Assessment of Ability of Trap and Treat® Carbon-Based Injectants to Actively Biodegrade BTEX In-Situ

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Introduction

- In Kentucky, Carbon based Trap and Treat® products have been used successfully to reduce BTEX concentrations in groundwater at LUST sites in cases where using more conventional methods alone (e.g., excavation, MDPE) was not feasible or proved insufficient to meet goals.

- Existing evidence in the form of decreasing concentrations of benzene and Terminal Electron Acceptors (TEAs) in groundwater suggests that these products may act to support anaerobic biodegradation of BTEX compounds; however, uncertainty remains as to whether these products merely sequester BTEX within the carbon matrix or actually support biodegradation within the matrix thereby regenerating the carbon in-situ.

- Given the expanding use of these products, additional evidence is needed to better characterize their potential to biodegrade BTEX compounds in-situ.

- Kentucky USTB worked with collaboratively with HMB, AST and RPI to design and execute this study.

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Study Objectives

- Characterize a typical Kentucky LUST site pre and post-treatment using soil microbial analysis techniques to assess the potential of a carbon-based BOS 200 Trap and Treat® product to biodegrade BTEX in-situ.

- Assess microbial diversity pre-injection with emphasis given to identification of microbes capable of biodegrading BTEX compounds

- Identify the presence or absence of microbes that carry genes which code for enzymes capable of degrading BTEX compounds

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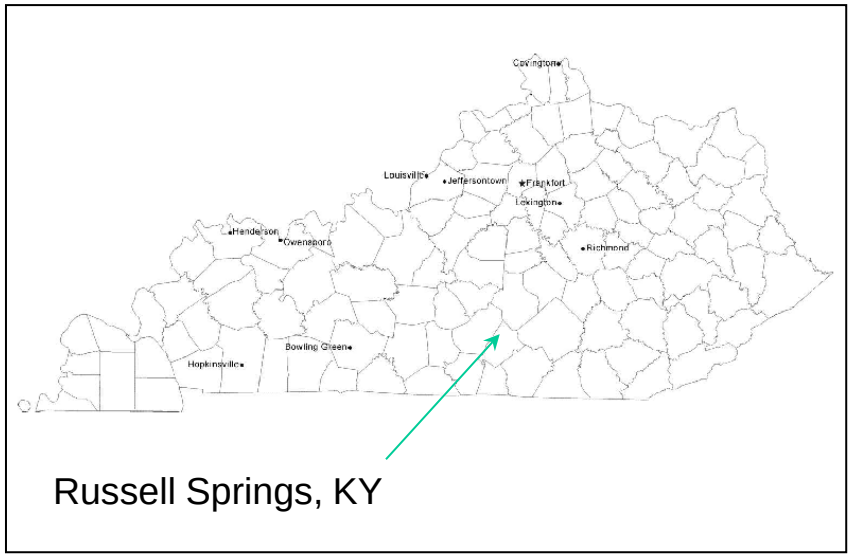
Setting and Site History

- Site Location: Russell Springs, KY
- Land use: rural/ residential
- Overburden: sandy/silty clay with some gravel
- Bedrock: Limestone (7 – 18 feet bgs)
- Depth to groundwater: 6-10 feet bgs

- Four (4) small gasoline USTs closed in 2000 – 2001
- Release was detected near dispensers in 2001 (soil benzene > 9 ppm)
- Subsequent site investigation identified extensive soil and groundwater contamination (no LNAPL evident)
- No other corrective action activities performed prior to carbon injections
- Pre-treatment soil benzene levels ranged from 0.1 – 15.8 ppm
- Pre-treatment groundwater benzene levels ranged from ND to > 8 ppm



https://www.waterproofpaper.com/printable-maps/kentucky.shtml

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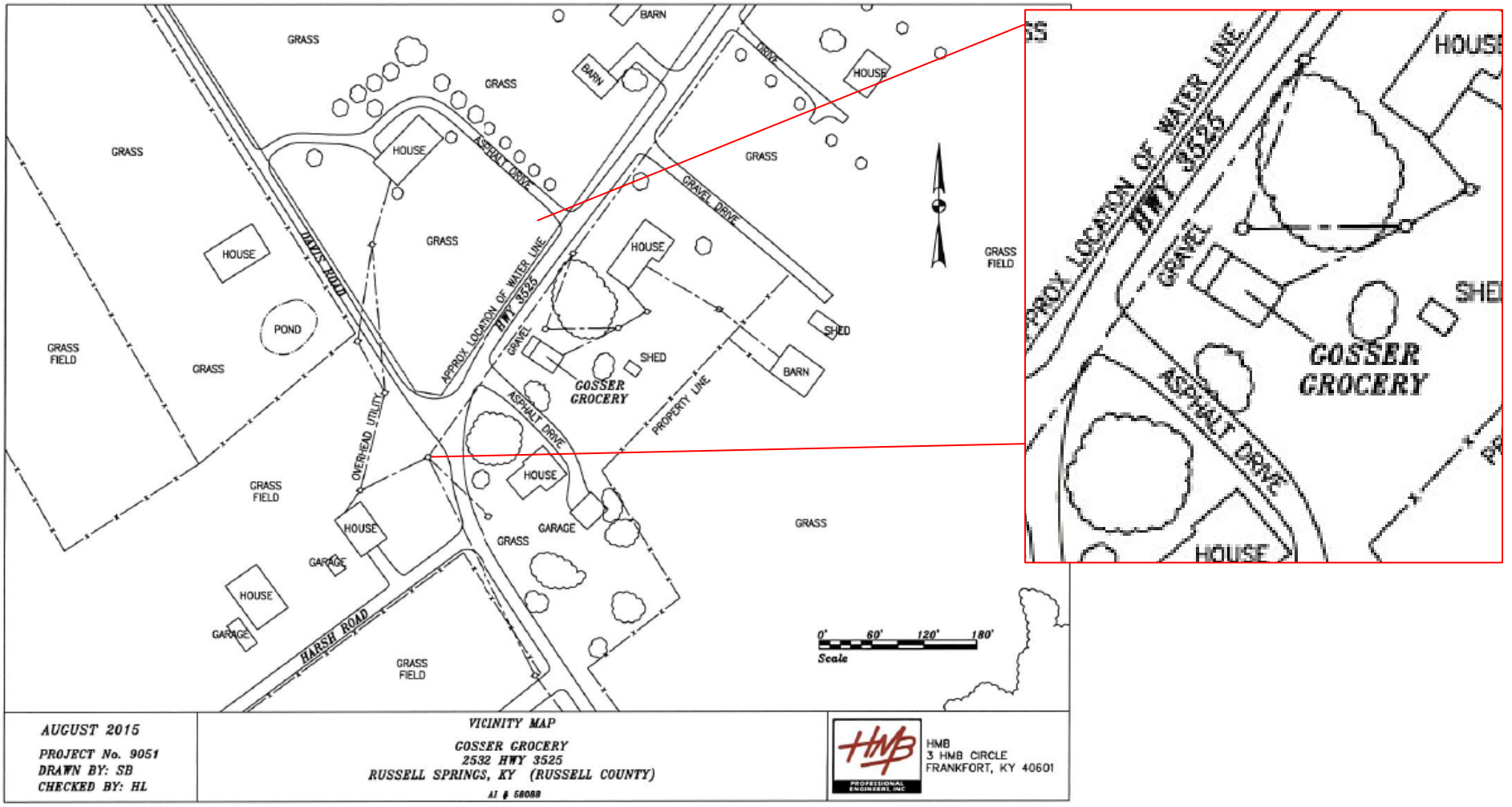
Site Map



