Single-Well Push-Pull Tests to Assess Aerobic Cometabolism of Isobutene as a Surrogate for 1,4-Dioxane

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April 10, 2018
Aerobic cometabolism of 1,4-dioxane

• 1,4-Dioxane:
  – Cyclic ether
  – Widespread groundwater contaminant due to use as stabilizer for 1,1,1-trichloroethane
  – Median maximal plume concentration: 365 ppb (Adamson, 2014)
  – Resistant to conventional pump and treat remediation strategies

• Aerobic cometabolism: use of a primary substrate (electron donor) to stimulate expression of enzymes that transform both primary substrate and contaminant of interest with oxygen as the electron acceptor
1,4-dioxane transformation by isobutane-utilizing microorganisms

- Primary substrate: isobutane
- Monooxygenase enzyme oxidizes 1,4-dioxane
- Model bacteria: *Rhodococcus rhodochrous* ATCC 21198
- Biostimulation and bioaugmentation in aquifer microcosms

April 10, 2018
Single-well push-pull tests

- MW2 at the OSU motor pool
- 2”, 13’ deep, clay aquifer
- Injection via peristaltic pump:
  - Bromide (KBr)—tracer
  - Nitrate (KNO₃)—inorganic nutrient
  - Isobutane—primary substrate
  - Hydrogen peroxide—oxygen
- Extraction via 1L bailer
Site Constraints

- Active lot
- Unproductive wells
  - Maximum injection/extraction rate in MW2: 400 mL/min
- 40 ppb arsenic
- **No 1,4-dioxane present!**
1,4-Dioxane Surrogate: Isobutene

- No growth of isobutane-utilizers on isobutene
- Monooxygenase enzyme transforms isobutene (like 1,4-dioxane)
- Formation of epoxide: isobutene oxide
## Push-Pull Tests in MW2

<table>
<thead>
<tr>
<th>Well test in MW2</th>
<th>Date Test Started</th>
<th>Volume Injected (L)</th>
<th>Injection Rate (mL/min)</th>
<th>Injection Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Isobutane</td>
</tr>
<tr>
<td>Transport</td>
<td>09/26/17</td>
<td>23</td>
<td>260</td>
<td>7.7</td>
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<tr>
<td>Biostimulation 1</td>
<td>10/26/17</td>
<td>39</td>
<td>270</td>
<td>1.8</td>
</tr>
<tr>
<td>Biostimulation 2</td>
<td>11/07/17</td>
<td>33</td>
<td>245</td>
<td>4.3</td>
</tr>
<tr>
<td>Biostimulation 3</td>
<td>11/15/17</td>
<td>32</td>
<td>300</td>
<td>5.7</td>
</tr>
<tr>
<td>Biostimulation 4</td>
<td>11/29/17</td>
<td>22</td>
<td>300</td>
<td>6.0</td>
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<tr>
<td>Biostimulation 5</td>
<td>02/09/18</td>
<td>36</td>
<td>450</td>
<td>2.4</td>
</tr>
<tr>
<td>Cometabolism 1 (Isobutene alone)</td>
<td>02/16/18</td>
<td>36</td>
<td>300</td>
<td>0.8</td>
</tr>
<tr>
<td>Biostimulation 6</td>
<td>03/16/18</td>
<td>35</td>
<td>220</td>
<td>2.5</td>
</tr>
<tr>
<td>Cometabolism 2 (Isobutene and isobutane)</td>
<td>03/28/18</td>
<td>25</td>
<td>240</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Analytical Methods

- *Isobutane* and *Isobutene*: headspace analysis, GC-FID or GC-MS
- *Isobutene Oxide*: liquid analysis, GC-MS with heated purge and trap
- *Bromide* and *Nitrate*: liquid analysis, IC
- *Oxygen*: liquid analysis, YSI dissolved oxygen probe
- *Hydrogen Peroxide*: liquid analysis, Indigo test strips
Transport test

- Injection: 23 L
- Continuous extraction: 50 L

\[ C^* = \frac{C/C_0}{Br/Br_0} \]
Isobutane Biostimulation

- Injection solution: Isobutane, nitrate, bromide, hydrogen peroxide
- Natural drift tests: injection followed by daily extraction of 4L
Isobutane Biostimulation

\[ C^* = \frac{C}{C_0} \frac{Br}{Br_0} \]

<table>
<thead>
<tr>
<th>Well test in MW2</th>
<th>Date Test Started</th>
<th>Days since previous test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biostimulation 1</td>
<td>10/26/17</td>
<td></td>
</tr>
<tr>
<td>Biostimulation 2</td>
<td>11/07/17</td>
<td>12</td>
</tr>
<tr>
<td>Biostimulation 3</td>
<td>11/15/17</td>
<td>8</td>
</tr>
<tr>
<td>Biostimulation 4</td>
<td>11/29/17</td>
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</tr>
<tr>
<td>Biostimulation 5</td>
<td>02/09/18</td>
<td>72</td>
</tr>
</tbody>
</table>
Isobutene Cometabolism Test 1

- Injection solution: Isobutene, nitrate, bromide, hydrogen peroxide
  - Resting cell test
- Isobutene decreases relative to bromide tracer
- Isobutene transformation was slower than isobutane transformation from recent biostimulation test
- Isobutene oxide formed
Biostimulation Test 6

- Immediately after isobutene cometabolism test
- Injection solution: Isobutane, nitrate, bromide, hydrogen peroxide, **30 ppb isobutene oxide**
- Isobutane consumption not significantly slower than in biostimulation 5
- Decrease of isobutene oxide relative to bromide tracer – biotically driven
Isobutene Cometabolism Test 2

- Active cell test – primary substrate present
- Injection solution: Isobutane, isobutene, nitrate, bromide, hydrogen peroxide
- No inhibition of isobutene transformation by isobutane (unlike 1,4-dioxane)
Resting vs Active Cometabolism

- Faster isobutene and isobutene oxide transformation in active cometabolism test
- Potential implications for 1,4-dioxane transformation products

<table>
<thead>
<tr>
<th>Test</th>
<th>Isobutene injected (mg/L)</th>
<th>Maximum Isobutene oxide (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cometabolism 1– Resting</td>
<td>0.8</td>
<td>0.11</td>
</tr>
<tr>
<td>(Isobutene alone)</td>
<td></td>
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</tr>
<tr>
<td>Cometabolism 2– Active</td>
<td>1.1</td>
<td>0.135</td>
</tr>
<tr>
<td>(Isobutane and isobutene)</td>
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</tr>
</tbody>
</table>

April 10, 2018
Summary and Conclusions

• Isobutane-utilizing microorganisms were biostimulated in situ
• In situ cometabolic activity demonstrated by transformation of isobutene to isobutene oxide
• Cometabolic transformation did not diminish activity of microbial community
• Faster isobutene and isobutene oxide transformation in the presence of isobutane—implications for 1,4-dioxane mineralization
Future Work

- Molecular analysis: qPCR for SCAM enzyme
- Push-pull tests with labeled 1,4-dioxane
- Reactive-transport modeling
- Assessment of other primary substrates
Acknowledgements

• SERDP ER-2303
• DoD NDSEG
• OSU Motor pool
• Jon Laurence, Eileen Lukens, Gillian Williams, Grant Kresge
• Chapman Professional Development Grant

Questions?
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