In Situ Bioreactors for In-Well Groundwater Remediation

Eric J. Raes, P.E., LSRP
Kerry Sublette

The Bio-Sep *In Situ* Bioreactor (ISBR)

- Enhancement of *in situ* bioremediation in groundwater with compact bioreactor installed in-well
- Overcomes common limitations of bioremediation of groundwater
  - Low contaminant concentrations
    - A threshold concentration of substrate is required for growth
  - Substrate inhibition
    - At high concentrations some biodegradable contaminants can be toxic to the organisms that have the ability to degrade them

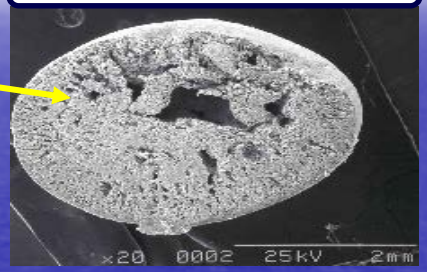
In the beginning…..

- Adsorptive surface
- High surface area

Bio-Trap® Sampler with Bio-Sep® Beads

Nomex and PAC

X-Section of Bio-Sep® Bead

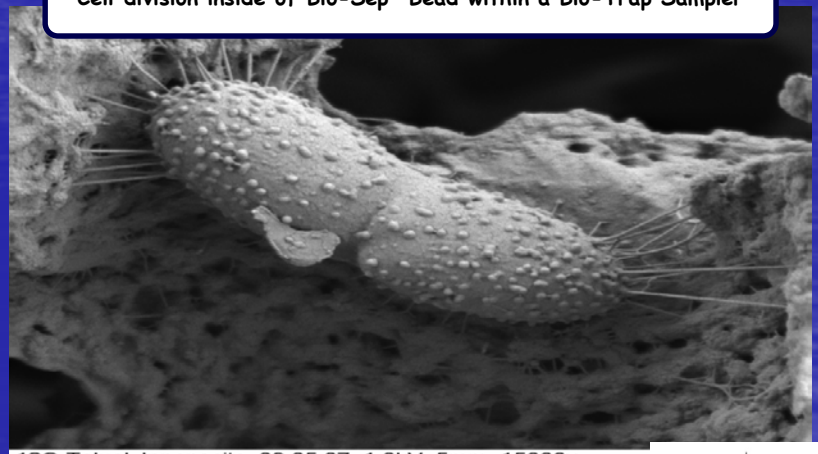


Interior of Bio-Sep® Bead

The Bio-Trap® Sampler:

- Rapidly colonized by indigenous bacteria forming active biofilms
- Thousands used worldwide for over a decade for forensic analysis of groundwater microbiology

Cell division inside of Bio-Sep® Bead within a Bio-Trap Sampler

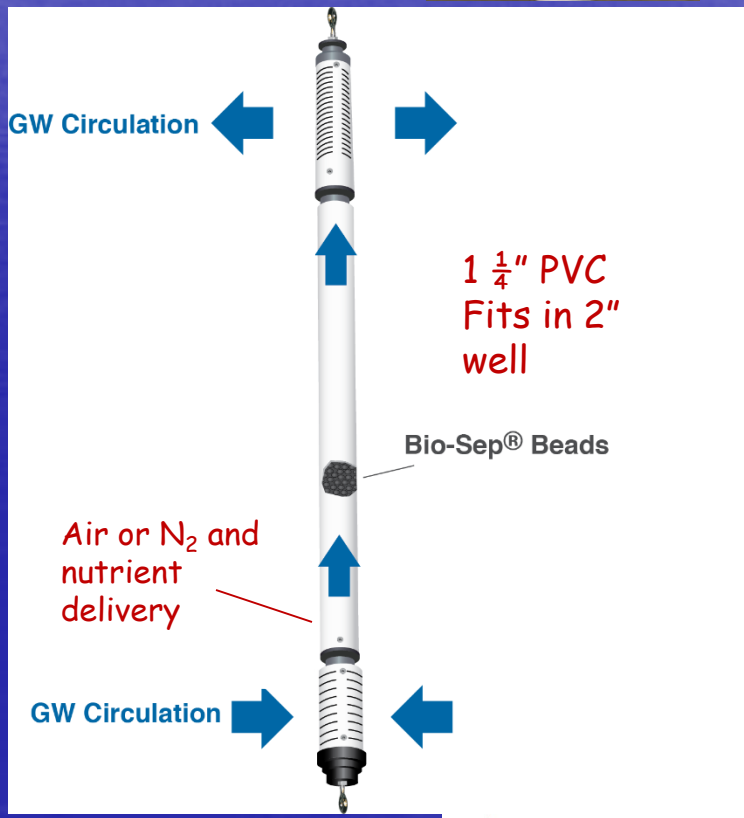


microbialinsights

THE UNIVERSITY of TULSA

The Bio-Sep ISBR

- Bio-Sep beads provide an incredible surface area for microbial growth
- Gas sparging (air or N_2) creates an airlift for circulation of groundwater through the bioreactor.
  - Contaminated groundwater is treated as it moves through the column of Bio-Sep beads
- Nutrient addition (N, P, electron donors, electron acceptors) support growth of desired indigenous microbes
- Water exiting the reactor carries contaminant-degrading microbes into the aquifer



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Topside control

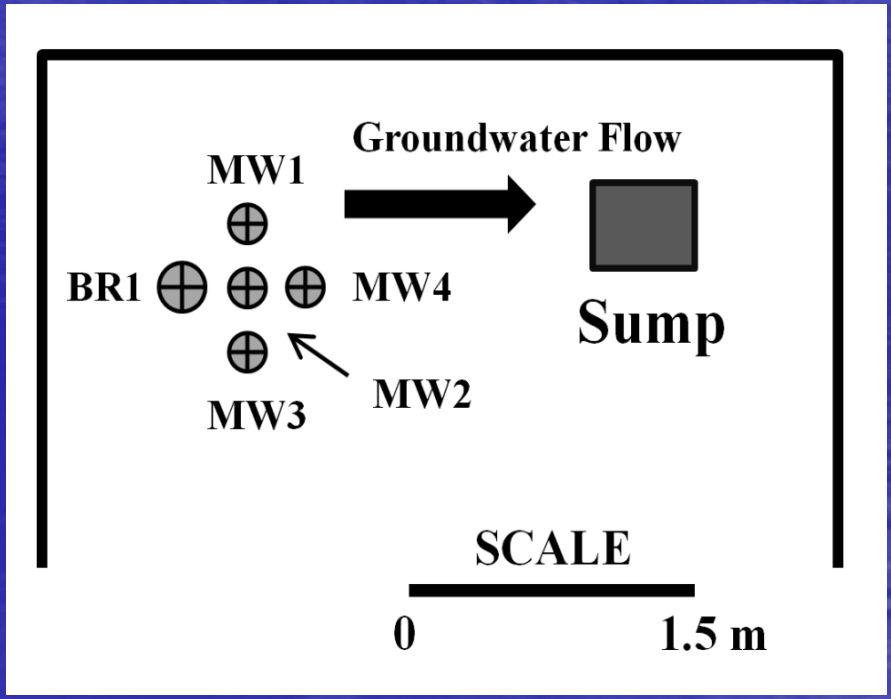
- Nutrient reservoirs and pumps
- Air pump
- Air flow control