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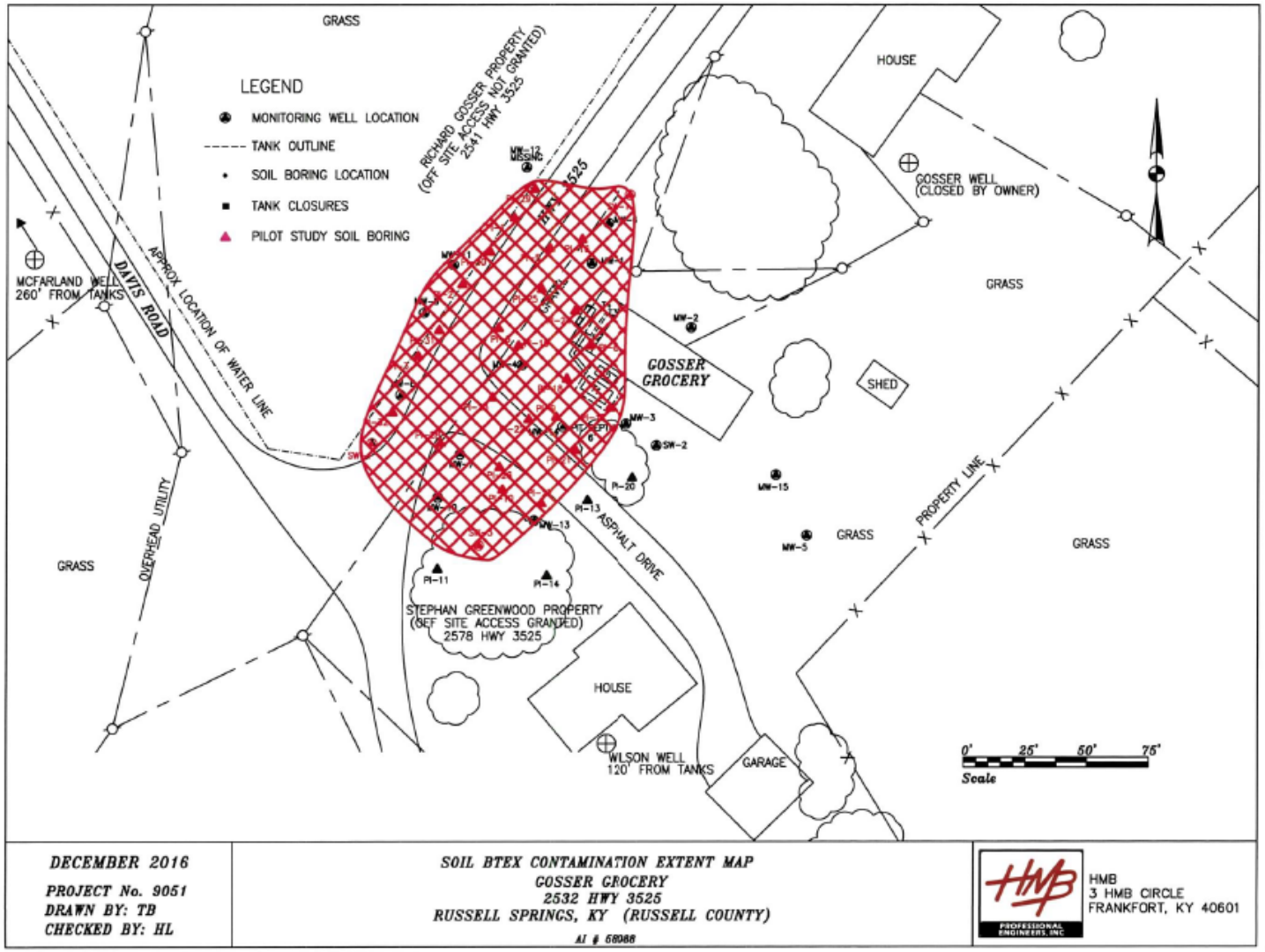
Benzene in Soil



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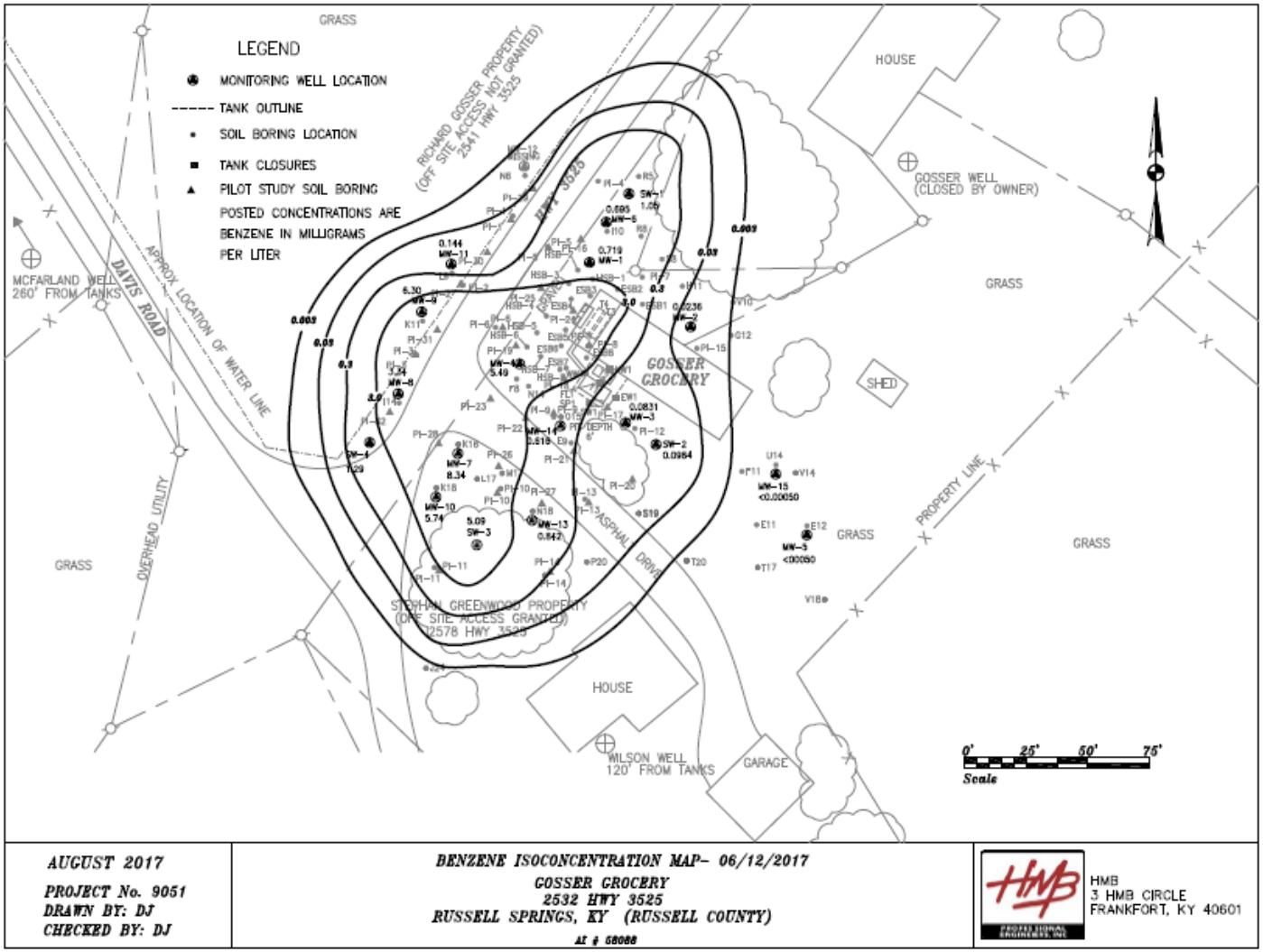
Benzene in Groundwater (mg/L)



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Study Design

- Using dual tube Geoprobe® sampling, obtain pre and post-injection soil samples for microbial and BTEX analysis
- Obtain four (4) soil microbial samples pre-injection and post-injection (3 from contaminated area and 1 control)
- Obtain one sample of microbial mix to be co-injected with carbon
- Analyze pre-injection soil samples using NextGen Sequencing and qPCR techniques for population diversity and functional gene identification, respectively
- Analyze post-injection soil samples using qPCR

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Microbial Testing Methods

NextGen Sequencing:

- Used to simultaneously sequence sample DNA from multiple microbes
- DNA sample is broken into pieces containing different numbers of base pairs
- These fragments are exponentially amplified using PCR (Polymerase Chain Reaction) to form DNA Library
- DNA fragments are multiplied again on a substrate forming clusters, each containing the same size fragments
- Fluorescently tagged nucleotides are read by the sequencer as they are added to the various cluster fragments
- The fragments are analyzed to determine the microbes with which they are associated

qPCR:

- Uses gene specific primers to target genes of interest
- Target gene sequences are exponentially amplified using PCR
- Fluorescent probes added to the PCR mixture bind to the target DNA strands
- The level of fluorescence increases above a background level as more cycles of DNA replication occur and more copies are made of the target gene
- The more copies present in the original sample, the fewer cycles are required to achieve fluorescence above background
- A standard curve along with the number of cycles required to reach threshold fluorescence determine the number of bacterial cells present in the original sample

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Methods (Pre-Injection)

Fall 2016:

- Installed 32 soil borings and 4 sentry wells throughout known area of contamination
- Three (3) soil samples collected from each soil boring for BTEX analysis
  - Two (2) highest PID readings per boring plus one at bedrock interface
- Groundwater samples from fourteen (14) existing and four (4) new monitoring wells analyzed for BTEX
- Obtained soil samples for microbial testing at three (3) contaminated and one (1) control location
  - Stainless steel tool cleaned after each sample collected
  - Samples placed in sterile plastic containers provided by lab
- Also sampled microbial mixture (Positive Control)
- Microbial samples tested using NextGen Sequencing and qPCR to assess diversity and presence of BTEX metabolizing genes

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Methods (Post-Injection)

Summer 2017:

- Inject of 26,000 lbs BOS 200® with microbial mix and sulfate at 302 injection points spanning 7 – 17 feet bgs (or refusal) spaced on 5 foot triangular grid

Winter 2017/2018:

- Collected second round of soil samples for qPCR testing at four (4) locations sampled previously

Summer 2018:

- Conducted third and final round of microbial sampling to target carbon zones (results pending)

Future Work:

- Obtain, analyze (for BTEX) and compare final round of soil samples to pre-treatment samples

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Pre-Injection Soil (> 2 ppm benzene)

			BTEX Results (ppm)			
Sample ID	Depth (ft.)	Date Collected	Benzene	Toluene	Ethylbenzene	Xylene
Pre-Injection Soils						
PI-2	9-11	11/3/2016	2.29	0.22	3.22	10.6
PI-6	10-12	11/1/2016	2.8	2.65	0.817	4.18
PI-9	10-12	11/2/2016	2.25	2.97	6.72	30.7
PI-23	12-14	11/1/2016	2.28	<0.485	0.444	2.04
PI-24	10-12	11/2/2016	15.8	60.8	25.1	105
PI-25	10-12	11/2/2016	5.88	8.77	12.3	53.3
PI-26	6-8	11/2/2016	2.3	1.46	4.74	11.3
PI-30	12-14	11/3/2016	2.42	0.0547	2.42	0.348
PI-31	10-12	11/3/2016	3.63	2.7	8.62	22.2

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Pre-Injection Groundwater (> 1 ppm benzene)

			BTEX Results (ppm)			
Sample ID	Screened Interval (ft.)	Date Collected	Benzene	Toluene	Ethylbenzene	Xylene
Pre-Injection Groundwater						
MW-4	5.5-15.5	11/7/2016	4.78	<0.250	0.43	<0.0750
MW-7	7-17	11/8/2016	5.46	<1.00	0.939	1.18
MW-8	5-13	11/8/2016	2.46	<0.125	0.311	0.0432
MW-9	5-11.5	11/7/2016	8.45	<0.5	1.05	0.452
MW-10	5-18	11/8/2016	4.11	<0.250	0.767	0.0976
MW-14	5-17	11/8/2016	1.66	<0.125	0.0407	0.405
SW-1	9-19	11/8/2016	1.32	<0.125	0.0215	0.138
SW-3	5.8-15.8	11/8/2016	2.13	<0.125	0.443	0.0504
SW-4	7.11-17.11	11/8/2016	1.07	<0.1	0.134	0.0384

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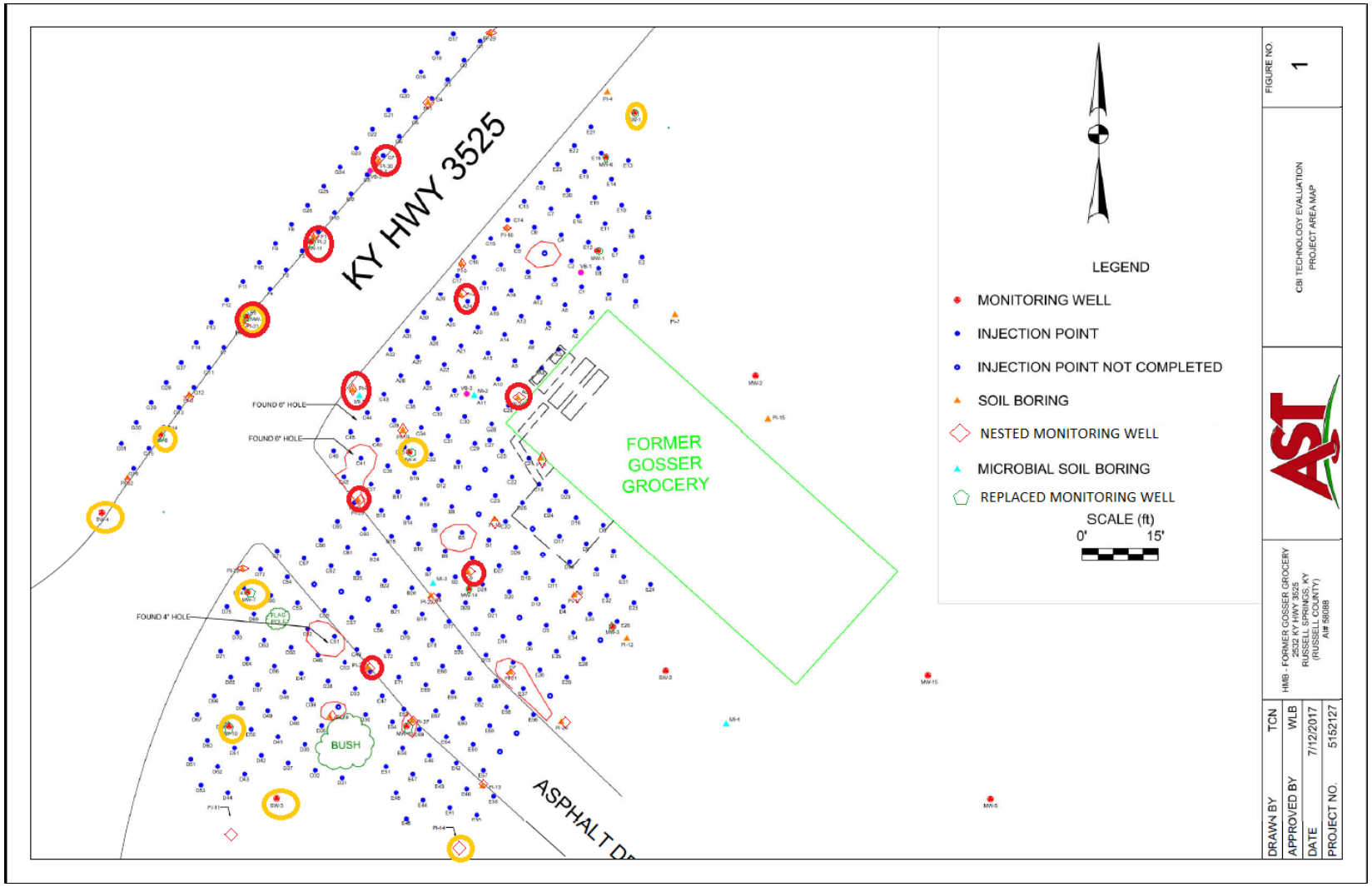
Highest Soil and Groundwater Benzene Concentrations



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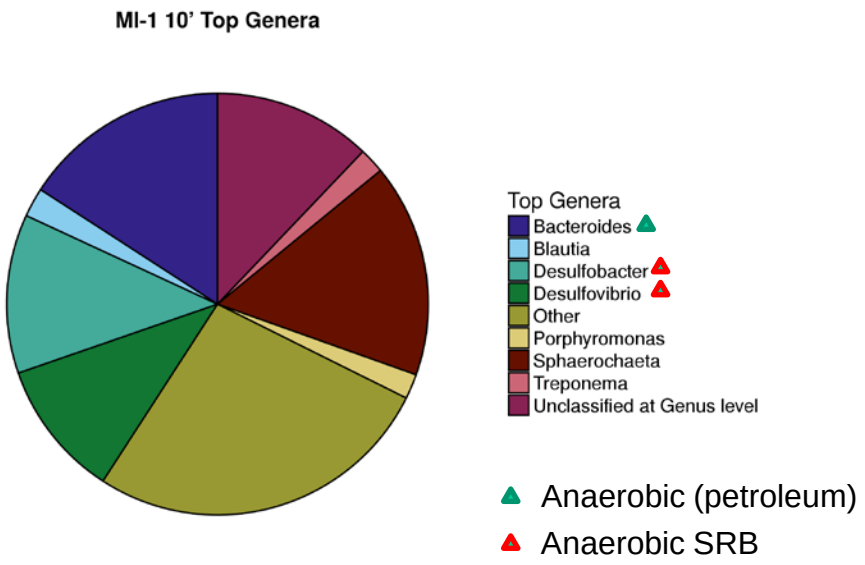
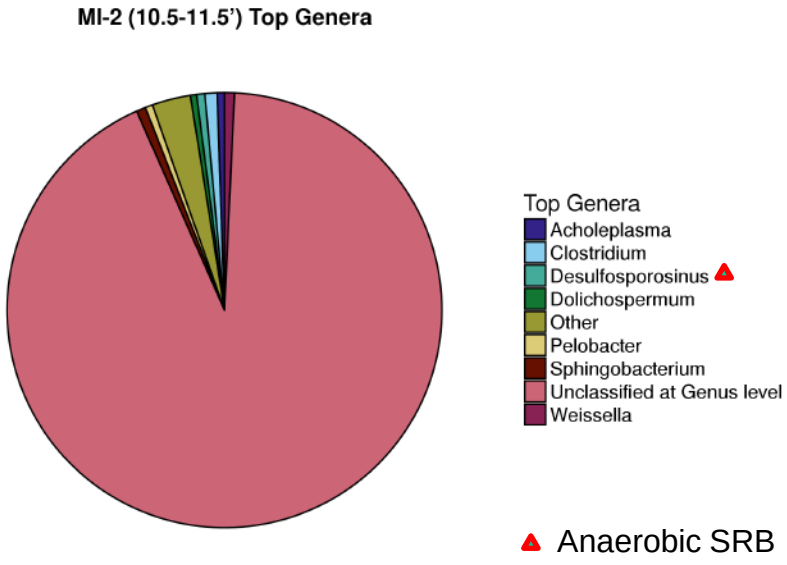
Pre-Injection NextGen Sequencing Results

