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Use of ^{14}C Assays to Determine Rate Constants for Degradation of Chlorinated Ethenes and 1,4-Dioxane

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Webinar for tersus environmental

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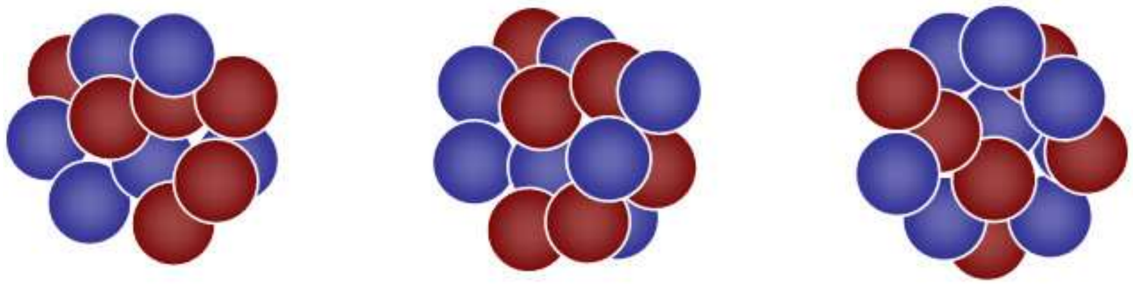
Background – ^{14}C

- **Isotopes of carbon: 12, 13, 14**
- **Focus today is on the radioactive isotope, carbon-14**
- **Half life is 5730 years**
- **Emits *β* energy during decay**



carbon-12	carbon-13	carbon-14
98.9%	1.1%	<0.1%
6 protons	6 protons	6 protons
6 neutrons	7 neutrons	8 neutrons
	Measured by compound specific isotope analysis (CSIA)	**Measured by photons emitted from scintillators excited by *β* energy from ^{14}C decay**

^{14}C has been around

- Use of ***radiocarbon dating*** extends back to 1946: Developed by Willard Libby at the University of Chicago

- Use of ^{14}C-labeled substrates to determine ***degradation pathways*** and measure ***degradation rates*** has been around for at least 4 decades
  - Lignin Biodegradation Importance and Historical Research Perspective: T. KentKirk, 1976
  - Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene to Ethylene under Methanogenic Conditions: D. L. Freedman and J. M. Gossett, 1989
- Biodegradation studies performed with numerous other ^{14}C-labeled compounds: chlorinated methanes, fuel hydrocarbons, PAHs, munitions, MTBE, . .

Current Applications

- ^{14}C assays can be an effective tool for determining rate constants when the degradation products are difficult to discern from other sources, e.g., CO_2, CH_4, and organic acids
- Lab determined rate constants can be helpful for validating rate estimates from concentration versus distance data
- Other tools that provide lines of evidence for degradation include CSIA and biomarkers, but those are not as easily convertible to rate constants
- Bottom line: ^{14}C assays can provide estimates of rates constants for biotic and abiotic degradation based on product formation

A Few Basics

- Work must be done in a lab licensed to handle radioactive materials
- A limited vendors provide ^{14}C-labeled material; custom synthesis $$$
- ^{14}C compounds usually delivered in a solvent (e.g., acetonitrile, butanol) making purification essential
- Prepare a stock solution (e.g., in buffered DDI water)
- Methods are needed for separating the parent compound from ^{14}C degradation products
- Follow ^{14}C distribution by counting samples in liquid scintillation cocktail using a liquid scintillation counter
- Use of controls to account for background activity is essential

Example Applications

Current applications of ^{14}C assays of interest to the remediation community that will be covered today:

- Aerobic co-oxidation of TCE
- Degradation rates for TCE and cDCE in crushed rock or soil microcosms
  - Ambient conditions
  - Improvement in rates with gentle heating
- Degradation rates for TCE to assess back diffusion from rock
- Aerobic biodegradation of 1,4-dioxane

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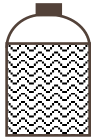
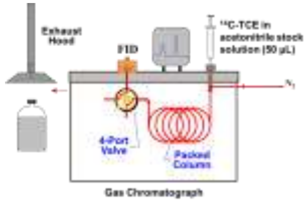
^{14}C Assay for Co-oxidation of TCE



Collect GW in 160 mL serum bottles

Ship to lab; warm overnight; measure VOCs



Add GC-purified ^{14}C-TCE

Analyze 3 mL samples for ^{14}C using LSC (weekly)

Calculate 1st order rate

Controls:

- Filter-sterilized groundwater (FSGW)

Total ^{14}C:

- Initial total ^{14}C is critical

Total ^{14}C products:

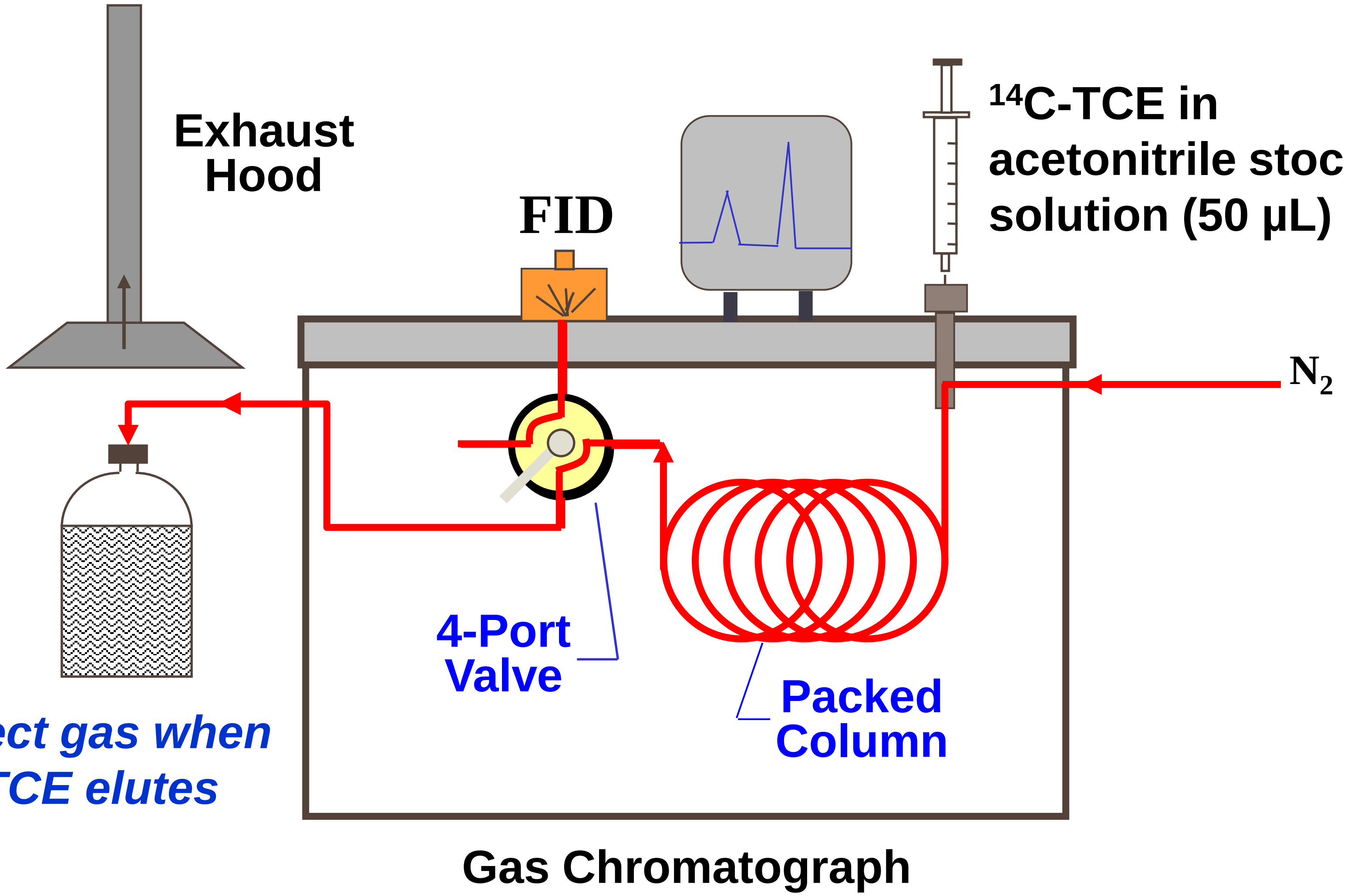
- Remove 3 mL aqueous samples, weekly
- Raise pH>10 (NaOH) to retain $^{14}CO_2$
- Sparge samples for 30 min with N_2 to remove TCE