Bio-Sep ISBR Applications

- Petroleum hydrocarbons
  - Aerobic
  - Anaerobic
- Chlorinated hydrocarbons
  - Anaerobic
- Fuel oxygenates (MTBE, TBA)
- Emerging contaminants (1,4-Dioxane)

Case Study – Aerobic ISBR – Low Concentrations of Hydrocarbons

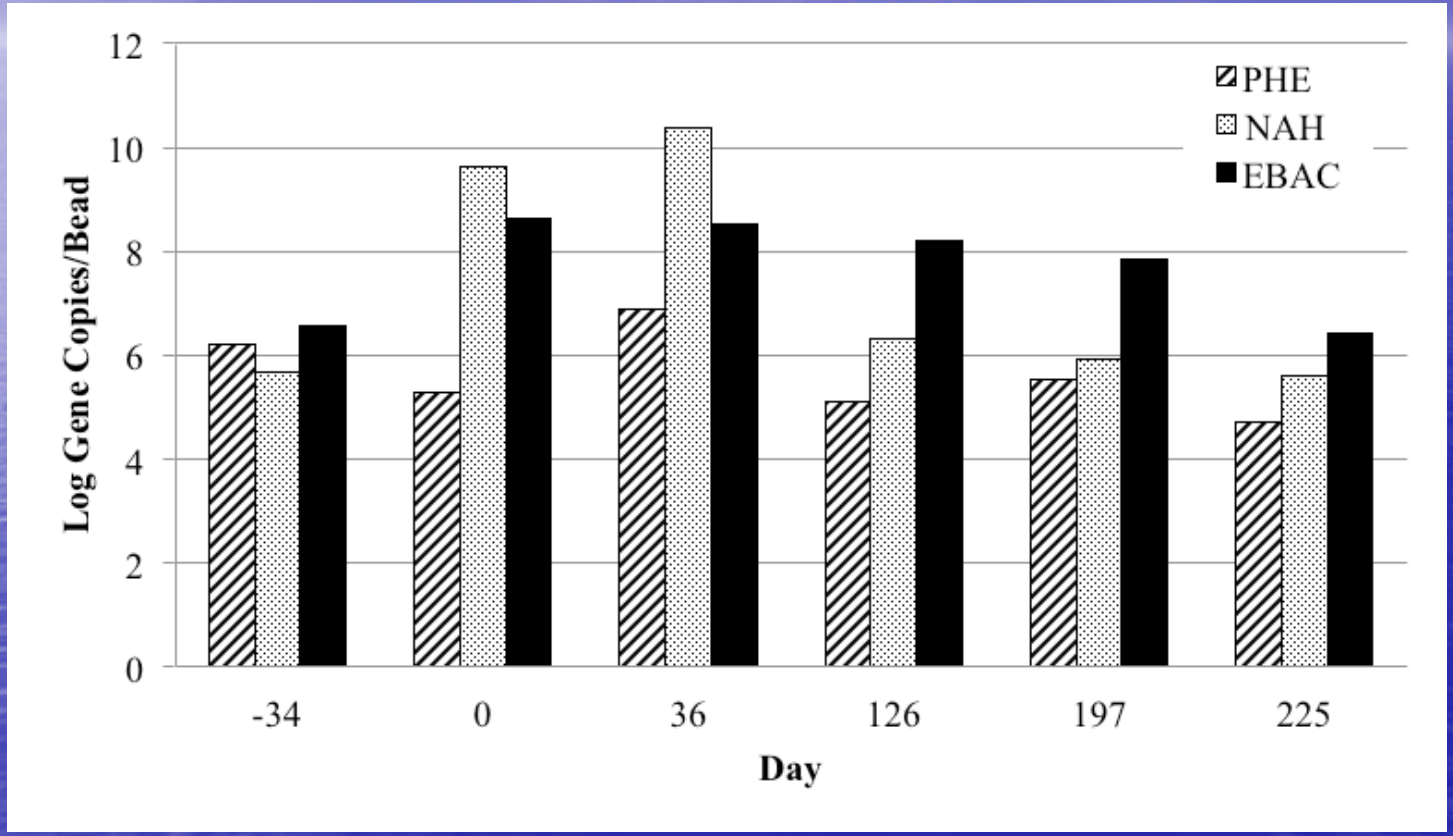
- Fuel oil release impacting soil and groundwater beneath a private residence
- The bioreactor well & four monitoring wells were installed in the basement
- Wells spaced 1 ft. apart



