Application of CSIA in 1,4-Dioxane Studies: Latest Developments

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(North Carolina State University)
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The motivation for this work….

- Little is known about 1,4-dioxane degradation at field sites
- CSIA is a uniquely powerful method for proving biodegradation in the field, BUT:
  - **At many sites, 1,4-dioxane concentrations are too low for CSIA**
  - **Enrichment factors for 1,4-dioxane during degradation are not well described**
- Development of a method for applying CSIA at lower concentrations of 1,4-dioxane will allow for field studies on 1,4-dioxane biodegradation
- Determination of enrichment factors for 1,4-dioxane biodegradation will allow meaningful interpretation of CSIA data from field sites

Co-Authors:

Development of CSIA Method for 1,4-Dioxane:

- Humam El Mugammar and Ramon Aravena, University of Waterloo
- Mike Nickelsen, ECT_2

Development of enrichment factors (reactions with pure cultures):

- Mike Hyman and Christy Smith, North Carolina State University

Field demonstration of CSIA applied during aerobic cometabolic biodegradation of 1,4-dioxane:

- Jacob Chu, Haley & Aldrich

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1. CSIA on Low Concentrations of 1,4-Dioxane

Method Development

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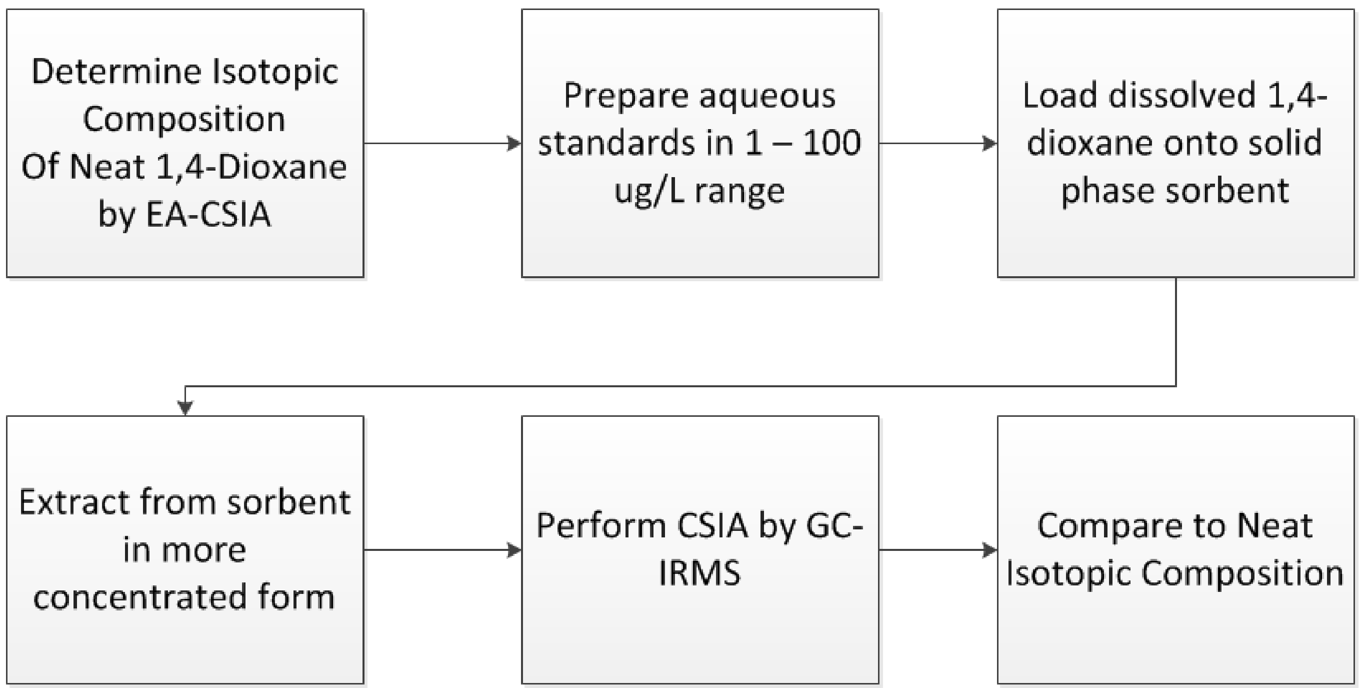
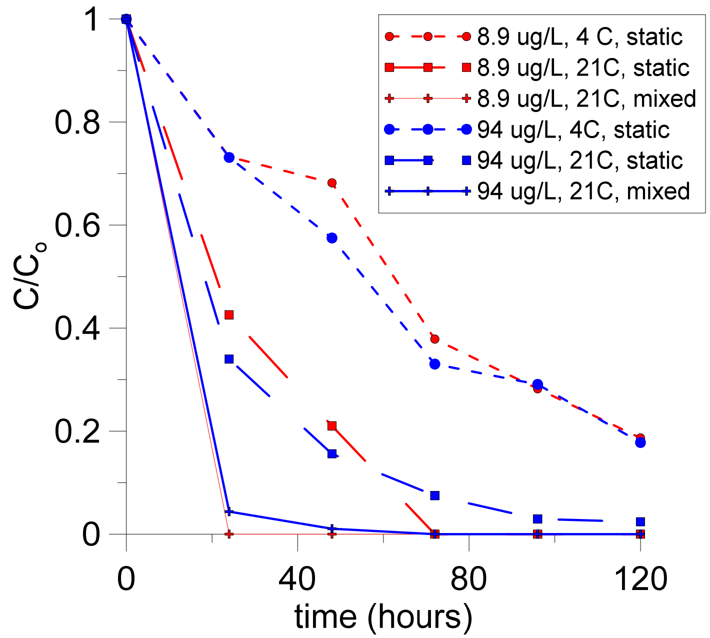
CSIA Method Development