End-of-incubation products:

- Confirm $^{14}CO_2$ by ppt with $Ba(OH)_2$
- Determine percent $^{14}CO_2$

^{14}C Assay for Co-oxidation of TCE

Purification of ^{14}C-TCE



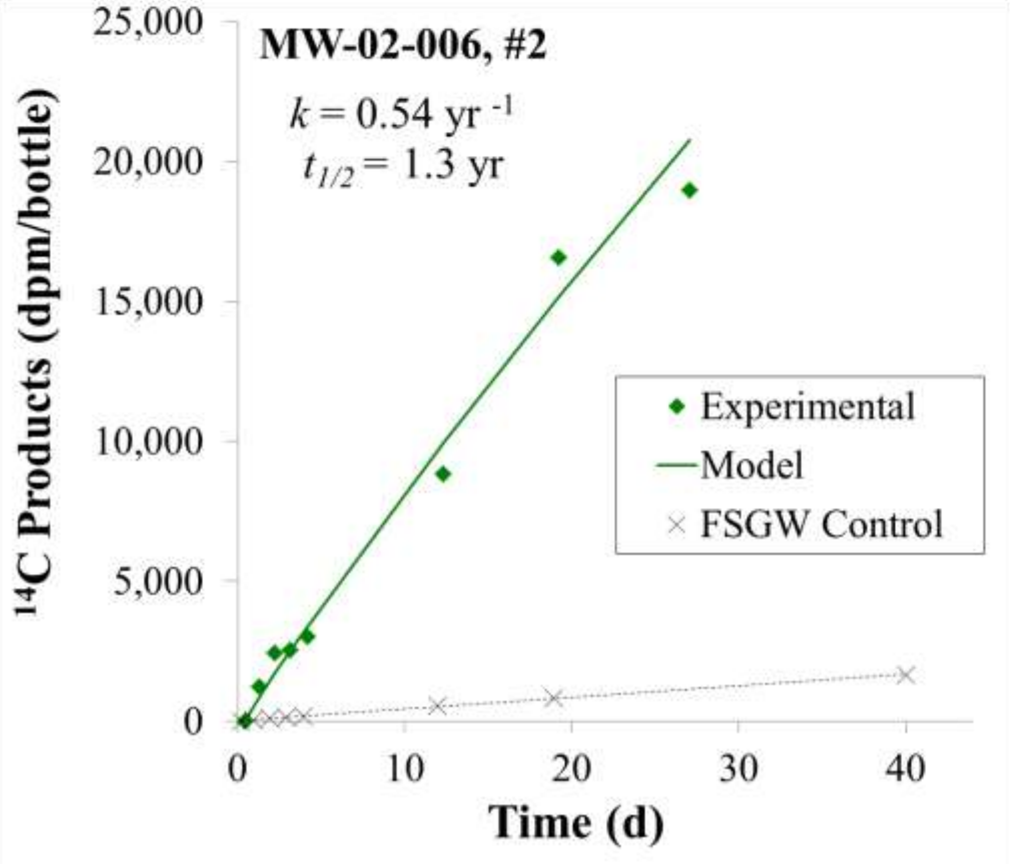
Inject gas when TCE elutes

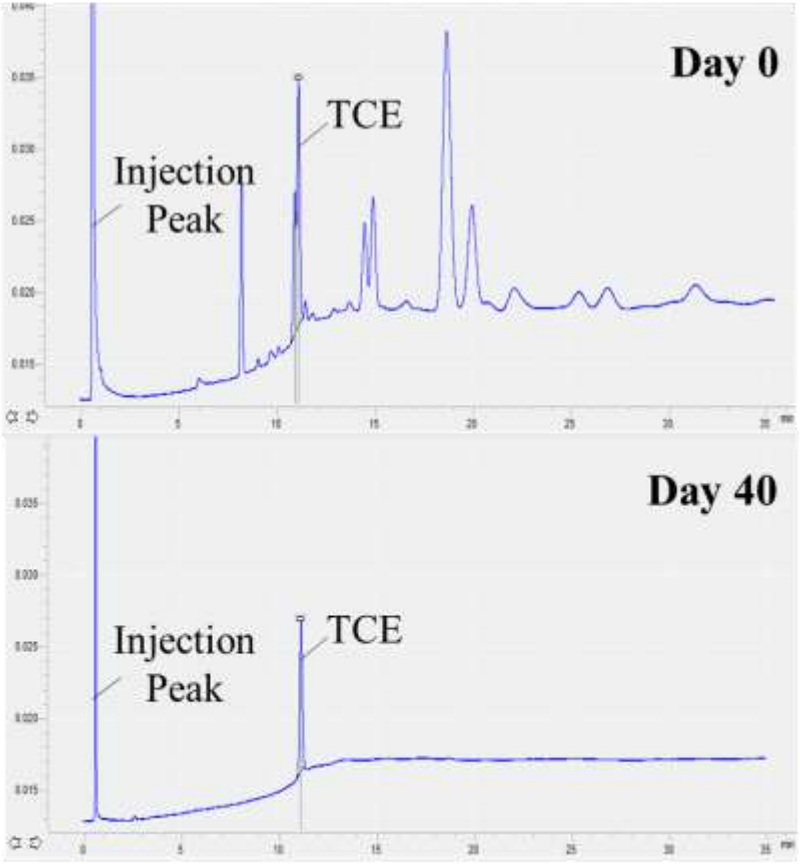
^{14}C Assay for Co-oxidation of TCE

Sites Selected for GW Sampling

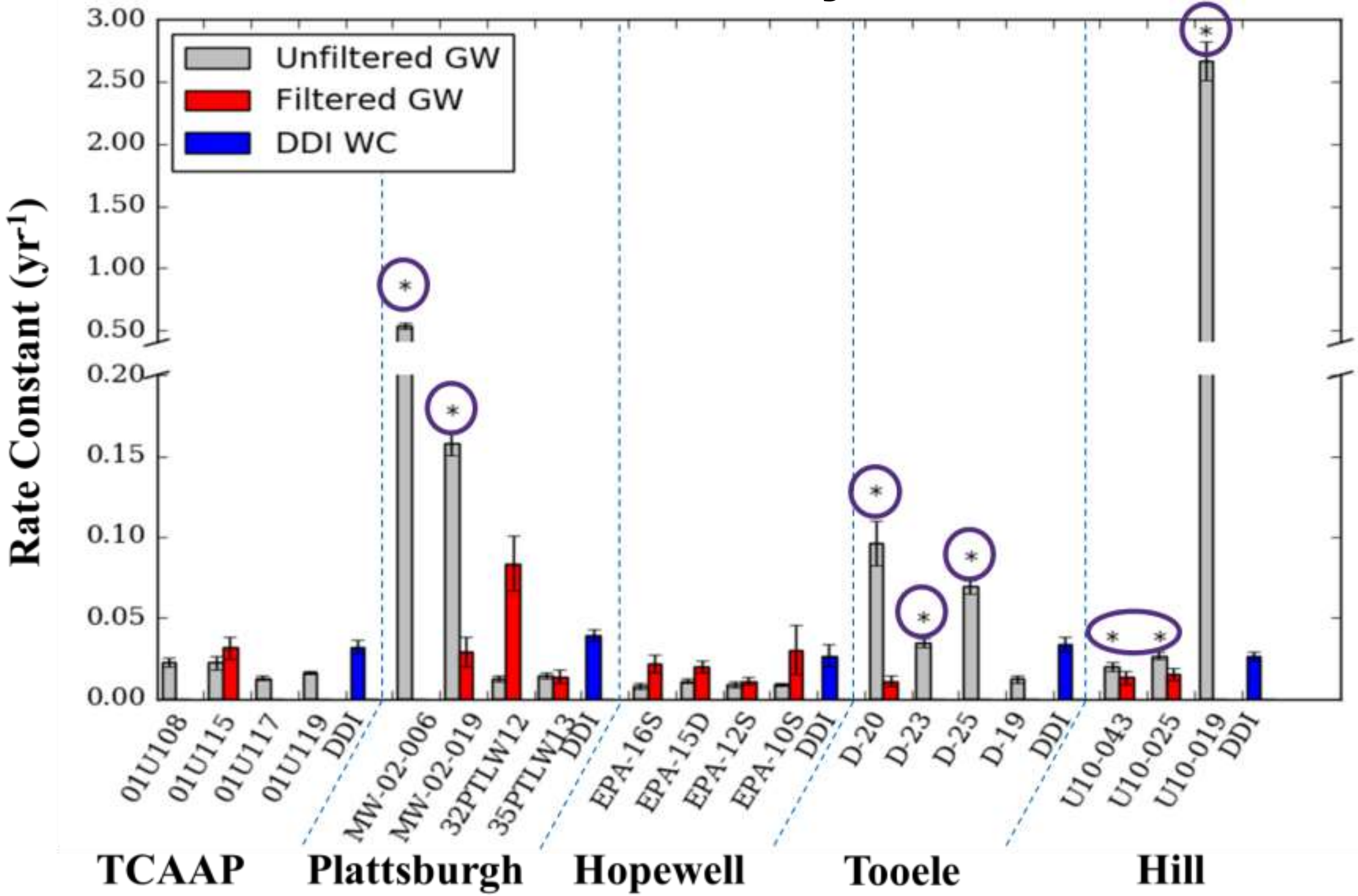
^{14}C Assay for Co-oxidation of TCE

Results: Plattsburgh AFB, NY





^{14}C Assay for Co-oxidation of TCE



^{14}C Assay for Co-oxidation of TCE Summary

- ^{14}C assay provides quantitative evidence for aerobic TCE co-oxidation; provides rate constants (with confidence intervals) that can be used as a line of evidence to assess MNA
- Capable of predicting 1st order rate constants for TCE degradation as low as 0.0066 yr^{-1} = half-life up to 105 yr
- ^{14}C product distribution was 37-97% $^{14}CO_2$ with remainder as soluble and non-volatile products
- Results are published in:

  Mills, J. C.; Wilson, J. T.; Wilson, B. H.; Wiedemeier, T. H.; Freedman, D. L., Quantification of TCE co-oxidation in groundwater using a ^{14}C–assay. *Groundwater Monitoring & Remediation,* **2018,** *38* (2), 57-67*.*
