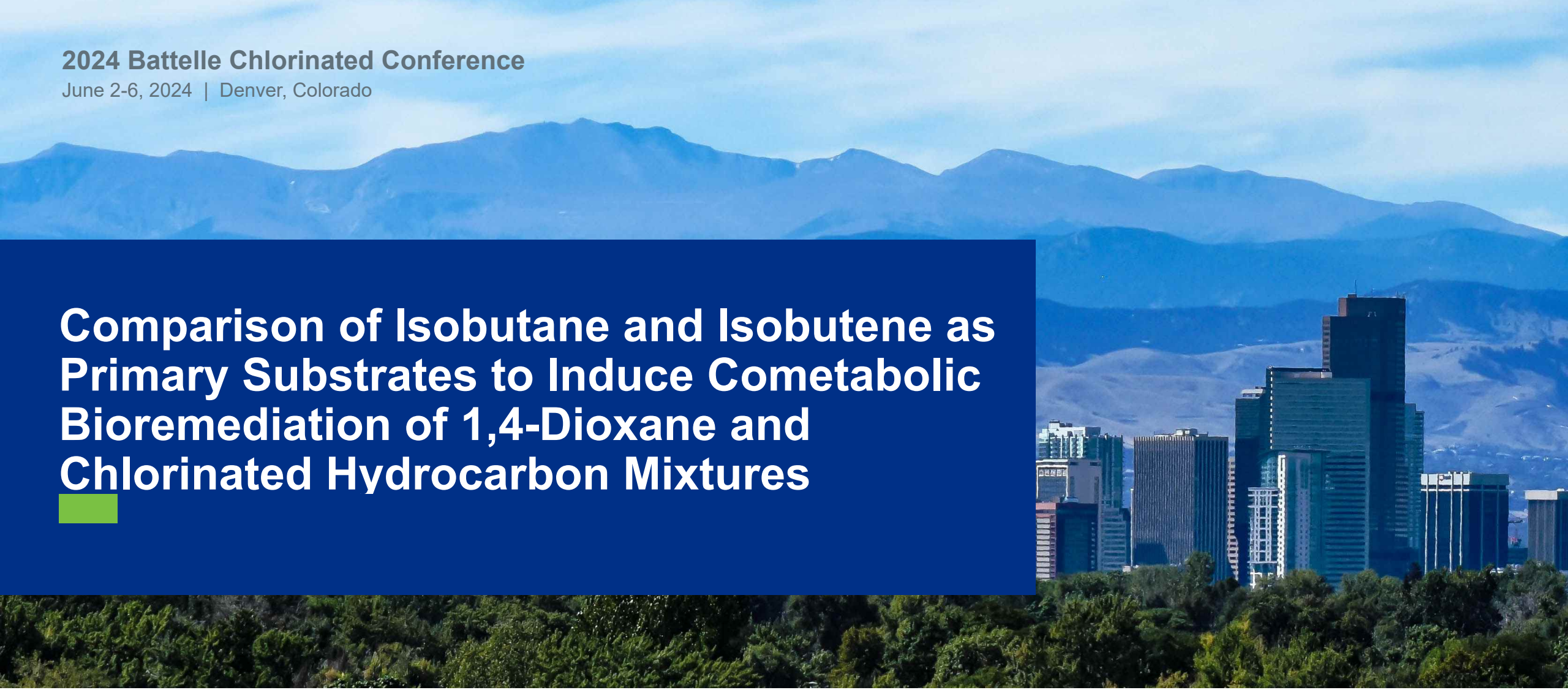
2024 Battelle Chlorinated Conference
June 2-6, 2024 | Denver, Colorado



Comparison of Isobutane and Isobutene as Primary Substrates to Induce Cometabolic Bioremediation of 1,4-Dioxane and Chlorinated Hydrocarbon Mixtures

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CDM Smith

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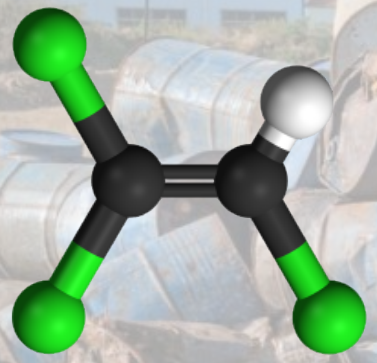
June 6, 2024

Oregon State University College of Engineering

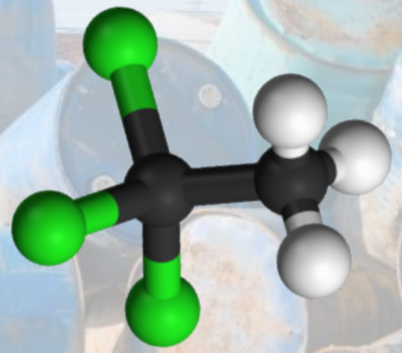
CDM Smith

Background and Objectives

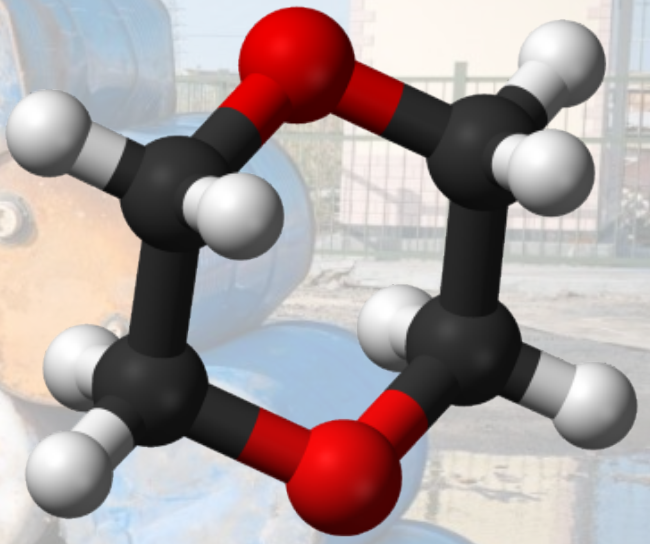
Chlorinated aliphatic hydrocarbons (CAHs) & 1,4-Dioxane



