Evaluation of a Novel Multiple Primary Substrate (MPS) Cometabolic Approach for *In Situ* Bioremediation of 1,4-Dioxane and Chlorinated Solvents in Groundwater

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14. ABSTRACT
The key objective of this ESTCP project was to utilize aerobic cometabolism to treat both 1,4-D and CVOCs in groundwater at North Island Naval Air Station, Operable Unit 11. During this demonstration, two gaseous compounds were used as primary substrates for specific groups of cometabolic microorganisms with different abilities to degrade 1,4-D and CVOCs in the impacted aquifer. An off-the-grid, solar powered hybrid treatment system to distribute oxygen gas, two substrate gases (methane and isobutane), inorganic nutrients, and bioaugmentation cultures isolated from the site (ENV493 and ENV494M) was designed, constructed, and operated for ~17 months.

The off-the-grid solar powered system proved to be reliable and simple to operate and maintain. However, the site conditions at NASNI, and particularly the high concentrations of 1,1-DCE, prevented overall remedial objectives from being met. large quantities of ENV493 were grown and added to the test plot on 3 occasions to aid in the biodegradation relevant COCs. While this resulted in substantial degradation of 1,1-DCE, residual concentrations persisted, possibly adsorbed to sediments, or entering the plot during recirculation phases. We hypothesize that the residual 1,1-DCE continued to cause enhanced rates of death of ENV493 and any native cometabolic organisms that oxidized it to 1,1-DCE-epoxide, thus limiting degradation of many of the other target COCs.

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LIST OF ACRONYMS AND ABBREVIATIONS

Acronym	Full text
%	Percent
µg/L	micrograms per liter
ABL	activity-based labeling
ABPP	activity-based protein profiling
Ah	Ampere hour
AMO	Ammonia Monooxygenase
AOC	Area of Concern
APTIM	Aptim Federal Services, LLC
BDAG	Biotechnology Development and Applications Group
bgs	below ground surface
CF	Chloroform
cis-DCE	*cis*-1,2-Dichloroethene
COC	chemicals of concern or chain of custody
CP	chemically pure
CVOC	chlorinated volatile organic compound
1,4-D	1,4-dioxane
DAP	diammonium phosphate
1,1-DCA	1,1-dichloroethane
1,2-DCA	1,2-dichloroethane
1,1-DCE	1,1-dichloroethene
DC	direct current
DO	dissolved oxygen
DoD	United States Department of Defense
EC	electric conductivity
EDB	1,2-dibromoethane
ERD	enhanced reductive dechlorination
ESTCP	Environmental Security Technology Certification Program
EW	extraction well
ft	feet or foot
GAC	granular activated carbon
GPM	gallons per minute
GW	groundwater
HCl	hydrochloric acid
HPT	Hydraulic Profiling Tool boring
HSA	hollow stem auger
ID	inner diameter
in	inch(es)
ISCO	*in-situ* chemical oxidation
ISCR	*in-situ* chemical reduction
IW	injection well
IWTP	industrial waste treatment plant
K	hydraulic conductivity
kg	Kilograms

Acronym	Full text
L	Liter(s)
Lbs.	Pounds
LPM	liters per minute
LTM	Long Term Monitoring
MCL	Maximum Contaminant Level
mg/L	milligram(s) per liter
mL	milliliter(s)
MPS	multiple primary substrate
MSL	mean sea level
NASNI	Naval Air Station North Island
NAVFAC	Naval Facilities Engineering Systems Command
Navy	United States Department of the Navy
NCSU	North Carolina State University
NDMA	*N*-Nitrosodimethylamine
ng/L	nanogram(s) per liter
NL	notification level
NPV	Net Present Value
O&M	operation and maintenance
OD	outer diameter
ORP	Oxidation-Reduction Potential
OSU	Oregon State University
OU	Operable Unit
OWTP	oily waste treatment plant
P&T	pump and treat
PCE	Tetrachloroethene
PI	Principal Investigator
PLC	programmable logic controller
pMMO	Particulate Methane Monooxygenase
PMW	performance monitoring well
PPE	personal protective equipment
PPO	Propane Monooxygenase
PSI	pounds per square in
PTW	pump test well
PVC	Polyvinyl chloride
QC	quality control
qPCR	quantitative polymerase chain reaction
RDO	rugged dissolved oxygen probe
RPM	Remedial Project Manager
S/D	shallow/deep (well pairs)
SAIC	Science Applications International Corporation
SCADA	supervisory control and data acquisition
SCAM	short chain alkane monooxygenase
SCFM	standard cubic ft per minute
SERDP	Strategic Environmental Research and Development Program
SMMO	soluble methane monooxygenase

Acronym	Full text
SWMU	Solid Waste Management Unit
1,1,1-TCA	1,1,1-trichloroethane
TCE	Trichloroethene
USEPA/EPA	United States Environmental Protection Agency
VC	vinyl chloride
VOA	volatile organic analysis (vials)
VOC	volatile organic compound

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ABSTRACT

1,4-Dioxane (1,4-D) has emerged as a chemical in groundwater throughout the United States. This compound was widely used as a stabilizer in 1,1,1-trichloroethane (1,1,1-TCA) and is frequently found comingled with 1,1,1-TCA and other chlorinated volatile organic compounds (CVOCs), including 1,1,2-trichloroethene (TCE) in aquifers. Unfortunately, 1,4-D is not treated by the most common bioremediation approach for CVOCs, which entails adding carbon substrates to aquifers to stimulate anaerobic reductive dechlorination. Presently, the most effective technology for removing comingled 1,4-D and CVOCs from groundwater is pump-and-treat (P&T) with multiple system components often including air-strippers and advanced oxidation processes to address this mix of chemicals. However, this approach is expensive, requiring the installation of significant infrastructure, including numerous extraction wells (EWs) and a treatment facility. In addition to high capital costs, annual Operation and Maintenance (O&M) costs are typically high, and these systems are often operated for decades.

The key objective of this Environmental Security Technology Certification Program (ESTCP) project was to utilize aerobic cometabolism to treat both 1,4-D and CVOCs in groundwater at North Island Naval Air Station (NASNI) Operable Unit 11 (OU-11). Cometabolism typically occurs when one or more broad-specificity oxygenase enzymes are induced in bacteria - enzymes that allow such bacteria to grow on a primary substrate (e.g., methane, propane, isobutane), yet also to biodegrade a range of other non-growth compounds, including many United States Department of Defense (DoD) chemicals of concern (COCs). During this demonstration, two gaseous compounds were used as primary substrates for specific groups of cometabolic microorganisms with different abilities to degrade 1,4-D and CVOCs in the OU-11 plume. A hybrid treatment system was designed, consisting of 5 biosparging wells to add oxygen gas, and a groundwater recirculation system consisting of 1 central EW and 5 surrounding IWs to add the two substrate gases (methane and isobutane), inorganic nutrients (diammonium phosphate) and bioaugmentation cultures isolated from the site (ENV493 and ENV494M). Importantly, the treatment system was designed to be completely "off-the-grid", running on solar power and gas pressure – thus making the approach efficient, sustainable and cost-effective.

The results of the microcosm studies as well as enrichment and pure culture tests indicated that all relevant CVOCs and 1,4-D in the NASNI OU-11 area groundwater could be successfully treated to below project performance objective criteria by adding two cometabolic substrates and two different bioaugmentation cultures. One critical finding of the laboratory testing was that 1,1-dichloroethene (1,1-DCE) inhibits the cometabolic oxidation of other COCs at the site by native bacteria, likely due to formation of a reactive epoxide that increases the rate of cell death. However, data also indicated that when added in sufficient quantity, bioaugmentation cultures could degrade the 1,1-DCE to low levels, thus allowing complete degradation of all other site COCs. The two cultures isolated from the site (ENV493 and ENV494M) were determined to be the best candidates for bioaugmentation based on their continued activity under site conditions. Therefore, it was determined that ENV493 and isobutane would be added to biodegrade 1,1-DCE, 1,4-D, and *cis*-1,2-dichloroethene (*cis*-DCE), while methane and ENV494M would be used to transform residual TCE during the field demonstration. The bioaugmentation cultures were grown in the required quantities for field application in Aptim Federal Service, LLC (APTIM's) commercial fermentation facility (Lawrenceville, NJ).

A monitoring network was installed consisting of 4 monitoring well pairs screened in two separate intervals of the treatment zone, as well as two upgradient and one downgradient monitoring well. The cometabolic system was operated in three main phases: (1) oxygen-only sparging with groundwater recirculation; (2) system operation with sparging of oxygen and addition of cometabolic gases, inorganic nutrients, and bioaugmentation cultures to recirculating groundwater (3) post-treatment monitoring. The cometabolic treatment system operated as planned over the course of the study, rapidly distributing oxygen and cometabolic substrates throughout the treatment area. Over the course of the demonstration, which lasted ~ 19 months, concentrations of 1,1-DCE declined appreciably, from ~ 1,000 micrograms per liter (µg/L) at the conclusion of Phase 1 to ~ 180 µg/L after ~ 12 months of Phase 2 operation, an average decline of 82 percent (%). Similarly, TCE declined by an average of 73% over the same time interval, from ~ 800 µg/L on average to 220 µg/L. COCs present at lower concentrations, including *cis*-DCE, 1,1- dichloroethane (1,1-DCA), and 1,4-D, declined by less, averaging 19%, 37%, and 16%, respectively. The most likely reason for the limited decline of these compounds was persistence of 1,1-DCE at ~ 180 µg/L in the test plot. Multiple strategies to overcome this issue, including isobutanol addition to enhance the growth of isobutane-degrading bacteria without stimulating the oxidase that degrades 1,1-DCE, (creating reactive epoxides) and multiple rounds of bioaugmentation were not successful at reducing 1,1-DCE to sufficiently low concentration to allow degradation of all other COCs including 1,4-D as observed in laboratory studies.

In summary, the off-the-grid solar powered system proved to be reliable, simple to operate and maintain, and economical for addition of necessary amendments to the aquifer. For many applications, this type of system is expected to be less expensive to install and operate than a conventional P&T system. However, the site conditions at NASNI, and particularly the high concentrations of 1,1-DCE, prevented overall remedial objectives from being met. Laboratory microcosm and pure culture studies indicated that once 1,1-DCE was completely biodegraded, all other relevant COCs would degrade rapidly. This was achieved in the laboratory using high densities of the bioaugmentation culture ENV493. The same was attempted in the field trial, as large quantities of ENV493 were grown and added to the test plot (on 3 occasions), along with isobutanol to increase cell growth but not induce the oxygenase responsible for 1,1-DCE oxidation (and cell toxicity). While this resulted in substantial degradation of 1,1-DCE, residual concentrations persisted, possibly adsorbed to sediments or entering the plot during recirculation phases. We hypothesize that the residual 1,1-DCE continued to cause enhanced rates of death of ENV493 and any native cometabolic organisms that oxidized it to 1,1-DCE-epoxide, thus limiting degradation of many of the other target COCs.

EXECUTIVE SUMMARY

Introduction

1,4-D, which was widely used as a stabilizer in chlorinated solvents, has emerged as an important chemical in groundwater throughout the United States. Typical of many ethers, 1,4-D is miscible in water, and has both a low Henry's Law constant and a low organic carbon partitioning coefficient, making it difficult to treat by typical remediation approaches, such as air-stripping and carbon adsorption. Moreover, because of its use as a solvent stabilizer, 1,4-D is frequently comingled with CVOCs in aquifers, including 1,1,1-TCA and TCE, yet it is not treated by the most common bioremediation approach for these chemicals, which entails adding carbon substrates to aquifers to stimulate anaerobic reductive dechlorination. Presently, the most effective technology for removing comingled 1,4-D and CVOCs from groundwater is pump-and-treat with multiple treatment components, such as air-stripping (for CVOCs) and advanced oxidation (for 1,4-D). However, this *ex-situ* technology is expensive, requiring the installation of significant infrastructure, including numerous EWs and a treatment facility.

Aerobic cometabolism has shown significant promise for the *in situ* treatment of both CVOCs and 1,4-D. Cometabolic organisms grow aerobically on a supplied substrate rather than the target COCs, often allowing good degradation kinetics, minimal impacts to aquifer geochemistry, and the ability to achieve relevant maximum contaminant levels (MCLs). The application of multiple cometabolic substrates to is an advance on this process, which allows better targeting of cometabolic strains based on their degradation capabilities. However, to meet current DoD needs, this and all new remedial technologies must be efficient, sustainable, and cost effective. The development and field validation of an off-the-grid system capable of meeting these needs was also a key goal of this field demonstration.

Objectives

The overall objective of this project was to demonstrate that a novel multiple primary substrate cometabolic bioremediation technology can meet DoD needs for reliable, flexible, and cost-effective treatment of groundwater contaminated with comingled 1,4-D and CVOCs. This field demonstration was conducted in the shallow aquifer at NASNI OU-11. Based on the results of laboratory treatability testing, gaseous primary substrates (isobutane and methane), oxygen, inorganic nutrients, and two bioaugmentation cultures were added to the aquifer to promote 1,4-D and CVOC biodegradation. The ability of the technology to achieve appreciable reduction of 1,4- D and CVOC concentrations in the groundwater and the cost effectiveness of the technology was evaluated.

Specific objectives of this project were as follows:

- Assess degradation of target COCs (1,4-D and TCE, other CVOCs) within the treatment zone during active treatment;
- Document the selective degradation of 1,4-D by isobutane-degrading bacteria;
- Determine which organisms and/or genes are involved in the cometabolic biodegradation processes using state-of-the art molecular and proteomic analysis of field samples;

- Evaluate distribution of gases within the target treatment zone; and
- Assess oxygen and gaseous primary substrate consumption within the target treatment zone to optimize gaseous amendment delivery mass and frequencies.

Technology Description

Cometabolic biodegradation typically occurs when one or more broad-specificity oxygenase enzymes are induced in bacteria - enzymes that allow such bacteria to grow on a primary substrate (e.g., methane, propane, isobutane), yet also to biodegrade a range of other non-growth compounds, including many DoD COCs. The application of this approach for remediation typically entails the addition of a specific primary growth substrate (often an alkane gas) and oxygen to an aquifer with or without accompanying inorganic nutrients and bioaugmentation cultures. In this demonstration project, we utilized ***multiple*** primary substrates (MPS) to stimulate the aerobic degradation of 1,4-D and CVOCs by distinct groups of widely distributed bacteria. The application of the MPS approach can be highly flexible based upon the site conditions and the mixture of COCs present. MPS addition can be applied *in situ* using several different configurations based on site conditions, including biosparging, groundwater recirculation with active gas addition, passive gas addition, or a hybrid approach as was utilized in this project.

There are multiple reasons that cometabolism (primary or multiple substrates) should be considered at DoD sites, including the following: (1) the approach is widely applicable for groundwater CVOCs (tetrachloroethene [PCE] excluded) and anaerobic degradation intermediates (e.g., *cis*-DCE and vinyl chloride (VC)), as well as a wide range of other DoD COCs including 1,4-D, methyl tertiary-butyl ether, *N*-Nitrosodimethylamine (NDMA), 1,2-Dibromoethane (EDB), and 1,2,3-trichloropropane; (2) the technology is very well suited for dilute plumes because the cometabolic organisms are not required to grow on the COC, but rather utilize the substrate gas that is supplied to the aquifer; (3) very low treatment levels (e.g., low nanograms per liter [ng/L] concentrations) can be achieved for some pollutants; and (4) groundwater remains aerobic, minimizing issues such as mobilization of metals (e.g., iron, arsenic and manganese), production of hydrogen sulfide, and large shifts in pH, as sometimes observed when high concentrations of carbon substrates are added to aquifers for anaerobic treatment of CVOCs and other COCs.

During this demonstration, two gaseous compounds were used as primary substrates for specific groups of cometabolic microorganisms with different abilities to degrade 1,4-D and CVOCs in the OU-11 plume. A hybrid system was designed, consisting of 5 biosparging wells to add oxygen gas and a groundwater recirculation system consisting of 1 central EW and 5 surrounding IWs to add the two substrate gases (methane and isobutane), inorganic nutrients (diammonium phosphate), and bioaugmentation cultures isolated from the site (ENV493 and ENV494M). Importantly, the treatment system was designed to be completely "off-the-grid", running on solar power and gas pressure – thus making the approach efficient, sustainable, and cost-effective.

Performance Assessment

Remediation Effectiveness

One of the primary objectives of this demonstration was to assess the effectiveness of applying aerobic cometabolism to treat groundwater impacted with comingled 1,4-D and CVOCs. Groundwater data collected during the demonstration showed that concentrations of 1,1-DCE at the four pair of treatment zone monitoring wells declined appreciably, from an average concentration of 1,017 µg/L at the conclusion of Phase 1 to an average concentration of 181 µg/L after ~ 12 months of Phase 2 operation, an average decline of 82%. Similarly, TCE declined by an average of 73% over the same time interval, from 810 µg/L on average to 220 µg/L. COCs present at lower concentrations, including *cis*-DCE, 1,1-DCA, and 1,4-D, declined by less, averaging 19%, 37%, and 16%, respectively. The most likely reason for the limited decline of these compounds was persistence of 1,1-DCE in the test plot continued to cause enhanced rates of death of ENV493 and any native cometabolic organisms that oxidized it to 1,1-DCE-epoxide, thus limiting degradation of many of the other target COCs. Multiple strategies to overcome this issue, including isobutanol addition to enhance the growth of isobutane-degrading bacteria without stimulating the oxidase that degrades 1,1-DCE, (creating reactive epoxides) and multiple rounds of bioaugmentation were not successful at reducing 1,1-DCE to sufficiently low concentration to allow degradation of all other COCs including 1,4-D as observed in laboratory studies.

Maintaining Aerobic Conditions

Maintaining aerobic conditions within the treatment zone was critical, as cometabolism using an alkane/alkene gas substrate is an aerobic process. Therefore, the extent to which sparged oxygen gas could be distributed (both vertically and horizontally) within the treatment zone was important to the overall success of the remedial approach. Oxygen sparging cycles were only required approximately once every two weeks during Phase 1, and approximately once every two to three days during Phase 2 of the demonstration to maintain aerobic conditions in the aquifer. Dissolved oxygen (DO) concentrations generally ranged between 5 milligrams per liter (mg/L) and 40 mg/L at the 8 treatment zone monitoring wells the downgradient monitoring well, and the single EW during Phases 1 and 2 of the demonstration, exceeding the 1 mg/L target concentration.

Distribution of MPS Gases

Optimization of the MPS gas delivery mass and frequencies increase the effectiveness of the aerobic cometabolic treatment technology by supplying appropriate amounts of the cometabolic gases, while ensuring that high dissolved gas concentrations do not lead to continuous competitive inhibition. Therefore, the extent to which the cometabolic gases could be distributed (both vertically and horizontally) via the groundwater recirculation system, and the dissolved gases consumed within the treatment zone, were important to the overall success of the remedial approach. Dissolved gas data collected during the demonstration were used to optimize MPS gas addition cycle concentrations, durations, and frequencies, and to minimize the effects of competitive inhibition.

While one of the performance objectives for this demonstration was to observe >200 µg/L dissolved isobutane and methane in at least 6 of the 8 treatment zone monitoring wells during the first month of Phase 2 system operation (to maximize growth of isobutane-oxidizing bacteria), optimal dissolved isobutane and methane concentrations between approximately 10 µg/L and 100 µg/L at the EW were targeted during the majority of Phase 2 to support continued biomass

growth, while minimizing competitive inhibition within the treatment zone. Dissolved isobutane concentrations exceeded the target 200 µg/L in seven of the eight treatment zone monitoring wells within 21 days of initiating isobutane addition, and at the eighth monitoring well within 35 days. Dissolved methane concentrations exceeded the target of 200 µg/L in six of the eight treatment zone monitoring wells within 14 days of initiating methane addition. Furthermore, dissolved isobutane concentrations measured at the EW were typically between the target of approximately 10 µg/L and 100 µg/L during the periods of Phase 2 when consumption of isobutane was observed, and dissolved methane concentrations were generally between 10 µg/L and 100 µg/L at EW (EW-1) throughout most of Phase 2 operation. These data indicated that, apart from periods of time where isobutane concentrations were observed to increase (because of toxicity associated with the 1,1-DCE epoxide), consumption of these gases were occurring within the treatment zone.

System Reliability

Reliability of the oxygen biosparging, groundwater recirculation, and amendment delivery systems were essential during the demonstration, as the regular addition of oxygen, cometabolic gases, and nutrients is critical to the treatment and cost effectiveness of any cometabolic treatment approach. The off-the grid solar power system provided consistent power to the treatment system throughout the entire 499 days of the demonstration, with only minor O&M required. The cometabolic treatment system operated as designed, and no major system or equipment failures were experienced during the demonstration, apart from unexpected damage caused by Pocket Gophers to subsurface groundwater recirculation, nutrient injection, and oxygen injection lines. Importantly, there were no apparent issues with biofouling of the IWs, EW or oxygen biosparging wells during the demonstration. In general, the off-the-grid solar powered treatment system operated for >95% of the time, proved to be highly reliable, simple to operate and maintain, and economical for *in situ* cometabolic treatment.

Ease of Use

The level of effort required to maintain effective 1,4-D and CVOC treatment using the aerobic cometabolism technology will determine, in part, the cost effectiveness of this approach for treating plumes with these comingled COCs. System O&M requirements, which primarily consisted of regular system checks and changeout of the oxygen cylinders, were not substantial during the demonstration. System checks (which entailed collecting manual system pressure and flow data, performing regular system maintenance, and performing leak checks) were generally performed every 2-4 weeks in under 3 hours per visit. Change out of the racks of oxygen cylinders was conducted approximately every 2-3 months and was typically performed in under 6 hours. The 4 cylinders of liquified isobutane and 12 cylinders of compressed methane did not require replacement during 12 months of Phase 2 cometabolic biosparging due to the general efficiency of this treatment approach. The ability to communicate remotely with the system (and adjust system operating parameters), as well as programmed logging capabilities of the Supervisory Control and Data Acquisition (SCADA) system reduced the number of site visits required. Furthermore, other than soil cuttings and development water generated during well installations, there was no waste generated during application of this *in situ* technology. The off-the-grid solar powered system provided continuous power for operation of the treatment system and proved simple to operate and maintain. In addition to the Principal Investigator (PI) and project engineer, two field technicians were trained to operate and conduct system O&M in one day.

Identification of Degradative Genes/Proteins

The ability to identify the microorganism being stimulated, as well as to confirm that bacteria express the expected monooxygenases after stimulation by each gaseous primary substrate, advances the understanding of microbial communities and monooxygenase enzyme stimulation to specifically target treatment at this and other sites with mixed 1,4-D and CVOC plumes. Analysis of target cometabolic organisms/genes/enzymes by quantitative polymerase chain reaction (qPCR) was performed and proteomic analysis using an activity-based protein profiling (ABPP) approach capable of simultaneously identifying multiple types of active monooxygenases in environmental samples was also be used to confirm that bacteria express the expected monooxygenases after stimulation by each gaseous primary substrate.

The qPCR data collected indicated that a limited number of wells showed meaningful (approximately 2 orders of magnitude) increases in the target cometabolic organisms/genes during Phase 2 of the demonstration. Furthermore, the results of the ABPP analysis targeting isobutane-oxidizing monooxygenases generally agreed with the qPCR data. The relatively small magnitude of the increases and the apparent limited distribution of the of the target organisms were unexpected, as the aquifer was bioaugmented twice with ENV493 and once with ENV494M during the period when these samples were collected. The limited increases could be due to the formation and subsequent toxicity of the reactive 1,1-DCE epoxide that reduced the populations appreciably and/or the adsorption of ENV493 (and to a lesser extent, ENV494M) to sediments (rather than being planktonic). Regardless, the data indicate that we were able to successfully identify and/or quantify the genes and active enzymes via both qPCR and ABPP.

Cost Assessment

The expected cost drivers for installation and operation of a cometabolic biosparging system to treat a full-scale CVOC and 1,4-D plume, and those that will determine the cost/selection of this technology over other options, include the following:

- Depth of the plume below ground surface (bgs);
- Width, length, and thickness of the plume;
- Aquifer lithology and hydrogeology;
- Passive and sustainable power (solar);
- Length of time for clean-up (e.g., necessity for accelerated clean-up);
- The presence of indigenous bacteria capable of cometabolically degrading CVOCs and 1,4-D;
- Concentrations of chemicals and alternate electron acceptors; and
- Presence of comingled COCs (1,1-DCE, in particular).

A cost analysis of a cometabolic biosparging system and a traditional groundwater treatment approach (P&T) to treat a full-scale CVOC and 1,4-D plume was performed. These two technologies were selected for comparison because they can be applied as treatment barrier for CVOC and 1,4-D plume capture. The base case presents a situation where a shallow aquifer, consisting of homogeneous silty sands, is contaminated with TCE and 1,4-D, with small quantities (<10 µg/L) of 1,1-DCE. The contaminated groundwater extends from 10 to 50 feet (ft) bgs, along the direction of groundwater flow for 800 ft, and is 400 ft in width. The costing for the template

site assumes that the source zone has been treated and that there is no continuing impacts to groundwater. The cost analyses comparing the above approaches are presented below based on a 30-year operating scenario.

The estimated total costs for the cometabolic biosparging barrier alternative over 30 years are $3,536,000 with a total Net Present Value (NPV) of lifetime costs of $3,664,741. The capital cost including design, work plan, installation of biosparging and monitoring wells, installation of the solar power system, and fabrication, installation, and start-up of the biosparging system is $446,900. The NPV of the O&M is $2,224,660 for the 30 years of treatment. The O&M costs primarily include the labor and material costs associated with weekly inspections and battery replacement every five years. The costs for materials and other consumables are negligible with this alternative. The NPV of the 30 years of monitoring and reporting costs is $993,181.

The estimated total cost for the P&T alternative over 30 years is $8,005,853 with a total NPV of lifetime costs of $8,260,617. The capital cost including design, work plan, installation of injection well (IW)/EW and monitoring wells, construction of the groundwater treatment system, and system start up and testing is $2,114,836. The NPV of the O&M is $5,152,600. The O&M costs include the labor costs associated with system O&M, costs for equipment repair and replacement, electrical costs, and cost for the replacement and disposal of the granular activated carbon (GAC). The NPV of the 30 years of monitoring and reporting costs is $993,181.

Overall, the cometabolic biosparging barrier alternative ranks lowest in estimated total remedy cost and lowest in NPV of lifetime costs. The estimated capital cost for this approach is the lowest of the two alternatives because of the limited infrastructure required and the relative ease of installation. The estimated long-term O&M costs are also the lowest of the two alternatives. As with P&T, total remedy costs will increase if the treatment needs to extend beyond 30 years.

Summary

In summary, the off-the-grid solar powered system proved to be reliable, simple to operate and maintain, and economical for addition of necessary amendments to the aquifer. For many applications, this type of system is expected to be less expensive to install and operate than a conventional P&T system. However, the site conditions at NASNI, and particularly the high concentrations of 1,1-DCE, prevented overall remedial objectives from being met. Laboratory microcosm and pure culture studies indicated that once 1,1-DCE was completely biodegraded, all other relevant COCs would degrade rapidly. This was achieved in the laboratory using high densities of the bioaugmentation culture ENV493. The same was attempted in the field trial, as large quantities of ENV493 were grown and added to the test plot (on 3 occasions), along with isobutanol to increase cell growth but not induce the oxygenase responsible for 1,1-DCE oxidation (and cell toxicity). This resulted in substantial degradation of 1,1-DCE (> 80% on average), but residual concentrations persisted, possibly via desorption/back-diffusion from sediments or entering the plot during recirculation phases. We hypothesize that the residual 1,1-DCE continued to cause enhanced rates of death of ENV493 and any native cometabolic organisms that oxidized it to 1,1-DCE-epoxide, thus limiting degradation of many of the other target COCs.

1.0 INTRODUCTION

1.1 BACKGROUND

1,4-D, which was widely used as a stabilizer in chlorinated solvents, has emerged as an important chemical in groundwater throughout the United States. Typical of many ethers, 1,4-D is miscible in water, and has both a low Henry's Law constant and a low organic carbon partitioning coefficient, making it difficult to treat by typical remediation approaches, such as air-stripping and carbon adsorption (Mohr et al., 2010). Moreover, because of its use as a solvent stabilizer, 1,4-D is frequently comingled with CVOCs in aquifers (Anderson et al., 2012), yet it is not treated by the most common bioremediation approach for these COCs, which entails adding carbon substrates to aquifers to stimulate reductive dechlorination.

Chlorinated solvents, including 1,1,1-TCA which contained 1,4-D as a stabilizer, have been widely used by the DoD. The widespread occurrence of 1,4-D in groundwater could result in the re-evaluation and re-opening of chlorinated solvent sites. Thus, the DoD has highlighted the need to evaluate the fate of this compound in the environment. As discussed in the 2013 Summary Report from the Strategic Environmental Research and Development Program (SERDP) and ESTCP Workshop on Long Term Management of Contaminated Groundwater Sites (SERDP & ESTCP, 2013), one critical demonstration need is "developing cost-effective remedial strategies to address contaminant mixtures at contaminated sites." The treatment of 1,4-D comingled with chlorinated solvents was identified as a major priority.

Presently, the most effective technology for removing comingled 1,4-D and CVOCs from groundwater is P&T with multiple treatment components to address this mix of COCs. However, this *ex-situ* technology is expensive, requiring the installation of significant infrastructure, including numerous EWs and a treatment facility. In addition to high capital costs, annual O&M costs (including groundwater filtration, air strippers and/or granular activated carbon maintenance, and energy) are typically high, and these systems are often operated for decades. Furthermore, treated groundwater typically must either be re-injected into the aquifer (requiring additional wells or infiltration galleries), or sent to a local publicly owned treatment works. The capital cost for installation of P&T infrastructure and continuing O&M costs for treatment of large volumes of groundwater are anticipated to be prohibitively expensive at some DoD facilities.

During this demonstration project, which is partially based on recent data from SERDP Project ER-2303, *Evaluation of Branched Hydrocarbons as Stimulants for In Situ Cometabolic Biodegradation of 1,4-Dioxane and Its Associated Co-Contaminants*, we utilized multiple gaseous substrates to stimulate the aerobic degradation of 1,4-D and CVOCs by distinct groups of widely distributed bacteria. One group of bacteria that grows on isobutane has been shown to biodegrade 1,4-D to $\leq$1 µg/L (Rolston et al., 2019). This activity is consistently observed in both commercially sourced and newly isolated isobutane-oxidizing bacteria, as well as in aquifer microcosms. Isobutane-oxidizers can also biodegrade a range of CVOCs including 1,1,1-TCA and VC, but have not been shown to biodegrade TCE to the same extent. Other cometabolic organisms, including those that grow on methane, ethene, propane and isobutene, among other substrates, have been observed to degrade TCE. Thus, the concept of this effort is to use multiple substrates to target different COCs in a mixed plume.

This field demonstration of cometabolic bioremediation was performed at the NASNI, Coronado, CA, where groundwater is impacted with 1,4-D, TCE, and several other CVOCs. During this demonstration, isobutane was added to the aquifer to specifically promote biodegradation of 1,4-D and select CVOCs, and methane was used to stimulate bacteria capable of rapidly degrading specific CVOCs, including TCE, which is not effectively biodegraded by isobutane-oxidizing bacteria. In addition to adding these two gaseous substrates, the aquifer was bioaugmented with two bacterial cultures (isobutane oxidizer *Rhodococcus aetherivorans* ENV493 and mixed methanotrophic consortium ENV494M) to optimize degradation of all relevant COCs in the NASNI OU-11 groundwater. These cultures were isolated from the site at APTIM's laboratory and were determined to be the best candidates for bioaugmentation during site-specific treatability testing (**Section 5.3**). This approach represents a significant advance for traditional single gas cometabolic bioremediation technology in that it utilizes a new understanding of microbial communities and monooxygenase enzyme stimulation and inhibition to specifically target DoD sites with mixed 1,4-D and CVOCs.

1.2 OBJECTIVE OF THE DEMONSTRATION

The overall objective of this project was to demonstrate that a novel MPS cometabolic bioremediation technology can meet DoD needs for reliable, flexible, and cost-effective treatment of groundwater impacted with comingled 1,4-D and CVOCs. This field demonstration was conducted in the shallow aquifer at NASNI OU-11. Based on the results of laboratory treatability testing (**Section 5.3**), gaseous primary substrates (isobutane and methane) plus oxygen was added to the aquifer to support 1,4-D and CVOC cometabolic biodegradation by native bacteria and bioaugmentation cultures that have been isolated from the site. The ability of the technology to achieve appreciable reduction of 1,4-D and CVOC concentrations in the groundwater and the cost of the technology was evaluated.

Specific objectives of this project are as follows:

- Assess degradation of target COCs (1,4-D and TCE, other CVOCs) within the treatment zone during active treatment;
- Document the selective degradation of 1,4-D by isobutane-degrading bacteria;
- Determine which organisms and/or genes are involved in the cometabolic biodegradation processes using state-of-the art molecular and proteomic analysis of field samples;
- Evaluate distribution of gases within the target treatment zone; and
- Assess oxygen and gaseous primary substrate consumption within the target treatment zone to optimize gaseous amendment delivery mass and frequencies.

1.3 REGULATORY DRIVERS

CVOCs, including TCE, *cis*-DCE, 1,1-DCE, and 1,1-DCA continue to be primary COCs for the DoD. Some of these compounds are known to be carcinogenic or potentially carcinogenic to humans and are regulated in drinking and groundwater by both the U.S. Environmental Protection Agency (USEPA) and the state of California.

Although there currently is no federal MCL for 1,4-D, concerns about the carcinogenicity of the compound have led several states to institute their own regulatory standards. The California State Water Board, for example, has established a drinking water notification level (NL) of 1 µg/L for this compound. Applicable USEPA and California groundwater standards for these COCs are provided in **Table 1.1**.

Table 1.1. Applicable Groundwater Standards

Constituents	USEPA MCL (µg/L)	California MCL or NL (µg/L)
Trichloroethene (TCE)	5	5
cis-1,2-dichlorethene (*cis*-DCE)	70	6
1,1-dichloroethene (1,1-DCE)	7	6
1,1-dichloroethane (1,1-DCA)	None	5
1,4-dioxane (1,4-D)	None	1*

*California State Water Board notification level. No MCL currently established.

Concentrations of 1,4-D and total CVOCs in some areas of OU-11 have been measured as high as 8,000 µg/L and 15,000 µg/L, respectively. The demonstration area is located in the downgradient and slightly side-gradient portion of the groundwater plume, with 1,4-D concentrations ranging between approximately 100 µg/L and 1,000 µg/L and total CVOC concentrations both ranging between approximately 500 µg/L and 6,000 µg/L. COC concentrations and distribution are discussed in detail in **Sections 4.3** and **5.2.1.3**.

2.0 TECHNOLOGY

The following subsections detail the technology implemented including advantages and disadvantages.

2.1 TECHNOLOGY DESCRIPTION

Cometabolic biodegradation typically occurs when one or more broad-specificity oxygenase enzymes are induced in bacteria - enzymes that allow such bacteria to grow on a primary substrate (e.g., methane, propane, butane, isobutane), yet also to biodegrade a range of other non-growth compounds, including many DoD COCs (**Figure 2.1**). The application of this approach for remediation typically entails the addition of a specific growth substrate (often an alkane gas) and oxygen to an aquifer with or without accompanying inorganic nutrients and bioaugmentation cultures. Cometabolic treatment is mature, cost effective, and can be safely applied *in situ* using a number of different configurations based on site conditions, including biosparging (e.g., Steffan et al., 2003; Lippincott et al., 2015; Hatzinger and Lippincott, 2019); groundwater recirculation with active gas addition (Semprini et al., 1994; Hatzinger et al., 2018; Chu et al., 2018) and passive gas addition in groundwater wells (Begley et al., 2012). A graphic providing generalized examples of the two most common *in situ* treatment designs (biosparging and groundwater recirculation) is provided in **Figure 2.2**.

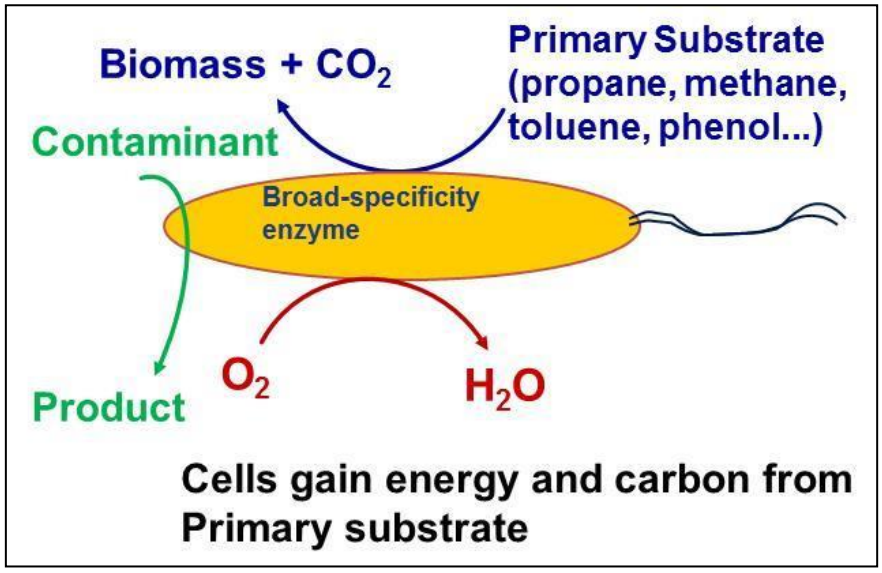


Figure 2.1. General concept of "cometabolism". Bacteria grow on one or more primary substrates that are typically added *in situ* or to a bioreactor. These substrates induce specific broad-specificity enzymes (often oxygenases) which subsequently degrade the COCs.

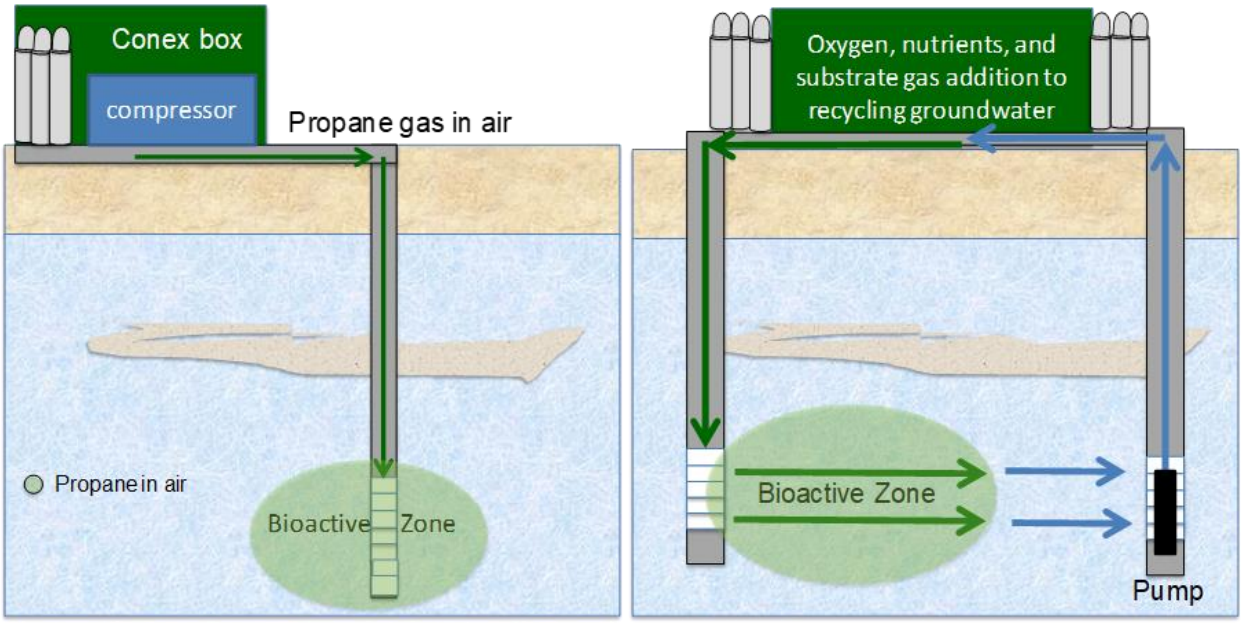


Figure 2.2. Graphic examples of two types of systems used for *in situ* aerobic cometabolic treatment. The biosparging system on the left operates by adding a substrate gas (e.g., propane) in air through a series of *in situ* gas IWs. No groundwater is pumped. The groundwater recirculation system on the right adds the gaseous substrate to groundwater that is pumped to the surface and then reinjected.

2.2 TECHNOLOGY DEVELOPMENT

1,4-D, one of the key chemicals impacting groundwater in this demonstration, can be biodegraded aerobically by bacteria via two distinct processes. Several strains have now been isolated that directly metabolize 1,4-D as a sole source of carbon and energy (e.g., Parales et al., 1994; Kim et al., 2009; Mahendra and Alvarez-Cohen, 2005). However, these bacteria typically grow very slowly, have low affinities for 1,4-D (i.e., high K_s values), and are unlikely to be effective for *in situ* bioremediation of environmentally relevant concentrations of 1,4-D (≤100 µg/L) at many sites (Barajas-Rodriguez et al., 2018). Moreover, recent studies suggest that 1,4-D degradation by this growth-linked process is inhibited by various CVOCs (Mahendra et al., 2013). A much wider range of bacteria can cometabolically degrade 1,4-D after growth on primary substrates including propane, ethane, isobutane, and tetrahydrofuran (Zenker et al., 2000; Burback and Perry, 1993; Mahendra and Alvarez-Cohen, 2006; Vainberg et al., 2006; Hatzinger et al., 2018; Deng et al., 2018; Rolston et al., 2019). Microbial growth on these substrates induces expression of monooxygenase enzymes that fortuitously degrade 1,4-D.

One of the primary advantages of aerobic cometabolic treatment of CVOCs and other target COCs is that the geochemistry of an aerobic aquifer is largely preserved, with few secondary groundwater impacts. In addition, for CVOCs, accumulation of VC or other carcinogenic metabolites is not

anticipated based on known metabolic pathways. Moreover, this process has been recently shown to treat various COCs to very low final concentrations (e.g., < 20 ng/L for EDB in a recent study at Joint Base Cape Cod; Hatzinger et al., 2018). Members of the project team recently applied a propane biosparging approach during an Air Force Civil Engineer Center-funded field demonstration to treat 1,4-D at Site 24, Vandenberg Air Force Base, California (Lippincott et al., 2015). The results showed for the first time that 1,4-D can be treated to regulatory levels *in situ* by applying propane biosparging. This process was effective in the presence of low concentrations (≤ 50 µg/L) of several CVOCs.

However, in more typical situations where higher CVOC concentrations are present with 1,4-D, we anticipate that 1,4-D biodegradation using propane as the sole primary substrate may have been adversely impacted by competitive effects due to concurrent CVOC degradation (e.g., Hand et al., 2015; Mahendra et al., 2013). Small differences in gaseous primary substrates (e.g., ethane *vs.* propane) are now known to have large impacts on the cometabolic capabilities of stimulated microorganisms. Treatment strategies using multiple gaseous substrates to biostimulate different groups of microorganisms with different abilities to degrade 1,4-D and distinct subsets of CVOCs could provide a reliable, flexible, and ultimately more effective treatment than cometabolic biosparging systems based on a single primary substrate such as propane.

In this demonstration project, we utilized ***multiple*** gaseous substrates to stimulate the aerobic degradation of 1,4-D and CVOCs by distinct groups of widely distributed bacteria (**Figure 2.3**). One group of bacteria, typified by *Rhodococcus rhodochrous* ATCC 21198 (Process A in **Figure 2.3**), grows on isobutane and can biodegrade 1,4-D to ≤1 µg/L (Rolston et al., 2019). This activity is consistently observed in both commercially sourced and newly isolated isobutane-oxidizing bacteria. Current data also suggest that these organisms are widely distributed and readily stimulated in groundwater aquifers (SERDP ER-2303, 2022). Strain 21198 can also biodegrade a range of CVOCs including 1,1,1-TCA and VC, but is largely unreactive towards TCE, both in tests with individual CVOCs and CVOC mixtures. Conversely, many other cometabolic strains, including methanotrophs and propanotrophs readily degrade TCE as well as some of the other COVCs. In the demonstration treatment system, isobutane was used to specifically promote biodegradation of 1,4-D and some CVOCs, and methane was used to stimulate bacteria capable of rapidly degrading CVOCs such as TCE that are not effectively biodegraded by isobutane-oxidizing bacteria (Process B in **Figure 2.3**). The project was built on previous successful demonstrations of *in situ* cometabolic biosparging with single cometabolic gases for the treatment of 1,4-D (e.g., Lippincott et al., 2015) and NDMA (Hatzinger and Lippincott, 2019) from several project team members. This is the first application of a targeted gaseous MPS approach for the cometabolic degradation of mixed COCs.

The application of the MPS approach can be highly flexible based upon the site conditions and the mixture of chemicals present. For example, at sites with a large dilute plume of TCE and 1,4- D, a simple biosparging barrier could be utilized consisting of a row of wells cross-gradient to groundwater flow. Isobutane, methane, and oxygen could then be applied to these sparge wells in sequence or via separate lines in individual wells. The isobutane would be used to specifically stimulate 1,4-D degradation to below regulatory limits, while a second cometabolic gas would be used to specifically remove TCE. Recent data from APTIM's laboratory (pure culture, microcosm, and pure enzyme studies with Soluble Methane Monooxygenase [sMMO]) suggest that methanotrophs do not typically biodegrade 1,4-D at environmentally relevant concentrations

(**Figure 2.4**; Hatzinger et al., 2018). For source area treatment with 1,4-D and TCE or other CVOCs, a series of biosparging wells could be placed throughout the target treatment zone, and the selected MPS gases could be added either in sequence or together. In this case, a mixture of methane and isobutane would be expected to treat the target compounds without competitive interactions. For sites where biosparging is not possible due to site geology or other factors, a groundwater recirculation system can be used with active gas addition to the flowing water stream. This approach has proven effective for both EDB (Hatzinger et al., 2018) and 1,4-D (Chu et al., 2018). Thus, this MPS technology has many different potential application approaches.

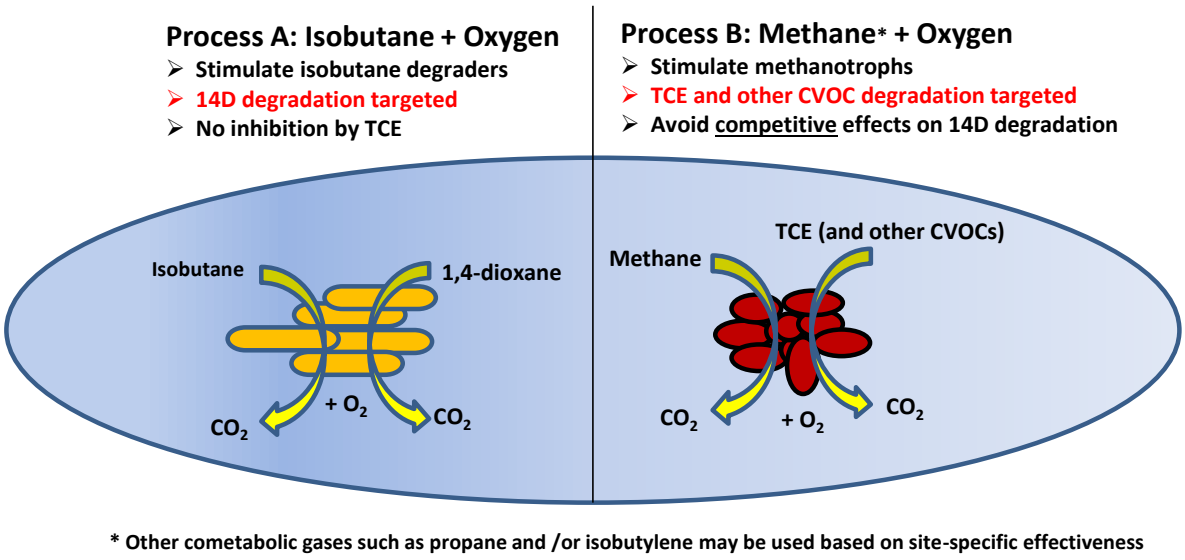


Figure 2.3. Schematic of MPS Cometabolic Bioremediation Approach. Isobutane was added to the aquifer to specifically promote biodegradation of 1,4-D and some CVOCs, and methane was used to stimulate bacteria capable of rapidly degrading CVOCs such as TCE that are not effectively biodegraded by isobutane-oxidizing bacteria. The mixture of gases used to target COCs are based on site-specific treatability tests.

As previously noted, the addition of single alkanes, including methane, propane, and *n*-butane has been used successfully in the past to treat various COCs, including chlorinated solvents and fuel oxygenates (Wilson and Wilson 1985; Semprini and McCarty, 1991; Semprini, 1997; Fournier et al., 2009). However, in environments containing CVOCs, one drawback with these earlier applications was the production of reactive metabolites (e.g., TCE epoxide) that can bind to cellular DNA and other biomolecules, potentially limit the efficacy of the process depending on both CVOC type and concentration (Oldenhuis et al., 1991; Halsey et al., 2005; Rui et al., 2004; Yeager et al., 2001; Alvarez-Cohen and McCarty, 1991; Hand et al., 2015; Hatzinger et al., 2011; Mahendra et al., 2013). In addition, with multiple COCs as substrates for a given enzyme (e.g., 1,4-D and TCE as substrates for propane monooxygenase), enzyme kinetics and competitive inhibition may cause one compound to be selectively degraded, while another remains. Results from SERDP project ER-2303 indicate that isobutane-oxidizing bacteria do not degrade TCE at any appreciable rate and consequently the presence of TCE does not impact 1,4-D biodegradation. The ability of ATCC 21198 to simultaneous transform a mixture of 1,4-D and CVOCs, while continuing to utilize isobutane indicates the stimulation of isobutane utilizers is of value for the *in*

situ treatment of sites with mixtures of 1,4-D and CVOCs, including TCE, 1,1-DCE and 1,1,1-TCA. The results from these demonstrations (and the lessons learned) were utilized during the design of this field trial. **The critical objective of this demonstration was to determine whether multiple gaseous amendments distributed within an impacted aquifer can stimulate indigenous bacteria to biodegrade 1,4-D and co-mingled CVOCs to target levels (i.e., MCLs).**

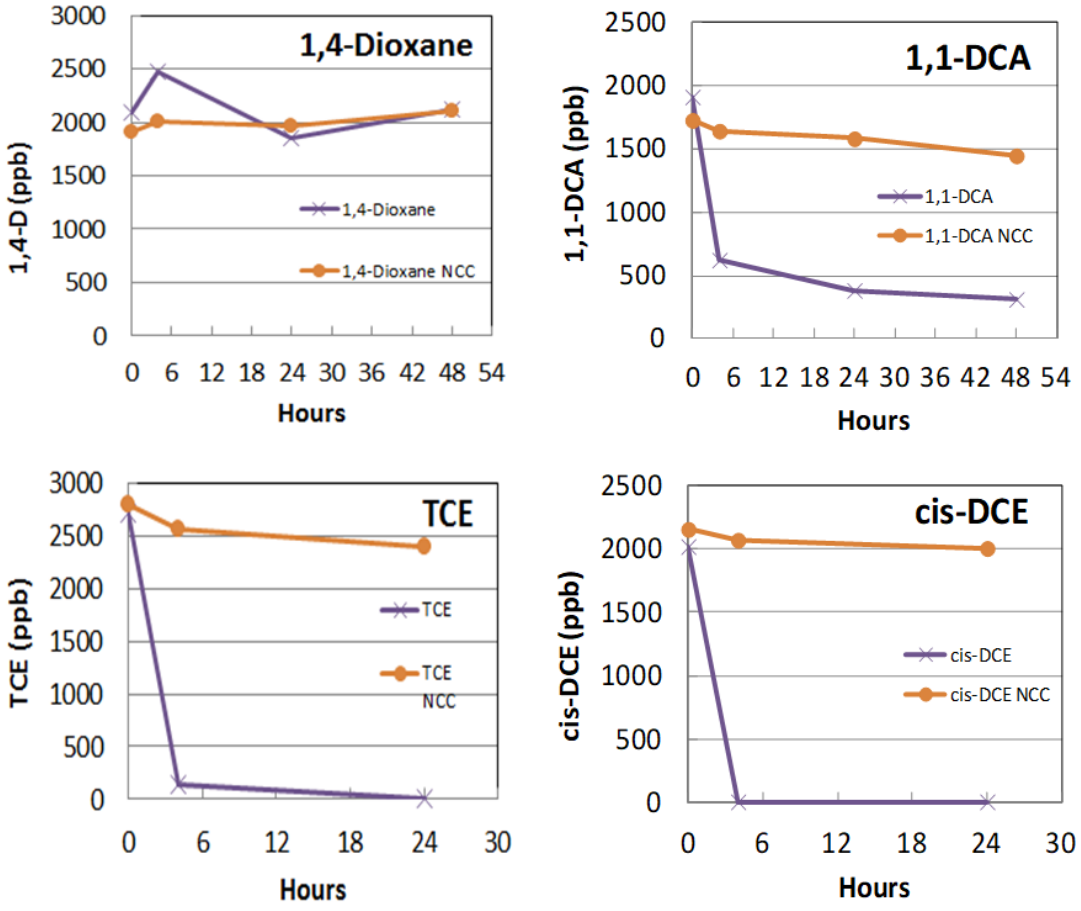


Figure 2.4. Biodegradation of 1,4-D, TCE, 1,1-DCA, and *cis*-DCE by the methanotroph *Methylocella palustris* expressing sMMO. Similar results were obtained for other pure cultures and for the pure sMMO enzyme. NCC = no cell control.

2.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

2.3.1 Advantages

As previously discussed, many sites implement P&T to remove comingled 1,4-D and CVOCs from groundwater. However, this *ex-situ* technology is expensive, requiring the installation of significant infrastructure, including numerous EWs and a treatment facility. Cometabolic biodegradation of comingled 1,4-D and CVOCs has several advantages over the current P&T technology. Importantly, the technology results in complete degradation of target COCs, and it can be applied *in situ*, thereby reducing the risk of chemical exposure, reducing COC/media disposal

costs, and eliminating groundwater recovery costs. Unlike bioremediation processes that require the degradative bacteria to metabolize and grow on the target COC, the cometabolic approach allows bacteria to grow on the available co-substrate (i.e., isobutane, methane), allowing them to degrade the COCs (CVOCs) to sub-ng/L concentrations, as previously described in **Section 2.1** and references cited therein. Such low treatment levels are typically not attainable with metabolic systems because there is insufficient carbon and energy for growth at low COC concentrations (Alexander, 1994; Schmidt et al., 1985). Furthermore, the cometabolic technology is very flexible and can be applied in a wide range of configurations (source area treatment, *in situ* permeable barriers, re-circulation systems, etc.), and it relies on the use of low-cost substrates. With the application of MPS, cometabolism may now be targeted to allow the simultaneous treatment of multiple comingled COCs (chlorinated ethenes, chlorinated ethanes, NDMA; Tovanabootr et al., 2001; Battelle, 2001, and 1,4-D; Lippincott et al., 2015), without appreciably impacting aquifer geochemistry.

In addition to P&T, *in situ* treatment technologies including anaerobic bioremediation (i.e., enhanced reductive dechlorination, [ERD]), *in situ* chemical oxidation (ISCO) and *in situ* chemical reduction (ISCR) have been widely and successfully used for treating groundwater impacted with high concentrations of CVOCs. ERD and ISCR approaches, however, are generally ineffective for treating 1,4-D, thus requiring a second treatment technology to address the 1,4-D. While ISCO has the potential to treat both 1,4-D and CVOCs, it requires direct contact between the oxidant and the dissolved COCs for reaction to occur, is subject to uneven distribution over large areas, and has limited longevity. ISCO is performed by adding a strong oxidant (typically permanganate, persulfate, or Fenton's reagent) to the subsurface to oxidize target pollutants. Typically, however, the majority of the added oxidant actually oxidizes non-target species including natural organic matter and reduced minerals, leading to high dosing requirements, thus making treatment using ISCO technically impractical and cost prohibitive at many sites. ISCO requires direct contact between the oxidant and the dissolved COCs for oxidation to occur. Even modest heterogeneities in the aquifer materials make effective distribution of chemical oxidants in the subsurface unlikely. Furthermore, chemical oxidants are short-lived in the subsurface, and typically require multiple applications to treat COC rebound or influx of upgradient COCs.

2.3.2 Disadvantages

In addition to its many advantages, the cometabolic technology has some potential disadvantages. For example, successful application of the technology generally requires the presence of indigenous alkane- or alkene-gas oxidizing bacteria that can degrade the target COCs. At some sites, indigenous bacteria able to degrade target CVOCs may not be abundant and/or may not be able to be successfully stimulated. In these cases, bioaugmentation may be required. Due to the addition of carbon substrates, it is also possible that inorganic nitrogen can become limiting at sites, so nutrient addition is often required at sites. However, this is easily done via liquid (e.g., diammonium phosphate [DAP]) or gaseous (ammonia) amendments. Also, at some sites achieving and demonstrating adequate distribution of injected gases (propane, oxygen and ammonia) may be challenged by site hydrogeology. These same conditions, however, would likely also limit the implementation of other *in situ*, and possibly *ex situ* technologies. Finally, successful application of the technology could be inhibited by the presence of certain chemicals. For example, some chlorinated ethenes (i.e., TCE and 1,1-DCE in particular) form epoxides during cometabolic degradation that are reactive and bind to cellular materials, potentially increasing death rates of the

microbial populations carrying out the reaction. As a result, high concentrations of comingled COCs at a site may result in poor remedial performance. The potential for such inhibition can be assessed by performing laboratory treatability testing.

3.0 PERFORMANCE OBJECTIVES

Performance objectives and results are summarized in **Table 3.1**, and details are provided in **Sections 3.1** through **3.6**.

Table 3.1. Performance Objectives

Performance Objective	Data Requirements	Success Criteria	Results
Quantitative Performance Objectives			
Determine remediation effectiveness	Baseline and treatment phase 1,4-D and CVOC concentrations in groundwater	>95% reduction in aqueous 1,4-D, TCE, *cis*-DCE, 1,1-DCE, and 1,1-DCA concentrations in at least 6 of the 8 treatment zone performance monitoring wells (PMWs) within the 12-month treatment period	Average reductions in the 8 treatment zone PMWs: • 82% reduction in 1-1-DCE • 73% reduction in TCE • 19% reduction in *cis*-DCE • 37% reduction in 1,1-DCA • 16% reduction in 1,4-D
Maintain aerobic conditions within the treatment zone	Groundwater DO concentration measurements at treatment zone PMWs during active treatment	Consistently (>75% of the time) maintain groundwater DO concentrations >1 mg/L in at least 6 of the 8 treatment zone PMWs during Phase 2 system operation	• DO consistently > 1 mg/L in treatment zone PMWs during Phase 2 operation
Adequate distribution of MPS gases in the treatment zone	Groundwater dissolved gas concentrations at PMWs during the first month of Phase 2 system operation	>200 µg/L dissolved isobutane & methane observed in at least 6 of the 8 treatment zone PMWs during the first month these gases were added to the aquifer	• Dissolved isobutane > 200 µg/L in 7 of 8 treatment zone PMWs within 21 days • Dissolved methane > 200 µg/L in 6 of 8 treatment zone PMWs within 14 days
Determine treatment system reliability	System operational data recorded by a data acquisition system	>90% system operation as designed	• The treatment system operated as designed >95% of the time • The off-the-grid solar power system provided consistent power through the demonstration
Qualitative Performance Objectives			
Ease of Use	Feedback from field technician on usability of technology and training time required	Minimal O&M and minimal technician training requirements	• The treatment system proved to be easy to operate and maintain • Training on operation and O&M completed in 1 day
Identification of degradative genes/proteins	qPCR and proteomic analysis conducted on groundwater samples	Identify active enzymes degrading 1,4-D and/or CVOCs	• We were able to successfully identify and/or quantify the active enzymes via qPCR and proteomic analysis

3.1 DETERMINE REMEDIATION EFFECTIVENESS

One of the primary objectives of this demonstration was to assess the effectiveness of applying aerobic cometabolism to treat groundwater impacted with comingled 1,4-D and CVOCs. Understanding the extent to which the target COC mass was removed, and the subsequent impact on groundwater quality, was critical in this evaluation.

This performance objective was considered met if the concentration of 1,4-D, TCE, *cis*-DCE, 1,1-DCE, and 1,1-DCA were each reduced a minimum of 95% from their equilibrated levels (achieved during Phase 1) within the 12-month treatment (Phase 2) timeframe in at least 6 of the 8 (≥75%) PMWs located within the treatment zone. The collection of numerous complete and reliable sets of groundwater 1,4-D and CVOC data at different time points throughout the demonstration were performed to evaluate this performance objective. These include two baseline groundwater sampling events, two Phase 1 sampling events, seven Phase 2 sampling events conducted during 12-months of treatment system operation, and one post-treatment sampling event performed approximately three months after system shutdown. Groundwater 1,4-D and CVOC concentrations were measured at eight PMWs and the EW within the target treatment zone, and one monitoring well downgradient of the treatment zone, as well as two upgradient (including one control) monitoring wells. Treatment phase (Phase 2) 1,4-D and CVOC data collected from wells within and downgradient the treatment zone was compared to baseline and Phase 1 data and data collected from the control well to evaluate treatment effectiveness. The data collected were used to determine the extent of 1,4-D and CVOC reduction during treatment.

Concentrations of 1,1-DCE at the four pair of treatment zone PMWs declined appreciably, from an average concentration of 1,017 µg/L at the conclusion of Phase 1 to an average concentration of 181 µg/L after ~ 12 months of Phase 2 operation, an average decline of 82%. Similarly, TCE declined by an average of 73% over the same time interval, from 810 µg/L on average to 220 µg/L. COCs present at lower concentrations, including *cis*-DCE, 1,1-DCA, and 1,4-D, declined by less, averaging 19%, 37%, and 16% respectively. The most likely reason for the limited decline of these compounds was persistence of 1,1-DCE in the test plot continued to cause enhanced rates of death of ENV493 and any native cometabolic organisms that oxidized it to 1,1-DCE-epoxide, thus limiting degradation of many of the other target COCs. Multiple strategies to overcome this issue, including isobutanol addition to enhance the growth of isobutane-degrading bacteria without stimulating the oxidase that degrades 1,1-DCE, (creating reactive epoxides) and multiple rounds of bioaugmentation were not successful at reducing 1,1-DCE to sufficiently low concentration to allow degradation of all other COCs including 1,4-D as observed in laboratory studies. Overall, the concentration of chlorinated ethenes (1,1-DCE in particular) at the NASNI site were likely too high for technology to achieve the desired 95% removal metric. Therefore, this performance objective was not achieved.

3.2 MAINTAIN AEROBIC CONDITIONS WITHIN THE TREATMENT ZONE

Achieving and maintaining aerobic conditions within the treatment zone was critical during the demonstration, as cometabolism using an alkane/gas substrate is an aerobic process. Therefore, the extent to which sparged oxygen gas could be distributed (both vertically and horizontally) and target DO concentrations maintained within the treatment zone were critical to the overall success of the remedial approach.

This quantitative performance objective was considered met if groundwater DO concentrations were consistently maintained (>75% of the time) at >1 mg/L in at least 6 of the 8 treatment zone PMWs during Phase 2 system operation. The DO data collected throughout the demonstration were used to optimize oxygen sparge cycle durations and frequencies throughout the demonstration. This performance objective was evaluated based on measured DO concentrations measured within the treatment zone. DO concentrations were measured at all 11 monitoring wells during baseline sampling, nine performance groundwater sampling events performed throughout Phases 1 and 2, and the post treatment groundwater sampling event.

DO concentrations measured during Phase 2 of the demonstration were greater than 1.0 mg/L at 6 of the 8 treatment zone PMWs (PMW-1D, PMW-2D, PMW-03S/D and PMW-04S/D), EW-1 and downgradient well DMW-1 at between 75 and 100 percent of the measurements collected during Phase 2 of the demonstration. Eighty-six percent of the DO measurements collected at the eight treatment zone PMWs during Phase 2 were > 1.0 mg/L, with 79 percent of the measurements > 5 mg/L, with average DO concentrations ranging between 3.69 mg/L and 26.56 mg/L. Therefore, the objective of maintaining aerobic conditions in the aquifer was clearly achieved.

3.3 ADEQUATE DISTRIBUTION OF MPS GASES WITHIN THE TREATMENT ZONE

Optimization of the MPS gas delivery mass and frequencies increase the effectiveness of the aerobic cometabolic treatment technology by supplying appropriate amounts of the cometabolic gases, while ensuring that high dissolved gas concentrations do not lead to continuous competitive inhibition. Therefore, the extent to which the cometabolic gases could be distributed (both vertically and horizontally) via the groundwater recirculation system, and the dissolved gases consumed within the treatment zone, were important to the overall success of the remedial approach. During the demonstration, isobutane and methane gases were added to the aquifer through the groundwater recirculation system to stimulate microbial growth and activity. The data collected during the demonstration were used to optimize MPS gas addition cycle concentrations, durations, and frequencies, to minimize the effects of competitive inhibition, and reduce overall operating costs.

The success criteria for this performance objective were to observe >200 µg/L dissolved isobutane and methane in at least 6 of the 8 treatment zone PMWs during the first month these gases were added to the aquifer. During Phase 2 of the demonstration, dissolved isobutane concentrations exceeded the target 200 µg/L in seven of the eight treatment zone PMWs within 21 days of initiating isobutane addition, and at the eighth monitoring well within 35 days. Additionally, dissolved methane concentrations exceeded the target of 200 µg/L in six of the eight treatment zone PMWs within 14 days of initiating methane addition. Therefore, this performance objective was achieved.

3.4 DETERMINE TREATMENT SYSTEM RELIABILITY

Reliability of the oxygen biosparging, groundwater recirculation, and amendment delivery systems was essential during the demonstration, as the regular addition of oxygen, cometabolic gases, and nutrients is critical to the treatment and cost effectiveness of any cometabolic treatment approach.

This performance objective was considered met if the treatment system operated as designed >90% of the time. The system was considered operating as designed if the oxygen sparge, MPS gas addition, and groundwater recirculation cycles start and stop as programmed, and if the mass of gaseous amendments delivered during each cycle was within +/- 10% of the designed loading rate. Data collected for assessing this performance objective included the regular downloading of system operational logs recorded by a data acquisition system. System operations data (gas and recirculation flows, pressures, cycle frequencies, and downtime) were downloaded on a regular basis via remote access to the treatment system computer data files.

The off-the grid solar power system provided consistent power to the treatment system throughout most of the 499 days of the demonstration and was only down for seven days for the replacement of a charge controller and circuit breaker. The cometabolic treatment system operated as designed, required minimal O&M, and no major system or equipment failures were experienced during the demonstration, apart from unexpected damage caused by Pocket Gophers to subsurface groundwater recirculation, nutrient injection, and oxygen injection lines, as discussed in **Section 5.7.7**. The system was down for ten days while the subsurface lines were replaced.

In general, the off-the-grid solar powered treatment system proved to be highly reliable, simple to operate and maintain, and economical for *in situ* cometabolic treatment. Importantly, there were no apparent issues with biofouling of the IWs, EW or oxygen biosparging wells during the demonstration. Systems operation data collected during the demonstration showed that the treatment system operated as designed >95% of the time (482 out of 499 days). Therefore, this performance objective was achieved.

3.5 EASE OF USE

The level of effort required to maintain effective 1,4-D and CVOC treatment using the aerobic cometabolism technology will determine, in part, the cost effectiveness of this approach for treating plumes with these comingled COCs. This qualitative performance objective was evaluated with respect to the level of effort, time, and resources required to operate and maintain the treatment system. This performance objective was considered met if a single field technician was able to effectively collect system and groundwater measurements, and O&M requirements were similar to, or less than, other treatment approaches. Data required for assessing this qualitative performance objective included feedback from the field technician on system O&M and the time required to address potential operational issues. The O&M information collected throughout the demonstration was considered when assessing the overall ease of implementation of this approach.

In addition to the PI and project engineer, two field technicians were trained to operate and conduct system O&M in one day. System O&M requirements, which primarily consisted of regular system checks and changeout of the oxygen cylinders, were not substantial during the demonstration. System checks (which entailed collecting manual system pressure and flow data, performing regular system maintenance, and performing leak checks) were generally performed every 2-4 weeks in under 3 hours per visit. Change out of the oxygen 16-packs was conducted approximately every 2-3 months and was typically performed in under 6 hours. The 4 cylinders of liquified isobutane and 12 cylinders of compressed methane did not require replacement during 12 months of Phase 2 cometabolic biosparging due to the general efficiency of this treatment approach. The ability to communicate remotely with the system (and adjust system operating parameters), as well

as programmed logging capabilities of the SCADA system reduced the number of site visits required. Furthermore, other than soil cuttings and development water generated during well installations, there was no waste generated during application of this *in situ* technology (even purge water generated during groundwater sampling was mixed with nutrients and injected back into the aquifer via the nutrient injection system). The off-the-grid solar powered system provided continuous power for operation of the treatment system and proved simple to operate and maintain. Based on the minimal O&M and minimal technician training requirements that were documented, this performance objective was achieved.

3.6 IDENTIFICATION OF DEGRADATIVE GENES/PROTEINS

The ability to identify the microorganism being stimulated as well as to confirm that bacteria express the expected monooxygenases after stimulation by each gaseous primary substrate advances the understanding of microbial communities and monooxygenase enzyme stimulation to specifically target treatment at this and other sites with mixed 1,4-D and CVOC plumes.

This performance objective was considered met if we could identify and, where primers are available, quantify the genes and active enzymes involved in the degradation of 1,4-D and CVOCs via qPCR and/or ABPP (qualitative). This performance objective was evaluated based on microbial analysis performed by Microbial Insights (Knoxville, TN) and North Carolina State University (NCSU) on groundwater samples collected during four groundwater sampling events. Groundwater samples for microbial analysis were collected at six select monitoring wells during baseline sampling, Phase 1 and Phase 2 of the demonstration. Analysis of target cometabolic organisms/genes/enzymes by qPCR was performed and proteomic analysis using an ABPP approach capable of simultaneously identifying multiple types of active monooxygenases in environmental samples was also be used to confirm that bacteria express the expected monooxygenases after stimulation by each gaseous primary substrate.

The qPCR data collected indicated that a limited number of wells showed meaningful (approximately 2 orders of magnitude) increases in the target cometabolic organisms/genes during Phase 2 of the demonstration. Furthermore, the results of the ABPP analysis targeting isobutane-oxidizing monooxygenases performed by NCSU generally agreed with the qPCR data. The relatively small magnitude of the increases and the apparent limited distribution of the of the target organisms were unexpected, as the aquifer was bioaugmented twice with ENV493 and once with ENV494M during the period when these samples were collected. The limited increases could be due to the formation and subsequent toxicity of the reactive 1,1-DCE epoxide that reduced the populations appreciably and/or the adsorption of ENV493 (and to a lesser extent, ENV494M) to sediments (rather than being planktonic). Regardless, the data indicate that we were able to successfully identify and/or quantify the genes and active enzymes via both qPCR and ABPP. Therefore, this performance objective was achieved.

4.0 SITE DESCRIPTION

A key to the success of any field demonstration project is the selection of an appropriate demonstration site. Site selection included an initial review of the *in situ* conditions at several CVOC-impacted DoD facilities. Site data evaluated for each candidate location included the following: (1) basic aquifer conditions (e.g., geology, plume depth, geochemistry, hydrology etc.); (2) CVOC concentrations and plume characteristics; and (3) basic infrastructure (e.g., site access, presence of wells, roads, etc.). Ideal characteristics for evaluation of the demonstration site were as follows:

- 1,4-D concentrations > 50 µg/L
- CVOC concentrations > 50 µg/L
- Plume bottom depth < 50 ft bgs
- Permeable (spargeable) site hydrogeology
- Previously delineated plume
- Neutral groundwater pH
- Absence of dense non-aqueous phase liquid
- Basic infrastructure (roads, existing wells).

Based on the data review, OU-11 at NASNI was selected as the optimal site for hosting the field demonstration. Three potential demonstration location were identified during the review, and a site visit to NASNI was made on December 19, 2017. The visit included a meeting with Michael Pound, the facility's Lead Remedial Project Manager (RPM), site walks and evaluation, and discussions with APTIM personnel who have worked on the base for many years. Logistics and timing of the field demonstration were discussed during this visit.

Upon completion of the site visit and data evaluation, the optimal location for the field demonstration within OU-11 was determined to be in a vegetated area located in a side-gradient portion of the plume (**Figure 4.1**). A Site Selection Memo was submitted to ESTCP on September 30, 2017 (NAVFAC and APTIM, 2017), and four comments from ESTCP on the memo were received on February 14, 2018. A response to comments memorandum was submitted to ESTCP on March 1, 2018, and site selection was approved by ESTCP on July 6, 2018.

4.1 SITE LOCATION AND HISTORY

NASNI is located in the city of Coronado, San Diego County, California. As shown in **Figure 4.2**, the base is located at the north end of the Silver Strand Peninsula that separates San Diego Bay from the Pacific Ocean and is bounded by the city of Coronado on the East, the Pacific Ocean to the south, San Diego Bay to the north, and the inlet to San Diego Bay to the West (Battelle and Accord Engineering, 2017).

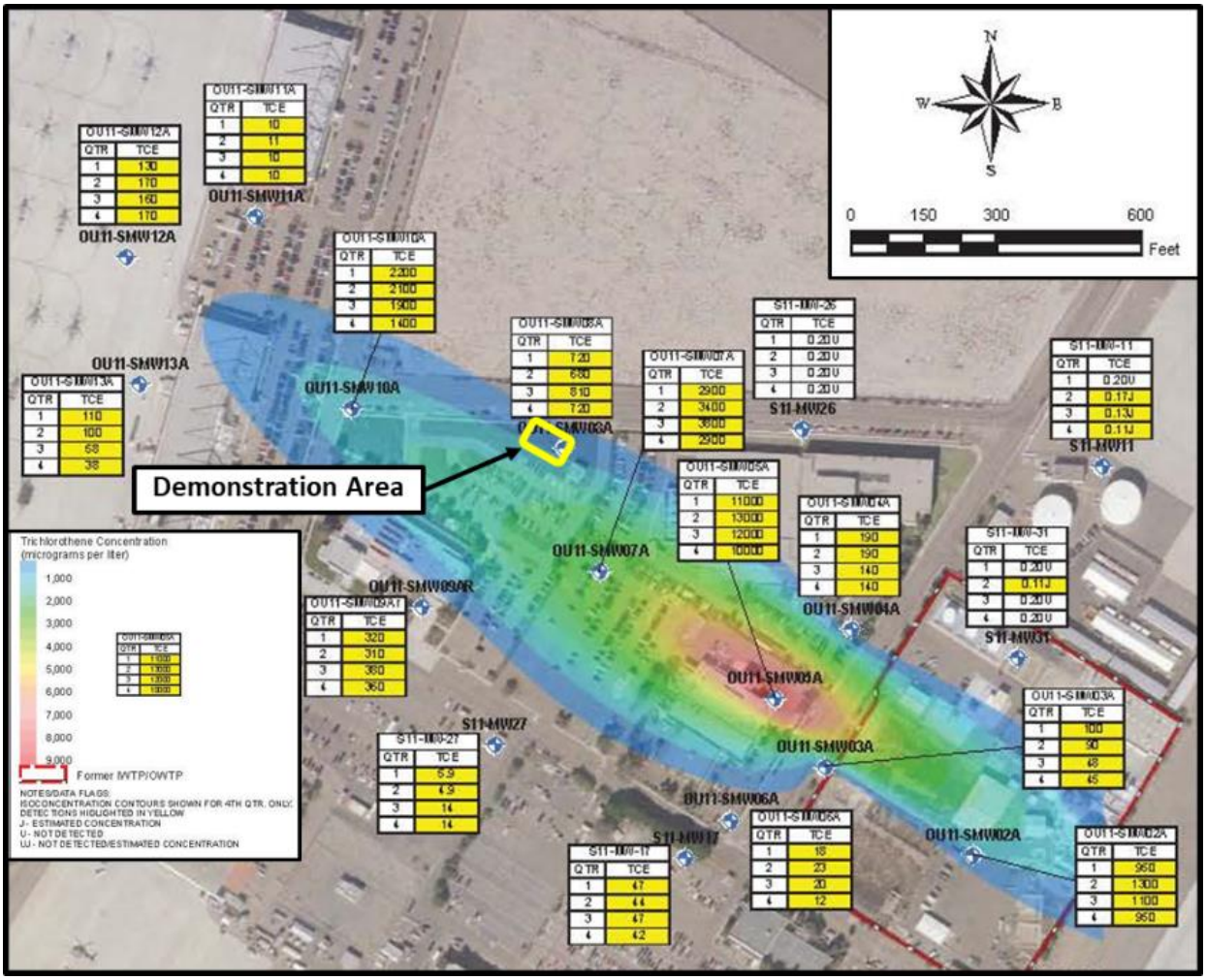


Figure 4.1. Demonstration Location Within the TCE Plume. (Source: Battelle and Accord Engineering, 2017).

NASNI was commissioned in 1917 and is currently an active military base. The United States Department of the Army and the United States Department of the Navy (Navy) both conducted aviation training activities there from 1912 to 1935. After 1935, NASNI was occupied exclusively by the Navy (Bechtel, 2003). The mission of NASNI is to provide services and material that support operation of aviation activities and units of the Navy, as well as other units designated by the Chief of Naval Operations. Fleet helicopter and jet aircraft squadrons are based at NASNI. The base is also the headquarters of several naval staffs and the site of various school commands. NASNI includes 12 naval departments, nine special assistants, and 63 tenant activities (Bechtel, 2003).

OU-11 is a 10-acre area in the center of NASNI whose boundary is that of the industrial waste treatment complex [the former industrial waste treatment plant (IWTP)/oily waste treatment plant (OWTP)], currently designated IWTP2. OU 11 comprises all the area within the industrial waste treatment complex. The former IWTP (constructed in 1972) and the OWTP (constructed in 1977) processed and disposed hazardous and oil wastes generated by NASNI and other San Diego-area naval operations. The IWTP was operated from 1972 until IWTP2 was constructed in 1994. The

former IWTP processed organic and inorganic contaminated rinse waters from plating, stripping, and other processes. At its peak operating volume, the former IWTP treated approximately 860,000 gallons of contaminated wastewater per day through its network of Solid Waste Management Units (SWMUs). The former OWTP operated from 1977 until 1988, when it was replaced by the Oil Recovery Plan, which is located within the IWTP2 facility (Bechtel, 2003).

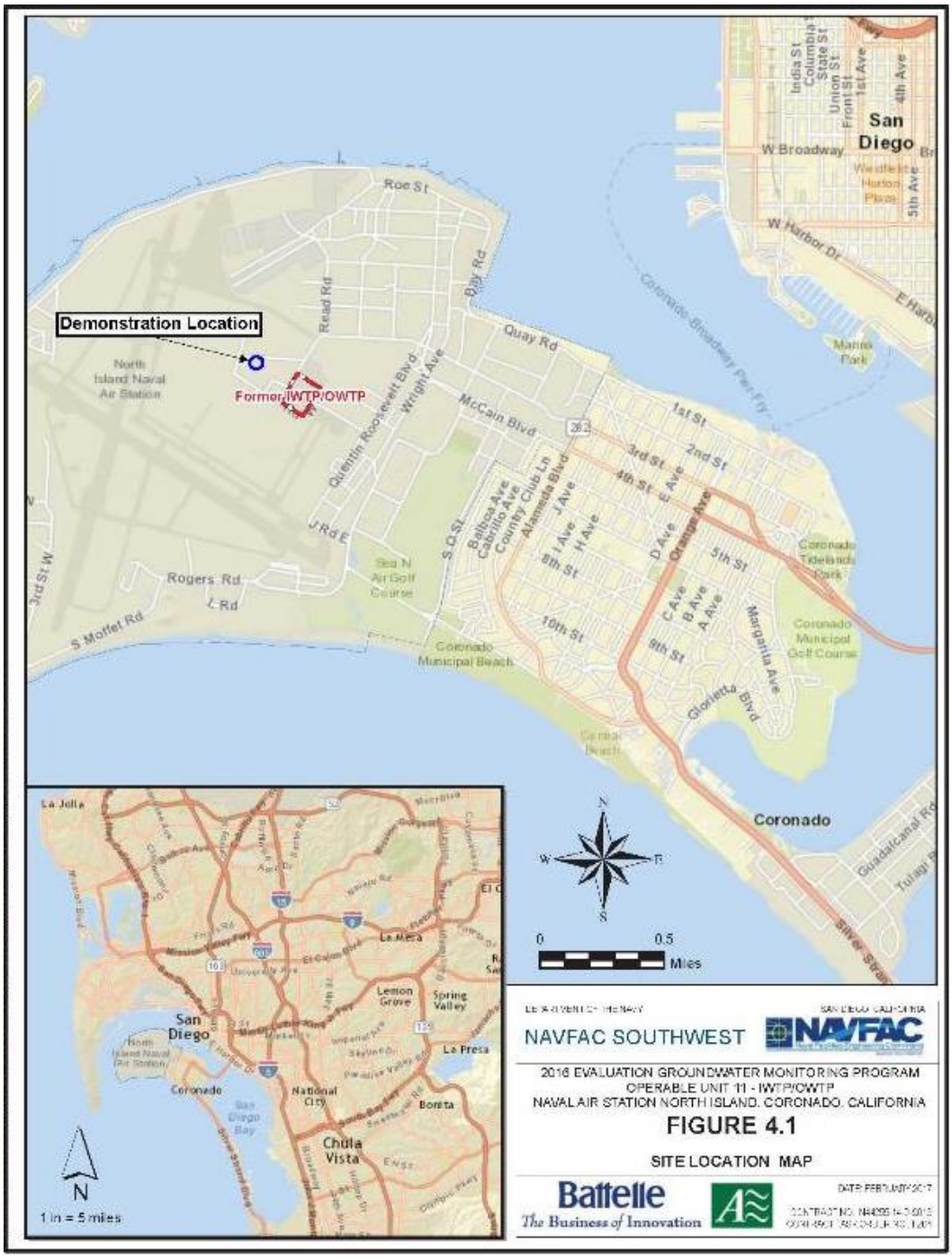


Figure 4.2. Site Location Map. (Source: Battelle and Accord Engineering, 2017).

As a result of a Resource Conservation and Recovery Act (RCRA) facility assessment (DHS, 1989) conducted at NASNI, 34 SWMUs and two areas of concern (AOCs) were identified as areas or processes pertinent to the former IWTP and OWPT operations. There are currently seven active SWMUs and one active AOC that are part of the industrial waste treatment complex located within the boundary of OU-11 that are not included as part of OU-11 because they are currently in use as part of the IWTP2 (Bechtel, 2003).

Historic activities at OU-11 are the source of a groundwater plume containing both 1,4-D and CVOCs located in the center portion of NASNI. The comingled plume extends from within OU 11 to the northwest for approximately 2,000 ft. The extent of the TCE plume and the location of the Demonstration Area are shown in **Figure 4.1**. The extent of the 1,4-D impacts in groundwater, as well as most of the other COCs, is similar to TCE. The Demonstration Area is located on the northern edge of the plume, approximately 1,100 ft downgradient (northwest) of the source area (**Figure 4.1**).

4.2 SITE GEOLOGY/HYDROGEOLOGY

NASNI is located on generally flat land, with an average elevation of approximately 20 ft above mean sea level (MSL). The elevation of in the Demonstration Area is approximately 25-26 ft above MSL. The majority of NASNI covered with buildings and concrete or asphalt pavement. Therefore, much of the precipitation is lost as runoff (Bechtel, 2003).

The geologic unit underlying OU-11 is the Bay Point Formation. The stratigraphy indicates relatively flat, thin silt and clay layers within larger sand layers. Some of the clay layers are discontinuous (Bechtel, 2003). As shown in **Figure 4.3**, the hydrogeology of the Demonstration Area consists of an unconfined upper water-bearing zone comprised primarily of permeable sand/silty sand to a depth of approximately 40 ft bgs, with a depth to water of approximately 25 ft bgs. This unit is underlain by a four- to five-ft thick low permeability silt layer, designated the 'A Silt' layer, that acts as a semi-confining layer. Deeper aquifers at the site are under confining or semi-confining conditions and are partially or completely saline. Wells installed within the upper water-bearing zone are typically designated "A Wells", while wells installed within subsequently deeper water-bearing zones are typically designated "B, C or D Wells", respectively. The treatment zone for the demonstration is within the upper water-bearing zone (approximately 25-40 ft bgs).

The identified flow direction of groundwater in the upper water-bearing zone is generally to the northwest with an average horizontal groundwater gradient of 0.0006 ft per ft (Barajas & Associates, Inc., 2009). Groundwater in the unconfined aquifer in the vicinity of OU 11 flows northwest at the rate of approximately 19 to 33 ft per year (Science Applications International Corporation [SAIC], 1995). The groundwater gradient measured in the upper water-bearing zone during the 3rd quarter of 2016 is presented in **Figure 4.4**.

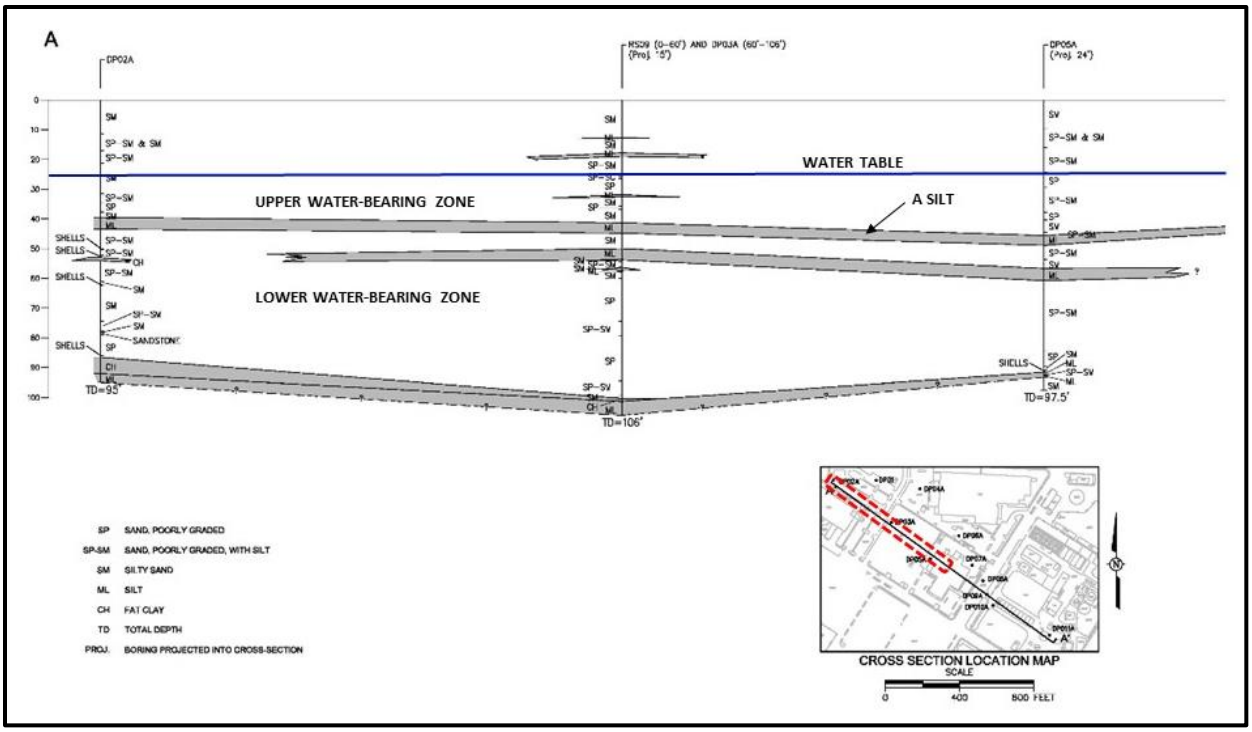


Figure 4.3. Cross Section Adjacent to Demonstration Area. Portion of Cross Section A-A', located approximately 100 ft southwest of the demonstration location (modified from Shaw, 2012).

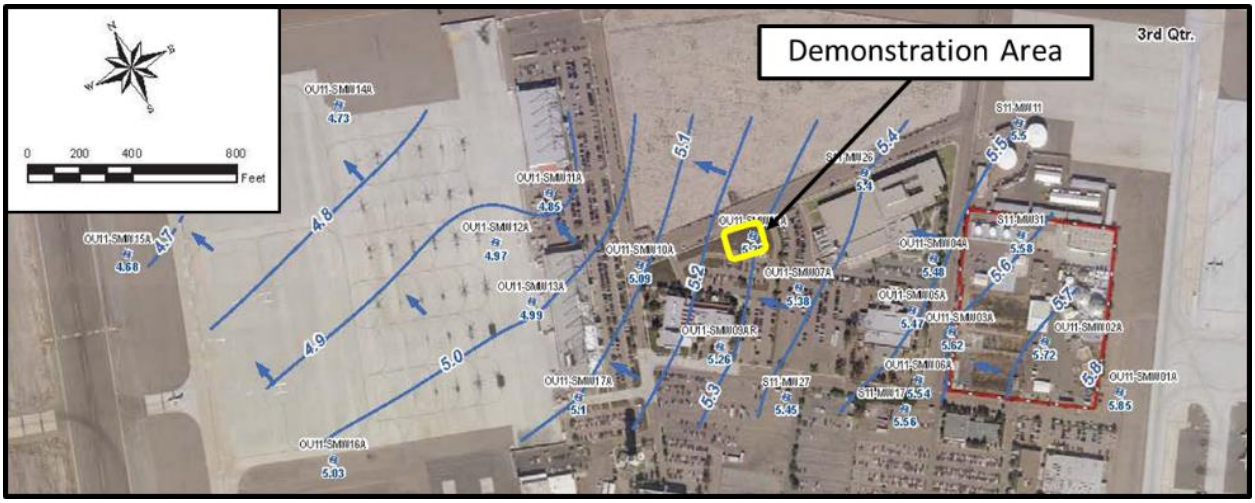


Figure 4.4. Groundwater Gradient in the Upper Water-Bearing Zone. Water table elevations measured during the 3rd quarter of 2016. (Source: Battelle and Accord Engineering, 2017).

4.3 CONTAMINANT DISTRIBUTION

The COCs in OU-11 include primarily 1,4-D, TCE, *cis*-DCE, 1,1-DCE, and 1,1-DCA. Concentrations of 1,4-D and total CVOCs in some areas of OU-11 have been measured as high as 8,000 µg/L and 15,000 µg/L, respectively. As shown in **Figure 4.1**, the Demonstration Area is in the downgradient and slightly side-gradient portion of the groundwater plume, with 1,4-D and CVOC concentrations both ranging between approximately 1,000 µg/L and 3,000 µg/L. Isoconcentration contours for 1,4-D and TCE within the upper water-bearing zone in the Demonstration Area are provided in **Figures 4.5** and **4.6**, respectively. The contours were generated using 2016 groundwater data collected as part of the OU-11 Evaluation Groundwater Monitoring Program (Battelle and Accord Engineering, 2017). Groundwater COC distribution within the Demonstration Area was further evaluated during site characterization activities and is discussed in more detail in **Section 5.2.1**.

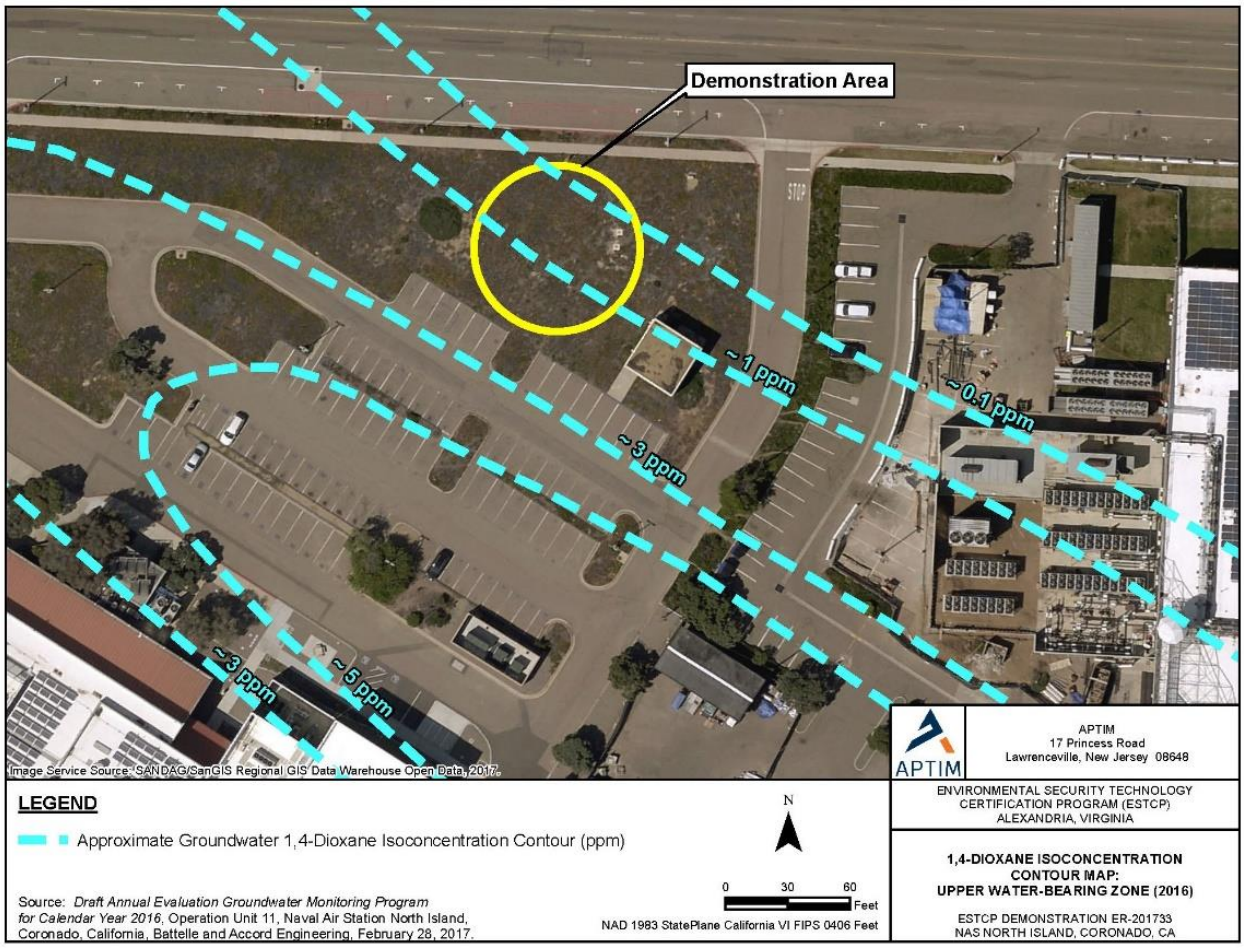


Figure 4.5. 1,4-D Isoconcentration Contour Map: Upper Water-Bearing Zone (2016). (Source: Battelle and Accord Engineering, 2017).

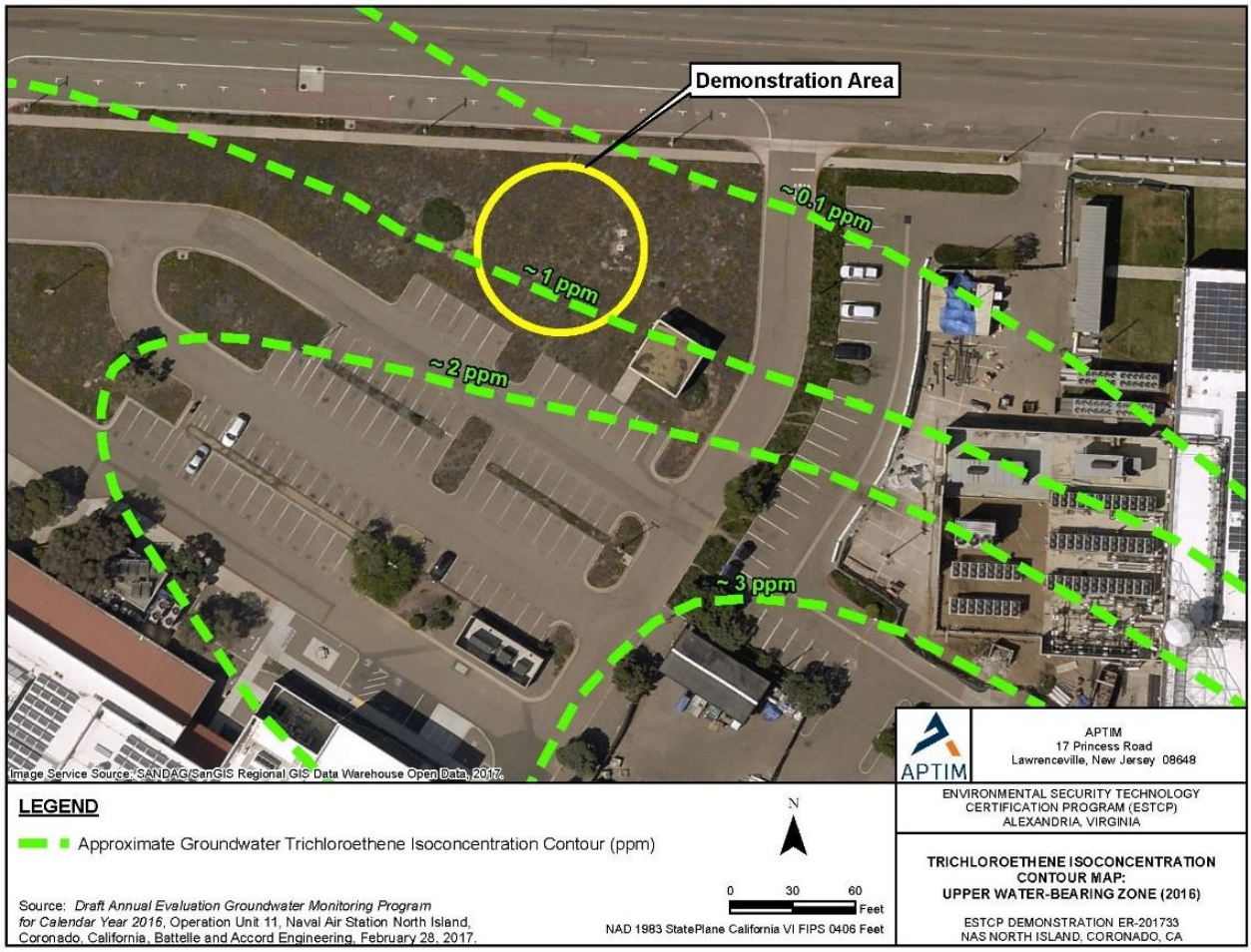


Figure 4.6. TCE Isoconcentration Contours in the Demonstration Area. (Source: Battelle and Accord Engineering, 2017).

5.0 TEST DESIGN

The following subsections provide detailed description of the system design and testing that was conducted to address the performance objectives described in **Section 3.0**.

5.1 EXPERIMENTAL DESIGN

As previously discussed, this field demonstration of cometabolic bioremediation entailed the use of multiple primary gaseous substrates to stimulate the aerobic degradation of comingled 1,4-D and CVOCs in groundwater by distinct groups of widely distributed bacteria. During this demonstration, isobutane was added to the aquifer to specifically promote biodegradation of 1,4- D and select CVOCs, and methane was used to stimulate bacteria capable of rapidly degrading specific CVOCs, including TCE, which is not effectively biodegraded by isobutane-oxidizing bacteria. As this is an aerobic process, oxygen was also added to the aquifer to maintain aerobic conditions. In addition to adding gaseous substrates, the aquifer was bioaugmented with two bacterial cultures (ENV493 and ENV494M). Nutrients (DAP) and isobutanol (a carbon source) were also added to optimize *in situ* growth of the bioaugmentation cultures, and possibly indigenous bacteria capable degradation of relevant COCs in NASNI OU-11 groundwater. The two bioaugmentation cultures were isolated from the demonstration site and were determined to be the best candidates for bioaugmentation during laboratory treatability testing (**Section 5.3**). This approach represents a significant advance for traditional single gas cometabolic bioremediation technology in that it utilizes a new understanding of microbial communities and monooxygenase enzyme stimulation and inhibition to specifically target groundwater with mixed 1,4-D and CVOCs.

The field implementation of the above treatment approach was unique in that it used a hybrid approach encompassing both biosparging and groundwater recirculation (~ 8-12 hours per day) to deliver and distribute amendments within the aquifer. The addition of oxygen to the aquifer was accomplished through direct sparging of pure oxygen through five dedicated biosparging wells, while the addition of the primary gaseous substrates, the bioaugmentation cultures, nutrients and isobutanol occurred using a groundwater recirculation system. The groundwater recirculation system also provided mixing of groundwater and a degree of hydraulic control within the treatment zone. The primary reason for not adding oxygen via the groundwater recirculation system was that the estimated mass of oxygen required to convert the anoxic aquifer at the site into an aerobic aquifer while maintaining aerobic conditions during treatment, was greater than what could have been added to the recirculated groundwater. This hybrid approach was intended to create a biologically active treatment zone to support 1,4-D and CVOC cometabolic biodegradation by native bacteria and commercially grown bacteria that had been isolated from the site (e.g., ENV493 and ENV494M), and then readded to the aquifer to rapidly increase the density of degrading organisms as previously mentioned.

Furthermore, the biosparging, groundwater recirculation and amendment delivery systems were designed to operate completely “off-the-grid” using solar energy and pressure from gas cylinders to supply necessary amendments. The systems were controlled automatically using a SCADA system, that allowed both on-site and remote system monitoring, operational set-point control, and alarm notification and acknowledgment. The gases were added to the aquifer under an optimized flow rate and frequency designed to maintain target dissolved concentrations of the gases to facilitate biomass growth, and ultimately treatment of the target COCs. Dissolved isobutane and

methane were added at concentrations that were designed to minimize continuous competitive substrate inhibition. In addition to maximizing treatment effectiveness, primary substrate gas delivery optimization was intended to reduce overall operating costs. Oxygen sparging frequency was optimized to maintain DO concentrations of 1.0 mg/L or greater within the treatment zone to ensure an aerobic environment was maintained. Details of system design, operation and safety features are provided in **Section 5.4**.

A network of 11 groundwater monitoring wells were monitored throughout the demonstration to evaluate aquifer conditions, including dissolved primary substrate gases (isobutane and methane), DO, nutrients (nitrate), isobutanol, and COC concentrations (as well as other relevant geochemical parameters) in areas within, upgradient and downgradient of the treatment zone. The design and layout of the monitoring well network is provided in **Section 5.4.1**. As detailed in **Section 5.7**, COC concentrations measured within and downgradient of the treatment zone during the various phases of the demonstration was compared with concentrations measured during baseline sampling, and at the upgradient "control" well to evaluate treatment performance.

5.2 BASELINE CHARACTERIZATION

Prior to site selection, APTIM reviewed existing site investigation documents and all available hydrogeologic, COC concentrations, and geochemical data for the OU-11 site. While this data was helpful in the selection of a potential demonstration location within the large, comingled plume, additional data was required to assist with design of the field demonstration. Baseline characterization activities included detailed site characterization and laboratory treatability testing as described in the following subsections.

5.2.1 Site Characterization Activities

While existing site characterization data for OU-11 at NASNI was available, additional site assessment was necessary to further develop the hydrogeological conceptual site model and provide essential data for system design. Specifically, site characterization activities were designed to fully delineate the vertical and horizontal extents of COCs within the demonstration area, evaluate aquifer lithology and hydraulic properties, and to assist in sparge well, EW/IWs, and biosparging system design. As discussed in greater detail below, site characterization activities included a direct-push technology (e.g., Geoprobe®) for soil and groundwater investigation, the installation and pump testing and monitoring wells, the implementation of a short-term pump test, and the implementation of a short-term oxygen sparging test. The hydrogeologic data collected during site characterization activities was used in developing a numerical groundwater model of the Demonstration Area (**Section 5.2.1.7**).

The work also included the collection of soil and groundwater samples to be utilized in laboratory treatability testing (microcosm studies). These microcosm tests with site materials were conducted at Oregon State University (OSU) in Corvallis, Oregon and APTIM's Biotechnology Development and Applications Group (BDAG) Laboratory in Lawrenceville, NJ, with microbial analytical support from NCSU in Raleigh, North Carolina, as detailed in **Section 5.3**.

Prior to implementation of site characterization activities, APTIM submitted a Draft Site Characterization Work Plan to Michael Pound, the Lead RPM for NASNI, on April 3, 2018, for review. While there were no comments received on the draft work plan, a revised Site

Characterization Work Plan that included changes to the number and types of wells to be installed was submitted to Michael Pound on August 10, 2018. The document was approved without comment.

Field activities were conducted using appropriate Level D personal protective equipment (PPE). Underground utility clearances were obtained for all intrusive site activities. Clearance of all underground utilities were arranged through the appropriate Navy representatives, facility personnel, and local utility companies. APTIM subcontracted a utility clearance contractor to identify the locations of underground utilities in the Demonstration Area and clear the proposed locations of all borings. Additionally, all borehole locations were cleared to five ft-bgs using a hand auger.

5.2.1.1 Hydraulic Profiling Borings

Three hydraulic profiling borings were advanced within the Demonstration Area (**Figure 5.1**) on August 29-30, 2018, using a Geoprobe® Hydraulic Profiling Tool (HPT)-Groundwater (GW) Sampler. This subsurface profiling tool combines an HPT, an Electric Conductivity (EC) sensor, and a discrete zone groundwater sampler (**Figure 5.2**). The HPT is a logging tool that measures the pressure required to inject a flow of water into the soil as a probe is advanced into the subsurface and is an excellent indicator of formation permeability. The hydraulic conductivity (K) of the subsurface is estimated by software using an empirical relationship developed for the tool (**Figure 5.3**). The HPT provided real-time on-screen logs of the subsurface permeability, allowing on-site decisions.

Data collected with the EC sensor was useful in classifying soil type and stratigraphy at each boring location. In general, silts and clays exhibit higher electrical conductivity readings than sands and gravels (**Figure 5.3**). The EC logs, in conjunction with HPT logs, were correlated across the study area to map the thickness and elevation of the varying lithologic units of interest and determine the optimal depths to collect discrete groundwater samples (**Section 5.2.1.3**). As shown in **Figure 5.3**, the high permeability material identified by the HPT exhibited low electrical conductivity, and the lower permeability material exhibited higher electrical conductivity. Like the HPT, the EC sensor provided real-time on-screen logs, allowing on-site decisions.

The Geoprobe® HPT-GW Sampler was advanced to a depth of approximately 43-44 ft bgs at three locations (HPT-1 through HPT-3) in the demonstration area (**Figure 5.1**). The tool was advanced approximately 3-5 ft into the A Silt layer, which was clearly indicated by peaks on both the HPT and EC logs for boring HPT-1 presented in **Figure 5.3**. These logs include EC (microsiemen per meter), HPT pressure (pounds per square inch [psi]), HPT flow (milliliter per minute), estimated K values (ft/day), and HPT pressure corrected for hydrostatic head (psi). The logs collected from the three HPT borings are provided in **Appendix B**, and indicate the following:

- The water table was encountered at approximately 24-25 ft bgs,
- Relatively permeable soils (estimated K's generally between 20 ft/day and 50 ft/day) were encountered between approximately 24 and 35 ft bgs,
- Soils with slightly higher permeability (estimated K's generally between 70 ft/day and 90 ft/day) were encountered between approximately 35 and 40 ft-bgs, and

- The A Silt layer, consisting of soils with relatively low permeability (estimated K's < 10 ft/day), was encountered at approximately 40 ft bgs, and is a minimum of 3 ft in thickness (this unit was not fully penetrated during drilling).

These results are consistent with the previous site characterization data collected at OU-11.

Figure 5.1. HPT Boring, Soil Core, and Pump Test Well Locations.

Figure 5.2. Geoprobe® Hydraulic Profiling Tool-Groundwater Sampler.

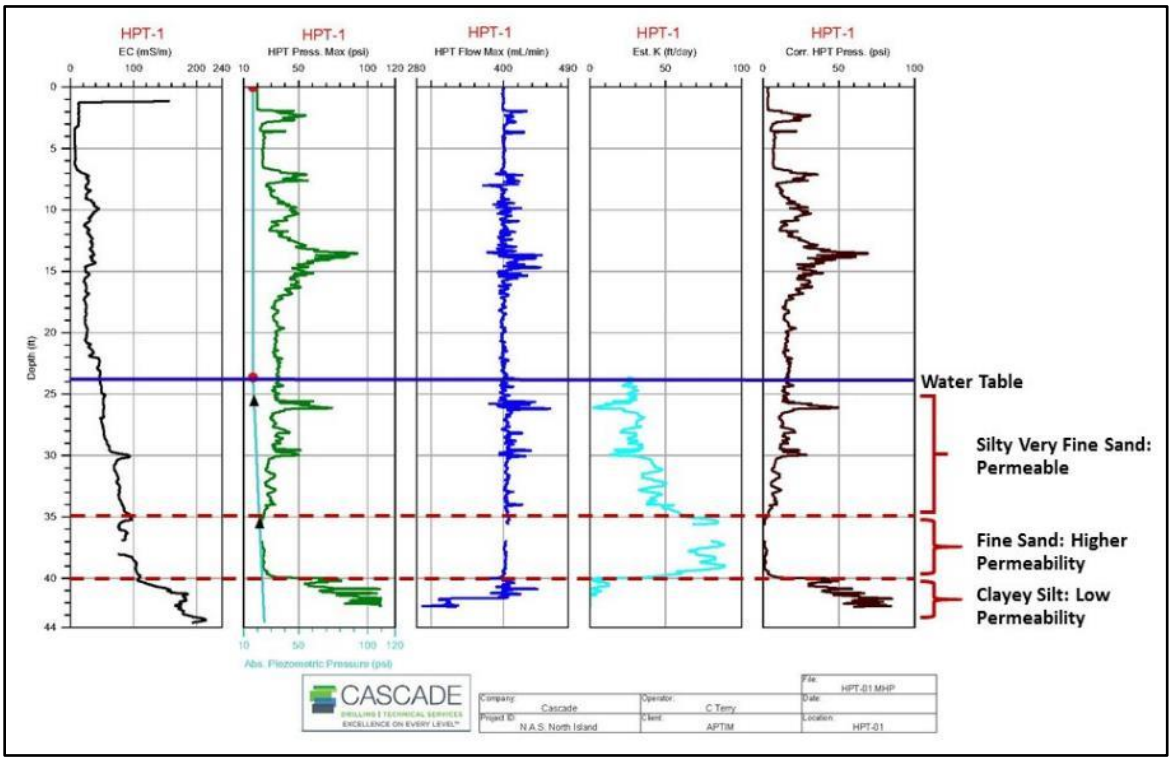


Figure 5.3. HPT Log from Boring HPT-1 and Correlation to Continuous Soil Core SC-1.

5.2.1.2 Continuous Soil Core Collection

Continuous direct-push soil coring was performed at one location (SC-1) to a depth of 45 ft within the site characterization area on August 29, 2018. The soil boring was advanced 15 ft from boring location HPT-1 (**Figure 5.1**) at the start of direct-push activities, followed by the advancement of the HPT-GW Sampler at boring location HPT-1. Data collected from visual inspection of the soil core was used to assess the lithology and to identify discrete lower permeability zones, particularly the depth and thickness of the A Silt layer. This allowed APTIM's on-site geologist to correlate the remaining HPT and EC probe readings to the lithology observed in the continuous soil core. Screening of the soil cores using a photoionization detector did not identify any potential zones with the presence of higher volatile organic compound (VOC) concentrations.

The HPT and EC logs, along with the lithologic log from soil cores SC-1 (**Appendix B**), were used to create the generalized geologic cross section presented in **Figure 5.4**. The cross section shows three distinct lithologic units within the upper layer approximately 45 ft bgs.

- **Layer 1: Very Fine silty Sand**: This unit exhibited estimated K values between ~ 20-50 ft/day, as discussed above and shown in **Figure 5.3**. This layer extends from the ground surface, to ~35 ft bgs with the bottom 10 ft being saturated.
- **Layer 2: Fine Sand**: While not being appreciably visually distinct from Layer 1, this layer exhibited less silt and slightly higher estimated K values, ranging between ~ 70-90 ft/day. Because of the observed higher permeability, this layer was differentiated from Layer 1 during development of the conceptual site model. This layer extends from ~ 35-40 ft bgs and is fully saturated.
- **Layer 3: A Silt**. This layer consists primarily of silt with ~10 percent very fine sand and exhibits a relatively low conductivity (estimated K's <10 ft/day). While not fully penetrated during drilling, this layer is estimated to extend from ~ 40-45 ft bgs, based on existing boring logs and cross sections in this area of the site.

After logging and screening, sections of saturated soil core material were collected for use during laboratory treatability testing, as described in **Section 5.3**. Boring SC-1 was subsequently over-drilled with larger direct-push rods and used for the installation of PMW-1D (**Section 5.2.1.4**).

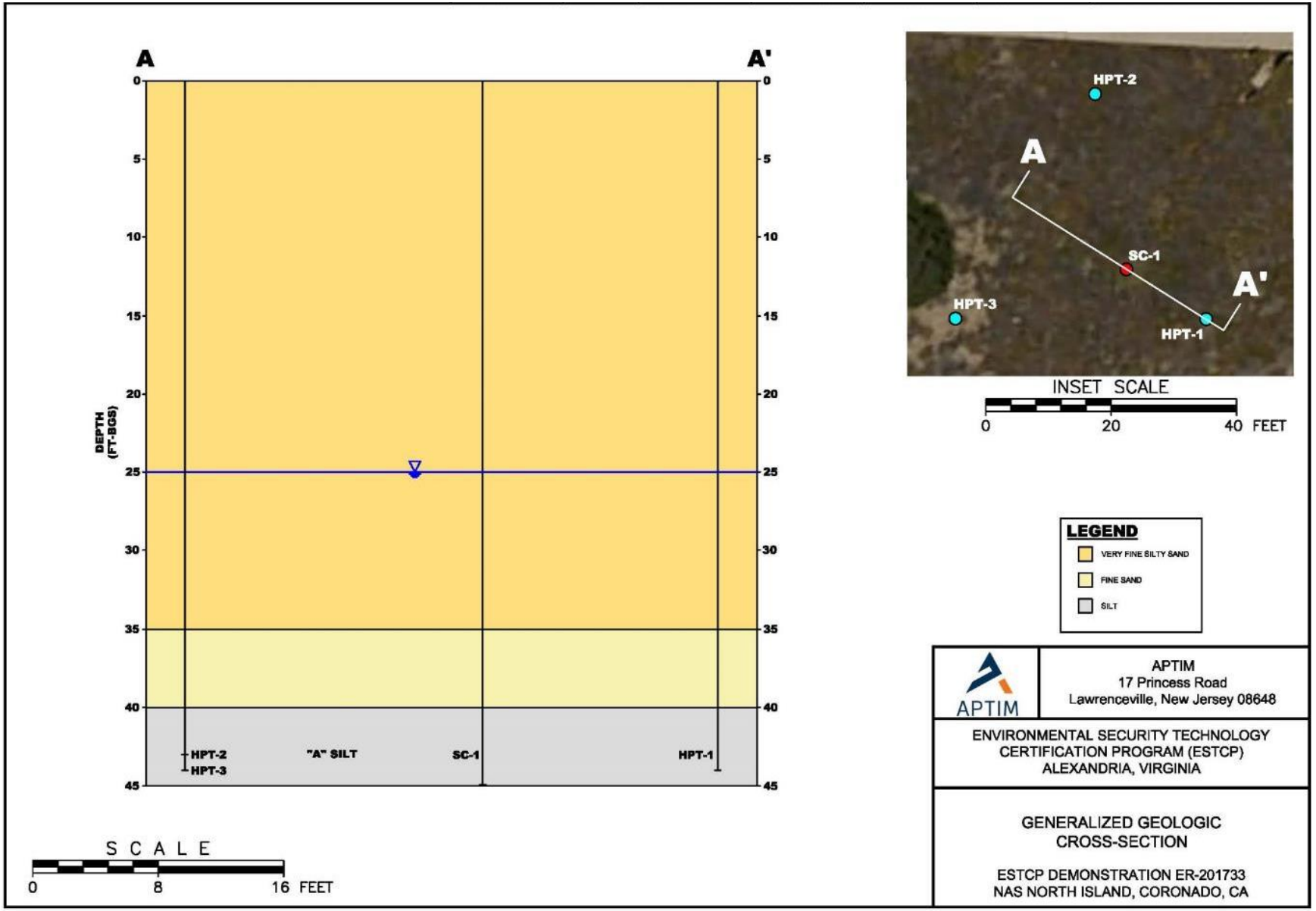


Figure 5.4. Generalized Geologic Cross Section.

5.2.1.3 Discrete Groundwater Sampling

During advancement of the HPT-GW Sampler, discrete groundwater samples were collected at three depths at each of the three HPT borings (**Figure 5.1)**. Sample collection at boring HPT-1 proved to be difficult with the downhole bladder pump that is used for groundwater depths that are beyond the limits of a peristaltic pump. Therefore, separate borings were advanced approximately three ft away from HPT borings HPT-2 and HPT-3, and a Geoprobe® Screen Point sampler and dedicated tubing with a stainless-steel check valve were used to collect discrete samples (typically 1.5 to 2.5 ft intervals) at three depths at each of these locations. The samples were generally evenly spaced across the saturated thickness of the shallow aquifer, with the deepest samples being collected just above the top of the A Silt layer at each location. The Screen Point Sampling and drill rods were decontaminated between samples.

The nine discrete groundwater samples collected from the three locations were analyzed for CVOCs (USEPA Method 8260B), 1,4-D (USEPA Method 8260 SIM GC/MS 5973), and dissolved gases (USEPA Method 3810 RSK-175) to assess the horizontal and vertical extent of COCs. The discrete sample interval was purged until extracted groundwater was clear to the extent possible. Samples of the purged groundwater were collected, and up to three sets of geochemical parameters [pH, DO, ORP, conductivity, temperature, and turbidity] were measured with a multi-parameter water quality meter (YSI 6820), prior to sample collection. One complete set of duplicate samples, one rinsate blank (collected from the decontaminated Screen Point Sampler), and trip blanks were also collected. Samples were placed on ice in a cooler and shipped overnight to APTIM's Analytical and Treatability Laboratory in Lawrenceville, New Jersey. The CVOC samples were analyzed on a 24-hour turnaround, thus facilitating a quick decision-making process to guide ongoing site characterization activities.

Table 5.1 presents a summary of detected CVOCs and 1,4-D concentrations in the nine discrete groundwater samples collected from the three HPT boring locations. As previously discussed, the primary CVOCs in OU-11 groundwater include TCE, *cis*-DCE, 1,1-DCE, and 1,1-DCA. Concentrations of VC, 1,2-dichloroethane (1,2-DCA) and/or PCE marginally above California and/or USEPA MCLs were also observed in two of the nine samples. The data indicate that concentrations generally increase with depth, and that the bulk of the COCs reside within the lower two-thirds of the aquifer ~30-40 ft bgs). Based on the data, treatment was focused on the bottom 10 ft of the 15 ft-thick overburden aquifer.

Table 5.1. Summary of Discrete Interval Groundwater Sampling Data

Boring ID	HPT-1			HPT-2			HPT-3		
Depth (ft, bgs)	29	34	39	27.5-29	32.5-34	38-39.5	25.5-28	32-33.5	38-39.5
Compound/Concentration	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
TCE	<5	155	1360	<5	323	851	7	210	2108
1,1-DCE	<5	135	1168	<5	311	667	15	339	2140
1,4-Dioxane	0.94J	69.8	427	234	107	240	50.2	415	1107
cis-1,2-DCE	<5	72	328	<5	100	89	30	293	613
1,1-DCA	<5	13	136	<5	34	58	<5	34	198
Vinyl Chloride	<5	<5	6	<5	<5	<5	<5	<5	15
Chloroform	<5	<5	5	2J	1J	3J	<5	2J	7
1,2-DCA	<5	<5	<5	<5	<5	<5	<5	1J	6
PCE	<5	<5	4J	<5	<5	<5	<5	<5	6
trans, 1,2-DCE	<5	<5	3J	<5	<5	<5	<5	<5	3J
1,1,2-TCA	<5	<5	<5	<5	<5	<5	<5	<5	1J

Note: Shaded concentrations exceed California NL (1,4-D) or MCL and/or EPA MCL.

5.2.1.4 Well Installations

One pump testing well (PTW-1) and four PMWs (PMW-1S, PMW-1D, PMW-2S and PMW-2D) were installed in the Demonstration Area, as shown on **Figures 5.1** and **5.5**. This group of wells were installed between September 4 and 7, 2018, and were used for aquifer pump testing as detailed in **Section 5.2.1.5**.

All five wells were installed using direct-push drilling techniques and 3.75-inch (in) outer diameter (OD), 3.0-in inner diameter (ID) direct-push rods. Drilling and well construction was performed by a licensed California driller and supervised by an APTIM geologist. Pump testing well PTW-1 was installed at the same boring location where HPT-1 was advanced (**Figure 5.1**) by over-drilling this boring with the larger direct-push tooling. PTW-1 is constructed of 2.0-in ID Schedule 40 polyvinyl chloride (PVC) casing, with 10 ft (two five-ft long sections) of pre-packed screen (2.8-in OD), as shown in **Figure 5.6**. As shown on **Figure 5.5**, this well was screened across the bottom two thirds of the aquifer, where discrete groundwater sampling data indicated most of the COCs reside. This well was used as an EW during pump testing and was also used as a monitoring well during the field demonstration.

The four PMWs were constructed of 1.5-in ID Schedule 40 PVC casing, with five-ft long pre-packed screens (2.8-in OD). As shown on **Figures 5.1** and **5.5**, the two shallow/deep PMW well pairs were installed approximately 15 and 30 ft downgradient of the PTW. The discrete screened intervals of these well pairs allow for monitoring of two discrete vertical groundwater zones at each location and were used to evaluate horizontal and vertical hydraulic conductivities during pump testing. They also facilitated the assessment of potential vertical hydraulic gradients. As shown in **Figure 5.5**, the wells span the bottom two thirds of the aquifer, with approximately one ft of vertical separation between screen intervals at each shallow/deep PMW well pairs.

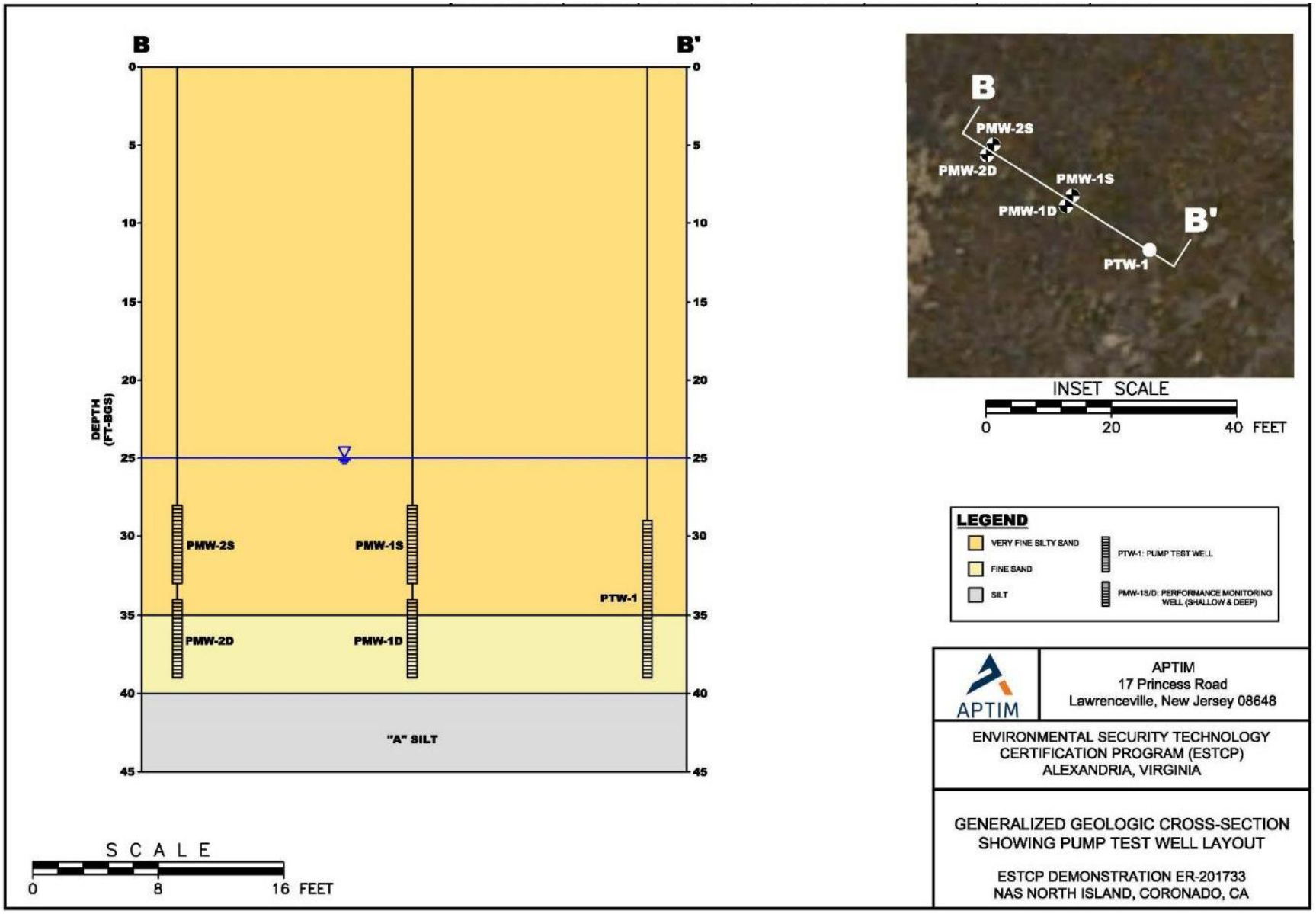


Figure 5.5. Generalized Geologic Cross Section Showing Pump Test Well Layout.

Figure 5.6. Installation of Well PTW-1 Prepacked Screens.

All five well borings were advanced to the pre-determined depths using 3.75-in OD direct-push rods with an expendable point. Once the target depth was reached, the well was assembled and lowered through the rods (**Figure 5.6**). Once the wells were installed to their target depths, the rod string was slowly retracted, allowing native formation material to collapse around the pre-packed screen, until the leading end of the rods was at the top of the well screen. If required, additional filter sand (20/40 mesh silica sand) was added to two ft above the top of the screen, as the rod string was being retracted. A three-ft seal consisting of granular bentonite was added from the top of the filter pack at wells PTW-1, PMW-1S and PMW-2S in the same manner. An additional four to five ft of bentonite seal (in lieu of cement-bentonite grout) was installed at wells PMW-1D and PMW-2D to minimize the potential of grout impacting the neighboring shallow well screens. A cement-bentonite grout was installed via tremie pipe into the remaining annular space to within one ft of the ground surface.

Each well was completed with an eight-in steel flush-mount well vault set in an 18-in square concrete pad (**Figure 5.7**), and a locking well cap was installed on each well. Upon completion,

the wells were fully developed to remove fine grained sediments from the well and sand pack. Initial development consisted of pumping until little or no sediment entered the well based on visual observation of water removed from the well. Following initial development, the wells were surged and continuously pumped using a submersible pump. The well was developed until the column of water was free of visible sediment. All five wells were used for performance monitoring during the demonstration. A summary of as-built well construction details for these wells is provided in **Table 5.2**, and well installation logs are provided in **Appendix B**.

Figure 5.7. Photograph of Pump Testing Well Plot Looking Downgradient.

Table 5.2. As-Built Site Characterization Well Construction Details.

Location ID	Approximate Distance from Pump Test Well (feet)	Well Inner Diameter (inches)	Borehole Diameter (inches)	Screen Interval (ft bgs)	Screen Length[1] (feet)	Filter Pack Interval[2] (feet bgs)	Filter Pack Length (feet)	Bentonite Seal Interval[3] (feet bgs)	Bentonite Seal Length (feet)	Grout Interval[4] (feet bgs)	Bentonite Grout Length (feet)
Pump Test Well											
PTW-1	NA	2	3.75	29.0 - 39.0	10.0	26.0 - 39.5	13.5	23.0 - 26.0	3.0	3.0 - 23.0	20.0
Performance Monitoring Wells											
PMW-1S	15	1.5	3.75	28.3 - 33.3	5.0	25.9 - 34.0	8.1	22.9 - 25.9	3.0	3.0 - 22.9	19.9
PMW-1D	15	1.5	3.75	34.0 - 39.0	5.0	31.8 - 39.5	7.7	28.6 - 31.8	3.2	3.0 - 28.6	25.6
PMW-2S	30	1.5	3.75	27.7 - 32.7	5.0	25.5 - 33.1	7.6	22.5 - 25.5	3.0	3.0 - 22.5	19.5
PMW-2D	30	1.5	3.75	33.8 - 38.8	5.0	29.0 - 39.2	10.2	26.0 - 29.0	3.0	3.0 - 26.0	23.0

Notes:

[1] Pre-packed screen wells for pump testing well and performance monitoring wells.

[2] Filter pack interval includes pre-packed screen and 20/40 mesh clean silica sand above the screen.

[3] Bentonite seal consists of 1/4" coated bentonite tablets for deep performance monitoring wells, and granular bentonite for all other wells.

[4] Grout composed of cement bentonite grout.

bgs = below ground surface; NA = not applicable

5.2.1.5 Aquifer Pump Testing

A one-day stepped rate aquifer pump test was performed at PTW-1 on September 19, 2018. Pump testing was performed to evaluate horizontal and vertical hydraulic conductivities, estimate aquifer transmissivity and storativity, and evaluate pumping well radius of influence. Data collected during testing was used to determine extraction and IW spacing and pumping rates for the groundwater recirculation system. During this test, water levels in the PTW, the four newly installed PMWs (PMW-1S, PMW-1D, PMW-2S and PMW-2D), and existing well OU11-SMW08A were monitored. Data loggers were installed in each of these wells to rapidly record groundwater elevation data during the testing. Manual water level measurements were also collected periodically from each of the wells, as well as OU11-SMW08B (this well is screened within a deeper aquifer).

Based on available hydrogeologic data, and drawdown data collected during the development of well PTW-1, the pumping rate selected for the first step was 1.0 gallons per minute (GPM). Subsequent pumping rates were 1.5, 2.0 and 3.0 GPM. Each step lasted between 40 and 210 minutes (depending on observed drawdown at the pumping and monitoring wells). The testing was performed by extracting groundwater from PTW-1 at each of the pumping rates using a submersible pump. The corresponding water level drawdown in nearby monitoring wells was measured as a function of time. After the final pumping step was performed, groundwater extraction was terminated and the recovery of water levels in the PTW and PMWs (PMW-1S, PMW-1D, PMW-2S and PMW-2D) was recorded by the data loggers overnight. Pumping and recovery data was analyzed using pumping test analysis software (ATESOLV Pro) to estimate the hydraulic properties of the aquifer. Estimated hydraulic conductivities at the five monitoring wells ranged between 94 ft/day and 123 ft/day. Results of the pump testing analysis are provided in **Appendix C**.

A total of approximately 700 gallons of groundwater was extracted during pump testing. The wastewater generated was pumped temporarily into 55-gallon drums and transported to the on-site waste treatment facility for processing.

5.2.1.6 Oxygen Sparge Testing

As discussed in **Section 5.1**, this field demonstration is unique in that it utilized a hybrid approach encompassing both biosparging and groundwater recirculation to deliver and distribute amendments within the aquifer. The addition of oxygen to the aquifer was accomplished through direct sparging of pure oxygen through dedicated biosparging wells, and not by means of the groundwater recirculation system. The primary reason for not adding oxygen via the groundwater recirculation system is that the estimated mass of oxygen required to convert the anoxic aquifer at the site into an aerobic aquifer, and maintain aerobic conditions during treatment, is greater than what can be added to the recirculated groundwater.

While existing sparging data (operating pressures, flows, and general air distribution) from a pilot-scale air sparging test in the OU-11 source area was available from previous work onsite (OHM, 1998), it was determined that an oxygen sparging test within the Demonstration Area was warranted to confirm that the treatment zone is spargeable, and to collect necessary data for design of the oxygen sparging design parameters (e.g., breakout and operating pressures, and area of influence for well spacing).

A short-term oxygen sparging test was performed on December 6, 2019, using the existing pump testing well network (**Figures 5.1** and **5.5**). A single compressed oxygen cylinder was mobilized to the site and fitted with an appropriate two-stage regulator and gas tubing made of compatible materials. A threaded PVC fitting was glued onto well PMW-1D, and a temporary sparging well head was threaded onto the fitting prior to testing. The well head included a pressure gauge and manual pressure relief valve.

Oxygen flow rates were measured using a gas-specific mass flow meter and controlled using a needle valve. The sparge tests involved sparging at continuous rate of approximately 5.5 standard cubic ft per minute (SCFM) for approximately 50 minutes. Wells PMW-1S, PMW-2S and PMW-2D and PTW-1 were monitored in the field for DO and groundwater elevation prior to (baseline) and during oxygen sparging to determine the horizontal and vertical influence of the oxygen sparging. DO concentrations were measured using a down-hole DO probe, and groundwater elevations were measured manually at all wells during the test.

While primarily designed to determine oxygen sparging breakout and operating pressures at a well installed within the deeper portion of the aquifer (PMW-1D), the DO and groundwater elevation data collected during this short test were informative as to both the vertical and horizontal area of influence that oxygen sparging exerted on the aquifer. As shown in **Figure 5.8**, an increase in DO concentrations were observed in wells PMW-1S and PMW-2S during and/or after oxygen sparging. These wells are both screened slightly above the sparging well (in the upper half of the treatment zone) and are located the ft and 15 ft away, respectively (**Figures 5.1** and **5.5**). It should be noted that baseline DO concentrations are thought to be artificially high due to shorter equilibration times during measurements.

Depth to groundwater data collected during sparge testing are presented in **Figure 5.9**. The data shows that mounding of groundwater occurred at all four observation wells and ranged between 0.65 ft (PMW-2S) and 1.62 ft (PMW-1S) during sparging. The measured increase in water table elevations at the three wells located 15 ft away from the sparging well ranged from 0.65 (PMW-2S) ft to 0.68 ft (PTW-1) during sparging. While these three wells are all located 15 ft away from the sparging well (**Figure 5.1**), they are screened at different intervals within the aquifer (treatment zone) and have varying screen lengths (five and 10 ft, as shown on **Figure 5.5**). Thus, the observation that the mounding measured at each of these wells was essentially the same during sparging suggests that the aquifer is relatively homogenous. Furthermore, groundwater elevation quickly returned to baseline conditions after oxygen sparging was stopped, suggesting that no appreciable oxygen gas pocketing occurred during the test.

Observed oxygen flow breakout and operational pressures were between approximately seven and eight psi, which is substantially below the estimated overburden pressure (26 psi), and optimal for the design of the oxygen biosparging system. The data collected during oxygen sparge testing indicate that oxygen can be successfully distributed throughout the 10-ft thick treatment interval by sparging at wells screened near the bottom of the aquifer. Furthermore, the sparge testing results indicate that an area of influence of approximately 15 ft can be obtained during sparging at a rate of 5.5 SCFM. This data was used to design of the biosparging system, as detailed in **Section 5.4**.

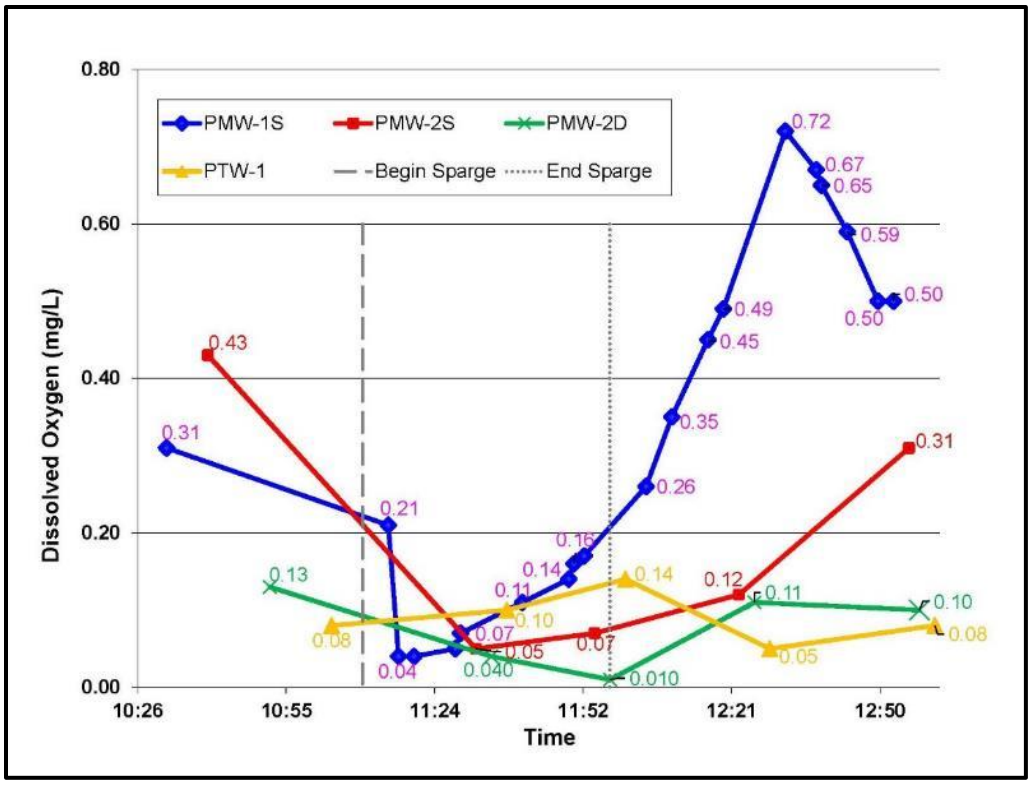


Figure 5.8. Dissolved Oxygen Concentrations During Sparge Testing.

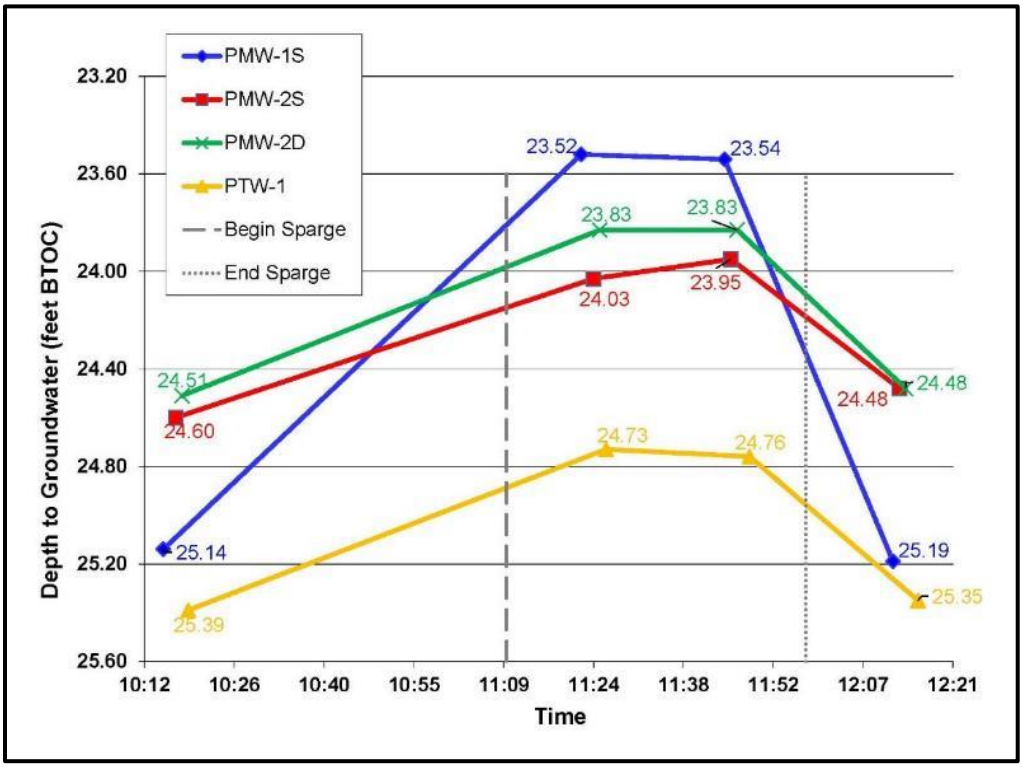


Figure 5.9. Depth to Water Measurements During Sparge Testing.

5.2.1.7 Groundwater Modeling

Data collected from site characterization activities and the analysis of pump testing data were used to construct a numerical three-dimensional groundwater model (MODFLOW) of the Demonstration Area. The model was used to simulate groundwater recirculation (extraction and reinjection) using different well configurations and pumping rates. Results of the simulations indicate that the extraction of groundwater from a central EW, and reinjection into five surrounding IWs (located approximately 15 ft from the EW and equidistant from each other) would create a groundwater treatment cell that would largely isolate the treatment zone from the surrounding aquifer (**Figure 5.10**). While the model showed that a small portion of groundwater from outside the treatment cell will be captured by the EW, most of the groundwater reaching the EW will originate from within the treatment cell and undergo recirculation.

Based on the groundwater modeling results, and the results of aquifer pump testing activities (**Section 5.2.1.5**), extraction rates during system operation are anticipated to be between 5 and 10 GPM at the EW, and re-injection of groundwater will be evenly distributed to each of the five IWs. Details of the groundwater model setup and modeling results are provided in **Appendix D**.

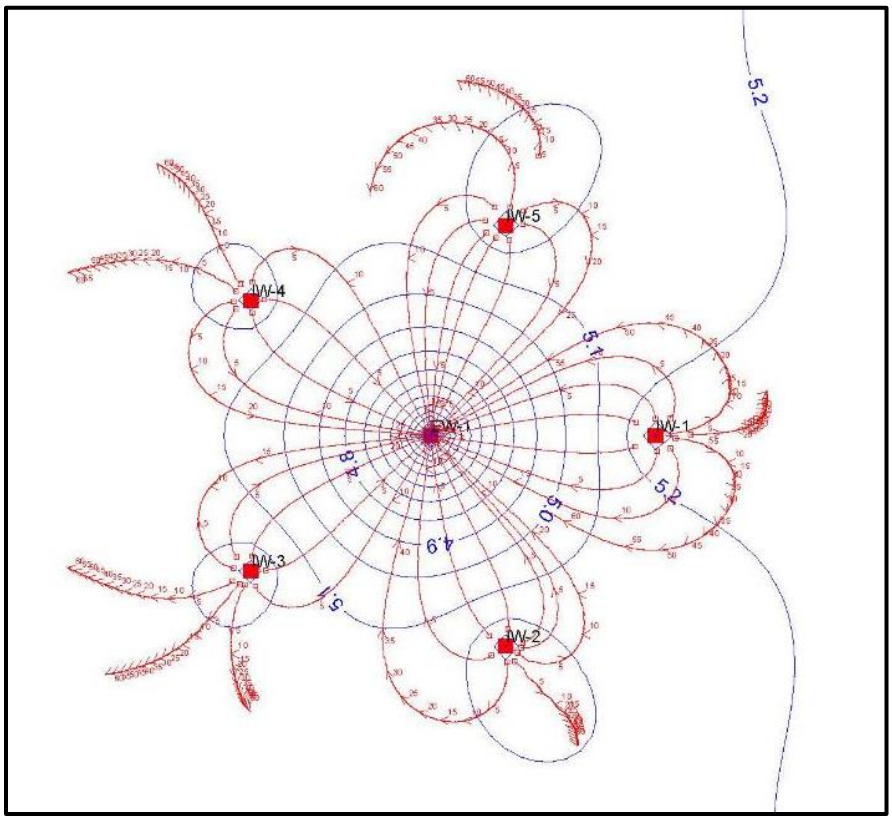


Figure 5.10. Groundwater Model Simulation of the Groundwater Recirculation Cell.
Particle tracking shows the estimated flow path and travel times of reinjected groundwater during recirculation at 7 GPM for 8 hours per day over 60 days.

5.3 LABORATORY TREATABILITY STUDY RESULTS

Laboratory treatability testing, performed at OSU located in Corvallis, OR and APTIM's BDAG laboratory in Lawrenceville, NJ consisted of microcosm testing with site materials and studies with pure and mixed cultures derived from those samples. A Laboratory Treatability Study Plan was submitted to ESTCP for review on February 9, 2018, and was approved on March 5, 2018. The relevant results of the testing are briefly summarized herein, with complete details provided in the approved Treatability Study Report located in **Appendix E.**

Microcosm studies were conducted to determine the most effective strategy to treat the different COCs in groundwater at OU-11, including relevant CVOCs (TCE, 1,1-DCE, *cis*-DCE), and 1,4-D. Treatment of chloroform (CF), which is present at the site below relevant MCLs, was also evaluated. Isobutane was selected as a primary growth substrate to promote aerobic cometabolism of the COC mixture present, except for TCE. Isobutane utilizers have been observed to effectively biodegrade 1,1-DCE, *cis*-DCE and 1,4-D. Isobutene and methane were both evaluated as second growth substrates. Isobutene utilizers and methanotrophs can typically cometabolize TCE at much higher rates that isobutane utilizers and are also likely to transform some of the other CVOCs present in int site groundwater.

Microcosms were prepared at OSU with aquifer solids and groundwater obtained during OU-11 site characterization activities at the demonstration site. Each microcosm received 15 mL of mixed core material and 50 mL of OU11-PTW-1 groundwater with the COCs that were present. After microcosm construction, the aqueous concentrations of the COCs in the groundwater were: TCE (768 µg/L); 1,1-DCE (761 µg/L); *cis*-DCE (368 µg/L); 1,4-D (405 µg/L) and CF (5 µg/L). Native isobutane utilizers were not stimulated in the microcosms over an incubation period of more than three months. Isobutene degrading organisms also were not stimulated. Additional testing suggested that the presence and/or transformation of 1,1-DCE is inhibitory to the stimulation of native microbes that biodegrade isobutane or isobutene. This is likely due to the formation of a reactive epoxide resulting from the initial transformation of 1,1-DCE or other physiological changes as reported by Mahendra et al. (2013).

One of the two sets of microcosms that had received isobutane was subsequently bioaugmented with the isobutane-utilizing culture *Rhodococcus rhodochrous* strain ATCC 21198 (21198), while the second set was bioaugmented with an isobutane-utilizing strain (*Rhodococcus aetherivorans* ENV493) that was enriched from the NASNI groundwater at BDAG prior to the initiation of the microcosms. Once added to microcosms, both ENV493 and 21198 were able to rapidly transform 1,1-DCE as well as 1,4-D, *cis*-DCE, and CF (ENV493 data presented in **Figure 5.11** and **Figure 5.12**). Successive additions of isobutane were consumed by ENV493, indicating that the native bacterium could grow under the aquifer conditions in the test zone. The activity of the second organism, 21198, decreased over time, indicating that the conditions were potentially unfavorable to its long-term growth and activity.

Isobutane-utilizing bacteria, including ENV493 and 21198, typically do not biodegrade TCE at high rates, and TCE remained in the microcosms after all other relevant COCs were degraded. As a result, a set of microcosms was bioaugmented with a methanotrophic mixed culture (ENV494M), also isolated at BDAG from NASNI groundwater, in an attempt to treat the remaining TCE. TCE was successfully degraded by the bioaugmented methane-utilizing culture and successive

additions of methane were consumed over time, showing that this native culture survived well under site conditions (**Figure 5.13**).

The results of the microcosm studies as well as enrichment and pure culture tests indicate that all relevant COCs in the NASNI OU-11 area groundwater can be successfully treated to below project performance objective criteria using a novel strategy that involves addition of two cometabolic substrates and two different bioaugmentation cultures. A summary of the relevant results is provided in **Table 5.3**. The two cultures isolated from the site (ENV493 and ENV494M) were determined to be the best candidates for bioaugmentation based on their continued activity under site conditions. Therefore, it was determined that ENV493 and isobutane would be added to biodegrade 1,1-DCE, 1,4-D, *cis*-DCE and CF, while methane and ENV494M would be used to transform residual TCE during the field demonstration. The bioaugmentation cultures were grown in the required quantities for field application in APTIM's commercial fermentation facility (Lawrenceville, NJ).

Additional studies to optimize the addition of the growth substrates and cultures in the field were performed by APTIM. Experiments showed that all relevant COCs were degraded when the cultures were added in sequence (ENV493 then ENV494M) or together (**Figure 5.14**). Experiments also showed that ENV493 grows rapidly on isobutanol in the presence of 1,1-DCE, without inducing the short chain alkane monooxygenase (SCAM) required to biodegrade isobutane and relevant COCs. Therefore, the addition of isobutanol prior to and/or immediately after bioaugmentation with ENV493 during the field demonstration was anticipated to maximize the growth and distribution of the culture within the aquifer without formation of reactive 1,1-DCE epoxide. Thus, the laboratory studies provided a clear strategy towards achieving project objectives. Additionally, data collected during column studies performed by OSU (**Appendix G**) were used to inform operation of the system during the field demonstration.

