PASSIVE COMETABOLIC TREATMENT OF 1,4-DIOXANE, CIS-DICHLOROETHENE, 1,1,1-TRICHLOROETHANE IN CONTINOUS FLOW COLUMNS PACKED WITH GELLAN GUM HYDROGEL BEADS CO-ENCAPSULATED WITH *RHODOCOCCUS RHODOCHROUS* 21198 AND SLOW-RELEASE-COMPOUNDS

Mohammad Azizian, Michael Hyman and Lewis Semprini

Clean Water Conference, September 1, 2020

Contaminants of Concern (CoC)

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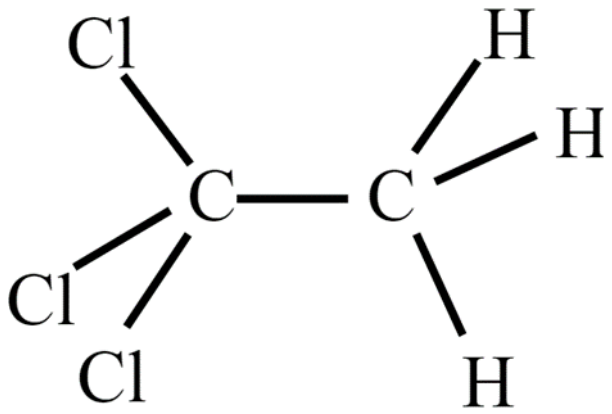
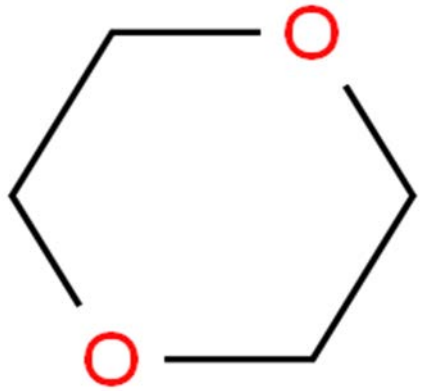


1,1,1-Trichloroethane (1,1,1-TCA)

Industrial Solvent

MCL 200 µL

Production ceased in the US in 2001



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

Solvent Stabilizer

Action level 0.35 µg/L

Probable human carcinogen

Miscible in water

Aerobic cometabolism summary

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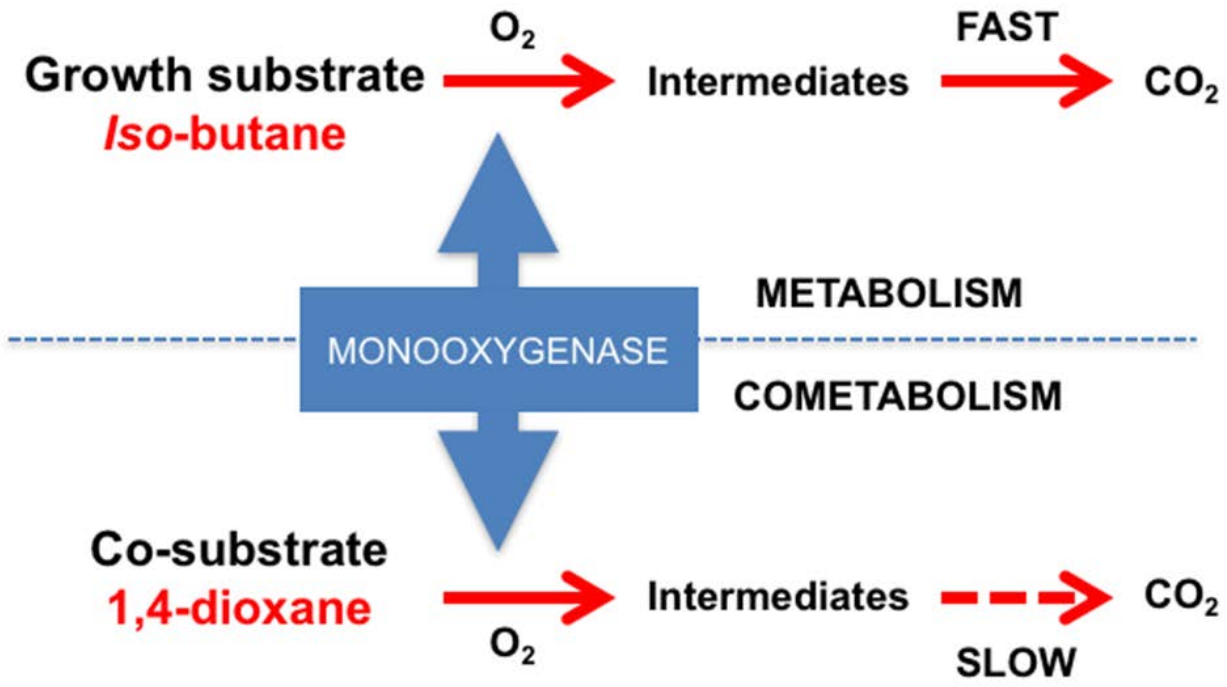

