Development of Passive and Sustainable Cometabolic Systems to Treat Complex Contaminant Mixtures by Encapsulating Microbial Cultures and Slow Release Substrates in Hydrogels

SRP Progress in Research Webinar Series, April 29, 2022

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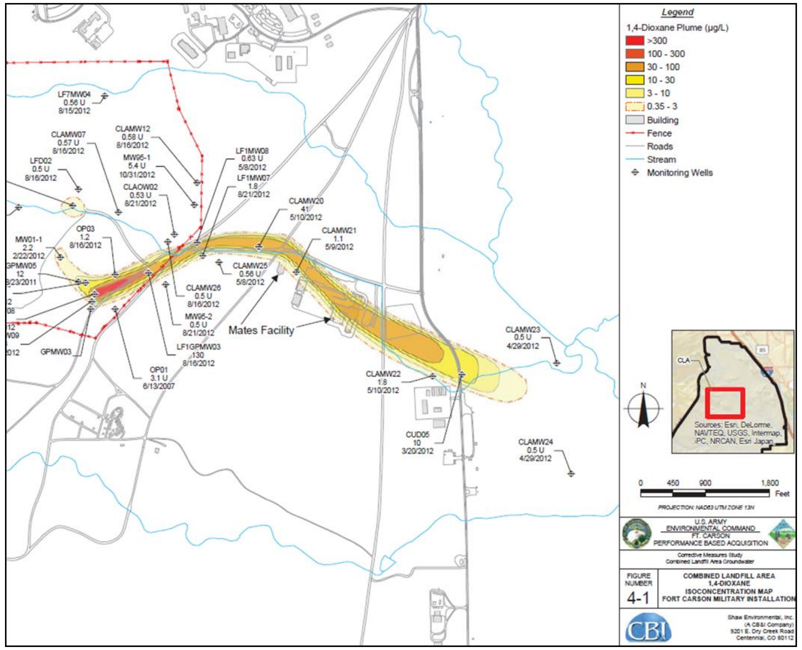
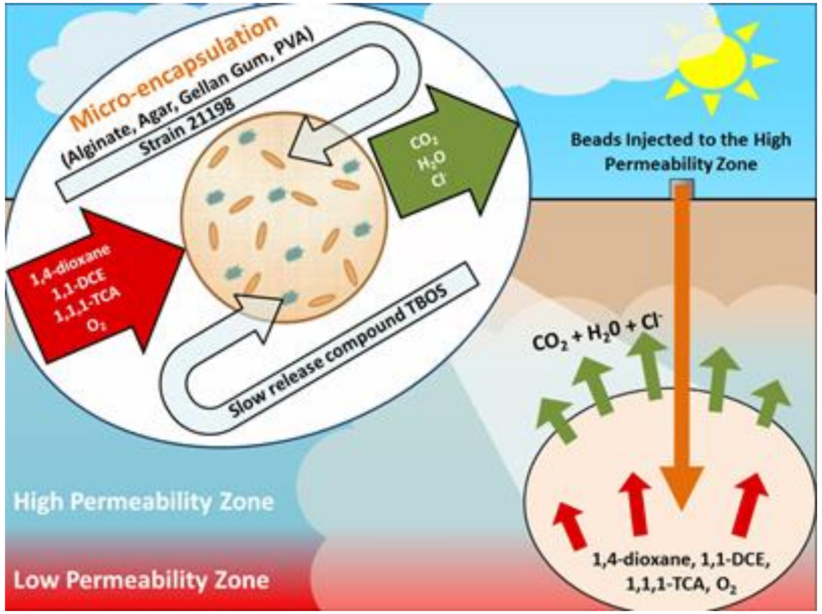
Long 1,4-dioxane groundwater plume





- **Problem:** Extensive groundwater plumes of contaminant mixtures of chlorinated solvents and 1,4-dioxane exist that are costly to treat using existing technologies

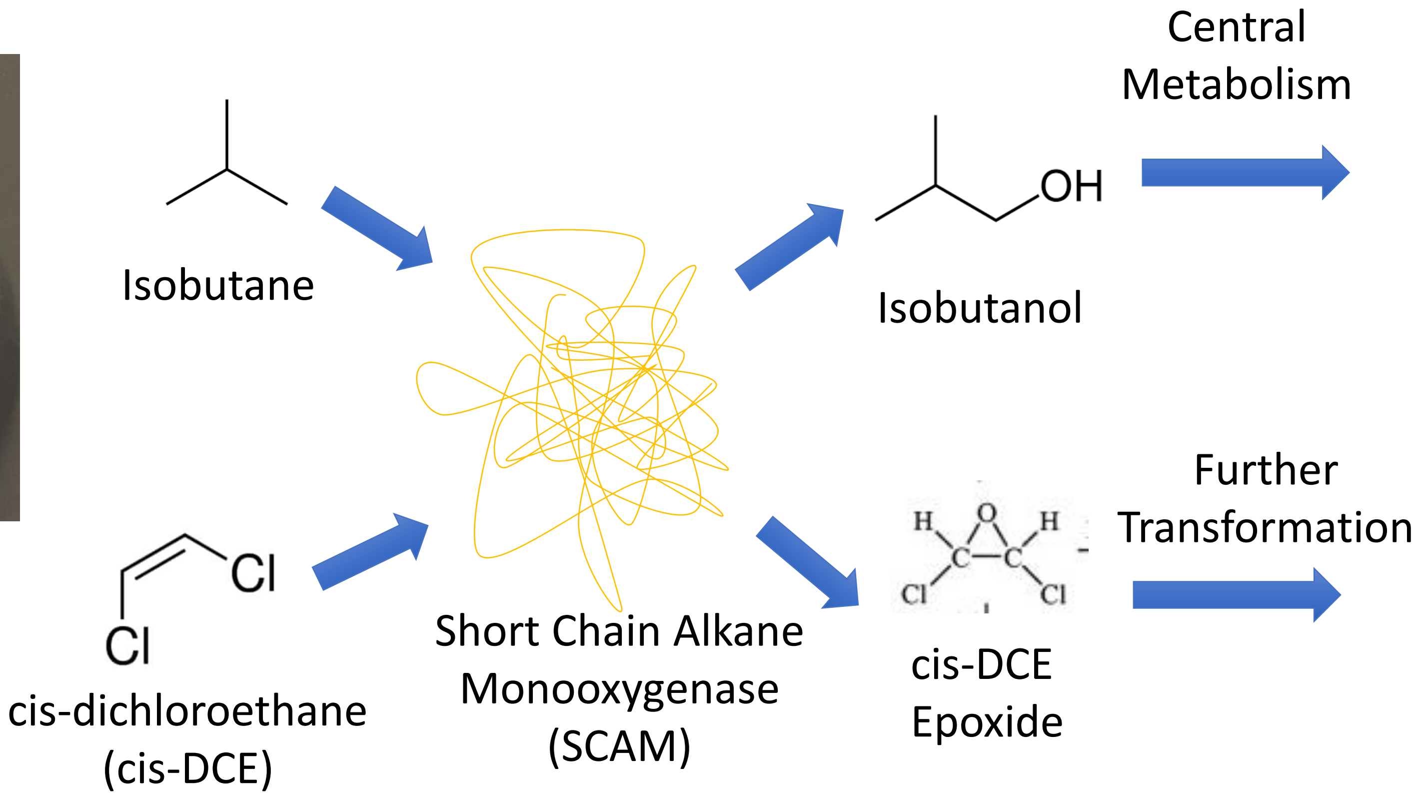
- **Solution:** Create passive systems for treating these plumes via aerobic cometabolism by co-encapsulating pure cultures of bacteria with a slow release compound (SRC) in hydrogel beads

Preliminary Work Funded by SERDP (Semprini and Hyman 2022, Final Report Project ER-2716)

Microbial Culture Used in This Work

Rhodococcus rhodochrous ATCC 21198

Cometabolism



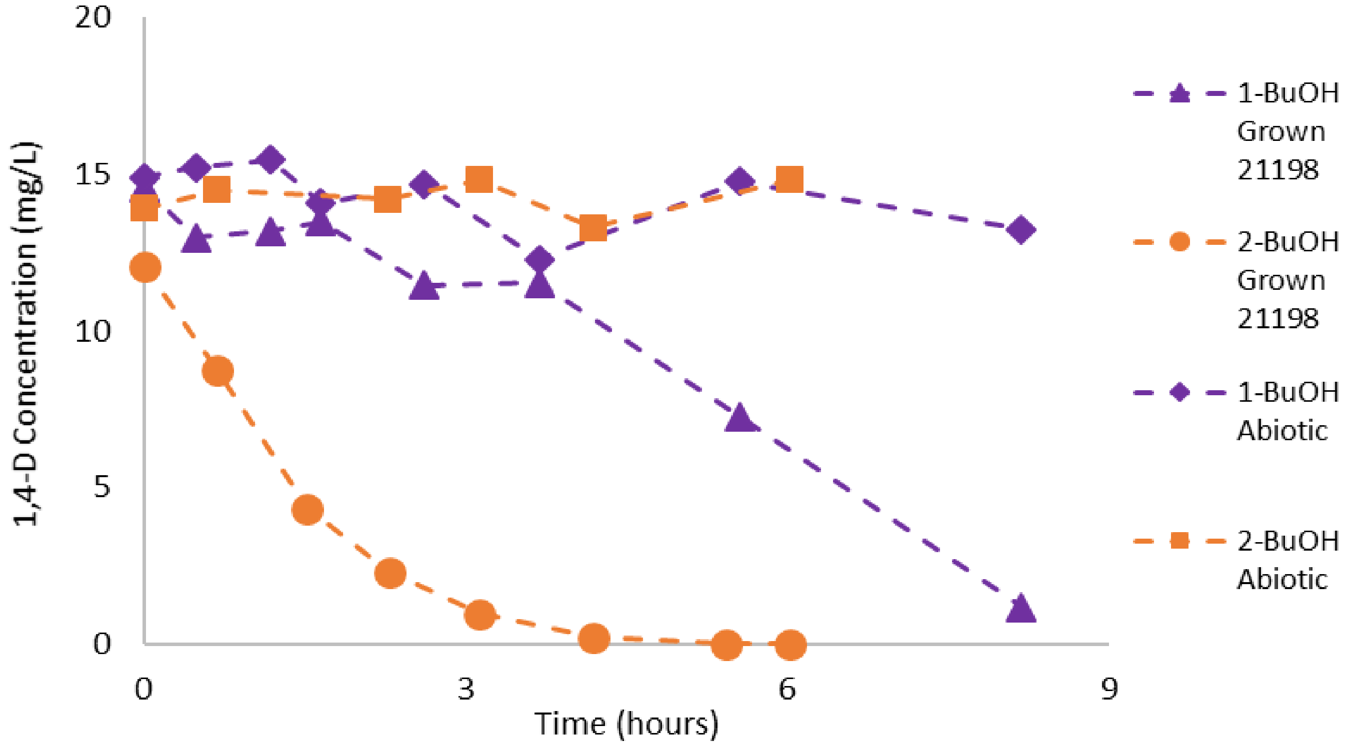
Previous Work with ATCC 21198:

Bioremediation
- CAHs
- 1,4-dioxane

Other
- Lipid storage

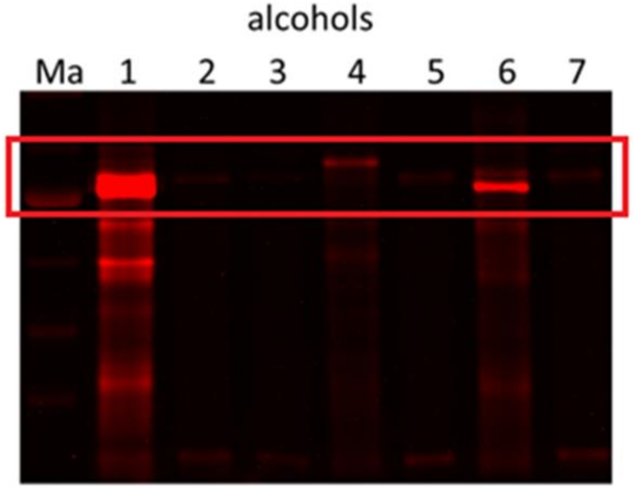
SCAM-Inducing Alcohols

1,4-D transformation by 5 mg **1-Butanol versus 2-Butanol** grown 21198



4 hour transformation lag time for 1-butanol grown 21198

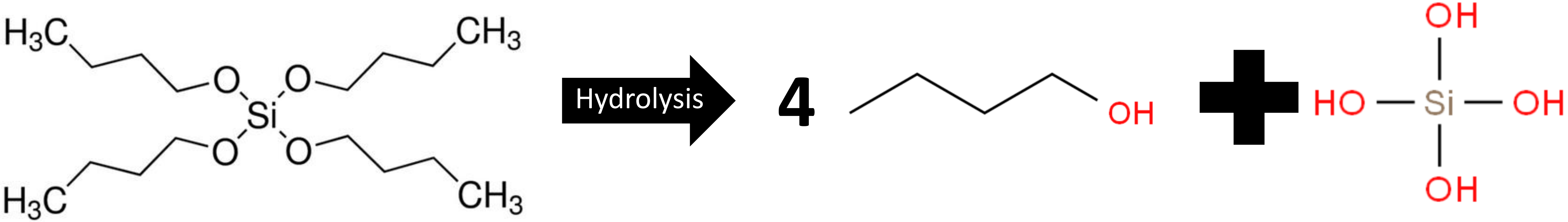
Induction Mechanism?
Exposure to 1,4-dioxane



Ma = markers
1 = isobutane
2 = ethanol
3 = 1-propanol
4 = 2-propanol (faint)
5 = 1-butanol
6 = 2-butanol
7 = isobutanol

(Murnane et al., Journal of Contaminant Hydrology, 2022)

SRC Evaluation – TBOS



TBOS
(tetrabutyl orthosilicate)

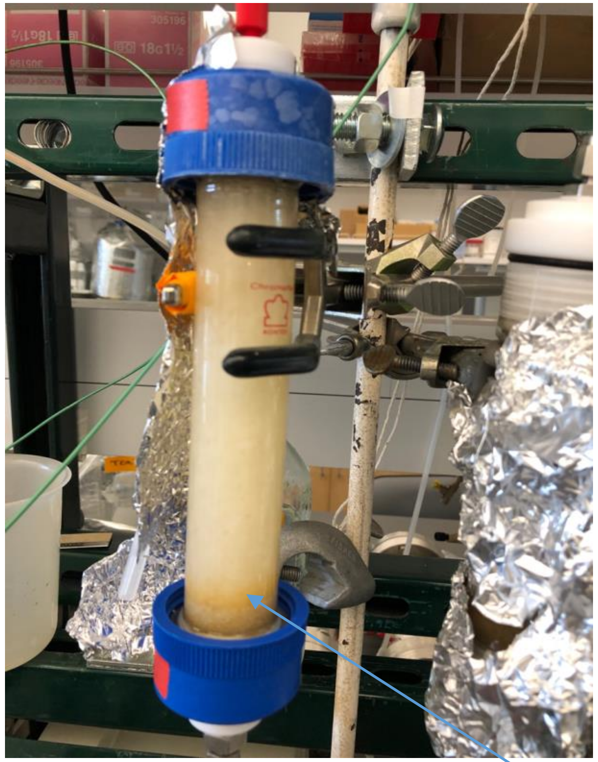
1-Butanol

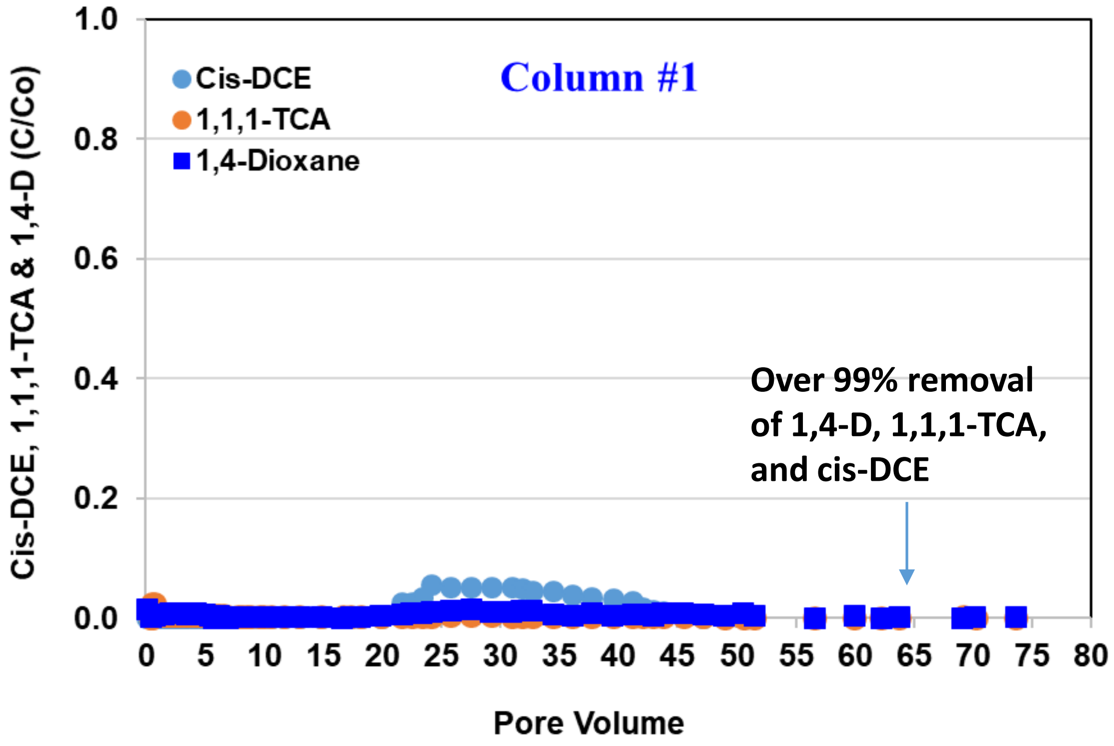
Silicic Acid

Continuous Flow Packed Column Tests with TBOS/21198 Gellan-Gum Beads cis-DCE, 1,1,1-TCA and 1,4-Dioxane (250 µg/L)

(from Azzian and Semprini, ES&T Engineering, In Press)

