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

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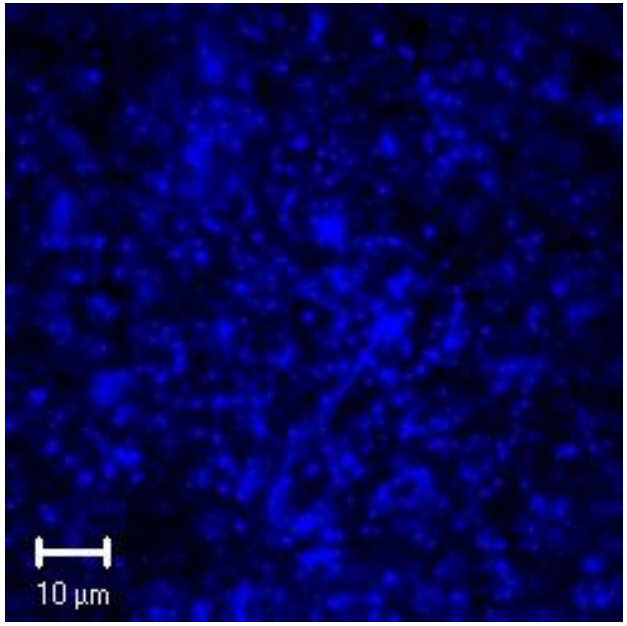
What's In It For Me?

- 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**



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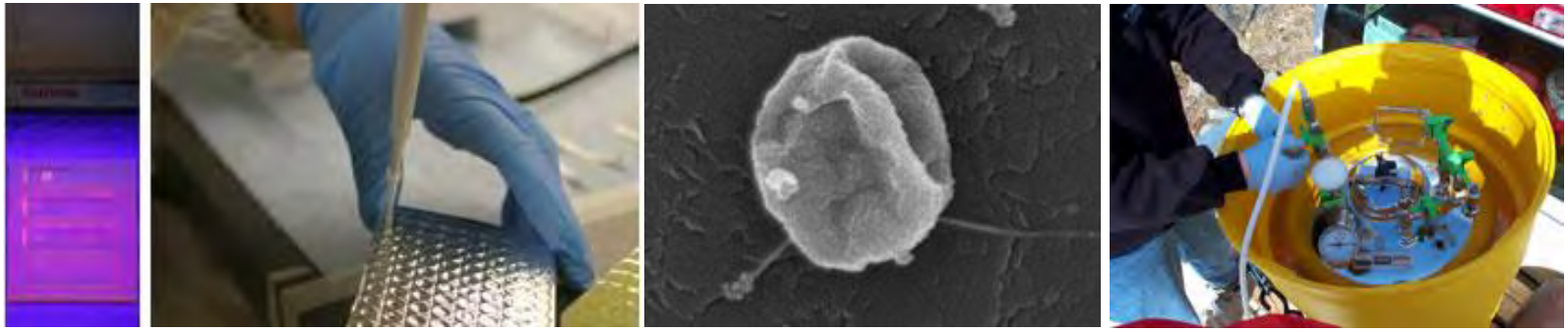
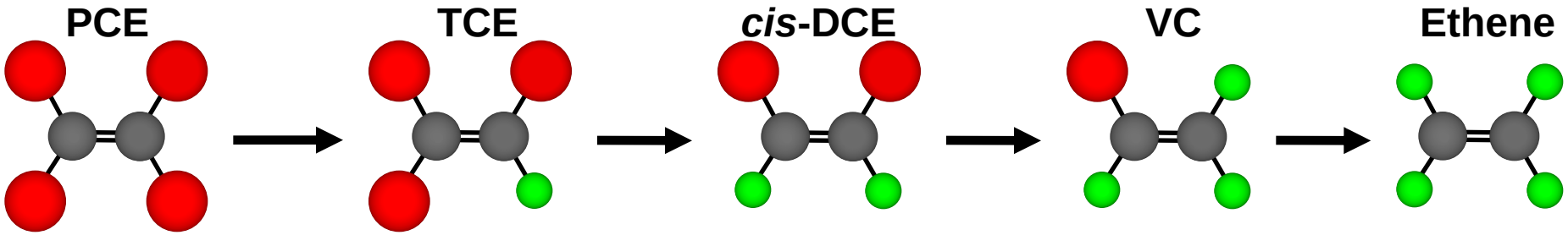
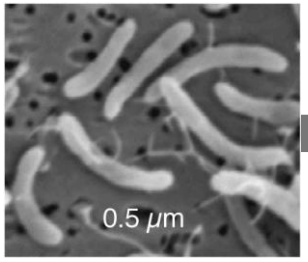
Purpose of MBTs

- **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**



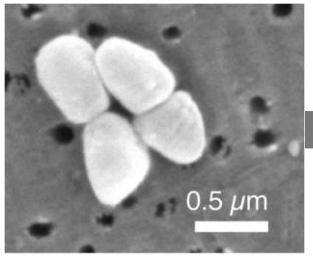
Chlorinated Ethene Reductive Dechlorination