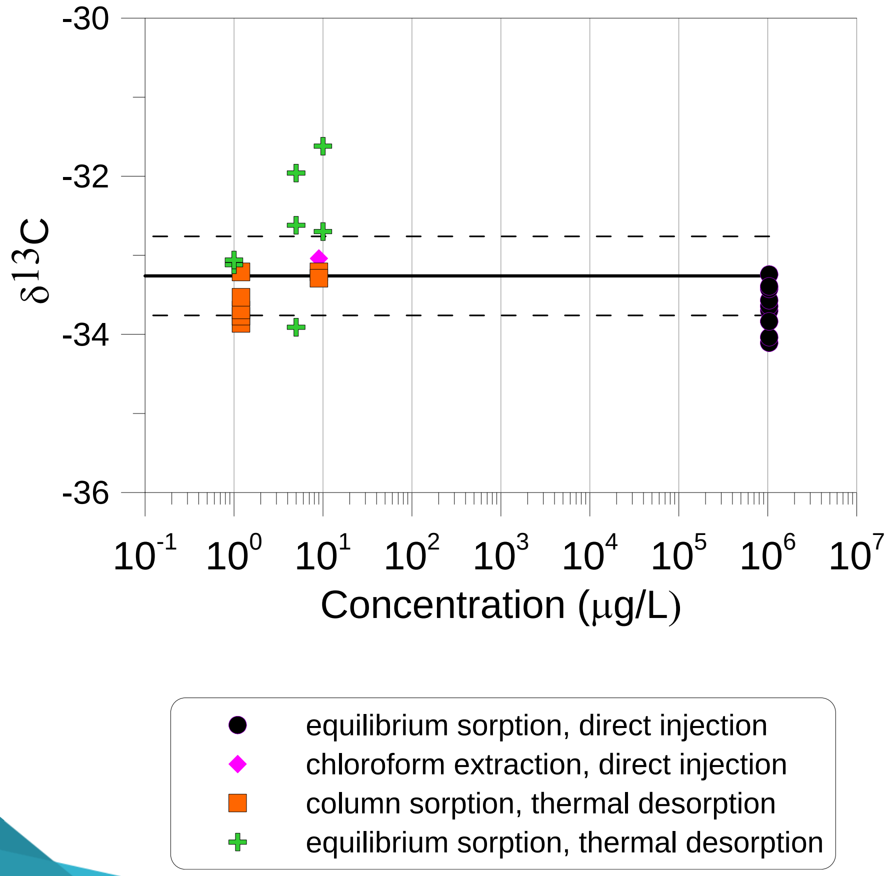


Sorption of 1,4-Dioxane onto Ambersorb 560™



- 0.3 grams A560
- 40 cc 1,4-dioxane at:
  - 8.9 ug/L or
  - 94 ug/L
- Temperatures: 4ºC, 21ºC
- Static and mixed
- Findings:
  - 21ºC preferred over 4ºC
  - Mixing preferred over static
  - Only 24 hours needed for sorption

Method Performance



- No fractionation during partial sorption
- Concentrations as low as 0.6 ug/L have been analyzed for hydrogen!
- Low concentrations require multiple successive extraction steps to concentrate 1,4-dioxane onto resin
- Currently working to optimize low concentration extraction process

2. Carbon and hydrogen enrichment factors for aerobic cometabolic degradation of 1,4-dioxane

Reactions with pure cultures

Overview of Degradation Reactions with Pure Cultures

Culture	Substrate
R. rhodochrous ATCC 21198	1A. Propane 1B. Propane 1C. Propane + 1-butyne
R. rhodochrous ATCC 21198	2A. Isobutane 2B. Isobutane 2C. Isobutane + 1-butyne
P. tetrahydrofuranoxidans K1	3A. Tetrahydrofuran 3B. Tetrahydrofuran 3C. Tetrahydrofuran + 1-butyne

Starting 1,4-dioxane concentration in all reactions: 476 mg/L

Abiotic control reactions (no cells) performed in addition to biologically-active controls (1-butyne addition)