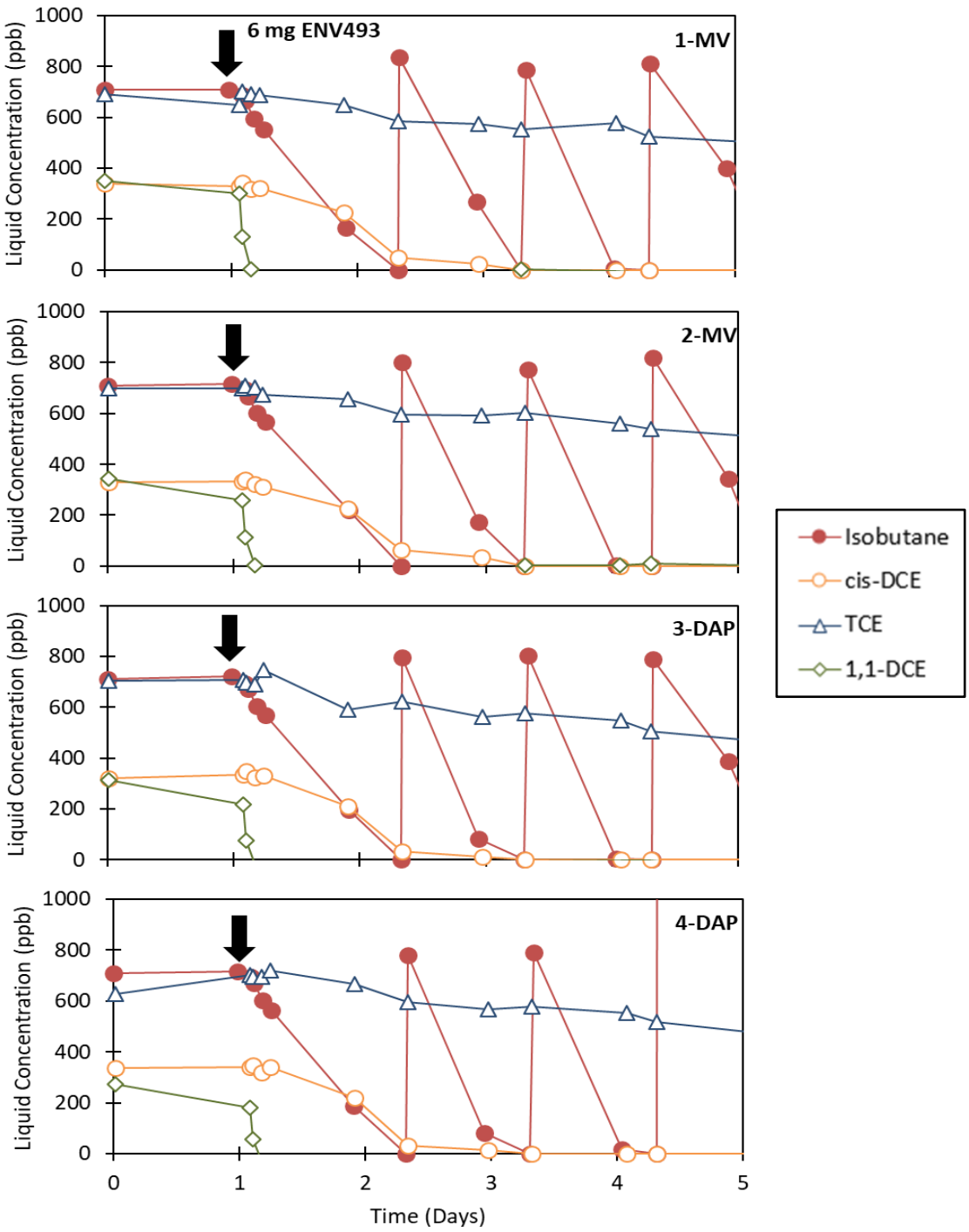


Figure 5.11. Chlorinated Ethene Transformation After Bioaugmentation with ENV493. Data from microcosm set 12 showed isobutane utilization. Microcosms 1 and 2 had MV for inorganic nutrients and Microcosms 3 and 4 had DAP as an inorganic nutrient source.

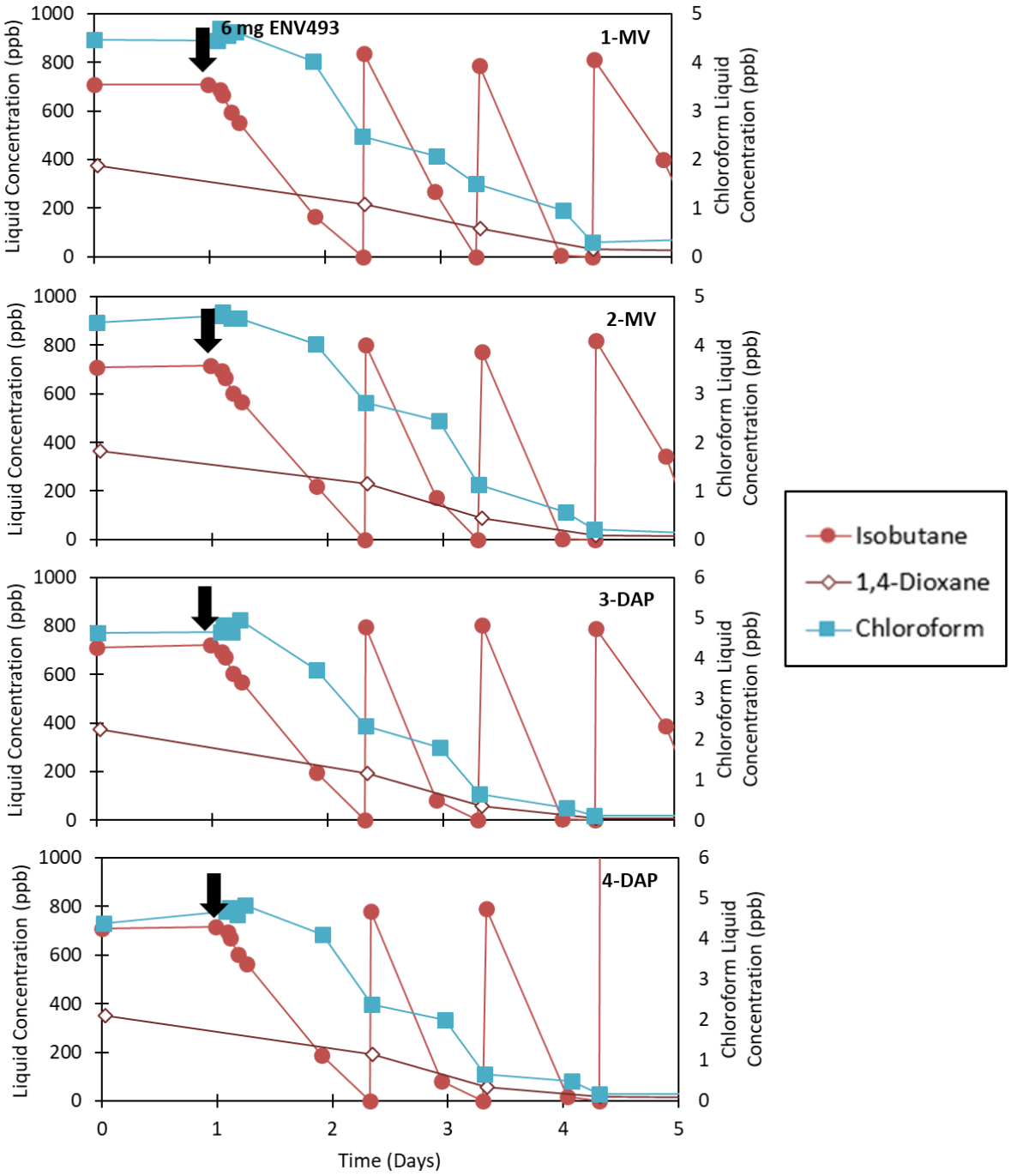


Figure 5.12. Chloroform and 1,4-D Transformation After Bioaugmentation with ENV493. Data from microcosm set 12 showed isobutane utilization. Microcosms 1 and 2 had MV for inorganic nutrients and Microcosms 3 and 4 had DAP as an inorganic nutrient source.

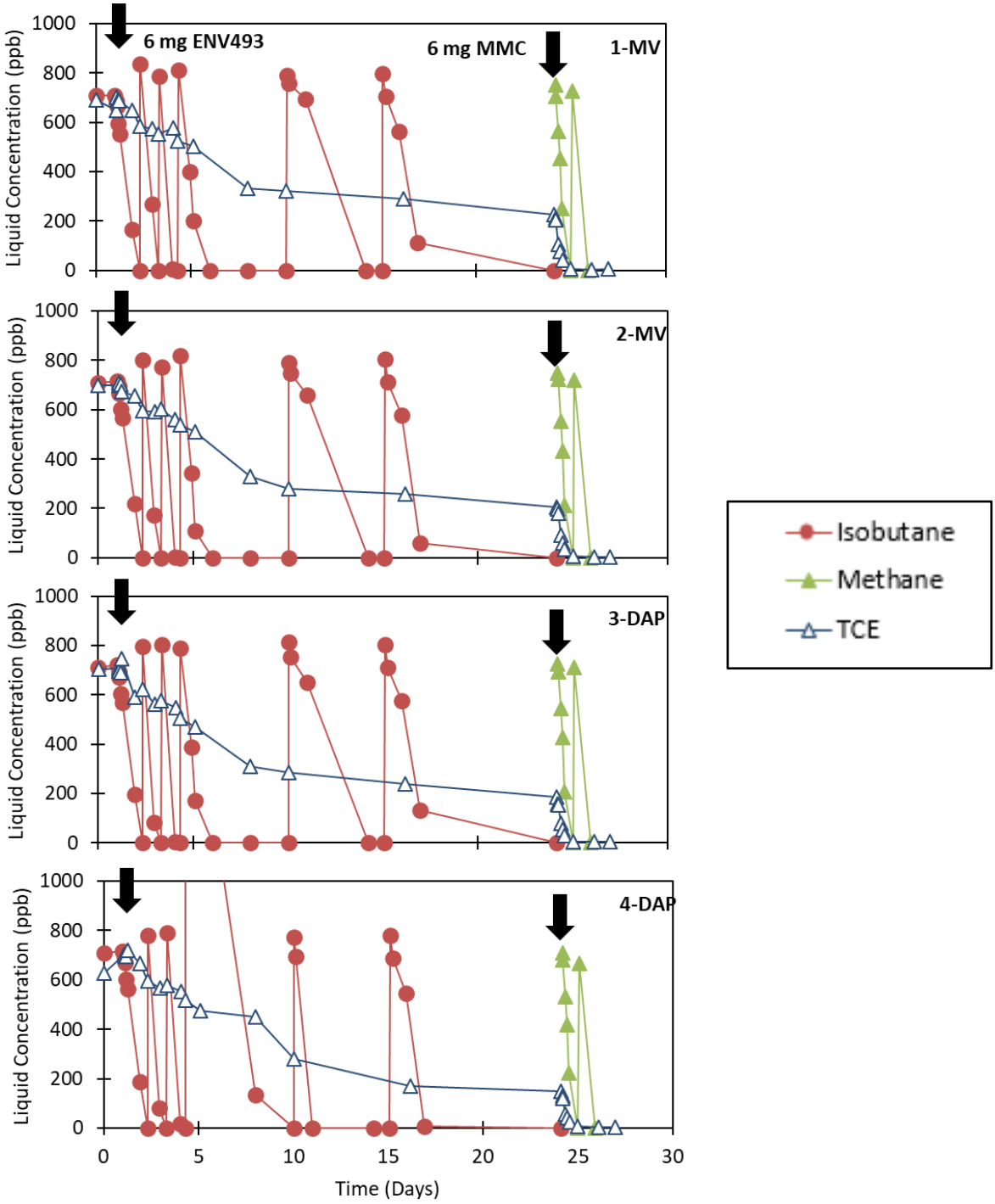


Figure 5.13. TCE Transformation After Bioaugmentation with ENV494M. Slow treatment of TCE was observed after bioaugmentation with ENV493 grown on isobutane. The remaining TCE was rapidly degraded after bioaugmentation with the mixed methanotroph culture ENV494M and addition of methane on day 25.

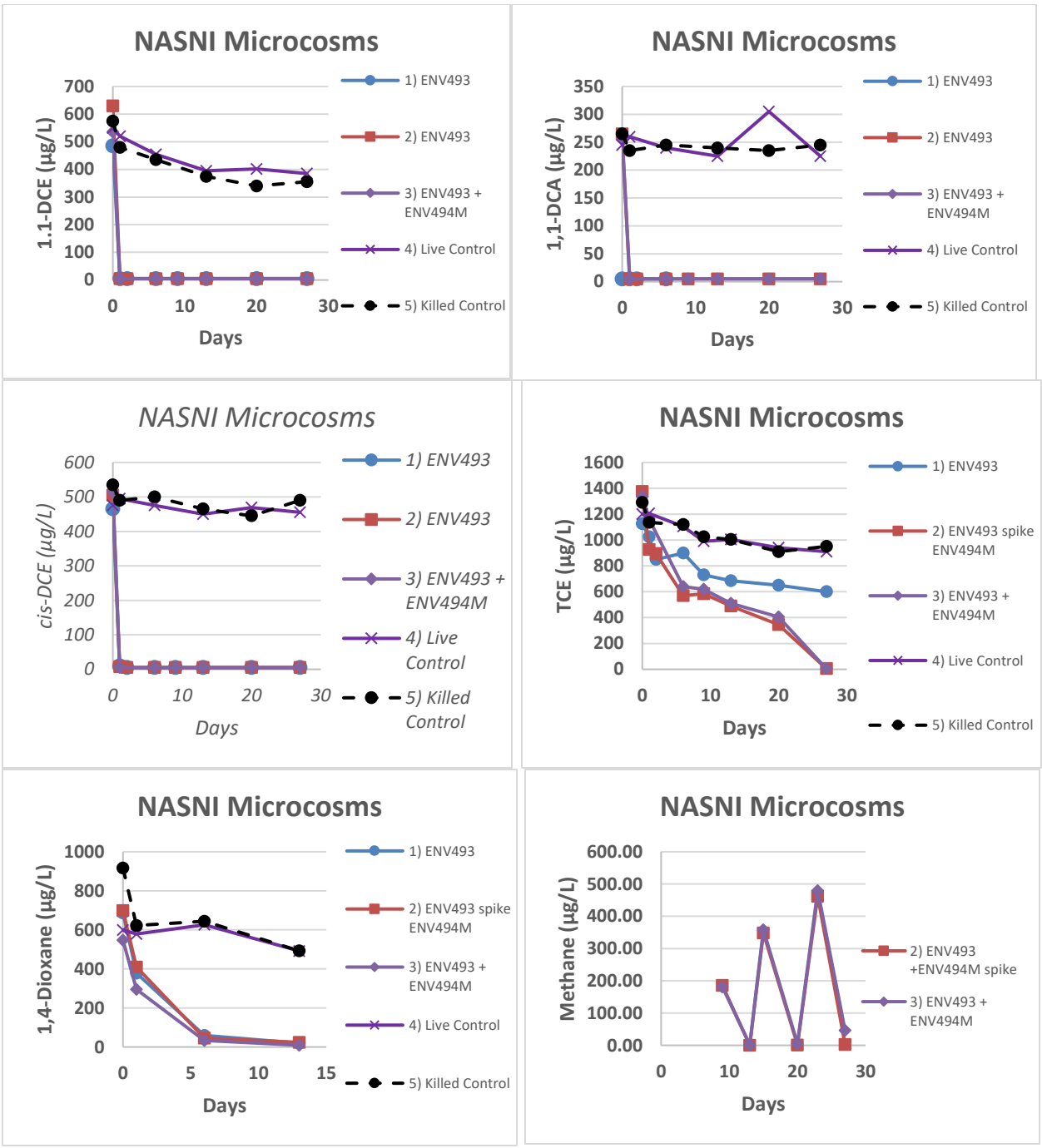


Figure 5.14. CVOC and 1,4-D degradation in NASNI groundwater only microcosms bioaugmented with ENV493 and ENV494M. The treatment labeled *ENV493 + ENV494M* received both cultures together while that labeled *ENV493 spike ENV494M* received the cultures in sequence. Methane degradation also was monitored.

Table 5.3. Qualitative Results of Microcosm Studies

Culture	Primary Substrate	Enriched from NASNI	Treats COC				
			1,1-DCE	cis-DCE	Chloroform	1,4-Dioxane	TCE
ENV493	Isobutane	Yes	Yes	Yes	Yes	Yes	No
ENV494M	Methane	Yes	Yes	Yes	NE	No	Yes
ELW1	Isobutene	No	Yes	Yes	NE	No	Partial degradation observed
21198	Isobutane	No	Yes	Yes	Yes	Yes	No

NE=Not evaluated in microcosms.

5.4 DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS

The following subsections detail the design, layout, and installation of the various demonstration components. All field activities associated with system installation were conducted using appropriate Level D PPE. APTIM submitted a Site Approval Request and a Laydown Area Request to NASNI for review and approval, along with other required work permits prior to starting work on site. Prior to beginning intrusive activities, a utility survey was conducted through the Base as part of the approval process for APTIM's Subsurface Operations Permit application along with the California 811 ticket that was updated throughout the project. In addition to contacting California 811, APTIM also contracted a third-party utility locater to perform a utility mark-out in the Demonstration Area. The utility clearance contractor cleared the proposed well locations and located underground utilities within the primary laydown/work area. Additionally, all borehole locations were cleared to five ft bgs using a hand auger or other soft-dig technologies prior to drilling.

Based on previous *in situ* treatments performed at NASNI, the reinjection of groundwater that includes amendments to promote *in situ* treatment is allowed under section 3020(b) of RCRA with the condition that treatment is intended to substantially reduce hazardous constituents in groundwater (either before or after reinjection). Therefore, a discharge to groundwater permit was not required for the demonstration.

5.4.1 Demonstration Well Design and Layout

The designed treatment zone for this demonstration was generally cylindrical in shape, approximately 35 ft in diameter and 12 ft in height, as shown in **Figures 5.15** and **5.16**. The demonstration well layout is provided in **Figure 5.15** and included a total of 22 wells. The groundwater recirculation well network consisted of six wells: one groundwater EW and five groundwater IWs. The EW (EW-1) was in the center of the treatment zone, with the five groundwater IWs (IW-1 through IW-5) positioned radially 15 ft out from the EW. The oxygen biosparging well network consisted of five oxygen biosparging wells, also positioned radially 15 ft out from the EW (equidistant between the groundwater IWs). The monitoring well network consisted of a total of 11 monitoring wells located within, upgradient and downgradient of the treatment zone as follows:

- Three shallow/deep (S/D) pairs of PMW wells (PMW 1S/D, PMW-3S/D and PMW 4S/D) located within the treatment zone. Each of the well pairs was used to monitor two separate intervals within the treatment zone thickness (see cross-section C-C', **Figure 5.16**). Well pair PMW-1S/D was installed during site characterization activities as detailed in **Section 5.2.1.4**;

- S/D well pair MW-2S/D (installed during site characterization activities) is located slightly downgradient and towards the edge of the treatment zone (**Figure 5.15**). These wells were used to evaluate potential impacts of treatment across the target treatment zone interval near the outside edge of the treatment zone;

- Monitoring well PTW-1 (previously used as a pump test well, as discussed in **Section 5.2.1.5**) is located slightly upgradient of the treatment zone. This well had a 10 ft screen and was used to evaluate potential impacts of treatment near the upgradient edge of the target treatment zone;

- Monitoring well DMW-1 is located approximately 20 ft downgradient of the treatment zone. This well had a 10 ft screen and was used to monitor across the target treatment zone interval, as shown on cross-section C-C' on **Figure 5.16**. This well was located outside the influence of the groundwater recirculation and biosparging wells, and was used to evaluate downgradient impacts of treatment; and

- Monitoring well OU11-SMW08A (installed prior to this demonstration) is located approximately 30 ft upgradient of the treatment zone. This well had a five-ft screen located near the bottom of the target treatment interval (similar to the deep wells in the well pairs discussed above) and served as a control well.

All performance monitoring and biosparging wells installed during this demonstration were installed using direct-push technology drilling techniques. The EW and IWs were installed using hollow stem auger (HSA) drilling techniques. Installation of the 16 demonstration wells not installed prior to or during site characterization activities was completed between August 31 and September 22, 2020, as detailed in **Section 5.4.2** and **Section 5.4.3**. Drilling and well construction was performed by a California-licensed driller and supervised by an APTIM geologist, and as-built well construction details are provided in **Table 5.4,** and well installation logs are provided in **Appendix B**.

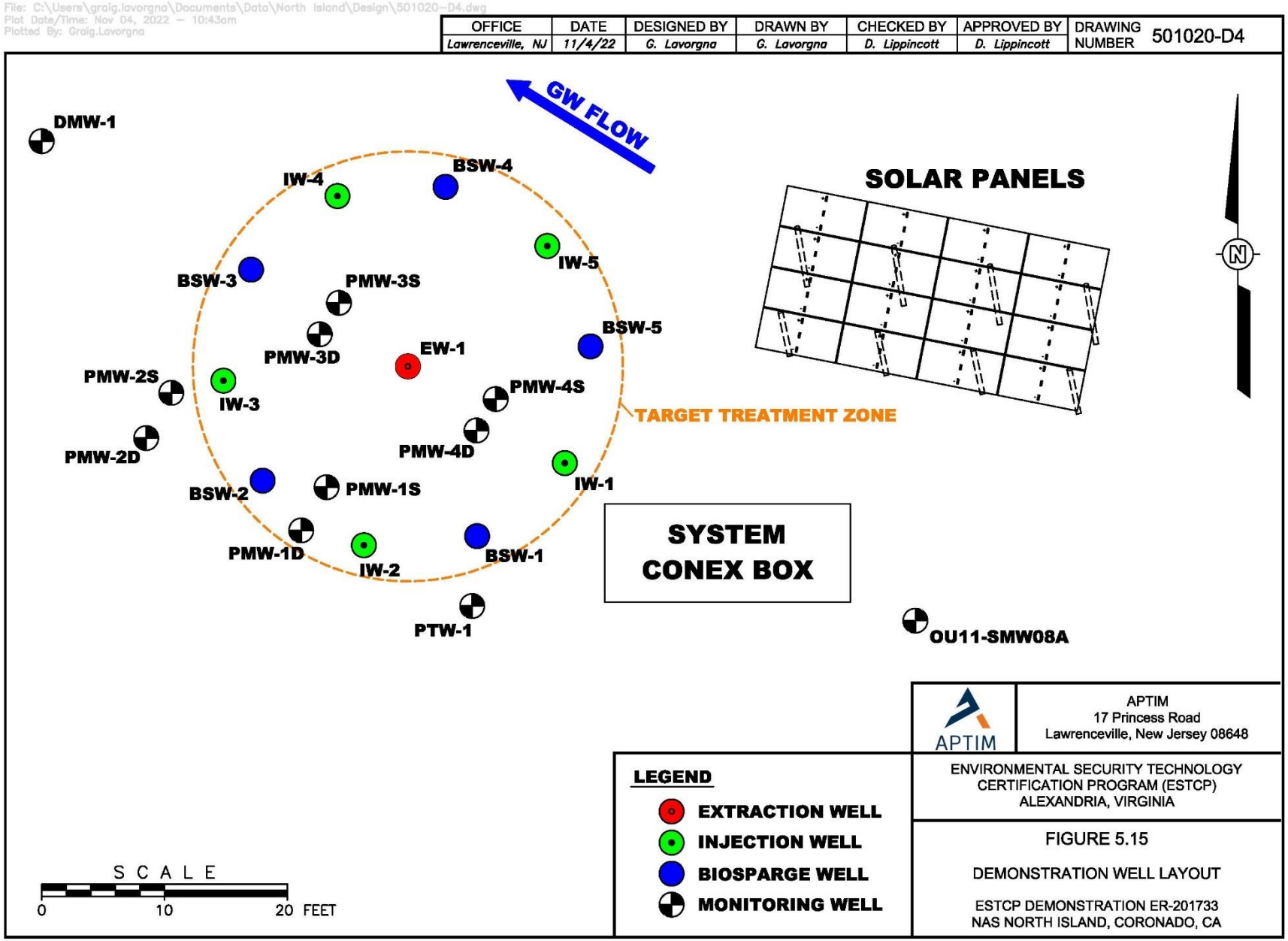


Figure 5.15. Demonstration Well Layout.

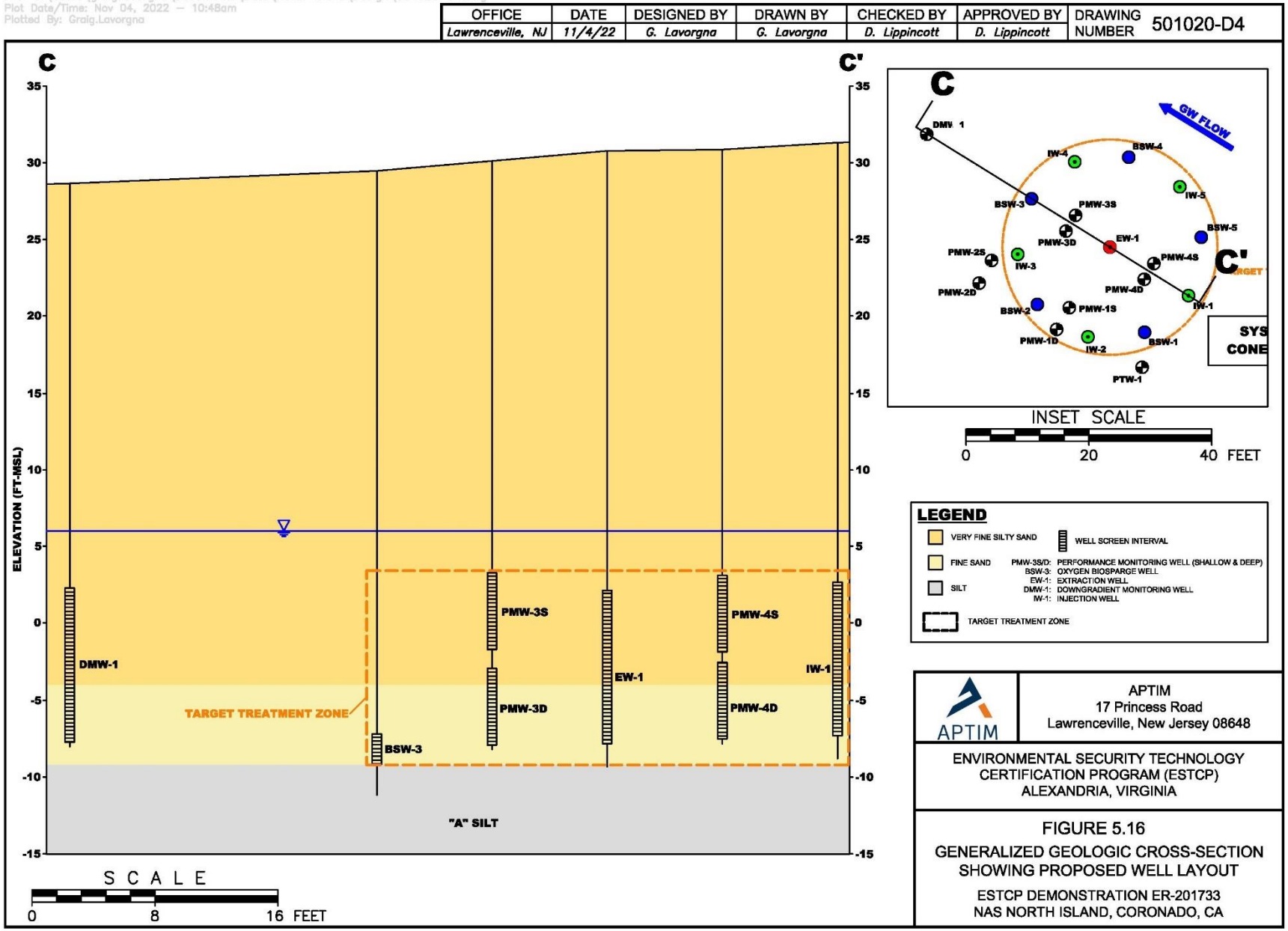


Figure 5.16. Generalized Geologic Cross Section Showing Demonstration Well Layout.

Table 5.4. Summary of As-Built Well Construction Details

Location ID	Installation Date	Approximate Distance from Extraction Well (feet)	Well Inner Diameter (inches)	Borehole Diameter (inches)	Sump Interval (ft bgs)	Sump Length (feet)	Screen Interval (ft bgs)	Screen Length[1] (feet)	Filter Pack Interval (feet bgs)	Filter Pack Length (feet)	Bentonite Seal Interval[2] (feet bgs)	Bentonite Seal Length (feet)	Grout Interval[3] (feet bgs)	Bentonite Grout Length (feet)
Extraction Well														
EW-1	9/20/2022	NA	4	10	38.6 - 40.1	1.5	28.6 - 38.6	10.0	25.6 - 40.1	14.5	22.6 - 25.6	3.0	3.0 - 22.6	19.6
Injection Wells														
IW-1	9/14/2020	15	4	10	39.1 - 40.1	1	29.1 - 39.1	10.0	26.1 - 40.1	14.0	23.1 - 26.1	3.0	3.0 - 23.1	20.1
IW-2	9/10/2020	15	4	10	38.9 - 40.4	1.5	28.9 - 38.9	10.0	25.9 - 40.4	14.5	22.9 - 25.9	3.0	3.0 - 22.9	19.9
IW-3	9/11/2020	15	4	10	38.2 - 39.4	1.2	28.2 - 38.2	10.0	25.2 - 39.4	14.2	22.2 - 25.2	3.0	3.0 - 22.2	19.2
IW-4	9/11/2020	15	4	10	36.9 - 38.0	1.1	26.9 - 36.9	10.0	23.9 - 38.0	14.1	20.9 - 23.9	3.0	3.0 - 20.9	17.9
IW-5	9/14/2020	15	4	10	37.2 - 38.2	1	27.2 - 37.2	10.0	24.2 - 38.2	14.0	21.2 - 24.2	3.0	3.0 - 21.2	18.2
Oxygen Biosparge Wells														
BSW-1	8/31/2020	15	1.5	3.75	40.0 - 41.55	1.55	38.0 - 40.0	2.0	36.4 - 41.55	5.2	25.0 - 36.4	11.4	3.0 - 25.0	22.0
BSW-2	8/31/2020	15	1.5	3.75	40.3 - 41.55	1.25	38.3 - 40.3	2.0	36.8 - 41.55	4.8	24.9 - 36.8	11.9	3.0 - 24.9	21.9
BSW-3	9/1/2020	15	1.5	3.75	38.7 - 40.0	1.3	36.7 - 38.7	2.0	35.0 - 40.0	5.0	24.8 - 35.0	10.2	3.0 - 24.8	21.8
BSW-4	9/1/2020	15	1.5	3.75	37.8 - 39.0	1.2	35.8 - 37.8	2.0	35.0 - 39.0	4.0	23.0 - 35.0	12.0	3.0 - 23.0	20.0
BSW-5	9/2/2020	15	1.5	3.75	39.25 - 40.55	1.3	37.3 - 39.3	2.0	36.3 - 40.6	4.3	24.3 - 36.3	12.0	3.0 - 24.3	21.3
Performance Monitoring Wells														
DMW-1	9/2/2020	55	1.5	3.75	36.4 - 36.7	0.3	26.4 - 36.4	10.0	23.4 - 36.7	13.3	20.4 - 23.4	3.0	3.0 - 20.4	17.4
PMW-1S	9/4/2018	10	1.5	3.75	NA	NA	28.3 - 33.3	5.0	25.9 - 34.0	8.1	22.9 - 25.9	3.0	3.0 - 22.9	19.9
PMW-1D	9/5/2018	10	1.5	3.75	NA	NA	34.0 - 39.0	5.0	31.8 - 39.5	7.7	28.6 - 31.8	3.2	3.0 - 28.6	25.6
PMW-2S	9/4/2018	25	1.5	3.75	NA	NA	27.7 - 32.7	5.0	25.5 - 33.1	7.6	22.5 - 25.5	3.0	3.0 - 22.5	19.5
PMW-2D	9/5/2018	25	1.5	3.75	NA	NA	33.8 - 38.8	5.0	29.0 - 39.2	10.2	26.0 - 29.0	3.0	3.0 - 26.0	23.0
PMW-3S	9/2/2020	5	1.5	3.75	31.8 - 32.1	0.3	26.8 - 31.8	5.0	23.8 - 31.8	8.0	20.8 - 23.8	3.0	3.0 - 20.8	17.8
PMW-3D	9/2/2020	5	1.5	3.75	38.0 - 38.3	0.3	33.0 - 38.0	5.0	30.0 - 38.3	8.3	27.0 - 30.0	3.0	3.0 - 27.0	24.0
PMW-4S	9/1/2020	10	1.5	3.75	32.7 - 33.0	0.3	27.7 - 32.7	5.0	24.4 - 32.7	8.3	21.3 - 24.4	3.1	3.0 - 21.3	18.3
PMW-4D	9/2/2020	10	1.5	3.75	38.4 - 38.7	0.3	33.4 - 38.4	5.0	30.4 - 38.4	8.0	27.4 - 30.4	3.0	3.0 - 27.4	24.4
PTW-1	9/5/2018	5	2	3.75	NA	NA	29.0 - 39.0	10.0	26.0 - 39.5	13.5	23.0 - 26.0	3.0	3.0 - 23.0	20.0
OU11-SMW08A	8/2/2011	40	2	8	NA	NA	36.0 - 41.0	5.0	34.0 - 42.0	8.0	31.0 - 34.0	3.0	3.0 - 31.0	28.0

Notes:

[1] Pre-packed screens for biosparge and performance monitoring wells. Continuously wrapped stainless steel screens for extraction and injection wells.

[2] Bentonite seal consists of granular bentonite for the shallow performance monitoring wells, and 1/4" coated bentonite tablets for all other wells.

[3] Grout composed of cement bentonite grout, installed to within 3' of the ground surface.

ft bgs = feet below ground surface; NA = not applicable

5.4.1.1 Biosparging Well Installation

Figure 5.15 presents the locations of the five vertical oxygen biosparging wells (BSW-1 through BSW-5), which were installed radially 15 ft out from EW-1. As represented by BSW-3 on **Figure 5.16**, these wells were installed near the bottom of the target treatment zone, with screen intervals just above the "A" Silt layer. A typical oxygen biosparging well construction diagram is presented as **Figure 5.17**.

Each biosparging well was constructed from one-in Schedule 40 PVC casing, with a two-ft long pre-packed screen. A one-ft sump (PVC casing) was installed below the well screens to collect potential sediment that may enter the wells during cycling of the biosparging wells. Borings for each well were advanced to their pre-determined depth using 3.75-in OD direct-push rods with an expendable point. Once the well was lowered to depth within the rods, the expendable point was dislodged, and the rod string slowly retracted, allowing the surrounding formation to collapse until the leading end of the rods was at the top of the well screen. Additional filter sand (20/40 mesh silica sand) was added to approximately one ft above the top of the screen as the rod string was being retracted, as required. A seal consisting of coated ¼-in bentonite tablets (Cetco® coated tablets) was installed from the top of the collapsed formation to the water table (approximately 25 ft-bgs) as the rod string was retracted further. The extended bentonite seal was used instead of bentonite-cement grout throughout portions of the annular space to eliminate the possibility of grout impacting neighboring well screens (located as close as five ft away). The coated ¼-in bentonite tablets are designed to fall through water columns without "bridging" and are small enough to be installed within the annular space of the borehole (providing a continuous annular seal). A cement-bentonite grout was then installed via tremie pipe into the remaining annular space to within three ft of the ground surface as the remaining rod string was removed. During grout placement, the bottom of the tremie pipe remained submerged in the grout column.

The wells were completed with 11-in by 18-in polymer composite flush-mount well vaults set in and surrounded by compacted surface soil. Upon completion, the wells were fully developed to remove fine grained sediments from the well and sand pack. Initial development consisted of pumping until little or no sediment entered the well based on visual observation of water removed from the well. Following initial development, the wells were surged and continuously pumped using a submersible pump. The well was developed until the column of water was free of visible sediment.

5.4.1.2 Performance Monitoring Well Installation

PMW pairs PMW-3S/D and PMW-4S/D and downgradient monitoring well DMW-1 were constructed using the same materials and installation techniques as well pairs PMW-1S/D and PMW-2S/D (**Section 5.2.1.4**). As discussed in **Section 5.4.1**, and shown on **Figures 5.15** and **5.16**, the PMWs were grouped in pairs and were constructed with five ft screens, which allowed the monitoring of two discrete vertical aquifer intervals at each location. The vertical and horizontal distribution of these wells (along with PMW-1S/D, PMW-2S/D and PTW-1) were designed for the assessment of gas distribution and groundwater quality and spanned the vertical extent of the plume identified during the site characterization direct-push investigation.

There was a minimum of one ft of vertical spacing between screen intervals at each well pair, and the bottom of the deepest well was installed approximately one ft above the bottom of the "A" Silt layer. All monitoring wells were completed with 8-in diameter steel flush-mount well vaults set in

concrete, and well development was accomplished as described in **Section 5.4.1.1**. Well construction details are provided in **Table 5.4**, and a typical monitoring well construction diagram is presented as **Figure 5.18**.

5.4.1.3 Groundwater Extraction and Injection Well Installation

Figure 5.15 presents the locations of groundwater EW (EW-1) and the five vertical groundwater IWs (IW-1 through IW-5), which were installed radially 15 ft out from EW-1. As represented by EW-1 and IW-1 on **Figure 5.16**, these wells were installed to approximately two ft above the "A" Silt layer, each with a 10-ft screen interval and a 1.0-1.5 ft sump. HSA drilling techniques were used to install these wells. Each well was constructed with four-in Schedule 40 PVC casing and a 10-ft long stainless-steel .040-in slotted wire wrap screen.

Borings for each well were advanced to their pre-determined depth using 10.25-in OD (6.25-in ID) hollow stem augers. Once at the desired depth, the wells were assembled and installed through the augers, and the auger string slowly retracted while adding filter sand (20/40 mesh silica sand) to two ft above the top of the screen. A seal consisting of coated ¼-in bentonite tablets (Cetco® coated tablets) was installed from the top of the sand filter pack to approximately 22 ft-bgs, as the auger string was retracted further. A cement-bentonite grout was then installed via tremie pipe into the remaining annular space to within three ft of the ground surface as the remaining auger string was removed. During grout placement, the bottom of the tremie pipe remained submerged in the grout column.

The wells were completed with 11-in by 18-in polymer composite flush-mount well vaults set in and surrounded by compacted surface soil, and well development was accomplished as described in **Section 5.4.1.1**. Well construction details are provided in **Table 5.4**, and a typical groundwater EW/IW construction diagram is presented as **Figure 5.19**.

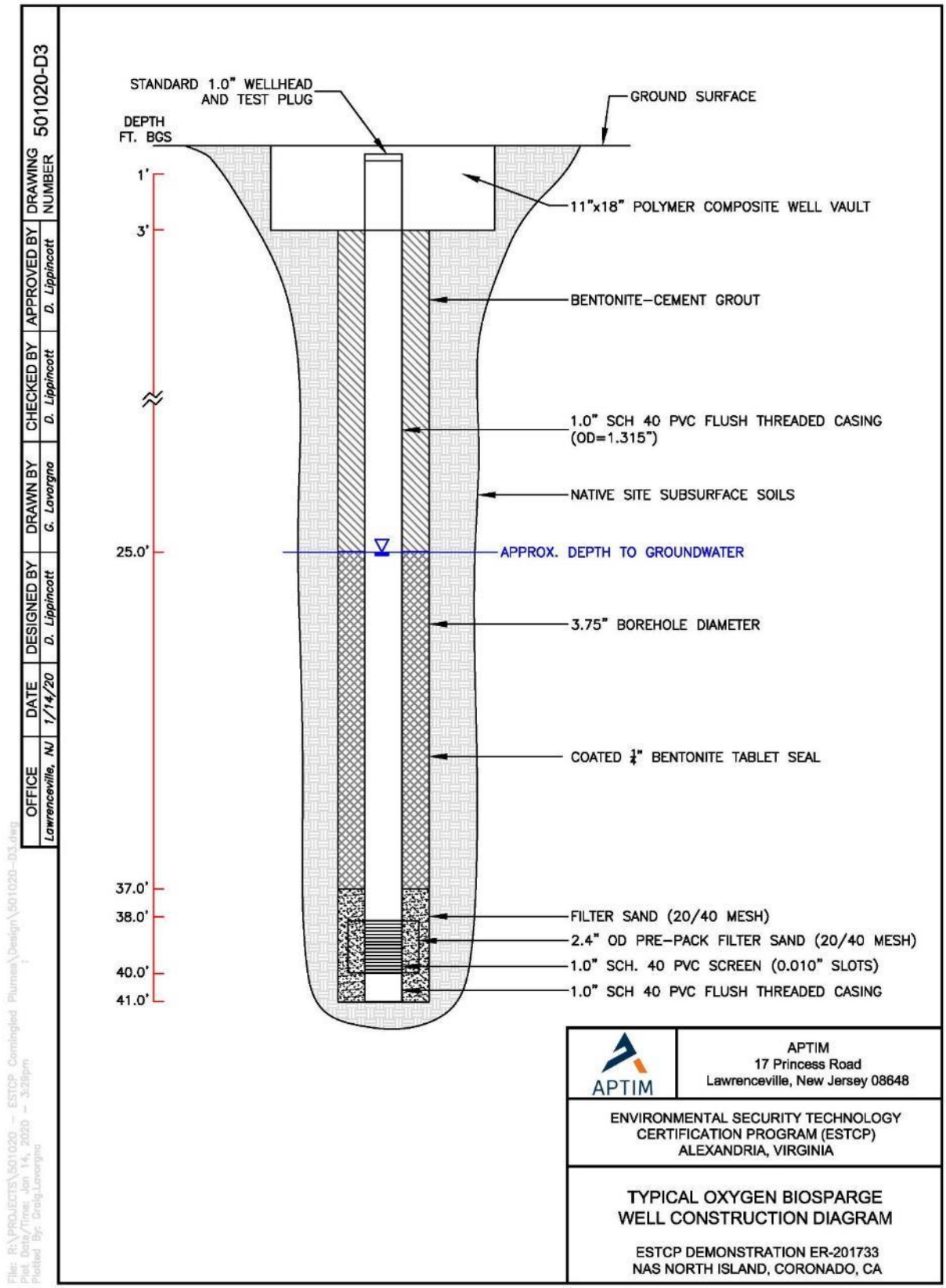


Figure 5.17. Typical Biosparging Well Construction Diagram.

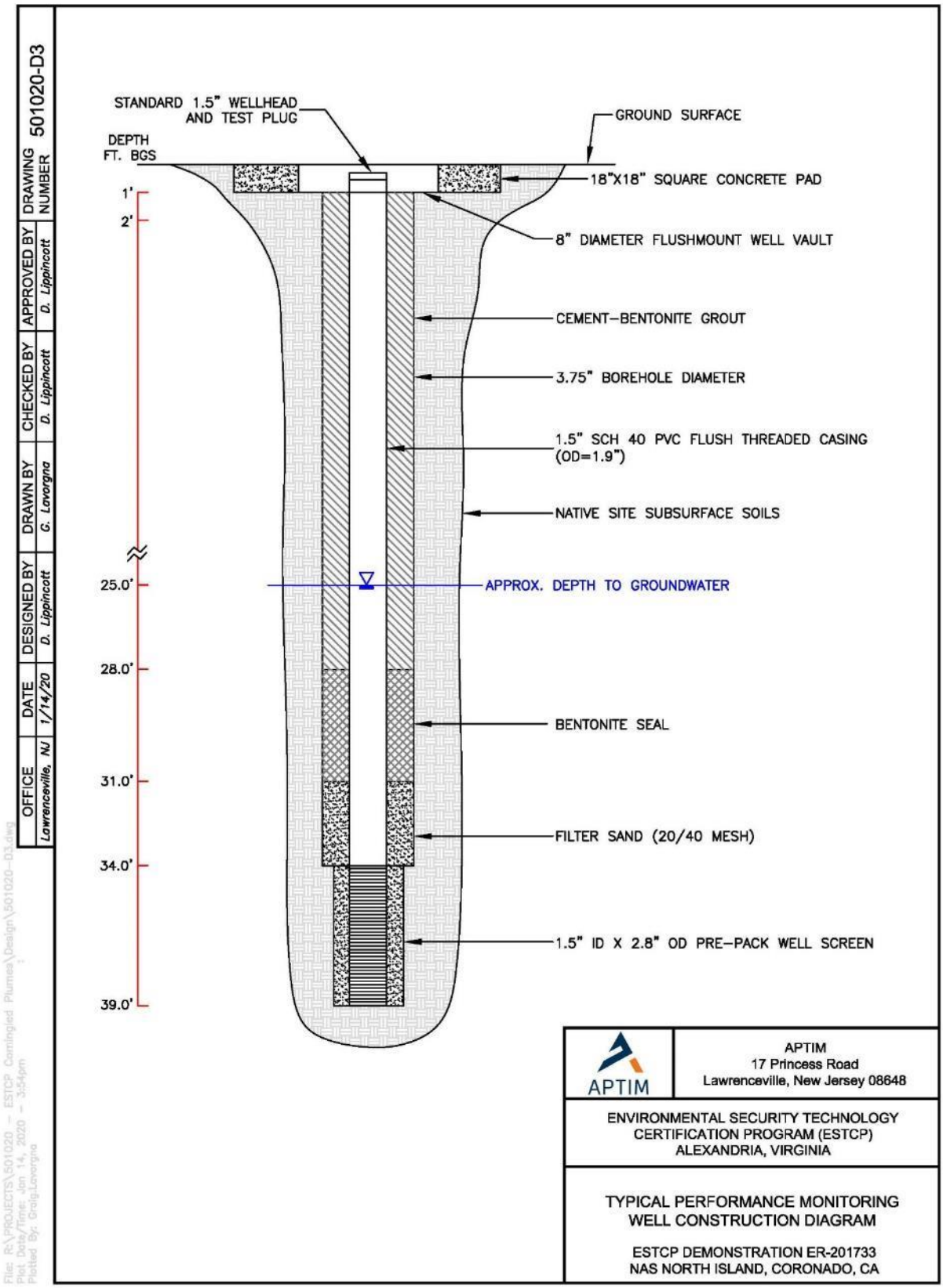


Figure 5.18. Typical Performance Monitoring Well Construction Diagram.

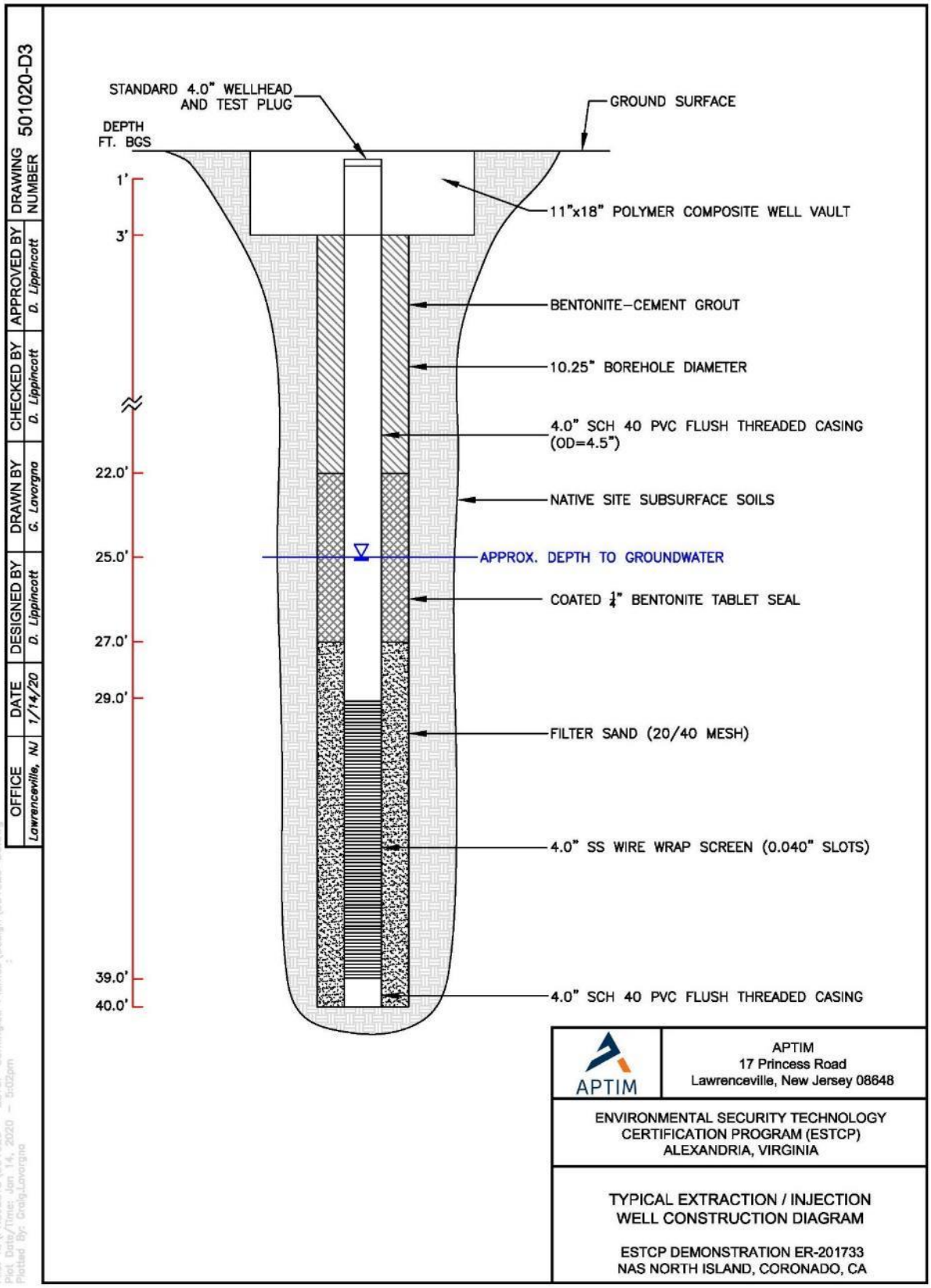


Figure 5.19. Typical Groundwater Extraction/Injection Well Construction Diagram.

5.4.2 Cometabolic Bioremediation System Design and Installation

The hybrid cometabolic bioremediation system consisted of a groundwater recirculation system, a gas amendment delivery system, a liquid amendment delivery system, a biofouling mitigation system, and an oxygen biosparging system. These systems were designed to operate completely "off-the-grid" using solar energy and pressure from gas cylinders to supply necessary amendments. APTIM made modifications to an existing off-the-grid cometabolic biosparging system that completed operation at the former Myrtle Beach Air Force Base as part of ESTCP project ER-201629. These modifications included the addition of a groundwater recirculation system, liquid amendment injection systems, biofouling mitigation system, and changes to the existing gas delivery system. The modified system (as detailed below) was then transported in December 2020 to Calcon Systems Inc. in San Ramon, California, for the installation of an additional control panel and the wiring of components for the newly installed groundwater recirculation system. The completed system was delivered to NASNI on February 16, 2021, where it was off-loaded and placed on leveled lengths of 6 in x 6 in pressure treated lumber within the demonstration area.

A piping and instrumentation diagram for the system is presented as **Figure 5.20**, and a system enclosure layout drawing is included as **Figure 5.21**. The remediation system was controlled automatically using a SCADA system that allowed both on-site and remote system monitoring of system operational parameters (groundwater and gas flows, pressures, and injection durations and frequencies), operational set-point control, and alarm notification and acknowledgment.

As presented in **Figure 5.21**, cometabolic bioremediation system was housed within a 20-ft long enclosure (e.g., Conex box). The enclosure had a partition wall, separating the enclosure into two spaces. The smaller of the two spaces was the system control room which was rated as a non-hazardous atmosphere and housed the SCADA system with integrated computer, electrical control panel, solar power distribution systems, and a combination air conditioner/heater. The larger space included groundwater and gas piping/fittings, mass flow controllers, well control solenoid valves and other system process components, and was rated as a Class 1, Division 2 atmosphere, due to the presence of flammable sparge gases flowing through the piping in this portion of the enclosure. All electrical components and connections in this portion of the enclosure were intrinsically safe to meet the hazardous atmosphere classification. This space was monitored with gas detectors which continuously measured oxygen, methane, and isobutane levels within the enclosure. The system would shut down and notify appropriate personnel if elevated levels of gas were detected. A cellular-based remote telemetry system was incorporated into the SCADA system to allow remote control and monitoring of the system, as well as alarm condition callouts when needed. The SCADA system was connected to two system programmable logic controller (PLC) panels that controlled the various systems. A photograph of the PLC panels (adjacent to the battery charge controllers) is provided in **Figure 5.22**, and a screenshot of the SCADA system interface screen is provided in **Figure 5.23**.

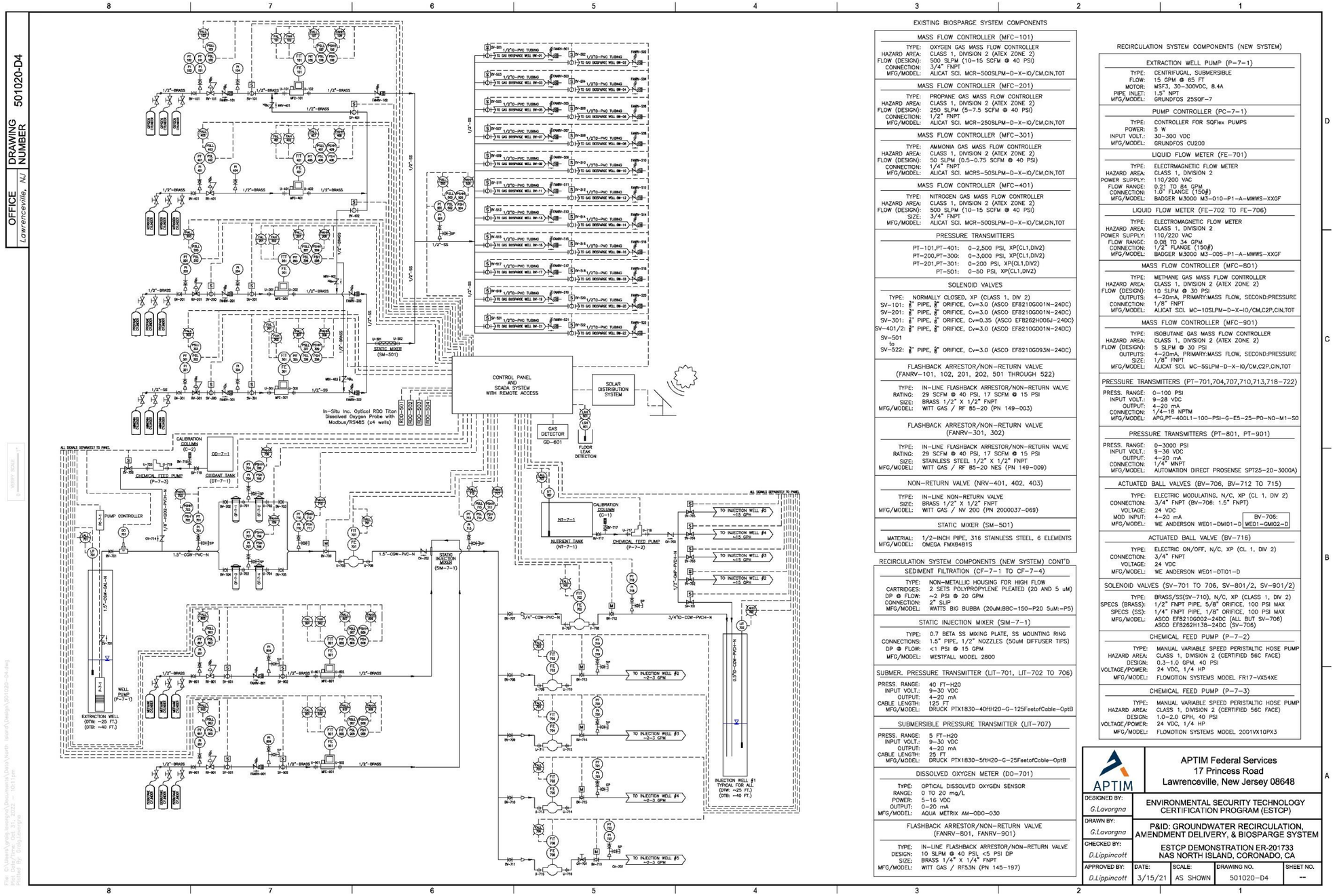
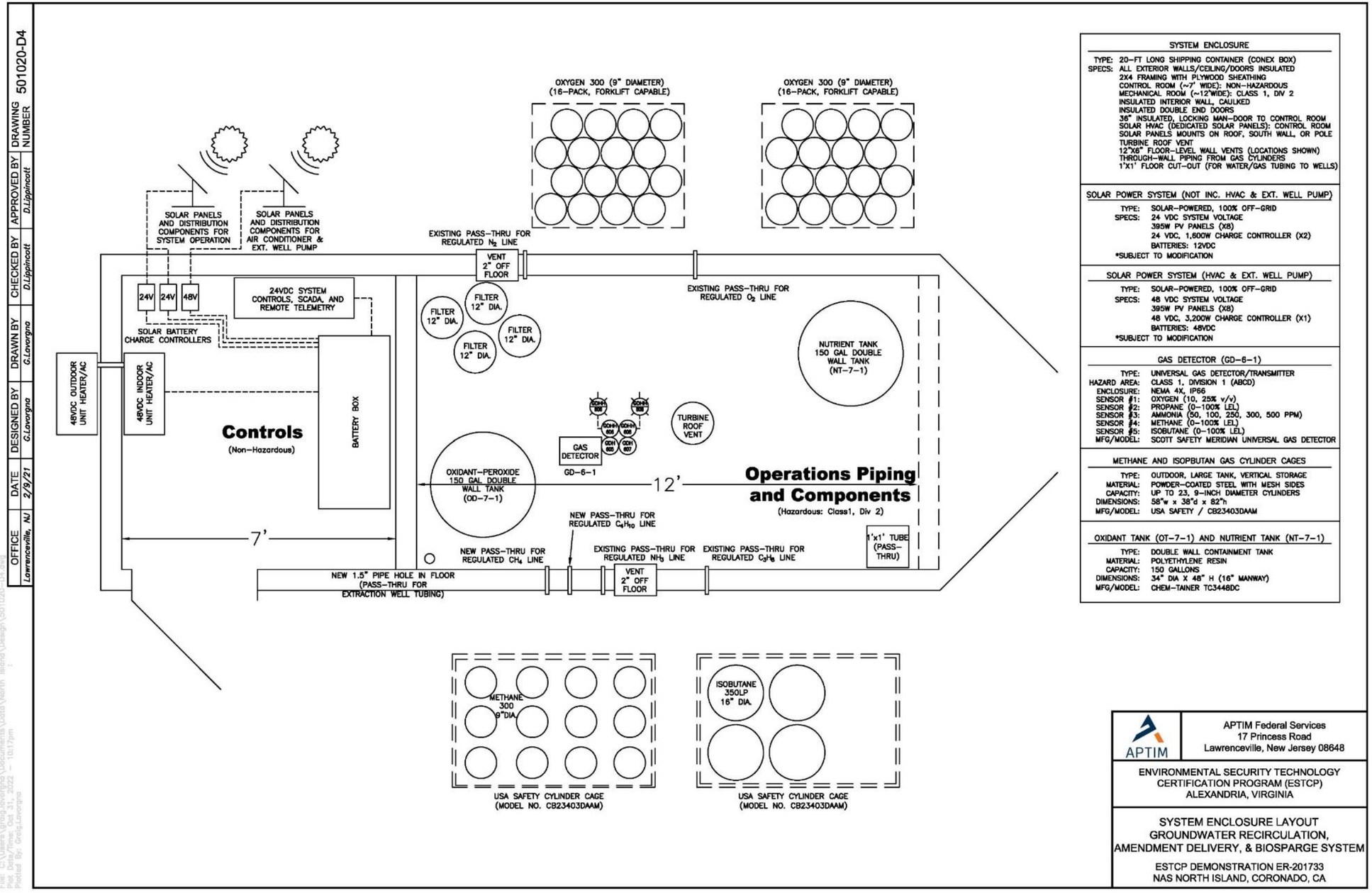


Figure 5.20. Cometabolic Bioremediation System Piping and Instrumentation Diagram.



SYSTEM ENCLOSURE

TYPE: 20-FT LONG SHIPPING CONTAINER (CONEX BOX)
SPECS: ALL EXTERIOR WALLS/CEILING/DOORS INSULATED
2X4 FRAMING WITH PLYWOOD SHEATHING
CONTROL ROOM (~7' WIDE): NON-HAZARDOUS
MECHANICAL ROOM (~12'WIDE): CLASS 1, DIV 2
INSULATED INTERIOR WALL, CAULKED
INSULATED DOUBLE END DOORS
36" INSULATED, LOCKING MAN-DOOR TO CONTROL ROOM
SOLAR HVAC (DEDICATED SOLAR PANELS): CONTROL ROOM
SOLAR PANELS MOUNTS ON ROOF, SOUTH WALL, OR POLE
TURBINE ROOF VENT
12"X6" FLOOR-LEVEL WALL VENTS (LOCATIONS SHOWN)
THROUGH-WALL PIPING FROM GAS CYLINDERS
1'X1' FLOOR CUT-OUT (FOR WATER/GAS TUBING TO WELLS)

SOLAR POWER SYSTEM (NOT INC. HVAC & EXT. WELL PUMP)

TYPE: SOLAR-POWERED, 100% OFF-GRID
SPECS: 24 VDC SYSTEM VOLTAGE
395W PV PANELS (X8)
24 VDC, 1,600W CHARGE CONTROLLER (X2)
BATTERIES: 12VDC
*SUBJECT TO MODIFICATION

SOLAR POWER SYSTEM (HVAC & EXT. WELL PUMP)

TYPE: SOLAR-POWERED, 100% OFF-GRID
SPECS: 48 VDC SYSTEM VOLTAGE
395W PV PANELS (X8)
48 VDC, 3,200W CHARGE CONTROLLER (X1)
BATTERIES: 48VDC
*SUBJECT TO MODIFICATION

GAS DETECTOR (GD-6-1)

TYPE: UNIVERSAL GAS DETECTOR/TRANSMITTER
HAZARD AREA: CLASS 1, DIVISION 1 (ABCD)
ENCLOSURE: NEMA 4X, IP66
SENSOR #1: OXYGEN (10, 25% v/v)
SENSOR #2: PROPANE (0-100% LEL)
SENSOR #3: AMMONIA (50, 100, 250, 300, 500 PPM)
SENSOR #4: METHANE (0-100% LEL)
SENSOR #5: ISOBUTANE (0-100% LEL)
MFG/MODEL: SCOTT SAFETY MERIDIAN UNIVERSAL GAS DETECTOR

METHANE AND ISOPBUTAN GAS CYLINDER CAGES

TYPE: OUTDOOR, LARGE TANK, VERTICAL STORAGE
MATERIAL: POWDER-COATED STEEL WITH MESH SIDES
CAPACITY: UP TO 23, 9-INCH DIAMETER CYLINDERS
DIMENSIONS: 58"w x 38"d x 82"h
MFG/MODEL: USA SAFETY / CB23403DAAM

OXIDANT TANK (OT-7-1) AND NUTRIENT TANK (NT-7-1)

TYPE: DOUBLE WALL CONTAINMENT TANK
MATERIAL: POLYETHYLENE RESIN
CAPACITY: 150 GALLONS
DIMENSIONS: 34" DIA X 48" H (16" MANWAY)
MFG/MODEL: CHEM-TAINER TC3448DC

DRAWING NUMBER	APPROVED BY	CHECKED BY	DRAWN BY	DESIGNED BY	DATE	OFFICE
501020-D4	D.Lippincott	D.Lippincott	G.Lovergna	G.Lovergna	2/9/21	Lawrenceville, NJ

APTIM Federal Services
17 Princess Road
Lawrenceville, New Jersey 08648

ENVIRONMENTAL SECURITY TECHNOLOGY CERTIFICATION PROGRAM (ESTCP)
ALEXANDRIA, VIRGINIA

SYSTEM ENCLOSURE LAYOUT
GROUNDWATER RECIRCULATION, AMENDMENT DELIVERY, & BIOSPARGE SYSTEM

ESTCP DEMONSTRATION ER-201733
NAS NORTH ISLAND, CORONADO, CA

File: C:\Users\greig.lovergno\Documents\Data\North Island\Design\501020-D4.dwg
Plot Date/Time: Oct 31, 2022 - 10:17pm
Plotted By: Greig.Lovergno

Figure 5.21. Cometabolic Bioremediation System Enclosure Layout

Figure 5.22. Photograph of the Solar Battery Charging System and PLC Panels.

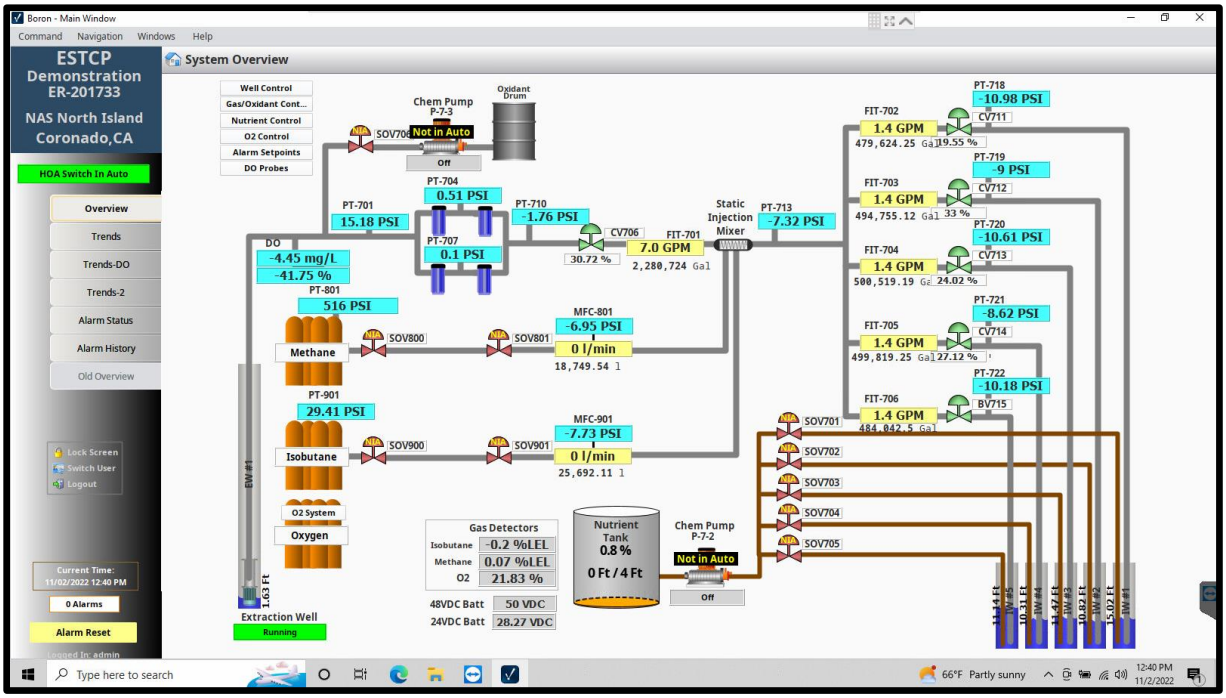


Figure 5.23. Screenshot of the SCADA System Interface Screen.

5.4.2.1 Solar Power System

As previously mentioned, the "off-the-grid" bioremediation system was powered by solar energy, including two separate solar power distribution systems. The first solar power distribution system was a 24-volt direct current (DC) system which powered the SCADA system and associated system process control components (pumps, solenoid valves, mass flow controllers, gas detector, etc.). Eight 395-watt solar panels were securely mounted to a commercially sourced solar panel rack adjacent to the enclosure (**Figures 5.15** and **5.24**), and were utilized to charge eight, 440 ampere hour (Ah) 12V batteries for the system (**Figure 5.25**). The second solar power distribution system was rated at 48-volt DC and was used to power the air conditioner/heater in the control room and the submersible pump located within EW (EW-1). This system consisted of eight 395-watt solar panels mounted to the same solar panel rack (**Figure 5.24**) and two 100 Ah and one 69 Ah 48-volt batteries (**Figure 5.25**). The reason for implementing two separate solar power systems was to ensure that if the air conditioner/heater or submersible pump potentially drained its battery bank, it would not shut-down the SCADA/control system and lose system operability. The orientation of the solar panels was due south, with a fixed installation angle of approximately 30 degrees from horizontal to maximize year-round sun exposure. Construction of the solar panel array consisting of all sixteen 395-watt solar panels was performed in the demonstration area during the week of February 8, 2021 (**Figure 5.24**).

Figure 5.24. Photograph of the Solar Panel Array. The sixteen 395W solar panels charged 24V and 48V battery banks that powered the hybrid cometabolic treatment system.

Figure 5.25. Photograph of the 24V and 48V Solar Power Battery Systems.

5.4.2.2 Groundwater Recirculation System

As shown on **Figure 5.20** and discussed in **Sections 5.2.1.7** and **5.4.1**, groundwater recirculation was accomplished by extracting groundwater from a central EW (EW-1), and re-injecting evenly into each of the surrounding five IWs (IW-1 through IW-5). A DC-powered submersible pump installed in EW-1 was used to extract groundwater at the designed flowrate (~8-12 GPM). Liquid flow meters (with digital output to the SCADA) worked in tandem with modulating ball valves to keep the total flow and individual flow to the five IWs at the operator input value. The pumped groundwater was directed through two pairs of cartridge filters to protect the flow meters and injection wells from solids in the water. Groundwater levels in the EW and IWs were monitored using submersible pressure transmitters tied into the PLC/SCADA system and would shut down the system if alarm set-point levels were reached. A photograph of the groundwater recirculation system within the enclosure is provided in **Figure 5.26**.

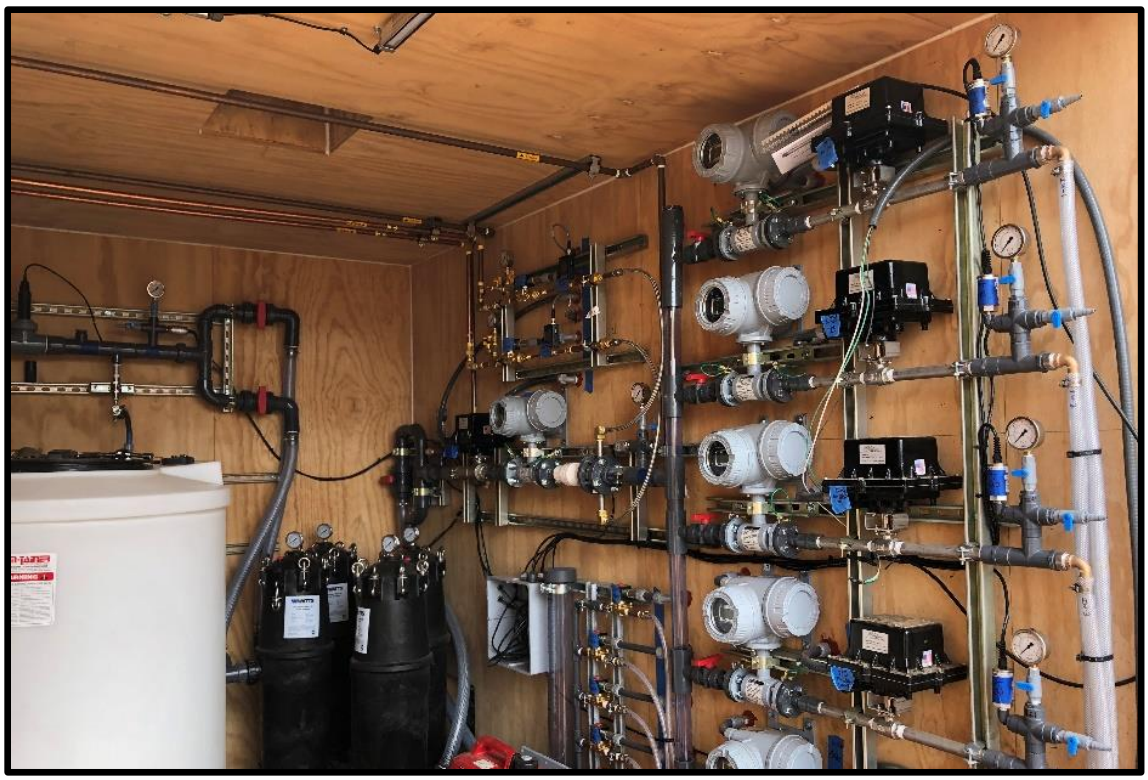


Figure 5.26. Photograph of the groundwater recirculation system. The system includes a submersible pump (not shown), cartridge filters, liquid flow meters and modulating ball valves to control groundwater extraction and reinjection rates.

5.4.2.3 Methane and Isobutane Gas Delivery System

The methane and isobutane gas delivery system relied on the regulated cylinder gas pressures to inject directly into the subsurface (in the case of oxygen) or the groundwater recirculation process water stream (methane and isobutane). The addition of methane and isobutane at specified rates was controlled by individual mass flow controllers and were added to the process water through static injection mixers prior to reinjection into the five IWs. As shown in **Figure 5.21**, compressed methane and liquid isobutane gas cylinders were stored outside of the system enclosure in locked cylinder cages to keep them securely staged and inaccessible to non-authorized personnel. The cylinders were "ganged" together with manifolds and equipped with regulators as needed to provide the appropriate delivery pressures and flows. A total of 12 cylinders, each containing 14.93 pounds (lbs.) (360 cubic ft) of Chemically Pure (CP) grade (99.5% pure) compressed methane, were stored in one of the cages. Six of the cylinders were ganged together, and six were kept in reserve. A total of 4 cylinders, each containing 111 lbs. (740 cubic ft) of CP grade (99.0% pure) liquid isobutane and ganged together, were stored in the second cage.

As presented on **Figure 5.20**, flow of each of the gases was controlled by individual automatic solenoid valves and digital mass flow controllers. Each mass flow controller had an integrated valve that automatically adjusted to maintain a specified flow entered by the operator on the SCADA system control screen. Pressure and flow indicators and transmitters were placed throughout the system to monitor system operational conditions, trigger alarms, or shut-down the system, if necessary. Flame arrestors/non-return valves were incorporated upstream of the mass flow controllers to protect the system from potential flashbacks and stop the unwanted backflow

of gases. Individual gas monitors, one for oxygen and two for lower explosive limit (specific to isobutane and methane) were also incorporated into the system enclosure to monitor the atmosphere and shut-down the system if alarm set-points were reached. A photograph of the methane and isobutane gas delivery system within the enclosure is provided in **Figure 5.27**.

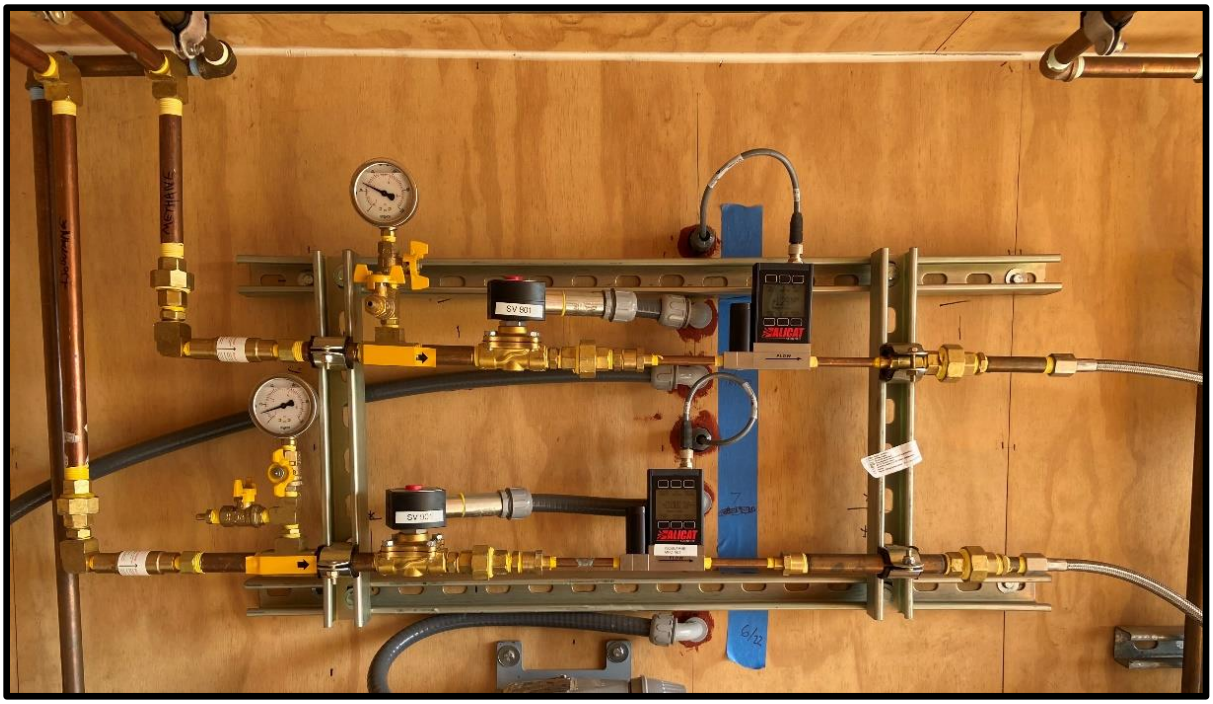


Figure 5.27. Photograph of the Isobutane and Methane Gas Delivery System. Delivery of the gases to the recirculated groundwater were controlled by solenoid valves and digital mass flow controllers within the enclosure.

5.4.2.4 Liquid Amendment Delivery System

Inorganic nutrients (DAP in solution) were added to the process stream via a chemical feed pump in short pulses (typically <1 hour) as needed (approximately once per week) during the demonstration. The chemical feed pump transferred the nutrient solution at a set rate and duration from a 150-gallon double-walled mix tank to each well individually, controlled by automatic solenoid valves.

As discussed in **Section 5.3**, laboratory testing indicated that ENV493 grows rapidly on isobutanol in the presence of 1,1-DCE, without inducing the monooxygenase (SCAM) required to biodegrade isobutane and relevant COCs. Therefore, the liquid amendment delivery system was used to add isobutanol immediately prior to and/or after bioaugmentation with the ENV493 culture was performed (**Section 5.5.3.2**) to maximize the growth and distribution of the culture within the aquifer without formation of reactive 1,1-DCE epoxide.

Additionally, the liquid amendment delivery system was used to inject the bioaugmentation cultures (ENV493 and ENV4M) into the aquifer, as detailed in **Section 5.5.3.1**. A photograph of the liquid amendment delivery system is provided in **Figure 5.28**.

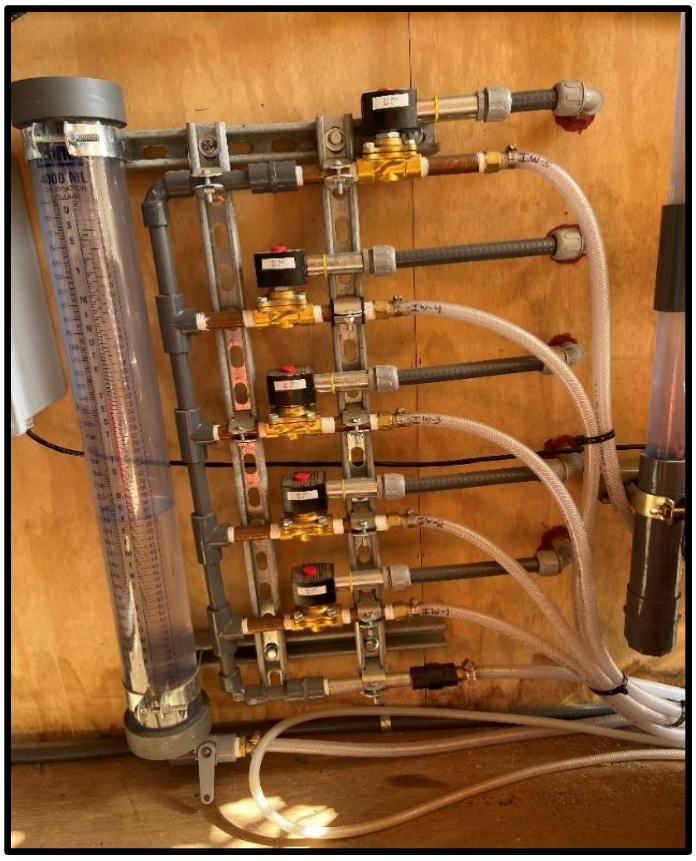


Figure 5.28. Photograph of the Liquid Nutrient Delivery System. The delivery system was connected to a 150-gallon liquid nutrient tank within the Enclosure. The manifold system consisted of individual solenoid valves to control flow of liquid nutrients to the five IWs.

5.4.2.5 Biofouling Mitigation System

A biofouling mitigation system was designed into the groundwater recirculation system to reduce performance degradation due to potential biofouling of the various system components and/or the IWs. As shown on **Figures 5.20** and **5.35**, a compatible chemical feed pump was used to pulse a small amount of oxidant (35% hydrogen peroxide or equivalent) from a 150-gallon double-walled oxidant holding tank into the system at the end of each operational day. The volume of oxidant injected was enough to fill the process piping (downstream of BV-101), filter housings, flow meters and IW screen intervals (and surrounding sand pack), but not a large enough volume to appreciably distribute the oxidant out into the formation. The design oxidant concentration in the process water (~400-800 mg/L) allowed for complete decomposition to oxygen and water overnight, prior to startup of the recirculation system the following morning. A photograph of the biofouling mitigation system is provided in **Figure 5.29**.

Figure 5.29. Photograph of the Biofouling Mitigation System. A peristaltic metering pump was connected to a 150-gallon double-walled oxidant holding tank containing 35% hydrogen peroxide within the Enclosure. A small amount of oxidant was delivered to the groundwater recirculation system and IWs at the end of each operational day to prevent potential biofouling.

5.4.2.6 Oxygen Biosparging System

Pure oxygen gas was added directly to the subsurface via the five biosparging wells. As presented on **Figure 5.20**, oxygen flow was controlled by individual automated solenoid valves and a gas-specific digital mass flow controllers with an integrated valve that automatically adjusted to maintain a specified flow entered by the operator on the SCADA system control screen. Oxygen was supplied by compressed oxygen cylinders housed within movable steel racks (6-packs and/or 16-packs of oxygen cylinders) stored outside of the enclosure (**Figure 5.21**). The oxygen flow was directed to a gas distribution manifold system, which directed the oxygen to each of the five biosparging wells using individually controlled solenoid valves to allow sparging one well at a time. A length of ¾-in ID by one-in OD reinforced PVC tubing was run from each of the five solenoid valves to the corresponding well head. Tubing runs exited the enclosure box through a one-ft by one-ft access port in the floor (**Figure 5.21**) and ran underground in a shallow trench to the appropriate well head.

Pressure and flow indicators and transmitters were installed throughout the system to monitor system operational conditions and trigger alarms or shut-down the system, if necessary. Flashback arrestors were installed throughout the gas distribution system to protect from potential flashbacks and stop the unwanted backflow of oxygen. A photograph of the oxygen biosparging system is provided in **Figure 5.30**.

A DO probe was incorporated into the piping of the recirculation system, prior to the filters (see **Figure 5.20**). The DO sensor was connected to the biosparging system control panel, allowing for viewing and continuous logging of DO concentrations in the recirculated water during and between oxygen sparging cycles. Additionally, dedicated optical rugged dissolved oxygen (RDO) were installed in wells PMW-3S, PMW-3D, PMW-4S and PMW-4D, allowing for continuous viewing and logging of DO concentrations in these wells. The data from the in-line DO sensor and the four RDO probes were used to determine the frequency and duration of oxygen biosparging pulses to maintain DO concentrations in the treatment zone above the target concentration of 1.0 mg/L (**Section 3.2**).

Figure 5.30. Photograph of the Oxygen Biosparging System. Reinforced gas tubing was connected to the gas distribution manifold system within the enclosure. The manifold system consisted of individual solenoid valves to direct flow to the 5 biosparging wells (with the capability to sparge at up to 22 wells).

5.4.2.7 System Installation Activities

Immediately following delivery of the enclosure, shallow trenches (~ 10 to 12-in deep) were excavated between the enclosure and the system wells (extraction, injection, and oxygen biosparging) and the solar panel array. Custom well heads that included a pressure gauge and flashback arrestor were then installed on each of the five biosparging wells (**Figure 5.31**). Custom well heads for the EW and IWs that included the required tubing/piping and wiring pass-throughs were also installed (**Figure 5.32**). Groundwater process tubing, electrical conduit (for solar and pump power, level transducer, and RDO probe wire), and oxygen biosparging gas tubing were all installed within the trenches (**Figure 5.33**).

Figure 5.31. Photograph of Oxygen Biosparging Well Head Assembly. The custom well heads included a pressure gauge and a flashback arrestor. Each of the five well heads were connected to the biosparging system using reinforced gas tubing installed in shallow trenches.

Figure 5.32. Photograph of an Injection Well Head Assembly. The custom well heads were liquid-tight and included pass-throughs for groundwater re-injection, liquid nutrient injection, and a submersible pressure transmitter. Each of the 5 well heads were connected to the groundwater recirculation system using reinforced gas tubing installed in shallow trenches.

APTIM personnel worked on-site with the Calcon engineer to make all the necessary electrical connections for the off-the-grid solar power system (**Figure 5.24**), including the 24-volt and 48-volt battery systems (**Figures 5.25**). The Calcon engineer also made the final control wire connections for four down-well RDO probes which were installed in wells PMW-3S, PMW-3D, PMW-4S and PMW-4D. All electrical and control wiring was installed within dedicated conduit in shallow trenches. Once all connections were made between the piping on the system enclosure, well heads, and the solar panel array, the trenches were backfilled using the overburden excavated soil.

Figure 5.33. Photograph of Conduit and Hose Installations within Shallow Trenches.

APTIM procured and installed gas cylinders, cylinder cages and gas manifolds/regulators. As shown in **Figure 5.34,** the liquid isobutane and compressed methane cylinders were stored outside in locked, steel cylinder cages on the north side of the system enclosure. The compressed oxygen cylinders were delivered to the site in movable steel racks (6-packs and/or 16-packs) and stored on opposite side of the enclosure for safety (**Figure 5.35**).

Figure 5.34. Photograph of Methane and Isobutane Storage. Compressed methane and liquified isobutane cylinders were stored in steel cages on the northern side of the enclosure. The cylinders were "ganged" together with manifolds and equipped with regulators to provide the appropriate delivery pressures and flows.

Figure 5.35. Photograph of Oxygen Gas Storage. 6-Packs of oxygen cylinders were located on the southern side of the enclosure box system. These manifolded racks of cylinders were subsequently replaced with 3 racks containing 16-packs of oxygen when they became available from the vendor.

Upon completion of the biosparging system installation activities, the Calcon Engineer assisted APTIM with system shake-down and startup activities on March 11 and 12, 2021. A photograph of the completed system is provided in **Figure 5.36**.

Figure 5.36. Photograph of the Completed Solar-Powered Hybrid Cometabolic Treatment System.

5.5 FIELD TESTING

The field demonstration was performed over a period of approximately 20 months, and included the following major activities/phases:

- Baseline Sampling
- Phase 1: Groundwater Recirculation with Oxygen Biosparging (7 weeks)
- Phase 2: Cometabolic Treatment and Performance Monitoring (12 months)
- Phase 3: Post Treatment Sampling
- Decommissioning

Table 5.5 summarizes the pertinent events that occurred within each of these activities/phases, along with their timing and duration. Each of these activities/phases are described in detail in the following subsections.

Table 5.5. Summary of Demonstration Activities/Phases

Event Description	Day from System Startup	Start Date	Duration	Comments
Baseline				
Baseline Sampling Event #1	-91	12/14/2020	2 Days	Baseline Groundwater Sampling (11 Wells)
Baseline Sampling Event #2	-35	2/8/2021	2 Days	Baseline Groundwater Sampling (11 Wells)
Phase 1: Groundwater Recirculation with Oxygen Biosparging (50 Days)				
Begin Phase 1 System Startup	0	3/15/2021	-	Begin groundwater recirculation and oxygen sparging
Down-Well Groundwater DO and Water Level Monitoring	0	3/15/2021	1 Day	Start Up DO Monitoring (11 monitoring wells & EW-1)
Down-Well Groundwater DO and Water Level Monitoring	1	3/16/2021	1 Day	Start Up DO Monitoring (11 monitoring wells & EW-1)
Down-Well Groundwater DO and Water Level Monitoring	2	3/17/2021	1 Day	Start Up DO Monitoring (11 monitoring wells & EW-1)
Down-Well Groundwater DO and Water Level Monitoring	3	3/18/2021	1 Day	Start Up DO Monitoring (11 monitoring wells & EW-1)
Down-Well Groundwater DO and Water Level Monitoring	4	3/19/2021	1 Day	Start Up DO Monitoring (11 monitoring wells & EW-1)
Phase 1 Groundwater Sampling Event #1	22	4/6/2021	2 Days	Groundwater Sampling (11 monitoring wells & EW-1)
Phase 1 Groundwater Sampling Event #2	43	4/27/2021	2 Days	Groundwater Sampling (11 monitoring wells & EW-1)
Phase 2: Cometabolic Treatment and Performance Monitoring (371 Days)				
Begin Phase 2 / ENV493 Bioaugmentation Event #1	51	5/5/2021	1 Day	First bioaugmentation with isobutane oxidizing culture ENV493
Isobutanol Addition #1	51	5/5/2021	12 Days	Begin 12 days of isobutanol addition
Performance Groundwater Sampling Event #1	64	5/18/2021	2 Days	Groundwater Sampling (11 monitoring wells & EW-1)
Isobutane Addition #1	64	5/18/2021	41 Days	Begin 41 days of isobutane addition
Dissolved Gas Groundwater Sampling Event #1	67	5/21/2021	1 Day	Groundwater Sampling (8 monitoring wells & EW-1)
Dissolved Gas Groundwater Sampling Event #2	71	5/25/2021	2 Days	Groundwater Sampling (8 monitoring wells & EW-1)
Dissolved Gas Groundwater Sampling Event #3	78	6/1/2021	1 Day	Groundwater Sampling (9 monitoring wells & EW-1)
Dissolved Gas Groundwater Sampling Event #4	85	6/8/2021	1 Day	Groundwater Sampling (9 monitoring wells & EW-1)
Dissolved Gas Groundwater Sampling Event #5	92	6/15/2021	1 Day	Groundwater Sampling (6 monitoring wells & EW-1)
Performance Groundwater Sampling Event #2	98	6/21/2021	2 Days	Groundwater Sampling (11 monitoring wells & EW-1)
ENV494M Bioaugmentation Event	99	6/22/2021	1 Day	Bioaugmentation with mixed methanotroph culture ENV494M
Start Methane Addition	99	6/22/2021	323 Days	Begin 323 days of methane addition
Stop Isobutane Addition	105	6/28/2021	-	Temporarily halt isobutane addition
Dissolved Gas Groundwater Sampling Event #6	113	7/6/2021	1 Day	Groundwater Sampling (6 monitoring wells & EW-1)
Dissolved Gas Groundwater Sampling Event #7	126	7/19/2021	1 Day	Groundwater Sampling (6 monitoring wells & EW-1)
Dissolved Gas Groundwater Sampling Event #8	155	8/17/2021	1 Day	Groundwater Sampling (7 monitoring wells & EW-1)
Isobutanol Addition #2	164	8/26/2021	28 Days	Begin 28 days of isobutanol addition
Performance Groundwater Sampling Event #3	183	9/14/2021	1 Day	Groundwater Sampling (11 monitoring wells & EW-1)
ENV493 Bioaugmentation Event #2	183	9/14/2021	1 Day	Second Bioaugmentation with ENV493
Isobutane Addition #2	205	10/6/2021	76 Days	Resume 76 days of isobutane addition
Performance Groundwater Sampling Event #4	246	11/16/2021	2 Days	Groundwater Sampling (11 monitoring wells & EW-1)
Performance Groundwater Sampling Event #5	274	12/14/2021	1 Day	Groundwater Sampling (11 monitoring wells & EW-1)
Stop Isobutane Addition	280	12/20/2021	-	Temporarily halt isobutane addition
Isobutanol Addition #3	281	12/21/2021	45 Days	Begin 45 days of isobutanol addition
ENV493 Bioaugmentation Event #3	304	1/13/2022	1 Day	Third (and final) Bioaugmentation with ENV493
Isobutane Addition #3	205	10/6/2021	27 Days	Resume 27 days of isobutane addition
Performance Groundwater Sampling Event #6	345	2/23/2022	1 Day	Groundwater Sampling (11 Wells & EW-1)
Stop Isobutane Addition	353	3/3/2022	-	Stop isobutane addition for the remainder of the project
Performance Groundwater Sampling Event #7	386	4/5/2022	1 Day	Groundwater Sampling (11 Wells & EW-1)
End Phase 2	422	5/11/2022	-	Stop oxygen sparging and methane addition/Continue groundwater recirculation
Phase 3: Post Treatment Monitoring				
Post Treatment Sampling Event	499	7/27/2022	1 Day	Groundwater Sampling (11 monitoring wells & EW-1)

5.5.1 Baseline Sampling

Baseline groundwater samples were collected from all 11 monitoring wells twice prior to system startup, and from EW-1 during startup. Baseline sampling events were performed on December 14-15, 2020, and February 8-9, 2021. Samples were analyzed for VOCs, 1,4-D, dissolved gases (methane, ethane, ethene, propane, acetylene, and isobutane), and anions (chloride, nitrate, nitrite, bromide, phosphate, and sulfate) by APTIM's analytical laboratory in Lawrenceville, NJ. Geochemical parameters (DO, ORP, pH, specific conductivity, and temperature) at each well were measured with a multi-parameter meter in the field during sample collection. Quantification of target cometabolic organisms/genes SCAM, Propane Monooxygenase [PPO], Ammonia

Monooxygenase [AMO], sMMO, and Particulate Methane Monooxygenase [pMMO]) by qPCR was performed by Microbial Insights (Knoxville, TN) on samples collected from six select wells (PMW-3S/D, PMW-4S/D, DMW-1, and OU11-SMW08A) during the second baseline sampling event. Proteomics analysis was also performed at NCSU on samples from the same six select wells and focused on the use of activity-based labeling (ABL) of monooxygenases expressed by native bacteria. Sampling and analytical methods, sampling locations, and a list of the number and types of samples collected are provided in **Section 5.6**. The results for each well for each parameter are provided in **Appendix F** on a well-by-well basis. The data collected during these two events were used as baseline measurements of site conditions prior to system operation.

5.5.2 Phase 1: Groundwater Recirculation with Oxygen Biosparging

Phase 1 of the field demonstration included operation of the groundwater recirculation and oxygen biosparging systems. Startup of these two systems was initiated on March 15, 2021 (day 0) and Phase 1 operations continued through May 4, 2021 (day 50) (**Table 5.5**). Groundwater recirculation consisted of pumping 10 GPM from EW-1 and reinjecting the groundwater equally into the five IWs (IW-1 through IW-5). The groundwater recirculation system was initially operated for nine hours per day (during daylight hours when solar power operation is optimized). The groundwater recirculation rate was increased to 12.5 GPM on March 16, 2021, and the pumping duration was gradually increased to 13 hours per day to maximize mixing of groundwater within the treatment zone. Approximately 405,000 gallons (an estimate 12.5 treatment zone pore volumes) of groundwater were recirculated during Phase 1 operation.

An oxygen sparge cycle consisting of oxygen flowing at 10 SCFM sequentially into each biosparging wells for approximately five minutes was performed on March 15, 2021. Five additional oxygen sparge cycles were performed between March 18 and March 19, 2021, at rates that varied from 3 SCFM to 8.5 SCFM for up to five minutes per sparge well to evaluate influence of the sparging at various flow rates. Additional oxygen sparge cycles consisting of oxygen flowing at 10 SCFM sequentially into each of the five biosparging wells for between 3 and 5 minutes were performed approximately every two weeks for the remainder of Phase 1 to maintain aerobic conditions within the aquifer. Approximately 740 cubic ft (52.7 lbs.) of oxygen were sparged into aquifer during Phase 1 operation.

DO concentrations were measured daily at all 11 monitoring wells using a down-well DO probe during the first week of operation. Additionally, DO concentrations were continuously recorded by the SCADA system for the dedicated RDO sensors located in wells PMW-3S, PMW-3D, PMW-4S and PMW-4D, and the in-line DO sensor on the recirculation system piping during startup.

The biofouling mitigation system was also operated to reduce potential biofouling of the process piping and IWs. A chemical feed pump pulsed two liters (L) of oxidant (35% hydrogen peroxide) into the system at the end of each operational day. The volume of oxidant injected was calculated to be sufficient to fill the process piping, filters, flow meters and IW screen intervals (and surrounding sand pack), but not a large enough volume to distribute the oxidant out into the formation. The hydrogen peroxide concentration in the process water was set at ~600 mg/L, a concentration high enough to temporarily inhibit microbial growth, and low enough to allow for complete decomposition to oxygen and water overnight. No nutrients (DAP) were added to the

recirculated groundwater during this phase of the project. However, a nominal amount of isobutane and methane were added as part of system testing activities.

Two comprehensive Phase 1 groundwater sampling events were conducted on days 22 and 43, as summarized in **Table 5.5**. Groundwater samples were collected from all 11 demonstration monitoring wells and EW-1. Samples were analyzed for VOCs, 1,4-D, dissolved gases, and anions by APTIM's analytical laboratory in Lawrenceville, NJ. Geochemical parameters were measured with a multi-parameter meter at each well in the field during sample collection. Quantification of target cometabolic organisms/genes (SCAM, PPO, AMO, sMMO, and pMMO) by qPCR was performed by Microbial Insights (Knoxville, TN) on samples collected from six select wells (PMW-3S/D, PMW-4S/D, DMW-1, and OU11-SMW08A) during the sampling event performed on day 43 (at the end of Phase 1). Proteomics analysis was also performed at NCSU on samples from the same six select wells and focused on the use of ABL of monooxygenases expressed by native bacteria. Sampling and analytical methods, sampling locations, and a list of the number and types of samples collected are provided in **Section 5.6**, and the results discussed in **Section 5.7**. Sampling results for each well for each parameter are provided in **Appendix F** on a well-by-well basis.

System operations data (gas/water flows, pressures, cycle frequencies, and downtime) and DO data collected from the four dedicated RDO probes and the in-line DO sensor on the system piping were recorded and stored in data files by the SCADA system on the system laptop.

5.5.3 Phase 2: Cometabolic Treatment and Performance Monitoring

Phase 2 of the field demonstration included the injection of bioaugmentation cultures and the operation of the groundwater recirculation, amendment delivery, and the oxygen biosparging systems. Phase 2 of the field demonstration began on May 5, 2021 (day 51) and continued through May 11, 2022 (day 422) (**Table 5.5**).

5.5.3.1 Bioaugmentation Injections

A total of four bioaugmentation injection events were performed during Phase 2 of the field demonstration. For each bioaugmentation event, approximately 500 L of the culture (ENV493 or ENV494M) was grown in APTIM's commercial fermentation facility in Lawrenceville, NJ. The cultures were concentrated approximately 6- to 7-fold (resulting in volumes of ~ 70 – 80 L) and placed in 18-L soda kegs with air in the headspace. The kegs were placed on ice and shipped overnight to the site for bioaugmentation. Once onsite, the contents of the kegs were emptied into one or two 55-gallon drums containing site groundwater (provided by a temporary slip stream on the groundwater recirculation system) and the mixture was pumped at a rate of approximately 0.75 GPM into each of the five groundwater IWs (IW-1 through IW-5) in equal volumes using the nutrient injection pump as the groundwater recirculation system was operating. The bioaugmentation events are summarized as follows:

- ENV 493 Bioaugmentation Event #1: Performed on May 5, 2021 (day 51) and marked the start of Phase 2. During this event, the aquifer was bioaugmented with 76 L of concentrated ENV493 (an isobutane-oxidizing pure culture that was isolated from site groundwater);

- ENV494M Bioaugmentation Event #1: Performed on June 22, 2021 (day 99). During this event, the aquifer was bioaugmented with 70 L of concentrated ENV494M (a mixed-methanotrophic culture isolated from site groundwater);
- ENV493 Bioaugmentation Event #2: Performed on September 14, 2021 (day 183). During this event, the aquifer was bioaugmented for a second time with 80 L of concentrated ENV493.; and,
- ENV493 Bioaugmentation Event #3: Performed on January 13, 2022 (day 304). During this event, the aquifer was bioaugmented for the third time with 76 L of concentrated ENV493.

This second and third bioaugmentation events with ENV493 were performed because groundwater sampling data showed that 1.1.-DCE biodegradation rates had declined as had rates of utilization of both isobutane and oxygen in the aquifer. The data suggested that the reactive epoxide of 1,1-DCE had caused appreciable population declines in the bioaugmented culture. The concentrations of 1,1-DCE had also fallen appreciably between bioaugmentation events, so it was clear that the culture was active in the aquifer, but likely added at a density too low to degrade all of the remaining 1,1-DCE. Subsequent bioaugmentation events were designed to overcome this limitation, and promote complete biodegradation of 1,1-DCE, which, based on laboratory data, would then allow ENV493 to grow in the aquifer (absent of 1,1-DCE toxicity), and degrade the remaining COCs, including 1,4-D.

5.5.3.2 Isobutanol, Isobutane, and Methane Addition

Because the ENV493 culture was determined by APTIM to grow rapidly on isobutanol as a carbon source, daily additions of isobutanol to the recirculated groundwater were initiated immediately prior to and/or after each of the three ENV493 bioaugmentations to assist with growth of the culture within the aquifer. The isobutanol was added to the nutrient solution mix tank at concentrations between approximately 0.5 to 2 percent and added to the process stream via the chemical feed pump in short pulses (~1 hour) as follows:

- Isobutanol Addition Period #1: The first isobutanol addition was conducted from day 51 (the day of the first ENV493 bioaugmentation) through day 62. A total of 18 L were added, with one L of isobutanol was added each day for the first six days, followed by two L of isobutanol for the next six days.
- Isobutanol Addition Period #2: The second isobutanol addition was conducted from day 164 (19 days before ENV493 bioaugmentation event #2) through day 191. A total of 54 L of isobutanol were added, with between 0.25 and 3.0 L added per day; and
- Isobutanol Addition Period #3: The third isobutanol addition was conducted from day 281 (23 days before ENV493 bioaugmentation event #3) through day 325. A total of 36 L of isobutanol were added, with between 0.25 and 3.0 L added per day.

Isobutane addition to the groundwater recirculation process stream was performed over three periods, one after each of the ENV493 bioaugmentation events, as follows:

- Isobutane Addition Period #1: The first isobutane addition was conducted from day 64 through day 105. Isobutane was typically added to the process stream at a rate of 0.15

liters per minute (LPM) for 10 hours per day, to achieve a final injection concentration of 8 mg/L (0.23 kilograms [kg]/day);

- Isobutane Addition Period #2: The second isobutane addition was conducted from day 205 through day 280. Isobutane was typically added to the process stream at a rate of 0.15 to 0.25 LPM for between 6.0 to 7.5 hours per day, to achieve daily additions between 0.13 and 0.28 kg/day; and,
- Isobutane Addition Period #3: The third isobutane addition was conducted from day 327 through day 353. Isobutane was typically added to the process stream at a rate of 0.15 to 0.25 LPM for between 6.0 to 7.5 hours per day, to achieve daily additions between 0.13 and 0.28 kg/day.

Isobutane addition was halted for each of the periods when dissolved isobutane concentration data indicated that consumption of isobutane was no longer occurring within the treatment zone. A total of approximately 910 cubic ft (63.6 kg) of isobutane were added to the aquifer during Phase 2 operation.

Methane addition to the groundwater recirculation process stream was initiated on June 22, 2021 (day 99), the same day that bioaugmentation with ENV494M was performed. Methane was initially added to the process stream at a rate of 0.57 LPM for 10 hours per day, to achieve a final injection concentration of 8 mg/L (0.23 kg/day). Methane addition rates were reduced to 0.1 LPM for 10 hours per day on July 24, 2021 (day 131) when it was observed that dissolved methane concentrations were continuing to climb within the aquifer (as detailed in **Section 5.7.4**). Methane addition was reduced to 0.1 LPM for 6 hours per day on August 20, 2021 (day 158). Methane addition subsequently varied between 0.1 and 0.6 LPM for between 6 and 9 hours per day through May 11, 2022 (day 422, the end of Phase 2), to achieve daily additions between 0.02 and 0.22 kg/day. Changes to methane injection concentrations and duration throughout the demonstration were based on observed dissolved methane concentrations measured at EW-1 and/or the PMWs, as discussed in **Section 5.7.4**. A total of approximately 1,200 cubic ft (22.6 kg) of methane were added to the aquifer during Phase 2 operation.

5.5.3.3 Groundwater Recirculation System Operation

Groundwater recirculation continued throughout Phase 2 of the demonstration (day 51 through day 422). Groundwater recirculation rates and durations were modified throughout demonstration as follows:

- Day 51 through 175: 12.5 GPM for between 11 and 13 hours per day.
- Day 176 through day 186: 10 GPM for 13 hours per day
- Day 187 through day 222: 5-8 GPM for between 11 and 13 hours per day
- Day 223 through day 208: 10 GPM for between 9 and 13 hours per day
- Day 209 through day 366: 5 GPM for 6 to 10 hours per day
- Day 367 to 422: 8 GPM for 6 to 7 hours per day

As with Phase 1 operation, groundwater recirculation during Phase 2 consisted of pumping from EW-1 and reinjecting the groundwater equally into the five IWs. Changes in recirculation rates and duration were made to evaluate the impacts on varying pore volume exchange rates within the treatment zone on treatment performance. Approximately 1,850,000 gallons (an estimate 58 treatment zone pore volumes) of groundwater were recirculated during Phase 2 operation.

5.5.3.4 Oxygen Biosparging

During Phase 2 operation, oxygen sparge cycles typically consisted of oxygen flowing at between 6 and 10 SCFM sequentially into each of the five biosparging wells for five minutes once every two to five days to maintain aerobic conditions within the aquifer. Oxygen sparging was typically performed every one to two days during and for several days after each of the three isobutanol additions (**Section 5.5.3.2**), due to the additional biological oxygen demand created by the addition of this carbon substrate. The final oxygen sparging cycle was performed on May 11, 2022. DO concentrations were measured at the monitoring wells during each of the groundwater sampling events (**Section 5.5.3.6**). Additionally, DO concentrations were continuously recorded by the SCADA system for the dedicated RDO sensors located in wells PMW-3S, PMW-3D, PMW-4S and PMW-4D, and the in-line DO sensor on the recirculation system piping during Phase 2 of the demonstration. These data were used to determine adjustments to oxygen sparging cycle injection rates and frequencies to maintain aerobic conditions in the aquifer. A total of approximately 27,000 cubic ft (2,240 lbs.) of oxygen were sparged into aquifer during Phase 2 operation.

5.5.3.4 Nutrient Addition

DAP was also pulsed into the aquifer approximately once per week, as described in **Section 5.3.5**. As described in **Section 5.4.2.4**, inorganic nutrients (DAP in solution) were added to the process stream via the liquid amendment delivery system in short pulses (typically <1 hour) approximately once per week during Phase 2 operation. Each nutrient mix included approximately 60 lbs. of technical grade DAP mixed into solution with 140 gallons of potable water (a solution concentration of ~ 51 grams per liter) within a 150-gallon double-walled mix tank. Approximately 20-25 gallons of nutrient solution was added to the aquifer per week, with additional injections performed prior to and immediately after each of the bioaugmentation events (**Section 5.5.3.1**). A total of 700 lbs. of DAP were added to the aquifer during Phase 2 operation.

5.5.3.5 Biofouling Mitigation System Operation

During Phase 2 operation, the biofouling mitigation system was operated at the end of each groundwater recirculation cycle, as detailed in **Section 5.5.2**. A small volume (~ 0.7 to 1.5 liters per day) of 35% hydrogen peroxide was injected into the groundwater recirculation system at the end of each operational day. The hydrogen peroxide concentration in the process water was typically set at between 400 and 800 mg/L. A total of approximately 90 gallons of 35% hydrogen peroxide was injected during Phase 2 operation.

5.5.3.6 Groundwater Monitoring

Phase 2 groundwater sampling events included seven comprehensive sampling rounds on days 64, 98, 183, 246, 274, 345, and 386, as summarized in **Table 5.5**. Groundwater samples were collected from all 11 demonstration monitoring wells and EW-1 during each event. Samples were analyzed for VOCs, 1,4-D, dissolved gases, isobutanol, and anions by APTIM's analytical laboratory in Lawrenceville, NJ. Geochemical parameters were measured with a multi-parameter meter at each

well in the field during sample collection. Quantification of target cometabolic organisms/genes (SCAM, PPO, AMO, sMMO, and pMMO) by qPCR was performed by Microbial Insights (Knoxville, TN) on samples collected from six select wells (PMW-3S/D, PMW-4S/D, DMW-1, and OU11-SMW08A) during the sampling events performed on days 64 (13 days after the first bioaugmentation with ENV493) and 274. Proteomics analysis was also performed at NCSU on samples from the same six select wells and focused on the use of ABL of monooxygenases expressed by native bacteria.

In addition to the comprehensive sampling events, eight smaller groundwater sampling events were conducted to evaluate dissolved gas distribution and consumption, and concentration trends of target COCs within the treatment zone. As summarized on **Table 5.5**, these events occurred on days 67, 71, 78, 85, 92, 113, 126, and 155 of the demonstration. Between 6 and 9 monitoring wells were sampled for dissolved gases during these events. VOCs, 1,4-D and/or isobutanol samples also were collected during several of these events. Regular samples (typically weekly or bi-weekly) for dissolved gases, and at times isobutanol and VOCs, were also collected from EW-1 during Phase 2 system operation. Sampling and analytical methods, sampling locations, and a list of the number and types of samples collected are provided in **Section 5.6**, and the results discussed in **Section 5.7**. The results for each well for each parameter are provided in **Appendix F** on a well-by-well basis. The data collected during these events were used to evaluate the efficacy of the cometabolic treatment.

5.5.3.7 System Operation and Maintenance

System O&M consisted of regular (every 2-4 weeks) system checks to collect manual system pressure and flow data, perform regular system maintenance, mix nutrients, and perform leak checks. Changeout of the oxygen 6-packs and/or 16-packs required the use of an off-road forklift and occurred approximately every 3-5 months. The 4 cylinders of liquified isobutane and 12 cylinders of compressed methane did not require replacement during the demonstration. System operations data (gas flows, pressures, cycle frequencies, and downtime) and DO data collected from the four dedicated RDO probes were recorded and stored in data files by the SCADA system on the system laptop.

5.5.4 Post Treatment Sampling

While the addition of oxygen, isobutane, methane, isobutanol, and nutrients had stopped by the end of Phase 2 (day 422), the groundwater recirculation system continued to operate intermittently between days 423 and 499 (approximately 2 ½ months) to maintain a degree of hydraulic control within the treatment zone. During this time, groundwater was typically recirculated at 8 GPM for 6.5 hours per day, approximately 70 percent of the time. A total of approximately 170,000 gallons of groundwater was recirculated during this period.

Post treatment groundwater samples were collected from all 11 demonstration monitoring wells and EW-1 on July 27, 2022 (day 499). Samples were analyzed for VOCs, 1.4-D, dissolved gases, and anions by APTIM's analytical laboratory. Geochemical parameters were measured with a multi-parameter meter at each well in the field during sample collection. Sampling and analytical methods, sampling locations, and a list of the number and types of samples collected are provided in **Section 5.6**, and the results discussed in **Section 5.7**. The results for each well for each parameter

are provided in **Appendix F** on a well-by-well basis. The data collected during this event were used to evaluate potential continued COC degradation or rebound.

5.5.5 Decommissioning

The treatment system was decommissioned after completion of the demonstration. All subsurface electrical conduit, wiring and tubing was removed and properly disposed. Project equipment, including dedicated RDO sensors, the submersible pump, the dosing pumps, gas regulators and manifolds, and well heads were removed and cleaned. The crate of solar panels and other sensitive items were packed and secured inside the Conex box, and other items were packed inside the steel gas cages for transport off-site to APTIM's Lawrenceville, NJ office, as it is anticipated that the solar powered biosparging system will be used during ESTCP Project ER21-5086. All gas cylinders were disconnected and returned to the gas rental company. The wells installed during the pilot test were all abandoned in accordance with the California Department of Water Resources Well Standards. Solid and liquid wastes generated during the well abandonment activities were properly containerized, characterized, and disposed.

5.6 SAMPLING METHODS

Groundwater sampling within and surrounding the treatment zone were conducted to measure COC concentrations and distribution, measure gas amendment concentrations and distribution, quantify target cometabolic organisms/genes, and assess subsurface geochemical conditions. All activities were conducted in accordance with the site-specific health and safety documents contained in the Site-Specific Safety and Health Plan, presented in the ESTCP-approved Demonstration Plan for the project.

5.6.1 Groundwater Sampling

Groundwater samples were collected utilizing a peristatic pump and low-flow techniques. Prior to each sampling event, the well ID and sample interval were confirmed and recorded on a field data collection form. Groundwater depth measurements were then collected using an electronic water level probe (Solinst 101 water level meter, or equivalent) prior to purging and collecting groundwater samples. Water depth measurements were obtained from the surveyed mark on the top of the well casing and recorded to the nearest 0.01-ft on the field forms. Depth to water measurements were used to determine water table elevations and hydraulic gradient within the demonstration area, including the potential for water table mounding during gas injection cycles.

Dedicated Teflon tubing was used to sample each of the wells, which did not require decontamination between sampling events. A peristaltic pump with dedicated tubing was used to withdraw water from the wells at a typical flow rate between 0.1 to 0.25 liters per minute. The water level in the well was monitored and recorded during purging. Drawdown during purging was limited to <0.3 ft.

Purged water was pumped through a flow cell connected to an in-line multi-parameter groundwater meter (YSI 6920 or equivalent). Parameters, including temperature, conductivity, DO, ORP, turbidity, and pH were measured as a function of pumping time, and the values recorded on a field sheet approximately every 5 minutes. Water was purged from the well until all parameters were stable for three consecutive readings, or for a maximum of 30 minutes (to minimize the volume of

groundwater removed from the formation in the closely spaced well intervals). Stability was defined as variation of <1% for pH, <3% for temperature and specific conductivity, and <10% for DO, ORP, and turbidity. When parameters were stable according to the above guidelines, the sampling time was recorded, and all samples were collected.

The procedures used in collecting groundwater samples during the demonstration are described below. After the well parameters stabilized during low flow purging, or the well was purged for 30 minutes (whichever came first), samples were collected from the tubing prior to the flow cell, in the following order (as applicable), using the following procedures:

- VOCs: Three (3) 40 milliliter (mL) glass volatile organic analysis (VOA) vials with hydrochloric acid (HCl) (pH <2) preservative and with Teflon-lined caps were filled directly from the groundwater purge stream. The bottles were filled leaving zero head space (the bottles were filled to the top resulting in a convex meniscus). The vials were then be capped and placed on adequate ice for shipment.

- 1,4-D: Two (2) 40 mL glass VOA vials with HCl (pH <2) preservative and with Teflon-lined caps were filled directly from the groundwater purge stream. The bottles were filled leaving zero head space (the bottles were filled to the top resulting in a convex meniscus). The vials were then capped and placed on adequate ice for shipment.

- Dissolved gases (methane, ethane, ethene, propane, isobutane): Two (2) 40 mL glass VOA vials with HCl (pH <2) preservative and with Teflon-lined caps were filled directly from the groundwater purge stream. The bottles were filled leaving zero head space (the bottles were filled to the top resulting in a convex meniscus). The vials were then be capped and placed on adequate ice for shipment.

- Anions: One (1) 100 mL sample jar (plastic, no chemical preservatives) was filled to the top with water. The jar was then capped and placed on adequate ice for shipment. This sample was used for analysis of nitrate, nitrite, sulfate, phosphate, chloride, and bromide.

- Cometabolic Organisms / Genes: One (1) 1 L sample jar (plastic, no chemical preservatives) was filled to the top with water. The jar was then capped and placed on adequate ice for shipment. This sample was used for analysis of the following:

    - PPO
    - sMMO
    - pMMO
    - SCAM
    - AMO

- Proteomics: Two (2) 1 L plastic sample jars (plastic, no chemical preservatives) were filled to the top with water and treated on site by adding a solution (1 mL) containing 1,7-octadiyne in dimethyl sulfoxide. The bottles were capped and intermittently shaken by hand prior to storage on adequate ice for shipment.

5.6.2 Groundwater Analysis

Groundwater samples were submitted to APTIM's Analytical and Testing Laboratory in Lawrenceville, NJ for analysis of VOCs, 1,4-D, dissolved gases, and anions. The samples for quantification of target cometabolic organisms/genes were submitted to Microbial Insights, located in Knoxville, TN, and the samples for proteomics analysis were submitted to NCSU for analysis. The analytical methods for groundwater samples collected during the field demonstration are summarized in **Table 5.6**. Field geochemical parameters, including pH, DO, specific conductivity, ORP, temperature, and turbidity were measured at the site during well purging using a field meter, as described in **Section 5.5.**

Table 5.6. Analytical Methods for Groundwater Samples Collected during the Demonstration

Analyte[1]	Method/ Laboratory	Preservative	Bottle	Hold time
VOCs	EPA 8260 APTIM	4°C with HCl	40 mL VOA vial (x3), no headspace	14 days
1,4-Dioxane	EPA 8260/SIM APTIM	4°C with HCl	40 mL VOA vial (x3), no headspace	14 days
Dissolved Gases	EPA 3810, RSK175 APTIM[2]	4°C with HCl	40 mL VOA vial (x2), no headspace	14 days
Anions	EPA 300.0 APTIM	4°C	100 mL (x1) polyethylene screw-cap	2 days (NO_3, PO_4); 28 days all others
Cometabolic Organisms / Genes	Census DNA (qPCR) Microbial Insights	4°C	1 L (x1) polyethylene screw-cap	NA[3]
Proteomics	Activity-Based Labeling (ABL) NCSU	4°C with DMSO	1 L (x2) polyethylene screw-cap	NA[3]
Redox Potential	Field Meter	--	--	NA[3]
Dissolved Oxygen	Field Meter	--	--	NA[3]
pH	Field Meter	--	--	NA[3]
Specific Conductivity	Field Meter	--	--	NA[3]
Temperature	Field Meter	--	--	NA[3]

[1]All analyses are in groundwater

[2]Not a standard EPA Method.

[3]NA, Not applicable

5.6.3 Numbers and Types of Samples

The numbers and types of groundwater samples collected during the demonstration are summarized in **Table 5.7**. The table also provides the number of sampling events during each of the demonstration phases, including baseline sampling, system startup, and the three operational phases.

Table 5.7. Total Number and Types of Samples Collected During the Demonstration

Phase	Duration	Matrix	Number of Events	Number of Samples Per Event[3]	Analyte	Location
Baseline Sampling	Two-time event: Prior to system startup	Groundwater	2	12	VOCs	11 performance monitoring wells and EW-1
				12	1,4-Dioxane	
				12	Anions	
				12	Dissolved Gases	
				12	Field Parameters[1]	
			1	6	Cometabolic organisms/genes[2] and proteomics	PMW-3S, PMW-3D, PMW-4S, PMW-4D, DMW-1, and OU11-SMW08A
Phase 1: Groundwater Recirculation and Oxygen Sparging	1 week (System Start Up)	Groundwater	5	12	Dissolved Oxygen	11 performance monitoring wells and EW-1
	2 months	Groundwater	2	12	VOCs	11 performance monitoring wells and EW-1
				12	1,4-Dioxane	
				12	Isobutanol	
				12	Anions	
				12	Dissolved Gases	
				12	Field Parameters[1]	
			1	6	Cometabolic organisms/genes[2] and proteomics	PMW-3S, PMW-3D, PMW-4S, PMW-4D, DMW-1, and OU11-SMW08A
Phase 2: System Operation and Monitoring	12 months	Groundwater	8	7-10	Dissolved Gases	PMW-1D, PMW-2S, PMW-3S, PMW-3D, PMW-4S, PMW-4D, and EW-1 Locations added during select events: PMW-1S, PMW-2D and OU11-SMW08A
				7-10	Field Parameters[1]	
			5	7-10	VOCs	
			7	12	VOCs	11 performance monitoring wells and EW-1
				12	1,4-Dioxane	
				12	Anions	
				12	Dissolved Gases	
				12	Field Parameters[1]	
			8	12	Isobutanol	
			2	6	Cometabolic organisms/genes[2] and proteomics	PMW-3S, PMW-3D, PMW-4S, PMW-4D, DMW-1, and OU11-SMW08A
Phase 3: Post Treatment Monitoring	One-time event: 2.5 months after system shutdown	Groundwater	1	12	VOCs	11 performance monitoring wells and EW-1
				12	1,4-Dioxane	
				12	Anions	
				12	Dissolved Gases	
				12	Field Parameters[1]	

Notes:

[1] Field parameters include pH, temperature, dissolved oxygen, oxidation/reduction potential, and specific conductivity.

[2] Census DNA (qPCR) was performed by Microbial Insights and proteomics analysis was performed by NCSU.

[3] Sample total does not include duplicate or QA/QC samples.

5.6.4 Decontamination and Purge Water Waste Handling and Disposal

Sampling and measuring equipment that were reused in multiple wells were decontaminated prior to use. This includes water level indicators, multi-parameter water quality meters and flow cells, DO probes, water level transducers, and any other instrumentation or material potentially exposed to COCs. Decontamination of sampling and measurement equipment included the following:

- Initial wash using Alconox, or other approved detergent;
- Rinse with potable water; and
- Air dry or drying using clean towel.

Decontamination fluids were pumped to 55-gallon drums, to await proper disposal. The use of peristaltic pumps with dedicated down-hole tubing precluded the need for decontamination of these components.

Purged groundwater during sampling, groundwater extracted during well development, and decontamination of equipment generated liquid investigation derived waste. These fluids were containerized in 55-gallon drums and temporarily staged at the IWTP at NASNI. The contents of the drums were sampled for waste characterization and subsequent off-site disposal.

5.6.5 Quality Assurance for Groundwater Sampling and Analysis

5.6.5.1 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 can have an appreciable impact on data quality and are performed regularly. In addition, preventative maintenance is important to the efficient collection of data. For preventative maintenance purposes, critical spare parts were obtained from the instrument manufacturer.

All field and laboratory instruments were calibrated according to manufacturers' specifications. All APTIM laboratory instruments were calibrated in accordance with established Standard Operating Procedures. Calibration was performed prior to initial use, during periods of extended use, and after periods of non-use. Certified standards were used for all calibrations and calibration check measurements. A calibration logbook was maintained by APTIM field and laboratory quality assurance personnel.

5.6.5.2 Quality Control Samples

Internal quality control (QC) data provides information for identifying and defining qualitative and quantitative limitations associated with measurement data. Analysis of the following types of QC samples provided the primary basis for quantitative evaluation of field data quality:

Field QC Samples:

- Trip blanks to evaluate the presence of contamination from handling errors or cross-contamination during transport; and
- Field duplicates to assess the homogeneity of samples received by the laboratory as well as the homogeneity of COCs in the matrix.

5.6.5.3 Trip Blanks

Trip blanks were prepared by the analytical laboratory with purified water for groundwater samples. The water was sent to the site in the same containers to be used for collection of the samples. Trip blanks were submitted at a frequency of one trip blank per shipment of samples for VOC analysis. For non-VOC analyses, no trip blanks were deemed necessary, and none were submitted.

5.6.5.4 Field Duplicate Samples

Field duplicate samples were analyzed for all parameters, except quantification of target cometabolic organisms/genes, to evaluate the accuracy of the analytical process. Each duplicate was run at a frequency of at least 5% of the total number of environmental samples. A comparison of the detected concentrations in the duplicate samples was performed to evaluate precision.

5.6.5.5 Sample Documentation

APTIM Lawrenceville, NJ project staff coordinated shipment and receipt of sample bottles, coolers, ice packs, chain of custody (COC) forms, and custody seals. Upon completion of sampling, the COC was filled out and returned with the samples to the APTIM and Microbial Insights laboratories. An electronic copy of each COC form was placed in the project database. An important consideration for the collection of environmental data is the ability to demonstrate that the analytical samples have been obtained from predetermined locations and that they have reached the laboratory without alteration. Evidence of collection, shipment, laboratory receipt, and laboratory custody until disposal was documented to accomplish this. Documentation was accomplished through a COC Record that recorded each sample and the names of the individuals responsible for sample collection, transport, and receipt. A sample was considered in custody if it was:

- in a person's actual possession;
- in view after being in physical possession;
- sealed so that no one can tamper with it after having been in physical custody; or
- in a secured area, restricted to authorized personnel.

Sample custody was initiated by field personnel upon collection of samples. Samples were packaged appropriately to prevent breakage or leakage during transport and shipped to the laboratory via commercial carrier.

5.6.5.6 Sample Identification

A discrete well number was assigned to each sample. This discrete identifier was placed on each bottle and recorded, along with other pertinent data, in a field notebook and/or field forms dedicated to the project. The sample identification number designated the sample location (e.g.,

"PMW-1S"). The bottle label also contained the site name, the sampling date and time, any preservatives added to the bottle, and the initials of the sampler.

5.6.5.7 Chain-of Custody Forms

The COC Record that was used by APTIM's laboratory is shown in **Figure 5.37**. All samples collected for off-site analysis were physically inspected by the field sampler prior to shipment.

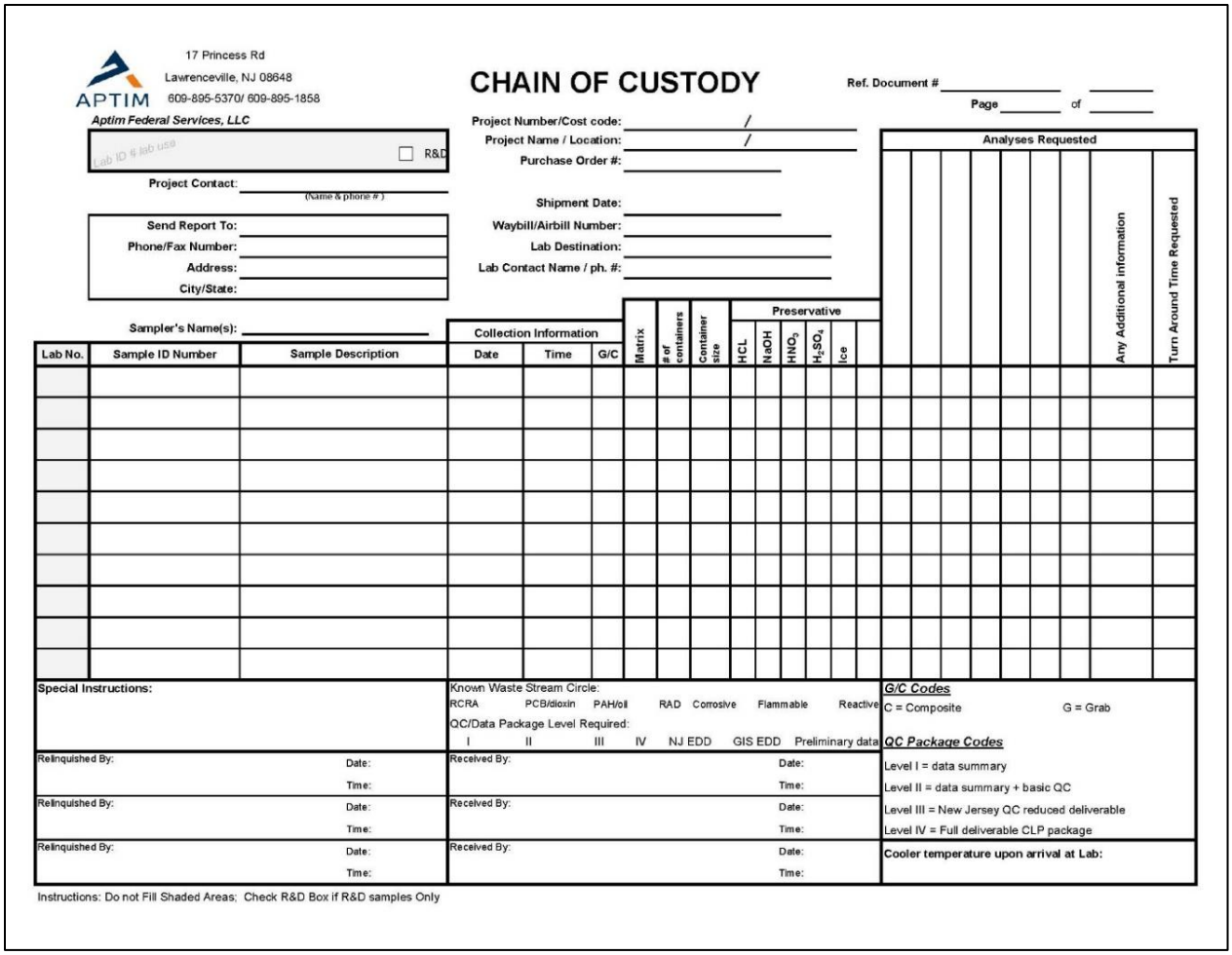
Everyone who had samples in their possession were required to sign the COC Record. Preparation of the COC Record was as follows:

- The COC Record was initiated in the field by the person collecting the sample, for every sample. Every sample was assigned a unique identification number on the COC Record.
- The record was completed in the field to indicate project, sampling person, etc.
- The person delivering the samples for shipment signed the COC record as "Relinquished By _____".
- The original COC Record was sealed in a watertight bag, taped to the top (inside) of the shipping container, and the shipping container was sealed prior to being given to the commercial carrier.
- Upon receipt by the off-site laboratory, the laboratory QC Coordinator, or designated representative, opened the shipping container(s), compared the contents with the COC Record, and signed and dated the record. Discrepancies were noted on the COC Record.
- COC Records were maintained with the project files and became part of the data package.

5.6.5.8 Laboratory Sample Receipt

Following sample receipt, the laboratory manager or qualified personnel:

- Examined all samples and determined if proper temperature had been maintained during transport. If samples had been damaged during transport, the remaining samples were carefully examined to determine whether they were affected;
- Compared samples received against those listed on the COC record;
- Verified that sample holding times were not exceeded;
- Signed and dated the COC record;
- Recorded samples in the laboratory sample log-in book containing, at a minimum, the following information:
  - Project identification number
  - Sample numbers
  - Type of samples
  - Date and time received
- Placed the COC Record in the project file.



17 Princess Rd
Lawrenceville, NJ 08648
609-895-5370/ 609-895-1858

APTIM

Aptim Federal Services, LLC

CHAIN OF CUSTODY

Ref. Document # _____ _____
Page _____ of _____

Lab ID # lab use ☐ R&D

Project Number/Cost code: _____ /
Project Name / Location: _____ /
Purchase Order #: _____

Project Contact: _____ (Name & phone #)

Send Report To: _____
Phone/Fax Number: _____
Address: _____
City/State: _____

Shipment Date: _____
Waybill/Airbill Number: _____
Lab Destination: _____
Lab Contact Name / ph. #: _____

Sampler's Name(s): _____

Lab No.	Sample ID Number	Sample Description	Collection Information: Date	Time	G/C	Matrix	# of containers	Container size	Preservative: HCL	NaOH	HNO_3	H_2SO_4	Ice	Analyses Requested							Any Additional information	Turn Around Time Requested

Special Instructions:

Known Waste Stream Circle:
RCRA PCB/dioxin PAH/oil RAD Corrosive Flammable Reactive

QC/Data Package Level Required:
I II III IV NJ EDD GIS EDD Preliminary data

G/C Codes
C = Composite G = Grab

QC Package Codes
Level I = data summary
Level II = data summary + basic QC
Level III = New Jersey QC reduced deliverable
Level IV = Full deliverable CLP package

Relinquished By:	Date: / Time:	Received By:	Date: / Time:
Relinquished By:	Date: / Time:	Received By:	Date: / Time:
Relinquished By:	Date: / Time:	Received By:	Date: / Time:

Cooler temperature upon arrival at Lab:

Instructions: Do not Fill Shaded Areas; Check R&D Box if R&D samples Only

Figure 5.37. Chain of Custody (COC) Form Used by APTIM's Laboratory. The commercial waybill served as an extension of the COC Record between the final field custodian and receipt by the off-site laboratory.

5.6.5.9 Other Documentation

Following sample receipt at the laboratory, the Laboratory Manager or sample custodian clearly documented the processing steps applied to the sample. The analytical data from laboratory QC samples were identified with each batch of related samples. The laboratory logbook included the time, date, and name of the person who logged each sample into the laboratory system. This documentation was thorough enough to allow tracking of the sample analytical history without aid from the analyst, if needed. At a minimum, laboratory documentation procedures provided the following:

- Recording in a clear, comprehensive manner using indelible ink.
- Corrections to data and logbooks made by drawing a single line through the error and initialing and dating the correction.
- Consistency before release of analytical results by assembling and cross-checking the information on the sample tags, custody records, bench sheets, personal and instrument

logs, and other relevant data to verify that data pertaining to each sample are consistent throughout the record.

- Observations and results identified with the project number, date, and analyst and reviewer signatures on each line, page, or book as appropriate.
- Data recorded in bound books or sheaf of numbered pages, instrument tracings or hard copy, or computer hard copy.
- Data tracking through document consolidation and project inventory of accountable documents: sample logbook, analysis data book, daily journal, instrument logbook, narrative, and numerical final reports, etc.

5.7 SAMPLING RESULTS

The results from the performance monitoring conducted during baseline sampling, the two operational phases, and post treatment sampling are summarized in the following subsections. As discussed in **Section 5.5.3**, and as summarized in **Table 5.5**, most of the performance sampling was performed during Phase 2 of the demonstration. The results for each well for each parameter are provided in **Appendix F** on a well-by-well basis.

5.7.1 CVOCs and 1,4-D

As discussed in **Section 1.2**, the overall objective of this work was to treat groundwater impacted with comingled CVOCs and 1,4-D using a novel MPS cometabolic bioremediation technology. To evaluate effectiveness, CVOC and 1,4-D concentrations in groundwater were monitored. The PMW network (**Figure 5.15**) consisted of a total of 11 monitoring wells located within, upgradient and downgradient of the treatment zone as detailed in **Section 5.4.1**. Eight of the 11 monitoring wells (PMW-1S/D, PMW-2S/D, PMW-3S/D, and PMW-4S/D) were shallow/deep well pairs located within or at the edge of the treatment zone and were used to monitor two separate intervals within the vertical thickness of the treatment zone.

5.7.1.1 Baseline and Phase 1

As shown in **Table 5.8**, baseline CVOC groundwater data collected from the four S/D well pairs (PMW-1S/D, PMW-2S/D, PMW-3S/D and PMW-4S/D) on day -34 indicated that 1,1-DCE, TCE *cis*-DCE, and 1,1-DCA concentrations in groundwater were generally an order of magnitude higher in the deeper portion of the treatment zone. This is consistent with the discrete-interval groundwater sampling data collected during site characterization activities (**Section 5.2.1.3**). The data presented in **Figures 5.38** through **5.41** show that CVOC concentrations in the shallow/deep well pairs generally equilibrated due to mixing of the groundwater within the treatment zone during Phase 1 of the demonstration (days 0 through 50). Baseline 1,4-D concentration data presented in **Table 5.8** and **Figure 5.42** show less of a concentration difference between the S/D well pairs, and concentrations taking slightly longer (~60 days of groundwater recirculation) to equilibrate.

Table 5.8. Comparison of Baseline Sampling Results at the Shallow/Deep Well Pairs

Compound	4 Shallow Wells (~28-33' bgs)		4 Deep Wells (~34-39'bgs)		Magnitude of Difference (Avg. Deep / Avg. Shallow)
	Concentration Range (µg/L)	Average Concentration (µg/L)	Concentration Range (µg/L)	Average Concentration (µg/L)	
1,1-DCE	14 - 227	91	1,805 - 2,898	2482	27.3
TCE	12.5 - 190	70	1,621 - 2,076	1998	28.5
cis-DCE	3.4 - 128	46	619 - 1,317	872	19.0
1,1-DCA	<1 - 24	8	121 - 198	169	21.1
1,4-Dioxane	55 - 376	146	249 - 699	453	3.1

5.7.1.2 Phase 2

1,1-DCE

The groundwater monitoring data show that, once equilibrated, 1,1-DCE concentrations decreased appreciably, with typical decreases between ~25 and 55% at six of the eight treatment zone PMWs and EW-1 during the first few weeks of Phase 2 operation (days 51 through 98, shown on **Figure 5.38**), after the first bioaugmentation and isobutanol and isobutane addition. However, data from several subsequent sampling events (performed on days 98 through 183) indicated that the decline of 1,1-DCE concentrations had stalled. As discussed in **Section 5.7.3**, isobutane consumption in the aquifer also had appreciably slowed during this time, despite adequate concentrations of dissolved oxygen and inorganic nutrients. Therefore, isobutane addition was temporarily halted on day 105.

The initial decline in 1,1-DCE suggested that the treatment approach (bioaugmentation, isobutanol and then isobutane addition) had been successful, but that ENV493 (as well as other potential indigenous isobutane-oxidizers) had not reached the *in situ* densities necessary to degrade 1,1-DCE to non-inhibitory concentrations. Thus, as detailed in **Section 5.5.3.1**, a second bioaugmentation was performed on day 183, along with a larger addition of isobutanol (**Section 5.5.3.2)**, and resumption of isobutane addition on day 205 (after groundwater data indicate that the isobutanol had been consumed). CVOC data collected at EW-1 between days 199 and 219 showed that 1,1-DCE concentrations declined further from 457 µg/L to 180 µg/L in the EW (**Figure 5.38**, panel F). 1,1-DCE concentrations also decreased appreciably (between 73 and 85%) at the eight treatment zone PMWs between the end of Phase 1 (sampling performed on day 43) and day 246 **(Figure 5.38**, panels A through D). However, data collected from EW-1 between days 219 and 298, and at treatment zone PMWs on day 274, indicated that the decline of 1,1-DCE concentrations had again stalled. As discussed in **Section 5.7.3**, dissolved isobutane concentrations generally increased between days 253 and 281, suggesting that 1,1-DCE concentrations had not yet reached non-inhibitory levels after the second bioaugmentation, and isobutane addition was temporarily halted for a second time on day 280.

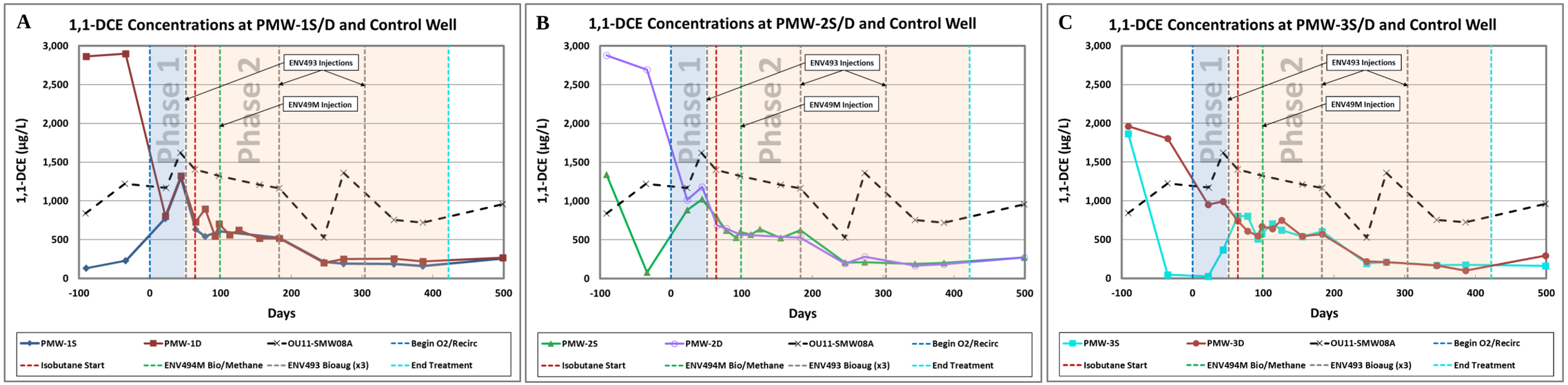


Figure 5.38. 1,1-DCE Concentrations at the Demonstration Monitoring Wells. Control well OU11-SMW08A data plotted with PMW-1S/D (panel A), PMW-2S/D (panel B), PMW-3S/D (Panel C), PMW-4S/D (panel D), PTW-1 and DMW-1 (panel E), and EW-1 (panel F).

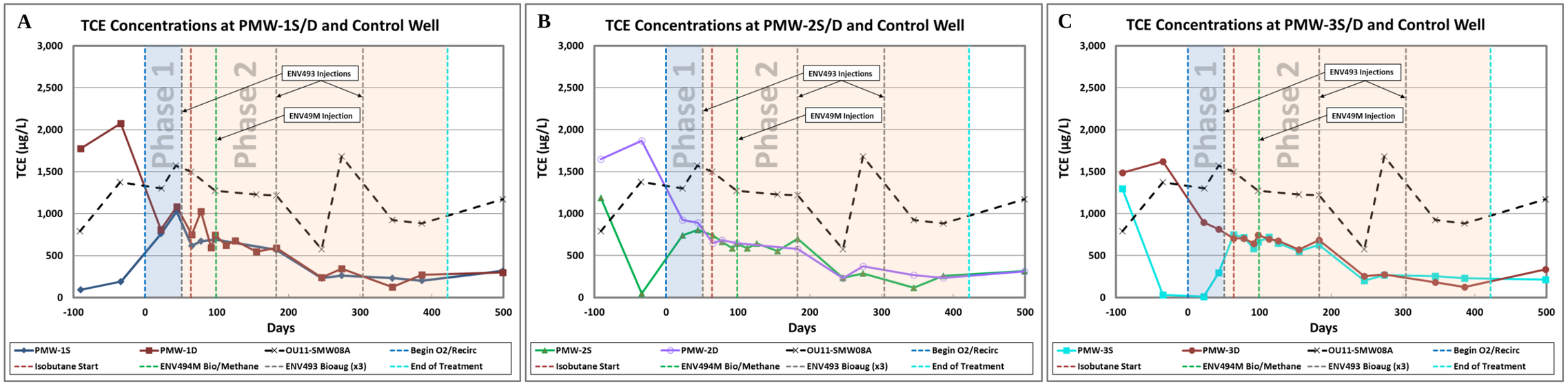


Figure 5.39. TCE Concentrations at the Demonstration Monitoring Wells. Control well OU11-SMW08A data plotted with PMW-1S/D (panel A), PMW-2S/D (panel B), PMW-3S/D (Panel C), PMW-4S/D (panel D), PTW-1 and DMW-1 (panel E), and EW-1 (panel F).

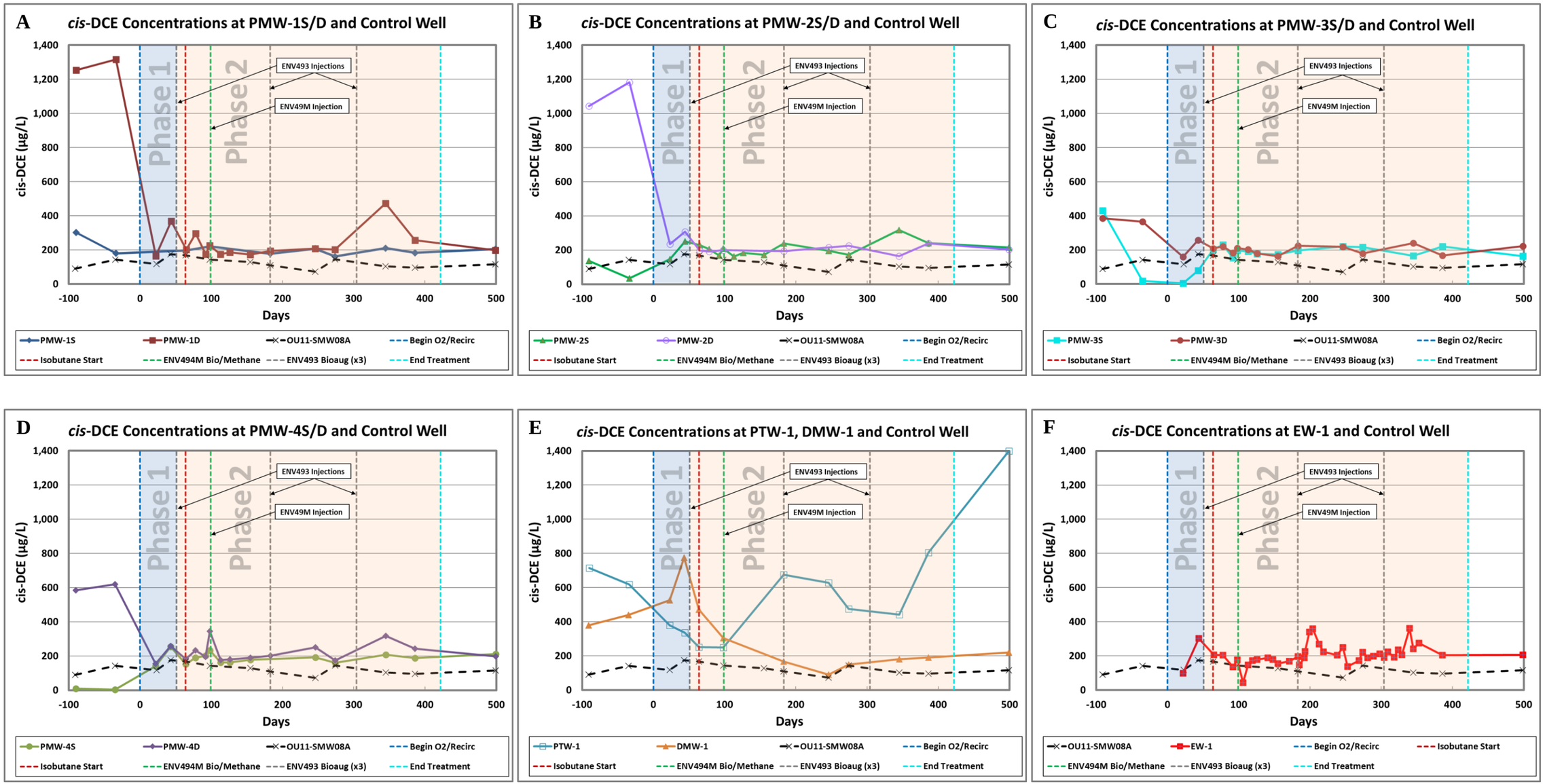


Figure 5.40. *cis*-DCE Concentrations at the Demonstration Monitoring Wells. Control well OU11-SMW08A data plotted with PMW-1S/D (panel A), PMW-2S/D (panel B), PMW-3S/D (Panel C), PMW-4S/D (panel D), PTW-1 and DMW-1 (panel E), and EW-1 (panel F).

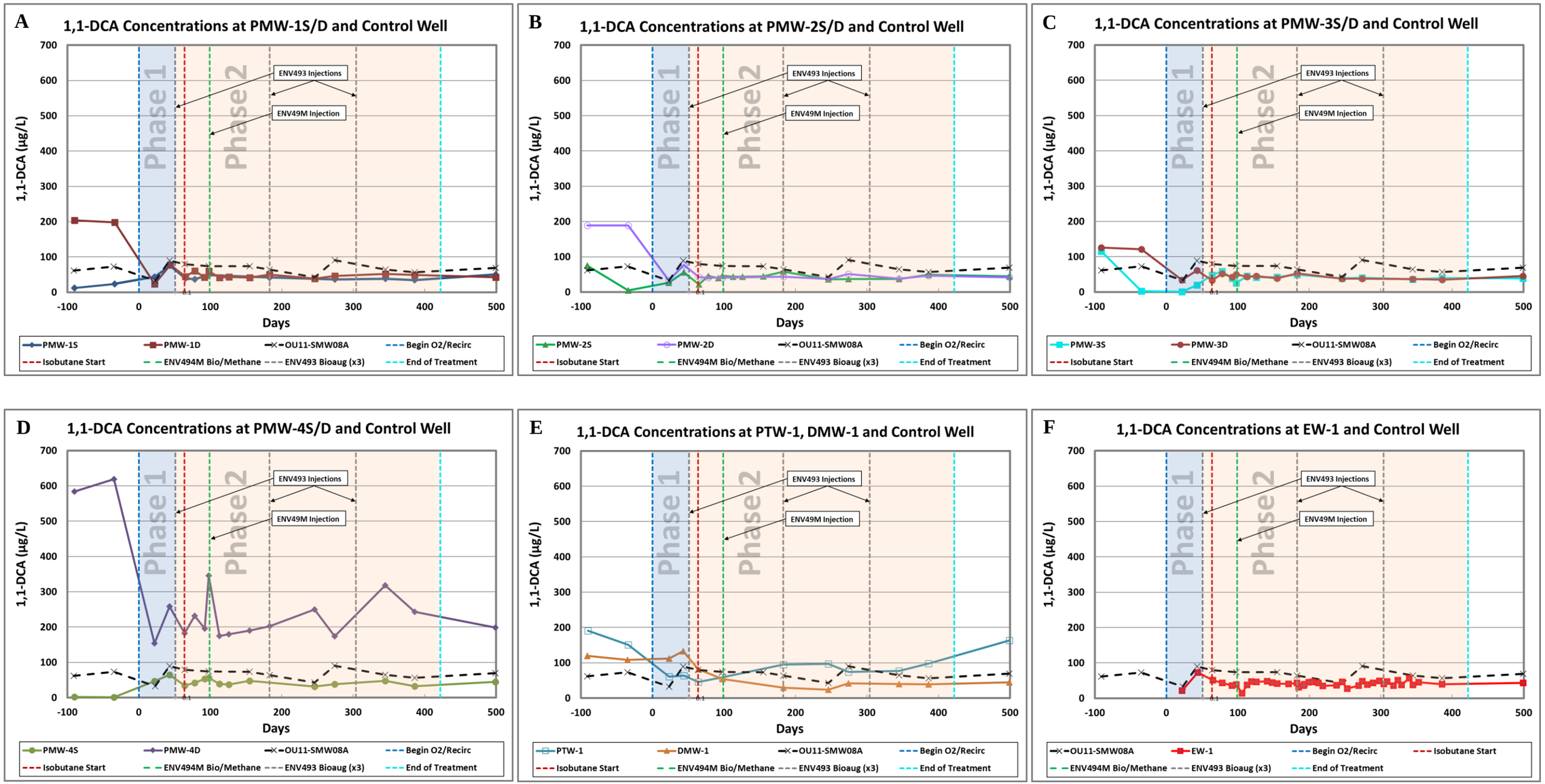


Figure 5.41. 1,1-DCA Concentrations at the Demonstration Monitoring Wells. Control well OU11-SMW08A data plotted with PMW-1S/D (panel A), PMW-2S/D (panel B), PMW-3S/D (Panel C), PMW-4S/D (panel D), PTW-1 and DMW-1 (panel E), and EW-1 (panel F).