ISBR Timeline

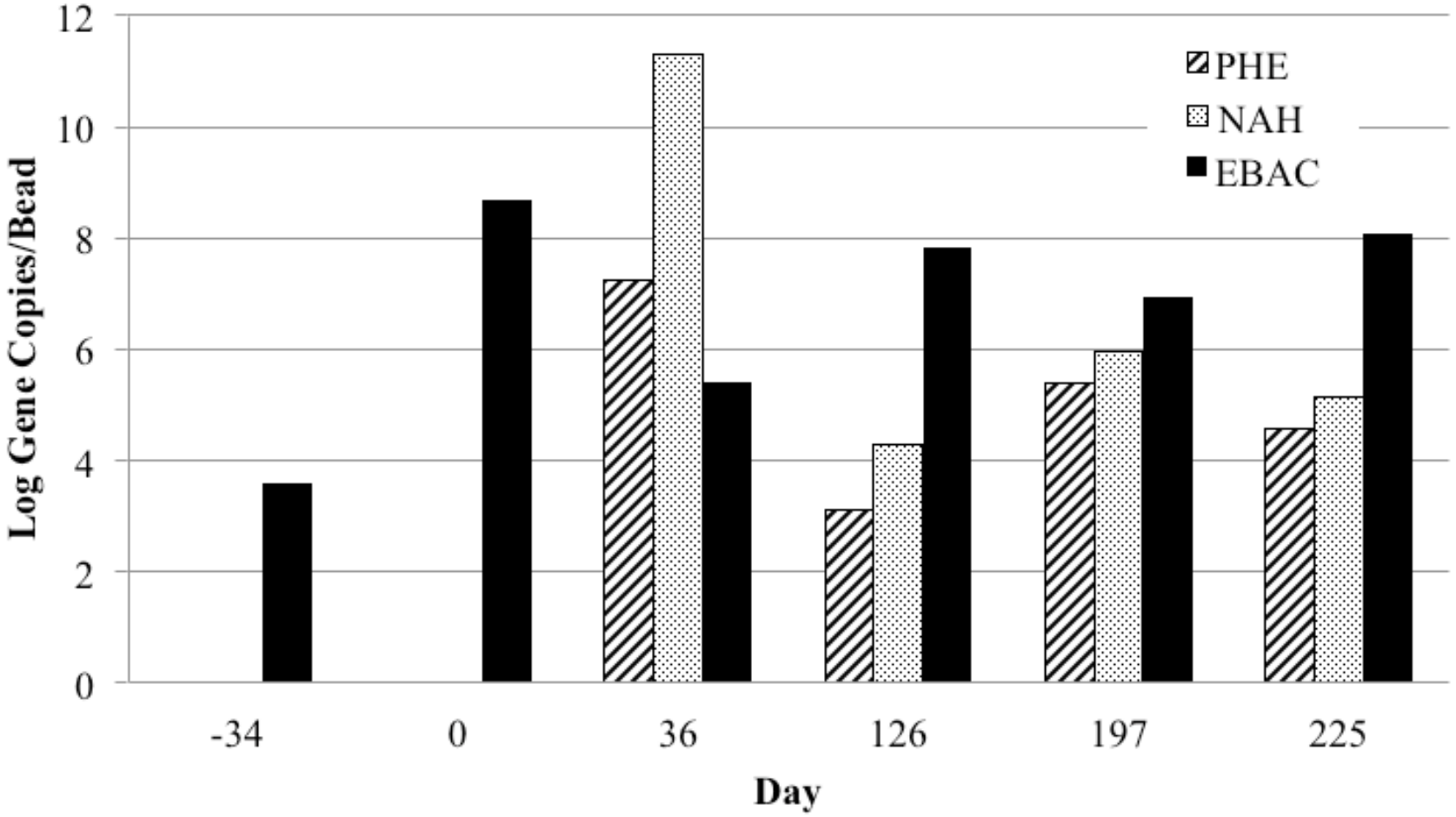
- Days -67 through -34: Air sparging only (no Bio-Sep)
- Days -34 through 0: Air sparging and nutrient delivery (no Bio-Sep)
- Day 0 onward: Complete bioreactor system operational (Bio-Sep beads added)
- Bio-traps® (Microbial Insights, Inc) used through out testing to monitor microbiology of bioreactor well and monitoring wells

qPCR Analysis of BR1 Bio-traps During ISBR Testing



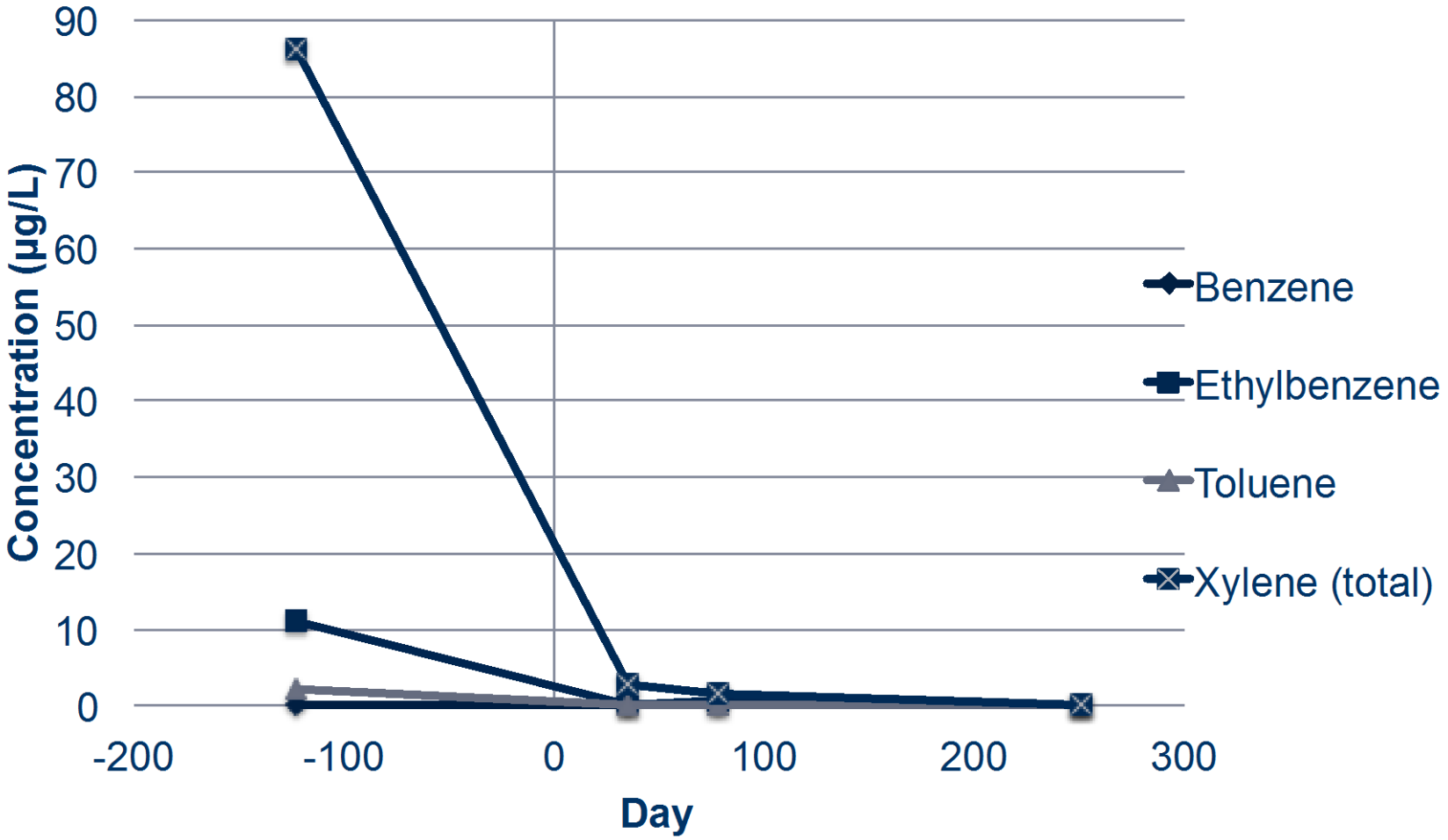
Didn't see much difference in hydrocarbon catabolic genes with DNA. This is common observation since hydrocarbon degraders are ubiquitous in the environment.

