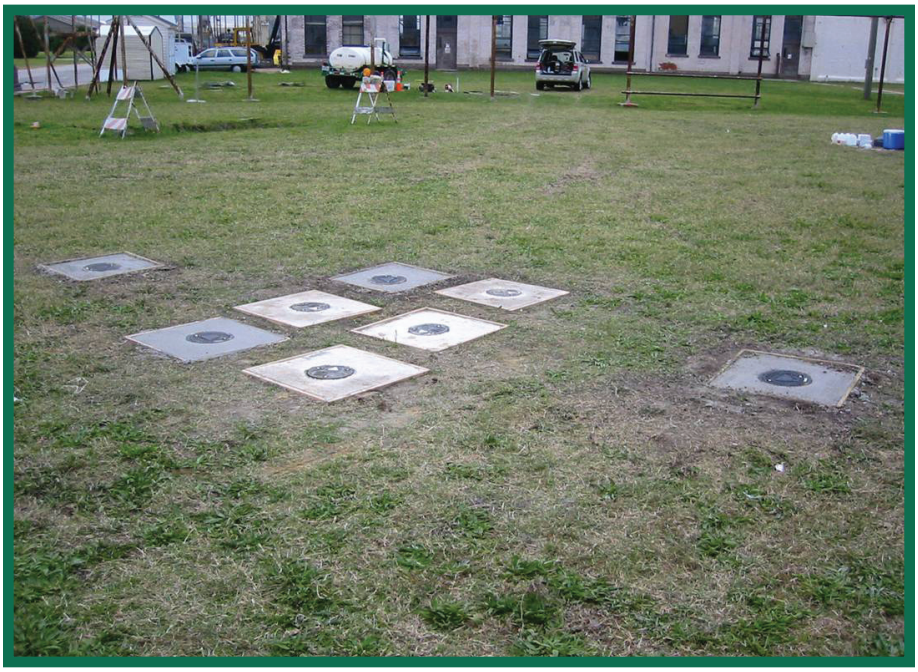
ESTCP
Cost and Performance Report

(ER-0516)



Enhancing Natural Attenuation through Bioaugmentation with Aerobic Bacteria that Degrade Cis-1,2-Dichloroethene

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ACRONYMS AND ABBREVIATIONS

1,2-DCA	1,2-dichloroethane
AFB	Air Force Base
AFCEE	Air Force Center for Engineering and the Environment
bgs	below ground surface
cDCE	cis-1,2-dichloroethene
cfu	colony forming units
CMO	cyclohexanone monooxygenase
DCA	1,2-dichloroethane
DNA	deoxyribonucleic acid
DO	dissolved oxygen
DoD	Department of Defense
EISB	enhanced in situ bioremediation
ESTCP	Environmental Security Technology Certification Program
GIT	Georgia Institute of Technology
ISO	isocitrate lyase
KH_2PO_4	potassium monobasic orthophosphate
K_2HPO_4	potassium dibasic orthophosphate
MCL	maximum contaminant level
MNA	monitored natural attenuation
MSM	minimal salts medium
NPV	net present value
O.D.	optical density
O&M	operation and maintenance
ORP	oxidation-reduction potential
P&T	pump and treat
PCE	tetrachloroethene
PTA	pilot test area
PVC	polyvinyl chloride
qPCR	quantitative polymerase chain reaction
RI	remedial investigation
rRNA	ribosomal ribonucleic acid

ACRONYMS AND ABBREVIATIONS (continued)

SERDP	Strategic Environmental Research and Development Program
SJCA	St. Julien's Creek Annex
SRS	Savannah River Site
TCE	trichloroethene
tDCE	trans-1,2-dichloroethene
UIC	underground injection control
UNI	universal
USEPA	U.S. Environmental Protection Agency
UST	underground storage tank
VC	vinyl chloride
VOA	volatile organic analysis
VOC	volatile organic compound

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Technical material contained in this report has been approved for public release.

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1.0 EXECUTIVE SUMMARY

1.1 BACKGROUND

Monitored natural attenuation (MNA) and enhanced in situ bioremediation (EISB) remedies hold the promise of reducing the costs associated with the cleanup of Department of Defense (DoD) sites impacted by chlorinated solvents. However, there are many DoD sites where tetrachloroethene (PCE) and trichloroethene (TCE) are undergoing only partial dechlorination to cis-1,2-dichloroethene (cDCE), even when sufficient electron donor is present, either because of the absence of required bacteria (*Dehalococcoides*) or aerobic conditions.

Under sponsorship from the Strategic Environmental Research and Development Program (SERDP) (Project ER-1168), a novel aerobic bacterium (*Polaromonas* sp. strain JS666) that uses cDCE as a sole carbon and energy source was isolated and characterized (Coleman et al., 2002a,b). Since it requires no exotic growth factors, JS666 is a promising bioaugmentation culture for aerobic sites where cDCE is recalcitrant. The microorganism will grow and thrive where oxygen and cDCE are colocated, and JS666 also degrades 1,2-dichloroethane (DCA) and cometabolizes TCE and vinyl chloride (VC). Ideal groundwater conditions for JS666 include dissolved oxygen (DO) levels between 0.01 milligrams per liter (mg/L) and 8 mg/L; low ionic strength (conductivity <15 millisiemens per centimeter [mS/cm]); a pH of 6.5 to 8; and relatively low concentrations of TCE, 1,2-DCA, and VC (<500 micrograms per liter [µg/L]).

1.2 OBJECTIVES OF THE DEMONSTRATION

The goal of this first field demonstration was to evaluate the effectiveness of JS666 in biodegrading cDCE. The demonstration was conducted at Site 21, St. Julien's Creek Annex (SJCA) in Chesapeake, VA. This site had several relatively well-characterized groundwater plumes of chlorinated volatile organic compounds (VOC), primarily cDCE, TCE, and VC; appropriate site conditions; and a suitable on-site support network. In the vicinity of the pilot test area (PTA), groundwater flow is towards the west. Shallow groundwater typically ranges from 2 to 7 ft below ground surface (bgs). Estimates of the hydraulic gradient and groundwater velocity for the Columbia aquifer are 0.004-0.01 ft/ft and 72 ft/year, respectively (CH2M HILL, 2008). Preliminary baseline sampling indicated that the groundwater pH was in the 6 to 6.3 range and that buffering would be required.

1.3 DEMONSTRATION RESULTS

The principal results of the project include:

- Greater cDCE reductions were observed in many of the wells in the bioaugmented plots compared to the control plots, as evidenced by analysis of VOCs and carbon stable isotopes. However, cDCE biodegradation in the bioaugmentated plots was likely limited by lack of oxygen and inhibited by high levels of TCE in some areas.

- Reductions in average cDCE concentrations of up to 44% were observed in the bioaugmentation plot receiving oxygen and buffer, and up to 25% in the bioaugmentation plot receiving only buffer.
- qPCR and microcosm results demonstrated the spread, in-situ survival and sustained activity of the JS666 organisms in the bioaugmented plots. However, it was difficult to tell whether growth was occurring because bacterial densities did not consistently increase over time.
- Addition of the aerobic culture via injection wells was straightforward, Aeration of the test plots using the Waterloo Emitter was easy but not effective in distributing oxygen beyond the injection wells. Injection of buffer was also easy but was time-consuming and required reapplication due to the soluble nature of the buffer employed.
- The cost assessment showed a 47% cost savings compared to pump and treat (P&T), assuming no aeration or buffering is required and sufficient oxygen is present in the groundwater naturally.

1.4 IMPLEMENTATION ISSUES

At full-scale, an underground injection control (UIC) permit may be required for the injection of bacteria, buffer amendments (if needed), and extraction and re-injection of contaminated groundwater (if needed). Buffer addition will be required if the pH of the groundwater is low (pH<6.5), and the amendment process may be time-consuming and require repeating if a soluble buffer is used. Aeration may also be required if the ambient dissolved oxygen is not sufficient to support biodegradation. However, JS666 does not tolerate oxygen concentrations above 10 mg/L; thus, care must be taken not to achieve concentrations above this level. JS666 can degrade cDCE metabolically and TCE and VC cometabolically. As the concentration of TCE increases, the rate of cDCE degradation decreases due to competitive inhibition. Therefore, JS666 will perform better when there are lower concentrations of TCE (<500 µg/L) in groundwater. To mitigate the effects of competitive inhibition, due to high TCE concentrations to some extent, higher densities of JS666 can be employed.

2.0 INTRODUCTION

2.1 BACKGROUND

MNA and EISB remedies hold the promise of reducing the costs associated with cleanup of DoD sites impacted by chlorinated solvents. However, there are many DoD sites where PCE and TCE are undergoing only partial dechlorination to cDCE, even when sufficient electron donor is present or added. *Dehalobacter*, *Desulfitobacterium*, *Dehalospirillum*, *Desulfomonile*, *Desulfuromonas*, and *Enterobacter* are found widely in the environment, and can dechlorinate PCE and TCE to cDCE, but are incapable of further dechlorinating cDCE to VC or ethene (Geosyntec, 2005). As a result, there are a significant number of plumes at DoD and related sites where PCE and TCE have been dechlorinated to cDCE, but where the cDCE persists and migrates uncontrolled in groundwater rather than undergoing further dechlorination to ethene (the desired end product in MNA and ESIB remedies).

Dehalococcoides are the only known group of microorganisms that can dechlorinate cDCE via VC to ethene. While *Dehalococcoides* are present at many sites, they are not ubiquitous in the environment (Hendrickson et al., 2002). Furthermore, anaerobic bioremediation/ bioaugmentation may not be the best remediation strategy at sites with large cDCE plumes in aerobic aquifers. Instead, aerobic biotreatment of the cDCE may be more cost-effective, provided that this process can be induced to occur over the target treatment area.

Until recently, aerobic biodegradation of cDCE was thought to occur cometabolically, requiring the addition of an appropriate primary substrate, such as methane, propane, or toluene, to stimulate the co-oxidation of cDCE, and these processes were generally determined to have limited feasibility for large-scale field application. However, research conducted under sponsorship from the SERDP Project ER-1168 has isolated and described a novel aerobic bacterium (*Polaromonas* sp. strain JS666) that uses cDCE as sole carbon and energy source (Coleman et al., 2002a,b). Since it requires no exotic growth factors, JS666 is a promising bioaugmentation culture for aerobic sites where cDCE is recalcitrant. In essence, this microorganism can be used to achieve MNA without any further intervention other than adding it to groundwater because the microorganism will grow and thrive where oxygen and cDCE are colocated.

2.2 OBJECTIVES OF THE DEMONSTRATION

The goal of this field demonstration was to evaluate the effectiveness and robustness of JS666 as a bioaugmentation culture to enhance the biodegradation of cDCE. No field demonstrations of this technology have been conducted to date. The demonstration described herein represents the first demonstration of the effectiveness of JS666 for degrading cDCE in the field.

The objectives of the field demonstration were to:

- Assess JS666's ability to degrade cDCE and other chlorinated ethenes/ethanes in-situ
- Evaluate the ability of JS666 to compete with indigenous microorganisms

- Evaluate the use of molecular markers to detect the spread of JS666 in groundwater
- Evaluate the effectiveness of isotopes to detect and quantify cDCE biodegradation
- Provide reliable technical data relevant to field-scale aerobic biotreatment using JS666, including documenting benefits of the technology in terms of expected reduction in the duration and cost of remediation of sites where cDCE persists in groundwater.

2.3 REGULATORY DRIVERS

The U.S. Environmental Protection Agency (USEPA) maximum contaminant level (MCL) for cDCE in drinking water is 70 µg/L, 5 µg/L for TCE, and 2 µg/L for VC. While several sites have observed successful dechlorination of PCE and/or TCE plumes to ethene, there are a significant number of DoD and related sites where PCE and/or TCE plumes have been dechlorinated to cDCE, but where the cDCE persists and migrates uncontrolled in groundwater rather than undergoing further dechlorination to ethene. Groundwater cDCE concentrations at these sites can be considerably higher than the USEPA MCL. The JS666 technology strives to reduce cDCE concentrations below the MCL.

3.0 TECHNOLOGY

The following sections provide an overview of the technology (Section 3.1), technology development (Section 3.2), and advantages and limitations of the technology (Section 3.3). A detailed description of these items is provided in the Final Report (Geosyntec, 2010).

3.1 TECHNOLOGY DESCRIPTION

Through research conducted under SERDP sponsorship (ER-1168), a novel aerobic bacterium (*Polaromonas* sp. strain JS666) was isolated that is able to use cDCE as the sole carbon and energy source under aerobic conditions. It converts cDCE to carbon dioxide and water without the addition of exotic co-factors (Coleman et al., 2002a,b). This organism was found in only one of 37 samples screened for ability to aerobically oxidize cDCE. Thus, while not necessarily unique, it appears to be relatively rare. Since it requires no exotic growth factors, JS666 is a promising bioaugmentation culture for aerobic sites where cDCE is recalcitrant. In essence, this microorganism can be used to achieve MNA without any further intervention other than adding it to groundwater because the microorganism will grow and thrive when oxygen and cDCE are colocated. Though cDCE and 1,2-dichloroethane (1,2-DCA) are the only known solvents (thus far) to serve as growth substrates for JS666, this microorganism can co-metabolize several other chloroethenes (TCE, trans-1,2-dichloroethene [tDCE], and VC) while growing on cDCE.

In the laboratory phase of study, the relative kinetics and mutual effects of binary mixtures of cDCE at ~2 mg/L in the presence of lesser concentrations (50 to 450 μg/L) of VC, TCE, or 1,2-DCA were investigated. Although the co-presence of VC, TCE, or 1,2-DCA reduced the maximum degradation rate of cDCE, the rate remained substantial and cDCE could be completely degraded, as could the co-substrates. Co-presence of VC or TCE caused cDCE degradation rates to be halved, but the effect was not proportional to concentrations of VC or TCE. On the other hand, degradation of the co-substrate was either improved (VC) or unaffected (TCE) by the presence of cDCE (Geosyntec, GIT, and Cornell University, 2008).

The patterns of 1,2-DCA degradation in the presence of cDCE were different than those observed with VC and TCE. Clearer signs of true competition were observed with cDCE degradation in the presence of 1,2-DCA. cDCE was modestly inhibited by 1,2-DCA in a roughly linear decline with increasing 1,2-DCA concentration to 0.6 mg/L, and 1,2-DCA degradation was markedly inhibited by the much higher 1.8 mg/L cDCE concentration. These results were consistent with the observation that JS666 can grow on 1,2-DCA, but not on VC or TCE (Geosyntec, GIT, and Cornell University, 2008).

During laboratory studies, no evidence was found to suggest that the ability to degrade cDCE can be transferred from JS666 to indigenous bacteria. Therefore, it is necessary to ensure that site conditions are suitable for the JS666 strain so that it can grow and thrive (Geosyntec, GIT, and Cornell University, 2008).

3.1.1 Expected Applications of the Technology

JS666 can be incorporated into passive, active, or semipassive bioremediation systems or it can be injected once into groundwater with appropriate conditions to facilitate natural attenuation (otherwise known as enhanced attenuation).

Ideal conditions for JS666 include:

- Groundwater DO levels as low as 0.01 mg/L and as high as 8 mg/L
- Groundwater with low ionic strength (conductivity <15 mS/cm)
- Groundwater pH of 6.5 to 8
- Relatively low concentrations of TCE, 1,2-DCA and VC (<500 µg/L) in groundwater.

3.2 TECHNOLOGY DEVELOPMENT

3.2.1 JS666 Growth

A variety of laboratory experiments were conducted to establish factors that allow optimal cell growth for production purposes. Results of these experiments indicated that the JS666 culture could be effectively grown for field application. In addition, cells stored or stockpiled over a short period of time rapidly recovered the ability to degrade cDCE (Geosyntec, GIT, and Cornell University, 2008).

A reactor system for growing 64-liter (L) batches of cells was designed and used to grow JS666 in the lab. Once grown to an ideal density, the cultures were harvested and the concentrated cells were either frozen at -80 °C or diluted with cold (4°C) minimal medium to a total volume of 18 L for transport to the site.