- Fortuitous biotransformation of target pollutant(s)
- Separates carbon and energy acquisition from biotransformation
- Potential to treat very low contaminant concentrations (**ppb to ppt**)

- Active microorganisms are often widely distributed
- Monooxygenases are common catalysts in aerobic systems
- Active bacteria can often concurrently degrade multiple contaminants

Objective

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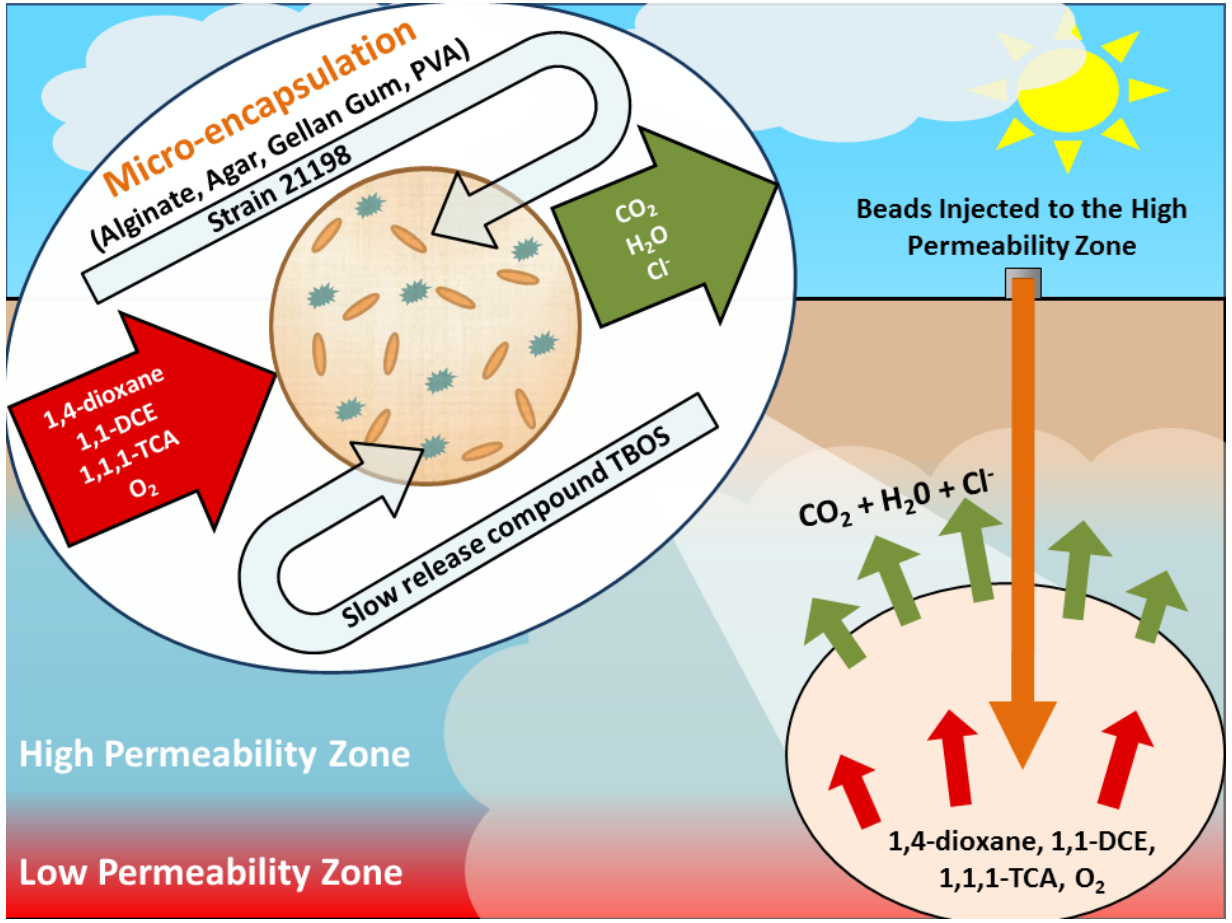
To develop novel aerobic **cometabolic** processes based on **co-encapsulation** of **slow release compounds (SRCs)** and **isobutane**-metabolizing bacteria to treat **COC mixtures** of interest



