FINAL REPORT

Post-Remediation Evaluation of EVO Treatment - How Can We Improve Performance?

ESTCP Project ER-201581

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Solutions-IES, Inc.

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Enhanced Reductive Dechlorination (ERD) with Emulsified Vegetable Oil (EVO) has been used at hundreds of Department of Defense (DoD) sites to remediate chlorinated solvents, chromate, uranium, perchlorate, and explosives. This report presents the results of detailed field, laboratory, and design evaluations at two sites (SA17 and OU2) treated with EVO and can be used to improve the performance of ERD with EVO at other sites. Major sections of the report are: (1) project scope and objectives; (2) SA17 assessment results; (3) OU2 assessment results; (4) conclusions and lessons learned; (5) conceptual model of ERD treatment with EVO; and (5) estimating base requirement for aquifer pH control.

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ACRONYMS AND ABBREVIATIONS

*c*DCE	*cis*-1,2-Dichloroethene
COC	Contaminant of Concern
CVOCs	Chlorinated Volatile Organic Compounds
DI	De-Ionized
DO	Dissolved Oxygen
DoD	Department of Defense
DPDO	Defense Property Disposal Office
ELAP	Environmental Laboratory Accreditation Program
EOS®	Emulsified Oil Substrate
ERD	Enhanced Reductive Dechlorination
FDEP	Florida Department of Environmental Protection
GCTLs	Groundwater Cleanup Target Levels
IDW	Investigatory Derived Waste
IRA	Interim Remedial Action
ISCO	*in situ* Chemical Oxidation
LTM	Long Term Monitoring
mg/L	Milligrams per Liter
µg/L	Micrograms per Liter
µS/cm	MicroSiemens per centimeter
mV	Millivolts
NADCs	Natural Attenuation Default Concentrations
NAVFAC SE	Navy Facilities Engineering Command - Southeast
NTC	Naval Training Center
OU2	Operable Unit 2
ORP	Oxidation-Reduction Potential
PAHs	Polynuclear Aromatic Hydrocarbons
PCE	Tetrachloroethene
QA/QC	Quality Assurance/Quality Control
RAOs	Remedial Action Objectives
Resolutions	Resolutions Consultants
SA17	Study Area 17

SAP	Sampling and Analysis Plan
SIES	Solutions-IES, Inc.
SOP	Standard Operating Procedures
SU	Standard Units
SWCTL	Surface Water Cleanup Target Level
TCE	Trichloroethene
TOC	Total Organic Carbon
VC	Vinyl Chloride

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EXECUTIVE SUMMARY

Enhanced Reductive Dechlorination (ERD) with Emulsified Vegetable Oil (EVO) has been used at hundreds of Department of Defense (DoD) sites to remediate chlorinated solvents, chromate, uranium, perchlorate, and explosives. This process commonly involves injecting EVO, nutrients, pH buffer or base, and microbial cultures to adjust biogeochemical conditions in the immediate vicinity of the contaminant, so that:

a. Sufficient levels of fermentable organic substrates are present to support microbial growth and contaminant biodegradation.
b. The aquifer pH is appropriate for microbial growth and contaminant biodegradation.
c. Critical microorganisms are present in sufficient numbers with the required genetic capability to degrade the pollutants.

PROJECT SCOPE AND OBJECTIVES

This report presents the results of detailed field, laboratory, and design evaluations at two sites treated with EVO as part of project 201581-PR "Post-Remediation Evaluation of EVO Treatment – How Can We Improve Performance?" supported by the Environmental Security Technology Certification Program (ESTCP). The information presented in this report can be used to improve the performance of ERD with EVO at other sites.

The two sites evaluated in this project are both located at the former Naval Training Center (NTC) Orlando: (1) Study Area 17 (SA17); and (2) Operable Unit 2 (OU2). At both sites, the remediation systems were initially successful, resulting in substantial reductions in trichloroethene (TCE) concentrations. However, concentrations of cis-1,2-dichloroethene (*c*DCE) and vinyl chloride (VC) increased in some wells due to TCE degradation and remain elevated. Results from the project evaluations were used to: (a) identify the reason(s) why the ERD systems failed to meet cleanup goals; and (b) develop new approaches and/or procedures to improve performance.

SA17 ASSESSMENT RESULTS

At SA17, two different depth intervals of a TCE source area were treated with EVO to stimulate ERD; Zone B extending from 15 to 30 ft bgs and Zone C extending from 30 to 50 ft bgs. Bioremediation performance in Zone B at SA17 source area was good with 2.8 to 4.6 Order of Magnitude (OoM) reductions in TCE. While *c*DCE and VC removal were lower, sum of organic chlorine (ΣCl) declined by 0.8 to 3 OoM indicating a substantial portion of the parent compound was reduced to non-toxic end-products. TCE removal was also good in Zone C source area at SA17. However, higher levels of *c*DCE and VC accumulated with ΣCl declining by only 0.5 to 1.5 OoM. EVO distribution in both Zones B and C at SA17 was limited by: (a) injection of too little EVO; and (b) development of stagnation zones during injection. *c*DCE and VC removal in Zone C was inhibited by the low pH due to injection of too little base to neutralize acidity produced during ERD and the background acidity of the aquifer. While *Dhc* populations were low at many locations, substantial populations of *Dhc* capable of growing on VC developed at locations with sufficient substrate and appropriate pH, indicating ERD was not limited by absence of required microorganisms. There was no evidence of significant lower permeability zones near the target treatment zone that would result in substantial back diffusion of contaminants, limiting treatment.

In summary, the primary factors limiting bioremediation performance at SA17 were inadequate levels of fermentable substrate and low pH due to injection of too little substrate, too little base to increase pH, and limited distribution of these materials throughout the target treatment zone.

OU2 ASSESSMENT RESULTS

Bioremediation was less effective in reducing chlorinated solvent concentrations downgradient of the EVO Permeable Reactive Barrier (PRB) at OU2. TCE concentrations in individual monitoring wells declined by 0 to 3.2 OoM at OU2 (median reduction of 0.5 OoM) with production of large amounts of *c*DCE. ΣCl removal at OU2 varied from 0.1 to 0.7 OoM with a median reduction of 0.2 OoM, which is lower than reported for other ERD projects. Effective distribution of EVO at OU2 was limited by: (a) injection of too little EVO; and (b) the presence of high TCE concentrations with and/or immediately adjoining lower permeability zones. Conversion of *c*DCE to ethene was inhibited by the low pH due to injection of too little base to neutralize acidity produced during ERD and the background acidity of the aquifer. While *Dhc* populations were low at many locations, substantial populations of *Dhc* capable of growing on VC developed at locations with sufficient substrate and appropriate pH, indicating ERD was not limited by absence of required microorganisms. While back diffusion of contaminants out of the underlying low permeability unit does occur downgradient of the OU2 PRB, the short travel distance from the PRB to the discharge point would greatly limit the impact of this process. In summary, the primary factors limiting bioremediation performance at OU2 were inadequate levels of fermentable substrate and low pH. The low substrate concentrations and low pH were due to injection of too little substrate, too little base to increase pH, and challenges in distributing these materials within and adjoining lower permeability units.

LESSONS LEARNED

1. Parent compound (TCE) removal was relatively good in the SA 17 source area in both Zones B and C, even though the amount of EVO and base injected was much less than that required for optimum treatment. This indicates that ERD with EVO is a fairly robust technology and good parent compound removal can be achieved with a less-than-perfect design.
2. For the most effective treatment, amendments need to be distributed throughout the entire target treatment zone. If the treatment amendments are not uniformly distributed, CVOCs can persist in untreated zones, increasing the treatment duration.
3. Generating strongly reducing conditions with methane production is a poor indicator of effective substrate distribution. Once produced in an EVO treated zone, methane is relatively unreactive and can be transported away from the residual oil.
4. EVO was not effectively distributed throughout the target treatment zone at both SA17 and OU2. The most likely causes of limited oil distribution include:
    - Common rules of thumb used for designing EVO injections can greatly under-estimate the actual oil requirement. Oil retention tests should be run to generate more accurate estimates of the actual amount of EVO required for effective treatment.
    - Addition of alkaline materials to increase aquifer pH can significantly increase oil retention, reducing contact efficiency. If possible, the alkaline materials should be added after the EVO is injected to reduce these impacts. When groundwater ionic strength is high due to background geochemistry and/or amendment addition, oil retention tests should be run with solutions representative of the groundwater geochemistry.

- The common practice of simultaneously injecting all wells to reduce injection time can result in stagnation zones, leaving some areas untreated. Injecting every other well in a group, then injecting the remaining wells in a second group can improve amendment distribution.
- While not directly addressed in this project, recirculation systems can be used to more effectively distribute substrate, pH buffers, microorganisms, and the target contaminants, improving treatment.

5. pH less than 6 can significantly reduce *c*DCE reduction to VC and ethene. Low pH can result from a variety of factors including low background pH, HCl release during dechlorination, and VFA/carbonic acid produced during substrate fermentation.

   - When the aquifer pH is less than 6.3, site characterization should include measurement of inorganic carbon, mineral acidity, and aquifer buffering capacity (pHBC). With this information, designers can generate reasonable estimates of the amount of alkaline material require to maintain pH within a suitable range.
   - In some cases, the amount of base required to maintain an appropriate pH can equal or exceed the amount of organic substrate required.

6. Dechlorinator populations including *Dhc* and *bvcA*/*vcrA* can increase and decrease with time due to temporal variations in amount of organic substrate and/or contaminant concentrations. Low dechlorinators numbers do not necessarily indicate absence of required organisms, but can result from unfavorable geochemical conditions.\

7. Remedial performance at SA17 and OU2 has improved over time as the treatment system has been modified based on our improved understanding of site conditions and in situ bioremediation processes. Site managers should recognize that it may not be practical to remediate a contaminated site with a single EVO injection. It may be more efficient and effective to employ an iterative process where a lower cost remedial system is installed, followed by monitoring and site characterization to identify treatment issues, and then the system is modified to improve performance.