Pore Volumes - 0 To 33
20 mg/L DO
HRT = 2 days





Pore Volumes – 50 to 80
50 mg/L H_2O_2
HRT = 0.5 days

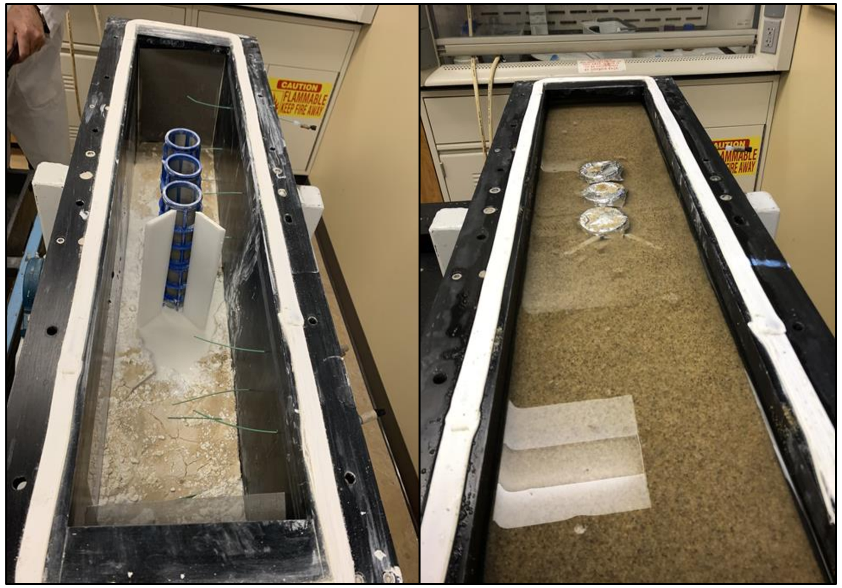
Only Column Entrance Stimulated Due to Lack of DO

Whole Column is Stimulated Excess DO in the Column Effluent

Physical Aquifer Model (PAM)

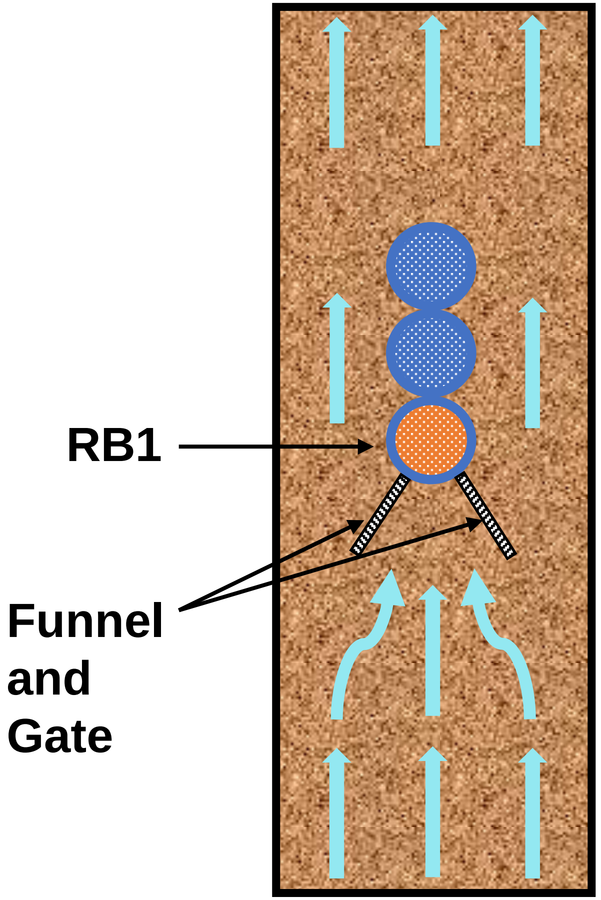
Introduction:

- 100L sand packed confined aquifer model
- 3 vertical columns, 1 slurry-packed (RB1) with gellan-gum beads co-encapsulated with ATCC 21198 and TBOS
- "Funnel and Gate" PRB design



Top view of the PAM before packing with sand (left) and after (right)

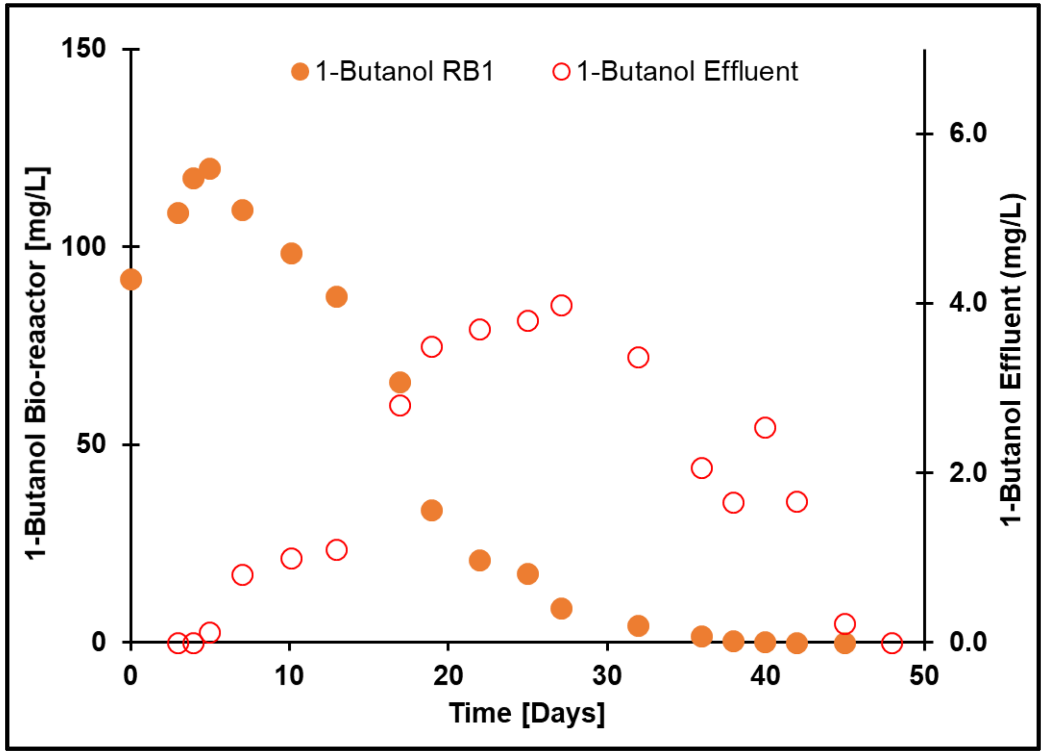
Slurry-Packing the permeable bioactive reactive barrier

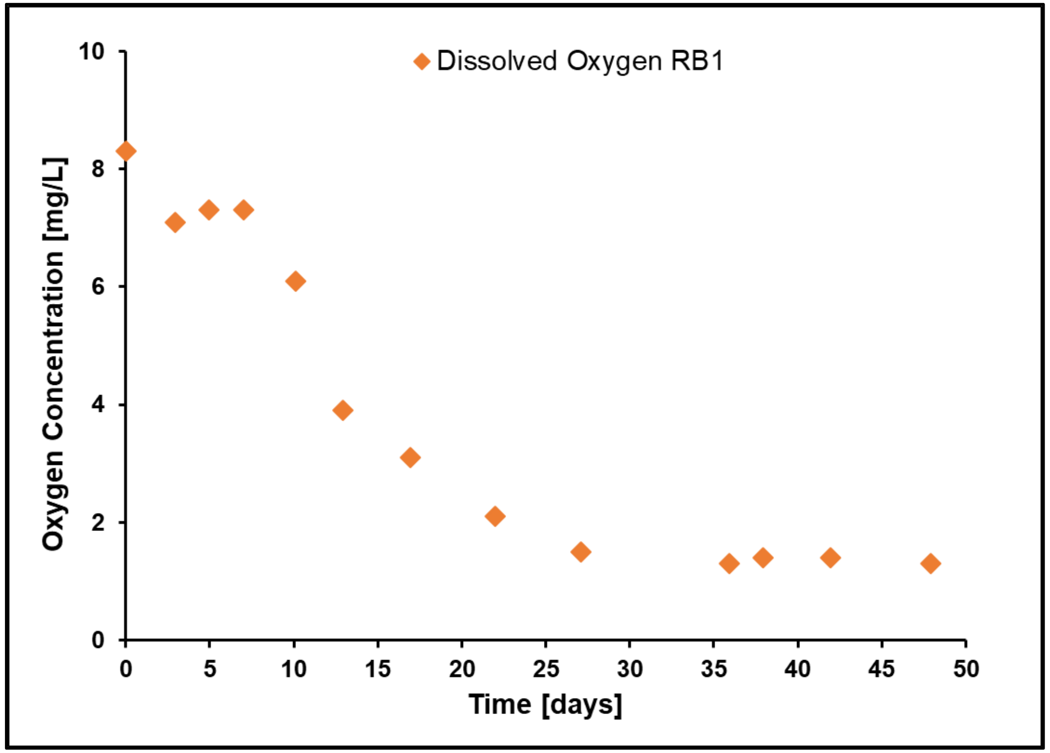


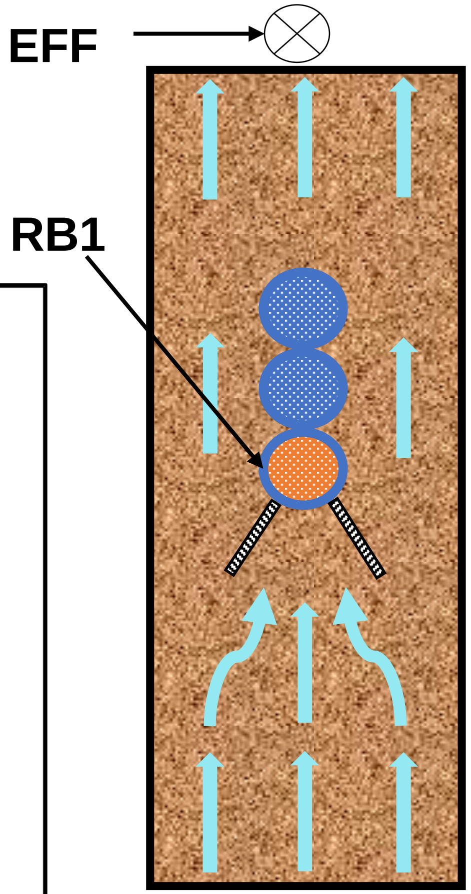
Physical Aquifer Model (PAM)

