Monitoring Gene Expression to Evaluate the Effectiveness of Oxygen Infusion

microbialinsights

Operating Retail Gas Station

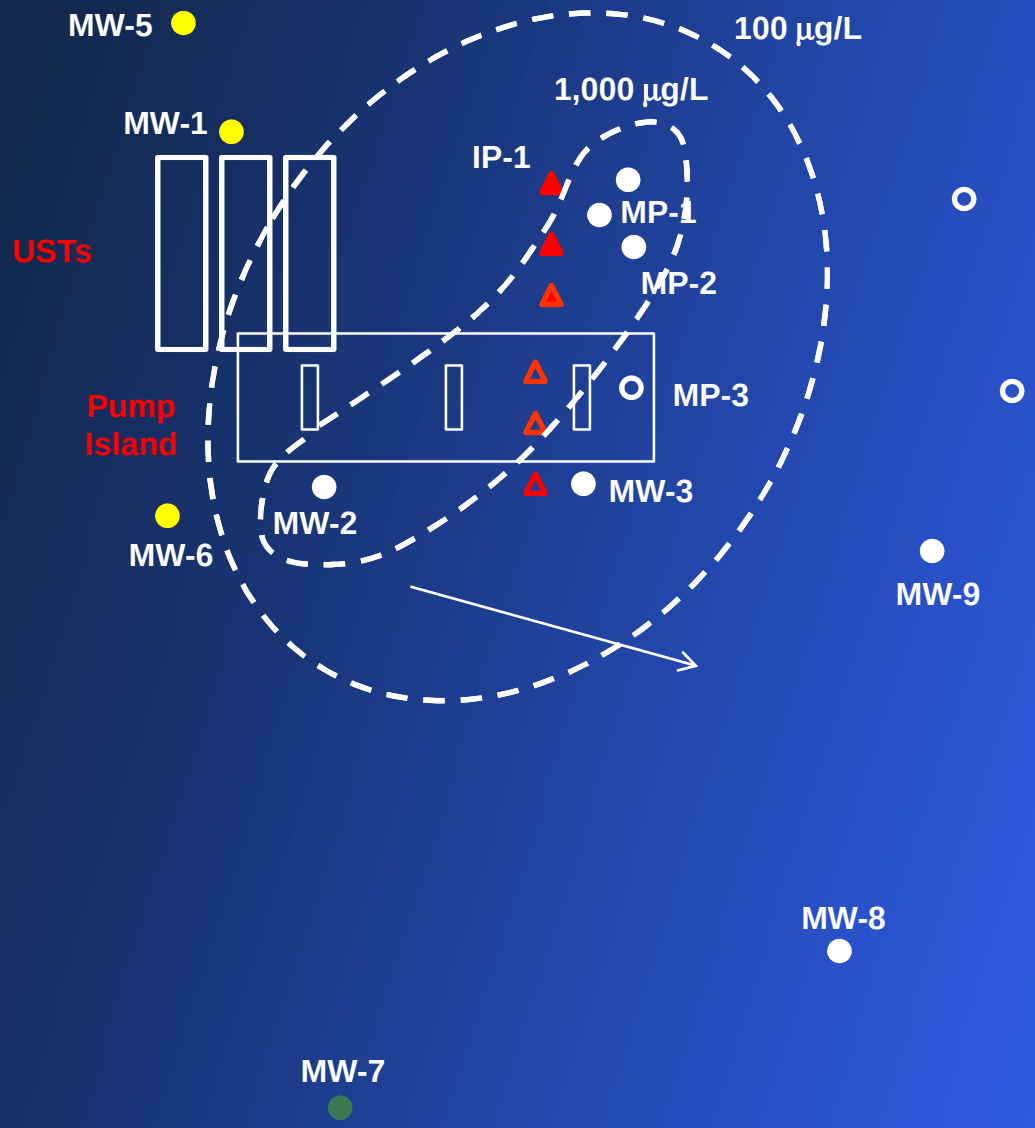
- **Shallow aquifer impacted by gasoline.**

- **Primary Contaminants**
  - Benzene, toluene, ethylbenzene, xylenes (BTEX)
  - Methyl *tert*-butyl ether (MTBE)

Considering oxygen injection in the source zone to stimulate biodegradation.

