Stable carbon and hydrogen isotope ratios for assessing fate and transport of 1,4-dioxane

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Agenda

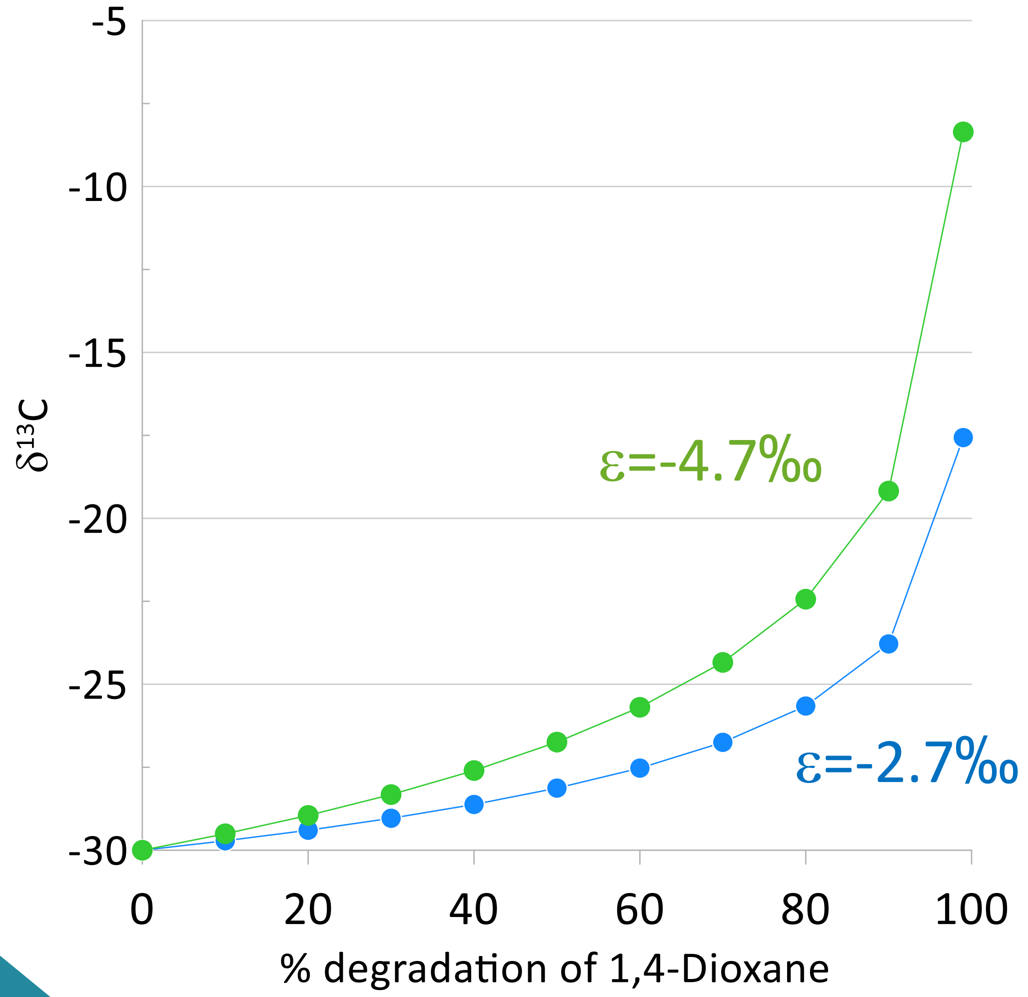
1. Background: Biodegradation and CSIA
2. Lessons learned: CSIA applied during bioremediation pilot test
3. Masking of isotopic enrichment at field sites
4. Example site
5. Conclusions

Pseudo-1st order degradation rates for 1,4-dioxane

Degradation process	half-life (d)	experimental conditions	reference:
aerobic cometabolic	0.45	Bio-stimulation pilot test using groundwater recirculation	Chu et al., 2018
	19.3 to 33	Bio-augmentation pilot test with propane sparging	Lippincott et al., 2015
natural attenuation	600	Median of 22 sites (Site-wide values)	Adamson et al., 2015
	1,500	Median of 131 wells (well-specific values)	

Chu, M. Y. J., Bennett, P. J., Dolan, M. E., Hyman, M. R., Peacock, A. D., Bodour, A., … Goltz, M. N. (2018). Concurrent Treatment of 1,4-Dioxane and Chlorinated Aliphatics in a Groundwater Recirculation System Via Aerobic Cometabolism. *Groundwater Monitoring and Remediation*, *38*(3), 53–64.
Lippincott, D., Streger, S. H., Schaefer, C. E., Hinkle, J., Stormo, J., & Steffan, R. J. (2015). Bioaugmentation and propane biosparging for in situ biodegradation of 1,4-dioxane. *Groundwater Monitoring and Remediation*, *35*(2), 81–92.
Adamson, D. T., Anderson, R. H., Mahendra, S., & Newell, C. J. (2015). Evidence of 1,4-dioxane attenuation at groundwater sites contaminated with chlorinated solvents and 1,4-dioxane. *Environmental Science and Technology*, *49*(11), 6510–6518. https://doi.org/10.1021/acs.est.5b00964

Rayleigh equation for estimating % degradation



- Simplified form: $\delta^{13}C_t = \delta^{13}C_o + \varepsilon \ln f$
  - $\delta^{13}C_t$ = isotope ratio in sample at time t
    - this is what we measure in well samples
  - $\delta^{13}C_o$ = isotope ratio at time t=0
    - this is the isotope ratio before biodegradation begins (source term)
  - ε is the "enrichment factor"
    - Degradation reactions in laboratory
  - f is the "fraction remaining"
    - $(1-f)$x100 = %degradation
- % degradation can be calculated if $\delta^{13}C_o$, ε , and $\delta^{13}C_t$ are know

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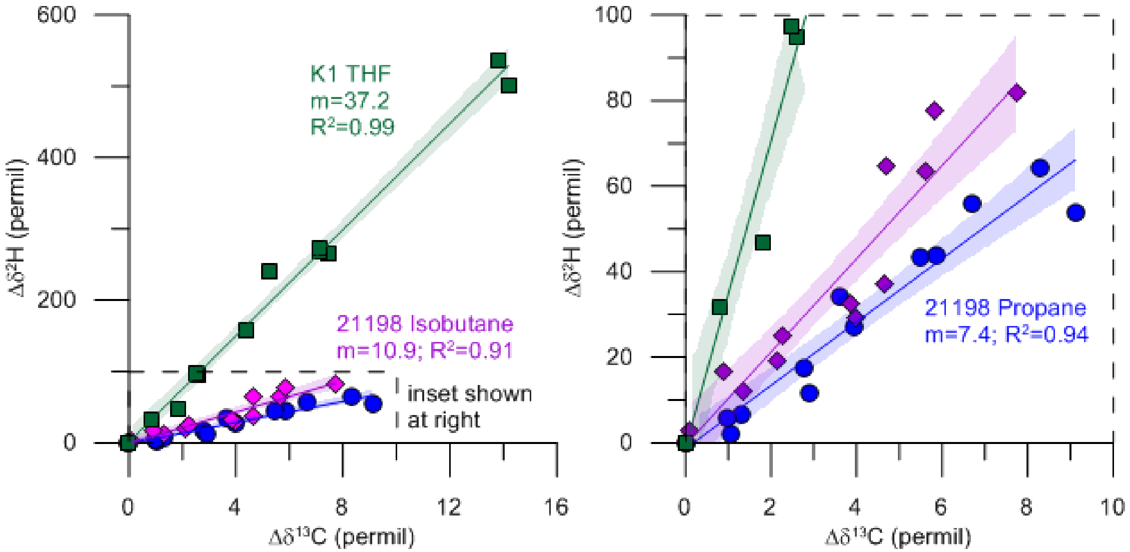
Enrichment trends from reactions with pure cultures

