Oregon State University
COLLEGE OF ENGINEERING | School of Chemical, Biological, and Environmental Engineering

Column Study of Aerobic Cometabolism of Chlorinated Solvents and 1,4-Dioxane with Co-Encapsulated Bacteria and Slow-Release Substrate in Hydrogel Beads

Kaden Bennett

Battelle Chlorinated Conference 2024
6/6/24

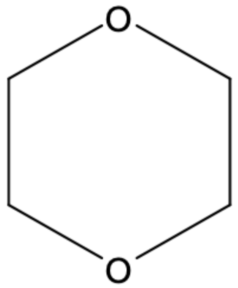
Groundwater Contaminants

1. Adamson et al. (2017)

2. "Chemical Search | IRIS | US EPA"

1,4-Dioxane

- Present in over 21% of US public water systems [1]
- Listed as probable human carcinogen by EPA [2]
- Used as a solvent stabilizer for chlorinated solvents



1,4-Dioxane
(1,4-D)

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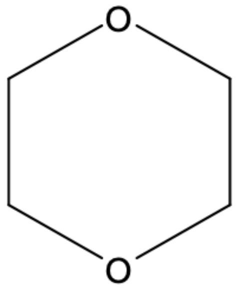
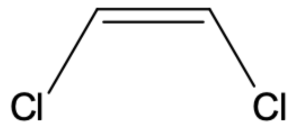


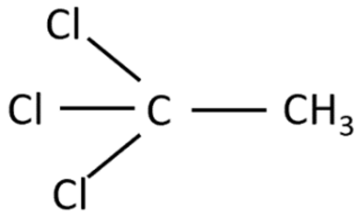
1,4-Dioxane
(1,4-D)

Chlorinated Solvents

- Chlorine-containing compounds often used as industrial solvents & degreasers
- Acutely toxic & many listed as possible or confirmed human carcinogens by EPA



cis-1,2-Dichloroethylene
(cis-DCE)

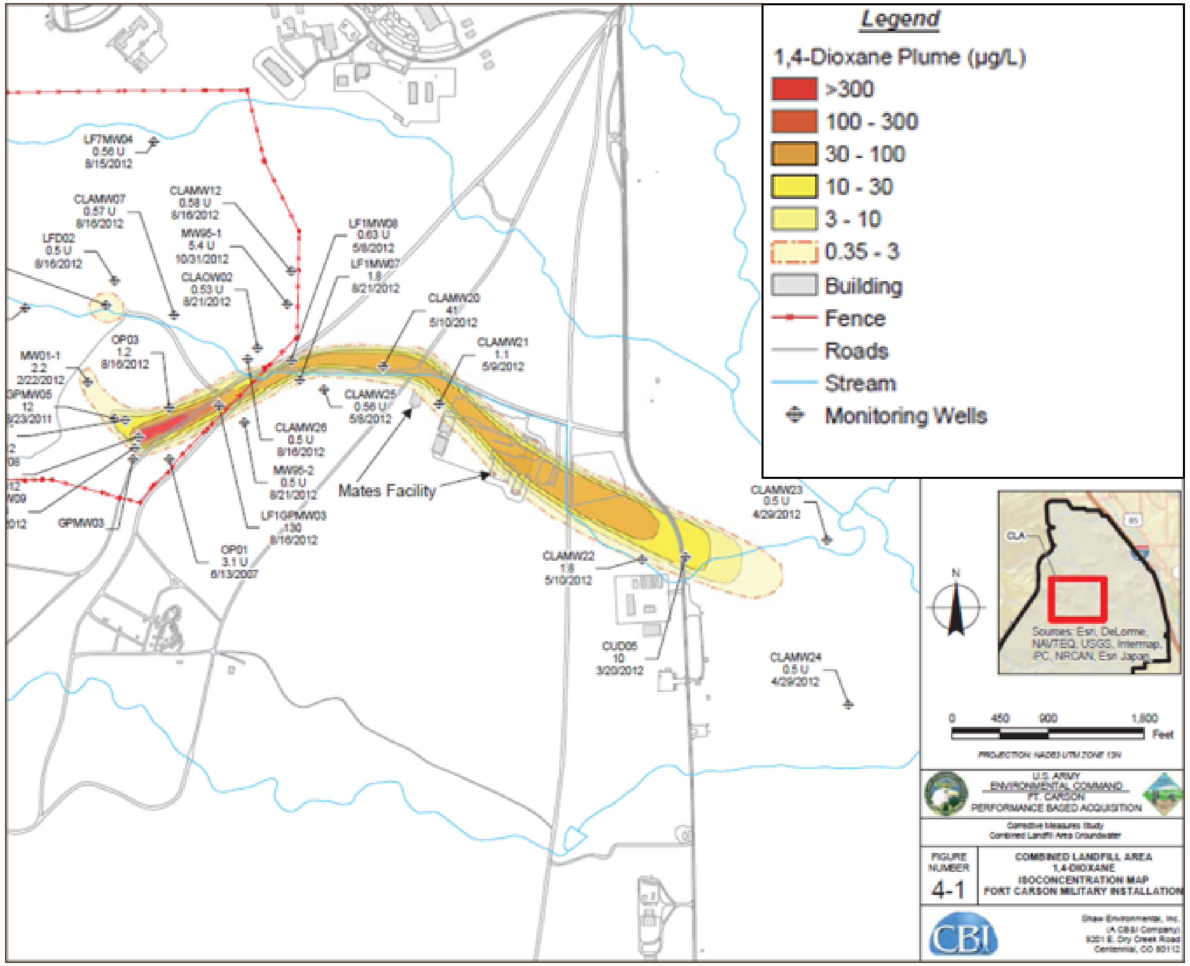


1,1,1-Trichloroethane
(1,1,1-TCA)

Mixed Groundwater Contaminant Plumes

<u>Co-occurrence of 1,4-D and Chlorinated Solvents</u>

- 1,4-D and chlorinated solvents co-occur at many groundwater sites



Groundwater Plume in Fort Carson, CO

Background | Experimental Design | Results | Conclusion

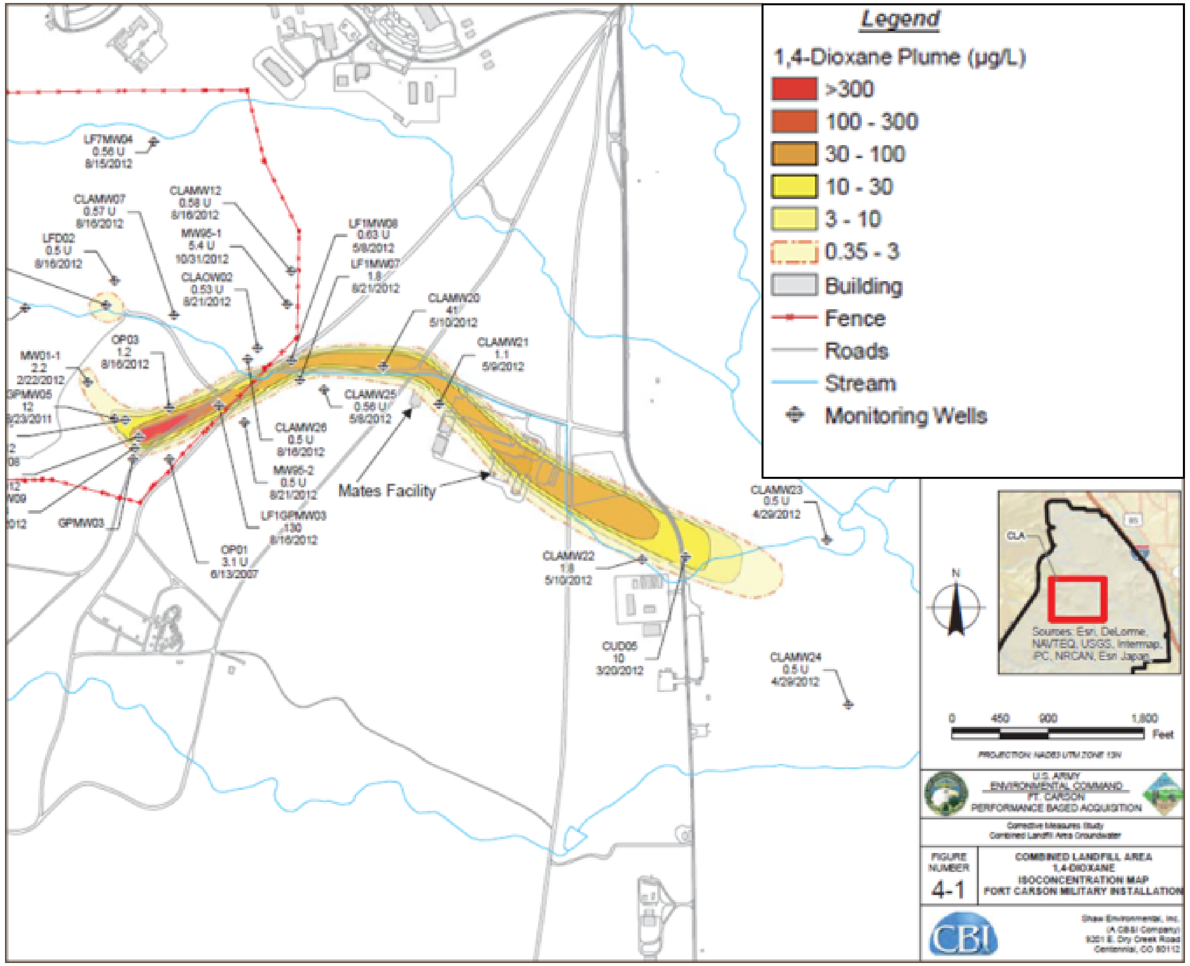
Mixed Groundwater Contaminant Plumes

<u>Co-occurrence of 1,4-D and Chlorinated Solvents</u>

- 1,4-D and chlorinated solvents co-occur at many groundwater sites

- 195 1,4-D contaminated GW sites in California
  - 95% with measured chlorinated solvents
  - 76% with measured 1,1,1-TCA

(Adamson et al., 2014)



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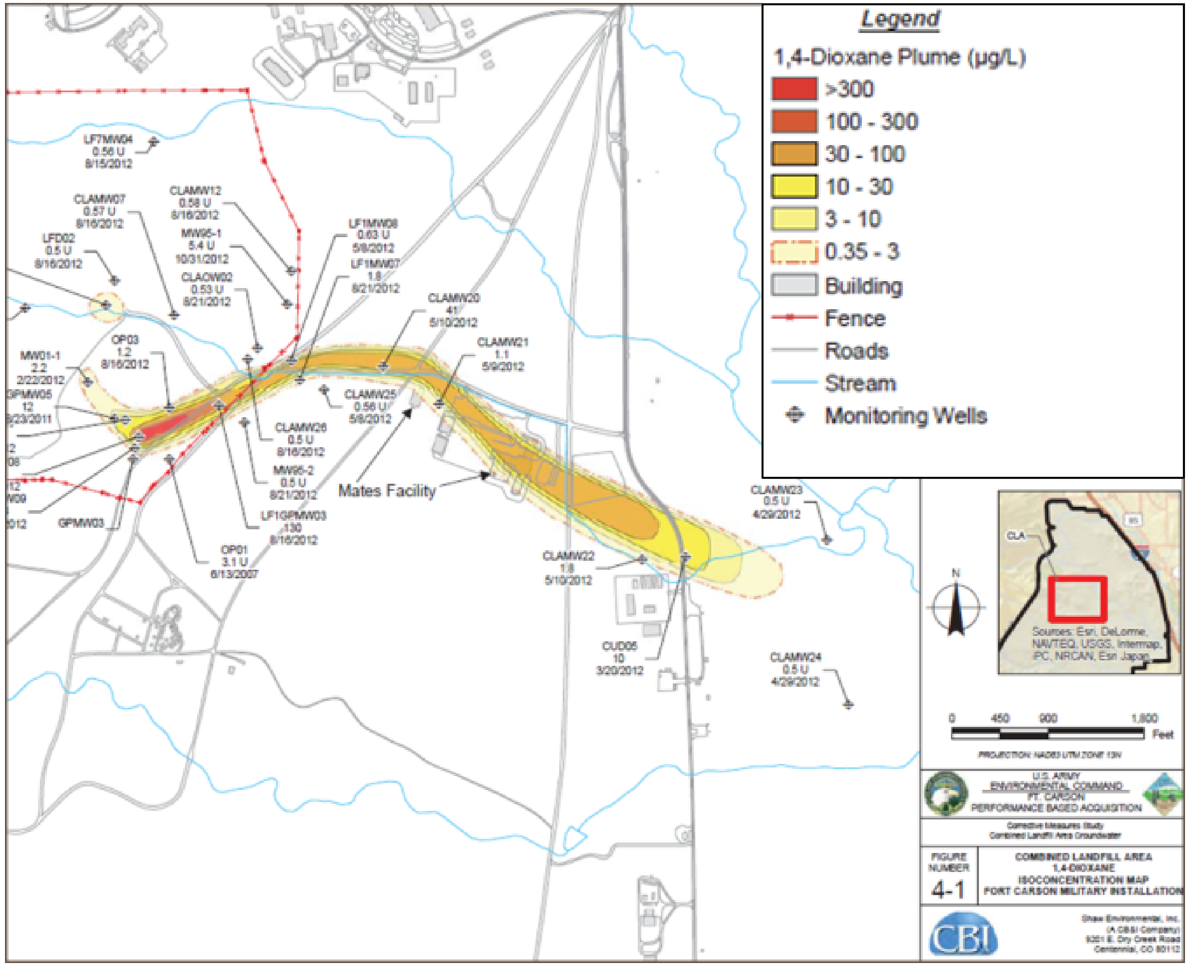
  (Adamson et al., 2014)
- Different treatment technologies for 1,4-D and chlorinated solvents
- Often not feasible to remediate entire plumes