OSU Patent Pending

Rhodococcus rhodochrous 21198

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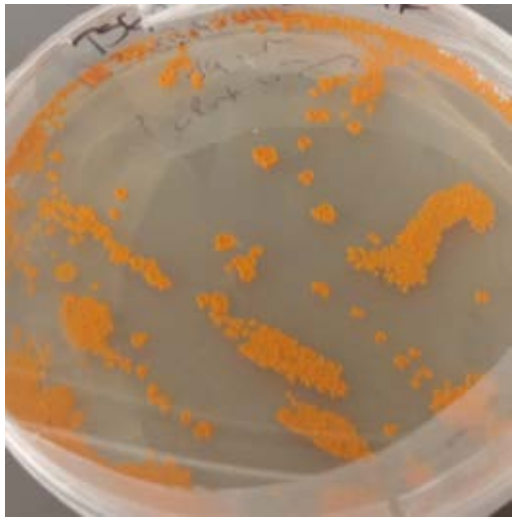
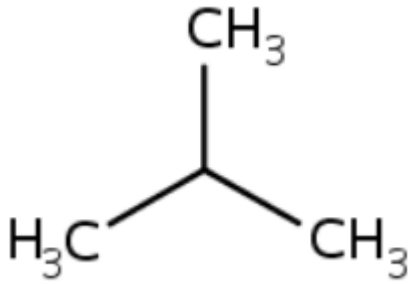
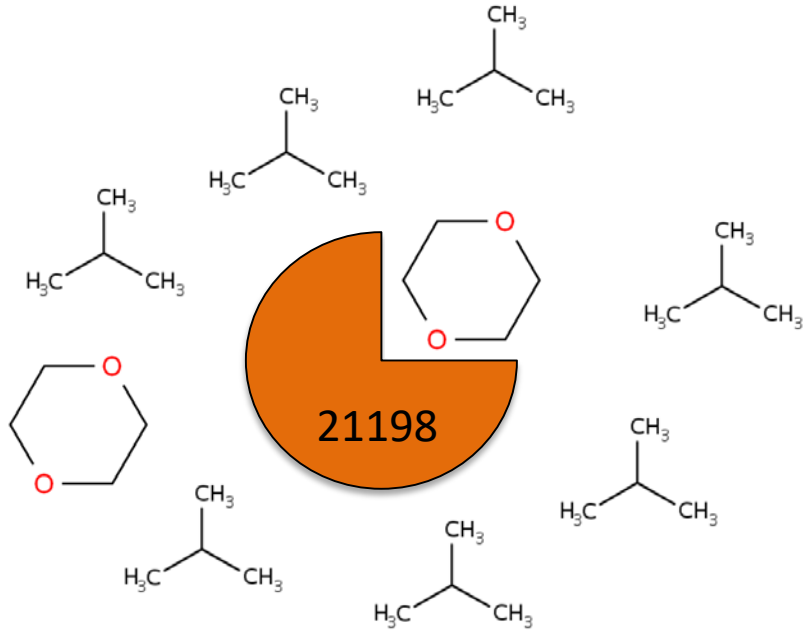


Rhodococcus rhodochrous ATCC® 21198™ is an aerobic, gram-positive bacterium isolated from soil.



21198 expresses a monooxygenase enzyme when grown on Isobutane (C_4H_{10})



Cometabolism: the fortuitous oxidation of non-growth supporting compounds (1,4-D) by microorganisms grown on primary substrates (isobutane, 2-butanol, etc.)

SRC Evaluation – T2BOS

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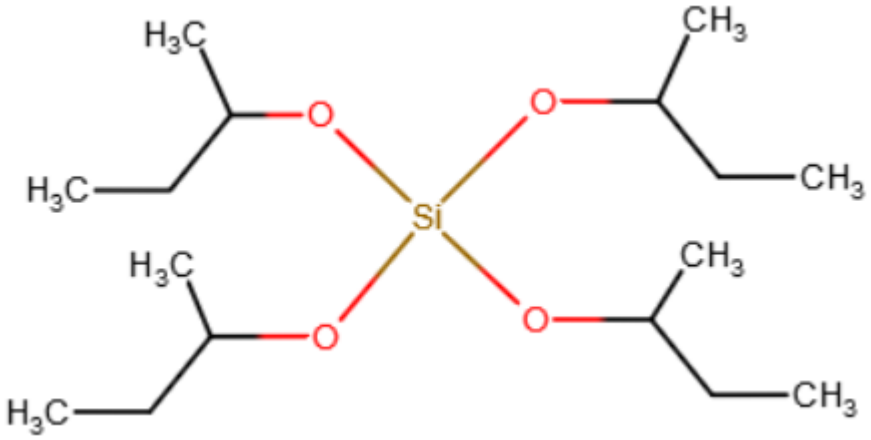
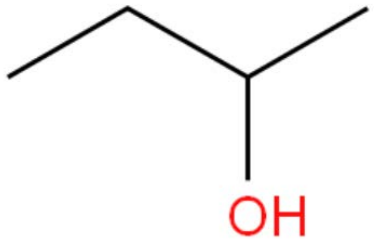


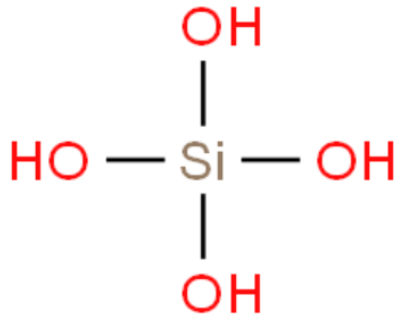
T2BOS
(tetra-s-butyl orthosilicate)

Hydrolysis

4



2-Butanol



Silicic Acid

Produces 2-butanol - which induces SCAM
Branched group – slower rate of abiotic hydrolysis compared to linear TBOS

