An *in situ* bioreactor for the treatment of groundwater impacted by petroleum hydrocarbons

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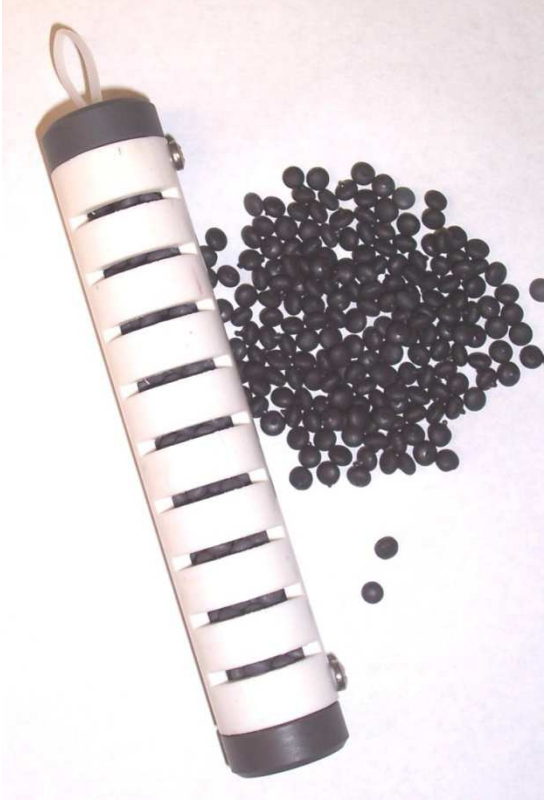
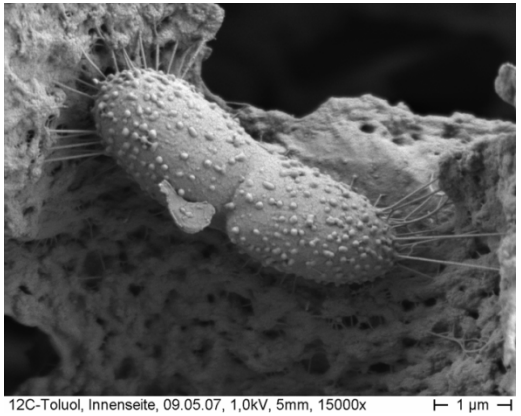
Adapting Bio-Sep technology for remediation

- Bio-Sep beads have long been used a diagnostic tool to monitor *in situ* microbial activity
- Recently, trials have begun to test the effectiveness of a bioreactor utilizing Bio-Sep beads to bolster indigenous microbial populations and enhance bioremediation



What is a Bio-Trap?

- Passive sampling tool for microorganisms
  - Simplest form consists of Bio-Sep beads in a housing that allows contact between the beads and groundwater
- Collects only **environmentally competent, active microbes**
  - Organisms must grow and reproduce within the beads to be detected
- **Integrative** sample (vs. snapshot)
  - Typical incubation 30 – 45 days
- Analyzed using molecular biological tools and stable isotope analysis



How do Bio-Traps work? Bio-Sep beads

- 3-4 mm in diameter
- 25 % Nomex, 75% PAC
- 74% porosity
- 600 m^2 of surface area/g
- Surrounded by ultrafiltration-like membrane with 1-10 micron holes
- Autoclavable
- Cleaned of fossil biomarkers by heating to 270 ^oC
- Biofilms form rapidly on Bio-Sep beads

Characteristics of Bio-Sep beads useful for treatment applications

- Provide favorable conditions for growth and reproduction
- Concentrate nutrients and hydrocarbons
  - Increasing local concentrations above threshold levels
  - Reducing concentrations of inhibitory aromatic hydrocarbons in the aqueous phase
- High surface area
- Rapid formation of biofilms
- Release new microbes into the aquifer once carrying capacity is reached

Aerobic bioreactor design

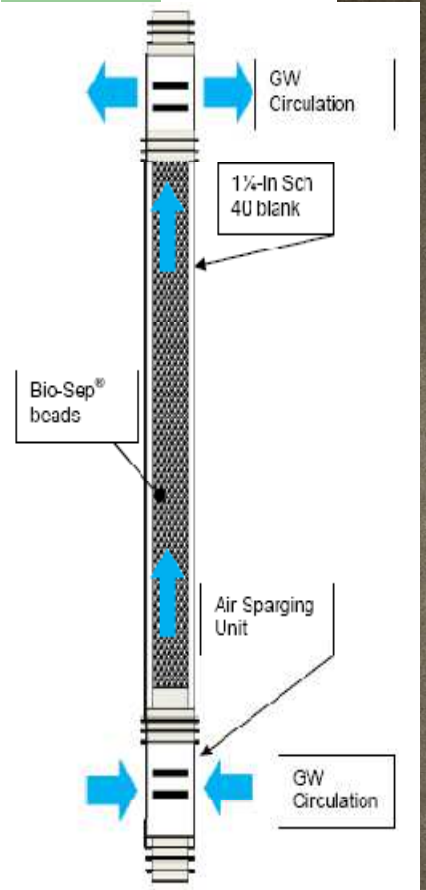
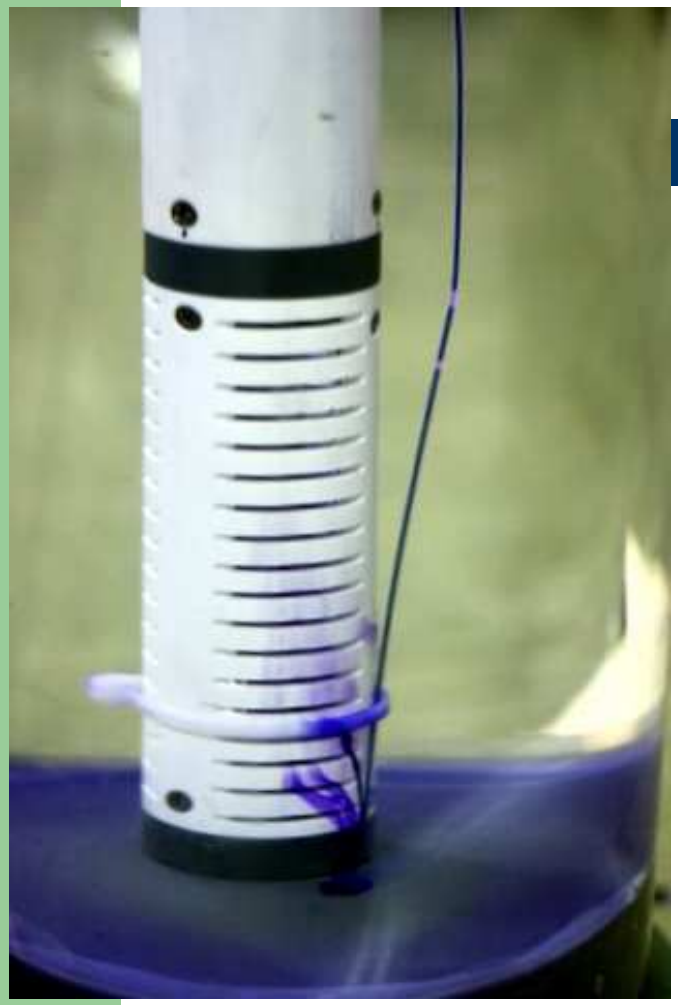


