An *In Situ* Bioreactor for Treatment of Hydrocarbon-Impacted Groundwater

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Bioremediation of Contaminated Groundwater

- "*In situ* bioremediation of groundwater has become one of the most widely used technologies for contaminated site treatment because of its relatively low cost, adaptability to site specific conditions, and efficacy when properly implemented" – US EPA

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

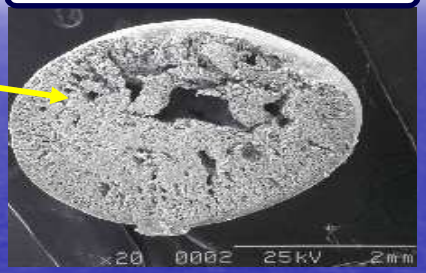
- Enhancement of *in situ* bioremediation in groundwater with compact bioreactor installed in-well
- Overcomes common limitations of bioremediation of groundwater
  - Low contaminant concentrations
    - A threshold concentration of substrate is required for growth
  - Substrate inhibition
    - At high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

In the beginning…..

Bio-Trap® Sampler with Bio-Sep® Beads

Nomex and PAC

X-Section of Bio-Sep® Bead

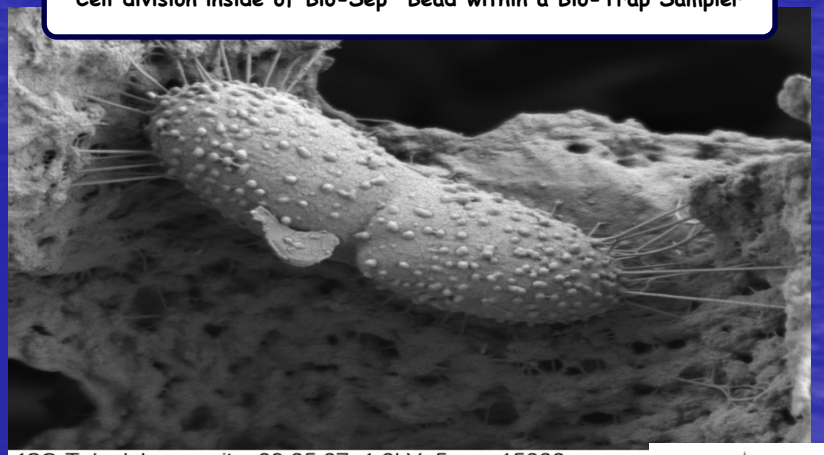


Interior of Bio-Sep® Bead

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



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Properties of Bio-Sep® Beads Useful for Treatment Applications

- Adsorptive surface
  - Concentrates contaminants present at low concentrations
  - Reduces aqueous phase concentrations at high contaminant concentrations
- High porosity and surface area
  - Rapidly colonized by indigenous microbes
  - Release microbes into the aquifer once carrying capacity of the beads is reached

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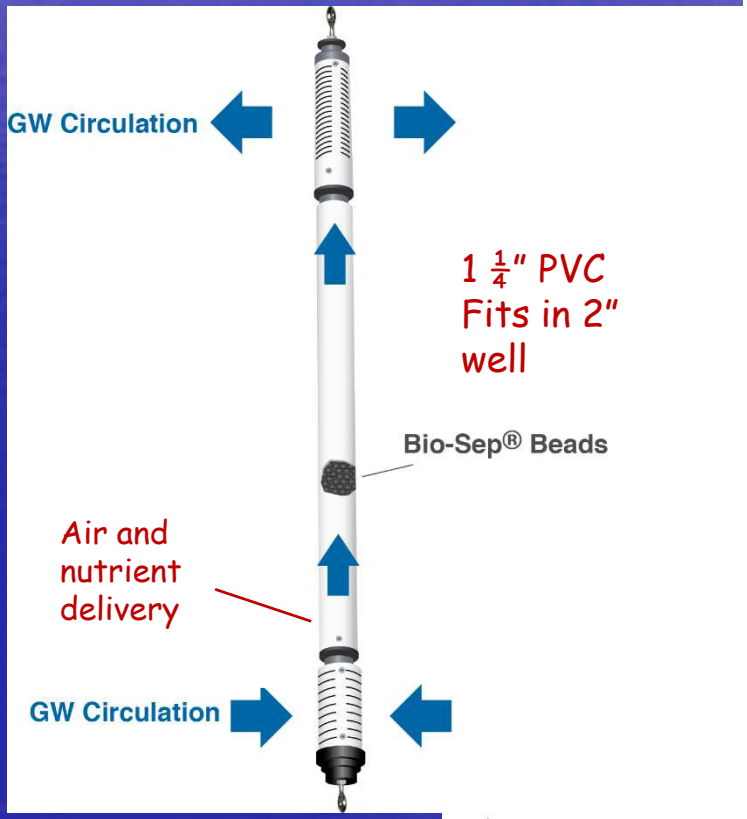
The Aerobic Bio-Sep ISBR

- Bio-Sep beads provide an incredible surface area for microbial growth
- Air sparging 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) support growth of desired indigenous microbes
- Water exiting the reactor carries contaminant-degrading microbes into the aquifer



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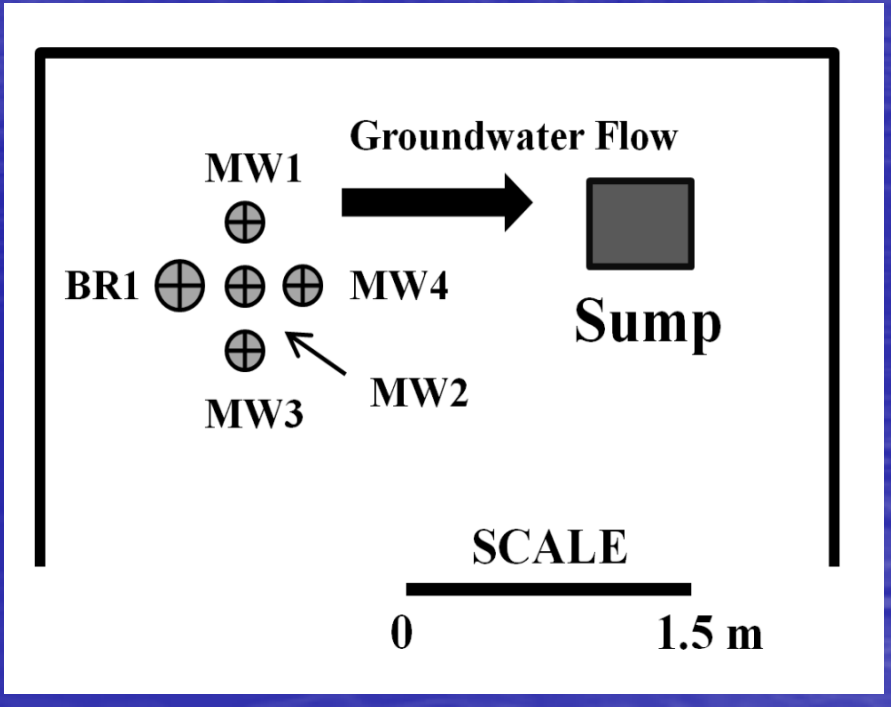
Topside control

