IN SITU MICROCOSM STUDY REPORT
SANTA FE COUNTY JUDICIAL COMPLEX
327 SANDOVAL STREET
SANTA FE, NEW MEXICO

FACILITY #: 53763
RELEASE ID #: 4597
WPID #: 4072

Prepared for:

New Mexico Environment Department
Petroleum Storage Tank Bureau
2905 Rodeo Park Drive East, Building 1
Santa Fe, NM 87505

Prepared by:

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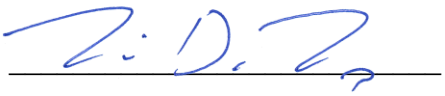
February 2020

EA Project No. 6347002.03

STATEMENT OF FAMILIARITY

I, the undersigned, am personally familiar with the information submitted in this report and the attached documents and attest that it is true and complete.

Signature: 

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Date: February 28, 2020

1. INTRODUCTION

EA Engineering, Science, and Technology, Inc., PBC (EA) has prepared this *In Situ* Microcosm (ISM) Study Report for the Santa Fe County Judicial Complex (SFCJC) State Lead Site (the site) located at 327 Sandoval Street in Santa Fe, New Mexico (Figure 1). The work was completed under EA's Professional Services Contract number 19-667-3200-0007 and in accordance with applicable requirements of New Mexico Administrative Code, Title 20, Chapter 5, Part 119 and the *Work Plan for CRP Development, File Review, Baseline Groundwater Monitoring, In Situ Microcosm Study, Benzene Plume Delineation, FRP Development, and Discharge Permit, Santa Fe County Judicial Complex State Lead Site, 327 Sandoval Street, Santa Fe, New Mexico,* approved by the New Mexico Environment Department (NMED) Petroleum Storage Tank Bureau (PSTB) on June 27, 2019 under work plan identification (WPID) number 4072.

Bio-Trap® ISM units were installed in monitoring wells TWN-03, SFCMW-10, CMW-01, MW-1R, MW-11, and MW-4R to assess the potential for biodegradation of benzene, toluene, ethylbenzene, and xylenes (BTEX), the polycyclic aromatic hydrocarbon (PAH) naphthalene, petroleum alkanes ($C_5 – C_{16}$), 1,2-dibromoethane (ethylene dibromide or EDB), and 1,2-dicholoroethane (ethylene dichloride or EDC) in groundwater under amended and unamended site conditions (Figure 1). Bio-Trap® ISM units are field-deployed passive samplers that provide a very large surface upon which bacteria of interest can grow; they may be amended with various augmentations, such as different electron acceptors or donors. They can also be used to monitor contaminant concentrations and anions relevant to monitored natural attenuation (MNA) bacterial processes.

At the SFCJC, the following types of Bio-Trap® ISM units were installed:

- **MNA control units containing no amendments.** These units were used to assess the potential for MNA of EDB and EDC under current *in situ* conditions and to serve as a baseline for comparison with amendments, including the addition of nitrate and sulfate electron acceptors. (Note: nitrate and sulfate may also serve as nutrient sources for bacterial growth, as nitrogen and sulfur are essential components of enzymes and structural proteins.)

- **BioStim® units amended with nitrate or nitrate and sulfate to serve as electron acceptors.** These units were used to determine if biostimulation of indigenous bacteria using nitrate or nitrate and sulfate results in greater bacterial numbers than the control MNA units.

- **BioAug® units amended with sulfate as the electron acceptor and SDC-9 bacterial culture.** The SDC-9 culture is a proprietary mixture of anaerobic bacteria, including *Dehalobacter* and *Dehalococcoides* spp., which have been shown to use EDC and EDB as electron acceptors under anaerobic conditions. Oxygen exposure can irreversibly inhibit dechlorination, growth, and viability of these obligate anaerobes (Amos *et al*, 2008). BioAug® units were used to determine if bioaugmentation will result in greater numbers of bacteria than the control Bio-Trap® and BioStim® units.

Multiple Bio-Trap® ISM units may be deployed in the same monitoring well to simultaneously evaluate different treatment options. In addition, Bio-Traps® may be analyzed to determine concentrations of contaminants of concern (COCs) in addition to dissolved gases and anions commonly used to evaluate MNA processes. The following is a summary of deployed Bio-Trap® ISMs (Table 1):

- MW-11: Standard Bio-Trap® (Standard Bio-Traps® are not analyzed for site COCs)
- TWN-03: MNA, BioStim®, and BioAug® (BioStim® with nitrate plus sulfate and BioAug® with sulfate and SDC-9 culture)
- SFCMW-10: MNA and BioStim® (BioStim® with nitrate plus sulfate)
- CMW-01: MNA and BioStim® (BioStim® with nitrate plus sulfate)
- MW-1R: MNA and BioStim® (BioStim® with nitrate and nitrate plus sulfate)
- MW-4R: Standard Bio-Trap®

Forty-eight (48) days after the Bio-Trap® ISM units were deployed, they were retrieved and sent to Microbial Insights for the following analyses:

- QuantArray®, which quantifies genes associated with total numbers of Eubacteria and sulfate-reducing bacteria
- QuantArray-Petro®, which quantifies genes associated with bacteria that degrade BTEX, PAHs, and petroleum alkanes
- CENSUS®, which quantifies genes associated with *Dehalobacter* and *Dehalococcoides* spp.
- In addition, the Bio-Trap® ISM units were analyzed to determine concentrations of dissolved gases and anions that provide information pertinent to assessing geochemical conditions, and in some, concentrations of BTEX, naphthalenes, EDB, and EDC were also measured.

The Microbial Insights Bio-Trap® ISM Study report with the results is provided in Appendix A. Field notes for deployment and retrieval of the Bio-Trap® ISM units were provided to the NMED PSTB in the Baseline Groundwater Monitoring Report dated December 10, 2019. Results of the baseline groundwater monitoring event are shown on Figures 1 through 4.

2. BIO-TRAP® ISM STUDY RESULTS

This section provides a summary of the Bio-Trap® ISM study results. The results are discussed separately for each well.

It is important to note that while bacterial DNA and genes of interest may be detected in any given sample, suitable geochemical conditions are essential before the desired processes will occur at appreciable rates. For example, if site conditions are anoxic (generally dissolved oxygen (DO) concentrations less than 0.5-1.0 milligram per liter [mg/L]), then significant aerobic processes are unlikely to occur. Conversely, anaerobic processes will not occur in the presence of appreciable quantities of DO because oxygen is toxic to strict anaerobes.

It is also important to note that some bacteria involved in BTEX and petroleum alkane degradation, such as Pseudomas spp., are capable of both aerobic and anaerobic growth. Bacteria such as these grow optimally with oxygen but can switch to other electron acceptors, such as nitrate or metals, when oxygen is depleted. These bacteria are referred to as facultative anaerobes.

2.1. MW-11: Standard Bio-Trap® ISM Unit - DeVargas Plume

MW-11 is located in the northern portion of the SFCJC site in the parking lot of the 200 West DeVargas Condominium complex in what has been referred to as the DeVargas Plume (Figure 1). Samples collected from the well during the baseline groundwater monitoring event showed a BTEX concentration of 193 µg/L, an EDB concentration of 0.74 µg/L, and a total naphthalenes concentration of 411µg/L (Figure 3). The DO concentration measured during purging and sampling of the well was 0.03 mg/L, which is considered too low to support aerobic bacterial growth but will allow anaerobic bacterial growth.

Concentrations of genes associated with aerobic BTEX degradation were moderate and within the same general ranges as gene copy numbers associated with anerobic BTEX degradation.

Genes for aerobic and anaerobic PAH degradation were not detected.

Gene copies associated with aerobic alkane degradation were low or not detected, while genes associated with anaerobic alkane degradation were not detected.

Bacteria and genes associated with degradation of EDB were not detected.

MNA may be a viable treatment option for residual BTEX contamination but may not be viable for remediation of total naphthalenes, alkanes, or EDB. Addition of SDC-9 culture may encourage in situ degradation of the EDB.

2.2 TWN-3: MNA, BioStim®, and BioAug® ISM Units – DeVargas Plume

TWN-3 is also located in the DeVargas Plume approximately 30 feet northwest of MW-11 (Figure 1). Baseline groundwater monitoring results showed a benzene concentration of 29 µg/L, a BTEX concentration of 44.1 µg/L, an EDB concentration of 4.4 µg/L, an EDC

concentration of 1.6 µg/L, and a total naphthalenes concentration of 5.0 µg/L (Figure 3). However, Bio-Trap® results showed that BTEX, EDC, and total naphthalenes were not detected at TWN-3. The DO concentration measured during purging and sampling of the well was 5.68 mg/L. This concentration will readily support aerobic bacterial growth but will severely inhibit anaerobic bacterial growth.

The fact that methane concentrations were extremely low (up to 4.4 µg/L) supports the assertion that conditions are oxic. Genes associated with the aerobic and anaerobic degradation of BTEX were detected at moderate to high concentrations, indicating that the plume has the genetic capacity for aerobic and anaerobic BTEX degradation. However, aerobic degradation potentials of BTEX and PAHs were not different between the amended (BioStim®) and unamended (MNA) Bio-Traps®. Although genes coding for enzymes responsible for anaerobic degradation of BTEX were detected in all three units, it is unlikely that these enzymes are active, given the significant concentrations of DO.

Concentrations of genes associated with aerobic PAH degradation were low and genes associated with anaerobic PAH degradation were low or not detected in all units.

Concentrations of genes associated with aerobic alkane degradation were moderate among all units and genes associated with anaerobic alkane degradation were not detected. The simultaneous presence of moderate concentrations of genes associated with aerobic and anaerobic petroleum degradation may be due to the presence of facultative anaerobes.

The CENSUS® analysis detected elevated concentrations of SDC-9 culture in the BioAug® treatment, which is very likely because the BioAug® treatment included SDC-9 culture; it does not necessarily indicate or confirm that these organisms are active. It is unclear why low concentrations of sulfate-reducing bacteria (APS) and SDC-9 genes were detected in the MNA and BioStim® units because the DO concentration at TWN-3 is too high to allow for the growth of these obligate anaerobes. It is possible that this is an artifact of having deployed the BioAug® unit near the MNA and BioStim® units (note that enumeration of APS genes also captures numbers of SDC-9 genes). Regardless, the fact that the enzyme responsible for EDC degradation (1,2-DCA reductase [DCAR]) was not detected in any unit, supports the conclusion that dehalogenating bacteria were not active at the site.

Collectively, it appears that nutrient amendment will not increase the BTEX degradation capacity at this location, and bioaugmentation with SDC-9 is recommended after oxygen depletion.

2.3 SFCMW-10: MNA and BioStim® ISM Units – SFCJC Plume

SFCMW-10 is located directly southeast of the SFCJC within the SFCJC plume (Figure 1). Baseline groundwater monitoring results showed a benzene concentration of 83 µg/L, a BTEX concentration of 145 µg/L, and a total naphthalenes concentration of 3,010 µg/L (Figure 3). Bio-Trap® results for BTEX and total naphthalene concentrations were generally consistent with laboratory analytical results. The DO concentration at this location was 0.05 mg/L when measured during the baseline groundwater monitoring event, which is too low to support significant aerobic microbial growth.

Low to moderate concentrations of bacterial genes associated with aerobic and anaerobic BTEX metabolism were present in both units. Gene copies related to BTEX degradation did not appear to be substantially different between the two units, although certain BTEX-degrading genes were present in the BioStim® unit but not in the MNA, and genes involved in anaerobic degradation of BTEX and naphthalene were detected at somewhat higher concentrations in the BioStim® unit.

Genes associated with aerobic and anaerobic PAH degradation were higher in the BioStim® unit.

Moderate concentrations of aerobic alkane-degrading genes were present in both units but were slightly higher in the BioStim® unit. Genes for anaerobic alkane degradation were not detected.

Sulfate-reducing bacteria (APS) concentrations were moderate in both units but concentrations did not vary between the two. APS can produce significant concentrations of hydrogen sulfide as an end product of sulfate reduction and there are some reports that hydrogen sulfide accumulation may become toxic to bacteria, even to APS. Although significant numbers of APS were detected, hydrogen sulfide was not detected. This is likely because hydrogen sulfide reacts almost immediately with reduced metals and rapidly precipitates out of solution due to extremely low solubility.

Collectively, these data suggest that nutrient amendment may enhance biodegradation of petroleum hydrocarbons at this location. Hydrogen sulfide toxicity is unlikely in this and other anoxic wells at the site.

2.4 CMW-01: MNA and BioStim® ISM Units – Capital 66 Plume

CMW-01 is located in the parking lot of the Saveur Bistro Restaurant (former Capital 66) in the Capital 66 plume (Figure 1). Baseline groundwater monitoring results showed a benzene concentration of 150 µg/L, a BTEX concentration of 165.2 µg/L, an EDB concentration of 0.25 µg/L, and a total naphthalenes concentration of 4.7 µg/L (Figure 3). Bio-Trap® results for benzene and EDB were consistent with laboratory results. The DO concentration at this location was 3.01 mg/L when measured during the baseline groundwater monitoring event, which will support aerobic growth and inhibit anaerobic growth.

Concentrations of genes associated with aerobic degradation of BTEX, PAHs, and alkanes were low to moderate in both units and were not substantially different between the two units.

Concentrations of genes associated with anaerobic BTEX degradation were similar between units and anaerobic alkane-degrading genes were not detected.

Numbers of APS were also moderate, but it is unlikely these bacteria are active given the concentrations of DO and nitrate at this well. *Dehalobacter* spp. and 1,2-DCA reductase (DCAR) were not detected.

Collectively, these data indicate that nutrient amendment is unlikely to increase degradation of BTEX, and EDB degradation would be possible once oxygen is depleted.

2.5 MW-1R: MNA, BioStim® with Nitrate, and BioStim® with Nitrate Plus Sulfate ISM Units – Design Center Plume

MW-1R is located at the southeast corner of the 210 & 218 Montezuma Avenue building within the Design Center Plume (Figure 1). Baseline groundwater monitoring results showed a benzene concentration of 1,700 µg/L, a BTEX concentration of 27,200 µg/L, an EDB concentration of 0.52 µg/L, and a total naphthalenes concentration of 1,210 µg/L. Bio-Trap® results for benzene and total naphthalenes were substantially lower than analytical laboratory results. The DO concentration at this location was 0.0 mg/L when measured during the baseline groundwater monitoring event, which is considered too low to support aerobic bacterial growth but will allow uninhibited anaerobic growth.

The *in-situ* sulfate concentration was less than 2.5 mg/L, which is two to three orders of magnitude less than sulfate concentrations measured at other locations. This indicates that sulfate-reducing bacteria are active at this location, although APS concentrations did not appear to be substantially different between the two BioStim® units.

In general, concentrations of genes associated with aerobic degradation of BTEX, PAHs, and alkanes were moderate to high, and higher in the MNA unit.

Concentrations of genes associated with anaerobic BTEX degradation were mixed. The concentration of benzoyl coenzyme A reductase (BCR) was highest in the MNA unit, the concentration of benzylsuccinate synthase (BSS) was highest in the BioStim® unit amended with nitrate, and benzene carboxylase (ABC) was highest in the BioStim® unit with nitrate, but not detected in the unamended MNA unit.

No genes for anaerobic PAH degradation were detected and genes for aerobic PAH degradation were very low and only detected in the MNA unit.

Genes for aerobic alkane degradation were very low and only detected in the MNA unit.

Total numbers of APS comprised a large portion of the total bacterial community, and cell numbers did not vary between units. It appears that nutrient amendment at this location will not increase concentrations of most genes associated with anaerobic BTEX, PAH, or petroleum alkane degradation.

2.6 MW-4R: Standard Bio-Trap® ISM Unit - Design Center Plume

MW-4R is located just south of the median in Cerrillos Road between the Design Center and the Bardacke Attorney General Complex in the Design Center plume (Figure 1). Baseline groundwater monitoring results showed a benzene concentration of 1,800 µg/L, a BTEX concentration of 25,300 µg/L, an EDB concentration of 0.52 µg/L, and a total naphthalenes concentration of 1,210 µg/L. The DO concentration at this location was 0.05 mg/L when measured during the baseline groundwater monitoring event, which is considered too low to support significant aerobic growth but will allow for anaerobic growth.

Concentrations of some genes associated with aerobic BTEX degradation were moderate while others were low. Gene copies for anaerobic BTEX degradation were low to moderate.

Concentrations of genes for aerobic alkane degradation were low and genes for anaerobic alkane degradation were not detected.

Bacteria and genes associated with EDB degradation were not detected. Anaerobic conditions would be required for degradation of EDB, and bioaugmentation would be necessary.

3. SUMMARY AND CONCLUSIONS

DeVargas Plume

- In MW-11, EDB and total naphthalenes concentrations were above the standards. Aerobic and anaerobic degradation may be viable treatment options for residual BTEX contamination but may not be viable for remediation of total naphthalenes and alkanes. Carbon absorption with subsequent degradation could be a potential remedial alternative for naphthalene. Anaerobic conditions and bioaugmentation with SDC-9 culture may encourage *in situ* degradation of the EDB.

- In TWN-3, benzene and EDB concentrations were above the standards. Aerobic and anaerobic degradation may be viable treatment options for residual BTEX. Nutrient amendment appears to increase the BTEX degradation capacity at this location. Anaerobic conditions and bioaugmentation with SDC-9 culture may encourage *in situ* degradation of the EDB.

SFCJC Plume

- In SFCMW-10, benzene and total naphthalene concentrations were above the standards. Aerobic and anaerobic degradation may be viable treatment options for residual BTEX and PAHs. Nutrient amendment may enhance biodegradation of petroleum hydrocarbons at this location. Hydrogen sulfide toxicity is unlikely in this and other anoxic wells at the site.

Capital 66 Plume

- In CMW-1, benzene and EDB concentrations were above the standards. Aerobic and anaerobic degradation may be viable treatment options for residual BTEX. Nutrient amendment is unlikely to increase degradation of BTEX. Anaerobic conditions and bioaugmentation with SDC-9 culture may encourage *in situ* degradation of the EDB.

Design Center Plume

- In MW-1R, benzene, EDB and total naphthalene concentrations were above the standards. Aerobic degradation may be a viable treatment option for residual BTEX and PAHs. Total numbers of APS comprised a large portion of the total bacterial community, and cell numbers did not vary between units. It appears that nutrient amendment at this location will not increase concentrations of most genes associated with anaerobic BTEX, PAH, or petroleum alkane degradation. Anaerobic conditions and bioaugmentation with SDC-9 culture may encourage *in situ* degradation of the EDB and EDC.

- In MW-4R, benzene, EDB, and total naphthalene concentrations were above the standards. Aerobic and anaerobic degradation may be viable treatment options for residual BTEX and to a lesser degree for PAHs. Bacteria and genes associated with EDB degradation were not detected. Anaerobic conditions and bioaugmentation with SDC-9 culture may encourage *in situ* degradation of the EDB and EDC.

EA will develop remedial injection strategies for each of the four plumes at the SFCJC based on results of the Bio-Trap® ISM Study. These strategies will be presented in the Final Remediation Plan for the site.

FIGURES

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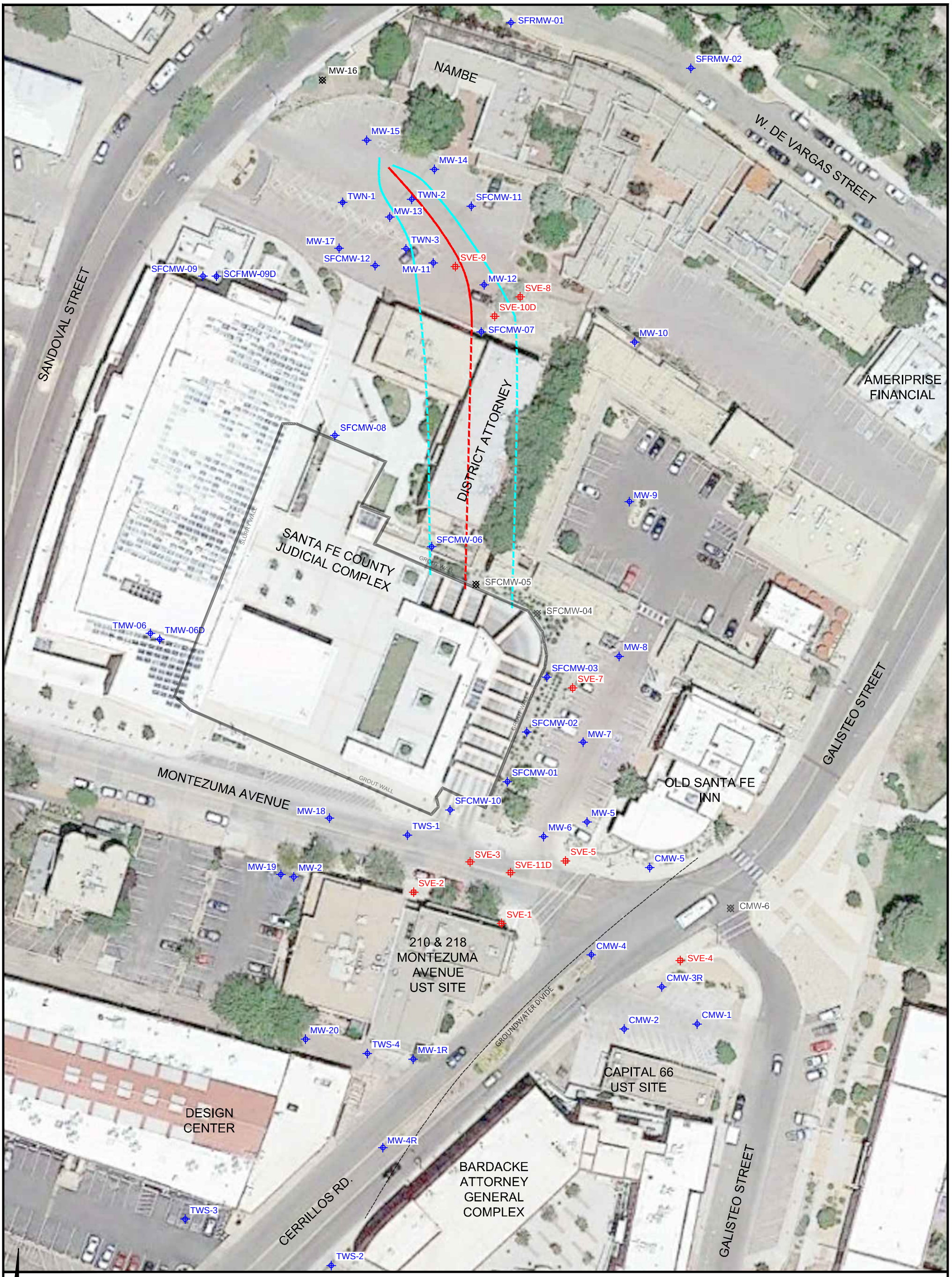

LEGEND:

- MONITORING WELL
- DESTROYED MONITORING WELL
- SOIL VAPOR EXTRACTION WELL
- HORIZONTAL SVE WALL
- HORIZONTAL HOT AIR INJECTION WELL

SOURCE: SOUDER, MILLER, & SSOCIATES. 2018 AUGUST.

