PILOT STUDY TO EVALUATE TOLUENE SOURCE AREA BIOREMEDIATION USING AN AEROBIC IN-SITU BIOREACTOR® (ISBR)

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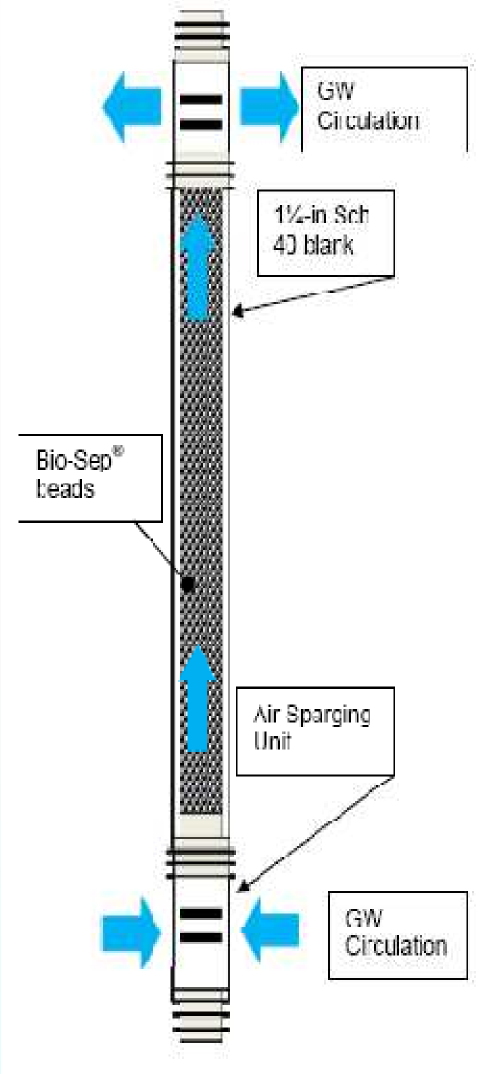
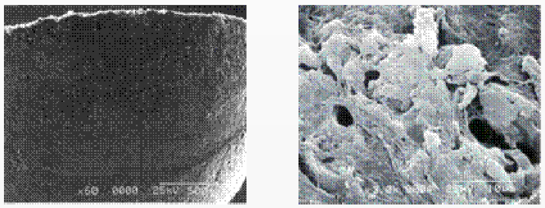
Operation of an Aerobic Bioreactor at Hydrocarbon Sites

- 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

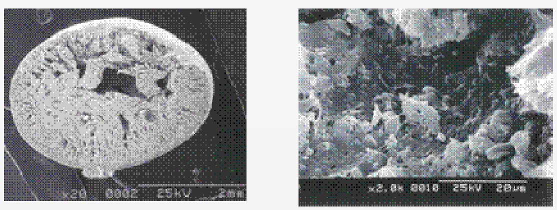


Aerobic Bioreactor Design

Exterior of Bio-Sep



Interior of Bio-Sep



•Developed by Kerry Sublette Ph. D. @ the Univ. of Tulsa

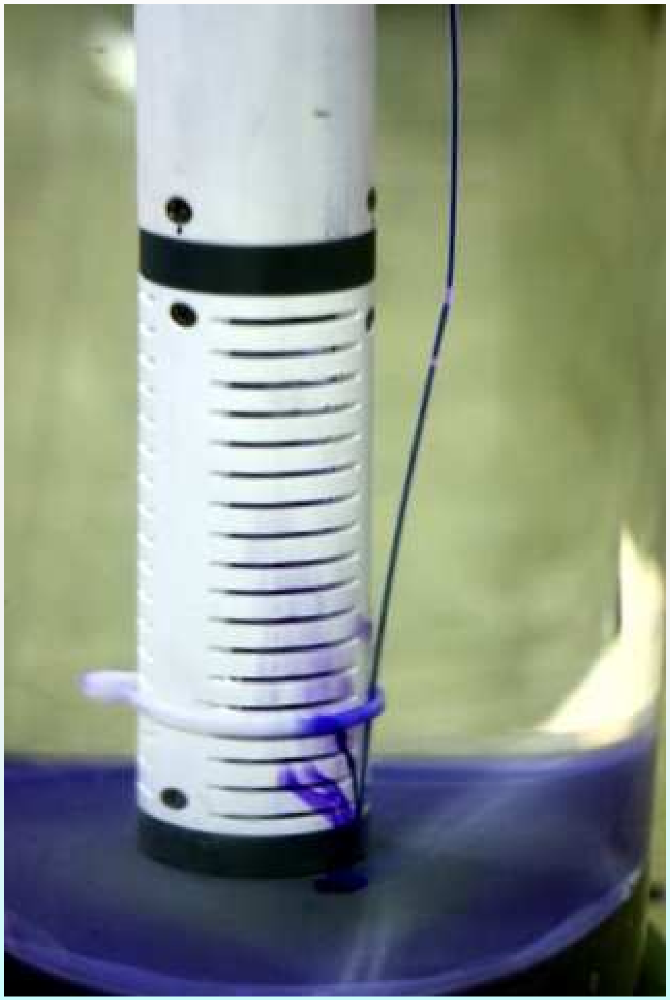
•Fits in standard 2” well

•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 lines connected to surface equipment

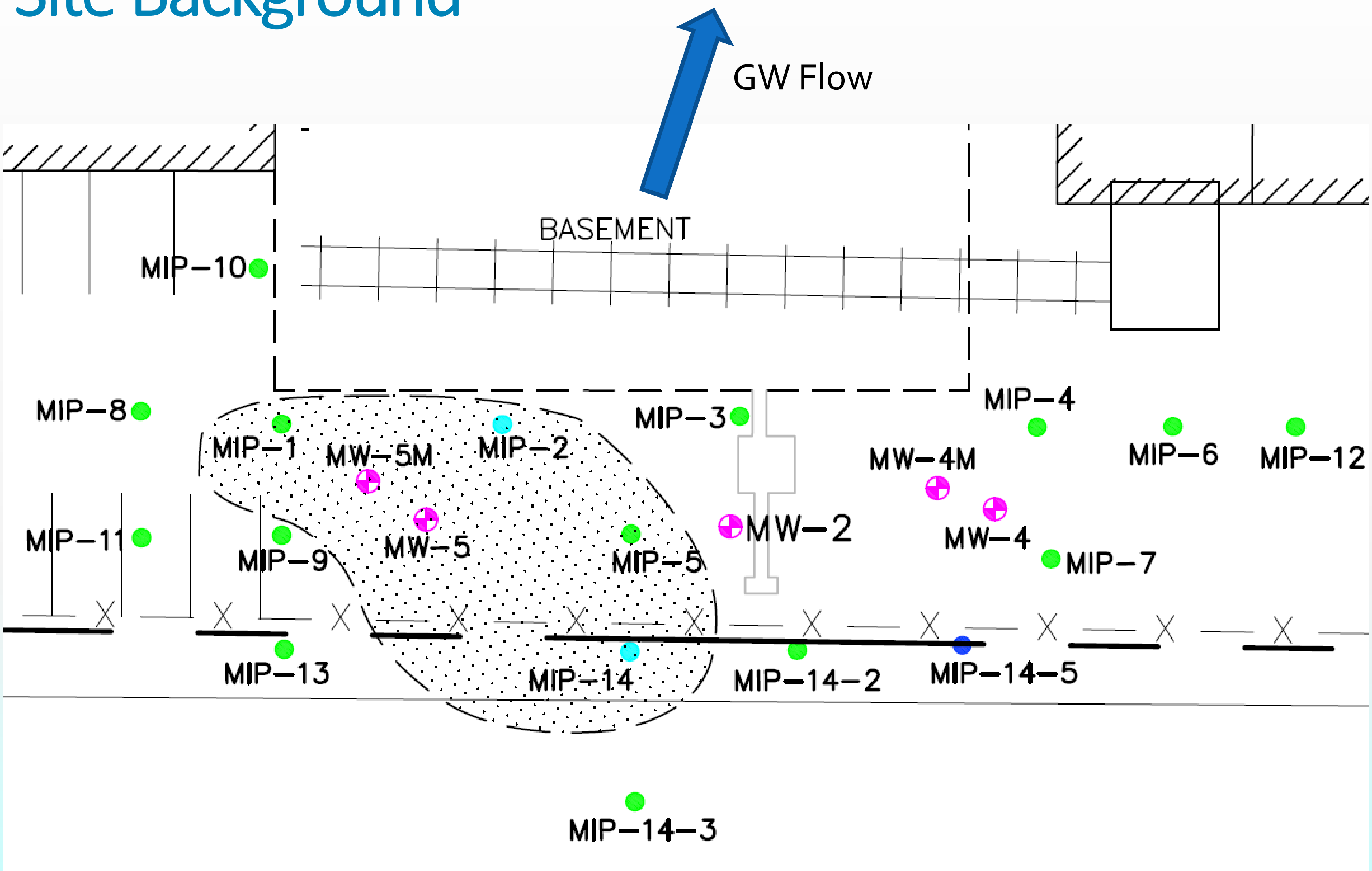
Aerobic Bioreactor Flow Pattern



Site Background

- Former Industrial site in northern New Jersey
- Toluene UST was removed in October 2001
- Soil and groundwater contamination and **free phase toluene product** has historically been observed in the pilot study area
- An attempt was made to recover free product but was largely unsuccessful
- The Aerobic In-Situ Bio-Reactor (ISBR) treatment system was installed and activated in August 2011