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

Case Study – Aerobic ISBR – Low Concentrations of Hydrocarbons

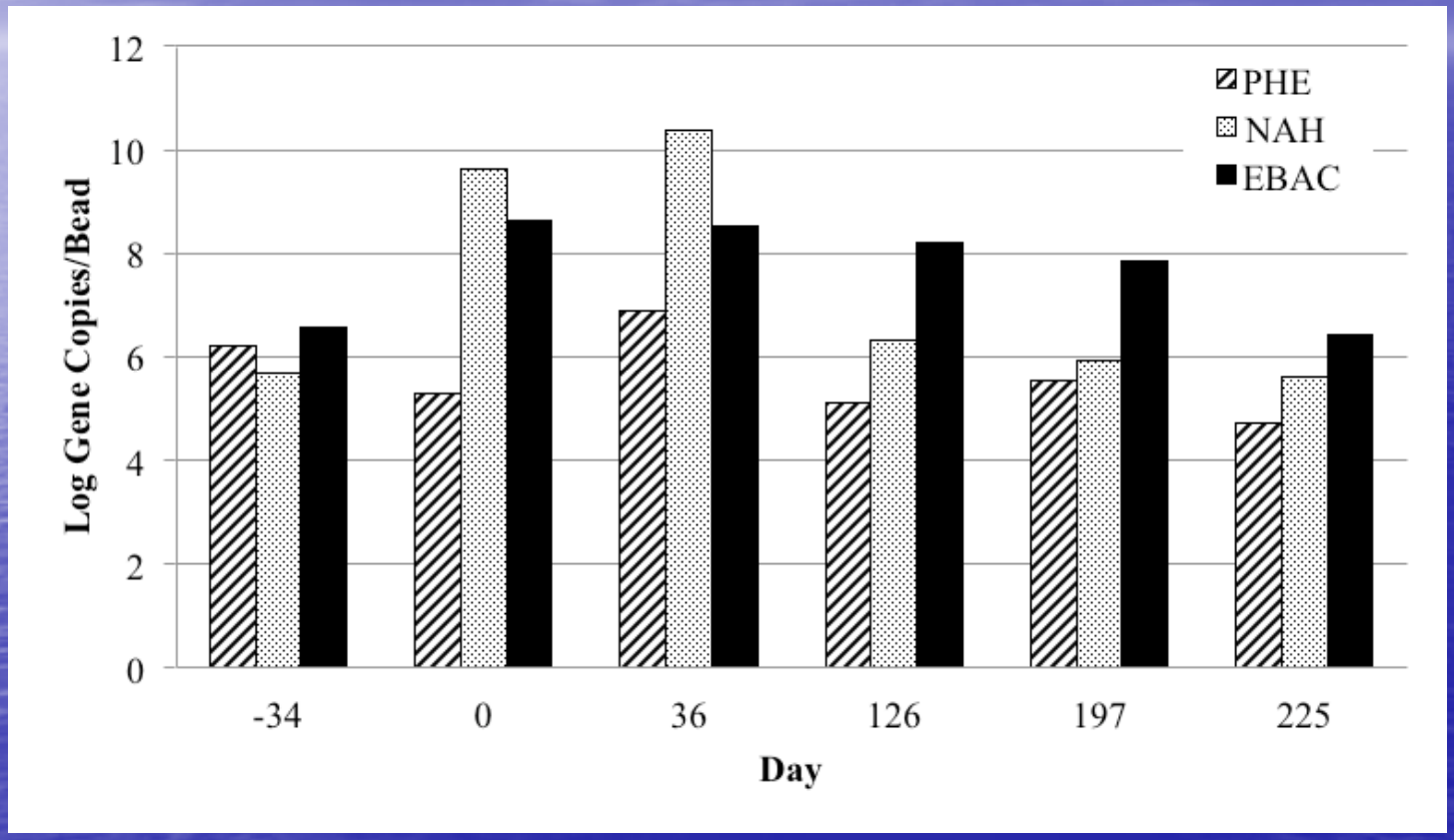
- Fuel oil release impacting soil and groundwater beneath a private residence
- The bioreactor well & four monitoring wells were installed in the basement
- Wells spaced 1 ft. apart



