FINAL REPORT

Assessment of Post Remediation Performance of a Biobarrier Oxygen Injection System at a Methyl Tert-Butyl Ether (MTBE)-Contaminated Site, Marine Corps Base Camp Pendleton San Diego, California

ESTCP Project ER-201588

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14. ABSTRACT

This Environmental Security Technology Certification Program (ESTCP)-funded project was performed to evaluate the long-term performance of monitored natural attenuation (MNA) at a site where natural attenuation of methyl tert-butyl ether (MTBE) is being used as a polishing step following in situ bioremediation. To achieve this goal, two primary objectives were identified:

-Evaluate the current microbial activity supporting natural attenuation of MTBE using a combination of conventional contaminant concentration data and geochemistry trend analyses and advanced molecular biological tools (MBTs), including metaproteomics and metagenomics.

-Assess the long-term impact of the biobarrier system on formation permeability.

In addition to evaluating data collected using conventional monitoring techniques, this project applied metagenomics and metaproteomics to improve the understanding of long-term impacts of the remedy on biodegradation at the site. Use of these advanced MBTs for quantification and detection of biomarkers, especially deoxyribonucleic acid (DNA) and peptides (protein fragments) in environmental samples has been rapidly expanding over the last few decades.

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ACRONYMS AND ABBREVIATIONS

Bgs	below ground surface
BTEX	benzene, toluene, ethylbenzene and total xylenes
COC	contaminant of concern
CSM	conceptual site model
CVOC	chlorinated volatile organic compound
DIPE	diisopropyl ether
DO	dissolved oxygen
DoD	U.S. Department of Defense
DON	Department of the Navy
EPA	Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
ETBE	ethyl-tert butyl ether
HIBA	2-hydroxy isobutyric acid
IAS	in situ air sparge
ID	identification
LC-MS/MS	liquid chromatography mass spectrometry
MBT	molecular biology tool
MCB	Marine Corps Base
MCL	maximum contaminant level
MCX	Marine Corps Exchange
MHP	2-methyl-2-hydroxy-1-propanol
MNA	monitored natural attenuation
MtBE	methyl tert-butyl ether
ORP	oxidation reduction potential
qPCR	quantitative polymerase chain reaction
ROI	radius of influence
RT	retention time
RWQCB	Regional Water Quality Control Board
SVE	soil vapor extraction
SWRCB	California State Water Resources Control Board
TAME	tertiary amyl methyl ether
TBA	tert-butyl alcohol

TBF	tert-butyl formate
TDS	total dissolved solids
TPH-G	total petroleum hydrocarbon quantified as gasoline
UST	underground storage tank

Acknowledgements

This report represents the results and conclusions from a collaborative effort between scientists and engineers at Naval Facilities Engineering and Expeditionary Warfare Center (NAVFAC EXWC) (Port Hueneme, CA) and Battelle Memorial Institute (Columbus, OH). This demonstration project was funded by the Environmental Security Technology Certification Program (ESTCP), with the goal of evaluating the long-term performance of natural attenuation of methyl tert butyl ether (MTBE) after shutdown of a biobarrier system. The utility of advanced molecular biology tools was evaluated in assessing natural attenuation. In addition, the long-term impact of the biobarrier system on formation permeability was assessed via slug tests.

Researchers for this project included Ms. Kenda Neil (Principle Investigator, NAVFAC EXWC), Dr. Katarzyna H. Kucharzyk (Battelle), Dr. Heather V. Rectanus (Battelle), Dr. Craig Bartling (Battelle), Pamela Chang (Battelle), and Tanwir Chaudhry (NAVFAC EXWC). Several personnel at Battelle Memorial Institute, including Larry Mullins and Angela Minard-Smith, were instrumental in assisting with metagenomic and metaproteomic data analysis and interpretation. We gratefully acknowledge Dr. Thomas McHugh (GSI Environmental, Inc.) and Steve Rosansky (Battelle) for their help with in-depth review of the final project report and offering valuable suggestions. Other site personnel that provided support include Derek Payne (Battelle). Laboratory extractions and analyses of groundwater samples for metagenomics were conducted by Nick Fackler (Battelle), Angela Minard-Smith (Battelle) and Nick Skomrock (Battelle). Slug test data analysis was performed by Michael Meyer (Battelle) with support of Dr. Heather V. Rectanus (Battelle).

Finally, the project team wishes to thank Dr. Andrea Leeson and the support staff from the ESTCP program office for their help and guidance throughout this demonstration.

EXECUTIVE SUMMARY

This Environmental Security Technology Certification Program (ESTCP)-funded project was performed to evaluate the long-term performance of monitored natural attenuation (MNA) at a site where natural attenuation of methyl tert-butyl ether (MTBE) is being used as a polishing step following in situ bioremediation. To achieve this goal, two primary objectives were identified:

- Evaluate the current microbial activity supporting natural attenuation of MTBE using a combination of conventional contaminant concentration data and geochemistry trend analyses and advanced molecular biological tools (MBTs), including metaproteomics and metagenomics.
- Assess the long-term impact of the biobarrier system on formation permeability.

In addition to evaluating data collected using conventional monitoring techniques, this project applied metagenomics and metaproteomics to improve the understanding of long-term impacts of the remedy on biodegradation at the site. Use of these advanced MBTs for quantification and detection of biomarkers, especially deoxyribonucleic acid (DNA) and peptides (protein fragments) in environmental samples has been rapidly expanding over the last few decades.

Unlike conventional MBTs, such as qPCR or microarrays, metagenomics provides insight into gene sequence information for whole communities. Metagenomic sequencing of environmental samples provides a comprehensive picture of all bacterial and archaeal sequences within a sample, not just those microorganisms targeted with qPCR assays. Providing a larger snapshot of microbial community composition not only allows detection of microorganisms related to the degradation of a specific chemical, but also has a potential to link composition of microbial consortia and geochemical characteristics of the site (Pérez-de-Mora, Zila et al. 2014).

Metaproteomics provides the most direct measure of microbial activity through detection of proteins of interest, providing information on molecular processes used by microorganisms. It can identify proteins encoded by genes in the metagenome and give a snapshot of community metabolic activities at the moment of sampling. The characterization of a proteome can be accomplished by interpreting mass-spectrometry-based peptide sequencing using data derived from 16S rRNA gene sequencing. In environmental metaproteomics, a predicted protein database constructed from metagenomic information of the exact same sample is required to assign peptide sequence information to proteins from which the peptides were derived.

Metagenomic and metaproteomics are cutting-edge environmental microbiological tools that are rapidly developing. With the availability of metagenomic sequences and the increasing number of complete individual bacterial and archaeal genome sequences, it is now possible to apply postgenomic techniques (particularly proteomics) to complex microbial communities. Combined, these powerful tools provide a capability to reveal the presence of specific proteins within the microbial community to provide direct evidence of specific pathways involved in the degradation of contaminants.

The site selected for this demonstration is the 22 Area Marine Corps Exchange (MCX) Gas Station site, located at the Marine Corps Base (MCB) Camp Pendleton, California. The treatment system,

consisting of a set of two biosparging biobarriers, was installed in 2004. Each barrier was comprised of a number of sparging wells used to inject oxygen into the aquifer. During operation of these biobarriers (from 2004 through 2010), MTBE concentrations in groundwater declined significantly such that only dilute levels of MTBE (i.e., 5 µg/L to 40 µg/L) remained. In 2010 and 2012, regulatory agencies agreed to discontinue operation of the mid-plume and leading-edge biobarrier, respectively. However, since low-levels of MTBE still existed at the site that exceeded the State of California's secondary maximum contaminant level (MCL) for MTBE (5 µg/L), the site was transitioned to MNA after shutdown of the biobarriers.

The technical approach for the evaluation at 22 MCX Area Gas Station was designed to test two specific hypotheses including:

1. Current microbial activity supports degradation of the remaining MTBE dissolved in groundwater, indicating that MNA is occurring.
2. Formation permeability within the ROI of the biobarriers decreased over time due to biofouling as a direct result of injecting oxygen into the aquifer.

Historical data combined with analytical results from two rounds of sampling performed during this demonstration were used to test the first hypothesis. The data collected consisted of results from conventional chemical (i.e., COC) concentrations and geochemical analyses (i.e., groundwater quality, anions/cations, etc.) and with metagenomics and metaproteomics. The second hypothesis was tested by performing several slug tests to assess the long-term impact of the biobarrier system on formation permeability and comparing the results to historical data measured before the biobarrier system was in operation.

To provide for a more comprehensive study, a decision was made to perform supplemental sampling and analysis at 13 Area Gas Station site, located approximately 2.5 miles from the 22 Area MCX Gas Station site. This second site, at which a soil vapor extraction and biosparging system is currently in operation to treat high concentrations of MTBE, was used as a positive control to allow for a comparison of measured metagenomic and metaproteomic results to assess where natural attenuation presumably is occurring to treat low residual concentrations.

To test the first hypothesis described above, a tiered approach was used to evaluate MNA at the 22 Area MCX Gas Station that relied on multiple, converging lines of evidence. This evaluation included evaluating contaminant concentrations (primary line of evidence) and geochemical trends (secondary line of evidence), as well as demonstrating and validating metagenomic and metaproteomic methods to determine MTBE degradation microbiology and activity at the site (tertiary line of evidence for MNA). The second hypothesis was tested by performing slug tests at several wells located at the 22 Area MCX Gas Station and comparing the results to similar data collected before the biobarriers were installed and in operation. Methods, results, and key findings for each are as follows.

Contaminant Concentrations – Primary Line of Evidence

A statistical analysis was conducted on the MTBE data at the 22 Area MCX Gas Station to determine if the plume has been increasing, decreasing or stable since the biosparging system was shut off. Additionally, concentrations of other contaminants of concern (COCs) including TPH-G, benzene, toluene, ethylbenzene and total xylenes (BTEX) and five oxygenates including:

MTBE, di-isopropyl ether (DIPE), ethyl tertiary butyl ether (ETBE), tertiary amyl methyl ether (TAME), and the intermediate degradation product, TBA, were analyzed and evaluated.

MTBE was detected in samples collected from five monitoring wells during both sampling events; however, the other oxygenates were not detected. During the first sampling event, MTBE concentrations ranged from 1.8 µg/L (at 22-MM-07) to 9.0 µg/L (at 22-BMW-15) and during the second sampling event MTBE concentrations ranged from 0.68 µg/L (at 22-MM-08) to 20 µg/L (at 22-BMW-0). The wells with the highest MTBE concentrations are located in between the biobarriers. These results are similar to historical data collected after shutdown of barriers prior to this investigation, at which time MTBE concentrations ranged from to 11 to 5.3 µg/L, indicating that MNA could be occurring to prevent further spread of the MTBE.

To better evaluate MTBE concentration trends, the Mann-Kendall Test was used to evaluate historical data along with the results from this investigation. Overall site trends (combining data collected, before, during and after the biosparge system was in operation), trends during active biosparging, and trends during the MNA phase of the remedy were evaluated. As expected, the overall site trends and the active treatment trends demonstrated a significant decrease in COCs. However, the analysis performed on data collected after the biosparge system was discontinued did not show a clear decreasing trend. Within the mid-plume biobarrier, the analysis showed the MTBE concentration at 22-BMW-11 is stable – neither increasing nor decreasing. Between the biobarriers, at wells 22-BMW-15 and 22-DMM-05, the analysis revealed no trend in the data. At the leading edge biobarrier well, 22-BMW-3, the analysis indicated a stable MTBE trend after system shutdown in 2012. Although not decreasing, these stable trends indicate that the rate of contaminant loading (advection and dissolution) is balanced with the rate of contaminant attenuation (degradation and sorption). ***Finding:*** Hence, these data provide the first line of evidence that MNA is sufficiently occurring to prevent migration and increased concentrations of the remaining MTBE.

Concentrations of the intermediate TBA were either near the detection limit or no TBA was detected over the last 15 years of monitoring, which may indicate that MTBE is not being degraded through this pathway. However, the absence of TBA does not rule out MTBE degradation via other degradation pathways. ***Finding:*** Thus, the presence/absence of intermediates did not provide a line of evidence for assessing MNA at the 22 Area MCX Gas Station.

For comparison purposes, samples also were collected from the 13 Area Gas Station and analyzed for COCs and intermediates. Concentrations of MTBE were confirmed to be much higher ranging from 42,000 µg/L to 2,800 µg/L; however, none of the other oxygenates (i.e., DIPE, ETBE, and TAME) were detected. A Mann-Kendall evaluation, performed as part of the annual groundwater monitoring program, confirmed a significantly decreasing trend of MTBE in most wells. ***Finding:*** These results are a strong line of evidence that treatment activities are effectively reducing MTBE concentrations, and therefore likely that MTBE degrading microorganisms would be detected via metagenomic and metaproteomic analyses.

Geochemical Trends – Secondary Line of Evidence

The secondary line of evidence is not intended to provide direct evidence that MTBE is/has been biodegraded. Rather, these data are collected to delineate biogeochemical processes at the site and

infer microbial activity related to contaminant biodegradation. This evaluation required analysis of a variety of geochemical parameters including such as oxidation-reduction potential (ORP), dissolved oxygen (DO), terminal electron acceptor indicators (e.g., nitrate, ferrous iron, sulfate/sulfide, methane), and various other anions and cations. Key results include:

- **DO and ORP:** Based on DO readings, the 22 Area MCX Gas Station was predominately anoxic during both sampling events. The exceptions were the wells located between the biobarriers, which showed oxic conditions during the first sampling event. The ORP varied spatially and temporally without any evident relationship to other redox parameters. Based on ORP data alone, the second sampling event indicated the site was anoxic except for well 22-BMW-3 located in the leading edge of the biobarrier where the ORP was 200 mV. However, the DO measured in well 22-BMW-3 was zero. Overall, ORP data did not indicate whether the wells were predominately oxic or anoxic because there was too much variation in data.

- **Nitrate**: Overall, nitrate levels were below their detection limits. Given the non-detectable levels of nitrate in the wells upgradient of the mid-plume barrier, nitrate is not expected to support MTBE biodegradation.

- **Ferrous Iron**: Both rounds of sampling showed that ferrous iron concentrations were near or below their detection limits (0.05 mg/L) at all locations. This indicated either iron reducing conditions have not been reached at the site or ferrous iron was removed as a precipitate with sulfide produced during sulfate reduction. Given the high levels of sulfate (>100 mg/L) and low levels of methane (<0.5 mg/L) in most wells, it is highly likely that iron reducing conditions have not occurred at this site.

- **Sulfate**: The levels of sulfate at the site did not indicate sulfate reduction has or is occurring. Furthermore, the levels of sulfide were non-detect. As such, it was not expected for sulfate reduction to support MTBE degradation.

- **Methane**: Methane levels were at or below the detection limit of 0.010 mg/L. As sites with dissolved methane concentrations near or greater than 0.5 ppm are termed methanogenic (Wilson, Smith et al. 1986), the 22 Area MCX Gas Station was not considered to be methanogenic.

Finding: Overall, the site appeared to be anoxic with no demonstrable levels of iron reduction, sulfate reduction or methanogenesis. As such, the geochemical data do not provide positive evidence that biodegradation may be contributing to MNA of MTBE.

For comparison purposes, geochemical data from the 13 Area Gas Station was used to evaluate the biosparging system performance. Historical process monitoring results indicate that while the biosparing system has been operating, the majority of the monitoring wells within 15 ft of a biosparge well have had DO concentrations greater than 2.0 mg/L, which is a recognized threshold for aerobic bioremediation to occur. However, only 2 of the 5 wells sampled during this demonstration had a DO value above this concentration, presumably due to rapid depletion of oxygen during the 24 hours that transpired between turning the system off and the time the samples

were collected. ***Finding:*** The rapid depletion of DO is a strong line of evidence that biodegradation (of MTBE or other petroleum constituents) is occurring.

ORP readings were varied at the site and did not indicate a trend in the source area and did not exhibit an apparent relationship with DO readings. In the mid-plume and at the leading edge of the plume, oxygenation by the biosparge system is limited, and overall the ORP levels indicated anoxic conditions during the second sampling event. Terminal electron acceptors for anaerobic processes (i.e., ferrous iron, sulfate/sulfide, and methane) in the source area reflect active biosparging in the source area and MNA in the downgradient portion of the plume. ***Finding:*** The electron acceptors in the mid-plume and leading edge wells indicate that natural attenuation of MTBE is contributing to contaminant decreases outside of the source area (active biosparge treatment zone).

Metagenomics and Metaproteomics – Tertiary Line of Evidence

At sites where MNA is difficult to demonstrate, tertiary lines of evidence may be necessary. For this project, the combination of metagenomics and metaproteomics was used to evaluate the microbial activity supporting natural attenuation. Metagenomic analysis can reveal the presence of MTBE-degrading species, which indicates the potential for bioremediation. Proteomics can reveal the presence of proteins, in known MTBE degradation pathways[1], which are actively degrading MTBE. The presence of these proteins is direct evidence that MTBE degradation is occurring and provides direct evidence that this metabolic pathway is active.

Groundwater samples were collected from each sample location from the 22 Area MCX Gas Station and 13 Area Gas Station sites for metagenomic processing and analysis. A list of most common direct and cometabolic MTBE degraders was compiled and used together with a custom-designed SILVA 16S reference database in order to perform analyses of microbial organisms in samples. The identified microorganisms served as a foundation to build a database for mass spectrometry (MS) spectra searching using BLASTn software, and were filtered to retain only those that provided the highest probability for a positive match of microorganism sequence to the database. The filtered results were then grouped to determine relative abundance of specific MTBE-degrading microbial species including: 1) direct, 2) cometabolic, 3) anaerobic and 4) aerobic degraders. This classification helped to address questions regarding the type of MTBE metabolism occurring at the site.

Metaproteomic analysis were performed using groundwater collected from the same locations as the samples for metagenomics analyses. Specific proteins were identified from liquid chromatography-mass spectrometer spectra by searching against a database of proteins sequences constructed from the results of the metagenomic sequencing. The data were queried against this database and searched against the library of known enzymes involved in MTBE degradation such as: monooxygenases, alkene hydroxylases, esterases and dehydrogenases and were statistically evaluated using Protein Pilot.

[1] Proteins responsible for the degradation of MTBE could be present; however, their contribution to MTBE degradation may not be known and documented in the literature.

Metagenomic Results

Samples collected from the 22 Area MCX Gas Station showed a diversity of both direct and cometabolic MTBE degraders. The metagenomes from samples located between the biobarriers were dominated by full MTBE mineralizers, which points towards a potential for its complete degradation. Although direct metabolizers were not present with high percentage in samples at upgradient and leading edge locations, the sequencing data showed higher abundance of cometabolic species that have an ability to carry out partial degradation of MTBE with parallel utilization of other substrates such as C_5 - C_8 n-alkanes.

A vast majority of microorganisms detected were aerobic MTBE degraders (both direct and cometabolizers). This is not unexpected since the site underwent sparging activities in the past. Moreover, the portion of the site that exhibited the highest relative abundance of aerobic species was between biobarriers and in the mid-plume location. ***Finding:*** These results suggest that the biobarrier installation and oxygen sparging activities impacted the microbiology of the site, enriching the aerobic population of MTBE degraders.

Metagenomic results of samples collected at the 13 Area Gas Station provide direct evidence to support MTBE aerobic degradation. The genera measured in source area well 1327-MW-01R indicate that the microbial population consists of highly abundant aerobic direct and cometabolic MTBE species. This finding is in agreement with rapidly decreasing DO concentrations and a decreasing trend of contaminant concentrations. On the contrary, sequencing data collected at the leading edge of the plume show little to no abundance of MTBE degrading species. However, within the mid-plume location, direct and cometabolic species are present, but with much lower abundance in comparison to the source zone. This result is in agreement with the geochemical data that show little or no DO, and ORP levels that indicated anoxic conditions within these two locations.

Finding: At both sites, the metagenomics line of evidence demonstrated the presence of MTBE-degrading species of microorganisms. Higher concentrations of aerobic microbes were observed in areas where active aeration is occurring or had occurred at the site.

Metaproteomic Results

Degradation activity was evaluated at both sites using shotgun metaproteomics where this activity is revealed by the presence of specific MTBE-degrading proteins. Data was categorized to determine: 1) presence of proteins from known MTBE degradation pathways (aerobic/anaerobic, cometabolic and direct MTBE mineralization), and 2) presence of proteins of known MTBE-degrading microorganisms. These two groups of proteins serve as indicators of MTBE degradation. While presence of proteins of known MTBE degradation pathways provides direct evidence of degradation, detection of proteins from known MTBE-degrading microorganisms serves as indirect evidence of degradation.

Overall, no MTBE degradation proteins were identified at the 22 Area MCX Gas Station; however, a few proteins from cometabolic MTBE-degrading microorganisms were present. In contrast, both groups of protein indicators were found at the 13 Area Gas Station. As such, the proteomic data do not provide positive evidence of active MNA of MTBE at the 22 Area MCX Gas Station, but confirm active MTBE degradation at the 13 Area Gas Station. These positive indicators of

bioremediation at the 13 Area Gas Station confirm that this site can serve a positive control for metaproteomic analysis.

Finding: These results demonstrate that no MTBE degradation was ongoing at the 22 Area MCX Gas Station. The proteomic data show direct evidence of MTBE degradation at the 13 Area Gas Station.

In summary, metageonomics and metaproteomics are two innovative techniques that have the potential to provide robust lines of evidence that degradation of COCs at a site continue to occur after an active remedy has been applied. At present, these techniques serve to augment conventional data, but may not be able to replace and/or reduce the frequency of application of conventional techniques at this time. The cost for these analyses for this demonstration were $350 and $1,800 per sample for the metagenomics and metaproteomic analyses, respectively, based on analysis of a batch of 7 samples. Cost for metagenomic analysis is not anticipated to decrease as the quantity of samples increases; however, the cost for the proteomic analysis will decrease as the number of samples increases. For instance, had 50 samples been analyzed during this demonstration, the resulting cost per sample would have been $750. It is expected as some of the implementation issues are overcome, including lack of widespread regulatory acceptance, lack of commercial laboratories that perform these types of analyses, techniques are refined, and confidence in the data improves, it is expected that cost will decrease substantially.

Assessment of Formation In n Permeability at 22 Area MCX Gas Station

Slug tests were performed in two of the same wells where slug tests were conducted in 2001 (i.e., 22-MM-07 and 22-MM-08), which are upgradient of the mid-plume biobarrier. Well 22-BMW-08 was selected as the third well for slug testing based on its proximity to, and similarity of construction with, well 22-MM-06, which was destroyed in 2012. To replicate the analysis performed on the slug tests conducted in 2001, the 2016 slug tests were analyzed following the Bouwer and Rice (Bouwer and Rice 1976) and Cooper et al. (Cooper, Bredehoeft et al. 1967) methods to estimate hydraulic conductivity. Both methods used to evaluate the slug test data rely on graphical curve matching to estimate the hydraulic conductivity of the formation adjacent to the well. To the extent possible, the curve matching approach shown in IT, 2001 (IT Corporation 2001) was replicated during evaluation of the 2016 data.

The geometric means of hydraulic conductivity values derived from multiple slug tests using multiple data reduction solutions were compared on a well-by-well basis with equivalent hydraulic conductivity values derived in 2001. This comparison shows that hydraulic conductivity values derived for each of the three wells based on 2001 and 2016 data were within a factor of two.

Finding: Given the variability inherent in slug testing and slug test data reduction, a factor of two difference is small enough to conclude that the hydraulic conductivity values are not meaningfully different before and after biosparging.

1. INTRODUCTION

1.1 BACKGROUND

The Department of Defense (DoD) has funded numerous pilot- and field-scale demonstrations of in situ remediation technologies over the past 20 years. While most of these projects included careful monitoring and assessment over the first few months up to a few years, these projects rarely included monitoring of long-term performance and addressed long-term impact issues. As a result, there is a lack of data from field sites evaluating the duration of treatment effects and the potential for long-term rebound. Given that active restoration efforts have been performed for over two decades, there is now an opportunity to collect long-term performance data and evaluate the conditions that foster and/or limit performance of different in situ remediation technologies.

One particular challenge is the treatment and subsequent long-term monitoring of sites contaminated with methyl tert-butyl ether (MTBE). MTBE has been used as a gasoline additive since the late 1970s as a replacement for tetraethyl lead to enhance fuel combustion efficiency and to lower emissions of carbon dioxide and other air pollutants. It is extremely water soluble and rapidly moves through soil and aquifers. Thus, leaks from underground storage tanks (USTs) used to store gasoline containing this additive have resulted in large MTBE-contaminated groundwater plumes that have become a concern for human health as a potential carcinogen. While great efforts have been made to remove MTBE from contaminated soil and groundwater, large dilute MTBE plumes remain at many sites.

The goal of this project was to evaluate long-term performance data at a site where natural attenuation of MTBE is being used as a polishing step following in situ bioremediation. In addition to evaluating data collected using conventional techniques, including analysis of contaminants of concern (COCs) and geochemical parameters, this project applied advanced molecular biological tools (MBTs), specifically metagenomics and metaproteomics, to improve the understanding of long-term impacts of the remedy on biodegradation at the site. In situ measurements of aquifer permeability also were included to evaluate the potential influence the remedy has had on groundwater hydraulics.

The site selected for this Environmental Security Technology Certification Program (ESTCP) demonstration is the 22 Area Marine Corps Exchange (MCX) Gas Station site, located at the Marine Corps Base (MCB) Camp Pendleton, California. The treatment system, consisting of a set of two biobarriers (**Figure 1-1**), was installed in 2004. Each barrier was comprised of a number of sparging wells used to inject oxygen into the aquifer. The system reduced concentrations of MTBE to between 5 µg/L to 40 µg/L. In 2010 and 2012, regulatory agencies agreed to discontinue operation of the mid-plume and leading-edge biobarrier, respectively. However, since low-levels of MTBE still existed at the site, which exceeded the State of California's secondary maximum contaminant level (MCL) for MTBE (5 µg/L), the site was transitioned to monitored natural attenuation (MNA) after shutdown of the biobarriers.

Use of Advanced MBTs for Assessing Biodegradation

At sites relying on MNA after active remediation is completed, performance assessment is typically conducted via measuring changes in contaminant concentration as a primary line of evidence. Geochemical and molecular biological analyses often supplement the assessment by serving as secondary and tertiary lines of evidence. The secondary line of evidence provides an evaluation of the geochemical environment to delineate biogeochemical processes and infer microbial activity at the site. The tertiary assessment informs on presence or absence of microbial populations potentially involved in biodegradation. Often conventional MBTs, such as quantitative polymerase chain reaction (qPCR) or microarrays, are used to determine gene abundance and assess if specific microbial populations are present in the aquifer. However, conventional MBTs provide only a measure of targeted microorganisms and do not provide a holistic understanding of the microbial community.

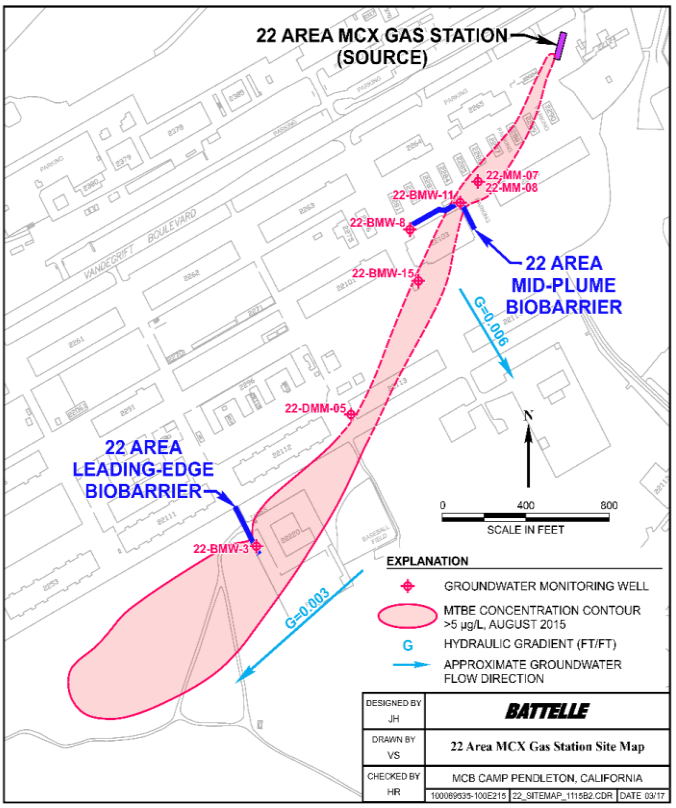

Figure 1-1. 22 MCX Gas Station Site Map

In the case of MTBE degradation, the value of conventional MBTs is limited because a wide variety of microorganisms can perform MTBE biotransformation. Moreover, conventional MBTs do not provide a *direct* measurement of microbial *activity*. Rather only the presence of the genes of the targeted microorganism(s) is provided, and gene presence reflects only the *potential* for microbial degradation. Because a lack of a global understanding of the microbial community as well as a direct measure of microbial activity cannot be achieved using conventional MBTs, there are critical data gaps to definitively confirm that biodegradation is occurring. Recognizing these information data gaps, this project worked to expand the MNA toolbox and include next-generation sequencing technology and high resolution mass spectrometry – specifically 16S metagenomics and metaproteomics – as tertiary lines of evidence to support direct measurement of the microbial community and activity in the subsurface.

Formation Permeability Assessment

At the 22 Area MCX Gas Station, historical data has indicated that there is a widening of the plume footprint as the plume moved towards the leading edge of the biobarrier although no increases in injection pressures were noted during the operation of the biobarrier. Thus, this project sought to improve the understanding of the impact of the biobarrier on the subsurface environment. Given that microbial growth within the radius of influence (ROI) of a biobarrier can lead to biofouling, evaluating changes to the porous media was assessed. These changes can be manifested by a

reduction in permeability of the formation, which can influence groundwater flow through the aquifer. Rather than collecting and analyzing soil samples for permeability, the results of which are oftentimes inaccurate due to sampling techniques and small sample sizes, hydraulic conductivity is often used to assess changes in the ease of groundwater flow. Measurements of hydraulic conductivity were determined by performing field slug tests.

1.2 OBJECTIVE OF THE DEMONSTRATION

The goal of this demonstration was to evaluate the long-term performance of natural attenuation of MTBE after shutdown of a biobarrier system. To achieve this goal, two primary objectives were identified:

- Evaluate current microbial activity supporting natural attenuation of MTBE combining conventional contaminant concentration and geochemistry trend analyses with the use of advanced MBTs – specifically metaproteomics and metagenomics.
- Assess the long-term impact of the biobarrier system on formation permeability.

These two primary objectives are presented in further detail in Section 3.0.

1.3 REGULATORY DRIVERS

The 22 Area MCX Gas Station is a leaking UST site with known groundwater impacts; hence, it is subject to various state regulations for petroleum sites including oversight by the California State Water Resources Control Board (SWRCB). In 2012, the SWRCB issued a policy to determine criteria for when site closure is appropriate for low-threat petroleum UST sites (SWRCB Resolution No. 2012-0016). The policy provides guidelines to cease long-term monitoring and to achieve no further action status at the site. The California SWRCB criteria for achieving low-threat closure are cited in the policy at the following address: http://www.swrcb.ca.gov/ust/lt_cls_plcy.shtml#policy081712.

Following shutdown of the biobarrier system, it was determined by the Regional Water Quality Control Board (RWQCB) San Diego that long-term monitoring was needed at the 22 Area MCX Gas Station. Long-term monitoring was expected to proceed until the appropriate remedial action objectives (RAOs) were met and low-threat closure could be achieved. The RAOs for groundwater at the site were defined as:

- Conduct active groundwater remediation through engineered applications until “low-risk” criteria are met;
- Monitor dissolved concentrations of fuel constituents and compare to proposed cleanup goals; and
- Monitor dissolved hydrocarbon plumes for migration.

Because groundwater in this area of MCB Camp Pendleton is designated as a potential source of drinking water, the cleanup goals for constituents of concern cannot exceed MCLs for drinking water, which is 13 µg/L for MTBE. SWRCB’s Non-Degradation Policy applies to the impacted aquifer beneath the site (SWRCB Resolution No. 68-16). In addition, the Water Quality Control Plan for the San Diego Basin, hereafter referred to as the Basin Plan ((San Diego Department of

Environmental Health 2014), includes a narrative secondary water quality goal designed to protect water resources against undesirable taste and odor. The secondary MCL for MTBE is 5 µg/L. Because secondary MCLs defined in the Basin Plan for toluene, ethylbenzene, xylenes, and MTBE are lower than the respective primary MCLs, groundwater cleanup goals for these constituents must meet the respective secondary MCLs.

The SWRCB policy cited above does support the closure of low-threat UST sites where contaminant levels in groundwater are above clean-up criteria, but otherwise present little or no risk. As part of a petition for site closure, RWQCB San Diego requested additional information to demonstrate the "stability and immobility of the plume's leading edge." For this site, a demonstration of the on-going degradation of MTBE and its ability to stabilize the plume under a range of site conditions is part of the requirement to achieve low-threat closure and is the main regulatory driver for conducting this study.

2.0 TECHNOLOGY

2.1 TECHNOLOGY DESCRIPTION

MTBE degradation and the fundamentals of two advanced MBTs – metaproteomics and metagenomics – are described to better understand the natural microbial processes that are occurring to degrade MTBE.

2.1.1 MTBE DEGRADATION

Biodegradation of MTBE can occur either by direct metabolism or co-metabolism with another chemical substrate (Deeb, Scow et al. 2000). While pathways for aerobic and anaerobic degradation have been elucidated, the aerobic degradation pathway has been documented to a greater extent at numerous field sites (Deeb, Scow et al. 2000; Hristova, Gebreyesus et al. 2003), and is therefore the primary focus of this discussion.

In direct metabolism, microbial organisms utilize MTBE as a sole carbon and energy source for growth in a series of reactions (**Figure 2-1**). First, the MTBE ether bond is cleaved to form tert-butyl alcohol (TBA) and formaldehyde as main metabolic intermediates. Next, TBA transforms into 2-methyl-2-hydroxy-1-propanol and 2-hydroxyisobutyric acid (HIBA). Other MTBE degradation intermediates include 2-propanol, acetone, hydroxyacetone or 2,3-dihydroxy-2-methypropionate and lactate (Steffan, McClay et al. 1997). MTBE-utilizing bacteria include aerobes, such as *Methylibium petroleiphilum* PM1 (Hanson, Ackerman et al. 1999), *Aquincola tertiaricarbonis* L108 (Müller, Rohwerder et al. 2008), and *Hydrogenophaga flava* ENV 735 (Streger, Vainberg et al. 2002) and anaerobes such as *Aquincola* (Müller, Rohwerder et al. 2008) and *Cupriavidus*. Only a few pure strains of aerobes have been cultured to date and have been observed to grow on MTBE at a relatively slow rate (Deeb, Scow et al. 2000).

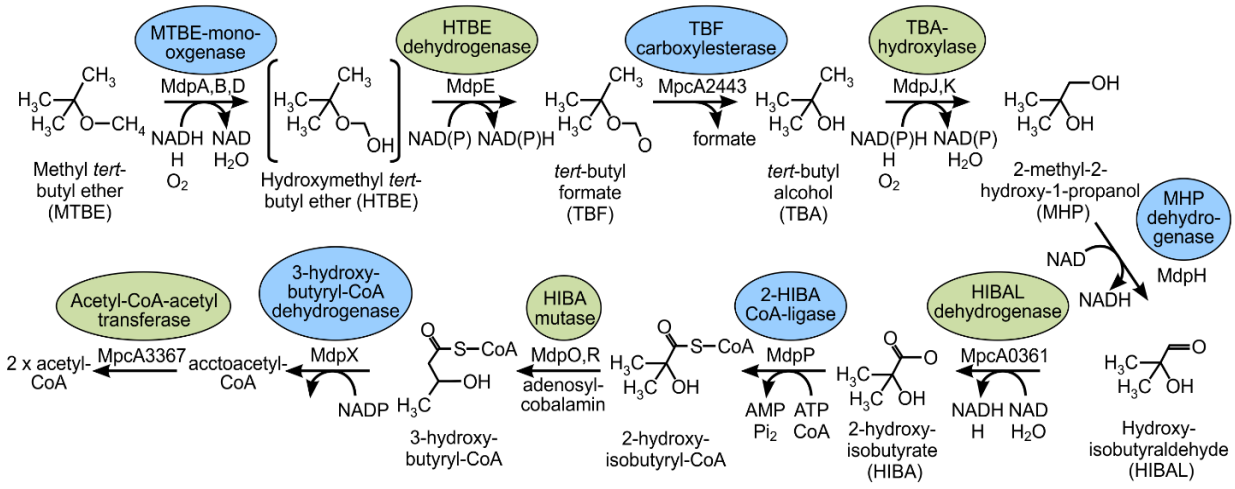

Figure 2.0-1. Peptides Involved in Degradation of Gasoline and Aromatic Compounds in *M. petropleiphilum* PM1(Hristova, Gebreyesus et al. 2003; Schmidt, Battaglia et al. 2008)

In cometabolic scenarios, oxidation of MTBE occurs in conjunction with degradation of another chemical substrate such as n-alkanes and branched alkanes e.g.., propane, butane or methane (Wilson, Smith et al. 1986; Ferreira, Malandain et al. 2006), but not with aromatic compounds (Deeb, Scow et al. 2000). Aerobic cometabolic MTBE degradation by alkane-oxidizing bacteria, such as *Pseudomonas*, *Rhodococcus*, *Mycobacterium*, *Enterobacter* and *Achromobacter* has been confirmed (Smith, O'Reilly et al. 2003; Schmidt, Schirmer et al. 2004; Eixarch and Constantí 2010). **Figure 2-2** illustrates cometabolic oxidation of MTBE by *Mycobacterium austroafricanum* JOB5, which uses propane as a carbon source. In the first reaction, MTBE is oxidized by the same enzyme responsible for propane oxidation – short chain alkane monooxygenase (SCAM). Next, the unstable hemiacetal is oxidized to tert-butyl formate (TBF) by alcohol dehydrogenase (ADH) followed by esterase (EST)-catalyzed hydrolysis of TBF to TBA (Smith, O'Reilly et al. 2003; Digabel and Fayolle-Guichard 2015).

The intermediate product, TBA, often accumulates and increases the toxicity of the aquifer. Therefore, before an MTBE bioremediation strategy can be used, an assessment of the risks associated with the accumulation of its breakdown products is essential. In addition to TBA, intermediate products of direct metabolism or co-metabolism of MTBE include TBF, HIBA, 2-methyl-2-hydroxyl-1-propanol and acetone (Fayolle, Vandecasteele et al. 2001; Digabel and Fayolle-Guichard 2015). Persistence of these intermediate species can be variable and depends on the rate-limiting step in their production and degradation, geochemical conditions, and the composition of the in situ microbial community (Ferreira, Malandain et al. 2006; Nava, Morales et al. 2007; Müller, Rohwerder et al. 2008).

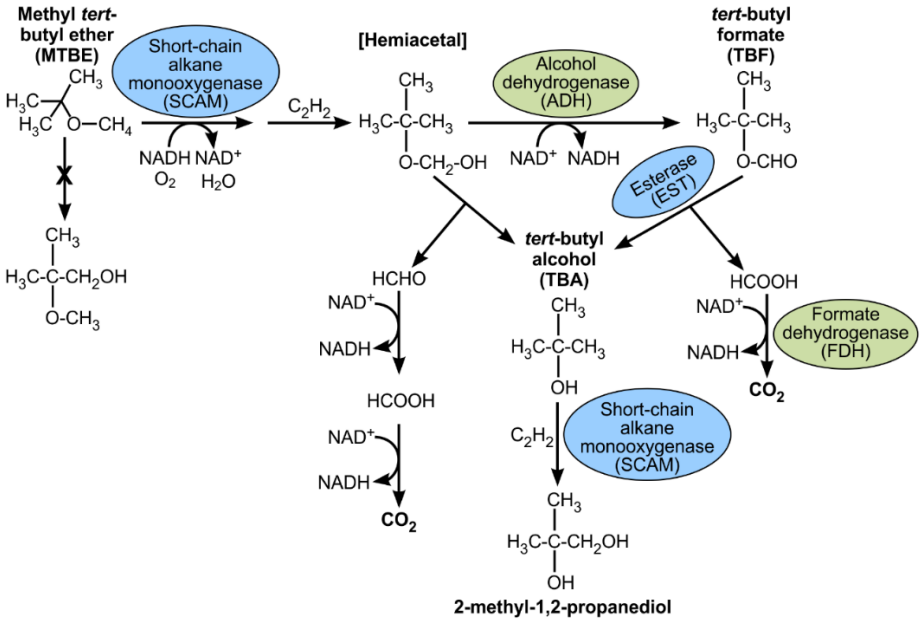

Figure 2.0-2. Initial Reactions and Corresponding Enzymes Determined in Cometabolic Degradation of MTBE by Propane-Grown *Mycobacterium austroafricanum* JOB (Smith, O'Reilly et al. 2003; Nava, Morales et al. 2007)

Several enzymes, summarized in **Table 2-1**, are involved in direct and cometabolic degradation of MTBE and other oxygenates such as TBA.

Table 2.0-1. Selected MTBE Degradation Enzymes

Microorganism	MTBE Mineralization	Reaction	Enzyme	Growth Compound
Direct metabolism				
Hydrogenophaga flava ENV735	TBA stoichiometric accumulation	MTBE → TBA	Constitutive MO	MTBE and TBA
Mycobacterium austroafricanum	TBA stoichiometric accumulation	MTBE → TBA TBA → HIBA HIBA → CO_2	MO No-heme MO No-heme Co^{2+} dependent	MTBE and TBA
Co-metabolism				
Rhodococcus rubber	TBA stoichiometric accumulation	MTBE → TBA TBA → CO_2	Cytochrome P450	Ethanol
Gordonia terrae ENV425 strain	TBA stoichiometric accumulation	MTBE → TBA	P450 monooxygenase	Propane
Graphium sp.	TBA stoichiometric accumulation	MTBE → TBA TBF → TBA	Cytochrome P450 Biotic/Abiotic	n-Butane
Xanthobacter sp.	TBA stoichiometric accumulation	MTBE → TBA	Cytochrome P450	Alkanes C_3-C_6
Mycobacterium vaccae JOB5	TBF and TBA accumulation	MTBE → TBA TBA → MPDiol	MO	Propane
M1-P Pseudomonas sp.	TBA accumulation	MTBE → TBA TBA → CO_2	N.D.	Pentane
Pseudomonas aeruginosa	TBA accumulation	MTBE → TBA	N.D.	Pentane
Pseudomonas putida GPo1	TBA accumulation	MTBE → TBA	MO	Octane

(Adapted from (Nava, Morales et al. 2007)
N.D. Not determined
MO - Monooxygenase,
MP-Diol- 2 methyl, 1,2 propanediol,

Three classes of enzymes in particular have been proposed to play a significant role (Ferreira, Malandain et al. 2006; Kane, Chakicherla et al. 2007; Müller, Rohwerder et al. 2008; Bastida, Rosell et al. 2010; Schuster, Purswani et al. 2013), including:

- **Alkane monooxygenase**: These monooxygenases are specific for *n*-alkanes (C_5 to C_{12}) and alkanes-related molecules (Smith, O'Reilly et al. 2003). The monooxygenases are a part of an alkane degradation system organized in two operons: *alkBFGHIJK* and *alkST* region. The non-heme monooxygenase (AlkB) requires rubredoxin with a non-heme iron atom and a flavin adenine dinucleotide (FAD)-dependent rubredoxin reductase to transfer electron from dioxygen to the substrate (Ferreira, Malandain et al. 2006). AlkB has a broad substrate specificity with ability to oxidase MTBE but not TBA.
- **Esterase**: The role of esterase is essential in early steps of the MTBE metabolic pathway, functioning to hydrolyze TBF to TBA. In some studies (Eixarch and Constantí 2010; Chen, Chen et al. 2011) TBF was not always detected as an intermediate, possibly due to either

rapid degradation by an efficient esterase or the degradation through dismutation reaction rather than dehydrogenation.

- **Dehydrogenase**: A cluster of *mpd* genes is involved in the conversion of 2-methyl 1,2-propanediol to HIBA, including the enzymes alcohol dehydrogenase and aldehyde dehydrogenase, which are strongly expressed during the growth of *Mycobacterium austroafricanum* strains on MTBE (Ferreira, Malandain et al. 2006).

2.1.2 MOLECULAR BIOLOGICAL TOOLS

Use of MBTs for quantification and detection of biomarkers, especially deoxyribonucleic acid (DNA), peptides (protein fragments), proteins and lipids, in environmental samples has been rapidly increasing over the last few decades. MBTs are being used by remediation professionals to aid remedial design, assess remedial performance, and perform long-term monitoring of biologically-based degradation technologies. The goal of MBT application is measuring temporal and special changes of microorganisms and their activity. The quantitative level of information is invaluable to understand and interpret contaminant biodegradation.

Conventional MBTs typically used in microbial diagnostics include qPCR and microarrays. qPCR provides information on the abundance of target organisms and specific genes. Genes commonly identified using this technique typically are the functional genes responsible for the production of enzymes (proteins) that can break down the contaminant of interest (Morey, Ryan et al. 2006; Pradervand, Weber et al. 2010). qPCR can therefore determine if the organisms responsible for biodegradation are abundant and if the potential for biotic MNA pathways exist at a site. To use qPCR effectively, the practitioner must know the specific organism and gene that should be present if biodegradation is occurring.

Microarrays differ from qPCR in that they are less specific. They provide the capability to simultaneously detect and semi-quantitatively measure thousands of biomarker genes. By utilizing microarrays to evaluate the microbial community at a site, information on the community's ability to degrade contaminants can be determined (Morey, Ryan et al. 2006; Git, Dvinge et al. 2010). This technique allows multiple genes to be simultaneously monitored using one microarray; however, each microarray must be designed to target a specific population (Git, Dvinge et al. 2010). This tool is useful to assess the overall change of a community in response to physical, chemical, and biological changes resulting from application of a remedy.

Most recent efforts have extended genome-based science by large-scale genome sequencing, often called metagenomics, which provides an insight into whole community sequence information on microbial members from various ecological communities. Integration of a protein component of these microbial communities (metaproteomics or whole community proteomics) seeks to identify functional expression of the metagenome and gives a snapshot of community metabolic activities at the moment of sampling. While metagenomic sequencing can define the microbial and/or gene composition and inform about the potential molecular machinery, it does not reveal details on its actual function. Metaproteomics provides the most direct measure of microbial activity. It allows detection of proteins of interest, providing information on molecular processes utilized by microorganisms to sustain the metabolic processes required for life (Daffonchio, Ferrer et al.

2013). These two techniques represent the cutting-edge of experimental genome science and each are rapidly developing.

A typical metagenome analysis of environmental samples, whether through global (referred to as "shotgun") or amplicon-targeting of specific genes, provides a comprehensive picture of all bacterial and archaeal sequences within a sample, not just those microorganisms targeted with qPCR assays, without the need to culture microorganisms in the laboratory. Providing a larger snapshot of microbial community composition versus focusing only on a single species' metabolism aids in the understanding of contaminant degradation in a dynamic environmental setting. High-throughput sequencing of bacterial and archaeal 16S rDNA not only allows detection of microorganisms related to the degradation of a specific chemical, but also has a potential to link composition of microbial consortia and geochemical characteristics of the site (Pérez-de-Mora, Zila et al. 2014).

In metagenomic workflow (**Figure 2-3**), the amplification of 16S rDNA genes is performed with specific primers and is followed by analysis with a selected sequencing platform (e.g., Illumina). To analyze the sequencing data, a microbial taxonomy dependent approach is applied to search sequences against a 16S rRNA reference database (e.g., Ribosomal Database Project [RDP] or SILVA), and sequences that fail to match the reference database are discarded. Further analysis of taxonomic abundance and microbial diversity can be performed using various techniques (e.g., Shannon diversity index) to inform on sample richness.

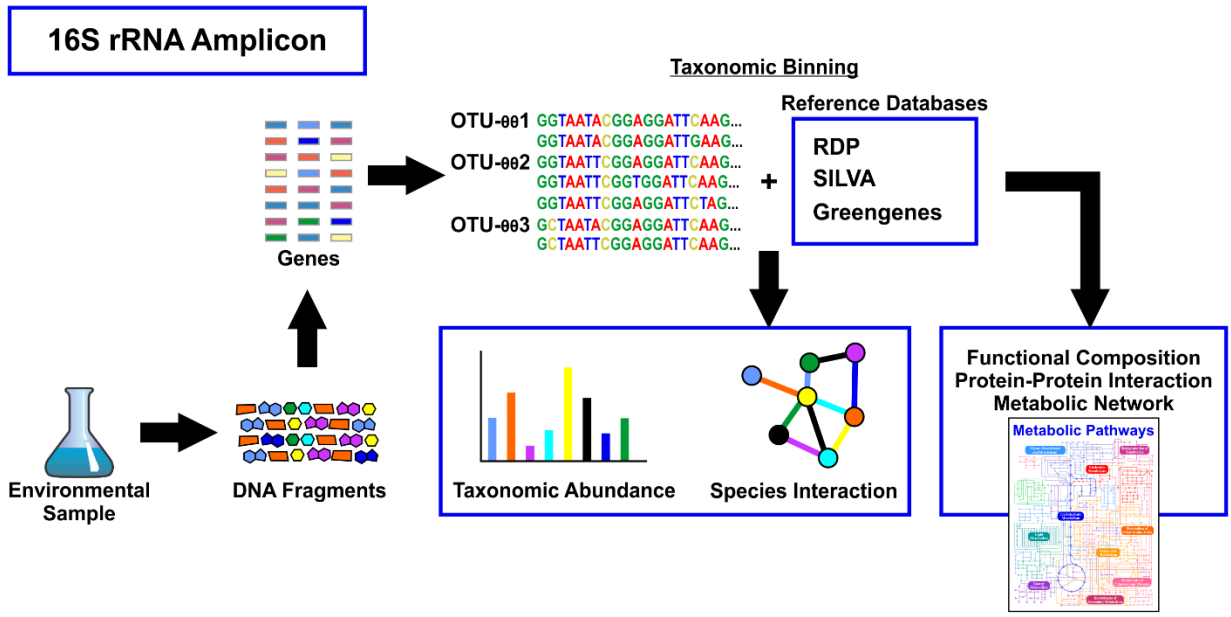

Figure 2.0-3. Metagenomic Workflow for Environmental Samples

With the availability of metagenomic sequences and the increasing number of complete individual bacterial and archaeal genome sequences that have been identified and catalogued, it is now possible to apply postgenomic techniques (particularly proteomics) to complex microbial communities. Metaproteomics provides insight into the functionality of environmental genomes and helps to achieve a major goal of environmental microbiology – the ability to link individual

microbial species to specific function. It further describes the community at its functional level by characterization of the global proteome in the sample (shotgun proteomics) or targeting specific proteins of interest (Daffonchio, Ferrer et al. 2013). The large-scale characterization of a proteome is accomplished by comparing measured protein or peptide data with predicted protein or peptide data derived from the 16S sequencing of the metagenome. In environmental metaproteomics, a predicted protein database constructed from metagenomic information of the exact same sample is required to properly assign peptide sequence information to proteins from which the peptides were derived. The following requirements need to be met for successful proteomic measurement: high throughput processing of samples, detection of protein/peptide in samples, large dynamic range of peptide detection, instrumentation ability to deal with very complex mixtures, accurate mass measurements for peptides of interest, and ability to structurally characterize peptide sequences (Zhang, VerBerkmoes et al. 2006; Keller and Hettich 2009; VerBerkmoes, Denef et al. 2009). The proteomic workflow (**Figure 2-4**) consists of protein extraction, protein trypsin digestion that results in formation of shorter tryptic peptides, separation of peptides with mass spectrometry and data analysis.

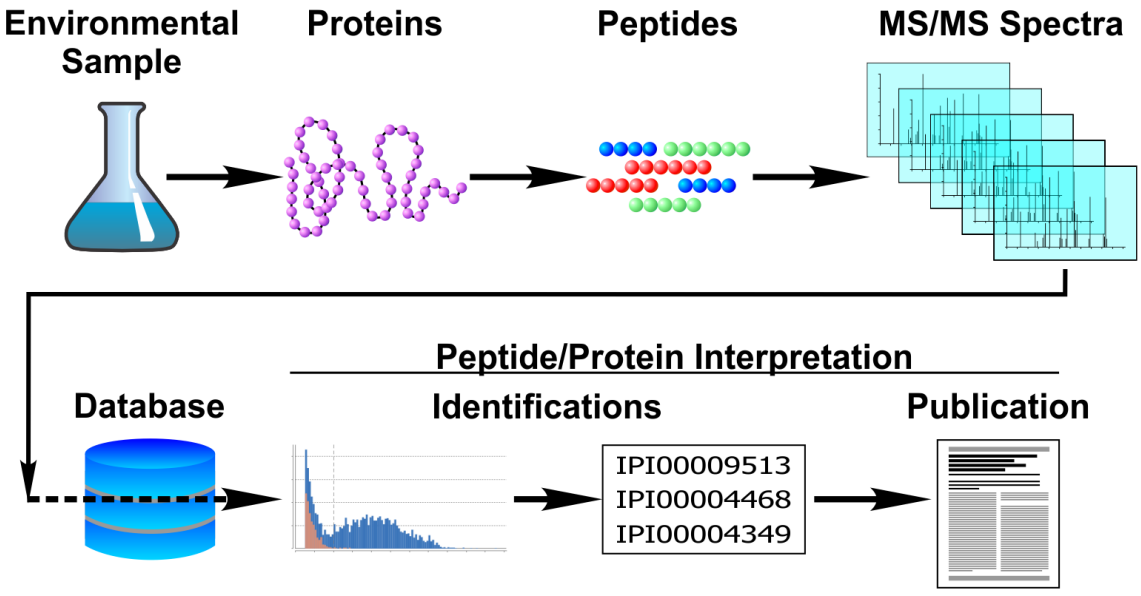

Figure 2.0-4. Metaproteomic Workflow for Environmental Samples

Recently published studies, the combination of metagenomics and metaproteomics can provide valuable insights into the activity of different microbial groups and specific proteins (including those involved in biodegradation) in different environments (Lo, Denef et al. 2007; Wilmes, Andersson et al. 2008; Denef, VerBerkmoes et al. 2009; Goltsman, Denef et al. 2009). Combined these powerful tools provides a capability to reveal the presence of specific proteins compared to the abundance of microbial species in the metagenome. The resulting data are especially useful in scenarios where qPCR does not provide sufficient evidence of the presence of genes involved in specific pathways related to the degradation of contaminants. The combined metagenomics/metaproteomic approach also can assign functional attributes (such as gene expression) to microbial communities for better understanding of degradation activity on site.

2.1.3 APPLICATION OF MBTS TO EVALUATE MTBE DEGRADATION

MBTs have enabled the identification of key microorganisms and functional genes involved in MTBE degradation in environmental samples (Aslett, Haas et al. 2011; Hicks, Schmidt et al. 2014). Specific qPCR tools and associated primers that enumerate MTBE degrading *Methylibium petroleiphilum* PM1 and other functional genes involved in aerobic and anaerobic degradation exist and provide information about MTBE degradation steps (**Figure 2-1**) and detoxification potential (Nakatsu, Hristova et al. 2006; Kane, Chakicherla et al. 2007).

Metagenomic and proteomic technologies can be used to facilitate an understanding of MTBE biodegradation processes including evaluating the microbial MTBE–degrading community and identifying proteins present in the sample at the time of collection. Estimates of the activity of specific contaminant-degrading microorganisms has provided definitive evidence of biological degradation. For this project, MTBE biodegradation activity was evaluated with the use of proteomics and metagenomics during long-term monitoring after operation of the biobarriers had been discontinued.

2.1.4 SLUG TEST PERFORMANCE

To determine horizontal hydraulic conductivity, a common field test called a slug test can be performed. During a slug test, the water level in a well is changed rapidly by displacement, and then the rate of water-level response to that change is monitored until equilibrium conditions return. The water-level data, along with subsurface and groundwater properties, are used to calculate the horizontal hydraulic conductivity K in feet per day (ft/d). A slug test requires a rapid ("instantaneous") water-level change and measurement of the water-level response at high frequency. A rapid change in water level can be induced in many ways, including injecting or withdrawing water, increasing or decreasing air pressure in the well casing, or adding a mechanical device such as a plastic rod to displace water. Water-level changes can be measured with many methods, including steel tape, electric tape, airline, wireline/float, and submersible pressure transducers. One of the most common methods in use is displacing groundwater with a mechanical slug, measuring groundwater levels with a submersible in-well pressure transducer, and recording water levels with a data logger. This method combines ease of use, accuracy, and rapidity of water-level measurement. This project utilized a mechanical slug that enabled the measurement of groundwater level changes upon introducing the slug (slug-in) and withdrawing the slug (slug-out).

2.2 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

2.2.1 ADVANTAGES AND LIMITATIONS OF METAPROTEOMICS AND METAGENOMICS

The two main advantages of the application of advanced MBTs such as metaproteomics and metagenomics are: 1) the ability to measure microbial activity (and not just potential for activity), and 2) the ability to generate detailed information on hundreds of microorganisms, genes, and proteins in one assay. The process of obtaining the information does not require culturing of the microorganisms and performing molecular end-point assays. While culture-based approaches such as quantitative qPCR have successfully identified genes involved in MTBE degradation,

metagenomics approaches provide the ability to study an entire genome of complex microbial ecosystems. Sequenced-based metagenomics offer significant promise to identify novel functionalities residing within the unculturable fraction of the microbiome.

The assessment of MTBE-degrading populations is usually based on the enumeration of 16S rRNA genes using qPCR to identify populations of MTBE degraders and other microorganisms of interest, as well as target specific degradation genes such as HIBA mutase, P450 cytochrome monooxygenase or MTBE monooxygenases. While 16S rRNA gene and specific gene copy numbers provide useful abundance information, these measures do not necessarily correlate with microbial activity, which is a major limitation of qPCR technology. To measure values that are directly correlated to microbial activity, metaproteomics can be used to identify and quantify proteins. These proteins provide important information on community activity, such as which microbial organisms are most active, and what proteins are expressed (including contaminant-degrading proteins) (Arsène-Ploetze, Bertin et al. 2015).

Limitations associated with this technology are related to 1) 16S sequencing, 2) composition of the proteome to be analyzed, mainly concerning protein expression levels and 3) limitations of the analytical equipment. Limitations are summarized as follows:

16S Sequencing

- The inability to identify a known microorganism is limited due to the lack of established references in the database. However, as research continues, it is expected that the database of known organisms will continue to expand and be much more robust in the future.

- The short length of reads from the 16S Illumina sequencing platform may present a limitation, although 16S rDNA reads as short as 100 base pairs (bp) can be sufficient for an accurate taxonomic characterization of microbial communities. Those read-length limitations may decrease with use of sequencers that produce longer read lengths (e.g., HiSeq2500 or comparable).

- Another limitation may be related to characteristics of the 16S rDNA. The genes coding for it are referred to as 16S rRNA gene and are used in reconstructing phylogenies, due to the slow rates of evolution of this region of the gene. Diversity metrics and classification accuracy depends on which region of the gene is being used. 16S rDNA gene fragments extracted from metagenomes will randomly cover different areas of the gene, thus providing a mixed taxonomic and evolutionary signal. Using different regions may allow reconstructing the whole 16S rDNA sequence which could improve diversity analyses.

Composition of the Proteome

- Sample preparation for proteomic analysis is one of the most important and difficult steps in the analytical workflow. The vast diversity of protein molecular sizes, charge states, conformational states, post-translational modifications etc., make it unfeasible to use a single sample preparation protocol that captures the entire proteome for a given microbial community. Thus, use of a protocol that allows isolation of protein content from a

biological sample and eliminates non-specific contaminants (e.g., keratins, fatty acids, plastic polymers, nucleic acids and salt clusters) should be developed and tested prior to sample analysis.

- Proteins are not expressed in equal amounts and there may be large differences in protein levels in proteomes in samples collected from the same site. A proteomic analysis has to employ proper technologies for the detection of all proteins or proteins of interest. In a small sample volume that is usually used in a proteomic analysis, a large percentage of the expressed proteins occur in low abundance levels and cannot be readily detected during analysis. These proteins are usually of particular interest in environmental samples because many times proteins associated with contaminant degradation occur in small quantities and are a very small fraction of the total expressed proteins. The practical protein amount for LC-MS/MS-TOF analysis lies in the femtomol (10^{-15} [fmol]) range. However, due to losses during protein extraction and sample clean up and dilution, the sufficient protein concentration for detection should be in the low picomol (10^{-12} [pmol]) to high fmol range. This limitation can be partially addressed by collecting a greater volume of groundwater for analysis; however, this requires additional field time.

- Detection of proteins at low concentrations (low-abundance) may be limited by presence of other proteins present in high concentrations (high abundance). The successful search for low-abundance proteins may be mitigated by use of chromatography for separation of high-abundance proteins and precipitation for elution of proteins of interest prior to analytical detection. However, complete removal of high-abundance proteins may not be recommended because they may trap the low-abundance proteins along with their associated fragments and peptides, which will be lost and not detected. An alternative approach that relies on 2-dimensional chromatography coupled with tandem mass spectrometry may be considered as an alternative method to overcome this limitation.

Analytical Equipment and Methodology

- Success in the identification of proteins may vary with the sensitivity of the mass spectrometer, completeness of the database and presence of post-translational modifications. Of the most sensitive mass spectrometers, electrospray ionization and laser desorption ionization-based instruments have the ability to detect peptides with low detection limits. To mitigate issues related to the lack of sensitivity, proper instrumentation needs to be used.

- Selection of the proteomic method for the experiment strongly depends on the success of the sample to be analyzed and the goal of the study. The risk of application of using an incorrect method is mitigated by preliminary work performed for the enrichment of the low-abundance proteins, extraction of proteins and tuning of the analytical equipment.

2.2.2 ADVANTAGES AND LIMITATIONS OF SLUG TESTING

Slug tests are often used in place of aquifer tests to determine hydraulic conductivities. The field methodology and analysis procedures to perform these tests are well known and documented (Cooper, Bredehoeft et al. 1967; Bouwer and Rice 1976). Advantages of slug tests include:

- Low cost and short test duration
- Easily applied to existing monitoring wells; and
- Appropriate for use in the lithology of the site.

The main advantage for using slug tests in this project was to collect similar data to historical data generated by performing slug tests in 2001, prior to installation of the biobarriers. The results of the 2001 slug tests provided baseline hydraulic conductivity values from which to assess this project's slug test results.

There are a few limitations associated with slug tests:

- Represents the area immediately surrounding the well which is a small portion of the aquifer;
- May not be representative of the average hydraulic conductivity of the aquifer due to heterogeneities; and
- Influenced by well filter packs in well bore hole.

3.0 TECHNICAL APPROACH OVERVIEW

The technical approach for this demonstration was designed to test two specific hypotheses including:

1. Current microbial activity supports degradation of the remaining MTBE dissolved in groundwater.
2. Formation permeability within the ROI of the biobarriers decreased over time due to biofouling as a direct result of injecting oxygen into the aquifer.

Historical data combined with analytical results from two rounds of sampling performed during this demonstration were used to test the first hypothesis. The data collected consisted of results from conventional chemical (i.e., COC) concentrations and geochemical analyses (i.e., groundwater quality, anions/cations, etc.) along with advanced MBTs, specifically metagenomics and metaproteomics. In addition, a second nearby Camp Pendleton site, 13 Area Gas Station, was identified and used as a positive control for the advanced MBT analyses. The 13 Area Gas Station has similar lithologic and hydrogeologic conditions to the 22 Area MCX Gas Station. However, MTBE concentrations are much higher and a biosparge system is currently operating to address the plume. Differences in the composition of the microbial community between the two sites can provide valuable insight with respect to applying advanced MBTs to monitor natural attenuation as compared to monitoring active remediation.

The second hypothesis was tested by performing several slug tests to assess the long-term impact of the biobarrier system on formation permeability and comparing the results to historical data measured before the biobarrier system was in operation. These hypotheses are discussed in detail below.

3.1 EVALUATE MICROBIAL ACTIVITY SUPPORTING NATURAL ATTENUATION OF MTBE

The first hypothesis was tested using a multiple line of evidence approach that included metaproteomics and metagenomics in addition to chemical and geochemical analyses to evaluate natural attenuation of MTBE at the 22 Area MCX Gas Station. Results of analyses of groundwater samples collected from locations with active MTBE degradation were compared to results from locations where no MTBE degradation activity is known to be occurring. Specifically:

- The first two lines of evidence for MNA evaluated the plume behavior (i.e., decreasing, stable or increasing) and then assessed the geochemical data. In the first line of evidence, a statistical analysis was conducted using contaminant concentration data collected since the biobarrier system was shut off. Then, geochemical data were assessed to delineate biogeochemical processes at the site and infer microbial activity related to contaminant biodegradation. Neither of these lines of evidence directly demonstrate microbial activity; it can only be inferred.
- The **potential** for MTBE degradation was evaluated based on the diversity of microorganisms detected in groundwater samples determined using a 16S metagenomic sequencing approach. Based on the information summarized in **Table D-1** on microbial species, metagenomic data were grouped to determine relative abundance of specific MTBE-

degrading microbial species qualified into four main groups: 1) direct, 2) cometabolic, 3) anaerobic, and 4) aerobic.

- The occurrence of degradation **activity** was evaluated using metaproteomics. Proteomic data were searched by using specific targets representing enzyme classes related to MTBE degradation (e.g., monooxygenases, alkene hydroxylases, esterases and dehydrogenases). Positive activity was revealed by the presence of specific MTBE-degrading proteins. General proteins from MTBE-degrading bacteria were used as indicators for the population to be alive; however, they were not used as direct evidence of MTBE degradation. Global proteomic analyses were performed on the samples, and activity was determined by the presence or absence of MTBE-degrading peptides. No quantitative measurements were performed.

3.1.1 DATA REQUIREMENTS TO EVALUATE MICROBIAL ACTIVITY SUPPORTING NATURAL ATTENUATION OF MTBE

Two rounds of samples were collected from seven monitoring wells at the 22 Area MCX Gas Station site. The sampling events were conducted in March and August 2016. One additional round of sampling conducted in May 2016 was performed at three wells that did not meet the DNA extraction criteria (i.e., insufficient DNA was collected during the previous round). The wells that were sampled are shown in **Figure 1-1** and included:

- Two wells upgradient of the mid-plume biobarrier (22-MM-07 and 22-MM-08)
- Two wells within the mid-plume biobarrier section (22-BMW-11 and 22-BMW-8)
- Two wells in between the mid-plume and leading edge biobarriers (22-BMW-15 and 22-DMM-05)
- One well located within the leading edge biobarrier section (22-BMW-3).

A comparison of community compositions was performed across all wells sampled to evaluate the presence of the microorganisms necessary for MTBE biodegradation with respect to observed trends in contaminant concentration and geochemistry. Then, peptide signatures from known MTBE degradation pathways and MTBE-degrading microorganisms were used to assess MTBE degradation activity.

Two rounds of samples were collected from five monitoring wells located at the control site (13 Area Gas Station) where MTBE remediation with air sparging is ongoing. The wells sampled included:

- Three wells located in the source zone (1327-MW-01R, 1327-RW-07 and 1327-MW-07R)
- One mid-plume well (1327-MW-23)
- One well located at the leading edge of the plume (1327-MW-39).

As with the 22 Area MCX Gas Station, metagenomic and metaproteomic analyses were performed to assess the potential for MTBE degradation and the results were compared to contaminant concentrations and geochemistry observations.

3.1.2 SUCCESS CRITERIA TO EVALUATE MICROBIAL ACTIVITY SUPPORTING NATURAL ATTENUATION OF MTBE

The first hypothesis described in Section 3.0 was considered true if:

- Concentrations of MTBE are decreasing or stable over time and geochemical parameters indicate that the environment is conducive to biodegradation through a known degradation pathway,
- Direct metabolizing and co-metabolizing microbial organisms were detected with higher than 0.01% abundance within the MTBE-degrading population (Atlas, Stoeckel et al. 2015), and
- Peptides from known MTBE degradation pathways (aerobic, anaerobic, cometabolic and direct MTBE mineralization) were determined.

The hypothesis was not proven for the 22 Area MCX Gas Station. After shutdown of the biobarriers, concentration trends for MTBE no longer decreased and became stable, and site geochemistry shifted from oxic to anoxic. The intermediate product, TBA, was not detected during biosparing or after system shutdown; therefore, TBA was not used to support that biodegradation of MTBE was occurring. Thus, the only conventional line of evidence that supports MNA are the contaminant concentrations. Although the contaminant concentration trends are not decreasing, these stable trends indicate that the rate of contaminant loading (advection and dissolution) is balanced with the rate of contaminant attenuation (degradation, dispersion, and sorption).

Metagenomics did detect the presence of several MTBE-degrading microorganisms, including *Methylibium petroleiphilum* PM, *Aquincola tertiaricarbonis* L108, and *Hydrogenophaga flava* ENV, in the groundwater samples. However, the detected MTBE-degraders were aerobic microorganisms, indicating MTBE degradation would be possible under aerobic conditions. While proteomic data identified a variety of peptides related to the cellular metabolism of MTBE degraders, no peptide signatures of MTBE-degrading proteins were detected.

3.2 DETERMINE THE LONG-TERM IMPACT OF THE BIOBARRIER SYSTEM ON FORMATION PERMEABILITY

The results of this project postulate that the formation permeability within the ROI of the biobarriers decreased over time due to an increase in biomass and mineral precipitate production as a direct result of injecting oxygen into the aquifer. This hypothesis stemmed from a noted widening of the plume footprint as the plume moves towards the leading edge of the biobarrier. Although no increase in injection pressures was noted during the operation of the biobarrier, microbial growth within the ROI of the biobarrier could have led to biofouling and changes to the porous media. Changes to the porous media would be manifested in a reduction in permeability of the formation, thereby reducing the ability for groundwater to flow through the biobarrier. Thus, widening of the plume may have been due to long-term impacts of the biobarrier. This project proposed to evaluate if formation permeability was altered within the ROI of the biobarrier.

3.2.1 DATA REQUIREMENTS FOR ASSESSMENT OF IMPACT OF BIOBARRIERS ON FORMATION PERMEABILITY

Rather than collecting and analyzing soil samples for permeability and then analyzing the uncertain results of small sample sizes, a direct measurement of hydraulic conductivity was proposed to assess the changes in groundwater flow. Hydraulic conductivity changes can be directly correlated to permeability changes when the dynamic viscosity and density of the fluid do not change. Hydraulic conductivity can be evaluated through a variety of tests. Because slug tests had been performed at the 22 Area MCX Gas Station in 2001, slug tests were proposed in 2016 as the method to assess changes in hydraulic conductivity and evaluate the hypothesis.

Mechanical slug tests were performed during the second 2016 sampling event upgradient of the mid-plume biobarrier at 22-MM-07 and 22-MM-08 and within the ROI of the mid-plume biobarrier at 22-BMW-08 to provide assessment of temporal and spatial changes in hydraulic conductivity. Both slug-in and slug-out tests were repeated three times each to provide information on the adjacent formation (i.e., clogging, etc.). Prior to the slug test, the depth to static groundwater was measured with a Solinst oil/water interface probe. The slug test data were analyzed following the Bouwer and Rice (Bouwer and Rice 1976) and Cooper et al. (Cooper, Bredehoeft et al. 1967) methods to estimate permeability.

3.2.2 SUCCESS CRITERIA FOR ANALYZING IMPACT OF BIOBARRIERS ON FORMATION PERMEABILITY

Slug tests were performed in 2016 to estimate the hydraulic conductivities upgradient and within the mid-plume biobarrier. The results of the slug tests were compared to the 2001 slug tests to assess if changes in the formation occurred. If the hydraulic conductivity of the well within the ROI of the biobarriers decreased while the hydraulic conductivities in upgradient wells remained the same, then the hypothesis would be proven correct.

The hydraulic conductivity values derived based on 2016 data were not substantially different from those derived based on 2001 data, indicating that operation of the biosparge system did not have a long-term impact on the aquifer by reducing formation permeability. The geometric means of hydraulic conductivity values derived from multiple tests using multiple data reduction solutions were compared on a well-by-well basis with “representative K” values presented by IT (IT Corporation 2001). This comparison shows that hydraulic conductivity values derived for each well based on 2001 and 2016 data were within a factor of two. Given the variability inherent in slug testing and slug test data reduction, a factor of two difference is small enough to conclude that the hydraulic conductivity values are not meaningfully different before and after biosparging. These results indicate that the operation of the biosparge system did not have a long-term impact on the aquifer by reducing formation permeability.

4.0 SITE DESCRIPTION

4.1 SITE SELECTION

The following criteria were used to select a suitable test site for this study (ESTCP 2015):

1. Historical in situ restoration technologies implemented at the site were demonstrated with sufficient data to allow for a thorough comparison of monitoring data collected during active remediation and during this study.
2. Restoration technologies were implemented in situ at full scale and were believed to be successful.
3. The site was accessible for additional monitoring planned as part of this study and was not substantially disturbed since the completion of active remediation.
4. The objective of the restoration project was to treat contaminated groundwater.
5. Active remediation was completed at least three years ago.
6. On-going natural attenuation is needed as a polishing phase to attain numerical clean-up criteria throughout the plume.

Based on the abovementioned criteria, the 22 Area MCX Gas Station was selected as the test site.

Although not initially proposed in the work plan, the 13 Area Gas Station site was identified as a positive control because MTBE contamination is currently undergoing biodegradation with the application of biosparging. Differences in the composition of the microbial community and protein abundance between the two sites can provide valuable insight with respect to applying advanced MBTs to monitor natural attenuation compared to monitoring active remediation as well as to better understand shifts that may occur after active remediation is ceased and long-term monitoring is commenced.

The relative locations and vicinity of the two sites is provided in **Figure 4-1**. Despite slight differences in local geological settings, the 22 Area MCX Gas Station and the 13 Area Gas Station sites are similar. High concentrations of MTBE, benzene, and total petroleum hydrocarbons quantified as gasoline (TPH-G) have been detected in groundwater of the 13 Area Gas Station site originating from UST releases of gasoline during the same timeframe as those detected at the 22 Area MCX Gas Station. However, MTBE concentrations at the 13 Area Gas Station are three orders of magnitude higher than at the 22 Area MCX Gas Station. The graphical timeline of activities at both selected sites is presented in **Figure 4-2**.

Five wells were selected for groundwater sampling at the 13 Area Gas Station. The goal of obtaining the additional metagenomic and proteomic data at this site was to provide a reference point for the abundance of microorganisms involved in MTBE degradation as well as a representation of proteins expressed during active degradation.

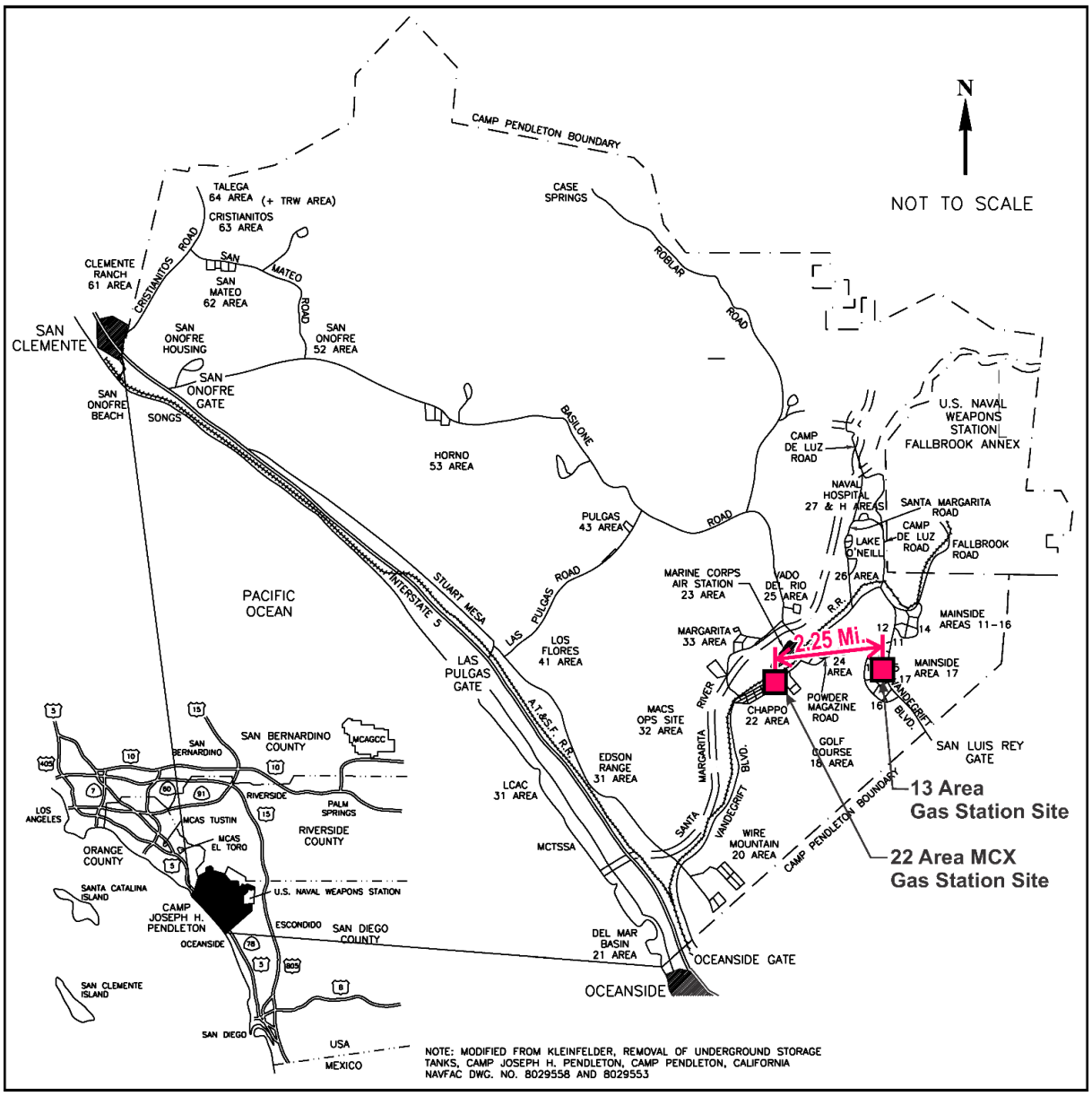

Figure 4-1. 22 Area MCX Gas Station Site and 13 Area Gas Station Site Location Map

4.2 SITE LOCATION AND HISTORY

MCB Camp Pendleton is located in San Diego County, California, and covers approximately 120,000 acres of land bordered on the west by the Pacific Ocean (IT Corporation 2002) (**Figure 4-1**). The 13 Area Gas Station and 22 Area MCX Gas Stations are located in the southeastern portion of MCB Camp Pendleton off of Vandegrift Boulevard approximately 2.2 miles apart. Both sites are in use as commercial gasoline service stations and the adjacent land use includes commercial structures and military installations. Both gas stations were installed in the 1940s and 1950s with UST replacements performed in 1996 through 1997. Remediation began in 1995 and continues to the present.

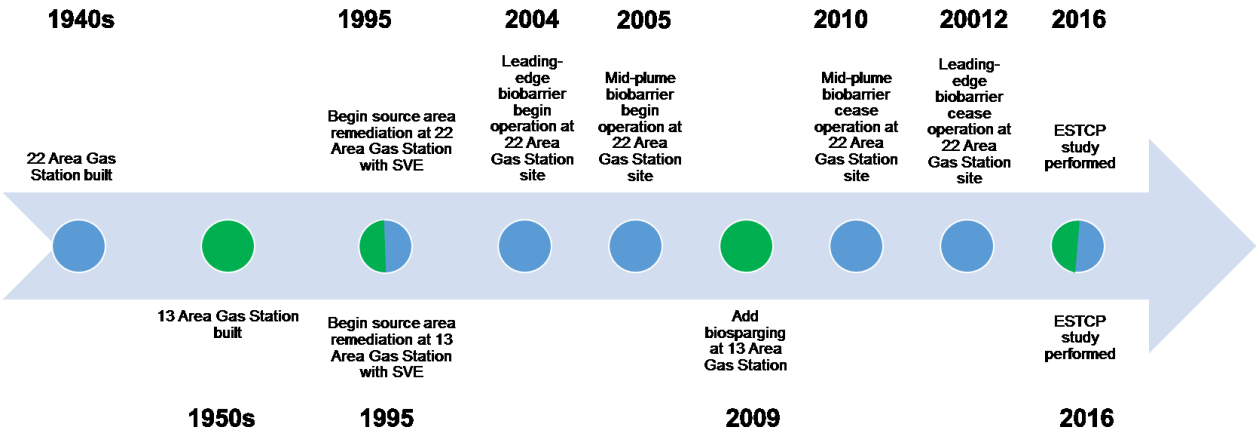

Figure 4-2. Graphical Timeline of Activities at the 22 Area MCX Gas Station (Blue circles) and 13 Area Gas Station (Green circles)

Remedial actions at both sites have included the successful implementation of a series of in situ technologies to address source zone contamination. At the 22 Area MCX Gas Station, an estimated 51,255 lbs of petroleum hydrocarbon mass was removed from the source area during the implementation of in situ air sparge/soil vapor extraction. Downgradient contamination consisted solely of MTBE. The resultant dissolved-phase MTBE plume was later treated with a two-stage biobarrier system consisting of a 400-ft-long mid-plume biobarrier (installed in 2005) and a 250-ft-long leading-edge biobarrier (installed in 2004) (**Figure 4-1** and **Figure 4-2**). During operation of these biobarriers (from 2004 through 2010), MTBE concentrations in groundwater declined significantly such that only dilute levels of MTBE (i.e., 5 µg/L to 40 µg/L) remained. In 2010, regulatory agencies agreed that the energy requirements and costs associated with operation of the mid-plume barrier were not justified based on the minimal risk the groundwater concentrations in that area posed to human health and the environment. Consequently, operation of the mid-plume biobarrier was discontinued in 2010. The same conclusion was made regarding the leading-edge biobarrier, and operation was discontinued in June 2012. However, low-level dissolved-phase MTBE still exists at concentrations that exceed the secondary MCL for MTBE (5 µg/L) in some areas. Historical information and data collected at the 22 Area MCX Gas Station site are summarized in the *Semiannual Groundwater Monitoring Report for 22 Area MCX Gas Station Marine Corps Exchange Gas Station, Marine Corps Base Camp Pendleton, California* (CB&I 2016b).

The 13 Area Gas Station site has a similar site history to the 22 Area MCX Gas Station except for the remediation system, which currently remains operational. As of December 2016, an estimated 462,497 lbs of petroleum hydrocarbons have been removed from the subsurface. The remedial actions from 1995 through 2009 used a soil vapor extraction and total fluid recovery pump system. Beginning in 2009, a biosparge system was installed and the total fluid recovery pump system was shut down. The soil vapor extraction and biosparge systems have operated continuously from 2009 through 2016. As of July 2015, MTBE concentrations at the site were as high as 23,000 µg/L. Historical information and data collected at the 13 Area Gas Station site are summarized in

the *Annual Groundwater Monitoring Report for 13 Area Gas Station Marin Corps Base Camp Pendleton, California* (CB&I 2016b).

4.3 SITE-GEOLOGY/HYDROGEOLOGY

A brief description of the pertinent geology and hydrogeology of the 22 Area MCX Gas Station and 13 Area Gas Station sites is provided in this section. More in-depth descriptions are provided in the *Final Corrective Action Plan 22 Area MCX Marine Corps Exchange Gas Station Marine Corps Base Camp Pendleton, California* (IT Corporation 2002) and the *Final Corrective Action Plan 13 Area Marine Corps Base Camp Pendleton, California* (IT Corporation 2001), respectively.

Lithology comprising the subsurface of the 22 Area MCX Gas Station ranges from well-graded medium sands to clays and includes intermediate textured soils of silt, silty sand, sandy silt, clayey sand, clayey silt, silty clay, and sandy clay. The materials occur as interfingered beds ranging from several inches to tens of feet in thickness, consistent with a Quaternary fluvial depositional environment. Five main sand zones (A through E) are present with MTBE contamination detected in upper sand zones B and C (extending from approximately 12 to 43 ft below ground surface [bgs]), which were interpreted as coarser sands and act as a conduit for MTBE migration. Regional groundwater flows to the southwest and is encountered generally between 9 and 15 ft bgs. Except for the leading-edge portion of the plume, the site is covered by asphalt with runoff from the site directed towards the storm drain system installed throughout the 22 Area MCX Gas Station. Based on slug test data obtained in 2001, hydraulic conductivity at the 22 Area MCX Gas Station site was estimated to range from 0.35 to 49 ft/day.

Lithology comprising the subsurface of the 13 Area Gas Station site include fine-grained sands with interbedded siltstone layers, which are uncemented to moderately indurated and have relatively low primary permeability. In the area of the site, the La Jolla Group rocks generally strike northerly and have a westerly dip direction (into the ridge west of the site). Dip angles ranging from 11 to 15 degrees were measured in core samples recovered during site assessment. The uppermost groundwater surface in the eastern portion of the site was observed at a considerable higher elevation than in the western portion. The differences in water levels, along with stratigraphic discontinuities observed in boring logs, led to the interpretation that a structural feature, such as a fault, creates variable hydraulic conductivity to groundwater flow beneath the site. The interpretation of a controlling structural feature was supported by seismic refraction and seismic reflection geophysical surveys. The geophysical survey report concluded that a high-angle fault with a north-south strike approximately bisects the site. East of the fault that bisects the site, the uppermost groundwater is within an unconfined perched zone that is approximately 25 feet thick. Groundwater flows west towards the fault. West of the fault, groundwater occurs in an unconfined lower zone and flows west away from the fault. Depth to groundwater is generally between 12 to 20 ft bgs on the east side of the fault and between 42 to 140 ft bgs on the west side of the fault. Also noteworthy, most of the 13 Area site is covered by asphalt, which is graded to the east (towards Vandegrift Blvd.), thus directing runoff from the site and surrounding areas to the storm drain system. Based on an average hydraulic gradient of 0.06 feet per foot (ft/ft), an estimated hydraulic conductivity of 2.6×10^{-4} centimeters per second, and an estimated effective porosity of 0.34, the average groundwater velocity in the shallow zone is estimated to be 0.13 ft per day.

4.4 CONTAMINANT DISTRIBUTION

The 22 Area MCX Gas Station and 13 Area Gas Station sites had UST releases at approximately the same time with active remediation being applied at the same start time; however, the current length and width of the groundwater plumes are very different. The MTBE contamination travelled through the 22 Area MCX Gas Station subsurface faster than the MTBE at the 13 Area Gas Station site primarily because of differences in site lithology. As previously mentioned, the MTBE travelled through coarse sands in the 22 Area MCX Gas Station, whereas the MTBE travelled through tighter sandy silts in the 13 Area Gas Station.

At the 22 Area MCX Gas Station, the MTBE contaminant plume extends at least 1,500 feet downgradient of the site, with a plume width of approximately 450 ft at its widest point. The first detection of MTBE in groundwater was in July 1996 due to the addition of MTBE to the groundwater monitoring program. During this monitoring event, MTBE was detected in monitoring wells with concentrations ranging from 54 to 3,090 µg/L, no TPH or other fuel oxygenates were detected. Contamination was detected from 12 to 43 ft bgs. As of 2015, after substantial treatment, the highest MTBE concentration detected was 23 µg/L. **Figure 4-3** shows the MTBE levels at selected wells for 2015-2016. The corresponding groundwater elevation contours for 2015 are provided in **Figure 4-4**.

At the 13 Area Gas Station, the MTBE contaminant plume extends approximately 850 ft downgradient of the gas station source area, with a width of approximately 200 ft at its widest point. As illustrated in **Figure 4-5**, contamination extends approximately 200 ft north, 800 ft west, 400 ft south, and 200 ft east of the source area. The first detection of MTBE in groundwater at this site was in April 1996, also due to the addition of MTBE to the groundwater monitoring program. The maximum concentration of MTBE detected at this site was 1,060,000 µg/L in 2001. Groundwater contamination extends from approximately 4 ft bgs to 133 feet bgs. Free product was reported with a maximum thickness of 10 ft in the source area; however, active remediation at the site during the past 20 years has reduced the thickness to a sheen. Similarly, active remediation substantially reduced concentrations of MTBE in groundwater with BTEX, TPH and TBA also being detected at the site. As of July 2015, the maximum concentration of MTBE detected at the site was 23,000 µg/L.

Groundwater contamination beneath the site extends over a fault that bisects the site into a western portion and an eastern portion. Similar to the 22 Area MCX Gas Station, the depth to groundwater on the western portion ranges from 42 to 140 ft bgs and on the eastern portion ranges from 12 to 20 ft bgs. Soil and groundwater contamination has been detected from 12 to 140 ft bgs. Contamination on the east side of the fault (the upper zone of groundwater) has remained relatively stable since groundwater monitoring began in 1995. Concentrations of contaminants have fluctuated on the west side of the fault (lower zone of groundwater) primarily because groundwater is being recharged by groundwater from the upper zone flowing westward across the fault. MTBE concentrations and corresponding concentration contours, as well as flow direction and hydraulic gradient reported from groundwater sampling events conducted in 2014 through 2016, are provided in **Figure 4-6**.