Desulfitobacterium sp. strain Viet1

Desulfitobacterium sp. strain PCE1



Desulfuromonas michiganensis

Sulfurospirillum, Desulfitobacterium, Dehalobacter, Geobacter

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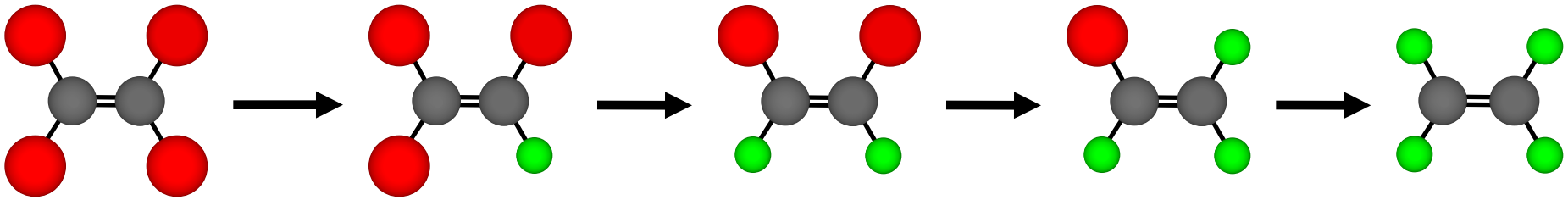
Dehalococcoides (Dhc) Involved in Reductive Dechlorination



Dehalococcoides ethenogenes strain 195

Maymó-Gatell et al. 1997. Science 276:1568

YIELD

Dehalococcoides sp. strain FL2

He et al. 2005. Environ. Microbiol. 7:1442

YIELD

Dehalococcoides sp. strain BAV1

Müller et al., 2004, AEM, 70:4880

Dehalococcoides sp. strain VS

Sung et al., 2006, AEM, 72:1980

Dehalococcoides sp. strain GT

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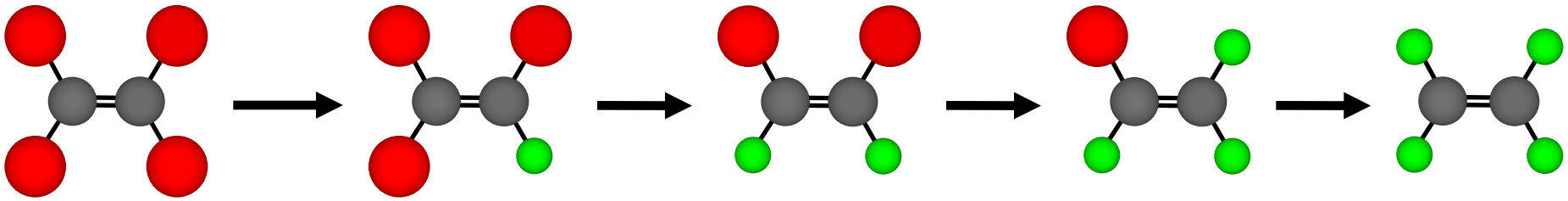
16S rRNA Gene Targets for Dhc

- 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

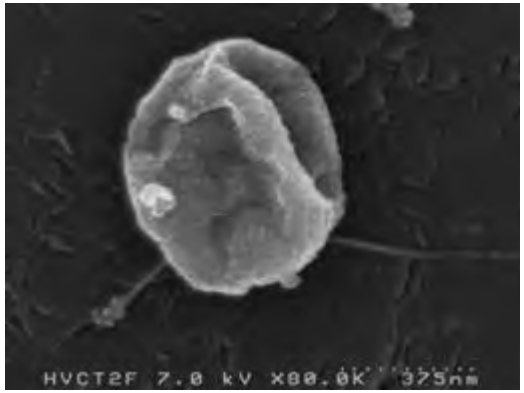
The 16S rRNA molecule has insufficient information to infer physiological traits

Dhc Reductive Dehalogenases



tceA

Dehalococcoides ethenogenes strain 195



Dehalococcoides sp. strain FL2

Dehalococcoides sp. strain BAV1

bvcA

vcrA

Dehalococcoides sp. strain VS

Dehalococcoides sp. strain GT

Dehalococcoides sp. strain KB-1/VS

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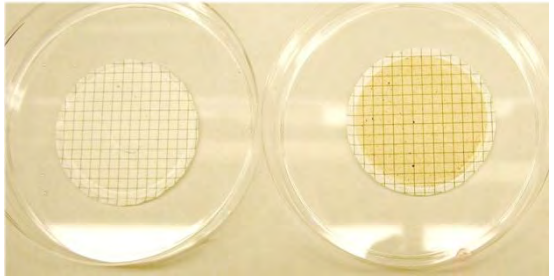
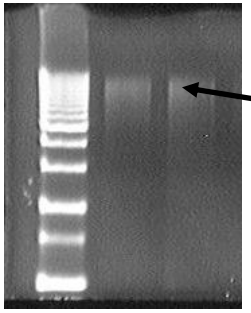
qPCR Sensitivity: Detection vs. Quantification