60 30 0 60

SCALE IN FEET

SANTA FE COUNTY JUDICIAL COMPLEX
SANTA FE, NEW MEXICO

FIGURE 1
SITE LAYOUT

PROJECT #:	6347002	PROJECT PHASE:	02	PROJECT MANAGER:	MM

320 Gold Avenue, SW Suite 1300
Albuquerque, NM 87102
Phone: (505) 224-9013

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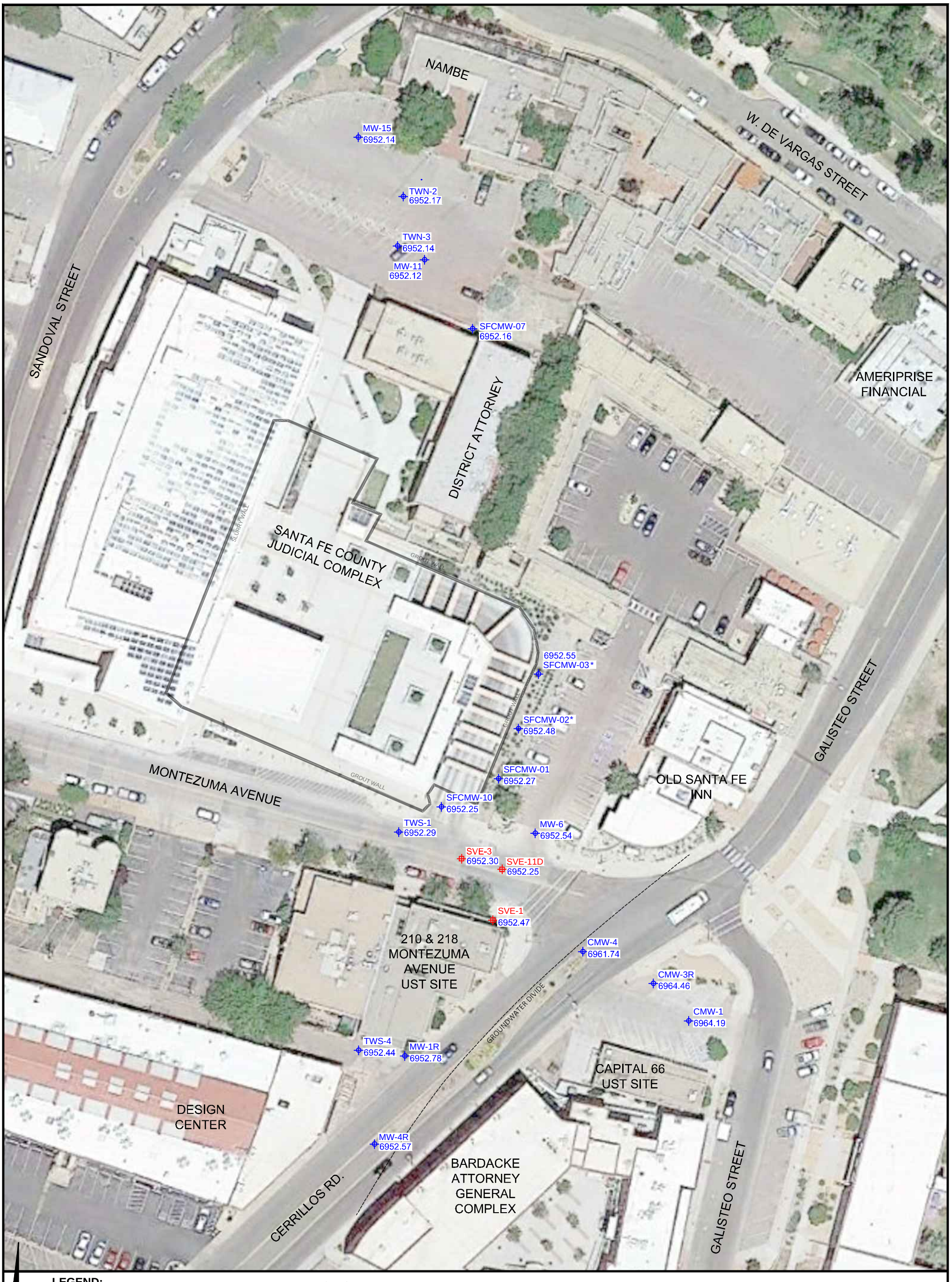

LEGEND:

- MONITORING WELL
- DESTROYED MONITORING WELL
- SOIL VAPOR EXTRACTION WELL
- GROUNDWATER CONTOUR, FEET AMSL

NOTES:

* DATA COLLECTED IN OCTOBER

6952.30 POTENTIOMETRIC SURFACE ELEVATION IN FEET ABOVE MEAN SEA LEVEL (AMSL)

SOURCE: SOUDER, MILLER, & ASSOCIATES. 2018 AUGUST.

60 30 0 60

SCALE IN FEET

SANTA FE COUNTY JUDICIAL COMPLEX
SANTA FE, NEW MEXICO

FIGURE 2
POTENTIOMETRIC ELEVATION MAP
SEPTEMBER 2019

PROJECT #:	6347002	PROJECT PHASE:	02	PROJECT MANAGER:	MM

320 Gold Avenue, SW Suite 1300
Albuquerque, NM 87102
Phone: (505) 224-9013

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MW-15	
BENZENE	<1.0
BTEX	<1.5
MTBE	<1.0
EDB	<0.0094
EDC	**26**
TOTAL NAPH	<4.0

TWN-2	
BENZENE	3.1
BTEX	3.1
MTBE	<1.0
EDB	**0.14**
EDC	**6.3**
TOTAL NAPH	<4.0

TWN-3	
BENZENE	**29**
BTEX	44.1
MTBE	<1.0
EDB	**4.4**
EDC	1.6
TOTAL NAPH	5.0

MW-11	
BENZENE	<2.5
BTEX	193
MTBE	<5.0
EDB	**0.74**
EDC	<5.0
TOTAL NAPH	**411**

SFCMW-07	
BENZENE	<1.0
BTEX	<1.5
MTBE	<1.0
EDB	0.021
EDC	<1.0
TOTAL NAPH	<4.0

SFCMW-03	
BENZENE	<1.0
BTEX	<1.5
MTBE	<1.0
EDB	<1.0
EDC	<1.0
TOTAL NAPH	**188**

SFCMW-10	
BENZENE	**83**
BTEX	145
MTBE	<10
EDB	<10
EDC	<10
TOTAL NAPH	**3,010**

SFCMW-01	
BENZENE	<2.5
BTEX	52
MTBE	<5.0
EDB	<5.0
EDC	<5.0
TOTAL NAPH	**490**

SFCMW-02	
BENZENE	<1.0
BTEX	<1.5
MTBE	<1.0
EDB	<1.0
EDC	<1.0
TOTAL NAPH	**367**

TWS-1	
BENZENE	<1.0
BTEX	<1.5
MTBE	<1.0
EDB	<1.0
EDC	<1.0
TOTAL NAPH	<4.0

MW-6	
BENZENE	<2.0
BTEX	185
MTBE	<2.0
EDB	<2.0
EDC	<2.0
TOTAL NAPH	**71**

SVE-3	
BENZENE	**20**
BTEX	1,801
MTBE	<10
EDB	<10
EDC	<10
TOTAL NAPH	**730**

SVE-1	
BENZENE	<2.0
BTEX	<3.0
MTBE	<2.0
EDB	<2.0
EDC	<2.0
TOTAL NAPH	<8.0

SVE-11D	
BENZENE	<1.0
BTEX	<1.5
MTBE	<1.0
EDB	<1.0
EDC	<1.0
TOTAL NAPH	<4.0

CMW-4	
BENZENE	<1.0
BTEX	700
MTBE	<1.0
EDB	<0.0096
EDC	<1.0
TOTAL NAPH	**82**

TWS-4	
BENZENE	**410**
BTEX	4,970
MTBE	<1.0
EDB	**0.056**
EDC	**6.9**
TOTAL NAPH	**308**

MW-1R	
BENZENE	**1,700**
BTEX	27,200
MTBE	<10
EDB	**0.52**
EDC	<10
TOTAL NAPH	**1,210**

CMW-3R	
BENZENE	<1.0
BTEX	31
MTBE	<1.0
EDB	<1.0
EDC	<1.0
TOTAL NAPH	19.5

CMW-1	
BENZENE	**150**
BTEX	165.2
MTBE	<1.0
EDB	**0.25**
EDC	<1.0
TOTAL NAPH	4.7

MW-4R	
BENZENE	**1,800**
BTEX	25,300
MTBE	<1.0
EDB	**2.2**
EDC	<1.0
TOTAL NAPH	**597**

NAMBE
W. DE VARGAS STREET
SANDOVAL STREET
AMERIPRISE FINANCIAL
DISTRICT ATTORNEY
SANTA FE COUNTY JUDICIAL COMPLEX
SLURRY WALL
GROUT WALL
GALISTEO STREET
OLD SANTA FE INN
MONTEZUMA AVENUE
210 & 218 MONTEZUMA AVENUE UST SITE
GROUNDWATER DIVIDE
CAPITAL 66 UST SITE
DESIGN CENTER
CERRILLOS RD.
BARDACKE ATTORNEY GENERAL COMPLEX

LEGEND:

- MONITORING WELL
- SOIL VAPOR EXTRACTION WELL
- 5 µg/L — BENZENE CONTOUR
- 30 µg/L — TOTAL NAPTHALENES CONTOUR
- 0.05 µg/L — EDB CONTOUR
- 5 µg/L — EDC CONTOUR

NOTES:

ALL CONCENTRATIONS REPORTED IN MICROGRAMS PER LITER (µg/L).

BOLD INDICATES CONCENTRATION ABOVE THE NEW MEXICO WATER QUALITY CONTROL COMMISSION (NMWQCC) STANDARD.

BTEX	BENZENE, TOLUENE, ETHYLBENZENE, AND XYLENES
MTBE	METHYL TERTIARY-BUTYL ETHER
EDB	ETHYLENE DIBROMIDE
EDC	ETHYLENE DICHLORIDE
TOTAL NAPH	TOTAL NAPHTHALENES

60 30 0 60

SCALE IN FEET

SANTA FE COUNTY JUDICIAL COMPLEX
SANTA FE, NEW MEXICO

FIGURE 3
DISTRIBUTION OF DISSOLVED PHASE HYDROCARBONS
SEPTEMBER 2019

PROJECT #:	6347002	PROJECT PHASE:	02	PROJECT MANAGER:	MM

320 Gold Avenue, SW Suite 1300
Albuquerque, NM 87102
Phone: (505) 224-9013

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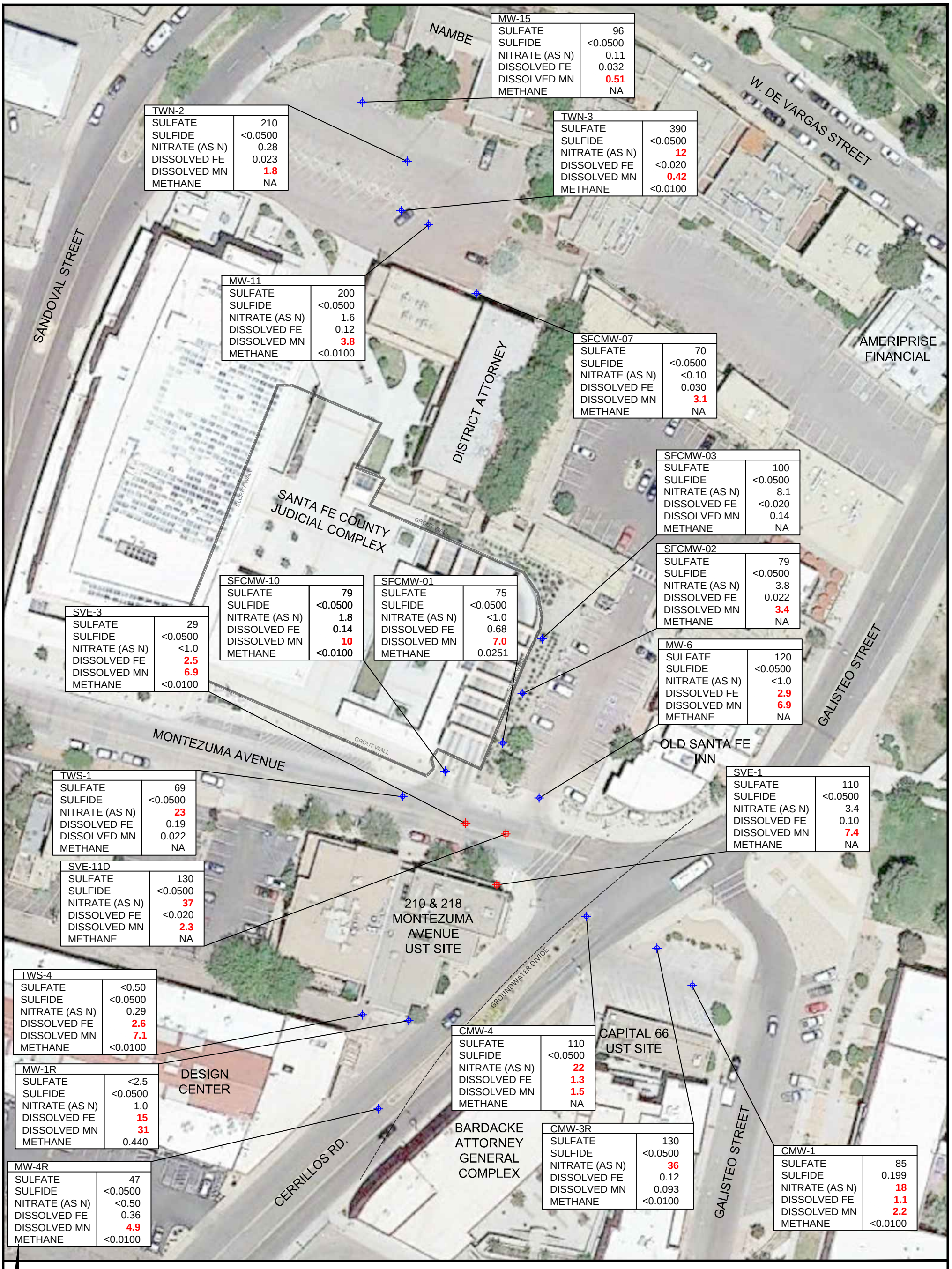

MW-15	
SULFATE	96
SULFIDE	<0.0500
NITRATE (AS N)	0.11
DISSOLVED FE	0.032
DISSOLVED MN	**0.51**
METHANE	NA

TWN-2	
SULFATE	210
SULFIDE	<0.0500
NITRATE (AS N)	0.28
DISSOLVED FE	0.023
DISSOLVED MN	**1.8**
METHANE	NA

TWN-3	
SULFATE	390
SULFIDE	<0.0500
NITRATE (AS N)	**12**
DISSOLVED FE	<0.020
DISSOLVED MN	**0.42**
METHANE	<0.0100

MW-11	
SULFATE	200
SULFIDE	<0.0500
NITRATE (AS N)	1.6
DISSOLVED FE	0.12
DISSOLVED MN	**3.8**
METHANE	<0.0100

SFCMW-07	
SULFATE	70
SULFIDE	<0.0500
NITRATE (AS N)	<0.10
DISSOLVED FE	0.030
DISSOLVED MN	**3.1**
METHANE	NA

SFCMW-03	
SULFATE	100
SULFIDE	<0.0500
NITRATE (AS N)	8.1
DISSOLVED FE	<0.020
DISSOLVED MN	0.14
METHANE	NA

SFCMW-02	
SULFATE	79
SULFIDE	<0.0500
NITRATE (AS N)	3.8
DISSOLVED FE	0.022
DISSOLVED MN	**3.4**
METHANE	NA

SFCMW-10	
SULFATE	79
SULFIDE	<0.0500
NITRATE (AS N)	1.8
DISSOLVED FE	0.14
DISSOLVED MN	**10**
METHANE	<0.0100

SFCMW-01	
SULFATE	75
SULFIDE	<0.0500
NITRATE (AS N)	<1.0
DISSOLVED FE	0.68
DISSOLVED MN	**7.0**
METHANE	0.0251

SVE-3	
SULFATE	29
SULFIDE	<0.0500
NITRATE (AS N)	<1.0
DISSOLVED FE	**2.5**
DISSOLVED MN	**6.9**
METHANE	<0.0100

MW-6	
SULFATE	120
SULFIDE	<0.0500
NITRATE (AS N)	<1.0
DISSOLVED FE	**2.9**
DISSOLVED MN	**6.9**
METHANE	NA

TWS-1	
SULFATE	69
SULFIDE	<0.0500
NITRATE (AS N)	**23**
DISSOLVED FE	0.19
DISSOLVED MN	0.022
METHANE	NA

SVE-1	
SULFATE	110
SULFIDE	<0.0500
NITRATE (AS N)	3.4
DISSOLVED FE	0.10
DISSOLVED MN	**7.4**
METHANE	NA

SVE-11D	
SULFATE	130
SULFIDE	<0.0500
NITRATE (AS N)	**37**
DISSOLVED FE	<0.020
DISSOLVED MN	**2.3**
METHANE	NA

TWS-4	
SULFATE	<0.50
SULFIDE	<0.0500
NITRATE (AS N)	0.29
DISSOLVED FE	**2.6**
DISSOLVED MN	**7.1**
METHANE	<0.0100

CMW-4	
SULFATE	110
SULFIDE	<0.0500
NITRATE (AS N)	**22**
DISSOLVED FE	**1.3**
DISSOLVED MN	**1.5**
METHANE	NA

MW-1R	
SULFATE	<2.5
SULFIDE	<0.0500
NITRATE (AS N)	1.0
DISSOLVED FE	**15**
DISSOLVED MN	**31**
METHANE	0.440

CMW-3R	
SULFATE	130
SULFIDE	<0.0500
NITRATE (AS N)	**36**
DISSOLVED FE	0.12
DISSOLVED MN	0.093
METHANE	<0.0100

MW-4R	
SULFATE	47
SULFIDE	<0.0500
NITRATE (AS N)	<0.50
DISSOLVED FE	0.36
DISSOLVED MN	**4.9**
METHANE	<0.0100

CMW-1	
SULFATE	85
SULFIDE	0.199
NITRATE (AS N)	**18**
DISSOLVED FE	**1.1**
DISSOLVED MN	**2.2**
METHANE	<0.0100

LEGEND:

MONITORING WELL

SOIL VAPOR EXTRACTION WELL

NOTES:

ALL CONCENTRATIONS REPORTED IN MICROGRAMS PER LITER (µg/L).

BOLD INDICATES CONCENTRATION ABOVE THE NEW MEXICO WATER QUALITY CONTROL COMMISSION (NMWQCC) STANDARD.

