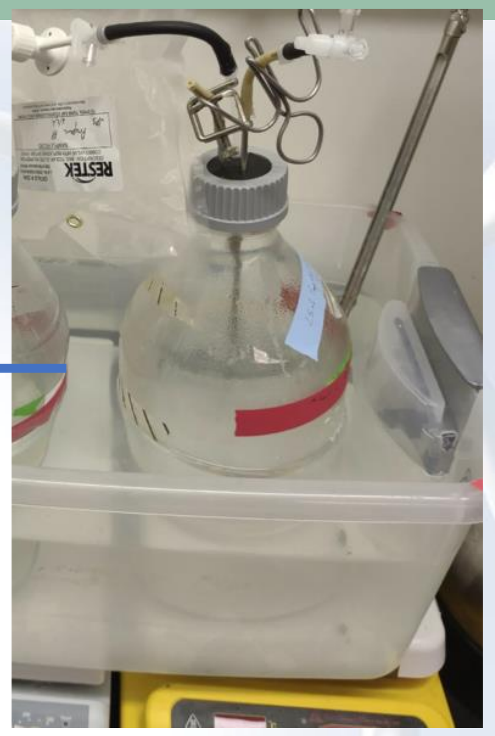


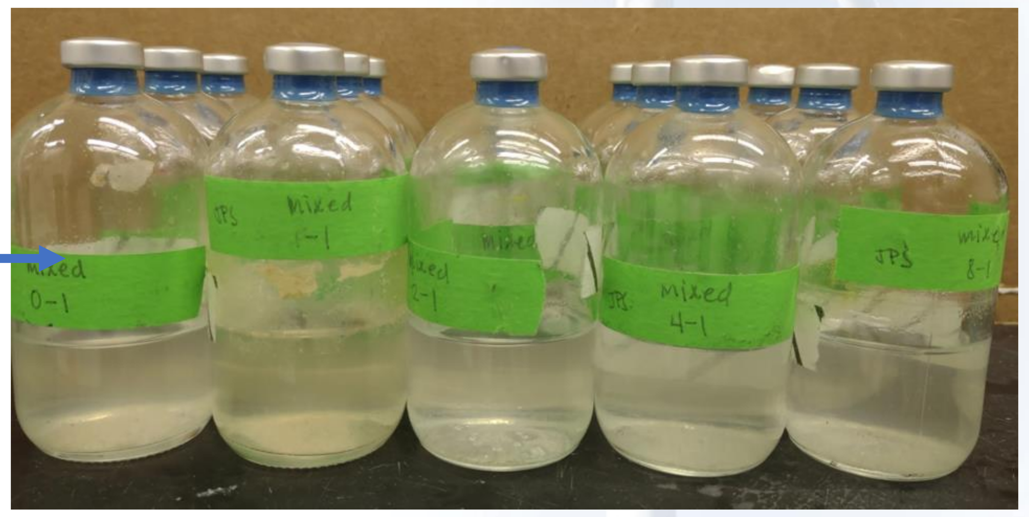
(Scow, 2016)

Methodology

- Studies were conducted using two microbial cultures.
  - *Mycobacterium austroafricanum* JOB5.
  - A soil-derived propane-oxidizing mixed culture.
- Mother bottles- fed with propane and oxygen.
- Cultures then exposed to acetylene gas at 5% v/v for differing lengths of time.
  - No exposure (0 days) or 1, 2, 4, 8 days
- Cultures spiked with propane, oxygen, and 50 µM TCE to verify if microbial growth and TCE cometabolic capacities were altered.