- Rates correlate well to rate estimates based on qPCR data for monooxygenases

  Wilson, J. T.; Mills, J. C.; Wilson, B. H.; Ferrey, M. L.; Freedman, D. L., Taggart, D. Using qPCR assays to predict rates of cometabolism of TCE in aerobic groundwater. *Groundwater Monitoring & Remediation*, 2019, 39 (2), 53-63.

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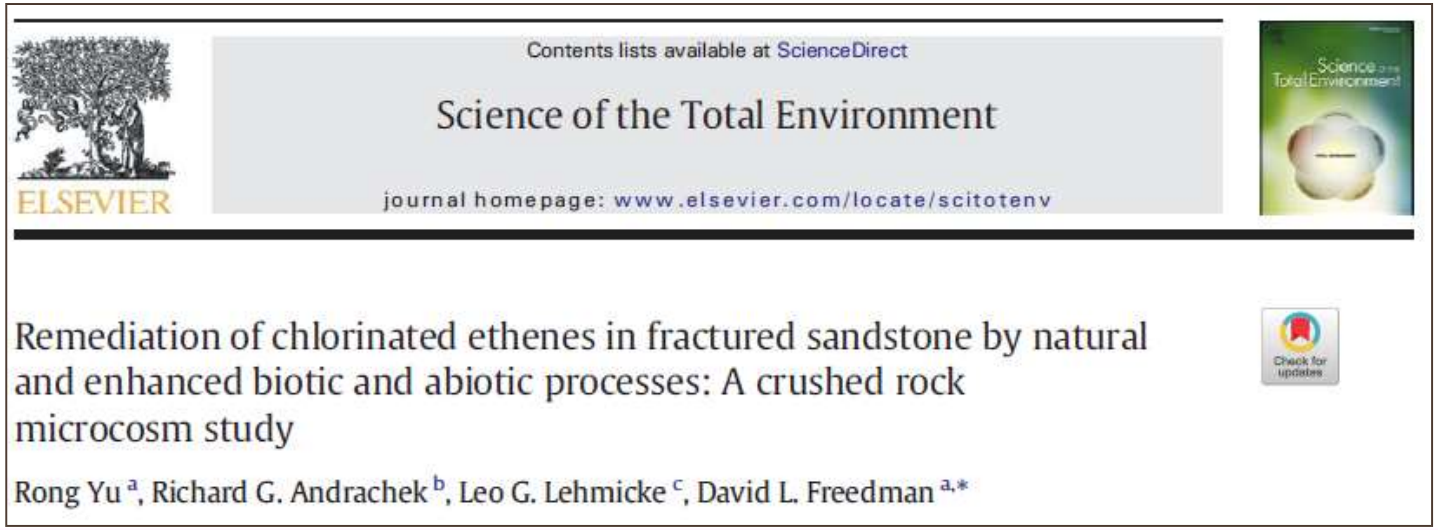
^{14}C assay for degradation of cDCE and TCE

- Crushed rock from fractured sandstone site
- Added ~20 g crushed rock + 50 mL GW
- Experimental design
  - ✓ 11 treatments
  - ✓ 12 bottles per treatment; triplicates sacrificed at 4 time intervals
  - ✓ One set received ^{14}C-TCE, another ^{14}C-cDCE
- Prepared in anaerobic chamber
- Full results in →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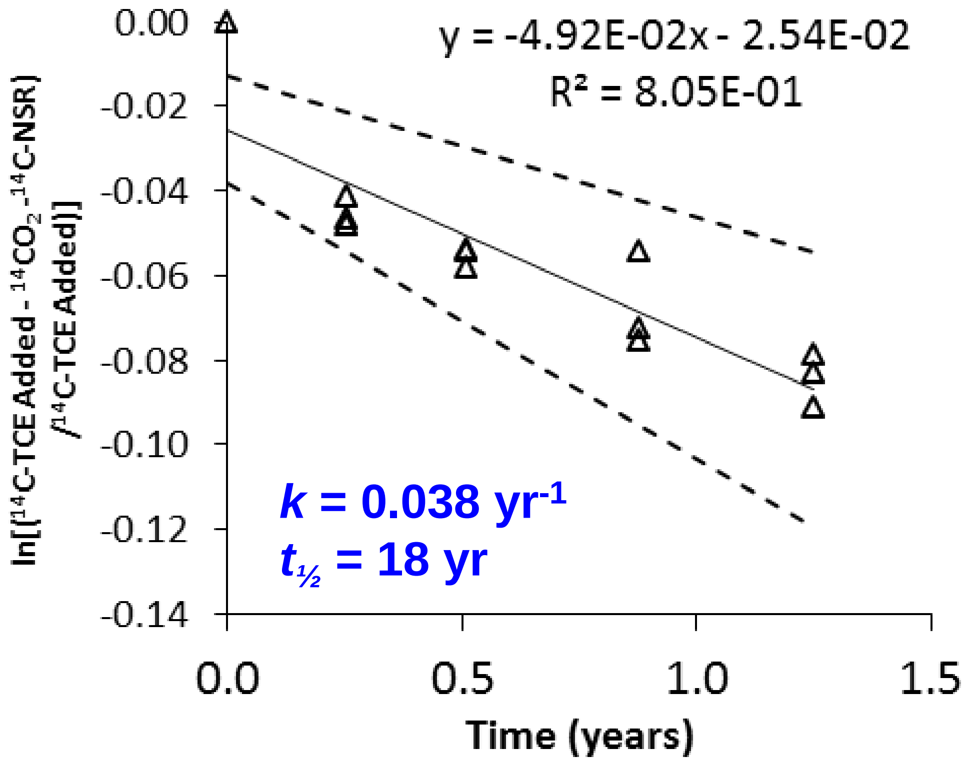
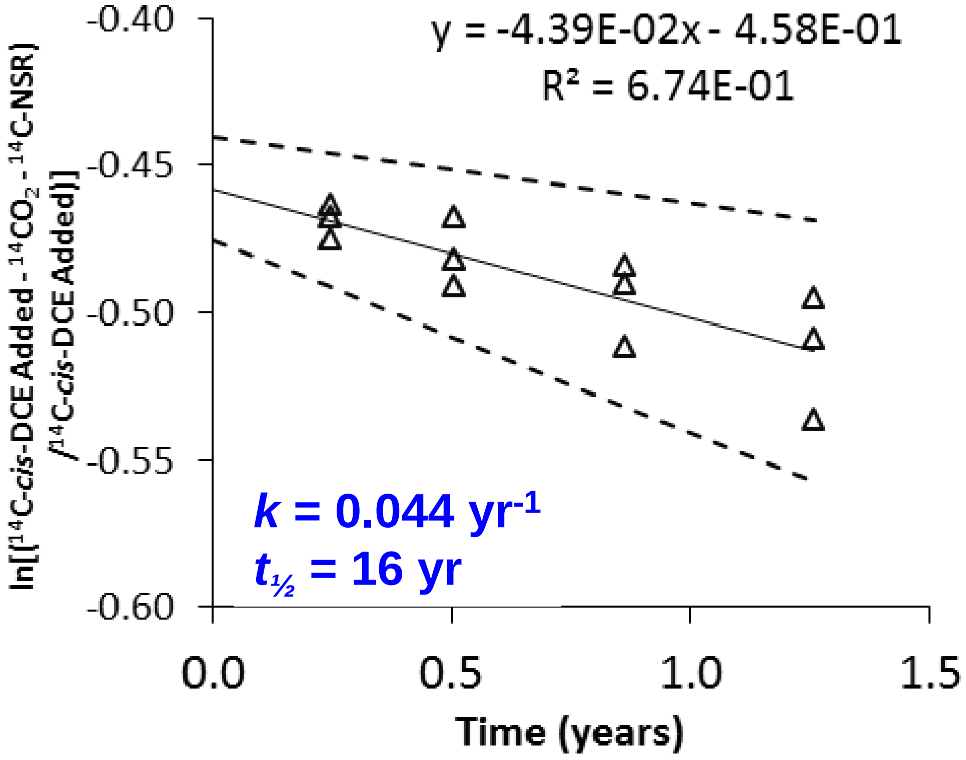
Remediation of chlorinated ethenes in fractured sandstone by natural and enhanced biotic and abiotic processes: A crushed rock microcosm study