NITRATE (AS N)	NITRATE (AS NITROGEN)
DISSOLVED FE	DISSOLVED IRON
DISSOLVED MN	DISSOLVED MANGANESE
NA	NOT ANALYZED

60 30 0 60

SCALE IN FEET

SANTA FE COUNTY JUDICIAL COMPLEX
SANTA FE, NEW MEXICO

FIGURE 4
DISTRIBUTION OF DISSOLVED METALS, ANIONS, AND METHANE
SEPTEMBER 2019

PROJECT #:	6347002	PROJECT PHASE:	02	PROJECT MANAGER:	MM

320 Gold Avenue, SW Suite 1300
Albuquerque, NM 87102
Phone: (505) 224-9013

EA ENGINEERING, SCIENCE, AND TECHNOLOGY, INC. PBC

TABLE

TABLE 1. BIO-TRAP SAMPLING REGIMEN
***IN-SITU* MICROCOSM STUDY**
SANTA FE COUNTY JUDICIAL COMPLEX, SANTA FE, NEW MEXICO

Plume	Monitoring Well	Standard Bio-Trap®	MNA Bio-Trap®	Bio Stimulation Bio-Trap®	Bio Augmentation Bio-Trap®	Bio-Trap® Stimulant(s)	Culture
De Vargas	MW-11	1		--	--	--	--
	TWN-03	--	1	--	1	Sulfate	SDC-9
				1	--	Nitrate, Sulfate	--
Santa Fe County Judicial Complex	SFCMW-10		1	1	--	Nitrate, Sulfate	--
Capital 66	CMW-01		1	1	--	Nitrate, Sulfate	--
Design Center	MW-1R		1	1	--	Nitrate	--
				1	--	Nitrate, Sulfate	--
	MW-4R	1		--	--	--	--
Total		**2**	**4**	**5**	**1**		

APPENDIX A
MICROBIAL INSIGHTS BIO TRAP® *IN SITU* MICROCOSM STUDY

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SITE LOGIC Report

Bio-Trap In Situ Microcosm Study

Contact: Michael McVey
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Phone: 505-224-9013

Email: mmcvey@eaest.com

MI Identifier: 039QK

Report Date: December 5, 2019

Project: NMED Santa Fe County ID 4072, 6347002.03

Comments:

Executive Summary

Bio-Trap® *In Situ* Microcosm (ISM) units were deployed in wells TWN-03, SFCMW-10, CMW-01, and MW-1R in order to evaluate the potential for enhanced biodegradation of BTEX hydrocarbons, naphthalene, 1,2-dibromoethane, and 1,2-dichloroethane under current and amended site conditions. The ISM assembly deployed in well TWN-03 consisted of three Bio-Trap units: (i) a control MNA unit containing no exogenous amendment, (ii) a BioStim unit amended with electron acceptors nitrate and sulfate, and (iii) a BioAug unit amended with sulfate and the SDC-9 bioaugmentation culture. Wells SFCMW-10 and CMW-01 each contained a control MNA unit and a BioStim unit amended with nitrate and sulfate. MW-1R contained three ISM Bio-Traps: (i) a control MNA unit, (ii) a BioStim unit amended with nitrate and sulfate, and (iii) a BioStim unit amended with nitrate. Unamended standard Bio-Traps were deployed in wells MW-11 and MW-4R. Following an in-well deployment period of 48 days, Bio-Trap units were recovered for QuantArray®-Petro analysis of targeted functional genes involved in aerobic and anaerobic biodegradation of BTEX compounds, polycyclic aromatic hydrocarbons (PAHs), and *n*-alkanes (C_5 – C_{16} in length) and CENSUS analysis to quantify *Dehalobacter* and 1,2-DCA reductase. Results for contaminant concentrations, dissolved gases, and anions are presented in Tables 1 and 2. QuantArray®-Petro and CENSUS results are presented in Tables 3 - 15 and Figures 1 – 21. Background information on the spectrum of petroleum biodegradative processes indicated by QuantArray®-Petro analysis is provided at the end of this report as a guide to interpretation of the microbial and functional gene data. Key observations derived from this study are described below.

TWN-03 MNA, BioStim, and BioAug Units

- Overall, QuantArray®-Petro results indicated that electron acceptor amendment with nitrate and sulfate did not enhance the genetic potential for the aerobic and anaerobic biodegradation of BTEX, PAHs, and alkanes over that observed under MNA conditions during the 48-day in-well deployment period.
- Notable increases in concentration were observed for genes encoding toluene/benzene dioxygenase (TOD) and toluene ring hydroxylating monooxygenases (RMO) under bioaugmentation conditions, suggesting an increased genetic potential for the aerobic biodegradation of BTEX.
- QuantArray® analysis of total eubacteria (EBAC) showed high levels of biomass, on the order of 10^8 cells/bead, present in the MNA and BioStim units deployed in well TWN-03. EBAC concentrations increased by an order of magnitude to 10^9 cells/bead under bioaugmentation with SDC-9, a *Dehalococcoides*-containing culture known to degrade 1,2-dibromoethane and 1,2-dichloroethane among other halogenated compounds. Sulfate-reducing bacteria (APS) were detected at higher concentrations in the BioStim (10^4 cells/bead) and BioAug (10^7 cells/bead) units relative to the MNA unit.
- Contaminant analysis indicated that BTEX hydrocarbons, naphthalene, 1,2-dibromoethane, and 1,2-dichloroethane were below the detection limit in all three Bio-Trap units deployed in well TWN-03.
- Methane concentrations were less than 5 µg/L in each of the units. Nitrate and sulfate concentrations were slightly elevated in the BioStim and BioAug units compared to the MNA unit.

Aerobic Biodegradation of BTEX

- QuantArray® analysis indicated that microorganisms with the genetic potential for aerobic biodegradation of BTEX were present in the MNA, BioStim, and BioAug units deployed in well TWN-03. In general, this aerobic biodegradative potential ranged from low to high and was based on the detection of a number of genes encoding key enzymes involved in the aerobic catalysis of BTEX degradation. While some genetic enhancement for aerobic

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BTEX biodegradation was observed under bioaugmentation conditions, this potential was not increased under biostimulation compared to MNA conditions.

- The degradative functional gene phenol hydroxylase (PHE) was detected at substantial concentrations of 10^6 (MNA unit) or 10^5 cells/bead (BioStim and BioAug units). The PHE enzyme catalyzes a critical early oxidation step in the aerobic biodegradation of benzene, toluene, and xylene compounds.
- RMO levels increased two orders of magnitude from 10^4 to 10^6 cells/bead under bioaugmentation conditions relative to the control unit.
- In addition, the TOD concentration increased by one order of magnitude in the BioAug unit (10^3 cells/bead) compared to the MNA unit (10^2 cells/bead). TOD tends to be present at lower concentrations than PHE and is not as good an indicator of aerobic BTEX biodegradation potential as PHE.
- No enhancement of the genetic potential for aerobic BTEX biodegradation was observed under biostimulation with electron acceptors nitrate and sulfate.

Anaerobic Biodegradation of BTEX

- A genetic potential for anaerobic BTEX biodegradation was observed in all three ISM units with the detection of genes encoding benzoyl coenzyme A reductase (BCR) and benzylsuccinate synthase (BSS). However, this potential was not enhanced under either biostimulation or bioaugmentation conditions. BCR is involved in the anaerobic metabolism of many aromatic hydrocarbons, while BSS is responsible for initiating the anaerobic biodegradation of toluene and other alkyl-substituted benzenes. The benzene carboxylase (ABC) gene, which is involved in anaerobic benzene biodegradation, was not detected in any of the ISM units deployed in well TWN-03.

Biodegradation of PAHs and Alkanes

- The key naphthalene dioxygenase (NAH) gene in aerobic PAH biodegradation was detected only in the MNA unit at a concentration of 10^3 cells/bead, while the gene encoding naphthalene-inducible dioxygenase (NidA) was detected in both the MNA and BioStim units.
- Contaminant analysis indicated that naphthalene concentrations were below the detection limit at well TWN-03.
- QuantArray®-Petro results indicated a low genetic potential for the aerobic biodegradation of naphthalene under bioaugmentation conditions.
- Alkane monooxygenase gene ALK was measured at a concentration of 10^5 cells/bead in the MNA unit and 10^4 cells/bead in the BioStim and BioAug units.
- In general, the genetic potential for the anaerobic biodegradation of PAHs and alkanes was limited in all three ISM units deployed in well TWN-03.

Anaerobic Dehalogenation

- CENSUS analysis for *Dehalococcoides* demonstrated the *in situ* survival of the SDC-9 culture. A high concentration of *Dehalococcoides* (10^7 cells/bead) was detected in the BioAug unit, which was four to five orders of magnitude greater than the *Dehalococcoides* concentrations present in the MNA and BioStim units. *Dehalococcoides* has been shown to degrade 1,2-dibromoethane and is also capable of the dichloroelimination of 1,2-dichloroethane.
- *Dehalobacter* spp. was below the detection limit in all three units deployed in TWN-03. *Dehalobacter* spp. has been shown to perform the dichloroelimination of 1,1,2-trichloroethane and 1,2-dichloroethane to vinyl chloride and ethene, respectively.

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- The 1,2-dichloroethane reductase gene was detected on the order of 10^3 cells/bead in the MNA unit but was below the detection limit in the BioStim and BioAug units. This assay targets the 1,2-dichloroethane reductase gene in members of *Desulfitobacterium* and *Dehalobacter* genera, which dechlorinate 1,2-dichloroethane to ethene.

SFCMW-10 MNA and BioStim Units

- In general, QuantArray®-Petro analysis indicated that certain genes involved in the anaerobic biodegradation of BTEX and naphthalene were detected at higher concentrations under biostimulation with nitrate and sulfate compared to MNA conditions.
- The MNA and BioStim units deployed in well SFCMW-10 both contained relatively high levels of biomass (EBAC), on the order of 10^7 cells/bead, indicating that well site parameters were conducive to microbial growth under conditions of monitored natural attenuation and biostimulation. With respect to specific microbial groups, concentrations of sulfate-reducing bacteria (APS) were similar in both ISM Bio-Trap units.
- Contaminant analysis indicated that naphthalene was the primary contaminant measured in both ISM Bio-Traps deployed in SFCMW-10. Low concentrations of benzene, toluene, and xylenes were also detected in both units. No reductions in contaminant concentrations were observed under biostimulation conditions relative to MNA conditions during the 48-day deployment period.
- Methane concentrations were slightly higher the Bio-Traps deployed in SFCMW-10 (15-22 µg/L) compared to the concentrations in samples from TWN-03. Low levels (<2 µg/L) of ethane and ethene were detected in the MNA and BioStim units.

Aerobic Biodegradation of BTEX

- The following genes involved in aerobic BTEX biodegradation were detected in both the MNA and BioStim units: TOD (below the practical quantitation limit), PHE (10^5 cells/bead), RDEG (10^4 cells/bead), and EDO (10^3 cells/bead). However, the concentrations of these genes were not increased under biostimulation conditions.
- The gene encoding biphenyl/isopropylbenzene dioxygenase (BPH4) was detected at a moderate concentration of 10^3 cells/bead in the BioStim unit, but was below the detection limit in the MNA unit.

Anaerobic Biodegradation of BTEX

- QuantArray®-Petro analysis demonstrated that the BCR gene was detected in both ISM units, but the concentrations were an order of magnitude lower in the BioStim unit (10^4 cells/bead) relative to the MNA unit (10^5 cells/bead). However, the BSS gene was detected at a concentration of 10^4 cells/bead under biostimulation conditions, while this gene was not detected under MNA conditions. Overall, these results indicate a higher genetic potential for anaerobic BTEX biodegradation under nitrate and sulfate amendment.

Biodegradation of PAHs and Alkanes

- The genetic potential for aerobic naphthalene biodegradation was low in both the MNA and BioStim units deployed in well SFCMW-10.
- Levels of the ALK gene increased from 10^4 cells/bead in the MNA unit to 10^5 cells/bead in the BioStim unit, suggesting an enhanced potential for aerobic alkane biodegradation under amendment with nitrate and sulfate.
- The genetic potential for the anaerobic biodegradation of naphthalene was higher under biostimulation conditions compared to MNA conditions. The gene encoding naphthalene carboxylase (ANC) was detected at a concentration

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of 10^3 cells/bead in the BioStim unit, while this gene was below the detection limit in the MNA unit. Naphthalene carboxylase initiates the only known pathway for anaerobic naphthalene biodegradation.

Anaerobic Dehalogenation

- CENSUS analysis indicated that *Dehalobacter* spp. was below the detection limit in both units deployed in SFCMW-10.
- Moderate concentrations of the 1,2-dichloroethane reductase gene (10^4 – 10^5 cells/bead) were detected both the MNA and BioStim units, indicating that the genetic potential to degraded 1,2-dichloroethane to ethene was present in this location.

CMW-01 MNA and BioStim Units

- Overall, QuantArray®-Petro analysis indicated that electron acceptor amendment with nitrate and sulfate did not enhance the genetic potential for the aerobic and anaerobic biodegradation of BTEX and PAHs.
- The MNA and BioStim units deployed in well CMW-01 both contained relatively high levels of biomass (EBAC), on the order of 10^8 cells/bead, suggesting no enhancement of bacterial growth under biostimulation conditions. With respect to specific microbial groups, concentrations of sulfate-reducing bacteria (APS) were similar in both ISM Bio-Trap units and were measured at 10^6 cells/bead.
- Contaminant analysis indicated that benzene was the primary contaminant measured in both ISM Bio-Traps deployed in CMW-01. No reduction was observed in the benzene concentration under biostimulation conditions relative to MNA conditions during the 48-day deployment period.
- Methane and nitrate concentrations were elevated in the BioStim unit compared to the MNA unit while the sulfate concentration was the same (100 mg/L) in both units.

Aerobic Biodegradation of BTEX

- The degradative functional gene PHE, biomarker for aerobic BTEX biodegradation, was detected at a high concentration of 10^6 cells/bead in both the MNA and BioStim units. Other genes indicating a genetic capacity for aerobic BTEX biodegradation—namely, RDEG and RMO—were also detected in both ISM Bio-Traps. These results demonstrated a genetic potential for aerobic BTEX biodegradation under both MNA and biostimulation conditions, but no enhancement of this potential was observed under nitrate and sulfate amendment.

Anaerobic Biodegradation of BTEX

- The genetic potential for anaerobic TEX biodegradation was moderate in the MNA unit based on the detection of genes BCR (10^5 cells/bead) and BSS (10^4 cells/bead). The BCR gene was detected in the BioStim unit at a concentration that was an order of magnitude lower than the BCR concentration detected in the MNA unit, indicating that electron acceptor amendment did not enhance the genetic potential for anaerobic TEX biodegradation. ABC gene concentrations were below the detection limit in both ISM units.

Biodegradation of PAHs and Alkanes

- The gene for naphthalene dioxygenase (NAH) was detected in both the MNA unit (10^4 cells/bead) and BioStim unit (10^3 cells/bead), indicating a potential for aerobic naphthalene biodegradation. However, this potential was not enhanced under biostimulation conditions.

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- Electron acceptor amendment increased the genetic potential for the anaerobic biodegradation of alkanes over that observed under MNA conditions, as demonstrated by the detection of the alkylsuccinate synthase (ASSA) gene (10^3 cells/bead) in the BioStim unit only.

Anaerobic Dehalogenation

- CENSUS analysis indicated that *Dehalobacter* spp. and the 1,2-dichloroethane reductase gene were below the detection limit in both units deployed in CMW-01.

MW-1R MNA and BioStim Units

- Overall, QuantArray®-Petro analysis indicated shifts in the microbial community following electron acceptor amendment (nitrate+sulfate and nitrate alone) as several aerobic genes decreased while some anaerobic genes increased compared to MNA conditions.
- Total eubacterial biomass (EBAC) concentrations were an order of magnitude lower in the BioStim units compared to the MNA unit. With respect to specific microbial groups, concentrations of sulfate-reducing bacteria (APS) were similar in all three ISM Bio-Trap units, *i.e.*, 10^5 cells/bead.
- No contaminants of concern were detected in the MNA unit while BTEX and naphthalene were present in the two BioStim units. This unexpected result may be due to vertical heterogeneity in the distribution of contaminants in the vicinity of MW-1R.
- Methane concentrations in the two BioStim units were one to two orders of magnitude higher than the concentrations in the MNA unit as well as the ISM units deployed in TWN-03, SFCMW-10, and CMW-01, suggesting that conditions were more reducing in these two units. Low concentrations of ethene (<4 µg/L) were also detected. Ethene was below the detection limit in all three units deployed in MW-1R.

Aerobic Biodegradation of BTEX

- QuantArray®-Petro results demonstrated that a genetic potential for aerobic BTEX biodegradation existed under both MNA conditions and biostimulation conditions based on the detection of PHE, RDEG, RMO, and EDO. However, amendment with nitrate+sulfate or nitrate alone did not enhance this potential during the deployment period. For example, PHE gene concentrations were two orders of magnitude lower under biostimulation conditions compared to MNA conditions. The concentration of another key indicator for aerobic BTEX biodegradation, RDEG, was lower by an order of magnitude in both BioStim units.

Anaerobic Biodegradation of BTEX

- BCR and BSS genes were detected in all three ISM Bio-Traps deployed in well MW-1R. However, the concentrations of BCR and BSS were an order of magnitude lower in the BioStim unit amended with nitrate+sulfate, while the BCR concentration was an order of magnitude lower in the BioStim unit amended with nitrate alone.
- The gene encoding benzene carboxylase (ABC), however, was detected at a concentration of 10^3 cells/bead in the nitrate+sulfate unit and 10^4 cells/bead in the nitrate unit, while the ABC concentration in the MNA unit was below the detection limit.

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Biodegradation of PAHs and Alkanes

- In general, the genetic potential for the aerobic and anaerobic biodegradation of naphthalene was limited at well MW-1R under current MNA conditions. QuantArray®-Petro target genes involved in naphthalene biodegradation processes were either not detected or detected below the practical quantitation limit.
- Neither amendment with nitrate+sulfate nor amendment with nitrate alone enhanced the genetic potential for aerobic or anaerobic naphthalene biodegradation.
- The ALK gene involved in aerobic alkane biodegradation was detected at 10^4 cells/bead, but the ALK concentration was two orders of magnitude lower under biostimulation conditions.

Anaerobic Dehalogenation

- CENSUS analysis indicated that *Dehalobacter* spp. was below the detection limit in all three units deployed in MW-1R.
- Concentrations for the gene encoding 1,2-DCA reductase, which catalyzes the reductive dechlorination of the chlorinated ethane 1,2-DCA, increased under biostimulation with nitrate+sulfate (10^5 cells/bead) and with nitrate alone (10^5 cells/bead) by an order of magnitude compared to MNA conditions (10^4 cells/bead).

MW-11 Standard Bio-Trap

- In general, the genetic potential for the aerobic and anaerobic biodegradation of BTEX hydrocarbons was moderate at this well site, whereas the genetic potential for aerobic and anaerobic naphthalene biodegradation was limited.
- QuantArray®-Petro analysis of total eubacteria (EBAC) showed high levels of biomass on the order of 10^7 cells/bead present in the bio-trap collected from monitoring well MW-11. Populations of sulfate-reducing bacteria were detected at 10^6 cells/bead.

Aerobic Biodegradation of BTEX

- The genetic potential for the aerobic biodegradation of BTEX was moderate based on the concentrations of target QuantArray® genes.
  - Key functional genes PHE and RDEG were detected at concentrations of 10^5 cells/bead and 10^4 cells/bead, respectively.
  - EDO was detected at 10^4 cells/bead, while the concentration of xylene/toluene monooxygenase (TOL) was below the practical quantitation limit.

Anaerobic Biodegradation of BTEX

- All three key functional genes involved in anaerobic BTEX biodegradation were detected at notable concentrations in the MW-11 sample: BCR (10^4 cells/bead), BSS (10^5 cells/bead), and ABC (10^3 cells/bead).

Biodegradation of PAHs and Alkanes

- The phenanthrene dioxygenase (PHN) gene was detected at a concentration of 10^3 cells/bead, while NAH and NidA were not detected. These findings indicated a low-to-moderate genetic potential for the aerobic biodegradation of PAHs.
- The ALK gene was measured at a low concentration of 10^2 cells/bead.

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- None of the QuantArray® functional genes for anaerobic PAH and alkane biodegradation were detected in the MW-11 sample.

Anaerobic Dehalogenation

- CENSUS analysis indicated that *Dehalobacter* spp. and the 1,2-dichloroethane reductase gene were below the detection limit in the standard bio-trap deployed in MW-11.

MW-4R Standard Bio-Trap

- In general, the genetic potential for the aerobic and anaerobic biodegradation of BTEX hydrocarbons was moderate at this well site, whereas the genetic potential for aerobic and anaerobic naphthalene biodegradation was low.
- QuantArray®-Petro analysis of total eubacteria (EBAC) showed high levels of biomass on the order of 10^7 cells/bead present in the bio-trap sample collected from monitoring well MW-4R. However, sulfate-reducing bacteria were not detected.

Aerobic Biodegradation of BTEX

- The genetic potential for the aerobic biodegradation of BTEX was moderate based on the concentrations of target QuantArray® genes.
  - Key functional genes PHE (10^5 cells/bead), RDEG (10^5 cells/bead), and RMO (10^4 cells/bead) were detected at noteworthy concentrations.
  - The EDO concentration was 10^4 cells/bead, while TOD and TOL concentrations were below the detection limit.

Anaerobic Biodegradation of BTEX

- Only the BCR (10^3 cells/bead) and BSS (10^3 cells/bead) genes were detected in the MW-4R sample, indicating a low-to-moderate genetic potential for anaerobic TEX biodegradation at this well site.