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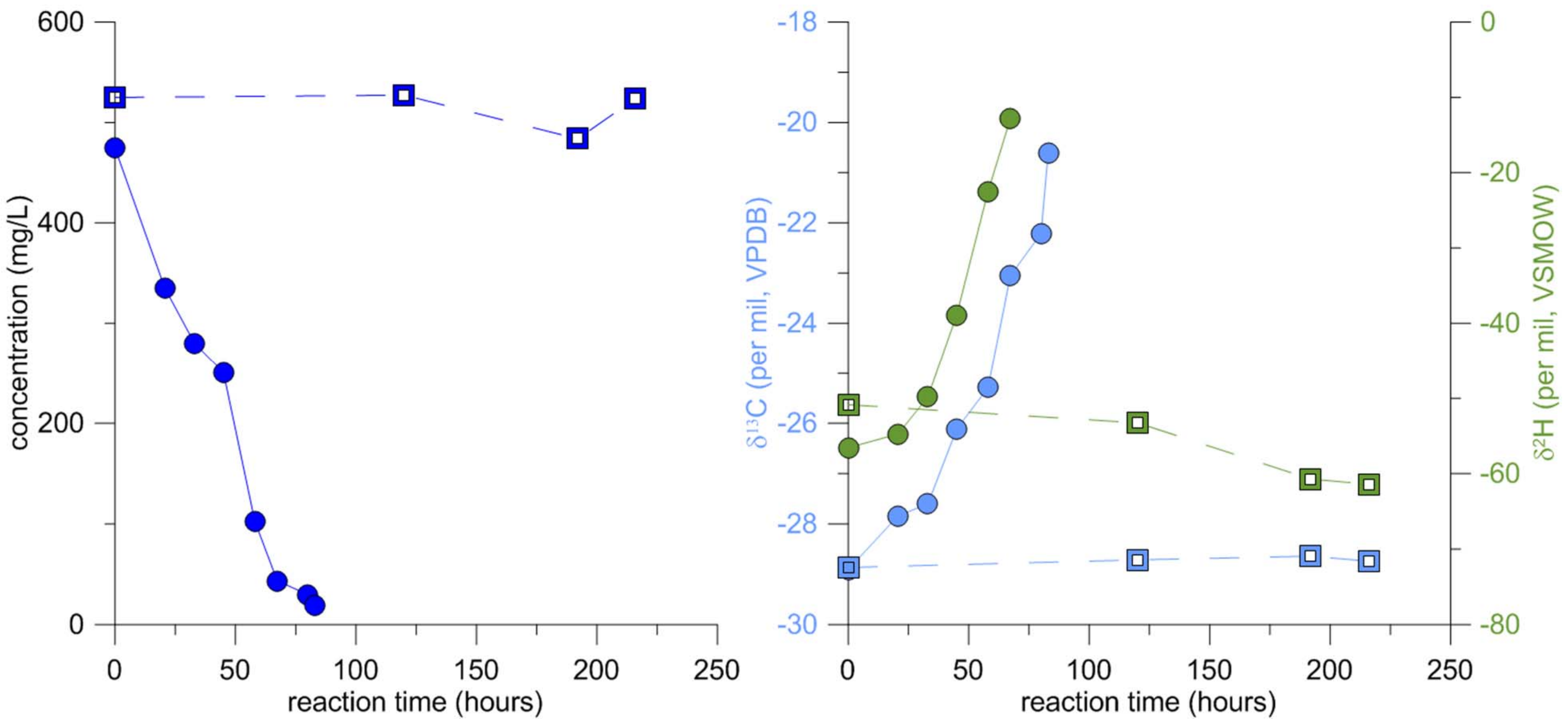
1A. Rhodococcous rhodochrous ATCC 21198: propane