3.2.2 Microcosm Studies

Microcosms were constructed with subsurface materials from five sites: Savannah River Site (SRS), Hill Air Force Base (AFB), Robins AFB, Fort Lewis, and Aerojet. In neutral-pH-buffered microcosms constructed from all five site materials, high concentrations (~60 mg/L) of cDCE were completely degraded within 10 to 15 days when inoculated with JS666 culture at 4 x 105 cells per milliliter (mL). Without inoculation, no significant cDCE degradation was observed. Studies were also undertaken to determine effective inoculum density, using three levels of cell density. In microcosms constructed with SRS soil and minimal salts medium (MSM), and with a more realistic initial cDCE concentration (0.6 mg/L), complete degradation was observed in about 5 days at 4×10^5 cells/mL and 4×10^4 cells/mL, and in about 20 days at the 4×10^3 cells/mL inoculum level. Therefore, a minimum of 10^4 cells/mL was the suggested inoculum level for field application. All of the microcosm studies suggested that JS666 would survive and remain active in subsurface environments (Geosyntec, GIT, and Cornell University, 2008).

3.2.3 Molecular Probe Development

To track the distribution and growth of JS666 in the field, two deoxyribonucleic acid (DNA)-based probes were developed at Cornell University: (1) isocitrate lyase (ISO) (based on the isocitrate lyase gene of JS666) and (2) cyclohexanone monooxygenase (CMO) (based on the cyclohexanone monooxygenase gene of JS666). Additionally, a putative universal (UNI) probe was employed that was intended to target the 16S ribosomal ribonucleic acid (rRNA) gene of eubacteria (Bach et al., 2002). ISO and CMO were intended to be JS666-specific, while UNI was intended to capture most eubacteria and could thus serve as a "normalize" if necessary.

The ISO probe was used in microcosms constructed with soil and groundwater from five field sites. Preliminary results revealed a strong correlation between the presence of JS666 and degradation of cDCE, suggesting the probe would be a useful tool for tracking JS666 movement in subsurface environments (Geosyntec, GIT, and Cornell University, 2008). When early field results indicated that the ISO probe was not absolutely specific to JS666 (i.e., some positive results were occasionally observed in control wells), a second JS666-specific probe, CMO, was developed.

3.3 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

Groundwater remediation approaches for VOC-impacted sites have historically employed groundwater extraction and ex situ treatment (i.e., P&T). Unfortunately, these approaches have been largely ineffective in significantly improving groundwater quality, even after decades of continuous operation (National Research Council, 1994). As a result, remediation technologies such as MNA and EISB have received significant attention because they are less intrusive, more effective, and less costly.

The main advantages of aerobic biotreatment using JS666 over other treatment technologies include:

- Potential for lower overall costs than alternative technologies such as groundwater P&T that have high operation and maintenance (O&M) costs
- Potential for achieving cDCE biodegradation without any further intervention other than adding JS666 to groundwater (i.e., JS666 does not require exotic co-factors to survive)
- cDCE (and potentially other VOCs) will be destroyed rather than transferred to another medium.

The main limitations of aerobic biotreatment using JS666 are:

- The presence of co-contaminants (e.g., TCE and VC) at concentrations that may be inhibitory to bioremediation by the JS666 culture
- Aerobic groundwater with a near-neutral pH is required for optimal growth and activity of the JS666 culture

- Low pH groundwater requires the addition of buffer, which can be time-consuming.

4.0 PERFORMANCE OBJECTIVES

The performance objectives are provided in Table 1. Each objective is discussed briefly in the following sections. A detailed discussion of each objective is provided in the Final Report (Geosyntec, 2010).

Table 1. Performance objectives.

Type of Performance Objective	Primary Performance Criteria	Expected Performance	Actual Performance Objective Met? (to be completed following demonstration)
Qualitative	1) Reduce cDCE concentrations	Greater reduction of cDCE concentrations in bioaugmented plots than in control plots	Yes, in some wells
	2) Spread and growth of JS666	Growth and spatial distribution of JS666 away from injection wells. Higher numbers of JS666 in bioaugmented plots than in control plots.	Yes
	3) Growth and degradation rates higher where oxygen levels are higher	Bioaugmentation plot with oxygen shows higher activity and higher numbers of JS666	Degradation rates are higher; cannot distinguish whether growth rates are higher
	4) Ease of use	Technology is easy to implement	Yes if only bioaugmentation and aeration; buffering is more time-consuming
Qualitative	1) Reduce cDCE concentrations	>75% reduction of cDCE concentrations in bioaugmented plots	No
	2) Greater reduction of cDCE in bioaugmented plots compared to control plot	Greater then 2x reduction of cDCE in bioaugmented plots compared to control plots	No
	3) Lower costs compared to P&T	Average cost savings of 30-50% over P&T	Yes, if not buffer or aeration required

4.1 REDUCTION IN cDCE CONCENTRATIONS

A key performance objective was to obtain greater reductions in cDCE concentrations in the bioaugmentation plots than in the control plots so that the effect of the JS666 bacteria (rather than the addition of buffer and/or oxygen) could be assessed. This objective was met for many but not all of the bioaugmented wells.

When cDCE concentration reductions in groundwater were quantitatively evaluated, the objectives were to achieve greater than 75% reduction in bioaugmentation plots over background concentrations and twice the reduction of cDCE concentrations in bioaugmented plots versus control plots. Although there were substantial cDCE declines in some of the bioaugmented wells, the percent reduction was less than 75% relative to baseline concentrations, and the reduction in

the bioaugmented plots was not twice that of the control plots, likely due to TCE inhibition and/or oxygen limitation. Therefore, this performance objective was not met.

4.2 GROWTH AND SPATIAL DISTRIBUTION OF JS666

The qualitative objective associated with the growth and distribution of JS666 was to observe the movement of JS666 away from the injection well. Achieving this objective is important so that the culture can be distributed throughout the treatment area. The further the culture can be distributed, fewer injection wells are required for full-scale implementations.

This objective was evaluated through use of the molecular probes and microcosm assays. Successful distribution was indicated by the presence and activity of JS666 in bioaugmented plots but not in control plots or background wells. JS666 also spread downgradient and transgradient from the injection wells in the bioaugmented plots and was not identified in the upgradient or control wells to any significant degree.

4.3 IMPACT OF OXYGEN LEVELS ON GROWTH AND DEGRADATION RATES

For this performance objective, we originally planned to compare the impact of higher oxygen levels (relative to ambient) on the rate of cDCE degradation and growth of JS666 between the bioaugmented plots. Despite the higher TCE concentrations in Bioaugmentation Plot #1, more biodegradation was observed in Bioaugmentation Plot #1 as illustrated by the higher degree of δ13C enrichment. The higher degree of δ13C enrichment may have been due to more biodegradation as a result of the added oxygen in IW-01. Both Bioaugmentation Plot #1 and Plot #2 had relatively low levels of JS666 according to quantitative polymerase chain reaction (qPCR) measurements. Therefore, the effect of oxygen on JS666 growth could not be evaluated.

4.4 EASE OF USE

The ease of use of the bioaugmentation culture, buffer and aeration equipment is an important factor in maintaining low operation costs for this technology. Ideally, the culture and amendment delivery can be conducted with minimal special training for operators and in a short period of time. The ease of use of this technology was evaluated based on our experience in the field with these bacteria and amendments.

Based on our experience with the field demonstration, bioaugmentation was easy, requiring no special measures, as was aeration and buffer amendment. Buffer injections were, however, time-consuming due to the lower permeability of this aquifer. Nevertheless, this performance objective was met and would definitely be met at sites with groundwater pH in the 6.5 to 8 range.

4.5 COST COMPARISON

The final quantitative objective was to compare the cost of a JS666 bioaugmentation remedy to a P&T system over a 30-year time frame. A present value cost comparison between the two technologies was conducted, as discussed in Section 8.0. The criterion chosen for success was a present value cost-savings of 30-50% for the JS666 technology compared to P&T. The cost

analysis showed a projected cost savings of 47%, assuming no aeration or buffering is required. Thus, under these assumptions, the JS666 technology is cost-effective when compared to P&T.

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5.0 SITE DESCRIPTION

In the following sections, the site location and history (Section 5.1), site geology/hydrogeology (Section 5.2), and contaminant distribution (Section 5.3) are briefly discussed. Detailed descriptions of the site are provided in the Final Report (Geosyntec, 2010), and in the Remedial Investigation (RI) Report for Site 21 (CH2M HILL, 2008).

5.1 SITE LOCATION AND HISTORY

The site is located on SJCA Navy Depot, Site 21, in Chesapeake, VA. SJCA began operations in 1849 as a naval ammunitions facility, although ordnance operations ceased in 1977. SJCA currently acts as a radar-testing range and houses various administrative and warehousing facilities for the nearby Norfolk Naval Shipyard and other local naval activities (CH2M HILL, 2008).

The site is located in a former industrial area in the south-central portion of SJCA. Buildings at the site were historically used as machine, vehicle, and locomotive maintenance shops including paint shops, degreasing shops, electrical shops, and munitions loading facilities. However, many of the older buildings have been demolished. Outdoor areas were used for equipment and chemical storage. Solvents and other chemicals used at the site were reportedly dumped on the ground outside the buildings for dust and weed control. A former fuel service station was also located at the site. Two abandoned underground storage tanks (UST) with a history of leakage are located at the former fuel station (CH2M HILL, 2008).

5.2 SITE GEOLOGY/HYDROGEOLOGY

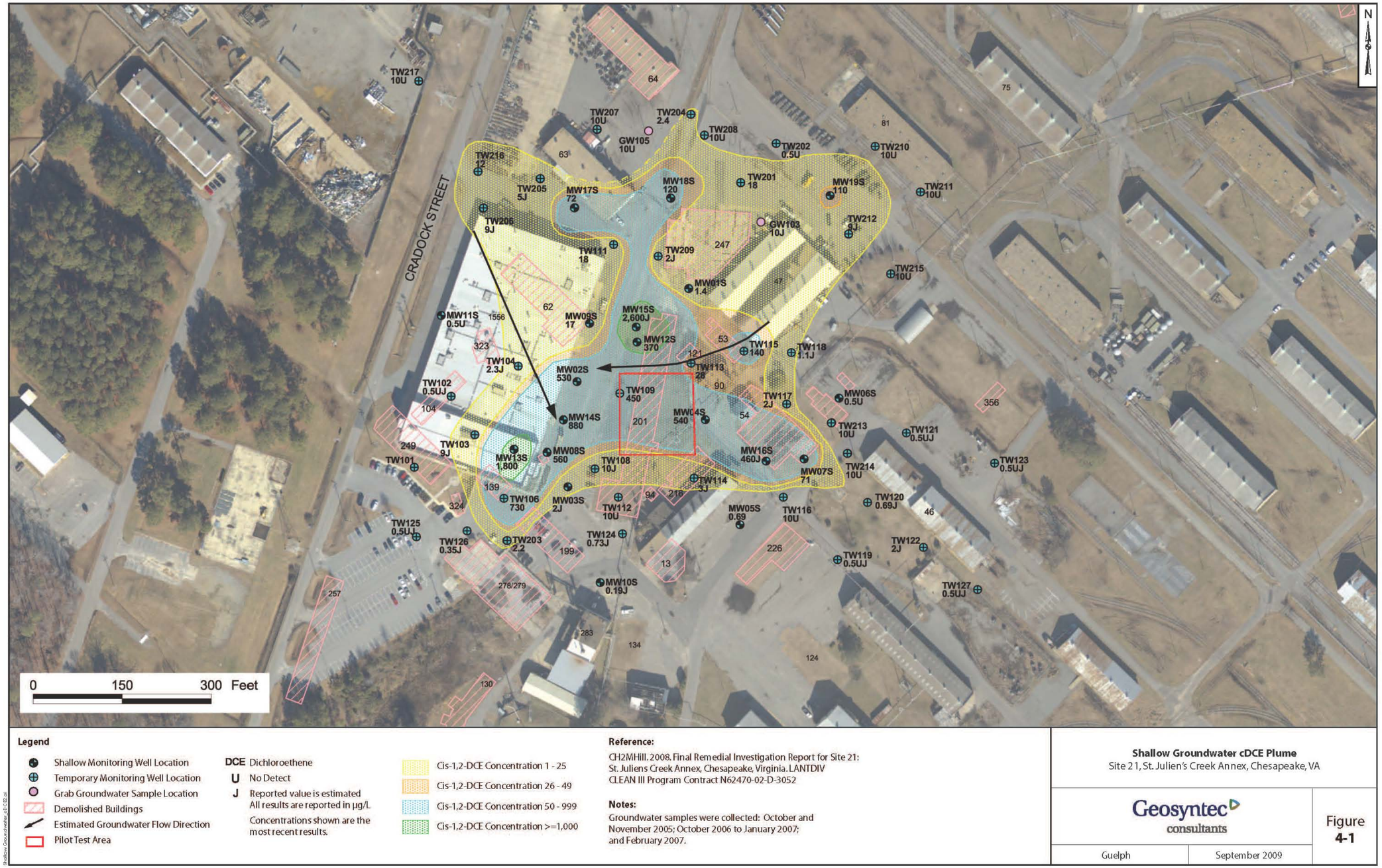
Several geologic units are present beneath SJCA. The Columbia Group, composed of Holocene deposits and undifferentiated Pleistocene deposits, is the uppermost geologic unit in the area and is approximately 60-ft thick. The upper 20 to 40 ft comprises the Columbia aquifer. Beneath the site, the Columbia aquifer consists of brown and tan fine to coarse silty sand, ranging in thickness from approximately 13 to 20 ft. The lower 20 to 40 ft of the Columbia Group consists of relatively impermeable silt, clay, and sandy clay (CH2M HILL, 2008).

Groundwater at the site flows southwest in the eastern portions of the site and southeast in the western portions of the site toward the storm sewer system east of Building 1556. In the vicinity of the PTA, groundwater flow is towards the west. Shallow groundwater typically ranges from 2 to 7 ft bgs (CH2M HILL, 2008). Estimates of the hydraulic gradient and groundwater velocity for the Columbia aquifer are 0.004-0.01 ft/ft and 72 ft/year, respectively (CH2M HILL, 2008).

5.3 CONTAMINANT DISTRIBUTION

Based on historical records and field investigation data, several source areas have been identified at the site. Upon review of this data, a potentially favorable demonstration area was identified near existing monitoring well MW04S where only cDCE was present at elevated concentrations and moderately aerobic conditions prevailed (Figure 1). To confirm that appropriate groundwater conditions for a field demonstration were present in this area, a groundwater sample was collected from well MW04S in December 2007 and analyzed for VOCs and select geochemical

parameters. Results of these analyses confirmed that suitable groundwater conditions exist. TCE, cDCE, and VC concentrations were <10 µg/L, 780 µg/L, and 2 µg/L, respectively. Concentrations of other VOCs were either near or below analytical quantitation limits. The groundwater pH at well MW04S was observed to be 5.88 which, although being slightly lower than desired, could be adjusted through use of a buffering agent (phosphate buffer). The DO and the oxidation-reduction potential (ORP) levels were observed to be 1.65 mg/L and 79 millivolts (mV), respectively, and were indicative of moderately aerobic groundwater conditions.



Legend

- Shallow Monitoring Well Location
- Temporary Monitoring Well Location
- Grab Groundwater Sample Location
- Demolished Buildings
- Estimated Groundwater Flow Direction
- Pilot Test Area

DCE Dichloroethene
U No Detect
J Reported value is estimated
All results are reported in µg/L
Concentrations shown are the most recent results.

- Cis-1,2-DCE Concentration 1 - 25
- Cis-1,2-DCE Concentration 26 - 49
- Cis-1,2-DCE Concentration 50 - 999
- Cis-1,2-DCE Concentration >=1,000

Reference:
CH2MHill. 2008. Final Remedial Investigation Report for Site 21: St. Juliens Creek Annex, Chesapeake, Virginia. LANTDIV CLEAN III Program Contract N62470-02-D-3052

Notes:
Groundwater samples were collected: October and November 2005; October 2006 to January 2007; and February 2007.

Shallow Groundwater cDCE Plume
Site 21, St. Julien's Creek Annex, Chesapeake, VA

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Figure 4-1

Figure 1. Shallow groundwater cDCE plume.

6.0 TEST DESIGN

The following sections provide an overview of the conceptual experimental design, site-specific treatability studies, the design and layout of the technology components, field activities, groundwater sampling methods, analytical methods, and test results. A detailed description of these items is provided in the Final Report (Geosyntec, 2010).