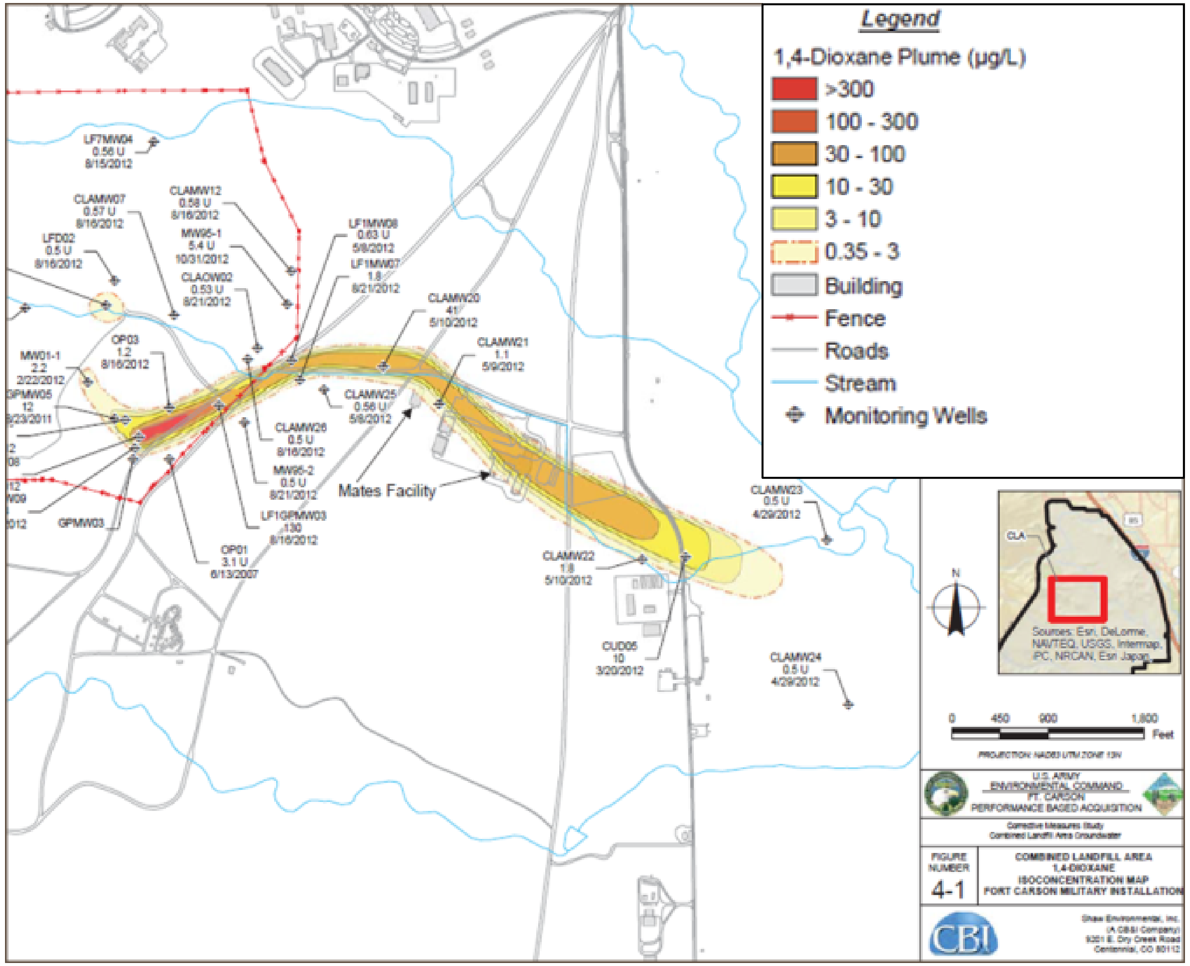
Groundwater Plume in Fort Carson, CO

Mixed Groundwater Contaminant Plumes

<u>Containment of Groundwater Plumes</u>

- Often not feasible to remediate entire plumes

- **Goal:** Develop long-term containment technology for mixed contaminant plumes of 1,4-D and chlorinated solvents.



Groundwater Plume in Fort Carson, CO

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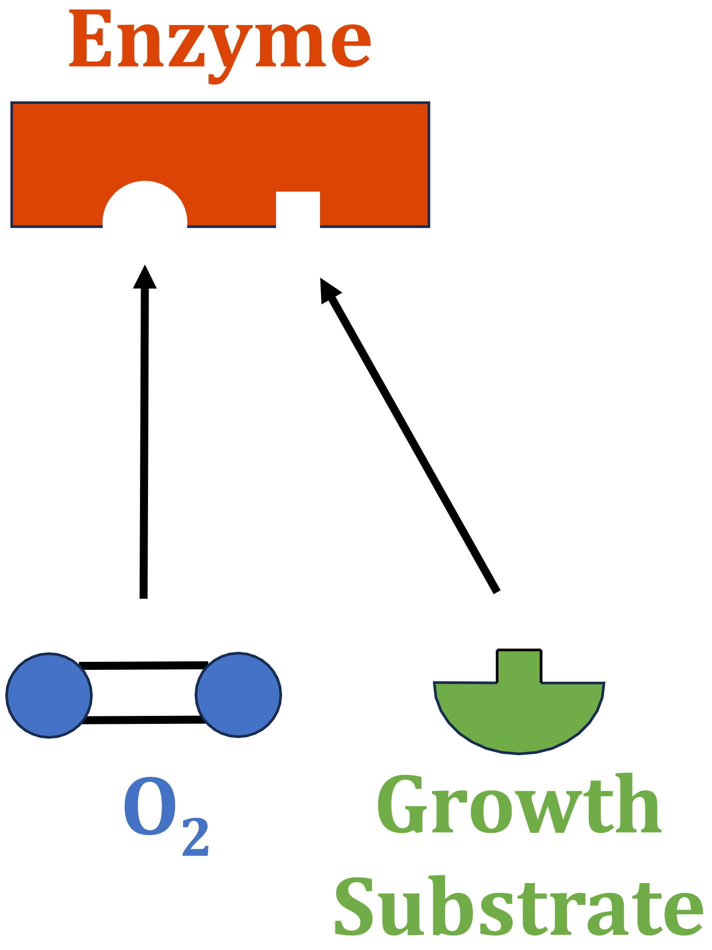
Bioremediation of Chlorinated Solvents & 1,4-Dioxane

One promising treatment option: **Aerobic Cometabolism**

Aerobic Cometabolism

Aerobic Cometabolism

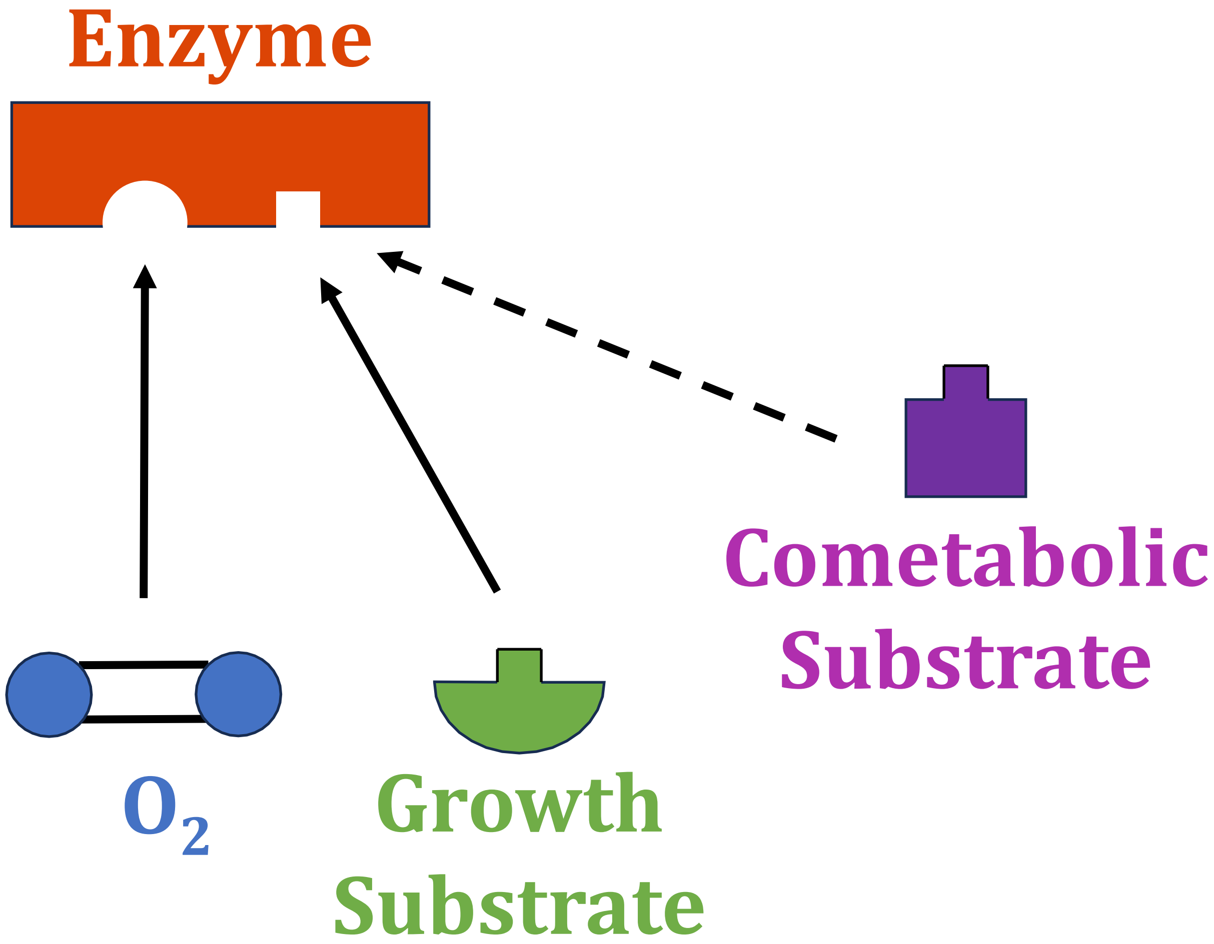
- Some aerobic microbes have non-specific metabolic enzymes



Aerobic Cometabolism

Aerobic Cometabolism

- Some aerobic microbes have non-specific metabolic enzymes
- Fortuitous transformation of cometabolic substrate (non-growth)



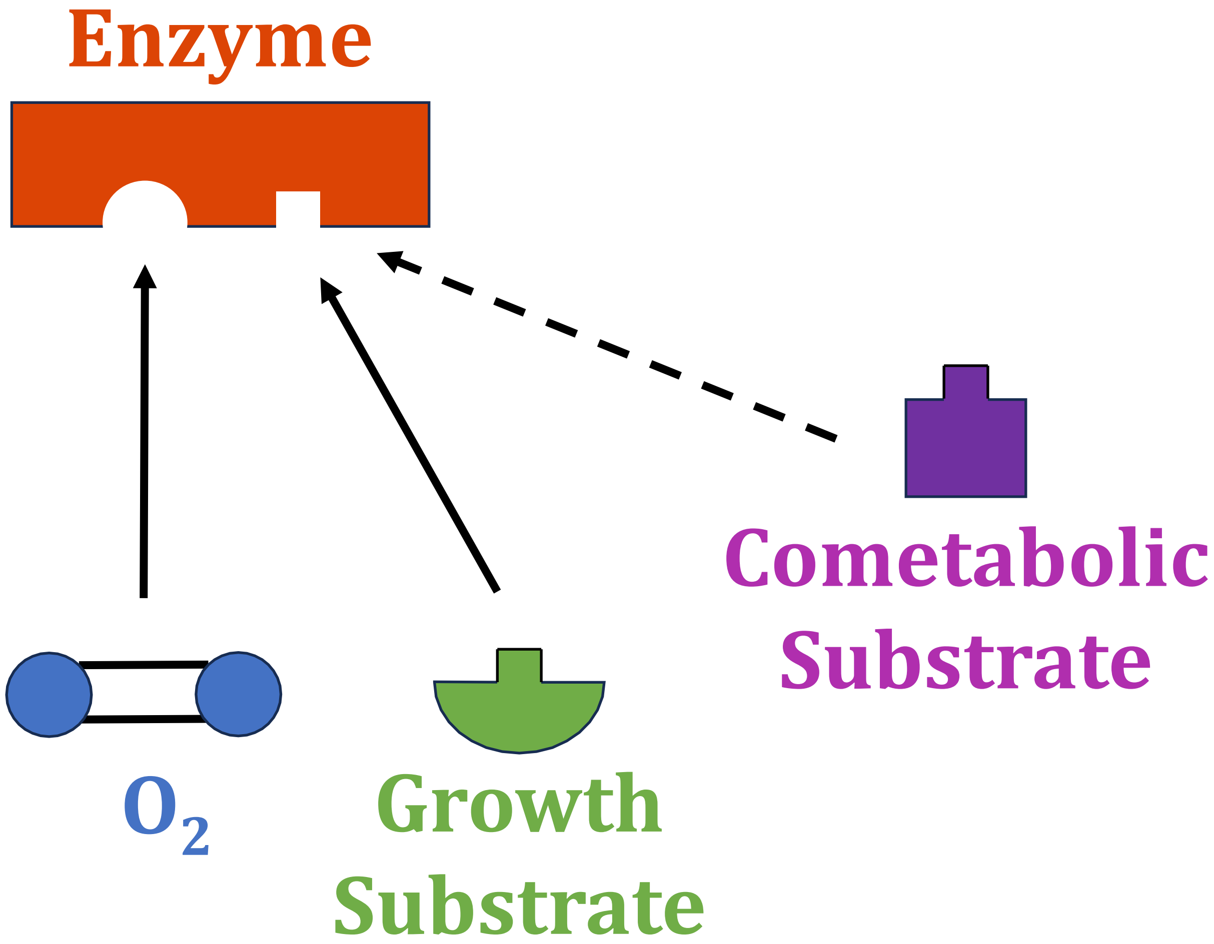
Aerobic Cometabolism

Aerobic Cometabolism

- Some aerobic microbes have non-specific metabolic enzymes

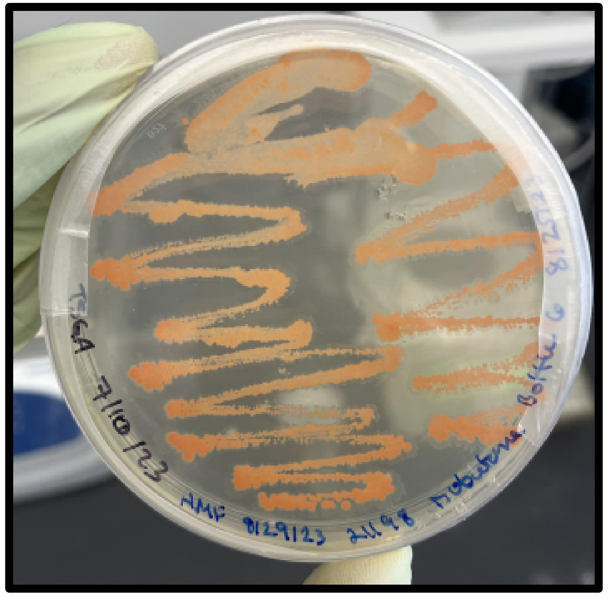
- Fortuitous transformation of cometabolic substrate (non-growth)

- **Benefit:** Continued transformation at low concentrations of cometabolic substrate



Our Cometabolic Bacterium

Rhodococcus rhodochrous
ATCC Strain 21198



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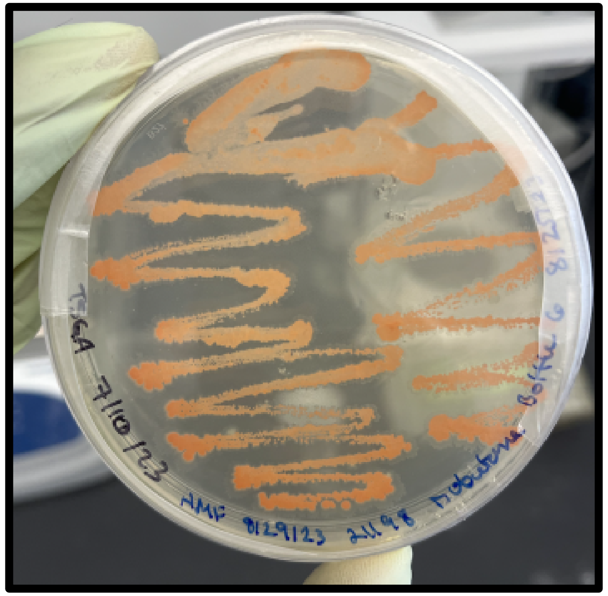
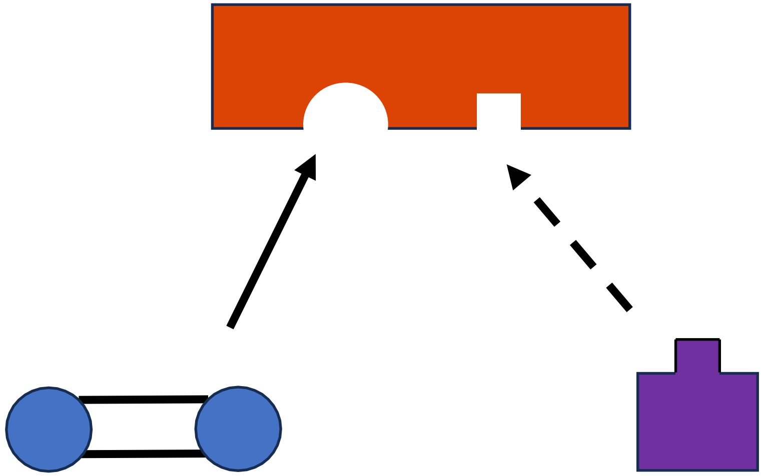