Site Background



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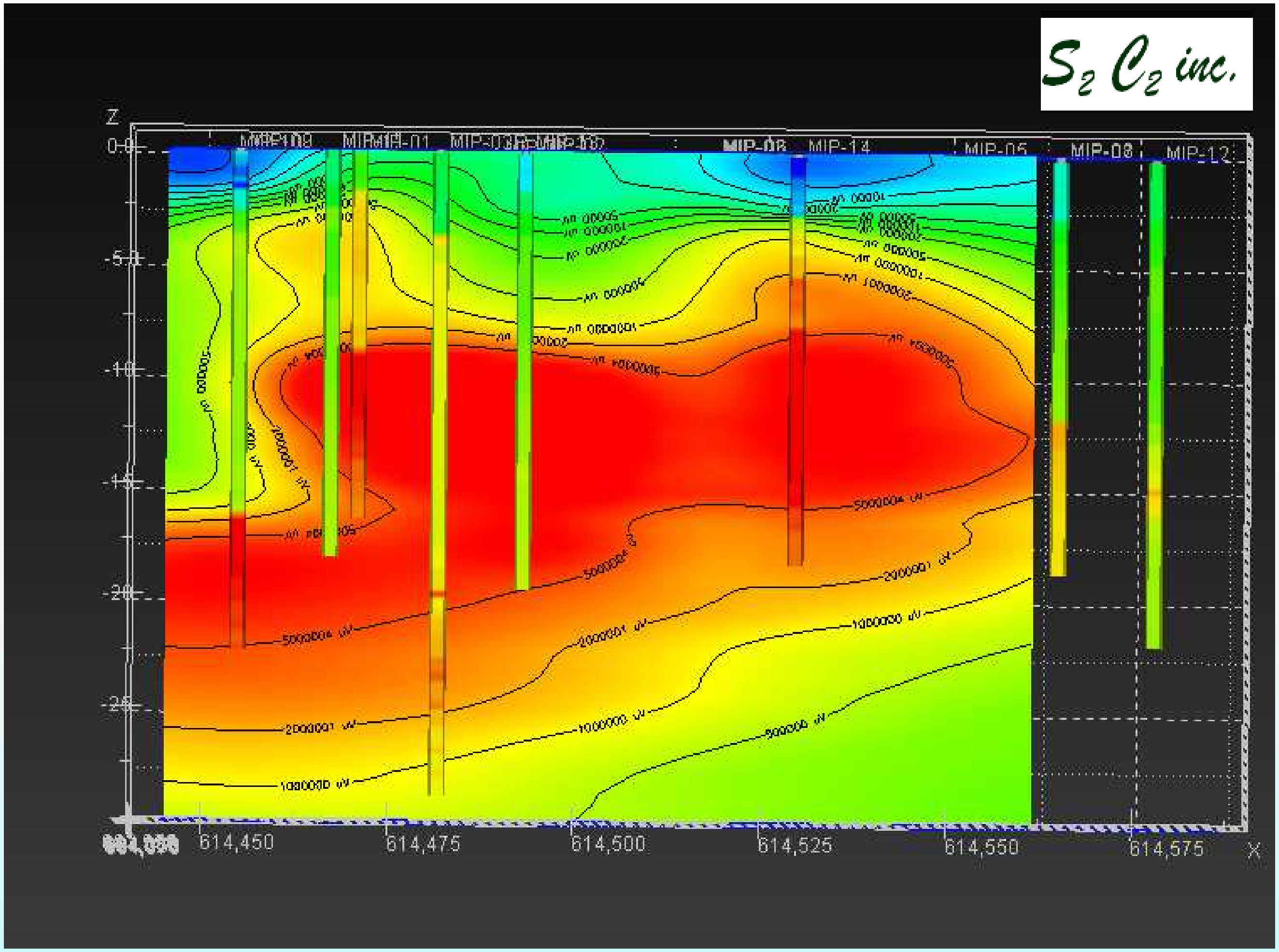
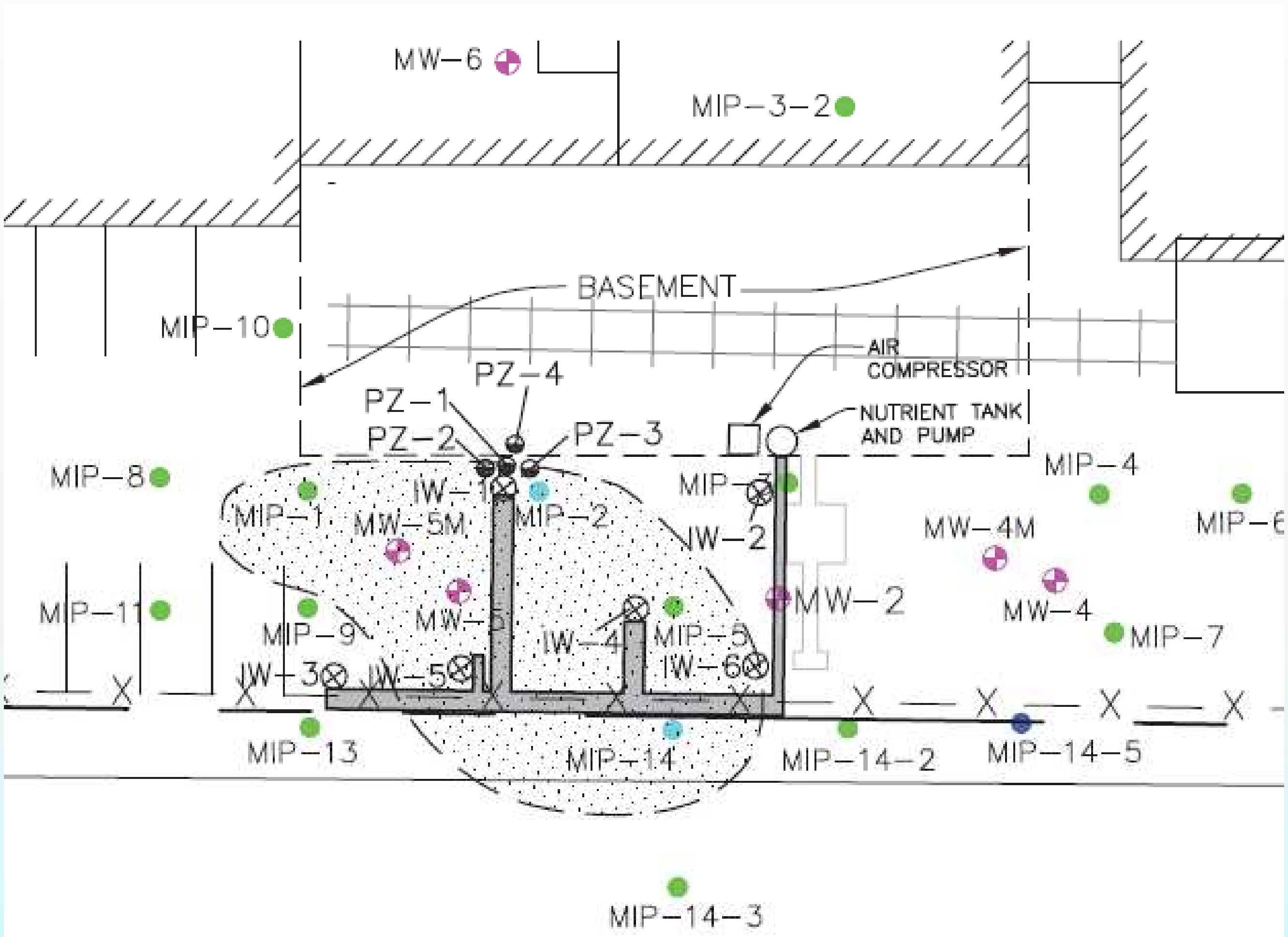
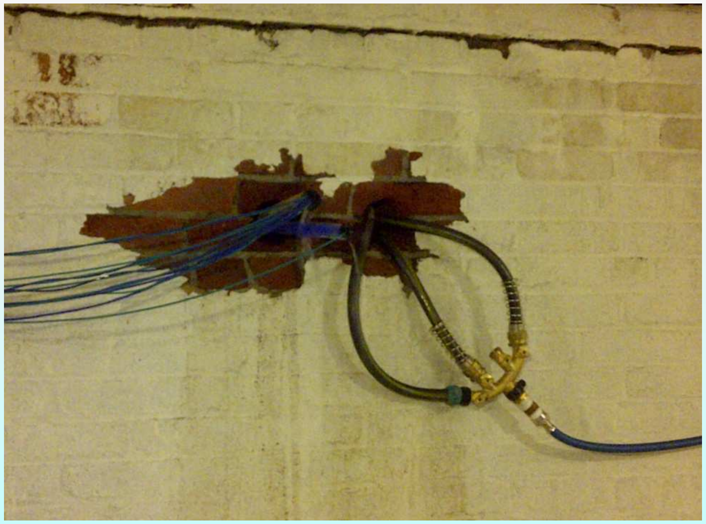


Aerobic Bioreactor Installation



Aerobic Bioreactor Installation



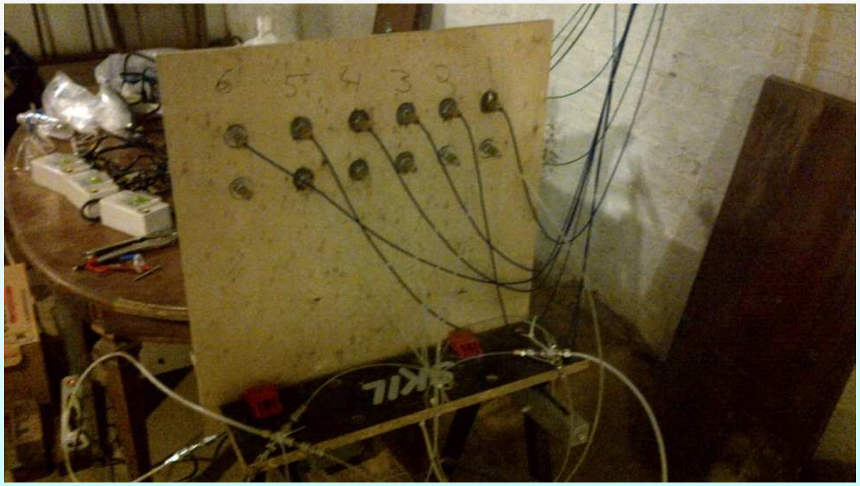
•Six (6) nutrient solution reservoirs. Nutrients include ammonium ion (NH4+), phosphate and Bromide (tracer)

•One compressor attached to a manifold distributing supplied air to six (6) different lines

•Nutrients (green lines) and air (blue lines) joined together and fed through existing hole in the basement wall;

•Installed a dedicated potable water line that was attached to a splitter to supply water to three separate ISBR treatment well locations

Aerobic Bioreactor Installation



Then: Surface equipment supporting the bioreactor

Now: *Bio-Enhance* Control Box

Aerobic Bioreactor Installation

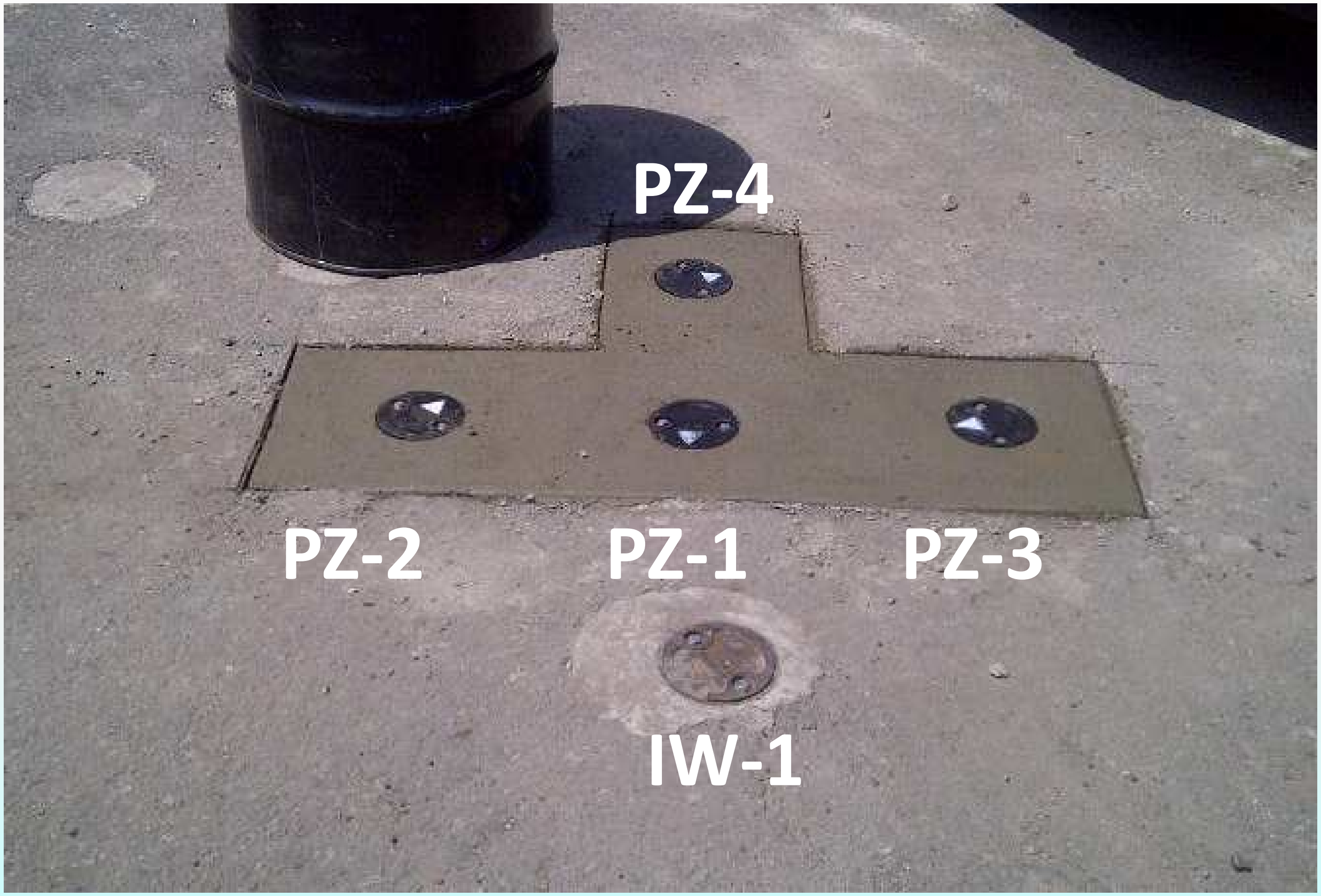
•Teflon tubing exits the basement wall and into a trench. Each pair of Teflon tubing per ISBR well was encased in 1 inch PVC conduit