RT-qPCR Analysis of BR1 Bio-traps During ISBR Testing

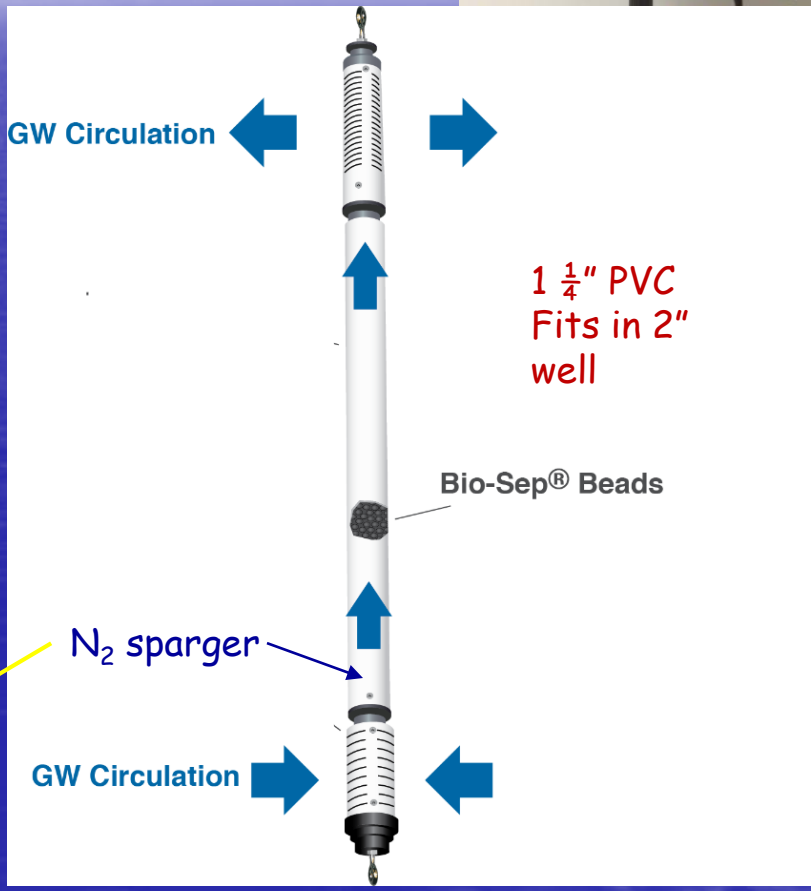


No expression of hydrocarbon catabolic genes until the complete bioreactor system was in service.