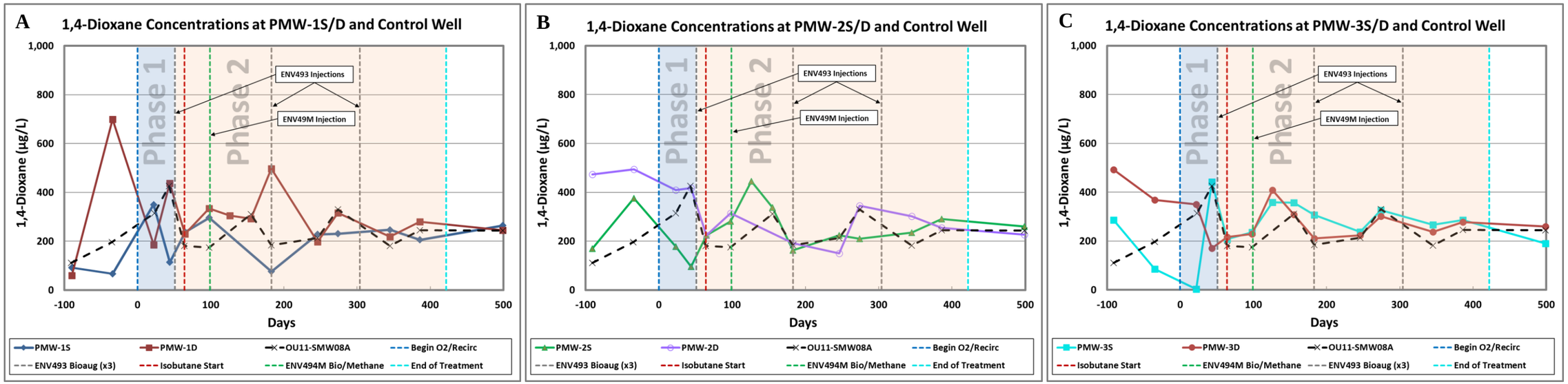


Figure 5.42. 1,4-D Concentrations at the Demonstration Monitoring Wells. Control well OU11-SMW08A data plotted with PMW-1S/D (panel A), PMW-2S/D (panel B), PMW-3S/D (Panel C), PMW-4S/D (panel D), PTW-1 and DMW-1 (panel E), and EW-1 (panel F).

As detailed in **Section 5.3.3.1**, a third bioaugmentation with ENV493 was performed on day 304, along with isobutanol addition (**Section 5.5.3.2**). Unfortunately, as shown on **Figure 5.38**, further appreciable decreases in 1,1-DCE concentrations were not observed after the third bioaugmentation. As summarized on **Table 5.9**, 1,1-DCE concentrations decreased between 73 and 90% at the four treatment zone PMW pairs during Phase 2, with an average decrease of 82%. 1,1-DCE concentrations decreased 82 percent at EW-1 during this same period.

As shown on **Figure 5.38** (panel E) and **Table 5.9**, an appreciable decrease in 1,1-DCE concentrations was observed at downgradient monitoring well DMW-1 during Phase 2, with concentrations dropping from 2,064 µg/L on day 43 to 298 µg/L on day 386 (an 86% decrease). 1,1-DCE concentrations measured at monitoring well PTW-1 (located at the upgradient edge of the target treatment zone) during Phase 2 were generally higher than the four S/D well pairs within the treatment zone, and as expected, showed no appreciable downward trend. Data collected at upgradient control well OU11-SMW08A (**Figures 5.38**, all six panels) showed that, while there were modest fluctuations, there was no downward trend in 1,1-DCE concentrations during Phase 2, indicating that COC flux into the treatment zone was relatively steady during the field demonstration.

TCE

Prior to bioaugmentation with ENV494M and the start of methane addition on day 99, appreciable decreases in TCE did not occur at the treatment zone PMWs and EW-1 during the first few weeks of Phase 2 operations (days 51 through 98, shown on **Figure 5.39**). This was expected, as laboratory treatability testing (**Section 5.3**) using site soils and groundwater indicated that the continued presence of 1,1-DCE was inhibitory to the cometabolic degradation of TCE (and other CVOCs and 1,4-D) by both isobutane and methane oxidizers, and that isobutane oxidizers did not have a high affinity for degrading TCE. However, data from the subsequent Phase 2 groundwater sampling events (performed on days 113 through 386), indicated that TCE concentrations decreased appreciably after bioaugmentation with ENV494M and the start of methane addition. As summarized on **Table 5.9**, TCE concentrations decreased between 22 and 85 percent at the four S/D treatment zone monitoring well pairs during Phase 2, with an average decrease of 73 percent. TCE concentrations decreased 73 percent at EW-1 during this same period.

As shown on **Figure 5.39** and **Table 5.9**, an appreciable decrease in TCE concentrations was observed at downgradient monitoring well DMW-1 during Phase 2, with concentrations dropping from 1,339 µg/L on day 43 to 379 µg/L on day 386 (a 72 percent decrease). As with 1,1-DCE, TCE concentrations measured at monitoring well PTW-1 (located at the upgradient edge of the target treatment zone) during Phase 2 were generally higher than the four S/D well pairs within the treatment zone, and as expected, showed no appreciable downward trend. Data collected at upgradient control well OU11-SMW08A (**Figure 5.39**, all six panels) showed that, while there were modest fluctuations, there was no downward trend in TCE concentrations during Phase 2, indicating that COC flux into the treatment zone was relatively steady during the field demonstration.

Table 5.9. Summary of CVOC and 1,4-D Concentration Changes During Phase 2

Well ID	Type of Well	1,1-DCE (µg/L)		Percent	TCE (µg/L)		Percent	cis-DCE (µg/L)		Percent	1,1-DCA (µg/L)		Percent	1,4-D (µg/L)		Percent
		Day 43	Day 386	Change	Day 43	Day 386	Change	Day 43	Day 386	Change	Day 43	Day 386	Change	Day 43	Day 386	Change
PMW-1S	Shallow Treatment Zone	1,298	158	-88	1,028	201	-80	302	183	-39	75.2	34.4	-54	114	206	81
PMW-1D	Deep Treatment Zone	1,321	220	-83	1,082	271	-75	368	257	-30	77.2	48.9	-37	437	281	-36
PMW-2S	Shallow Treatment Zone	1,025	204	-80	807	256	-68	251	240	-4	55.6	49.0	-12	95.7	291	204
PMW-2D	Deep Treatment Zone	1,181	186	-84	891	235	-74	307	239	-22	76.8	46.8	-39	417	255	-39
PMW-3S[1]	Shallow Treatment Zone	366	175	-52	295	230	-22	78.8	164	108	ND[3]	39.1	NM	442	286	-35
PMW-3D	Deep Treatment Zone	990	99.6	-90	813	123	-85	257	167	-35	61.0	34.8	-43	170	278	64
PMW-4S	Shallow Treatment Zone	990	148	-85	787	159	-80	253	188	-26	64.8	32.9	-49	425	279	-34
PMW-4D	Deep Treatment Zone	966	256	-73	780	281	-64	259	243	-6	51.8	42.4	-18	462	280	-39
Average of Treatment Zone Wells		**1,017**	**181**	**-82**	**810**	**220**	**-73**	**259**	**210**	**-19**	**66.1**	**41.3**	**-37**	**320**	**270**	**-16**
DMW-1	Downgradient	2064	298	-86	1339	379	-72	775	191	-75	133	39.00	-71	398	596	50
PTW-1	Slightly Upgradient	1096	934	-15	804	672	-16	336	805	140	64.2	98.2	53	455	633	39
EW-1	Extraction Well	1132	194	-83	934	248	-73	301	205	-32	72.8	39.7	-45	449	294	-35
OU11-SMW08A[2]	Upgradient Control	1622	722	-55	1573	882	-44	175	95.7	-45	89.4	56.5	-37	426	245	-42

Notes:

[1]CVOC concentrations measured at PMW-3S on day 44 were significantly lower than those measured during the previous and subsequent sampling events.

[2]CVOC and 1,4-D concentrations measured at Control Well OU11-SMW08A fluctuated moderately throughout the demonstration and were at or near their highest point of the demonstration on day 44, and their lowest point on 386, which caused the calculated percent change to be artificially negative.

[3]1,1-DCA was below the analaytical detection limit of 20 ug/L for this diluted sample, but was measured is subsequent samples collected at this well.

NM=Not measured

ND=Non Detect (below the analyticel detection limit for that sample)

cis-DCE and 1,1-DCA

The groundwater monitoring data show that, once equilibrated, *cis*-DCE and 1,1-DCA concentrations did not show decreasing trends at the treatment zone PMWs and EW-1 during Phase 2 of the demonstration (**Figures 5.40** and **5.41**, and **Table 5.9**). The lack of degradation suggests that the continued presence of 1,1-DCE was inhibitory to the cometabolic degradation of these compounds. The fact that their relative decrease was less than observed for 1,1-DCE may reflect either enzyme affinity (i.e., relative affinity of the different CVOCs to the SCAM enzyme) or that each was present at a much lower molar concentration than 1,1-DCE in site groundwater. Thus, 1,1-DCE was preferentially degraded by the SCAM enzyme and the resulting reactive epoxide deactivated the enzyme and/or killed the cells. *cis*-DCE concentrations decreased at downgradient well DMW-1 during Phase 2 of the demonstration, possibly due to the mixing of groundwater within the treatment zone.

1,4-D

The groundwater monitoring data show that, once equilibrated, 1,4-D concentrations did not show decreasing trends at the treatment zone PMWs and EW-1 during Phase 2 of the demonstration (**Figure 5.42** and **Table 5.9**). The lack of degradation suggests that, like *cis*-DCE and 1,1-DCA, the continued presence of 1,1-DCE was inhibitory to the cometabolic degradation of 1,4-D, with enzyme affinities and/or relative concentrations determining degradation rates.

5.7.1.3 Post Treatment Sampling

As discussed in **Section 5.5.4**, while the addition of oxygen, isobutane, methane, and nutrients had stopped by the end of Phase 2 (day 422), the groundwater recirculation system continued to operate intermittently between days 323 and 499 (approximately 2 ½ months) to maintain a degree of hydraulic control within the treatment zone.

As shown in **Figures 5.38** through **5.42**, post treatment groundwater data collected on July 27, 2022 (day 499) indicated that there were no appreciable changes in CVOC and 1,4-D concentrations at treatment zone PMWs, downgradient monitoring well DMW-1, or EW-1 during this post treatment period. However, there were spikes in 1,1-DCE, TCE, *cis*-DCE, 1,1-DCA and 1,4-D concentrations at monitoring well PTW-1, located slightly upgradient of the treatment area. These data suggest that this well was partially impacted during Phase 2 but was far enough outside of the target treatment zone that target COC concentrations rebounded after amendment addition ceased. Data collected at upgradient control well OU11-SMW08A showed that, while there were modest fluctuations, there was no appreciable change in target COC concentrations during Phase 2, indicating that COC flux into the treatment zone was relatively steady during the field demonstration.

5.7.2 Dissolved Oxygen

Groundwater samples were collected and analyzed in the field for DO at the 11 monitoring wells and EW-1 during baseline, Phase 1, Phase 2, and the post treatment monitoring events, as detailed in **Section 5.5** and summarized in **Table 5.7**. DO concentrations measured at the 11 monitoring wells and EW-1 during Phase 2 are summarized in **Table 5.10** and provided as box plots on **Figure 5.43**. DO concentration data are provided in **Appendix F** on a well-by-well basis. Results of the data collected are summarized as follows:

- DO concentrations ranged between 0.02 mg/L and 41.02 mg/L at the eight treatment zone PMWs during Phase 2, with average concentrations ranging between 3.69 mg/L and 26.56 mg/L (as summarized in **Table 5.10**);
- DO concentrations were greater than 1.0 mg/L at six of the eight treatment zone PMWs (PMW-1D, PMW-2D, PMW-03S/D and PMW-04S/D), EW-1 and downgradient well DMW-1 at between 75 and 100 percent of the measurements collected during Phase 2 of the demonstration, achieving the second performance objective summarized in **Table 3.1** and detailed in **Section 3.2**. DO concentrations were >1.0 mg/L 46 percent and 73 percent of the time at wells PMW-1S and PMW-02S (both near the edge of the treatment zone) during Phase 2 of the demonstration, respectively. Eighty-six percent of the DO measurements collected at the eight treatment zone PMWs during Phase 2 were > 1.0 mg/L, with 79 percent of the measurements > 5 mg/L;
- DO concentrations ranged between 0.12 mg/L and 1.56 mg/L at control well OU11-SMW08A during Phase 2 of the demonstration, indicating that this well was outside the influence of the oxygen sparging system;
- DO concentrations at upgradient monitoring well PTW-1 typically measured below 3 mg/L during Phase 2, indicating that this well was at the outer edge of the treatment zone;
- As discussed in **Sections 5.5.2** and **5.5.3**, oxygen sparging cycles were only required approximately once every two weeks during Phase 1, and approximately once every two to three days during Phase 2 to maintain aerobic conditions in the aquifer, indicating that biological oxygen demand increased after bioaugmentation and amendment addition; and,
- DO concentrations decreased in the treatment zone during and after each of the isobutanol injections, due to the additional biological oxygen demand created by the addition of this carbon substrate (additional oxygen sparge cycles were performed during these periods to maintain target DO concentrations within the treatment zone).

Table 5.10. Summary of DO Concentration Data Collected During Phase 2

Well ID	Well Type	Well Location	Minimum Concentration (mg/L)	Maximum Concentration (mg/L)	Average Concentration (mg/L)	Median Concentration (mg/L)	Average Readings > 1.0 mg/L (percent)	Average Readings > 5.0 mg/L (percent)
PMW-1S	Monitoring	Treatment Zone	0.03	15.72	3.69	1.23	50.00	30.00
PMW-1D	Monitoring	Treatment Zone	0.02	25.17	14.52	17.71	78.57	78.57
PMW-2S	Monitoring	Treatment Zone	0.12	29.36	14.22	17.05	71.43	64.29
PMW-2D	Monitoring	Treatment Zone	4.44	34.97	23.80	28.61	100.00	90.00
PMW-3S	Monitoring	Treatment Zone	10.49	34.18	23.20	23.71	100.00	100.00
PMW-3D	Monitoring	Treatment Zone	11.23	41.02	26.56	24.54	100.00	100.00
PMW-4S	Monitoring	Treatment Zone	0.44	22.77	12.07	14.85	92.31	69.23
PMW-4D	Monitoring	Treatment Zone	0.14	34.64	15.58	16.69	92.86	78.57
PTW-1	Monitoring	Upgradient	0.19	22.52	4.93	2.11	66.67	16.67
OU11-SMW08A	Monitoring	Upgradient	0.12	1.56	0.53	0.39	12.50	0.00
DMW-1	Monitoring	Downgradient	4.17	26.72	20.00	23.44	100.00	83.33
EW-1	Extraction	Treatment Zone	4.50	29.56	16.73	14.73	100.00	91.67

-Shaded data represent achievement of performance objective.

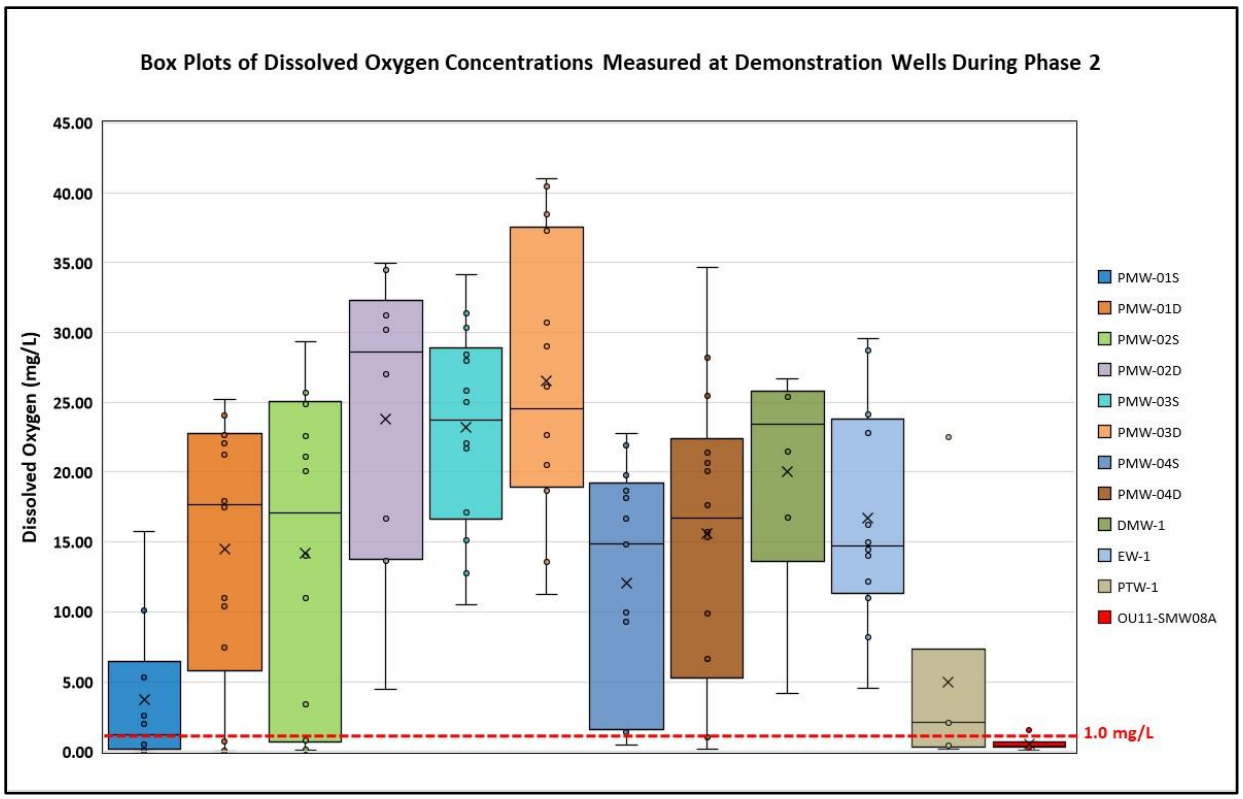


Figure 5.43. Box Plots of DO Concentrations Measured at Demonstration Monitoring Wells During Phase 2

5.7.3 Dissolved Isobutane

Groundwater samples were collected and analyzed for dissolved isobutane at the 11 demonstration monitoring wells and EW-1 during Phase 1, Phase 2, and the post treatment monitoring events, as detailed in **Section 5.5** and summarized in **Table 5.7**. As discussed in **Section 5.5.3.2**, isobutane addition to the groundwater recirculation process stream was performed over three separate time periods, each occurring after ENV493 bioaugmentation events. Changes to isobutane injection concentrations and duration throughout Phase 2 of the demonstration were made primarily based on observed dissolved isobutane concentrations measured at EW-1.

Dissolved isobutane concentrations measured at the 11 demonstration monitoring wells are plotted on **Figure 5.44**, and concentrations measured at the control well and EW-1 are plotted on **Figure 5.45**. Dissolved isobutane concentration data are provided in **Appendix F** on a well-by-well basis. Results of the data collected are summarized as follows:

- Dissolved isobutane was observed in all eight treatment zone PMWs, PTW-1, and EW-1 during each of the three addition periods, with concentrations ranging from <10 µg/L to > 1,500 µg/L (PMW-4S on day 98);
- Dissolved isobutane concentrations exceeded the target 200 µg/L in seven of the eight treatment zone PMWs within 21 days of initiating isobutane addition (achieving one of the demonstrations quantitative performance objectives), and at the eighth monitoring well (PMW-3S) within 35 days;

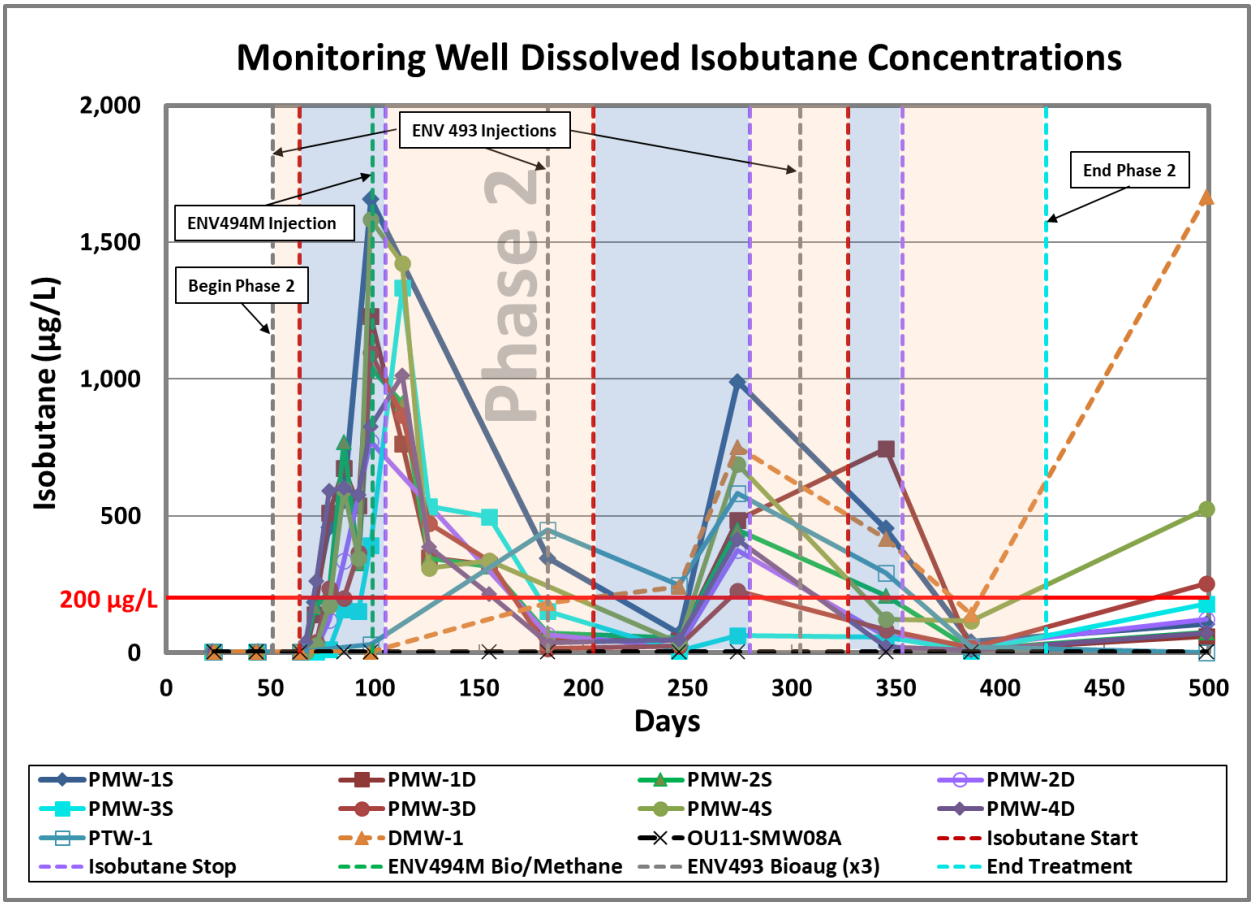


Figure 5.44. Dissolved Isobutane Concentrations at the Monitoring Wells. Phase 2 began on day 51 with the first ENV493 bioaugmentation injection. The three periods of isobutane addition are highlighted in blue. The performance objective of achieving a target dissolved isobutane concentration of >200 µg/L in at least six of the eight treatment zone PMWs during the first month of isobutane addition was achieved when seven of the eight wells exceeded 200 µg/L within 21 days.

- Dissolved isobutane concentrations generally increased in the treatment zone PMWs between days 85 and 98, indicating consumption of isobutane had slowed during that time, despite adequate concentrations of dissolved oxygen and inorganic nutrients. Therefore, isobutane addition was temporarily halted on day 105;
- Dissolved isobutane concentrations at the treatment zone PMWs and EW-1 measured between days 98 and 183 confirmed slow consumption of isobutane;
- Residual isobutane consumption was observed after isobutanol addition was resumed on day 164 and continued after the second bioaugmentation performed on day 183 and daily isobutane addition resumed on day 205. These data suggest that the addition of isobutanol aided in the growth of isobutane-oxidizing bacteria already present in the aquifer (either indigenous, or from the first ENV493 bioaugmentation).
- Dissolved isobutane concentrations measured in the treatment zone PMWs and EW-1 remained low (typically <50 µg/L) between days 183 and 253 (**Figures 5.44** and **5.45**), indicating that isobutane being added daily to the aquifer was also being rapidly consumed in the aquifer at this time.

- Dissolved isobutane concentrations measured in the monitoring wells and EW-1 were increasing again by day 269, indicated consumption of isobutane had slowed for a second time. Therefore, isobutane addition was temporarily halted again on day 280;
- No appreciable consumption of isobutane was observed after the third ENV493 bioaugmentation event on day 304, and the restart of isobutane addition on day 327. Therefore, isobutane addition was halted for the last time on day 353;
- Dissolved isobutane concentrations generally increased in the treatment zone PMWs and EW-1 during the post treatment sampling event on day 499. The reason for this spike in concentrations is not clear but may reflect dissolution of a pocket of gas into groundwater over time; and,
- Dissolved isobutane concentrations at downgradient well DMW-1 increased an order of magnitude (from 141 µg/L to 1667 µg/L) between days 386 and 499, suggesting a slug of dissolved isobutane traveled downgradient from the treatment zone after Phase 2 treatment ended on day 422.

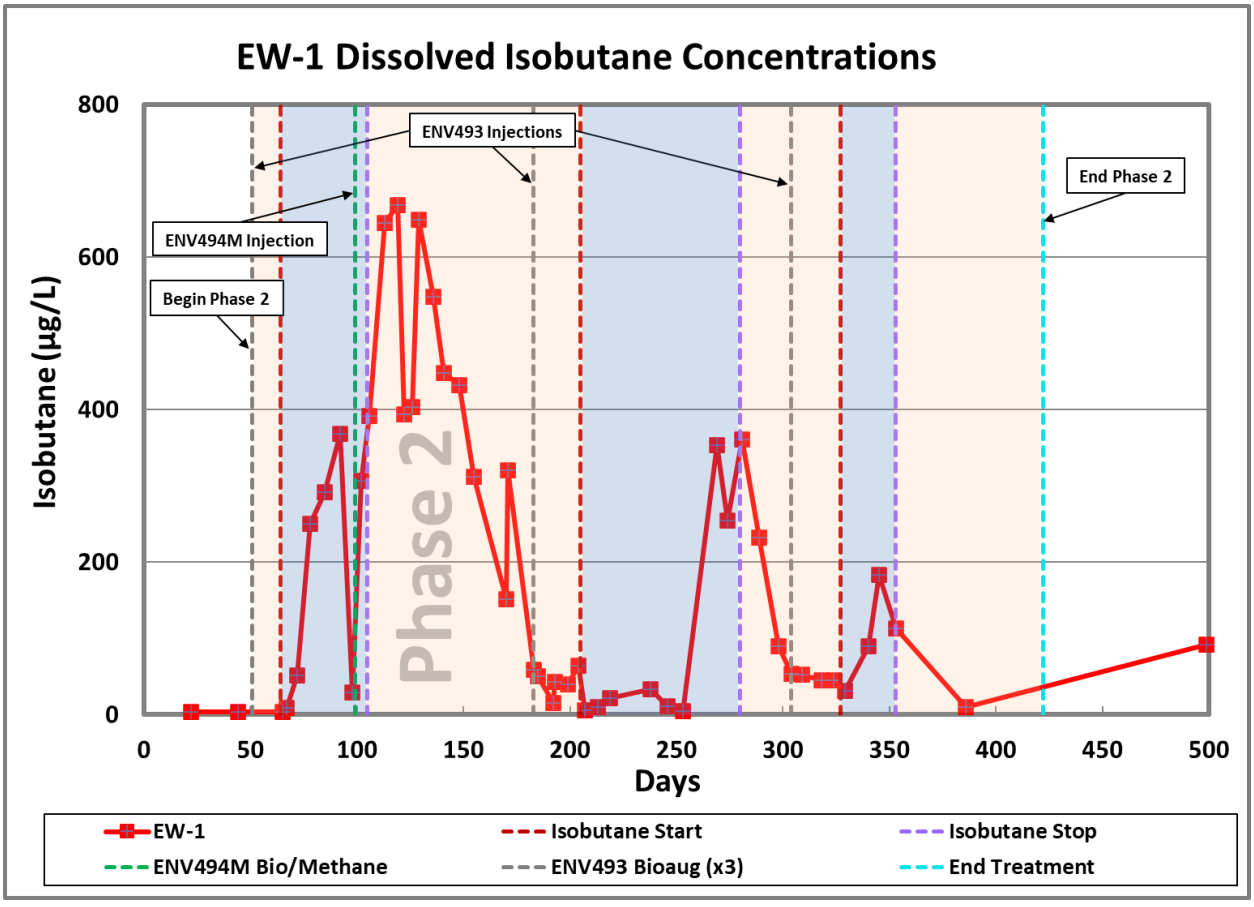


Figure 5.45. Dissolved Isobutane Concentrations at the Extraction Well. Phase 2 began on day 51 with the first ENV493 bioaugmentation injection. The three periods of isobutane addition are highlighted in blue. Dissolved isobutane concentrations increased during each injection period, followed by consumption of the isobutane that slowed over time. Isobutane addition was subsequently halted and additional ENV493 bioaugmentation injections were performed when isobutane consumption rates slowed.

5.7.4 Dissolved Methane

As detailed in **Section 5.5.3.2**, regular methane additions to the groundwater recirculation process stream were initiated on June 22, 2021 (day 99), the same day that bioaugmentation with ENV494M was performed, and continued through May 11, 2022 (day 422). Changes to methane injection concentrations and duration throughout Phase 2 of the demonstration were made primarily based on observed dissolved methane concentrations measured at EW-1.

Dissolved methane concentrations measured at the 11 demonstration monitoring wells are plotted on **Figure 5.46**, and concentrations measured at the control well and EW-1 are plotted on **Figure 5.47**. Dissolved methane concentration data are provided in **Appendix F** on a well-by-well basis. Results of the data collected are summarized as follows:

- Dissolved methane was observed in all eight treatment zone PMWs, PTW-1, and EW-1 during Phase 2 operation, with concentrations ranging from <10 µg/L to >3,015 µg/L (PMW-4S on day 113);
- Dissolved methane concentrations exceeded the target of 200 µg/L in six of the eight treatment zone PMWs within 14 days of initiating methane addition (achieving one of the demonstrations quantitative performance objectives);
- Methane addition rates were reduced on day 131 (**Section 5.5.3.2**) when dissolved methane concentrations were measured above 1,000 µg/L at several of the treatment zone PMWs and EW-1 on days 108 through 126;
- Dissolved methane concentrations were generally below 100 µg/L in most of the treatment zone PMWs and EW-1 throughout the majority of Phase 2 operation;
- A spike in dissolved methane concentrations was observed in several of the treatment zone PMWs and EW-1 on day 345. The reason for this spike in concentrations is not clear, as no appreciable changes in methane addition or system operation were made around that time; and,
- Unlike dissolved isobutane, dissolved methane concentrations did not increase in the treatment zone PMWs during the post treatment sampling event on day 499; however, an increase in dissolved methane concentrations (3.8 µg/L to 99 µg/L) was observed at EW-1 between days 389 and 499.

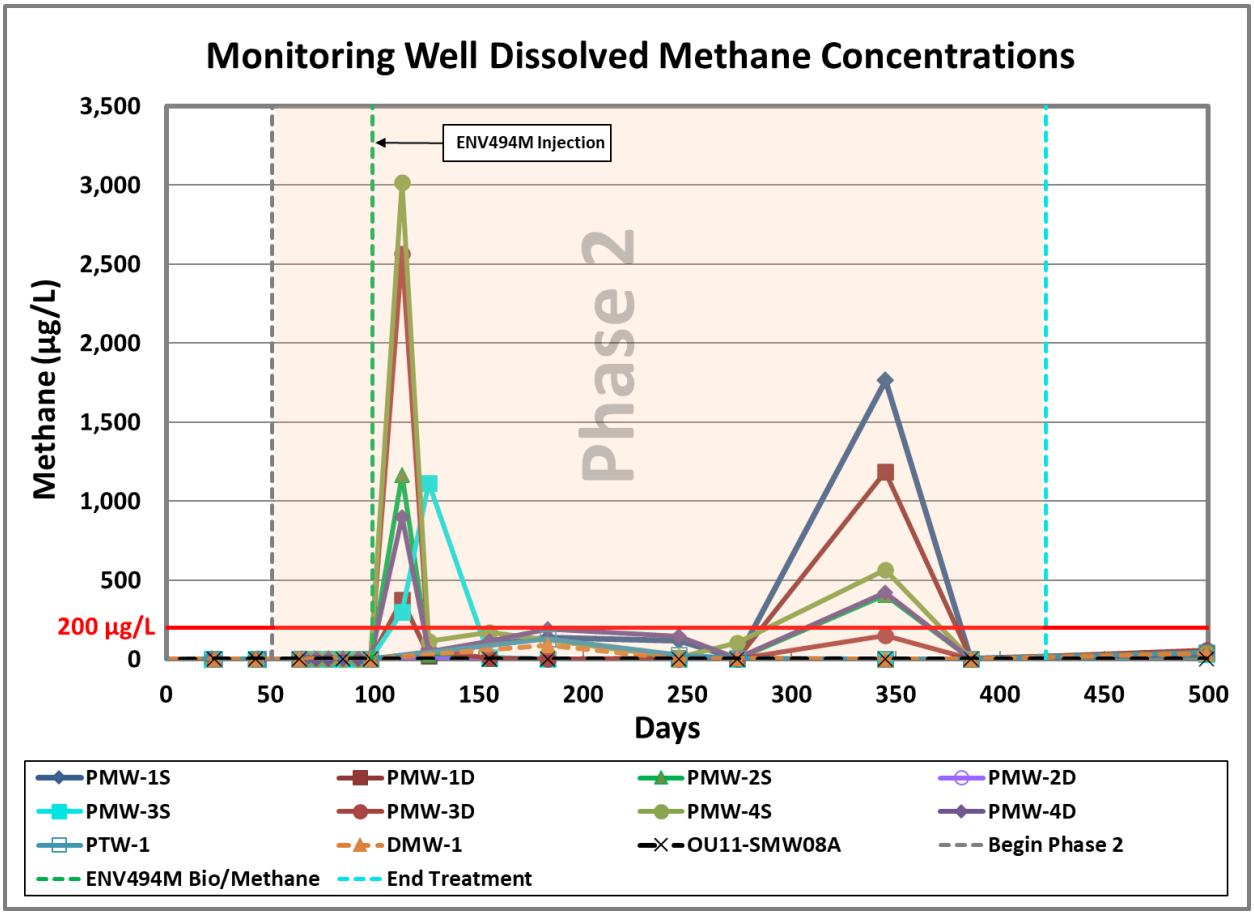


Figure 5.46. Dissolved Methane Concentrations at the Monitoring Wells. Phase 2 began on day 51 with the first ENV493 bioaugmentation injection. One continuous period of methane addition was performed from day 99 (when the aquifer was bioaugmented with ENV494M) through day 422. The performance objective of achieving a target dissolved methane concentration of >200 µg/L in at least six of the eight treatment zone PMWs during the first month of isobutane addition was achieved when six of the eight wells exceeded 200 µg/L within 14 days.

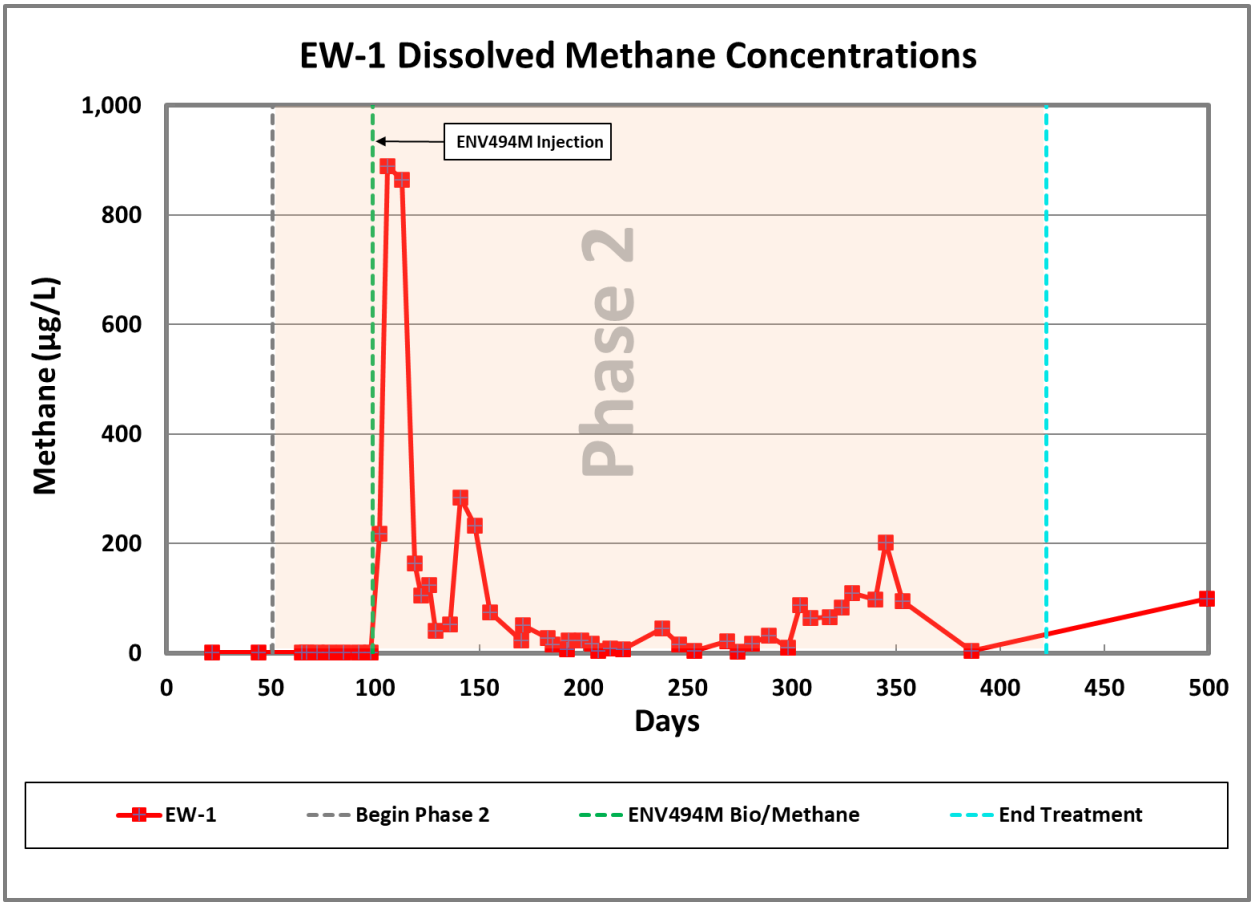


Figure 5.47. Dissolved Methane Concentrations at the Extraction Well. Phase 2 began on day 51 with the first ENV493 bioaugmentation injection. One continuous period of methane addition was performed from day 99 (when the aquifer was bioaugmented with ENV494M) through day 422. After the first two weeks of methane addition, dissolved methane concentrations were generally around or below 100 µg/L at the extraction well for the majority of Phase 2.

5.7.5 Isobutanol

Groundwater samples were collected and analyzed for isobutanol at the 11 demonstration monitoring wells and EW-1 during Phase 1, Phase 2, and the post treatment monitoring events, as detailed in **Section 5.5** and summarized in **Table 5.7**. As discussed in **Section 5.5.3.2**, isobutanol addition to the groundwater recirculation process stream was performed over three separate time periods, each occurring immediately prior to and/or after each of the three ENV493 bioaugmentation events to assist with growth of the culture within the aquifer. Isobutanol concentrations measured at the 11 demonstration monitoring wells are plotted on **Figure 5.48**, and concentrations measured at the control well and EW-1 are plotted on **Figure 5.49**. Isobutanol concentration data are provided in **Appendix F** on a well-by-well basis. Results of the data collected are summarized as follows:

- Isobutanol was observed in three of the treatment zone PMWs (PMW-1S, PMW-1D and PMW-4D) and EW-1 during Phase 2 operation, with concentrations ranging from <10 mg/L to 95 mg/L (PMW-1D on day 65);

- The biggest spike in isobutanol concentrations was observed during the first isobutanol addition (performed days 51 through 62), when 18 L were added over a 12-day period;
- The data show that isobutanol was rapidly consumed during and after each of the three additions periods; and,
- Isobutanol was not observed in downgradient well DMW-1 or control well OU11-SMW08A during the demonstration.

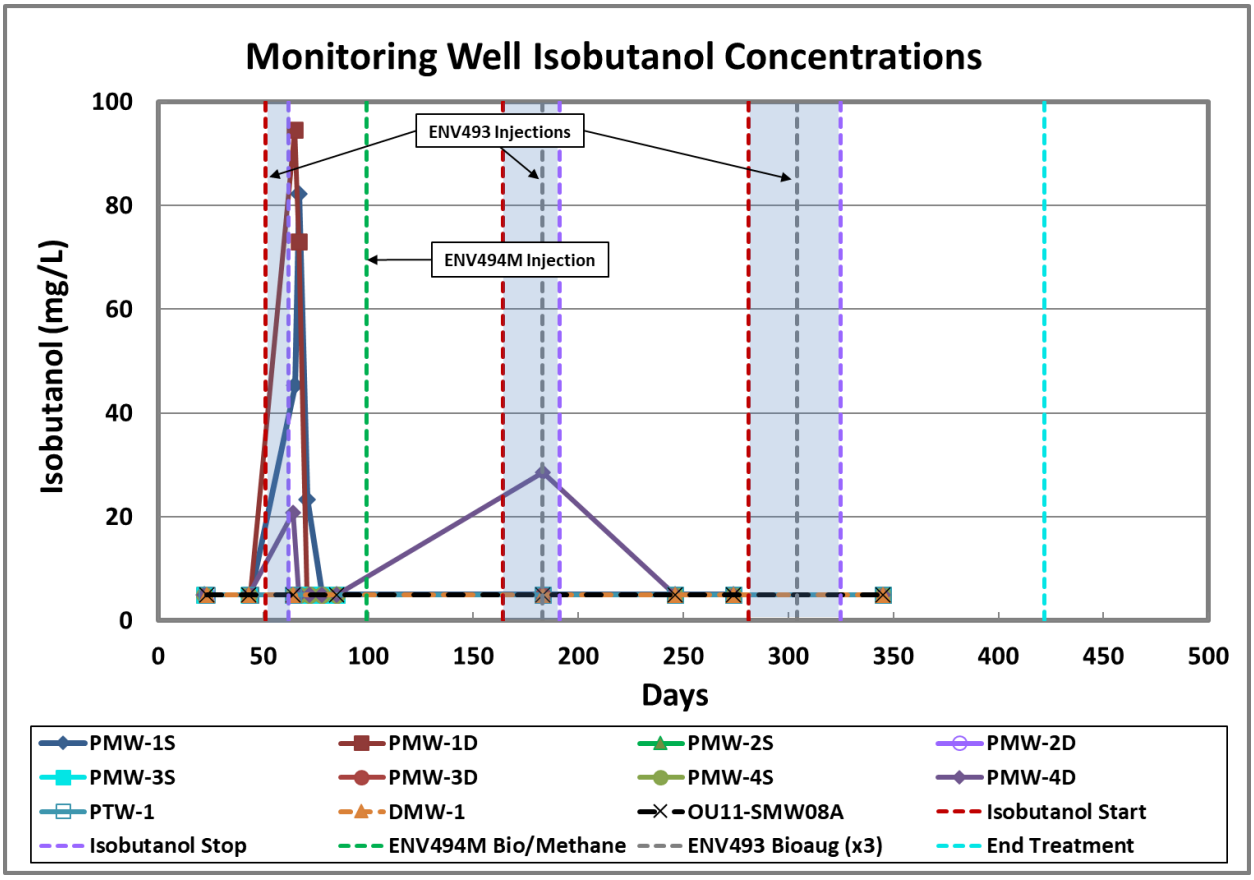


Figure 5.48. Isobutanol Concentrations at the Monitoring Wells.

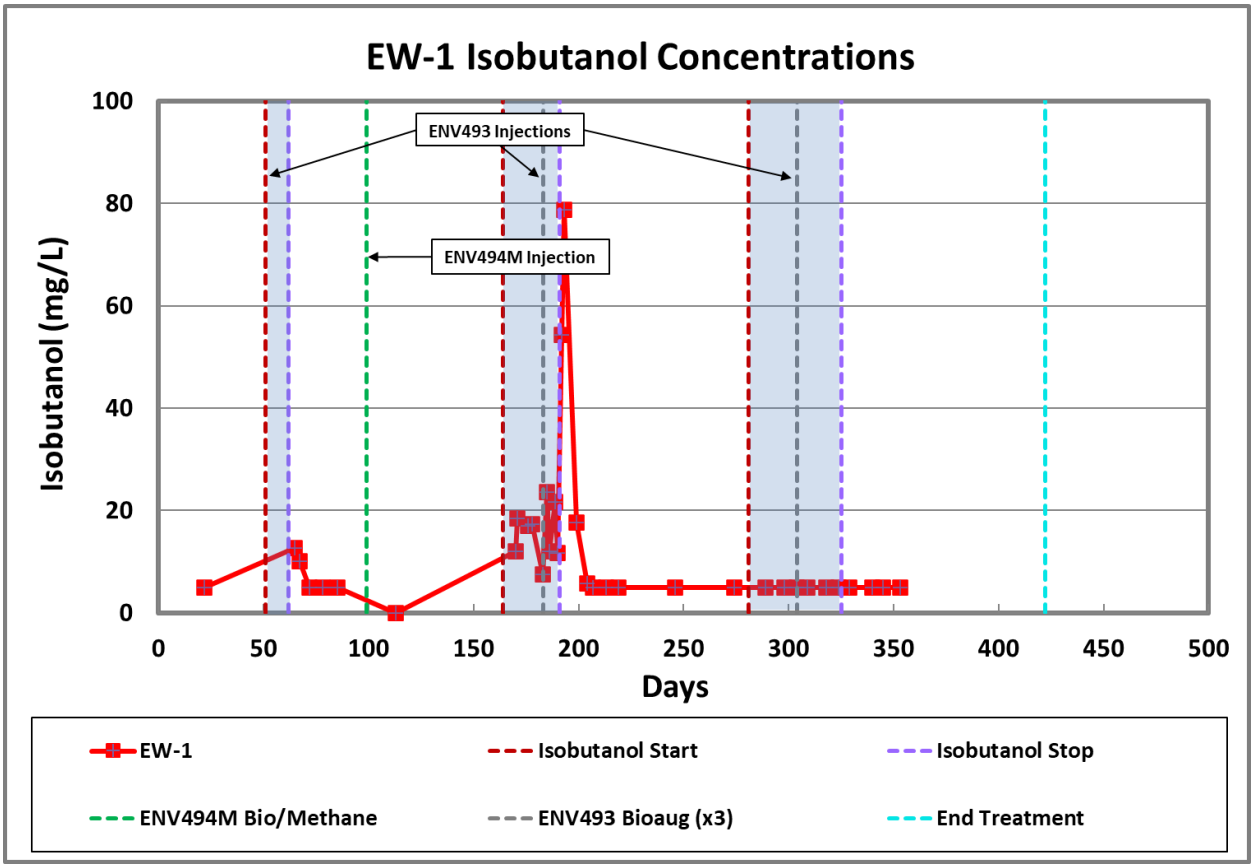


Figure 5.49. Isobutanol Concentrations at the Extraction Well.

5.7.6 Cometabolic Organisms/Genes

Groundwater samples were collected and analyzed for microbial analysis at six select monitoring wells (treatment zone downgradient monitoring wells PMW-3S/D and PMW-4S/D, downgradient well DMW-1, and control well OU11-SMW08A) once during baseline, once during Phase 1, and twice during Phase 2, as detailed in **Section 5.5** and summarized in **Table 5.7**. Quantification of target cometabolic organisms/genes (SCAM, PPO, AMO, sMMO, and pMMO) by qPCR was performed by Microbial Insights (Knoxville, TN), and proteomics analysis was also performed at NCSU and focused on the use of ABL of monooxygenases expressed by native bacteria.

Target cometabolic organism/gene data are provided in **Appendix F** on a well-by-well basis and plotted in **Figures 5.50** through **5.52**. Results of the data collected are summarized as follows:

- Only well PMW-4D showed a meaningful increase in SCAM (approximately two orders of magnitude) during the demonstration (**Figure 5.50**). These results were unexpected, as the aquifer was bioaugmented twice with ENV493 during the period when these samples were collected. However, the formation and subsequent toxicity of the reactive 1,1-DCE epoxide likely reduced the population appreciably. The adsorption of ENV493 to sediments (rather than being planktonic), also may partially account for these data;

- A two to three order of magnitude increase in sMMO was observed at wells PMW-3S, PMW-3D, PMW-4D and DMW-1 during the demonstration (**Figure 5.51**), indicating that the bioaugmentation with ENV494M and/or addition of methane addition led to an increase in the methanotroph population; and,
- A two order of magnitude increase in pMMO was observed at well PMW-3D during the demonstration (**Figure 5.52**). No appreciable increase in pMMO was observed in the other five select wells.

The results of the ABL analysis performed by NCSU are presented in **Appendix H**. The results of this work generally agree with the SCAM data presented in **Figure 5.50**.

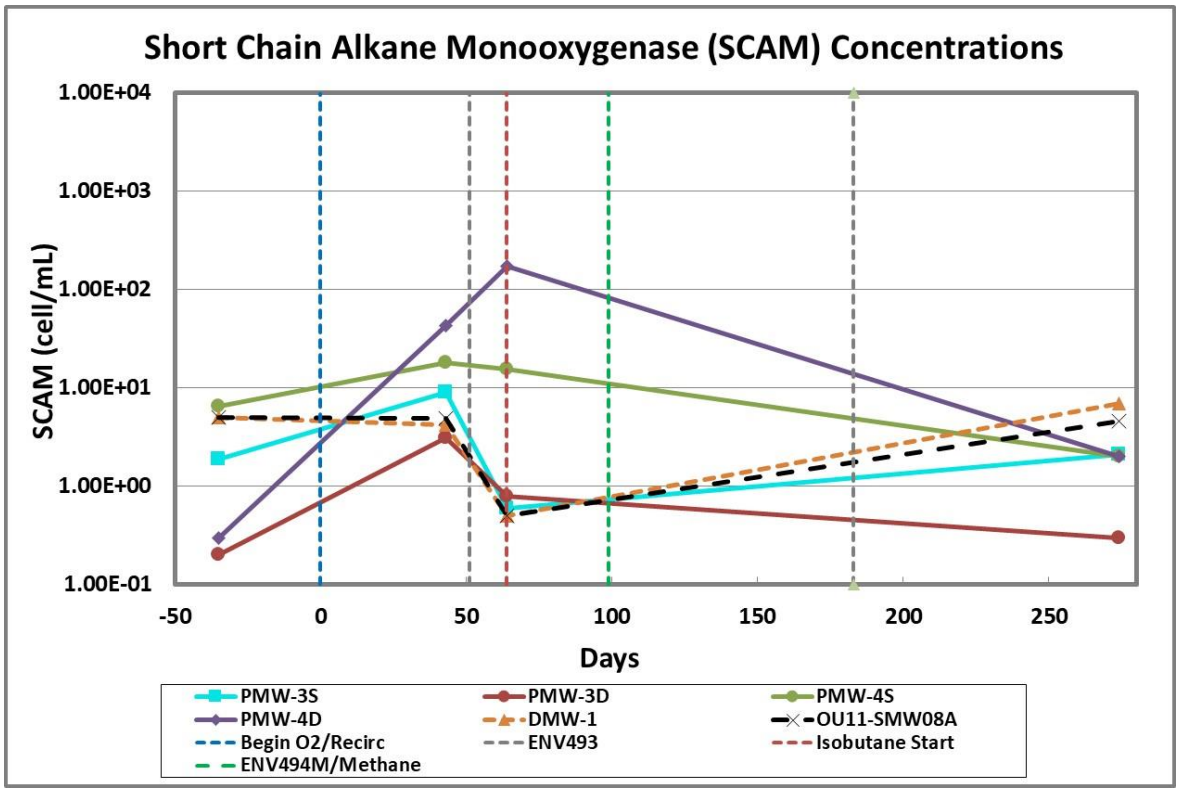


Figure 5.50. SCAM Concentrations at Selected Monitoring Wells.

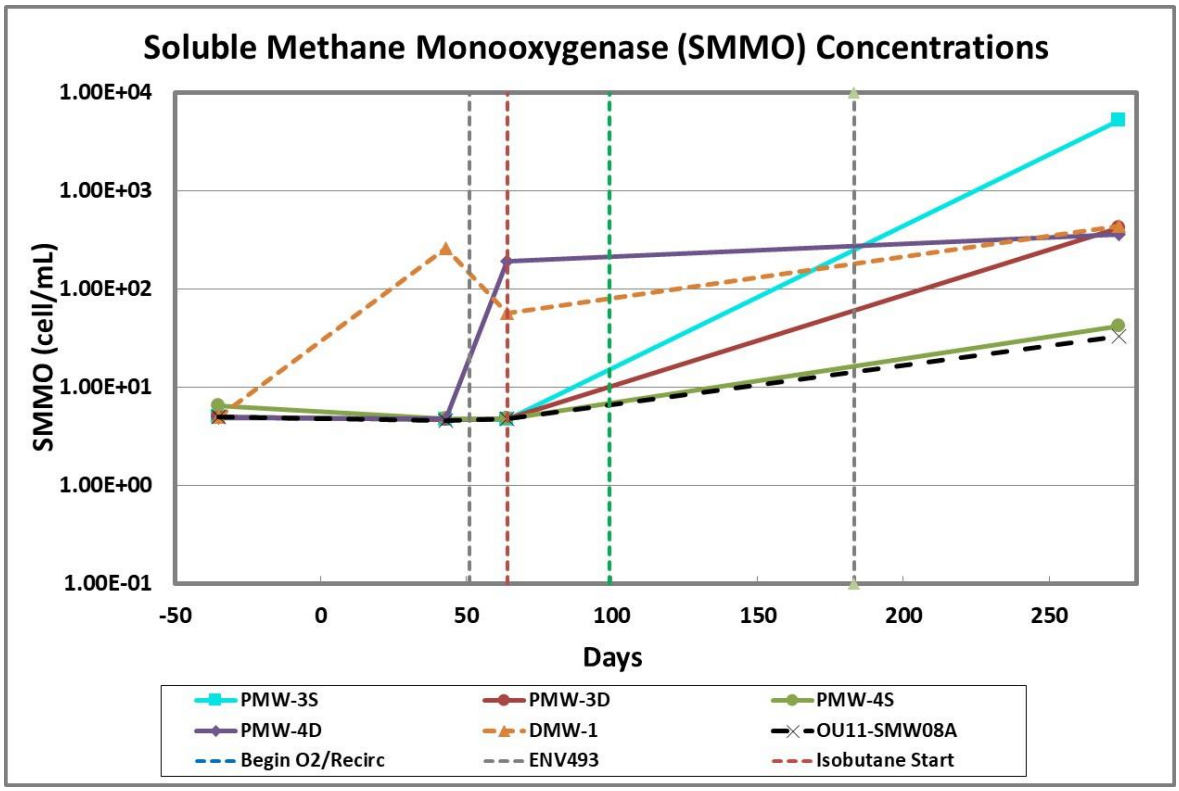


Figure 5.51. sMMO Concentrations at Selected Monitoring Wells.

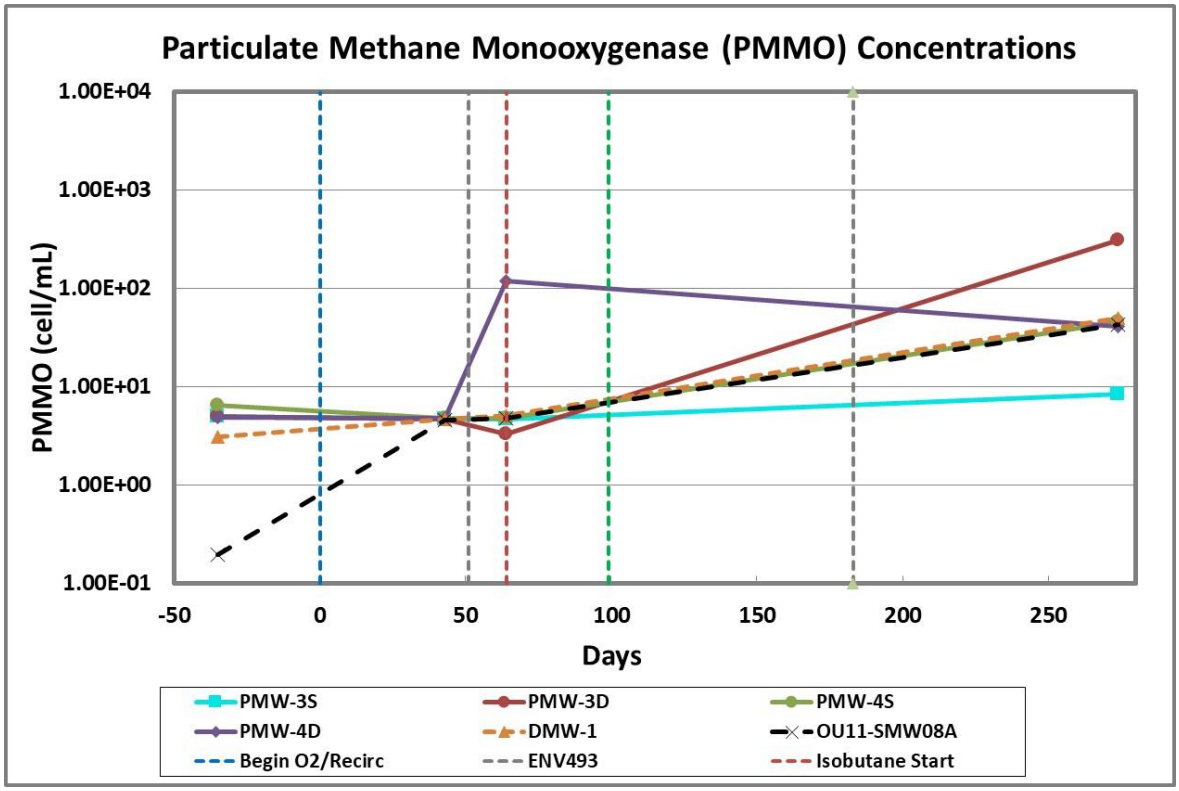


Figure 5.52. PMMO Concentrations at Selected Monitoring Wells.

5.7.7 System O&M

As detailed in **Section 5.5.3**, system O&M primarily consisted of regular (every 2-4 weeks) system checks (to collect manual system pressure and flow data, perform regular system maintenance, and perform leak checks) and changeout of the oxygen 16-packs approximately every 2-3 months. System operations data (gas flows, pressures, cycle frequencies, and downtime) and DO data collected from the four dedicated RDO probes were recorded and stored in data files by the SCADA system on the system laptop. Results of system O&M are summarized as follows:

- The off-the grid solar power system provided consistent power to the treatment system throughout most of the 499 days of the demonstration and was only down for seven days for the replacement of a charge controller and circuit breaker;
- System checks were typically performed in under 3 hours;
- Changeout of the oxygen racks were typically performed in under 4 hours, and required the use of an off-road forklift;
- The 12 cylinders of compressed methane and 4 cylinders of liquified isobutane did not require replacement during the demonstration;
- Intermittent biofouling of the groundwater recirculation filter cartridges occurred during the demonstration. This appears to have been associated primarily with isobutanol addition, and the presumed increase in cell biomass in the test plot;
- There were no apparent issues with biofouling of the IWs, EW or oxygen biosparging wells;
- Remote communication with the system and the logging capabilities of the SCADA system reduced the number of site O&M visits required. However, additional trips to the site (approximately 6 times during the demonstration) were required to reset the computer or Wi-Fi after remote communication was lost; and
- The treatment system operated as designed for >95% of the time.

No major system or equipment failures were experienced during the demonstration. An unexpected issue encountered during the demonstration was damage caused to subsurface tubing by Pocket Gophers present at the NASNI site. The gophers damaged groundwater recirculation, nutrient injection, and oxygen injection lines during the 3rd quarter of 2021, requiring excavation and repair of damaged tubing. The complete excavation and replacement of all subsurface tubing with reinforced tubing wrapped in rodent resistant material was performed during the 4th quarter of 2021. Michael Pound, the Site Remedial Project Manager, arranged for a pest control service to set and monitor traps, which yielded several gophers. The system was down for a total of 10 days to repair and replace the tubing.

6.0 PERFORMANCE ASSESSMENT

Performance objectives were established for this demonstration to provide a basis for evaluating the use of a MPS cometabolic bioremediation technology for the treatment of groundwater impacted with comingled 1,4-D and CVOCs. The performance objectives are provided in **Table 3.1** and discussed in **Sections 3.1** to **3.6** in this document. The data for each given objective are provided in **Section 5.7** and **Appendix D**. As summarized in **Section 3.0**, five of the six performance objectives established for this demonstration were achieved. The following subsections provide a summary and assessment of the data supporting the performance objectives.

6.1 DETERMINE REMEDIATION EFFECTIVENESS

One of the primary objectives of this demonstration was to assess the effectiveness of applying aerobic cometabolism to treat groundwater impacted with comingled 1,4-D and CVOCs. Understanding the extent to which the target COC mass was removed, and the subsequent impact on groundwater quality, was critical in this evaluation.

Groundwater data collected during the demonstration included two baseline sampling events, two Phase 1 sampling events, seven Phase 2 sampling events conducted during 12-months of treatment system operation, and one post-treatment sampling event performed approximately three months after system shutdown. Groundwater 1,4-D and CVOC concentrations were measured at eight PMWs and the EW within the target treatment zone, and one monitoring well downgradient of the treatment zone, as well as two upgradient (including one Control) monitoring wells. Treatment phase (Phase 2) 1,4-D and CVOC data collected from wells within and downgradient of the treatment zone were compared to baseline and Phase 1 data and data collected from the control well to evaluate treatment effectiveness. The data collected were used to determine the extent of 1,4-D and CVOC reduction during treatment.

As detailed in **Section 5.7.1**, concentrations of 1,1-DCE at the four pair of treatment zone PMWs declined appreciably, from an average concentration of 1,017 µg/L at the conclusion of Phase 1 to an average concentration of 181 µg/L after ~ 12 months of Phase 2 operation, an average decline of 82%. Similarly, TCE declined by an average of 73% over the same time interval, from 810 µg/L on average to 220 µg/L. COCs present at lower concentrations, including *cis*-DCE, 1,1-DCA, and 1,4-D, declined by less, averaging 19%, 37%, and 16%, respectively.

Ultimately, the quantitative performance objective of reducing CVOCs and 1,4-D by 95% (as discussed in **Section 3.1**) was not achieved despite good delivery of oxygen, inorganic nutrients, and cometabolic substrates throughout the treatment zone. This metric was readily achievable in the laboratory, but only when microcosms were bioaugmented with high densities of a culture isolated from the site (ENV493). The 95% metric could not be achieved in the laboratory via biostimulation alone. In fact, no degradation of any of the COCs was observed under biostimulation conditions. Laboratory studies indicated that the concentration of 1,1-DCE in site groundwater was toxic to native isobutane-degrading organisms, likely due to the formation of a very reactive epoxide. However, when enough ENV493 was added to degrade all the 1,1-DCE in the laboratory, the other CVOCs as well as 1,4-D were subsequently biodegraded to below detection.

In the field, three large-scale bioaugmentation injection events were conducted with ENV493 to achieve cell densities throughout the treatment zone to enhance cometabolic degradation of 1,1-DCE, which presumably would have then resulted in degradation of the remaining CVOCs and 1,4-D to > 95%. Isobutanol was also added to the treatment zone as a noninducing substrate to allow growth of ENV493 or other native isobutane degraders without induction of the SCAM enzyme that converts 1,1-DCE to a toxic epoxide. This was done to increase the cell population above the threshold necessary for complete 1,1-DCE degradation. As noted above, our efforts resulted in a > 80% decline in 1,1-DCE concentrations within the treatment zone, but we could not get below ~ 200 µg/L. The reasons that residual 1,1-DCE persisted in the treatment zone may include (1) continual desorption/back diffusion of 1,1-DCE from site sediments, thus replenishing the dissolved concentration (2) 1,1-DCE entering the treatment zone during groundwater recirculation, and (3) insufficient distribution of ENV493 throughout the treatment zone, thus leaving some areas with high 1,1-DCE concentrations which subsequently continued to distribute throughout the areas with ENV493 during groundwater recirculation. We hypothesize that the residual 1,1-DCE continued to cause enhanced rates of death of ENV493 and any native cometabolic organisms that oxidized it to 1,1-DCE-epoxide, thus limiting degradation of many of the other target COCs.

One of the lessons learned from this demonstration is that 1,1-DCE represents a significant challenge for cometabolic treatment at field scale, and is likely to inhibit the activity of organisms that can degrade many other CVOCs and 1,4-D. The "threshold" concentration of 1,1-DCE above which cometabolism should be used is not clear and may be site and organism specific. This is a good area for future laboratory studies. However, based on the field data, if one is unable to biostimulate native bacteria to cometabolically biodegrade the COCs at a site with 1,1-DCE (i.e., via air/oxygen, nutrient and cometabolic gas addition only), then cometabolic treatment in the field is contraindicated. Overall, cometabolism is an excellent approach to treat dilute plumes (i.e., tens to a few hundred µg/L) with a variety of different CVOCs and emerging chemicals, including 1,4-D, but the toxicity of epoxides from chlorinated ethenes, with 1,1-DCE being perhaps the most problematic, is likely to limit the effectiveness of this approach at sites with high chlorinated ethene concentrations (e.g., several hundred µg/L to mg/L). Overall, the concentration of chlorinated ethenes (1,1-DCE in particular) at the NASNI site were likely too high for the technology to achieve the desired 95% removal metric.

6.2 MAINTAIN AEROBIC CONDITIONS WITHIN THE TREATMENT ZONE

Maintaining aerobic conditions within the treatment zone was critical, as cometabolism using an alkane/alkene gas substrate is an aerobic process. Therefore, the extent to which sparged oxygen gas could be distributed (both vertically and horizontally) within the treatment zone was critical to the overall success of the remedial approach.

As discussed in **Sections 5.5.2** and **5.5.3**, oxygen sparging cycles were only required approximately once every two weeks during Phase 1, and approximately once every two to three days during Phase 2 of the demonstration to maintain aerobic conditions in the aquifer. As detailed in **Section 5.7.2**, DO concentrations were greater than 1.0 mg/L at six of the eight treatment zone PMWs (PMW-1D, PMW-2D, PMW-03S/D and PMW-04S/D), EW-1 and downgradient well DMW-1 at between 75 and 100 percent of the measurements collected during Phase 2 of the demonstration, achieving the second performance objective of maintaining aerobic conditions

within the treatment zone. Eighty-six percent of the DO measurements collected at the eight treatment zone PMWs during Phase 2 were > 1.0 mg/L, with 79 percent of the measurements > 5 mg/L, with average DO concentrations ranging between 3.69 mg/L and 26.56 mg/L (**Table 5.10**). DO concentrations decreased in the treatment zone during and after each of the isobutanol injections, due to the additional biological oxygen demand created by the addition of this carbon substrate (additional oxygen sparge cycles were performed during these periods to maintain target DO concentrations within the treatment zone).

6.3 ADEQUATE DISTRIBUTION OF MPS GASES WITHIN THE TREATMENT ZONE

Optimization of the MPS gas delivery mass and frequencies increase the effectiveness of the aerobic cometabolic treatment technology by supplying appropriate amounts of the cometabolic gases, while ensuring that high dissolved gas concentrations do not lead to continuous competitive inhibition. Therefore, the extent to which the cometabolic gases could be distributed (both vertically and horizontally) via the groundwater recirculation system, and the dissolved gases consumed within the treatment zone, were important to the overall success of the remedial approach.

As discussed in **Section 5**, isobutane and methane gases were added to the aquifer through the groundwater recirculation system to stimulate microbial growth and activity. The data collected during the demonstration were used to optimize MPS gas addition cycle concentrations, durations and frequencies, and to minimize the effects of competitive inhibition. While one of the performance objectives for this demonstration was to observe >200 µg/L dissolved isobutane and methane in at least 6 of the 8 treatment zone PMWs during the first month of Phase 2 system operation (to maximize growth of isobutane-oxidizing bacteria), optimal dissolved isobutane and methane concentrations between approximately 10 µg/L and 100 µg/L at EW-1 were targeted during the majority of Phase 2 to support continued biomass growth, while minimizing competitive inhibition within the treatment zone. Notably, in addition to maximizing treatment effectiveness, MPS gas delivery optimization will reduce overall operating costs.

As detailed in **Section 5.7.3**, dissolved isobutane concentrations exceeded the target 200 µg/L in seven of the eight treatment zone PMWs within 21 days of initiating isobutane addition, and at the eighth monitoring well within 35 days. As detailed in **Section 5.7.4**, dissolved methane concentrations exceeded the target of 200 µg/L in six of the eight treatment zone PMWs within 14 days of initiating methane addition. Therefore, this performance objective of observing >200 µg/L dissolved isobutane and methane in at least six of the eight treatment zone PMWs during the first month these gases were added to the aquifer was achieved.

Furthermore, dissolved isobutane concentrations measured at EW-1 were typically between the target of approximately 10 µg/L and 100 µg/L during the periods of Phase 2 when consumption of isobutane was observed, and dissolved methane concentrations were generally between 10 µg/L and 100 µg/L at EW-1 throughout most of Phase 2 operation. These data indicated that, apart from periods of time where isobutane concentrations were observed to increase (as discussed in **Section 5.7.3**), consumption of these gases were occurring within the treatment zone.

6.4 DETERMINE TREATMENT SYSTEM RELIABILITY

Reliability of the oxygen biosparging, groundwater recirculation, and amendment delivery systems was essential during the demonstration, as the regular addition of oxygen, cometabolic gases, and nutrients is critical to the treatment and cost effectiveness of any cometabolic treatment approach.

As detailed in **Section 5.7.7**, the off-the grid solar power system provided consistent power to the treatment system throughout most of the 499 days of the demonstration and was only down for seven days for the replacement of a charge controller and circuit breaker. The cometabolic treatment system operated as designed, and no major system or equipment failures were experienced during the demonstration, apart from unexpected damage caused by Pocket Gophers to subsurface groundwater recirculation, nutrient injection, and oxygen injection lines, as discussed in **Section 5.7.7**. The system was down for ten days while the subsurface lines were replaced.

In general, the off-the-grid solar powered treatment system proved to be highly reliable, simple to operate and maintain, and economical for *in situ* cometabolic treatment. Importantly, there were no apparent issues with biofouling of the IWs, EW or oxygen biosparging wells during the demonstration. Systems operation data collected during the demonstration showed that the treatment system operated as designed >95% of the time (482 out of 499 days). Therefore, the performance objective of the treatment system operating as designed for >90% of the time was achieved.

6.5 EASE OF USE

The level of effort required to maintain the hybrid cometabolic treatment system was assessed during the demonstration, as ease of use and low maintenance requirements are critical to the long-term treatment and cost effectiveness of this approach.

As detailed in **Section 5.7.7**, system O&M requirements, which primarily consisted of regular system checks and changeout of the oxygen cylinders, were not substantial during the demonstration. System checks (which entailed collecting manual system pressure and flow data, performing regular system maintenance, and performing leak checks) were generally performed every 2-4 weeks in under 3 hours per visit. Change out of the oxygen 16-packs was conducted approximately every 2-3 months and was typically performed in under 6 hours. The 4 cylinders of liquified isobutane and 12 cylinders of compressed methane did not require replacement during 12 months of Phase 2 cometabolic biosparging due to the general efficiency of this treatment approach. The ability to communicate remotely with the system (and adjust system operating parameters), as well as programmed logging capabilities of the SCADA system reduced the number of site visits required. However, additional trips to the site (approximately 6 times during the demonstration) were required to reset the computer or Wi-Fi after remote communication was lost.

The off-the-grid solar powered system provided continuous power for operation of the treatment system and proved simple to operate and maintain. Importantly, there were no apparent issues with biofouling of the IWs, EW or oxygen biosparging wells during the demonstration.

In addition to the PI and project engineer, two field technicians were trained to operate and conduct system O&M in one day. Site checks were typically performed by a single technician, and cylinder changeouts were typically performed by one technician and one forklift operator. O&M requirements were assessed to be lower than more active remediation technologies, such as P&T. Furthermore, other than soil cuttings and development water generated during well installations, there was no waste generated during application of this *in situ* technology (even purge water generated during groundwater sampling was mixed with nutrients and injected back into the aquifer via the nutrient injection system).

6.6 IDENTIFICATION OF DEGRADATIVE GENES/PROTEINS

The ability to identify the microorganism being stimulated, as well as to confirm that bacteria express the expected monooxygenases after stimulation by each gaseous primary substrate, advances the understanding of microbial communities and monooxygenase enzyme stimulation to specifically target treatment at this and other sites with mixed 1,4-D and CVOC plumes.

As detailed in **Section 5.7.6**, groundwater samples for microbial analysis were collected at six select monitoring wells during baseline sampling, Phase 1 and Phase 2 of the demonstration. Analysis of target cometabolic organisms/genes/enzymes by qPCR was performed and proteomic analysis using an ABPP approach capable of simultaneously identifying multiple types of active monooxygenases in environmental samples was also be used to confirm that bacteria express the expected monooxygenases after stimulation by each gaseous primary substrate.

The qPCR data collected indicated that a limited number of wells showed meaningful (approximately 2 orders of magnitude) increases in the target cometabolic organisms/genes during Phase 2 of the demonstration. Furthermore, the results of the ABPP analysis targeting isobutane-oxidizing monooxygenases performed by NCSU generally agreed with the qPCR data. The relatively small magnitude of the increases and the apparent limited distribution of the of the target organisms were unexpected, as the aquifer was bioaugmented twice with ENV493 and once with ENV494M during the period when these samples were collected. The limited increases could be due to the formation and subsequent toxicity of the reactive 1,1-DCE epoxide that reduced the populations appreciably and/or the adsorption of ENV493 (and to a lesser extent, ENV494M) to sediments (rather than being planktonic). Regardless, the data indicate that we were able to successfully identify and/or quantify the genes and active enzymes via both qPCR and ABPP.

7.0 COST ASSESSMENT

7.1 COST MODEL

To evaluate the cost of a potential cometabolic biosparging system to treat a full-scale CVOC and 1,4-D plume, and compare it against other remedial approaches, costs associated with various aspects of the demonstration were tracked throughout the course of the project. **Table 7.1** summarizes the various cost elements and total cost of the demonstration project. The costs have been grouped by categories as recommended in the Federal Remediation Technologies Roundtable Guide to Documenting Cost and Performance for Remediation Projects (FRTR, 1998). Many of the costs shown in this table are a product of the innovative and technology validation aspects of this project and would not be applicable to a typical site application. Therefore, a separate "discounted costs" column that excludes or appropriately discounts these costs has been included in **Table 7.1** to provide a cost estimate for implementing this technology at the same scale as the demonstration (i.e., pilot scale).

Costs associated with the demonstration were tracked from August 2017 to July 2022. The total cost of the demonstration was $1,479,242 which included approximately $438,400 in capital costs, $346,600 in O&M costs, and $694,242 in demonstration-specific costs (cost related to ESTCP-specific requirements, site selection, site characterization/testing and reporting). It should be noted that costs incurred by Naval Facilities Engineering and Expeditionary Warfare Center for project oversight and technical support were not included in this analysis, and only those costs incurred by APTIM to perform the demonstration were included.

7.1.1 Capital Costs

Capital costs (primarily system design and installation) accounted for $438,400 (or 30%) of the total demonstration costs. As indicated in **Table 7.1**, these costs exceed what would be expected (the discounted cost) during a typical remediation project due to the innovative nature of the technology and the associated complexity of the system design and fabrication/installation.

7.1.2 O&M Costs

O&M costs accounted for $346,600 (or 23%) of the total demonstration cost. These costs consisted primarily of groundwater monitoring (including analytical) and system O&M. System O&M costs were $124,300, or 8.4% of the total demonstration cost. As detailed in **Section 5.5**, extensive performance monitoring activities were conducted to effectively validate this technology, including two baseline groundwater sampling events, 7 performance monitoring events, eight dissolved gas distribution sampling events, and one post-treatment monitoring event.

7.1.3 Demonstration-Specific Costs

Other demonstration-specific costs (costs not expected to be incurred during non-research-oriented remediation projects) accounted for approximately $572,442 (or 39%) of the total demonstration cost. These costs included site selection, additional laboratory microcosm and proteomics testing, ESTCP demonstration reporting and meeting (Interim Progress Report) requirements, technology transfer efforts, and preparation of a final technical that is more extensive than that of typical technical reports.

Table 7.1. Demonstration Cost Components

Cost Element	Details	Tracked Demonstration Costs	Discounted Costs[1]
CAPITAL COSTS			
System Design	Labor	$32,400	$32,400
	Equipment & Materials	$800	$800
System Installation (system and monitoring well installation, surveying, sparge/GW ext. system with controls, piping, trenching, system materials)	Labor	$108,400	$108,400
	Travel	$7,600	$7,600
	Equipment & Materials	$113,500	$113,500
	Subcontracts (driller, surveyor, system fabrication/install)	$175,700	$175,700
	Subtotal	**$438,400**	**$438,400**
OPERATION AND MAINTENANCE COSTS			
Groundwater Sampling	Labor	$20,000	$50,000
	Equipment & Materials	$4,400	$5,000
	In-House Analytical (labor and materials)	$53,100	$0
	Outside Analytical (OSU and NCSU)	$75,100	$50,000
	Subcontracts (sampling)	$69,700	$0
System O&M (including testing & start-up)	Labor	$49,200	$49,200
	Equipment & Materials	$40,400	$40,400
	Subcontracts (system O&M)	$34,700	$34,700
	Subtotal	**$346,600**	**$229,300**
OTHER TECHNOLOGY-SPECIFIC COSTS			
Site Selection	Labor	$19,000	$0
Site Characterization (DPT investigation)	Labor (including in-house analytical)	$55,000	$5,500
	Travel	$3,100	$3,100
	Materials	$400	$400
	Subcontractor (driller, surveyor)	$16,400	$16,400
Treatability Studies and Proteomics	Labor (including in-house analytical)	$22,700	$50,000
	Equipment & Materials	$200	$5,000
	Subcontractor (OSU and NCSU)	$266,100	$0
Test Well Installation and Sparge Testing	Labor	$46,300	$46,300
	Travel	$1,700	$1,700
	Equipment & Materials	$4,000	$4,000
	Subcontractor (driller & outside lab)	$14,200	$14,200
System and Well Decommissioning	Labor	$19,800	$15,000
	Equipment & Materials	$3,000	$2,500
	Subcontractor (system decom & waste disposal)	$29,000	$25,000
Quarterly Reporting & IPR/Project Meetings	Labor	$39,000	$0
	Travel	$4,500	$0
	Subcontractor (OSU and NCSU)	$34,000	$0
Technology Transfer (presentations, papers)	Labor	$24,000	$0
	Travel	$4,500	$0
Demonstration Plan/Work Plan	Labor	$28,200	$25,000
Final Report	Labor	$59,142	$25,000
	Subtotal	**$694,242**	**$239,100**
	TOTAL COSTS	**$1,479,242**	**$906,800**

Notes:

[1]Discounted costs are defined as estimated costs to implement this technology at the same scale as the demonstration. These costs do not include the technology validation apects of this ESTCP demonstrations, such as site selection, treatability studies, extensive groundwater sampling, ESTCP demonstration reporting and meeting (IPR) requirements, and preparation of technical and cost and performance reports.

7.2 COST DRIVERS

The expected cost drivers for installation and operation of a cometabolic biosparging system to treat a full-scale CVOC and 1,4-D plume, and those that will determine the cost/selection of this technology over other options, include the following:

- Depth of the plume bgs;
- Width, length, and thickness of the plume;
- Aquifer lithology and hydrogeology;
- Passive and sustainable power (solar);
- Length of time for clean-up (e.g., necessity for accelerated clean-up);
- The presence of indigenous bacteria capable of cometabolic degradation of CVOCs and 1,4-D;
- Concentrations of chemicals and alternate electron acceptors (e.g., nitrate [NO_3^-], sulfate [SO_4^{2-}], and O_2); and
- Presence of comingled COCs (1,1-DCE, in particular).

7.3 COST ANALYSIS

A cost analysis of a cometabolic biosparging system and a traditional groundwater treatment approach [P&T] to treat a full-scale CVOC and 1,4-D plume was performed. These two technologies were selected for comparison because they can be applied as treatment barrier for CVOC and 1,4-D plume capture. The base case presented in Krug et al. (2009) is used as a template for the cost analysis of the above technologies/approaches. The base case presents a situation where a shallow aquifer, consisting of homogeneous silty sands, is contaminated with TCE and 1,4-D, with little (<25 µg/L) 1,1-DCE. The contaminated groundwater extends from 10 to 50 ft bgs, along the direction of groundwater flow for 800 ft, and is 400 ft in width (**Figure 7.1**). The specific base case site characteristics, including aquifer characteristics and design parameters for each of the remedial approaches analyzed, are summarized in **Table 7.2**. The costing for the template site assumes that the source zone has been treated and that there is no continuing source of groundwater COCs. The cost analyses comparing the above approaches are presented below based on a 30-year operating scenario.

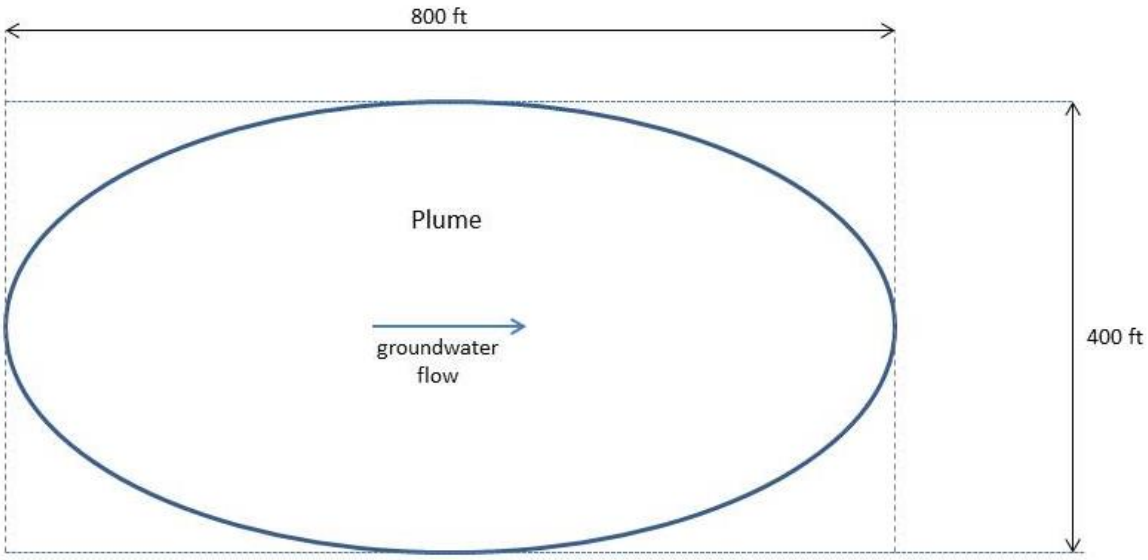


Figure 7.1. Base Case Plume Characteristics

Table 7.2. Summary of Base Case Site Characteristics and Design Parameters

Design Parameter	Units	Alternative	
		Cometabolic Biosparging Barrier	Pump and Treat
Width of Plume	feet	400	400
Length of Plume	feet	800	800
Depth to Water	feet	10	10
Vertical Saturated Thickness	feet	40	40
Porosity	dimensionless	0.25	0.25
Gradient	dimensionless	0.008	0.008
Hydraulic Conductivity	ft/day	2.8	2.8
Groundwater Seepage Velocity	ft/year	33	33
Nitrate Concentration	mg/L	15	15
Dissolved Oxygen Concentration	mg/L	5	5
Number of Barriers	each	1	1
Number of Monitoring Wells	each	10	10
Number of Biosparge Wells	each	44	0
Number of Extraction/Injection Wells	each	0	9

The following subsections provide cost estimates for implementation of each of the two treatment approaches for the base case. The cost estimates provide insight into the comparative capital, O&M, and long-term monitoring (LTM) costs to better identify cost drivers for each technology/approach. Total costs and the NPV of future costs were calculated for each of the treatment approaches. Future costs (O&M and LTM costs) are discounted using a -0.3% real discount rate to determine the NPV estimates of these costs (OMB Circular A-94, 2020). Specifically excluded from consideration are the costs of pre-remedial investigations and treatability studies, assuming the costs for these activities would be similar for each alternative. The cost estimates for each of the alternatives also assume the long-term performance monitoring costs are identical for each alternative. Monitoring is assumed to be at a quarterly frequency for the first five years and an annual frequency thereafter.

7.3.1 Cometabolic Biosparging Barrier

The cometabolic biosparging barrier alternative assumes that a row of biosparging wells will be installed at the downgradient edge and perpendicular to the axis of the plume (**Figure 7.2**). The system will include 22 biosparging well pairs (one shallow and one deep well at each location to sufficiently treat the entire 40 ft plume depth) installed with 20-ft spacing across the 400-ft-wide plume. The biosparging wells would be installed by direct-push drilling methods and consist of one-inch diameter PVC well materials. The system will be powered by solar only (off-the-grid).

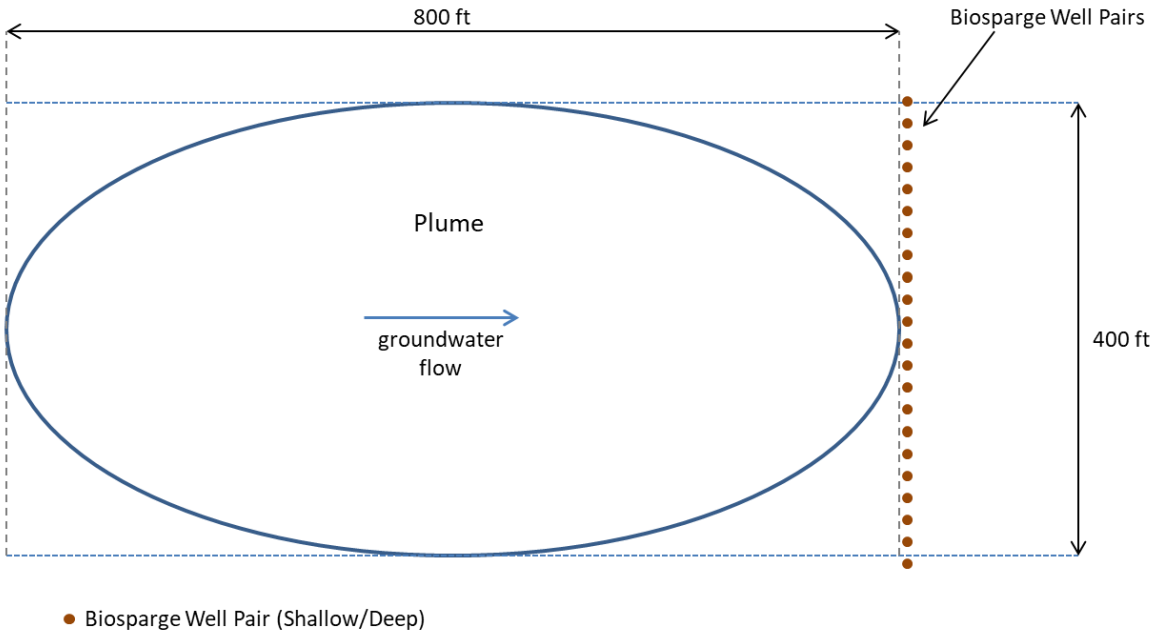


Figure 7.2. Cometabolic Biosparging Barrier Alternative for Plume Cutoff

As summarized in **Table 7.3**, the estimated total costs for the cometabolic biosparging barrier alternative over 30 years are $3,536,000 with a total NPV of lifetime costs of $3,664,741. The capital cost including design, work plan, installation of biosparging and monitoring wells, installation of the solar power system, and fabrication, installation, and start-up of the biosparging system is $446,900. The NPV of the O&M is $2,224,660 for the 30 years of treatment. The O&M

costs primarily include the labor and material costs associated with weekly inspections and battery replacement every five years. The costs for materials and other consumables are negligible with this alternative. The NPV of the 30 years of monitoring and reporting costs is $993,181.

Table 7.3. Cost Components for Cometabolic Biosparging Barrier

	Year Cost is Incurred							NPV of Costs*	Total Costs
	1	2	3	4	5	6	6 to 30		
CAPITAL COSTS									
System Design	70,000	-	-	-	-	-		70,000	70,000
Well Installation	130,500	-	-	-	-	-		130,500	130,500
System Installation	226,400	-	-	-	-	-		226,400	226,400
Start-up and Testing	20,000	-	-	-	-	-		20,000	20,000
SUBCOST ($)	446,900	-	-	-	-	-		**446,900**	**446,900**
OPERATION AND MAINTENANCE COSTS									
System Operation and Maintenance	69,170	70,170	70,170	70,170	75,170	70,170	32,227 every year	2,224,660	2,129,100
SUBCOST ($)	**69,170**	**70,170**	**70,170**	**70,170**	**75,170**	**70,170**		**2,224,660**	**2,129,100**
LONG TERM MONITORING COSTS									
Sampling/Analysis/Reporting (Quarterly through 5 years then Annually)	74,500	74,500	74,500	74,500	74,500	23,500	23,500 every year	993,181	960,000
SUBCOST ($)	**74,500**	**74,500**	**74,500**	**74,500**	**74,500**	**23,500**		**993,181**	**960,000**
TOTAL COST ($)	**590,570**	**144,670**	**144,670**	**144,670**	**149,670**	**93,670**		**3,664,741**	**3,536,000**

Notes:
NPV - Net Present Value
* - NPV calculated based on a -0.3% discount rate

This alternative ranks lowest in estimated total remedy cost and lowest in NPV of lifetime costs (see **Table 7.6**). The estimated capital cost for this approach is the lowest of the two alternatives because of the limited infrastructure required and the relative ease of installation. The estimated long-term O&M costs are also the lowest of the two alternatives, which helps make this the least expensive of the alternatives. As with the other alternative, total remedy costs will increase if the treatment needs to extend beyond 30 years.

7.3.2 Active Pump and Treat (P&T)

The groundwater P&T system alternative is like the other technology in that a downgradient barrier is installed (**Figure 7.3**). The system includes a row of four EWs and five IWs, which would be used to create a groundwater capture zone at the downgradient edge perpendicular to the axis of the plume (**Figure 7.3**). The extracted groundwater would be treated above-ground by air stripping followed by treatment with GAC. The treated groundwater would be re-injected providing hydraulic control and mass removal at the downgradient edge of the plume. The P&T system would be maintained for a period of 30 years. This alternative also assumes 30 years of associated O&M and LTM costs.

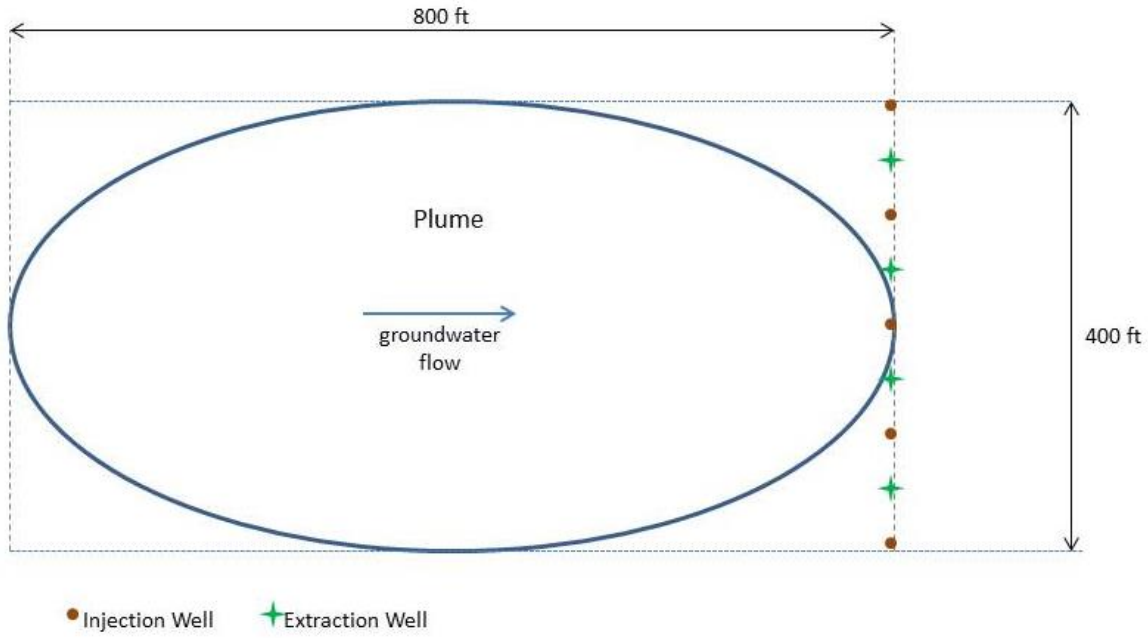


Figure 7.3. P&T Alternative for Plume Cutoff

As summarized in **Table 7.4**, the total cost for this alternative over 30 years is $8,005,853 with a total NPV of lifetime costs of $8,260,617. The capital cost including design, work plan, installation of IWs/EWs and monitoring wells, construction of the groundwater treatment system, and system start up and testing is $2,114,836. The NPV of the O&M is $5,152,600. The O&M costs include the labor costs associated with system O&M, costs for equipment repair and replacement, electrical costs, and cost for the replacement and disposal of the GAC. The NPV of the 30 years of monitoring and reporting costs is $993,181.

Table 7.4. Cost Components for P&T

	Year Cost is Incurred							NPV of Costs*	Total Costs
	1	2	3	4	5	6	6 to 30		
CAPITAL COSTS									
System Design	99,387	-	-	-	-	-		99,387	99,387
Well Installation	141,185	-	-	-	-	-		141,185	141,185
System Installation	1,845,389	-	-	-	-	-		1,845,389	1,845,389
Start-up and Testing	28,875	-	-	-	-	-		28,875	28,875
SUBCOST ($)	2,114,836	-	-	-	-	-		**2,114,836**	**2,114,836**
OPERATION AND MAINTENANCE COSTS									
System Operation and Maintenance	158,519	164,569	164,569	164,569	164,569	164,569	145,244 every year	5,152,600	4,931,017
SUBCOST ($)	**158,519**	**164,569**	**164,569**	**164,569**	**164,569**	**164,569**		**5,152,600**	**4,931,017**
LONG TERM MONITORING COSTS									
Sampling/Analysis/Reporting (Quarterly through 5 years then Annually)	74,500	74,500	74,500	74,500	74,500	23,500	23,500 every year	993,181	960,000
SUBCOST ($)	**74,500**	**74,500**	**74,500**	**74,500**	**74,500**	**23,500**		**993,181**	**960,000**
TOTAL COST ($)	**2,347,855**	**239,069**	**239,069**	**239,069**	**239,069**	**188,069**		**8,260,617**	**8,005,853**

Notes:
NPV - Net Present Value
* - NPV calculated based on a -0.3% discount rate

This alternative ranks highest in both estimated total remedy cost and NPV of lifetime costs (**Table 7.5**). The estimated capital costs for this alternative are higher than the other alternative because of the higher costs associated with constructing a groundwater treatment system. The high O&M costs associated with operating the P&T system are what makes this alternative the more expensive of the two alternatives. As with the other approach, total remedy costs will increase if the treatment needs to extend beyond 30 years.

Table 7.5. Summary of Costs for Treatment Alternatives.

Alternative	Capital Costs	NPV of 30 Years of O&M Costs	NPV of 30 Years of Monitoring Costs	NPV of 30 Years of Total Remedy Costs	Total 30-Year Remedy Costs
Cometabolic Biosparging Barrier	$447	$2,225	$993	$3,665	$3,536
Pump and Treat	$2,115	$5,153	$993	$8,261	$8,006

Notes:
All costs are in thousands of dollars
NPV - Net Present Value; current value of future costs based on a -0.3% annual discount rate
O&M - Operation and Maintenance

8.0 IMPLEMENTATION ISSUES

The primary end-users of this technology are expected to be DoD and commercial site managers, and their contractors, consultants, and engineers. The general concerns of these end users are likely to include the following: (1) technology applicability and performance under local site conditions; (2) safety; (3) secondary groundwater impacts, and (4) technology cost compared to other remedial options. These implementation issues are addressed in the following subsections.

8.1 TECHNOLOGY APPLICABILITY AND PERFORMANCE UNDER LOCAL SITE CONDITIONS

The primary objective of cometabolic treatment for groundwater impacted with comingled 1,4-D and CVOC is to supply one or more gaseous co-substrates (i.e., alkane or alkene gases depending on the mix of CVOCs), oxygen, and a nitrogen source (i.e. ammonia gas or DAP depending on system design) to an aquifer for growth of cometabolic organisms. There are number of different approaches to achieve this end whose applicability primarily depends on site geology/hydrogeology and plume characteristics. These approaches include (1) oxygen/air- and co-substrate gas biosparging; (2) groundwater recirculation with above-ground co-substrate gas and oxygen addition, (3) bubble-free gas diffusion systems, and (4) trenches with air and co-substrate gas injection lines, among others (Steffan et al., 2003). Hybrid/combined versions of these approaches are also possible. The critical objective with any of these approaches is to distribute the co-substrate and oxygen gases evenly and consistently throughout the desired treatment area. A hybrid approach was utilized here in which oxygen was added via sparge wells and co-substrate gases (methane and isobutane), inorganic nutrients (DAP), and bioaugmentation cultures were added via groundwater recirculation.

During ESTCP project ER-200828 (*Field Demonstration of Propane Biosparging for In Situ Remediation of N-Nitrosodimethylamine (NDMA) in Groundwater*), members of the project team performed biosparging by injecting propane gas into a groundwater aquifer in a stream of air. Data from that ESTCP field test clearly indicated that propane biosparging was an effective approach to reduce the concentrations of NDMA in a groundwater aquifer by 3 to 4 orders of magnitude, and that concentrations in the low ng/L range can be achieved with continuous treatment. These results are consistent with data achieved in pure culture studies as well as with various bioreactor tests (Hatzinger and Lippincott, 2019; Hatzinger et al., 2017).

Members of the project team also recently tested a groundwater recirculation design for treatment of EDB in groundwater using ethane gas and pure oxygen (Hatzinger et al., 2018). In this case, groundwater was pumped from an existing EW at 10-12 gallons per minute, amended with oxygen, ethane gas, and inorganic nutrients, and then re-injected into an IW (approximately 60 ft upgradient), forming a closed loop. Effective gas distribution was observed in system monitoring wells and the biodegradation of ethane and EDB were documented throughout the demonstration plot. EDB reached concentrations below the stringent Massachusetts MCL of 0.02 µg/L. The one potential O&M issue with this approach is biofouling of the system components (piping, filters, EW/IWs, etc.), as was observed in the IW tubing when ethane concentrations were increased from 2 mg/L in the injected water to 4 mg/L during one phase of the study.

A study also examined the use of bubble-free gas diffusion systems to supply oxygen and propane to a groundwater aquifer (Shaw Environmental, 2013). This approach was significantly less successful than either biosparging or groundwater recirculation for two main reasons (1) the inability to adequately control the oxygen:propane ratio with the system used and (2) the inability to supply and distribute enough oxygen in the aquifer to overcome the highly reducing geochemical conditions. Gas distribution can be a significant limitation with this type of system.

As previously noted, the hybrid technology utilized during this demonstration consisted of biosparging pure oxygen into the aquifer, with the addition of isobutane and methane gases, DAP, and bioaugmentation cultures in recycled groundwater. This approach clearly shows the flexibility of the overall technology. In this case, biosparging of oxygen was utilized to ensure that we could maintain aerobic anoxic conditions in the treatment zone. Due to the relatively low solubility of oxygen, this is the most effective means to achieve elevated DO concentrations in groundwater. Conversely, groundwater recirculation was utilized to add isobutane and methane, as well as DAP and bioaugmentation cultures. This type of addition is suitable for liquid amendments (DAP and cultures) and provides good control over the concentration of added co-substrate gases (isobutane and methane). As previously discussed, isobutanol was also added as a liquid using this approach.

Biosparging, groundwater recirculation and hybrid cometabolic treatment approaches can generally be used cost-effectively in deep as well as shallow aquifers. Aquifer depth is one of the limiting factors for fully passive designs, which become increasingly expensive due to close spacing of injection points and/or technically impractical (e.g., for passive trench barriers) as the depth to the water table increases (Stroo and Ward, 2009). A semi-passive pumping design has fewer limitations with depth. Similarly, wide plumes are more readily treated with active or semi-passive approaches than with fully passive designs, as a few wells (and high sparging rates) can often be used to distribute co-substrate over a large area, rather than closely spaced wells or injection points [see Stroo and Ward (2009) for further comparisons of different amendment designs].

One of the lessons learned from this demonstration is that 1,1-DCE represents a significant challenge for cometabolic treatment at field scale, and is likely to inhibit the activity of organisms that can degrade many other CVOCs and 1,4-D. The “threshold” concentration of 1,1-DCE above which cometabolism should be used is not clear and may be site and organism specific. This is a good area for future laboratory studies. However, based on the field data, if one can’t biostimulate native bacteria to cometabolically biodegrade the COCs at a site with 1,1-DCE (i.e., via air/oxygen, nutrient and cometabolic gas addition only), then cometabolic treatment in the field is contraindicated. Overall, cometabolism is an excellent approach to treat dilute plumes (i.e., tens to a few hundred ug/L) with a variety of different CVOCs and emerging COCs, including 1,4-D, but the toxicity of epoxides from chlorinated ethenes, with 1,1-DCE being perhaps the most problematic, is likely to limit the effectiveness of this approach at sites with high chlorinated ethene concentrations (e.g., several hundred µg/L to mg/L). Overall, the concentration of chlorinated ethenes (1,1-DCE in particular) at the NASNI site were likely too high for technology to achieve the desired 95% removal metric.

8.2 SAFETY

Because methane, isobutane and other co-substrate gases are flammable, specific safety measures must be considered when designing, installing, and monitoring an *in situ* cometabolic treatment system. However, it is relatively straight forward for a competent engineer to design a system that is safe for operation. All electrical equipment and wiring in the system enclosure supplying co-substrate gases should be intrinsically safe, and the gas cylinders/tanks should be stored outside of the enclosure. In addition to local regulations, there are numerous codes, standards, and regulations associated with the storage, handling and use of flammable gases and oxygen from the following agencies that should be researched and followed during the design, construction, and operation of cometabolic treatment system:

- National Fire Protection Agency (NFPA)
- American National Standards Institute (ANSI)
- Occupational Safety & Health Administration (OSHA)
- National Institute of Standards and Technology (NIST)
- Compressed Gas Association (CGA)
- American Society for Testing and Materials (ASTM)
- Underwriters Laboratories (UL)
- American Gas Association (AGA) codes and Standards

During this demonstration, compressed oxygen was stored on one side of the enclosure and isobutane and methane on the other side of the enclosure. The biosparging system and pumping components were housed within a 20-ft long Conex box. The box had a partition wall separating the enclosure into two spaces. The smaller of the two spaces was the system control room, which was rated as a non-hazardous atmosphere, and housed the PLC/SCADA system with integrated computer, electrical control panel, solar power charging and distribution systems, and a combination air conditioner/heater. The larger space, which included gas piping/fittings, mass flow controllers, piping for the groundwater recirculation system, tanks for adding DAP to the groundwater, well control solenoid valves and other system process components, was rated as a Class 1, Division 2 atmosphere, due to the presence of flammable sparge gases flowing through the process piping in this portion of the enclosure. All electrical components and connections in this portion of the enclosure were intrinsically safe to meet the hazardous atmosphere classification. This space was monitored with three separate gas detectors, which continuously measured oxygen, isobutane, and methane levels within the enclosure, and were programmed to shut-down the system and notify appropriate personnel if 10% of the LEL for isobutane or methane or 23% oxygen were measured within the enclosure.

While the same safety practices would exist for both pilot scale and full-scale systems, it is likely that a full-scale system would not utilize oxygen cylinders and would instead use an air compressor or an oxygen generator (with an air compressor). Furthermore, the storage of larger quantities of flammable gases and/or oxygen might require additional safeguards and/or permitting.

8.3 SECONDARY GROUNDWATER IMPACTS

One of the significant advantages of an aerobic treatment system of this type is that there are typically very few negative impacts to groundwater geochemistry, particularly in comparison to *in situ* anaerobic treatment approaches (e.g., enhanced reductive dechlorination, or ERD), where large amounts of carbon substrate are applied to treat COCs. ERD results in a variety of secondary groundwater issues, including mobilization of iron, manganese, and/or arsenic, production of sulfide and methane, and elevated total organic carbon. These changes are often acceptable in source areas which are heavily impacted by CVOCs, but less so in a larger, dilute plumes where the groundwater resources are more likely to be part of a drinking water aquifer. As noted in **Sections 5.7.2** and **6.2**, the aquifer was generally aerobic in the demonstration area prior to treatment and DO throughout the treatment area typically ranged between 5 mg/L and 40 mg/L during the treatment phase (Phase 2) of this demonstration. Therefore, appreciable changes in aquifer geochemistry (including ORP and pH) were not observed during the demonstration.

8.4 TECHNOLOGY COSTS

The expected cost drivers for the installation and operation of an *in situ* MPS biosparging system for the treatment of groundwater impacted with comingled 1,4-D and CVOCs and comparisons to other remedial approaches are provided in **Section 7.0**.

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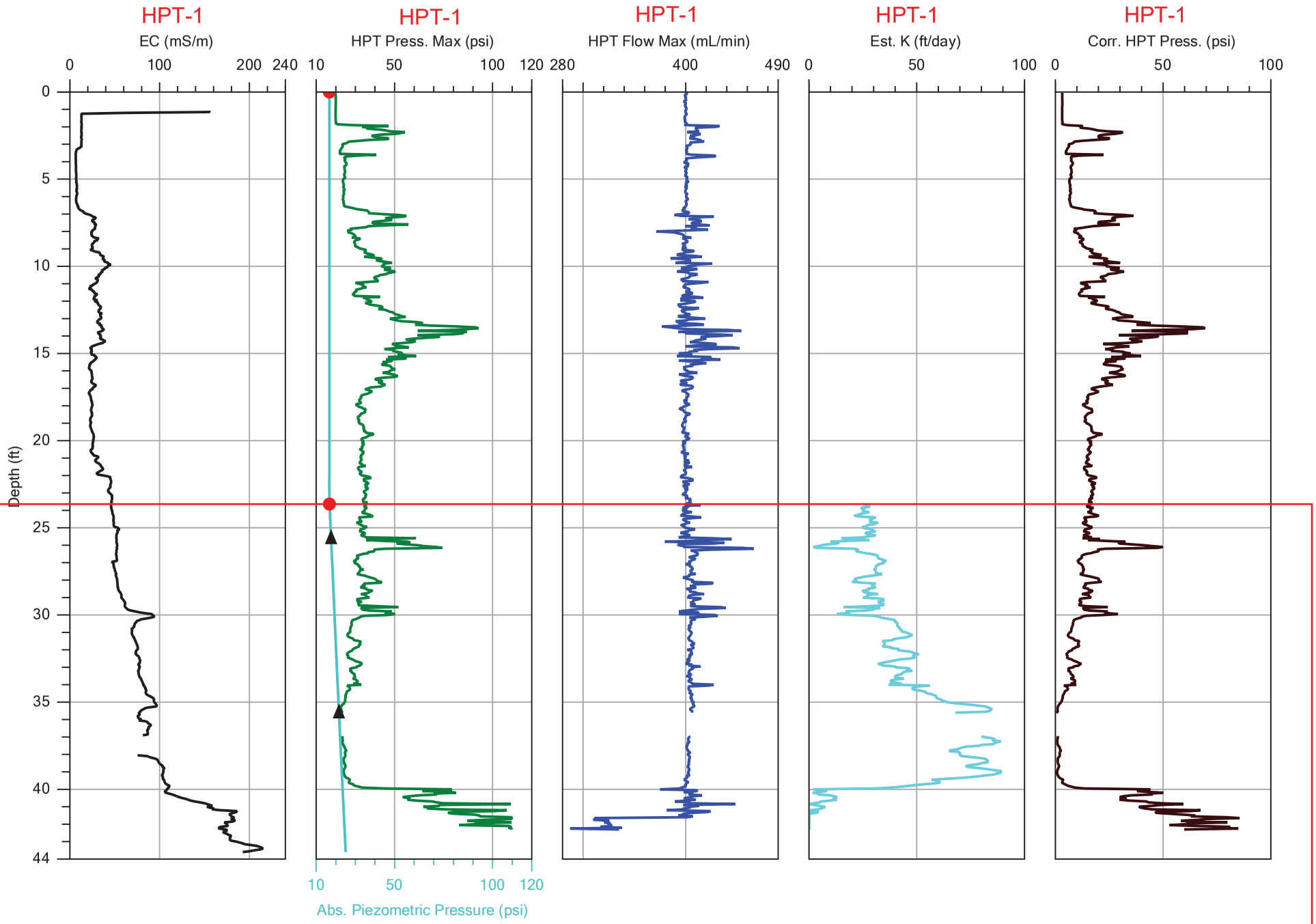
Appendix A
Points of Contact

Points of Contact

POINT OF CONTACT Name	ORGANIZATION Name Address	CONTACT INFORMATION Phone E-mail	ROLE IN PROJECT
Anthony S. Danko, PhD, PE	NAVFAC EXWC	805-982-4805 direct anthony.danko@navy.mil	Principal Investigator
Paul B. Hatzinger, PhD	APTIM Federal Services 17 Princess Road Lawrenceville, NJ 08648	609-895-5356 direct 267-337-4003 cell paul.hatzinger@aptim.com	Co-Principal Investigator
David R. Lippincott, PG	APTIM Federal Services 17 Princess Road Lawrenceville, NJ 08648	609-895-5380 direct 609-605-0883 cell david.lippincott@aptim.com	Project Manager
Graig M. Lavorgna	APTIM Federal Services 17 Princess Road Lawrenceville, NJ 08648	609-895-5343 direct 908-309-7651 cell graig.lavorgna@aptim.com	Project Engineer
Lewis Semprini, PhD	Oregon State University School of Chemical, Biological and Environmental Engineering Corvallis, Oregon 97331	541-737-6895 direct lewis.semprini@oregonstate.edu	Technical Support
Michael Hyman, Ph.D.	North Carolina State University Department of Plant and Microbial Biology 4545 Thomas Hall Raleigh, NC 27695	919-515-7814 direct mrhyman@ncsu.edu	Technical Support
Andrea Leeson, PhD	SERDP/ESTCP 4800 Mark Center Drive Suite 17D03 Alexandria, VA 22350-3605	703-696-2118 direct 703-696-2114 fax andrea.leeson@gmail.com	ESTCP Environmental Restoration Program Manager
Michael J. Pound	NAVFAC Southwest	619-556-9901 direct michael.pound@navy.mil	NAS North Island Site Lead

Appendix B
HPT, Soil Boring, and Well Installation Logs



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DRILLING | TECHNICAL SERVICES
EXCELLENCE ON EVERY LEVEL™

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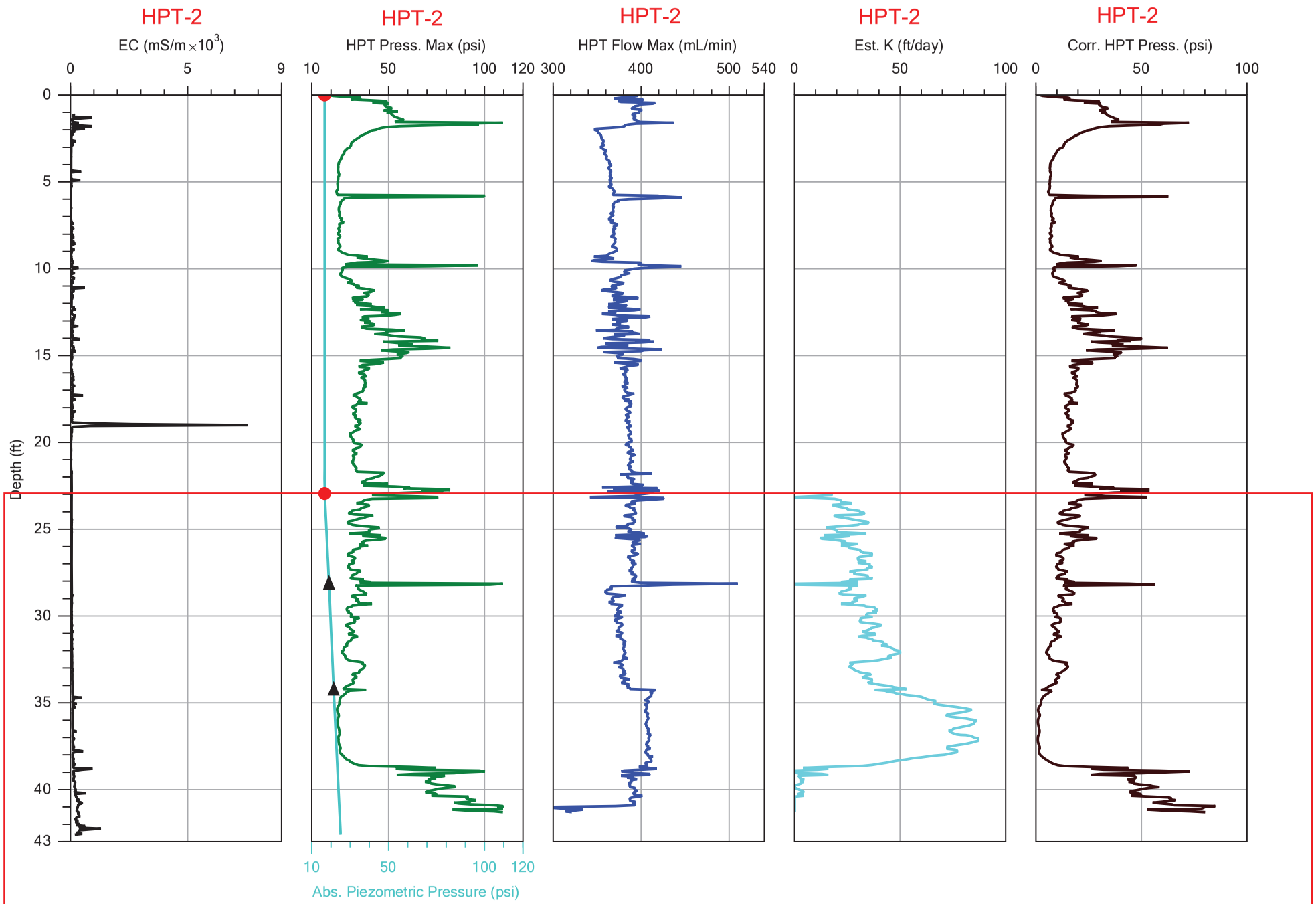
		Location: HPT-01



CASCADE
DRILLING | TECHNICAL SERVICES
EXCELLENCE ON EVERY LEVEL™

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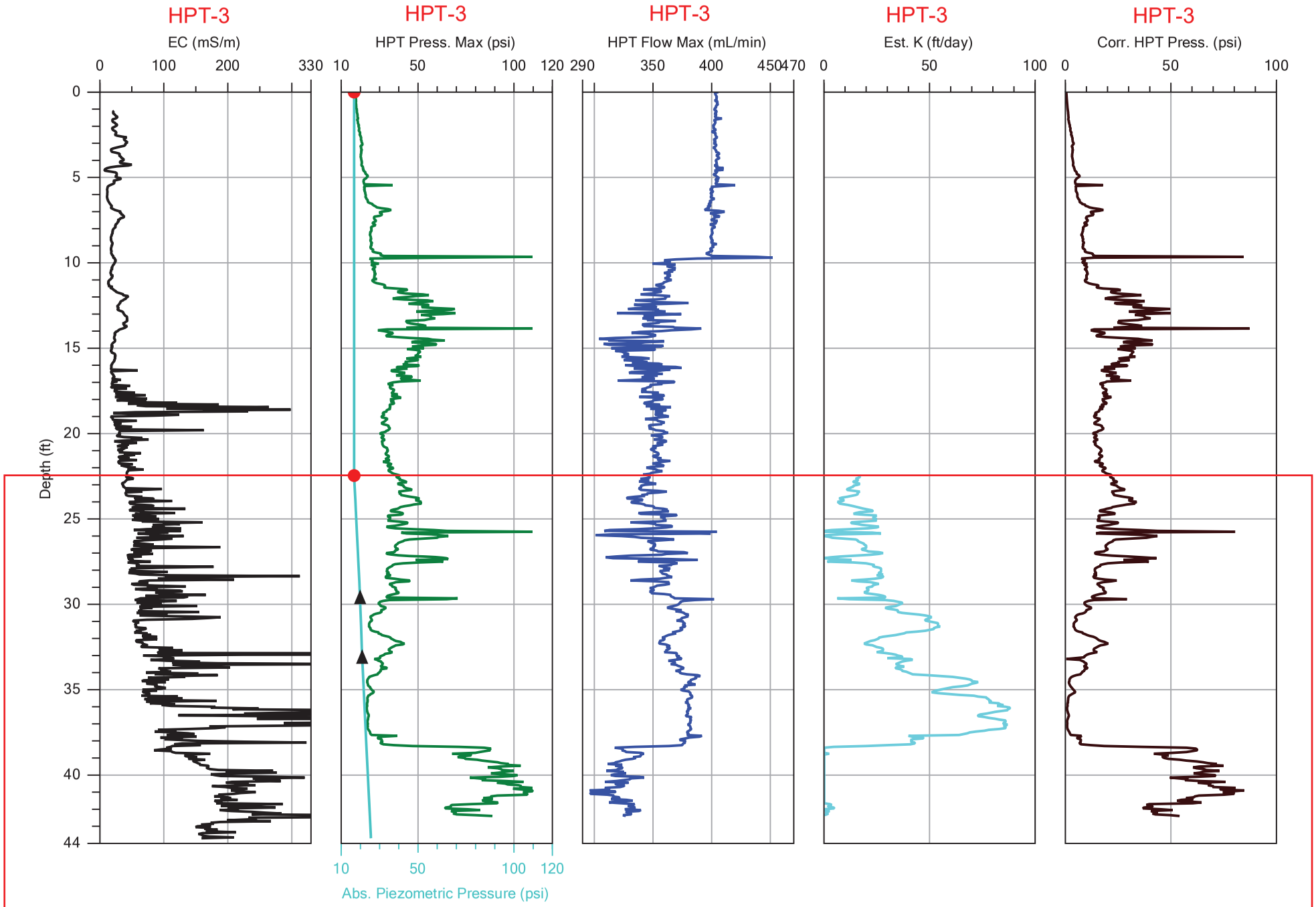
Project ID: N.A.S. North Island	Client: APTIM	Location: HPT-02

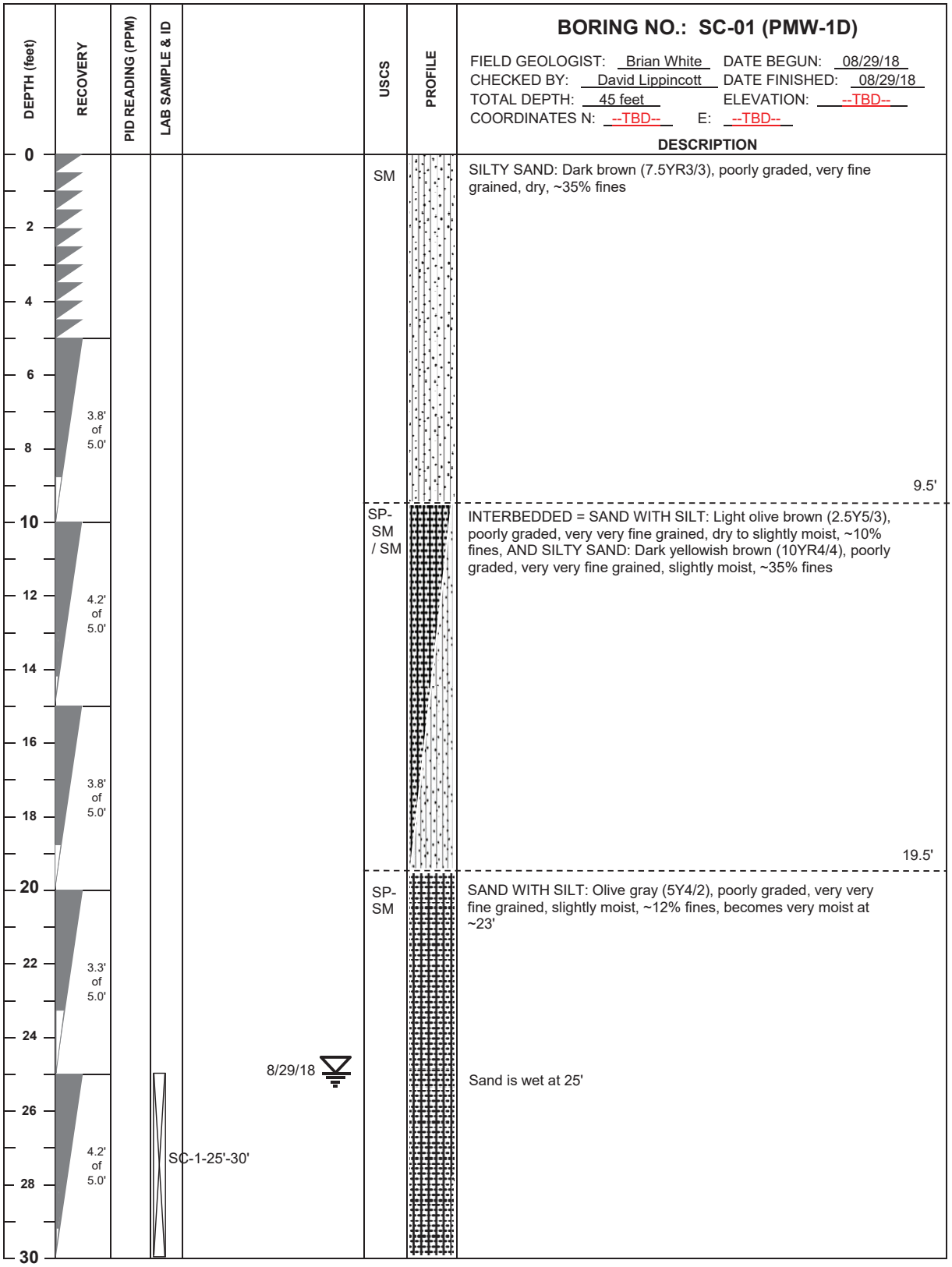


CASCADE
DRILLING | TECHNICAL SERVICES
EXCELLENCE ON EVERY LEVEL™

Company: Cascade	Operator: C Terry	File: HPT-03.MHP
Project ID: N.A.S. North Island	Client: APTIM	Date: 8/30/2018
		Location: HPT-03

BORING NO.: SC-01 (PMW-1D)

FIELD GEOLOGIST: Brian White DATE BEGUN: 08/29/18
CHECKED BY: David Lippincott DATE FINISHED: 08/29/18
TOTAL DEPTH: 45 feet ELEVATION: --TBD--
COORDINATES N: --TBD-- E: --TBD--

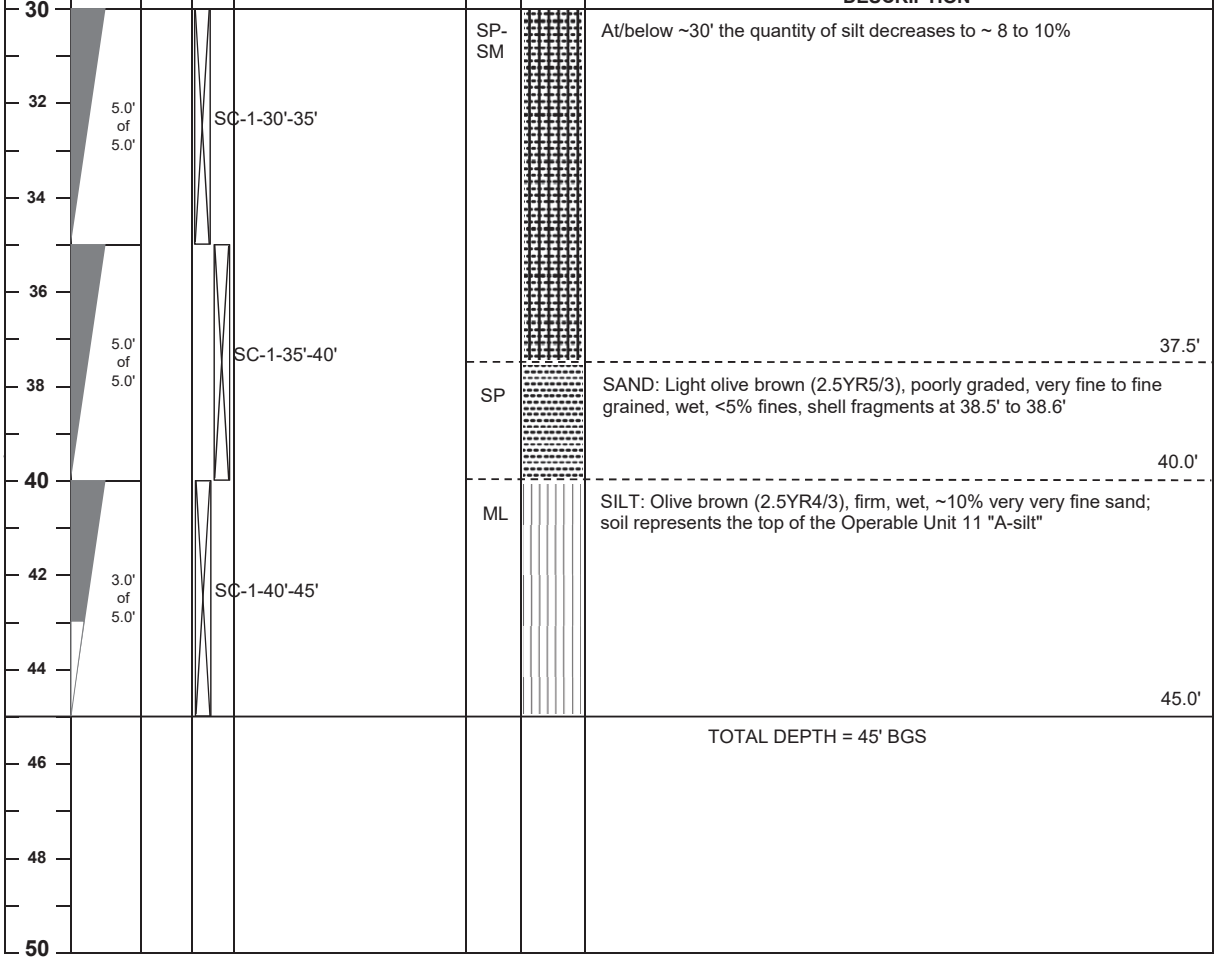


LOCATION: Operable Unit 11, Naval Air Station North Island, Coronado, California
CLIENT: U.S. Navy
APTIM PROJECT NO.: 501020

APTIM

BORING NO.: SC-01 (PMW-1D)

FIELD GEOLOGIST: Brian White DATE BEGUN: 08/29/18
CHECKED BY: David Lippincott DATE FINISHED: 08/29/18
TOTAL DEPTH: 45 feet ELEVATION: --TBD--
COORDINATES N: --TBD-- E: --TBD--



DRILLING DETAILS:

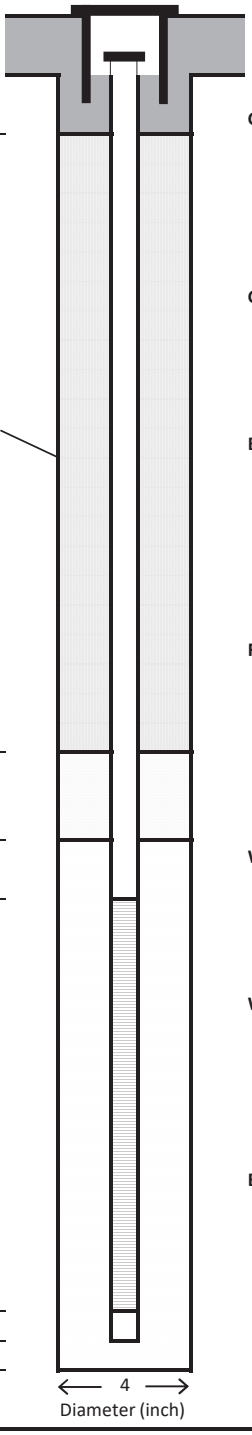
1. Direct push continous core soil boring (SC-01) drilled by Cascade Drilling Services using Geoprobe 6600 with 1.5" inside diameter core barrel. Borehole repushed using Geoprobe 8040 DT to install monitoring well PMW-1D (see well construction detail).
2. Borehole handaugered to 5' below grade before drilling.
3. Soil samples were collected for treatability testing, see boring log for depths.
4. No obvious soil staining or odor was detected.
5. Soil color classification (i.e., olive yellow [5Y6/3]) are from Munsell Soil Color Chart: 1994 Edition; describing hydrated/wet soil.

APTIM

MONITORING WELL CONSTRUCTION DETAILS

Project Name/No.:	ESTCP Demonstration Project / 501020
Location:	Operable Unit 11, NAS North Island
Client:	U.S. Navy
Drilling Co.:	Cascade Drilling Services
Driller:	Daniel Ortiz
Drill Rig/Method:	Geoprobe 8040 DT/Direct Push

Well Number:	**PTW-1**
Start/End Date(s):	9/5/2018
Logged/Checked By:	Brian White / David Lippincott
Northing:	--TBD--
Easting:	--TBD--
Surf. Elev.:	--TBD--
	(NAD 83 / NAVD 88)



Top of Grout (feet):	3.0
Top of Bentonite (feet):	23.0
Top of Filter Pack (feet):	26.0
Top of Screen Casing (feet):	29.0
Bottom of Screen (feet):	39.0
Bottom of Well Casing (feet):	39.3
Bottom of Borehole (feet):	39.5

NOTE: Well Diagram Not to Scale

Protection:

Well Head:	8" Diameter Iron Bolt-Down
	Traffic-Rated Well Vault
Casing:	Locking Well Cap

Ground Seal:

Dimensions:	18" Square
	(extends down 3' in center)
Type:	Concrete

Grout:

Type:	Cement w/~5% Bentonite
Installation:	Tremie Pipe/Gravity
Volume (feet^3):	5.23

Bentonite:

Type:	1/4" TR30 (Time Release)
Installation:	Inside Drill Casing/Gravity
Volume (feet^3):	0.79

Filter Pack:

Material:	Sand
Size:	#1/20
Installation:	Inside Drill Casing/Gravity
Volume (feet^3):	3.53

Well Casing:

Type:	Schedule 40 PVC
Diameter (inches):	2.0

Well Screen Casing:

Type:	Schedule 40 PVC
Diameter (inches):	2.0
Slot Size:	Wire Mesh
Slot Type:	Factory Pre-Pack

End Cap:

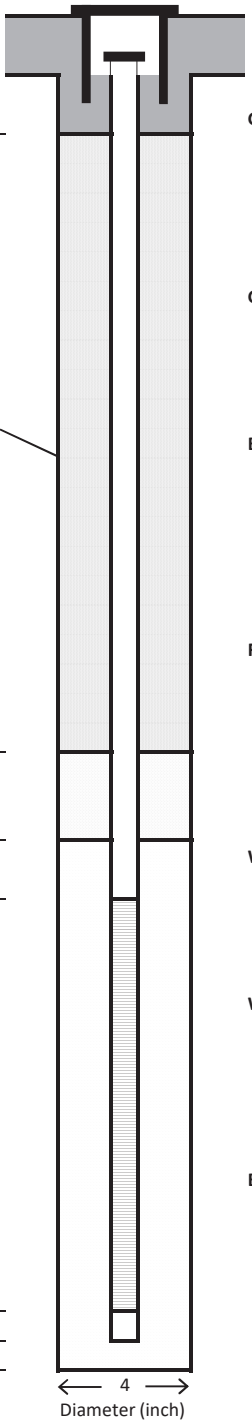
Type:	Schedule 40 PVC
Dimensions:	2.0 " Diameter x 0.30' Length

APTIM

MONITORING WELL CONSTRUCTION DETAILS

Project Name/No.:	ESTCP Demonstration Project / 501020
Location:	Operable Unit 11, NAS North Island
Client:	U.S. Navy
Drilling Co.:	Cascade Drilling Services
Driller:	Daniel Ortiz
Drill Rig/Method:	Geoprobe 8040 DT/Direct Push

Well Number:	**PMW-1S**
Start/End Date(s):	9/4/2018
Logged/Checked By:	Brian White / David Lippincott
Northing:	--TBD--
Easting:	--TBD--
Surf. Elev.:	--TBD--
	(NAD 83 / NAVD 88)



Top of Grout (feet):	3.0
Top of Bentonite (feet):	22.9
Top of Filter Pack (feet):	25.9
Top of Screen Casing (feet):	28.3
Bottom of Screen (feet):	33.3
Bottom of Well Casing (feet):	33.7
Bottom of Borehole (feet):	34.0

NOTE: Well Diagram Not to Scale

Protection:

Well Head:	8" Diameter Iron Bolt-Down
	Traffic-Rated Well Vault
Casing:	Locking Well Cap

Ground Seal:

Dimensions:	18" Square
	(extends down 3' in center)
Type:	Concrete

Grout:

Type:	Cement w/~5% Bentonite
Installation:	Tremie Pipe/Gravity
Volume (feet^3):	5.97

Bentonite:

Type:	1/4" TR30 (Time Release)
Installation:	Inside Drill Casing/Gravity
Volume (feet^3):	0.90

Filter Pack:

Material:	Sand
Size:	#1/20
Installation:	Inside Drill Casing/Gravity
Volume (feet^3):	2.43

Well Casing:

Type:	Schedule 40 PVC
Diameter (inches):	1.5

Well Screen Casing:

Type:	Schedule 40 PVC
Diameter (inches):	1.5
Slot Size:	Wire Mesh
Slot Type:	Factory Pre-Pack

End Cap:

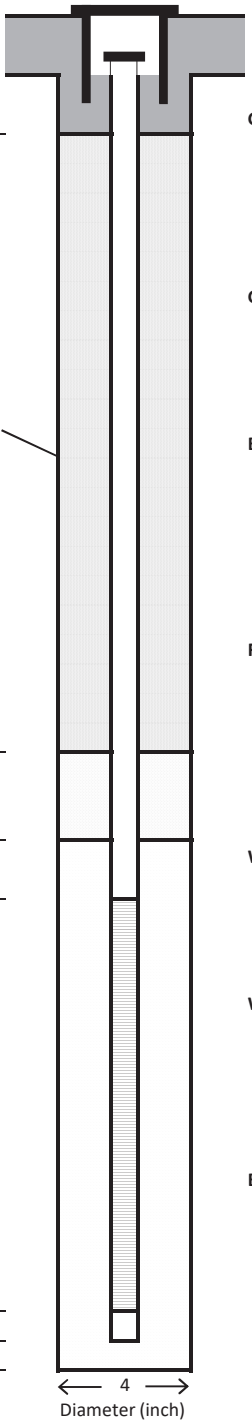
Type:	Schedule 40 PVC
Dimensions:	1.5 " Diameter x 0.35' Length

APTIM

MONITORING WELL CONSTRUCTION DETAILS

Project Name/No.:	ESTCP Demonstration Project / 501020
Location:	Operable Unit 11, NAS North Island
Client:	U.S. Navy
Drilling Co.:	Cascade Drilling Services
Driller:	Daniel Ortiz
Drill Rig/Method:	Geoprobe 8040 DT/Direct Push

Well Number:	**PMW-1D**
Start/End Date(s):	9/5/2018
Logged/Checked By:	Brian White / David Lippincott
Northing:	--TBD--
Easting:	--TBD--
Surf. Elev.:	--TBD--
	(NAD 83 / NAVD 88)



Top of Grout (feet):	3.0
Top of Bentonite (feet):	28.6
Top of Filter Pack (feet):	31.8
Top of Screen Casing (feet):	34.0
Bottom of Screen (feet):	39.0
Bottom of Well Casing (feet):	39.4
Bottom of Borehole (feet):	39.5

NOTE: Well Diagram Not to Scale

Protection:

Well Head:	8" Diameter Iron Bolt-Down
	Traffic-Rated Well Vault
Casing:	Locking Well Cap

Ground Seal:

Dimensions:	18" Square
	(extends down 3' in center)
Type:	Concrete

Grout:

Type:	Cement w/~5% Bentonite
Installation:	Tremie Pipe/Gravity
Volume (feet^3):	7.68

Bentonite:

Type:	1/4" TR30 (Time Release)
Installation:	Inside Drill Casing/Gravity
Volume (feet^3):	0.96

Filter Pack:

Material:	Sand
Size:	#1/20
Installation:	Inside Drill Casing/Gravity
Volume (feet^3):	2.31

Well Casing:

Type:	Schedule 40 PVC
Diameter (inches):	1.5

Well Screen Casing:

Type:	Schedule 40 PVC
Diameter (inches):	1.5
Slot Size:	Wire Mesh
Slot Type:	Factory Pre-Pack

End Cap:

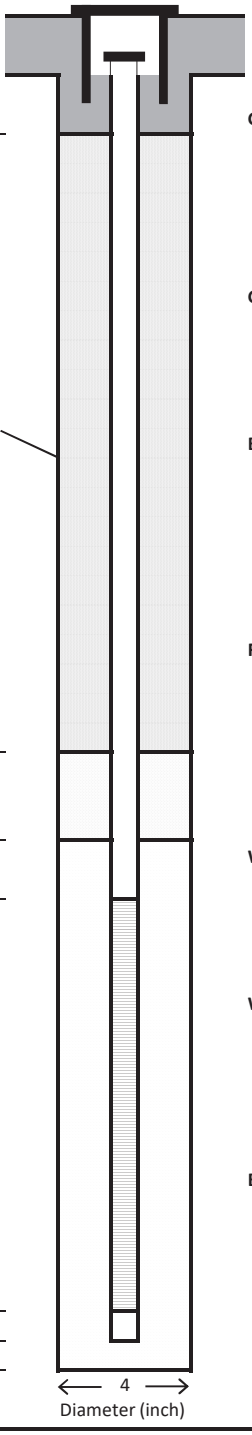
Type:	Schedule 40 PVC
Dimensions:	1.5 " Diameter x 0.35' Length

APTIM

MONITORING WELL CONSTRUCTION DETAILS

Project Name/No.:	ESTCP Demonstration Project / 501020
Location:	Operable Unit 11, NAS North Island
Client:	U.S. Navy
Drilling Co.:	Cascade Drilling Services
Driller:	Daniel Ortiz
Drill Rig/Method:	Geoprobe 8040 DT/Direct Push

Well Number:	**PMW-2S**
Start/End Date(s):	9/4/2018
Logged/Checked By:	Brian White / David Lippincott
Northing:	--TBD--
Easting:	--TBD--
Surf. Elev.:	--TBD--
	(NAD 83 / NAVD 88)



Top of Grout (feet):	3.0
Top of Bentonite (feet):	22.5
Top of Filter Pack (feet):	25.5
Top of Screen Casing (feet):	27.7
Bottom of Screen (feet):	32.7
Bottom of Well Casing (feet):	33.1
Bottom of Borehole (feet):	33.1

NOTE: Well Diagram Not to Scale

Protection:

Well Head:	8" Diameter Iron Bolt-Down
	Traffic-Rated Well Vault
Casing:	Locking Well Cap

Ground Seal:

Dimensions:	18" Square
	(extends down 3' in center)
Type:	Concrete

Grout:

Type:	Cement w/~5% Bentonite
Installation:	Tremie Pipe/Gravity
Volume (feet^3):	5.85

Bentonite:

Type:	1/4" TR30 (Time Release)
Installation:	Inside Drill Casing/Gravity
Volume (feet^3):	0.90

Filter Pack:

Material:	Sand
Size:	#1/20
Installation:	Inside Drill Casing/Gravity
Volume (feet^3):	2.28

Well Casing:

Type:	Schedule 40 PVC
Diameter (inches):	1.5

Well Screen Casing:

Type:	Schedule 40 PVC
Diameter (inches):	1.5
Slot Size:	Wire Mesh
Slot Type:	Factory Pre-Pack

End Cap:

Type:	Schedule 40 PVC
Dimensions:	1.5 " Diameter x 0.35' Length

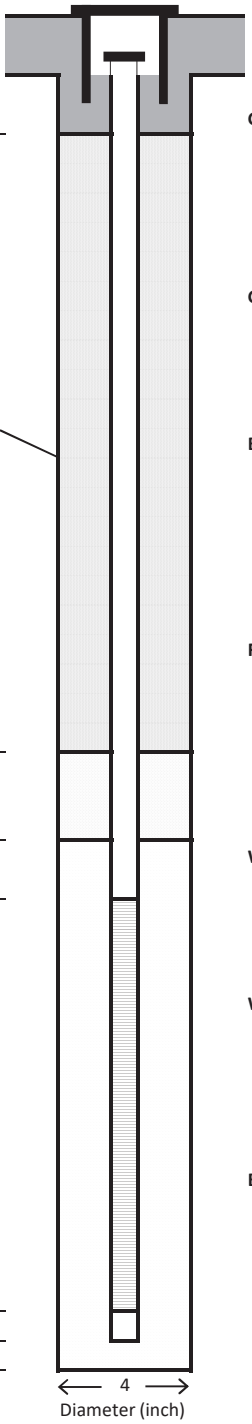
APTIM

MONITORING WELL CONSTRUCTION DETAILS

Project Name/No.:	ESTCP Demonstration Project / 501020
Location:	Operable Unit 11, NAS North Island
Client:	U.S. Navy
Drilling Co.:	Cascade Drilling Services
Driller:	Daniel Ortiz
Drill Rig/Method:	Geoprobe 8040 DT/Direct Push

Well Number:	**PMW-2D**
Start/End Date(s):	9/5/2018
Logged/Checked By:	Brian White / David Lippincott
Northing:	--TBD--
Easting:	--TBD--
Surf. Elev.:	--TBD--

(NAD 83 / NAVD 88)



Top of Grout (feet):	3.0
Top of Bentonite (feet):	26.0
Top of Filter Pack (feet):	29.0
Top of Screen Casing (feet):	33.8
Bottom of Screen (feet):	38.8
Bottom of Well Casing (feet):	39.2
Bottom of Borehole (feet):	39.2

NOTE: Well Diagram Not to Scale

Protection:

Well Head:	8" Diameter Iron Bolt-Down
	Traffic-Rated Well Vault
Casing:	Locking Well Cap

Ground Seal:

Dimensions:	18" Square
	(extends down 3' in center)
Type:	Concrete

Grout:

Type:	Cement w/~5% Bentonite
Installation:	Tremie Pipe/Gravity
Volume (feet^3):	6.90

Bentonite:

Type:	1/4" TR30 (Time Release)
Installation:	Inside Drill Casing/Gravity
Volume (feet^3):	0.90

Filter Pack:

Material:	Sand
Size:	#1/20
Installation:	Inside Drill Casing/Gravity
Volume (feet^3):	3.06

Well Casing:

Type:	Schedule 40 PVC
Diameter (inches):	1.5

Well Screen Casing:

Type:	Schedule 40 PVC
Diameter (inches):	1.5
Slot Size:	Wire Mesh
Slot Type:	Factory Pre-Pack

End Cap:

Type:	Schedule 40 PVC
Dimensions:	1.5 " Diameter x 0.35' Length

APTIM

WELL CONSTRUCTION DIAGRAM (Above ground)

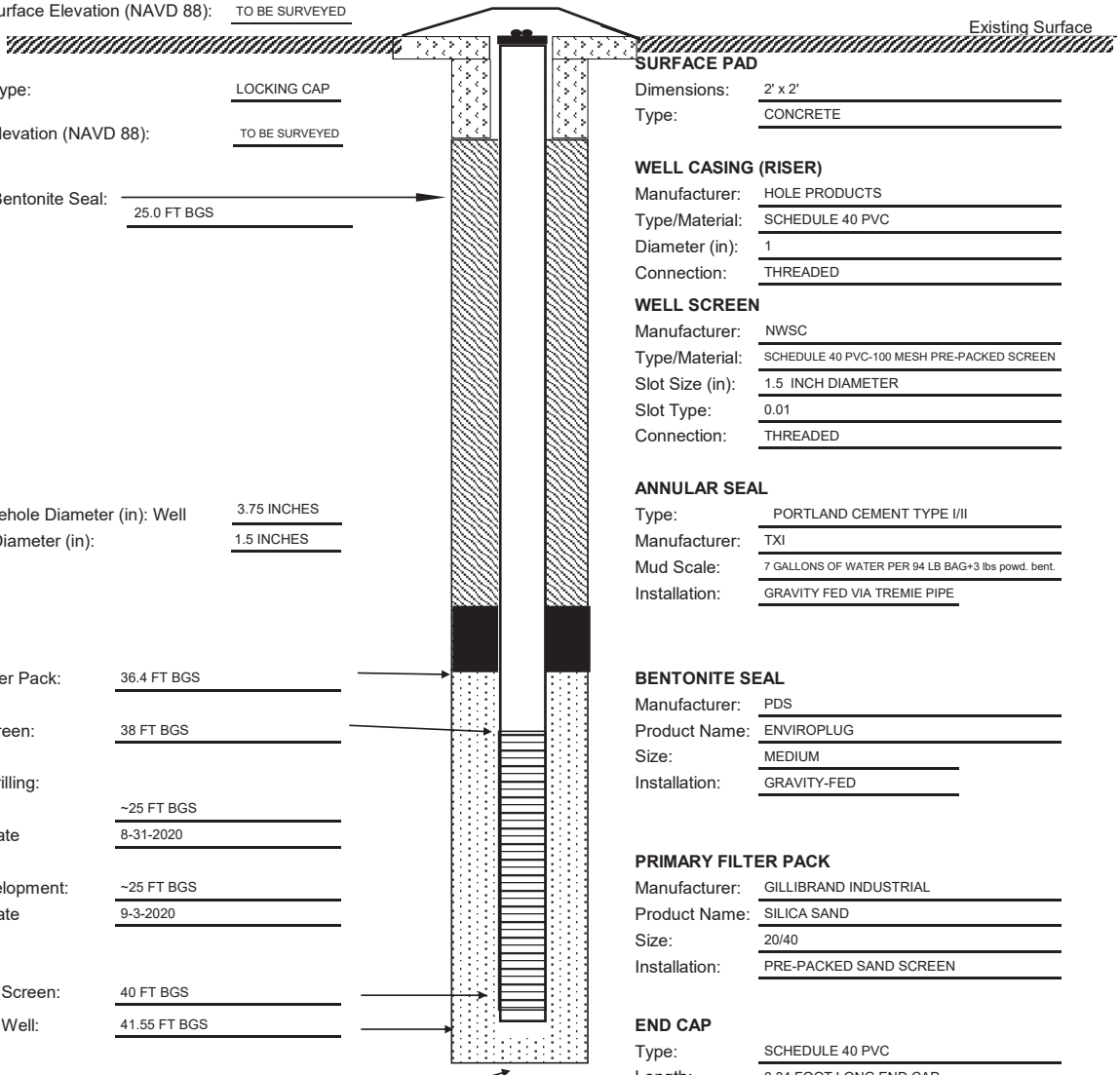
SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	BSW-1
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	DPT (8040 DT)
PROJECT NO.:	501020	**DATE(S):**	8-31-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	KEVIN WARD	**NORTHING:**	1836964.25
SCIENTIST:	BRIAN WHITE	**EASTING:**	6267811.27

PROTECTIVE CASING

Type: FLUSH MOUNT, 8-INCH DIAMETER MANHOLE
Dimensions: 8 INCHES
Length: NA
Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP
Casing Elevation (NAVD 88): TO BE SURVEYED
Top of Bentonite Seal: 25.0 FT BGS
Well Borehole Diameter (in): 3.75 INCHES
Well Casing Diameter (in): 1.5 INCHES
Top of Filter Pack: 36.4 FT BGS
Top of Screen: 38 FT BGS
During Drilling: ~25 FT BGS
Date: 8-31-2020
Post Development: ~25 FT BGS
Date: 9-3-2020
Bottom of Screen: 40 FT BGS
Bottom of Well: 41.55 FT BGS
Borehole Depth: 41.25 FT BGS

SURFACE PAD

Dimensions: 2' x 2'
Type: CONCRETE

WELL CASING (RISER)

Manufacturer: HOLE PRODUCTS
Type/Material: SCHEDULE 40 PVC
Diameter (in): 1
Connection: THREADED

WELL SCREEN

Manufacturer: NWSC
Type/Material: SCHEDULE 40 PVC-100 MESH PRE-PACKED SCREEN
Slot Size (in): 1.5 INCH DIAMETER
Slot Type: 0.01
Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE I/II
Manufacturer: TXI
Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.
Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PDS
Product Name: ENVIROPLUG
Size: MEDIUM
Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: GILLIBRAND INDUSTRIAL
Product Name: SILICA SAND
Size: 20/40
Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC
Length: 0.34 FOOT LONG END CAP

BACKFILL MATERIAL

Type: 20/40 SILICA SAND

Comments: SILT TRAP (0.34 FT LONG END CAP PLUS 1 FT LONG SUMP) AT BOTTOM OF WELL.
ADDED ~4 GALLONS

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	BSW-2
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	DPT (8040 DT)
PROJECT NO.:	501020	**DATE(S):**	8-31-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	KEVIN WARD	**NORTHING:**	1836968.77
SCIENTIST:	BRIAN WHITE	**EASTING:**	6267793.81

PROTECTIVE CASING

Type: FLUSH MOUNT, 8-INCH DIAMETER MANHOLE

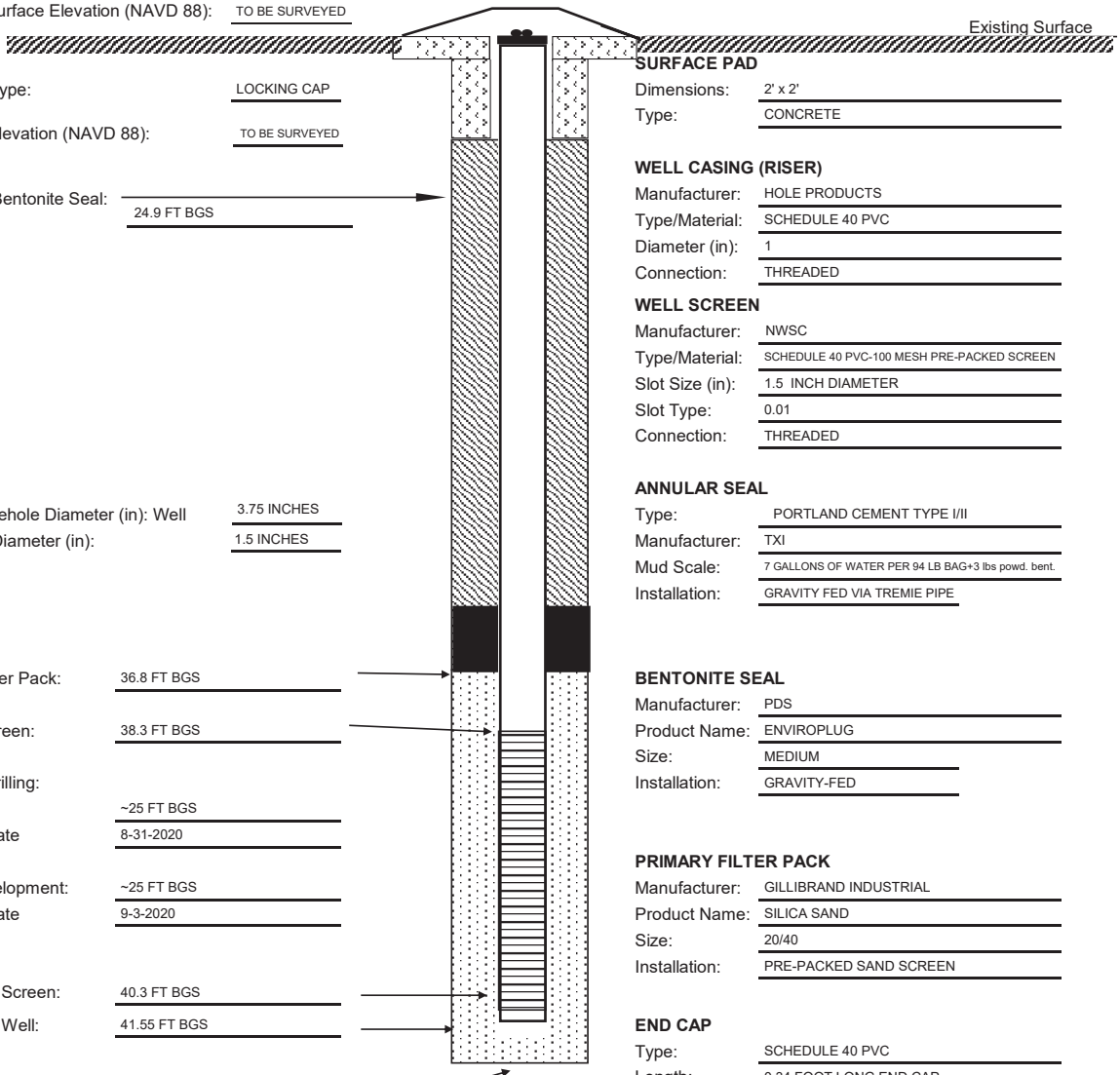
Dimensions: 8 INCHES

Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 24.9 FT BGS

Well Borehole Diameter (in): 3.75 INCHES

Casing Diameter (in): 1.5 INCHES

Top of Filter Pack: 36.8 FT BGS

Top of Screen: 38.3 FT BGS

During Drilling: ~25 FT BGS

Date: 8-31-2020

Post Development: ~25 FT BGS

Date: 9-3-2020

Bottom of Screen: 40.3 FT BGS

Bottom of Well: 41.55 FT BGS

Borehole Depth: 41.6 FT BGS

SURFACE PAD

Dimensions: 2' x 2'

Type: CONCRETE

WELL CASING (RISER)

Manufacturer: HOLE PRODUCTS

Type/Material: SCHEDULE 40 PVC

Diameter (in): 1

Connection: THREADED

WELL SCREEN

Manufacturer: NWSC

Type/Material: SCHEDULE 40 PVC-100 MESH PRE-PACKED SCREEN

Slot Size (in): 1.5 INCH DIAMETER

Slot Type: 0.01

Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE I/II

Manufacturer: TXI

Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.

Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PDS

Product Name: ENVIROPLUG

Size: MEDIUM

Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: GILLIBRAND INDUSTRIAL

Product Name: SILICA SAND

Size: 20/40

Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC

Length: 0.34 FOOT LONG END CAP

BACKFILL MATERIAL

Type: 20/40 SILICA SAND

Comments: SILT TRAP (0.34 FT LONG END CAP PLUS 1 FT LONG SUMP) AT BOTTOM OF WELL.

ADDED ~4 GALLONS

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	BSW-3
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	DPT (8040 DT)
PROJECT NO.:	501020	**DATE(S):**	9-1-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	KEVIN WARD	**NORTHING:**	1836985.95
SCIENTIST:	BRIAN WHITE	**EASTING:**	6267792.87

PROTECTIVE CASING

Type: FLUSH MOUNT, 8-INCH DIAMETER MANHOLE

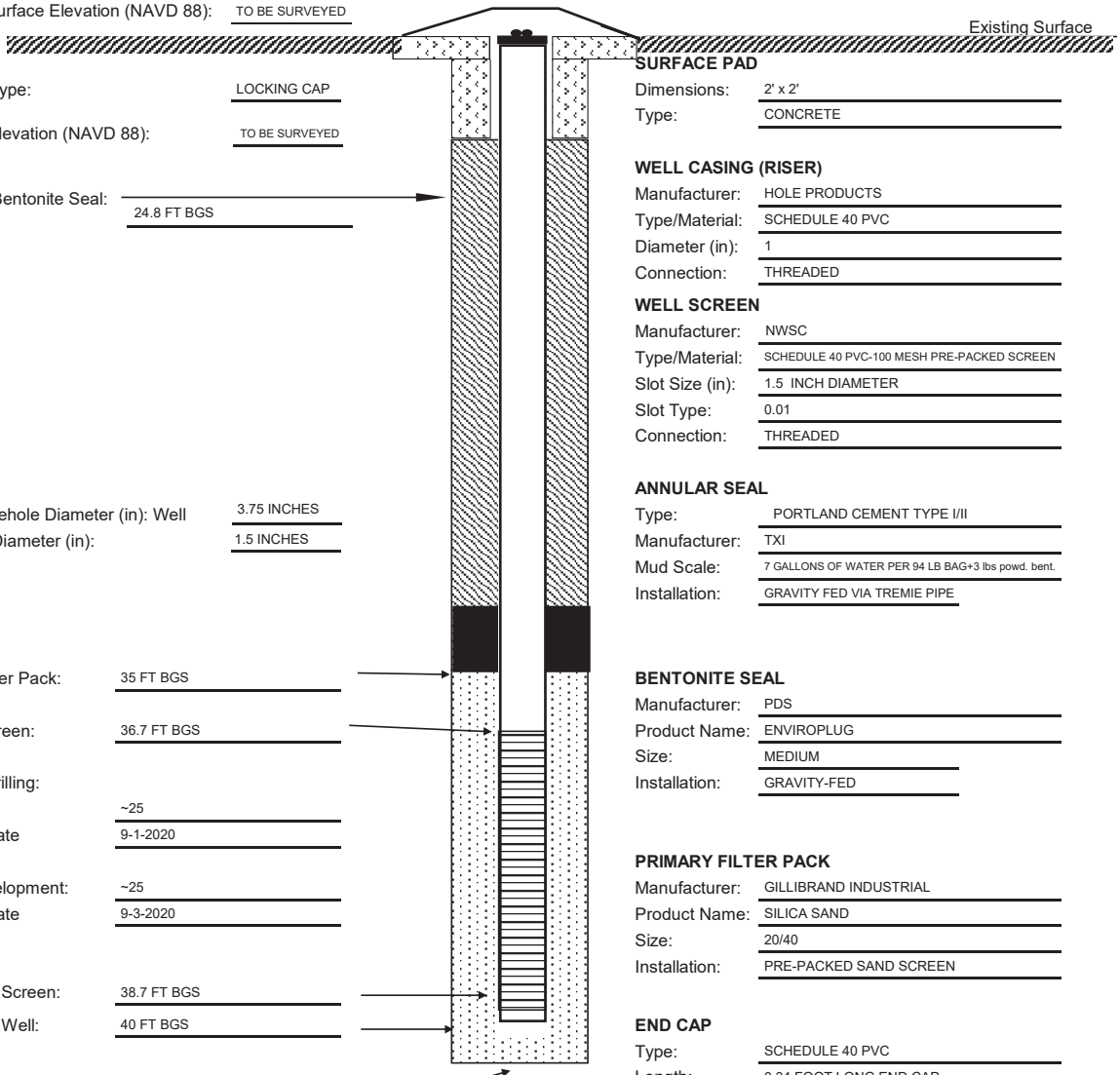
Dimensions: 8 INCHES

Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 24.8 FT BGS

Well Borehole Diameter (in): 3.75 INCHES

Casing Diameter (in): 1.5 INCHES

Top of Filter Pack: 35 FT BGS

Top of Screen: 36.7 FT BGS

During Drilling: ~25

Date: 9-1-2020

Post Development: ~25

Date: 9-3-2020

Bottom of Screen: 38.7 FT BGS

Bottom of Well: 40 FT BGS

Borehole Depth: 40 FT BGS

SURFACE PAD

Dimensions: 2' x 2'

Type: CONCRETE

WELL CASING (RISER)

Manufacturer: HOLE PRODUCTS

Type/Material: SCHEDULE 40 PVC

Diameter (in): 1

Connection: THREADED

WELL SCREEN

Manufacturer: NWSC

Type/Material: SCHEDULE 40 PVC-100 MESH PRE-PACKED SCREEN

Slot Size (in): 1.5 INCH DIAMETER

Slot Type: 0.01

Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE I/II

Manufacturer: TXI

Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.

Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PDS

Product Name: ENVIROPLUG

Size: MEDIUM

Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: GILLIBRAND INDUSTRIAL

Product Name: SILICA SAND

Size: 20/40

Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC

Length: 0.34 FOOT LONG END CAP

BACKFILL MATERIAL

Type: 20/40 SILICA SAND

Comments: SILT TRAP (0.34 FT LONG END CAP PLUS 1 FT LONG SUMP) AT BOTTOM OF WELL.

ADDED ~4 GALLONS

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	BSW-4
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	DPT (8040 DT)
PROJECT NO.:	501020	**DATE(S):**	9-1-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	KEVIN WARD	**NORTHING:**	1836992.7
SCIENTIST:	BRIAN WHITE	**EASTING:**	6267808.71

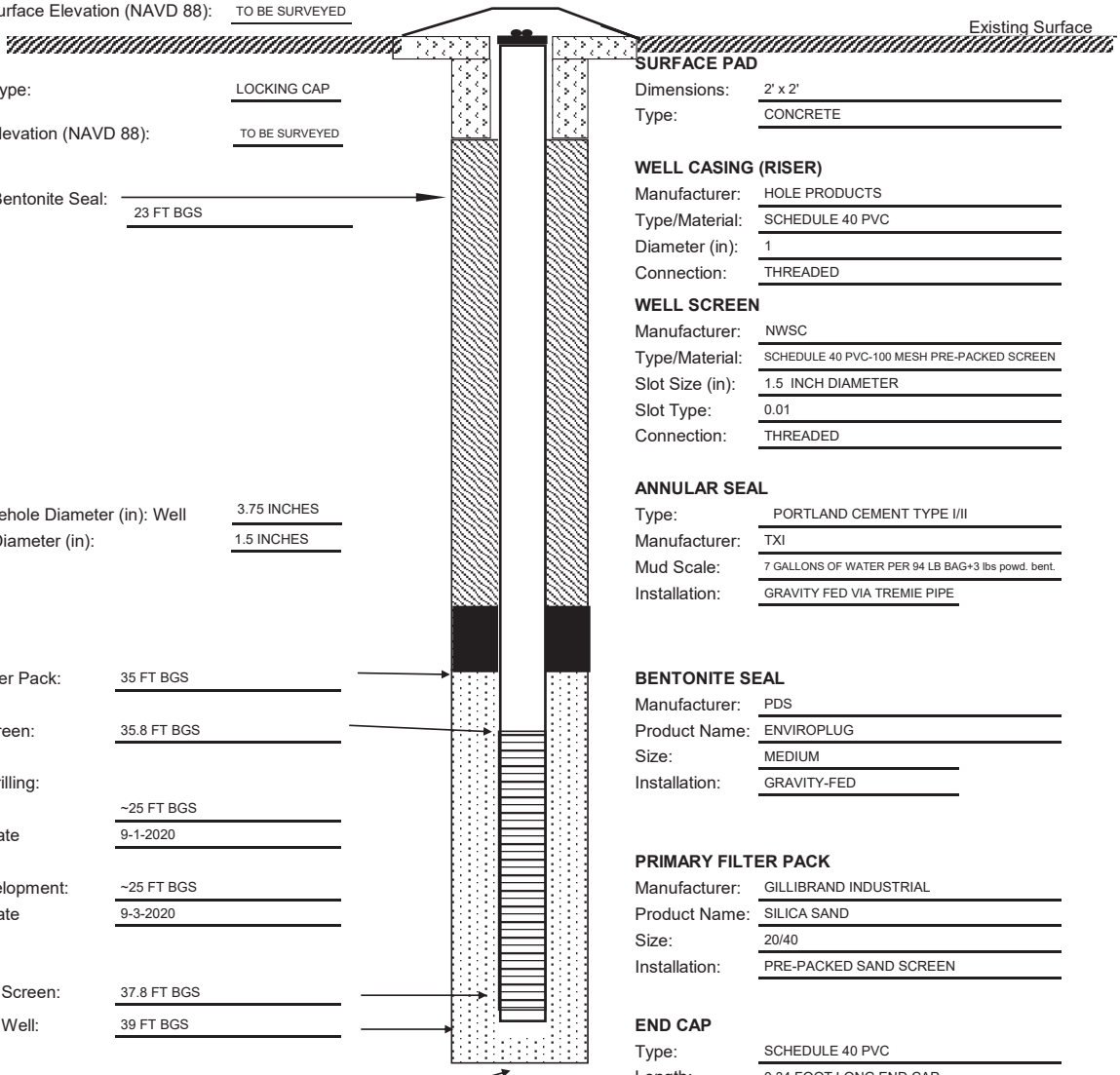
PROTECTIVE CASING

Type: FLUSH MOUNT, 8-INCH DIAMETER MANHOLE
Dimensions: 8 INCHES
Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 23 FT BGS

Well Borehole Diameter (in): 3.75 INCHES
Well Casing Diameter (in): 1.5 INCHES

Top of Filter Pack: 35 FT BGS

Top of Screen: 35.8 FT BGS

During Drilling: ~25 FT BGS
Date: 9-1-2020

Post Development: ~25 FT BGS
Date: 9-3-2020

Bottom of Screen: 37.8 FT BGS

Bottom of Well: 39 FT BGS

Borehole Depth: 39

SURFACE PAD

Dimensions: 2' x 2'
Type: CONCRETE

WELL CASING (RISER)

Manufacturer: HOLE PRODUCTS
Type/Material: SCHEDULE 40 PVC
Diameter (in): 1
Connection: THREADED

WELL SCREEN

Manufacturer: NWSC
Type/Material: SCHEDULE 40 PVC-100 MESH PRE-PACKED SCREEN
Slot Size (in): 1.5 INCH DIAMETER
Slot Type: 0.01
Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE I/II
Manufacturer: TXI
Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.
Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PDS
Product Name: ENVIROPLUG
Size: MEDIUM
Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: GILLIBRAND INDUSTRIAL
Product Name: SILICA SAND
Size: 20/40
Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC
Length: 0.34 FOOT LONG END CAP

BACKFILL MATERIAL

Type: 20/40 SILICA SAND

Comments: SILT TRAP (0.34 FT LONG END CAP PLUS 1 FT LONG SUMP) AT BOTTOM OF WELL.
ADDED ~4 GALLONS

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	BSW-5
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	DPT (8040 DT)
PROJECT NO.:	501020	**DATE(S):**	9-2-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	KEVIN WARD	**NORTHING:**	1836979.7
SCIENTIST:	BRIAN WHITE	**EASTING:**	6267820.51

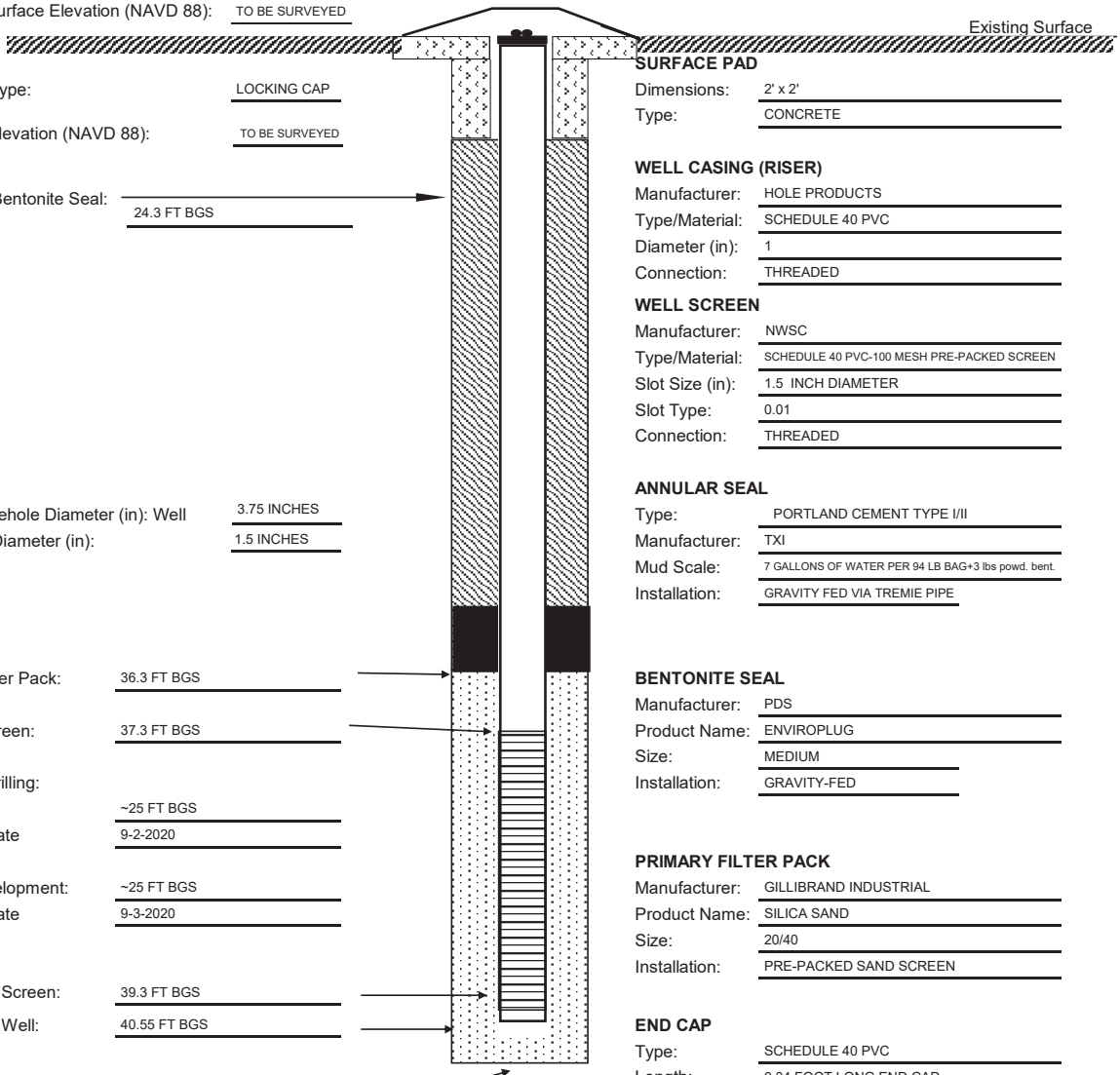
PROTECTIVE CASING

Type: FLUSH MOUNT, 8-INCH DIAMETER MANHOLE
Dimensions: 8 INCHES
Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 24.3 FT BGS

Well Borehole Diameter (in): 3.75 INCHES
Well Casing Diameter (in): 1.5 INCHES

Top of Filter Pack: 36.3 FT BGS

Top of Screen: 37.3 FT BGS

During Drilling: ~25 FT BGS
Date: 9-2-2020

Post Development: ~25 FT BGS
Date: 9-3-2020

Bottom of Screen: 39.3 FT BGS

Bottom of Well: 40.55 FT BGS

Borehole Depth: 40.55 FT BGS

SURFACE PAD

Dimensions: 2' x 2'
Type: CONCRETE

WELL CASING (RISER)

Manufacturer: HOLE PRODUCTS
Type/Material: SCHEDULE 40 PVC
Diameter (in): 1
Connection: THREADED

WELL SCREEN

Manufacturer: NWSC
Type/Material: SCHEDULE 40 PVC-100 MESH PRE-PACKED SCREEN
Slot Size (in): 1.5 INCH DIAMETER
Slot Type: 0.01
Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE I/II
Manufacturer: TXI
Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.
Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PDS
Product Name: ENVIROPLUG
Size: MEDIUM
Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: GILLIBRAND INDUSTRIAL
Product Name: SILICA SAND
Size: 20/40
Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC
Length: 0.34 FOOT LONG END CAP

BACKFILL MATERIAL

Type: 20/40 SILICA SAND

Comments: SILT TRAP (0.34 FT LONG END CAP PLUS 1 FT LONG SUMP) AT BOTTOM OF WELL.
ADDED ~4 GALLONS

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	DMW-1
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	DPT (8040 DT)
PROJECT NO.:	501020	**DATE(S):**	9-2-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	KEVIN WARD	**NORTHING:**	1836996.42
SCIENTIST:	ALLEN WILLMORE	**EASTING:**	6267775.83

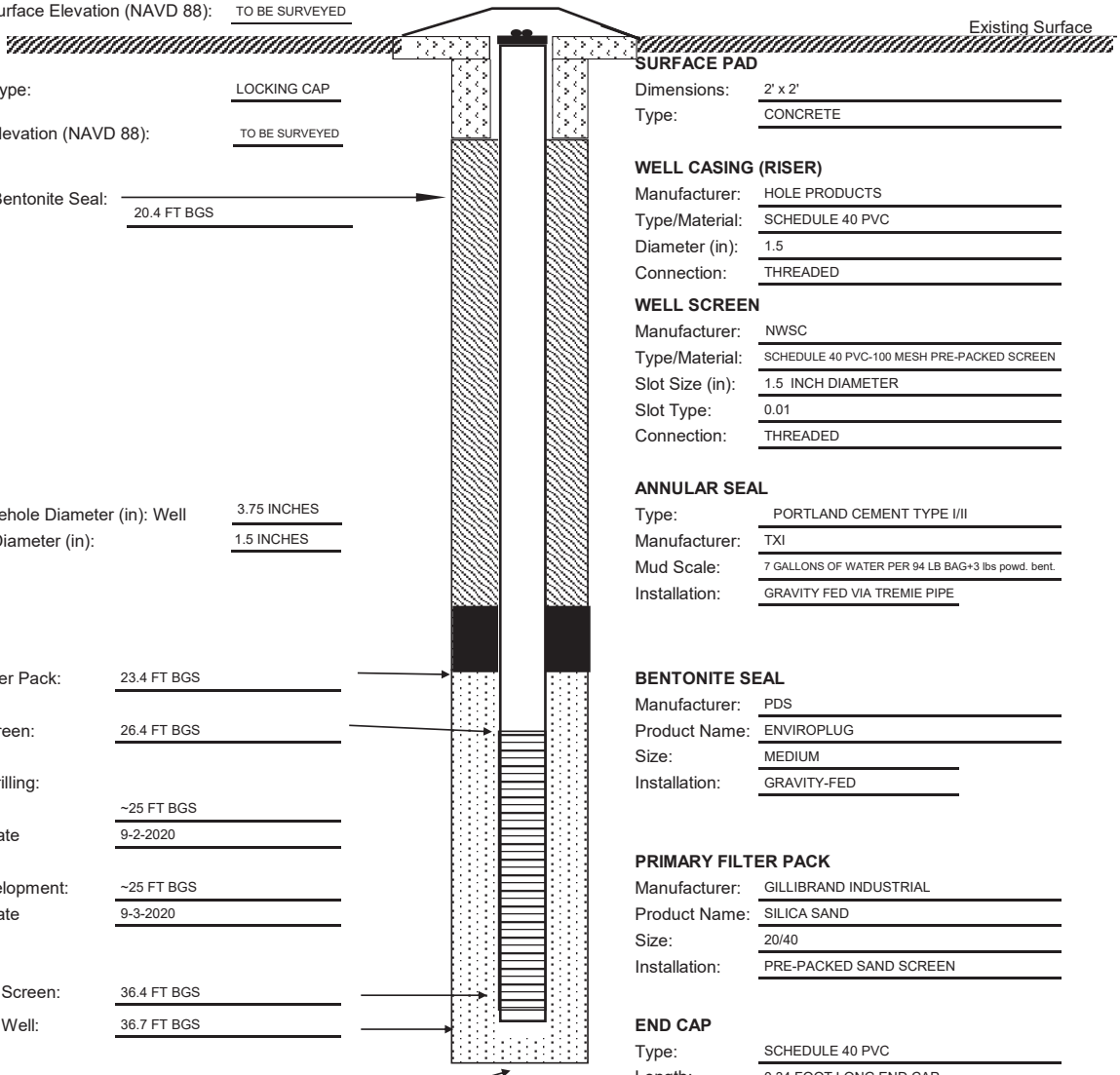
PROTECTIVE CASING

Type: FLUSH MOUNT, 8-INCH DIAMETER MANHOLE
Dimensions: 8 INCHES
Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 20.4 FT BGS

Well Borehole Diameter (in): 3.75 INCHES
Well Casing Diameter (in): 1.5 INCHES

Top of Filter Pack: 23.4 FT BGS

Top of Screen: 26.4 FT BGS

During Drilling: ~25 FT BGS
Date: 9-2-2020

Post Development: ~25 FT BGS
Date: 9-3-2020

Bottom of Screen: 36.4 FT BGS

Bottom of Well: 36.7 FT BGS

Borehole Depth: 36.7

SURFACE PAD

Dimensions: 2' x 2'
Type: CONCRETE

WELL CASING (RISER)

Manufacturer: HOLE PRODUCTS
Type/Material: SCHEDULE 40 PVC
Diameter (in): 1.5
Connection: THREADED

WELL SCREEN

Manufacturer: NWSC
Type/Material: SCHEDULE 40 PVC-100 MESH PRE-PACKED SCREEN
Slot Size (in): 1.5 INCH DIAMETER
Slot Type: 0.01
Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE I/II
Manufacturer: TXI
Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.
Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PDS
Product Name: ENVIROPLUG
Size: MEDIUM
Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: GILLIBRAND INDUSTRIAL
Product Name: SILICA SAND
Size: 20/40
Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC
Length: 0.34 FOOT LONG END CAP

BACKFILL MATERIAL

Type: 20/40 SILICA SAND

Comments:

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	EW-1
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	CME-85
PROJECT NO.:	501020	**DATE(S):**	9-10-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	RYAN KRUGER	**NORTHING:**	1836963.51
SCIENTIST:	ALLEN WILLMORE	**EASTING:**	6267802.06

PROTECTIVE CASING

Type: FLUSH MOUNT, 10-INCH DIAMETER MANHOLE

Dimensions: 10 INCHES

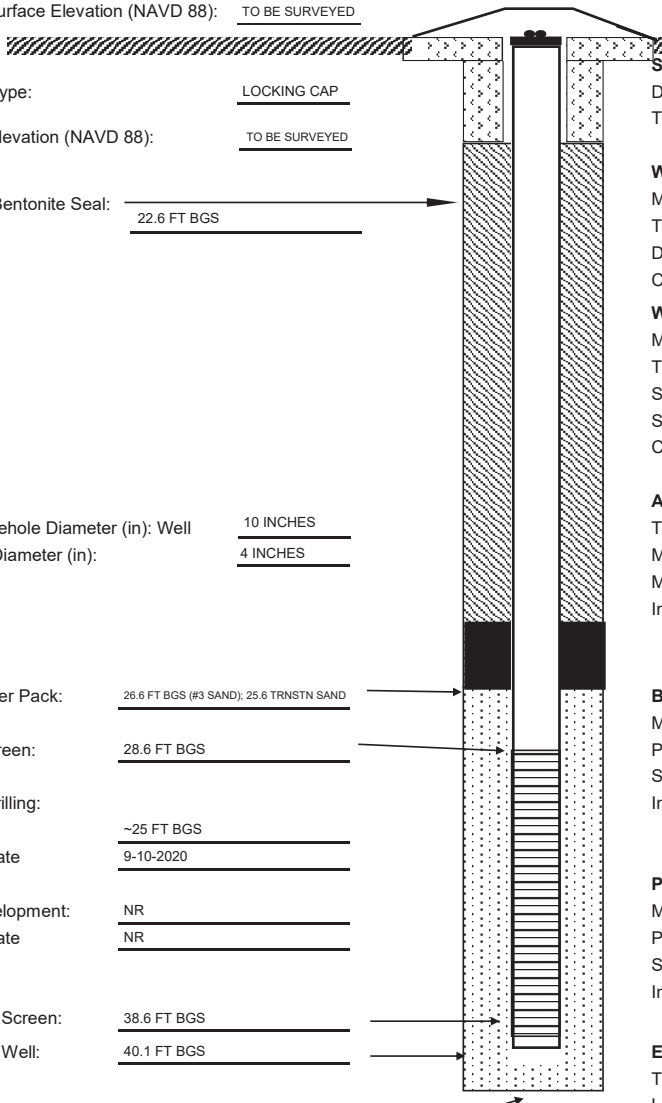
Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE

Existing Surface



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 22.6 FT BGS

Well Borehole Diameter (in): 10 INCHES

Well Casing Diameter (in): 4 INCHES

Top of Filter Pack: 26.6 FT BGS (#3 SAND); 25.6 TRNSTN SAND

Top of Screen: 28.6 FT BGS

During Drilling: ~25 FT BGS

Date: 9-10-2020

Post Development: NR

Date: NR

Bottom of Screen: 38.6 FT BGS

Bottom of Well: 40.1 FT BGS

Borehole Depth: 40.1 FT BGS

SURFACE PAD

Dimensions: 11" x18"

Type: COMPOSITE WELL VAULT

WELL CASING (RISER)

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.

Type/Material: SCHEDULE 40 PVC

Diameter (in): 4

Connection: THREADED

WELL SCREEN

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.

Type/Material: STAINLESS STEEL WRAP

Slot Size (in): 4 INCH DIAMETER

Slot Type: 0.02

Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE II

Manufacturer: QUIK GROUT

Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.

Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PEL-PLUG

Product Name: ENVIROPLUG

Size: MEDIUM

Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: CEMEX LAPIS LUSTRE

Product Name: SILICA SAND

Size: #3

Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC

Length: 1.5 FOOT LONG SUMP AND END CAP

BACKFILL MATERIAL

Type: #3 SILICA SAND

Comments: END CAP AND SUMP FASTENED TOGETHER WITH STAINLESS STEEL BOLTS.

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	IW-1
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	CME-85
PROJECT NO.:	501020	**DATE(S):**	9-14-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	RYAN KRUGER	**NORTHING:**	1836970.2
SCIENTIST:	ALLEN WILLMORE	**EASTING:**	6267818.41

PROTECTIVE CASING

Type: FLUSH MOUNT, 10-INCH DIAMETER MANHOLE

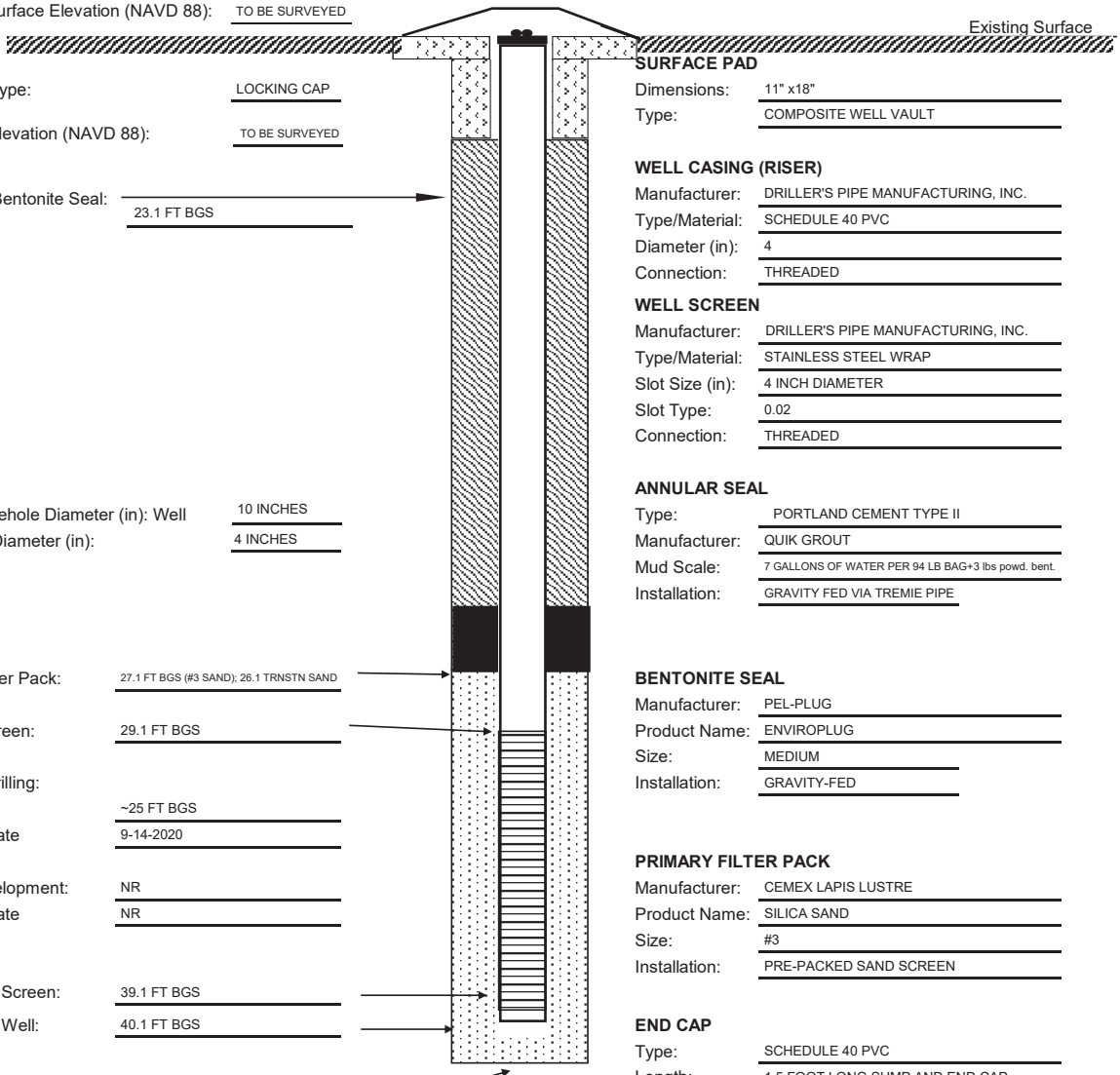
Dimensions: 10 INCHES

Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 23.1 FT BGS

Well Borehole Diameter (in): 10 INCHES

Well Casing Diameter (in): 4 INCHES

Top of Filter Pack: 27.1 FT BGS (#3 SAND); 26.1 TRNSTN SAND

Top of Screen: 29.1 FT BGS

During Drilling: ~25 FT BGS

Date: 9-14-2020

Post Development: NR

Date: NR

Bottom of Screen: 39.1 FT BGS

Bottom of Well: 40.1 FT BGS

Borehole Depth: 40.1 FT BGS

SURFACE PAD

Dimensions: 11" x18"

Type: COMPOSITE WELL VAULT

WELL CASING (RISER)

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.

Type/Material: SCHEDULE 40 PVC

Diameter (in): 4

Connection: THREADED

WELL SCREEN

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.

Type/Material: STAINLESS STEEL WRAP

Slot Size (in): 4 INCH DIAMETER

Slot Type: 0.02

Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE II

Manufacturer: QUIK GROUT

Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.

Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PEL-PLUG

Product Name: ENVIROPLUG

Size: MEDIUM

Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: CEMEX LAPIS LUSTRE

Product Name: SILICA SAND

Size: #3

Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC

Length: 1.5 FOOT LONG SUMP AND END CAP

BACKFILL MATERIAL

Type: #3 SILICA SAND

Comments: END CAP AND SUMP FASTENED TOGETHER WITH STAINLESS STEEL BOLTS.

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	IW-2
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	CME-85
PROJECT NO.:	501020	**DATE(S):**	9-10-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	RYAN KRUGER	**NORTHING:**	1836963.51
SCIENTIST:	ALLEN WILLMORE	**EASTING:**	6267802.06

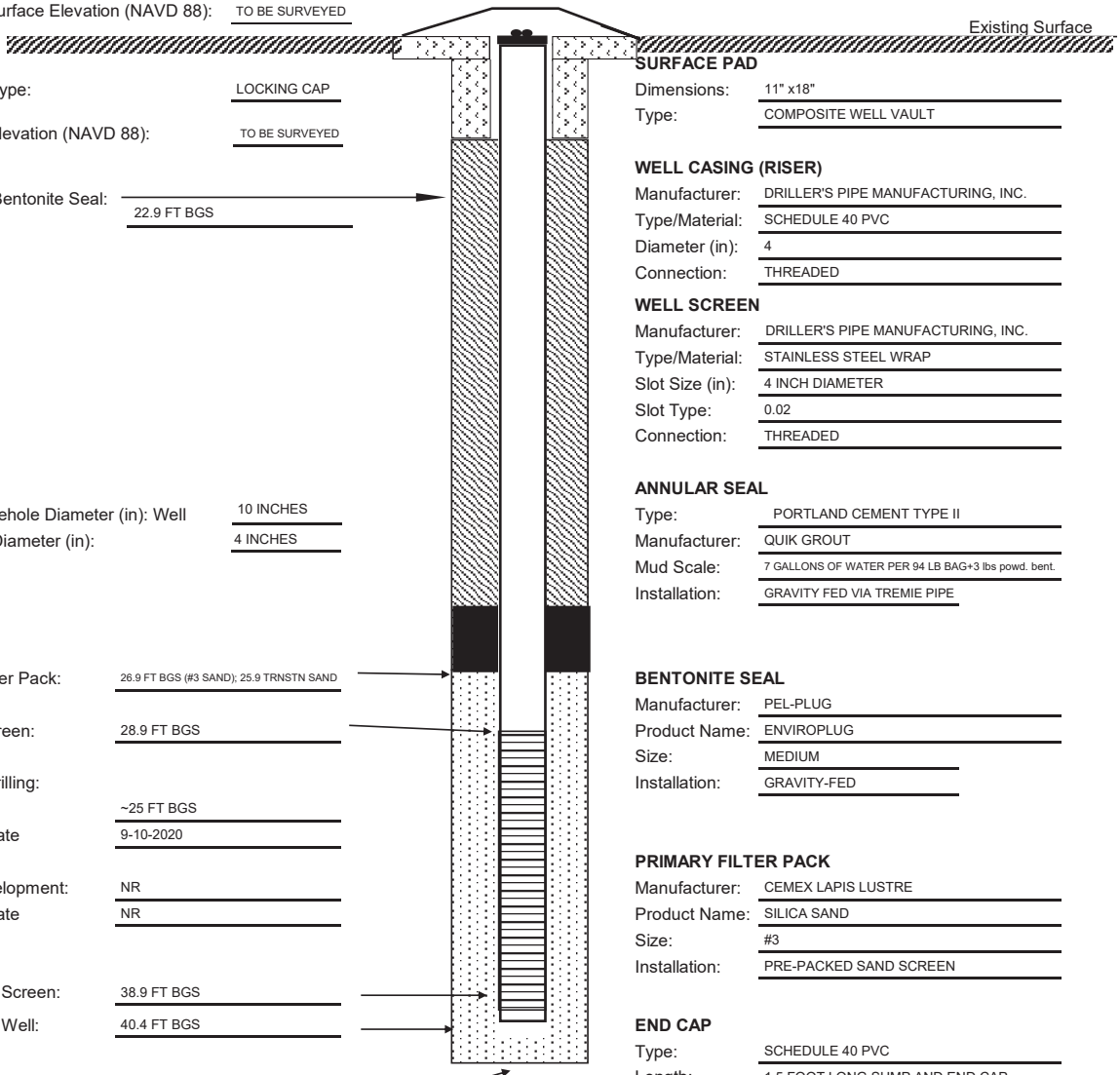
PROTECTIVE CASING

Type: FLUSH MOUNT, 10-INCH DIAMETER MANHOLE
Dimensions: 10 INCHES
Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 22.9 FT BGS

Well Borehole Diameter (in): 10 INCHES
Well Casing Diameter (in): 4 INCHES

Top of Filter Pack: 26.9 FT BGS (#3 SAND); 25.9 TRNSTN SAND

Top of Screen: 28.9 FT BGS

During Drilling: ~25 FT BGS
Date: 9-10-2020

Post Development: NR
Date: NR

Bottom of Screen: 38.9 FT BGS

Bottom of Well: 40.4 FT BGS

Borehole Depth: 40.4 FT BGS

SURFACE PAD

Dimensions: 11" x18"
Type: COMPOSITE WELL VAULT

WELL CASING (RISER)

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.
Type/Material: SCHEDULE 40 PVC
Diameter (in): 4
Connection: THREADED

WELL SCREEN

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.
Type/Material: STAINLESS STEEL WRAP
Slot Size (in): 4 INCH DIAMETER
Slot Type: 0.02
Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE II
Manufacturer: QUIK GROUT
Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.
Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PEL-PLUG
Product Name: ENVIROPLUG
Size: MEDIUM
Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: CEMEX LAPIS LUSTRE
Product Name: SILICA SAND
Size: #3
Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC
Length: 1.5 FOOT LONG SUMP AND END CAP

BACKFILL MATERIAL

Type: #3 SILICA SAND

Comments: END CAP AND SUMP FASTENED TOGETHER WITH STAINLESS STEEL BOLTS.

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	IW-3
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	CME-85
PROJECT NO.:	501020	**DATE(S):**	9-11-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	RYAN KRUGER	**NORTHING:**	1836976.92
SCIENTIST:	ALLEN WILLMORE	**EASTING:**	6267792.87

PROTECTIVE CASING

Type: FLUSH MOUNT, 10-INCH DIAMETER MANHOLE

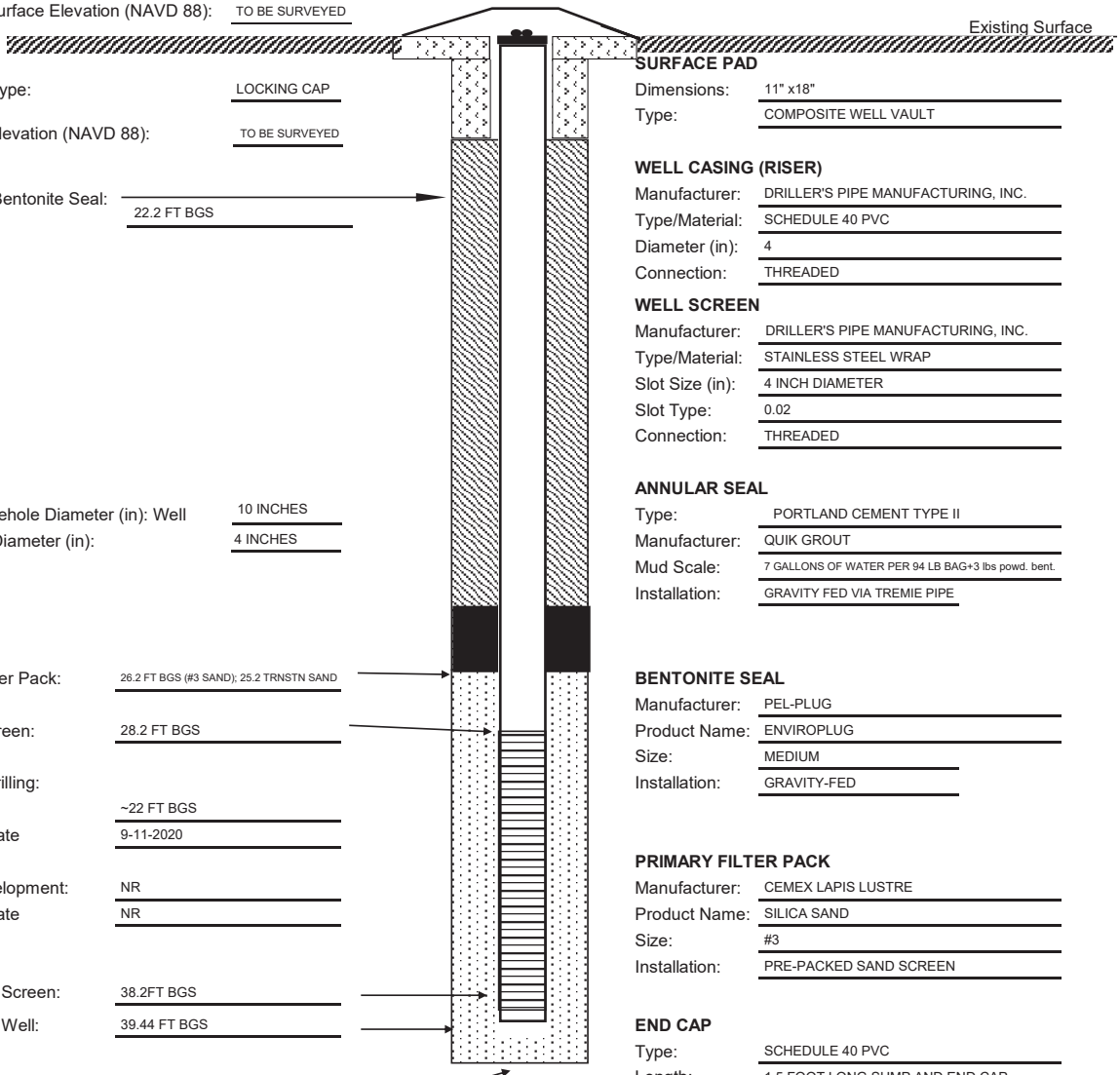
Dimensions: 10 INCHES

Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 22.2 FT BGS

Well Borehole Diameter (in): 10 INCHES

Well Casing Diameter (in): 4 INCHES

Top of Filter Pack: 26.2 FT BGS (#3 SAND); 25.2 TRNSTN SAND

Top of Screen: 28.2 FT BGS

During Drilling: ~22 FT BGS

Date: 9-11-2020

Post Development: NR

Date: NR

Bottom of Screen: 38.2FT BGS

Bottom of Well: 39.44 FT BGS

Borehole Depth: 39.44 FT BGS

SURFACE PAD

Dimensions: 11" x18"

Type: COMPOSITE WELL VAULT

WELL CASING (RISER)

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.

Type/Material: SCHEDULE 40 PVC

Diameter (in): 4

Connection: THREADED

WELL SCREEN

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.

Type/Material: STAINLESS STEEL WRAP

Slot Size (in): 4 INCH DIAMETER

Slot Type: 0.02

Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE II

Manufacturer: QUIK GROUT

Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.

Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PEL-PLUG

Product Name: ENVIROPLUG

Size: MEDIUM

Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: CEMEX LAPIS LUSTRE

Product Name: SILICA SAND

Size: #3

Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC

Length: 1.5 FOOT LONG SUMP AND END CAP

BACKFILL MATERIAL

Type: #3 SILICA SAND

Comments: END CAP AND SUMP FASTENED TOGETHER WITH STAINLESS STEEL BOLTS.

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	IW-4
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	CME-85
PROJECT NO.:	501020	**DATE(S):**	9-11-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	RYAN KRUGER	**NORTHING:**	1836991.95
SCIENTIST:	ALLEN WILLMORE	**EASTING:**	6267799.91

PROTECTIVE CASING

Type: FLUSH MOUNT, 10-INCH DIAMETER MANHOLE

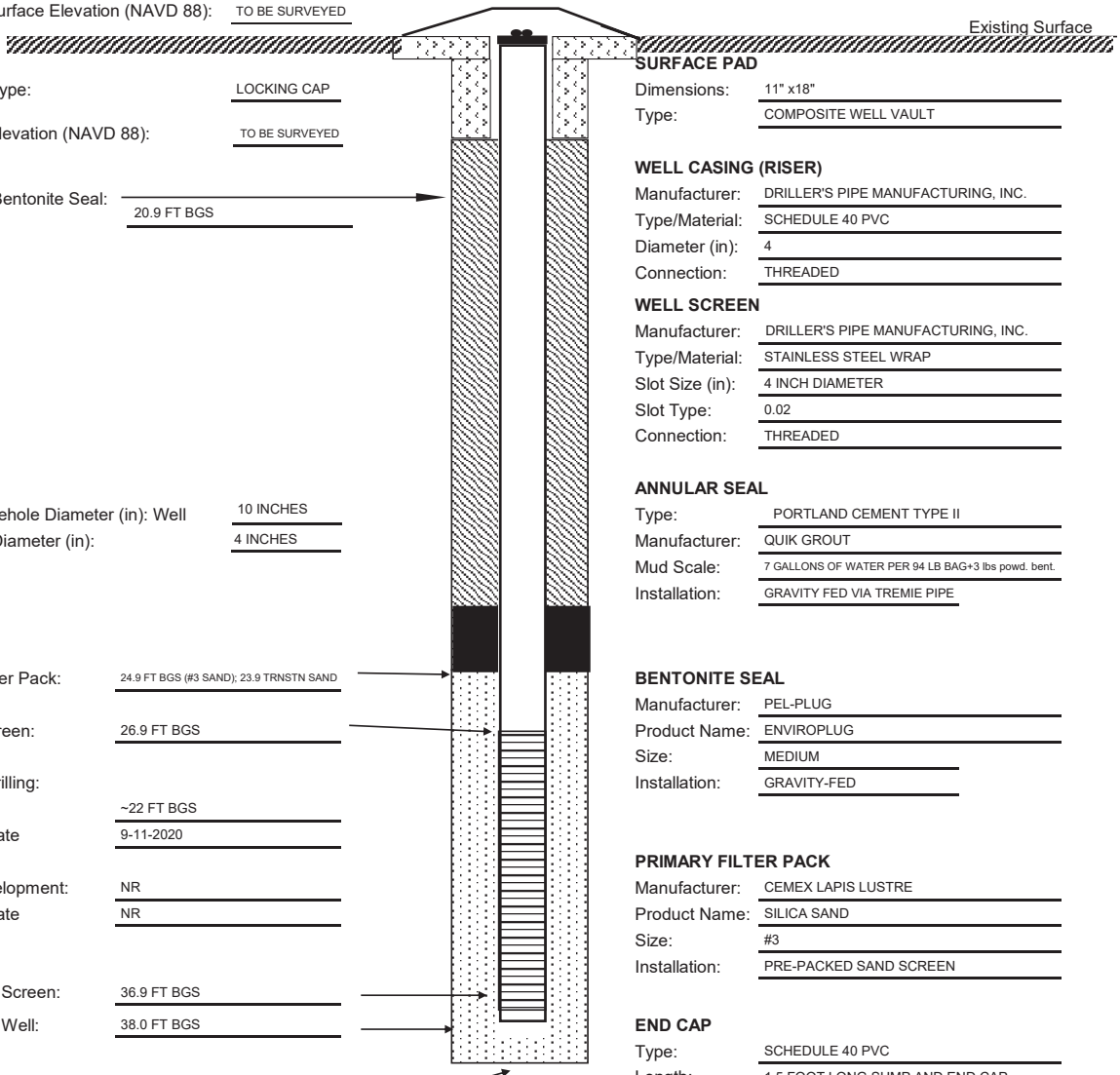
Dimensions: 10 INCHES

Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 20.9 FT BGS

Well Borehole Diameter (in): 10 INCHES

Well Casing Diameter (in): 4 INCHES

Top of Filter Pack: 24.9 FT BGS (#3 SAND); 23.9 TRNSTN SAND

Top of Screen: 26.9 FT BGS

During Drilling: ~22 FT BGS

Date: 9-11-2020

Post Development: NR

Date: NR

Bottom of Screen: 36.9 FT BGS

Bottom of Well: 38.0 FT BGS

Borehole Depth: 38.0 FT BGS

SURFACE PAD

Dimensions: 11" x18"

Type: COMPOSITE WELL VAULT

WELL CASING (RISER)

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.

Type/Material: SCHEDULE 40 PVC

Diameter (in): 4

Connection: THREADED

WELL SCREEN

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.

Type/Material: STAINLESS STEEL WRAP

Slot Size (in): 4 INCH DIAMETER

Slot Type: 0.02

Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE II

Manufacturer: QUIK GROUT

Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.

Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PEL-PLUG

Product Name: ENVIROPLUG

Size: MEDIUM

Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: CEMEX LAPIS LUSTRE

Product Name: SILICA SAND

Size: #3

Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC

Length: 1.5 FOOT LONG SUMP AND END CAP

BACKFILL MATERIAL

Type: #3 SILICA SAND

Comments: END CAP AND SUMP FASTENED TOGETHER WITH STAINLESS STEEL BOLTS.

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	IW-5
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	CME-85
PROJECT NO.:	501020	**DATE(S):**	9-14-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	RYAN KRUGER	**NORTHING:**	1836987.88
SCIENTIST:	ALLEN WILLMORE	**EASTING:**	6267816.99

PROTECTIVE CASING

Type: FLUSH MOUNT, 10-INCH DIAMETER MANHOLE

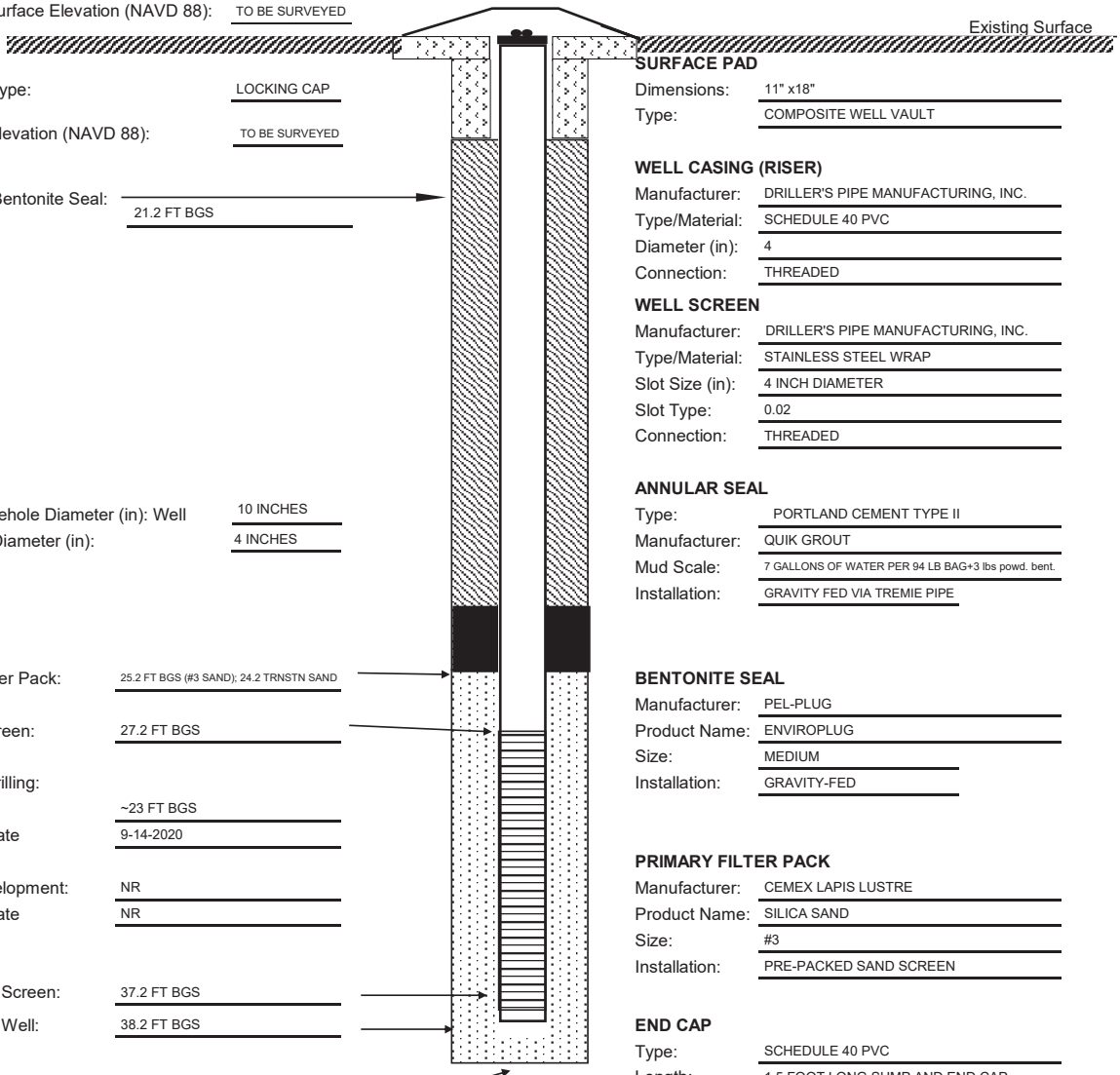
Dimensions: 10 INCHES

Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 21.2 FT BGS

Well Borehole Diameter (in): 10 INCHES

Well Casing Diameter (in): 4 INCHES

Top of Filter Pack: 25.2 FT BGS (#3 SAND); 24.2 TRNSTN SAND

Top of Screen: 27.2 FT BGS

During Drilling: ~23 FT BGS

Date: 9-14-2020

Post Development: NR

Date: NR

Bottom of Screen: 37.2 FT BGS

Bottom of Well: 38.2 FT BGS

Borehole Depth: 38.2 FT BGS

SURFACE PAD

Dimensions: 11" x18"

Type: COMPOSITE WELL VAULT

WELL CASING (RISER)

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.

Type/Material: SCHEDULE 40 PVC

Diameter (in): 4

Connection: THREADED

WELL SCREEN

Manufacturer: DRILLER'S PIPE MANUFACTURING, INC.

Type/Material: STAINLESS STEEL WRAP

Slot Size (in): 4 INCH DIAMETER

Slot Type: 0.02

Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE II

Manufacturer: QUIK GROUT

Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.

Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PEL-PLUG

Product Name: ENVIROPLUG

Size: MEDIUM

Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: CEMEX LAPIS LUSTRE

Product Name: SILICA SAND

Size: #3

Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC

Length: 1.5 FOOT LONG SUMP AND END CAP

BACKFILL MATERIAL

Type: #3 SILICA SAND

Comments: END CAP AND SUMP FASTENED TOGETHER WITH STAINLESS STEEL BOLTS.

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	PMW-3D
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	DPT (8040 DT)
PROJECT NO.:	501020	**DATE(S):**	9-2-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	KEVIN WARD	**NORTHING:**	1836980.69
SCIENTIST:	ALLEN WILLMORE	**EASTING:**	6267798.47

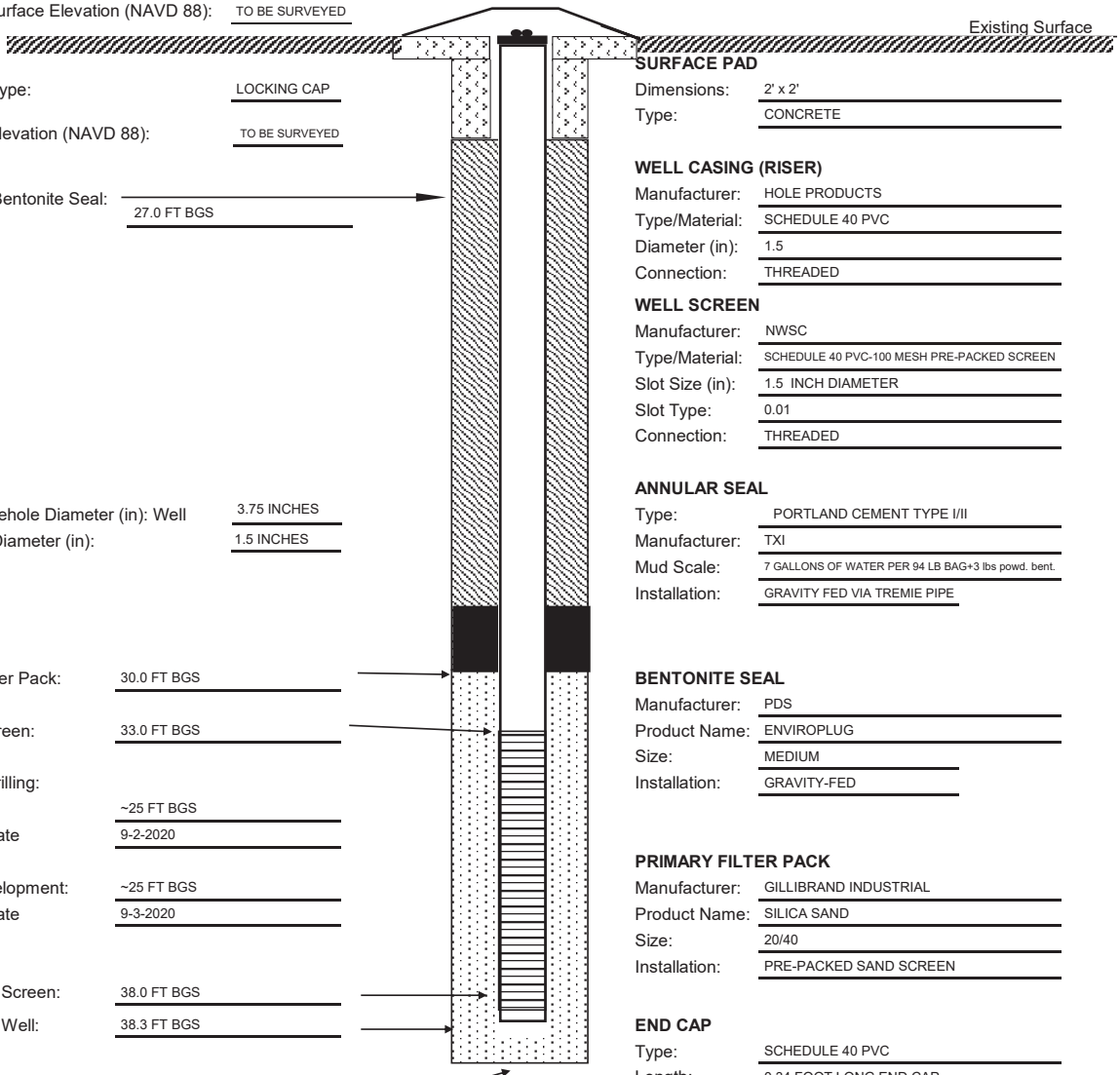
PROTECTIVE CASING

Type: FLUSH MOUNT, 8-INCH DIAMETER MANHOLE
Dimensions: 8 INCHES
Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 27.0 FT BGS

Well Borehole Diameter (in): 3.75 INCHES
Well Casing Diameter (in): 1.5 INCHES

Top of Filter Pack: 30.0 FT BGS

Top of Screen: 33.0 FT BGS

During Drilling: ~25 FT BGS
Date: 9-2-2020

Post Development: ~25 FT BGS
Date: 9-3-2020

Bottom of Screen: 38.0 FT BGS

Bottom of Well: 38.3 FT BGS

Borehole Depth: 38.3

SURFACE PAD

Dimensions: 2' x 2'
Type: CONCRETE

WELL CASING (RISER)

Manufacturer: HOLE PRODUCTS
Type/Material: SCHEDULE 40 PVC
Diameter (in): 1.5
Connection: THREADED

WELL SCREEN

Manufacturer: NWSC
Type/Material: SCHEDULE 40 PVC-100 MESH PRE-PACKED SCREEN
Slot Size (in): 1.5 INCH DIAMETER
Slot Type: 0.01
Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE I/II
Manufacturer: TXI
Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.
Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PDS
Product Name: ENVIROPLUG
Size: MEDIUM
Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: GILLIBRAND INDUSTRIAL
Product Name: SILICA SAND
Size: 20/40
Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC
Length: 0.34 FOOT LONG END CAP

BACKFILL MATERIAL

Type: 20/40 SILICA SAND

Comments:

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	PMW-3S
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	DPT (8040 DT)
PROJECT NO.:	501020	**DATE(S):**	9-2-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	KEVIN WARD	**NORTHING:**	1836983.25
SCIENTIST:	ALLEN WILLMORE	**EASTING:**	6267800.03

PROTECTIVE CASING

Type: FLUSH MOUNT, 8-INCH DIAMETER MANHOLE

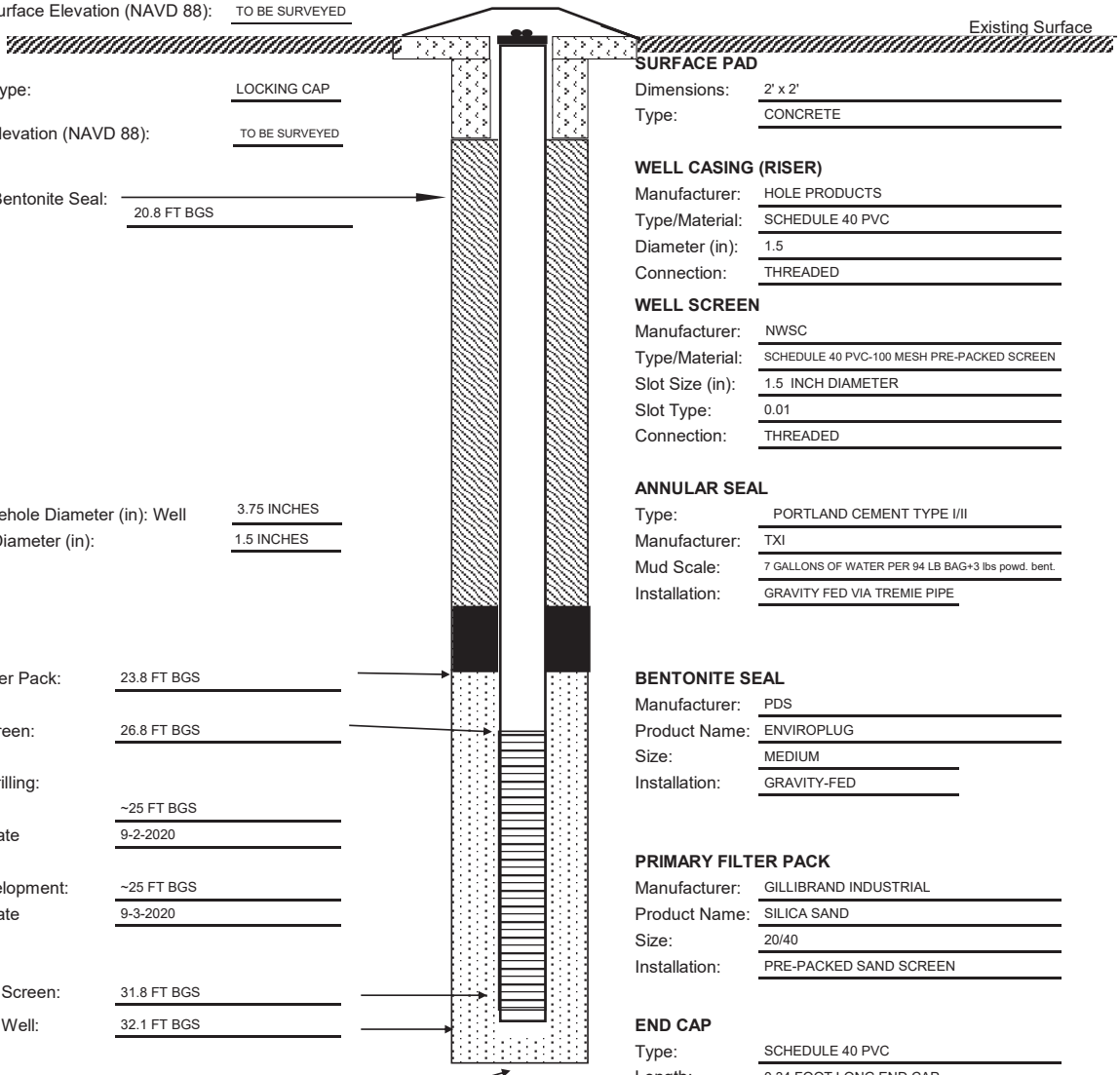
Dimensions: 8 INCHES

Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 20.8 FT BGS

Well Borehole Diameter (in): 3.75 INCHES

Casing Diameter (in): 1.5 INCHES

Top of Filter Pack: 23.8 FT BGS

Top of Screen: 26.8 FT BGS

During Drilling: ~25 FT BGS

Date: 9-2-2020

Post Development: ~25 FT BGS

Date: 9-3-2020

Bottom of Screen: 31.8 FT BGS

Bottom of Well: 32.1 FT BGS

Borehole Depth: 32.1

SURFACE PAD

Dimensions: 2' x 2'

Type: CONCRETE

WELL CASING (RISER)

Manufacturer: HOLE PRODUCTS

Type/Material: SCHEDULE 40 PVC

Diameter (in): 1.5

Connection: THREADED

WELL SCREEN

Manufacturer: NWSC

Type/Material: SCHEDULE 40 PVC-100 MESH PRE-PACKED SCREEN

Slot Size (in): 1.5 INCH DIAMETER

Slot Type: 0.01

Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE I/II

Manufacturer: TXI

Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.

Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PDS

Product Name: ENVIROPLUG

Size: MEDIUM

Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: GILLIBRAND INDUSTRIAL

Product Name: SILICA SAND

Size: 20/40

Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC

Length: 0.34 FOOT LONG END CAP

BACKFILL MATERIAL

Type: 20/40 SILICA SAND

Comments:

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	PMW-4D
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	DPT (8040 DT)
PROJECT NO.:	501020	**DATE(S):**	9-2-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	KEVIN WARD	**NORTHING:**	1836972.85
SCIENTIST:	ALLEN WILLMORE	**EASTING:**	6267811.22

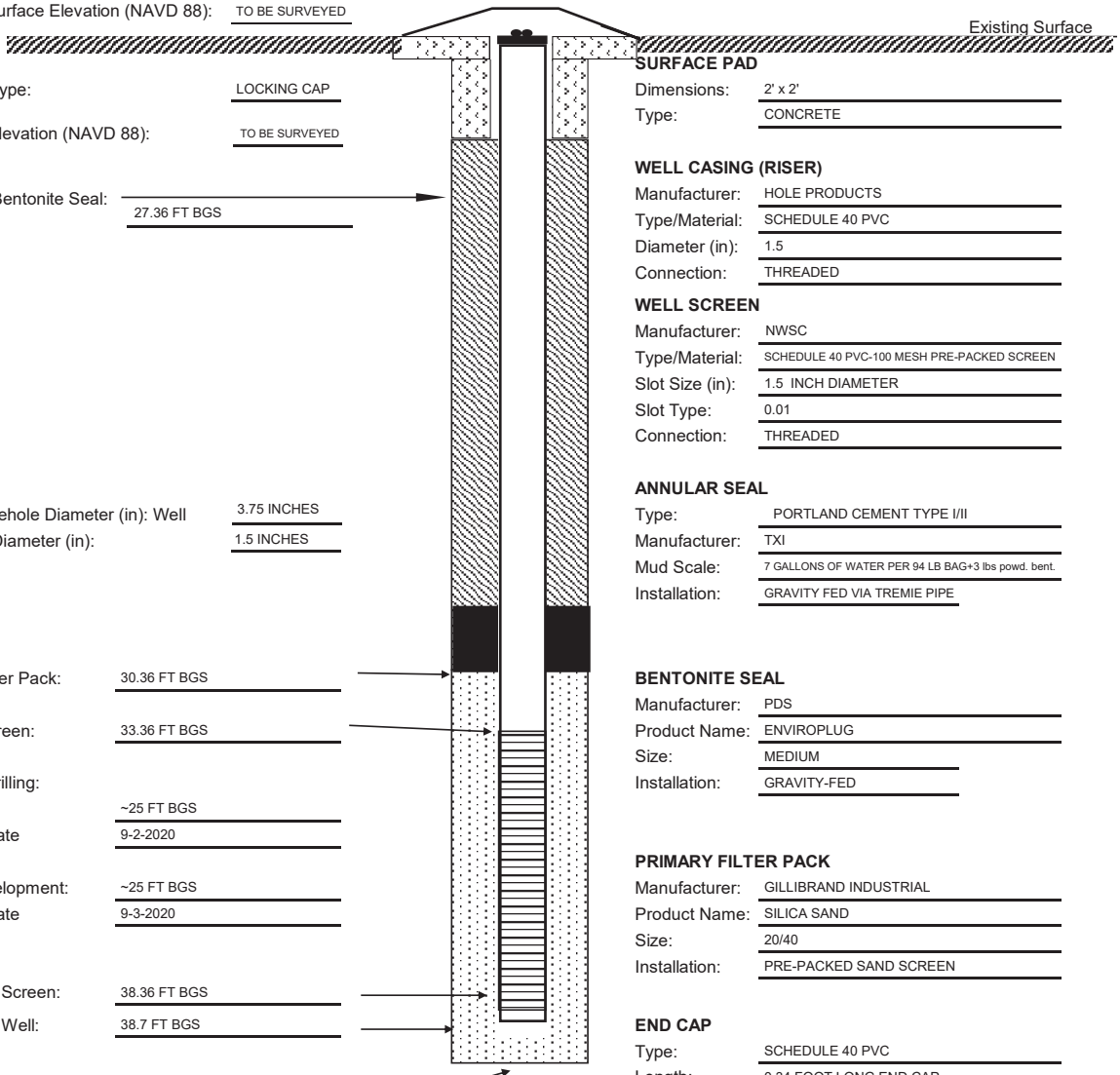
PROTECTIVE CASING

Type: FLUSH MOUNT, 8-INCH DIAMETER MANHOLE
Dimensions: 8 INCHES
Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 27.36 FT BGS

Well Borehole Diameter (in): 3.75 INCHES
Well Casing Diameter (in): 1.5 INCHES

Top of Filter Pack: 30.36 FT BGS

Top of Screen: 33.36 FT BGS

During Drilling: ~25 FT BGS
Date: 9-2-2020

Post Development: ~25 FT BGS
Date: 9-3-2020

Bottom of Screen: 38.36 FT BGS

Bottom of Well: 38.7 FT BGS

Borehole Depth: 38.7

SURFACE PAD

Dimensions: 2' x 2'
Type: CONCRETE

WELL CASING (RISER)

Manufacturer: HOLE PRODUCTS
Type/Material: SCHEDULE 40 PVC
Diameter (in): 1.5
Connection: THREADED

WELL SCREEN

Manufacturer: NWSC
Type/Material: SCHEDULE 40 PVC-100 MESH PRE-PACKED SCREEN
Slot Size (in): 1.5 INCH DIAMETER
Slot Type: 0.01
Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE I/II
Manufacturer: TXI
Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.
Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PDS
Product Name: ENVIROPLUG
Size: MEDIUM
Installation: GRAVITY-FED

PRIMARY FILTER PACK

Manufacturer: GILLIBRAND INDUSTRIAL
Product Name: SILICA SAND
Size: 20/40
Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC
Length: 0.34 FOOT LONG END CAP

BACKFILL MATERIAL

Type: 20/40 SILICA SAND

Comments: RECEIVED TABLE AFTER WELL INSTALLED.

WELL CONSTRUCTION DIAGRAM (Above ground)

SITE:	NORTH ISLAND NAVAL BASE-OU11	**WELL/BORING ID:**	PMW-4S
PROJECT NAME:	REMEDIAL DEMOSTRATION AT OU11	**DRILLING METHOD:**	DPT (8040 DT)
PROJECT NO.:	501020	**DATE(S):**	9-1-2020
DRILLING CONTRACTOR:	CASCADE	**SURVEYOR:**	FROM R. WONG SURVEY
DRILLER:	KEVIN WARD	**NORTHING:**	1836975.41
SCIENTIST:	BRIAN WHITE	**EASTING:**	6267812.79

PROTECTIVE CASING

Type: FLUSH MOUNT, 8-INCH DIAMETER MANHOLE

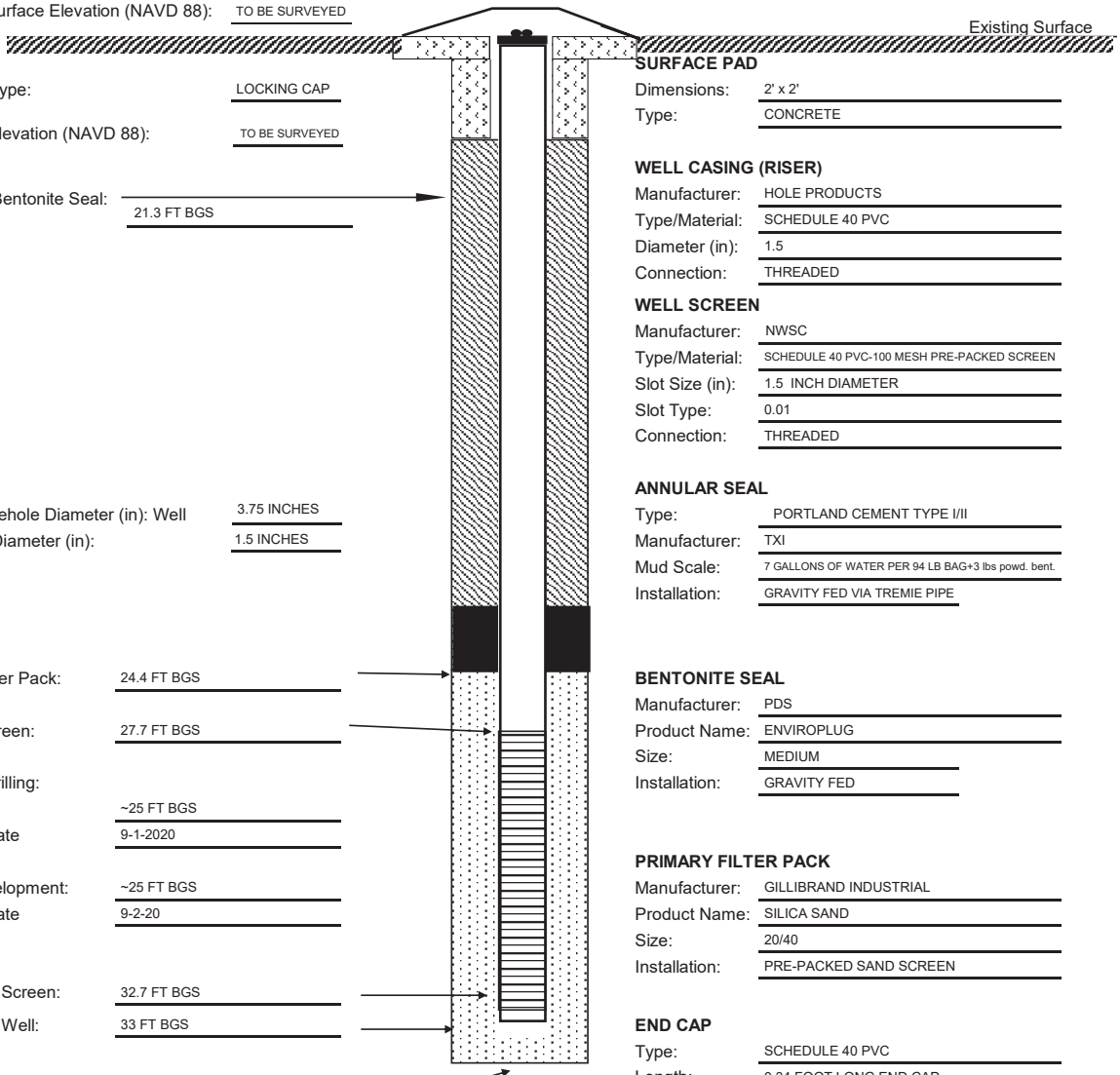
Dimensions: 8 INCHES

Length: NA

Ground Surface Elevation (NAVD 88): TO BE SURVEYED

NOT TO SCALE

Bollards: NONE



Well Lid Type: LOCKING CAP

Casing Elevation (NAVD 88): TO BE SURVEYED

Top of Bentonite Seal: 21.3 FT BGS

Well Borehole Diameter (in): 3.75 INCHES

Well Casing Diameter (in): 1.5 INCHES

Top of Filter Pack: 24.4 FT BGS

Top of Screen: 27.7 FT BGS

During Drilling: ~25 FT BGS

Date: 9-1-2020

Post Development: ~25 FT BGS

Date: 9-2-20

Bottom of Screen: 32.7 FT BGS

Bottom of Well: 33 FT BGS

Borehole Depth: 33

SURFACE PAD

Dimensions: 2' x 2'

Type: CONCRETE

WELL CASING (RISER)

Manufacturer: HOLE PRODUCTS

Type/Material: SCHEDULE 40 PVC

Diameter (in): 1.5

Connection: THREADED

WELL SCREEN

Manufacturer: NWSC

Type/Material: SCHEDULE 40 PVC-100 MESH PRE-PACKED SCREEN

Slot Size (in): 1.5 INCH DIAMETER

Slot Type: 0.01

Connection: THREADED

ANNULAR SEAL

Type: PORTLAND CEMENT TYPE I/II

Manufacturer: TXI

Mud Scale: 7 GALLONS OF WATER PER 94 LB BAG+3 lbs powd. bent.

Installation: GRAVITY FED VIA TREMIE PIPE

BENTONITE SEAL

Manufacturer: PDS

Product Name: ENVIROPLUG

Size: MEDIUM

Installation: GRAVITY FED

PRIMARY FILTER PACK

Manufacturer: GILLIBRAND INDUSTRIAL

Product Name: SILICA SAND

Size: 20/40

Installation: PRE-PACKED SAND SCREEN

END CAP

Type: SCHEDULE 40 PVC

Length: 0.34 FOOT LONG END CAP

BACKFILL MATERIAL

Type: 20/40 SILICA SAND

Comments:

Borehole Log/Well Completion Log

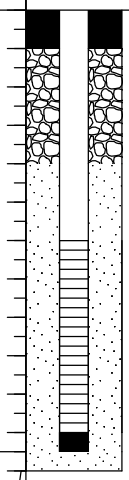
Project Name: OU-11 Well Installation	Project Number: 1066-002	**Borehole Number:** **SMW-08A**	
Borehole Location: Naval Air Station North Island	*Northing:* 1836957.379	*Easting:* 6267846.931	Sheet *1* of *2*
Drilling Agency: Test America		Driller: Matt Byers	
Drilling Equipment: CME 85		Date Started: 8/2/11	Total Depth (feet): 42.0
Drilling Method: HOLLOW STEM AUGER	Number of Soil Samples: 0	Date Finished: 8/2/11	
Drilling Fluid: None/Tap Water	Borehole Diameter (in): 8	Depth to Water (feet): *Drilling:* 27.5	*Static: 25.9*
Completion Information: Well box in concrete pad flush with grade. Materials as described below.		Elevation (feet MSL) : 31.49	
		Logged By: Nia Nikmanesh	Checked By: Paul Sones

Depth (feet)	Samples: Number	Type	Blow Count	Percent Recovery	Time	Field Analyses: PID/FID (ppm) Sample	Additional Tests	Log: Graphic	USCS or Rock Type	Lithologic Description	Well Details	Remarks
0–4										Hand auger to 5' bgs		Concrete Quickset from 0.5 to 3' bgs
5				20	13:55	PID = 0			SP	SAND, 7.5YR 4/4, very fine grained, dry, loose, poorly graded		2" Sch 40 PVC blank from 0.5 to 36' bgs
8				30	13:58	PID = 0						
10				30	14:01	PID = 0						Bentonite grout from 3 to 31' bgs (9.2 cubic ft)
13				80	14:06	PID = 0				color grades to 10YR 5/6, some plant debris		
15				80	14:10	PID = 0			SP	SAND, 10YR 5/6, fine grained, dry, poorly graded		
18				60	14:14	PID = 0				color grades to 10YR 6/4		
20				70	14:21	PID = 0						
23				80	14:25	PID = 0				moist		
25				50	14:29	PID = 0			SP	SAND, 10YR 6/3, very fine to fine grained, moist, loose, poorly graded		
28				80	14:34	PID = 0			SP	SAND, 10YR 5/2, very fine grained, moist to wet, poorly graded		

Borehole Log/Well Completion Log
(Continuation Sheet)

Project Name: OU-11 Well Installation	Project Number: 1066-002	**Borehole Number:** **SMW-08A**
Borehole Location: Naval Air Station North Island		Sheet *2* of *2*

Depth (feet)	Samples: Number	Type	Blow Count	Percent Recovery	Time	Field Analyses: PID/FID (ppm) Sample/Background	Additional Tests	Log: Graphic	USCS or Rock Type	Lithologic Description	Well Details	Remarks
30				90	14:36	PID = 0						
31												
32												
33				90	14:40	PID = 0						
34												
35				90	14:45	PID = 0						
36												
37												
38				90	14:49	PID = 0						
39												
40												
41				113	14:54	PID = 0						
42									ML	Sandy SILT, 10YR 5/2, moist to wet, very fine grained sand, no odor		



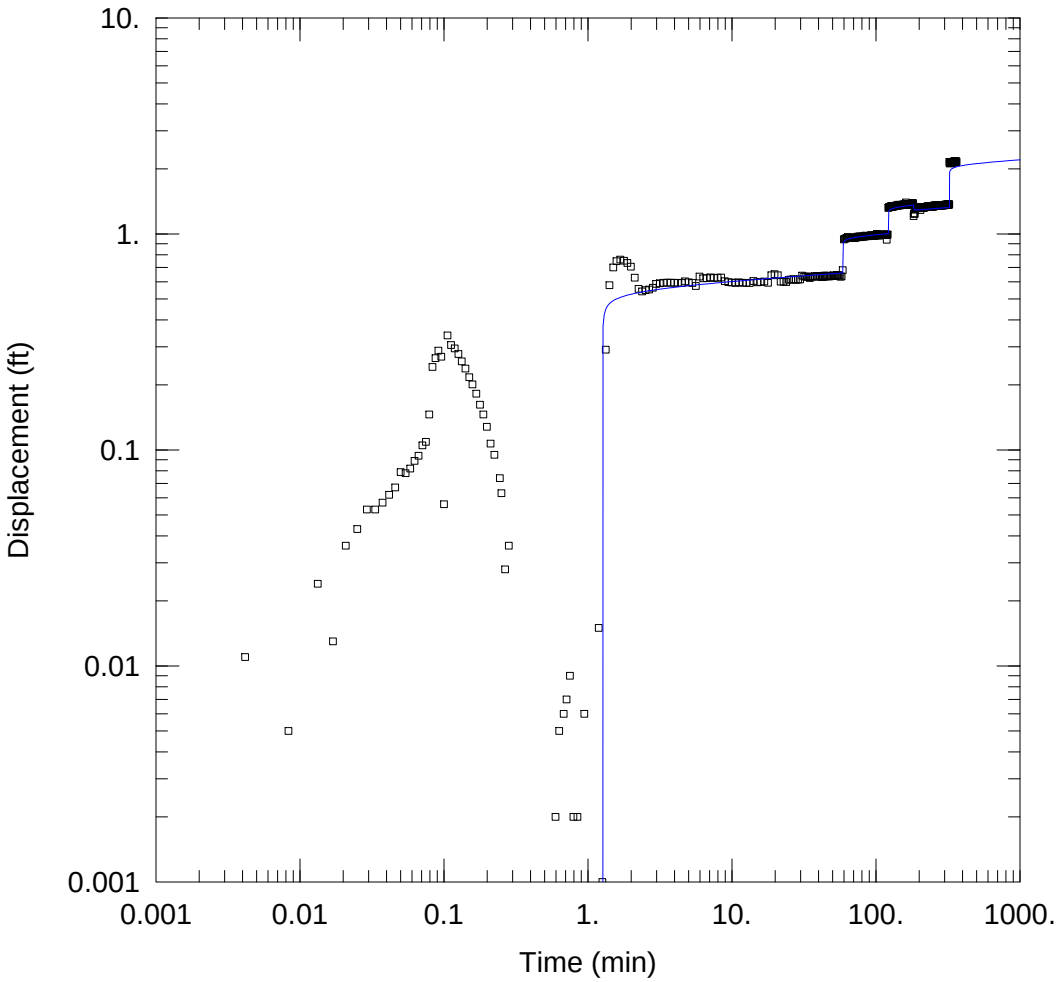
Bentonite pellets from 31 to 34' bgs (1 cubic ft)

#16-30 filter pack from 34 to 42' bgs (2.7 cubic ft)

0.010" Sch 40 PVC Screen 2" from 36 to 41' bgs

Screw cap

Appendix C
Pump Test Analysis



WELL TEST ANALYSIS

Data Set: C:\Users\james.teo\Documents\Federal\Navy\North Island\aqtesolv analysis\PTW-01.aqt
Date: 10/18/18 Time: 15:42:37

PROJECT INFORMATION

Company: APTIM
Client: ESTCP
Project: 501020
Location: North Island
Test Well: PTW-01
Test Date: March 9, 2018

AQUIFER DATA

Saturated Thickness: 18. ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Pumping Wells

Well Name	X (ft)	Y (ft)
PTW-01	6267810.42	1836958.47

Observation Wells

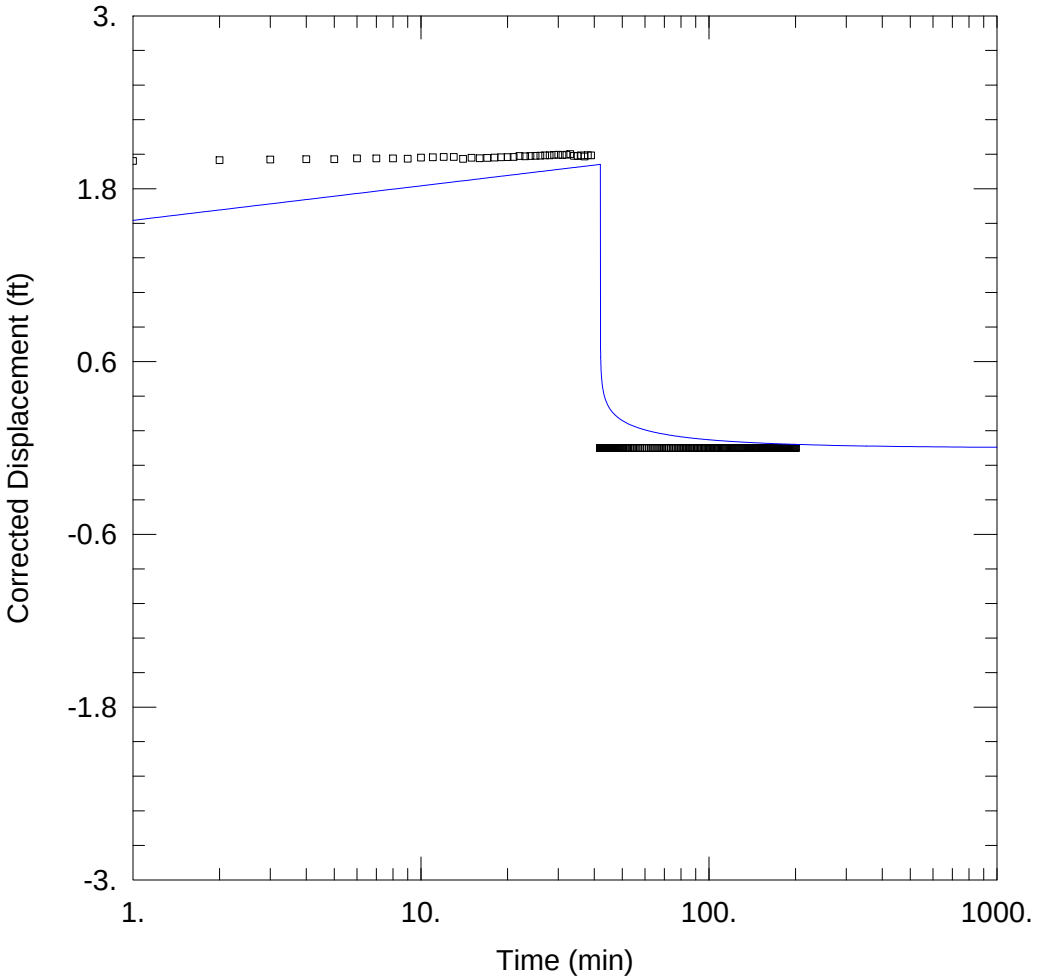
Well Name	X (ft)	Y (ft)
▫ PTW-01	6267810.42	1836958.47

SOLUTION

Aquifer Model: Confined
Solution Method: Theis (Step Test)

T = 505.2 ft^2/day
S = 0.0003633
Sw = 0.
C = 0. min^2/ft^5
P = 2.

Step Test Model: Jacob-Rorabaugh
$s(t) = 3.963Q + 0.Q^{2.}$



WELL TEST ANALYSIS

Data Set: C:\...\PTW-01_recoverytheis.aqt
Date: 10/31/18 Time: 14:17:42

PROJECT INFORMATION

Company: APTIM
Client: ESTCP
Project: 501020
Location: North Island
Test Well: PTW-01
Test Date: March 9, 2018

WELL DATA

Pumping Wells

Well Name	X (ft)	Y (ft)
PTW-01	6267810.42	1836958.47

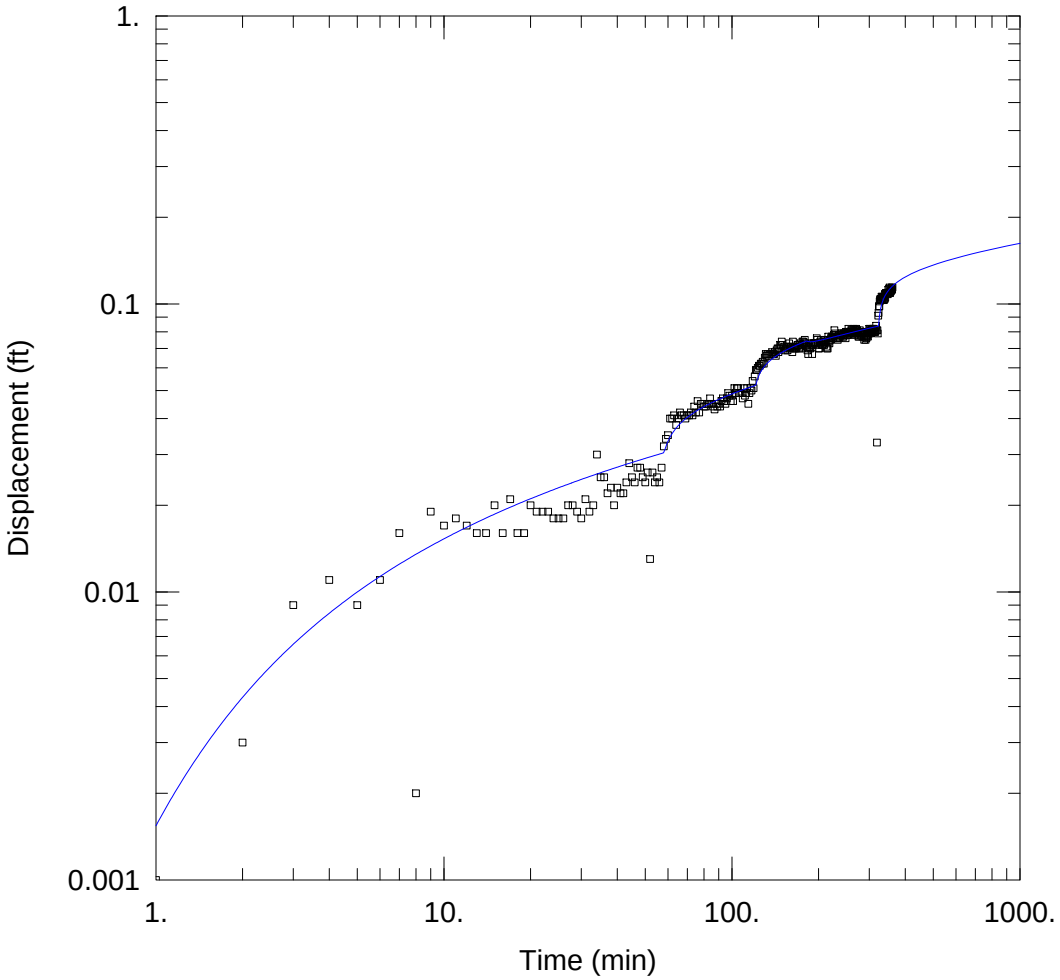
Observation Wells

Well Name	X (ft)	Y (ft)
▫ PTW-01	6267810.42	1836958.47

SOLUTION

Aquifer Model: Unconfined
Solution Method: Theis

T = 442. ft^2/day
S = 0.000507
Kz/Kr = 1.
b = 18. ft



WELL TEST ANALYSIS

Data Set: C:\Users\james.teo\Documents\Federal\Navy\North Island\aqtesolv analysis\PMW_01s.aqt
Date: 10/19/18 Time: 11:15:46

PROJECT INFORMATION

Company: APTIM
Client: ESTCP
Project: 501020
Location: North Island
Test Well: PTW-01
Test Date: March 9, 2018

AQUIFER DATA

Saturated Thickness: 18. ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Pumping Wells

Well Name	X (ft)	Y (ft)
PTW-01	6267810.42	1836958.47

Observation Wells

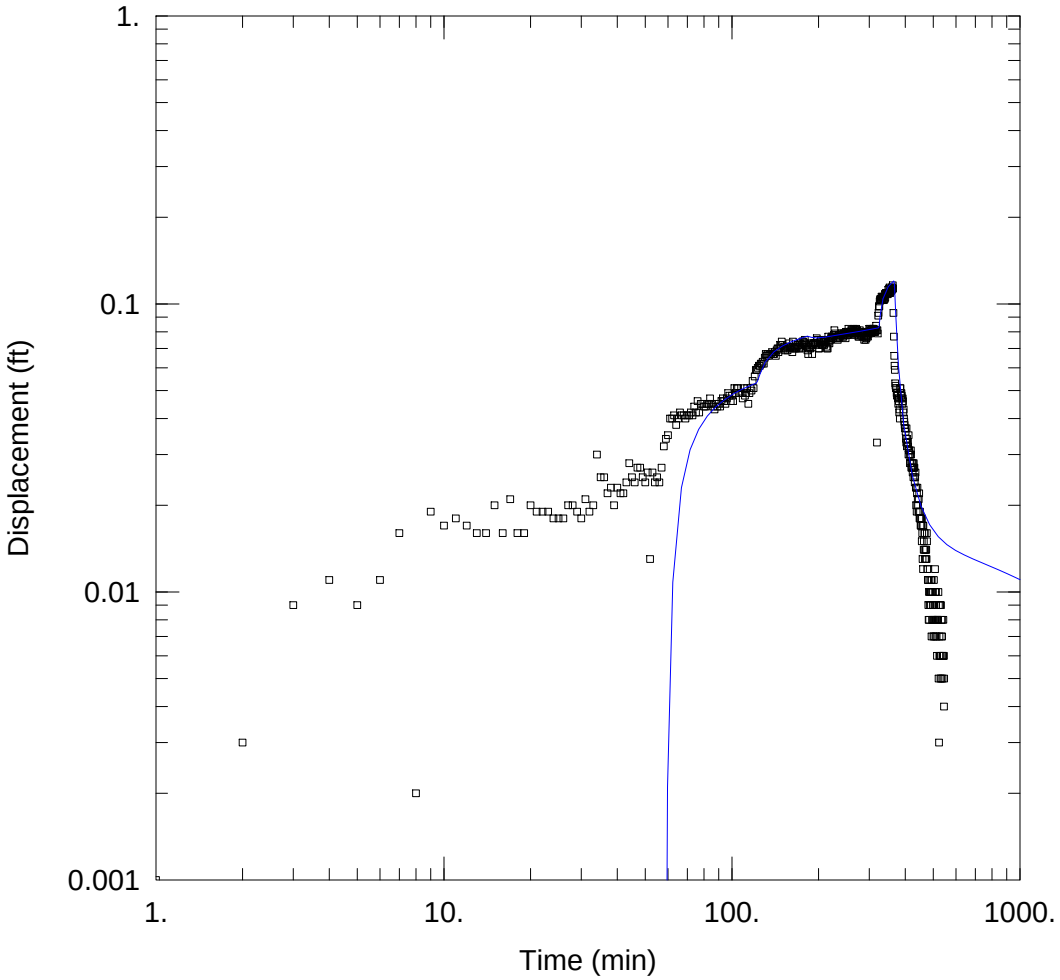
Well Name	X (ft)	Y (ft)
PMW-01s	6267846.931	1836957.379

SOLUTION

Aquifer Model: Confined
Solution Method: Theis (Step Test)

T = 1698.1 ft^2/day
S = 0.004329
Sw = 0.
C = 0. min^2/ft^5
P = 2.

Step Test Model: Jacob-Rorabaugh
$s(t) = 0.01141Q + 0.Q^{2.}$



WELL TEST ANALYSIS

Data Set: C:\...\PMW_01s_recovery_neuman_unconfined.aqt
Date: 11/01/18 Time: 14:17:36

PROJECT INFORMATION

Company: APTIM
Client: ESTCP
Project: 501020
Location: North Island
Test Well: PTW-01
Test Date: March 9, 2018

AQUIFER DATA

Saturated Thickness: 18. ft

WELL DATA

Pumping Wells

Well Name	X (ft)	Y (ft)
PTW-01	6267810.42	1836958.47

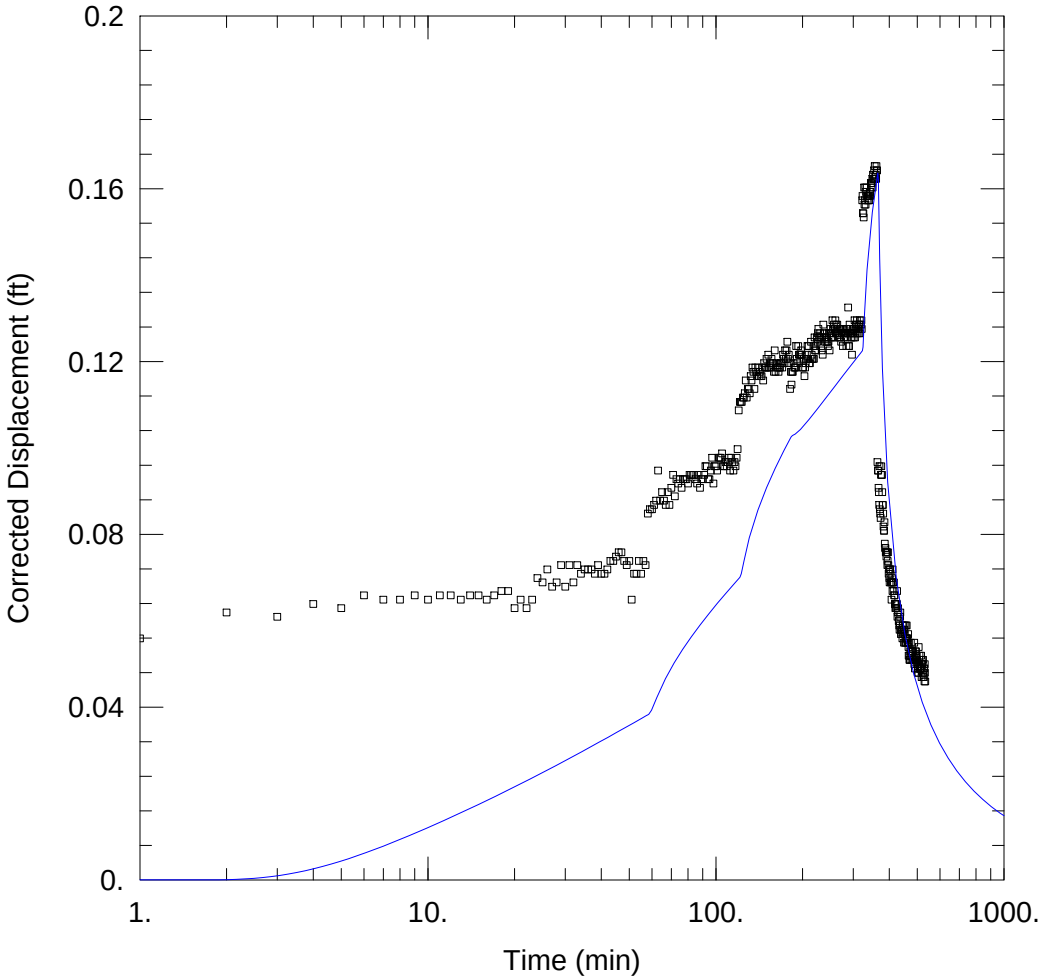
Observation Wells

Well Name	X (ft)	Y (ft)
▫ PMW-01s	6267846.931	1836957.379

SOLUTION

Aquifer Model: Unconfined
Solution Method: Neuman

T = 602.5 ft^2/day
Sy = 0.1
S = 0.005908
ß = 0.1



WELL TEST ANALYSIS

Data Set: C:\...\PMW_01d_recovery_theis_unconfined.aqt
Date: 11/01/18 Time: 14:14:32

PROJECT INFORMATION

Company: APTIM
Client: ESTCP
Project: 501020
Location: North Island
Test Well: PTW-01
Test Date: March 9, 2018

WELL DATA

Pumping Wells

Well Name	X (ft)	Y (ft)
PTW-01	6267810.42	1836958.47

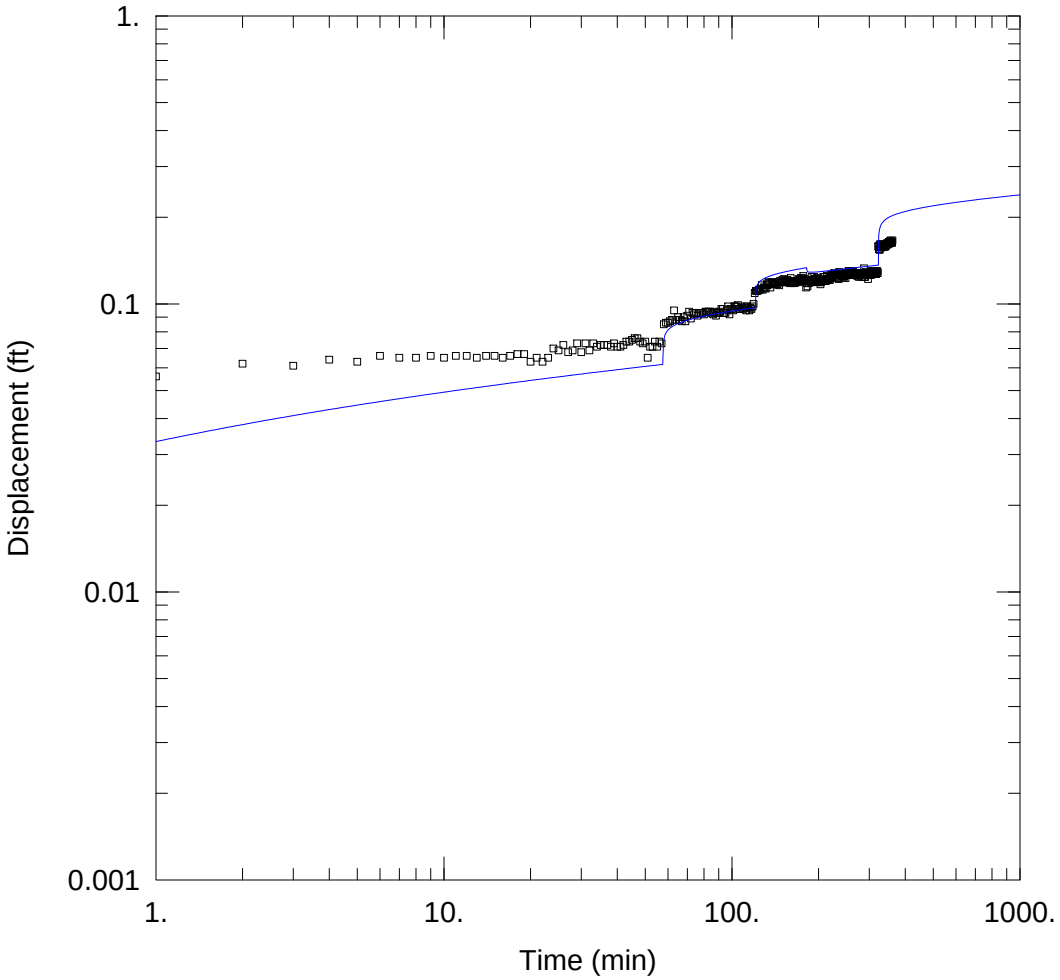
Observation Wells

Well Name	X (ft)	Y (ft)
▫ PMW-01d	6267794.789	1836959.415

SOLUTION

Aquifer Model: Unconfined
Solution Method: Theis

T = 878.1 ft^2/day
S = 0.04365
Kz/Kr = 0.1321
b = 18. ft



WELL TEST ANALYSIS

Data Set: C:\Users\james.teo\Documents\Federal\Navy\North Island\aqtesolv analysis\PMW_01d.aqt
Date: 10/19/18 Time: 10:46:44

PROJECT INFORMATION

Company: APTIM
Client: ESTCP
Project: 501020
Location: North Island
Test Well: PTW-01
Test Date: March 9, 2018

AQUIFER DATA

Saturated Thickness: 18. ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Pumping Wells

Well Name	X (ft)	Y (ft)
PTW-01	6267810.42	1836958.47

Observation Wells

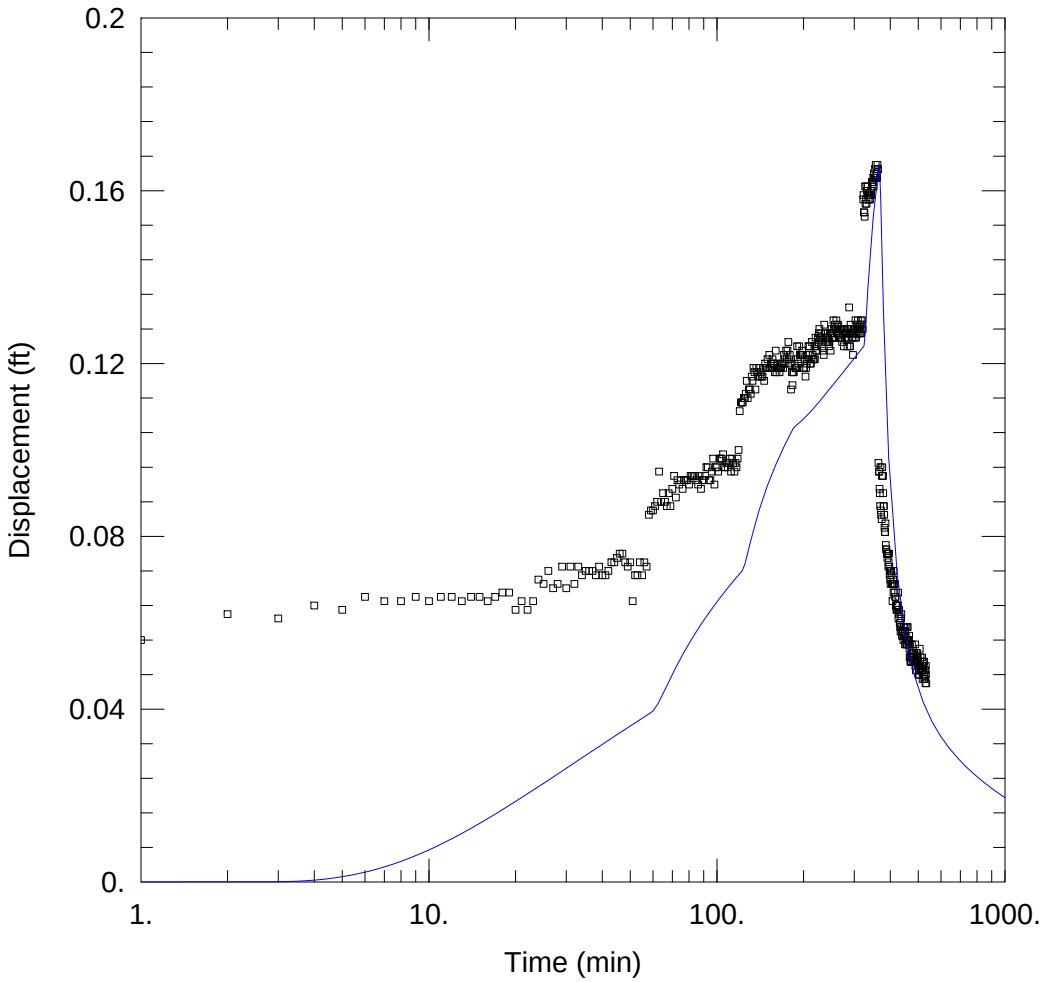
Well Name	X (ft)	Y (ft)
▫ PMW-01d	6267794.789	1836959.415

SOLUTION

Aquifer Model: Confined Solution Method: Theis (Step Test)

T = 2205.7 ft^2/day S = 0.0001437
Sw = 0. C = 0. min^2/ft^5
P = 2.

Step Test Model: Jacob-Rorabaugh $s(t) = 0.2464Q + 0.Q^{2.}$



WELL TEST ANALYSIS

Data Set: C:\...\PMW_01d_recovery_neuman_unconfined.aqt
Date: 11/01/18 Time: 13:59:09

PROJECT INFORMATION

Company: APTIM
Client: ESTCP
Project: 501020
Location: North Island
Test Well: PTW-01
Test Date: March 9, 2018

AQUIFER DATA

Saturated Thickness: 18. ft

WELL DATA

Pumping Wells

Well Name	X (ft)	Y (ft)
PTW-01	6267810.42	1836958.47

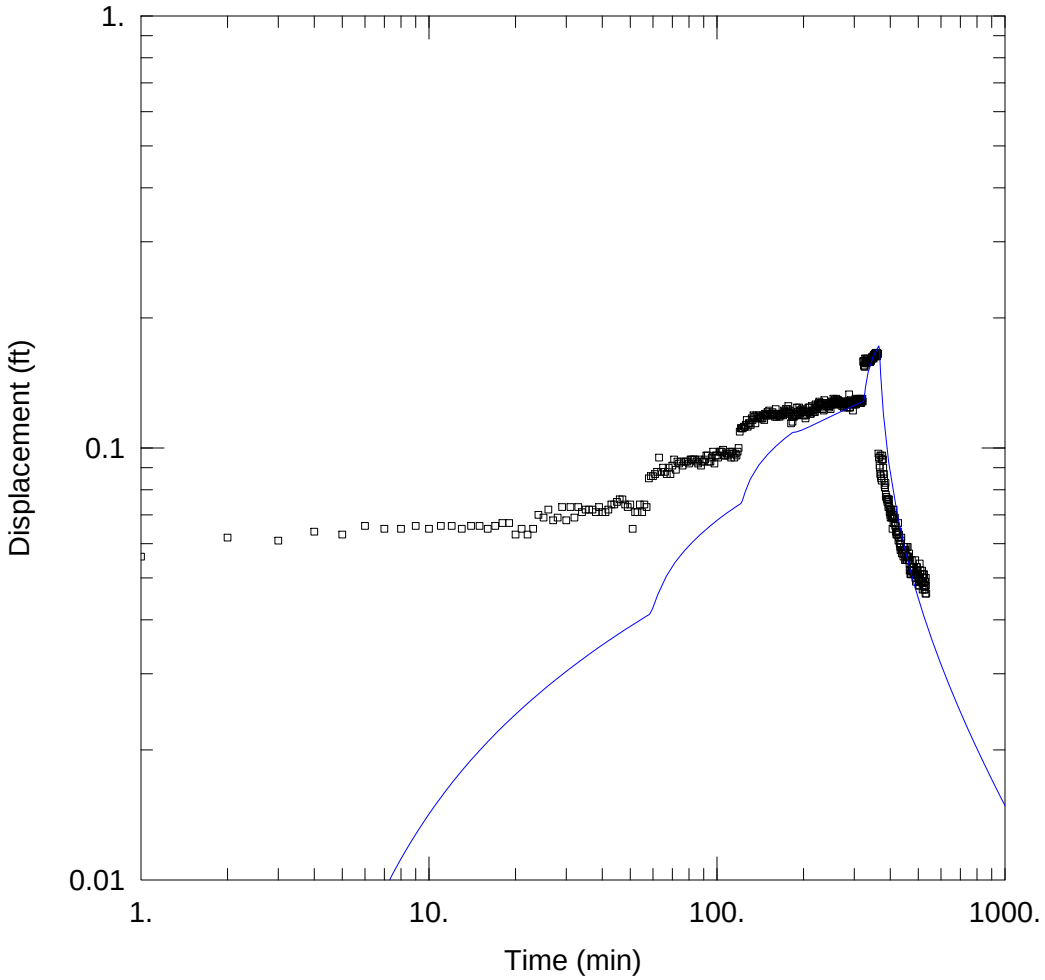
Observation Wells

Well Name	X (ft)	Y (ft)
PMW-01d	6267794.789	1836959.415

SOLUTION

Aquifer Model: Unconfined
Solution Method: Neuman

T = 554. ft^2/day
S = 0.05957
Sy = 0.1
ß = 0.1



WELL TEST ANALYSIS

Data Set: C:\...\PMW_01d_recovery_theis_confined.aqt
Date: 11/01/18 Time: 13:55:02

PROJECT INFORMATION

Company: APTIM
Client: ESTCP
Project: 501020
Location: North Island
Test Well: PTW-01
Test Date: March 9, 2018

AQUIFER DATA

Saturated Thickness: 18. ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Pumping Wells

Well Name	X (ft)	Y (ft)
PTW-01	6267810.42	1836958.47

Observation Wells

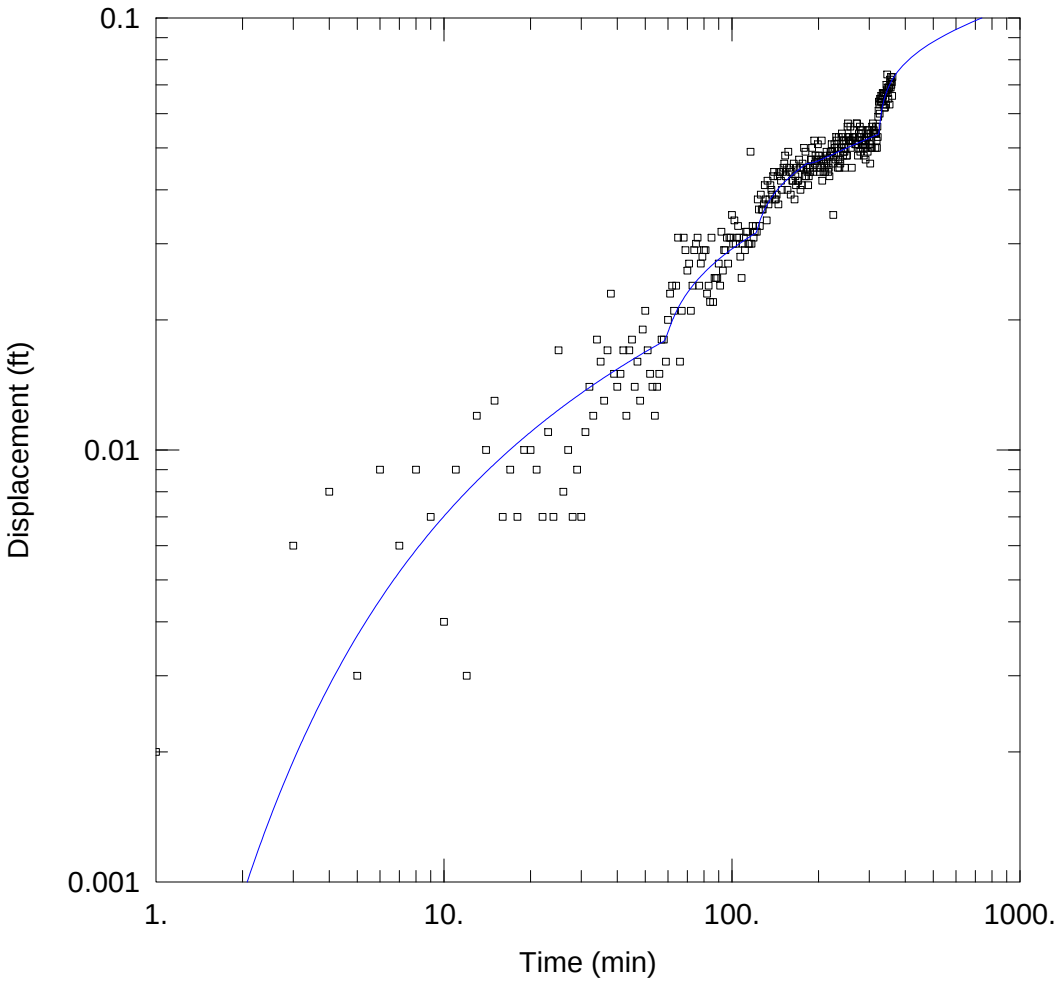
Well Name	X (ft)	Y (ft)
▫ PMW-01d	6267794.789	1836959.415

SOLUTION

Aquifer Model: Confined
Solution Method: Theis (Step Test)

T = 878.1 ft^2/day
S = 0.03867
Sw = 0.075
C = 0. min^2/ft^5
P = 2.

Step Test Model: Jacob-Rorabaugh
$s(t) = 0.Q + 0.Q^{2.}$



WELL TEST ANALYSIS

Data Set: C:\Users\james.teo\Documents\Federal\Navy\North Island\aqtesolv analysis\PMW_02s.aqt
Date: 10/19/18 Time: 11:29:40

PROJECT INFORMATION

Company: APTIM
Client: ESTCP
Project: 501020
Location: North Island
Test Well: PTW-01
Test Date: March 9, 2018

AQUIFER DATA

Saturated Thickness: 18. ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Pumping Wells

Well Name	X (ft)	Y (ft)
PTW-01	6267810.42	1836958.47

Observation Wells

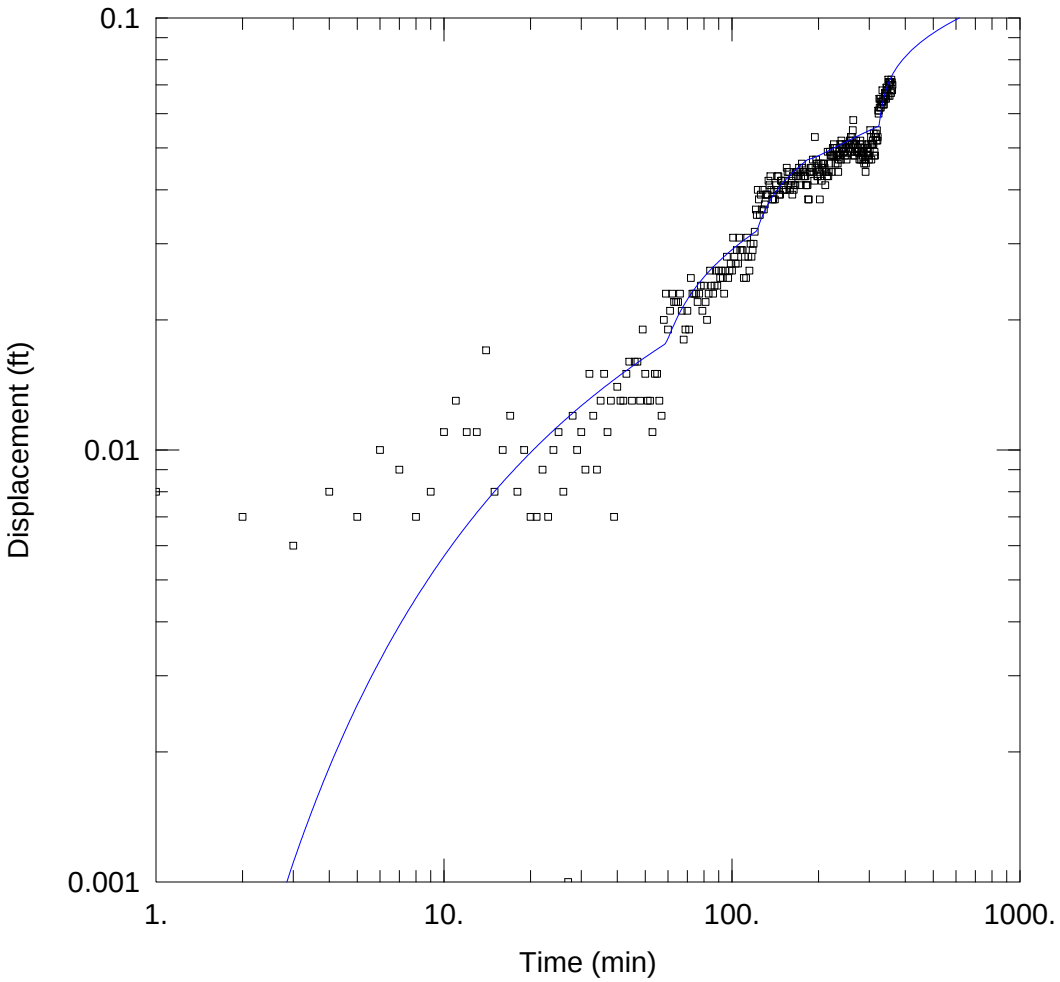
Well Name	X (ft)	Y (ft)
PMW-02s	6267780.178	1836962.69

SOLUTION

Aquifer Model: Confined
Solution Method: Theis (Step Test)

T = 2205.7 ft^2/day
S = 0.01872
Sw = 0.
C = 0. min^2/ft^5
P = 2.

Step Test Model: Jacob-Rorabaugh
$s(t) = 0.001052Q + 0.Q^{2.}$



WELL TEST ANALYSIS

Data Set: C:\Users\james.teo\Documents\Federal\Navy\North Island\aqtesolv analysis\PMW_02d.aqt
Date: 10/19/18 Time: 11:01:35

PROJECT INFORMATION

Company: APTIM
Client: ESTCP
Project: 501020
Location: North Island
Test Well: PTW-01
Test Date: March 9, 2018

AQUIFER DATA

Saturated Thickness: 18. ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Pumping Wells

Well Name	X (ft)	Y (ft)
PTW-01	6267810.42	1836958.47

Observation Wells

Well Name	X (ft)	Y (ft)
▫ PMW-02d	6267780.176	1836962.69

SOLUTION

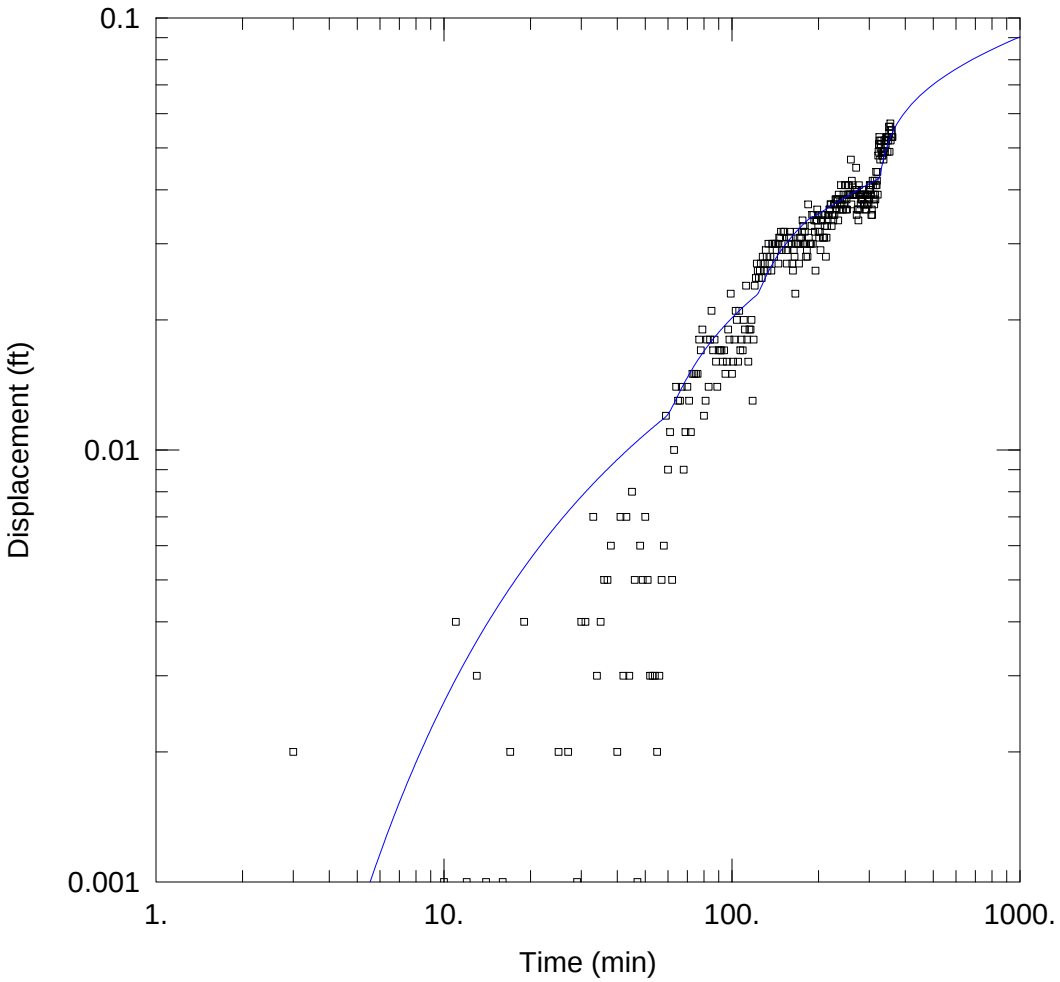
Aquifer Model: Confined
Solution Method: Theis (Step Test)

T = 1905.3 ft^2/day
Sw = 0.
P = 2.

S = 0.02272
C = 0. min^2/ft^5

Step Test Model: Jacob-Rorabaugh

$s(t) = 0.0002737Q + 0.Q^{2.}$



WELL TEST ANALYSIS

Data Set: C:\Users\james.teo\Documents\Federal\Navy\North Island\aqtesolv analysis\SMW-08a.aqt
Date: 10/19/18 Time: 11:33:22

PROJECT INFORMATION

Company: APTIM
Client: ESTCP
Project: 501020
Location: North Island
Test Well: PTW-01
Test Date: March 9, 2018

AQUIFER DATA

Saturated Thickness: 18. ft Anisotropy Ratio (Kz/Kr): 0.1

WELL DATA

Pumping Wells

Well Name	X (ft)	Y (ft)
PTW-01	6267810.42	1836958.47

Observation Wells

Well Name	X (ft)	Y (ft)
SMW-08a	6267846.931	1836957.379

SOLUTION

Aquifer Model: Confined
Solution Method: Theis (Step Test)

T = 2137.8 ft^2/day
Sw = 0.
P = 2.

S = 0.031
C = 0. min^2/ft^5

Step Test Model: Jacob-Rorabaugh

$s(t) = 6.522E\text{-}6Q + 0.Q^{2.}$

Appendix D
Groundwater Modeling Report

GROUNDWATER MODELING REPORT

ESTCP Project ER-201733
Evaluation of a Novel Multiple Primary Substrate (MPS) Cometabolic Approach for In Situ Bioremediation of 1,4-Dioxane and Chlorinated Solvents in Groundwater

North Island Naval Air Station
Coronado, California

January 2020

Prepared by

APTIM

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3.0 Results 6
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Figure 1 MODFLOW Grid Area in Plan View

Figure 2 MODFLOW Grid Area in Cross Section

Figure 3 MODFLOW Simulation Results, Model Layer 2, 7 gpm, 15-Foot Spacing

1.0 Overview and Objectives

APTIM Federal Services, LLC developed a groundwater flow model to simulate transient groundwater pathlines during cyclic pumping of one recovery well and five injection wells for the Naval Air Station North Island ESTCP project.

The groundwater model was set up to include the area of the proposed recovery and injection wells. The groundwater model is fully three-dimensional (3D) and includes the site groundwater flow system from the top of saturation (groundwater table) to an elevation of -14 feet at the base. The groundwater model is designed to simulate the circulation of injected groundwater from the injection wells to the recovery well.

The groundwater modeling was performed using the U.S. Geological Survey (USGS) MODFLOW 2005 (Harbaugh, 2005) numerical groundwater flow model. The Groundwater Vistas 6 software system (Rumbaugh and Rumbaugh, 2011, Environmental Simulations, Inc.) was used as the graphical user's interface for model pre- and post-processing.

2.0 Groundwater Flow Model Setup

This section describes the model setup including the model region, model grid, and the hydrogeologic properties and conditions.

2.1 Model Area, Grid, and Layers

The model area is divided into a rectangular block-centered finite-difference grid centered on the area of well PTW-01, which is located near the planned extraction well EW-1. The model region was oriented with its long axis (direction along the rows) extending from southeast to northwest to parallel the direction of natural groundwater flow at the site. The model area is subdivided in 142 columns and 128 rows of grid cells. The columns and rows are numbered from the north corner of the model area starting with Column 1 and Row 1. The grid cell widths are 1 foot throughout the model area. The MODFLOW model approximates the solution of the groundwater flow equations at the center of each grid cell using the finite-difference numerical solution method. The grid cell widths were selected to provide increased accuracy and detailed representation of the simulated hydraulic head distribution. **Figure 1** shows the model grid in plan view.

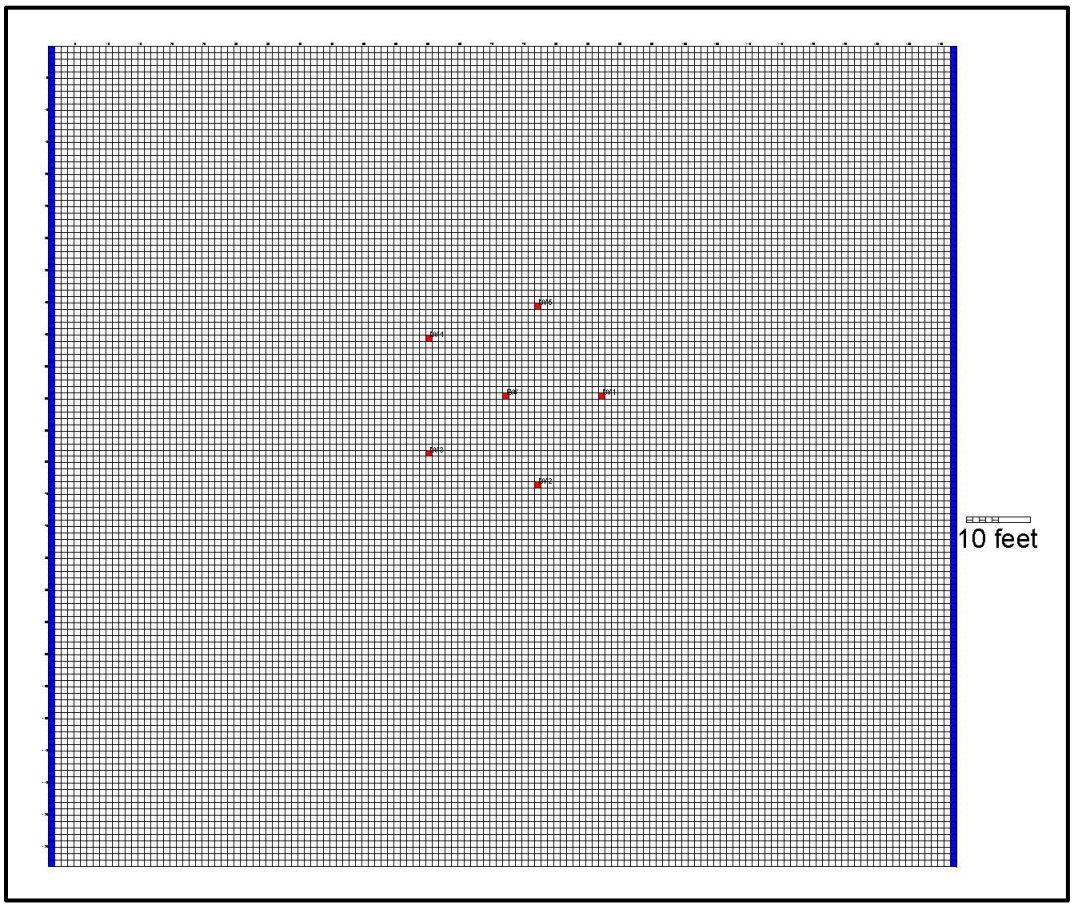

Figure 1. MODFLOW Grid Area in Plan View

As shown in **Figure 2**, the model consists of 5 layers to represent the elevation range of the site groundwater flow system. The top elevation of the model is 30 feet msl at the top of Model Layer 1 and represents the ground surface. The bottom elevation of the model is -14 feet msl at the base of Model Layer 5 and represents the base of the permeable zone. Model Layer 1 extends from elevation 30 feet to elevation 0 feet at the base. Model Layer 2 extends from 0 feet to -5 feet elevation. Model Layer 3 extends from -5 feet to -7 feet elevation. Model Layer 4 extends from -7 feet to -9 feet elevation. Model Layer 5 extends from -9 feet to the base of the model at -14 feet (depth of 44 feet below ground surface. Each model layer is of uniform thickness throughout the model area.

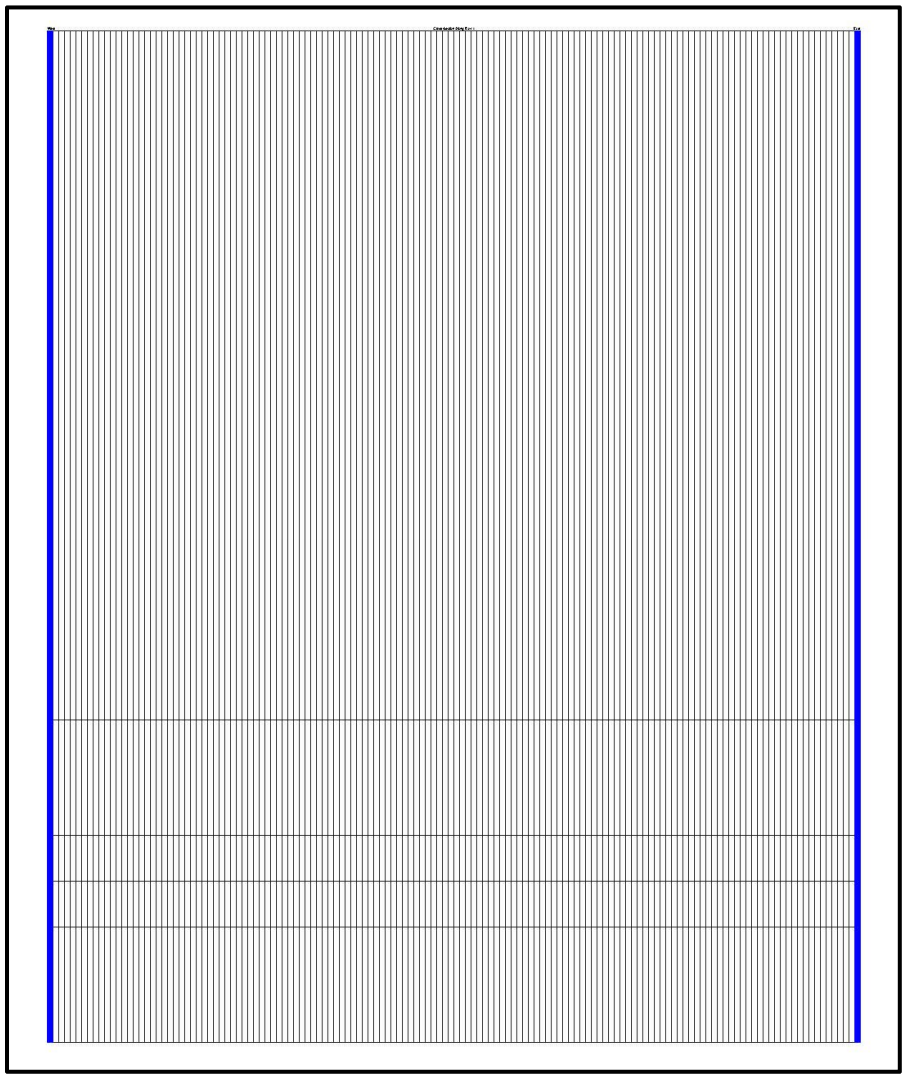

Figure 2. MODFLOW Grid Area in Cross Section

Model Layer 1 was designated as unconfined (Type 1). Model Layers 2 through 5 were designated as unconfined (Type 3) with variable transmissivity dependent on the saturated thickness at each point.

2.2 Hydrogeologic Properties and Conditions

This section presents the hydrogeologic properties and conditions used in preparing the groundwater flow model. These include the hydraulic conductivities (horizontal and vertical), model boundary conditions, and pumping conditions of the extraction and recovery wells.

The horizontal hydraulic conductivity values were based on hydraulic testing of well PTW-01 and evaluation of recovery in wells PMW-01s, PMW-01d, PMW-02s, PMW-02d, and SMW-08a, which were located within 36 feet of the location of well PTW-01. The horizontal hydraulic conductivity of Model Layers 1 and 2 was assigned to be 110 feet per day (ft/day). Model Layers 3 and 4 were assigned hydraulic conductivity of 130 ft/day. Model Layer 5 was assigned a hydraulic conductivity value of 0.5 ft/day. Based on the time unit of hours used in the modeling, the hydraulic conductivities were 4.6 ft/hour in Model Layers 1 and 2, 5.4 ft/hour in Model Layers 3 and 4, and 0.02 ft/hour in Model Layer 5. The assigned hydraulic conductivity values were uniform throughout each model layer. The vertical hydraulic conductivity was assigned to be one-tenth of the horizontal hydraulic conductivity. During development of the MODFLOW model simulations, the hydraulic conductivity values were decreased to 35 ft/day (1.46 ft/hour) in Model Layers 1 and 2 and to 50 ft/day (2 ft/hour) in Model Layers 3 and 4. The hydraulic conductivity values of Model Layer 5 were not changed.

The storativity was assigned to be 0.003 1/foot throughout the model. The porosity was assigned to be 0.3 (dimensionless). The specific yield of Model Layer 1 was assigned to be 0.1.

Boundary conditions were assigned to the model boundaries to represent the hydrologic conditions of the site. The southeast boundary of the model (Column 142) was assigned a constant-head elevation of 5.225 feet in all five model layers. The northwest boundary of the model (Column 1) was assigned a constant-head elevation of 5.135 feet in all of the model layers. The constant-head boundary conditions result in a southeast to northwest direction of groundwater flow in the absence of extraction or injection pumping. The northeast and southwest boundaries of the model are approximately parallel to the natural groundwater flow direction and were assigned as general head boundary conditions in all of the model layers. The starting heads throughout the model grid were assigned to be 5.3 feet.

Recharge was not assigned to the model area because of its small size and short durations of the simulations.

The injection wells were located in a pentagon-shaped configuration around the extraction well EW-1 with equal distances between each of the injection wells and between the injection wells and well EW-1. The simulations included cases in which the distances between the wells were 25 feet, 20 feet, and 15 feet. Pumping rates for the proposed extraction well and injection wells were assigned in Model Layers 1 through 4. No pumping was simulated in Model Layer 5. The

extraction well (EW-1) pumping rates ranged from 5 to 7 gallons per minute (gpm) in the simulations. In the model units of feet and hour 5 gpm is equivalent to 40 cubic feet per hour. The 7 gpm simulations are equivalent to 56 cubic feet per hour. The combined injection pumping rates of the five injection wells IW-1 through IW-5 were assigned to be equal to the extraction pumping rate in each simulation. The pumping rates were apportioned between the model layers in each well relative to the thickness of each model layer. For example, in the 7 gpm case, the extraction rates from Model Layers 1 and 2 were each 18 cubic feet per hour and from Model Layers 3 and 4 were each 10.1 cubic feet per hour. Each injection well had injection rates of 3.6 cubic feet per hour in each of Model Layers 1 and 2 and of 2.0 cubic feet per hour in each of Model Layers 3 and 4.

The model simulations were transient with cycles of extraction and injection pumping separated by intervals of no pumping. Each interval of pumping and each non-pumping interval was modeled as a MODFLOW stress period consisting of 10 time steps. The cycles of pumping included 8 hours of extraction and injection pumping followed by 16 hours of no pumping. The simulations included 1,448 hours of simulated time, which is equivalent to 60 days and 8 hours. Each day consisted of an 8-hour pumping stress period followed by a 16-hour non-pumping stress period. The final 8-hour stress period of the simulation duration included pumping of the extraction well and injection wells.

.

3.0 Results

The simulations included modeling of the hydraulic head distribution in each model layer and particle tracking of groundwater flow lines with the MODPATH model. For each simulation, locations were selected as starting points for the particle tracking. The time of travel was plotted adjacent to each flow line and shown in days since the beginning of the simulation.

The model simulations showed development of a cone of depression surrounding extraction well EW-1 and hydraulic mounds around each injection well. The simulated hydraulic head distribution and particle path lines in model layer 2 are shown in **Figure 3** for the case in which the injection wells and extraction well are 15 feet apart and the total extraction pumping rate and total injection rate were 7 gpm. The cone of depression of extraction well EW-1 has approximately 1.7 feet of drawdown. The travel times for the modeled path lines range from 5 to 15 days for points located between the injection wells and the extraction well and are greater than 60 days for points located on the opposite sides of the injection wells.