ISBR Timeline

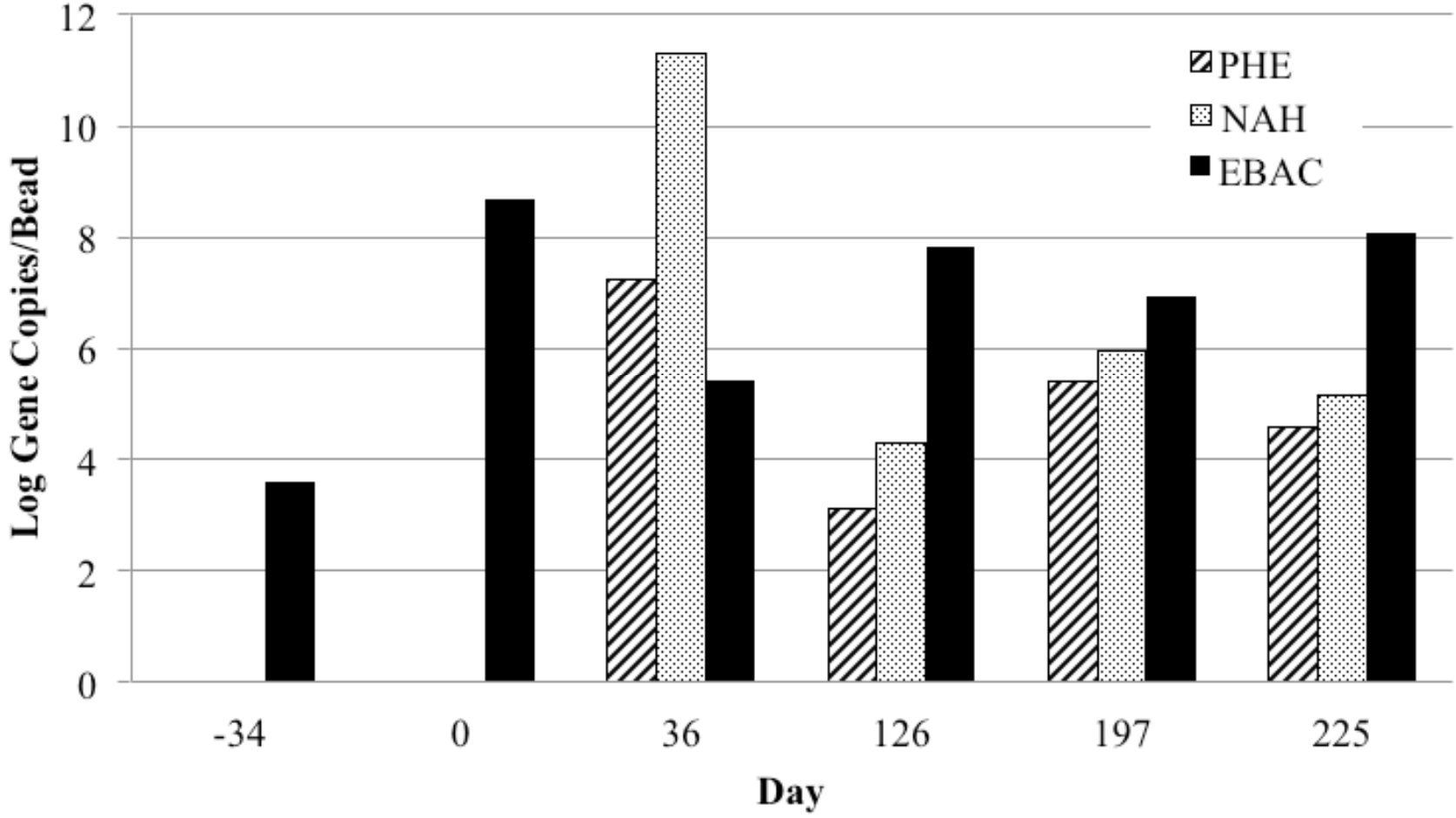
- Days -67 through -34: Air sparging only (no Bio-Sep)
- Days -34 through 0: Air sparging and nutrient delivery (no Bio-Sep)
- Day 0 onward: Complete bioreactor system operational (Bio-Sep beads added)
- Bio-traps® (Microbial Insights, Inc) used through out testing to monitor microbiology of bioreactor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

qPCR Analysis of BR1 Bio-traps During ISBR Testing



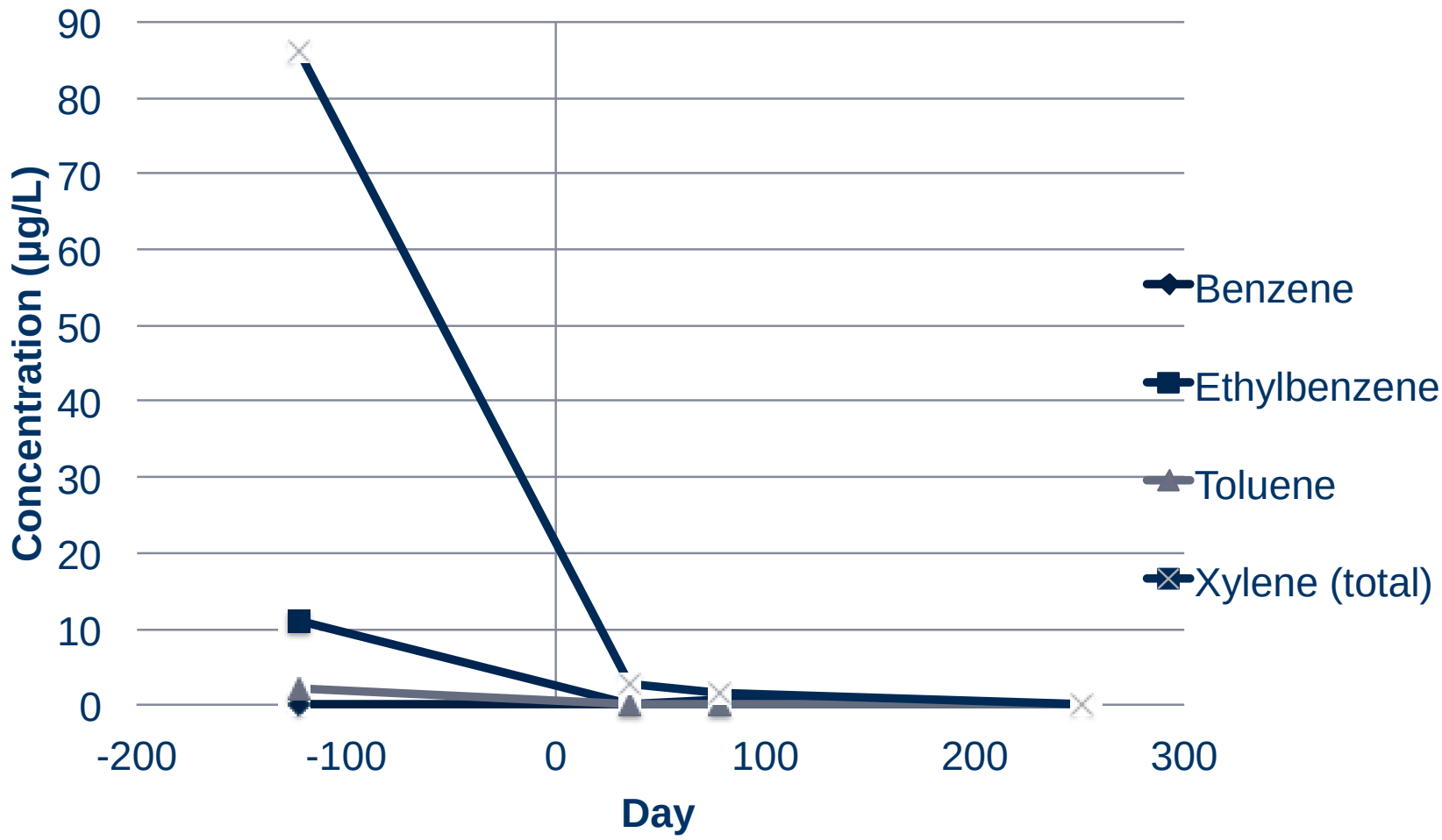
Didn't see much difference in hydrocarbon catabolic genes with DNA. This is common observation since hydrocarbon degraders are ubiquitous in the environment.