Mother Culture Bottle



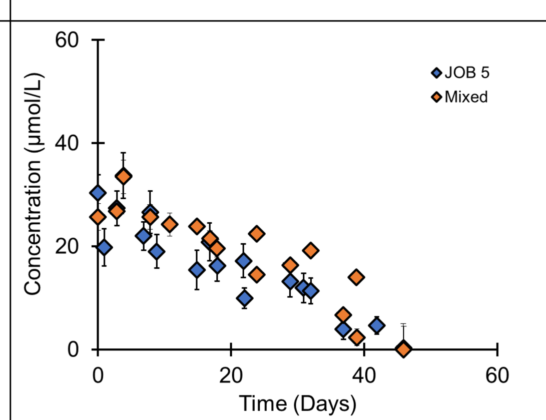
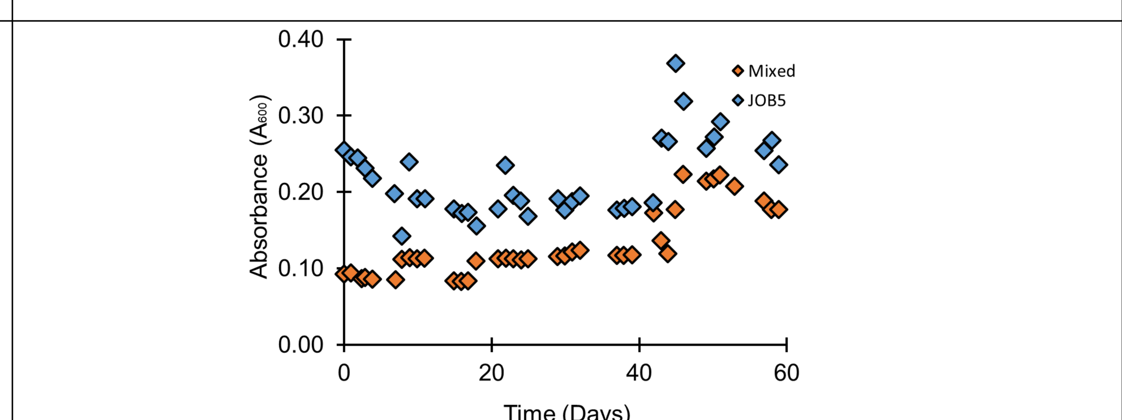
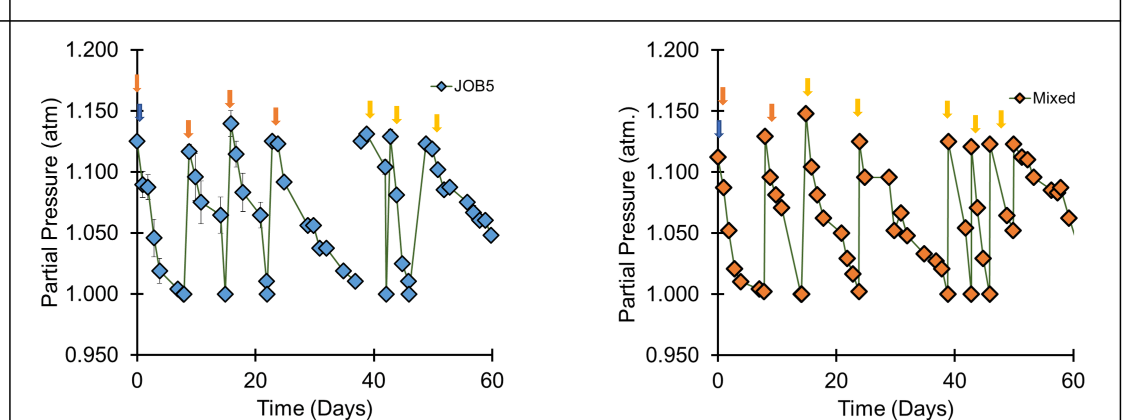