microbialinsights

Benzene Concentrations

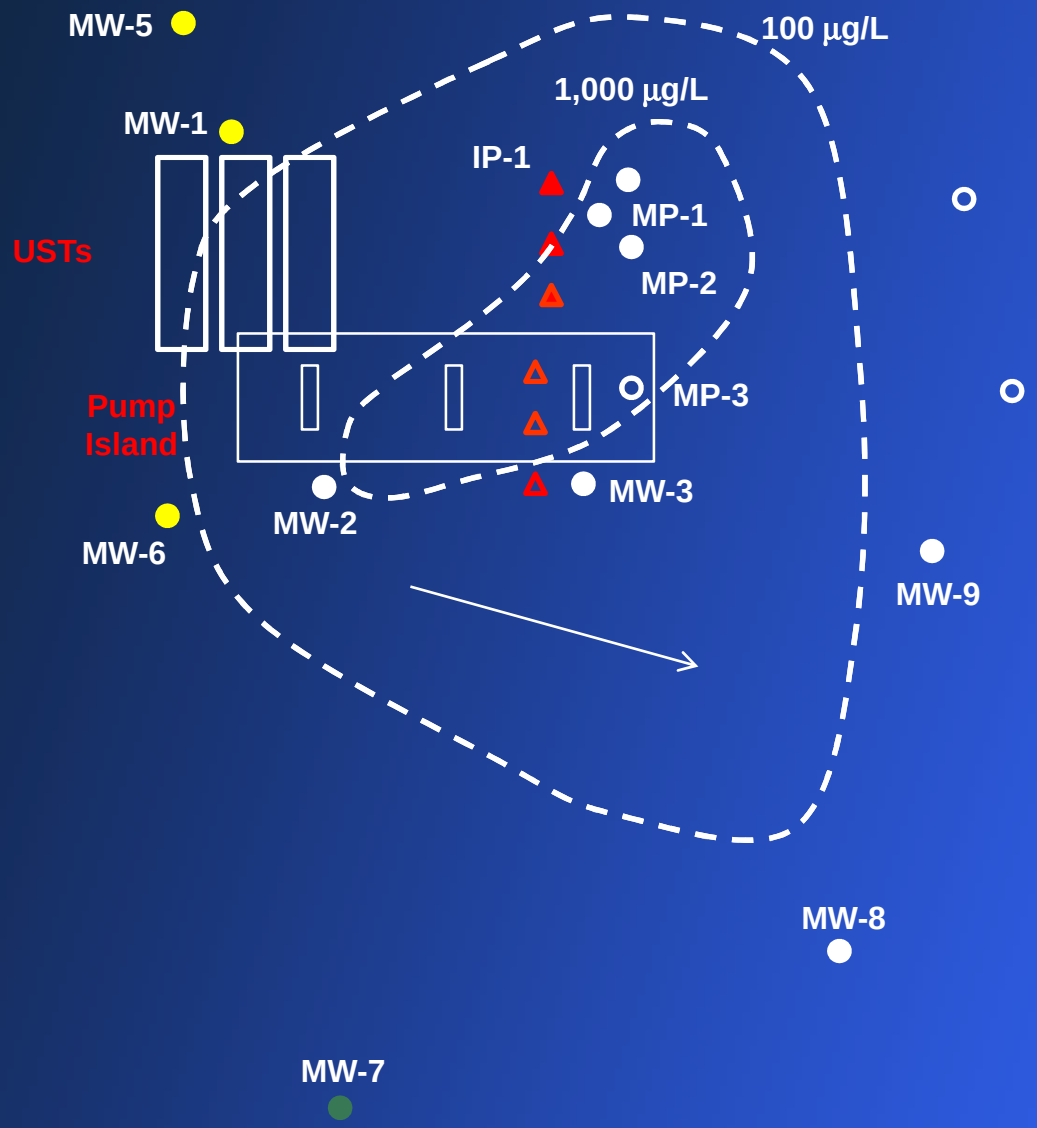


Prior to System Activation

- ✓ Open markers – wells had not been installed.
- ✓ Low BTEX concentrations at MW-1, MW-5 and MW-6.
- ✓ Greatest benzene concentrations associated with the pump island.
- ✓ Benzene concentrations ranged from 600 µg/L (IP-1) to 10,000 µg/L (IP-3) at the injection points.