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



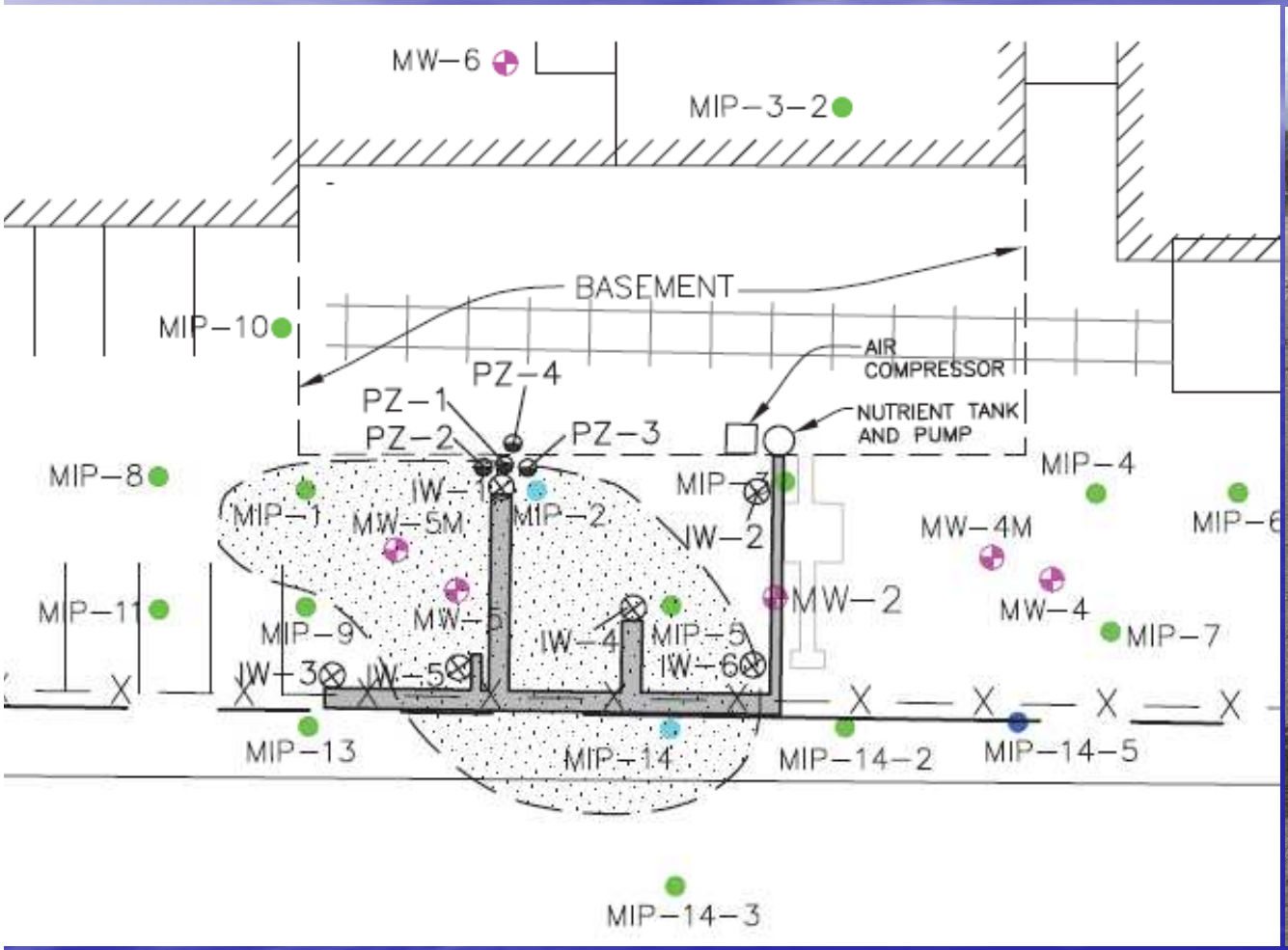
Conclusions

- Biodegradation stimulated at low hydrocarbon concentration
- Stimulation required the presence of Bio-Sep® beads in the ISBR
- Stimulation in hydrocarbon biodegradation was not confined to the well housing the ISBR but was evident in downgradient monitoring wells as far away as 60 cm within 36 days after the ISBR was fully operational.
- All lines of evidence indicated that the ISBR system effectively increased the numbers and activity of indigenous microbial degraders and enhanced biodegradation of BTEX components

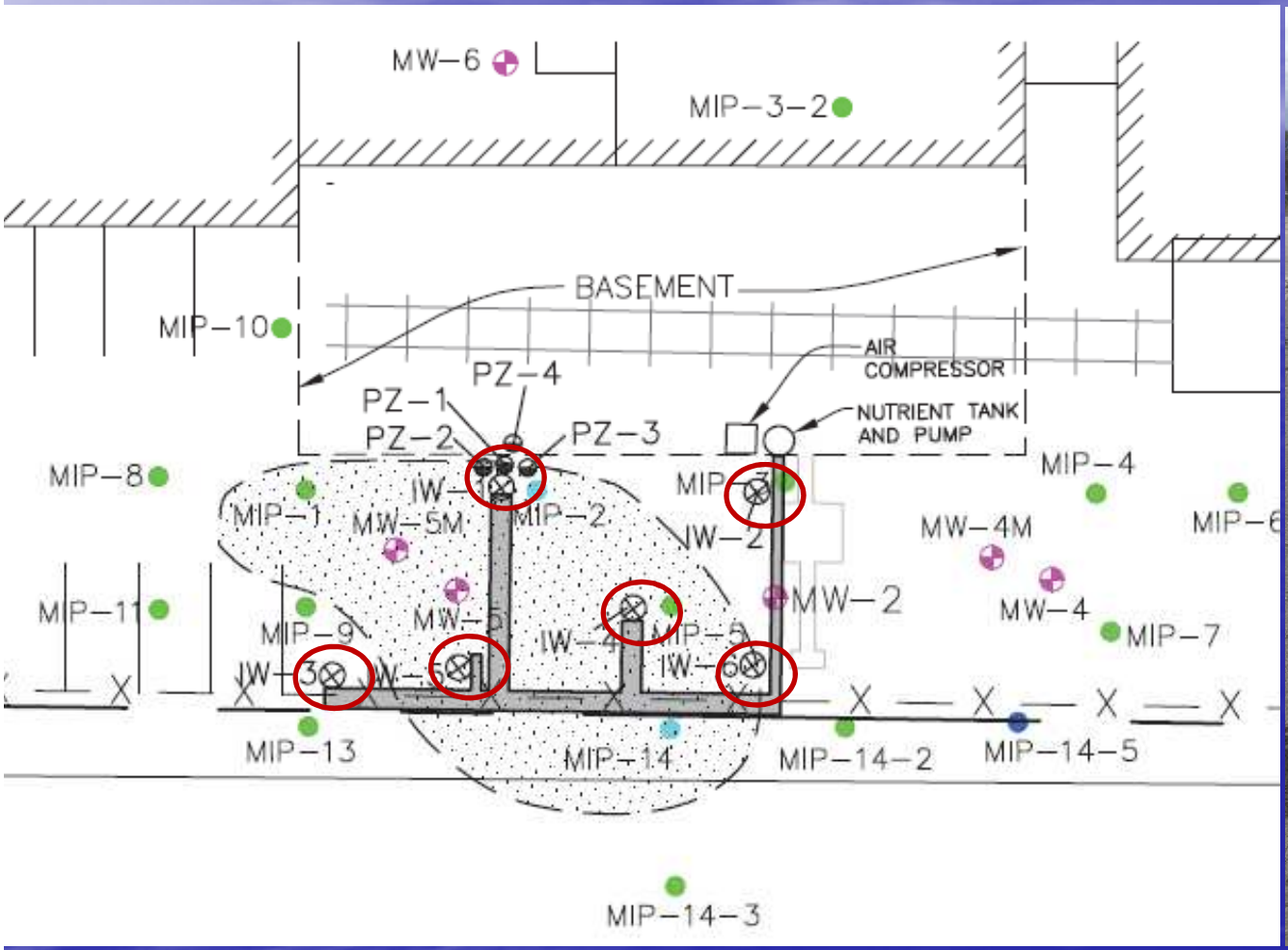
Case Study – Aerobic ISBR – Inhibitory Concentrations of Aromatic Hydrocarbons