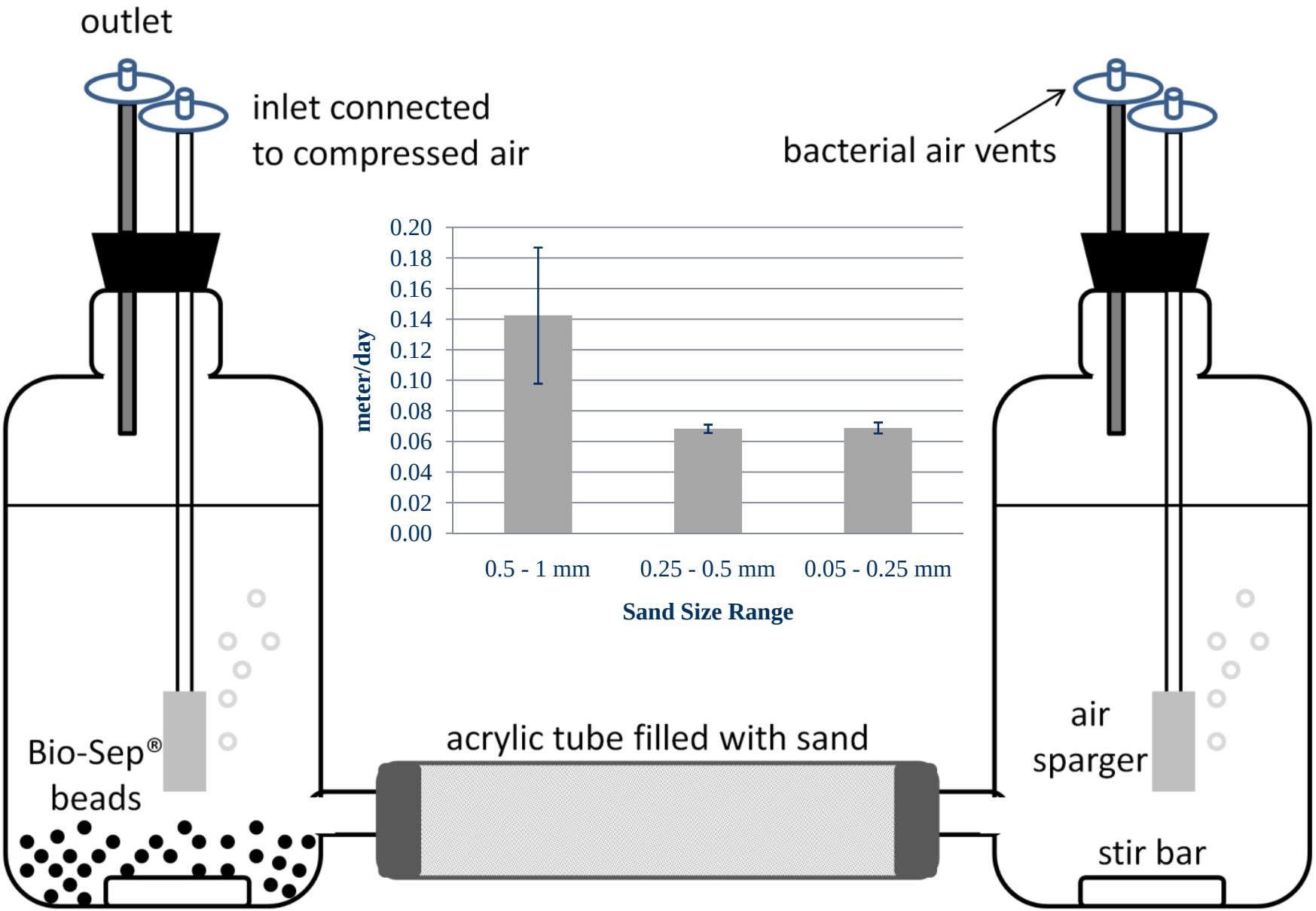
Figure X: Aerobic ISBR

- Fits in standard 2” well
- Packed bed bioreactor containing Bio-Sep beads open for fluid flow at top and bottom
- Air sparging into bottom of packed bed creates air lift for circulation of groundwater
- Air sparging and nutrient/sampling lines connected to surface equipment

Aerobic bioreactor flow pattern



Microbial release and transport

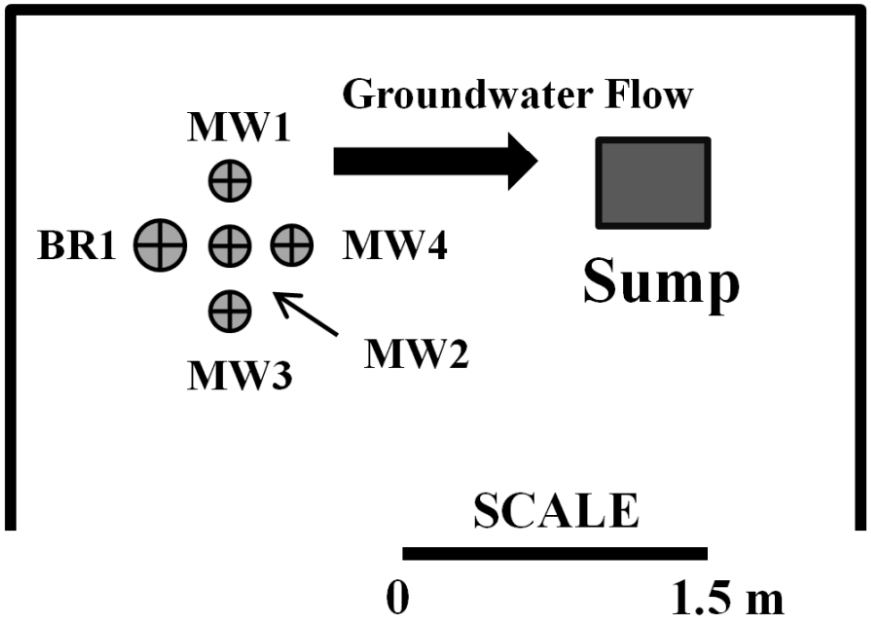


Operation of an aerobic bioreactor at hydrocarbon sites

- Bio-Sep beads, nutrient addition, and air sparging encourage microbial growth and reproduction
- Contaminated groundwater is treated as it moves through the column of Bio-Sep beads
- Water exiting the reactor carries hydrocarbon-degrading microbes into the aquifer

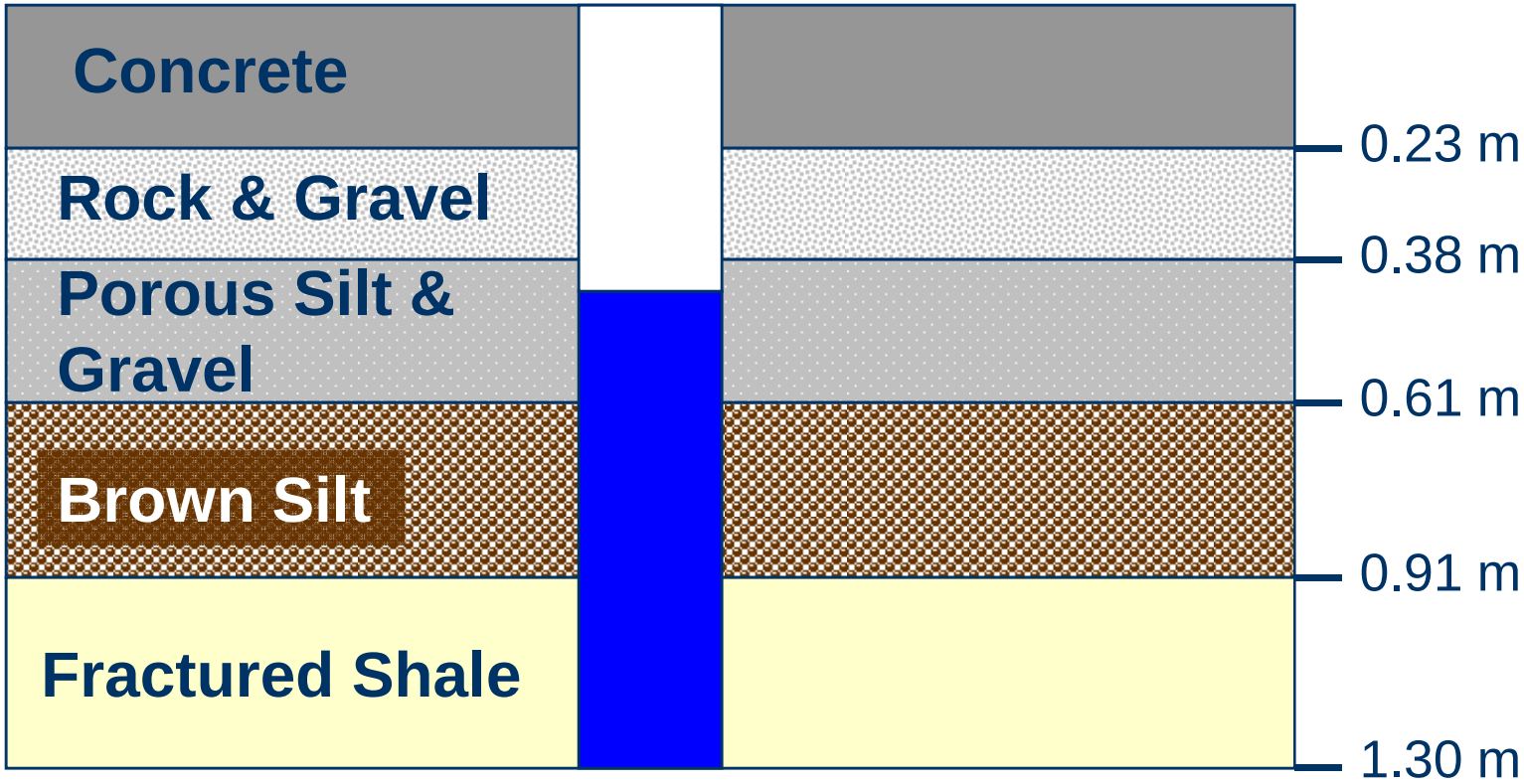
Residential site impacted with heating oil