microbialinsights

MTBE Concentrations

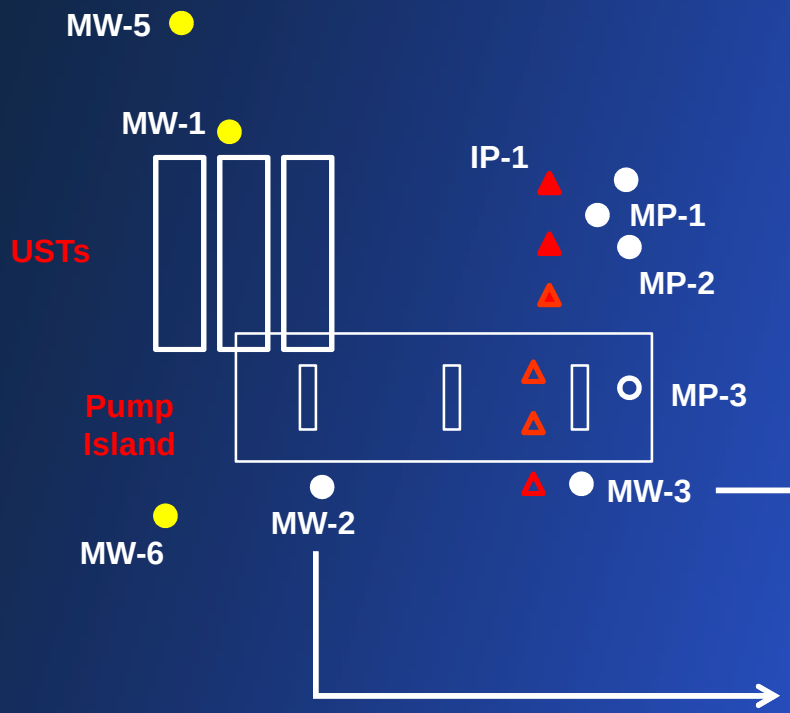


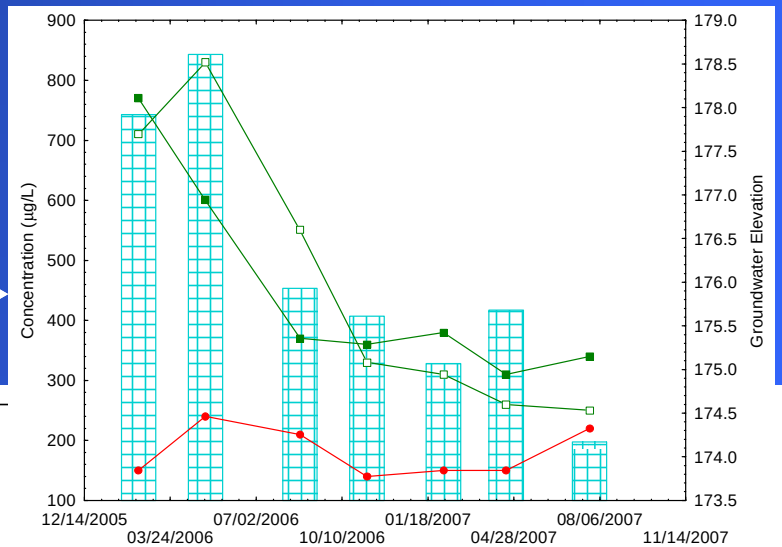
Prior to System Activation

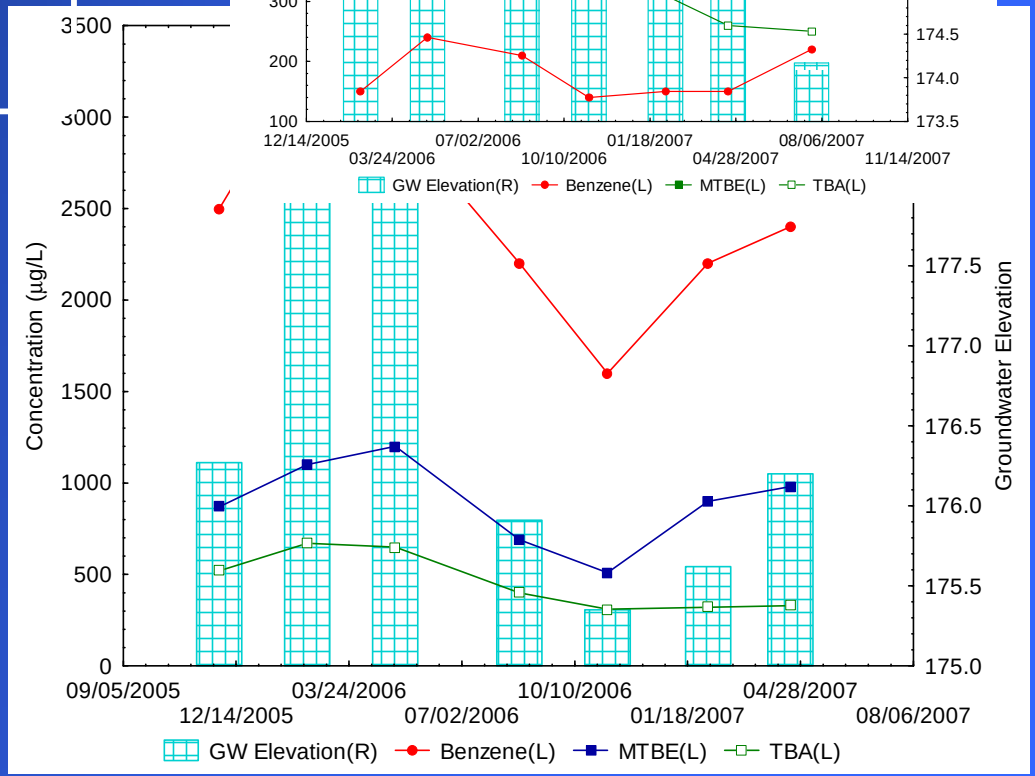
- ✓ MTBE concentrations ranged from 280 µg/L (IP-1) to 5,500 µg/L (IP-3) at the injection points.

microbialinsights

Contaminant Concentrations





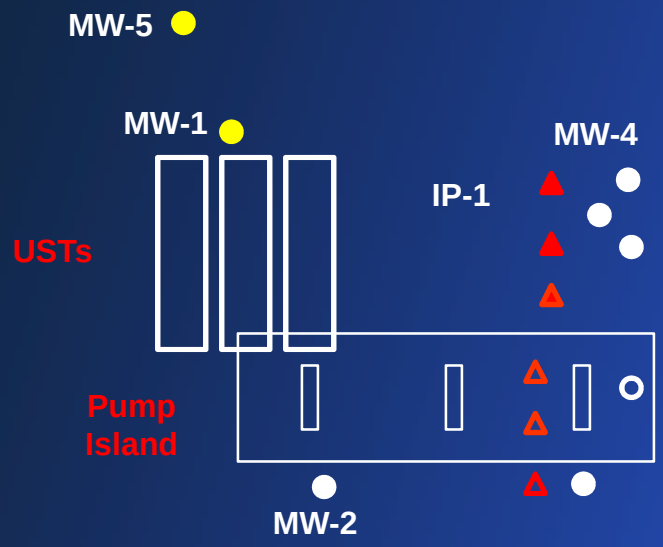


Prior to System Activation

✓ Dissolved benzene, MTBE, and TBA concentrations fluctuated with groundwater elevation.

MW-7

Geochemical Conditions



Impacted

Well	DO	NO_3^-	Fe^{2+}	SO_4^{2-}	CH_4
MW-4	0.72	ND	2.00	ND	1.79

Impacted

Well	DO	NO_3^-	Fe^{2+}	SO_4^{2-}	CH_4
MW-2	0.85	ND	1.02	2.53	1.55

Background

Well	DO	NO_3^-	Fe^{2+}	SO_4^{2-}	CH_4
MW-7	1.93	3.31	ND	306	0.244

MW-7

Prior to System Activation

- ✓ Background – mildly aerobic.
- ✓ Impacted Wells
  - ✓ Reducing Conditions
  - ✓ Available DO, nitrate, ferric iron, and to some extent sulfate consumed.
  - ✓ Addition of an electron acceptor (e.g. oxygen) may promote biodegradation.

microbialinsights

Study Design

- Oxygen injection at three wells (IP-1 through IP-3)

- Two wells located downgradient are used for monitoring (MP-1 and MP-2)

- Traditional monitoring parameters
  - Dissolved oxygen (DO) concentrations
  - Contaminant concentrations

microbialinsights

Site Specific Question

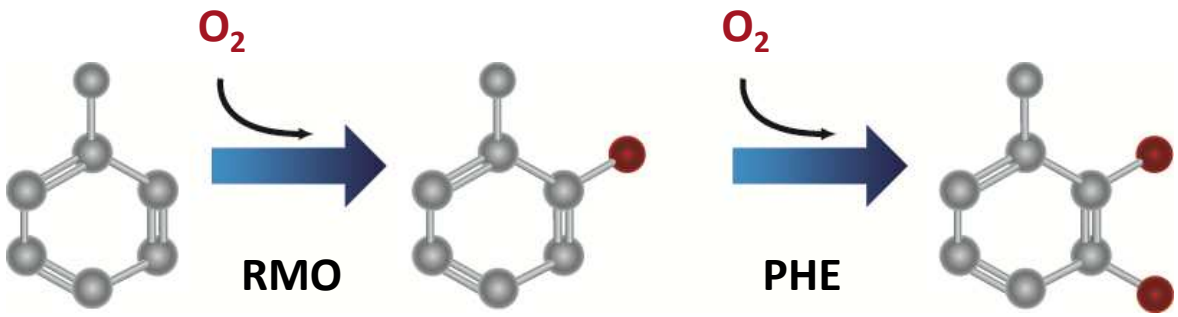
Will oxygen addition promote the activity of bacteria capable of aerobic biodegradation of BTEX and MTBE?