(ATCC 21198)

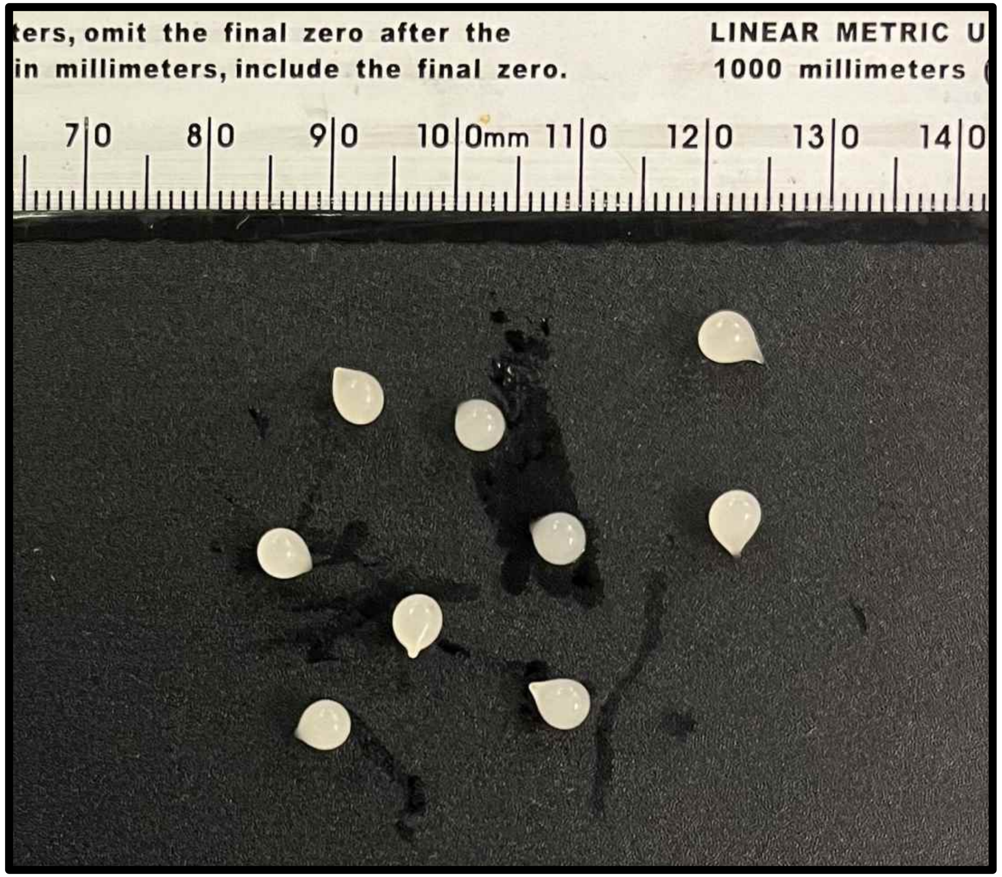
Short-Chain Alkane Monooxygenase



O_2

1,4-Dioxane
+
Chlorinated Solvents

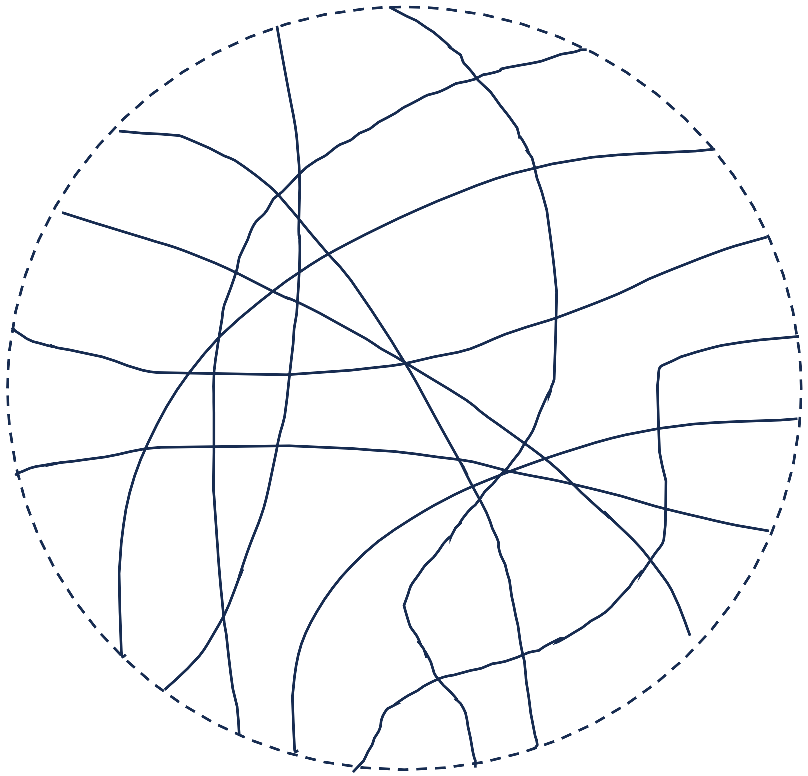
Hydrogels



- Water-based gels held together by polymers
- Polymers in our hydrogels:
  - Polyvinyl alcohol (synthetic)
  - Sodium alginate (natural organic)

Hydrogels

Hydrogel Bead Diagram



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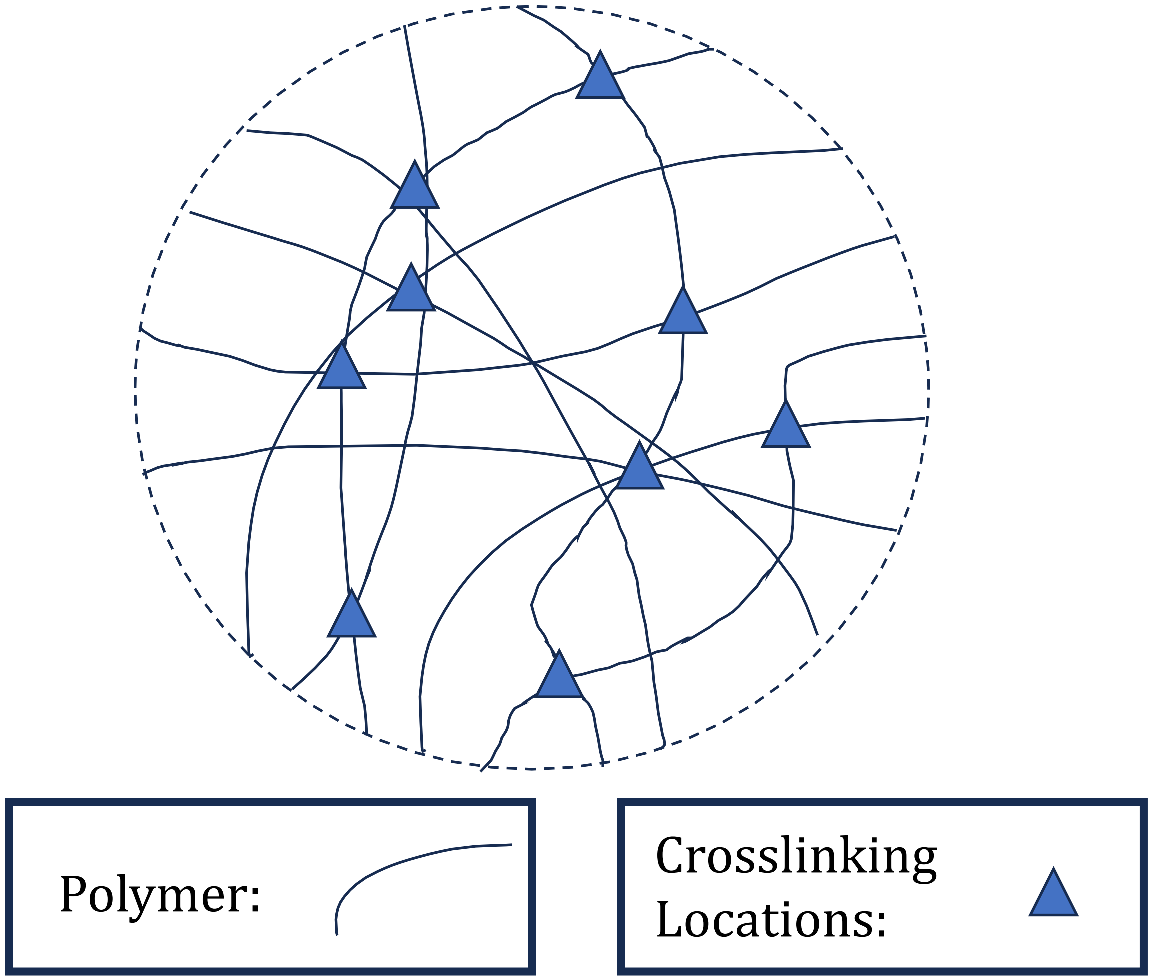


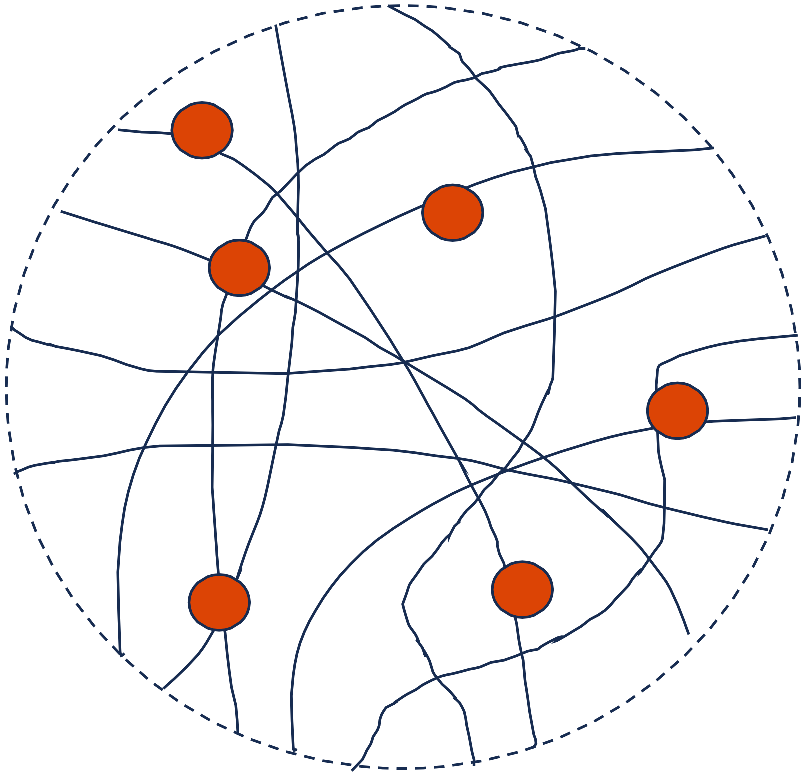
Hydrogels

- Water-based gels held together by polymers
- Polymers in our hydrogels:
  - Polyvinyl alcohol (synthetic)
  - Sodium alginate (natural organic)
- Crosslinked with calcium ions and boric acid

Hydrogel Beads

Polymer:

Bacteria (ATCC 21198):

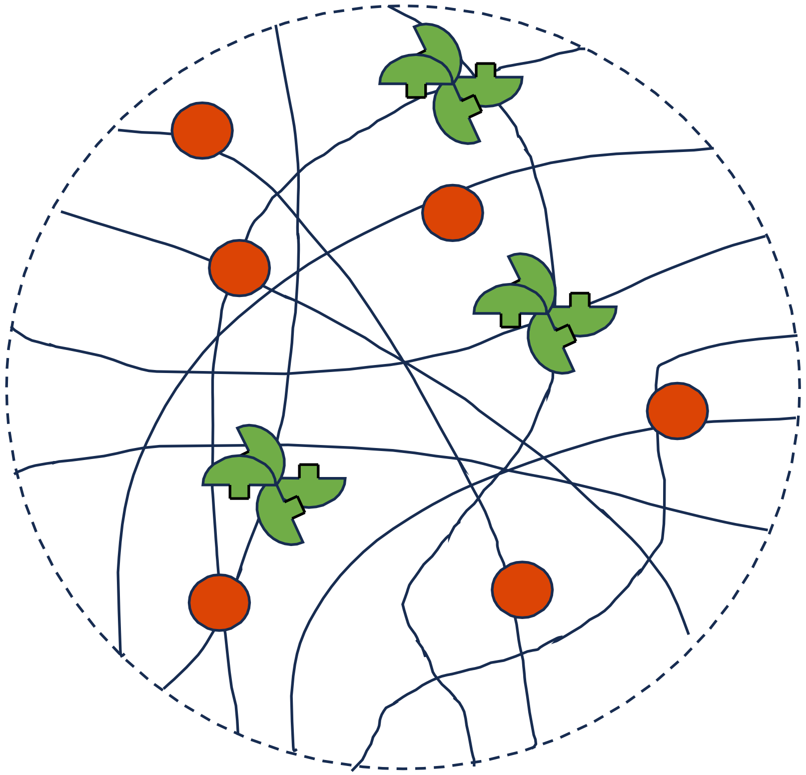


Hydrogel Beads

Polymer:

Bacteria (ATCC 21198):

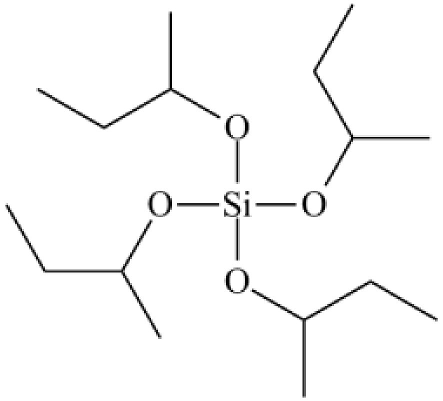
Slow-Release Substrate:



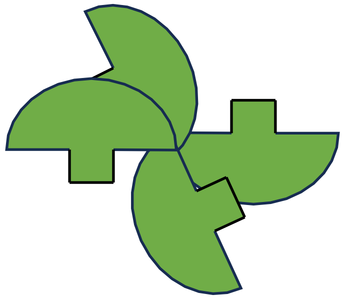
Background | Experimental Design | Results | Conclusion

