Culture Testing for a Dual-Biofilm Reactive Barrier for Treatment of Chlorinated Benzenes in Wetland Groundwater and Sediment

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in cooperation with
USEPA, Region III and
NIEHS (National Institute of Environmental Health Sciences)
Background

- Chemical plant 1966-2002; EPA Superfund site since 2002
- USGS wetland study with EPA since 2009 to characterize wetland and evaluate bioremediation
- NIEHS research study started Oct 2014 in collaboration with JHU and Geosyntec
Biodegradation Pathways

Anaerobic (reductive dechlorination)
- CB serves as terminal electron acceptor
- Separate e- donor required
- Rate decreases with decreasing number of Cl

Aerobic (oxidation)
- O₂ required as electron acceptor
- CBs utilized as C and e- donor
- Rate increases with decreasing number of Cl
- Short-lived intermediates

Drinking Water MCL µg/L

* Parent contaminant
Conceptual model for contamination and dual-biofilm reactive barrier in wetland
Anaerobic Culture WBC-2

- Enriched from wetland sediment at APG to degrade chlorinated ethanes and ethenes
- Sediment-free culture since 2002
- Readily available in large quantities

Aerobic Culture 15B

- Wetland groundwater from DP-15B at SCD
- Tryptone-yeast extract media
- Fed with CB, 12DCB, 14DCB, and 124TCB
- Incubated aerobically on shaker
Methods: GAC Seeding

- Treatments in duplicate
- 10 g of GAC (Calgon Carbon Corp. Filtrasorb 600-Unsorted) for each
- 10 mL of media or culture in media
- Soaked GAC for 3 days for seeding (anaerobic glove box for WBC-2)
- DNA extracted and frozen until genetic analysis

### Anaerobic

- WBC-2 Media only
- WBC-2 in media + GAC
- WBC-2 in media + GAC + TeCA

### Aerobic

- Groundwater filter samples from 15B
- 15B Media only
- 15B in media + GAC
Workflow for Characterizing Microbial Communities

1. Extract DNA
2. Amplify 16S rRNA gene
   - Gold standard taxonomic gene for microbiology
3. Characterize community composition—diversity, abundance, & identity of organisms
4. Illumina iTag Sequencing

Microbial Community

Illumina iTag Sequencing (magic box)
GAC with WBC-2: Classes >1% Abundance

- Bacilli
- Actinobacteria
- Mollicutes
- Bacteroidia
- Thermotogae
- Gammaproteobacteria
- Betaproteobacteria
- Deltaproteobacteria
- Synergistia
- Clostridia
- Dehalococcoidetes
- Anaerolineae
- Chloroflexi
GAC with WBC-2: Chloroflexi, Order

- Significant increase in Dehalococcoidales on GAC.
- Decrease in Anaerolineales on GAC.
GAC with WBC-2: Genus

1. **Anaerolineae**
   - f__Anaerolinaceae;Other
   - f__Anaerolinaceae;g__C1_B004
   - f__Anaerolinaceae;g__T78
   - f__Anaerolinaceae;g__WCHB1-05

2. **Dehalococcoidetes**
   - f__Dehalococcoidaceae;g__Dehalococcoides
   - f__Dehalococcoidaceae;g__Dehalogenimonas

3. **Deltaproteobacteria**
   - f__Geobacteraceae;Other
   - f__Geobacteraceae;g__
   - f__Geobacteraceae;g__Geobacter

Genus T78 and Dehalococcoides were predominant in both the culture and the WBC-2-seeded GAC.
GAC with 15B: Classes >1% Abundance

Relative Abundance (%)

GW.1  GW.2  15B.1  15B.2  +GAC.1  +GAC.2

- Sphingobacteria
- Bacilli
- Actinobacteria
- Gammaproteobacteria
- Betaproteobacteria
- Alphaproteobacteria
• **Significant increase in the Betaproteobacteria group Burkholderiales on GAC.**
• **Moderate decrease in the Alphaproteobacteria group Rhizobiales on GAC; greater decrease in Caulobacterales.**
GAC with 15B: Proteobacteria, Genus