Results: Initial Biostimulation

- Groundwater Amended with H_2O_2
- Decrease in 1-Butanol
- Dissolved Oxygen Consumption



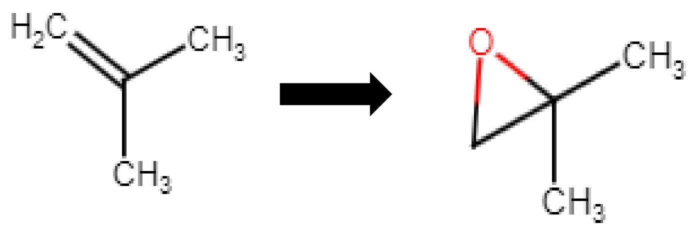


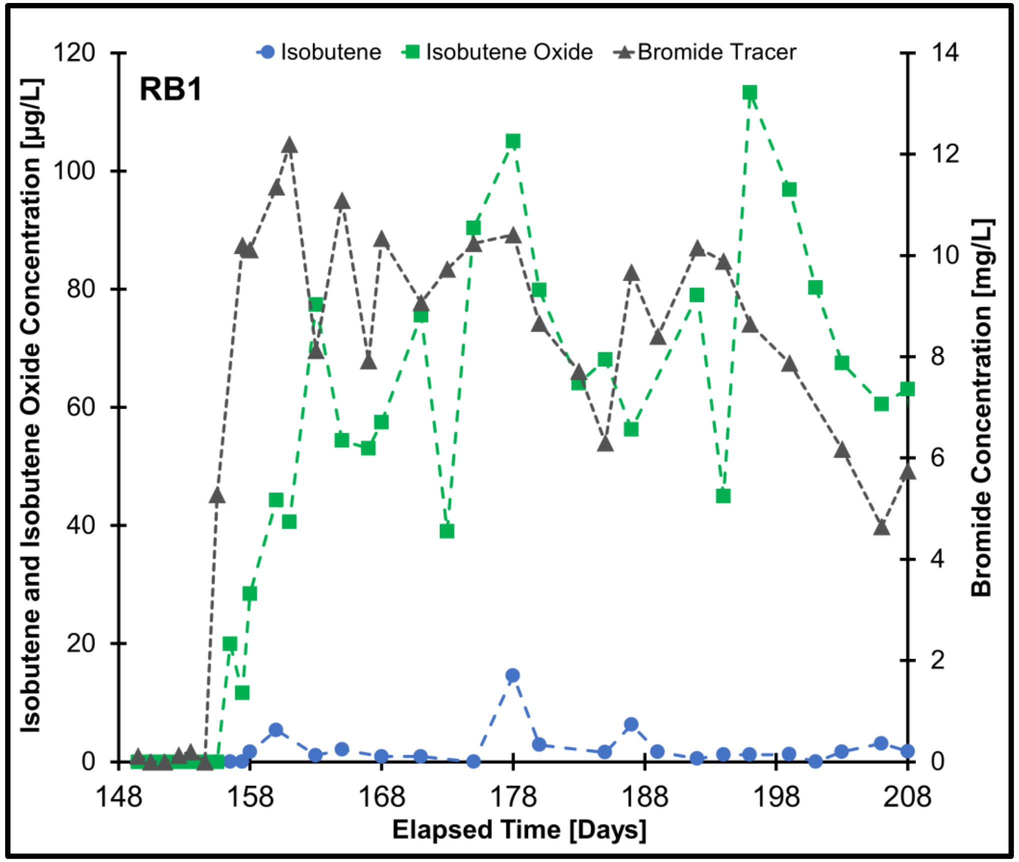


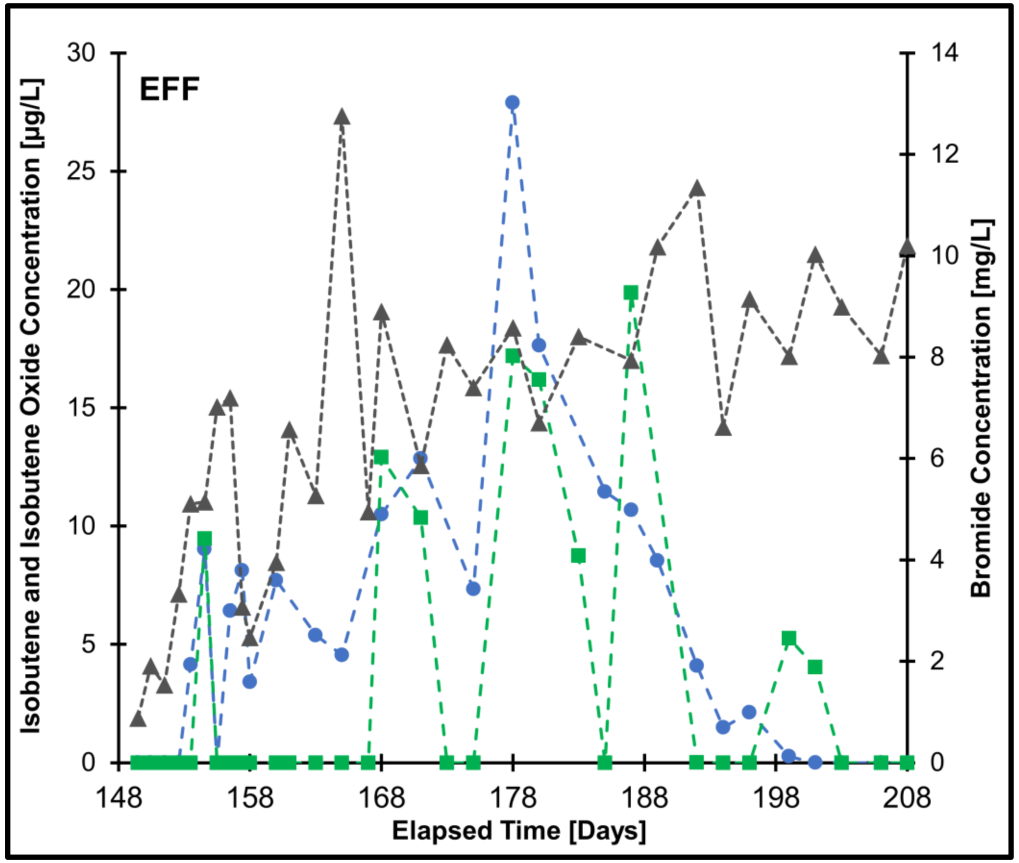
Physical Aquifer Model (PAM)

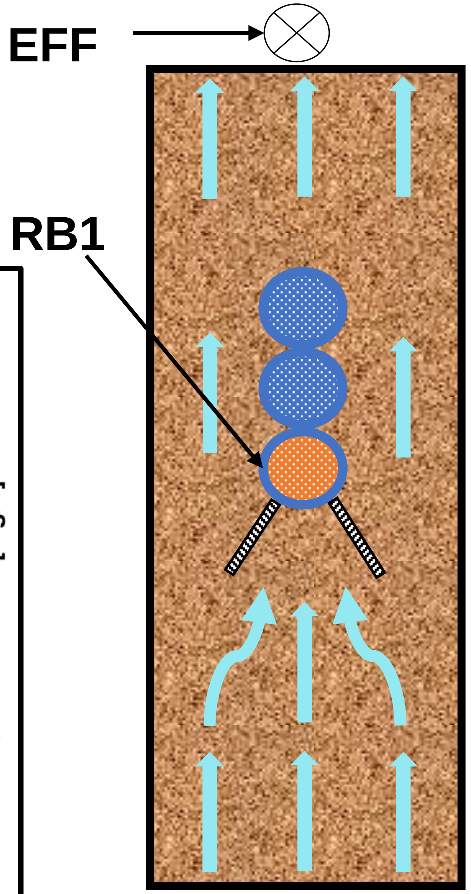
Results: Aerobic Cometabolism

- Isobutene is a surrogate for 1,4-dioxane
- Presence of **Isobutene Oxide** indicates aerobic cometabolism