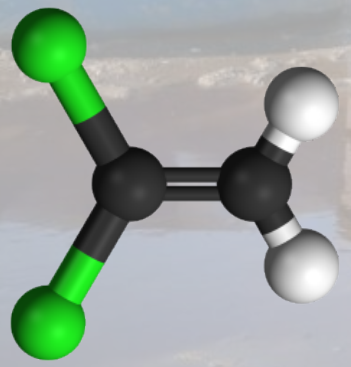
Trichloroethene
(TCE)



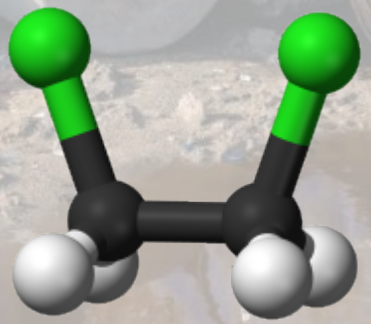
1,1,1-
Trichloroethane
(1,1,1-TCA)



1,4-Dioxane



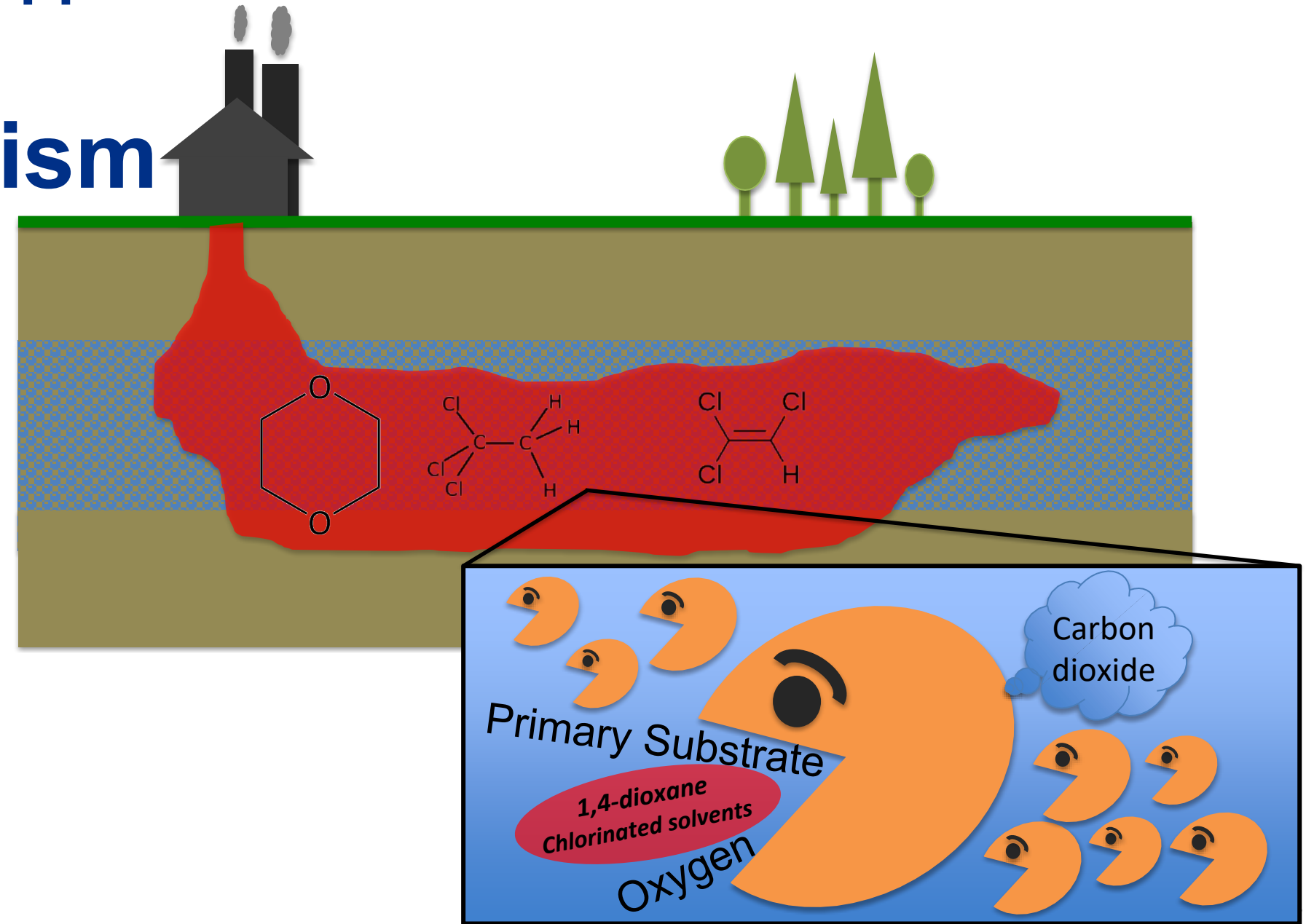
1,1-Dichloroethene
(1,1-DCE)



