Case Studies of Advances in Bioremediation of Organics: Part 1

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Outline

• Chlorinated Solvents
  – Molecular Tools
  – Electrokinetic Bioremediation

• 1,4-Dioxane
  – $^{14}$C Assay
  – Aerobic Cometabolism using Multiple Primary Substrates
Case Study – qPCR and 16S Sequencing at NAS JAX

Site Background

• Operable Unit 3 (OU3) occupies 134 acres on eastern side of installation
• Industrial/commercial land use
• Fleet Readiness Command (FRC) (formerly NADEP) primary tenant on installation since 1940s
• Former dry cleaner facility located within OU3 property
• 7 identified groundwater plumes (Areas A – G)
• Buildings 780 and 106 also sources of contamination
Case Study – qPCR and 16S Sequencing at NAS JAX
Enhanced In Situ Bioremediation Application

- 50 DPT injection locations
- 2 injection intervals per location
- 145,000 gallons of an 0.7% emulsified vegetable oil solution (EDS-ERTM)
- Average flow rate of 1.8 gpm
- 100 liters of KB-1® and KB-1® Plus injected
- Bromide tracer used
- 10 performance monitoring wells
- 4 soil gas probes
Case Study – qPCR & 16S Sequencing at NAS JAX
C VOC Results

Legend
- Performance Monitoring Well
- Soil Gas Probe
- UIC Monitoring Well
- Temporary DPT Injection Location

Target Treatment Area (TTA)

Chloroethenes/Methane (µg/L)
log10 (vcrA)

PZ-01

Legend
- PCE
- TCE
- cDCE
- VC
- Ethene
- Methane
- vcrA

PZ-02

Graphs show the performance monitoring data for wells PZ-01 and PZ-02 with various compounds monitored over time.
Case Study – CVOC qPCR & 16S Sequencing at NAS JAX

Microbial Composition

Upgradient Well MW-40S

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MW-40S</th>
<th>PZ-02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehalococcoides</td>
<td>0.01%</td>
<td>5%</td>
</tr>
<tr>
<td>Geobacter</td>
<td>0.09%</td>
<td>24%</td>
</tr>
<tr>
<td>Methylobacter</td>
<td>9%</td>
<td>9%</td>
</tr>
</tbody>
</table>

Pilot Test Well PZ-02

• Anticipate • Innovate • Accelerate •
**Electrokinetic (Ek) Bioremediation**

- Low permeability silts and clays \( (K < 10^{-7} \text{ m/s}) \) present challenge for amendment distribution
- This technology leverages the electrical properties of the amendments to promote distribution
- Technology applies an electric current to facilitates electromigration and electro-osmosis
- Successfully demonstrated in Denmark to treat PCE
  - Generated lactate flow of 3 to 5 cm/day through clay
Ek-Bio Demonstration at NAS Jacksonville, FL  
(ESTCP ER-201325)

• Potassium lactate and KB-1 (2 Stages)
• Stage 1
  - 100 gallons, 60% lactate
  - 4 L KB-1 into each of 8 supply wells & 2 L KB-1 into each of 9 electrode wells
  - Introduced over 6 months
PCE at 15 – 40 mg/L in clay

NC – not characterized; SP – sand; CL – clay
Ek-Bio Demonstration at NAS Jacksonville, FL (ESTCP ER-201325)

Results (cont.)

Supply Wells in Yellow
Electrode Wells in RED
Monitoring Well in Grey

- Anticipate - Innovate - Accelerate -
• Chlorinated Solvents
  – Molecular Tools
  – Electrokinetic Bioremediation

• 1,4-Dioxane
  – $^{14}$C Assay
  – Aerobic Cometabolism using Multiple Primary Substrates
1,4-Dioxane – ESTCP 201730

• Many 1,4-dioxane plumes appear to attenuate; how to prove?

• Multiple lines of evidence approach, including:
  - Concentration trend analysis; plume mass estimates; CSIA; biomarkers

• More direct evidence of aerobic biodegradation may be needed
  - Challenge: Aerobic biodegradation of 1,4-dioxane yields CO₂, biomass, and possibly soluble intermediates; how to document product formation?

• \(^{14}\)C assays quantify products and allow for measurement of a rate coefficient

Adamson et al., 2014

n = 103 sites where dioxane and chlorinated solvents co-occur
What is BioPIC (ESTCP ER-201129)?