- Fuel oil release impacting soil and groundwater beneath a private residence
- Low, but persistent [BTEX]
- The bioreactor well and four monitoring wells were drilled in the basement
- Wells spaced 1 ft apart
- Fractured shale aquifer

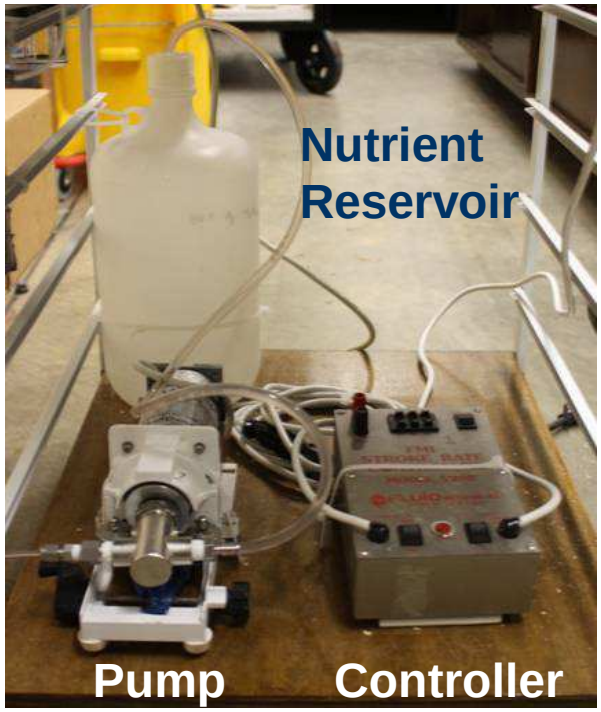


Well cross-section

Average Depth to Groundwater: 1.2 ft



New Jersey site



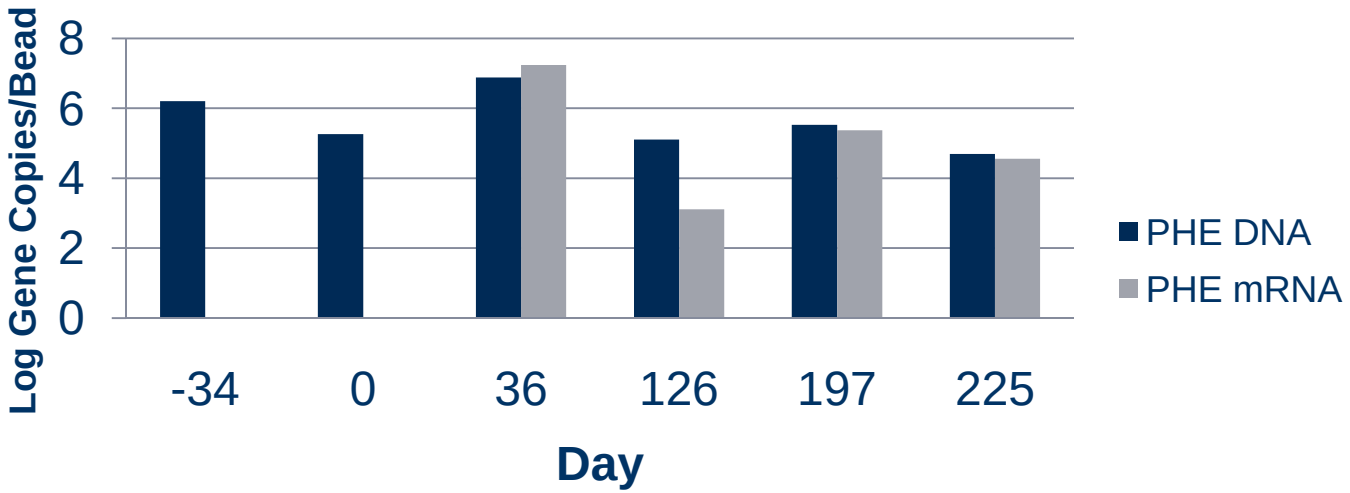
Bio-Trap Sampling

- Bio-traps were deployed monthly in each of the 5 wells
- DNA and mRNA analyses
  - total eubacteria
  - functional genes associated with aromatic hydrocarbon degradation
    - Phenol Hydroxylase (PHE)
    - Naphthalene Dioxygenase (NAH)

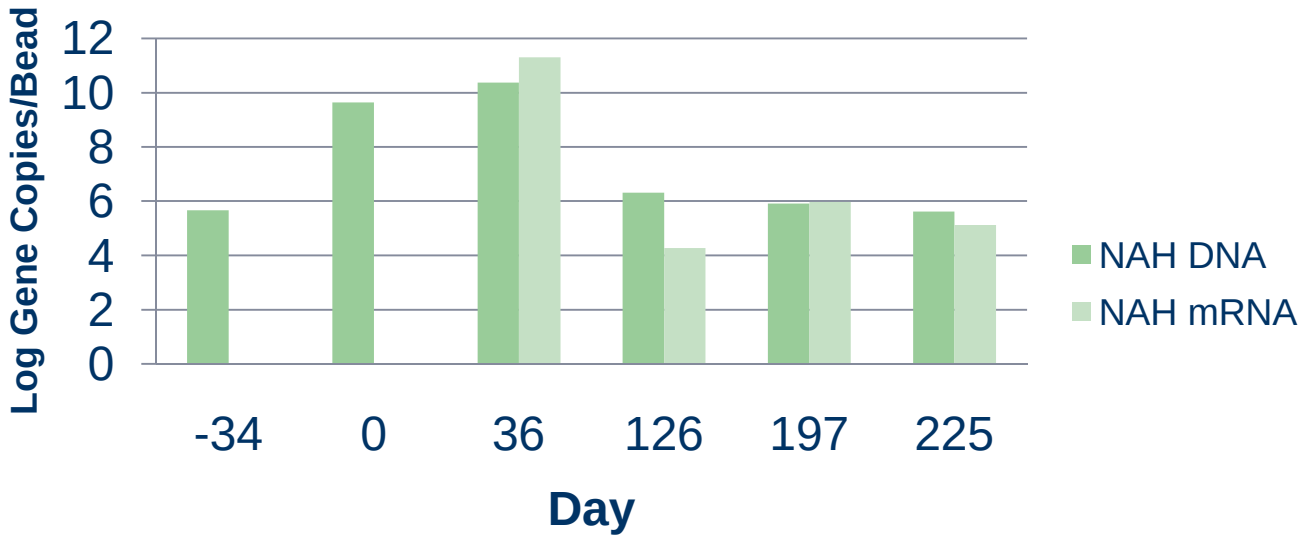
Bioreactor Timeline

- Days -67 through -34: Air sparging only
- Days -34 through 0: Air sparging and nutrient delivery
- Day 0 onward: Complete bioreactor system operational