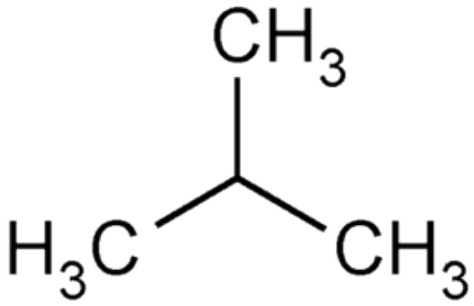
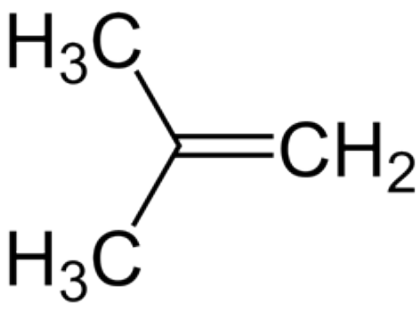
1,2-Dichloroethane
(1,2-DCA)

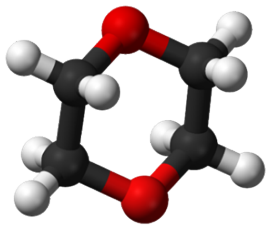
Chlorine
Oxygen
Carbon
Hydrogen

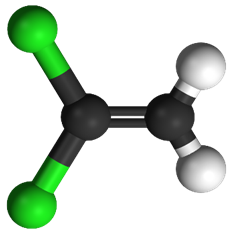
Bioremediation approach:
Aerobic Cometabolism

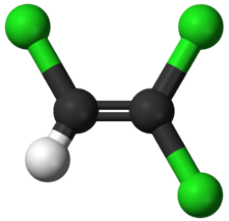


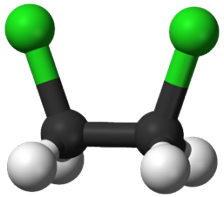
Objective: Primary Substrate Comparison

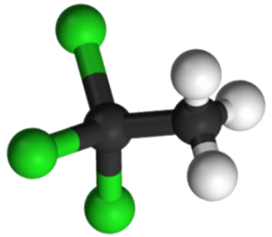
		
Primary Substrate	**Isobutane**	**Isobutene**
Model bacterium	*Rhodococcus rhodochrous* strain 21198	*Mycobacterium* sp. strain ELW1





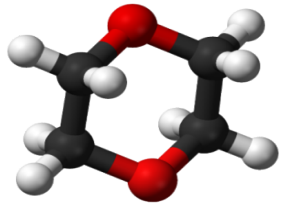




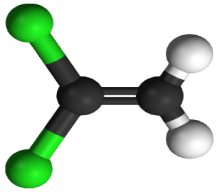


Methods

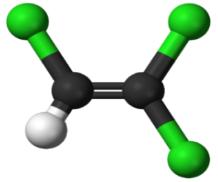
Groundwater Microcosms:



1,4-Dioxane



1,1-Dichloroethene (1,1-DCE)



Trichloroethene (TCE)

Bioaugmentation

- Strain 21198 or strain ELW1 added with respective primary substrate to microcosms

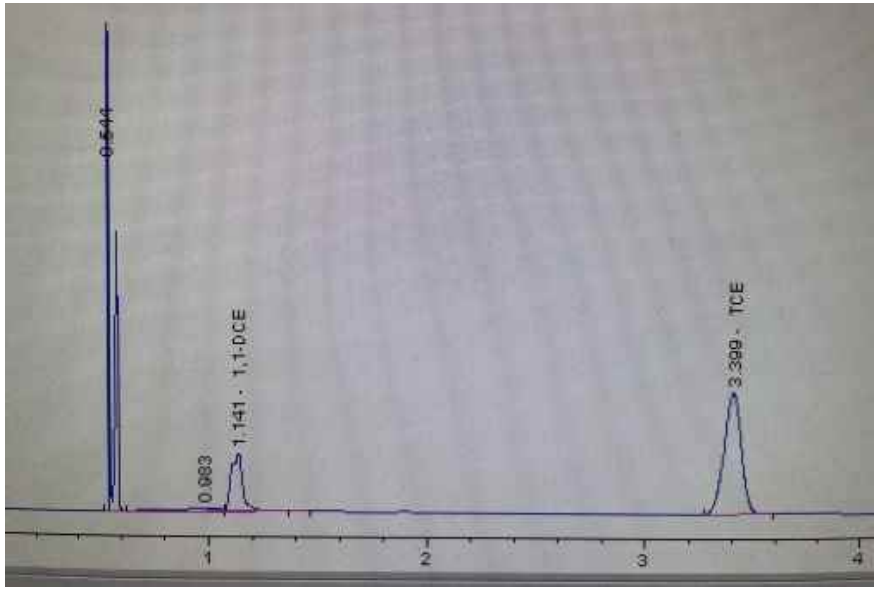
Biostimulation

- Isobutane or isobutene added to microcosms
- No addition of model bacteria

Analytical Methods

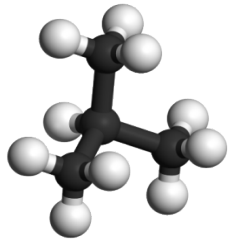
Gas chromatography (GC)

- GC-ECD: chlorinated hydrocarbons
- GC-FID: isobutane, isobutene
- GC-MS with heated purge and trap: 1,4-dioxane



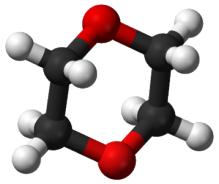
Modeling:
Michaelis-Menten/Monod and First-Order Kinetics