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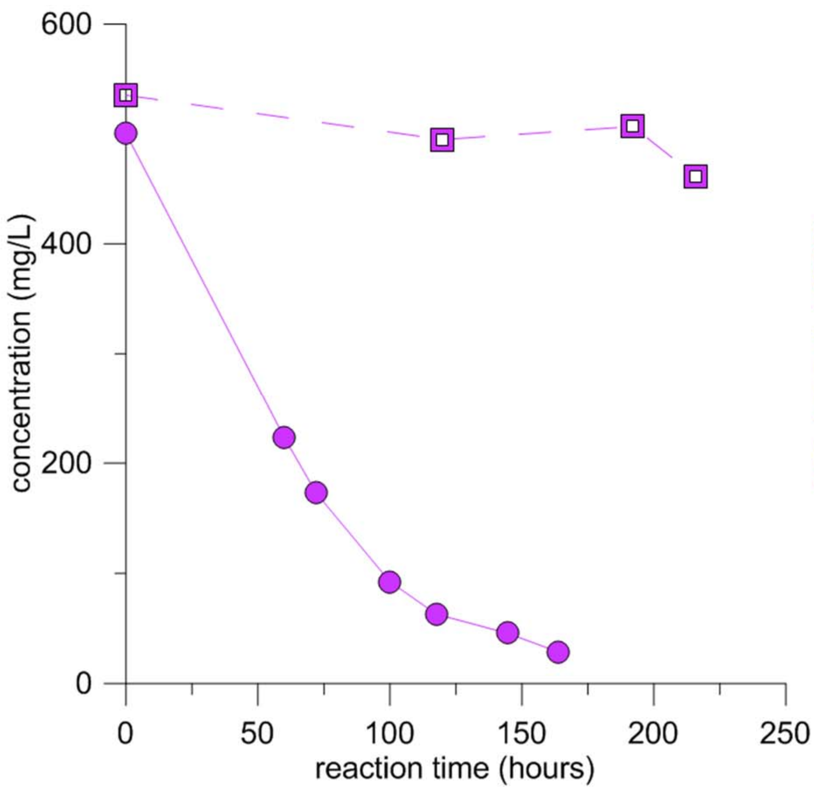
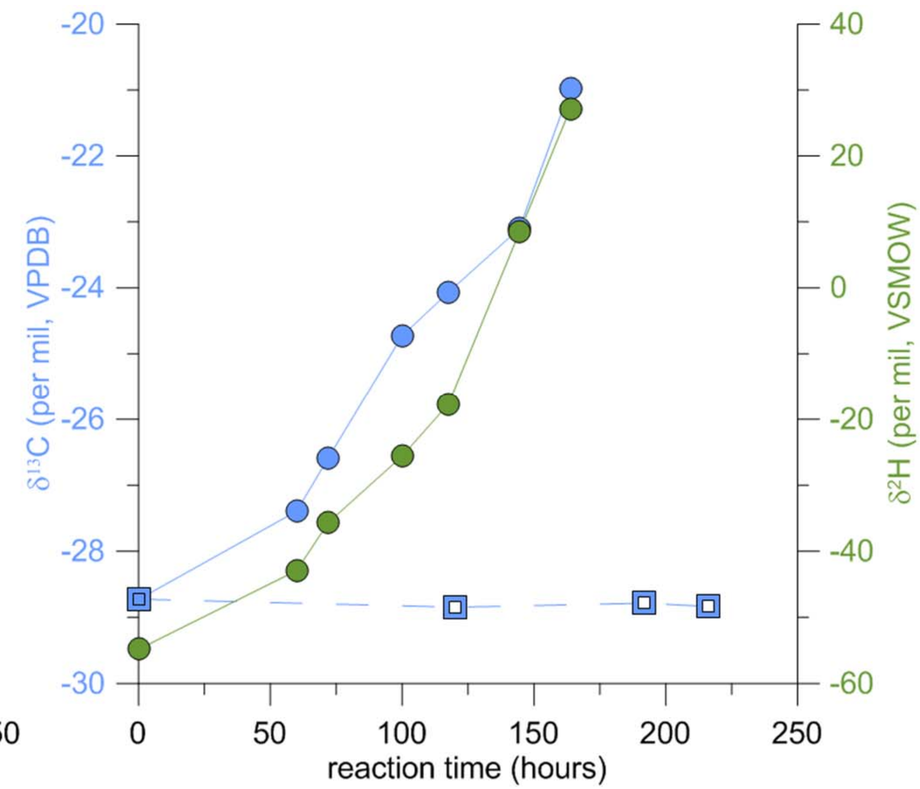
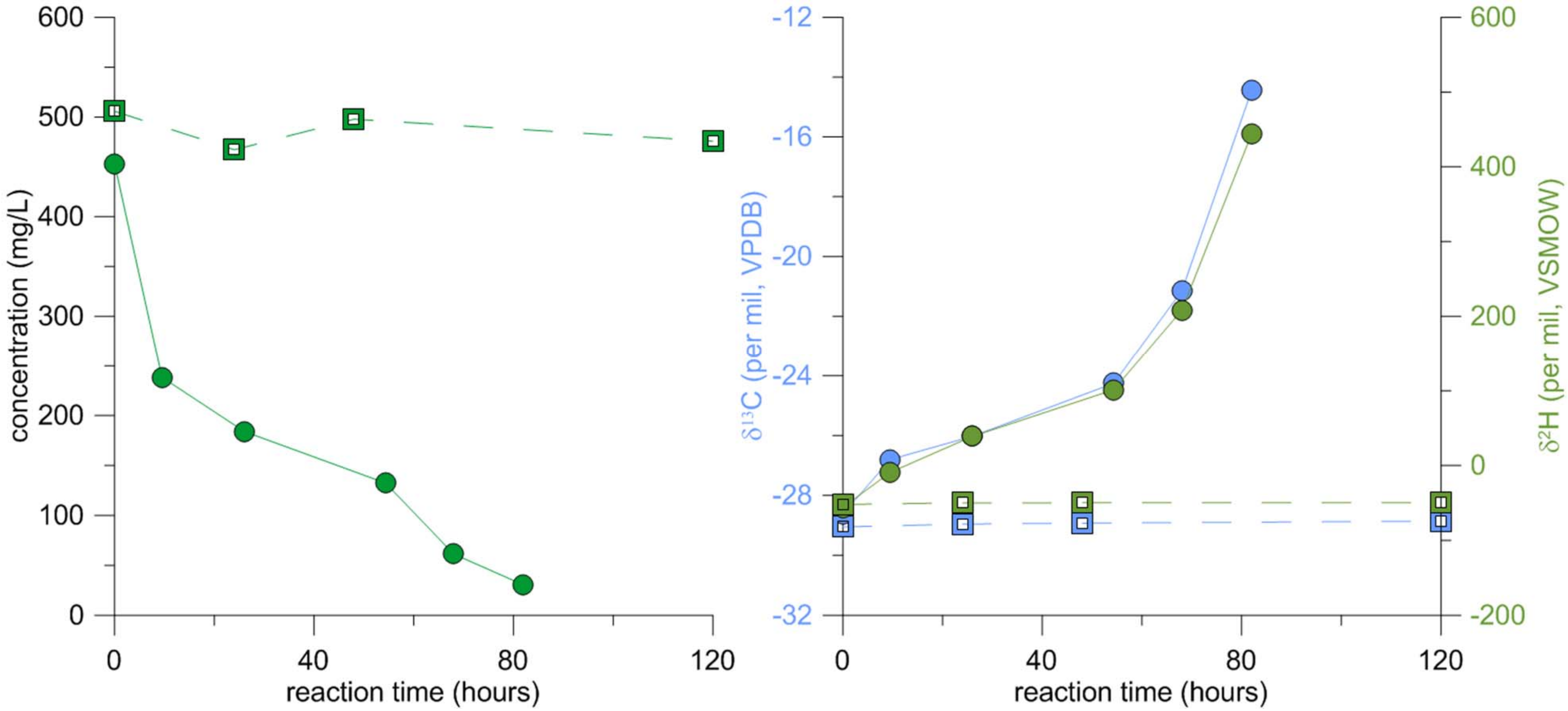
2A. Rhodococcous rhodochrous ATCC 21198: Isobutane