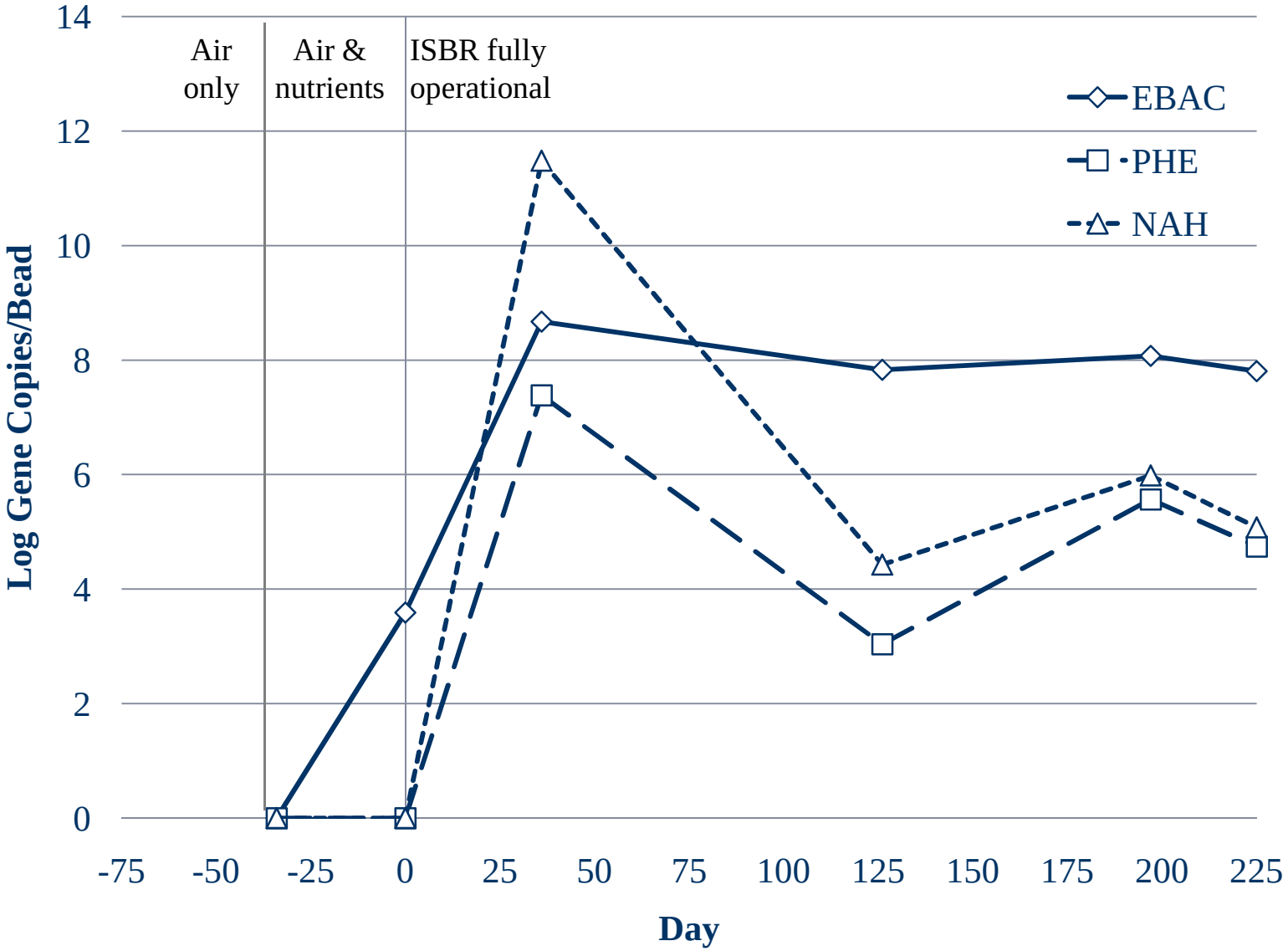
Comparison of PHE DNA and mRNA in BR1



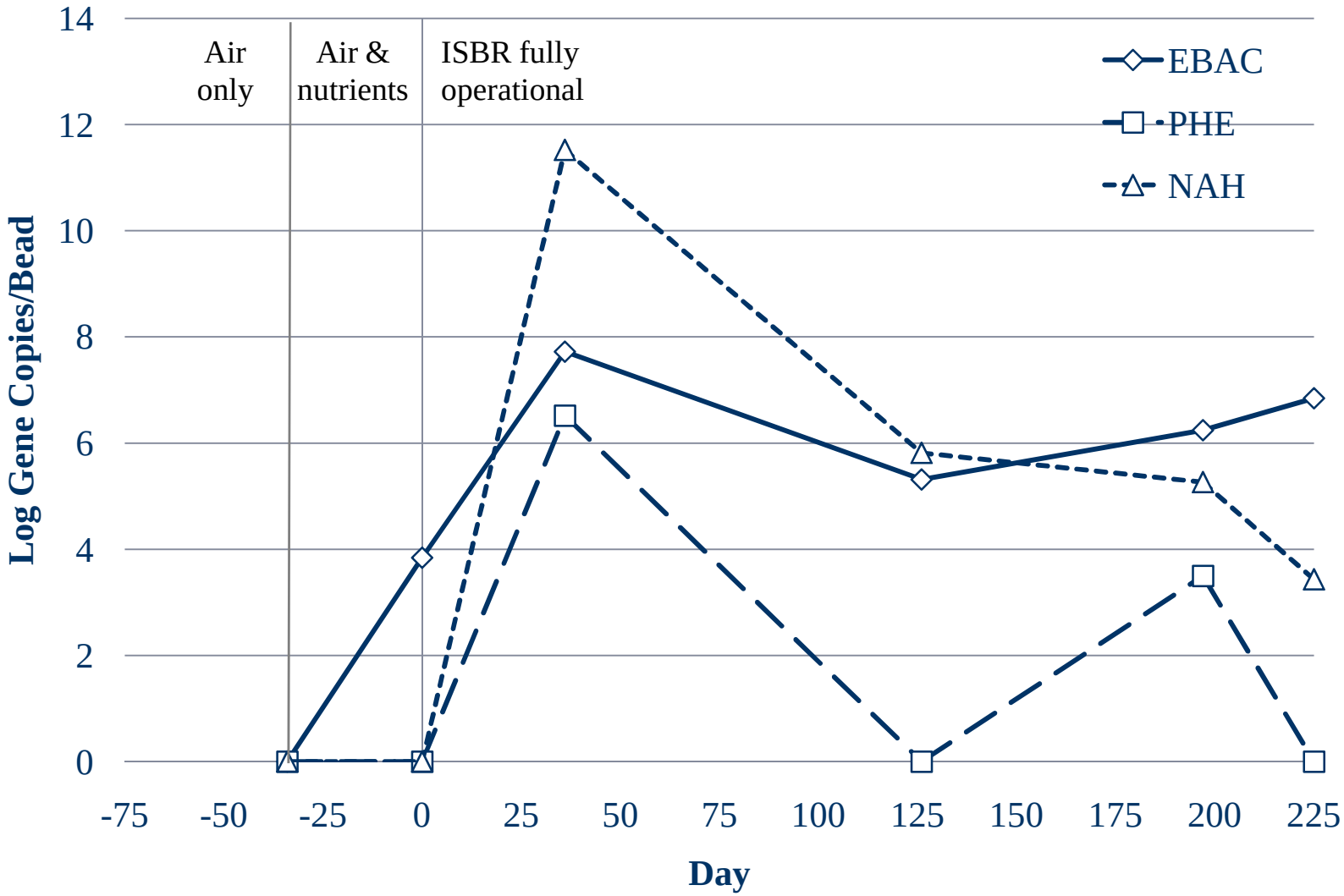
Comparison of NAH DNA and mRNA in BR1