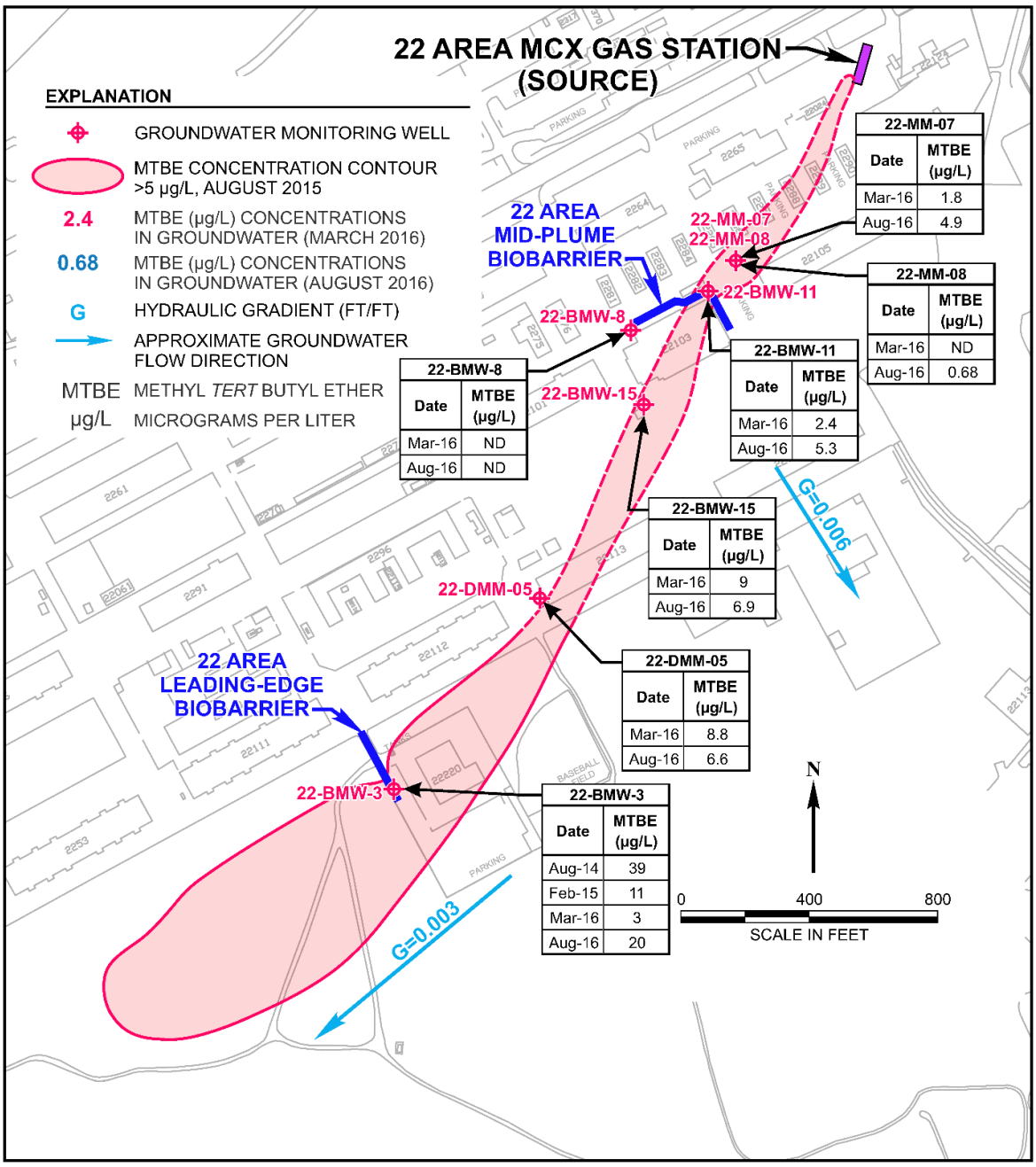

Figure 4-3. MTBE Concentrations in Groundwater at the 22 Area MCX Gas Station Site (August/September 2014, February 2015, March 2016 and August 2015)

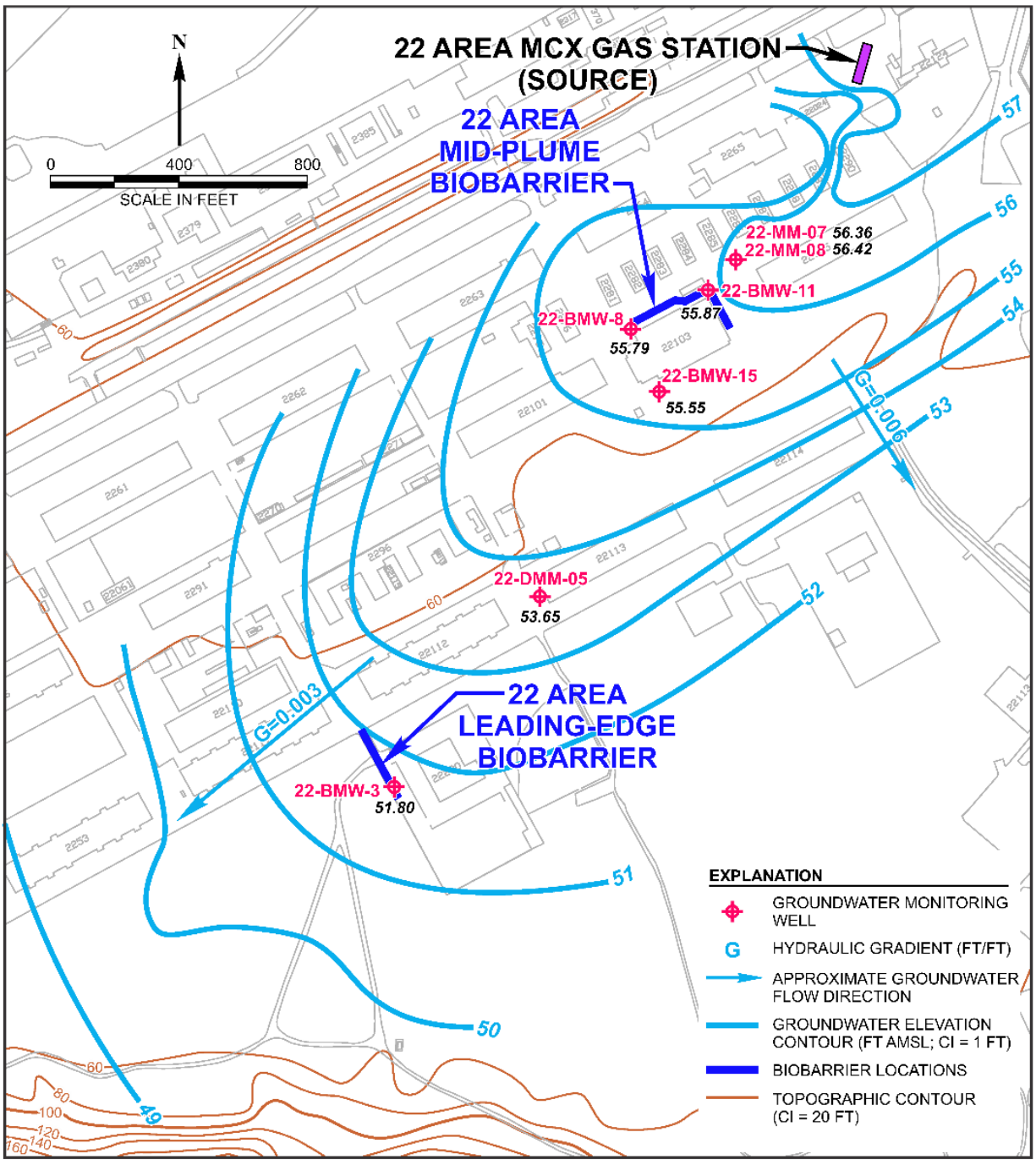

Figure 4-4. Elevation Contours, 22 Area MCX Gas Station Site, July 2015

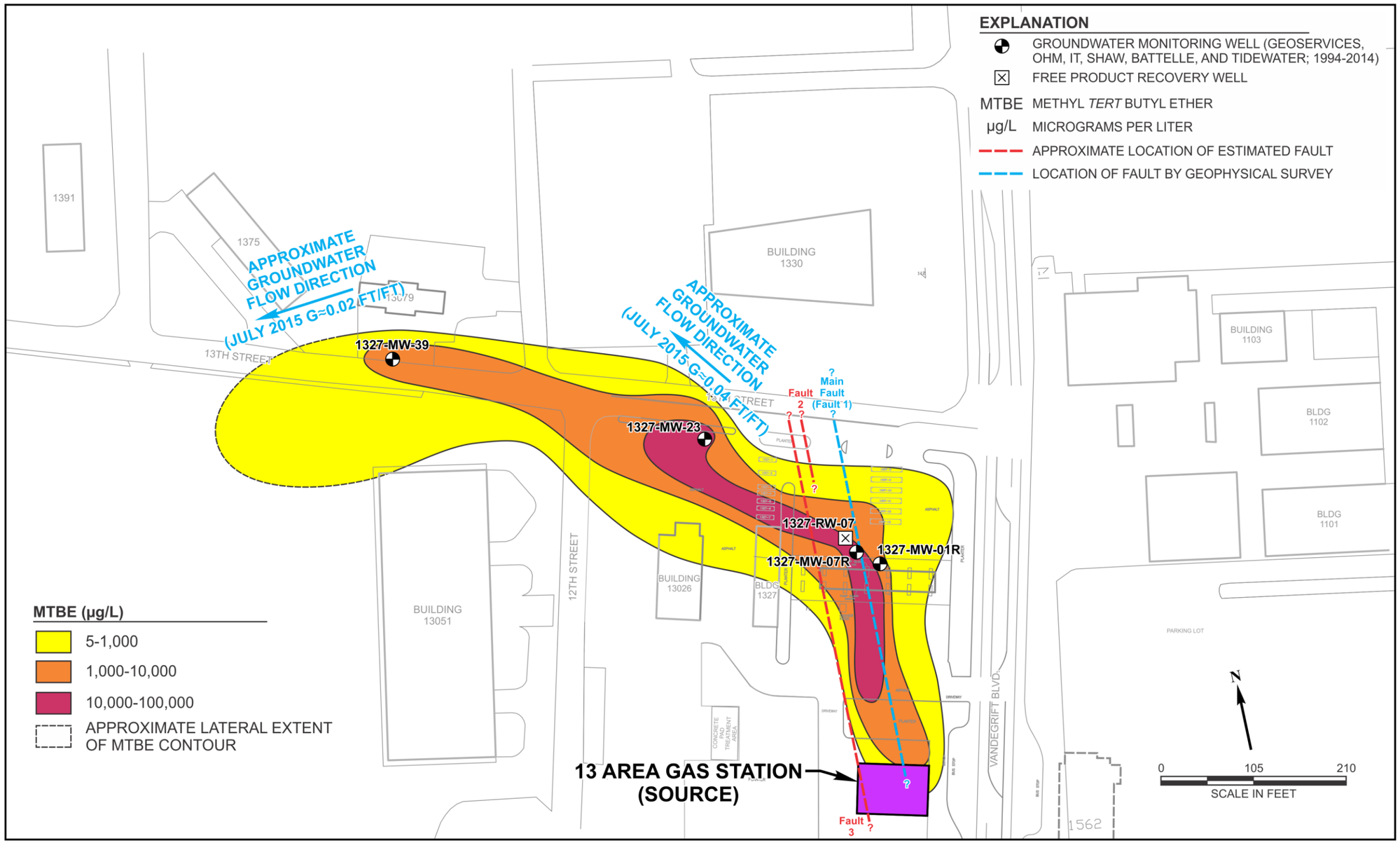

Figure 4-5. 13 Area Gas Station MCB Camp Pendleton

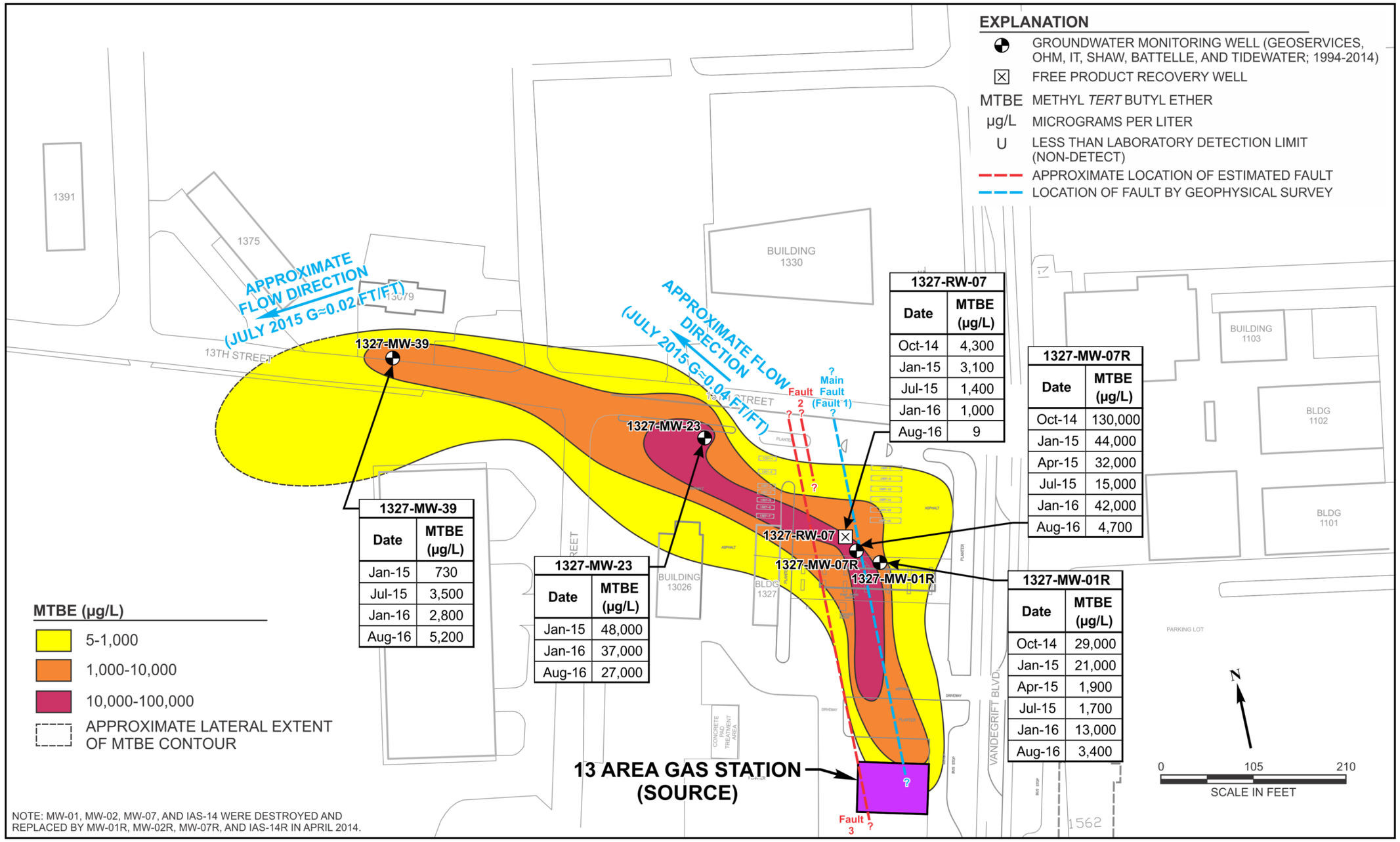

Figure 4-6. MTBE Concentrations from 2014 through 2016 at 13 Area Gas Station MCB Camp Pendleton

5.0 TEST DESIGN

This section provides a brief overview of the data evaluation and sampling conducted to address technical objectives stated in Section 3.0. Detailed descriptions of data collected during the field demonstration are included in subsections below.

5.1 CONCEPTUAL EXPERIMENTAL DESIGN

This ESTCP demonstration evaluated the long-term performance of MTBE natural attenuation after shutdown of the biobarrier system at the 22 Area MCX Gas Station. In addition, data from 13 Area Gas Station was used as a positive control for demonstration and validation of metagenomic and metaproteomic analyses. The conceptual experimental design approach is depicted graphically in **Figure 5-1.**

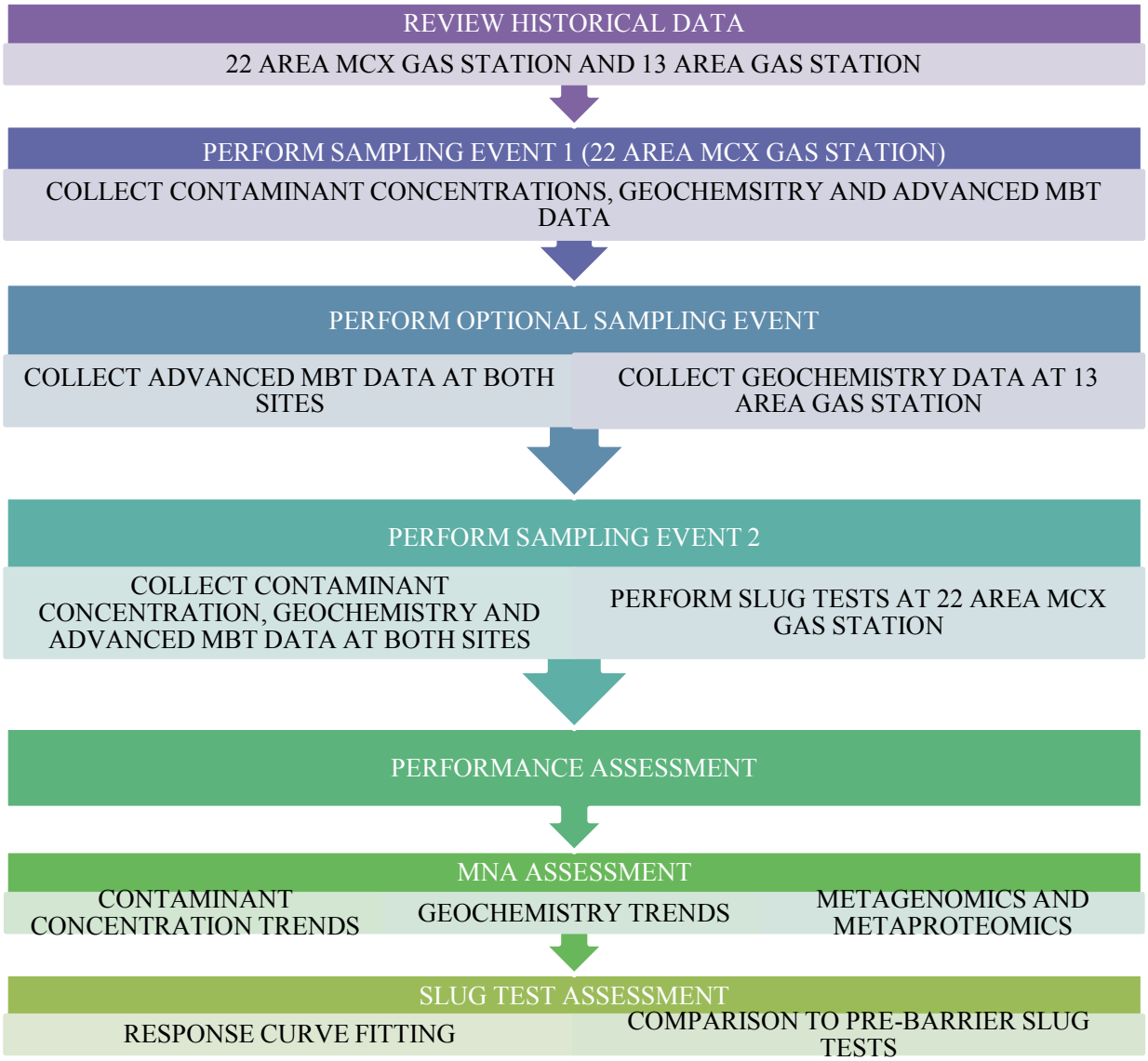

Figure 5-1. Conceptual Design for the Demonstration

Historical data collected during operation of the biobarrier system at the 22 Area MCX Gas Station were reviewed and provided the basis for selecting wells to be sampled for assessing natural attenuation. The previous locations of slug tests were reviewed and a subset was selected for this demonstration. To provide a positive control, data from the 13 Area Gas Station was reviewed and the site was incorporated into the project.

Field sampling was performed in March (sampling event 1), May (optional sampling event) and August (sampling event 2) 2016. During the first sampling event, samples were collected from seven monitoring wells at the 22 Area MCX Gas Station site. Analyses were performed for contaminant concentrations geochemistry and advanced MBTs. However, DNA and protein concentrations collected at three wells were insufficient, thus an optional round of sampling was performed. In addition, five samples were collected from monitoring wells at the 13 Area Gas Station to demonstrate and validate application of the advance MBTs during active remediation of MTBE. For discussion purposes in the remainder of this report, the optional sampling event is considered to be a subset of the first sampling event and is therefore results of those samples are presented as part of first sampling event. The second sampling event included both the 22 Area MCX Gas Station and the 13 Area Gas Station. As part of the second sampling event, mechanical slug tests were performed.

The performance assessment evaluated two hypotheses. First, a tiered approach was used that relied on multiple, converging lines of evidence to evaluate microbial activity supporting natural attenuation of MTBE. Advanced MBTs were used as an innovative tool, within the tiered MNA assessment, to evaluate current microbially-mediated processes at the site. Second, long-term impacts of the biobarrier on formation permeability were evaluated within the radius of influence of the mid-plume biobarrier at the 22 Area MCX Gas Station comparing slug tests between 2001 and 2016. The resulting dataset was used to:

- Evaluate contaminant concentrations and geochemical trends that indicate natural attenuation of MTBE.
- Demonstrate and validate metagenomic and metaproteomic methods to accurately determine MTBE degradation microbiology and activity at the site.
- Assess and evaluate hydraulic conductivity on site.

Data analysis and interpretation are described in Sections 5.0 and 6.0.

5.2 DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS

The following subsections detail the design and elements of each of the technologies demonstrated.

5.2.1 GROUNDWATER SAMPLING DESIGN

An evaluation of natural attenuation relies on a multiple lines of evidence approach. To support the MNA evaluation, groundwater samples were collected at the 22 Area MCX Gas Station. Samples were collected at locations upgradient of the mid-plume biobarrier as well as within the MNA zone within the two biobarriers and between the two biobarriers – all within the footprint of the contaminated plume.

Groundwater samples were collected at five locations at the 13 Area Gas Station during the optional sampling event and second sampling event. Samples were not collected from this site in March. Samples were collected at three source area wells within the ROI of the biosparing system and two wells outside of the ROI of remediation system, one of which was in the mid-plume and one at the leading edge of the plume.

As in other MNA protocols (Wiedemeier 1999; U.S. EPA. 2012; Lebron, Weidemeier et al. 2105), the following three-tiered approach was used to evaluate microbial activity supporting natural attenuation of MTBE at the 22 Area MCX Gas Station MNA of MTBE (AIP. 2007):

- Contaminant Concentrations (Primary)
- Geochemistry (Secondary)
- Advanced MBT Data (Tertiary)

The primary line of evidence to assess MNA of MTBE is to analyze the historic contaminant concentrations within the context of hydrogeologic data. To evaluate this line of evidence, a statistical analysis was conducted on the MTBE data at the 22 Area MCX Gas Station to determine if the plume has been increasing, decreasing or stable since the biosparging system was shut off. Additionally, the production of intermediate degradation products, such as TBA, was evaluated.

The secondary line of evidence is not intended to provide direct evidence that MTBE is/has been biodegraded. Rather, these data are collected to delineate biogeochemical processes at the site and infer microbial activity related to contaminant biodegradation. This evaluation requires analysis of a variety of geochemical parameters such as oxidation-reduction potential (ORP), dissolved oxygen (DO) and terminal electron acceptor indicators (e.g., nitrate, ferrous iron, sulfate/sulfide, methane). These data were used to determine the predominant geochemical (redox) environment and ascertain what MTBE degradation pathway(s) could be occurring at the site.

At sites where MNA is difficult to demonstrate, tertiary lines of evidence may be necessary. Applicable tools can include laboratory specific microcosm studies and conventional molecular microbial community analyses (e.g., qPCR, 16S rRNA sequencing). However, advancement of the genome-based science, which relies on high-throughput DNA sequencing is quickly becoming an accepted approach to sequence complex environmental samples and acquire information on microbial dynamics, shifts and composition. These genomic technologies provide insight into composition of the microbial community without the need to perform microcosm studies. For this project, the combination of metagenomics and metaproteomics was used to evaluate the microbial activity supporting natural attenuation.

Metagenomics. Metagenomic analysis can reveal the presence of MTBE-degrading species, which indicates the potential for bioremediation. For this demonstration, metagenomic data were grouped to determine relative abundance of specific MTBE-degrading microbial species into three main groups: 1) direct, 2) cometabolic, 3) anaerobic/aerobic (**Table 5-1**). This classification helped to address questions regarding the type of MTBE metabolism occurring at the site. For example, if the data shows a high relative abundance of direct aerobic MTBE-degraders such as *Methylobium,* the likelihood for mineralization of MTBE is probable because these microbial species facilitate direct MTBE degradation, as shown in **Figure 2-1**. On the contrary, if the relative

abundance of cometabolic species is high, the potential for full mineralization of MTBE would be low since its degradation would occur in parallel with other substrates (e.g., C_5 to C_8 *n*-alkanes), as shown in **Figure 2-2**.

The ultimate goal of metagenomics is to link functional and phylogenetic information to the geochemical, physical and other biological parameters that characterize contaminant degradation in specific environment. Moreover, correlation of the metagenomic data to proteomic results can bring better understanding to the specific ***activity*** of the microbial populations at the site.

Table 5-1. Designation of Known MTBE Degraders into Four Groups Dependent on Respiration and MTBE Degradation Types

	Aerobic	Anaerobic	Direct MTBE Degrader	Cometabolic MTBE Degrader	Reference
Achromobacter	x		x		(Eixarch and Constantí 2010)
Acinetobacter	x			x	(Mo, Lora et al. 1997)
Aquincola	x		x		(Müller, Rohwerder et al. 2008)
Arthrobacter		x		x	(Liu, Speitel et al. 2001)
Bacillus	x		x		(Hanson, Ackerman et al. 1999)
Cupriavidus		x	x		(Aslett, Haas et al. 2011)
Delftia	x			x	(Bastida, Rosell et al. 2010)
Enterobacter	x		x		(Chen, Chen et al. 2011)
Gordonia	x			x	(Hernandez-Perez, Fayolle et al. 2001)
Hydrogenophaga	x		x		(Streger, Vainberg et al. 2002)
Kocuria	x		x		(Lalević, Jović et al. 2012)
Methylobium	x		x		(Hristova, Gebreyesus et al. 2003)
Mycobacterium	x		x		(Rohwerder, Breuer et al. 2006)
Nocardioides	x			x	(Chen, Chen et al. 2011)
Ochrobactrum	x			x	(Hunkeler, Butler et al. 2001)
Paucibacter	x		x		(Aslett, Haas et al. 2011)
Polaromomas	x		x		(Auffret, Yergeau et al. 2015)
Pseudomonas	x			x	(Smith, O'Reilly et al. 2003; Johnson and Hyman 2006; Kane, Chakicherla et al. 2007)
Pseudonocardia	x			x	(Vainberg, McClay et al. 2006)
Pseudo-xanthomonas	x		x		(Le Digabel and Fayolle-Guichard 2015)
Rhodobacter	x			x	(Bastida, Rosell et al. 2010)
Rhodoferax	x		x		(Kane, Chakicherla et al. 2007)
Sinorhizobium		x		x	(Ferreira, Malandain et al. 2006)
Sphingo-bacterium	x			x	(Li, Zhang et al. 2014)
Sphingomonas	x			x	(Fayolle, Vandecasteele et al. 2001)
Sphingopyxis		x		x	(Sun, Sun et al. 2012)
Variovorax	x		x		(Müller, Rohwerder et al. 2008)

Proteomics. Proteomics can reveal activity related to the biodegradation of a contaminant in the system. The positive indicator of active MTBE biodegradation is the presence of proteins involved in known MTBE degradation pathways. The presence of these proteins is direct evidence that MTBE degradation is occurring and provides direct evidence that this metabolic pathway is active. In this case, the detection of proteins is limited by known degradation pathways[2]. It is possible that other pathways exist, but have not been documented in the literature. The identification of other proteins (e.g., structural proteins for cellular metabolism) from known MTBE degraders also is an indication that MTBE-degrading bacteria are present and active. However, the absence of MTBE degradation proteins and cellular metabolism proteins of MTBE degrading microorganisms is not necessarily indicative of a lack of MTBE degradation. Other degradation pathways and proteins may exist, but at present, are unknown or have not been documented in the literature. It also is noted that the presence of proteins involved in co-metabolism is not direct evidence of MTBE metabolism as such enzymes have primary specificity toward other substrates; however, their presence can be considered indirect evidence that cometabolic degradation of MTBE is occurring.

Due to limitations associated with the current detection limits inherent to mass spectrometry based proteomic analysis, absence of proteins could be due to one of the following:

- **True absence of peptide types targeted in the sample**. In this case, no MTBE degradation proteins are identified which indicates lack of ongoing MTBE degradation.
- **Presence of peptide at levels below detection limits.** If few (e.g., <<10 peptides) are identified, MTBE degrading organisms may or may not be present. The true detection limit is an inherent property that is unique to each protein, matrix, and mass spectrometer.
- **Presence of interfering substances**. In highly diverse samples proteins may not be identified at high numbers (e.g., > 50 peptides) due to interference of other molecular compounds such as humic or fulvic acids that cause false negative DNA and protein detection.

5.2.2 SLUG TESTS

Slug tests were performed in two of the same wells where slug tests were conducted in 2001 (i.e., 22-MM-07 and 22-MM-08) which are upgradient of the mid-plume biobarrier. Well 22-BMW-08 was selected as the third well for slug testing based on its close proximity to, and similarity of construction with, well 22-MM-06, which was destroyed in 2012. Well 22-BMW-08 is located within the ROI of the mid-plume biobarrier, is screened through the same depth internal (5-10 ft bgs) as 22-MM-06, and was logged as exhibiting the same lithology as 22-MM-06. The results of slug testing in these wells was used to perform a temporal comparison to assess the differences in formation permeability pre-biosparging and post-biosparging. The results were also used to evaluate potential changes in hydraulic conductivity between the upgradient wells (22-MM-07 and 22-MM-08) and the well (22-BMW-08) within the ROI of the mid-plume biobarrier.

[2] A library of MTBE degradation peptides was compiled from *Methylobium petropleiphilum* PM1 species that is known for direct MTBE degradation. Sequences of main proteins involved in the MTBE degradation pathway were identified for this species and are included in the library.

Three slug insertion (i.e., slug-in) and withdrawal (i.e., slug-out) tests were conducted in each of the three wells (i.e., 22-BMW-08, 22-MM-07, and 22-MM-08). This provided a total of 18 sets of test results for the 2016 dataset. This approach of conducting both slug-in and slug-out tests, and conducting multiple tests in each well, was consistent with the approach used to generate the 2001 dataset (IT Corporation 2001). The rationale for this approach is to develop a dataset that could be used to assess test repeatability, which can be affected by the way the test well was constructed, and is sensitive to wells that recover quickly, as was observed in the test wells.

To maximize the comparability between K values derived based on 2001 and 2016 slug test data, the 2016 field procedures were matched as closely as possible to those used in 2001. This included constructing similar slugs and measuring water level response using a water-level data logger (i.e., pressure transducer) on a logarithmic interval.

To replicate the analysis performed on the slug tests conducted in 2001 (see **Appendix F**), the 2016 slug tests were analyzed following the Bouwer and Rice (Bouwer and Rice 1976) and Cooper et al. (Cooper, Bredehoeft et al. 1967) methods to estimate hydraulic conductivity. The Bouwer and Rice solution can account for partial penetration of a well in an aquifer, which is the case for all three of the wells tested. Assumptions of the Bower and Rice solution include the following:

- The aquifer is unconfined, homogeneous, continuous, uniform thickness;
- The water table is horizontal over the area influenced by the test;
- The lower boundary is an impermeable layer;
- The flow to the well is quasi-steady state by disregarding the compressibility of the aquifer; and
- The instantaneous change in water level was due to withdrawal or addition of a slug in the well.

This method was developed to solve for transmissivity and storativity in confined aquifers, but can also be used in unconfined aquifers. Hydraulic conductivity can be calculated by dividing transmissivity by saturated aquifer thickness. The bottom of the aquifer interpreted by IT (IT Corporation 2001) was 25 ft bgs in the vicinity of 22-BMW-08 and 22-MM-07, and 45 ft bgs in 22-MM-08. This depth and the depth to groundwater at the time of testing was used to calculate the saturated aquifer thickness.

Both methods used to evaluate the slug test data rely on graphical curve matching to estimate the hydraulic conductivity of the formation adjacent to the well. To the extent possible, the curve matching approach shown in IT, 2001 (IT Corporation 2001) was replicated during evaluation of the 2016 data. For this project, AQTESOLV v4.5 was used to analyze the data.

5.3 SAMPLING METHODS AND FIELD PROCEDURES

5.3.1 GROUNDWATER SAMPLING AND ANALYSES

Groundwater samples were collected by Lynco Environmental in accordance with the Site Assessment and Mitigation Manual (San Diego Department of Environmental Health 2014) as

part of the Navy's routine sampling efforts. In addition, groundwater samples for proteomics and metagenomic analyses were collected. Samples were analyzed for the basic field parameters, detailed in **Table 5-2**.

Table 5-2. Analytical Methods for the Demonstration

Analyte (in groundwater)	Method	Preservative	Bottle (number of containers)	Hold time
ORP	Field Meter	--	--	NA
Dissolved Oxygen	Field Meter	--	--	NA
pH	Field Meter	--	--	NA
Conductivity	Field Meter	--	--	NA
Anions (Cl^-, F^-, NO_3^{2-}, Ortho-PO_4, SO_4^{2-})	EPA 300.0	4 °C	100 mL polyethylene screw-cap	2 days (NO_3, PO_4); 28 days other
BTEX	EPA Method 624/8260	$Na_2S_2O_3$, 4 °C	(3) 40 ml VOA Vials	7 days
TPH-G/BTEX/Five Oxygenates	EPA Method 624/8260	$Na_2S_2O_3$, 4 °C	(3) 40 ml VOA Vials	7 days
Total Organic Carbon	EPA Method SW9060 / SM5310C	H_2SO_4, pH<2, 4 °C	(2) 40 ml VOA Vials	28 days
TDS	SM2540C	4 °C	500 mL polyethylene screw-cap	7 days
Sulfide	SM4500-S D	ZnOAC, NaOH, pH>9, 4 °C	(2) 250 mL polyethylene screw-cap	7 days
Methane	MODIFIED Method RSK-175 GC/FID	HCl, pH<2, 4 °C. If CO_2, 4 °C	(2) 20 ml VOA Vials	14 days preserved
Metals (Ca, Mg, K, Na)	EPA Method SW6020/SW 6020A	4 °C	100 mL polyethylene screw-cap	2 days (NO_3, PO_4); 28 days all other
COD	EPA Method 410.4	H_2SO_4, pH<2, 4 °C	250 mL polyethylene screw-cap	28 days
BOD	SM5120B	4 °C	500 mL polyethylene screw-cap	48 hours
Alkalinity	SM2320B	4 °C	250 mL polyethylene screw-cap, no headspace	14 days
Ferrous Iron	SM3500-FeB	4 °C	500 mL polyethylene screw-cap	14 days
Metagenomics	Next Generation Sequencing	-80 °C, filtered	4 L polyethylene screw-cap	Up to 6 months
Proteomics	LC-QTOF-MS/MS	-80 °C, lyophilized	4 L polyethylene screw-cap	Up to 6 months

Groundwater analyses included:

- **Concentrations of contaminants of concern,** performed by Alpha Analytical Laboratory (Sparks, NV), including: TPH-G, benzene, toluene, ethylbenzene and total xylenes (BTEX) and five oxygenates including MTBE, di-isopropyl ether (DIPE), ethyl tertiary butyl ether (ETBE), tertiary amyl methyl ether (TAME), and TBA;

- **Site geochemistry,** performed by Alpha Analytical Laboratory (Sparks, NV): including: ethane, ethene, methane, alkalinity, anions (Cl, F, NO_3, NO_2, ortho-PO_4, SO_4), biological oxygen demand, sulfide (S^{2-}), total phosphorus, chemical oxygen demand, total organic carbon (water only), total dissolved solids (TDS), cations (Ca, Mg, K, Na), ferrous iron (Fe^{2+});
- **Metaproteomics and metagenomics** performed in the Battelle Sequencing and Mass Spectrometry Laboratories (Columbus, OH).

All groundwater monitoring wells were sampled using low-flow purge sampling outlined in the Site Assessment and Mitigation Manual (San Diego Department of Environmental Health 2014). After groundwater levels were recorded, groundwater samples were collected using a low-flow bladder pump lowered to the midpoint between the water level and the screen bottom. Attached to the pump was a length of dual-bonded, Teflon®-lined tubing and safety line sufficient to reach ground surface. After stabilization was achieved, field parameters (pH, turbidity, specific conductance, temperature, ORP, and DO) were measured with a Horiba U-52 water quality meter. Measurements for each well were recorded on groundwater sample collection logs. Samples were collected in accordance with **Table 5-2** and **Table 5-3**. Containers were placed on ice in a cooler for delivery to Alpha Analytical, Inc., which is a California-accredited and Department of the Navy (DON)-approved stationary laboratory and the Battelle laboratory. Support documentation for sampling and analysis is provided in **Appendix C.**

Table 5-3. Total Number and Types of Samples Collected during the Demonstration

Event	Occurrence	Number of Samples	Analyte	Location
Performance Assessment Sampling 1	1 event, **2 days**	**7**	**Suite of analyses listed in Table 5-1**	**7 Wells at the 22 Area MCX Gas Station**: Upgradient of mid-plume biobarrier (22-MM-07 and 22-MM-08); Mid-plume biobarrier (22-BMW-08 and 22-BMW-11); between the biobarriers (22-BMW-15 and 22-DMM-05); leading-edge biobarrier (22-BMW-3)
Optional Sampling	1 event, **2 days**	**8**	**Suite of analyses listed in Table 5-1**	**3 Wells at the 22 Area MCX Gas Station**: 22-MM-07, 22-BMW-15, and 22-BMW-11 **5 Wells at the 13 Area Gas Station:** Source area (1327-MW-01R, 1327-MW-07R, 1327-RW-07); Mid Plume (1327-MW-23); Leading edge of plume (1327-MW-39)
Performance Assessment Sampling 2	1 event, **3 days**	**12**	**Suite of analyses listed in Table 5-1**	**7 Wells at the 22 Area MCX Gas Station**: Upgradient of mid-plume biobarrier (22-MM-07 and 22-MM-08); Mid-plume biobarrier (22-BMW-08 and 22-BMW-11); between the biobarriers (22-BMW-15 and 22-DMM-05); leading-edge biobarrier (22-BMW-3) **5 Wells at the 13 Area Gas Station:** Source area (1327-MW-01R, 1327-MW-07R, 1327-RW-07); Mid Plume (1327-MW-23); Leading edge of plume (1327-MW-39)

5.3.2 METAGENOMICS METHODOLOGY

Approximately 1 L of groundwater from each of the sample locations was filtered through 0.45-µm membrane filters for metagenomics sample processing. DNA was extracted from the filters using MoBio Laboratories PowerWater DNA Isolation Kit. In some cases, due to low concentration yields, an additional volume of groundwater (2 to 3 L) was used for a second round of extractions. The isolated genetic material was quantified by fluorimetry (Qubit 2.0) and qPCR using adaptor flanked primers targeting the 16S region of rDNA. The resulting amplified products were tagged with a sample-specific index sequence and sequenced using an Illumina MiSeq.

A list of common direct and cometabolic MTBE degraders was compiled (**Appendix D**, **Table D-2**) and used together with a custom-designed SILVA 16S reference database in order to perform analyses of microbial organisms in samples. The identified microorganisms served as a foundation to build a database for mass spectrometry spectral searching. After the 16S sequencing, the zipped FASTQ files were transferred to the Galileo high performance computing system for analysis. FASTQ files were unzipped using gunzip. Paired reads were assembled and quality filtered using

Pear v.0.9.6 software. Paired parameters required a minimum overlap of 50 nucleotides, a minimum length of 250 and quality of 30 or greater. For each assembled FASTQ read file, quality plots were prepared using the fastx toolbox (fastx_quality_stats and fastq_quality_boxplot.sh). Assembled and quality filtered reads were converted to FASTA format and run through BLASTn v.2.2.31 software using a custom created 16S reference database containing 68,710 bacterial and archaeal nucleotide sequences. BLASTn results were filtered to retain only those hits that had ≥ 97% identity to their reference sequence and a match length at least 80% of the original read length (Mason, Hazen et al. 2012; Sharpton 2014; Atlas, Stoeckel et al. 2015). The BLASTn filtering parameters were selected to provide the highest probability for a positive match of microorganism sequence to the database. The BLASTn filtering parameters were selected to provide the highest probability for a positive match of microorganism sequence to the database.

Because the 16S reference database contained full genome sequences for some microorganisms, it was possible for a single assembled read to match equally well to more than one position in a genome. This is because many microorganisms have several copies of the 16S gene in their genome. In this instance, BLASTn returned more than one hit for a read. Identical duplicate hits were removed from the BLASTn hits list to remove redundancy in the organism identifications using a custom Perl script (RemoveDuplicateBlastHits.pl). An additional filter was applied to the results to remove those identifications that were 1/1,000 of the hits using a custom script (fpos.py).

The resulting filtered BLASTn hits were assigned their associated TaxID and identified by organism name. These identifications were tabulated in an Excel spreadsheet listing the count of hits, organism name and taxID for each organism identified. Based on this information, the diversity for each sample was calculated using the Shannon diversity[3] index. The metagenomic data were reduced to Krona plots (Krona v.2.5) and tables (**Appendix D**) in which the columns represent genus and species, the rows show percent abundance. This is analogous to species-sample matrices in ecology of higher organisms, and hence many of the statistical tools are available to identify correlations and statistical significant patterns are transferrable.

For each metagenome sample, organisms were grouped into four categories representing the types of MTBE metabolizers (aerobic/anaerobic and direct vs cometabolic). The number of reads associated with the groups were totaled and compared across each of the samples using a Dirichlet-multinomial model. Data management and analyses were conducted using the R open source statistical software Version 3.3.2. The Human Microbiome Project (HMP) package provided the functions to conduct the statistical evaluations of the metagenome compositions.

5.3.3 METAPROTEOMICS METHODOLOGY

Proteins were extracted from lyophilized groundwater, reduced, alkylated, trypsin-lys-C digested, and subjected to liquid chromatography mass spectrometry (LC-MS/MS) using a Nano 415 LC system in line with an ABI Sciex Triple TOF 5600 high resolution MS instrument (**Figure 5-2**).

[3] The Shannon diversity index, which is a quantitative measure that reflects how many different microbial species are in an analyzed community, was determined for each sample. In any given sample, the Shannon diversity index informs only on microbial richness and does not provide information on community composition

During processing, the entire sample was subjected to protein extraction. In bottom-up metaproteomics, protein and peptide concentration determination is a prerequisite for optimal protein digestion and optimal sample loading amount. The protein and peptide concentrations were calculated with a tryptophan assay (Wiśniewski and Gaugaz 2015). For LC-MS/MS analysis, shotgun (non-targeted qualitative) metaproteomics runs were performed, as well as runs where tryptic peptides from proteins involved in contaminant degradation were targeted. Conserved tryptic peptides were selected based on multiple sequence alignment of known protein sequences. The targeted approach allows for enhanced sensitivity of detection for proteins of interest.

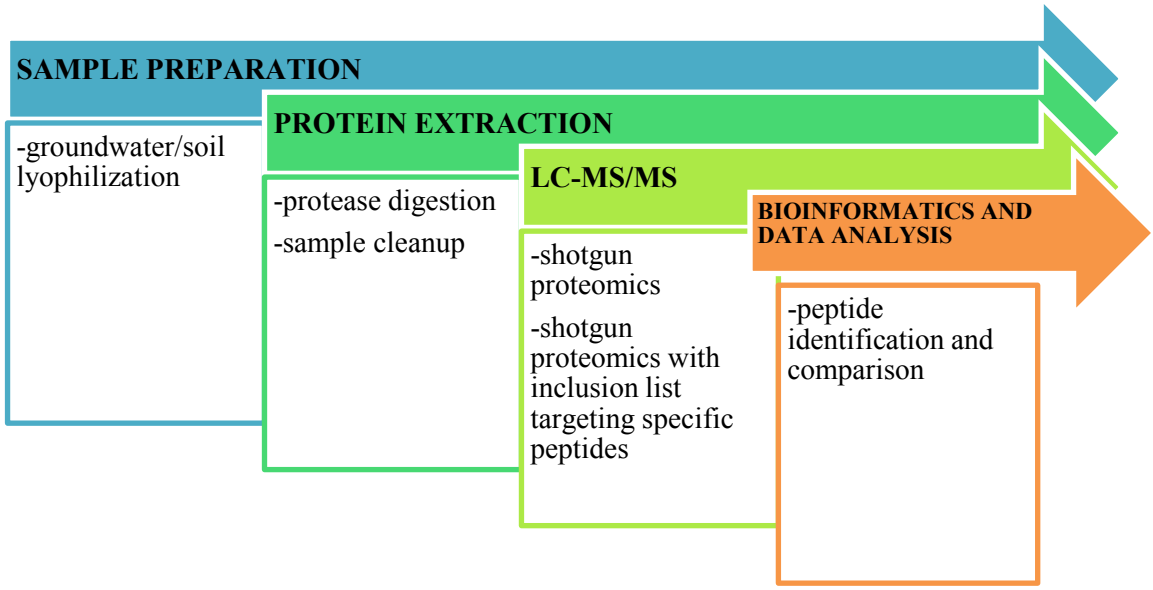

Figure 5-2. Steps Involved in Proteomic Analysis of Groundwater Samples

Proteins were identified from LC-MS/MS spectra by searching against a database of proteins sequences constructed from the results of the 16S metagenomic sequencing performed in the project. Protein sequences from taxa representing 0.01% of the relative microbial population or more were retrieved from National Center for Biology Information (NCBI) (reference sequence database [RefSeq] – sequences only). In addition, sequences of protein contaminants typical for proteomic experiments (e.g., keratin and trypsin) were added to the database. The shotgun LC-MS/MS data were queried against this database and searched against the library of known enzymes involved in MTBE degradation such as: monooxygenases, alkene hydroxylases, esterases and dehydrogenases (Smith, O'Reilly et al. 2003; Ferreira, Malandain et al. 2006; Schuster, Purswani et al. 2013). Only peptides with the "Protscore" for a particular protein higher than 1.3 were considered true positives. For one sample (1327-MW-01R) in source zone area multiple MTBE degradation pathway proteins were detected. Thus, the search database was supplemented with *Methylibium petroleiphilum* PM1 megaplasmid-containing proteins (which were not included in the original database built upon RefSeq entries only) to perform a targeted search. Statistical analyses of proteomic data were performed using Protein Pilot (confidence score and false discovery rate).

5.3.4 FIELD PROCEDURE FOR SLUG TESTS

On August 18, 2016 slug tests were performed in 22-MM-07 and 22-MM-08 upgradient of the mid-plume biobarrier and one well (i.e., 22-BMW-08) within the ROI of the mid-plume biobarrier.

Slug-in and slug-out tests were performed in each of the three wells (i.e., 22-BMW-08, 22-MM-07, and 22-MM-08) using slugs constructed for the casing size of the wells. A 1-inch-diameter by 5-ft-long slug was utilized for the tests in wells 22-MM-08 and 22-MM-07. A 3-inch-diameter by 5-ft-long slug was utilized in 22-BMW-08. Each slug was sealed on both ends with polyvinyl chloride caps, and the slugs were weighted with clean sand. A stainless-steel eyelet was screwed into each cap and lined with Teflon® tape to prevent leakage. The slugs were suspended on a rope. Both slug-in and slug-out tests were repeated three times each to provide information on the well construction and overall test repeatability. Prior to the slug test, the depth to static groundwater was measured with a Solinst oil/water interface probe.

Water-level response was measured with an In-Situ® Level Troll 700 water-level data logger (i.e., pressure transducer) on a logarithmic interval. Measurements were collected until the water level returned to pre-test levels. Data were stored via In-Situ's proprietary WinSitu software program and compiled via Microsoft® Excel's software program for input into the program Aqtesolv v4.5 for analysis.

5.4 SAMPLING RESULTS AND FIELD MEASUREMENTS

5.4.1 CONTAMINANT CONCENTRATION RESULTS

22 MCX GAS STATION

MTBE was detected at five monitoring wells during the first and second sampling events (**Table 5-4**) while all other oxygenates (i.e., DIPE, ETBE, TAME, and TBA) were non-detect. During the first sampling event, MTBE concentrations ranged from 1.8 µg/L (at 22-MM-07) to 9.0 µg/L (at 22-BMW-15) and during the second sampling event MTBE concentrations ranged from 0.68 µg/L (at 22-MM-08) to 20 µg/L (at 22-BMW-3). The wells with the highest MTBE concentrations are located between the biobarriers and in the leading edge biobarrier. **Appendix B** provides tables of the historic concentrations of TPH (as diesel and gasoline), BTEX, MTBE and other fuel oxygenates at the wells sampled for this project. Concentration versus time graphs are provided for MTBE in **Appendix C** (**Figure 1** to **Figure 7**).

As shown in **Appendices B** and **C**, MTBE concentrations during active treatment decreased on average two orders of magnitude and the intermediate product TBA was not detected. Prior to active treatment with the biobarriers, MTBE concentrations ranged from 119 µg/L to 1.420 µg/L. After active treatment, MTBE concentrations ranged from to 5.3 to 11 µg/L. Although final concentrations were greater than the remedial goal of 5 µg/L, this concentration data indicated that the biobarrier operated as designed.

Historic MTBE concentration trends were evaluated using the Mann-Kendall test as shown in **Table 5-4**. The Mann-Kendall test is a nonparametric method to determine if concentrations are consistently increasing, decreasing, or stable. The overall trend of all the historic data was assessed for all monitoring wells. Trends were evaluated during the timeframes of active remediation and MNA at the site. Exceptions for the trend analyses occurred at 22-MM-07 and 22-BMW-08 where a sufficient quantity of data were not collected. At 22-MM-07, only three samples were collected between 2002 and 2016, and Mann Kendall requires at least four data points. At 22-BMW-08, groundwater samples were not collected after shutdown of the mid-plume biobarrier until 2016,

preventing the analysis of a trend at this location. **Figure 5-3** graphically depicts results for 22-DMM-05 representative well for the time period evaluated.

Table 5-4. Summary of MTBE and TBA Results from 2016 Sampling at the 22 Area MCX Gas Station

Location	Monitoring Well	Sampling Date	MTBE (µg/L)	TBA[1] (µg/L)
Upgradient of Mid Plume Biobarrier	22-MM-07	3/8/2016	1.8	ND
		8/17/2016	4.9	ND
	22-MM-08	3/8/2016	ND	ND
		8/17/2016	0.68	ND
Within Mid Plume Biobarrier	22-BMW-11	3/9/2016	2.4	ND
		8/17/2016	5.3	ND
	22-BMW-8	3/8/2016	ND	ND
		8/17/2016	ND	ND
Between Biobarriers	22-BMW-15	3/8/2016	9.0	ND
		8/17/2016	6.9	ND
	22-DMM-05	3/8/2016	8.8	ND
		8/17/2016	6.6	ND
Leading Edge Biobarrier	22-BMW-3	3/8/2016	3	ND
		8/17/2016	20	ND

ND – non detect

[1] Primary intermediate product of MTBE degradation

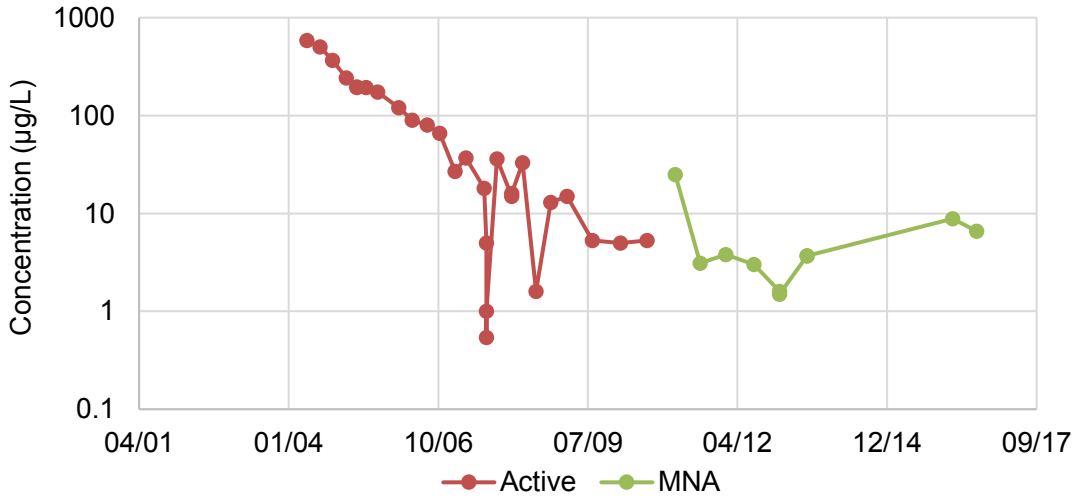

Figure 5-3. Trends of MTBE Concentration over Time for a Representative Well (22-DMM-05) at 22 MCX Gas Station Evaluated with Mann Kendall Analysis

Results of the Mann-Kendall analyses are summarized as follows:

- **Overall Site Trends:** When trend analyses are performed on the MTBE concentrations measured prior to biobarrier operation until 2016, after the barriers had been shut down, all wells except 22-BMW-08 show a decreasing trend with a confidence factor of greater than 95%. A decreasing trend was seen in 22-BMW-08, however the confidence factor was lower (>90%) due to fewer groundwater samples collected from the well. These trends demonstrated the overall remedial strategy at the 22 Area MCX Gas Station has significantly decreased the concentration of MTBE in site groundwater.
- **Active Treatment Trends:** Mann-Kendall trend analyses were performed on the data collected during active treatment (**Table 5-5)**. Similar to the overall MTBE trends, the trends during active treatment demonstrated a significant decrease in contaminant concentrations. This decreasing trend was seen at every well, demonstrating the success of the biobarriers in reducing contaminant concentrations at the 22 Area MCX Gas Station.

Table 5-5. Summary of MTBE Degradation Trends in Site Historical Data Including Period of Active Remediation

Location	Monitoring Well	Time	Phase	Coeff. of Variation	MK Statistic (S)	CF	MTBE Trend
Upgradient	22-MM-07	2002-2016	N/A	Insufficient data collected			
	22-MM-08	2002-2016	N/A	1.9	-439	>99.9%	Decreasing
Within Mid-Plume Biobarrier	22-BMW-11	2005-2016	Overall	1.81	-148	98.2%	Decreasing
		2005-2010	Active	1.69	-101	99.4%	Decreasing
		2010-2016	MNA	0.48	-6	64.8%	Stable
	22-BMW-08	2002-2016	Overall	4.58	-59	91.10%	Probably Decreasing
		2005-2010	Active	2.05	-91	99.9%	Decreasing
		2010-2016	MNA	Insufficient data collected			
Between Biobarriers	22-BMW-15	2005-2016	Overall	1.60	-170	98.70%	Decreasing
		2005-2010	Active	1.43	-207	>99.9%	Decreasing
		2010-2016	MNA	0.47	15	92.50%	Probably Increasing
	22-DMM-05	2005-2016	Overall	1.7	-474	>99.9%	Decreasing
		2005-2010	Active	1.42	-287	>99.9%	Decreasing
		2010-2016	MNA	1.15	2	53.50%	No Trend
Leading Edge Biobarrier	22-BMW-3	2004-2016	Overall	1.29	-227	99.60%	Decreasing
		2004-2012	Active	1.19	-112	97.60%	Decreasing
		2012-2016	MNA	0.76	-10	78.40%	Stable

MK Statistic – Mann Kendall Statistic
CF- Confidence Factor

- **MNA Trends (After Termination of Active Treatment):** Focusing the Mann-Kendall trend analysis on data collected after discontinuing operation of the biobarriers enabled evaluation of contaminant concentration trends during the MNA phase of the remedy. Within the mid-plume biobarrier, the analysis showed the MTBE concentration at 22-BMW-11 is stable – neither increasing nor decreasing. Between the biobarriers, at wells 22-BMW-15 and 22-DMM-05, the analysis revealed no trend in the data. At the leading edge biobarrier well, 22-BMW-3, the analysis indicated a stable MTBE trend after system shutdown in 2012. These stable trends indicate that the rate of contaminant loading (advection and dissolution) is balanced with the rate of contaminant attenuation (degradation and sorption). However, given the low levels of MTBE at the site, it is possible that the scatter within the data is a primary reason for not seeing a trend with MTBE concentration during the MNA timeframe.

As noted previously, MTBE can be degraded via direct and cometabolic processes following a variety of different pathways with the predominant degradation pathway producing TBA under both aerobic and anaerobic conditions. Concentrations of TBA were either near the detection limit or no TBA was detected over the last 15 years of monitoring. However, the absence of TBA does not rule out MTBE degradation via other degradation pathways. Other intermediates of MTBE biotransformation include HIBA, TBF, 2-methyl-2-hydroxy-1-propanol (MHP), and acetone (AIP. 2007). Of these other intermediate degradation products, HIBA is the only one which might accumulate during active remediation (Wilson 2003). However, analytical methods for HIBA had not been developed during the time the biobarriers were in operation, and therefore, was not analyzed. Thus, intermediates did not provide a line of evidence for assessing remedy progress at the 22 Area MCX Gas Station.

13 AREA GAS STATION

The 13 Area Gas Station served as a positive control for evaluating the microbial activity supporting natural attenuation of MTBE at the 22 Area MCX Gas Station. As such, groundwater from the 13 Area Gas Station was analyzed for MTBE and other oxygenates during the sampling events. In the first sampling event, MTBE was detected in the monitoring wells (**Table 5-6**) with concentrations ranging from 2,800 µg/L to 42,000 µg/L. The highest MTBE concentrations were located west of the fault at 1327-MW-07R and mid-plume at 1327-MW-23, respectively, as shown in **Figure 4-6**. In addition to MTBE, ETBE, TAME, and the intermediate TBA were detected during the first sampling event. In the second sampling event, MTBE concentrations, ranging from 5,700 µg/L to 27,000 µg/L, were detected and TBA was present in all wells except 1327-RW-07. None of the other oxygenates (i.e., DIPE, ETBE, and TAME) were detected. As in the first sampling event, 1327-MW-07R and 1327-MW-23 had the highest MTBE concentrations.

The *Annual Groundwater Monitoring Report for 13 Area Gas Station* (CB&I 2016b*)* presents results of Mann-Kendall tests performed using data from 33 wells with sufficient detection frequency of MTBE to test for concentrations trends. The results of the tests showed that statistically significant MTBE decreasing trends at most wells. These results are a strong line of evidence that treatment activities are effectively reducing MTBE concentrations. Therefore, it was expected that MTBE degrading microorganisms would be detected via metagenomic and metaproteomic analyses.

Table 5-6. Summary of MTBE and TBA Results from 2016 Sampling at the 13 Area Gas Station

Location	Monitoring Well	Sampling Date	MTBE (µg/L)	TBA (µg/L)	DIPE (µg/L)	ETBE (µg/L)	TAME (µg/L)
Source Area	1327-MW-01R	1/27/2016	13,000	2,100	ND	5.8	ND
		8/18/2016	3,400	450	ND	ND	ND
	1327-RW-07	1/27/2016	1,000	36,000	ND	ND	ND
		8/18/2016	8.6	ND	ND	ND	ND
	1327-MW-07R	1/27/2016	42,000	11,000	ND	ND	140
		8/18/2016	4,700	4,400	ND	ND	ND
Mid Plume	1327-MW-23	1/27/2016	37,000	2,100	ND	ND	ND
		8/18/2016	27,000	2,100	ND	ND	ND
Leading Edge	1327-MW-39	1/27/2016	2,800	270	ND	ND	19
		8/18/2016	5,200	1,300	ND	ND	ND

ND – non detect

5.4.2 GEOCHEMISTRY RESULTS

22 MCX GAS STATION

Table 5-7 lists geochemical data beginning with parameters representing terminal electron acceptors, parameters indicting whether the aquifer supports microbial activity followed by general parameters. Additionally, data on historic concentrations of DO and ORP during system operation were plotted for each well and are shown in **Appendix C (Figure C-8** through **Figure C-14)**. The timing of biosparge system operation was graphed to contrast the site conditions with the time when sparging was not active. Overall, the historic data show that the biobarriers created oxic conditions to support aerobic biodegradation of MTBE.

Multiple lines of evidence were used to interpret the redox conditions and assess what biogeochemical processes might be occurring at the site. The geochemical data contained conflicting information – suggesting both aerobic and anaerobic processes could be occurring in the same well. The following discussion describes each redox parameter and analyzes what the parameters indicated in terms of biogeochemical processes:

- **DO and ORP:** Based on DO readings, the site was predominately anoxic for both sampling events. The exceptions were the wells located between the biobarriers, which showed oxic conditions during the first sampling event. The ORP readings supported 22-BMW-15 could be oxic. This is an example of conflicting redox data. The ORP varied spatially and temporally without any evident relationship to other redox parameters. Based on ORP data alone, second sampling event indicated the site was anoxic except for wells 22-DMM-05 and 22-BMW-3. However, the DO measured in these wells was zero. This is another example of conflicting data. Overall, ORP data did not indicate whether the wells were predominately oxic or anoxic (oxygen depleted) because there was too much variation in data.

- **Nitrate**: Overall, nitrate levels were below their detection limits. During first sampling event, wells within the mid-plume biobarrier and in between the biobarriers had detectable, low levels of nitrate. Given the non-detectable levels of nitrate in the wells upgradient of the mid-plume barrier, nitrate is not expected to support MTBE biodegradation.

- **Ferrous Iron**: Both rounds of sampling showed that ferrous iron concentrations were at or below detection limits (0.05 mg/L) at all locations. The exception is 22-MW-08 where trace levels of iron were detected. This indicated either iron reducing conditions have not been reached at the site or ferrous iron was removed as a precipitate with sulfide produced during sulfate reduction. Given the high levels of sulfate (>100 mg/L) and low levels of methane (<0.5 mg/L) in most wells, it is highly likely that iron reducing conditions have not occurred at this site.

- **Sulfate**: As noted in the above bullet, the levels of sulfate at the site did not indicate sulfate reduction has or is occurring. Furthermore, the levels of sulfide were non-detect. Additionally, several studies with ^{14}C-labeled MTBE in sulfate reducing conditions indicate no evidence of loss of MTBE mass over a course of a year (Johnson, Bruce et al. 2010). As such, it was not expected for sulfate reduction to support MTBE degradation.

- **Methane**: Methane levels were at or below the detection limit of 0.010 mg/L. As sites with dissolved methane concentrations near or greater than 0.5 ppm are termed methanogenic (Wilson, Smith et al. 1986), the 22 Area MCX Gas Station was not considered to be methanogenic.

Overall, the site appeared to be anoxic with no demonstrable levels of iron reduction, sulfate reduction or methanogenesis. As such, the geochemical data do not provide positive evidence that biodegradation may be contributing to MNA of MTBE.

Table 5-7. Summary of Geochemical Results from 2016 Sampling at the 22 Area MCX Gas Station

Geochemical Parameter	Sampling Date	Upgradient of Mid Plume Biobarrier		Within Mid Plume Biobarrier		Between Biobarriers		Leading Edge
		22-MM-07	22-MM-08	22-BMW-11	22-BMW-8	22-BMW-15	22-DMM-05	22-BMW-3
ORP (mV)	3/8/2016	2	-292	24	50	145	10	80
	8/17/2016	-122	-211	-36	-37	-33	39	201
DO (mg/L)	3/8/2016	0	0	0	0	5.29	5.17	0
	8/17/2016	0	0	0	0	0	0	0
Nitrate (mg/L)	3/8/2016	<0.25	<0.25	0.36	0.28	1	1.2	<0.5
	8/17/2016	<0.25	0.25	<0.25	<0.25	<0.25	<0.25	<0.25
Ferrous Iron (mg/L)	3/8/2016	<0.05	0.15	<0.05	<0.05	<0.05	0.067	<0.05
	8/17/2016	<0.05	0.14	<0.05	<0.05	<0.05	<0.05	<0.05
Sulfate (mg/L)	3/8/2016	110	120	110	77	94	110	150
	8/17/2016	97	140	140	160	110	160	150
Sulfide (mg/L)	3/8/2016	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	8/17/2016	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Methane (mg/L)	3/8/2016	<0.010	0.14	<0.010	<0.010	<0.010	0.014	0.013
	8/17/2016	0.012	0.018	<0.010	0.013	0.013	<0.010	<0.010
pH	3/8/2016	7.18	7.18	6.81	7.04	7.16	6.83	7.20
	8/17/2016	7.67	7.64	7.77	7.8	7.74	7.53	7.53
Alkalinity (mg/L)	3/8/2016	460	330	480	380	580	350	500
	8/17/2016	500	440	390	440	460	450	480
TOC (mg/L)	3/8/2016	2.8	2.6	3.2	4.3	3.7	7.5	3.2
	8/17/2016	3.1	2.8	2.2	2.4	2.3	3.1	3.1
Conductivity (mS/cm)	3/8/2016	1.33	0.93	1.35	0.74	1.49	1.03	1.64
	8/17/2016	1.64	1.35	1.54	1.70	1.62	0.69	1.87
Turbidity (NTU)	3/8/2016	4.51	6.1	6.7	6.9	6.1	8.1	9.2
	8/17/2016	2.3	0	0	0	0	7.1	0
TDS (mg/L)	3/8/2016	900	820	910	670	1,100	850	1,100
	8/17/2016	890	800	850	970	910	1,000	1,100

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Geochemical data from the 13 Area Gas Station was used to evaluate the biosparging system performance with DO being the main parameter used in the assessment. The parameters were measured at the 13 Area Gas Station after temporarily turning off the biosparge system for 24 hours. Results are presented in **Table 5-8.** Similarly to the 22 Area MCX Gas Station, the geochemical parameters are listed beginning with parameters representing terminal electron acceptors, then parameters indicting whether the aquifer supports microbial activity followed by general parameters.

Table 5-8. Summary of Geochemical Results from 2016 Sampling at the 13 Area Gas Station

Geochemical Parameter	Sampling Date	Source Area			Mid Plume	Leading Edge
		1327-MW-01R	1327-RW-07	1327-MW-07R	1327-MW-23	1327-MW-39
ORP (mV)	4/25/2016	166	-66	170	-79	-43
	8/17/2016	-47	-49	10	36	-69
DO (mg/L)	4/25/2016	3.21	0	0	0	0
	8/17/2016	0	0.14	0.00	1.13	0.48
Nitrate (mg/L)	4/25/2016	<0.25	<0.25	0.38	<0.25	<0.25
	8/17/2016	<0.25	0.33	<0.25	<0.25	<0.025
Ferrous Iron (mg/L)	4/25/2016	<0.05	0.16	0.06	6.2	16
	8/17/2016	<0.05	0.62	0.082	6.2	14
Sulfate (mg/L)	4/25/2016	43	120	63	0.97	0.57
	8/17/2016	29	110	54	<0.50	2.2
Sulfide (mg/L)	4/25/2016	<0.1	<0.1	<0.1	<0.1	<0.1
	8/17/2016	<0.1	<0.1	<0.1	<0.1	<0.1
Methane (mg/L)	4/25/2016	NA	NA	NA	NA	NA
	8/17/2016	0.089	0.031	0.017	0.14	4.1
pH	4/25/2016	7.20	7.10	6.78	6.86	6.86
	8/17/2016	7.53	6.94	6.93	5.53	6.95
Alkalinity (mg/L)	4/25/2016	NA	NA	NA	NA	NA
	8/17/2016	780	370	460	150	310
TOC (mg/L)	4/25/2016	19	19	8.1	13	9.3
	8/17/2016	23	13	16	14	9.3
Conductivity (mS/cm)	4/25/2016	1.69	7.73	5.88	3.73	6.03
	8/17/2016	1.91	9.03	7.03	4.32	6.98
Turbidity (NTU)	4/25/2016	69.1	25.7	6.99	Over the detection limit	138
	8/17/2016	2.1	5.6	87.6	82.3	6.3
TDS (mg/L)	4/25/2016	NA	NA	NA	NA	NA
	8/17/2016	1,100	5,400	3,900	2,500	4,100

A DO concentration of 2 mg/L and greater is the threshold considered sufficient to support bioremediation. As noted by CBI&I in the *Annual Groundwater Monitoring Report for 13 Area Gas Station* (CB&I 2016b), the majority of the monitoring wells within 15 ft of a biosparge well had DO concentrations greater than 2.0 mg/L during process monitoring, which indicates the system is performing according to expectations. For this project, monitoring wells were sampled 24 hours after the biosparge system was temporarily shut down, and only 1327-MW-01R demonstrated a DO concentration above the threshold. The rapid depletion of DO in the other source wells (1327-RW-07 and 1327-MW-07R) indicated that aerobic degradation of

contaminants was occurring, and the aquifer was electron acceptor limited given the high level of contamination in the source.

ORP readings were varied at the site and did not indicate a trend in the source area. While the DO concentrations also did not have a trend associated with their values, there was no apparent relationship between the DO and ORP readings. It appears that some areas were rapidly depleted of oxygen in the source area where at other locations oxygenated groundwater remained for several days after system shutdown. In the mid-plume and at the leading edge of the plume, oxygenation by the biosparge system is limited, and overall the ORP levels indicated anoxic conditions during the second sampling event.

Terminal electron acceptors for anaerobic processes (i.e., ferrous iron, sulfate/sulfide, and methane) in the source area reflect active biosparging. Concentrations of ferrous iron and methane were at or below their detection limits, and sulfate concentrations were not depleted within the source area. In contrast, the mid-plume and leading edge wells indicate that natural attenuation of the contamination is occurring via anaerobic biodegradation. Concentrations of ferrous iron has increased in wells in the downgradient plume (mid-plume and leading edge), and methane was detected at the leading-edge well. Additionally, sulfate concentrations were depleted downgradient of the source area. The electron acceptors in the mid-plume and leading edge wells indicate that natural attenuation of MTBE is contributing to contaminant decreases outside of the source area (active biosparge treatment zone).

5.4.3 METAGENOMICS RESULTS

Final results based on the sequencing data for each sample collected from the 22 Area MCX Gas Station and the 13 Area Gas Station, were analyzed. The data tables and Krona plots as well as sample file statistics are listed in **Appendix D**. Since metagenomic data of samples 22-BMW-11, 22-BMW-15 and 22-MM-07 collected during the first sampling event did not meet the required DNA extraction criteria, only corresponding samples collected during the optional sampling were used for data analysis.

The taxonomic compositions of microbial communities from the both sampling events were analyzed at a genus level. A comparison was conducted across sampling events to evaluate if the metagenome composition was different between any of the sampling events. The comparison across events for both sites found insufficient evidence that there were significant differences between metagenomes with respect to the composition of MTBE metabolizers (**Figure 5-4**). A probability (p-value) of 0.21 indicates the lack of significant difference between the first and second sampling event for samples collected at either of the sites. In all cases the p-value of the significant difference between metagenomes was rejected with $p > 0.05$.

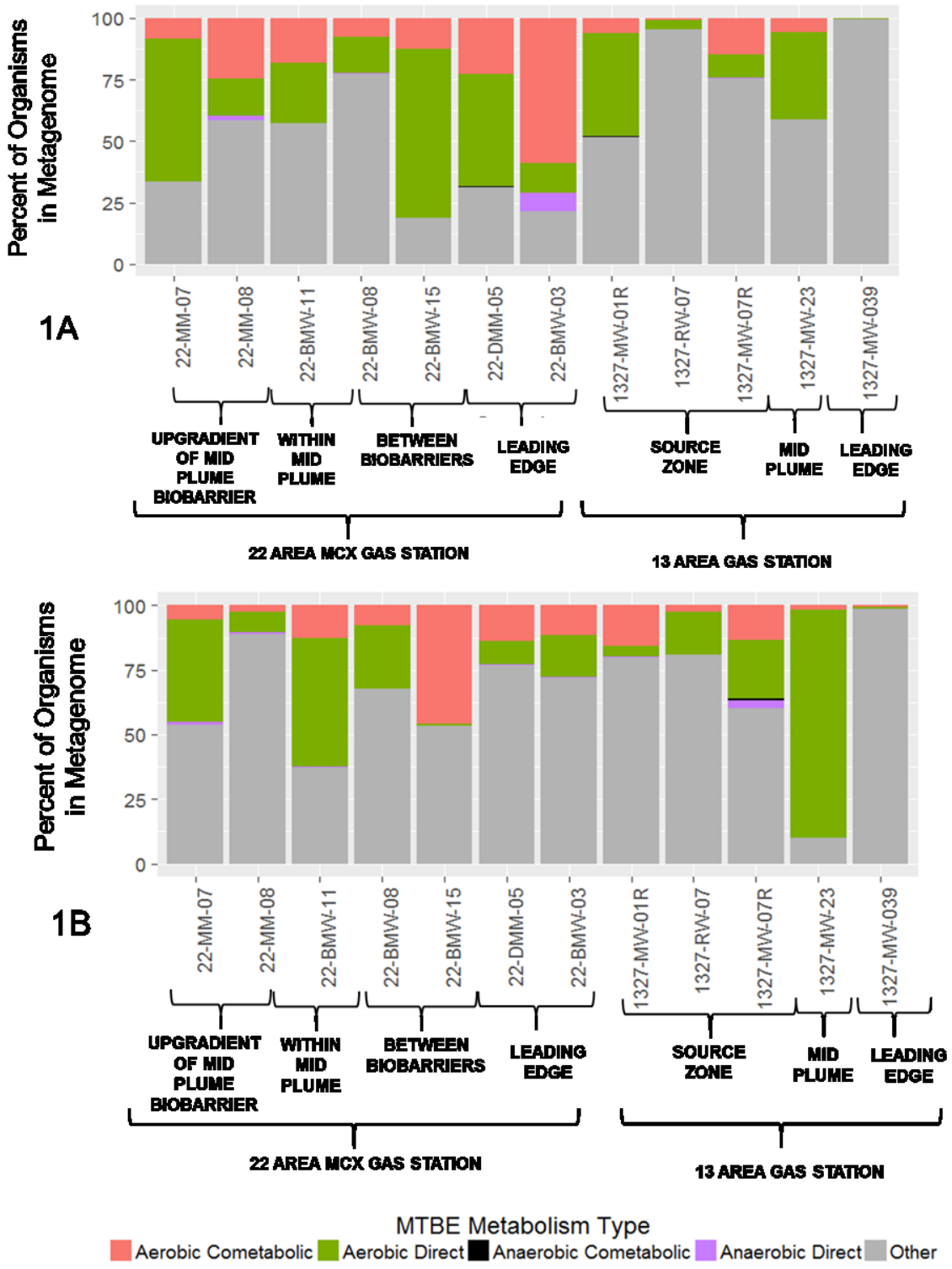

Figure 5-4. Percent Abundance of Microorganisms in Samples from the 22 Area MCX Gas Station and the 13 Area Gas Station where 1A is the First Sampling Event and 1B is the Second Sampling Event. MTBE-degrading Microorganisms were Categorized Depending on Aerobic/Anaerobic and Direct versus Cometabolic Degradation.

Data from the 22 Area MCX Gas Station was compared to the data from the 13 Area Gas Station using a Dirichlet- multinomial model and a significant difference (p-value = 0.0028) was found with respect to composition of MTBE-degrading microorganisms. At the 13 Area Gas Station when source zone metagenomes were compared to metagenomes from the leading edge of the plume and the mid-plume at 13 Area Gas Station, the statistical analysis showed a significant difference in microbial composition with a p-value of 0.016. A comparison within 22 Area MCX Gas Station wells showed that there were no significant differences between the upgradient, leading edge, between biobarriers and mid-plume metagenomes (p-value = 0.29). However, this evaluation is likely underpowered due to a small number of samples analyzed at a given well type and may not provide meaningful difference between the groups if such difference exists. Since a significant difference was not detected, pairwise comparisons between different well types at 22 Area MCX Gas Station were not conducted.

The Shannon diversity index at the 22 Area MCX Gas Station was comparable among all analyzed locations and showed little to no difference in richness of the microbial composition (H typically ranges from 1.5 to 3.5 for environmental samples). However, at the 13 Area Gas Station, the Shannon diversity index showed higher diversity of microorganisms (H ranging from 2.6 to 3.3) in the source zone area wells in comparison to the leading edge and mid-plume locations (H ranging from 1.4 to 1.7). This result indicates lower microbial diversity outside of the source zone area of the plume.

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Aerobic/Anaerobic Respirators. The majority of microorganisms detected at the 22 Area MCX Gas Station were aerobic with a small percentage of anaerobic or facultative anaerobic bacteria which are predominantly MTBE co-metabolizing species (**Figure 5-4**). At the locations upgradient from the biobarrier, aerobic species of *Acinetobacter, Bacillus, Pseudoxanthomonas* and *Sphingomonas* were dominant and a small percentage of anaerobic species of *Rhodoferax* and *Pseudomonas* were detected.

Similarly, the mid-plume biobarrier and the area between the biobarriers were characteristic of an abundance of aerobic species from genus *Pseudoxanthomonas* and *Sphingomonas* with only a small percentage of anaerobic or facultative anaerobic MTBE degraders such as *Aquinicola, Cupriavidus* or *Virovorax.*

At the leading edge of the biobarrier, the presence of both aerobic and anaerobic MTBE degraders with a dominance of cometabolic *Cupriavidus, Rhodoferax* and *Variovorax* species were detected suggesting MTBE cometabolism as a main degradation mechanism.

Direct versus Cometabolic MTBE Degraders. MTBE direct mineralizers were detected in all 22 Area MCX Gas Station samples with relative abundance ranging from 8 to 15% (**Appendix D**, **Tables D3 – D12** and **Tables D18- D24**). Cometabolic MTBE degraders were present in all sampled locations with highest relative abundance detected in the leading edge biobarrier samples.

The upgradient of the biobarrier and mid-plume biobarrier areas were dominated by the abundance of species from genus *Bacillus* (~2%), *Cupriavidus* (~7%), *Variovorax* (~8%) and

Pseudoxanthomonas (10 to 20%) (**Appendix D, Table D-3 to Table D-6**) that are known to degrade MTBE to TBA. These species are equipped with most, if not all enzymes, to support MTBE mineralization. The cometabolic bacteria detected in these two areas were predominantly of genus: *Acidobacteria* (ETBE degradation to TBA), *Pseudomonas* (variety of species degrade MTBE and BTEX when grown on pentane, partial MTBE degradation to HIBA grown on pentane, cometabolic MTBE degradation when grown on C_5 – C_8 n-alkanes), and *Sphingomonas* (partial MTBE degradation).

The area of highest abundance of direct MTBE mineralizers was detected in between the two biobarriers. Species of *Bacillus* and *Pseudoxanthomonas* were of the highest relative abundance (**Appendix D, Table D-7 and Table D-8)** with *Pseudoxanthomonas* representing approximately 66% of the microbial population in sample 22-BMW-15 (**Figure 5-5**). The cometabolic microorganisms present were classified into genus *Acinetobacter,* which performs partial oxidation of alkyl ethers; *Nocardioides,* which performs partial MTBE degradation using propane as a carbon source; and *Sphingomonas*, which also performs partial MTBE degradation.

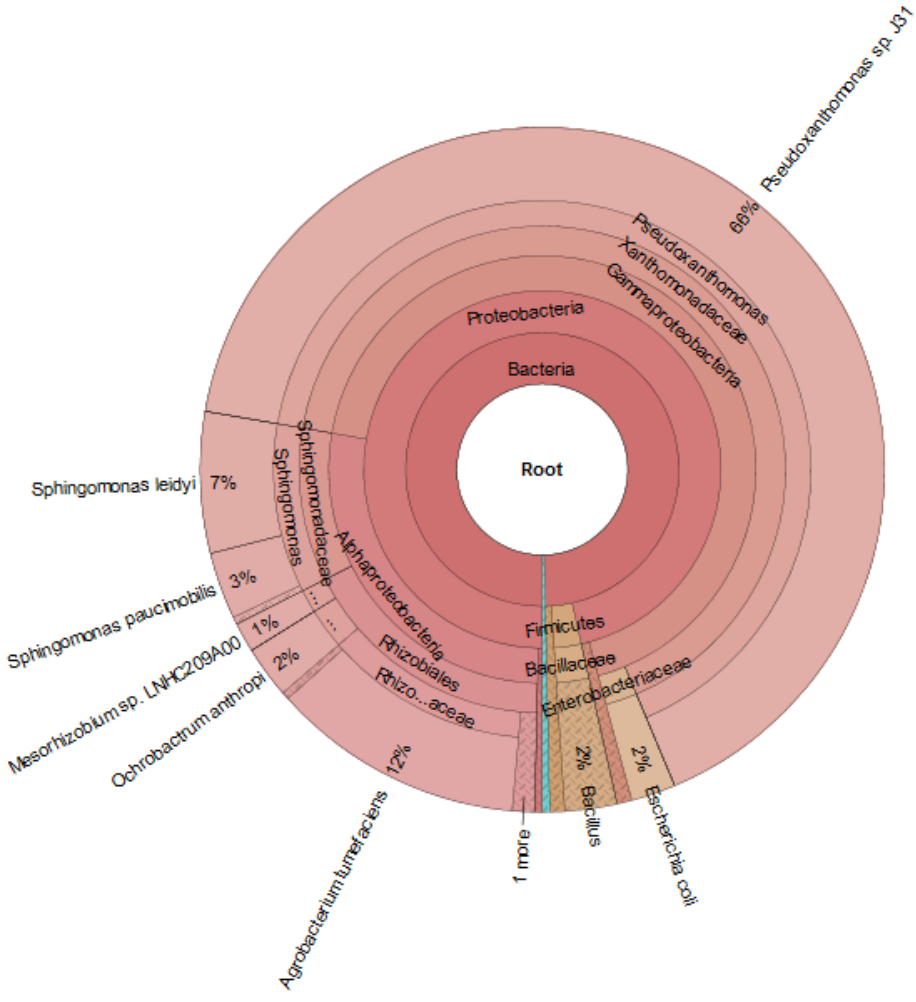

Figure 5-5. Krona Plot Illustrating Abundance of Microorganisms in the 22-BMW-15 Sample Collected in Between Biobarriers at the 22 Area MCX Gas Station

The leading edge biobarrier area showed dominance of cometabolic degraders from the genera *Pseudomonas* and *Sphingomonas* as illustrated in **Figure 5-6**. Microorganisms of these two genera represent a variety of species that are able to degrade MTBE and BTEX when grown on pentane, partially degrade MTBE to HIBA when grown on pentane and facilitate cometabolic MTBE degradation when grown on C_5-C_8 n-alkanes. However, the direct MTBE mineralizers within the leading edge biobarrier represented only 15% of the entire population with species from genus *Pseudoxanthomonas* and *Bacillus* (**Appendix D, Figure D-7**).

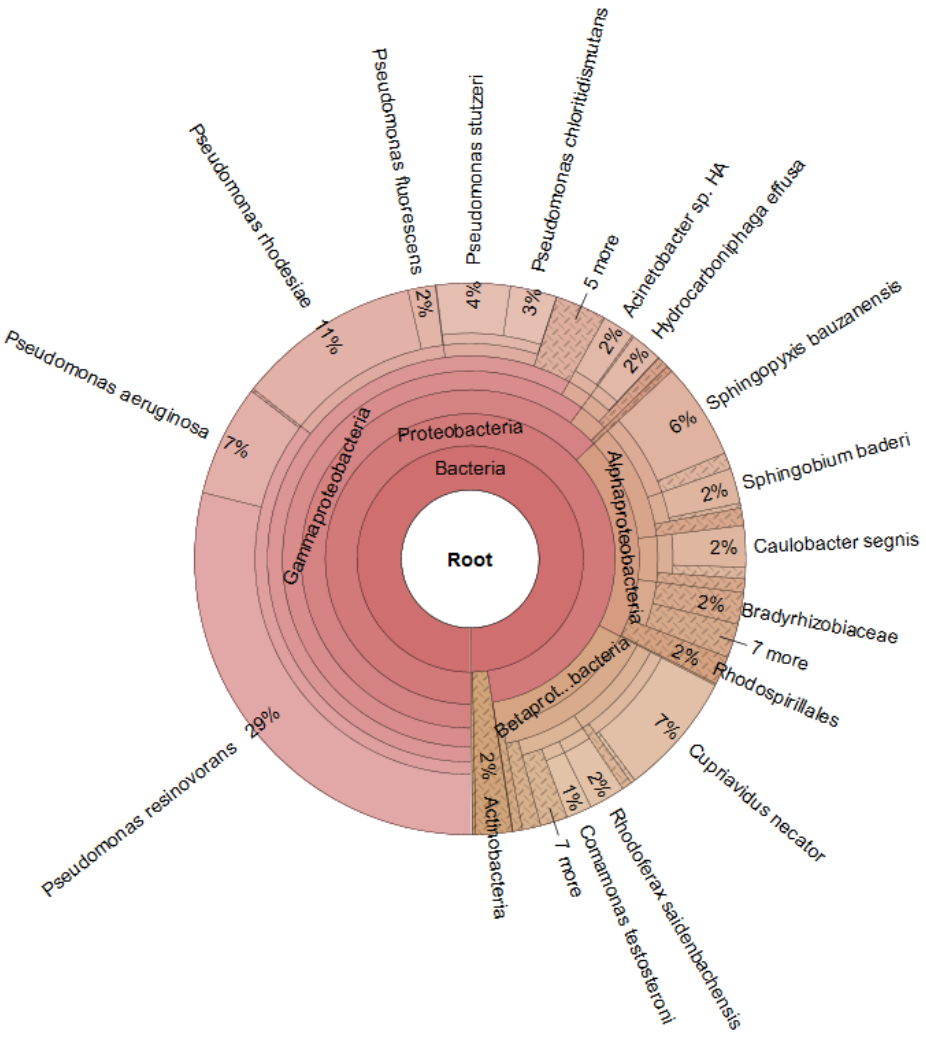

Figure 5-6. Krona Plot Illustrating Abundance of Microorganisms in the 22-BMW-3 Sample Collected in the Leading Edge of the Plume at the 22 Area MCX Gas Station

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Aerobic versus Anaerobic Respirators. The majority of MTBE degrading microorganisms detected at the 13 Area Gas Station were aerobic with a small percentage of anaerobic or facultative anaerobic MTBE cometabolizing species (**Figure 5-4**). Within the source zone and mid-plume locations, species of *Bacillus, Hydrogenophaga, Methylobium, Mycobacterium,* and *Nocardioides*

were the most abundant (**Appendix D, Tables D-13** to **D-17** and **Tables D-25** to **D-29**). A small percentage of anaerobic species from genera *Aquincola* and *Vorivarax* was also present. Thus, the dominant metabolism of MTBE in this area is aerobic.

Similarly, the mid-plume biobarrier was characteristic of high relative abundance of aerobic species from genera *Bacillus* and *Rhodobacter* detected during the first sampling event. A high relative abundance of *Mycobacterium* genus (86%). Specifically *Mycobacterium austroafricanum* that is known for aerobic direct MTBE degradation at gasoline spills sites, was observed in the samples collected during second sampling event.

In the leading edge of the biobarrier 1.7% of total microorganisms were MTBE-degraders of which 0.8% were aerobic species of genera: *Bacillus, Hydrogenophaga, Methylobium* and *Mycobacterium* (**Appendix D, Table D-29** and **Figure D-12**).

Direct versus Cometabolic MTBE Degraders. MTBE direct mineralizers were present in all samples of the 13 Area Gas Station with lowest relative abundance, approximately 0.2% of total microorganisms, in the leading edge of the plume (**Appendix D, Tables D-17** and **D-29**). Cometabolic MTBE degraders were present in all sampled locations with highest relative abundance in the source zone area (up to 24%) and leading edge area of the plume (up to 30%).

The source zone at the 13 Area Gas Station was dominated by the abundance of species from genera *Bacillus* (~6%), *Hydrogenophaga* (~7.5%), *Methylobium* (35%), *Variovorax* (~8%) and *Sphingopyxis* (15%) that are capable of biodegradation of MTBE to TBA and contain most if not all of the necessary enzymes for full mineralization of MTBE (**Appendix D, Table D-25 through Table D-27)**. **Figure 5-6** illustrates the microbial community composition in source zone sample 1327-MW-01R. The cometabolic bacteria in source zone wells were predominantly of genera: *Mycobacterium* (found to grow on MTBE and TBA), *Nocardioides, Rhodoferax* and *Rhodobacter*, which perform partial MTBE degradation with cyclohexane.

The mid-plume sample was rich in direct MTBE metabolizers with up to 30% of total MTBE degraders representing this fraction during the first sampling event; however, the number of direct metabolizers dropped to less than 2% when the next sampling was performed 6 months later. The variety of species represented genera *Bacillus, Variovorax* and *Sphingopyxis*. The dominant cometabolic fraction of the microbial population was represented by species of genera: *Mycobacterium, Nocardioides* and *Rhodobacter*. The leading edge population showed the least percentage abundance of direct MTBE species (0.3%) and cometabolic (0.3%) species.

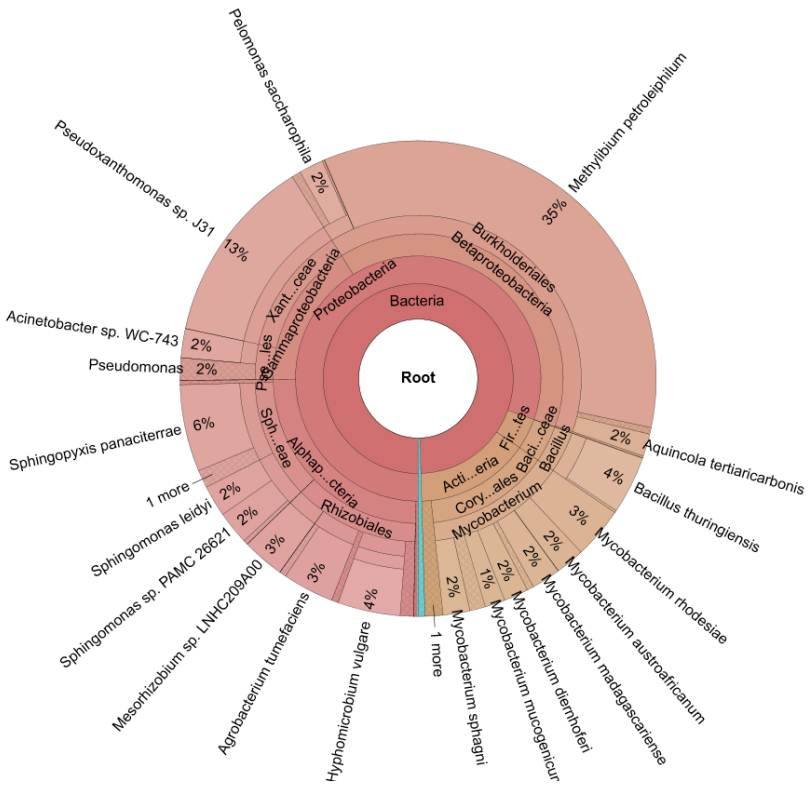

Figure 5-7. Krona Plot Illustrating Abundance of Microorganisms in the 1327-MW-01R Sample Collected in the Leading Edge of the Plume at the 13 Area Gas Station

SUMMARY FINDINGS OF METAGENOMIC DATA

Overall, metagenomes at the 22 Area MCX Gas Station showed a diversity of both direct and cometabolic MTBE degraders. The metagenomes of samples located between the biobarriers were dominated by full MTBE mineralizers which points towards a potential for its complete degradation. Although direct metabolizers were not present with high percentage in samples of upgradient and leading edge locations, the sequencing data showed higher abundance of cometabolic species that have an ability to carry out partial degradation of MTBE with parallel utilization of other substrates such as C_5 - C_8 n-alkanes.

A vast majority of microorganisms detected with 16S sequencing were aerobic MTBE degraders, both direct and cometabolizers. This is not unexpected since the site underwent sparging activities in the past. Moreover, the portion of the site that exhibited the highest relative abundance of aerobic species was between biobarriers and in the mid-plume location. This finding suggests that the biobarrier installation and oxygen sparging activities impacted the microbiology of the site, enriching the aerobic population of MTBE degraders.

Metagenomic results of samples collected at 13 Area Gas Station provide direct evidence to support MTBE aerobic degradation. The genera measured in source area well 1327-MW-01R indicate that the microbial population consists of highly abundant aerobic direct and cometabolic

MTBE species. This finding is in agreement with a decreasing trend of contaminant concentrations. On the contrary, sequencing data collected at the leading edge of the plume show little to no abundance of MTBE degrading species. However, within the mid-plume location, direct and cometabolic species are present, but with much lower abundance in comparison to the source zone. This result is in agreement with the geochemical data that show little or no DO, and ORP levels that indicated anoxic conditions within these two locations.

5.4.4 METAPROTEOMICS

Shotgun proteomics was performed on samples collected from both sites during all sampling events. Data was compiled in **Table 5-10** to determine: 1) presence of proteins from known MTBE degradation pathways (aerobic/anaerobic, cometabolic and direct MTBE mineralization), and 2) presence of proteins of known MTBE-degrading microorganisms. These two groups of proteins serve as indicators of MTBE degradation. While presence of proteins of known MTBE degradation pathways informs on activity of the degradation processes, detection of proteins from known MTBE-degrading microorganisms serves as indirect evidence of degradation.

Table 5-9. Summary of Protein Indicators of MTBE Biodegradation in Samples from the 22 Area MCX Gas Station and the 13 Area Gas Station during Sampling Events 1 and 2

Indicator	22 Area MCX Gas Station		13 Area Gas Station	
	Sampling Event 1	Sampling Event 2	Sampling Event 1	Sampling Event 2
Presence of proteins involved in known MTBE degradation pathways	None	None	2/5 samples (Source Area)	None
Presence of proteins from known MTBE degraders (e.g., membrane proteins and porins)	Few proteins identified from cometabolic degraders		5/5 samples (Direct and cometabolic degraders)	3/5 samples (Direct and cometabolic degraders)

Overall, no MTBE degradation proteins were identified at the 22 Area MCX Gas Station; however, a few proteins from cometabolic MTBE-degrading microorganisms were present. In contrast, both groups of protein indicators were found at the 13 Area Gas Station. As such, the proteomic data do not provide positive evidence of active MNA of MTBE at the 22 Area MCX Gas Station, but confirm active MTBE degradation at the 13 Area Gas Station. These positive indicators of bioremediation at the 13 Area Gas Station confirm that this site can serve a positive control for metaproteomic analysis. A comprehensive review of results at each site is provided below.

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Tables 5-10 and **5-11** show the number of peptides identified from direct and cometabolic MTBE-degrading microorganisms and categorize them as anaerobic- or aerobic-based degradation for each sampling well. **Appendix E** shows a list of all proteins detected in samples from the 22 Area MCX Gas Station.

During the first sampling event, the peptides detected at all of the sampling locations were derived from aerobic cometabolic MTBE-degrading microorganisms. For example, proteins from aerobic cometabolic MTBE metabolizer, *Nocardioides,* were found in upgradient and leading edge wells and suggest potential for cometabolic degradation. Some proteins from aerobic cometabolic MTBE-degrader, *Pseudomonas spp.,* were also present including a dehydrogenase, an aldehyde dehydrogenase and monooxygenases. However, the number of identified peptides was small, which indicated limited to negligible MTBE degradation is occurring.

During the second sampling event, a few peptides derived from aerobic direct MTBE-degraders were detected in addition to the peptides of aerobic cometabolic MTBE-degrading microorganisms. Both types of peptides were detected in all locations except for the mid-plume biobarrier. A few membrane structural proteins from the genera *Pseudomonas, Methylibium and Cupriavidus* were identified and suggested potential for either aerobic direct or cometabolic degradation.

Overall, although a few proteins from MTBE metabolizers were identified at the site, no peptides from known MTBE degradation pathways were detected. This can be related to either lack of ongoing MTBE degradation or presence of these peptides below the method detection limit.

Table 5-10. Number of Peptides from MTBE-Degrading Microorganisms Identified in Samples from the 22 Area MCX Gas Station during Sampling Event 1

Sampling Event 1							
	Upgradient of Mid Plume Biobarrier		Within Mid Plume Biobarrier	Between Biobarriers		Within Leading Edge	
	22-MM-7	22-MM-8	22-BMW-11	22-BMW-8	22-BMW-15	22-DMM-05	22-BMW-3
Electron Acceptor							
Aerobic	5	4	1	4	1	8	2
Anaerobic	0	0	0	0	0	0	0
Energy Source							
Direct	0	0	0	0	0	0	0
Cometabolic	5	4	1	4	1	8	2

Table 5-11. Number of Peptides from MTBE-Degrading Microorganisms Identified in Samples from the 22 Area MCX Gas Station during Sampling Event 2

Sampling Event 2							
	Upgradient of Mid Plume Biobarrier		Within Mid Plume Biobarrier	Between Biobarriers		Leading Edge	
	22-MM-7	22-MM-8	22-BMW-11	22-BMW-8	22-BMW-15	22-DMM-05	22-BMW-3
Electron Acceptor							
Aerobic	5	2	0	6	4	4	6
Anaerobic	0	0	0	0	0	0	4
Energy Source							
Direct	1	0	0	2	1	0	4
Cometabolic	4	2	0	4	3	4	6

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Tables 5-12 and **5-13** show peptides of aerobic/anaerobic, direct and cometabolic MTBE-degrading microorganisms detected at the 13 Area Gas Station during two rounds of sampling. During the first sampling event, the majority of identified peptides were associated with aerobic direct MTBE-degraders, including proteins associated with the direct MTBE degradation pathway and only a few peptides derived from cometabolic species. However, during the second sampling event, the number of identified peptides decreased in samples where only a few peptides of aerobic direct MTBE-degraders were identified. A more detailed discussion of proteomic results is provided below.

Table 5-12. Number of Peptides from MTBE-degrading Microorganisms Identified in Samples from the 13 Area Gas Station during Sampling Event 1

Sampling Event 1					
	Source Zone			Mid Plume	Leading Edge
	1327-MW-01R	1327-RW-07	1327-MW-07R	1327-MW-23	1327-MW-39
Electron Acceptor					
Aerobic	519	26	16	49	1
Anaerobic	0	0	0	0	0
Energy Source					
Direct	502	26	13	45	1
Cometabolic	17	0	3	4	0

Table 5-13. Number of Peptides from MTBE-degrading Microorganisms Identified in Samples from the 13 Area Gas Station during Sampling Event 2

Sampling Event 2					
	Source Zone			Mid Plume	Leading Edge
	1327-MW-01R	1327-RW-07	1327-MW-07R	1327-MW-23	1327-MW-39
Electron Acceptor					
Aerobic	1	6	0	0	12
Anaerobic	0	0	1	0	1
Energy Source					
Direct	1	5	1	0	10
Cometabolic	0	1	0	0	3

During the first sampling event, numerous proteins associated with MTBE-degradation pathways were detected in source zone area wells and are listed in **Table E-1 Appendix E**. The majority of proteins from the MTBE degradation pathway were detected in the 1327-MW-01R well. Proteins derived from direct aerobic MTBE degraders such as *Methylibium petroleiphilum*, *Aquincola tertiaricarbonis* and *Mycobacterium austroafricanum* were found. Seven out of ten known proteins from the *Methylibium petroleiphilum* MTBE degradation pathway (**Figure 5-8**) were detected and include the following:

- MdpE (HTBF dehydrogenase),
- MdpH (MHP dehydrogenase),
- MdpJ (TBA hydroxylase),
- MdpK (TBA hydroxylase),
- MdpO (HIBA mutase),
- MdpR (HIBA mutase).