Slow-Release Substrate

Tetra-sec-butyl Orthosilicate



$$\xrightarrow[\text{Hydrolysis}]{+\ H_2O}$$

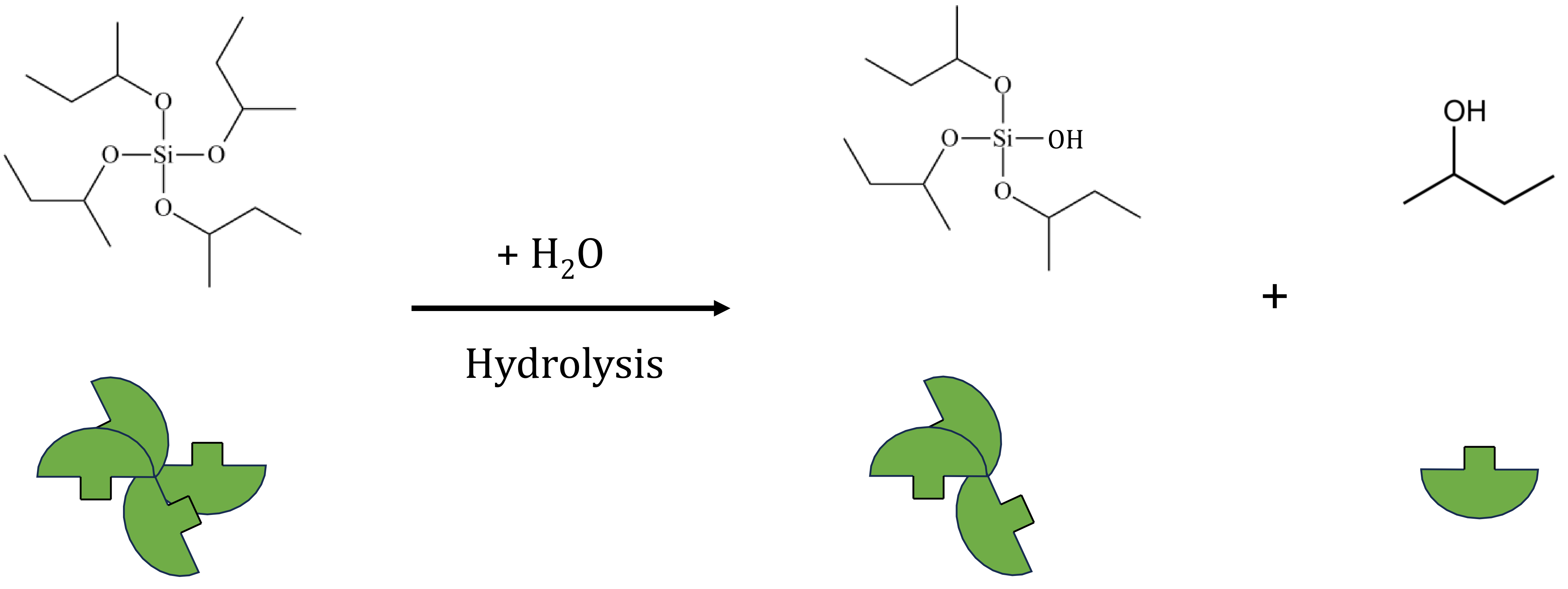


Slow-Release Substrate

Slow-Release Substrate

Tetra-sec-butyl Orthosilicate

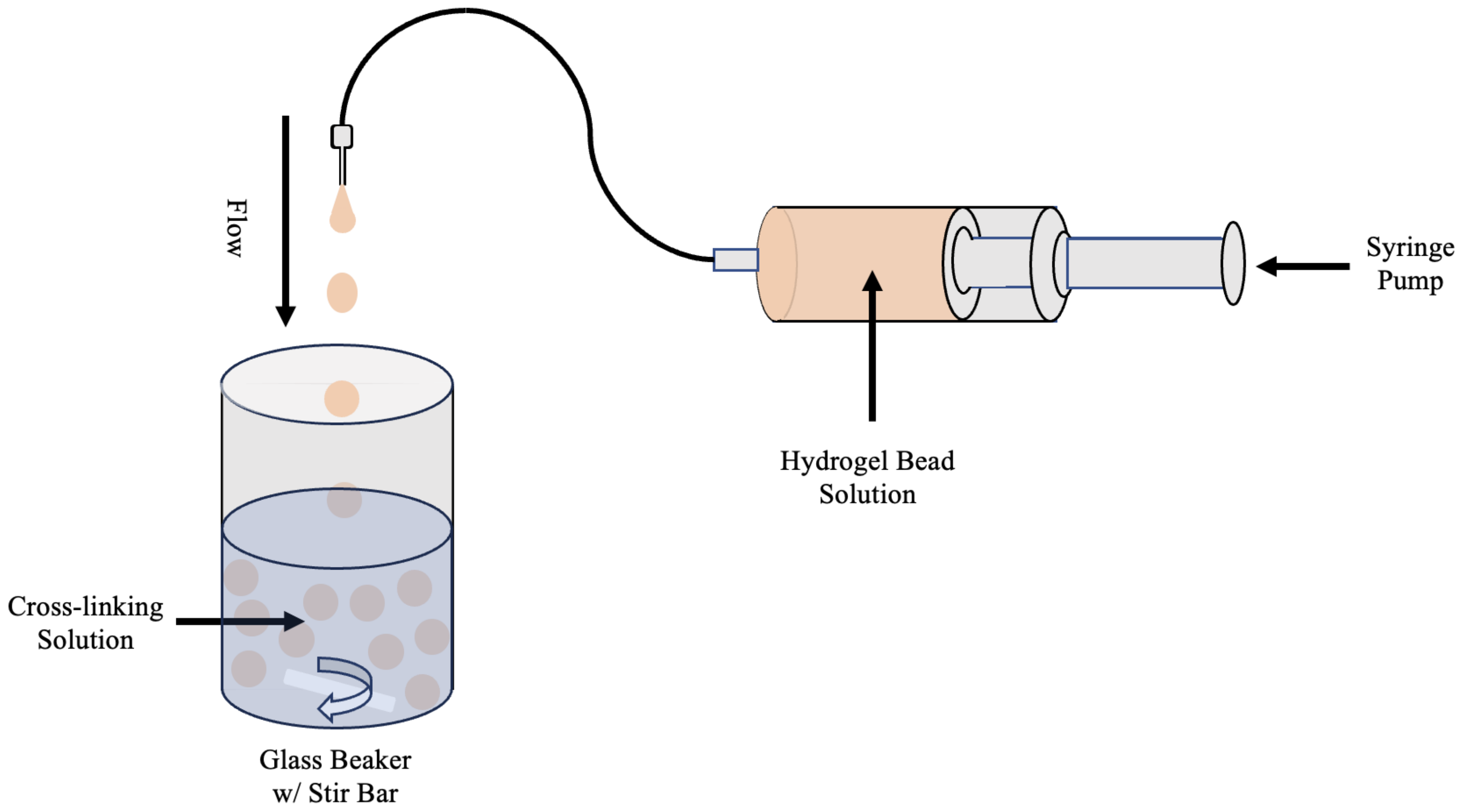
2-Butanol



Slow-Release Substrate

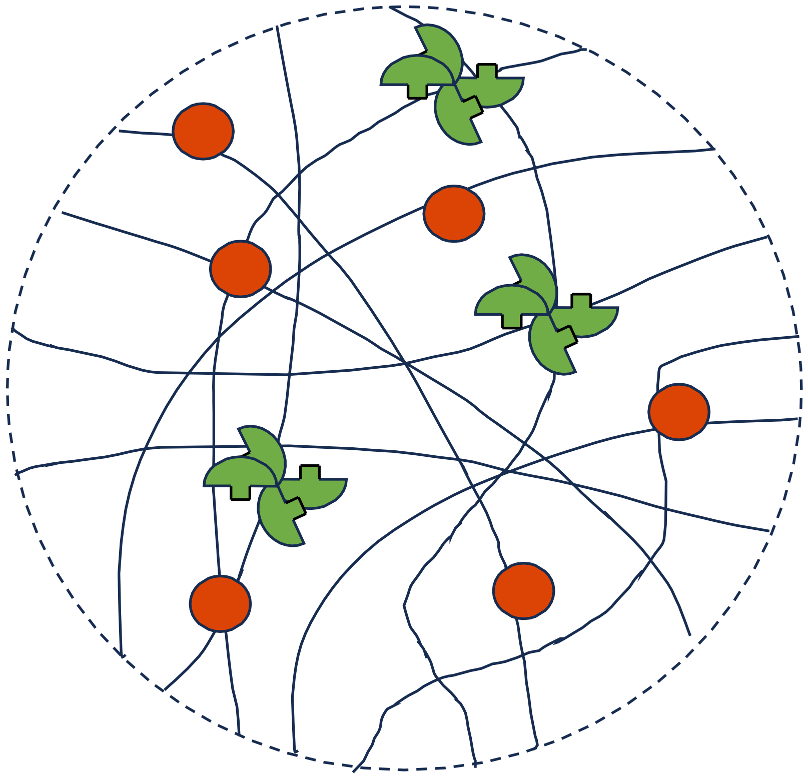
Growth Substrate

Hydrogel Bead Creation

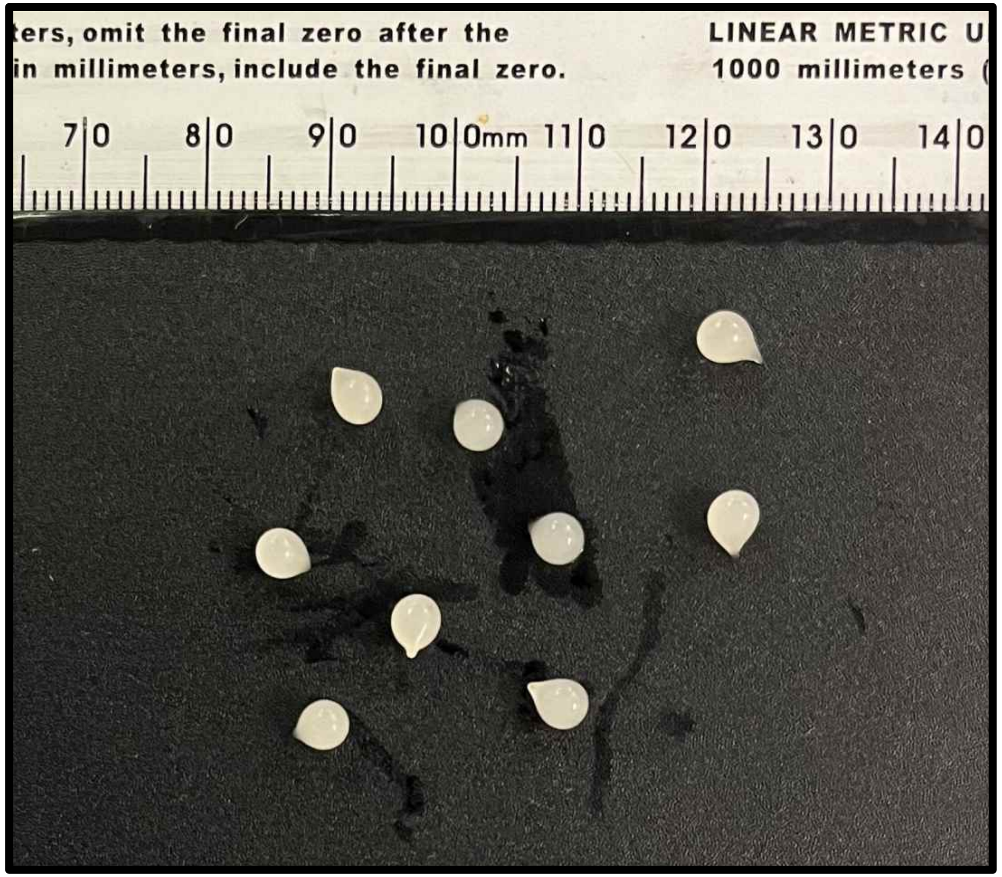


Hydrogel Beads

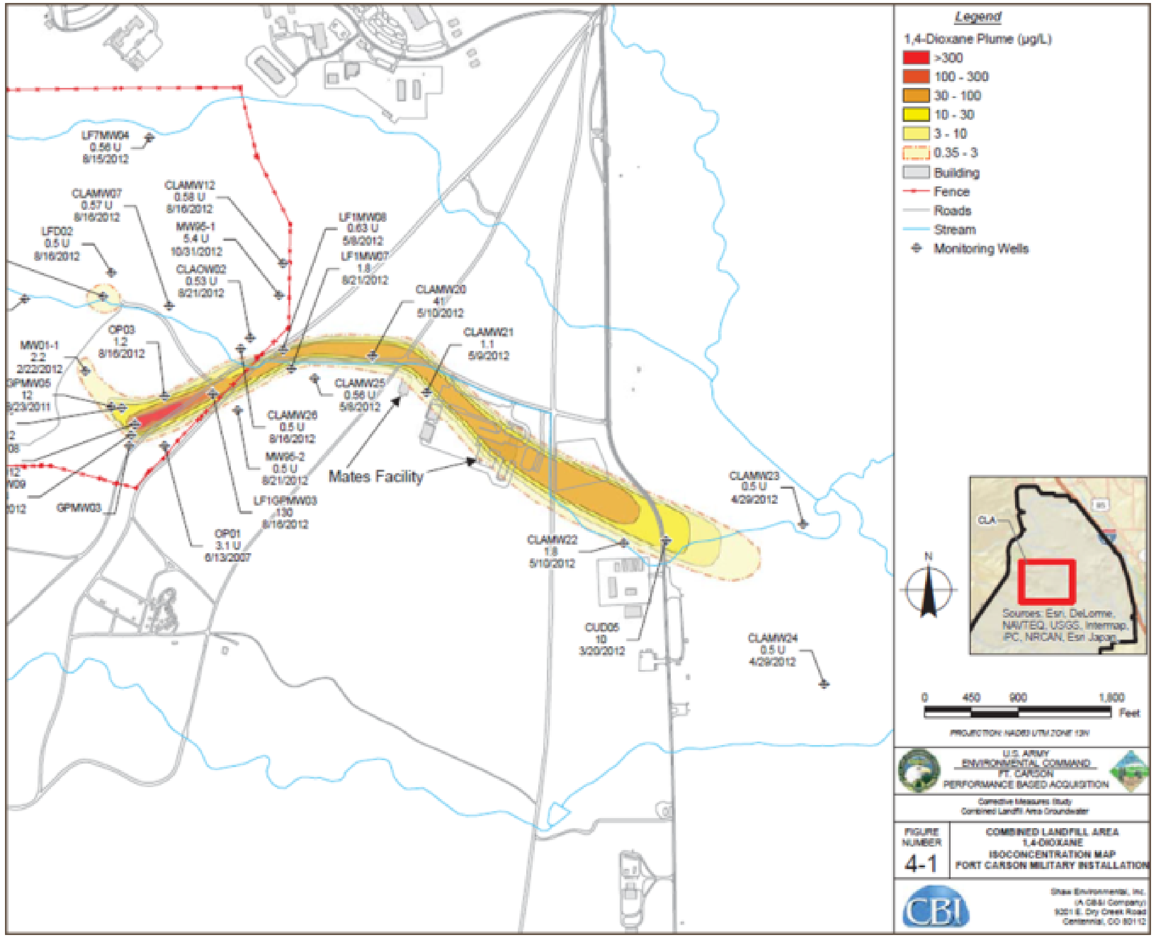
Slow-Release Substrate:



Bacteria (ATCC 21198):