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

strain	substrate	ε_C (‰)	ε_H (‰)
*Mycobacterium 1A**	propane	-2.0	-26
*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

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



*Bennett, P. & Aravena, R. (2017). **Extending the application of compound-specific isotope analysis to low concentrations of 1,4-dioxane**. *SERDP ER-2535 Final Report*.

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*

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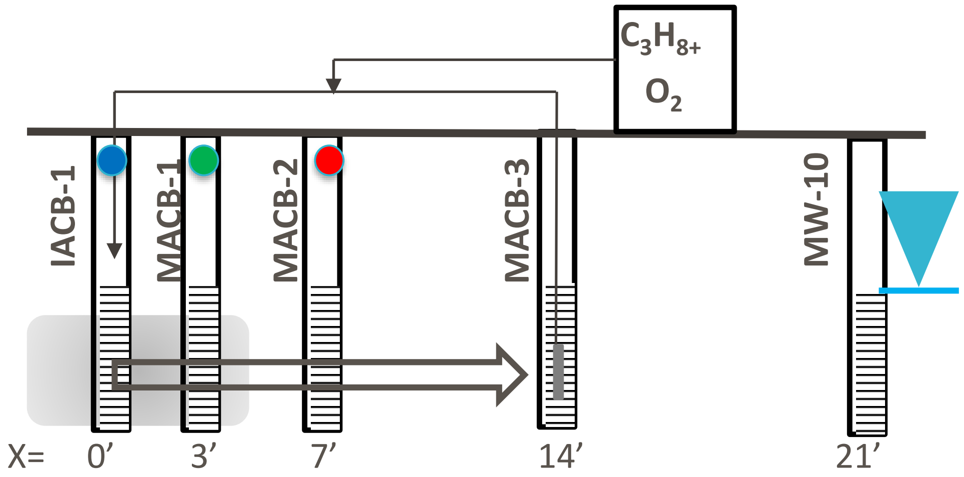
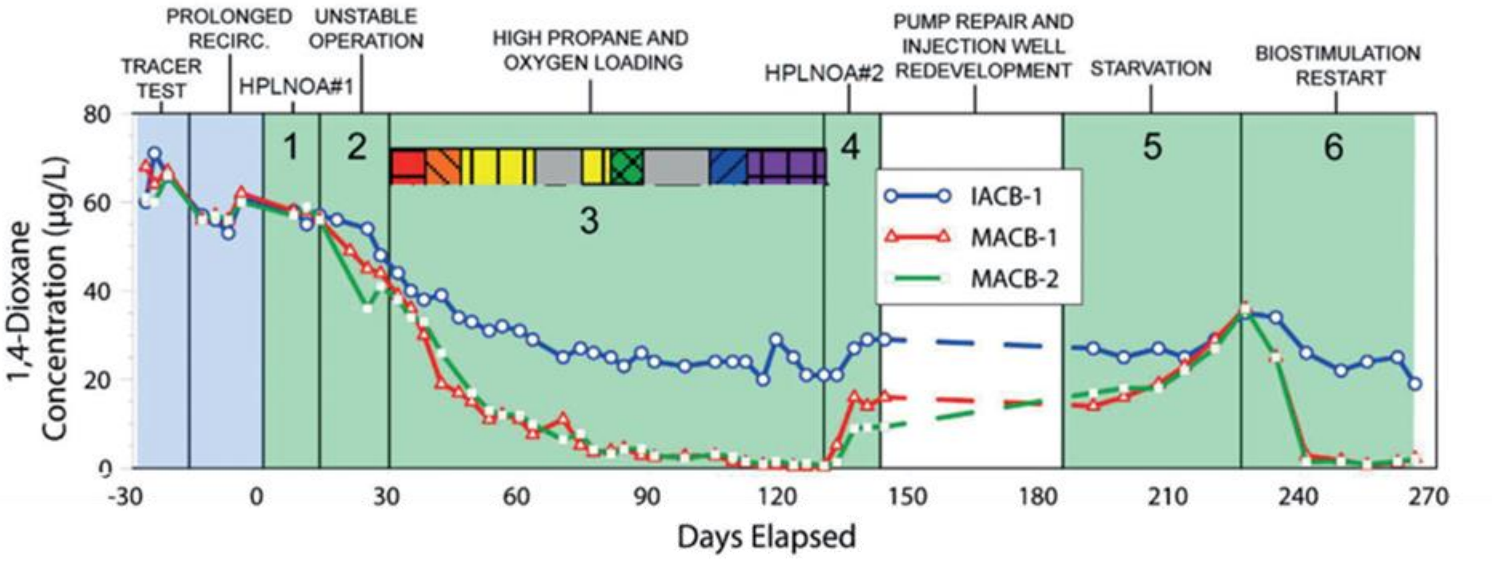
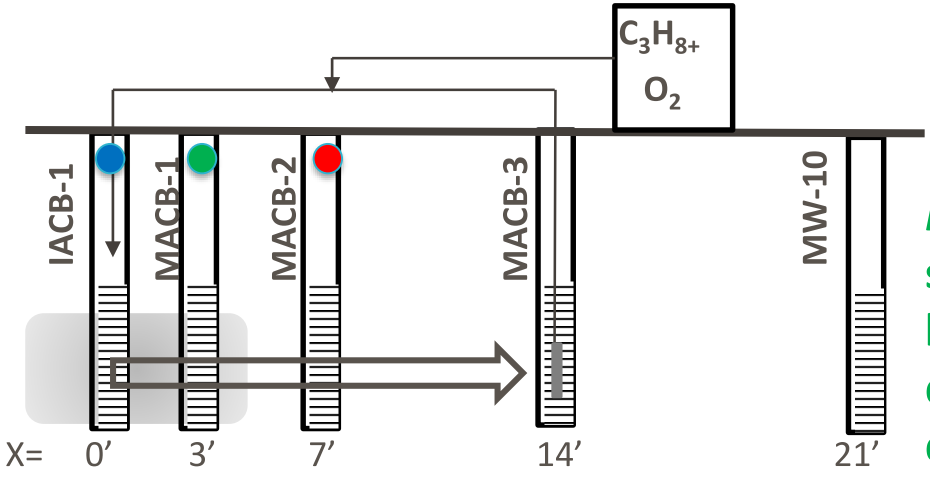
Bioremediation pilot test, McClellan AFB