Radius of influence?

microbialinsights

CENSUS-Expression - Aerobic BTEX

Target	Code	Contaminant	Environmental Relevance
Phenol hydroxylase	qPHE	BTEX	• Catalyzes continued (and sometimes initial) hydroxylation of BTEX compounds
Toluene dioxygenase	qTOD	BTEX	• Catalyzes initial oxidation of benzene and toluene
MTBE utilizing PM1	qPM1	MTBE	• Targets *Methylibium petroleiphilum* PM1. one of the few bacteria isolated that is capable of growth on MTBE



microbialinsights

Bio-Trap® Samplers

- Passive sampling tool that collects microbes over time
- Unique sampling matrix, bio-sep beads, which mimics environmental conditions
- Can be analyzed using a variety of molecular based approached (DNA, RNA and PLFA)

microbialinsights

What is the difference between DNA and RNA?

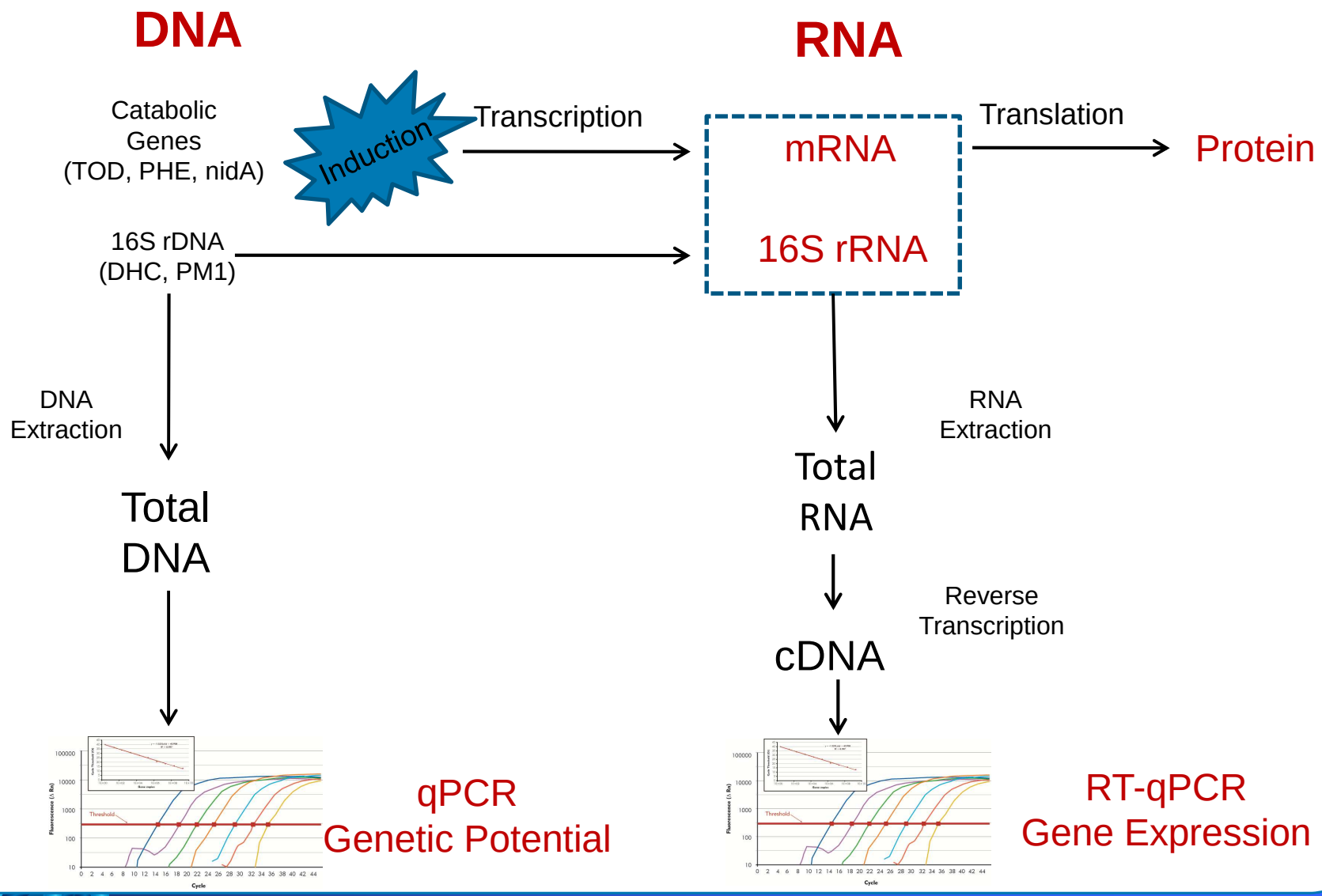
DNA

- Genetic Potential
- Permanent Master Copy
- Like a comprehensive reference book