Rong Yu [a], Richard G. Andrachek [b], Leo G. Lehmicke [c], David L. Freedman [a,*]

^{14}C assay for degradation of cDCE and TCE





- Unamended microcosms → *in situ* conditions
- Rate constants based on ^{14}C products formed
- No detectable reductive dechlorination
- Enrichment in δ^{13}C-*cis*-DCE also observed

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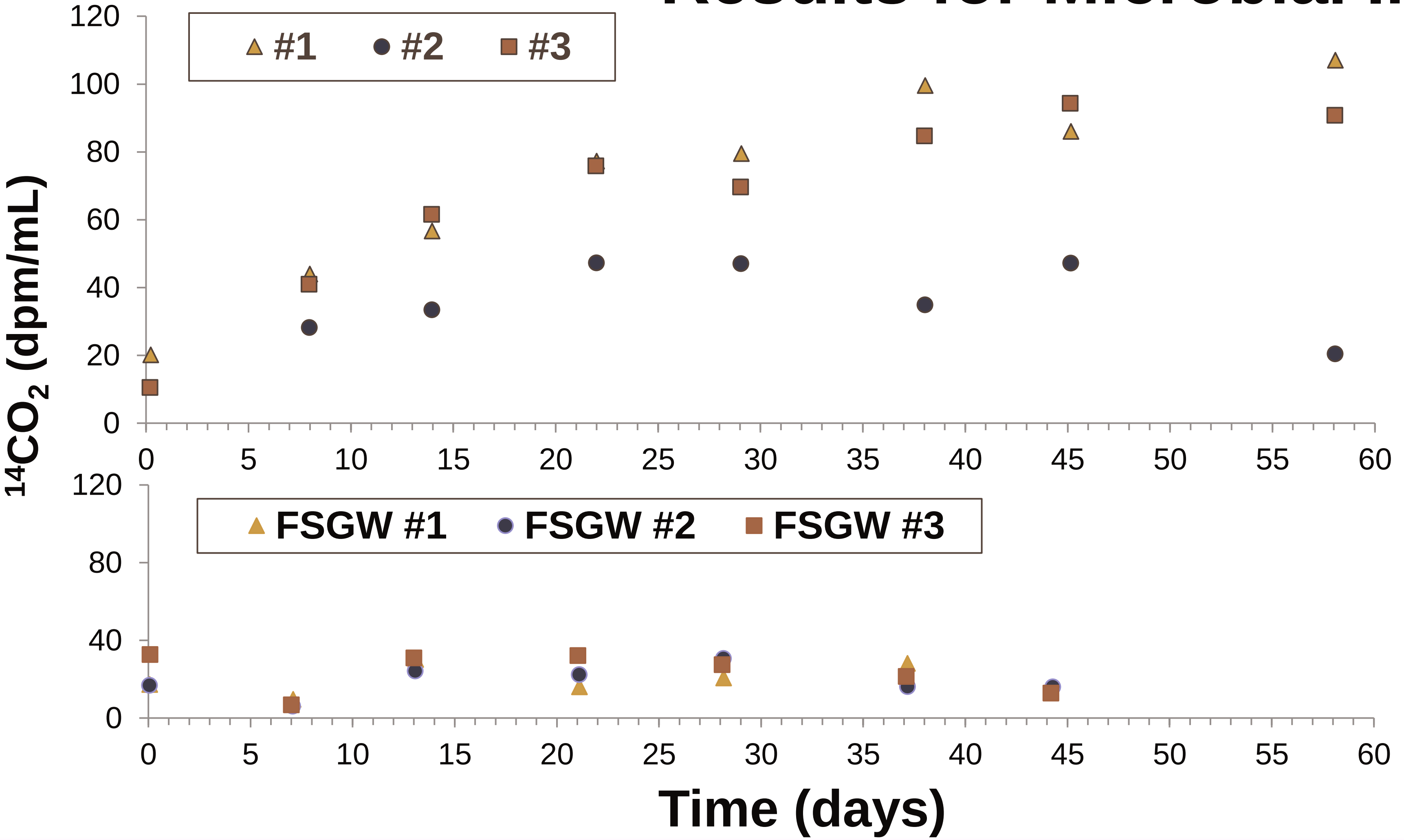
^{14}C assay for TCE degradation rate: Results for Microbial Insights

- Single sample of soil received
- GW from 4 wells
- Added ~10 g soil + 94 mL filter sterilized GW from each well
- Triplicate serum bottles for each well
- Prepared in anaerobic chamber
- Removed from chamber, sparged with N_2 to remove H_2
- Injected purified ^{14}C-TCE
- Measured VOCs by GC/FID
- Measured $^{14}CO_2$ by alkaline + acid sparging

^{14}C assay for TCE degradation rate: Results for Microbial Insights



^{14}C assay for TCE degradation rate: Microbial Insights

- Average degradation rate coefficient:
  - k = 0.15 yr^{-1} (95% CI = 0.11 to 0.18 yr^{-1})
  - $t_{½}$ = 4.8 yr (3.9-6.2 yr)
- Includes effect of adsorption
- Adjustment to field conditions

$$r_{aquifer} = r_{microcosm} \times \frac{\left(\frac{M_{rock}}{V_{water}}\right)_{aquifer}}{\left(\frac{M_{rock}}{V_{water}}\right)_{microcosm}} = r_{microcosm} \times \frac{\left(\frac{bulk\ density\ of\ rock}{porosity}\right)_{aquifer}}{\left(\frac{M_{rock}}{V_{water}}\right)_{microcosm}}$$

Example Applications

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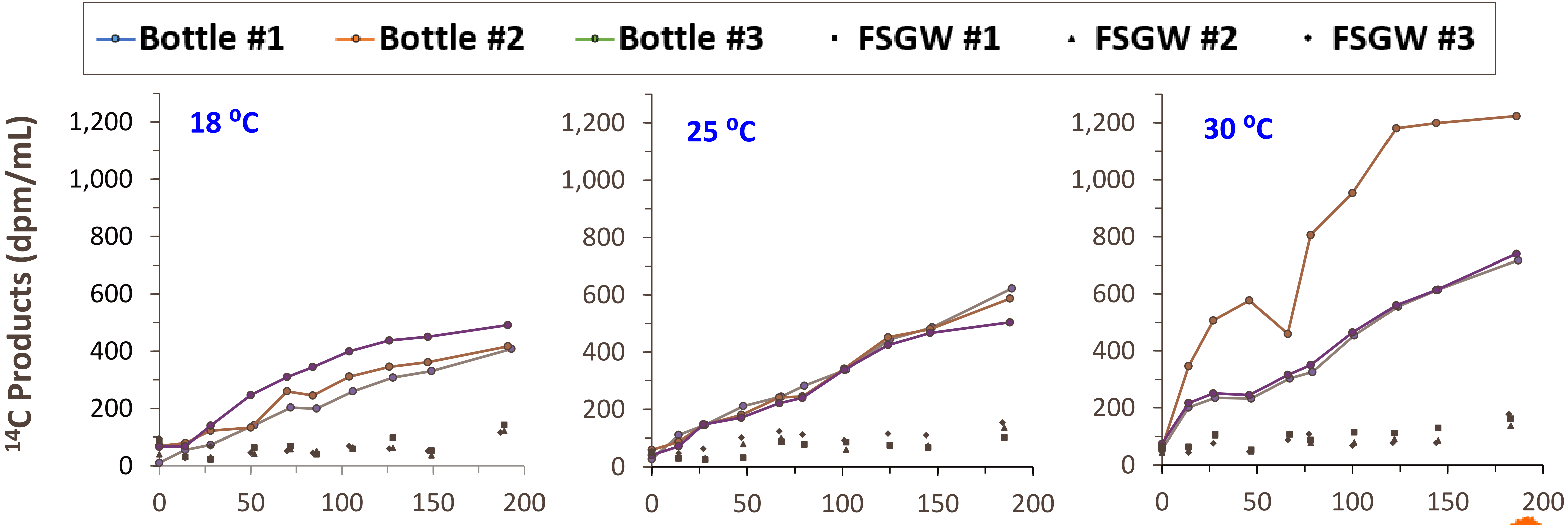
Improvement in Rates of TCE Degradation with Gentle Heating