Bioremediation Strategies



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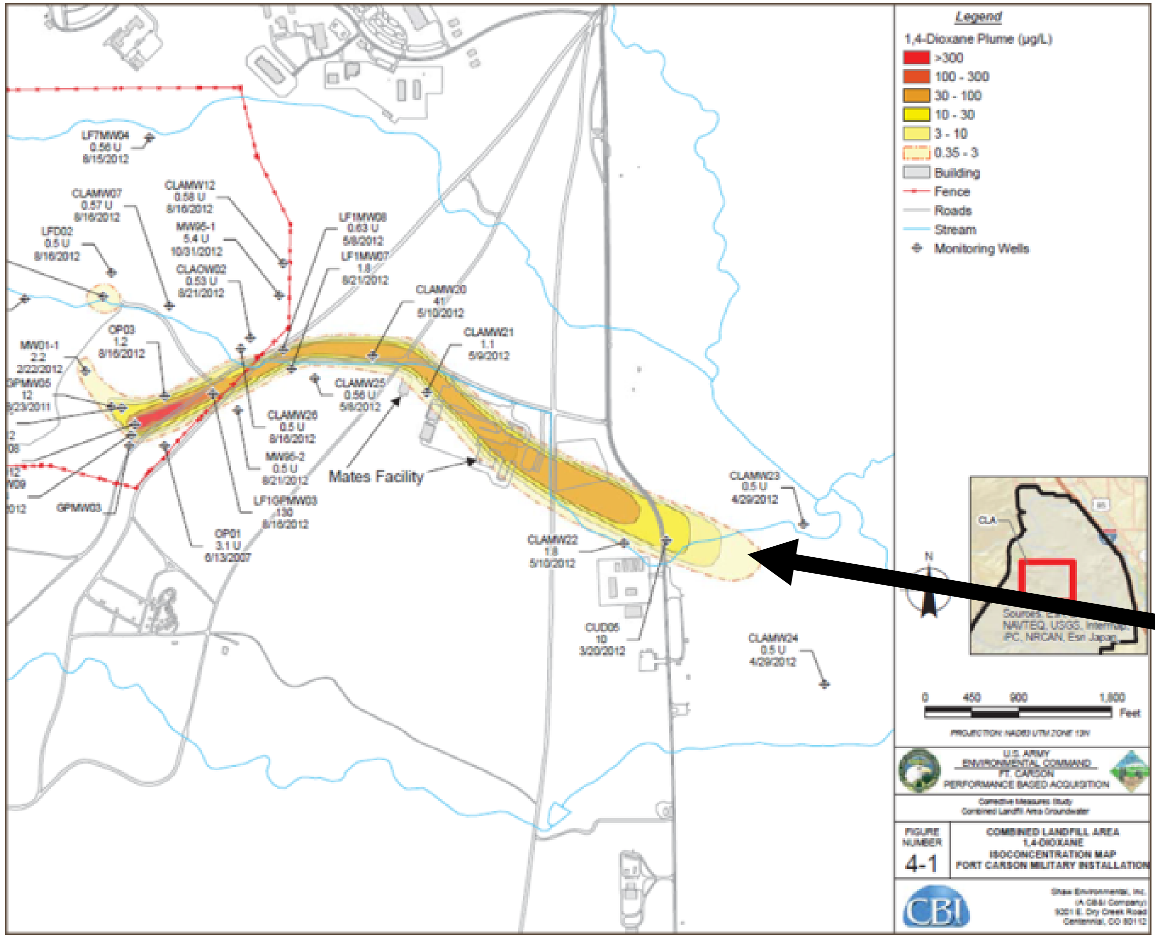
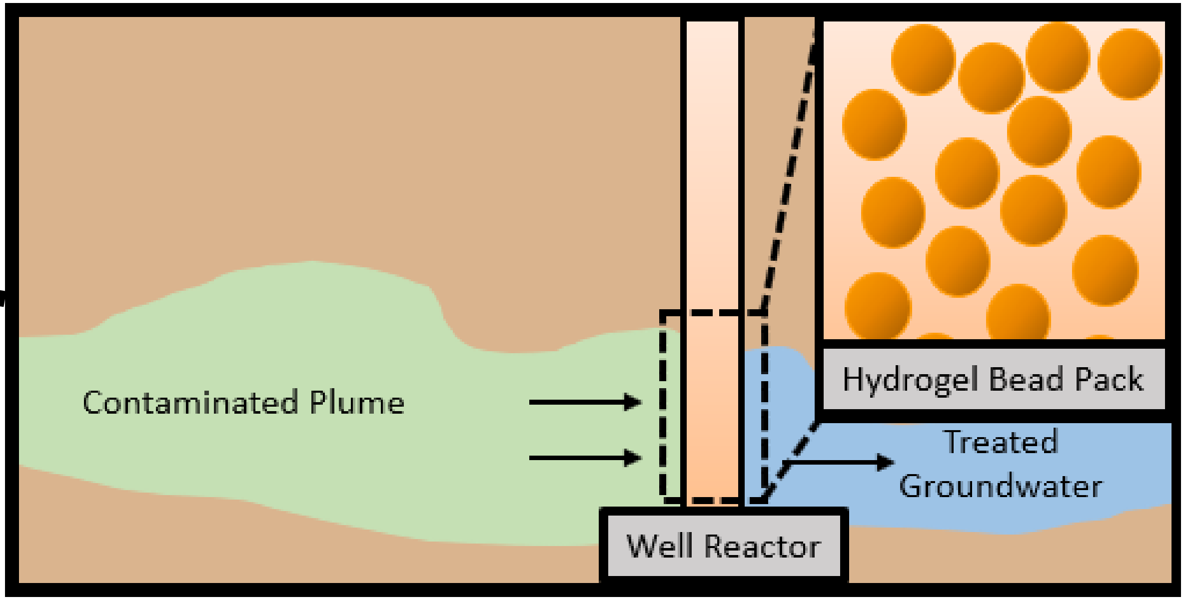


Proposal: Use hydrogel beads to create well reactors or permeable reactive barriers to treat mixed groundwater plumes of 1,4-D and chlorinated solvents.

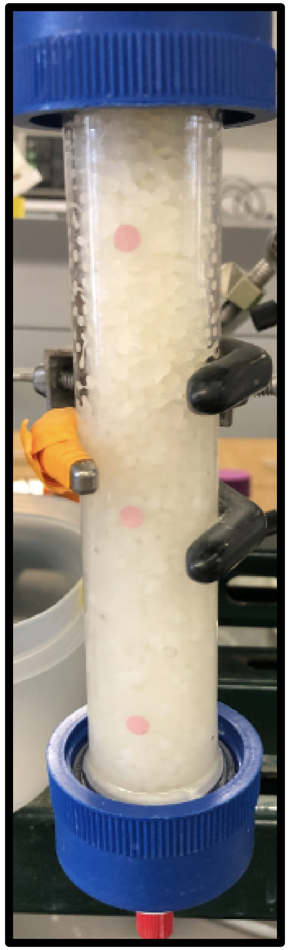


Packed Column Design

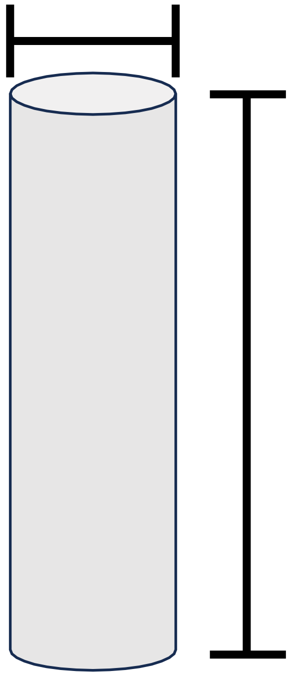
Column pack

Packed with 54 g of hydrogel beads containing:

- ATCC 21198
- Slow-release substrate



Diameter = 2.5 cm



Length = 15.9 cm

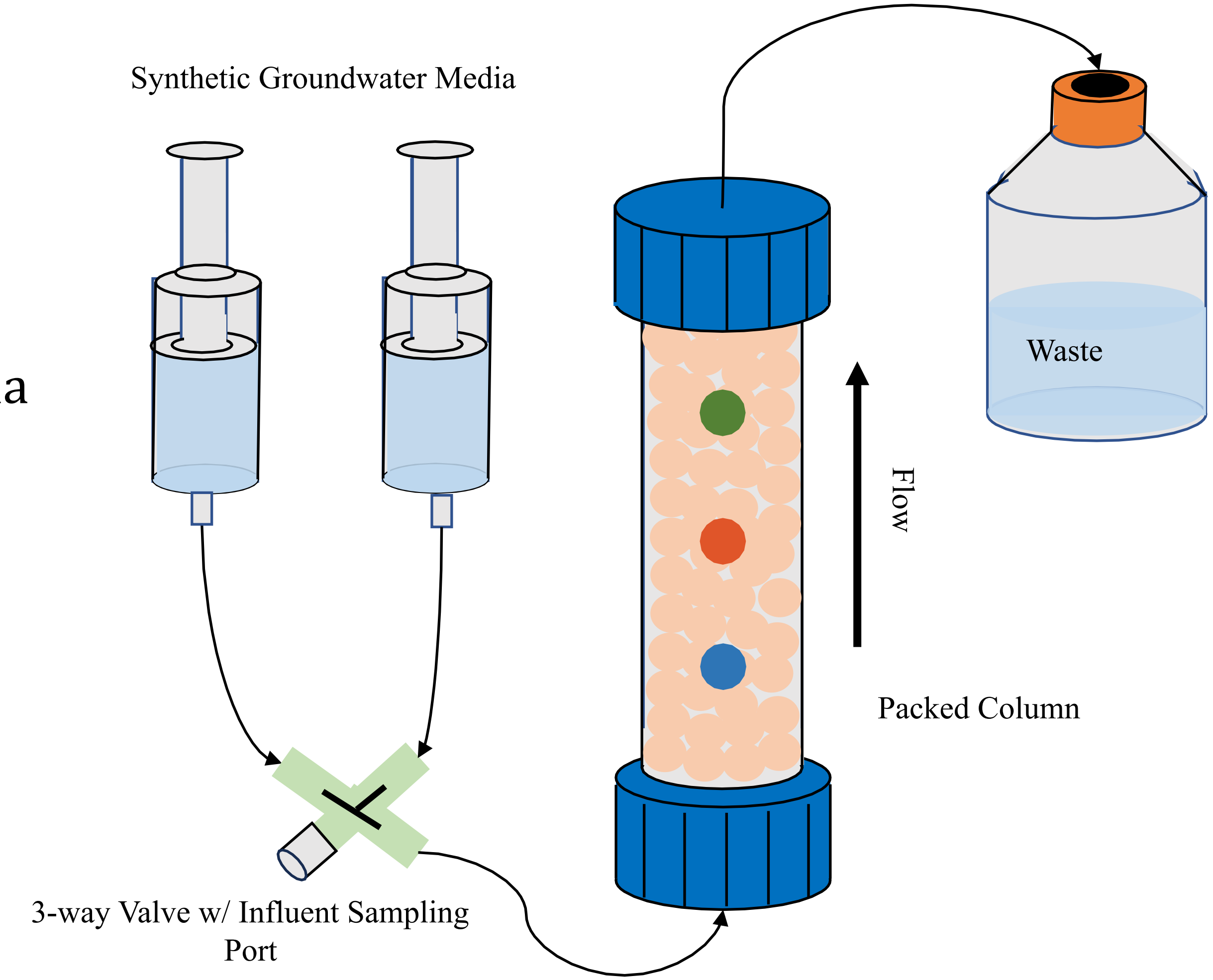
Volume = 78 mL

Packed Column Design

Synthetic Groundwater Media

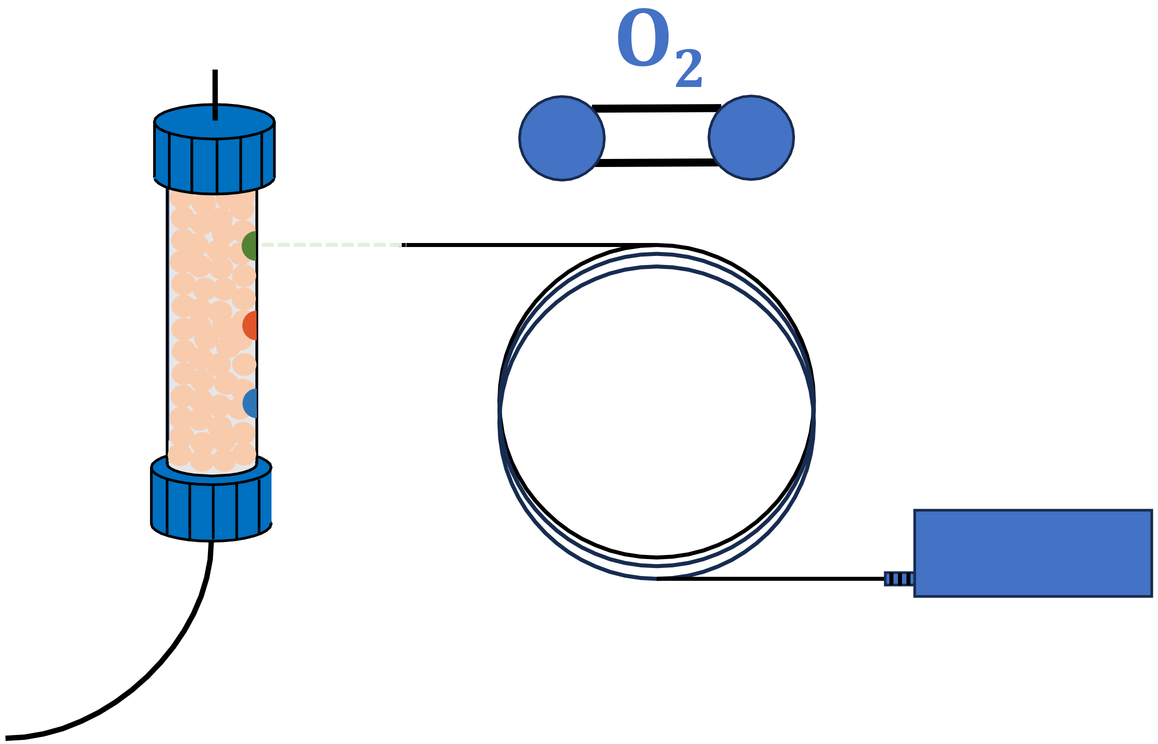
Composed of:

- Dissolved groundwater minerals
- Dilute nitrogen and phosphorus media
- Contaminants
  - 1,4-D
  - cis-DCE
  - 1,1,1-TCA
- Oxygen source
  - Hydrogen peroxide (H_2O_2)
  - Dissolved oxygen (DO)