Biodegradation of PAHs and Alkanes

- The genetic potential for aerobic and anaerobic naphthalene biodegradation was low at MW-4R. Functional genes involved in PAH biodegradation were not detected in this sample.
- The ALK gene was detected at a low concentration of 10^2 cells/bead.

Anaerobic Dehalogenation

- CENSUS analysis indicated that *Dehalobacter* spp. and the 1,2-dichloroethane reductase gene were below the detection limit in the standard bio-trap deployed in MW-4R.

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Overview of Approach

Site managers have frequently turned to laboratory microcosms or small pilot studies to evaluate bioremediation. However, duplication of *in situ* conditions in the laboratory is difficult and the results often do not correlate to the field. Pilot studies are performed in the field but are often prohibitively expensive as an investigative tool. Bio-Trap studies serve as cost-effective, *in situ* microcosms providing microbial, chemical, and geochemical evidence to evaluate biodegradation as a treatment mechanism and to screen remedial alternatives.

Typically each Bio-Trap Unit will contain samplers to evaluate the following:

Geochemical Fingerprint (GEO)	•40 mL VOA vial with a nylon screened cap designed for assessment of a variety of geochemical parameters including anions and metabolic acids.
Contaminant of Concern (COC)	•Passive diffusion bag designed for analysis of a variety of COCs including chlorinated solvents and petroleum hydrocarbons.
Microbial Populations (MICRO)	•PVC cassette containing Bio-Sep® beads, which provide a large surface area for microbial attachment and were designed for analysis by a variety of molecular biological tools (MBTs).

How does it work?

The MICRO sampler (microbial populations) contains Bio-Sep® beads, an engineered composite of Nomex® and powdered activated carbon which provides an incredibly large surface area (~600 m^2/g) that is readily colonized by subsurface microorganisms. In addition to a matrix for microbial growth, the Bio-Sep® beads can be "baited" with amendments including electron donors (e.g. hydrogen releasing compounds) to investigate biostimulation approaches to enhance biodegradation. The Bio-Trap units also contain a COC (contaminant of concern) sampler to measure contaminant concentrations, daughter product formation, and dissolved gases and a GEO (geochemical fingerprint) sampler for quantification of geochemical parameters (nitrate, iron, sulfate, etc.), chloride production, and metabolic acids (pyruvic, lactic, acetic, propionic, etc.).

Bio-Trap® *In Situ* Microcosm studies at chlorinated solvent sites typically include three types of Bio-Trap Units deployed within a monitoring well. Each Bio-Trap Unit corresponds to one of the three most common remedial options: monitored natural attenuation (MNA), Biostimulation (BioStim), and Bioaugmentation (BioAug). All three Bio-Trap Units contain COC and GEO samplers for chemical and geochemical analyses. The key difference between the Bio-Trap Units is in the MICRO sampler.

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Types of Bio-Trap Units typically deployed and MICRO sampler configurations:

Control (MNA)	•Bio-Sep® beads contain no additional amendment and represent current aquifer conditions.
Biostimulation (BioStim)	•An amendment supplier is used to release the desired specified electron donor (sodium lactate, molasses, EVO, etc.) or electron acceptor (oxygen release compound, sulfate, etc.).
Bioaugmentation (BioAug)	•Bio-Sep® beads are pre-inoculated with a bioaugmentation culture, such as *Dehalococcoides*. These units can also be baited with an additional amendment.

MNA Unit: The purpose of the Control Bio-Trap Unit is to quantify contaminant degrading bacteria and daughter product formation under monitored natural attenuation (MNA) conditions and to serve as a baseline for comparison to BioStim and/or BioAug Units.

Following in-well deployment, DNA or phospholipid fatty acids (PLFA) can be extracted from the Bio-Sep beads for further analysis. For example, DNA extracted from the Bio-Sep beads can be used in CENSUS analysis of *Dehalococcoides* (DHC) and vinyl chloride reductase (*bvcA* and *vcrA*) genes to evaluate the potential for complete reductive dechlorination of PCE to ethene under MNA conditions. The VOC and anion samplers can be used to determine concentrations of contaminants, daughter products, dissolved gases, terminal electron acceptors, and chloride.

BioStim Unit: The Biostimulation Bio-Trap Unit is designed to test the hypothesis that electron donor addition will stimulate growth of dechlorinating bacteria and enhance biodegradation. As with the MNA Unit, the BioStim Unit contains COC and GEO samplers for chemical analyses. The BioStim Unit contains an amendment supplier to release the desired amendment over the incubation time.

BioAug Unit: The Bioaugmentation Bio-Trap Unit is designed to evaluate bioaugmentation as a treatment technology. The MICRO sampler contains Bio-Sep® beads pre-inoculated with the desired commercial culture. An amendment supplier may also be used to deliver an amendment. As with the MNA and BioStim Units, the BioAug Unit also contains a COC and GEO samplers for chemical analyses.

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The QuantArray® Approach

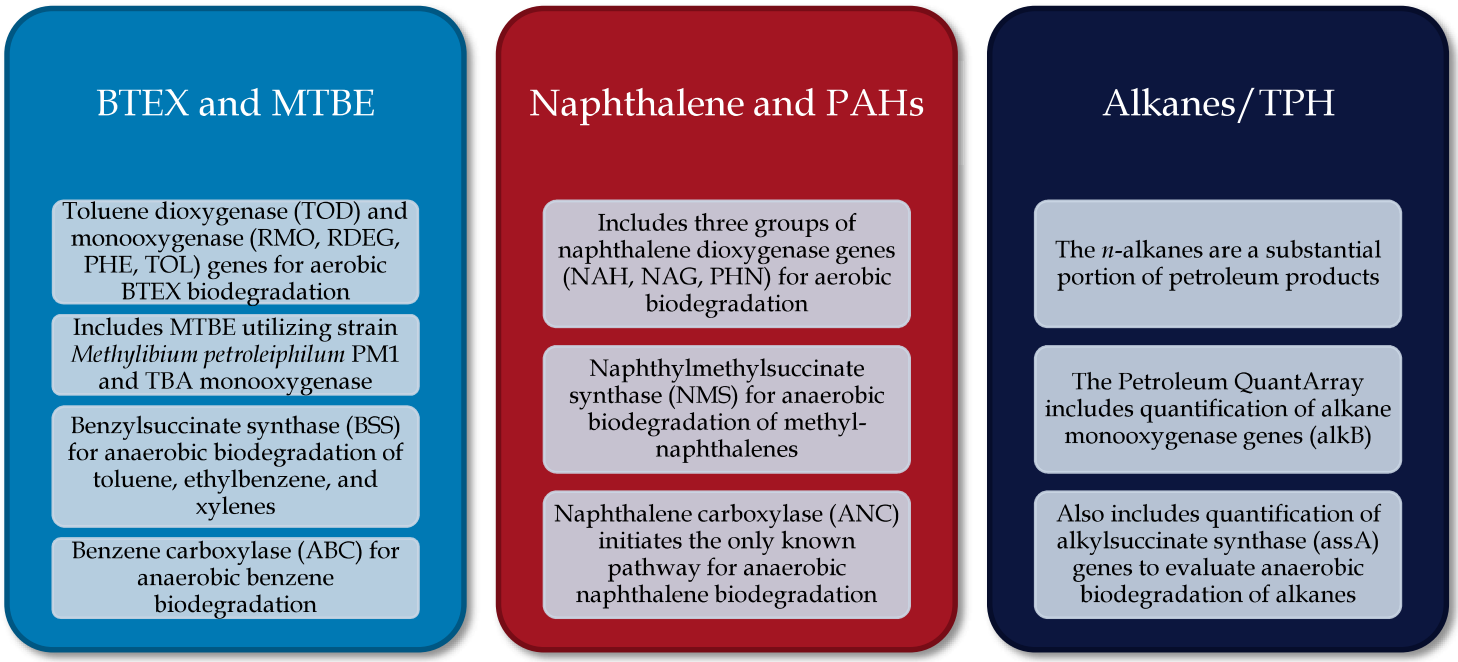
Comprehensive evaluation of biodegradation potential at petroleum impacted sites is inherently problematic due to two factors:

(1) Petroleum products are complex mixtures of hundreds of aliphatic, aromatic, cyclic and heterocyclic compounds
(2) Even for common classes of contaminants like benzene, toluene, ethylbenzene, and xylenes (BTEX), biodegradation can proceed by a multitude of pathways.

The Petroleum QuantArray has been designed to address both of these issues by providing the simultaneous quantification of the specific functional genes responsible for both aerobic and anaerobic biodegradation of BTEX, PAHs, and a variety of short and long chain alkanes.

Thus, when combined with chemical and geochemical groundwater monitoring programs, the QuantArray allows site managers to simultaneously yet economically evaluate the potential for biodegradation of a spectrum of petroleum hydrocarbons through a multitude of aerobic and anaerobic pathways to give a much more clear and comprehensive view of contaminant biodegradation.

The Petroleum QuantArray is used to quantify specific microorganisms and functional genes to evaluate aerobic and anaerobic biodegradation of the following classes of compounds present in petroleum products:



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How do QuantArrays® work?

The QuantArray in many respects is a hybrid technology combining the highly parallel detection of microarrays with the accurate and precise quantification provided by qPCR into a single platform. The key to highly parallel qPCR reactions is the nanoliter fluidics platform for low volume, solution phase qPCR reactions.

How are QuantArray® results reported?

One of the primary advantages of the Petroleum QuantArray is the simultaneous quantification of a broad spectrum of different microorganisms and key functional genes involved in a variety of pathways for chlorinated hydrocarbon biodegradation. However, highly parallel quantification combined with the various metabolic and cometabolic capabilities of different target organisms can complicate data presentation. Therefore, in addition to Summary Tables, QuantArray results will be presented as Microbial Population Summary and Comparison Figures to aid in data interpretation and subsequent evaluation of site management activities.

Types of Tables and Figures:

Microbial Population Summary	•Figure presenting the concentrations of QuantArray target gene concentrations (e.g. toluene dioxygenase) relative to typically observed values.
Summary Tables	• Tables of target population concentrations grouped by biodegradation pathway and contaminant type.
Comparison Figures	•Depending on the project, sample results can be presented to compare changes over time or examine differences in microbial populations for along a transect of the dissolved plume.

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Results

Table 1. Summary of the results obtained for *In Situ* Microcosm Units.

Sample Information	TWN-03 MNA	TWN-03 Bio-Stim Nitrate, Sulfate	TWN-03 Bio-Aug SDC-9, Sulfate	SFCMW-10 MNA	SFCMW-10 Bio-Stim Nitrate, Sulfate
Treatment	MNA	BioStim	BioStim	MNA	BioStim
Sample Date	11/12/2019	11/12/2019	11/12/2019	11/12/2019	11/12/2019
MI ID	039QK1	039QK2	039QK3	039QK4	039QK5
Contaminant of Concern (µg/L)					
Benzene	<1.0	<1.0	<1.0	**8.7**	**15.9**
Toluene	<1.0	<1.0	<1.0	**1.2**	**1.5**
Ethylbenzene	<1.0	<1.0	<1.0	<1.0	<1.0
Xylene (total)	<3	<3	<3	**6**	**14.7**
Naphthalene	<2.0	<2.0	<2.0	**69.9**	**159**
1,2-Dibromoethane	<1.0	<1.0	<1.0	<1.0	<1.0
1,2-Dichloroethane	<1.0	<1.0	<1.0	<1.0	<1.0
Dissolved Gases (µg/L)					
Methane	**4.4**	**2.8**	**2.7**	**15**	**22**
Ethane	<1.0	<1.0	<1.0	**1.0**	**1.3**
Ethene	<1.0	<1.0	<1.0	**1.7**	**1.8**
Anions (mg/L)					
Chloride	**210**	**270**	**270**	**260**	**260**
Nitrate	**12**	**51**	**32**	<0.5	<0.5
Nitrite	<0.5	<0.5	**1.6**	<0.5	<0.5
Sulfate	**200**	**330**	**340**	**94**	**88**
OrthoPhosphate	<1.5	<1.5	<1.5	<1.5	<1.5

Legend: NA = Not analyzed NS = Not sampled J = Estimated result below PQL but above LQL I = Inhibited <= Result not detected.

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Table 2. Summary of the results obtained for *In Situ* Microcosm Units.

Sample Information	CMW-01 MNA	CMW-01 Bio-Stim Nitrate, Sulfate	MW-1R MNA	MW-1R Bio-Stim Nitrate, Sulfate	MW-1R Bio-Stim Nitrate
Treatment	MNA	BioStim	MNA	BioStim	BioStim
Sample Date	11/12/2019	11/12/2019	11/12/2019	11/12/2019	11/12/2019
MI ID	039QK6	039QK7	039QK8	039QK9	039QK10
Contaminant of Concern (µg/L)					
Benzene	**155**	**171**	<1.0	**15.5**	**48.4**
Toluene	<1.0	<1.0	<1.0	**6.0**	**36.4**
Ethylbenzene	<1.0	<1.0	<1.0	**10.1**	**270**
Xylene (total)	<3	<3	<3	**150**	**640**
Naphthalene	<2.0	<2.0	<2.0	**20.8**	**118**
1,2-Dibromoethane	<1.0	<1.0	<1.0	<1.0	<1.0
1,2-Dichloroethane	<1.0	<1.0	<1.0	<1.0	<1.0
Dissolved Gases (µg/L)					
Methane	**5.5**	**18**	**10**	**200**	**650**
Ethane	<1.0	<1.0	<1.0	**2.7**	**3.6**
Ethene	<1.0	<1.0	<1.0	<1.0	<1.0
Anions (mg/L)					
Chloride	**500**	**570**	**410**	**790**	**1000**
Nitrate	**78**	**110**	**0.8**	<0.5	<0.5
Nitrite	**8.6**	**4.3**	<0.5	<0.5	<0.5
Sulfate	**100**	**100**	**44**	**61**	**3.8**
OrthoPhosphate	<1.5	<1.5	<1.5	<1.5	<1.5

Legend: NA = Not analyzed NS = Not sampled J = Estimated result below PQL but above LQL I = Inhibited <= Result not detected.

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Results

Table 3: Summary of the QuantArray®-Petro results obtained for samples TWN-03 MNA, TWN-03 Bio-Stim Nitrate, Sulfate, TWN-03 Bio-Aug SDC-9, Sulfate, SFCMW-10 MNA, and SFCMW-10 Bio-Stim Nitrate, Sulfate.

Sample Name	TWN-03 MNA	TWN-03 Bio-Stim Nitrate, Sulfate	TWN-03 Bio-Aug SDC-9, Sulfate	SFCMW-10 MNA	SFCMW-10 Bio-Stim Nitrate, Sulfate
Sample Date	**11/12/2019**	**11/12/2019**	**11/12/2019**	**11/12/2019**	**11/12/2019**
Aerobic BTEX and MTBE	cells/bead	cells/bead	cells/bead	cells/bead	cells/bead
Toluene/Benzene Dioxygenase (TOD)	**3.85E+02**	<2.50E+02	**1.47E+03**	**1.11E+02 (J)**	**1.28E+02 (J)**
Phenol Hydroxylase (PHE)	**1.67E+06**	**3.44E+05**	**5.07E+05**	**1.27E+05**	**2.19E+05**
Toluene 2 Monooxygenase/Phenol Hydroxylase (RDEG)	**1.76E+06**	**6.29E+05**	**2.43E+05**	**4.71E+04**	**3.82E+04**
Toluene Ring Hydroxylating Monooxygenases (RMO)	**2.79E+04**	**1.17E+04**	**3.72E+06**	<2.50E+02	<2.50E+02
Xylene/Toluene Monooxygenase (TOL)	**2.15E+01 (J)**	<2.50E+02	<2.50E+02	**9.50E+00 (J)**	<2.50E+02
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)	**8.23E+04**	**5.78E+04**	<2.50E+02	**5.82E+03**	**4.87E+03**
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)	**4.26E+03**	**1.96E+03**	**2.49E+03**	<2.50E+02	**1.03E+03**
Methylibium petroleiphilum PM1 (PM1)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
TBA Monooxygenase (TBA)	**8.62E+03**	**1.10E+03**	**1.31E+03**	<2.50E+02	<2.50E+02
Aerobic PAHs and Alkanes					
Naphthalene Dioxygenase (NAH)	**1.18E+03**	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Naphthalene-inducible Dioxygenase (NidA)	**9.88E+03**	**3.95E+03**	<2.50E+02	<2.50E+02	<2.50E+02
Phenanthrene Dioxygenase (PHN)	<2.50E+02	<2.50E+02	<2.50E+02	**6.47E+01 (J)**	<2.50E+02
Alkane Monooxygenase (ALK)	**1.07E+05**	**1.72E+04**	**5.65E+04**	**4.48E+04**	**1.08E+05**
Alkane Monooxygenase (ALMA)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Anaerobic BTEX					
Benzoyl Coenzyme A Reductase (BCR)	**1.53E+05**	**1.64E+04**	**1.77E+05**	**1.14E+05**	**8.37E+04**
Benzylsuccinate Synthase (BSS)	**1.27E+05**	**9.52E+04**	**4.39E+05**	<2.50E+02	**1.04E+04**
Benzene Carboxylase (ABC)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Anaerobic PAHs and Alkanes					
Naphthylmethylsuccinate Synthase (MNSSA)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Naphthalene Carboxylase (ANC)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	**1.08E+03**
Alkylsuccinate Synthase (ASSA)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Other					
Total Eubacteria (EBAC)	**4.48E+08**	**1.62E+08**	**1.42E+09**	**8.36E+07**	**9.86E+07**
Sulfate Reducing Bacteria (APS)	**9.52E+03**	**2.65E+04**	**1.49E+07**	**4.71E+05**	**3.65E+05**
CENSUS Targets					
1,2 DCA Reductase (DCAR)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Dehalobacter spp. (DHBt)	**4.14E+03**	<2.50E+02	<2.50E+02	**1.86E+05**	**3.89E+04**
Dehalococcoides (DHC)	**3.38E+03**	**9.46E+02**	**1.44E+07**		

Legend:
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NS = Not Sampled
J = Estimated Gene Copies Below PQL but Above LQL
I = Inhibited
< = Result Not Detected

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Table 4: Summary of the QuantArray®-Petro results obtained for samples CMW-01 MNA, CMW-01 Bio-Stim Nitrate, Sulfate, MW-1R MNA, MW-1R Bio-Stim Nitrate, Sulfate, and MW-1R Bio-Stim Nitrate.

