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-1\textsuperscript{st} order degradation rates for 1,4-dioxane

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


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)
  - $\varepsilon$ is the “enrichment factor”
    - Degradation reactions in laboratory
  - $f$ is the “fraction remaining”
    - $(1-f) \times 100 = \%$degradation

- % degradation can be calculated if $\delta^{13}C_o$, $\varepsilon$, and $\delta^{13}C_t$ are known
Enrichment trends from reactions with pure cultures

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

<table>
<thead>
<tr>
<th>strain</th>
<th>substrate</th>
<th>$\varepsilon_C$ (‰)</th>
<th>$\varepsilon_H$ (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycobacterium 1A</em></td>
<td>propane</td>
<td>-2.0</td>
<td>-26</td>
</tr>
<tr>
<td><em>R. rhodochrous</em> ** ATCC 21198*</td>
<td>propane</td>
<td>-2.7±0.3</td>
<td>-21±2</td>
</tr>
<tr>
<td></td>
<td>isobutane</td>
<td>-2.5±0.3</td>
<td>-28±6</td>
</tr>
<tr>
<td><em>P. tetrahydrofuran-oxidans K1</em></td>
<td>THF</td>
<td>-4.7±0.9</td>
<td>-147±22</td>
</tr>
</tbody>
</table>

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


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.
CSIA on 1,4-dioxane during biodegradation

Samples collected for CSIA ($\delta^{13}C$ and $\delta^2H$ of 1,4-dioxane) on day 90 and day 270.
Enrichment was smaller than anticipated at MACB-1

<table>
<thead>
<tr>
<th></th>
<th>IACB-1</th>
<th>MACB-1</th>
<th>MACB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,4-D (µg/L)</td>
<td>26</td>
<td>3.6</td>
<td>4.2</td>
</tr>
<tr>
<td>residual 1,4-D (f)</td>
<td>1</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>δ\textsuperscript{13}C measured (‰)</td>
<td>-33.7</td>
<td>-33.2</td>
<td>-30.2</td>
</tr>
<tr>
<td>δ\textsuperscript{13}C expected (‰)</td>
<td></td>
<td>-29.8</td>
<td>-30.0</td>
</tr>
<tr>
<td>Day 270</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,4-D (µg/L)</td>
<td>24</td>
<td>0.68</td>
<td>0.82</td>
</tr>
<tr>
<td>residual 1,4-D (f)</td>
<td>1.00</td>
<td>0.028</td>
<td>0.034</td>
</tr>
<tr>
<td>δ\textsuperscript{13}C measured (‰)</td>
<td>-28.9</td>
<td>-25.4</td>
<td>-24.1</td>
</tr>
<tr>
<td>δ\textsuperscript{13}C expected (‰)</td>
<td></td>
<td>-21.8</td>
<td>-22.1</td>
</tr>
</tbody>
</table>

Expected δ\textsuperscript{13}C values calculated from Rayleigh equation and microcosm-based enrichment factor for \textit{Mycobacterium 1A}: -2.0 ‰
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
Hypothetical scenario: degradation in shallow plume

Depletion in heavy isotope with increased degradation can occur:

How heterogeneity can mask enrichment (EPA, 2008)
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 $\xi_C$ & $\xi_H$

• 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 δ¹³C = -30‰
Initial δ²H = -33.1‰
¹³C enrichment factor = -2.7
²H 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 $\delta^2H = -33.1\%$

$^{13}C$ enrichment factor = -4.7
$^2H$ 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.)
Example site

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

<table>
<thead>
<tr>
<th>Well</th>
<th>1,4-D (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW1</td>
<td>970</td>
</tr>
<tr>
<td>MW2</td>
<td>86</td>
</tr>
<tr>
<td>MW3</td>
<td>130</td>
</tr>
<tr>
<td>MW4</td>
<td>14</td>
</tr>
<tr>
<td>MW5</td>
<td>1.4 J</td>
</tr>
</tbody>
</table>

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.
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