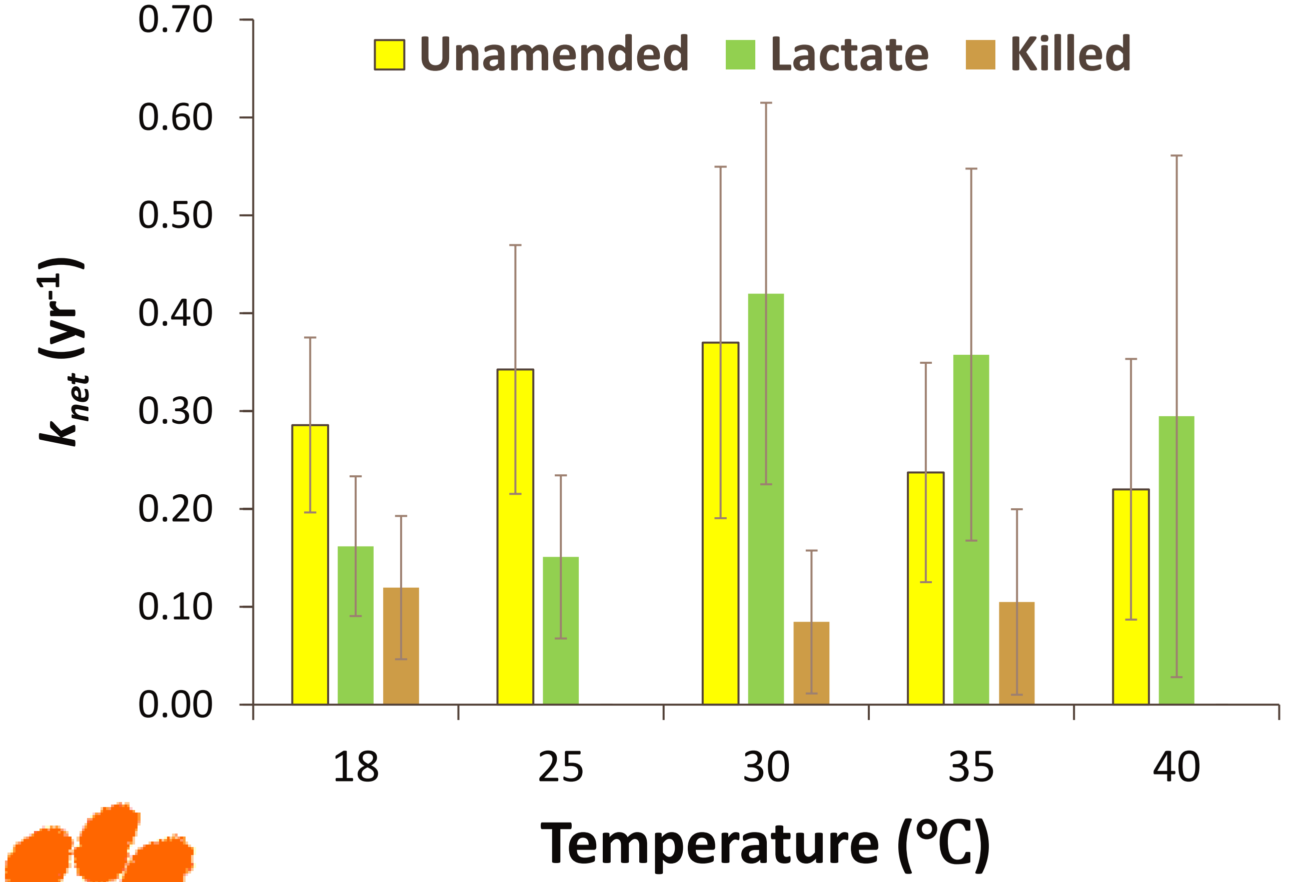
- Hypothesis: Gentle heating (e.g., up to ~20 ºC above ambient) will significantly increase the rate of biologically mediated abiotic degradation of TCE
- Microcosms prepared with crushed sandstone + GW
- Anaerobic preparation and incubation
- Purified ^{14}C-TCE added
- Incubated at 5 temperatures; range = 18-40 ºC
- Monitored rate of ^{14}C product formation
- Net rates = $k_{microcosms} - k_{FSGW\ controls}$

Improvement in Rates of TCE Degradation with Gentle Heating



- Lactate-amended rate of ^{14}C product accumulation increased with temperature
- FSGW = filter sterilized groundwater (control)

Rates of TCE Degradation with Gentle Heating



Treatment	u (kJ/mol)	θ	Temp (ºC)
Unamended	16.0	1.02	18, 25, 30
Lactate	53.5	1.08	18, 25, 30
Killed control	-	-	-

$$k_1 = k_2 \cdot \theta^{(T_1 - T_2)}$$

- **Rate constants estimated based on ^{14}C products**
- **Expected trend observed for 18-30 ºC**
- **Rapid initial heating appeared to inhibit activity at 35 and 40 ºC**

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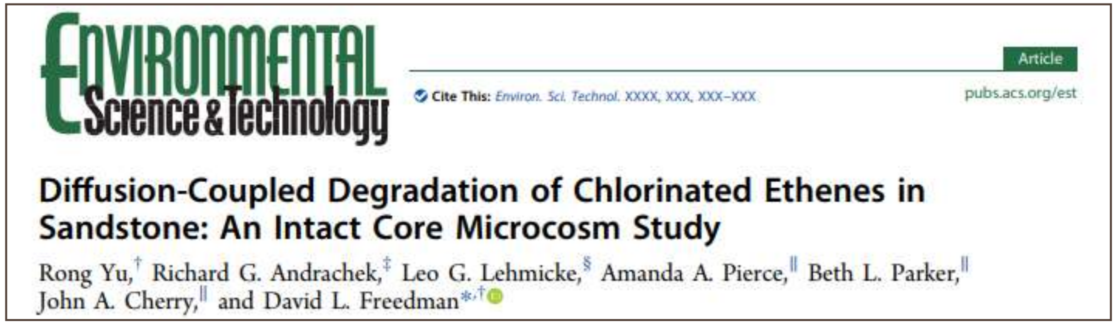
^{14}C-TCE intact anaerobic rock core microcosms: SERDP Project ER-2622

- In order to model back diffusion from low permeability zones contaminated with TCE, need good estimates of degradation rates within the low permeability zone
- Developed a novel type of intact rock core microcosm to assess degradation within rock
- Prior results reported for sandstone without ^{14}C-added
  - ✓ Enrichment in δ^{13}C-TCE and cDCE
- SERDP experimental design
  - ✓ 3 sites
  - ✓ 4 treatments in quadruplicate
  - ✓ One set received ^{14}C-TCE, another set only TCE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Diffusion-Coupled Degradation of Chlorinated Ethenes in Sandstone: An Intact Core Microcosm Study

Rong Yu,[†] Richard G. Andrachek,[‡] Leo G. Lehmicke,[§] Amanda A. Pierce,[∥] Beth L. Parker,[∥] John A. Cherry,[∥] and David L. Freedman*,[†]