The uniqueness of Mdp peptides has not yet been confirmed (i.e., BLASTp was not performed to determine if peptides are unique to proteins of interest). Additionally, MdpA, the enzyme responsible for the conversion of MTBE to TBA, was targeted using an inclusion list that consisted of theoretical MdpA tryptic peptide masses listed in **Table E-2** in **Appendix E**. This list was based upon alignment of known MdpA protein sequences published in literature and available in the NCBI database. This targeted strategy increases the sensitivity for detection of targeted peptides that may not be detected during a shotgun proteomics run. However, the MdpA protein was not identified using this targeted strategy.

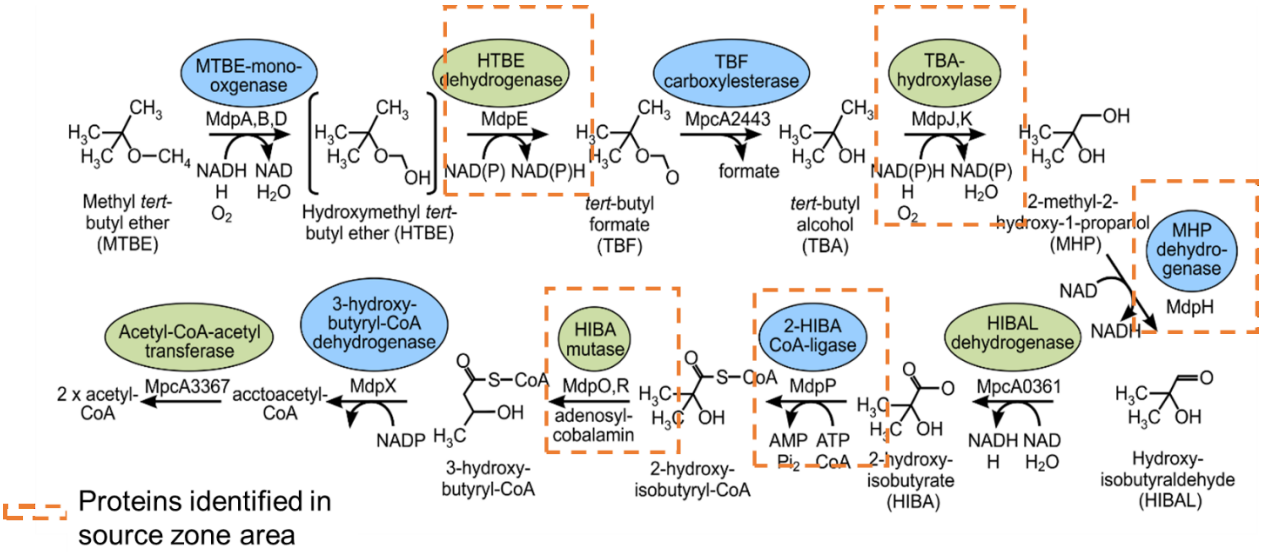

Figure 5-8. Proteins Involved in MTBE Degradation of *M. petropleiphilum* PM1 (Hristova, Gebreyesus et al. 2003; Schmidt, Battaglia et al. 2008)

During the first sampling event, the remaining two source zone area wells, 1327-RW-07 and 1327-MW-07R, also showed presence of proteins from *Methylibium petroleiphilum*. However, no Mdp proteins from the MTBE degradation pathway were identified. Rather membrane proteins (i.e., porins or membrane transporters) were found and are known to be involved in phenol degradation and transport of both ions and small molecules across a cellular membrane. However, their role (if any) in direct MTBE degradation is unknown. Moreover, in the source zone areas phenol degrading proteins and cytochrome P450, that catalyzes hydroxylation of methoxy and ethoxy residues in fuel oxygenates, were also detected and suggest a potential for BTEX degradation. Presence of proteins of known cometabolic MTBE degraders such as *Nocardioides* also suggest some MTBE cometabolic degradation could be occurring.

The number of proteins of MTBE-degrading microorganisms was low in samples collected during the first sampling event at the mid-plume and leading edge. No MTBE proteins related to the Mdp or cometabolic pathways were found but a handful of *Aquinicola sp.* and *Methylibium sp.* membrane proteins and porins were detected. It is worth noting that the mid-plume and leading-edge wells are located within the area of limited influence of the biosparge system. Thus the degradation of MTBE as well as diversity of microbial community differed significantly ($p = 0.016$) in comparison to the source zone location.

The total number of proteins detected throughout the site were lower during the second sampling event in comparison to the first sampling event. Although no proteins associated with the MTBE degradation pathway were detected, proteomic data showed general cellular metabolism and structural proteins from direct aerobic MTBE degraders *Mycobacterium* spp, *Methylibium petroleiphilum,* and *Aquincola tertiaricarbonis.* A variety of proteins from *Methanosaeta concilii*,which is a known obligate anaerobic archaea populating sites with gasoline contamination, were also found.

The difference in number of detected proteins at the 13 Area Gas Station during two sampling events may be due to the fluctuations in groundwater levels noted between two sampling events. The groundwater elevation fluctuates an average of two feet during the year. These changes in groundwater level may affect the concentration of planktonic microbial biomass available for collection. It is possible that with this decrease in the groundwater level, the bulk of the microbial biomass is tightly bound to the sediment or present within the sediment porous spaces. However, to explicitly prove that this was the case, additional rounds of sampling and data analysis would need to be performed.

SUMMARY FINDINGS FOR METAPROTEOMICS

Proteomic results do not suggest degradation of MTBE in samples from the 22 Area MCX Gas Station. The lack of identification of MTBE-degrading proteins from either direct or cometabolic MTBE degradation pathways shows that the microbial community demonstrated by the metagenomics analyses to be capable of degrading MTBE, is currently either not metabolizing the compound or that the concentration of the MTBE degrading proteins is below the detection level of the proteomics experiment.

The presence of proteins catalyzing MTBE degradation, specifically MHP dehydrogenase, TBA hydroxylase and HIBA mutase in the source area supports the use of the 13 Area Gas Station as a positive control for metaproteomic analyses. In the first sampling event, the results from the source area are in a good agreement with the contaminant concentration data and geochemical parameters[4] and demonstrate that **active** direct aerobic MTBE degradation is occurring. In contrast, proteomic data collected during the second sampling event show less evidence of contaminant degradation. This is may be due to the change in groundwater level during the second sampling event compared to the first sampling event. Similar to the 22 Area MCX Gas Station, the absolute quantification of proteins involved in MTBE degradation would help to fully understand an ongoing metabolism.

5.4.5 SLUG TEST RESULTS TO ASSESS IMPACT OF BIOBARRIERS ON FORMATION PERMEABILITY

Slug tests were performed in 2001 in wells 22-MM-06, 22-MM-07, and 22-MM-08 to estimate hydraulic conductivity K (ft/day) in the formation adjacent to each well. Results of the 2001 slug tests are summarized in **Table 5-14** and **Appendix F** provides the response curves as well as a write-up of the results. These 2001 results serve as the baseline to evaluate the second hypothesis in the project that postulates the formation permeability within the ROI of the biobarriers decreased over time due to increased biomass production as a direct result of injecting oxygen into the aquifer. To evaluate this hypothesis, slug tests were performed in 2016 to estimate the hydraulic conductivities upgradient and within the mid-plume biobarrier. The results of the 2016 slug tests at 22-BMW-08, 22-MM-07, and 22-MM-08 are presented in **Table 5-15**.

[4] The geochemical parameters at the 13 Area Gas Station were measured after 24 hours of biosparge system shutdown. Depletion of DO is expected to occur quickly because of the high levels of MTBE.

Table 5-14. 2001 Slug Test Hydraulic Conductivity Results

Well ID	Slug Position	Test Number	Cooper et al.				Bouwer and Rice	
			T (ft^2/d)	S	Fit	K (ft/d)	K (ft/d)	Fit
22-MM-06[1]	In	Test 1	0.4988	0.006003	Good	56.92	40.74	Good
	Out		0.8642	0.000103	Poor	98.61	19.81	Poor
22-MM-07	In	Test 1	0.5279	0.000113	Good	51.12	32	Good
		Test 2	0.2471	0.003331	Good	23.93	23.39	Good
	Out	Test 1	1.025	1.00E-10	Poor	99.26	62.12	Good
		Test 2	0.4668	3.22E-05	Poor	45.2	64.57	Good
22-MM-08	In	Test 1	0.0319	1.00E-10	Good	4.18	1.41	Good
	Out		0.01443	1.00E-10	Poor	1.89	0.7	Good

Table 5-15. 2016 Slug Test Hydraulic Conductivity Results

Well ID	Slug Position	Test No.	Cooper et al.				Bouwer and Rice	
			T (ft^2/d)	S	Fit	K (ft/d)	K (ft/d)	Fit
22-BMW-8	In	Test 1	121.3	0.00741	Good	8.1	41.13	Good
		Test 2	75.5	0.02371	Good	5.0	40.98	Good
		Test 3	164	0.01321	Poor	10.9	33.39	Good
	Out	Test 1	343.7	1.576E-07	Good	22.9	47.74	Good
		Test 2	287.2	1.395E-06	Good	19.1	45.72	Good
		Test 3	335.5	2.133E-08	Good	22.3	39.33	Good
22-MM-07	In	Test 1	967.1	7.746E-06	Good	60.3	38.49	Good
		Test 2	1,556.7	1.0E-10	Poor	97.0	36.63	Good
		Test 3	1,630.1	1.0E-10	Good	101.6	27.83	Good
	Out	Test 1	754.6	2.687E-05	Good	47.0	158.1	Good
		Test 2	1,523.4	1.0E-10	Good	94.9	228.4	Good
		Test 3	2,055.8	1.0E-10	Good	128.1	179.1	Good
22-MM-08	In	Test 1	56.2	1.0E-10	Fair	1.6	1.485	Good
		Test 2	20.6	1.834E-05	Fair	0.6	1.337	Good
		Test 3	25.6	6.259E-06	Fair	0.7	1.305	Good
	Out	Test 1	54.3	1.0E-10	Good	1.5	1.542	Good
		Test 2	34.5	1.92E-07	Fair	1.0	1.376	Good
		Test 3	22.9	6.259E-06	Fair	0.6	1.347	Good

The raw 2016 slug test data were imported into AQTESOLV and analyzed using the same solution methods used to analyze the 2001 data, including nonlinear inversion to estimate transmissivity (T) by the Cooper et al. (Cooper, Bredehoeft et al. 1967) method and visual straight line matching to estimate K by the Bouwer-Rice method. The response curves are provided in **Appendix G**. As seen by the response curves, the initial displacement caused by the withdrawal or insertion of

the slug was an approximate 1 to 2 feet of change in water level in the well. After displacement, water levels in wells 22-BMW-8 and 22-MM-07 recovered to pre-test levels in 2 to 4 minutes. This relatively rapid recovery matches that observed in 2001 and is consistent with the logged soil types in the screened interval of these wells – silty sand. In contrast, 7 to 10 minutes was required for recovery of well 22-MM-08. This again is consistent with the findings from 2001 and with the logged soil type in the screened interval of this well – silt with sand. **Figure 5-9** shows the third slug-out test for 22-MM-08 where equilibrium was reached after 7 minutes. Also noted in the 2016 results, the slug-in tests exhibited oscillating behavior while the slug-out tests showed a regular water level rebound. **Figure 5-10** shows examples of the oscillating behavior at 22-BMW-08.

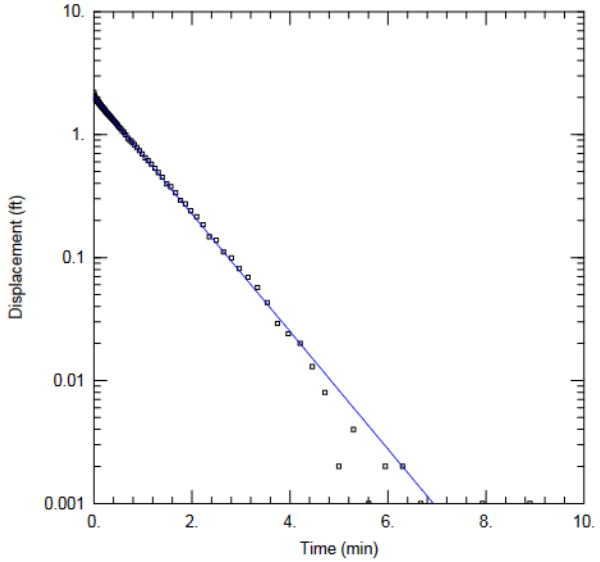

Figure 5-9. Response Curve for the Slug-out Test 3 for 22-MM-08 Where Equilibrium was Reached after Seven Minutes

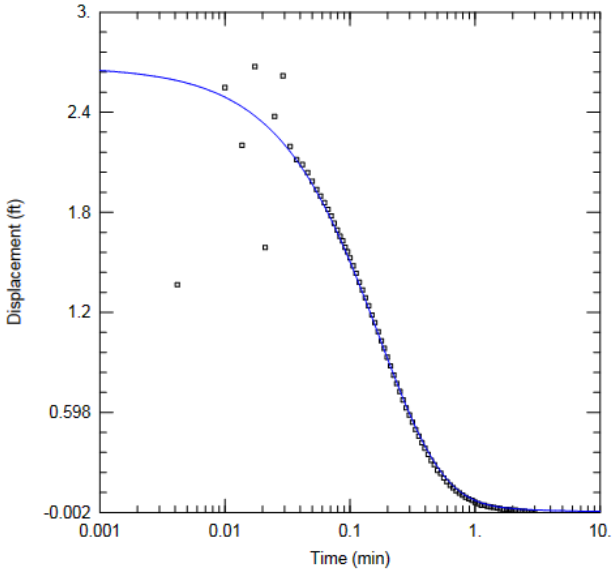

Figure 5-10. Response Curve for the Slug-out Test 1 at 22-BMW-08

(The oscillations occurred directly after insertion [prior to 0.03 minutes]).

22-BMW-08 Slug Test Results

Three complete slug tests (slug-in and slug-out) were conducted. Slug-out tests using Cooper et al., showed a geometric mean hydraulic conductivity of 12.9 ± 2.0 ft/day (**Table 5-16**). The slug-in tests evaluated with Cooper et al., had a geometric mean of 7.6± 3.0 ft/day. The geometric means of the slug-out and slug-in tests estimated using the Bouwer-Rice method were more similar, with values of 44.1 ± 4.4 and 38.3 ± 4.4 ft/dy, respectively. The overall geometric mean for 22-BMW-08 is 22.9 ± 15.3 ft/day.

Table 5-16. Summary of the Geometric Means of the Estimated Hydraulic Conductivity at 22-BMW-08 Using the Cooper et al., and Bouwer-Rice Methods

Tests Performed	Geometric mean (ft/day)	
	Cooper et al.	Bouwer -Rice
Slug-out Tests	12.9 ± 2.0	44.1 ± 4.4
Slug-in Tests	7.6 ± 3.0	38.3 ± 4.4
Combined	22.9 ± 15.3	

22-MM-07 Slug Test Results

For well 22-MW-07, the three slug-out tests evaluated using Cooper et al., showed a geometric mean hydraulic conductivity of 83.0 ± 40.8 ft/day (**Table 5-17**). The three slug-in tests evaluated with Cooper et al., had a geometric mean of 76.5± 26.0 ft/day. The geometric means of the slug-out and slug-in tests estimated using the Bouwer-Rice method were 168.3 ± 14.9 and 34.0 ± 5.7 ft/dy, respectively. The overall geometric mean for 22-BMW-08 is 81.5 ± 63.5 ft/day.

Table 5-17. Summary of the Geometric Means of the Estimated Hydraulic Conductivity at 22-MM-07 Using the Cooper et al., and Bouwer-Rice Methods

Tests Performed	Geometric mean (ft/day)	
	Cooper et al.	Bouwer -Rice
Slug-out Tests	83.0 ± 40.8	168.3± 14.9
Slug-in Tests	76.5 ± 26.0	34.0± 5.7
Combined	81.5 ± 63.5	

22-MM-08 Slug Test Results

Three slug tests were also performed at well 22-MM-08. The difference between 22-MM-08 and the other two wells is the screening interval. 22-MM-08 is screened at a lower depth, from 35 to 40 ft bgs, in a silt with sand, compared to the sand above. This difference in lithology is reflected in the estimated hydraulic conductivity values of 22-MM-08; the overall geometric mean is 1.13

± 0.37 ft/day (**Table 5-18**). This represents an order of magnitude reduction in permeability when compared to 22-MM-07, which is located upgradient of the mid-plume biobarrier.

The Bouwer-Rice method resulted in a very consistent estimated geometric mean for K of 1.4 ft/day for both slug-in and slug out tests. The curve matches for the Bouwer-Rice method for 22-MM-08 were the strongest of the 2016 data. The strong fit of the calculated and measured data is most likely a combination of the short well screen (5 ft) and not intercepting the water table. Both items reduce the impact of the well on the test results. The results from the Cooper et al., method were also more consistent in this well, with the geometric mean of K values for slug-out tests at 0.97 ± 0.44 ft/day and for slug-in tests at 0.86 ± 0.53 ft/day. The overall geometric mean K value for this well was estimated as 1.1 ± 0.37 ft/day.

Table 5-18. Summary of the Geometric Means of the Estimated Hydraulic Conductivity at 22-MM-08 Using the Cooper et al., and Bouwer-Rice Methods

Tests Performed	Geometric mean (ft/day)	
	Cooper et al.	Bouwer -Rice
Slug-out Tests	0.97 ± 0.44	1.4 ± 0.11
Slug-in Tests	0.86 ± 0.53	1.4 ± 0.10
Combined	1.1 ± 0.37	

Summary of 2016 Slug Test Results

Overall, the 2016 slug test results show an acceptable fit between the calculated type curves and measured data for most of the results. However, the Bouwer-Rice method produced better curve fits to the data compared to the Cooper et al., method. At 22-BMW-08 and 22-MM-07, double-line responses reduced the quality of fit for the Bouwer-Rice method, which is typical for wells screened across the water table. A double-line response shows influence of the filter pack on the response, and a correction is applied within Aqtesolv when evaluating wells screen across a water table.

In addition to reviewing the fit of the measured to the calculated data, the reproducibility of the triplicates for the slug-in and slug-out tests were also reviewed. For 22-BMW-08, there was good reproducibility with the triplicate tests and the geometric means having small standard deviations. For 22-MM-07, there was a wider range of estimated K values between replicates, especially using the Cooper et al., method. For 22-MM-08, there was good reproducibility in the triplicate sets when analyzed with the Bouwer-Rice Method, less so with the Cooper et al., method.

Summary of 2001 Slug Test Results

The results of the 2001 slug tests are summarized in **Table 5-19**. Overall fewer slug tests were conducted during 2001 with duplicate slug tests performed only in 22-MM-07. Only one slug test (in and out) was performed at each of 22-MM-06 and 22-MM-8. As such, there is limited information on the variability within the 2001 slug test data for the wells examined for this project. However, slug tests were performed on five different wells in 2001 with the intent of evaluating the hydraulic conductivity around the mid-plume biobarrier location. The slug tests results

estimated the hydraulic conductivity of the shallow alluvium (20 ft bgs) at 43 ft/day and of the deeper alluvium (40ft) at 2.0 ft/day. In the bullets below, the three wells serving as baselines for this project are discussed in terms of the model fit between the type curves and measured data.

Table 5-19. Summary of the Geometric Means of the Estimated Hydraulic Conductivities for the 2001 Slug Tests

Well Location	Geometric Mean
	ft/day
22-MM-06	48.2 ± 11.4
22-MM-07	39.3 ± 18.8
22-MM-08	1.6 ± 1.8

- For 22-MM-06, one complete slug-in and slug-out test was conducted. The slug-in tests had good fits for both methods (Cooper et al., and Bouwer-Rice) while the slug-out tests had poor fits for both methods. The geometric mean shown in **Table 5-19** was calculated removing the results with poor fits.
- For 22-MM-07, two complete slug-in and slug-out tests were conducted. As noted in the 2016 results, this well is screened across the water and the well filter pack impacted the slug test results. Specifically, the slug-out duplicates had poor fits with the Cooper et al., method and were removed from the geomean calculation. Even with removing the poor fits, slug test results had a wide range of estimated hydraulic conductivities due to the impact of the well filter pack and the resultant double-line response in the response curves.
- For 22-MM-08, one complete slug-in and slug-out test was conducted. The slug-in tests had good fits for both the Cooper et al., and Bouwer-Rice methods. For the slug-out tests, the fit was poor for the Cooper et al., method but good for the Bouwer-Rice method. It is assumed the poor fit resulted from the well only partially penetrating the aquifer. The geometric mean calculated with the good fit results is 1.6 ± 1.8 ft/day.

Comparison of 2001 to 2016 Slug Test Results

The geometric means of hydraulic conductivity values derived from multiple slug tests using multiple data reduction solutions (**Tables 5-17** through **5-19**) can be compared on a well-by-well basis with equivalent hydraulic conductivity values derived in 2001 (**Table 5-19**). This comparison shows that hydraulic conductivity values derived for each of the three wells based on 2001 and 2016 data were within a factor of two. Given the variability inherent in slug testing and interpretation, a factor of two difference is small enough to conclude that the hydraulic conductivity values are not meaningfully different before and after biosparging.

6.0 PERFORMANCE ASSESSMENT

This section includes a detailed assessment of technology performance based on quantitative data presented in Section 5. Data were reviewed to determine whether the hypotheses tested were true. The evaluation of each of the hypotheses is discussed below, with references to the relevant supporting results in Section 5.

6.1 EVALUATION OF MICROBIAL ACTIVITY SUPPORTING NATURAL ATTENUATION OF MTBE

The first hypothesis was tested using a multiple line of evidence approach that included chemical and geochemical analyses as well as metagenomics and metaproteomics to evaluate natural attenuation at of MTBE at the 22 Area MCX Gas Station.

The first two lines of evidence for natural attenuation, including an evaluation of plume behavior (i.e., decreasing, stable or increasing) and geochemical data, are discussed in section 6.1.1 and evaluated the plume behavior (i.e., decreasing, stable or increasing) and then assessed the geochemical data. Metagenomics and metaproteomics were used as a tertiary line of evidence to assess attenuation of MTBE at the 22 Area MCX Gas Station. The advanced MBTs helped to evaluate the potential for MTBE degradation as well as MTBE degradation activity. The **potential** for MTBE degradation was evaluated based on the diversity of microorganisms detected in groundwater samples determined using a 16S metagenomic sequencing approach and is discussed in Section 6.1.2. The occurrence of degradation **activity** was evaluated using metaproteomics and is detailed in Section 6.1.3.

6.1.1 ASSESSMENT OF NATURAL ATTENUATION WITH CONVENTIONAL MONITORING

Hypothesis: Concentrations of MTBE are decreasing or stable over time and geochemical parameters indicate that the environment at the 22 Area MCX Gas Station is conducive to MTBE biodegradation.

Finding 1: *The contaminant concentration data provide the first line of evidence that natural attenuation is sufficiently occurring to prevent migration and increased concentrations of the remaining MTBE.*

In the first line of evidence, a statistical analysis was conducted to evaluate MTBE concentration trends. For this analysis, the Mann-Kendall Test was used to evaluate historical data along with the results from this investigation. Overall site trends (combining data collected, before, during and after the biosparge system was in operation), trends during active biosparging, and trends during the MNA phase of the remedy were evaluated. As expected, the overall site trends and the active treatment trends demonstrated a significant decrease in COCs. However, the analysis performed on data collected after the biosparge system was discontinued did not show a clear decreasing trend. Within the mid-plume biobarrier, the analysis showed the MTBE concentration at 22-BMW-11 is stable – neither increasing nor decreasing. Between the biobarriers, at wells 22-BMW-15 and 22-DMM-05, the analysis revealed no trend in the data. At the leading edge

biobarrier well, 22-BMW-3, the analysis indicated a stable MTBE trend after system shutdown in 2012. Although not decreasing, these stable trends indicate that the rate of contaminant loading (advection and dissolution) is balanced with the rate of contaminant attenuation (degradation and sorption).

Finding 2: *The presence/absence of intermediates did not provide a line of evidence for assessing natural attenuation at the 22 Area MCX Gas Station.*

Concentrations of the intermediate TBA were either near the detection limit or no TBA was detected over the last 15 years of monitoring, which may indicate that MTBE is not being degraded through this pathway. However, the absence of TBA does not rule out MTBE degradation via other degradation pathways.

Finding 3: *Overall, the 22 Area MCX Gas Station appeared to be anoxic with no demonstrable levels of iron reduction, sulfate reduction or methanogenesis. As such, the geochemical data do not provide positive evidence that biodegradation may be contributing to natural attenuation of MTBE.*

The secondary line of evidence is not intended to provide direct evidence that MTBE is/has been biodegraded. Rather, these data are collected to delineate biogeochemical processes at the site and infer microbial activity related to contaminant biodegradation. This evaluation required analysis of a variety of geochemical parameters including oxidation-reduction potential (ORP), dissolved oxygen (DO), terminal electron acceptor indicators (e.g., nitrate, ferrous iron, sulfate/sulfide, methane), and various other anions and cations.

Based on DO readings, the 22 Area MCX Gas Station was predominately anoxic during both sampling events. The ORP varied spatially and temporally without any evident relationship to other redox parameters. As such, ORP data did not indicate whether the wells were predominately oxic or anoxic. Negligible to non-detect levels in nitrate, ferrous iron, and methane as well as the lack of consumption of sulfate indicated little anaerobic microbial activity. Thus, the geochemical data do not indicate that specific biogeochemical processes occur at the site, and it cannot be inferred that MTBE biodegradation is actively occurring.

6.1.2 ASSESSMENT OF MICROBIAL ACTIVITY WITH METAGENOMICS

Hypothesis: The presence of microbial species determined using metagenomics supports the biodegradation potential of MTBE at the 22 Area MCX Gas Station.

Finding 1*: Metagenomics supports biodegradation* ***potential*** *of MTBE at the 22 Area MCX Gas Station.*

Summary: This hypothesis was developed to demonstrate the potential for MTBE biodegradation at the 22 Area MCX Gas Station using 16S metagenomic sequencing. To perform assessment of the current state of MTBE degradation at the 22 Area MCX Gas Station, a total of 14 metagenomes were investigated (two sampling rounds of seven wells each). This included analysis of specific groups of microorganisms: aerobic/anaerobic, direct mineralizers, cometabolic degraders, involved in the degradation of MTBE and its daughter products (e.g., TBA, HIBA, TBF, 2-methyl-

2-hydroxyl-1-propanol, and acetone). All of the 14 metagenomes showed the presence of a broad diversity of MTBE degrading bacteria with a varied percentage of relative abundance of direct MTBE metabolizers to the cometabolic MTBE degraders shown in **Figure 5-4**. The majority of identified MTBE degraders were aerobic cultures which highlights the potential for aerobic direct or cometabolic mineralization processes.

The highest potential for direct mineralization of MTBE was detected in between biobarriers where a high abundance of MTBE degraders was found. Presence of *Methylobium petroleiphilum* PM1, *Hydrogenophaga flava* ENV735*, Mycobacterium austroafricanum* and *Pseudoxanthomonas* species further confirms this observation. For example, bacteria of genus *Pseudoxanthomonas* have been detected at the site and are capable to degrade MTBE using it as a sole carbon source and are capable to degrade other gasoline components such as octane, BTEX, cyclohexanol, cyclohexane, and isooctane (Digabel and Fayolle-Guichard 2015). This increased relative abundance of MTBE-degrading species in between the biobarriers is most likely linked to the oxygen injection activities at the site during the past remedy implementation phase (**Figure 6-1**). These results suggest that the biobarrier installation and oxygen sparging activities impacted the microbiology of the site, enriching the aerobic population of MTBE degraders.

The mid-plume biobarrier area and leading edge biobarrier sampling locations showed higher relative abundance of cometabolic MTBE degraders in comparison to the direct mineralizers. This observation points towards preferential metabolism of C_5 to C_8 n-alkanes versus utilization of MTBE as a carbon source. The presence of propane degraders (*Nocardioides sp., Xhanthobacter, Mycobacterium sp.*), as well as species utilizing butane (*Arthrobacter*), ethanol (*Gordonia terrae*), pentene (*Rhodococcus* sp., *Pseudomonas aeruginosa*) and hydrocarbon mixtures (*Pseudomonas* sp.) proves existence of a robust microbial population capable of degradation of mixed gasoline components.

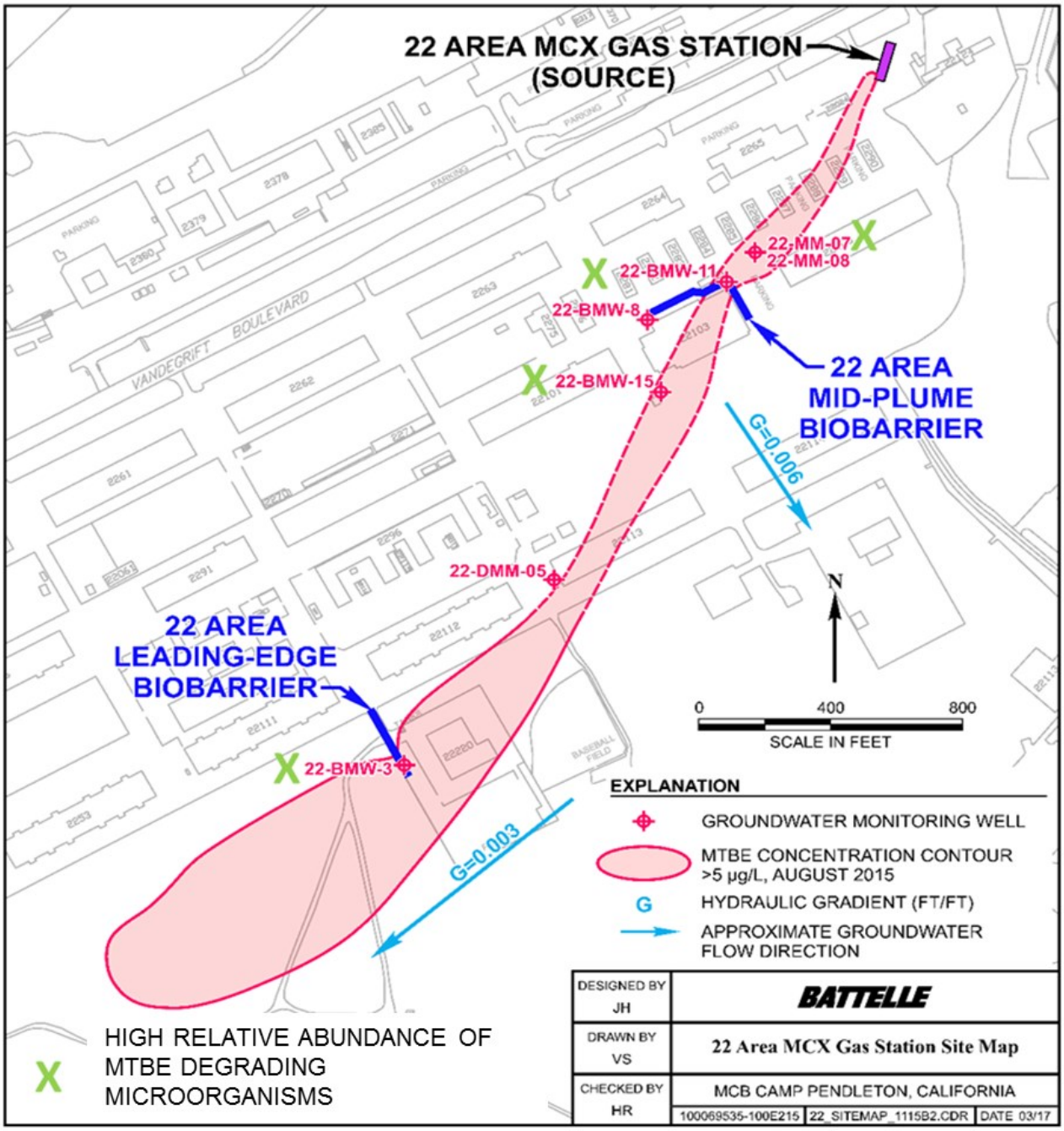

Figure 6-1. Location of Wells with High Percentage Relative Abundance of MTBE Degrading Microbial Species Detected during Sampling Events 1 and 2 at 22 Area MCX Gas Station

***Finding 2**: Metagenomic data show differences between diversity of microbial MTBE degraders at the 22 Area MCX Gas Station and the 13 Area Gas Station.*

This hypothesis was developed to compare microbial diversity at the 22 Area MCX Gas Station and 13 Area Gas Station, which served as a positive control. Similar to the 22 Area MCX Gas Station, 13 Area Gas Station metagenomes were categorized depending on the MTBE degradation respiration (aerobic/anaerobic, direct mineralizing and cometabolic). The analyzed metagenomes showed the presence of a broad diversity of MTBE degrading bacteria with a varied percentage of relative abundance of direct MTBE metabolizers to the cometabolic MTBE degraders. At both sites, the majority of the MTBE degraders were aerobic, which shows potential for aerobic direct or cometabolic mineralization processes.

No significant difference was found between metagenomes across both sites when comparing the first and second sampling events. However, a significant difference was found when metagenomes from the 13 Area Gas Station source zone area and the 22 Area MCX Gas Station were compared. This significant difference was found with respect to the composition of MTBE microbial metabolizers. At the 13 Area Gas Station a higher relative abundance of direct aerobic mineralizers was observed in the metagenome. These results were expected as the source zone served as a positive control in this study.

Although the microbial diversity related to MTBE degradation at the 22 Area MCX Gas Station and 13 Area Gas Station showed significant differences, both direct MTBE degrading species as well as cometabolic microorganisms were present at both sites. The presence of MTBE degrading species indicates potential for full and cometabolic degradation of this contaminant.

6.1.3 ASSESSMENT OF MICROBIAL ACTIVITY WITH PROTEOMICS

Hypothesis: Metaproteomic results support active degradation of MTBE at the 22 Area MCX Gas Station.

***Finding 1**: Metaproteomic results do not support on-going degradation of MTBE at 22 Area MCX Gas Station.*

Summary: Degradation activity was evaluated using metaproteomics where this activity is revealed by the presence of specific MTBE-degrading proteins. Proteins derived from MTBE-degrading bacteria were used as indicators for the population to be alive, however these proteins were not used as direct evidence of MTBE degradation. Global proteomics was performed to maximize the number of peptide identifications at the 22 Area MCX Gas Station. The results from both sampling events showed a negligible number of proteins from MTBE-specific pathways and suggest little to no on-going degradation of the contaminant.

The negligible biodegradation may be caused by several factors:

- The cellular yield of microorganisms that utilize MTBE as a sole organic carbon source is very low;

- The presence of more easily biodegradable organic compounds in the subsurface can either inhibit MTBE biodegradation or promote cometabolic MTBE biodegradation;
- The oxygen concentration in the subsurface is too low to promote aerobic MTBE biodegradation;
- The species of microorganisms responsible for anaerobic transformation of MTBE may not be present;
- It is possible that there is a threshold of the contaminant concentration below which the contaminant is no longer bioavailable for microbial degradation (Trindade, Sobral et al. 2002).

Finding 2: *Metaproteomics shows direct evidence of on-going degradation of MTBE at the 13 Area Gas Station and provides evidence of biologically-mediated contaminant degradation.*

Summary: This hypothesis was developed to demonstrate if active degradation of MTBE is ongoing at the 13 Area Gas Station and validate the use of metaproteomics to provide a direct line of evidence of biodegradation. Data collected from the source zone area samples and presented in Section 5.4.4 show presence of proteins associated with the MTBE-degradation pathway and support active MTBE degradation at the site. The majority of identified proteins originate from the direct aerobic MTBE-degrader, *Methylibium petroleiphilum*, and include seven out of ten Mdp proteins involved in the MTBE full mineralization. These results prove that the hypothesis is true and that metaproteomics can provide meaningful data for environmental analyses.

The next step in environmental metaproteomic analysis would be to link the concentration of specific peptides to the degradation rate of MTBE using the multiple reaction monitoring (MRM) assay to quantify peptide targets. This ESTCP demonstration focused only on a qualitative determination of MTBE degradation with proteomics.

6.1.4 CONCORDANCE BETWEEN LINES OF EVIDENCE

Hypothesis: Metagenomic and metaproteomic results (tertiary line of evidence) are consistent with other primary and secondary lines of evidence and provide an improved understanding of MTBE degradation at the 22 Area MCX Gas Station.

Finding 1*: Metagenomic and metaproteomic results were consistent with other primary and secondary lines of evidence for the 22 Area MCX Gas Station and for the 13 Area Gas Station used as a control site.*

Summary: A multiple line of evidence approach was used to evaluate MNA at the 22 Area MCX Gas Station and included evaluating contaminant concentrations (primary line of evidence), geochemical trends (secondary line of evidence), and demonstrate and validate metagenomic and metaproteomic methods to determine MTBE degradation microbiology and activity at the site (tertiary line of evidence) (**Table 6-1**). The 13 Area Gas Station was used as a positive control for the metagenomic and metaproteomic data analyses due to actively ongoing degradation of MTBE.

The analysis of contaminant concentrations at the 22 Area MCX Gas Station on the data collected after the biosparge system was discontinued did not show a clear decreasing trend. For example, at the leading edge biobarrier well, the analysis showed a *stable* MTBE trend after system shutdown in 2012. Analysis of the mid-plume biobarrier data also indicated the MTBE concentration is stable – neither increasing nor decreasing. Between the biobarriers, the analysis revealed no trend in the data. These stable trends provide evidence that the rate of MTBE loading is balanced with the rate of contaminant attenuation. In contrast, the MTBE concentration trends evaluated at the 13 Area Gas Station demonstrate ongoing degradation of the COCs with generally decreasing trends. This observation is not surprising since this site is currently undergoing remediation efforts.

The secondary line of evidence parameters such as DO, ORP and terminal electron acceptors analyzed at the 22 Area MCX Gas Station, do not provide positive evidence of an ongoing natural attenuation of MTBE. The analyses of site geochemical parameters showed that the site is predominantly anoxic with no sulfate or iron reduction or methanogenesis occurring at the time of sample collection. On the contrary, DO concentrations measured at the 13 Area Gas Station support aerobic conditions at the site consistent with an ongoing MTBE bioremediation (**Table 6-2**).

Metagenomic data from both sites, showed statistically comparable presence of a broad diversity of MTBE degrading bacteria with a varied percentage of relative abundance of direct MTBE metabolizers to cometabolic MTBE degraders. The majority of identified MTBE degraders at the 22 Area MCX Gas Station and 13 Area Gas Station were aerobic cultures, which highlights the potential for aerobic direct or cometabolic mineralization processes. These data suggest potential for MTBE degradation at the 22 Area MCX Gas Station due to presence of a diverse population of microbial species. In comparison, species richness at the 13 Area Gas Station was higher indicating that highly abundant aerobic direct and cometabolic microbial species are present at the site and may be involved in MTBE degradation at the moment of sampling. The highest abundance of these species was present at the source zone area where active air sparging is currently ongoing.

Metaproteomic data for samples from 22 Area MCX Gas Station showed the presence of proteins from MTBE-degrading microorganisms, but no proteins involved in MTBE degradation were detected at any wells. This finding supports negligible MTBE degradation at the site.

Metaproteomic data collected from the 13 Area Gas Station show presence of both protein groups (MTBE degradation pathway and MTBE-degrading microorganisms proteins) and provide direct evidence to an ongoing MTBE degradation. Detailed analysis of proteomic data showed that seven out of ten known proteins involved in the direct mineralization of MTBE were present in source zone area well. However, to provide a linkage between concentration of these specific MTBE peptides and degradation rates, additional experiments need to be performed.

Table 6-1. Lines of Evidence for On-Going MTBE Degradation in the 22 Area MCX Gas Station

Line of Evidence		Summary	Indicative of On-Going Degradation?	Level of Confidence
Primary	**MTBE concentration trend**	Generally stable after termination of active remediation	Possible	Low
	MTBE degradation products	Not detected	Possible	Low
Secondary	**Geochemical data**	Suggests little or no aerobic or anaerobic degradation	No	Medium
Tertiary	**Metagenomic data**	Indicates potential for aerobic or anaerobic degradation	Possible	Medium
	Metaproteomic data	Indicates some potential for aerobic degradation; no evidence of anaerobic degradation	No[1]	Medium
Overall Finding		Taken together, the available lines of evidence indicate little or no on-going degradation of MTBE in the 22 Area.		

Note: 1) Metaproteomic results interpreted in conjunction with geochemical data indicating anoxic conditions throughout the 22 Area MCX Gas Station plume (i.e., an absence of aerobic degradation).

Table 6-2. Lines of Evidence for On-Going MTBE Degradation in the 13 Area Gas Station

Evidence for MTBE Degradation	Summary	Indicative of On-Going Degradation?	Level of Confidence
MTBE concentration trend	Generally decreasing	Yes	Medium
MTBE degradation products	TBA detected in most wells	Yes	High
Geochemical data	Evidence of anaerobic metabolism including iron reduction, sulfate reduction, and methanogenesis	Yes	Medium
Metagenomic data	Indicates potential for aerobic or anaerobic degradation	Possible	High
Metaproteomic data	MTBE degrading proteins detected in source area wells.	Yes	High
Overall Finding	Taken together, the available lines of evidence provide strong evidence of on-going degradation of MTBE in the 13 Area.		

6.2 ASSESSMENT OF THE LONG-TERM IMPACT OF THE BIOBARRIER SYSTEM ON FORMATION PERMEABILITY

Hypothesis: Operation of the biosparge system had a long-term impact on the aquifer by reducing the formation permeability.

***Finding**: The hydraulic conductivity values derived based on 2016 slug-test data were not substantially different from those derived based on 2001 slug-test data, indicating that operation of the biosparge system did not have a long-term impact on the aquifer by reducing formation permeability.*

Summary: To answer this question, the present day hydraulic conductivity of the aquifer in the vicinity of the biosparge system was estimated using a series of slug tests performed in three monitoring wells, with the resulting hydraulic conductivity values compared to pre-biosparging hydraulic conductivity values estimated by others (IT Corporation 2001). To maximize the comparability between K values derived based on 2001 and 2016 slug-test data, the 2016 field procedures and data analysis techniques were matched as closely as possible to those used in 2001.

The geometric means of hydraulic conductivity values derived from multiple tests using multiple data reduction solutions were compared on a well-by-well basis with "representative K" values presented by IT, 2001. This comparison shows that hydraulic conductivity values derived for each well based on 2001 and 2016 data were within a factor of two. Given the variability inherent in slug testing and slug-test data reduction, a factor of two difference is small enough to conclude that the hydraulic conductivity values are not meaningfully different before and after biosparging.

7.0 COST ASSESSMENT

The primary objective of this project was to investigate the long-term effectiveness of a set of two biobarriers and assess the extent that natural attenuation continues to remove the remaining MTBE contamination at the site. A secondary objective was to assess the impact of operation of the biobarrier on the formation permeability. Cost elements that pertain to this evaluation and the cost benefits of the techniques used are summarized in this section.

7.1 COST MODEL

At sites relying on MNA after active remediation, assessments of MNA performance are typically monitored via changes in contaminant concentration as the primary line of evidence. Geochemical analyses including measurements of ferrous iron, dissolved gas (methane), dissolved manganese, and sulfate are performed to help demonstrate secondary lines of evidence for degradation. Tertiary analyses, including analyses of various biological data, are intended to aid evaluation of MNA at sites where primary and secondary results alone are not adequate to assess performance. Tools that support tertiary analyses can include laboratory specific microcosm studies and analysis of conventional MBTs. Although microcosm studies have been used to demonstrate that microorganisms at a site can degrade a contaminant, they tend to be expensive, time consuming, and often yield equivocal results. As a consequence, they are rarely performed as part of an MNA evaluation. During this demonstration project, tertiary analyses consisted of applying metagenomics and proteomic techniques to acquire information on microbial diversity and activity. **Table 7-1** lists the elements of the cost model that was developed for this project. Additional details for each cost element are described in the remainder of this section.

7.1.1 PROJECT PLANNING AND PREPARATION

Project planning for the field demonstration included labor hours for site selection, review of existing site data, identification of pertinent data gaps, development of the project work plan, quality assurance plan and health and safety documents (i.e., Sampling and Analysis Plan, Accident Prevention Plan, Activity Hazard Analysis, and Health and Safety Plan), and arrange any required subcontracts and/or purchased services. Alpha Analytical Laboratories was contracted to perform conventional groundwater analyses (see **Table 5-1**), and Battelle performed advanced MBTs. Although project planning costs were tracked during this project, activities are considered standard practice for performing long-term monitoring of natural attenuation at a site. Sections of field documents that relate to tertiary sample collection, including the groundwater sampling plan for metagenomics and proteomic analyses, require input from staff having specialized expertise performing these types of sampling and analyses. Even so, analyses using advanced MBTs should not add substantial additional cost (less than 10%) to project planning and preparation activities.

A small amount of additional effort was required to identify the 13 Area Gas Station control site. Costs related to identification and planning of sampling activities at this site were not tracked separately from those required at the 22 Area MCX Gas Station site. It is estimated that less than 5% of the total costs of project planning and preparation were associated with the 13 Area Gas Station. Activities associated with this effort included identifying the site, reviewing historical data, and selecting appropriate locations to perform the requisite sampling.

Table 7-1. Cost Model of the Demonstration

Cost Element	Sub Category	Tracked Data	Approximate Demonstration Cost
Project Planning and Preparation	Work plan, quality assurance plan, health and safety plan	• Labor hours	$21,400
	Subcontracts and procurement	• Labor hours	$14,400
Field Sampling and Analysis	Groundwater Sampling and Geochemical Analysis	• Labor hours • Equipment rental costs (e.g. low flow pump) • Expendable material costs (e.g. gloves, Teflon tubing, calibration standards) • Analytical costs	$20,400
	Groundwater sampling and application of advanced MBTs	• Labor (specialized) hours • Equipment rental costs • Expendable material costs • Analytical costs	$53,400
	Slug Tests	• Labor hours • Equipment rental (e.g. water level probe, data logger) • Expendable materials	$23,400
	Waste Disposal	• Sampling IDW • Laboratory hazardous waste disposal	$10,400
	Data evaluation for conventional groundwater analyses	• Labor hours	$48,400
Data Evaluation and Reporting	Data evaluation for MBTs	• Labor hours • Specialized software	$28,400
	Report Development	• Labor hours	$38,800
	Peer reviewed publications	• Peer reviewed publications	$55,000
		Total Cost	**$314,000**

7.1.2 FIELD SAMPLING AND ANALYSIS

Field sampling and analysis include labor hours to perform the investigation, rental or use rate costs for sampling equipment, cost for expendable materials such as gloves, Teflon® tubing, health and safety equipment, and other miscellaneous items required to support the field effort. Sampling and analysis costs were tracked; however, similar to project planning and preparation, primary and secondary sampling activities are common practice at sites where MNA is being applied. These activities included collecting groundwater samples for analysis of the following:

1. Primary line of evidence - COCs including TPH-G, BTEX and five oxygenates including: MTBE, DIPE, ETBE, TAME, and TBA. However, it is noted that for a typical evaluation

of MNA, in many cases analysis of only the COCs (i.e., MTBE and BTEX) may be necessary, which would reduce the analytical cost by about 30%,

2. Secondary line of evidence - Geochemical parameters including ethane, ethene, alkalinity, anions (Cl, F, NO_3, NO_2, ortho-PO_4, SO_4), BOD, sulfide (S^{2-}), total phosphorus, COD, TOC, TDS, cations (Ca, Mg, K, Na), and ferrous iron (Fe^{2+}).

The number of samples collected per event is listed in **Table 5-2**. Lynco Environmental was contracted to collect the groundwater samples and ship them overnight to Alpha Analytical for analysis.

A small amount of additional labor was required to collect samples required for the metagenomics and metaproteomic analyses. These types of analyses are novel and not yet commonly used for long-term monitoring assessment. Specialized techniques as described in Section 5 were employed. It is estimated that sampling for these analyzed increased field labor costs by about 2%.

Metagenomics and metaproteomic analyses were performed at Battelle laboratories. Costs for these analyses were tracked on a sample batch basis (10 samples/batch) using real project costs. This step utilizes specialized personnel with knowledge and training to prepare samples for analyses, run the sequencer and mass spectrometer, and analyze the data using bioinformatic tools.

The field investigation also included slug testing for assessment of changes in aquifer conductivity and its potential to impact plume migration. Principal costs include labor and rental/use rates for data loggers and water level probes. Costs are highly dependent on the number of wells analyzed.

Additional field sampling costs for this demonstration were incurred to sample the Area 13 Gas Station control site. A control site and associated costs to sample it would not be required during a typical investigation, but were required for this demonstration. The costs presented in Table 7-2 represent total field sampling and analytical costs incurred during the demonstration. A breakdown of these costs is estimated based on the number of samples collected at each site and an estimate of the level of effort to perform the various investigation activities.

Table 7-2. Breakdown of Field Sampling Costs

Cost Element	Sub Category	13 Area Gas Station	22 Area MCX Gas Station
Groundwater Sampling and Geochemical Analysis	Labor	$500	3,750
	Equipment/materials	$50	$100
	Analytical	$2,000	$13,000
Groundwater sampling and application of advanced MBTs	Labor	$500	$3,750
	Equipment/materials	$140	$300
	Analytical	$15,000	$33,700
Slug Tests[1]	Labor hours	$0[1]	$13,500
	Equipment/materials	$0[1]	$1,500
	TOTAL:	**$18,190**	**$69,600**

3. Slug tests were not performed at the 13 Area Gas Station site.

7.1.3 DATA EVALUATION AND REPORTING

Geochemical and biological data obtained during the investigation were reviewed and processed to generate information on ongoing MNA of MTBE and evaluate potential changes to plume migration and formation permeability due to the installation of the two biobarriers. The level of effort was not tracked separately for evaluation and reporting of the more conventional data versus evaluation and reporting of the metagenomics and metaproteomic data. However, it is anticipated that evaluation of data from application of the advanced MBTs increases the total labor cost for the reporting effort by about 10 to 15 percent.

Analysis of metagenomic data requires personnel who are able to perform bioinformatic analysis of samples, filter sequencing data, perform quality control of the resulting output data, build a project-specific database, and create Krona plots and output Excel files with microbial specific information, all of which increase complexity and level of effort.

Analysis of the metaproteomic data requires a similar level of effort and training as the analysis of metagenomics data. Personnel with specialized expertise are required to transfer the data into the Protein Pilot software, screen the data against the database, provide quality assurance/quality control of the data and assemble spreadsheets containing the data outputs. These trained personnel require several hours of labor to generate desired output data files. Costs are highly dependent on the number of samples analyzed.

For the purpose of this cost assessment, real project costs were used, though it was assumed that staff already have the requisite training to perform the metagenomics and metaproteomic data reduction and evaluation. These trained personnel require several hours with the specific software to generate the desired output data. The cost for this element was based on the labor required, and units are reported on a per sample basis.

The analysis of slug test data requires personnel that are able to perform hydrogeologic evaluations including curve fitting and statistical analyses of data. For the purpose of the cost assessment, real project costs were used, though it was assumed that the necessary staff did not need to undergo extra training (i.e., operation of Aqtesolv software) needed for slug test analyses (Cooper, Bredehoeft et al. 1967; Bouwer and Rice 1976) and performing Mann Kendall analyses. These trained personnel require several hours using the specific software to generate the desired output data. The cost for this element was based on the labor required, and units reported on a per site basis.

All information is assembled in a report (i.e., this document), which provides an overview of project objectives and activities, methods employed, data collected, a comprehensive evaluation of results, and documents relevant conclusions and recommendations. Labor hours and associated costs to perform the evaluation and develop this report were not tracked separately for 13 Area Gas Station and 22 Area MCX Gas Station. It is assumed that the additional labor required to evaluate the data generated from 13 Area Gas Station, and incorporate into this report represents no more than a total of 10 percent of this cost element.

7.2 COST DRIVERS

The primary drivers associated with long-term monitoring of MNA include the size of the site, proximity of the site to nearby receptors, regulatory requirements, and nature and diversity of COCs. These drivers dictate the number of sampling locations, the COCs (and degradation products) for which groundwater must be analyzed, and frequency and duration of sampling events.

Implementation of advanced MBTs during the long-term monitoring and assessment phase of the project also are impacted by the factors as described above. Although there are currently no regulatory requirements that specifically mandate advanced MBTs be used to assess a site, the data provided by the MBTs are meant to supplement and possibly replace other forms of data that provide lines of evidence that MNA is occurring and to estimate a removal rate. Hence, the total sampling and analytical cost is driven by number of sample locations at a site and total number of samples collected (i.e., a greater number of samples equates to a higher cost). It should be noted however that the individual cost per sample for analyses with advanced MBTs may decrease based on a greater number of total samples requiring analyses since the lab work is highly specialized and cost efficiencies generally can be realized for a larger quantity of analyses.

The main cost drivers for the slug tests that were performed as part of this demonstration relate to the size of the site and site heterogeneity. A greater number of tests are generally required at sites at which a remedy is applied across multiple lithologic units or where lithology changes significantly from one area of a site to another.

7.3 COST ANALYSIS

With the exception of metagenomics and metaproteomics, the techniques used to assess the performance of the biobarrier and continued potential for natural attenuation are common and costs to apply these techniques are well documented in the literature (Lo, Denef et al. 2007; Rabus 2013; Fouhy, Stanton et al. 2015). As discussed in Section 7.2, costs are highly dependent on the number of samples collected (and slug tests performed), frequency of sampling, and number/types of analytes, which are primarily dictated by the nature/diversity of the COCs, size of the site, proximity of receptors, and regulatory requirements. Hence, it is not the intent of this demonstration report to generate a life-cycle cost estimate for a hypothetical site at which these techniques are applied to evaluate remedial performance and subsequent natural attenuation of the remaining COCs to achieve site RAOs.

Table 7-3 provides a cost comparison of conventional MBTs (e.g., qPCR) to the advanced MBTs. As indicated in the last column of the table, many of these techniques have only limited commercial availability and/or are available through a university or other research laboratory. As such, application costs remain relatively high. It is expected as these techniques mature, they will become more widely available and the analytical cost per sample will decrease substantially. For comparison purpose, the cost of the metagenomics and metaproteomic analyses based on cost data collected during this demonstration were $300 and $1,800 per sample, respectively[5], assuming analysis of a batch of 10 samples. The cost of the metaproteomic analyses included use of an

[5] Analytical cost only. Does not include any costs associated with sample collection.

existing metaproteomic platform, but assumed development of a workflow specific for MTBE. Cost for metagenomic analysis is not anticipated to decrease as the quantity of samples increases; however, the cost for the proteomic analysis will decrease as the number of samples increases. Since this demonstration, due to the restructuring of prices at Battelle's laboratories, the proteomic cost decreased significantly to approximately $1,000 a sample assuming analysis of a batch of 10 samples. Moreover, had 50 samples been analyzed in batches of 25 during this demonstration, the resulting cost per sample would have been $300 and $750 for the metagenomics and metaproteomic analyses, respectively, including data analysis with bioinformatic tools.

Table 7-3. Cost Comparison of Conventional to Advanced (Omic) MBTs

Molecular Tool	Identity/ Potential Activity/ Expressed Activity [a]	Quantitative, Qualitative (QA/QL)	Cost Range ($) [b]	Availability [C]
Conventional MBTs				
Compound specific isotope analysis	E	QA	100 to 2,500	C/R
Quantitative polymerase chain reaction	I/P/E	QA	275 to 425	WC
Microarrays	I/P/E	QL	1,250 to 5,000	C/R
Stable isotope probing	I/P/E	QA/QL	1,500 and up	C/R
Enzyme activity probes	E	QA	250 to 2,500	C/R
Advanced (omic) MBTs[d]				
Metagenomics (16S Sequencing)	I	QL	150 to 500	WC/R
Shotgun Metaproteomics	E	QL	1,000 to 1,500	C/R
MRM Metaproteomics	E	QA	500 and up	C/R

Adapted from ITRC (2011)

[a] I - identity of microorganisms (i.e., genus or species), P - potential activity (i.e., genetically capable of completing the activity), E - expressed activity (i.e., actually completing the activity at a given time).

[b] Estimated price per sample. Low end represents compound specific restricted analysis.

[c] WC - widely commercially available, C- minimally commercially available, R - available through university or other research laboratory.

[d] The cost of advanced omic MBTs represents cost from two commercial laboratories and Battelle metagenomic and proteomic lab. These costs are based on current costs from 2017 and higher number of batches (20 samples). These costs elements are reduced since the methods are maturing and proteomic analyses becomes more routinely used.

8.0 IMPLEMENTATION ISSUES

This section focuses on the advanced MBTs that were used to facilitate assessment of MNA. The advanced MBTs and conventional groundwater analyses (contaminant concentration and geochemistry) used are commonly employed for these types of assessments and implementation issues are well understood. The primary end users of advanced MBTs are expected to be DoD site managers, consultants and their contractors, consultants and engineers. The general concerns of these end users are likely to include the following: (1) regulatory acceptance; (2) insufficient confidence in results and access to specialized laboratories; and (3) technology cost compared to other more conventional monitoring options. These implementation issues are addressed in the following sections.

8.1 REGULATORY ACCEPTANCE

Currently metagenomics data is used as a tertiary line of evidence to indicate that site conditions are favorable for biodegradation to occur (e.g. sufficient diversity and quantity of microorganisms). Metagenomic results expand upon the type of data generated by techniques such as qPCR, which already has received widespread regulatory acceptance. Hence it is expected that metagenomics results will receive a similar degree of regulatory acceptance. Nonetheless, this approach and associated data may be relatively unfamiliar to regulators since it only has recently been applied at environmental sites and because it requires an advanced understanding of microbial processes.

Proteomics is not commonly used due to its high current cost associated with operation of specialized instrumentation, but is expected to become a powerful monitoring tool as the technology advances and the costs of analyses are reduced. At present, proteomics can be used to provide a direct line of evidence that biodegradation is actively occurring based on the detection of proteins that are produced during the degradation process. However, in the future, it is conceivable that proteomics could provide a direct measure of degradation rates based on the concentrations of proteins that are measured in a sample, which could eliminate or reduce the need to measure concentrations of COCs. It is therefore expected that regulatory acceptance of this technology will in part be based on the application and end use of the resulting data.

As with any new technology, detailed demonstration and validation are required to ensure accuracy and precision of results for both techniques before widespread regulatory acceptance can be obtained. Standardized methods and procedures for sample collection and shipping, analytical methods, QA/QC and data evaluation must be further developed and validated to help ensure regulatory acceptance. In addition, technology transfer through SERDP/ESTCP, peer reviewed journal articles, webinars, conferences, and other meetings will play an important role to facilitate understanding and acceptance of these powerful tools.

8.2 INSUFFICIENT CONFIDENCE IN RESULTS AND ACCESS TO SPECIALIZED LABORATORIES

Obtaining quantitative results from advanced MBTs is challenging mainly due to high inherent variability of results. The variation in results obtained from the analyses of the same sample at

different laboratories can be significant, although in some cases close agreement between result sets have been reported. These variations reduce the confidence in the results from biological analyses and result from the relatively young state of the practice and lack of QA/QC guidelines for environmental applications. Only few analytical environmental laboratories even offer advanced MBTs, and quantitative proteomics is not yet commercially available.

There are variety of unique issues that affect the confidence of results in relation to the environmental applications of advanced MBTs. For example, the biomass is soils or aquifers is far lower in comparison to what is present in culture studies and the biomarkers of interest are present with low abundance. The biomarkers of interest are also not well understood and may be more variable. Finally, the background of the professionals involved in performing analysis with the advanced MBTs is a significant issue. Engineers and environmental scientists usually have little to no training in molecular biology and biochemistry. As a result, even in cases where the tools have proven to be of benefit, acceptance by practitioners and regulators has been slow and difficult.

8.3 TECHNOLOGY COST COMPARED TO OTHER MONITORING OPTIONS

Costs to apply advanced MBTSs are high compared to conventional technologies (refer to Section 7), but are expected to decrease substantially as the technologies continue to advance. Although costs per sample currently range from several hundred dollars to about $1,000 for these types of analyses, MBTs help to answer a variety of management questions and facilitate decision making that can result in a reduction of the life-cycle cost of a remedy. For instance, MBTs such as metagenomics and metaproteomics, may be used instead of laborious microcosm studies to definitively state if microorganisms of interest are performing required activities and are actively degrading specific contaminant. Not only will this likely result in a direct cost savings to the project since microcosm studies can be more costly than the MBT analyses, but it also reduces the time required for assessment because microcosm studies generally take 60 to 90 days to perform.

During remediation efforts, MBTs help to design the remedy, to optimize remedial strategies, and to troubleshoot unsuccessful treatment approaches. Results can be used to determine when to reapply amendments to optimize growth and distribution of the target organisms, which can help to minimize the time required for the active portion of the remedy. Conceivably, in the near future, metaproteomics may provide the necessary means to directly calculate degradation rates, which then can be augmented during the active portion of the remedy to facilitate removal of COCs, thereby reducing application time and life-cycle cost.

Metagenomics and metaproteomics can facilitate long-term monitoring efforts by confirming that active degradation is occurring across the site, and eventually may aid to estimate the rate of degradation to decide if site specific cleanup goals can be achieved within a desired timeframe. This could result in less frequent monitoring events and or a reduced number of analytes, which may reduce the life-cycle long-term monitoring cost and may support more rapid site closure. As metagenomics and metaproteomics are increasingly used in environmental applications, and as more laboratories begin to offer these analyses, competition increases, and techniques are refined, costs are expected to decrease.

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APPENDICES

APPENDIX A

POINTS OF CONTACT

Table A-1. Points of Contact for ESTCP Project Demonstration

POINT OF CONTACT	ORGANIZATION	PHONE/E-MAIL	PROJECT ROLE
Kenda Neil	NAVFAC EXWC 1000 23rd Avenue Port Hueneme, CA 93043	(805) 982-6060 (office) (805) 982-5798 (fax) (805) 377-6505 (cell) Kenda.Neil@navy.mil	Project Manager
Dr. Kate Kucharzyk	Battelle Memorial Institute 505 King Ave. Columbus, OH 43201	(614) 424-5489 (office) Kucharzyk@battelle.org	Principal Investigator
Dr. Heather Rectanus	Battelle Memorial Institute 505 King Ave. Columbus, OH 43201	(760) 801-5596 RectanusH@battelle.org	Task Lead and Deputy Program Manager for ESAT Contract
Pamela Chang	Battelle Memorial Institute 505 King Ave. Columbus, OH 43201	(614) 424-5978 (office) (714) 313-9067 (cell) ChangP@battelle.org	Field Sampling and Analyses Task Lead
Dr. Craig Bartling	Battelle Memorial Institute 505 King Ave. Columbus, OH 43201	(614) 424-5377 BartlingC@battelle.org	Proteomics and Metagenomics Task Lead

APPENDIX B

SUMMARY OF HISTORICAL ANALYTICAL RESULTS

Table B-1. Historical Analytical Results for Selected Groundwater Samples - 22 Area Wells (1995 to 2016)

Analyte			TPH as Diesel	TPH as Gasoline	Benzene	Ethylbenzene	Toluene	Total Xylenes	MTBE	DIPE	ETBE	TAME	TBA
Method	Date Sampled	Sample Number	CA LUFT 8015M	CA LUFT 8015M	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260
Unit			(mg/L)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)
Location Code													
22-BMW-3	11/11/2003	842092-0356	NA	NA	NA	NA	NA	NA	246 D	5 U	5 U	5 U	20 U
22-BMW-3	3/1/2004	101877-0038	NA	NA	NA	NA	NA	NA	218	5 U	0.5 J	1 J	13 J
22-BMW-3	8/9/2004	101877-0223	NA	NA	NA	NA	NA	NA	173	5 U	0.4 J	5 U	20 UJ
22-BMW-3	11/1/2004	101877-0330	NA	NA	NA	NA	NA	NA	312	5 U	0.6 J	5 U	20 UJ
22-BMW-3	1/31/2005	101877-0462	NA	NA	NA	NA	NA	NA	180	25 U	25 U	25 U	100 U
22-BMW-3	4/13/2005	101877-0573	NA	NA	NA	NA	NA	NA	93	5 UJ	5 U	5 U	20 U
22-BMW-3	6/14/2005	101877-0678	NA	NA	NA	NA	NA	NA	5	5 U	5 U	5 U	20 U
22-BMW-3	8/30/2005	101877-0763	NA	NA	NA	NA	NA	NA	0.6 J	5 U	5 U	5 U	20 U
22-BMW-3	1/19/2006	1085-040	NA	NA	NA	NA	NA	NA	95 J	2 UJ	2 UJ	2 UJ	25 UJ
22-BMW-3	4/18/2006	1085-148	NA	NA	NA	NA	NA	NA	20	2 U	2 U	2 U	25 U
22-BMW-3	7/31/2006	1085-305	NA	NA	NA	NA	NA	NA	170 J	50 U	50 UJ	50 UJ	200 U
22-BMW-3	10/23/2006	1085-376A	NA	NA	NA	NA	NA	NA	150 J	50 U	50 U	50 UJ	200 U
22-BMW-3	2/1/2007	1085-495	NA	NA	NA	NA	NA	NA	230	120 U	120 J	120 U	500 U
22-BMW-3	4/13/2007	1085-588	NA	NA	NA	NA	NA	NA	34	5 U	0.65 J	5 U	20 U
22-BMW-3	4/13/2007	1085-589(Dup)	NA	NA	NA	NA	NA	NA	37	5 U	0.65 J	5 U	20 U
22-BMW-3	8/15/2007	1085-724	NA	NA	NA	NA	NA	NA	1.7 J	5 U	0.51 J	5 U	20 U
22-BMW-3 (top)[a]	8/31/2007	1085-788	NA	NA	NA	NA	NA	NA	130	50 UJ	50 U	50 UJ	200 UJ
22-BMW-3 (middle)[a]	8/31/2007	1085-789	NA	NA	NA	NA	NA	NA	120	50 UJ	50 U	50 UJ	200 UJ
22-BMW-3 (bottom)[a]	8/31/2007	1085-790	NA	NA	NA	NA	NA	NA	110	50 UJ	50 U	50 UJ	200 UJ
22-BMW-3	11/8/2007	1085-861	NA	NA	NA	NA	NA	NA	0.8 J	5 U	0.35 J	5 UJ	20 UJ
22-BMW-3	2/8/2008	1085-984	NA	NA	NA	NA	NA	NA	28	5 U	0.51 J	5 U	20 U
22-BMW-3	4/24/2008	1085-1062	NA	NA	NA	NA	NA	NA	40	5 U	0.34 J	5	20 U
22-BMW-3	7/23/2008	922036-0007	NA	NA	NA	NA	NA	NA	0.9	1 U	1 U	1 U	10 UJ
22-BMW-3	11/5/2008	922036-0120	NA	NA	NA	NA	NA	NA	62	1 U	1 U	1 U	10 UJ
22-BMW-3	2/13/2009	922036-0130	NA	NA	NA	NA	NA	NA	25	1 U	1 U	1 U	10 UJ
22-BMW-3	4/27/2009	922036-0198	NA	NA	NA	NA	NA	NA	92	1 U	1 U	1 U	10 UJ
22-BMW-3	8/3/2009	62473-0009	NA	NA	NA	NA	NA	NA	73	1 U	1 U	1 U	10 U

(Data highlighted in red are from March and August 2016 sampling events).

Table B-1. Historical Analytical Results for Selected Groundwater Samples - 22 Area Wells (1995 to 2016) (continued)

Analyte			TPH as Diesel	TPH as Gasoline	Benzene	Ethylbenzene	Toluene	Total Xylenes	MTBE	DIPE	ETBE	TAME	TBA
Method	Date Sampled	Sample Number	CA LUFT 8015M	CA LUFT 8015M	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260
Unit			(mg/L)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)
Location Code													
22-BMW-3	10/23/2009	62473-0068	NA	NA	NA	NA	NA	NA	13	1 U	1 U	1 U	10 UJ
22-BMW-3	2/5/2010	62473-0132	NA	NA	NA	NA	NA	NA	49	1 U	1 U	1 U	10 UJ
22-BMW-3	4/14/2010	62473-0190	NA	NA	NA	NA	NA	NA	44	1 U	1 U	1 U	10 U
22-BMW-3	8/4/2010	922073-052	NA	NA	NA	NA	NA	NA	34	1 U	1 U	1 U	10 U
22-BMW-3	10/22/2010	922073-098	NA	NA	NA	NA	NA	NA	9.2	1 U	1 U	1 U	10 U
22-BMW-3	2/8/2011	922073-0142	NA	NA	NA	NA	NA	NA	4.8	1 U	1 U	1 U	10 U
22-BMW-3	2/8/2011	922073-0143 (Dup)	NA	NA	NA	NA	NA	NA	4.5	1 U	1 U	1 U	10 U
22-BMW-3	4/22/2011	922073-500	NA	NA	NA	NA	NA	NA	29	1 U	1 U	1 U	10 U
22-BMW-3	4/22/2011	922073-501 (Dup)	NA	NA	NA	NA	NA	NA	28	1 U	1 U	1 U	10 U
22-BMW-3	7/25/2011	922073-715	NA	NA	NA	NA	NA	NA	23 J	1 U	1 U	1 U	10 U
22-BMW-3	10/18/2011	922073-832	NA	NA	NA	NA	NA	NA	0.7	1 U	1 U	1 U	10 UJ
22-BMW-3	1/12/2012	4267-013	NA	NA	NA	NA	NA	NA	1.4	1 U	1 U	1 U	10 U
22-BMW-3	4/18/2012	4267-224	NA	NA	NA	NA	NA	NA	3.6	1 U	1 U	1 U	10 U
22-BMW-3	7/20/2012	4267-348	NA	NA	NA	NA	NA	NA	18	2 U	2 U	2 U	11 U
22-BMW-3	10/5/2012	4267-412	NA	NA	NA	NA	NA	NA	16	3 U	3 U	3 U	12 U
22-BMW-3	1/7/2013	None	NA	NA	NA	NA	NA	NA	2.8	1 U	1 U	1 U	10 UJ
22-BMW-3	4/4/2013	None	NA	NA	NA	NA	NA	NA	23 J	1 U	1 U	1 U	10 UJ
22-BMW-3	7/10/2013	None	NA	NA	NA	NA	NA	NA	18	1 U	1 U	1 U	13 J
22-BMW-3	10/3/2013	None	NA	NA	NA	NA	NA	NA	22	1 UJ	1 UJ	1 U J	10 UJ
22-BMW-3	8/29/2014	4931-22-BMW-3	NA	NA	NA	NA	NA	NA	39	1 U	1 U	1 U	10 U
22-BMW-3	8/29/2014	4931-22-BMW-3 (Dup)	NA	NA	NA	NA	NA	NA	39	1 U	1 U	1 U	10 U
22-BMW-3	2/6/2015	22-BMW-3	NA	NA	0.5 U	0.5 U	0.5 U	1.4	11	1 UJ	1 U	1 U	10 UJ
22-BMW-3	2/6/2016	22-BMW-3 (Dup)	NA	NA	0.5 U	0.5 U	0.5 U	1.91	11	1 UJ	1 U	1 U	10 UJ
22-BMW-3	3/7/2016	22-BMW-3	NA	0.05 U	0.5 U	0.5 U	0.5 U	0.5 U	3	1 UJ	1 U	1 U	10 U
22-BMW-3	8/17/2016	22-BMW-3	NA	0.05 U	0.5 U	0.5 U	0.5 U	0.5 U	20	1 U	1 U	0.51	10 U
22-BMW-11	11/10/2003	842092-0351	NA	NA	NA	NA	NA	NA	119 D	5 U	0.4 J	5 U	20 U
22-BMW-11	3/11/2004	101877-0101	NA	NA	NA	NA	NA	NA	12	5 U	5 U	5 U	20 UJ

(Data highlighted in red are from March and August 2016 sampling events).

Table B-1. Historical Analytical Results for Selected Groundwater Samples - 22 Area Wells (1995 to 2016) (continued)

Analyte			TPH as Diesel	TPH as Gasoline	Benzene	Ethylbenzene	Toluene	Total Xylenes	MTBE	DIPE	ETBE	TAME	TBA
Method	Date Sampled	Sample Number	CA LUFT 8015M	CA LUFT 8015M	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260
Unit			(mg/L)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)
Location Code													
22-BMW-11	5/18/2004	101877-0185	NA	NA	NA	NA	NA	NA	99	5 U	0.6 J	5 U	20 UJ
22-BMW-11	8/12/2004	101877-0263	NA	NA	NA	NA	NA	NA	167	5 U	0.9 J	5 U	20 UJ
22-BMW-11	11/5/2004	101877-0366	NA	NA	NA	NA	NA	NA	73	5 U	5 U	5 U	20 UJ
22-BMW-11	2/7/2005	101877-0514	NA	NA	NA	NA	NA	NA	14	5 U	0.4 J	5 U	20 U
22-BMW-11	4/15/2005	101877-0599	NA	NA	NA	NA	NA	NA	15	5 U	1 J	5 U	20 U
22-BMW-11	6/17/2005	101877-0703	NA	NA	NA	NA	NA	NA	2 J	5 U	0.3 J	5 U	20 UJ
22-BMW-11	9/1/2005	101877-0786	NA	NA	NA	NA	NA	NA	148	5 U	1 J	5 U	20 U
22-BMW-11	1/18/2006	1085-026	NA	NA	NA	NA	NA	NA	3.2 J	2 UJ	2 U	2 U	25 U
22-BMW-11	4/20/2006	1085-172	NA	NA	NA	NA	NA	NA	4.8 J	2 U	2 UJ	2 UJ	25 UJ
22-BMW-11	7/27/2006	1085-286	NA	NA	NA	NA	NA	NA	2.2 J	5 U	5 U	5 U	20 UJ
22-BMW-11	10/19/2006	1085-351A	NA	NA	NA	NA	NA	NA	31	5 U	5 U	5 U	20 U
22-BMW-11	1/30/2007	1085-473	NA	NA	NA	NA	NA	NA	1.3 J	5 U	5 U	5 U	20 UJ
22-BMW-11	1/30/2007	1085-474 (Dup)	NA	NA	NA	NA	NA	NA	1.1 J	5 U	5 U	5 U	20 UJ
22-BMW-11	4/11/2007	1085-577	NA	NA	NA	NA	NA	NA	5.5	5 U	5 U	5 U	20 UJ
22-BMW-11	8/13/2007	1085-705	NA	NA	NA	NA	NA	NA	1.4 J	5 U	5 U	5 U	20 U
22-BMW-11 (top)[a]	8/28/2007	1085-778	NA	NA	NA	NA	NA	NA	0.2 J	5 U	5 U	5 U	20 U
22-BMW-11 (middle)[a]	8/28/2007	1085-779	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 U
22-BMW-11 (bottom)[a]	8/28/2007	1085-780	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 U
22-BMW-11	11/7/2007	1085-854	NA	NA	NA	NA	NA	NA	0.9 J	5 UJ	5 UJ	5 UJ	20 U
22-BMW-11	2/6/2008	1085-967 (MS/MSD)	NA	NA	NA	NA	NA	NA	6.5	5 U	0.21 J	5 U	20 U
22-BMW-11	4/23/2008	1085-1055	NA	NA	NA	NA	NA	NA	6.8	5 U	5 U	5 U	20 UJ
22-BMW-11	4/23/2008	1085-1056 (Dup)	NA	NA	NA	NA	NA	NA	6.5 J	5 U	5 UJ	5 UJ	20 UJ
22-BMW-11	7/25/2008	922036-0024	NA	NA	NA	NA	NA	NA	17	1 U	1 U	1 U	10 UJ
22-BMW-11	10/28/2008	922036-0093	NA	NA	NA	NA	NA	NA	11	1 U	1 U	1 U	10 UJ
22-BMW-11	2/17/2009	922036-0146	NA	NA	NA	NA	NA	NA	2.4	1 U	1 U	1 U	10 UJ
22-BMW-11	4/29/2009	922036-0210	NA	NA	NA	NA	NA	NA	3	1 U	1 U	1 U	10 UJ
22-BMW-11	8/5/2009	62473-0023	NA	NA	NA	NA	NA	NA	15	1 U	1 U	1 U	10 U
22-BMW-11	10/27/2009	62473-0083	NA	NA	NA	NA	NA	NA	1	1 U	1 U	1 U	10 UJ

(Data highlighted in red are from March and August 2016 sampling events).

Table B-1. Historical Analytical Results for Selected Groundwater Samples - 22 Area Wells (1995 to 2016) (continued)

Analyte			TPH as Diesel	TPH as Gasoline	Benzene	Ethylbenzene	Toluene	Total Xylenes	MTBE	DIPE	ETBE	TAME	TBA
Method	Date Sampled	Sample Number	CA LUFT 8015M	CA LUFT 8015M	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260
Unit			(mg/L)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)
Location Code													
22-BMW-11	2/8/2010	62473-0140	NA	NA	NA	NA	NA	NA	1.5	1 U	1 U	1 U	10 U
22-BMW-11	4/19/2010	62473-0213	NA	NA	NA	NA	NA	NA	1.4	1 U	1 U	1 U	10 UJ
22-BMW-11	8/6/2010	922073-069	NA	NA	NA	NA	NA	NA	14	1 U	1 U	1 U	10 U
22-BMW-11	10/28/2010	922073-114	NA	NA	NA	NA	NA	NA	11	1 U	1 U	1 U	10 U
22-BMW-11	2/10/2011	922073-0152	NA	NA	NA	NA	NA	NA	9	1 U	1 U	1 U	10 UJ
22-BMW-11	4/23/2011	922073-517	NA	NA	NA	NA	NA	NA	8.4	1 U	1 U	1 U	10 UJ
22-BMW-11	10/24/2011	922073-847	NA	NA	NA	NA	NA	NA	12	1 U	1 U	1 U	10 UJ
22-BMW-11	10/24/2011	922073-848 (Dup)	NA	NA	NA	NA	NA	NA	12	1 U	1 U	1 U	10 UJ
22-BMW-11	1/13/2012	4267-025	NA	NA	NA	NA	NA	NA	4.6	1 U	1 U	1 U	10 U
22-BMW-11	4/19/2012	4267-233	NA	NA	NA	NA	NA	NA	6.8	1 U	1 U	1 U	10 U
22-BMW-11	7/21/2012	4267-356	NA	NA	NA	NA	NA	NA	13	1 U	1 U	1 U	10 U
22-BMW-11	1/8/2013	None	NA	NA	NA	NA	NA	NA	18	1 U	1 U	1 U	10 UJ
22-BMW-11	7/11/2013	None	NA	NA	NA	NA	NA	NA	9.7	1 U	1 U	1 U	10 UJ
22-BMW-11	3/8/2016	22-BMW-11	NA	0.050 U	0.5 U	0.5 U	0.5 U	0.5 U	2.4	1 UJ	1 U	1 U	10 UJ
22-BMW-11	817/2016	22-BMW-11	NA	0.050 U	0.5 U	0.5 U	0.5 U	0.5 U	5.3	1 U	1 U	1 U	10 U
22-BMW-15	2/8/2005	101877-0521	NA	NA	NA	NA	NA	NA	194	5 U	0.4 J	5 U	20 U
22-BMW-15	4/14/2005	101877-0591	NA	NA	NA	NA	NA	NA	158	5 UJ	5 U	5 U	20 U
22-BMW-15	6/16/2005	101877-0693	NA	NA	NA	NA	NA	NA	111	5 U	5 U	5 U	20 UJ
22-BMW-15	8/31/2005	101877-0779	NA	NA	NA	NA	NA	NA	118	5 U	5 U	5 U	20 U
22-BMW-15	1/23/2006	1085-057	NA	NA	NA	NA	NA	NA	66 J	2 U	2 U	2 U	25 UJ
22-BMW-15	4/20/2006	1085-167	NA	NA	NA	NA	NA	NA	81 J	2 U	2 UJ	2 UJ	25 UJ
22-BMW-15	7/28/2006	1085-300	NA	NA	NA	NA	NA	NA	90 J	50 U	50 U	50 UJ	200 UJ
22-BMW-15	10/23/2006	1085-370A	NA	NA	NA	NA	NA	NA	47	5 U	0.34 J	5 U	20 U
22-BMW-15	1/31/2007	1085-483	NA	NA	NA	NA	NA	NA	2.1 J	5 U	0.32 J	5 U	20 UJ
22-BMW-15	4/12/2007	1085-582	NA	NA	NA	NA	NA	NA	2.3 J	5 UJ	0.22 J	5 U	20 UJ
22-BMW-15	8/14/2007	1085-712	NA	NA	NA	NA	NA	NA	8.7	5 U	5 U	5 U	20 UJ
22-BMW-15 (top)[a]	8/28/2007	1085-775	NA	NA	NA	NA	NA	NA	76 J	50 UJ	50 UJ	50 UJ	200 UJ

(Data highlighted in red are from March and August 2016 sampling events).

Table B-1. Historical Analytical Results for Selected Groundwater Samples - 22 Area Wells (1995 to 2016) (continued)

Analyte			TPH as Diesel	TPH as Gasoline	Benzene	Ethylbenzene	Toluene	Total Xylenes	MTBE	DIPE	ETBE	TAME	TBA
Method	Date Sampled	Sample Number	CA LUFT 8015M	CA LUFT 8015M	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260
Unit			(mg/L)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)
Location Code													
22-BMW-15 (middle)[a]	8/28/2007	1085-776	NA	NA	NA	NA	NA	NA	72 J	50 UJ	50 UJ	50 UJ	200 UJ
22-BMW-15 (bottom)[a]	8/28/2007	1085-777	NA	NA	NA	NA	NA	NA	0.7 J	5 U	5 U	5 U	20 U
22-BMW-15	11/7/2007	1085-855	NA	NA	NA	NA	NA	NA	1.4 J	5 UJ	5 UJ	5 UJ	20 U
22-BMW-15	2/7/2008	1085-978	NA	NA	NA	NA	NA	NA	0.9 J	5 U	5 U	5 U	20 U
22-BMW-15	4/23/2008	1085-1058	NA	NA	NA	NA	NA	NA	3.9 J	5 U	5 UJ	5 UJ	20 UJ
22-BMW-15	7/24/2008	922036-0014	NA	NA	NA	NA	NA	NA	6.9	1 U	1 U	1 U	10 UJ
22-BMW-15	7/24/2008	922036-0015 (Dup)	NA	NA	NA	NA	NA	NA	6.8	1 U	1 U	1 U	10 UJ
22-BMW-15	11/4/2008	922036-0112	NA	NA	NA	NA	NA	NA	2.7	1 U	1 U	1 U	10 UJ
22-BMW-15	11/4/2008	922036-0113 (Dup)	NA	NA	NA	NA	NA	NA	3.2	1 U	1 U	1 U	10 UJ
22-BMW-15	2/18/2009	922036-0157	NA	NA	NA	NA	NA	NA	1.6	1 U	1 U	1 U	10 UJ
22-BMW-15	4/29/2009	922036-0208	NA	NA	NA	NA	NA	NA	1.3	1 U	1 U	1 U	10 UJ
22-BMW-15	8/6/2009	62473-0027	NA	NA	NA	NA	NA	NA	1.4	1 U	1 U	1 U	10 UJ
22-BMW-15	10/27/2009	62473-0082	NA	NA	NA	NA	NA	NA	3.7	1 U	1 U	1 U	10 U
22-BMW-15	2/8/2010	62473-0136	NA	NA	NA	NA	NA	NA	5.5	1 U	1 U	1 U	10 U
22-BMW-15	4/16/2010	62473-0208	NA	NA	NA	NA	NA	NA	0.7	1 U	1 U	1 U	10 UJ
22-BMW-15	8/6/2010	922073-072	NA	NA	NA	NA	NA	NA	1.8	1 U	1 U	1 U	10 U
22-BMW-15	2/10/2011	922073-0153	NA	NA	NA	NA	NA	NA	2.2	1 U	1 U	1 U	10 UJ
22-BMW-15	7/27/2011	922073-726	NA	NA	NA	NA	NA	NA	1.6 J	1 U	1 UJ	1 U	10 U
22-BMW-15	1/14/2012	4267-033	NA	NA	NA	NA	NA	NA	6.7	1 U	1 U	1 U	10 U
22-BMW-15	7/21/2012	4267-354	NA	NA	NA	NA	NA	NA	9.9	1 U	1 U	1 U	10 U
22-BMW-15	7/21/2012	4267-355 (Dup)	NA	NA	NA	NA	NA	NA	10	1 U	1 U	1 U	10 U
22-BMW-15	1/8/2013	None	NA	NA	NA	NA	NA	NA	11	1 U	1 U	1 U	10 UJ
22-BMW-15	7/10/2013	None	NA	NA	NA	NA	NA	NA	11	1 U	1 U	1 U	10 UJ
22-BMW-15	3/9/2016	22-BMW-15	NA	0.05 U	0.5 U	0.5 U	0.5 U	0.5 U	9	1 UJ	1 U	1 U	10 UJ
22-BMW-15	8/17/2016	22-BMW-15	NA	0.05 U	0.5 U	0.5 U	0.5 U	0.5 U	6.9	1 U	1 U	1 U	10 U
22-DMM-05	5/31/2002	821816-0673	NA	NA	NA	NA	NA	NA	307	5 U	0.5 J	0.3 J	20 U
22-DMM-05	9/3/2002	821816-0733	NA	NA	NA	NA	NA	NA	461	5 U	0.9 J	5 U	20 U
22-DMM-05	10/25/2002	821816-0799	NA	NA	NA	NA	NA	NA	732	5 U	0.9 J	5 U	20 U

(Data highlighted in red are from March and August 2016 sampling events).

Table B-1. Historical Analytical Results for Selected Groundwater Samples - 22 Area Wells (1995 to 2016) (continued)

Analyte			TPH as Diesel	TPH as Gasoline	Benzene	Ethylbenzene	Toluene	Total Xylenes	MTBE	DIPE	ETBE	TAME	TBA
Method	Date Sampled	Sample Number	CA LUFT 8015M	CA LUFT 8015M	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260
Unit			(mg/L)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)
Location Code													
22-DMM-05	3/13/2003	842092-0096	NA	NA	NA	NA	NA	NA	13	5 U	5 U	5 U	20 U
22-DMM-05	3/13/2003	842092-0097 (Dup)	NA	NA	NA	NA	NA	NA	12	5 U	5 U	5 U	20 U
22-DMM-05	5/5/2003	842092-0133	NA	NA	NA	NA	NA	NA	209	5 U	0.7 J	5 U	20 U
22-DMM-05	8/18/2003	842092-0227	NA	NA	NA	NA	NA	NA	497	5 U	1 J	0.6 J	20 UJ
22-DMM-05	11/4/2003	842092-0306	NA	NA	NA	NA	NA	NA	515 D	130 U	130 U	130 U	500 U
22-DMM-05	3/5/2004	101877-0070	NA	NA	NA	NA	NA	NA	86	5 U	5 U	5 U	20 U
22-DMM-05	5/14/2004	101877-0167	NA	NA	NA	NA	NA	NA	583	5 U	1 J	0.7 J	20 UJ
22-DMM-05	8/11/2004	101877-0251	NA	NA	NA	NA	NA	NA	503	5 U	0.9 J	0.4 J	20 UJ
22-DMM-05	11/2/2004	101877-0339	NA	NA	NA	NA	NA	NA	367	5 U	0.5 J	5 U	20 U
22-DMM-05	2/2/2005	101877-0480	NA	NA	NA	NA	NA	NA	242	50 U	50 U	50 U	200 U
22-DMM-05	4/14/2005	101877-0584	NA	NA	NA	NA	NA	NA	197	5 UJ	0.3 J	5 U	20 U
22-DMM-05	4/14/2005	101877-0585 (Dup)	NA	NA	NA	NA	NA	NA	194	5 UJ	0.3 J	5 U	20 U
22-DMM-05	6/15/2005	101877-0688	NA	NA	NA	NA	NA	NA	193	5 U	0.4 J	5 U	20 U
22-DMM-05	8/30/2005	101877-0768	NA	NA	NA	NA	NA	NA	174	5 U	0.4 J	5 U	20 U
22-DMM-05	1/19/2006	1085-037	NA	NA	NA	NA	NA	NA	120 J	2 U	2 U	2 U	25 U
22-DMM-05	4/19/2006	1085-160	NA	NA	NA	NA	NA	NA	90	2 U	2 U	2 U	25 U
22-DMM-05	7/28/2006	1085-296	NA	NA	NA	NA	NA	NA	80 J	50 U	50 UJ	50 UJ	200 UJ
22-DMM-05	10/20/2006	1085-361A	NA	NA	NA	NA	NA	NA	66 J	50 U	50 U	50 UJ	200 U
22-DMM-05	1/31/2007	1085-484	NA	NA	NA	NA	NA	NA	27 J	5 U	5 U	5 U	20 UJ
22-DMM-05	4/12/2007	1085-583	NA	NA	NA	NA	NA	NA	37 J	5 UJ	5 U	5 U	20 UJ
22-DMM-05	8/14/2007	1085-713	NA	NA	NA	NA	NA	NA	18	5 U	5 U	5 U	20 UJ
22-DMM-05 (top)[a]	8/28/2007	1085-772	NA	NA	NA	NA	NA	NA	5 J	5 U	5 U	5 U	20 U
22-DMM-05 (middle)[a]	8/28/2007	1085-773	NA	NA	NA	NA	NA	NA	0.5 J	5 U	5 U	5 U	20 U
22-DMM-05 (bottom)[a]	8/28/2007	1085-774	NA	NA	NA	NA	NA	NA	1 J	10 UJ	10 UJ	10 UJ	40 UJ
22-DMM-05	11/7/2007	1085-856	NA	NA	NA	NA	NA	NA	36	5 U	0.2 J	5 UJ	20 UJ
22-DMM-05	2/12/2008	1085-998	NA	NA	NA	NA	NA	NA	15	5 U	5 U	5 U	20 U
22-DMM-05	2/12/2008	1085-999 (Dup)	NA	NA	NA	NA	NA	NA	16	5 U	5 U	5 U	20 U

(Data highlighted in red are from March and August 2016 sampling events).

Table B-1. Historical Analytical Results for Selected Groundwater Samples - 22 Area Wells (1995 to 2016) (continued)

Analyte			TPH as Diesel	TPH as Gasoline	Benzene	Ethylbenzene	Toluene	Total Xylenes	MTBE	DIPE	ETBE	TAME	TBA
Method	Date Sampled	Sample Number	CA LUFT 8015M	CA LUFT 8015M	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260
Unit			(mg/L)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)
Location Code													
22-DMM-05	4/25/2008	1085-1072	NA	NA	NA	NA	NA	NA	33	5 UJ	5 U	5 UJ	32 J
22-DMM-05	7/24/2008	922036-0012	NA	NA	NA	NA	NA	NA	1.6	1 U	1 U	1 U	10 U
22-DMM-05	10/31/2008	922036-0102	NA	NA	NA	NA	NA	NA	13	1 U	1 U	1 U	10 UJ
22-DMM-05	2/16/2009	922036-0136	NA	NA	NA	NA	NA	NA	15	1 U	1 U	1 U	10 UJ
22-DMM-05	8/5/2009	62473-0018	NA	NA	NA	NA	NA	NA	5.3	1 U	1 U	1 U	10 U
22-DMM-05	2/10/2010	62473-0160	NA	NA	NA	NA	NA	NA	5	1 U	1 U	1 U	10 U
22-DMM-05	8/6/2010	922073-071	NA	NA	NA	NA	NA	NA	5.3	1 U	1 U	1 U	10 U
22-DMM-05	2/10/2011	922073-0151	NA	NA	NA	NA	NA	NA	25	1 U	1 U	1 U	10 UJ
22-DMM-05	7/26/2011	922073-722	NA	NA	NA	NA	NA	NA	3.1 J	1 U	1 UJ	1 U	10 U
22-DMM-05	1/13/2012	4267-024	NA	NA	NA	NA	NA	NA	3.8	1 U	1 U	1 U	10 U
22-DMM-05	7/21/2012	4267-353	NA	NA	NA	NA	NA	NA	3	1 U	1 U	1 U	10 U
22-DMM-05	1/8/2013	None	NA	NA	NA	NA	NA	NA	1.6	1 U	1 U	1 U	10 UJ
22-DMM-05	1/8/2013	None (Dup)	NA	NA	NA	NA	NA	NA	1.5	1 U	1 U	1 U	10 UJ
22-DMM-05	7/11/2013	None	NA	NA	NA	NA	NA	NA	3.7	1 U	1 U	1 U	10 UJ
22-DMM-05	7/11/2013	None (Dup)	NA	NA	NA	NA	NA	NA	3.7	1 U	1 U	1 U	10 UJ
22-DMM-05	3/9/2016	22-DMM-05	NA	0.05 U	0.5 U	0.5 U	0.5 U	0.5 U	8.8	1 U	1 U	1 U	10 UJ
22-DMM-05	8/17/2016	22-DMM-05	NA	0.05 U	0.5 U	0.5 U	0.5 U	0.5 U	6.6	1 U	1 U	1 U	10 U
22-BMW-8	11/10/2003	842092-0348	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 U
22-BMW-8	3/10/2004	101877-0096	NA	NA	NA	NA	NA	NA	1 J	5 U	5 UJ	5 U	20 UJ
22-BMW-8	5/18/2004	101877-0182	NA	NA	NA	NA	NA	NA	68	5 U	0.8 J	5 U	20 UJ
22-BMW-8	8/11/2004	101877-0257	NA	NA	NA	NA	NA	NA	2 J	5 U	5 U	5 U	20 UJ
22-BMW-8	11/4/2004	101877-0356	NA	NA	NA	NA	NA	NA	0.6 J	5 U	5 U	5 U	20 U
22-BMW-8	2/3/2005	101877-0491	NA	NA	NA	NA	NA	NA	0.5 J	5 U	5 U	5 U	20 U
22-BMW-8	4/15/2005	101877-0603	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 U
22-BMW-8	6/16/2005	101877-0690	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 UJ
22-BMW-8	8/31/2005	101877-0772	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 U
22-BMW-8	8/31/2005	101877-0773	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 U
22-BMW-8	1/18/2006	1085-028	NA	NA	NA	NA	NA	NA	1 UJ	2 UJ	2 U	2 U	25 U

(Data highlighted in red are from March and August 2016 sampling events).