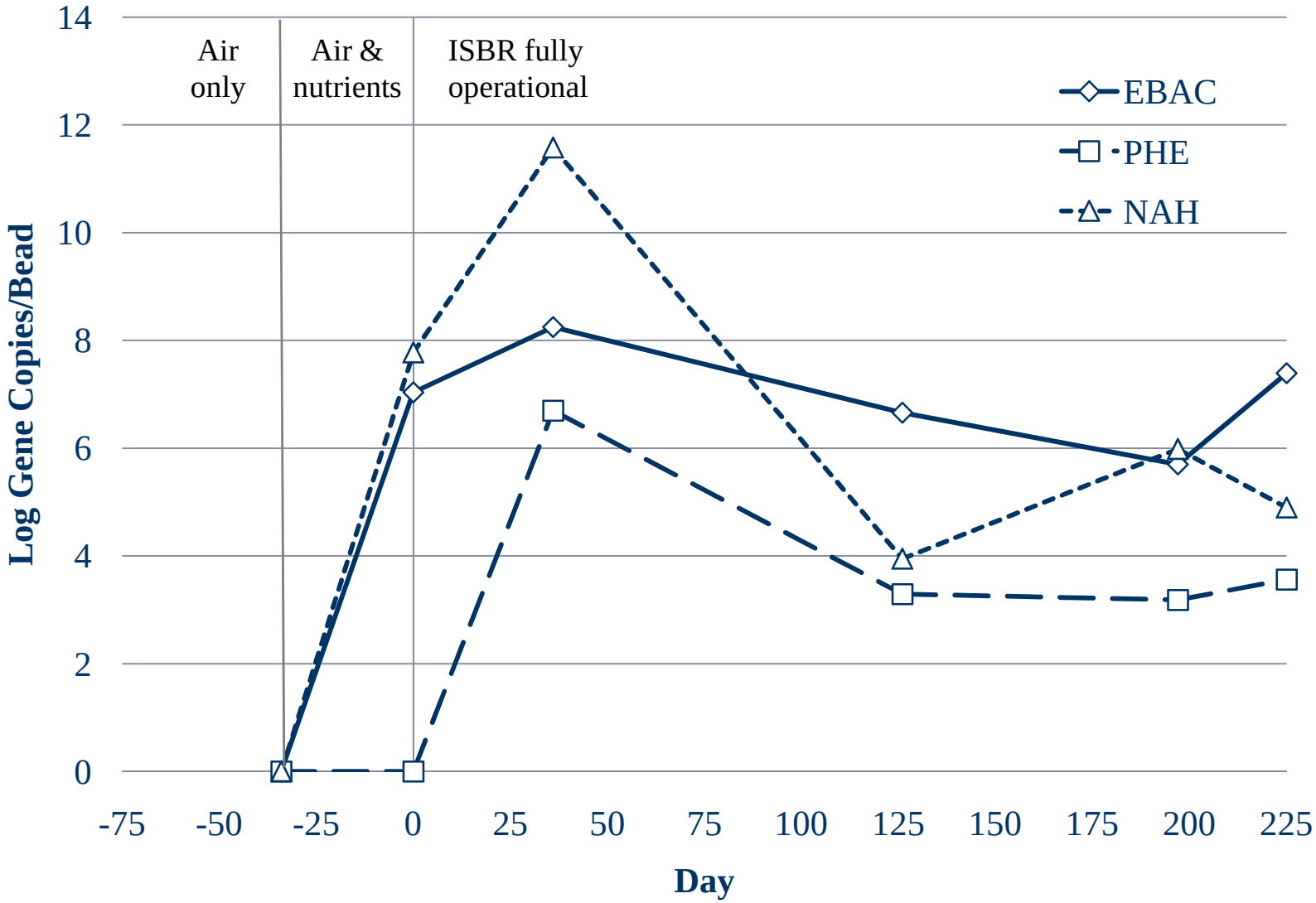
RT-qPCR results for BR1



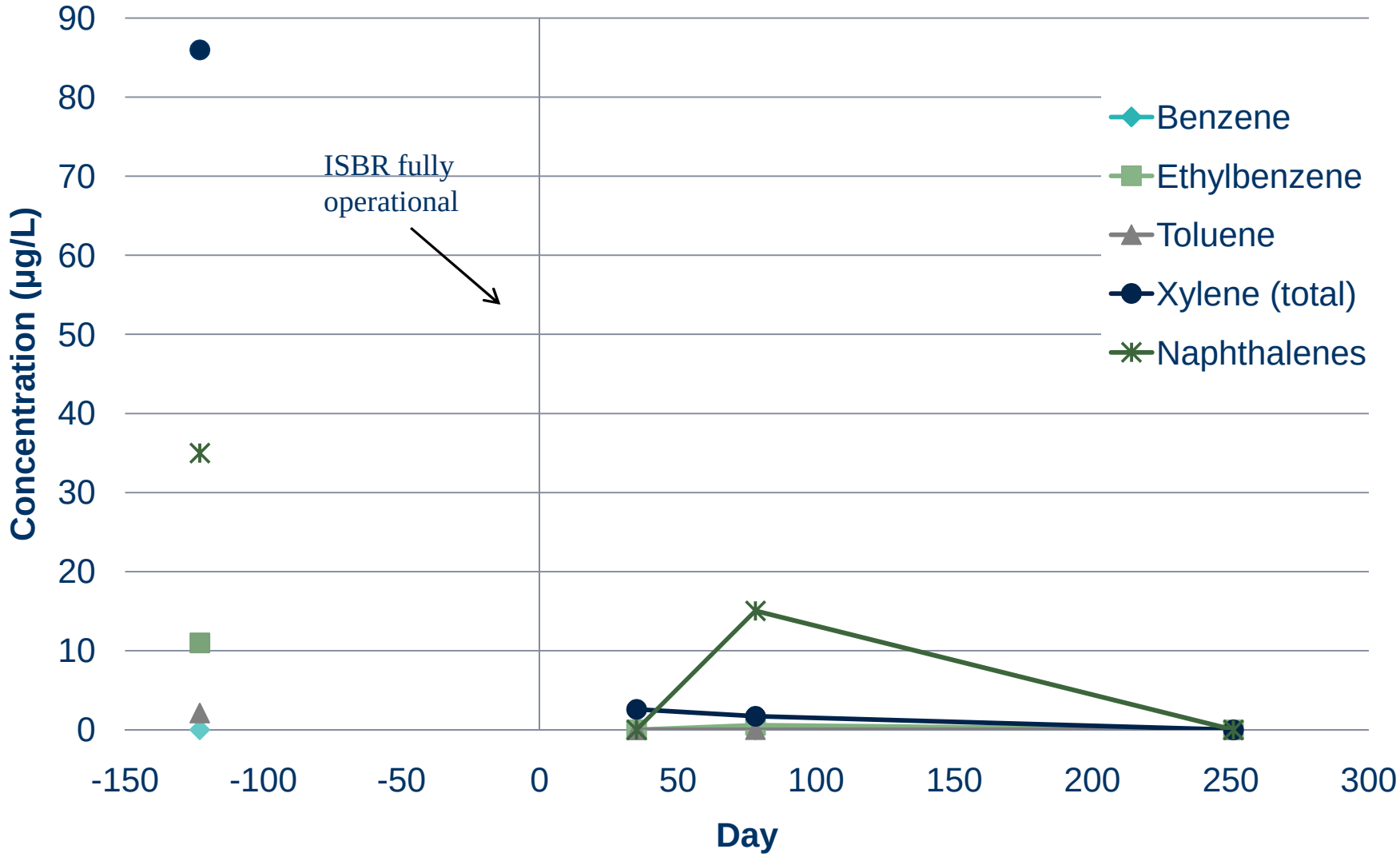
RT-qPCR results for MW2, 1 ft downgradient from BR1