Aerobic Bioreactor Installation

IW Well

- PVC conduit connected to the well at the top of the casing, and air/nutrient lines connect to the top of ISBR unit

Aerobic Bioreactor Installation



Pilot Study Monitoring

<u>Bio-Trap® Samples:</u>

•Polymerase Chain Reaction (PCR) analysis for total bacteria counts and functional gene expression

•Stable Isotope Probing (SIP)- ^{13}C-labeled toluene

<u>Groundwater Samples:</u>

•Field parameters (dissolved oxygen [DO], oxidation/reduction potential [ORP], conductivity and pH);

•Nutrients and bromide tracer; and

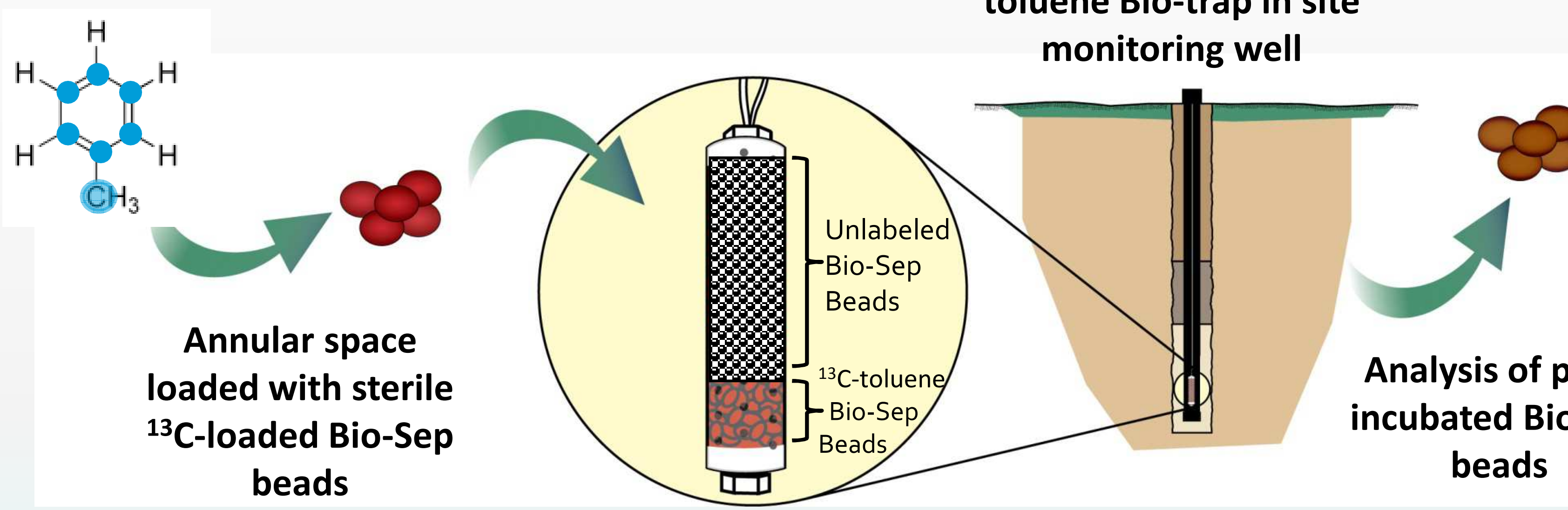
•TCL VOCs (VO+10)

Pilot Study Monitoring

Overview of Bio-Trap Stable Isotope Probing (SIP) Approach

^{13}C-labeled toluene

In situ incubation of ^{13}C-toluene Bio-trap in site monitoring well



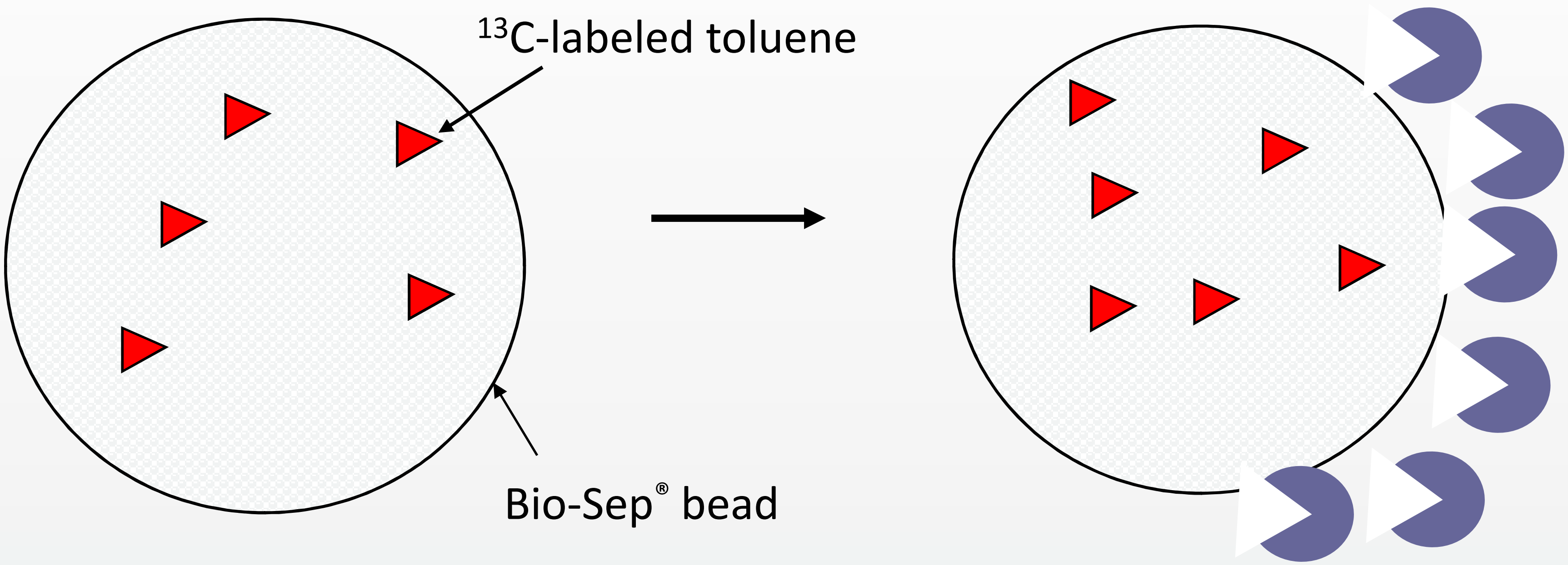
Annular space loaded with sterile ^{13}C-loaded Bio-Sep beads

Analysis of post-incubated Bio-Sep beads

Bio-trap filled with both regular Bio-Sep beads and a small amount of ^{13}C-toluene labeled Bio-Sep beads

Pilot Study Monitoring

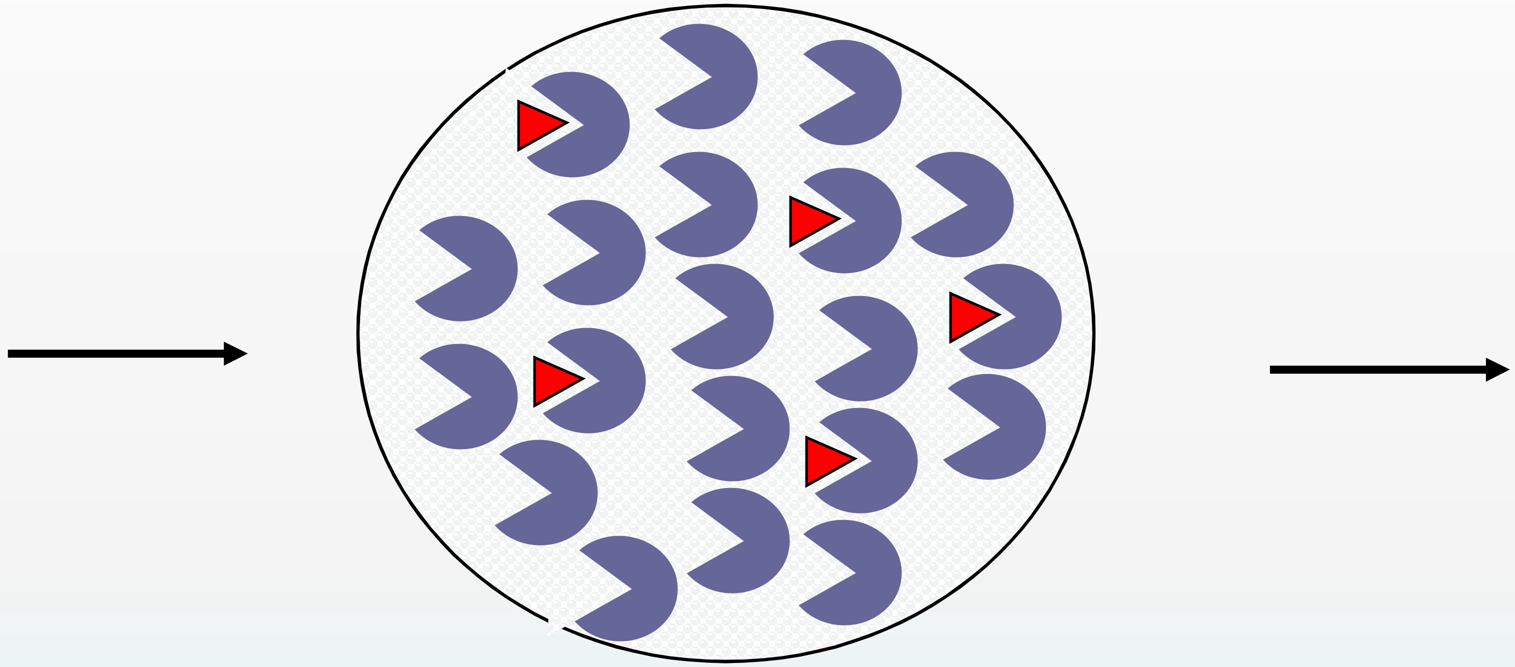
Stable isotope probing with Bio-Sep beads