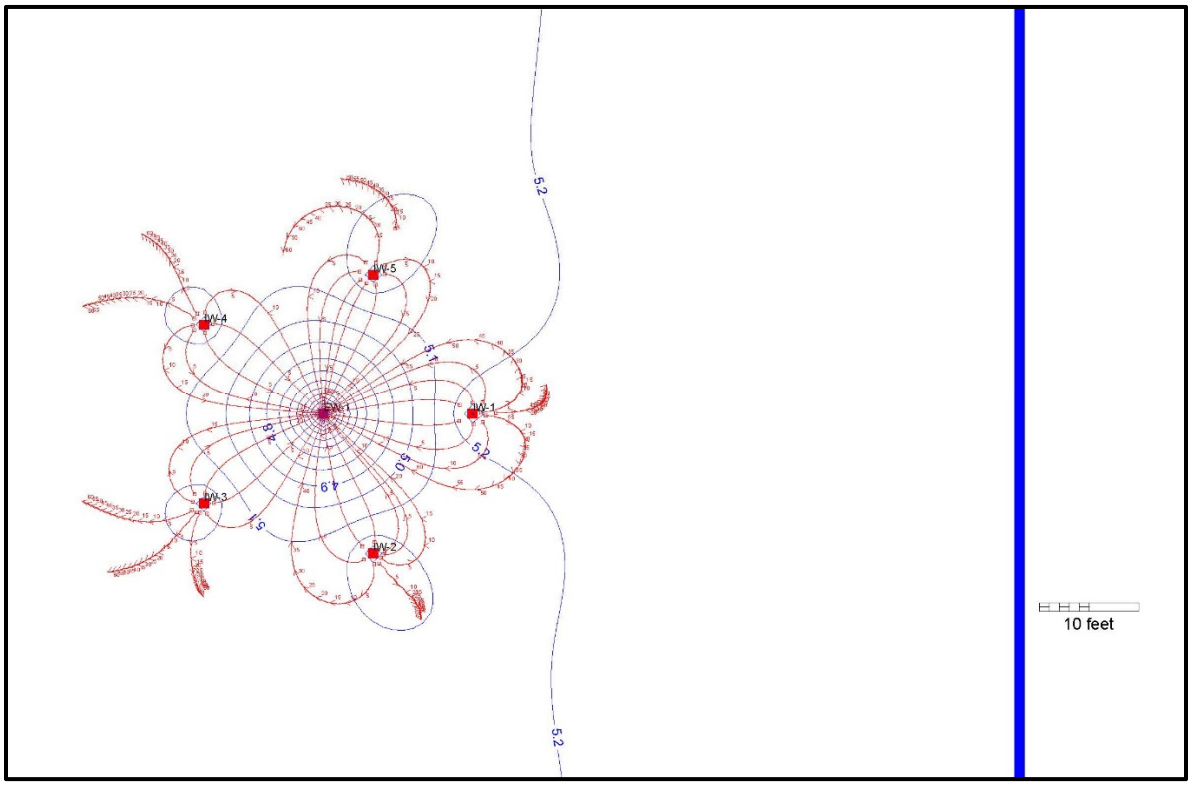

Figure 3. MODFLOW Simulation Results, Model Layer 2, 7 gpm, 15-Foot Spacing

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Harbaugh, A.W., 2005, MODFLOW-2005, The U.S. Geological Survey Modular Ground-Water Model—The Ground-Water Flow Process, U.S. Geological Survey Techniques and Methods 6-A16.

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Appendix E
Treatability Study Report

Evaluation of a Novel Multiple Primary Substrate (MPS) Biosparging Technology for In Situ Bioremediation of 1,4-Dioxane and Chlorinated Solvents in Groundwater

ESTCP PROJECT ER-201733
Treatability Testing Report

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LIST OF ACRONYMS AND ABBREVIATIONS

Acronym	Full text
µg/L	micrograms per liter
µL	microliter(s)
µm	micron
ABPP	activity-based protein profiling
APTIM	Aptim Federal Services, LLC.
°C	degrees Celsius
CAHs	chlorinated aliphatic hydrocarbons
CH_4	methane
cis-DCE	*cis*-1,2-dichloroethene
cm	centimeter(s)
COC(s)	contaminant(s) of concern
Cu	copper
14-D	1,4-Dioxane
1,1-DCE	1,1-dichloroethene
1,2-DCE	1,2-dichloroethene
DAP	diammonium phosphate
DO	dissolved oxygen
ECD	electron capture detector
ESTCP	Environmental Security Technology Certification Program
FID	flame ionization detector
GW	groundwater
GC	gas chromatograph
GC-MS	gas chromatography-mass spectrometry
L	liter(s)
LEL	lower explosive limit
min	minutes
mg	milligram(s)
mg/L	milligrams per liter
mL	milliliter(s)
mL/min	milliliter(s) per minute
mm	millimeter(s)
MPS	multiple primary substrate
N	nitrogen
N_2	nitrogen gas
NAS	Naval Air Station
NCSU	North Carolina State University
O_2	oxygen
OSU	Oregon State University
pMMO	particulate methane monooxygenase
qPCR	quantitative polymerase chain reacation
rpm	revolutions per minute
SERDP	Strategic Environmental Research and Development Program
sMMO	soluble methane monooxygenase
1,1,1-TCA	1,1,1-trichloroethane
TCD	thermal conductivity detector
TCE	1,1,2-trichloroethene
USEPA	United States Environmental Protection Agency
VOC	volatile organic compound

EXECUTIVE SUMMARY

Microcosm studies were conducted to determine the most effective strategy to treat several different contaminants of concern (COCs) in groundwater at OU-11, Naval Air Station, North Island (NANSI), San Diego, CA. COCs of interest in the groundwater included 1,1,2-trichloroethene (TCE), 1,1-dichlorethene (1,1-DCE), *cis*-1,2-dichlorethene (*cis*-DCE), chloroform (CF), and 1,4-dioxane (1,4-D). Isobutane and isobutene were selected as primary growth substrates to promote aerobic cometabolism of the COC mixture present. Isobutane utilizers have been observed to effectively biodegrade 1,1-DCE, *cis*-DCE and 1,4-D, while isobutene utilizers are typically very effective at degradation of both 1,1-DCE and TCE.

Microcosms were prepared with aquifer solids and groundwater obtained during OU-11 site characterization activities at the demonstration site. Each microcosm received 15 mL of mixed core material and 50 mL of OU11-PTW-01 groundwater with the COCs that were present. After microcosm construction, the aqueous concentrations of the COCs in the groundwater were: TCE (768 µg/L); 1,1-DCE (761 µg/L); *cis*-DCE (368 µg/L) and 1,4-D (405 µg/L) and CF (5 µg/L). Neither native isobutane nor isobutene biodegraders were stimulated in the microcosms over an incubation period of more than 3 months. Additional testing suggested that the presence and/or transformation of 1,1-DCE is inhibitory to the stimulation of native microbes that biodegrade both isobutane and/or isobutene. This is likely due to the formation of a toxic epoxide resulting from the initial transformation of 1,1-DCE.

One of the two sets of microcosms that had received isobutane was subsequently bioaugmented with the isobutane-utilizing culture *Rhodococcus rhodochrous* strain ATCC 21198 (21198), while the second set was bioaugmented with an isobutane-utilizing strain (ENV493) that was enriched from the NASNI groundwater prior to the initiation of the microcosms. This pure culture has been identified as being very closely related to both *Rhodococcus ruber* and *Rhodococcus aetherivorans* species. Once added to microcosms, both ENV493 and 21198 were able to rapidly transform 1,1-DCE as well as 1,4-D, *cis*-DCE, and CF. Successive additions of isobutane were consumed by ENV493, indicating that it could grow under the aquifer conditions in the test zone. The activity of the second organism, 21198, decreased over time, indicating that the conditions were potentially unfavorable to its long-term growth and activity.

Isobutane-utilizing bacteria, including ENV493 and 21198, typically do not biodegrade TCE at high rates, and TCE remained in the microcosms after all other relevant COCs were degraded. One set of the microcosms was bioaugmented with the isobutene-degrading bacterium ELW1, which successfully cometabolized the remaining TCE. However, isobutene consumption became slower with successive additions. A second set of microcosms was bioaugmented with a methanotrophic mixed culture (ENV494M), isolated from NASNI groundwater, to cometabolize that remaining TCE. TCE was successfully degraded by the bioaugmented methane-utilizing culture and successive additions of methane were consumed over time, showing that this native culture survived well under site conditions.

The results of the microcosm studies as well as enrichment and pure culture tests indicate that all relevant COCs in the NASNI OU-11 area groundwater can be successfully treated to below project

performance objective criteria using a novel strategy that involves addition of two cometabolic substrates and two different bioaugmentation cultures. As a result, our project team recommends a GO decision for field implementation of this technology at NASNI.

The two cultures that have been isolated from the site (ENV493 and ENV494M) are the best candidates for bioaugmentation. ENV493 and isobutane will be added to biodegrade 1,1-DCE, 1,4-D, *cis*-DCE and CF, while methane and ENV494M will be used to transform residual TCE. The bioaugmentation cultures can be grown in the required quantities for field application in APTIM's commercial fermentation facility (Lawrenceville, NJ). Studies are ongoing to optimize the addition of the growth substrates and cultures in the field. This multiple primary substrate approach with or without bioaugmentation (based on site conditions), is likely to have broad applicability at DoD sites containing mixed chlorinated solvents and 1,4-D.

1.0 INTRODUCTION

1,4-Dioxane (1,4-D), which was widely used as a stabilizer in chlorinated solvents, has emerged as an important groundwater contaminant throughout the United States. Typical of many ethers, 1,4-D is miscible in water, and has both a low Henry's Law constant and a low organic carbon partitioning coefficient, making it difficult to treat by typical remediation approaches, such as air-stripping and carbon adsorption (Mohr et al., 2010). Moreover, because of its use as a solvent stabilizer, 1,4-D is frequently co-mingled with chlorinated volatile organic compounds (CVOCs) in aquifers (Anderson et al., 2012), yet it is not treated by the most common bioremediation approach for these contaminants, which entails adding carbon substrates to aquifers to stimulate reductive dechlorination.

Chlorinated solvents, including 1,1,2-trichloroethene (TCE) and 1,1,1-trichloroethane (1,1,1-TCA), which contained 1,4-D as a stabilizer, have been widely used by DoD. While 1,1,1-TCA is thought to be the likely source of most 1,4-D plumes, 64% of 1,4-D detections at USAF installations are associated with only TCE (Anderson et al., 2012). The widespread occurrence of 1,4-D in groundwater could result in the re-evaluation and re-opening of chlorinated solvent sites. Thus, the DoD has highlighted the need to evaluate the fate of this compound in the environment. As discussed in the 2013 Summary Report from the SERDP and ESTCP Workshop on Long Term Management of Contaminated Groundwater Sites (SERDP & ESTCP, 2013), one critical demonstration need is "developing cost-effective remedial strategies to address contaminant mixtures at contaminated sites". The treatment of 1,4-D comingled with chlorinated solvents was identified as a major priority.

This report presents microcosm work conducted at OSU since October 2018. Microcosms constructed before October 2018 (Sets 1-8) at OSU that consisted of groundwater only with a small amount of sediment from the wells SMW-08A and SMW-10A are summarized in Section 4.0. The full data and modeling associated with the groundwater only microcosms can be found in the MS thesis of Krippaehne (2018), which is attached as **Appendix D**. Results from microcosms constructed since the beginning of October 2018 (Sets 9-12) using aquifer core material and groundwater collected during site investigation work are provided herein. Culture enrichment, isolation and testing as well as an additional microcosm testing conducted at APTIM in support of this project are summarized in Section 3.1, and details are provided in **Appendix B**.

The microcosm studies reported herein were conducted to optimize the field approach for ESTCP Project ER-201733*: Evaluation of A Novel Multiple Primary Substrate (MPS) Biosparging Technology for In Situ Bioremediation of 1,4-D and Chlorinated Solvents in Groundwater*. The key objective of this project is to demonstrate that a novel multiple primary substrate (MPS) cometabolic biosparging technology can meet DoD needs for reliable, flexible, and cost-effective treatment of groundwater contaminated with co-mingled 1,4-D and CVOCs. The primary objective of this approach is to use different cometabolic substrates (and the microbial populations that biodegrade them) to target specific COCs. The field demonstration is planned for the Naval Air Station North Island (NASNI), San Diego, CA, where groundwater is contaminated with 1,4-D, TCE, and several other chlorinated aliphatic hydrocarbons (CAHs), including 1,1-DCE, *cis*-

DCE, and CF, among others. This multiple-substrate approach represents a significant advance for traditional single gas biosparging in that it utilizes a new understanding of microbial communities and monooxygenase enzymes to specifically target DoD sites with mixed 1,4-D and CAHs.

2.0 TREATABILITY STUDY OBJECTIVES

Laboratory microcosm studies were conducted with aquifer samples obtained from the site during preliminary site characterization activities. The primary objectives of the microcosm studies were (1) to determine the efficacy of oxygen and different combinations of gaseous primary substrates (e.g., isobutane, isobutene, methane) to stimulate cometabolic treatment of 1,4-D and target CAHs by indigenous microbial populations and (2) to evaluate the need for bioaugmentation with cometabolic cultures and identify the most effective cultures for this purpose. As part of the second objective, enrichment and isolation of cometabolic cultures from site groundwater was undertaken.

2.1 Contaminants of Concern

As previously noted, the primary COCs at the OU-11 site are TCE, 1,4-D, 1,1-DCE and *cis*-DCE (**Table 1**). Trace amounts of several other halogenated compounds (< 7 µg/L) were also detected in some of the samples during site characterization work. **Table 1** summarizes the primary contaminants at the demonstration site, and shows the concentrations measured in the microcosms. The first column summarizes contaminant concentration ranges measured in discrete interval groundwater samples collected from borings HPT-1, HPT-2, and HPT-3 during site characterization activities. The second column shows the starting contaminant concentrations for microcosm Sets 9-12 that contained soil core material and groundwater collected from monitoring well OU11-PTW-01 that was installed within the demonstration area. Therefore, **Table 1** provides the expected concentration ranges in the demonstration plot location that was selected after the site characterization work was complete.

Table 1. Primary Contaminant Concentrations in Microcosm Studies and Field Samples

Contaminant	Site Characterization Discrete Groundwater Samples (µg/L)	Microcosms Containing Groundwater and Soil Core Material (µg/L)
TCE	<5 - 2108	768
[1] 1,1-DCE	<5 - 2140	761
1,4-Dioxane	0.94 - 1107	405
Chloroform	<5 - 7	5
cis-1,2-DCE	<5 - 613	378
PCE	4 - 6	2

[1]A trace amount of Freon-113 was detected in microcosm samples. This compound co-eluted with 1,1-DCE during GC analysis at OSU, and the two compounds are reported together as 1,1-DCE. 1,1-DCE represents ~ 60% of the total instrument response in column 3. Freon-113 was not measured above the detection limit (5 µg/L) in any of the discrete groundwater samples collected during site characterization activities.

3.0 METHODS

General protocols previously developed by Oregon State University (OSU) during ESTCP ER-199810 (*Use of Cometabolic Air Sparging to Remediate Chloroethene-Contaminated Groundwater Aquifers*) were utilized for microcosm testing (Tovanabootr and Semprini, 1998; Timmons et al., 2001). Models developed for simulating cometabolic transformations that include microbial growth and decay, inhibition among CAHs and the primary substrate, and transformation toxicity (Semprini et al., 2007; Rolston, 2017) were also applied to a subset of the microcosm studies. Since the microcosm protocol was developed for evaluating gaseous cometabolic substrates, it was easily adapted for the present study.

3.1. Bioaugmentation Cultures

Four different cultures were evaluated for bioaugmentation purposes during the microcosm testing. The culture identities and growth conditions are summarized in **Table 2**

Culture	Phylogenetic Information	Primary Substrates Investigated	Pure Culture	Enriched from NAS North Island	[1]Vol. Primary Substrate per Growth Reactor Addition (mL)	[1]Number of Substrate Additions	[1]Media Type	[1]Media Volume (mL)
21198	*Rhodococcus rhodochrous ATCC® 21198™*	Isobutane	Yes	No	45	2	MV	300
ENV493	*Rhodococcus sp.*	Isobutane	Yes	Yes	45	2	MV	300
ELW1	*Mycobacterium sp. ELW1*	Isobutene	Yes	No	50	2	MV	300
ENV494M	--	Methane	No	Yes	60	3	OB3b	200

. Three of the cultures grew on branched hydrocarbons: *Rhodococcus rhodochrous* ATCC® 21198 on isobutane, *Mycobacterium* sp. ELW1 on isobutene (Kottegoda et al., 2015), and ENV493 on isobutane. The ENV493 culture was enriched and purified from the NASNI OU-11 groundwater by scientists at APTIM. Details concerning the isolation of this culture, its ability to biodegrade relevant COCs, as well as additional testing data in OU-11 site groundwater are provided in **Appendix B**. In addition to the cultures that grew on branched hydrocarbons, a mixed methanotrophic culture (ENV494M) was enriched from the NASNI site samples by APTIM scientists. The details concerning the enrichment of this culture and its' degradative abilities are also provided in **Appendix B**.

Table 2. Summary of the Cultures Bioaugmented into Microcosms

Culture	Phylogenetic Information	Primary Substrates Investigated	Pure Culture	Enriched from NAS North Island	[1]Vol. Primary Substrate per Growth Reactor Addition (mL)	[1]Number of Substrate Additions	[1]Media Type	[1]Media Volume (mL)
21198	*Rhodococcus rhodochrous ATCC® 21198™*	Isobutane	Yes	No	45	2	MV	300
ENV493	*Rhodococcus sp.*	Isobutane	Yes	Yes	45	2	MV	300
ELW1	*Mycobacterium sp. ELW1*	Isobutene	Yes	No	50	2	MV	300
ENV494M	--	Methane	No	Yes	60	3	OB3b	200

[1]Growth reactor conditions

All of the cultures were grown in minimal media, at 30°C, in butyl septa-sealed 710 mL Wheaton bottles. When growing ENV493, 21198, and ELW1, two additions of primary substrate were made. Three additions were made when growing ENV494M. The minimal media used to grow

ENV494M did not contain copper, in order to promote the expression of soluble methane monooxygenase (sMMO; expressed in the absence of Cu), as opposed to particulate methane monooxygenase (pMMO; expressed with Cu). The headspace of all growth reactors was air. See Table 2 for the volume of substrate, volume of media, and growth media types used. The composition of all growth media is presented in the **Appendix C**.

Culture	Phylogenetic Information	Primary Substrates Investigated	Pure Culture	Enriched from NAS North Island	[1]Vol. Primary Substrate per Growth Reactor Addition (mL)	[1]Number of Substrate Additions	[1]Media Type	[1]Media Volume (mL)
21198	*Rhodococcus rhodochrous ATCC® 21198™*	Isobutane	Yes	No	45	2	MV	300
ENV493	*Rhodococcus sp.*	Isobutane	Yes	Yes	45	2	MV	300
ELW1	*Mycobacterium sp. ELW1*	Isobutene	Yes	No	50	2	MV	300
ENV494M	--	Methane	No	Yes	60	3	OB3b	200

The contents of the reactors used to grow each culture for bioaugmentation were transferred to 500 mL centrifuge bottles and centrifuged at 8,000 rpm for 10 minutes. The supernatant was decanted, and the cell pellet in each bottle was washed in 200 mL of phosphate buffer, resuspended in ~ 5 mL of phosphate buffer, and used immediately or stored at 4°C in polypropylene centrifuge tubes. Total suspended solids (TSS) analysis was performed on the concentrated cell solution as described in the Standard Methods for the Examination of Water and Wastewater (Eaton, 1995).

3.2 Methods for Microcosm Setup

Soil core material and 16 L of groundwater collected from well OU11-PTW-01 by APTIM during site characterization activities were shipped overnight on ice in coolers to OSU. After receipt from APTIM, the soil core material and groundwater were stored at 4 °C in the dark. The original caps on the 1-L amber glass bottles containing the groundwater were removed and replaced with butyl septa sealed plastic caps to minimize potential loss of CVOCs during storage.

The microcosms were constructed in a chemical fume hood to minimize human exposure to volatile constituents in the soil core material and groundwater. All microcosms were constructed in sterile, 155 mL glass Wheaton bottles with butyl septa. Each microcosm contained 15 mL of homogenized soil core material. Core material consisted of an equal volume mix from soil cores obtained from three aquifer intervals; 25'-30', 30'-35', and 35'-40' below ground surface. Immediately before microcosm construction, core material was homogenized in a sterilized beaker with a sterilized stainless steel spatula. **Figure 1** shows isobutane microcosms after the addition of soil core material.

Figure 1. Photograph of Isobutane Microcosm Construction. Photograph taken after the addition of homogenized soil core material and before the addition of groundwater.

Table 3 provides details of the microcosm construction. Sets of microcosms were constructed to evaluate isobutane (Sets 9 and 12), isobutene (Set 10) and propane (Set 11) as primary substrates for cometabolic treatment of the target contaminants. Treatments were set up in triplicate, and controls were set up in either duplicate or triplicate. Each microcosm received 50 mL of groundwater collected from well OU11-PTW-01. Groundwater was introduced into the microcosm from the 1-L sample bottle by drawing 50 mL of groundwater through a sterile, 10 cm long piece of size 16 silicone tubing into a sterile 60 mL syringe. Groundwater was added from the bottom of the bottle to minimize volatile losses of CVOCs. The caps were tightened, and subsequent samples and additions were made through the septa. **Figure 2** shows the amber sample bottle from APTIM, syringe and silicone tubing, and the microcosms after the groundwater addition.

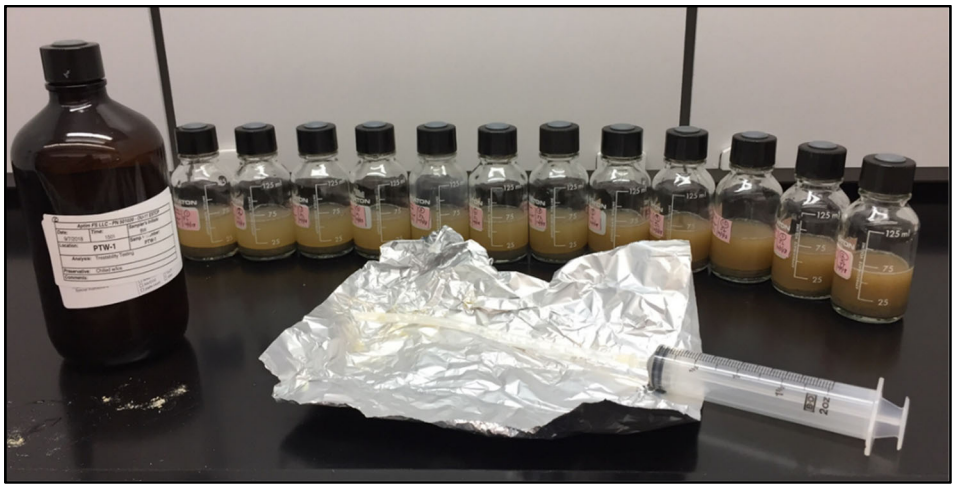

Figure 2. Photograph of Fully-Constructed Isobutane Microcosms.

An inorganic nutrient solution was added to each bottle. The solution was prepared by adding 0.16 g disodium-dihydrate EDTA, 5.9 g diammonium phosphate, and 0.06 g cobalt sulfate heptahydrate to 0.5 L of deionized water and autoclaving. Two hundred microliters of nutrient solution were added through the septa into all microcosms.

Killed controls, acetylene-inhibited controls, and propyne-inhibited controls (acetylene and propyne are inhibitors of specific monooxygenase enzymes) were prepared. Killed (poisoned) controls were constructed by adding 2.5 mL of 1.0 M sodium azide through the septa of appropriate microcosms. Acetylene controls were constructed by adding 1.0 mL and 0.52 mL acetylene gas to bottles that were treated with isobutane and propane, respectively. For the isobutene microcosm set, propyne controls were also prepared by adding 1.3 mL of propyne to a subset of isobutene microcosms. Azide, acetylene, or propyne were added 1 day before addition of primary substrate. Primary substrate was added through the septa one day after initial microcosm construction. **Table 3** provides a summary of initial microcosm additions and the resulting concentrations.

Table 3. Microcosm Construction Details

Microcosm Set Number	Treatment	Gaseous Substrate Volume	Monooxygenase Inhibitor Volume	Poison Volume (1.0 M sodium azide)	Sustrate Liquid Concentration	Substrate Headspace Concentration	Sodium Azide Concentration	Ammonium Nitrogen Concentration	Co^{2+} Concentration	Inhibitor Liquid Concentration	Percent of the LFL of Mixture of Substrate and Inhibitor, if present
		mL	mL	mL	µmol/L	Vol%	mM	ppm	ppb	µmol/L	--
Set 9 & [1]Set 12	Isobutane Poison Controls, Bottles 1-3	1.3	0	2.5	10	1.2%	50	10	100	0	67%
	Isobutane Monoxygenase Inhibitor Controls (Acetylene), Bottles 4-6	1.3	1.0	0	10	1.2%	0	10	100	268	93%
	Isobutane, Bottle 7-12	1.3	0	0	10	1.2%	0	10	100	0	67%
Set 10	Isobutene Poison Controls, Bottles 1-2	1.2	0	2.5	57	1.1%	50	10	100	0	61%
	Isobutene Monooxygenase Inhibitor Controls (Propyne), Bottles 3-4	1.2	1.3	0	57	1.1%	0	10	100	554	85%
	Isobutene, Bottles 5-13	1.2	0	0	57	1.1%	0	10	100	0	61%
Set 11	Propane Poison Controls, Bottles 1-2	1.6	0	2.5	23	1.5%	50	10	100	0	71%
	Propane Monoxygenase Inhibitor Controls (Acetylene), Bottles 3-4	1.6	0.5	0	23	1.5%	0	10	100	140	85%
	Propane, Bottle 5-7	1.6	0	0	23	1.5%	0	10	100	0	71%

[1]Set 12 active bottles were constructed exactly like Set 9 except for the type of nutrients. Two had 0.2 mL of MV media and two had 0.2 mL of diammonium phosphate (DAP) media. There were no acetylene or poison controls.

All microcosms contain: 15 mL, wet volume basis, of homogenized core material from three depths
50 mL groundwater
105 mL air headspace
0.2 mL DAP media + Cobalt

4.0 GROUNDWATER-ONLY MICROCOSM RESULTS

As previously noted, a subset of preliminary microcosm studies (Sets 1-8) were conducted with groundwater collected from wells SMW-08A and SMW-10A (no soil core material). The bottles did contain a small amount of sediment which was obtained during sampling of the groundwater. Details on the results of these groundwater-only studies, including modeling, can be found in the MS thesis of Krippaehne (2018) provided in **Appendix D**. The key findings of the initial groundwater-only studies are summarized as follows (modified from Krippaehne, 2018):

- Native isobutane-utilizers could not be stimulated in the presence of site CVOCs and 1,4-D. It was hypothesized that the formation of the 1,1-DCE epoxide via activation of monooxygenase enzymes by isobutane was toxic and inhibited biostimulation of the native microbial community.

- Native isobutane-utilizers were able to be stimulated in the absence of CVOCs when 1,1-DCE and TCE were removed from solution via sparging. Isobutane uptake was observed after 12 to 69 days of incubation. Effective long-term cometabolic treatment of groundwater for the removal of key CVOCs and 1,4-D that were added after isobutane uptake was achieved. This further supports the hypothesis that 1,1-DCE is inhibitory via epoxide formation and must be treated prior to other COCs.

- Bioaugmentation with isobutane-grown 21198 was a successful mechanism to achieve rapid 1,1-DCE transformation, moderate 1,4-D transformation, and slow TCE transformation. Bioaugmentation effectively promoted long-term cometabolic treatment of groundwater for the removal of CAHs and 1,4-D, though 1,1-DCE toxicity and slow TCE transformations remained as process limitations. These results are consistent with results observed with pure-culture studies of isobutane-grown 21198.

- Bioaugmentation with isobutene-grown ELW1 was also a successful means to achieve degradation of target CVOCs. It was observed that aqueous concentrations of isobutene > approximately 500 µg/L inhibited transformation of the COCs (competitive inhibition). However, once the aqueous concentration of isobutene decreased below ~500 µg/L, rapid 1,1-DCE transformation was observed, followed by slower, but eventually complete, TCE degradation. 1,4-D was slowly transformed in the bioaugmented system. Rates of isobutene uptake and COC transformations were drastically reduced with successive additions of primary substrate and COCs, which strained the long-term viability of the bioaugmented system.

- The addition of inorganic nutrients was important for the stimulation of native isobutane utilizers, though the lack of substantial aquifer solids could have contributed to the necessity of nutrient additions.

The results from the groundwater-only microcosms were used to inform the design of the microcosm study using site soil and groundwater, as detailed in Section 3.

5.0 SOIL AND GROUNDWATER MICROCOSM RESULTS

Results from microcosm studies constructed with site soil and groundwater (Sets 9-12) are provided in the following subsections.

5.1 Isobutane Microcosms

Microcosm Set 9 was constructed to determine if native isobutane utilizing microorganisms could be stimulated in site soil and groundwater. As shown in **Figure 3**, isobutane uptake was not observed over a period of greater than 100 days, indicating stimulation of isobutane utilizers did not occur. Also, no treatment of COCs was observed, with measured concentrations relatively similar to those in acetylene and azide treated controls. These data are consistent with the groundwater only microcosms as summarized in **Section 4.0**.

A decrease in TCE concentration in both the treatments and controls is thought to be the result of sorption to aquifer material. The data for the azide and acetylene controls are provided in **Appendix A**, **Figures 16 and 17**, respectively. Because the indigenous isobutane-utilizing cultures could not be stimulated to degrade COCs (presumably due to 1,1-DCE epoxide formation), the isobutane utilizing cultures ENV493 and 21198 were bioaugmented into separate sets of microcosms. These cultures are both known to biodegrade 1,1-DCE, *cis*-DCE and 1,4-D, but not TCE.

5.1.1 Bioaugmentation with ENV493

Microcosms bottles 7-9 in Set 9 were bioaugmented with 6 mg (based on TSS) of the ENV493 culture on day 107. This inoculum correlates to an optical cell density (OD_{660}) of ~ 0.2, which is estimated to be an achievable cell density in the field through bioaugmentation injections. As shown on **Figure 4**, rapid biodegradation of isobutane was subsequently observed in all three bottles. 1,1-DCE was rapidly transformed over the course of several hours, and *cis*-DCE was transformed over the course of a few days, concurrent with utilization of multiple additions of isobutane. There was minimal transformation of TCE by ENV493 as expected based on pure culture studies (See **Appendix B**). Isobutane consumption stalled with the second addition of isobutane into bottle 8, requiring an additional 3 mg addition of ENV493 on day 119 to treat the remaining *cis*-DCE and to utilize the remaining isobutane. 1,4-D and chloroform were also successfully treated by ENV493 in all three bottles, as shown in **Figure 5**. These data are consistent with the pure culture and groundwater microcosm studies conducted at APTIM using this organism (**Appendix B**).

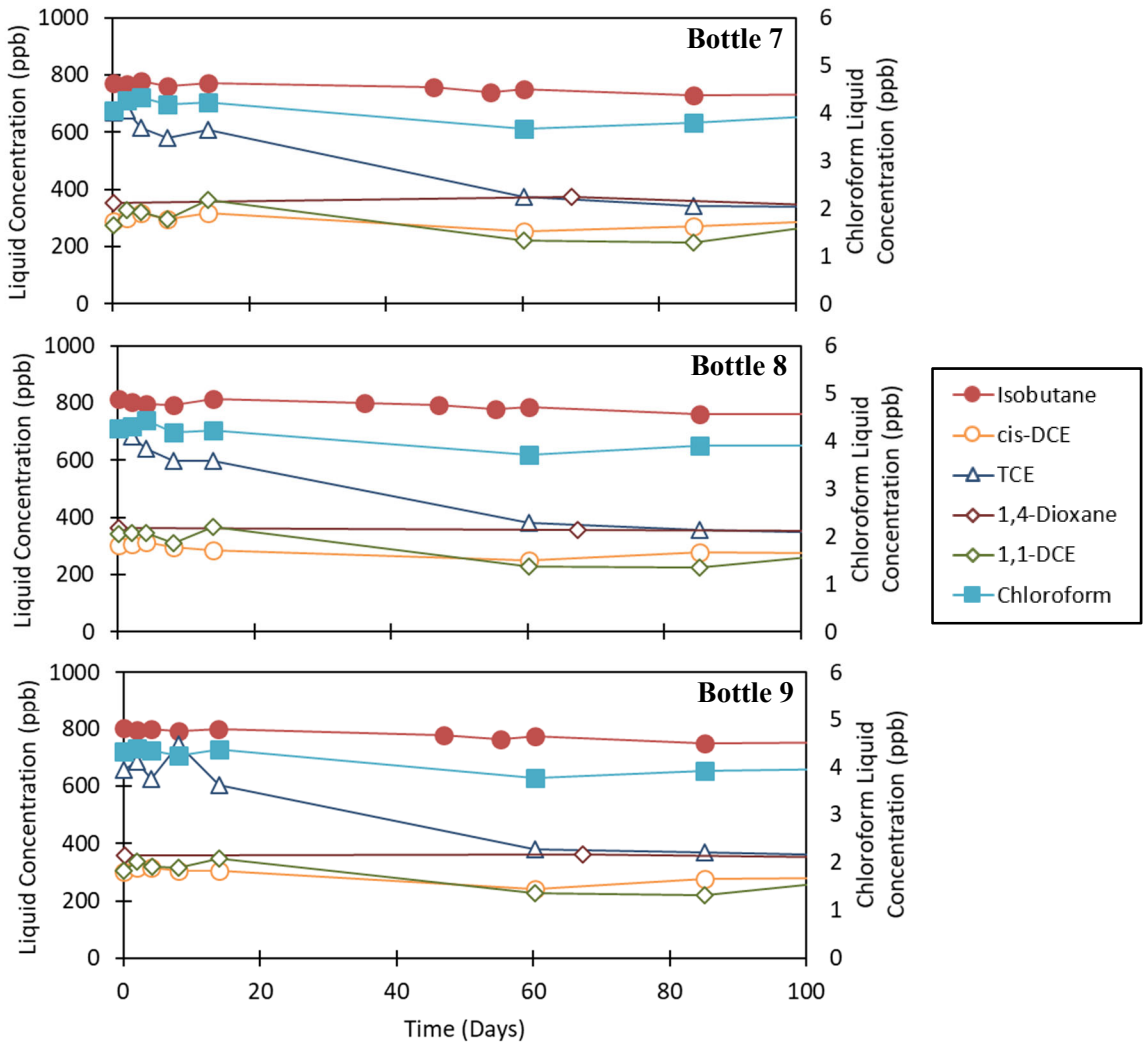

Figure 3. Aqueous Concentrations of Isobutane and COCs in Isobutane Microcosms. Microcosm bottles 7-9 in microcosm Set 9 showed no stimulation of native isobutane utilizing microorganisms over the course of more than 100 days.

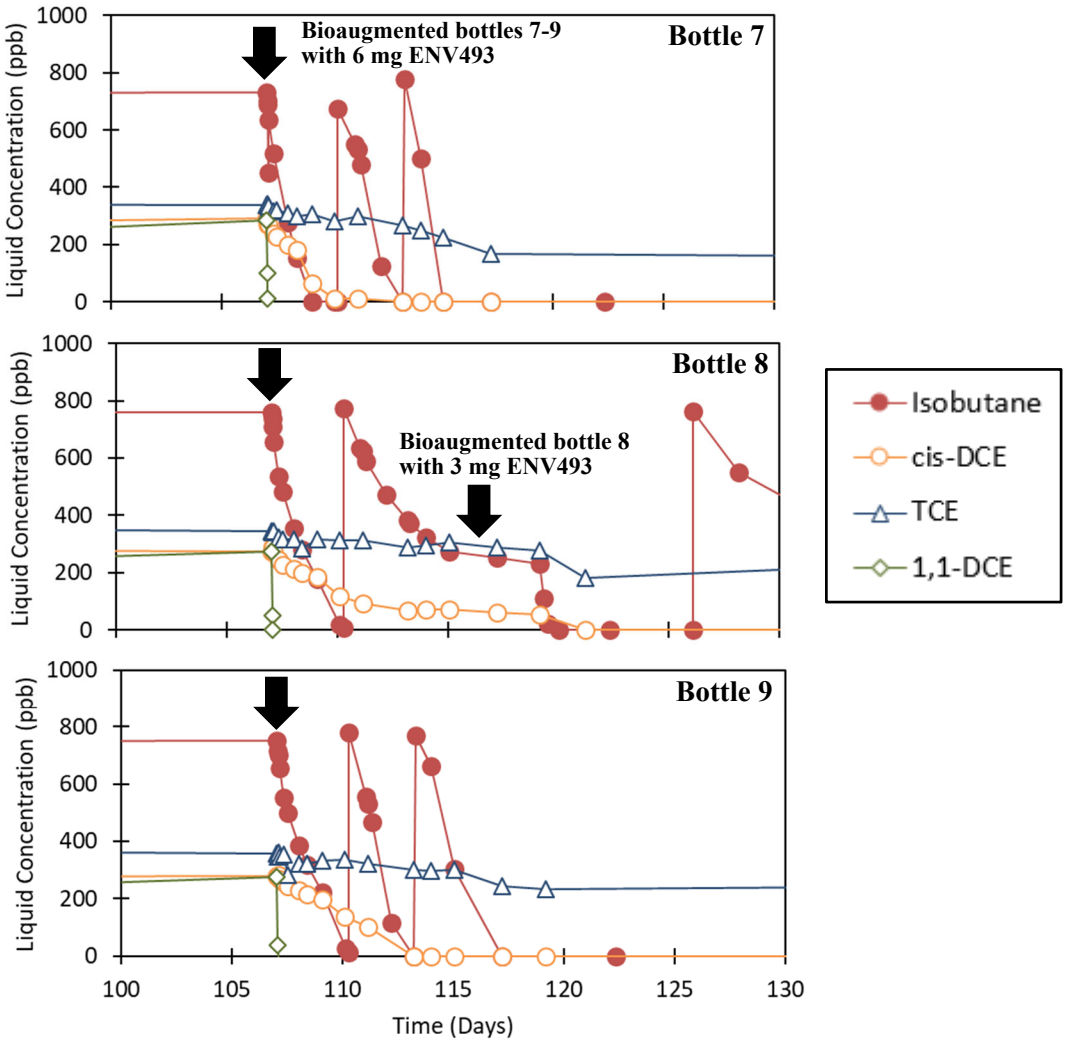

Figure 4. Chlorinated Ethene Transformation and Isobutane Utilization After Bioaugmentation with ENV493. Microcosms bottles 7-9 in Set 9 were bioaugmented with 6 mg of ENV493 on day 107. Microcosm bottle 8 was bioaugmented with an additional 3 mg of ENV493 on day 119.

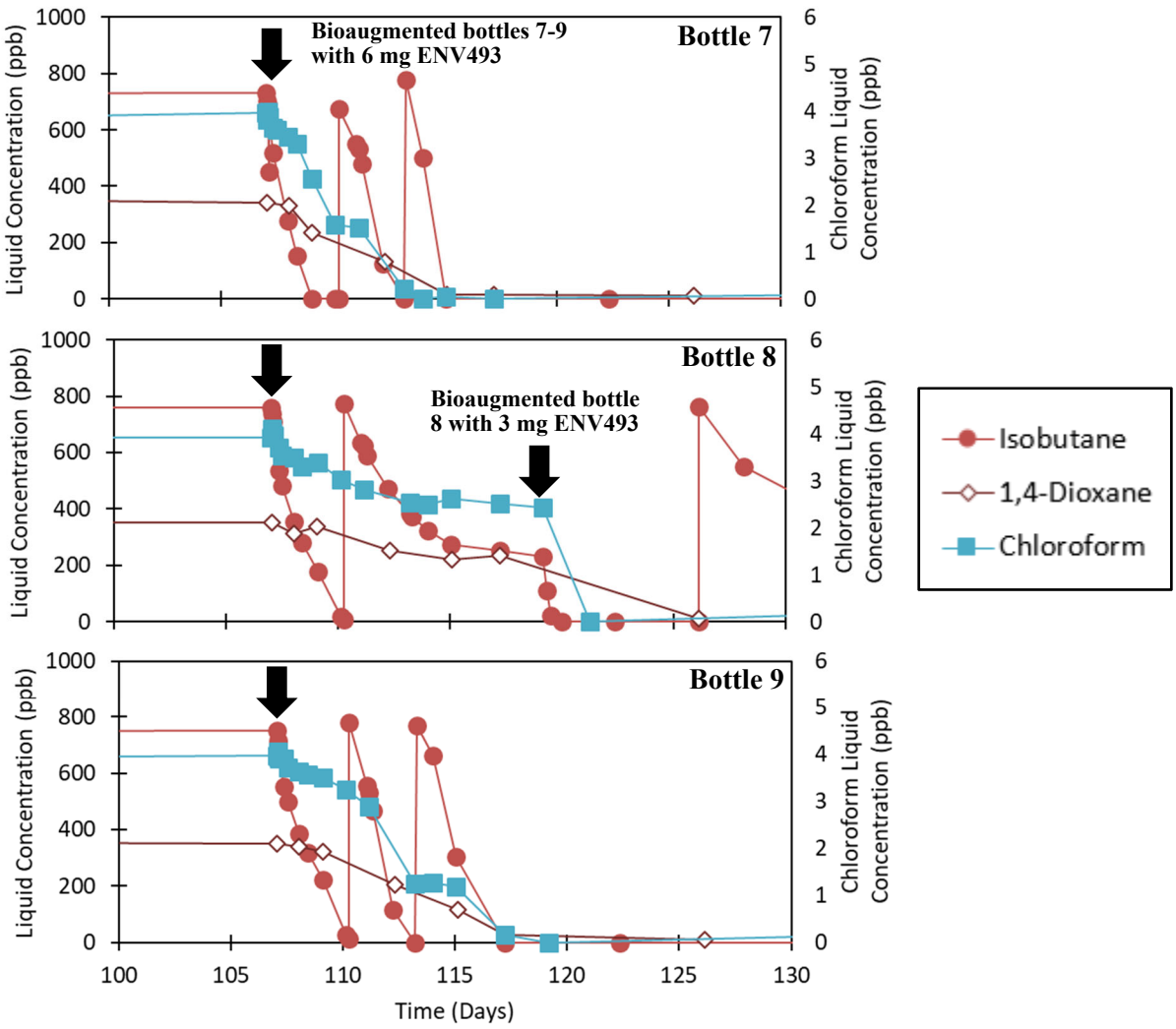

Figure 5. Chloroform and 1,4-D Transformation and Isobutane Utilization After Bioaugmentation with ENV493.

5.1.2 Bioaugmentation of ENV493-treated Microcosms with ELW1

As discussed in Section 5.1.1, after bioaugmentation with ENV493 and three additions of isobutane, minimal transformation of TCE was observed in bottles 7-9 in Set 9. On Day 132, isobutene was added to the headspace of these microcosms along with 12.8 mg of the ELW1 culture to enhance TCE biodegradation. As shown in **Figure 6,** TCE was rapidly transformed after uptake of the isobutene (note: variations in the isobutene data are due to analytical problems with GC-FID analysis). Uptake of isobutene significantly decreased with successive additions of isobutene, indicating reduced activity of the ELW1 culture. Additionally, the TCE concentration increased slightly in the microcosms after initial transformation by ELW1. This is most likely due to desorption of TCE from the aquifer material in the microcosms.

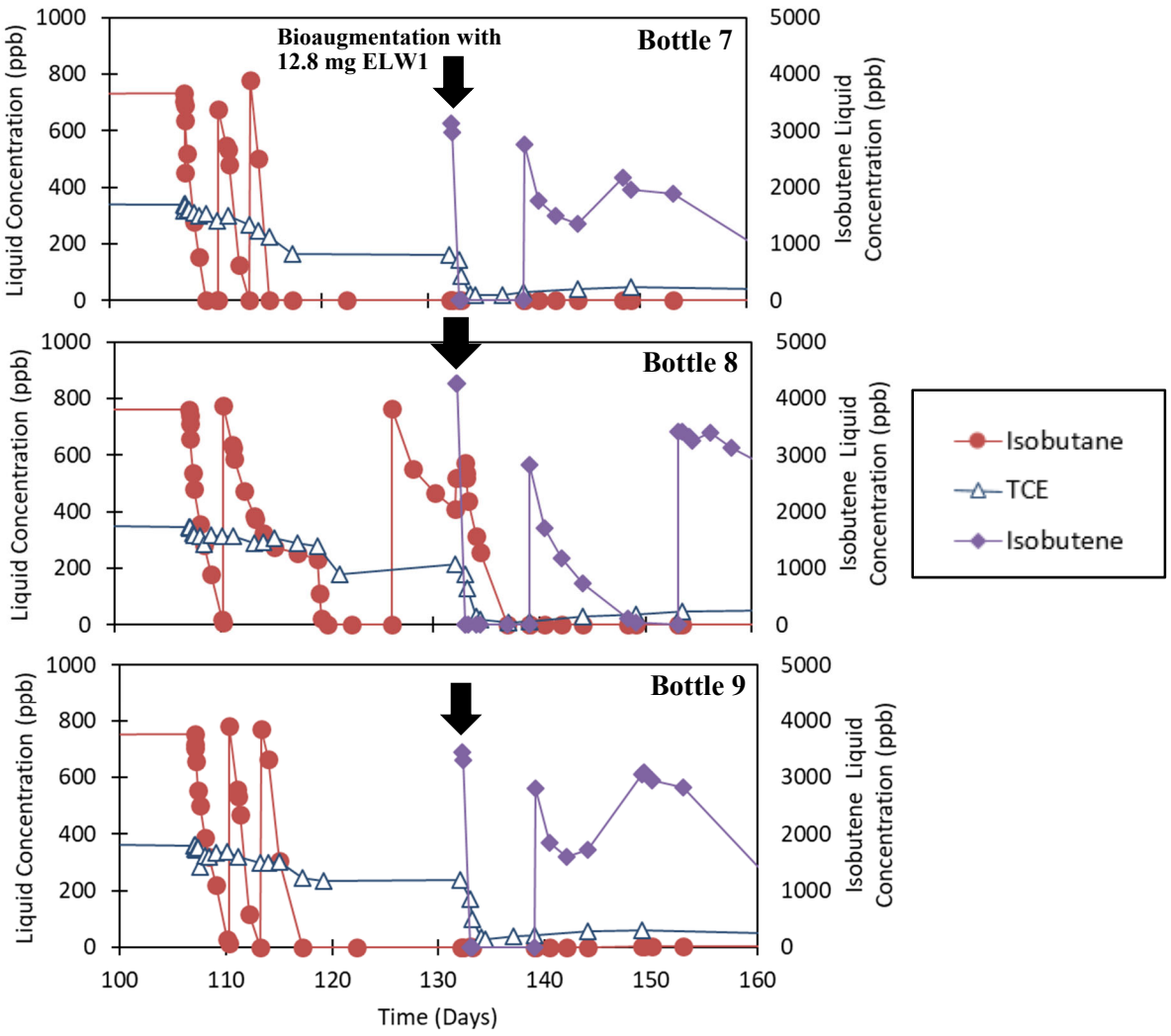

Figure 6. TCE Transformation After Bioaugmentation with ELW1. A total of 12.8 mg of ELW1 was added to bottles 7-9 in Set 9 on day 132 along with isobutene.

5.1.3 Bioaugmentation with 21198

A separate triplicate set of isobutane microcosms (Set 9: bottles 10, 11, and 12) were bioaugmented with 6 mg of the isobutane-utilizing 21198 culture after 70 days of incubation. The concentration of cells added was similar to that used for ENV493 (Section 5.1.1). Rapid biodegradation of isobutane was subsequently observed in all three bottles (**Figure 7**). 1,1-DCE was rapidly transformed over the course of several hours. While *cis*-DCE was significantly reduced, transformation stalled after a few days, with approximately 50 µg/L remaining in the microcosm bottles. Isobutane consumption stalled with the second addition in each of the bottles, and multiple bioaugmentations with 21198 were required to maintain isobutane uptake and for complete transformation of *cis*-DCE. As anticipated with this culture, there was no observable transformation of TCE. Transformation of 1,4-D and chloroform were achieved after the third bioaugmentation of 21198 (**Figure 8**).

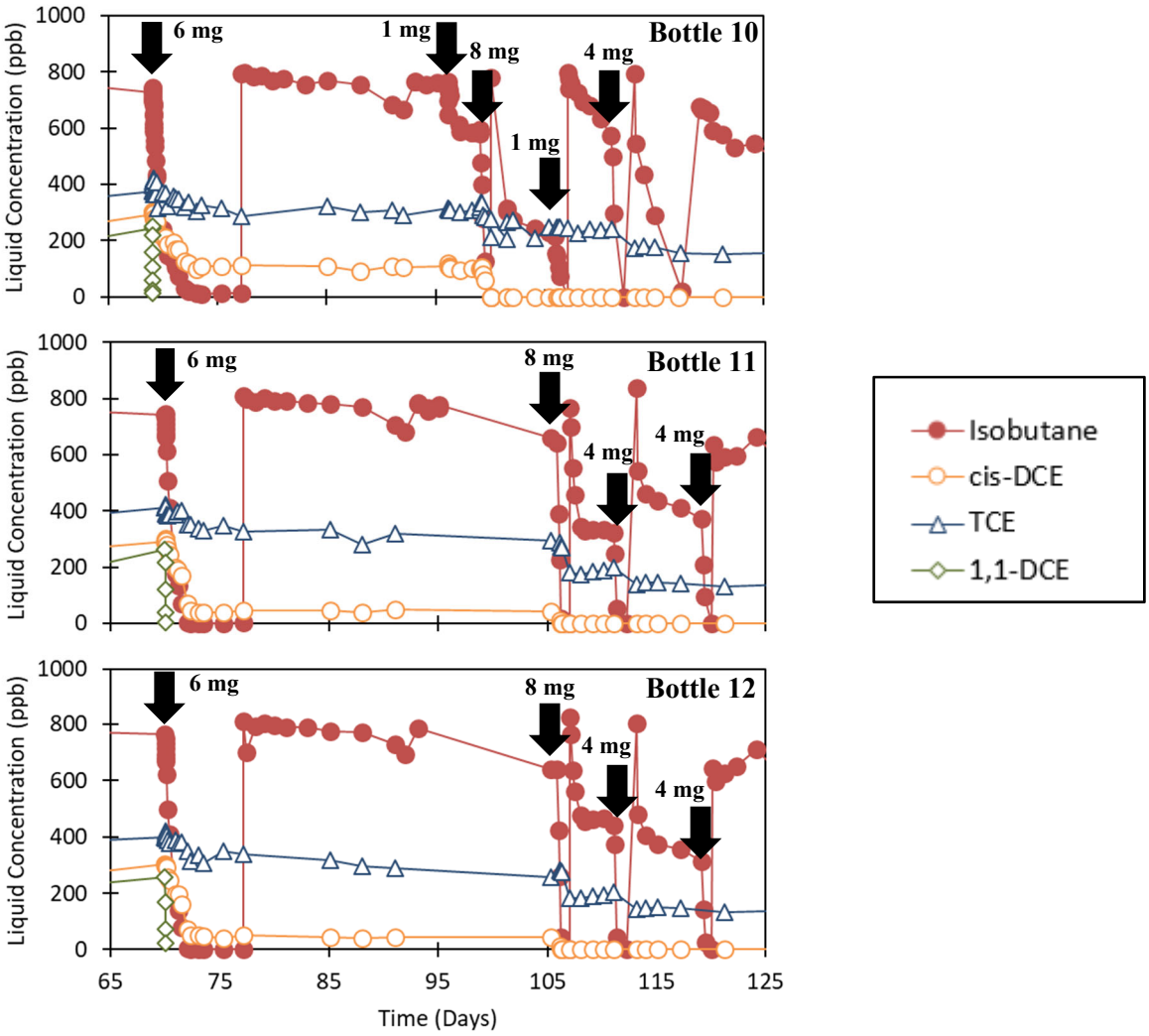

Figure 7. Chlorinated Ethene Transformation and Isobutane Utilization After Multiple Bioaugmentations with 21198.

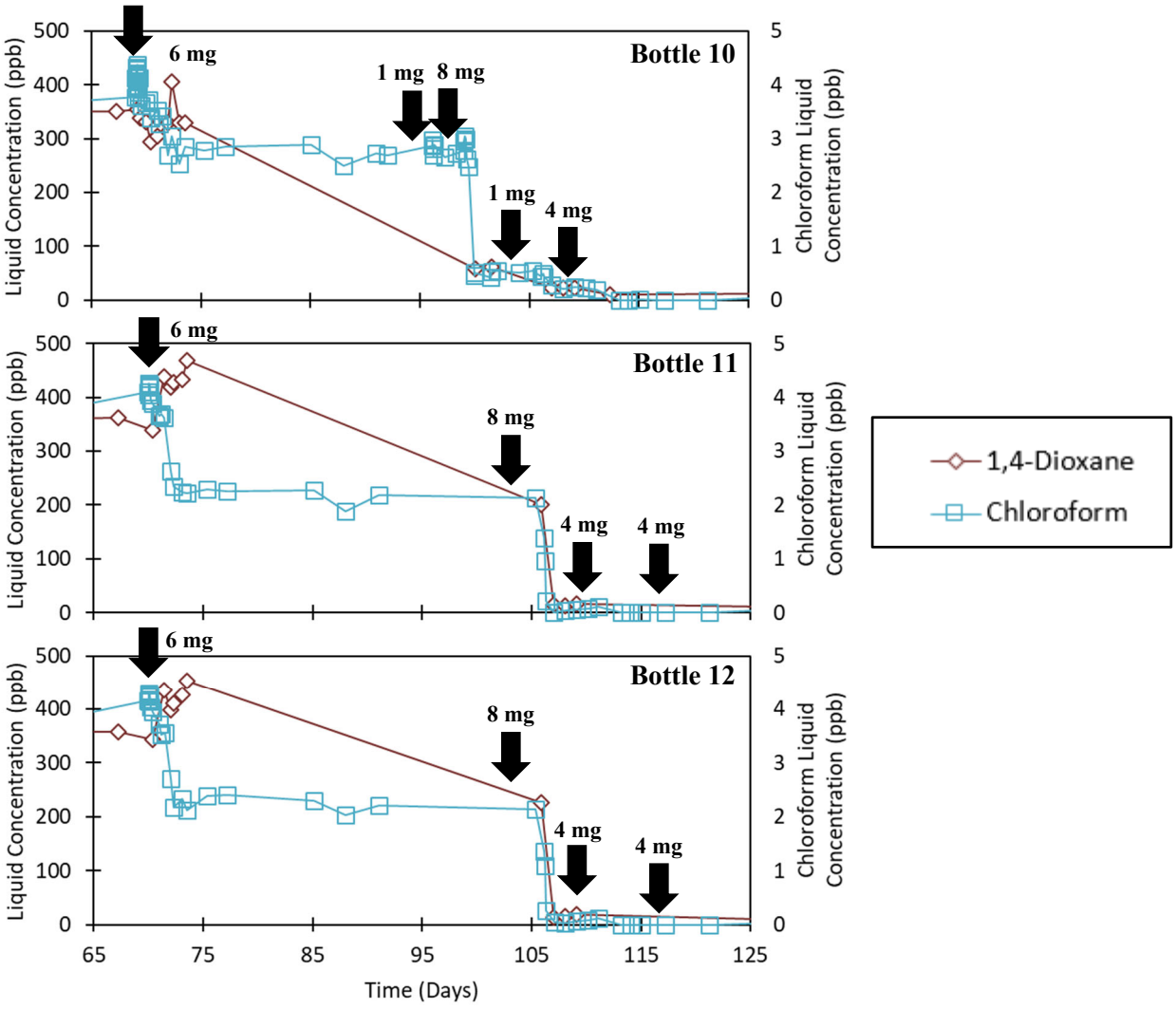

Figure 8. Chloroform and 1,4-D Transformation and Isobutane Utilization After Multiple Bioaugmentations with 21198.

5.1.4 Bioaugmentation of 21198-treated Microcosms with ELW1

As discussed in Section 5.1.3, after multiple bioaugmentations with 21198 and several additions of isobutane, minimal transformation of TCE was observed in microcosm bottles 10 through 12 of Set 9. On Day 132, Isobutene was added to the headspace of these microcosms along with 12.8 mg of the ELW1 culture to enhance TCE biodegradation. As shown by **Figure 6,** TCE was transformed from a concentration of ~150 µg/L to ~40-60 µg/L in the three microcosm bottles after uptake of the isobutene (note: variations in the isobutene data are due to analytical problems with GC-FID analysis). No further TCE transformation was observed. Uptake of isobutene stalled or significantly decreased with a second addition of isobutene, indicating significantly reduced activity of the ELW1 culture. TCE concentration increased slightly in the microcosms after initial transformation by ELW1. This is most likely due to desorption of TCE from the aquifer material within the microcosms.

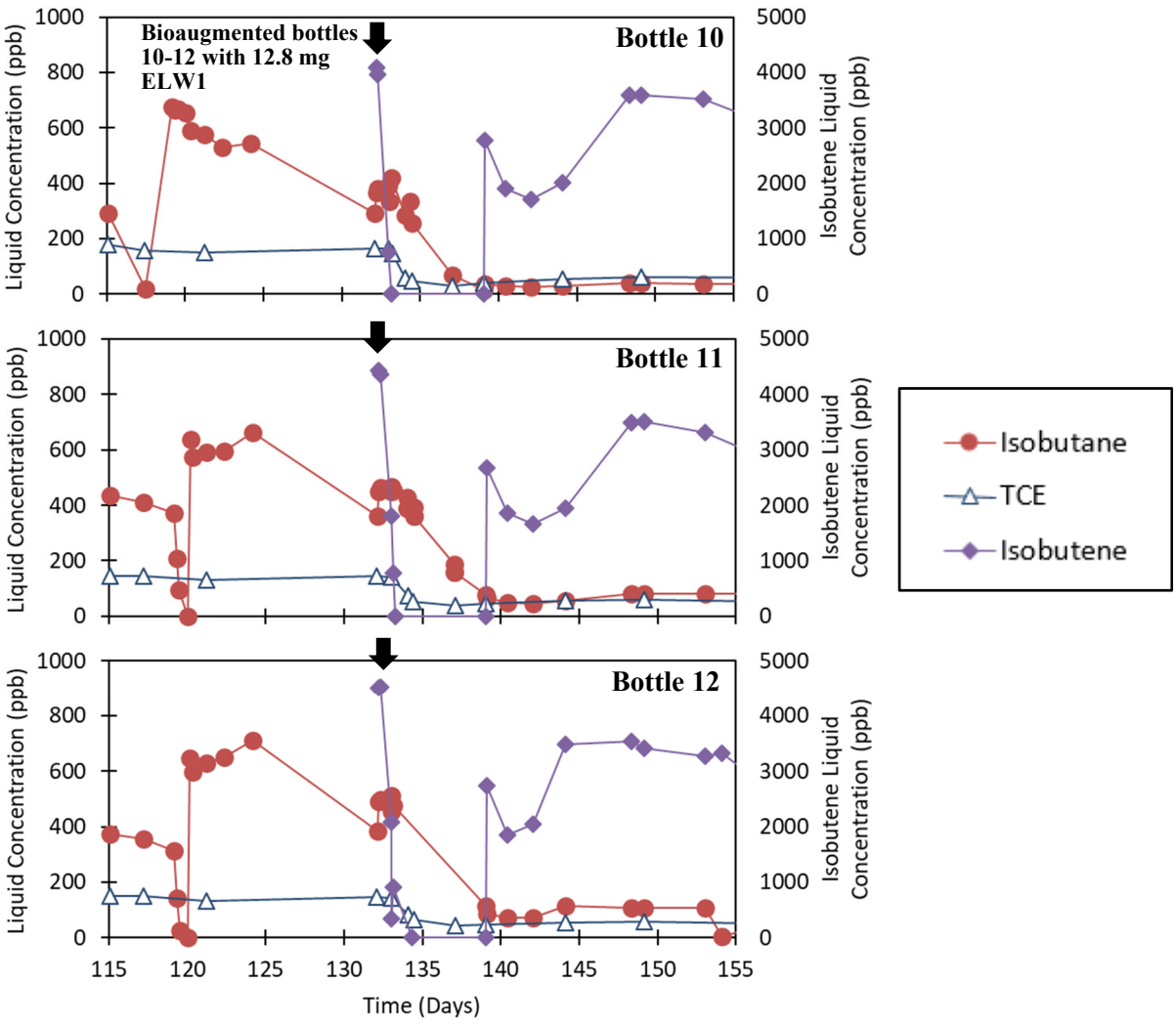

Figure 9. Partial TCE Transformation After Bioaugmentation with ELW1. 12.8 mg of ELW1 was added to bottles 10-12 in Set 9 on day 132 along with isobutene.

5.1.5 Bioaugmentation of ENV493-treated Bottles with ENV494M

Because the ELW1 culture did not completely degrade TCE in the Set 9 microcosms (**Figure 9**), even after further addition of isobutene, an additional microcosm study was conducted with ENV494M (the mixed methanotroph culture isolated from the site) to assess this cultures' ability for fully degrading TCE. Further information on the characteristics of the culture, which is native to NASNI is provided in **Appendix B**.

The additional microcosms (Set 12) were constructed to replicate initial ENV493 bioaugmentation results (**Figure 4** & **5**), and to test the ability of the ENV494M culture to treat TCE after the other COCs were removed by ENV493. Set 12 consisted of four microcosm bottles, two utilizing MV media as a source of inorganic nutrients (bottles 1-MV and 2-MV) and two using diammonium phosphate (DAP) for inorganic nutrients (bottles 3-DAP and 4-DAP). Unlike the previous study, there was no initial incubation period with isobutane to attempt stimulation of native

microorganisms. Rather, all four microcosms were bioaugmented with 6 mg of ENV493 immediately after microcosm construction.

As shown in **Figure 10**, the initial ENV493 bioaugmentation results (see **Figure 4**) were replicated. These results included rapid treatment of 1,1-DCE, with *cis*-DCE gradually decreasing in concentration, and successive utilization of isobutane. **Figure 11** shows that the decreases in 1,4-D and CF concentrations in the ENV493-bioaugmented microcosms were also similar to the initial Set 9 bioaugmented with ENV493 (see **Figure 5**), with both compounds completely degraded with repeated additions of isobutane. The COC degradation rate and isobutane uptake was similar for the two different nutrient types used.

As shown in **Figure 11**, slow transformation of TCE was observed after bioaugmentation with ENV493, as was the case with the earlier Set 9 microcosms (Section 5.1.1) and pure culture studies with ENV493 provided in **Appendix B**. TCE concentrations decreased from ~700 µg/L to ~200 µg/L over a period of 25 days with repeated additions and consumption of isobutane. All four bottles were bioaugmented with the ENV494M culture, and methane was added to the headspace on Day 25 to enhance removal of TCE. The residual TCE was treated rapidly over the course of one day after bioaugmentation, with a final TCE concentration of ~5 ppb measured in all four microcosms after addition of the mixed methanotrophs **(Figure 11**). Rapid utilization of methane (two additions) was also observed.

The data from this microcosm study (**Figures 10 - 12**) as well as enrichment and pure culture tests (**Appendix B**) indicate that the target COCs in the NASNI OU-11 area groundwater can be successfully treated to below project performance objective criteria by adding isobutane gas and bioaugmentation culture ENV493 (1,1-DCE, 1,4-D, *cis*-DCE and CF) as well as methane gas and ENV494M (TCE).

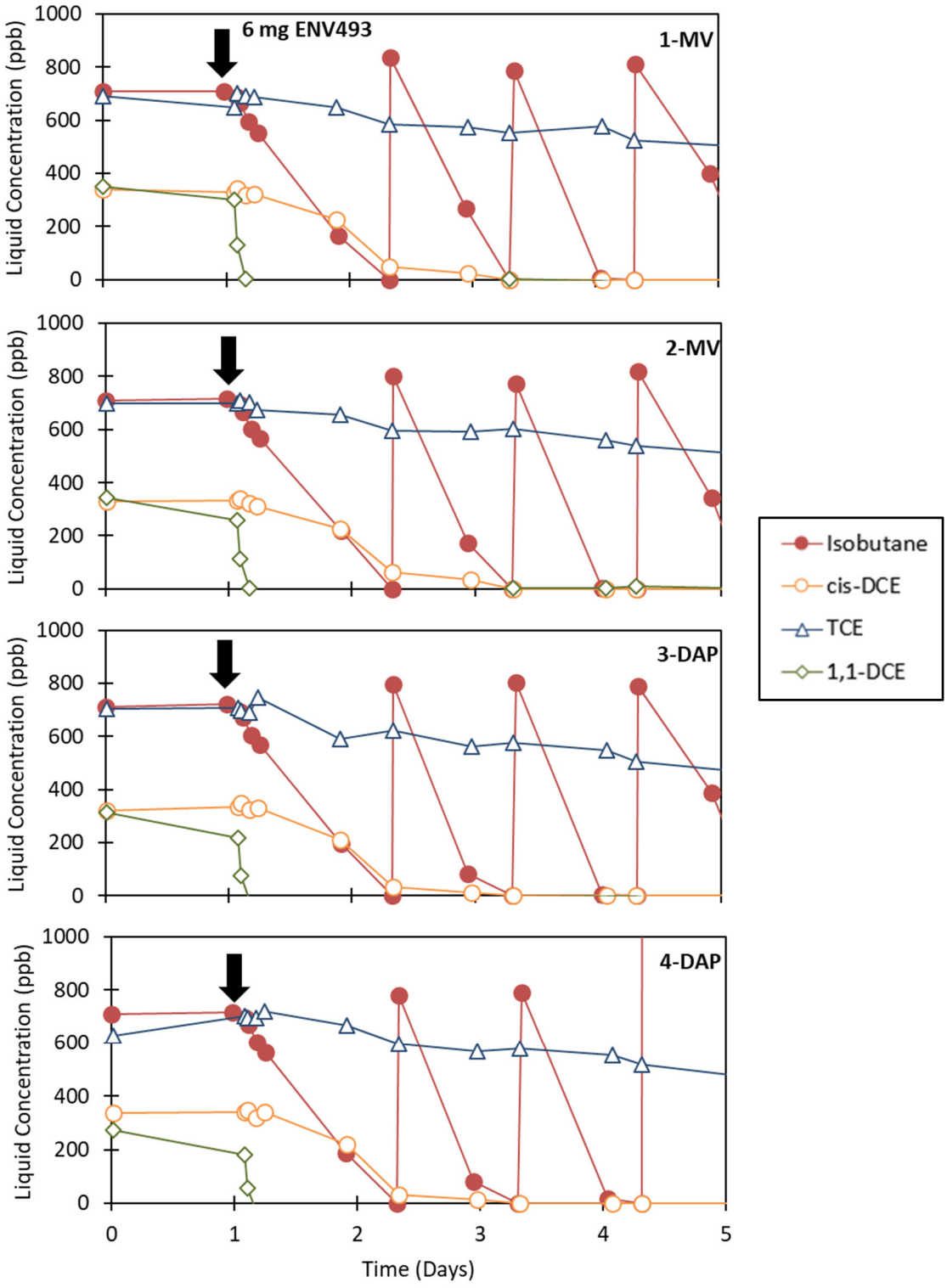

Figure 10. Chlorinated Ethene Transformation and Isobutane Utilization After Bioaugmentation with ENV493 in Microcosm Set 12. Microcosms 1 and 2 have MV for inorganic nutrients and Microcosms 3 and 4 have DAP as an inorganic nutrient source.

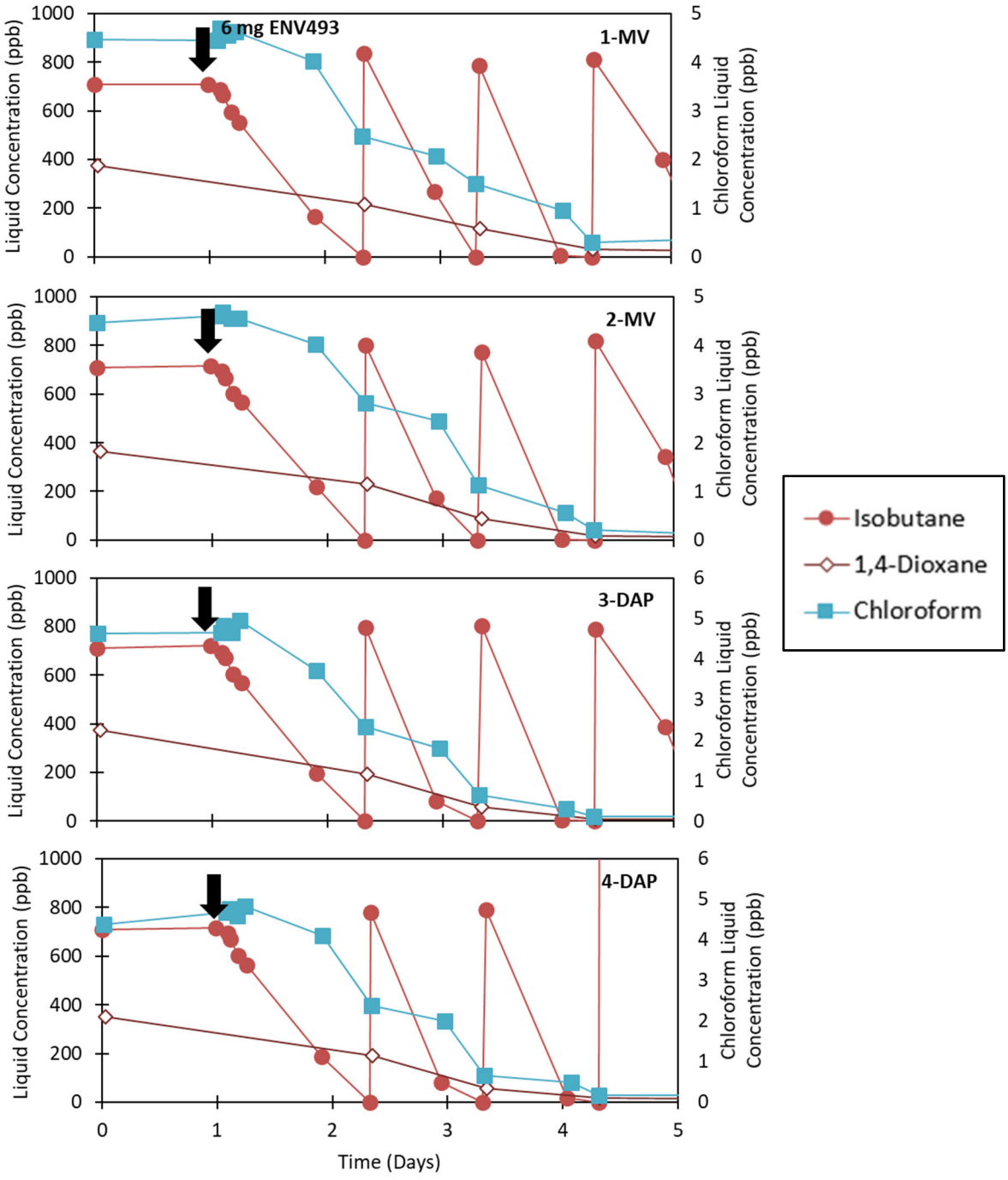

Figure 11. Chloroform and 1,4-D Transformation and Isobutane Utilization After Bioaugmentation with ENV493 in Microcosm Set 12.

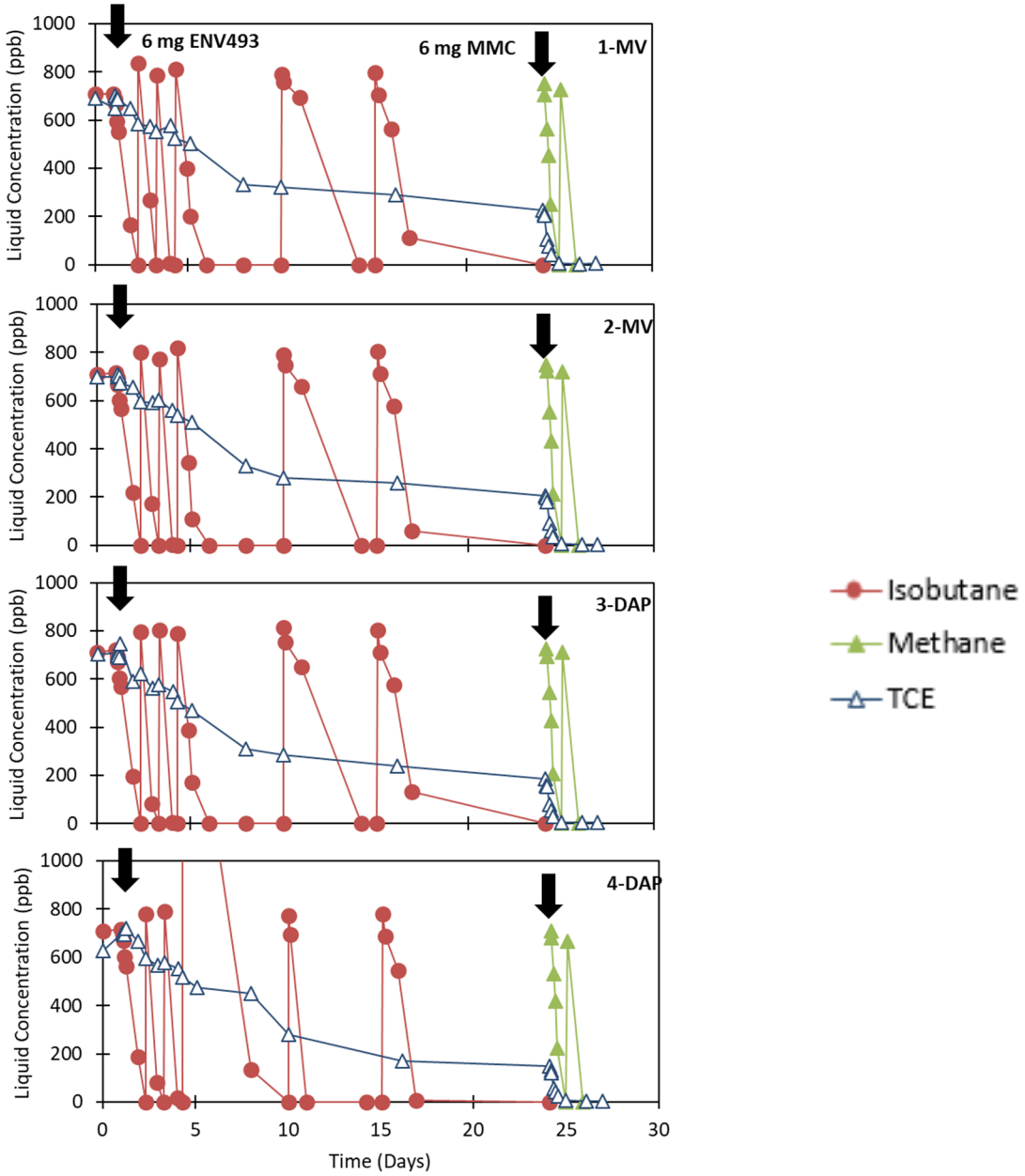

Figure 12. TCE Transformation After Bioaugmentation with ENV494M. Slow treatment of TCE was observed after bioaugmentation with ENV493 grown on isobutane. The remaining TCE was rapidly degraded after bioaugmentation with the mixed methanotroph culture ENV494M and addition of methane on day 25.

5.2 Isobutene Microcosms

Microcosm Set 10 was constructed to determine if native isobutene utilizing microorganisms could be stimulated in site soil and groundwater. As shown in **Figure 3**, there was no isobutene uptake, oxygen consumption, or carbon dioxide production over the course of more than 100 days.

Additionally, no significant changes in relevant COCs were observed over the course of the study (**Figure 19** and **Figure 20** in **Appendix A**). Results of this study indicated that stimulation of native isobutene utilizers did not occur, potentially due to 1,1-DCE epoxide formation, as was the case with the isobutane microcosms. Because of the positive results from the microcosm studies employing isobutane and bioaugmentation, we did not conduct additional studies with isobutene as a primary substrate.

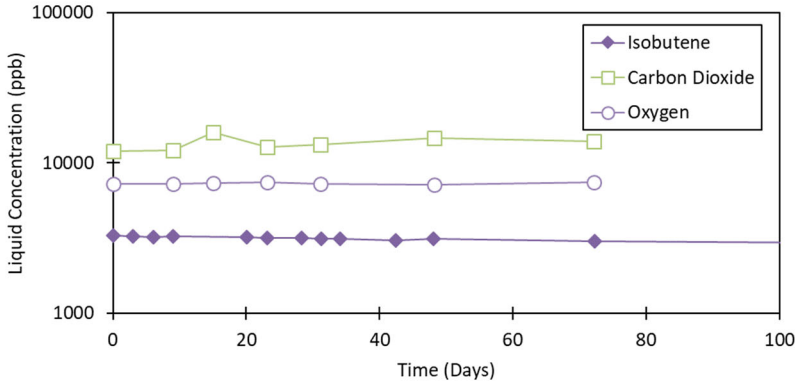

Figure 13. Aqueous Concentrations of Isobutene, Oxygen and Carbon Dioxide in Isobutene Microcosms. Microcosm Set 10 showed no stimulation of native isobutene utilizing microorganisms over the course of more than 100 days.

5.3 Propane Microcosms

Microcosm Set 11 was constructed to determine if native propane utilizing microorganisms could be stimulated in site soil and groundwater. As shown in **Figure 14**, there was no propane uptake, oxygen consumption, or carbon dioxide production over the course of more than 100 days. Additionally, no significant changes in relevant COCs were observed over the course of the study (**Figure 21** in **Appendix A**). Results of this study indicated that stimulation of native propane utilizers did not occur, potentially due to 1,1-DCE epoxide formation, as was the case with the isobutane microcosms. Because of the positive results from the microcosm studies employing isobutane and bioaugmentation, we did not conduct additional studies with propane as a primary substrate.

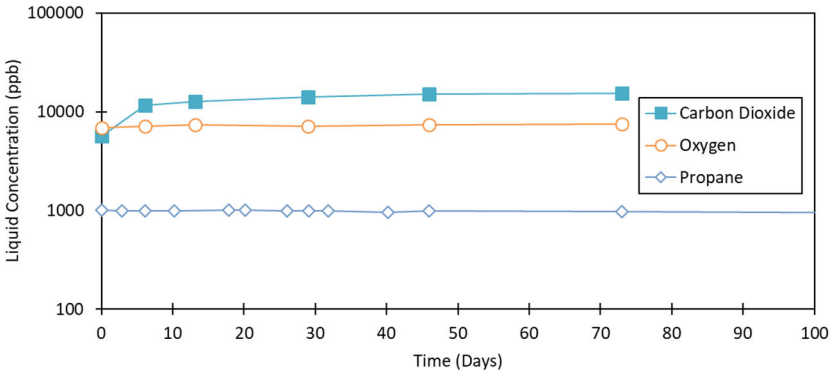

Figure 14. Aqueous Concentrations of Propane, Oxygen and Carbon Dioxide in Propane Microcosms. Microcosm Set 11 showed no stimulation of native propane utilizing microorganisms over the course of more than 100 days.

5.4 Final 1,4-D Concentration in Treated Microcosms

Aqueous samples for 1,4-D analysis were collected from microcosms bottles 7-10 of Set 9 on day 161 of the study (**Figures 4-6**). Two 1 mL samples were collected from each microcosm bottle, and one sample from each bottle was spiked with 0.35 µg/L of 1,4-D. All samples were analyzed via GC/MS using a heated purge and trap system to remove the CVOCs from solution and introduce them to the GC/MS. The purpose of this experiment was to determine if 1,4-D could be degraded cometabolically to below 0.35 µg/L (which is the drinking water concentration representing a 1 x 10^{-6} increased cancer risk level based on USEPA evaluation; USEPA 2014) , and that it could be quantified accurately at very low levels using this analytical method.

Results of the analysis are shown in **Figure 15**. Final 1,4-D concentrations in the four un-spiked microcosm bottles ranged from 0.06 to 0.66 µg/L, with 3 of the four bottles being well below 0.35 µg/L. Concentrations in the spiked bottles ranged from 0.53 to 1.08 µg/L. The amount of spike recovered (e.g., the difference between 1,4-D concentrations in the spiked bottles and the un-spiked bottles) ranged from 0.35 to 0.43 µg/L, with an average spike recovery of 0.40 µg/L. This mass balance analysis of the standard addition shows the low 1,4-D concentrations were effectively measured, and the results of this experiment show that 1,4-D can be treated by aerobic cometabolism to below a level of 0.35 µg/L by ENV493. We should note that the performance objectives of this study do not require in situ 1,4-D treatment to below 0.35 µg/L, but the microcosm data do indicate that under laboratory conditions, this is achievable via cometabolism.

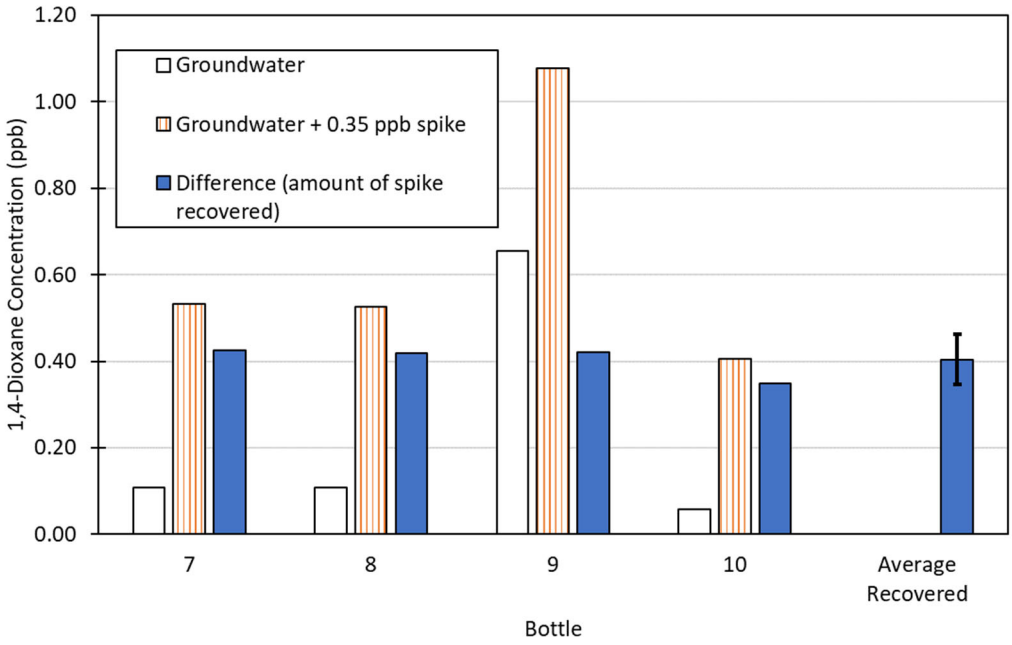

Figure 15. Standard Addition of 0.35 µg/L 1,4-D into a 1.0 mL Liquid Injection Sample Using Purge and trap GC/MS. Error bar is the distribution based 95% confidence interval on the mean of the amount of spike recovered.

6.0 TREATABILITY STUDY SUMMARY AND CONCLUSIONS

The pure cultures ELW1 and 21198 were effective at cometabolizing the COC mixture present at the site but they did not have the ability to utilize multiple additions of primary substrate in the aquifer material matrix. The ability to continuously transform the primary substrate would be required in the field demonstration. The cultures ENV493 and ENV494M, which were isolated from the site, were effective at cometabolizing the COC mixture present at the site and showed the ability to transform multiple additions of primary substrate and survive under site conditions. The results of the treatability study indicate that the most successful approach to treat the entire mixture of COCs at NANSI OU-11 is to bioaugment with ENV493 with isobutane as a primary substrate to treat 1,4-D, 1,1-DCE, *cis*-DCE, and CF, and to bioaugment with ENV494M with methane as a primary substrate to treat residual TCE. **Table 4** gives a summary of the microcosm study conclusions. It should be noted that a recent study conducted by APTIM showed that it may be possible to add both cultures simultaneously in the field. All relevant COCs were degraded when the cultures were added in sequence (ENV493 then ENV494M) or together (**Appendix B**). Additional column studies are ongoing at OSU that will provide optimization data for the field process.

Table 4. Qualitative results of microcosm studies.

			Treates COC				
Culture	**Primary Substrate**	**Survives in Groundwater**	**1,1-DCE**	**cis-DCE**	**Chloroform**	**1,4-Dioxane**	**TCE**
ENV493	Isobutane	Yes, uptakes multiple spikes of substrate	Y	Y	Y	Y	N
ENV494M	Methane	Yes, uptakes multiple spikes of sustrate	--	--	--	--	Y
ELW1	Isobutene	Yes*, uptakes multiple spikes of substrate in low COC GW	Y	Pure culture evidence of transformation	--	N	Observed Treatment from 150 to 50 ppb
21198	Isobutane	Yes*, uptakes multiple spikes of substrate in low COC GW	Y	Y	Y	Y	N

Yes(Y): treatment is to below detection for 1,1-DCE, *cis*-DCE, and chloroform and to below 1 ppb for 1,4-D.

No(N), No treatment

Yes*: In the MS thesis by Krippaehne (2018), utilization of multiple additions of substrate by both ELW1 and 21198 was observed in the lower concentration groundwater with suspended sediment. This data is shown in Appendix D and discussed above in the Microcosm Sets 1-8 Discussion.

7.0 GO/NO GO DECISION

Completion of treatability testing marks a Go/No Go decision point for this project. The data presented in this treatability study report indicate that all relevant COCs at the NASNI OU-11 site can be biodegraded to below project performance objective criteria using an approach that entails addition of two primary substrates (isobutane and methane) and two bioaugmentation cultures (ENV493 and ENV494M). These cultures can be grown in the required quantities for field application in APTIM's commercial fermentation facility (Lawrenceville, NJ). As a result, our project team recommends a GO decision for field implementation of this technology art NASNI.

While the original remedial approach for this project included the use of biosparging to deliver multiple gaseous cometabolic substrates to the aquifer to stimulate indigenous bacteria capable of degrading comingled 1,4-D and CVOCs, the treatability testing results indicate that native organisms could not be stimulated in the microcosms over an incubation period of more than 3 months. Additional testing suggested that the presence and/or transformation of 1,1-DCE is inhibitory to the stimulation of native microbes that biodegrade various cometabolic gases (i.e., isobutane, isobutene, methane and propane). This is likely due to the formation of a toxic epoxide resulting from the initial transformation of 1,1-DCE. The revised approach will involve recirculation of groundwater, with gaseous amendments added to the groundwater prior to reinjection, and bioaugmentation with two cultures enriched from site groundwater.

The primary reasons for the change in remedial design are; 1) the need to distribute the bioaugmentation culture required to address the high concentrations of 1,1-DCE that is likely to inhibit growth of indigenous bacteria to densities required, and 2) challenges associated with obtaining electrical service in the demonstration area. The use of groundwater recirculation will allow for effective distribution of the gaseous amendments as well as the bioaugmentation culture. Furthermore, groundwater recirculation (~8-10 hours per day) and gaseous amendment delivery can be effectively implemented using off-the-grid solar power. The application of groundwater recirculation with the addition of cometabolic gases to the reinjected groundwater has recently been shown to be effective at treating 1,4-D (Chu et al., 2018) and EDB (Hatzinger et all., 2018).

8.0 PRELIMINARY FIELD DEMONSTRATION DESIGN

Discrete groundwater sampling, high resolution profiling and continuous soil core data collected during site characterization activities, as well as existing site data, were used to create the conceptual site model presented in **Figure 16**. The model indicates that the majority of COCs reside within the lower 10 feet (30-40 feet below ground surface) of an unconfined aquifer in the demonstration area. The aquifer is underlain by a low permeability silt unit that acts as a confining or semi-confining layer between the shallow aquifer and deeper aquifer units. A series of stepped pump tests were performed using one pumping well and four monitoring wells installed during site characterization activities, as well as two existing monitoring wells.

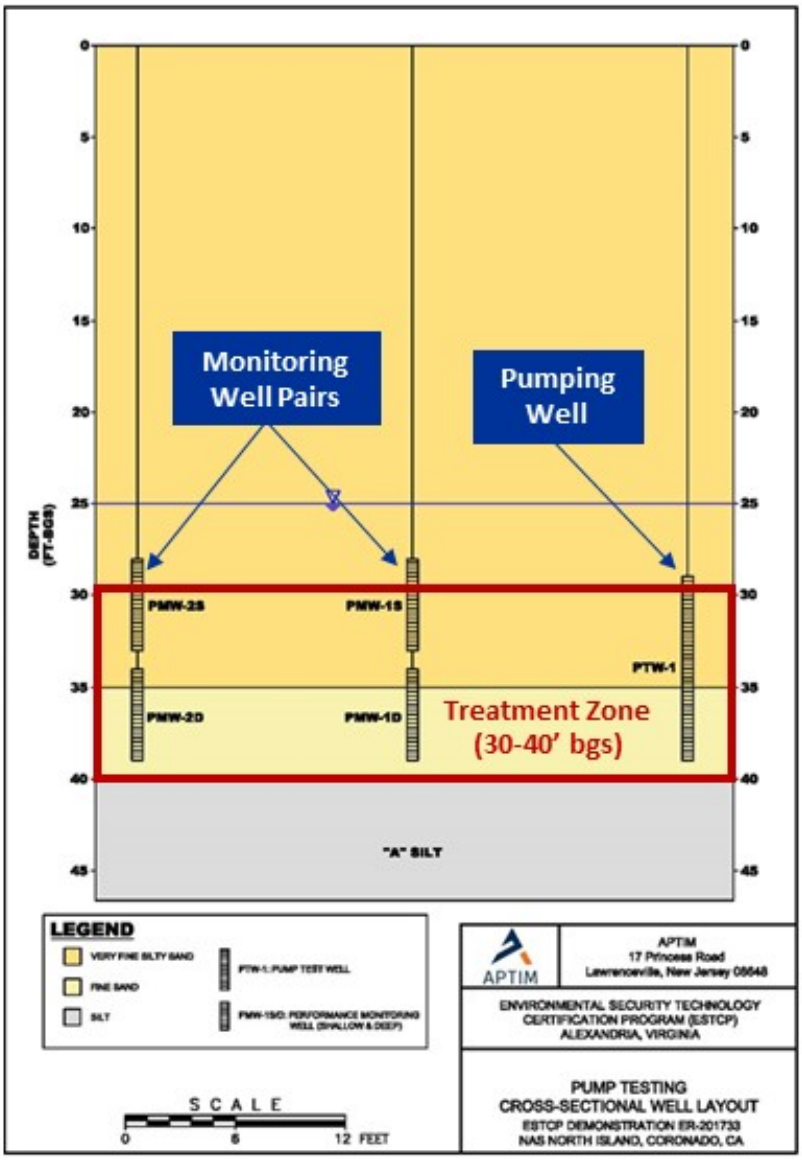

Figure 16. Conceptual Site Model and Pump Testing Cross-Sectional Well Layout.

The site characterization data and hydrogeologic data collected during pump testing were used to construct a numerical 3-dimensional groundwater model (MODFLOW) of the demonstration area. The model was used to simulate groundwater recirculation (extraction and reinjection) using different well configurations and pumping rates. Results of the simulations indicate that the extraction of groundwater from a central extraction well, and reinjection into 5 surrounding injection wells (located ~15 feet from the extraction well and equidistant from each other) would create a groundwater treatment cell that would largely isolate the treatment zone from the surrounding aquifer (**Figure 17**). While the model showed that a small portion of groundwater from outside the treatment cell will be captured by the extraction well, most of the groundwater reaching the extraction well will originate from within the treatment cell and undergo recirculation. Extraction rates are anticipated to be between 5 and 10 gpm at the extraction well, and evenly distributed to each of the five injection wells.

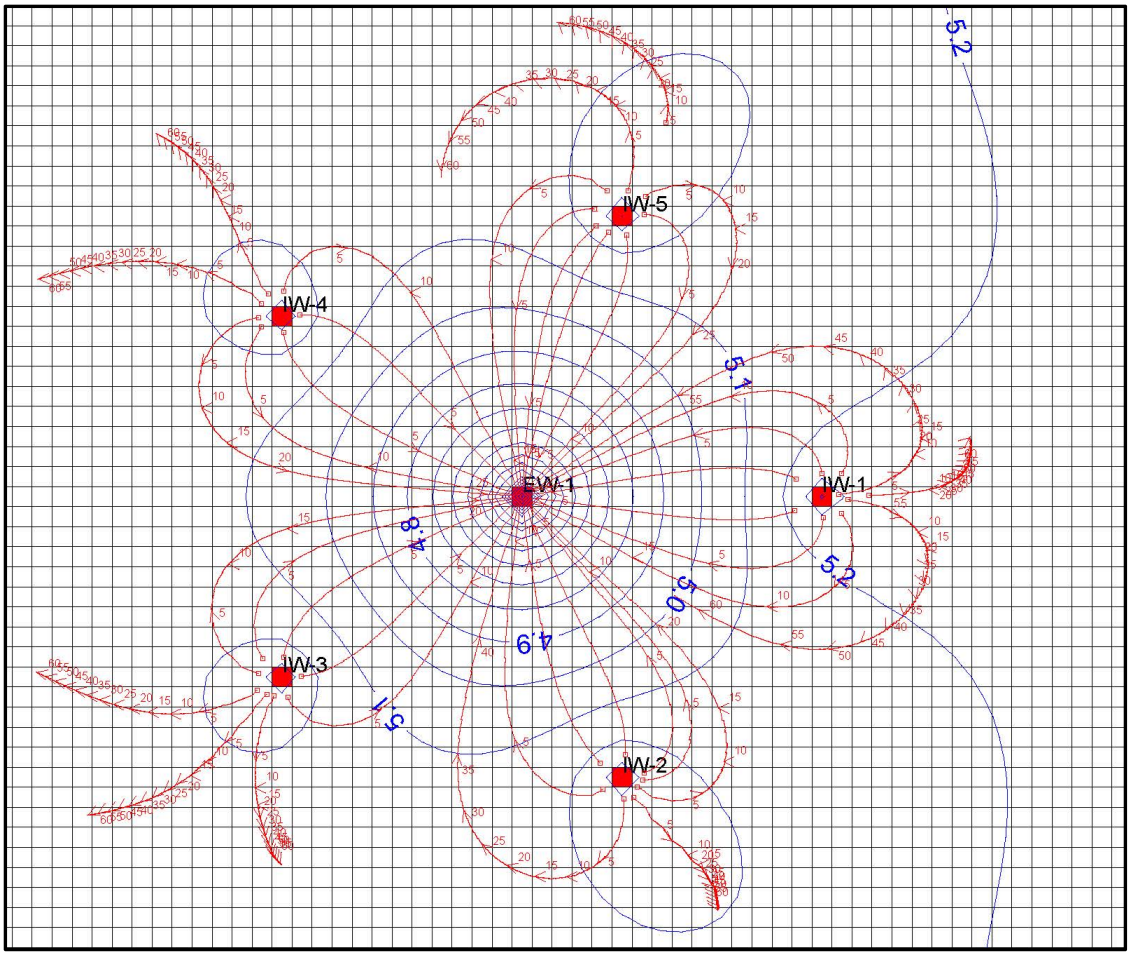

Figure 17. Groundwater Model Simulation of Groundwater the Recirculation Cell. Particle tracking shows the estimated flow path and travel times of reinjected groundwater during recirculation at 7 gpm for 8 hours per day over 60 days.

During groundwater recirculation, gaseous amendments (oxygen and alkane/alkene gases) will be dissolved into the extracted groundwater at the surface (as part of an amendment delivery system) prior to reinjection. Inorganic nutrients (nitrogen and phosphorous) and bioaugmentation cultures will also be added in batch. A general Process Flow Diagram of the groundwater recirculation and amendment addition system is presented in **Figure 18**. The system will be constructed off-site within a small shipping container (Conex box) or trailer, and delivered to the site during demonstration installation activities. Because access to electrical service at the demonstration site has proven to be a major issue, the groundwater recirculation pumps and system process control and monitoring equipment will be powered by an off-the-grid solar energy system.

During operation, pure oxygen, isobutane and methane will be added to the recirculated groundwater as needed to provide dissolved oxygen (DO) and substrate to maintain target dissolved gas levels within the treatment zone. Cylinders of oxygen, isobutane and methane gases will be stored on site in cages. The cylinders will be "ganged" together and equipped with regulators as needed to provide the appropriate delivery pressures and flows. Mass flow controllers/meters will be used to control the gas flow rates, and solenoid valves will be used to direct the gases to nozzles that inject the gases into the recirculated groundwater prior to downstream mixing and reinjection at the five injection wells. A similar design was recently used successfully by Aptims's biotechnology group to provide ethane gas, oxygen and nutrients to recirculating groundwater for in situ treatment of EDB (Hatzinger et al., 2018). The final groundwater recirculation and amendment delivery system design and operating parameters will be detailed in the Demonstration Work Plan.

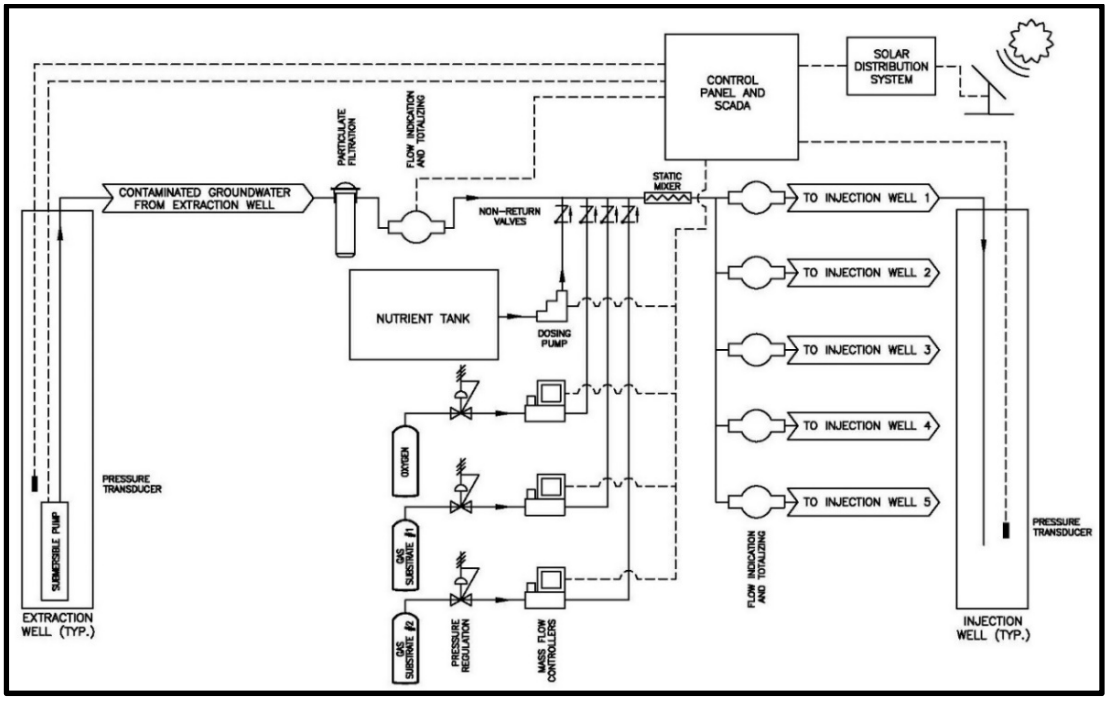

Figure 18. General Process Flow Diagram of the Groundwater Recirculation and Amendment Addition System.

Performance groundwater monitoring wells will be installed within, upgradient and downgradient of the treatment cell, as shown in **Figure 19**. Most of the monitoring wells (which include wells installed during site characterization activities) will be installed in pairs, with discrete 5-foot screen intervals located within the upper and lower portions of the approximately 10-foot thick treatment zone (~30-40 feet below ground surface), respectively. The conceptual demonstration well layout provide in **Figure 19** includes one upgradient "background" monitoring well, seven monitoring wells within the treatment cell, and two monitoring wells located downgradient of the treatment cell. In addition to monitoring for target COCs (i.e., 1,4-D and CVOCs), the wells will be sampled regularly for dissolved oxygen, pH, inorganic nutrients (nitrate, ammonia, phosphate) and dissolved cometabolic gases which will be monitored and optimized to promote the survival and growth of the bioaugmentation cultures. Groundwater samples will also be collected for microbial analysis, including quantification of target cometabolic organisms/genes/enzymes by quantitative polymerase chain reaction (qPCR) by North Carolina State University (NCSU). Additionally, analysis by NCSU using proteomic methods will focus on the use of activity-based protein profiling (ABPP), which can simultaneously identify multiple types of active monooxygenases in environmental samples. These microbial analysis will provide data on the distribution, quantification and activity levels of bioaugmented and indigenous bacteria. System operation, including groundwater recirculation and gas addition rates will be optimized throughout the demonstration based on these groundwater data. The final groundwater sampling plan and schedule will be detailed in the Demonstration Work Plan.

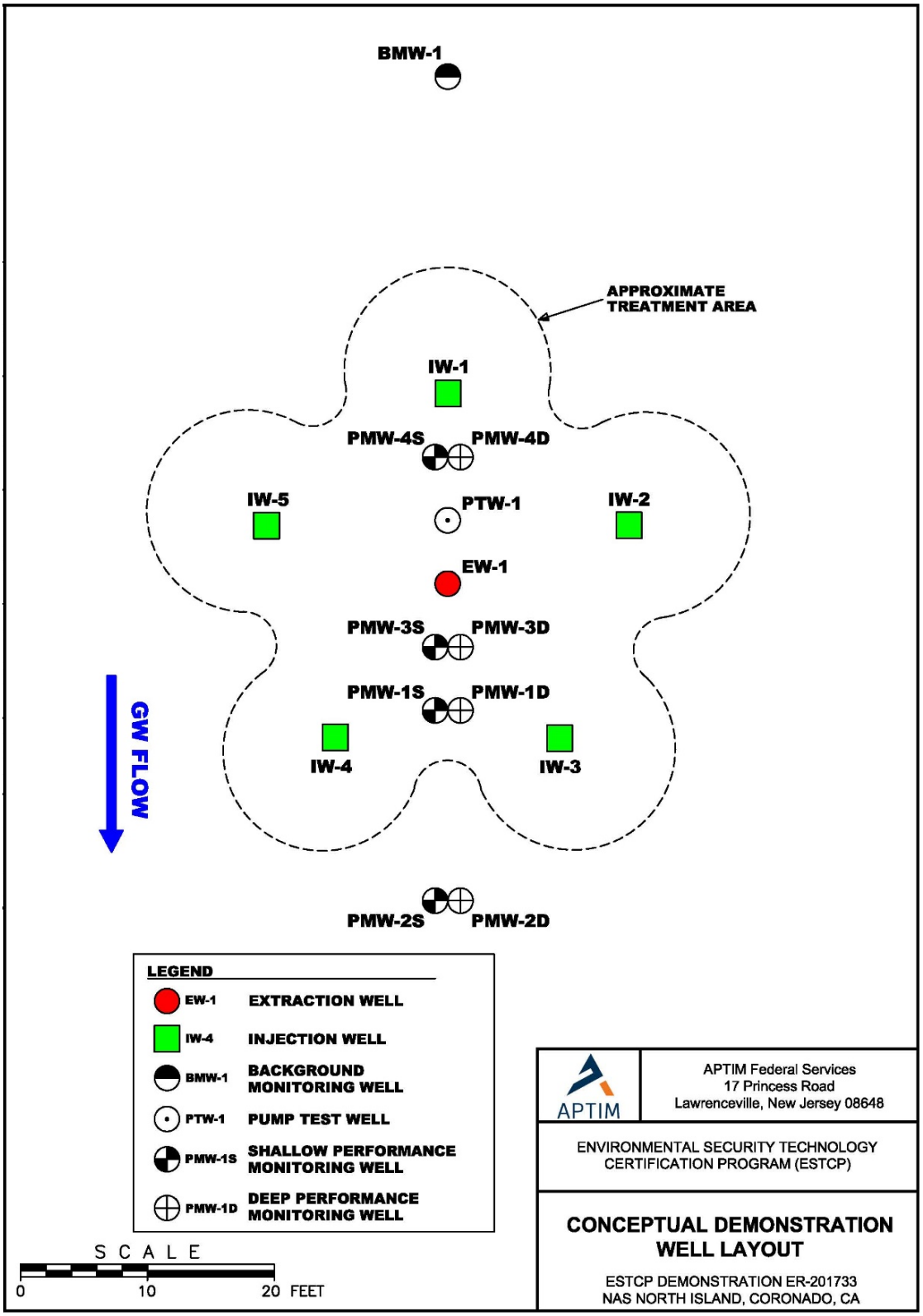

Figure 19. Conceptual Demonstration Well Layout.

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APPENDIX A

ADDITIONAL MICROCOSM INFORMATION AND DATA

Appendix A: Additional Microcosm Information and Data

Set 9 (Isobutane→Isobutene) Supplemental Data

Azide Poisoned Controls

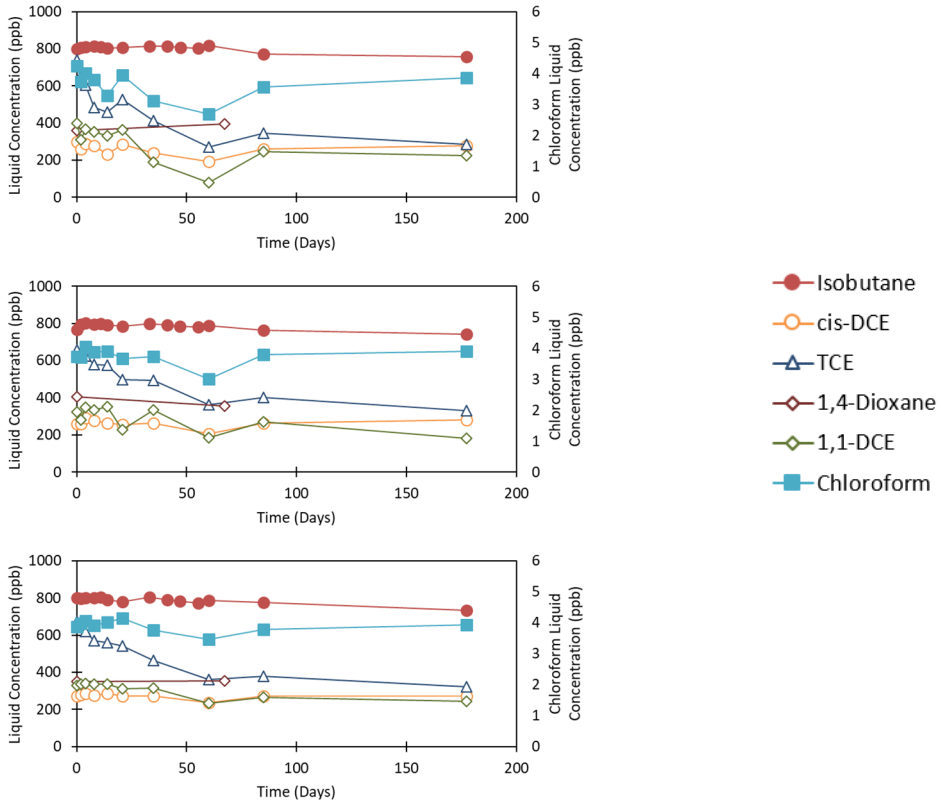

Figure 16. Set 9 poison controls. 50 mM sodium azide was added to inhibit all microbial activity and prevent biotic treatment of COCs. Over the course of 180 days, there was no uptake of isobutane or transformation of COCs. TCE did decrease over time, this was most likely due to sorption to the core material.

Acetylene Controls

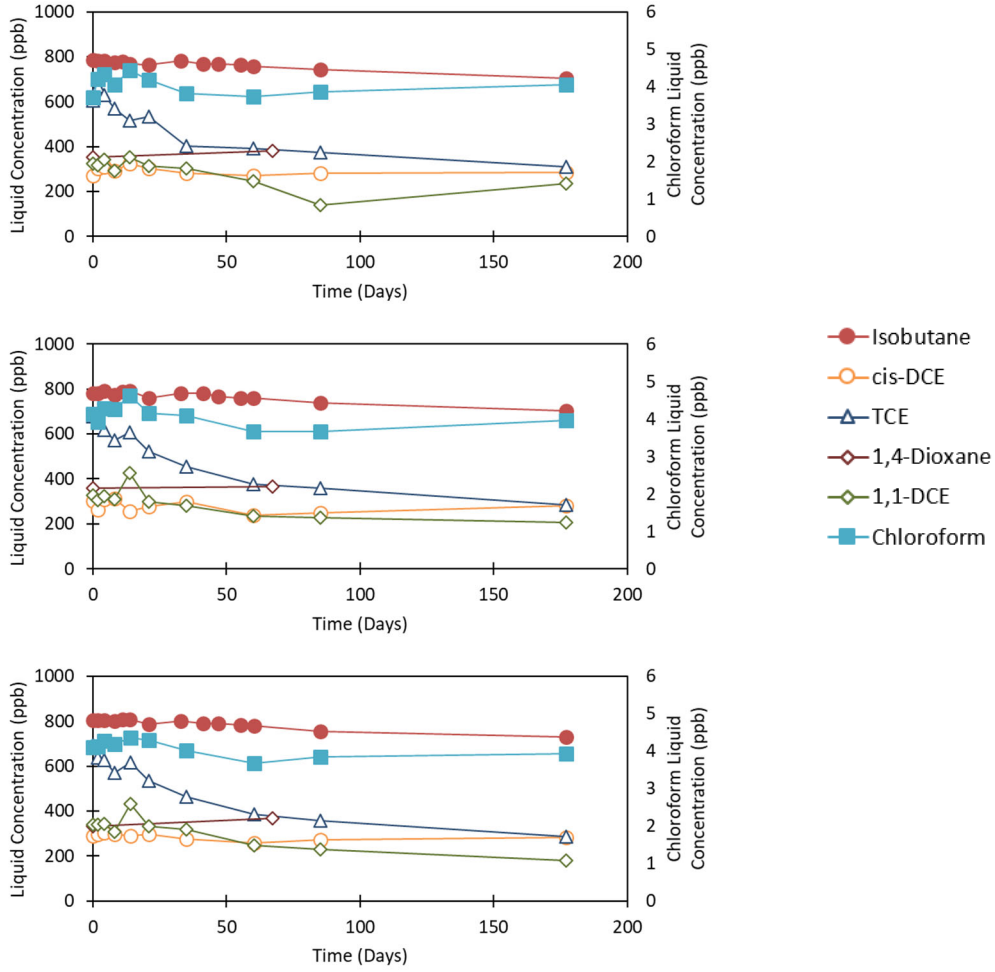

Figure 17. Set 9 acetylene controls. Acetylene was added such that the liquid concentration was 170 µM. Acetylene has been shown to inhibit the monooxygenase enzymes responsible for CAH cometabolism by isobutane utilizing microorganisms. Over the course of 180 days, there was no significant change in isobutane or COC concentrations as compared to the poison controls.

PCE and 1,1-DCE/Freon-113

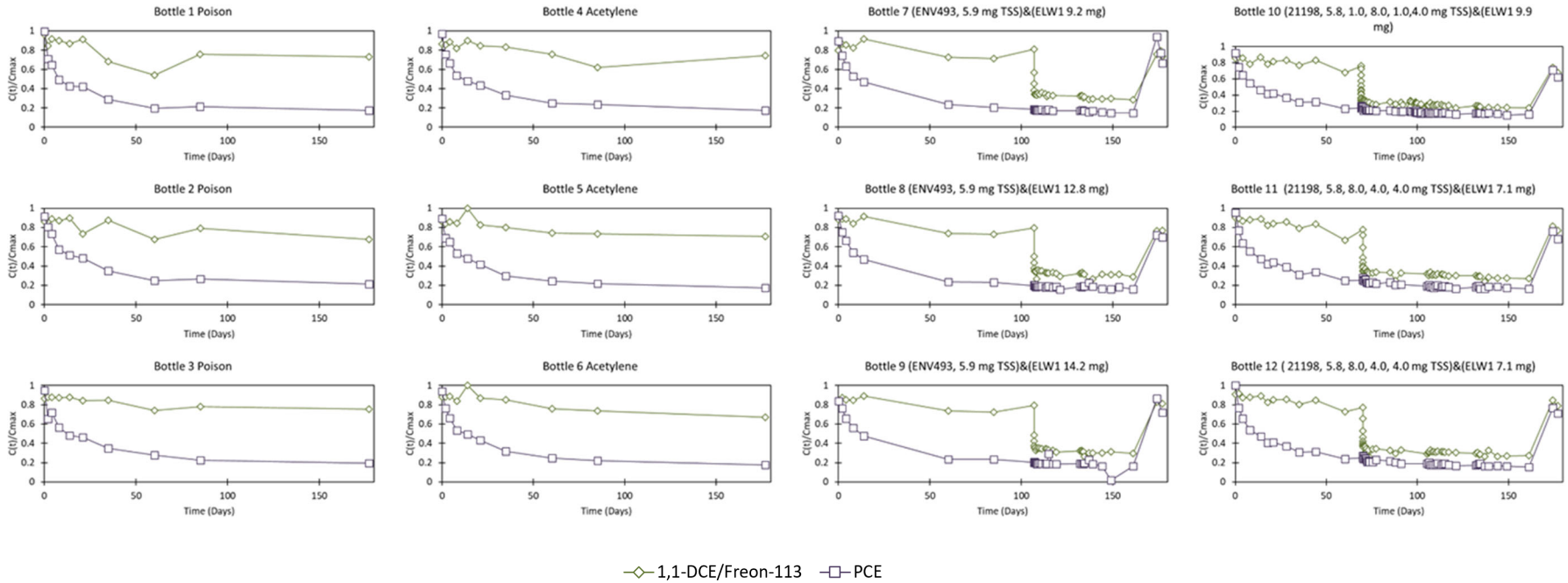

Figure 18. PCE, 1,1-DCE, and Freon-113 data. 1,1-DCE and Freon-113 co-elute on the GC column used. About 40% of the instrument response measured for Freon-113 and 1,1-DCE is due to Freon-113 as can be seen in Bottles 7-12 after treatment of 1,1-DCE after 110 days. PCE and Freon-113 are recalcitrant to aerobic cometabolism by monooxygenase producing microorganisms. PCE slowly decreases over time, which is likely due to sorption to the aquifer materials. The main source of evidence for sorption, is the steady concentrations of 1,1-DCE and Freon-113 (both of which are expected to show less sorption to solids) over long periods of time showing there is minimal VOC loss through the septa or from sampling. The increase in concentrations on day 160 is due to exchange of groundwater. In this set of plots Cmax is 1.8 ppb for PCE and 430 ppb for 1,1-DCE and Freon-113.

Set 10 (Isobutene) Supplemental Data

COC concentrations over time

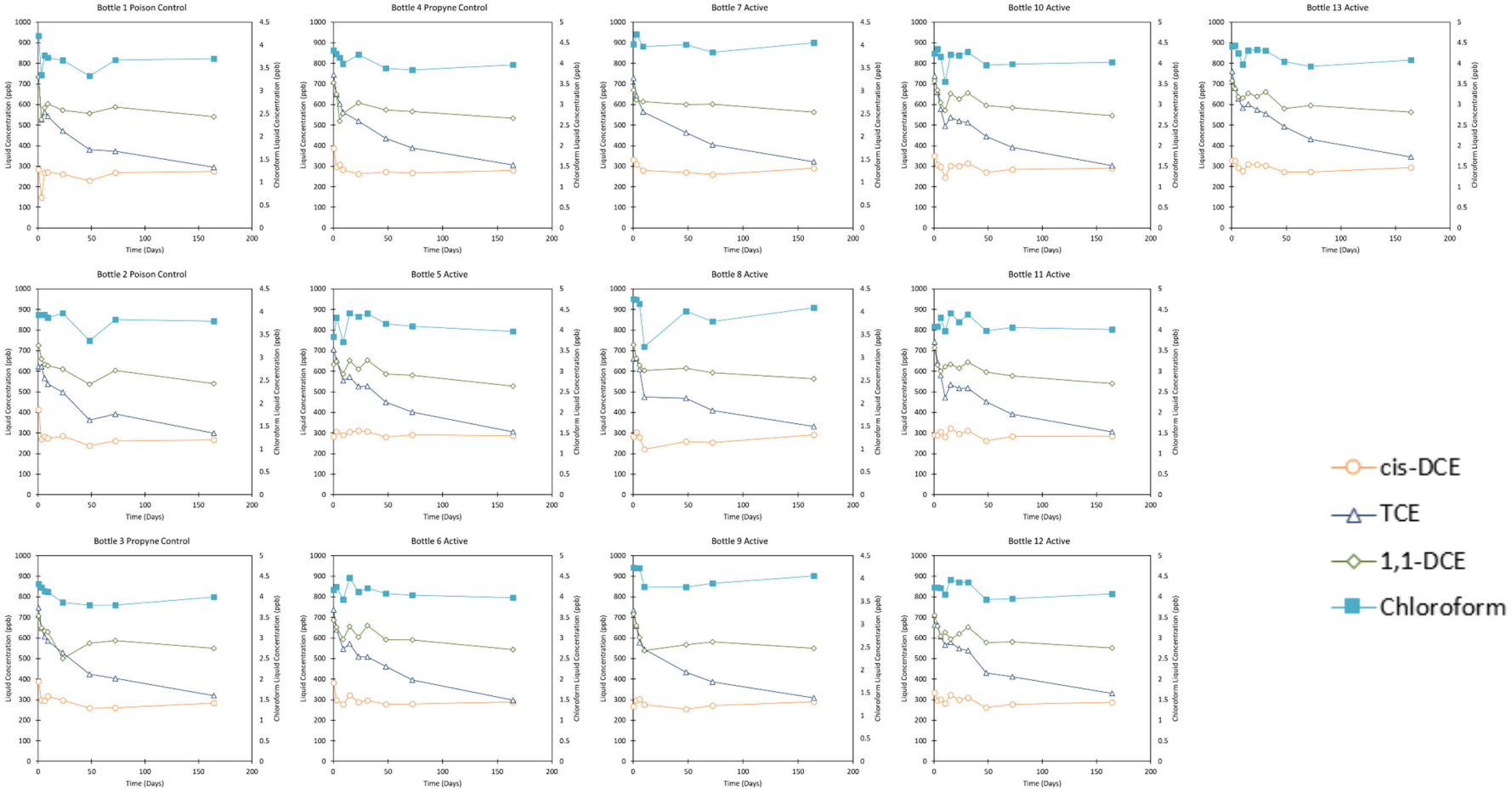

Figure 19. Contaminant concentrations in native isobutene microcosms. There has been no transformation of contaminants over 180 days. Decreases in TCE concentration are most likely due to contaminant sorption to the aquifer solids. Sodium azide poison controls and propyne monooxygenase inhibitor controls are indistinguishable from the potentially active microcosms.

Substrate (Isobutene) concentrations Over Time

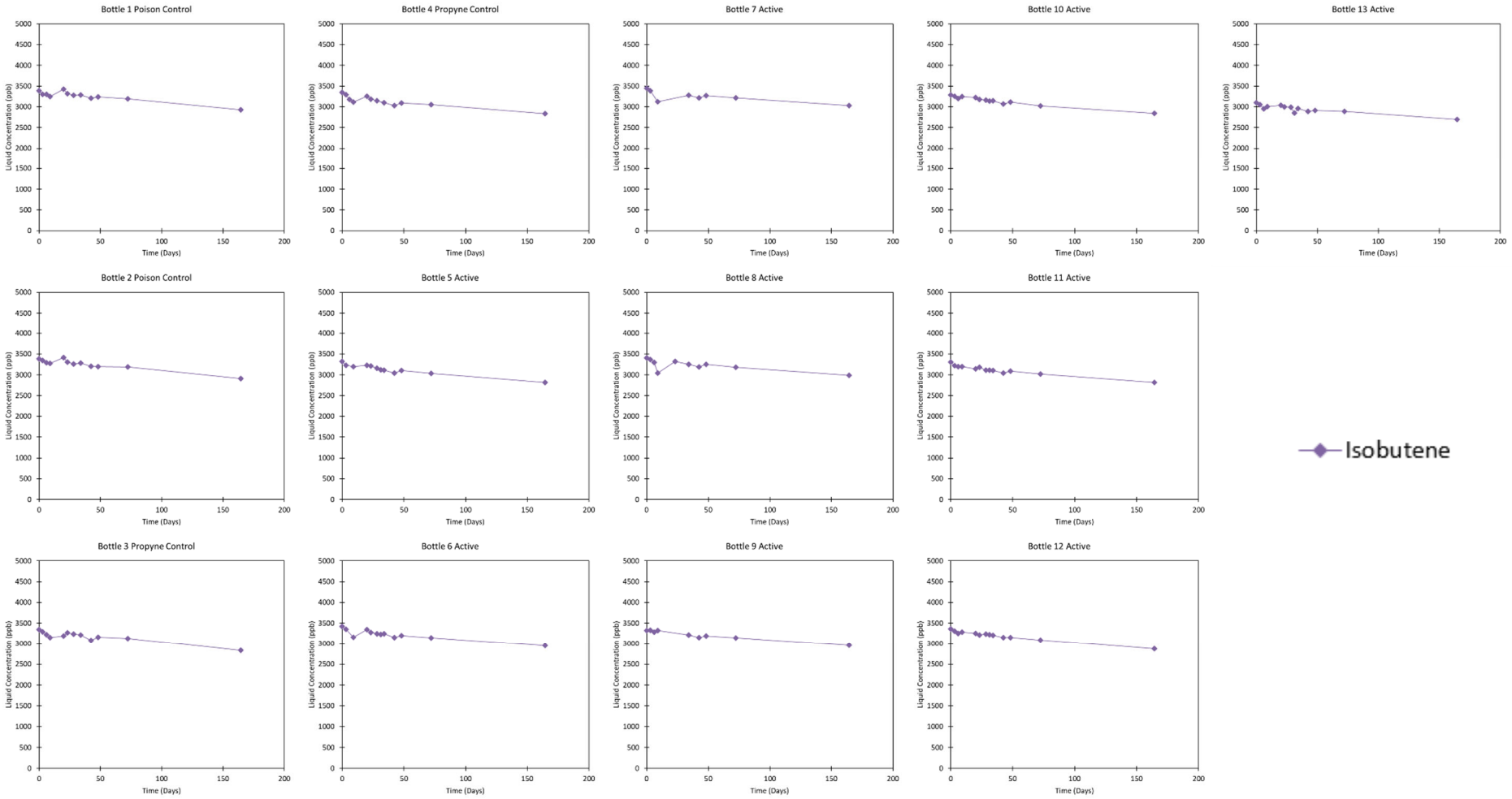

Figure 20. Substrate concentration in isobutene microcosms showing slight decreases in all bottles including poison and propyne controls. The decreases in isobutene over time are consistent in all microcosms including poison controls, this is likely due to loss through the septa over time.

Set 11 Supplemental Data

Propane Microcosms COCs and Substrate

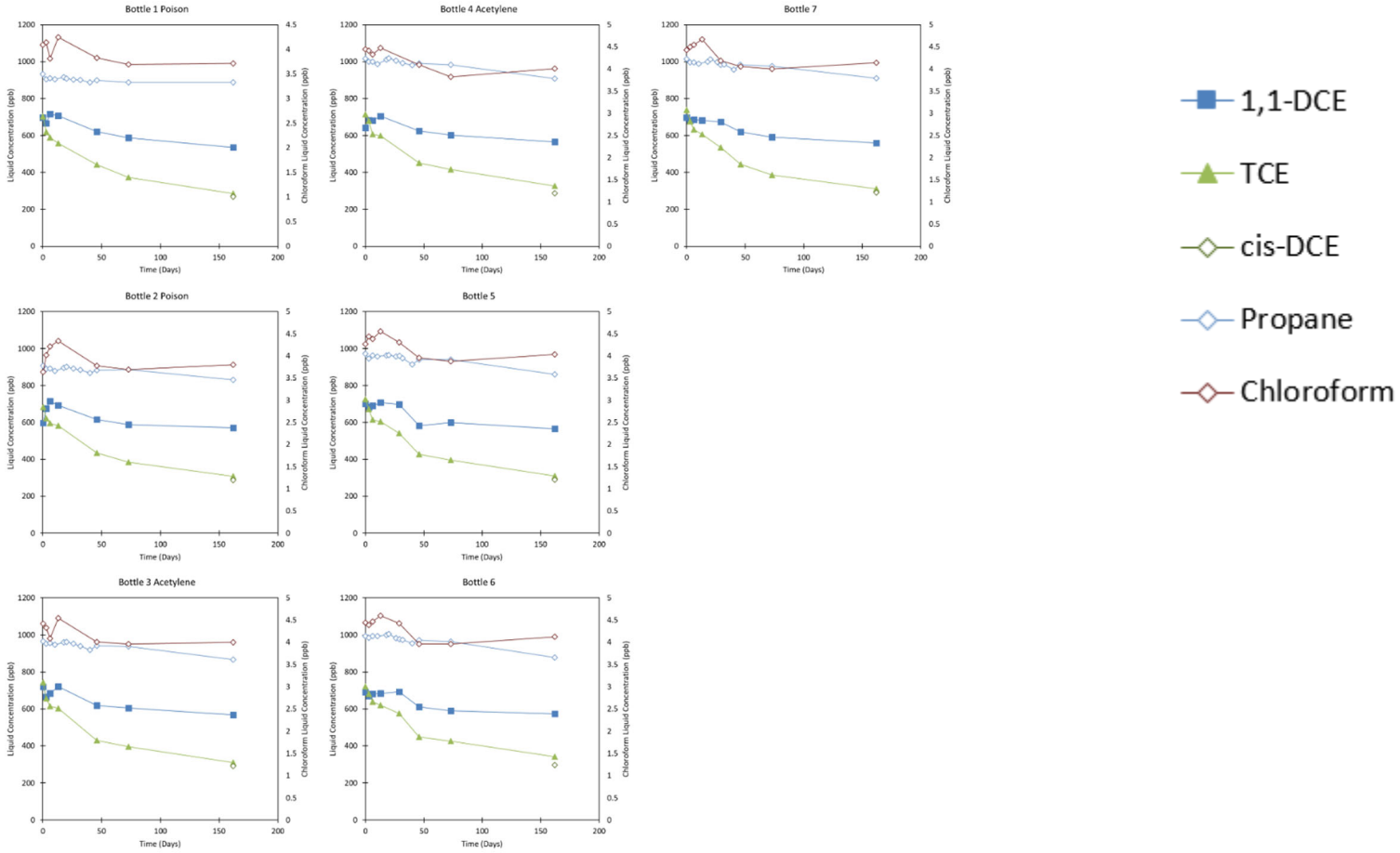

Figure 21. Native set of propane microcosms. There has been no transformation of contaminants over 180 days. Decreases in TCE concentration are most likely due to contaminant sorption to the aquifer solids. Sodium azide poison controls and acetylene monooxygenase inhibitor controls are indistinguishable from the potentially active microcosms.

APPENDIX B

APTIM ENRICHMENT CULTURES AND MICROCOSMS

APPENDIX B: SUMMARY OF APTIM CULTURE ENRICHMENT AND MICROCOSM WORK

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1.0 MICROBIAL ENRICHMENT CULTURES

1.1. Methods

Groundwater was received from Naval Air Station North Island (NASNI) on February 23, 2018 in 1 L polyethylene bottles from wells SMW-08A and SMW-10A. Groundwater from each well was homogenized aerobically at room temperature by combining water from the individual containers into one large sterile container each for SMW-08A and SMW-10A. Two types of microbial enrichments were performed; 1) groundwater only enrichments, and 2) enrichments in bacteriological growth medium. For the enrichments in groundwater only, homogenized site water (30 mL) was transferred into 125 mL Erlenmeyer flasks. The flasks received either 3% isobutane (v/v), 3% propane (v/v), 3% propylene (v/v), or 10% methane (v/v) in the headspace. Diammonium phosphate (DAP) (50 mg/L) was added as a source of inorganic nutrients to some of the flasks. For the enrichments prepared in bacteriological growth medium, 1 mL of homogenized site groundwater was added to either Basal Salts Medium (BSM; Hareland et al., 1975) amended with either isobutane, propane, or propylene in the headspace, or OB3b Medium (Cornish et al., 1984) amended with methane in the headspace. For enrichments that became turbid (indicative of microbial growth), 1 mL of the enrichment was passed into 100 mL of fresh medium (same type as original enrichment). Groundwater only enrichments that became turbid were also passed into growth medium.

In order to determine which gasses were being consumed, and at what rate, 2 mL of the groundwater only enrichments with DAP were placed into 8 mL serum vials after 1 month and sealed with Teflon lined septa and crimp caps. Groundwater only enrichments not containing DAP showed no signs of growth after 1 month, indicating that groundwater collected from the site is likely nutrient limited. The vials were spiked with 1 % of their respective alkane/alkene gas to the headspace and time zero and subsequent headspace samples were analyzed using a GC-FID. As shown on Figure B1, all four target gasses had been consumed within 15 days, indicating that indigenous bacteria present at the site are capable of growing on all the gasses tested. Isobutane was consumed at the fastest rate (within 2 days) indicating that isobutane-metabolizing bacteria are abundant at the site.

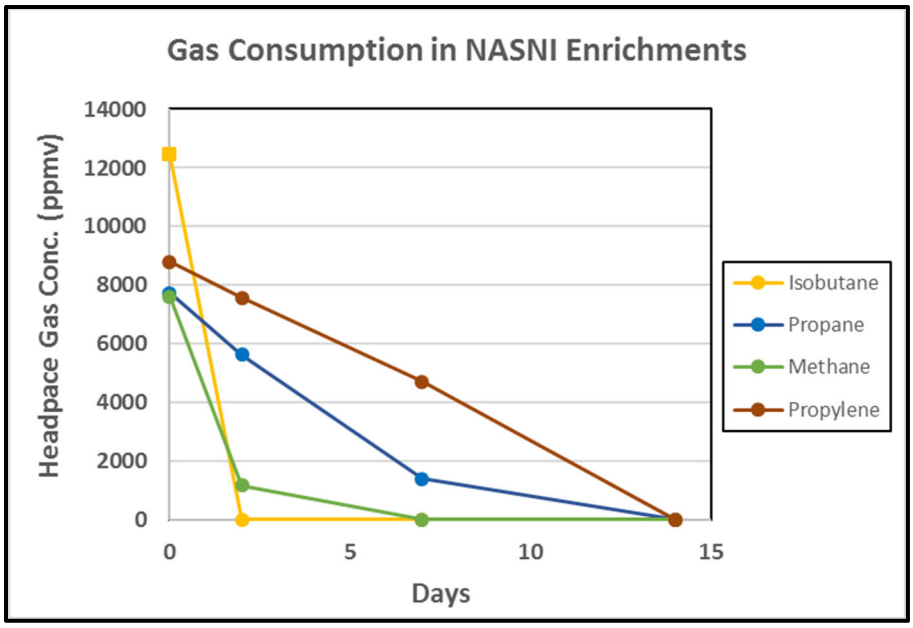


Figure B1. Indigenous bacteria are present at NASNI are capable of growth on all gasses.

Following three successful passes into fresh medium, the ability of the enrichment cultures to biodegrade target CAHs and 1, 4-D was examined. Enrichments from well location 08A did not continue to grow during the third pass. Enrichments amended with propylene also did not grow after multiple passes. Selected cultures that did grow after multiple passes were washed via centrifugation (6,000 rpm X 20 min), resuspended in fresh growth medium, and placed in serum vials which were then sealed with Teflon-lined septa and aluminum crimp caps. These vials were spiked with individual and pairs of various target contaminants of concern (COCs) found at the Site to evaluate biodegradation and potential inhibition. COCs of interest in the Site groundwater include 1,1,2-trichloroethene (TCE), 1,1-dichlorethene (1,1-DCE), *cis*-1,2-dichlorethene (*cis*-DCE), 1,1-dichloroethane (1,1-DCA), and 1,4-dioxane (1,4-D). Substrate gases were not added to the vials. Samples were taken immediately after the initial contaminant spike (i.e., time zero), and at set time points thereafter. VOC samples were analyzed via EPA 8260 for aqueous samples, or on the GC-FID for headspace samples (100 µl). Aqueous 1,4-D samples were analyzed by modified EPA 8260 (GC-MS with heated purge-and-trap).

1.2. Results of 1,4-D and VOC degradation tests

Results of the enrichment testing are provided in **Figures B2** through **B4**. Isobutane enrichments were able to completely degrade 1,1-DCE within the first 5 hours of incubation (**Figure B2**). 1,4-D was completely degraded by itself, or in the presence of TCE or 1,1-DCE. However, TCE was not degraded. Propane enrichments behaved very similarly to the isobutane enrichments, and were able to degrade 1,1-DCE as well as *cis*-DCE and 1,1-DCA within 24 hours, but not TCE. Propane enrichments also degraded 1,4-D alone and with all groundwater VOCs present (**Figure B3**). Conversely, methane enrichments were able to degrade TCE and roughly 53% of the *cis*-DCE (**Figure B4**). However, there was no significant degradation of 1,1-DCE, 1,1-DCA, or 1,4-D by the methane enrichments. **These data clearly show that multiple primary substrate gases are capable of stimulating indigenous bacteria, and that these bacteria, when bioaugmented into groundwater, are capable of degrading 1, 4-D and target VOCs.**

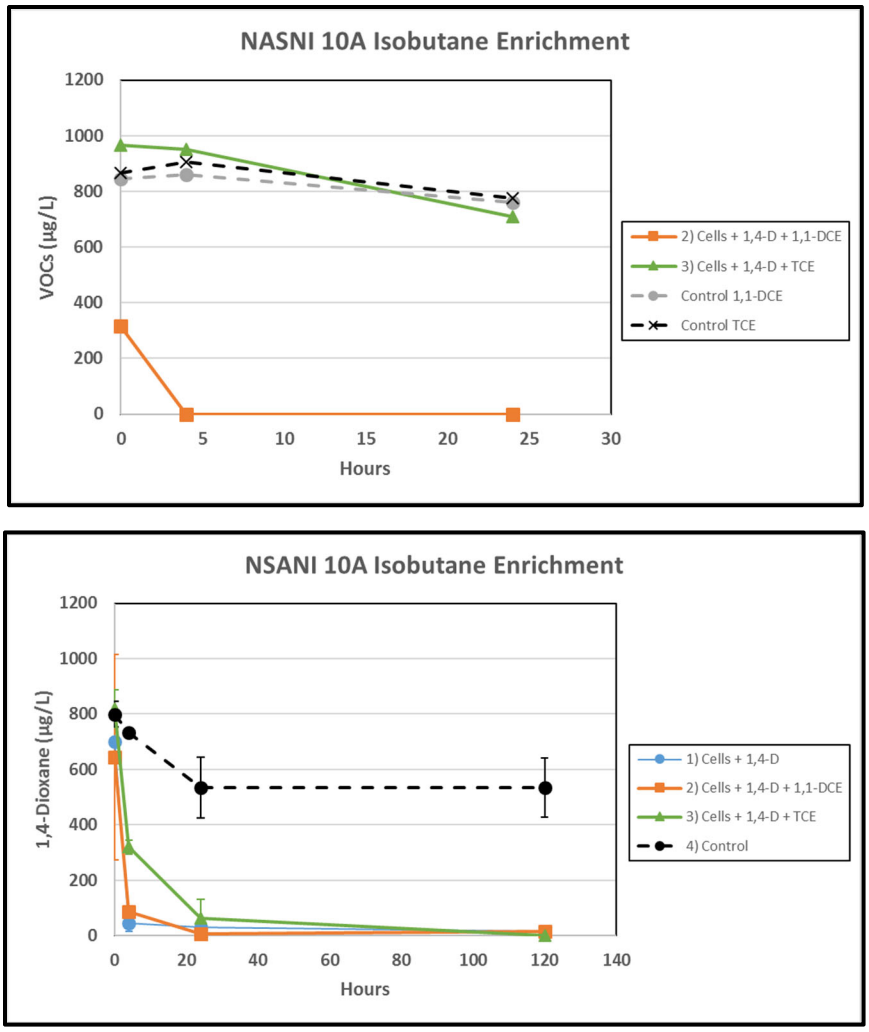


Figure B2. Biodegradation of VOCs (1,1-DCE or TCE; top panel) and 1,4-D (bottom panel) by an isobutane enrichment culture from NASNI site groundwater. The isobutane enrichment rapidly biodegraded 1,1,-DCE and 1,4-D (paired or separately), but not TCE.

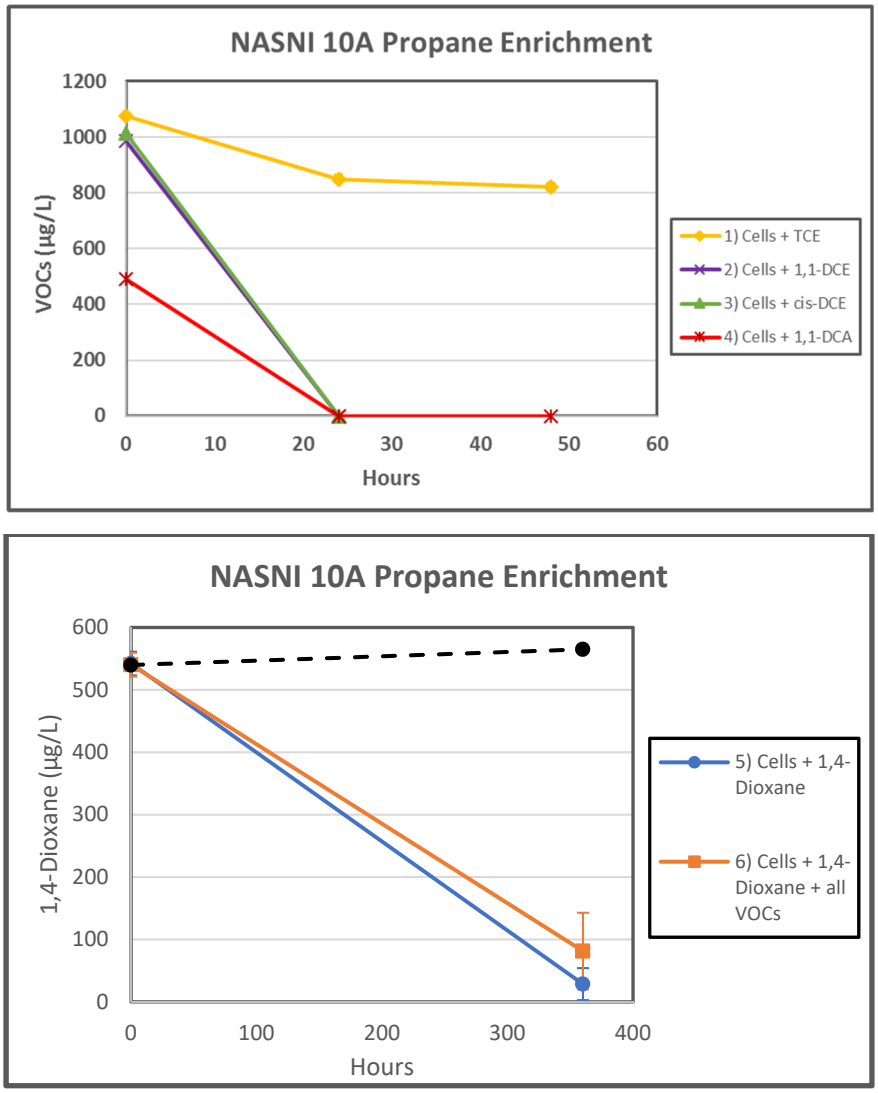


Figure B3. Biodegradation of VOCs (top panel) and 1,4-D (bottom panel) by a propane enrichment culture from NASNI site groundwater. The propane enrichment rapidly biodegraded 1,1,-DCE, *cis*-DCE, 1,1-TCA and 1,4-D (all tested separately), but not TCE.

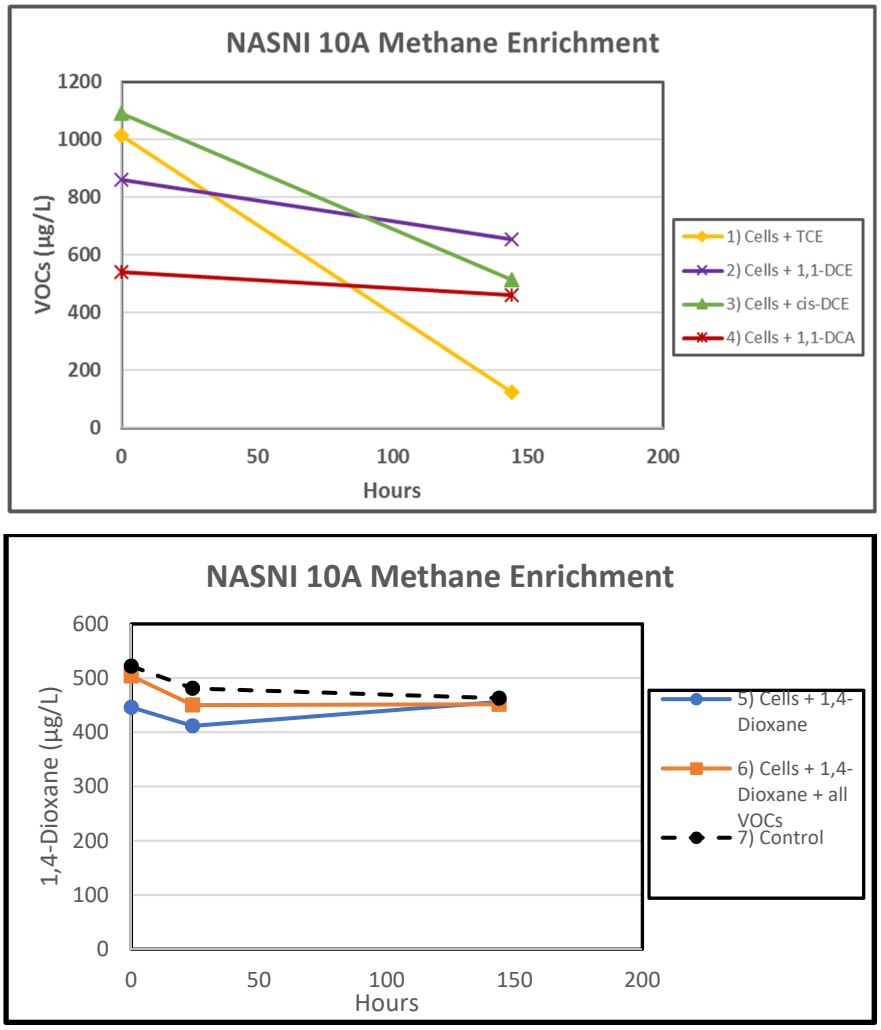


Figure B4. Biodegradation of VOCs (top panel) and 1,4-D (bottom panel) by a methane enrichment culture from NASNI site groundwater. The methane enrichment biodegraded TCE, and *cis*-DCE but not 1,1,-DCE, 1,1-DCA, or 1,4-D (tested separately and with all other VOCs).

1.3 Enrichment culture growth tests

Initial testing from the soil and groundwater microcosms indicated that 1,1-DCE is an inhibiting factor for stimulation and growth of cometabolic bacteria capable of degrading 1,4-D and other COCs at the site (see main Treatability Study Report). The enrichments described above were subsequently tested for their ability to grow in the presence of 1,1-DCE. Since the isobutane-oxidizing bacterial enrichment grew quickly and was able to rapidly degrade 1,1-DCE, it was chosen for initial testing. The isobutane enrichment was grown to an optical density of 0.45 and passed 1:100 into a 160 mL serum bottle containing 100 mL BSM media spiked to site groundwater concentration levels of 500 µg/L or 5000 µg/L 1,1-DCE. Bottles were also spiked with 5% isobutane to monitor substrate consumption. Controls containing no 1,1-DCE to measure uninhibited growth, as well as a no cell control to measure headspace VOC losses were also prepared.

The data confirm that 1,1-DCE is inhibitory to the growth of the isobutane-oxidizing enrichment culture (**Figure B5)**. No isobutane consumption, degradation of 1,1-DCE or growth of the culture were observed in bottles amended with either concentration of 1,1-DCE. However, bottles not containing 1,1-DCE grew exponentially and completely consumed isobutane by day 3.

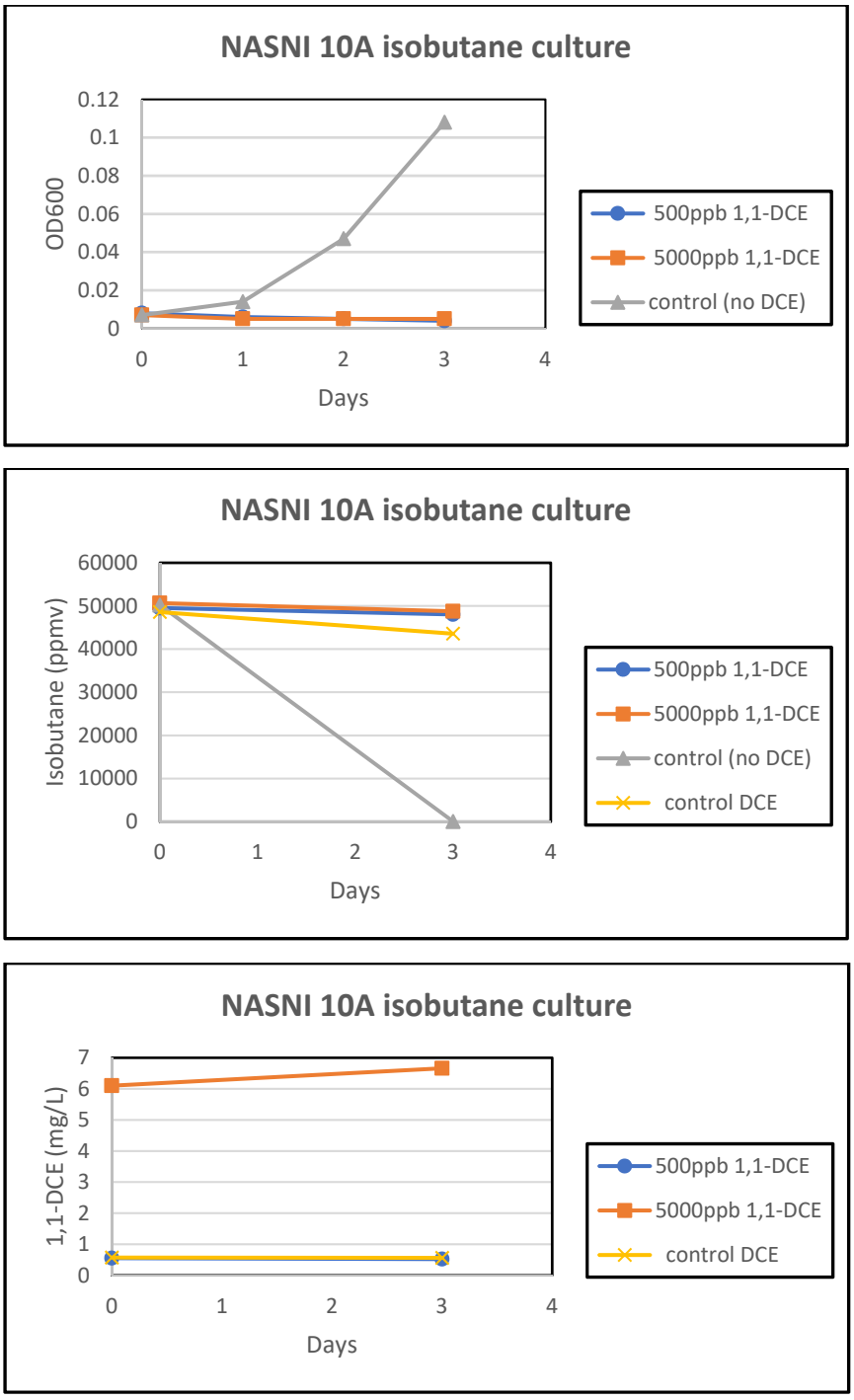


Figure B5. Growth of the isobutane enrichment in the presence or absence of 1,1-DCE (top panel) and isobutane consumption (middle panel) and 1,1-DCE (bottom panel) by the culture.

2.0 PURE CULTURE ISOLATION

2.1 Methods

Small volumes of each enrichment were plated onto their various media prepared with 15% agar to form solid plates. Isobutane-grown bacteria were plated onto BSM agar plates containing 1000 mg/L isobutyl alcohol (a soluble breakdown product in isobutane metabolism), or R2A (Reasoner's 2A agar). Methanotroph enrichments were plated onto OB3b noble agar plates and incubated in a BD GasPak system amended with 10% (v/v) methane as a sole carbon substrate. Single colonies were harvested, aseptically passed back into liquid media, and amended with 3% (v/v) isobutane or 10 % methane, respectively. Following three successful passes into fresh media the cultures were again plated on media to check for purity and then tested to determine their potential to biodegrade target VOCs and 1,4-D.

2.2 Results

A pure isobutane-degrading culture was quickly isolated and tested. This new culture was labeled ENV493 and was found to rapidly biodegrade 1,4-D and all target VOCs except TCE, which was only partially degraded (~38%). A sample plate of ENV493 was sent to Accugenix (Newark, DE) for identification. ENV493 was identified by Accugenix as within 98.8 % similar to both *Rhodococcus ruber* and *Rhodococcus aetherivorans*. Additional lab testing by Dr. Hyman at North Carolina State University confirmed this strain to be *Rhodococcus aetherivorans.*

Characterization of ENV493 included assays designed to determine which hydrocarbons were growth substrates and which enzymes were expressed during growth on the various substrates. ENV493 was observed to grow on ethane, propane and n-butane as well as dextrose and isobutane. Enzyme expression patterns with different alkane growth substrates and dextrose suggest multiple oxygenases are generally expressed and that and some different oxygenases occur with the different substrates (**Figure B6)**.

For field applications of cometabolic organisms, microbial consortia are typically grown in batch reactors on a carbon source such as lactate and then switched to the enzyme inducing gaseous substrates. To evaluate whether this process would be successful, ENV493 was grown on lactate (~12 mM) and then switched to isobutane or ethane. After growth on lactate, isobutane was quickly consumed by the culture (**Figure B7**). However, ethane which was added at a concentration used was 3% (v/v), was not consumed. Several 125 mL Erlenmeyer flasks were then prepared with ENV493 and ethane concentrations ranging from 3% - 20% (v/v). It was determined that ENV493 would only grow at concentrations of at least 20% (v/v) ethane in the headspace, which may reflect the Km of the enzyme(s) responsible for degradation of this gas.