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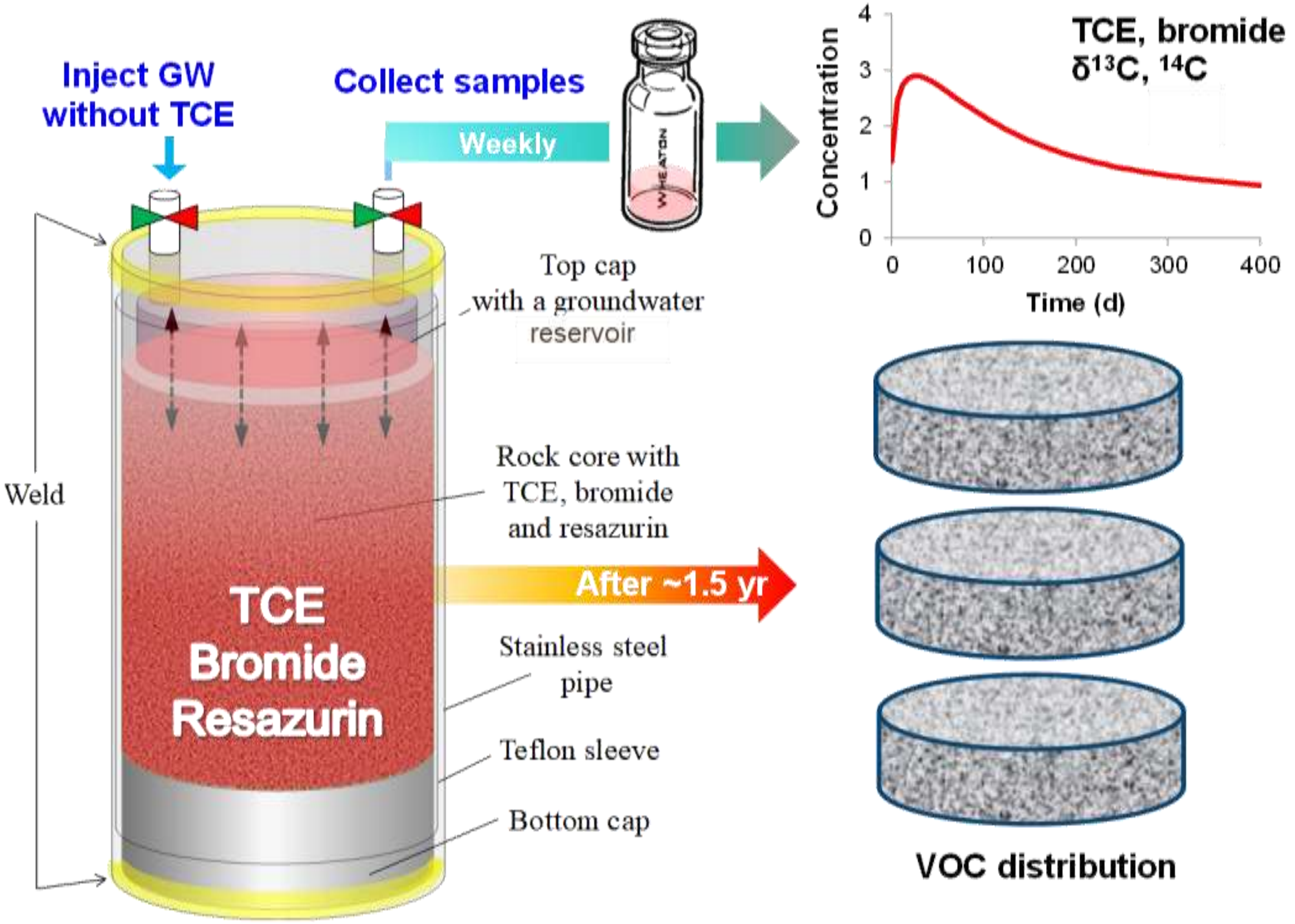
^{14}C-TCE intact anaerobic rock core microcosms: SERDP Project ER-2622



Schematic design of intact rock microcosm

Sample core

SERDP Project ER-2622

^{14}C-TCE + TCE added (+ resazurin + Br^-)

TCE added (+ resazurin + Br^-)

Unamended
Lactate Amended
Lac + SO_4^{2-} Amended
Abiotic ($HgCl_2$)
Container Control

Unamended
Lactate Amended
Lac + SO_4^{2-} Amended
Abiotic ($HgCl_2$)
Container Control

Site #1 intact rock core microcosms

- Rock type: dolomite
- Fractured bedrock contaminated with TCE

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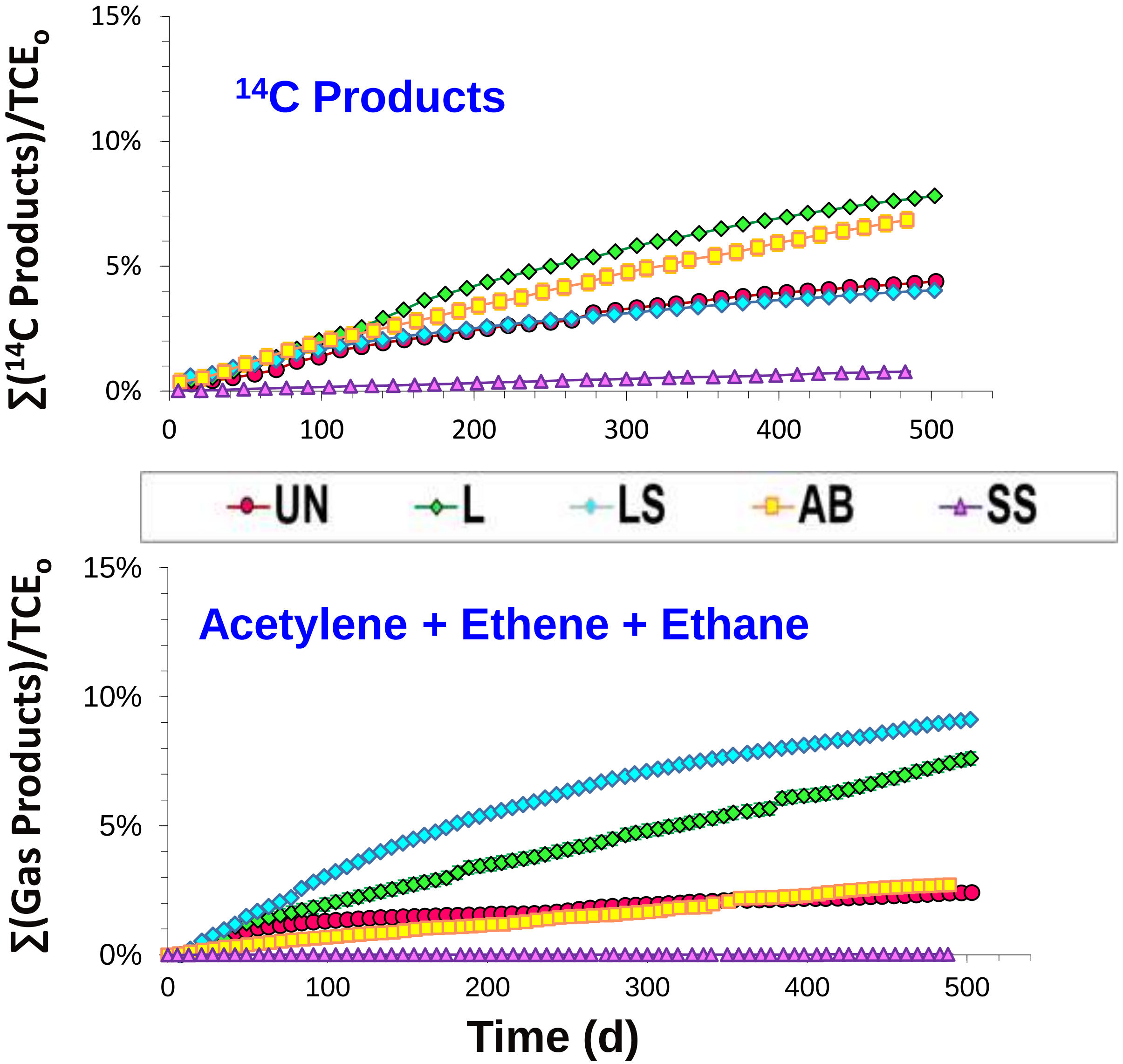
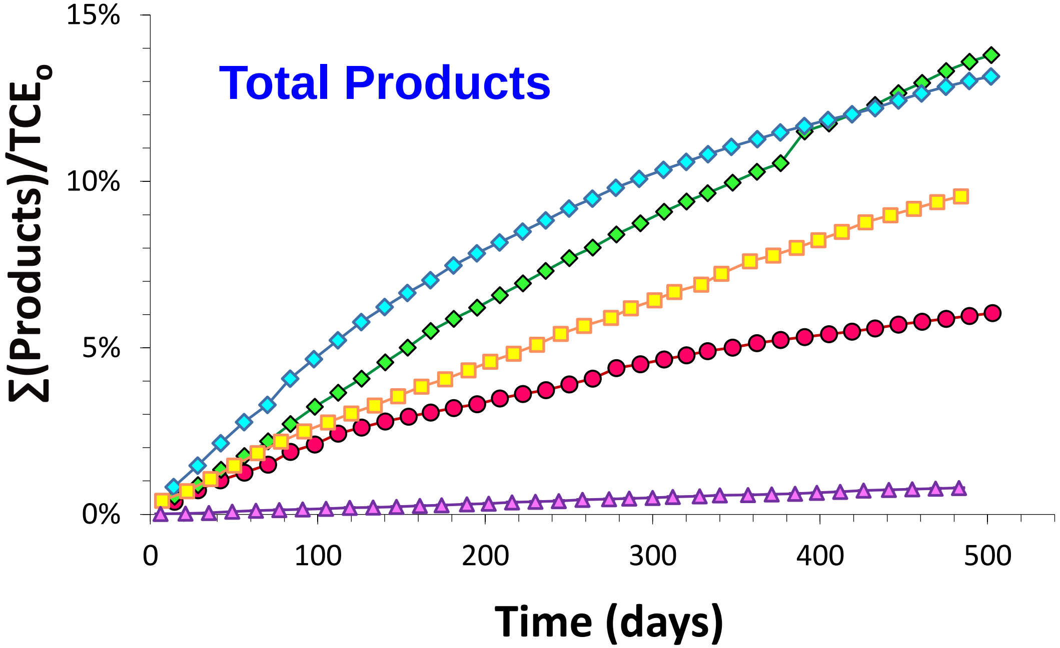
SERDP Project ER-2622