MI-1



MI-2



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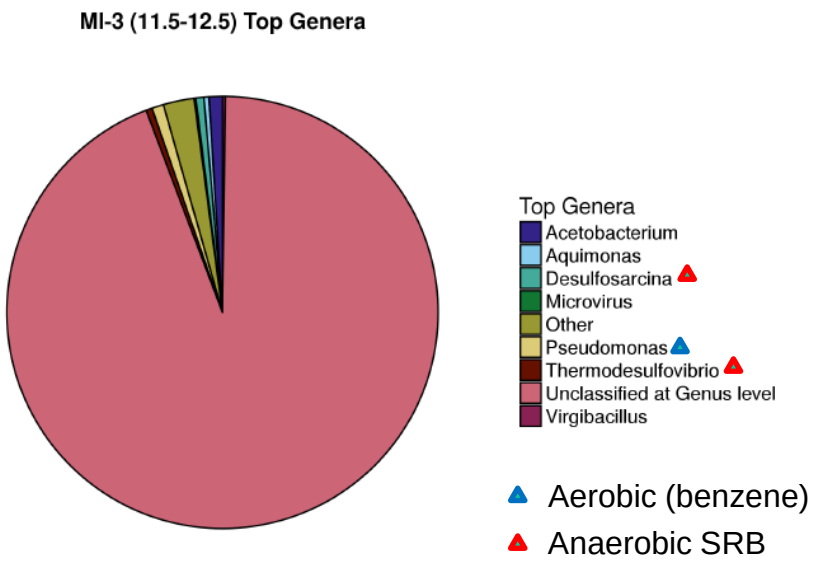
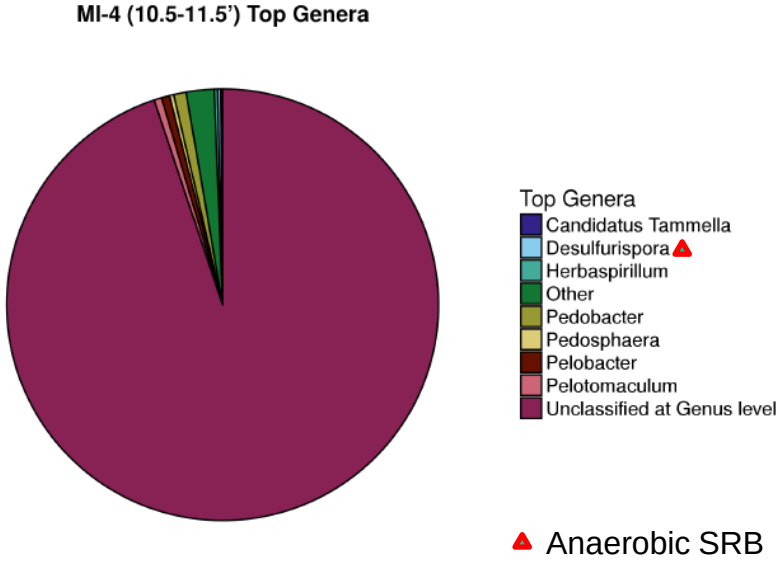
Pre-Injection NextGen Sequencing Results

MI-3



MI-4 (Control)



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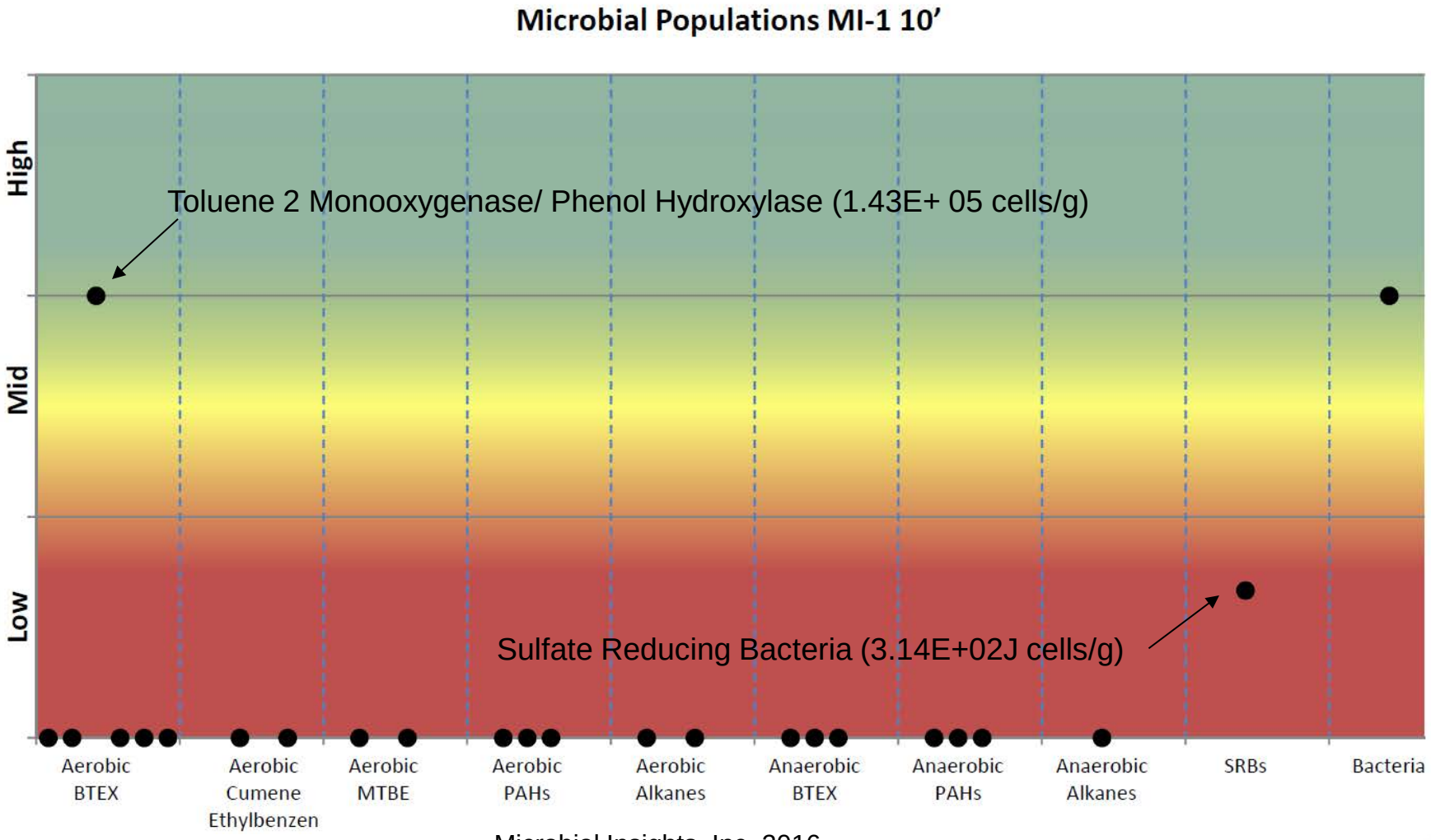
Pre-injection qPCR Results