6.1 CONCEPTUAL EXPERIMENTAL DESIGN

For this demonstration, the site was instrumented to create four test plots within the PTA: a bioaugmentation plot receiving JS666, oxygen and buffer (Plot #1); a bioaugmentation plot receiving JS666 and buffer (Plot #2); a control plot receiving buffer (Plot #3); and a control plot receiving oxygen and buffer (Plot #4), as shown in Figures 2 and 3. The intent of the two bioaugmentation plots was to establish the effect of adding JS666 and additional oxygen on the rate of biodegradation, while the corresponding control plots were intended to account for the effects of buffer and buffer and oxygen on the results in the bioaugmentation plots. Two upgradient wells (MW-11 and MW-7) served as background controls to monitor the groundwater in the absence of amendments.

6.2 BASELINE CHARACTERIZATION

Prior to the injection of any amendments, groundwater samples were collected from each of the demonstration wells to determine baseline concentrations. Samples were collected following sampling protocols established for the site in the Demonstration Plan.

6.3 TREATABILITY STUDIES

Treatability tests included site-specific microcosm studies and titration experiments, which were conducted at Cornell University. Microcosm studies are described below, while titration experiments are discussed in the Final Report (Geosyntec, 2010).

6.3.1 Microcosm Studies with Site Groundwater

Microcosms using site groundwater were prepared in 160-mL serum bottles. Because of the low initial pH of the site groundwater (pH of 5.65), the groundwater was buffered to a pH of 7 using a phosphate buffer. Each microcosm was set up in duplicate and then dosed with cDCE (final nominal concentration of approximately 11 mg/L). JS666 inoculum was obtained from active transfer cultures exhibiting growth on cDCE. Microcosms were inoculated with JS666 to achieve roughly either 3.5×10^8 ("1X") or 3.5×10^7 ("0.1X") organisms per bottle. An uninoculated control was also run in duplicate for comparison.

All 1X- and 0.1X-inoculated microcosms with buffered groundwater degraded all the cDCE present within 2 and 4 days, respectively, as shown in Figure 4. There was no degradation in any of the uninoculated controls.

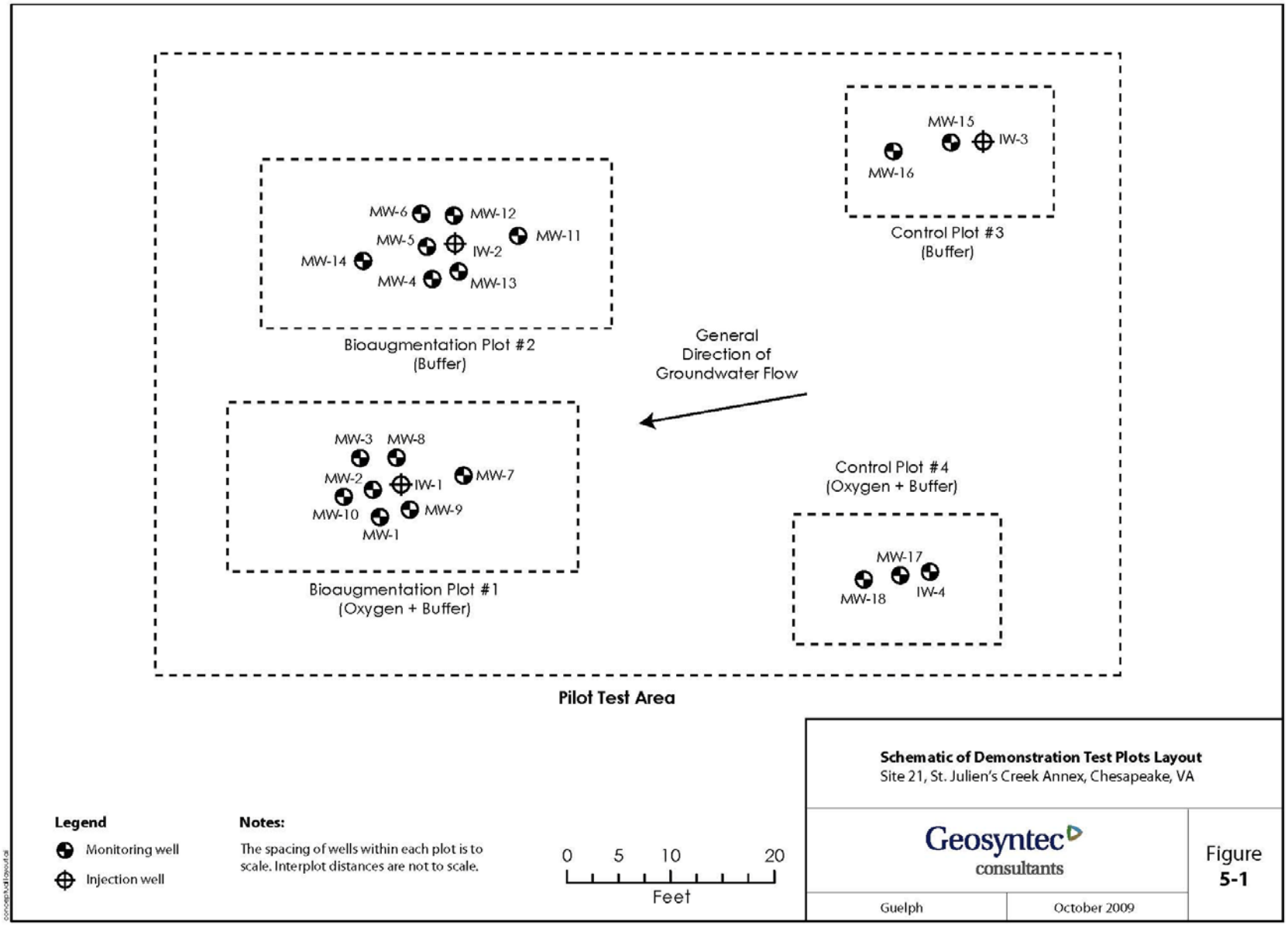


Figure 2. Schematic of demonstration test plots layout.

Figure 3. PTA well locations.

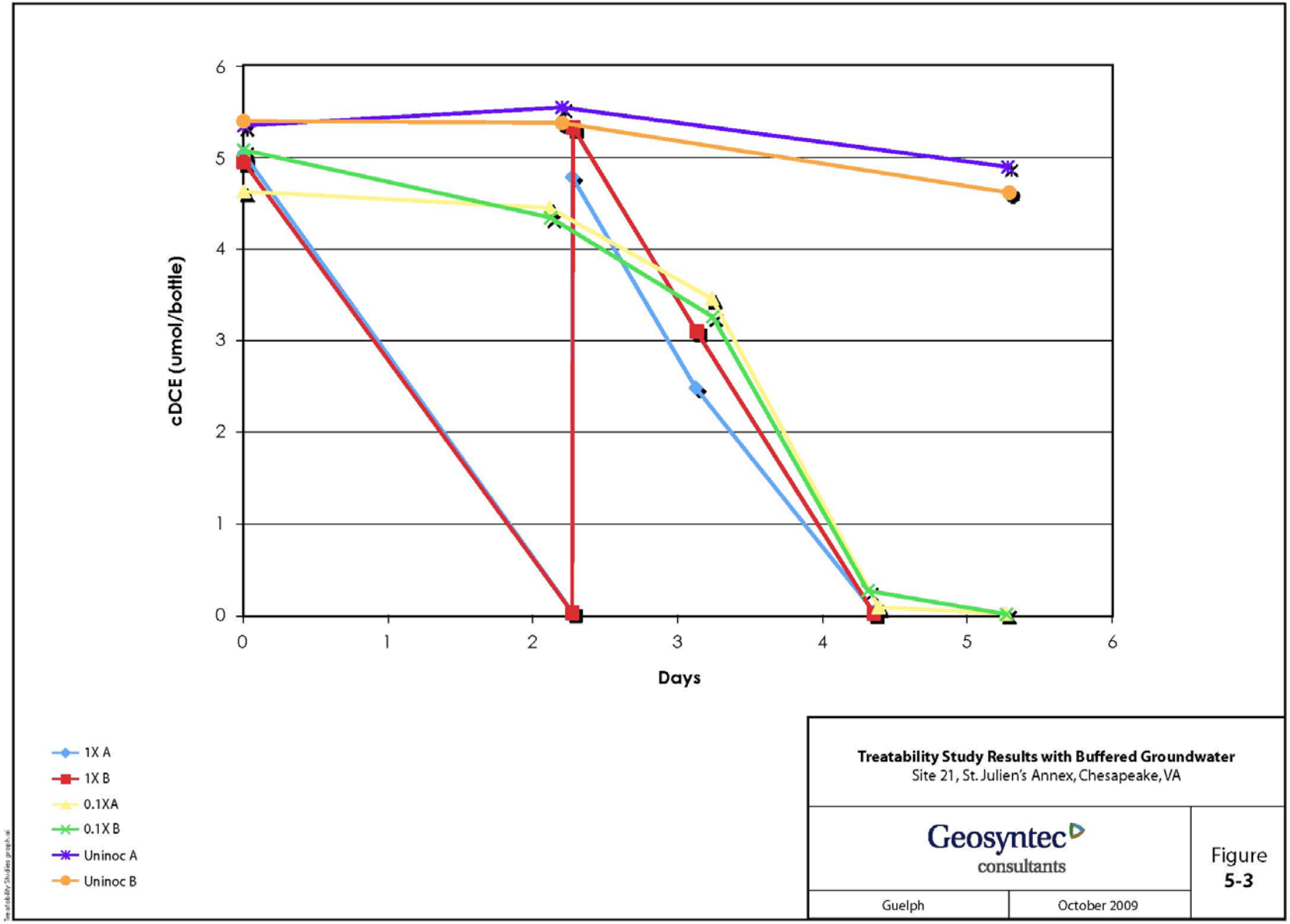


Figure 4.Treatability study results with buffered groundwater.

6.4 DESIGN AND LAYOUT OF TECHNOLOGY COMPONENTS.

The monitoring network for each of the bioaugmentation plots consisted of one fully screened injection well and seven fully screened monitoring wells (one well upgradient of the injection well, two wells transgradient to the injection well, and four wells downgradient of the injection well). The control plots were comprised of a smaller well network of one fully screened injection well and two fully screened downgradient monitoring wells, located upgradient and transgradient to the bioaugmentation plots (Figures 2 and 3).

In an attempt to confirm groundwater direction and flow velocities before all wells were installed, well installations were performed in two separate mobilizations. During the first mobilization, the four injection wells and the first row of downgradient monitoring wells in the bioaugmentation plots were installed, and a conservative tracer study was performed as discussed in Section 5.3.2 of the Final Report (Geosyntec, 2010). The remaining demonstration wells were installed during the second mobilization, following the tracer study.

The results of the tracer tests confirmed the monitoring wells in the bioaugmentation plots were positioned downgradient from the injection wells. In Plot #1, the residence time between IW-01 and MW-02 was estimated to be between 13 and 14 days. In Plot # 2, the residence time between IW-02 and MW-05 was approximately 12 days. The groundwater flow rate was estimated from the results of the tracer test to be between 72 and 84 ft/year, which is similar to the rate of 72 ft/year estimated by CH2M Hill (Section 5.2).

Down-well Waterloo Emitters were deployed in injection wells IW-01 and IW-04 to promote aerobic conditions within Plots #1 and #4. The emitters consisted of silicone tubing coiled around a 4-ft long polyvinyl chloride (PVC) frame. Two emitters were joined together in each well to target the majority of the screened interval. Each series of emitters was connected to an air cylinder and pressure regulating valve, which provided a constant supply of oxygen to the emitters. The air cylinders and regulating valves were housed within the protective well vaults. Compressed air was used instead of compressed oxygen because JS666 is sensitive to oxygen levels greater than 10 mg/L.

6.5 FIELD ACTIVITIES

Field activities following well installation consisted of buffer injections, aeration, and bioaugmentation. Two bioaugmentations were performed during the demonstration—one in October 2008 and one in February 2009. The monthly field events consisted of groundwater sampling and buffer injections, with the exception of the final field event (May 2009) where only groundwater sampling was conducted. The Gantt Chart presented in Figure 5 outlines the schedule for each monthly sampling and buffer injection event. Specifics of the field operations are discussed in detail in the Final Report (Geosyntec, 2010) and are discussed briefly in the following sections.

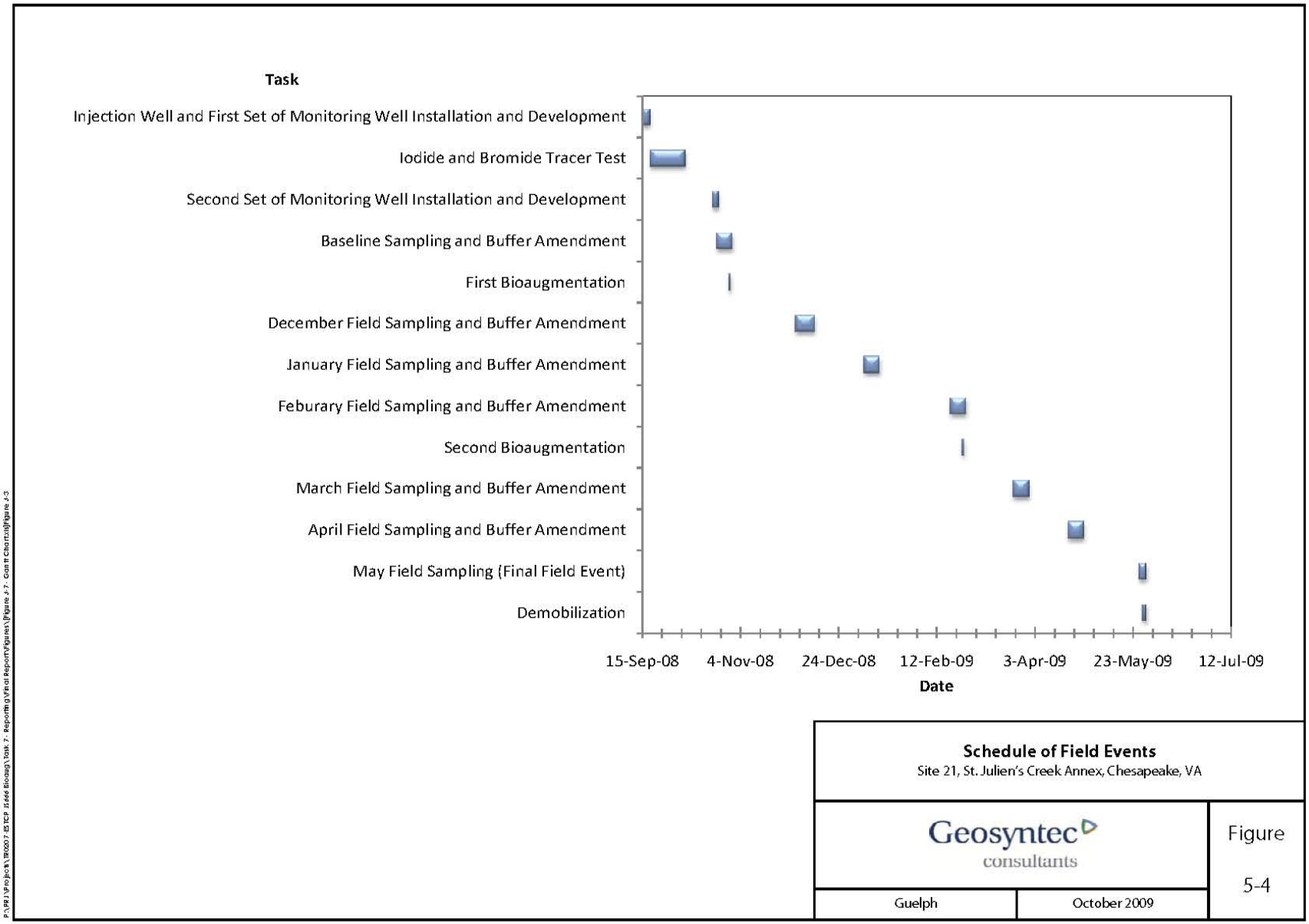


Figure 5. Schedule of field events.

6.5.1 Buffer Amendments and Aeration

A phosphate buffer consisting of potassium monobasic orthophosphate (KH_2PO_4) and potassium dibasic orthophosphate (K_2HPO_4) was added monthly to the injection well of each test plot to raise the groundwater pH to 7.1-7.2, as JS666 loses its activity below a pH of 6.5. Groundwater from each injection well was extracted into dedicated poly tanks, amended with the phosphate buffer, and re-injected.

Air was added to the injection wells in Bioaugmentation Plot #1 and Control Plot #4 using the down-well diffusers described in Section 6.4. The emitter tubing was pressurized to between 10 and 15 psi with the air canister to allow for the diffusion of oxygen into the groundwater. The emitters were removed during the sampling of their respective injection wells and, at the end of each field event, the emitter discharge tubing needle valves were opened for 5-7 seconds to purge the air in the silicone tubing.