BTEX Concentrations Over Time

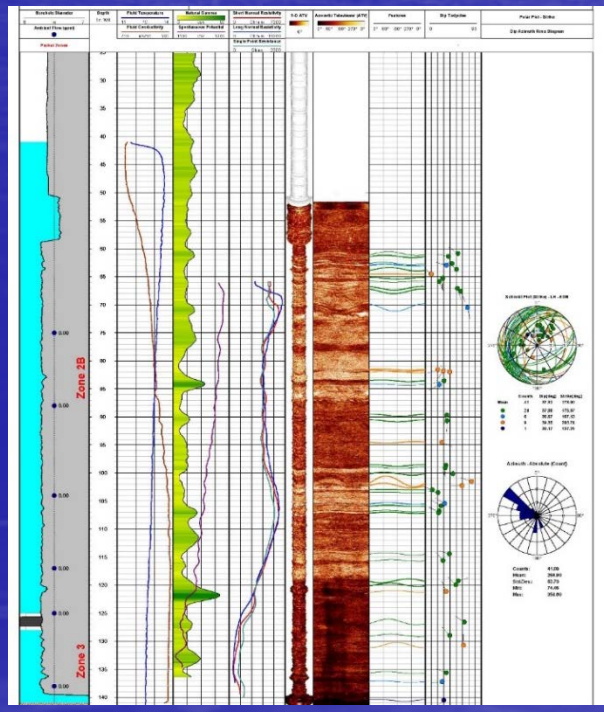


Anaerobic ISBRs, the same but different



Case Study – Anaerobic ISBR

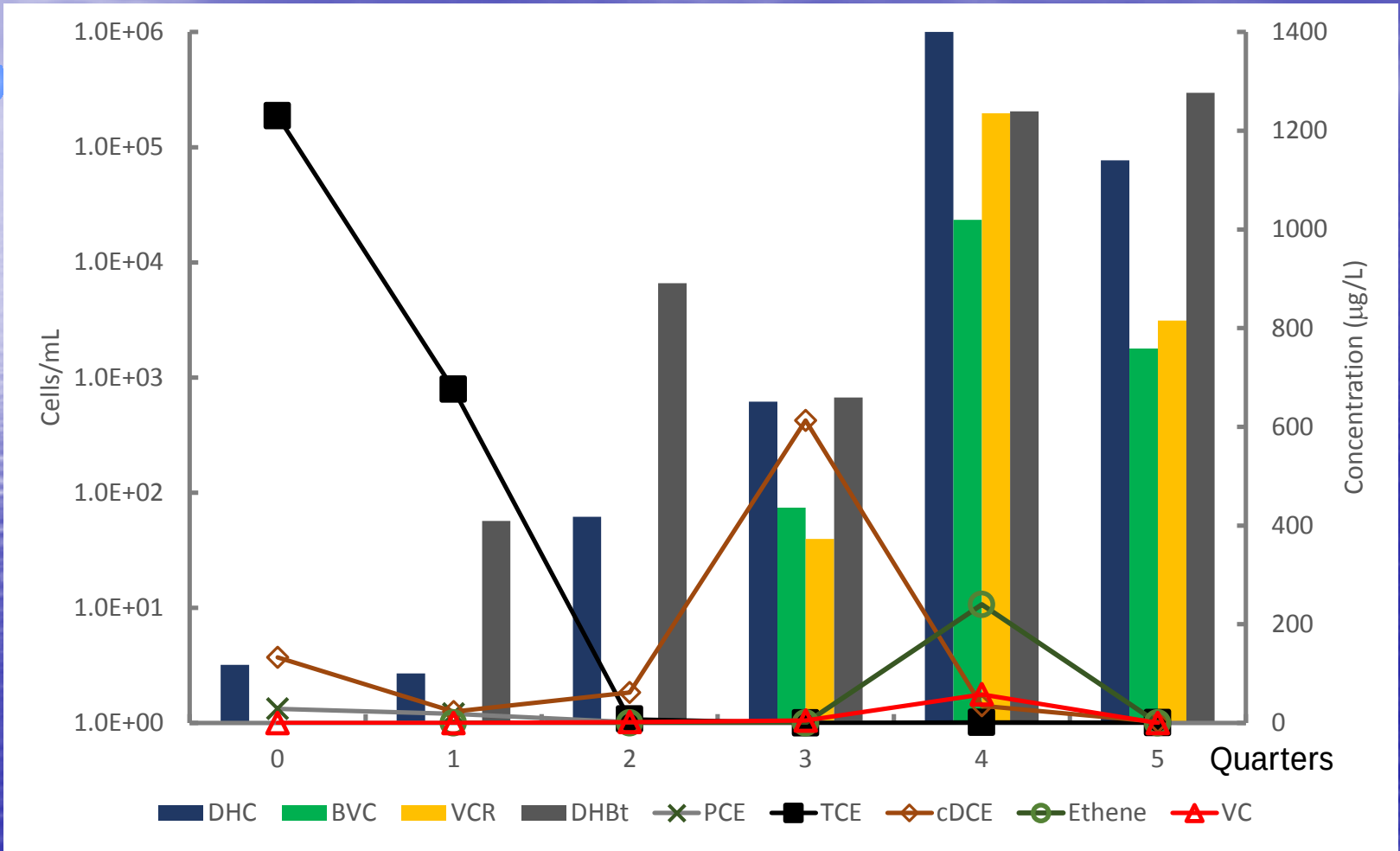
- Chlorinated solvent impacted site
- Fractured bedrock aquifer
- Deep groundwater impacts (140' bgs)
- Unfavorable geochemistry
  - Low but measureable DO
  - DO increase with rain event



Case Study – Anaerobic Bioreactor

- ISBR installed at a depth of 30' BGS
- "Liquid carbon" electron donor
- Groundwater monitoring
  - Contaminant concentrations
  - Geochemistry
  - qPCR for *Dehalococcoides* and functional genes for reductive dechlorination (bio-traps and groundwater)

Groundwater Contaminants & Microbiology



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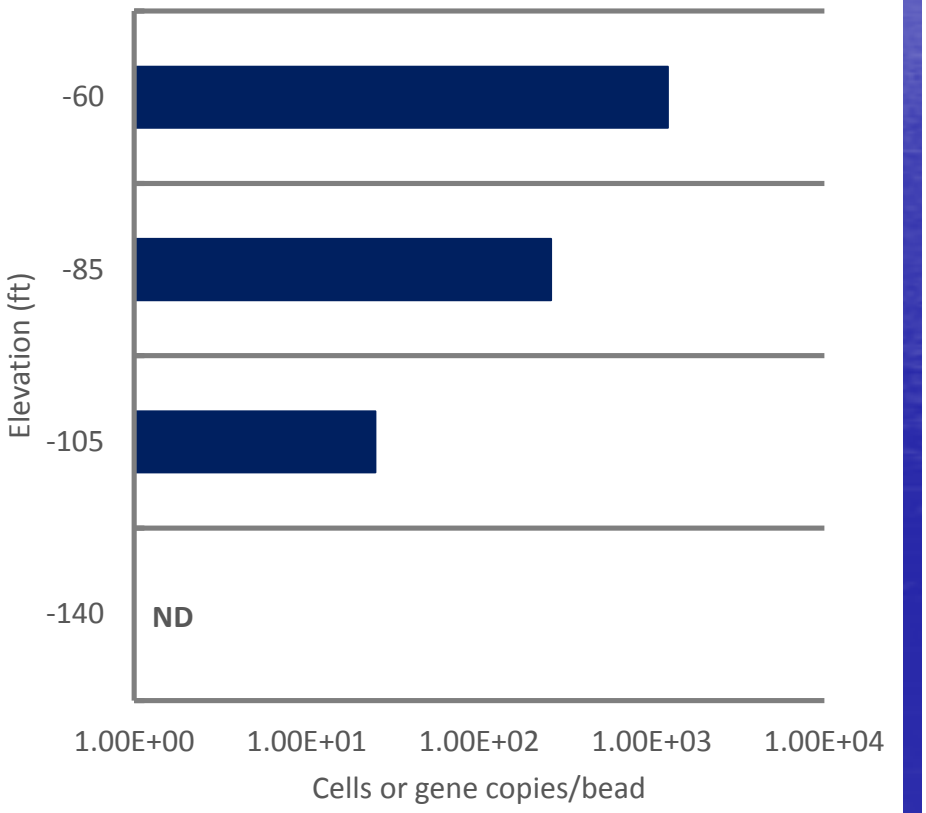
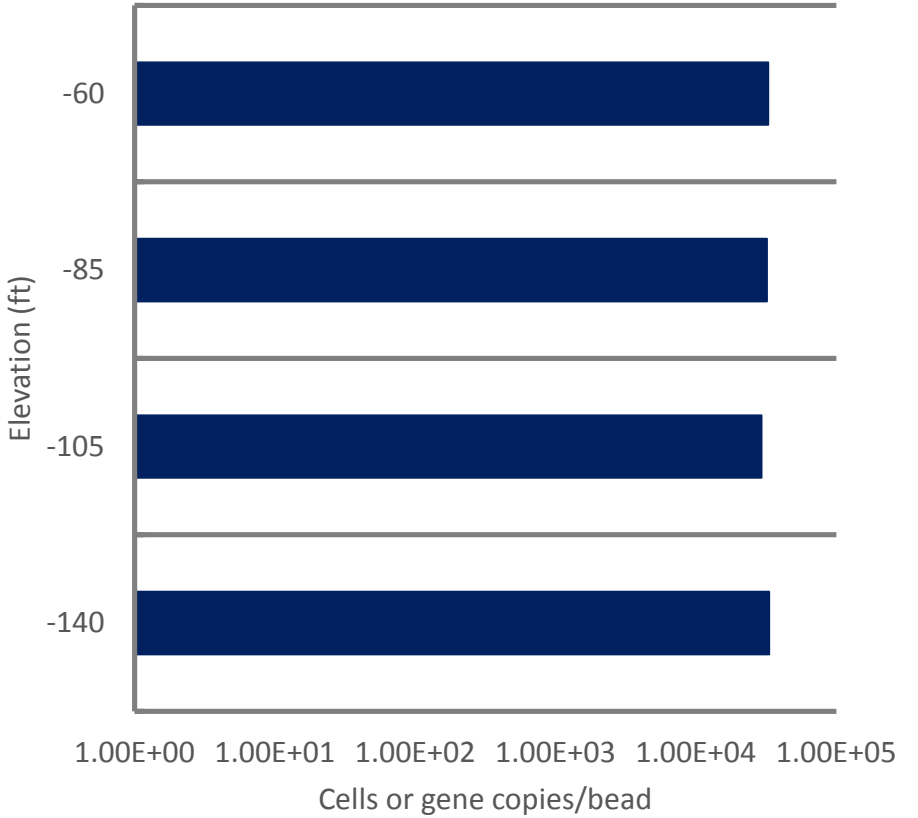
Dehalococcoides (DHC) Concentration with Depth