In addition to testing growth substrate ranges, ENV493 was evaluated to determine if growth on different substrates resulted in different patterns of VOC degradation. ENV493 was grown on dextrose (10 mM), butane 3% (v/v), or ethane 20% (v/v). As before, following three successful passes into fresh medium, the ability of the culture to biodegrade target VOCs and 1,4-D was examined. When grown on dextrose, ENV493 degraded *cis*-DCE, 1,1-DCA, and 1,4-D separately, but not TCE or 1,1-DCE (**Figure B8**). After growth on dextrose, however, the culture did not degrade any of the contaminants when all were present together as a mixture - perhaps due to inhibition by 1,1-DCE epoxide. ENV493 grown on butane degraded 1,1-DCE, *cis*-DCE, and 1,1-DCA but not TCE when all were present in a mixture, which differs from the dextrose-grown cells

(**Figure B9**). TCE also was not degraded by butane-grown ENV493 when supplied individually. When grown on ethane, the degradation pattern of ENV493 was similar to that observed with butane-grown cells - all site COCs were biodegraded except for TCE (**Figure B10**).

The data clearly show that ENV493 has a wide range of growth substrates and will cometabolize all critical site contaminants with the exception of TCE. The mixed methanotroph culture (ENV494M) that was enriched from site MW-10A groundwater was capable of degrading TCE. The two cultures were sent from APTIM to OSU for inclusion in microcosm testing, and a separate set of groundwater microcosms was prepared at APTIM to assess the effectiveness of these two cultures together.

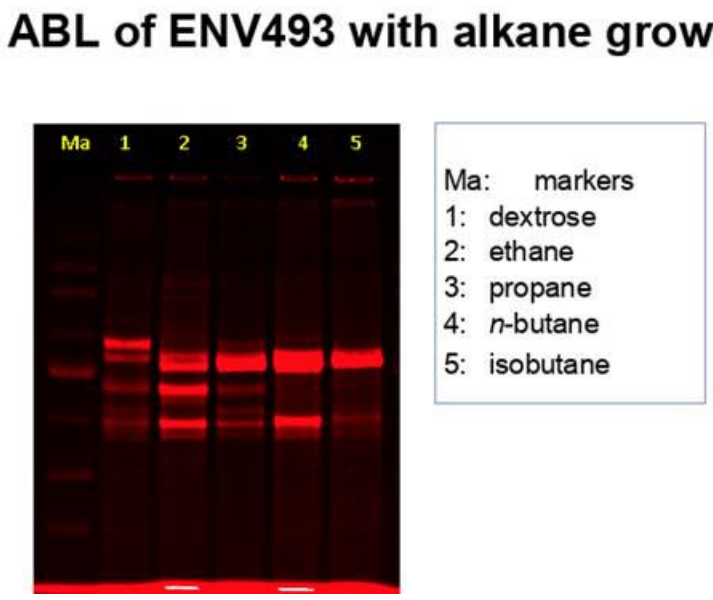
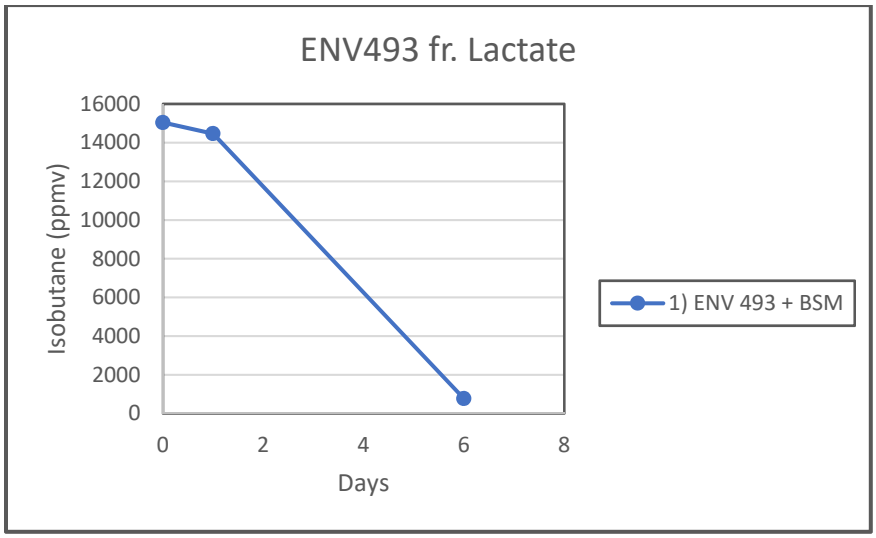


Figure B6. Enzyme expression patterns expressed by ENV493 on alkane growth substrates dextrose, ethane, propane, butane, and isobutane. Each band represents a presumed monooxygenase enzyme.



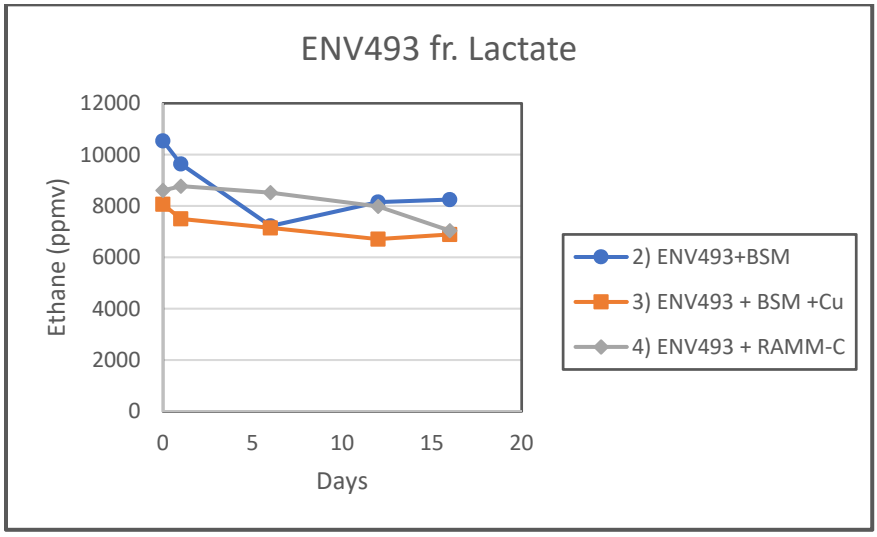


Figure B7. ENV493 Isobutane or ethane utilization after growth on lactate.

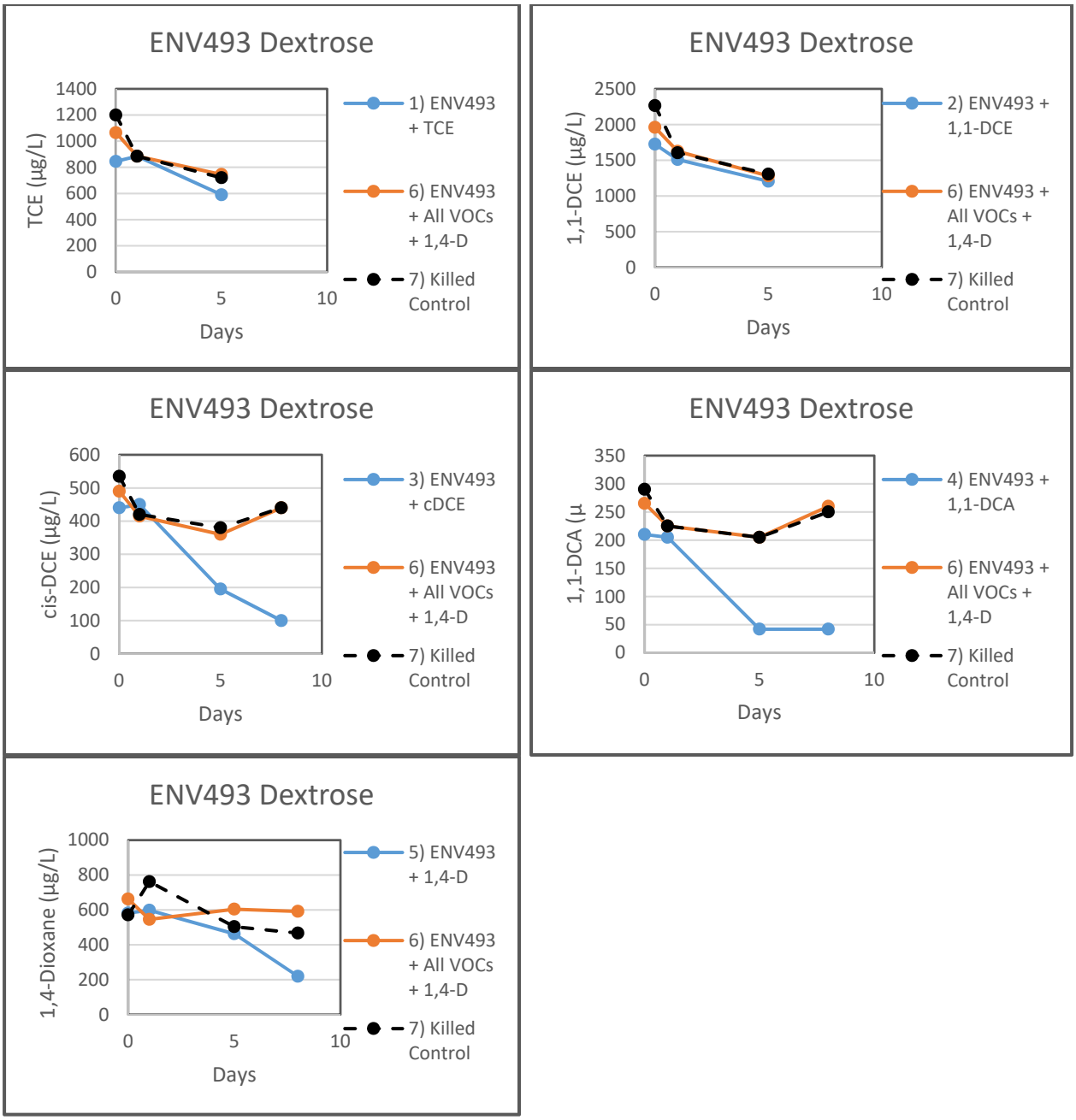


Figure B8. Evaluation of 1,4-D and VOC degradation for ENV493 grown on dextrose.

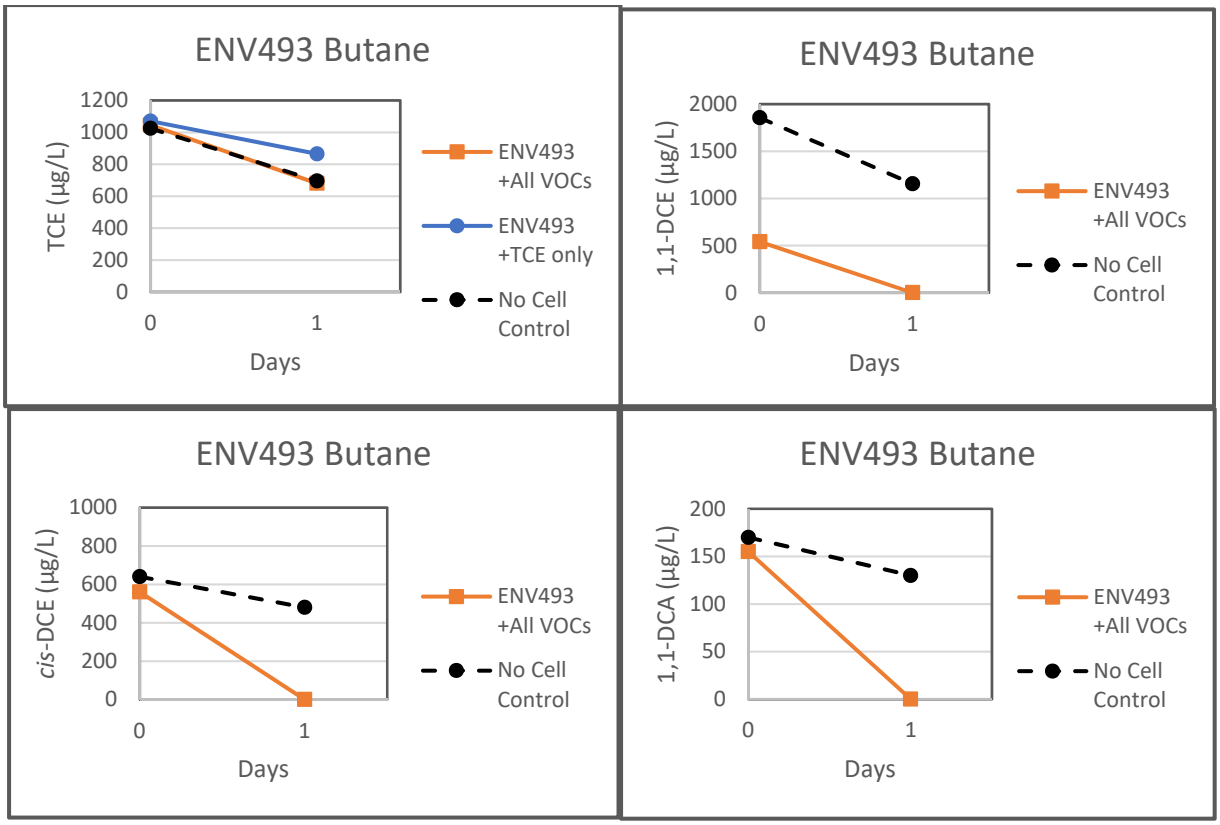


Figure B9. Evaluation of 1,4-D and VOC degradation for ENV493 grown on butane.

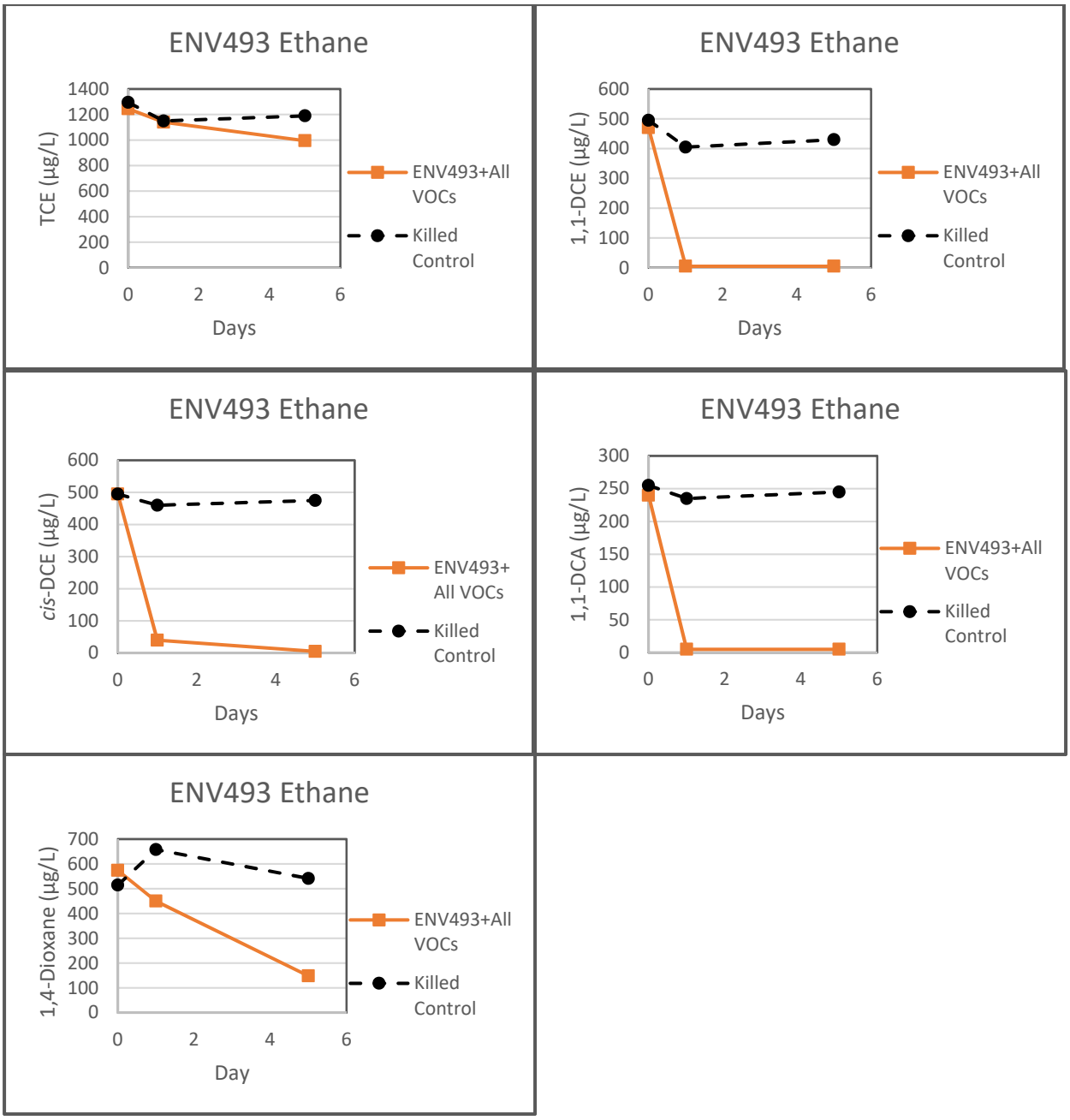


Figure B10. Evaluation of 1,4-D and VOC degradation for ENV493 grown on ethane.

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3.0 GROUNDWATER MICROCOSMS WITH ENRICHMENT CULTURES

3.1 Methods

Groundwater only microcosms were prepared in glass serum bottles (approximate volume, 160 mL). Approximately 100 mL SMW-08A site groundwater (sparged for 15 min to remove residual VOCs) was added to each of the bottles, then each was spiked with 1000 µg/L TCE, 1000 µg/L 1,1-DCE, 500 µg/L *cis*-DCE, 200 µg/L 1,1-DCA, and 500 µg/L 1,4-D. The bottles also received 50 mg/L DAP as a source of nutrients for biological degradation activities. The bottles were sealed with Teflon-lined butyl rubber stoppers and crimp caps. Five treatments were prepared in duplicate as follows.

Treatment 1: Bioaugmentation with ENV493 only. ENV493 grown on 3% (v/v) isobutane was washed and spiked to a concentration of OD_{600} ~ 0.2, which is the average field scale concentration achievable. This treatment assessed the ability of ENV493 to biodegrade key COCs in the site groundwater, including 1,1-DCE and 1,4-D.

Treatment 2: Bioaugmentation with ENV493 followed by ENV494M. The bottles initially received ENV493 to an OD_{600} ~ 0.2. Once COCs that are degradable by ENV493 were below detection, ENV494M was added at an OD between 0.15 and 0.2 to biodegrade residual TCE and any other VOCs.

Treatment 3: Bioaugmentation with ENV493 and ENV494M. This treatment was identical to Treatment 2 except that both ENV493 and ENV494M were added together, each at OD_{600} ~ 0.2 at the start of treatment. This treatment determined if all contaminants could be degraded at the same time, or if inhibitory contaminant 1,1-DCE needed to be removed first.

Treatment 4: Live Control. This treatment received groundwater and DAP only.

Treatment 5: Killed Control. This treatment was amended with a mercuric chloride solution to a final concentration in groundwater approximately 2000 mg/L as a microbial inhibitor, and used to evaluate abiotic loss of VOCs;

Microcosm bottles were incubated in the dark with gentle shaking on an orbital shaker at room temperature. At each sampling event, 10 mL of liquid was removed through the setpta using a glass gastight syringe equipped with a 25-gauge needle, preserved with HCl, and analyzed for VOCs via EPA 8260, and 1,4-D by modified EPA 8260 (GC-MS with heated purge-and-trap). To prevent a vacuum and to maintain aerobic conditions in the microcosms, the liquid volume removed was replaced with sterile-filtered air to maintain desired oxygen concentrations in the headspace. Headspace oxygen levels (100 µL sample) were periodically taken and measured on a gas chromatography/thermal conductivity detector (GC-TCD). DAP was added a second time on day 15 to ensure that inorganic nutrients were not limiting. ENV494M was spiked into Treatment 2 on day 2, once 1,1-DCE, 1,1-DCA, and *cis*-DCE were below detection limits. Methane gas (2% v/v) was added to the headspace of Treatments 2 and 3 on day 9, 15, and 23 to stimulate TCE degradation by ENV494M.

3.2 Microcosm Results

The results of the microcosm study are provided in **Figure B11**. The target VOCs, 1,1-DCE, 1,1-DCA, and *cis*-DCE, were all degraded below detection limits (5 µg/L) by ENV493 treatments (Treatments 1, 2 and 3) within 2 days. Similar losses were not apparent in the live or killed

controls. TCE was completely degraded below detection (5 µg/L) after 27 days in the treatments amended with ENV494M regardless of whether the culture was added after 1,1-DCE degradation or at the start of the study. This indicates that there was no inhibition by 1,1-DCE on the mixed methanotroph culture, which could be due to the rapid depletion of 1,1-DCE by ENV493 within 2 days.

Methane gas was added to the treatments with ENV494M on multiple occasions and was consistently consumed after each addition, indicating ENV494M was very active. Headspace methane concentrations were converted to aqueous concentrations using Henry's Law, and data are presented as calculated aqueous concentrations in **Figure B11**. TCE was not completely degraded in the absence of ENV494M in any of the treatments. However, in Treatment 1 (ENV493 only), losses of TCE (47%) were significantly greater than for the live or killed control samples showing some level of degradative activity. 1,4-D decreases greater than 95% were achieved in all treatments that received ENV493 (Treatment 1, 2, and 3) after 13 days, achieving the performance objectives for the site as discussed in the demonstration plan. 1,4-D was not degraded in either live or killed controls. The fact that the Live controls tracked exactly with the killed controls indicates that nutrient addition alone will not stimulate indigenous bacteria to degrade target contaminants.

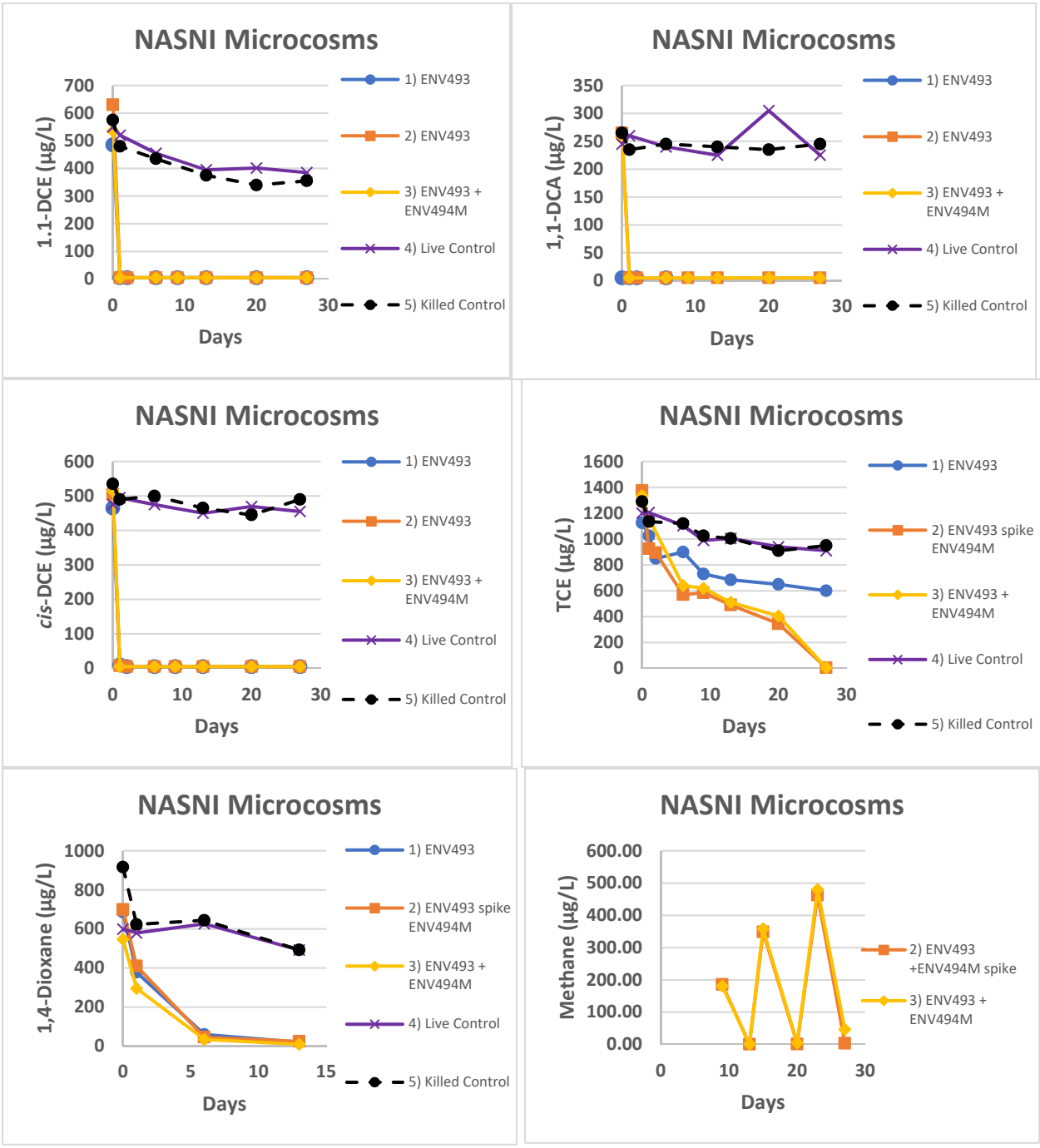

Figure B11. VOC and 1,4-D degradation in NASNI groundwater only microcosms bioaugmented with ENV493 and ENV494M. Methane degradation also was monitored.

4.0 SUMMARY

Two cultures (ENV493 and ENV494M) were enriched from the NASNI groundwater that together were capable of aerobic biodegradation of 1,4-D and the target VOCs present at the Site. While these organisms are indigenous in the site groundwater, they are not apparently present at sufficient density to overcome the toxicity associated with the epoxide formed when 1,1-DCE is oxidized via a monooxygenase enzyme. The addition of ENV493 to site groundwater at field-relevant bioaugmentation doses allows biodegradation of 1,1-DCE below toxic levels and subsequently results in rapid biodegradation of 1,4-D and all other relevant VOCs except TCE. The addition of ENV494M either with or subsequent to ENV493 addition provides a catalyst for TCE to be biodegraded. Thus, the data provide a strategy for degradation of all relevant COCs at the NASNI site.

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APPENDIX C

GROWTH MEDIA COMPOSITION

21198, ELW1, and ENV493 media

Both ELW1 and JOB5 are grown in Mv media. We normally prepare this as a 10X solution.

Mv Media

		1.5x	(1980mL) 2x
Soln 1 (10X)			
To 900 mL of ddH_20 add		30g	40
20.0 g	NH_4Cl	1.125	1.5
0.75 g	$MgCl_2 \cdot 6H_2O$	1.5	2.0
1.0 g	$(NH_4)_2SO_4$	3	4.0
2.0 mL	Trace Elements		
Bring volume up to 100mL			

Soln 2 (100X)
To 500 mL of ddH_2O add

77.5 g	K_2HPO_4
42.5 g	NaH_2PO_4

$NaH_2PO_4 \cdot H_2O \rightarrow 48.9g$

Media Preparation

Autoclave solutions separately. To make 1X media, autoclave 890mL of ddH_2O. When coo add 100mL of solution #1 and 10mL of solution #2.

Trace Elements

To 500 mL of ddH_2O add

25 g	EDTA
11 g	$ZnSO_4 \cdot 7H_2O$
2.27 g	$CaCl_2$
2.53 g	$MnCl_2 \cdot 4H_2O$
2.5 g	$FeSO_4 \cdot 7H_2O$
0.55 g	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ Ammonium Heptamolybdate tetrahydrate
0.753 g	$CuSO_4 \cdot 5H_2O$
0.855 g	$CoCl_2 \cdot 6H_2O$

39.

Adjust pH to 6.0 with KOH

ENV494M Media

Nitrate Salts media for methane oxidizing bacteria

Part A	Mix in 1 L water:		
media conc. g		stock solution concentration	volume of stock per L
1	KNO3	25g/100ml	4 ml
1	MgSO4.7H2O	25g/100ml	4 ml
0.134	CaCl2.H2O	13.4g/100ml	1 ml
0.025	KH2PO4	2.5g/100ml	1 ml

Autoclave Part A
When Part A has returned to room temp add Parts B and C which have been autoclaved, and cooled, separately

Part B			
media conc. g		stock solution concentration	volume of stock per L
0.7	Na2HPO4.7H2O	7g/100ml	10 ml

Part C
2 ml trace elements solution

Mix in 1 L of water: g		
1.29	Na2EDTA	
1.69	FeSO4.7H2O	
0.8	ZnSO4.7H2O	
0.005	MnCl2.2H2O	
0.03	H3BO3	
0.05	CoCl2.6H2O	
0.002	NiCl2.6H2O	
0.05	Na2MoO4.2H2O	
0.75	CuCl2.2H2O	add Cu for pMMO omit Cu for sMMO

APPENDIX D

MS THESIS: KRYSTA J. KRIPPAEHNE (2018)

AN ABSTRACT OF THE THESIS OF

Krysta J. Krippaehne for the degree of Master of Degree in Environmental Engineering presented on September 19, 2018.

Title: Cometabolism of 1,4-Dioxane and Chlorinated Aliphatic Hydrocarbons by Pure Cultures of *Rhodococcus rhodochrous* 21198 and *Mycobacterium* ELW1 and in Groundwater Microcosms Fed Isobutane and Isobutene as Growth Substrates.

Abstract approved: _____

Lewis Semprini

1,4-dioxane and chlorinated aliphatic hydrocarbons (CAHs) such as trichloroethylene (TCE) and 1,1-dichloroethene (1,1-DCE) are hazardous compounds commonly found in soil and groundwater. Bioremediation through aerobic cometabolism is a potential option for the remediation of these contaminated sites. The purpose of this study was to examine the use of different primary substrates to stimulate bacteria that can potentially perform concurrent aerobic cometabolism of 1,4-dioxane and chlorinated solvents. Batch pure-culture rate tests were conducted utilizing isobutane, ethane, and propane-grown *Rhodococcus rhodochrous* ATCC® 21198™ and isobutene-grown *Mycobacterium* sp. ELW1. In support of a field demonstration, microcosms containing a groundwater and sediment slurry from the Naval Air Station North Island in San Diego, California, were also performed using isobutane and isobutene as cometabolic growth substrates. This site is contaminated with CAHs (predominately TCE and 1,1-DCE) and 1,4-dioxane due to historical improper storage of chemical waste.

The effectiveness of the cometabolic transformation of CAHs and 1,4-dioxane was evaluated through the determination of zero order degradation rates of TCE, 1,1-DCE,

cis-1,2-dicholoroethylene (c-DCE), 1,2-dichloroethane (1,2-DCA), 1,1,1-trichloroethane (1,1,1-TCA), and 1,4-dioxane and transformation capacities of c-DCE and 1,1-DCE by microorganisms grown on different substrates. The general trend in rates indicated isobutane-grown 21198 > ethane-grown 21198 > propane-grown 21198. The rates for TCE were comparable among all growth substrates with 21198. The TCE degradation rate of isobutene-grown ELW1 was double the rates observed for 21198 grown on all substrates. 1,4-dioxane, 1,2-DCA, and 1,1,1-TCA were not readily transformed by ELW1. Transformation of mixtures of CAHs and 1,4-dioxane were consistent with the single compound tests.

Isobutane and isobutene were selected as primary growth substrates to promote cometabolism in microcosm studies. After 52 days of isobutene exposure, isobutene uptake was observed in a native microcosm system in the presence of TCE, 1,1-DCE, and 1,4-dioxane. Once nearly complete uptake of isobutene was achieved, rapid 1,1-DCE transformation and slow TCE degradation were observed. After 130 days of isobutane exposure, no primary substrate uptake and minimal TCE and 1,1-DCE degradation were observed. It is hypothesized that the presence and transformation of 1,1-DCE is inhibitory to the stimulation of native microbes. This is due to the formation of a toxic epoxide resulting from the activation of a monooxygenase by the primary substrate and the observation that 1,1-DCE was transformed prior to isobutane utilization. In order to circumvent the toxic effects of 1,1-DCE, a variety of strategies were implemented, including sparging to remove the chlorinated compounds through air stripping, bioaugmentation with isobutane and isobutene-utilizing cultures, and isobutanol addition to cultivate an active isobutane-utilizing population without the activation of the monooxygenase enzyme responsible for 1,1-DCE transformation. Sparging and bioaugmentation were effective strategies in the stimulation of the microcosms to transform 1,4-dioxane, 1,1-DCE, and TCE.

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September 19, 2018
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Cometabolism of 1,4-Dioxane and Chlorinated Aliphatic Hydrocarbons by Pure Cultures of *Rhodococcus rhodochrous* 21198 and *Mycobacterium* ELW1 and in Groundwater Microcosms Fed Isobutane and Isobutene as Growth Substrates

by
Krysta J. Krippaehne

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented September 19, 2018
Commencement June 2019

Master of Science thesis of Krysta J. Krippaehne presented on September 19, 2018

APPROVED:

Major Professor, representing Environmental Engineering

Head of the School of Chemical, Biological, and Environmental Engineering

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Krysta J. Krippaehne, Author

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First and foremost, I would like to thank my major professor, Dr. Lewis Semprini, for his mentorship over the last year and a half. Your patience and encouragement as I faced frustrations with the many roadblocks this project encountered were always so appreciated. I am thankful for everything you've taught me about environmental engineering, research, and perseverance. I feel very lucky to have had the opportunity to be a part of your lab group. You cultivate an environment of kindness, generosity, curiosity, and determination that made coming to work all the more enjoyable.

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LIST OF ABBREVIATIONS

µg/L	micrograms per liter
µL	microliter(s)
ABPP	activity-based protein profiling
ATCC	American Type Culture Collection
BGS	below ground surface
^{o}C	degrees Celsius
CAH	chlorinated aliphatic hydrocarbon
c-DCE	cis-1,2-dichloroethene
CAH(s)	chlorinated aliphatic hydrocarbon(s)
COC(s)	contaminant(s) of concern
CO_2	carbon dioxide
1,4-D	1,4-dioxane
1,1-DCE	1,1-dichloroethene
1,2-DCE	1,2-dichloroethene
ECD	electron capture detector
FID	flame ionization detector
GC	gas chromatograph
GC-MS	gas chromatography-mass spectrometry
L	liter(s)
MCL	maximum contaminant level
mg	milligram(s)
mg/L	milligrams per liter
mL	milliliter(s)
MTBE	methyl tertiary butyl ether
m/z	mass-to-charge ratio
N	nitrogen
NAS	Naval Air Station
O_2	oxygen
OHPHE	hydroxyphenanthrene
P	phosphorous
pMMO	particulate methane monooxygenase
PrMO	propane monooxygenase
RCF	relative centrifugal force
rpm	revolutions per minute
SCAM	short-chain alkane monooxygenase
sMMO	soluble methane monooxygenase
TBA	tert-butyl alcohol
1,1,1-TCA	1,1,1-trichloroethane
TCD	thermal conductivity detector
TCE	trichloroethylene
TSS	total suspended solids
USEPA/EPA	United States Environmental Protection Agency

1.0 INTRODUCTION AND OBJECTIVES

Historical use and improper storage and disposal of chlorinated solvents have resulted in widespread groundwater contamination with chlorinated aliphatic hydrocarbons (CAHs) such as trichloroethylene (TCE), 1,1-dichloroethylene (1,1-DCE), cis-1,2-dichloroethylene (c-DCE), 1,1,1-trichlolorethane (1,1,1-TCA), and 1,2-dichloroethane (1,2-DCA), and the solvent stabilizer, 1,4-dioxane (1,4-D). These compounds often exist in as mixtures in the subsurface. While 1,4-dioxane contamination has most notably been connected to the use of 1,1,1-TCA, it has also been found to be a common co-contaminant with TCE (Anderson et al., 2012). This is likely due to the substitution of TCE with 1,1,1-TCA in the mid-1960s as a result of increased regulations restricting the use of TCE (Mohr et al., 2010).

These compounds are listed by the Environmental Protection Agency (EPA) as known or possible human carcinogens (US EPA, 2017a), and stringent drinking water standards have promoted a drive for the remediation of these sites. Due to the unique chemical and physical properties of these compounds, remediation can be difficult. Bioremediation, or the use of microbial metabolic processes to break down contaminants, has been shown to be an effective strategy for CAH and 1,4-dioxane remediation (Frascari et al., 2015). Aerobic cometabolism is a specific bioremediation process by which microorganisms are fed a primary growth substrate for use as a carbon and energy source, while fortuitous transformation of contaminants of concern (COCs) is observed via the activation of monooxygenase enzymes by the primary growth substrate (Bradley, 2003).

Effective primary growth substrates for the promotion of aerobic cometabolism include compounds such as methane, ethane, propane, and butane (Dolinová et al., 2017). Many studies have reported successful transformations of 1,4-dioxane and a variety of chlorinated compounds including TCE, 1,1-DCE, c-DCE, 1,1,1-TCA, and 1,2-DCA via aerobic cometabolism (Alvarez-Cohen and Speitel, 2001; Mahendra and Alvarez-Cohen, 2006). An increasingly prevalent issue involves the implementation of aerobic cometabolism at sites contaminated with more complex mixtures of CAHs and 1,4-dioxane.

Multiple pure-cultures have been identified that are capable of performing cometabolism. Two such cultures include *Rhodococcus rhodochrous* American Type Culture Collection (ATCC) 21198 (hereby referred to as 21198) and *Mycobacterium* sp. ELW1 (hereby referred to as ELW1).

21198 has been shown to grown on a variety of gaseous short-chain alkanes including isobutane, ethane, propane, and n-butane (Babu and Brown, 1984; Hou et al., 1983; MacMichael and Brown, 1987). 21198 has been found to transform 1,4-dioxane and a variety of CAHs including 1,2-DCA, 1,1-DCA, 1,1,2-TCA, 1,1,1-TCA, 1,1-DCE via cometabolism (Bennett et al., 2018; Rich, 2015; Rolston, 2017; Thankitkul, 2016). ELW1 is an isobutene-utilizer that has been found to cometabolize phenanthrene, vinyl chloride, c-DCE, and TCE (Kottegoda et al., 2015; Rich, 2015; Schrlau et al., 2017).

A volatile organic compound plume was discovered at Naval Air Station (NAS) North Island in San Diego, California in 2005. Historical use and improper storage of chlorinated solvents resulted in contamination of groundwater with CAHs (predominately TCE and 1,1-DCE) and 1,4-dioxane. Due to the composition of the contaminant mixture located on NAS North Island, aerobic cometabolism and the implementation of a multiple primary substrate biosparging approach were proposed for the remediation of this site.

The objectives of this study were to:

1. Evaluate the cometabolic abilities of isobutane, ethane, and propane-grown 21198 and isobutene-grown ELW1 to transform single-compounds and mixtures of CAHs and 1,4-dioxane;
2. Use microcosms with groundwater and aquifer solids from NAS North Island in San Diego, California to evaluate the use of isobutane and isobutene as primary growth substrates to promote cometabolism of mixtures of 1,4-dioxane and CAHs by native microbial communities;
3. Evaluate the effectiveness of bioaugmentation to native microcosms containing mixtures of COCs with isobutane-grown 21198 and isobutene-grown ELW1;
4. Evaluate the long-term effectiveness of cometabolism in microcosms containing 1,4-dioxane and exposed to increasing concentrations of 1,1-DCE and TCE.

2.0 LITERATURE REVIEW

2.1 Chlorinated Aliphatic Hydrocarbons

Chlorinated aliphatic hydrocarbons (CAHs), including TCE, c-DCE, 1,1-DCE, 1,1,1-TCA, and 1,2-DCA are common groundwater contaminants existing from the improper disposal of chlorinated solvents. Chlorinated solvents have been used most often in degreasing and dry-cleaning operations (Mohr et al., 2010). Other industrial applications have included use in adhesives, pharmaceuticals, textile processing, and coating solvents (Ward and Stroo, 2010). These solvents gained popularity due to their effectiveness, noncorrosivity, nonflammability, and ease of recycling (Doherty, 2000). The physical structures of primary CAHs are shown in Figure 2.1, and key physical and chemical properties for TCE, c-DCE, 1,1-DCE, 1,1,1-TCA, and 1,2-DCA are shown in Table 2.1.

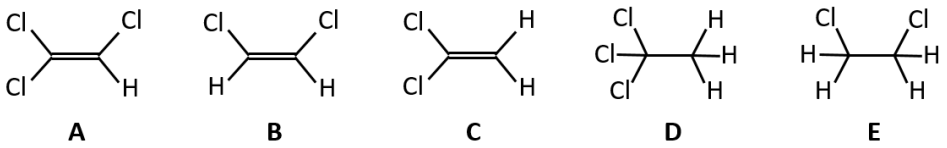

Figure 2.1. *Chemical structure of TCE (A), c-DCE (B), 1,1-DCE (C), 1,1,1-TCA (D), and 1,2-DCA (E).*

Table 2.1. *Physical and chemical properties, including molar mass, solubility (PUBCHEM), Henry's constant (Mackay and Shiu, 1981), and octanol-water partition coefficient (log KOW) (US EPA, 1992, 1995) of TCE, c-DCE, 1,1-DCE, 1,1,1-TCA, and 1,2-DCA.*

	TCE	c-DCE	1,1-DCE	1,1,1-TCA	1,2-DCA
Molar mass (g/mol)	131.38	96.94	96.94	133.40	98.95
Solubility @ 25 °C (mg/L)	1,280	3,500	2,420	1,290	8,600
Henry's constant @ 20°C (dimensionless)	0.296	0.122	0.857	0.548	0.179
Log K_{OW}	2.53	1.86	1.48	2.47	1.45

While the physical and chemical properties of CAHs made them ideal for industrial applications, they have also contributed to their proliferation throughout the environment and impacted their fate and transport in aquifers. Most chlorinated solvents have densities greater than that of water, promoting their downward movement through the subsurface (Ward and Stroo, 2010). When groundwater encounters a CAH source, solvents dissolve into solution up to their solubility limits and proceed to travel along the downward gradient of groundwater flow. The hydrogeological features of the subsurface can also greatly impact the fate and transport of CAHs through aquifers. Chlorinated solvent plumes have been found to travel great distances beyond their source.

Many CAHs have been found to have adverse effects on human health and/or have been identified as known or possible human carcinogens. As such, the USEPA has established drinking water maximum contaminant levels (MCLs) for a variety of compounds, including TCE (5 µg/L), c-DCE (70 µg/L), 1,1-DCE (7 µg/L), 1,1,1-TCA (200 µg/L), and 1,2-DCA (5 µg/L).

2.2 1,4-Dioxane

As shown in Figure 2.2, 1,4-dioxane is a cyclic ether with a highly stable structure (Mohr et al., 2010). The commercial sale of 1,4-dioxane began in 1929. 1,4-dioxane has many industrial uses including the application as a constituent in pharmaceuticals and pesticides and as a solvent in paints, stains, resins, oils, and adhesives (US EPA, 2006). Production and use of 1,4-dioxane increased in the 1950s and early 1960s due to its use as a stabilizer in 1,1,1-TCA (Agency for Toxic Substances and Disease Registry, 2012). 1,1,1-TCA actively reacts with aluminum. To counteract this reaction, 1,4-dioxane was added at approximately 4% (Ward and Stroo, 2010).

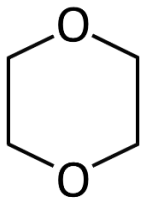

Figure 2.2. *Chemical structure of 1,4-dioxane.*

1,4-dioxane is hydrophilic, fully miscible in water and has a low octanol-water partition coefficient (0.27), which makes sorption to organic matter unlikely (US EPA, 2006). Due to these physical and chemical properties, 1,4-dioxane contaminant plumes have the ability to travel great distances with the flow of groundwater. The low Henry's constant (3×10^{-6} atm·m^3/mol) and vapor pressure of 1,4-dioxane (37 mm Hg at 25°C) also prevent volatilization into the vapor phase, complicating remediation via air stripping (Mohr, 2001).

While 1,4-dioxane contamination has most notably been connected to the use of 1,1,1-TCA, it has also been found to be a common co-contaminant with TCE (Anderson et al., 2012). This is likely due to the substitution of TCE with 1,1,1-TCA in the mid-1960s due to increased regulations restricting the use of TCE (Mohr et al., 2010). Anderson et al. studied the co-occurrence of 1,4-dioxane and TCE at U.S. Air Force installations (Anderson et al., 2012). They found that 93.7% of all 1,4-dioxane detections were concurrently detected with 1,1,1-TCA

and/or TCE, and 64.4% of all 1,4-dioxane detections were independently connected to TCE detections.

1,1-DCE is often also present at 1,4-dioxane contaminated sites due to its formation during the abiotic transformation of 1,1,1-TCA (Vogel, 1987). A survey conducted by Adamson et al. found that 1,4-dioxane was present at 68% of 1,1-DCE contaminated sites, and 97% of surveyed sites with 1,1-DCE contamination and no 1,1,1-TCA or TCE detection were not analyzed for 1,4-dioxane (Adamson et al., 2014). This finding suggests that the absence of 1,1,1-TCA and TCE should not be accepted as proof that 1,4-dioxane contamination is not present.

1,4-dioxane has been classified by the US EPA as a probable human carcinogen (US EPA, 2006). While there is currently no established federal drinking water MCL for 1,4-dioxane, it is included in the US EPA's Third Unregulated Contaminant Monitoring Rule (US EPA, 2016). In addition, numerous states have established drinking water and groundwater guidelines (US EPA, 2017b).

2.3 Bioremediation and Aerobic Cometabolism

Bioremediation is the practice of applying microbiological processes to the clean-up of contaminated soil and groundwater through engineered systems (Mandelbaum et al., 1997). Biostimulation, the modification of a natural aquifer through the addition of limiting nutrients, oxygen, and/or primary substrates, and bioaugmentation, the addition of a known COC-degrading microorganism to a natural aquifer, are common forms of bioremediation (Adams et al.). Bioremediation can be applied through direct metabolism wherein microbes utilize a particular COC as the sole carbon and energy source, or through cometabolism wherein microbes utilize a separate primary substrate as a carbon and energy source, while COCs are fortuitous transformed via enzymes induced by the primary substrate (Frascari et al., 2015). A variety of compounds can be employed as primary growth substrates to induce cometabolism, including butane, isobutane, propane, methane, ethane, ethene, isobutene, n-pentane, n-hexane, propene, toluene, and ammonia (CITATION). Specific studies conducted with the use of butane, isobutane, ethane, propane, isobutene, and methane as primary growth substrates are summarized in Sections 2.4.2 through 2.4.7.

2.3.1 Butane

Numerous studies have been conducted utilizing butane as a primary growth substrate for aerobic cometabolism (Doughty et al., 2005; Hamamura et al., 1997; Jitnuyanont et al., 2001;

Kim et al., 1997, 2000, 2002; Semprini et al., 2007a, 2007b, 2009; Tovanabootr and Semprini, 1998). Tovanabootr and Semprini researched the use of butane to stimulate the cometabolism of TCE in batch aquifer microcosms (Tovanabootr and Semprini, 1998). A substrate utilization lag time of 20 days was observed, and an average of 3 days were required for complete utilization. The stimulated butane-utilizers were not able to transform TCE. Kim et al. studied the cometabolism of a variety of CAHs by a butane-grown mixed culture (Kim et al., 2000). Chloromethane, dichloromethane, chloroform, chloroethane, 1,1-DCA, 1,2-DCA, 1,1,1-TCA, 1,1,2-TCA, vinyl chloride, 1,1-DCE, and c-DCE were all capable of transformation via cometabolism. TCE was not readily transformed by the butane enrichment culture. CAH transformations were inactivated by the presence of acetylene, suggesting a monooxygenase is likely responsible for the lack of transformations.

Kim et al. further conducted kinetic and inhibition studies for the cometabolism of 1,1,1-TCA, 1,1-DCE, and 1,1-DCA by a butane-grown mixed culture (Kim et al., 2002). They determined the maximum degradation rates and half-saturation coefficients via single compound kinetic tests (Table 2.2). Direct linear plots were used to identify the types of inhibition. Competitive and mixed inhibition were found to be the prominent inhibition types at work in the system. CAHs were found to be weak inhibitors of butane utilization, while butane was found to be a strong inhibitor of CAH transformation. The results also suggested that 1,1-DCE was a stronger inhibitor than 1,1,1-TCA and 1,1-DCA.

Table 2.2. *Summary of half saturation coefficients (K_S) and maximum degradation rates for butane-grown mixed culture.*

Compound	K_S (μmol/L)	Max Rate (μmol S/mg TSS/h)
1,1,1-TCA	12	0.19
1,1-DCE	1.5	1.3
1,1-DCA	19	0.49
Butane	19	2.6

Jitnuyanont et al. studied the bioaugmentation of butane-utilizing microorganisms to promote cometabolism of 1,1,1-TCA in groundwater microcosms (Jitnuyanont et al., 2001). Two microcosms containing groundwater and aquifer solids from Moffett Field, Sunnyvale, California, were established with butane as the primary substrate. No butane uptake was observed in either microcosm after 57 days of incubation. Bioaugmentation potential was tested via inoculation of one microcosm with a butane-utilizing enrichment from the Hanford

DOE site. Butane uptake was observed three days after the inoculation. The bioaugmented microcosm initially demonstrated effective 1,1,1-TCA transformation, but the transformation ability decreased after prolonged exposure. After 80 days of incubation, the non-bioaugmented microcosm began to uptake butane. The transformation of 1,1,1-TCA was initially limited but improved with time. The transformation yields (0.04 mg 1,1,1-TCA/mg butane) and the microbial compositions of the bioaugmented and non-bioaugmented systems were found to be similar after 440 days of incubation.

Semprini et al. have researched the bioaugmentation of butane-utilizing microorganisms for the transformation of 1,1-DCE, 1,1-DCA, and 1,1,1-TCA (Semprini et al., 2007a). A butane-enriched culture consisting primarily of *Rhodococcus* sp.-type microorganisms was used to bioaugment laboratory microcosms containing aquifer solids and groundwater from the Moffett Field Test Facility. 1,1-DCE was most quickly transformed, followed by 1,1-DCA and then 1,1,1-TCA. The transformation of 1,1,1-TCA appeared to be the strongest inhibitor of butane uptake. Accelerated removal rates with subsequent butane and CAH additions were observed over 100 days of incubation. Butane uptake was not observed in non-bioaugmented microcosms over 83 days of incubation.

Semprini et al. conducted further studies into the bioaugmentation of butane-utilizing microorganisms for the transformation of 1,1-DCE, 1,1-DCA, and 1,1,1-TCA (Semprini et al., 2007b). Rapid 1,1-DCE transformation with high product toxicity and poor inhibition by butane were demonstrated in microcosm experiments and modeling studies. Model simulations of laboratory data determined a 1,1,-DCE transformation capacity 0.175 µmol/mg cells. 1,1,1-TCA transformation was much slower and strongly inhibited by butane.

2.3.2 Isobutane

Studies have been conducted that demonstrate the successful use of isobutane as the sole carbon and energy source for the growth microorganism *Rhodococcus rhodochrous* (Babu and Brown, 1984; MacMichael and Brown, 1987). Bennett et al. quantified 1,4-dioxane biodegradation in isobutane-grown *Rhodococcus rhodochrous* strain 21198 (Further discussed in Section 2.5) (Bennett et al., 2018). Resting isobutane-grown cells were able to transform ≥95% of the initial 1,4-dioxane within 80 to 220 hours. The inhibition of 1,4-dioxane degradation by

the presence of 1-butyne, a known monooxygenase blocker, suggests monooxygenase enzymes are responsible for the transformation.

Rich conducted a kinetic analysis of the aerobic degradation of chlorinated ethanes and ethenes by isobutane-grown 21198 (Rich, 2015). This research was conducted via pure cultures studies in batch reactors. 21198 was able to cometabolize a variety of CAHs. The rates of transformation were ordered as follows: vinyl chloride > 1,1-DCE > c-DCE > TCE, and 1,2-DCA > 1,1,2-TCA > 1,1,1-TCA > 1,2,3-TCP. Thankitkul further studied the kinetics of cometabolic transformations of CAHs by isobutane-grown 21198 (Thankitkul, 2016). The transformation capacities were ranked as follows: 1,2-DCA > 1,1-DCA > 1,1,2-TCA > 1,1,1-TCA > 1,1-DCE. The zero order transformation rates were ranked as follow: 1,1-DCA > 1,2-DCA > 1,1-DCE > 1,1,2-TCA > 1,1,1-TCA.

Rolston researched the use of isobutane to promote the aerobic cometabolism of 1,4-dioxane in native microbial aquifer communities and by 21198 (Rolston, 2017). Rolston also investigated the potential inhibition of 1,4-dioxane transformation by the presence of TCE and the importance of inorganic nutrients. Isobutane was found to successfully stimulate native microbes after a lag time of approximately one week, with subsequent 1,4-dioxane transformation. Bioaugmentation with 21198 was also effective, with 1,4-dioxane transformation observed after the concentration of isobutane reached below 0.15 mg/L. Primary substrate inhibition was observed in both biostimulated and bioaugmented microcosms.

TCE was minimally transformed relative to 1,4-dioxane in both the native and bioaugmented microcosms. Results found that neither the rates of isobutane and 1,4-dioxane transformation nor the length of the biostimulation lag period were inhibited by the presence of TCE at a liquid concentration of 200 μg/L. The importance of inorganic nutrients was assessed through the addition of spent growth media with the bioaugmentation inoculum. Higher 1,4-dioxane degradation rates were maintained when compared to microcosms devoid of spent media, suggesting nutrient addition is important for sustained 1,4-dioxane transformation.

2.3.3 Ethane

Few studies have been conducted regarding the use of ethane as a primary growth substrate for aerobic cometabolism of 1,4-dioxane and chlorinated solvents. Freedman and Herz evaluated

the use of ethane as a primary substrate for aerobic cometabolism of vinyl chloride (Freedman and Herz, 1996). An ethane-grown enrichment culture was capable of utilizing vinyl chloride as a primary substrate and was able to transform it below 2 µg/L. Verce and Freedman modeled the kinetics of vinyl chloride cometabolism by novel ethane-grown Pseudomonas sp. Strain EA1 (Verce and Freedman, 2000). EA1 was isolated from an enrichment culture that had been fed ethane and vinyl chloride over two years. A yield of 0.99 mg TSS per mg ethane was determined. EA1 cometabolized vinyl chloride at a maximum rate of 0.35 µmol/mg VSS/d when ethane was utilized as the primary substrate. Primary substrate inhibition was not observed, though the presence of ethane reduced the total mass of vinyl chloride transformed. Hatzinger et al. researched the use of ethane to promote aerobic cometabolism of 1,4-dioxane in aquifers (Hatzinger et al., 2017). Through natural aquifer microcosms, aquifer enrichment cultures, and pure cultures, ethane was found to promote cometabolism of 1,4-dioxane. 1,4-dioxane concentrations within aquifer microcosms decreased from 370 µg/L to 32 µg/L over 49 days.

2.3.4 Propane

Deng et al. examined the cometabolism of 1,4-dioxane and 1,1-DCE by novel propane-utilizing *Azoarcus* sp. DD4 (Deng et al., 2018). Field sample microcosms containing approximately 10 mg/L 1,4-dioxane and 3.3 mg/L 1,1-DCE were bioaugmented with 0.024 mg of DD4 protein and 8 mg/L propane. 12.6% of propane was consumed over the first 7 days, with minimal 1,4-dioxane and 1,1-DCE degradation. Accelerated propane uptake and 1,1-DCE transformation were observed until propane was completely consumed on day 18, while only 23% of the 1,4-dioxane had been degraded. The concentration of 1,1-DCE decreased from 0.92 mg/L to below the detection limit of 0.02 mg/L by day 23, after a second addition of propane. The 1,4-dioxane concentration rapidly decreased to 0.17 mg/L on day 32 following complete 1,1-DCE transformation. In summary, high concentrations of 1,1-DCE significantly inhibited primary growth substrate uptake and delayed the removal of 1,4-dioxane and 1,1-DCE.

2.3.5 Isobutene

Rich conducted a kinetic analysis of the aerobic degradation of chlorinated ethenes by novel isobutene-grown ELW1 (further described in Section 2.6) (Rich, 2015). ELW1 was able to cometabolize a number of CAHs. The rates of transformation were ordered as follows: vinyl

chloride > c-DCE > TCE. Rich also found that the addition of formate increased the transformation capacities of ELW1.

Schrlau et al. researched the capabilities of isobutene-grown ELW1 to cometabolize phenanthrene, a polycyclic aromatic hydrocarbon (Schrlau et al., 2017). ELW1 was found to fully transform phenanthrene. Transformation products included 1-hydroxyphenanthrene (OHPHE), 3-OHPHE, 4-OHPHE, 9-OHPHE, 1,9-OHPHE, and trans-9,10-OHPHE. Minor metabolites, including 1-OHPHE, 3-OHPHE, 4-OHPHE, 9-OHPHE, 1,9-OHPHE, were found to be toxic to embryonic zebrafish. Phenanthrene transformation was moderately inhibited by 1-octyne, suggesting that an alkene monooxygenase is likely the responsible transformation enzyme.

2.3.6 Methane

Methanotroph metabolism is supported by the production of methane monooxygenase enzymes (MMO) (Balasubramanian and Rosenzweig, 2007). These enzymes can either be expressed in the soluble (sMMO) or particulate form (pMMO) (Ayala and Torres, 2004). When grown in the presence of copper, particulate methane monooxygenase is expressed, while the soluble methane monooxygenase is expressed in copper-deficient environments. Burrows et al. found that raising the concentration of Cu^{2+} from 1 µmol/L to 5 µmol/L promoted a transition from the production of sMMO to pMMO (Burrows et al., 1984).

The specific expression of the MMO enzymes can affect the capabilities of methane-grown microorganisms to transform compounds of interest. Jahng and Wood found that methane-grown *Methylosinus trichosporium* OB3b was able to degrade TCE and chloroform when sMMO was expressed (Jahng and Wood, 1994). TCE transformation was found by Tsien et al. to be significantly less in cultures grown with greater than 0.25 µmol/L of copper added (Tsien et al., 1989).

Chang and Alvarez-Cohen studied the biodegradation of a variety of CAHs by methane-oxidizing cultures (Chang and Alvarez-Cohen, 1996). Methane-grown cultures were able to degrade chloroform, dichloromethane, 1,1,1-TCA, TCE, 1,1-DCE, c-DCE, t-DCE, and vinyl chloride. The maximum transformation rates of TCE and c-DCE for a chemostat-grown mixed methane-oxidizing culture were estimated to be 73 and 86 µmol per mg TSS per day, respectively. The half saturation coefficients for TCE and c-DCE were 47 and 31 µmol/L, respectively.

Mahendra and Alvarez-Cohen researched the kinetics of 1,4-dioxane biodegradation by a variety of monooxygenase-expressing bacteria, including the methane-grown OB3b (Mahendra and Alvarez-Cohen, 2006). OB3b was found to only degrade 1,4-dioxane when the soluble methane monooxygenase was expressed. With an initial 1,4-dioxance concentration of 50 mg/L, the degradation rate was 0.38 mg 1,4-dioxane per mg protein per hour.

2.3.7 1,1-DCE Toxicity

As shown in Figure 2.3, an epoxide is formed via the transformation of 1,1-DCE by the monooxygenase enzyme activated by isobutane. Epoxides are electrophilic and have the ability to modify cellular components including DNA, RNA, lips, proteins, and other small molecules, often resulting in cellular inactivation (Rui et al., 2004). This epoxide has been found to exhibit strong toxicity to microorganisms grown on a variety of substrates.

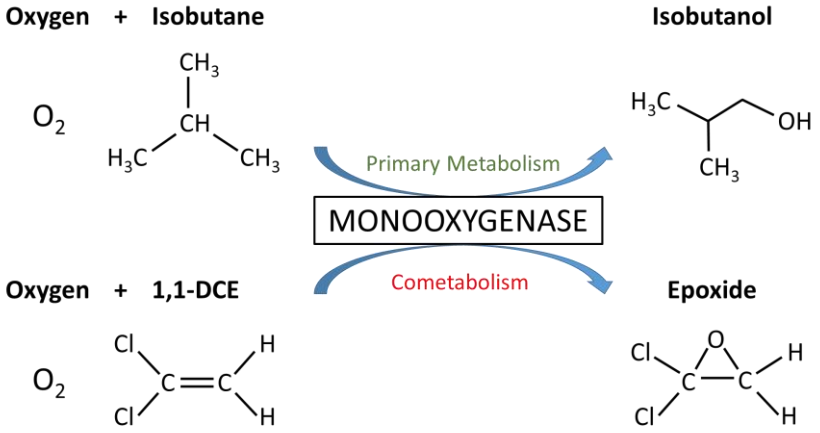

Figure 2.3. *Depiction of primary metabolism of isobutane and cometabolism of 1,1-DCE to their respective transformation products.*

1,1-DCE toxicity has been observed in various cometabolism systems established for laboratory and field-scale implementation. Anderson and McCarty reported on the effect of chlorinated ethenes on growth rates for a mixed methanotrophic culture (Anderson and McCarty, 1996). They found that growth rates were reduced by approximately 20% in the presence of 0.05 mg/L 1,1-DCE, 1 mg/L t-DCE, or 1 mg/L TCE. Growth rate data were modeled, and transformation product toxicity was shown to have the greatest impact on the growth rate reduction. Dolan and McCarty investigated 1,1-DCE transformation product toxicity in a mixed methanotrophic culture (Dolan and McCarty, 1995). Results indicated that cell inactivation was caused by 1,1-DCE transformation products rather than 1,1-DCE itself.

Kim et al. studied the transformation of CAH mixtures, including 1,1-DCE, by a butane-grown mixed culture (Kim et al., 2002). The transformation capacity of 1,1-DCE was found to be 0.52 µmol/mg TSS. Semprini et al. further examined the transformation capacity of butane-grown microorganisms through modeling of microcosm data. Model simulations of laboratory data determined a 1,1,-DCE transformation capacity 0.175 µmol/mg cells (Semprini et al., 2007b). These results show greater product toxicity of 1,1-DCE than reported by Kim et al. Chang and Alvarez-Cohen reported a transformation capacity of 0.36 µmol/mg TSS for methane-grown pure culture *Methylosinus tricosproium* OB3b (Chang and Alvarez-Cohen, 1996).

2.4 Rhodococcus rhodochrous

Rhodococci are prolific in natural systems and have been isolated from numerous environmental sources including soil, groundwater, and marine sediments (Bell et al., 1998). The metabolic diversity and distinctive enzymatic abilities of *Rhodococci* have permitted an array of applications in the environmental, pharmaceutical, energy, and chemical industries (van der Geize and Dijkhuizen, 2004). The hydrophobic nature of their cell wells contributes to their ability to uptake hydrophobic substrates (Bell et al., 1998). Rhodococcus cells also have the ability to survive in starvation conditions (Martinkova et al., 2009).

Rhodococcus rhodochrous strain ATCC 21198, formally known as *Nocardia paraffinica*, was isolated from soil (Tanaka et al., 1973). This is a gram-positive, aerobic microorganism that experiences optimum growth between 25 to 37°C at a pH between 6.0 and 9.0. 21198 has been shown to grown on a variety of gaseous short-chain alkanes including isobutane, ethane, propane, and n-butane (Babu and Brown, 1984; Hou et al., 1983; MacMichael and Brown, 1987). Rolston estimated the growth yield for isobutane-grown 21198 to be 0.8 mg TSS per mg isobutane (Rolston, 2017). Figure 2.4 shows the results of activity-based protein profiling (ABPP) for the detection of the short chain alkane monooxygenase (SCAM) and the propane monooxygenase (PrMO) in dextrose, ethane, propane, n-butane, and isobutane-grown 21198. The expression of these enzymes is suggested to be the cause of the fortuitous transformations of CAHs and 1,4-dioxane. The expression of 59 kDa is greatest for isobutane-grown 21198, followed by ethane and butane-grown 21198. Propane-grown 21198 minimally expresses this enzyme. PrMO is only expressed by propane-grown 21198 and minimally expressed by isobutane-grown 21198.

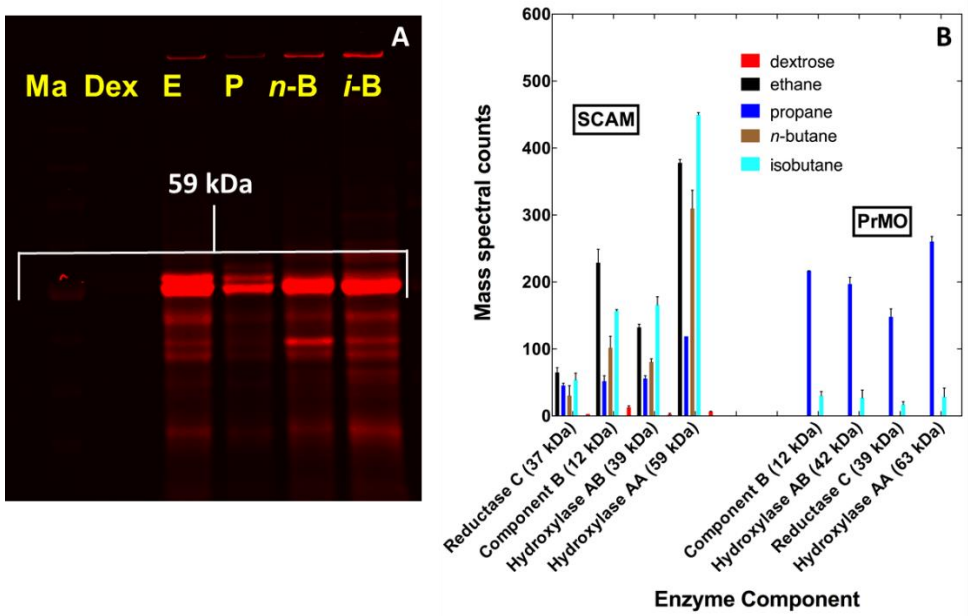

Figure 2.4. *ABPP using 1,7-octadiyne as a probe for detection of SCAM in dextrose, ethane, propane, n-butane, and isobutane-grown 21198 (Hyman).*

Rhodococci have been found to be capable of degrading polychlorinated biphenyls, benzene, toluene, naphthalene, 1,4-dioxane and a range of CAHs including TCE, vinyl chloride, chloroform, 1,1-DCE, and 1,1,1-TCA (van der Geize and Dijkhuizen, 2004; Mahendra and Alvarez-Cohen, 2006; Malachowsky et al., 1994). As discussed in Section 2.3.2, isobutane-grown 21198 has been found to transform 1,4-dioxane and a variety of CAHs including 1,2-DCA, 1,1-DCA, 1,1,2-TCA, 1,1,1-TCA, 1,1-DCE via cometabolism (Bennett et al., 2018; Rich, 2015; Rolston, 2017; Thankitkul, 2016).

2.5 ELW1

ELW1 was isolated from stream sediment collected from the campus of North Carolina State University by Dr. Michael Hyman of North Carolina State University (Kottegoda et al., 2015). The sample was cultured using isobutene as the singular source of carbon and energy. Results from PCR amplification and 16S rRNA sequencing were compared with the Ribosomal Database Project database. ELW1 was found to be most closely related to several strains of *Mycobacterium*, including *Mycobacterium sphagni*, *Mycobacterium* sp. Strain CP-2, and numerous strains of *Mycobacterium* with recognized hydrocarbon-oxidizing capabilities such as *Mycobacterium petroleophilum* ATCC 21497, *Mycobacterium petroleophilum* ATCC 21498, and *Myocbacterium* sp. Strain TA27.

The growth yield was estimated to be 0.38 mg (dry weight) per mg isobutene consume. ELW1 was unable to grown on smaller branched alkanes, such as isobutane and isopentane, or any of the aromatic compounds, C_1 to C_6 n-alkanes, or C_2 to C_5 straight-chain, branched, or chlorinated alkenes tested. Various alkynes were tested to determine their capabilities for monooxygenase inhibition. Propyne and 1-butyne showed strong initial inhibition with decreased effectiveness over time. 1-octyne was an effective inhibitor during long term testing.

Previous studies emphasized the importance of cobalt or cobalamin in the metabolism of methyl tertiary butyl ether (MTBE) and tert-butyl alcohol (TBA) by MTBE and TBA-oxidizing microorganisms (Hyman, 2013). Kottegoda et al. conducted studies of ELW1 growth in both cobalt supplemented and cobalt deficient medium (Kottegoda et al., 2015). It was hypothesized that the degradation pathway of isobutene would be similar to that of MTBE and TBA and thus also exhibit a dependence on the presence of cobalt. ELW1 growth on isobutene was severely impacted by the absence of cobalt ions. These results suggest the metabolism pathway of isobutene by ELW1 involves a cobalt/cobalamin-dependent transformation of the end metabolite product.

The ability of ELW1 to transform environmental COCs has been minimally studied. As discussed in Section 2.4.6, Schrlau et al. researched the capabilities of ELW1 to cometabolize phenanthrene, a polycyclic aromatic hydrocarbon (Schrlau et al., 2017). ELW1 was found to fully transform phenanthrene. Transformation products included 1-hydroxyphenanthrene (OHPHE), 3-OHPHE, 4-OHPHE, 9-OHPHE, 1,9-OHPHE, and trans-9,10-OHPHE. Phenanthrene transformation was moderately inhibited by 1-octyne, suggesting that an alkene monooxygenase is likely the responsible transformation enzyme. Rich conducted a kinetic analysis of the aerobic degradation of chlorinated ethenes by ELW2 (Rich, 2015). ELW1 was able to cometabolize a number of CAHs. The rates of transformation were ordered as follows: vinyl chloride > c-DCE > TCE.

2.6 Naval Air Station North Island Site

The NAS North Island was commissioned in 1917 and has since been used by as a naval hub for the United States Navy (Commander, Navy Installations Command). A volatile organic compound groundwater plume was discovered in the central portion of the island in 2005 (Figure 2.5). Former sludge basins and settling ponds that were located near the NAS North

Island Industrial Waste Treatment Plant were identified as the source of the plume (Aptim Federal Services, LLC, 2018). The site hydrogeology consists of approximately 41 feet of unconfined sand/silty sand underlain by a four- to five-foot thick low permeability silt deposit that acts as a semi-confining layer. The depth to groundwater resides at approximately 25 feet below ground surface (BGS). The proposed treatment zone for this project is within the saturated zone between 25 to 41 BGS. Chlorinated volatile organic compounds and 1,4-dioxane concentrations within the proposed treatment zone range from approximately 1,000 µg/L to 5,000 µg/L. Table 2.3 includes concentration details for specific CAHs and 1,4-dioxane measured in two monitoring wells (SMW-08A and SMW-10A) near the proposed treatment zone. Figure 2.6 identifies the specific locations of the groundwater monitoring wells, SMW-08A and SMW-10A, used in this study.

Table 2.3. *Liquid concentrations of key CAHs and 1,4-dioxane measured at monitoring wells SMW-08A and SMW-10A in December 2016* (Battelle and Accord Engineering, Inc., 2017).

	Liquid Concentration (µg/L)	
Compound	**SMW-08A**	**SMW-10A**
PCE	2.1	12
TCE	720	1,400
1,1,1-TCA	ND	ND
1,1-DCA	46	310
1,2-DCA	1.3	16
c-DCE	37	3,200
t-DCE	1.1	4.5
1,1-DCE	510	3,100
Chloroform	3.2	9.9
Vinyl Chloride	0.75	13
1,4-dioxane	130	3,300

ND: not detected

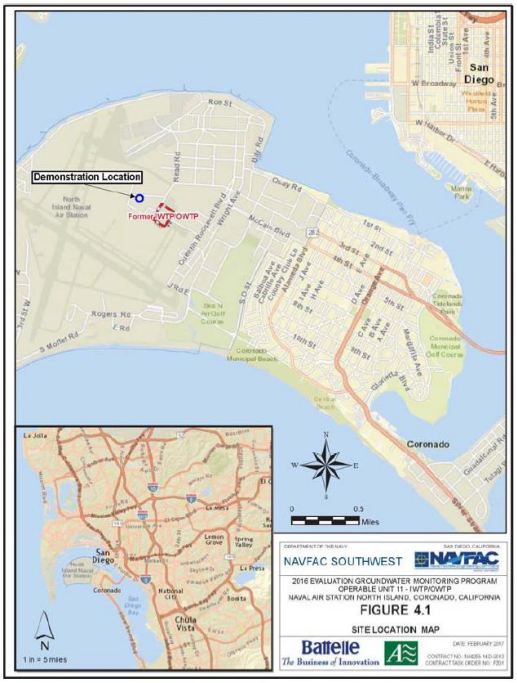

Figure 2.5. *NAS North Island location map (Aptim Federal Services, LLC, 2018).*

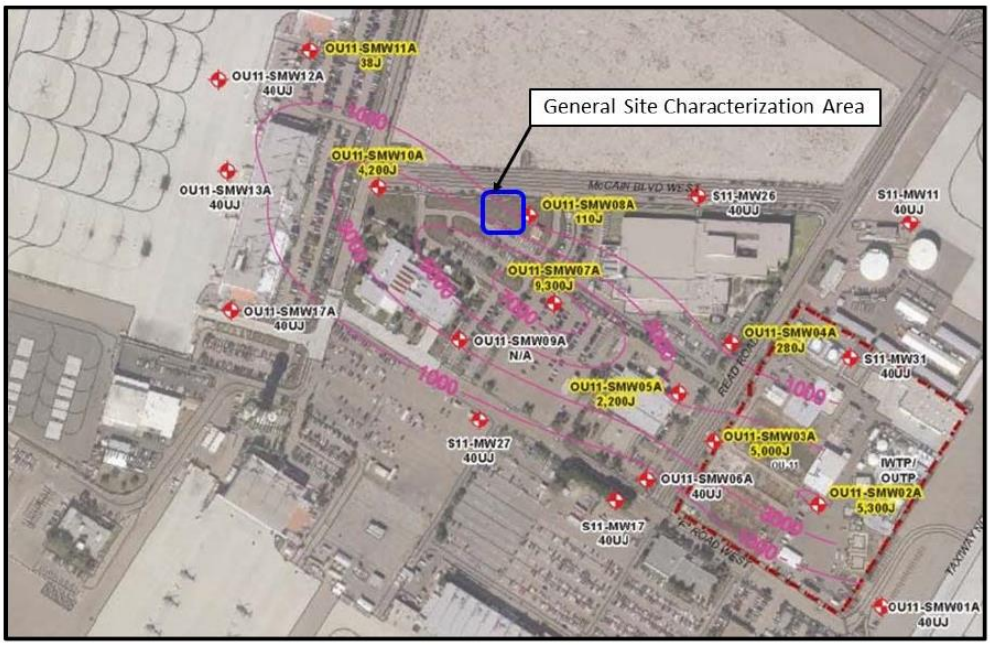

Figure 2.6. *1,4-dioxane isoconcentration contour map at NAS North Island, including monitoring well locations, from December 2015 (Aptim Federal Services, LLC, 2018).*

Due to the composition of the contaminant mixture located on the NAS North Island, aerobic cometabolism has been proposed for the remediation of this site. As described in Sections 2.3.1 through 2.3.6, a variety of primary substrates have been studied for the remediation of CAHs and 1,4-dioxane via cometabolism. The implementation of a multiple primary substrate

biosparging approach could be beneficial at NAS North Island due to the mixture of contaminants present. For instance, methane and/or propane could be better utilized to remediate CAHs, while isobutane could be used to transform 1,4-dioxane.

3.0 MATERIALS AND METHODS

3.1 Chemicals

All chemicals used for experimentation are listed in Table 3.1.

Table 3.1. *Summary of chemical names, CAS numbers, manufactures, and purities used during experimentation.*

Name	CAS No.	Manufacturer	Purity
Ethane	74-84-0	Adlrich Chemical Company	99+%
Isobutane	75-28-5	Gas Innovations	99.99%
Isobutanol	78-83-1	Mallinckrodt AR	99.9%
Isobutene	115-11-7	Aldrich	99%
Propane	74-98-6	Airgas	99%
Propyne	74-99-7	Aldrich Chemical Company	97%
1,4-Dioxane	123-91-1	Baker Analytical	100%
1,1-DCA	75-34-3	Acros	99+%
1,2-DCA	107-06-2	TCI America	>99.5% (GC)
1,1-DCE	75-34-4	Aldrich	99%
cis-DCE	156-59-2	TCI	min. 99.0%
trans-DCE	156-60-5	TCI America	98% (GC)
1,1,1-TCA	71-55-6	Baker Analytical	95.5%
TCE	79-01-6	Macron Fine Chemicals	AR/ACS

3.2 Analytical Methods

All headspace measurements were analyzed via 100 µL headspace samples taken by Hamilton 1700 Series gas-tight syringes to be run through gas chromatographic (GC) analysis. Liquid samples for the analysis of 1,4-dioxane and/or isobutanol were extracted from microcosm bottles or rate test sample vials. Microcosm liquid samples (0.5 mL) were first run on an Eppendorf 5415C Centrifuge at 9,500 revolutions per minute (rpm) for two minutes to separate larger suspended solids. All liquid samples were filtered through 0.2 µm polyvinylidene fluoride syringe tip filters (Phenomenex AF6-5205-12) and stored at 4°C. Liquid isobutanol samples for GC analysis were analyzed using a 25 µL Hamilton 1700 Series gas-tight syringe.

3.2.1 1,4-Dioxane

1,4-dioxane was measured using liquid samples run on a Hewlett Packard 6890 Series GC containing a Restk 30 mx 0.2 mm Rtx-VMS capillary column and a Hewlett Packard 5973 Mass Selective Detector. The gas chromatography-mass spectrometry (GC-MS) was preceded by a Tekmar Dohrmann 3100 sample purge and trap concentrator and an AQUA Tek 70 Liquid Autosampler. Samples were diluted in 40 mL VOA vials to below 50 ppb using 25 to 40 mL of

nano-pure water. An internal standard of 5 µg/L of deuterated 1,4-dioxane (m/z 96) was added to each sample to compare peak areas with 1,4-dioxane (m/z 88). A single ion scan was used to detect low concentrations of 1,4-dioxane. The GC-MS was calibrated by creating a standard curve of varying 1,4-dioxane (m/z 88) concentrations with a constant internal standard of 5 µg/L deuterated 1,4-dioxane (m/z 96).

3.2.2 Chlorinated Aliphatic Hydrocarbons

Chlorinated aliphatic hydrocarbons, including TCE, 1,1-DCE, 1,2-DCA, 1,1,1-TCA, were measured using a Hewlett Packard 6890 Series GC with an electron capture detector (ECD) and an Agilent DB-624 UI 30 mx 0.53 mm capillary column. The carrier gas was helium and ran at a flow rate of either 10 or 15 mL/min, with a constant oven temperature of 50°C. Cis-DCE was measured using a Hewlett Packard 6890 Series GC with a flame ionization detector (FID) and an Agilent 30 mx 0.53 mm capillary column. The carrier gas was helium and ran at a flow rate of 15 mL/min, with a constant oven temperature of 150°C. The GC was calibrated using external standards made from saturated solutions of CAHs.

3.2.3 Alkane and Alkene Hydrocarbons

Alkane and alkene hydrocarbons, including isobutane, propane, ethane, and isobutene, were measured using a Hewlett Packard 6890 Series GC with a FID and an Agilent 30 mx 0.53 mm capillary column. The carrier gas was helium and ran at a flow rate of 15 mL/min, with a constant oven temperature of 150°C. The GC was calibrated by creating a standard curve with varying concentrations of hydrocarbons.

3.2.4 Oxygen

Oxygen (O_2) was measured using a Hewlett Packard 5890 Series II GC with a thermal conductivity detector (TCD) and a Supelco 60/80 Caroboxen 1000 capillary column. The carrier gas was helium and ran at a flow rate of 30 mL/min, with a constant oven temperature of 40°C. The GC was calibrated by creating an external standard via the injection of varying volumes of air, assuming 21% O_2 in atmospheric air.

3.2.5 Carbon Dioxide

Carbon dioxide (CO_2) was measured using a Hewlett Packard 5890 Series II GC with a TCD and a Supelco 60/80 Caroboxen 1000 stainless steel capillary column. The carrier gas was argon and

ran at a flow rate of 30 mL/min, with a constant oven temperature of 220°C. The GC was calibrated by creating an external standard via the injection of varying volumes of 10% CO_2.

3.2.6 Isobutanol

Isobutanol was measured using 5 µL liquid injections on a Hewlett Packard 5890 Series II GC with a FID and a Supelco 80/100 Carbopack-C packed column (6ft x 1/8 in.). The carrier gas was nitrogen at a constant column pressure of 40 pounds per square inch, with a constant oven temperature of 105°C. Before samples were run, the oven temperature was set to 150°C for approximately 10 minutes in order to clear the column. The GC was calibrated using an external standard made from a stock solution of isobutanol.

3.2.7 Biomass

Biomass was quantified using total suspended solids (TSS) analysis. A 0.45 µm mixed cellulose ester membrane (Advantec A045A047A) and a glass filtration apparatus were used to filter 0.5 or 0.8 mL of concentrated cell solution. The membrane was then placed into a 103°C drying oven for 15 minutes to obtain the dry weight of cells present in the cell solution.

3.2.8 Calculations

The total masses (M_T) of CAHs were calculated assuming equilibrium between liquid and gas phase partitioning. The dimensionless Henry's constant (H_{cc}) was evaluated using Equation 1 and specifies the ratio of the gas concentration (C_g) to the liquid concentration (C_l). The total mass was calculated using Equation 2, where V_l is the liquid volume of the reactor, and V_g is the gas volume of the reactor. The Henry's constants using for experimentation are reported in Table 3.2.

$$H_{cc} = \frac{C_g}{C_l} \tag{1}$$

$$M_T = (C_l \cdot V_l) + (C_g \cdot V_g) \tag{2}$$

Table 3.2. *Henry's constants for select CAHs used during experimentation (Mackay and Shiu, 1981).*

Compound	Henry's constant @ 20°C (dimensionless)
TCE	0.296
c-DCE	0.122
1,1-DCE	0.857
1,1,1-TCA	0.548
1,2-DCA	0.179

Zero order degradation rates (r) were calculated by taking the slope of the first three to four points on a plot of the total mass of a compound per initial quantity of biomass (X_0, in mg TSS) over time (t), as shown in Equation 3.

$$r = \frac{M_T / X_0}{t} \tag{3}$$

The transformation capacity (T_C) of a compound was calculated using Equation 4, where M_i is the initial total mass (µmol) and M_f is the final total mass (µmol). Compounds that exhibited higher transformation capacities required multiple additions of the CAH of interest over time until the biomass was exhausted and transformation was no longer observed.

$$T_C = \frac{\sum M_i - M_f}{X_0} \tag{4}$$

3.3 Pure Culture Studies

3.3.1 Inoculation

Autoclaved 720 mL glass Wheaton bottles containing 300 mL of autoclaved media solution (1X MV media - recipe shown in Appendix A) were used to grow 21198 and ELW1 in batch pure cultures. Both 21198 and ELW1 were obtained from Michael Hyman at North Carolina State University. 21198 and ELW1 were incubated on minimal salts media growth plates (recipe shown in Appendix B) at 30°C in 3.5 L canisters with 1-2% v/v isobutane/air and isobutene/air, respectively. All work with pure cultures was performed under a laminar flow hood to prevent contamination. A colony of 21198 or ELW1 were aseptically removed from a media plate to inoculate the growth reactor bottles. The bottles were then sealed with autoclaved butyl septa and plastic caps, injected with the primary growth substrate of choice, and stored at 30°C on a shaker table at 200 rpm until significant growth was observed (Table 3.3). The specified volume of primary growth substrate was chosen to keep the levels above the upper explosive limit .

Table 3.3. *Required volume of primary growth substrate and the typical number of days of incubation prior to refreshing.*

	21198			ELW1
	Isobutane	**Propane**	**Ethane**	**Isobutene**
Volume of Gas Added (mL)	45	45	60	50
Days of Incubation Before Refreshing	4-5	5-6	6-10	6-7

After significant growth was observed, the bottles were opened for approximately 15 minutes in a laminar flow hood to allow for oxygen replenishment, and the required volume of primary growth substrate was re-injected into the headspace. The bottles were returned to the shaker table for an additional 24 hours of growth time to allow the cells to reach late exponential growth phase. At the time of refreshing, heterotrophic growth plates (recipe shown in Appendix B) were streaked with cell solution to determine the purity of the culture prior to use.

3.3.2 Harvesting

The growth reactor cell solution was centrifuged in 50 mL centrifuge tubes using a Beckman Coulter Allegra 21 Centrifuge for 15 minutes at 4,000 relative centrifugal force (RCF) in order to concentrate the cells. The cells were then rinsed in the centrifuge for 15 minutes at 4,000 RCF with 50 mmol/L monosodium phosphate buffer (pH 7). The cells were then resuspended in five to ten mL of buffer to reach the desired biomass concentration (between 7 and 20 mg TSS/L) and stored at 4°C prior to use. The cells were typically stored for no longer than one to two days prior to experimental use.

3.3.3 Rate Test Experimental Design

10 mL of 1X MV media were added to autoclaved 27 mL glass vials. The vials were sealed with autoclaved butyl rubber septa and metal crimp tops. Typical tests were comprised of four vials, including one with 2 mL of acetylene or propyne added to the headspace to act as a monooxygenase blocker, one without cells (abiotic), and two duplicate 'active' vials. Based on the results of the TSS test, the required volume of concentrated cell solution was calculated in order to obtain a desired cell mass. Between 4 and 14 mg of TSS were added to the active and acetylene or propyne control vials. The acetylene or propyne was allowed to interact with the cells for approximately one hour prior to adding the contaminant interest to ensure the monooxygenase enzymes were effectively blocked. CAHs were added to the vials via injection of a saturated solution of the compound of interest. 1,4-dioxane was added to the vials via

injection of a 1,000 mg/L stock solution. CAHs were added to the vials and allowed to equilibrate on a shaker table at 200 rpm for approximately 15 minutes prior to initial headspace measurements on the GC-ECD. The cells were added to the active bottles after equilibration of the CAHs had been achieved.

Table 3.4 provides a summary of rate test experimental design parameters for single-compound tests. Table 3.5 provides a summary of rate test experimental design parameters for mixture tests. The tables describe the compound tested, the growth substrate and culture used, the volume of compound added, the initial liquid concentration of the compound, the mass of TSS added, and the resulting biomass concentration. Tests were performed at different biomass concentrations depending on the anticipated rate of contaminant transformation.

Table 3.4. *Summary of rate test experimental design parameters, including the volume of solution added, initial liquid concentration, mass of TSS, and biomass concentration for single-compound tests.*

Compound	Growth Substrate/ Culture	Volume of Soln. Added (µL)	Initial Liq. Conc. (µmol/L)		Mass of TSS Added (mg)	Biomass Conc. (mg/L)
			Avg.	SD		
1,4-D	Isobutane-grown 21198	175 µL of 1,000 ppm stock	168	24.2	5.0	~ 500
	Ethane-grown 21198		244	16.7	5.0	467
	Propane-grown 21198		235	10.4	5.0	467
	Isobutene-grown ELW1		176	1.1	5.1	496
TCE	Isobutane-grown 21198	10	10.2	0.5	12.9	1,212
	Ethane-grown 21198		10.3	0.6	11.8	1,050
	Propane-grown 21198		12.7	0.5	12.8	1,146
	Isobutene-grown ELW1		9.3	0.2	13.0	1,200
1,1-DCE	Isobutane-grown 21198	20	24.4	0.8	4.0	393
		60	69.8	1.4		
	Ethane-grown 21198	20	25.3	0.1	3.9	391
		60	70.3	2.4		
	Propane-grown 21198	20	27.3	0.6	4.2	405
		60	71.8	0.8		
	Isobutene-grown ELW1	20	27.2	0.6	4.0	389
		60	76.3	2.0		
c-DCE	Isobutane-grown 21198	80	292	1.1	11.8	1,073
	Ethane-grown 21198		293	7.2	10.8	982
	Propane-grown 21198		269	4.6	9.5	881
1,2-DCA	Isobutane-grown 21198	40	242	0.5	11.8	1,073
	Ethane-grown 21198		241	3.7	12.8	1,193
	Propane-grown 21198		237	4.8	12.9	1,173
1,1,1-TCA	Isobutane-grown 21198	100	39.1	1.2	13.1	1,197
	Ethane-grown 21198		41.0	0.2	12.8	1,193
	Propane-grown 21198		38.9	1.7	13.1	1,179

Table 3.5. *Summary of rate test experimental design parameters, including the volume of solution added, initial liquid concentration, mass of TSS, and biomass concentration for mixture tests.*

Growth Substrate/ Culture	Compound	Volume of Soln. Added (µL)	Initial Liq. Conc. (µmol/L)		Mass of TSS Added (mg)	Biomass Conc. (mg/L)
			Avg.	SD		
Isobutane-grown 21198	1,4-D	50 µL 1,000 ppm stock	51.4	0.2	5.0	476
	TCE	5	4.71	0.14		
	1,1-DCE	5	2.98	0.13		
	1,1,1-TCA	5*	0.51	0.027		
	1,2-DCA	5	8.78	0.57		
Ethane-grown 21198	1,4-D	50 µL 1,000 ppm stock	66.1	0.8	5.1	487
	TCE	5	4.27	0.05		
	1,1-DCE	5	2.77	0.05		
	1,1,1-TCA	5	4.71	0.04		
	1,2-DCA	5	5.77	0.02		
Propane-grown 21198	1,4-D	50 µL 1,000 ppm stock	49.7	1.0	5.0	478
	TCE	5	4.59	0.16		
	1,1-DCE	5	2.79	0.05		
	1,1,1-TCA	5*	0.014	0.001		
	1,2-DCA	5	8.25	0.03		
Isobutene-grown ELW1	1,4-D	50 µL 1,000 ppm stock	60.3	7.7	4.9	484
	TCE	5	4.17	0.003		
	1,1-DCE	5	2.58	0.10		
	1,1,1-TCA	5	5.21	0.29		
	1,2-DCA	5	6.18	0.07		
Isobutene-grown ELW1	1,4-D	50 µL 1,000 ppm stock	71.8	4.3	5.5	536
	TCE	5	4.74	0.02		
	1,1-DCE	5	3.56	0.02		
	1,1,1-TCA	5	6.58	0.10		
	1,2-DCA	5	6.98	0.16		
	Isobutene	~1000	258	0.2		

*Likely less than 5 µL added to solution; inaccurate syringe needle

3.4 Microcosm Studies

Groundwater used for microcosm construction was obtained from monitoring wells SMW-08A and SMW-10A located on the NAS North Island in San Diego, California. The groundwater was stored at 4°C in one liter plastic bottles. Aquifer cores were not available for experimentation; therefore, groundwater sampling was conducted to include trace amounts of aquifer sediments. The minor amount of aquifer solids present in the groundwater appeared to consist of sand and silt. Analyses for particle size, nutrient content, and organic matter were not conducted.

Groundwater was shaken to suspend the aquifer solids, and 50 mL were measured into autoclaved 155 mL glass Wheaton bottles (Figure 3.1). The bottles were sealed with autoclaved butyl septa and plastic caps, and the caps were wrapped with parafilm. All microcosms were incubated at 20°C on a 200 rpm shaker table.

Table 3.6 shows a summary of the days of the experiment additions or amendments were made to each microcosm. Seven sets of microcosms were operated and will be further described in the following sections. Sets 1 through 4 utilized isobutane as a primary growth substrate and incorporated experimentation with nitrogen sparging to remove CAHs and with bioaugmentation with isobutane-grown 21198. Sets 5 and 7 examined the use of isobutanol as a primary growth substrate. As shown in Figure 2.3, isobutanol is the product of isobutane transformation by SCAM. The purpose of this approach was to determine if isobutane-utilizers could be could stimulated in the presence of isobutane and CAHs via the direct addition of the isobutane metabolite, isobutanol. Set 6 explored the use of isobutene as a primary growth substrate and incorporated bioaugmentation with isobutene-grown ELW1. Each microcosm set is further described in Sections 3.4.1 through 3.4.7.

Microcosms were analyzed for oxygen content prior to re-spikes of primary substrate and COCs. Pure oxygen was added to the headspace if oxygen content was low (less than ~3,000 umol/L gas concentration). Microcosms received nutrients via the addition of 200 µL of 1X MV media. This quantity of media corresponds to a concentration of approximately 620 µmol N/L and 240 µmol P/L. Monooxygenase inhibitors acetylene (for isobutane microcosms) or propyne (for isobutene microcosms) were added to control microcosms at 2% headspace concentration.

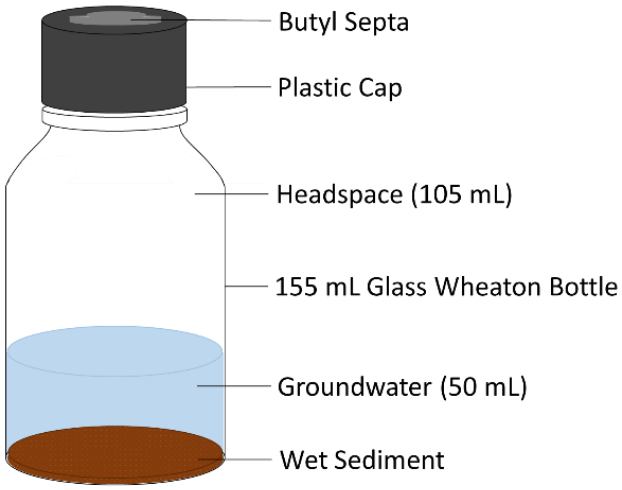

Figure 3.1. *Microcosm schematic diagram.*

Table 3.6. *Summary of days of the experiment additions or amendments were made to each microcosm.*

	MW[1]	Sparged[2]	Media[3]	Isobutane[4]	Isobutene[5]	Isbutanol[6]	Acetylene[7]	Propyne[8]	21198 Bioaug.[9]	ELW1 Bioaug.[10]
Set 1 - Native Groundwater and Isobutane										
B 1/2	SMW-08A	--	--	0	--	--	--	--	--	--
B 3/4	SMW-08A	--	0	0	--	--	--	--	--	--
B 5/6	SMW-08A	--	0	0	--	--	0	--	--	--
B 7/8	SMW-08A	--	--	--	--	--	--	--	--	--
Set 2 - Sparged Groundwater and Isobutane										
B 1	SMW-08A	0	24	0	--	--	--	--	--	--
B 2	SMW-08A	0	24	0,32	--	--	--	--	--	--
B 3	SMW-08A	0	0,65	0,19,20,25,33, 40,47,61,82	--	--	--	--	--	--
B 4	SMW-08A	0	0,65	0,19,20,25,33, 40,47,61,82	--	--	--	--	--	--
B 5/6	SMW-08A	0	0	0	--	--	0	--	--	--
Set 3 - Bioaugmentation with 0.5 mg of Isobutane-Grown 21198										
B 1	SMW-08A	--	0,58	0,5,12,13,14, 26,33,40,54,75	--	--	--	--	0	--
B 2	SMW-08A	--	0,58	0,12,14,18,26, 33,40,54,75	--	--	--	--	0	--
B 3/4	SMW-08A	--	0	0	--	--	0	--	0	--
Set 4 - Bioaugmentation with 0.7 mg of Isobutane-Grown 21198										
B 1	SMW-08A	--	0,53	0,7,9,21,28, 35,49,70	--	--	--	--	0	--
B 2	SMW-08A	--	0,53	0,7,9,21,28, 35,49,70,103	--	--	--	--	0	--
B 3/4	SMW-08A	--	0	0	--	--	0	--	0	--
Set 5 - Isobutane Data Confirmation and Isobutanol Experimentation										
B 1/2	SMW-08A	--	0	0	--	--	--	--	--	--
B 3	SMW-08A	23,51	0	0*,23,51, 76	--	--	--	--	--	--
B 4	SMW-08A	23,51	0	0*,23,51	--	--	--	--	--	--
B 5/6	SMW-08A	--	0	0	--	23,43,55, 58,63, 70	--	--	--	--
B 7/8	SMW-08A	--	0	0	--	--	0	--	--	--
B 9/10	SMW-10A	--	0	0	--	--	--	--	--	--
Set 6 - Native Groundwater with Isobutene										
B 1	SMW-08A	--	0	--	0, 67, 74, 83	--	--	--	--	--
B 2	SMW-08A	--	0	--	0	--	--	--	--	--
B 3/4	SMW-08A	25,29	0	--	0,25,29	--	--	--	--	--
B 5/6	SMW-08A	--	0	--	0,40, 67	--	--	--	--	33
B 7/8	SMW-08A	--	0	--	0	--	0	20	--	--
Set 7 - Sparged Groundwater with Isobutane/Isobutene and Isobutanol Experimentation										
B 1/2	SMW-08A	0	0	0	--	--	--	--	--	--
B 3/4	SMW-08A	0	0	--	0	--	--	--	--	--
B 5-8	SMW-08A	--	0	17	--	0,10,12, 30, 37, 39	--	--	--	--

Notes:
1. Indicates the monitoring well from which groundwater was used
2. Indicates the day of experiment that groundwater was sparged with nitrogen
3. Indicates the day of experiment that 0.2 mL of 1X MV media was added to the microcosm
4. Indicates the day of experiment that 1.26 mL of isobutane was added to the microcosm
5. Indicates the day of experiment that 1.26 mL of isobutene was added to the microcosm
6. Indicates the day of experiment that 0.3 mL of 8,333.3 ppm isobutanol was added to the microcosm
7. Indicates the day of experiment that 2.1 mL of acetylene were added to the microcosm
8. Indicates the day of experiment that 2.1 mL of propyne were added to the microcosm
9. Indicates the day of experiment that the microcosm was bioagumented with 0.5 mg (set 3) or 0.7 mg (set 4) of 21198
10. Indicates the day of experiment that the microcosm was bioaugmented with 0.9 g of ELW1

-- : not applicable for the listed microcosm

* : 15 mL of isobutane were added added to the microcosm

3.4.1 Set 1: Native groundwater, isobutane

Table 3.7 indicates the initial liquid concentrations of isobutane, 1,1-DCE, TCE, and 1,4-D in bottles 1 through 8 in microcosm set 1. The initial liquid concentration of 1,1-DCE is variable for each bottle due to the volatilization of 1,1-DCE during the open-system transfer of groundwater from the plastic 1 L storage bottle to the microcosm bottles. Media addition was omitted from bottles 1 and 2 to determine if nutrients were required for the system.

Table 3.7. *Initial liquid concentrations and total masses of isobutane, 1,1-DCE, TCE, and 1,4-Dioxane in set 1, bottles 1 through 8.*

	Isobutane		1,1-DCE		TCE		1,4-Dioxane	
Bottle #	Initial Liq. Conc. (ug/L)	Total Mass (µmol)	Initial Liq. Conc. (ug/L)	Total Mass (µmol)	Initial Liq. Conc. (ug/L)	Total Mass (µmol)	Initial Liq. Conc. (ug/L)	Total Mass (µmol)
1	563	51	93	0.13	292	0.18	190	0.11
2	575	52	222	0.32	451	0.28	177	0.10
3	539	49	220	0.32	417	0.26	161	0.09
4	578	52	137	0.20	380	0.23	275	0.16
5	537	48	148	0.21	365	0.23	301	0.17
6	567	51	76	0.11	315	0.19	247	0.14
7	0	0	202	0.29	398	0.25	295	0.17
8	0	0	68	0.10	295	0.18	173	0.10

3.4.2 Set 2: Sparged groundwater, isobutane

In order to determine if native isobutane-utilizers could be stimulated in the absence of CAHs, groundwater was sparged with nitrogen to remove CAHs from solution. Table 3.8 indicates the initial liquid concentrations of isobutane, 1,1-DCE, TCE, and 1,4-D in bottles 1 through 6 in microcosm set 2. Media addition was initially omitted from bottles 1 and 2 to determine if nutrients were required for the system. Media was later added to bottles 1 and 2 on day 24 of incubation. On day 25, TCE was reintroduced to bottles 3 through 6 to a liquid concentration of approximately 300 µg/L after three to four additions of isobutane to bottles 3 and 4. 1,4-dioxane was also added at a concentration of approximately 3,500 µg/L on day 25. On day 33, 1,1-DCE was reintroduced to bottles 3 through 6 to a liquid concentration of approximately 150 µg/L. The concentration of TCE was gradually increased over five subsequent additions, culminating in a liquid concentration of approximately 4.5 mg/L.

Table 3.8. *Initial liquid concentrations and total masses of isobutane, 1,1-DCE, TCE, and 1,4-Dioxane in set 2, bottles 1 through 6.*

	Isobutane		1,1-DCE		TCE		1,4-Dioxane	
Bottle #	Initial Liq. Conc. (ug/L)	Total Mass (µmol)	Initial Liq. Conc. (ug/L)	Total Mass (µmol)	Initial Liq. Conc. (ug/L)	Total Mass (µmol)	Initial Liq. Conc. (ug/L)	Total Mass (µmol)
1	572	51	0	0	0.9	0.001	137	0.08
2	589	53	0	0	2.6	0.002	105	0.06
3	559	50	0	0	1.1	0.001	111	0.06
4	538	48	0	0	1.3	0.001	116	0.07
5	570	51	0	0	1.4	0.001	114	0.06
6	551	50	0	0	1.8	0.001	129	0.07

3.4.3 Set 3: Bioaugmentation with 0.5 mg isobutane-grown 21198

Table 3.9 indicates the initial liquid concentrations of isobutane, 1,1-DCE, TCE, and 1,4-D in bottles 1 through 4 in microcosm set 3. Media was added to all bottles to supply nutrients. 0.5 mg of isobutane-grown 21198 (cultured as described in Sections 3.3.1 and 3.3.2) was added to all bottles. Five to six additions of isobutane were made to bottles 1 and 2 over the first 18 days of incubation. The concentration of TCE was gradually increased over the course of 75 days with five subsequent additions, culminating in a liquid concentration of approximately 4.5 mg/L. On day 18, 0.2 mL of 1,000 mg/L 1,4-dioxane was added to bottles 1 through 4, for an initial liquid concentration of 3,000 to 4,000 µg/L. The concentrations of 1,1-DCE and 1,4-dioxane were kept constant with subsequent re-spikes.

Table 3.9. *Initial liquid concentrations and total masses of isobutane, 1,1-DCE, TCE, and 1,4-Dioxane in set 3, bottles 1 through 4.*

	Isobutane		1,1-DCE		TCE		1,4-Dioxane	
Bottle #	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)
1	595	54	145	0.21	265	0.16	113	0.06
2	608	55	176	0.25	300	0.19	138	0.08
3	600	54	191	0.28	293	0.18	115	0.07
4	604	54	235	0.34	329	0.20	119	0.07

3.4.4 Set 4: Bioaugmentation with 0.7 mg isobutane-grown 21198

Table 3.10 indicates the initial liquid concentrations of isobutane, 1,1-DCE, TCE, and 1,4-D in bottles 1 through 4 in microcosm set 3. Media was added to all bottles to supply nutrients. 0.7 mg of isobutane-grown 21198 (cultured as described in Sections 3.3.1 and 3.3.2) was added to all bottles. Four additions of isobutane were made to bottles 1 and 2 over the first 20 days of incubation. The concentration of 1,1-DCE was gradually increased over the course of 103 days with four subsequent additions to bottle 1 and five additions to bottle 2, culminating in a liquid concentration of approximately 1530 µg/L and 830 µg/L, respectively. On day 21, 0.2 mL of

1,000 mg/L 1,4-dioxane was added to bottles 1 through 4, for an initial liquid concentration of 3,000 to 4,000 µg/L. The concentration of 1,4-dioxane were kept constant with subsequent re-spikes. The concentration of TCE in bottles 1 and 2 was increased from approximately 230 µg/L to approximately on day 950 µg/L on day 35.

Table 3.10. *Initial liquid concentrations and total masses of isobutane, 1,1-DCE, TCE, and 1,4-Dioxane in set 4, bottles 1 through 4.*

	Isobutane		1,1-DCE		TCE		1,4-Dioxane	
Bottle #	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)
1	613	55	66	0.10	228	0.14	132	0.07
2	622	56	76	0.11	228	0.14	115	0.07
3	592	53	81	0.12	233	0.14	87	0.05
4	619	56	110	0.16	276	0.17	134	0.08

3.4.5 Set 5: Isobutane data confirmation and isobutanol experimentation

The microcosms in set 5 were established to confirm the results from sets 1 and 2, as well as study the addition of isobutanol as a primary growth substrate. As shown in Figure 2.3, isobutanol is the product of isobutane transformation by SCAM. The purpose of this approach was to determine if isobutane-utilizers could be could stimulated in the presence of isobutane and CAHs via the direct addition of the isobutane metabolite, isobutanol.

Table 3.11 indicates the initial liquid concentrations of isobutane, 1,1-DCE, TCE, and 1,4-D in bottles 1 through 10 in microcosm set 5. Media was added to all bottles to supply nutrients. 0.2 mL of 1,000 mg/L 1,4-dioxane was also added to bottles 1 through 8, for an initial liquid concentration of 3,000 to 4,000 µg/L. Bottles 3 and 4 were sparged with nitrogen on day 23 to remove CAHs and again on day 51 to further remove CAHs that had desorbed back into solution. Isobutane was re-added to the headspace of bottles 3 and 4 following the sparging events. Isobutanol was added to bottles 5 and 6 on day 23 at an initial liquid concentration of approximately 45 mg/L. Five additional re-spikes of isobutanol were added to these bottles between days 43 and 70. Oxygen and carbon dioxide were subsequently monitored in these bottles.

Table 3.11. *Initial liquid concentrations and total masses of isobutane, 1,1-DCE, TCE, and 1,4-Dioxane in set 5, bottles 1 through 10.*

	Isobutane		1,1-DCE		TCE		1,4-Dioxane	
Bottle #	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)
1	580	59	160	0.23	319	0.17	3,282	1.86
2	616	63	151	0.21	333	0.18	3,648	2.07
3	6,609	686	163	0.23	346	0.19	3,764	2.14
4	6,724	689	149	0.21	337	0.18	3,706	2.10
5	590	60	150	0.21	364	0.20	3,900	2.21
6	619	63	180	0.26	346	0.19	3,423	1.94
7	624	64	145	0.21	322	0.17	3,523	2.00
8	610	62	155	0.22	332	0.18	3,352	1.90
9	598	61	385	0.55	338	0.18	2,495	1.42
10	642	66	429	0.61	326	0.18	2,339	1.33

3.4.6 Set 6: Native groundwater with isobutene

The microcosms in set 6 were established to determine if native isobutene-utilizers could be stimulated in the presence of CAHs and to evaluate the use of bioaugmentation with isobutene-grown ELW1

Table 3.12 indicates the initial liquid concentrations of isobutene, 1,1-DCE, TCE, and 1,4-D in bottles 1 through 8 in microcosm set 6. Media was added to all bottles to supply nutrients. 0.2 mL of 1,000 mg/L 1,4-dioxane was also added to bottles 1 through 8, for an initial liquid concentration of 3,000 to 4,000 µg/L. Bottles 3 and 4 were sparged with nitrogen on day 25 to remove CAHs and again on day 29 to further remove CAHs that had desorbed back into solution. Isobutene was re-added to the headspace of bottles 3 and 4 following the sparging events. 0.9 mg of isobutene-grown ELW1 (cultured as described in Sections 3.3.1 and 3.3.2) was added to bottles 5 and 6 on day 33 of incubation. On day 40, bottles 5 and 6 were re-spiked with isobutene, TCE, and 1,1-DCE to liquid concentrations of approximately 3,100 µg/L, 530 µg/L and 270 µg/L, respectively.

Table 3.12. *Initial liquid concentrations and total masses of isobutane, 1,1-DCE, TCE, and 1,4-Dioxane in set 6, bottles 1 through 8.*

	Isobutene		1,1-DCE		TCE		1,4-Dioxane	
Bottle #	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)
1	2,905	50	225	0.33	373	0.23	3,469	1.97
2	3,025	52	234	0.34	347	0.21	3,229	1.83
3	2,987	51	240	0.35	381	0.23	3,596	2.04
4	3,021	52	205	0.30	404	0.25	3,432	1.95
5	3,174	54	224	0.32	390	0.24	3,433	1.95
6	3,109	53	222	0.32	396	0.24	3,423	1.94
7	2,979	51	218	0.32	395	0.24	3,770	2.14
8	3,101	53	228	0.33	393	0.24	3,460	1.96

3.4.7 Set 7: Sparged groundwater with isobutane/isobutene and isobutanol experimentation

The microcosms in set 7 were established to confirm the results from sets 2 and 5. As shown in Figure 2.3, isobutanol is the product of isobutane transformation by SCAM. The purpose of this approach was to determine if isobutane-utilizers could be could stimulated in the initial absence of isobutane and in the presence of CAHs via the direct addition of the isobutane metabolite, isobutanol.

Table 3.13 indicates the initial liquid concentrations of isobutane, 1,1-DCE, TCE, and 1,4-D in bottles 1 through 8 in microcosm set 7. Media was added to all bottles to supply nutrients. Isobutane was added to the headspaces of bottles 1 and 2 at an initial liquid concentration of approximately 580 µg/L, and isobutene was added to headspaces of bottles 3 and 4 at an initial liquid concentration of approximately 2,800 µg/L. Isobutanol was added to bottles 5 through 8 at an initial liquid concentration of approximately 45 mg/L. 0.2 mL of 1,000 mg/L 1,4-dioxane was also added to bottles 5 through 8, for an initial liquid concentration of 3,000 to 4,000 µg/L. Two subsequent additions of approximately 45 mg/L isobutanol were added to bottles 5 through 8 on days 10 and 13 before isobutane was added to the headspace on day 17 at an initial liquid concentration of approximately 600 µg/L. Three additional re-spikes of isobutanol were added to these bottles between days 30 and 45. Oxygen and carbon dioxide were subsequently monitored in these bottles.

Table 3.13. *Initial liquid concentrations of isobutane, 1,1-DCE, TCE, and 1,4-Dioxane in set 7, bottles 1 through 8.*

Bottle #	Isobutane		Isobutene		1,1-DCE		TCE		1,4-Dioxane	
	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)	Initial Liq. Conc. (µg/L)	Total Mass (µmol)
1	580	52	0	0	0	0	3.1	0.002	278	0.16
2	587	53	0	0	0	0	0.9	0.001	303	0.17
3	0	0	2,867	49	0	0	0.7	0.000	213	0.12
4	0	0	2,913	50	0	0	0.7	0.000	264	0.15
5	0	0	0	0	175	0.25	384	0.24	3,000-4,000	1.7-2.2
6	0	0	0	0	196	0.28	432	0.27	3,000-4,000	1.7-2.2
7	0	0	0	0	208	0.30	404	0.25	3,000-4,000	1.7-2.2
8	0	0	0	0	188	0.27	400	0.25	3,000-4,000	1.7-2.2

4.0 RESULTS AND DISCUSSION

4.1 Pure Culture Studies

Resting cell rate tests were performed with isobutane, ethane, and propane-grown 21198 and isobutene-grown ELW1 in order to identify potential effective primary substrates to promote the cometabolic transformation of COCs. As previously shown in Figure 2.4, the relative quantity of monooxygenase expression of 21198 has been found to vary based on the primary growth substrate. The results indicate that the isobutane-grown 21198 produces the greatest quantity of SCAM expression, followed by ethane and then propane-grown 21198. It was hypothesized that isobutane-grown 21198 would demonstrate the most effective transformation of COCs (i.e. fastest zero order degradation rate and highest transformation capacity), followed by ethane and then propane-grown 21198. As noted in section 2.5, minimal studies have been conducted to investigate the abilities of isobutene-grown ELW1 to cometabolize CAHs and 1,4-dioxane. ELW1 was employed in this study as a model isobutene-utilizer to determine its potential effectiveness for CAH and 1,4-dioxane cometabolism.

Resting cell rate tests included single-compound analyses for TCE, 1,1,1-TCA, 1,1-DCE, c-DCE, 1,2-DCA, and 1,4-dioxane as well as mixtures of the afore listed COCs. The results from these experiments are presented in Sections 4.1.1 to 4.1.5. Data plots for all conducted rate tests are included in Appendix C.

4.1.1 Isobutane-grown 21198

Figure 4.1 depicts the results from a typical rate test, showing the total mass of 1,1-DCE per mg TSS over time for isobutane-grown 21198. The transformation of 1,1-DCE occurs rapidly. The degradation rate moderately changes between high and low initial 1,1-DCE concentrations (30.5 ± 4.4 µmol/mg TSS/d at an initial concentration of approximately 25 µmol/L versus 39.3 ± 5.8 µmol/mg TSS/d at an initial concentration of approximately 70 µmol/L). The transformation capacity for 1,1-DCE was determined to be 0.66 µmol/mg TSS at a high initial concentration and 0.51 µmol/mg TSS at a low initial concentration. Acetylene actively blocked the degradation of all COCs tested, suggesting monooxygenase enzymes are involved in the transformations of these compounds.

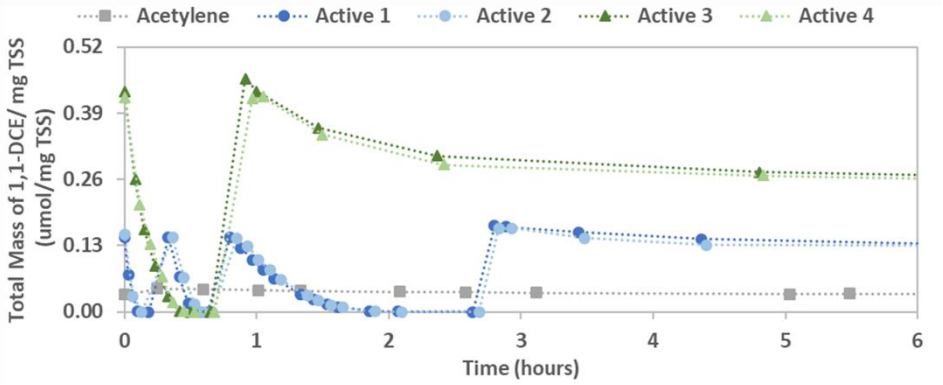

Figure 4.1. *Total mass of 1,1-DCE per mg TSS over time for isobutane-grown 21198. Approximately 4 mg of cells were used for experimentation.*

As shown in Figure 4.2, the zero order degradation rates of CAHs and 1,4-dioxane by isobutane-grown 21198 were ordered in the following rank: 1,1-DCE > 1,2-DCA > 1,4-dioxane > c-DCE > 1,1,1-TCA > and TCE. These results are consistent with the findings of Thankitkul (Thankitkul, 2016). These results are also fairly similar to those of Kim et al. in that more chlorinated compounds (TCE and 1,1,1-TCA) exhibited slower rates of transformation than less chlorinated compounds (1,1-DCE, c-DCE, and 1,2-DCA) in a butane-grown mixed culture (Kim et al., 2000).

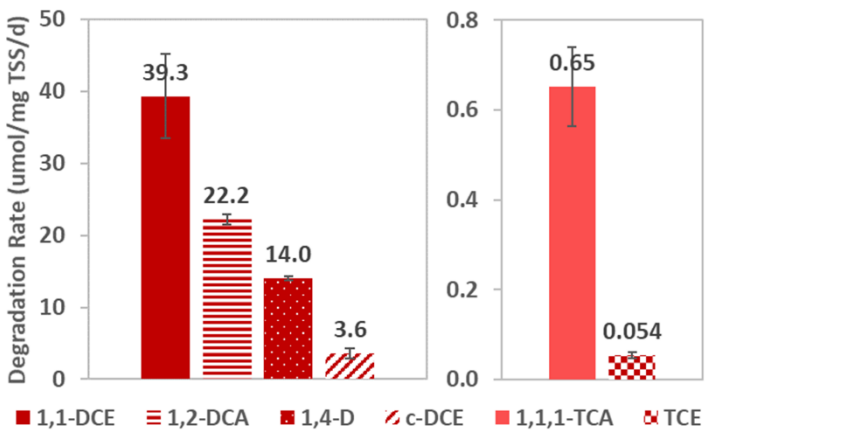

Figure 4.2. *Comparison of zero order degradation rates of 1,1-DCE, 1,2-DCA, 1,4-dioxane, c-DCE, 1,1,1-TCA, and TCE for isobutane-grown 21198. Error bars indicate the standard deviation of a two sample average.*

4.1.2 Ethane-grown 21198

Figure 4.3 depicts the results from a typical rate test, showing the total mass of 1,1-DCE per mg TSS over time for ethane-grown 21198. The transformation of 1,1-DCE occurs rapidly. The degradation rate is similar between high and low initial 1,1-DCE concentrations (25.2 ± 0.3 µmol/mg TSS/d at an initial concentration of approximately 25 µmol/L versus 26.7 ± 0.7 µmol/mg TSS/d at an initial concentration of approximately 70 µmol/L). The transformation

capacity for 1,1-DCE was determined to be 0.78 µmol/mg TSS at a high initial concentration and 0.58 µmol/mg TSS at a low initial concentration. Acetylene actively blocked the degradation of all COCs tested, suggesting monooxygenase enzymes are involved in the transformations of these compounds.

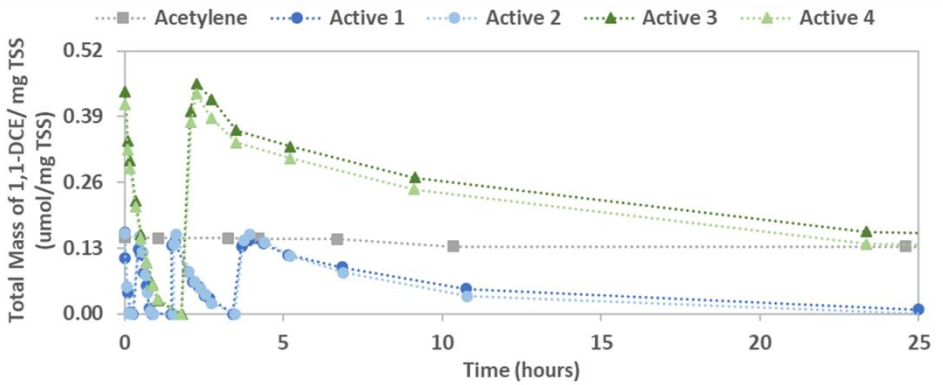

Figure 4.3. *Total mass of 1,1-DCE per mg TSS over time for ethane-grown 21198. Approximately 4 mg of cells were used for experimentation.*

As shown in Figure 4.4, the zero order degradation rates of CAHs and 1,4-dioxane by ethane-grown 21198 were ordered in the following rank: 1,1-DCE > 1,4-dioxane > 1,2-DCA > c-DCE > 1,1,1-TCA > and TCE.

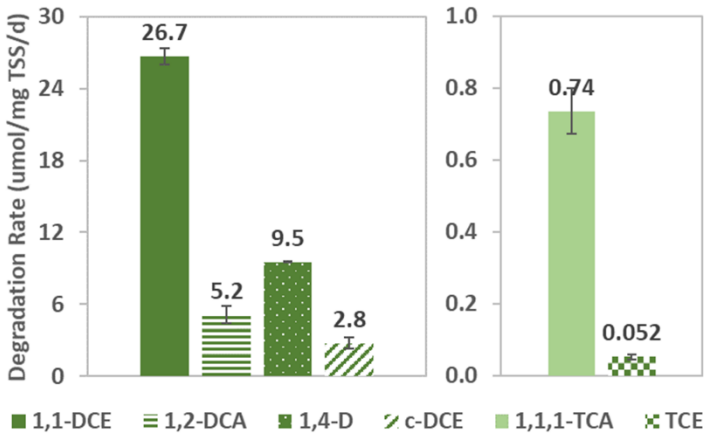

Figure 4.4. *Comparison of zero order degradation rates of 1,1-DCE, 1,2-DCA, 1,4-dioxane, c-DCE, 1,1,1-TCA, and TCE for ethane-grown 21198. Error bars indicate the standard deviation of a two sample average.*

Figure 4.5 depicts the zero order primary substrate uptake rates of isobutane and ethane for ethane-grown 21198. The rate of isobutane uptake was greater than the rate of ethane uptake. The rates of primary substrate uptake were on similar orders of magnitude to the transformation rates of less chlorinated compounds, 1,1-DCE and 1,2-DCA, but were much greater than the transformation rates of more chlorinated compounds, TCE and 1,1,1-TCA.

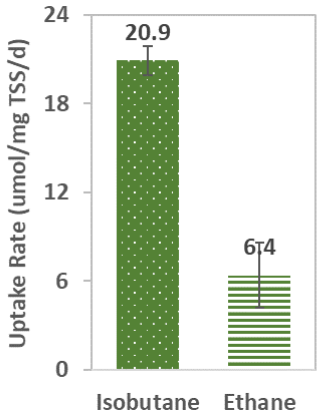

Figure 4.5. *Zero order primary substrate uptake rates of isobutane and ethane for ethane-grown 21198. Error bars indicate the standard deviation of a two sample average.*

4.1.3 Propane-grown 21198

Figure 4.6 depicts the results from a typical rate test, showing the total mass of 1,1-DCE per mg TSS over time for propane-grown 21198. The transformation of 1,1-DCE occurs moderately quickly. The degradation rate is similar between high and low initial 1,1-DCE concentrations (9.3 ± 0.9 µmol/mg TSS/d at an initial concentration of approximately 27 µmol/L versus 11.9 ± 1.6 µmol/mg TSS/d at an initial concentration of approximately 72 µmol/L). The transformation capacity for 1,1-DCE was determined to be 0.32 µmol/mg TSS at a high initial concentration and 0.30 µmol/mg TSS at a low initial concentration. Acetylene actively blocked the degradation of all COCs tested, suggesting monooxygenase enzymes are involved in the transformations of these compounds.

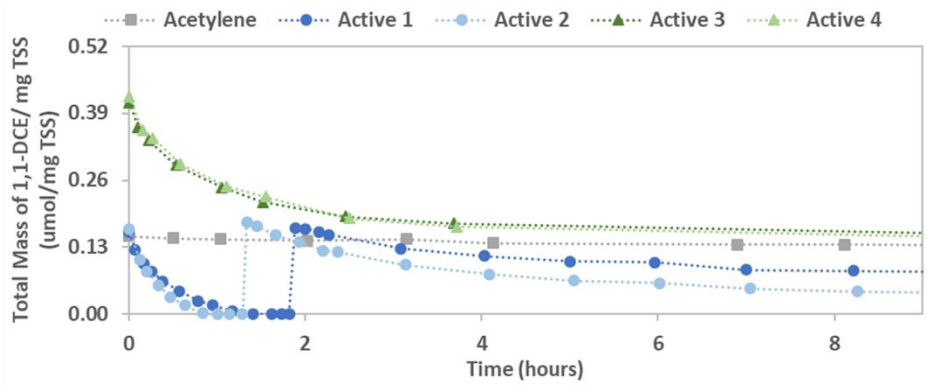

Figure 4.6. *Total mass of 1,1-DCE per mg TSS over time for propane-grown 21198. Approximately 4 mg of cells were used for experimentation.*

As shown in Figure 4.7, the zero order degradation rates of CAHs and 1,4-dioxane by propane-grown 21198 were ordered in the following rank: 1,1-DCE > 1,4-dioxane > 1,2-DCA > c-DCE > 1,1,1-TCA > and TCE.

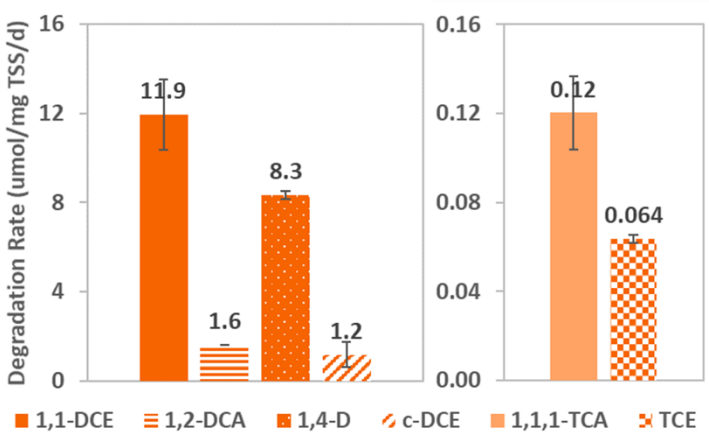

Figure 4.7. *Comparison of zero order degradation rates of 1,1-DCE, 1,2-DCA, 1,4-dioxane, c-DCE, 1,1,1-TCA, and TCE for propane-grown 21198. Error bars indicate the standard deviation of a two sample average.*

Figure 4.8 depicts the zero order primary substrate uptake rates of isobutane and propane for propane-grown 21198. The rate of isobutane uptake was greater than the rate of propane uptake. The rates of primary substrate uptake were on similar orders of magnitude to the transformation rates of less chlorinated compounds, 1,1-DCE and 1,2-DCA, but were much greater than the transformation rates of more chlorinated compounds, TCE and 1,1,1-TCA.

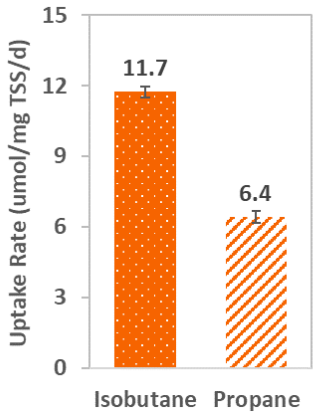

Figure 4.8. *Zero order primary substrate uptake rates of isobutane and propane for propane-grown 21198. Error bars indicate the standard deviation of a two sample average.*

4.1.4 Isobutene-grown ELW1

Figure 4.9 depicts the results from a typical rate test, showing the total mass of 1,1-DCE per mg TSS over time for isobutene-grown ELW1. The transformation of 1,1-DCE occurs moderately quickly. The degradation rate is similar between high and low initial 1,1-DCE concentrations (9.7 ± 0.4 µmol/mg TSS/d at an initial concentration of approximately 27 µmol/L versus 6.2 ± 1.0 µmol/mg TSS/d at an initial concentration of approximately 76 µmol/L). The transformation

capacity for 1,1-DCE was determined to be 0.30 µmol/mg TSS at a high initial concentration and 0.33 µmol/mg TSS at a low initial concentration. Acetylene was only moderately effective at blocking the degradation of 1,1-DCE. Propyne was used for later experimentation with TCE and 1,4-dioxane in order to provide improved monooxygenase inhibition.

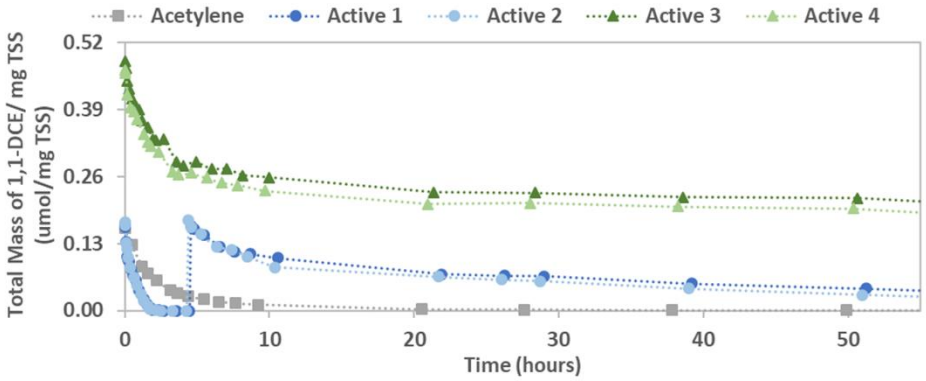

Figure 4.9. *Total mass of 1,1-DCE per mg TSS over time for isobutene-grown ELW1. Approximately 4 mg of cells were used for experimentation.*

As shown in Figure 4.10, the zero order degradation rates of CAHs and 1,4-dioxane by isobutene-grown ELW1 were ordered in the following rank: 1,1-DCE > 1,4-dioxane > and TCE. The transformation of 1,1-DCE was much faster than that of both 1,4-dioxane and TCE. These rates are similar to those observed in other systems, in that more chlorinated compounds (TCE) are more slowly transformed than less chlorinated compounds (1,1-DCE).

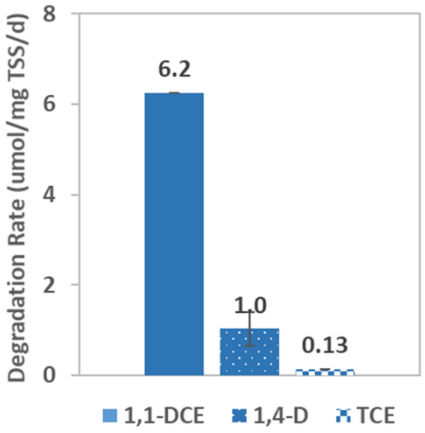

Figure 4.10. *Comparison of zero order degradation rates of 1,1-DCE, 1,4-dioxane, and TCE for isobutene-grown ELW1. Error bars indicate the standard deviation of a two sample average.*

Individual rate tests with 1,1,1-TCA, 1,2-DCA, and c-DCE were not performed with ELW1. Two mixture tests were conducted; one with TCE, 1,1,1-TCA, 1,1-DCE, 1,2-DCA, and 1,4-dioxane and one with TCE, 1,1,1-TCA, 1,1-DCE, 1,2-DCA, 1,4-dioxane, and isobutene. As shown in Figure

4.11, 1,1-DCE was rapidly transformed in the absence of isobutene, while TCE was more slowly degraded. 1,1,1-TCA, 1,2-DCA, and 1,4-dioxane were not readily transformed by ELW1. Figure 4.12 demonstrates metabolism in the presence of the primary substrate, isobutene. Isobutene significantly inhibited the transformation of CAHs. Rapid 1,1-DCE transformation did not begin until the total mass of isobutene reached below 1.5 µmol (approximately 10 µmol/L or 560 µg/L). These results were also observed in microcosm studies further discussed in Section 4.2.8 in which microcosms were bioaugmented with 0.9 mg of ELW1. As with the mixture in the absence of isobutene, 1,1,1-TCA, 1,2-DCA, and 1,4-dioxane were not readily transformed by ELW1 (1,4-dioxane data not shown).

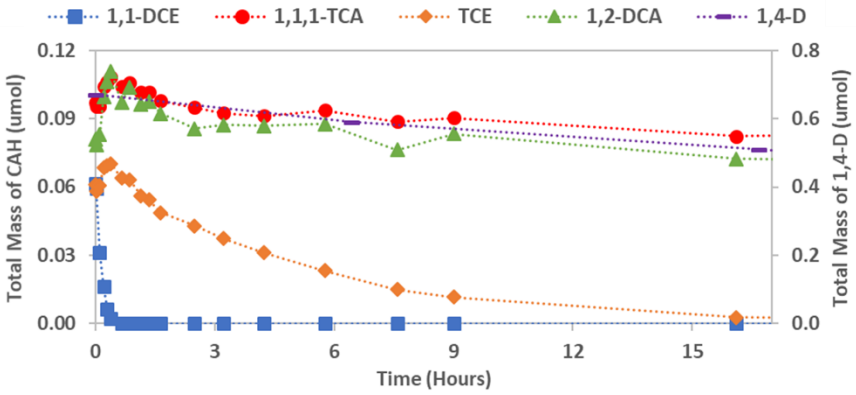

Figure 4.11. *Total mass of 1,1-DCE, 1,1,1-TCA, TCE, 1,2-DCA, and 1,4-dioxane over time for isobutene-grown ELW1.*

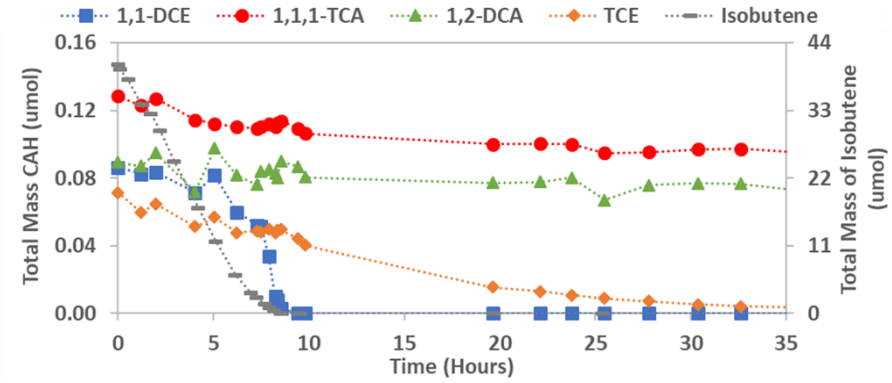

Figure 4.12. *Total mass of 1,1-DCE, 1,1,1-TCA, TCE, 1,2-DCA, and isobutene over time for isobutene-grown ELW1 (1,4-dioxane data not shown).*

4.1.5 Comparisons between growth substrates

A comparison of zero order degradation rates of 1,4-dioxane, TCE, 1,1,1-TCA 1,2-DCA, 1,1-DCE, and c-DCE for isobutane, ethane, and propane-grown 21198 and isobutene-grown ELW1 is shown in Figure 4.13. The general trend in rates for 1,4-dioxane, 1,2-DCA, 1,1-DCE, and c-DCE shows isobutane-grown 21198 > ethane-grown 21198 > propane-grown 21198. The rates for

TCE are comparable among all growth substrates with 21198. The TCE degradation rate of isobutene-grown ELW1 is double the rates observed for 21198 grown on all substrates. The 1,4-dioxane transformation rate by ELW1 was eight to 14 times less than that of 21198 grown on all substrates. 1,1,1-TCA rates were comparable for isobutane and ethane-grown 21198, but the rate of transformation with propane-grown 21198 was much lower.

As shown in Figure 2.4, the results CAH and 1,4-dioxane transformation rates by 21198 generally reflect the relative quantity of SCAM detected in isobutane, ethane, and propane-grown culture. The highest quantity of SCAM was detected in isobutane-grown cultures (approximately 450 mass spectral counts for 59 kDa), followed by ethane-grown (approximately 375 mass spectral counts for 59 kDa) and then propane-grown (approximately 110 mass spectral counts for 59 kDa) cultures. The rates of CAH transformations were generally highest for isobutane-grown 21198, followed by ethane and then propane-grown cultures.

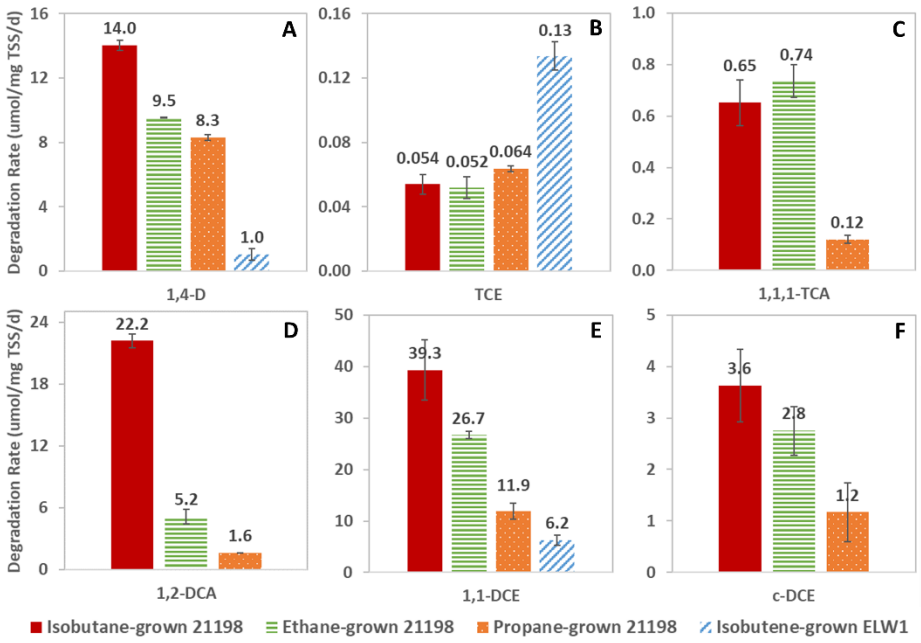

Figure 4.13. *Zero order degradation rates of 1,4-D (A), TCE (B), 1,1,1-TCA (C), 1,2-DCA (D), 1,1-DCE (E), and c-DCE (F) for isobutane-, ethane-, and propane-grown 21198 and isobutene-grown ELW1. Error bars indicate the standard deviation of a two sample average.*

Figure 4.14 shows the transformation capacities of 1,1-DCE and c-DCE for isobutane, ethane, and propane-grown 21198 and the transformation capacity of 1,1-DCE for isobutene-grown ELW1. The transformation capacities for isobutane and ethane-grown 21198 are comparable for both 1,1-DCE and c-DCE and greater than those for propane-grown 21198. Isobutene-grown

ELW1 had the lowest transformation capacity for 1,1-DCE but was comparable with propane-grown 21198. Transformation capacities for 1,2-DCA were attempted to be obtained. Minimum transformation capacities were 1.7 µmol/mg TSS, 0.95 µmol/mg TSS, and 1.8 µmol/mg TSS for isobutane, ethane, and propane-grown 21198, respectively. These are greater than those obtained for 1,1-DCE. These results could be explained by the likely formation of an epoxide intermediate in the transformation of chlorinated ethenes. The transformations of chlorinated ethanes, like 1,2-DCA, likely do not involve a toxic epoxide intermediate, which could allow microbial communities to be capable of transforming a greater quantity of these compounds.

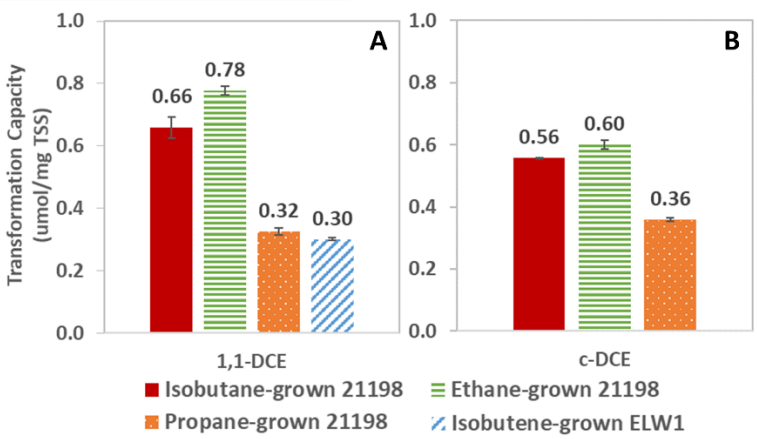

Figure 4.14. *Transformation capacities of 1,1-DCE (A) and c-DCE (B) for isobutane, ethane, and propane-grown 21198 and isobutene-grown ELW1. Error bars indicate the standard deviation of a two sample average.*

These results can be compared to previous studies conducted with mixed and pure cultures of butane, methane, and propane grown cultures (summarized in Table 4.1). The general trend of more chlorinated compounds exhibiting slower transformation rates can be observed from cultures grown on nearly all of these primary substrates. Butane-grown cultures also exhibited similar trends to the results from isobutane-grown 21198 presented in this thesis.

Table 4.1. *Summary of half-saturation constants (K_S), maximum transformation rates, and transformation capacities (T_C) for TCE, 1,1,1-TCA, 1,1-DCA, c-DCE, and 1,2-DCA by various mixed and pure cultures grown on butane, methane, and propane in previous studies.*

Compound	Organism/Condition	Growth Substrate	Temp. (°C)	Initial Conc. (µmol/L)	K_S (µmol/L)	Max Rate (µmol S/mg TSS/d)	T_C (µmol/ mg TSS)	Source
TCE	Mixed culture	Butane	--	19	--	0.24		Kim et al. 2000
TCE	*M. trichosporium* OB3b PP358	Methane/Formate	22	0.46-60.9	82.2	158.3		Aziz et al., 1999
TCE	*M. trichosporium* OB3b	Methane	30	30.4-685	145	418		Oldenhuis et al., 1991
TCE	Mixed culture, chemostat	Methane	20		47	73	4.1	Chang and Alvarez-Cohen, 1996
TCE	Mixed culture, chemostat	Methane	20		29.2	7.8	0.381	Chang and Alvarez-Cohen, 1995
TCE	Mixed culture, chemostat	Methane/Formate	20		52.9	31.7	0.761	Chang and Alvarez-Cohen, 1995
TCE	*Mycobacterium vaccae* JOB5	Propane	30		4.4	0.43		Alvarez & Speitel, 2001
TCE	Mixed culture, chemostat	Propane	20		39.7	3.4	0.049	Chang and Alvarez-Cohen, 1995
1,1,1-TCA	Mixed culture	Butane	--	25	--	0.72		Kim et al. 2000
1,1,1-TCA	Mixed culture	Butane	20		12	4.56		Kim et al. 2002
1,1,1-TCA	*M. trichosporium* OB3b	Methane	30		214	35		Oldenhuis et al., 1991
1,1-DCE	Mixed culture	Butane	--	37	--	8.16		Kim et al. 2000
1,1-DCE	Mixed culture	Butane	20		1.5	31.2	0.52	Kim et al. 2002
1,1-DCE	*M. trichosporium* OB3b PP358	Methane/Formate	22	0.10-35.1	>35.1	>77.4		Aziz et al., 1999
1,1-DCE	*M. trichosporium* OB3b	Methane	30		5	9		Oldenhuis et al., 1991
c-DCE	Mixed culture	Butane	--	87	--	4.8		Kim et al. 2000
c-DCE	Mixed culture, chemostat	Methane	20		31	86	5.9	Chang and Alvarez-Cohen, 1996
c-DCE	*M. trichosporium* OB3b PP358	Methane/Formate	22	0.52-57.8	11.3	98.0		Aziz et al., 1999
c-DCE	*M. trichosporium* OB3b	Methane	30		30	262		Oldenhuis et al., 1991
1,2-DCA	Mixed culture	Butane	--	52	--	0.24		Kim et al. 2000
1,2-DCA	*M. trichosporium* OB3b	Methane	30		77	94		Oldenhuis et al., 1991

Figure 4.15 shows the total mass of 1,1-DCE, 1,1,1-TCA, TCE, 1,2-DCA, and 1,4-dioxane over time for isobutane, ethane, and propane-grown 21198 and isobutene-grown ELW1 for a mixture of COCs. The observed rates between each compound for each substrate are consistent with those observed in single compound studies. 1,1-DCE and 1,2-DCA are most rapidly transformed, followed by slower 1,4-dioxane, and 1,1,1-TCA transformation and minimal TCE degradation for 21198 grown on all substrates. The transformations by propane-grown 21198 were slower than those for isobutane and ethane-grown 21198. 1,1-DCE was transformed rapidly for isobutene-grown ELW1. TCE was more readily transformed by ELW1 than 21198 grown on any substrate. 1,4-dioxane, 1,1,1-TCA, and 1,2-DCA were not readily degraded by ELW1 compared to 21198.

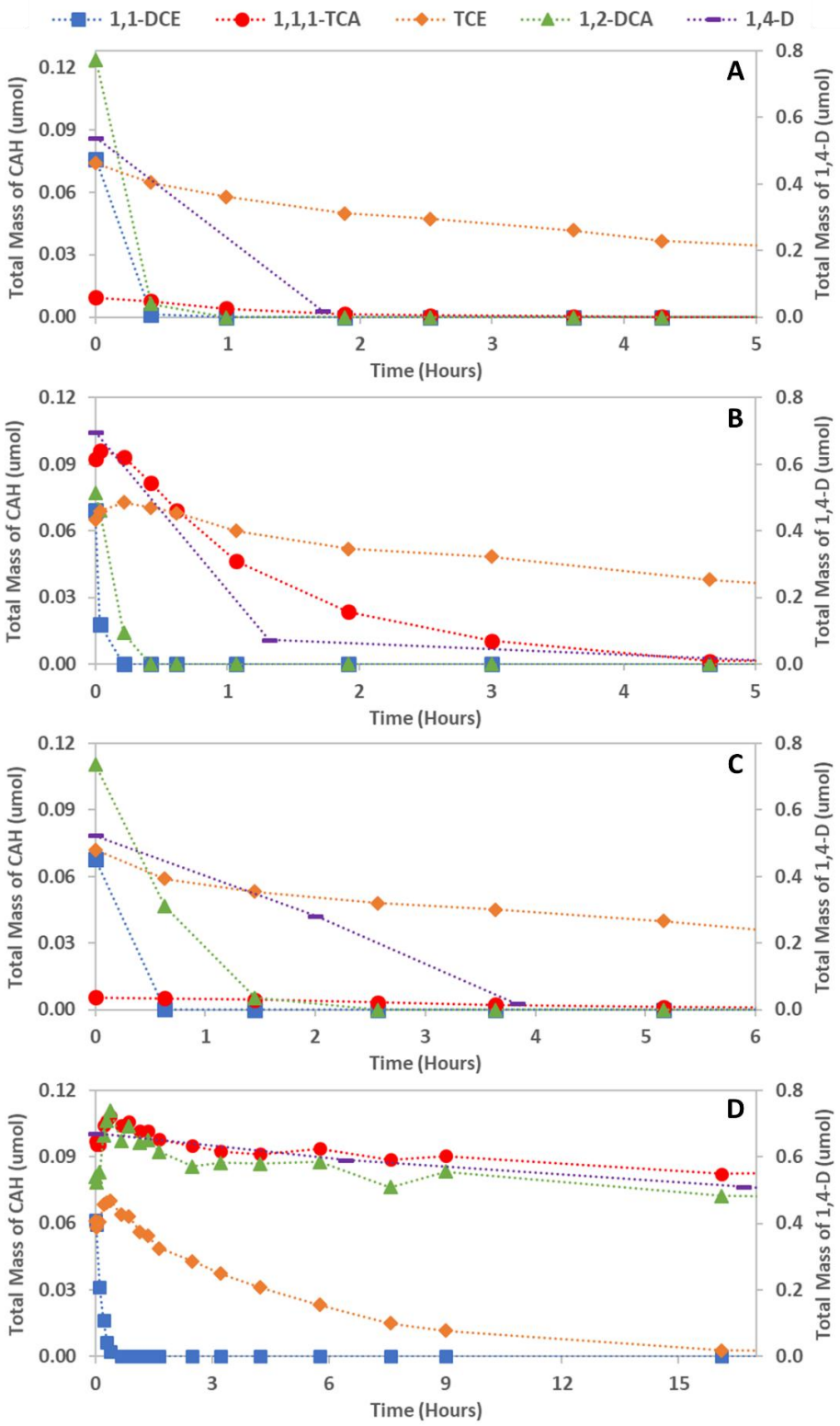

Figure 4.15. *Total mass of 1,1-DCE, 1,1,1-TCA, TCE, 1,2-DCA, and 1,4-dioxane in isobutane (A), ethane (B), and propane (C) grown 21198, and isobutene grown ELW1 (D) for COC mixtures. Approcimately 5 mg of cells were used in each experiment.*

4.2 Microcosm Studies

Microcosm studies were conducted using groundwater and sediment from NAS North Island in San Diego, California. As discussed in Section 2.8, this site is contaminated with CAHs (predominately TCE and 1,1-DCE) and 1,4-dioxane due to historical improper storage of chemical waste. Aerobic cometabolism has been proposed for the remediation of this site due to the composition of the contaminant mixture. The purpose of this section was to identify an effective primary substrate, or combination of multiple primary substrates, that was capable of performing concurrent transformations of CAHs and 1,4-dioxane.

Figure 4.16 depicts the microcosm experimental process for long-term microcosm studies utilizing isobutane and isobutene as primary growth substrates. Early isobutane experimentation (further discussed in Section 4.2.1) demonstrated the difficulty of achieving stimulation of native microbial communities in the presence of CAHs, particularly 1,1-DCE. In order to circumvent this challenge, three main strategies were employed, including the removal of CAHs via sparging (Section 4.2.2), the bioaugmentation with isobutane-grown 21198 (Section 4.2.3), and the addition of isobutanol (Section 4.2.4). Isobutene ultimately demonstrated successful stimulation of the native microbial community in the presence of CAHs (Section 4.2.6), though the removal of CAHs via sparging (Appendix D) and bioaugmentation with isobutene-grown ELW1 (Section 4.2.8) were also employed.

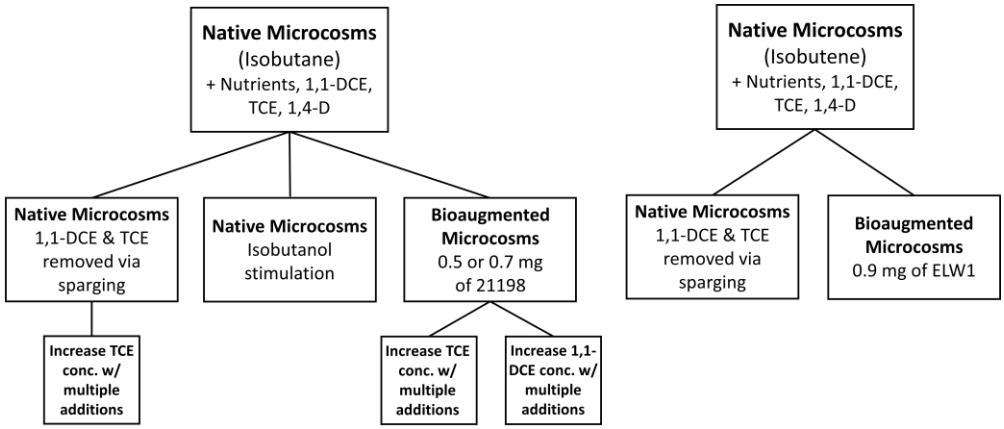

Figure 4.16. *Experimental microcosm road map indicating the general experimental process for long-term microcosm studies utilizing isobutane and isobutene as primary growth substrates.*

4.2.1 Isobutane in the presence in CAHs

Eight microcosms were initially established with isobutane in the presence of CAHs. Bottles 1 and 2 did not have nutrients added; bottles 3 and 4 had nutrients added; bottles 5 and 6 were

acetylene controls; and bottles 7 and 8 did not have isobutane added to the headspace. As shown in Figure 4.17A, isobutane uptake was not observed in any of the microcosms over 130 days of incubation in the presence of CAHs.