6.5.2 Bioaugmentation #1

The first bioaugmentation was performed on October 29, 2008. Approximately 8 L of culture (density of 1.8 x 109 colony forming units [cfu]/mL according to optical density [O.D.] measurements) added to each of the test plots following the injection of 500 L of buffer. Then, the remaining 1500 L of buffer was injected.

6.5.3 Aeration of Buffer

From February 2009 onward, the extracted groundwater from all four plots was oxygenated to a DO concentration just below 10 mg/L to promote biodegradation. The extracted groundwater was oxygenated using dedicated air diffusion stones connected to an oxygen tank and lowered to the bottom of each poly tank. An oxygen probe placed just under the surface of the water in the tank was used to assess when the desired DO concentration of the extracted groundwater had been reached.

6.5.4 Bioaugmentation #2

Because the pH was not optimal after the first bioaugmentation, the activity of the bacteria was not as high as desired. Consequently, a second bioaugmentation was performed on February 25, 2009. For this bioaugmentation, 9 L of culture (density of 2.3 x 109 cfu/mL based on O.D. measurements) was injected into each bioaugmentation plot; 4.5 L of culture was first dispersed in 1400 L of buffer; 150-300 L of buffer with culture was first injected, then the remaining 4.5 L of culture was co-injected directly into the well, followed by the remaining buffer/culture solution. This approach was taken in an attempt to improve the distribution of the bacteria in the subsurface.

6.6 SAMPLING METHODS

Samples were collected and analyzed following protocols established in the Technology Demonstration Plan and described in the Section 5.5 of the Final Report (Geosyntec, 2010). A summary of the total number and types of samples collected is presented in Table 5-4 of the Final Report (Geosyntec, 2010). Laboratory analytical methods used during the demonstration

are summarized in Table 2 of this report, and detailed descriptions of the isotope analyses, microcosm activity assays, and probe assays are presented in Section 5.6 of the Final Report (Geosyntec, 2010).

6.7 SAMPLING RESULTS

In the following sections, the analytical results are summarized. Water level elevation data can be found in Section 6.7.1, field parameters in Section 6.7.2, geochemical parameters in Section 6.7.3, a summary of the isotopic analyses in Section 6.7.4, volatile organic compound data in Section 6.7.5, and microcosm assay and probe assay results in Section 6.7.6. A complete compilation of the analytical data is presented in Appendix E of the Final Report (Geosyntec, 2010). All data were validated using USEPA data qualifiers for organic and inorganic data (USEPA 540-R-08-01 and 540-R-04-004). A summary of the data validation results and findings is presented in Appendix G of the Final Report (Geosyntec, 2010).

6.7.1 Water Level Elevation Data

Water levels were collected prior to sampling each well to help identify any changes in the direction of groundwater flow. A complete compilation of measured water level elevations is presented in Appendix C of the Final Report (Geosyntec, 2010). However, due to the limited amount of data collected and because of the close proximity of the wells, groundwater flow directions could not be confidently predicted. As a result, field parameter (pH and specific conductivity) data for each sampling event was used to assess groundwater flow directions. Estimated groundwater flow directions are shown on the cDCE and qPCR/microcosm assay figures referenced below.

6.7.2 Field Parameters

Following the initial buffer injections, increases in groundwater pH and specific conductivity (0.11 to 0.69 pH units and 148 to 1001 µS/cm, respectively, as measured during the December sampling event) were observed in the injection wells and immediately downgradient monitoring wells for all plots. Slight increases in these parameters were also observed in a few of the transgradient monitoring wells in the bioaugmentation plots. For all plots, the increased pH and specific conductivity levels were generally sustained throughout the project duration as a result of continued buffer injections. No significant changes in pH were observed in the upgradient wells for either bioaugmentation plot.

In Plots #1 and #4, groundwater ORP and DO concentrations increased significantly in injection wells IW-01 and IW-04 (which were both equipped with oxygen emitters) throughout most of the demonstration. In IW-01, dissolved oxygen levels increased from 0.53 mg/L to levels generally above 2.97 mg/L, and ORP levels increased from 24.7 mV to levels generally above 100 mV. However, the DO increases were predominately limited to the injection wells themselves. DO levels in the downgradient wells remained relatively unchanged, with concentrations generally ranging from 0.08 mg/L to 1.24 mg/L throughout the demonstration. ORP levels in the downgradient wells ranged between -382.2 mV and 34.8 mV.

Table 2. Summary of sample handling and laboratory analytical details.

Parameter	Analytical Method	Method Number	Analytical Laboratory	Quantitation/ Reporting Limit[1]	Sample Container	Preservative	Holding Time
Field Parameters (DO, ORP, pH, conductivity, temperature)	Field probes	Field	NA	Varies	NA	NA	NA
VOCs (TCE, cDCE, tDCE, 1,2-DCA, VC)	Gas Chromatography/ Mass spectrometry	EPA 8260B	CAS	1-20 µg/L	3 x 40 mL VOA	HCl to pH<2, cool to <6°C	14 days
Dissolved Hydrocarbon Gases (methane, ethane, ethene)	Gas chromatography/ flame ionizing detector	RSK-175	CAS	1-2 µg/L	3 x 40 mL VOA	HCl to pH<2, cool to <6°C	14 days
Tracers (bromide, iodide)	Ion-selective electrode	Field	NA	0.005-0.4 mg/L	120 mL plastic	cool to <6°C	28 days
Alkalinity	Titration	EPA 310.1, SM 2320B	CAS	2-40 mg/L	250 mL plastic	cool to <6°C	14 days
Dissolved Metals (Fe^{2+}, Mn^{2+})	Inductively-coupled plasma	EPA 6010B	CAS	0.01-0.1 mg/L	250 mL plastic	Filter on-site, HNO^3 to pH<2	180 days
cDCE Carbon Isotopes (^{13}C, ^{12}C)	Gas chromatography/ combustion/ isotope ration mass spectrometry	NA	U of T	10 µg/L	8 x 40 mL VOA	1 mL 12N HCl, cool	NA
JS666 Activity	Microcosm activity assay	NA	Cornell	0.5% loss of cDCE per day	2 x 1 L plastic*	cool to <4°C	14 days
JS666 Detection	Molecular probe	NA	Cornell	3,000 copies/mL	120 mL plastic*	cool to <4°C	14 days

VOA = volatile organic analysis
HCl = hydrochloric acid

In Plots #2 and #3, increases in both DO and ORP were observed in wells IW-02 and IW-03 only immediately following buffer injection (likely because of elevated DO concentrations in the injected buffers as a result of mixing and/or aeration during buffer preparation). By the following event, DO and ORP had returned to pre-buffer injection levels, which ranged from 0.17 mg/L to 0.57 mg/L and -376.7 mV and 162.1 mV, respectively. In the downgradient wells, DO concentrations were generally less than 1 mg/L, and ORP levels were predominately negative.

6.7.3 Geochemical Parameters

Throughout the study duration, significant increases in groundwater alkalinity were observed in all plots. Increases in alkalinity were predominately observed in wells immediately downgradient of the injection wells, with smaller increases in the transgradient wells. No significant change in alkalinity was observed in the upgradient wells for either bioaugmentation plot, indicating that downgradient increases were attributed to microbial activity stimulated by buffer addition and/or JS666 bioaugmentation.

Concentrations of dissolved manganese in the four injection wells and some of the downgradient monitoring wells decreased over the study duration, most likely due to increasing pH levels in these wells, and thus formation of manganese hydroxides. Dissolved manganese concentrations in other monitoring wells, including the upgradient monitoring wells MW-07 and MW-11, varied slightly but ultimately returned to near baseline concentrations during the final sampling event. Concentrations of dissolved iron in almost all monitoring wells were more variable than dissolved manganese. However, the four injection wells all showed reductions in dissolved iron over the project duration, likely as a result of addition of air or aerated buffer.

Methane concentrations in Plots #1, #2, and #3 varied for most wells, with levels ranging from 43 to 940 µg/L, but the levels were generally not indicative of deeply reduced conditions. The exceptions to this observation were the methane concentrations in all wells in Plot #4, which increased over the project duration. Methane concentrations in the two downgradient monitoring wells, MW-17 and MW-18, increased from 960 µg/L to 2800 µg/L and from 2200 µg/L to 12,000 µg/L, respectively. The reason for this is not clear.

6.7.4 Isotopic Analyses

Results of cDCE isotope analyses are presented in Figures F-1a through F-4c in Appendix F of the Final Report (Geosyntec, 2010). Bar charts showing changes in δ13C in cDCE compared to the Month 1 sampling event can be found in Figure 6. Trends observed in the control and bioaugmentation plots are presented below.

6.7.4.1 <u>Control Plots</u>

All monitoring wells in both Control Plots #3 and #4 showed substantial isotopic enrichment between the first two sampling dates, consistent with significant biodegradation of cDCE in those areas of the plume. Thereafter however, while concentration levels increase and decrease over time in these wells, δ13C values for cDCE showed little or only a small degree of continued

enrichment (IW-3, MW-15, MW-16, MW-18) or there was a reversal of the enrichment trend, and δ13C values became less enriched (IW-4, MW-17).

Figure 6 presents the data in a different way by showing the change in δ13C relative to the δ13C levels during the Month 1 sampling event for all wells in Control Plots #3 and #4. Between Month 4 and Month 6, MW-15, IW4, and MW-17 became less enriched, while IW-3 and MW-18 showed enrichment.

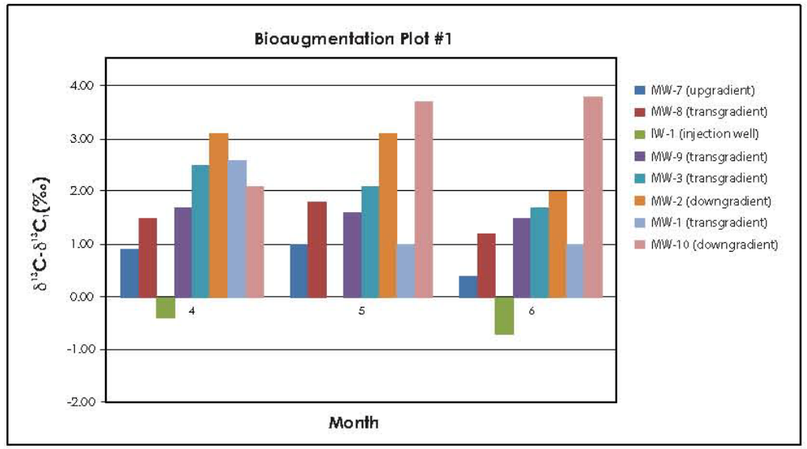
Taken together these results indicate that the main control on cDCE concentrations in the control plots was not biodegradation but fluctuations due to pumping and/or groundwater transport processes. The possible exception is MW-18 where the changes in VOC concentrations and isotope signatures track quite closely and suggest biodegradation may be occurring in this control well to a greater extent than any of the others. This conclusion is supported as well by the fact that MW-18 shows the most enriched δ13C value (-15 .2 permil) in any of the control wells on the second to last sampling date. Higher VC levels and a lower ORP in this well suggest reductive dechlorination was occurring rather than degradation attributable to JS666.

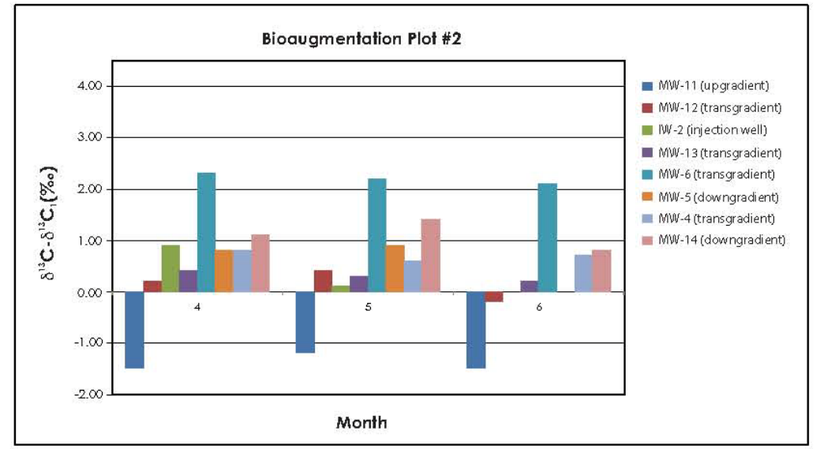
6.7.4.2 Bioaugmentation Plots #1 and #2

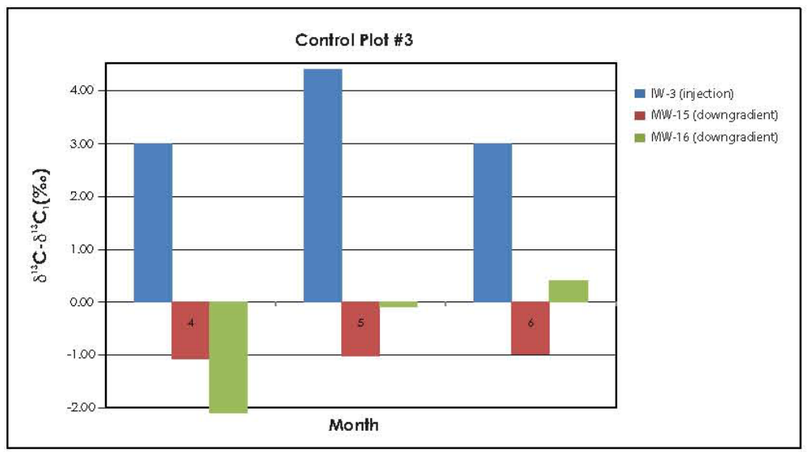
All wells in Bioaugmentaton Plot #1 showed trends of isotopic enrichment over the study consistent with the effects of biodegradation. The most consistent trends and most pronounced isotopic enrichments (up to 4-5 permil) were observed in downgradient wells MW-2, MW-3, and MW-10.

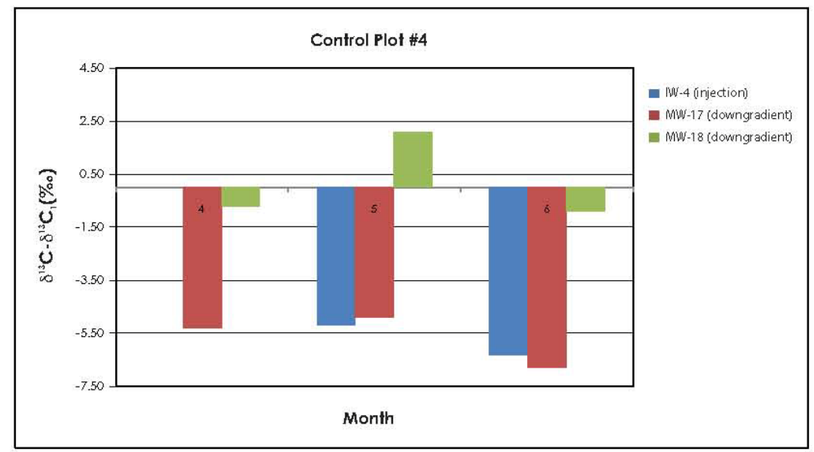
With the exception of the upgradient well MW-11, all wells in Bioaugmentation Plot #2 showed trends of isotopic enrichment over the study consistent with the effects of biodegradation. Well MW-11 showed substantial isotopic enrichment between the first two sampling dates, but thereafter showed a general reversal of the enrichment trend, with δ13C values becoming less enriched.

Figure 6 shows changes in δ13C relative to Month 1 values (i.e., $\delta^{13}C_t$ - $\delta^{13}C_1$) for Months 4 to 6. The monitoring wells in Bioaugmentation Plot #1 show substantial enrichment, while the monitoring wells in the corresponding Control Plot #4 do not (with the exception of MW-18). These results suggest that biodegradation was occurring primarily because of the addition of JS666 rather than the addition of buffer. Figure 6 also indicates that there was modestly more overall enrichment in Bioaugmentation Plot #2 relative to Control Plot #3, suggesting a modest effect of JS666 relative to buffer alone. Plots #2 and #3 did not receive air via the Waterloo emitter and, therefore, may have been oxygen-limited. In conclusion, the carbon isotope results support a significant degree of biodegradation in downgradient wells in Bioaugmentation Plots #1 and #2 and more biodegradation in bioaugmentation plots relative to control plots that received buffer but not JS666.