Extract Community DNA

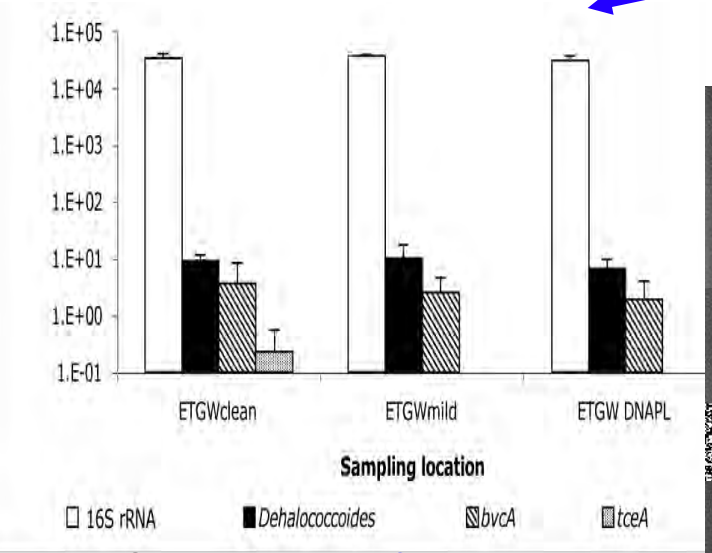
qPCR

PCR

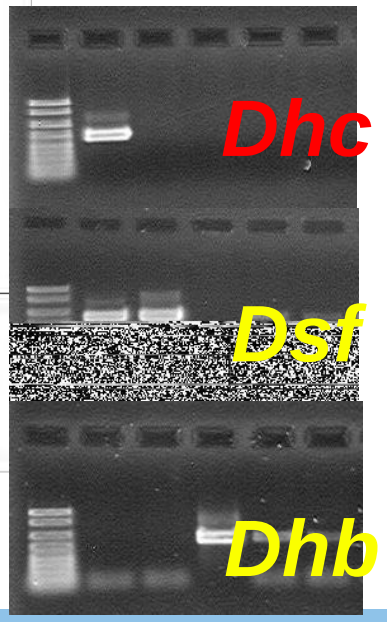


Genomic DNA

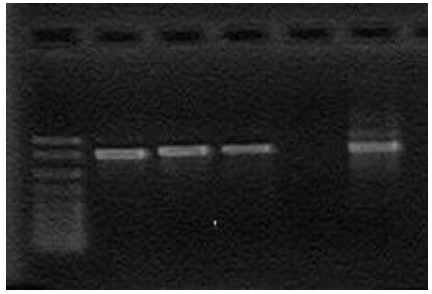
Amplification with universal 16S rRNA gene-targeted primers (for nested PCR)



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



Dechlorinator targeted primers



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

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Dhc Rules of Thumb in the Field

Dhc 16S rRNA gene copies per L	Interpretation
10^3 or lower	Suboptimal *Dhc* to sustain dechlorination rates
10^4 – 10^6	May sustain appreciable dechlorination rates
10^7 or greater	Often associated with high rates of dechlorination and ethene production

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

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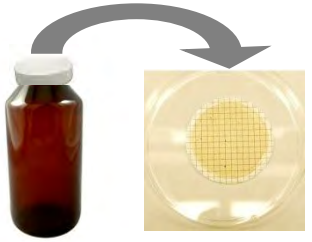
Groundwater Sampling

Shipping groundwater samples is problematic

- Heavy, costly
- Leakage/breakage
- Biomarker integrity
- Groundwater disposal



DNA

RNA

vs.

DNA

RNA

Improved procedure: Filtration in the field

- Economical
- No leakage/breakage
- Biomarker stability?
- GW remains on-site

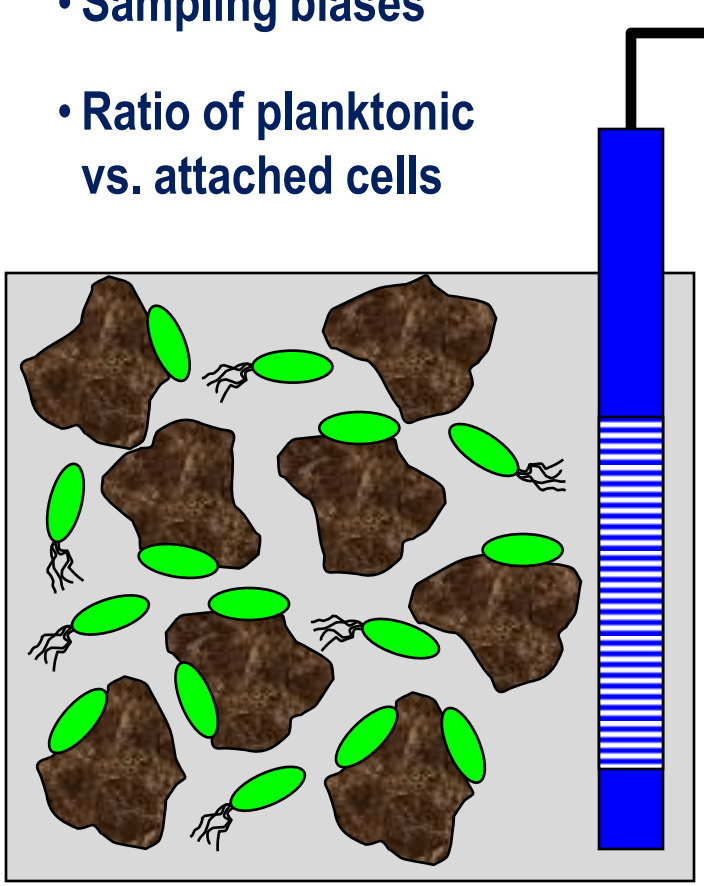
It is not practical to perform vacuum filtration in the field

ESTCP Project ER-0518:

Sterivex™ filters are a viable alternative!

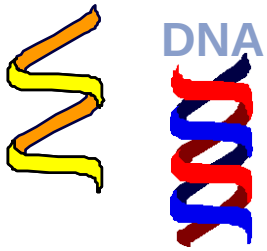
Sampling Considerations

- Sampling biases
- Ratio of planktonic vs. attached cells



RNA

DNA



Results

- Biomarker stability?
- Efficiency of each step?
- Method biases?
- No standards!
- No guidelines for results interpretation