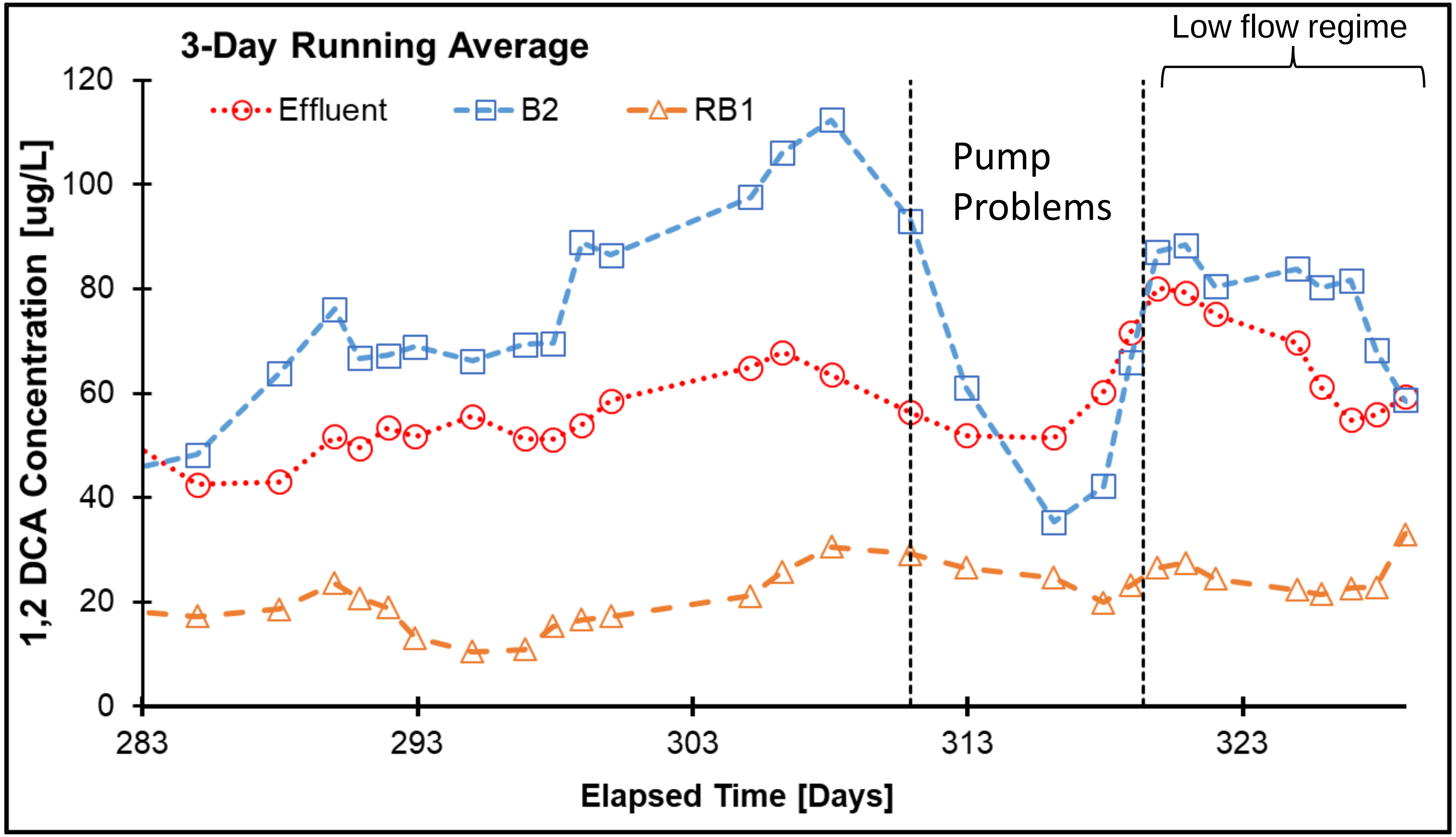


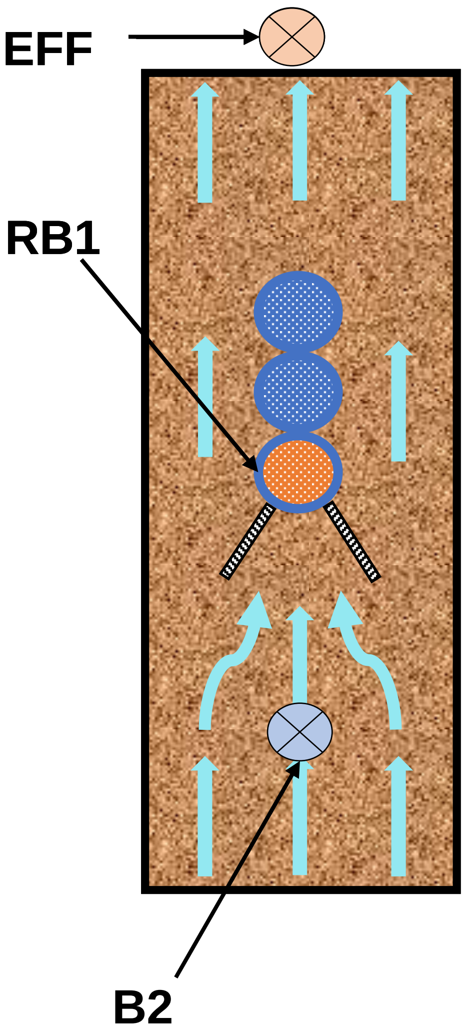


Physical Aquifer Model (PAM)

Results: CAH Bioremediation

- 1,2-dichloroethane was chosen since its transformation rate in batch studies was similar to 1,4-dioxane

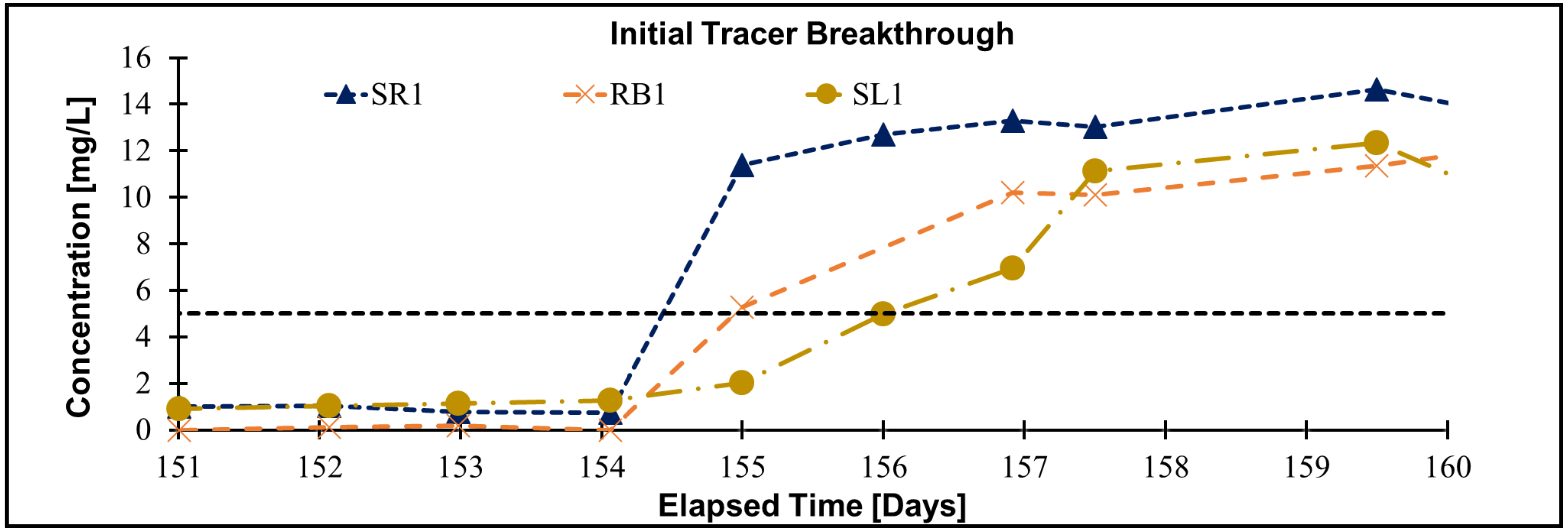


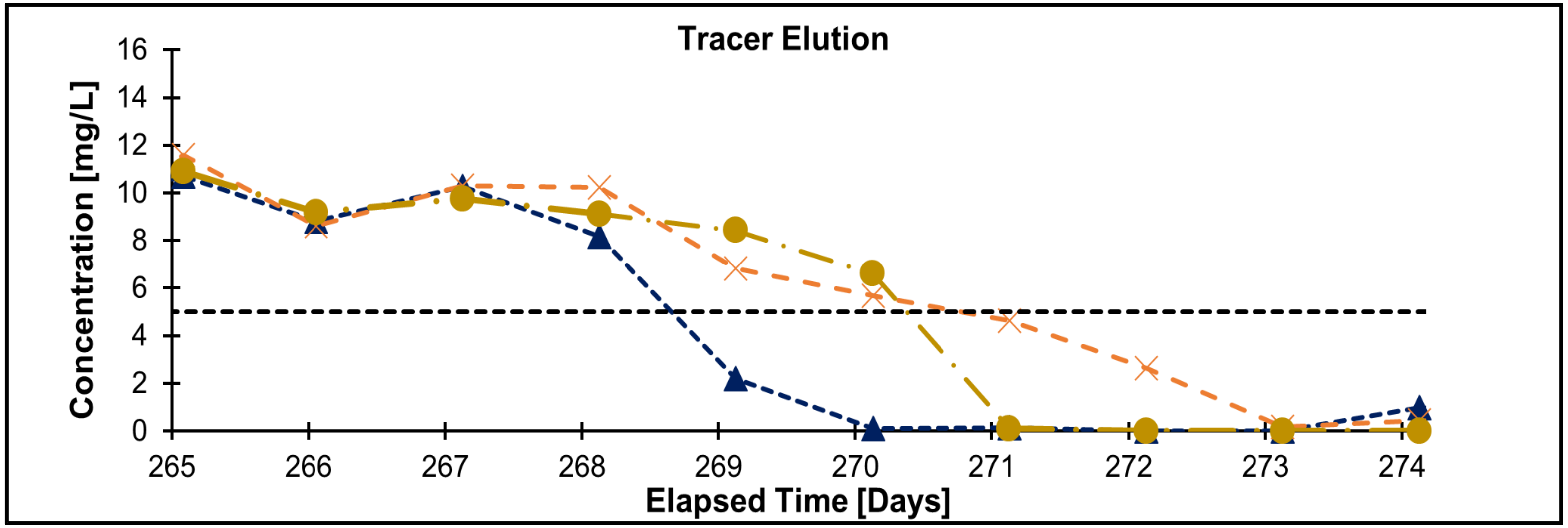


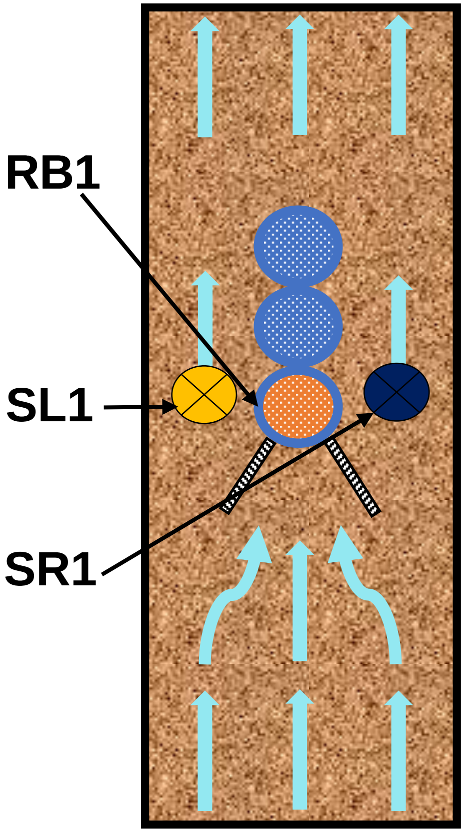
Physical Aquifer Model (PAM)