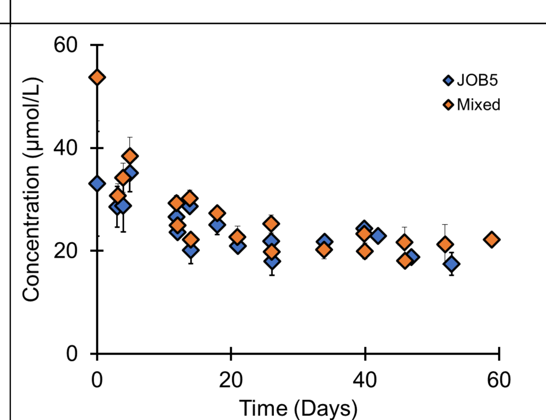
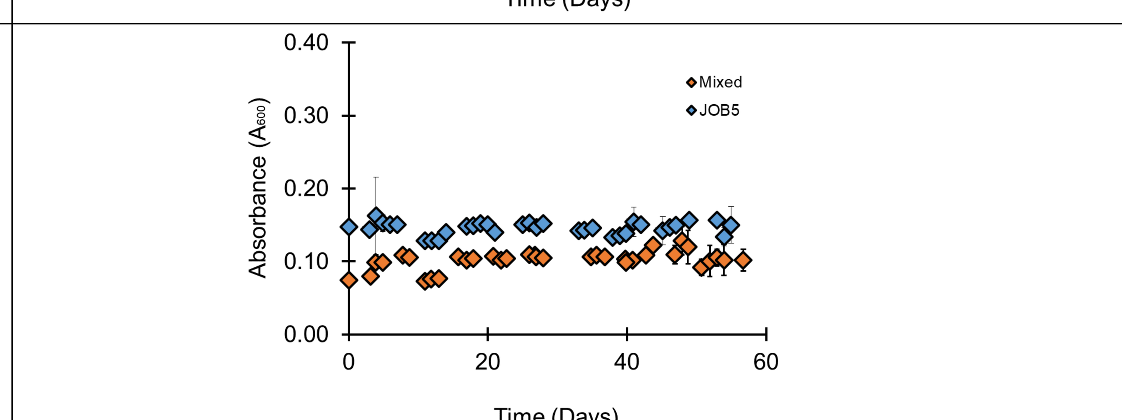
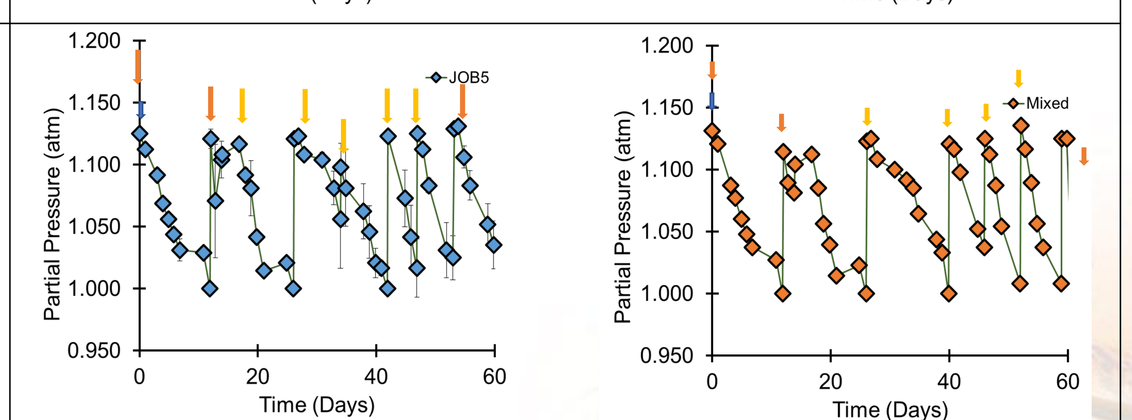