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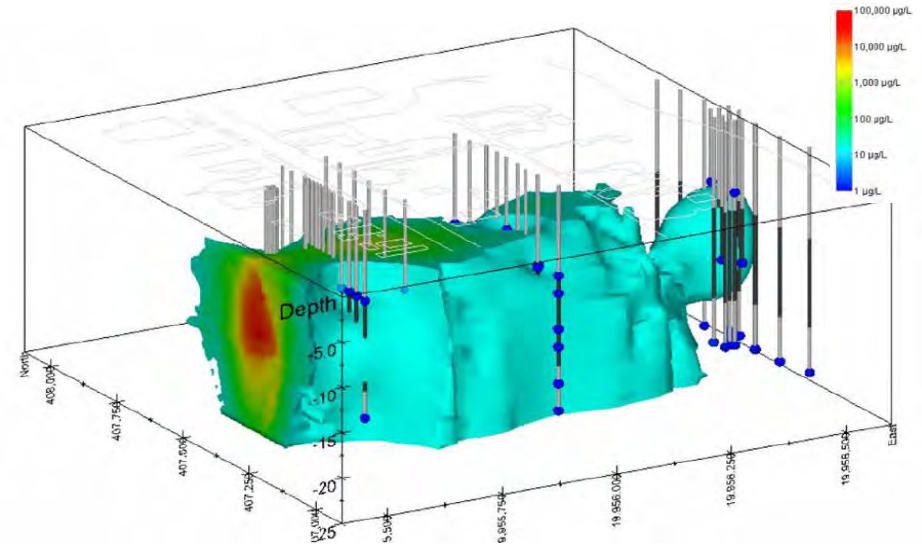
Groundwater Sampling SOP (ER-0518)

- **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 biodeg products observed**
  - **Vertical stratification**
    - **Where possible, use discrete sampling zones and avoid sampling wells with extended screen intervals**



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Sampling Frequency

- **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

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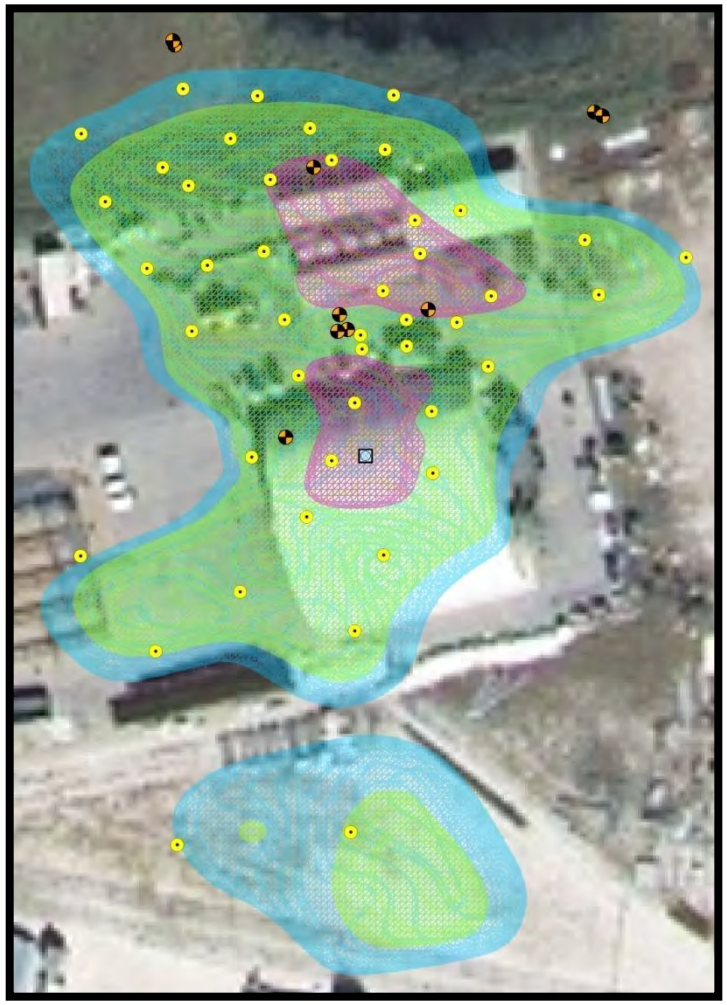
Cost for Not Using MBTs

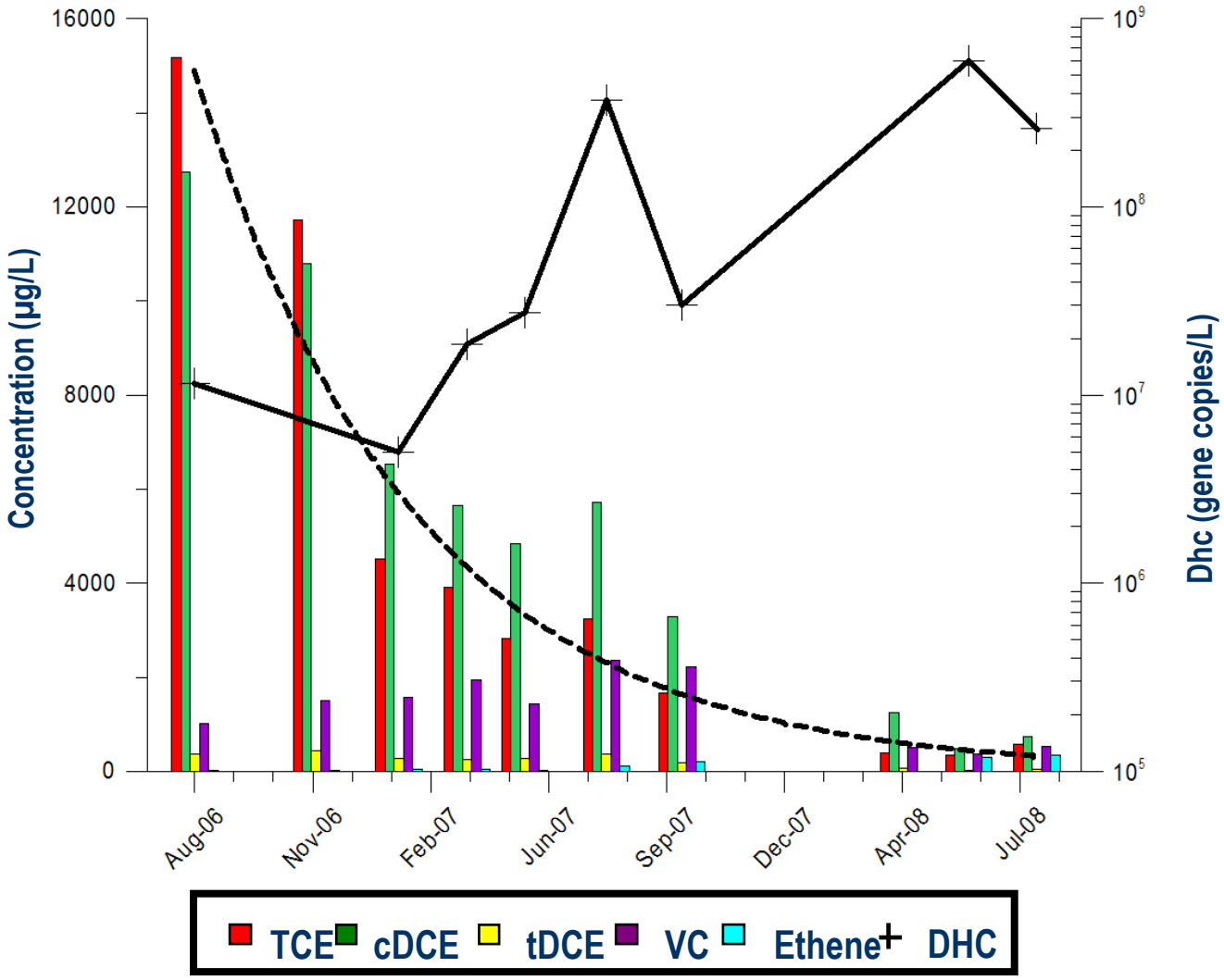
- Unnecessary bench tests
- Poorly designed pilot tests
- Inefficient full-scale treatment
  - Application of bioaugmentation and/or biostimulation when MNA would be appropriate
  - Bioaugmentation when sufficient Dhc are present to meet remediation goals
  - Failure to bioaugment when Dhc populations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