The total mass of TCE decreased by 60 to 70 percent over the span of the incubation (Figure 4.17B). Because this phenomenon occurred in the acetylene controls, the observed decrease in TCE was likely due to abiotic transformations or due to sorption to sediment and/or the rubber septa rather than biotical activity. Jeffers et al. reported an abiotic hydrolysis half-life of 1.0 x 10^6 years for TCE (Jeffers et al., 1989). Due to this slow rate, it is unlikely that the loss of TCE can be accounted for by abiotic transformations. As noted in Table 2.1, the octanol-water partition coefficient (log K_{OW}) for TCE is 2.53. The log K_{OW} represents the partitioning of a compound into water and octanol phases and is often used to signify the hydrophobicity of the compound and describe the partitioning of the compound between water and organic solids. Compounds with a higher log K_{OW} are more apt to partition into organic or solid phases rather than the aqueous phase, as demonstrated by TCE. The loss in total mass of TCE over time can likely be attributed to sorption to sediment and/or the rubber septa.

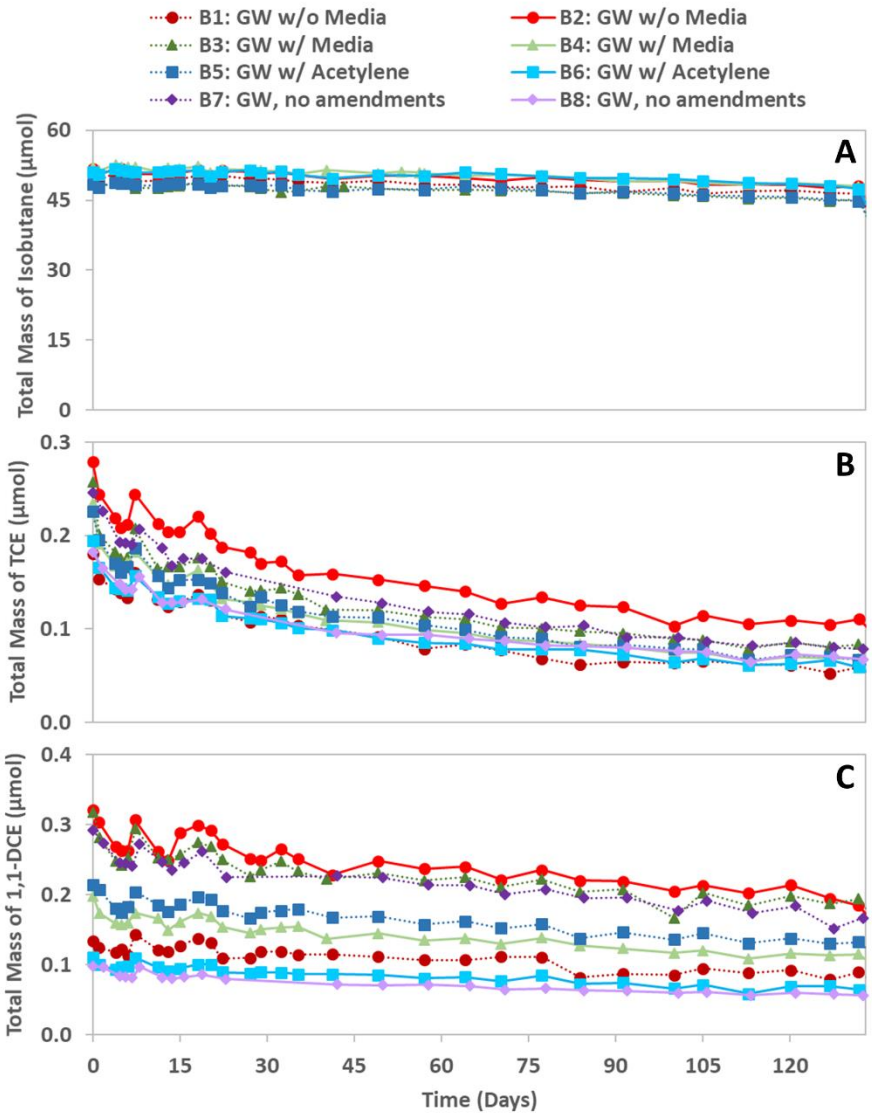

Figure 4.17. *Total mass of isobutane (A), TCE (B), and 1,1-DCE (C) over time in native microcosms incubated with isobutane in the presence of CAHs (set 1). Bottles 1 and 2 do not have added nutrients; bottles 3 and 4 have added nutrients; bottles 5 and 6 are acetylene controls; and bottles 7 and 8 do not have isobutane added.*

These results were confirmed in subsequent microcosms wherein isobutane uptake was not observed after 22 to 105 days of incubation in the presence of CAHs (Figure 4.18). The lack of activity is hypothesized to be due to the toxicity of the 1,1-DCE epoxide formed from the activation of the monooxygenase enzyme. Minimal isobutane may have been utilized, but the microbial population was not able to grow due to immediate toxicity exposure from concurrent 1,1-DCE transformation.

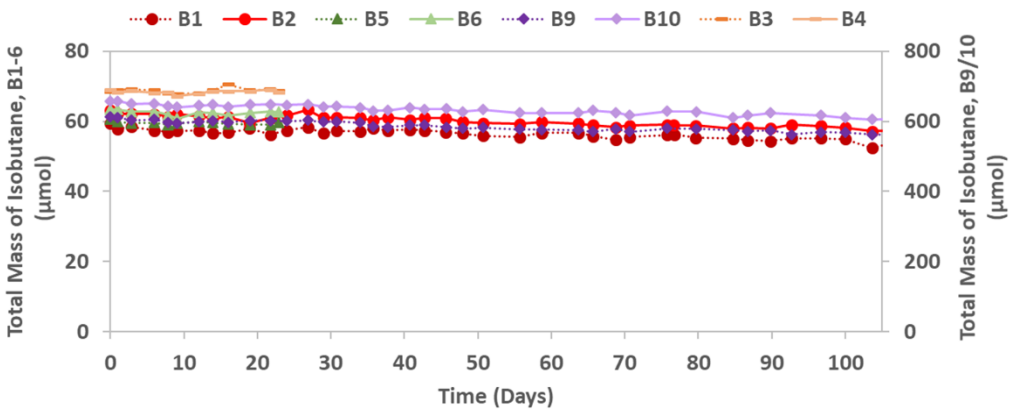

Figure 4.18. *Total mass of isobutane over time in native microcosms incubated with isobutane in the presence of CAHs (set 5). All bottles have nutrients added. Bottles 1 through 6 were constructed with groundwater from SMW-08A, and bottles 9 and 10 were constructed with groundwater from SMW-10A (with approximately two times the total mass of 1,1-DCE compared to SMW-08A).*

4.2.2 Isobutane in the absence in CAHs

Based on the results described in Section 4.2.1, it was hypothesized that the presence of 1,1-DCE was inhibitory to the stimulation of native isobutane-utilizers due to the toxicity of the 1,1-DCE epoxide. In order to test this hypothesis, the groundwater used for experimentation described in this section was sparged with nitrogen to remove CAHs from solution prior to the addition of isobutane to the headspace of the microcosm. Six microcosms were established with isobutane in the absence of CAHs from day 0 (set 2). Microcosms 1 and 2 did not have added nutrients; microcosms 3 and 4 had added nutrients; and microcosms 5 and 6 were acetylene controls. Figure 4.19 depicts data from the long-term incubation of groundwater with isobutane in the initial absence of CAHs. As described in greater detail later in this section, isobutane uptake was observed after 12 days of incubation in the absence of CAHs. 1,1-DCE and TCE were reintroduced to the system, and subsequent respikes of isobutane, 1,4-dioxane, and CAHs were performed over 125 days of incubation, with gradually increasing concentrations of TCE.

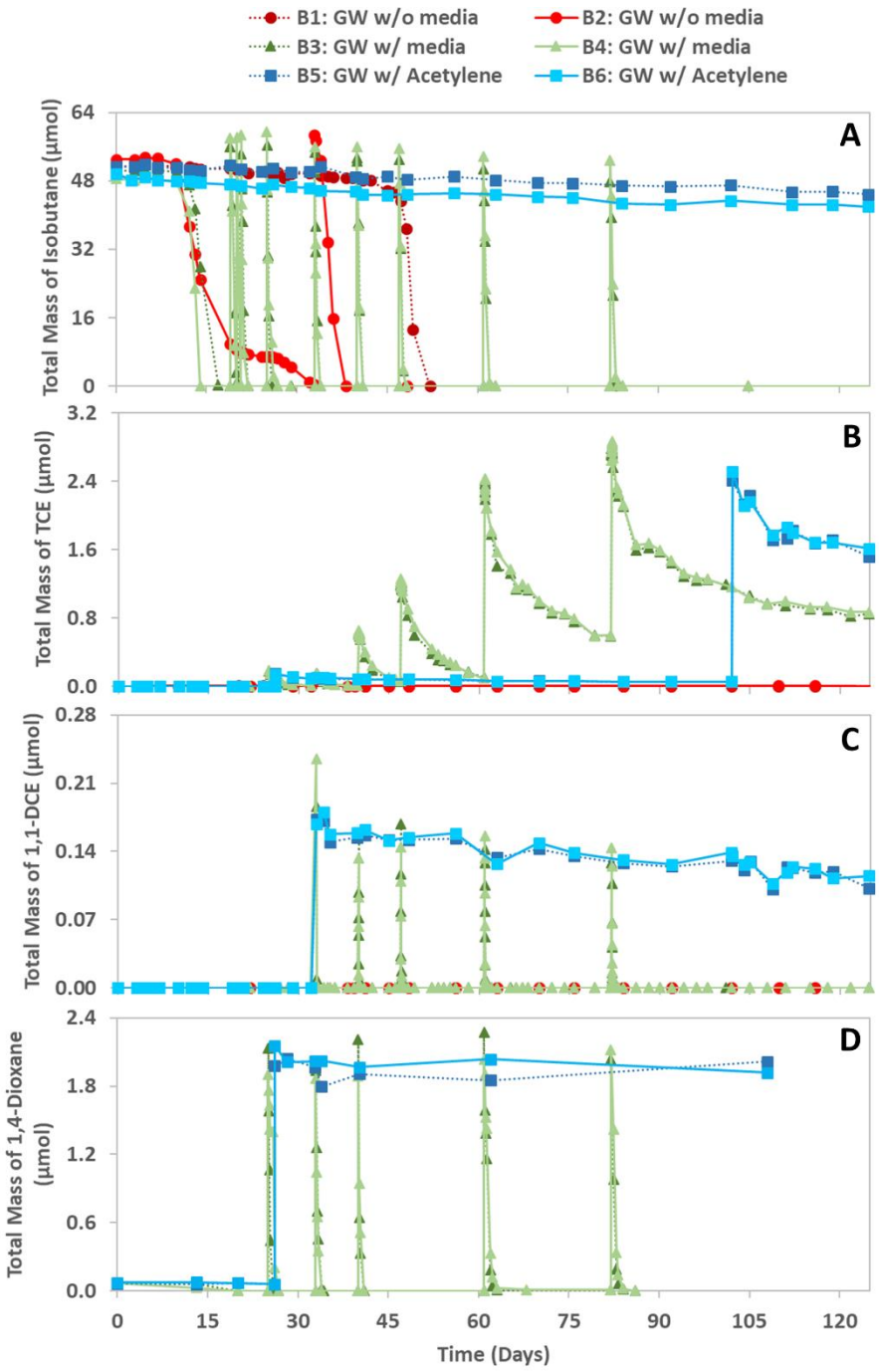

Figure 4.19. *Total mass of isobutane (A), TCE (B), 1,1-DCE (C), and 1,4-dioxane (D) over time in native microcosms initially sparged to remove CAHs. Nutrients were added to bottles 1 and 2 on day 24. Bottles 3 through 6 had nutrients added on day 0. Bottles 5 and 6 were acetylene controls.*

As shown in Figure 4.20, isobutane uptake was observed in microcosms 3 and 4 (those with nutrients added) after approximately 12 days of incubation. Isobutane uptake observed in microcosms 1 and 2 (without added nutrients) is further discussed in Section 4.2.8. Microcosms

3 and 4 continued to support repeated isobutane additions and demonstrated an increase in uptake rate with subsequent additions of isobutane.

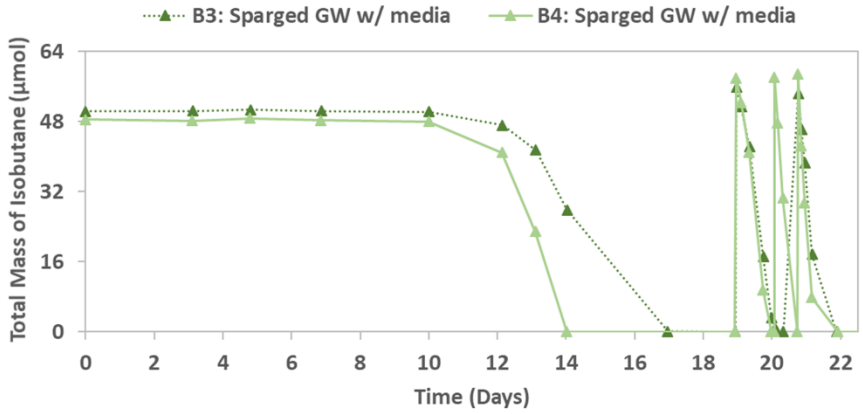

Figure 4.20. *Total mass of isobutane over time in native microcosms with added nutrients and initially sparged to remove CAHs (set 2, bottles 3 and 4).*

Isobutane, TCE, and 1,4-dioxane were added to microcosms 3 and 4 on day 25 (data not shown). Isobutane and 1,4-dioxane were transformed within one day, while TCE was more slowly degraded over four days. As shown in Figure 4.21, 1,1-DCE was introduced into the system on day 33, along with another spike of isobutane, TCE, and 1,4-dioxane. 1,1-DCE was transformed very rapidly (within 2 hours), followed by isobutane uptake and 1,4-dioxane degradation within one day. TCE was more slowly transformed over five days.

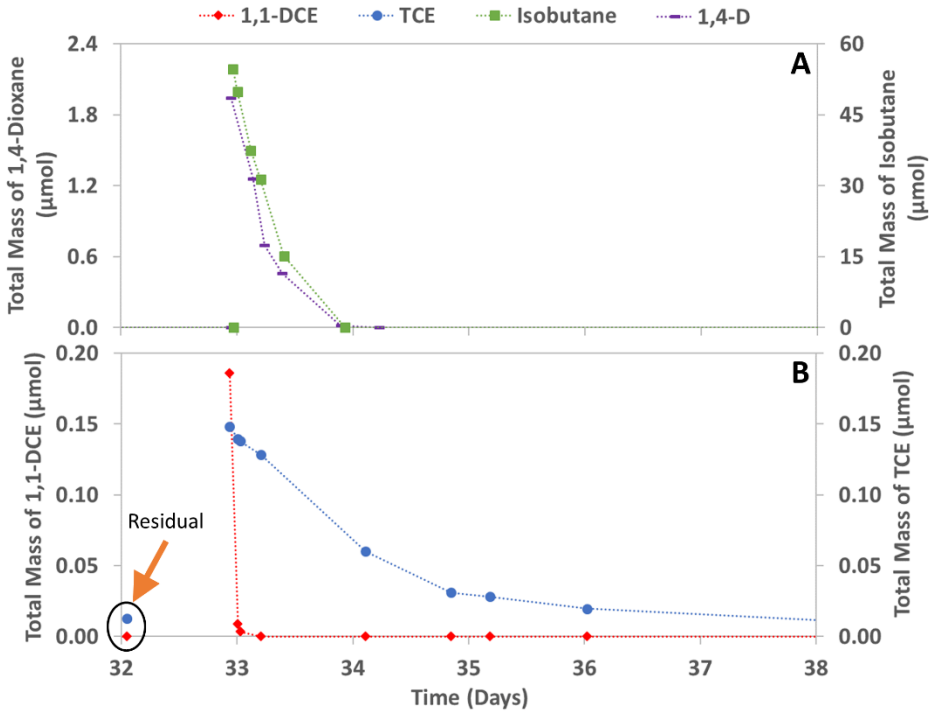

Figure 4.21. *Total mass of 1,4-dioxane and isobutane (A), and 1,1-DCE and TCE (B) over time in one native microcosm with added nutrients on day 0 and initially sparged to remove CAHs (set 2, bottle 3). This depicts an early spike of isobutane and COCs added to this microcosm on day 33.*

Four subsequent additions of isobutane, TCE, 1,1-DCE, and 1,4-dioxane were made between days 40 and 82, with gradual increases in TCE concentrations, culminating in a liquid concentration of 4.5 mg/L. The final spike of primary substrate and COCs on day 82 is depicted in Figure 4.22. Rapid 1,1-DCE transformation continued to be observed, followed by concurrent isobutane uptake and 1,4-dioxane degradation. TCE was slowly degraded by the native microbial community. The final measured concentration of TCE was approximately 1.3 mg/L (an approximately 70% overall reduction).

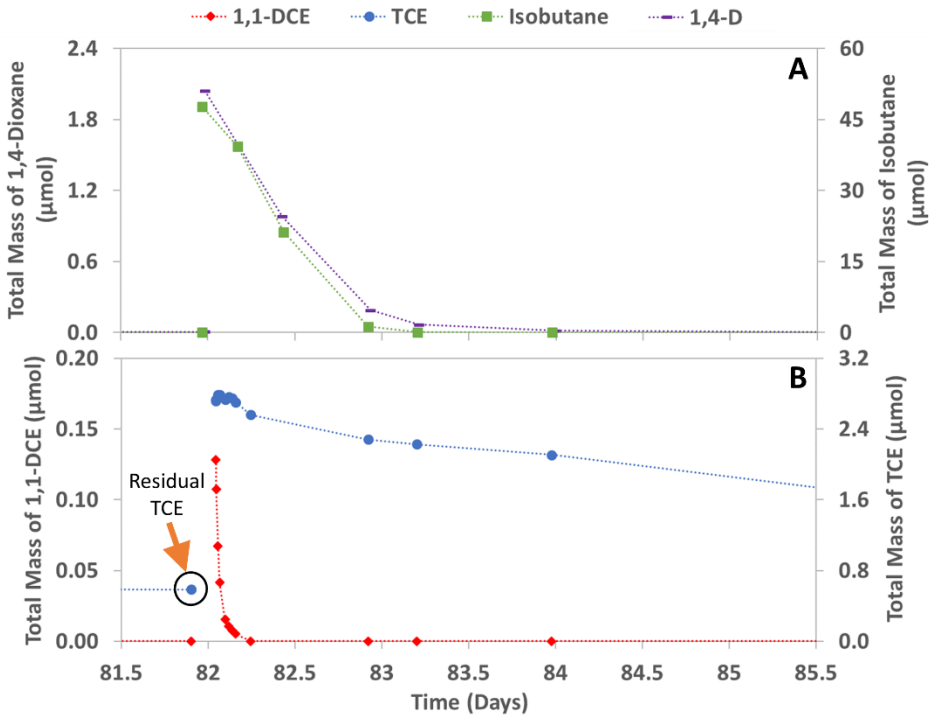

Figure 4.22. *Total mass of 1,4-dioxane and isobutane (A), and 1,1-DCE and TCE (B) over time in one native microcosm with added nutrients on day 0 and initially sparged to remove CAHs (set 2, bottle 3). This depicts the last spike of isobutane and COCs added to this microcosm on day 82.*

To verify that the observed decrease in TCE concentration in microcosms 3 and 4 was due to biological activity, TCE was added to the acetylene control bottles 5 and 6 at a comparable concentration to the last two TCE spikes in microcosms 3 and 4 (Figure 4.23). Over the span of 10 days, the TCE concentrations in microcosms 5 and 6 decreased by approximately 25%, while the concentrations in microcosms 3 and 4 decreased by approximately 45%. This suggests that while there is some TCE concentration decrease likely due to sorption to sediment and/or the rubber septa, there is also a biological component to the decrease.

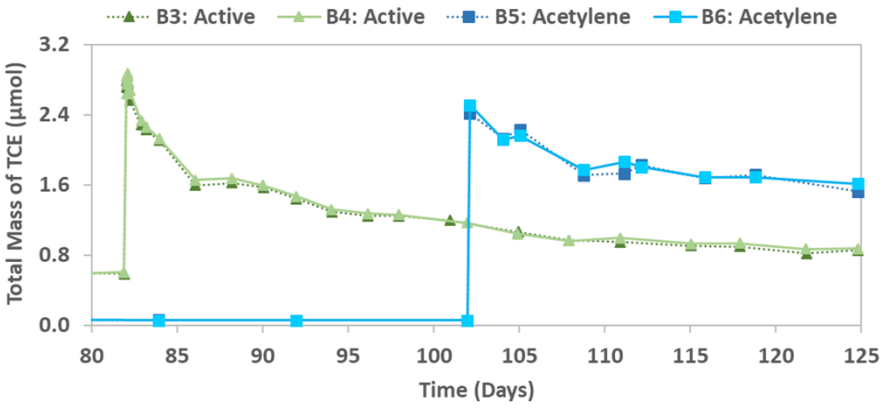

Figure 4.23. *Comparison of total mass of TCE over time in native active and acetylene control microcosms that were initially sparged to remove CAHs (set 2, bottles 3-6). TCE was added to the acetylene control bottles on day 102 in order to compare the change in total mass over time to the change observed in the active bottles.*

To verify the results from the initially sparged microcosms with added nutrients (set 2, bottles 3 and 4), two additional microcosms were established with isobutane in the absence of CAHs (set 7, bottles 1 and 2). As shown in Figure 4.24, isobutane uptake was not observed over 60 days of incubation. The lack of activity could be explained by a very small initial biomass, either from a lack of native isobutane-utilizers present at NAS North Island and/or due to the minimal quantity of aquifer solids present in the microcosm, or due to slow growth rates. There is also the possibility that the set 2 microcosms were contaminated with 21198 over the course of the experimentation and that native isobutane-utilizers were not responsible for the observed activity.

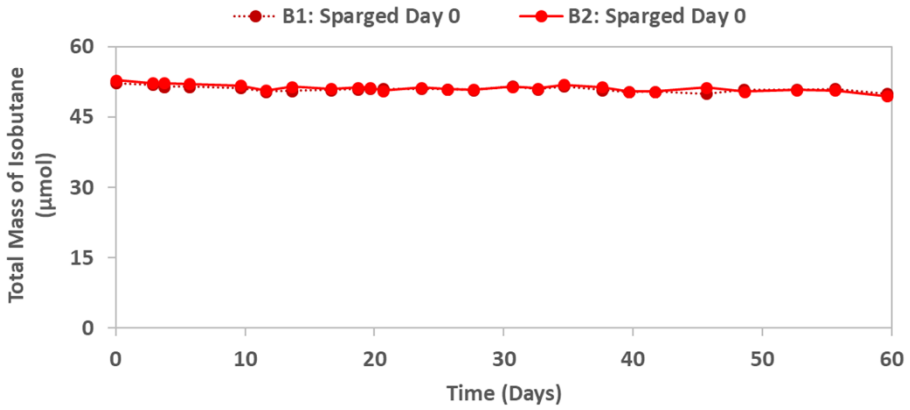

Figure 4.24. *Total mass of isobutane over time in native microcosms sparged to remove CAHs on day 0.*

To further confirm the results from the active native microcosms with added nutrients initially sparged to remove CAHs (set 2, bottles 3 and 4), two additional microcosms were established with isobutane initially in the presence of CAHs (set 5, bottles 3 and 4). These microcosms were

sparged with nitrogen on day 23 to remove CAHs from solution. They were again sparged on day 51 to further remove CAHs that had desorbed from the aquifer solids. After the second sparging event, the liquid concentrations of 1,1-DCE and TCE were approximately 1 to 5 µg/L and approximately 20 to 30 µg/L, respectively.

As shown in Figure 4.25, isobutane uptake was observed in set 5, bottle 3 46 days after the initial sparging event and after 69 days of isobutane incubation. This microcosm consumed a second addition of isobutane at a faster rate. The 1,1-DCE concentration dropped to below detection levels, and the TCE concentration was 9 µg/L on day 106. The 1,4-dioxane concentration also decreased from 3.8 mg/L to 49 µg/L (data not shown). These results support the assertion that native isobutane-utilizers can be stimulated in the absence of CAHs following initial concurrent exposure to isobutane, CAHs, and 1,4-dioxane.

Isobutane uptake has not been observed in bottle 4 after 106 days of incubation. As with bottles 1 and 2 from set 7 that were sparged to remove CAHs on day 0, these results could be explained by a very small initial biomass, either from a lack of native isobutane-utilizers present at NAS North Island and/or due to the minimal quantity of aquifer solids present in the microcosm, or due to slow growth rates.

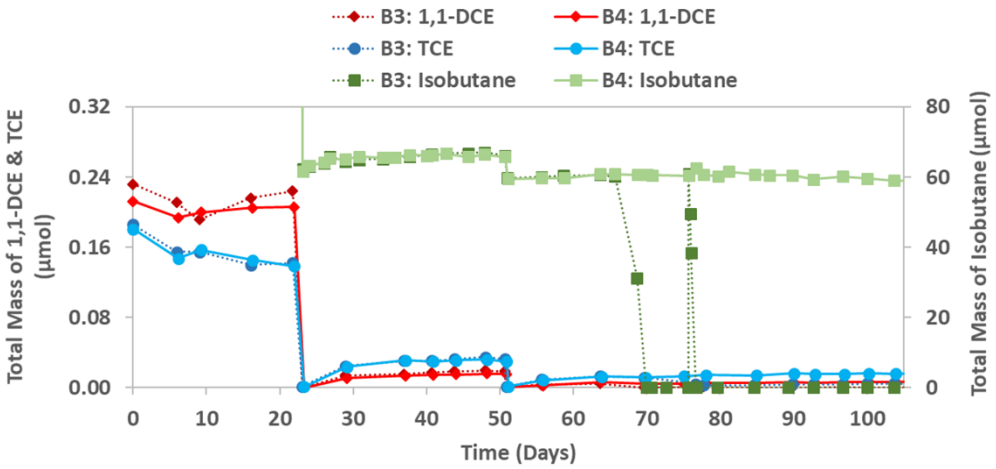

Figure 4.25. *Total mass of 1,1-DCE, TCE, and isobutane over time in native microcosms sparged to remove CAHs on days 23 and 51 (set 5, bottles 3 and 4). Complete isobutane uptake was observed in bottle 3 on day 69. Isobutane was added to bottle 3 on day 76, and subsequent uptake was observed within one day.*

4.2.3 Isobutane bioaugmentation with 21198

Another proposed method to circumvent to toxic effects of the 1,1-DCE epoxide was the implementation of bioaugmentation with isobutane-grown 21198. As discussed in Section

4.1.1, isobutane-grown 21198 is capable of transforming 1,1-DCE at a fast rate but with a low transformation capacity (0.66 µmol/mg TSS). The purpose of the approach described in this section was to add a sufficient quantity of 21198 biomass to fully transform the amount of 1,1-DCE present in the system.

Set 3 was comprised of four microcosms that were bioaugmented with 0.5 mg of 21198, and set 4 was comprised of four microcosms that were bioaugmented with 0.7 mg of 21198. Microcosms 1 and 2 were described as “active”, while microcosms 3 and 4 were acetylene controls. All microcosms had nutrients added to the system. Figure 4.26 depicts data from the long-term incubation of groundwater with isobutane in the presence of CAHs and bioaugmented with 0.5 mg of isobutane-grown 21198. As described in greater detail later in this section, 1,1-DCE transformation was observed immediately and rapidly, followed by isobutane uptake, 1,4-dioxane transformation, and slow TCE transformation. Subsequent respikes of isobutane, 1,4-dioxane, and CAHs were performed over 120 days of incubation, with gradually increasing concentrations of TCE.

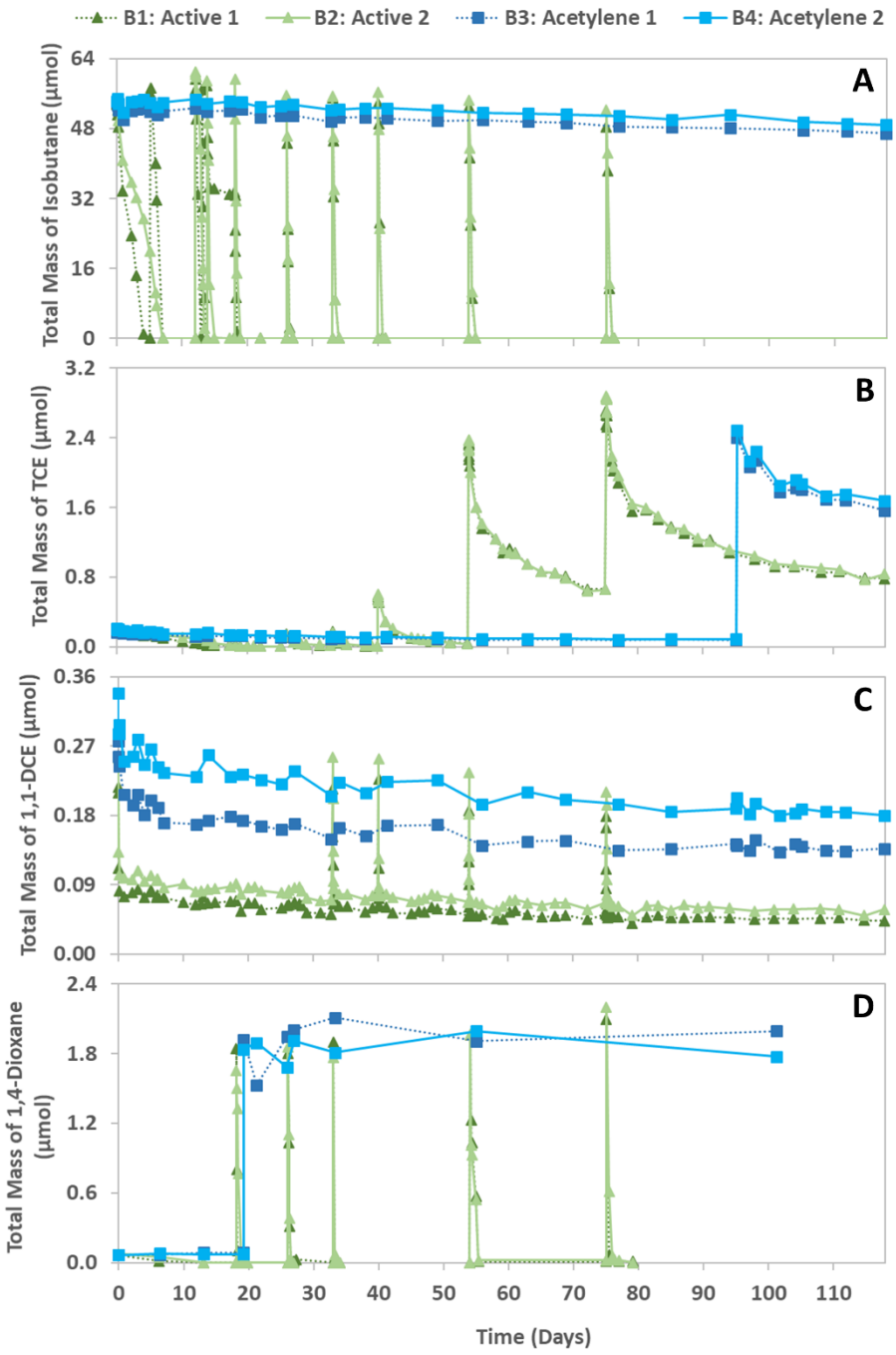

Figure 4.26. *Total mass of isobutane (A), TCE (B), 1,1-DCE (C), and 1,4-dioxane (D) over time in microcosms bioaugmented with 0.5 mg of 21198 (set 3). Nutrients were added to all microcosms on day 0. Bottles 3 and 4 were acetylene controls. Bottles 1 and 2 were respiked with isobutane, 1,1-DCE, 1,4-dioxane, and gradually increasing concentrations of TCE over 120 days of incubation.*

As shown in Figure 4.27, 1,1-DCE was immediately transformed over two to four hours, and isobutane was consumed within four to seven days. The rate of isobutane uptake increased with subsequent additions. Isobutane uptake in bottle 3 stalled around day 15 due to lack of oxygen and resumed on day 18 after oxygen was added to the headspace. TCE was slowly

transformed over 19 days with the addition of four to five spikes of isobutane. Though minimal 1,4-dioxane was initially present in the groundwater, the concentration decreased from approximately 110 to 140 µg/L to below the detection limit over the first 1 to 2 spikes of isobutane (Figure 4.28). 1,4-dioxane was added at a concentration of approximately 3 mg/L on day 18 and was transformed to below the detection limit within one day.

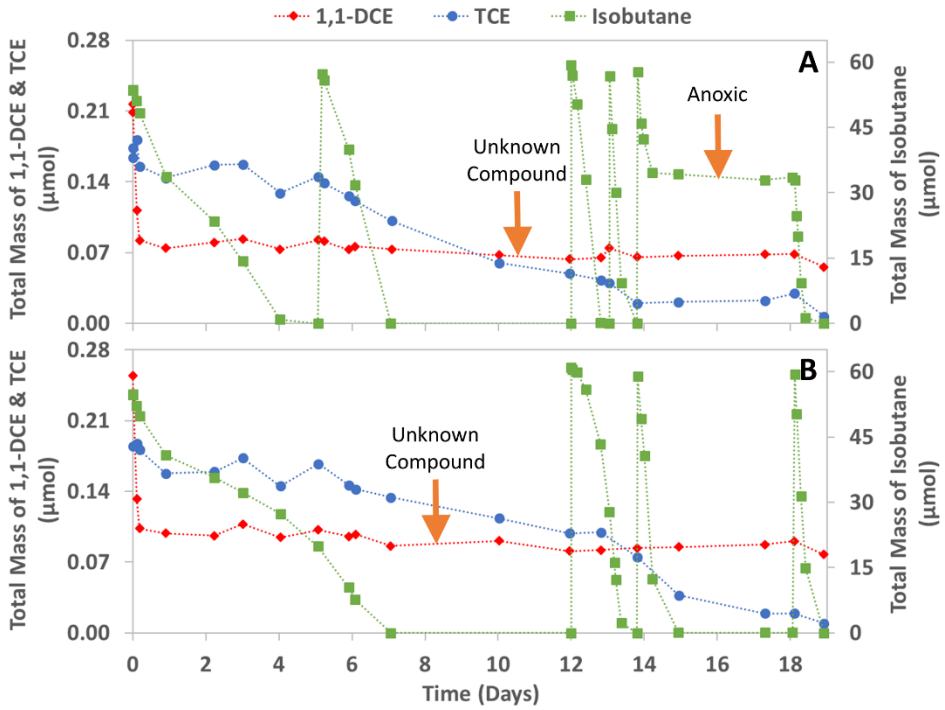

Figure 4.27. Total mass of isobutane, 1,1-DCE, and TCE over time set 3, bottles 1 (A) and 2 (B) bioaugmented with 0.5 mg 21198. The residual 1,1-DCE was hypothesized to be an unknown compound that coelutes with 1,1-DCE (further discussed below)

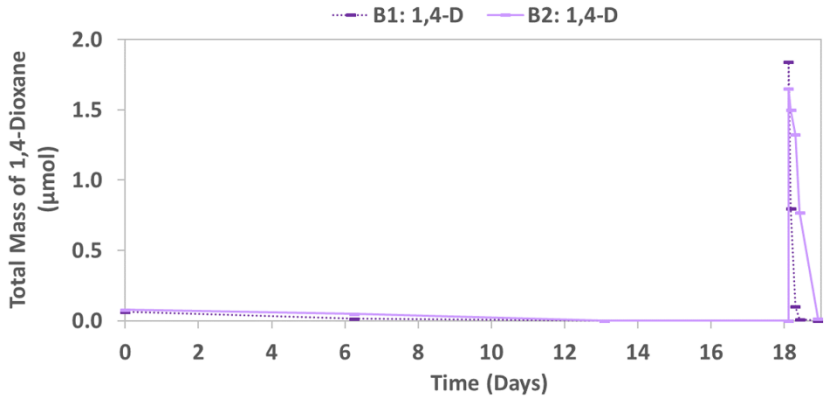

Figure 4.28. *Total mass of 1,4-dioxane in set 3, bottles 1 and 2 bioaugmented with 0.5 mg 21198.*

The total mass of 1,1-DCE decreased from 0.2 to 0.25 µmol to 0.07 to 0.08 µmol. It is hypothesized that the remaining 0.07 to 0.08 µmol was not 1,1-DCE but rather an unknown

compound that coelutes with 1,1-DCE and was not able to be degraded by 21198. Based on the 1,1-DCE transformation capacity obtained for isobutane-grown 21198 and reported in Section 4.1.5 (0.66 µmol 1,1-DCE per mg TSS), 0.5 mg of 21198 should have been able to fully transform the initial mass of 1,1-DCE present in the microcosms. GC analyses were performed for chloroform, carbon tetrachloride, vinyl chloride, and t-DCE to compare retention times with that of 1,1-DCE. None of the retention times for these compounds matched that of 1,1-DCE, suggesting they are not the unknown compound. Multiple GC-MS analyses were conducted to try and identify this compound. These analyses were unsuccessful, likely because the concentration of the mystery compound is below the limits of detection on the GC-MS.

Isobutane, TCE, and 1,4-dioxane were added to the active microcosms 1 and 2 on day 26. Isobutane and 1,4-dioxane were transformed within one day, while TCE was more slowly degraded over six days (data not shown). As shown in Figure 4.29, 1,1-DCE was re-added to the system on day 33, along with another spike of isobutane, TCE, and 1,4-dioxane. 1,1-DCE was transformed very rapidly (within 1 to 2 hours), followed by isobutane uptake and 1,4-dioxane degradation within one day. TCE was more slowly transformed over six days.

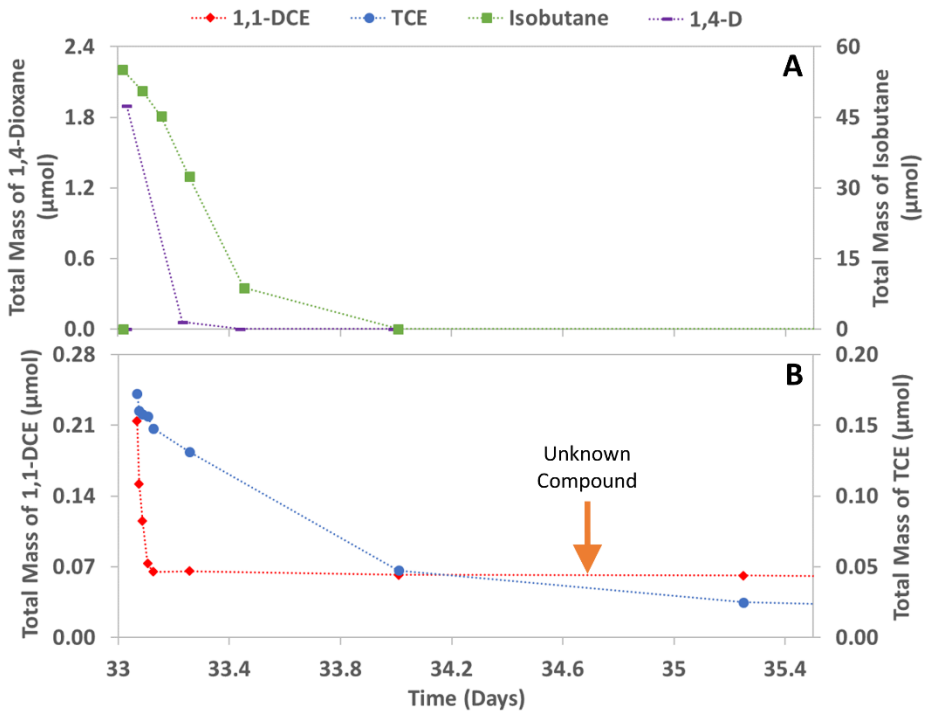

Figure 4.29. *Total mass of 1,4-dioxane and isobutane (A), and 1,1-DCE and TCE (B) over time in one microcosm bioaugmented with 0.5 mg of isobutane-grown 21198 (set 3, bottle 1). This depicts an early spike of isobutane and COCs added to this microcosm on day 33.*

Three subsequent additions of isobutane, TCE, 1,1-DCE, and 1,4-dioxane were made between days 40 and 75, with gradual increases in TCE concentrations, culminating in a liquid concentration of 4.4 to 4.6 mg/L. The final spike of primary substrate and COCs on day 75 is depicted in Figure 4.30. Rapid 1,1-DCE transformation continued to be observed, followed by concurrent isobutane uptake and 1,4-dioxane degradation. TCE was able to be slowly degraded by the established microbial community. The final measured concentration of TCE was approximately 1.1 mg/L (an approximately 75% overall reduction).

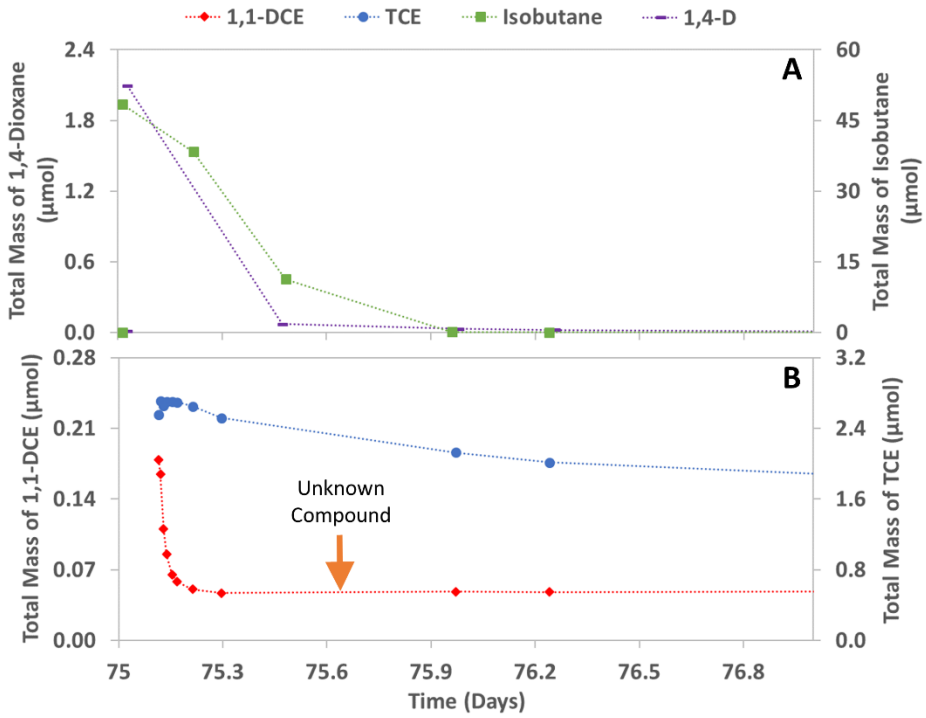

Figure 4.30. *Total mass of 1,4-dioxane and isobutane (A), and 1,1-DCE and TCE (B) over time in one microcosm bioaugmented with 0.5 mg of isobutane-grown 21198 (set 3, bottle 1). This depicts the final spike of isobutane and COCs added to this microcosm on day 75 after gradual increases in TCE concentration.*

As with the set 2 microcosms, TCE was added to the acetylene control bottles 3 and 4 at a comparable concentration to the last TCE spike in bottles 1 and 2 in order to verify that the observed decrease in TCE concentration in bottles 1 and 2 was due to biological activity. Over the span of 10 days, the TCE concentrations in bottles 3 and 4 decreased by approximately 25%, while the concentrations in the active bottles 1 and 2 decreased by approximately 50% (Figure 4.31). This suggests that while there is some TCE concentration decrease likely due to sorption to sediment and/or the rubber septa, there is also a biological component to the decrease.

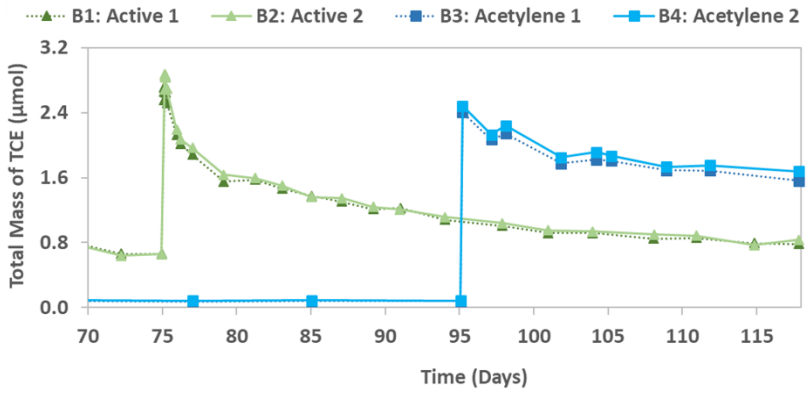

Figure 4.31. *Comparison of total mass of TCE over time in microcosms bioaugmented with 0.5 mg of 21198. Active and acetylene control microcosms that were initially sparged to remove CAHs (set 3, bottles 3-6). TCE was added to the acetylene control bottles on day 102 in order to compare the change in total mass over time to the change observed in the active bottles.*

Set 4 was comprised of four microcosms that were bioaugmented with 0.7 mg of 21198. Microcosms 1 and 2 were described as “active”, while microcosms 3 and 4 were acetylene controls. All microcosms had nutrients added to the system. Figure 4.32 depicts data from the long-term incubation of groundwater with isobutane in the presence of CAHs and bioaugmented with 0.7 mg of isobutane-grown 21198. The initial hypothesis regarding the residual mass of 1,1-DCE remaining in the set 3 microcosms was that not enough biomass was added to fully transform the 1,1-DCE, despite the transformation capacity determined in pure culture tests. The set 4 microcosms were bioaugmented with a greater initial biomass in order to fully transform the present mass of 1,1-DCE. As described in greater detail later in this section, 1,1-DCE transformation was observed immediately and rapidly, followed by isobutane uptake, 1,4-dioxane transformation, and slow TCE transformation. Subsequent respikes of isobutane, 1,4-dioxane, and CAHs were performed over 115 days of incubation, with gradually increasing concentrations of 1,1-DCE.

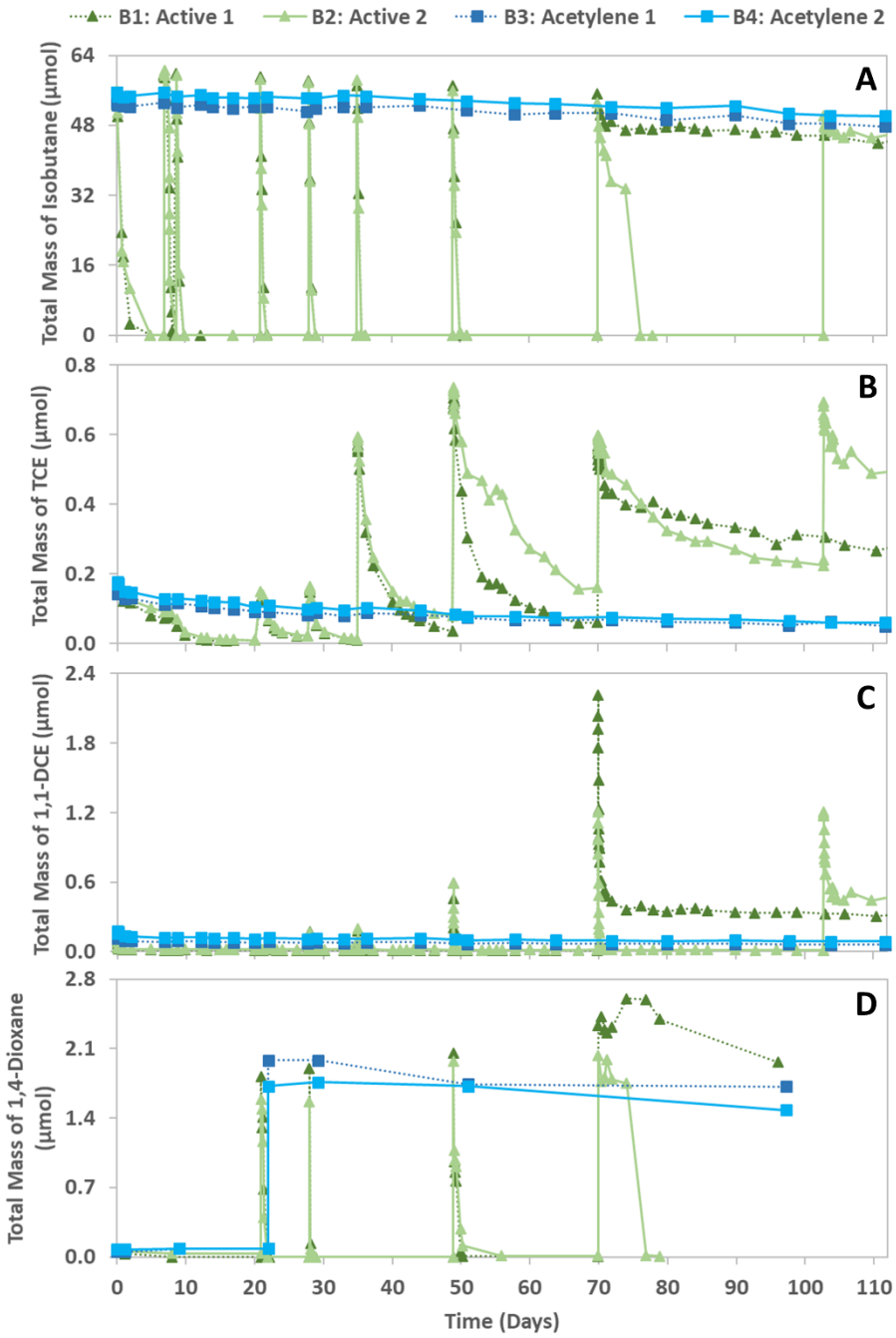

Figure 4.32. *Total mass of isobutane (A), TCE (B), 1,1-DCE (C), and 1,4-dioxane (D) over time in microcosms bioaugmented with 0.7 mg of 21198 (set 4). Nutrients were added to all microcosms on day 0. Bottles 3 and 4 were acetylene controls. Bottles 1 and 2 were respiked with isobutane, TCE, 1,4-dioxane, and gradually increasing concentrations of 1,1-DCE over 115 days of incubation.*

As shown in Figure 4.33, 1,1-DCE was immediately transformed within one hour. The total mass of 1,1-DCE decreased from approximately 0.1 µmol to approximately 0.015 µmol. The recurring presence of residual mass of 1,1-DCE supported the hypothesis that the residual was another unknown compound that was coeluting with 1,1-DCE and unable to be degraded by 21198.

Isobutane was consumed at a faster rate with greater initial biomass. Complete isobutane uptake was observed within two to five days. TCE was slowly transformed over 15 days with the addition of three spikes of isobutane. Though minimal 1,4-dioxane was initially present in the groundwater, the concentration decreased from approximately 115 to 130 µg/L to below the detection limit over the first 2 spikes of isobutane (data not shown).

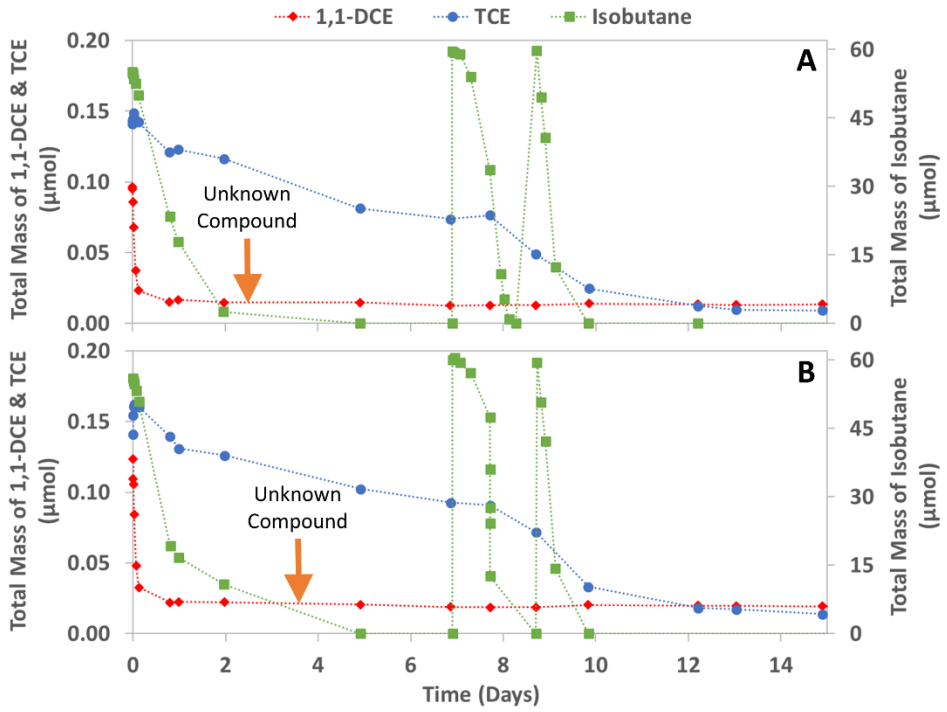

Figure 4.33. *Total mass of isobutane, 1,1-DCE, and TCE over time in set 4, bottles 1 (A) and 2 (B) bioaugmented with 0.7 mg 21198.*

Isobutane, TCE, and 1,4-dioxane were added to bottles 1 and 2 on day 21. Isobutane and 1,4-dioxane were transformed within one day, while TCE was more slowly degraded over seven days (data not shown). As shown in Figure 4.34, 1,1-DCE was re-added to the system on day 28, along with another spike of isobutane, TCE, and 1,4-dioxane. 1,1-DCE was transformed very rapidly (within 1 to 2 hours), followed by isobutane uptake and 1,4-dioxane degradation within one day. TCE was more slowly transformed over seven days.

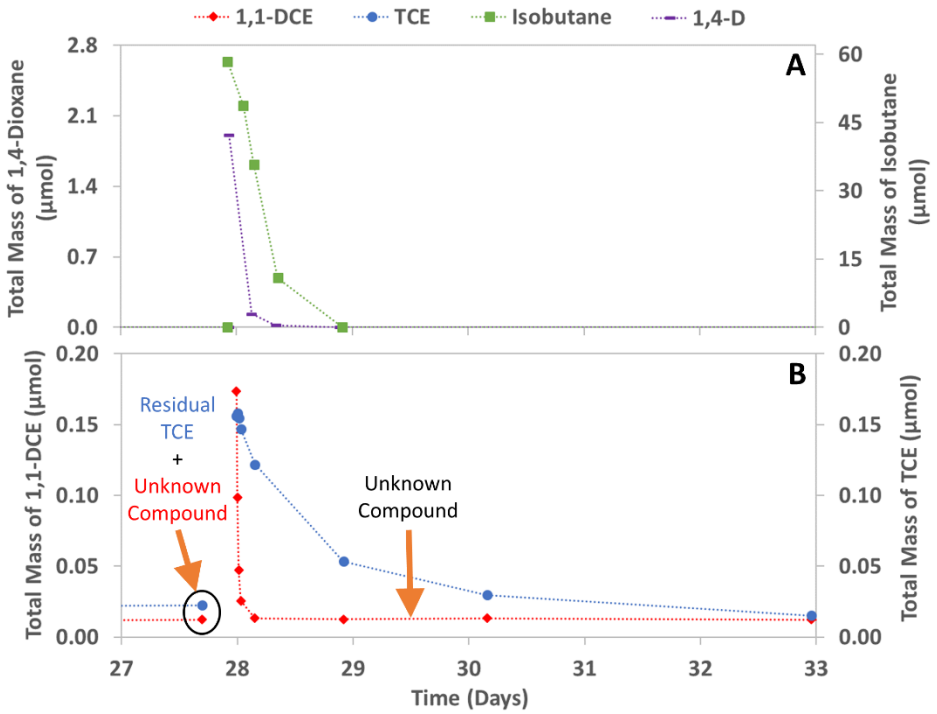

Figure 4.34. *Total mass of 1,4-dioxane and isobutane (A), and 1,1-DCE and TCE (B) over time in one microcosm bioaugmented with 0.7 mg of isobutane-grown 21198 (set 4, bottle 1). This depicts an early spike of isobutane and COCs added to this microcosm on day 28.*

Three subsequent additions of isobutane, TCE, 1,1-DCE, and 1,4-dioxane were made to bottle 1 between days 35 and 70, with gradual increases in 1,1-DCE concentration, culminating in a liquid concentration of 1.5 mg/L. The final spike of primary substrate and COCs on day 70 is depicted in Figure 4.35. 1,1-DCE transformation occurred more slowly and completely stalled at a concentration of approximately 200 µg/L (0.3 µmol total mass). Isobutane uptake and 1,4-dioxane and TCE degradation were minimally observed. The total mass of 1,1-DCE transformed was approximately 1.9 µmol. The biomass present in the system was likely exhausted by the transformation of this quantity of 1,1-DCE.

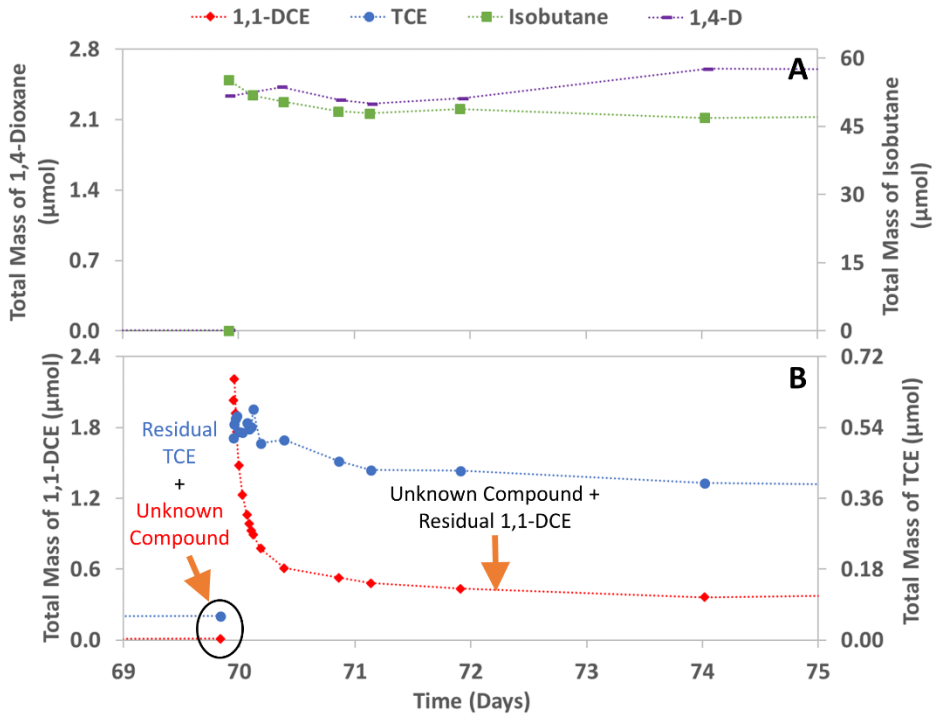

Figure 4.35. *Total mass of 1,4-dioxane and isobutane (A), and 1,1-DCE and TCE (B) over time in one microcosm bioaugmented with 0.7 mg of isobutane-grown 21198 (set 4, bottle 1). This depicts the final spike of isobutane and COCs added to this microcosm on day 70 after gradual increases in 1,1-DCE concentration. The presence of the unknown compound cannot be visualized due to the scale of the plot. 1,1-DCE was not able to be fully transformed.*

Three subsequent additions of isobutane, TCE, 1,1-DCE, and 1,4-dioxane were made to bottle 2 between days 35 and 70, with gradual increases in 1,1-DCE concentration, culminating in a liquid concentration of 0.97 mg/L. The second-to-last spike of primary substrate and COCs on day 70 is depicted in Figure 4.36. 1,1-DCE transformation occurred more slowly. Minimal isobutane uptake and 1,4-dioxane degradation were observed over four days following the respike. Between days 74 and 76, complete isobutane uptake and 1,4-dioxane transformation resumed, followed by slow TCE transformation. The system was able to moderately recover following the exposure to a high concentration of 1,1-DCE.

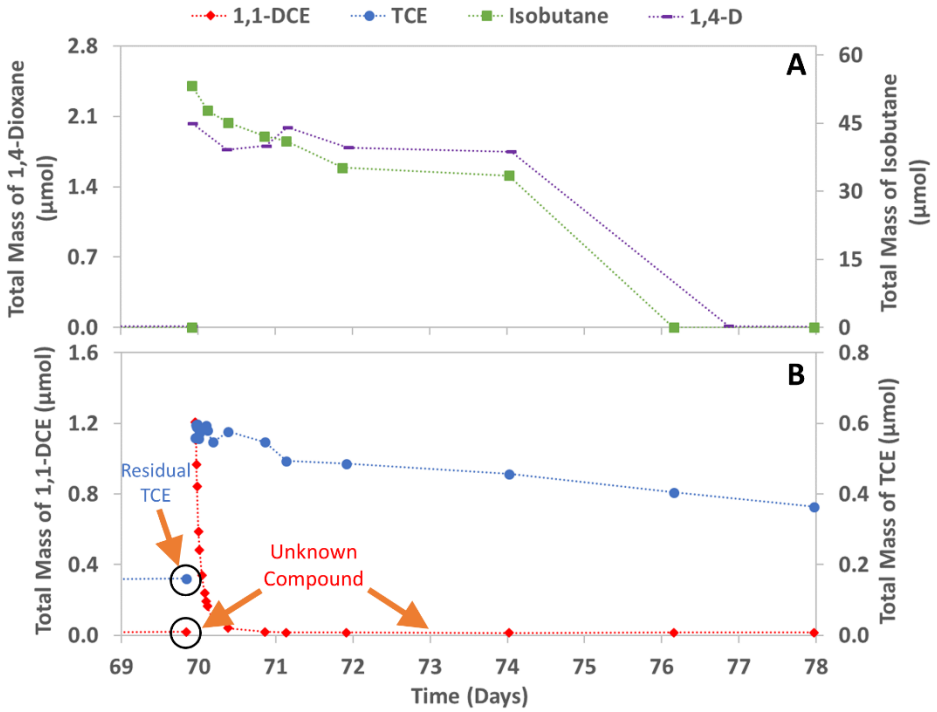

Figure 4.36. *Total mass of 1,4-dioxane and isobutane (A), and 1,1-DCE and TCE (B) over time in one microcosm bioaugmented with 0.7 mg of isobutane-grown 21198 (set 4, bottle 2). This depicts a spike of isobutane and COCs added to this microcosm on day 70 after gradual increases in 1,1-DCE concentration. The presence of the unknown compound cannot be visualized due to the scale of the plot.*

Cometabolism of 1,1-DCE was achieved by the bioaugmented culture and resulted in the rapid initial decrease of 1,1-DCE prior to isobutane utilization. This outcome was consistent with pure culture rate test results utilizing isobutane-grown 21198 that showed rapid transformation of 1,1-DCE but with a low transformation capacity. Based on the quantity of biomass added to the system and the calculated transformation capacity, 0.5 and 0.7 mg of 21198 should have been able to transform 0.33 and 0.46 µmol of 1,1-DCE, respectively. At a slow rate, TCE was also moderately transformed. Isobutane uptake exhibited a shorter lag time using a bioaugmented system, and 1,4-dioxance transformation occurred at a similar rate to isobutane uptake. These results were also consistent with pure culture rate test results with 21198. In summary, bioaugmentation with isobutane-grown 21198 was shown to successfully transform 1,1-DCE and promote sustained cometabolism over 115 days.

4.2.4 Isobutane competitive inhibition

As illustrated in the previous sections, 1,1-DCE toxicity and inhibition resulted in difficulty to stimulate native microbial communities to perform cometabolism. The experimental approach described in this section was conducted in an attempt to competitively inhibit the

transformation of 1,1-DCE via the addition of a high concentration of isobutane. Isobutane was initially added to two native microcosms established in the presence of CAHs at a liquid concentration of 6.6 and 6.7 mg/L (set 5, bottles 3 and 4), approximately ten times greater than the isobutane liquid concentrations in other microcosms. As shown in Figure 4.37, isobutane uptake was not observed over the course of 22 days. This suggests that a high concentration of isobutane cannot competitively inhibit the transformation of 1,1-DCE and preferentially uptake isobutane.

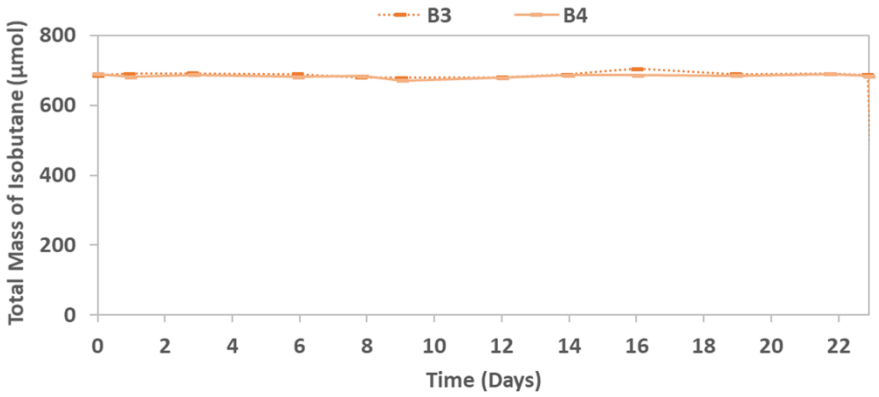

Figure 4.37. *Total mass of isobutane over time in two native microcosms established in the presence of CAHS with approximately ten times greater starting mass of isobutane than other microcosms.*

4.2.5 Isobutane and isobutanol

As shown in Figure 2.3, isobutanol is the product of isobutane transformation by SCAM. The purpose of this approach was to determine if isobutane-utilizers could be could stimulated in the presence of isobutane and CAHs via the direct addition of the isobutane metabolite, isobutanol.

After approximately 20 days of incubation without isobutane uptake in the presence of CAHs and 1,4-dioxane, isobutanol was added to two native microcosms (set 5, bottles 5 and 6). Six additions of approximately 30 to 45 µmol of isobutanol were added to the microcosms over a span of 60 days (Figure 4.38A). Subsequent oxygen consumption and carbon dioxide production were observed, indicating that isobutanol-utilizers were present and active in the system (Figure 4.38B). The total mass of oxygen consumed was approximately 570 µmol, and the total mass of isobutanol consumed was approximately 220 µmol. Based on the stoichiometry from the redox reaction of isobutanol and oxygen, approximately 95 µmol of isobutanol would need to be consumed to produce the observed oxygen consumption. The discrepancy between the

quantity of oxygen consumed for the amount of isobutanol utilized could be explained by isobutanol being used for the production of new cell mass rather than complete oxidation.

Isobutane uptake was not observed over 82 days of incubation following the first addition of isobutanol (Figure 4.38A), and minimal 1,1-DCE and TCE degradation occurred (Figure 4.38C). These results indicate that direct isobutanol-utilizers cannot necessarily utilize isobutane, and that isobutane-utilizers cannot be stimulated via the addition of isobutanol.

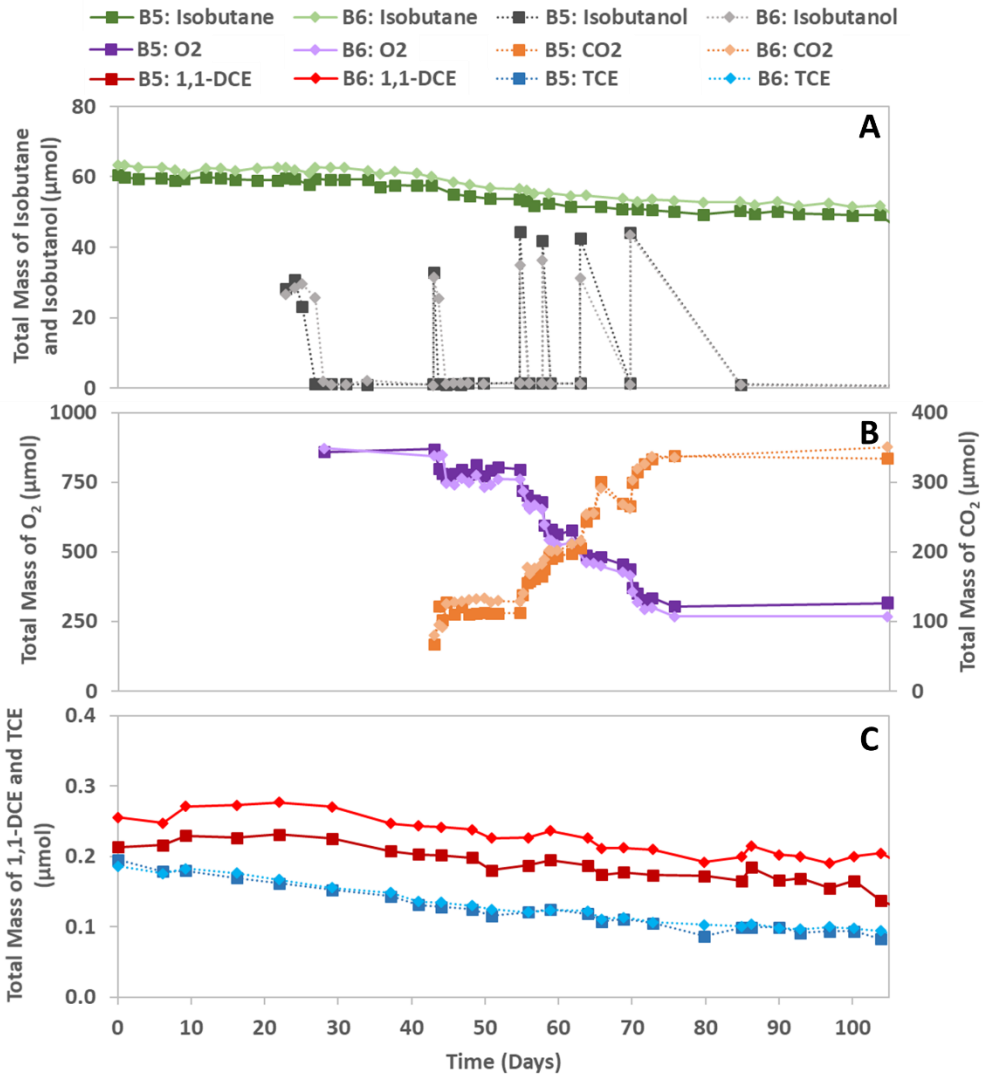

Figure 4.38. *Total mass of isobutane and isobutanol (A), O_2 and CO_2 (B), and 1,1-DCE and TCE (C) in isobutanol-amended microcosms initially in the presence of isobutane. Isobutanol was initially added to microcosms on day 23.*

An additional set of isobutanol-amended microcosms was established initially in the presence of CAHs and in the absence of isobutane. The purpose of this approach was to prevent the activation of the isobutane-induced monooxygenase enzyme responsible for the transformation

of 1,1-DCE into the toxic epoxide and to stimulate an isobutanol-utilizing microbial community prior to the addition of isobutane. It was hypothesized that a healthy isobutanol-enriched culture could exhibit monooxygenase induction following isobutane exposure. To investigate this hypothesis, three additions of 20 to 45 µmol of isobutanol were made to four microcosms in the presence of CAHs and in the absence of isobutane over the first 12 days of incubation (Figure 4.39A).

Subsequent oxygen consumption and carbon dioxide production were observed, indicating that isobutanol-utilizers were present in the system (Figure 4.39B). Approximately 60 µmol of isobutane were added to the microcosms on day 17. Isobutane uptake was not observed over 42 days of incubation (Figure 4.39A). Minimal 1,1-DCE and TCE degradation was observed (Figure 4.39C). Three additions of isobutanol were made between days 30 and 39. Corresponding oxygen consumption and carbon dioxide production were observed. This indicates that isobutanol-utilizers were not negatively impacted by the potential isobutane-induced monooxygenase production and subsequent 1,1-DCE epoxide formation. These results suggest that direct isobutanol-utilizers cannot necessarily utilize isobutane, and that isobutane-utilizers cannot be stimulated via the addition of isobutanol.

The total mass of oxygen consumed was approximately 790 µmol, and the total mass of isobutanol consumed was approximately 190 µmol. Based on the stoichiometry from the redox reaction of isobutanol and oxygen, approximately 130 µmol of isobutanol would need to be consumed to produce the observed oxygen consumption. The discrepancy between the quantity of oxygen consumed for the amount of isobutanol utilized could be explained by isobutanol being used for the production of new cell mass rather than complete oxidation.

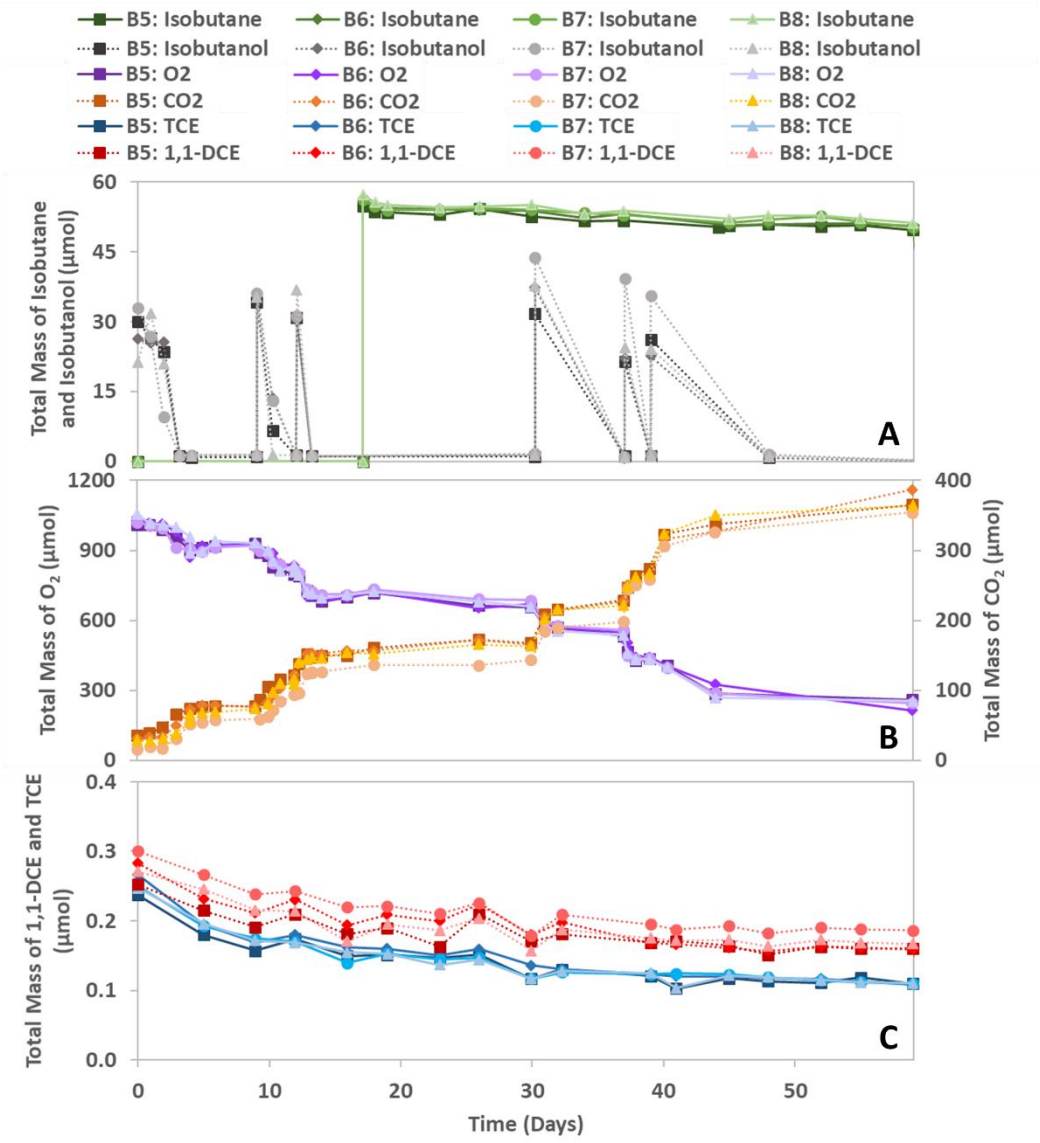

Figure 4.39. *Total mass of isobutane and isobutanol (A), O_2 and CO_2 (B), and 1,1-DCE and TCE (C) in isobutanol-amended microcosms initially in the absence of isobutane. Isobutanol was initially added to microcosms on day 0.*

4.2.6 Isobutene in the presence of CAHs

Due to the 1,1-DCE toxicity and inhibition, a different primary growth substrate was desired that would potentially better perform cometabolic transformations of CAHs. Preliminary work conducted by Rich suggested that a model isobutene-utilizer, ELW1, could transform TCE better than the model isobutane-utilizer, 21198 (Rich, 2015). To explore the use of isobutene as a primary growth substrate, eight microcosms were established in the presence of CAHs (set 6). All microcosms had nutrients added. Microcosms 7 and 8 were propyne controls. Isobutene uptake was not observed in microcosms 3 or 4 over 25 days or in microcosms 5 or 6 over 33 days of incubation in the presence of CAHs and 1,4-dioxane (Figure 4.40). Microcosms 3 and 4

were subsequently sparged (further discussed in Appendix D), and microcosms 5 and 6 were bioaugmented with isobutene-grown ELW1 (further discussed in Section 4.2.8).

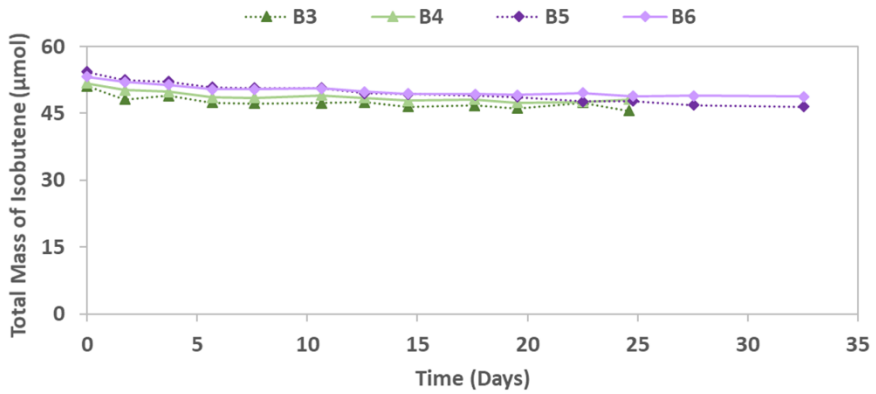

Figure 4.40. *Total mass of isobutene over time in native isobutene microcosms established in the presence of CAHs (set 6, bottles 3 through 6). Microcosms 3 and 4 were sparged to remove CAHs on day 25, and microcosms 5 and 6 were bioaugmented with 0.9 mg of isobutene-grown ELW1 on day 33.*

Isobutene uptake was observed in one native isobutene microcosm established in the presence of CAHs (set 6, bottle 1) after 52 days of incubation in the presence of CAHs (Figure 4.41). The long lag phase suggests either native isobutene-utilizers exist in low quantities in the subsurface at NAS North Island and/or exhibit slow growth rates. It took approximately 8 days for complete isobutene uptake (50 µmol). CAH transformation was strongly inhibited by the presence of isobutene. Rapid 1,1-DCE degradation was observed once the liquid concentration of isobutene was below 500 µg/L. Slower TCE degradation followed the transformation of 1,1-DCE. These results are consistent with those observed in pure culture studies with isobutene-grown ELW1 (Figure 4.12).

Three subsequent additions of isobutene, 1,1-DCE, and TCE showed a similar response, with increasing rates of isobutene uptake and TCE transformation with the second and third respike. As shown in Figure 4.42A/C, the degradation rates of isobutene and TCE approximately doubled for the second and third spike of primary substrate and CAHs. The rate of 1,1-DCE transformation greatly increased between the first and second spikes, but decreased slightly between the second, third, and fourth spikes (Figure 4.42B). 1,4-dioxane has been minimally transformed over the course of the four respikes of isobutene and CAHs (Figure 4.43).

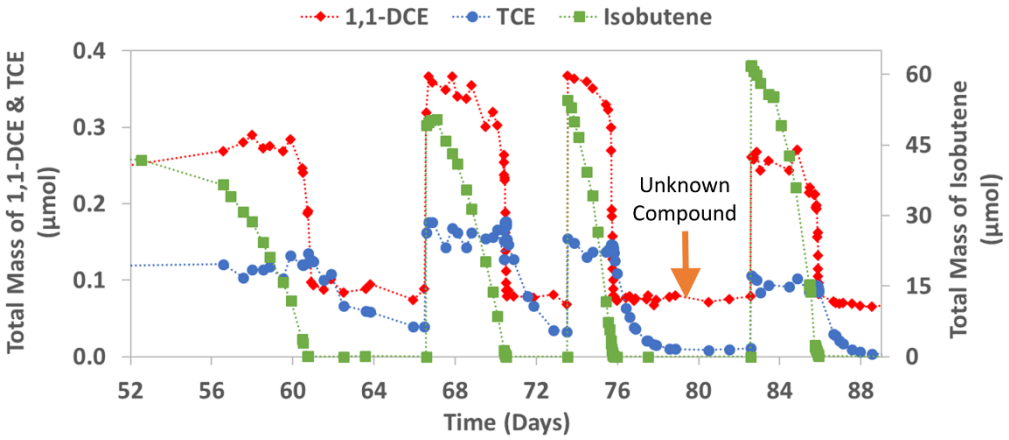

Figure 4.41. *Total mass of isobutene, 1,1-DCE, and TCE over time in the native isobutene microcosm established in the presence of CAHs and 1,4-dioxane (set 6, bottle 1). The residual mass of 1,1-DCE remaining after the transformation is likely the unknown compound discussed in Section 4.2.3.*

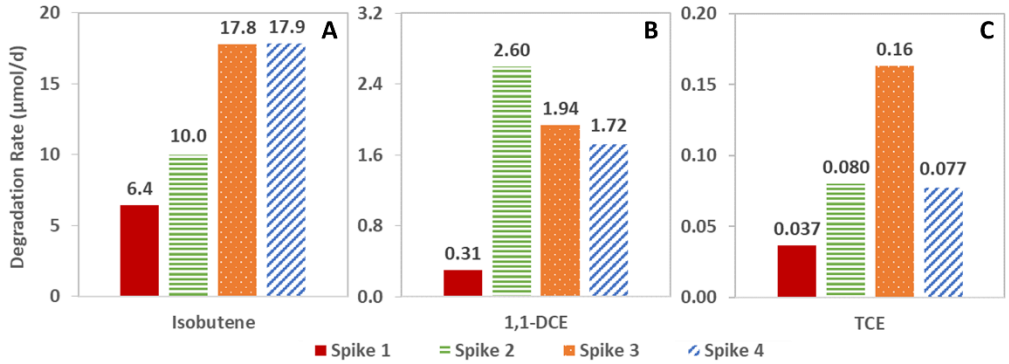

Figure 4.42. *Comparison of degradation rates of isobutene (A), 1,1-DCE (B), and TCE (C) in the native isobutene microcosm established in the presence of CAHs over four spikes of primary substrate and CAHs (set 6, bottle 1).*

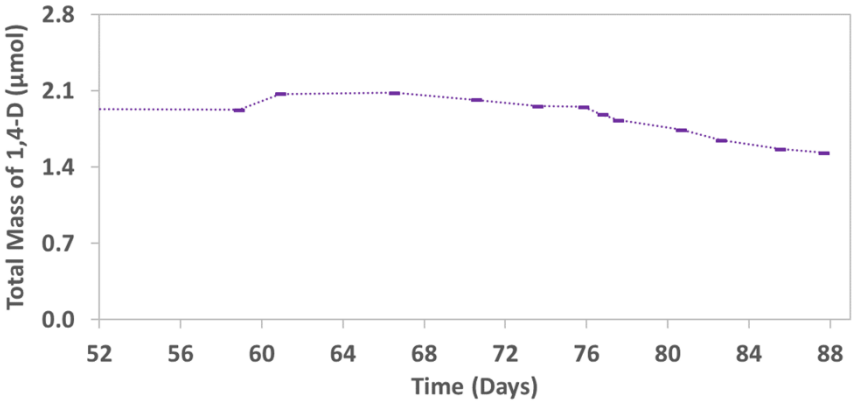

Figure 4.43. *Total mass of 1,4-dioxane over time in the native isobutene microcosm established in the presence of CAHs and 1,4-dioxane (set 6, bottle 1).*

Isobutene uptake was also observed in a second native isobutene microcosm established in the presence of CAHs (set 6, bottle 2) after 89 days of incubation in the presence of CAHs (Figure

4.44). The rate of isobutene uptake was much slower than the initial observed rate in bottle 1. As with bottle 1, CAH transformation was strongly inhibited my isobutene. 1,1-DCE was not fully transformed, and TCE was not transformed.

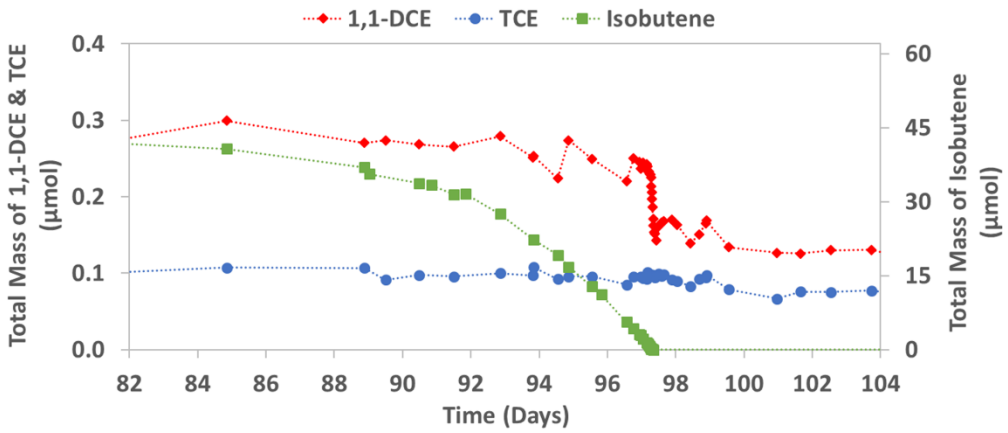

Figure 4.44. *Total mass of isobutene, 1,1-DCE, and TCE over time in a second native isobutene microcosm established in the presence of CAHs (set 6, bottle 2).*

The strong inhibition of 1,1-DCE transformation by the presence of isobutene is advantageous because a microbial population can be established during primary substrate utilization prior to the transformation of 1,1-DCE to the toxic epoxide. The complete transformation of TCE is also significant when compared to isobutane-enriched systems that minimally transformed TCE.

4.2.7 Isobutene bioaugmentation with ELW1

After 33 days of incubation without observable isobutane uptake, two native isobutene microcosms established in the presence of CAHs (set 6, bottles 5 and 6) were bioaugmented with 0.9 mg of isobutene-grown ELW1 (cultured as described in Sections 3.3.1 and 3.3.2). The biomass inoculum quantity was chosen based on the 1,1-DCE transformation capacity reported in Section 4.1.5 (0.30 µmol/mg TSS). Sufficient biomass was desired to be able to fully transform the 1,1-DCE present in the microcosms. 0.9 mg of ELW1 should have been able to transform 0.27 µmol of 1,1-DCE, the approximate starting mass of 1,1-DCE in bottles 5 and 6.

The total mass of isobutene, 1,1-DCE, and TCE over time are presented in Figure 4.45). CAH transformation was strongly inhibited by the presence of isobutene, as was observed in the native microcosm. Rapid 1,1-DCE transformation was observed after isobutene uptake, and slower TCE transformation followed. Slow 1,4-dioxane degradation was observed in both microcosms between days 33 and 67 (Figure 4.46).

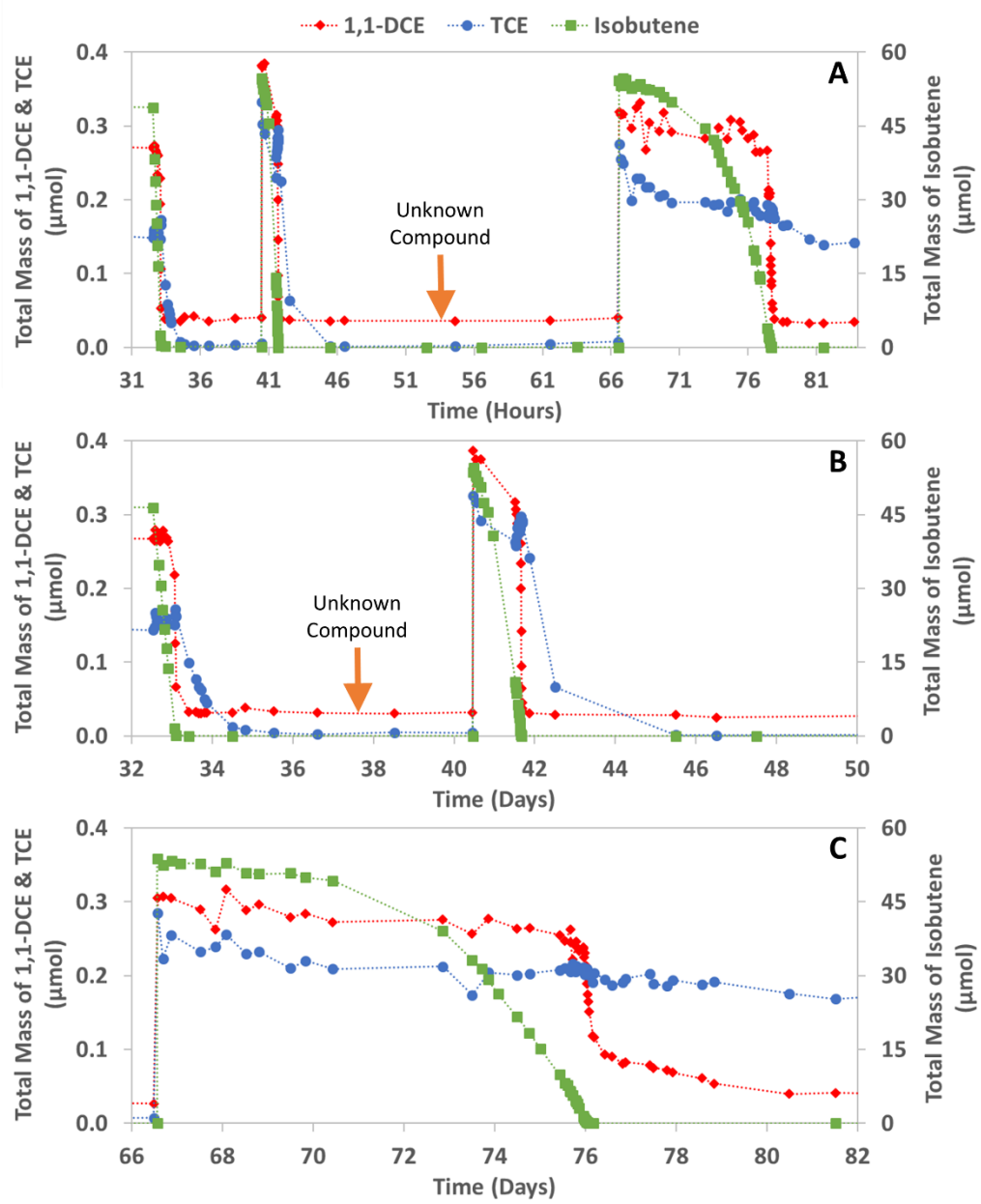

Figure 4.45. *Total mass of isobutene, 1,1-DCE, and TCE over time in ELW1 bioaugmented microcosm 5 (A). The first and second spikes spanning 16 days (A) and the third spike spanning 16 days (B) are shown to observe the inhibition of CAH transformations by the presence of isobutene.*

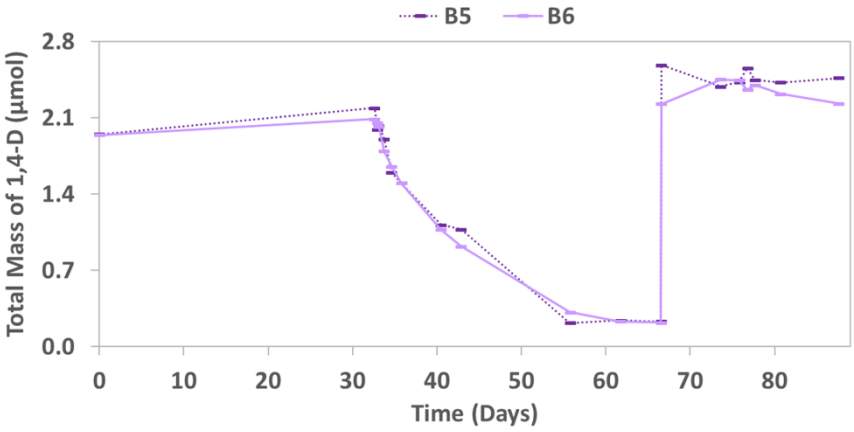

Figure 4.46. *Total mass of 1,4-dioxane over time in native isobutene microcosms established in the presence of CAHs and bioaugmented with 0.9 mg of isobutene-grown ELW1 on day 33 (set 6, bottles 5 and 6).*

The initial rate of isobutene uptake was much greater than that of the active native microcosm discussed in Section 4.2.6. The rate of isobutene uptake significantly decreased with subsequent spikes, and the rates of 1,1-DCE and TCE transformation between the second and third spikes decreased by 87% and 99%, respectively (Figure 4.47A-C). The dramatic differences in results from the native microcosm and the bioaugmented microcosms suggest that a different and more robust microbial community had been enriched from the native aquifer. Microbial analyses are currently being conducted by Dr. Michael Hyman from North Carolina State University to attempt to isolate the microorganism that was stimulated in the native isobutene microcosm.

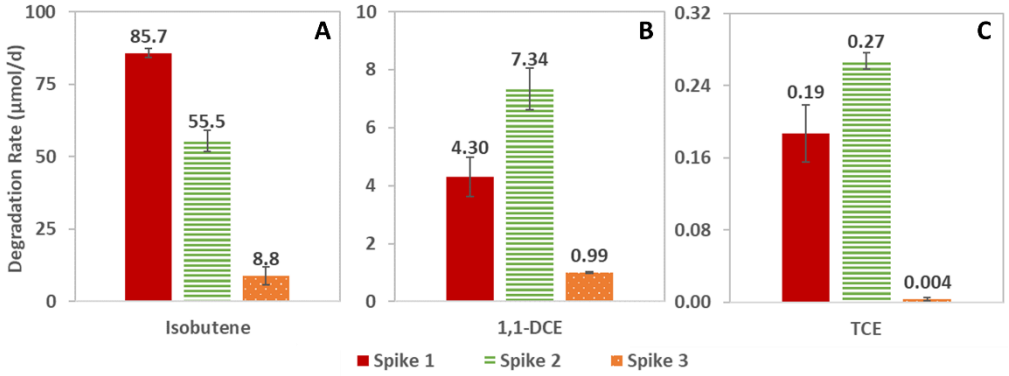

Figure 4.47. *Comparison of degradation rates of isobutene (A), 1,1-DCE (B), and TCE (C) in ELW1 bioaugmented microcosms over three spikes of primary substrate and CAHs. Error bars indicate the standard deviation of a two sample average.*

4.2.8 Nutrient assessment

In order to assess the importance of nutrient addition to the microcosm system, media addition was omitted from two microcosms established with isobutane in the absence of CAHs (set 2,

bottles 1 and 2), and media was added to two microcosms established with isobutane in the absence of CAHs (set 2, bottles 3 and 4). After approximately 12 days of incubation in the absence of CAHs, isobutane uptake was observed in bottles 2 through 4 (Figure 4.48). Isobutane uptake was not observed in bottle 1. Isobutane uptake in bottle 2 that did not have nutrients initially added stalled around day 20, and 0.2 mL of media was subsequently added to bottles 1 and 2 on day 24. This quantity of media corresponds to a concentration of approximately 620 µmol N/L and 240 µmol P/L. Isobutane uptake in bottle 2 resumed, and the system was able to consume an additional spike of isobutane at a faster rate. Twenty days after media was added to bottle 1, isobutane uptake was observed.

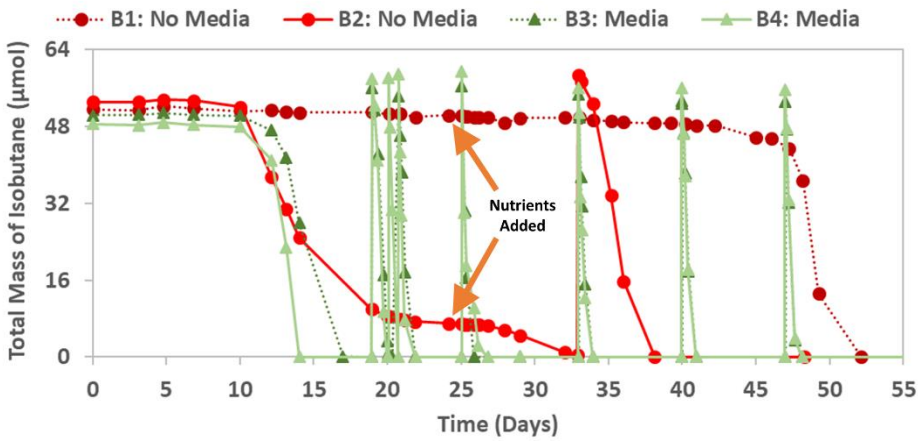

Figure 4.48. *Liquid concentration of isobutane over time in microcosms initially sparged to remove CAHs (set 2). Microcosms 3 and 4 received nutrients on day zero, while microcosms 1 and 2 received nutrients on day 24.*

The importance of nutrients to the success of bioremediation installations has been widely studied. Brockman et al. examined the effect nitrogen and phosphorous addition on in situ bioremediation of TCE (Brockman et al., 1995). The addition of nitrogen and phosphorous was found to increase the biomass count by an order of magnitude and increased the TCE biodegradative potential by three orders of magnitude. Palumbo and Scarborough also found that the addition of nitrogen and phosphorous increased methanotrophic populations by a factor of six and increased TCE mineralization (Palumbo and Scarborough, 1995). The findings of previous investigations and the microcosm results from this study indicate that nutrient addition is likely important to the stimulation of native microbes at the NAS North Island, though minimal aquifer solids were present in the system.

5.0 CONCLUSIONS

Pure-culture rate tests utilizing isobutane, ethane, and propane-grown 21198 and isobutene-grown ELW1 were conducted to assess these cultures' abilities to cometabolize CAHs and 1,4-dioxane in both single-compound and mixture analyses. Microcosm studies were also conducted using aquifer solids and groundwater from NAS North Island in San Diego, California to determine the potential for cometabolic stimulation of native microbial communities utilizing isobutane and isobutene as primary growth substrates. The main conclusions drawn from pure-culture studies are presented in Section 5.1, and the highlights from microcosms studies are described in Section 5.2.

5.1 Rate Test Studies

- For single-compound rate tests with 21198, isobutane-grown 21198 generally demonstrated faster rates of transformation than ethane and propane-grown 21198, though the rates of TCE transformation were similar for all substrates. More chlorinated compounds (TCE and 1,1,1-TCA) exhibited slower rates of transformation than less chlorinated compounds (c-DCE, 1,1-DCE, and 1,2-DCA). For all substrates the rates of transformation were ranked as follows: 1,1-DCE > 1,2-DCA > c-DCE > 1,1,1-TCA > TCE.
- ELW1 transformed TCE approximately twice as fast as 21198 grown on the substrates tested, though 21198 grown on all substrates transformed 1,4-dioxane much more readily than ELW1.
- Rate tests performed with mixtures of contaminants demonstrated similar results to single-compound tests. Transformation rates were generally fastest for isobutane-grown 21198, followed by ethane-grown 21198 and then propane-grown 21198. Isobutene-grown ELW1 was not able to readily transform 1,4-dioxane, 1,2-DCA, or 1,1,1-TCA, but was much more effective at transforming TCE.

5.2 Microcosm Studies

- Native isobutane-utilizers could not be stimulated in the presence of CAHs and 1,4-dioxane. It was hypothesized that the likely formation of the 1,1-DCE epoxide via activation of monooxygenase enzymes by isobutane was toxic and inhibitory to biostimulation of the native microbial community.

- Native isobutane-utilizers were able to be stimulated in the absence of CAHs. When 1,1-DCE and TCE were removed from solution via sparging, isobutane uptake was observed after 12 to 69 days of incubation. Effective long-term cometabolic treatment of groundwater for the removal of CAHs and 1,4-dioxane was achieved, though slow TCE transformation was a process limitation.
- Bioaugmentation with isobutane-grown 21198 was a successful mechanism to achieve rapid 1,1-DCE transformation, moderate 1,4-dioxane transformation, and slow TCE transformation. Bioaugmentation effectively promoted long-term cometabolic treatment of groundwater for the removal of CAHs and 1,4-dioxane, though 1,1-DCE toxicity and slow TCE transformations remained as process limitations, as observed in the native system. The rapid transformation of 1,1-DCE, effective transformation of 1,4-dioxane, and slow TCE transformation were consistent with results observed with pure-culture studies of isobutane-grown 21198.
- Native isobutane-utilizers could not be stimulated via the addition of a high concentration of isobutane to competitively inhibit the transformation of 1,1-DCE.
- Native isobutane-utilizers could not be stimulated via the addition of isobutanol. Isobutanol consumption was observed in the presence of CAHs, but isobutane uptake was not observed. These results indicated that native isobutanol-utilizers were likely not isobutane-utilizers.
- Native isobutene-utilizers were able to be stimulated in the presence of CAHs and 1,4-dioxane. Isobutene strongly inhibited the transformation of CAHs. Once the liquid concentration of isobutene reached below 500 µg/L, rapid 1,1-DCE transformation was observed, followed by slower, but eventually complete, TCE degradation. 1,4-dioxane was minimally transformed by the native isobutene-utilizers. The dimensionless Henry's constant of isobutene (8.7) is much lower than that of isobutane (49.3); consequently, the initial liquid concentration of isobutene in the microcosms was much higher (~ 3,000 µg/L) than that of isobutane (~600 µg/L). The higher liquid concentration of the isobutene could have resulted in greater bioavailability and contributed to the strong inhibition of isobutene on the transformation of CAHs.
- Bioaugmentation with isobutene-grown ELW1 is a successful mechanism to achieve contaminant degradation. As observed in the native microcosm systems, isobutene strongly inhibited the transformation of CAHs. Once the liquid concentration of isobutene

reached below 500 µg/L, rapid 1,1-DCE transformation was observed, followed by slower, but eventually complete, TCE degradation. 1,4-dioxane was slowly transformed in the bioaugmented system. Rates of isobutene uptake and CAH transformations were drastically reduced with successive additions of primary substrate and COCs, which strained the long-term viability of the bioaugmented system. The strong inhibition of CAH transformation by isobutene and the rapid transformation of 1,1-DCE, minimal transformation of 1,4-dioxane, and slow TCE transformation were consistent with results observed with pure-culture studies of isobutene-grown 21198.

- The addition of inorganic nutrients was important for the stimulation of native isobutane utilizers, though the lack of substantial aquifer solids could have contributed to the necessity of nutrient additions.

6.0 FUTURE WORK

Future studies should be conducted with a greater initial mass of aquifer solids. Aquifer cores were not provided for the experimental studies described in this thesis. Experimentation was solely conducted using groundwater that contained trace amounts of sediment. The limited quantity of aquifer solids present for the experimentation described in this thesis could explain the importance of nutrient addition and the extended lag times observed in the native sparged isobutane microcosms and the native isobutene microcosms. Future studies should also focus on the potential use of other primary substrates such as methane and propane to determine their ability for potential concurrent transformation of 1,4-dioxane and CAHs. Future studies should also be performed in columns, rather than microcosms, to more accurately reflect natural transient subsurface conditions.

An objective of this study was to evaluate the potential use of a duel-substrate approach in which multiple primary substrates would be used to effectively transform contaminant mixtures. Based on the results presented in this thesis, further microcosm studies should be conducted utilizing isobutene first to promote the complete transformation of 1,1-DCE and TCE and then introducing isobutane to stimulate the transformation of 1,4-dioxane.

Numerical models for the cometabolism of primary substrates, 1,4-dioxane, and CAHs should also be developed for both pure-culture and microcosm results to be able to better understand and predict field-scale application and success. This would include a more detailed study of the inhibition kinetics of primary substrates and COCs and determination of kinetic parameters such as maximum transformation rates and half-saturation constants.

Microbial analyses should also be performed to compare the microbial communities established in the native and bioaugmented microcosms. A more detailed analysis should be conducted on the native isobutene microcosm to isolate the microorganism that was stimulated and enriched. If the microorganism is found to be different from ELW1, pure culture rate tests and microcosm bioaugmentation should be performed to evaluate its potential for field application.

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8.0 APPENDICES

A. Recipe for mineral salts media (growth media)

Mineral salts medial trace element solution:

Compound	Mass added to 500 mL nano-pure H_2O (g)
EDTA	25
$ZnSO_4 \cdot 7H_2O$	11
$CaCl_2$	2.27
$MnCl_2 \cdot 4H_2O$	2.53
$FeSO_4 \cdot 7H_2O$	2.5
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.55
$CuSO_4 \cdot 5H_2O$	0.753
$CoCl_2 \cdot 6H_2O$	0.855

1. Adjust pH to 6.0 with KOH
2. Store in refrigerator

Solution 1 (concentrated by 10x):

Compound	Quantity added to 1,000 mL nano-pure H_2O
NH_4Cl	20 g
$MgCl_2 \cdot 6H_2O$	0.75 g
$(NH_4)_2SO_4$	1.0 g
Trace element soln.	2.0 mL

Solution 2 (concentrated by 100x):

Compound	Mass added to 1,000 mL nano-pure H_2O (g)
K_2HPO_4	155
NaH_2PO_4	85

1. Autoclave Solution 1 and Solution 2 separately
2. Mix 1X concentration by dilution with autoclaved nano-pure water

B. Heterotrophic and minimal media plates

Heterotrophic growth plates:

Compound	Mass added to 1,000 mL nano-pure H_2O (g)
Tryptic soy	3
Glucose	10
Agar	15

1. Autoclave solution
2. Store at 4°C prior to use
3. Wrap in parafilm after streaking and store at 30°C

Minimal media plates:

Compound	Quantity added to 890 mL nano-pure H_2O
Agar	15 g
Solution 1	100 mL
Solution 2	10 mL

1. Autoclave agar and nano-pure water
2. Add Solution 1 and Solution 2 once the agar mixture is cool to the touch
3. Store at 4°C prior to use

C. Pure Culture Rate Test Results

C.1 Isobutane-grown 21198 Pure Culture Rate Test Results

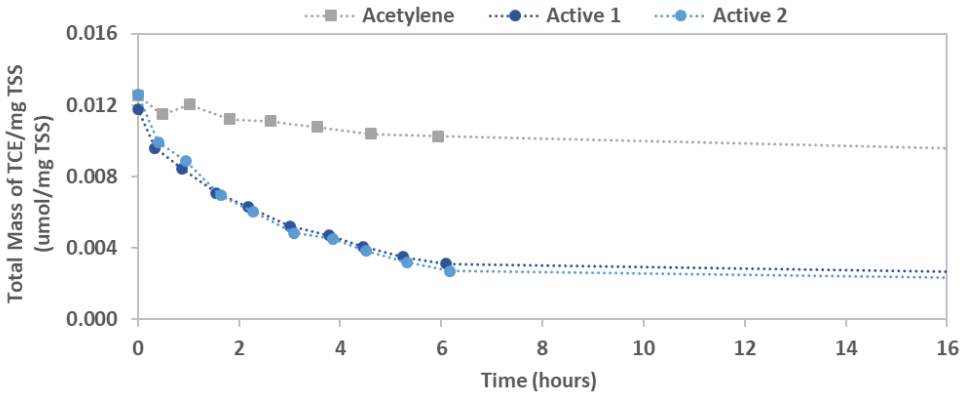


Figure A.1. *Total mass of TCE per mg TSS over time for isobutane-grown 21198.*

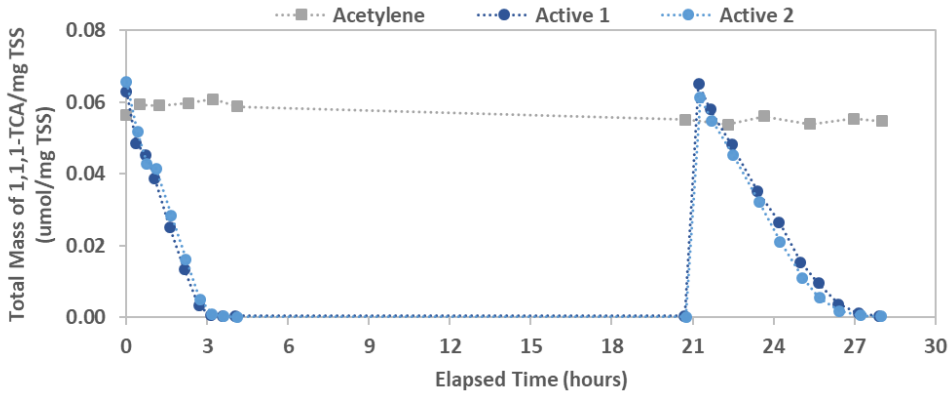


Figure A.2. *Total mass of 1,1,1-TCA per mg TSS over time for isobutane-grown 21198.*

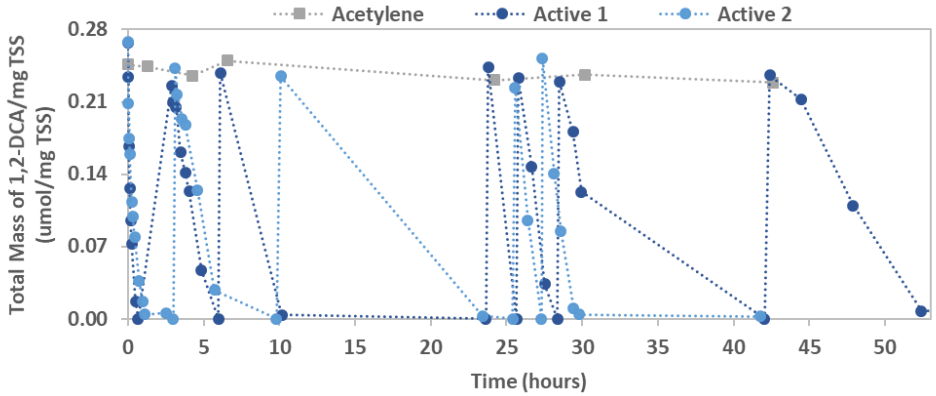


Figure A.3. *Total mass of 1,2-DCA per mg TSS over time for isobutane-grown 21198.*

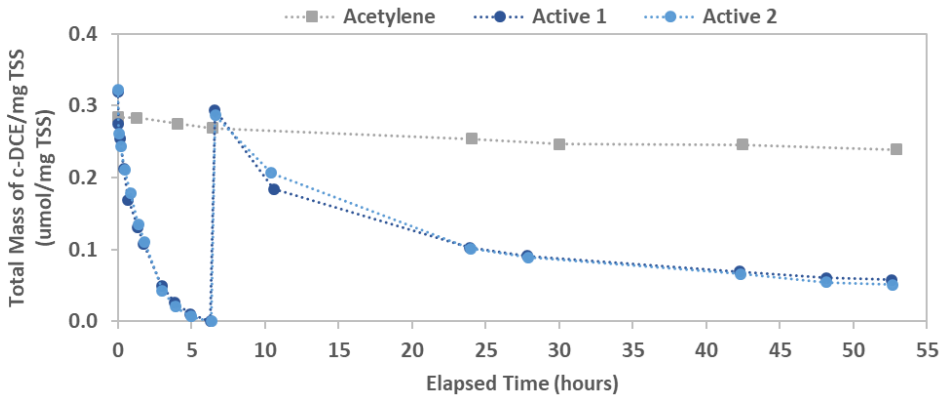


Figure A.4. *Total mass of c-DCE per mg TSS over time for isobutane-grown 21198.*

C.2 Ethane-grown 21198 Pure Culture Rate Test Results

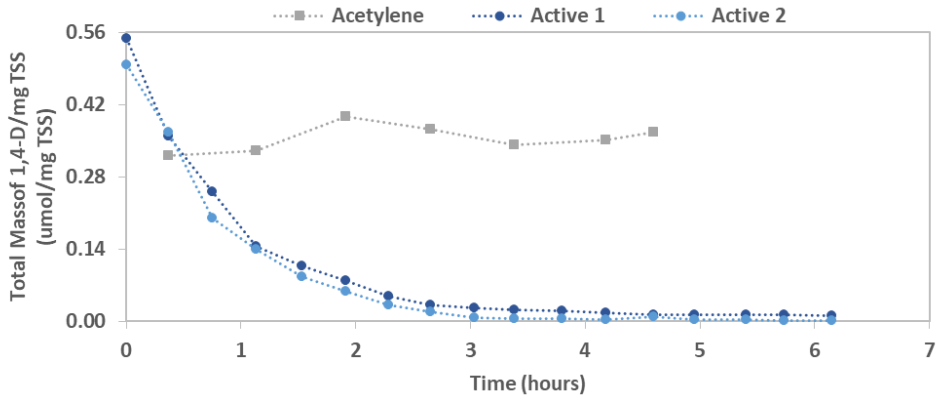


Figure A.5. *Total mass of 1,4-dioxane per mg TSS over time for ethane-grown 21198.*

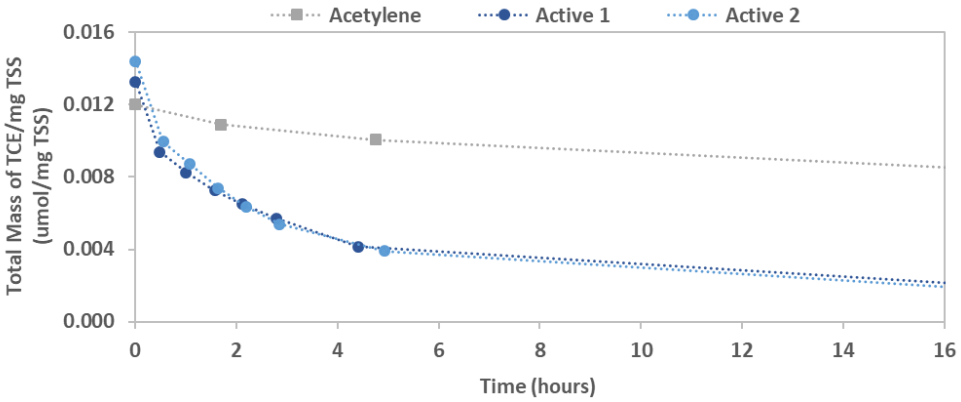


Figure A.6. *Total mass of TCE per mg TSS over time for ethane-grown 21198.*

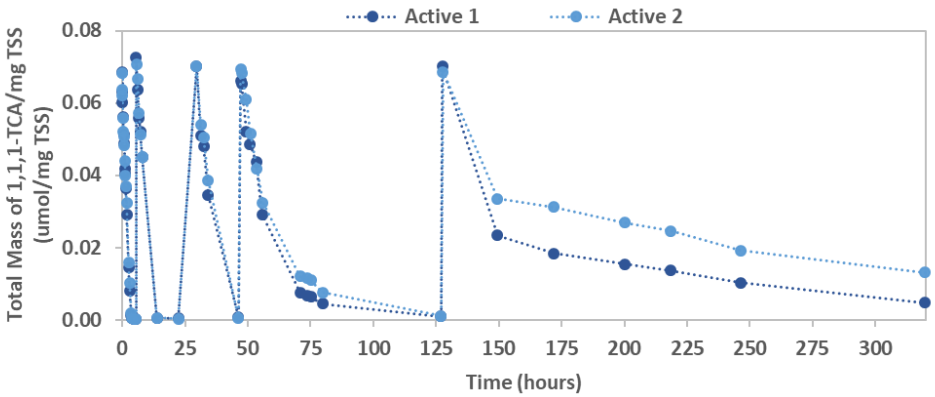


Figure A.7. *Total mass of 1,1,1-TCA per mg TSS over time for ethane-grown 21198.*

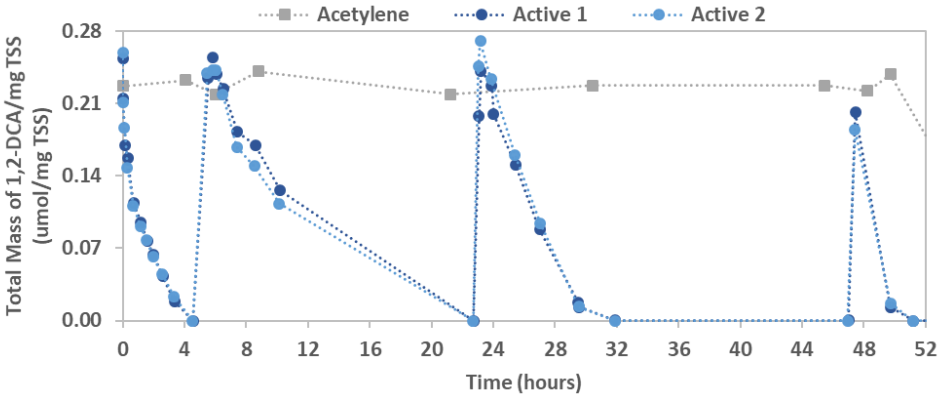


Figure A.8. *Total mass of 1,2-DCA per mg TSS over time for ethane-grown 21198.*

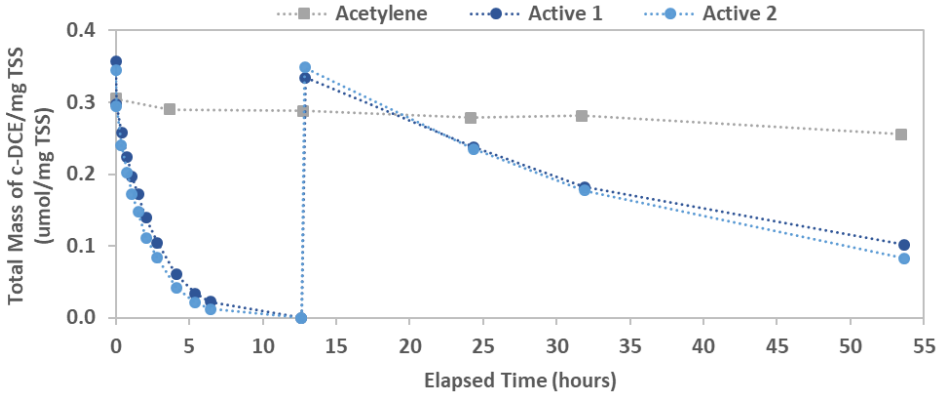


Figure A.9. *Total mass of c-DCE per mg TSS over time for ethane-grown 21198.*

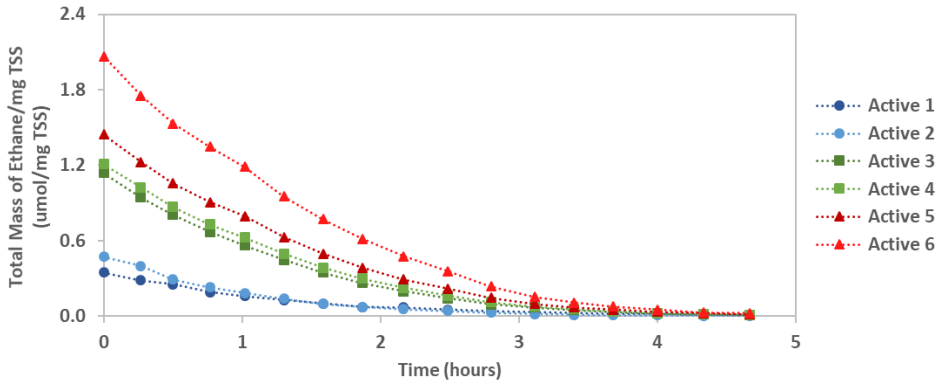


Figure A.10. *Total mass of ethane per mg TSS over time for ethane-grown 21198.*

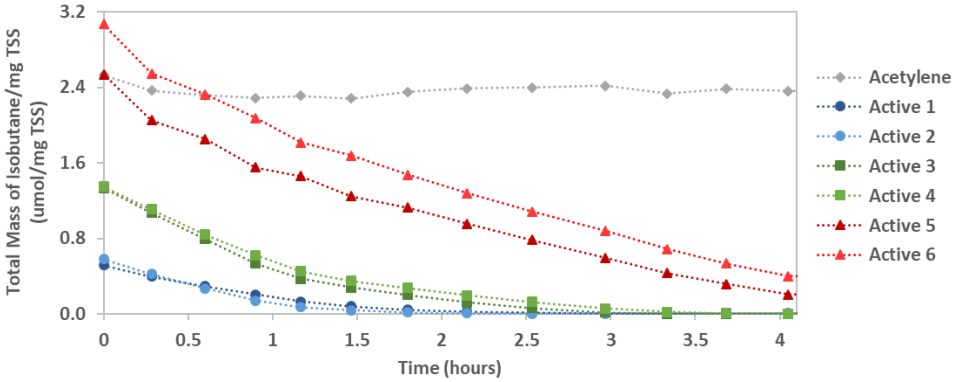


Figure A.11. *Total mass of isobutane per mg TSS over time for ethane-grown 21198.*

C.3 Propane-grown 21198 Pure Culture Rate Test Results

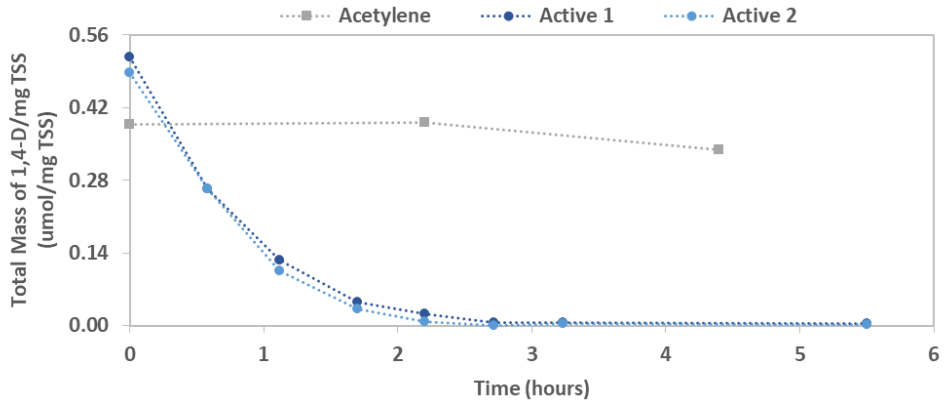


Figure A.12. *Total mass of 1,4-dioxane per mg TSS over time for propane-grown 21198.*

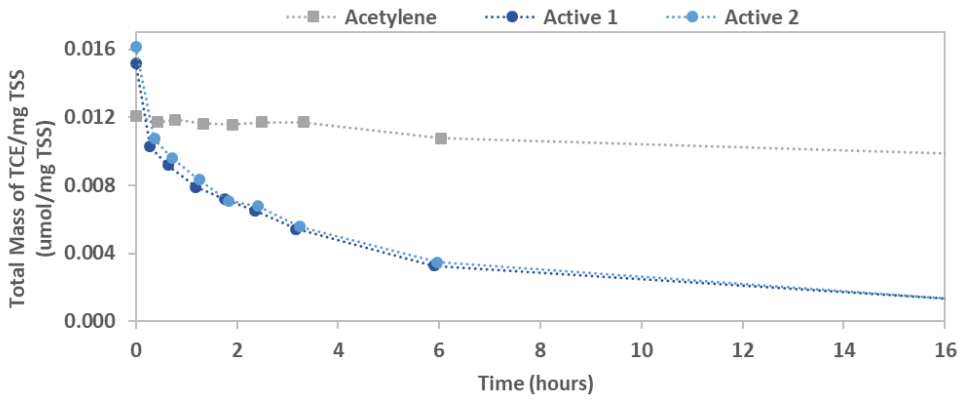


Figure A.13. *Total mass of TCE per mg TSS over time for propane-grown 21198.*

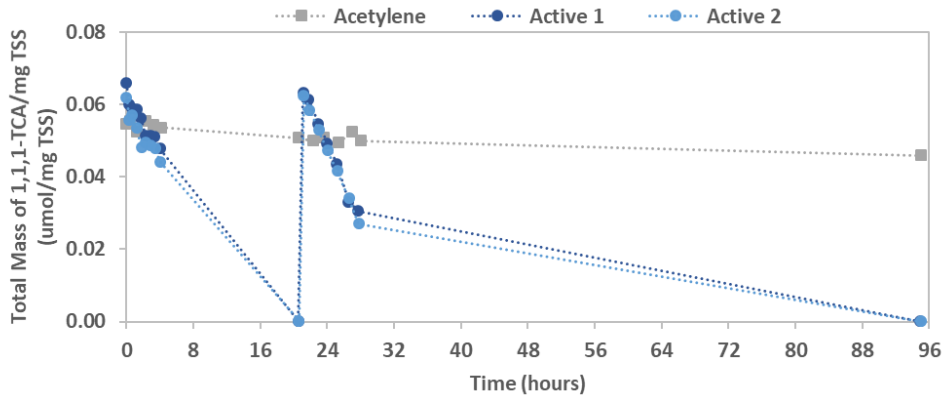


Figure A.14. *Total mass of 1,1,1-TCA per mg TSS over time for propane-grown 21198.*

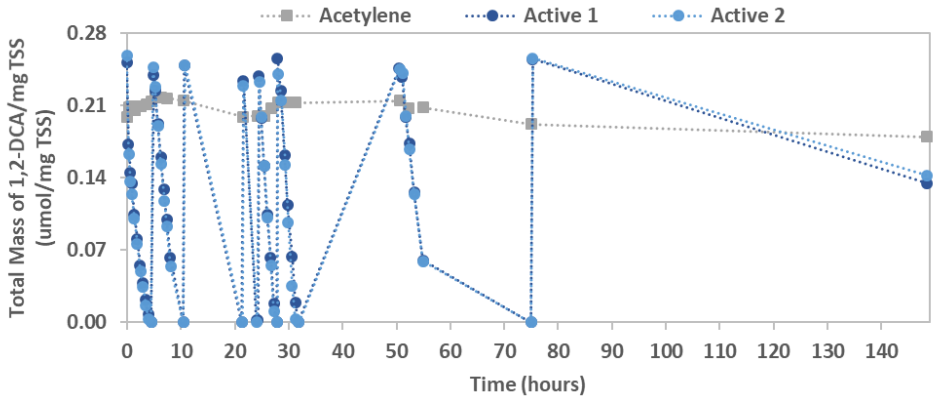


Figure A.15. *Total mass of 1,2-DCA per mg TSS over time for propane-grown 21198.*

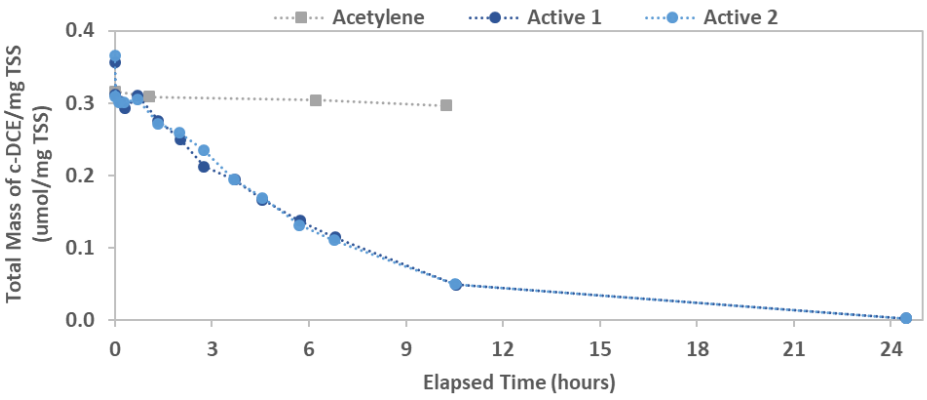


Figure A.16. *Total mass of c-DCE per mg TSS over time for propane-grown 21198.*

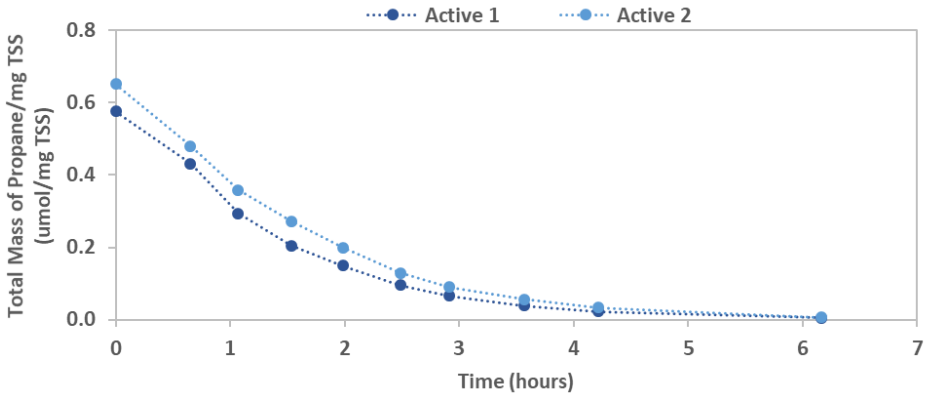


Figure A.17. *Total mass of propane per mg TSS over time for propane-grown 21198.*

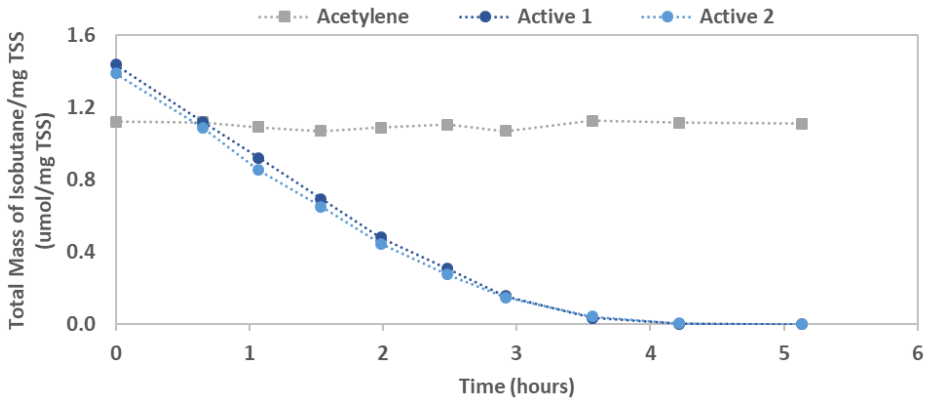


Figure A.18. *Total mass of isobutane per mg TSS over time for propane-grown 21198.*

C.4 Propane-grown 21198 Pure Culture Rate Test Results

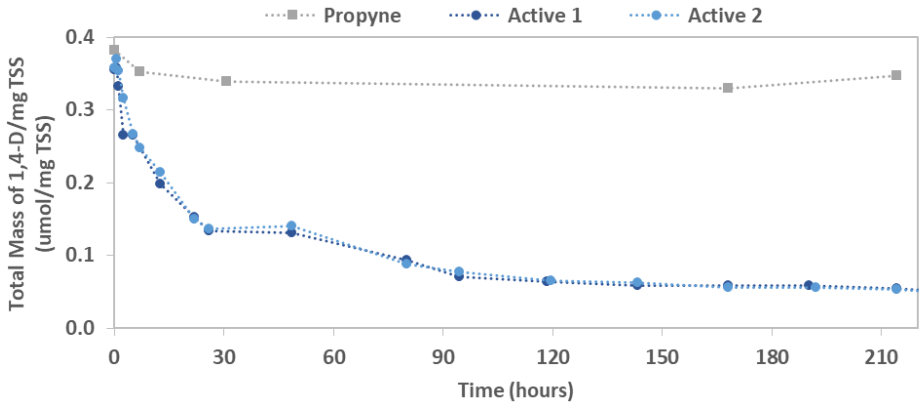


Figure A.19. *Total mass of 1,4-dioxane per mg TSS over time for isobutene-grown ELW1.*

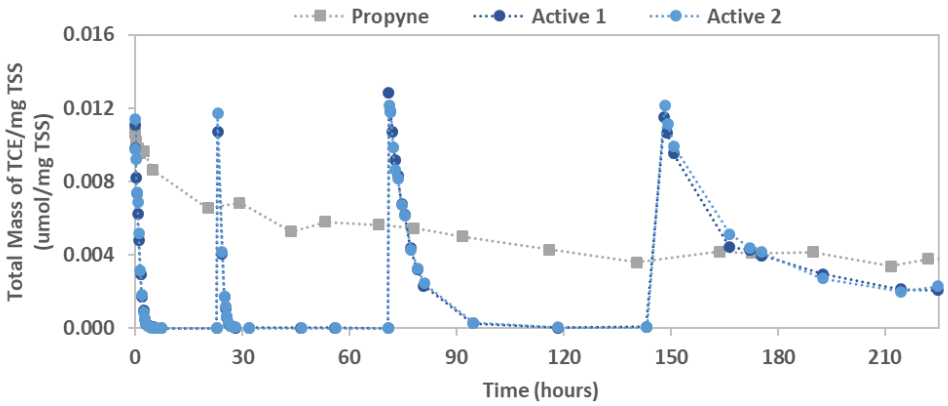


Figure A.20. *Total mass of TCE per mg TSS over time for isobutene-grown ELW1.*

D. Isobutene in the absence of CAHs

After 25 days of isobutene exposure in the presence of CAHs, native microcosms initially established in the presence of CAHs and isobutene (set 6 bottles 3 and 4) were sparged with nitrogen to remove 1,1-DCE and TCE from solution. Isobutene uptake was not observed in the relative absence of CAHs after 50 days of isobutene incubation that followed initial concurrent CAH/isobutene exposure (Figure A.21). A small residual of CAHs remained in these bottles at liquid concentrations of approximately 10 µg/L (1,1-DCE) and 40 µg/L (TCE) due to desorption from sediments. Isobutene uptake was also not observed after 60 days of isobutene exposure in the absence of CAHs (Figure A.22). The absence of microbial activity can likely be attributed to a low prevalence of isobutene-utilizing bacteria in the natural system and/or a very slow growth rate of isobutene-utilizers.