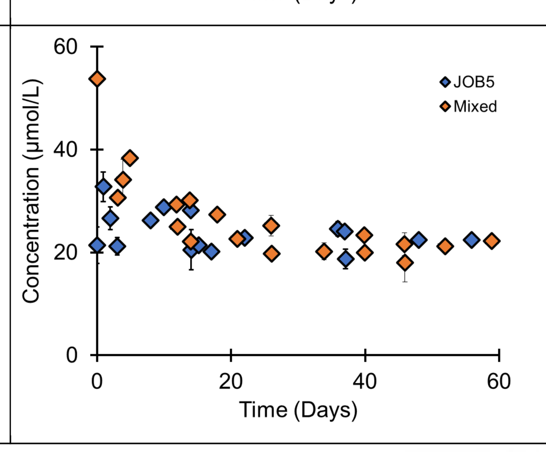
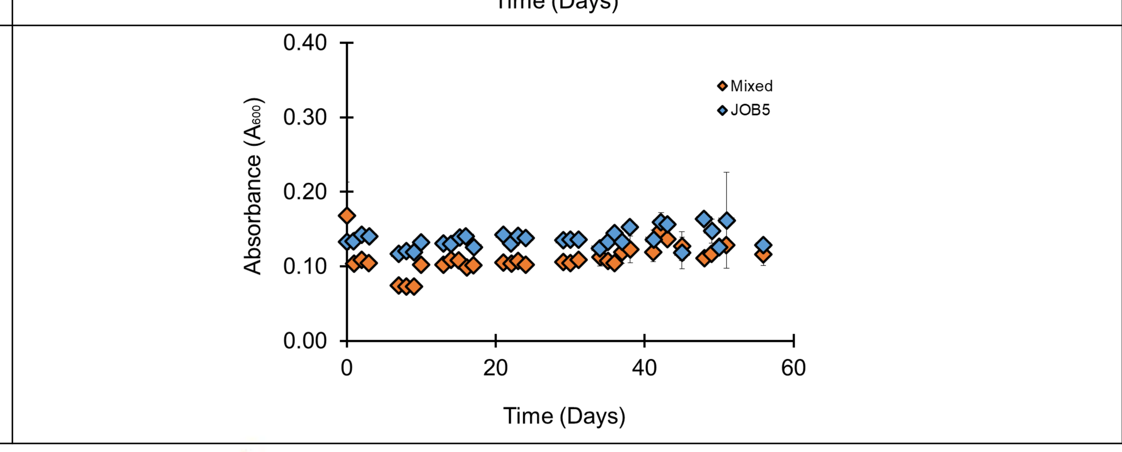
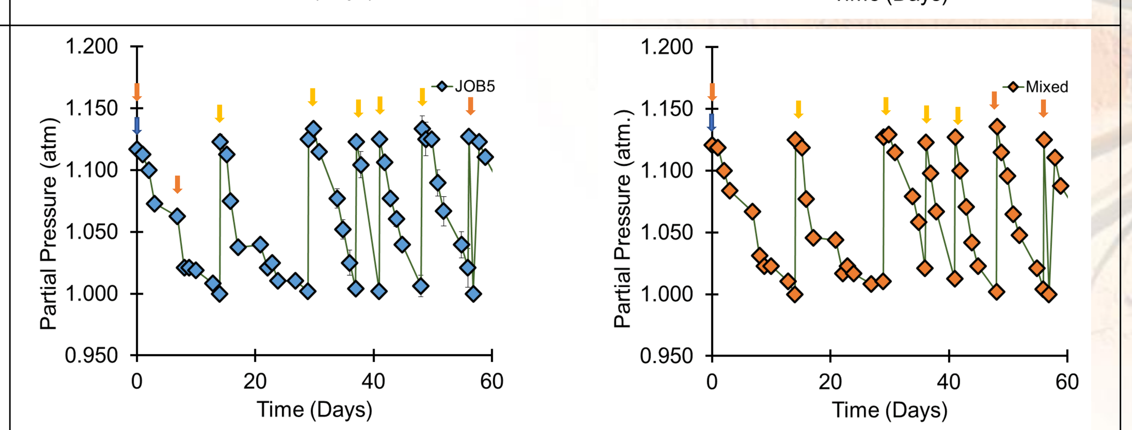