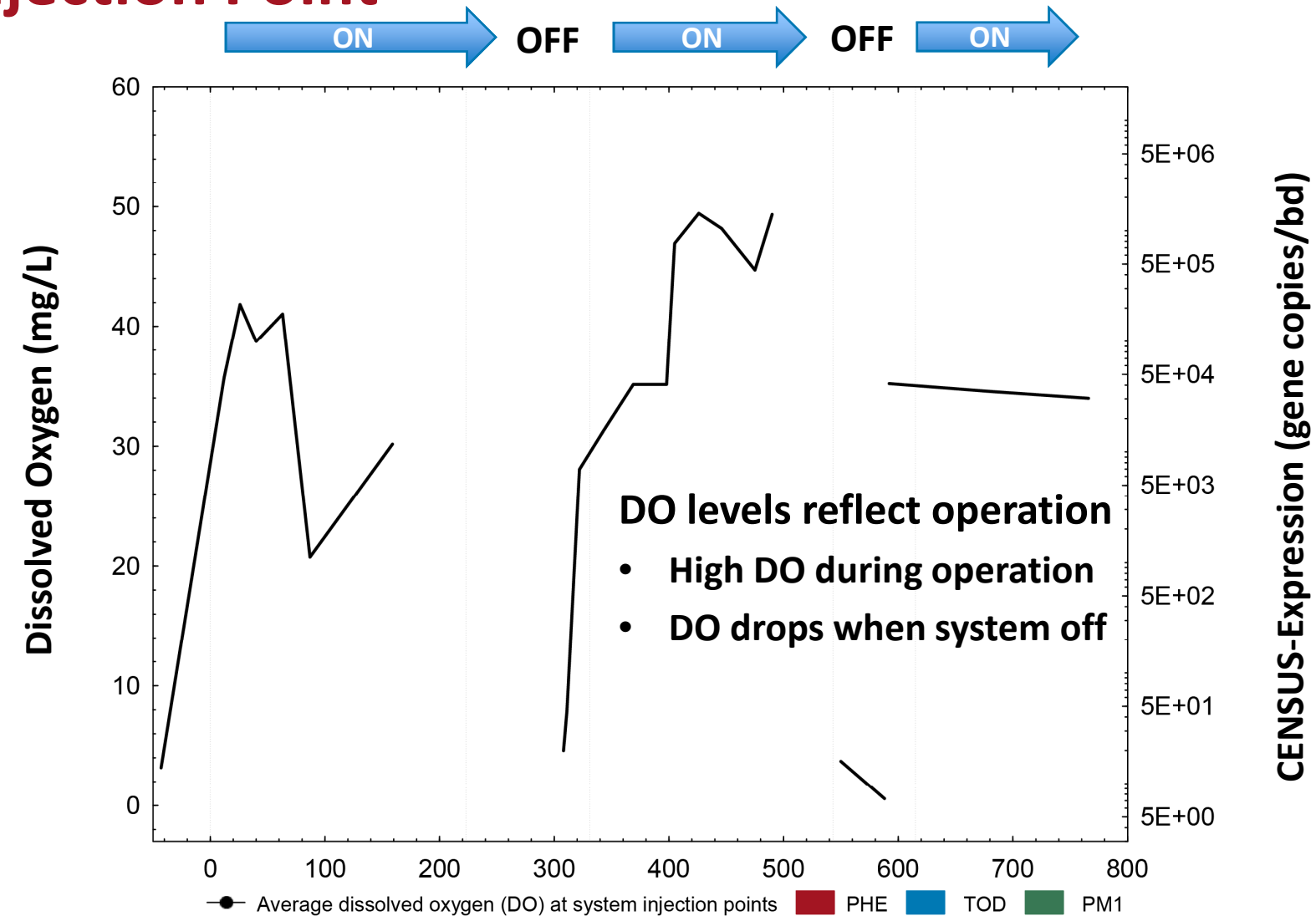
RNA

- Gene Expression
- Disposable, working copy
- Like the Xerox of a page needed to complete an assignment