- Former industrial site in northern New Jersey
- Toluene UST was removed in October 2001
- Previous remedial efforts failed to achieve treatment goals
- Free phase toluene product observed historically in the pilot study area
- Toluene concentrations up to **430 mg/L** (inhibitory)

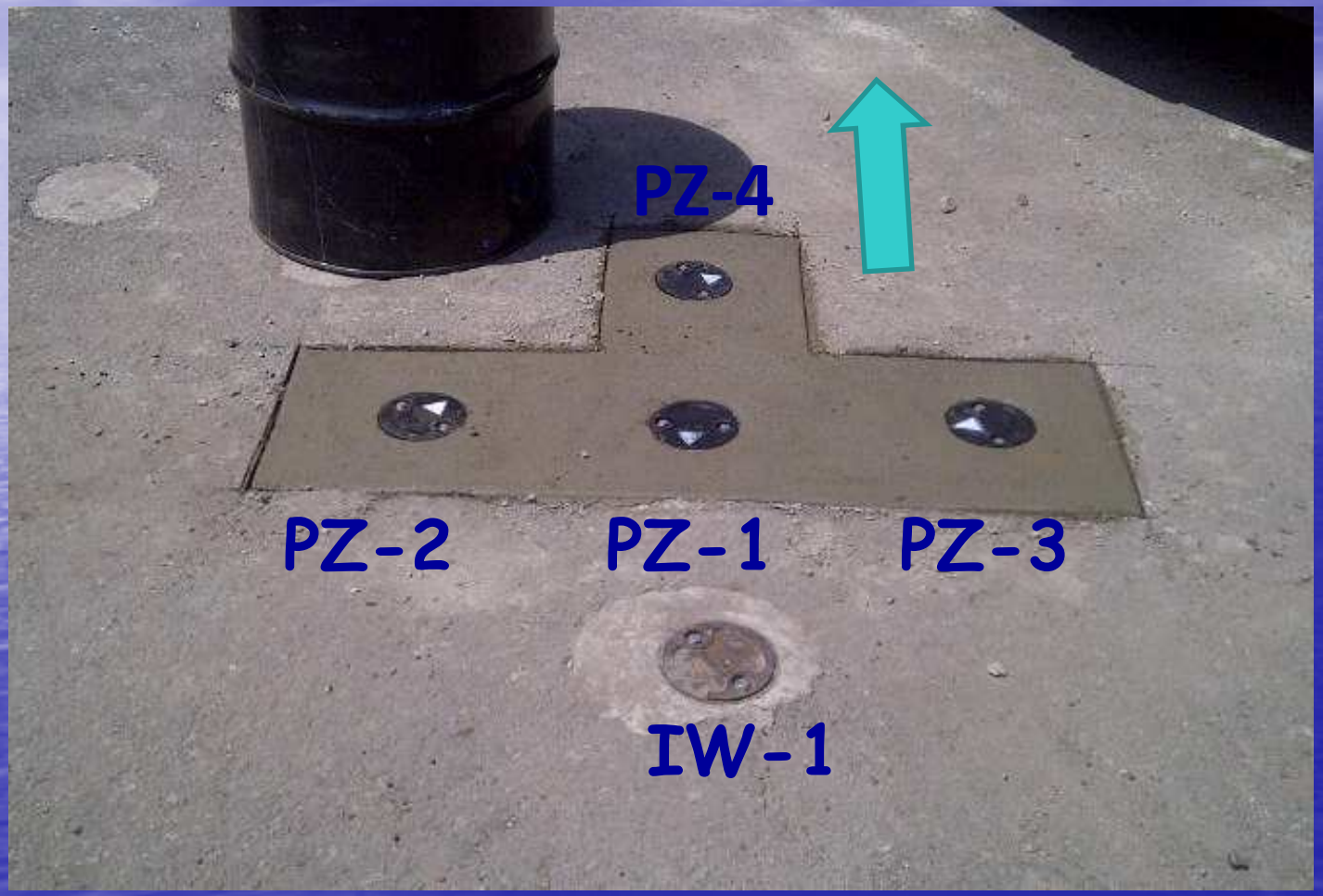
Aerobic ISBR - Pilot Study



Aerobic ISBR - Pilot Study



Pilot Study Results - IW-1 and Piezometers



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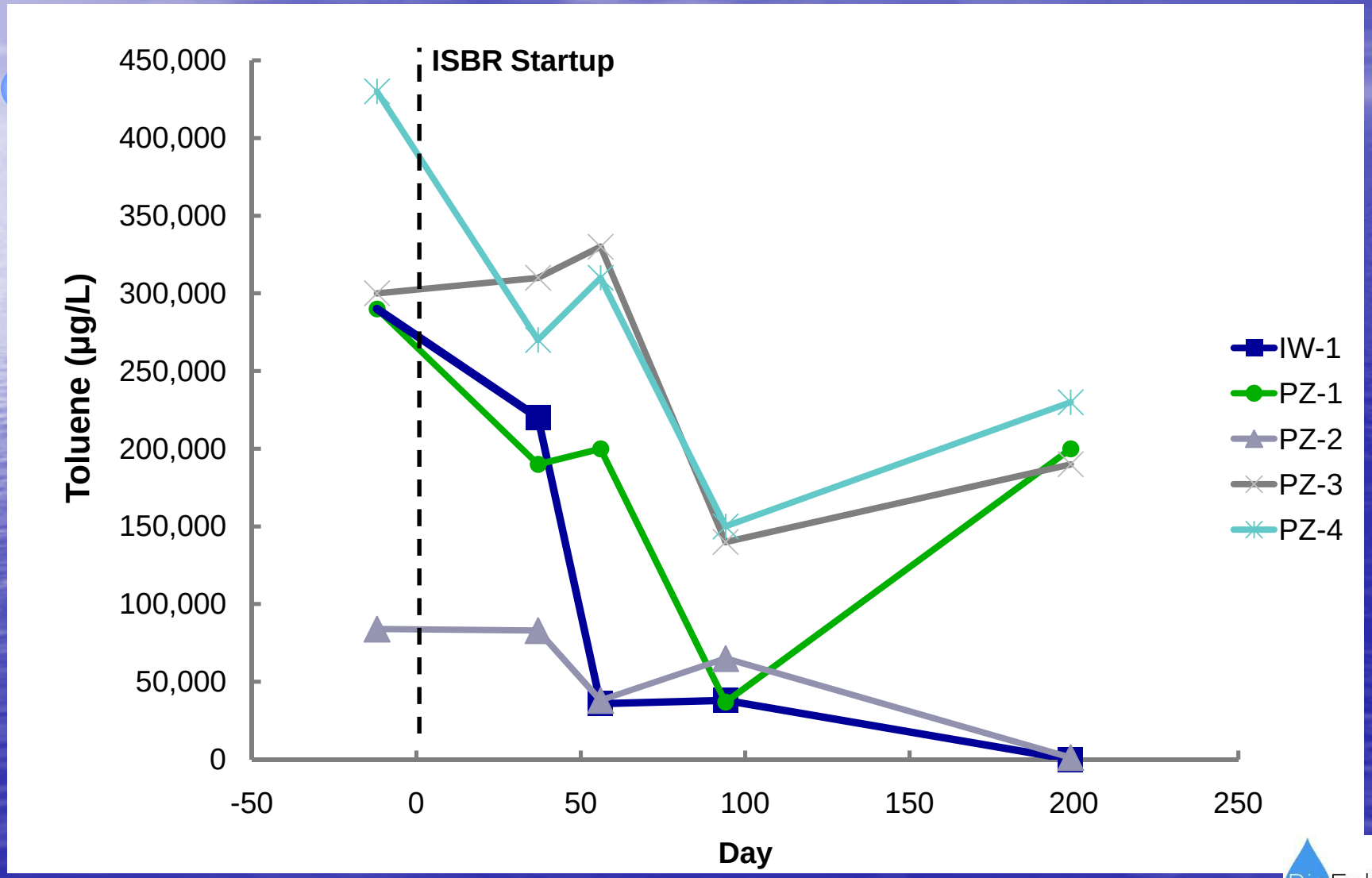