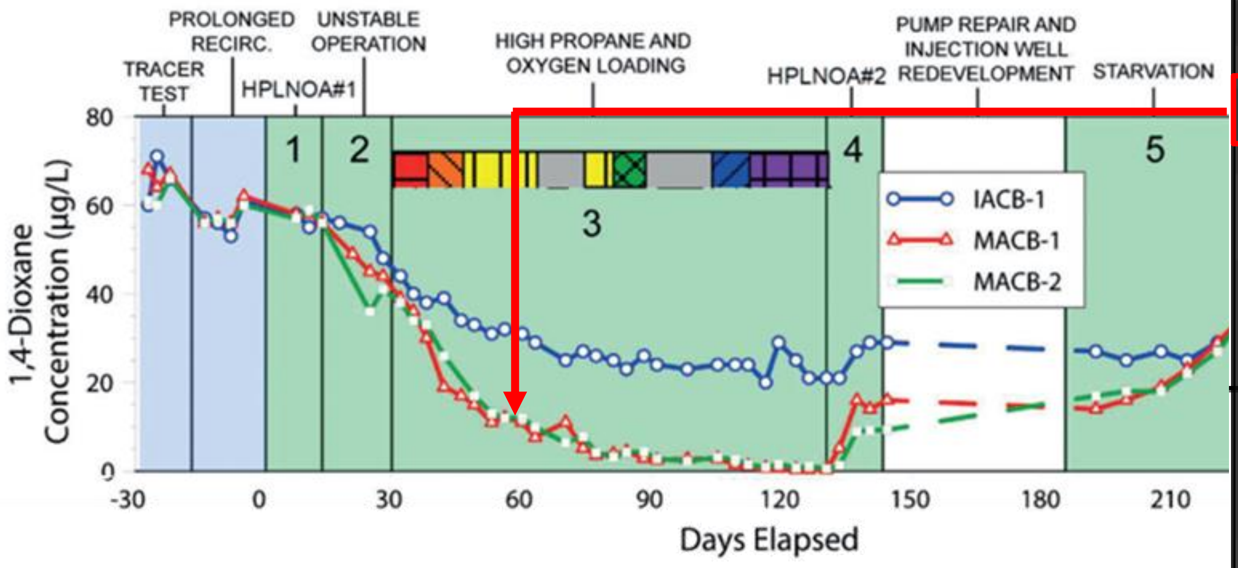
AFCEC-funded pilot test of GW recirculation with propane and oxygen injection to stimulate aerobic cometabolic biodegradation of 1,4-dioxane (Chu et al., 2018)



Growth of *Mycobacterium* due to propane injection

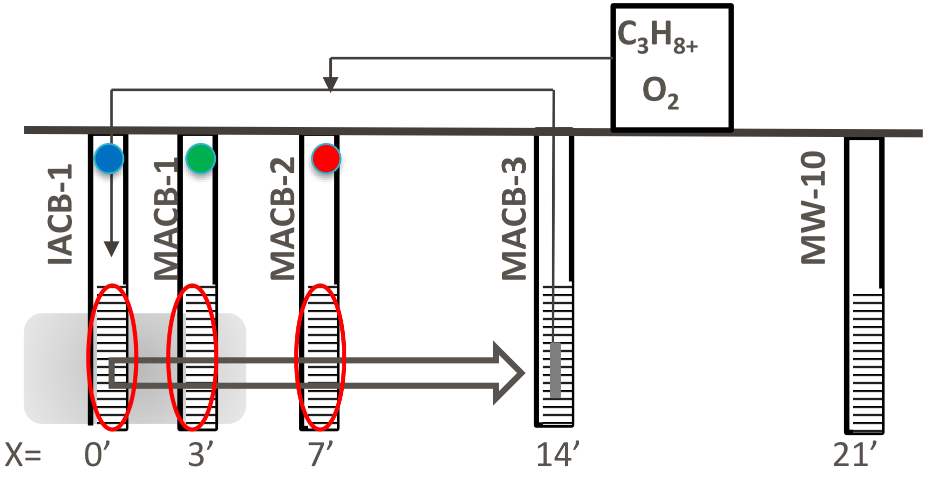


Mycobacterium strains are known to degrade 1,4-dioxane.