Daughter Triplicate Bottles Started After Varying Lengths of Exposure to Acetylene

Phase 1: Batch Scale Experimentation

Culture	Color
M. austroafricanum JOB5	◆ (blue)
Soil-Derived Propane Consuming Mixed Culture	◆ (orange)

Symbol	Meaning
↓ (blue)	TCE Addition
↓ (orange)	Oxygen Addition
↓ (yellow)	Propane Addition

Treatment	TCE Concentration		Optical Density	Partial Pressure in Bottles
0 Days Acetylene Exposure		TCE consumption rate: Mixed = 1.76 µmol TCE/(L day) JOB5 = 0.76 µmol TCE/(L day)		
4 Days Acetylene Exposure		TCE consumption rate: Mixed = 0.52 µmol TCE/(L day) JOB5 = 0.36µmol TCE/(L day)		
8 Days Acetylene Exposure		TCE consumption rate: Mixed = 0.25 µmol TCE/(L day) JOB5 = 0.07µmol TCE/(L day)		

Key Findings and Next Steps

Key Findings

- •Acetylene at 5% v/v partially inhibits microbial growth and TCE cometabolism.
- •A time-dependent relationship exists between acetylene exposure and inhibition.
- •Our enriched mixed culture exhibits greater TCE cometabolism rates and lower ODs than the pure culture. It therefore may be less likely to bioclog *in situ.*

Next Steps

- The soil columns will employ varying flow regimes:
  - Groundwater (GW) + TCE
  - GW + TCE + Propane
  - GW + TCE + Propane + Acetylene
- Goal:
  - Optimize operational parameters
  - Increase the real-world relevance

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In situ manganese biomineralization in granular media for contaminant removal

Eleanor Fadely, Gaïtan Géhin, Lena Ray, Verónica Morales, Jasquelin Peña

University of California – Davis, Department of Civil and Environmental Engineering, Davis, CA 95616.

1. Background

Goal: Perform *in situ* immobilization and/or transformation of heavy metal contaminants by stimulating soil bacteria to produce manganese oxides.

Biogenic manganese oxides: Highly reactive microbially-mediated mineral precipitates capable of oxidizing and adsorbing heavy metals (e.g., Pb, Cu, Co, Zn, Ni).

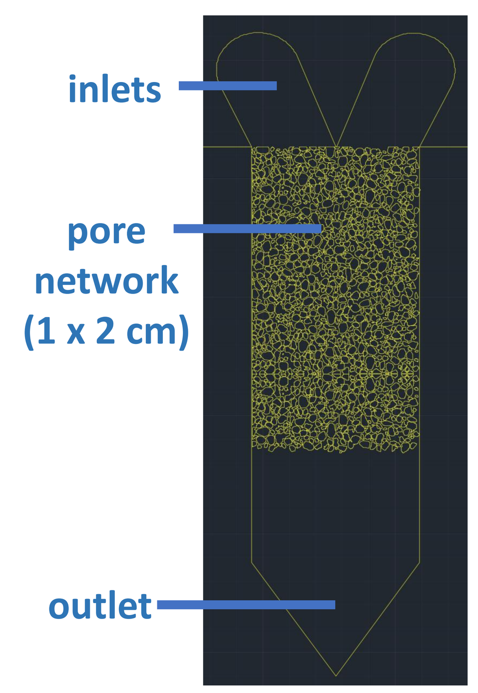
2. Research Objectives

1. Determine the optimal hydrodynamic conditions necessary for *Pseudomonas putida GB-1* to uniformly colonize a porous medium.
2. Optimize pore-scale nutrient and reagent delivery to maximize Mn biomineralization and promote spatial homogeneity.
3. Use pore-scale data to upscale Mn biomineralization process to column reactors and field tests.