microbialinsights

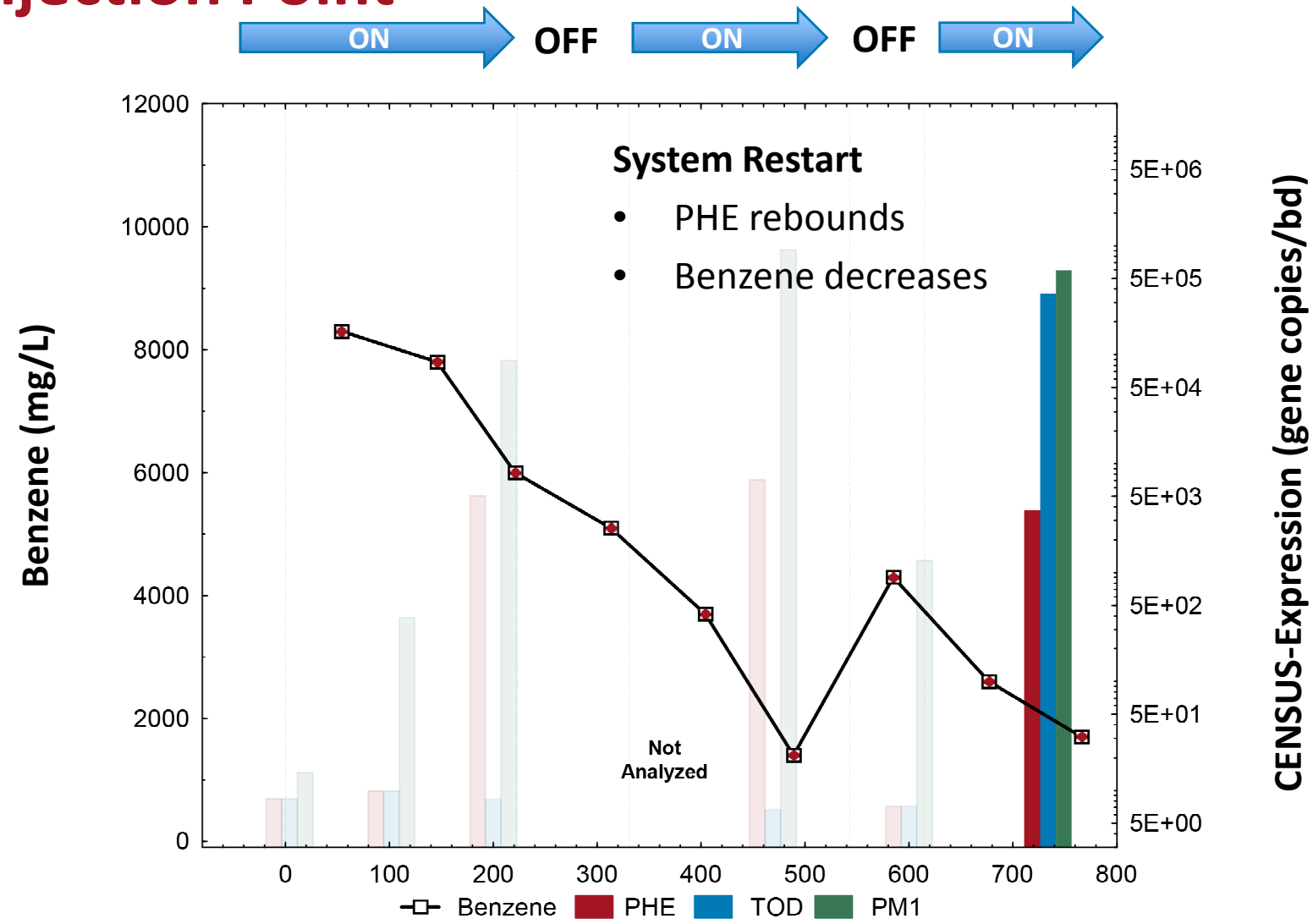

DNA
RNA
Catabolic Genes (TOD, PHE, nidA)
Induction
Transcription
mRNA
Translation
Protein
16S rDNA (DHC, PM1)
16S rRNA
DNA Extraction
RNA Extraction
Total DNA
Total RNA
Reverse Transcription
cDNA
qPCR Genetic Potential
RT-qPCR Gene Expression