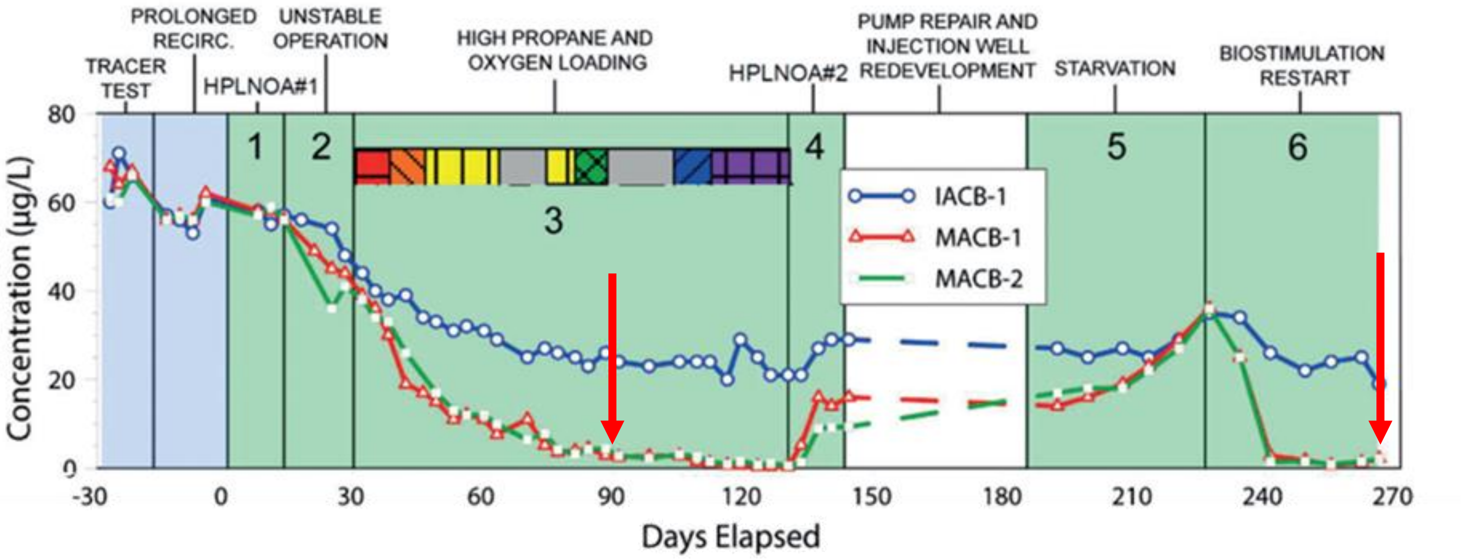


Sample from MACB-1 on 11/16/2015		
Species Level	**% of Profile**	**Respiration**
methyloversatilis universalis	11.61	Aerobic
pseudomonas spp.	9.81	Aerobic
methylibium petroleiphilum	9.21	Aerobic
sulfuritalea spp.	7.34	Facultative
massilia timonae	5.43	Aerobic
spongiibacter sp.	4.7	Aerobic
alkalibacter spp.	3.02	Anaerobic
hydrogenophaga spp.	2.49	
ideonella sp.	2.1	Aerobic
mycobacterium spp.	2.04	Aerobic
rhodocyclus tenuis	1.83	
alkalilimnicola spp.	1.62	Facultative
nitrosospira spp.	1.39	Aerobic
simplicispira sp.	1.24	
zoogloea oryzae	1.23	
herbaspirillum spp.	1.2	
pseudomonas veronii	1.19	Aerobic
ralstonia spp.	1.18	Variable
zoogloea resiniphila	1.14	Aerobic
comamonas spp.	0.93	Aerobic
pseudomonas sp.	0.88	Aerobic

CSIA on 1,4-dioxane during biodegradation



Samples collected for CSIA ($\delta^{13}C$ and $\delta^{2}H$ of 1,4-dioxane) on day 90 and day 270



Enrichment was smaller than anticipated at MACB-1

Day 90	IACB-1	MACB-1	MACB-2
1,4-D (µg/L)	26	3.6	4.2
residual 1,4-D (f)	1	0.14	0.16
$\delta^{13}C$ measured (‰)	-33.7	-33.2	-30.2
$\delta^{13}C$ expected (‰)		**-29.8**	**-30.0**
Day 270			
1,4-D (µg/L)	24	0.68	0.82
residual 1,4-D (f)	1.00	0.028	0.034
$\delta^{13}C$ measured (‰)	-28.9	-25.4	-24.1
$\delta^{13}C$ expected (‰)		**-21.8**	**-22.1**

Expected $\delta^{13}C$ values calculated from Rayleigh equation and **microcosm-based enrichment factor for *Mycobacterium* 1A: -2.0 ‰**

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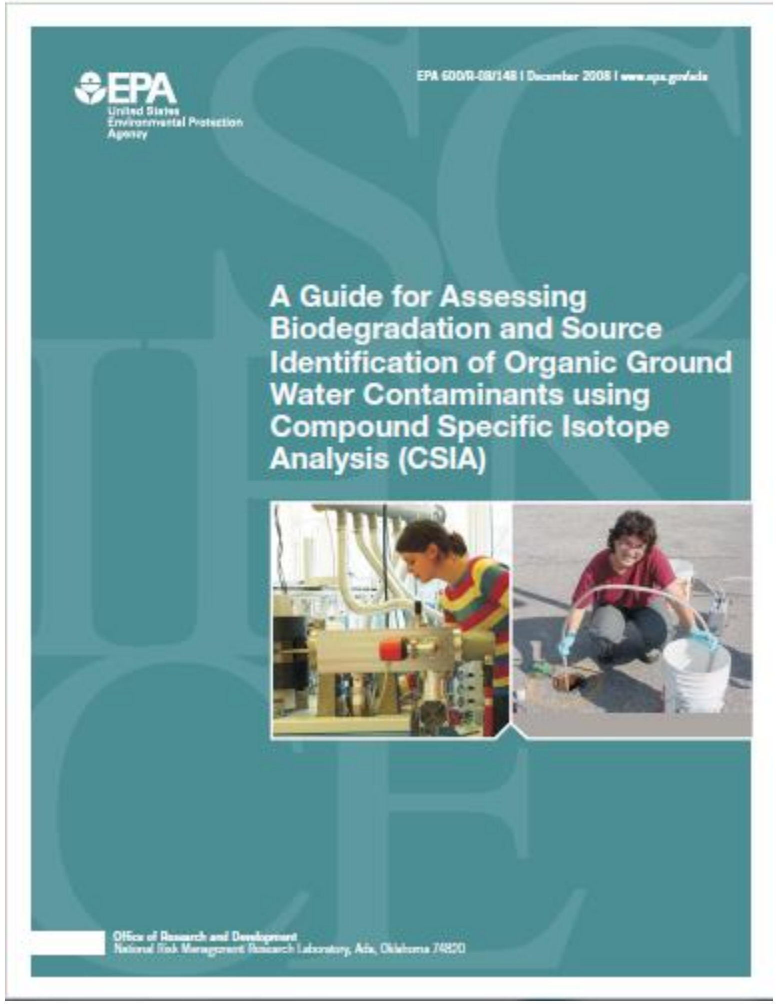
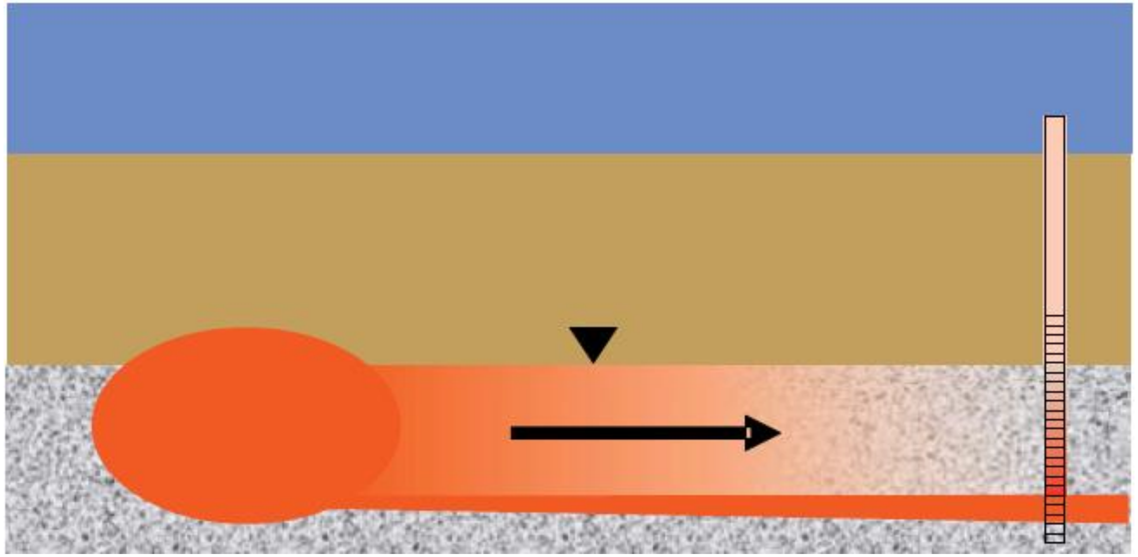
Masking of isotopic enrichment