ESTIMATING BASE REQUIREMENT FOR AQUIFER pH CONTROL

At both sites evaluated in this project, low pH inhibited ERD of TCE to non-toxic end-products with accumulation of cDCE and VC. The low pH was due to: (a) low background pH of the aquifer; (b) acidity produced during ERD; and (3) injection of too little base to raise the pH to appropriate levels. To aid in the design of ERD projects at other sites, an MS Excel based design tool is presented to provide preliminary estimates of the amount of base required to maintain a neutral pH during ERD. The design tool approach and calculations were presented in **Appendix D**. Required input for the design tool include: (1) treatment zone dimensions and design life; (2) site characteristics including K, porosity, hydraulic gradient, contaminant concentrations and electron acceptors produced or consumed during ERD; (3) background pH, total inorganic carbon, mineral acidity, and pH buffering capacity (pHBC); (5) mass of organic substrate and base; and (6) target pH. The design tool calculates the amount of base required to: a) raise the pH of the aquifer material and influent groundwater, and b) neutralize acidity produced during reductive dechlorination and substrate fermentation.

CONCEPTUAL MODEL OF ERD TREATMENT WITH EVO

Conclusions and Lessons Learned in this project were integrated with prior laboratory and field studies to generate a general conceptual model of ERD with EVO and pH buffer (**Appendix F**). This conceptual model provides a relatively consise summary of our current understanding of ERD with EVO including: (1) ERD microbiology and organohalide respiration; (2) environmental requirements for efficient dechlorination; (3) EVO properties, transport and retention in the subsurface; (4) EVO consumption during ERD; (5) aquifer pH and buffering; and (6) injection system design.

1.0 INTRODUCTION

Enhanced Reductive Dechlorination (ERD with Emulsified Vegetable Oil (EVO) has been used at hundreds of Department of Defense (DoD) sites to remediate chlorinated solvents, chromate, uranium, perchlorate, and explosives (Solutions-IES, 2006; Borden et al., 2008). The technology has been very successful at some sites, with extensive documentation of treatment performance in source areas (Moretti, 2005; Borden et al., 2007; Riha et al., 2009; Zhong et al., 2015) and in permeable reactive barrier (PRB) applications (Hunter, 2005; Borden, 2007b; Kovacich et al., 2007; Liang et al., 2013; Kuo et al., 2013; Watson et al., 2013). However, at some sites, remediation systems did not meet cleanup goals.

In this project, detailed field, laboratory, and design evaluations were conducted at two sites treated with EVO. The remediation systems were initially successful, resulting in substantial reductions in trichloroethene (TCE) concentrations. However, concentrations of cis-1,2-dichloroethene (*c*DCE) and vinyl chloride (VC) increased due to TCE degradation and remain elevated. Results from the project evaluations were used to: (a) identify the reason(s) why the ERD systems failed to meet cleanup goals at these two sites; and (b) develop new approaches and/or procedures to improve performance.

1.1 TECHNOLOGY DESCRIPTION

Chlorinated solvents in groundwater are a frequently encountered problem at DoD facilities. ERD can be effective for transforming more highly chlorinated species to less chlorinated species. Chlorinated solvents amenable to ERD include tetrachloroethene (PCE), TCE, *c*DCE, VC, 1,1,1-trichloroethane (1,1,1-TCA), 1,1,2-trichloroethane (1,1,2-TCA), 1,2-dichloroethane (1,2-DCA), carbon tetrachloride (CT), and chloroform (CF). For example, chlorinated ethenes, such as PCE and TCE, can be biologically degraded into non-toxic end products by a series of reactions. The typical biodegradation sequence for reductive dechlorination of these compounds is shown below:

$$\text{PCE} \rightarrow \text{TCE} \rightarrow \textit{c}\text{DCE} \rightarrow \text{VC} \rightarrow \text{ethene } (C_2H_4)$$

To enhance *in situ* biodegradation, biogeochemical conditions in the immediate vicinity of the contaminant are adjusted to ensure the following:

a. Sufficient levels of fermentable organic substrates are present to support microbial growth and contaminant biodegradation.
b. The aquifer pH is appropriate for microbial growth and contaminant biodegradation.
c. Critical microorganisms are present in sufficient numbers with the required genetic capability to degrade the pollutants.

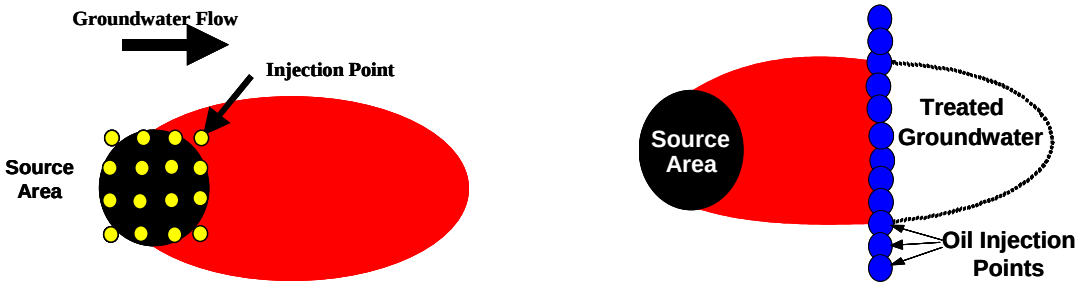
Fermentable organic substrates are commonly added to provide a carbon source for cell growth and as an electron donor for energy generation. The choice of substrate and injection method depend on the contaminant type and distribution in the aquifer, hydrogeology, and remediation objectives. Substrate can be added using conventional well installations, by direct-push technology, or by excavation and backfill. Slow-release products composed of edible oils or solid substrates tend to stay in place for an extended treatment period. Soluble substrates or soluble fermentation products of slow-release substrates can potentially migrate via advection and diffusion, providing broader but shorter-lived treatment zones.

As the emulsified oil slowly biodegrades over time, it provides a continuous source of volatile fatty acids (VFAs) to support anaerobic biodegradation of the target contaminants. Degradation of the soybean oil ($C_{56}H_{100}O_6$) results in removal of oxygen and production of acetic acid (CH_3COOH) and molecular hydrogen (H_2). This reaction is illustrated below.

$$C_{56}H_{100}O_6 + 50\ H_2O \rightarrow 28\ CH_3COOH + 44\ H_2$$

CH_3COOH can be used as an electron donor for PCE and TCE dechlorination to *c*DCE, and for removal of other competing electron acceptors (oxygen - O_2, nitrate - NO_3, ferric iron – Fe(III), and sulfate - SO_4). However, reduction of *c*DCE to ethene requires H_2 as an electron donor. As shown above, one mole of soybean oil can be fermented to produce approximately 44 moles of hydrogen.

Implementation of the EVO process involves preparation or purchase of the emulsion and injection into the treatment zone. Common injection system designs include area treatment and permeable reactive barriers (PRBs). Grids of injection wells are commonly used to treat source areas. Downgradient of the source area, injection wells may be aligned in rows, generally perpendicular to groundwater flow, to form a biologically active barrier which intercepts a plume (**Figure 1**).



Source Area Injection System Design Barrier Injection System Design

Figure 1. Common Injection System Designs

To be effective as a barrier or source treatment, the EVO must be distributed vertically and horizontally throughout the target treatment zone. If the EVO is not effectively distributed, contaminated soil and groundwater will not come into contact with the substrate and could remain untreated. In low permeability environments, it may be difficult to distribute the EVO throughout the treatment zone. This difficulty may be further amplified when groundwater velocity is low, resulting in limited distribution and higher emulsion concentrations in the immediate vicinity of the injection wells.

Geochemical factors such as levels of competing electron acceptors and presence/absence of inhibitory compounds can have a major impact on the efficacy of anaerobic bioremediation. In most cases, competing electron acceptors (O_2, NO_3, Fe(III), and SO_4) can be depleted by injecting additional oil. However, high levels of competing electron acceptors may reduce substrate longevity, increasing long-term operation and maintenance costs. Elevated levels of heavy metals (e.g., Cu, Hg, Zn) and some organic compounds can inhibit anaerobic biodegradation processes.

Dechlorinating bacteria appear to be particularly sensitive to pH with dechlorination of *c*DCE and VC to ethene inhibited below a pH of 6 (Rowlands, 2004; Vainberg et al., 2006; Eaddy, 2008). pH may be low during ERD due to several different factors including low background pH, release of free protons (H^+) during reductive dechlorination, and production of hydrochloric acid (HCl), carbonic acid (H_2CO_3), and VFAs during substrate fermentation. If the aquifer buffering capacity is low, the pH may decline inhibiting contaminant biodegradation.

Microbial populations are routinely monitored to ensure that critical microorganisms with the required genetic capability are present. Microorganisms capable of reducing PCE and TCE to *c*DCE are relatively common in the subsurface and are present at most sites. However, the only known organisms that can gain metabolic energy and grow on the reduction of *c*DCE and VC to ethene are strains of *Dehalococcoides (Dhc)* (Löffler et al., 2013). At sites where the required microorganisms are absent, commercially available bioaugmentation cultures may be added to the aquifer for improved treatment. Additional information on aquifer bioaugmentation can be found in ESTCP (2005) and Stroo et al. (2013).

The primary costs associated with ERD using EVO include injection point installation, reagent purchase, and labor for injection. These costs are affected by the mass of contaminants in the aquifer, the subsurface lithology, the depth to groundwater, and the extent of contamination. ERD treatment performance is primarily related to the presence of microorganisms capable of contaminant biodegradation, the ability to distribute the emulsion throughout the treatment zone, and the establishment of appropriate biogeochemical conditions.

1.2 STUDY OBJECTIVES AND PERFORMANCE CRITERIA

ERD operates by adjusting environmental conditions to optimize microbial growth and contaminant biotransformation. At some sites, ERD with EVO has been very effective resulting in high removal efficiency with efficient conversion of TCE to non-toxic end products. However, at the two sites examined in this project, substantial amounts of cDCE and VC accumulated. Potential causes include:

1. An inherent limitation of the technology for the specific site conditions including unusual or excessively complex hydrogeology.
2. A limitation in the design and/or implementation of the technology at these sites to provide appropriate biogeochemical conditions near the contaminant, including presence of required microorganisms, fermentable substrate, and/or pH.