Gellan Gum Encapsulation

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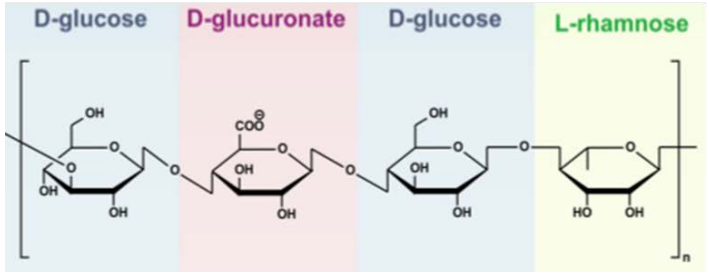
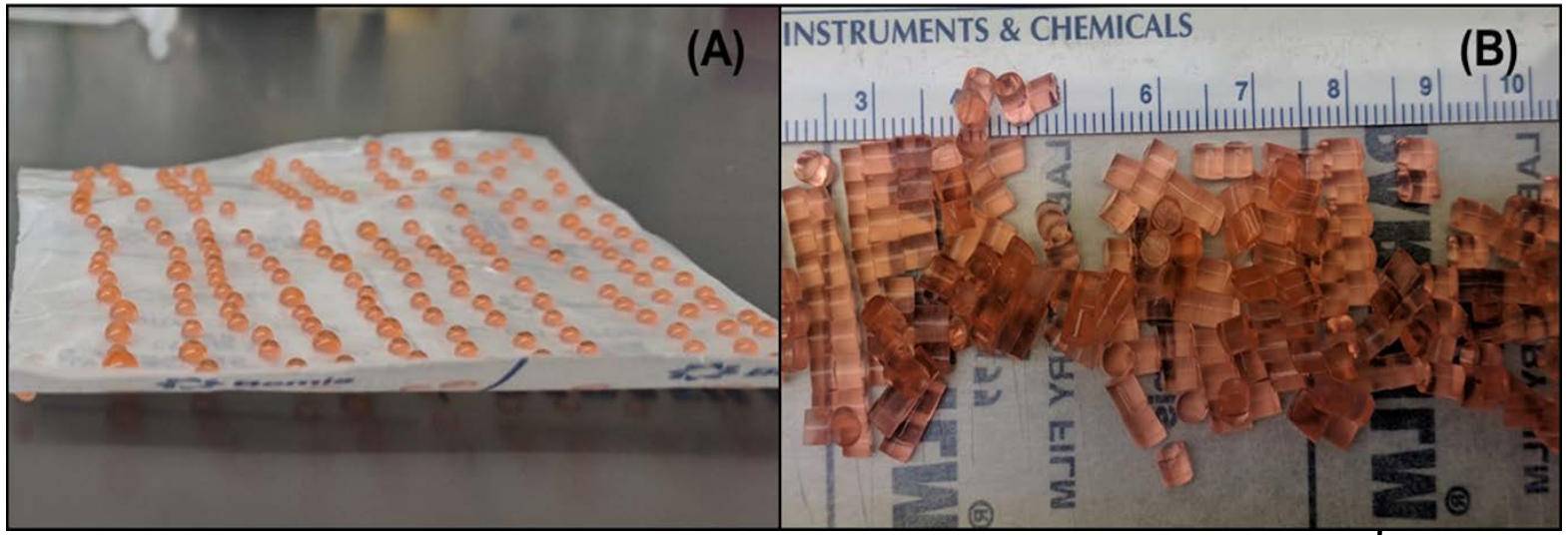
- Natural Occurring Polysaccharide Hydrogels
  - Gellan Gum
    - Thermally and ionically activated hydrogel