(Monitored by Bio-traps)

Pre-ISBR



Post-ISBR



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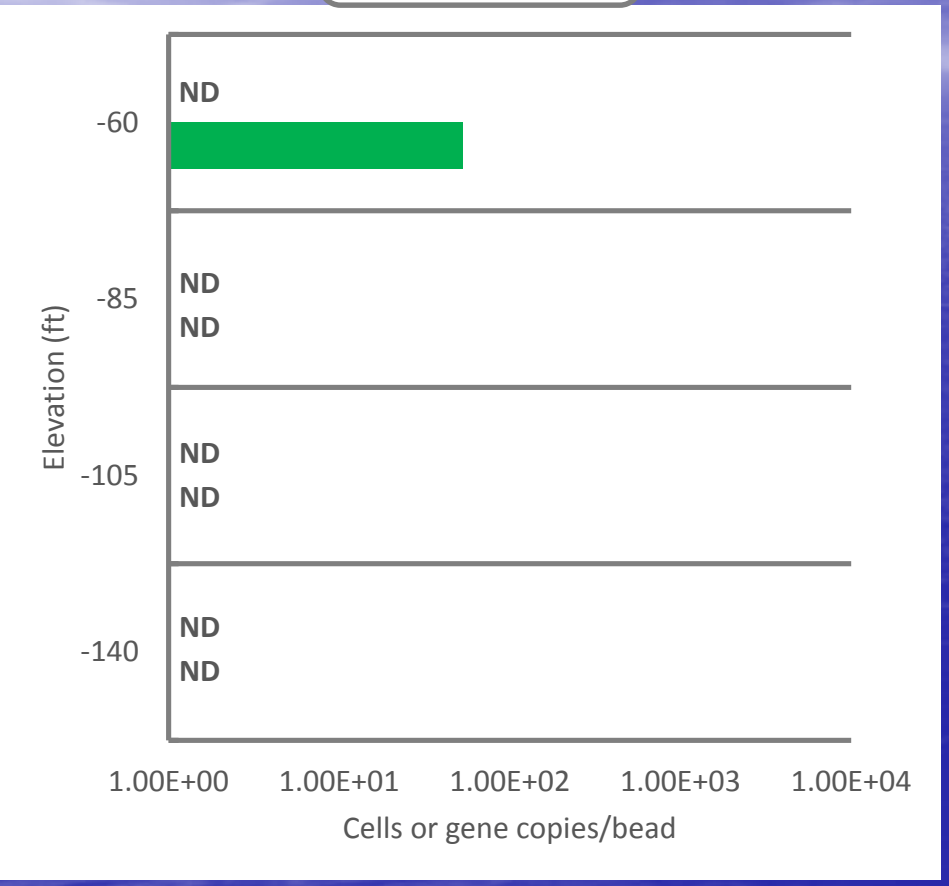
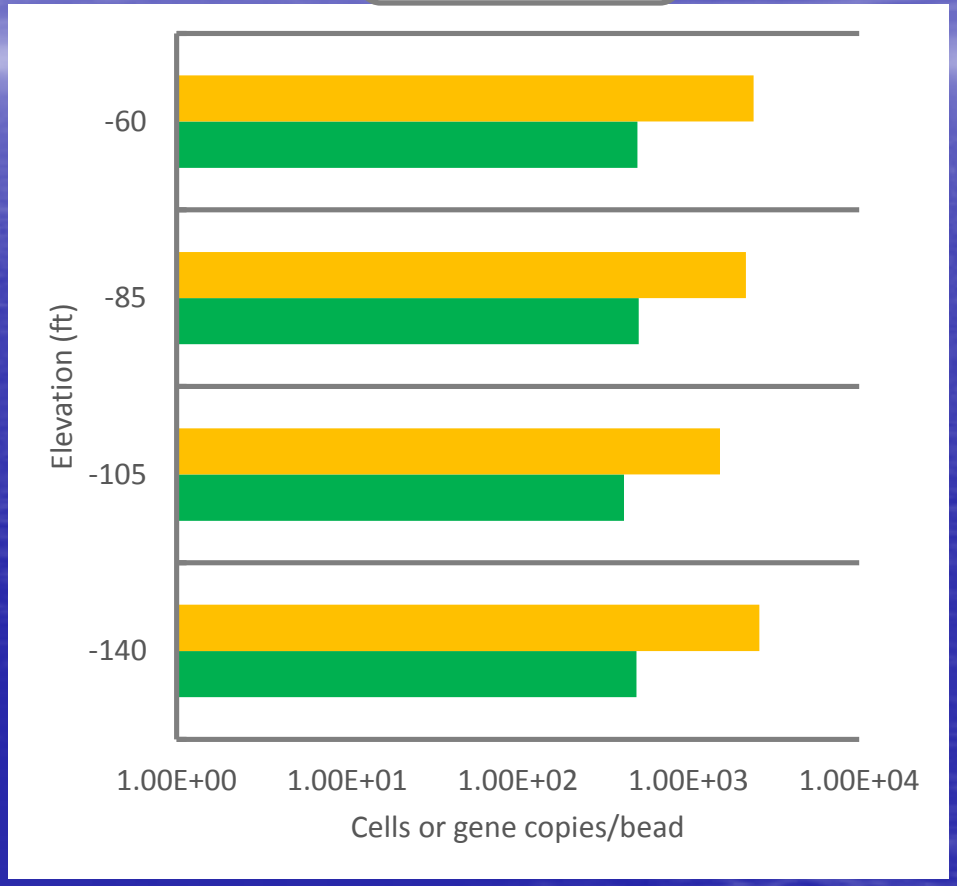
Vinyl Chloride RDases with Depth