1. ^{13}C-labeled toluene adsorbed to PAC within the beads. Approximately 15% of the loaded toluene contains ^{13}C stable isotope (red triangle = ^{13}C)
2. Substrate-baited Bio-Sep beads attract microorganisms; microenvironment provides optimal conditions for colonization

Pilot Study Monitoring

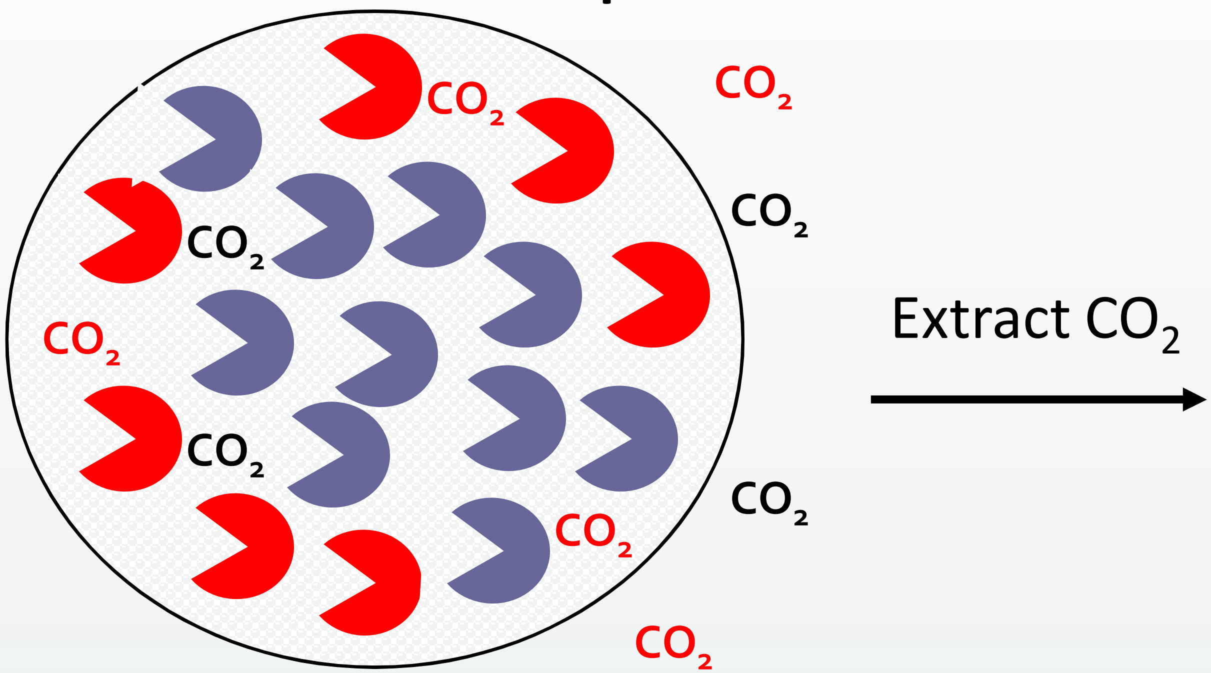
Microbes utilize the target compound



3. Indigenous microbes cultivated inside the Bio-Sep® bead utilize the ^{13}C-labeled toluene .

Pilot Study Monitoring

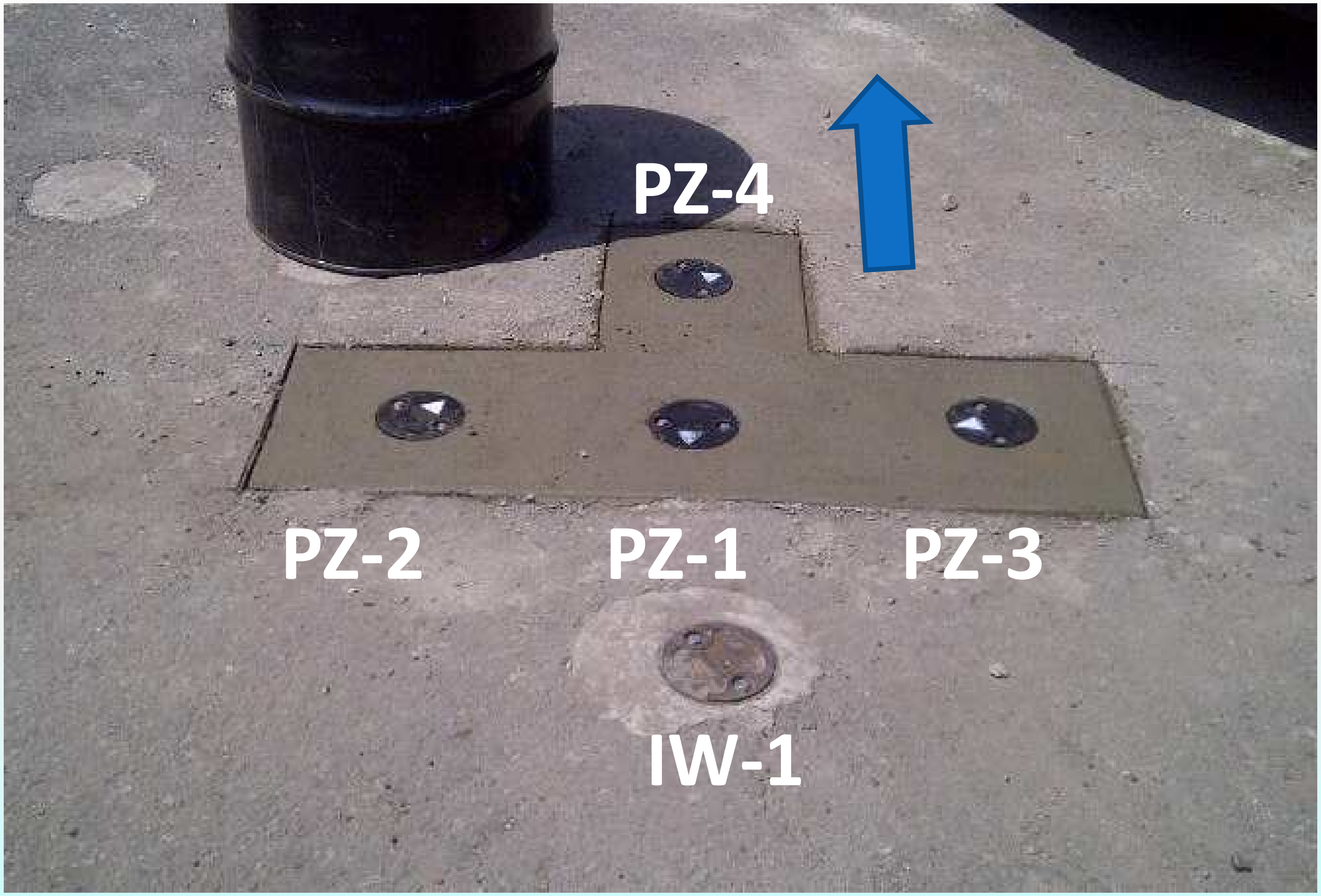
^{13}C Incorporation into biomass and CO_2



4. ^{13}C is incorporated into new cells growing in the beads and in CO_2, the desired end product of mineralization of the ^{13}C-toluene.

5. Extract CO_2 from beads and look for ^{13}C in the CO_2.

Pilot Study Results - IW-1 and Piezometers



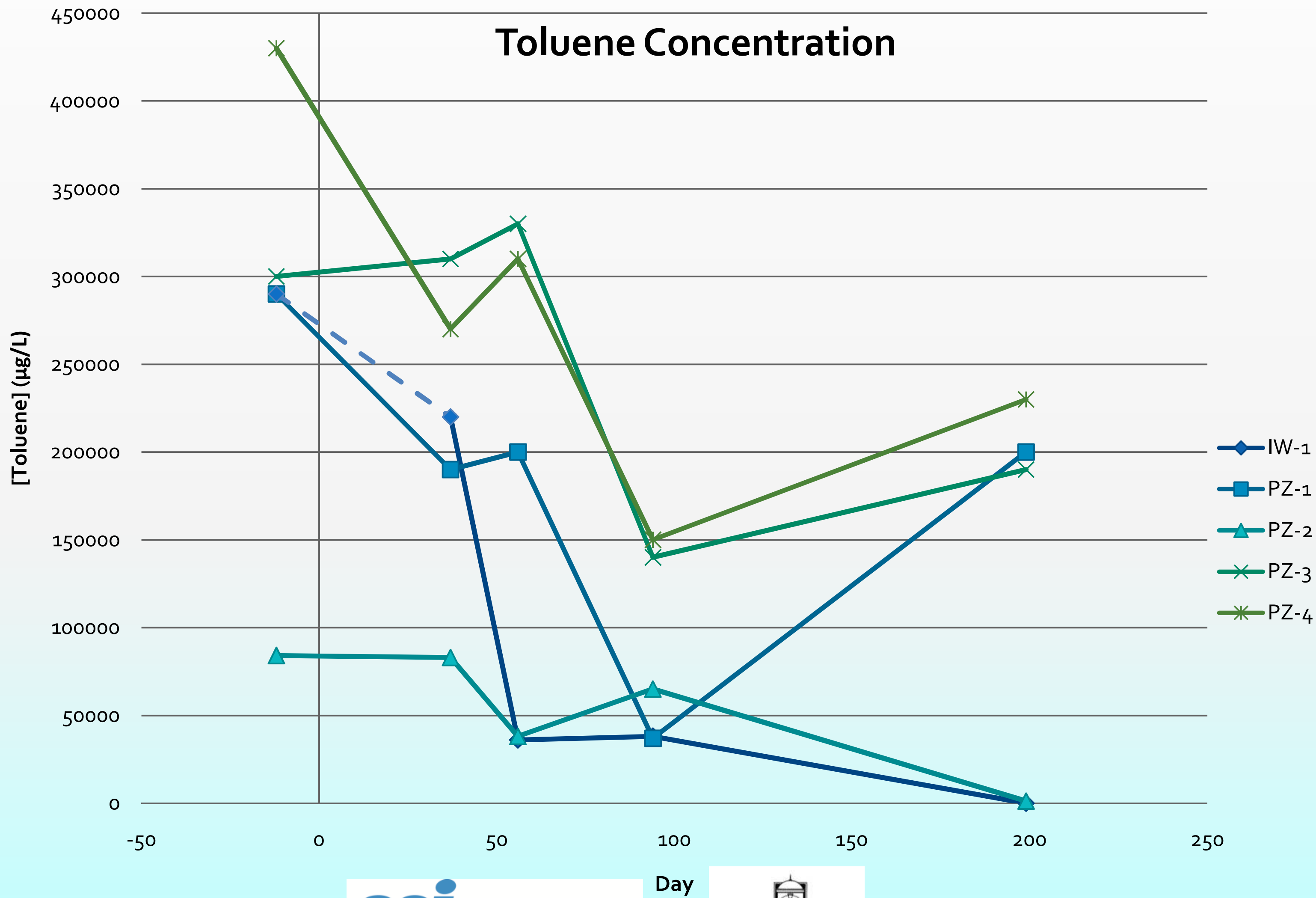
Pilot Study Results - IW-1 and Piezometers

The following decreases in VOC concentrations have been observed:

- IW-1: Toluene (220,000 µg/L with occasional free product to 17 µg/L)

- PZ-1: Toluene (290,000 µg/L to 37,000 µg/L)
  Benzene (870 µg/L to ND)
- PZ-2: Toluene (84,000 µg/L to 1,200 µg/L)
- PZ-3: Toluene (300,000 µg/L to 190,000 µg/L)
- PZ-4: Toluene (430,000 µg/L to 230,000 µg/L)