Injection Point



microbialinsights

Injection Point



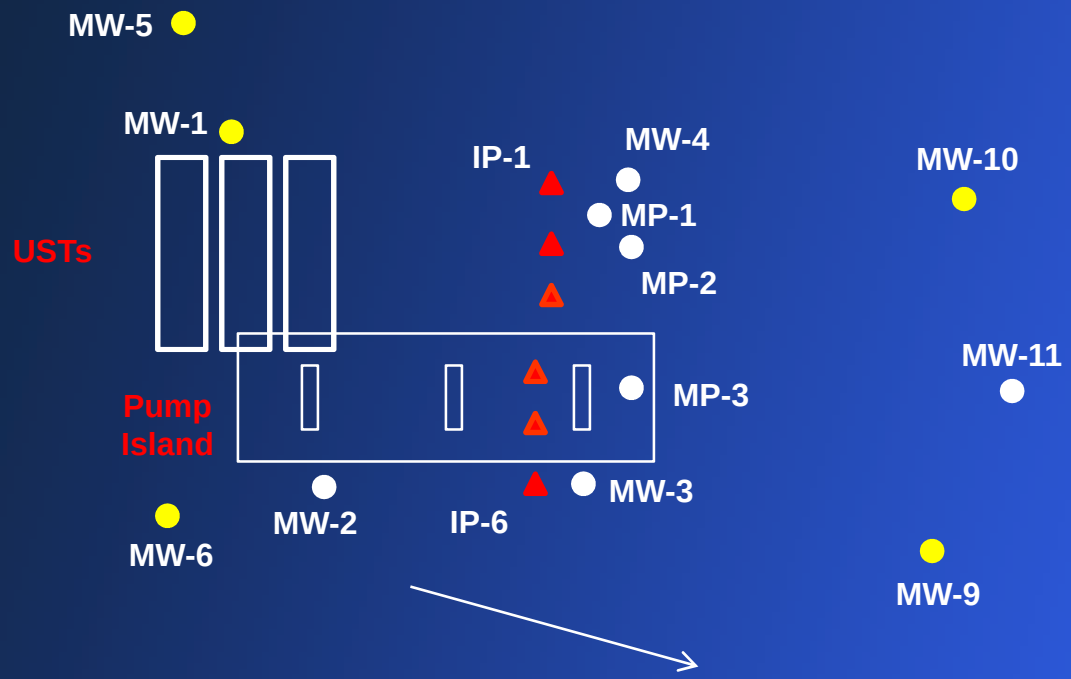
microbialinsights

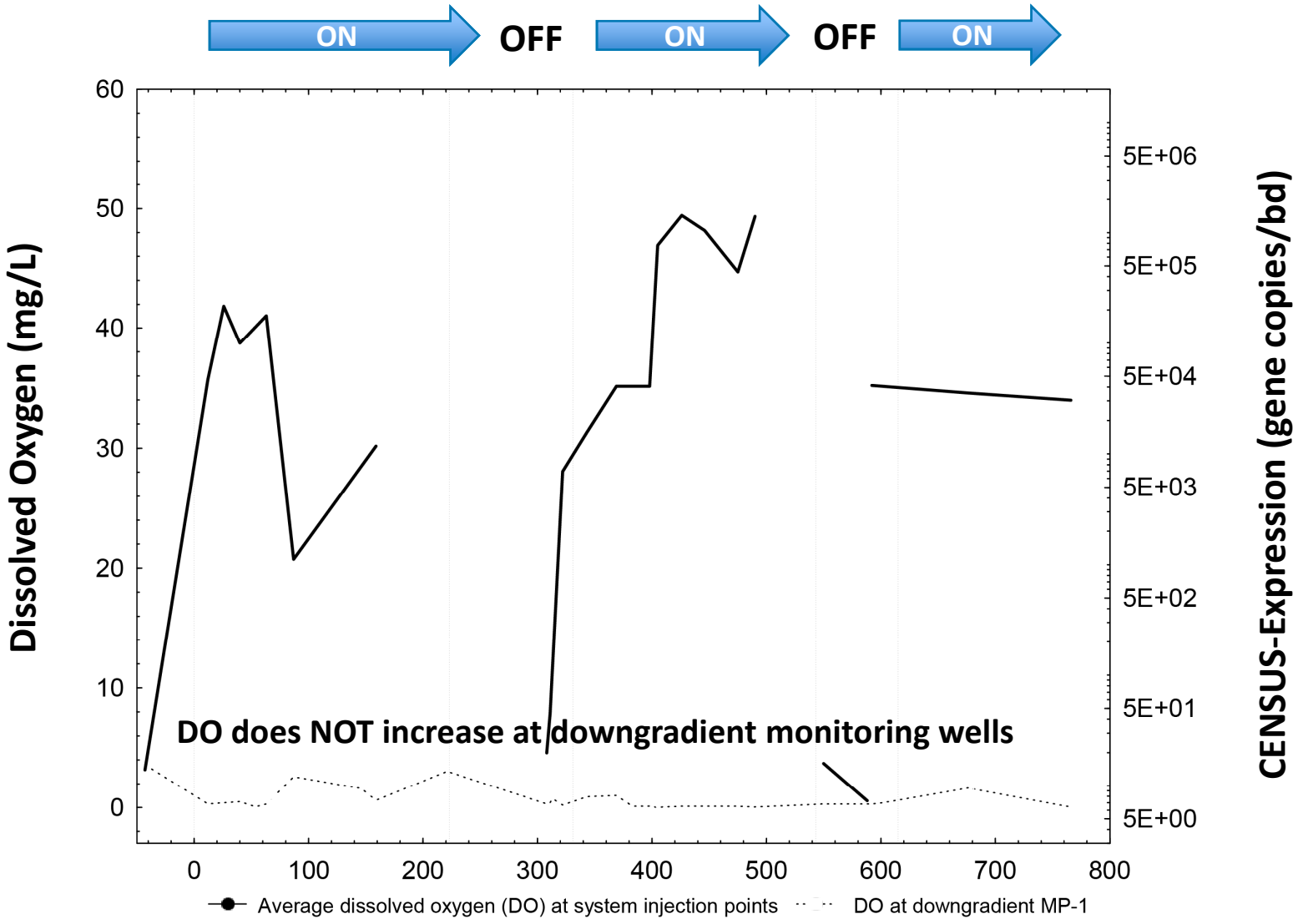
Summary of Injection Points

- Chemical Lines of Evidence
  - BTEX concentrations decreased
  - MTBE concentrations decreased without accumulation of TBA
- Geochemical Lines of Evidence
  - DO levels increased substantially at injection point
  - Subsurface conditions conducive to aerobic BTEX and MTBE biodegradation
- Biological Lines of Evidence
  - Substantially increased expression of phenol hydroxylase (PHE) and toluene dioxygenase (TOD) genes during system operation (and decreases during downtime).
    - Active expression of genes encoding enzymes responsible for BTEX biodegradation
  - Increased PM1 16S rRNA
    - Activity of known MTBE/TBA utilizing organism
- Conclusions
  - Oxygen addition promoted aerobic bioremediation