- Can occur from:
  - Variations in isotopic composition of source material
  - Heterogeneity/well blending can mask isotope effects (Section 4.5 of EPA Guidance)→
- Some degradation pathways may have small isotopic enrichment
- The potential for "false negatives" from CSIA is an important consideration for assessing the fate of 1,4-dioxane in groundwater

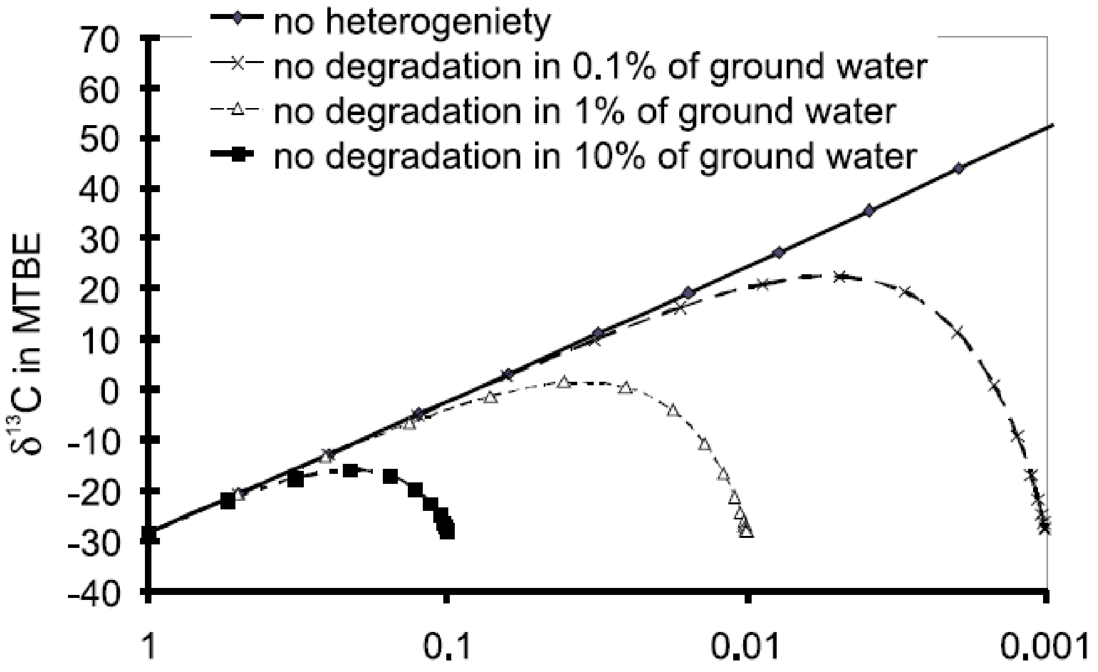


How heterogeneity can mask enrichment (EPA, 2008)

Hypothetical scenario: degradation in shallow plume



Depletion in heavy isotope with increased degradation can occur:

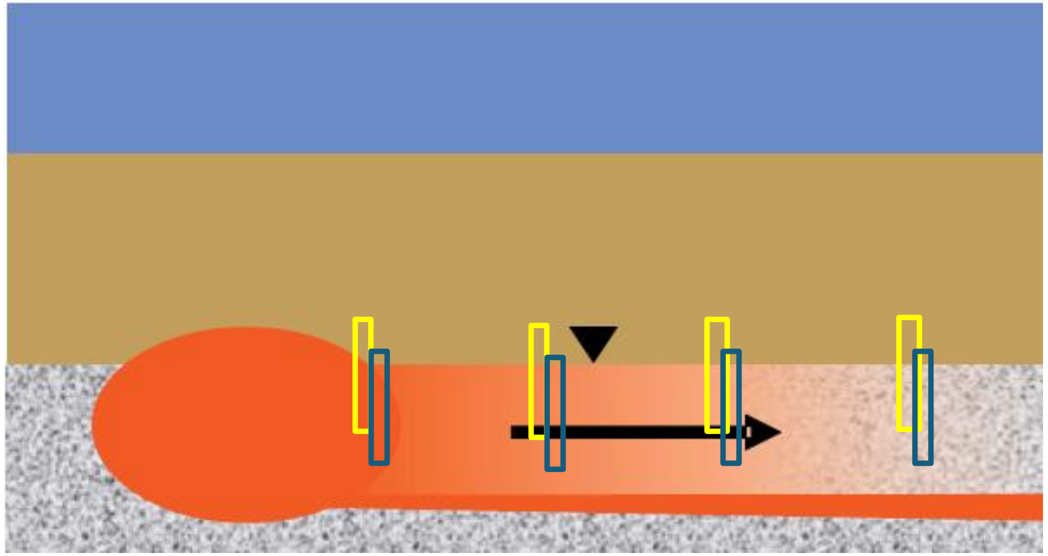


Simulations of heterogeneity

Hypothetical scenarios:

1: no heterogeneity (yellow wells)

2: heterogeneity (blue wells)



Modeling Method (BIOCHLOR-ISO)

- Scenario 1 (degradation only)
  - model degradation using published values for ε_C & ε_H
- Scenario 2: (degradation + heterogeneity)
  - assume no degradation for the "bottom" of the plume
  - Use mixing equations and Scenario 1 output to calculate CSIA results at each well for Scenario 2