3A. Pseudonocardia tetrahydrofuranoxidans K1: THF



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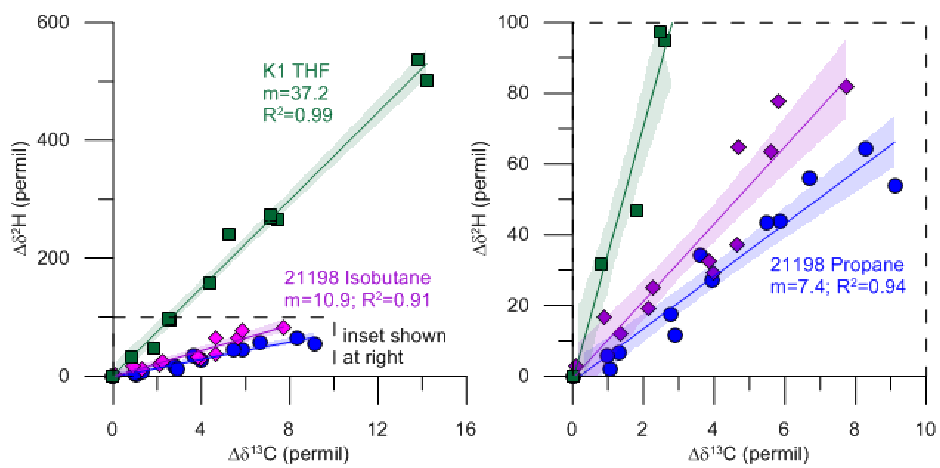
Enrichment Trends from Reactions with Pure Cultures

Dual-isotope plots show distinct slope for each reaction condition:



Enrichment factors (ε) are distinct for different reaction conditions:

strain	substrate	ε_C (‰)	ε_H (‰)
R. rhodochrous ATCC 21198	propane	-2.7±0.3	-21±2
	isobutane	-2.5±0.3	-28±6
P. tetrahydrofuran-oxidans K1	THF	-4.7±0.9	-147±22

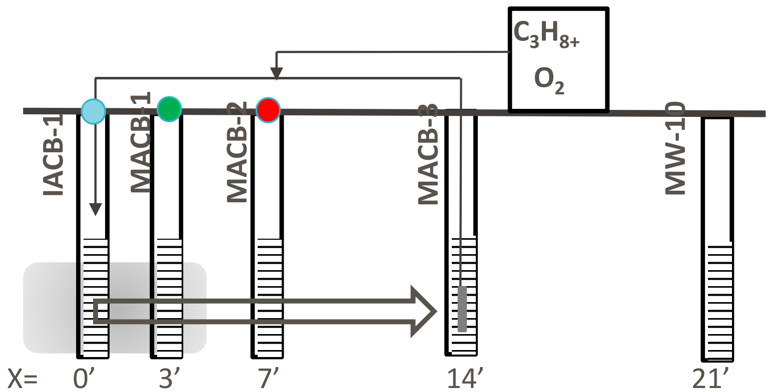
Practical implication: need to analyze carbon and hydrogen isotope ratios for demonstrating biodegradation of 1,4-dioxane.