Groundwater VOCs and Dhc: SAMW-02



Dhc data indicated no need to bioaugment!

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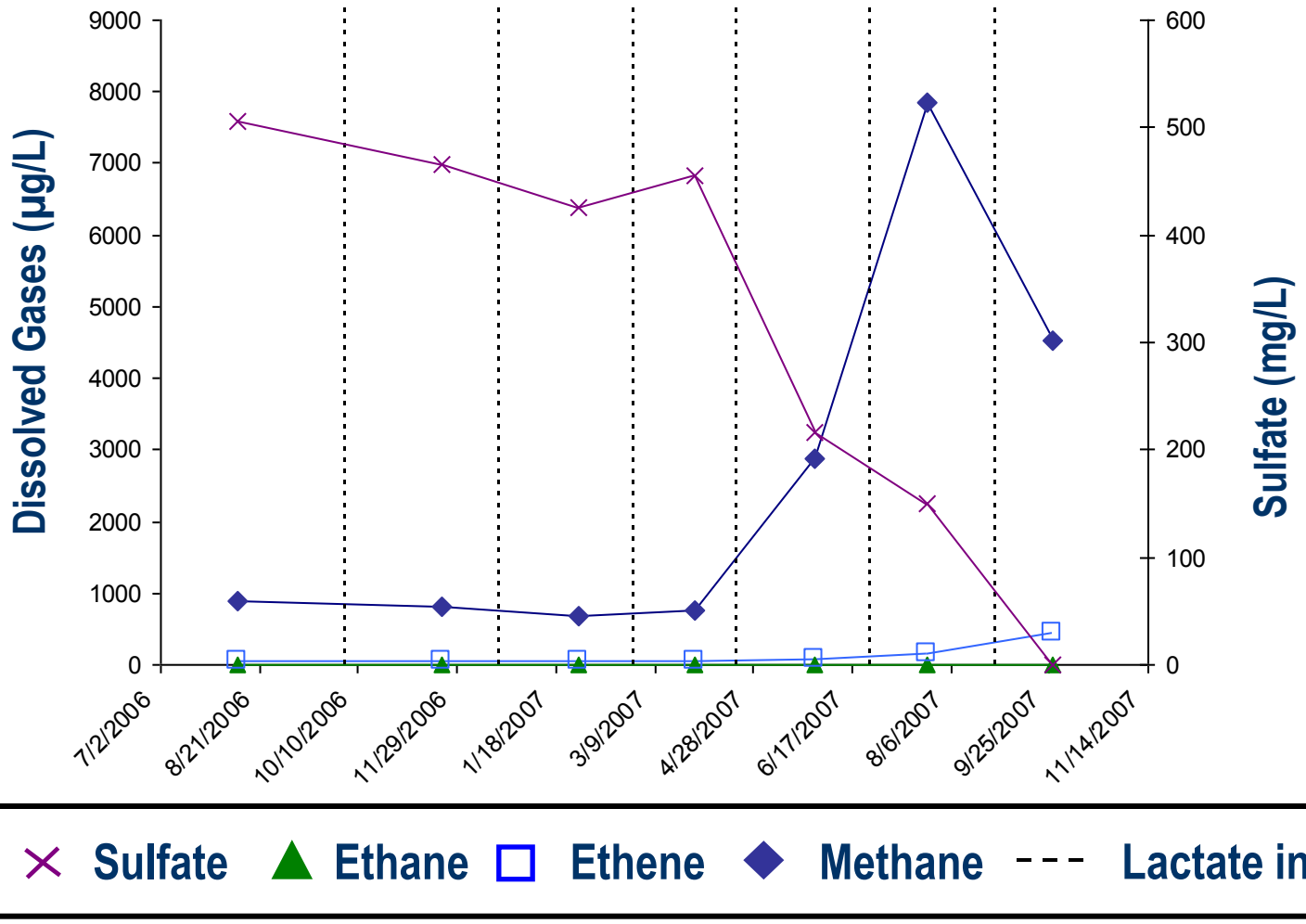
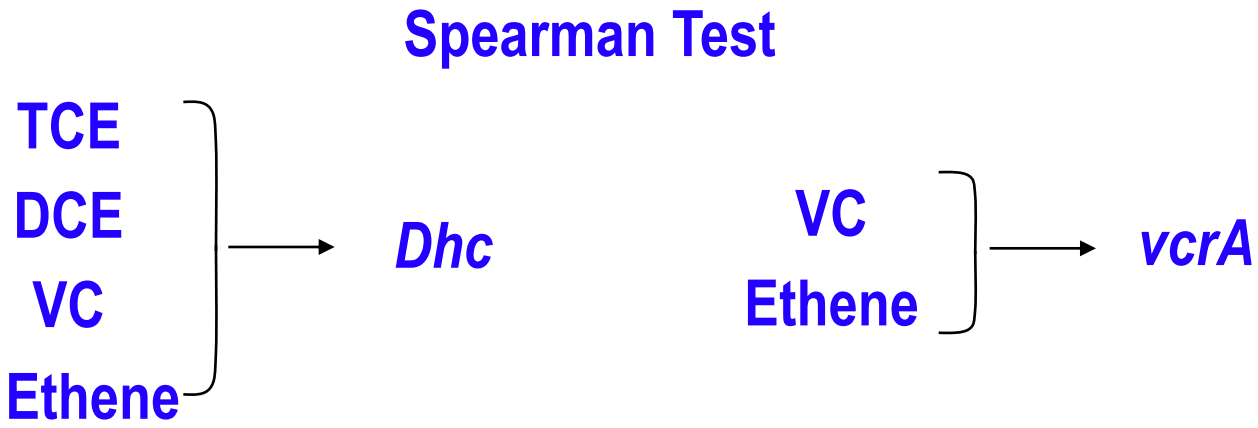
Groundwater DHGs and Sulfate: SAMW-02