Note:
Error in $\delta^{13}C$ values is +/- 0.5 permil.

Changes in $\delta^{13}C$ in cDCE in Groundwater
Site 21, St. Julien's Creek Annex
Chesapeake, Virginia

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Figure 5-5

Figure 6. Changes in $\delta^{13}C$ in cDCE in groundwater.

6.7.5 Volatile Organic Compound Data

6.7.5.1 cDCE

In Bioaugmentation Plot #1, cDCE concentrations in the upgradient unamended well MW-7 increased after October 2008, with an average cDCE concentration of 2533 µg/L from October 2008 to April 2009. Average cDCE concentrations in downgradient wells decreased 7-44% relative to upgradient cDCE concentrations. (Note that May 2009 data were excluded due the malfunctioning of the air cylinder supplying the Waterloo Emitters.) Of the eight wells in bioaugmentation Plot #1, IW-01, MW-01, MW-02, and MW-03 showed the greatest degree of cDCE removal (Figure 7a).

In Bioaugmentation Plot #2, cDCE concentrations in downgradient wells generally decreased 14-25% relative to average cDCE concentrations in upgradient well MW-11(Figure 7b). The exceptions to this trend were MW-4 and MW-13 where average cDCE concentrations increased relative to those in MW-11.

By contrast, in Control Plot #3, cDCE concentrations remained relatively the same throughout the demonstration (Figure 7c). In Control Plot #4 (which received an emitter and buffer), wells MW-17 and MW-18 showed initial reductions in cDCE up until the February sampling event, when cDCE rebounded to near baseline conditions (Figure 7d). However cDCE concentrations did decrease again immediately following the second bioaugmentation when aeration of the buffer-amended groundwater was initiated.

6.7.5.2 TCE

Almost all wells in Bioaugmentation Plot #1 (with the exception of MW-01 and MW-09) exhibited considerable reductions in TCE over the course of the demonstration (Figure 5-11a, Final Report [Geosyntec, 2010]). TCE concentrations in the upgradient well, MW-07, remained relatively constant throughout the demonstration. Given the high rates of TCE removal in the control plots (discussed below), the TCE reduction in this plot is likely due to biodegradation by bacteria other than JS666.

All downgradient wells in Bioaugmentation Plot #2 exhibited considerable reductions in TCE following the first bioaugmentation, and levels remained low throughout the remainder of the demonstration (Figure 5-11b, Final Report [Geosyntec, 2010]). TCE concentration in the upgradient well, MW-11, fluctuated but was generally considerably higher than in downgradient wells. Given the high rates of TCE removal in the Control Plot #3 (discussed below), the TCE reduction in this bioaugmentation plot is likely due to biodegradation by bacteria other than JS666.

All wells in Plot #3 and Plot #4 exhibited considerable and sustained reductions in TCE over the course of the demonstration (Figures 5-11c and 5-11d, Final Report [Geosyntec, 2010]), suggesting the addition of buffer alone had stimulated TCE biodegradation.

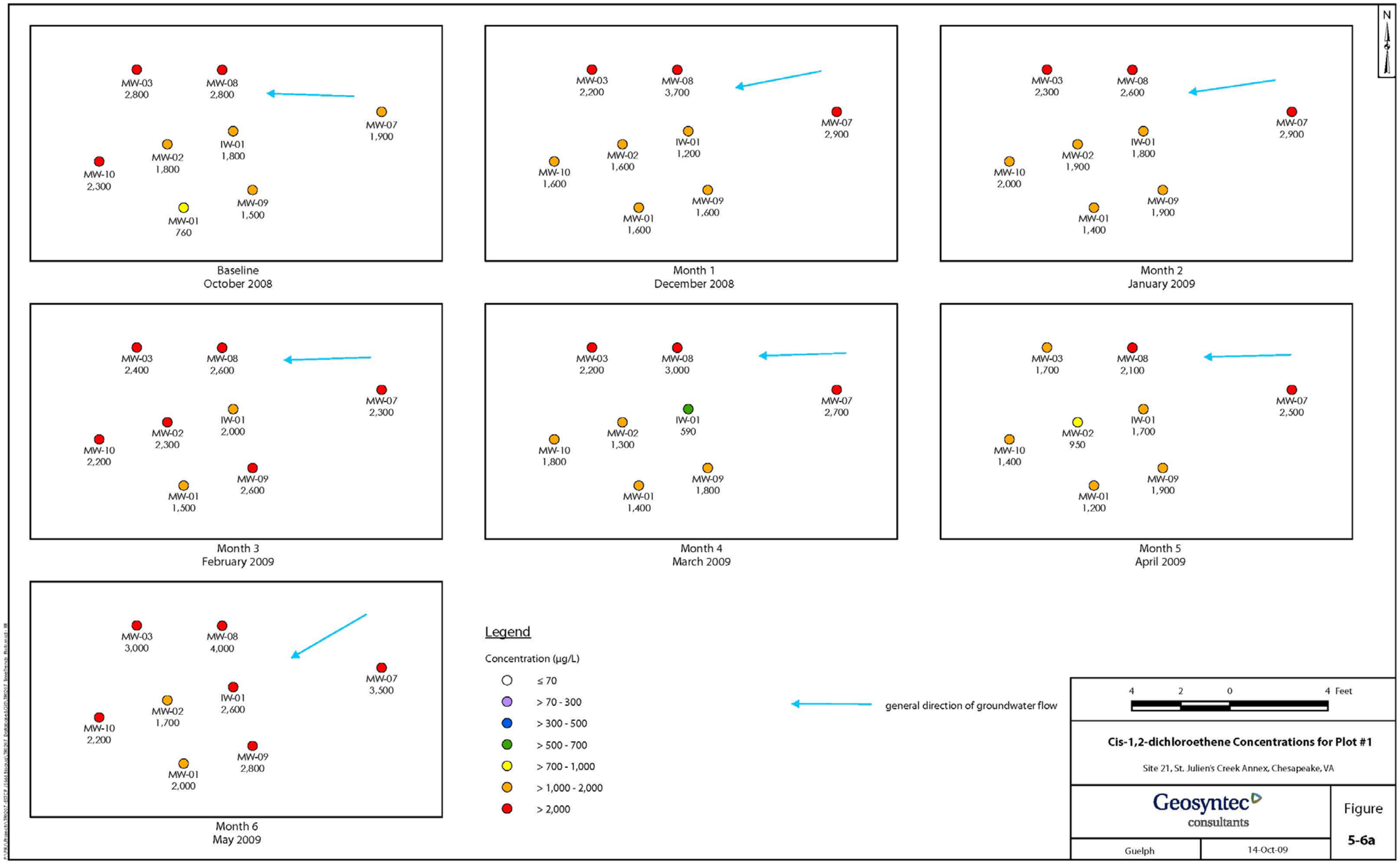

Figure 7a. Cis-1,2-dichloroethene concentrations for Plot #1.

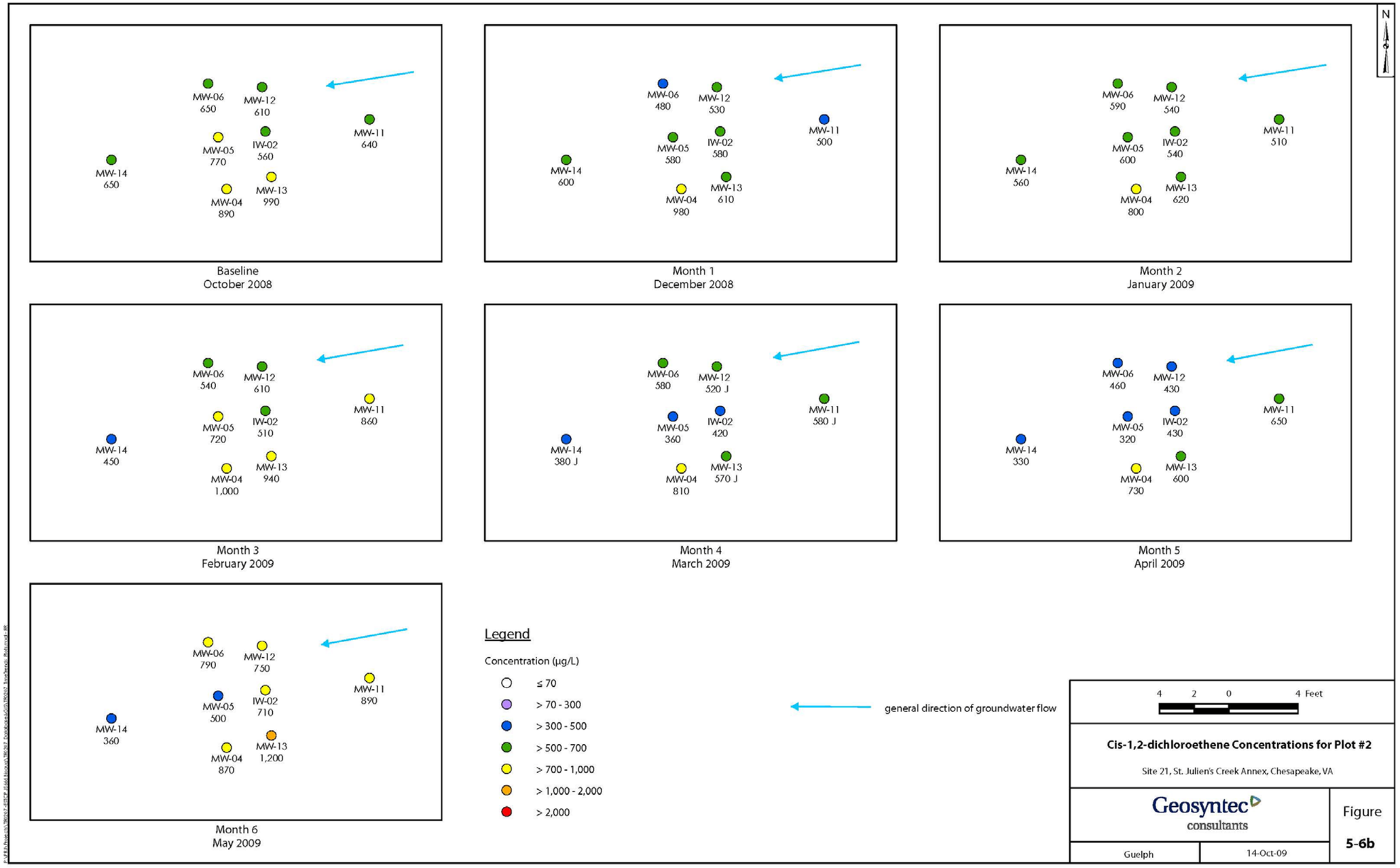

Figure 7b. Cis-1,2-dichloroethene concentrations for Plot #2.

Figure 7c. Cis-1,2-dichloroethene concentrations for Plot #3.

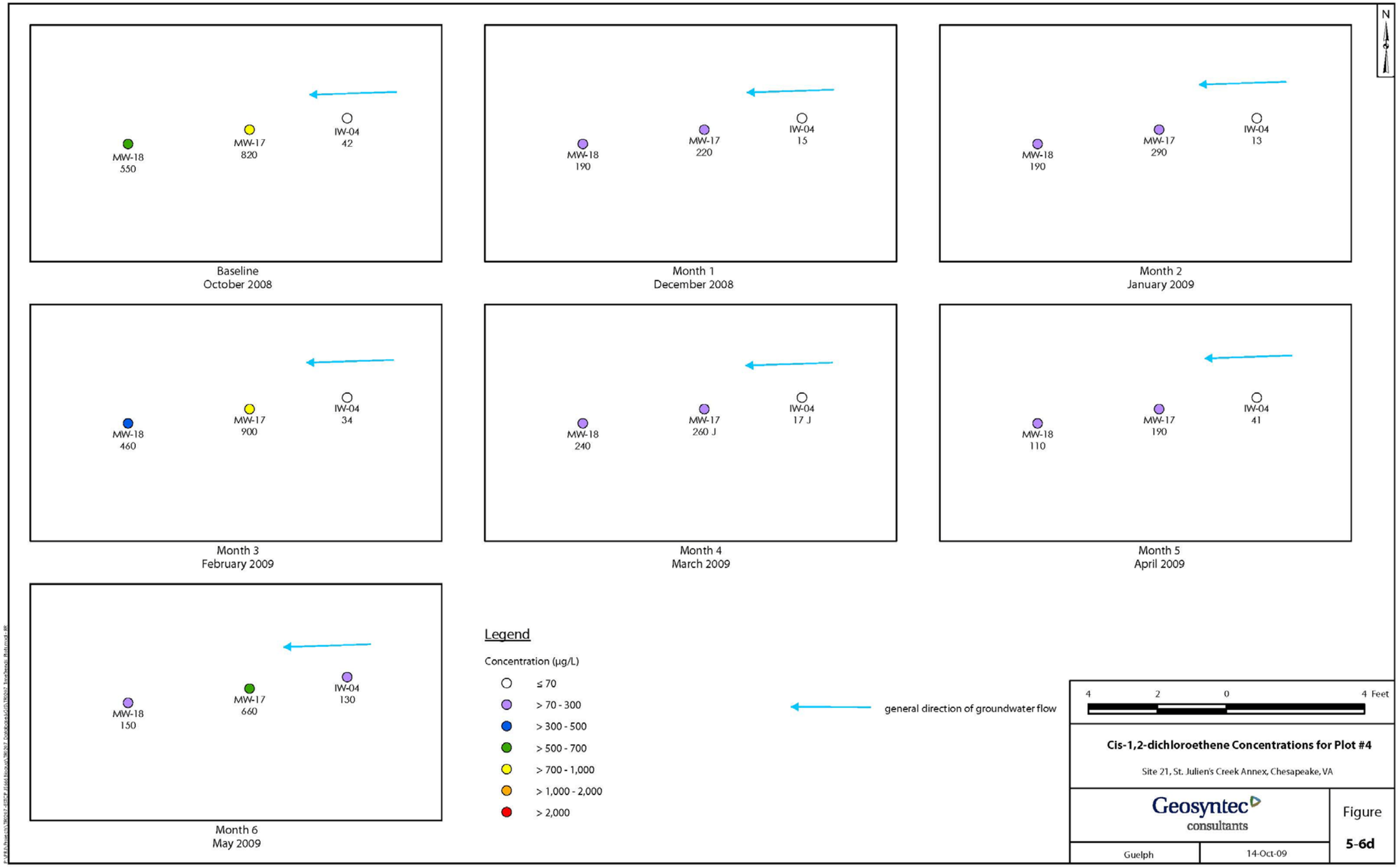

Figure 7d. Cis-1,2-dichloroethene concentrations for Plot #4.

6.7.5.3 VC

VC concentrations fluctuated over the demonstration period and were generally low in Plots #1 to #3 (Table E-2 in Appendix E, Final Report [Geosyntec, 2010]), but were highest in MW-18 in Plot #4 (Figure 5-13, Final Report [Geosyntec, 2010]). MW-18 also had more reduced conditions and a higher pH relative to the other wells. Thus, some of the TCE and cDCE declines in Plot #4 may have been due to reductive dechlorination rather than aerobic processes.

6.7.6 Probe Assay and Microcosm Assay Results

6.7.6.1 Probe Assay—Inoculum Levels

qPCR results for lab and field samples of the inoculum culture indicated JS666 inoculum densities of approximately 10^8 per mL for both bioaugmentation events.

6.7.6.2 Probe Assay—Monitoring JS666 Transport

qPCR data are presented graphically for wells in Plots #1 to #4 over the course of the demonstration on Figures 8a to 8d, respectively. The qPCR data represents ISO probe values (if only ISO data were collected) or the lower of ISO and CMO values (if both probes were used). A table of the plotted data can be found in the Final Report (Geosyntec, 2010) along with figures illustrating quantitative data for ISO, CMO and UNI probes.

In Figures 8a to 8d, qPCR results are coded for each sampling location and event as follows: "0" for nondetectable levels; "+" for results considered detectable, but not quantifiable (i.e., reasonable agreement in most qPCR plate-wells, but less than 20 copies/rxn); "++" for quantifiable levels below 104 copies per mL (20≤copies/rxn≤60); and "+++" for levels above 104 per mL.