Primary substrate



$$\frac{dPS}{dt} = \frac{-K_{max,PS} * X * PS}{K_{s,PS} + PS}$$

Cometabolic substrate



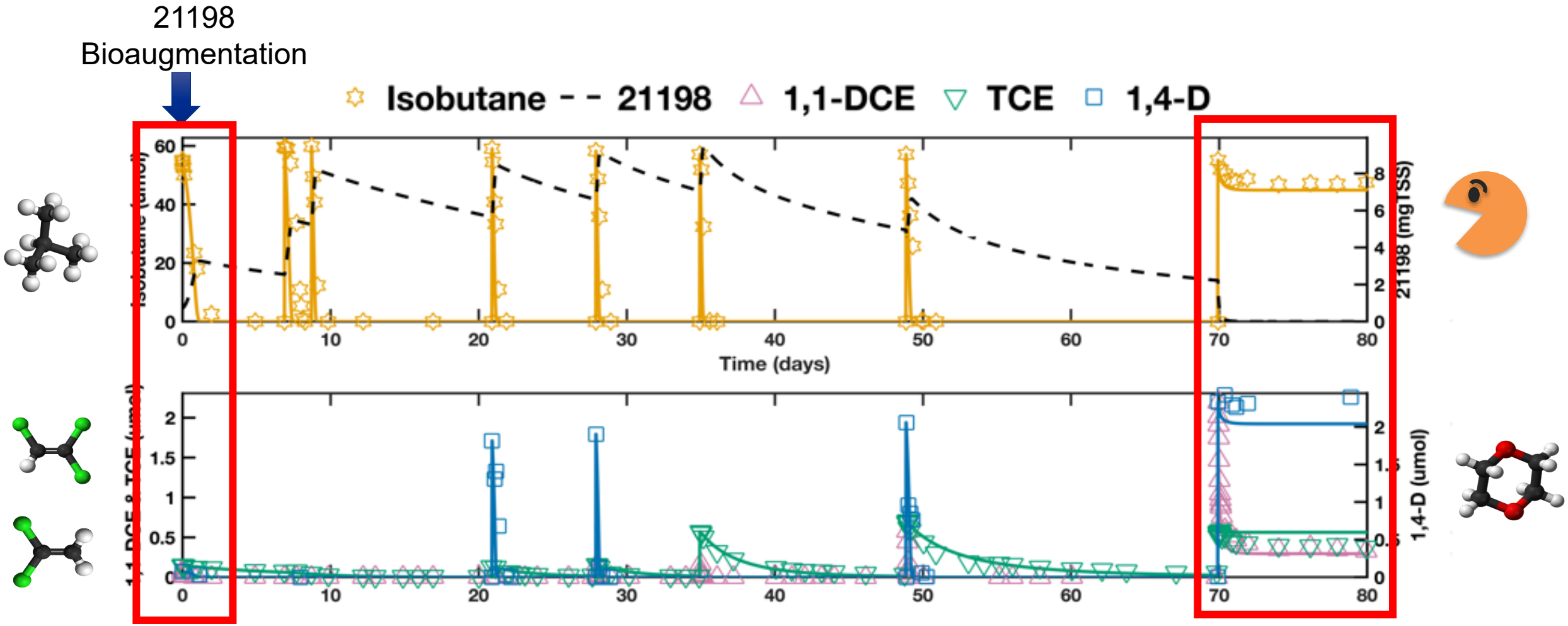
$$\frac{dCS}{dt} = \frac{-K_{max,CS} * X * CS}{K_{s,CS}\left(1+\frac{C_I}{K_I}\right)+CS} \quad \text{OR} \quad \frac{dCS}{dt} = \frac{-K_{FO,CS} * X * CS}{1+\frac{C_I}{K_I}}$$

Biomass

$$\frac{dX}{dt} = Y * \frac{dPS}{dt} - \sum \frac{1}{T_{c,n}} * \frac{dCS_n}{dt} - bX$$