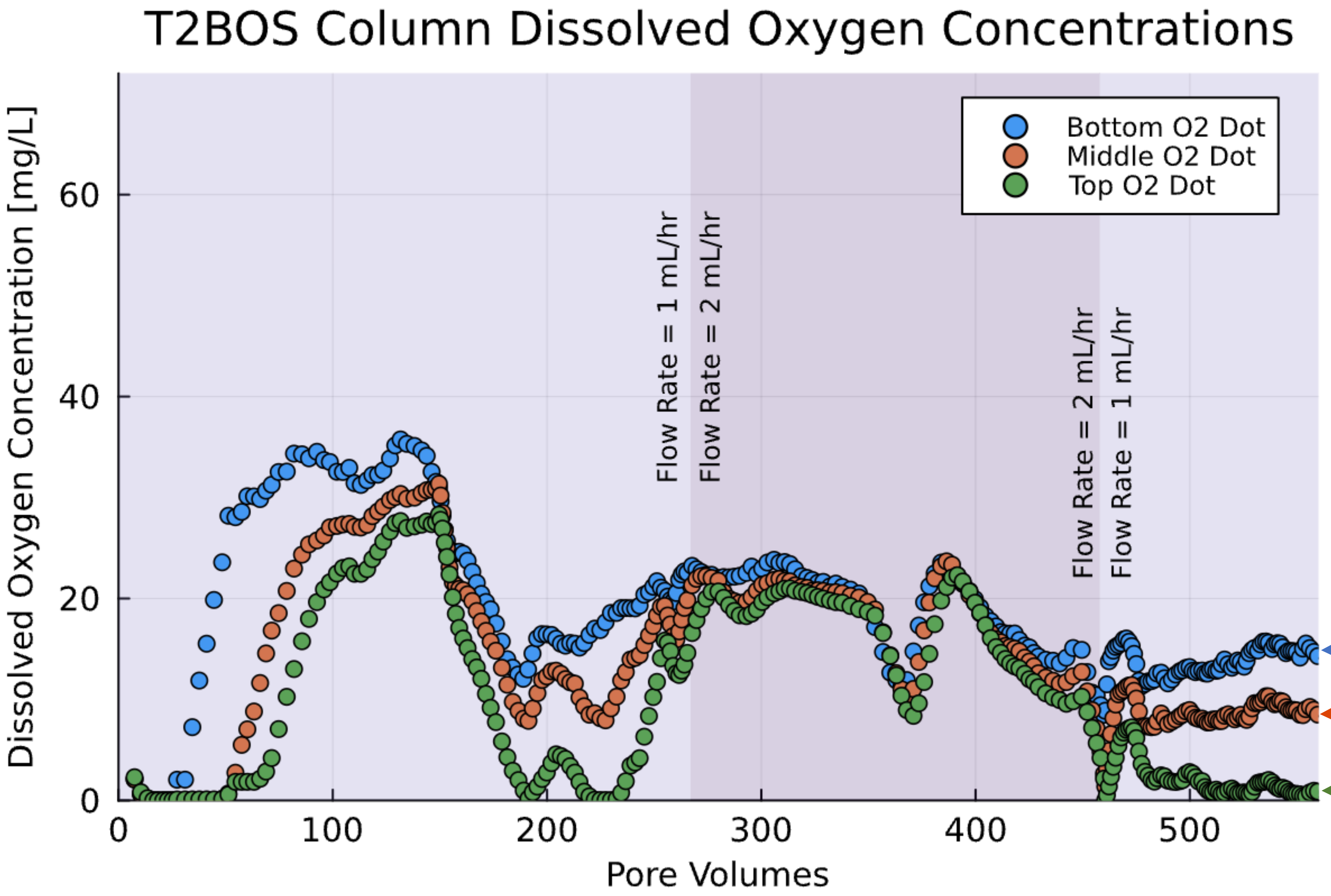
Packed Column Design

Fluorescent Oxygen Sensor Spots



Oxygen Sensor Spots spaced at ¼, ½, ¾ intervals along column

Oxygen Concentrations



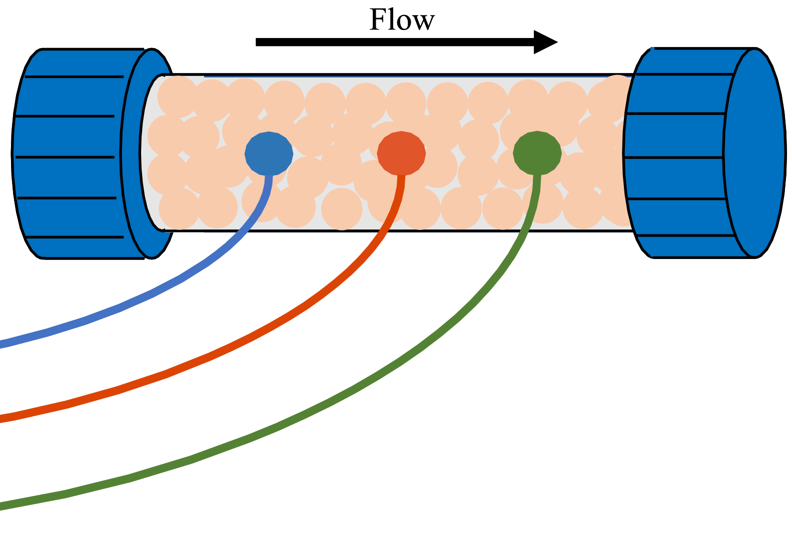
<u>Pore Volume (PV)</u>

Measurement of Operational Time

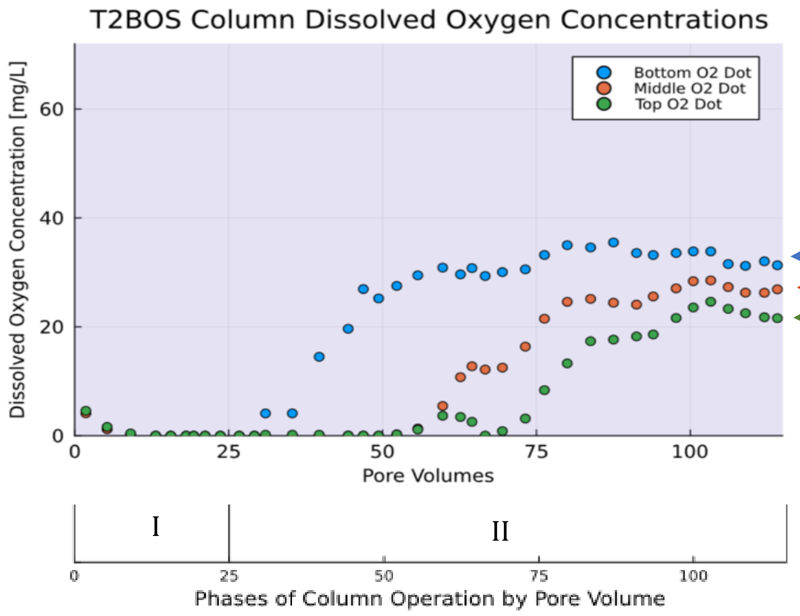
595 Pore Volumes → 553 days

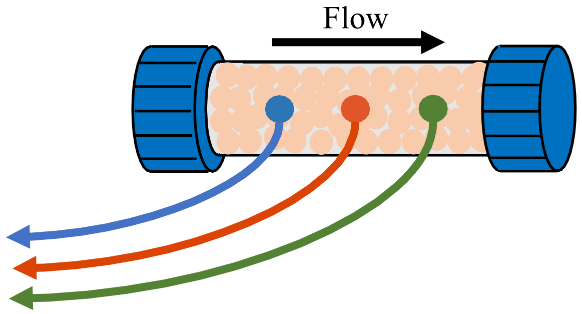
<u>Hydraulic Residence Time</u>

25.8 hours @ 1 mL/hr flow rate



Biostimulation of ATCC 21198 Within Hydrogel Beads





Operational Phase	Oxygen Source
Phase I	9 ppm DO
Phase II	9 ppm DO + 125 ppm H_2O_2

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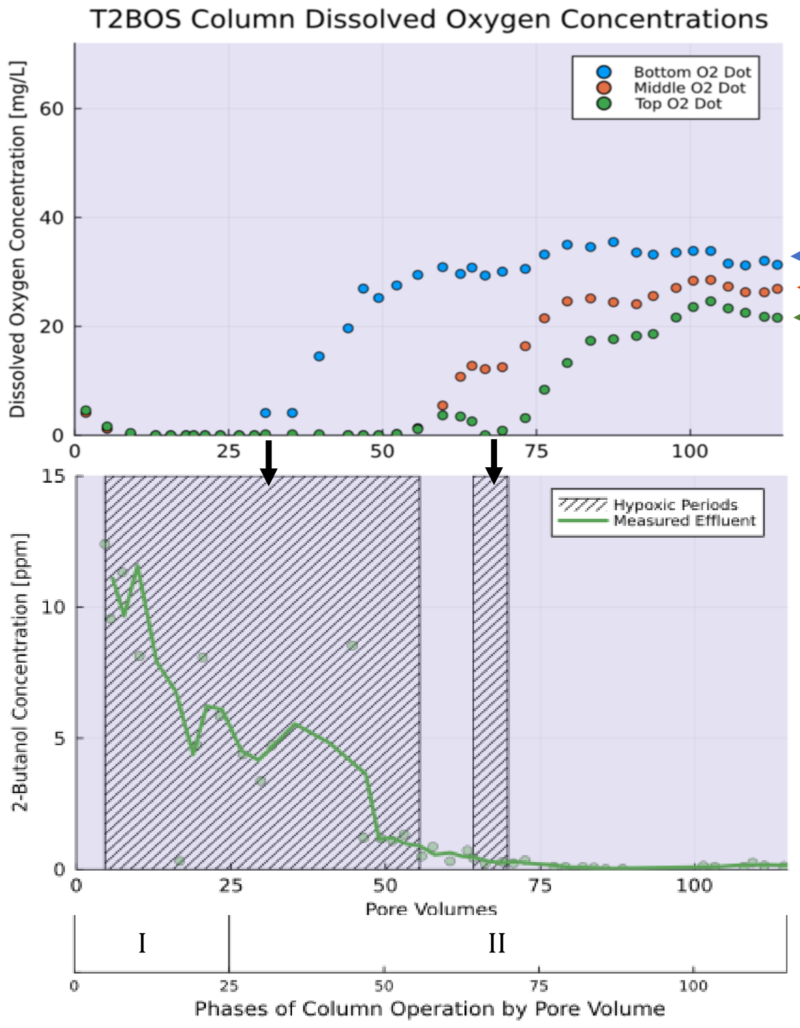

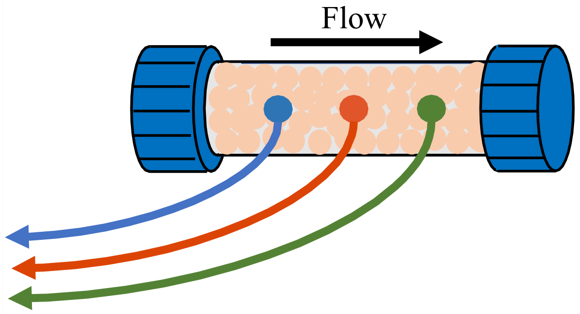

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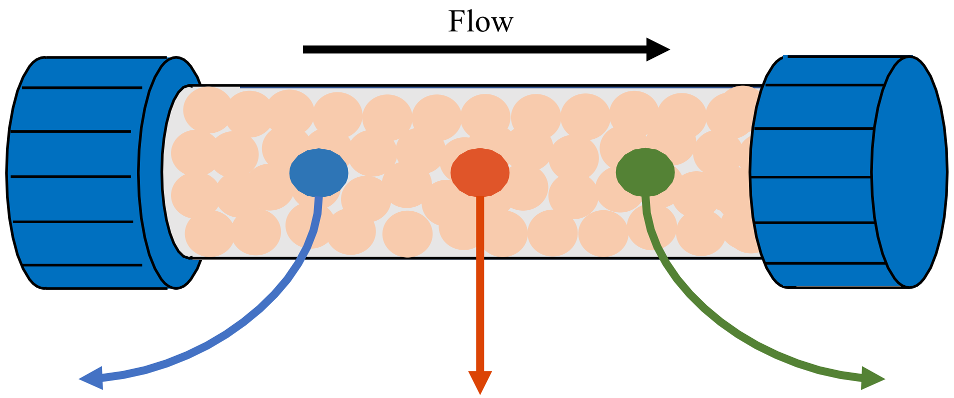