Results: Preliminary Tracer Data

- Bromide tracer indicates minimal changes to flow







Research Goal: Develop Stronger, Longer Lasting, and Mass Producible Hydrogel Beads that Co-Encapsulate ATCC 21198 and SRCs

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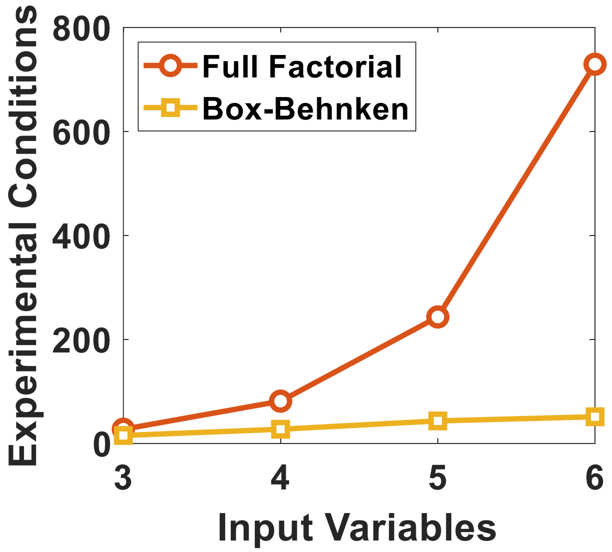
Optimization by Design of Experiments (DOE)

We **cannot** predict how cells will perform in hydrogel complexes.

Use DOE to develop empirical models of system while reducing number of experiments



Inputs

Polymer [% w/v]

Xlinking time [t]

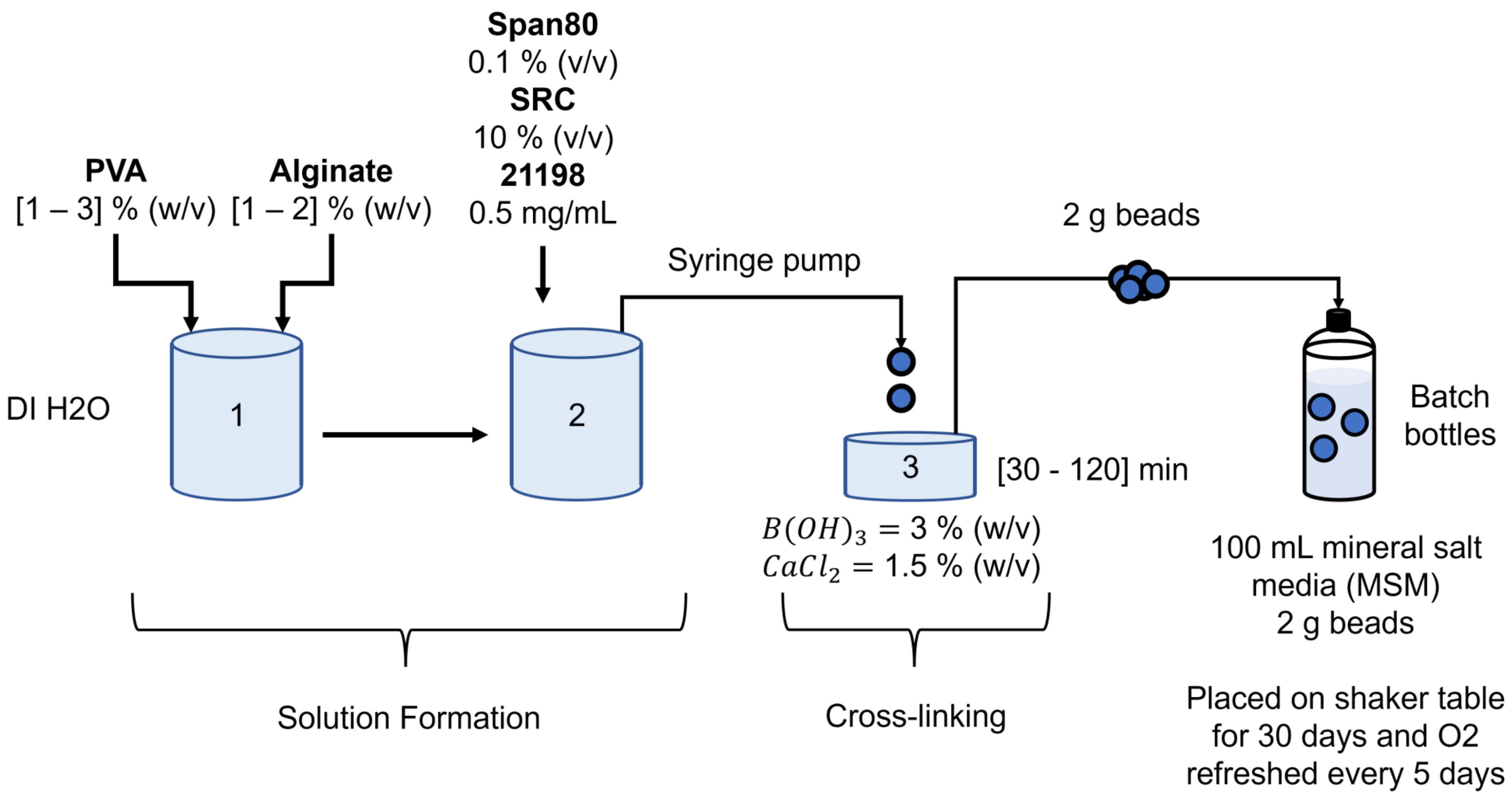
SYSTEM

Responses

Durability:
Compression Tests

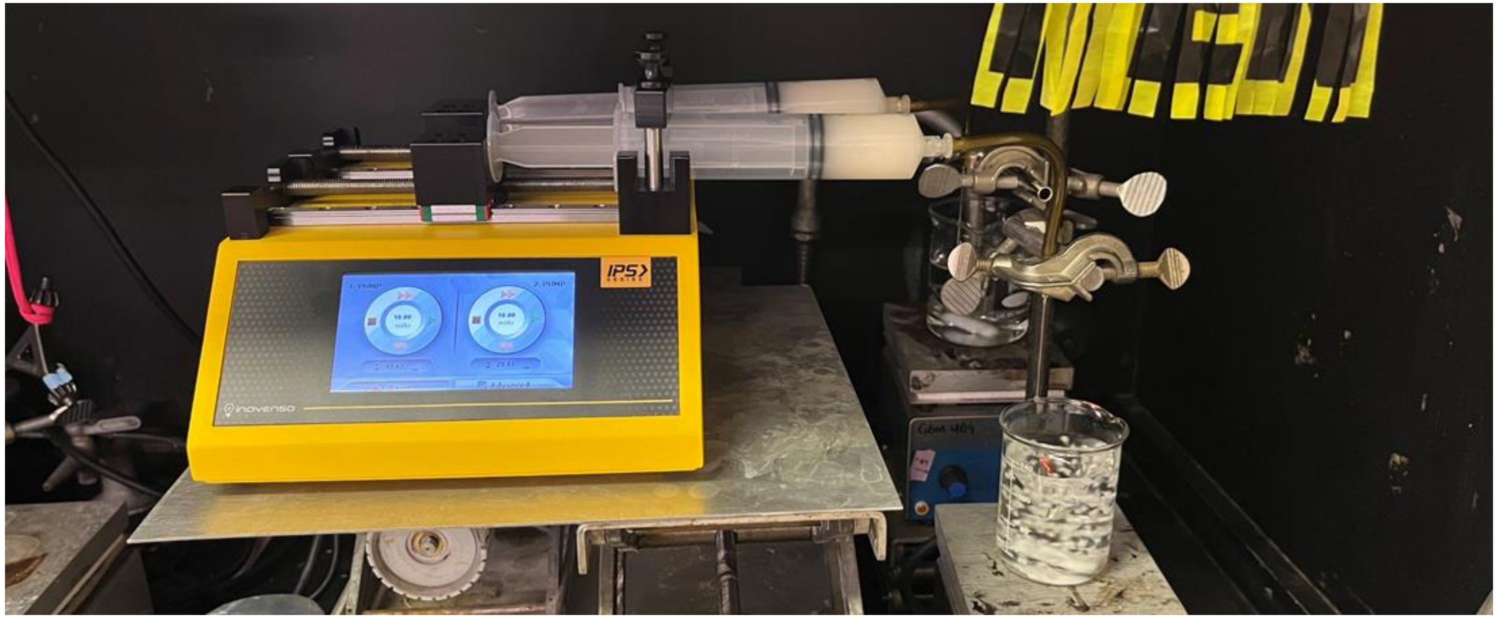
Toxicity:
Rate of substrate utilization (O2)
Co-metabolism rate (cDCE)