microbialinsights

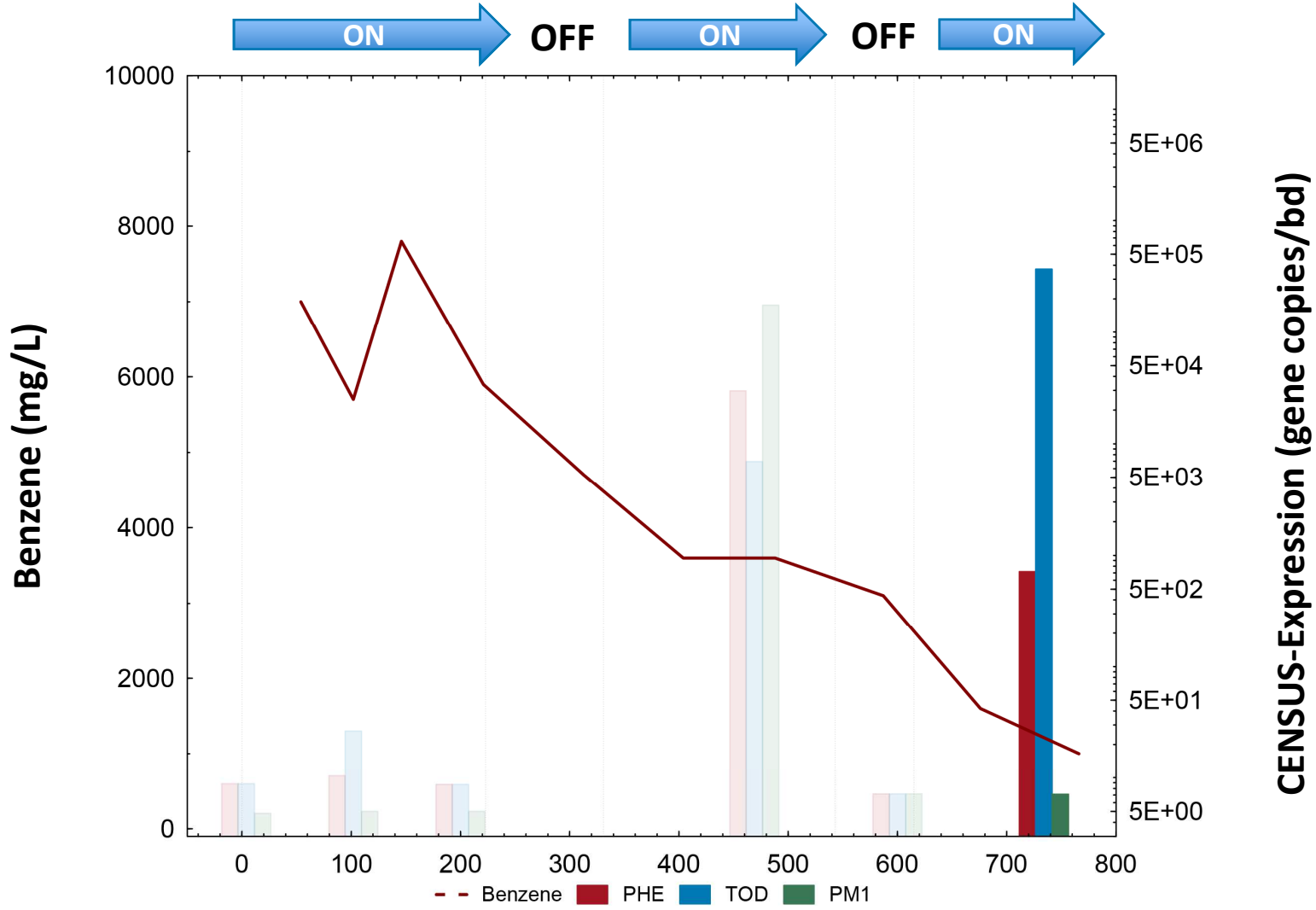
Site History





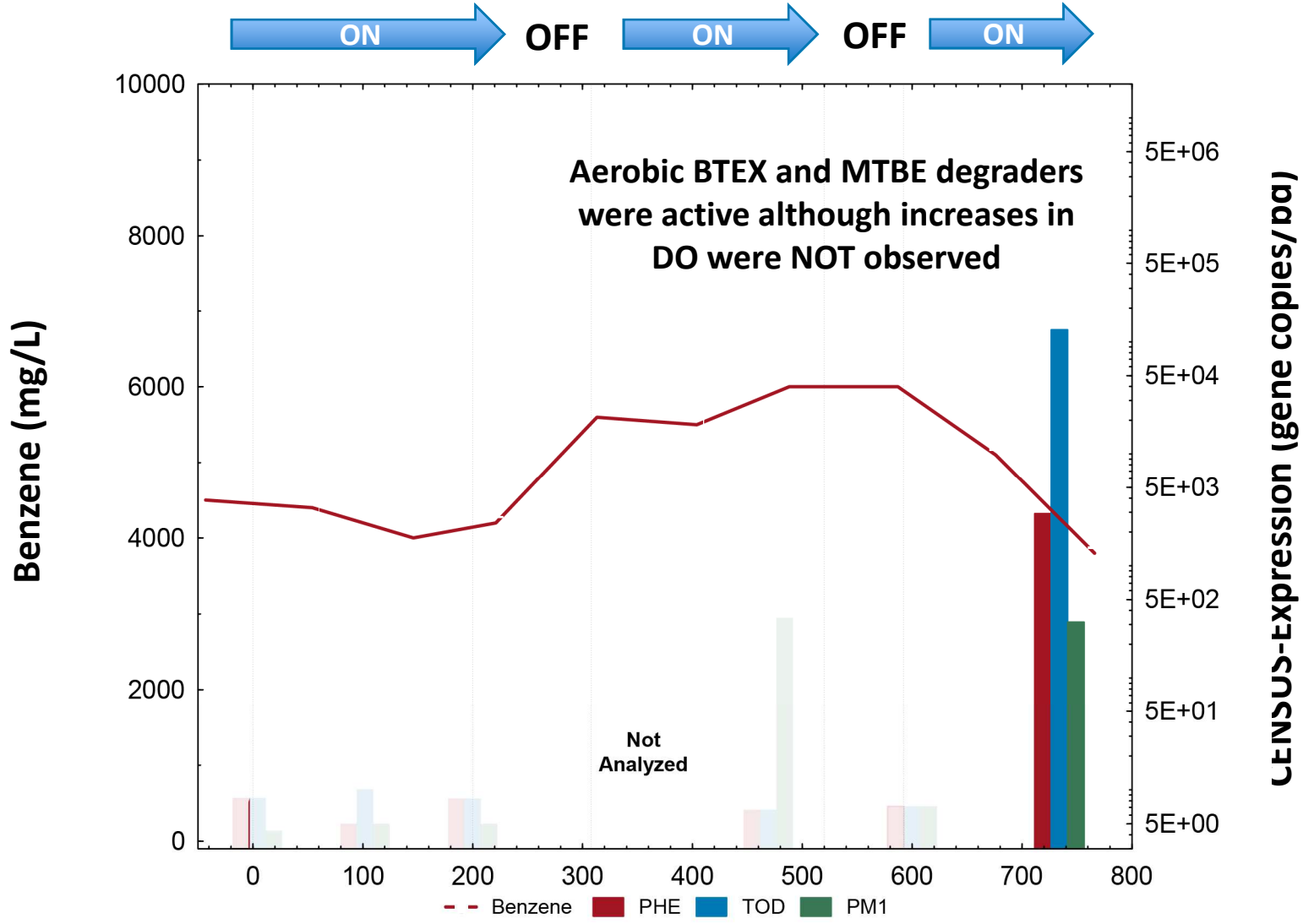
microbialinsights

Downgradient



microbialinsights

Further Downgradient



microbialinsights

Summary of Monitoring Points

- Chemical Lines of Evidence
  - MP-1: Benzene and MTBE concentrations are decreasing
  - MP-2: Benzene concentration initially increase but then begin to decrease
- Geochemical Lines of Evidence
  - DO levels did NOT increase appreciably during system operation.
- Biological Lines of Evidence
  - Definite lag before increased transcription of PHE and PM1 in downgradient monitoring points.
  - However, transcription of PHE and TOD increased by 2 – 4 orders of magnitude during the last sampling event.
- Conclusions
  - Oxygen addition will promote aerobic bioremediation at downgradient locations despite lack of an appreciable increase in DO.
  - Using these tools helped the regulators to see that system was having the desired impact even though COC levels did not initially decrease and DO levels were not high.

microbialinsights

Final Conclusions

- **No EMDs**
  - DO Levels did NOT increase downgradient during system operation.
  - Likely conclude system is not effective downgradient.
  - Increased cost for additional injection points or alternate treatment.
- **EMDs – CENSUS Expression TOD, PHE, and PM1**
  - BTEX and MTBE degrading bacteria activity linked to system operation and decreases in contaminant concentrations.
  - After an initial lag period BTEX and MTBE/TBA degrading bacteria became more active downgradient.
  - Oxygen addition will promote aerobic bioremediation at downgradient locations despite lack of an appreciable increase in DO.

Closer Look

"Monitoring Gene Expression to Evaluate Oxygen Infusion at a Gasoline-Contaminated Site"

September 2010 issue of *Environmental Science & Technology* (v. 44 no. 17: 6829-6834)

microbialinsights