Pilot Study Results - IW-1 and Piezometers



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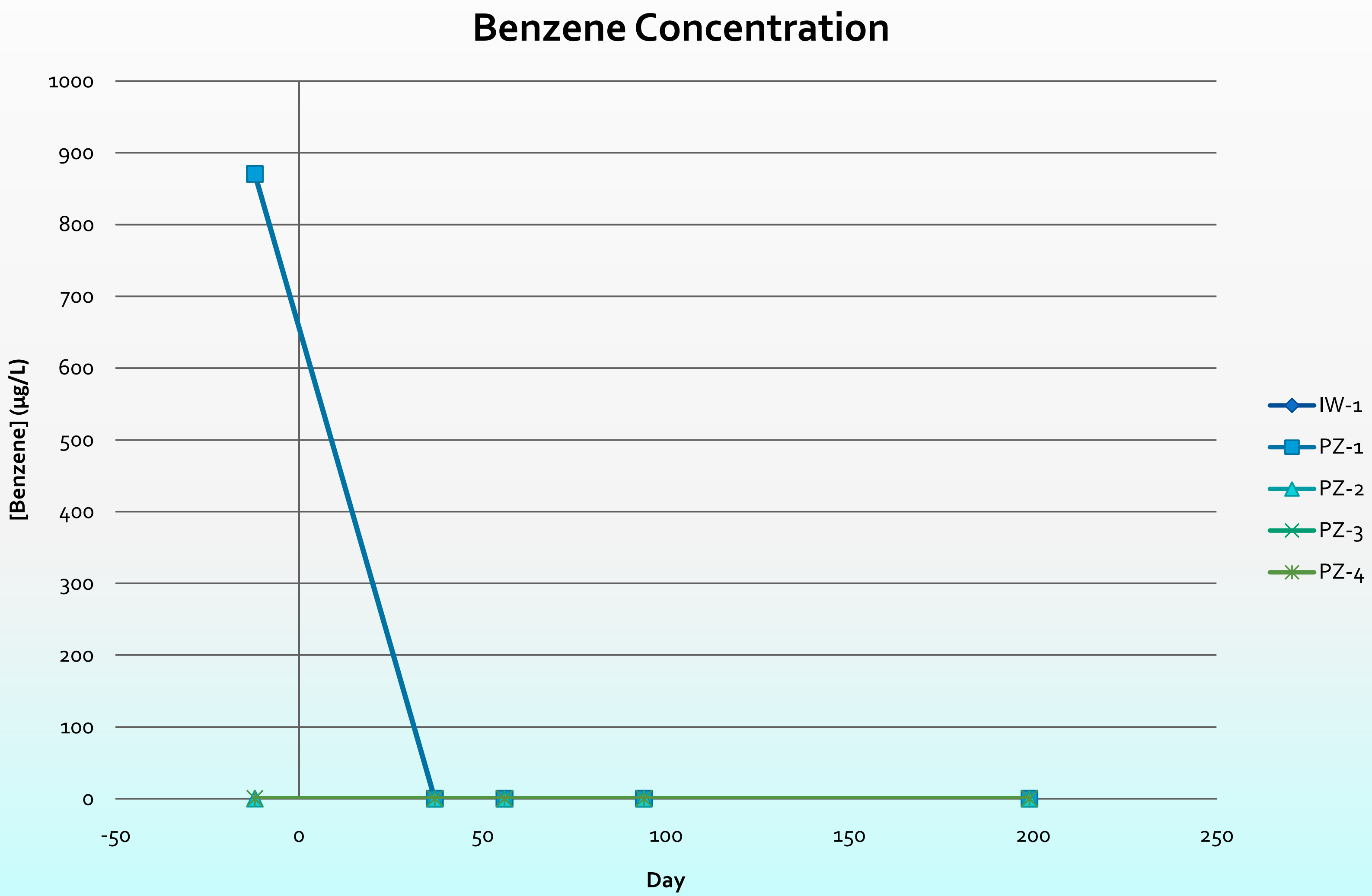

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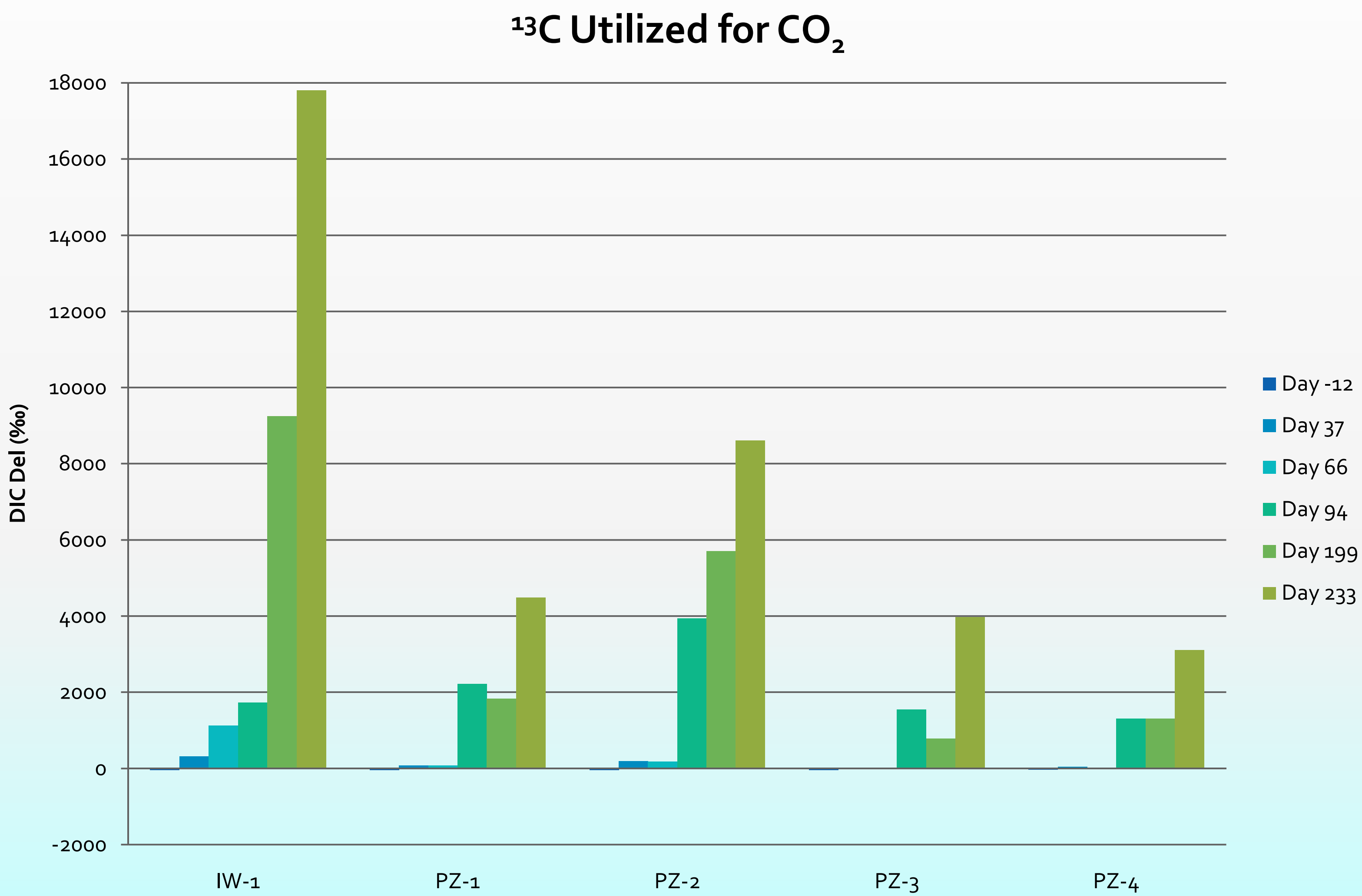
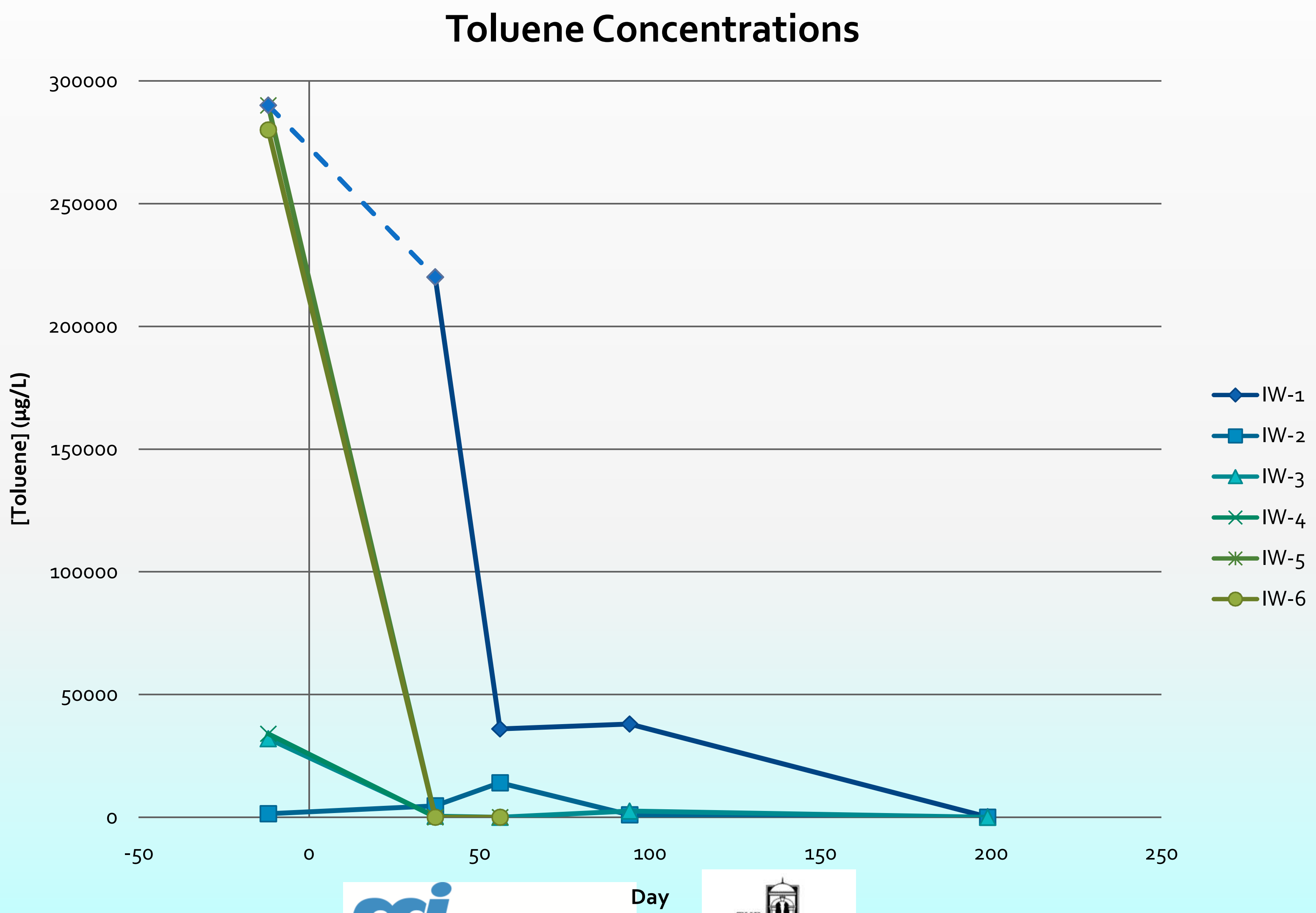