Smaller enrichment factors – Scenario 1

GW Velocity = 1 ft/d

Half Life = 1.7 yr

Log(Koc) = 1.24

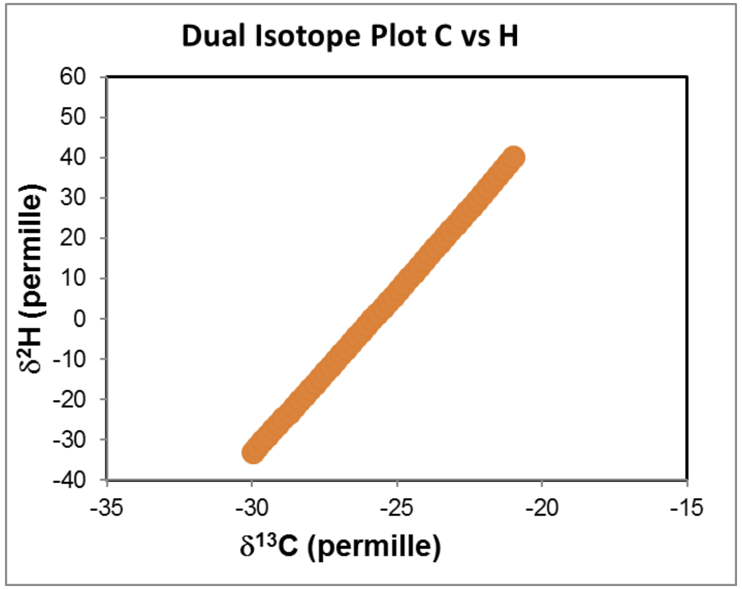
R = 1.07

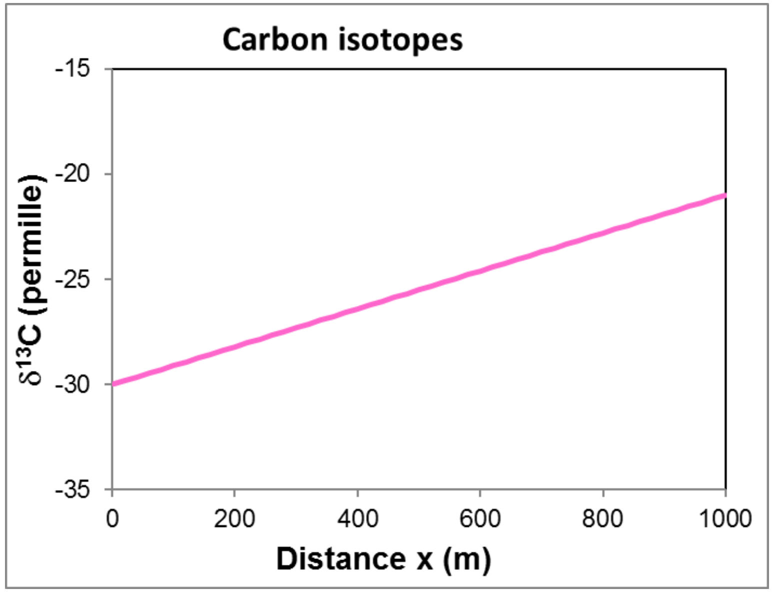
Initial $\delta^{13}C$ = -30‰

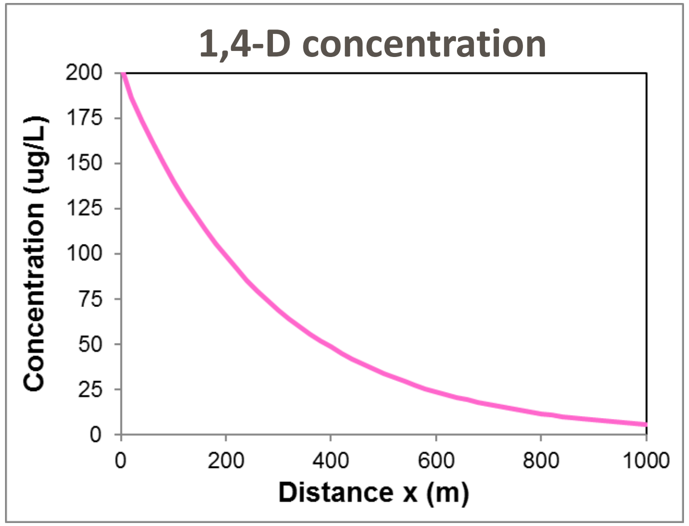
Initial $\delta^{2}H$ = -33.1‰

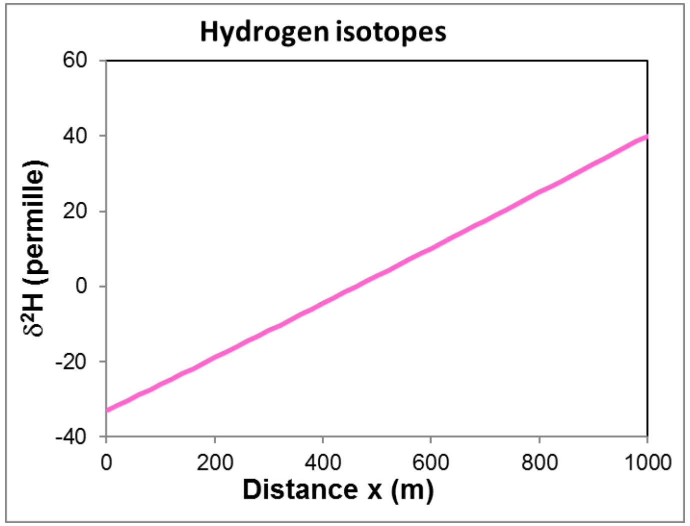
^{13}C enrichment factor = -2.7

^{2}H enrichment factor = -21









Smaller enrichment factors – Scenario 2

GW Velocity = 1 ft/d

Half Life = 1.7 yr

Log(Koc) = 1.24

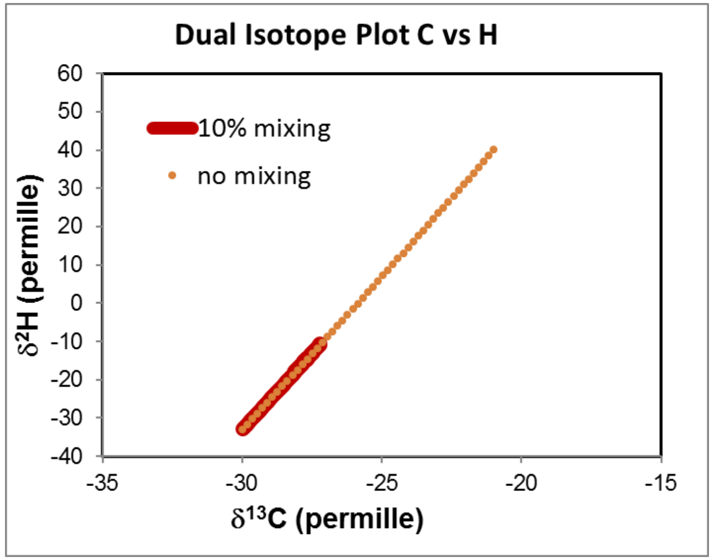
R = 1.07

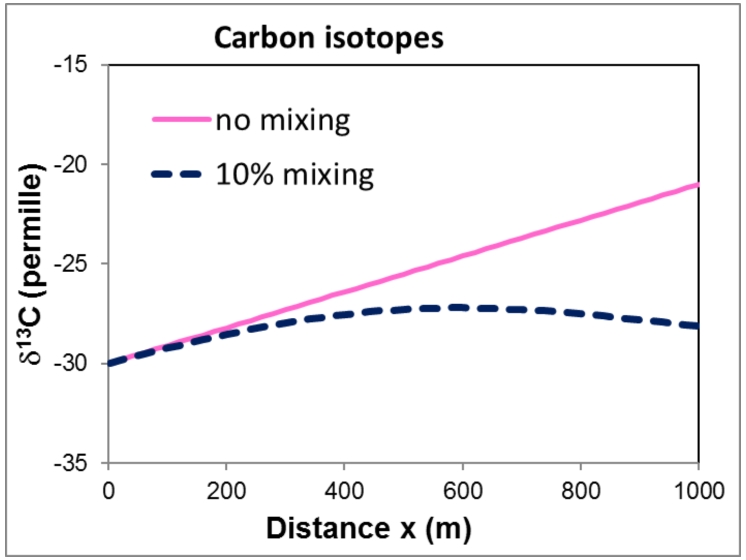
Initial $\delta^{13}C$ = -30‰

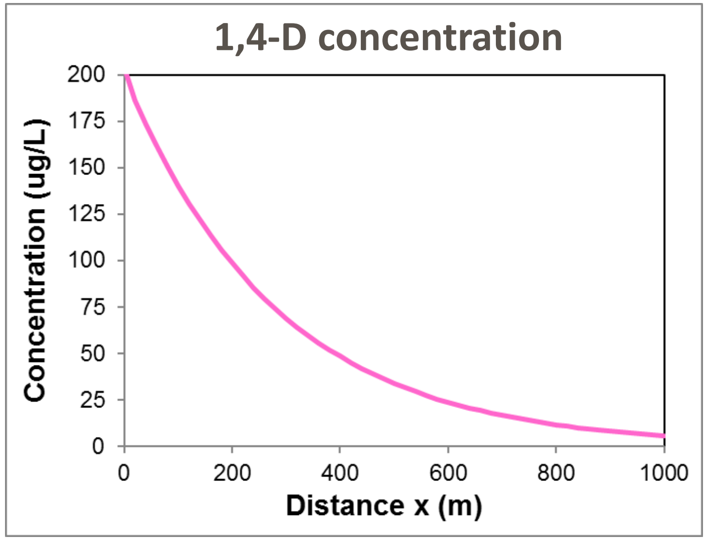
Initial $\delta^{2}H$ = -33.1‰

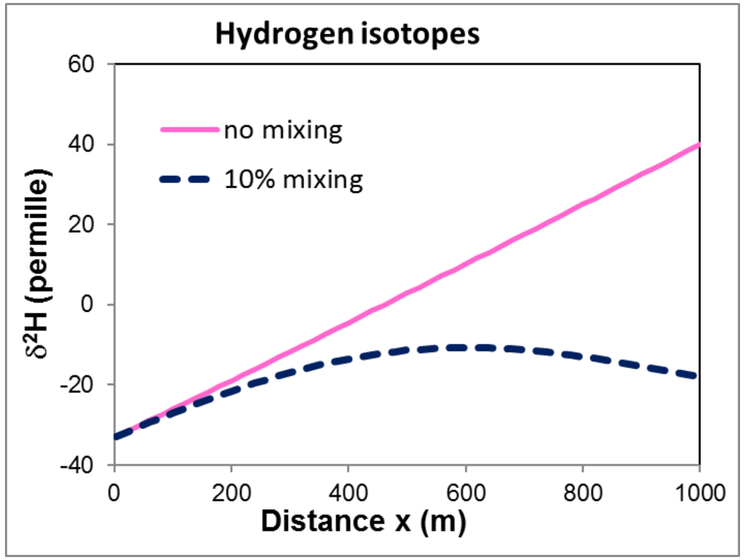
^{13}C enrichment factor = -2.7

^{2}H enrichment factor = -21









Larger enrichment factors – Scenario 2

GW Velocity = 1 ft/d

Half Life = 1.7 yr

Log(Koc) = 1.24

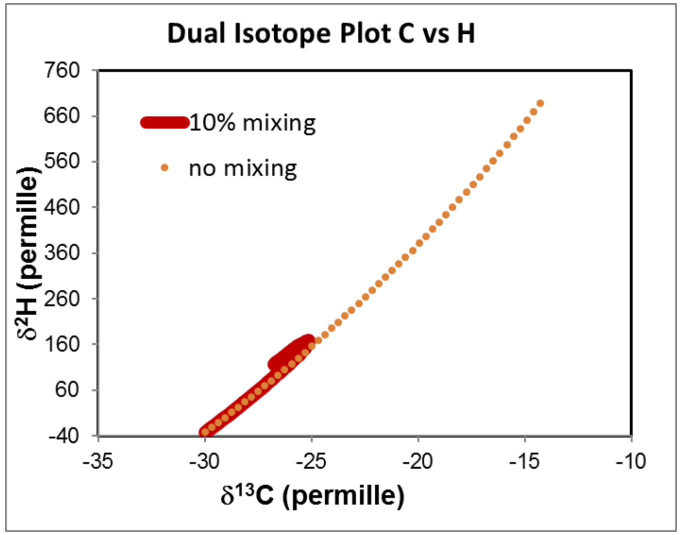