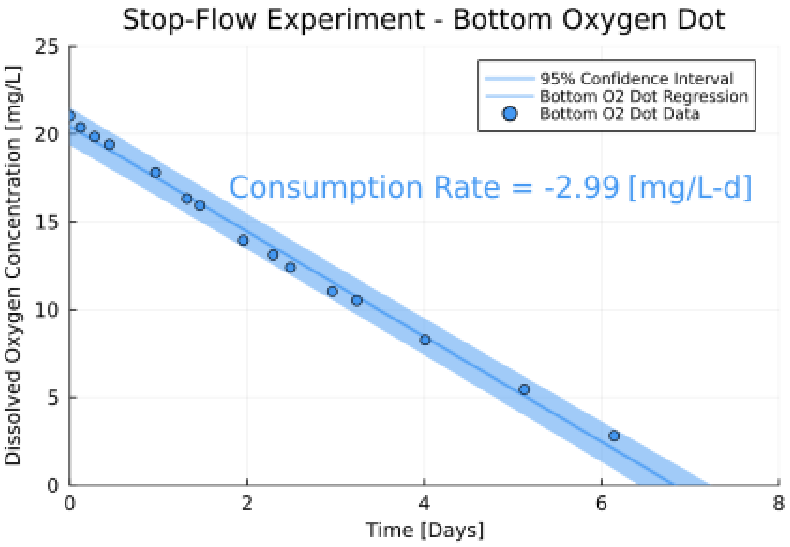

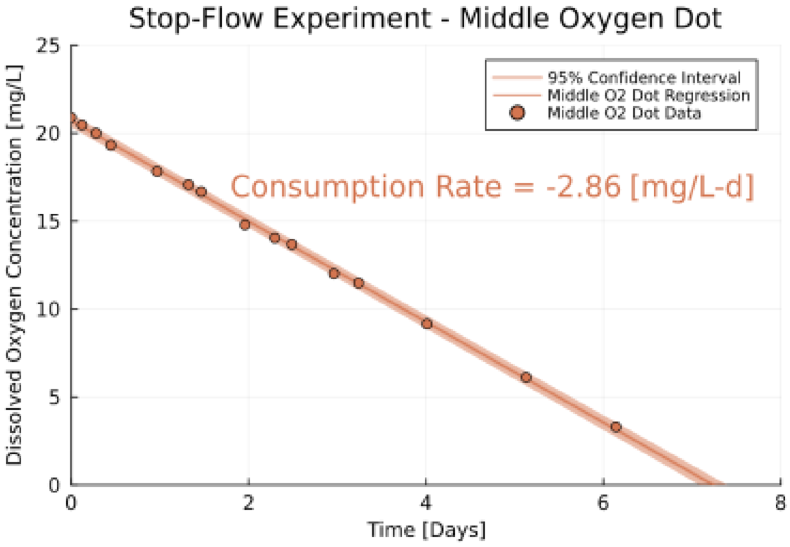

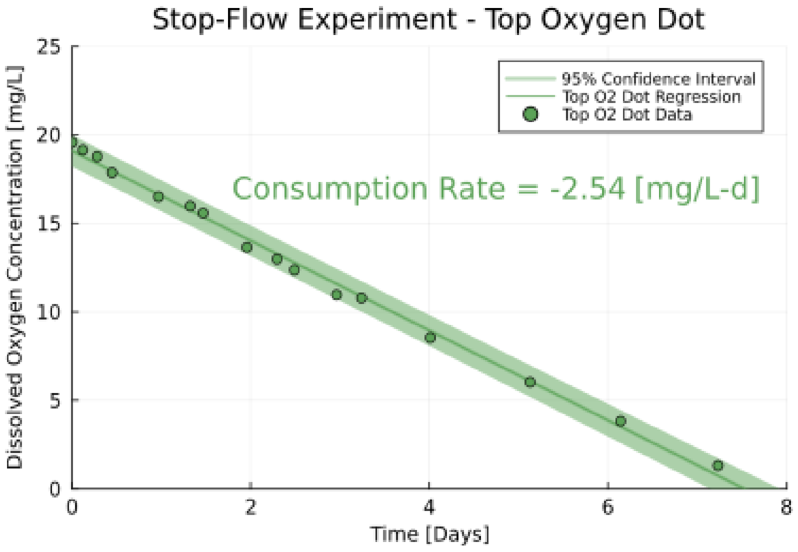

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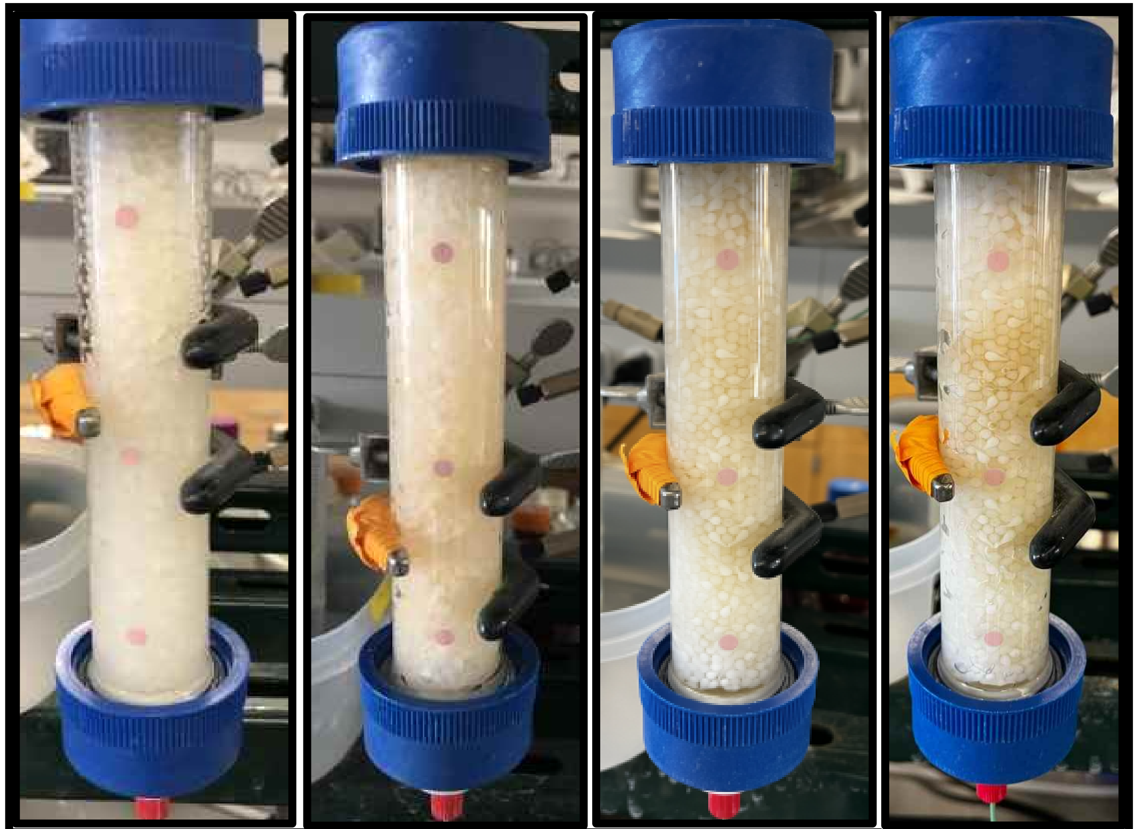
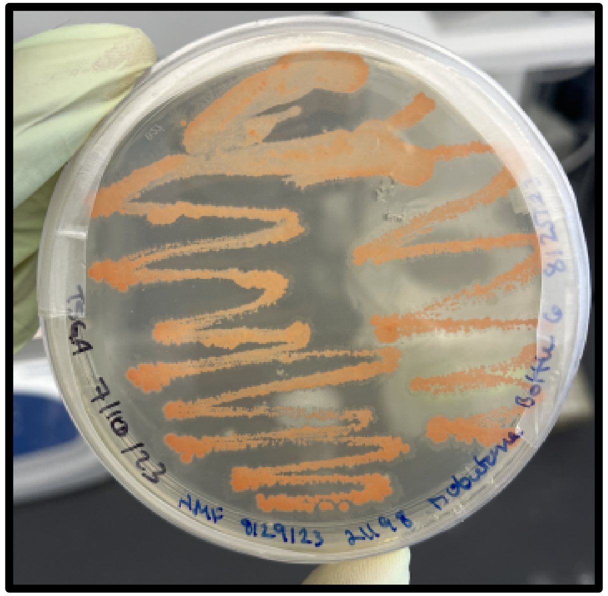


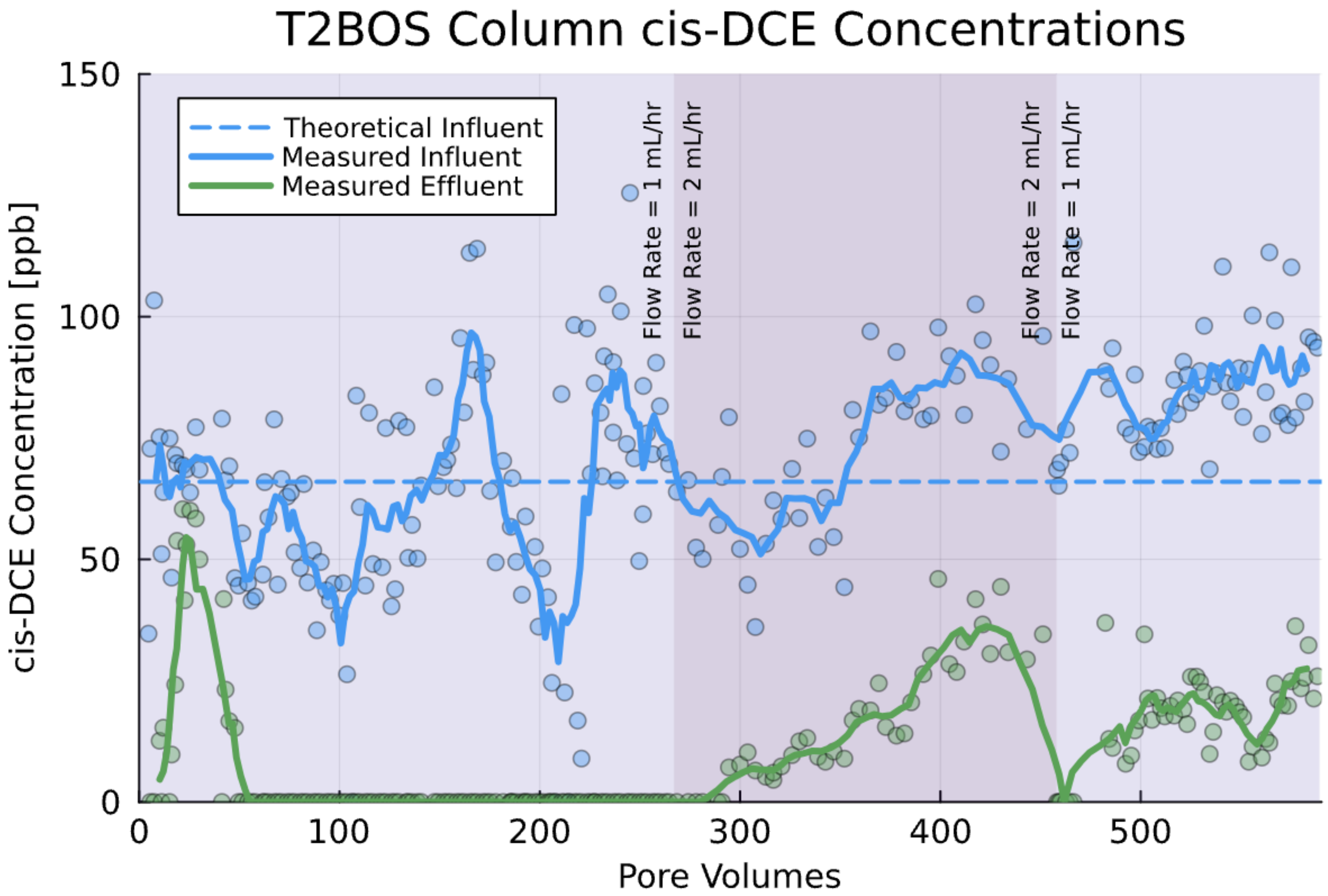
0 PV	95 PV	440 PV	585 PV



(ATCC 21198)