1. Alphaproteobacteria (Orders Caulobacterales, Rhizobiales)
   - f__Caulobacteraceae;Other
   - f__Caulobacteraceae;g__Brevundimonas
   - f__Brucellaceae;g__Ochrobactrum
   - f__Phyllobacteriaceae;Other
   - f__Phyllobacteriaceae;g__
   - f__Rhizobiaceae;Other
   - f__Rhizobiaceae;g__
   - o__Rhizobiales;Other;Other

2. Betaproteobacteria (Order Burkholderiales)
   - f__Alcaligenaceae;Other
   - f__Alcaligenaceae;g__
   - f__Comamonadaceae;g__Comamonas

3. Gammaproteobacteria
   - f__Pseudomonadaceae;g__Pseudomonas
   - f__Xanthomonadaceae;g__Stenotrophomonas

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cited by others, i.e. Vogt et al., 2002, “Two Pilot Plant Reactors Designed for the In Situ Bioremediation of Chlorobenzene-contaminated Ground Water...”
15B Isolate 16S rRNA Identification  
(Spiked with mixture of 124TCB, 14DCB, 12DCB, CB)

<table>
<thead>
<tr>
<th>Isolate Name</th>
<th>Isolated From</th>
<th>Top GenBank BlastN Hit</th>
<th>Score</th>
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<tr>
<td>15B-YJ-3</td>
<td>Full 15B in 10% media</td>
<td>Pseudomonas genticula</td>
<td>1724</td>
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<tr>
<td>15B-YJ-1</td>
<td>10% media plate isolate, directly from plate</td>
<td>Pseudomonas genticula</td>
<td>1818</td>
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<td>15B-YJ-5</td>
<td>10% media plate isolate transferred to liquid media</td>
<td>Pseudomonas genticula</td>
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<td>10% media plate isolate transferred to liquid media</td>
<td>Pseudomonas genticula</td>
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<td>15B-YJ-2</td>
<td>1% media plate isolate, directly from plate</td>
<td>Pseudomonas genticula</td>
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<tr>
<td>YJ-15B-1</td>
<td>100% media plate isolate with DNAPL CBs</td>
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<tr>
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Microcosm Results: Aerobic 15B Seeded on GAC

- Distinct decrease in CBs with culture in mineral media compared to DIW
- Delay in sorption to GAC with aerobic biofilm
- Slightly faster overall CB removal in biofilm-GAC
Microcosm Results:
Anaerobic WBC-2 Biofilm on GAC

- Slight decrease in CBs with culture in mineral media compared to DIW Rapid sorption to GAC with and without anaerobic biofilm
- Distinctly faster overall CB removal in biofilm-GAC
Conclusions

- GAC provides an effective growth matrix for the anaerobic WBC-2 and aerobic 15B cultures
- GAC enhanced the abundance of microbial groups/species involved in biodegradation; thus a good delivery and support matrix for the cultures
  - Dehalococcoides significantly increased (~doubled) in WBC-2 seeded GAC
  - Burkholderiales group doubled in 15B seeded GAC
  - Pseudomodales in 15B-seeded GAC community
- Biodegradation occurred in conjunction with sorption by GAC seeded with the cultures
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- Pilot test remediation

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**NIH National Institute of Environmental Health Sciences**

- R01 Program – Strengthens Remediation and Detection Mandates
  - Biogeochemical Interactions Affecting Bioavailability for in situ Remediation of Hazardous Substances (R01)

- **DETECTION and MONITORING**
  - bioavailability measures

- **REMEDICATION** – using biogeochem for effective remediation design

- **RISK:** Identify Factors in Uptake
- **HEALTH:** Reduced Exposure

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**United States Environmental Protection Agency**
Questions?