Sample Name	CMW-01 MNA	CMW-01 Bio-Stim Nitrate, Sulfate	MW-1R MNA	MW-1R Bio-Stim Nitrate, Sulfate	MW-1R Bio-Stim Nitrate
Sample Date	**11/12/2019**	**11/12/2019**	**11/12/2019**	**11/12/2019**	**11/12/2019**
Aerobic BTEX and MTBE	cells/bead	cells/bead	cells/bead	cells/bead	cells/bead
Toluene/Benzene Dioxygenase (TOD)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Phenol Hydroxylase (PHE)	**3.26E+06**	**4.02E+06**	**1.11E+06**	**9.08E+04**	**9.38E+04**
Toluene 2 Monooxygenase/Phenol Hydroxylase (RDEG)	**3.86E+06**	**5.31E+06**	**6.65E+05**	**7.16E+04**	**6.37E+04**
Toluene Ring Hydroxylating Monooxygenases (RMO)	**3.30E+05**	**2.07E+05**	**9.62E+04**	**3.42E+04**	**4.82E+04**
Xylene/Toluene Monooxygenase (TOL)	**5.30E+00 (J)**	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)	<2.50E+02	<2.50E+02	**7.99E+05**	**9.37E+04**	**6.75E+04**
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Methylibium petroleiphilum PM1 (PM1)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	**1.27E+03**
TBA Monooxygenase (TBA)	**1.52E+03**	**1.15E+02 (J)**	**6.06E+02**	**1.18E+03**	<2.50E+02
Aerobic PAHs and Alkanes					
Naphthalene Dioxygenase (NAH)	**1.63E+04**	**9.94E+03**	**8.20E+01 (J)**	<2.50E+02	<2.50E+02
Naphthalene-inducible Dioxygenase (NidA)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Phenanthrene Dioxygenase (PHN)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Alkane Monooxygenase (ALK)	**2.64E+05**	**4.24E+05**	**2.49E+04**	**4.22E+02**	**4.52E+02**
Alkane Monooxygenase (ALMA)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Anaerobic BTEX					
Benzoyl Coenzyme A Reductase (BCR)	**1.04E+05**	**6.20E+04**	**3.08E+04**	**4.28E+03**	**1.29E+03**
Benzylsuccinate Synthase (BSS)	**8.32E+04**	**5.62E+04**	**2.24E+05**	**7.16E+04**	**6.58E+05**
Benzene Carboxylase (ABC)	<2.50E+02	<2.50E+02	<2.50E+02	**9.88E+03**	**1.75E+04**
Anaerobic PAHs and Alkanes					
Naphthylmethylsuccinate Synthase (MNSSA)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Naphthalene Carboxylase (ANC)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Alkylsuccinate Synthase (ASSA)	<2.50E+02	**1.78E+03**	<2.50E+02	<2.50E+02	<2.50E+02
Other					
Total Eubacteria (EBAC)	**2.33E+08**	**2.44E+08**	**1.21E+08**	**3.23E+07**	**5.79E+07**
Sulfate Reducing Bacteria (APS)	**1.20E+06**	**1.17E+06**	**3.62E+05**	**3.16E+05**	**4.51E+05**
CENSUS Targets					
1,2 DCA Reductase (DCAR)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Dehalobacter spp. (DHBt)	<2.50E+02	<2.50E+02	**7.90E+04**	**1.27E+05**	**6.48E+05**

Legend:
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Table 5: Summary of the QuantArray®-Petro results obtained for samples MW-11 and MW-4R

Sample Name Sample Date	MW-11 11/12/2019	MW-4R 11/12/2019
Aerobic BTEX and MTBE	cells/bead	cells/bead
Toluene/Benzene Dioxygenase (TOD)	<2.50E+02	<2.50E+02
Phenol Hydroxylase (PHE)	**1.91E+05**	**2.30E+05**
Toluene 2 Monooxygenase/Phenol Hydroxylase (RDEG)	**7.87E+04**	**1.08E+05**
Toluene Ring Hydroxylating Monooxygenases (RMO)	<2.50E+02	**1.51E+04**
Xylene/Toluene Monooxygenase (TOL)	**7.60E+00 (J)**	<2.50E+02
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)	**4.19E+04**	**7.68E+04**
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)	<2.50E+02	<2.50E+02
Methylibium petroleiphilum PM1 (PM1)	**5.85E+03**	**1.54E+04**
TBA Monooxygenase (TBA)	<2.50E+02	**1.10E+03**
Aerobic PAHs and Alkanes		
Naphthalene Dioxygenase (NAH)	<2.50E+02	<2.50E+02
Naphthalene-inducible Dioxygenase (NidA)	<2.50E+02	<2.50E+02
Phenanthrene Dioxygenase (PHN)	**2.34E+03**	<2.50E+02
Alkane Monooxygenase (ALK)	**5.92E+02**	**5.69E+02**
Alkane Monooxygenase (ALMA)	<2.50E+02	<2.50E+02
Anaerobic BTEX		
Benzoyl Coenzyme A Reductase (BCR)	**9.97E+04**	**3.77E+03**
Benzylsuccinate Synthase (BSS)	**1.18E+05**	**2.84E+03**
Benzene Carboxylase (ABC)	**3.04E+03**	<2.50E+02
Anaerobic PAHs and Alkanes		
Naphthylmethylsuccinate Synthase (MNSSA)	<2.50E+02	<2.50E+02
Naphthalene Carboxylase (ANC)	<2.50E+02	<2.50E+02
Alkylsuccinate Synthase (ASSA)	<2.50E+02	<2.50E+02
Other		
Total Eubacteria (EBAC)	**5.00E+07**	**4.10E+07**
Sulfate Reducing Bacteria (APS)	**2.84E+06**	<2.50E+02
CENSUS Targets		
1,2 DCA Reductase (DCAR)	<2.50E+02	<2.50E+02
Dehalobacter spp. (DHBt)	<2.50E+02	<2.50E+02

Legend:
NA = Not Analyzed
NS = Not Sampled
J = Estimated Gene Copies Below PQL but Above LQL
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< = Result Not Detected

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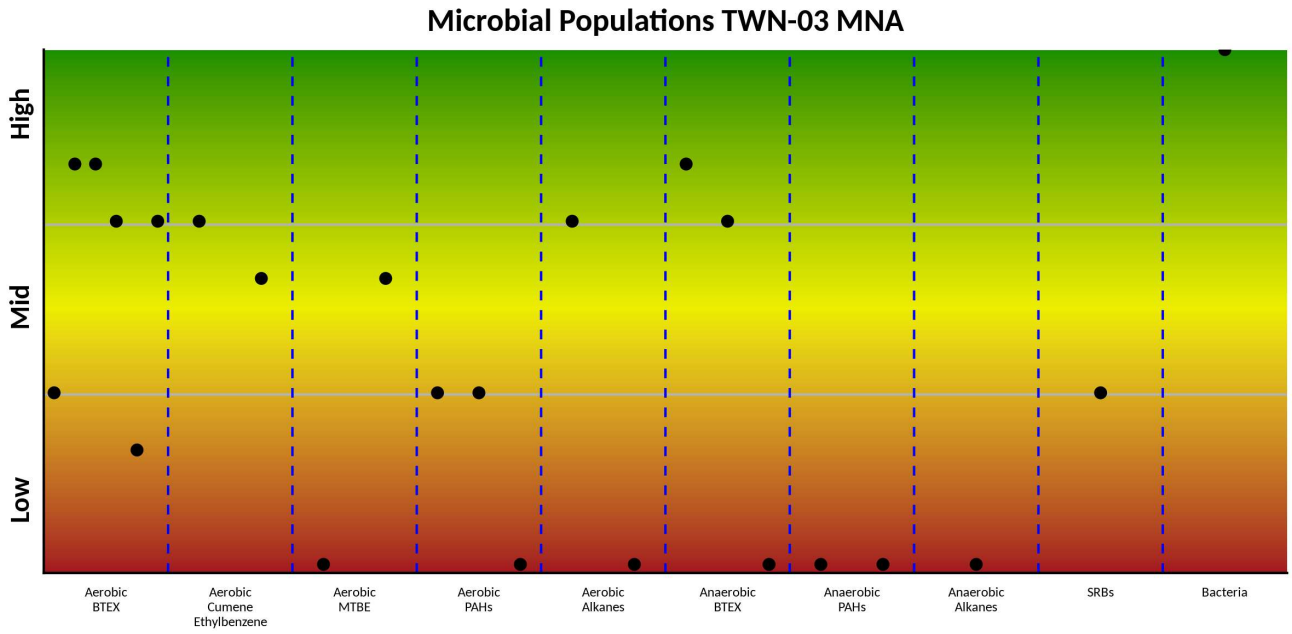

Figure 1: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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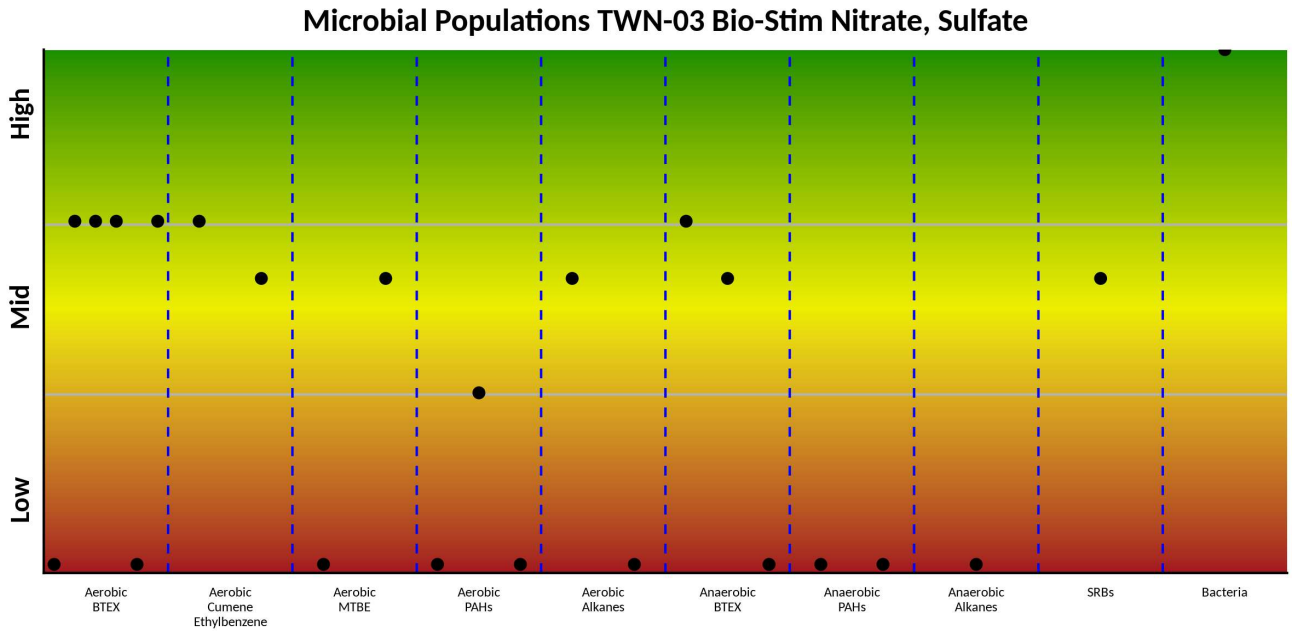

Figure 2: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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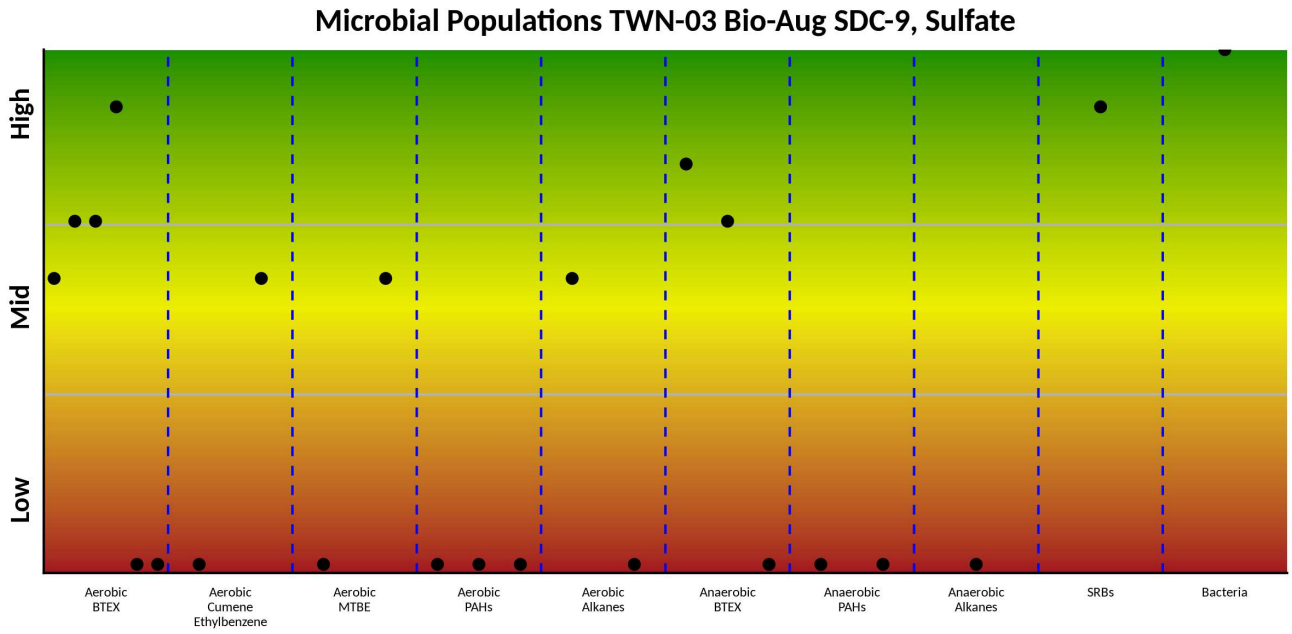

Figure 3: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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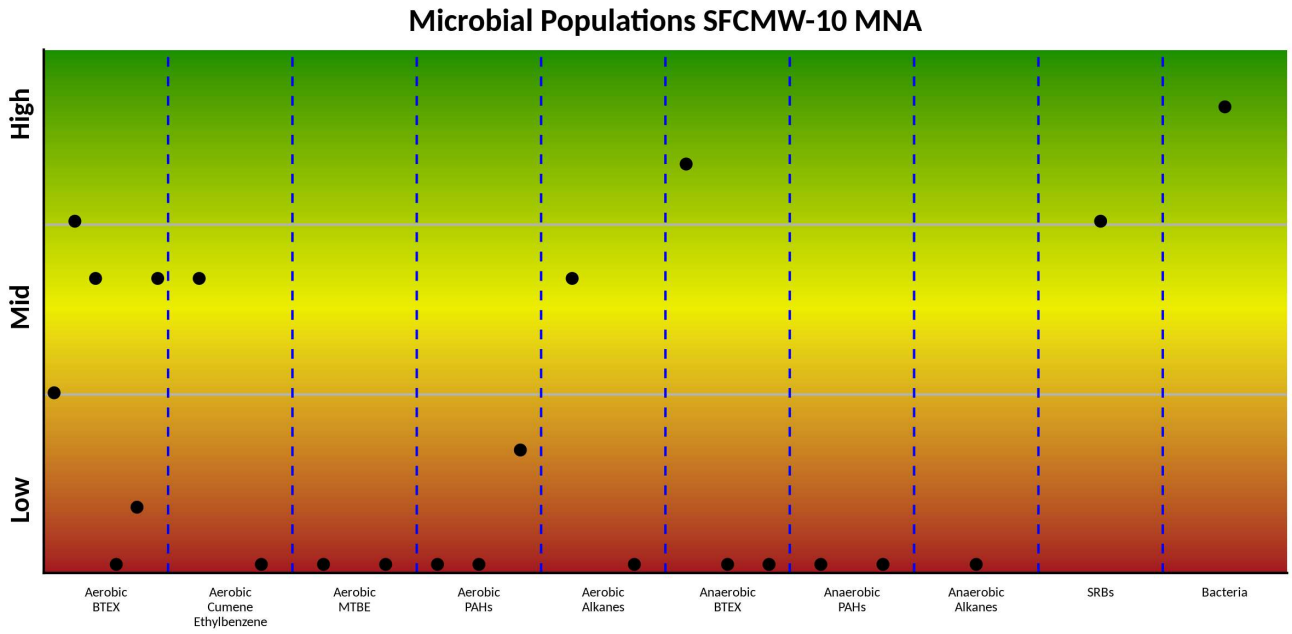

Figure 4: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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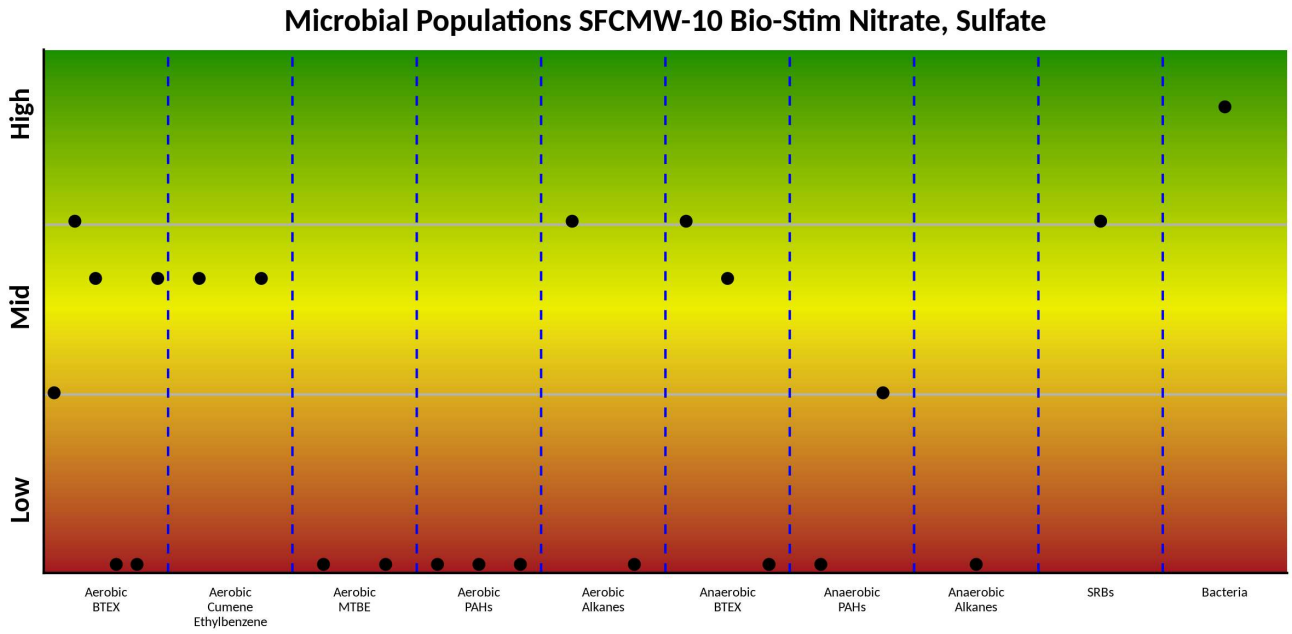

Figure 5: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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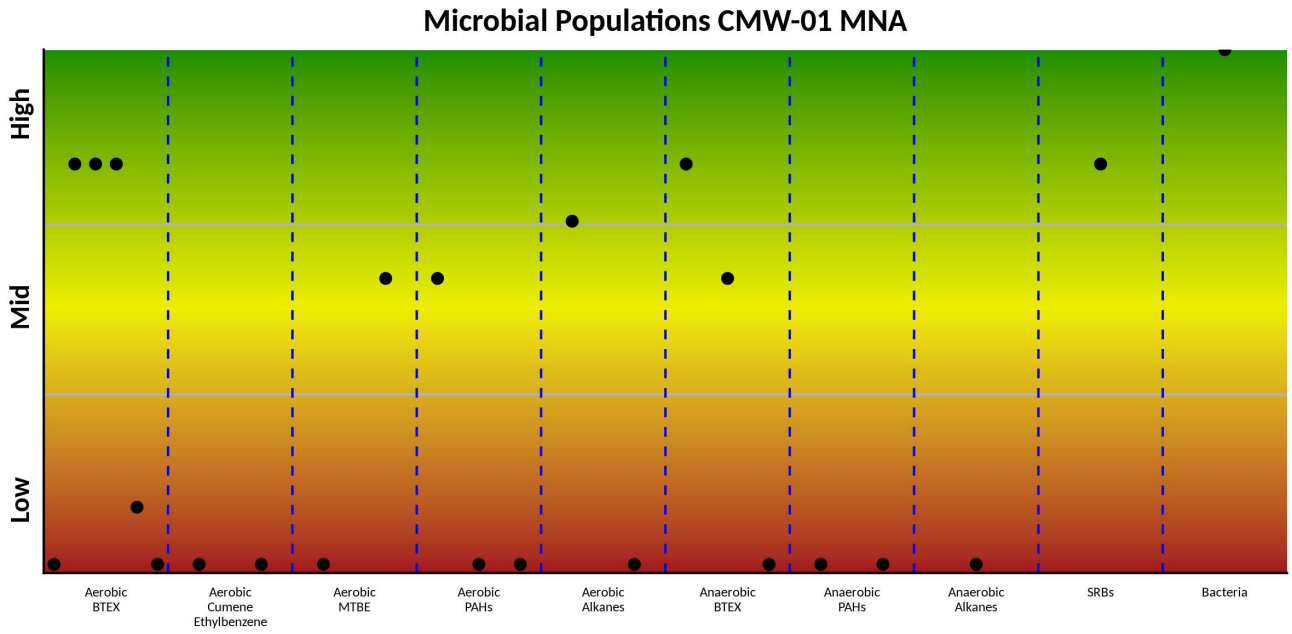

Figure 6: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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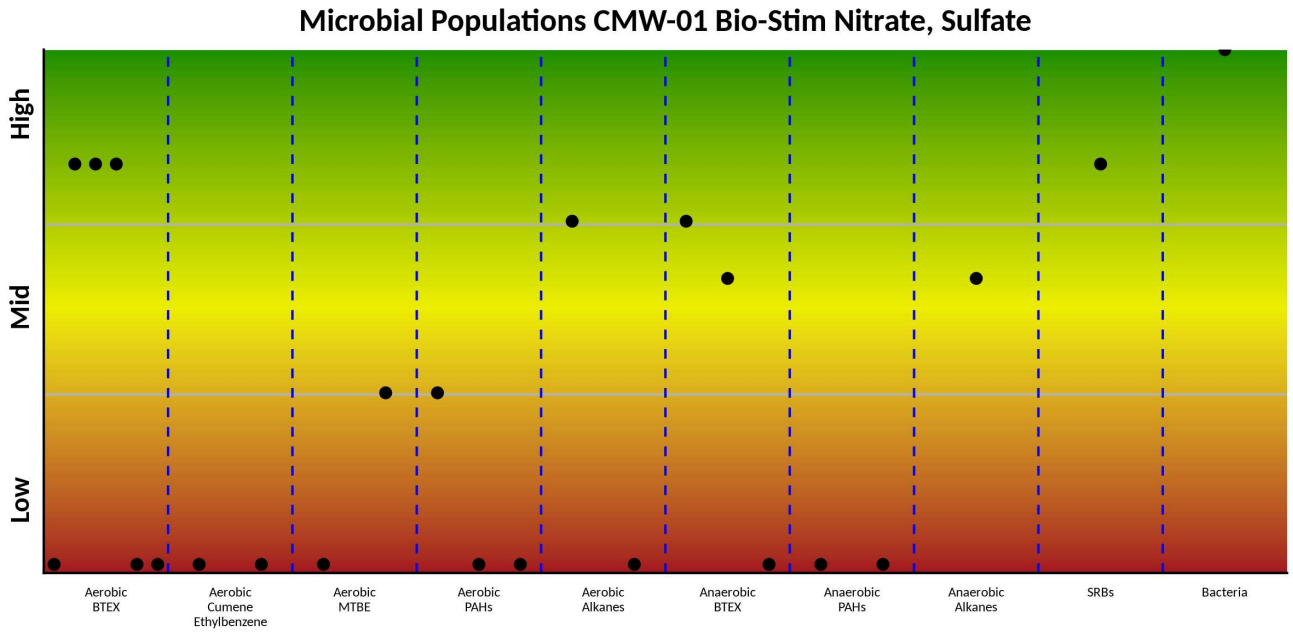