Pilot Study Results - ISBR Wells

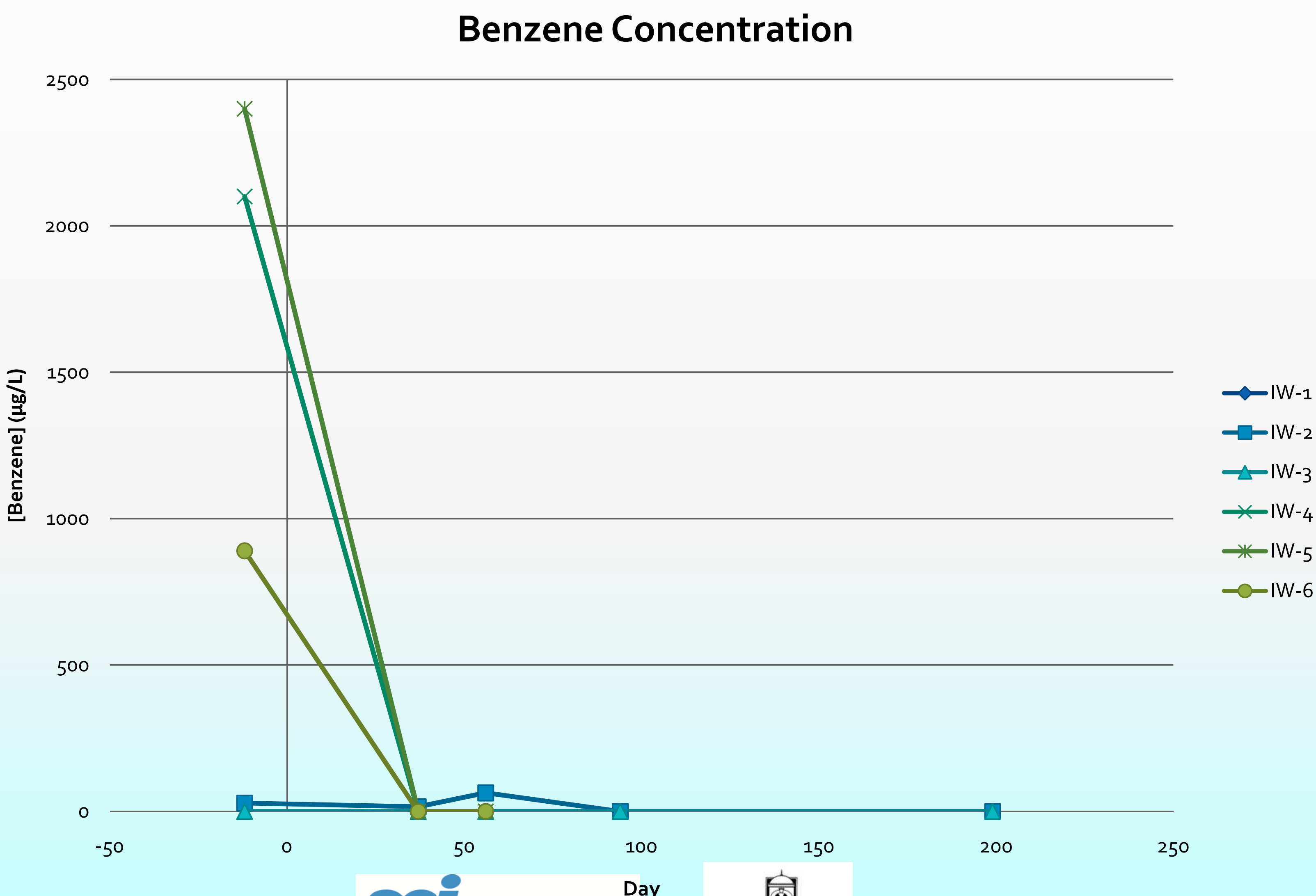
The following decreases in VOC concentrations have been observed:

- IW-2: Toluene (14,000 µg/L to **1.3** µg/L)
  Benzene (63 µg/L to ND)
- IW-3: Toluene (32,000 µg/L to **10** µg/L)
- IW-4: Toluene (340,000 µg/L to **1.3** µg/L)
  Benzene (2,100 µg/L to **ND**)
- IW-5: Toluene (290,000 µg/L to **1.2** µg/L)
  Benzene (2,400 µg/L to **ND**)
- IW-6: Toluene (280,000 µg/L to **ND**)
  Benzene (890 µg/L to **ND**)