Co-Encapsulated Cell and SRCs Long Term Cometabolic Transformation Performance

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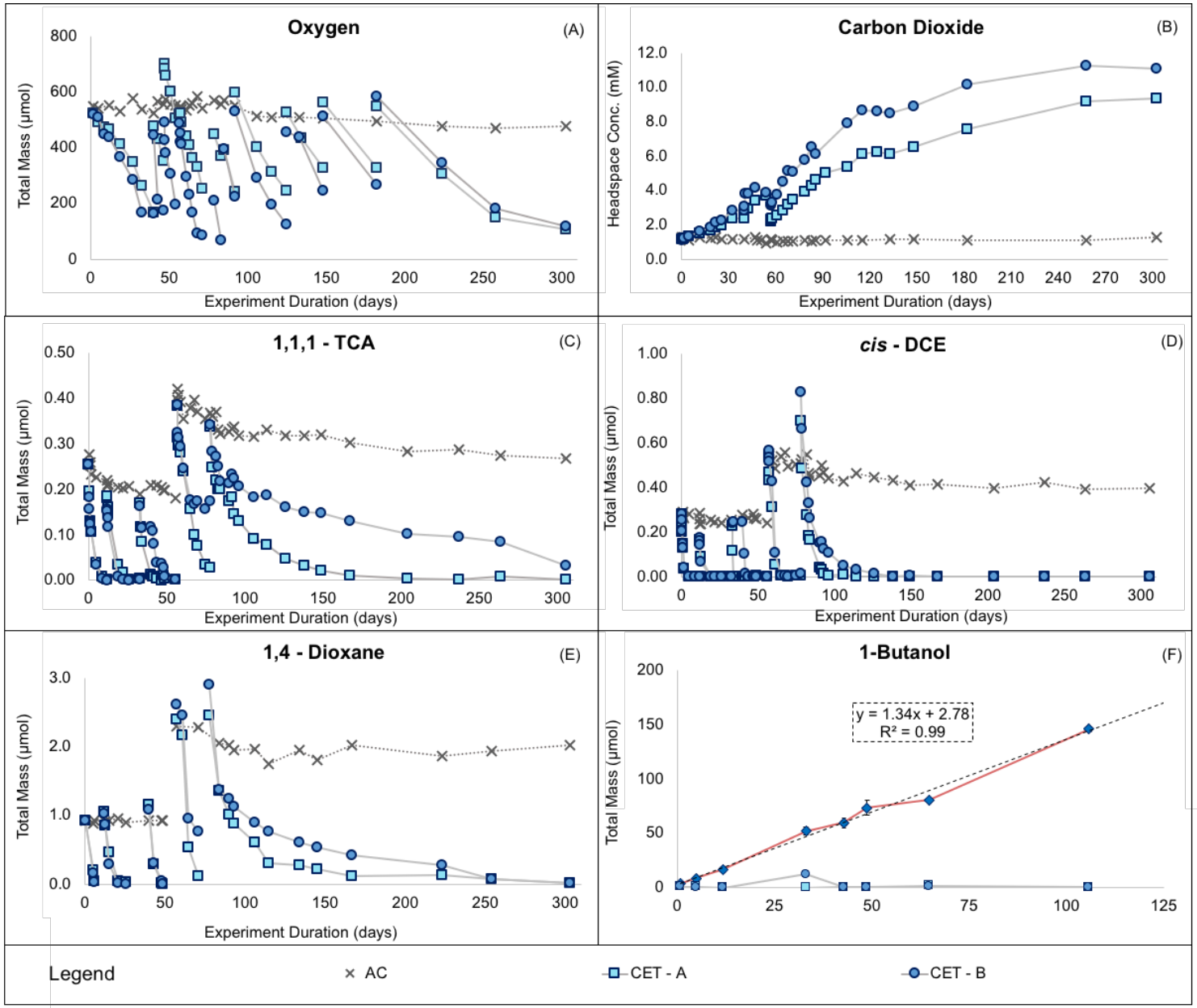
- Gellan gum beads
- Two batches of beads
  - 1) 0.5 mg_{TSS}/g_{bead} and 8% TBOS
  - 2) 0.5 mg_{TSS}/g_{bead} and 8% T_2BOS
  - 3) 2 g of beads per batch reactor

TBOS and 21198 Co-Encapsulated in Gellan gum Cometabolic Performance



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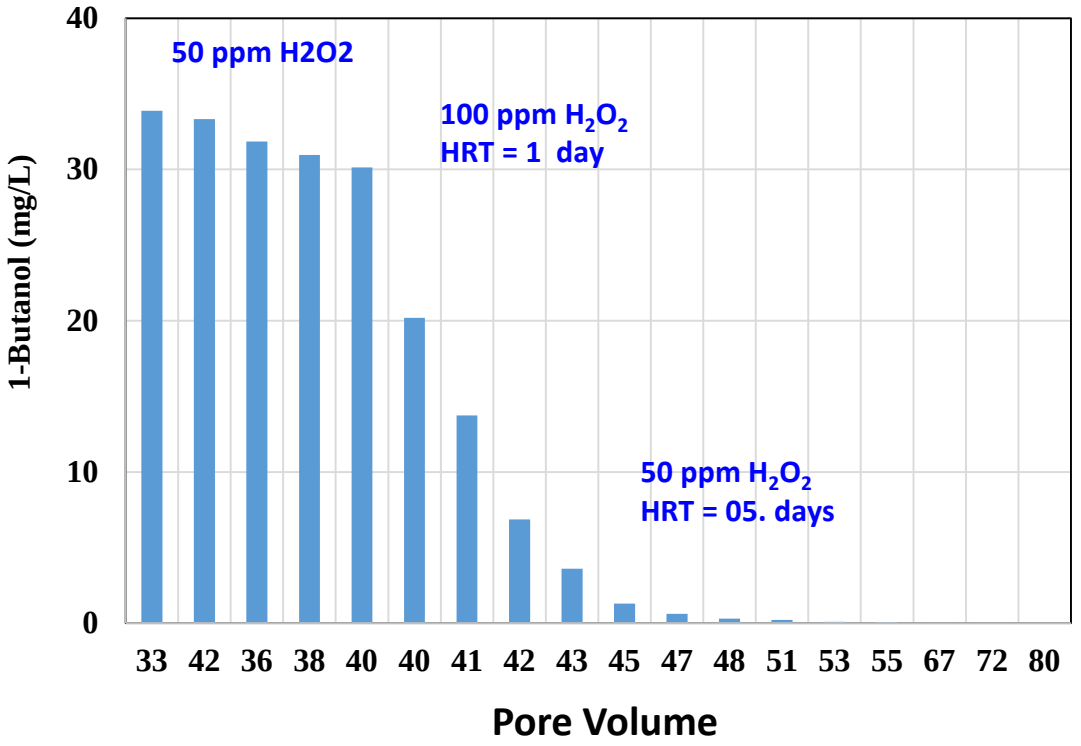
- **Fast zero-order rates of abiotic TBOS hydrolysis**
- **High Rates O_2 utilization and CO_2 production**
- **Successive transformation of 1,1,1-TCA, cis-DCE and 1,4-dioxane**
- **Cometabolic activity over a period of 300 days**
- **Slowing in transformation rates with time**

from: Rasmussen et al. Environmental Processes and Impacts (2020)