For all of the plots, there is no evidence of JS666 during the baseline sampling event (October 2008) prior to the addition of JS666 and buffer (and oxygen in some cases). In general, there are almost no qPCR detections in the control plots (#3 and #4) where no JS666 was added, with the exception of a few sporadic low-level qPCR hits. Likewise, there are no qPCR detections in upgradient wells MW-07 or MW-11 (with the exception of one low-level hit in MW-11 in January 2009). Taken together these data indicate that there is no significant native population of JS666.

The qPCR data for Bioaugmentation Plot #1 is shown in Figure 8a. The highest levels were generally observed in January 2009 (2 months after the first bioaugmentation) and typically levels were highest in the MW-08, which is transgradient to the injection well. qPCR data show that JS666 bacteria have migrated at least 6 ft downgradient.

The qPCR data for Bioaugmentation Plot #2 is presented in Figure 8b. The best distribution of JS666 was generally observed in March 2009 (one month after the second bioaugmentation). The qPCR counts declined in the months following. qPCR data show that JS666 bacteria have migrated at least 8 ft downgradient. Growth is not clearly observed throughout the demonstration either due to oxygen limitation or the cells washing out of the test area.

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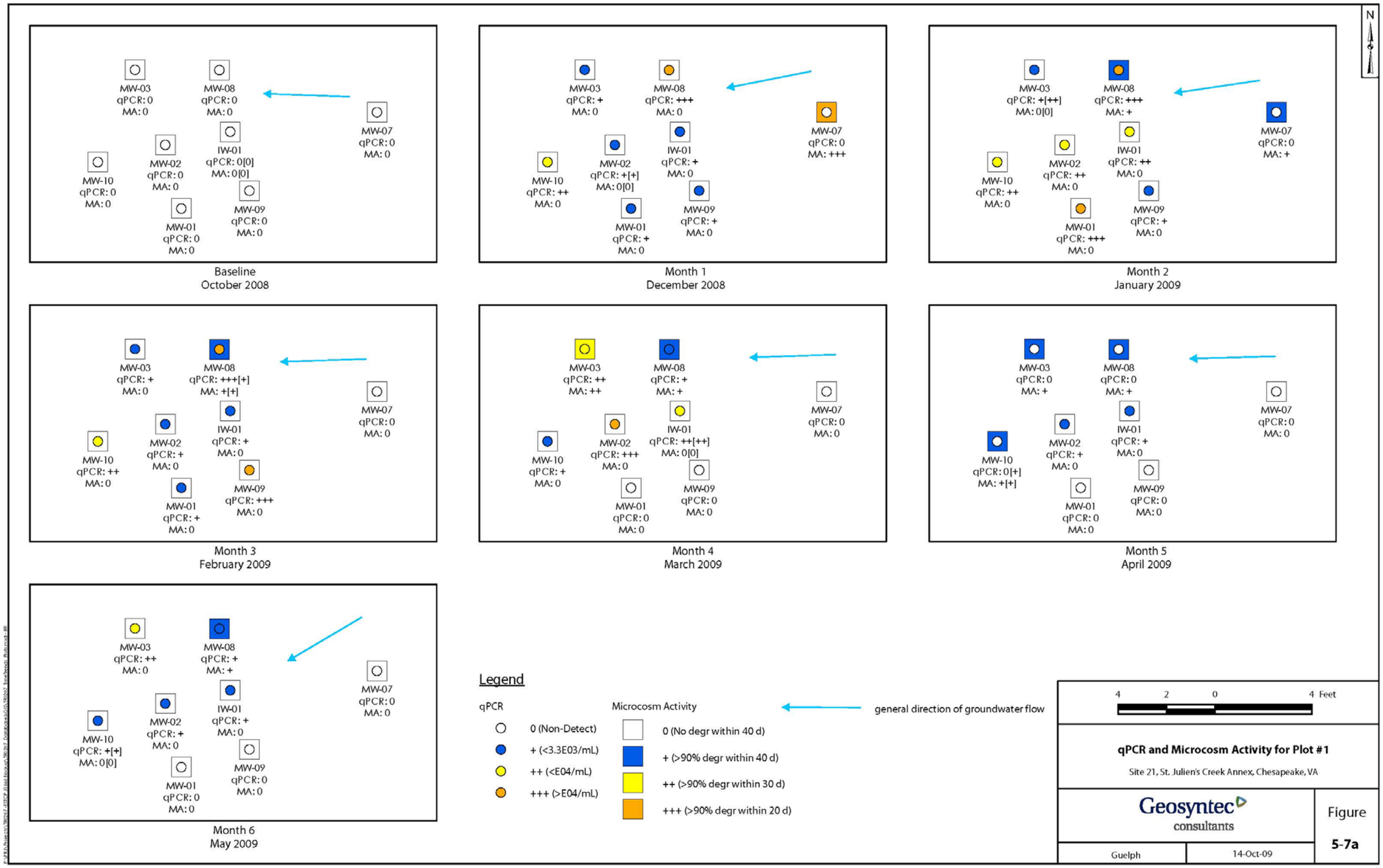

Figure 8a. qPCR and microcosm activity for Plot #1.

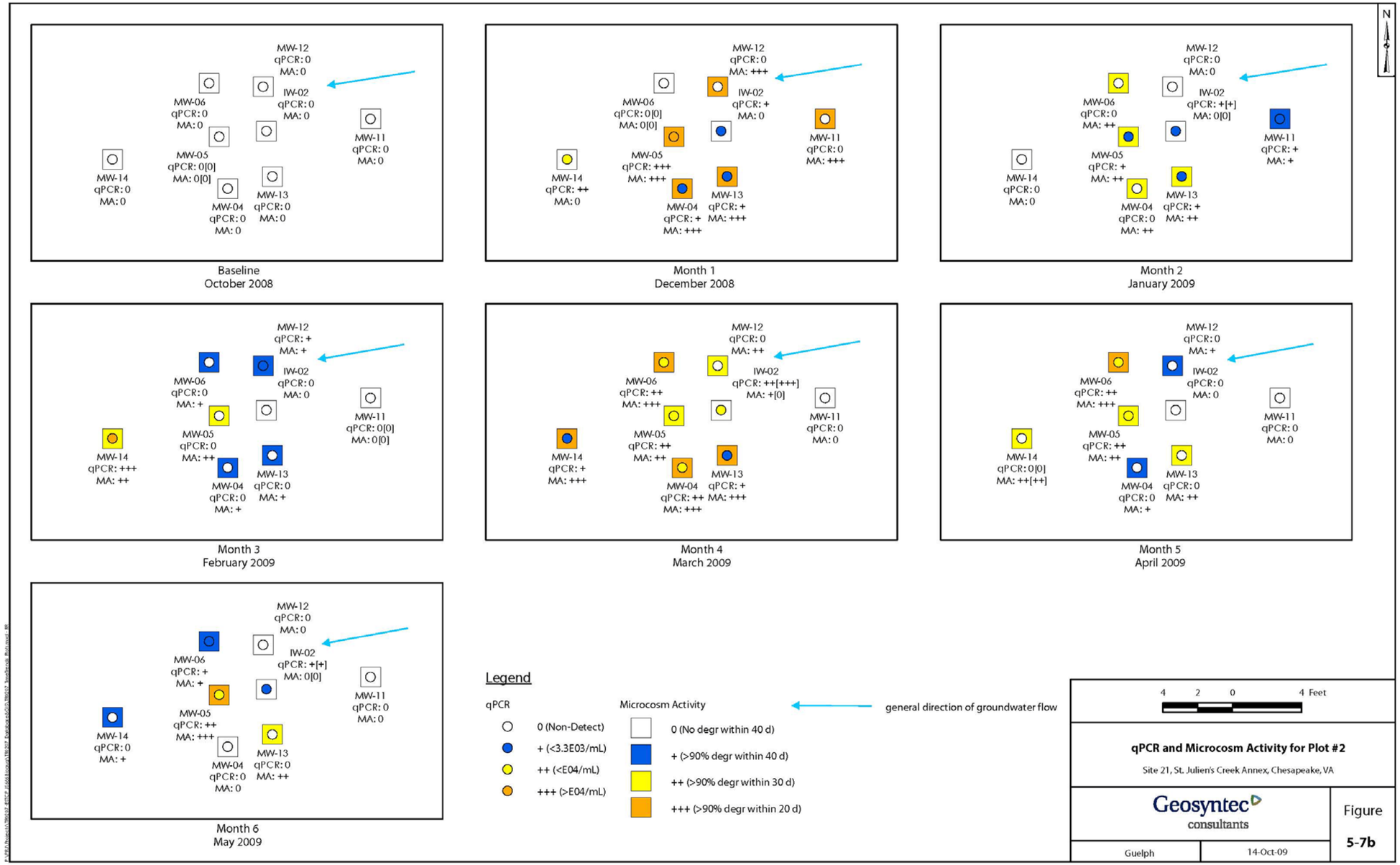

Figure 8b. qPCR and microcosm activity for Plot #2.

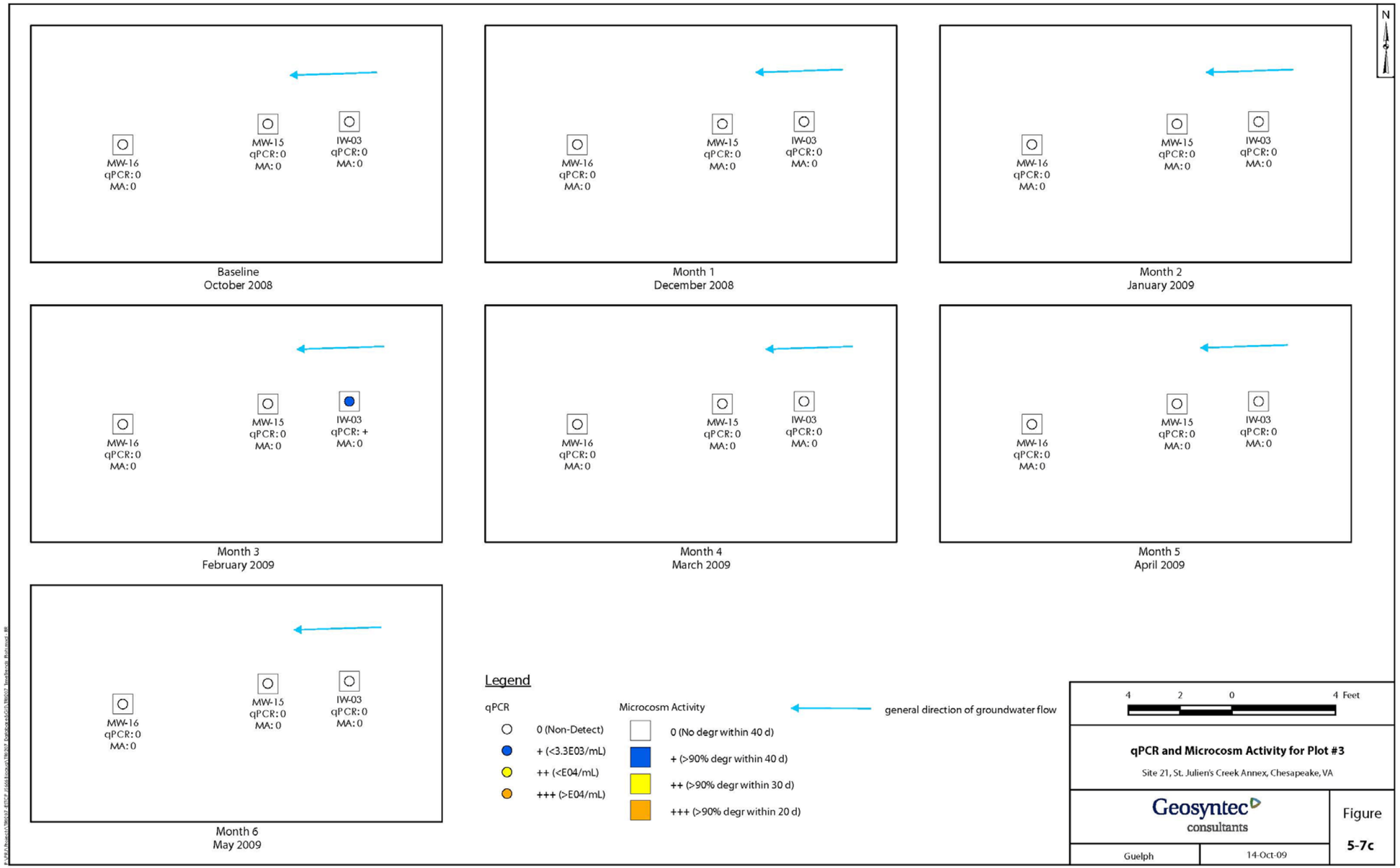

Figure 8c. qPCR and microcosm activity for Plot #3.

Figure 8d. qPCR and microcosm activity for Plot #4.

6.7.6.3 Microcosm Assay—Monitoring

For the seven sampling events, groundwater samples were collected from each of the wells, along with two quality-control duplicates. These groundwater samples were used to conduct microcosm assays in triplicate. Generally only two of the three microcosm replicates were sampled, unless there was significant variability (in which case the third replicate would be sampled as a tie-breaker). Virtually all microcosms that showed degradation of cDCE also showed degradation of TCE (and usually also of VC).

Results of the microcosm assays for each of the plots over the course of the demonstration are shown in Figures 8a through 8d. Microcosm activity results have been coded as follows: "0" if no cDCE degradation occurred over 40 days of monitoring; "+" if cDCE was degraded within 40 days; "++" if within 30 days; "+++" if within 20 days; and "++++" if within 10 days. For this purpose, "degradation" was considered to be greater than 90% disappearance of cDCE.

Through examination of Figures 8a through 8d, it is evident that no microcosm activity was evident in any of the plots prior to bioaugmentation and buffer addition (Baseline, October 2008). Likewise, there is no microcosm activity in samples collected from wells in the Control Plots #3 and #4 during any of the sampling events.

In Bioaugmentation Plot #1, although JS666 was typically present according to the qPCR assays, microcosm activity was not generally evident or was evident at very low levels in downgradient wells. The exception to this was the activity measured in MW-03 during the March 2009 sampling event (>90% degradation within 30 days), which corresponded to a JS666 density of between 3.3×10^3 and 10^4 cfu/mL. The low microcosm activity is likely attributable to the high TCE concentrations (greater than 1000 µg/L) in Plot #1. High TCE concentrations (i.e., greater than 500 µg/L) can inhibit cDCE biodegradation, as illustrated in additional microcosm studies discussed in Section 5.7.6 of the Final Report (Geosyntec, 2010).

In Bioaugmentation Plot #2, the highest microcosm activity was generally observed one month following each of the two bioaugmentations (in December 2008 and then in March 2009) and then decreased in the months following. Higher microcosm activity was observed in groundwater samples from Bioaugmentation Plot #2 compared to Bioaugmentation Plot #1, likely due to the lower concentrations of TCE in groundwater samples in Bioaugmentation Plot #2. Note that isotopic analyses indicated more biodegradation in Bioaugmentation Plot #1 relative to Bioaugmentation Plot #2, so microbial activity may have been higher in Plot #1 than measured in the laboratory using groundwater samples, which likely had lower levels of JS666 than the surrounding aquifer matrix.

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7.0 PERFORMANCE ASSESSMENT

7.1 REDUCTION IN cDCE CONCENTRATIONS

7.1.1 Qualitative

A key performance objective was greater reductions of cDCE concentrations in bioaugmentation plots versus control plots. To evaluate this objective, cDCE data from bioaugmentation plots were compared to data from control plots and from background (upgradient) wells. cDCE data in Bioaugmentation Plots #1 and #2 and in Control Plots #3 and #4 are shown in Figures 7a to 7d and summarized in Table 3. Comparison of cDCE concentrations over time in the bioaugmented plots to the control plots reveals some reduction in cDCE in several wells (e.g., MW-1, MW-2, MW-3, MW-5, MW-6, MW-10, MW-12, and MW-14), indicating the effectiveness of JS666 bioaugmentation. Isotopic enrichment in groundwater samples in the bioaugmented wells compared to the upgradient and Control Plots #3 and #4 wells further corroborates the effect of JS666 bioaugmentation on cDCE degradation as discussed in Section 6.7.4. Therefore, greater cDCE reductions were observed in many of the wells in the bioaugmented plots compared to the control plots. cDCE biodegradation was likely limited by lack of oxygen in Bioaugmentation Plot #2 and inhibited by high levels of TCE in Bioaugmentation Plot #1, as discussed in Section 6.7.6.

Table 3. Percent removal of cDCE in wells in bioaugmentation Plots #1 and #2.