RT-qPCR results for MW4, 2 ft downgradient from BR1



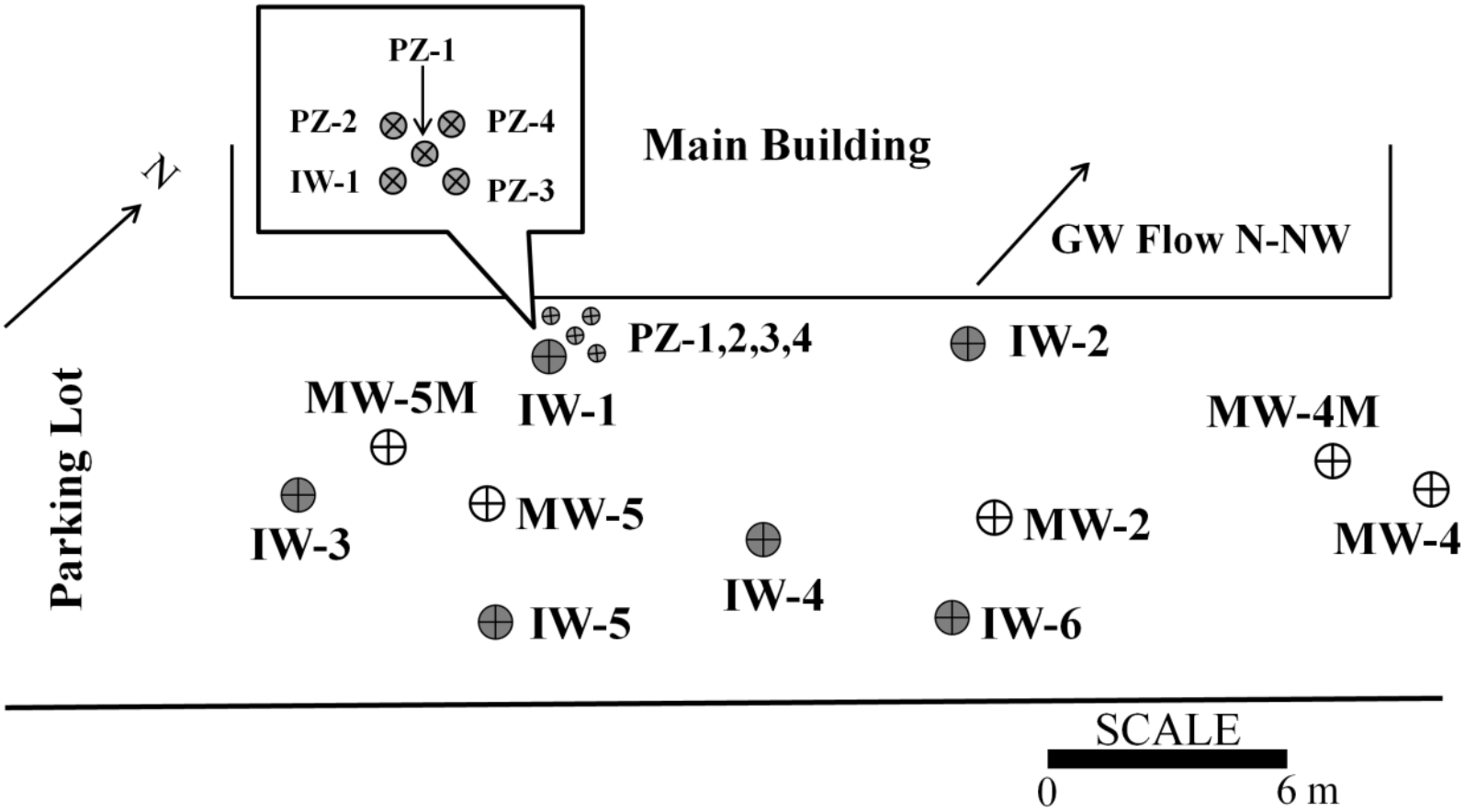
Concentrations of BTEX and Naphthalenes Over Time



New Jersey site summary

- Increased concentration of degraders and increased expression of functional genes involved in degradation
- Microbial community responded to changes in treatment; effect is seen in both the bioreactor well and the monitoring wells
- All constituents of concern were either not detected or below groundwater quality criteria by the end of the study

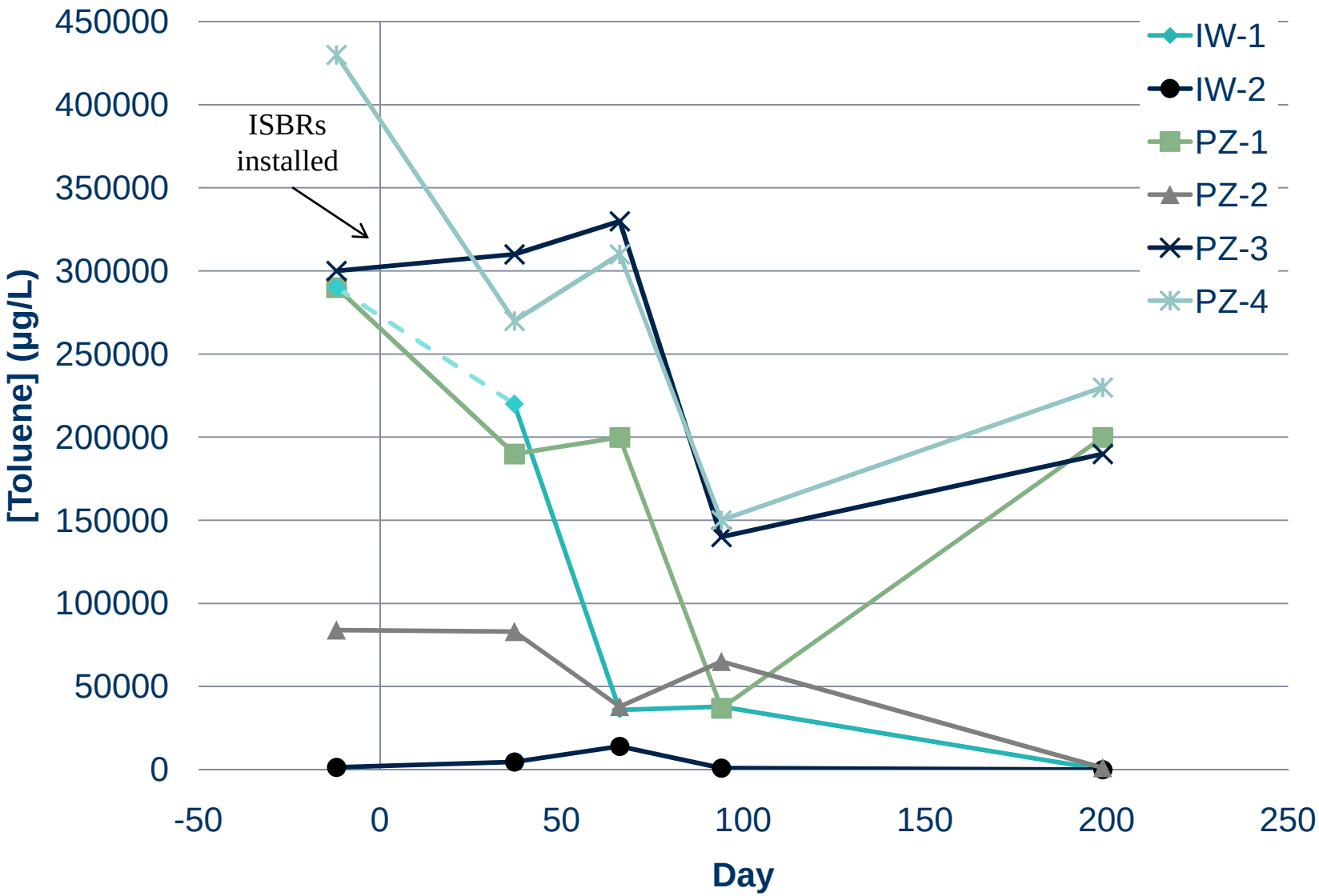
Historic toluene release at a former industrial site