- Bioremediation Pathway Identification Criteria
- Updated protocol for evaluating natural attenuation
- Guides users in the selection of MNA, biostimulation and/or bioaugmentation or other remedial technology
- Spreadsheet driven (Excel™)
- Currently limited to chlorinated ethenes
  - Development of ‘BioPic 2.0’ for 1,4-Dioxane and associated cVOCs (ESTCP 201730)

To Obtain BioPIC
Search under ER-201129 Report at SERDP-ESTCP
1,4-Dioxane – ESTCP 201730

**GOAL:** Develop tool that will walk RPMs through this process for evaluating MNA

- Extract Rate Constant from Field Data or $^{14}$C Assay
- Use Model to Forecast Conc. at Point of Compliance
- Consider Active Remedial Options
- Max Conc. $\leq$ Standards
  - No
  - Yes: MNA Plausible. Support with Second Lines of Evidence
1,4-Dioxane – ESTCP 201730

- Geographic diversity
  - ≥ 4 states; East coast, West coast, Midwest
- Mix of Department of Defense and industrial sites (7 sites)
- All exhibit a decrease in C/C₀ 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: Δ¹⁴C products; Δ1,4-dioxane; VOCs; ΔO₂
  - Also included CSIA and relevant biomarkers
Basic Test Procedure: $^{14}$C Assay

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

Measure initial conditions: $^{14}$C, 1,4-DX, VOCs, O$_2$

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

Calculate $k_{net} = k_{GW} - k_{FSGW}$

and net 95% Confidence Interval

End of incubation analyses: $^{14}$C products, 1,4-DX, VOCs, O$_2$
1,4-Dioxane – ESTCP 201730

100 mL GW → HCl, N₂ → SPE → NaOH → Liquid Scintillation Counter, count ¹⁴CO₂ (A)

Solid Phase Extraction (SPE), retains DX → Liquid Scintillation Counter, count ¹⁴C-nonvolatile products (B)

Concentrate by lyophilization → HPLC → Collect fractions → Liquid Scintillation Counter quantify non ¹⁴CO₂ metabolites (e.g., glyoxylate)

∑(A+B)

Time (weeks)
Overall

- 36 well samples analyzed
- 12 have statistically significant rate coefficients
- Maximum rate coefficient* = 0.096 yr\(^{-1}\)
  Median rate coefficient = 0.0061 yr\(^{-1}\)

**1,4-Dioxane – ESTCP 201730**

\[ k_{\text{net}} = 0.096 \text{ yr}^{-1} \]
\[ t_{\frac{1}{2}} = 7.2 \text{ yr} \]
(95% CI = 6.3-9.6 yr)

- **36 well samples analyzed**
- **12 have statistically significant rate coefficients**
- **Maximum rate coefficient* = 0.096 yr\(^{-1}\)**
  Median rate coefficient = 0.0061 yr\(^{-1}\)
- Highest rate constants at the lowest VOCs and 1,1-DCE
- Rate constants found at
  - 6.3 µM VOCs
  - 29 µg/L 1,1-DCE (0.30 µM)
- VOCs likely slow in situ rates
1,4-Dioxane – ESTCP 201733

Multiple Primary Substrates: Isobutane + Methane

Process A: Isobutane + Oxygen
- Stimulate Isobutane degraders
- 1,4-D targeted
- 1,1-DCE and select other CVOCs

Process B: Methane + Oxygen
- Stimulate methanotrophs
- TCE targeted
- Select other CVOCs
1,4-Dioxane – ESTCP 201733

NAS North Island: Operable Unit 11

1. Waste disposal–surface impoundments
2. Shallow aquifer (25’-40’ BGS)
3. Neutral pH
4. Comingled 1,4-D and CVOCs (mg/L)

1,4-D Plume

TCE Plume

• Anticipate • Innovate • Accelerate •
• Sandy Aquifer: 25’- 40’ bgs
• Low permeability unit at 40’
1,4-Dioxane – ESTCP 201733

Discrete groundwater sampling results

• Concentrations generally increasing with depth

• Plume located from ~30’ to 40’ bgs

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<th>HPT-2</th>
<th>HPT-3</th>
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<tr>
<td>Depth (ft, bgs)</td>
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<td>34</td>
<td>39</td>
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<tr>
<td>Compound/Concentration</td>
<td>µg/L</td>
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<td>0.94J</td>
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1,4-Dioxane – ESTCP 201733

Microcosm Test

Objectives

1. Can indigenous organisms be stimulated to degrade 1,4-D and target cVOCs?
   - Evaluating several alkane/alkene gases

2. Are low levels achievable?

3. Nutrients required/beneficial?

4. Bioaugmentation required?
Isobutane Treatments

- No isobutane uptake in 107 days
- Suspect 1,1-DCE toxicity
- ENV493 added on day 107
- Rapid transformation of 1,1-DCE
- Uptake of isobutane (3 days)
Isobutane Treatments

- ENV493 added on day 107
- 3 successive spikes of isobutane
- Rapid transformation of 1,4-dioxane, cis-DCE and chloroform
1,4-Dioxane – ESTCP 201733

Isobutane Treatments

- Limited cometabolism of TCE by ENV493
- Mixed methanotroph culture ENV494M and methane added
- TCE degraded and methane uptake observed
Both Cultures Isolated from the Site
1,4-Dioxane – ESTCP 201733

Preliminary MODFLOW Modeling Simulation

**Groundwater Modeling**

- Groundwater recirculation
  - 1 extraction well
  - 5 injection wells
  - 15’ well spacing
- ~8-10 gpm – 8 hr/day
- Substrate gas & nutrient addition
- Bioaugmentation

7 gpm; 8 hrs day; 60 days
Acknowledgements

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Thank you!