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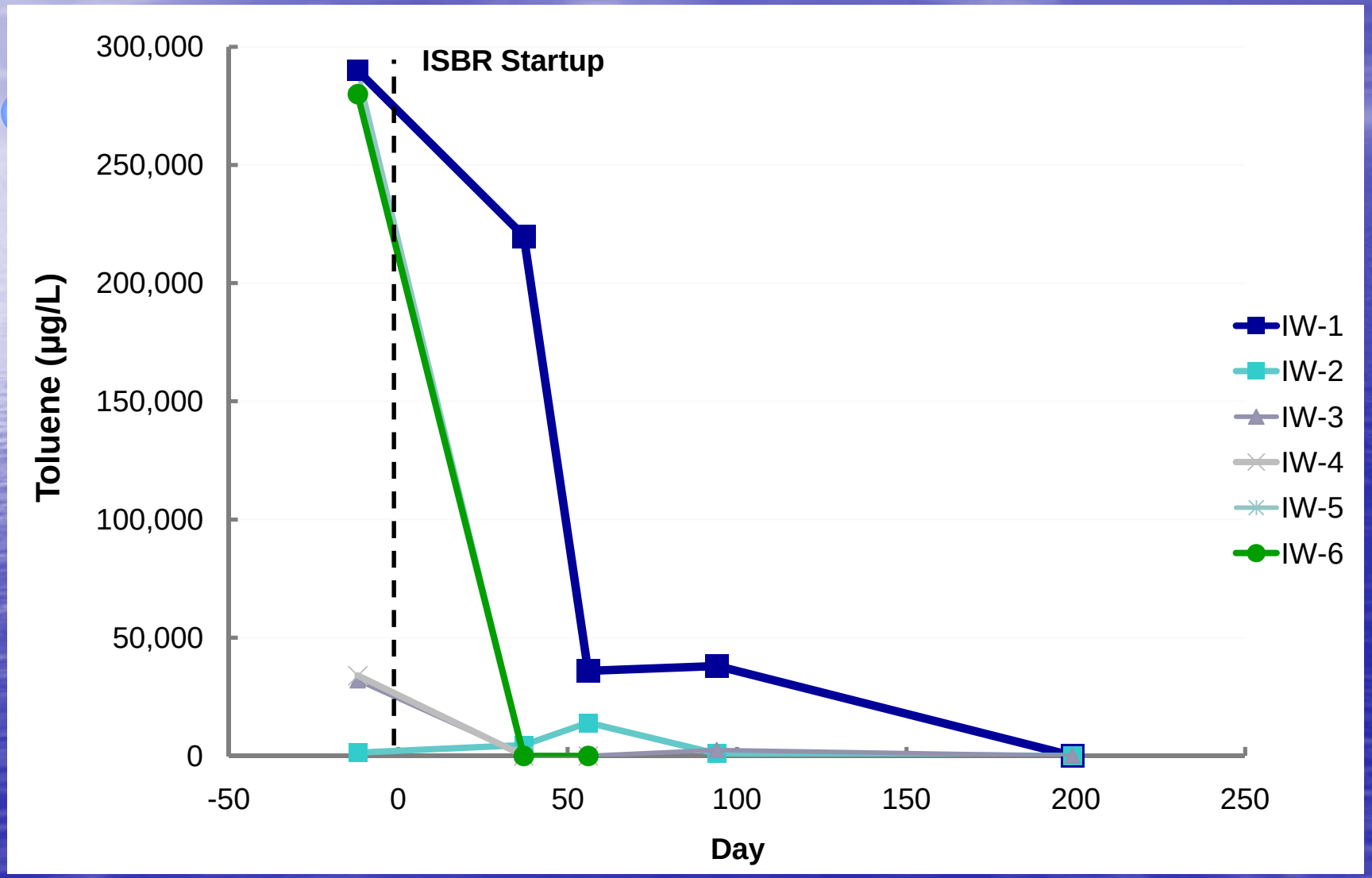