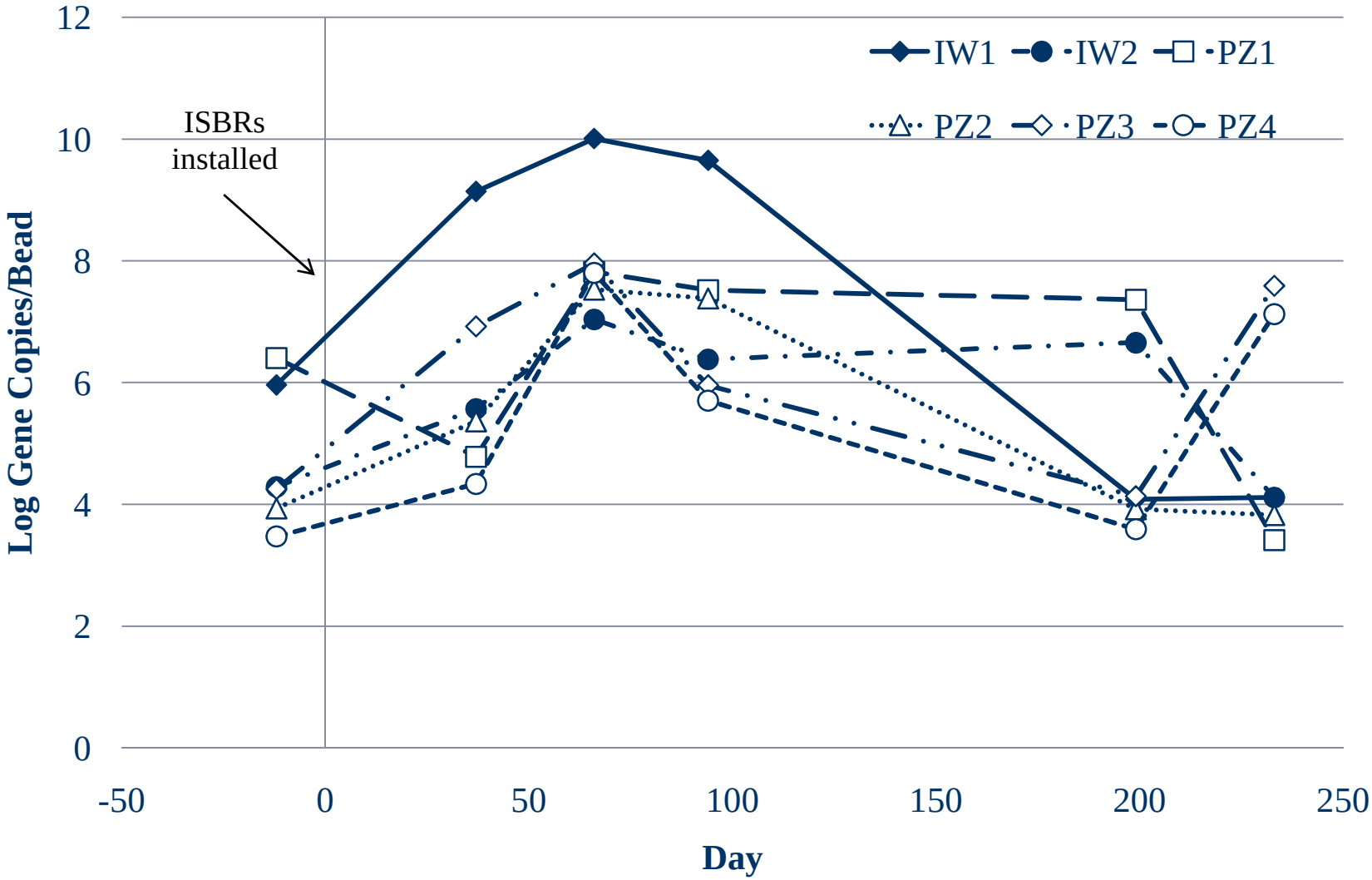
Historic toluene release at a former industrial site

- Historic chemical releases, with up to 430 mg/L toluene
- Toluene concentrations above 250 mg/L shown to inhibit microbial activity
- Aquifer with high content of fine silt and clay
- Groundwater samples analyzed for contaminant concentrations
- Bio-traps deployed in each of the wells
  - RNA analysis and SIP