R = 1.07

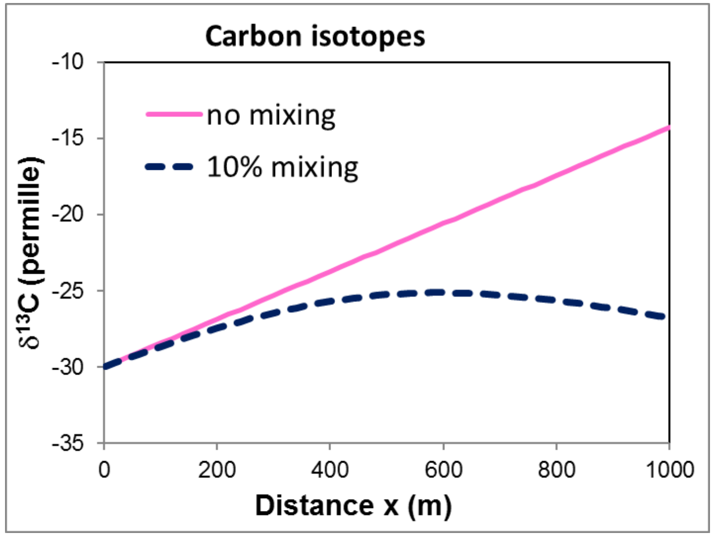
Initial $\delta^{13}C$ = -30‰

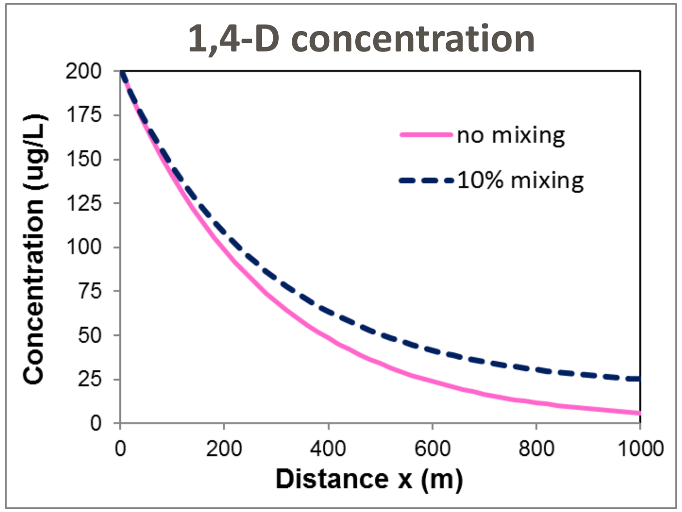
Initial $\delta^{2}H$ = -33.1‰

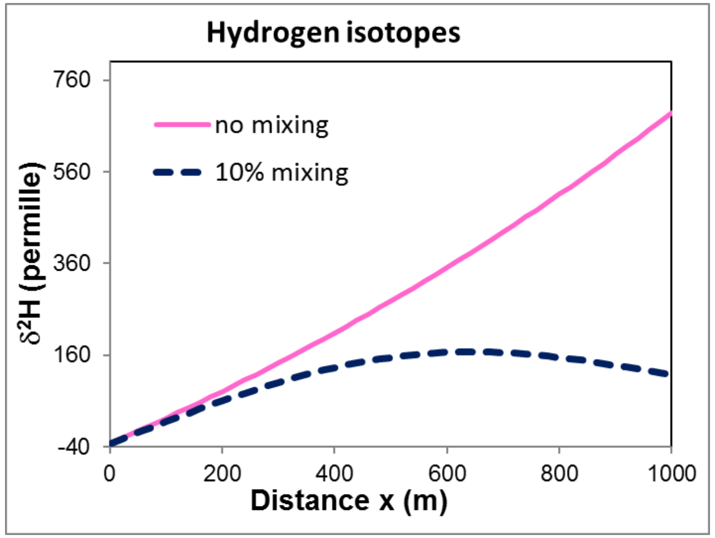
^{13}C enrichment factor = -4.7

^{2}H enrichment factor = -147









Implications for fate and transport assessments

- At sites where 1,4-dioxane degradation is occurring, it may be difficult to observe isotopic enrichment
- Quantitative estimates of degradation based on CSIA are likely to be underestimates in most cases
- Dual isotope trends are expected to be an important line of evidence for degradation of 1,4-dioxane
- Likelihood of successful CSIA applications increase with:
  - High resolution sampling
  - Knowledge of spatial and temporal redox conditions
  - Other supporting lines of evidence (advanced microbial tools, etc.)

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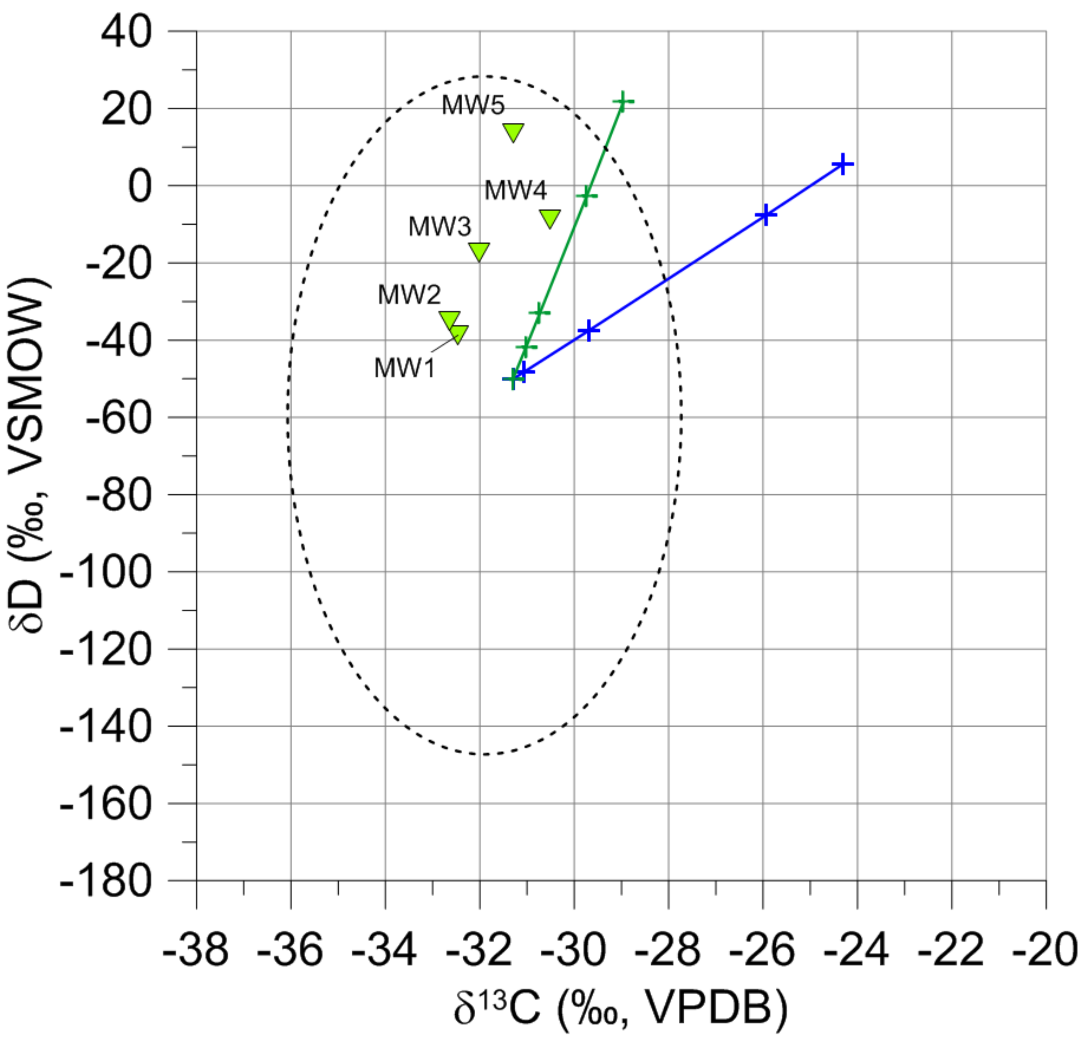
Example site

- Rayleigh degradation curves:
  - THF-grown culture
  - Propane-grown culture

Well	1,4-D (µg/L)
MW1	970
MW2	86
MW3	130
MW4	14
MW5	1.4 J

Bennett et. al., 2018. Enrichment with Carbon-13 and Deuterium during Monooxygenase-Mediated Biodegradation of 1,4-Dioxane. Environmental Science & Technology Letters 5(3): 148-153



Conclusions

- Dual isotope plot is critical for applying CSIA toward:
  - performance monitoring of remediation systems,
  - MNA assessments
  - fate and transport evaluations
- While CSIA is a powerful line of evidence for degradation, it may be difficult to quantify degradation rates based on CSIA evidence alone
- Absence of isotopic enrichment should not be used to infer absence of degradation.

Acknowledgements

- SERDP Grant ER-2535 (Bennett): CSIA method development
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- Dr. Hunter Anderson at AFCEC
- Dr. Adria Bodour at AFCEC
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