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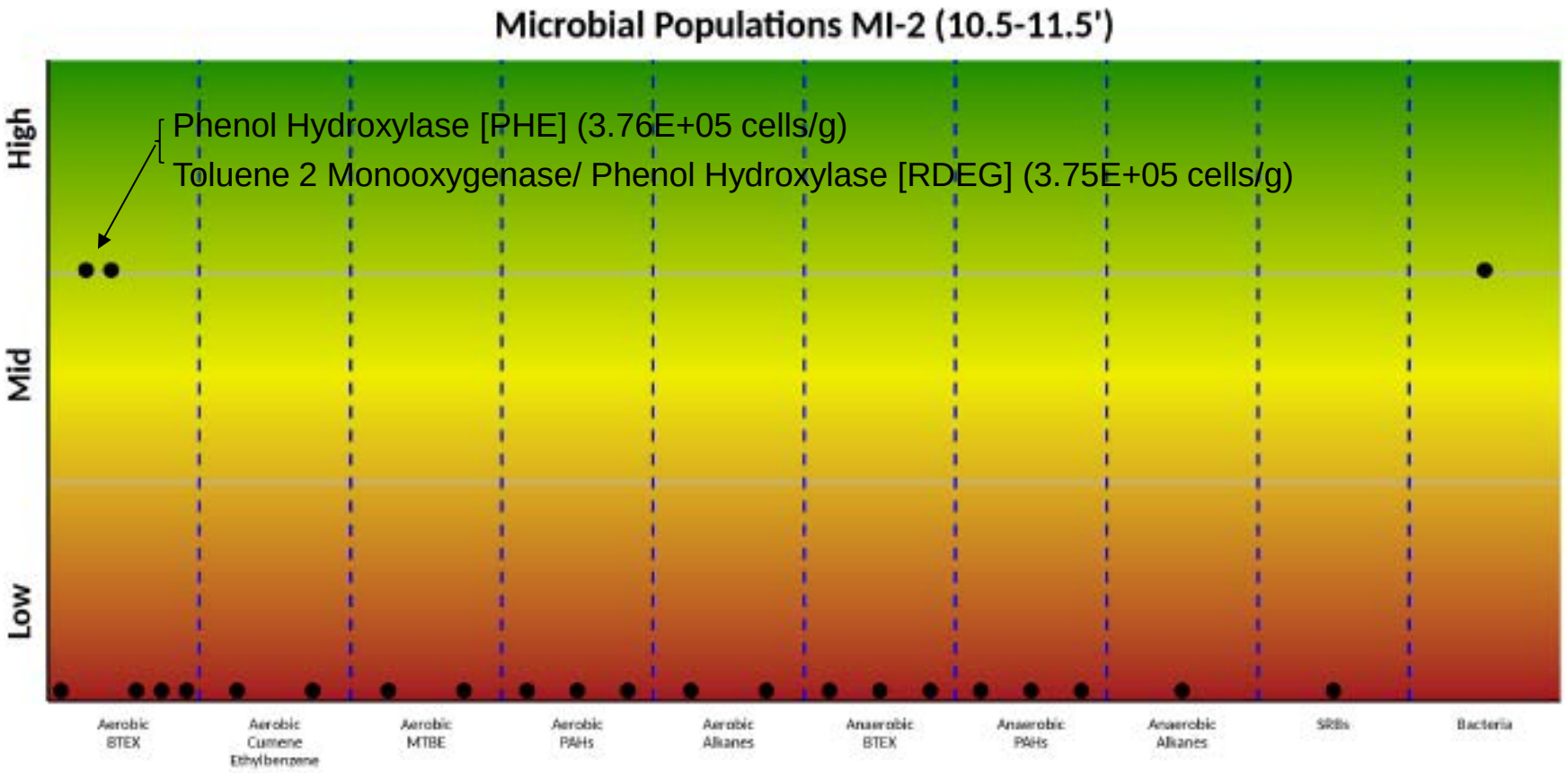
Pre-injection qPCR Results



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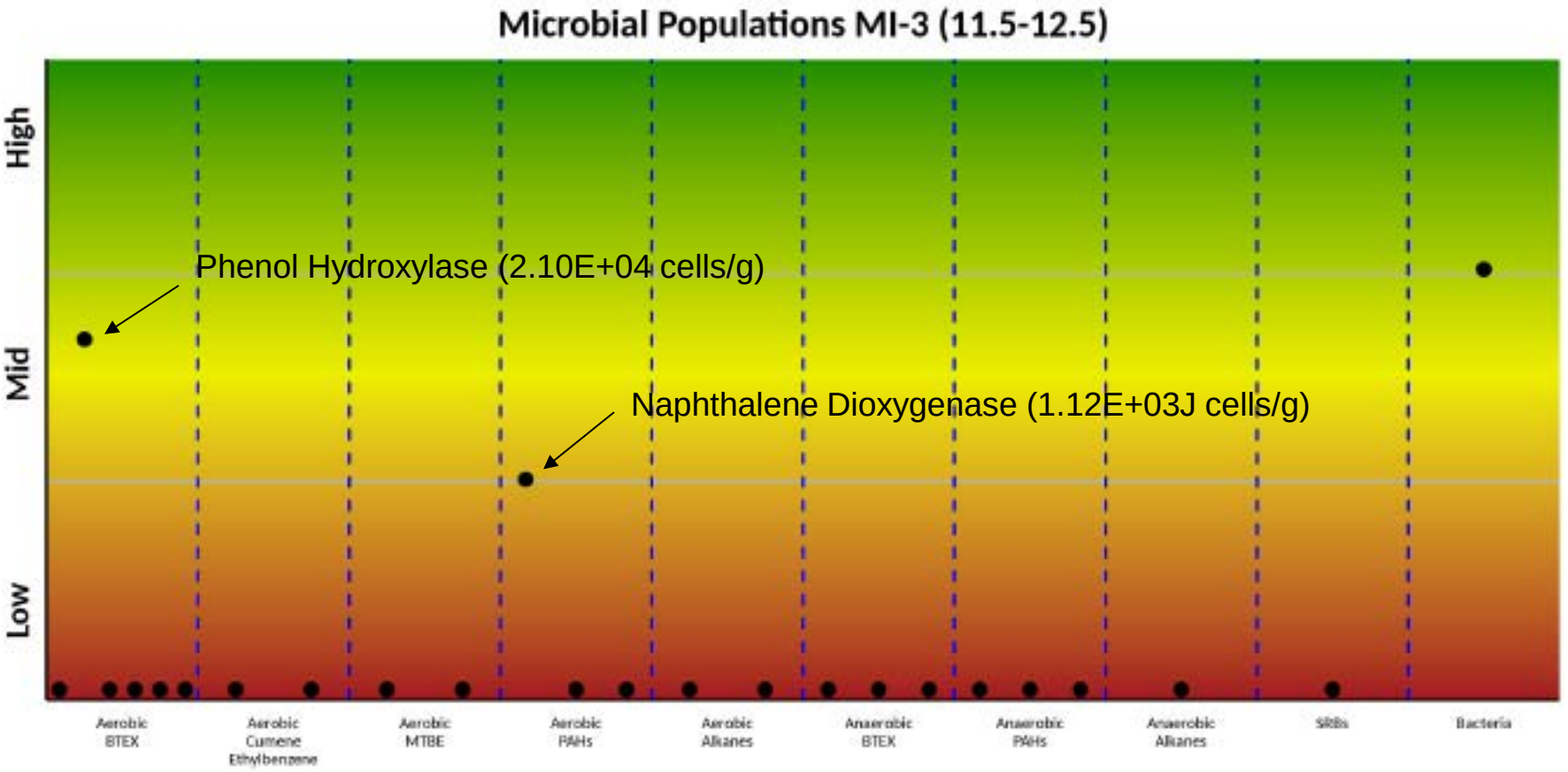
Pre-injection qPCR Results



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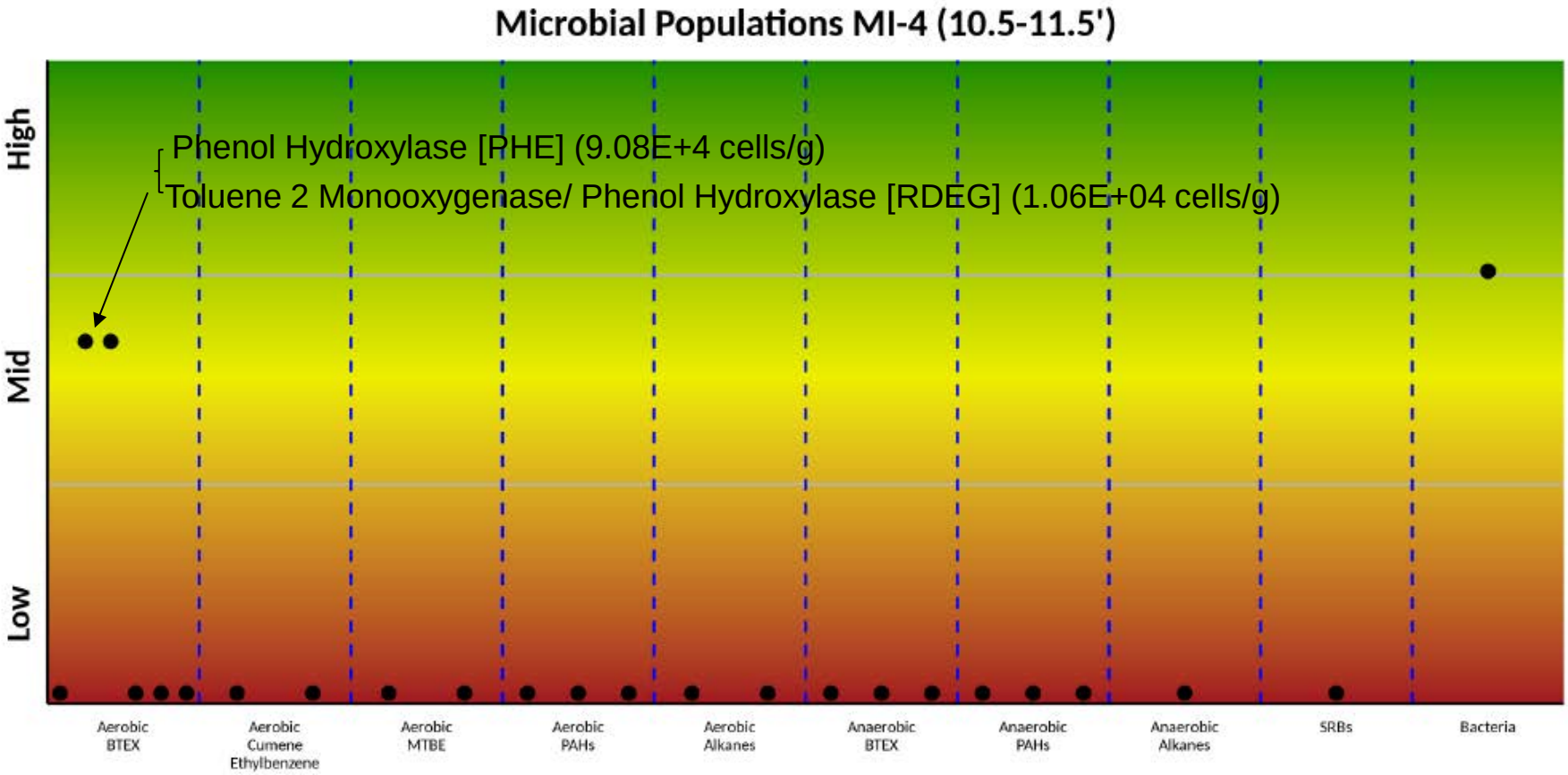