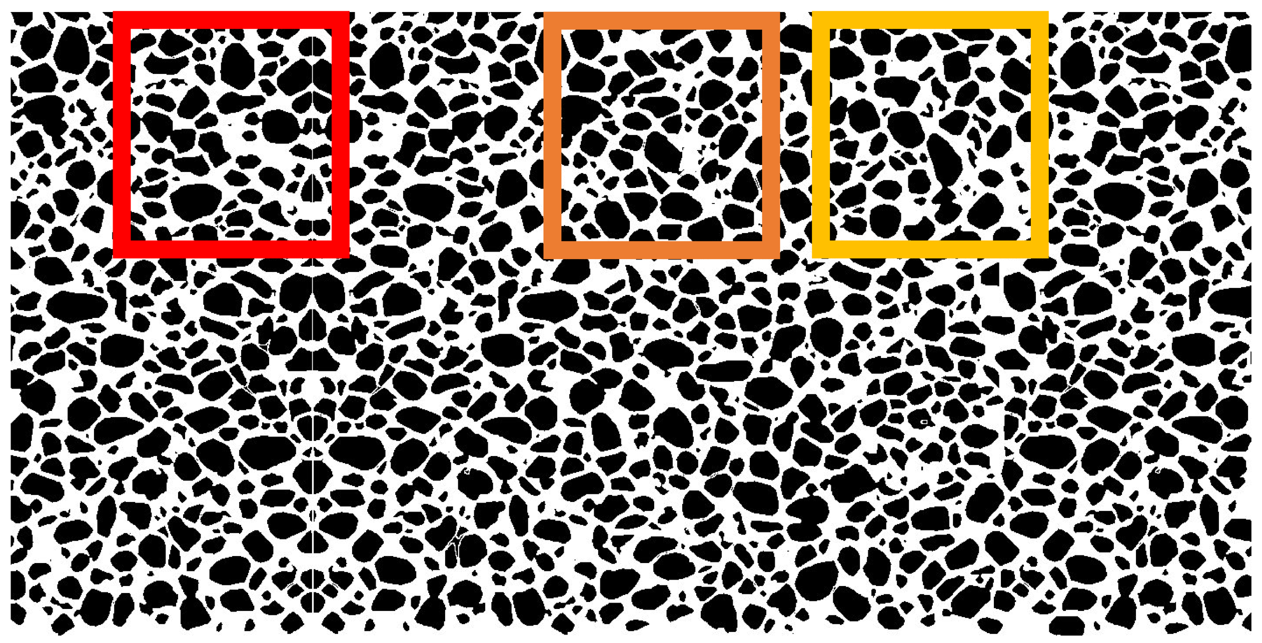
3. Plans for Year 7

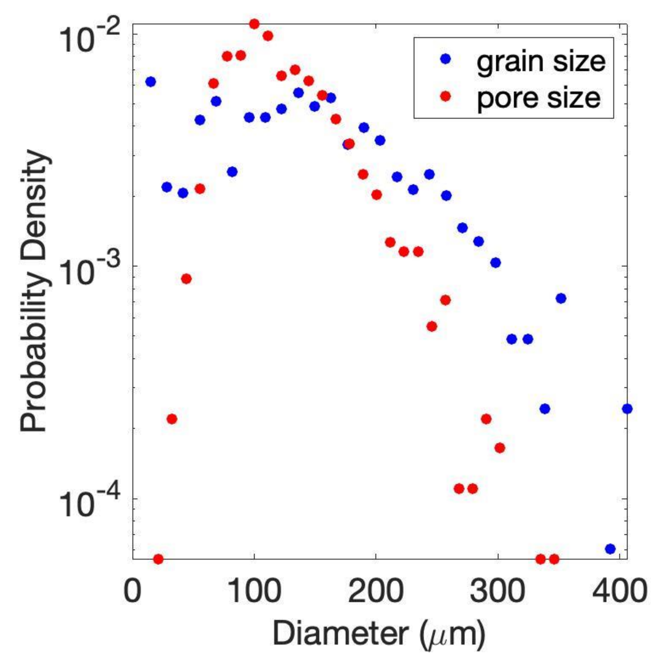
1. Use bioreporter fluorescence to determine Mn oxide concentration.
2. Produce time-lapse movies of Mn biomineralization for quantifying reaction kinetics and observing oxide distribution under different environmental conditions.
3. Continue developing relationships with industry partners and begin experiments with field samples.

4. Porous Medium Characterization



Reactor Specifications	
Porosity (--)	0.5
Pore volume (µL)	2.1
Fluid residence time (s)	
Flow rate = 0.1 mL/h	76.6
Flow rate = 0.5 mL/h	15.3
Flow rate = 1 mL/h	7.7
NOTE: Medium geometry based on Ottawa silica test sand adapted from Wang et al. (2019).	