Continuous Flow Packed Column Tests with TBOS/21198 Beads

H_2O_2 Addition to Utilize the 1-Butanol Produced and to Achieve Cometabolic Transformation Potential Throughout the Entire Column

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



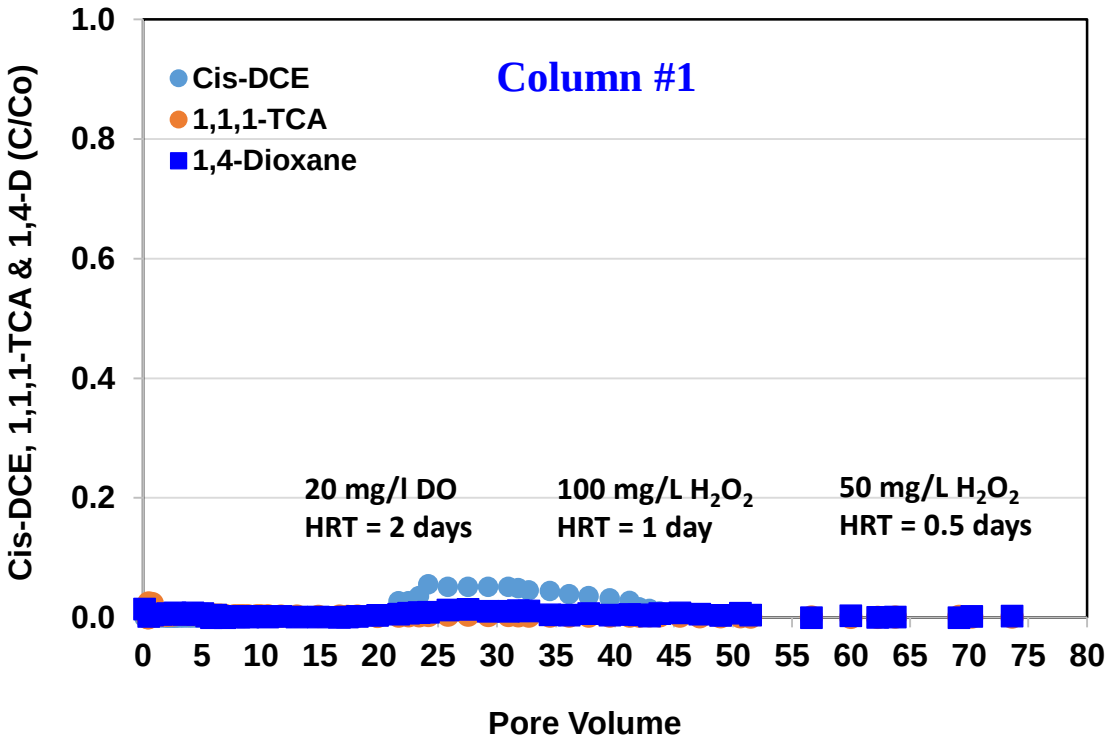
Pore Volumes – 50 to 80
50 mg/L H_2O_2
HRT = 0.5 days