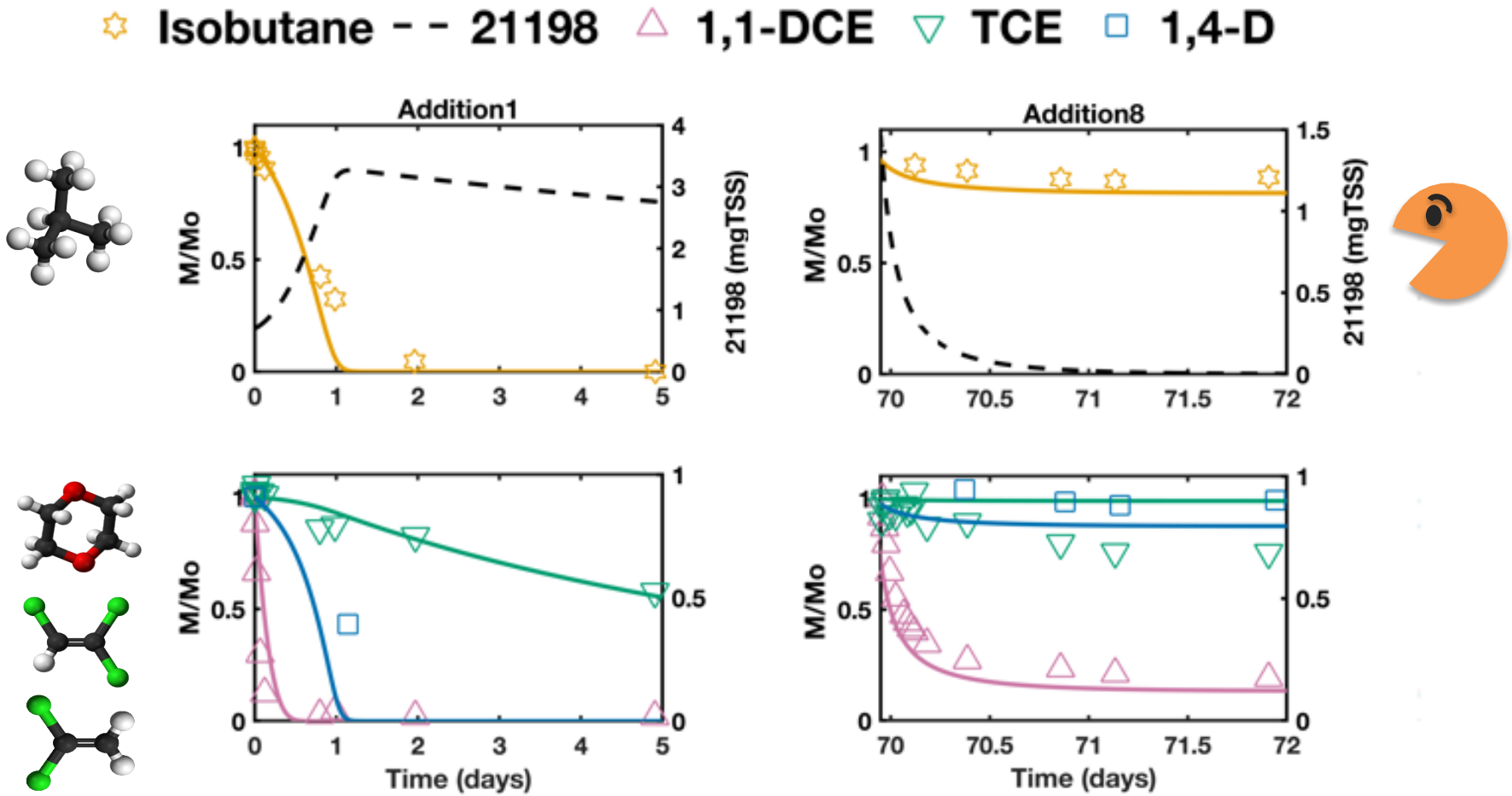
Results

21198 Bioaugmentation in Groundwater Microcosms



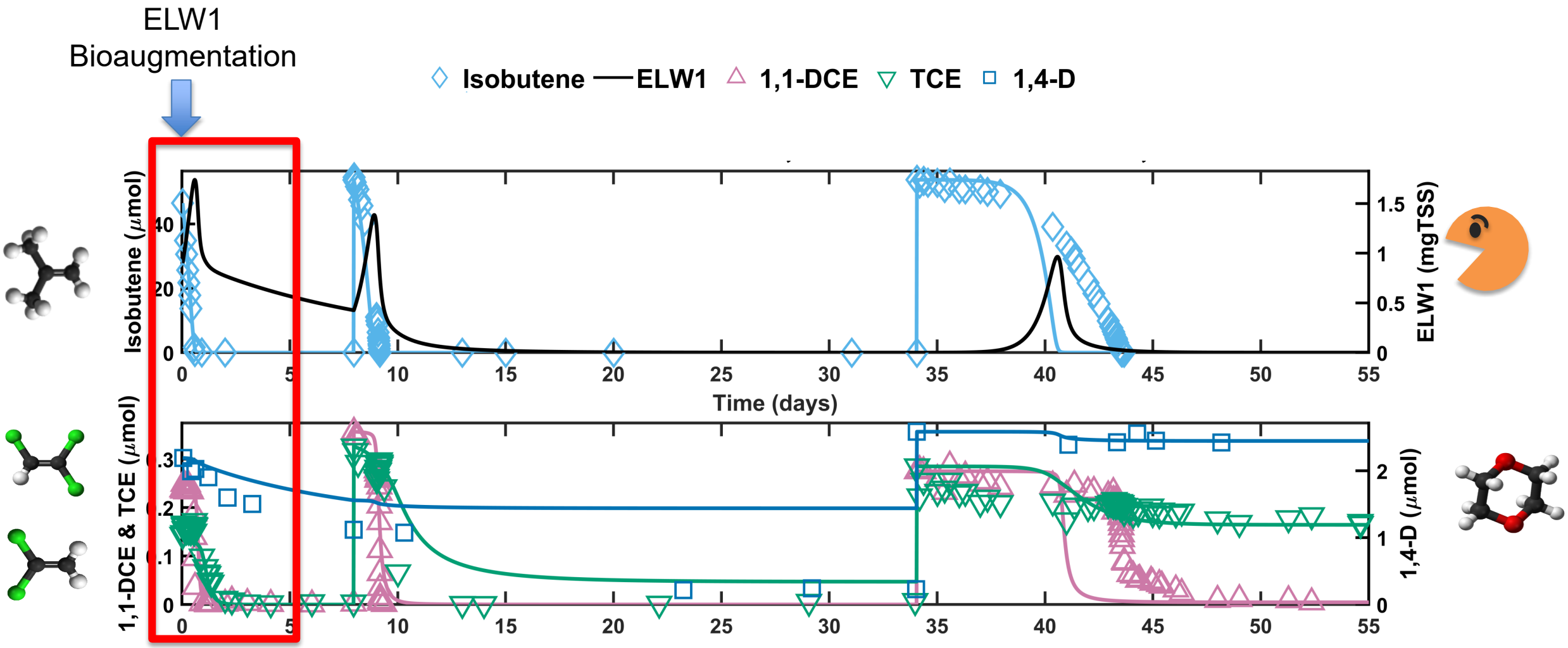
After bioaugmentation, transformation of multiple additions of isobutane and contaminants over 80 days