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

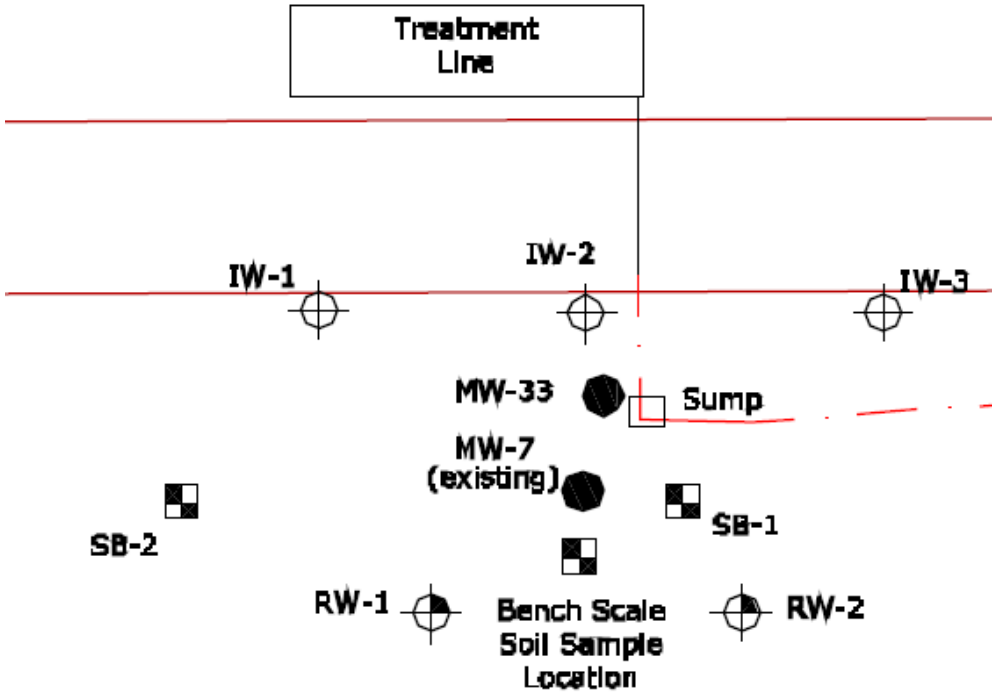
TCE rate (yr^{-1})
DCE rate (yr^{-1})
VC rate (yr^{-1})
→ *Dhc*

DCE rate (yr^{-1})
VC rate (yr^{-1})
→ *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