The overall objectives of this project are to: (a) identify the reason(s) why the ERD systems at the former Naval Training Center (NTC) Orlando Study Area 17 (SA17) and Operable Unit 2 (OU2) sites was less than desired; and (b) develop new approaches and/or procedures to improve performance. Specific objectives of this work include the following.

a. Evaluate the performance of ERD using EVO for treatment of TCE at SA17 and OU2.
b. Determine if there were unusual site conditions that prevented effective implementation of ERD at these sites.

c. Identify limitations in the design and/or implementation of ERD at these sites to provide appropriate biogeochemical conditions near the contaminant, including appropriate levels of required microorganisms, fermentable substrate and/or pH.

d. Develop alternative design and implementation methods to improve performance at this and other sites.

1.3 PERFORMANCE CRITERIA

Performance criteria used to determine if ERD was effective for treating TCE and if biogeochemical conditions were appropriate for ERD are summarized in **Table 1**.

Table 1. Performance Criteria

Substrate levels adequate for complete dechlorination • TOC > 20 mg/L • Total VFAs excluding acetate > 2 mg/L
pH appropriate for complete dechlorination (6.0 <pH < 8.0)
Generate strongly reducing conditions (SO_4 < 10 mg/L, CH_4 > 3 mg/L)
Microbial population adequate for complete dechlorination • *Dhc* counts > 10^4 cells/mL • *vcrA* or *bvcA* > 10^3 cells/mL
TCE reduced to ethene with little accumulation of *c*DCE or VC • Parent compound (TCE) concentration reduced by greater than two Orders of Magnitude (OoM) • ΣCl reduced by greater than two OoM • Cl# < 0.5
Uniform treatment with few wells failing to meet performance criteria • Over 90% of monitoring points meet performance criteria

1.3.1 Substrate Levels Adequate for Complete Dechlorination

Effective in situ treatment of TCE by ERD requires adequate levels of fermentable organic carbon to produce acetate and H_2 and acetate as electron donors for dehalorespiration. Acetate can be used by some microorganisms to reduce TCE to *c*DCE and reduce levels of competing electron acceptors. However, H_2 is required for *Dhc* to reduce *c*DCE to ethene. Existing guidance suggests that maintaining TOC > 50 mg/L in monitoring wells within the treatment zone should be sufficient for soluble substrate systems (Suthersan et al., 2002; AFCEE, 2007). However, lower levels of TOC may be acceptable for slow release substrates (e.g. mulch, vegetable oil) (Stroo et al., 2014). A significant fraction of this TOC should be VFAs, other than acetate, which can be fermented releasing H_2 for *c*DCE reduction to ethene.

The performance criteria for substrate is to maintain at least 20 mg/L TOC in the active treatment zone and 2 mg/L total VFAs excluding acetate.

1.3.2 Circumneutral pH

Most microorganisms function most efficiently in near neutral conditions. However, dechlorinating bacteria appear to be particularly sensitive to pH with dechlorination of *c*DCE and VC to ethene inhibited below a pH of 6 (Rowlands, 2004; Vainberg et al., 2006; Eaddy, 2008) resulting in a significant decline in degradation rates (Duhamel, 2002; McCarty et al., 2007). Low pH is a particular concern during ERD, since chlorine atoms are replaced with hydrogen, releasing hydrochloric acid (HCl) (Vogel and McCarty, 1985; Mohn and Tiedje, 1992).

The performance criteria for pH is to maintain the pH of the active treatment zone between 6.0 and 8.0 throughout the active treatment period.

1.3.3 Strongly Reducing Conditions

ERD requires the establishment of strongly reducing conditions for growth of *Dhc*. In general, this requires depletion of sulfate to below 10 mg/L and generation of methane.

The performance criteria for reducing conditions is to maintain sulfate below 10 mg/L and generate substantial methane (CH_4 > 3 mg/L).

1.3.4 Microbial Population Adequate for Complete Dechlorination

Efficient conversion of TCE to non-toxic end-products (e.g. ethene) requires the presence of microorganisms with the genetic potential to gain metabolic energy through the reduction of *c*DCE to VC to ethene through a process referred to as organohalide respiration (Löffler et al., 2013). The only known organisms capable of mediating this process are strains of *Dhc* (Löffler et al. 2013). However, not all strains of *Dhc* can grow using VC as an electron acceptor (Maymó-Gatell et al., 2001). The number of *Dhc* can be determine by quantitative polymerase chain reaction (qPCR). Some strains with the ability to respire VC can be quantified by monitoring for the *vcrA* (Müller et al., 2004) and *bvcA* (Krajmalnik-Brown et al., 2004) genes. However, there are other VC reducing genes that are not detected by these assays (e.g., Ritalahti et al., 2006; Scheutz et al., 2008).

The performance criteria for microbial populations is the presence of *Dhc* counts > 10^4 cells/mL and *vcrA* or *bvcA* > 10^3 cells/mL.

1.3.5 TCE Reduced to Non-Toxic End-Products

The primary objective of this technology is to reduce TCE to non-toxic end-products with TCE, *c*DCE, and VC concentrations in groundwater reduced below regulatory levels. Important indicators of treatment performance include Orders of Magnitude (OoM) reductions in parent compound concentration, OoM reductions in Sum of Organic Chlorine (ΣCl) and reducing the Chlorine Number (Cl#) to near zero. ΣCl is the total amount of organic chlorine and Cl# is the average number of chlorine atoms per ethene. These parameters are calculated as:

$$\Sigma Cl = 4* [PCE] + 3* [TCE] +2* [DCE] +1* [VC]$$

$$Cl\# = \Sigma Cl / ([PCE] + [TCE] +[DCE] + [VC])$$

where [ ] indicates the concentration in micro-moles per liter (µM).

The performance criteria for TCE reduction to non-toxic end-products are

- TCE reduced by over 2 OoM
- ΣCl reduced by over 2 OoM
- Cl# < 0.5

OoM reductions in TCE concentrations were calculated by comparing monitoring results in individual wells immediately before injection to the most complete round of recent sampling results.

1.3.6 Uniform Treatment

In many ERD projects, chlorinated solvents are effectively reduced to non-toxic end-products in one or more locations. However, at other locations, treatment is less effective with smaller reductions in parent compounds and/or accumulation of regulated daughter products (e.g. *c*DCE and VC). These spatial variations in treatment performance are commonly attributed to non-uniform distribution of remediation amendments including organic substrates, pH buffer, nutrients and microorganisms.

The performance criteria for uniform treatment is for over 90% of monitoring points meet all other performance criteria.

1.4 STUDY APPROACH AND METHODS

Detailed post-treatment evaluations of two ERD systems were conducted at the former NTC Orlando: (1) SA17 (source area treatment); and (2) OU2 (PRB). At both SA17 and OU2, EVO addition was effective in stimulating conversion of TCE to *c*DCE and VC at most monitored locations. However, at many locations, treatment appeared to 'stall' at *c*DCE or VC and further conversion to ethene was limited. The following activities were conducted at each site to identify the cause of the apparent *c*DCE stall and develop new approaches to improve performance:

- Detailed hydrogeological characterizations were conducted to determine if unusual or excessively complex site conditions prevented effective implementation of ERD.
- Historic and current monitoring data were compiled and analyzed to determine treatment performance and identify geochemical factors limiting performance.
- Detailed evaluations were conducted of important factors that can limit performance including potential back diffusion of contaminants from low permeability zones, subsurface microbiology, amount of EVO injected, injection system hydraulics, and pH.

2.0 SA17 ASSESSMENT RESULTS

The hydrogeology, contaminant distribution, remedial activities, and monitoring results from site SA17 are summarized here and presented in more detail in Appendix A. The SA17 is located in the McCoy Annex at Former NTC Orlando (**Figure 2**). Groundwater at the site is impacted with TCE released when the site was used for motor pool storage and maintenance.