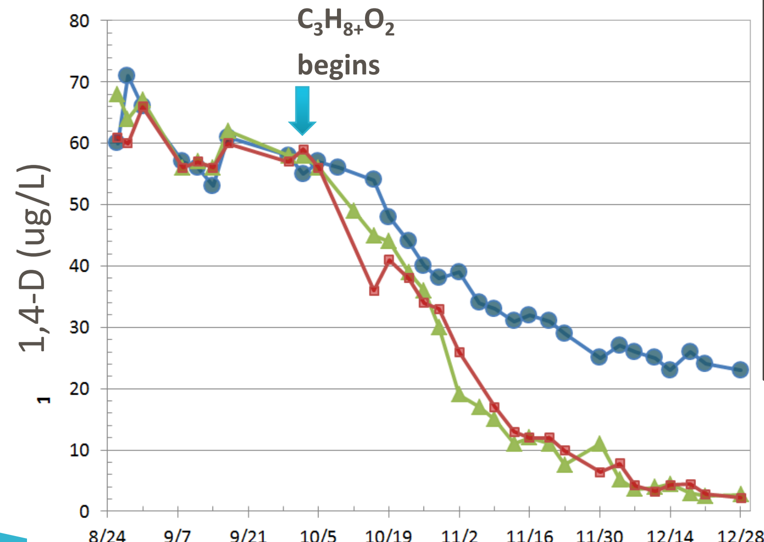
Bennett, P., Hyman, M., Smith, C., El Mugammar, H., Chu, M.-Y., Nickelsen, M., & Aravena, R. (2018). **Enrichment of carbon-13 and deuterium during monooxygenase-mediated biodegradation of 1,4-dioxane**. *Environmental Science & Technology Letters*, https://doi.org/10.1021/acs.estlett.7b00565

3. Assessing biodegradation of 1,4-dioxane in the field

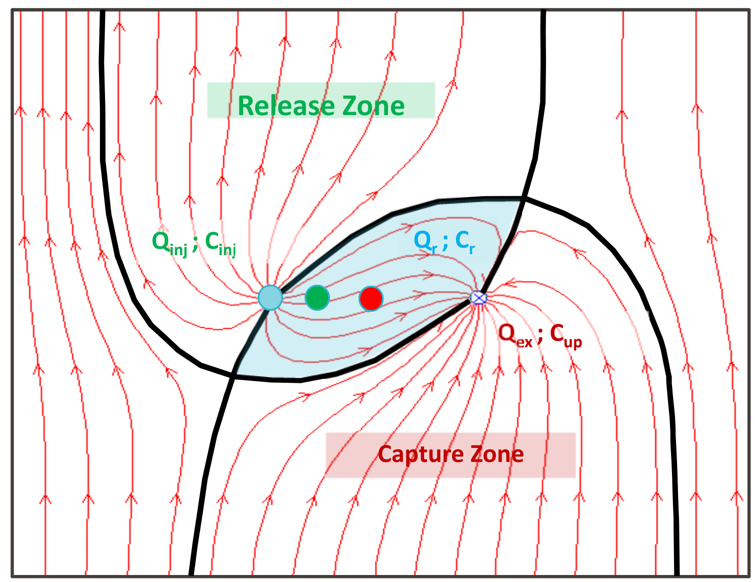
a. Pilot test of Aerobic Cometabolic Biodegradation
b. Survey of field sites

Aerobic Cometabolic Pilot – McClellan AFB





GW recirculation with propane and oxygen injection to create an underground ACB bio-reactor.



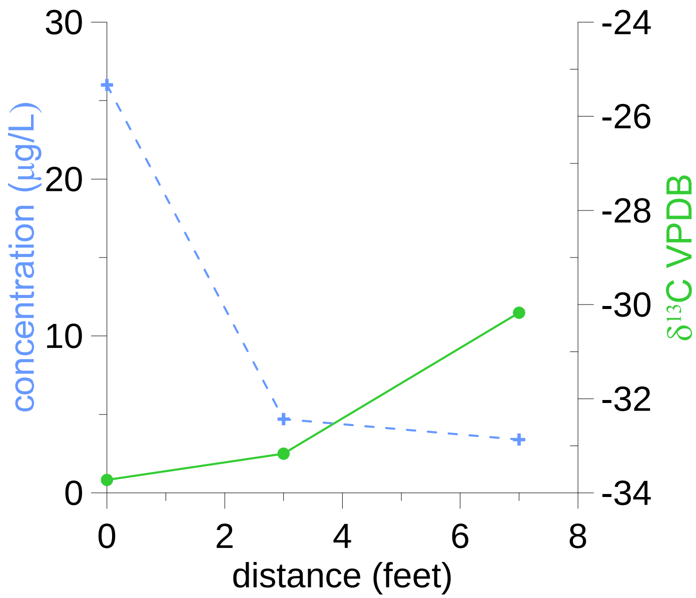
C_{up} = background 1,4-D concentration in GW