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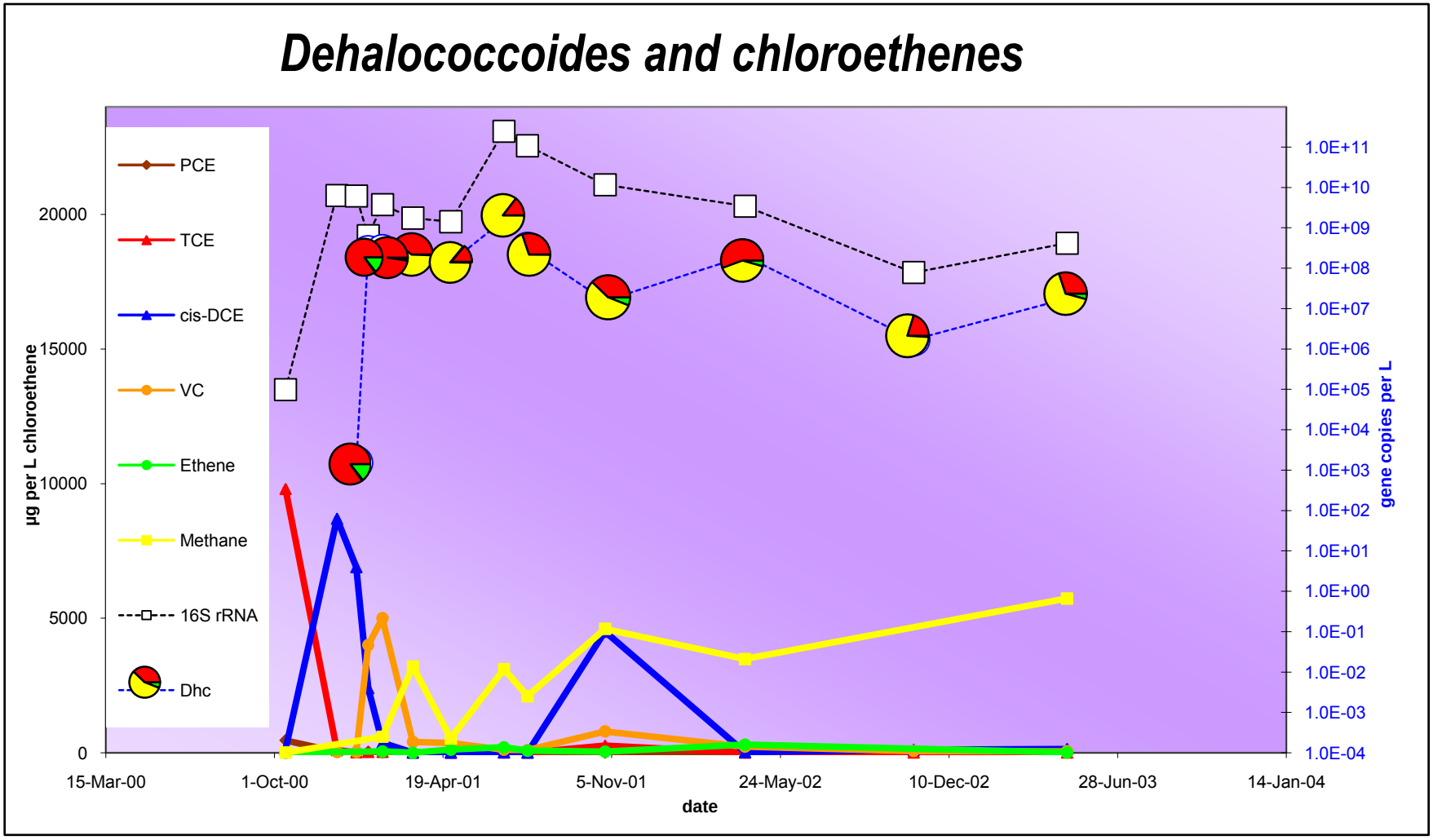
VOC and Dhc Data



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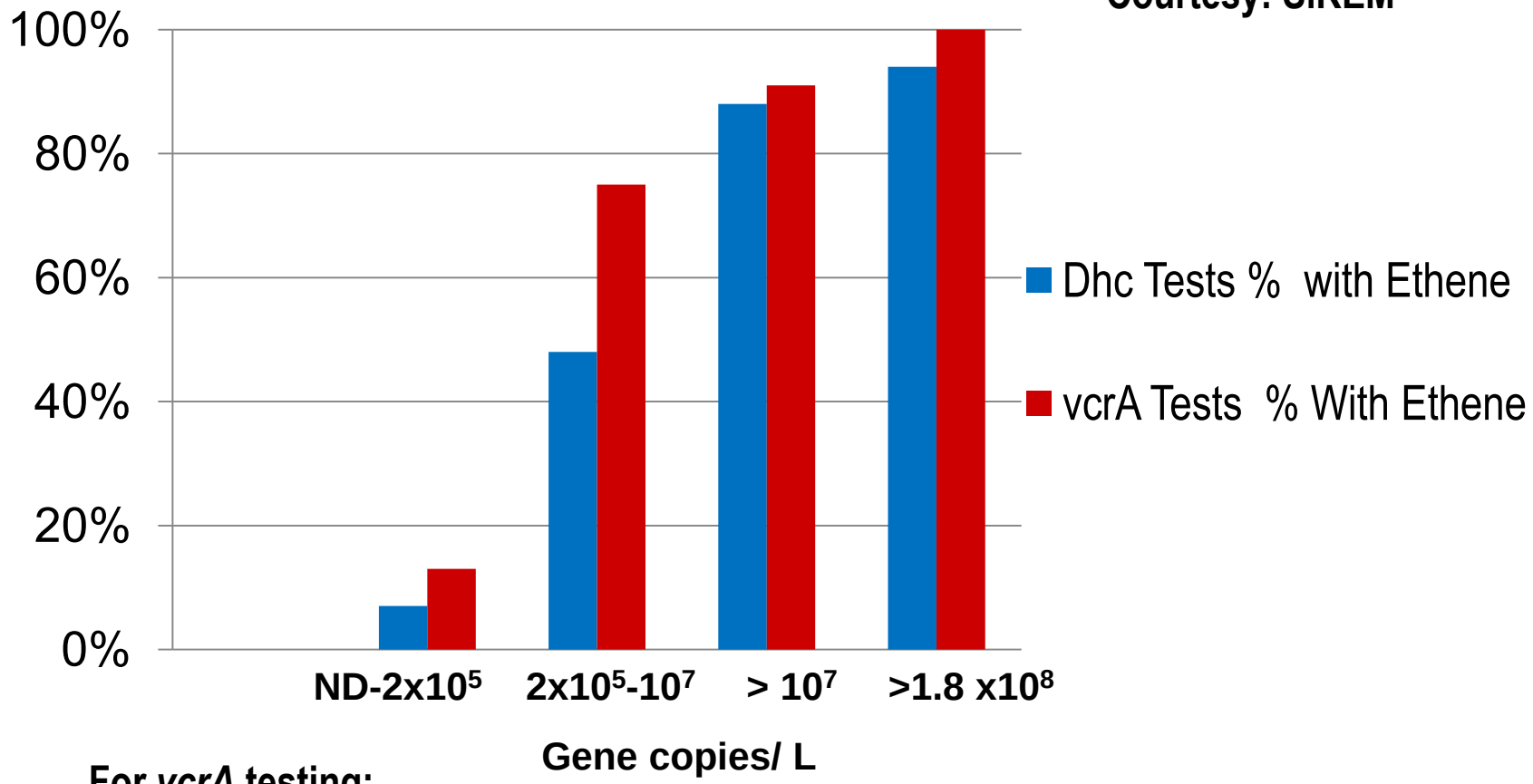
Correlation of Dhc or RDases to VOCs