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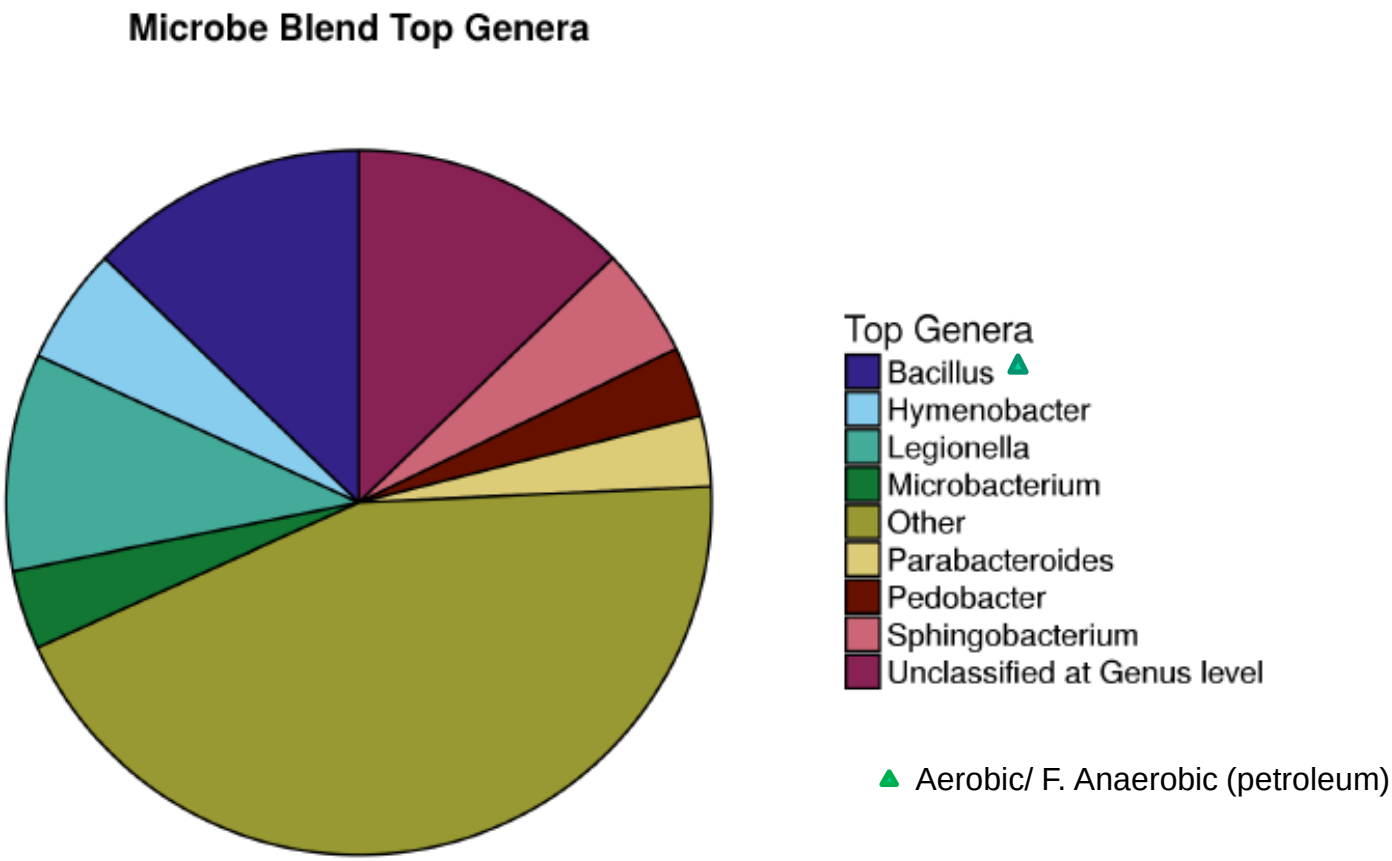
NextGen Sequencing Microbial Blend



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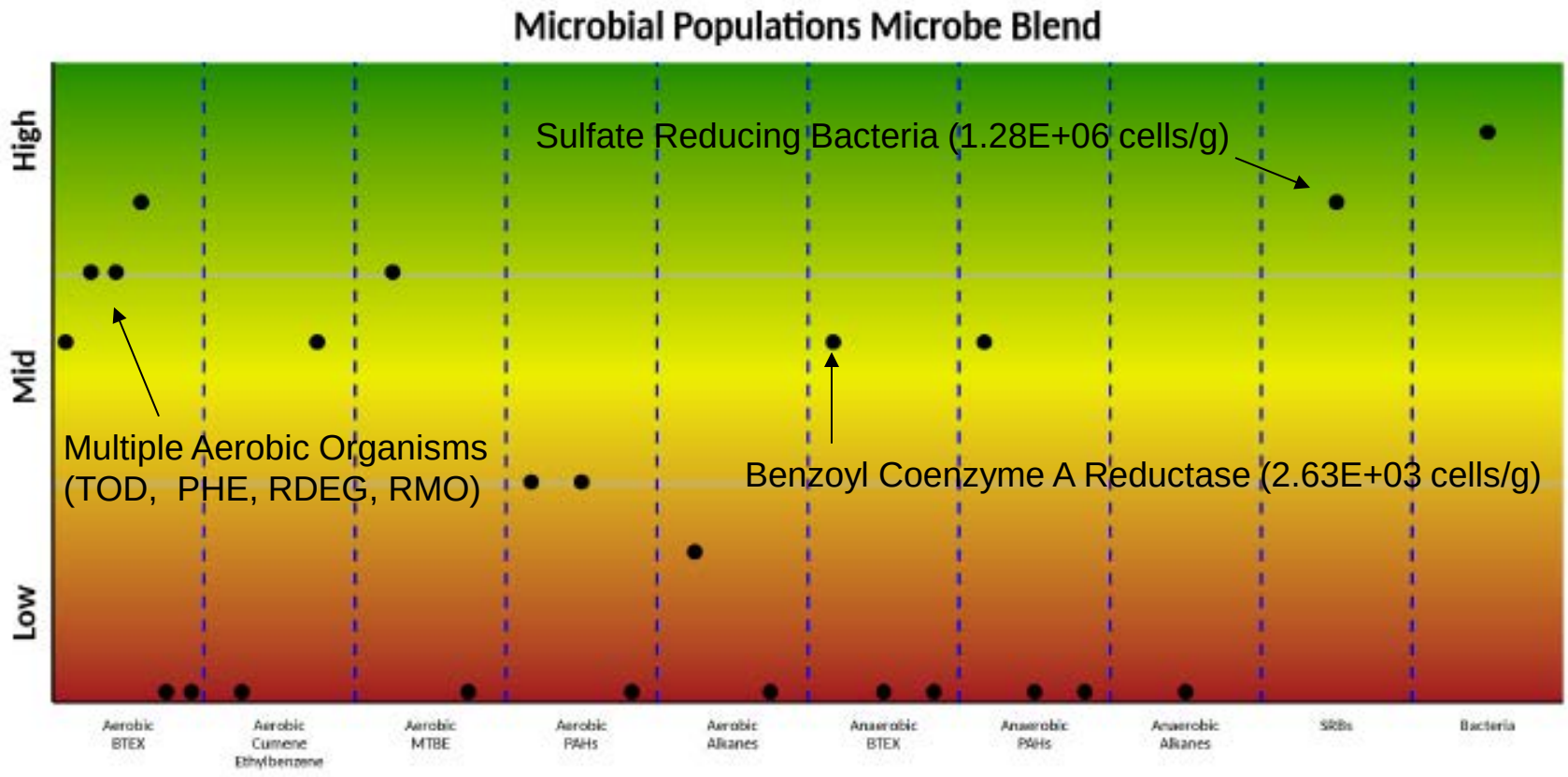
qPCR Microbial Blend