C_{inj} = 1,4-D concentration in injected GW

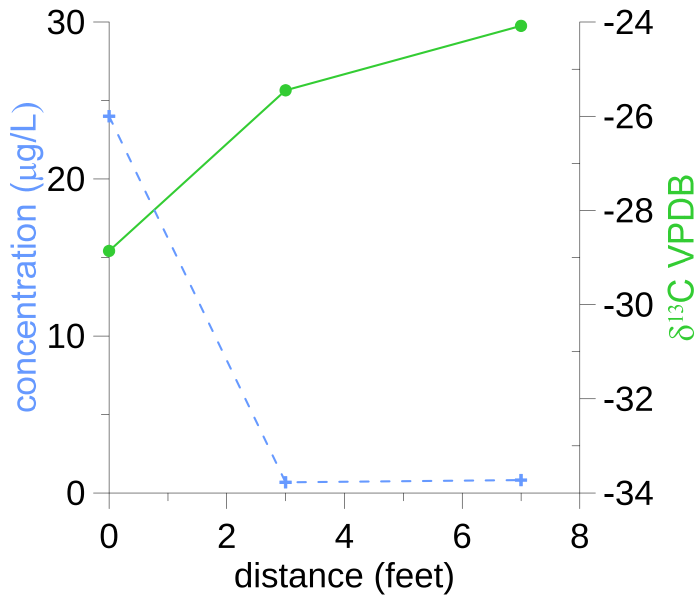
C_r = 1,4-D concentration at the end of recirculation zone