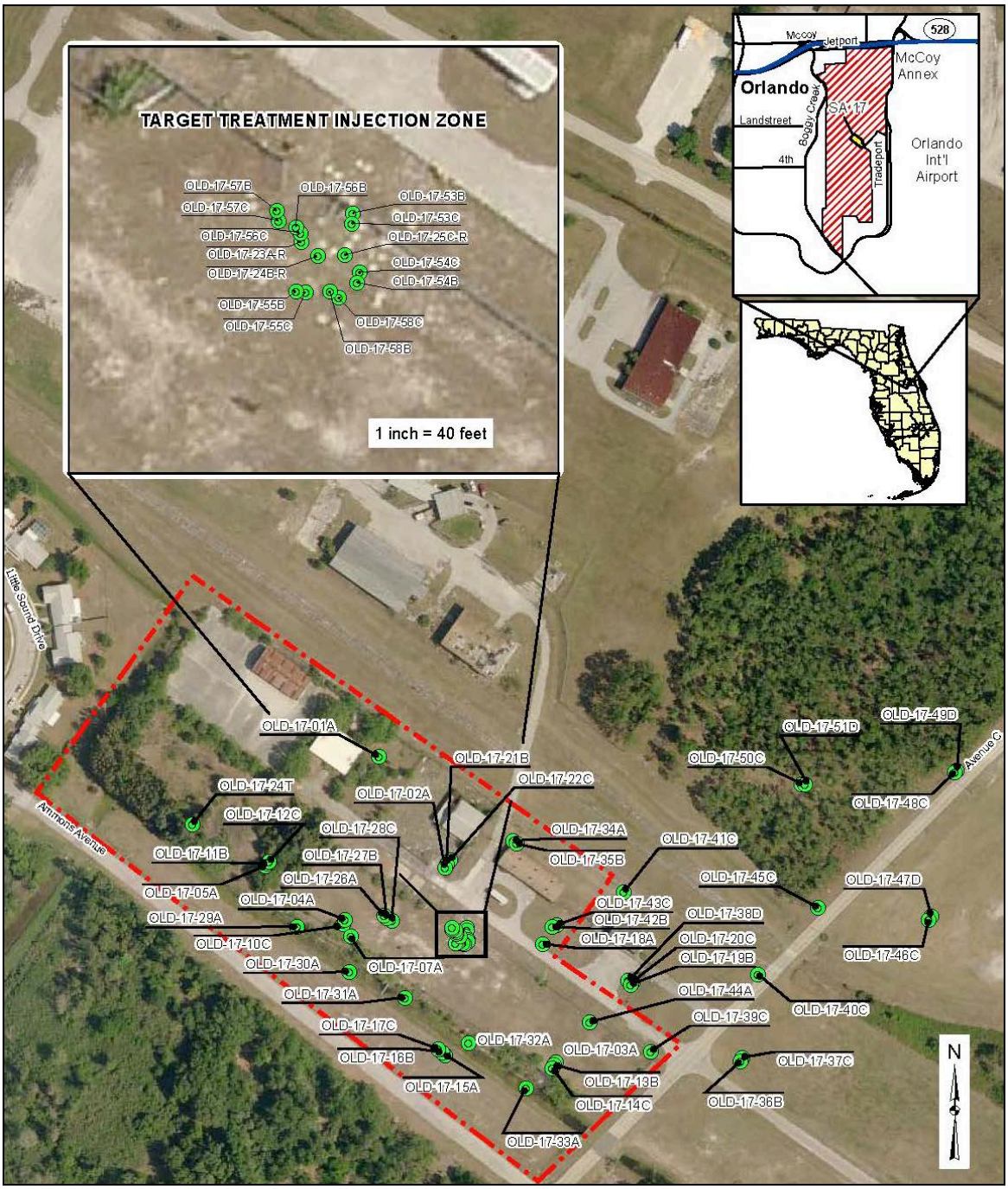


Figure 2. SA17 Monitor Well Location Map (BFA 2012)

2.1 HYDROGEOLOGY

The primary aquifers in the area include the surficial aquifer and the Floridian aquifer, separated by the Miocene Hawthorne Group which acts as a confining unit separating the two aquifers (Adamski and German 2004). Soils in the upper 30 feet of the unconfined aquifer consists of fine sand with multiple discontinuous layers of silty sand, ranging from 1 to over 5 ft thick. Beneath the lower silty sand is a layer of fine to coarse grained sand that extends from 30 to 50 ft bgs. The confining unit of the Hawthorne Group occurs at approximately 55 ft bgs and is considered to be the bottom of the surficial aquifer (HLA, 1999). The water table is typically encountered at 4 to 6 ft bgs.

The subsurface has been delineated into four different zones designated Zone A (5 to 15 ft bgs), Zone B (15 to 30 ft bgs), Zone C (30 to 50 ft bgs), and Zone D (>50 ft bgs). CH2M Hill (2006) report that, in some locations, a thin layer of lower permeability silty sand separates Zones A and B, and a thin semi-confining silty sand separates Zones B and C. Zone D is the upper Hawthorn Aquifer and is separated from the surficial aquifer by a confining clay.

In February 2015, hydraulic conductivity (K) profiles were measured using a hydraulic profiling tool (HPT) at five locations in the northern portion of the ERD treatment area (**Figure 4**). The three K profiles shown in **Figure 3** are reasonably consistent, showing a more heterogeneous zone extending from the water table (~5.5 ft bgs) to ~15 ft bgs (Zone A) with an average K of 6.3 ft/d, underlain by a more homogeneous, lower K (average K = 3.9 ft/d) region. The average K in the Zone C (=5.8 ft/d) is somewhat higher and reasonably constant with depth. There is no evidence of significant lower K zones in any boring from 5 to 45 ft bgs that would limit vertical water movement.

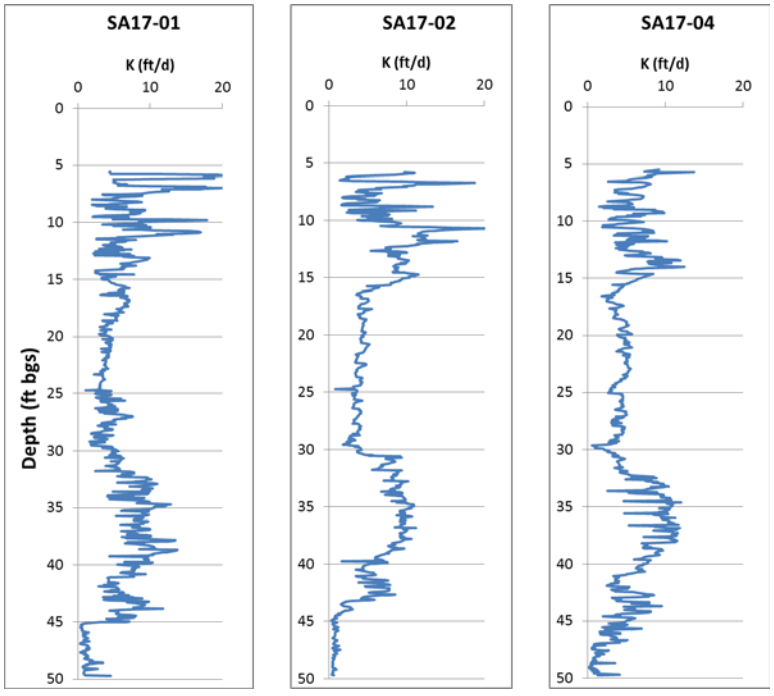


Figure 3. HPT Hydraulic Conductivity (K) Profiles Measured in February 2015.

In the shallow A Zone, groundwater is influence by the large drainage channel to the south and small drainage ditch to the north. During the wet summer season, flow is radially to the north and south towards these drainage features. In the deeper C Zone, flow is to the east. Contaminant migration indicates a northerly component to the deep groundwater flow further east from the site. Groundwater flow in the intermediate B Zone is influenced by both the shallow and deep systems. In most areas, a downward vertical hydraulic gradient of 0.007 to 0.020 ft/ft exists within the surficial aquifer. Near the drainage ditch, there is an upward gradient of approximately 0.25 ft/ft and groundwater discharges to the ditch. In the C Zone, horizontal gradient ranges from 0.003 to 0.004 ft/ft. Assuming an effective porosity of 0.25, the average seepage velocity in the C zone is about 30 ft/yr.

In summary, there were significant spatial variations in K typical of many aquifers. However, there is no evidence of unusual or excessively complex site conditions that would have prevented effective implementation of ERD.

2.2 CONTAMINANT DISTRIBUTION AND REMEDIAL ACTIVITIES

Between 2000 to 2002, organic contaminants in saturated soil and groundwater were treated by in situ chemical oxidation (ISCO). This involved the injection of 100,000 lb of hydrogen peroxide (H_2O_2) and trace quantities of metallic salts over a roughly 100 ft by 300 ft area in four injection events. ISCO was effective in reducing dissolved phase TCE concentrations by 88%. However, TCE concentrations rebounded over time. Following the ISCO treatment, a Membrane Interface Probe (MIP) investigation identified areas with residual chlorinated volatile organic compounds (CVOCs) (CH2M Hill, 2006). Confirmatory laboratory analyses indicated the presence of residual contamination with maximum CVOCs concentrations up to 577,000 µg/L. Groundwater sampling indicated highly localized impacts, with the majority of contaminant mass between 15 and 35 ft bgs, and extending approximately 50 feet laterally. The maximum TCE concentration prior to ERD was 47,300 µg/L in OLD-17-53C1. *c*DCE and VC concentrations were much lower with maximum concentrations of 2,500 µg/L *c*DCE in OLD-17-53C2 and 820 µg/L VC in OLD-17-20C.

Prior to implementing the ERD project, CH2M Hill (2006) implemented an extensive microbial and geochemical characterization. Results showed that the effect of the ISCO treatment had dissipated and the aquifer was moderately reducing with some nitrate, some dissolved iron, low to moderate methane levels and 1 to 4 nanomolar (nM) dissolved H_2. Groundwater at SA17 was somewhat acidic, with pH ranging from 5.4 to 6.3, and total alkalinity ranging from 8.6 to 111 milligrams per liter (mg/L). *Dhc* was detected in both the control and baited (lactate) Bio-Traps.

In 2006, EVO was injected at the site to stimulate ERD. The selected design included separate injection and extraction systems in B and C zones. Groundwater was extracted from a central well, amended with EVO, and distributed between six injection wells located in a rough circle surrounding the center well. Injection wells were screened from 15 to 25 ft (B zone) and 30 to 40 ft bgs (C zone) with a 5-foot vertical gap between the injection intervals. The EVO used in this injection (EOS® 598B42) contained 60% soybean oil, 4% soluble substrate, 10% emulsifiers and vitamin B-12. Injection and monitor well locations are shown in **Figure 4**.

During the field implementation, soils within B zone were found to be less permeable than anticipated, and less EOS® was injected than planned. To more effectively treat B zone, CH2M HILL performed a "polishing" injection of EVO in 2008 using direct-push technology in the area around OLD-17-55B and OLD-17-56B. In February 2012, Solutions-IES (2013) injected additional EVO and a pH buffered EVO formulation (AquabupH™) through the previously installed injection wells in both zones B and C to replenish the previous treatment and raise the aquifer pH to a level appropriate for ERD. AquabupH™ used in the injection contained 39% soybean oil, 4% soluble substrate, 7% emulsifiers and 10% $Mg(OH)_2$. Details of the 2006 and 2012 injections are provided in **Appendix B**.

