Investigating the in situ biodegradation of BTEX at an active refinery using bio-traps and molecular biological tools

Katherine Clark
Microbial Insights, Inc.

Kerry Sublette
Department of Chemical Engineering
University of Tulsa
What are Bio-Trap® samplers?

Passive sampling tool for microbes

Collects active microbes

Integrated sample vs. “snapshot”

Analyzed using molecular biological tools, analytical chemistry, and stable isotope analysis
Bio-Sep® beads

- 3-4 mm in diameter
- 25% Nomex, 75% PAC
- Cleaned of fossil biomarkers by heating to 270 °C
- 74% porosity
- 600 m² of surface area/g
- Biofilms form rapidly on Bio-Sep® beads
Molecular biological tools (MBTs)

- Culture independent technologies
- Use biomarkers to gain information about microbial populations and activity:
  - Phospholipid Fatty Acids (PLFA)
  - DNA (genetic potential)
  - RNA (gene expression)
- Stable Isotope Probing (SIP) tracks $^{13}$C from a labeled contaminant as it is mineralized ($\text{CO}_2$ or methane) or metabolized (PLFA, DNA)
Assessing BTEX biodegradation potential at a refinery using molecular biological tools
Assessment of *in situ* BTEX biodegradation using MBTs

- **Phase I:** Groundwater survey using bio-traps and groundwater sampling
- **Phase II:** Microbial activity in the vadose zone studied in soil cores
- **Phase III:** SIP used to provide direct proof of benzene biodegradation in groundwater
Site map

- Non-amended bio-trap sampling only
- Non-amended and $^{13}$C-benzene bio-trap sampling, core sampling
Specific qPCR targets

- **16S rRNA genes**
  - EBAC: total eubacteria
  - PM1: aerobic MTBE degradation

- **Functional genes—Taxonomic**
  - *nirK* and *nirS*: denitrifiers
  - APS: sulfate-reducing bacteria
  - MGN: methanogens

- **Functional genes:**
  - NAH, PHE, TOD: aerobic hydrocarbon degradation
qPCR analysis of DNA extracted from bio-traps in groundwater

- All gene targets detected in all 25 wells
- Genetic potential for aerobic oxidation, sulfate reduction, denitrification, and methanogenesis present across the site

<table>
<thead>
<tr>
<th>Correlations</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>log [EBAC]</td>
<td>log [nirS]</td>
<td>+0.61</td>
</tr>
<tr>
<td>log [EBAC]</td>
<td>log [APS]</td>
<td>+0.55</td>
</tr>
<tr>
<td>log [EBAC]</td>
<td>log [MGN]</td>
<td>+0.71</td>
</tr>
<tr>
<td>log [EBAC]</td>
<td>log [NAH]</td>
<td>+0.64</td>
</tr>
<tr>
<td>log [EBAC]</td>
<td>log [PM1]</td>
<td>+0.60</td>
</tr>
<tr>
<td>[Toluene]</td>
<td>log [PM1]</td>
<td>+0.51</td>
</tr>
</tbody>
</table>
Groundwater BTEX concentrations

[BTEX] ranged between non-detect and 74 mg/L

Well

log ([BTEX])

Dissolved Oxygen (mg/L)

Well
# Geochemistry correlations

<table>
<thead>
<tr>
<th>Correlations</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>log [BTEX] [Fe$^{+2}$]</td>
<td>+0.54</td>
<td>0.009</td>
</tr>
<tr>
<td>log [BTEX] [Methane]</td>
<td>+0.51</td>
<td>0.014</td>
</tr>
<tr>
<td>log [BTEX] pH</td>
<td>-0.65</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Alkalinity ORP</td>
<td>+0.70</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Alkalinity [Ammonia-N]</td>
<td>+0.85</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
RT-qPCR analysis of RNA extracted from soil cores

- Gene targets associated with denitrifiers and sulfate reducers present in all 8 locations
- Expression of aerobic hydrocarbon oxidation genes detected in 5 locations
- Methanogenic gene target detected in 3 locations
Eubacteria rRNA in the vadose zone and groundwater [BTEX]
Overview of Bio-trap stable isotope probing (SIP) approach

\[ ^{13}\text{C}_6\text{-benzene} \]

Annular space loaded with sterile \(^{13}\text{C}\)-loaded Bio-Sep\(^{\text{\textregistered}}\) beads

Bio-trap filled with \(^{13}\text{C}_6\text{-benzene labeled Bio-Sep}\(^{\text{\textregistered}}\) beads

\(\text{In situ} \) incubation of \(^{13}\text{C}_6\text{-benzene bio-trap in site monitoring well}\)

Analysis of post-incubated Bio-Sep\(^{\text{\textregistered}}\) beads
Stable isotope probing with Bio-Sep® beads
\[ \delta^{13}C \text{ [\%o]} = \left( \frac{(^{12}C/^{12}C)_{\text{Sample}}}{(^{12}C/^{12}C)_{\text{Standard}}} - 1 \right) \times 1000 \]
Relative proportion of fatty acids from the PLFA analysis of $^{13}$C$_6$-benzene-amended beads from the well MW10 bio-trap.
$^{13}\text{C Utilized for CO}_2$ and BTEX Concentration

DIC $^{13}\text{C} (%)$

Well

$\log [\text{BTEX}]$ (µg/L)

DIC Del

$\log$ BTEX

0

10 16 18 19 22 1 25 26
Relationship between SIP results and proximity to the river
Geochemistry comparison between river and non-river associated sites

<table>
<thead>
<tr>
<th>Parameter</th>
<th>River (n= 10)</th>
<th>Non-River (n=15)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORP (mV)(^1)</td>
<td>-58.1 ± 89.2</td>
<td>-96.4 ± 64.8</td>
<td>0.26</td>
</tr>
<tr>
<td>Dissolved Oxygen(^1)</td>
<td>1.0 ± 1.2</td>
<td>0.57 ± 0.32</td>
<td>0.06</td>
</tr>
<tr>
<td>(mg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methane (µg/L)(^2)</td>
<td>4320 ± 2563</td>
<td>6934 ± 3212</td>
<td>0.04</td>
</tr>
<tr>
<td>Alkalinity (mg/L)(^1)</td>
<td>522 ± 204</td>
<td>444 ± 89</td>
<td>0.11</td>
</tr>
<tr>
<td>Ferrous iron (mg/L)(^2)</td>
<td>6.5 ± 5.8</td>
<td>9.1 ± 7.5</td>
<td>0.36</td>
</tr>
</tbody>
</table>

\(^1\)Mann-Whitney U test  
\(^2\)Student’s t test
Results of *in situ* BTEX biodegradation assessment

- A significant, diverse microbial community active in groundwater and vadose zone
- Likely BTEX biodegradation mechanisms: aerobic oxidation, denitrification, sulfate reduction, methanogenesis, and possibly Fe$^{+3}$ reduction
- Microbial distribution in the vadose zone is more influenced by structural characteristics than groundwater hydrocarbon concentrations
- SIP provided direct evidence of benzene biodegradation in 6 of the 8 wells sampled
Questions?