Pre-ISBR



Post-ISBR



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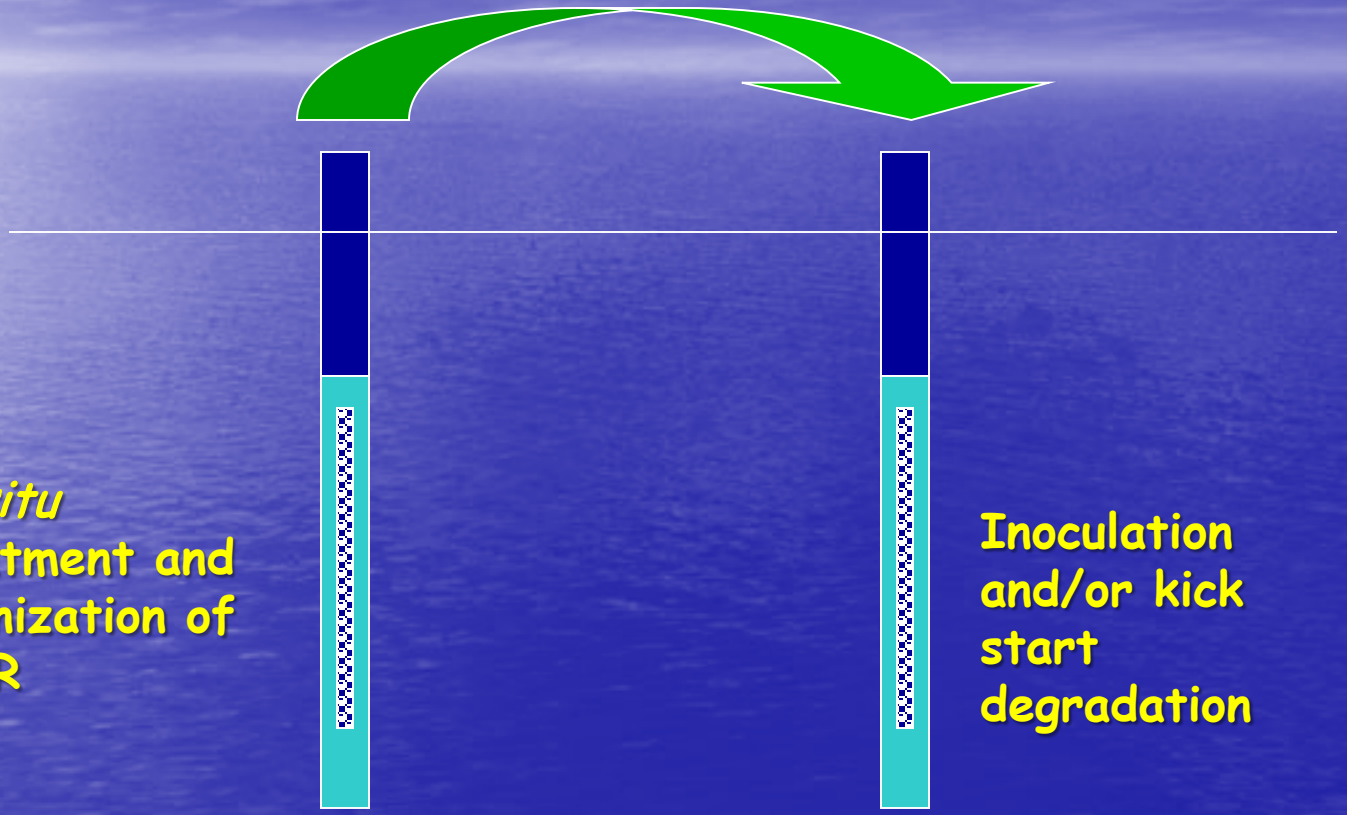
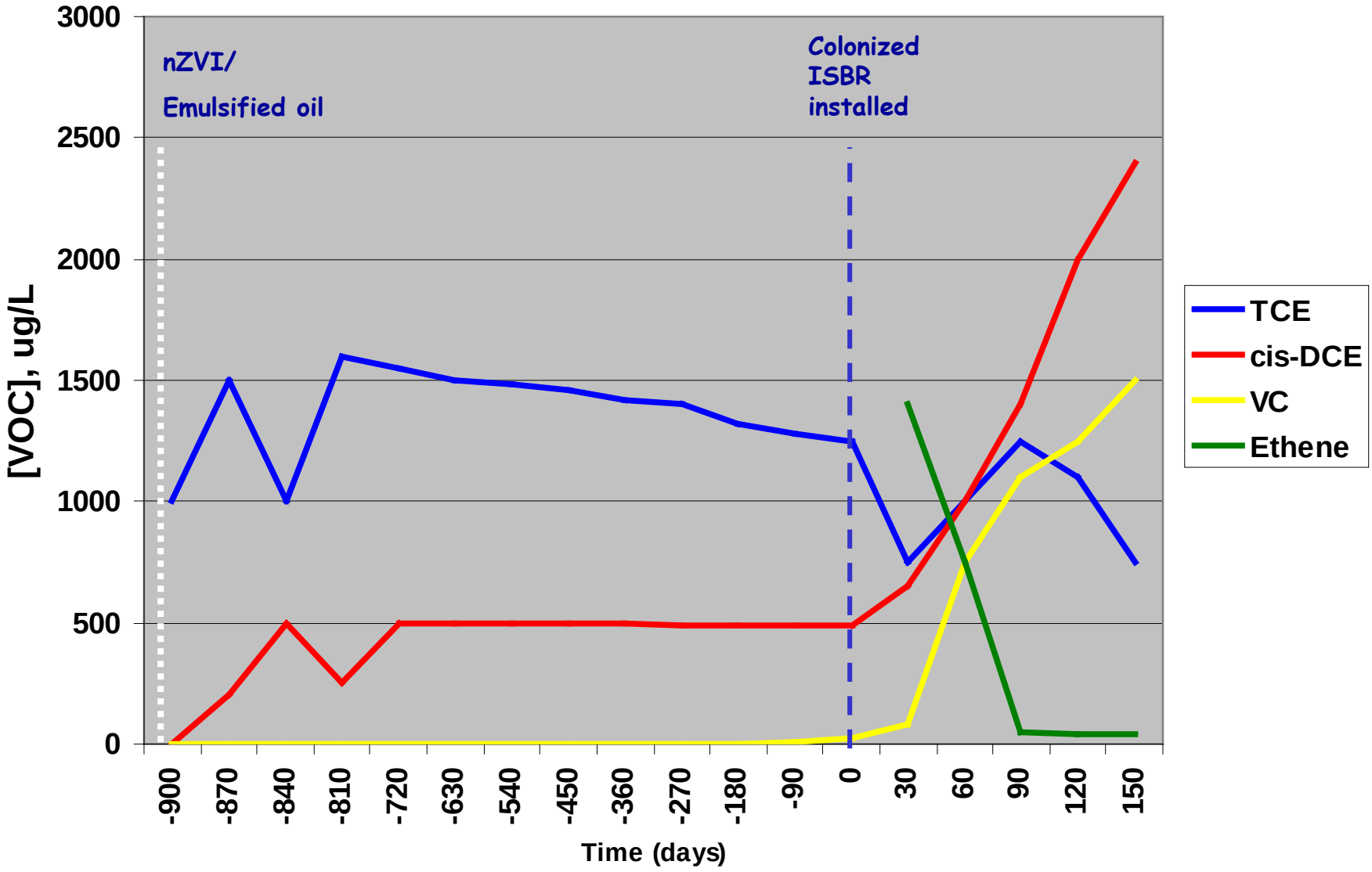
ISBRs Will Transfer Degraders from One Well to Another