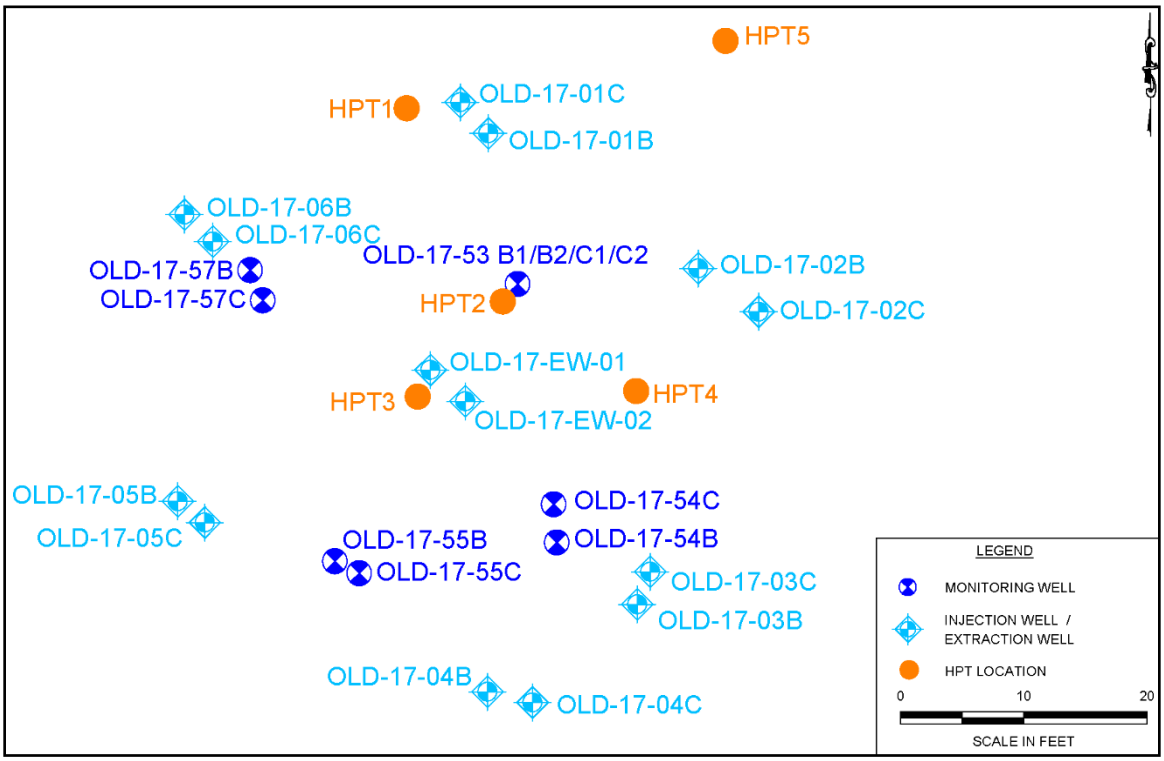


Figure 4. Injection and Monitor Wells in the Vicinity of SA17 Bioremediation Project.

2.3 REMEDIAL SYSTEM PERFORMANCE

Performance of the ERD remediation system was evaluated using historical groundwater monitoring data (CH2M HILL, 2010; BFA, 2012; Resolution, 2013; Solutions-IES, 2013b) and data collected as part of this project. Groundwater monitoring data collected as part of this project included samples from monitor and injection wells in the treatment zone and analyzed for VOCs, methane, ethane, and ethene (MEE), nitrate (NO_3), sulfate (SO_4), geochemical indicators (O_2, pH, oxidation-reduction potential (ORP), acidity, alkalinity), major cations and anions (Na, K, Ca, Mg, Fe, Mn, Cl, Br), molecular biological tools (major dechlorinators and genes), and VFAs. As the first step in this process, all available data was compiled into a single master database and reviewed to identify trends in important parameters, important biological and geochemical factors that might influence ERD, and outliers.

2.3.1 Biogeochemistry

Prior to implementation of the ERD system in 2006, the aquifer was slightly reducing, with moderate to high dissolved iron (1 to 5 mg/L) and some CH_4 (typically 0.1 to 2 mg/L). Sulfate levels at some locations were above 200 mg/L, likely due to prior injection of sulfuric acid during ISCO. Shortly after EVO injection, SO_4 concentrations declined in all wells and remained between 5 and 20 mg/L for the duration of monitoring. Sulfide levels were occasionally monitored and were consistently below 1 mg/L. There is no indication that elevated levels of sulfate or sulfide inhibited reductive dechlorination.

Shortly after the first EVO injections in 2006, there was a sharp increase in TOC in many of the B and C zone wells (**Figure 5**). However, in a few wells, TOC never increased above baseline suggesting poor EVO distribution in some areas. In Zone B, TOC declined rapidly in all wells, with the average TOC concentration dropping below 10 mg/L within one year. In Zone C, TOC declined somewhat more gradually. However, average TOC in zone C wells was near 10 mg/L at 2-3 years after injection. TOC increased somewhat in several of the B and C zones wells following the 2012 reinjection, then quickly declined to near background levels. In wells that were monitored for VFAs, there was a spike in VFAs immediately after injection, then quickly declined. With the exception of OLD-17-54C, total VFAs were less than 2 mg/L in all monitor wells within 9 months of the 2012 injections (data not shown). In summary, TOC levels were below optimum (< 20 mg/L) in most wells for most monitoring events. Low levels of bioavailable substrate likely limited reductive dechlorination.

Methane (CH_4) levels were less variable than TOC, gradually increasing with time in all wells. There was no apparent correlation between average CH_4 and TOC concentrations. CH_4 was elevated in some wells where TOC was low, while CH_4 was lower in some wells with high TOC. This indicates that the presence of methane is not a good indicator of bioavailable carbon. TOC is consumed relatively quickly in the subsurface and only remains elevated close to residual vegetable oil. In contrast, once CH_4 is produced, it is relatively unreactive and can migrate significant distances away from the residual vegetable oil (Borden et al., 2015).

In Zone B, the average pH was 5.8 prior to injection, and then gradually declined to ~5.5. After the 2012 AquabupH injections, pH increased in Zone B, remaining at or above 6 through 2016. An important exception to this general trend was well OLD-17-57B, where pH remained low with an average value of 5.7. TOC also remained low in OLD-17-57B, indicating this well was not effectively contacted. In Zone C, the initial pH was lower (pH~5.2), but pH remained constant or increased slightly following the 2006 injection. Average pH increased in Zone C following the AquabupH injection reaching a maximum of 5.9 at six months after injection, then gradually declined to near 5 in 2016. While AquabupH injection did temporarily increase the average pH in Zone C, results were variable with pH ranging from 5.0 to 7.3 at six months after injection. Overall, these results indicate that pH was below optimum in all wells from 2006 to 2011. Buffer addition was effective in raising the pH in Zone B and maintaining it at an appropriate level for four years. However, buffer addition was less effective in Zone C, and pH declined below appropriate levels within one year after injection.

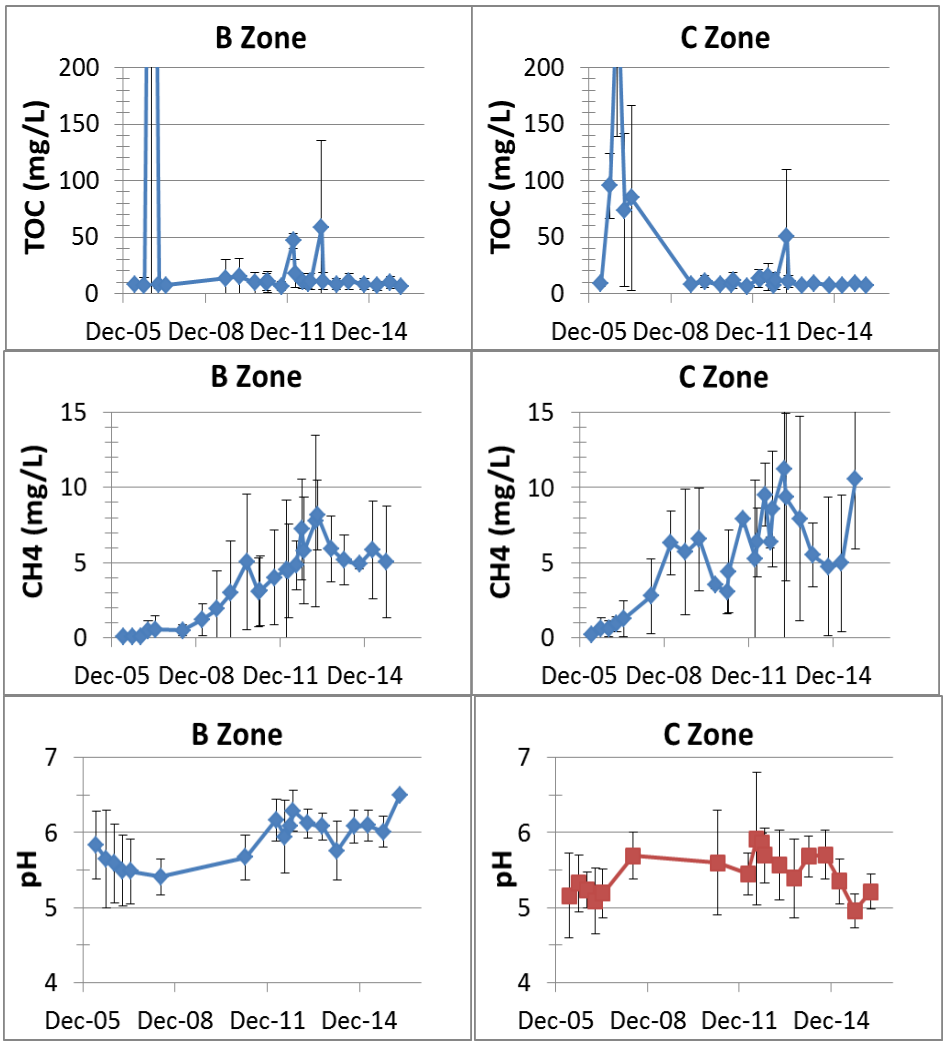


Figure 5. Average CH_4, TOC, and pH in SA17 B and C Zones Monitor Wells.

(Error bars are ±1 standard deviation).

2.3.2 Microbiology

Dehalogenating bacteria including *Dhc*, *Dhb*, and functional genes (*tceA*, *bvcA*, *vcrA*) were not routinely monitored prior to 2011. However, sampling of lactate baited biotraps in 2005 prior to EVO injection indicated low, but detectable numbers (10^1 to 10^3 *Dhc* cells/biotrap bead, AGVIQ-CH2M HILL JV-II, 2006). Microbial monitoring results are presented in detail in **Appendix B, Table B-4**.

