Bioaugmentation With KB-1™ for *In Situ* Treatment of Chlorinated Ethenes

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State Coalition for Remediation of Dry Cleaners Conference
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Presentation Overview

- Background on *Dehalococcoides*
  - Role in reductive dechlorination
  - Distribution
  - Detection

- Introduction to KB-1™ Dechlorinator

- Kelly AFB, TX – Bioaugmentation Case study with KB-1™
Role of *Dehalococccoides* (DHC)

- *Dehalococccoides* **ONLY KNOWN** microorganisms that mediate complete dechlorination of chloroethenes to ethene

- At Sites Where DHC is absent dechlorination stalls at *cis*-DCE (Hendrickson et al., 2001)

*SEM of DHC Courtesy S. Zinder*
Distribution of *Dehalococcoides*

- In contrast to bacteria that biodegrade hydrocarbons, evidence exists that DHC bacteria are **NOT** ubiquitous in the environment
  - Molecular surveys (Hendrickson et al., 2002)
  - Incomplete dechlorination observed at hundreds of sites under natural and enhanced conditions
Field Detection of *Dehalococcoides*

Gene-Trac Testing for:
- Presence/absence indigenous DHC
- Monitoring KB-1 dispersion *in situ*
- Offered commercially by SiREM
What is KB-1™ Dechlorinator?

- Mixed anaerobic culture for *in situ* bioaugmentation of chloroethene sites
- Natural culture enriched from a TCE DNAPL site
- Contains several strains of *Dehalococcoides*
- Free of known pathogens
- **NOT** genetically modified

*DAPI Stained KB-1 500X magnification*
DGGE Analysis of KB-1

A = *Dehalococcoides* sp.
B = *Dehalococcoides* sp.
F = *Spirochaetes*
G = *Sulfuricurvum* (sulfur oxidizer)
H = Beta *Proteobacteria*
I = Related to Rice Paddy Soil Bacterium (Candidate Division WS3)
J = same as I
P = *Spirochaetes*
Q = *Dehalococcoides* sp.
R = *Desulfovibrio*
## KB-1™ Consistently Tests Negative for Common Pathogens

<table>
<thead>
<tr>
<th>Organism Tested For</th>
<th>Disease(s) Caused</th>
<th>Test result</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em> sp.</td>
<td>Typhoid fever, gastroenteritis</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Listerioses</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Vibrio</em> sp.</td>
<td>Cholera, gastroenteritis</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Campylobacter</em> sp.</td>
<td>Bacterial diarrhea</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Clostridia</em> sp., <em>(Hemolytic)</em></td>
<td>Food poisoning, Botulism, tetanus, gas gangrene</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Bacillus anthracis</em></td>
<td>Anthrax</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa,</em></td>
<td>Wound infection</td>
<td>Negative</td>
</tr>
<tr>
<td><em>Yersinia</em> sp.</td>
<td>Bubonic Plague, intestinal infection</td>
<td>Negative</td>
</tr>
<tr>
<td>Pathogenic Yeast</td>
<td>Candidiasis, Yeast infection etc.</td>
<td>Negative</td>
</tr>
<tr>
<td>Fecal coliforms</td>
<td>Indicator organisms for many human pathogens viruses etc., diarrhea, urinary tract infections</td>
<td>Negative</td>
</tr>
<tr>
<td>Enterococci</td>
<td>Various opportunistic infections</td>
<td>Negative</td>
</tr>
</tbody>
</table>
KB-1 Production

Grown under sterile conditions in 100L pressure vessels
Transferred to 20 L vessels for field injection
Kelly AFB Case Study

Groundwater Chemistry

- Predominantly PCE, some TCE and cis-DCE
- No Vinyl Chloride or ethene
- Total chlorinated VOCs ~2 mg/L
- Nitrate = 24 mg/L; sulfate = 16 mg/L
- Dissolved iron = 0.06 mg/L
- Aerobic and oxidizing groundwater (DO ~2 mg/L, positive ORP)
Site Geology and Hydrogeology

- Unconsolidated alluvial deposits over clay
- Water bearing unit is clayey-gravel
- Thickness ranges from 20 to 40 feet
- Depth to water ~10-15ft bgs
- Saturated thickness ~15-25 feet
- Ambient GW velocity of 3 ft/day
In Situ Pilot Test

