Nucleic Acid-Based Tools for Monitoring Bioremediation at Chlorinated Solvent Sites

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What’s In It For Me?

• Learn the science behind MBTs
• Learn when to use MBTs
• Learn how to sample groundwater for MBTs
• Learn the “Rules of Thumb” for MBT data
• Learn how to save time and money with a smarter bioremediation design and operation—do I bioaugment?
Types of MBTs

• Nucleic acid probes (DNA or RNA)
  ▪ Various targets including
    • 16S rRNA gene
    • Functional genes (e.g., RDase, Hydrogenase, Oxygenase, etc.)

• Protein biomarkers
• Lipid biomarkers
Purpose of MBTs

• Reduce remediation costs and increase effectiveness by
  ▪ Supporting sites where MNA is being evaluated
  ▪ Predicting sites where biostimulation will succeed
  ▪ Identifying sites where bioaugmentation is required
Chlorinated Ethene Reductive Dechlorination

- **PCE** → **TCE** → **cis-DCE** → **VC** → **Ethene**

- **Desulfitobacterium sp. strain Viet1**
- **Desulfitobacterium sp. strain PCE1**
- **Desulfuromonas michiganensis**

**Sulfurospirillum**, **Desulfitobacterium**, **Dehalobacter**, **Geobacter**
Dehalococcoides (Dhc) Involved in Reductive Dechlorination

Dehalococcoides ethenogenes strain 195

Dehalococcoides sp. strain FL2
He et al. 2005. Environ. Microbiol. 7:1442

Dehalococcoides sp. strain BAV1
Müller et al., 2004, AEM, 70:4880
Sung et al., 2006, AEM, 72:1980

Dehalococcoides sp. strain VS

Dehalococcoides sp. strain GT
16S rRNA Gene Targets for Dhc

- 16S rRNA found in all bacteria
- rRNA part of the ribosome; critical for protein biosynthesis
- Contains variable regions which allows for the differentiation of bacterial species
- *Dhc* has one 16S rRNA gene per cell
- *Dhc* 16S rRNA gene count = number of *Dhc* cells

The 16S rRNA molecule has insufficient information to infer physiological traits
**Dhc Reductive Dehalogenases**

- **tceA**
  - *Dehalococcoides ethenogenes* strain 195
  - *Dehalococcoides* sp. strain FL2
  - *Dehalococcoides* sp. strain BAV1

- **bvcA**
  - *Dehalococcoides* sp. strain VS
  - *Dehalococcoides* sp. strain GT

- **vcrA**
  - *Dehalococcoides* sp. strain KB-1/VS
qPCR Sensitivity: Detection vs. Quantification

Extract Community DNA

qPCR

PCR

Amplification with universal 16S rRNA gene-targeted primers (for nested PCR)

Dechlorinator targeted primers

Sensitive quantification of dechlorinating bacteria (~10^3 copies/L)

Sensitive detection of dechlorinating bacteria (~10^1 copies/L)

Genomic DNA
**Dhc Rules of Thumb in the Field**

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MBT Sampling

• Groundwater sampling preferred
  ▪ Difficulties with repetitive soil sampling
  ▪ Spatial variability in soil
• Sampling method influences results
• Use SOP that complements VOC sampling methods
Groundwater Sampling

Shipping groundwater samples is problematic
- Heavy, costly
- Leakage/breakage
- Biomarker integrity
- Groundwater disposal

Improved procedure: Filtration in the field
- Economical
- No leakage/breakage
- Biomarker stability?
- GW remains on-site

ESTCP Project ER-0518: Sterivex™ filters are a viable alternative!
Sampling Considerations

- Sampling biases
- Ratio of planktonic vs. attached cells

Results

- Biomarker stability?
- Efficiency of each step?
- Method biases?
- No standards!
- No guidelines for results interpretation
• Low-flow purge
  ▪ Wait for stabilization of geochemical parameters to obtain a sample representative of formation groundwater

• Surging
  ▪ Increases particulate matter in sample for recovery of associated (i.e., attached) biomass

• Field filtration
  ▪ Sterivex™ filters for biomass collection in the field
    • Economical, no leakage/breakage, groundwater remains onsite

• Shipping
  ▪ Secure samples for overnight shipment to laboratory
  ▪ Maintain samples at 4°C

**Sampling protocol should be defined and maintained for the duration of the monitoring effort for a particular site**
Sampling Locations

- Key sampling locations should include
  - Source area(s)
  - Downgradient plume locations where biodegradation products observed
  - Vertical stratification
    - Where possible, use discrete sampling zones and avoid sampling wells with extended screen intervals
• **Seasonal variability**
  - Geochemical conditions and biomarker abundance can be affected by seasonal changes (e.g., rain events, temperature changes, etc.)...be aware!