Well ID	Average cDCE Concentration (µg/L)	% Removal	Well ID	Average cDCE Concentration (µg/L)	% Removal
MW-07 (upgradient)	2533	--	MW-11 (upgradient)	620	--
IW-01	1459	42	IW-02	497	20
MW-01	1420	44	MW-04	864	-39
MW-02	1580	38	MW-05	516	17
MW-03	2400	42	MW-06	532	14
MW-08	2350	7	MW-12	526	15
MW-09	2400	23	MW-13	674	-9
MW-10	1800	29	MW-14	464	25

Note: Average cDCE concentrations were calculated from October 2008 to April 2009 for upgradient wells and from December 2008 to April 2009 for downgradient wells.

7.1.2 Quantitative

When cDCE concentration reductions were quantitatively evaluated, the objective was to achieve greater than 75% reduction in cDCE in bioaugmentation plots over background concentrations and twice the reduction of cDCE concentrations in bioaugmented plots versus control plots.

Table 3 presents percent removals of cDCE based on average upgradient and downgradient concentrations. Although reductions in average cDCE concentrations of up to 44% were observed in Bioaugmentation Plot #1 and up to 25% were observed in Bioaugmentation Plot #2 relative to average upgradient cDCE concentrations, the objective of a 75% reduction was not achieved. The reduction was also evaluated by plotting normalized concentrations in each well for a selected event (April 2009) relative to baseline concentrations for both bioaugmentation

plots and control plots, as shown in Figures 9a and 9b. Note that the cDCE concentrations from the May 2009 sampling event were not used because of problems with the air cylinder supplying the Waterloo Emitter. None of the wells in either bioaugmentation plot showed cDCE reductions of 75% or more (i.e., a normalized C/Co of 0.25 or less) relative to baseline. Furthermore, although cDCE concentrations declined in many of the bioaugmentation wells (i.e., all downgradient wells in Plot #2); the reductions were not typically twice that observed in the control plot wells.

In addition to high TCE concentrations in Bioaugmentation Plot #1, the reason for not meeting the performance objective may be due to the difficulty in achieving good dissolved oxygen distribution. A diffusive gas emitter device (the Waterloo Emitter) was employed, and elevated oxygen levels were generally limited to the vicinity of the injection well only. The emitter was supplied with compressed air instead of compressed oxygen because JS666 is sensitive to oxygen levels above 10 mg/L. Air sparging might have been more effective in distributing oxygen a further distance from the injection well. This approach was initially discounted as preliminary lithologic data had suggested the subsurface was heterogeneous and good oxygen distribution would not be achieved. Furthermore, the ambient dissolved oxygen concentrations were very low in Bioaugmentation Plot #2, which undoubtedly influenced the performance in that plot.

7.2 GROWTH AND SPATIAL DISTRIBUTION OF JS666

The objective associated with the growth and distribution of JS666 was to determine the extent of transport of JS666 away from the injection well. JS666 was enumerated in groundwater samples using two molecular probes (one based on the isocitrate lyase gene and one based on the cyclohexanone monooygenase gene). In addition, JS666 activity and presence was also evaluated through microcosm assays conducted using groundwater from the wells in each of the plots. Successful distribution was indicated by the presence and activity of JS666 in bioaugmented plots but not in control plots or background wells. Growth of JS666 was demonstrated by observing higher counts of JS666 with time in bioaugmented plots.

Following bioaugmentation, qPCR and microcosm results demonstrated in situ survival and activity over the course of the demonstration in the bioaugmentation plots (Figures 8a and 8b). Though the levels of JS666 were low (i.e., 3×10^3 to 10^4 cfu/mL), they were adequate to effect cDCE degradation, if suitable environmental conditions (adequate oxygen, pH and absence of inhibitory levels of TCE) were present. In general, there were very few qPCR detections in the control plots where no JS666 was added. Likewise there were no qPCR detections in either of the upgradient wells (MW-7 and MW-11), except for one instance of a 3.3×10^3 cfu/mL detection in MW-11. Thus, the pilot tests were successful in demonstrating the spread of the JS666 organisms in the bioaugmented plots. It was difficult to tell whether growth was occurring because bacterial densities did not consistently increase over time.

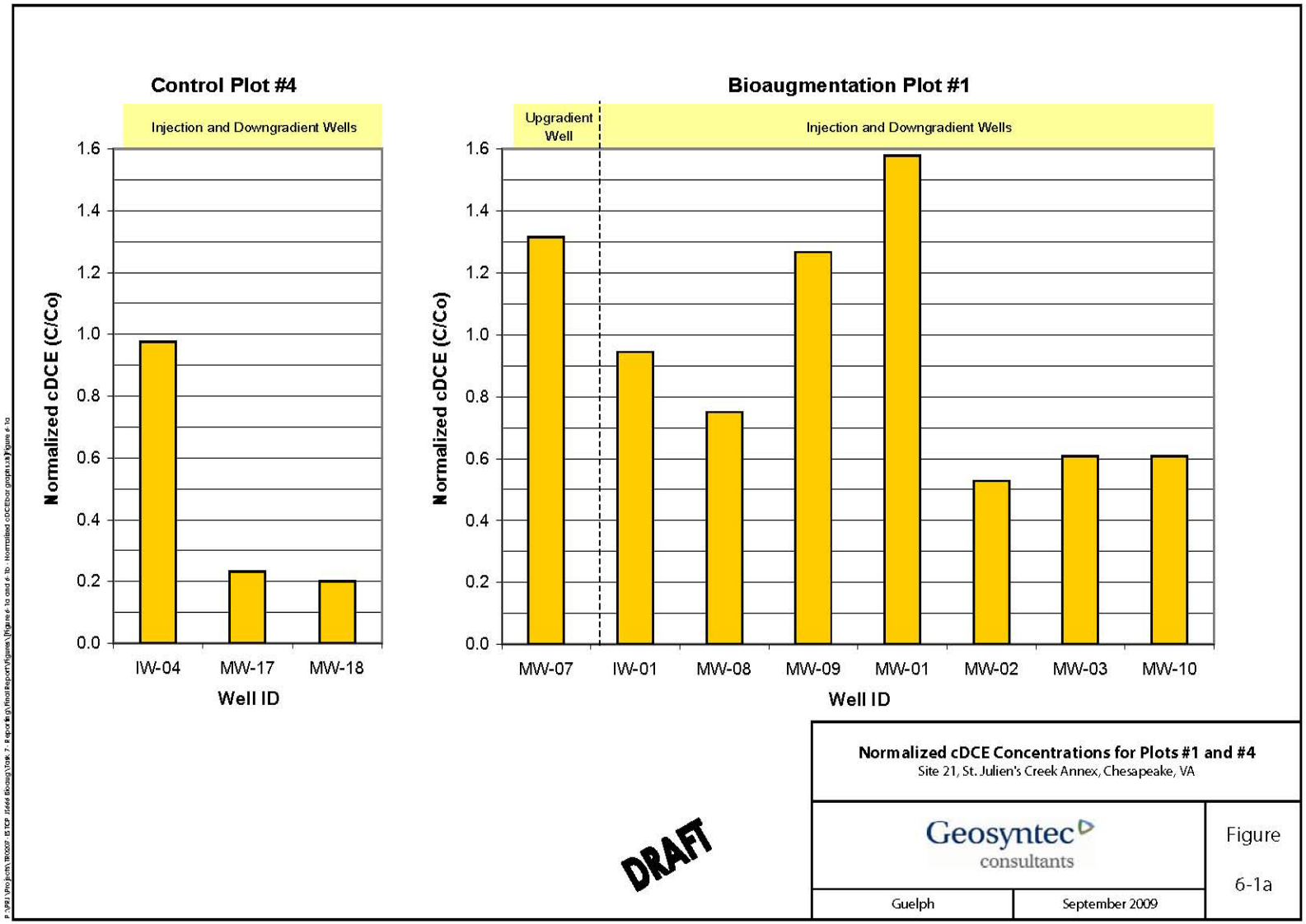

Figure 9a. Normalized cDCE concentrations for Plots #1 and #4.

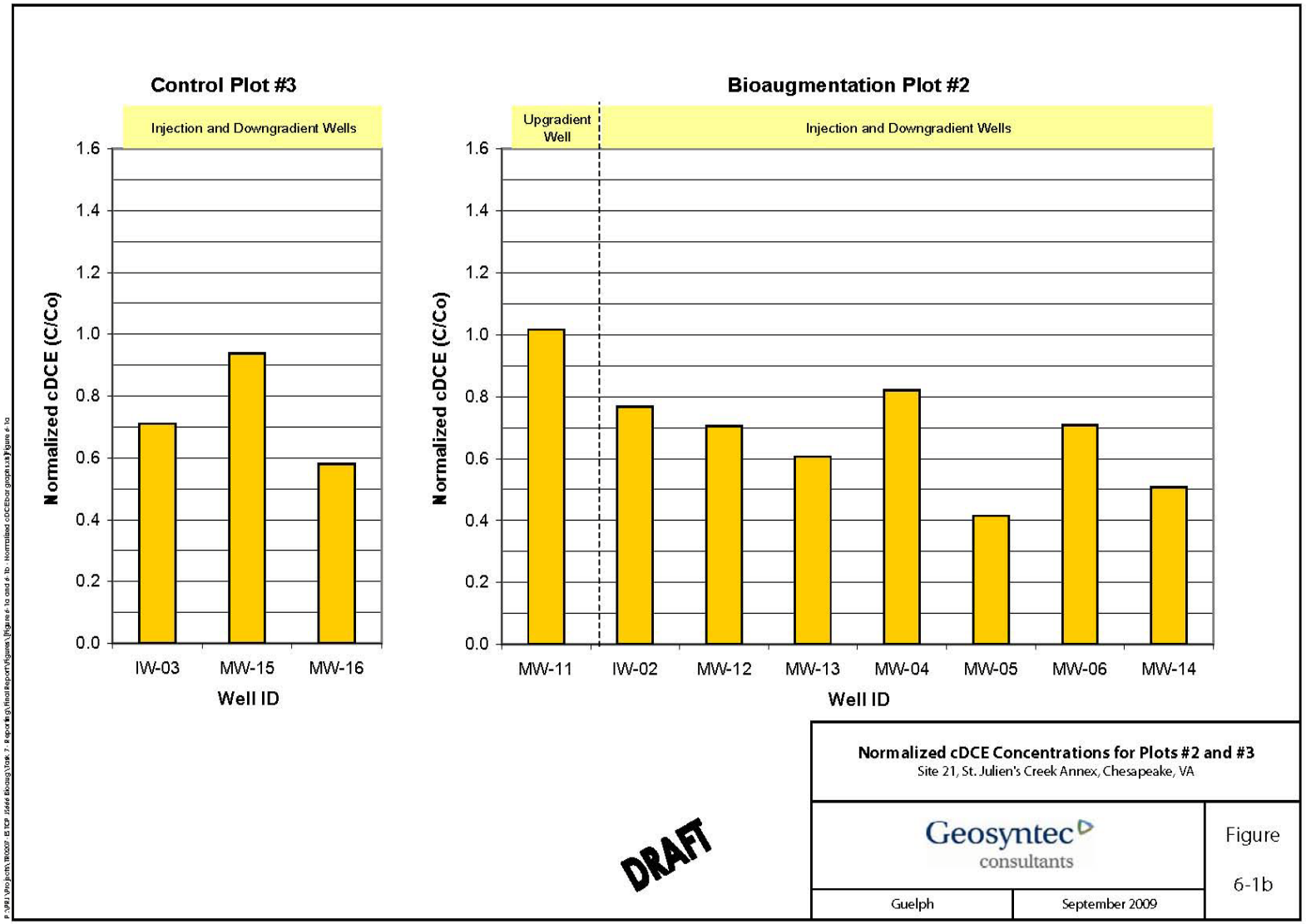

Figure 9b. Normalized cDCE concentrations for Plots #2 and #3.

The microcosms were apparently more sensitive detectors of JS666 than was qPCR—i.e., positive microcosm activity (if one uses 40 days to degradation as a measure) was observed in downgradient samples in many instances where qPCR was negative (Figures 8a and Figure 8b). The fact that such positive microcosm results occurred only in samples from locations downgradient of bioaugmentation (rather than in control plots, for example) is meaningful. It should be noted that microcosm assays were conducted at 22°C (compared to 17°C of groundwater) and were not oxygen-limited. On the other hand, field DO levels were quite low. These results demonstrated that the JS666 cells were transported through the subsurface and maintained their activity.

7.3 IMPACT OF OXYGEN LEVELS ON GROWTH AND DEGRADATION RATES

To evaluate the impact of oxygen levels on growth and degradation rates, we originally planned to compare the impact of higher oxygen levels (relative to ambient) on the growth of JS666 and rate of cDCE degradation between the bioaugmented plots with similar VOC concentrations. Despite the higher TCE concentrations in Bioaugmentation Plot #1, more biodegradation was observed as illustrated by the higher degree of δ13C enrichment in Bioaugmentation Plot #1, as discussed in Section 6.7.4. The higher degree of δ13C enrichment may have been due to more biodegradation as a result of the added oxygen in IW-01. Both Bioaugmentation Plot #1 and Plot #2 had relatively low levels of JS666 according to qPCR measurements. Therefore, the addition of oxygen appeared to increase the rate of cDCE degradation. However, increased JS666 growth rates could not definitely be identified in Plot #1 using qPCR data because the values were close to detection limits.

7.4 EASE OF USE

The ease of use of this technology was evaluated based on our experience in the field with the JS666 bacteria. Addition of the culture via injection wells was straightforward because it was an aerobic culture. Therefore, no special procedures were required to exclude oxygen during the injection. Because the native groundwater pH was low at the demonstration site, buffer was required. To distribute the buffer throughout the injection area, groundwater was extracted, amended with buffer, and then reinjected. Although the procedure was simple, it was time-consuming and needed to be repeated due to the soluble nature of the buffer employed. Aeration using the Waterloo Emitter was easy (only requiring change out of the compressed cylinder approximately monthly) but was not effective in distributing oxygen beyond the injection well. Ideally, JS666 should be employed in an aquifer with measurable dissolved oxygen (e.g., above 0.5-1 mg/L) or perhaps in an active recirculation system where oxygen can be metered into the injection stream continually.

7.5 COMPARISON OF RESULTS TO PREVIOUS STUDIES

This bioaugmentation technology was compared to P&T over a 30-year time period. Results of the cost comparison are presented in Section 8.0. A present value cost savings of 30-50% compared to P&T would represent a successful demonstration. The cost analysis shows a projected cost savings of 47%, assuming no aeration or buffering is required and sufficient oxygen is present in the groundwater naturally. Thus, under these assumptions, this technology is cost-effective when compared to P&T.

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8.0 COST ASSESSMENT

This section presents an overview of a cost assessment to implement EISB of cDCE-impacted groundwater using JS666 as a bioaugmentation culture. A detailed discussion of the cost assessment is provided in the Final Report (Geosyntec, 2010). Section 8.1 briefly describes a cost model that was developed for the application of EISB using JS666; Section 8.2 presents a summary of the cost drivers for the application of the technology; and Section 8.3 presents an overview of the cost model analysis with a comparison to a conventional P&T system.

8.1 COST MODEL

A cost model was developed to assist remediation professionals in understanding costing implications associated with the JS666 EISB technology. The cost model identified the major cost elements required to implement the EISB approach at a typical site with cDCE-impacted shallow groundwater. A summary of the cost elements is presented in Table 4, along with the associated cost for each element as incurred during the current technology demonstration. The cost model focused on pilot-scale treatment of contaminated groundwater. Specifically excluded from consideration are the costs of pre-remediation investigations (e.g., plume delineation, risk determination, and related needs), treatability studies, permitting, source zone treatment, and post remediation and decommissioning.

Table 4. Cost model for EISB using JS666.