- ***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

Courtesy: SiREM



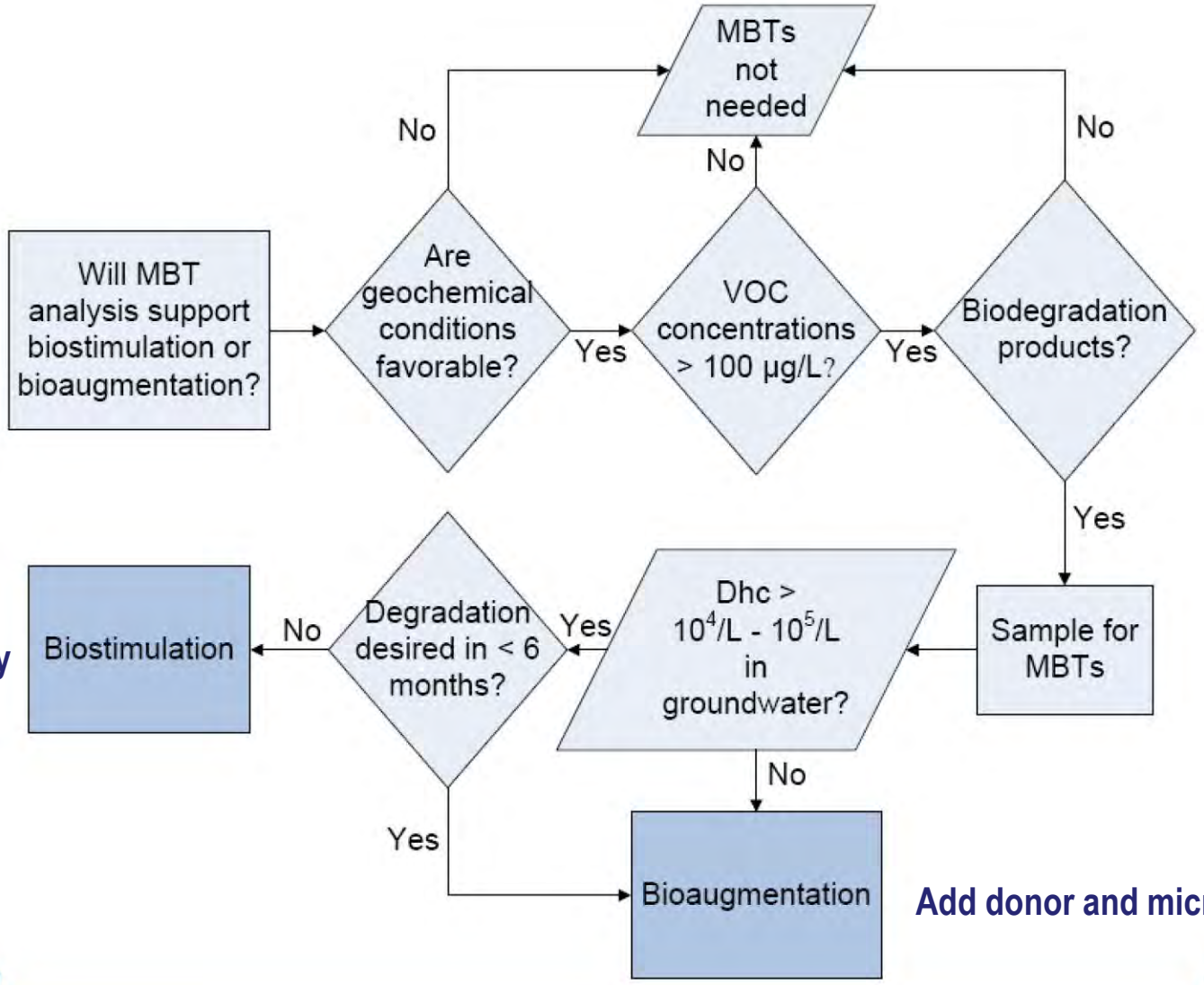
For *vcrA* testing:

- **below 2 x10^5/L ethene not normally detected**
- **above 1 x10^7/L ethene commonly detected**
- **above 1.8 x10^8/L ethene always detected**

N= 121 Samples *vcrA*

N=244 samples Dhc

Biostimulation/Bioaugmentation Flowchart



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Reminder-Dhc Rules of Thumb

***Dhc* 16S rRNA gene copies per L**	**Interpretation**
10^3 or lower	Suboptimal *Dhc* to sustain dechlorination rates
10^4 – 10^6	May sustain appreciable dechlorination rates
10^7 or greater	Often associated with high rates of dechlorination and ethene production

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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