Table B-1. Historical Analytical Results for Selected Groundwater Samples - 22 Area Wells (1995 to 2016) (continued)

Analyte			TPH as Diesel	TPH as Gasoline	Benzene	Ethylbenzene	Toluene	Total Xylenes	MTBE	DIPE	ETBE	TAME	TBA
Method	Date Sampled	Sample Number	CA LUFT 8015M	CA LUFT 8015M	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260
Unit			(mg/L)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)
Location Code													
22-BMW-8	4/21/2006	1085-179	NA	NA	NA	NA	NA	NA	1 UJ	2 U	2 UJ	2 UJ	25 UJ
22-BMW-8	4/21/2006	1085-180 (Dup)	NA	NA	NA	NA	NA	NA	1 UJ	2 U	2 UJ	2 UJ	25 UJ
22-BMW-8	7/28/2006	1085-299	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 U
22-BMW-8	10/23/2006	1085-368A	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 U
22-BMW-8	10/23/2006	1085-369A (Dup)	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 U
22-BMW-8	1/31/2007	1085-482	NA	NA	NA	NA	NA	NA	5 UJ	5 U	5 U	5 U	20 UJ
22-BMW-8	8/13/2007	1085-708	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 UJ
22-BMW-8	8/13/2007	1085-709 (Dup)	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 UJ
22-BMW-8	2/7/2008	1085-977	NA	NA	NA	NA	NA	NA	5 U	5 U	5 U	5 U	20 U
22-BMW-8	2/17/2009	922036-0149	NA	NA	NA	NA	NA	NA	0.5 U	1 U	1 U	1 U	10 UJ
22-BMW-8	8/5/2010	62473-0021	NA	NA	NA	NA	NA	NA	0.6	1 U	1 U	1 U	10 U
22-BMW-8	2/10/2010	62473-0155	NA	NA	NA	NA	NA	NA	1.4	1 U	1 U	1 U	10 U
22-BMW-8	3/8/2016	1085-713	NA	0.05 U	0.5 U	0.5 U	0.5 U	0.5 U	0.5 U	5 U	5 U	5 U	10 UJ
22-BMW-8	8/17/2016	22-BMW-8	NA	0.05 U	0.5 U	0.5 U	0.5 U	0.5 U	0.5 U	1 U	1 U	1 U	10 U
22-MM-07	3/6/2002	821816-0527	NA	NA	NA	NA	NA	NA	1420	5 U	4 J	1 J	20 U
22-MM-07	3/8/2016	22-MM-07	NA	0.05 U	0.5 U	0.5 U	0.5 U	0.5 U	1.8	1 U	1 U	1 U	10 UJ
22-MM-07	8/17/2016	22-MM-07	NA	0.05 U	0.5 U	0.5 U	0.5 U	0.5 U	4.9	1 U	1 U	1 U	10 U
22-MM-08	3/6/2002	821816-0528	NA	NA	NA	NA	NA	NA	55	5 U	5 U	5 U	20 U
22-MM-08	6/6/2002	821816-0721	NA	0.05	0.5 U	5 U	5 U	15 U	29	5 U	5 U	5 U	20 U
22-MM-08	9/6/2002	821816-0753	NA	0.05	0.5 U	5 U	5 U	15 U	32	5 U	5 U	5 U	20 U
22-MM-08	11/12/2002	821816-0874	NA	0.06	0.5 U	5 U	5 U	15 U	47	5 U	5 U	5 U	20 U
22-MM-08	3/11/2003	842092-0075	NA	0.021	0.5 U	5 U	0.4 J	5 U	27	5 U	5 U	5 U	20 U
22-MM-08	5/7/2003	842092-0149	NA	0.012	0.5 U	5 U	5 U	5 U	21	5 U	5 U	5 U	20 U
22-MM-08	8/20/2003	842092-0245	NA	0.008	0.5 U	5 U	5 U	15 U	8	5 U	5 U	5 U	20 UJ
22-MM-08	11/6/2003	842092-0329	NA	0.005	0.5 U	5 U	5 U	15 U	5	5 U	5 U	5 U	20 U
22-MM-08	3/11/2004	101877-0103	NA	0.007	0.5 U	5 U	5 U	5 U	3 J	5 U	5 U	5 U	20 UJ
22-MM-08	5/18/2004	101877-0189	NA	0.005	0.5 U	5 U	5 U	5 U	2 J	5 U	5 U	5 U	20 UJ
22-MM-08	8/12/2004	101877-0268	NA	0.006	0.5 U	5 U	5 U	5 U	1 J	5 U	5 U	5 U	20 U
22-MM-08	11/5/2004	101877-0370	NA	0.007	0.5 U	5 U	5 U	5 U	15	5 U	5 U	5 U	20 UJ

(Data highlighted in red are from March and August 2016 sampling events).

Table B-1. Historical Analytical Results for Selected Groundwater Samples - 22 Area Wells (1995 to 2016) (continued)

Analyte			TPH as Diesel	TPH as Gasoline	Benzene	Ethylbenzene	Toluene	Total Xylenes	MTBE	DIPE	ETBE	TAME	TBA
Method	Date Sampled	Sample Number	CA LUFT 8015M	CA LUFT 8015M	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260	EPA 8260
Unit			(mg/L)	(mg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)
Location Code													
22-MM-08	2/4/2005	101877-0506	NA	0.006	0.5 U	5 U	5 U	5 U	1 J	5 U	5 U	5 U	20 U
22-MM-08	4/18/2005	101877-0610	NA	0.005	0.5 U	5 U	5 U	5 U	6	5 U	5 U	5 U	20 U
22-MM-08	4/18/2005	101877-0611	NA	0.005	0.5 U	5 U	5 U	5 U	6	5 U	5 U	5 U	20 U
22-MM-08	6/20/2005	101877-0711	NA	NA	NA	NA	NA	NA	0.8 J	5 U	5 U	5 U	20 UJ
22-MM-08	9/1/2005	101877-0789	NA	NA	NA	NA	NA	NA	0.5 J	5 U	5 U	5 U	20 U
22-MM-08	1/18/2006	1085-021	NA	NA	NA	NA	NA	NA	0.7 J	2 UJ	2 U	2 U	25 U
22-MM-08	1/18/2006	1085-022 (MS/MSD)	NA	NA	NA	NA	NA	NA	0.7 J	2 UJ	2 U	2 U	25 U
22-MM-08	4/24/2006	1085-188	NA	NA	NA	NA	NA	NA	1 UJ	2 U	2 UJ	2 UJ	25 UJ
22-MM-08	7/26/2006	1085-278	NA	NA	0.5 U	5 U	5 U	5 U	0.9 J	5 U	5 U	5 U	20 U
22-MM-08	10/18/2006	1085-343A	NA	NA	0.5 U	5 U	5 U	5 U	0.5 J	5 UJ	5 UJ	5 UJ	20 U
22-MM-08	10/18/2006	1085-344A (Dup)	NA	NA	0.5 U	5 U	5 U	5 U	0.6 J	5 UJ	5 UJ	5 UJ	20 U
22-MM-08	1/26/2007	1085-462	NA	NA	0.5 U	5 U	5 U	5 U	0.4 J	5 U	5 U	5 U	20 U
22-MM-08	4/11/2007	1085-575	NA	NA	0.5 U	5 U	5 U	5 U	0.2 J	5 U	5 U	5 U	20 UJ
22-MM-08	4/11/2007	1085-576(Dup)	NA	NA	0.5 U	5 U	5 U	5 U	5 U	5 U	5 U	5 U	20 UJ
22-MM-08	8/9/2007	1085-691	NA	NA	0.5 U	5 U	5 U	5 U	5 U	5 UJ	5 U	5 U	20 U
22-MM-08	11/6/2007	1085-847	NA	NA	0.5 U	5 U	5 U	5 U	4.8 J	5 U	5 U	5 U	20 U
22-MM-08	2/5/2008	1085-960	NA	NA	0.5 U	5 U	5 U	5 U	1.1 J	5 U	5 U	5 U	20 U
22-MM-08	4/23/2008	1085-1053	NA	NA	0.5 U	5 U	0.26 J	5 U	5 U	5 U	5 U	5 U	20 UJ
22-MM-08	7/25/2008	922036-0025	NA	NA	NA	NA	NA	NA	0.5 U	1 U	1 U	1 U	10 UJ
22-MM-08	10/30/2008	922036-0097	NA	NA	0.5 U	0.5 U	0.5 U	1 U	0.5 U	1 U	1 U	1 U	10 UJ
22-MM-08	2/19/2009	922036-0160	NA	NA	0.5 U	0.5 U	0.5 U	1 U	0.5 U	1 U	1 U	1 U	10 UJ
22-MM-08	4/30/2009	922036-0215	NA	NA	0.5 U	0.5 U	0.5 U	1 U	0.5 U	1 U	1 U	1 U	10 UJ
22-MM-08	8/7/2009	62473-0044	NA	NA	0.5 U	0.5 U	0.5 U	1 U	0.5 U	1 U	1 U	1 U	10 UJ
22-MM-08	10/26/2009	62473-0076	NA	NA	0.5 U	0.5 U	0.5 U	1 U	0.5 U	1 U	1 U	1 U	10 UJ
22-MM-08	10/26/2009	62473-0077 (Dup)	NA	NA	0.5 U	0.5 U	0.5 U	1 U	0.5 U	1 U	1 U	1 U	10 UJ
22-MM-08	2/10/2010	62473-0167	NA	NA	0.5 U	0.5 U	0.5 U	1 U	0.5 U	1 U	1 U	1 U	10 U
22-MM-08	4/19/2010	62473-0219	NA	NA	0.5 U	0.5 U	0.5 U	1 U	0.5 U	1 U	1 U	1 U	10 UJ
22-MM-08	3/8/2016	22-MM-08	NA	0.05 U	0.5 U	0.5 U	0.5 U	1 U	0.5 U	1 U	1 U	1 U	10 UJ
22-MM-08	8/17/2016	22-MM-08	NA	0.05 U	0.5 U	0.5 U	0.5 U	0.5 U	0.68	1 U	1 U	1 U	10 U

(Data highlighted in red are from March and August 2016 sampling events).

APPENDIX C

SAMPLING METHODS AND GEOCHEMISTRY PARAMETERS

C-1 QUALITY ASSURANCE FOR GROUNDWATER SAMPLING AND ANALYSIS

Calibration Procedures and Frequency. Calibration refers to the checking of physical measurements of both field and laboratory instruments against accepted standards. It also refers to determining the response function for an analytical instrument, which is the measured net signal as a function of the given analyte concentration. These determinations have a significant impact on data quality and are performed regularly. In addition, preventative maintenance is important to the efficient collection of data. The calibration policies and procedures set forth apply to all test and measuring equipment. For preventative maintenance purposes, critical spare parts are obtained from the instrument manufacturer.

Field Measurements (Groundwater). All field and laboratory instruments were calibrated according to manufacturers' specifications. Calibration was performed prior to initial use and after periods of non-use. A logbook is maintained by Lynco Environmental collection personnel similarly for laboratory instrumentation. Groundwater was assessed for dissolved oxygen, pH, temperature, oxidation/reduction potential, and conductivity with a field meter. Depth to groundwater measurements were taken using a water interface probe.

Dissolved Oxygen, Temperature, pH, Conductivity and Oxidation/Reduction Potential. Prior to sampling, the well or sampling point identification was checked and recorded along with the date and time on the field sampling sheet. Groundwater samples were collected using bladder pumps connected to a compressor via a pump-specific controller. Samples were measured for dissolved oxygen, temperature, pH, conductivity and redox potential using a multi- probe water quality meter (e.g., YSI Model 6920, or similar). In order to minimize aeration of the sample, a continuous flow-through cell was used to provide a sampling chamber for the meter. A sufficient volume of water from the well or groundwater sampling point was purged before sample collection to ensure that a sample representative of the formation was obtained based on standard low-flow procedures. A field sheet was prepared for each well to document standardization of parameters prior to sampling.

Field quality control samples including source water blanks, equipment rinsate samples, trip blanks, and field duplicates were used to measure total process performance as follows:

- Source water blanks: one per sampling event
- Equipment rinsate samples: one per day
- Trip blanks: one per cooler
- Field duplicates: one every 10 samples
- Matrix spike matrix spike duplicates: one every 20 samples

C-1.1 DECONTAMINATION PROCEDURES

Decontamination was a five-step process completed on all field equipment to avoid cross-contamination between wells or samples and to ensure the health and safety of field personnel.

Decontamination water was collected in an appropriate container and disposed. The following sequence was used to clean equipment and sampling devices prior to and between each use:

- Rinse with potable water.
- Wash with Liquinox™ detergent and tap water and clean with a stiff-bristle brush.
- Rinse three times with deionized water.
- Rinse with reagent-grade methanol.
- Place the sampling equipment or non-dedicated equipment on a clean surface and air dry.

For the submersible pump and slug equipment, the outside of the equipment and the associated tubing or piping was cleaned using the above steps. Subsequently, the pump and slug equipment and the associated tubing or piping was purged sequentially using the same washing solutions. A minimum pump decontamination purge volume of five times the pump volume was used for detergent and clean water purge, based on the volume capacity of the hose and pump.

C-1.2 SAMPLE DOCUMENTATION

A project-specific field logbook were used to provide daily records of significant events, observations, and measurements during field investigations. The field logbook also was used to document all sampling activities. All logbook entries were made with indelible ink to provide a permanent record. Logbooks were kept in the possession of the field team leader during the on-site work and all members of the field team had access to the notebook. These notebooks were maintained as permanent records. Any errors found in the logbook were verified, crossed-through, and initialed by the person discovering the error.

The field logbooks are intended to provide sufficient data and observations to reconstruct events that occurred during field activities. Field logbooks were permanently bound and pre-paginated; the use of designated forms should be used whenever possible to ensure that field records are complete.

- Name, date, and time of entry
- Names and responsibilities of field crew members
- Name and titles of any site visitors
- Descriptions of field procedures, and problems encountered
- Number and amount of samples taken at each location
- Details of sampling location, including sampling coordinates
- Sample identification numbers of all samples collected
- Date and time of collection
- Sample collector
- Sample collection method
- Decontamination procedures

- Field instrument calibration and maintenance
- Field measurements (e.g., DO, ORP, temperature, pH, and conductivity) and general observations.

C-2 MTBE DEGRADATION TRENDS OVER TIME AT 22 MCX GAS STATION SITE

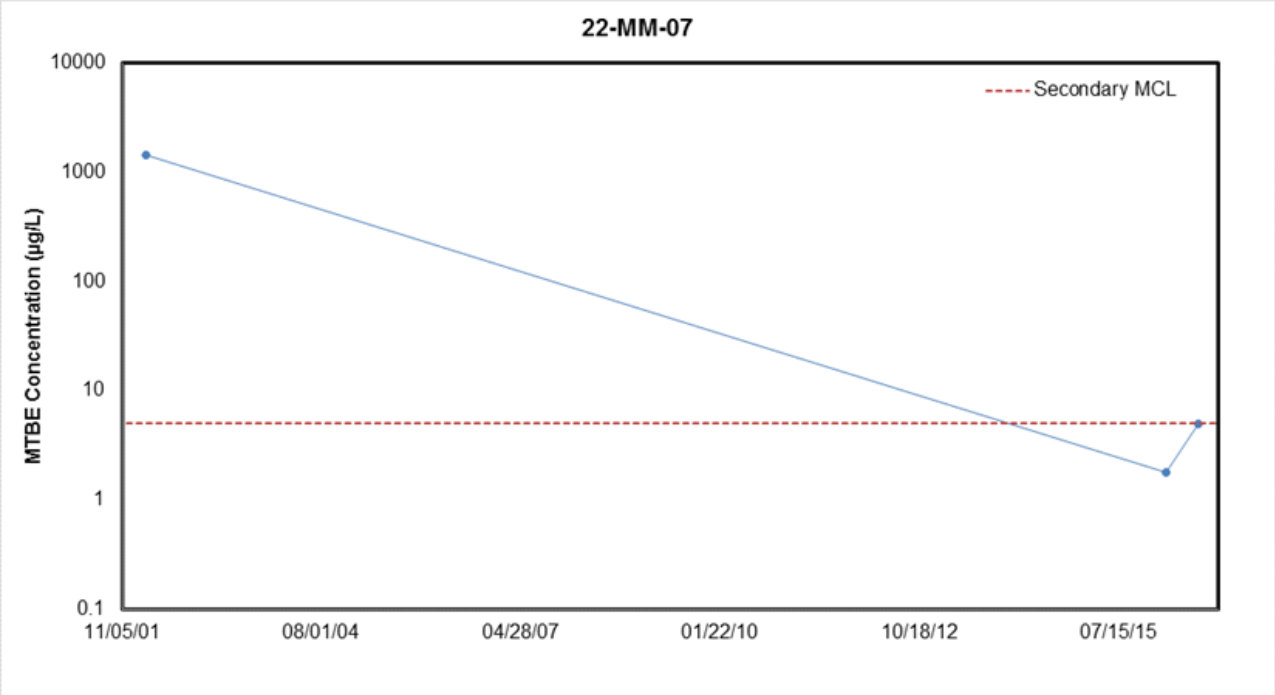


Figure C-1. MTBE Degradation Trend in Well 22-MM-07 at the 22 MCX Gas Station Site

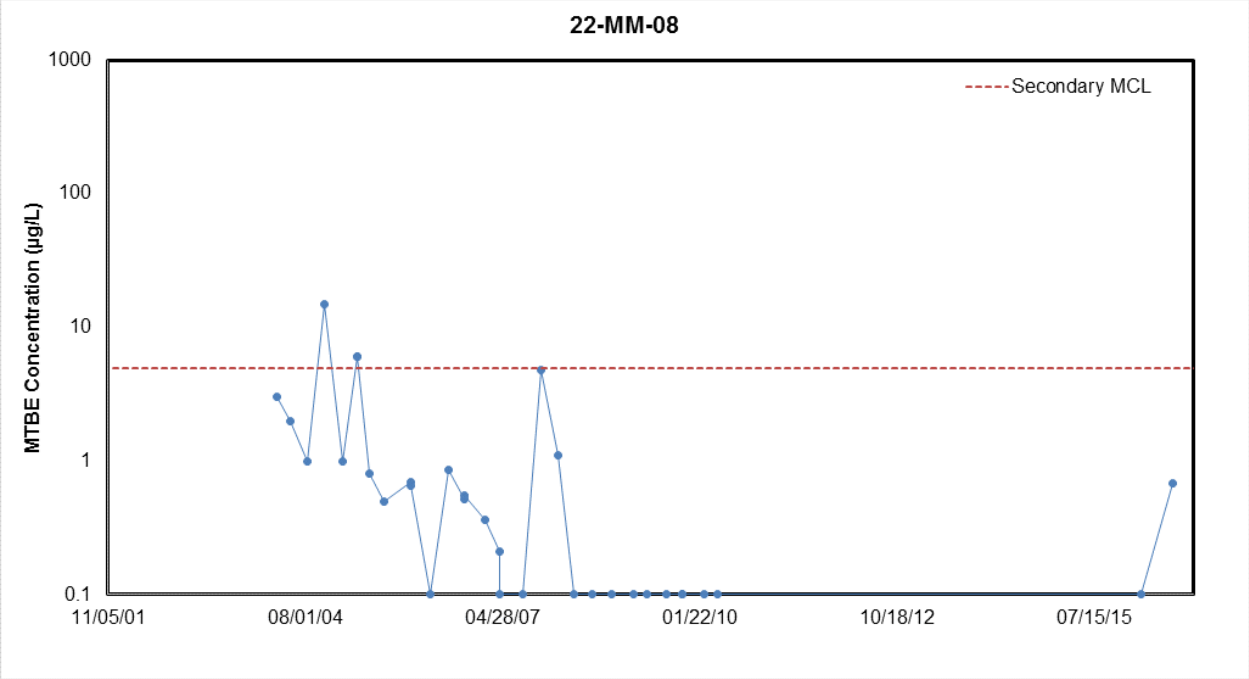


Figure C-2. MTBE Degradation Trend in Well 22-MM-08 at the 22 MCX Gas Station Site

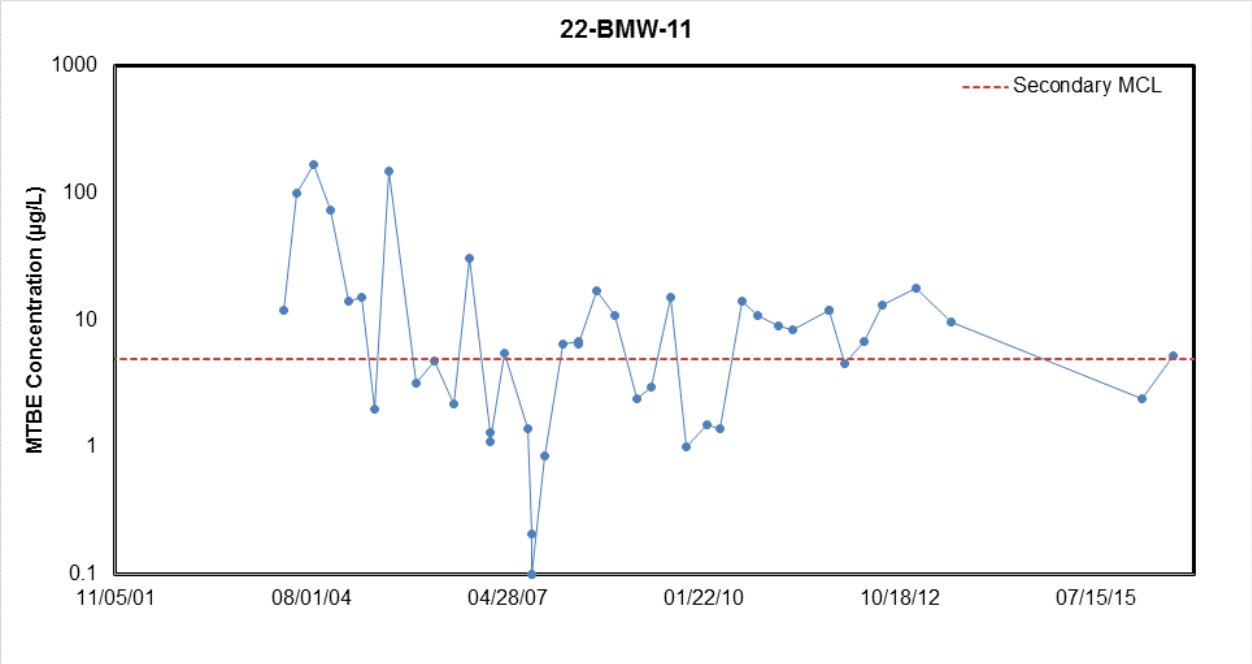


Figure C-3. MTBE Degradation Trend in Well 22-BMW-11 at the 22 MCX Gas Station Site

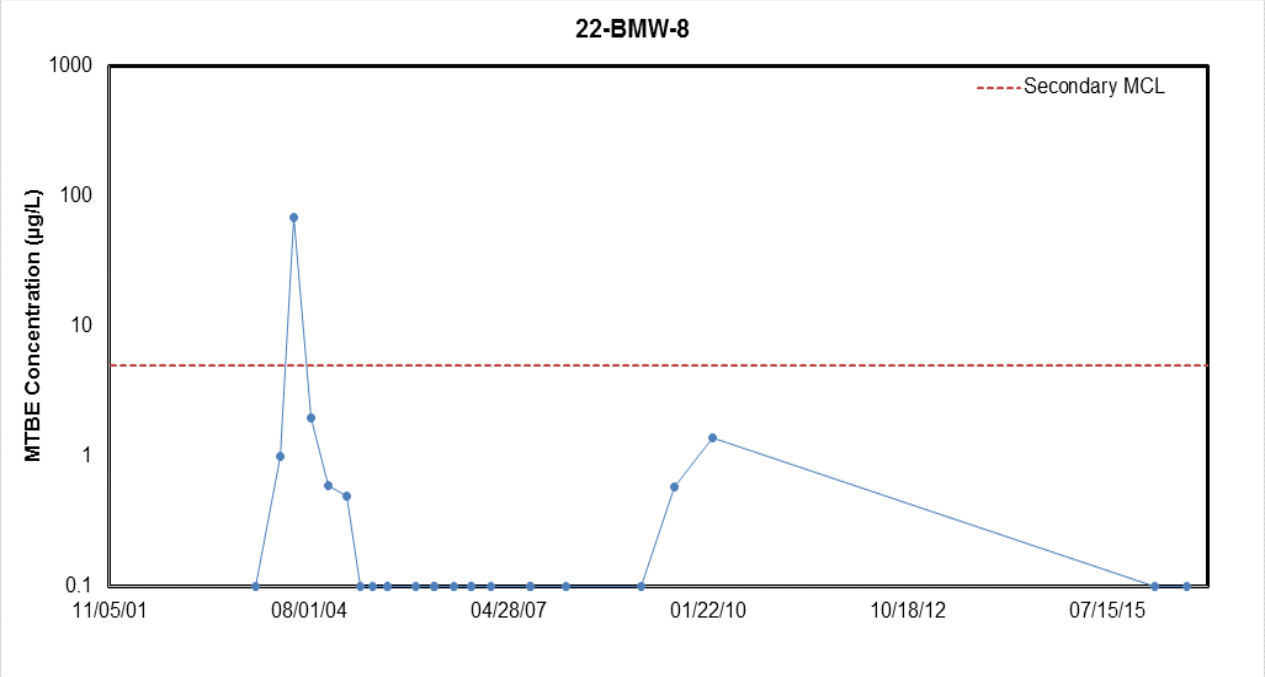


Figure C-4. MTBE Degradation Trend in Well 22-BMW-8 at the 22 MCX Gas Station Site

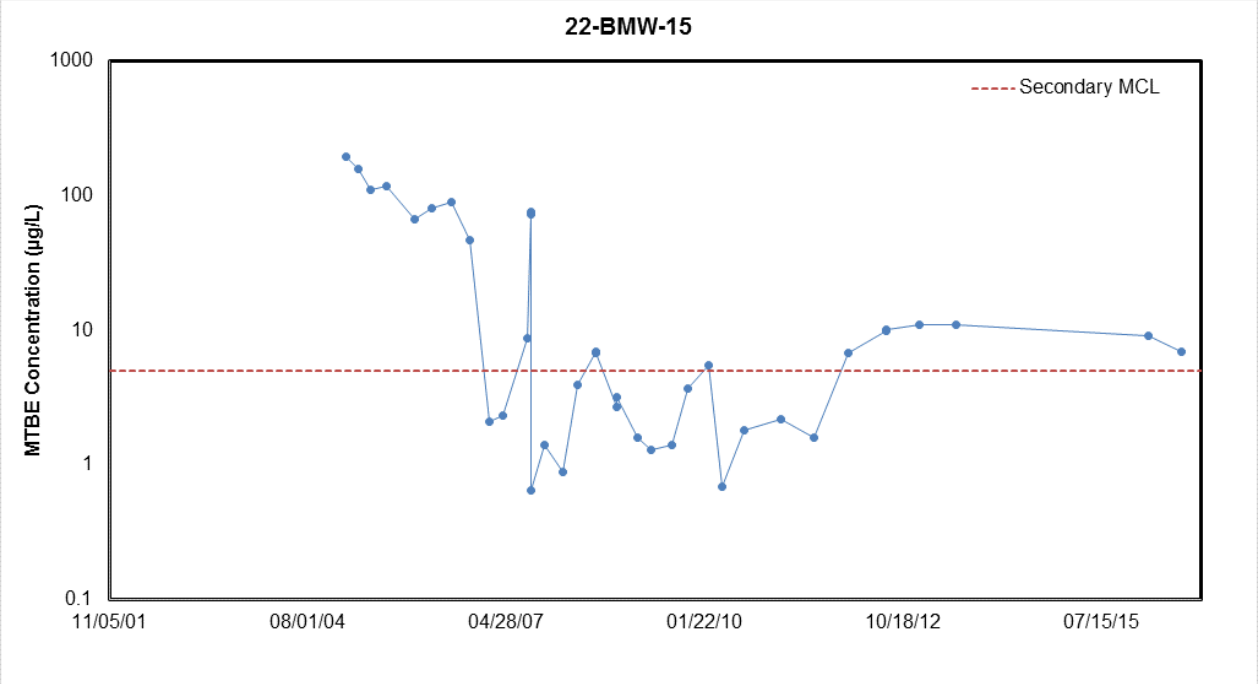


Figure C-5. MTBE Degradation Trend in Well 22-BMW-15 at the 22 MCX Gas Station Site

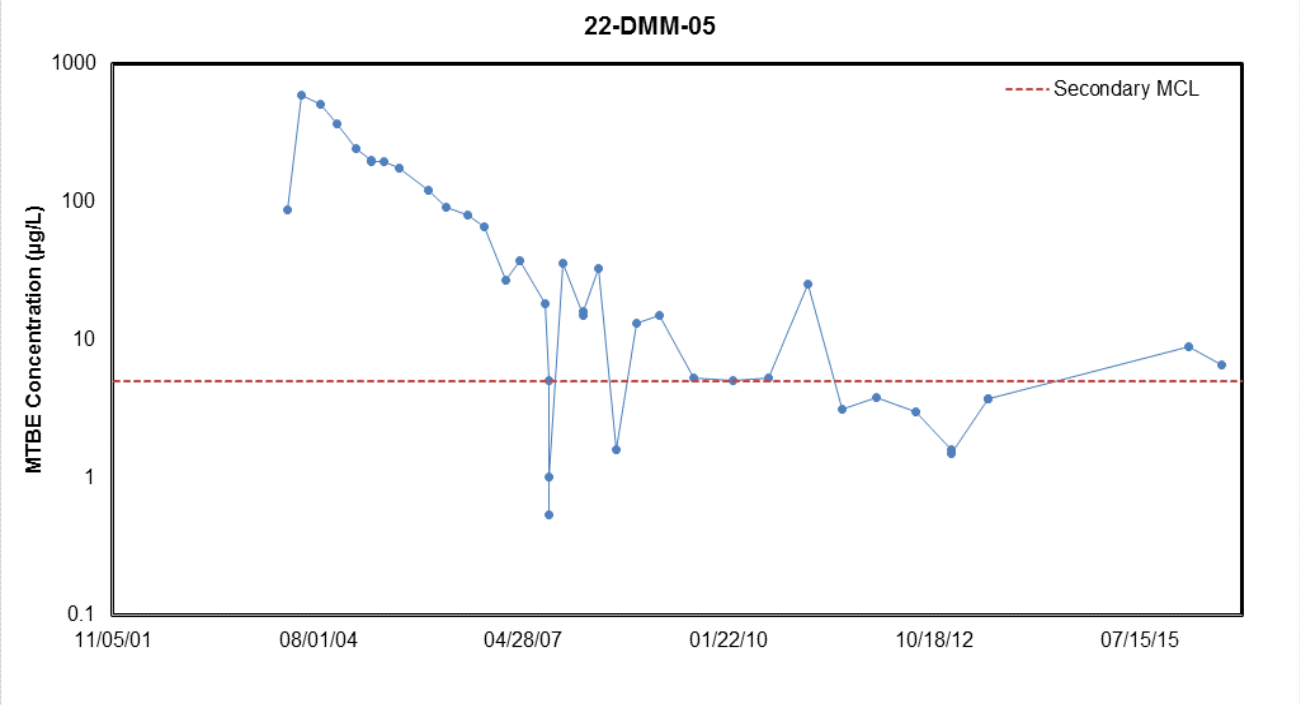


Figure C-6. MTBE Degradation Trend in Well 22-DMM-05 at the 22 MCX Gas Station Site

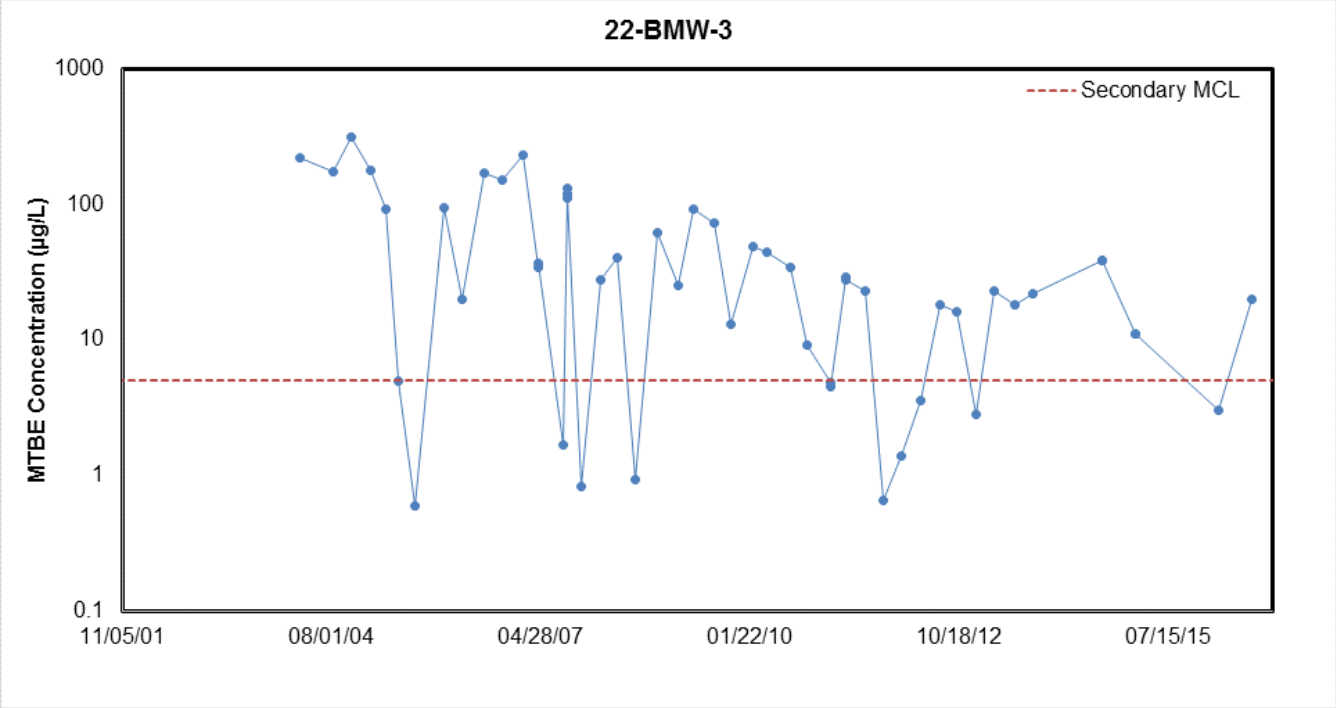


Figure C-7. MTBE Degradation Trend in Well 22-BMW-3 at the 22 MCX Gas Station Site

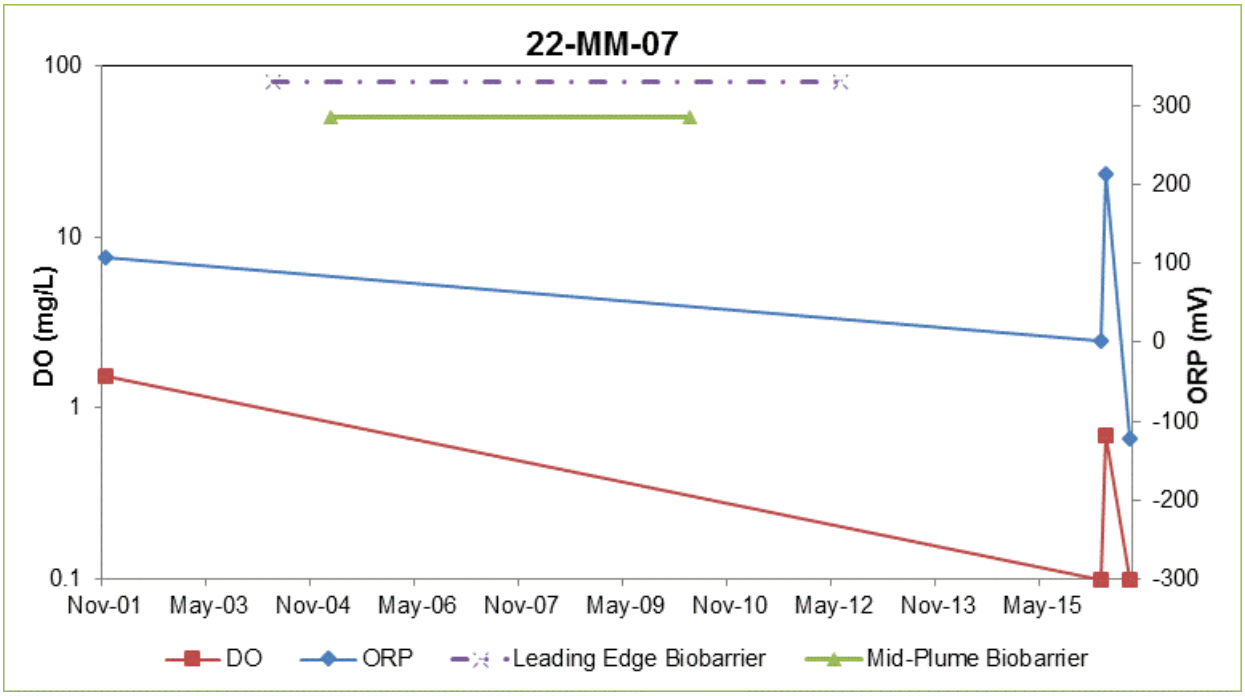


Figure C-8. DO and ORP Trend in Well 22-MM-07 at the 22 MCX Gas Station Site

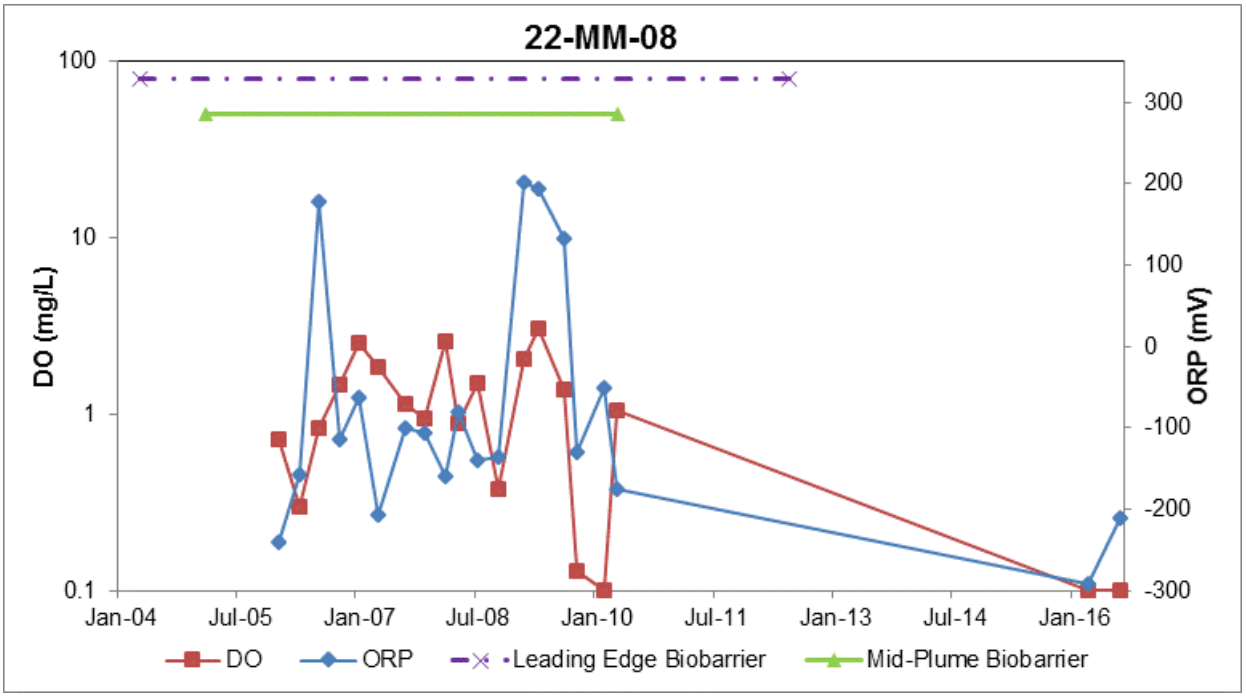


Figure C-9. DO and ORP Trend in Well 22-MM-08 at the 22 MCX Gas Station Site

Figure C-10. DO and ORP Trend in Well 22-BMW-11 at the 22 MCX Gas Station Site

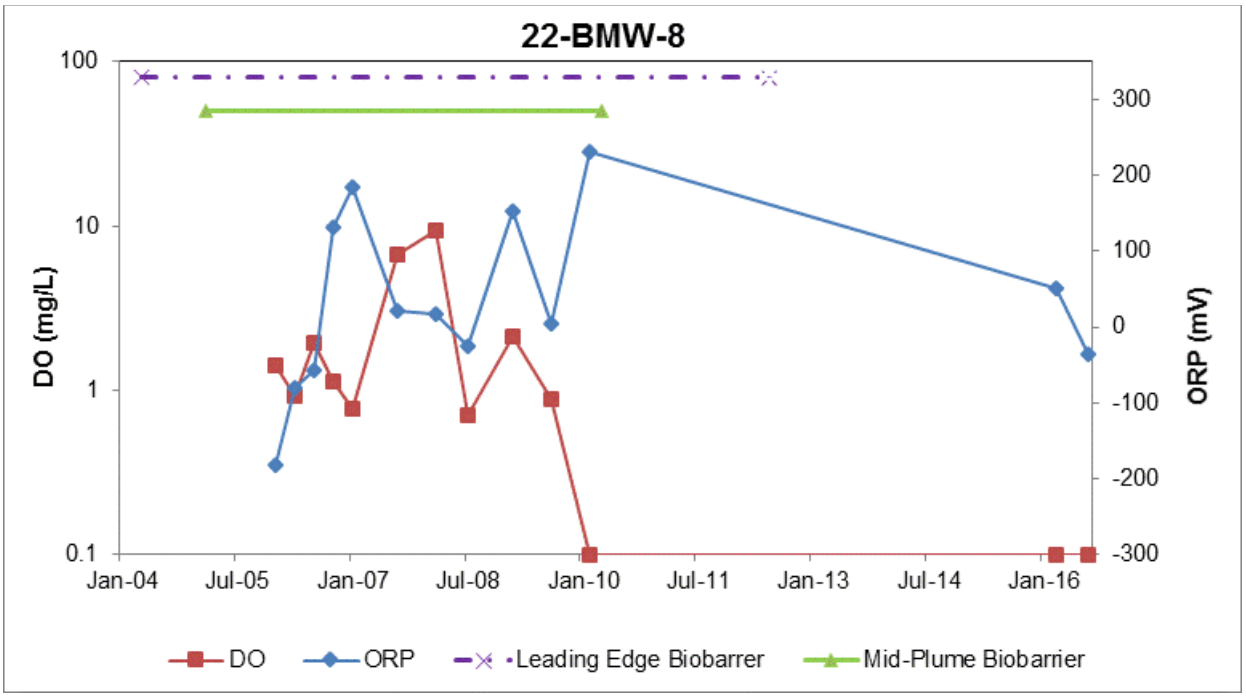


Figure C-11. DO and ORP Trend in Well 22-BMW-8 at the 22 MCX Gas Station Site

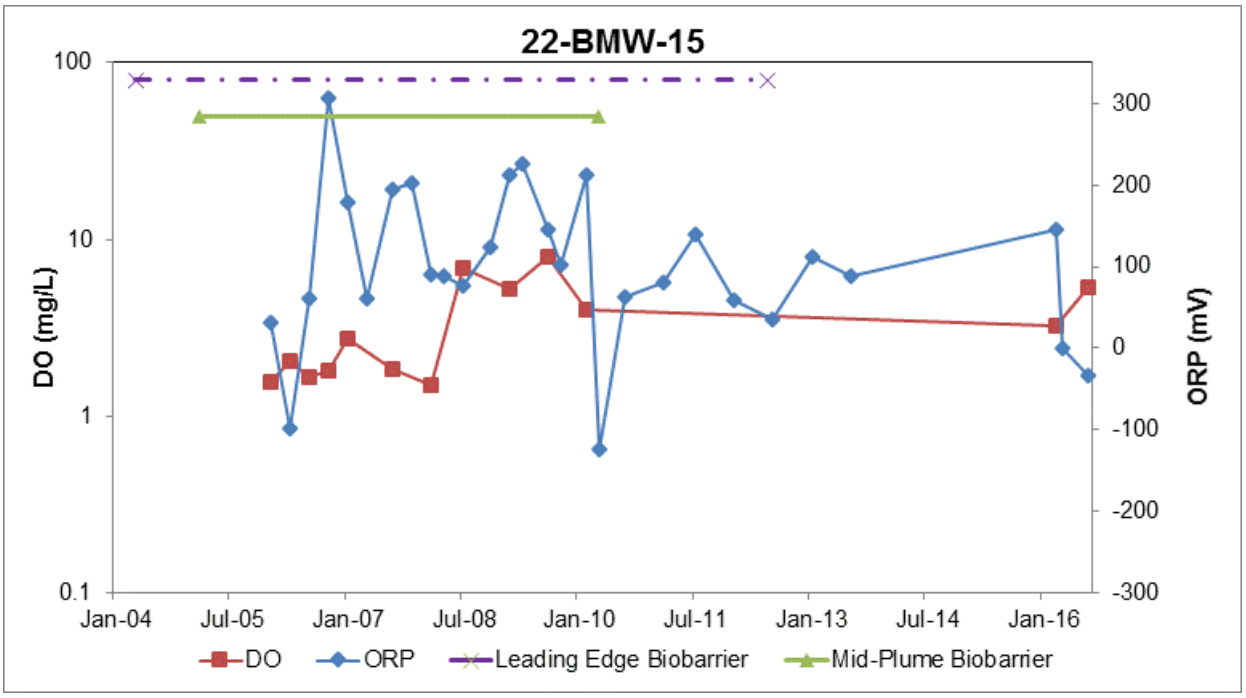


Figure C-12. DO and ORP Trend in Well 22-BMW-15 at the 22 MCX Gas Station Site

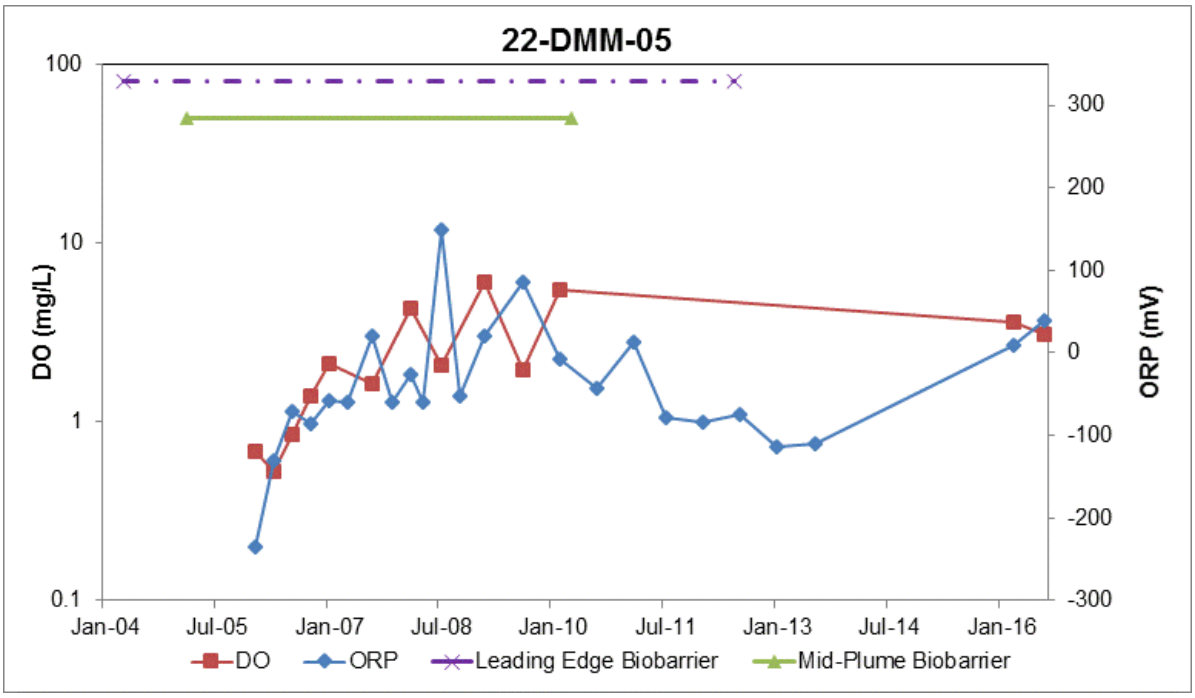


Figure C-13. DO and ORP Trend in Well 22-DMM-05 at the 22 MCX Gas Station Site

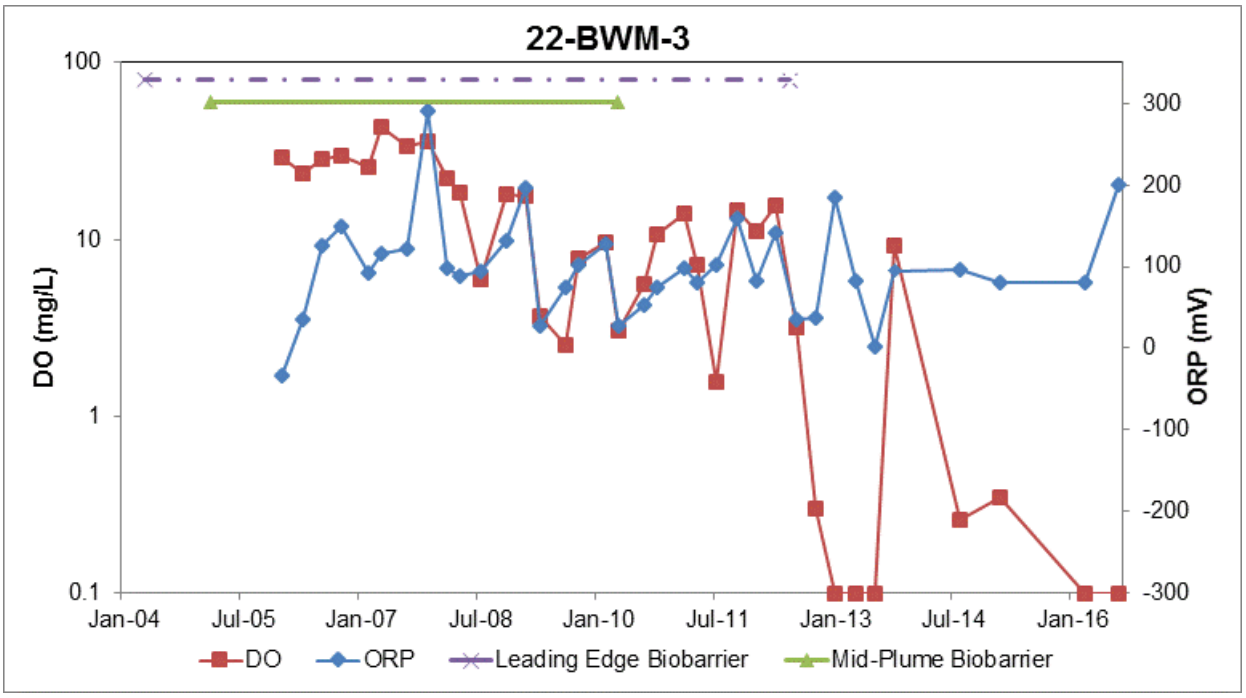


Figure C-14. DO and ORP Trend in Well 22-BMW-3 at the 22 MCX Gas Station Site

APPENDIX D

DETERMINATION OF KEY MICROBIAL PLAYERS WITH METAGENOMICS

Table D-1. Literature Representation of Relevant Genes, Species and Metabolism Type of Microorganisms Involved in MTBE Degradation Process

Species	Reference(s)	Relevant Genes (if detected)	Degradation (cometabolic, etc.)
Acidoobacteria (phyla)	Lui et al., 2009		Identified in MTBE enriched culture
Aquincola tertiaricarbonis L108	Rosell et al., 2007 Chen et al., 2011	Hydrolysis-reaction (ether bond cleavage) for MTBE	MTBE mineralization
Aquincola tertiaricarbonis L108, L(10), and CIP I-2052	Lechner et al., 2007		TBA mineralization
Aquincola tertiaricarbonis L108	Kane et al., 2007 Li et al., 2015		MTBE mineralization
Aquincola tertiaricarbonis L108	Muller et al., 2008 Aslett et al., 2011		TBA mineralization
Aquincola tertiaricarbonis L108	Schuster et al., 2013	*ethABCD* constitutively expressed, not regulated by *ethR ethB*	MTBE mineralization
Aquincola tertiaricarbonis L108	Jechalke et al., 2011	P450 (CYP249A1) / *ethABCD*	
Aquincola tertiaricarbonis L108	Schaefer et al., 2007	*mdpJ/K* induced when cells grown on TBA and 2-HIBA	
Arthrobacter ATCC 27778	Lui et al., 2001		Cometabolism of MTBE with butane
Arthrobacter spp	Eixarch et al., 2010		Partially degrade MTBE
MG strain (Arthrobacter spp)	Purswani et al., 2008		MTBE; co-metabolic degradation in presence of yeast extract and ethanol
Achromobacter xyloxidans MCM 1/1	Chen et al., 2011		MTBE mineralization
Achromobacter xyloxidans MCM 1/1	Barbera et al., 2011		MTBE degradation in resting cells
Achromobacter xyloxidans MCM 1/1	Eixarch et al., 2010		MTBE degradation
M10 strain (Acinetobacter spp)	Purswani et al., 2008		MTBE degradation, MTBE as sole source
Bacillus sp NKNU01	Chen et al., 2011		MTBE degradation
Bacillus spp	Eixarch et al., 2010		Partially degrade MTBE
Cupriavidus spp	Aslett et al., 2011	*Mdp* genes detected in metagenome	Incorporates 13C DNA SIP from 13C TBA
T3 strain (Gordonia spp)	Purswani et al., 2008		MTBE degradation alone
EA strain (Gordonia spp)	Purswani et al., 2008		MTBE; co-metabolic degradation in presence of yeast extract and ethanol
Hydrogenophaga flava ENV735	Hatzinger et al., 2001		MTBE and TBA mineralization

Table D-1. Literature Representation of Relevant Genes, Species and Metabolism Type of Microorganisms Involved in MTBE Degradation Process (continued)

Species	Reference(s)	Relevant Genes (if detected)	Degradation (cometabolic, etc.)
Hydrogenophaga flava ENV735	Streger et al., 2002 Li et al., 2015		MTBE mineralization
Hydrogenophaga flava ENV735	Ref 10 in Chen		MTBE mineralization
Hydrogenophaga spp	Bastida et al., 2010		Along with other species, found in gasoline impacted site
ENV425	Steffan et al., 1997	P450	Cometabolic mineralization of MTBE when strain grown on propane
Enterobacter sp. NKNU02, NKNU01	Chen et al., 2011		MTBE degradation to TBA, acetic acid, propanol and propenoic acid in resting cells
Enterobacter clonacae MCM2/1	Barbera et al., 2011		MTBE degradation in resting cells
Exophiala dermatidis (a fungus)	Barbera et al., 2011		MTBE degradation in resting cells
Hydrogenophaga spp	Aslett et al., 2011	*Mdp* genes detected in metagenome	Incorporates 13C DNA SIP from 13C TBA
Kocuria sp	Lalevic et al., 2012		MTBE sole carbon source
Klebsiella sp. NKNU01	Chen et al., 2011		MTBE degradation
M1, related to Arthrobacter ATCC 27778	Li et al., 2014		MTBE sole carbon source
M7, M9; related to Pseudomonas putida	Li et al., 2014		MTBE sole carbon source
M5, M2-3, M3, related to Pseudoxanthomonas Mexicana	Li et al., 2014		MTBE sole carbon source
M4, related to Sinorhizobium arborus	Li et al., 2014		MTBE sole carbon source
M6, related to Delftia lacustris	Li et al., 2014		MTBE sole carbon source
M2-1; related to Bacillus horikoshii	Li et al., 2014		MTBE sole carbon source
M2-2, related to Microbacterium oxydans	Li et al., 2014		MTBE sole carbon source
M10; related to Sphingobacterium daejeonse	Li et al., 2014		MTBE sole carbon source
M8; related to Nubsella zeaxanthinifaciens	Li et al., 2014		MTBE sole carbon source
Methylibium petroleiphilum PM1	Joshi et al., 2015	*mdpC* (transcriptional activator); *mdpA* (MTBE monooxygenase) and *mdpJ* (TBA hydroxylase) on megaplasmid	MTBE and TBA mineralization

Table D-1. Literature Representation of Relevant Genes, Species and Metabolism Type of Microorganisms Involved in MTBE Degradation Process (continued)

Species	Reference(s)	Relevant Genes (if detected)	Degradation (cometabolic, etc.)
Methylibium petroleiphilum PM1	Schmidt et al., 2008	*mdpA* induced by MTBE; Thr59 distinguishes *MdpA* from alkane hydroxylases (hydrocarbon ruler of sorts, Rhodobacteraceae have this residue as well)	
Methylibium petroleiphilum PM1	Rosell et al., 2007	Oxidation of alkyl group of MTBE (based on CSIA)	MTBE and TBA mineralization
Methylibium petroleiphilum PM1	Chen et al., 2011		MTBE mineralization
Methylibium petroleiphilum PM1	Bastida et al., 2010		Along with other species, found in gasoline impacted site; enrich in 13C protein when cultures fed 13C-MTBE; see table below for mdpJ peptides detected
Methylibium sp. R8	Rosell et al., 2007	Oxidation of alkyl group of MTBE (based on CSIA)	MTBE and TBA mineralization
Methylibium sp strain T29	Szabo et al., 2015	MTBE degradation genes not on plasmid (actually on chromosome); cobalamine synthesis genes;	MTBE and TBA mineralization
Methylibium spp	Aslett et al., 2011	*Mdp* genes detected in metagenome, may belong to other organisms in this study	Incorporates 13C DNA SIP from 13C TBA
Mycobacterium austroafricanum IFP2012 and IFP2015	Francois et al., 2001 Lopes Ferreira et al., 2006 Chen et al., 2011 Eixarch et al., 2010		MTBE mineralization
Mycobacterium vaccae	Smith et al., 2003		Cometabolic MTBE degradation with propane
Mycobacterium duvalii TA5	Ohbubo et al., 2009		MTBE cometabolic degradation with various organic acids
Mycobacterium gilvum TA27	Ohbubo et al., 2009		MTBE cometabolic degradation with various organic acids
Mycobacterium chlorophenolicum TCE 28	Ohbubo et al., 2009		MTBE cometabolic degradation with various organic acids
Methanosarcina and Methanocorpusculum archaea	Sun et al., 2012	Anaerobic degradation	Anaerobic degradation based on DNA SIP

Table D-1. Literature Representation of Relevant Genes, Species and Metabolism Type of Microorganisms Involved in MTBE Degradation Process (continued)

Species	Reference(s)	Relevant Genes (if detected)	Degradation (cometabolic, etc.)
Nocardiodes sp	Bastida et al., 2010		Along with other species, found in gasoline impacted site
E7 strain (Nocardioides sp)	Purswani et al., 2008		MTBE degradation with MTBE as sole source
Ochrombactrum anthropi MCM5/1	Barbera et al., 2011		MTBE degradation in resting cells
Paucibacter spp.	Aslett et al., 2011	*Mdp* genes detected in metagenome, may belong to other organisms in this study	Incorporates 13C DNA SIP from 13C TBA
Pseudonocardia tetrahydrofuranoxydans K1	McKelvie et al., 2009	Different mechanism than PM1 and R8; K1 involves oxidation of methoxy group	Cometabolic degradation
Pseudomonas aeruginosa	Salazar et al., 2012		Cometabolic degradation of MTBE with hexane; TBA accumulated then gradually consumed
Pseudomonas putida GPo	Li et al., 2015		MTBE cometabolic with n-octane
Pseudomonas putida GPo	Hristova et al., 2007	*alkB* very similar in sequence to *mdpA* from PM1	
Pseudomonas sp. PM1 (referred to as "UC1" in primary reference)	Chen et al., 2011	Not impacted by BTEX	MTBE mineralization
Pseudomonas citronellolis UAM-Ps1	Bravo et al., 2014	*alkB*	Cometabolic degradation of MTBE to TBA with pentane, octane, or other hydrocarbons
Pseudomonas putida CAM	Steffan et al., 1997	P450-cam	Cometabolic degradation of MTBE to TBA when cells grown on camphor (hydrocarbon wax)
Pseudomonas mendocina KR-1	Smith et al., 2003	Likely alkane monooxygenase	Cometabolic degradation of MTBE to TBA when cells grown on alkanes
Pseudomonas sp NKNU01	Chen et al., 2011		MTBE degradation
Pseudomonas sp	Eixarch et al., 2010		Partially degrade MTBE
Polarimomas spp	Aslett et al., 2011	*Mdp* genes detected in metagenome, may belong to other organisms in this study	Incorporates 13C DNA SIP from 13C TBA
Rhodobacter sp	Bastida et al., 2010		Along with other species, found in gasoline impacted site
Rhodococcus ruber IFP 2001	Rosell et al., 2007	Hydrolysis-reaction of MTBE (ether bond cleavage) based on CSIA	ETBE mineralization

Table D-1. Literature Representation of Relevant Genes, Species and Metabolism Type of Microorganisms Involved in MTBE Degradation Process (continued)

Species	Reference(s)	Relevant Genes (if detected)	Degradation (cometabolic, etc.)
Rhodococcus ruber IFP 2001	Schuster et al., 2013 Jechalke et al., 2011	*EthABCD* catalyzes hydroxylation of methoxy of MTBE; *ethR* regulates	
Rhodococcus zopfii IFP 2001	Maladain et al., 2010		Grows on ETBE, degrades MTBE but cannot mineralize
Rhodococcus zopfii IFP 2005	Schuster et al., 2013	*EthABCD* catalyzes hydroxylation of methoxy of MTBE; *ethR* regulates	
Rhodococcus zopfii IFP 2005	Maladain et al., 2010		Grows on ETBE, degrades MTBE but cannot mineralize
Rhodococcus sp EH831	Lee et al., 2009		MTBE degradation inhibited in the presence of BTEX compounds
Rhodococcus sp	Chen et al., 2011		
Rhodococcus sp	Purswani et al., 2008		MTBE degradation, sole carbon source
Rhodococcus wratislaviensis IFP 2016, Rhodococcus aetherivorans IFP 2010	Auffret et al., 2009	*alkB* and *ethB*	Partially degraded when MTBE as sole carbon source, degraded to TBA in the presence of BTEX, not degraded in the presence of ETBE; octane increased degradation, BTEX reduced degradation
Rhodoferax spp	Aslett et al., 2011	*Mdp* genes detected in metagenome, may belong to other organisms in this study	Incorporates 13C DNA SIP from 13C TBA
Ruminococcaceae family	Sun et al., 2012	Anaerobic degradation	Anaerobic degradation based on DNA SIP
Gordonia sp. strain IFP 2009	Schuster et al., 2013	*EthABCD* catalyzes hydroxylation of methoxy of MTBE; *ethR* regulates	
Gordonia sp. strain IFP 2009	Maladain et al., 2010		Grows on ETBE, degrades MTBE but cannot mineralize
Sphingopyxis spp	Sun et al., 2012	Anaerobic degradation	Anaerobic degradation based on DNA SIP
Sphingomonadacea spp	Bastida et al., 2010		Along with other species, found in gasoline impacted site
Terrimonas spp	Lui et al., 2009		Identified in MTBE enriched culture
Thiothrix unzii	Bastida et al., 2010		Along with other species, found in gasoline impacted site
Variovorax paradoxus CL-8	Zaitsev et al., 2007		MTBE, TBA mineralization

Table D-2. DNA Concentration after Performing qPCR with 16S Primers

	DNA Concentration (ng/µl)		
Well ID	**Sampling Event 1**	**Optional Sampling Event**	**Sampling Event 2**
22-MM-07	Lower than blank	22.4	0.069
22-MM-08	0.154	-	32.0
22-BMW-11	0.048	25.5	Lower than blank
22-BWM-8	0.818	-	Lower than blank
22-BMW-15	Lower than blank	25.1	17.0
22-DMM-05	0.293	-	49.0
22-BMW-3	0.157	-	Lower than blank
1327-MW-01R	-	19.3	50.0
1327-RW-07	-	61.3	13.0
1327-MW-07R	-	104.4	31.0
1327-MW-23	-	25.2	0.055
1327-MW-39	-	32.8	55.0
Extraction blank	0.0064	-	-

Table D-3. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-DMM-07, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Pseudoxanthomonas	**53.64%**
sp.	53.64%
Agrobacterium	**15.95%**
tumefaciens	15.95%
Bacillus	**11.73%**
cereus	0.18%
pseudofirmus	1.64%
thuringiensis	9.90%
Sphingomonas	**5.42%**
leidyi	0.88%
melonis	1.13%
sp.	3.40%
Vogesella	**4.18%**
indigofera	4.18%
Mesorhizobium	**2.04%**
sp.	2.04%
Pseudomonas	**2.01%**
resinovorans	0.12%
sp.	1.89%
Aquamicrobium	**1.76%**
defluvii	1.76%
Acinetobacter	**0.70%**
sp.	0.70%
Aromatoleum	**0.64%**
aromaticum	0.64%
Agrococcus	**0.59%**
pavilionensis	0.59%
Leucobacter	**0.47%**
salsicius	0.47%
Sphingobium	**0.24%**
baderi	0.24%
Delftia	**0.19%**
sp.	0.19%
Bradyrhizobium	**0.14%**
japonicum	0.14%
Rubrivivax	**0.14%**
gelatinosus	0.14%

Genus species	Percent
Propionibacterium	**0.09%**
sp.	0.09%
Marmoricola	**0.02%**
sp.	0.02%
Clavibacter	**0.02%**
michiganensis	0.02%
Leifsonia	**0.02%**
sp.	0.02%
Microbacterium	**0.01%**
resistens	0.01%

Table D-4. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-DMM-08, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Pseudomonas	**23.44%**
alcaliphila	1.77%
balearica	0.30%
fluorescens	0.05%
geniculata	0.02%
mendocina	3.29%
monteilii	0.15%
pseudoalcaligenes	0.51%
putida	0.22%
resinovorans	4.42%
sp.	0.42%
stutzeri	12.30%
Dechlorosoma	**15.13%**
suillum	15.13%
Pseudoxanthomonas	**10.01%**
mexicana	0.88%
sp.	9.13%
Acidovorax	**4.81%**
avenae	0.08%
citrulli	0.05%
sp.	4.68%
Thiobacillus	**4.47%**
thioparus	4.47%
Bifidobacterium	**4.17%**
animalis	2.94%
longum	1.23%
Geobacter	**3.28%**
bemidjiensis	3.28%
Legionella	**3.14%**
drozanskii	3.14%
Magnetospirillum	**3.05%**
gryphiswaldense	2.57%
magneticum	0.48%
Caulobacter	**2.48%**
fusiformis	0.02%
segnis	1.53%
sp.	0.93%

Genus species	Percent
Shewanella	**2.37%**
oneidensis	0.01%
putrefaciens	2.00%
sp.	0.37%
Acinetobacter	**2.31%**
baumannii	0.06%
sp.	2.24%
Desulfosporosinus	**2.13%**
meridiei	2.03%
orientis	0.11%
Bacillus	**2.09%**
cereus	0.02%
pseudofirmus	0.22%
thuringiensis	1.85%
Phenylobacterium	**1.98%**
zucineum	1.98%
Cupriavidus	**1.86%**
metallidurans	0.31%
necator	1.51%
taiwanensis	0.03%
Rubrivivax	**1.55%**
gelatinosus	1.55%
Lactobacillus	**1.42%**
acidophilus	0.24%
casei	0.07%
gallinarum	0.02%
plantarum	0.83%
salivarius	0.26%
Sphingomonas	**1.17%**
leidyi	0.07%
sp.	0.25%
wittichii	0.86%
Agrobacterium	**1.10%**
tumefaciens	1.10%
Xanthobacter	**0.96%**
flavus	0.96%

Genus species	Percent
Comamonas	**0.69%**
testosteroni	0.69%
Lactococcus	**0.64%**
lactis	0.64%
Stenotrophomonas	**0.60%**
maltophilia	0.60%
Novosphingobium	**0.56%**
sp.	0.56%
Ralstonia	**0.40%**
pickettii	0.40%
Sphingopyxis	**0.25%**
baekryungensis	0.22%
soli	0.02%
Azospirillum	**0.23%**
oryzae	0.23%
Brevundimonas	**0.19%**
bacteroides	0.09%
subvibrioides	0.10%
Terriglobus	**0.19%**
roseus	0.19%
Methylosinus	**0.19%**
trichosporium	0.19%
Nocardioides	**0.18%**
sp.	0.18%
Bradyrhizobium	**0.18%**
elkanii	0.18%
Sulfuritalea	**0.18%**
hydrogenivorans	0.18%
Acetivibrio	**0.18%**
cellulolyticus	0.18%
Azoarcus	**0.17%**
sp.	0.17%
Propionibacterium	**0.16%**
sp.	0.16%
Terrimonas	**0.16%**
lutea	0.16%

Table D-4 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-DMM-08, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Sphingorhabdus	**0.15%**
flavimaris	0.15%
Afipia	**0.15%**
sp.	0.15%
Lysinibacillus	**0.13%**
sphaericus	0.13%
Escherichia	**0.13%**
coli	0.13%
Mesorhizobium	**0.13%**
opportunistum	0.04%
sp.	0.08%
Mycobacterium	**0.11%**
chlorophenolicum	0.02%
engbaekii	0.01%
marinum	0.08%
Hydrocarboniphaga	**0.11%**
effusa	0.11%
Desulfotomaculum	**0.11%**
reducens	0.11%
Sphingobium	**0.11%**
baderi	0.02%
indicum	0.03%
lactosutens	0.03%
sp.	0.03%
Streptococcus	**0.11%**
tigurinus	0.11%
Methylibium	**0.09%**
petroleiphilum	0.09%
Flavobacterium	**0.07%**
branchiophilum	0.07%
Dehalogenimonas	**0.06%**
lykanthroporepellens	0.06%
Methylocystis	**0.06%**
sp.	0.06%

Genus species	Percent
Curvibacter	**0.05%**
lanceolatus	0.05%
Hyphomicrobium	**0.04%**
denitrificans	0.04%
Renibacterium	**0.03%**
salmoninarum	0.03%
Ramlibacter	**0.03%**
tataouinensis	0.03%
Rhodoferax	**0.02%**
fermentans	0.02%
Desulfovibrio	**0.02%**
magneticus	0.02%
Desulfomonile	**0.02%**
tiedjei	0.02%
Methyloversatilis	**0.02%**
discipulorum	0.02%
Aromatoleum	**0.01%**
aromaticum	0.01%

Table D-5. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-11, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Pseudoxanthomonas	**61.86%**
sp.	61.86%
Agrobacterium	**14.38%**
tumefaciens	14.38%
Bacillus	**11.87%**
cereus	0.20%
pseudofirmus	4.26%
thuringiensis	7.41%
Lysinibacillus	**3.16%**
sphaericus	3.16%
Bradyrhizobium	**1.87%**
japonicum	1.87%
Stenotrophomonas	**1.82%**
maltophilia	1.82%
Mesorhizobium	**1.51%**
sp.	1.51%
Pseudomonas	**1.10%**
aeruginosa	0.02%
fluorescens	0.01%
resinovorans	0.96%
rhodesiae	0.06%
sp.	0.02%
stutzeri	0.02%
syringae	0.01%
Acinetobacter	**0.69%**
baumannii	0.68%
sp.	0.01%
Ochrobactrum	**0.39%**
anthropi	0.39%
Ralstonia	**0.31%**
pickettii	0.31%
Staphylococcus	**0.26%**
aureus	0.20%
epidermidis	0.06%
Brevundimonas	**0.21%**
bullata	0.20%
subvibrioides	0.01%

Genus species	Percent
Sphingomonas	**0.07%**
leidyi	0.01%
melonis	0.01%
paucimobilis	0.04%
Achromobacter	**0.06%**
xylosoxidans	0.06%
Hydrocarboniphaga	**0.06%**
effusa	0.06%
Sinorhizobium	**0.06%**
arboris	0.06%
Aquabacter	**0.05%**
spiritensis	0.05%
Agrococcus	**0.04%**
pavilionensis	0.04%
Cupriavidus	**0.04%**
necator	0.04%
Parvibaculum	**0.04%**
lavamentivorans	0.04%
Shewanella	**0.02%**
putrefaciens	0.02%
Variovorax	**0.02%**
paradoxus	0.02%
Methylobacter	**0.02%**
luteus	0.02%
Verrucomicrobium	**0.01%**
spinosum	0.01%
Rhodoferax	**0.01%**
saidenbachensis	0.01%
Comamonas	**0.01%**
testosteroni	0.01%
Sphingobium	**0.01%**
baderi	0.01%
Azospirillum	**0.01%**
oryzae	0.01%
Methylocystis	**0.01%**
sp.	0.01%

Genus species	Percent
Caulobacter	**0.01%**
fusiformis	0.01%
Sphingopyxis	**0.01%**
bauzanensis	0.01%

Table D-5 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-11, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Pseudoxanthomonas	**45.88%**
sp.	45.88%
Agrobacterium	**12.68%**
tumefaciens	12.68%
Bacillus	**11.04%**
cereus	0.19%
megaterium	2.80%
thuringiensis	8.04%
Mesorhizobium	**5.33%**
sp.	5.33%
Ochrobactrum	**4.64%**
anthropi	4.62%
pituitosum	0.02%
Sphingomonas	**4.62%**
leidyi	0.82%
melonis	0.01%
sp.	3.79%
Acinetobacter	**2.56%**
baumannii	0.71%
radioresistens	0.28%
sp.	1.57%
Microbacterium	**2.39%**
resistens	2.39%
Stenotrophomonas	**2.12%**
maltophilia	2.12%
Rhodoferax	**1.97%**
saidenbachensis	1.97%
Pseudomonas	**1.55%**
chloritidismutans	0.01%
putida	0.09%
resinovorans	1.45%
Clavibacter	**1.33%**
michiganensis	1.33%
Isoptericola	**1.20%**
variabilis	1.20%
Variovorax	**0.48%**
paradoxus	0.48%

Genus species	Percent
Propionibacterium	**0.47%**
sp.	0.47%
Alcaligenes	**0.40%**
aquatilis	0.40%
Hyphomicrobium	**0.35%**
vulgare	0.35%
Comamonas	**0.30%**
testosteroni	0.30%
Dietzia	**0.23%**
psychralcaliphila	0.23%
Thiobacillus	**0.13%**
thioparus	0.13%
Corynebacterium	**0.09%**
durum	0.04%
mycetoides	0.04%
Leucobacter	**0.03%**
salsicius	0.03%
Ralstonia	**0.03%**
solanacearum	0.03%
Methylocystis	**0.03%**
sp.	0.03%
Aquabacter	**0.02%**
spiritensis	0.02%
Sphingopyxis	**0.02%**
bauzanensis	0.02%
Caulobacter	**0.02%**
segnis	0.02%
Brucella	**0.02%**
melitensis	0.02%
Methylobacter	**0.01%**
luteus	0.01%
Carbophilus	**0.01%**
carboxidus	0.01%
Sinorhizobium	**0.01%**
arboris	0.01%
Rubrivivax	**0.01%**
gelatinosus	0.01%

Genus species	Percent
Hydrocarboniphaga	**0.01%**
effusa	0.01%

Table D-6. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-8, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Methylobacter	**10.51%**
luteus	10.51%
Hydrocarboniphaga	**8.82%**
effusa	8.82%
Aquabacter	**8.75%**
spiritensis	8.75%
Methylocystis	**8.41%**
sp.	8.41%
Mycobacterium	**7.31%**
cookii	0.02%
rhodesiae	2.64%
sphagni	4.65%
Agrococcus	**6.99%**
pavilionensis	6.99%
Verrucomicrobium	**6.90%**
spinosum	6.90%
Caulobacter	**5.75%**
fusiformis	2.43%
segnis	2.97%
sp.	0.35%
Brevundimonas	**4.70%**
aveniformis	0.26%
bacteroides	0.86%
subvibrioides	3.52%
vesicularis	0.06%
Variovorax	**4.03%**
paradoxus	4.03%
Sphingomonas	**3.83%**
leidyi	0.19%
melonis	1.31%
sp.	2.01%
wittichii	0.32%
Pseudomonas	**3.61%**
aeruginosa	0.01%
alcaliphila	0.24%
balearica	0.29%

Genus species	Percent
Pseudomonas	**3.61%**
entomophila	0.02%
fluorescens	0.23%
geniculata	0.01%
hibiscicola	0.03%
monteilii	0.04%
pseudoalcaligenes	0.10%
putida	0.37%
resinovorans	1.60%
rhodesiae	0.04%
sp.	0.11%
stutzeri	0.52%
syringae	0.01%
Parvibaculum	**2.22%**
lavamentivorans	2.22%
Shewanella	**2.20%**
oneidensis	0.01%
putrefaciens	2.01%
sp.	0.18%
Hyphomicrobium	**1.70%**
sp.	0.03%
vulgare	1.68%
Methylobacterium	**1.39%**
extorquens	0.20%
radiotolerans	0.01%
sp.	1.18%
Sphingopyxis	**1.36%**
baekryungensis	0.51%
bauzanensis	0.16%
italica	0.06%
macrogoltabida	0.09%
soli	0.31%
ummariensis	0.24%
Rhodopseudomonas	**1.19%**
palustris	1.19%
Pseudoxanthomonas	**1.14%**
mexicana	0.95%

Genus species	Percent
Pseudoxanthomonas	**1.14%**
sp.	0.20%
Acinetobacter	**1.04%**
baumannii	0.04%
lwoffii	0.03%
sp.	0.96%
Acidovorax	**0.89%**
ebreus	0.30%
sp.	0.59%
Bradyrhizobium	**0.80%**
elkanii	0.76%
japonicum	0.04%
Novosphingobium	**0.66%**
aromaticivorans	0.51%
sp.	0.15%
Bacterium	**0.64%**
alphaproteobacterium	0.64%
Gemmobacter	**0.50%**
aquatilis	0.50%
Azospirillum	**0.46%**
lipoferum	0.02%
oryzae	0.43%
Hyphomonas	**0.35%**
polymorpha	0.35%
Leifsonia	**0.29%**
sp.	0.29%
Rubrivivax	**0.26%**
gelatinosus	0.26%
Sphingobium	**0.25%**
baderi	0.13%
lactosutens	0.06%
sp.	0.06%
Mesorhizobium	**0.25%**
opportunistum	0.16%
sp.	0.09%
Salinibacterium	**0.22%**
sp.	0.22%

Table D-6 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-8, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Microbacterium	**0.19%**
lacticum	0.14%
resistens	0.04%
Sphingorhabdus	**0.16%**
flavimaris	0.05%
marina	0.03%
wooponensis	0.08%
Sulfuricurvum	**0.15%**
kujiense	0.15%
Stenotrophomonas	**0.14%**
maltophilia	0.14%
Xanthobacter	**0.14%**
flavus	0.12%
tagetidis	0.02%
Methylococcus	**0.14%**
capsulatus	0.14%
Achromobacter	**0.11%**
xylosoxidans	0.11%
Methyloversatilis	**0.09%**
discipulorum	0.09%
Agrobacterium	**0.07%**
tumefaciens	0.07%
Clavibacter	**0.07%**
michiganensis	0.07%
Dechlorosoma	**0.07%**
suillum	0.07%
Alicycliphilus	**0.07%**
denitrificans	0.07%
Janthinobacterium	**0.06%**
lividum	0.06%
Cupriavidus	**0.06%**
metallidurans	0.03%
necator	0.03%
Enterobacter	**0.06%**
cloacae	0.06%
Aurantimonas	**0.06%**
manganoxydans	0.06%

Genus species	Percent
Pelomonas	**0.06%**
saccharophila	0.06%
Xanthomonas	**0.05%**
axonopodis	0.05%
Pseudonocardia	**0.05%**
ailaonensis	0.02%
hydrocarbonoxydans	0.02%
spinosa	0.01%
Legionella	**0.05%**
drancourtii	0.03%
longbeachae	0.02%
Methylibium	**0.05%**
petroleiphilum	0.05%
Rhizobium	**0.05%**
sp.	0.05%
Carbophilus	**0.04%**
carboxidus	0.04%
Mycoplana	**0.03%**
dimorpha	0.03%
Bacillus	**0.03%**
niacini	0.01%
subtilis	0.00%
thuringiensis	0.02%
Janibacter	**0.03%**
hoylei	0.03%
Nitrobacter	**0.03%**
vulgaris	0.03%
Moraxella	**0.03%**
osloensis	0.03%
Isoptericola	**0.03%**
variabilis	0.03%
Nevskia	**0.03%**
ramosa	0.03%
Micavibrio	**0.03%**
aeruginosavorus	0.03%
Paracoccus	**0.03%**
sp.	0.03%

Genus species	Percent
Aeromonas	**0.03%**
veronii	0.03%
Geothrix	**0.02%**
fermentans	0.02%
Nocardia	**0.02%**
nova	0.02%
Curvibacter	**0.02%**
lanceolatus	0.02%
Lactobacillus	**0.02%**
salivarius	0.02%
Modestobacter	**0.02%**
marinus	0.02%
Turneriella	**0.02%**
parva	0.02%
Comamonas	**0.02%**
testosteroni	0.02%
Psychrobacter	**0.01%**
sp.	0.01%
Magnetospirillum	**0.01%**
gryphiswaldense	0.01%
Starkeya	**0.01%**
novella	0.01%
Nocardioides	**0.01%**
sp.	0.01%
Methylotenera	**0.01%**
mobilis	0.01%
Methylosinus	**0.01%**
trichosporium	0.01%
Limnobacter	**0.01%**
sp.	0.01%
Kocuria	**0.01%**
rosea	0.01%
Planomicrobium	**0.01%**
okeanokoites	0.01%
Serratia	**0.01%**
liquefaciens	0.01%

Table D-7. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-15, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Pseudoxanthomonas	**45.88%**
sp.	45.88%
Agrobacterium	**12.68%**
tumefaciens	12.68%
Bacillus	**11.04%**
cereus	0.19%
megaterium	2.80%
thuringiensis	8.04%
Mesorhizobium	**5.33%**
sp.	5.33%
Ochrobactrum	**4.64%**
anthropi	4.62%
pituitosum	0.02%
Sphingomonas	**4.62%**
leidyi	0.82%
melonis	0.01%
sp.	3.79%
Acinetobacter	**2.56%**
baumannii	0.71%
radioresistens	0.28%
sp.	1.57%
Microbacterium	**2.39%**
resistens	2.39%
Stenotrophomonas	**2.12%**
maltophilia	2.12%
Rhodoferax	**1.97%**
saidenbachensis	1.97%
Pseudomonas	**1.55%**
chloritidismutans	0.01%
putida	0.09%
resinovorans	1.45%
Clavibacter	**1.33%**
michiganensis	1.33%
Isoptericola	**1.20%**
variabilis	1.20%
Variovorax	**0.48%**
paradoxus	0.48%

Genus species	Percent
Propionibacterium	**0.47%**
sp.	0.47%
Alcaligenes	**0.40%**
aquatilis	0.40%
Hyphomicrobium	**0.35%**
vulgare	0.35%
Comamonas	**0.30%**
testosteroni	0.30%
Dietzia	**0.23%**
psychralcaliphila	0.23%
Thiobacillus	**0.13%**
thioparus	0.13%
Corynebacterium	**0.09%**
durum	0.04%
mycetoides	0.04%
Leucobacter	**0.03%**
salsicius	0.03%
Ralstonia	**0.03%**
solanacearum	0.03%
Methylocystis	**0.03%**
sp.	0.03%
Aquabacter	**0.02%**
spiritensis	0.02%
Sphingopyxis	**0.02%**
bauzanensis	0.02%
Caulobacter	**0.02%**
segnis	0.02%
Brucella	**0.02%**
melitensis	0.02%
Methylobacter	**0.01%**
luteus	0.01%
Carbophilus	**0.01%**
carboxidus	0.01%
Sinorhizobium	**0.01%**
arboris	0.01%
Rubrivivax	**0.01%**
gelatinosus	0.01%

Genus species	Percent
Hydrocarboniphaga	**0.01%**
effusa	0.01%

Table D-8. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-DMM-05, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Sphingopyxis	**39.45%**
baekryungensis	4.12%
bauzanensis	34.75%
macrogoltabida	0.07%
panaciterrae	0.02%
soli	0.48%
terrae	0.02%
Sphingomonas	**11.67%**
azotifigens	0.09%
echinoides	0.01%
leidyi	0.13%
melonis	7.30%
sp.	3.66%
starnbergensis	0.13%
wittichii	0.35%
Nocardioides	**8.56%**
sp.	8.56%
Xanthobacter	**5.02%**
flavus	5.02%
Rhodopseudomonas	**3.38%**
palustris	3.38%
Pseudoxanthomonas	**2.67%**
mexicana	0.05%
sp.	2.56%
spadix	0.06%
Carbophilus	**2.63%**
carboxidus	2.63%
Bradyrhizobium	**2.46%**
elkanii	1.68%
japonicum	0.78%
Sphingorhabdus	**2.33%**
flavimaris	0.86%
marina	0.17%
wooponensis	1.30%
Microbacterium	**1.97%**
lacticum	0.01%
resistens	1.88%

Genus species	Percent
Microbacterium	**1.97%**
sp.	0.04%
testaceum	0.04%
Pseudomonas	**1.94%**
alcaliphila	0.33%
brassicacearum	0.12%
entomophila	0.03%
fluorescens	0.20%
protegens	0.23%
putida	0.37%
resinovorans	0.41%
rhodesiae	0.09%
stutzeri	0.16%
syringae	0.01%
Acidovorax	**1.86%**
avenae	0.19%
citrulli	0.24%
ebreus	0.27%
sp.	1.16%
Caulobacter	**1.40%**
fusiformis	0.09%
segnis	0.04%
sp.	1.26%
Rhodoferax	**1.18%**
ferrireducens	0.02%
saidenbachensis	1.16%
Mycobacterium	**1.13%**
austroafricanum	0.19%
avium	0.20%
brumae	0.05%
chubuense	0.03%
engbaekii	0.20%
madagascariense	0.14%
mucogenicum	0.04%
senegalense	0.18%
sp.	0.07%
wolinskyi	0.03%

Genus species	Percent
Legionella	**1.08%**
drozanskii	0.06%
pneumophila	1.02%
Brevundimonas	**1.06%**
bacteroides	0.55%
subvibrioides	0.38%
vesicularis	0.13%
Frankia	**0.97%**
sp.	0.97%
Thiobacillus	**0.74%**
thioparus	0.74%
Intrasporangium	**0.60%**
calvum	0.60%
Bacillus	**0.60%**
firmus	0.03%
funiculus	0.09%
horikoshii	0.01%
lentus	0.02%
licheniformis	0.07%
megaterium	0.02%
niacini	0.01%
pseudofirmus	0.01%
sp.	0.05%
thuringiensis	0.28%
Hyphomicrobium	**0.54%**
denitrificans	0.04%
nitrativorans	0.06%
sp.	0.10%
vulgare	0.34%
Pseudonocardia	**0.48%**
hydrocarbonoxydans	0.48%
Salinibacterium	**0.44%**
sp.	0.44%
Variovorax	**0.40%**
paradoxus	0.40%
Nitrobacter	**0.38%**
vulgaris	0.36%

Table D-8 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-DMM-05, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Nitrobacter	**0.38%**
winogradskyi	0.01%
Agrobacterium	**0.31%**
rubi	0.05%
tumefaciens	0.26%
Desulfosporosinus	**0.28%**
meridiei	0.25%
orientis	0.03%
Ramlibacter	**0.26%**
tataouinensis	0.26%
Mesorhizobium	**0.26%**
loti	0.22%
opportunistum	0.03%
sp.	0.01%
Acinetobacter	**0.26%**
baumannii	0.17%
sp.	0.08%
Candidatus	**0.25%**
Nitrospira	0.23%
Protochlamydia	0.02%
Comamonas	**0.24%**
testosteroni	0.24%
Methylocystis	**0.23%**
sp.	0.23%
Methylocella	**0.22%**
silvestris	0.22%
Novosphingobium	**0.22%**
aromaticivorans	0.17%
sp.	0.05%
Pelomonas	**0.19%**
saccharophila	0.19%
Leptothrix	**0.16%**
cholodnii	0.16%
Janthinobacterium	**0.16%**
lividum	0.13%
sp.	0.03%

Genus species	Percent
Prosthecomicrobium	**0.15%**
pneumaticum	0.15%
Arthrobacter	**0.15%**
phenanthrenivorans	0.15%
Pedobacter	**0.13%**
heparinus	0.09%
sp.	0.04%
Sphingobium	**0.12%**
lactosutens	0.12%
Geothrix	**0.10%**
fermentans	0.10%
Gordonia	**0.08%**
amicalis	0.03%
sp.	0.05%
Sulfuritalea	**0.07%**
hydrogenivorans	0.07%
Rhizobium	**0.07%**
leguminosarum	0.05%
sp.	0.02%
Paracoccus	**0.06%**
aminophilus	0.06%
Turneriella	**0.06%**
parva	0.06%
Clavibacter	**0.06%**
michiganensis	0.06%
Luteimonas	**0.05%**
mephitis	0.05%
Azospirillum	**0.05%**
lipoferum	0.01%
oryzae	0.03%
Phenylobacterium	**0.05%**
zucineum	0.05%
Nitrosovibrio	**0.05%**
tenuis	0.05%
Alcaligenes	**0.04%**
aquatilis	0.04%
Streptomyces	**0.04%**
coerulescens	0.04%

Genus species	Percent
Gemmatimonas	**0.04%**
phototrophica	0.04%
Mycoplana	**0.04%**
dimorpha	0.04%
Hydrogenophaga	**0.04%**
flava	0.04%
Dyadobacter	**0.04%**
fermentans	0.04%
Cellulomonas	**0.03%**
fimi	0.02%
iranensis	0.01%
Methylibium	**0.03%**
petroleiphilum	0.03%
Dechlorosoma	**0.03%**
suillum	0.03%
Stenotrophomonas	**0.03%**
maltophilia	0.03%
Paucibacter	**0.03%**
toxinivorans	0.03%
Afipia	**0.03%**
sp.	0.03%
Alicycliphilus	**0.03%**
denitrificans	0.03%
Agrococcus	**0.02%**
pavilionensis	0.02%
Methylobacterium	**0.02%**
sp.	0.02%
Microvirga	**0.02%**
subterranea	0.02%
Methylotenera	**0.02%**
mobilis	0.02%
Marmoricola	**0.02%**
sp.	0.02%
Xanthomonas	**0.02%**
axonopodis	0.02%
Sulfuricurvum	**0.02%**
kujiense	0.02%

Table D-8 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-DMM-05, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Herbaspirillum	**0.02%**
rubrisubalbicans	0.02%
Shewanella	**0.02%**
putrefaciens	0.02%
Sinorhizobium	**0.02%**
arboris	0.02%
Sporomusa	**0.02%**
paucivorans	0.02%
Ralstonia	**0.02%**
solanacearum	0.02%
Leifsonia	**0.01%**
sp.	0.01%
Knoellia	**0.01%**
subterranea	0.01%
Ochrobactrum	**0.01%**
pituitosum	0.01%
Parvibaculum	**0.01%**
lavamentivorans	0.01%
Aquincola	**0.01%**
tertiaricarbonis	0.01%
Verrucosispora	**0.01%**
gifhornensis	0.01%
Propionibacterium	**0.01%**
sp.	0.01%
Curtobacterium	**0.01%**
plantarum	0.01%
Polaromonas	**0.01%**
sp.	0.01%
Serratia	**0.01%**
proteamaculans	0.01%
Modestobacter	**0.01%**
marinus	0.01%

Table D-9. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-03, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Rhodoferax	**2.13%**
saidenbachensis	2.13%
Acinetobacter	**2.01%**
baumannii	0.11%
sp.	1.90%
Hydrocarboniphaga	**1.86%**
effusa	1.86%
Comamonas	**1.50%**
testosteroni	1.50%
Zavarzinia	**1.43%**
compransoris	1.43%
Acidovorax	**1.25%**
avenae	0.02%
citrulli	0.02%
sp.	1.20%
Bradyrhizobium	**1.22%**
elkanii	0.80%
japonicum	0.42%
Brevundimonas	**0.80%**
bacteroides	0.37%
bullata	0.13%
subvibrioides	0.28%
vesicularis	0.02%
Methylocystis	**0.80%**
sp.	0.80%
Salinibacterium	**0.66%**
sp.	0.66%
Rubrivivax	**0.61%**
gelatinosus	0.61%
Ralstonia	**0.59%**
eutropha	0.01%
pickettii	0.49%
syzygii	0.09%
Pseudoxanthomonas	**0.51%**
mexicana	0.22%
sp.	0.29%

Genus species	Percent
Mycobacterium	**0.49%**
abscessus	0.45%
diernhoferi	0.03%
rhodesiae	0.01%
Nitrobacter	**0.45%**
vulgaris	0.45%
Novosphingobium	**0.44%**
aromaticivorans	0.44%
Sphingomonas	**0.39%**
azotifigens	0.02%
leidyi	0.08%
melonis	0.18%
phyllosphaerae	0.03%
sp.	0.05%
wittichii	0.04%
Hyphomicrobium	**0.36%**
sp.	0.07%
vulgare	0.30%
Pseudonocardia	**0.34%**
hydrocarbonoxydans	0.34%
Agrococcus	**0.33%**
pavilionensis	0.33%
Methylibium	**0.32%**
petroleiphilum	0.32%
Agrobacterium	**0.30%**
tumefaciens	0.30%
Mesorhizobium	**0.26%**
opportunistum	0.03%
sp.	0.23%
Azospirillum	**0.23%**
lipoferum	0.09%
oryzae	0.14%
Aromatoleum	**0.23%**
aromaticum	0.23%
Clavibacter	**0.21%**
michiganensis	0.21%

Genus species	Percent
Variovorax	**0.20%**
paradoxus	0.20%
Sphingorhabdus	**0.19%**
flavimaris	0.03%
marina	0.09%
wooponensis	0.07%
Rhodopseudomonas	**0.18%**
palustris	0.18%
Shewanella	**0.17%**
putrefaciens	0.15%
sp.	0.02%
Achromobacter	**0.15%**
xylosoxidans	0.15%
Thiobacillus	**0.15%**
thioparus	0.15%
Sulfuritalea	**0.13%**
hydrogenivorans	0.13%
Methylococcus	**0.11%**
capsulatus	0.11%
Carbophilus	**0.11%**
carboxidus	0.11%
Xanthomonas	**0.11%**
axonopodis	0.11%
Enterobacter	**0.09%**
aerogenes	0.00%
cloacae	0.09%
Bacillus	**0.09%**
pseudofirmus	0.01%
subterraneus	0.03%
thuringiensis	0.05%
Stenotrophomonas	**0.07%**
maltophilia	0.07%
Mycoplana	**0.05%**
dimorpha	0.05%
Methyloversatilis	**0.05%**
discipulorum	0.05%

Table D-9 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-03, as Evaluated by 16S Sequence Analysis, Sampling Event 1