Figure 7: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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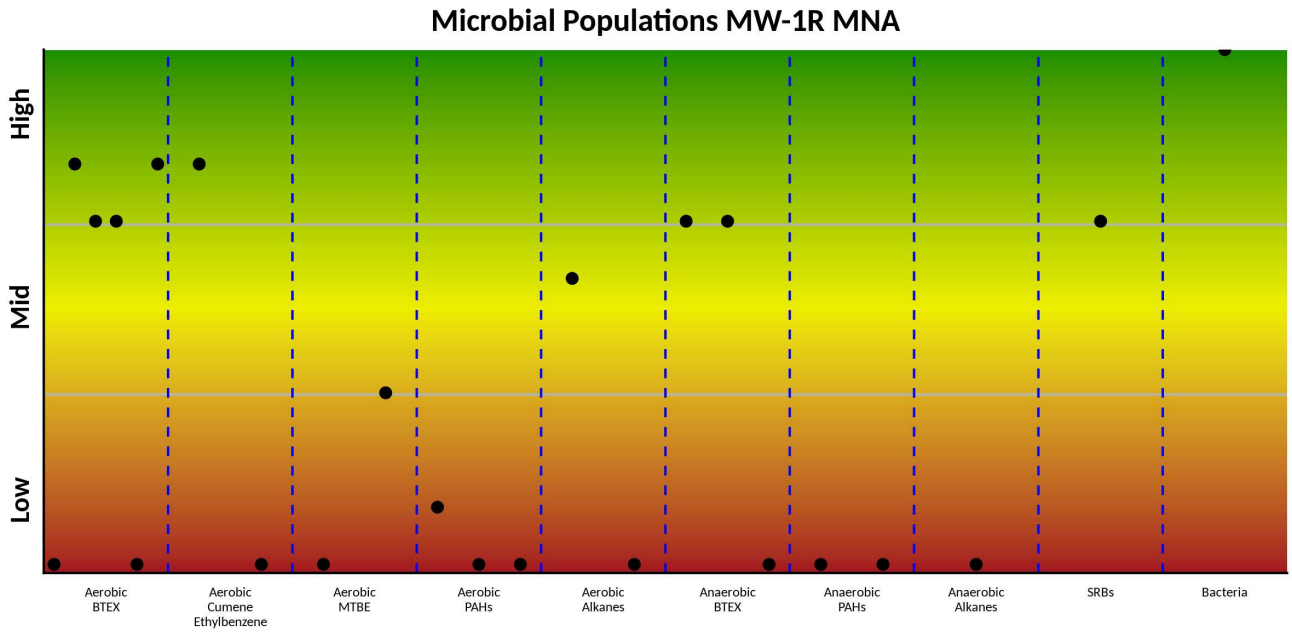

Figure 8: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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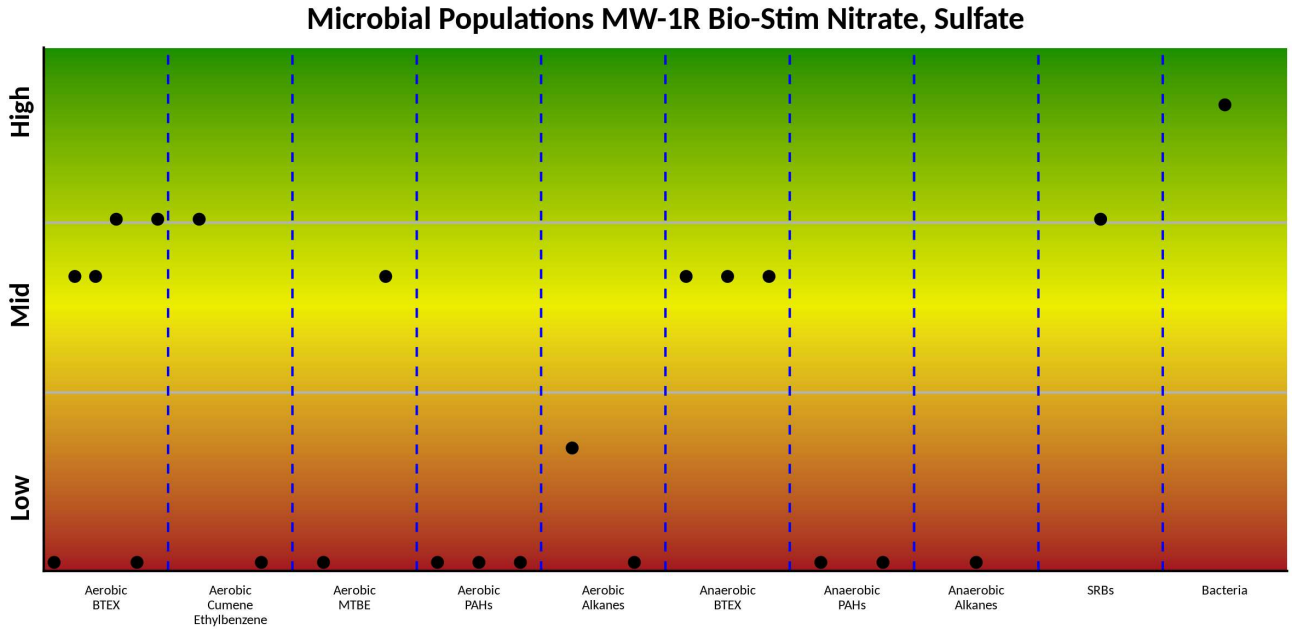

Figure 9: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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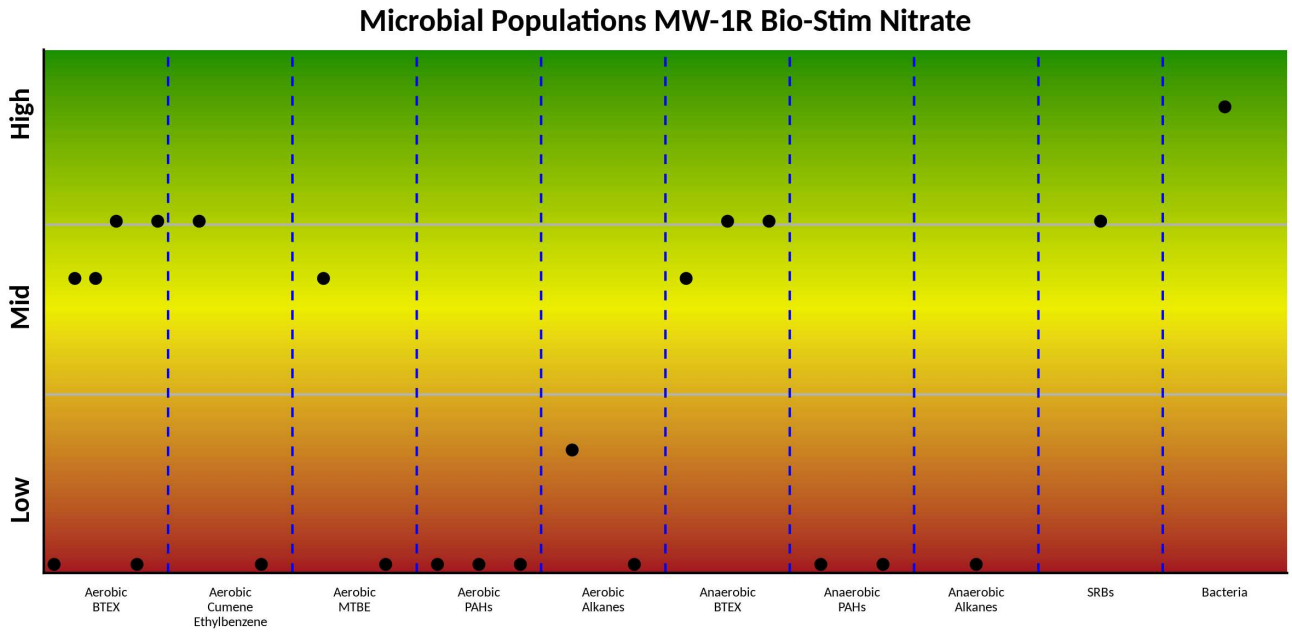

Figure 10: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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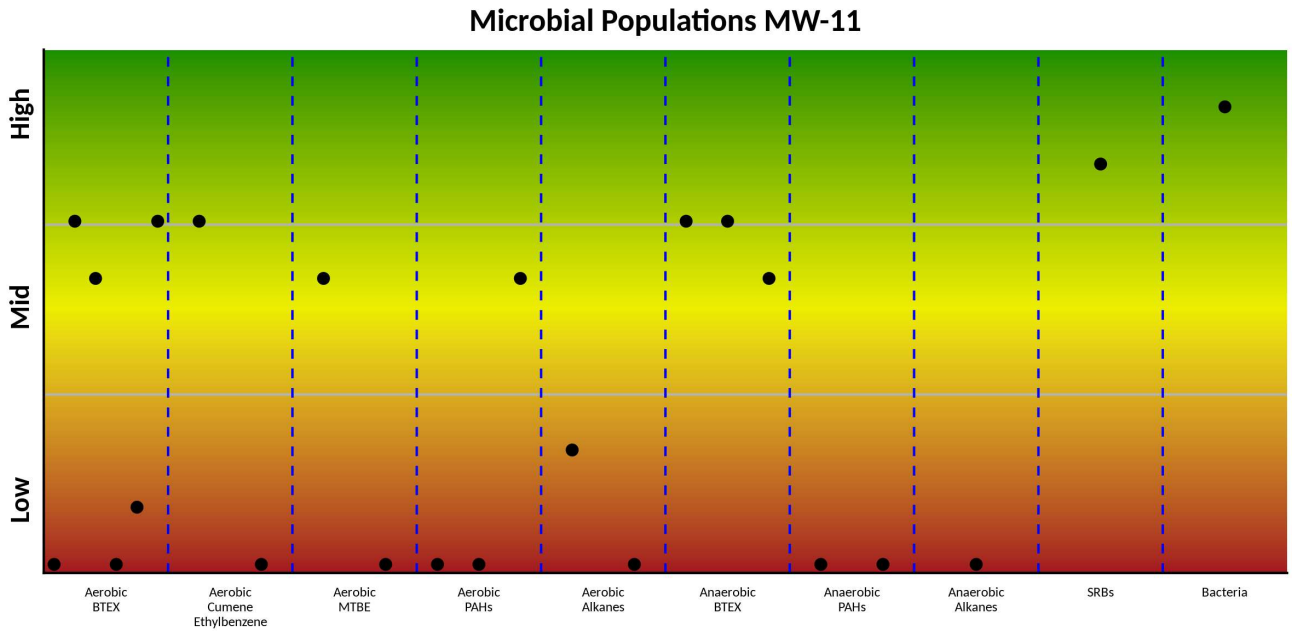

Figure 11: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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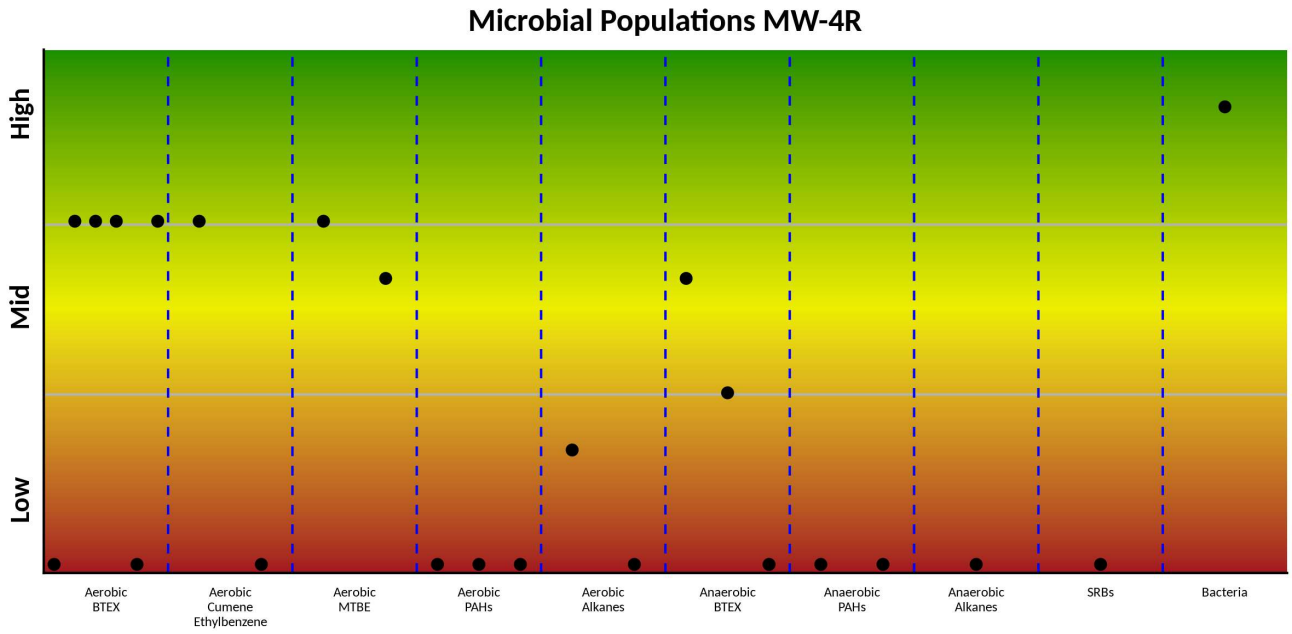

Figure 12: Microbial population summary to aid in evaluating potential pathways and biodegradation of specific contaminants.

Aerobic		Anaerobic	
BTEX	TOD, PHE, RDEG, RMO, TOL, EDO	BTEX	BCR, BSS, ABC
Cumene, Ethylbenzene	EDO, BPH4	Naphthalene/Methylnaphthalene	MNSSA, ANC
MTBE/TBA	PM1, TBA	Alkanes	assA
Naphthalene	NAH, NidA		
Phenanthrene	PHN		
Alkanes	ALK, ALMA		

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Table 6: Summary of the QuantArray®-Petro results for microorganisms responsible for aerobic biodegradation of BTEX and MTBE for samples TWN-03 MNA, TWN-03 Bio-Stim Nitrate, Sulfate, TWN-03 Bio-Aug SDC-9, Sulfate, SFCMW-10 MNA, and SFCMW-10 Bio-Stim Nitrate, Sulfate.

Sample Name	TWN-03 MNA	TWN-03 Bio-Stim Nitrate, Sulfate	TWN-03 Bio-Aug SDC-9, Sulfate	SFCMW-10 MNA	SFCMW-10 Bio-Stim Nitrate, Sulfate
Sample Date	11/12/2019	11/12/2019	11/12/2019	11/12/2019	11/12/2019
Aerobic BTEX and MTBE	cells/bead	cells/bead	cells/bead	cells/bead	cells/bead
Toluene/Benzene Dioxygenase (TOD)	**3.85E+02**	<2.50E+02	**1.47E+03**	**1.11E+02 (J)**	**1.28E+02 (J)**
Phenol Hydroxylase (PHE)	**1.67E+06**	**3.44E+05**	**5.07E+05**	**1.27E+05**	**2.19E+05**
Toluene 2 Monooxygenase/Phenol Hydroxylase (RDEG)	**1.76E+06**	**6.29E+05**	**2.43E+05**	**4.71E+04**	**3.82E+04**
Toluene Ring Hydroxylating Monooxygenases (RMO)	**2.79E+04**	**1.17E+04**	**3.72E+06**	<2.50E+02	<2.50E+02
Xylene/Toluene Monooxygenase (TOL)	**2.15E+01 (J)**	<2.50E+02	<2.50E+02	**9.50E+00 (J)**	<2.50E+02
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)	**8.23E+04**	**5.78E+04**	<2.50E+02	**5.82E+03**	**4.87E+03**
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)	**4.26E+03**	**1.96E+03**	**2.49E+03**	<2.50E+02	**1.03E+03**
Methylibium petroleiphilum PM1 (PM1)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
TBA Monooxygenase (TBA)	**8.62E+03**	**1.10E+03**	**1.31E+03**	<2.50E+02	<2.50E+02

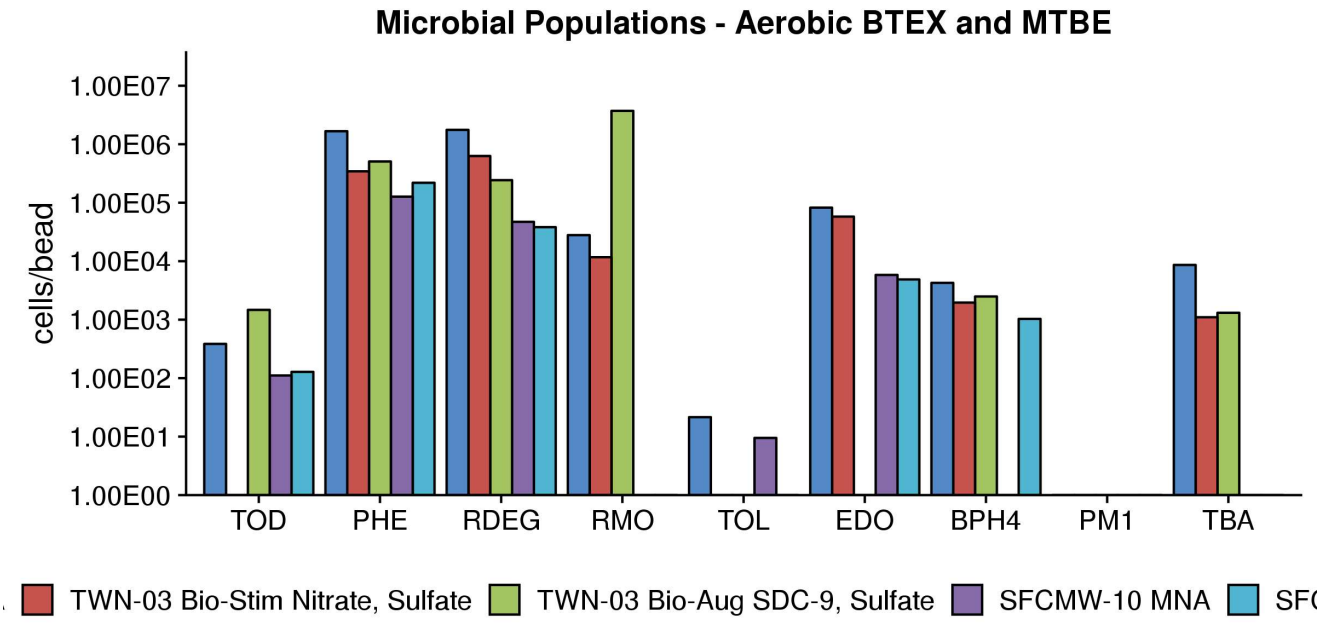

Figure 13: Comparison - microbial populations involved in aerobic biodegradation of BTEX and MTBE.

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Table 7: Summary of the QuantArray®-Petro results for microorganisms responsible for aerobic biodegradation of BTEX and MTBE for samples CMW-01 MNA, CMW-01 Bio-Stim Nitrate, Sulfate, MW-1R MNA, MW-1R Bio-Stim Nitrate, Sulfate, and MW-1R Bio-Stim Nitrate.