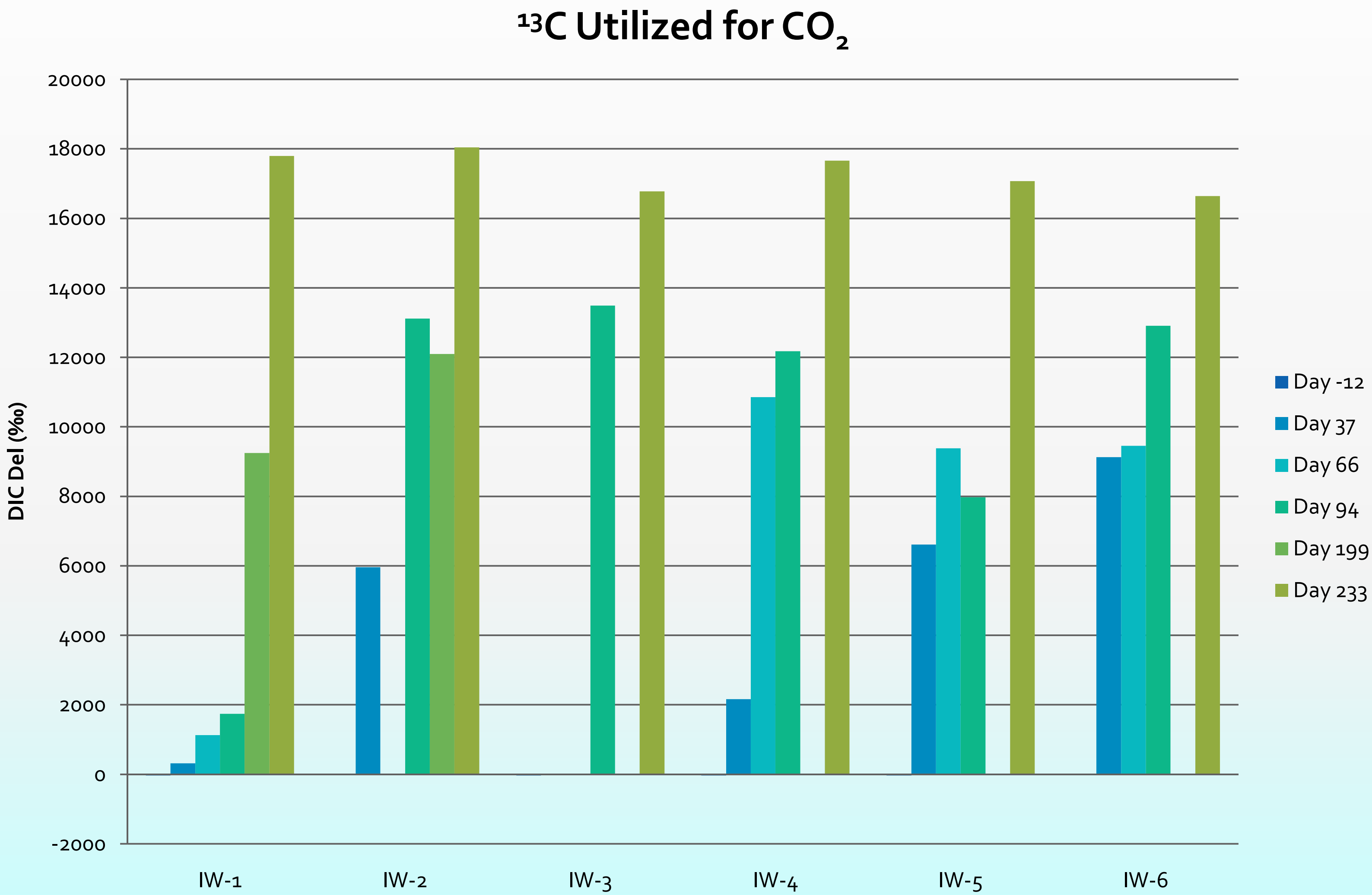
Pilot Study Results - ISBR Wells



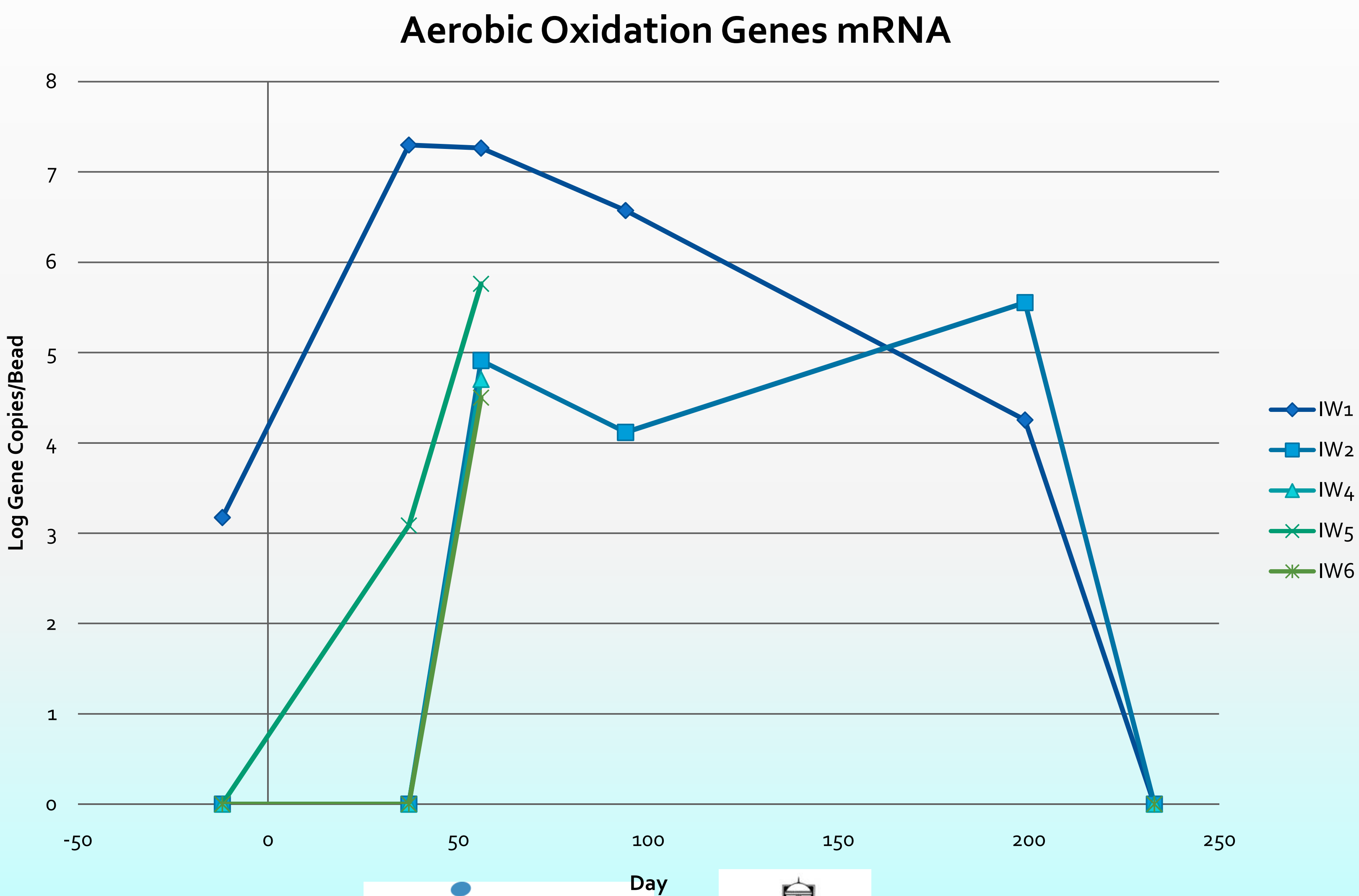
Pilot Study Results - ISBR Wells



Pilot Study Results - ISBR Wells



Pilot Study Results - ISBR Wells



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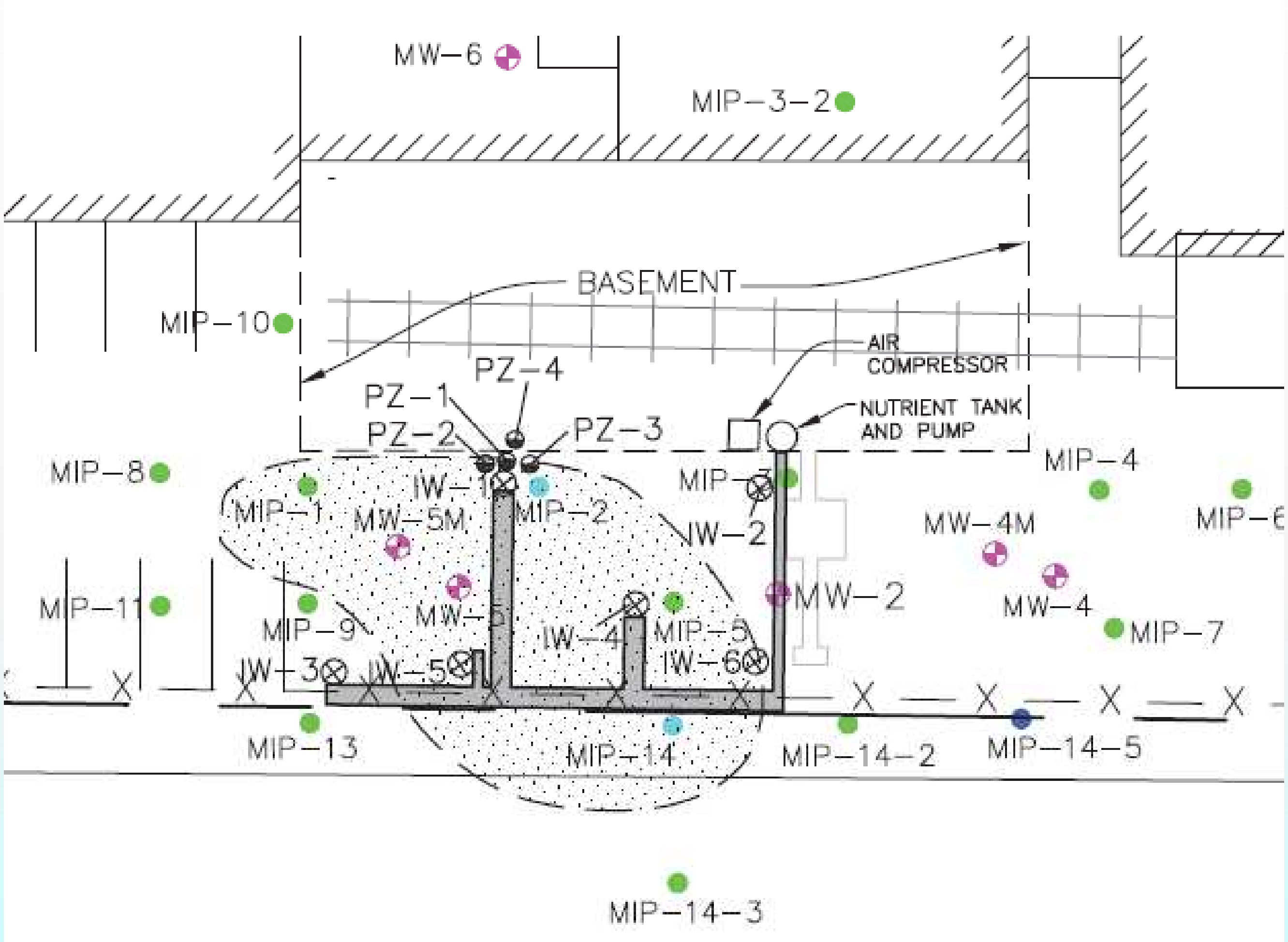
Aerobic Bioreactor Installation



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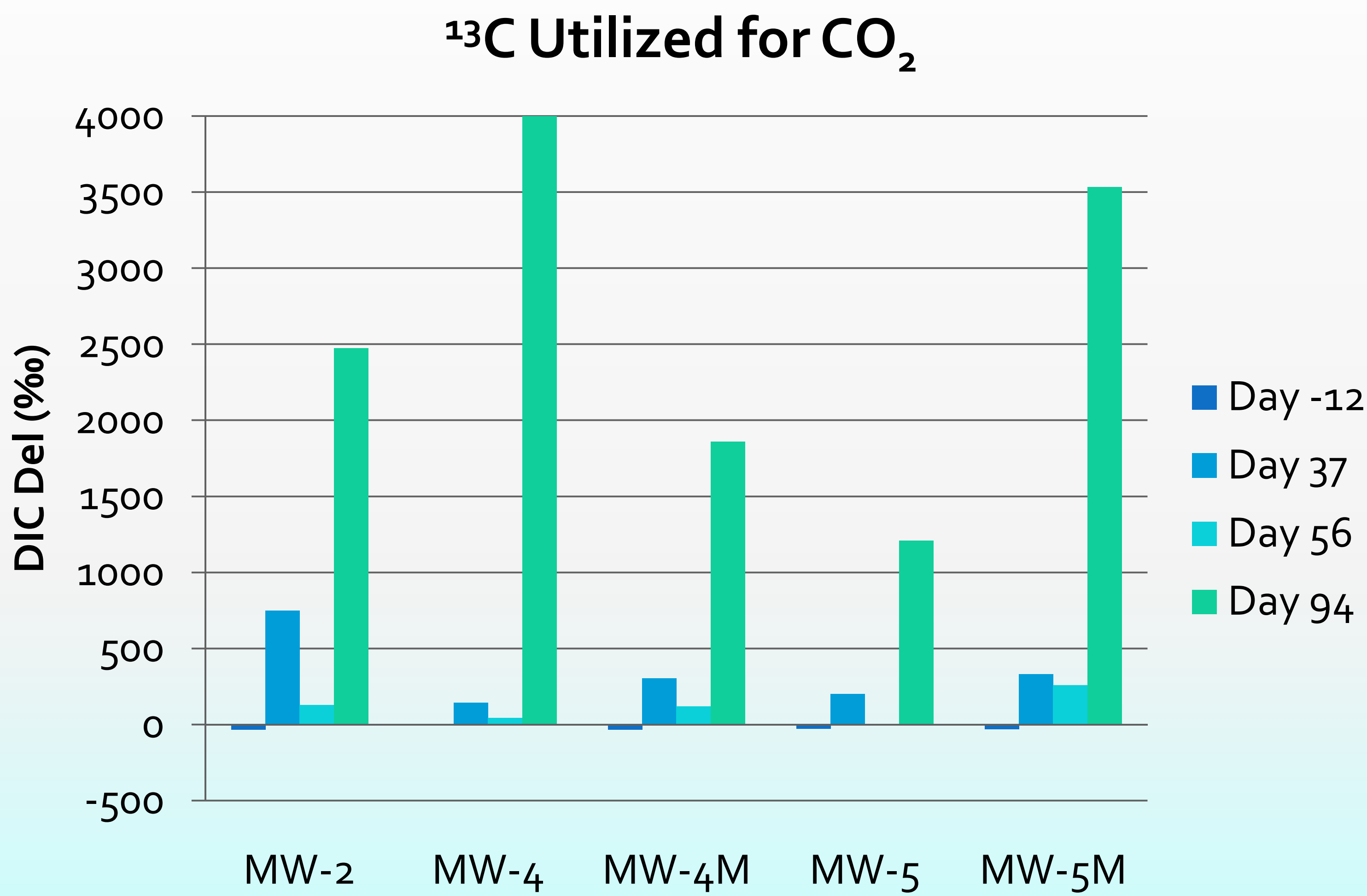
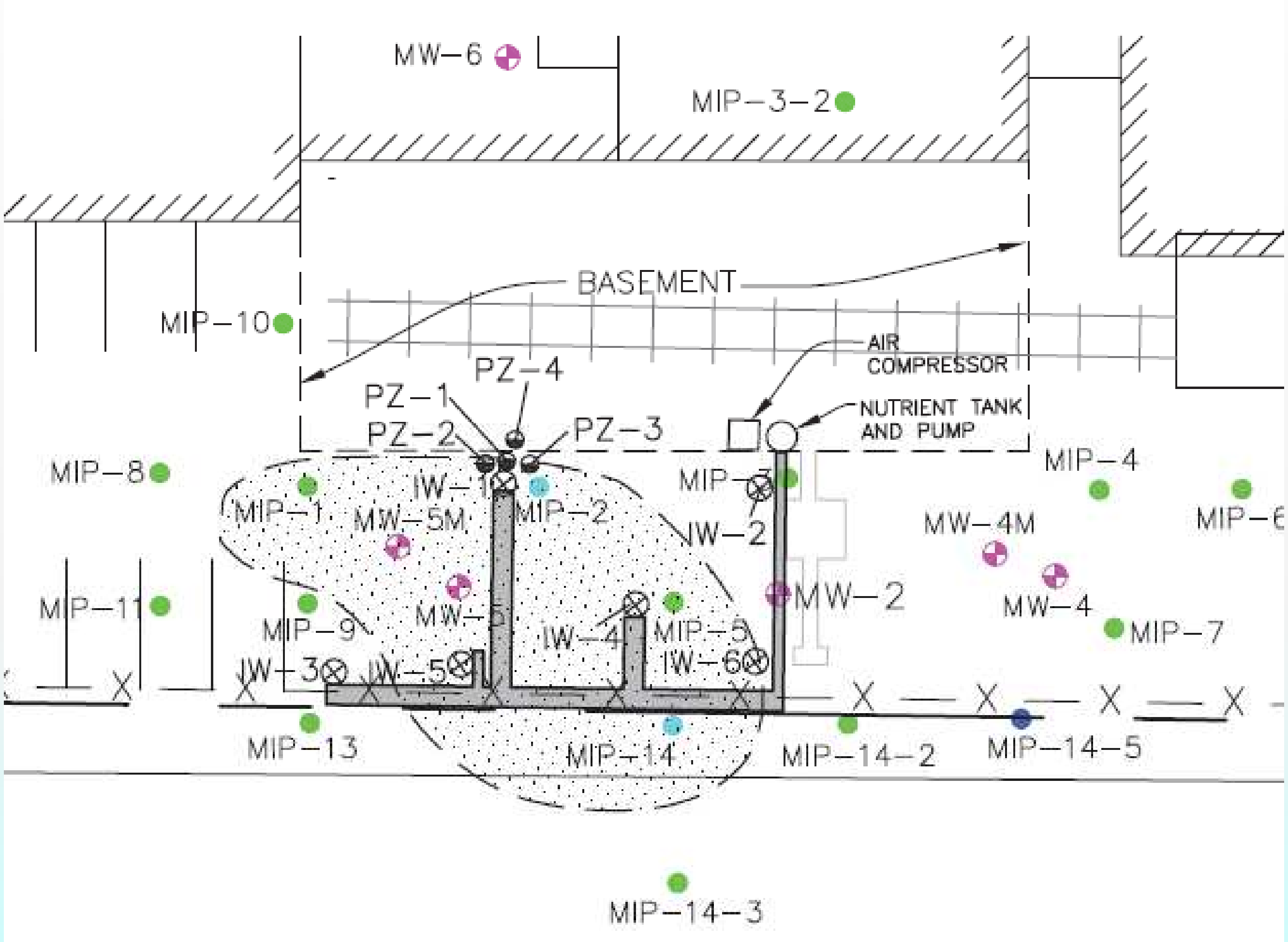
Pilot Study Results - Monitoring Well Results