Genus species	Percent
Gemmobacter	**0.05%**
aquatilis	0.05%
Pelomonas	**0.05%**
saccharophila	0.05%
Aquabacter	**0.05%**
spiritensis	0.05%
Pedobacter	**0.05%**
heparinus	0.05%
Modestobacter	**0.04%**
marinus	0.04%
Verrucomicrobium	**0.04%**
spinosum	0.04%
Alicycliphilus	**0.04%**
denitrificans	0.04%
Hyphomonas	**0.04%**
polymorpha	0.04%
Nevskia	**0.04%**
ramosa	0.04%
Sulfuricurvum	**0.03%**
kujiense	0.03%
Microbacterium	**0.03%**
lacticum	0.02%
resistens	0.01%
Delftia	**0.03%**
acidovorans	0.03%
Parvibaculum	**0.03%**
lavamentivorans	0.03%
Leifsonia	**0.02%**
sp.	0.02%
Aurantimonas	**0.02%**
manganoxydans	0.02%
Micavibrio	**0.02%**
aeruginosavorus	0.02%
Malikia	**0.02%**
spinosa	0.02%

Genus species	Percent
Staphylococcus	**0.02%**
aureus	0.02%
uncultured	**0.02%**
Sinorhizobium	0.02%
Ramlibacter	**0.02%**
tataouinensis	0.02%
Turneriella	**0.02%**
parva	0.02%
Limnobacter	**0.01%**
sp.	0.01%
Burkholderia	**0.01%**
vietnamiensis	0.01%

Table D-15. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-MM-07, as Evaluated by 16S Sequence Analysis, Optional Sampling Event

Genus species	Percent
Pseudoxanthomonas	**49.53%**
sp.	49.53%
Agrobacterium	**19.86%**
tumefaciens	19.86%
Bacillus	**8.48%**
cereus	0.24%
thuringiensis	8.24%
Sphingomonas	**8.01%**
echinoides	0.08%
leidyi	0.16%
sp.	7.77%
Bradyrhizobium	**4.16%**
japonicum	4.16%
Methanosaeta	**2.59%**
concilii	2.59%
Pseudomonas	**2.51%**
geniculata	2.51%
Lysinibacillus	**1.57%**
sphaericus	1.57%
Mesorhizobium	**1.26%**
sp.	1.26%
Thauera	**0.71%**
sp.	0.71%
Methylocystis	**0.55%**
sp.	0.55%
Delftia	**0.39%**
sp.	0.39%
Ochrobactrum	**0.16%**
anthropi	0.16%
Sulfuricurvum	**0.08%**
kujiense	0.08%
Magnetospirillum	**0.08%**
gryphiswaldense	0.08%
Clostridium	**0.08%**
beijerinckii	0.08%

Table D-16. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-11, as Evaluated by 16S Sequence Analysis, Optional Sampling Event

Genus species	Percent
Pseudoxanthomonas	**19.42%**
mexicana	1.16%
sp.	18.26%
Sphingomonas	**16.96%**
echinoides	0.14%
leidyi	0.87%
melonis	12.90%
sp.	3.04%
Sphingobium	**12.90%**
baderi	1.30%
lactosutens	11.59%
Stenotrophomonas	**11.74%**
maltophilia	11.74%
Brevundimonas	**7.54%**
bacteroides	5.80%
vesicularis	1.74%
Agrobacterium	**6.38%**
tumefaciens	6.38%
Bradyrhizobium	**6.09%**
japonicum	6.09%
Methylocystis	**3.48%**
sp.	3.48%
Rhizobium	**3.04%**
leguminosarum	3.04%
Pseudomonas	**3.04%**
geniculata	2.03%
resinovorans	1.01%
Sphingopyxis	**2.46%**
sp.	2.17%
terrae	0.14%
ummariensis	0.14%
Sulfuricurvum	**1.30%**
kujiense	1.30%
Mesorhizobium	**1.30%**
sp.	1.30%

Genus species	Percent
Acinetobacter	**1.16%**
baumannii	0.14%
sp.	1.01%
Thauera	**1.01%**
sp.	1.01%
Bacillus	**1.01%**
thuringiensis	1.01%
Enterobacter	**0.29%**
cloacae	0.29%
Ochrobactrum	**0.29%**
anthropi	0.29%
Methanosaeta	**0.14%**
concilii	0.14%
Caulobacter	**0.14%**
segnis	0.14%
Methylosinus	**0.14%**
trichosporium	0.14%
Alcaligenes	**0.14%**
aquatilis	0.14%

Table D-12. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-15, as Evaluated by 16S Sequence Analysis, Optional Sampling Event

Genus species	Percent
Pseudoxanthomonas	**65.96%**
sp.	65.96%
Agrobacterium	**11.93%**
tumefaciens	11.93%
Sphingomonas	**10.18%**
leidyi	6.67%
melonis	0.35%
paucimobilis	3.16%
Ochrobactrum	**2.46%**
anthropi	2.46%
Bacillus	**2.46%**
pseudofirmus	0.35%
sp.	1.05%
thuringiensis	1.05%
Escherichia	**2.11%**
coli	2.11%
Mesorhizobium	**1.40%**
sp.	1.40%
Hyphomicrobium	**1.05%**
vulgare	1.05%
Acidovorax	**0.70%**
ebreus	0.70%
Thermus	**0.35%**
islandicus	0.35%
Sulfuricurvum	**0.35%**
kujiense	0.35%
Carbophilus	**0.35%**
carboxidus	0.35%
Microbacterium	**0.35%**
sp.	0.35%
Methanosaeta	**0.35%**
concilii	0.35%

Table D-13. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-01R, as Evaluated by 16S Sequence Analysis, Optional Sampling Event

Genus species	Percent
Methylibium	**34.75%**
petroleiphilum	34.75%
Mycobacterium	**13.81%**
austroafricanum	1.97%
chlorophenolicum	0.46%
diernhoferi	1.67%
madagascariense	1.97%
mucogenicum	1.37%
rhodesiae	3.49%
sphagni	1.82%
wolinskyi	1.06%
Pseudoxanthomonas	**12.75%**
sp.	12.75%
Sphingopyxis	**7.13%**
baekryungensis	1.21%
panaciterrae	5.92%
Sphingomonas	**4.70%**
leidyi	2.12%
melonis	0.15%
sp.	2.28%
wittichii	0.15%
Hyphomicrobium	**4.25%**
vulgare	4.25%
Bacillus	**4.10%**
cereus	0.15%
thuringiensis	3.95%
Agrobacterium	**3.49%**
tumefaciens	3.49%
Mesorhizobium	**2.88%**
sp.	2.88%
Acinetobacter	**1.97%**
sp.	1.97%
Pelomonas	**1.67%**
saccharophila	1.67%
Aquincola	**1.52%**
tertiaricarbonis	1.52%

Genus species	Percent
Pseudomonas	**1.52%**
aeruginosa	0.15%
alcaliphila	0.15%
entomophila	0.15%
pseudoalcaligenes	0.15%
putida	0.91%
Microbacterium	**1.21%**
sp.	1.06%
testaceum	0.15%
Xanthobacter	**0.91%**
agilis	0.30%
tagetidis	0.61%
Sinorhizobium	**0.46%**
fredii	0.46%
Methanosaeta	**0.46%**
concilii	0.46%
Hydrogenophaga	**0.46%**
flava	0.46%
Rubrivivax	**0.46%**
gelatinosus	0.46%
Sulfuricurvum	**0.30%**
kujiense	0.30%
Caulobacter	**0.30%**
segnis	0.30%
Methylosinus	**0.30%**
trichosporium	0.30%
Ramlibacter	**0.15%**
tataouinensis	0.15%
Thauera	**0.15%**
sp.	0.15%
Comamonadaceae	**0.15%**
bacterium	0.15%
Methylocystis	**0.15%**
sp.	0.15%

Table D-14. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-RW-07, as Evaluated by 16S Sequence Analysis, Optional Sampling Event

Genus species	Percent
Sulfuricurvum	**81.05%**
kujiense	81.05%
Magnetospirillum	**3.37%**
gryphiswaldense	3.37%
Hydrogenophaga	**1.87%**
flava	1.87%
Gemmobacter	**1.69%**
aquatilis	1.69%
Methylocystis	**1.40%**
sp.	1.40%
Roseovarius	**1.39%**
sp.	0.15%
tolerans	1.24%
Hyphomicrobium	**1.24%**
denitrificans	0.05%
sp.	0.06%
vulgare	1.13%
Xanthobacter	**1.03%**
flavus	1.03%
Mycobacterium	**1.00%**
austroafricanum	0.32%
brumae	0.04%
madagascariense	0.17%
rhodesiae	0.45%
senegalense	0.03%
Sphingomonas	**0.62%**
wittichii	0.62%
Sphingopyxis	**0.56%**
baekryungensis	0.49%
sp.	0.07%
Methylosinus	**0.43%**
trichosporium	0.43%
Sphingobium	**0.32%**
baderi	0.32%
Bacterium	**0.30%**
alphaproteobacterium	0.30%

Genus species	Percent
Zavarzinia	**0.27%**
compransoris	0.27%
Parvibaculum	**0.26%**
lavamentivorans	0.26%
Hyphomonas	**0.24%**
adhaerens	0.04%
polymorpha	0.19%
Rhodobacter	**0.22%**
azotoformans	0.03%
capsulatus	0.11%
sphaeroides	0.08%
Alicycliphilus	**0.20%**
denitrificans	0.20%
Novosphingobium	**0.20%**
aromaticivorans	0.10%
sp.	0.10%
Aeromonas	**0.18%**
veronii	0.18%
Thiobacillus	**0.14%**
thioparus	0.14%
Simkania	**0.13%**
negevensis	0.13%
Burkholderia	**0.12%**
cenocepacia	0.12%
Sphaerochaeta	**0.12%**
globosa	0.12%
Sinorhizobium	**0.11%**
arboris	0.05%
fredii	0.07%
Pseudonocardia	**0.11%**
spinosispora	0.11%
Dehalogenimonas	**0.10%**
lykanthroporepellens	0.10%
Shewanella	**0.10%**
putrefaciens	0.07%
sp.	0.03%

Genus species	Percent
Carbophilus	**0.09%**
carboxidus	0.09%
Methylomonas	**0.08%**
rubra	0.08%
Thiomonas	**0.07%**
intermedia	0.07%
Rhizobium	**0.07%**
aggregatum	0.05%
sp.	0.02%
Parachlamydia	**0.06%**
acanthamoebae	0.06%
Bradyrhizobium	**0.06%**
japonicum	0.06%
Petrotoga	**0.06%**
mobilis	0.06%
Pseudoxanthomonas	**0.05%**
spadix	0.05%
Mesorhizobium	**0.05%**
opportunistum	0.02%
sp.	0.03%
Acidocella	**0.05%**
aminolytica	0.05%
Agrobacterium	**0.05%**
tumefaciens	0.05%
Salinibacterium	**0.05%**
sp.	0.05%
Antarctobacter	**0.05%**
heliothermus	0.05%
Mesotoga	**0.05%**
prima	0.05%
Bacillus	**0.04%**
cereus	0.00%
thuringiensis	0.04%
Treponema	**0.04%**
caldarium	0.04%

Table D-14 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-RW-07, as Evaluated by 16S Sequence Analysis, Optional Sampling Event

Genus species	Percent
Aquabacter	**0.04%**
spiritensis	0.04%
uncultured	**0.04%**
Pseudonocardia	0.04%
Methylibium	**0.04%**
petroleiphilum	0.04%
Beijerinckia	**0.03%**
indica	0.03%
Geobacter	**0.03%**
lovleyi	0.03%
Leptonema	**0.03%**
illini	0.03%
Rubrivivax	**0.02%**
gelatinosus	0.02%
Methylocella	**0.02%**
silvestris	0.02%
Muricauda	**0.02%**
ruestringensis	0.02%
Comamonadaceae	**0.02%**
bacterium	0.02%
Caulobacter	**0.02%**
segnis	0.02%
Serratia	**0.01%**
marcescens	0.01%
Actinoplanes	**0.01%**
sp.	0.01%
Pseudomonas	**0.01%**
resinovorans	0.01%
Oceanobacillus	**0.00%**
iheyensis	0.00%

Table D-15. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-07R, as Evaluated by 16S Sequence Analysis, Optional Sampling Event

Genus species	Percent
Sulfuricurvum	**33.97%**
kujiense	33.97%
Aeromonas	**16.43%**
hydrophila	0.06%
veronii	16.37%
Rhodobacter	**12.61%**
azotoformans	0.03%
capsulatus	12.54%
sphaeroides	0.04%
Hyphomonas	**4.72%**
adhaerens	0.02%
polymorpha	4.70%
Hydrogenophaga	**4.60%**
flava	4.60%
Shewanella	**4.09%**
oneidensis	0.02%
putrefaciens	3.38%
sp.	0.68%
Clostridium	**2.74%**
bifermentans	1.57%
botulinum	0.31%
propionicum	0.08%
saccharolyticum	0.03%
subterminale	0.62%
tertium	0.04%
viride	0.09%
Bacillus	**1.95%**
cereus	0.06%
thuringiensis	1.90%
Mycobacterium	**1.91%**
austroafricanum	0.20%
brumae	0.09%
chlorophenolicum	0.08%
diernhoferi	0.07%
madagascariense	0.09%
mucogenicum	0.04%

Genus species	Percent
Mycobacterium	**1.91%**
rhodesiae	1.29%
senegalense	0.02%
triplex	0.02%
wolinskyi	0.02%
Xanthobacter	**1.52%**
agilis	0.04%
flavus	1.44%
tagetidis	0.04%
Pseudomonas	**1.28%**
aeruginosa	0.02%
pseudoalcaligenes	0.48%
putida	0.17%
resinovorans	0.59%
sp.	0.02%
Magnetospirillum	**1.22%**
gryphiswaldense	1.16%
magneticum	0.07%
Hyphomicrobium	**1.16%**
denitrificans	0.02%
sp.	0.10%
vulgare	1.04%
Thauera	**0.92%**
sp.	0.92%
Gemmobacter	**0.81%**
aquatilis	0.81%
Methylocystis	**0.81%**
sp.	0.81%
Roseovarius	**0.67%**
sp.	0.10%
tolerans	0.57%
Sphingomonas	**0.61%**
melonis	0.03%
wittichii	0.58%
Bdellovibrio	**0.60%**
bacteriovorus	0.60%

Genus species	Percent
Azoarcus	**0.52%**
sp.	0.52%
Acinetobacter	**0.49%**
baumannii	0.09%
sp.	0.39%
venetianus	0.01%
Sphingopyxis	**0.40%**
baekryungensis	0.32%
macrogoltabida	0.01%
soli	0.03%
sp.	0.04%
Desulfotomaculum	**0.39%**
guttoideum	0.39%
Cupriavidus	**0.36%**
necator	0.36%
Methylibium	**0.35%**
petroleiphilum	0.35%
Agrobacterium	**0.28%**
tumefaciens	0.28%
Aromatoleum	**0.26%**
aromaticum	0.26%
Comamonas	**0.24%**
testosteroni	0.24%
Microbacterium	**0.24%**
lacticum	0.14%
resistens	0.02%
sp.	0.02%
testaceum	0.06%
Novosphingobium	**0.22%**
aromaticivorans	0.06%
sp.	0.17%
Sphingobium	**0.22%**
baderi	0.18%
lactosutens	0.05%
Ochrobactrum	**0.20%**
anthropi	0.20%

Table D-15 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-07R, as Evaluated by 16S Sequence Analysis, Optional Sampling Event

Genus species	Percent
Methylosinus	**0.19%**
trichosporium	0.19%
Rubrivivax	**0.17%**
gelatinosus	0.17%
Parvibaculum	**0.15%**
lavamentivorans	0.15%
Zavarzinia	**0.14%**
compransoris	0.14%
Bacterium	**0.12%**
alphaproteobacterium	0.12%
Carbophilus	**0.12%**
carboxidus	0.12%
Starkeya	**0.12%**
novella	0.12%
Arcobacter	**0.11%**
sp.	0.11%
Pseudonocardia	**0.10%**
ailaonensis	0.01%
oroxyli	0.01%
spinosispora	0.06%
yuanmonensis	0.02%
Azospirillum	**0.10%**
lipoferum	0.02%
sp.	0.09%
Thiobacillus	**0.10%**
thioparus	0.10%
Sinorhizobium	**0.09%**
arboris	0.03%
fredii	0.06%
Desulfomicrobium	**0.09%**
baculatum	0.09%
Rhizobium	**0.08%**
aggregatum	0.02%
sp.	0.06%
Bradyrhizobium	**0.08%**
elkanii	0.02%

Genus species	Percent
Bradyrhizobium	**0.08%**
japonicum	0.07%
Comamonadaceae	**0.07%**
bacterium	0.07%
Pseudoxanthomonas	**0.07%**
mexicana	0.03%
spadix	0.04%
Sphaerochaeta	**0.07%**
globosa	0.07%
Leptonema	**0.06%**
illini	0.06%
Caulobacter	**0.06%**
fusiformis	0.01%
segnis	0.03%
sp.	0.02%
Vogesella	**0.06%**
indigofera	0.06%
Alicycliphilus	**0.05%**
denitrificans	0.05%
Simkania	**0.04%**
negevensis	0.04%
Salinibacterium	**0.04%**
sp.	0.04%
Robiginitalea	**0.04%**
biformata	0.04%
Dehalogenimonas	**0.04%**
lykanthroporepellens	0.04%
Leucobacter	**0.04%**
salsicius	0.04%
Elizabethkingia	**0.04%**
meningoseptica	0.04%
Methanosaeta	**0.04%**
concilii	0.04%
Variovorax	**0.04%**
paradoxus	0.04%

Genus species	Percent
Methylomonas	**0.04%**
rubra	0.04%
Acidovorax	**0.03%**
sp.	0.03%
Methylocella	**0.03%**
silvestris	0.03%
Mesotoga	**0.03%**
prima	0.03%
Paraburkholderia	**0.03%**
phenoliruptrix	0.03%
Thiomonas	**0.03%**
intermedia	0.03%
Burkholderia	**0.03%**
cenocepacia	0.03%
Brevundimonas	**0.03%**
bacteroides	0.03%
Petrotoga	**0.03%**
mobilis	0.03%
Methylobacterium	**0.03%**
sp.	0.03%
Beijerinckia	**0.02%**
indica	0.02%
Mesorhizobium	**0.02%**
sp.	0.02%
Rhodoferax	**0.02%**
antarcticus	0.02%
Acidocella	**0.02%**
aminolytica	0.02%
Antarctobacter	**0.02%**
heliothermus	0.02%
Treponema	**0.02%**
caldarium	0.02%
Nocardia	**0.02%**
neocaledoniensis	0.02%

Table D-15 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-07R, as Evaluated by 16S Sequence Analysis, Optional Sampling Event

Genus species	Percent
Aquincola	**0.02%**
tertiaricarbonis	0.02%
Parachlamydia	**0.02%**
acanthamoebae	0.02%
Mycoplana	**0.02%**
dimorpha	0.02%
Aquabacter	**0.02%**
spiritensis	0.02%
Dechlorosoma	**0.02%**
suillum	0.02%
Nocardioides	**0.02%**
sp.	0.02%
Cellulomonas	**0.01%**
iranensis	0.01%
Serratia	**0.01%**
marcescens	0.01%
Legionella	**0.01%**
longbeachae	0.01%
Stenotrophomonas	**0.01%**
maltophilia	0.01%
Agrococcus	**0.01%**
pavilionensis	0.01%
Geobacter	**0.01%**
lovleyi	0.01%
Phaeospirillum	**0.01%**
molischianum	0.01%
Rhodococcus	**0.01%**
erythropolis	0.01%
Williamsia	**0.01%**
sp.	0.01%
Chryseobacterium	**0.01%**
sp.	0.01%
Streptomyces	**0.01%**
venezuelae	0.01%

Table D-16. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-23, as Evaluated by 16S Sequence Analysis, Optional Sampling Event

Genus species	Percent
Agrobacterium	**31.37%**
tumefaciens	31.37%
Bacillus	**15.69%**
thuringiensis	15.69%
Salmonella	**13.73%**
enterica	13.73%
Pseudoxanthomonas	**11.76%**
sp.	11.76%
Sulfuricurvum	**7.84%**
kujiense	7.84%
Sphingomonas	**5.88%**
melonis	1.96%
sp.	3.92%
Sphingopyxis	**3.92%**
indica	3.92%
Enterobacter	**3.92%**
cloacae	3.92%
Aeromonas	**1.96%**
veronii	1.96%
Hyphomonas	**1.96%**
polymorpha	1.96%
Methylocystis	**1.96%**
sp.	1.96%

Table D-17. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-39, as Evaluated by 16S Sequence Analysis, Optional Sampling Event

Genus species	Percent
Methanosaeta	**55.34%**
concilii	55.34%
Thauera	**22.30%**
sp.	22.30%
Methylocystis	**15.97%**
sp.	15.97%
Sulfuritalea	**1.17%**
hydrogenivorans	1.17%
Aeromonas	**0.81%**
hydrophila	0.80%
veronii	0.01%
Aromatoleum	**0.56%**
aromaticum	0.56%
Hyphomicrobium	**0.53%**
denitrificans	0.11%
sp.	0.05%
vulgare	0.36%
Geobacter	**0.47%**
lovleyi	0.16%
metallireducens	0.31%
Magnetospirillum	**0.42%**
gryphiswaldense	0.42%
Clostridium	**0.39%**
beijerinckii	0.31%
botulinum	0.03%
saccharobutylicum	0.01%
septicum	0.05%
Mycobacterium	**0.26%**
mucogenicum	0.26%
Carbophilus	**0.24%**
carboxidus	0.24%
Methylomonas	**0.21%**
rubra	0.21%
Gemmobacter	**0.15%**
aquatilis	0.15%

Genus species	Percent
Methanoculleus	**0.14%**
marisnigri	0.14%
Sphaerochaeta	**0.13%**
globosa	0.13%
Xanthobacter	**0.08%**
agilis	0.03%
flavus	0.05%
Sphingobium	**0.08%**
baderi	0.02%
lactosutens	0.06%
Sinorhizobium	**0.07%**
arboris	0.01%
fredii	0.05%
Pseudomonas	**0.06%**
balearica	0.02%
putida	0.01%
stutzeri	0.04%
Mesotoga	**0.06%**
prima	0.06%
Sulfuricurvum	**0.05%**
kujiense	0.05%
Sphingomonas	**0.05%**
melonis	0.02%
wittichii	0.03%
Rhizobium	**0.04%**
sp.	0.04%
Sphingopyxis	**0.04%**
macrogoltabida	0.04%
Dehalogenimonas	**0.03%**
lykanthroporepellens	0.03%
Mahella	**0.03%**
australiensis	0.03%
Hydrogenophaga	**0.03%**
flava	0.03%
Novosphingobium	**0.03%**
aromaticivorans	0.03%

Genus species	Percent
Pseudonocardia	**0.03%**
saturnea	0.03%
Bradyrhizobium	**0.03%**
japonicum	0.03%
Methylibium	**0.02%**
petroleiphilum	0.02%
Agrobacterium	**0.02%**
tumefaciens	0.02%
Desulfosporosinus	**0.02%**
orientis	0.02%
Arcobacter	**0.02%**
sp.	0.02%
Limnobacter	**0.01%**
sp.	0.01%
Acidovorax	**0.01%**
sp.	0.01%
Rhodobacter	**0.01%**
capsulatus	0.01%
Streptomyces	**0.01%**
venezuelae	0.01%
Nocardioides	**0.01%**
sp.	0.01%
alpha	**0.01%**
proteobacterium	0.01%
Dehalobacter	**0.01%**
sp.	0.01%
Treponema	**0.01%**
caldarium	0.01%
Salmonella	**0.01%**
enterica	0.01%

Table D-18. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-MM-07, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Acidovorax	**0.28%**
sp.	0.28%
Agrobacterium	**3.93%**
tumefaciens	3.93%
Alishewanella	**0.28%**
aestuarii	0.28%
Aromatoleum	**2.81%**
aromaticum	2.81%
Bacillus	**13.20%**
cereus	0.56%
subterraneus	0.56%
thuringiensis	12.08%
Bradyrhizobium	**0.28%**
elkanii	0.28%
Cupriavidus	**1.40%**
metallidurans	0.56%
necator	0.84%
Dechloromonas	**0.28%**
aromatica	0.28%
Geobacillus	**0.28%**
sp.	0.28%
Geobacter	**0.56%**
metallireducens	0.56%
Herminiimonas	**0.84%**
arsenicoxydans	0.84%
Hyphomicrobium	**0.56%**
denitrificans	0.56%
Mesorhizobium	**0.56%**
sp.	0.56%
Methanosaeta	**0.56%**
concilii	0.56%
Methylobacterium	**0.28%**
sp.	0.28%
Methylocystis	**0.28%**
sp.	0.28%
Methylomonas	**1.12%**
rubra	1.12%

Genus species	Percent
Paracoccus	**0.28%**
aminophilus	0.28%
Propionibacterium	**0.28%**
sp.	0.28%
Pseudomonas	**3.37%**
balearica	0.56%
chloritidismutans	0.28%
entomophila	0.28%
fluorescens	0.28%
putida	1.12%
sp.	0.28%
stutzeri	0.56%
Pseudoxanthomonas	**26.40%**
sp.	26.40%
Rubrivivax	**0.56%**
gelatinosus	0.56%
Sphingobium	**3.09%**
baderi	2.53%
lactosutens	0.56%
Sphingomonas	**1.97%**
paucimobilis	1.40%
sp.	0.56%
Streptococcus	**3.93%**
tigurinus	3.93%
Sulfuricurvum	**19.66%**
kujiense	19.66%
Sulfuritalea	**0.56%**
hydrogenivorans	0.56%
Thauera	**0.28%**
sp.	0.28%
Thiobacillus	**1.12%**
thioparus	1.12%
Vogesella	**10.96%**
indigofera	10.96%
Grand Total	**100.00%**

Table D-19. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-MM-08, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Acidovorax	**0.14%**
citrulli	0.08%
sp.	0.07%
Acinetobacter	**0.03%**
sp.	0.03%
Aromatoleum	**0.01%**
aromaticum	0.01%
Azoarcus	**0.03%**
sp.	0.03%
Bacillus	**0.02%**
thuringiensis	0.02%
Bradyrhizobium	**0.08%**
elkanii	0.08%
Comamonas	**0.01%**
testosteroni	0.01%
Cupriavidus	**1.05%**
metallidurans	0.35%
necator	0.70%
Dechlorosoma	**0.02%**
suillum	0.02%
Desulfomonile	**0.01%**
tiedjei	0.01%
Desulfosporosinus	**0.19%**
meridiei	0.19%
Enterobacter	**0.04%**
cloacae	0.04%
Geobacter	**0.12%**
bemidjiensis	0.12%
Hyphomicrobium	**0.13%**
denitrificans	0.02%
sp.	0.12%
Legionella	**0.02%**
drozanskii	0.02%
Magnetospirillum	**0.78%**
gryphiswaldense	0.78%
Mesorhizobium	**0.01%**
sp.	0.01%

Genus species	Percent
Methylibium	**0.13%**
petroleiphilum	0.13%
Methylocystis	**0.02%**
sp.	0.02%
Methyloversatilis	**0.03%**
discipulorum	0.03%
Microbacterium	**0.02%**
resistens	0.02%
Mycobacterium	**0.08%**
avium	0.01%
marinum	0.07%
Mycoplana	**0.01%**
dimorpha	0.01%
Phenylobacterium	**0.01%**
zucineum	0.01%
Pseudomonas	**2.45%**
balearica	0.17%
fluorescens	0.12%
mendocina	0.03%
monteilii	0.03%
putida	1.02%
resinovorans	0.52%
sp.	0.45%
stutzeri	0.11%
syringae	0.01%
Pseudoxanthomonas	**7.57%**
mexicana	0.02%
sp.	7.56%
Ramlibacter	**0.03%**
tataouinensis	0.03%
Rhodoferax	**0.04%**
fermentans	0.03%
saidenbachensis	0.01%
Rubrivivax	**0.42%**
gelatinosus	0.42%
Sphingobium	**0.08%**
baderi	0.04%

Genus species	Percent
Sphingobium	**0.08%**
indicum	0.01%
lactosutens	0.03%
Sphingomonas	**0.04%**
melonis	0.01%
sp.	0.01%
wittichii	0.01%
Sphingopyxis	**0.02%**
soli	0.02%
Sphingorhabdus	**0.23%**
flavimaris	0.20%
wooponensis	0.03%
Sulfuricurvum	**80.66%**
kujiense	80.66%
Sulfuritalea	**0.03%**
hydrogenivorans	0.03%
Thiobacillus	**4.01%**
thioparus	4.01%
Vogesella	**1.39%**
indigofera	1.39%
Xanthobacter	**0.03%**
flavus	0.03%
Grand Total	**100.00%**

Table D-17. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-11, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Pseudoxanthomonas	**41.69%**
sp.	41.69%
Methylocystis	**9.57%**
sp.	9.57%
Agrobacterium	**9.44%**
tumefaciens	9.44%
Pseudomonas	**8.01%**
aeruginosa	2.03%
fluorescens	1.30%
pseudoalcaligenes	0.33%
putida	4.12%
resinovorans	0.07%
rhodesiae	0.13%
stutzeri	0.03%
Bacillus	**7.28%**
anthracis	0.10%
cereus	0.07%
horikoshii	0.47%
pseudofirmus	0.17%
sp.	0.03%
thuringiensis	6.45%
Vogesella	**4.82%**
indigofera	4.82%
Sphingomonas	**4.29%**
leidyi	0.66%
melonis	0.03%
sp.	3.09%
wittichii	0.50%
Aromatoleum	**3.36%**
aromaticum	3.36%
Mesorhizobium	**1.63%**
sp.	1.63%
Methylibium	**1.33%**
petroleiphilum	1.33%
Methylomonas	**1.06%**
rubra	1.06%

Genus species	Percent
Methanosaeta	**1.00%**
concilii	1.00%
Mycoplana	**0.76%**
dimorpha	0.76%
Propionibacterium	**0.60%**
sp.	0.60%
Thauera	**0.53%**
sp.	0.53%
Lysinibacillus	**0.47%**
sphaericus	0.47%
Pelomonas	**0.37%**
saccharophila	0.37%
Geobacillus	**0.37%**
sp.	0.37%
Mycobacterium	**0.33%**
austroafricanum	0.03%
madagascariense	0.07%
mucogenicum	0.03%
rhodesiae	0.20%
Meiothermus	**0.27%**
silvanus	0.27%
Hyphomicrobium	**0.27%**
sp.	0.10%
vulgare	0.17%
Ochrobactrum	**0.23%**
anthropi	0.23%
Sulfuritalea	**0.20%**
hydrogenivorans	0.20%
Aquincola	**0.20%**
tertiaricarbonis	0.20%
Hydrogenophaga	**0.17%**
flava	0.17%
Cupriavidus	**0.17%**
necator	0.17%
Sulfuricurvum	**0.17%**
kujiense	0.17%

Genus species	
Magnetospirillum	**0.13%**
gryphiswaldense	0.13%
Aeromonas	**0.10%**
veronii	0.10%
Roseovarius	**0.10%**
tolerans	0.10%
Sphingopyxis	**0.10%**
baekryungensis	0.10%
Rhodoferax	**0.10%**
saidenbachensis	0.10%
Pseudomonas	**0.10%**
pictorum	0.10%
Methylotenera	**0.10%**
sp.	0.10%
Petrotoga	**0.07%**
mobilis	0.07%
Rubrivivax	**0.07%**
gelatinosus	0.07%
Hyphomonas	**0.07%**
polymorpha	0.07%
Variovorax	**0.07%**
paradoxus	0.07%
Stenotrophomonas	**0.03%**
maltophilia	0.03%
Dehalogenimonas	**0.03%**
lykanthroporepellens	0.03%
Acidovorax	**0.03%**
ebreus	0.03%
Leifsonia	**0.03%**
sp.	0.03%
Bradyrhizobium	**0.03%**
japonicum	0.03%
Shewanella	**0.03%**
putrefaciens	0.03%
Brevundimonas	**0.03%**
naejangsanensis	0.03%

Table D-20 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-11, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Sinorhizobium	**0.03%**
arboris	0.03%
Legionella	**0.03%**
pneumophila	0.03%
Smaragdicoccus	**0.03%**
niigatensis	0.03%
Nocardia	**0.03%**
neocaledoniensis	0.03%
Desulfomicrobium	**0.03%**
baculatum	0.03%
Novosphingobium	**0.03%**
sp.	0.03%
Grand Total	**100.00%**

Table D-21. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-8, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Sphingorhabdus	**29.69%**
wooponensis	29.69%
Pseudoxanthomonas	**21.20%**
sp.	21.20%
Acidovorax	**17.15%**
citrulli	0.12%
ebreus	17.03%
Pseudomonas	**4.61%**
aeruginosa	0.06%
alcaliphila	0.98%
balearica	0.25%
entomophila	0.12%
fluorescens	0.43%
pseudoalcaligenes	1.04%
putida	0.80%
resinovorans	0.06%
stutzeri	0.86%
Vogesella	**4.49%**
indigofera	4.49%
Ramlibacter	**2.95%**
tataouinensis	2.95%
Sphingomonas	**2.95%**
sp.	2.95%
Bacillus	**2.77%**
cereus	0.12%
thuringiensis	2.64%
Propionibacterium	**2.52%**
sp.	2.52%
Agrobacterium	**2.46%**
tumefaciens	2.46%
Mesorhizobium	**1.41%**
sp.	1.41%
Meiothermus	**1.29%**
silvanus	1.29%
Pelomonas	**1.23%**
saccharophila	1.23%

Genus species	Percent
Stenotrophomonas	**1.23%**
maltophilia	1.23%
Legionella	**0.68%**
longbeachae	0.68%
Mycobacterium	**0.68%**
madagascariense	0.06%
mucogenicum	0.61%
Rubrivivax	**0.55%**
gelatinosus	0.55%
Hyphomicrobium	**0.49%**
vulgare	0.49%
Hydrogenophilus	**0.43%**
hirschii	0.43%
Alcanivorax	**0.37%**
dieselolei	0.37%
Methylobacterium	**0.18%**
radiotolerans	0.18%
Brevundimonas	**0.12%**
naejangsanensis	0.12%
Delftia	**0.12%**
sp.	0.12%
Mesotoga	**0.12%**
prima	0.12%
Sulfuricurvum	**0.06%**
kujiense	0.06%
Sphingopyxis	**0.06%**
contaminans	0.06%
Alicycliphilus	**0.06%**
denitrificans	0.06%
Aquincola	**0.06%**
tertiaricarbonis	0.06%
Clostridium	**0.06%**
bifermentans	0.06%
Grand Total	**100.00%**

Table D-22. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-15, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Vogesella	**49.23%**
indigofera	49.23%
Pseudomonas	**45.82%**
aeruginosa	0.05%
alcaliphila	1.72%
entomophila	0.03%
fluorescens	13.97%
putida	29.03%
resinovorans	0.43%
rhodesiae	0.23%
stutzeri	0.35%
Rubrivivax	**2.16%**
gelatinosus	2.16%
Comamonadaceae	**0.62%**
bacterium	0.62%
Rhodoferax	**0.42%**
saidenbachensis	0.42%
Methylibium	**0.34%**
petroleiphilum	0.34%
Aromatoleum	**0.24%**
aromaticum	0.24%
Enterobacter	**0.18%**
cloacae	0.18%
Methylocystis	**0.12%**
sp.	0.12%
Hyphomicrobium	**0.11%**
vulgare	0.11%
Mycoplana	**0.08%**
dimorpha	0.08%
Stenotrophomonas	**0.07%**
maltophilia	0.07%
Acidovorax	**0.07%**
ebreus	0.04%
sp.	0.03%
Sphingobium	**0.06%**
baderi	0.04%
lactosutens	0.02%

Genus species	Percent
Mycobacterium	**0.06%**
mucogenicum	0.06%
Sphingomonas	**0.05%**
melonis	0.01%
wittichii	0.04%
Agrobacterium	**0.05%**
tumefaciens	0.05%
Cupriavidus	**0.04%**
necator	0.04%
Propionibacterium	**0.04%**
sp.	0.04%
Herbaspirillum	**0.04%**
sp.	0.04%
Rhizobium	**0.03%**
aggregatum	0.02%
sp.	0.02%
Sulfuritalea	**0.03%**
hydrogenivorans	0.03%
Sphingopyxis	**0.03%**
bauzanensis	0.03%
Alishewanella	**0.02%**
aestuarii	0.02%
Bradyrhizobium	**0.02%**
japonicum	0.02%
Parvibaculum	**0.02%**
lavamentivorans	0.02%
Bacillus	**0.01%**
firmus	0.01%
Janthinobacterium	**0.01%**
lividum	0.01%
Novosphingobium	**0.01%**
sp.	0.01%
Azospirillum	**0.01%**
oryzae	0.01%
Grand Total	**100.00%**

Table D-23. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-DMM-05, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Sphingorhabdus	**16.30%**
flavimaris	0.38%
wooponensis	15.92%
Bradyrhizobium	**15.98%**
elkanii	15.62%
japonicum	0.36%
Acidovorax	**13.58%**
citrulli	1.64%
ebreus	11.83%
sp.	0.11%
Pseudomonas	**11.98%**
aeruginosa	0.53%
alcaliphila	3.57%
balearica	1.16%
entomophila	0.03%
fluorescens	0.03%
hibiscicola	0.03%
mendocina	0.02%
pictorum	0.03%
putida	3.36%
resinovorans	2.09%
sp.	0.05%
stutzeri	1.10%
Sulfuritalea	**6.78%**
hydrogenivorans	6.78%
Paracoccus	**5.29%**
aminophilus	5.23%
denitrificans	0.02%
sp.	0.04%
Bacillus	**4.30%**
carboniphilus	0.06%
cereus	0.03%
drentensis	0.04%
firmus	0.25%
funiculus	2.51%
horikoshii	0.03%
lentus	0.08%

Genus species	Percent
Bacillus	**4.30%**
licheniformis	0.51%
megaterium	0.08%
niacini	0.24%
simplex	0.04%
sp.	0.15%
subterraneus	0.04%
thuringiensis	0.24%
Vogesella	**2.83%**
indigofera	2.83%
Mycobacterium	**2.43%**
austroafricanum	0.46%
avium	0.61%
brumae	0.10%
chlorophenolicum	0.07%
chubuense	0.02%
engbaekii	0.39%
madagascariense	0.12%
marinum	0.02%
mucogenicum	0.02%
rhodesiae	0.02%
scrofulaceum	0.04%
senegalense	0.36%
sinense	0.04%
sp.	0.02%
sphagni	0.03%
triplex	0.12%
Hyphomicrobium	**2.01%**
denitrificans	0.65%
nitrativorans	0.25%
sp.	0.55%
vulgare	0.55%
azotifigens	0.04%
leidyi	0.05%
melonis	0.42%
paucimobilis	0.01%

Genus species	Percent
Sphingomonas	**1.83%**
sp.	0.03%
starnbergensis	0.06%
wittichii	1.21%
Sphingobium	**1.69%**
baderi	0.71%
indicum	0.07%
lactosutens	0.49%
sp.	0.43%
Ramlibacter	**1.32%**
tataouinensis	1.32%
Nitrospira	**1.01%**
defluvii	1.01%
Thiobacillus	**0.98%**
thioparus	0.98%
Pseudoxanthomonas	**0.79%**
mexicana	0.45%
sp.	0.30%
spadix	0.03%
Sphingopyxis	**0.66%**
baekryungensis	0.32%
bauzanensis	0.22%
contaminans	0.03%
macrogoltabida	0.04%
soli	0.03%
ummariensis	0.02%
Novosphingobium	**0.61%**
aromaticivorans	0.29%
sp.	0.32%
Streptomyces	**0.45%**
armeniacus	0.04%
bottropensis	0.08%
coerulescens	0.15%
sampsonii	0.12%
thermodiastaticus	0.07%
Sulfuricurvum	**0.44%**
kujiense	0.44%

Table D-23 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-DMM-05, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Nitrobacter	**0.44%**
hamburgensis	0.04%
vulgaris	0.32%
winogradskyi	0.08%
Aromatoleum	**0.43%**
aromaticum	0.43%
Cupriavidus	**0.37%**
metallidurans	0.21%
necator	0.16%
Microbacterium	**0.36%**
lacticum	0.03%
resistens	0.21%
sp.	0.04%
testaceum	0.08%
Phaeospirillum	**0.35%**
molischianum	0.35%
Methylocystis	**0.33%**
sp.	0.33%
Methylobacterium	**0.33%**
extorquens	0.05%
sp.	0.29%
Methylibium	**0.33%**
petroleiphilum	0.33%
Desulfosporosinus	**0.30%**
meridiei	0.30%
Achromobacter	**0.28%**
xylosoxidans	0.28%
Propionibacterium	**0.28%**
sp.	0.28%
Stenotrophomonas	**0.24%**
maltophilia	0.24%
Methylocella	**0.24%**
silvestris	0.24%
Azoarcus	**0.24%**
sp.	0.24%

Genus species	Percent
Rhodoferax	**0.21%**
antarcticus	0.02%
fermentans	0.07%
ferrireducens	0.12%
Legionella	**0.19%**
drozanskii	0.12%
longbeachae	0.03%
santicrucis	0.04%
Terrimonas	**0.18%**
lutea	0.18%
Rubrivivax	**0.18%**
gelatinosus	0.18%
Alicycliphilus	**0.18%**
denitrificans	0.18%
Azospirillum	**0.16%**
brasilense	0.02%
lipoferum	0.05%
oryzae	0.09%
Comamonas	**0.15%**
testosteroni	0.15%
Hydrocarboniphaga	**0.13%**
effusa	0.13%
Pelomonas	**0.13%**
saccharophila	0.13%
Dechlorosoma	**0.12%**
suillum	0.12%
Nitrosovibrio	**0.12%**
tenuis	0.12%
Methyloversatilis	**0.11%**
discipulorum	0.11%
Parachlamydia	**0.10%**
acanthamoebae	0.10%
Rhizobium	**0.09%**
aggregatum	0.03%
sp.	0.07%
Pandoraea	**0.09%**
pnomenusa	0.09%

Genus species	Percent
Ralstonia	**0.09%**
pickettii	0.02%
solanacearum	0.05%
syzygii	0.02%
Carbophilus	**0.09%**
carboxidus	0.09%
Tistrella	**0.08%**
mobilis	0.08%
Herbaspirillum	**0.08%**
huttiense	0.08%
Solibacter	**0.08%**
usitatus	0.08%
Mesorhizobium	**0.07%**
opportunistum	0.05%
sp.	0.02%
Sphingobacterium	**0.07%**
sp.	0.07%
Gordonia	**0.07%**
polyisoprenivorans	0.05%
sp.	0.02%
Zavarzinia	**0.07%**
compransoris	0.07%
Rhodopseudomonas	**0.07%**
palustris	0.07%
Salinibacterium	**0.06%**
sp.	0.06%
Mycoplana	**0.06%**
dimorpha	0.06%
Enterobacter	**0.06%**
aerogenes	0.01%
cloacae	0.04%
Aquabacter	**0.05%**
spiritensis	0.05%
Leifsonia	**0.05%**
sp.	0.05%
Burkholderia	**0.05%**
cenocepacia	0.05%

Table D-23 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-DMM-05, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Agrobacterium	**0.05%**
rubi	0.01%
tumefaciens	0.04%
Geothrix	**0.05%**
fermentans	0.05%
Comamonadaceae	**0.05%**
bacterium	0.05%
Isoptericola	**0.05%**
variabilis	0.05%
Xanthomonas	**0.04%**
axonopodis	0.04%
Xanthobacter	**0.04%**
flavus	0.04%
Janthinobacterium	**0.04%**
lividum	0.04%
Clostridium	**0.04%**
beijerinckii	0.04%
Brevundimonas	**0.03%**
bacteroides	0.03%
Methylosinus	**0.03%**
trichosporium	0.03%
Variovorax	**0.03%**
paradoxus	0.03%
Protochlamydia	**0.03%**
amoebophila	0.03%
Nitrosospira	**0.03%**
multiformis	0.03%
Microvirga	**0.03%**
subterranea	0.03%
Rhodococcus	**0.02%**
jostii	0.02%
Agrococcus	**0.02%**
pavilionensis	0.02%
Nubsella	**0.02%**
zeaxanthinifaciens	0.02%
Methyloceanibacter	**0.02%**
caenitepidi	0.02%

Genus species	Percent
Blastococcus	**0.02%**
saxobsidens	0.02%
Alkaliphilus	**0.02%**
oremlandii	0.02%
Sinorhizobium	**0.02%**
Sp.	0.02%
Nocardioides	**0.02%**
sp.	0.02%
Paenibacillus	**0.02%**
sp.	0.02%
Hydrogenophaga	**0.02%**
flava	0.02%
Pelobacter	**0.02%**
propionicus	0.02%
Leucobacter	**0.02%**
salsicius	0.02%
Micromonospora	**0.02%**
sp.	0.02%
Acinetobacter	**0.02%**
baumannii	0.02%
Geobacter	**0.02%**
sp.	0.02%
Delftia	**0.02%**
acidovorans	0.02%
Leptospira	**0.01%**
licerasiae	0.01%
Kocuria	**0.01%**
flava	0.01%
Pseudonocardia	**0.01%**
kujensis	0.01%
Aquincola	**0.01%**
tertiaricarbonis	0.01%
Williamsia	**0.01%**
sp.	0.01%
Thiomonas	**0.01%**
intermedia	0.01%

Genus species	Percent
Modestobacter	**0.01%**
marinus	0.01%
Ochrobactrum	**0.01%**
pituitosum	0.01%
Aurantimonas	**0.01%**
manganoxydans	0.01%
Dyadobacter	**0.01%**
fermentans	0.01%
Parvibaculum	**0.01%**
lavamentivorans	0.01%
Grand Total	**100.00%**

Table D-24. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 22-BMW-3, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Vogesella	**56.55%**
indigofera	56.55%
Pseudoxanthomonas	**11.44%**
sp.	11.44%
Pseudomonas	**9.64%**
aeruginosa	0.10%
alcaliphila	0.11%
monteilii	0.04%
putida	4.72%
resinovorans	4.50%
sp.	0.18%
Sulfuricurvum	**7.06%**
kujiense	7.06%
Bacillus	**3.88%**
cereus	0.03%
pseudofirmus	1.24%
subterraneus	0.04%
thuringiensis	2.56%
Agrobacterium	**2.21%**
tumefaciens	2.21%
Methylocystis	**1.64%**
sp.	1.64%
Sphingomonas	**1.63%**
leidyi	0.30%
melonis	0.40%
sp.	0.93%
Meiothermus	**0.80%**
silvanus	0.80%
Carbophilus	**0.42%**
carboxidus	0.42%
Mesorhizobium	**0.35%**
loti	0.01%
sp.	0.34%
Acinetobacter	**0.35%**
baumannii	0.35%
Comamonas	**0.33%**
testosteroni	0.33%

Genus species	Percent
Zavarzinia	**0.33%**
compransoris	0.33%
Cupriavidus	**0.30%**
metallidurans	0.20%
necator	0.11%
Sphingopyxis	**0.25%**
baekryungensis	0.02%
bauzanensis	0.23%
Mycobacterium	**0.25%**
austroafricanum	0.06%
avium	0.19%
Hyphomicrobium	**0.23%**
vulgare	0.23%
Aromatoleum	**0.23%**
aromaticum	0.23%
Legionella	**0.19%**
drozanskii	0.11%
longbeachae	0.08%
Nocardioides	**0.18%**
sp.	0.18%
Streptococcus	**0.17%**
tigurinus	0.17%
Aquamicrobium	**0.17%**
defluvii	0.17%
Methylibium	**0.15%**
petroleiphilum	0.15%
Sphingobium	**0.14%**
baderi	0.08%
lactosutens	0.06%
Methylosinus	**0.13%**
trichosporium	0.13%
Sinorhizobium	**0.12%**
arboris	0.07%
fredii	0.05%
Geobacillus	**0.11%**
sp.	0.11%

Genus species	Percent
Corynebacterium	**0.11%**
ilicis	0.11%
Brevundimonas	**0.11%**
aveniformis	0.07%
bullata	0.04%
Geobacter	**0.10%**
bemidjiensis	0.04%
metallireducens	0.06%
Aquabacter	**0.08%**
spiritensis	0.08%
Magnetospirillum	**0.06%**
gryphiswaldense	0.06%
Propionibacterium	**0.05%**
sp.	0.05%
Caulobacter	**0.04%**
segnis	0.04%
Novosphingobium	**0.03%**
sp.	0.03%
Hyphomonas	**0.03%**
polymorpha	0.03%
Microbacterium	**0.03%**
lacticum	0.03%
Alcaligenes	**0.02%**
aquatilis	0.02%
Aquincola	**0.02%**
tertiaricarbonis	0.02%
Ochrobactrum	**0.01%**
anthropi	0.01%
Phenylobacterium	**0.01%**
zucineum	0.01%
Petrotoga	**0.01%**
mobilis	0.01%
Mesotoga	**0.01%**
prima	0.01%
Bradyrhizobium	**0.01%**
elkanii	0.01%
Grand Total	**100.00%**

Table D-25. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-01R, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Herminiimonas	**40.88%**
arsenicoxydans	40.88%
Pseudomonas	**15.46%**
aeruginosa	0.47%
alcaliphila	0.33%
balearica	0.02%
entomophila	0.23%
fluorescens	0.61%
hibiscicola	0.02%
monteilii	0.02%
pseudoalcaligenes	10.63%
putida	1.89%
resinovorans	0.23%
sp.	0.33%
stutzeri	0.62%
syringae	0.06%
Dechloromonas	**5.99%**
aromatica	5.99%
Acidovorax	**5.59%**
avenae	0.15%
citrulli	5.29%
ebreus	0.03%
sp.	0.12%
Aromatoleum	**3.79%**
aromaticum	3.79%
Alishewanella	**3.74%**
aestuarii	3.74%
Azoarcus	**3.32%**
sp.	3.32%
Clostridium	**3.08%**
ghonii	0.17%
subterminale	1.53%
viride	1.39%
Mycobacterium	**1.55%**
austroafricanum	0.04%
brumae	0.02%
chlorophenolicum	0.04%

Genus species	Percent
Mycobacterium	**1.55%**
diernhoferi	0.36%
madagascariense	0.03%
mucogenicum	0.07%
rhodesiae	0.88%
smegmatis	0.02%
wolinskyi	0.10%
Vogesella	**1.41%**
indigofera	1.41%
Arcobacter	**1.34%**
sp.	1.34%
Methylibium	**1.33%**
petroleiphilum	1.33%
Hyphomicrobium	**1.10%**
denitrificans	0.02%
sp.	0.67%
vulgare	0.42%
Comamonadaceae	**0.83%**
bacterium	0.83%
Rhodoferax	**0.65%**
fermentans	0.32%
saidenbachensis	0.33%
Aeromonas	**0.56%**
veronii	0.56%
Desulfovibrio	**0.55%**
magneticus	0.55%
Candidatus	**0.54%**
Solibacter	0.54%
Ramlibacter	**0.54%**
tataouinensis	0.54%
Hydrogenophaga	**0.53%**
flava	0.53%
Variovorax	**0.52%**
paradoxus	0.52%
Acetobacterium	**0.43%**
woodii	0.43%

Genus species	Percent
Chryseobacterium	**0.39%**
sp.	0.39%
Magnetospirillum	**0.37%**
gryphiswaldense	0.01%
magneticum	0.36%
Malikia	**0.35%**
spinosa	0.35%
Agrobacterium	**0.32%**
tumefaciens	0.32%
Sulfuritalea	**0.31%**
hydrogenivorans	0.31%
Aquincola	**0.31%**
tertiaricarbonis	0.31%
Sulfuricurvum	**0.23%**
kujiense	0.23%
Legionella	**0.21%**
longbeachae	0.14%
pneumophila	0.07%
Xanthobacter	**0.20%**
agilis	0.05%
flavus	0.02%
tagetidis	0.14%
Sphingomonas	**0.20%**
azotifigens	0.03%
leidyi	0.01%
melonis	0.02%
sp.	0.02%
starnbergensis	0.03%
wittichii	0.09%
Thauera	**0.19%**
sp.	0.19%
Microbacterium	**0.19%**
sp.	0.02%
testaceum	0.18%
Planomicrobium	**0.18%**
okeanokoites	0.18%

Table D-25 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-01R, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Acinetobacter	**0.16%**
lwoffii	0.05%
sp.	0.11%
Geobacter	**0.16%**
lovleyi	0.05%
sp.	0.11%
Rubrivivax	**0.15%**
gelatinosus	0.15%
Flavobacteria	**0.15%**
bacterium	0.15%
Enterobacter	**0.14%**
cloacae	0.14%
Bacillus	**0.14%**
horikoshii	0.04%
pseudofirmus	0.01%
thuringiensis	0.09%
Methylosinus	**0.13%**
trichosporium	0.13%
Rhodobacter	**0.12%**
azotoformans	0.05%
capsulatus	0.07%
Sphingobium	**0.10%**
baderi	0.06%
lactosutens	0.04%
Carbophilus	**0.10%**
carboxidus	0.10%
Gemmobacter	**0.10%**
aquatilis	0.10%
Dietzia	**0.10%**
psychralcaliphila	0.10%
Pseudoxanthomonas	**0.08%**
sp.	0.07%
spadix	0.02%
Methylocystis	**0.08%**
sp.	0.08%
Azospirillum	**0.08%**
sp.	0.08%

Genus species	Percent
Sphingopyxis	**0.07%**
baekryungensis	0.02%
contaminans	0.03%
macrogoltabida	0.01%
soli	0.01%
Comamonas	**0.07%**
testosteroni	0.07%
Cupriavidus	**0.06%**
metallidurans	0.03%
necator	0.03%
Methanosaeta	**0.06%**
concilii	0.06%
Stenotrophomonas	**0.05%**
maltophilia	0.05%
Dehalogenimonas	**0.05%**
lykanthroporepellens	0.05%
Ralstonia	**0.05%**
pickettii	0.05%
Novosphingobium	**0.05%**
sp.	0.05%
Bacterium	**0.05%**
proteobacterium	0.05%
Bdellovibrio	**0.04%**
exovorus	0.04%
Zavarzinia	**0.04%**
compransoris	0.04%
Paucibacter	**0.04%**
toxinivorans	0.04%
Bacteriovorax	**0.04%**
stolpii	0.04%
Methylomonas	**0.04%**
rubra	0.04%
Anaerobacterium	**0.03%**
chartisolvens	0.03%
Sinorhizobium	**0.03%**
arboris	0.02%
fredii	0.01%

Genus species	Percent
Paracoccus	**0.03%**
aminophilus	0.03%
Rhizobium	**0.02%**
sp.	0.02%
Methyloversatilis	**0.02%**
universalis	0.02%
Pelomonas	**0.02%**
saccharophila	0.02%
Desulfomicrobium	**0.02%**
baculatum	0.02%
Bradyrhizobium	**0.02%**
elkanii	0.02%
Actinosynnema	**0.02%**
mirum	0.02%
Salinibacterium	**0.01%**
sp.	0.01%
Hyphomonas	**0.01%**
polymorpha	0.01%
Thiobacillus	**0.01%**
thioparus	0.01%
Methanobacterium	**0.01%**
formicicum	0.01%
Xanthomonas	**0.01%**
axonopodis	0.01%
Thiothrix	**0.01%**
unzii	0.01%
Tsukamurella	**0.01%**
paurometabola	0.01%
Turneriella	**0.01%**
parva	0.01%
Kocuria	**0.01%**
rosea	0.01%
Acetivibrio	**0.01%**
ethanolgignens	0.01%
Alicycliphilus	**0.01%**
denitrificans	0.01%
Grand Total	**100.00%**

Table D-26. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-07R, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Pseudomonas	**10.44%**
aeruginosa	0.22%
alcaliphila	0.28%
balearica	0.31%
fluorescens	0.10%
hibiscicola	0.02%
pictorum	0.10%
pseudoalcaligenes	6.99%
putida	1.87%
resinovorans	0.25%
sp.	0.02%
stutzeri	0.27%
Mycobacterium	**9.88%**
austroafricanum	0.57%
brumae	0.28%
chlorophenolicum	0.41%
diernhoferi	0.39%
madagascariense	0.47%
mageritense	0.03%
mucogenicum	2.42%
murale	0.01%
rhodesiae	5.09%
senegalense	0.04%
sp.	0.01%
triplex	0.14%
wolinskyi	0.02%
Hyphomicrobium	**8.17%**
denitrificans	0.68%
sp.	1.00%
vulgare	6.48%
Clostridium	**7.42%**
bifermentans	6.59%
botulinum	0.14%
hungatei	0.01%
propionicum	0.03%
subterminale	0.53%
tertium	0.02%

Genus species	Percent
Clostridium	**7.42%**
thermoalcaliphilum	0.01%
viride	0.08%
Bacillus	**5.67%**
carboniphilus	0.02%
cereus	0.11%
firmus	0.02%
horikoshii	0.02%
megaterium	0.10%
pseudofirmus	0.02%
subterraneus	0.04%
thuringiensis	5.34%
Methylocystis	**4.62%**
sp.	4.62%
Xanthobacter	**3.84%**
agilis	0.03%
flavus	3.70%
tagetidis	0.11%
Hydrogenophaga	**3.64%**
flava	3.64%
Aromatoleum	**3.41%**
aromaticum	3.41%
Cupriavidus	**2.88%**
metallidurans	0.06%
necator	2.82%
Sulfuricurvum	**2.58%**
kujiense	2.58%
Azoarcus	**2.36%**
sp.	2.36%
Magnetospirillum	**2.24%**
gryphiswaldense	2.22%
magneticum	0.03%
Methylibium	**2.20%**
petroleiphilum	2.20%
Thauera	**2.04%**
sp.	2.04%

Genus species	Percent
Methylomonas	**2.01%**
rubra	2.01%
Roseovarius	**1.84%**
sp.	0.01%
tolerans	1.83%
Vogesella	**1.84%**
indigofera	1.84%
Hyphomonas	**1.70%**
adhaerens	0.01%
polymorpha	1.69%
Robiginitalea	**1.48%**
biformata	1.48%
Rhodobacter	**1.48%**
azotoformans	0.04%
capsulatus	1.44%
Methanosaeta	**1.05%**
concilii	1.05%
Rubrivivax	**0.99%**
gelatinosus	0.99%
Acinetobacter	**0.87%**
lwoffii	0.66%
sp.	0.03%
venetianus	0.18%
Aquincola	**0.80%**
tertiaricarbonis	0.80%
Pseudonocardia	**0.80%**
ailaonensis	0.02%
ammonioxydans	0.01%
antitumoralis	0.31%
asaccharolytica	0.03%
hispaniensis	0.34%
kujensis	0.02%
oroxyli	0.02%
spinosispora	0.03%
xishanensis	0.02%
Sphingomonas	**0.74%**
leidyi	0.01%

Genus species	Percent
Rhodoferax	**2.13%**
saidenbachensis	2.13%
Acinetobacter	**2.01%**
baumannii	0.11%
sp.	1.90%
Hydrocarboniphaga	**1.86%**
effusa	1.86%
Comamonas	**1.50%**
testosteroni	1.50%
Zavarzinia	**1.43%**
compransoris	1.43%
Acidovorax	**1.25%**
avenae	0.02%
citrulli	0.02%
sp.	1.20%
Bradyrhizobium	**1.22%**
elkanii	0.80%
japonicum	0.42%
Brevundimonas	**0.80%**
bacteroides	0.37%
bullata	0.13%
subvibrioides	0.28%
vesicularis	0.02%
Methylocystis	**0.80%**
sp.	0.80%
Salinibacterium	**0.66%**
sp.	0.66%
Rubrivivax	**0.61%**
gelatinosus	0.61%
Ralstonia	**0.59%**
eutropha	0.01%
pickettii	0.49%
syzygii	0.09%
Pseudoxanthomonas	**0.51%**
mexicana	0.22%
sp.	0.29%

Table D-26 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-07R, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Sphingomonas	**0.74%**
melonis	0.43%
paucimobilis	0.02%
sp.	0.05%
starnbergensis	0.03%
wittichii	0.20%
Thiobacillus	**0.68%**
denitrificans	0.01%
thioparus	0.66%
Rhodoferax	**0.68%**
antarcticus	0.05%
fermentans	0.38%
saidenbachensis	0.24%
Methylocella	**0.67%**
silvestris	0.67%
Microbacterium	**0.61%**
lacticum	0.25%
resistens	0.03%
testaceum	0.32%
Bradyrhizobium	**0.59%**
elkanii	0.03%
japonicum	0.56%
Pseudoxanthomonas	**0.58%**
mexicana	0.29%
sp.	0.04%
spadix	0.25%
Sinorhizobium	**0.57%**
arboris	0.15%
fredii	0.42%
Smaragdicoccus	**0.48%**
niigatensis	0.48%
Aeromonas	**0.47%**
veronii	0.47%
Novosphingobium	**0.45%**
sp.	0.45%
Acidovorax	**0.42%**
avenae	0.13%

Genus species	Percent
Acidovorax	**0.42%**
citrulli	0.13%
ebreus	0.04%
sp.	0.12%
Ramlibacter	**0.40%**
tataouinensis	0.40%
Enterobacter	**0.38%**
cloacae	0.37%
sp.	0.01%
Dehalogenimonas	**0.34%**
lykanthroporepellens	0.34%
Shewanella	**0.33%**
putrefaciens	0.30%
sp.	0.03%
Sphingobium	**0.32%**
baderi	0.09%
lactosutens	0.22%
sp.	0.01%
Stenotrophomonas	**0.27%**
maltophilia	0.27%
Sulfuritalea	**0.25%**
hydrogenivorans	0.25%
Sphingorhabdus	**0.23%**
marina	0.05%
wooponensis	0.18%
Sphingopyxis	**0.22%**
baekryungensis	0.13%
bauzanensis	0.02%
indica	0.01%
macrogoltabida	0.04%
soli	0.02%
Bacterium	**0.22%**
alphaproteobacterium	0.22%
Leucobacter	**0.22%**
salsicius	0.22%
Serratia	**0.19%**
marcescens	0.19%

Genus species	Percent
Salinibacterium	**0.17%**
sp.	0.17%
Paracoccus	**0.15%**
aminophilus	0.14%
denitrificans	0.01%
Legionella	**0.14%**
drozanskii	0.01%
longbeachae	0.09%
pneumophila	0.04%
Methanobacterium	**0.13%**
espanolae	0.01%
formicicum	0.10%
paludis	0.02%
Carbophilus	**0.12%**
carboxidus	0.12%
Citromicrobium	**0.10%**
sp.	0.10%
Methanosarcina	**0.10%**
horonobensis	0.08%
subterranea	0.02%
Williamsia	**0.09%**
sp.	0.09%
Rhodococcus	**0.09%**
ruber	0.09%
Mesorhizobium	**0.09%**
opportunistum	0.09%
Streptomyces	**0.09%**
mordarskii	0.03%
venezuelae	0.05%
Desulfotomaculum	**0.09%**
guttoideum	0.09%
Xanthomonas	**0.08%**
axonopodis	0.08%
Mesotoga	**0.08%**
prima	0.08%
Rhizobium	**0.07%**
sp.	0.07%

Table D-26 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-07R, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Phaeospirillum	**0.07%**
molischianum	0.07%
Methylobacterium	**0.07%**
extorquens	0.01%
sp.	0.06%
Saccharibacteria	**0.06%**
ap.	0.06%
Lysinibacillus	**0.06%**
sphaericus	0.06%
Desulfomonile	**0.06%**
tiedjei	0.06%
Agrobacterium	**0.06%**
tumefaciens	0.06%
Nocardia	**0.06%**
neocaledoniensis	0.06%
Comamonas	**0.06%**
testosteroni	0.06%
Nocardioides	**0.06%**
sp.	0.06%
Janibacter	**0.06%**
hoylei	0.06%
Methylosinus	**0.05%**
trichosporium	0.05%
Burkholderia	**0.05%**
cenocepacia	0.05%
Collimonas	**0.05%**
fungivorans	0.05%
Agrococcus	**0.04%**
pavilionensis	0.04%
Pseudonocardia	**0.04%**
sp.	0.02%
Sinorhizobium	0.03%
Geobacter	**0.04%**
lovleyi	0.04%
Simkania	**0.04%**
negevensis	0.04%
Alicycliphilus	**0.04%**
denitrificans	0.04%

Genus species	Percent
Parvibaculum	**0.03%**
lavamentivorans	0.03%
Arcobacter	**0.03%**
sp.	0.03%
Azospirillum	**0.03%**
sp.	0.03%
Erythrobacter	**0.03%**
litoralis	0.03%
Marmoricola	**0.03%**
sp.	0.03%
Comamonadaceae	**0.03%**
bacterium	0.03%
Mycoplana	**0.03%**
dimorpha	0.03%
Anaerobacterium	**0.03%**
chartisolvens	0.03%
Ochrobactrum	**0.03%**
anthropi	0.03%
Chryseobacterium	**0.03%**
sp.	0.03%
Gordonia	**0.03%**
bronchialis	0.03%
Achromobacter	**0.03%**
xylosoxidans	0.03%
Bdellovibrio	**0.03%**
bacteriovorus	0.03%
Desulfomicrobium	**0.02%**
baculatum	0.02%
Starkeya	**0.02%**
novella	0.02%
Ralstonia	**0.02%**
pickettii	0.02%
Hydrocarboniphaga	**0.02%**
effusa	0.02%
Aquabacter	**0.02%**
spiritensis	0.02%
Bactoderma	**0.02%**
rosea	0.02%

Genus species	Percent
Zavarzinia	**0.02%**
compransoris	0.02%
Moorella	**0.02%**
thermoacetica	0.02%
Exiguobacterium	**0.02%**
sp.	0.02%
Nitrosomonas	**0.02%**
nitrosa	0.02%
Leifsonia	**0.02%**
sp.	0.02%
Leptonema	**0.02%**
illini	0.02%
Actinosynnema	**0.02%**
mirum	0.02%
Nannocystis	**0.02%**
pusilla	0.02%
Caulobacter	**0.02%**
sp.	0.02%
Blastochloris	**0.02%**
viridis	0.02%
Leptothrix	**0.02%**
cholodnii	0.02%
Methyloversatilis	**0.02%**
discipulorum	0.02%
Paraburkholderia	**0.02%**
kururiensis	0.02%
Herbaspirillum	**0.02%**
huttiense	0.02%
Modestobacter	**0.02%**
marinus	0.02%
Petrotoga	**0.02%**
mobilis	0.02%
Muricauda	**0.01%**
ruestringensis	0.01%
Methanofollis	**0.01%**
liminatans	0.01%
Beijerinckia	**0.01%**
indica	0.01%

Table D-26 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-07R, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Desulfovibrio	**0.01%**
magneticus	0.01%
Methanomethylovorans	**0.01%**
hollandica	0.01%
Acetivibrio	**0.01%**
cellulolyticus	0.01%
Rhodopseudomonas	**0.01%**
palustris	0.01%
Dechloromonas	**0.01%**
aromatica	0.01%
Grand Total	**100.00%**

Table D-27. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-RW-07, as Evaluated by 16S Sequence Analysis during Sampling Event 2

Genus species	Percent
Sulfuricurvum	**14.10%**
kujiense	14.10%
Novosphingobium	**12.82%**
aromaticivorans	0.19%
sp.	12.63%
Roseovarius	**11.22%**
tolerans	11.22%
Mycobacterium	**9.01%**
abscessus	0.01%
austroafricanum	1.59%
brumae	0.27%
chlorophenolicum	0.03%
diernhoferi	0.30%
madagascariense	4.76%
mageritense	0.02%
rhodesiae	1.65%
senegalense	0.02%
smegmatis	0.06%
sp.	0.02%
triplex	0.05%
wolinskyi	0.24%
Magnetospirillum	**6.48%**
gryphiswaldense	6.15%
magneticum	0.33%
Methylocystis	**5.58%**
sp.	5.58%
Hyphomicrobium	**5.40%**
denitrificans	1.56%
sp.	0.85%
vulgare	2.99%
Aquincola	**3.42%**
tertiaricarbonis	3.42%
Mesotoga	**3.40%**
prima	3.40%
Petrotoga	**3.05%**
mobilis	3.05%

Genus species	Percent
Xanthobacter	**2.68%**
agilis	0.04%
flavus	2.54%
tagetidis	0.10%
Hydrogenophaga	**2.46%**
flava	2.46%
Hyphomonas	**2.23%**
adhaerens	0.14%
polymorpha	2.09%
Methylibium	**1.51%**
petroleiphilum	1.51%
Geobacter	**1.02%**
lovleyi	1.01%
metallireducens	0.01%
Sphingomonas	**0.93%**
leidyi	0.02%
melonis	0.06%
wittichii	0.86%
Sphingobium	**0.92%**
baderi	0.83%
lactosutens	0.09%
Thiobacillus	**0.88%**
thioparus	0.88%
Pseudoxanthomonas	**0.84%**
mexicana	0.04%
sp.	0.44%
spadix	0.36%
Bradyrhizobium	**0.67%**
japonicum	0.67%
Pseudomonas	**0.65%**
alcaliphila	0.05%
balearica	0.28%
fluorescens	0.03%
putida	0.15%
sp.	0.09%
stutzeri	0.05%

Genus species	Percent
Methylomonas	**0.58%**
rubra	0.58%
Legionella	**0.57%**
drozanskii	0.24%
longbeachae	0.09%
pneumophila	0.08%
rowbothamii	0.16%
Leifsonia	**0.56%**
sp.	0.56%
Bacillus	**0.49%**
cereus	0.02%
horikoshii	0.04%
subterraneus	0.05%
thuringiensis	0.38%
Simkania	**0.40%**
negevensis	0.40%
Rhodobacter	**0.40%**
azotoformans	0.04%
capsulatus	0.35%
sphaeroides	0.01%
Erythrobacter	**0.36%**
litoralis	0.36%
Sphaerochaeta	**0.35%**
globosa	0.35%
Rubrivivax	**0.34%**
gelatinosus	0.34%
Chryseobacterium	**0.32%**
sp.	0.32%
Paracoccus	**0.28%**
aminophilus	0.28%
Sinorhizobium	**0.27%**
arboris	0.19%
fredii	0.08%
Salinibacterium	**0.27%**
sp.	0.27%

Table D-27 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-RW-07, as Evaluated by 16S Sequence Analysis during Sampling Event 2

Genus species	Percent
Microbacterium	**0.26%**
lacticum	0.19%
resistens	0.03%
sp.	0.03%
Parvibaculum	**0.25%**
lavamentivorans	0.25%
Pseudonocardia	**0.24%**
ailaonensis	0.03%
antitumoralis	0.03%
oroxyli	0.03%
spinosispora	0.11%
yuanmonensis	0.04%
alpha	**0.24%**
proteobacterium	0.24%
Dehalogenimonas	**0.24%**
lykanthroporepellens	0.24%
Treponema	**0.24%**
caldarium	0.24%
Sphingopyxis	**0.22%**
baekryungensis	0.13%
macrogoltabida	0.02%
soli	0.02%
sp.	0.02%
witflariensis	0.04%
Enterobacter	**0.20%**
aerogenes	0.04%
cloacae	0.11%
sp.	0.05%
Methyloversatilis	**0.20%**
discipulorum	0.18%
universalis	0.02%
Agrococcus	**0.18%**
pavilionensis	0.18%
Sphingorhabdus	**0.17%**
marina	0.17%

Genus species	Percent
Thiomonas	**0.17%**
intermedia	0.17%
Methanosaeta	**0.16%**
concilii	0.16%
Alicycliphilus	**0.15%**
denitrificans	0.15%
Acidovorax	**0.15%**
ebreus	0.14%
sp.	0.01%
Antarctobacter	**0.13%**
heliothermus	0.13%
Methylocella	**0.13%**
silvestris	0.13%
Cellulomonas	**0.13%**
iranensis	0.13%
Zavarzinia	**0.11%**
compransoris	0.11%
Actinoplanes	**0.10%**
sp.	0.10%
Turneriella	**0.10%**
parva	0.10%
Methylosinus	**0.10%**
trichosporium	0.10%
Rhizobium	**0.10%**
aggregatum	0.04%
sp.	0.05%
Nocardioides	**0.09%**
sp.	0.09%
Acidocella	**0.09%**
aminolytica	0.06%
facilis	0.02%
Desulfomicrobium	**0.08%**
baculatum	0.08%
Clavibacter	**0.08%**
michiganensis	0.08%

Genus species	Percent
Muricauda	**0.08%**
ruestringensis	0.08%
Phaeospirillum	**0.07%**
molischianum	0.07%
Thauera	**0.07%**
sp.	0.07%
Moorella	**0.06%**
thermoacetica	0.06%
Thiothrix	**0.06%**
unzii	0.06%
Beijerinckia	**0.06%**
indica	0.06%
Paraburkholderia	**0.06%**
kururiensis	0.06%
Aeromonas	**0.05%**
veronii	0.05%
Clostridium	**0.05%**
beijerinckii	0.01%
termitidis	0.04%
Malikia	**0.05%**
spinosa	0.05%
Vogesella	**0.04%**
indigofera	0.04%
Mesorhizobium	**0.04%**
opportunistum	0.03%
sp.	0.01%
Carbophilus	**0.04%**
carboxidus	0.04%
Youngiibacter	**0.04%**
multivorans	0.04%
Parachlamydia	**0.04%**
acanthamoebae	0.04%
Klebsiella	**0.03%**
pneumoniae	0.03%

Table D-27 (cont.). Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-RW-07, as Evaluated by 16S Sequence Analysis during Sampling Event 2

Genus species	Percent
Isoptericola	**0.03%**
variabilis	0.03%
Agrobacterium	**0.03%**
tumefaciens	0.03%
Oceanobacillus	**0.03%**
iheyensis	0.03%
Serratia	**0.03%**
marcescens	0.03%
Gemmobacter	**0.02%**
aquatilis	0.02%
Starkeya	**0.02%**
novella	0.02%
Lachnoclostridium	**0.02%**
phytofermentans	0.02%
Dehalobacter	**0.02%**
sp.	0.02%
Methanobrevibacter	**0.02%**
arboriphilus	0.02%
Hydrocarboniphaga	**0.02%**
effusa	0.02%
Rhodoferax	**0.02%**
antarcticus	0.02%
Arcobacter	**0.02%**
sp.	0.02%
Mahella	**0.02%**
australiensis	0.02%
Paucimonas	**0.02%**
lemoignei	0.02%
Shewanella	**0.01%**
putrefaciens	0.01%
Aquabacter	**0.01%**
spiritensis	0.01%
Achromobacter	**0.01%**
xylosoxidans	0.01%
Leptonema	**0.01%**
illini	0.01%
Tistrella	**0.01%**
mobilis	0.01%
Methanoregula	**0.01%**
formicica	0.01%
Tsukamurella	**0.01%**
paurometabola	0.01%
Grand Total	**100.00%**

Table D-28. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-23, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Mycobacterium	**86.16%**
austroafricanum	0.02%
mucogenicum	85.98%
phlei	0.12%
senegalense	0.04%
Salmonella	**4.70%**
enterica	4.70%
Geobacter	**2.12%**
lovleyi	2.10%
metallireducens	0.02%
Pseudomonas	**1.69%**
aeruginosa	0.06%
balearica	1.36%
monteilii	0.05%
sp.	0.21%
Pseudoxanthomonas	**0.78%**
sp.	0.71%
spadix	0.07%
Enterobacter	**0.62%**
cloacae	0.62%
Methanosaeta	**0.58%**
concilii	0.58%
Agrobacterium	**0.56%**
tumefaciens	0.56%
Thiobacillus	**0.41%**
thioparus	0.41%
Treponema	**0.37%**
caldarium	0.37%
Bacillus	**0.33%**
thuringiensis	0.33%
Magnetospirillum	**0.30%**
gryphiswaldense	0.30%
Sphingopyxis	**0.25%**
bauzanensis	0.07%
indica	0.18%
Paraburkholderia	**0.14%**
kururiensis	0.14%

Genus species	Percent
Acetoanaerobium	**0.10%**
noterae	0.10%
Thiomonas	**0.09%**
intermedia	0.09%
Nocardioides	**0.09%**
sp.	0.09%
Achromobacter	**0.08%**
xylosoxidans	0.08%
Burkholderia	**0.07%**
anthina	0.07%
Sphingobium	**0.06%**
lactosutens	0.06%
Salinibacterium	**0.06%**
sp.	0.06%
Methanofollis	**0.06%**
liminatans	0.06%
Bordetella	**0.05%**
petrii	0.05%
Legionella	**0.05%**
rowbothamii	0.05%
Alicycliphilus	**0.05%**
denitrificans	0.05%
Acinetobacter	**0.04%**
venetianus	0.04%
Cellulomonas	**0.04%**
iranensis	0.04%
Sulfurospirillum	**0.03%**
cavolei	0.03%
Bradyrhizobium	**0.03%**
japonicum	0.03%
Lysinibacillus	**0.02%**
sphaericus	0.02%
Acidovorax	**0.02%**
ebreus	0.02%
Xanthobacter	**0.01%**
agilis	0.01%

Genus species	Percent
Vogesella	**0.01%**
indigofera	0.01%
Dehalobacter	**0.01%**
sp.	0.01%
Mesotoga	**0.01%**
prima	0.01%
Grand Total	**100.00%**

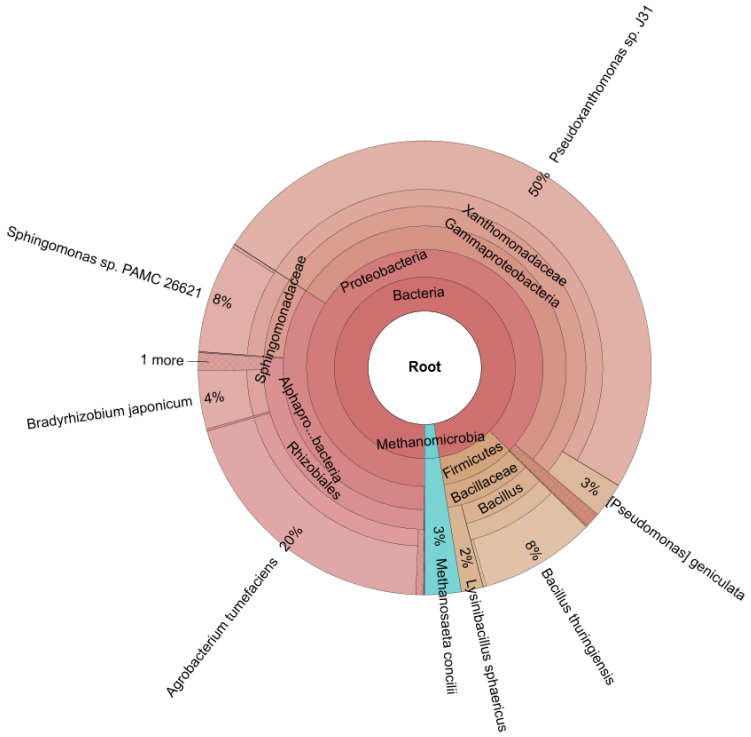
Table D-29. Reads That Could Be Identified to Various Taxa Identified as MTBE/TBA-Degradation Capable Organisms in Sample 1327-MW-39, as Evaluated by 16S Sequence Analysis, Sampling Event 2

Genus species	Percent
Methanosaeta	**42.46%**
concilii	42.46%
Methylomonas	**19.61%**
rubra	19.61%
Thauera	**7.16%**
sp.	7.16%
Methylotenera	**6.84%**
sp.	6.84%
Aromatoleum	**6.40%**
aromaticum	6.40%
Sulfuritalea	**5.07%**
hydrogenivorans	5.07%
Geobacter	**1.60%**
lovleyi	0.50%
metallireducens	1.10%
Methylocystis	**1.47%**
sp.	1.47%
Acidovorax	**1.09%**
ebreus	0.02%
sp.	1.08%
Hyphomicrobium	**1.00%**
denitrificans	0.43%
sp.	0.44%
vulgare	0.13%
Pseudomonas	**0.49%**
alcaliphila	0.03%
balearica	0.02%
chloritidismutans	0.01%
hibiscicola	0.12%
pictorum	0.03%
putida	0.05%
resinovorans	0.08%
stutzeri	0.14%
Methanoculleus	**0.48%**
marisnigri	0.48%
Methylocella	**0.47%**
silvestris	0.47%

Genus species	Percent
Dehalobacter	**0.44%**
sp.	0.44%
Stenotrophomonas	**0.41%**
maltophilia	0.41%
Novosphingobium	**0.36%**
aromaticivorans	0.36%
Carbophilus	**0.34%**
carboxidus	0.34%
Mesotoga	**0.32%**
prima	0.32%
Sphaerochaeta	**0.28%**
globosa	0.28%
Comamonas	**0.26%**
testosteroni	0.26%
Nocardioides	**0.22%**
sp.	0.22%
Sulfurimonas	**0.22%**
autotrophica	0.22%
Zavarzinia	**0.19%**
compransoris	0.19%
Sinorhizobium	**0.18%**
arboris	0.03%
fredii	0.09%
sp.	0.05%
Pseudoxanthomonas	**0.16%**
mexicana	0.02%
sp.	0.02%
spadix	0.11%
Sulfuricurvum	**0.16%**
kujiense	0.16%
Cupriavidus	**0.16%**
metallidurans	0.12%
necator	0.04%
Enterobacter	**0.15%**
cloacae	0.15%
Dehalogenimonas	**0.13%**
lykanthroporepellens	0.13%

Genus species	Percent
Afipia	**0.13%**
sp.	0.13%
Methylibium	**0.12%**
petroleiphilum	0.12%
Sphingomonas	**0.11%**
melonis	0.03%
phyllosphaerae	0.02%
wittichii	0.06%
Rhodobacter	**0.10%**
capsulatus	0.10%
Magnetospirillum	**0.08%**
gryphiswaldense	0.08%
Legionella	**0.08%**
pneumophila	0.08%
Thiobacillus	**0.07%**
thioparus	0.07%
Mahella	**0.07%**
australiensis	0.07%
Hydrogenophaga	**0.07%**
flava	0.07%
Xanthobacter	**0.06%**
agilis	0.02%
flavus	0.05%
Simkania	**0.06%**
negevensis	0.06%
Methyloversatilis	**0.05%**
discipulorum	0.05%
Sphingopyxis	**0.05%**
baekryungensis	0.02%
indica	0.03%
Sphingobium	**0.05%**
baderi	0.04%
lactosutens	0.01%
Bacterium	**0.05%**
proteobacterium	0.05%
Mesorhizobium	**0.05%**
opportunistum	0.05%

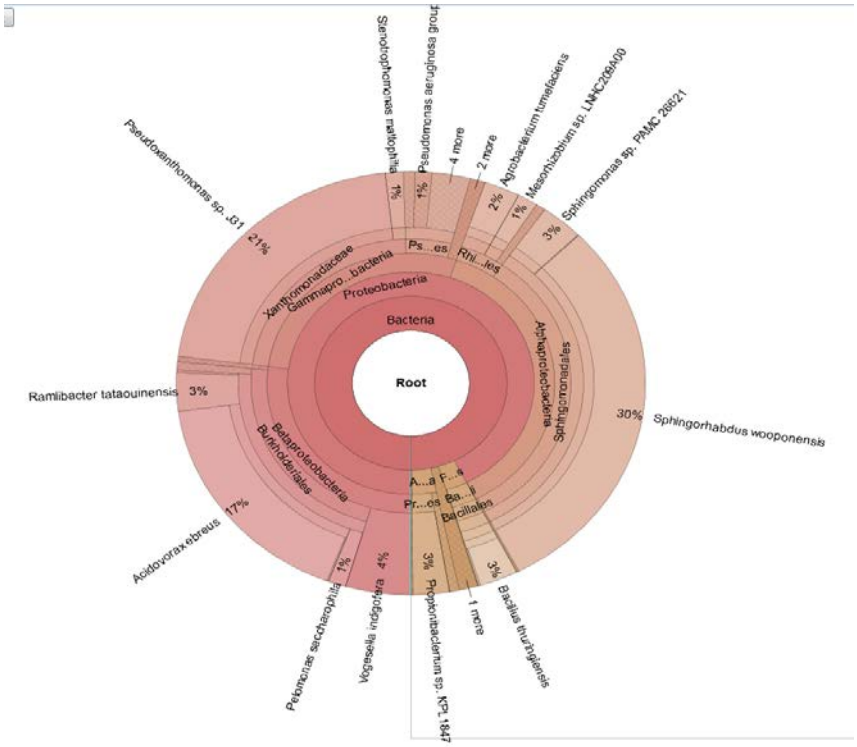
A



B

Figure D-1. Approximately Genus-level Depiction of Microbial Population for Sample 22-DMM-07 Collected during: A) Sampling Event 1 and B) Sampling Event 2

A



B

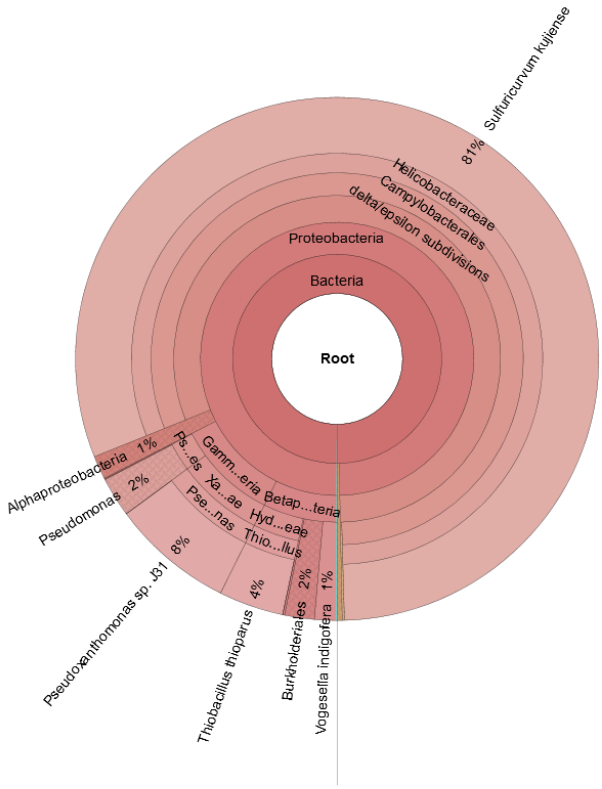
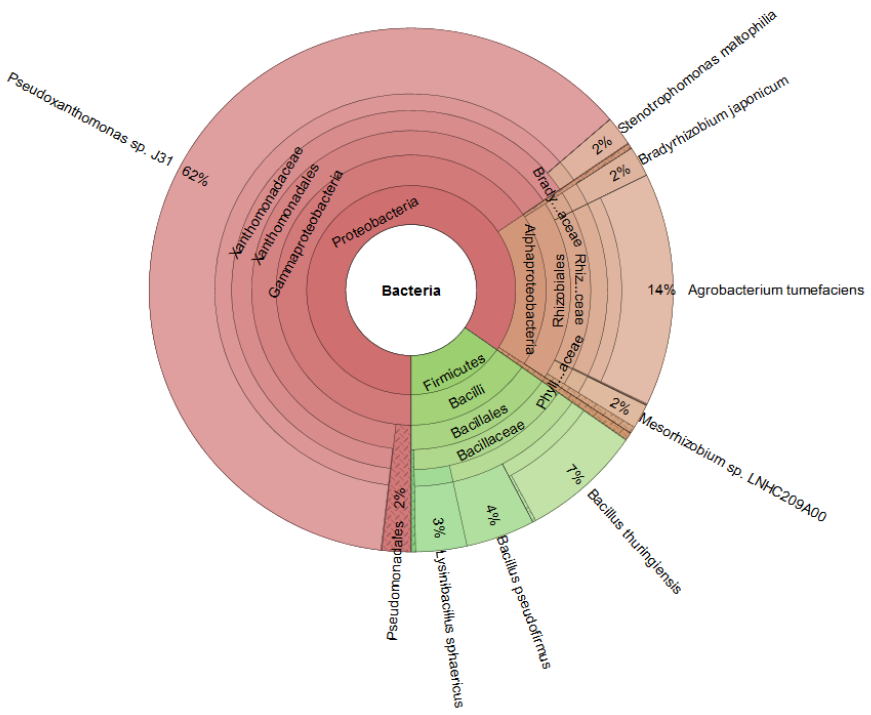


Figure D-2. Approximately Genus-level Depiction of Microbial Population for Sample 22-DMM-08 Collected during: A) Sampling Event 1 and B) Sampling Event 2

A



B

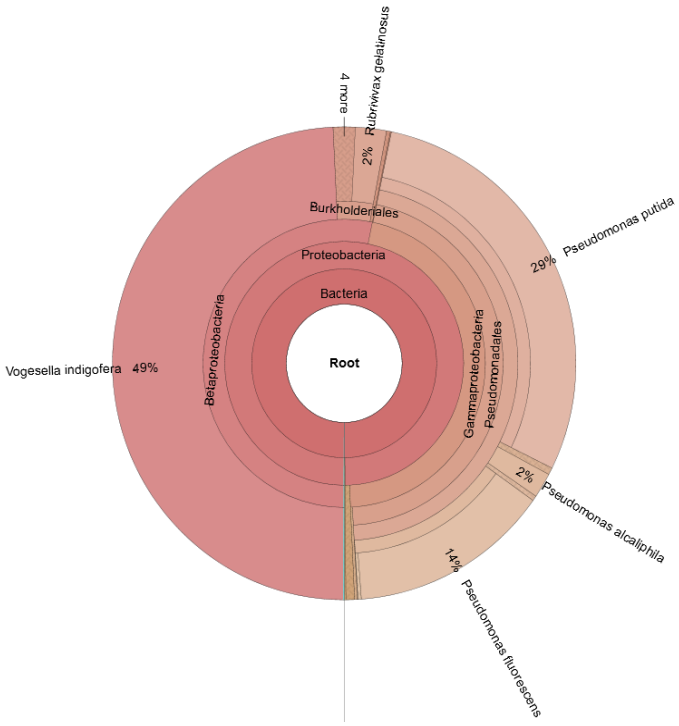
Figure D-3. Approximately Genus-level Depiction of Microbial Population for Sample 22-BMW-11 Collected during: A) Sampling Event 1 and B) Sampling Event 2

A

B

Figure D-4. Approximately Genus-level Depiction of Microbial Population for Sample 22-BMW-8 Collected during: A) Sampling Event 1 and B) Sampling Event 2

A



B

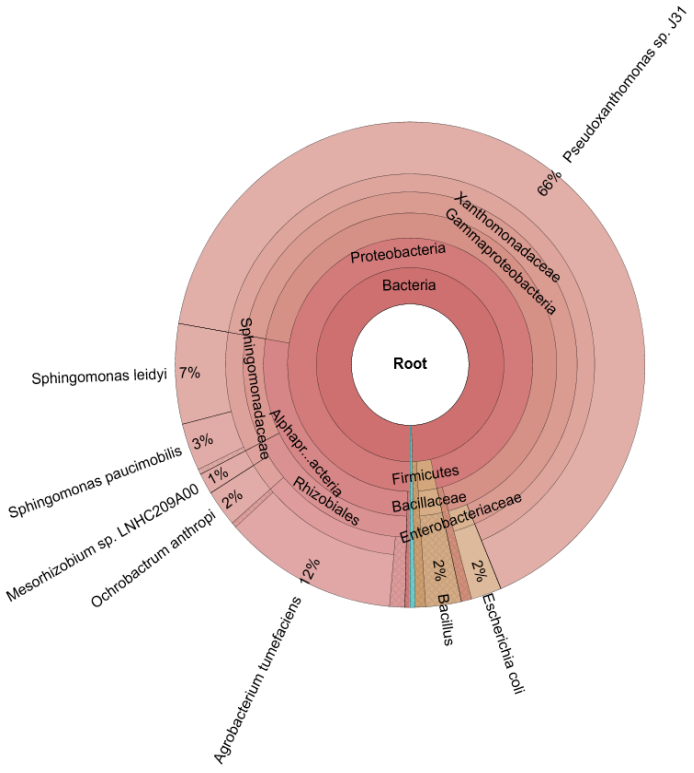
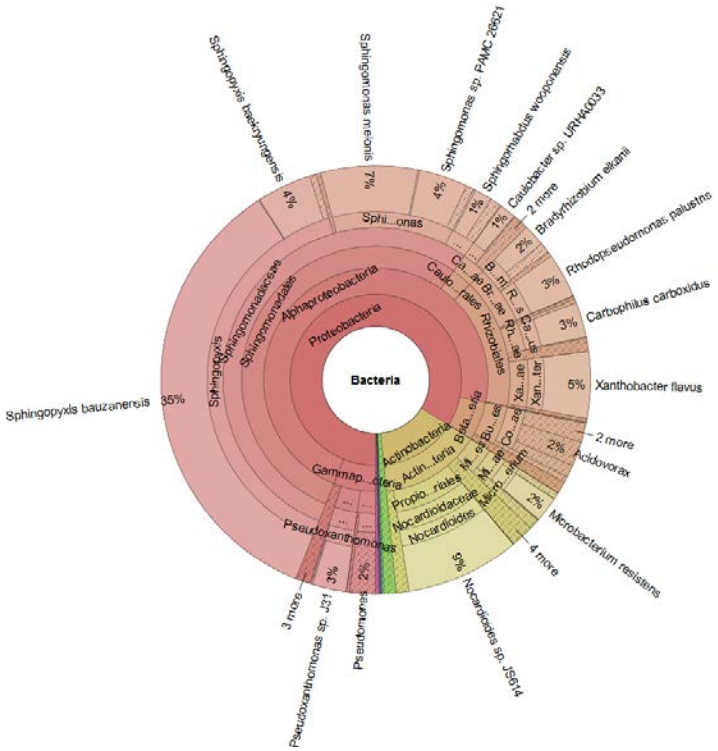


Figure D-5. Approximately Genus-level Depiction of Microbial Population for Sample 22-BMW-15 Collected during: A) Sampling Event 1 and B) Sampling Event 2