- **Accumulation of acetylene + ethene + ethane alone underestimates TCE degradation**
- **Transformation rates will be estimated based on a numerical model of the cores**
- **Outcome: TCE degradation rate constants that can be used to model back diffusion**

Example Applications

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Aerobic biodegradation of 1,4-dioxane

- Aerobic biodegradation of 1,4-dioxane yields CO_2, biomass, and possibly soluble intermediates; how to document *in situ*?
- ^{14}C assay developed as part of ESTCP Project ER-201730: *Development of a Quantitative Framework for Evaluating Natural Attenuation of 1,1,1-TCA, 1,1-DCA, 1,1-DCE, and 1,4-Dioxane in Groundwater*
- 7 sites selected, 4 wells per site
- 100 mL GW collected in 160 mL serum bottles + GW to prepare filter sterilized controls
- ^{14}C-1,4-dioxane purchased from Moravek Biochemicals (in butanol)
- Purified by HPLC
- Added to serum bottles: ~160,000 dpm + ~160 ppb 1,4-dioxane
- Assay evaluated with CB1190 and ENV487

Test Procedure

Collect GW samples:
Triplicate serum bottles + 2 L

↓

Ship overnight on ice

↓

Warm overnight to room temperature

↓

Prepare triplicate filter sterilized GW controls from 2 L sample

→

Add purified ^{14}C-1,4-dioxane to all bottles

↓

Measure initial conditions:
^{14}C, 1,4-DX, VOCs, O_2

↓

Sample weekly (5 mL) for 6 weeks:
measure ^{14}C products

↓

End of incubation analyses:
^{14}C products, 1,4-DX, VOCs, O_2

→

Calculate $k_{net} = k_{GW} - k_{FSGW}$
and net 95% Confidence Interval

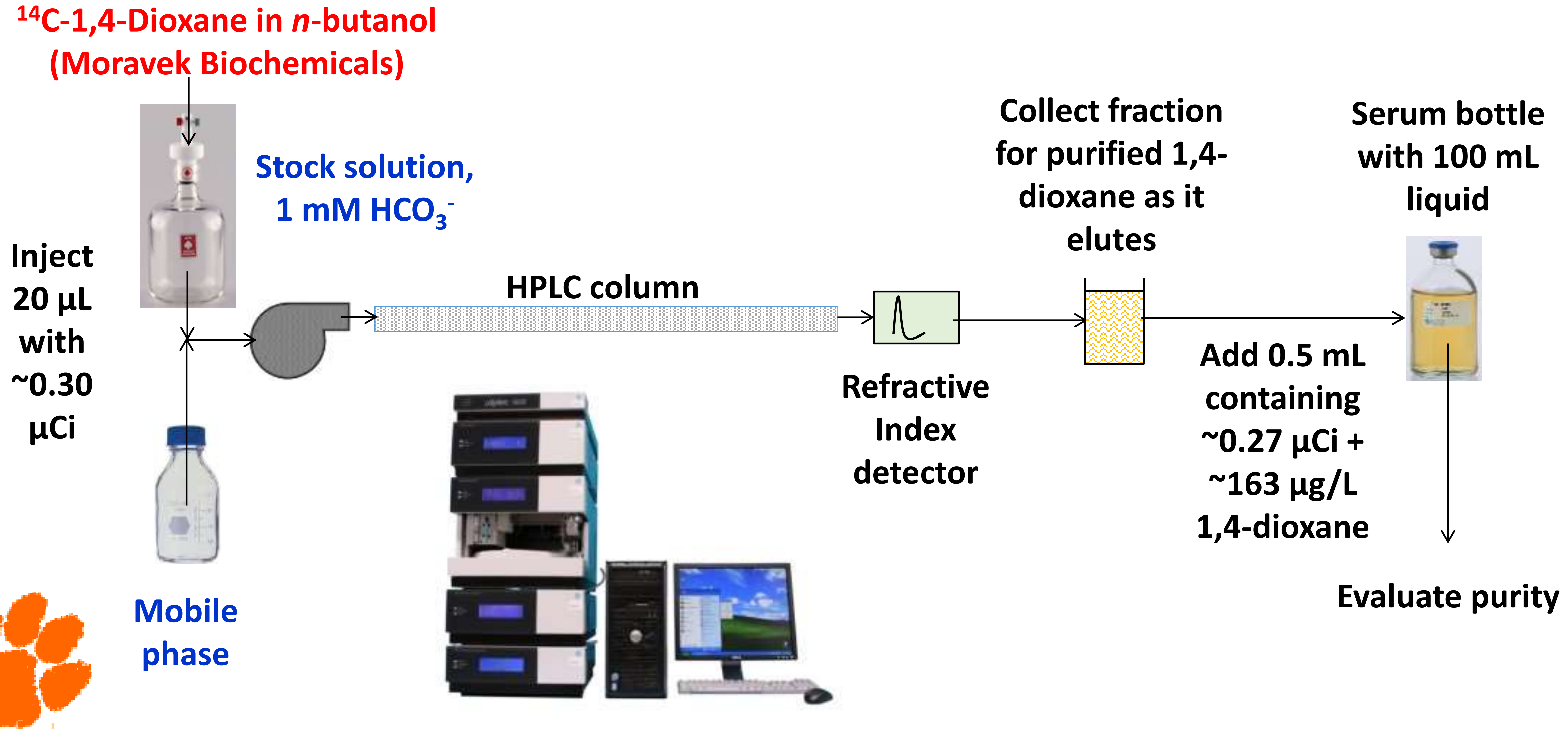
↓

Additional incubation

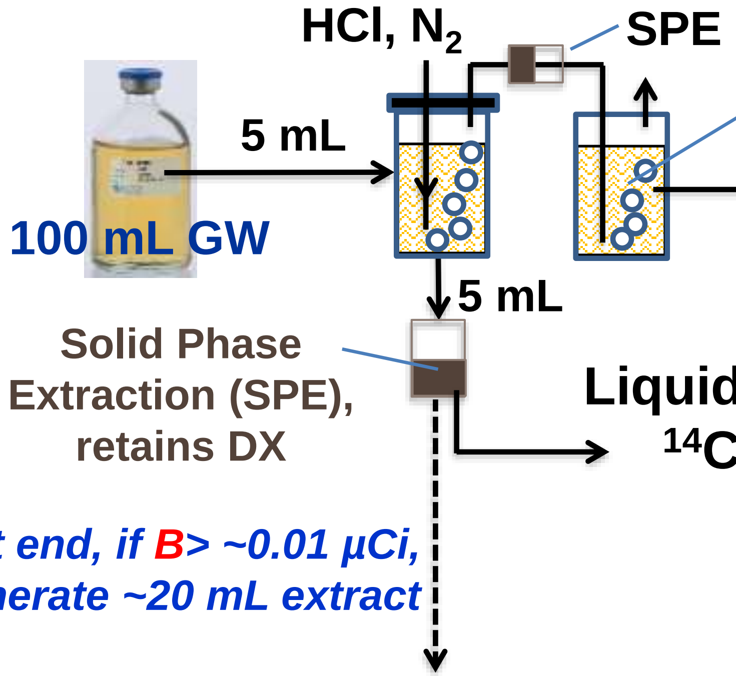
↓

Measure 1,4-DX; if change is significant, recheck ^{14}C products

Purification of ^{14}C-1,4-dioxane



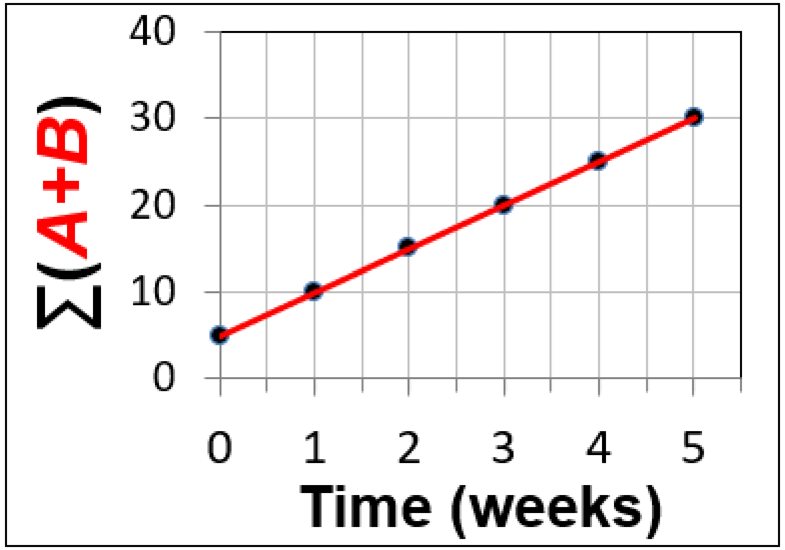
Analysis of ^{14}C-1,4-dioxane degradation products and rate



Liquid Scintillation Counter, count $^{14}CO_2$ (A)

Liquid Scintillation Counter, count ^{14}C-nonvolatile products (B)

At end, if B> ~0.01 µCi, generate ~20 mL extract



Concentrate by lyophilization ---> *100 µL* HPLC ---> Collect fractions ---> Liquid Scintillation Counter quantify non $^{14}CO_2$ metabolites (e.g., glyoxylate)

Aerobic biodegradation of 1,4-dioxane: 10 sites evaluated

Geographic diversity

- ≥ 4 states; East coast, West coast, Midwest

Mix of Department of Defense and industrial sites

All exhibit a decrease in C/C_o along plume axis