Aerobic Bioreactor Installation



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Question No. 1

- *What was the purpose of incorporating ^{13}C-toluene labeled Bio-Sep® beads with regular Bio-Sep® beads in the Bio-Trap® for this study?*

Question & Answer No. 1

- *What was the purpose of incorporating ^{13}C-toluene labeled Bio-Sep® beads with regular Bio-Sep® beads in the Bio-Trap® for this study?*

- Answer: SIP data provided direct evidence of increased toluene mineralization in the bioreactor wells and adjacent monitoring wells

Pilot Study Conclusions

- Within 60 days toluene and benzene concentrations in all bioreactor wells, except IW-1, met NJDEP groundwater quality standards. IW-1 met standards by Day 199

- Toluene concentrations decreased between 31-47% in PZ-1, 3, & 4 and by 99% in PZ-2--there is possibly a preferential flow path between IW-1 and PZ-2

- SIP data provided direct evidence of increased toluene mineralization in the bioreactor wells and adjacent monitoring wells

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Anerobic bioreactor design

- Fits in standard 2" well
- Packed bed bioreactor containing Bio-Sep beads open for fluid flow at top and bottom
- Circulation Element created by compressed gas N_2 sparging unit in bottom of packed bed.
- N_2 sparging and electron donor lines connected to surface equipment

Bio Enhance

Current and Future Operations

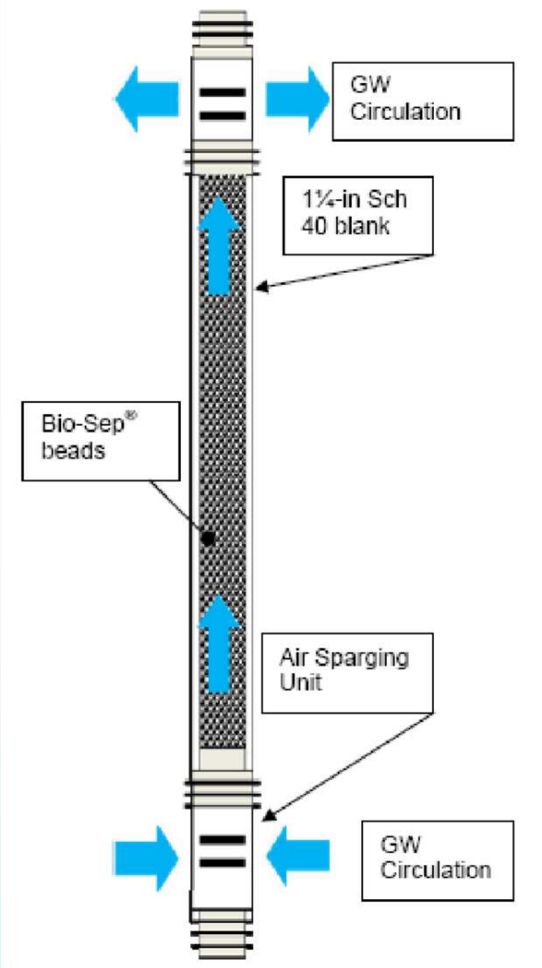
•Conduct Bi-weekly system checks

•Circulate water between wells during bi-weekly system checks

•May move bioreactors into different wells temporarily

•Sample wells every 3-6 months.

•Current cost to client to run system is less than $500* per month



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Bio Enhance

Permit Requirements & Costs

- Requirements Vary State by State
- Typically require DGW - Permit By Rule
- Costs:
  - Capital Cost: $4,000 per control box, up to 4 ISBR units can be run off a single box.
  - Monthly Rental: $500 per month per ISBR, $100 per month after 12 months. Discounted rates for multiple ISBR rentals and new constituents of concern.
  - Installation trenching, sampling and reporting are site specific

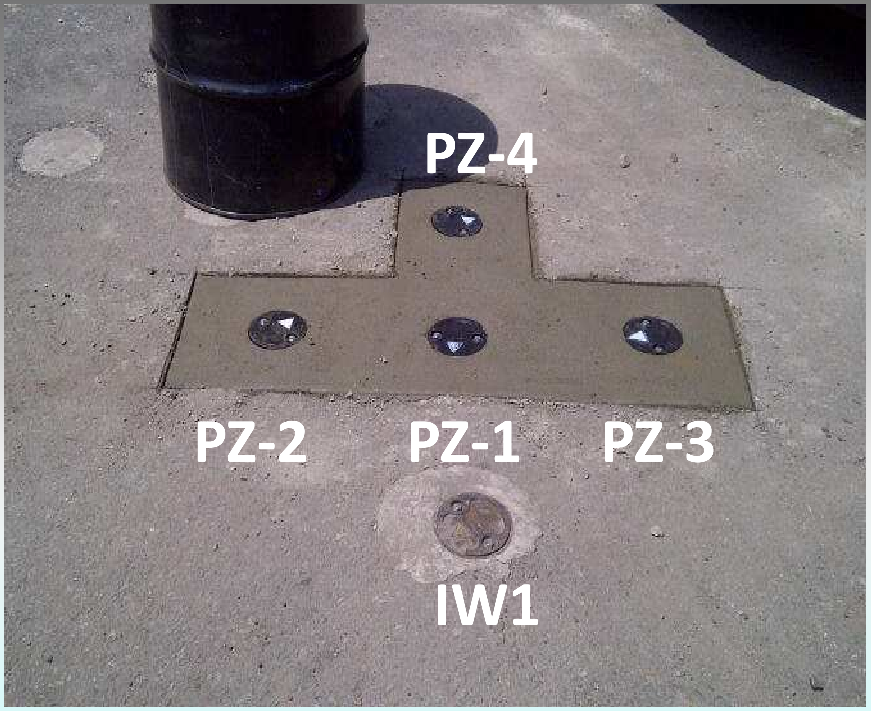
Applications

- Ideal Compliment to Chemical Oxidation
- Persistent, Low Levels of Residual Compounds, Such as MTBE, BTEX and Chlorinated Solvents
- Bio-Stimulation Injections, Electron Donor or Acceptors

Limitations of ISBR

- Aerobic operation limited to aquifers with low concentrations of reduced iron
  - Anaerobic operation accomplished by using N_2 to induce circulation and mixing and supplementing nutrient feed with electron acceptor
- Decreasing hydraulic conductivity of aquifer decreases radius of influence

Questions?



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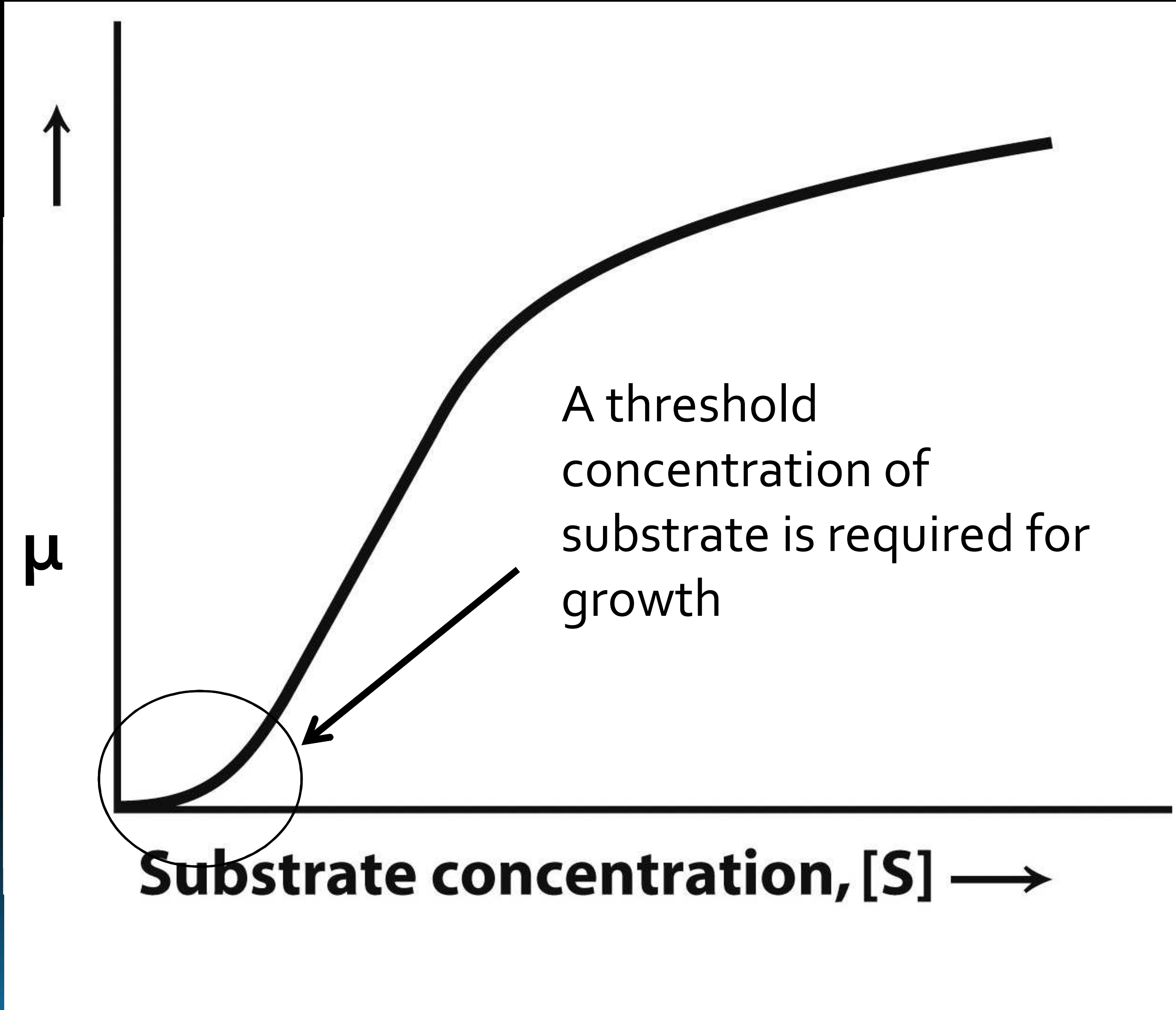
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A threshold concentration of substrate is required for growth
Substrate concentration, [S]