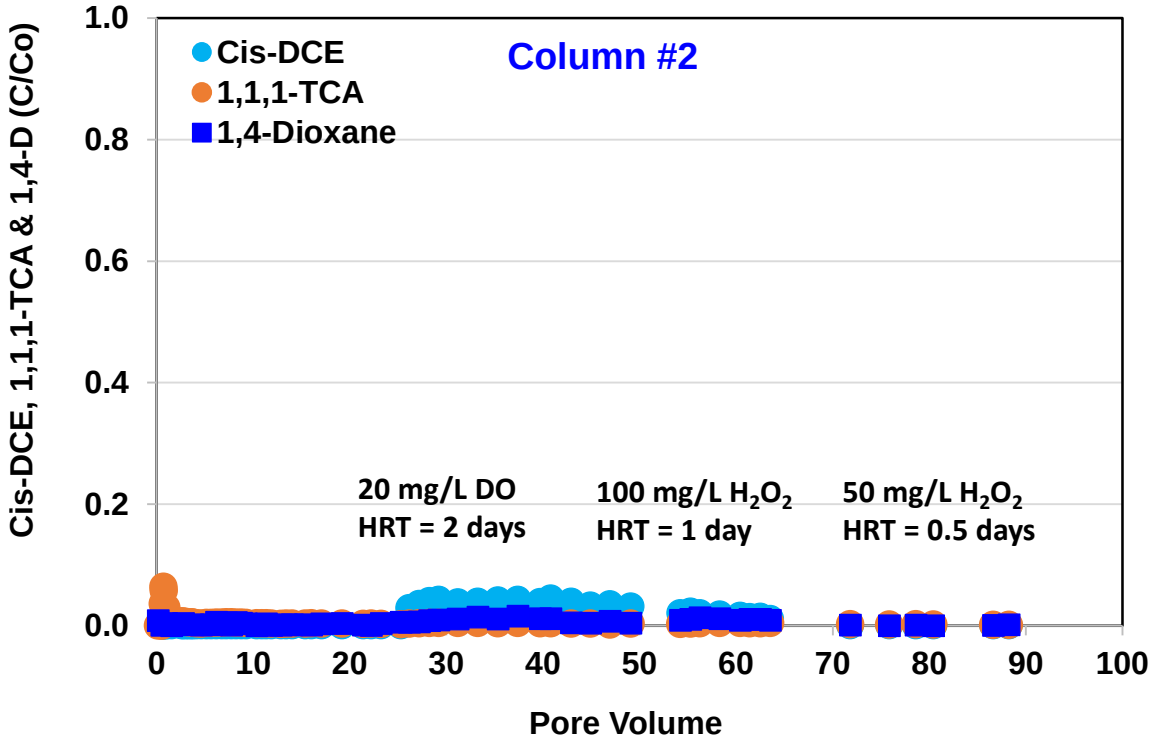
Only Column Entrance Stimulated
Due to Lack of DO

Whole Column is Stimulated
Excess DO in the Column Effluent

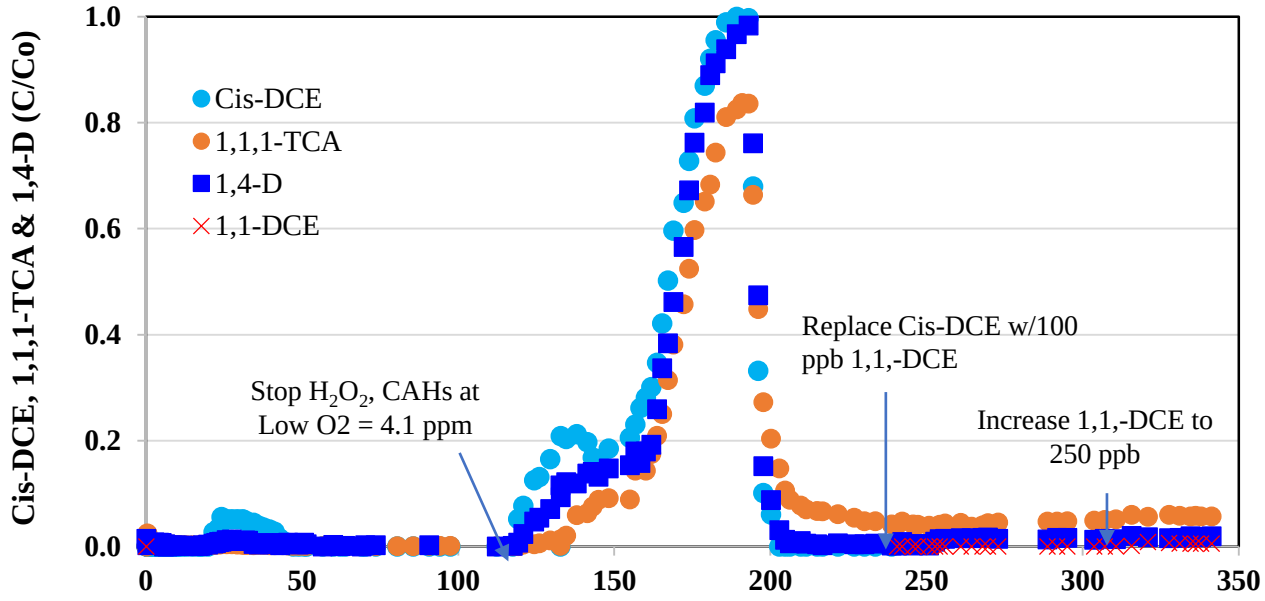
Continuous Flow Packed Columns Treating a Mixture of 1,4-Dioxane, 1,1,1-Trichloroethane and Cis-Dichloroethene:

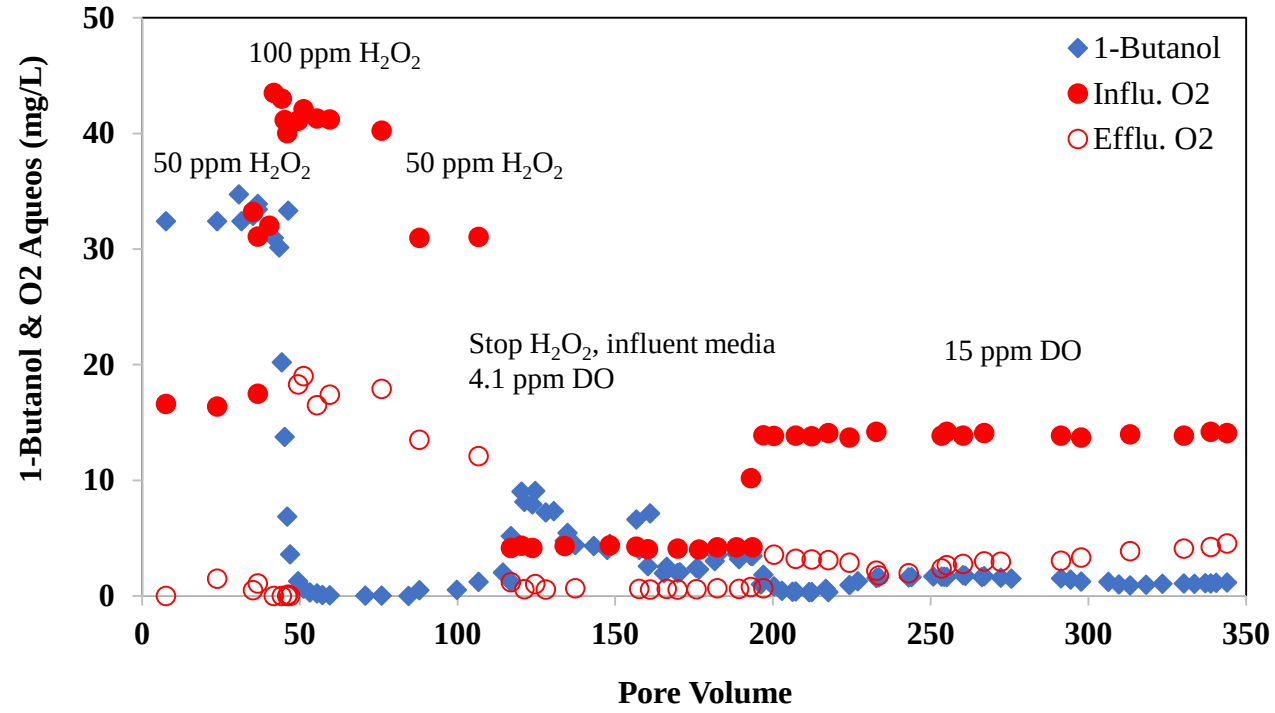
Co-Encapsulation with Strain 21198 and 10% TBOS





Compound	Influent Conc.(µg/L)	Effluent Conc. (µg/L)	Percent Transformed
1,4-Dioxane	250	1.1	99.6
1,1,1-TCA	250	0.2	99.9
Cis-DCE	250	0.3	99.8





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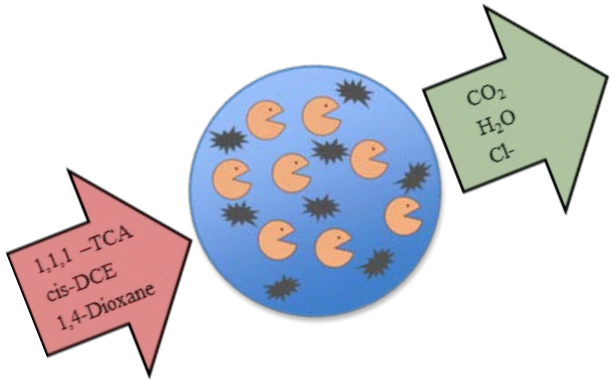
Continuous Flow Column 1,4-Dioxane, 1,1,1-Trichloroethane, Cis-Dichloroethene and 1,1-Dichloroethene: **Co-Encapsulation with Strain 21198 and 10% TBOS**

- **Long-term transformation of 250 µg/L of CoCs**
- **Transformation ceased at low influent DO concentrations**
- **Transformation recovered when the influent DO was increased**
- **1,1-DCE effectively transformed at 250 µg/L**
- **Capable of treating 175 ft of a GW plume at the operating conditions of the colum**

Conclusions



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- ATCC 21198 and SRCs were successfully encapsulated in gellan gum bead hydrogels for use in passive cometabolic treatment systems
- TBOS and T2BOS hydrolysis products, 1-butanol and 2-butanol, were utilized by co-encapsulated ATCC 21198 for cellular growth and SCAM enzyme production prior to diffusion from the beads.
- Different rates of alcohol release were achieved by varying the structure of the Orthosilicate with rates of TBOS > T2BOS. Rates of O_2 uptake are correlated with rates of hydrolysis.
- Cometabolism of 1,1,1-TCA, 1,2-cis-DCE, and 1,4-dioxane were achieved for a over 300 days in batch reactors with gellan gum beads encapsulated with ATCC 21198 and TBOS and T2BOS
- Continuous flow columns packed with beads achieved over 99.5% treatment of 1,1,1-TCA, 1,2-cis-DCE and 1,4-dioxane with a HRT of 12 hours

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Acknowledgments

SERDP
DOD • EPA • DOE
Project ER-2716

OSU

Mitchell Rasmussen
Riley Murnane
Alyssa Saito
Mohammad Azizian
Alisa Bealessio

NCSU

Michael Hyman
Weijue Chen
Christy Smith