21198 Bioaugmentation in Groundwater Microcosms



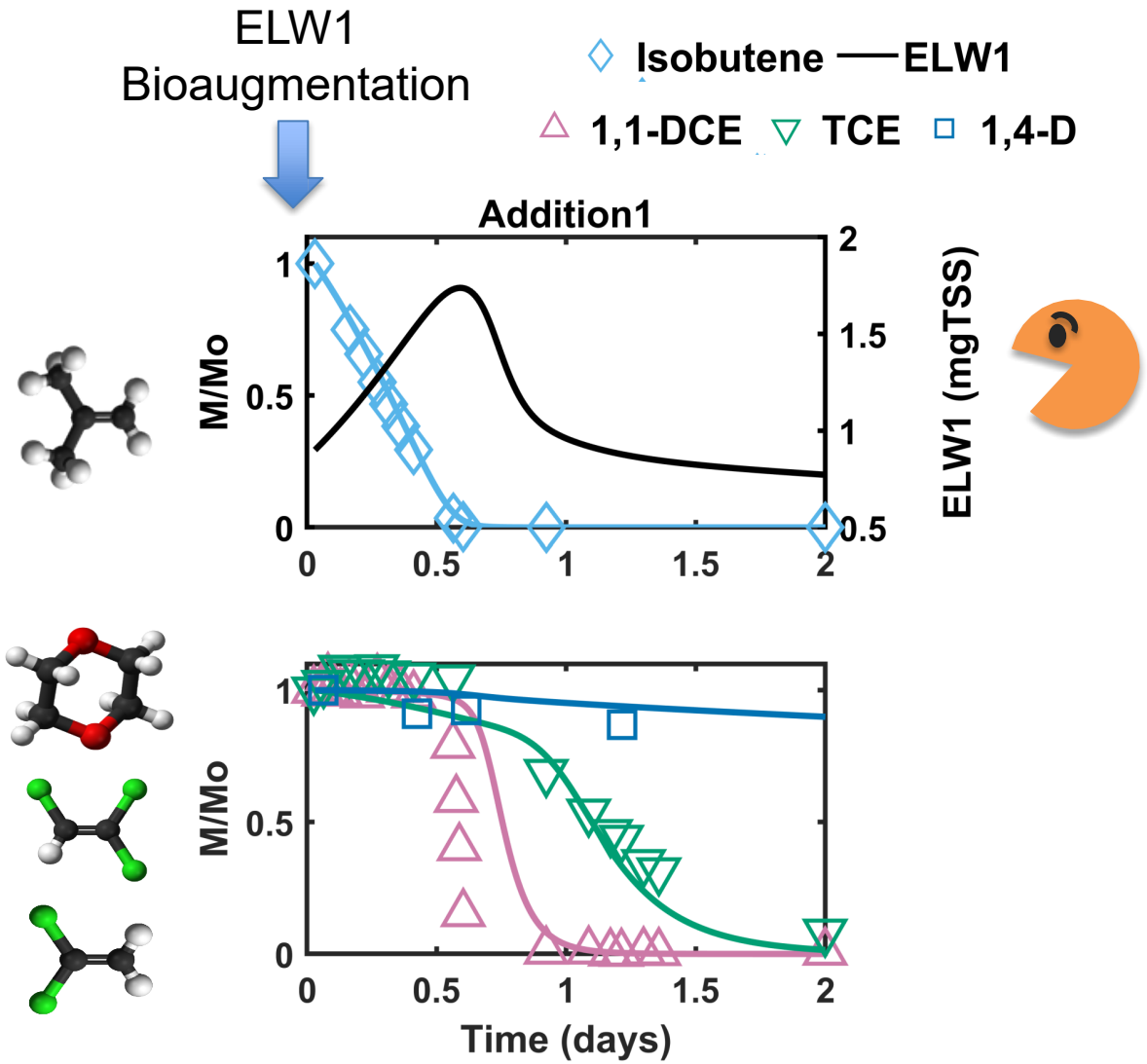
- 1,1-DCE cometabolized faster than isobutane consumed
- Inactivation of 21198 biomass when 1,1-DCE transformation capacity was reached

ELW1 Bioaugmentation in Groundwater Microcosms



After bioaugmentation, transformation of multiple additions of isobutene and contaminants over 55 days

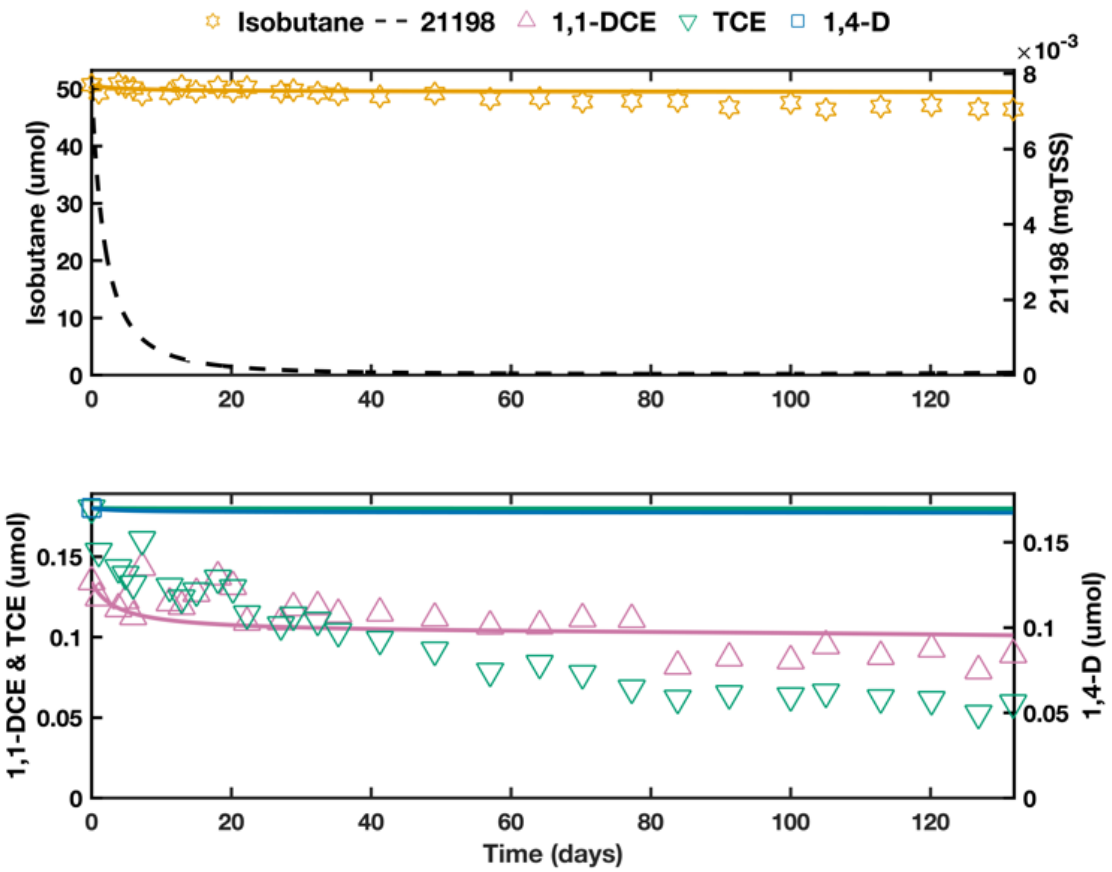
ELW1 Bioaugmentation in Groundwater Microcosms