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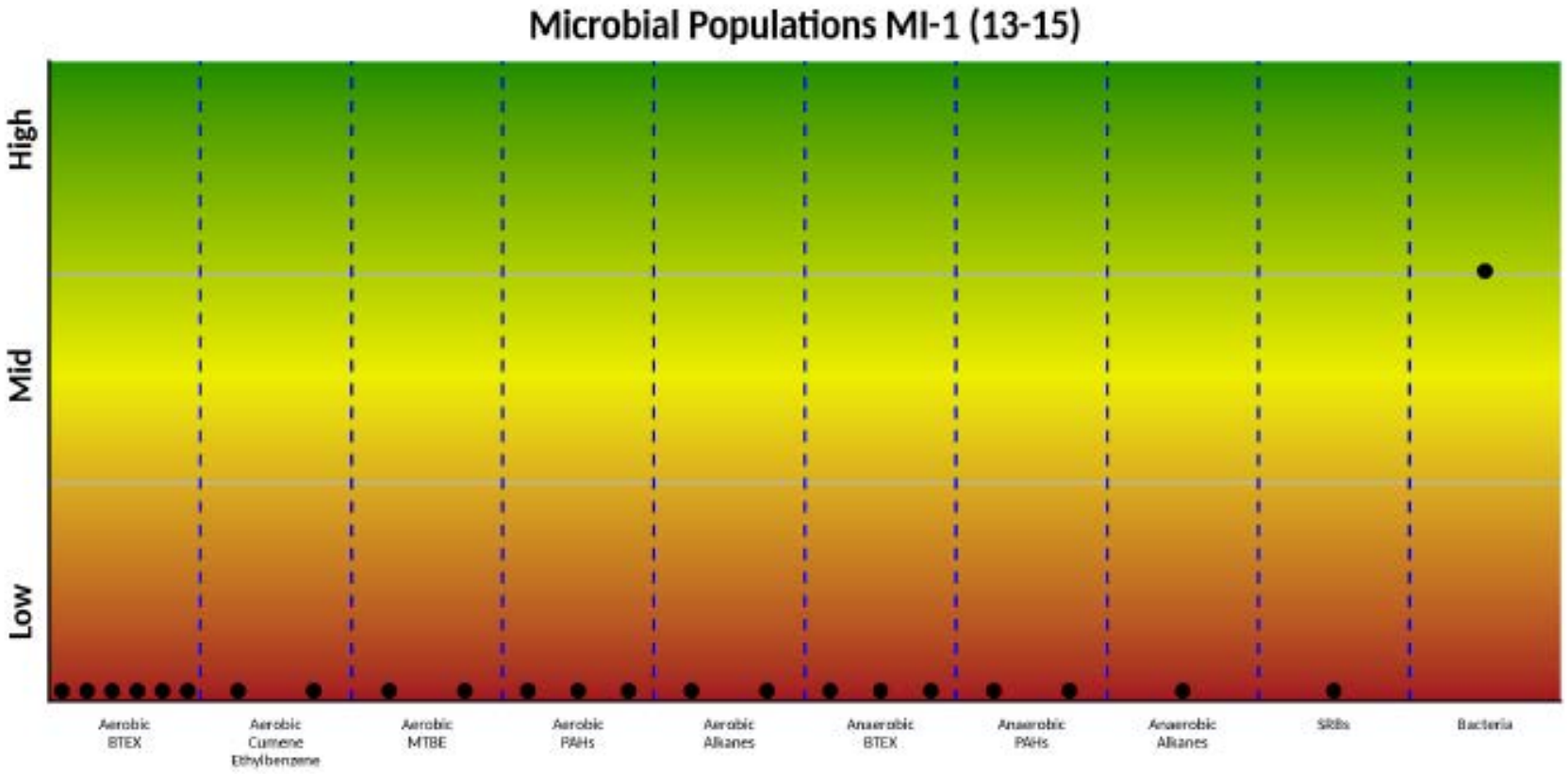
Post-Injection qPCR Results



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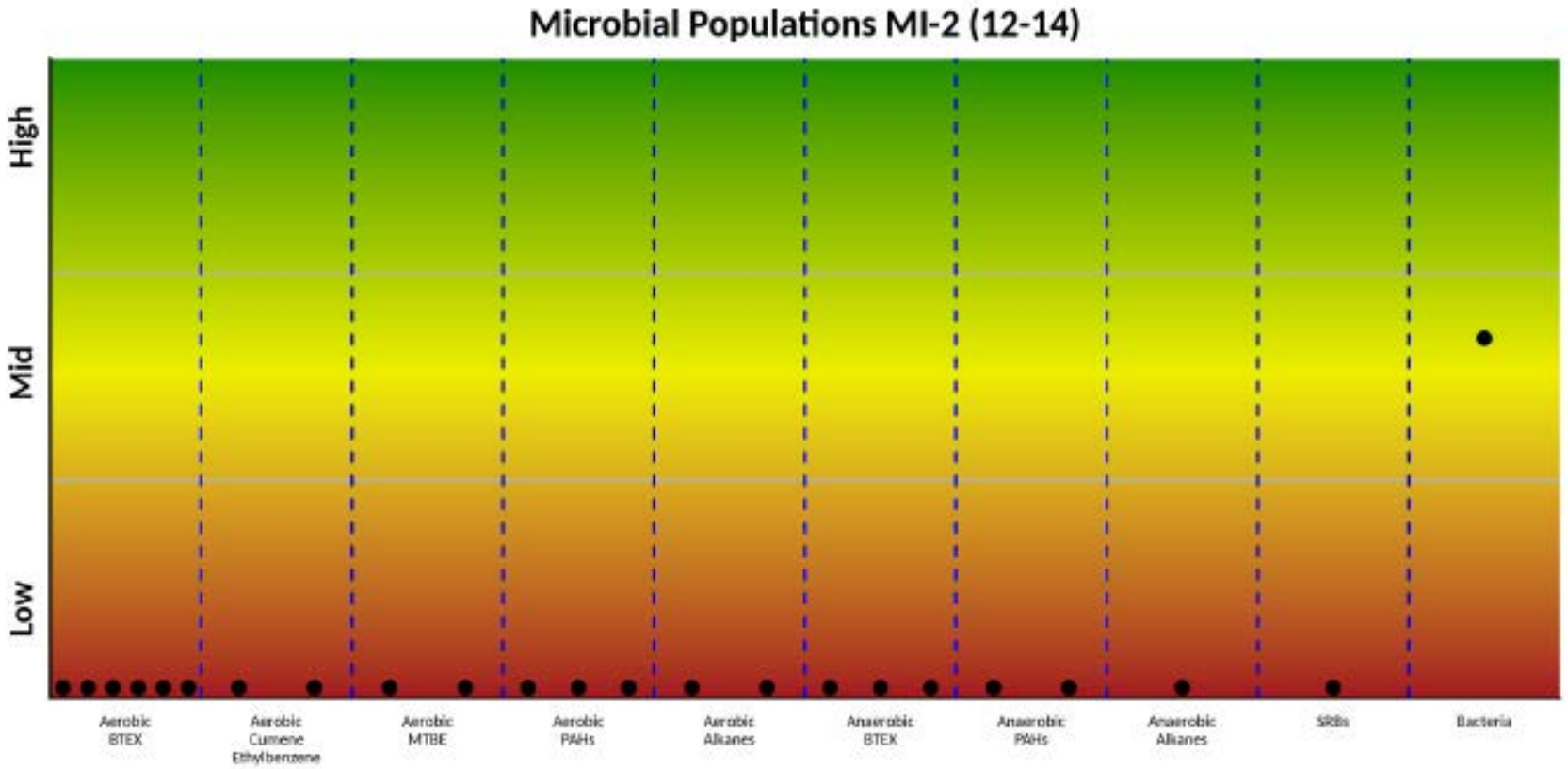
Post-Injection qPCR Results