A



B

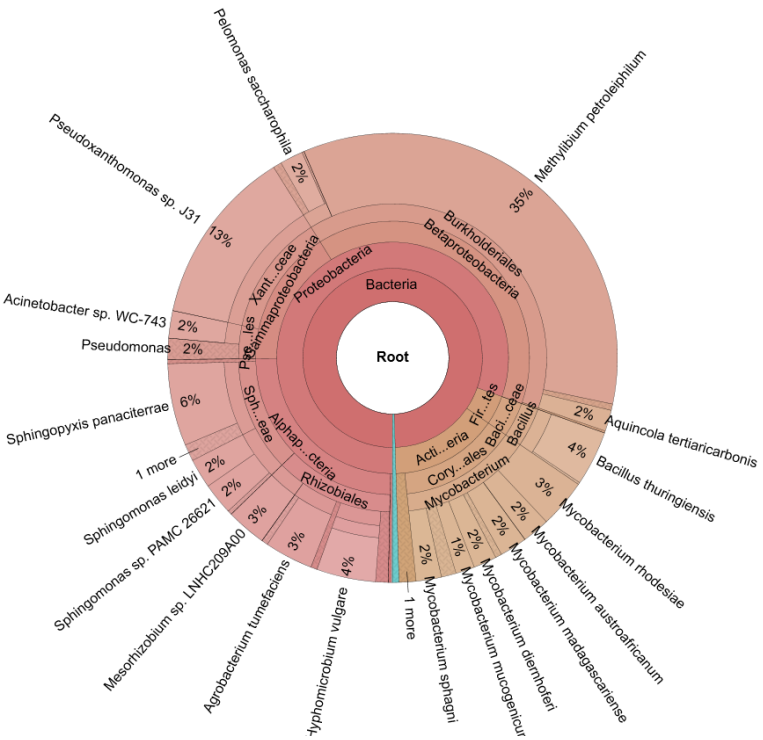
Figure D-6. Approximately Genus-level Depiction of Microbial Population for Sample 22-DMM-05 Collected during: A) Sampling Event 1 and B) Sampling Event 2

A

B

Figure D-7. Approximately Genus-level Depiction of Microbial Population for Sample 22-BMW-03 Collected during: A) Sampling Event 1 and B) Sampling Event 2

A



B

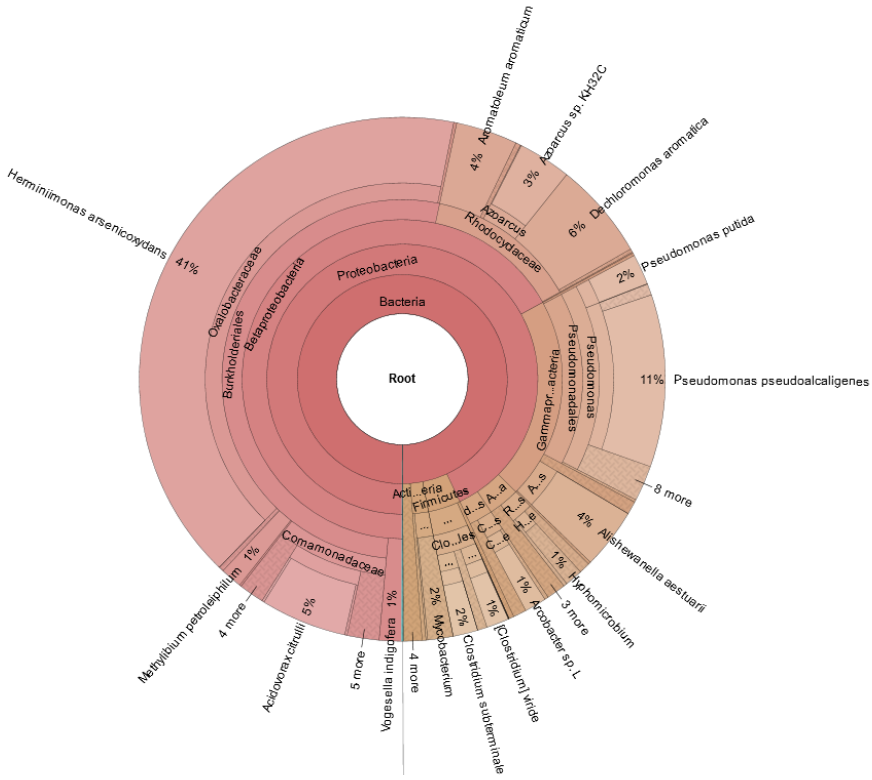


Figure D-8. Approximately Genus-level Depiction of Microbial Population for Sample 1327-MW-01R Collected during: A) Sampling Event 1 and B) Sampling Event 2

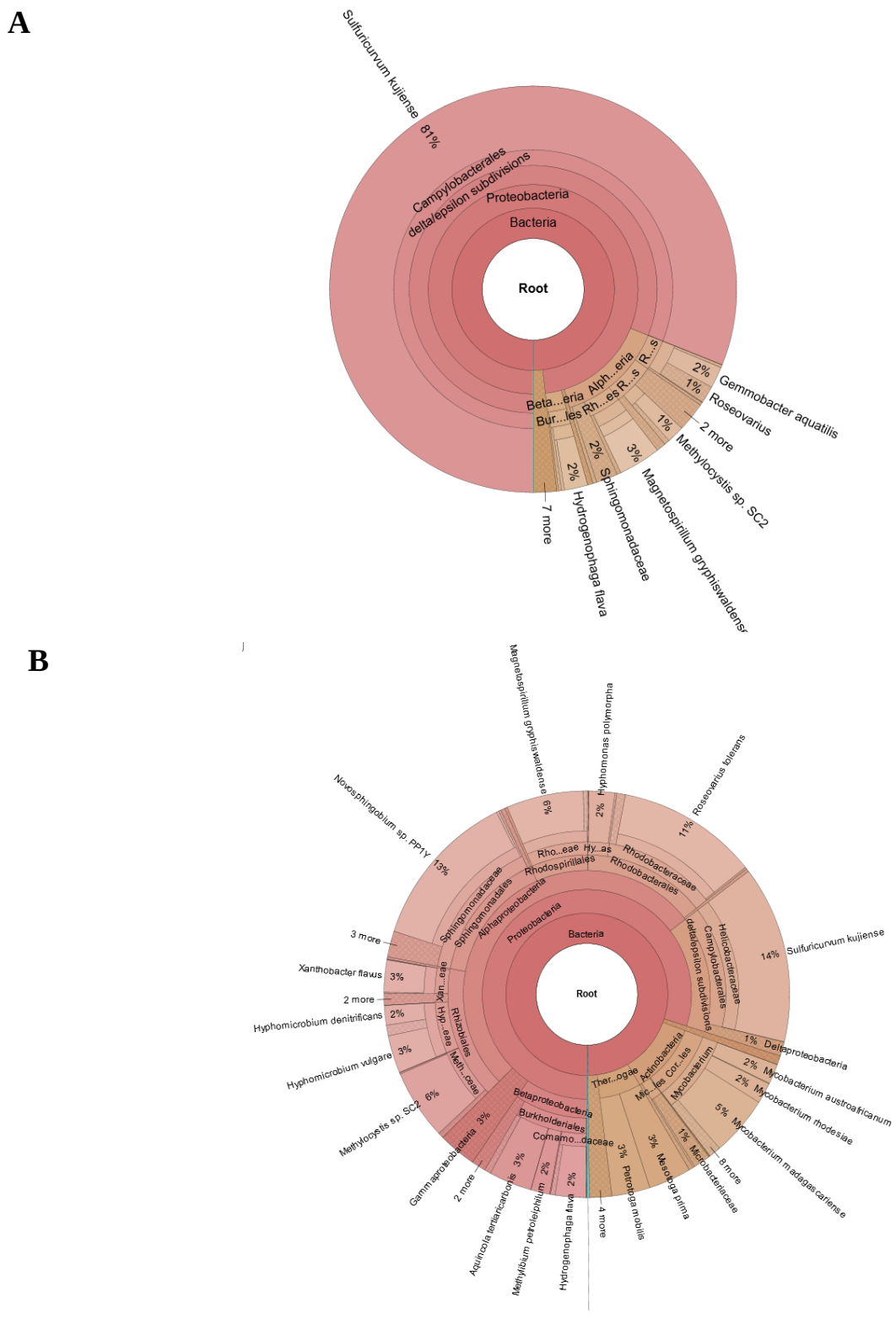
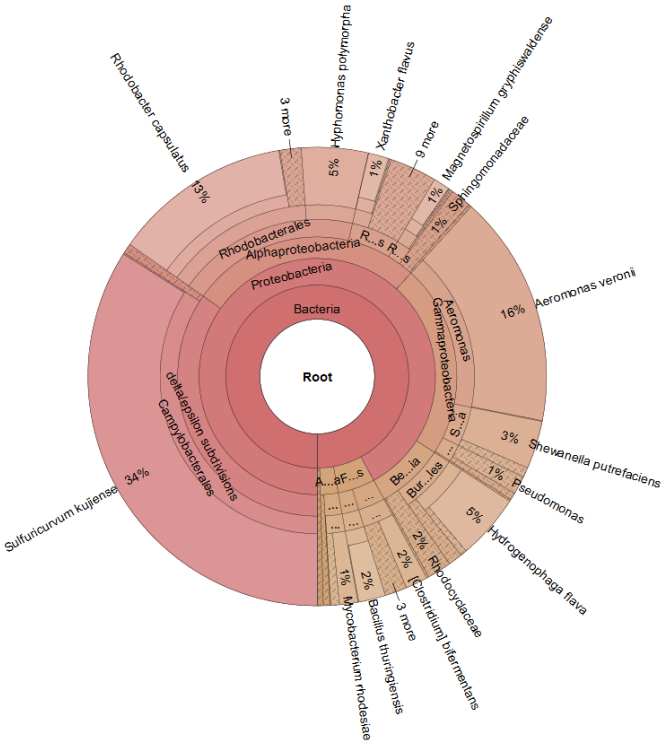


Figure D-9. Approximately Genus-level Depiction of Microbial Population for Sample 1327-RW-07 Collected during: A) Sampling Event 1 and B) Sampling Event 2

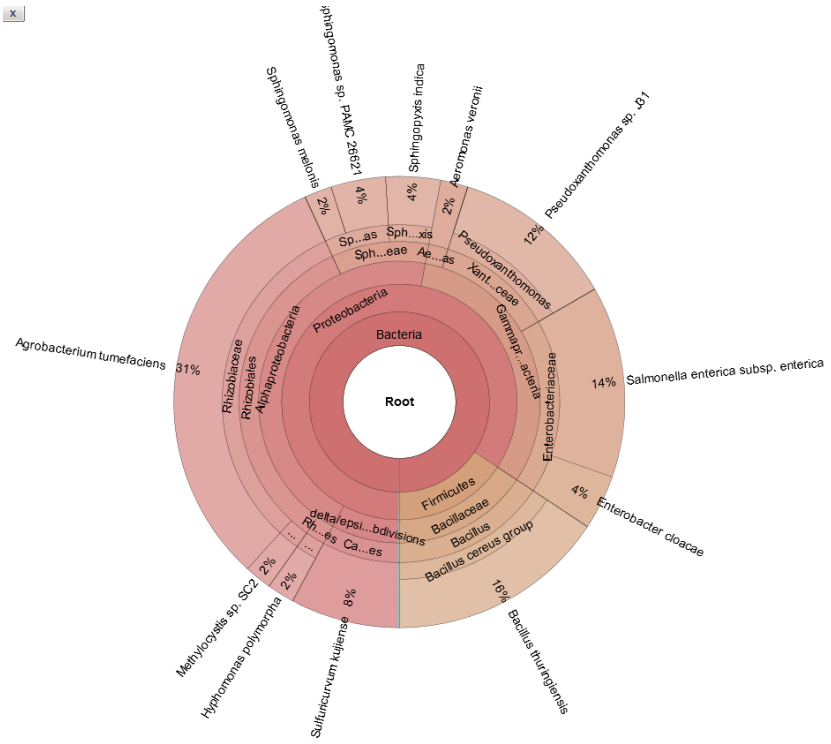
A



B

Figure D-10. Approximately Genus-level Depiction of Microbial Population for Sample 1327-MW-07R Collected during: A) Sampling Event 1 and B) Sampling Event 2

A



B

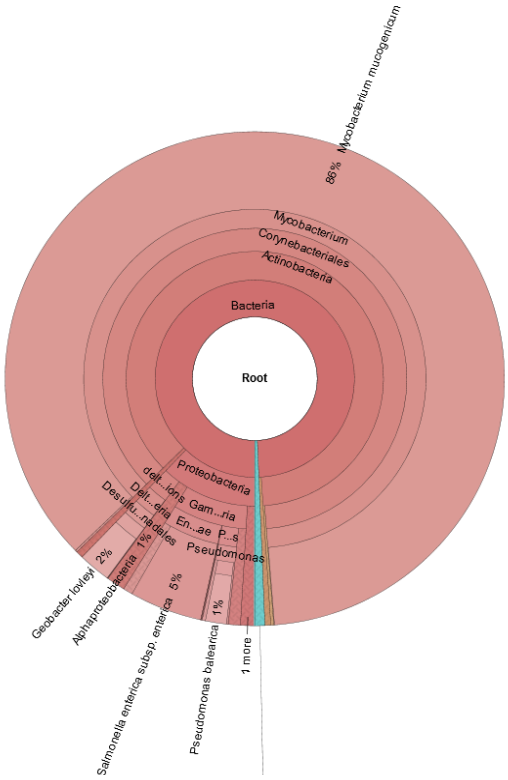
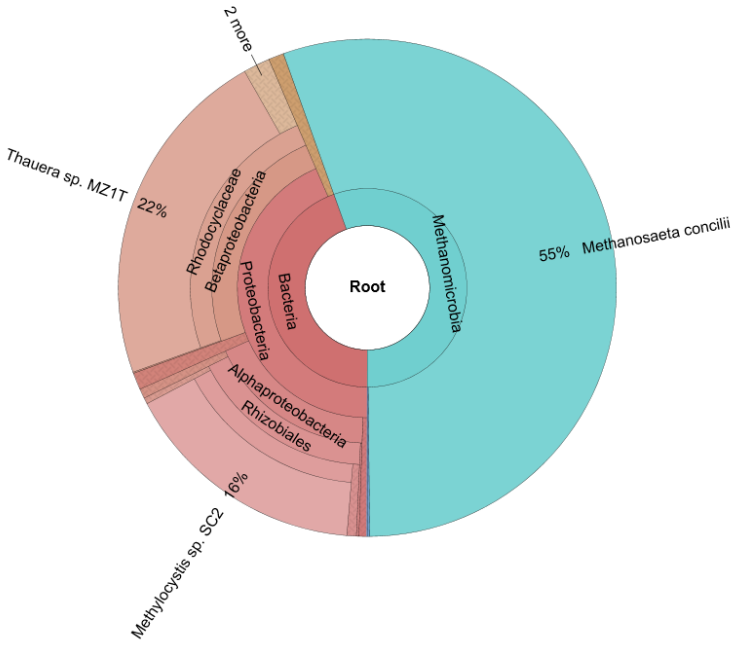


Figure D-11. Approximately Genus-level Depiction of Microbial Population for Sample 1327-MW-23 Collected during: A) Sampling Event 1 and B) Sampling Event 2

A



B

Figure D-12. Approximately Genus-level Depiction of Microbial Population for Sample 1327-MW-39 Collected during: A) Sampling Event 1 and B) Sampling Event 2

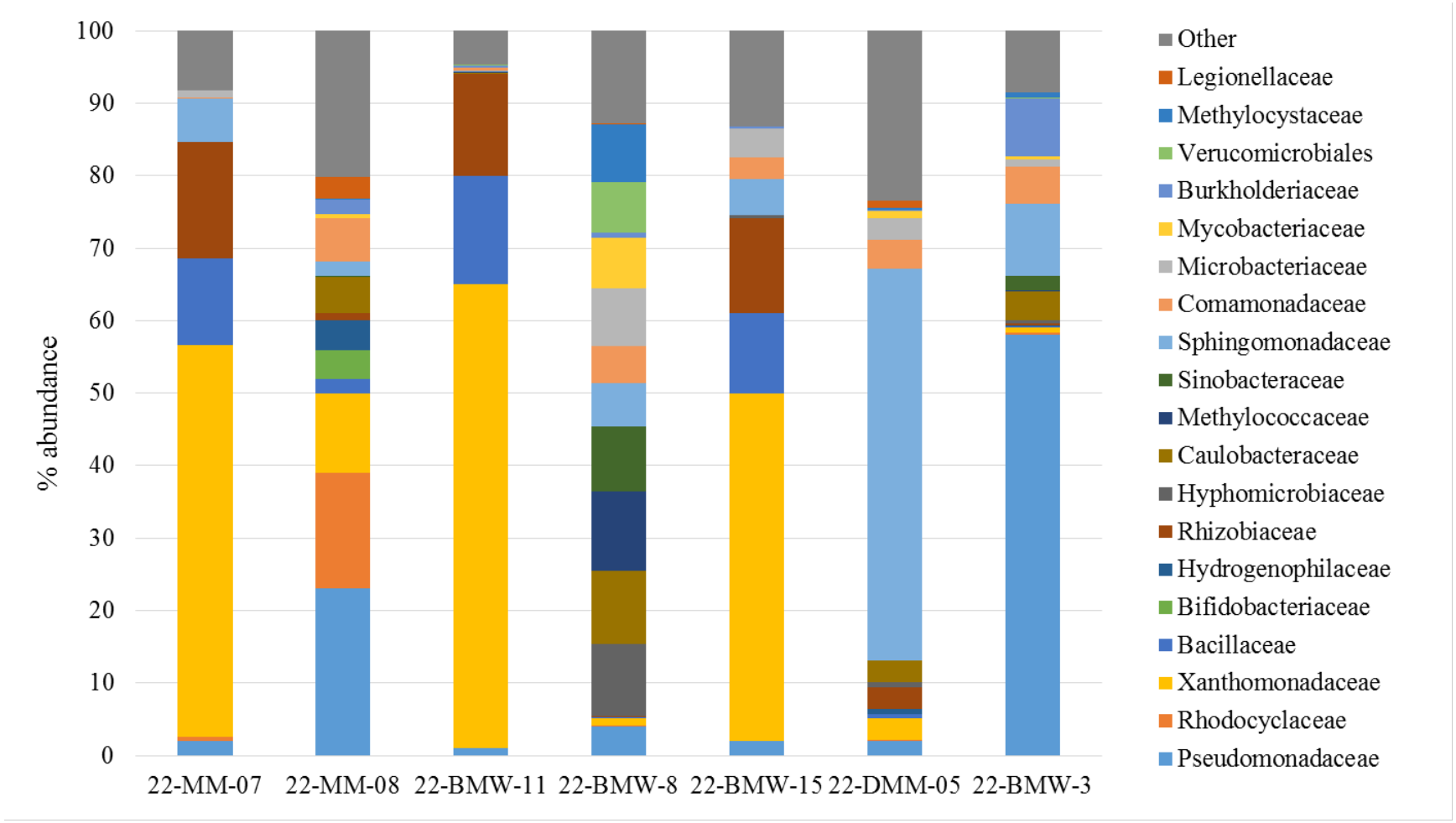


Figure D-13. Percentage Abundance of Microbial Families Found in Samples Collected from 22 Area MCX Gas Station Site during Sampling Event 1

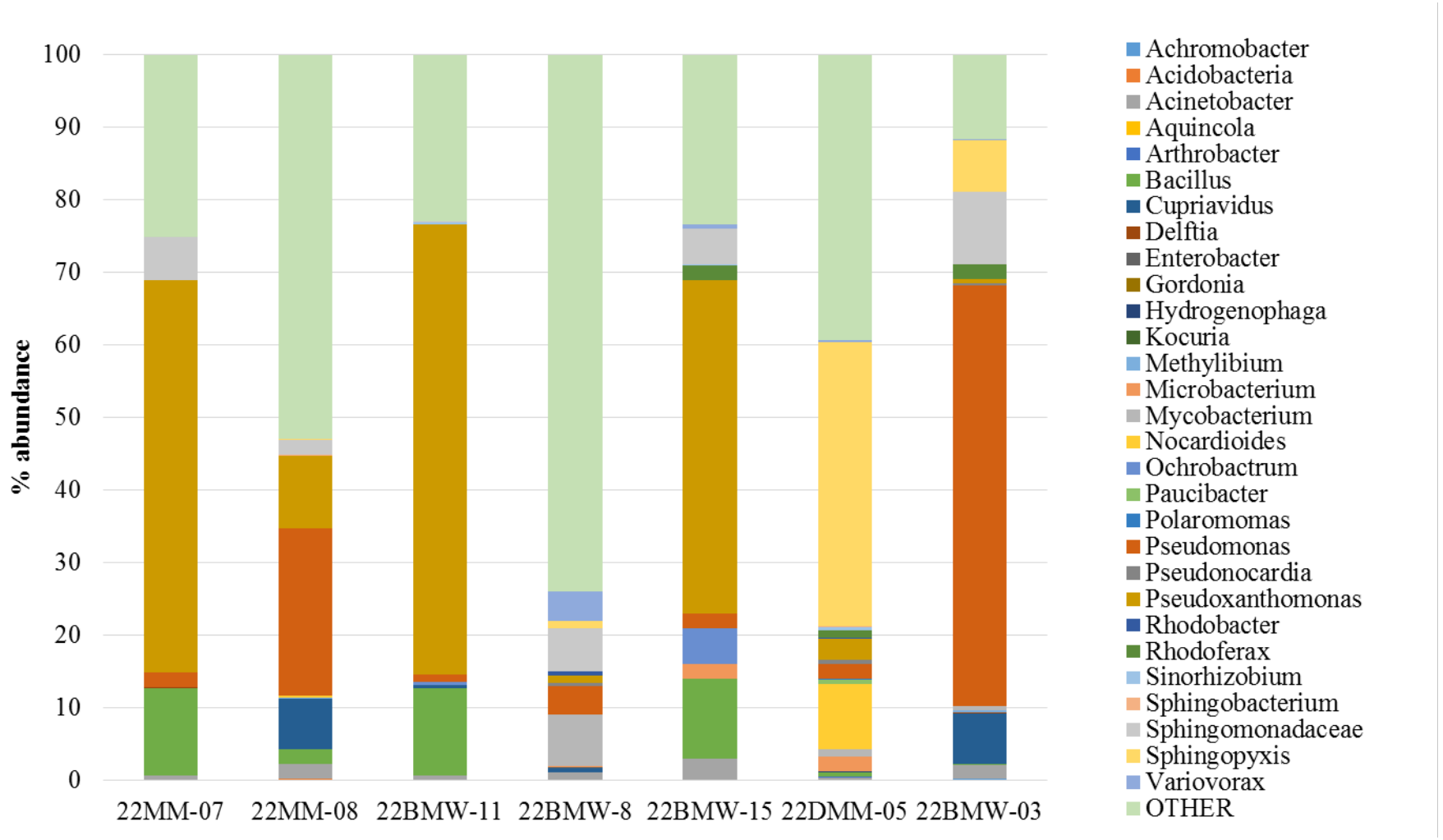


Figure D-14. Percentage Abundance of Microbial MTBE and TBA Degrading Microorganisms on Genus Level Detected in Samples Collected from 22 Area MCX Gas Station Site during Sampling Event 1

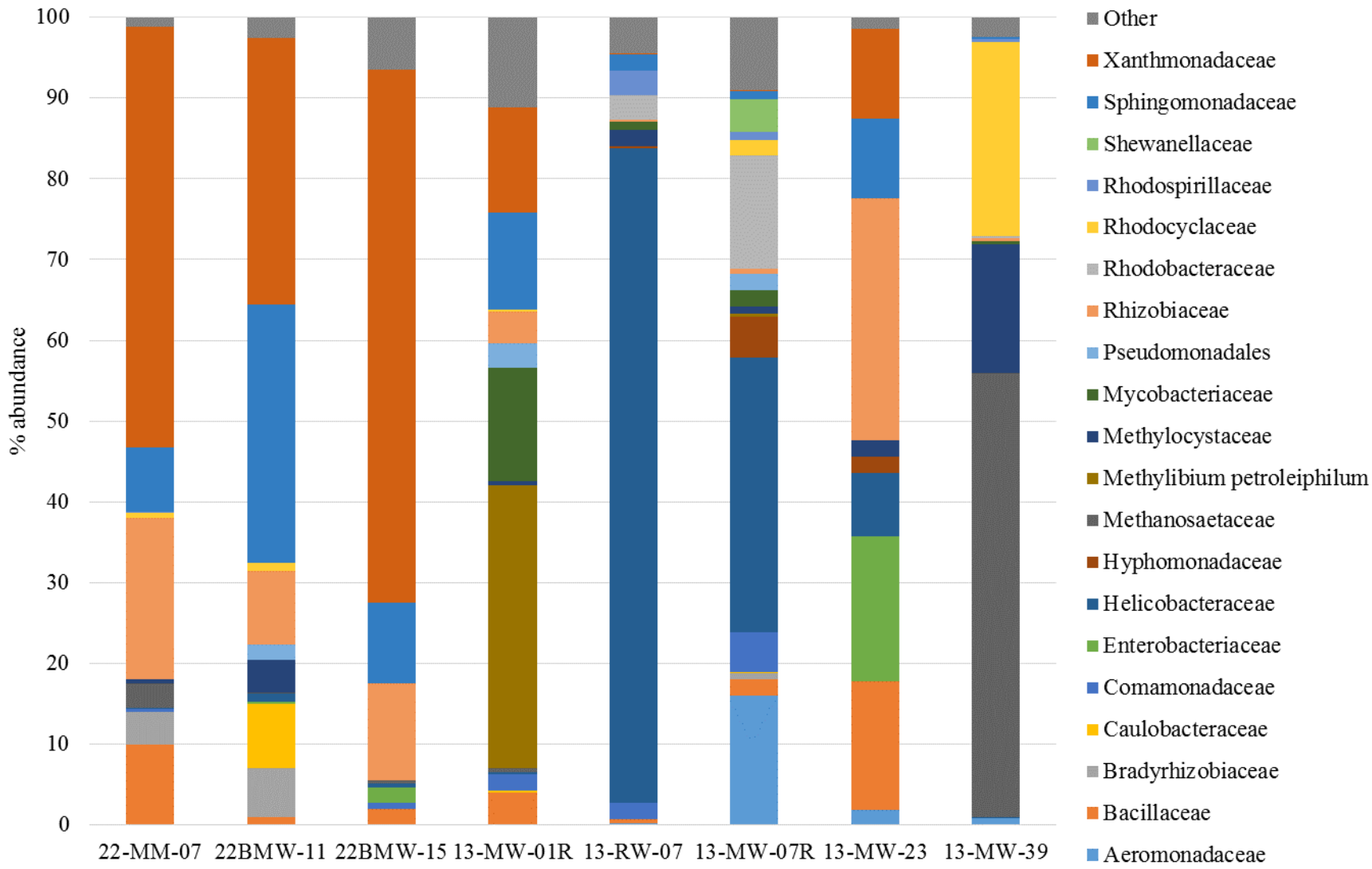


Figure D-15. Percentage Abundance of Microbial Families Found in Samples Collected from 22 Area MCX Gas Station and 13 Area Camp Pendleton Site during Optional Sampling Event

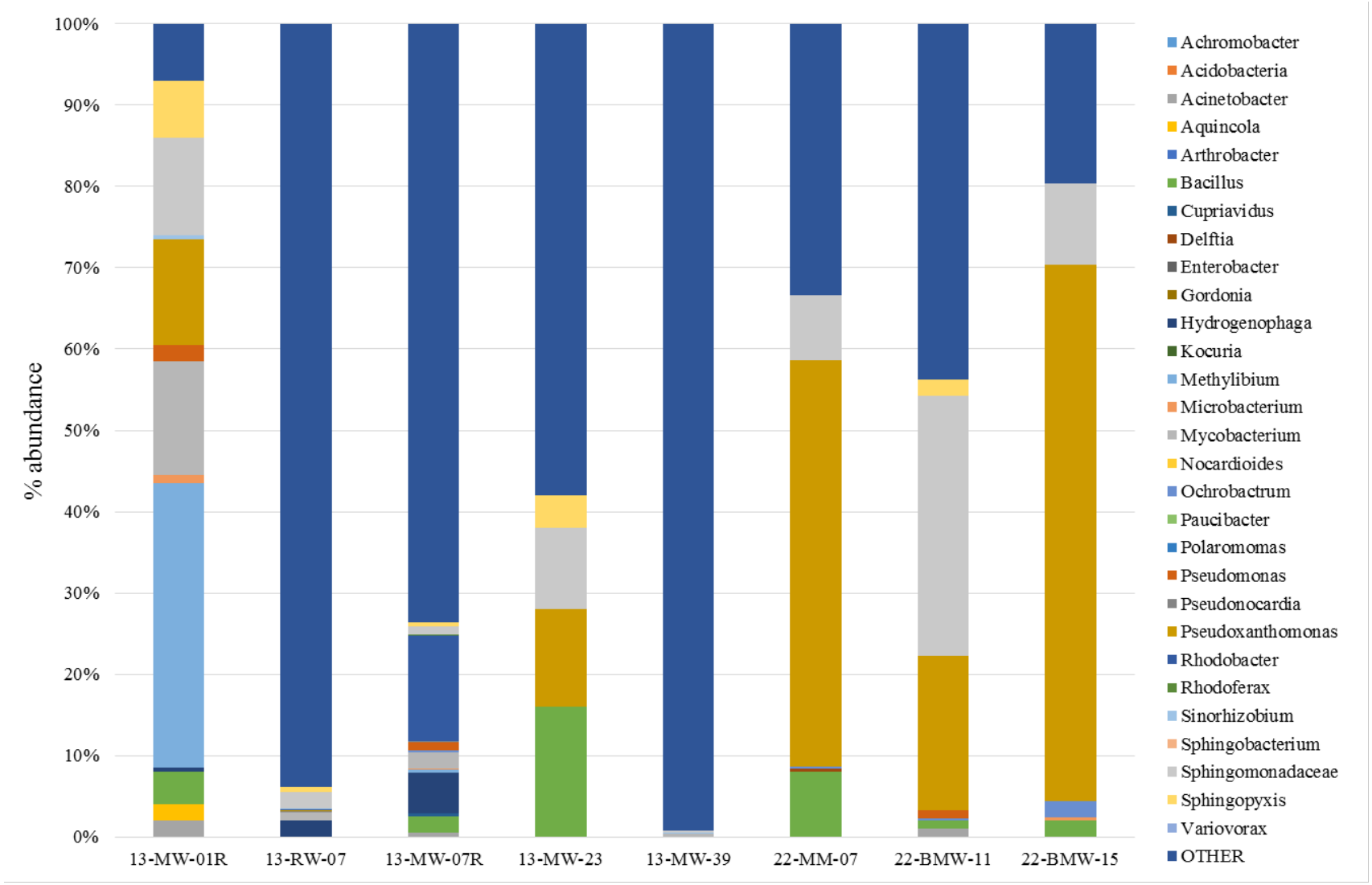


Figure D-16. Percentage Abundance of Microbial MTBE and TBA Degrading Microorganisms on Genus Level Detected in Samples Collected from 22 Area MCX Gas Station and 13 Area Camp Pendleton Site during Optional Sampling Event

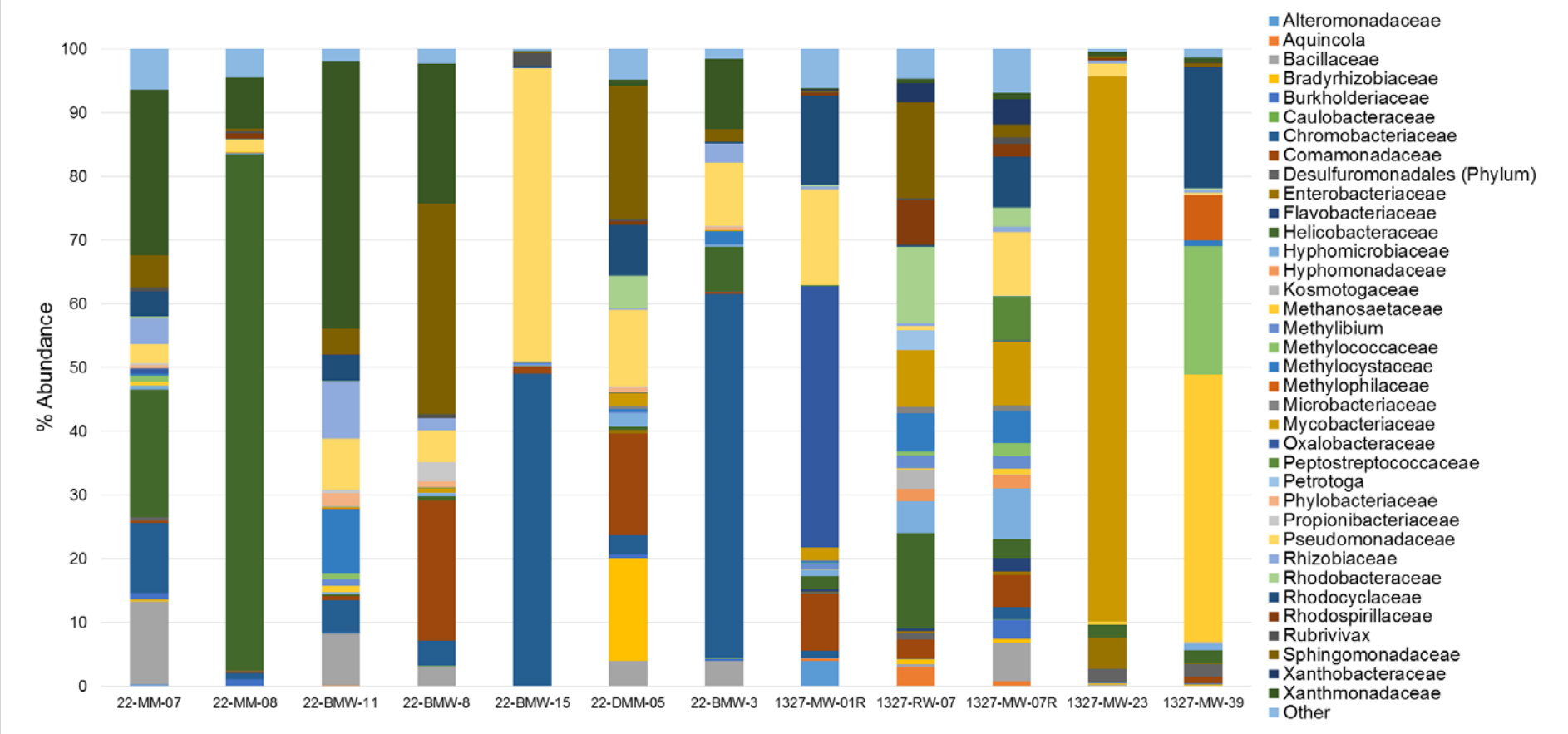


Figure D-17. Percentage Abundance of Microbial Families Found in Samples Collected from 22 Area MCX Gas Station and 13 Area Camp Pendleton Site during Sampling Event 2

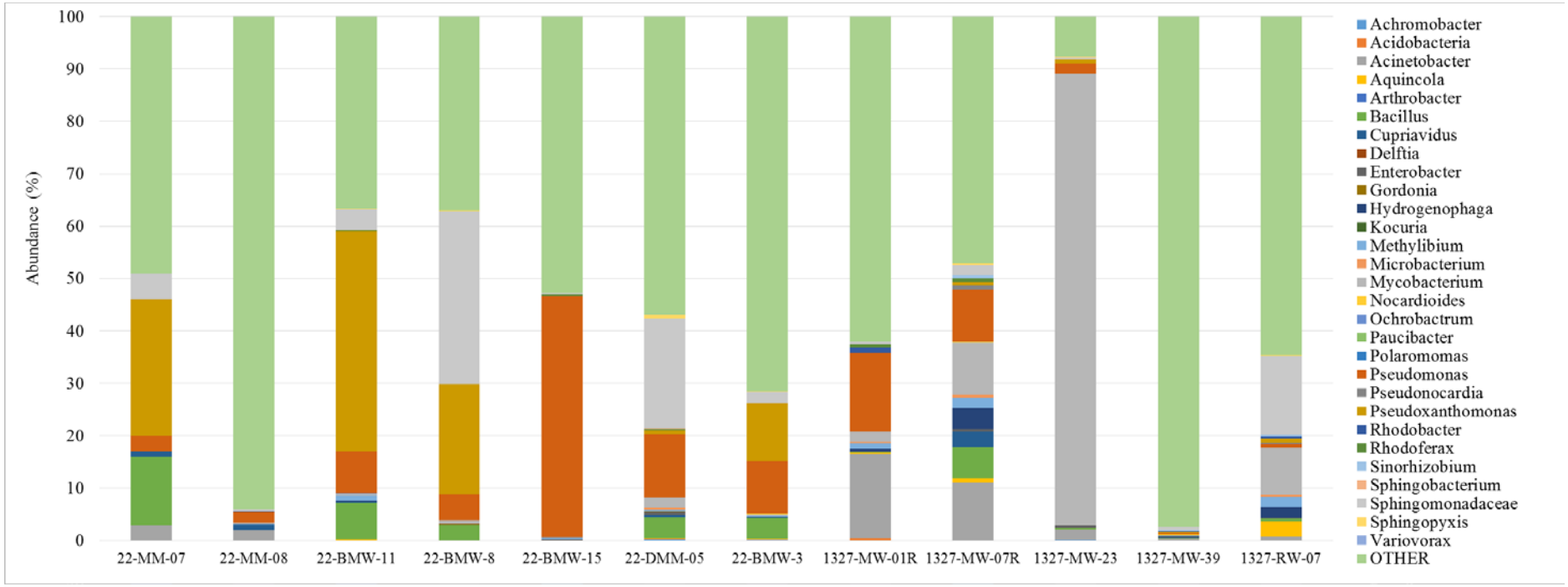


Figure D-18. Percentage Abundance of Microbial MTBE and TBA Degrading Microorganisms on Genus Level Detected in Samples Collected from 22 Area MCX Gas Station and 13 Area Camp Pendleton Site during Sampling Event 2

Table D-18. Results of Assembly and Quality Filtration of Reads for Samples Collected during Sampling Event 1

FASTQ File Name	# Reads	Maximum Assembled Read Length	Minimum Assembled Read Length	# Assembled Reads	% Assembled reads	# Discarded Reads	% Discarded Reads	# Unassembled Reads	% Unassembled Reads
22BMW-03	3,258,750	552	250	3,034,007	93.1%	8,518	0.3%	216,225	6.6%
22BMW-8	2,292,659	552	250	2,133,258	93.0%	3,555	0.2%	155,846	6.8%
22BMW-11	89,092	548	250	21,677	24.3%	63,553	71.3%	3,862	4.3%
22BMW-15	58,925	483	250	20,264	34.4%	36,229	61.5%	2,432	4.1%
22DMM-05	902,281	551	250	836,883	92.8%	6,497	0.7%	58,901	6.5%
22MM-07	546,711	548	250	102,805	18.8%	426,086	77.9%	17,820	3.3%
22MM-08	252,683	549	250	198,487	78.6%	39,383	15.6%	14,813	5.9%
TOTALS	**7,401,101**			6,347,381		583,821		469,899	

Table D-19. Number of Reads and Hits per Sample during the Database Search for Samples Collected during Sampling Event 1

Sample (assembled paired reads)	Total # Reads Received	# reads Assembled Minimum Length 250, quality ≥30, minimum overlap 50	% Assembled Reads	Total # of Reads Discarded	% Reads Discarded	# Unassembled Reads	% Unassembled Reads	# Reads Sent to BLASTn	# Filtered BLAST Hits (97% ID and 80% Read Length)	Hits (% of Total Reads)
22BMW-03	3,258,750	3,034,007	93.1%	8,518	0.3%	216,225	6.6%	3,034,007	1,218,784	40.17%
22BMW-8	2,292,659	2,133,258	93.0%	3,555	0.2%	155,846	6.8%	2,133,258	345,539	16.20%
22BMW-11	89,092	21,677	24.3%	63,553	71.3%	3,862	4.3%	21,677	17,200	79.35%
22BMW-15	58,925	20,264	34.4%	36,229	61.5%	2,432	4.1%	20,264	9,165	45.23%
22DMM-05	902,281	836,883	92.8%	6,497	0.7%	58,901	6.5%	836,883	274,308	32.78%
22MM-07	546,711	102,805	18.8%	426,086	77.9%	17,820	3.3%	102,805	51,347	49.95%
22MM-08	252,683	198,487	78.6%	39,383	15.6%	14,813	5.9%	198,487	40,349	20.33%
Average	1,057,300	906,769	62%	83,403	32.5%	67,128	5.4%	906,769	279,527	40.57%
TOTAL	7,401,101	6,347,381		583,821		469,899				

Table D-20. Results of Assembly and Quality Filtration of Reads for Samples Collected during Optional Sampling Event

FASTQ File Name	# Reads	Maximum Assembled Read Length	Minimum Assembled Read Length	# Assembled Reads	% Assembled Reads	# Discarded Reads	% Discarded Reads	# Unassembled Reads	% Unassembled Reads
13-MW-07R	1,075,617	551	250	356,031	33.10%	690	0.06%	718,896	66.84%
13-MW-23	52,358	552	250	1,542	2.95%	44,167	84.36%	6,649	12.70%
13-MW-39	3,991,063	552	250	1,362,255	34.13%	16,195	0.41%	2,612,613	65.46%
13-RW-07R	895,776	552	250	282,286	31.51%	3,820	0.43%	609,670	68.06%
22-BMW-11	102,939	469	250	2,870	2.79%	91,108	88.51%	8,961	8.71%
22-BMW-15	39,518	467	250	646	1.63%	35,962	91.00%	2,910	7.36%
22-MM-07	267,019	480	250	3,095	1.16%	247,239	92.59%	16,685	6.25%
22-MW-01R	72,483	551	250	6,028	8.32%	46,843	64.63%	19,612	27.06%

Table D-33. Number of Reads and Hits per Sample during the Database Search for Samples Collected during Optional Sampling Event

Sample (assembled paired reads)	Total # Reads Received	# Reads Assembled Minimum Length 250, quality ≥30, minimum overlap 50	% Assembled Reads	Total # of Reads Discarded	% Reads Discarded	# Unassembled Reads	% Unassembled Reads	# Reads Sent to BLASTn	# Filtered BLAST Hits (97% ID and 80% Read Length)	Hits (% of Total Reads)
22-MM-07	267,019	3,095	1.2%	247,239	92.6%	16,685	6.2%	3,095	1,284	0.48%
22-BMW-11	102,939	2,870	2.8%	91,108	88.5%	8,961	8.7%	2,870	690	0.67%
22-BMW-15	39,518	646	1.6%	35,962	91.0%	2,910	7.4%	646	286	0.72%
22-MW-01R	72,483	6,028	8.3%	46,843	64.6%	19,612	27.1%	6,028	664	0.92%
13-RW-07	895,776	282,286	31.5%	3,820	0.4%	609,670	68.1%	282,286	171,725	19.17%
13-MW-07R	1,075,617	356,031	33.1%	690	0.1%	718,896	66.8%	356,031	76,405	7.10%
13-MW-23	52,358	1,542	2.9%	44,167	84.4%	6,649	12.7%	1,542	63	0.12%
13-MW-39	3,991,063	1,362,255	34.1%	16,195	0.4%	2,612,613	65.5%	1,362,255	302,721	7.58%
Average	812,097	251,844	14%	60,753	52.7%	499,500	32.8%	251,844	69,230	4.60%

Table D-34. Results of Assembly and Quality Filtration of Reads for Samples Collected during Sampling Event 2

FASTQ File Name	# Reads	Maximum Assembled Read Length	Minimum Assembled Read Length	# Assembled Reads	% Assembled Reads	# Discarded Reads	% Discarded Reads	# Unassembled Reads	% Unassembled Reads
22-MM-07	124,345	2,757	2%	116,654	94%	4,934	4%	2,757	356
22-MM-08	518,953	499,165	96%	485	0%	19,303	4%	499,165	308,963
22-BMW-11	595,130	22,368	4%	550,143	92%	22,619	4%	22,368	3,008
22-BMW-8	96,102	8,853	9%	83,079	86%	4,170	4%	8,853	1,627
22-BMW-15	2,551,529	2,420,752	95%	8,918	0.3%	121,859	5%	2,420,752	1,477,280
22-DMM-05	915,521	880,973	96%	399	0.04%	34,149	4%	880,973	30,115
22-BMW-3	237,384	37,611	16%	191,648	81%	8,125	3%	37,611	14,140
1327-MW-01R	1,789,324	1,683,581	94%	15,640	1%	90,103	5%	1,683,581	155,002
1327-RW-07	1,489,303	1,405,266	94%	4,198	0.3%	79,839	5%	1,405,266	209,045
1327-MW-07R	1,113,272	1,051,508	94%	1,404	0.1%	60,360	5%	1,051,508	103,598
1327-MW-23	106,937	58,247	54%	44,222	41%	4,468	4%	58,247	18,480
1327-MW-39	726,884	681,237	94%	6,421	1%	39,226	5%	681,237	84,485
Average	962,139	825,040	65%	90,607	30%	46,492	5%	825,040	209,678
TOTAL	9,621,386	8,250,396		906,072		464,918		8,250,396	2,096,780

Table D-35. Number of Reads and Hits per Sample during the Database Search for Samples Collected during Sampling Event 2

Sample (assembled paired reads)	Total # Reads Received	# reads Assembled Minimum Length 250, quality ≥30, minimum overlap 50	% Assembled Reads	Total # of Reads Discarded	% Reads Discarded	# Unassembled Reads	% Un-assembled Reads	# Reads Sent to BLASTn	# Filtered BLAST Hits (97% ID and 80% Read Length)	Hits (% of Total Reads)
22-MM-07	124,345	2,757	2%	116,654	94%	4,934	4%	2,757	356	0.3%
22-MM-08	518,953	499,165	96%	485	0%	19,303	4%	499,165	308,963	60%
22-BMW-11	595,130	22,368	4%	550,143	92%	22,619	4%	22,368	3,008	1%
22-BMW-8	96,102	8,853	9%	83,079	86%	4,170	4%	8,853	1,627	2%
22-BMW-15	2,551,529	2,420,752	95%	8,918	0.3%	121,859	5%	2,420,752	1,477,280	58%
22-DMM-05	915,521	880,973	96%	399	0.04%	34,149	4%	880,973	30,115	3%
22-BMW-3	237,384	37,611	16%	191,648	81%	8,125	3%	37,611	14,140	6%
1327-MW-01R	1,789,324	1,683,581	94%	15,640	1%	90,103	5%	1,683,581	155,002	9%
1327-RW-07	1,489,303	1,405,266	94%	4,198	0.3%	79,839	5%	1,405,266	209,045	14%
1327-MW-07R	1,113,272	1,051,508	94%	1,404	0.1%	60,360	5%	1,051,508	103,598	9%
1327-MW-23	106,937	58,247	54%	44,222	41%	4,468	4%	58,247	18,480	17%
1327-MW-39	726,884	681,237	94%	6,421	1%	39,226	5%	681,237	84,485	12%
Average	962,139	825,040	65%	90,607	30%	46,492	5%	825,040	209,678	13%
TOTAL	9,621,386	8,250,396		906,072		464,918		8,250,396	2,096,780	

APPENDIX E

TARGETED PROTEOMICS OF DEGRADATION BIOMARKERS

Table E-1. MTBE-degrading Peptides Identified in 1327-MW-01R Sample

Gene	Confidence	Sequence
mdpH	99	DINLILDLANEEHYAAR
mdpH	99	GIGVVGFDLSPAATK
mdpH	99	GLGEENFTSVVK
mdpH	99	IEVSPAAVAQQVDVVVTSLPNPPIVR
mdpH	99	LYEILSVSGGR
mdpH	99	PGSTLIETSTIDPNTIR
mdpH	99	SAHFISGFQK
mdpH	96.68	VIEGDYGASFK
mdpH	99	GYEATAGIR
mdpH	89.85	DVYLGKQGLVAQARPGSTLIETSTIDPNTIR
mdpH	99	DINLILDLANEEHYAAR
mdpH	99	GIGVVGFDLSPAATK
mdpH	99	IEVSPAAVAQQVDVVVTSLPNPPIVR
mdpH	99	LYEILSVSGGR
mdpH	99	PGSTLIETSTIDPNTIR
mdpH	98.49	SAHFISGFQK
mdpJ	99	AAIVAAAPQR
mdpJ	99	DLQNAAPTNLEILR
mdpJ	99	GASLEFGIVQER
mdpJ	99	HFNDEVDPEHR
mdpJ	99	IDFLIGQTR
mdpJ	99	ILHEDLVAFR
mdpJ	99	LRDLQNAAPTNLEILR
mdpJ	99	LSDDEVWIR
mdpJ	99	RLSDDEVWIR
mdpJ	99	RPEFPVFDGYVLPK
mdpJ	99	YWQPVCLSQELTDVPK
mdpJ	99	LLEAPAEPPDTK
mdpJ	99	RPEFPVFDGYVLPKG
mdpJ	97.46	HSIAFGWR
mdpJ	70.65	RLDEIEASL
mdpJ	91.4	RPEFPVFDGY
mdpJ	99	VPGDYEAIVSQGPIAVHGLEHPGR
mdpJ	99	DLQNAAPTNLEILR
mdpJ	99	GASLEFGIVQER
mdpJ	99	IDFLIGQTR
mdpJ	99	ILHEDLVAFR
mdpJ	96.55	LLEAPAEPPDTK

Table E-1 (cont). MTBE-degrading Peptides Identified in 1327-MW-01R Sample

Gene	Confidence	Sequence
mdpJ	61.81	LRDLQNAAPTNLEILR
mdpJ	62.29	LSDDEVWIR
mdpJ	99	RLSDDEVWIR
mdpJ	99	RPEFPVFDGYVLPK
mdpK	99	QITYQGIGINAYEFVR
mdpK	99	SSAHAAFQEELAPLAAK
mdpK	99	GLDIAALLR
mdpK	99	YLIAVLR
mdpK	52.31	SSAHAAFQEELAPLAAK
mdpO	99	EVLLQDTPPQAIIDSIR
mdpP	99	VEPSESVHAAGAAALETFR
mdpR	99	IFEILEEVEK
mdpR	99	LSGTVQADILK
mdpR	99	TYTAADIADTPLEDIGLPGR
mdpR	88.97	IEIHPYDNTTAER
mdpR	99	LIEQGWFQK
mdpR	99	QIADFAYETALR
mdpR	64.62	TYTAADIADTPLEDIGLPGRYPFTR
mdpR	47.14	EYIYPIAPSVR
mdpR	62.29	LSGTVQADILK

Table E-2. Identified Potential mdpA Peptides to Target

Peptide	Notes	BlastP results
TGDGHGEPK		Unique to MTBE monooxygenase
DSGGSGETR		Identical to one other protein from *Nisaea denitrificans*
YAWAIGVLWPMLPVIGIA AAQITGEAAYYWLAPFLTI VIPILDLVIGTSQR	Contains Thr59, which is indicative of MTBE monoxygenase	Unique to MTBE monooxygenase
AAFYWLAPFLTFVVIPLLD MVIGSSQK	Contains Thr59	Unique to MTBE monooxygenase
NPPANAIQALEEDNYYK		Unique to MTBE monooxygenase
NPPESAIK		Identical to two other proteins, both hypothetical proteins from *Cladophialophora immunda*
ICLAVTAYGQYMIDHNR	Conserved in all entries	Unique to MTBE monooxygenase
DVSTPEDSSSAR		Unique to MTBE monooxygenase
DVATPEDSSSAR		Unique to MTBE monooxygenase
MGESIYAFALR		Also identical to alkane 1-monooxygease from *Pseudovibrio denitrificans*, Pseudovibrio sp. FO-BEG1, *Pseudovibrio axinellae*, uncultured bacterium
MGEGIYFFALR		Unique to MTBE monooxygenase
ELPYTGFIRPWR	Conserved in all entries	Unique to MTBE monooxygenase
PEHSWNTDHIASNLIYFHV QR		Unique to MTBE monooxygenase
PEHSWNTDHIASNVIYFHV QR		Unique to MTBE monooxygenase
HSDHHAFPTR	Conserved in all entries	Also identical to several alkane 1-monooxygease sequences (may not be unique to MTBE monooxygenase; need to check these entries for Thr59.
SYQALCSYSNVPTMPSGYP GMIWICHVPPLYR		Unique to MTBE monooxygenase
SYSDVPTMPSGYPGMIWL CHIPPLFR		Unique to MTBE monooxygenase
AIMDPLLLK		Unique to MTBE monooxygenase
AVMDPLLLK		Unique to MTBE monooxygenase
QYDGDITK		Unique to MTBE monooxygenase

APPENDIX F

SLUG TEST DATA

Calculation Brief
Slug Test Results
22 Area Camp Pendleton, CA
IT Project Number: 821816
By: TP Date: 06/08/01

Introduction

Slug tests were conducted at new monitoring wells at 22 Area, MCB Camp Pendleton, CA on June 4, 2000 in support of the site characterization.

Objective

The objective was to conduct slug tests on site wells and analyze the slug test data for hydraulic conductivity (K).

Field Procedures

A 2.7-inch-diameter slug constructed of flush-threaded 5-foot sections (one and two sections were used) of stainless steel pipe with steel caps at both ends was used to induce the initial displacement in the wells (the heights of the top and bottom caps were 4.5 and 2.5 inches, respectively, for a total length of the slug of 5.58 feet). The threads of the slug were sealed with Teflon® tape to prevent leakage, and the slug was weighted using a section of stainless steel pipe. The top of the slug was capped with a steel cap; the cap's moving eyelet was welded. The slug was suspended on a rope. A second slug made of 1-inch diameter stainless steel pipe 5.1 feet long with weld-seals at both ends was used for testing at 2-inch diameter wells.

Both slug-in and slug-out tests were conducted to provide information on the effect of the well construction and allow a more accurate analysis of results. The tests were repeated on wells that recovered fast, because data collection and analysis on rapidly recovering wells is more susceptible to errors; repeated tests can also provide indications on well construction (clogging, development, etc.).

Prior to the slug test, the depth to static groundwater was measured with an electrical sounder. Changes in water level were digitally recorded using 10 or 20 pounds per square inch (PSI) pressure transducers and a Hermit 2000 data logger. The time-displacement data were recorded in feet with a specified reference point of zero feet. Before the tests were started, the pressure transducer constants and test ID were verified; the test data were electronically downloaded and inspected after each test. All test activities were recorded in field logs. All equipment that came into contact with groundwater (slug, rope, pressure transducer, cable, and electric sounder) was decontaminated before each test using a solution of Alconox®, followed by a rinse with deionized (DI) water. The testing started with a slug-in test followed by a slug-out test.

Data Analysis Methods

The guidance for slug test analysis given by Butler (1998) was followed. The results of the representative tests were used to calculate an average K value for each well.

The time-displacement data were imported into a spreadsheet and the initial noisy portion (showing inertial effects immediately after the slug insertion) was removed. The initial displacement (H_0) caused by the insertion or withdrawal of the slug was estimated from semilog plots (log of time) of the recorded data.

The data were first analyzed using the Cooper et al. method (Cooper et al., 1967). This method solves for transmissivity (T) and storativity (S). K can be obtained from T by dividing T by the saturated thickness of the layer or by the screen length in case of a partially penetrating well. This method was developed for confined aquifers but can also be used for unconfined aquifers with sufficient accuracy.

The data were then analyzed using the Bouwer and Rice method (Bouwer and Rice, 1976; Bouwer, 1989) for K. This method accounts for partial penetration using a strictly radial flow approximation and solves directly for K (it does not include aquifer anisotropy and assumes pseudo-steady flow conditions around the tested well). The method uses a linear part of the semilog plot of data (log of displacement), therefore, the actual initial displacement during the test is not used for tests affected by flow from the sandpack. This method can be used for tests affected by flow from the sandpack.

AQTESOLV™ version 2.12 (HydroSOLVE, 1998,) was used for the analysis. The digitally recorded time-displacement data were electronically imported into AQTESOLV™. Nonlinear inversion was used to estimate T and S by the Cooper et al. method and visual straight line matching to estimate K by the Bouwer and Rice method.

The values of saturated thickness, screen length, height of water column in the well (for the Bouwer and Rice method; these parameters are not used by the Cooper et al. method), initial displacement, and casing and boring diameters were entered from Table 1. The value of the porosity of the sandpack used by AQTESOLV™ to calculate the effective radius of screen was set to zero, because the effective casing radius was calculated in Table 1 (this allowed to avoid using an assumed sandpack porosity value).

Results

The plots of the data with fit curves for the Cooper et al. method and Bouwer and Rice method are presented in Attachment 1. The well construction information and the analysis results are presented in Table 1.

The results for individual wells are discussed below. The Cooper et al. method is generally not very sensitive to S; therefore, the estimated S values should be used with caution.

The K values estimated from individual tests are presented in Table 1. The representative K value was calculated for each location as a mean of K values from tests that had a good fit between the observed and calculated data. The representative K values are presented with accuracy to two significant digits.

Well MM06

A total of two slug tests were conducted. The well is screened across the water table. The results from both methods were comparable; good fit of measured and calculated data was obtained from the slug-in test and poor fit from the slug-out test from both methods. The representative K value is 49 ft/d. The S value is approximately 0.006.

Well MM05

A total of two slug tests were conducted. The well is screened across the water table. The results from both methods were comparable; good fit of measured and calculated data was obtained from the slug-in test for the Cooper et al. method and for both tests from the Bouwer and Rice method. Sandpack drainage effects during the slug-out test resulted in a very poor fit of the Cooper et al. method. The representative K value is 0.35 ft/d. The S value is approximately 0.001.

Well MM02

A total of two slug tests were conducted. The well is screened across the water table. Well construction effects (probably bridging of the sandpack) were apparent from the data. The results from both methods were comparable; poor fit of measured and calculated data was obtained from the slug-out test for the Cooper et al. method and for both tests from the Bouwer and Rice method. Well construction effects during the slug-in test resulted in a very poor fit of the Cooper et al. method. The representative K value is 0.68 ft/d. The S value is approximately 0.004. The results for this well are less reliable than the results for the other wells tested.

Well MM07
A total of four slug tests were conducted. The well is screened across the water table. The results from both methods were comparable; good fit of measured and calculated data was obtained from the slug-in tests for the Cooper et al. method and for all tests from the Bouwer and Rice method. Sandpack drainage effects during the slug-out test resulted in a poor fit of the Cooper et al. method. The representative K value is 0.43 ft/d. The S value is approximately between 0.0001 and 0.003.

Well MM08
A total of two slug tests were conducted. The well is screened below the water table; therefore, no effects of sandpack drainage occurred. The results from both methods were comparable; good fit of measured and calculated data was obtained from the slug-in test for the Cooper et al. method and for both tests from the Bouwer and Rice method. A systematic error in the fit of the Cooper et al. method for the slug-out test resulted likely from a partial penetration effect. The representative K value is 2.0 ft/d. Estimation of the S value failed.

Conclusions

The results of the tests led to the following conclusions:

- The K values estimated for the shallow alluvium range from 0.35 to 49 ft/d in the tested area.
- At the MM07 – MM08 well cluster, higher K value (43 ft/d) was estimated for the upper (20 feet) zone compared to the K value (2.0 ft/d) estimated for the deeper (40 feet) zone of the shallow alluvium.
- A geometric mean K value of 4.0 ft/d was calculated from the test results. This value should be used with caution because it is based on five test locations only.

References

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Butler , J. J., Jr. 1998. The Design, Performance, and Analysis of Slug Tests, CRC Press LLC, Boca Raton, Florida, 252 pp.

Cooper, H. H., J. D. Bredehoeft, and S. S. Papadopulos. 1967. Response of a finite-diameter well to an instantaneous charge of water, Water Resources Research, vol. 3, no. 1, pp. 263-269.

Appendix G

2016 SLUG TEST RESULTS

Table 1
Well Construction and Slug Test Results
Area 22 MCAS Camp Pendleton

				survey			depth below ground			depth below TOC	
Well ID	Test No.	Test Type	Date	TOC elevation	ground elevation	height of TOC above ground	TOS	BOS	Aquifer Bottom	TD	DTW
							feet	feet	feet	feet	feet
MM06	T0S0	slug-in	6/4/01	65.58	66.18	-0.6	4	21	25	20.11	7.78
MM06	T0S1	slug-out	6/4/01	65.58	66.18	-0.6	4	21	25	20.11	7.78
MM05	T1S0	slug-in	6/4/01	64	64.8	-0.8	4	21	25	20.2	7.9
MM05	T1S1	slug-out	6/4/01	64	64.8	-0.8	4	21	25	20.2	7.9
MM02	T2S0	slug-in	6/4/01	61.8	62.57	-0.77	4	21	25	20.14	8.44
MM02	T2S1	slug-out	6/4/01	61.8	62.57	-0.77	4	21	25	20.14	8.44
MM07	T3S0	slug-in	6/4/01	64.94	65.54	-0.6	4	22	25	19.88	6.53
MM07	T3S1	slug-out	6/4/01	64.94	65.54	-0.6	4	22	25	19.88	6.53
MM07	T3S2	slug-in	6/4/01	64.94	65.54	-0.6	4	22	25	19.88	6.53
MM07	T3S3	slug-out	6/4/01	64.94	65.54	-0.6	4	22	25	19.88	6.53
MM08	T4S0	slug-in	6/4/01	65.01	65.54	-0.53	30	41	45	40.27	6.56
MM08	T4S1	slug-out	6/4/01	65.01	65.54	-0.53	30	41	45	40.27	6.56

Explanation:
BOS - Bottom of screen (sandpack).
TOS - Top of screen (sandpack).
TD - Depth to the well bottom.
H0 - Initial slug displacement.
DTW - Depth to water.
K - Hydraulic conductivity.
TOC - Top of casing.

Table 1
Well Construction and Slug Test Results
Area 22 MCAS Camp Pendleton

		depth below TOC										
Well ID	**Test No.**	TOS	BOS	Aquifer Bottom	Aquifer Lithology	Saturated Thickness	Water Column above TD	**H_0**	**Slug Length**	**Slug Diameter**	**Casing Diameter**	**Boring Diameter**
		feet	feet	feet		feet	feet	**feet**	**feet**	**inches**	**inches**	**inches**
MM06	**T0S0**	3.4	20.4	24.4	SM	16.62	12.33	**0.62**	**5.58**	**2.7**	**4**	**10**
MM06	**T0S1**	3.4	20.4	24.4	SM	16.62	12.33	**1.41**	**5.58**	**2.7**	**4**	**10**
MM05	**T1S0**	3.2	20.2	24.2	ML	16.3	12.3	**0.65**	**5.58**	**2.7**	**4**	**10**
MM05	**T1S1**	3.2	20.2	24.2	ML	16.3	12.3	**1.343**	**5.58**	**2.7**	**4**	**10**
MM02	**T2S0**	3.23	20.23	24.23	ML	15.79	11.7	**1**	**5.58**	**2.7**	**4**	**10**
MM02	**T2S1**	3.23	20.23	24.23	ML	15.79	11.7	**1.6**	**5.58**	**2.7**	**4**	**10**
MM07	**T3S0**	3.4	21.4	24.4	SM	17.87	13.35	**0.205**	**5.1**	**1**	**2**	**10**
MM07	**T3S1**	3.4	21.4	24.4	SM	17.87	13.35	**0.709**	**5.1**	**1**	**2**	**10**
MM07	**T3S2**	3.4	21.4	24.4	SM	17.87	13.35	**0.22**	**5.1**	**1**	**2**	**10**
MM07	**T3S3**	3.4	21.4	24.4	SM	17.87	13.35	**0.684**	**5.1**	**1**	**2**	**10**
MM08	**T4S0**	29.47	40.47	44.47	SM	37.91	33.71	**1.43**	**5.1**	**1**	**2**	**10**
MM08	**T4S1**	29.47	40.47	44.47	SM	37.91	33.71	**1.949**	**5.1**	**1**	**2**	**10**

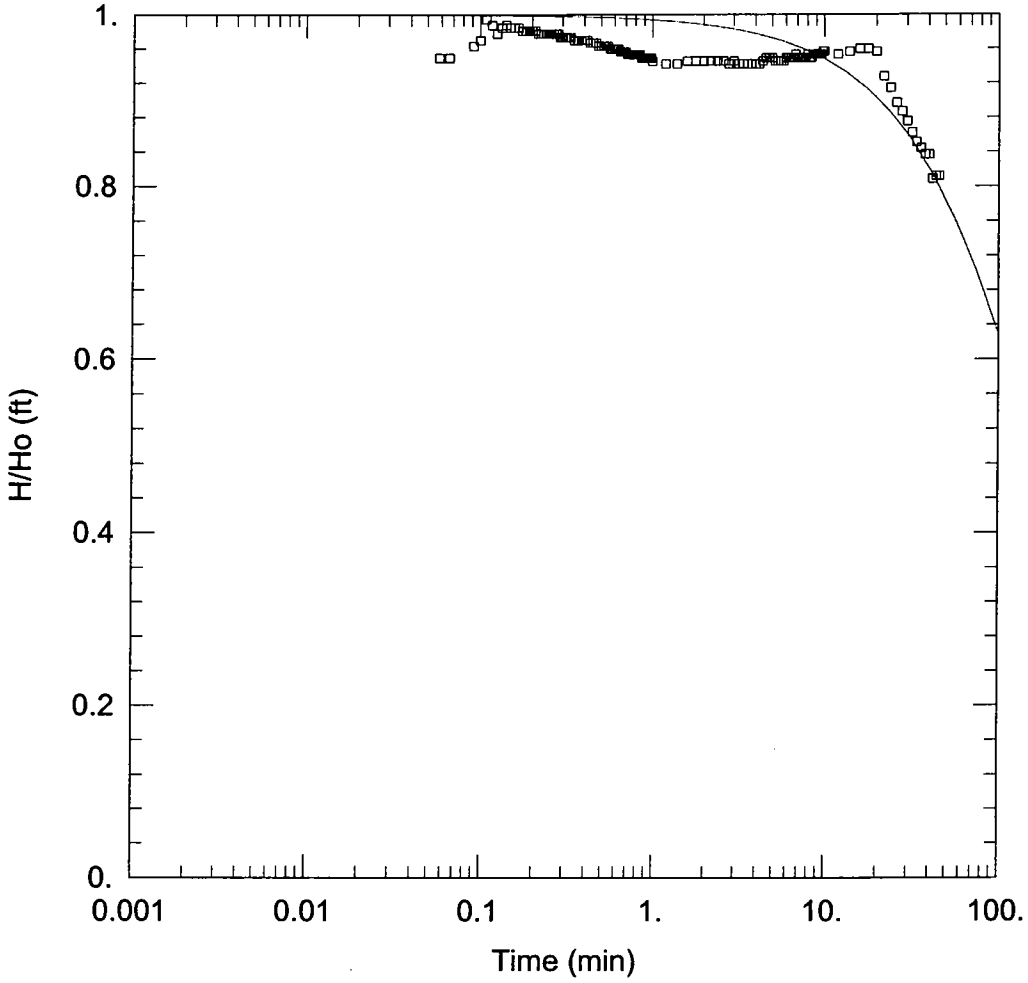
Table 1
Well Construction and Slug Test Results
Area 22 MCAS Camp Pendleton

Well ID	Test No.	Radius of Boring	Submerged Height of Slug	Water Column above BOS	Depth to TOS below Water	Effective Casing Radius
		feet	feet	feet	feet	feet
MM06	**T0S0**	0.417	5.58	12.62	0	0.338
MM06	**T0S1**	0.417	5.58	12.62	0	0.224
MM05	**T1S0**	0.417	5.58	12.3	0	0.330
MM05	**T1S1**	0.417	5.58	12.3	0	0.229
MM02	**T2S0**	0.417	5.58	11.79	0	0.266
MM02	**T2S1**	0.417	5.58	11.79	0	0.210
MM07	**T3S0**	0.417	5.10	14.87	0	0.208
MM07	**T3S1**	0.417	5.10	14.87	0	0.112
MM07	**T3S2**	0.417	5.10	14.87	0	0.201
MM07	**T3S3**	0.417	5.10	14.87	0	0.114
MM08	**T4S0**	0.417	5.10	33.91	22.91	0.083
MM08	**T4S1**	0.417	5.10	33.91	22.91	0.083

Table 1
Well Construction and Slug Test Results
Area 22 MCAS Camp Pendleton

		Cooper et al.				Bouwer and Rice		
Well ID	**Test No.**	**T**	**S**	Note	K	K	Note	Representative K
		ft^2/m	**-**		ft/d	ft/d		ft/d
MM06	**T0S0**	**0.4988**	**0.006003**	good fit	56.92	40.74	good fit	49
MM06	**T0S1**	**0.8642**	**0.000103**	poor fit	98.61	19.81	poor fit	
MM05	**T1S0**	**0.003358**	**0.001058**	good fit	0.39	0.31	good fit	0.35
MM05	**T1S1**	**NA**	**NA**	very poor fit	NA	0.34	good fit	
MM02	**T2S0**	**NA**	**NA**	very poor fit	NA	0.69	poor fit	0.68
MM02	**T2S1**	**0.004479**	**0.1**	poor fit, well construction effects	0.55	0.79	poor fit	
MM07	**T3S0**	**0.5279**	**0.000113**	good fit	51.12	32.00	good fit	43
MM07	**T3S1**	**1.025**	**1.00E-10**	poor fit	99.26	62.12	good fit	
MM07	**T3S2**	**0.2471**	**0.003331**	good fit	23.93	23.39	good fit	
MM07	**T3S3**	**0.4668**	**3.22E-05**	poor fit	45.20	64.57	good fit	
MM08	**T4S0**	**0.0319**	**1.00E-10**	good fit	4.18	1.41	good fit	2.0
MM08	**T4S1**	**0.01443**	**1.00E-10**	slightly poor fit	1.89	0.70	good fit	

Geometric mean	4.0



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM2T2S0.AQT
Date: 06/06/01 Time: 16:43:51

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

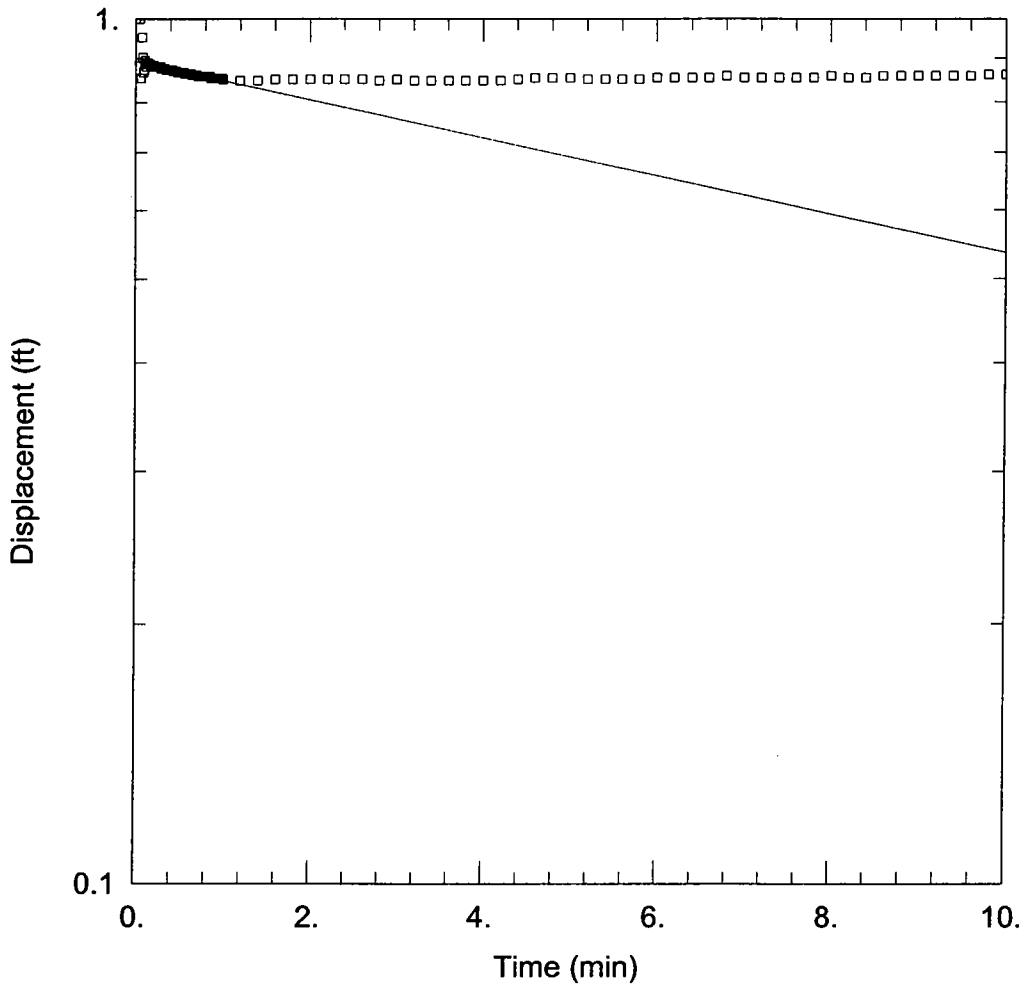
Saturated Thickness: 15.79 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.9 ft Water Column Height: 11.79 ft
Casing Radius: 0.266 ft Wellbore Radius: 0.417 ft
Screen Length: 11.79 ft

SOLUTION

Aquifer Model: Confined T = 0.001539 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 6.823E-09



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM2T2S0.AQT
Date: 06/06/01 Time: 16:45:20

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

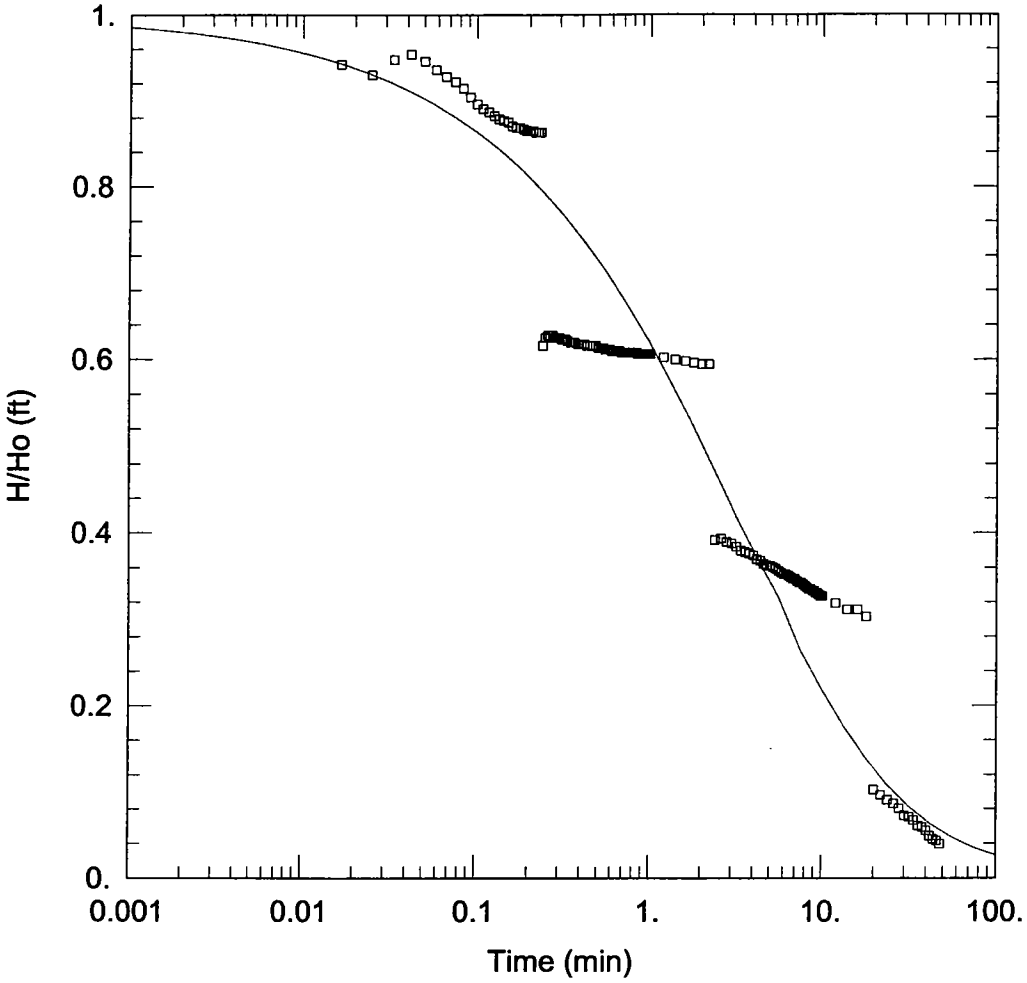
Saturated Thickness: 15.79 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 1. ft Water Column Height: 11.79 ft
Casing Radius: 0.266 ft Wellbore Radius: 0.417 ft
Screen Length: 11.79 ft

SOLUTION

Aquifer Model: Unconfined K = 0.0004815 ft/min
Solution Method: Bouwer-Rice y0 = 0.8921 ft



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM2T2S1.AQT
Date: 06/06/01 Time: 16:47:39

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

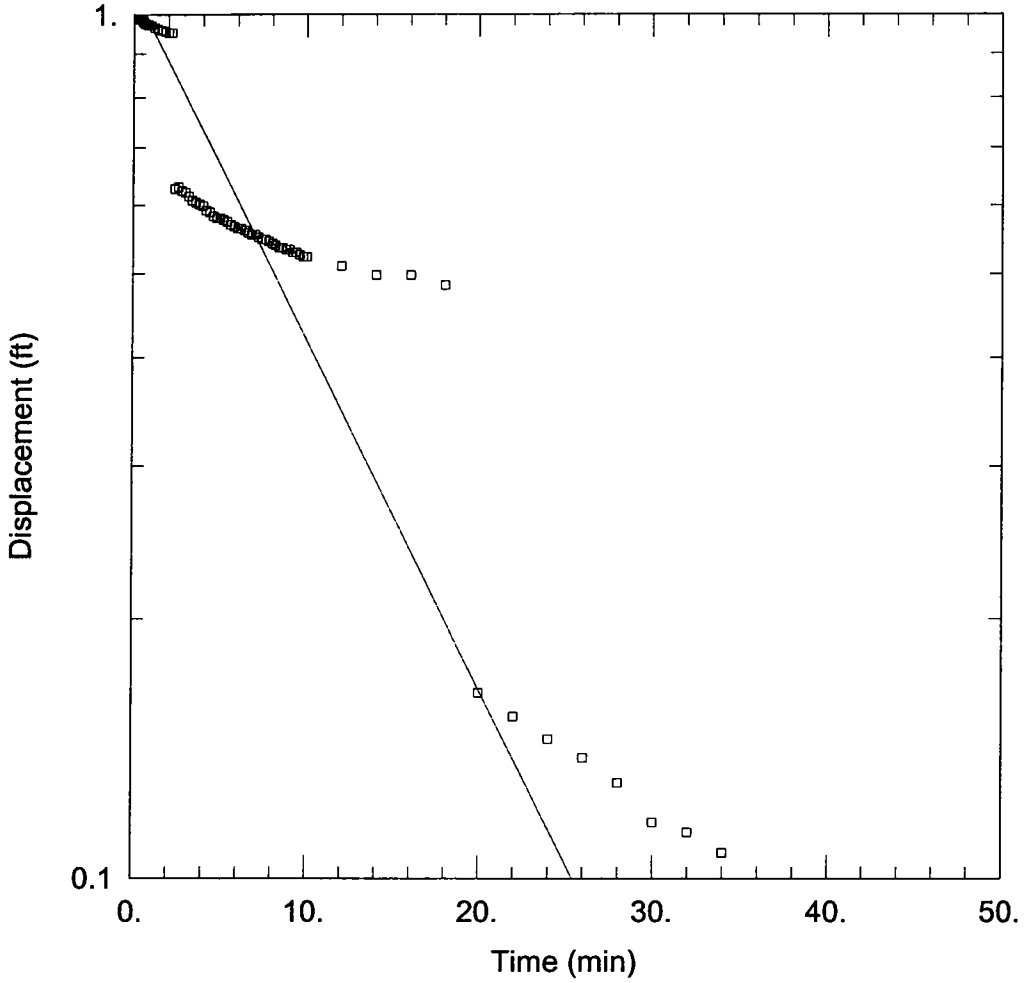
Saturated Thickness: 15.79 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 1.6 ft Water Column Height: 11.79 ft
Casing Radius: 0.21 ft Wellbore Radius: 0.417 ft
Screen Length: 11.79 ft

SOLUTION

Aquifer Model: Confined T = 0.004479 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 0.1



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM2T2S1.AQT
Date: 06/06/01 Time: 16:48:14

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

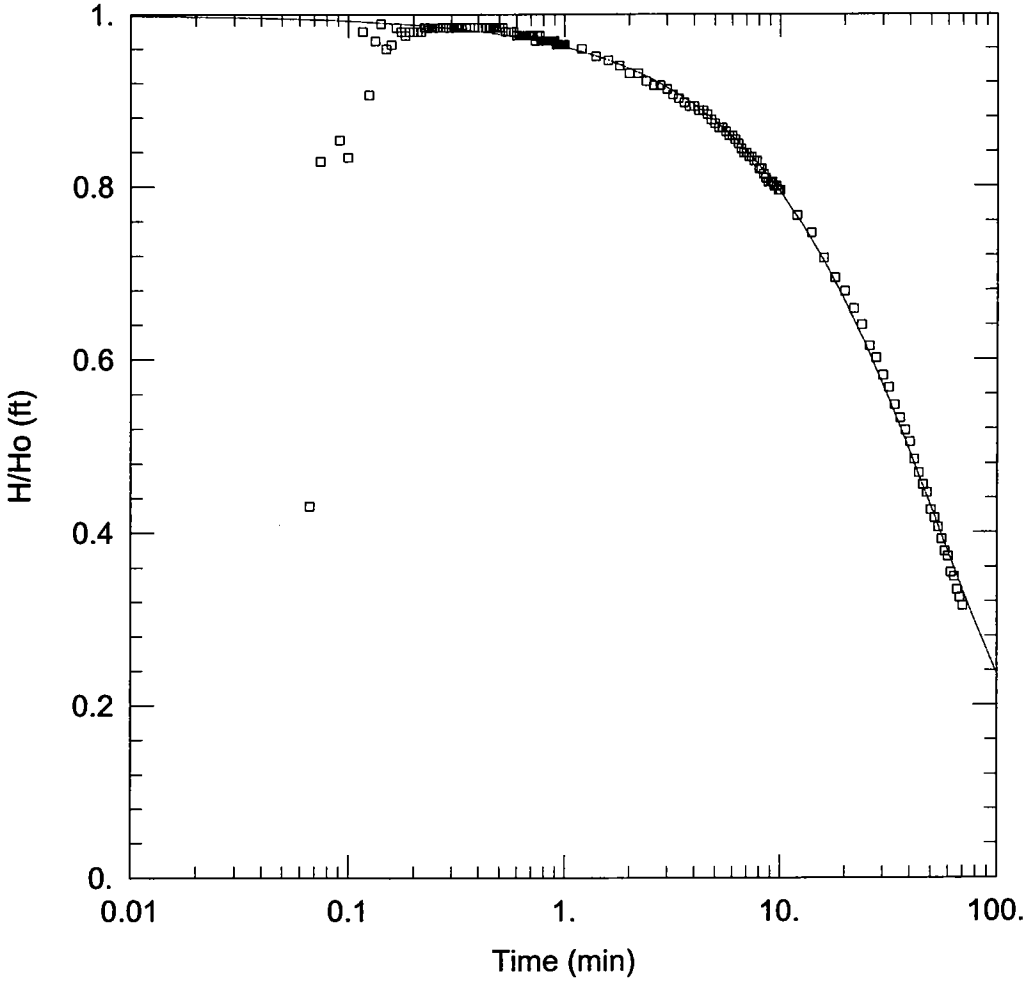
Saturated Thickness: 15.79 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 1.6 ft Water Column Height: 11.79 ft
Casing Radius: 0.21 ft Wellbore Radius: 0.417 ft
Screen Length: 11.79 ft

SOLUTION

Aquifer Model: Unconfined K = 0.0005484 ft/min
Solution Method: Bouwer-Rice y0 = 1.063 ft



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM5T1S0.AQT
Date: 06/06/01 Time: 16:37:58

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

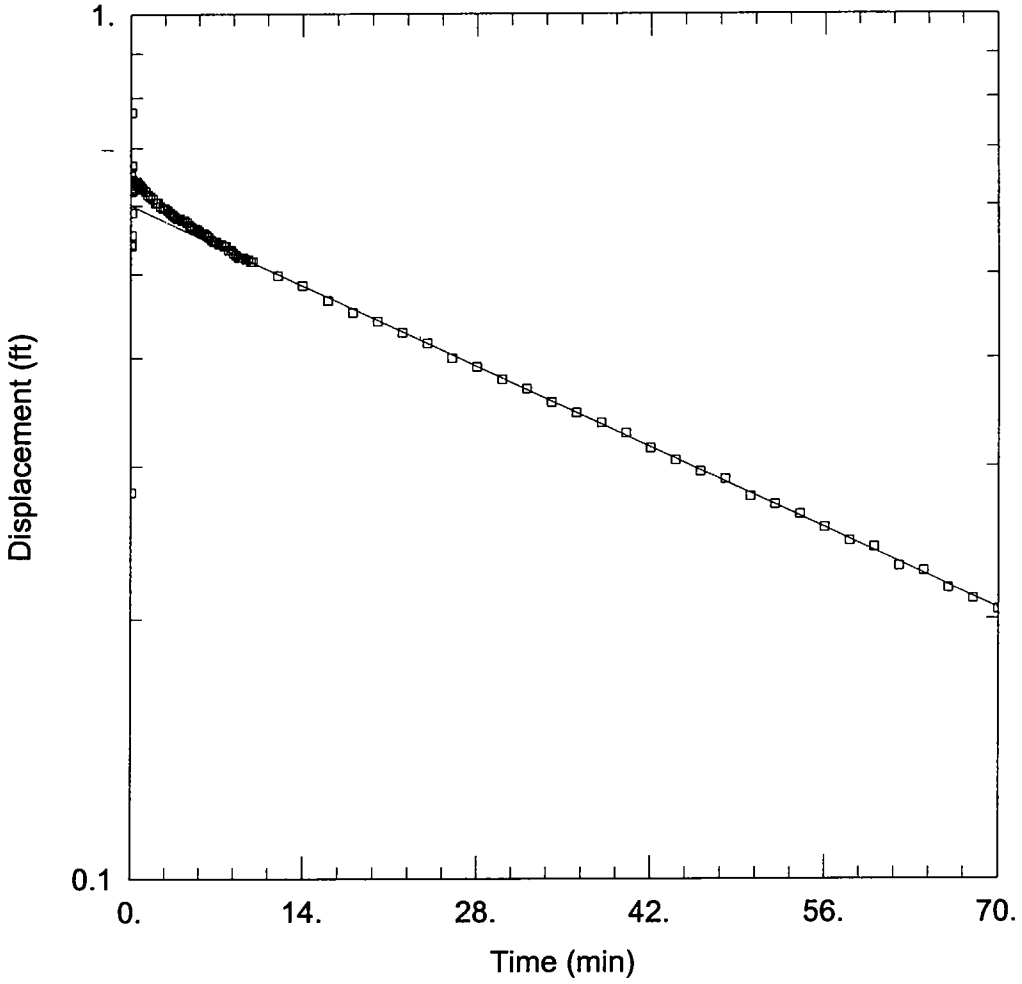
Saturated Thickness: 16.3 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.65 ft Water Column Height: 12.3 ft
Casing Radius: 0.33 ft Wellbore Radius: 0.417 ft
Screen Length: 12.3 ft

SOLUTION

Aquifer Model: Confined T = 0.003358 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 0.001058



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM5T1S0.AQT
Date: 06/06/01 Time: 16:38:46

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

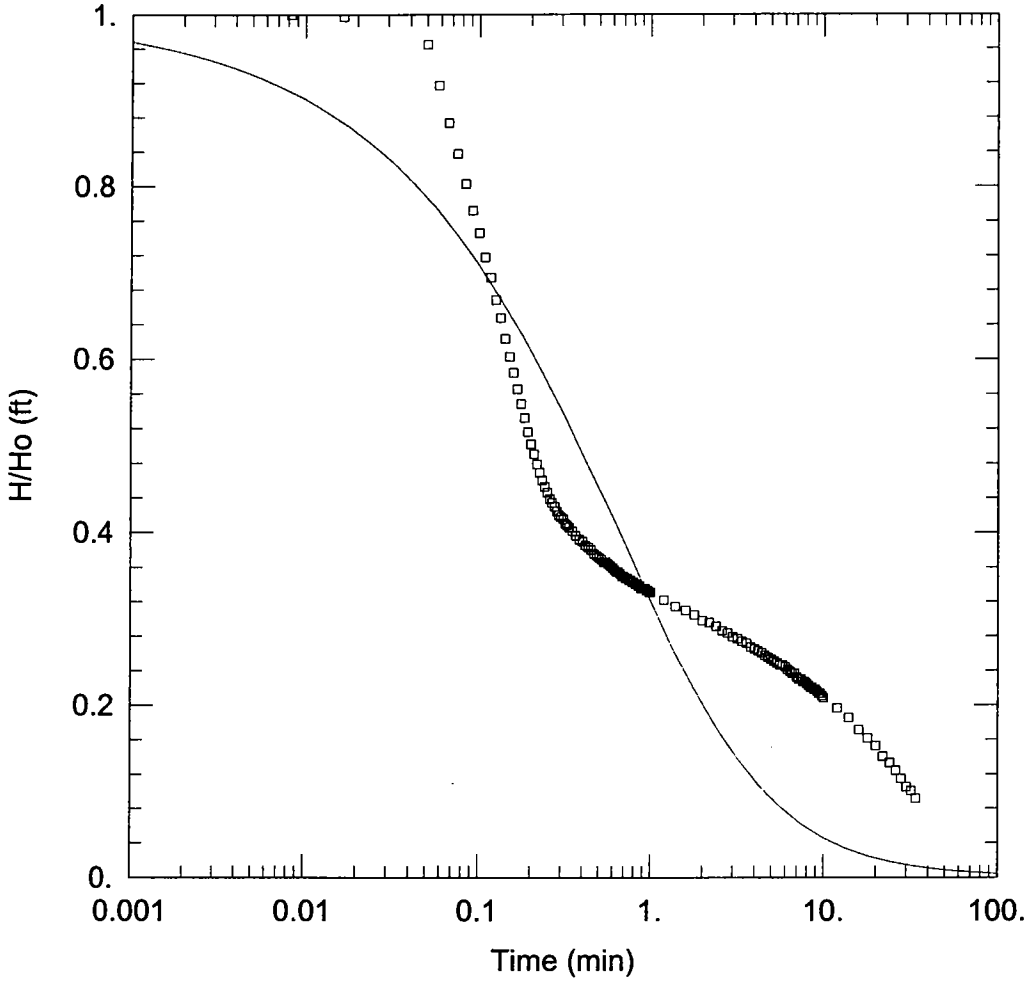
Saturated Thickness: 16.3 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.65 ft Water Column Height: 12.3 ft
Casing Radius: 0.33 ft Wellbore Radius: 0.417 ft
Screen Length: 12.3 ft

SOLUTION

Aquifer Model: Unconfined K = 0.0002162 ft/min
Solution Method: Bouwer-Rice y0 = 0.6006 ft



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM5T1S1.AQT
Date: 06/06/01 Time: 16:40:20

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

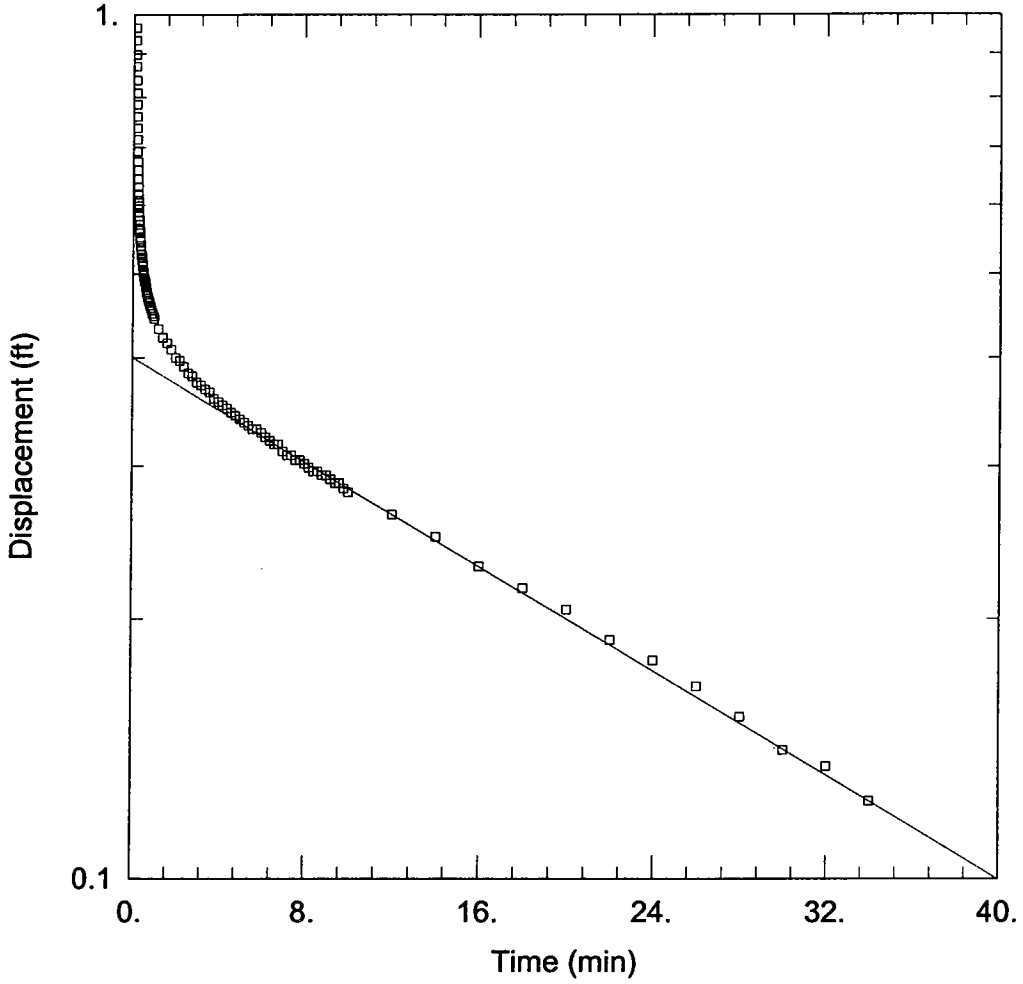
Saturated Thickness: 16.3 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 1.343 ft Water Column Height: 12.3 ft
Casing Radius: 0.229 ft Wellbore Radius: 0.417 ft
Screen Length: 12.3 ft

SOLUTION

Aquifer Model: Confined T = 0.03183 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 0.1



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM5T1S1.AQT
Date: 06/06/01 Time: 17:24:43