Prior to reinjection with EVO and buffer in 2011, dechlorinator populations were low in OLD-17-54B, OLD-17-54C, OLD-17-55C, and OLD-17-56C. However, *Dhc* counts greater than 10^4 cells/mL and *bvcrA* or *vcrA* reductase counts greater than 10^3 cells/mL were observed in OLD-17-55B and OLD-17-56B indicating substantial numbers of *Dhc* with the ability to reduce VC to ethene were present in the B zone. Following EVO and buffer addition, *Dhc* increased to over 10^5 and *bvcrA* / *vcrA* reductase increased to over 10^4 cells/mL in OLD-17-55B and OLD-17-56B.

In 2012, dechlorinator numbers began to decline in these wells, even though pH was near 6 and substantial levels of DCE and VC remained. The decline with dechlorinators with time is likely due to depletion of fermentable organic carbon. While 10-20 mg/L of TOC was present in these wells, propionic and other high molecular weight fatty acids that can be fermented releasing H_2 were below detection (data not shown). *Dhc* numbers remained below 10^3 in OLD-17-54B, presumably due to the rapid depletion of organic carbon in this well. In C zone wells OLD-17-55C and OLD-17-56C, *Dhc* numbers spiked immediately after injection, then declined as pH declined. In contrast, *Dhc* numbers increased over time in OLD-17-54C, reaching over 10^4 cells/mL of *Dhc* and *bvcrA* reductase. The increase in *Dhc* numbers coincided with an increase in pH.

These results indicate chloroethene degradation was not limited by absence of required microorganisms. Instead, microbial growth and chloroethene removal was limited by low levels of fermentable organic carbon and/or low pH.

2.3.3 Injection Well Sampling

Selected injection wells were sampled to determine if TCE was extensively degraded in wells where distribution of the reagents would be most effective. Monitoring results (**Appendix B, Table B-5)** indicate that TCE and its degradation products were being effectively treated in three of the injection wells (OLD-17-EW-01, OLD-17-EW-02 and OLD-17-IW-02C). In these three wells, pH is greater than 6, TOC concentrations were greater than 30 mg/L, and TCE and DCE have been substantially reduced. In two of these wells (OLD-17-EW-02 and OLD-17-IW-02C), relatively high numbers of dechlorinators are present. In the third well (OLD-17-EW-01) dechlorinator populations are relatively low, potentially because chlorinated ethene concentrations are too low to support an active population. In three wells, ERD is more limited and high concentrations of TCE, DCE, and/or VC persist. In two of these wells, ERD is probably limited by pH below optimum levels (pH is 4.9 in OLD-17-IW-01C and 5.7 in OLD-17-IW-02B). However, in OLD-17-IW-01B, pH = 6.1 and TOC = 86 mg/L, while *c*DCE=6,280 g/L with modest levels of ethene and low dechlorinator populations. The reason for the limited degradation in this well is not known.

2.3.4 Chlorinated Ethenes

Figure 6 illustrates the use of ΣCl and Cl# for evaluating ERD progress in OLD-17-56B. Prior to substrate EVO addition, TCE was the dominant chloroethene, Cl# was ~3 and ΣCl was approximately 3 times the TCE concentration in micro-moles per liter (µM). Following EVO addition in 2006, TCE declined below detection with a concurrent drop in Cl# from ~3 to ~1, and ΣCl declined from ~ 100 µM to 10 µM, indicating a 90% reduction in the amount of organic chlorine. However, from 2009 to 2015, ERD appeared to stall, with a very limited decline in *c*DCE and small increase in VC and ethene. The apparent stall in ERD is reflected in the near constant values of Cl# and ΣCl. In 2016, *c*DCE levels dropped sharply and ethene increased which is reflected in the decline in Cl# and ΣCl.

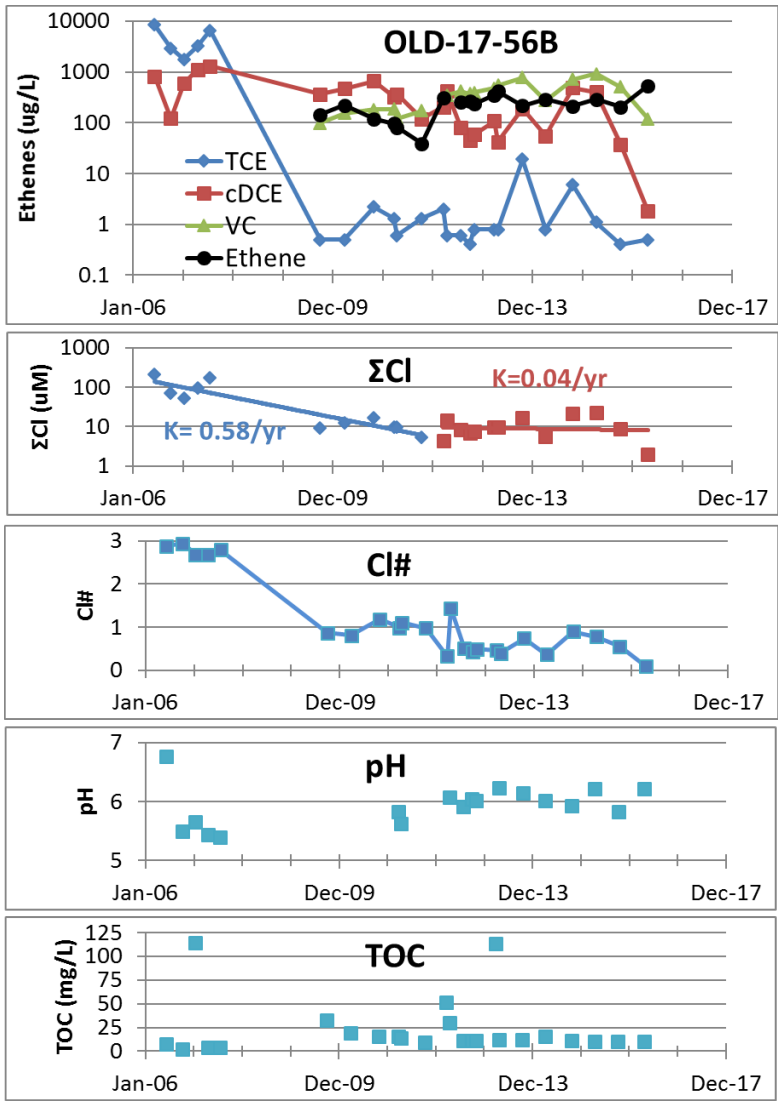


Figure 6. Variation in TCE, *c*DCE, VC, Ethene, ΣCl, Cl#, pH and TOC versus Time in OLD-17-56B.

Figure 7 shows the variation of ΣCl, Cl# and pH with time in Zones B and C monitor wells. In the B zone wells, ΣCl declined by one to two orders of magnitude between 2006 and 2009 as the Cl# decreased from 3 (indicating mostly TCE) to between 1 (indicating VC) and 2 (indicating *c*DCE). Between 2006 and 2011, pH was low which likely inhibited conversion of VC to ethene. AquabupH addition in 2012, increased the pH to near 6, followed by gradual declines in both ΣCl and Cl#.

In the C zone wells, ERD was much less effective. Following the EVO injection in 2006, the Cl# of all the C zone wells declined from 3 (TCE) to near 2 (*c*DCE), then stalled, presumably due to the low pH (average pH in Zone C was 5.3). Following AquabupH addition in 2012, there was a temporary increase in pH in most wells, but then pH declined again to below 5.5.

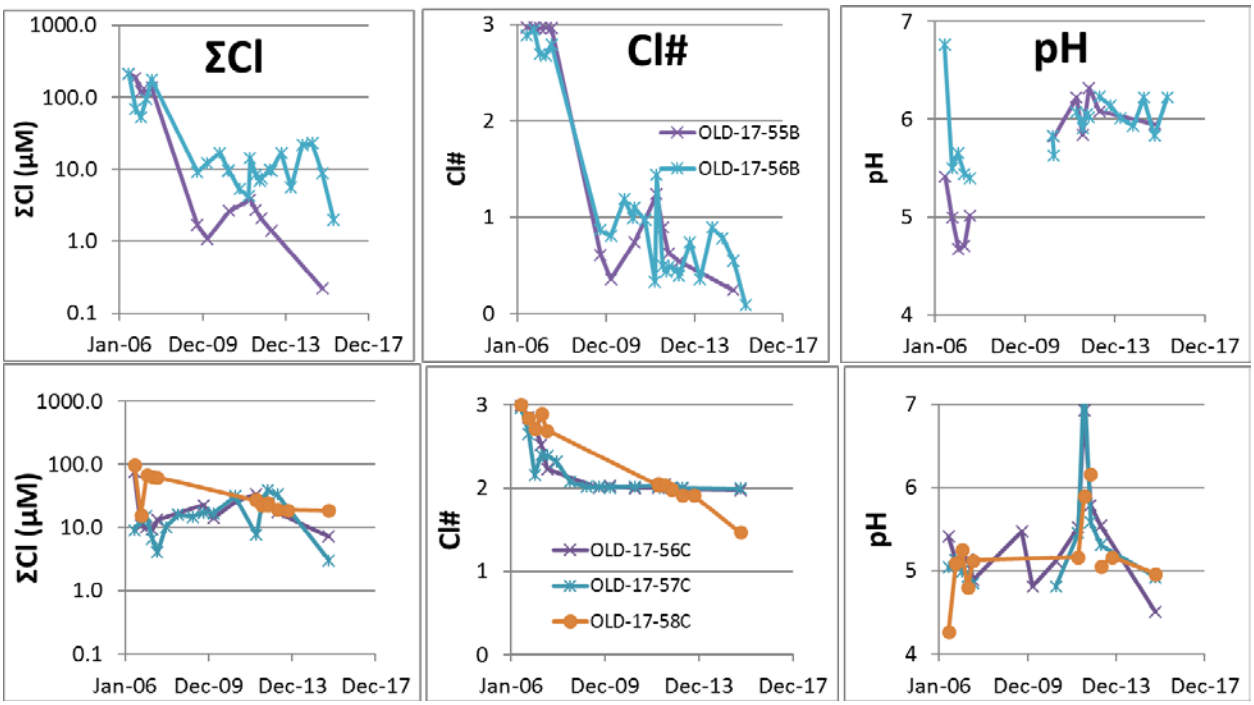


Figure 7. Variation in ΣCl, Cl# and pH in Selected Zones B and C Monitor Wells.