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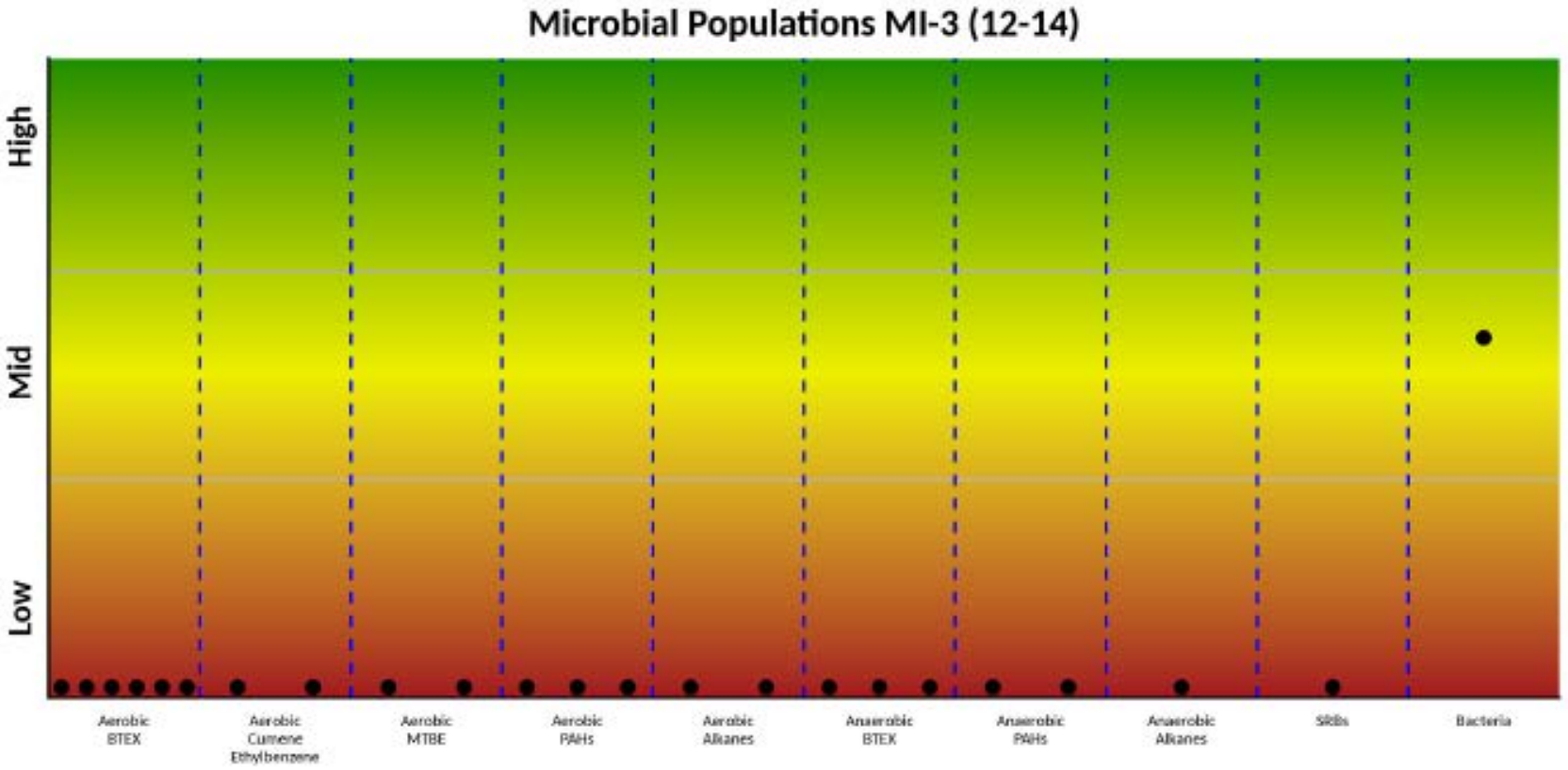

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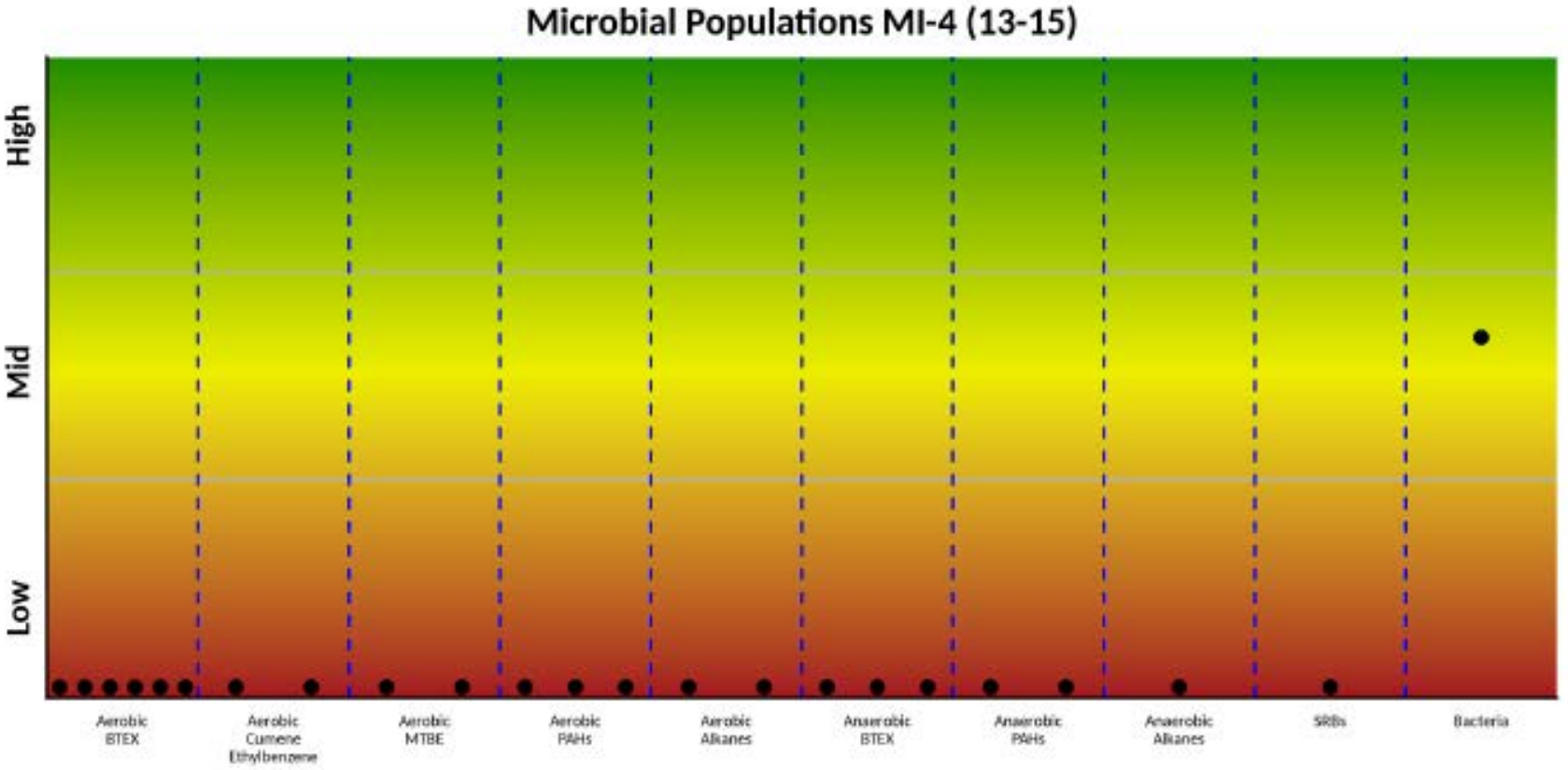

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Summary

- Study was conducted to assess potential of carbon-based BOS 200 Trap and Treat® product to biodegrade BTEX in-situ at a typical clay dominated Kentucky LUST site
- Soil and groundwater samples were analyzed to assess the distribution and concentration of BTEX prior to treatment (baseline conditions)
- Soil for microbial testing was obtained from three (3) contaminated and one (1) control location prior to treatment (baseline conditions)
- Pre-treatment microbial testing indicated the presence of mainly aerobic bacteria with sample MI-1 exhibiting the most diverse population of potential degraders
- Post-treatment microbial sampling was also completed at co-located borings
- While total bacterial numbers were good, post-treatment qPCR results indicated a lack of targeted microbes in all samples tested

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Discussion

- Potential reasons for lack of target bacteria in post-treatment samples may include:
  - Issues with sample collection, preservation or DNA extraction
  - Population has not stabilized post-injection
  - Injection has affected the bacterial population (unlikely given that control also exhibited low numbers of target bacteria post-injection)

- Results pending for microbial testing of carbon collected from cores (may show us something)

- Planned post-treatment soil sampling will provide clearer picture as to whether biodegradation is occurring at site

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QUESTIONS?

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