Sample Name	CMW-01 MNA	CMW-01 Bio-Stim Nitrate, Sulfate	MW-1R MNA	MW-1R Bio-Stim Nitrate, Sulfate	MW-1R Bio-Stim Nitrate
Sample Date	11/12/2019	11/12/2019	11/12/2019	11/12/2019	11/12/2019
Aerobic BTEX and MTBE	cells/bead	cells/bead	cells/bead	cells/bead	cells/bead
Toluene/Benzene Dioxygenase (TOD)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Phenol Hydroxylase (PHE)	**3.26E+06**	**4.02E+06**	**1.11E+06**	**9.08E+04**	**9.38E+04**
Toluene 2 Monooxygenase/Phenol Hydroxylase (RDEG)	**3.86E+06**	**5.31E+06**	**6.65E+05**	**7.16E+04**	**6.37E+04**
Toluene Ring Hydroxylating Monooxygenases (RMO)	**3.30E+05**	**2.07E+05**	**9.62E+04**	**3.42E+04**	**4.82E+04**
Xylene/Toluene Monooxygenase (TOL)	**5.30E+00 (J)**	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)	<2.50E+02	<2.50E+02	**7.99E+05**	**9.37E+04**	**6.75E+04**
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Methylibium petroleiphilum PM1 (PM1)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	**1.27E+03**
TBA Monooxygenase (TBA)	**1.52E+03**	**1.15E+02 (J)**	**6.06E+02**	**1.18E+03**	<2.50E+02

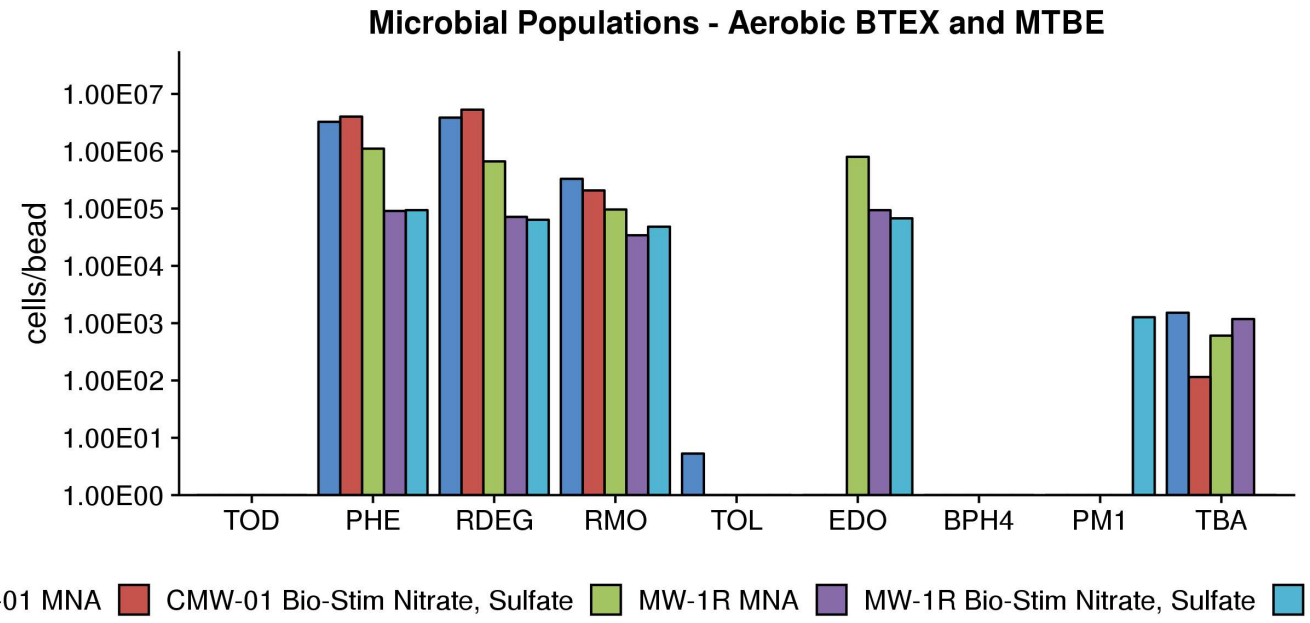

Figure 14: Comparison - microbial populations involved in aerobic biodegradation of BTEX and MTBE.

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Table 8: Summary of the QuantArray®-Petro results for microorganisms responsible for aerobic biodegradation of BTEX and MTBE for samples MW-11 and MW-4R.

Sample Name Sample Date	MW-11 11/12/2019	MW-4R 11/12/2019
Aerobic BTEX and MTBE	cells/bead	cells/bead
Toluene/Benzene Dioxygenase (TOD)	<2.50E+02	<2.50E+02
Phenol Hydroxylase (PHE)	**1.91E+05**	**2.30E+05**
Toluene 2 Monooxygenase/Phenol Hydroxylase (RDEG)	**7.87E+04**	**1.08E+05**
Toluene Ring Hydroxylating Monooxygenases (RMO)	<2.50E+02	**1.51E+04**
Xylene/Toluene Monooxygenase (TOL)	**7.60E+00 (J)**	<2.50E+02
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)	**4.19E+04**	**7.68E+04**
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)	<2.50E+02	<2.50E+02
Methylibium petroleiphilum PM1 (PM1)	**5.85E+03**	**1.54E+04**
TBA Monooxygenase (TBA)	<2.50E+02	**1.10E+03**

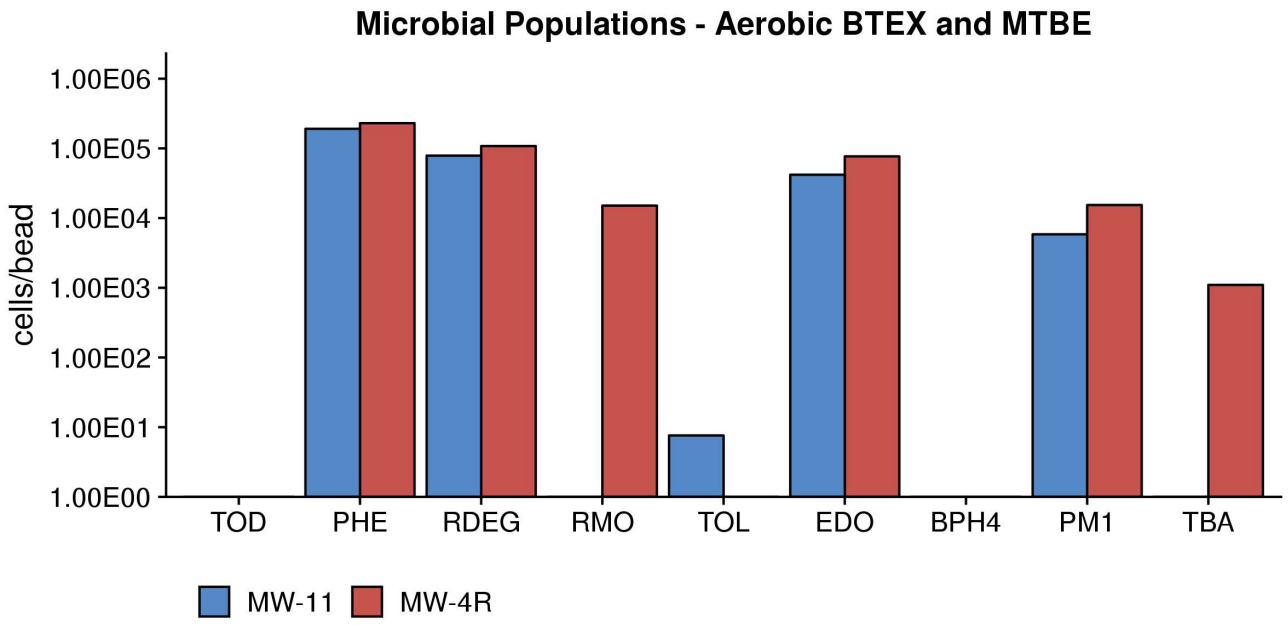

Figure 15: Comparison - microbial populations involved in aerobic biodegradation of BTEX and MTBE.

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Table 9: Summary of the QuantArray®-Petro results for microorganisms responsible for aerobic biodegradation of PAHs and alkanes for samples TWN-03 MNA, TWN-03 Bio-Stim Nitrate, Sulfate, TWN-03 Bio-Aug SDC-9, Sulfate, SFCMW-10 MNA, and SFCMW-10 Bio-Stim Nitrate, Sulfate.

Sample Name	TWN-03 MNA	TWN-03 Bio-Stim Nitrate, Sulfate	TWN-03 Bio-Aug SDC-9, Sulfate	SFCMW-10 MNA	SFCMW-10 Bio-Stim Nitrate, Sulfate
Sample Date	11/12/2019	11/12/2019	11/12/2019	11/12/2019	11/12/2019
Aerobic PAHs and Alkanes	cells/bead	cells/bead	cells/bead	cells/bead	cells/bead
Naphthalene Dioxygenase (NAH)	**1.18E+03**	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Naphthalene-inducible Dioxygenase (NidA)	**9.88E+03**	**3.95E+03**	<2.50E+02	<2.50E+02	<2.50E+02
Phenanthrene Dioxygenase (PHN)	<2.50E+02	<2.50E+02	<2.50E+02	**6.47E+01 (J)**	<2.50E+02
Alkane Monooxygenase (ALK)	**1.07E+05**	**1.72E+04**	**5.65E+04**	**4.48E+04**	**1.08E+05**
Alkane Monooxygenase (ALMA)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02

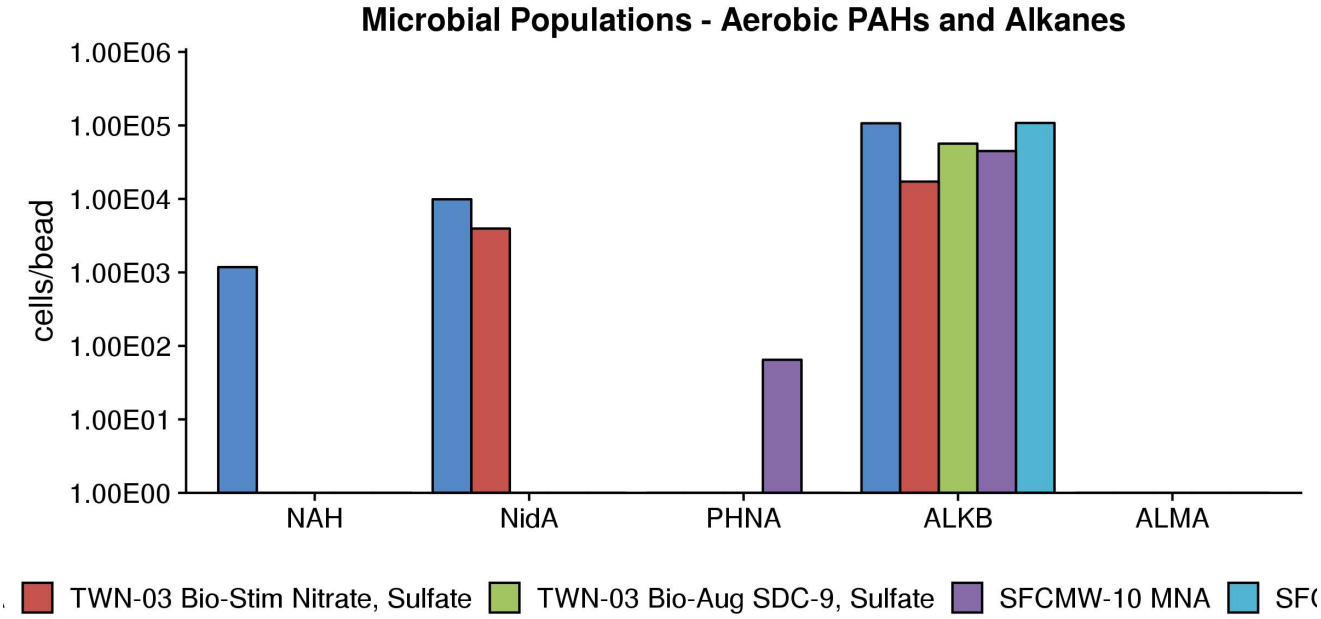

Figure 16: Comparison - microbial populations involved in aerobic biodegradation of PAHs and alkanes.

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Table 10: Summary of the QuantArray®-Petro results for microorganisms responsible for aerobic biodegradation of PAHs and alkanes for samples CMW-01 MNA, CMW-01 Bio-Stim Nitrate, Sulfate, MW-1R MNA, MW-1R Bio-Stim Nitrate, Sulfate, and MW-1R Bio-Stim Nitrate.

Sample Name	CMW-01 MNA	CMW-01 Bio-Stim Nitrate, Sulfate	MW-1R MNA	MW-1R Bio-Stim Nitrate, Sulfate	MW-1R Bio-Stim Nitrate
Sample Date	11/12/2019	11/12/2019	11/12/2019	11/12/2019	11/12/2019
Aerobic PAHs and Alkanes	cells/bead	cells/bead	cells/bead	cells/bead	cells/bead
Naphthalene Dioxygenase (NAH)	**1.63E+04**	**9.94E+03**	**8.20E+01 (J)**	<2.50E+02	<2.50E+02
Naphthalene-inducible Dioxygenase (NidA)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Phenanthrene Dioxygenase (PHN)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Alkane Monooxygenase (ALK)	**2.64E+05**	**4.24E+05**	**2.49E+04**	**4.22E+02**	**4.52E+02**
Alkane Monooxygenase (ALMA)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02

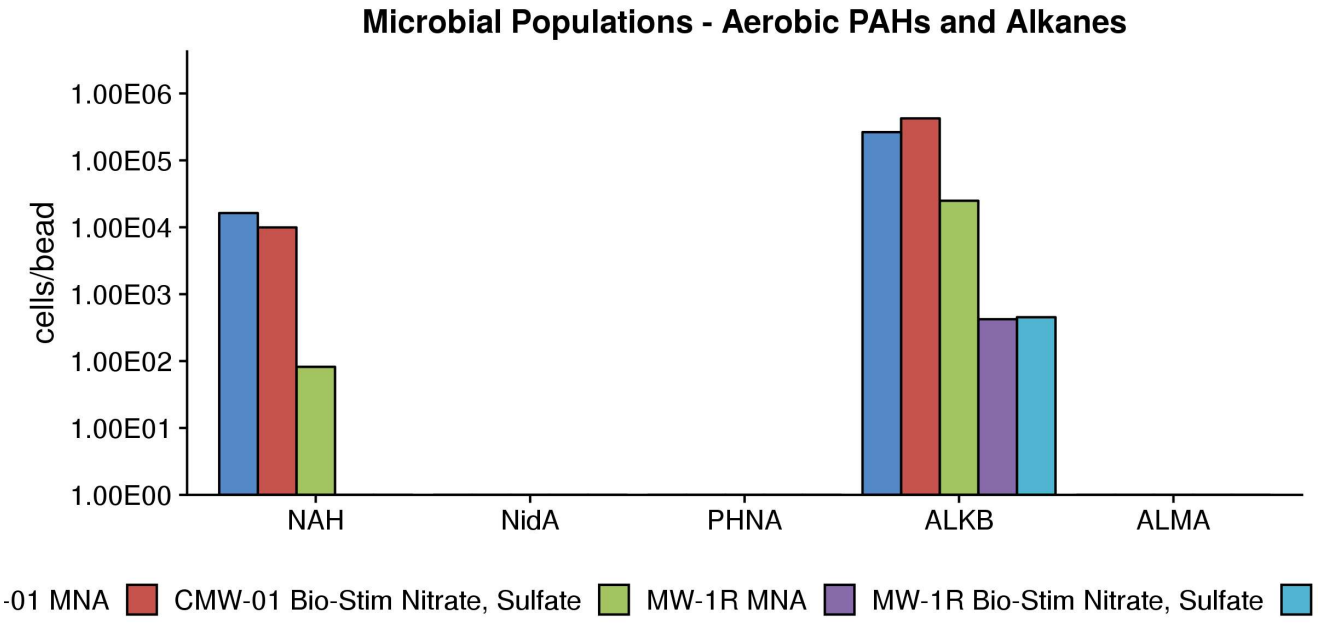

Figure 17: Comparison - microbial populations involved in aerobic biodegradation of PAHs and alkanes.

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Table 11: Summary of the QuantArray®-Petro results for microorganisms responsible for aerobic biodegradation of PAHs and alkanes for samples MW-11 and MW-4R.

Sample Name Sample Date	MW-11 11/12/2019	MW-4R 11/12/2019
Aerobic PAHs and Alkanes	cells/bead	cells/bead
Naphthalene Dioxygenase (NAH)	<2.50E+02	<2.50E+02
Naphthalene-inducible Dioxygenase (NidA)	<2.50E+02	<2.50E+02
Phenanthrene Dioxygenase (PHN)	**2.34E+03**	<2.50E+02
Alkane Monooxygenase (ALK)	**5.92E+02**	**5.69E+02**
Alkane Monooxygenase (ALMA)	<2.50E+02	<2.50E+02

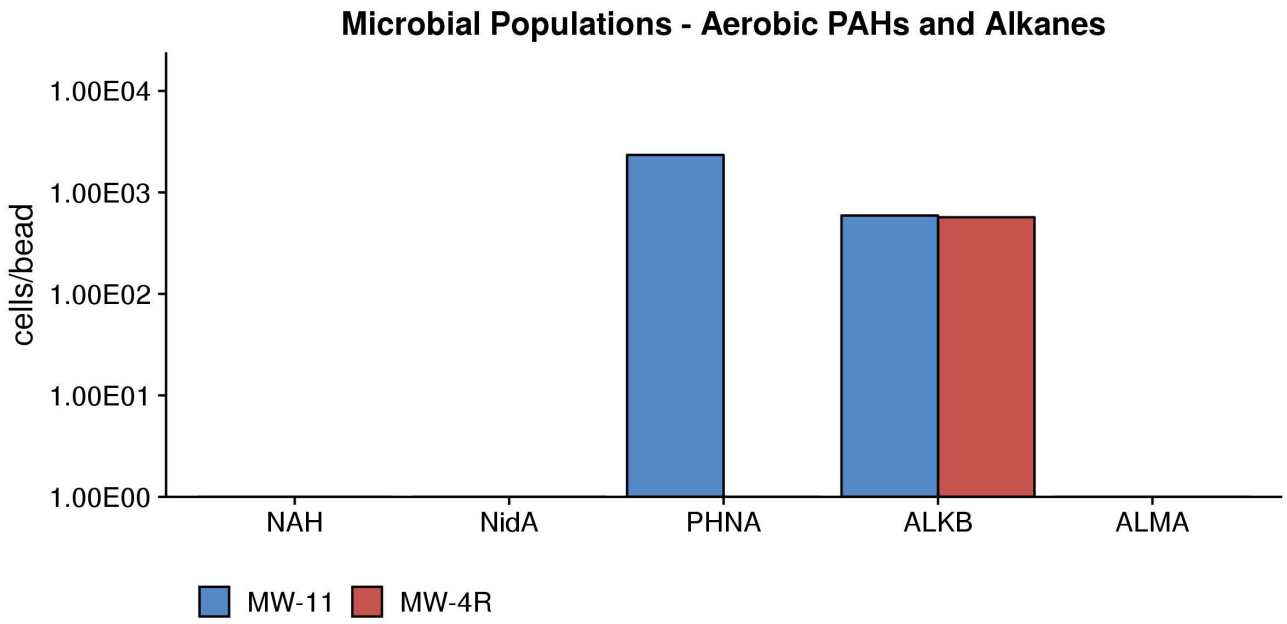

Figure 18: Comparison - microbial populations involved in aerobic biodegradation of PAHs and alkanes.

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Table 12: Summary of the QuantArray®-Petro results for microorganisms responsible for anaerobic biodegradation of BTEX, PAHs and alkanes for samples TWN-03 MNA, TWN-03 Bio-Stim Nitrate, Sulfate, TWN-03 Bio-Aug SDC-9, Sulfate, SFCMW-10 MNA, and SFCMW-10 Bio-Stim Nitrate, Sulfate.

Sample Name	TWN-03 MNA	TWN-03 Bio-Stim Nitrate, Sulfate	TWN-03 Bio-Aug SDC-9, Sulfate	SFCMW-10 MNA	SFCMW-10 Bio-Stim Nitrate, Sulfate
Sample Date	11/12/2019	11/12/2019	11/12/2019	11/12/2019	11/12/2019
Anaerobic BTEX	cells/bead	cells/bead	cells/bead	cells/bead	cells/bead
Benzoyl Coenzyme A Reductase (BCR)	**1.53E+05**	**1.64E+04**	**1.77E+05**	**1.14E+05**	**8.37E+04**
Benzylsuccinate Synthase (BSS)	**1.27E+05**	**9.52E+04**	**4.39E+05**	<2.50E+02	**1.04E+04**
Benzene Carboxylase (ABC)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Anaerobic PAHs and Alkanes					
Naphthylmethylsuccinate Synthase (MNSSA)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Naphthalene Carboxylase (ANC)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	**1.08E+03**
Alkylsuccinate Synthase (ASS)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02

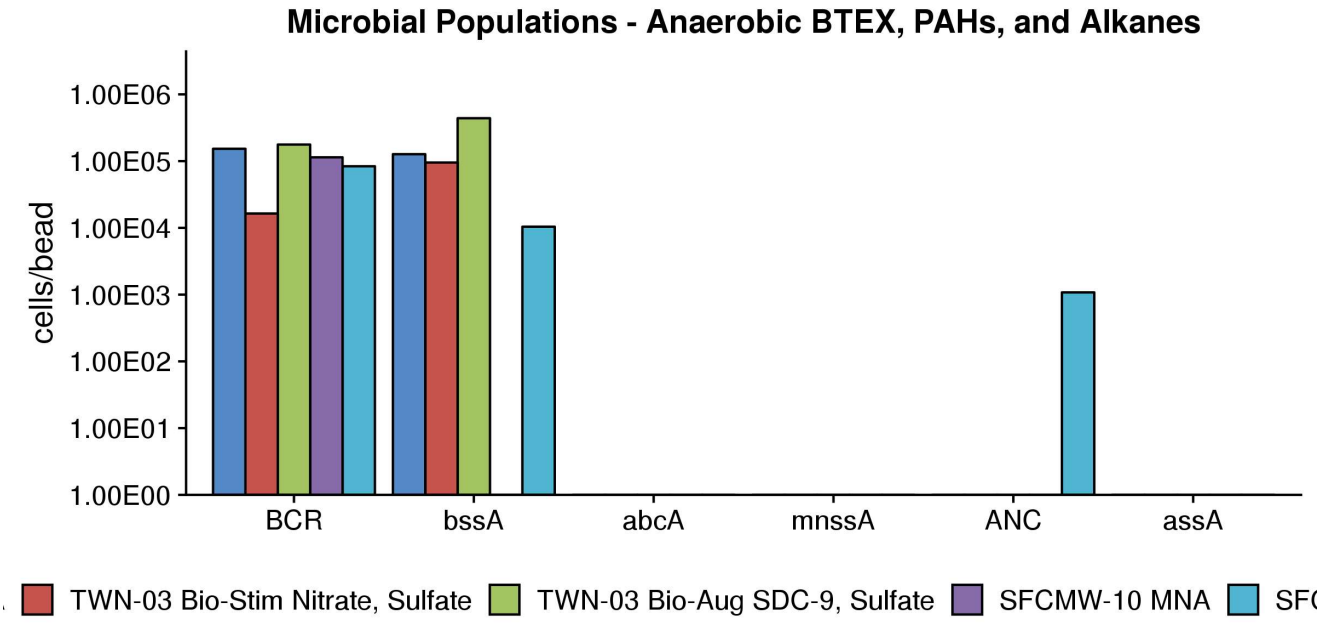

Figure 19: Comparison - microbial populations involved in anaerobic biodegradation of BTEX, PAHs and alkanes.