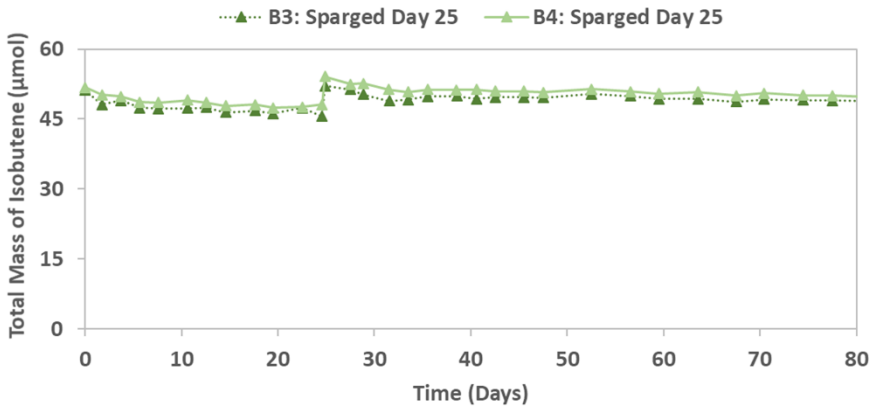


Figure A.21. *Total mass of isobutene over time in native microcosms initially established in the presence of CAHs (set 6, bottles 3 and 4). CAHs were sparged from solution on day 25, and isobutene was re-added to the headspace.*

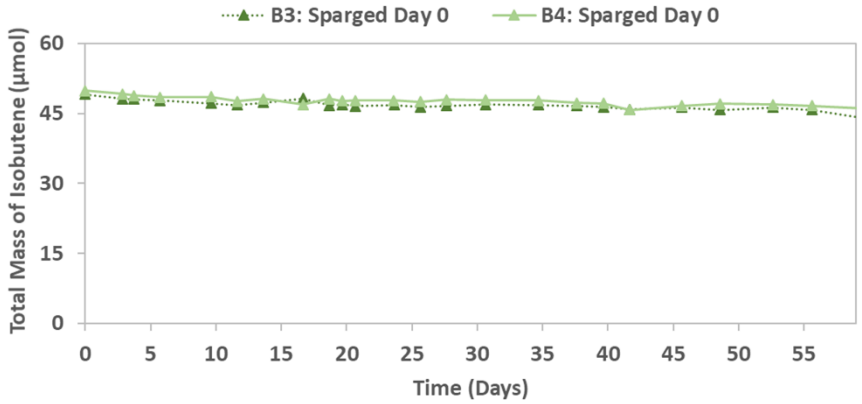


Figure A.22. *Total mass of isobutene over time in native microcosms established in the absence of CAHs (set 7, bottles 3 and 4). CAHs were sparged from solution on day 0 when isobutene was initially added to the headspace.*

Appendix F
Analytical and Field Parameter Results Summary Tables

Sample ID	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S
Sampling Date	12/15/2020	2/9/2021	4/6/2021	4/28/2021	5/19/2021	5/21/2021	5/25/2021	6/1/2021	6/8/2021	6/21/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022	
Days	-90	-34	22	44	65	67	71	78	85	98	183	246	274	345	386	499	
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	
VOCS (GC/MS)	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	
dichlorodifluoromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
chloromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
vinyl chloride	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5 U	
bromomethane	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
chloroethane	1.00 U	1.00 U	20.00 U	81.20 D	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
trichlorofluoromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1-dichloroethylene	129.48	227.20 D	774.60 D	1298.20 D	631.40 D	--	--	540.20 D	--	606.60 D	523.75 D	210.95 D	189.90 D	185.35 D	158.15 D	257.0 D	
methylene chloride	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
trans-1,2-dichloroethylene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1-dichloroethane	12.16	23.63	43.60 D	75.20 D	39.20 D	--	--	36.80 D	--	48.20 D	41.60 D	38.30 D	36.45 D	38.55 D	34.40 D	50.4 D	
2,2-dichloropropane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
cis 1,2- dichloroethylene	79.34	127.70	153.40 D	301.80 D	180.00 D	--	--	197.40 D	--	219.60 D	177.55 D	207.10 D	160.95 D	210.55 D	182.60 D	204.45 D	
bromochloromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
chloroform	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1,1-trichloroethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
carbon tetrachloride	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1-dichloropropene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
benzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,2-dichloroethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
trichloroethylene	92.27	189.60 D	759.60 D	1028.20 D	616.40 D	--	--	671.80 D	--	689.60 D	567.35 D	231.50 D	260.95 D	231.45 D	200.75 D	315.75 D	
1,2-dichloropropane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
dibromomethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
bromodichloromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
cis-1,3-dichloropropene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
toluene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
trans-1,3-dichloropropene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1,2-trichloroethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
tetrachloroethylene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5 U	
1,3-dichloropropane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
dibromochloromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2-dibromoethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
chlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1,1,2-tetrachloroethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
ethylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
xylenes (m/p)	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
o-xylene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
styrene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
bromoform	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
isopropyl benzene (cumene)	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
bromobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1,2,2-tetrachloroethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2,3-trichloropropane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
n-propyl benzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
2-chlorotoluene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
4-chlorotoluene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,3,5-trimethylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
tert-butylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2,4-trimethylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
sec-butylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,3-dichlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
4-isopropyltoluene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,4-dichlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2-dichlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
n-butylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2-dibromo-3-chloropropane	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
1,2,4-trichlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
hexachlorobutadiene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
naphthalene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2,3-trichlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
Methyl tertiary butyl ether	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
Acetone	5.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U	
carbon disulfide	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
2-butanone (MEK)	5.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U	
tetrahydrofuran (THF)	5.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	
4-methyl-2-pentanone (MIBK)	5.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U	
2-hexanone	5.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U	
2-chloroethyl vinyl ether	5.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U	

Sample ID	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S	PMW-1S
Sampling Date	12/15/2020	2/9/2021	4/6/2021	4/28/2021	5/19/2021	5/21/2021	5/25/2021	6/1/2021	6/8/2021	6/21/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-90	-34	22	44	65	67	71	78	85	98	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	92.22 D	67.50 D	350.40 D	114.21 D	238.11 D	--	--	--	--	296 D	76.06 D	227.88 D	231.00 D	247.04 D	205.80 D	264 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	0.76 J	0.69 J	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	1 U	135.32	118.27	2.65	1,764.96	2.04	45
Ethane	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.9 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.7 U	2.68 U	2.68 U	2.68 U	2.68 U	2.7 U	2.68 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	7.0 U	6.99 U	6.99 U	6.99 U	6.99 U	7.0 U	6.99 U
Isobutane	--	--	3.62 U	3.62 U	3.62 U	3.62 U	183.76	458.85	557.38	1656.01	346.17	69.25	989.58	454.57	39.61	104.82
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	--	--	5.00 U	5.00 U	45.31	82.22	23.35	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	--	--	--
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	224.71 D	202.87 D	346.95 D	315.56 D	325.14 D	320.30 D	318.91 D	341.58 D	339.22 D	352.7418 D	377.74 D	408.85 D	412.26 D	420.74 D	433.47 D	432.6006 D
Nitrite as N	0.20 U	0.20 U	1.29	0.19 J	0.20 U	0.20 U	1.89	1.31	0.20	0.2 U	0.19 J	0.20 U	0.20 U	0.20 U	0.20 U	0.2 U
Sulfate as SO4	313.54 D	284.84 D	314.77 D	290.07 D	299.67 D	282.81 D	281.45 D	301.31 D	314.19 D	303.979 D	306.60 D	321.41 D	324.18 D	302.39 D	303.04 D	348.0985 D
Bromide	0.20 U	0.20 U	4.29	5.98	0.20 U	14.74	0.20 U	3.80	4.43	0.2 U	1.15	2.50	0.20 U	3.11	3.40	4.2647
Nitrate as N	12.86	11.20	17.19	15.28	0.20 U	0.20 U	1.42	5.68	8.72	10.7034	6.28	2.29	6.21	0.89	3.00	4.9164
Phosphate as P, ortho	0.20 U	0.20 U	0.20 U	0.20 U	22.10 E	26.70 D	23.41 D	7.97	7.59	5.787	14.68	26.09 D	33.79 D	26.12 D	30.47 D	38.5526 D
FIELD PARAMETERS																
pH (SU)	6.25	6.74	7.12	6.98	6.81	6.63	6.94	6.28	6.48	6.61	6.93	6.88	6.86	7.52	6.85	6.98
Temperature (°C)	22.20	21	23.30	22.50	22.10	22.50	24.80	23.9	22.7	22.8	24.2	23.6	21.90	21.4	21.9	24.4
Dissolved Oxygen (DO; mg/L)	19.64	18.57	29.4	31.90	0.05	0.03	0.5	5.26	10.1	15.72	2.58	0.48	1.95	0.23	30.4	23.11
Redox Potential (ORP; mV)	356.8	185.5	171.1	157.6	-83.1	-37.5	-184.5	99.3	259.5	250.7	61.5	-60.4	189.60	-169.6	205.8	236.1
Specific Conductivity (mS/cm)	2.071	1.991	2.792	2.670	2.728	2.913	2.691	2.781	2.778	2.853	2.917	3281	3.46	3232	3.402	4750.18
Depth to Water (ft-btoc) prior to purging	25.2	25.5	25.6	25.60	25.70	25.73	25.71	25.91	25.75	25.63	25.8	25.73	25.50	25.50	25.76	25.73
Purge Rate (mL/min)	100	140	100	100	100	120	100	80	95	100	100	100	100	100	100	100
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Propane Monooxygenase (PPO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Ammonia Monooxygenase (AMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Particulate methane monooxygenase (PMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D
Sampling Date	12/15/2020	2/9/2021	4/6/2021	4/28/2021	5/19/2021	5/21/2021	5/25/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-90	-34	22	44	65	67	71	78	85	92	98	113	126	155	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
VOCS (GC/MS)	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
dichlorodifluoromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
vinyl chloride	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromomethane	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
chloroethane	20.00 U	20.00 U	20.00 U	82.00 D	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trichlorofluoromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloroethylene	2864.40 D	2898.40 D	805.60 D	1320.80 D	730.40 D	--	--	896.60 D	--	544.20 D	701.80 D	563.00 D	625.00 D	516.75 D	517.95 D	202.85 D	247.95 D	256.35 D	219.60 D	269.05 D
methylene chloride	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
trans-1,2-dichloroethylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloroethane	203.80 D	197.80 D	23.80 D	77.20 D	44.60 D	--	--	60.00 D	--	42.40 D	59.20 D	41.80 D	43.00 D	41.85 D	49.70 D	38.90 D	46.25 D	51.80 D	48.85 D	42.30 D
2,2-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
cis 1,2- dichloroethylene	1254.00 D	1316.80 D	164.40 D	368.00 D	201.40 D	--	--	296.20 D	--	173.80 D	224.40 D	175.00 D	186.20 D	172.30 D	193.00 D	207.20 D	201.45 D	473.40 D	256.85 D	198.50 D
bromochloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chloroform	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,1-trichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
carbon tetrachloride	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
benzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trichloroethylene	1776.60 D	2075.80 D	809.60 D	1081.60 D	748.80 D	--	--	1026.20 D	--	595.20 D	744.60 D	623.80 D	675.20 D	544.80 D	590.70 D	237.90 D	343.80 D	125.85 D	270.55 D	300.35 D
1,2-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
dibromomethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromodichloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
cis-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
toluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trans-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,2-trichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
tetrachloroethylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
dibromochloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dibromoethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,1,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
ethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
xylenes (m/p)	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
o-xylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
styrene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromoform	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
isopropyl benzene (cumene)	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,2,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,3-trichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
n-propyl benzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
2-chlorotoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
4-chlorotoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3,5-trimethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
tert-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,4-trimethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
sec-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
4-isopropyltoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,4-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
n-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dibromo-3-chloropropane	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
1,2,4-trichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
hexachlorobutadiene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
naphthalene	78.40 D	48.40 D	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,3-trichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
Methyl tertiary butyl ether	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
Acetone	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
carbon disulfide	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
2-butanone (MEK)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
tetrahydrofuran (THF)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
4-methyl-2-pentanone (MIBK)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
2-hexanone	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
2-chloroethyl vinyl ether	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U

Sample ID	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D	PMW-1D
Sampling Date	12/15/2020	2/9/2021	4/6/2021	4/28/2021	5/19/2021	5/21/2021	5/25/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-90	-34	22	44	65	67	71	78	85	92	98	113	126	155	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	59.8 D	698.80 D	185.80 D	437.24859 D	229.35 D	--	--	--	--	--	334.00 D	--	305.27 D	293.01 D	497.60 D	197.76 D	315.88 D	218.98 D	280.76 D	246 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	1.06	0.60 J	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	373.74	22.99	6.60	3.72	6.89	0.95 U	1,187.55	0.95 U	36.55
Ethane	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U
Isobutane	--	--	3.62 U	3.62 U	3.62 U	3.62 U	139.43	511.60	673.65	538.87	1229.44	763.28	347.43	320.59	44.50	44.05	485.71	746.03	9.36	60.07
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	--	--	5.00 U	5.00 U	94.58	73.06 U	5.00 U	5.00 U	5.00 U	--	--	--	--	--	5.00 U	5.00 U	5.00 U	--	--	--
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	448.64 D	401.41 D	345.1048 D	325.37 D	319.40 D	320.03 D	324.56 D	353.24 D	343.86 D	343.62 D	351.467 D	--	364.12 D	357.58 D	378.30 D	406.87 D	402.22 D	420.19 D	434.07 D	466.2712 D
Nitrite as N	1.43	2.79	1.137	0.14 J	0.20 U	0.20 U	1.59	1.03	0.26	0.20 U	0.2 U	--	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.2 U
Sulfate as SO4	295.32 D	259.96 D	314.5796 D	292.09 D	295.32 D	287.29 D	286.45 D	308.55 D	319.28 D	298.47 D	301.4334 D	--	307.32 D	301.28 D	313.69 D	319.48 D	315.77 D	274.95 D	313.61 D	349.8421 D
Bromide	0.20 U	8.05	5.1226	6.27	0.20 U	13.94	0.20 U	3.01	4.11	0.20 U	0.2 U	--	0.20 U	0.20 U	1.14	2.55	0.20 U	3.15	3.36	4.0028
Nitrate as N	19.84	15.06 D	17.9787	17.05 D	0.20 U	0.20 U	4.97	8.87	10.13	9.97	12.3764	--	12.48	14.57	6.68	5.04	7.61	0.12 J	3.40	5.6045
Phosphate as P, ortho	0.20 U	0.20 U	0.2 U	0.20 U	27.68 D	30.92 D	8.90	5.01	4.58	0.20 U	4.3193	--	6.23	10.30	14.76	28.13 D	31.52 D	26.77 D	32.77 D	35.3651 D
FIELD PARAMETERS																				
pH (SU)	7.59	7.38	7.15	7.17	7.22	7.07	7.6	6.86	7.06	7.09	7	6.93	7	7.03	6.98	6.98	6.97	6.77	6.9	7.01
Temperature (°C)	23.30	21.8	23.20	23.10	22.30	22.20	24.40	23.1	23.1	23.7	23.6	23.9	24.3	24.2	24.6	23.1	22.50	19.9	21.4	24.4
Dissolved Oxygen (DO; mg/L)	8.21	5.95	29.1	37.10	0.08	0.02	7.43	17.92	22.66	21.29	17.49	0.69	11.01	22.96	25.17	22.1	24.09	10.42	32.99	31.04
Redox Potential (ORP; mV)	281.9	116.8	146.5	166.1	-143.9	-124.9	108.5	231.1	193.8	167.2	130.7	-3.2	3	198.6	112.7	225.0	223.70	183.2	238.2	288.4
Specific Conductivity (mS/cm)	3.421	3.236	2.755	2.679	2.892	2.993	2.666	2.722	2.768	2.861	2.879	2.977	3.095	2.957	2.945	3.44	3.46	3806	3.397	4746.4
Depth to Water (ft-btoc) prior to purging	25.2	25.45	25.61	25.70	25.68	25.71	25.7	25.73	25.7	25.6	25.71	23.6	25.72	25.69	25.72	26.7	26.50	25.71	25.77	25.85
Purge Rate (mL/min)	200	190	180	120	140	180	100	120	90	120	100	100	120	100	100	100	100	100	100	120
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Propane Monooxygenase (PPO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Ammonia Monooxygenase (AMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Particulate methane monooxygenase (PMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S
Sampling Date	12/14/2020	2/9/2021	4/7/2021	4/28/2021	5/19/2021	5/21/2021	5/25/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-91	-34	23	44	65	67	71	78	85	92	98	113	126	155	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
VOCS (GC/MS)	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
dichlorodifluoromethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
chloromethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
vinyl chloride	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5 U
bromomethane	40.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
chloroethane	20.00 U	1.00 U	20.00 U	37.80 D	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5 U
trichlorofluoromethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1-dichloroethylene	1340.80 D	77.62	882.00 D	1024.80 D	783.20 D	--	--	620.20 D	--	526.00 D	602.60 D	567.80 D	639.60 D	522.00 D	625.65 D	206.20 D	213.00 D	187.45 D	203.70 D	272.1 D
methylene chloride	40.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
trans-1,2-dichloroethylene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1-dichloroethane	74.60 D	4.38	26.80 D	55.60 D	21.80 D	--	--	45.80 D	--	40.20 D	46.20 D	44.00 D	43.40 D	44.55 D	57.85 D	35.70 D	36.55 D	36.80 D	49.00 D	44.45 D
2,2-dichloropropane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
cis 1,2- dichloroethylene	136.00 D	34.25	146.60 D	251.20 D	228.00 D	--	--	206.00 D	--	167.80 D	208.40 D	162.00 D	183.60 D	172.15 D	239.45 D	194.95 D	171.40 D	316.05 D	239.50 D	215.15 D
bromochloromethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
chloroform	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,1-trichloroethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
carbon tetrachloride	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1-dichloropropene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
benzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2-dichloroethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
trichloroethylene	1185.60 D	46.71	740.60 D	806.80 D	741.60 D	--	--	664.80 D	--	587.40 D	644.20 D	587.60 D	641.80 D	555.40 D	698.45 D	231.75 D	288.05 D	116.40 D	256.05 D	314.95 D
1,2-dichloropropane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
dibromomethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
bromodichloromethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
cis-1,3-dichloropropene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
toluene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
trans-1,3-dichloropropene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,2-trichloroethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
tetrachloroethylene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,3-dichloropropane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
dibromochloromethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2-dibromoethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
chlorobenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,1,2-tetrachloroethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
ethylbenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
xylenes (m/p)	40.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
o-xylene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
styrene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
bromoform	40.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
isopropyl benzene (cumene)	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
bromobenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,2,2-tetrachloroethane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2,3-trichloropropane	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
n-propyl benzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
2-chlorotoluene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
4-chlorotoluene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,3,5-trimethylbenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
tert-butylbenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2,4-trimethylbenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
sec-butylbenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,3-dichlorobenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
4-isopropyltoluene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,4-dichlorobenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2-dichlorobenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
n-butylbenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2-dibromo-3-chloropropane	40.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
1,2,4-trichlorobenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
hexachlorobutadiene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
naphthalene	20.40 D	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2,3-trichlorobenzene	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
Methyl tertiary butyl ether	20.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
Acetone	100.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
carbon disulfide	40.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
2-butanone (MEK)	100.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
tetrahydrofuran (THF)	100.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
4-methyl-2-pentanone (MIBK)	100.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
2-hexanone	100.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
2-chloroethyl vinyl ether	100.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U

Sample ID	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S	PMW-2S
Sampling Date	12/14/2020	2/9/2021	4/7/2021	4/28/2021	5/19/2021	5/21/2021	5/25/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-91	-34	23	44	65	67	71	78	85	92	98	113	126	155	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	169.8 D	376 D	177.82 D	95.718403 D	223.85358 D	--	--	--	--	--	281.40 D	--	445.82 D	338.00 D	162.00 D	224.11 D	210.63 D	235.60 D	290.77 D	261 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	0.85 J	0.84 J	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	1163.61	25.94	2.38	0.95 U	1.36	0.95 U	406.27	0.95 U	25.09
Ethane	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U
Isobutane	--	--	3.62 U	3.62 U	3.62 U	26.98	168.38	225.74	769.08	326.25	1044.27	912.61	345.51	316.20	71.53	55.58	448.18	209.08	12.39	73.84
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	--	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	--	--	--	--	--	5.00 U	5.00 U	5.00 U	--	--	--
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	140.33 D	126.35 D	338.82 D	325.08 D	330.15 D	324.71 D	323.87 D	344.54 D	338.46 D	345.32 D	350.21 D	350.21 D	366.12 D	361.26 D	384.65 D	401.24 D	410.03 D	422.40 D	432.13 D	463.7981 D
Nitrite as N	0.20 U	0.20 U	1.41	0.22	3.46	3.47	2.32	1.45	0.13 J	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.2 U
Sulfate as SO4	262.43 D	234.98 D	311.32 D	298.65 D	303.08 D	290.12 D	295.68 D	303.45 D	312.98 D	300.82 D	301.09 D	301.09 D	306.43 D	305.94 D	313.23 D	316.02 D	322.64 D	266.80 D	317.44 D	350.1096 D
Bromide	0.20 U	0.20 U	3.79	5.29	0.20 U	6.32	0.20 U	3.53	4.03	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	1.14	2.46	0.20 U	3.73	3.22	3.5709
Nitrate as N	4.67	4.60	17.04	16.23	0.87	3.63	5.93	8.73	9.96	9.73	12.22	12.22	12.53	14.61	5.63	5.64	7.47	0.20 U	1.93	5.2638
Phosphate as P, ortho	0.20 U	0.20 U	0.20 U	0.20 U	7.84	10.53	7.40	4.74	4.44	0.20 U	4.05	4.05	5.76	8.71	15.86	76.08 D	39.47 D	28.44 D	31.62 D	36.6996 D
FIELD PARAMETERS																				
pH (SU)	7.43	6.95	7.23	7.17	7.22	7.09	7.64	6.31	7.04	7.04	6.9	6.95	7	7.04	6.85	7.30	6.96	7.3	7.09	7.08
Temperature (°C)	22.80	21.2	21.20	23.50	22.60	22.80	24.30	23.7	22.6	25.1	23.4	24	24.7	24.3	25.0	21.7	22.90	21.7	22.2	24.2
Dissolved Oxygen (DO; mg/L)	20.63	29	33.3	33.21	0.20	0.79	14.01	22.61	25.75	24.9	21.07	3.41	10.95	29.36	20.09	0.12	25.65	0.12	24.9	28.97
Redox Potential (ORP; mV)	263.9	207.4	193.7	234.7	207.2	131.1	157.5	226.4	260.1	198.7	197	144.2	84.8	208.7	126.6	-162.5	245.00	-162.5	228.2	163.6
Specific Conductivity (mS/cm)	1.474	2.192	2.782	2.598	2.774	2.884	2.625	2.755	2.746	2.851	2.658	2.973	3.076	2.92	2.977	3302	3.49	3302	3.648	7.801
Depth to Water (ft-btoc) prior to purging	24.6	24.86	25.09	25.12	25.05	24.93	25.01	25.04	25.05	24.9	24.9	24.6	25.05	25.00	25.0	-79.61	25.40	25.10	25.02	25.03
Purge Rate (mL/min)	90	100	100	100	160	100	100	100	90	80	100	100	100	100	100	25.10	100	100	100	200
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Propane Monooxygenase (PPO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Ammonia Monooxygenase (AMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Particulate methane monooxygenase (PMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D
Sampling Date	12/14/2020	2/9/2021	4/7/2021	4/28/2021	5/19/2021	5/21/2021	5/25/2021	6/1/2021	6/8/2021	6/21/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-91	-34	23	44	65	67	71	78	85	98	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
VOCS (GC/MS)	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
dichlorodifluoromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
chloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
vinyl chloride	10.20 JD	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5 U
bromomethane	20.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
chloroethane	20.00 U	20.00 U	20.00 U	79.60 D	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
trichlorofluoromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1-dichloroethylene	2878.40 D	2695.60 D	1014.80 D	1181.40 D	684.60 D	--	--	645.40 D	--	567.20 D	528.45 D	195.45 D	281.35 D	167.95 D	186.10 D	271.1 D
methylene chloride	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
trans-1,2-dichloroethylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1-dichloroethane	188.80 D	188.40 D	33.60 D	76.80 D	42.40 D	--	--	40.80 D	--	41.00 D	43.45 D	36.70 D	50.70 D	37.75 D	46.80 D	41.75 D
2,2-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
cis 1,2- dichloroethylene	1042.60 D	1182.80 D	234.20 D	307.20 D	197.00 D	--	--	191.80 D	--	198.40 D	192.70 D	215.80 D	225.75 D	163.50 D	239.05 D	203.85 D
bromochloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
chloroform	4.80 JD	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,1-trichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
carbon tetrachloride	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
benzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
trichloroethylene	1646.80 D	1867.80 D	922.80 D	890.80 D	648.20 D	--	--	684.20 D	--	647.00 D	577.90 D	230.80 D	371.70 D	265.35 D	235.00 D	310.2 D
1,2-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
dibromomethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
bromodichloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
cis-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
toluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
trans-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,2-trichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
tetrachloroethylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5 U
1,3-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
dibromochloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2-dibromoethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
chlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,1,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
ethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
xylenes (m/p)	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
o-xylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
styrene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
bromoform	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
isopropyl benzene (cumene)	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
bromobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,2,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2,3-trichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
n-propyl benzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
2-chlorotoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
4-chlorotoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,3,5-trimethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
tert-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2,4-trimethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
sec-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,3-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
4-isopropyltoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,4-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
n-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2-dibromo-3-chloropropane	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
1,2,4-trichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
hexachlorobutadiene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
naphthalene	20.00 D	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2,3-trichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
Methyl tertiary butyl ether	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
Acetone	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
carbon disulfide	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
2-butanone (MEK)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
tetrahydrofuran (THF)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
4-methyl-2-pentanone (MIBK)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
2-hexanone	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
2-chloroethyl vinyl ether	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U

Sample ID	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D	PMW-2D
Sampling Date	12/14/2020	2/9/2021	4/7/2021	4/28/2021	5/19/2021	5/21/2021	5/25/2021	6/1/2021	6/8/2021	6/21/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-91	-34	23	44	65	67	71	78	85	98	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	473.75 D	493.80 D	408.60 D	417.01331 D	223.7648 D	--	--	--	--	314.86 D	191.60 D	150.63 D	345.07 D	301.50 D	254.81 D	227 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	1.00	0.73 J	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	1.15	0.95 U	2.85	0.95 U	40.28
Ethane	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U
Isobutane	--	--	3.62 U	3.62 U	3.62 U	3.62 U	3.62 U	118.78	335.75	771.80	65.69	22.51	374.35	60.67	23.17	122.89
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	--	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	--	--	--
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	419.39 D	375.29 D	346.84 D	325.84 D	321.58 D	319.32 D	320.76 D	336.33 D	339.05 D	346.42 D	380.11 D	397.19 D	408.09 D	421.91 D	427.77 D	465.70 D
Nitrite as N	0.75	0.20 U	1.32	0.21	0.51	4.70	2.54	1.40	1.25	0.20 U	0.78	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U
Sulfate as SO4	319.13 D	281.30 D	324.86 D	293.70 D	303.77 D	290.74 D	294.48 D	299.82 D	317.29 D	300.85 D	313.77 D	313.79 D	321.95 D	328.47 D	293.65 D	350.92 D
Bromide	0.20 U	8.44	0.20 U	5.44	0.20 U	5.48	0.20 U	3.62	4.07	0.20 U	1.02	2.61	0.20 U	3.14	3.40	3.98
Nitrate as N	18.41	18.82	17.88	16.84	17.27	11.48	1.86	6.08	9.04	11.76	5.15	4.76	7.01	1.30	1.63	4.81
Phosphate as P, ortho	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	3.28	4.37	1.38	4.37	15.07	27.15 D	49.49 D	27.30 D	99.37 D	40.30 D
FIELD PARAMETERS																
pH (SU)	7.70	7.28	7.38	7.24	7.18	7.13	7.81	6.43	7.07	6.88	6.91	6.89	6.99	6.76	6.96	7.08
Temperature (°C)	22.70	20.7	22.40	23.60	22.00	23.10	24.40	23.6	22.6	24.3	25	22.7	21.50	19.8	20.4	24.7
Dissolved Oxygen (DO; mg/L)	20.57	21.29	41.3	36.29	31.22	13.61	4.44	16.68	30.22	27	31.53	34.50	34.97	13.84	95.78	33.78
Redox Potential (ORP; mV)	251.7	217.4	169.2	204.6	223.1	122.4	141.9	250.3	262.6	184.5	129.5	250.0	249.20	207.7	267.0	147.6
Specific Conductivity (mS/cm)	3.136	3.473	2.952	2.619	2.744	2.842	2.615	2.704	2.724	2.650	2.950	3.249	3.63	3824	3.693	7.821
Depth to Water (ft-btoc) prior to purging	24.5	24.65	24.85	24.94	24.75	24.93	24.85	24.92	24.95	25.8	24.9	24.89	25.40	24.82	25.04	24.98
Purge Rate (mL/min)	100	100	100	100	100	100	100	100	90	100	100	100	100	100	100	130
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Propane Monooxygenase (PPO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Ammonia Monooxygenase (AMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Particulate methane monooxygenase (PMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S
Sampling Date	12/14/2020	2/8/2021	4/6/2021	4/27/2021	5/18/2021	5/21/2021	5/26/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-91	-35	22	43	64	67	72	78	85	92	98	113	126	155	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
VOCS (GC/MS)	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
dichlorodifluoromethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
chloromethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
vinyl chloride	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5 U	
bromomethane	40.00 U	2.00 U	2.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
chloroethane	20.00 U	1.00 U	1.00 U	27.20 D	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
trichlorofluoromethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1-dichloroethylene	1866.80 D	45.42	26.84	366.40 D	802.20 D	--	--	801.80 D	--	509.80 D	577.80 D	704.80 D	622.80 D	537.20 D	604.50 D	190.00 D	211.40 D	170.40 D	174.80 D	162.2 D
methylene chloride	40.00 U	2.00 U	2.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
trans-1,2-dichloroethylene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1-dichloroethane	115.20 D	2.77	1.00 U	20.00 U	47.80 D	--	--	59.00 D	--	38.80 D	25.20 D	45.40 D	41.20 D	42.30 D	49.50 D	38.80 D	40.95 D	35.85 D	39.75 D	39.1 D
2,2-dichloropropane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
cis 1,2- dichloroethylene	430.00 D	17.94	4.44	78.80 D	203.60 D	--	--	231.20 D	--	154.40 D	196.80 D	191.60 D	178.20 D	174.40 D	197.95 D	220.70 D	216.20 D	165.00 D	219.80 D	164.35 D
bromochloromethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
chloroform	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,1-trichloroethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
carbon tetrachloride	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1-dichloropropene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
benzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2-dichloroethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
trichloroethylene	1298.20 D	32.09	12.64	295.20 D	749.60 D	--	--	716.20 D	--	584.60 D	658.80 D	721.20 D	647.00 D	551.80 D	627.25 D	203.30 D	265.45 D	253.00 D	230.05 D	212.35 D
1,2-dichloropropane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
dibromomethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
bromodichloromethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
cis-1,3-dichloropropene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
toluene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
trans-1,3-dichloropropene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,2-trichloroethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
tetrachloroethylene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,3-dichloropropane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
dibromochloromethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2-dibromoethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
chlorobenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,1,2-tetrachloroethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
ethylbenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
xylenes (m/p)	40.00 U	2.00 U	2.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
o-xylene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
styrene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
bromoform	40.00 U	2.00 U	2.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
isopropyl benzene (cumene)	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
bromobenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,1,2,2-tetrachloroethane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2,3-trichloropropane	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
n-propyl benzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
2-chlorotoluene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
4-chlorotoluene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,3,5-trimethylbenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
tert-butylbenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2,4-trimethylbenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
sec-butylbenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,3-dichlorobenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
4-isopropyltoluene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,4-dichlorobenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2-dichlorobenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
n-butylbenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2-dibromo-3-chloropropane	40.00 U	2.00 U	2.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U
1,2,4-trichlorobenzene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
hexachlorobutadiene	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
naphthalene	55.20 D	1.67	13.62	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.80 D	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
1,2,3-trichlorobenzene	20.00 U	1.00 U	1.24	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
Methyl tertiary butyl ether	20.00 U	1.00 U	1.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	626.40 D	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U
Acetone	100.00 U	5.00 U	5.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
carbon disulfide	40.00 U	2.00 U	2.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
2-butanone (MEK)	100.00 U	5.00 U	5.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
tetrahydrofuran (THF)	100.00 U	5.00 U	5.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
4-methyl-2-pentanone (MIBK)	100.00 U	5.00 U	5.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
2-hexanone	100.00 U	5.00 U	2.54	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U
2-chloroethyl vinyl ether	100.00 U	5.00 U	5.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U

Sample ID	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S	PMW-3S
Sampling Date	12/14/2020	2/8/2021	4/6/2021	4/27/2021	5/18/2021	5/21/2021	5/26/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-91	-35	22	43	64	67	72	78	85	92	98	113	126	155	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	285.60 D	85.57 D	3.07	441.96 D	207.68 D	--	--	--	--	--	235.57 D	--	358.80 D	357.40 D	306.40 D	236.93 D	327.16 D	267.00 D	286.43 D	189.95 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	2.21	1.04	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	300.93	1112.61	12.45	0.95 U	1.22	1.95	4.05	0.95 U	34
Ethane	0.39 J	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.9 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.7 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	7.0 U
Isobotane	--	--	3.62 U	3.62 U	3.62 U	3.62 U	3.62 U	13.72	158.94	153.59	393.56	1332.79	533.82	495.22	152.50	6.14	62.89	55.79	6.46	179.33
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	--	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	--	--	--	--	--	5.00 U	5.00 U	5.00 U	--	--	--
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	181.04 D	164.38 D	128.78 D	202.91 D	320.75 D	310.25 D	317.11 D	332.31 D	331.31 D	338.47 D	337.21 D	--	356.67 D	357.52 D	377.25 D	399.53 D	402.28 D	415.68 D	433.53 D	455.5409 D
Nitrite as N	0.33	0.20 U	0.20 U	0.20 U	0.52	2.00	4.02	2.11	0.28	0.20 U	0.20 U	--	0.20 U	0.20 U	0.35	0.20 U	0.20 U	0.20 U	0.20 U	0.2 U
Sulfate as SO4	291.62 D	268.87 D	260.29 D	270.78 D	301.00 D	288.52 D	306.38 D	300.29 D	312.31 D	300.02 D	295.87 D	--	305.61 D	308.43 D	312.85 D	314.53 D	318.75 D	326.82 D	306.52 D	385.6491 D
Bromide	0.20 U	0.20 U	0.20 U	0.20 U	2.58	8.54	0.20 U	3.85	4.25	0.20 U	0.20 U	--	0.20 U	0.20 U	1.25	2.86	0.20 U	3.66	2.93	4.4595
Nitrate as N	8.24	9.63	4.26	9.42	16.31	17.76	12.49	4.87	6.28	8.35	10.10	--	11.66	13.68	12.41	1.47	5.10	1.51	3.07	0.5266
Phosphate as P, ortho	0.20 U	0.12 J	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	--	4.45	4.45	7.16	10.71	21.65 D	28.19 D	64.24 D	45.4187 D
FIELD PARAMETERS																				
pH (SU)	7.14	7.18	7.01	6.99	6.96	7.00	6.65	6.94	6.98	7.02	6.78	6.90	6.97	7.02	7.01	6.89	6.92	6.89	7.02	7.08
Temperature (°C)	22.70	20.6	23.00	23.20	22.90	23.20	22.50	23.8	23	25.1	24.8	24.9	24.7	24.4	25.9	23.9	20.20	22.4	22.2	25.8
Dissolved Oxygen (DO; mg/L)	1.57	1.36	23.6	21.92	30.34	31.40	25.02	15.11	22.09	28	22.40	17.10	10.49	12.76	28.43	34.18	21.68	25.8	43.61	2.32
Redox Potential (ORP; mV)	241.2	177.1	193.7	154.4	186.4	251.4	258.5	213.4	223.7	181.9	199.8	229.8	64.2	201.1	121.5	224.2	238.60	150.8	241.5	94.3
Specific Conductivity (mS/cm)	1.817	2.402	1.480	1.705	2.513	2.689	2.375	2.622	2.656	2.785	2.581	2.884	2.984	2.861	2.904	3118	3.03	3294	3.648	7.993
Depth to Water (ft-btoc) prior to purging	25.8	25.21	26.45	25.60	25.57	25.71	26.01	25.3	25.2	25.6	25.1	25.70	25.9	25.60	24.78	25.65	24.60	24.7	25.4	24.69
Purge Rate (mL/min)	100	100	80	100	100	90	100	50	50	80	50	80	100	60	100	100	80	100	100	100
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--	1.90E+00 J	--	9.10E+00	6.00E-01 J	--	--	--	--	--	--	--	--	--	--	--	2.10E+00 J	--	--	--
Propane Monooxygenase (PPO)	--	1.46E+02	--	1.29E+02	2.61E+01	--	--	--	--	--	--	--	--	--	--	--	7.96E+00	--	--	--
Ammonia Monooxygenase (AMO)	--	5.00E+00 U	--	4.70E+00 U	4.70E+00 U	--	--	--	--	--	--	--	--	--	--	--	5.10E+00 U	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	5.00E+00 U	--	4.70E+00 U	4.70E+00 U	--	--	--	--	--	--	--	--	--	--	--	5.19E+03	--	--	--
Particulate methane monooxygenase (PMMO)	--	5.00E+00 U	--	4.70E+00 U	4.70E+00 U	--	--	--	--	--	--	--	--	--	--	--	8.40E+00	--	--	--

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D
Sampling Date	12/14/2020	2/8/2021	4/6/2021	4/27/2021	5/18/2021	5/21/2021	5/26/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-91	-35	22	43	64	67	72	78	85	92	98	113	126	155	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
VOCS (GC/MS)	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
dichlorodifluoromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
vinyl chloride	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromomethane	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
chloroethane	20.00 U	20.00 U	20.00 U	80.40 D	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trichlorofluoromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloroethylene	1964.00 D	1804.80 D	953.00 D	990.00 D	743.20 D	--	--	610.60 D	--	546.20 D	672.40 D	638.20 D	748.20 D	543.05 D	570.15 D	217.30 D	211.30 D	165.30 D	99.55 D	294.15 D
methylene chloride	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
trans-1,2-dichloroethylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloroethane	125.80 D	121.20 D	34.20 D	61.00 D	33.40 D	--	--	52.00 D	--	42.20 D	49.60 D	43.00 D	45.00 D	38.95 D	53.35 D	38.10 D	37.30 D	36.35 D	34.75 D	45.45 D
2,2-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
cis 1,2- dichloroethylene	385.20 D	366.60 D	159.80 D	257.00 D	208.00 D	--	--	221.60 D	--	183.80 D	211.00 D	203.80 D	179.80 D	163.15 D	224.75 D	218.55 D	180.00 D	239.50 D	167.40 D	223.15 D
bromochloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chloroform	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,1-trichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
carbon tetrachloride	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
benzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trichloroethylene	1486.80 D	1620.60 D	896.20 D	812.80 D	703.20 D	--	--	703.00 D	--	643.00 D	746.40 D	696.00 D	675.80 D	572.65 D	682.80 D	255.25 D	276.35 D	179.45 D	122.70 D	335.50 D
1,2-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
dibromomethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromodichloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
cis-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
toluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trans-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,2-trichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
tetrachloroethylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
dibromochloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dibromoethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,1,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
ethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
xylenes (m/p)	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
o-xylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
styrene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromoform	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
isopropyl benzene (cumene)	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,2,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,3-trichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
n-propyl benzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
2-chlorotoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
4-chlorotoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3,5-trimethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
tert-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,4-trimethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
sec-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
4-isopropyltoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,4-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
n-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dibromo-3-chloropropane	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
1,2,4-trichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
hexachlorobutadiene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
naphthalene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,3-trichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
Methyl tertiary butyl ether	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
Acetone	100.00 U	100.00 U	100.00 U	20.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
carbon disulfide	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
2-butanone (MEK)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
tetrahydrofuran (THF)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
4-methyl-2-pentanone (MIBK)	100.00 U	100.00 U	167.80 D	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
2-hexanone	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
2-chloroethyl vinyl ether	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U

Sample ID	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D	PMW-3D
Sampling Date	12/14/2020	2/8/2021	4/6/2021	4/27/2021	5/18/2021	5/21/2021	5/26/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-91	-35	22	43	64	67	72	78	85	92	98	113	126	155	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	492.80 D	368.52 D	350.87 D	170.45 D	216.77 D	--	--	--	--	--	228.54 D	--	408.44 D	308.40 D	210.80 D	223.50 D	301.38 D	238.00 D	277.88 D	260 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	0.89 J	0.92 J	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	2568.78	58.95	4.60	0.95 U	1.61	0.95 U	151.17	0.95 U	56.06
Ethane	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U
Isobutane	--	--	3.62 U	3.62 U	3.62 U	3.62 U	40.70	236.71	199.43	365.20	1096.69	867.93	472.71	337.51	14.41	25.38	226.68	82.66	19.94	253.73
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	--	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	--	--	--	--	--	5.00 U	5.00 U	5.00 U	--	--	--
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	421.91 D	372.61 D	348.45 D	319.22 D	336.63 D	318.36 D	323.05 D	342.24 D	339.65 D	345.27 D	351.07 D	--	365.58 D	365.89 D	381.38 D	403.58 D	410.75 D	420.41 D	430.01 D	465.9391 D
Nitrite as N	1.21	2.34	1.25	0.19 J	4.16	6.08	3.08	1.59	0.29	0.20 U	0.20 U	--	0.20 U	0.20 U	0.07 J	0.20 U	0.20 U	0.20 U	0.20 U	0.2 U
Sulfate as SO4	326.39 D	290.69 D	319.87 D	290.48 D	300.38 D	289.71 D	291.50 D	301.51 D	317.66 D	300.59 D	301.29 D	--	309.38 D	318.56 D	313.84 D	318.32 D	323.25 D	295.55 D	272.94 D	352.499 D
Bromide	0.20 U	8.61	5.59	5.37	2.21	6.68	0.20 U	3.73	4.56	0.20 U	0.20 U	--	0.20 U	0.20 U	1.14	2.53	0.20 U	3.32	2.62	4.562
Nitrate as N	24.11 D	22.65 D	17.91	16.61	6.70	4.67	3.62	8.04 D	9.62	10.40	12.33	--	12.45	14.81	2.11	5.02	7.20	0.35	0.05 J	5.3574
Phosphate as P, ortho	0.20 U	0.20 U	0.20 U	0.20 U	10.48	7.61	8.76	4.91	4.84	0.20 U	4.16	--	6.05	7.75	14.99	56.12 D	32.25 D	23.78 D	17.57	32.2836 D
FIELD PARAMETERS																				
pH (SU)	6.78	7.27	7.26	7.23	6.95	6.99	6.79	6.96	7.06	7.08	6.94	6.95	6.98	7.04	6.94	6.86	6.98	6.68	6.65	7.11
Temperature (°C)	22.70	21.6	23.00	22.50	22.60	23.30	23.50	23.7	23.2	24.5	25.5	24.7	24.9	24.6	25.2	23.5	22.10	20.4	22.5	25.5
Dissolved Oxygen (DO; mg/L)	14.63	13.43	37.2	40.06	18.70	19.00	22.95	26.13	41.02	30.73	20.53	13.6	11.23	29.02	40.46	37.25	38.47	22.69	5.77	35.34
Redox Potential (ORP; mV)	243.8	154.1	201.0	139.0	185.0	223.1	236.3	217.6	216.5	215.1	172.7	199.6	88.4	178.0	120.9	230.3	250.30	219.5	232.9	149.6
Specific Conductivity (mS/cm)	3.177	3.496	2.756	2.655	2.740	2.861	2.564	2.716	2.729	2.845	2.668	2.969	3.046	2.935	2.97	3.373	3.373	3813	3.249	7.819
Depth to Water (ft-btoc) prior to purging	24.5	24.72	24.72	24.95	24.85	25.19	25.26	25.15	25.2	25.2	25.2	25.05	25.35	25.15	24.95	24.92	24.70	24.92	24.84	25.18
Purge Rate (mL/min)	100	110	90	120	110	90	100	60	100	120	100	100	200	100	100	100	100	100	100	150
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--	2.00E-01 J	--	3.10E+00 J	8.00E-01 J	--	--	--	--	--	--	--	--	--	--	--	3.00E-01 J	--	--	--
Propane Monooxygenase (PPO)	--	2.15E+01	--	1.04E+01	3.20E+00 J	--	--	--	--	--	--	--	--	--	--	--	3.20E+00 J	--	--	--
Ammonia Monooxygenase (AMO)	--	2.00E-01 J	--	4.70E+00 U	4.80E+00 U	--	--	--	--	--	--	--	--	--	--	--	4.80E+00 U	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	5.00E+00 U	--	4.70E+00 U	4.80E+00 U	--	--	--	--	--	--	--	--	--	--	--	4.18E+02	--	--	--
Particulate methane monooxygenase (PMMO)	--	5.00E+00 U	--	4.70E+00 U	3.30E+00 J	--	--	--	--	--	--	--	--	--	--	--	3.09E+02	--	--	--

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S
Sampling Date	12/15/2020	2/8/2021	4/6/2021	4/27/2021	5/18/2021	5/21/2021	5/26/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-90	-35	22	43	64	67	72	78	85	92	98	113	126	155	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
VOCS (GC/MS)	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
dichlorodifluoromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chloromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
vinyl chloride	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromomethane	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
chloroethane	1.00 U	1.00 U	20.00 U	95.80 D	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trichlorofluoromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloroethylene	22.38	14.12	708.60 D	990.00 D	626.40 D	--	--	658.00 D	--	625.60 D	733.60 D	541.60 D	631.80 D	567.55 D	145.10 D	172.70 D	229.30 D	148.10 D	251.65 D
methylene chloride	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
trans-1,2-dichloroethylene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloroethane	2.13	1.00 U	46.60 D	64.80 D	34.40 D	--	--	41.80 D	--	53.20 D	56.60 D	39.00 D	37.60 D	47.35 D	31.55 D	37.80 D	47.80 D	32.85 D	44.90 D
2,2-dichloropropane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
cis 1,2- dichloroethylene	8.80	3.44	139.80 D	253.40 D	152.60 D	--	--	189.00 D	--	200.60 D	232.00 D	161.60 D	161.40 D	178.75 D	191.25 D	159.75 D	206.60 D	188.05 D	210.80 D
bromochloromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chloroform	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,1-trichloroethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
carbon tetrachloride	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloropropene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
benzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dichloroethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trichloroethylene	19.46	12.51	667.00 D	786.80 D	613.80 D	--	--	635.20 D	--	705.60 D	801.00 D	614.80 D	686.20 D	587.70 D	130.40 D	227.90 D	343.55 D	158.55 D	293.55 D
1,2-dichloropropane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
dibromomethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromodichloromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
cis-1,3-dichloropropene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
toluene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trans-1,3-dichloropropene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,2-trichloroethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
tetrachloroethylene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3-dichloropropane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
dibromochloromethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dibromoethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,1,2-tetrachloroethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
ethylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
xylenes (m/p)	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
o-xylene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
styrene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromoform	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
isopropyl benzene (cumene)	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,2,2-tetrachloroethane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,3-trichloropropane	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
n-propyl benzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
2-chlorotoluene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
4-chlorotoluene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3,5-trimethylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
tert-butylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,4-trimethylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
sec-butylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3-dichlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
4-isopropyltoluene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,4-dichlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dichlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
n-butylbenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dibromo-3-chloropropane	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
1,2,4-trichlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
hexachlorobutadiene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
naphthalene	1.00 U	1.00 U	20.00 U	88.80 D	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,3-trichlorobenzene	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
Methyl tertiary butyl ether	1.00 U	1.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
Acetone	5.00 U	5.00 U	544.20 D	20.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
carbon disulfide	2.00 U	2.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
2-butanone (MEK)	5.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
tetrahydrofuran (THF)	5.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
4-methyl-2-pentanone (MIBK)	5.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
2-hexanone	5.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
2-chloroethyl vinyl ether	5.00 U	5.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U

Sample ID	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S	PMW-4S
Sampling Date	12/15/2020	2/8/2021	4/6/2021	4/27/2021	5/18/2021	5/21/2021	5/26/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-90	-35	22	43	64	67	72	78	85	92	98	113	126	155	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	136.34 D	55.28 D	229.40 D	425.22 D	229.87 D	--	--	--	--	--	203.53499 D	--	312.50 D	338.60 D	197.26 D	272.59 D	342.03 D	279.01 D	225.40 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	2.94	1.54	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	3015.41	117.03	170.19	10.08	105.26	565.04	0.95 U	43.00
Ethane	0.64 J	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U
Isobutane	--	--	3.62 U	3.62 U	3.62 U	3.62 U	27.39	172.35	598.11	345.58	1584.361	1422.87	310.43	336.15	37.73	689.43	123.69	116.90	526.72
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	--	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	--	--	--	--	--	5.00 U	5.00 U	--	--	--
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	179.05 D	242.83 D	339.65 D	328.52 D	333.78 D	317.42 D	318.95 D	335.24 D	334.66 D	340.09 D	343.56 D	--	344.21 D	361.05 D	408.04 D	412.30 D	423.86 D	431.98 D	461.49 D
Nitrite as N	0.98	3.41	1.12	0.20 U	0.61	2.14	2.66	0.85	0.20 U	0.20 U	0.20 U	--	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U
Sulfate as SO4	284.96 D	140.51 D	321.53 D	291.37 D	301.13 D	291.32 D	293.83 D	300.17 D	311.87 D	299.61 D	298.41 D	--	286.66 D	318.02 D	355.32 D	364.67 D	346.19 D	315.12 D	349.91 D
Bromide	0.20 U	0.20 U	6.28	10.05	2.26	8.77	6.15	3.82	5.30	0.20 U	0.20 U	--	0.20 U	0.20 U	2.70	0.20 U	3.27	3.70	4.32
Nitrate as N	8.78	2.65	17.13	17.86	14.66	13.00	8.85	6.75	8.02	10.93	11.81	--	10.63	14.52	0.68	1.21	0.90	1.21	4.84
Phosphate as P, ortho	0.20 U	0.13 J	0.20 U	0.20 U	0.20 U	1.24	2.86	1.44	2.09	0.20 U	4.18	--	22.98 D	16.12 D	54.07 D	38.31 D	16.87 D	20.53 D	35.84 D
FIELD PARAMETERS																			
pH (SU)	6.98	7.13	6.01	6.94	7.02	7.16	6.59	7	7.07	7	7.01	6.93	6.95	7.03	6.76	7.01	7.18	6.91	7.08
Temperature (^{o}C)	21.70	22.1	23.90	22.30	22.00	22.00	22.90	23.7	24.2	25.5	23.4	24.4	25.3	26.1	23.3	21.10	21.0	22.9	25.4
Dissolved Oxygen (DO; mg/L)	0.86	0.69	22.9	29.45	22.77	18.15	14.85	9.95	16.64	18.69	21.89	9.31	1.35	19.75	0.44	1.39	1.67	31.93	24.1
Redox Potential (ORP; mV)	250.2	-145.1	156.9	190.4	278.0	210.7	237.4	209.9	221.3	219.6	216.2	170.4	70.1	179.2	-181.9	-180.70	-9.5	55.7	196.1
Specific Conductivity (mS/cm)	1.920	1.633	2.618	2.625	2.634	2.789	2.475	2.651	2.732	2.807	2.828	2.968	3.051	3.019	3471	3.36	3300	3.553	4727.11
Depth to Water (ft-btoc) prior to purging	25.5	25.98	25.8	26.14	25.92	26.12	26.51	26.2	26.55	25.95	26.55	25.4	26.35	25.60	25.2	26.25	25.5	25.63	25.68
Purge Rate (mL/min)	80	100	100	100	100	100	100	80	80	50	100	100	100	80	100	100	100	100	100
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--	6.50E+00 U	--	1.79E+01	1.55E+01	--	--	--	--	--	--	--	--	--	--	2.00E+00 J	--	--	--
Propane Monooxygenase (PPO)	--	8.63E+01	--	1.17E+02	1.07E+02	--	--	--	--	--	--	--	--	--	--	5.90E+00	--	--	--
Ammonia Monooxygenase (AMO)	--	6.50E+00 U	--	4.80E+00 U	4.80E+00 U	--	--	--	--	--	--	--	--	--	--	5.10E+00 U	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	6.50E+00 U	--	4.80E+00 U	4.80E+00 U	--	--	--	--	--	--	--	--	--	--	4.18E+01	--	--	--
Particulate methane monooxygenase (PMMO)	--	6.50E+00 U	--	4.80E+00 U	4.80E+00 U	--	--	--	--	--	--	--	--	--	--	4.65E+01	--	--	--

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D
Sampling Date	12/15/2020	2/8/2021	4/6/2021	4/27/2021	5/18/2021	5/21/2021	5/26/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022	
Days	-90	-35	22	43	64	67	72	78	85	92	98	113	126	155	183	246	274	345	386	499	
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	
VOCS (GC/MS)	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	
dichlorodifluoromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
chloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
vinyl chloride	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
bromomethane	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	
chloroethane	20.00 U	20.00 U	20.00 U	24.20 D	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
trichlorofluoromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,1-dichloroethylene	2554.20 D	2527.60 D	866.60 D	966.40 D	681.20 D	--	--	739.80 D	--	592.20 D	990.40 D	548.20 D	770.40 D	615.95 D	532.80 D	256.20 D	195.20 D	183.10 D	225.50 D	287.95 D	
methylene chloride	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	
trans-1,2-dichloroethylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,1-dichloroethane	173.20 D	169.60 D	60.40 D	51.80 D	42.60 D	--	--	48.60 D	--	43.80 D	66.00 D	44.20 D	39.80 D	49.25 D	49.55 D	45.95 D	39.55 D	42.05 D	42.40 D	43.50 D	
2,2-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
cis 1,2- dichloroethylene	583.80 D	618.60 D	154.40 D	258.80 D	182.40 D	--	--	232.20 D	--	195.80 D	345.20 D	175.00 D	179.80 D	190.20 D	202.00 D	250.00 D	173.40 D	317.85 D	243.30 D	198.40 D	
bromochloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
chloroform	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,1,1-trichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
carbon tetrachloride	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,1-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
benzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,2-dichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
trichloroethylene	2113.60 D	2427.60 D	814.20 D	780.40 D	660.80 D	--	--	849.60 D	--	681.40 D	1013.40 D	571.20 D	751.40 D	616.25 D	594.05 D	293.75 D	274.55 D	102.30 D	281.05 D	319.25 D	
1,2-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
dibromomethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
bromodichloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
cis-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
toluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
trans-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,1,2-trichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
tetrachloroethylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,3-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
dibromochloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,2-dibromoethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
chlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,1,1,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
ethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
xylenes (m/p)	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	
o-xylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
styrene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
bromoform	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	
isopropyl benzene (cumene)	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
bromobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,1,2,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,2,3-trichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
n-propyl benzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
2-chlorotoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
4-chlorotoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,3,5-trimethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
tert-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,2,4-trimethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
sec-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,3-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
4-isopropyltoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,4-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,2-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
n-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,2-dibromo-3-chloropropane	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	
1,2,4-trichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
hexachlorobutadiene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
naphthalene	157.60 D	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,2,3-trichlorobenzene	24.00 D	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
Methyl tertiary butyl ether	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	20.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
Acetone	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	
carbon disulfide	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	40.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	
2-butanone (MEK)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	
tetrahydrofuran (THF)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	
4-methyl-2-pentanone (MIBK)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	
2-hexanone	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	
2-chloroethyl vinyl ether	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	100.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	

Sample ID	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D	PMW-4D
Sampling Date	12/15/2020	2/8/2021	4/6/2021	4/27/2021	5/18/2021	5/21/2021	5/26/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	7/6/2021	7/19/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-90	-35	22	43	64	67	72	78	85	92	98	113	126	155	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	418.6 D	248.85 D	315.80 D	462.62 D	218.75 D	--	--	--	--	--		--	332.80 D	337.60 D	307.60 D	293.35 D	311.20 D	424.29 D	280.20 D	258 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	1.19	0.76 J	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	898.70	48.73	114.33	189.82	146.25	9.34	423.20 D	0.95 U	33.36
Ethane	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	0.20 U	1.87 U	1.87 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	270.76 D	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	3.24	2.68 U	2.68 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	0.40	6.99 U	6.99 U
Isobutane	--	--	3.62 U	3.62 U	3.62 U	38.09	261.01	592	602.17	575.75	825.58	1014.10	386.10	213.43	37.50	48.28	412.94	20.80 D	9.61	73.31
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	--	--	5.00 U	5.00 U	20.78	5.00 U	5.00 U	5.00 U	5.00 U	232.45 D	--	--	--	--	28.60	5.00 U	5.00 U	--	--	--
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	424.31 D	379.06 D	343.08 D	324.11 D	337.27 D	320.60 D	324.09 D	345.24 D	339.32 D	348.36 D	0.95 U	--	366.05 D	363.61 D	380.57 D	409.93 D	412.38 D	468.02	436.27 D	467.8398 D
Nitrite as N	2.20	3.45	1.28	0.17 J	1.49	2.81	2.37	1.49	0.33	0.20 U	1.87 U	--	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	1.87 U	0.20 U	0.2 U
Sulfate as SO4	293.73 D	259.16 D	309.51 D	299.32 D	300.65 D	292.67 D	294.48 D	301.96 D	313.23 D	301.83 D	2.22 U	--	311.60 D	314.36 D	306.87 D	333.63 D	328.38 D	2.22 U	316.77 D	353.1869 D
Bromide	0.20 U	8.08	0.20 U	5.25	3.53	6.70	6.31	3.44	4.66	0.20 U	2.68 U	--	0.20 U	0.20 U	1.13	2.61	0.20 U	2.68 U	3.44	4.1346
Nitrate as N	25.55 D	24.30 D	17.27	16.59	0.01 J	2.00	5.88	8.77	9.73	11.74	6.99 U	--	12.45	14.81	0.02 J	3.70	6.46	6.99 U	3.39	5.7051
Phosphate as P, ortho	0.20 U	0.20 U	0.20 U	0.20 U	11.86	11.75	7.49	4.98	4.38	0.20 U	1584.36	--	12.39	16.55 D	24.56 D	30.05 D	32.22 D	999.50	31.50 D	34.3532 D
FIELD PARAMETERS																				
pH (SU)	6.95	7.15	7.19	7.04	6.98	6.99	6.25	6.88	7.04	7.06	7.02	6.96	7.03	6.97	6.87	6.86	7.04	6.71	7.03	7.02
Temperature (°C)	22.50	22.1	23.80	22.40	22.20	21.10	23.10	23.4	24.3	24.4	24	25	24.8	25.1	25.3	23.7	22.60	21.2	24.0	25.6
Dissolved Oxygen (DO; mg/L)	0.55	0.71	28.8	37.20	1.20	6.62	25.44	28.23	34.64	21.43	15.35	1.02	9.89	20.66	0.14	15.72	20.09	17.65	126.73	31.96
Redox Potential (ORP; mV)	253.3	71.1	144.1	217.1	184.7	198.8	249.2	221.8	219.9	226.1	216.9	161.5	88.3	163.8	-204.6	131.9	150.70	180.4	214.9	241.5
Specific Conductivity (mS/cm)	3.362	3.202	2.754	2.640	2.719	2.862	2.548	2.697	2.768	2.856	2.914	3.004	3.133	3.02	3.267	3.406	3.27	3496	3.669	4746.63
Depth to Water (ft-btoc) prior to purging	25.0	25.17	25.15	25.48	25.25	25.57	25.73	26.7	25.73	25.7	25.8	25.5	25.82	25.67	25.46	25.35	25.40	25.41	25.68	25.73
Purge Rate (mL/min)	150	120	140	100	80	90	100	160	100	120	100	100	200	100	100	100	160	100	100	120
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--	3.00E-01 J	--	4.33E+01	1.73E+02	--	--	--	--	--	--	--	--	--	--	--	2.00E+00 J	--	--	--
Propane Monooxygenase (PPO)	--	4.90E+00 U	--	1.20E+02	2.94E+01 U	--	--	--	--	--	--	--	--	--	--	--	5.48E+01	--	--	--
Ammonia Monooxygenase (AMO)	--	4.90E+00 U	--	1.00E-01 J	2.94E+01 U	--	--	--	--	--	--	--	--	--	--	--	4.90E+00 U	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	4.90E+00 U	--	4.80E+00 U	1.94E+02	--	--	--	--	--	--	--	--	--	--	--	3.65E+02	--	--	--
Particulate methane monooxygenase (PMMO)	--	4.90E+00 U	--	4.80E+00 U	1.20E+02	--	--	--	--	--	--	--	--	--	--	--	4.10E+01	--	--	--

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	PTW-1		PTW-1		PTW-1		PTW-1		PTW-1		PTW-1		PTW-1		PTW-1		PTW-1		PTW-1		PTW-1		PTW-1	
Sampling Date	12/15/2020		2/9/2021		4/7/2021		4/28/2021		5/18/2021		6/21/2021		9/14/2021		11/16/2021		12/14/2021		2/23/2022		4/5/2022		7/27/2022	
Days	-90		-34		23		44		64		98		183		246		274		345		386		499	
Matrix	Water		Water		Water		Water		Water		Water		Water		Water		Water		Water		Water		Water	
VOCS (GC/MS)	µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L	
dichlorodifluoromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
chloromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
vinyl chloride	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5	U
bromomethane	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.0	U
chloroethane	20.00	U	20.00	U	20.00	U	84.80	D	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5	U
trichlorofluoromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1-dichloroethylene	2702.20	D	2240.00	D	1056.00	D	1095.80	D	795.00	D	852.40	D	1225.20	D	991.20	D	781.60	D	571.40	D	934.00	D	1952.4	D
methylene chloride	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.0	U
trans-1,2-dichloroethylene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1-dichloroethane	190.60	D	151.20	D	60.80	D	64.20	D	45.40	D	59.00	D	95.20	D	96.80	D	74.45	D	76.65	D	98.20	D	163.75	D
2,2-dichloropropane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
cis 1,2- dichloroethylene	713.40	D	617.60	D	377.80	D	336.40	D	250.80	D	249.00	D	674.65	D	628.80	D	475.60	D	442.20	D	805.00	D	1398.2	D
bromochloromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
chloroform	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1,1-trichloroethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
carbon tetrachloride	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1-dichloropropene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
benzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,2-dichloroethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
trichloroethylene	2048.60	D	1943.80	D	808.20	D	803.80	D	659.00	D	785.60	D	880.05	D	730.80	D	731.70	D	439.00	D	672.00	D	1321.2	D
1,2-dichloropropane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
dibromomethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
bromodichloromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
cis-1,3-dichloropropene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
toluene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
trans-1,3-dichloropropene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1,2-trichloroethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
tetrachloroethylene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5	U
1,3-dichloropropane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
dibromochloromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2-dibromoethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
chlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1,1,2-tetrachloroethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
ethylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
xylenes (m/p)	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.0	U
o-xylene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
styrene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
bromoform	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.0	U
isopropyl benzene (cumene)	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
bromobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1,2,2-tetrachloroethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2,3-trichloropropane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
n-propyl benzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
2-chlorotoluene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
4-chlorotoluene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,3,5-trimethylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
tert-butylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2,4-trimethylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
sec-butylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,3-dichlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
4-isopropyltoluene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,4-dichlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2-dichlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
n-butylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2-dibromo-3-chloropropane	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.0	U
1,2,4-trichlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
hexachlorobutadiene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
naphthalene	60.60	D	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2,3-trichlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
Methyl tertiary butyl ether	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
Acetone	100.00	U	100.00	U	561.60	D	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25	U
carbon disulfide	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10	U
2-butanone (MEK)	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25	U
tetrahydrofuran (THF)	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25	U
4-methyl-2-pentanone (MIBK)	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.0	U
2-hexanone	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.0	U
2-chloroethyl vinyl ether	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.0	U

Sample ID	PTW-1	PTW-1	PTW-1	PTW-1	PTW-1	PTW-1	PTW-1	PTW-1	PTW-1	PTW-1	PTW-1	PTW-1
Sampling Date	12/15/2020	2/9/2021	4/7/2021	4/28/2021	5/18/2021	6/21/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-90	-34	23	44	64	98	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	821.6 D	588.84 D	364.95 D	455.18 D	244.91 D	213.88 D	285.17 D	107.10 D	426.65 D	445.03 D	632.79 D	890 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	0.89 J	0.77 J	0.95 U	0.95 U	0.95 U	0.95 U	132.12	27.03	6.20	1.34	0.95 U	42
Ethane	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.9 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.7 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	7.0 U
Isobutane	--	--	3.62 U	3.62 U	3.62 U	29.41	448.86	246.35	583.46	290.24	20.74	1.81 **J**
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	--	--	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	--	--	--
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	399.60 D	354.18 D	303.10 D	323.22 D	317.04 D	350.78 D	352.43 D	340.93 D	346.99 D	311.13 D	--	389.6335 D
Nitrite as N	1.55	3.04	1.21	0.20 U	0.72	0.20 U	0.20	0.20 U	0.20 U	0.20 U	--	1.3432
Sulfate as SO4	337.06 D	295.93 D	318.75 D	296.34 D	305.30 D	304.19 D	293.95 D	298.78 D	308.78 D	297.99 D	--	314.6366 D
Bromide	0.20 U	8.86	3.31	5.15	1.88	0.20 U	1.07	2.19	0.20 U	3.04	--	3.7556
Nitrate as N	20.09 D	17.64 D	23.76 D	17.95 D	17.04	12.18	13.67	13.27	12.28	11.92	--	18.9937
Phosphate as P, ortho	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	4.07	0.13 J	0.20 U	4.00 U	0.20 U	--	0.2 U
FIELD PARAMETERS												
pH (SU)	7.00	7.32	7.17	7.15	7.09	7.23	7.17	7.14	7.14	6.93	7.19	7.33
Temperature (°C)	22.80	22	18.60	23.20	23.60	24.1	23.40	23.3	21.90	20.8	24.3	23.9
Dissolved Oxygen (DO; mg/L)	0.55	0.71	1.4	24.16	22.52	2.17	0.19	2.04	2.27	0.39	-28.02	0.017
Redox Potential (ORP; mV)	249.6	134.3	130.1	166.6	245.0	192.5	128.5	137.3	154.60	162.2	142.8	60.7
Specific Conductivity (mS/cm)	3.162	3.001	2.516	2.706	2.486	2.699	2.886	2.840	2.62	2559	2.614	6.34
Depth to Water (ft-btoc) prior to purging	25.3	25.54	25.55	1.61	25.60	25.87	25.82	25.82	25.82	25.81	25.87	25.95
Purge Rate (mL/min)	150	170	100	140	200	100	100	100	140	100	100	200
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--	--	--		--	--	--	--	--	--	--	--
Propane Monooxygenase (PPO)	--	--	--		--	--	--	--	--	--	--	--
Ammonia Monooxygenase (AMO)	--	--	--		--	--	--	--	--	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	--	--		--	--	--	--	--	--	--	--
Ethene Monooxygenase (EtnC)	--	--	--		--	--	--	--	--	--	--	--

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	DMW-1		DMW-1		DMW-1		DMW-1		DMW-1		DMW-1		DMW-1		DMW-1		DMW-1		DMW-1		DMW-1		DMW-1	
Sampling Date	12/14/2020		2/8/2021		4/7/2021		4/27/2021		5/18/2021		6/21/2021		9/14/2021		11/16/2021		12/14/2021		2/23/2022		4/5/2022		7/27/2022	
Days	-91		-35		23		43		64		98		183		246		274		345		386		499	
Matrix	Water		Water		Water		Water		Water		Water		Water		Water		Water		Water		Water		Water	
VOCS (GC/MS)	µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L		µg/L	
dichlorodifluoromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
chloromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
vinyl chloride	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5	U
bromomethane	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.0	U
chloroethane	20.00	U	20.00	U	20.00	U	38.80	D	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5	U
trichlorofluoromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1-dichloroethylene	1895.60	D	1459.80	D	1897.60	D	2063.80	D	1215.00	D	911.00	D	481.15	D	255.10	D	407.95	D	306.85	D	298.20	D	241.8	D
methylene chloride	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.0	U
trans-1,2-dichloroethylene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1-dichloroethane	119.80	D	108.00	D	112.00	D	132.80	D	82.20	D	54.20	D	29.65	D	23.60	D	41.60	D	39.70	D	38.95	D	44.8	D
2,2-dichloropropane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
cis 1,2- dichloroethylene	379.40	D	440.20	D	526.80	D	775.00	D	471.20	D	304.80	D	167.00	D	90.50	D	148.40	D	180.75	D	191.00	D	218.85	D
bromochloromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
chloroform	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1,1-trichloroethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
carbon tetrachloride	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1-dichloropropene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
benzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,2-dichloroethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
trichloroethylene	1476.20	D	1286.20	D	1543.60	D	1338.60	D	972.20	D	714.60	D	446.15	D	292.85	D	519.65	D	372.55	D	379.00	D	281.7	D
1,2-dichloropropane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
dibromomethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
bromodichloromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
cis-1,3-dichloropropene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
toluene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
trans-1,3-dichloropropene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1,2-trichloroethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
tetrachloroethylene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5	U
1,3-dichloropropane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
dibromochloromethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2-dibromoethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
chlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1,1,2-tetrachloroethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
ethylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
xylenes (m/p)	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.0	U
o-xylene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
styrene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
bromoform	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.0	U
isopropyl benzene (cumene)	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
bromobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,1,2,2-tetrachloroethane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2,3-trichloropropane	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
n-propyl benzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
2-chlorotoluene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
4-chlorotoluene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,3,5-trimethylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
tert-butylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2,4-trimethylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
sec-butylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,3-dichlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
4-isopropyltoluene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,4-dichlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2-dichlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
n-butylbenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2-dibromo-3-chloropropane	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.0	U
1,2,4-trichlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
hexachlorobutadiene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
naphthalene	41.00	D	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
1,2,3-trichlorobenzene	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
Methyl tertiary butyl ether	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	20.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U
Acetone	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25	U
carbon disulfide	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	40.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10	U
2-butanone (MEK)	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25	U
tetrahydrofuran (THF)	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25	U
4-methyl-2-pentanone (MIBK)	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.0	U
2-hexanone	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.0	U
2-chloroethyl vinyl ether	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	100.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.0	U

Sample ID	DMW-1	DMW-1	DMW-1	DMW-1	DMW-1	DMW-1	DMW-1	DMW-1	DMW-1	DMW-1	DMW-1	DMW-1
Sampling Date	12/14/2020	2/8/2021	4/7/2021	4/27/2021	5/18/2021	6/21/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022
Days	-91	-35	23	43	64	98	183	246	274	345	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	437 D	236.79 D	180.20 D	397.8429 D	335.88 D	312.50 D	149.00 D	124.59 D	293.40 D	617.04 D	596.36 D	190.40 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	1.74	1.15	0.95 U	0.95 U	0.95 U	0.95 U	86.64	1.30	4.76	0.81 J	0.95 U	38.59
Ethane	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U
Isobutane	--	--	3.62 U	3.62 U	3.62 U	3.62 U	183.16	240.72	752.72	417.53	140.51	1667.21
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	--	--	5 U	5 U	5 U	--	5.00 U	5.00 U	5.00 U	--	--	--
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	352.41 D	329.29 D	353.97 D	338.15 D	314.01 D	349.21 D	269.68 D	214.23 D	311.66 D	376.29 D	387.45 D	429.04 D
Nitrite as N	0.77	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U
Sulfate as SO4	321.53 D	287.49 D	317.91 D	300.72 D	307.40 D	300.44 D	303.09 D	296.47 D	306.64 D	322.79 D	322.14 D	328.10 D
Bromide	0.20 U	6.08	4.68	8.18	2.34	0.20 U	0.92	1.99	0.20 U	3.59	3.30	5.33
Nitrate as N	18.04	17.37	17.72	18.69	16.99 D	12.08	11.60	7.03	10.70	9.10	7.06	4.83
Phosphate as P, ortho	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	3.62	0.20 U	0.20 U	0.20 U	0.20 U	0.20 U	14.32 D
FIELD PARAMETERS												
pH (SU)	7.49	7.3	7.32	7.07	7.22	7.11	7.2	7.1	7.17	6.79	7.56	7.02
Temperature (°C)	22.60	22	23.00	23.30	24.40	24.80	24.40	22.5	20.00	21.2	23.5	24.8
Dissolved Oxygen (DO; mg/L)	14.85	14.87	14.7	19.99	25.42	4.17	25.47	21.46	16.76	26.72	31.47	25.26
Redox Potential (ORP; mV)	257.2	188.5	193.2	224.2	157.6	188.9	95.4	172.9	249.80	161.9	229	264.9
Specific Conductivity (mS/cm)	2.841	3.243	3.094	2.774	2.660	2.306	2.269	2483	2.50	2886	2.974	4079.18
Depth to Water (ft-btoc) prior to purging	23.4	23.28	23.47	23.50	24.34	23.30	23.48	23.3	23.59	23.60	23.45	23.62
Purge Rate (mL/min)	170	140	120	100	90	100	100	200	100	100	100	100
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--	5.00E+00 U	--	4.20E+00 J	5.00E-01 J	--	--	--	7.00E+00	--	--	--
Propane Monooxygenase (PPO)	--	5.00E+00 U	--	3.60E+01	1.27E+01	--	--	--	8.50E+00	--	--	--
Ammonia Monooxygenase (AMO)	--	5.00E+00 U	--	4.70E+00 U	5.10E+00 U	--	--	--	4.60E+00 U	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	5.00E+00 U	--	2.62E+02	5.69E+01	--	--	--	4.41E+02	--	--	--
Particulate methane monooxygenase (PMMO)	--	3.10E+00 J	--	4.70E+00 U	5.10E+00 U	--	--	--	5.02E+01	--	--	--

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A
Sampling Date	12/14/2020	2/8/2021	4/7/2021	4/27/2021	5/18/2021	6/8/2021	6/21/2021	7/1/2021	7/8/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022	
Days	-91	-35	23	43	64	85	98	108	115	155	183	246	274	345	386	499	
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	
VOCS (GC/MS)	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	
dichlorodifluoromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
chloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
vinyl chloride	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5 U	
bromomethane	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
chloroethane	20.00 U	20.00 U	20.00 U	66.00 D	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
trichlorofluoromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1-dichloroethylene	840.80 D	1222.40 D	1169.80 D	1621.80 D	1403.60 D	--	1323.20 D	--	--	1209.00 ED	1166.00 D	526.90 D	1362.00 D	756.20 D	721.75 D	959.0 D	
methylene chloride	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
trans-1,2-dichloroethylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1-dichloroethane	61.60 D	73.20 D	32.60 D	89.40 D	79.40 D	--	74.00 D	--	--	73.45 D	64.00 D	42.55 D	90.75 D	64.65 D	56.50 D	69.3 D	
2,2-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
cis 1,2- dichloroethylene	89.40 D	142.20 D	117.20 D	175.20 D	167.60 D	--	142.40 D	--	--	128.25 D	109.80 D	71.85 D	144.05 D	102.70 D	95.65 D	115.85 D	
bromochloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
chloroform	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1,1-trichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
carbon tetrachloride	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
benzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	
1,2-dichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
trichloroethylene	789.20 D	1375.20 D	1301.00 D	1573.40 D	1500.40 D	--	1272.40 D	--	--	1226.50 ED	1217.80 D	573.65 D	1681.00 D	924.80 D	881.95 D	1170.2 D	
1,2-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
dibromomethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
bromodichloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
cis-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
toluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
trans-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1,2-trichloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
tetrachloroethylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5 U	
1,3-dichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
dibromochloromethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2-dibromoethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
chlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1,1,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
ethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
xylenes (m/p)	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
o-xylene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
styrene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
bromoform	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
isopropyl benzene (cumene)	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
bromobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,1,2,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2,3-trichloropropane	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
n-propyl benzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
2-chlorotoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
4-chlorotoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,3,5-trimethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
tert-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2,4-trimethylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
sec-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,3-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
4-isopropyltoluene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,4-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2-dichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
n-butylbenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2-dibromo-3-chloropropane	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
1,2,4-trichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
hexachlorobutadiene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
naphthalene	89.00 D	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
1,2,3-trichlorobenzene	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
Methyl tertiary butyl ether	20.00 U	20.00 U	20.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	20.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.0 U	
Acetone	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U	
carbon disulfide	40.00 U	40.00 U	40.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	40.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.0 U	
2-butanone (MEK)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U	
tetrahydrofuran (THF)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	
4-methyl-2-pentanone (MIBK)	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U	
2-hexanone	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U	
2-chloroethyl vinyl ether	100.00 U	100.00 U	100.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	100.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.0 U	

Sample ID	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A	OU11-SMW08A
Sampling Date	12/14/2020	2/8/2021	4/7/2021	4/27/2021	5/18/2021	6/8/2021	6/21/2021	7/1/2021	7/8/2021	8/17/2021	9/14/2021	11/16/2021	12/14/2021	2/23/2022	4/5/2022	7/27/2022	
Days	-91	-35	23	43	64	85	98	108	115	155	183	246	274	345	386	499	
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	
1,4-Dioxane	111.21	D	196.35	D	314.40	D	425.99	D	180.83	D	--	174.60	D	--	--	311.00	D	184.20	D	213.08	D	330.41	D	181.56	D	245.10	D	244	D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	
Methane	0.89	J	0.72	J	0.95	U	0.95	U	0.95	U	0.95	U	0.95	U	377.51	152.35	0.95	U	0.95	U	0.87	J	0.95	U	0.95	U	0.95	U	0.95	U
Ethane	1.87	U	1.87	U	1.87	U	1.87	U	1.87	U	1.87	U	1.87	U	1.87	U	1.87	U	1.87	U	1.87	U	1.87	U	1.87	U	1.87	U	1.87	U
Ethene	2.22	U	2.22	U	2.22	U	2.22	U	2.22	U	2.22	U	2.22	U	2.22	U	2.22	U	2.22	U	2.22	U	2.22	U	2.22	U	2.22	U	2.22	U
Propane	2.68	U	2.68	U	2.68	U	2.68	U	2.68	U	2.68	U	2.68	U	2.68	U	2.68	U	2.68	U	2.68	U	2.68	U	2.68	U	2.68	U	2.68	U
Acetylene	6.99	U	6.99	U	6.99	U	6.99	U	6.99	U	6.99	U	6.99	U	6.99	U	6.99	U	6.99	U	6.99	U	6.99	U	6.99	U	6.99	U	6.99	U
Isobutane	--		--		3.62	U	3.62	U	3.62	U	3.62	U	3.62	U	802.48	yes	3.62	U	3.62	U	3.62	U	3.62	U	**3.62**	**U**	3.62	U
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	
Isobutanol	--	--	5.00	U	5.00	U	5.00	U	5.00	U	--			--	5.00	U	5.00	U	--	--	--	
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	
Chloride	451.69	D	392.46	D	434.98	D	422.43	D	435.72	D	424.59	D	327.82	D	--	--	422.31	D	425.87	D	428.55	D	433.05	D	431.17	D	429.50	D	428.72	D
Nitrite as N	2.18	3.27	3.13	0.73	1.91	0.62	0.20	U	--	--	0.20	U	0.82	1.40	0.20	U	0.20	U	0.20	U	2.18	
Sulfate as SO4	297.47	D	262.45	D	294.61	D	287.13	D	298.39	D	313.01	D	298.12	D	--	--	294.85	D	304.34	D	313.20	D	320.14	D	322.47	D	316.30	D	308.06	D
Bromide	0.20	U	7.84	3.62	5.86	4.11	4.72	0.20	U	--	--	0.20	U	1.58	2.83	0.20	U	3.47	2.42	3.86	
Nitrate as N	37.23	D	34.21	D	35.66	D	34.04	D	34.90	D	33.28	D	3.85	--	--	32.85	D	32.00	D	31.90	D	31.17	D	30.91	D	30.69	D	30.89	D
Phosphate as P, ortho	0.20	U	0.20	U	0.20	U	0.20	U	0.20	U	0.20	U	0.20	U	--	--	0.20	U	0.20	U	0.20	U	0.20	U	0.20	U	0.20	U	0.20	U
FIELD PARAMETERS																	
pH (SU)	7.06	7.19	7.13	7.09	7.00	7.21	7.05	--	--	7.19	7.14	6.92	7.14	7.08	7.76	7.46	
Temperature (°C)	22.50	22.3	23.40	23.90	23.60	23.90	26.20	--	--	23.8	23.6	25.0	20.70	20.8	23.3	25.4	
Dissolved Oxygen (DO; mg/L)	0.66	0.55	0.6	1.09	0.28	0.12	0.36	--	--	0.67	0.30	1.56	0.41	0.57	0.32	0.37	
Redox Potential (ORP; mV)	231.2	165.2	100.8	146.0	192.6	160.1	187.2	--	--	99.3	114.2	112.2	208.90	158.9	187.7	183.1	
Specific Conductivity (mS/cm)	3.443	3.721	3.364	3.409	3.361	3.287	3.398	--	--	3.289	3.335	3.504	3.35	3301	3.295	4317.75	
Depth to Water (ft-btoc) prior to purging	25.7	26.02	26.1	26.29	26.10	26.30	26.21	--	--	26.2	26.21	26.10	26.20	26.2	26.30	26.23	
Purge Rate (mL/min)	100	150	110	140	100	200	100	--	--	200	100	100	100	100	100	200	
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	
Short Chain Alkane monooxygenase (SCAM)	--	5.00E+00	U	--	4.90E+00	5.00E-01	J	--	--	--	--	--	--	--	4.60E+00	U	--	--	--	
Propane Monooxygenase (PPO)	--	1.95E+01	--	1.10E+02	3.30E+00	J	--	--	--	--	--	--	--	1.53E+01	--	--	--	
Ammonia Monooxygenase (AMO)	--	5.00E+00	U	--	4.60E+00	U	4.80E+00	U	--	--	--	--	--	--	4.60E+00	U	--	--	--	
Soluble Methane Monooxygenase (SMMO)	--	5.00E+00	U	--	4.60E+00	U	4.80E+00	U	--	--	--	--	--	--	3.35E+01	--	--	--	
Particulate methane monooxygenase (PMMO)	--	2.00E-01	J	--	4.60E+00	U	4.80E+00	U	--	--	--	--	--	--	4.33E+01	--	--	--	

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.
E - Estimated value, beyond linear range.

Sample ID	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1
Sampling Date	4/6/2021	4/28/2021	5/19/2021	5/21/2021	5/26/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	6/25/2021	6/29/2021	7/6/2021	7/12/2021	7/15/2021	7/19/2021	7/22/2021	7/29/2021	8/3/2021	8/10/2021	8/17/2021
Days	22	44	65	67	72	78	85	92	98	102	106	113	119	122	126	129	136	141	148	155
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
VOCS (GC/MS)	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
dichlorodifluoromethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
chloromethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
vinyl chloride	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
bromomethane	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	--	20.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	10.00 U	10.00 U
chloroethane	20.00 U	30.60 D	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
trichlorofluoromethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,1-dichloroethylene	917.40 D	1132.20 D	710.20 D	--	--	620.00 D	--	400.20 D	532.40 D	--	146.20 D	470.40 D	607.20 D	--	664.60 D	--	--	560.70 D	543.60 D	486.15 D
methylene chloride	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	--	20.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	10.00 U	10.00 U
trans-1,2-dichloroethylene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,1-dichloroethane	21.40 D	72.80 D	50.80 D	--	--	43.60 D	--	36.00 D	38.80 D	--	14.30 D	37.20 D	46.80 D	--	46.00 D	--	--	48.00 D	44.30 D	41.75 D
2,2-dichloropropane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
cis 1,2- dichloroethylene	98.80 D	301.40 D	207.00 D	--	--	204.80 D	--	135.80 D	175.80 D	--	43.70 D	147.20 D	172.40 D	--	178.20 D	--	--	189.10 D	178.40 D	154.60 D
bromochloromethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
chloroform	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,1,1-trichloroethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
carbon tetrachloride	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,1-dichloropropene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
benzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,2-dichloroethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
trichloroethylene	784.00 D	934.00 D	692.20 D	--	--	701.00 D	--	468.80 D	544.60 D	--	143.30 D	507.00 D	622.20 D	--	688.80 D	--	--	617.10 D	581.50 D	491.75 D
1,2-dichloropropane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
dibromomethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
bromodichloromethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
cis-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
toluene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
trans-1,3-dichloropropene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,1,2-trichloroethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
tetrachloroethylene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,3-dichloropropane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
dibromochloromethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,2-dibromoethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
chlorobenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,1,1,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
ethylbenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
xylenes (m/p)	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	--	20.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	10.00 U	10.00 U
o-xylene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
styrene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
bromoform	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	--	20.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	10.00 U	10.00 U
isopropyl benzene (cumene)	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
bromobenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,1,2,2-tetrachloroethane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,2,3-trichloropropane	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
n-propyl benzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
2-chlorotoluene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
4-chlorotoluene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,3,5-trimethylbenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
tert-butylbenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,2,4-trimethylbenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
sec-butylbenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,3-dichlorobenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
4-isopropyltoluene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,4-dichlorobenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,2-dichlorobenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
n-butylbenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,2-dibromo-3-chloropropane	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	--	20.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	10.00 U	10.00 U
1,2,4-trichlorobenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
hexachlorobutadiene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
naphthalene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
1,2,3-trichlorobenzene	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
Methyl tertiary butyl ether	20.00 U	20.00 U	20.00 U	--	--	20.00 U	--	20.00 U	20.00 U	--	10.00 U	20.00 U	20.00 U	--	20.00 U	--	--	5.00 U	5.00 U	5.00 U
Acetone	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	--	50.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	25.00 U	25.00 U
carbon disulfide	40.00 U	40.00 U	40.00 U	--	--	40.00 U	--	40.00 U	40.00 U	--	20.00 U	40.00 U	40.00 U	--	40.00 U	--	--	10.00 U	10.00 U	10.00 U
2-butanone (MEK)	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	--	50.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	25.00 U	25.00 U
tetrahydrofuran (THF)	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	--	50.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	25.00 U	25.00 U
4-methyl-2-pentanone (MIBK)	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	--	50.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	25.00 U	25.00 U
2-hexanone	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	--	50.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	25.00 U	25.00 U
2-chloroethyl vinyl ether	100.00 U	100.00 U	100.00 U	--	--	100.00 U	--	100.00 U	100.00 U	--	50.00 U	100.00 U	100.00 U	--	100.00 U	--	--	25.00 U	25.00 U	25.00 U

Sample ID	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1
Sampling Date	4/6/2021	4/28/2021	5/19/2021	5/21/2021	5/26/2021	6/1/2021	6/8/2021	6/15/2021	6/21/2021	6/25/2021	6/29/2021	7/6/2021	7/12/2021	7/15/2021	7/19/2021	7/22/2021	7/29/2021	8/3/2021	8/10/2021	8/17/2021
Days	22	44	65	67	72	78	85	92	98	102	106	113	119	122	126	129	136	141	148	155
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	266.72 D	449.07 D	233.88 D	--	--	--	--	--	364.61 D	--	475 D	--	--	--	386.20 D	--	--	--		333.60 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	0.95 U	217.74	888.83	863.32	163.23	105.59	123.65	40.45	51.62	283.17	232.96	74.47
Ethane	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U
Isobutane	3.62 U	3.62 U	3.62 U	8.70	51.94	250.00	292.21	367.92	29.41	306.98	391.83	644.23	667.88	393.48	402.96	649.40	548.37	448.46	432.05	312.05
ISOBUTANOL	mg/L	mg/L	mg/L		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	5.00 U		12.72	10.15	5.00 U	5.00 U	5.00 U	--	--			--			--	--	--			--
ANIONS	mg/L	mg/L	mg/L		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	344.98 D	326.85 D	330.05 D	324.06 D	326.20 D	342.72 D	343.31 D	347.20 D	347.4459 D	--	--	--	--	--	369.86 D	--	--	--	--	366.46 D
Nitrite as N	0.92	0.22	5.33	3.43	2.83	1.86	0.39	0.20 U	0.2 U	--	--	--	--	--	0.20 U	--	--	--	--	0.20 U
Sulfate as SO4	309.87 D	303.32 D	302.30 D	287.03 D	295.16 D	301.65 D	319.81 D	300.67 D	298.7035 D	--	--	--	--	--	313.67 D	--	--	--	--	306.14 D
Bromide	3.44	5.77	0.20 U	6.06	9.71	6.39	4.66	0.20 U	0.2 U	--	--	--	--	--	0.20 U	--	--	--	--	0.20 U
Nitrate as N	17.33	17.06	5.45	5.42	8.23	10.11 D	10.15	12.13	12.0254	--	--	--	--	--	12.61	--	--	--	--	15.23
Phosphate as P, ortho	0.20 U	0.20 U	7.71	9.16	6.22	3.37	3.99	0.20 U	3.7938	--	--	--	--	--	6.76	--	--	--	--	10.37
FIELD PARAMETERS																				
pH (SU)	7.09	7.29	7.60	7.47	6.27	--	7.35	7.11	7.21	--	--	7.01	--	--	7.08	--	--	--	--	7.18
Temperature (^{o}C)	20.90	23.8	22.90	21.00	22.50	--	23.60	23	26.0	--	--	22.7	--	--	25	--	--	--	--	25.3
Dissolved Oxygen (DO; mg/L)	23.15	27.45	14.0	4.50	16.26	--	28.71	22.81	15.0	--	--	8.18	--	--	12.15	--	--	--	--	24.1
Redox Potential (ORP; mV)	156.1	146.4	131.5	167.8	225.3	--	199	203.5	188.5	--	--	225.8	--	--	83.7	--	--	--	--	134.8
Specific Conductivity (mS/cm)	2.782	2.692	2.746	2.888	2.484	--	2.774	2.946	2.638	--	--	3.028	--	--	3.064	--	--	--	--	2.996
Depth to Water (ft-btoc) prior to purging	--			--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Purge Rate (mL/min)	--			--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Propane Monooxygenase (PPO)	--		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Ammonia Monooxygenase (AMO)	--		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Soluble Methane Monooxygenase (SMMO)	--		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Particulate methane monooxygenase (PMMO)	--		--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Notes:
U - The compound was not detected at the indicated PQL concentration.
J - Data indicates the presence of a compound that meets the identification criteria. The result is less than the quantitation limit but greater than zero. The concentration given is an approximate value.
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1
Sampling Date	9/1/2021	9/2/2021	9/7/2021	9/9/2021	9/14/2021	9/16/2021	9/17/2021	9/20/2021	9/21/2021	9/23/2021	9/24/2021	9/30/2021	10/5/2021	10/8/2021	10/14/2021	10/20/2021	11/8/2021	11/16/2021	11/23/2021	12/9/2021
Days	170	171	176	178	183	185	186	189	190	192	193	199	204	207	213	219	238	246	253	269
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
VOCS (GC/MS)	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
dichlorodifluoromethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chloromethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
vinyl chloride	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromomethane	--	10.00 U	--	--	10.00 U	10.00 U	--	--	--	10.00 U	10.00 U	10.00 U	10.00 U	--	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
chloroethane	--	5.00 U	--	--	5.00 U	7.00 D	--	--	--	5.55 D	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trichlorofluoromethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloroethylene	--	499.65 D	--	--	467.90 D	350.00 D	--	--	--	340.95 D	432.75 D	457.50 D	410.35 D	--	224.70 D	180.20 D	219.65 D	278.90 D	148.35 D	195.65 D
methylene chloride	--	10.00 U	--	--	10.00 U	10.00 U	--	--	--	10.00 U	10.00 U	10.00 U	10.00 U	--	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
trans-1,2-dichloroethylene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloroethane	--	40.80 D	--	--	43.15 D	31.30 D	--	--	--	35.70 D	39.55 D	45.55 D	45.80 D	--	43.35 D	34.40 D	36.85 D	46.00 D	26.90 D	35.90 D
2,2-dichloropropane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
cis 1,2- dichloroethylene	--	169.75 D	--	--	196.95 D	148.05 D	--	--	--	187.50 D	226.70 D	339.50 D	359.85 D	--	268.55 D	223.90 D	203.45 D	249.35 D	137.85 D	172.85 D
bromochloromethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chloroform	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,1-trichloroethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
carbon tetrachloride	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1-dichloropropene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
benzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dichloroethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trichloroethylene	--	537.30 D	--	--	547.75 D	397.60 D	--	--	--	379.90 D	449.25 D	333.55 D	299.20 D	--	244.40 D	197.90 D	245.20 D	311.40 D	183.90 D	273.40 D
1,2-dichloropropane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
dibromomethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromodichloromethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
cis-1,3-dichloropropene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
toluene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
trans-1,3-dichloropropene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,2-trichloroethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
tetrachloroethylene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3-dichloropropane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
dibromochloromethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dibromoethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
chlorobenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,1,2-tetrachloroethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
ethylbenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
xylenes (m/p)	--	10.00 U	--	--	10.00 U	10.00 U	--	--	--	10.00 U	10.00 U	10.00 U	10.00 U	--	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
o-xylene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
styrene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromoform	--	10.00 U	--	--	10.00 U	10.00 U	--	--	--	10.00 U	10.00 U	10.00 U	10.00 U	--	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
isopropyl benzene (cumene)	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
bromobenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,1,2,2-tetrachloroethane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,3-trichloropropane	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
n-propyl benzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
2-chlorotoluene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
4-chlorotoluene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3,5-trimethylbenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
tert-butylbenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,4-trimethylbenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
sec-butylbenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,3-dichlorobenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
4-isopropyltoluene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,4-dichlorobenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dichlorobenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
n-butylbenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2-dibromo-3-chloropropane	--	10.00 U	--	--	10.00 U	10.00 U	--	--	--	10.00 U	10.00 U	10.00 U	10.00 U	--	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
1,2,4-trichlorobenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
hexachlorobutadiene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
naphthalene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
1,2,3-trichlorobenzene	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
Methyl tertiary butyl ether	--	5.00 U	--	--	5.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U
Acetone	--	25.00 U	--	--	25.00 U	5.00 U	--	--	--	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	5.00 U	5.00 U	25.00 U	5.00 U	25.00 U
carbon disulfide	--	10.00 U	--	--	10.00 U	10.00 U	--	--	--	10.00 U	10.00 U	10.00 U	10.00 U	--	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U	10.00 U
2-butanone (MEK)	--	25.00 U	--	--	25.00 U	25.00 U	--	--	--	25.00 U	25.00 U	25.00 U	25.00 U	--	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
tetrahydrofuran (THF)	--	25.00 U	--	--	25.00 U	25.00 U	--	--	--	25.00 U	25.00 U	25.00 U	25.00 U	--	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
4-methyl-2-pentanone (MIBK)	--	25.00 U	--	--	25.00 U	25.00 U	--	--	--	25.00 U	25.00 U	25.00 U	25.00 U	--	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
2-hexanone	--	25.00 U	--	--	25.00 U	25.00 U	--	--	--	25.00 U	25.00 U	25.00 U	25.00 U	--	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U
2-chloroethyl vinyl ether	--	25.00 U	--	--	25.00 U	25.00 U	--	--	--	25.00 U	25.00 U	25.00 U	25.00 U	--	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U	25.00 U

Sample ID	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1
Sampling Date	9/1/2021	9/2/2021	9/7/2021	9/9/2021	9/14/2021	9/16/2021	9/17/2021	9/20/2021	9/21/2021	9/23/2021	9/24/2021	9/30/2021	10/5/2021	10/8/2021	10/14/2021	10/20/2021	11/8/2021	11/16/2021	11/23/2021	12/9/2021
Days	170	171	176	178	183	185	186	189	190	192	193	199	204	207	213	219	238	246	253	269
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
1,4-Dioxane	--	--	--	--	331.60 D	--	--	--	--	--	--	--	--	--	--	--	--	268.55 D	--	204.94 D
REDUCED GASES	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Methane	23.43	51.10	--	--	27.73	16.21	--	--	--	7.43	23.18	22.89	17.14	3.97	7.55	6.85	44.30	15.81	3.87	21.40
Ethane	1.87 U	1.87 U	--	--	1.87 U	1.87 U	--	--	--	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U
Ethene	2.22 U	2.22 U	--	--	2.22 U	2.22 U	--	--	--	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	--	--	2.68 U	2.68 U	--	--	--	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U
Acetylene	6.99 U	6.99 U	--	--	6.99 U	6.99 U	--	--	--	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U
Isobutane	151.04	320.74	--	--	59.34	50.56	--	--	--	15.04	42.82	39.37	64.06	5.67	10.24	21.47	33.79	11.33	4.79	353.78
ISOBUTANOL	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Isobutanol	11.94	18.44	16.94	17.34	7.57	23.65	11.98	21.64	11.73	54.30	78.67	17.60	5.78	5.00 U	5.00 U	5.00 U	--	5.00 U	--	--
ANIONS	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Chloride	--	--	--	--	386.88 D	--	--	--	--	--	--	--	--	--	--	--	--	412.53 D	--	--
Nitrite as N	--	--	--	--	0.15 J	--	--	--	--	--	--	--	--	--	--	--	--	0.20 U	--	--
Sulfate as SO4	--	--	--	--	315.71 D	--	--	--	--	--	--	--	--	--	--	--	--	320.72 D	--	--
Bromide	--	--	--	--	1.16	--	--	--	--	--	--	--	--	--	--	--	--	2.64	--	--
Nitrate as N	--	--	--	--	6.64	--	--	--	--	--	--	--	--	--	--	--	--	5.70	--	--
Phosphate as P, ortho	--	--	--	--	14.49	--	--	--	--	--	--	--	--	--	--	--	--	24.38 D	--	--
FIELD PARAMETERS																				
pH (SU)	--	--	--	--	7.17	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Temperature (^{o}C)	--	--	--	--	24.5	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Dissolved Oxygen (DO; mg/L)	--	--	--	--	14.45	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Redox Potential (ORP; mV)	--	--	--	--	96.1	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Specific Conductivity (mS/cm)	--	--	--	--	3.067	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Depth to Water (ft-btoc) prior to purging	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Purge Rate (mL/min)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Microbial Analyses	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL	cells/mL
Short Chain Alkane monooxygenase (SCAM)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Propane Monooxygenase (PPO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Ammonia Monooxygenase (AMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Particulate methane monooxygenase (PMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Notes:
U - The compound was not detected at the indicate
J - Data indicates the presence of a compound tha
-- Not Sampled.
D - Sample was diluted prior to analysis.

Sample ID	EW-1		EW-1		EW-1		EW-1		EW-1		EW-1		EW-1		EW-1		EW-1		EW-1		EW-1		EW-1		EW-1		EW-1	
Sampling Date	12/14/2021		12/21/2021		12/29/2021		1/7/2022		1/13/2022		1/18/2022		1/27/2022		2/2/2022		2/7/2022		2/18/2022		2/23/2022		3/3/2022		4/5/2022		7/27/2022	
Days	274		281		289		298		304		309		318		324		329		340		345		353		386		499	
Matrix	Water		Water		Water		Water		Water		Water		Water		Water		Water		Water		Water		Water		Water		Water	
VOCS (GC/MS)	µg/L		µg/L		µg/L																							
dichlorodifluoromethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
chloromethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
vinyl chloride	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
bromomethane	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	20.00	U	20.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U
chloroethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
trichlorofluoromethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,1-dichloroethylene	270.60	D	152.70	D	226.65	D	240.40	D	223.60	D	213.50	D	215.90	D	216.90	D	190.20	D	289.35	D	209.45	D	227.650	D	193.70	D	302.00	D
methylene chloride	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	20.00	U	20.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.0	U	10.00	U	10.00	U
trans-1,2-dichloroethylene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U	5.00	U	5.00	U
1,1-dichloroethane	47.95	D	38.70	D	43.05	D	48.90	D	40.90	D	46.90	D	35.40	D	50.55	D	37.50	D	56.80	D	37.60	D	44.85	D	39.65	D	43.75	D
2,2-dichloropropane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U	5.00	U	5.00	U
cis 1,2- dichloroethylene	222.45	D	189.15	D	198.50	D	211.30	D	190.75	D	224.20	D	191.60	D	235.30	D	205.15	D	360.40	D	240.80	D	274.600	D	204.95	D	206.90	D
bromochloromethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U	5.00	U	5.00	U
chloroform	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U	5.00	U	5.00	U
1,1,1-trichloroethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U	5.00	U	5.00	U
carbon tetrachloride	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U	5.00	U	5.00	U
1,1-dichloropropene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U	5.00	U	5.00	U
benzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,2-dichloroethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.0	U	5.00	U	5.00	U
trichloroethylene	366.40	D	290.95	D	307.60	D	337.50	D	316.20	D	365.30	D	335.10	D	362.25	D	226.65	D	313.85	D	208.60	D	236.400	D	247.60	D	338.55	D
1,2-dichloropropane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
dibromomethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
bromodichloromethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
cis-1,3-dichloropropene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
toluene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
trans-1,3-dichloropropene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,1,2-trichloroethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
tetrachloroethylene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,3-dichloropropane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
dibromochloromethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,2-dibromoethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
chlorobenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,1,1,2-tetrachloroethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
ethylbenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
xylenes (m/p)	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	20.00	U	20.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U
o-xylene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
styrene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
bromoform	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	20.00	U	20.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U
isopropyl benzene (cumene)	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
bromobenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,1,2,2-tetrachloroethane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,2,3-trichloropropane	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
n-propyl benzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
2-chlorotoluene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
4-chlorotoluene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,3,5-trimethylbenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
tert-butylbenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,2,4-trimethylbenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
sec-butylbenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,3-dichlorobenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
4-isopropyltoluene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,4-dichlorobenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,2-dichlorobenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
n-butylbenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,2-dibromo-3-chloropropane	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	20.00	U	20.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U
1,2,4-trichlorobenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
hexachlorobutadiene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
naphthalene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
1,2,3-trichlorobenzene	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
Methyl tertiary butyl ether	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	10.00	U	10.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U	5.00	U
Acetone	25.00	U	5.00	U	5.00	U	5.00	U	5.00	U	50.00	U	50.00	U	5.00	U	5.00	U	5.00	U	25.00	U	5.00	U	25.00	U	25.00	U
carbon disulfide	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	20.00	U	20.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U	10.00	U
2-butanone (MEK)	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	50.00	U	50.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U
tetrahydrofuran (THF)	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	50.00	U	50.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U
4-methyl-2-pentanone (MIBK)	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	50.00	U	50.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U
2-hexanone	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	50.00	U	50.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U
2-chloroethyl vinyl ether	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	50.00	U	50.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U	25.00	U

Sample ID	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1	EW-1
Sampling Date	12/14/2021	12/21/2021	12/29/2021	1/7/2022	1/13/2022	1/18/2022	1/27/2022	2/2/2022	2/7/2022	2/18/2022	2/23/2022	3/3/2022	4/5/2022	7/27/2022
Days	274	281	289	298	304	309	318	324	329	340	345	353	386	499
Matrix	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
1,4-DIOXANE	µg/L	µg/L	µg/L											
1,4-Dioxane	193.89 D	--	--	--	--	--	--	--	--	--	440.99 D	--	294.06 D	436 D
REDUCED GASES	µg/L	µg/L	µg/L											
Methane	2.86	16.55	32.11	10.28	86.85	63.94	65.39	82.78	108.85	97.83	201.29	94.67	3.83	99.39
Ethane	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U	1.87 U
Ethene	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U	2.22 U
Propane	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U	2.68 U
Acetylene	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U	6.99 U
Isobutane	254.92	361.02	232.01	89.91	54.05	53.05	45.26	45.47	31.58	89.39	183.68	113.15	10.54	91.92
ISOBUTANOL	mg/L	mg/L	mg/L											
Isobutanol	5.00 U	--	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	5.00 U	--	5.00 U	--	--
ANIONS	mg/L	mg/L	mg/L											
Chloride	419.05 D	--	--	--	--	--	--	--	--	--	433.30 D	--	437.28 D	464.0409 D
Nitrite as N	0.20 U	--	--	--	--	--	--	--	--	--	0.20 U	--	0.20 U	0.2 U
Sulfate as SO4	329.91 D	--	--	--	--	--	--	--	--	--	280.25 D	--	317.83 D	350.2808 D
Bromide	0.20 U	--	--	--	--	--	--	--	--	--	3.55	--	3.31	4.2866
Nitrate as N	7.65	--	--	--	--	--	--	--	--	--	3.10	--	3.40	5.8282
Phosphate as P, ortho	24.26 D	--	--	--	--	--	--	--	--	--	27.34 D	--	31.36 D	31.8592 D
FIELD PARAMETERS														
pH (SU)	6.97	--	--	--	--	--	--	--	--	--	7.23	--	7.14	7.08
Temperature (^{o}C)	21.70	--	--	--	--	--	--	--	--	--	22.0	--	22	25
Dissolved Oxygen (DO; mg/L)	29.56	--	--	--	--	--	--	--	--	--	11.01	--	22.19	28.5
Redox Potential (ORP; mV)	190.90	--	--	--	--	--	--	--	--	--	116.0	--	212.5	127.1
Specific Conductivity (mS/cm)	3.40	--	--	--	--	--	--	--	--	--	3285	--	1.713	7.805
Depth to Water (ft-btoc) prior to purging	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Purge Rate (mL/min)	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Microbial Analyses	cells/mL	cells/mL	cells/mL											
Short Chain Alkane monooxygenase (SCAM)	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Propane Monooxygenase (PPO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Ammonia Monooxygenase (AMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Soluble Methane Monooxygenase (SMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Particulate methane monooxygenase (PMMO)	--	--	--	--	--	--	--	--	--	--	--	--	--	--

Notes:
U - The compound was not detected at the indicate
J - Data indicates the presence of a compound tha
-- Not Sampled.
D - Sample was diluted prior to analysis.

APPENDIX G
OSU COLUMN STUDY SUMMARY

Appendix G – OSU Column Study Summary

Column Test Objective

Continuous-flow packed-column studies were performed to assess cometabolic transformation of CAHs and 1,4-D in support of a field demonstration. The primary objective of this work was to assist with field operational conditions and develop a model to predict and simulate biodegradation of mixed contaminants at the site.

Supporting Microcosm Experiments

The potential complementarity of the two cultures was best illustrated in the batch groundwater microcosms as described in **Section 5.3** in the final report. In summary, Isobutane-grown ENV493 rapidly transformed 1,4-D when bioaugmented in groundwater microcosms, as expected from previous studies with isobutane-utilizing microorganisms (Bennett et al., 2018; Rolston et al., 2022, 2019a). By contrast, ENV494M did not transform 1,4-D in either microcosms or in the column in this study in agreement with the limited success of 1,4-D cometabolism of 1,4-D by methanotrophic cultures documented in the literature (Hatzinger et al., 2017; Sadeghi et al., 2016; Xiong et al., 2020). However, ENV494M transformed TCE to a greater extent and with less potential inhibition than ENV493.

While the analysis at University of Oregon (where the column test was conducted) was confounded by the coelution of 1,1-DCE and Freon 113, both cultures transformed 1,1-DCE. As shown in **Figure G-1**, ENV493 appeared to transform 1,1-DCE more rapidly than ENV494M, with faster transformation of 1,1-DCE than isobutane, as observed previously. Microcosm data may suggest 1,1-DCE transformation was inhibited in the presence of methane. The relatively low concentrations of CF (~4 µg/L) were rapidly and completely transformed by ENV493 but only ~40% was transformed by ENV494M in the same 1-day time period. Previous studies have shown that CF can be cometabolized by bacteria grown on various short-chain n-alkanes (Cappelletti et al., 2012), with more effective transformation observed by butane-utilizers than methanotrophs (Kim et al., 1997).

Both cultures rapidly transformed *cis*-DCE to its epoxide (**Figure G-1**), which served as an additional line of evidence of cometabolic activity in both the microcosm and column studies. More rapid transformation of the epoxide in microcosms with higher biomass (300 mgTSS/L ENV 493 or 435 mgTSS/L ENV494M) demonstrated the *cis*-DCE epoxide was also biologically transformed, as previously reported for butane-grown microorganisms (Halsey et al., 2007; Kim and Semprini, 2005). Maximum peak areas for *cis*-DCE epoxide detected in ENV493 microcosms were approximately equal in the high and low biomass reactors, and peak areas were ~30% of the initial *cis*-DCE concentrations (µg/L). By contrast, *cis*-DCE epoxide peak areas in ENV494M-bioaugmented microcosms reached 50-70% of the initial *cis*-DCE concentration and appeared to be more slowly transformed than in ENV493 microcosms. The lower relative peak areas in ENV493 microcosms indicate that the epoxide was being produced and transformed simultaneously, whereas higher peak areas in the EN494M-bioaugmented microcosms suggest slower rates of biotic transformation. In all groundwater microcosms, the epoxide was transformed

more slowly than it was formed, with tailing at lower concentrations as observed for previous biotransformation of epoxide products, such as isobutene epoxide (Rolston et al., 2022).

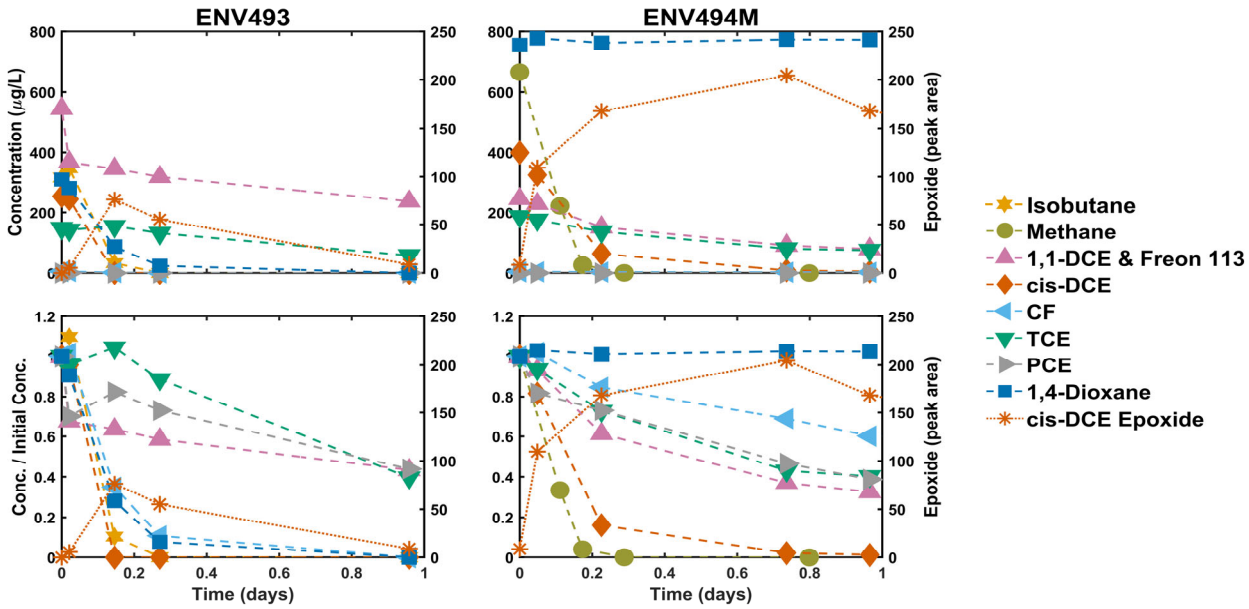


Figure G-1. Comparison of groundwater microcosms bioaugmented with isobutane-grown ENV493 (left) and methane-grown ENV494M (right), both measured (upper panel) and normalized concentrations (lower panel)

Column Test Design

A 600-mL stainless steel column was packed with sediment from the site. Experiments in the column were performed in two phases for the assessment of two primary substrates: the isobutane phase in which the column was bioaugmented with isobutane-utilizing *Rhodococcus* sp. ENV493, followed by the methane phase and bioaugmentation with a mixed methanotrophic culture, ENV494M (see **Table G-1** for phases). Both bacteria were enriched from site material. Isobutane or methane were dissolved in site groundwater contaminated with relatively high concentrations (~1 ppm) of CAHs and 1,4-D for continuous addition to the column.

Table G-1. Timing of tracer, isobutane, and methane phases and changes to operating conditions in the column study

Isobutane Phase	Day
ENV493 bioaugmentation; 80 mg/L hydrogen peroxide	0
Influent hydrogen peroxide concentration tripled to 250 mg/L	14
Recycle only (no influent)	52
Influent resumed	54
Influent and recycle stopped for 24 hours	82
Isobutane not included in influent	89
Recycle only (no influent)	98
Normal operations (influent, including isobutane, resumed and recycle on)	101
Recycle only (no influent)	110
End of isobutane phase	124
Methane Phase	Day
Second ENV494M bioaugmentation	0
Recycle only, ~24 hours	14
Sparged groundwater and methane, *cis*-DCE, and TCE only in influent	15
75 ul H_2O_2 in influent	17
Recycle only, ~24 hours	23
Influent resumed	24
Recycle only, ~48 hours	26
Influent resumed	28
Recycle only, ~36 hours	34
Influent resumed	35

Isobutane Phase

As shown in **Figure G-2**, Column effluent concentrations of isobutane, 1,1-DCE, *cis*-DCE, chloroform, TCE, and 1,4-dioxane dropped immediately after bioaugmentation with ENV493. *cis*-DCE concentrations were reduced the most relative to influent concentrations (approximately 70% reduced), followed by 1,4-D (~45% reduced), chloroform (~50% reduced), and TCE (~30% reduced), which generally followed expectations based on the batch tests and previous studies of CAH and 1,4-D transformation by isobutane utilizers (Rolston et al., 2022). The lowest concentration of 1,1-DCE observed in the effluent appeared to be only a ~30% reduction from influent concentrations, but the GC-ECD response represented both 1,1-DCE and Freon-113. Based on ENV493's ability to rapidly transform 1,1-DCE in batch single compound and mixture studies, most of the remaining mass detected was likely Freon-113. As *cis*-DCE concentrations decreased, *cis*-DCE epoxide was detected in the effluent, which offered an additional line of evidence that the COC reduction was due to cometabolic transformation. Finally, effluent isobutane concentrations dropped to ~50% of influent concentrations, suggesting growth of additional ENV493 biomass.

However, two days after bioaugmentation, effluent concentrations of all COCs and isobutane began to increase. Effluent concentrations had rebounded close to influent concentrations 5-7 days after bioaugmentation, and *cis*-DCE epoxide was only minimally detected (**Figure G-2**). While it

was anticipated that continuous addition of 1,1-DCE would negatively impact ENV493 biomass, the amount of 1,1-DCE added to the column in these two days in addition to the 1,1-DCE already present in the pore water at the time of bioaugmentation would have inactivated only ~80% of the bioaugmentation inoculum, not considering the additional ENV493 biomass produced from the isobutane consumption observed after bioaugmentation. However, some 1,1-DCE could have also desorbed from the sediment, which would have inactivated more biomass. While the cometabolic transformation of other COCs could have also inactivated biomass, previously determined transformation capacities in the presence of primary substrate in combination with the relatively little observed COC transformation suggested biomass was likely not inactivated via transformation toxicity.

While the effluent DO concentration was 5.75 mg/L two days before bioaugmentation and 80 mg/L H2O2 had been continuously added since ENV493 bioaugmentation, DO had dropped to 2 mg/L in samples taken from the middle intermediate port (the true DO concentration may have been lower as measured concentrations may have been skewed high due to analytical technique) (**Figure G-2**). In order to increase DO in the column, the H2O2 concentration in the influent was increased from 80 to 250 mg/L (beginning 14 days after bioaugmentation). DO measured in the middle port was relatively slow to respond, but as it increased beyond 3 mg/L by day 18, an increase in *cis*-DCE epoxide accompanied decreased COC concentrations. By day 33, DO in the middle port had increased to ~9 mg/L, effluent isobutane concentrations were ~90% less than influent concentrations, and *cis*-DCE was below detection in the effluent. The lowest observed concentrations other COCs were 67 µg /L 1,4-D, 93 µg/L 1,1-DCE + Freon 113, 0.7 µg /L CF, and 56 µg /L TCE.

While effluent isobutane concentrations remained low (< 2 mg/L) in the following 20 days of continuous flow operation, effluent COC concentrations began to increase toward their respective influent concentrations. The first "recycle only" (no influent) period occurred 52 days after bioaugmentation (**Figure G-2**). While little additional COC transformation was observed, measured DO in the middle port dropped back to ~3 mg/L after the ~36 hours without H2O2 addition. This illustrated the substantial continued oxygen demand of the system.

Separation of 1,1-DCE and Freon 113 via GC-MS analysis was achieved 56 days after bioaugmentation. The results demonstrated greater reduction of 1,1-DCE than Freon 113 relative to influent responses. Given that effluent samples showed very little contribution from 1,1-DCE peak area at the time separate analysis began and that 1,1-DCE has been readily transformed by ENV493 and other isobutane-utilizers, it is likely that 1,1-DCE concentrations in the column were near zero for the first 55 days of continuous operation.

In addition to significant reduction of 1,1-DCE, the most effective transformation appeared to be of *cis*-DCE, of which 50-75% was transformed over days 40-75 (**Figure G-2**). The formation of *cis*-DCE epoxide in the column effluent was also sustained over this period, with peak areas decreasing as *cis*-DCE concentrations steadily increased, both of which suggested a reduction in biological activity. Approximately 50% of chloroform was transformed between days 40 and 72. After reaching its lowest concentration on day 33, effluent concentrations of 1,4-D steadily increased and reached the influent concentration 78 days after bioaugmentation (**Figures G-2** and **G-3**). While the profile of effluent concentrations suggested that TCE transformation appeared to occur both after bioaugmentation and after the influent H2O2 concentration was increased, influent

concentrations of TCE were approximately equal to or greater than effluent concentrations for most of the isobutane phase, suggesting that the sediments acted as a source via desorption. This was also observed for PCE, which had greater effluent than influent concentrations for the entirety of the column experiment. Additional attempts to achieve greater degrees of cometabolic transformation through perturbation of column operation were unsuccessful.

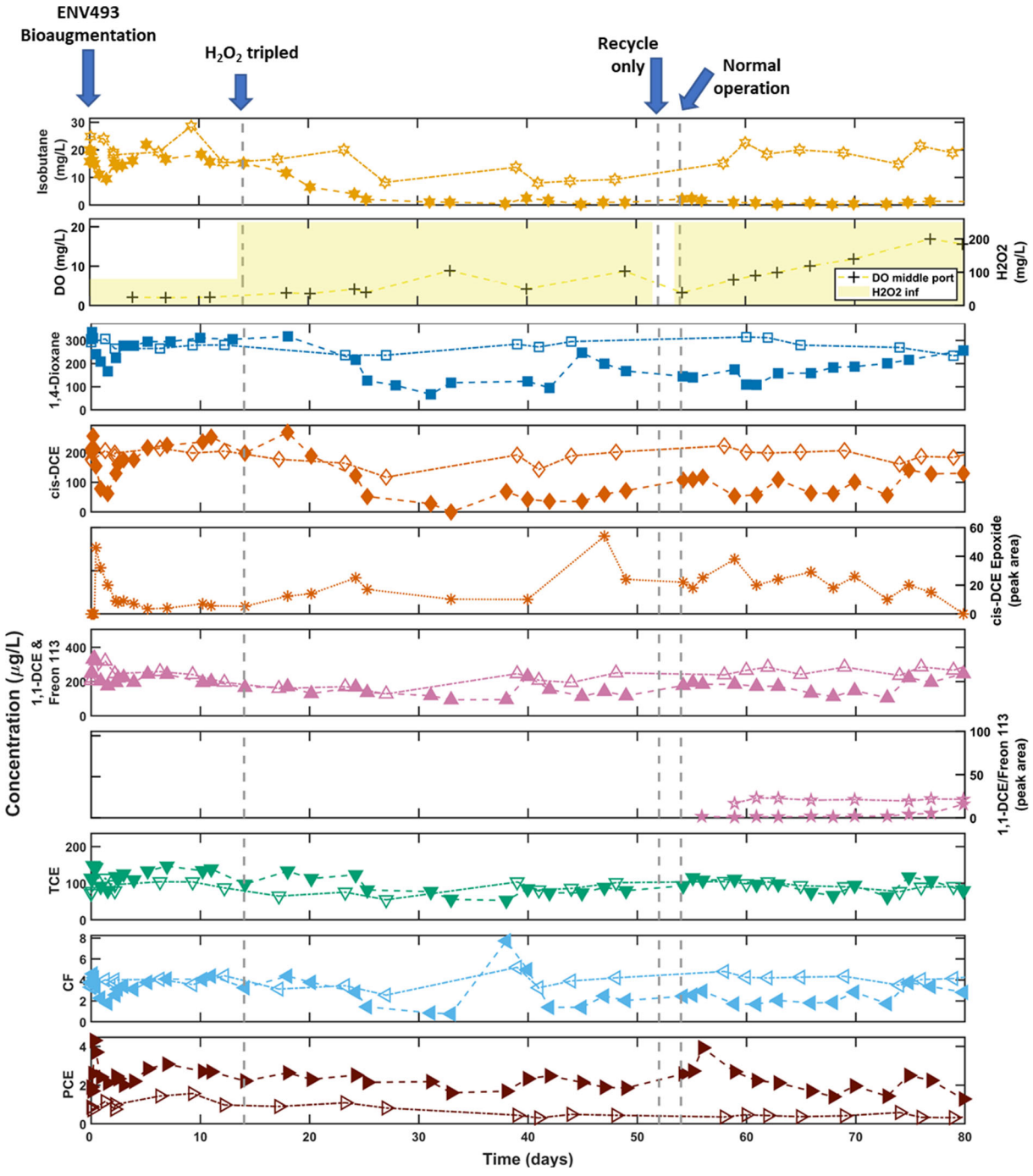


Figure G-2. Influent (open markers) and effluent (filled markers) concentrations in the continuous flow column 0-80 days after bioaugmentation with isobutane-grown ENV493.

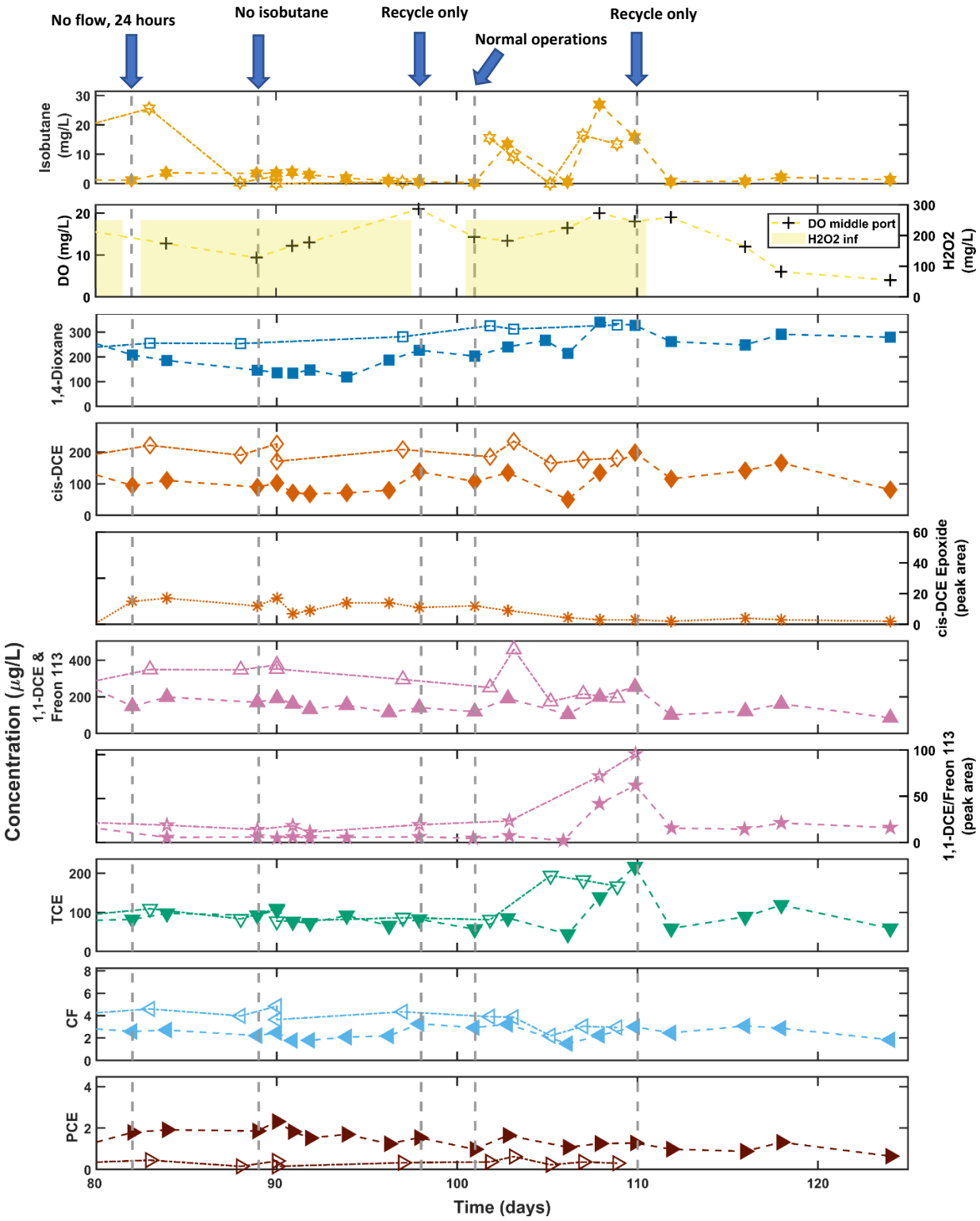


Figure G-3. Influent (open markers) and effluent (filled markers) concentrations in the continuous flow column 80-125 days after bioaugmentation with isobutane-grown ENV493.

Methane phase

As shown in **Figure G-4**, the effluent concentration of *cis*-DCE decreased immediately after bioaugmentation with ENV494M (a mixed methanotrophic culture isolated from the site), which was accompanied by an increase in its epoxide. However, the effluent concentration of *cis*-DCE never fell below 50% of the influent concentration and began to increase two days after bioaugmentation (and epoxide production decreased). 1,1-DCE also decreased approximately 50% relative to the influent, but little transformation of other CAHs was observed. A sample from the intermediate port three days after bioaugmentation showed a dissolved oxygen concentration of 5.5 mg/L, indicating oxygen limitation was not likely the cause of slowed transformation. Groundwater microcosms bioaugmented with the same batch of ENV494M culture illustrated the activity of the bioaugmentation culture was consistent with previous batches of ENV494M.

ENV494M was chosen for this study because of its previously demonstrated ability to rapidly transform TCE in batch microcosms after transformation of the lesser chlorinated compounds was achieved by isobutane-grown ENV493 (Laurance, 2019). In order to specifically investigate TCE transformation in the column in the absence of potential 1,1-DCE toxicity, the VOC mixture was sparged from the site groundwater and only TCE and *cis*-DCE were added back. However, greater extents of transformation of neither *cis*-DCE or TCE were observed. After the addition of sparged groundwater began, effluent concentrations of 1,1-DCE and CF were greater than influent concentrations (**Figure G-4**), suggesting that CAHs were slowly desorbing from the sediment. Just as in the isobutane phase, concentrations of PCE were greater in the effluent than in the influent throughout the methane phase of the column, also suggesting continued desorption of PCE from the sediment.

Isobutane was not included in the influent at any time during the methane phase, however background concentrations remaining from the isobutane phase were slow to desorb from the column, similar to observations of other hydrocarbons (toluene and xylene) in a previous column study (Borden and Kao, 1992). At the time methane was first added to the column, ~2 mg/L isobutane was detected in effluent samples (**Figure G-4**). By the time of the second bioaugmentation with ENV494M, isobutane concentrations had dropped one order of magnitude to less than 0.2 mg/L. Bioaugmentation with ENV494M may have resulted in decreased isobutane concentrations, but it is not conclusive form available data.

As shown in **Figure G-4**, methane concentrations were below detection during almost the entirety of the methane phase after bioaugmentation. Methane was only detected in the effluent or at the midpoint when oxygen concentrations dropped, and immediately disappeared when hydrogen peroxide was included in the influent again. This suggests there was an active community of methanotrophs in the column. Two pauses in H2O2 addition resulted in increased methane concentration in the column effluent: first in the beginning of the methane phase (SI) and again 18 days after the second bioaugmentation with ENV494M, as measured in both the middle port and in the effluent. In the second instance, the effluent concentration increased to 0.77 mg/L in the middle port and 0.47 mg/L in the effluent. Despite the evidence of methanotrophic activity, three "recycle only" periods showed little evidence of increased cometabolic activity. A possible reason for methane consumption without cometabolic COC is the change in enzyme expression from sMMO to pMMO.

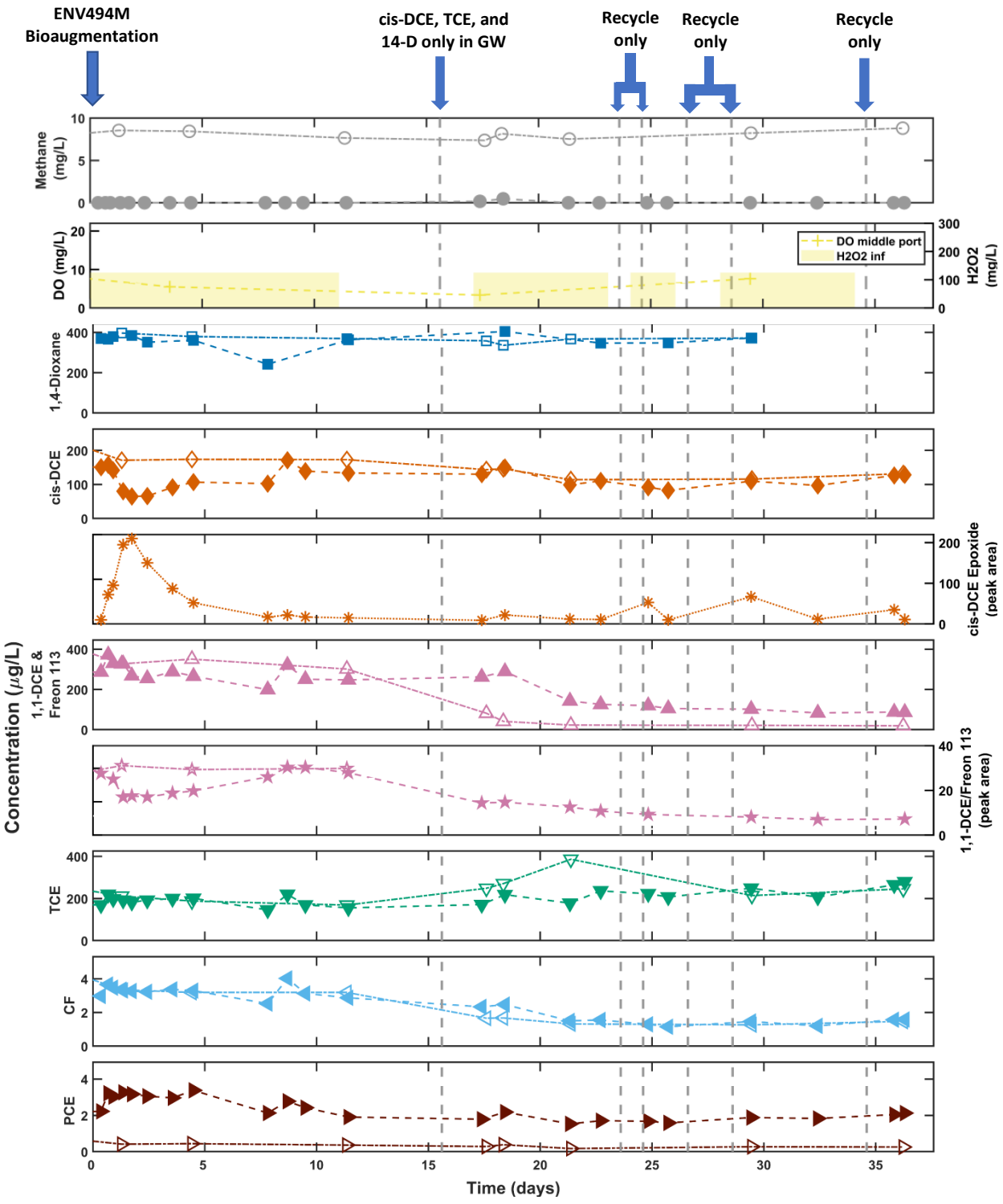


Figure G-4. Influent (open markers) and effluent (closed markers) concentrations in the continuous flow column after bioaugmentation with ENV494M (grown on methane with copper limitation to induce the expression of soluble methane monooxygenase)

Modeling the column (isobutane phase) as a CSTR

The simple modeling analysis was performed by treating the column as a continuous stirred-tank reactor (CSTR) because the recycle flowrate was 5x greater than the influent flowrate. As shown in **Figure G-5**, this resulted in relatively good agreement to experimental observations and illustrated the impact of continuous addition of the COC mixture. Rapid isobutane consumption and COC transformation proceeded immediately after bioaugmentation due to the relatively large mass of the ENV493 inoculum. However, the cumulative and irreversible influence of the transformation capacities of the four COCs included in the model eventually resulted in biomass inactivation and contaminant breakthrough even though 15 mg/L isobutane was continuously added mostly consumed, as observed experimentally. The removal of any individual COC increased the time before breakthrough of the mixture was simulated. COCs broke through according to their transformation rates. As the COCs with the least and greatest transformation rates, TCE and 1,1-DCE were the first and last COCs to breakthrough, respectively, in the simulation.

The simulation included competitive inhibition of all COC transformation by the presence of isobutane with KI equal to KS, isobutane. Removing isobutane inhibition did not result in greater extents of simulated COC transformation. The endogenous decay coefficient, b, was equal to 0.03 day-1 in this simulation, though the results were also sensitive to this value as the simulation was run for 125 days.

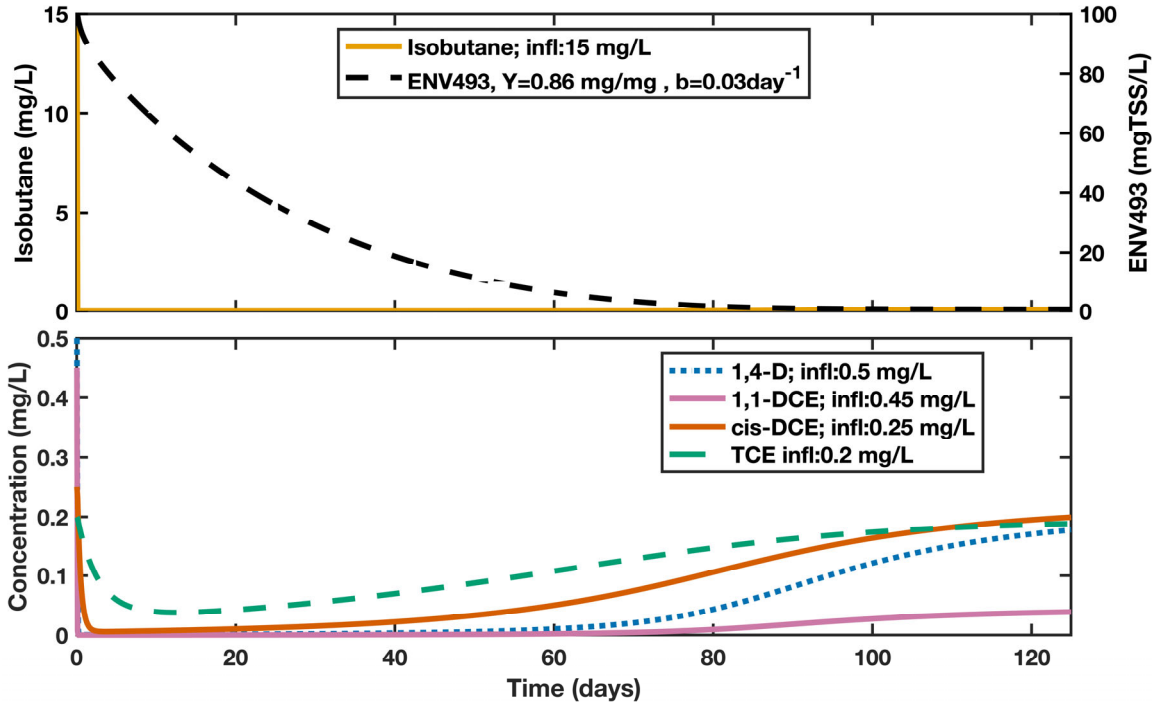


Figure G-5. Simulated isobutane consumption and contaminant transformation in the column after bioaugmentation with isobutane-grown ENV493

APPENDIX H
NCSU MOLECULAR ANALYSIS SUMMARY

NCSU Molecular Analyses Summary

Aim: The objective of the molecular analyses used in this project was to determine whether the *in situ* biostimulation of indigenous or augmented microorganisms with hydrocarbon gases and/or alcohols led to detectable changes in the abundance and distribution of these microorganisms in situ. The molecular analyses were directed at biomarkers for monooxygenase enzymes, including some enzymes capable of the cometabolic biotransformation of 1,4-dioxane and its associated chlorinated co-contaminants.

Approach: The project made use of two distinct but complementary molecular approaches. The first approach involved a conventional analyses of groundwater samples using quantitative polymerase chain reaction (qPCR) amplification of genes that encode structurally and catalytically distinct bacterial monooxygenases. These analyses targeted genes encoding the major hydroxylase subunits of soluble methane monooxygenase (SMMO), particulate methane monooxygenase (PMMO), ammonia monooxygenase (AMO), propane monooxygenase (PPO) and short chain alkane monooxygenase (SCAM). The two MMO enzymes are expressed by aerobic methane-oxidizing bacteria (methanotrophs). Although these microorganisms are not known to biodegrade 1,4-dioxane at environmentally relevant concentrations (<100 µg/L), they are widely distributed in ground water systems and can biodegrade some chlorinated solvents frequently detected in 1,4-dioxane-contaminated ground water. Ammonia monooxygenase is structurally closely related to pMMO and can catalyzing the cometabolic degradation of a number of chlorinated solvents. This enzyme is found in aerobic ammonia-oxidizing bacteria and was included in the analysis suite as the nutrients added to support the *in situ* growth and activity of indigenous and augmented microorganisms contained ammonia. Propane monooxygenase (PPO) and short chain alkane monooxygenase (SCAM) are two soluble diiron monooxygenases (SDIMOs). While PPO is widely distributed among gaseous alkane-oxidizing bacteria, there is no current evidence to suggest that this enzyme can oxidize 1,4-dioxane and its other common co-co-contaminants. In contrast, SCAM, which is sometimes misleadingly also described as a propane monooxygenase, is structurally and catalytically distinct from PPO and can oxidize a wide range of ground water contaminants, including 1,4-dioxane. Notably, some strains of alkane-oxidizing bacteria possess genes encoding both PPO and SCAM although current evidence suggests PPO is only strongly expressed in propane-grown cells whereas SCAM expression occurs consistently during growth of these strains on all gaseous alkanes.

qPCR is a well-established method that quantifies target gene abundance and can therefore provide an indirect but reasonable estimate of the abundance of microorganisms that harbor the target gene in their genomes. However, this technique has an important limitation and caveat in that gene abundance itself does not necessarily equate with gene expression (transcription) and hence the subsequent production of catalytically active enzymes (translation). In the context of the present application to biodegradation processes, the qPCR detection of genes that are not expressed can then lead to the false conclusion that the encoded enzymes participate in observed biodegradation activity.

The second molecular approach used in this study aimed to address the potential discrepancy between gene abundance and expression by focusing on the detection of catalytically active monooxygenases using activity-based labeling (ABL). This less well-established method involves the initial "probing" of groundwater samples with a terminal diyne. This probe is added to untreated ground water samples and diffuses into intact cells in the sample. Importantly, the probe remains unreactive unless it catalytically activated by a functioning monooxygenase enzyme. Once activated, the probe covalently attaches to the enzyme through one of the two terminal alkyne groups (**Figure H-1**). This reaction irreversibly inactivates the enzyme and generates an adduct that contains the inactivated enzyme and the probe residue which retains an unreacted terminal alkyne group. In subsequent steps using extracts of total cells harvested from the water sample, the unreacted alkyne group is rapidly and specifically reacted with an azide-containing reporter group using an *in vitro* copper-catalyzed alkyne/azide cycloaddition (CuAAC) reaction. In the version of ABL used in this present study, the CuAAC reaction covalently attaches a fluor to the enzyme:probe adduct which can then detected by first separating microbial protein using SDS-PAGE and then scanning the near infra-red fluorescence of the separated proteins within the gel (Fig. 1). Because the initial covalent attachment of the probe to the target monooxygenases requires catalytically active enzyme, this approach detects gene/enzyme expression rather than gene abundance. Furthermore, because SDS-PAGE separates proteins based on molecular mass, the migration of the fluorescently labeled proteins in the gel can provide a tentative identification of the catalytically active monooxygenases within a sample. Further refinements of this ABL approach that were not explored in this present study are that the labeled protein can be unequivocally identified through mass spectrometry-based amino acid sequencing and the abundance of individual monooxygenase proteins can be quantified by comparing sample fluorescence levels with known fluorescent standards.

Methods: The qPCR analyses used in this study were performed by Microbial Insights Inc. on standard 1 L water samples. The analyses were part of their Census product range and targeted (SMMO), particulate methane monooxygenase (PMMO), ammonia monooxygenase (AMO), propane monooxygenase (PPO) and short chain alkane monooxygenase (SCAM).

The ABL method used in this study was based on the approach described by Bennett *et al*., 2016. In each sampling event, two 1 L water samples were obtained from each site monitoring well in plastic containers. The water in each container was immediately treated by adding 1 mL of 0.1 M 17OD in DMSO (100 µM final concentration). After shaking the sample bottles by hand to ensure mixing, they were subsequently stored at 4 ° C and then transported to NCSU for analysis. On Receipt, each 1 L sample was centrifuged (10,000 x g for 10 min) and the resulting pellet was resuspended in phosphate buffer (200 µL) and disrupted by two cycles of bead beating (6.0 m/s, 45 sec). The resulting protein extract was concentrated using a 10 kDa cut-off protein concentrator tube. The volume of the residual protein suspension obtained after concentration was adjusted to a final volume of 50 µl with water before being added to a standard CuAAC reaction mixture which contained AlexaFluor 647 azide (8 µM) (1 µL of 0.6 mM stock solution in DMSO), $CuSO_4$ (2 mM) (1.5 µL 0.1 M aqueous solution) and sodium ascorbate (10 mM) (7.5 µL of 0.1 M aqueous solution) to give a final reaction volume of 75 µL. The tubes were briefly vortexed to mix the reactants and were then incubated at room temperature (23 ° C) in the dark for 1 h.

The reactions were then quenched by adding 3-butyn-1-ol (13 mM) (1 µL of a 1M aqueous solution).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE): SDS-PAGE analyses were conducted using 10% polyacrylamide gels (1.5 mm x10 cm x 10 cm) and a Laemmli discontinuous buffer system. Protein samples (≤25 µg total protein) were prepared for SDS-PAGE analysis by adding 6X sample buffer (375mM Tris-HCl, 9% SDS, 50% glycerol, 0.05% bromophenol blue, and 9% β-mercaptoethanol). The samples were heated at 90 ^{o}C for 5 minutes, and then centrifuged (12,000 rpm for 3 min). Solubilized protein samples were then loaded onto the gels and electrophoresed at room temperature for 30 min at a fixed current of 25-35 mA. To visualize fluorescently labeled polypeptides, the unfixed gel was immediately scanned with an excitation wavelength of 650 nm and a detection wavelength of 668 nm using an Odyssey 9120 IR scanner (LI-COR Biosciences, Lincoln, NE). The images were obtained and analyzed by Odyssey imaging system application software (Version 3.0). An infrared NIR marker protein ladder (Thermo Scientific, Waltham, MA) was used to estimate the mass of fluorescently labeled polypeptides.

Results:

qPCR:

ABL: The results of the ABL analyses for 5 sample rounds are shown in **Figures H-2** through **H-5**. In each case, these Figures show the detected NIR fluorescence of the unfixed gel obtained immediately after protein separation of the indicated samples by SDS-PAGE. These images show the fluorescence associated with fluorescent marker proteins as well as the protein-associated fluorescence for each well sampled during the same sampling event. In each case, the Figures also show an image of the same SDS-PAGE gel following destaining after fixation with Coomassie blue protein stain. These images show protein staining pattern for the fluorescent marker proteins and for each well sampled during the same sampling event.

No protein-associated fluorescence or protein staining material was detected for samples obtained during sampling rounds #1 and #2. A similar trend continued with the later sampling round except detectable fluorescence was consistently obtained for samples from well PMW4D in sampling rounds #3, #4 and #5. In all cases, the fluorescence detected in these samples was highly diffuse and appeared as a streak throughout the entire sample lane. The reason for this streaking is not clear at this time but is potentially associated with residual bacteria DNA within the extracted protein samples. Irrespective of the underlying cause of this streaking, the NIR fluorescence observed for samples taken from this monitoring well (PMW4D) were also associated with detected protein staining in the materials obtained during sampling rounds #3 (strong), #4 (faint), and #5 (strong). The only other sample in which streaking fluorescence was detected was for well PMW4S during sampling round #4. Like the result for PMW4D in this sampling round, this fluorescence was also associated with low levels of detected protein staining.

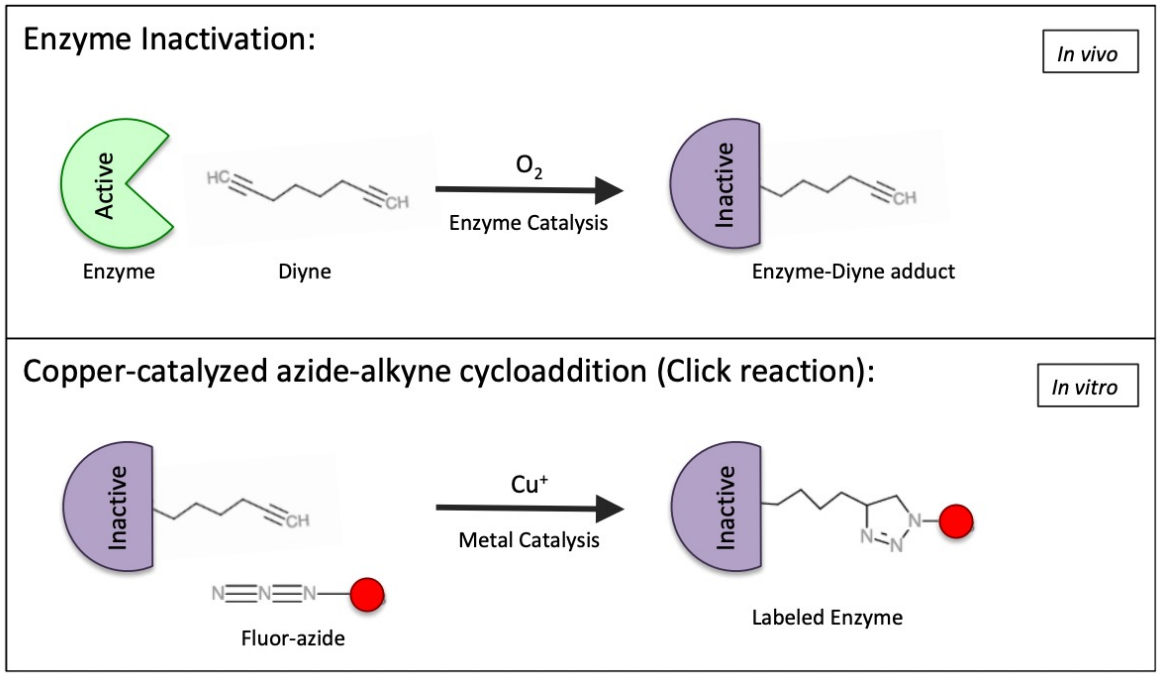
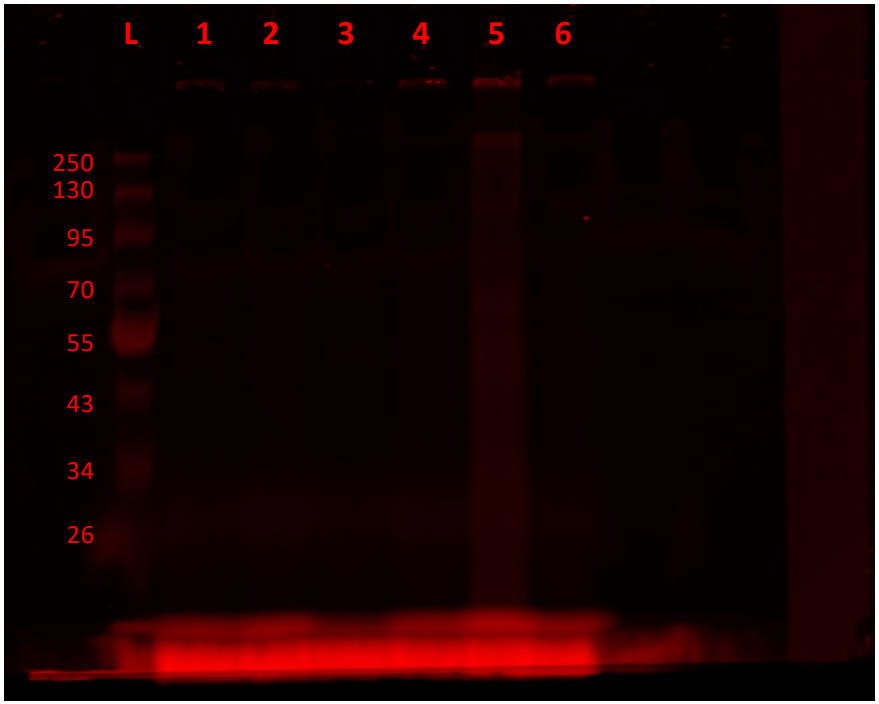
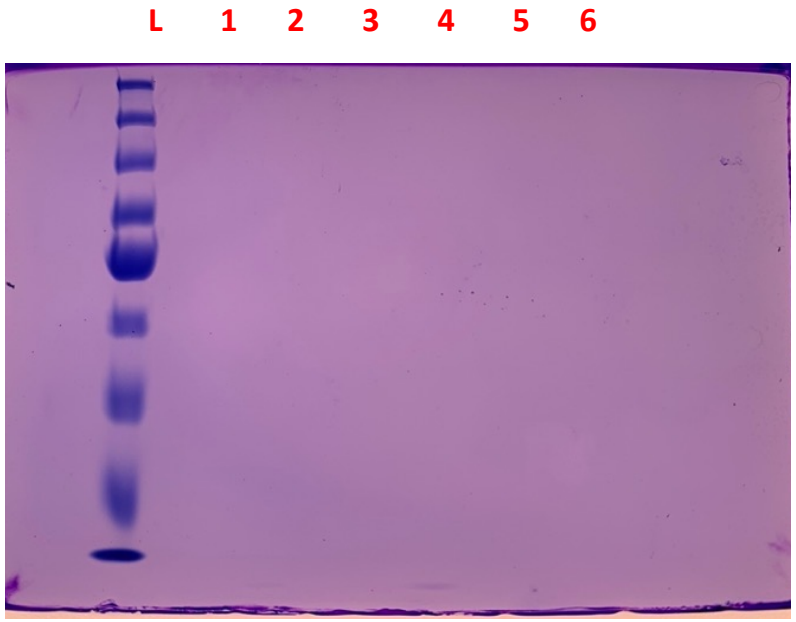


Figure H-1: Key steps in activity-based labeling of monooxygenaeses

Sample Round #1

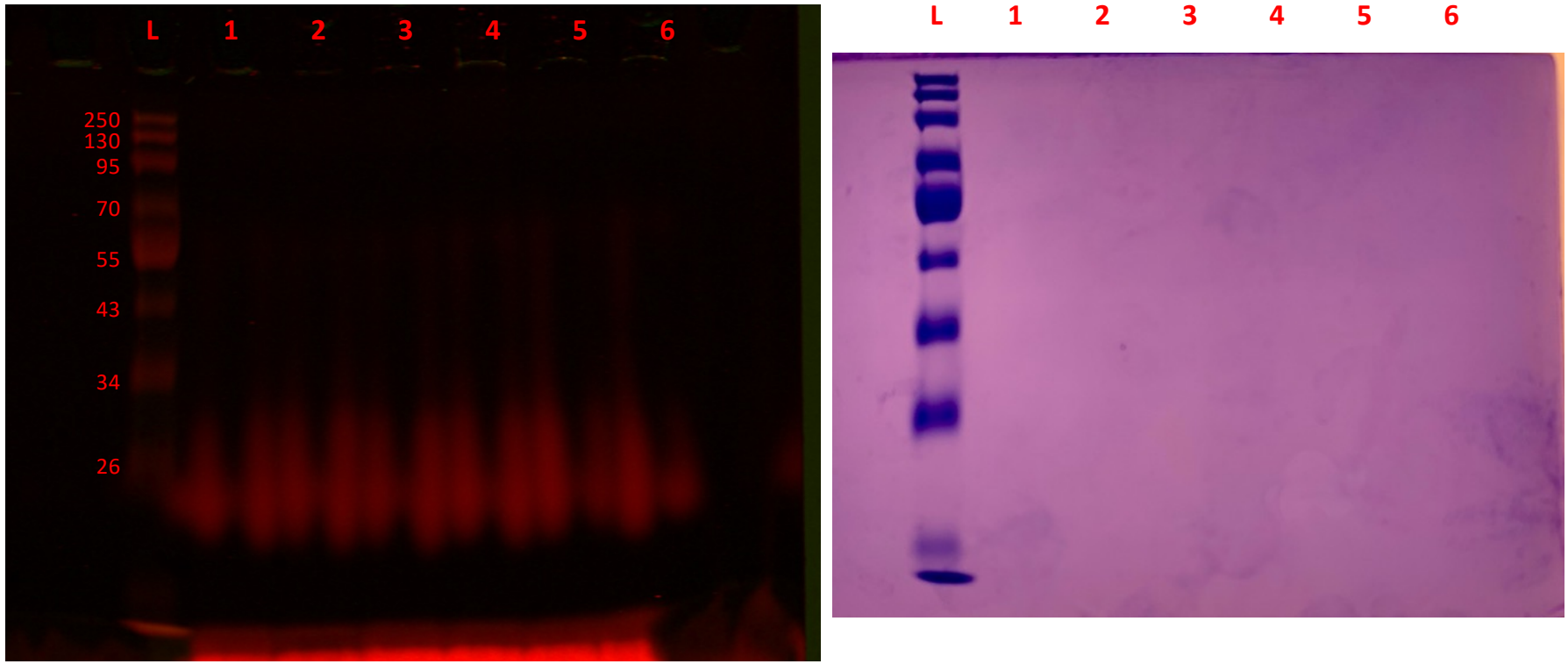




SDS PAGE Gel 12% of Water Samples 1.
L: Ladder, 1: PMW3D, 2: PMW3S, 3: PMW4D, 4: SMW08A, 5:PMW4S, 6: DMW1

Figure H-2: Resstults of ABL analysis for sample round #1

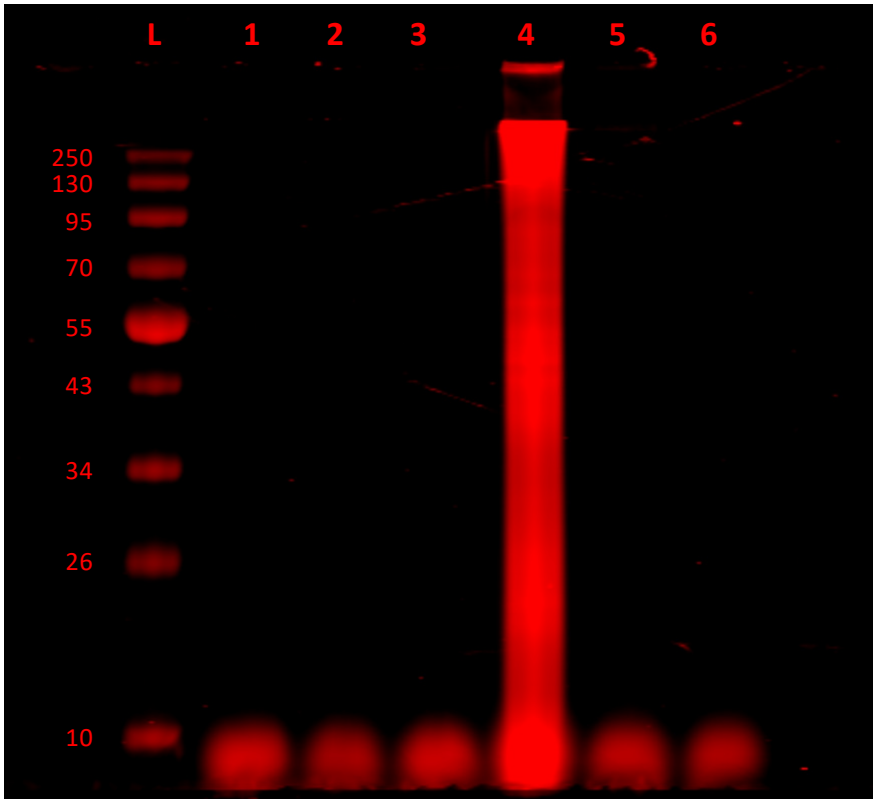
Sample Round #2

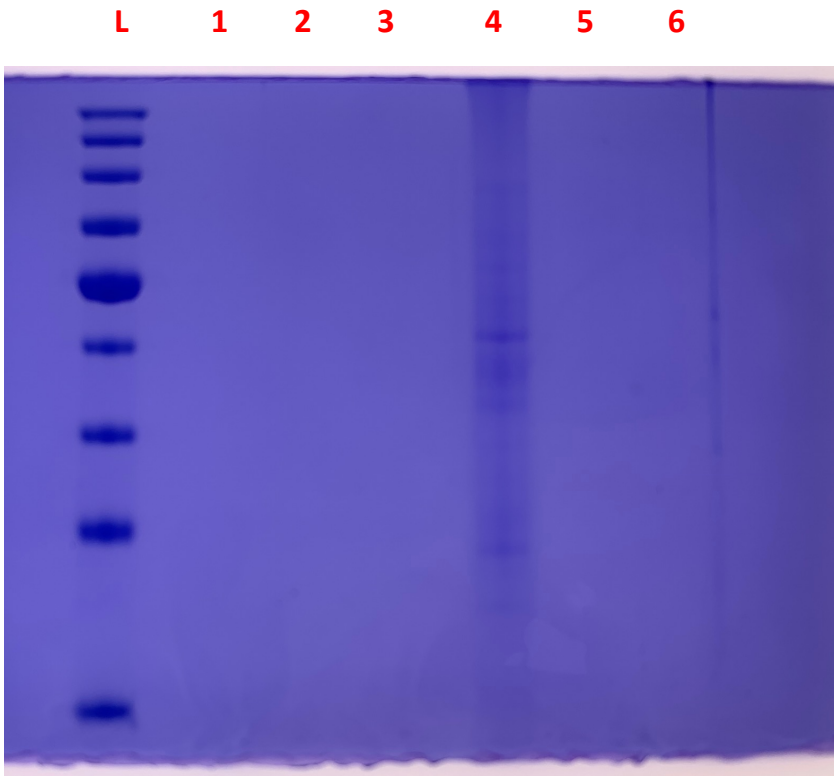


SDS PAGE Gel 12% of Water Samples 2.
L: Ladder, 1: PMW3S, 2: SMW08A, 3: PMWI, 4: PMW4D, 5: PMW3D, 6:PMW4S

Figure H-3: Resstults of ABL analysis for sample round #2

Sample Round #3

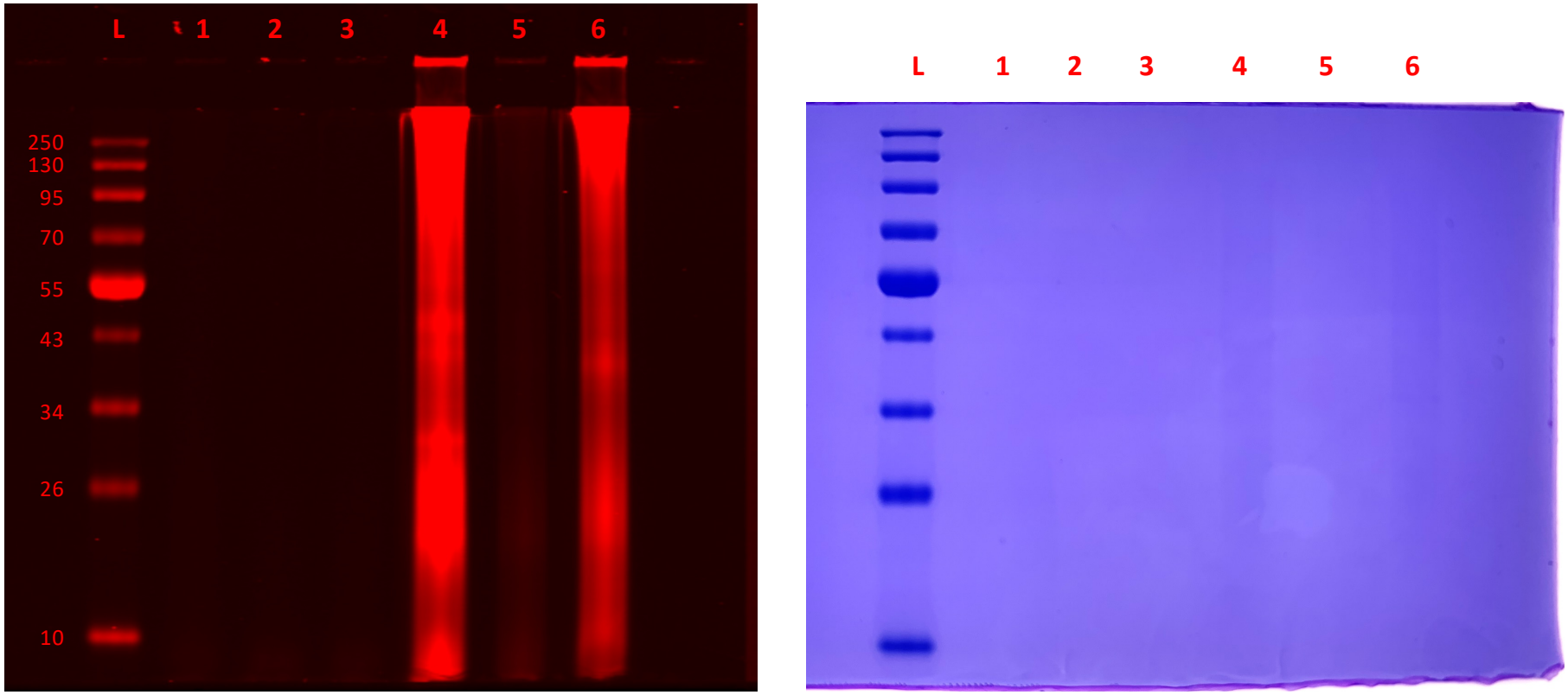




SDS PAGE Gel 12% of Water Samples 5.
L: Ladder, 1: PMW3S, 2:SMW08A, 3: PMWI, 4: **PMW4D**, 5: PMW3D, 6:PMW4S

Figure H-4: Resstults of ABL analysis for sample round #3

Sample Round #4



SDS PAGE Gel 12% of Water Samples 4.
L: Ladder, 1: PMW3S, 2:SMW08A, 3: PMWI, 4: **PMW4D**, 5: PMW3D, 6:**PMW4S**

Figure H-5: Resstults of ABL analysis for sample round #4