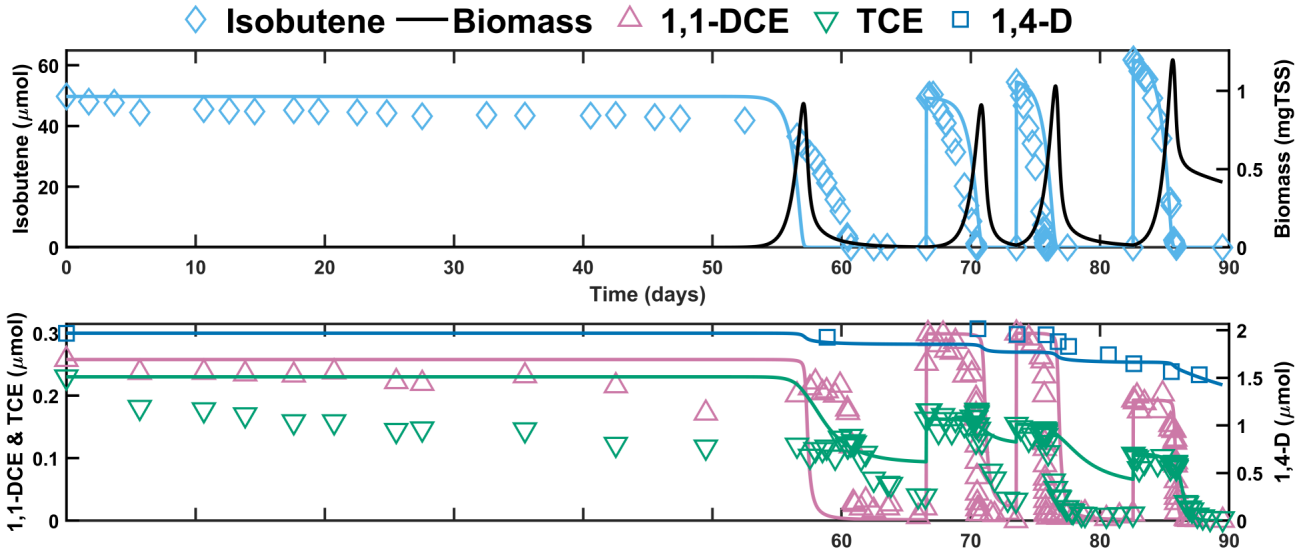


- Isobutene inhibits cometabolic transformation
- 1,1-DCE inhibits TCE transformation
- Minimal 1,4-dioxane transformation

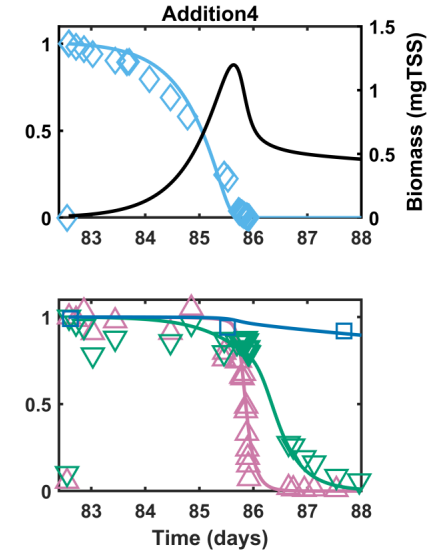
$$\frac{dCS}{dt} = \frac{-K_{max,CS} * X * CS}{K_{s,CS}\left(1 + \frac{C_I}{K_I}\right) + CS}$$