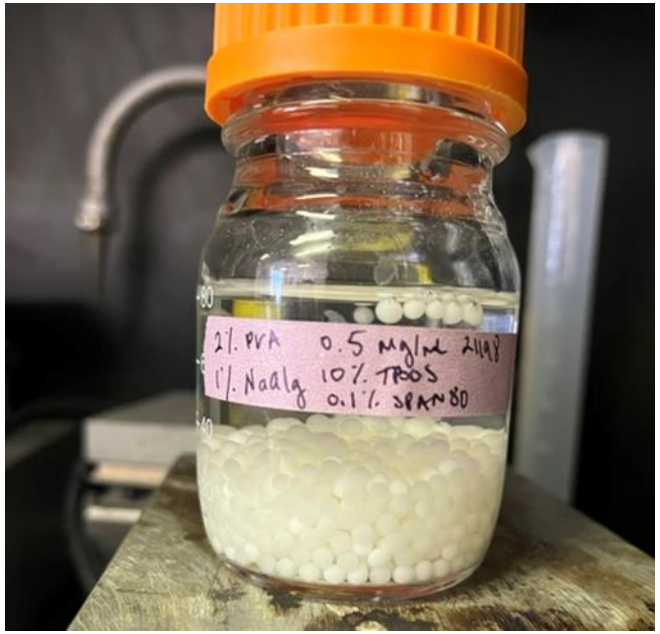
Materials and Methods: Bead process diagram



Hydrogel Beads Being Fabricated in the Lab

The fabrication process can be easily scaled up to mass produce the co-encapsulated hydrogel beads





Results: batch tests with ATCC 21198 immobilized in PVA-Alg hydrogel beads

Compression

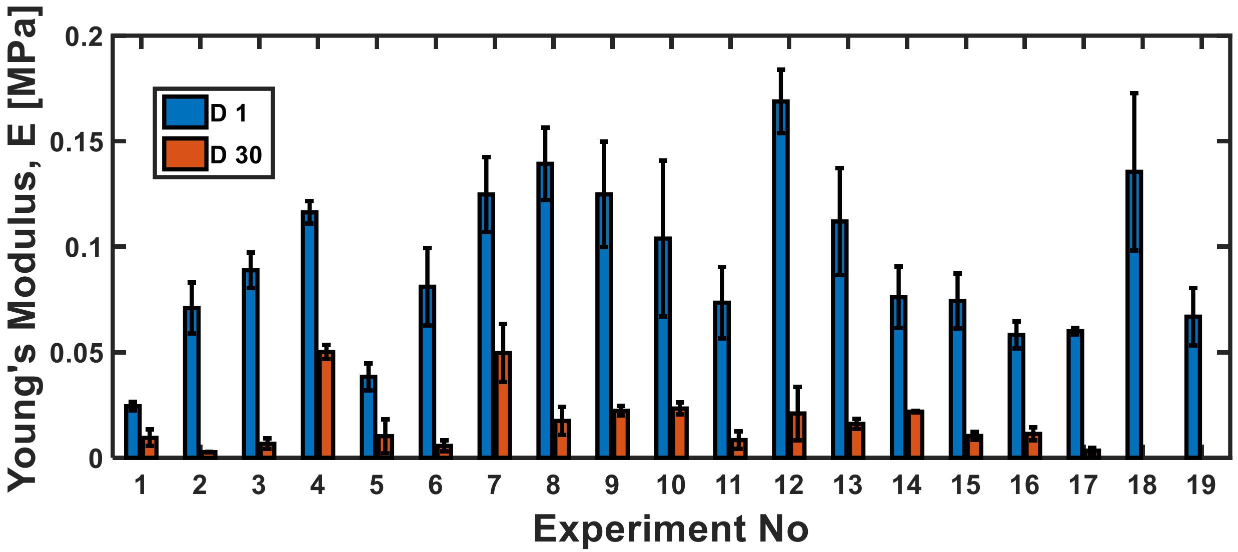
- General decrease of the Young's modulus after 30 day batch incubation period
    - Potential cause: growth of cells breaks down polymer matrix

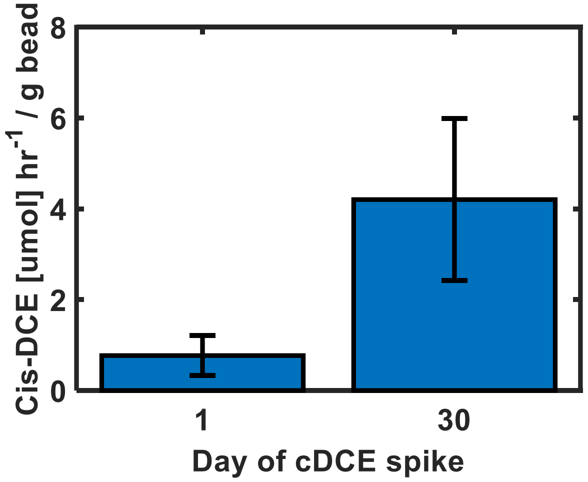
***cis*-dichlorethene (*cis*-DCE) transformation**

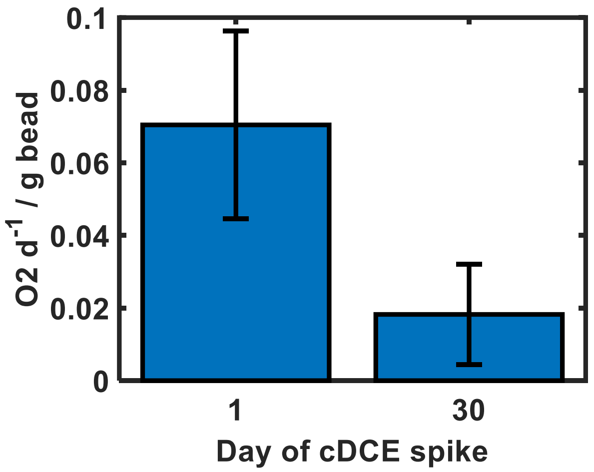
- Demonstrate cometabolism occurs for all bead types.
- Increase of cis-DCE rate after 30 day incubation period likely due to cell growth in hydrogels

Oxygen Utilization Rates

- Reduction of oxygen rate occurs after 30 days
    - Potential cause: Reduced rates of SRC hydrolysis
- Indicates substrate utilization of immobilized 21198 in batch bottle



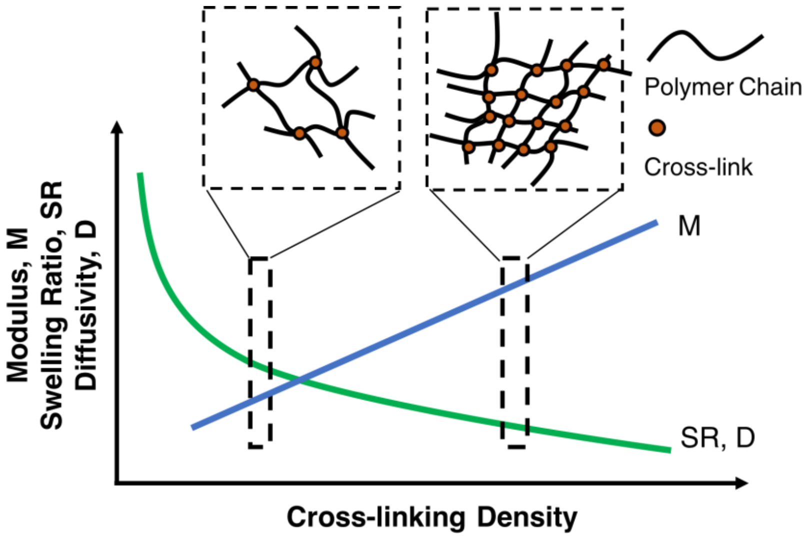


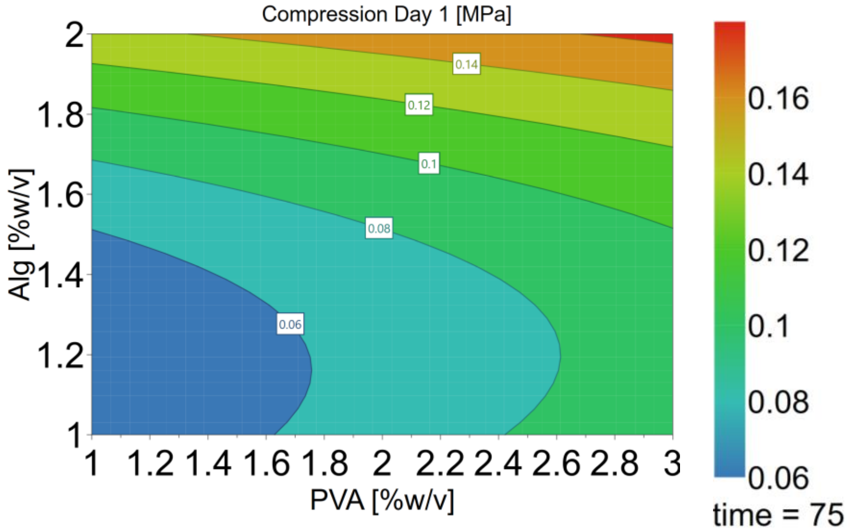


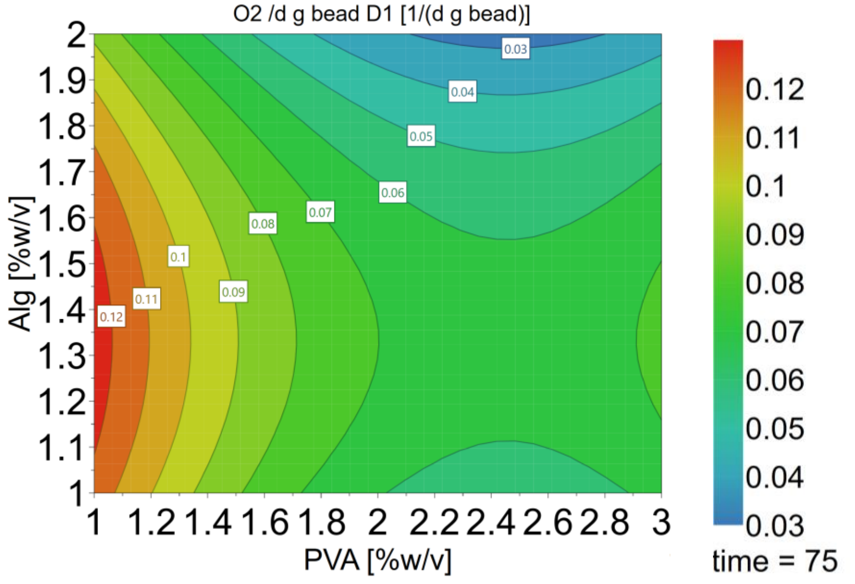
Results: DOE contour plots – Compression and O_2 Utilization

- Predictive models:
- **max compression** at **day 1** is achieved by maxing concentrations of PVA and Alg at xlink time = 75 min

- **min O_2 rates** at **day 1** is achieved with high concentrations of PVA and Alg at xlink time = 75 min.