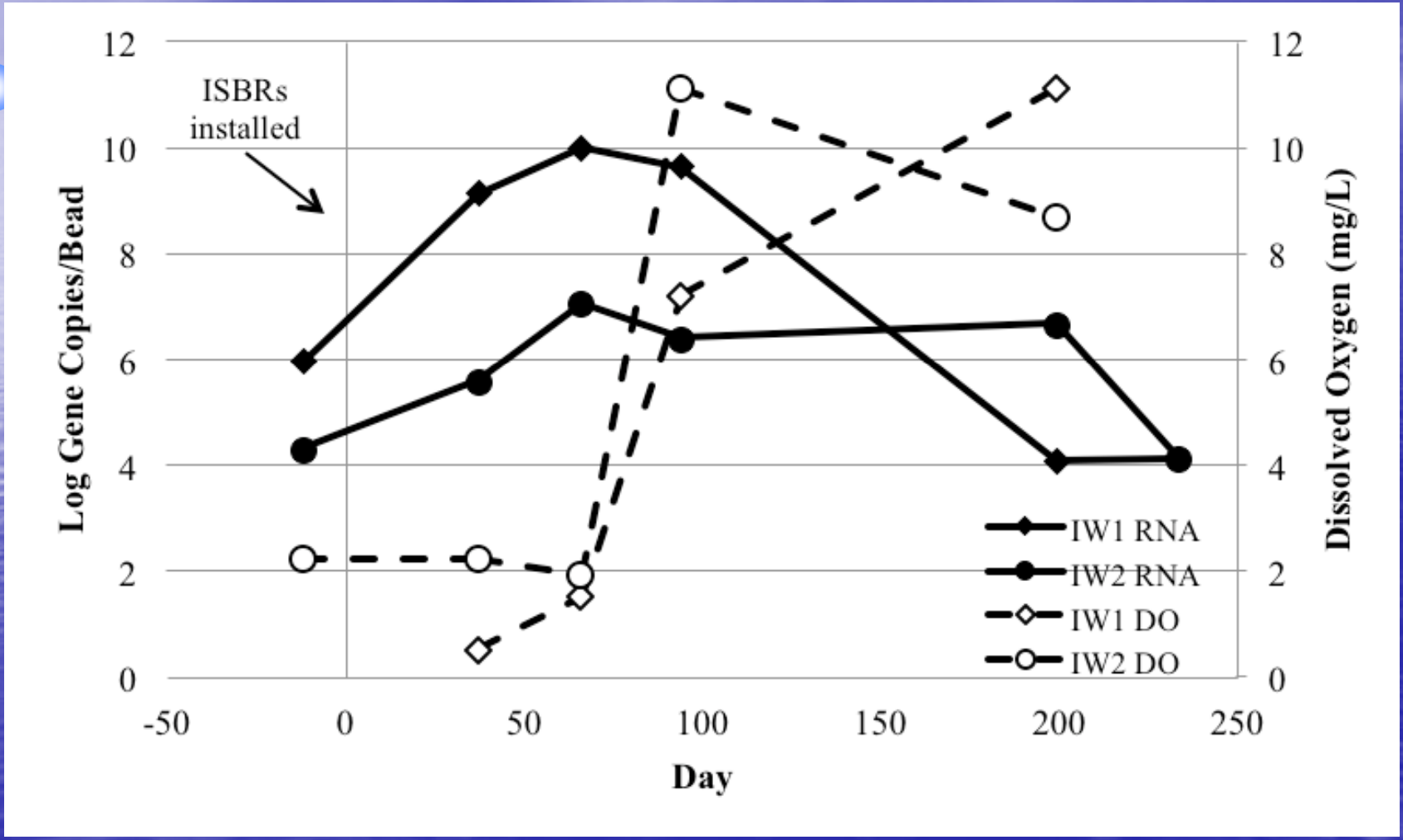
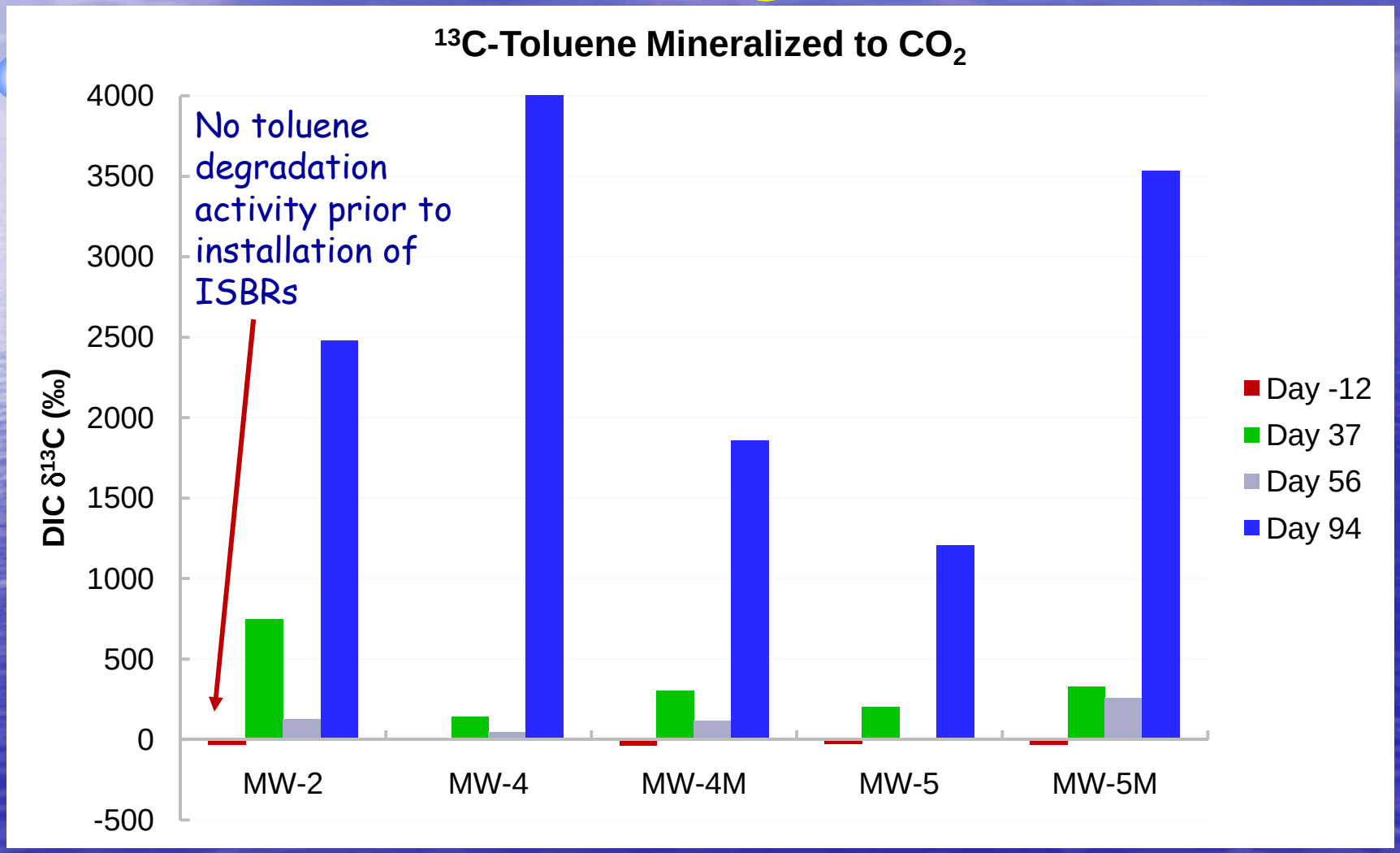
Pilot Study Results – All ISBR Wells