Biostimulation in Groundwater Microcosms



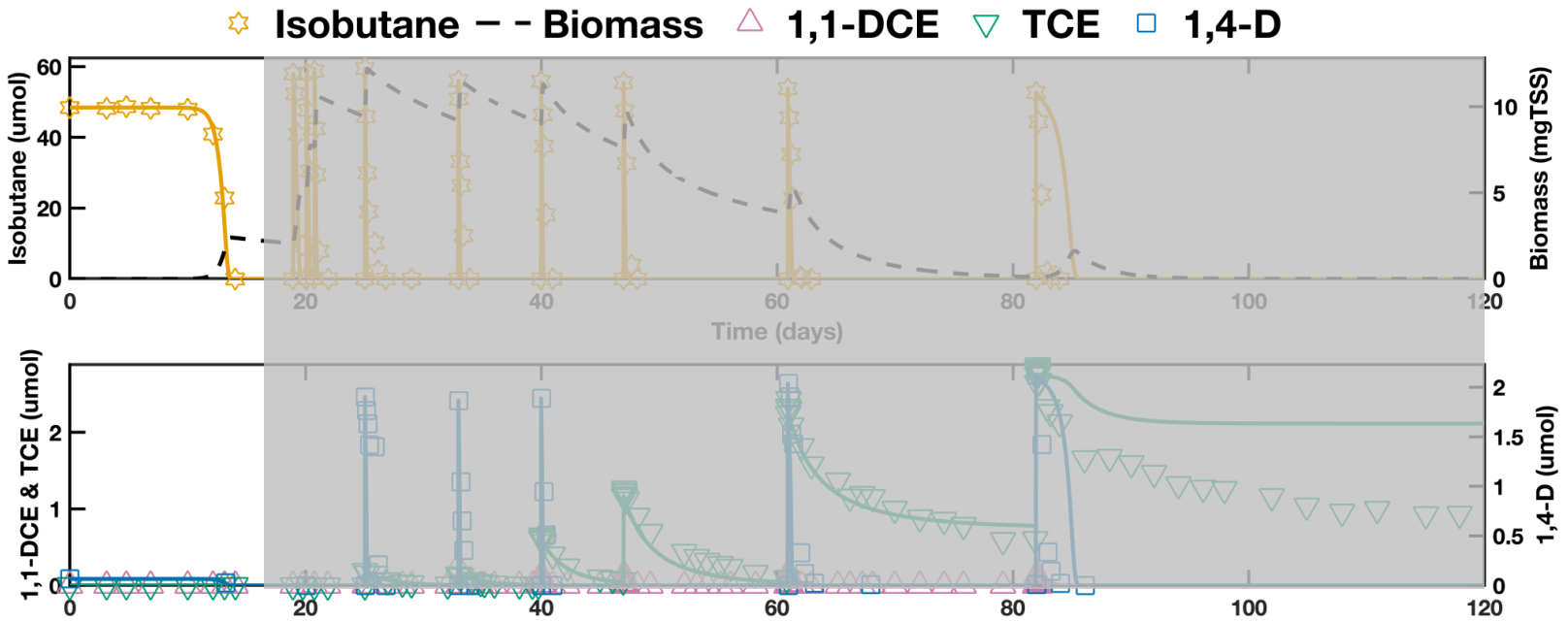


- Isobutene uptake after 55 days
- Contaminant transformation modeled with the same parameter values as ELW1



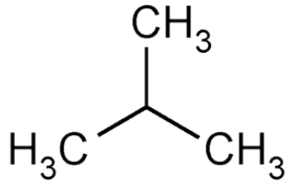
Biostimulation with Isobutane:
After 1,1-DCE was Sparged from Groundwater

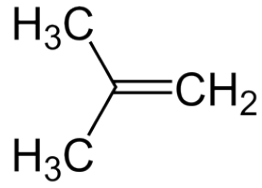
- Isobutane uptake after 12 days in the absence of 1,1-DCE
- Contaminant transformation similar to strain 21198



Conclusions

Comparison Summary

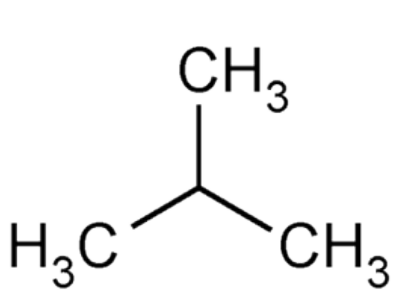




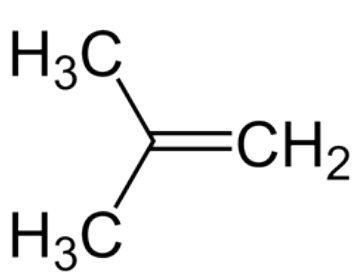
	Isobutane-utilizers	Isobutene-utilizers
1,4-dioxane cometabolism		
1,1-DCE cometabolism		
Primary substrate inhibition		
Biostimulation in the presence of 1,1-DCE		

Further Consideration

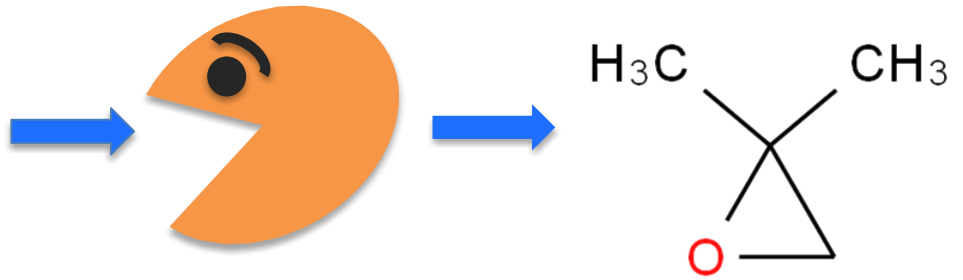
- Use of multiple primary substrates:
  - Isobutene can be a primary substrate but can also be cometabolized by isobutane-utilizing bacteria
  - Inhibition, transformation toxicity potential



Isobutane
(primary substrate)



Isobutene
(cometabolic substrate)



Isobutene epoxide
(transformation intermediate)

Key Take Aways

- Cometabolic transformations induced by growth on isobutane are **different** than by growth on isobutene
- Primary substrates induce similar cometabolic transformations by different bacteria:
  - Strain 21198 and isobutane-stimulated indigenous microbial community
  - Strain ELW1 and isobutene-stimulated indigenous microbial community
  - Kinetic parameter values were the same to model transformations in bioaugmented and biostimulated microcosms
- Cometabolic transformation of mixtures is complicated:
  - Inhibition interactions
  - Transformation toxicity

Acknowledgements

- Environmental Security Technology Certification Program
  - NAVFAC, APTIM
- DeVaan Fellowship for Clean Water Technology
- Jon Laurance (now Geosyntec)—data collection
- Mohammad Azizian (Oregon State University)—lab/instrument management
- Michael Hyman (North Carolina State University)—microbial cultures

Thank you!

To learn more, contact:

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