In situ treatment and colonization of ISBR

Inoculation and/or kick start degradation

Transfer of DHC in a TCE Plume



When to consider an ISBR

- Inhibitory contaminant concentrations
- Dilute plumes (persistent low levels of contaminants)
- Following ISCO
- Difficult situations
  - Limited physical access
  - Where one-time amendment injection is not feasible
  - Where bioremediation has failed previously

ISBR Limitations

- Aerobic operation limited to low concentrations of reduced iron (fouling)
- Radius of influence decreases with increasing hydraulic conductivity of aquifer matrix
- Works best with contaminants adsorbed by activated carbon

ISBR O&M and Costs

- O&M
  - System checks every 2-4 wks
  - Power
  - Nutrients
  - Water level (ISBR must be totally submerged to function)
- Costs
  - Life of project rental
    - $10,000 for one unit (ISBR and controller)
    - $15,000 for two units
    - Decreasing per unit costs with addition of more units at a given site

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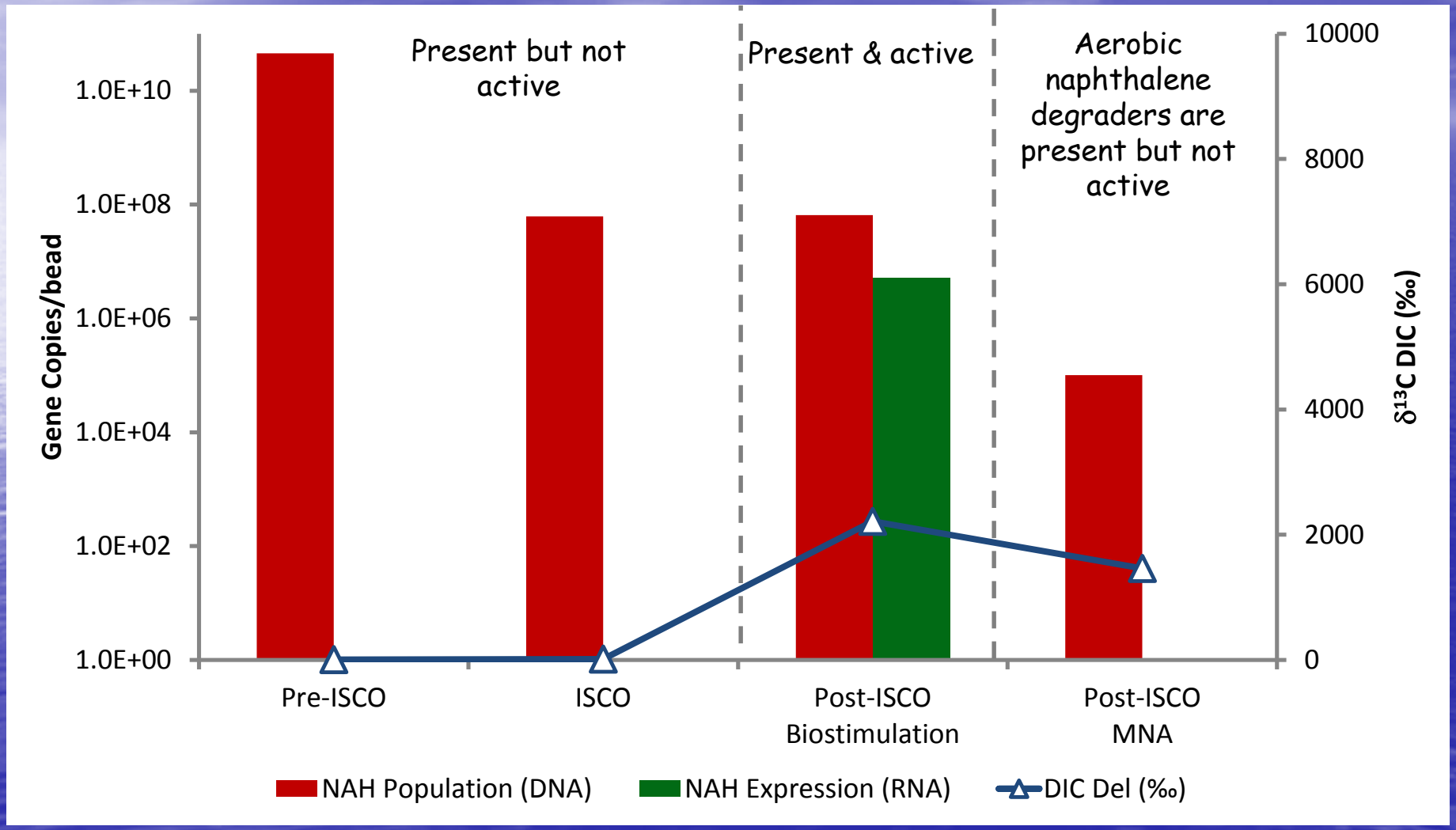
Eric Raes
BioEnhance, LLC
eric@bio-enhance.com
(973)219-6185

Kerry Sublette
BioEnhance, LLC
ksublette@microbe.com
(918)691-0639

Chemical Oxidation & Aerobic ISBR

Case study: Fuel oil release

Naphthalene degraders Pre- and Post-ISCO



Naphthalene degraders
ISBR – Post-ISCO