- Range of 1,4-dioxane concentrations: 163-11,000 µg/L; median = 169 µg/L
- Range of VOC co-contaminant concentrations: non-detect to 6 mg/L; 1,1-DCE from non-detect to 162 µg/L

3-5 wells sampled per site; repeat samples for 2 sites

Monitored: $\Delta^{14}C$ products; Δ1,4-dioxane; VOCs; ΔO_2

- ESTCP project (7/10 sites) also monitoring CSIA and relevant biomarkers

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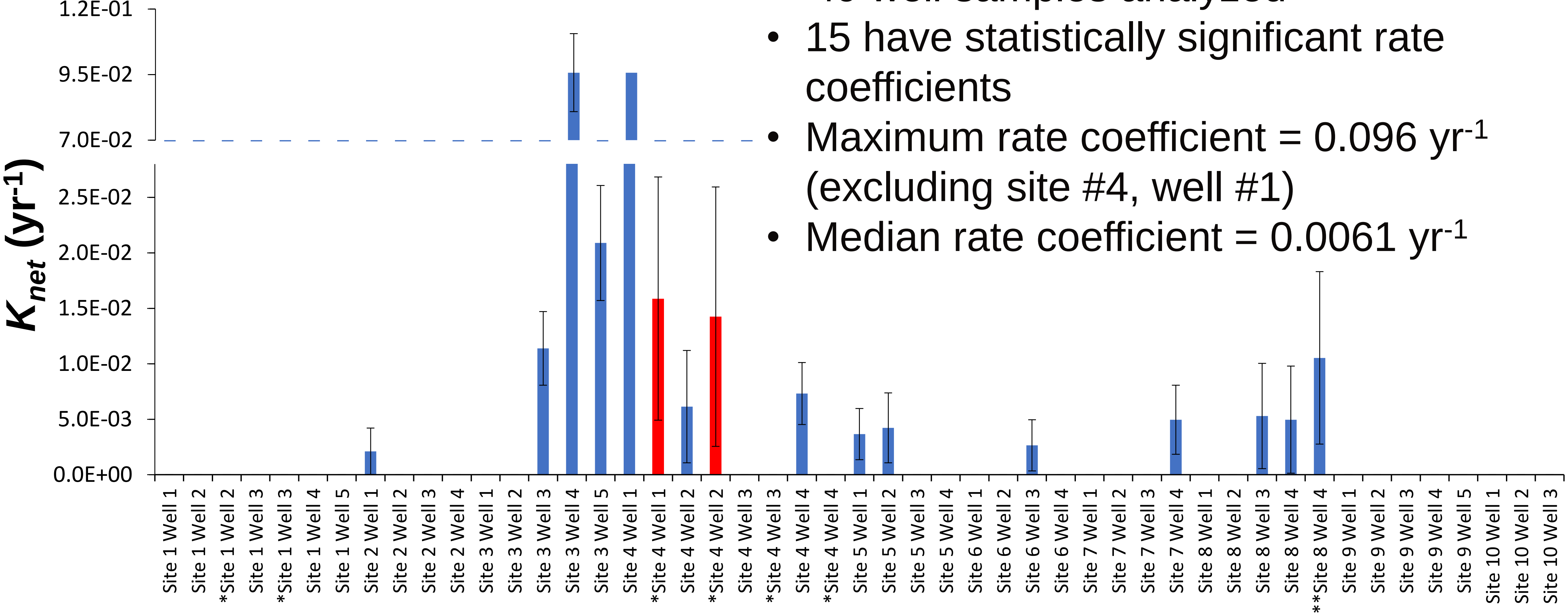

- 49 well samples analyzed
- 15 have statistically significant rate coefficients
- Maximum rate coefficient = 0.096 yr^{-1} (excluding site #4, well #1)
- Median rate coefficient = 0.0061 yr^{-1}

*** = resampled;** ** = nutrients added

Overall Evidence

	Site Conditions				Degradation	
Site	High levels of CVOCs?	Absence of co-substrate?	Low DO?	Low levels of 1,4-DX?	C vs. D, Biomarkers, CSIA	^{14}C Assay
#1	✓	✓	✓		++	-
#2		✓		✓	+	+
#3					++	++
#4		✓	✓		++	+
#5	✓	✓	✓		+	+
#6		✓		✓	+	+

- **^{14}C generally matches C vs. D, biomarkers, CSIA**
- **1,4-dioxane biodegradation not ubiquitous, but at least some evidence despite several unfavorable conditions**

Aerobic biodegradation of 1,4-dioxane

- Obtained rate constants in 15/49 well samples from 7/10 sites, but most are low
- Rate constants determined by ^{14}C assay are likely conservative
  - **Lack of solid-phase and/or nutrient supply may suppress rates**
  - **O_2 is not limiting in the assay, may be *in situ***
- ^{14}C assay may best be used as a screening step to be followed by microcosms with nutrients and/or soil
- VOCs reduce rates, but low levels are tolerable
- Reasonable reproducibility in repeat samples

Closing Thoughts

- ^{14}C assays have the potential to fill a critical need
  - Potential to determine rates of transformation (biotic and abiotic) when the products are not discernable *in situ*
  - Provide supporting evidence for MNA or success of active remediation
- Some issues
  - Assays are restricted to lab testing; how well do the results reflect *in situ* conditions?
  - Even short-term assays can take ~6 weeks
  - Longer-term incubation and rock core microcosms may be restricted to research applications
  - How well do the predicted rates correlate to faster and less costly lines of evidence, e.g., biomarkers and CSIA
- ^{14}C assays can be ordered through Microbial Insights

Who Did All the Work?

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Questions?