Column Test with Polyvinyl Alcohol/Alginate Hydrogel Beads (TBOS and ATCC 21198)

Oregon State University

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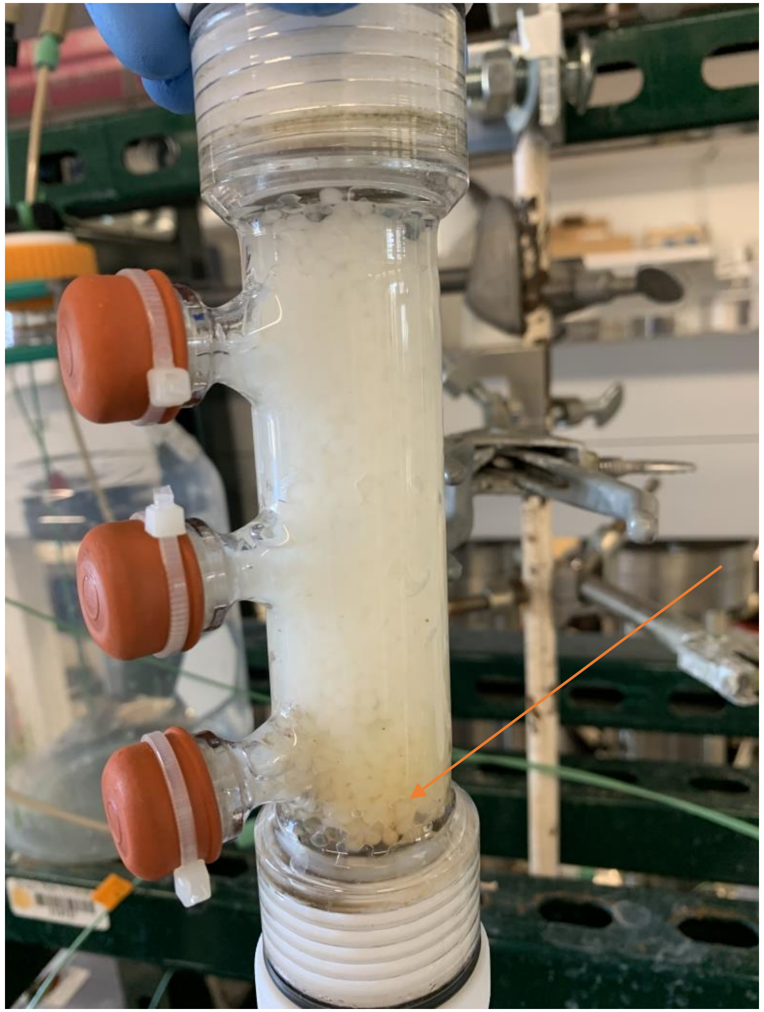
Introduction:

- **Column packed with 2.0% (w/v) polyvinyl alcohol and 2.2% (w/v) sodium alginate beads with 10% v/v TBOS**
- **Exposed immediately to ~150 ppb *cis*-DCE**
- **Side ports enabled gradient sampling**
- **The column influent was synthetic groundwater with added nutrients and H_2O_2**
- **Hydraulic Residence Time ~ 14 hours**

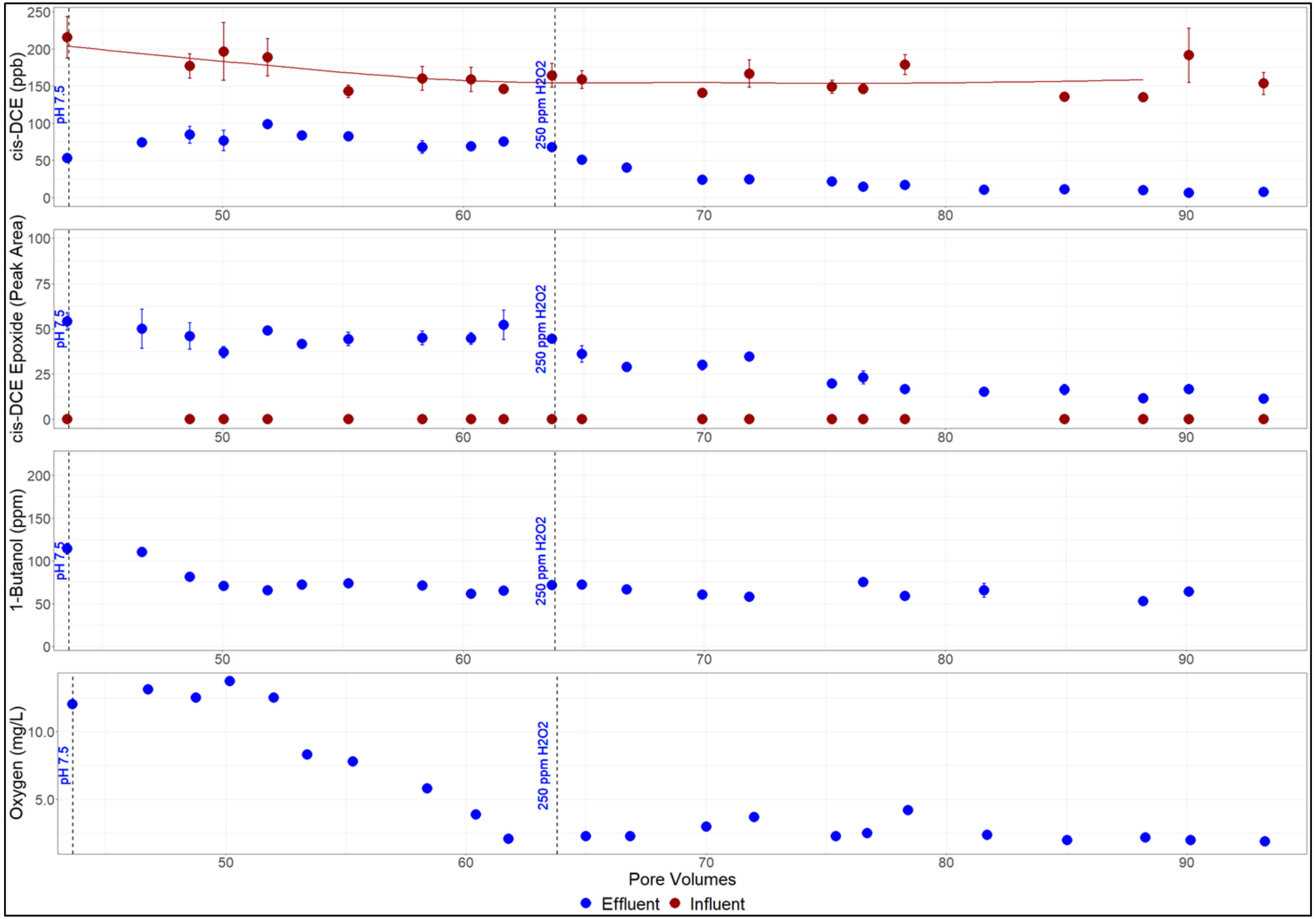
0 Pore Volumes



65 Pore Volumes

Effluent Concentration Histories from the Packed Column Fed Synthetic Groundwater Amended with Hydrogen Peroxide and *cis*-dichloroethene

- **High concentrations of 1-butanol observed in the column effluent**
- **Increasing pH of Synthetic GW resulted in an increase in dissolved oxygen utilization**
- **H_2O_2 addition is required**
- **Currently > 90% of cis-DCE is being treated**
- **cis-DCE epoxide formation and its transformation demonstrates the cometabolic transformation is occurring**



Summary

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Bead Optimization

- A hydrogel fabrication method was developed for the immobilization of ATCC 21198 and SRC (TBOS) in PVA-Alg beads that should permit for easier mass production
- The DOE method was successfully applied to experimentally investigate different polymer concentrations and cross linking times and the impact on bead compression, oxygen uptake, and cometabolism
- PVA-Alg hydrogel bead strength was correlated with increases in polymer concentration

Bead Application

- A funnel-and-gate treatment system was investigated in a large-scale physical aquifer model (PAM) with a reactive barrier constructed with gellan-gum hydrogel beads
- Cometabolism of a surrogate (isobutene) and 1,2-DCA has been observed in the PAM's reactive barrier ~ 1 year after the gellan-gum beads were emplaced
- Continuous flow column studies have been initiated with the PVA-Alg hydrogel beads with *cis*-DCE cometabolism increasing with longer term operation

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