Enrichment in ^{13}C in samples from Treatment Zone

December 2015 Results

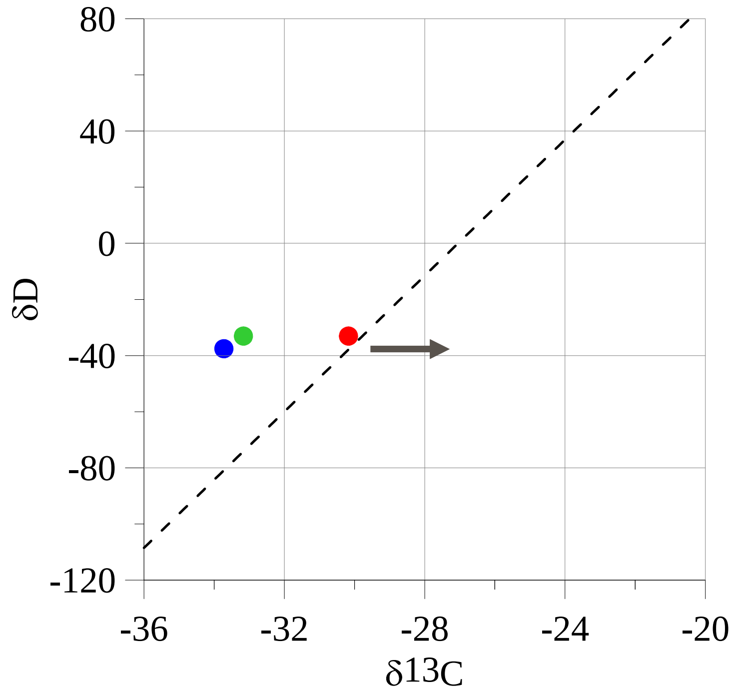


June 2016 Results

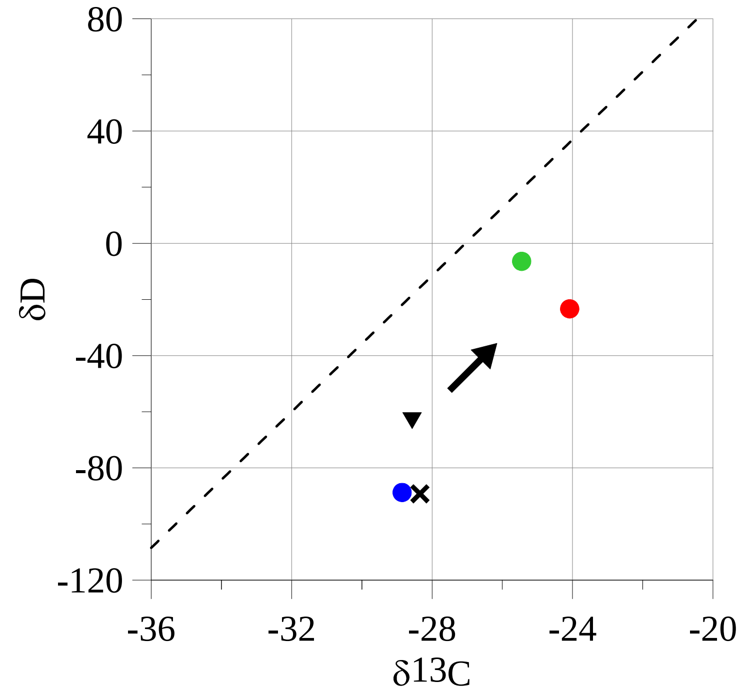


Dual Isotope Plots – Pilot Test Groundwater Samples

December 2015 Results

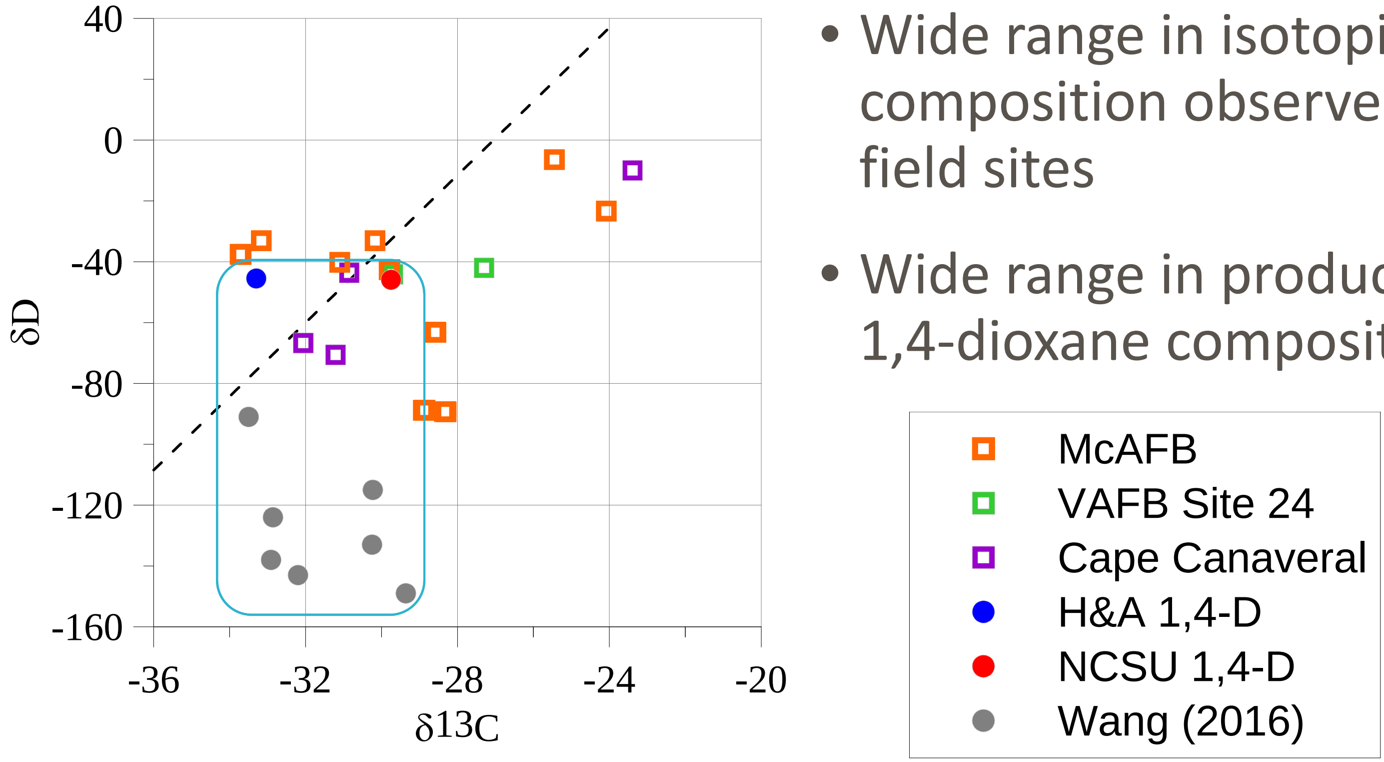


June 2016 Results



IACB-1 MACB-1 MACB-2 MACB-3 MW-10

Isotopic Composition of 1,4-Dioxane: Undegraded (Source) and Groundwater Samples



- Wide range in isotopic composition observed at field sites
- Wide range in product 1,4-dioxane composition

4. Summary and Conclusions

What we’ve learned
What we need to learn

What we've learned:

- Degradation of 1,4-dioxane on monooxygenase enzyme systems results in ^{13}C and ^{2}H enrichment trends that closely follow the Rayleigh model
- The degree of enrichment can vary with the type of monooxygenase
- For field implementation:
  - Need to analyze ^{13}C /^{12}C and ^{2}H/ ^{1}H and demonstrate enrichment in both isotopes
  - Analysis of monooxygenase biomarkers is valuable supporting information
  - Many samples (e.g. >10) are likely needed to demonstrate degradation at most sites

What we need to learn:

- The range in isotopic composition of 1,4-dioxane sources needs further characterization
- Other factors potentially affecting the isotopic composition of 1,4-dioxane require further study (e.g. evaporation, pH, etc.)
- For field implementation:
  - Simplified methods for low concentration CSIA (e.g. passive samplers downwell)
  - Whether natural degradation and isotopic enrichment occurs under anaerobic conditions

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- SERDP Grant ER-2535 (Bennett): CSIA method development
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