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

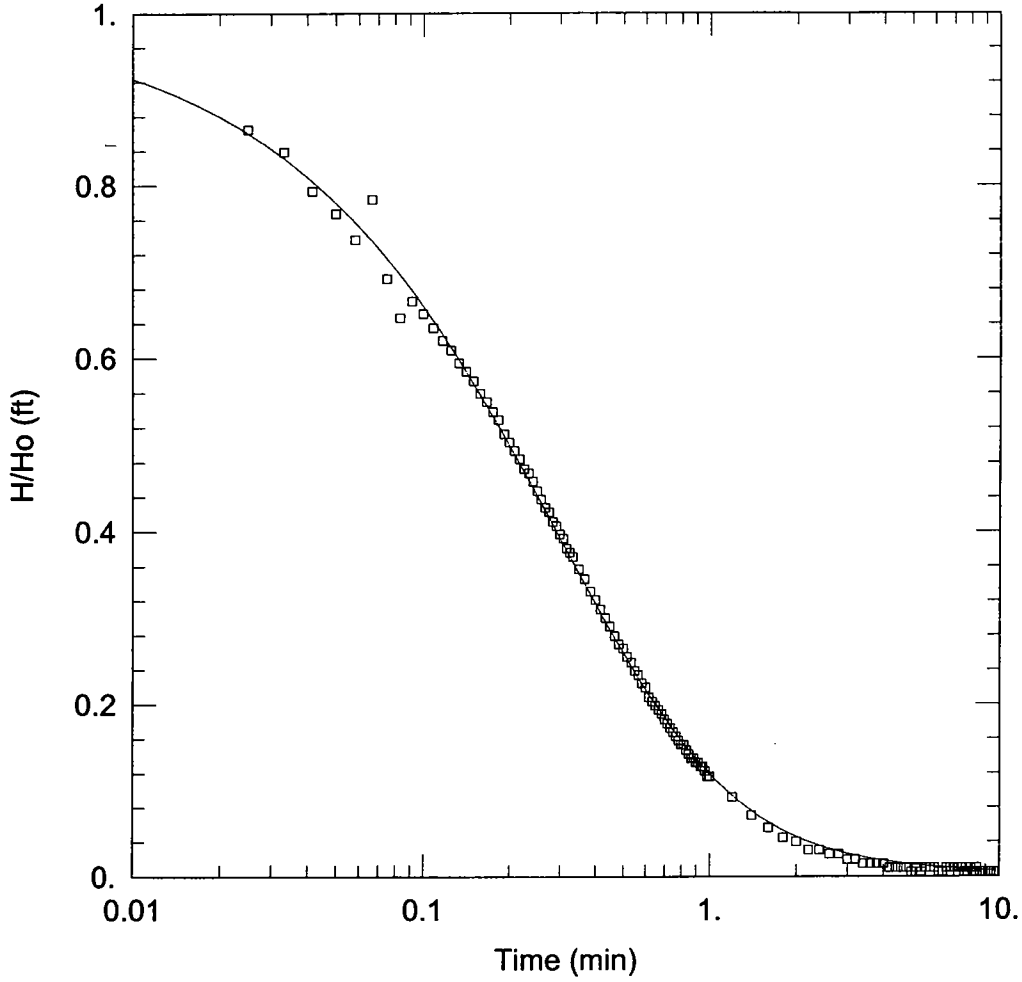
Saturated Thickness: 16.3 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 1.343 ft Water Column Height: 12.3 ft
Casing Radius: 0.229 ft Wellbore Radius: 0.417 ft
Screen Length: 12.3 ft

SOLUTION

Aquifer Model: Unconfined K = 0.0002361 ft/min
Solution Method: Bouwer-Rice y0 = 0.4003 ft



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM6T0S0.AQT
Date: 06/06/01 Time: 16:30:10

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

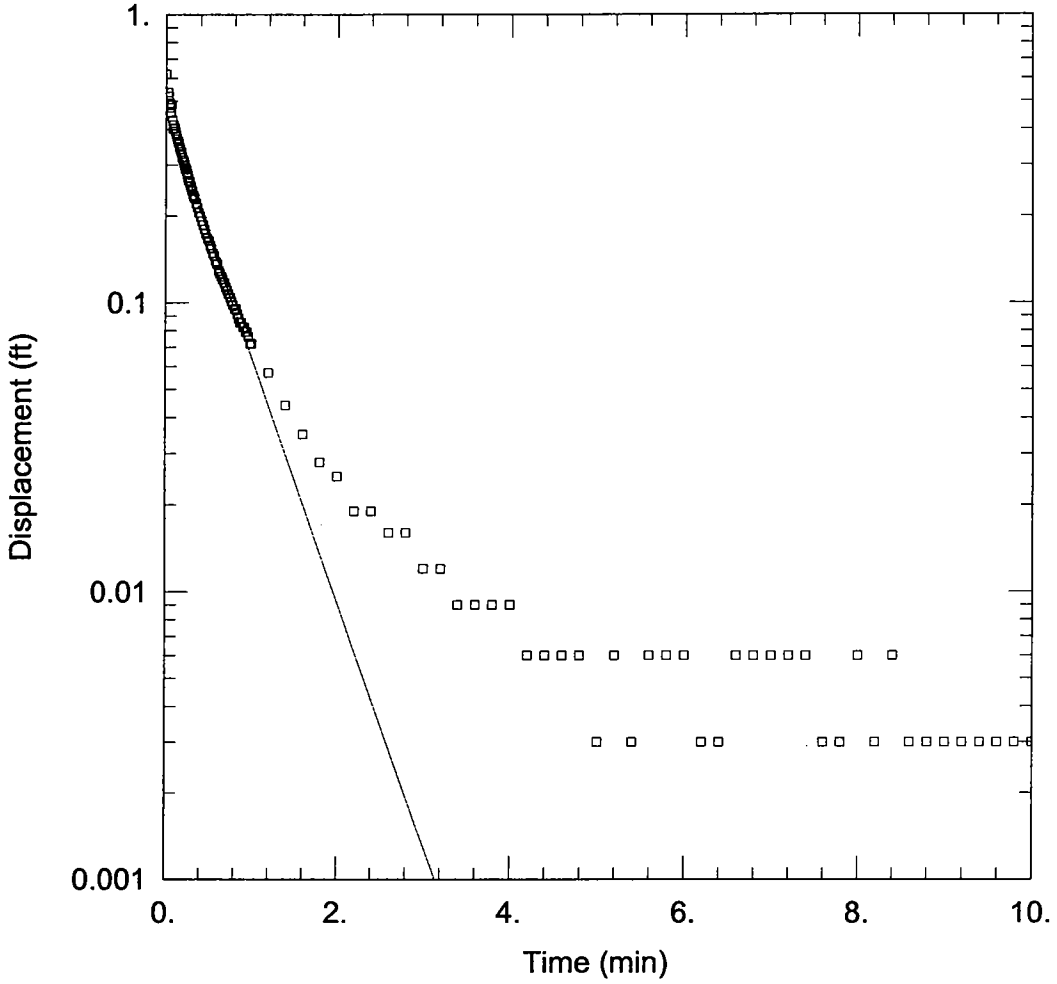
Saturated Thickness: 16.62 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.62 ft Water Column Height: 12.62 ft
Casing Radius: 0.338 ft Wellbore Radius: 0.417 ft
Screen Length: 12.62 ft

SOLUTION

Aquifer Model: Confined T = 0.4988 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 0.006003



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM6T0S0.AQT
Date: 06/06/01 Time: 16:30:58

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

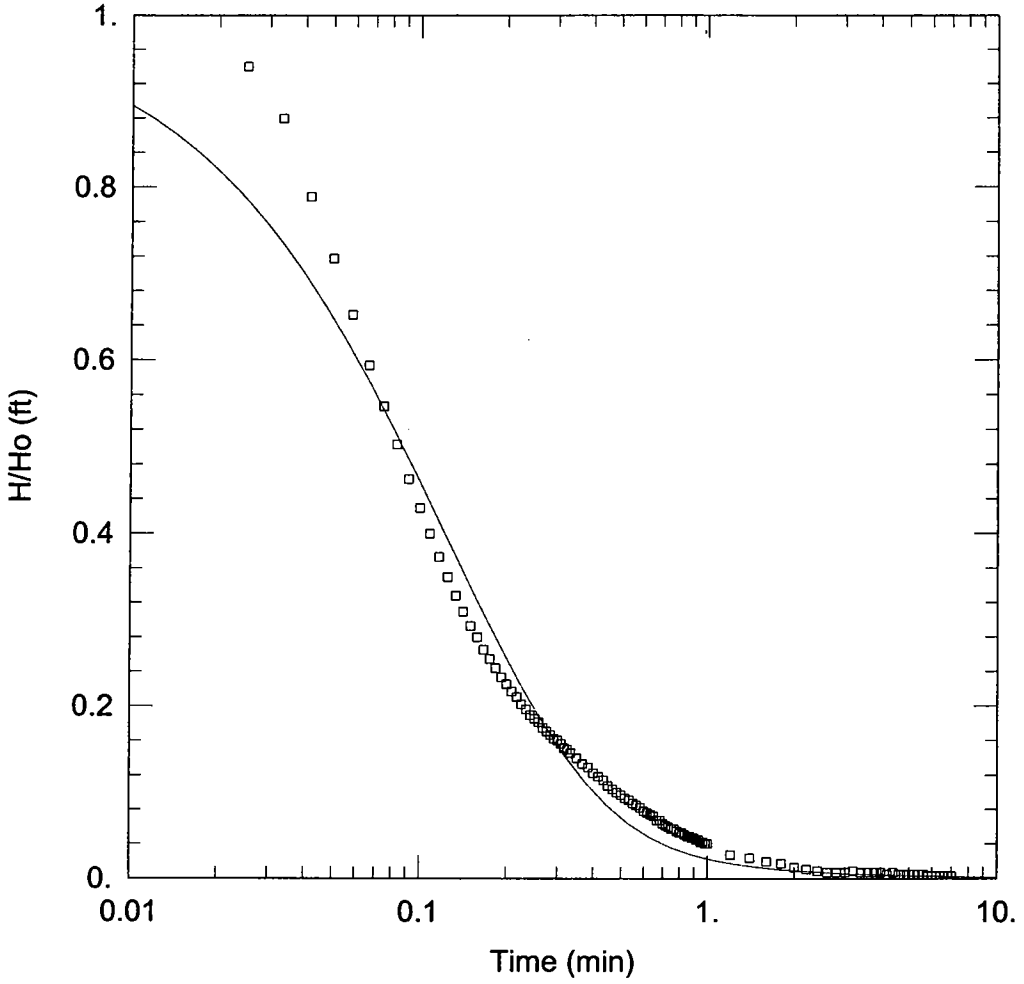
Saturated Thickness: 16.62 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.62 ft Water Column Height: 12.62 ft
Casing Radius: 0.338 ft Wellbore Radius: 0.417 ft
Screen Length: 12.62 ft

SOLUTION

Aquifer Model: Unconfined K = 0.02829 ft/min
Solution Method: Bouwer-Rice y0 = 0.4512 ft



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM6T0S1.AQT
Date: 06/06/01 Time: 16:32:36

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

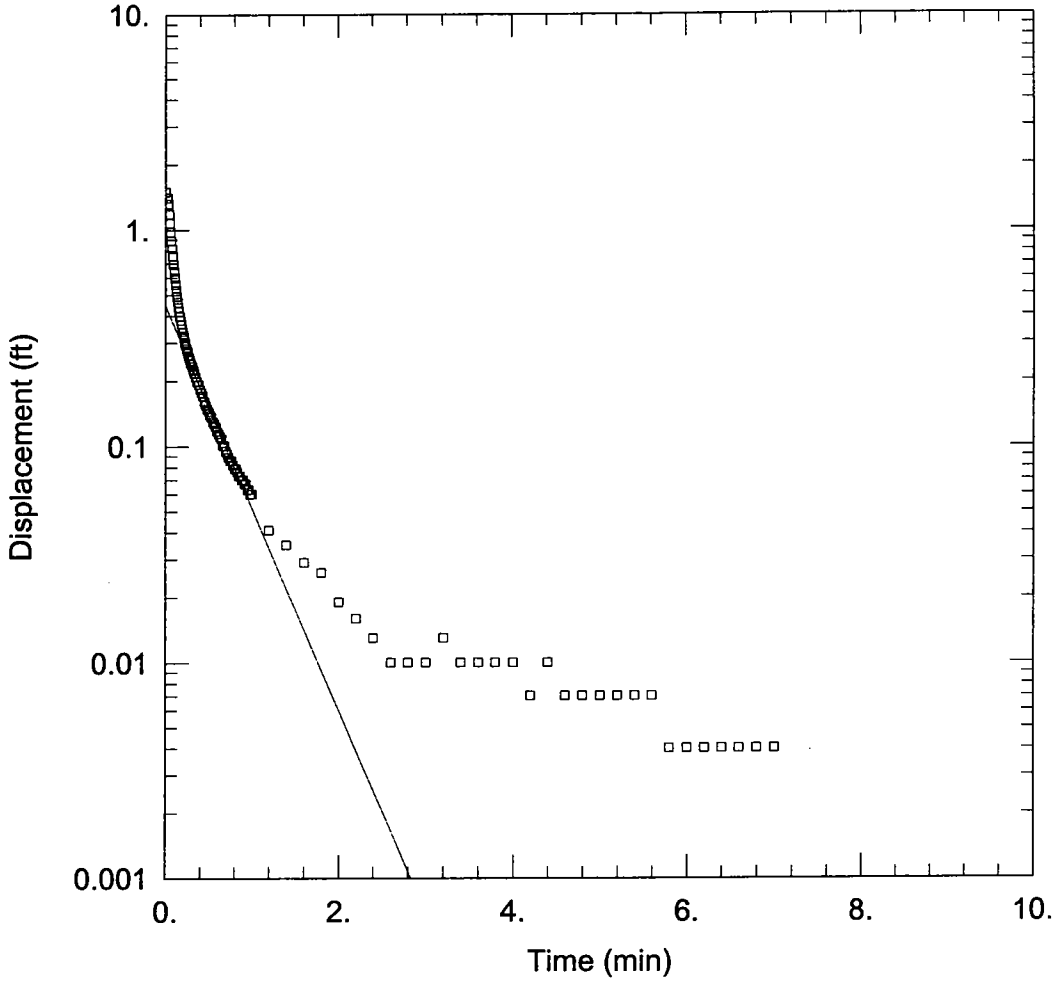
Saturated Thickness: 16.62 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 1.5 ft Water Column Height: 12.62 ft
Casing Radius: 0.224 ft Wellbore Radius: 0.417 ft
Screen Length: 12.62 ft

SOLUTION

Aquifer Model: Confined T = 0.8642 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 0.0001033



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM6T0S1.AQT
Date: 06/06/01 Time: 16:33:22

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

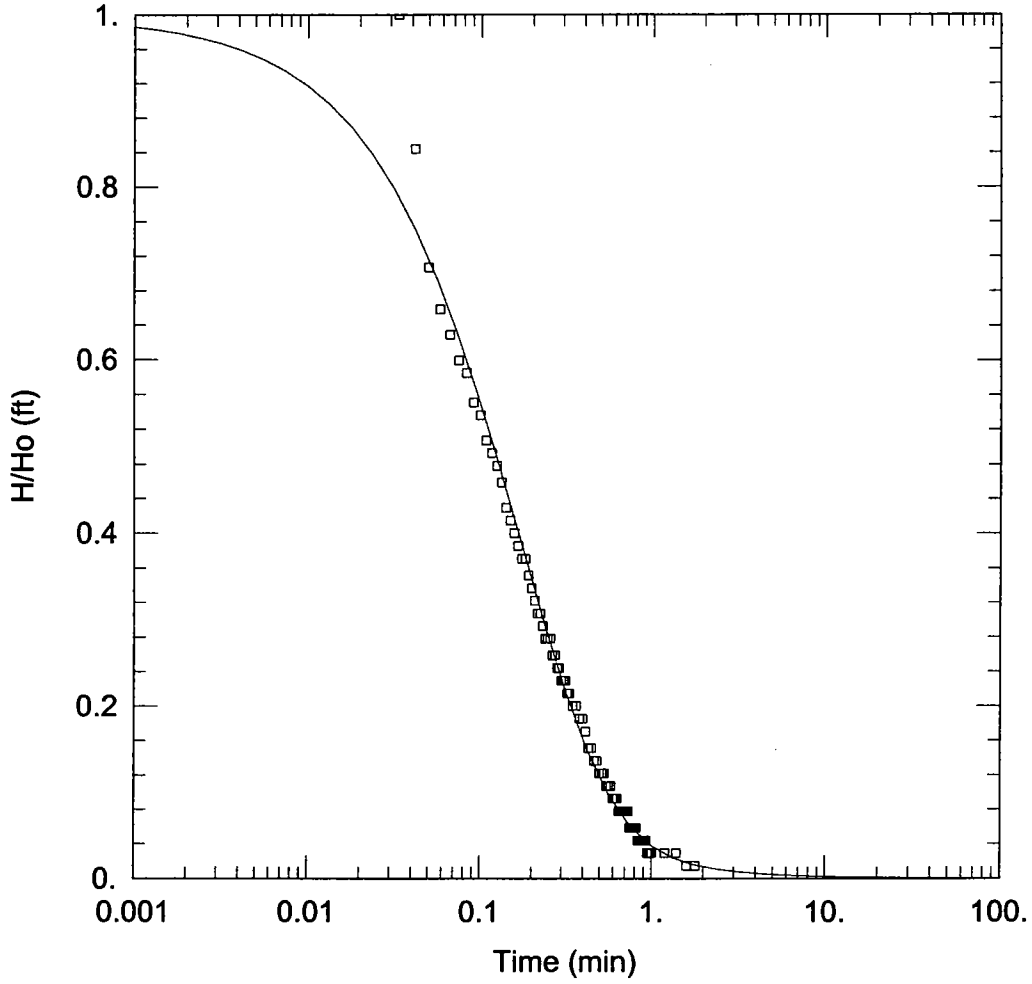
Saturated Thickness: 16.62 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 1.5 ft
Casing Radius: 0.224 ft
Screen Length: 12.62 ft
Water Column Height: 12.62 ft
Wellbore Radius: 0.417 ft

SOLUTION

Aquifer Model: Unconfined
Solution Method: Bouwer-Rice
K = 0.01376 ft/min
y0 = 0.4449 ft



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM7T3S0.AQT
Date: 06/06/01 Time: 16:50:12

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

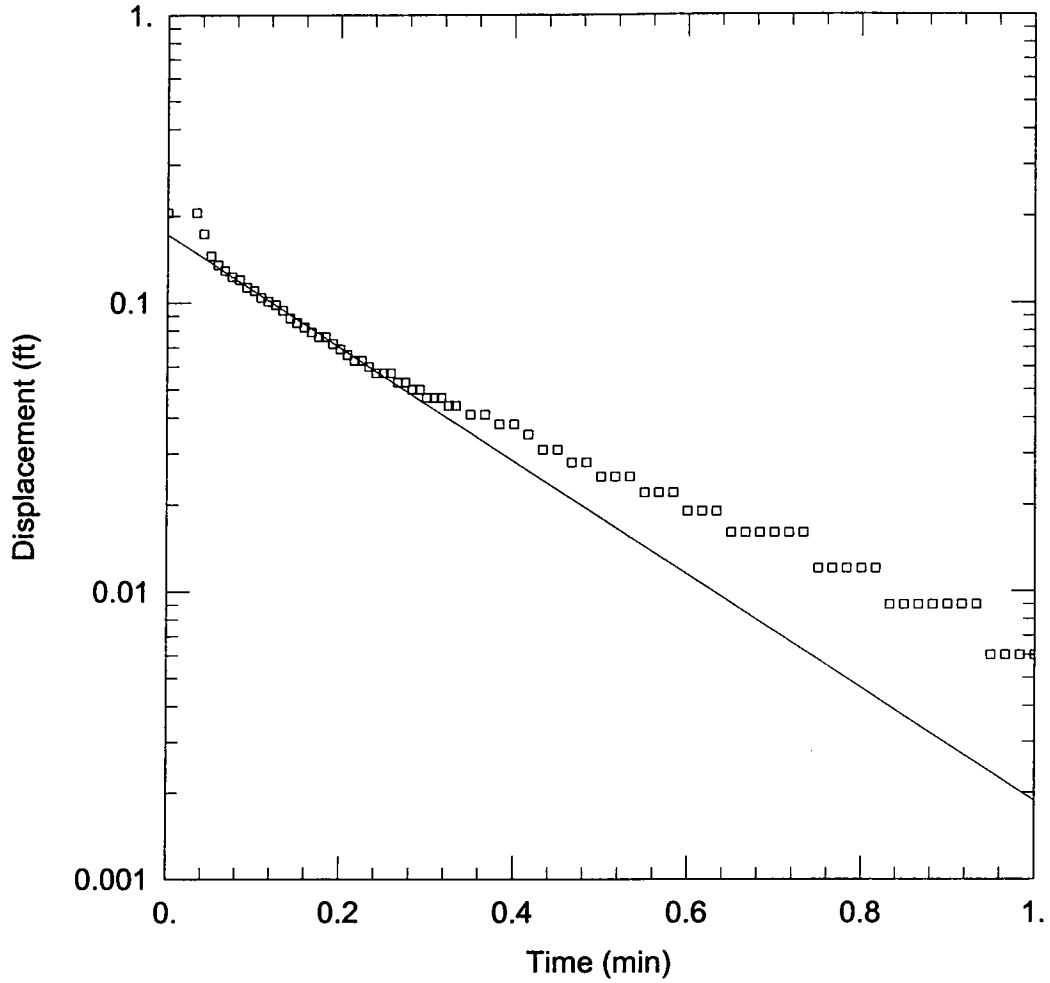
Saturated Thickness: 17.87 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.205 ft Water Column Height: 14.87 ft
Casing Radius: 0.208 ft Wellbore Radius: 0.417 ft
Screen Length: 14.87 ft

SOLUTION

Aquifer Model: Confined T = 0.5279 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 0.0001128



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM7T3S0.AQT
Date: 06/06/01 Time: 16:55:28

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

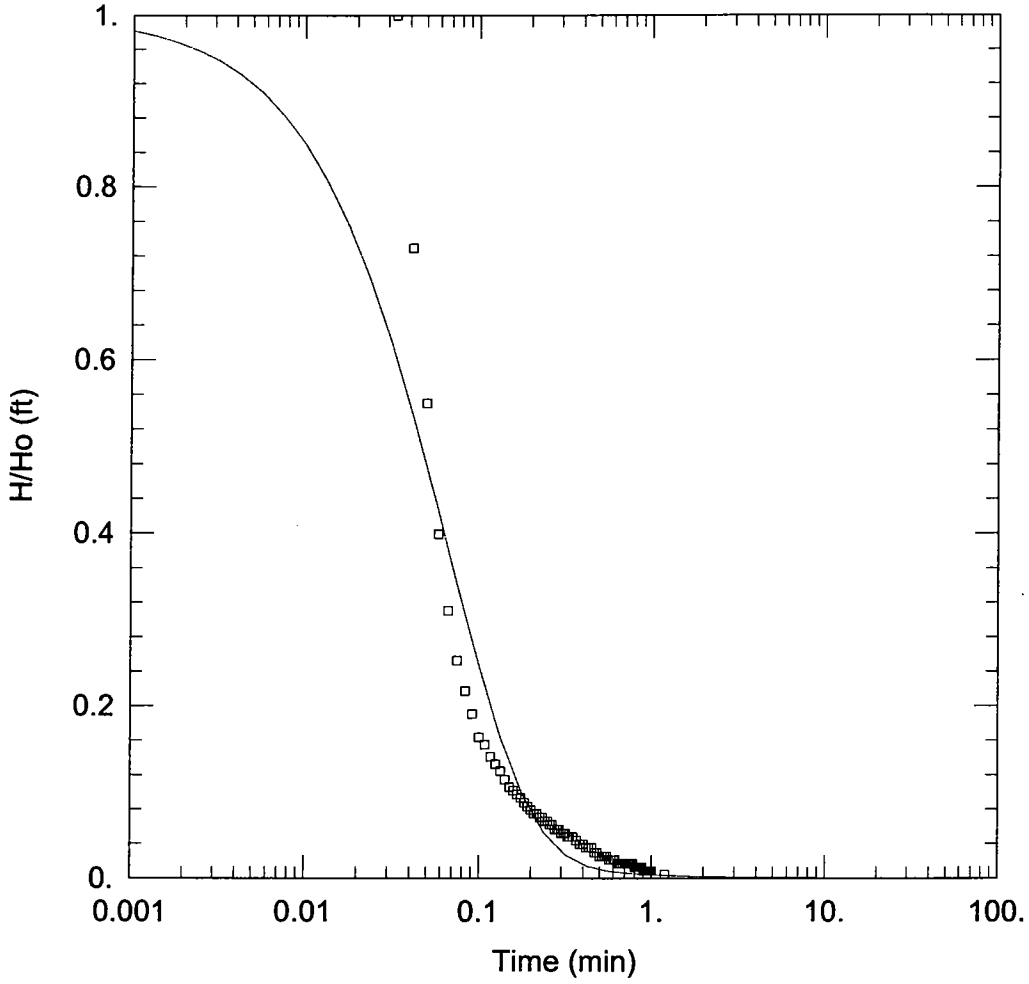
Saturated Thickness: 17.87 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.205 ft Water Column Height: 14.87 ft
Casing Radius: 0.208 ft Wellbore Radius: 0.417 ft
Screen Length: 14.87 ft

SOLUTION

Aquifer Model: Unconfined K = 0.02222 ft/min
Solution Method: Bouwer-Rice y0 = 0.1722 ft



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM7T3S1.AQT
Date: 06/06/01 Time: 16:57:44

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

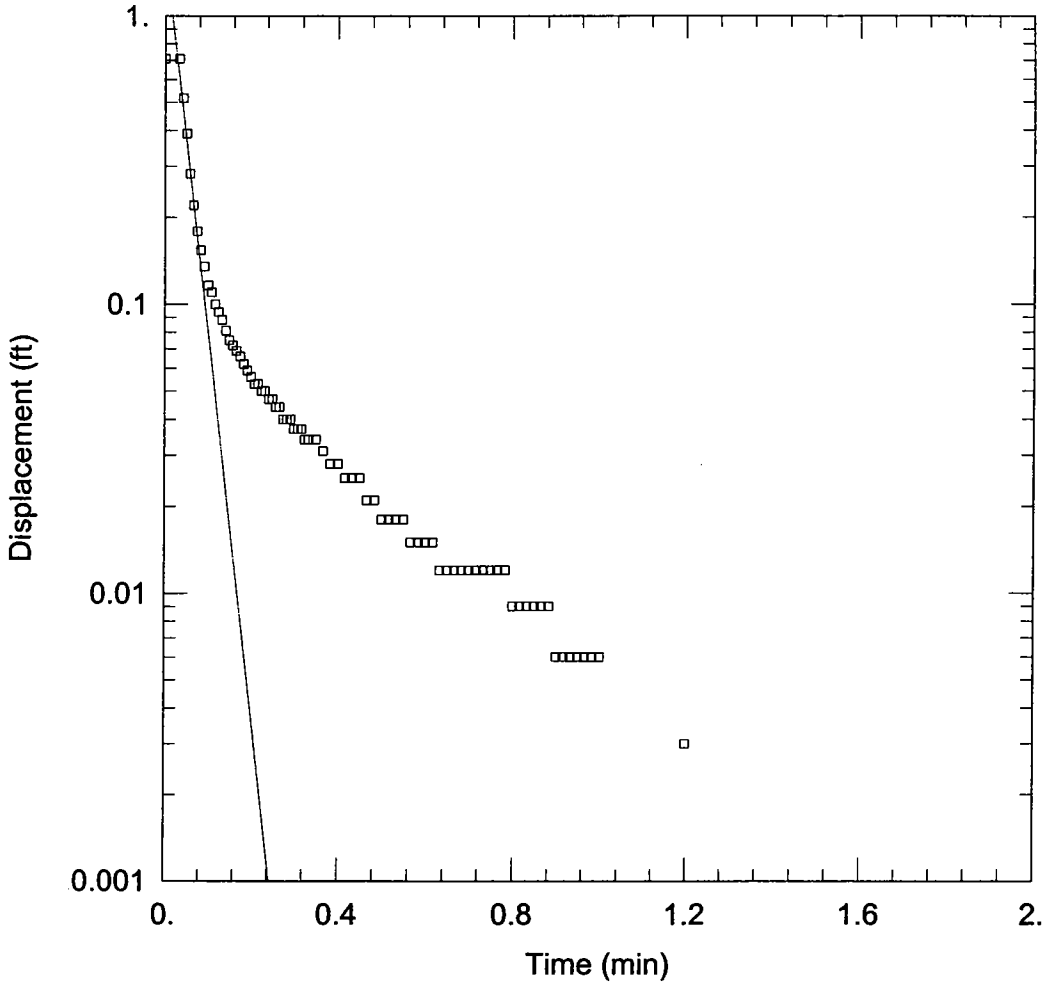
Saturated Thickness: 17.87 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.709 ft Water Column Height: 14.87 ft
Casing Radius: 0.112 ft Wellbore Radius: 0.417 ft
Screen Length: 14.87 ft

SOLUTION

Aquifer Model: Confined T = 1.025 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 1.E-10



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM7T3S1.AQT
Date: 06/06/01 Time: 16:58:46

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

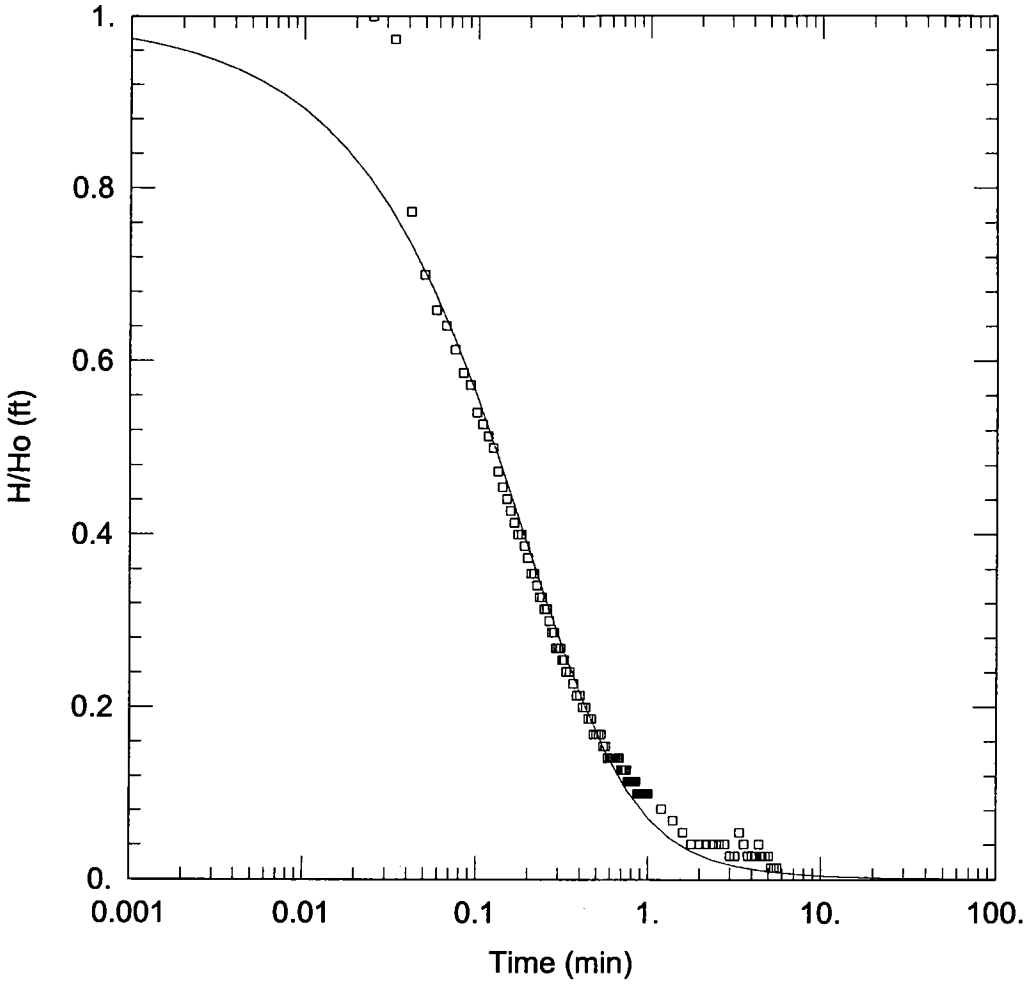
Saturated Thickness: 17.87 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.709 ft Water Column Height: 14.87 ft
Casing Radius: 0.112 ft Wellbore Radius: 0.417 ft
Screen Length: 14.87 ft

SOLUTION

Aquifer Model: Unconfined K = 0.04314 ft/min
Solution Method: Bouwer-Rice y0 = 1.657 ft



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM7T3S2.AQT
Date: 06/06/01 Time: 17:00:21

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

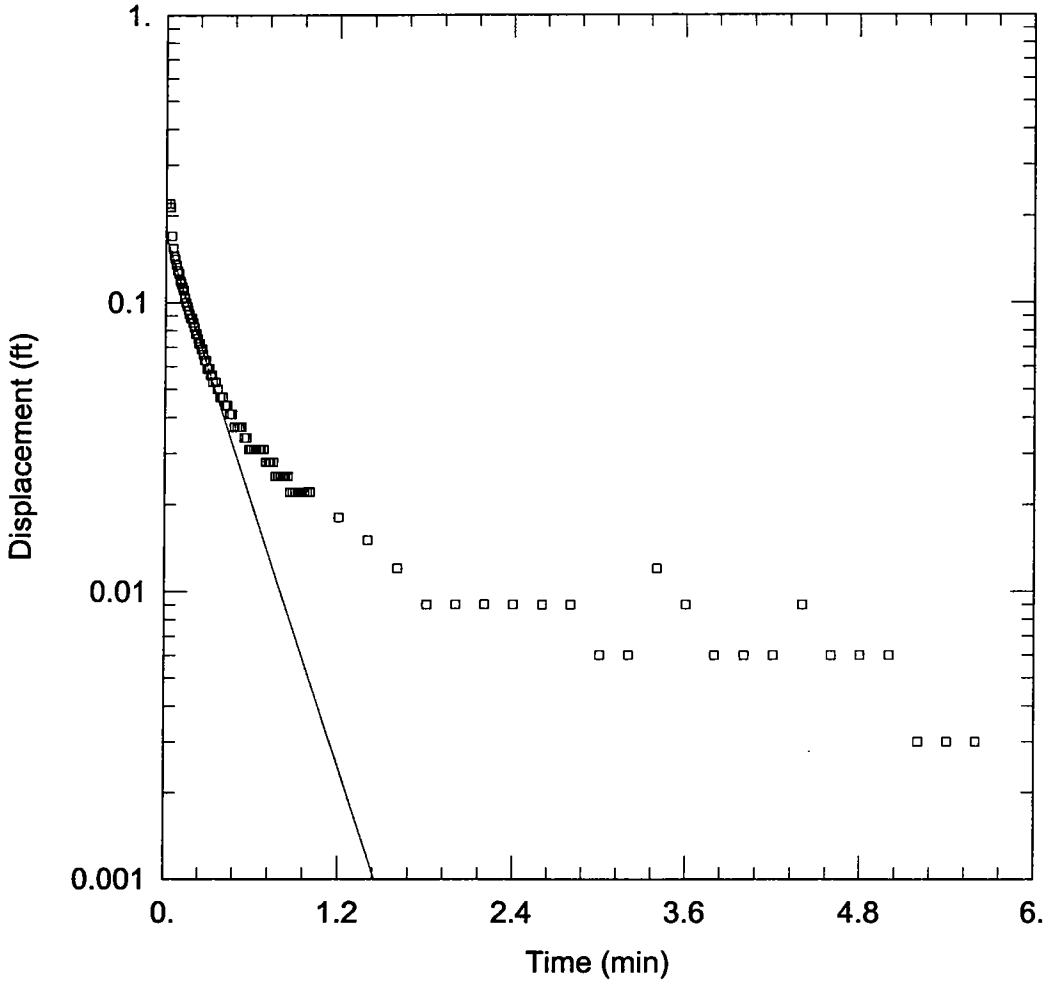
Saturated Thickness: 17.87 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.22 ft Water Column Height: 14.87 ft
Casing Radius: 0.201 ft Wellbore Radius: 0.417 ft
Screen Length: 14.87 ft

SOLUTION

Aquifer Model: Confined T = 0.2471 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 0.003331



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM7T3S2.AQT
Date: 06/06/01 Time: 17:01:20

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

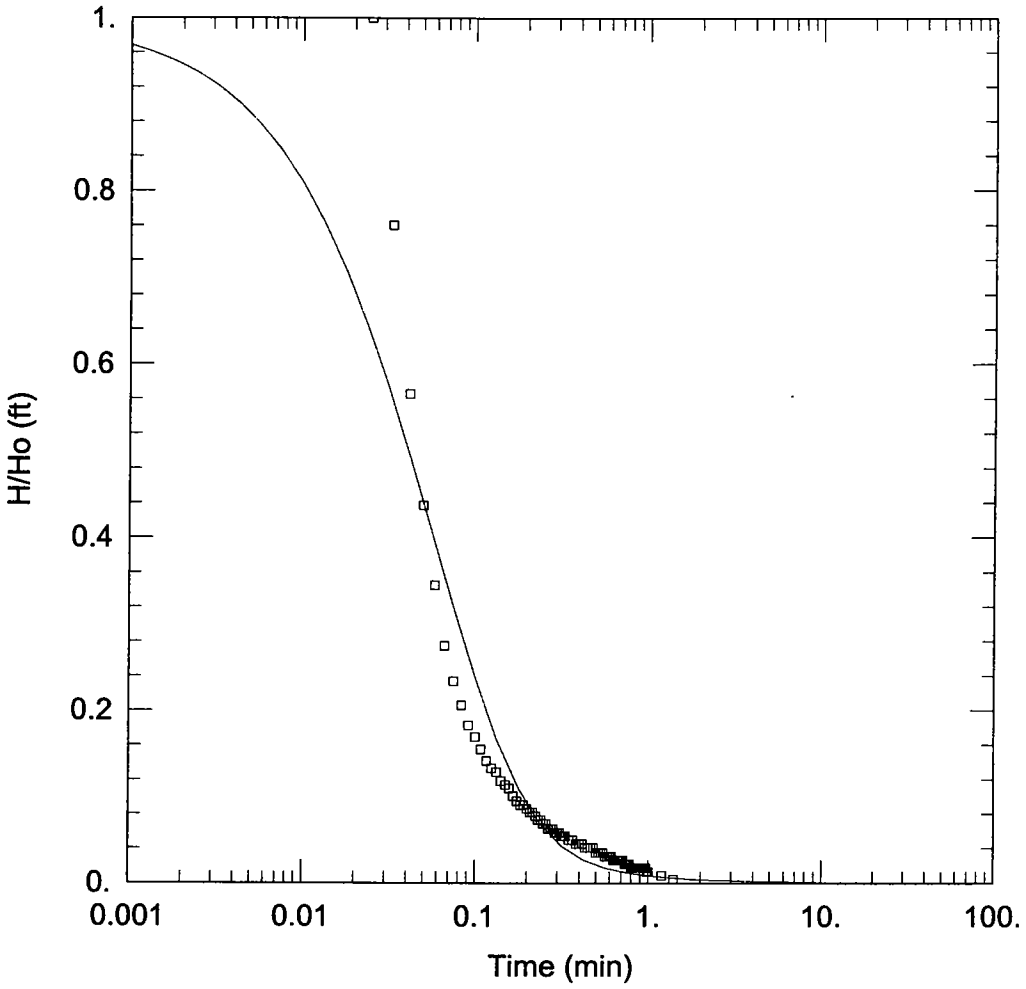
Saturated Thickness: 17.87 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.22 ft Water Column Height: 14.87 ft
Casing Radius: 0.201 ft Wellbore Radius: 0.417 ft
Screen Length: 14.87 ft

SOLUTION

Aquifer Model: Unconfined K = 0.01624 ft/min
Solution Method: Bouwer-Rice y0 = 0.1699 ft



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM7T3S3.AQT
Date: 06/06/01 Time: 17:11:48

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

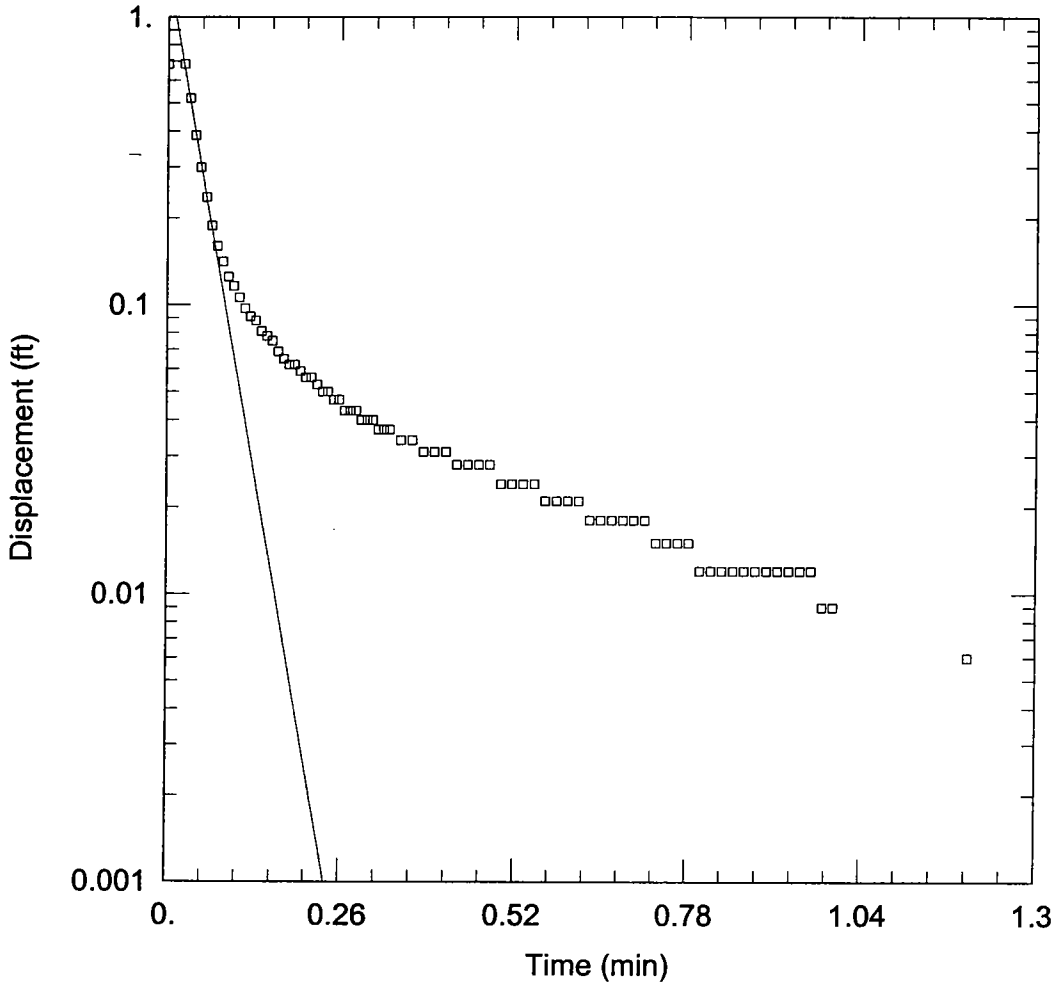
Saturated Thickness: 17.87 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.684 ft Water Column Height: 14.87 ft
Casing Radius: 0.114 ft Wellbore Radius: 0.417 ft
Screen Length: 14.87 ft

SOLUTION

Aquifer Model: Confined T = 0.4668 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 3.224E-05



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM7T3S3.AQT
Date: 06/06/01 Time: 17:12:27

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

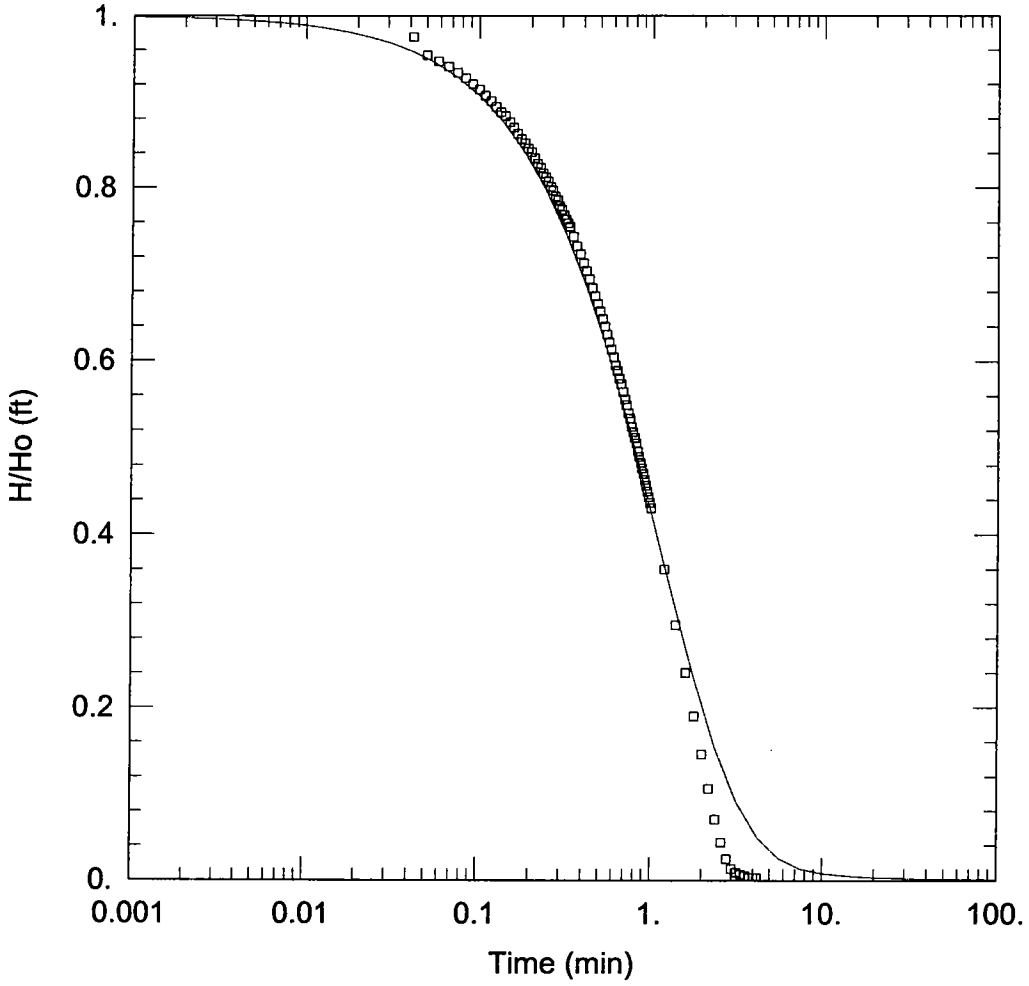
Saturated Thickness: 17.87 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 0.684 ft
Water Column Height: 14.87 ft
Casing Radius: 0.114 ft
Wellbore Radius: 0.417 ft
Screen Length: 14.87 ft

SOLUTION

Aquifer Model: Unconfined
K = 0.04484 ft/min
Solution Method: Bouwer-Rice
y0 = 1.401 ft



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM8T4S0.AQT
Date: 06/06/01 Time: 17:19:08

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

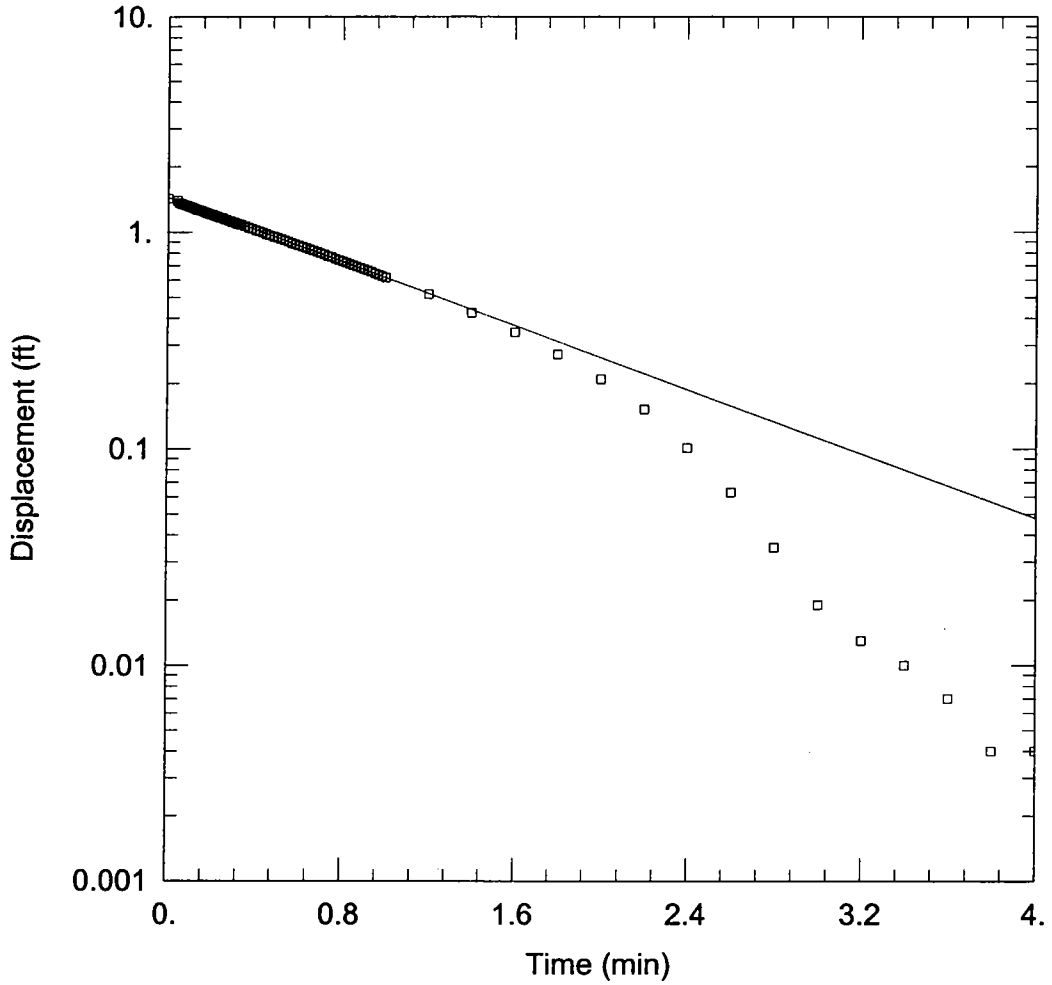
Saturated Thickness: 37.91 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 1.43 ft Water Column Height: 33.91 ft
Casing Radius: 0.083 ft Wellbore Radius: 0.417 ft
Screen Length: 11. ft

SOLUTION

Aquifer Model: Confined T = 0.0319 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 1.E-10



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM8T4S0.AQT
Date: 06/06/01 Time: 17:19:34

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

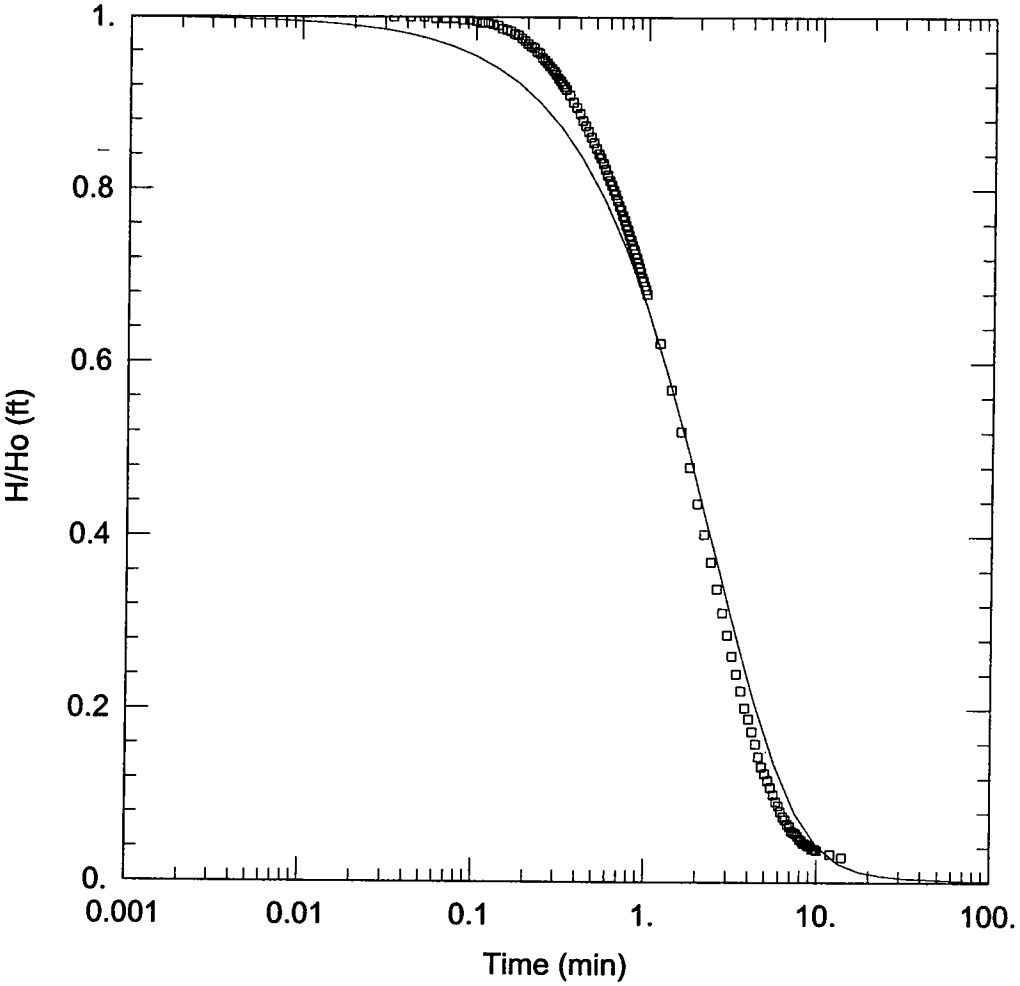
Saturated Thickness: 37.91 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 1.43 ft Water Column Height: 33.91 ft
Casing Radius: 0.083 ft Wellbore Radius: 0.417 ft
Screen Length: 11. ft

SOLUTION

Aquifer Model: Unconfined K = 0.0009781 ft/min
Solution Method: Bouwer-Rice y0 = 1.444 ft



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM8T4S1.AQT
Date: 06/06/01 Time: 17:20:56

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

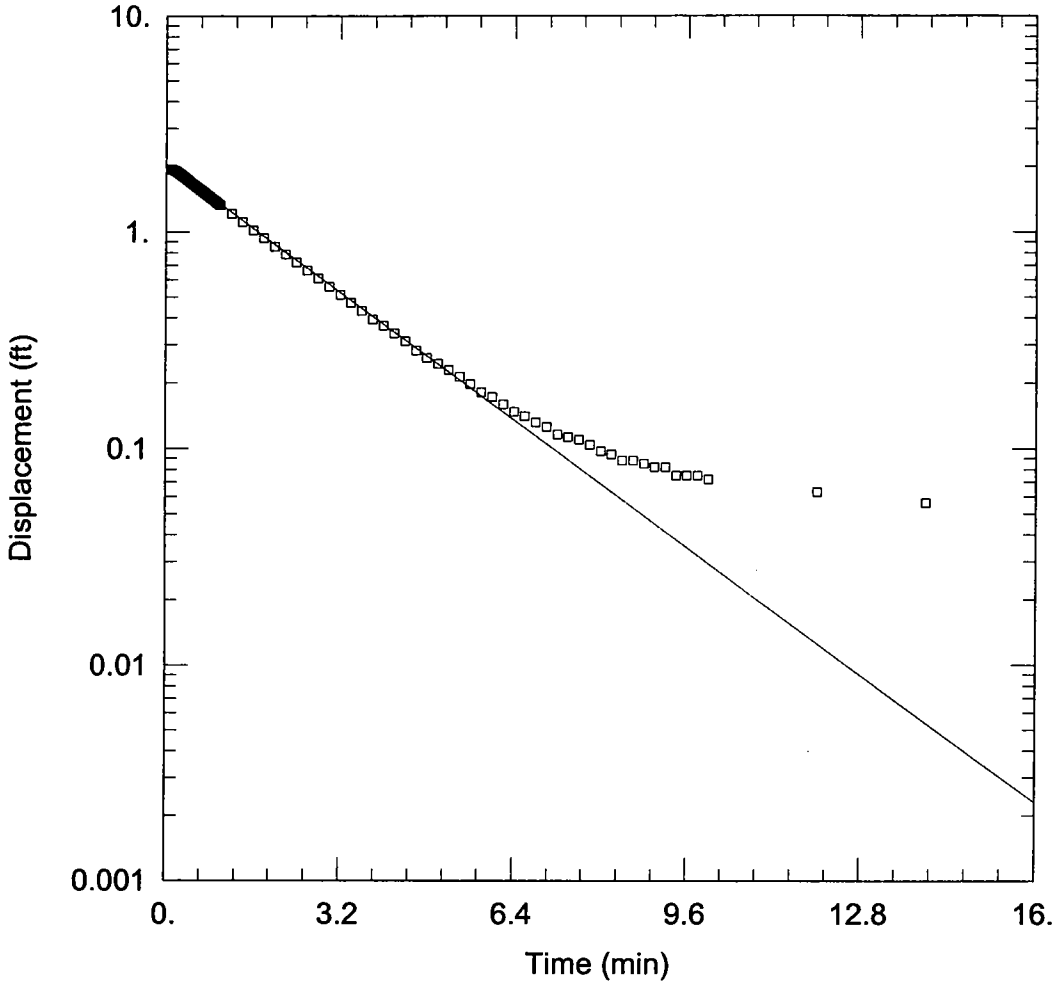
Saturated Thickness: 37.91 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 1.949 ft Water Column Height: 33.91 ft
Casing Radius: 0.083 ft Wellbore Radius: 0.417 ft
Screen Length: 11. ft

SOLUTION

Aquifer Model: Confined T = 0.01443 ft^2/min
Solution Method: Cooper-Bredehoeft-Papadopulos S = 1.E-10



WELL TEST ANALYSIS

Data Set: N:\COMMON\CAMPPE~1\DO116\SLUG\AREA22~1\MM8T4S1.AQT
Date: 06/06/01 Time: 17:21:26

PROJECT INFORMATION

Company: IT Corporation
Client: NAVY
Project: 821816
Test Location: Area 22, Camp Pendleton
Test Date: 6/5/01

AQUIFER DATA

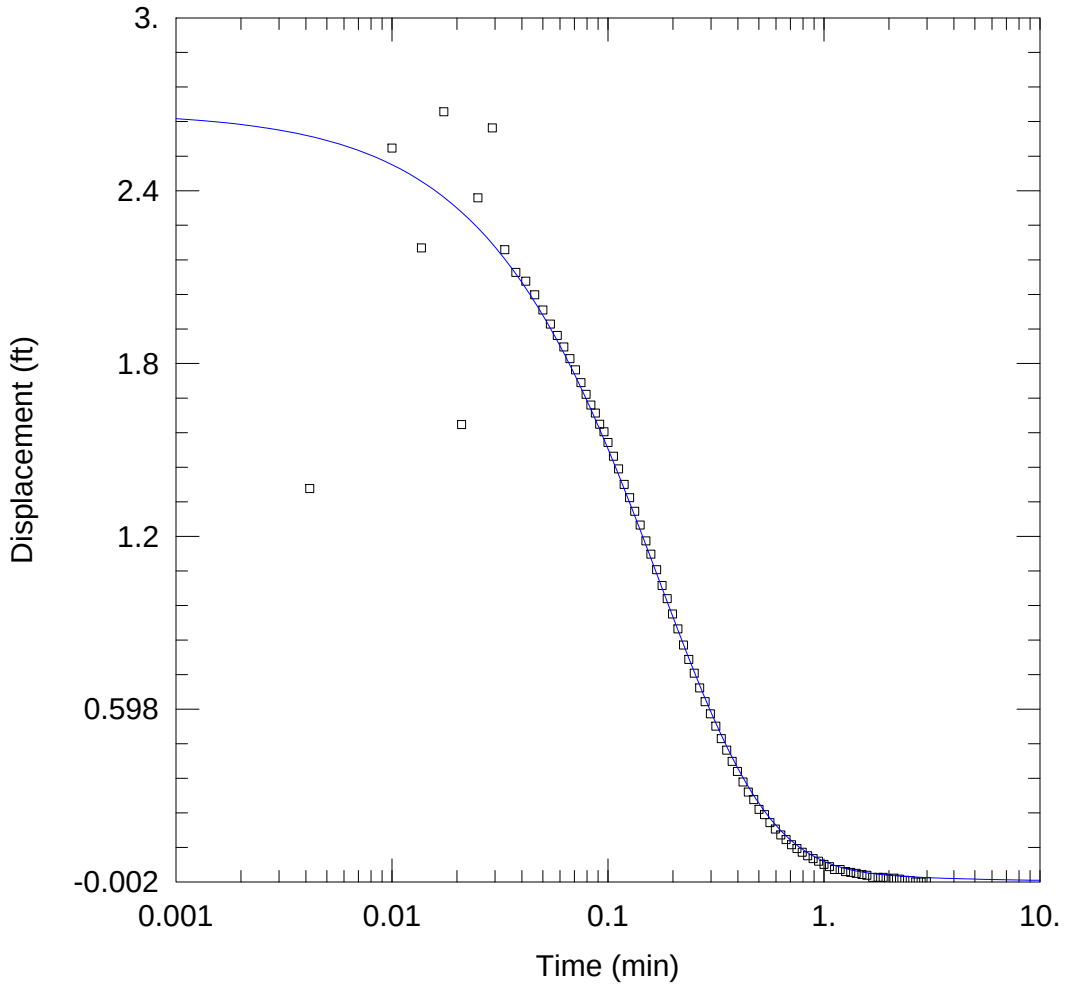
Saturated Thickness: 37.91 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Initial Displacement: 1.949 ft Water Column Height: 33.91 ft
Casing Radius: 0.083 ft Wellbore Radius: 0.417 ft
Screen Length: 11. ft

SOLUTION

Aquifer Model: Unconfined K = 0.0004868 ft/min
Solution Method: Bouwer-Rice y0 = 2.043 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-BMW-08test1out.aqt
Date: 03/08/17 Time: 15:31:49

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

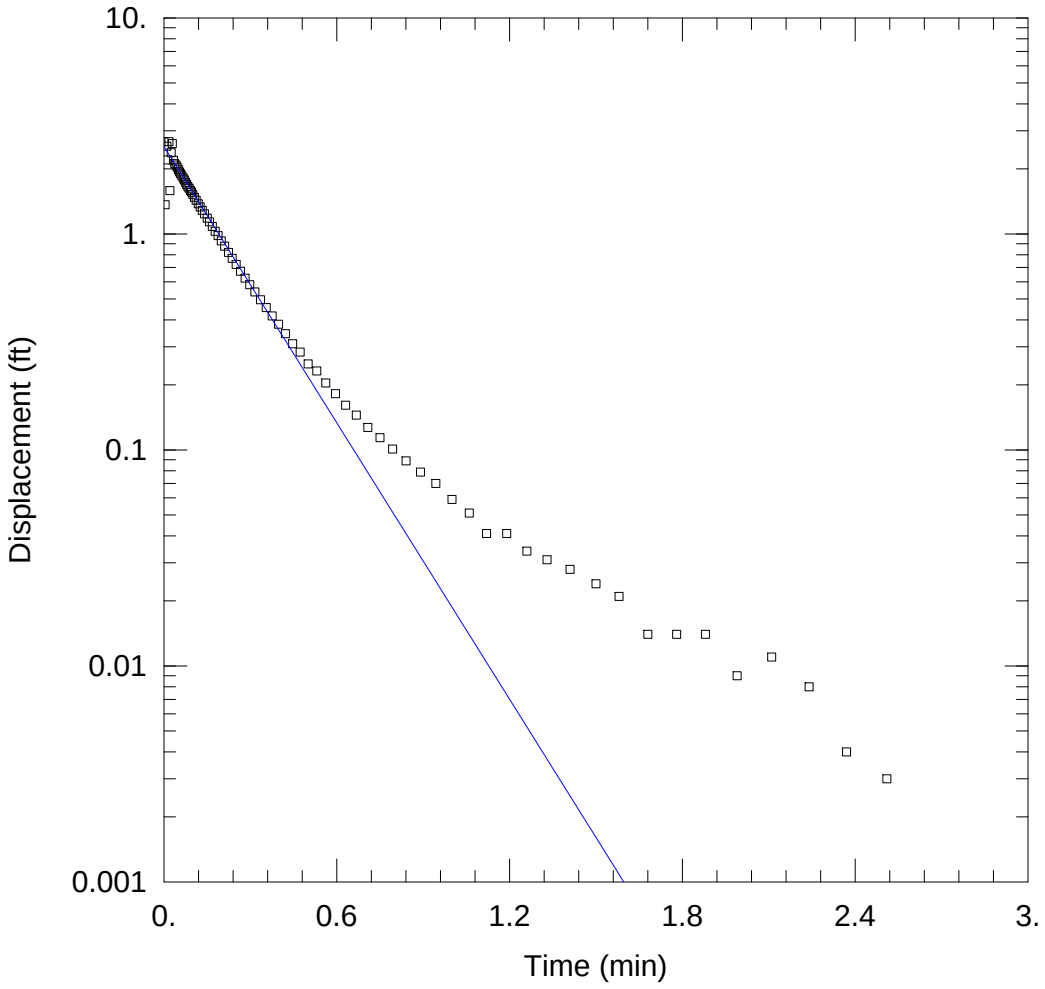
Saturated Thickness: 15.03 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-BMW-8)

Initial Displacement: 2.674 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft
Gravel Pack Porosity: 0.

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 343.7 ft^2/day
S = 1.576E-7



WELL TEST ANALYSIS

Data Set: C:\...\22-BMW-08test1outB.aqt
Date: 03/09/17 Time: 08:18:45

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

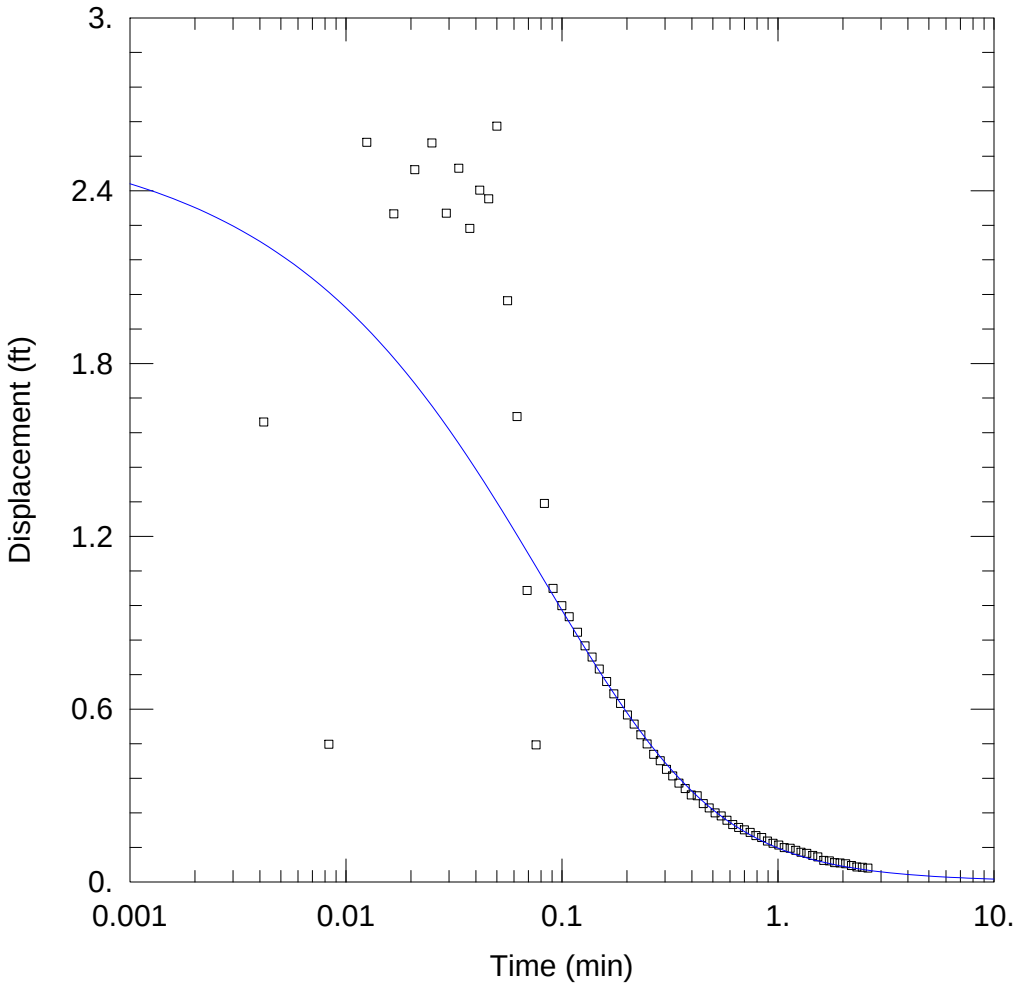
Saturated Thickness: 15.03 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-BMW-8)

Initial Displacement: 2.674 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined
Solution Method: Bouwer-Rice
K = 47.74 ft/day
y0 = 2.541 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-BMW-08test1in.aqt
Date: 03/08/17 Time: 15:18:24

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

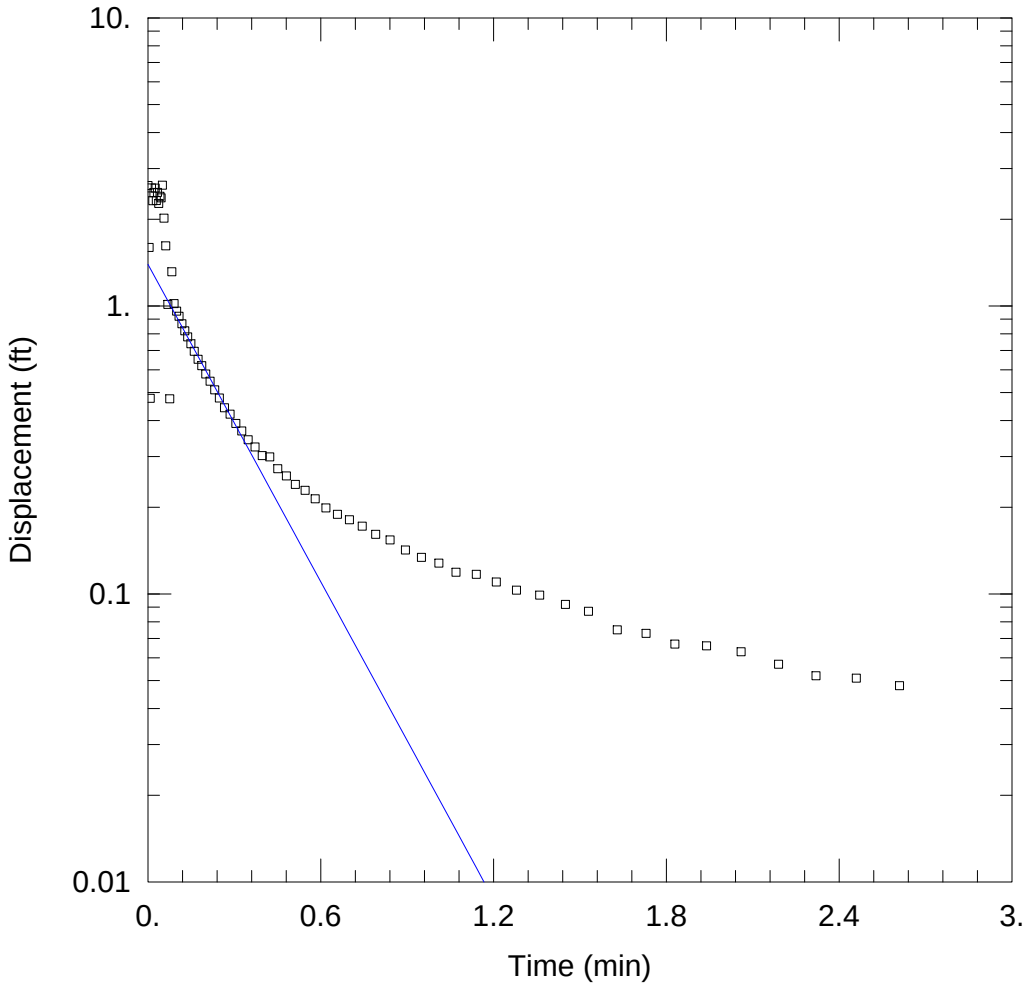
Saturated Thickness: 15.03 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-BMW-8)

Initial Displacement: 2.62 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
$T = 121.3\ ft^2/day$
S = 0.00741



WELL TEST ANALYSIS

Data Set: C:\...\22-BMW-08test1inB.aqt
Date: 03/09/17 Time: 08:18:58

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

Saturated Thickness: 15.03 ft Anisotropy Ratio (Kz/Kr): 0.1

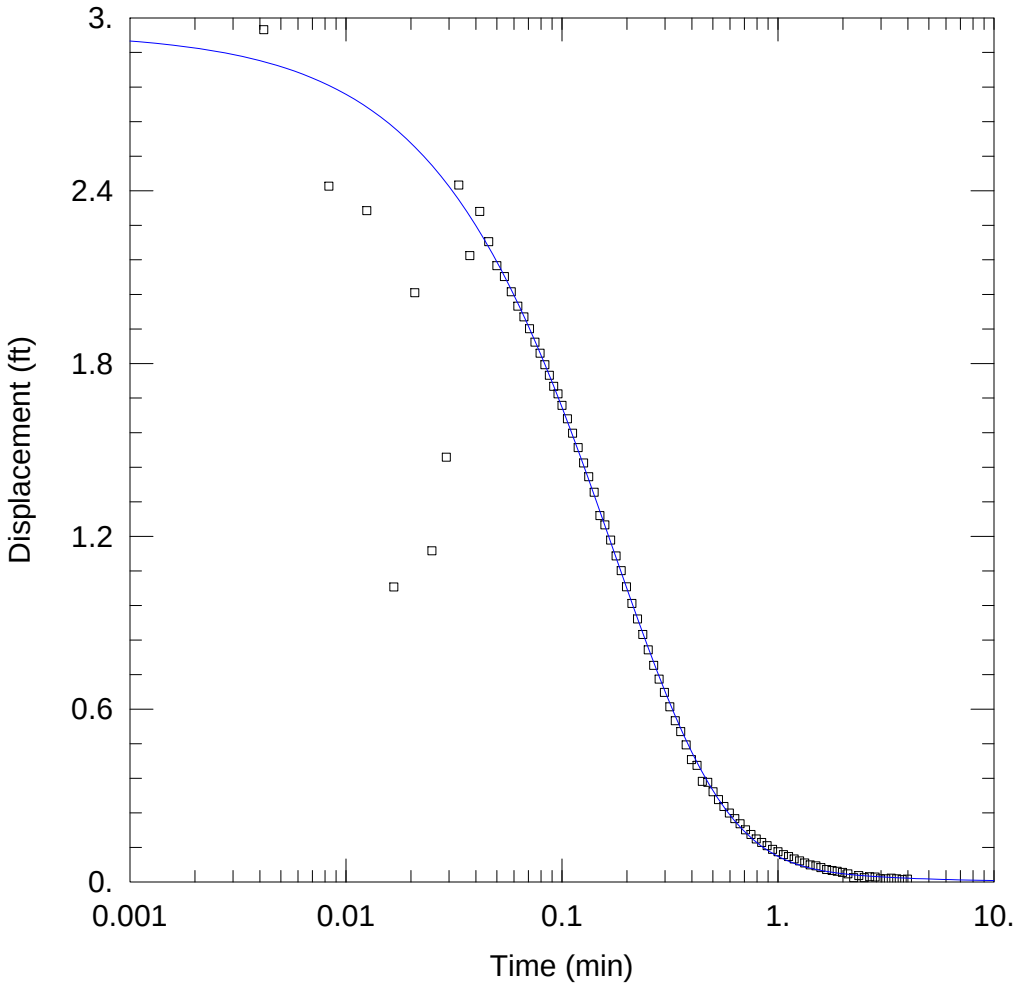
WELL DATA (22-BMW-8)

Initial Displacement: 2.62 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined Solution Method: Bouwer-Rice

K = 41.13 ft/day y0 = 1.394 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-BMW-08test2out.aqt
Date: 03/08/17 Time: 15:29:56

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

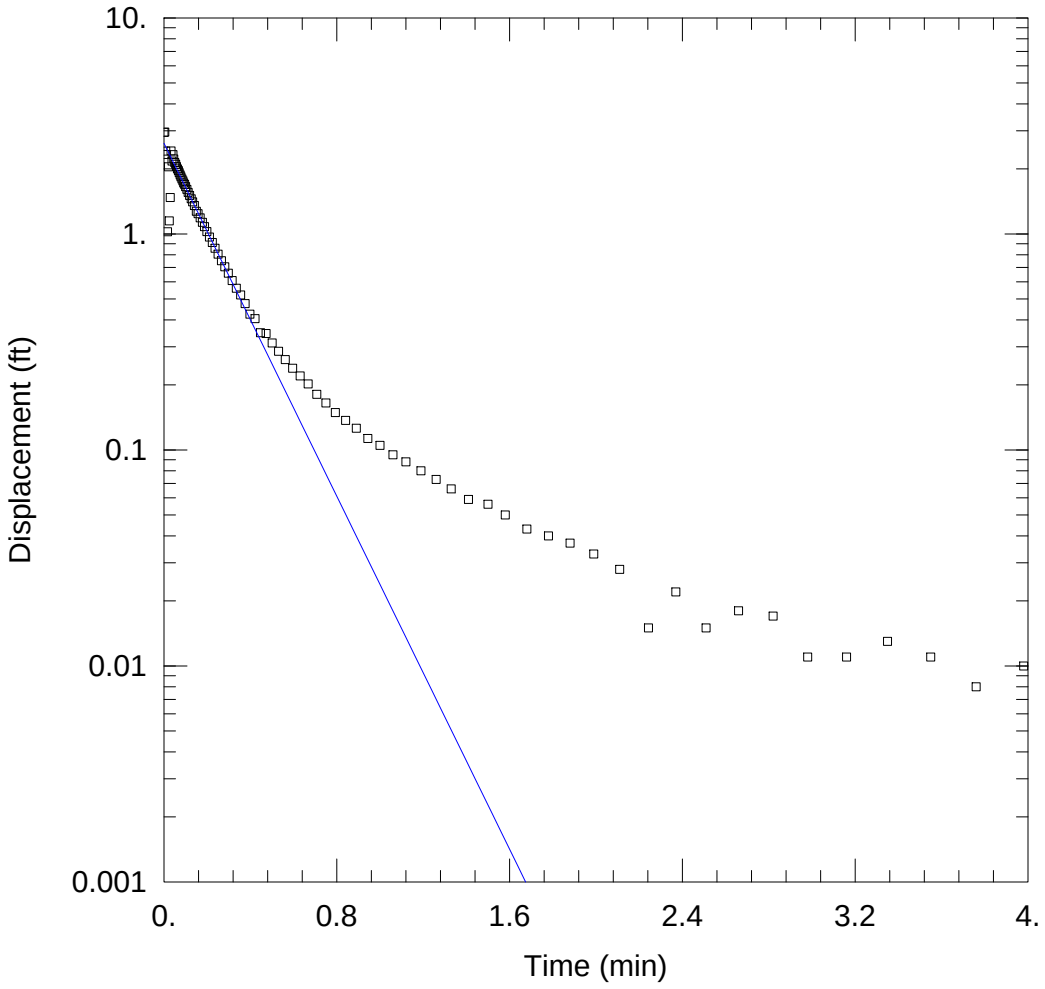
Saturated Thickness: 15.03 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-BMW-8)

Initial Displacement: 2.95 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 287.2 ft^2/day
S = 1.395E-6



WELL TEST ANALYSIS

Data Set: C:\...\22-BMW-08test2outB.aqt
Date: 03/09/17 Time: 08:19:18

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

Saturated Thickness: 15.03 ft Anisotropy Ratio (Kz/Kr): 0.1

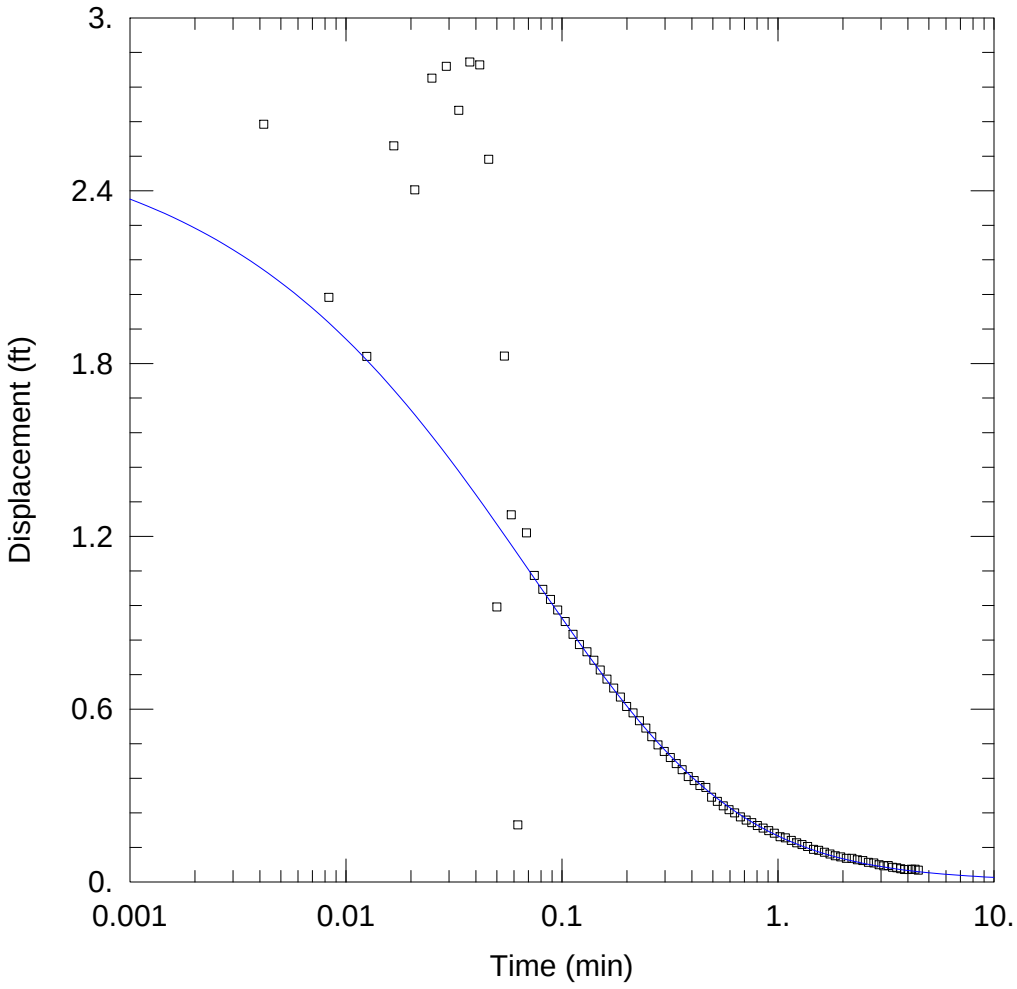
WELL DATA (22-BMW-8)

Initial Displacement: 2.95 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined Solution Method: Bouwer-Rice

K = 45.72 ft/day y0 = 2.629 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-BMW-08test2in.aqt
Date: 03/08/17 Time: 15:18:57

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

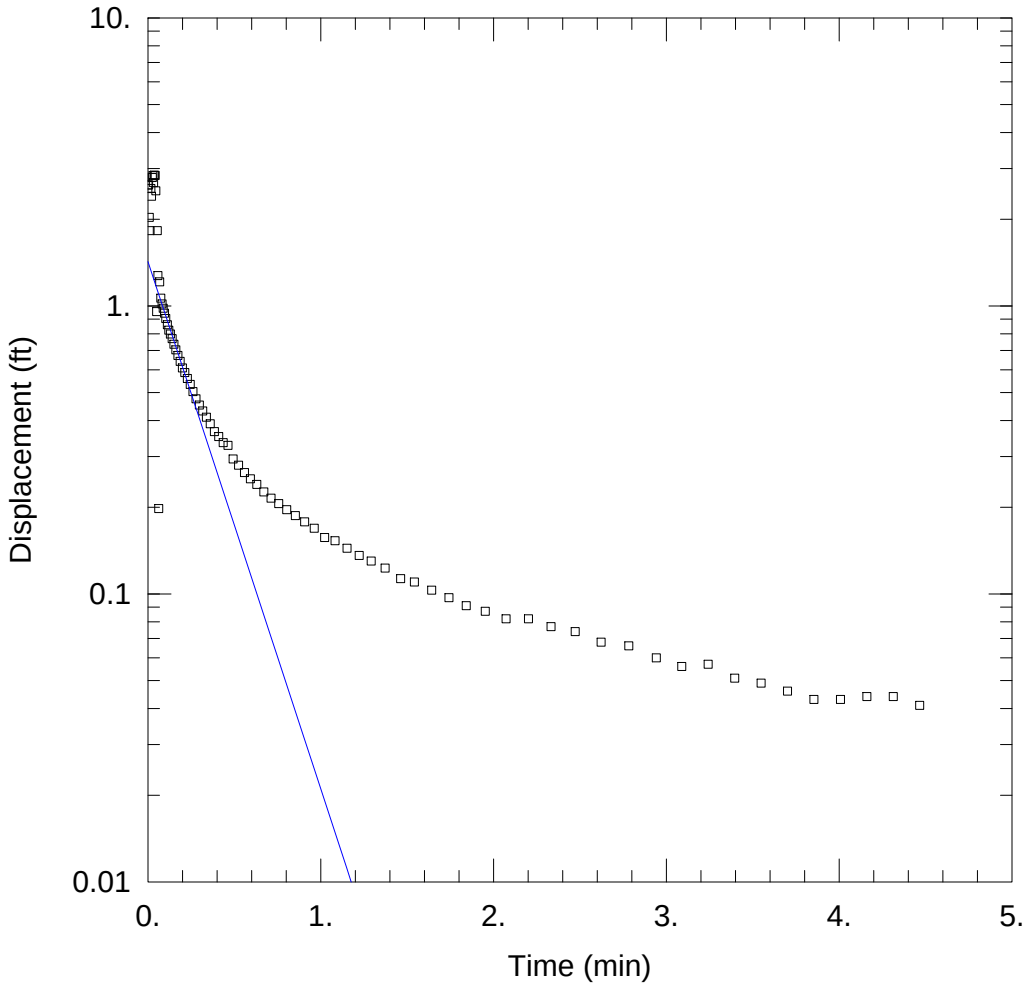
Saturated Thickness: 15.03 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-BMW-8)

Initial Displacement: 2.63 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 75.49 ft^2/day
S = 0.02371



WELL TEST ANALYSIS

Data Set: C:\...\22-BMW-08test2inB.aqt
Date: 03/09/17 Time: 08:19:09

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

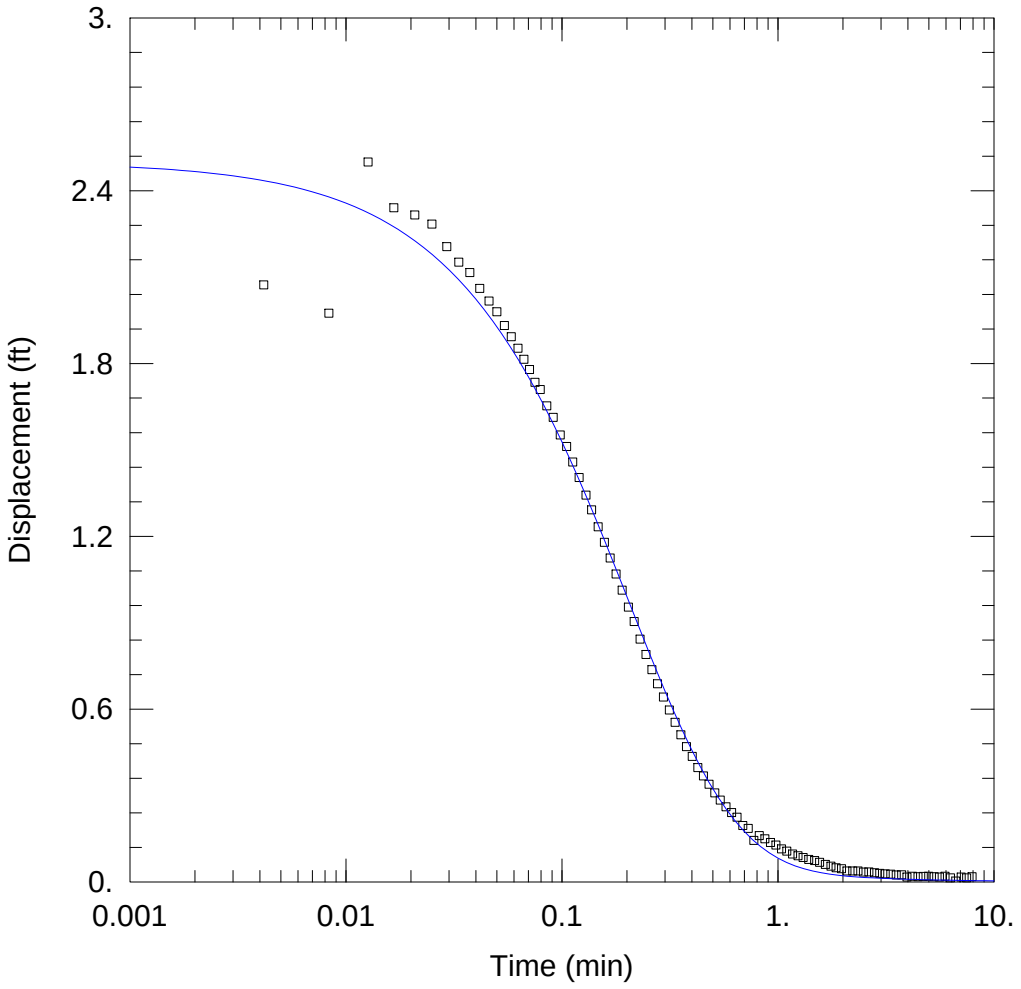
Saturated Thickness: 15.03 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-BMW-8)

Initial Displacement: 2.63 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined
Solution Method: Bouwer-Rice
K = 40.98 ft/day
y0 = 1.425 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-BMW-08test3out.aqt
Date: 03/08/17 Time: 15:31:25

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

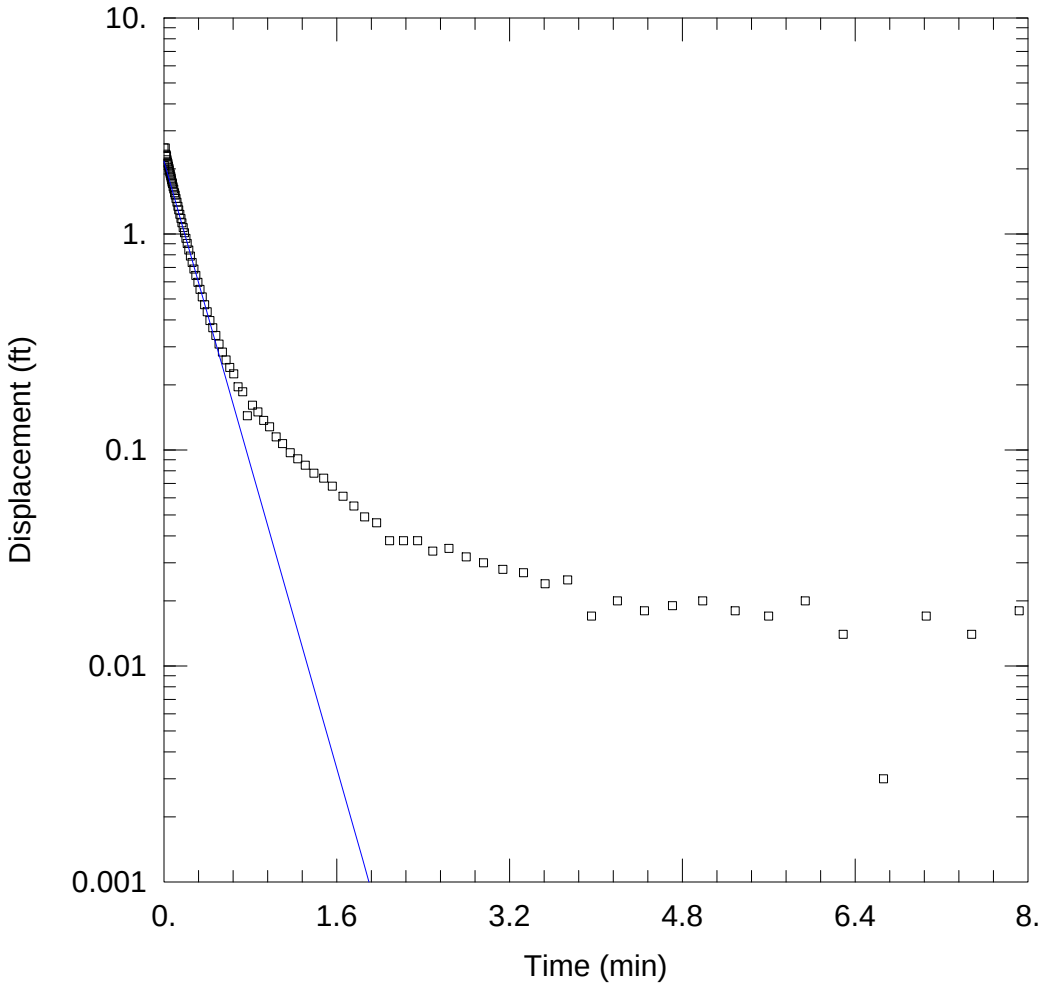
Saturated Thickness: 15.03 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-BMW-8)

Initial Displacement: 2.5 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft
Gravel Pack Porosity: 0.