2.3.5 Spatial Distribution of TCE and *c*DCE

As part of the site characterization work for this project, a MIP equipped with a halogen specific detector (XSD) was used to measure vertical profiles of total halogens at the same time the HPT profiler was used to measure K. These results indicated that total halogen concentrations were relatively low at most locations. However, in a boring adjoining OLD-17-53, a spike in XSD response was observed at about 30 ft bgs. **Figure 8** shows results of CVOCs analysis of soil samples collected from a continuous boring installed close to this location. Relatively high concentrations of TCE and *c*DCE were observed in the interval from 25 to 32 ft bgs. TCE concentrations in this interval varied from 5 to 7.5 µg/g which would result in aqueous concentrations of approximately 40,000 to 60,000 µg/L if 100% of the TCE was dissolved in the pore water. The maximum TCE concentration observed in the closest monitor well screen (OLD-17-53C1 screened from 30 to 35 ft bgs) was 47,200 µg/L in June 2006. Since 2012, TCE concentrations in this well screen have varied from 2.5 to 538 µg/L. However, TCE degradation products were higher with *c*DCE varying between 127 and 18,200 µg/L and VC varying between 117 and 822 µg/L. These concentrations are quite variable with TCE and *c*DCE concentrations declining to low levels, then spiking by two orders of magnitude. This variability is consistent with a residual source in this area.

The continued presence of *c*DCE, VC and smaller amounts of TCE in OLD-17-53C1 appears to be due to an untreated zone with relatively high residual TCE levels in the 25 to 32 ft. HPT profiles did not indicate a significant reduction in permeability in this interval, which would have limited reagent distribution. However, the B zone injection wells extended from 15 to 25 ft bgs and the C zone injection wells extended from 30 to 40 ft bgs, so the injection system design could have limited reagent distribution in the interval from 25 to 30 ft bgs.

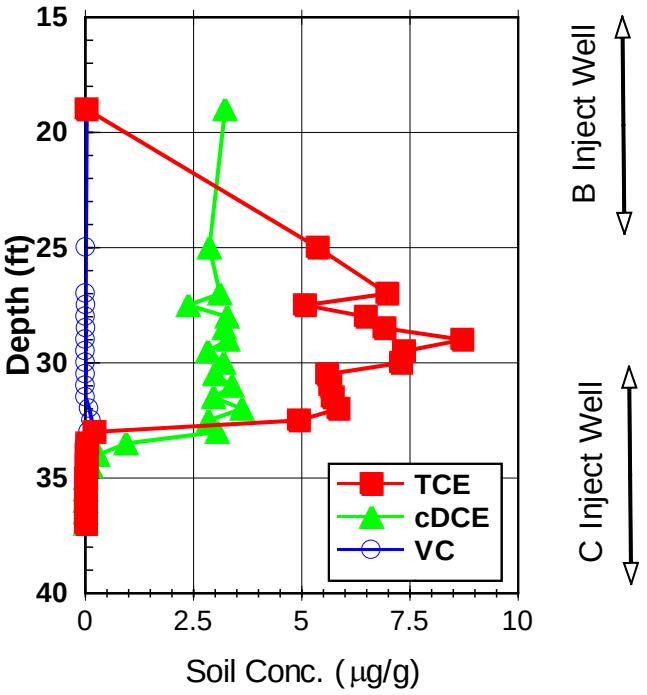


Figure 8. Concentrations of TCE, *c*DCE, and VC in Soil Samples Collected from a Boring Adjoining OLD-17-53.

2.4 EVALUATION OF FACTORS LIMITING REMEDIAL PERFORMANCE

2.4.1 Back Diffusion.

There is no evidence that the presence of lower permeability zones significantly reduced treatment efficiency or back diffusion of contaminants contributed to contaminant rebound after treatment. HPT profiles shown in **Figure 3** do not show any indication of substantial lower permeability zones between 15 to 40 ft bgs. Particle size distribution analysis of samples from two soil borings (SB1 and SB2) indicate the material from 15 to 40 ft bgs is predominantly medium sand with 0.5 to 6% silt+clay (**Appendix B, Table B-6**). The Hawthorne confining unit at is present at 45 - 50 ft bgs, which could slowly release dissolved contaminants that had diffused into this lower permeability zone. However, this unit 5 to 10 ft below the target treatment interval and is unlikely to substantially influence treatment performance from 25 to 35 ft bgs.

2.4.2 Evaluation of EVO Loading and Injection Volumes

The EVO design tool developed under ER-0626 (Borden et al., 2008; Weispfenning and Borden, 2008) was used to evaluate the 2006 and 2012 injections and determine if sufficient EVO and water were injected to achieve at least 60% contact efficiency for SA17. Details of this evaluation are presented in **Appendix B, Section B.4.2**.

Prior research has shown that oil droplet retention is influenced by the zeta potential of the oil droplets and the aquifer material (Coulibaly and Borden, 2004). To evaluate the effect of solution ionic strength on oil droplet retention by the aquifer material, the zeta potential of the EVO used in the injections (EOS 598B42) and aquifer material from SA17 were measured in deionized (DI) water and a solution of 200 mg/L $CaCl_2$ (approximately 73 mg/L Ca). The zeta potential of all materials was negative in the presence of both DI water and 200 mg/L $CaCl_2$ (**Appendix B, Table B-7**). However, the zeta potential of both the oil droplets and the aquifer material was more strongly negative in DI water than the $CaCl_2$ solution, indicating that oil droplet-sediment particle repulsion will be greater and oil retention will be lower in DI water.

Maximum oil retention by the aquifer material was measured in laboratory columns packed with aquifer material collected from 15 to 23 ft bgs (B zone) and 30 to 40 ft bgs (C zone) at SA17. The columns were first saturated, then 150 mL (~3 pore volumes [PV]) of a 20% EVO dilution, followed by ~3 PV of chase water. Measured oil retention in the B zone sediment flushed with 200 mg/L $CaCl_2$ was significantly higher than for the same material flushed with DI water consistent with reduced electrical repulsion between the oil droplets and sediment. These results demonstrate that ionic strength and cation concentration can have a major impact on zeta potential and oil retention.

Sampling of monitoring wells in both the B and C zones in 2015 showed low ionic strength groundwater with average concentrations of 9 mg/L Na, 2 mg/L K, 19 mg/L Ca and 7 mg/L Mg. Under these conditions, the oil droplets would be more strongly repelled by the aquifer material and oil retention would be low. However, monitoring of injection wells sampled in 2016 (**Appendix B, Table B-5**) found 10 to 33 mg/L Na, 2 to 4 mg/L K, 30 to 276 mg/L Ca, and 22 to 173 mg/L Mg, presumably due to addition of $Mg(OH)_2$ to raise the aquifer pH during the 2012 injections. The increased ionic strength likely increased oil retention by the aquifer material, reducing EVO spread during the 2012 injections.

Tables B-9 and **B-10** in **Appendix B** show the input parameters used in the original design of the 2006 and 2012 EVO injections (AGVIQ-CH2M HILL JV-II, 2006; Solutions-IES, 2011b) and the current best estimates for these parameters, based on additional site characterization results and laboratory column tests. Evaluation of the 2006 and 2012 injection volumes with the ESTCP EVO Design tool indicates that:

- Based on the original design assumptions, the total volume of diluted EVO and chase water should have been more than sufficient to effectively distribute the oil droplets throughout the treatment zone, achieving EVO contact efficiencies greater than 60%.
- The measured oil retention with DI water was over twice the value assumed in the original design. This under estimate of oil retention resulted in too little EVO being injected, which would have significantly reduced contact efficiency and treatment.
- Injection of a mixture of EVO and colloidal $Mg(OH)_2$ in 2012, likely increased the injection solution ionic strength and oil retention near the injection wells, further reducing EVO contact efficiency.

2.4.3 Hydraulic Design

The groundwater flow model, MODFLOW (Harbaugh et al., 2000) was used to simulate flow patterns during the 2006 and 2012 injections. EVO transport and retention was simulated using the reactive transport model RT3D (Clement, 1997) with a reaction module developed to simulate retention of colloidal oil droplets (Coulibaly et al., 2006). Injection volumes and flowrates used in model calibration are presented in **Appendix B, Sections B.2.1** and **B.2.2**. A maximum oil retention of 0.0027 g/g was used in all simulations for consistency. Details of the model simulations are provided in **Appendix B, Section B.4.3**.

Model simulations indicate that EVO distribution would have been poor following the 2006 injections, with much of the aquifer left untreated due to: (a) the small amount of EVO injected into Zone B; and (b) the gap between the Zones B and C injection well screens. Contact efficiency was better in Zone C due to the larger amount of EVO injected in this zone. However, there are significant gaps between treated zones due to the development of stagnation zones during simultaneous injection of all wells. Simulated EVO distribution was also poor in 2012 due to the small amount of EVO injected. EVO distribution could have been improved by: (a) injecting significantly more EVO to allow for the greater oil retention by the aquifer material; and (b) altering the injection sequence so half the wells (every other well) were injected in a group, followed by the remaining wells as a second group. This alternative injection approach would eliminate stagnation zones in both Zones B and C and should be reasonably effective in pushing EVO into the 5 ft gap between the B and C zones well screens.

2.4.4 Buffer Design

As part of this project, an Excel spreadsheet was developed to estimate the amount of base required to raise the aquifer pH to the desired level, and maintain it at that level for the design treatment period. The theoretical basis of the spreadsheet calculation procedures is presented in **Appendix D**. Details of the actual calculations for the 2012 injections at SA17 are presented in **Appendix B, Table B-11**.