Functional Gene RT-qPCR



Enhanced Biodegradation at Monitoring Wells



Stable isotope probing with ^{13}C-toluene bio-traps

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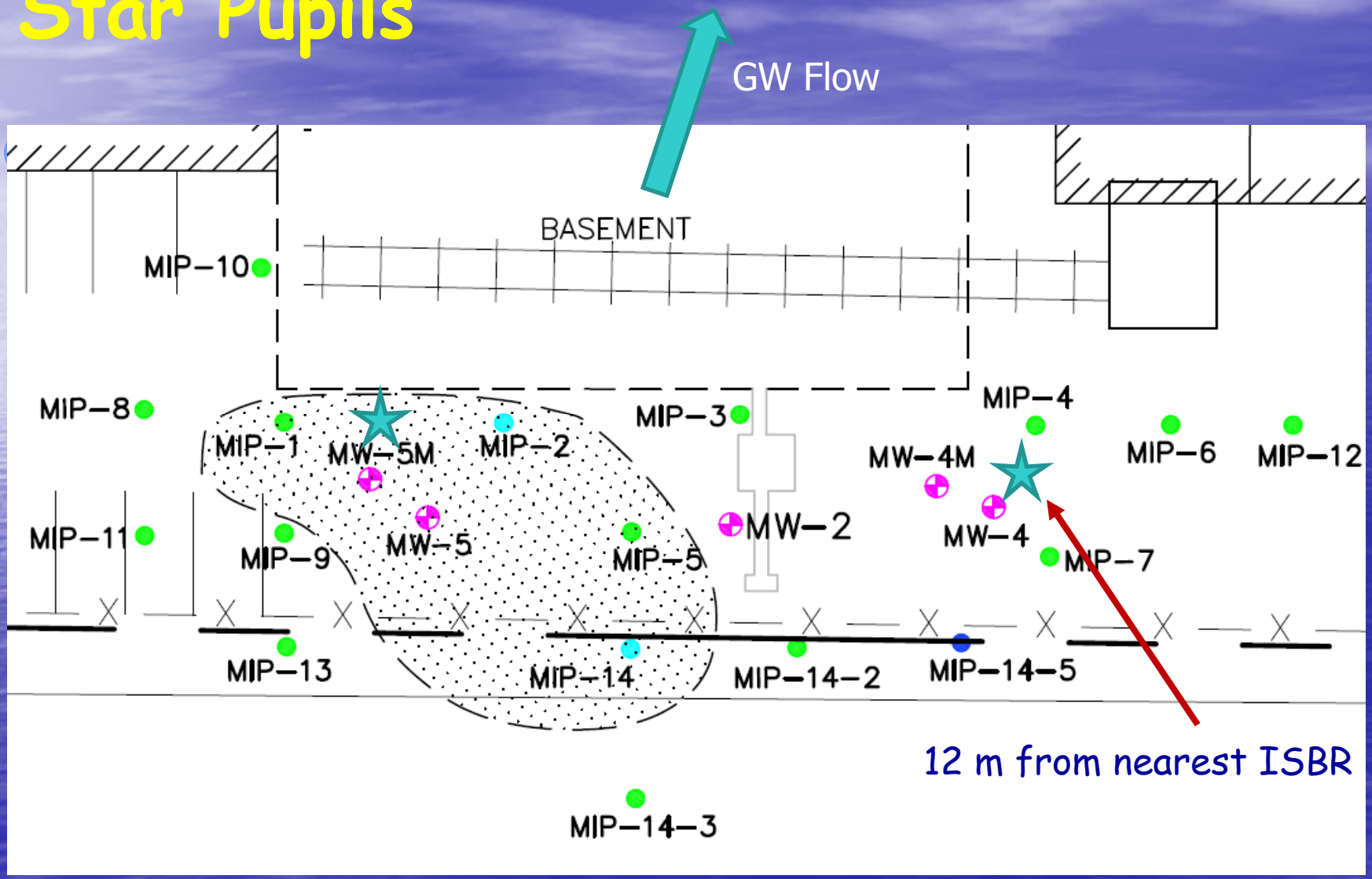
Star Pupils


GW Flow
BASEMENT
MIP–10
MIP–8
MIP–1
MW–5M
MIP–2
MIP–3
MIP–4
MW–4M
MIP–6
MIP–12
MIP–11
MIP–9
MW–5
MW–2
MIP–5
MW–4
MIP–7
MIP–13
MIP–14
MIP–14–2
MIP–14–5
12 m from nearest ISBR
MIP–14–3

Conclusions

- > 87% reduction in toluene concentrations in under two months under inhibitory conditions
- Enhanced biodegradation at ISRB and distant downgradient monitoring wells
  - Stable isotope probing (^{13}C incorporation into CO_2)
  - Expression of functional genes (RT-qPCR)

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

- O&M
  - System checks every 2-4 wks
  - Power
  - Nutrients
  - Water level (ISBR must be totally submerged to function)

The End