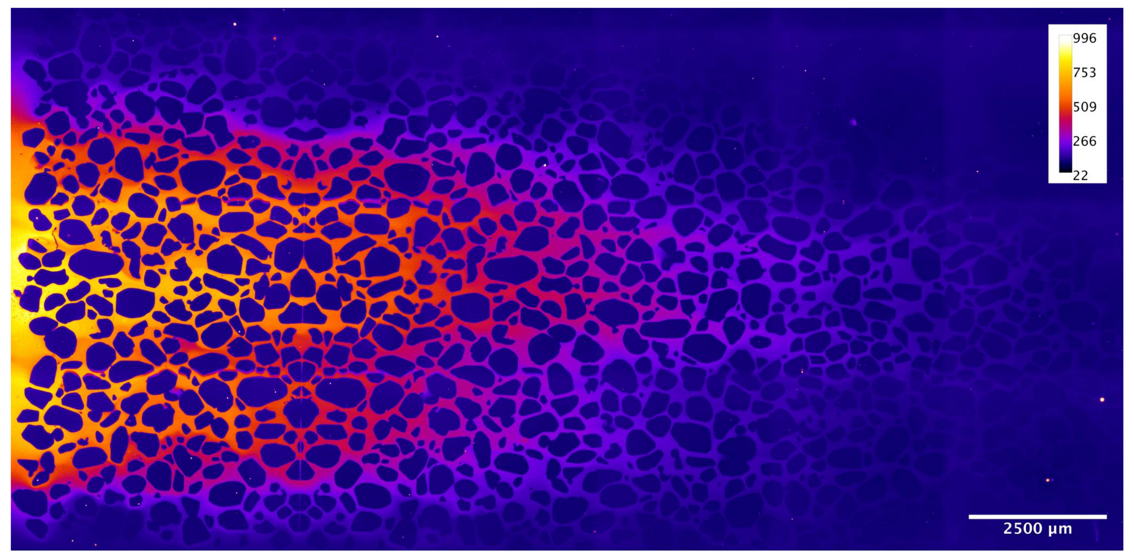




Left: Pore network used in reactors; Right: Probability density function of pore size and grain size distribution.

5. Flow-Field Characterization

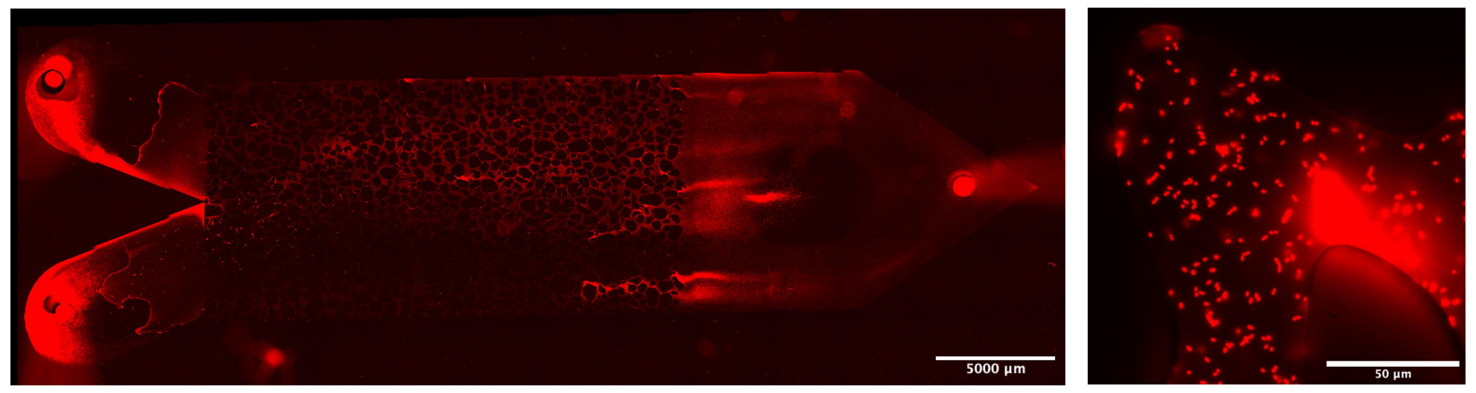
Qualitative assessment of reactant concentration gradient using fluorescent tracer.



Fluorescein concentration in reactor from inlet (left) to outlet (right).

6. Microbial Inoculation

Inoculation of reactor with fluorescent bioreporter strain of *P. putida* GB-1. Epifluorescence microscopy images used for quantitative analysis.