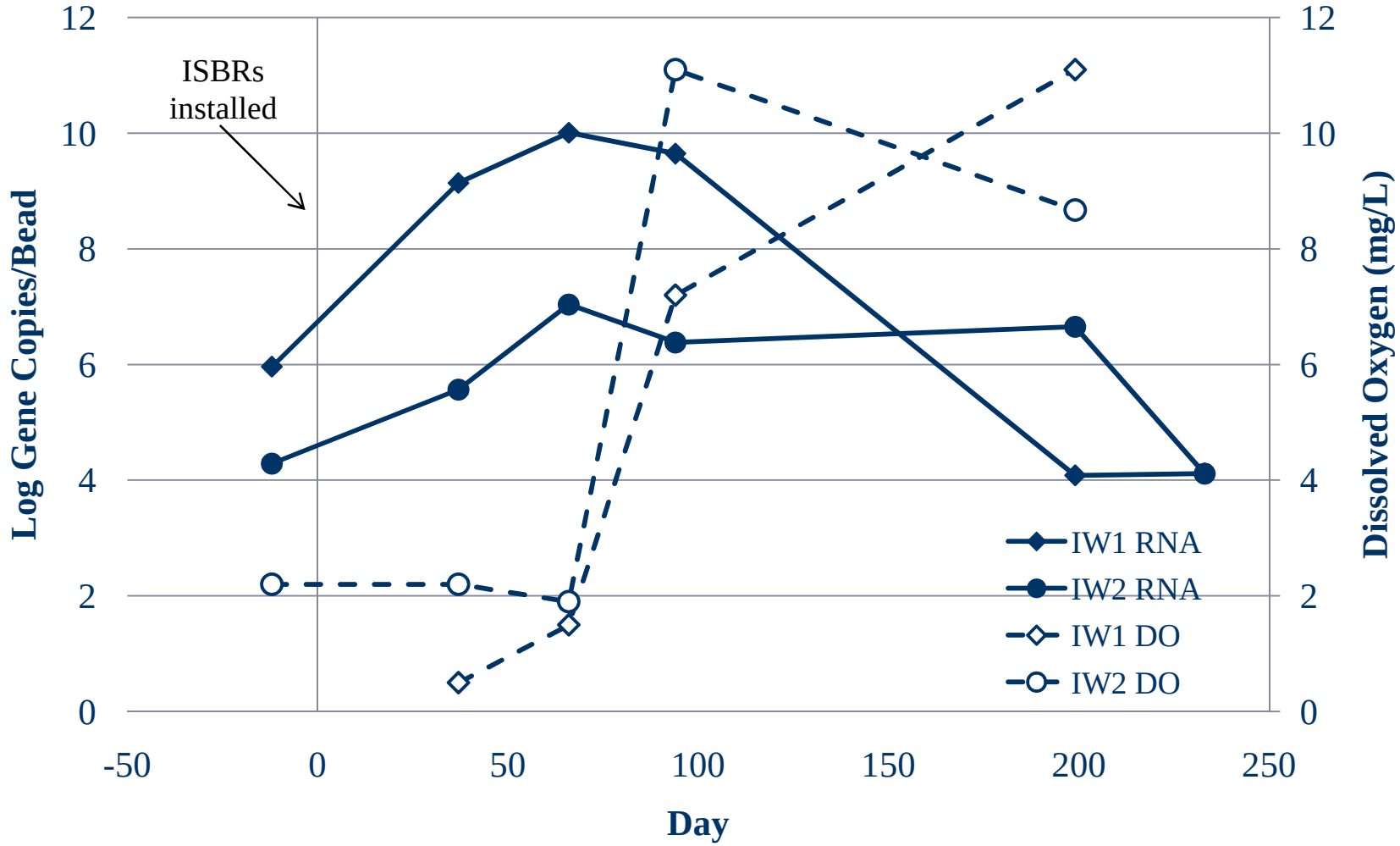
Toluene Concentration



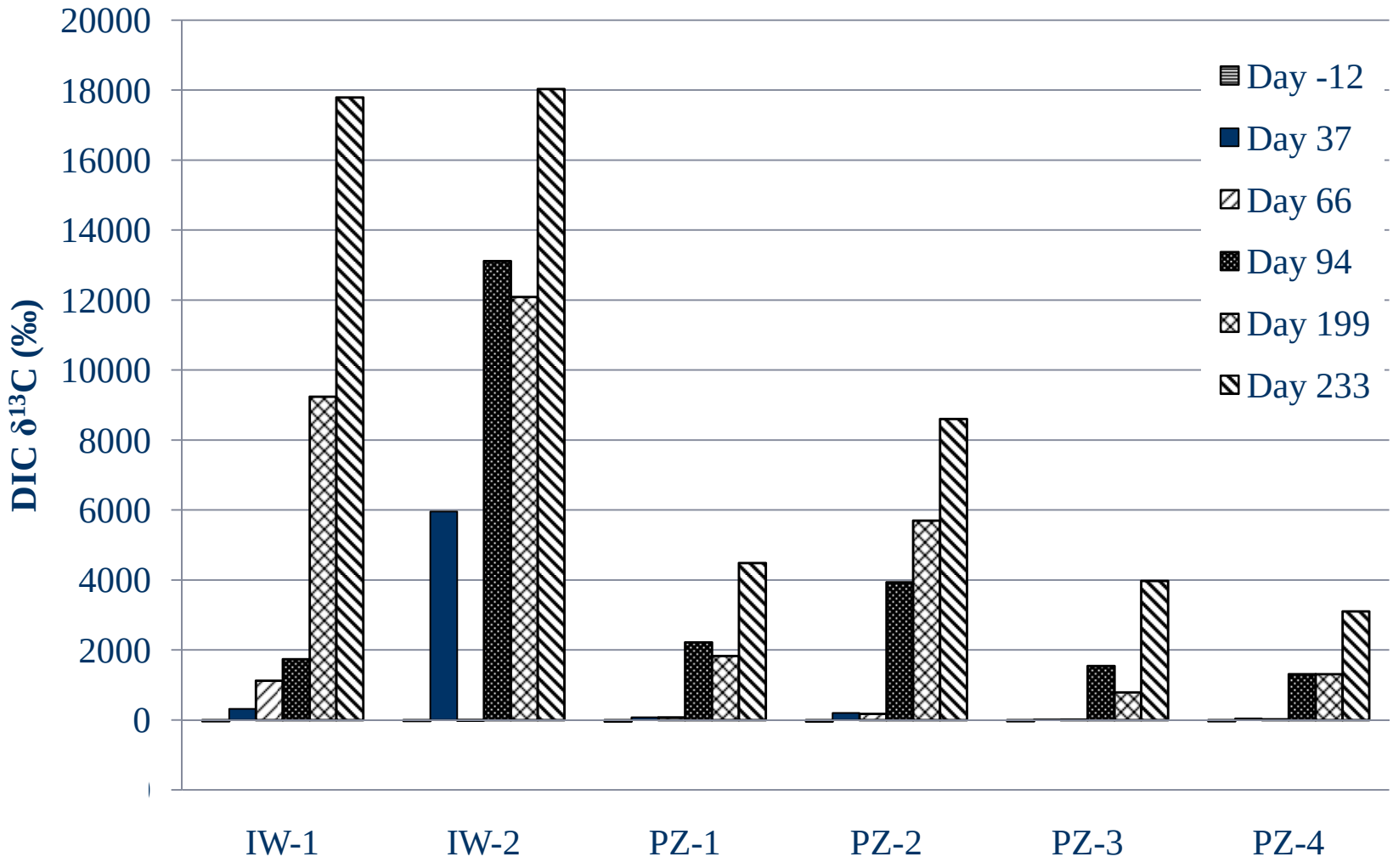
EBAC 16S rRNA genes detected by RT-qPCR



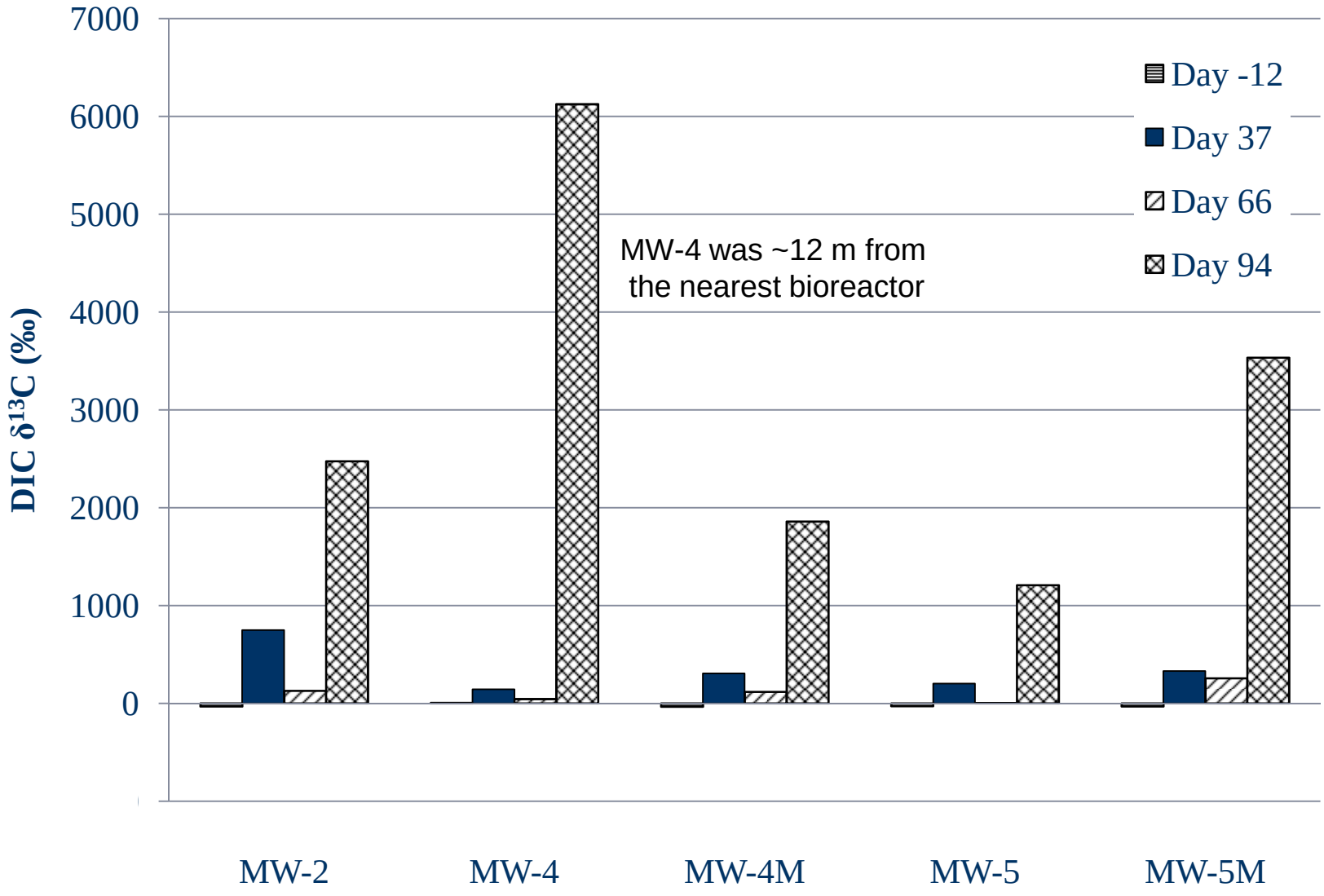
Total catabolic functional genes (NAH, PHE, TOD, RMO) detected by RT-qPCR and [DO]



DIC $\delta^{13}C$ values from IW-1, IW-2, and the PZ wells



DIC $\delta^{13}C$ values from the MW wells



Site summary

- By Day 199, toluene concentrations in all bioreactor wells met NJDEP groundwater standards
- Biodegradation was stimulated despite initial inhibitory toluene concentrations
- Toluene concentrations decreased between 31-47% in PZ-1, 3, & 4 and by 99% in PZ-2
- SIP data provided direct evidence of increased toluene mineralization in the bioreactor wells, adjacent PZ wells, and MW wells located throughout the site

ISBR now being commercialized by

Engineering & Land Planning Associates, Inc.

Acknowledgements

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Questions?