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 335.5 ft^2/day
S = 2.133E-8



WELL TEST ANALYSIS

Data Set: C:\...\22-BMW-08test3outB.aqt
Date: 03/09/17 Time: 08:19:38

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

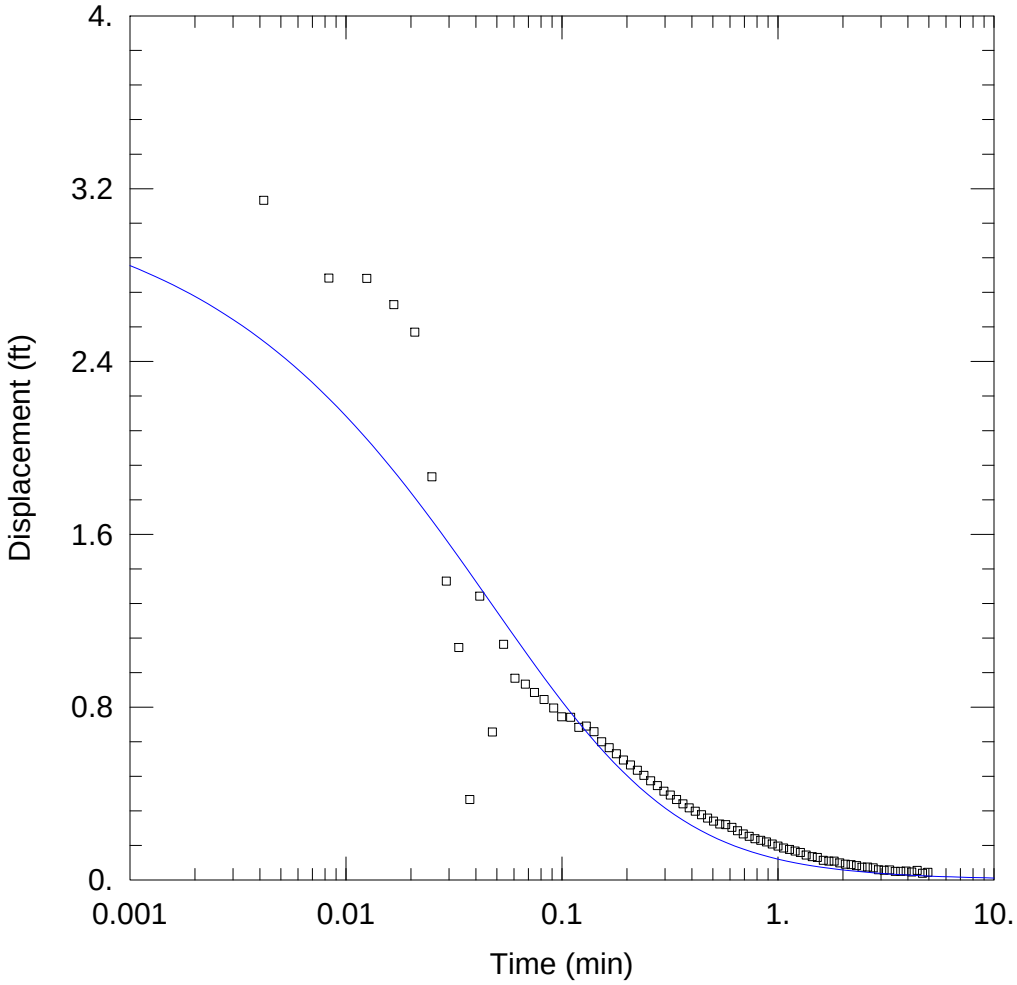
Saturated Thickness: 15.03 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-BMW-8)

Initial Displacement: 2.5 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined
Solution Method: Bouwer-Rice
K = 39.33 ft/day
y0 = 2.17 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-BMW-08test3in.aqt
Date: 03/08/17 Time: 15:17:19

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

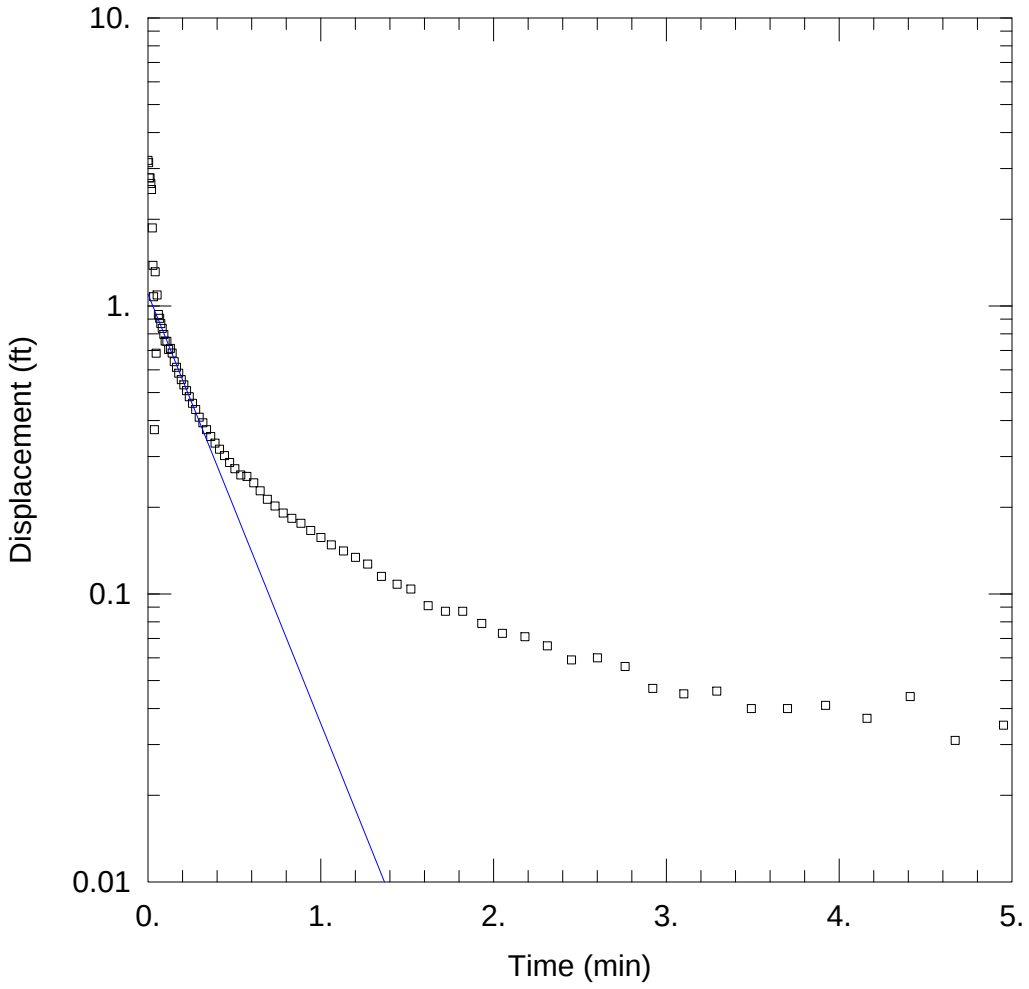
Saturated Thickness: 15.03 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-BMW-8)

Initial Displacement: 3.2 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 164. ft^2/day
S = 0.01321



WELL TEST ANALYSIS

Data Set: C:\...\22-BMW-08test3inB.aqt
Date: 03/09/17 Time: 08:19:28

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-BMW-8
Test Date: 8-18-2016

AQUIFER DATA

Saturated Thickness: 15.03 ft Anisotropy Ratio (Kz/Kr): 0.1

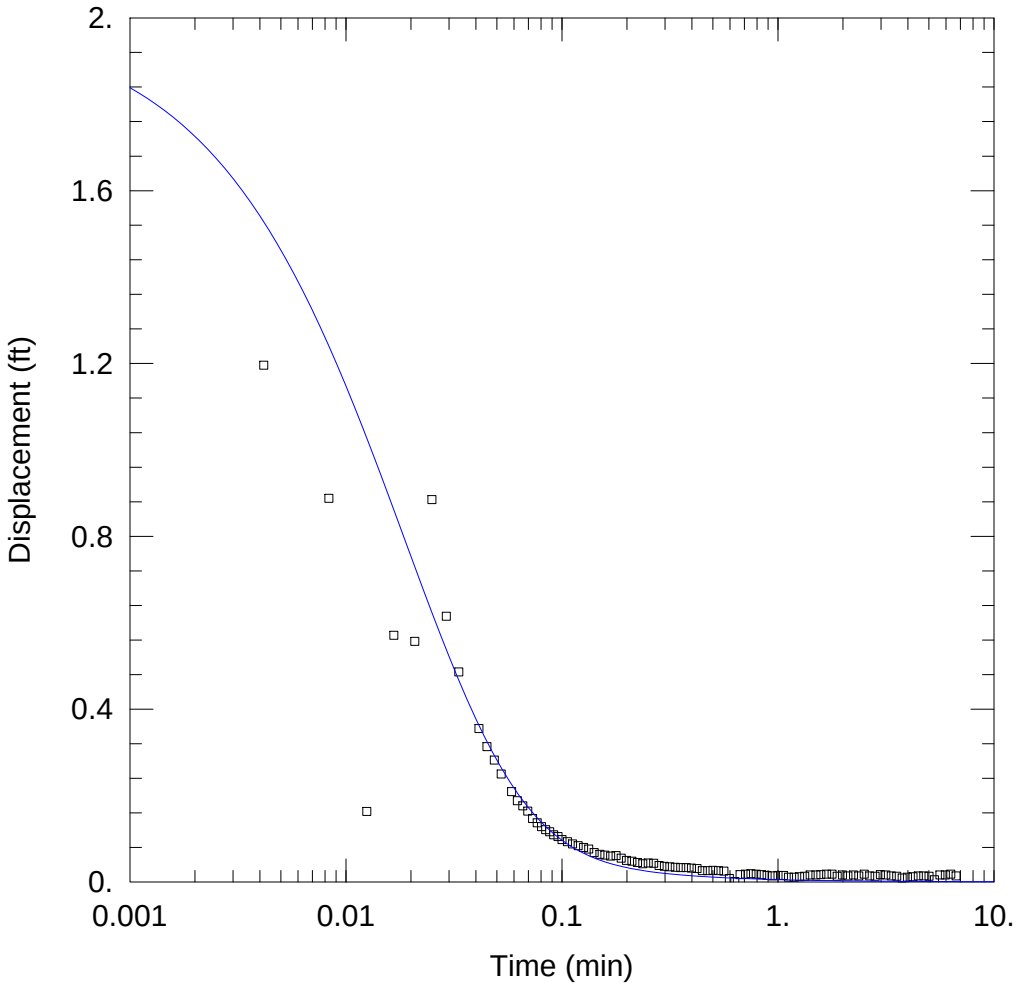
WELL DATA (22-BMW-8)

Initial Displacement: 3.2 ft
Total Well Penetration Depth: 15.03 ft
Casing Radius: 0.167 ft
Static Water Column Height: 15.03 ft
Screen Length: 15.03 ft
Well Radius: 0.5 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined Solution Method: Bouwer-Rice

K = 33.39 ft/day y0 = 1.1 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-07test1out.aqt
Date: 03/08/17 Time: 15:32:39

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

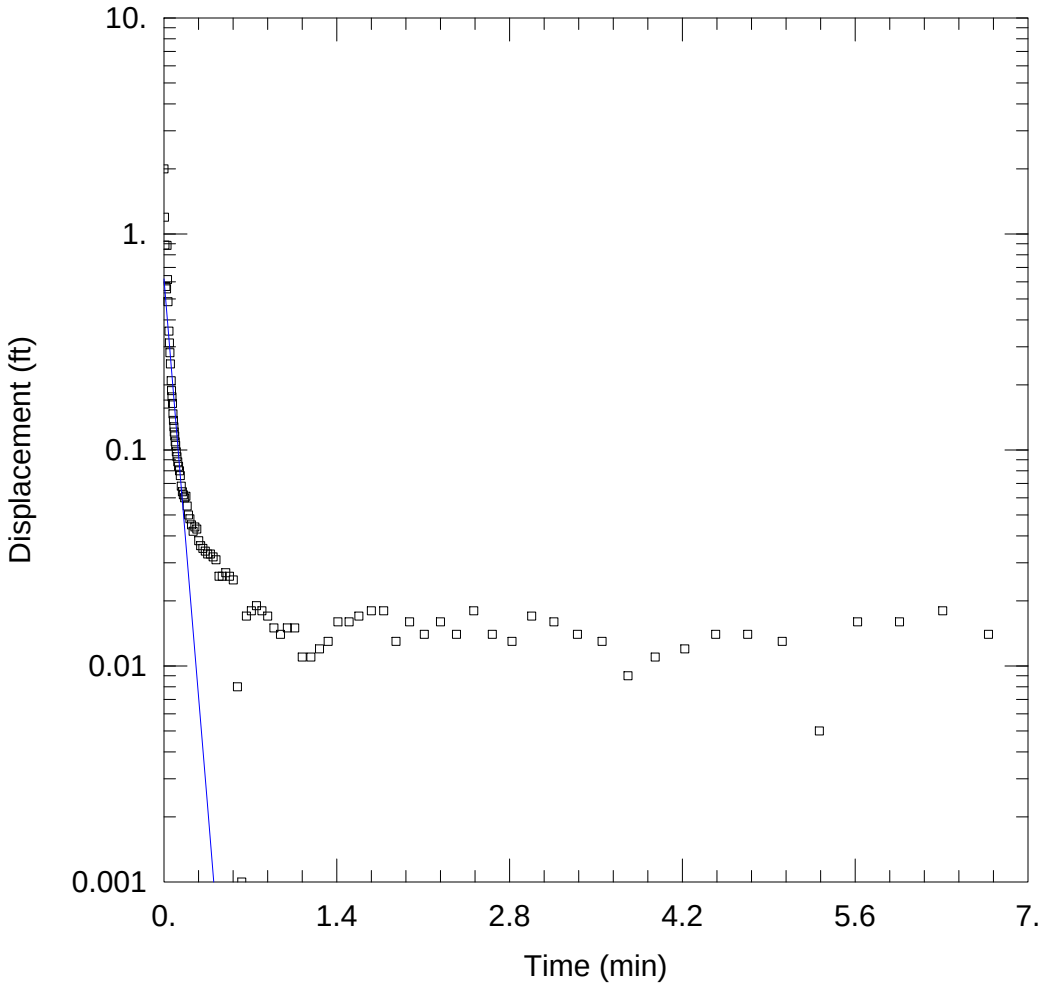
Saturated Thickness: 16.05 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-07)

Initial Displacement: 2. ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 754.6 ft^2/day
S = 2.687E-5



WELL TEST ANALYSIS

Data Set: C:\...\22-MM-07test1outB.aqt
Date: 03/09/17 Time: 08:20:00

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

Saturated Thickness: 16.05 ft Anisotropy Ratio (Kz/Kr): 0.1

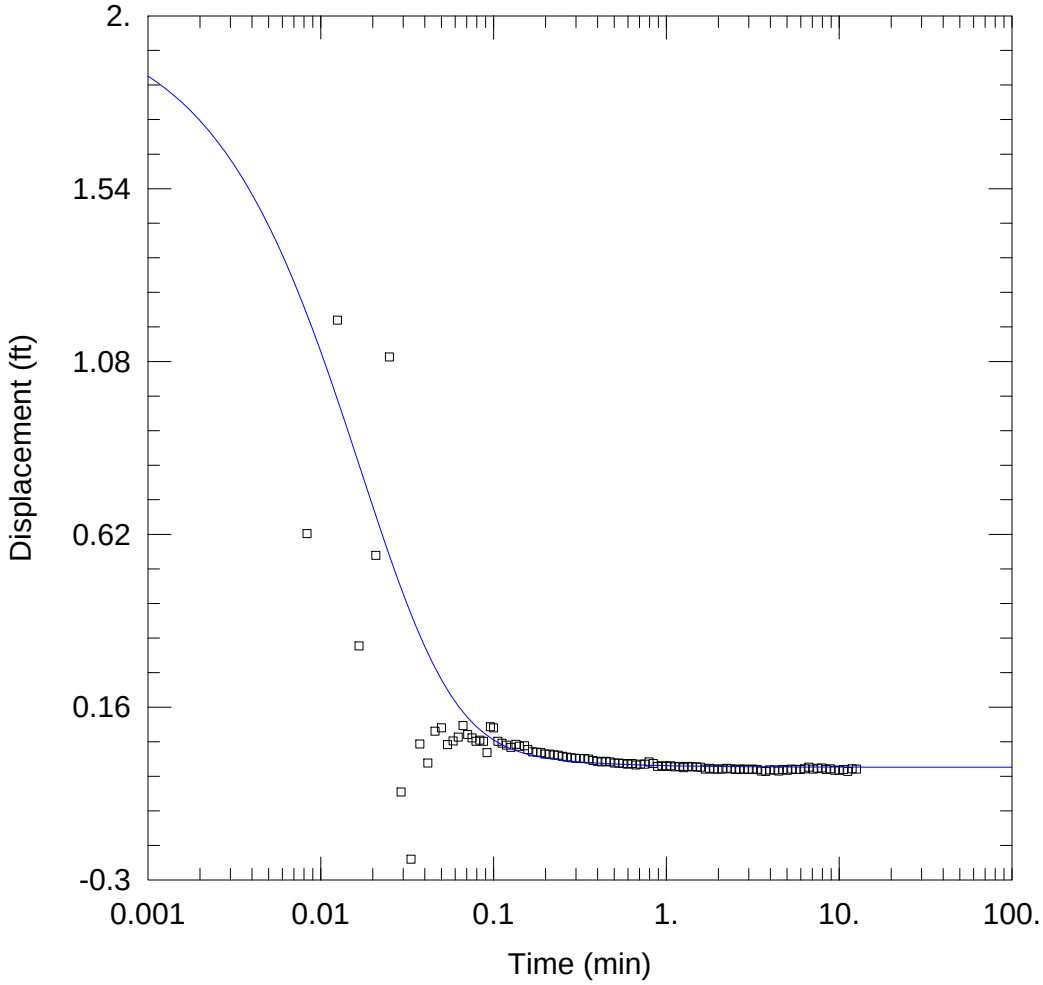
WELL DATA (22-MM-07)

Initial Displacement: 2. ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined Solution Method: Bouwer-Rice

K = 158.1 ft/day y0 = 0.6191 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MW-07test1in.aqt
Date: 03/08/17 Time: 15:19:22

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

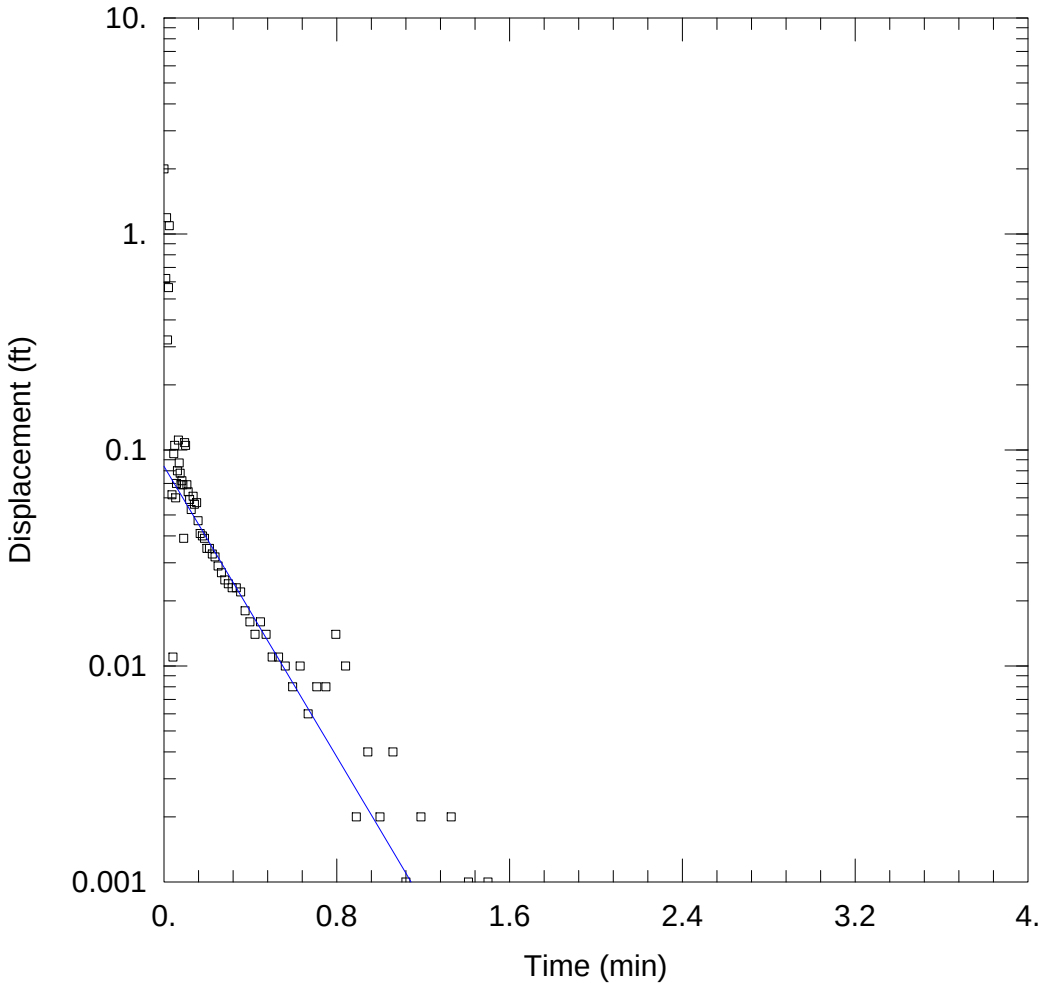
Saturated Thickness: 16.05 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-07)

Initial Displacement: 2. ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 967.1 ft^2/day
S = 7.746E-6



WELL TEST ANALYSIS

Data Set: C:\...\22-MM-07test1inB.aqt
Date: 03/09/17 Time: 08:19:46

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

Saturated Thickness: 16.05 ft Anisotropy Ratio (Kz/Kr): 0.1

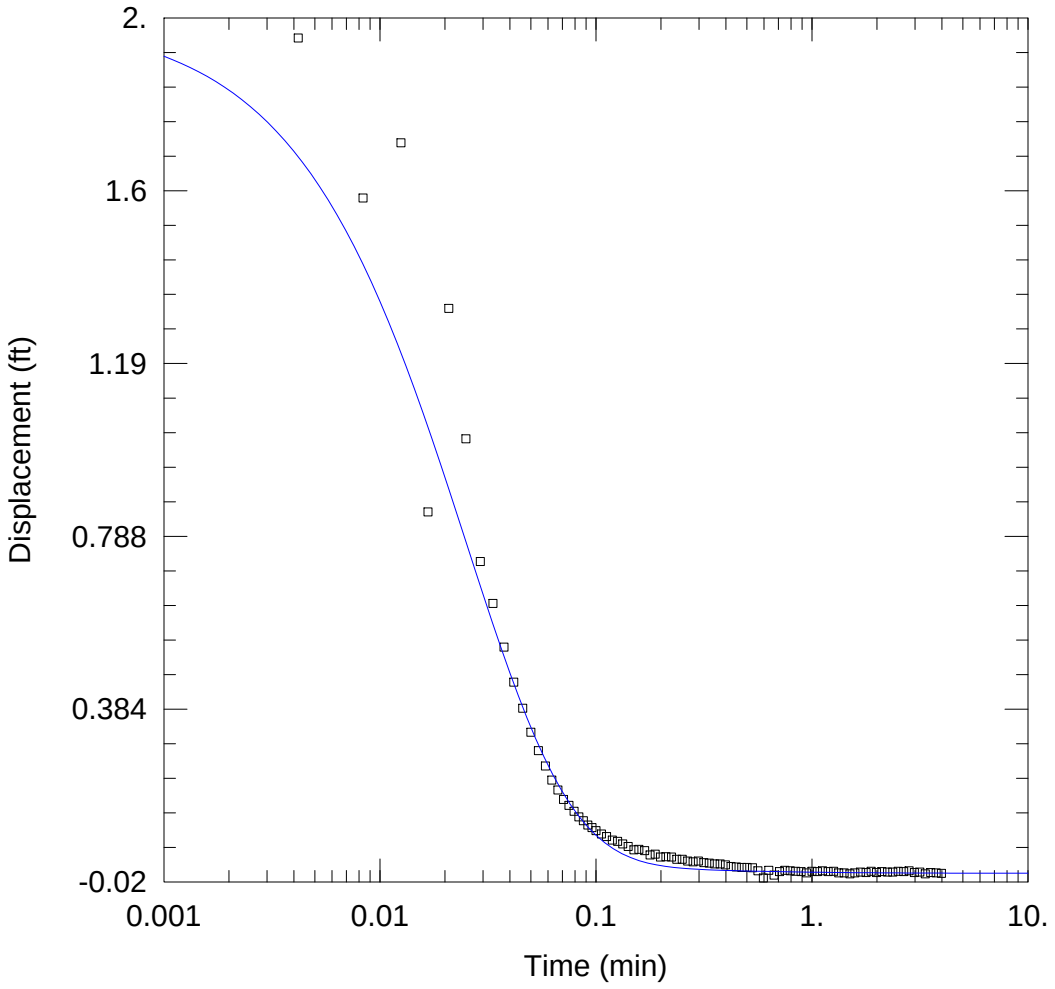
WELL DATA (22-MM-07)

Initial Displacement: 2. ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined Solution Method: Bouwer-Rice

K = 38.49 ft/day y0 = 0.08399 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-07test2out.aqt
Date: 03/08/17 Time: 15:33:07

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

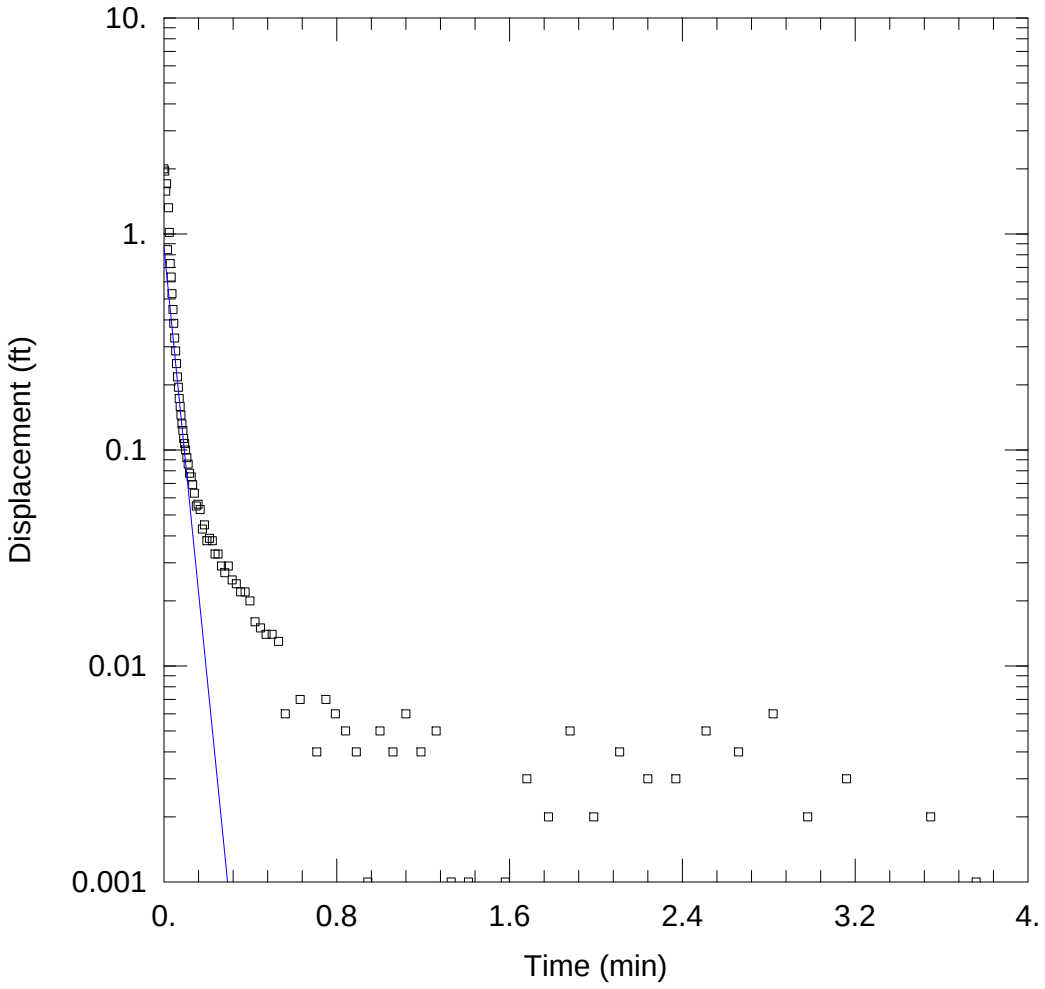
Saturated Thickness: 16.05 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-07)

Initial Displacement: 2. ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 1523.4 ft^2/day
S = 1.0E-10



WELL TEST ANALYSIS

Data Set: C:\...\22-MM-07test2outB.aqt
Date: 03/09/17 Time: 08:20:25

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

Saturated Thickness: 16.05 ft Anisotropy Ratio (Kz/Kr): 0.1

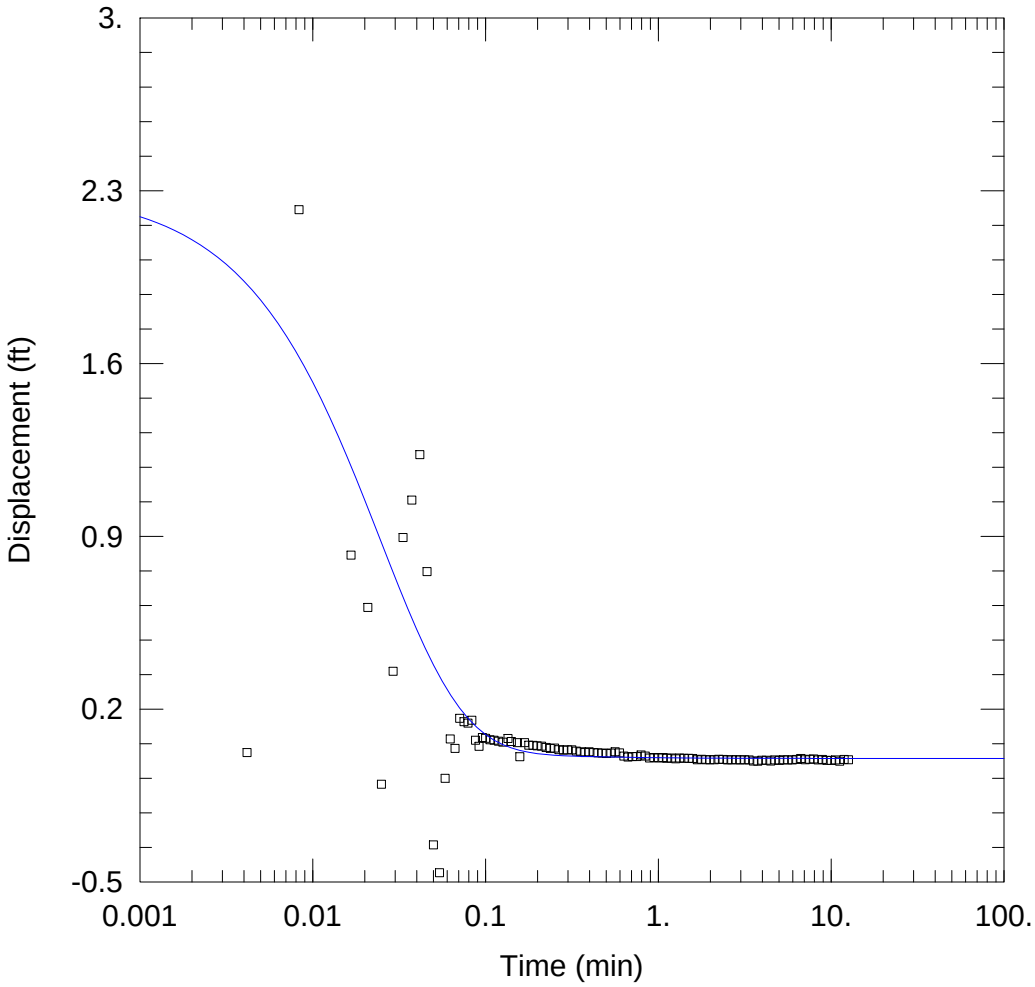
WELL DATA (22-MM-07)

Initial Displacement: 2. ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined Solution Method: Bouwer-Rice

K = 228.4 ft/day y0 = 0.8667 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-07test2in.aqt
Date: 03/08/17 Time: 15:33:33

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

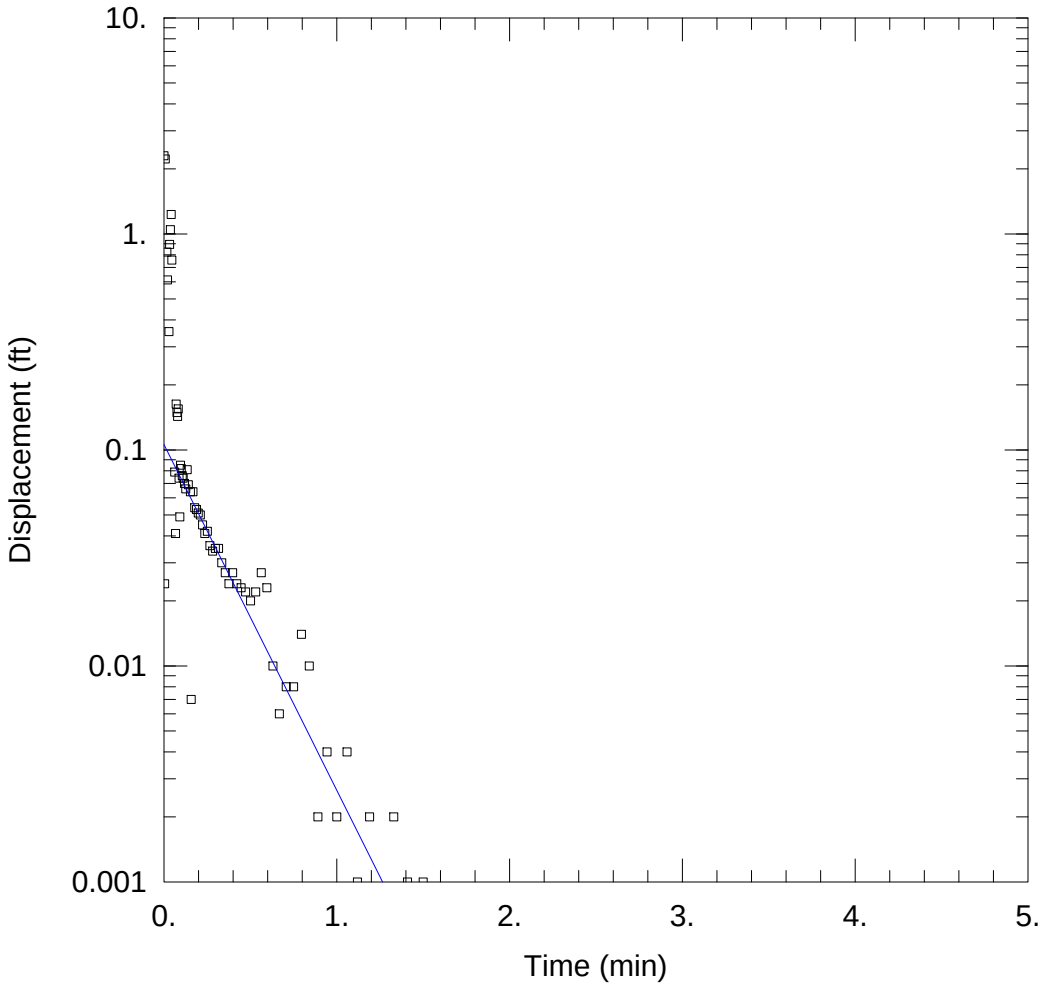
Saturated Thickness: 16.05 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-07)

Initial Displacement: 2.3 ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 1556.7 ft^2/day
S = 1.0E-10



WELL TEST ANALYSIS

Data Set: C:\...\22-MM-07test2inB.aqt
Date: 03/09/17 Time: 08:20:14

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

Saturated Thickness: 16.05 ft Anisotropy Ratio (Kz/Kr): 0.1

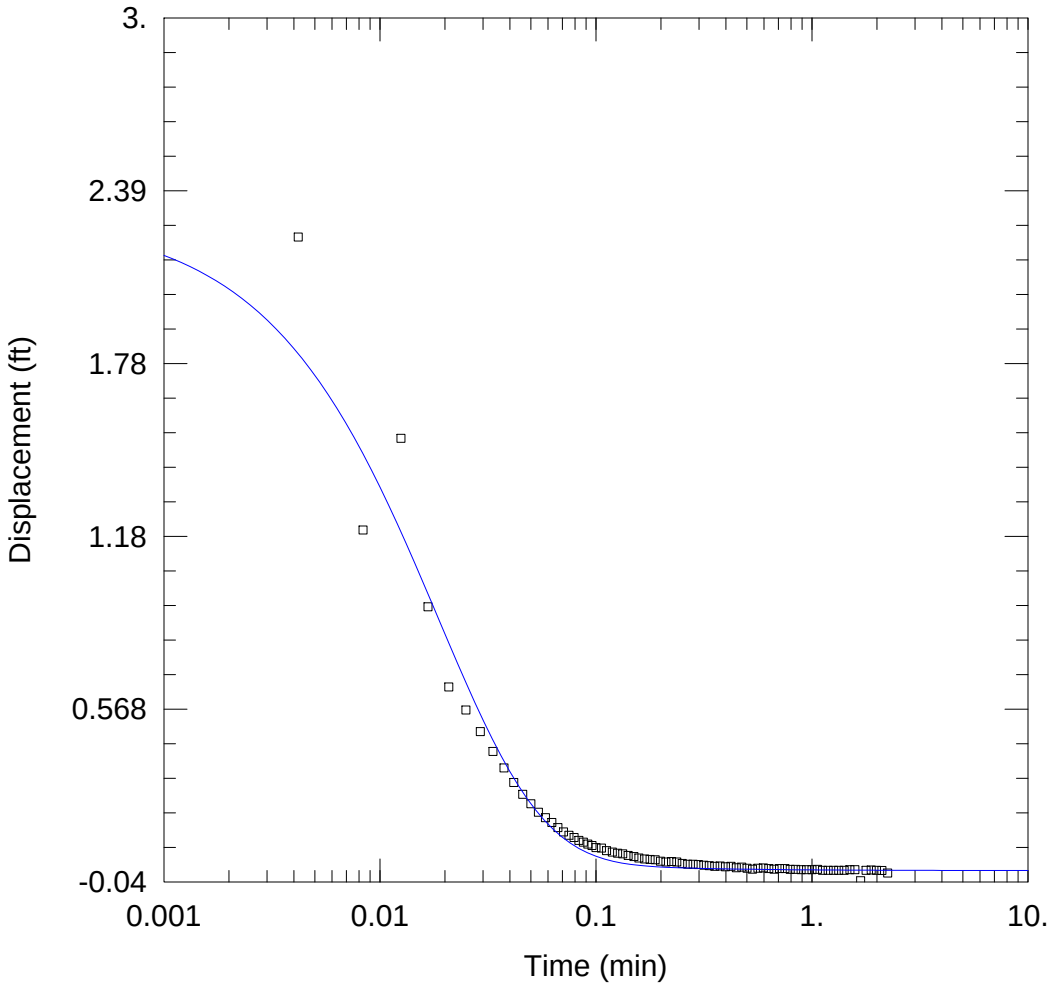
WELL DATA (22-MM-07)

Initial Displacement: 2.3 ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined Solution Method: Bouwer-Rice

K = 36.63 ft/day y0 = 0.1059 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-07test3out.aqt
Date: 03/08/17 Time: 15:24:55

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

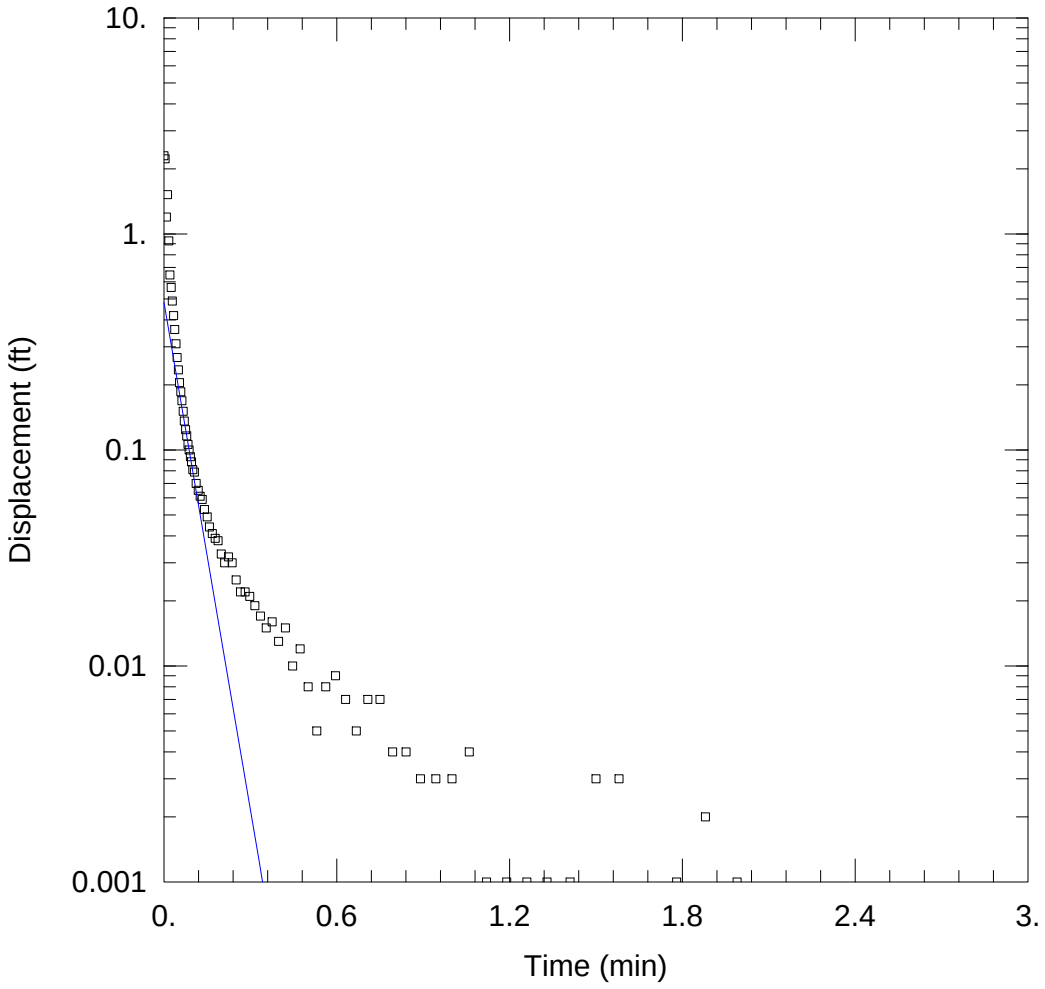
Saturated Thickness: 16.05 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-07)

Initial Displacement: 2.3 ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 2055.8 ft^2/day
S = 1.0E-10



WELL TEST ANALYSIS

Data Set: C:\...\22-MM-07test3outB.aqt
Date: 03/09/17 Time: 08:19:54

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

Saturated Thickness: 16.05 ft Anisotropy Ratio (Kz/Kr): 0.1

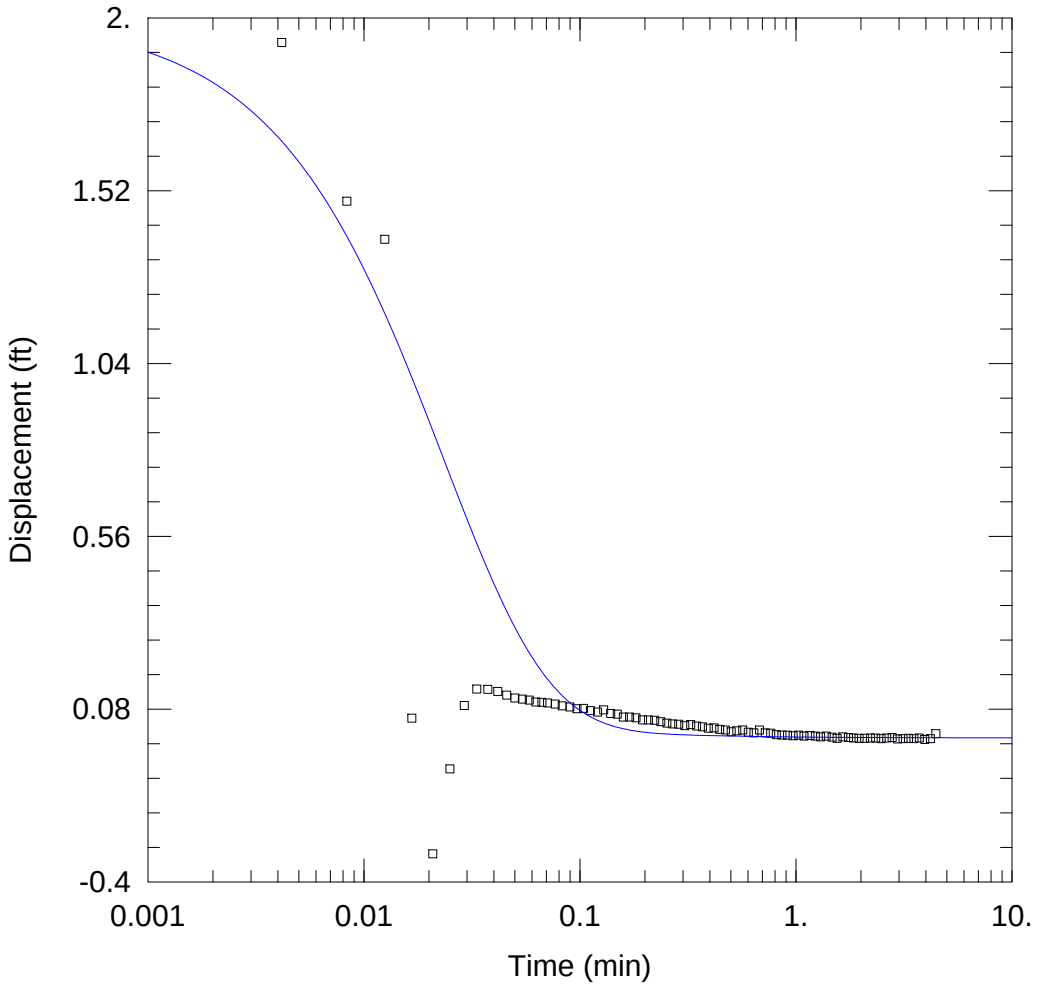
WELL DATA (22-MM-07)

Initial Displacement: 2.3 ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined Solution Method: Bouwer-Rice

K = 179.1 ft/day y0 = 0.4817 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-07test3in.aqt
Date: 03/08/17 Time: 15:34:09

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

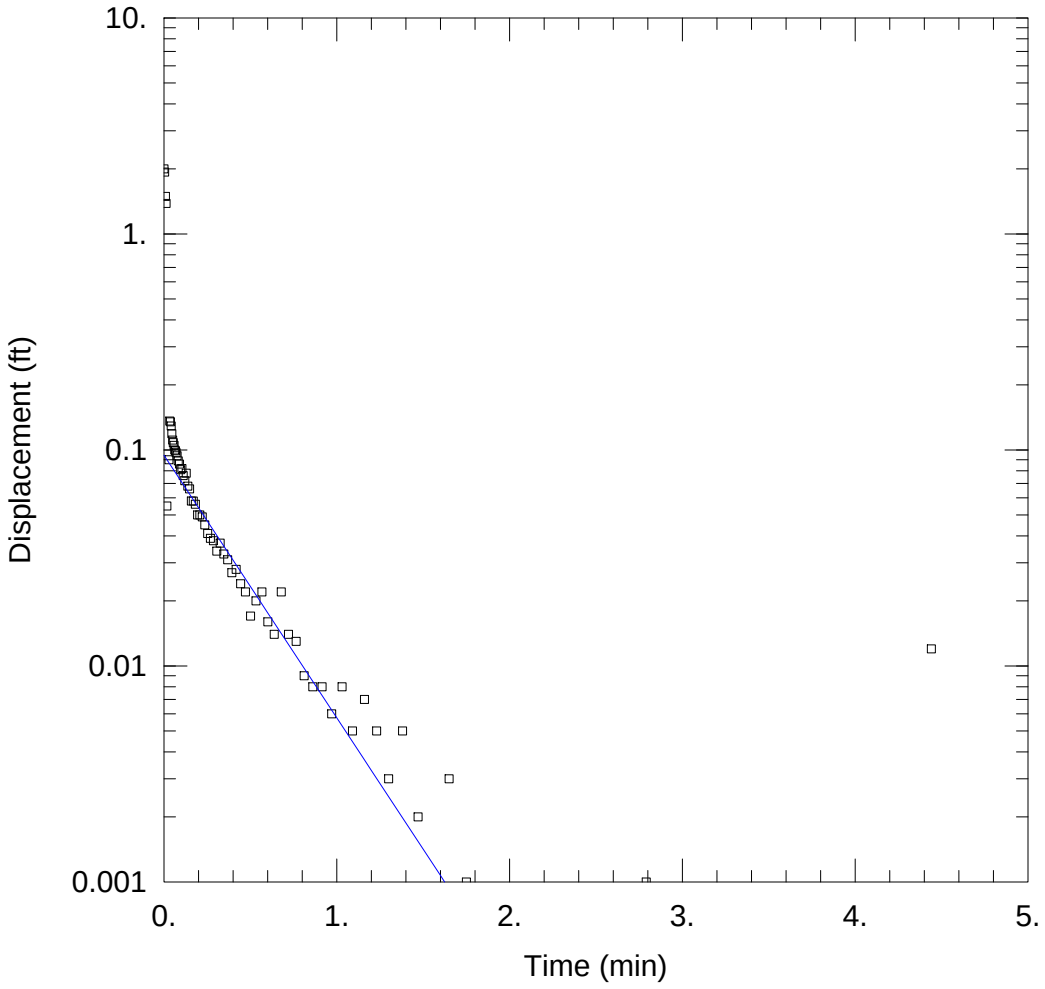
Saturated Thickness: 16.05 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-07)

Initial Displacement: 2. ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 1630.1 ft^2/day
S = 1.0E-10



WELL TEST ANALYSIS

Data Set: C:\...\22-MM-07test3inB.aqt
Date: 03/09/17 Time: 08:20:08

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-07
Test Date: 8-18-2016

AQUIFER DATA

Saturated Thickness: 16.05 ft Anisotropy Ratio (Kz/Kr): 0.1

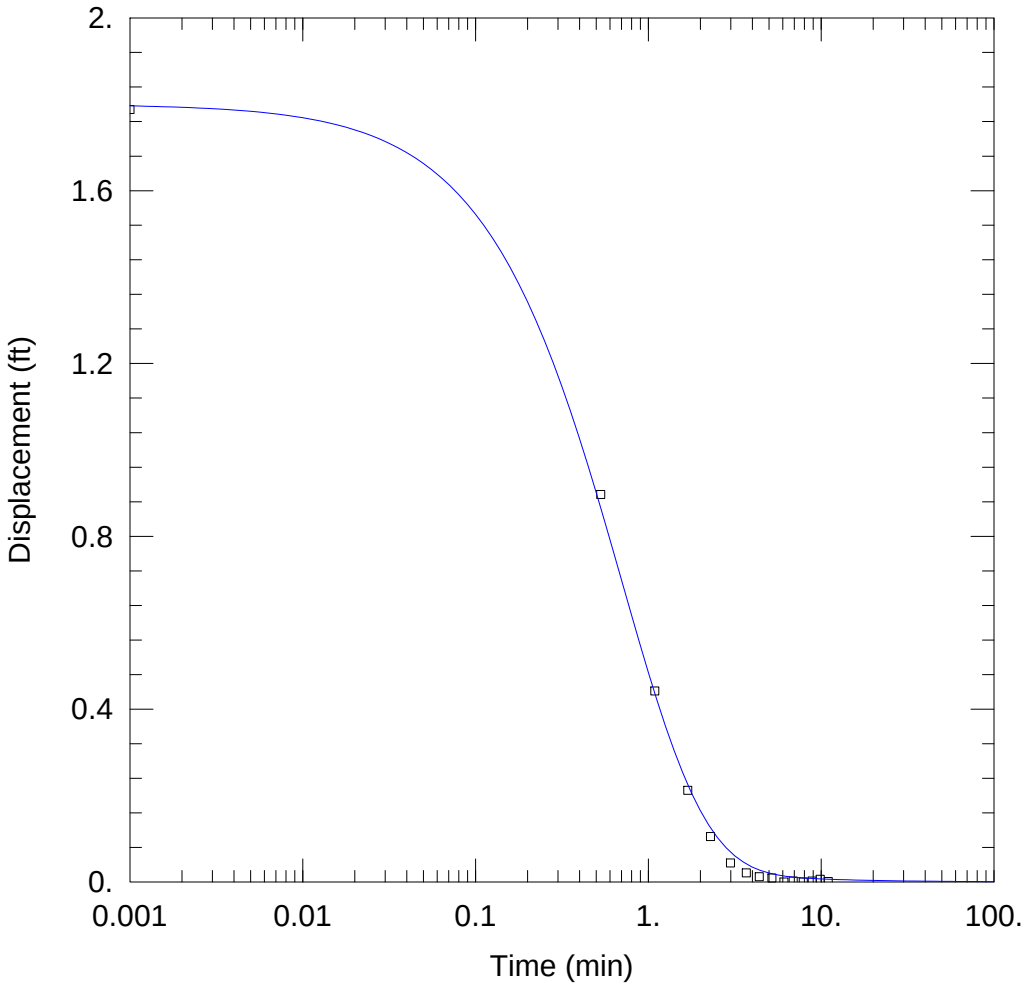
WELL DATA (22-MM-07)

Initial Displacement: 2. ft
Total Well Penetration Depth: 13.05 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 13.05 ft
Screen Length: 13.05 ft
Well Radius: 0.4167 ft
Gravel Pack Porosity: 0.3

SOLUTION

Aquifer Model: Unconfined Solution Method: Bouwer-Rice

K = 27.83 ft/day y0 = 0.09453 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-08test1out.aqt
Date: 03/08/17 Time: 15:23:08

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-08
Test Date: 8-18-2016

AQUIFER DATA

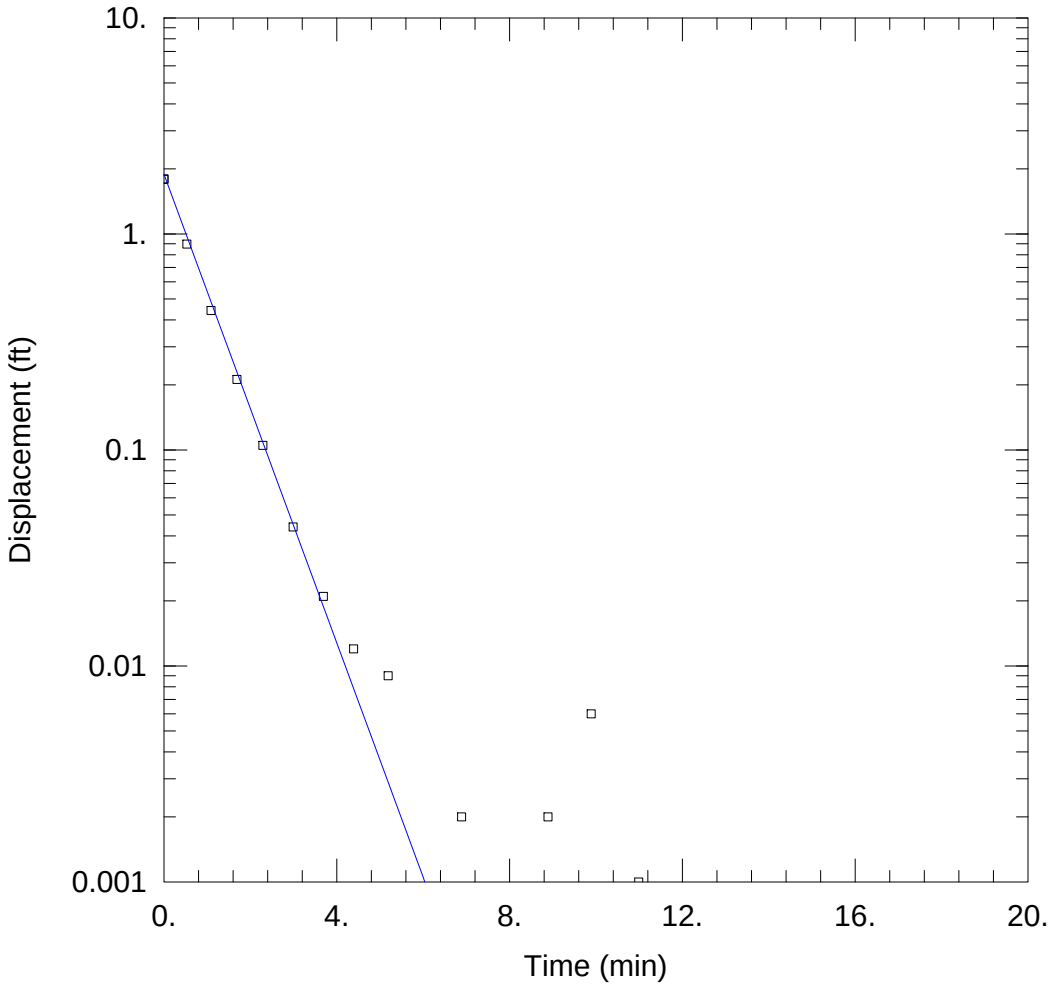
Saturated Thickness: 36.12 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MW-08)

Initial Displacement: 1.8 ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 54.27 ft^2/day
S = 1.0E-10



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MW-08test1outB.aqt
Date: 03/08/17 Time: 15:39:09

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-08
Test Date: 8-18-2016

AQUIFER DATA

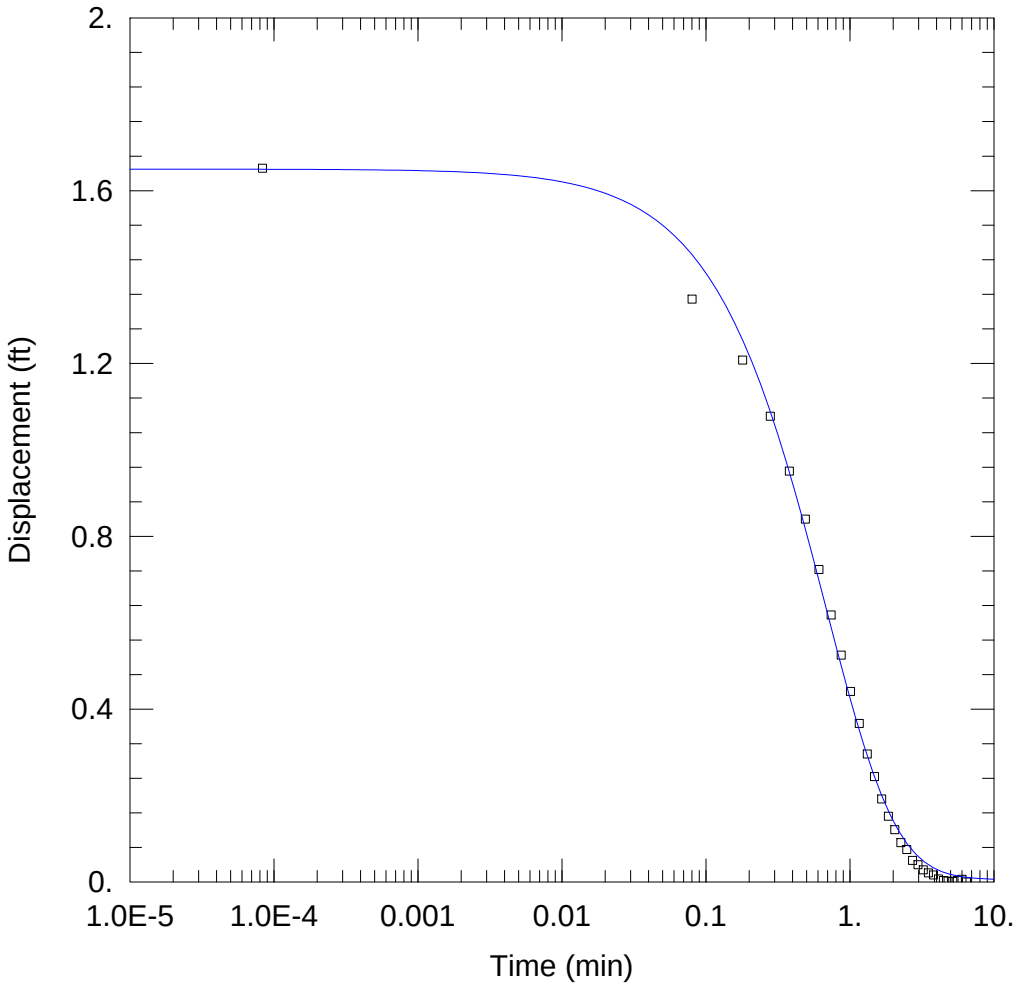
Saturated Thickness: 36.12 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MW-08)

Initial Displacement: 1.8 ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Unconfined
Solution Method: Bouwer-Rice
K = 1.542 ft/day
y0 = 1.888 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-08test1in.aqt
Date: 03/08/17 Time: 15:24:01

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-08
Test Date: 8-18-2016

AQUIFER DATA

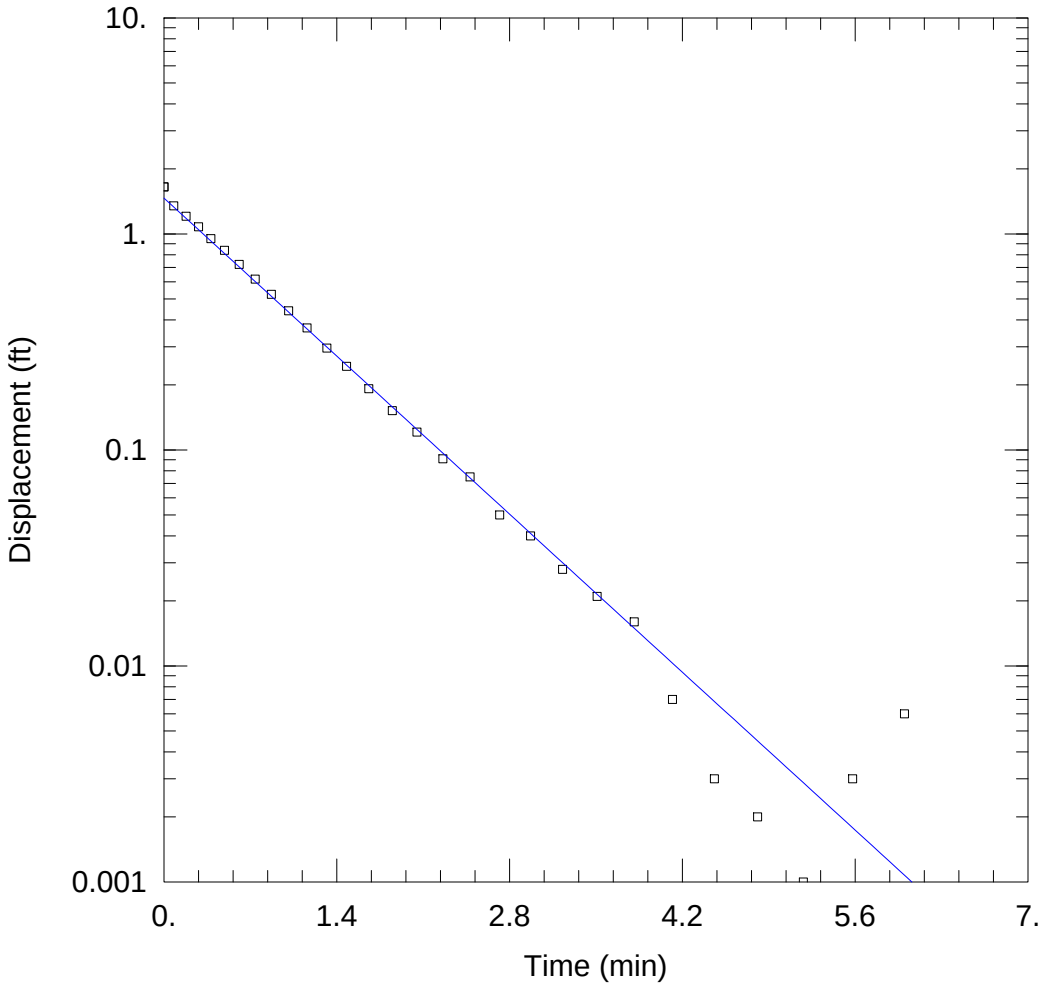
Saturated Thickness: 36.12 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-08)

Initial Displacement: 1.65 ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 56.23 ft^2/day
S = 1.0E-10



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-08test1inB.aqt
Date: 03/08/17 Time: 15:23:34

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-08
Test Date: 8-18-2016

AQUIFER DATA

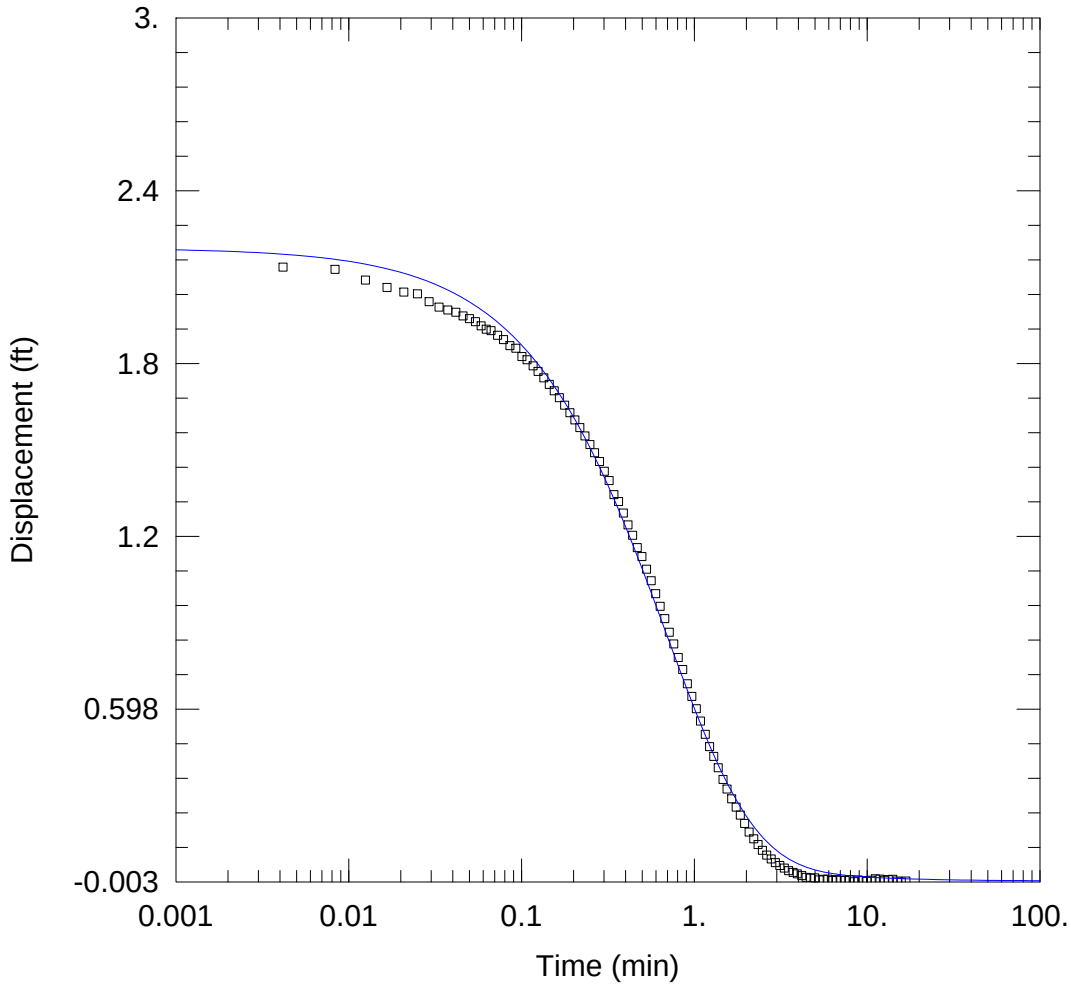
Saturated Thickness: 36.12 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MW-08)

Initial Displacement: 1.65 ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Unconfined
Solution Method: Bouwer-Rice
K = 1.485 ft/day
y0 = 1.464 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-08test2out.aqt
Date: 03/08/17 Time: 15:16:05

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-08
Test Date: 8-18-2016

AQUIFER DATA

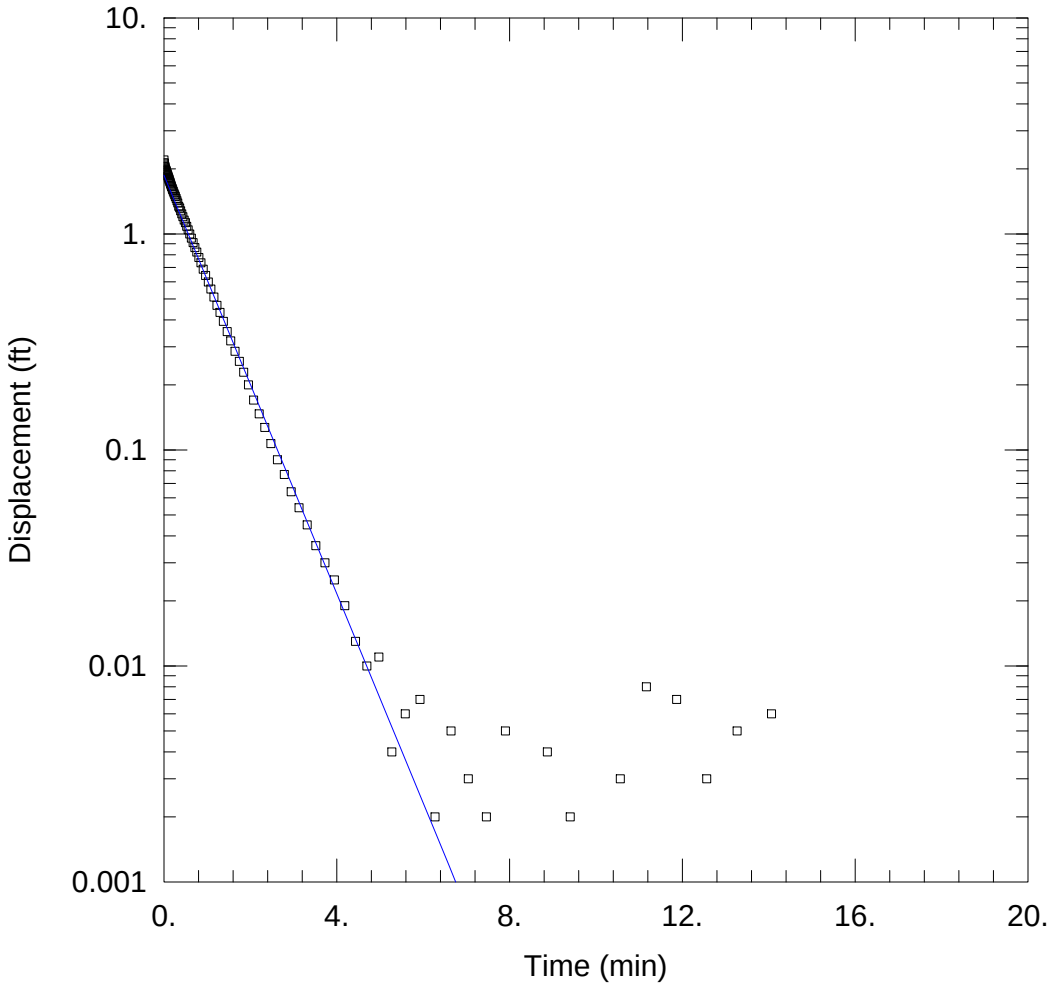
Saturated Thickness: 36.12 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-08)

Initial Displacement: 2.2 ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 34.47 ft^2/day
S = 1.92E-7



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-08test2outB.aqt
Date: 03/08/17 Time: 15:22:01

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-08
Test Date: 8-18-2016

AQUIFER DATA

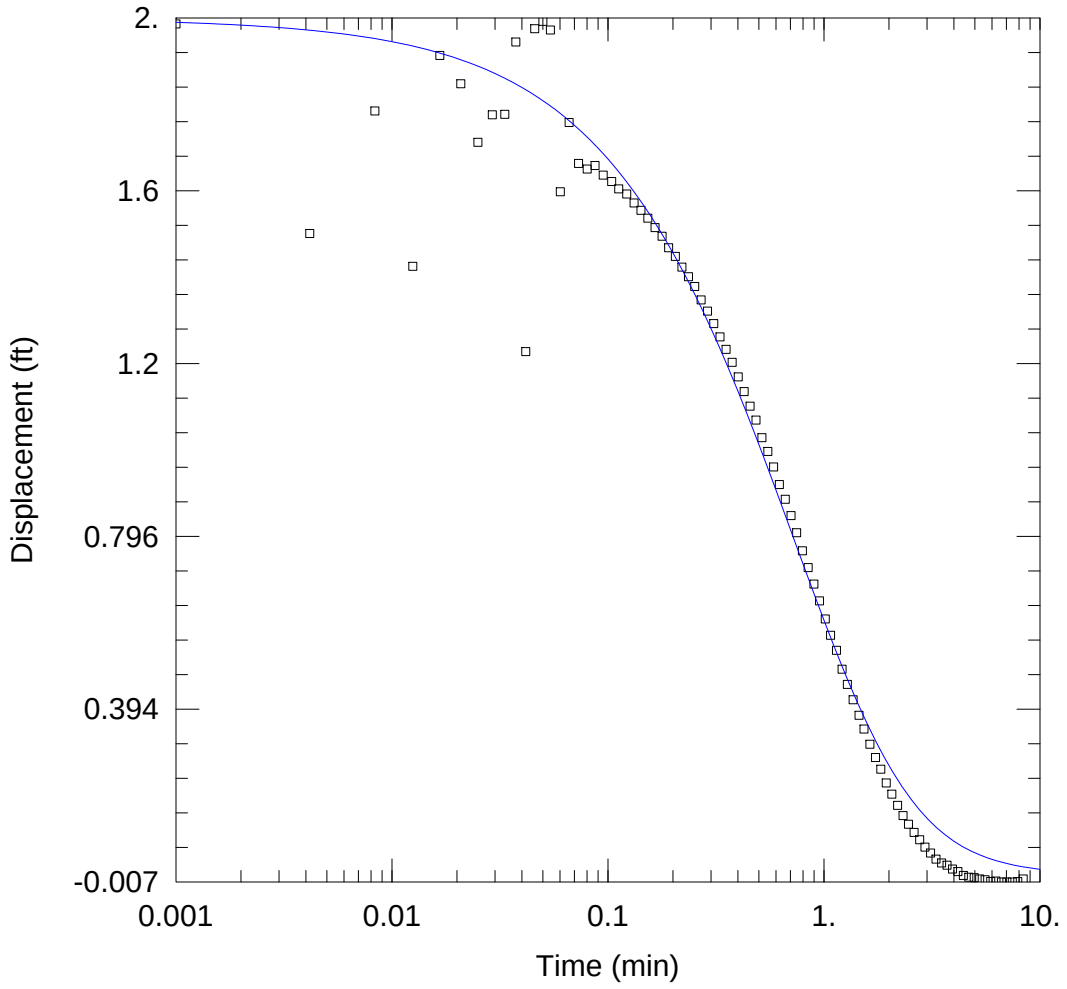
Saturated Thickness: 36.12 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-08)

Initial Displacement: 2.2 ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Unconfined
Solution Method: Bouwer-Rice
K = 1.376 ft/day
y0 = 1.869 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-08test2in.aqt
Date: 03/08/17 Time: 15:17:58

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-08
Test Date: 8-18-2016

AQUIFER DATA

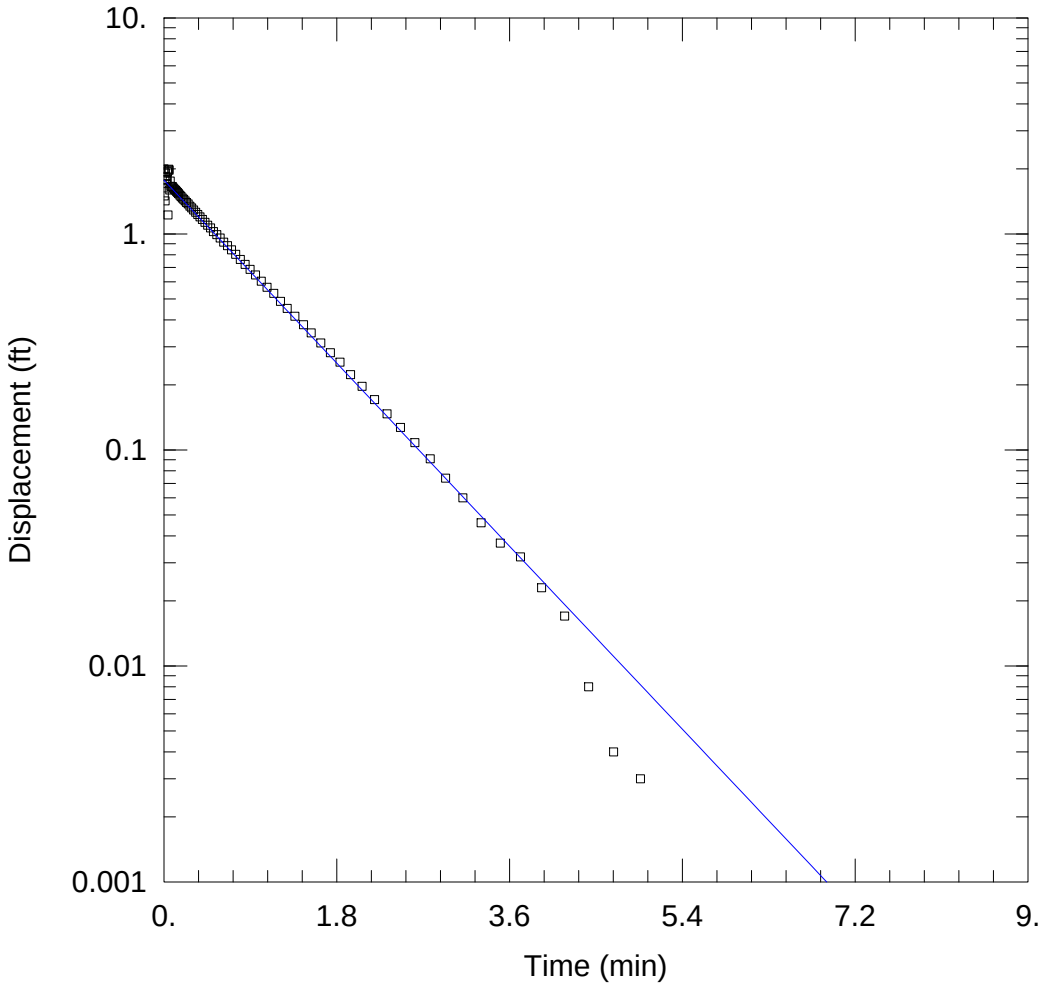
Saturated Thickness: 36.12 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-08)

Initial Displacement: 2. ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 20.56 ft^2/day
S = 1.834E-5



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-08test2inB.aqt
Date: 03/08/17 Time: 15:22:29

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MW-08
Test Date: 8-18-2016

AQUIFER DATA

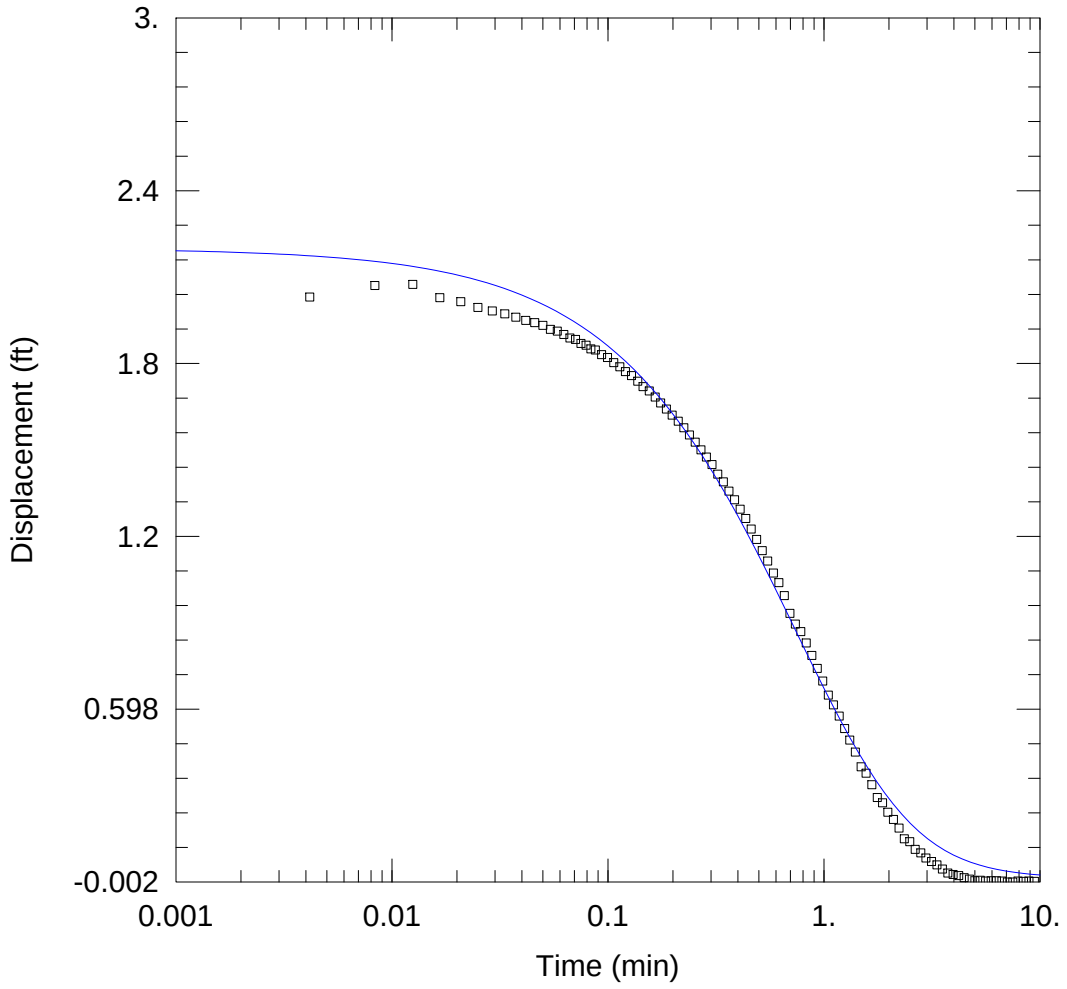
Saturated Thickness: 36.12 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-08)

Initial Displacement: 2. ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Unconfined
Solution Method: Bouwer-Rice
K = 1.337 ft/day
y0 = 1.772 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-08test3out.aqt
Date: 03/08/17 Time: 15:20:28

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-08
Test Date: 8-18-2016

AQUIFER DATA

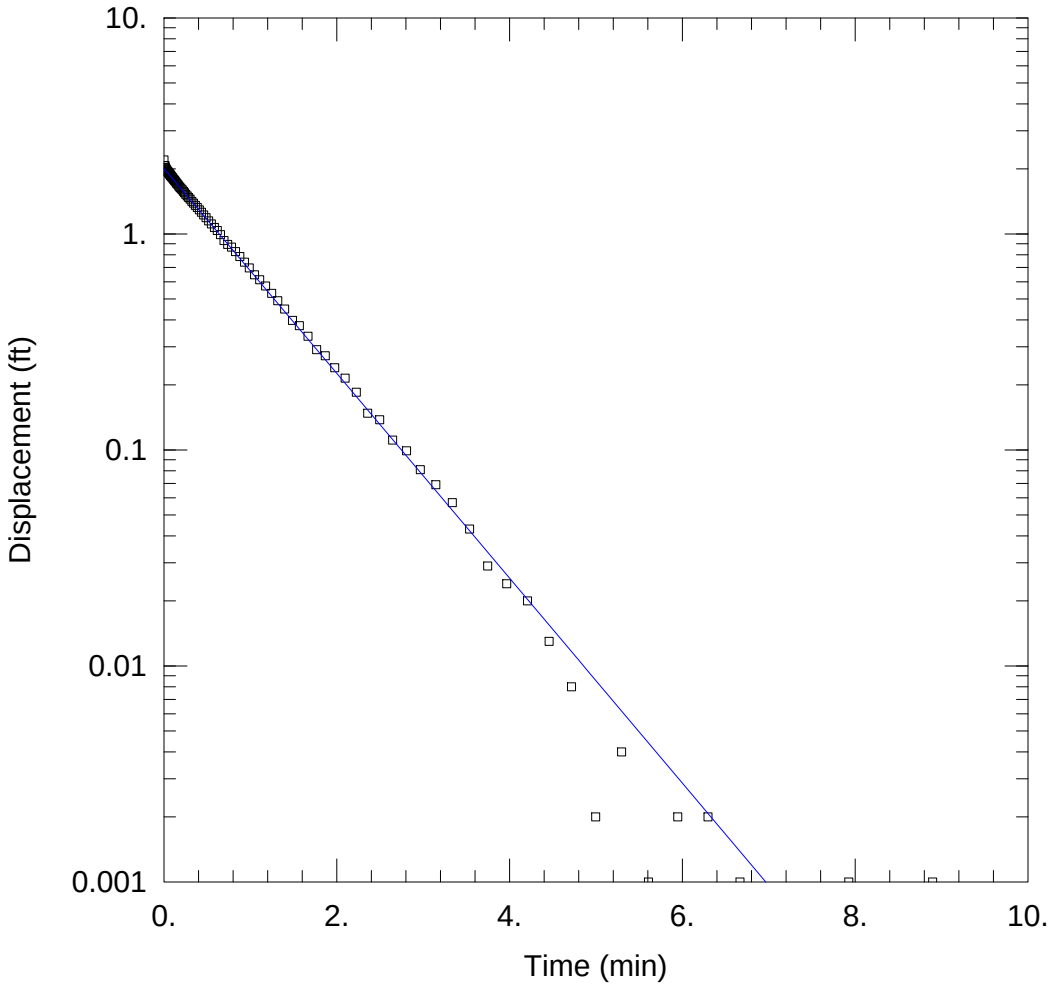
Saturated Thickness: 36.12 ft
Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-08)

Initial Displacement: 2.2 ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 22.85 ft^2/day
S = 6.259E-6



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-08test3outB.aqt
Date: 03/08/17 Time: 15:19:51

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-08
Test Date: 8-18-2016

AQUIFER DATA

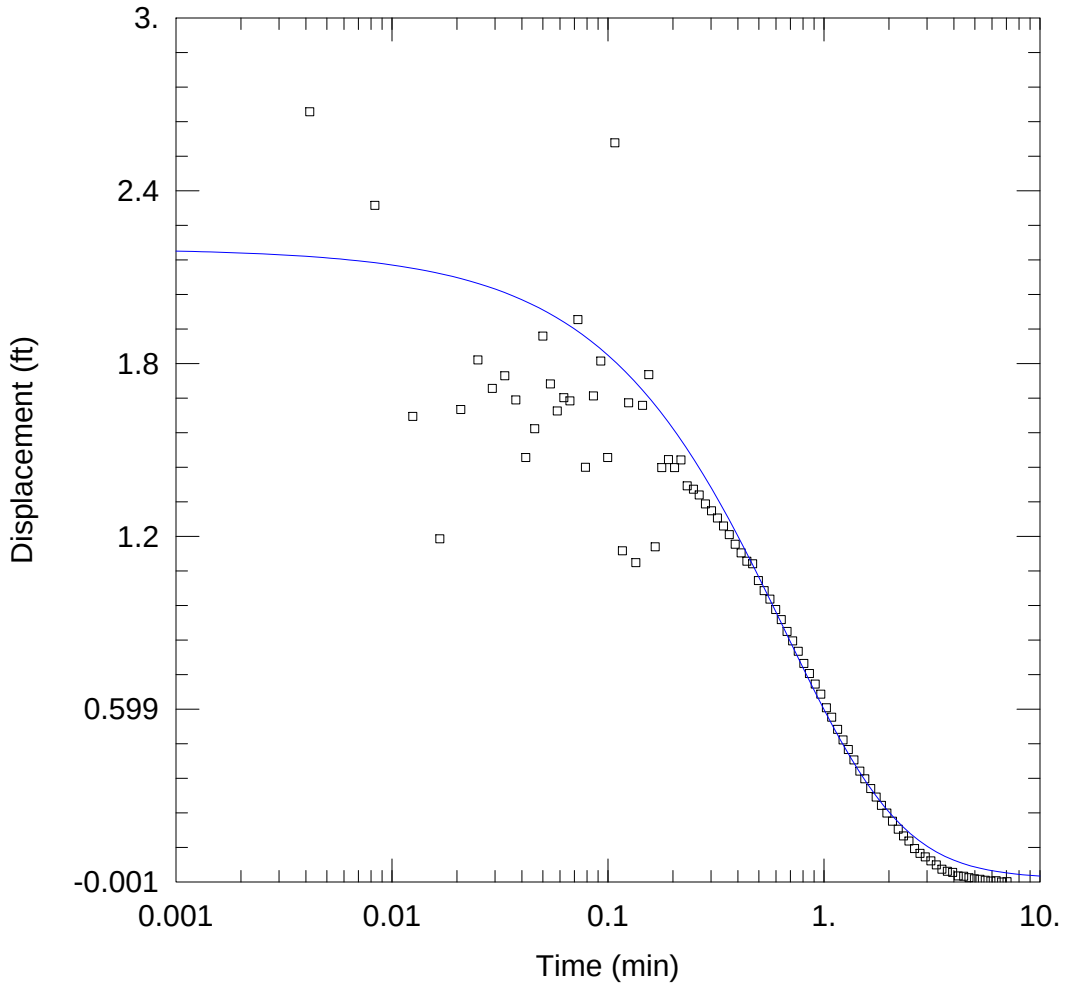
Saturated Thickness: 36.12 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-08)

Initial Displacement: 2.2 ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Unconfined
Solution Method: Bouwer-Rice
K = 1.347 ft/day
y0 = 2.007 ft



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-08test3in.aqt
Date: 03/08/17 Time: 15:21:31

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-08
Test Date: 8-18-2016

AQUIFER DATA

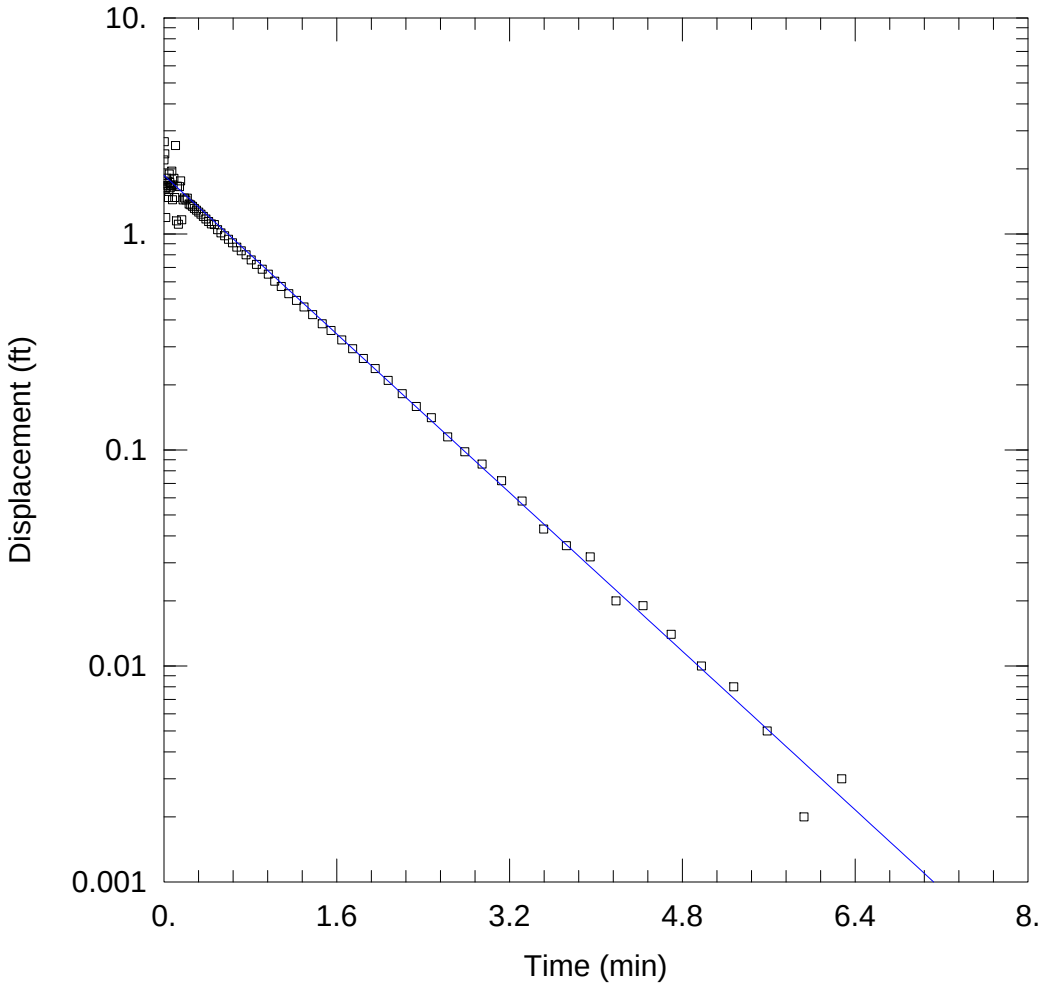
Saturated Thickness: 36.12 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-08)

Initial Displacement: 2.2 ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Confined
Solution Method: Cooper-Bredehoeft-Papadopulos
T = 25.6 ft^2/day
S = 6.259E-6



WELL TEST ANALYSIS

Data Set: C:\Users\meyerm\Documents\Projects\Slug Tests\March 2017\22-MM-08test3inB.aqt
Date: 03/08/17 Time: 15:21:05

PROJECT INFORMATION

Company: Battelle
Client: ESTCP
Location: MCBCP 22 Area
Test Well: 22-MM-08
Test Date: 8-18-2016

AQUIFER DATA

Saturated Thickness: 36.12 ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA (22-MM-08)

Initial Displacement: 2.2 ft
Total Well Penetration Depth: 32.12 ft
Casing Radius: 0.08333 ft
Static Water Column Height: 32.12 ft
Screen Length: 11. ft
Well Radius: 0.4167 ft

SOLUTION

Aquifer Model: Unconfined
Solution Method: Bouwer-Rice
K = 1.305 ft/day
y0 = 1.867 ft