Pore Volume (PV) – Measurement of Operational Time

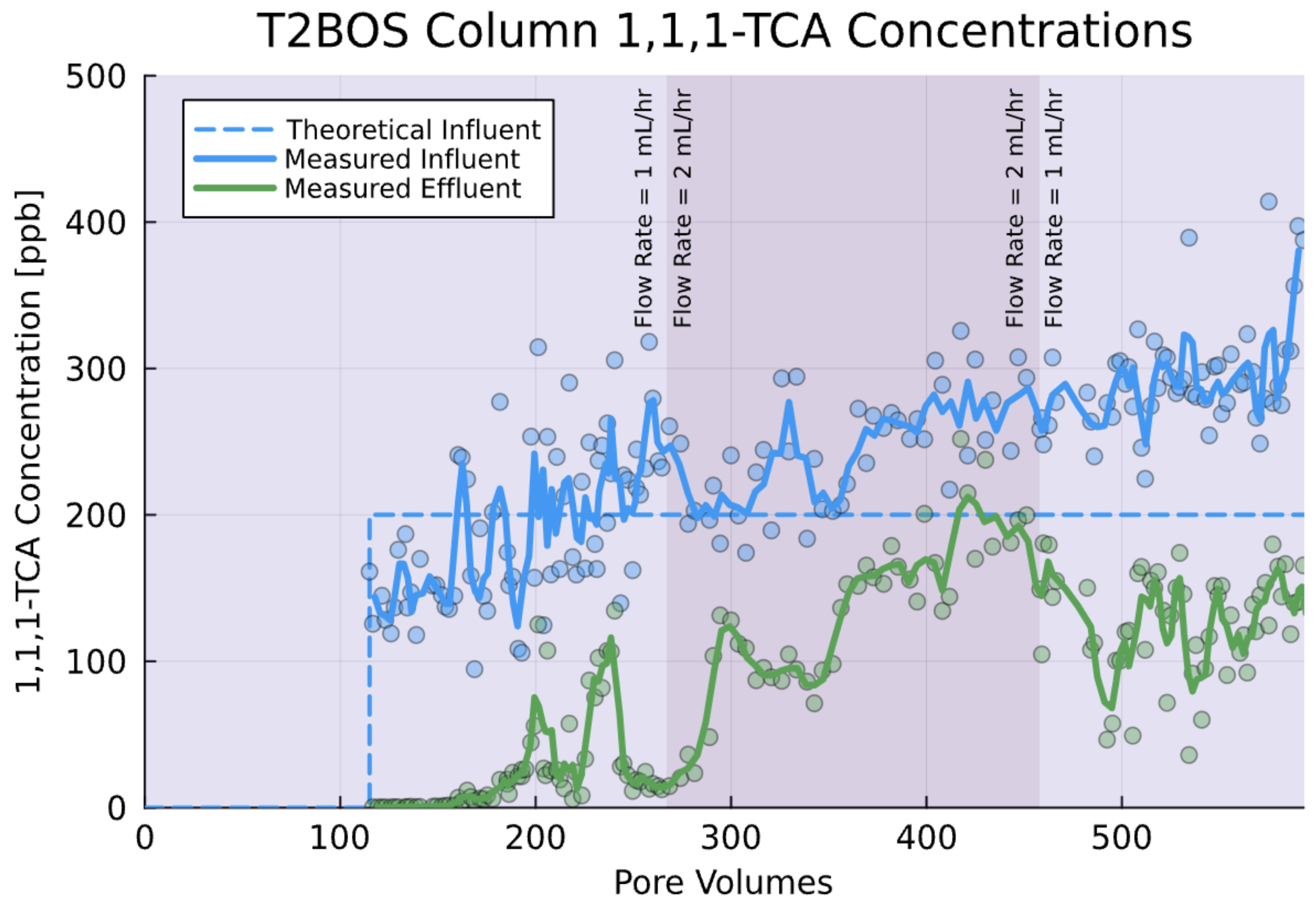
Contaminant Concentration Data



Max: >95% transformation of 66 ppb cis-DCE

Final: ~72% transformation of 66 ppb cis-DCE

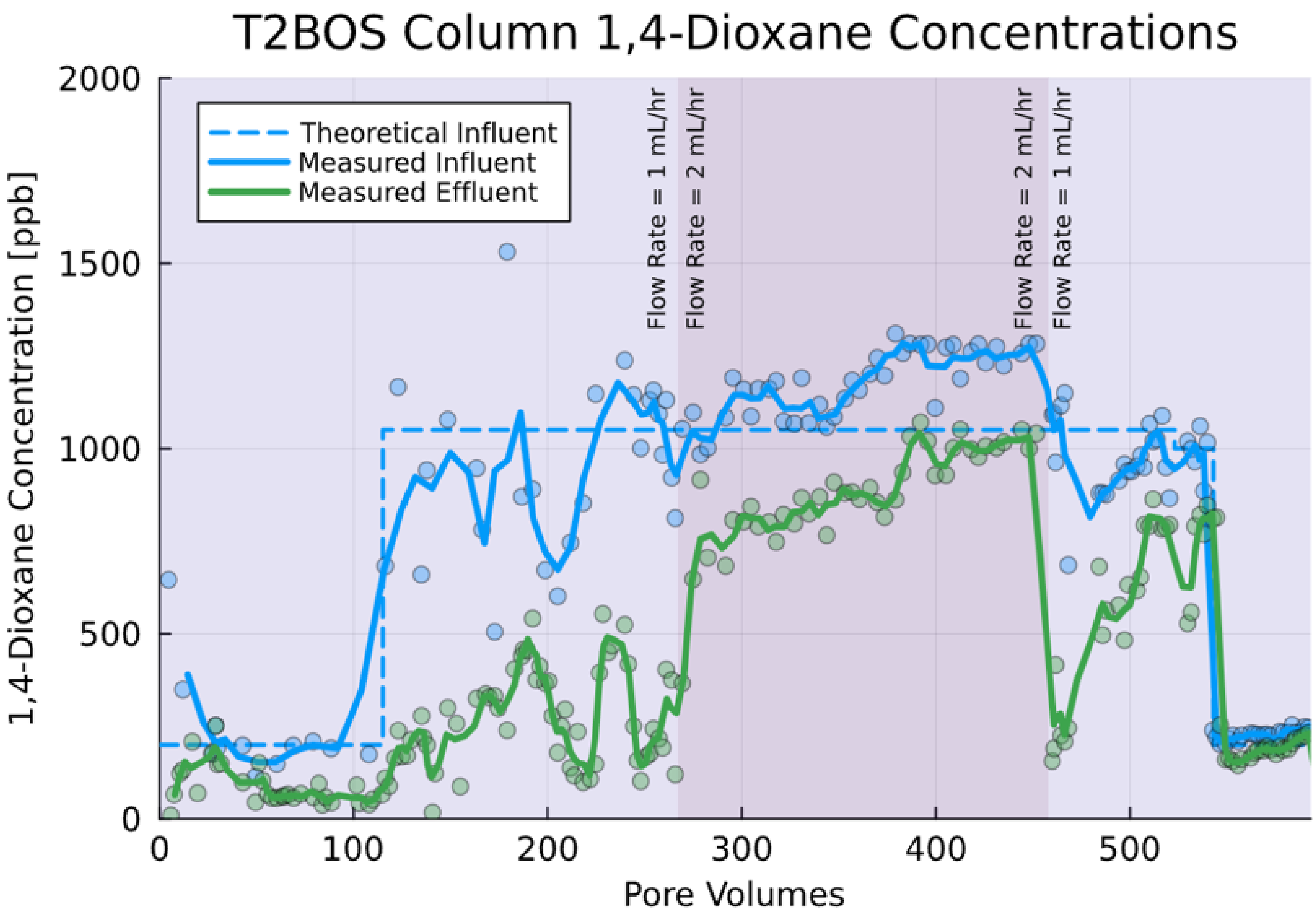
Contaminant Concentration Data



Max: >99% transformation of 200 ppb 1,1,1-TCA

Final: ~55% transformation of 200 ppb 1,1,1-TCA

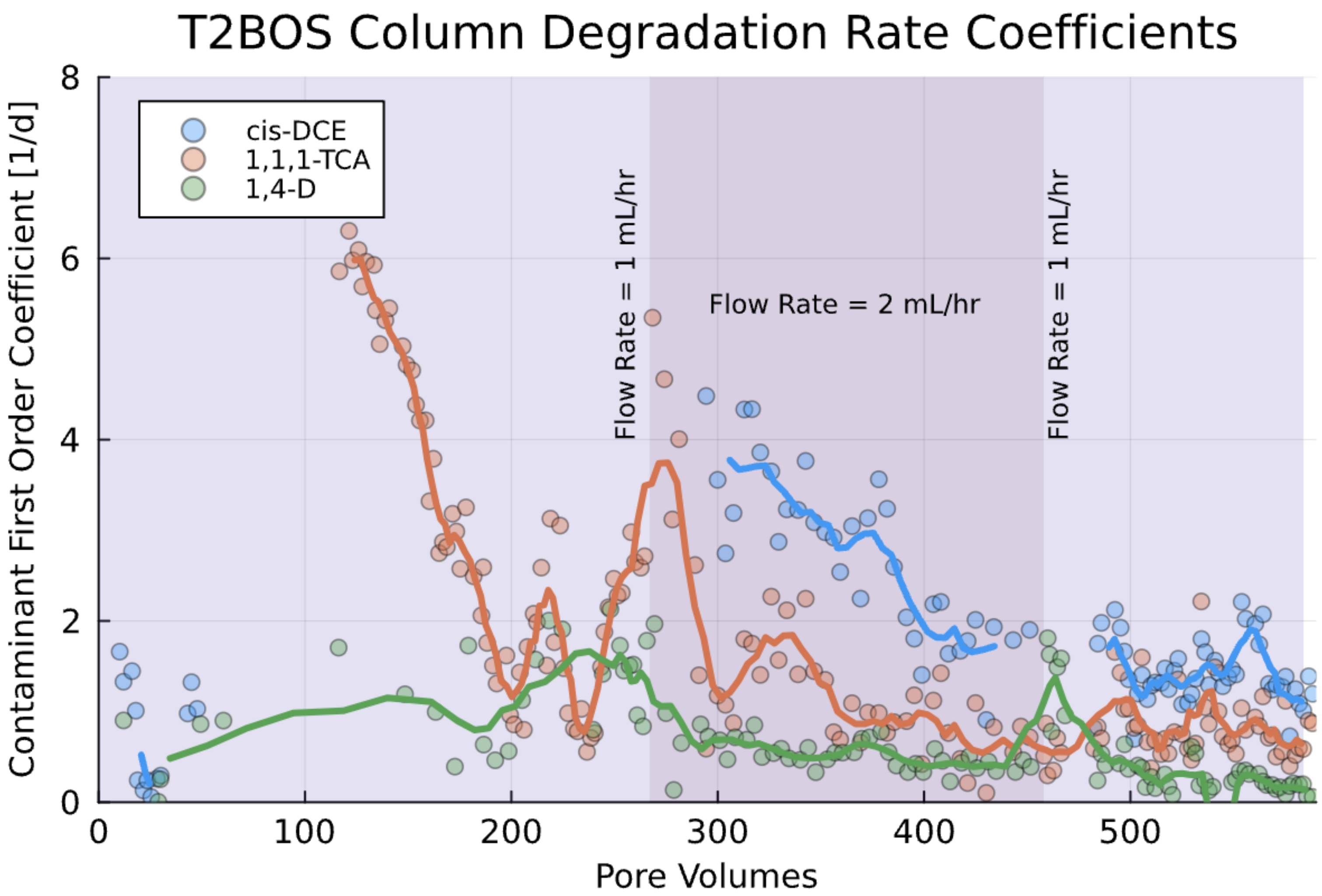
Contaminant Concentration Data



Max: ~84% transformation of 1100 ppb 1,4-D

Final: ~11% transformation of 200 ppb 1,4-D

Contaminant Treatment Rates



First Order Degradation Eqn.

$$C = C_0 e^{-kt}$$

1^{st} Order Coefficient: k [1/d]

Rate Constants

Contaminant	Max k [1/d]	Final k [1/d]
cis-DCE	> 4.8	1.2
1,1,1-TCA	5.7	0.75
1,4-D	1.7	0.11

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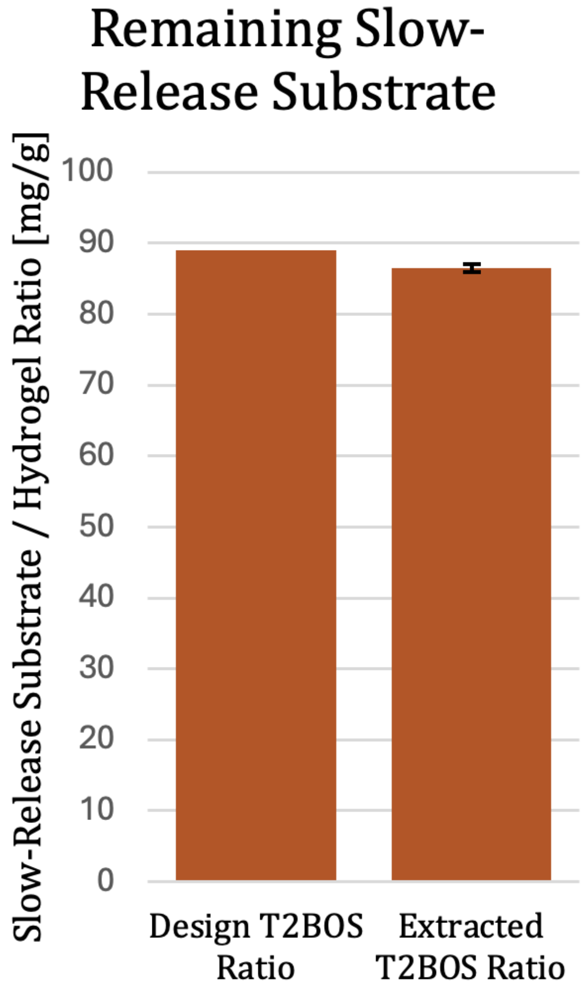
Column Dissection Results



Over 97% of the slow-release substrate remained

Column Dissection Results

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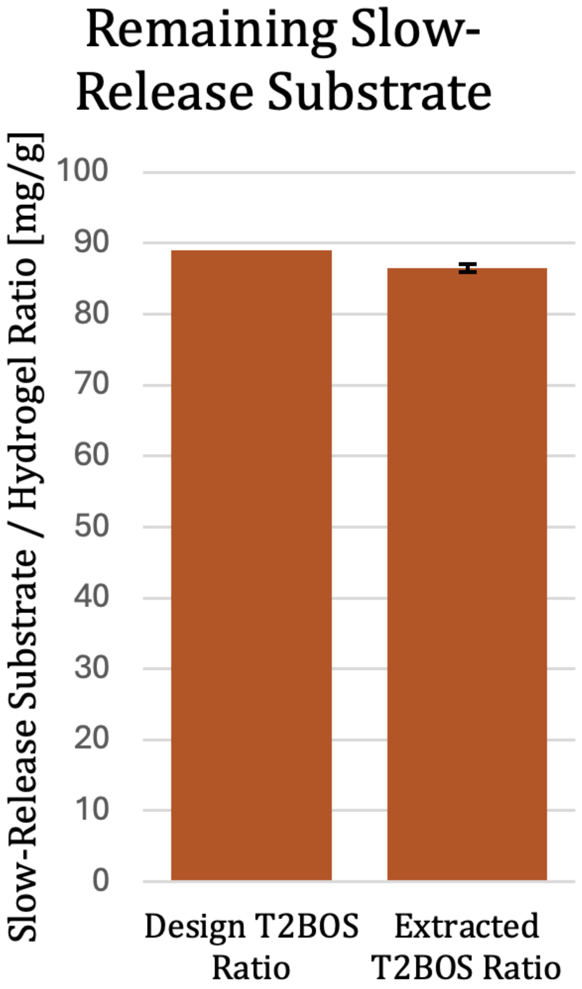
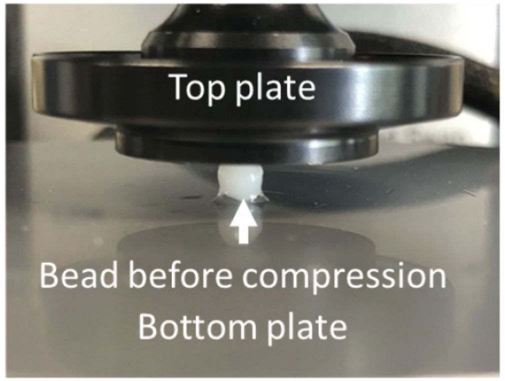
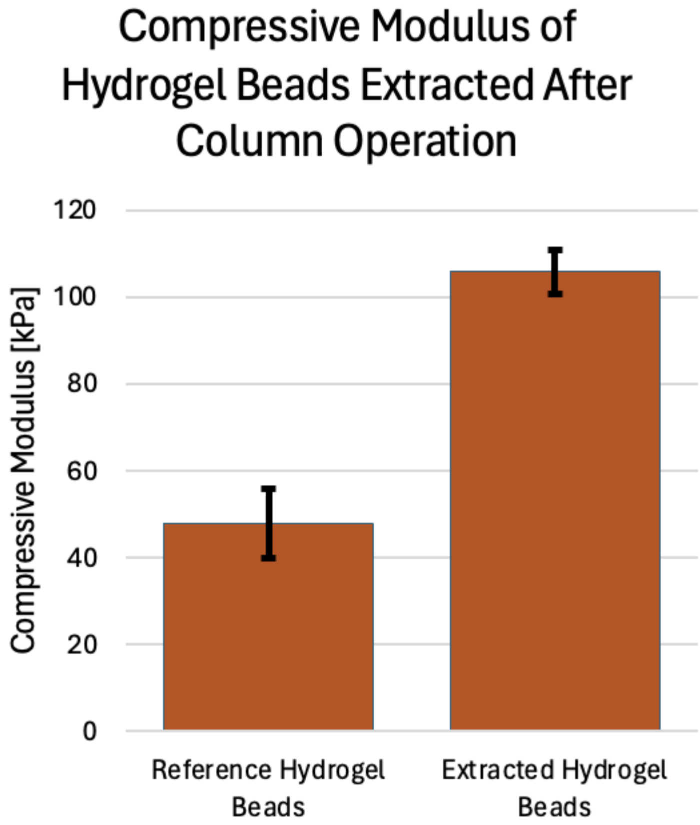


Rheometer Compression Test



Hydrogel beads became more durable within the packed column



(Harris et al, 2024)

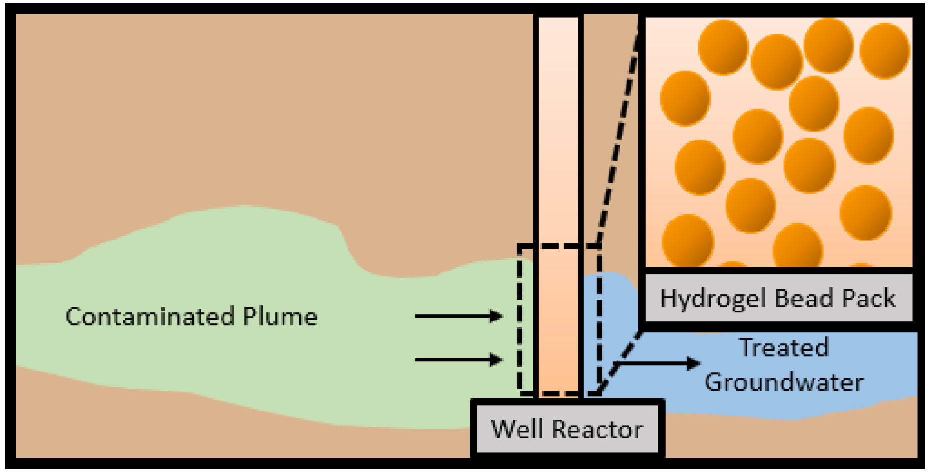
Conclusions and Future Work

Conclusions

- In situ oxygen sensors useful for quantifying biostimulation and DO consumption rates
- Feasibility of cometabolic treatment system with hydrogel-encapsulated bacteria to degrade chlorinated solvents and 1,4-dioxane
- Physical resilience of hydrogel beads under constant flow conditions
- Potential for long-term treatment (~18 months operation)

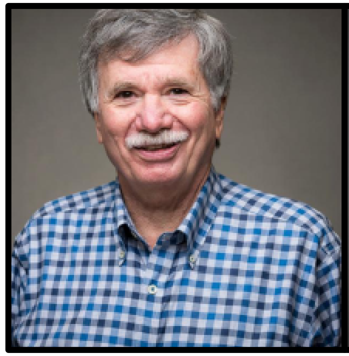
Future Work

- Explore declines in cometabolism
- Study enzyme activity in batch system after column dissection



Acknowledgements

Semprini Lab



Dr. Lewis Semprini

Dr. Mohammad Azizian

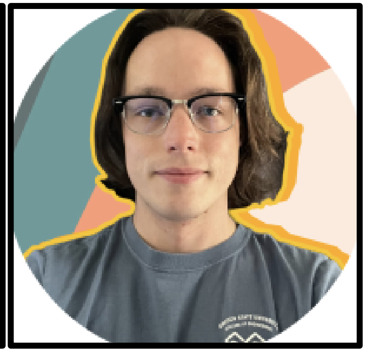
Dr. Juliana Huizenga

- Kestrel Bailey
- Marie Olland
- Brandon Beck

NIH R01 Grant Team

Dr. Kaitlin Fogg

Dr. Willie Rochefort



Conor Harris

- Dr. Michael Hyman (North Carolina State University)

NIEHS Grant: R01ES032707

NIH National Institute of Environmental Health Sciences

Superfund Research Program