- Closed-loop groundwater recirculation system was employed
- Pore Volume estimated at 64,000 L
- One P.V. in ca. 8 days
- Bromide used in conservative tracer, test used to calibrate transport model
Pre-Bioaugmentation

- Period of electron donor addition (Acetate/methanol) to develop reducing conditions appropriate for KB-1

- PCE dechlorination only to $cis$-1,2-DCE observed through electron donor addition

- No VC or ethene detected (stalled at $cis$-DCE)
Bioaugmentation In Progress
Post-Bioaugmentation

- Trace VC detected at B1 16 days after bioaugmentation
- Ethene detected at B1 72 days after bioaugmentation
- 115 days after bioaugmentation PCE, TCE and DCE were below MCLs at B1. VC was at 25 µg/L
- Time to appearance of VC and ethene similar to microcosms
VOC Results From B3

Grey area – system off, Orange dashed line – ED addition of acetate and methanol
Blue line - Bioaugmentation
Day 173-Electron Donor Phase

Avg. 
E1+E2

B1

PCE
TCE
cis-12DCE
VC
Ethene

umoles
Day 269 – 66 Days After Bioaugmentation

Avg. E1+E2

B1

0 5 10 umoles

PCE
TCE
cis-12DCE
VC
Ethene
Day 318 – 115 Days after Bioaugmentation

![Graph showing the concentration of different compounds over time.](image-url)

- **Avg. E1+E2**
- **B1**

- **Umoles**

- **Legend:**
  - PCE
  - TCE
  - cis-12DCE
  - VC
  - Ethene
## Summary of Field Half-Lives

<table>
<thead>
<tr>
<th>Days Post Bioaugmentation</th>
<th>PCE</th>
<th>TCE</th>
<th>cis-DCE</th>
<th>VC</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>1.0</td>
<td>3.0</td>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>93</td>
<td>1.0</td>
<td>2.5</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>115</td>
<td>1.0</td>
<td>2.5</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>142</td>
<td>1.0</td>
<td>2.5</td>
<td>0.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Injection and “Growth” of the KB1 Bioaugmentation Culture:
Illustration based on D.E. & VOC Monitoring Results

KB1 injected into ground 5/06/2000

Groundwater Gradient

Ground Surface

Nutrient Injection well (6" stainless steel, 0.040" slot screen)

1 of 3 Extraction wells (4" stainless steel, 0.010" v-wire wrap screen)
DHC Signature Sequence Found at Kelly Air Force Base

Variable Regions
= Conserved Regions

= Base Substitutions

= Consensus Sequence of DHC Strain 195

DHC kb1

MWB1 Groundwater Samples 6/2000

Kelly AFB Land Fill Trenches

DHC Strain 195

0 300 600 900 1200 1400

S1 S2 S3 S4 S5 S6 S7 S8 S9

= 100 nucleotides

S1 S2 S3 S4 S5 S6 S7 S8 S9

1434
Electron donors stimulated dechlorination only to *cis*-DCE in the control plots, and did not result in growth of an indigenous *Dehalococcoides*. 

*Dehalococcoides* associated with KB-1 only detected in bioaugmentation plot, and didn’t spread outside bioaugmentation plot.

Molecular results indicated that *Dehalococcoides* grew to higher cell densities in the bioaugmentation plot.
Conclusions

- Bioaugmentation with KB-1 stimulated complete dechlorination
- Electron donor alone didn’t stimulate complete dechlorination
- Dechlorination rates were faster in the field than in microcosms
- Many sites contain indigenous DHC, therefore bioaugmentation not required at all sites
- Bioaugmentation will be required when
  - *Dehalococcoides* not detected
  - Microcosm or field results show incomplete dechlorination
  - Indigenous populations of DHC are too low or incompetent to meet remedial goals within required time frames
Field Applications of KB-1

- Field demonstrations/applications, UIC permits approved in the U.S.
  - AeroJet Superfund Site, CA
  - Industrial Site, MA (Fractured Rock DNAPL)
  - Kelly AFB, TX (funded by RTDF/DoD ESTCP)
  - Caldwell Superfund Site, NJ (Frac. Rock, DNAPL)
  - Dover AFB, DE (DNAPL), in conjunction with NAVY (C. Lebron)
  - NASA KSC, FL (DNAPL – funded by ESTCP)
  - Industrial sites, AL, SC, PA, CA
  - Industrial site, OH (ISCO coupled with bioaug for frac rock)
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