Cost Element	Data Tracked during the Demonstration	Cost	
Capital Costs			
Design & planning	- Personnel required and associated labor	Labor	$41,200
		Expense	$10,800
Well installation	- Personnel required and associated labor - Mobilization costs - Drilling contractor cost	Labor	$12,100
		Expense	$48,200
O&M Costs			
Groundwater amendments	- Personnel required and associated labor for groundwater amendment activities - Mobilization costs - Costs for groundwater amendment chemicals (e.g. tracers, buffers) and equipment - JS666 culture costs - -Cost for aeration devices and equipment	Labor	$49,900
		Expense	$28,100
Performance Monitoring Costs			
Baseline characterization	- Personnel required and associated labor - Mobilization costs - Supplies and equipment for groundwater sampling - -Sample shipment and laboratory analytical costs	Labor	$4200
		Expense	$8200
Performance monitoring	- Personnel required and associated labor - Mobilization costs - Supplies and equipment for groundwater sampling - Sample shipment and laboratory analytical costs - Labor associated with data reporting	Labor	$58,100
		Expense	$54,100

While most of the identified cost elements are applicable to other remediation technologies, the groundwater amendments employed in this demonstration are fairly unique to the technology. The dose of the JS666 culture is relative to the size of the treatment area, so a larger treatment area will require a higher volume. The frequency and dose of other groundwater amendments (e.g., oxygen, pH buffer) will be dependent on site hydrology and geochemistry, but increased frequency and larger doses will ultimately result in higher operating costs.

8.2 COST DRIVERS

The costs to implement EISB of cDCE-impacted groundwater using JS666 will vary significantly from site to site. The key costs drivers are listed below, along with a brief discussion of their impact on cost.

8.2.1 Aquifer Geochemistry

- **Groundwater pH—**Relatively neutral pH (6.5 to 8) is required to provide optimal growth conditions for the JS666 culture. Sites where the groundwater pH is outside this range may require chemical alteration of the groundwater (e.g., addition of a buffer) to achieve a desirable pH. The added costs for buffer, amendment equipment, and labor required to inject the buffer will increase capital and operational costs of the technology. Ultimately, it may be possible to adapt JS666 to lower pH through selection of low-pH-tolerant variants.
- **Presence of other organic constituents—**Co-presence of VC, TCE, or 1,2-DCA can reduce the maximum degradation rate of cDCE. Thus the presence of co-contaminants may require additional bioaugmentation culture and longer time frames for remediation, which would increase operational costs.
- **DO—**JS666 has an absolute requirement for molecular oxygen but has been found to function at oxygen levels as low as 0.01 mg/L. Oxygen concentrations above 10 mg/L are inhibitory. Sites where groundwater DO levels are low may require additional amendments to increase groundwater DO to an ideal level (i.e., a minimum of 0.8 mg/L oxygen per 1 mg/L cDCE). The added costs for chemicals and/or oxygen delivery equipment will increase capital and operational costs of the technology.

8.2.2 Aquifer Geology and Hydrogeology

- **Hydraulic conductivity—**Microorganisms and other groundwater amendments may be more readily distributed in permeable media. Sites with a low hydraulic conductivity (K) will generally be more expensive because a greater number of injection points are required to treat a given area.
- **Geological heterogeneity—**High heterogeneity limits the uniform distribution of microorganisms and other groundwater amendments within the target treatment area. Thus treatment of sites with high heterogeneity will generally be more expensive as they may require a greater number of injection points or longer time frames for remediation.

- **Depth of impacted aquifer—**Deep groundwater plumes will be more expensive to treat as they require deeper injection and monitoring wells, which are more expensive to install.

8.2.3 Bioaugmentation System Design

- **Well size, depth, and number—**The cost of wells required to implement the technology is proportional to the depth of installation and number of wells required to treat a given area.
- **Cost of JS666 culture and other groundwater amendments—**The dose/cost of the JS666 culture is relative to the size of the treatment area. The frequency and dose of other groundwater amendments (e.g., oxygen, pH buffer) will also impact O&M costs.
- **Ability of the JS666 culture to migrate away from injection points—**The further the culture can be distributed from the injection points, the fewer injection points that are required to treat a given area. Fewer injection points will reduce the cost for well installation.

8.2.4 Available Infrastructure and Site Access

- **Available Infrastructure—**The availability of infrastructure (e.g., existing groundwater injection or monitoring wells, storage buildings, and utilities) can reduce the cost of technology implementation.
- **Site Access—**Sites having limited access for equipment and personnel (e.g., difficult terrain, overhead obstructions, or treatment beneath a building) may incur higher costs when implementing the technology.

8.3 COST ANALYSIS

The cost model was developed for a template site with cDCE-impacted shallow groundwater. A cost estimate was also prepared for a conventional P&T system to provide a point of comparison with the EISB approach using JS666. The cost model focused on treatment of a contaminated plume of groundwater. Specifically excluded from consideration are the costs of pre-remediation investigations (e.g., plume delineation, risk determination, and related needs), treatability studies, permitting, source zone treatment, and post remediation and decommissioning. Also excluded are costs for waste (e.g., soil cuttings and well development water), characterization, and disposal.

The specific site characteristics are similar to those observed at the test site used in the current technology demonstration. The template site assumes a homogenous silty sand aquifer to a depth of 18 ft bgs with a hydraulic conductivity of 7 ft/d, a horizontal gradient of 0.007 ft/ft and an effective porosity of 0.25. These aquifer characteristics result in a groundwater seepage velocity of approximately 72 ft/yr. Depth to water is 4 ft bgs. The plume of cDCE-impacted groundwater extends along the direction of groundwater flow for 500 ft and is 200 ft in width. Concentrations of cDCE, TCE, and VC in the plume are 1000 µg/L, 475 µg/L, and 15 µg/L,

respectively. Both alternatives were designed to achieve treatment to USEPA MCLs (70 µg/L, 5 µg/L, and 2 µg/L for cDCE, TCE, and VC, respectively).

The EISB using JS666 approach assumes 40 direct push injection points and six 2-inch monitoring wells screened within the saturated zone. The injection point layout assumes two transects of 20 injection points each, staggered injection point placement, 10-ft spacing between injection points, and a radius of influence of 2.5 ft, thus creating a biobarrier that measures 200-ft wide by 10-ft long (in the direction of groundwater flow). To facilitate the cost analysis, it was assumed that the groundwater pH and dissolved oxygen levels at the template site are suitable for growth of the JS666 culture and that no pH or buffer amendments are required. Assuming post-bioaugmentation degradation rates of -2.38/d, -2.23/d, and -2.55/d (estimated from laboratory microcosm tests; Geosyntec, GIT, and Cornell University, 2008) for cDCE, TCE, and VC, respectively, the residence times required for these compounds to be degraded to MCLs are approximately 1.1 days, 2 days, and 0.8 days, respectively, which are all considerably less than the estimated hydraulic residence time of 51 days for groundwater travelling through the biobarrier.

The P&T system assumes two groundwater extraction wells screened within the saturated zone and equipped with electrically operated submersible pumps. The maximum total groundwater extraction rate is assumed to be 2 gallons per minute. Extracted groundwater will be treated using granular activated carbon and then recharged into the shallow aquifer via an infiltration gallery.

Summaries of the costs for EISB using JS666 and the P&T alternatives are provided in Tables 5 and 6. The capital cost for the EISB using JS666 alternative, which includes installation of wells and bioaugmentation, is approximately $80,000. The annual monitoring cost is estimated to be $29,000 per year. The capital cost for the P&T alternative is $264,000, which is significantly higher than the capital cost for the EISB using JS666 alternative. The annual O&M costs of $56,000 per year are also higher than those of the EISB using JS666 alternative.

Life-cycle costs for the two technologies were calculated using net present value (NPV) of future costs and assuming a 30-year remediation time frame. O&M and long-term monitoring costs are discounted at a rate of 2.7% based on the real discount rate provided by the U.S. Office of Management and Budget for 30-year notes and bonds (Office of Management and Budget, 2008).

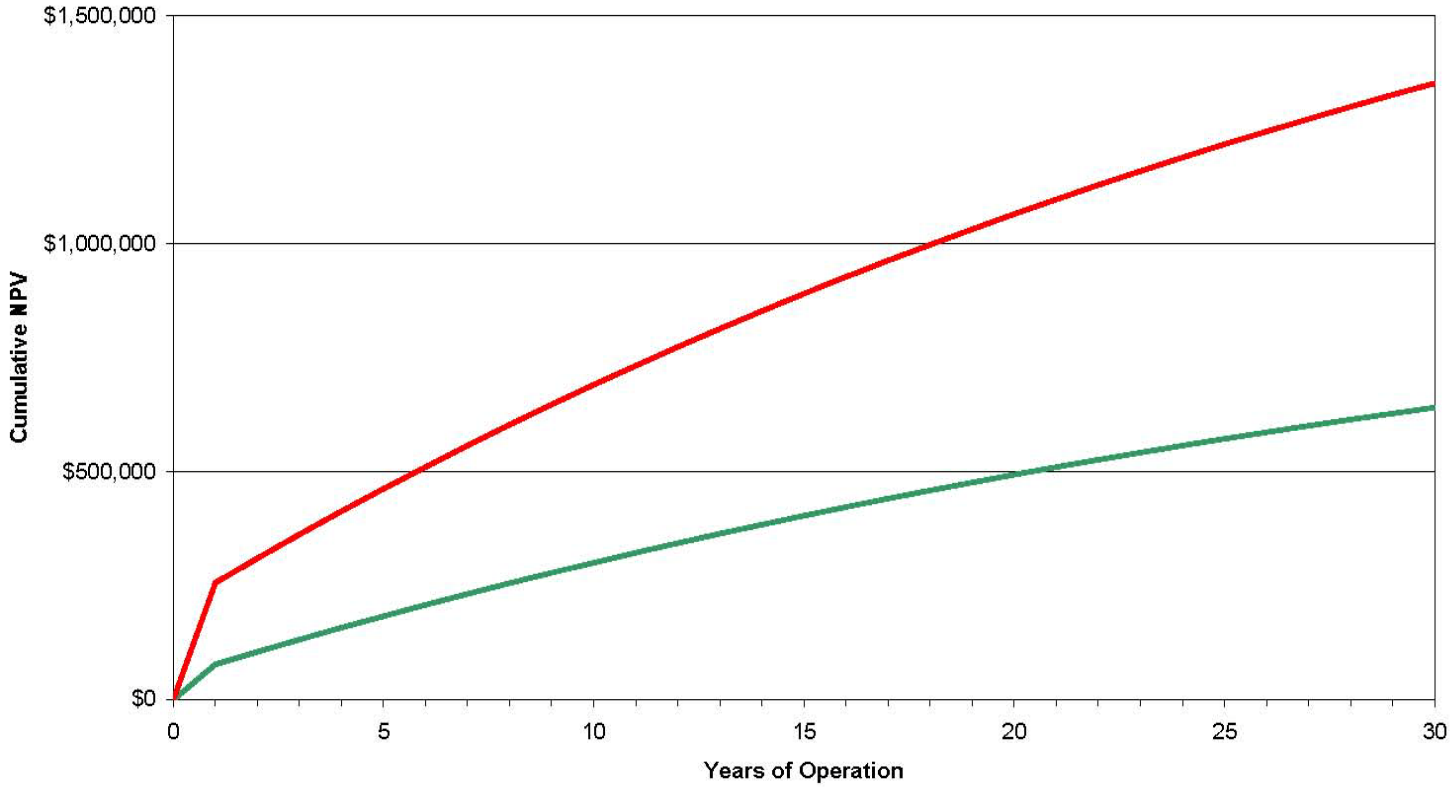
Figure 10 shows the cumulative NPV costs by year for the EISB using JS666 and P&T alternatives evaluated above. The total NPV cost for the EISB using JS666 alternative is estimated to be $641,000, and the total cost of the remedy over 30 years is estimated to be $922,000. The total NPV cost for the P&T alternative is estimated to be $1.352 million, and the total cost of the remedy over 30 years is estimated to be $1.901 million; both P&T cost estimates are significantly higher than those for the EISB using JS666 alternative.

Table 5. Cost for EISB using JS666.

Task Description	Unit	Unit Cost ($)	Quantity	Cost ($)	Cost ($) with 20% Contingency
Monitoring Well Drilling					
Six 2-inch monitoring wells, installed to 18 ft. (including mobilization, per diem, decontamination, and drums)	ea	1321	6	7926	9511
Drilling oversight (staff professional)	hr	85	108	9180	11,016
Travel, per diem	LS			4800	5760
Drilling Subtotal				**21,906**	**26,287**
JS666 Injection					
Forty injection points (including mobilization and per diem)	ea	500	40	20,000	24,000
JS666 culture	L	250	78	19,500	23,400
JS666 injection (staff professional)	hr	85	40	3400	4080
Travel, per diem	LS			1920	2304
First Year JS666 Injection Subtotal				44,780	53,736
Total Capital Costs (including contingency)					**80,026**
Annual Long-term Monitoring Cost					
Performance monitoring (including sampling and analysis)	sample	300	24	7200	8640
Reporting	LS			15,000	18,000
Annual Long-term Monitoring Cost Subtotal				**22,200**	**26,640**
Total Annual Long-term Monitoring Cost (including contingency)					**26,640**

Table 6. Cost for P&T.

Task Description	Unit	Unit Cost ($)	Quantity	Cost ($)	Cost ($) with 20% Contingency
Extraction Well Drilling					
Installation of two 4-inch extraction wells, installation to 18 ft. (including mobilization, per diem, decontamination, and drums)	ea	3200	2	6400	7680
Drilling oversight (staff professional)	hr	85	18	1530	1836
Travel, per diem	LS			1120	1344
Drilling Subtotal				**9050**	**10,860**
Treatment System Construction and Start-Up					
Design, planning, and procurement (professional)	hr	110	275	30,250	36,300
Piping, instrumentation, and process control equipment	LS			136,900	164,280
Infiltration gallery	LS			12,500	15,000
Construction supervision/oversight (staff professional)	hr	85	270	22,950	27,540
Start-Up testing (staff professional, technician)	hr	85	27	2295	2754
Travel, per diem	LS			6080	7296
Treatment System Construction and Start-Up Subtotal				180,725	253,170
Total Capital Costs (including contingency)					**264,030**
Annual O&M and Long-term Monitoring Cost					
Activated carbon changeout	ea	543	14	7602	9122
Process monitoring and maintenance (technician)	hr	55	208	11,440	13,728
Performance monitoring (including sampling and analysis)	sample	250	52	13,000	15,600
Reporting	LS			15,000	18,000
Annual O&M and Long-term Monitoring Cost Subtotal				**47,042**	**56,450**
Total Annual O&M Long-term Monitoring Cost (including contingency)					**56,450**



Legend

EISB

P&T

Notes:

NPV - Net Present Value

EISB - Enhanced In Situ Bioremediation

P&T - Pump and Treat

DRAFT

Cumulative NPV for EISB and Pump and Treat

Site 21, St. Julien's Creek Annex, Chesapeake, VA

Geosyntec consultants

Figure 7-1

Guelph | October 2009

Figure 10. Cumulative NPV for EISB and P&T.

9.0 IMPLEMENTATION ISSUES

This section provides information that will assist in future implementations of the technology. The following are four key issues related to implementation of the JS666 bioaugmentation technology:

9.1 PERMITTING

For this pilot test, only an injection notification letter was required by USEPA Region 3. At full-scale, an UIC permit may be required for the injection of bacteria and buffer amendments (if needed) and extraction and re-injection of contaminated groundwater.

9.2 BUFFER ADDITION

If the pH of the groundwater is low, buffer addition will be required. In a passive system, like the one demonstrated here, one way to distribute the buffer is to extract groundwater, amend it with buffer and re-inject. This process can be time-consuming for lower permeability aquifers. Furthermore, because the buffer is soluble, it must be re-amended periodically. If a site has low pH, a recirculation system may prove more effective for metering in buffer solution and maintaining it in the treatment zone. However, recirculation systems typically have higher O&M costs than passive systems.

9.3 AERATION

If the ambient dissolved oxygen is not sufficient to support biodegradation, then aeration is required to raise groundwater oxygen levels. JS666 does not tolerate oxygen concentrations above 10 mg/L; thus, care must be taken not to achieve concentrations above this level. There are several options for introducing oxygen. Air biosparging or diffusive emitters (expensive at full scale) can be used. Other means to introduce oxygen include the use of peroxides (either solid or liquid). Because of the possibility of achieving greater than 10 mg/L of DO locally, these products would need to be added some distance upgradient from where JS666 was injected to permit consumption of DO to levels that JS666 can tolerate.

9.4 CONTAMINANT INHIBITION

JS666 can degrade cDCE metabolically and TCE and VC cometabolically. However, as the concentration of TCE increases, the rate of cDCE degradation decreases due to competitive inhibition. Therefore, JS666 will perform better when there are lower concentrations of TCE (<500 µg/L) in groundwater. To mitigate the effects of competitive inhibition due to high TCE concentrations to some extent, higher densities of JS666 can be employed.

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10.0 REFERENCES

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APPENDIX A

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