• **Bioremediation field implementation**
  - Baseline and 1-2 months after injections
  - Quarterly in first 12-18 months
  - Collect with VOC, geochemical and TOC data
Cost

• Field labor
  ▪ Biomass collection can be performed concurrently with sampling events planned for assessment of contaminants
  ▪ Minimal additional time is needed for collection of samples for biomarker analysis

• Laboratory
  ▪ Microbiology labs specializing in biomarker analysis are typically independent from chemical laboratories used for other analyses
  ▪ Typical cost for quantification of *Dehalococcoides* in a sample of groundwater is approximately $250
Cost for Not Using MBTs

- Unnecessary bench tests
- Poorly designed pilot tests
- Inefficient full-scale treatment
  - Application of bioaugmentation and/or biostimulation when MNA would be appropriate
  - Bioaugmentation when sufficient Dhc are present to meet remediation goals
  - Failure to bioaugment when Dhc populations are insufficient
Case Study – NASA MLP/VAB Site

- **TCE Source Area**
  - (~4,000 gal release 1960’s)
- **Biostimulation**
  - Ethyl lactate
- **Performance monitoring**
  - Every other month
  - TCE, cDCE, VC, ethene
  - Dhc and vcrA
  - ~6 data points from each well

![Monitoring Well](30,000 \mu g/L TCE)
![Injection Well](3,000 \mu g/L TCE)
![Injection Well](1,000 \mu g/L TCE)
Groundwater VOCs and Dhc: SAMW-02

Dhc data indicated no need to bioaugment!
Groundwater DHGs and Sulfate: SAMW-02

Sulfate did not inhibit reductive dechlorination!
Correlation of Dhc/vcrA to VOCs and Ethene

- Correlation results:
  - Dhc or vcrA to TCE, DCE or VC
    - Weak correlation ($r_s < 0.33$) for all comparisons
  - Dhc or vcrA to ethene
    - $Dhc$ to ethene = strong correlation ($r_s = 0.66; n = 10; p = 0.05$)
    - $vcrA$ to ethene = strong correlation ($r_s = 0.67; n = 10; p = 0.05$)
Correlation of Dhc/vcrA to Dechlorination Rates

Spearman Test

\[ \text{TCE rate (yr}^{-1}\text{)} \quad \text{DCE rate (yr}^{-1}\text{)} \quad \text{VC rate (yr}^{-1}\text{)} \quad \text{Dhc} \quad \text{DCE rate (yr}^{-1}\text{)} \quad \text{VC rate (yr}^{-1}\text{)} \quad \text{vcrA} \]

- Correlation results:
  - Strong correlations (rs = 1.00) for all comparisons to Dhc
  - Medium correlations (rs = 0.50) for all comparisons to vcrA
- Limited validity to results (only three data points)
Case Study – Milledgeville

- TCE Source (18,000 ppb)
- Bioaugmentation Pilot Testing
  - 3 injection wells and 2 recovery wells oriented perpendicular to the prevailing direction of groundwater flow (southwest)
  - Soluble electron donor (lactate) and dechlorinating culture distributed by recirc
Dehalococcoides and chloroethenes

- PCE
- TCE
- cis-DCE
- VC
- Ethene
- Methane
- 16S rRNA
- Dhc
Correlation of Dhc or RDases to VOCs

• *Dhc or RDases to VOCs*
  - No correlations to *Dhc*
  - Strong correlation of *bvcA* (rs = 0, n = 10, p = 0.02)
  - Strong correlation of *vcrA* to cDCE (rs = -0.80, n = 10, p = 0.01)
  - Strong correlation of *tceA* to VC (rs = 0.76, n = 10, p = 0.02)
  - No other correlations identified
Survey: Correlation of Dhc/vcrA to Ethene

Gene copies/L

For vcrA testing:
- below 2 $\times 10^5$/L ethene not normally detected
- above 1 $\times 10^7$/L ethene commonly detected
- above 1.8 $\times 10^8$/L ethene always detected

Dhc Tests % with Ethene
vcrA Tests % with Ethene

N= 121 Samples vcrA
N=244 samples Dhc

Courtesy: SiREM
**Biostimulation/Bioaugmentation Flowchart**

1. **Will MBT analysis support biostimulation or bioaugmentation?**
   - No
   - Yes
   - Degradation desired in < 6 months?
     - No
     - Yes

2. **Are geochemical conditions favorable?**
   - No
   - Yes

3. **VOC concentrations > 100 μg/L?**
   - No
   - Yes

4. **Biodegradation products?**
   - No
   - Yes

5. **Dhc > 10^4/L - 10^5/L in groundwater?**
   - No
   - Yes

6. **Sample for MBTs**
   - Add donor only
   - Add donor and microbes
### Dhc 16S rRNA gene copies per L | Interpretation

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Summary

- MBTs are valuable tools to monitor biodegradation of chlorinated ethenes.
- SOPs are available for MBT sampling. Field filtration is reliable.
- Biomarker genes (bvcA, vcrA) are indicators of field dechlorination activity.
- Rules of thumb and draft guidance are available.
- Understand limitations of the data.
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