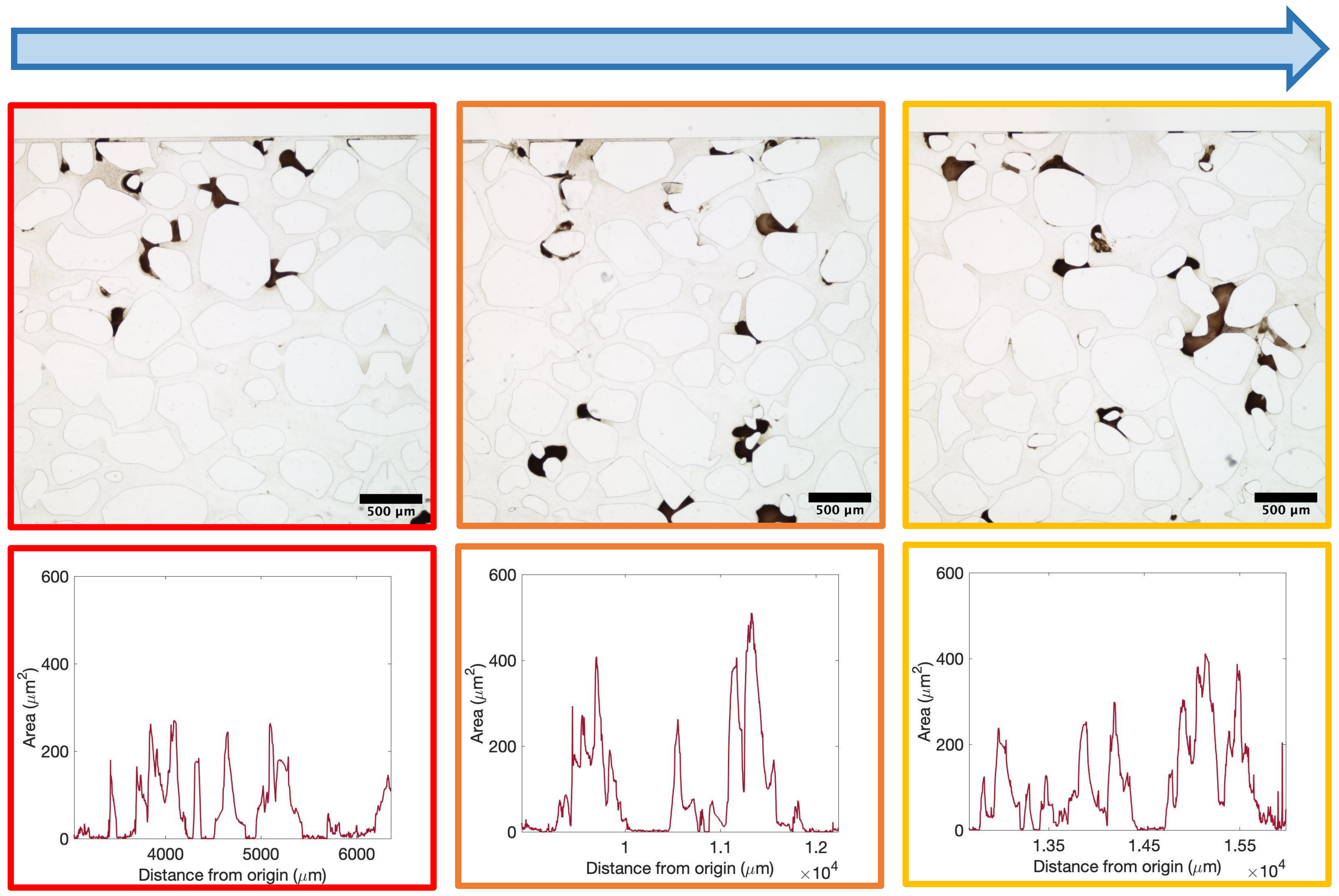


P. putida GB-1 in reactor in mCherry channel at 4x (left) and 100x (right) magnification.

7. Biomineral Production

Inoculated reactor with *P. putida* GB-1 in growth medium containing Mn(II), grew to stationary phase with intermittent feeding and starvation, observed distribution and size of Mn oxide aggregates.

Direction of fluid flow in microfluidic reactor



Top: Brightfield color images of Mn oxides in porous medium after inoculation and 64 hours of feeding/ starving.
Bottom: One-dimensional signal of total Mn oxide area at three positions in reactor pore network.

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