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Table 13: Summary of the QuantArray®-Petro results for microorganisms responsible for anaerobic biodegradation of BTEX, PAHs and alkanes for samples CMW-01 MNA, CMW-01 Bio-Stim Nitrate, Sulfate, MW-1R MNA, MW-1R Bio-Stim Nitrate, Sulfate, and MW-1R Bio-Stim Nitrate.

Sample Name	CMW-01 MNA	CMW-01 Bio-Stim Nitrate, Sulfate	MW-1R MNA	MW-1R Bio-Stim Nitrate, Sulfate	MW-1R Bio-Stim Nitrate
Sample Date	11/12/2019	11/12/2019	11/12/2019	11/12/2019	11/12/2019
Anaerobic BTEX	cells/bead	cells/bead	cells/bead	cells/bead	cells/bead
Benzoyl Coenzyme A Reductase (BCR)	**1.04E+05**	**6.20E+04**	**3.08E+04**	**4.28E+03**	**1.29E+03**
Benzylsuccinate Synthase (BSS)	**8.32E+04**	**5.62E+04**	**2.24E+05**	**7.16E+04**	**6.58E+05**
Benzene Carboxylase (ABC)	<2.50E+02	<2.50E+02	<2.50E+02	**9.88E+03**	**1.75E+04**
Anaerobic PAHs and Alkanes					
Naphthylmethylsuccinate Synthase (MNSSA)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Naphthalene Carboxylase (ANC)	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02	<2.50E+02
Alkylsuccinate Synthase (ASS)	<2.50E+02	**1.78E+03**	<2.50E+02	<2.50E+02	<2.50E+02

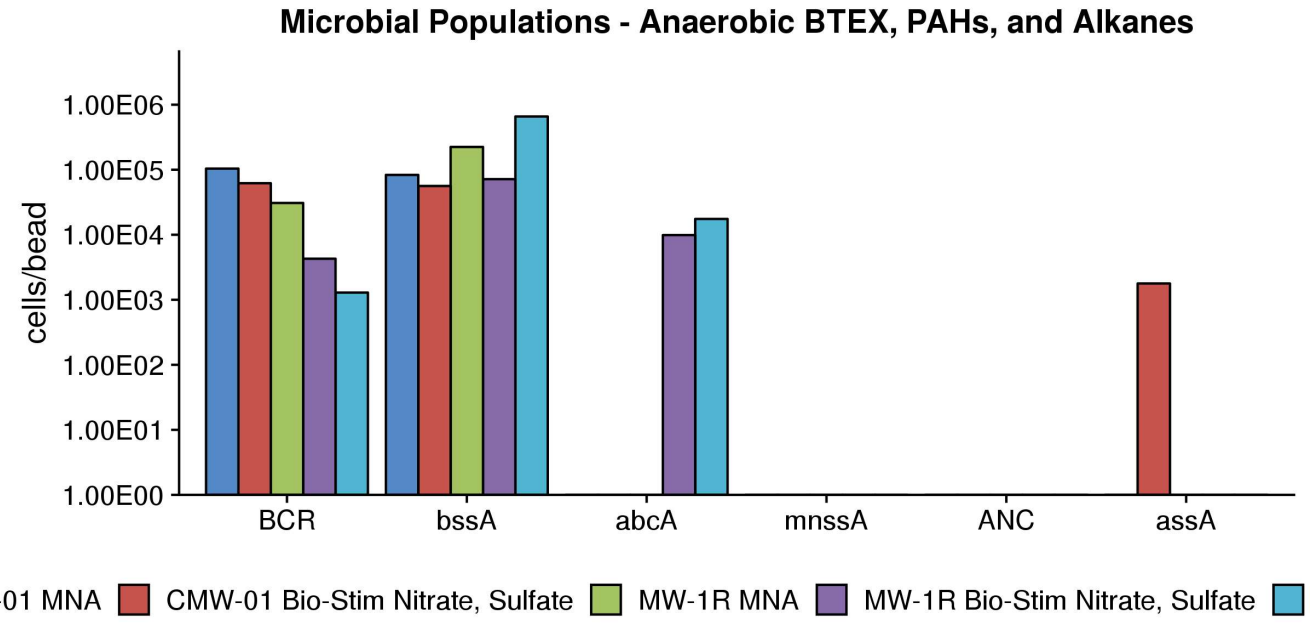

Figure 20: Comparison - microbial populations involved in anaerobic biodegradation of BTEX, PAHs and alkanes.

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Table 14: Summary of the QuantArray®-Petro results for microorganisms responsible for anaerobic biodegradation of BTEX, PAHs and alkanes for samples MW-11 and MW-4R.

Sample Name Sample Date	MW-11 11/12/2019	MW-4R 11/12/2019
Anaerobic BTEX	cells/bead	cells/bead
Benzoyl Coenzyme A Reductase (BCR)	**9.97E+04**	**3.77E+03**
Benzylsuccinate Synthase (BSS)	**1.18E+05**	**2.84E+03**
Benzene Carboxylase (ABC)	**3.04E+03**	<2.50E+02
Anaerobic PAHs and Alkanes		
Naphthylmethylsuccinate Synthase (MNSSA)	<2.50E+02	<2.50E+02
Naphthalene Carboxylase (ANC)	<2.50E+02	<2.50E+02
Alkylsuccinate Synthase (ASS)	<2.50E+02	<2.50E+02

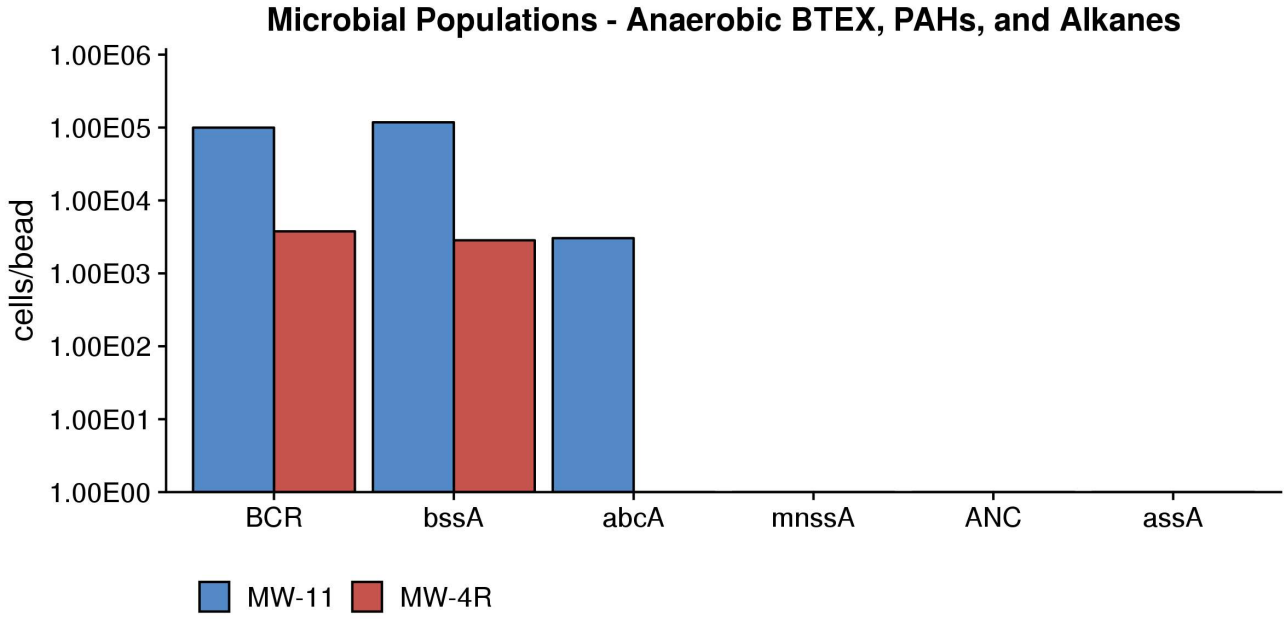

Figure 21: Comparison - microbial populations involved in anaerobic biodegradation of BTEX, PAHs and alkanes.

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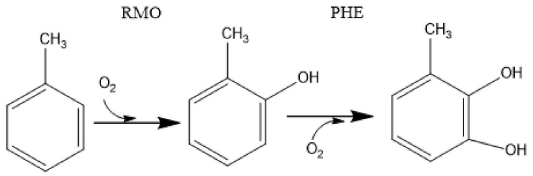
Interpretation

The overall purpose of the QuantArray®-Petro is to give site managers the ability to simultaneously yet economically evaluate the potential for biodegradation of a spectrum of contaminants found in petroleum products through a multitude of aerobic and anaerobic pathways to give a much more clear and comprehensive view of contaminant biodegradation. The following discussion describes interpretation of results in general terms and is meant to serve as a guide.

Aerobic Biodegradation - Benzene Toluene, Ethylbenzene, and Xylenes (BTEX): At sites impacted by petroleum products, aromatic hydrocarbons including BTEX are often contaminants of concern. Aerobic biodegradation of aromatic hydrocarbons has been intensively studied and multiple catabolic pathways have been well characterized. The substrate specificity of each pathway (range of compounds biodegraded via each pathway) is largely determined by the specificity of the initial oxygenase enzyme. The QuantArray®-Petro includes a suite of assays targeting the initial oxygenase genes of the known pathways for aerobic BTEX biodegradation.

Toluene/Benzene Dioxygenase (TOD): Toluene/benzene dioxygenase (TOD) incorporates both atoms of molecular oxygen into the aromatic ring. Although commonly called toluene dioxygenase, the substrate specificity of this enzyme is relaxed, allowing growth on toluene and benzene along with co-oxidation of a variety of compounds including ethylbenzene, *o*-xylene, *m*-xylene, and trichloroethene (TCE) when expressed.

Toluene/Benzene Monooxygenases (RMO/RDEG) and Phenol Hydroxylases (PHE): The next three known pathways for aerobic biodegradation of toluene (as well as benzene and xylenes) involve two steps: (1) an initial oxidation mediated by a toluene monooxygenase and (2) a second oxidation step catalyzed by a phenol hydroxylase. In these pathways, the toluene monooxygenases have been referred to as "ring hydroxylating monooxygenases" because they initiate biodegradation of toluene by incorporating oxygen directly into the aromatic ring rather than at a methyl group. The ring hydroxylating monooxygenases (RMOs) can be further described as toluene-2-monooxygenases, toluene-3-monooxygenases, or toluene-4-monooxygenases based upon where they attack the aromatic ring.



In General, phenol hydroxylases (PHE) catalyze the continued oxidation of phenols produced by RMOs. However, the difference between toluene monooxygenases (RMOs) and phenol hydroxylases (PHEs) is not absolute in terms of substrate specificity and catabolic function. For example, the TbmD toluene/benzene-2-monooxygenase [1] may be responsible for both the initial and second oxidation step [2].

The RMO, RDEG, and PHE assays target groups of genes encoding enzymes which perform the critical first and/or second steps in the aerobic biodegradation of BTEX compounds. In general terms, the RMO assay quantifies families of toluene-3-monooxygenase and toluene-4-monooxygenase genes. The RDEG assay is used to quantify groups of toluene-2-monooxygenase and phenol hydroxylase genes. Similarly, the PHE assay targets phenol hydroxylase genes and several benzene monooxygenase genes which catalyze both oxidation steps.

Toluene/Xylene Monooxygenase (TOL): The final known pathway for aerobic toluene biodegradation involves initial monooxygenase attack at the methyl group by a toluene/xylene monooxygenase.

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Ethylbenzene Dioxygenase (EDO): Similar to TOD, this group of aromatic oxygenases exhibits relatively broad specificity and is responsible for aerobic biodegradation of alkylbenzenes including ethylbenzene and isopropylbenzene or cumene [3].

Biphenyl Dioxygenase (BPH4): In environmental restoration, biphenyl dioxygenases are best known for cometabolism of polychlorinated biphenyls (PCBs). However, this subfamily includes benzene [4] and isopropylbenzene [5] dioxygenases from *Rhodococcus* spp.

Aerobic Biodegradation - MTBE and TBA: With increased use in the 1990s, the fuel oxygenate methyl *tert*-butyl ether (MTBE) has become one of the most commonly detected groundwater contaminants at gasoline contaminated sites. Pure cultures capable of utilizing MTBE as a growth supporting substrate have been isolated [6] and aerobic biodegradation of MTBE and the intermediate *tert*-butyl alcohol (TBA) has been reasonably well characterized. The QuantArray®-Petro includes quantification of two gene targets to assess the potential for aerobic biodegradation of MTBE and TBA.

Methylibium petroleiphilum PM1 (PM1): One of the few organisms isolated to date which is capable of utilizing MTBE and TBA as growth supporting substrates [6].

TBA Monooxygenase (TBA): Targets the TBA monooxygenase gene responsible for oxidation of TBA by *Methylibium petroleiphilum* PM1 [7].

Aerobic Biodegradation - Naphthalene and Other PAHs:

Naphthalene Dioxygenase (NAH): Naphthalene dioxygenase incorporates both atoms of molecular oxygen into naphthalene to initiate aerobic metabolism of the compound. However, the broad substrate specificity of naphthalene dioxygenase has been widely noted. When expressed, naphthalene dioxygenase is capable of catalyzing the oxidation of larger PAHs like anthracene, phenanthrene, acenaphthylene, fluorene, and acenaphthene. For a more comprehensive list of reactions mediated by naphthalene dioxygenases, see the University of Minnesota Biocatalysis/Biodegradation Database. (http://eawag-bbd.ethz.ch/naph/ndo.html, [8]).

Phenanthrene Dioxygenases (PHN): The PHN assays quantify phenanthrene/naphthalene dioxygenase genes from a diverse collection of microorganisms including *Pseudomonas*, *Burkholderia*, *Sphingomonas*, and *Acidovorax* spp. As with other naphthalene dioxygenases, substrate specificity is relatively broad and phenanthrene dioxygenases have been implicated in the biodegradation of naphthalene, phenanthrene, and anthracene and the co-oxidation of larger PAHs. Moreover, at least one research group has suggested that the PHN group of phenanthrene/naphthalene dioxygenases may be more environmentally relevant than the classical *nah*-like naphthalene dioxygenase [9].

Aerobic Biodegradation - *n*-alkanes: The *n*-alkanes are a substantial portion of petroleum products and are a component of TPH concentrations. The QuantArray®-Petro also includes quantification of alkane monooxygenase genes (ALK) which allow a wide range of *Proteobacteria* and *Actinomycetals* to grow on *n*-alkanes with carbon lengths from C_5 to C_{16} [10]. The QuantArray®-Petro also includes a second type of alkane hydroxylase (almA) which catalyzes the aerobic biodegradation of longer chain alkanes (C_{20}-C_{32}) by some *Alcanivorax* spp. considered dominant in marine systems [11].

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Anaerobic Biodegradation - Benzene, Toluene, Ethylbenzene, and Xylenes (BTEX): BTEX compounds are also susceptible to biodegradation under anoxic and anaerobic conditions although biodegradation pathways for each compound are not as well characterized as aerobic pathways. The QuantArray®-Petro includes sets of assays targeting a number of upper and lower pathway functional genes involved in the anaerobic catabolism of BTEX compounds for better evaluation of anaerobic biodegradation at petroleum contaminated sites.

Benzylsuccinate Synthase (BSS): Of the BTEX compounds, toluene biodegradation under anaerobic conditions is the most extensively studied and best characterized. The first step in this pathway, mediated by benzylsuccinate synthase (*bss*A) is the addition of fumarate onto the toluene methyl group to form benzylsuccinate. While additional pathways are possible, some bacterial isolates capable of anaerobic biodegradation of ethylbenzene and xylenes follow the same metabolic approach where the first step is the addition of fumarate.

Anaerobic Benzene Carboxylase (ABC): Although additional pathways are possible, the only pathway for anaerobic biodegradation of benzene elucidated to date is initiated by a benzene carboxylase enzyme.

Benzoyl Coenzyme A Reductase (BCR): Benzoyl-CoA is the central intermediate in the anaerobic biodegradation of many aromatic hydrocarbons. Benzoyl-CoA Reductase (BCR) is the essential enzyme for reducing the benzene ring structure.

Anaerobic Biodegradation - PAHs: The anaerobic biodegradation of PAHs involves analogous mechanisms to those described for anaerobic biodegradation of BTEX compounds. For example, the anaerobic biodegradation of methyl-substituted PAHs like 2-methylnaphthalene is initiated by fumarate addition to the methyl group while the only characterized pathway for anaerobic naphthalene biodegradation is initiated by a carboxylase.

Naphthylmethylsuccinate Synthase (MNSSA): MNSSA is analogous to the benzylsuccinate synthase described above for anaerobic biodegradation of toluene. Naphthylmethylsuccinate synthase catalyzes the addition of fumarate onto the methyl group of 2-methylnaphthalene [12].

Anaerobic Naphthalene Carboxylase (ANC): To date, the only pathway that has been characterized for anaerobic biodegradation of naphthalene is initiated by a naphthalene carboxylase enzyme [13].

Anaerobic Biodegradation - *n*-alkanes: As mentioned previously, the *n*-alkanes are a substantial portion of petroleum products and should be considered particularly when site cleanup goals include TPH reduction. The addition of fumarate is a common mechanism for activating and initiating biodegradation of a variety of petroleum hydrocarbons under anaerobic conditions including *n*-alkanes. The QuantArray®-Petro includes quantification of alkyl succinate synthase genes (assA) which have been characterized in nitrate reducing and sulfate reducing isolates utilizing *n*-alkanes from C_6 to at least C_{18} [14].

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Project Manager: MIKE MCVEY
Project Name: SFCTC
Project No.: 6347002.03

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☒ No Additional Samples

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Sample Information					MICRO										COC										GEO										
MI ID (Laboratory Use Only)	Monitoring Well	Unit Type	Date Deployed	Date Retrieved	QuantArray-Petro	CENSUS-DNA (DHBt, DCAR)									COC	Dissolved Gases (MEE)									Anions										
039QK1	TWN-03	MNA	9/25/19		X	X									X	X									X										
2	TWN-03	Bio-Stim Nitrate, Sulfate	9/25/19		X	X									X	X									X										
3	TWN-03	Bio-Aug SDC-9, Sulfate	9/25/19		X	X									X	X									X										
4	SFCMW-10	MNA	9/25/19		X	X									X	X									X										
5	SFCMW-10	Bio-Stim Nitrate, Sulfate	9/25/19		X	X									X	X									X										
6	CMW-01	MNA	9/25/19		X	X									X	X									X										
7	CMW-01	Bio-Stim Nitrate, Sulfate	9/25/19		X	X									X	X									X										
8	MW-1R	MNA	9/25/19		X	X									X	X									X										
9	MW-1R	Bio-Stim Nitrate, Sulfate	9/25/19		X	X									X	X									X										
10	MW-1R	Bio-Stim Nitrate	9/25/19		X	X									X	X									X										

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Project Name: SFCJC
Project No.: 6347002.03

Purchase Order No. 19582
Subcontract No.

Please Check One:
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☒ No Additional Samples

Report Type: ☒ Standard (‹ ☐ Comprehensive (15% surcharge) ☐ Historical (30% surcharge)

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Please contact us prior to submitting samples regarding questions about the analyses you are requesting at (865) 573-8188 (8:00 am to 4:00 pm M-F). After these hours please call (865) 300-8053.

Sample Information					MICRO										COC									GEO										
MI ID (Laboratory Use Only)	Monitoring Well	Unit Type	Date Deployed	Date Retrieved	QuantArray-Petro	CENSUS-DNA (DHBt, DCAR)									COC	Dissolved Gases (MEE)								Anions										
039QK 11	MW-11	Standard Bio-Trap	9/25/19		X	X																												
12	MW-4R	Standard Bio-Trap	9/25/19		X	X																												

Relinquished by: [signature] AJ Hessak 11/13/19

In order for analysis to be completed correctly, it is vital that chain of custody is filled out correctly & that all relative information is provided. Failure to provide sufficient and/or correct information regarding reporting, invoicing & analyses requested information may result in delays for which MI will not be liable. * additional cost and sample preservation are associated with RNA samples.