To provide guidance on amounts of base required during future injections, the base addition design tool was used to estimate the amount of NaOH, Na_2CO_3, $NaHCO_3$ or $Mg(OH)_2$ required to maintain the pH at different levels following the 2012 injections for both Zones B and C. Results presented in **Figure 9** indicate that amount of base required is very sensitive to the target pH. For target pH values less than 6.3, the majority of the CO_2 released from substrate fermentation during ERD remains in the protonated form (H_2CO_3*) and base demand is relatively low. Increasing the target pH to 7, results in the conversion of H_2CO_3* to HCO_3^- and H^+, requiring large amounts of base to neutralize the H^+ released. Further increases in pH above 7 does not require as much base since most of the H^+ has already been released from the H_2CO_3*.

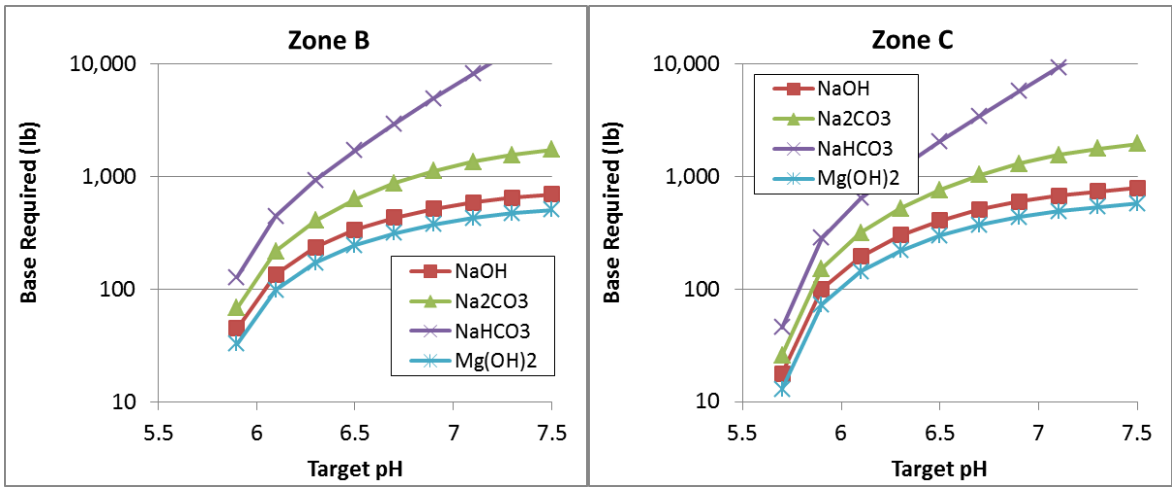


Figure 9. Required Base Addition for 2012 SA17 Zones B and C for Different Target pH Values.

In 2012, 53 lb of $Mg(OH)_2$ were injected into both Zones B and C. This small amount of base was sufficient to raise the pH of Zone B from ~5.6 to near 6.0, substantially improving treatment performance. However, in Zone C, the initial pH was lower (~5.3), and this small amount of base was not sufficient to raise the Zone C pH to an acceptable level.

Raising the pH to ~7 requires substantial amounts of base because of both the aquifer buffering capacity and conversion of H_2CO_3* to HCO_3^-. In general, it is not practical to raise the pH to near 7 with $NaHCO_3$ due to the small amount of H^+ consumed by this material at near neutral pH. NaOH and Na_2CO_3 can be effective for increasing pH to ~7. However, the high pH associated with these materials can kill bacteria near the injection zone. The total mass of $Mg(OH)_2$ required is less than NaOH, since two moles of OH^- are released per mole of $Mg(OH)_2$. However, $Mg(OH)_2$ has a very low aqueous solubility, so the material must be injected in a colloidal form. The pH of a pure slurry of $Mg(OH)_2$ is relatively high (~10.3) and can inhibit dechlorinators. However once injected, CO_3^{2-} precipitates on the surface of the $Mg(OH)_2$ particles forming a $MgCO_3$, coating maintaining the aquifer pH between 7 and 8 (Hiortdahl and Borden, 2014).

2.4.5 Overall Performance Evaluation

Results of the performance evaluation of SA17 are presented in **Table 2**. In summary, treatment of Zone B generally met performance criteria, even though EVO was not effectively distributed throughout this zone. Treatment of Zone C was less effective with greater production of *c*DCE and VC. The less effective treatment of Zone C was likely due to the lower pH (<5.5) and presence of residual TCE in the 25-30 ft bgs between the Zones B and C injection well screens.

Table 2. SA17 Performance Evaluation

Performance Criteria	Actual Performance
Substrate levels adequate for complete dechlorination • TOC > 20 mg/L • Total VFAs excluding acetate > 2 mg/L	TOC below 20 mg/L and total VFAs < 2 mg/L in most wells for much of the monitoring period. TOC concentrations never increased in some wells. EVO loading analysis and numerical modeling indicate poor TOC results due to: (a) injection of too little EVO; and (b) less than optimal injection design. DID NOT MEET PERFORMANCE CRITERIA.
pH appropriate for complete dechlorination (6.0 <pH < 8.0)	Buffer addition increased average pH in Zone B to near 6. However, average pH in Zone C < 5.5 indicating buffer addition was inadequate. PARTIALLY MET PERFORMANCE CRITERIA
Generate strongly reducing conditions • SO_4 < 10 mg/L • CH_4 > 3 mg/L	Substrate addition generated strongly reducing conditions throughout target treatment zone with SO_4 reduced to 5- 20 mg/L and CH_4 > 3 mg/L in all wells. MET PERFORMANCE CRITERIA
Microbial population adequate for complete dechlorination • *Dhc* counts > 10^4 cells/mL • *bvcA or vcrA* > 10^3 cells/mL	Dehalogenating bacteria were initially low, but increased following substrate and base addition. *Dhc* counts greater than 10^4 cells/mL and *bvcA or vcrA* counts greater than 10^3 cells/mL were observed in one or more wells in both Zones B and C indicating substantial numbers of *Dhc* with the ability to reduce VC to ethene were present. These results indicate chloroethene degradation was not limited by absence of required microorganisms. MET PERFORMANCE CRITERIA
TCE reduced to ethene with little accumulation of *c*DCE or VC • TCE reduced by > 2 OoM • ΣCl reduced by > 2 OoM • Cl# < 0.5	TCE removal exceed 2 OoM in over 90% of the wells in both Zones B and C indicating good parent compound removal. However, *c*DCE and VC accumulated with Cl# varying between 1 and 2 in most wells. Median ΣCl declined by 2.1 OoM in Zone B indicating relatively good removal of all chlorinated ethenes, but only declined 0.8 OoM in Zone C due to greater accumulation of *c*DCE and VC. DID NOT MEET PERFORMANCE CRITERIA
Uniform treatment with >90% of monitor wells meeting performance criteria.	Treatment was highly variable. In general, monitor wells in Zone B showed relatively good performance. Treatment was much less effective in Zone C, likely due to the low pH of this zone and the presence of residual TCE at 25-30 ft bgs. DID NOT MEET PERFORMANCE CRITERIA

3.0 OU2 ASSESSMENT RESULTS

The hydrogeology, contaminant distribution, remedial activities, and monitoring results from site OU2 are summarized here and presented in more detail in **Appendix B**. OU2 is located in the McCoy Annex at Former NTC Orlando. The site was used as a landfill from about 1960 to 1978. As part of landfill operations, 10 to 15 ft deep trenches were excavated, filled with waste to within 3 to 4 ft of the ground surface, backfilled with soil and seeded. Two shallow groundwater plumes covering approximately 23 acres have been identified that appear to be discharging from the former landfill and migrating towards a nearby drainage canal. Constituents of concerns (COCs) in groundwater included benzene, TCE, VC, and iron. TCE and its associated degradation compounds, *c*DCE and VC have been detected at concentrations in excess of the FDEP Groundwater Cleanup Target Levels (GCTLs). The plume intersects the adjacent drainage canal, but VOCs impacts to surface water above regulatory standards have not been detected. Installation of an EVO PRB was selected to prevent discharge of VOCs to the drainage canal (Tetra Tech, 2003). **Figure 10** shows the PRB layout and monitor well locations. As part of this project, one area in the northern and one area in the southern portions of the PRB were intensively characterized to better understand the factors controlling PRB performance.

3.1 HYDROGEOLOGY AND CONTAMINANT DISTRIBUTION

The primary aquifers in the area include the surficial aquifer and the Floridian aquifer, separated by the Miocene Hawthorne Group which acts as a confining unit separating the two aquifers (Adamski and German, 2004). The topography at the site is relatively flat. A drainage ditch runs from north to south along the eastern side of the site and appears to form a hydraulic barrier. Groundwater at the site flows to the east/southeast.

In the vicinity of the EVO barrier, the surficial aquifer extends from the water table at 6 to 8 ft bgs to approximately 35 ft bgs where the upper confining layer of the Hawthorne group is encountered (HLA, 1999). The surficial aquifer at the site is divided into two sub-units, labeled Zone A (0 to 25 ft bgs) and Zone B (25 to 40 ft bgs), which are reportedly separated by a semi-confining unit. A series of CPT borings installed in June 2016 indicate a clay unit over 10 ft thick is encountered at ~35 ft bgs. Overlying this clay is 1 to 2 ft of silt followed by interbedded clean sand, sand, and silty sand. In general, silty sand with thin clay layers is more common at 15 to 20 ft bgs with clean sand above and below. While not continuous, layers of clean sand or silty sand can be traced tens of feet horizontally.

In 2016, the HPT was used to measure vertical profiles of K at six locations. A cross-section of estimated K near OU2-43 is shown in **Figure 11**. K is relatively low in the Zone A from 10 to 20 ft bgs. From 20 to ~34 ft bgs (Zone B), K is variable ranging from 5 to over 50 ft/d. Between the clay layer at 35 ft and the overlying sand, there is a silty zone roughly 1 to 2 ft thick with K less than 1 ft/d. Groundwater flow is generally to the east towards the GOAA drainage canal with an average hydraulic gradient of ~0.002 ft/ft. Assuming an effective porosity of 0.2, groundwater velocity is expected to vary from 4 to over 150 ft/yr.

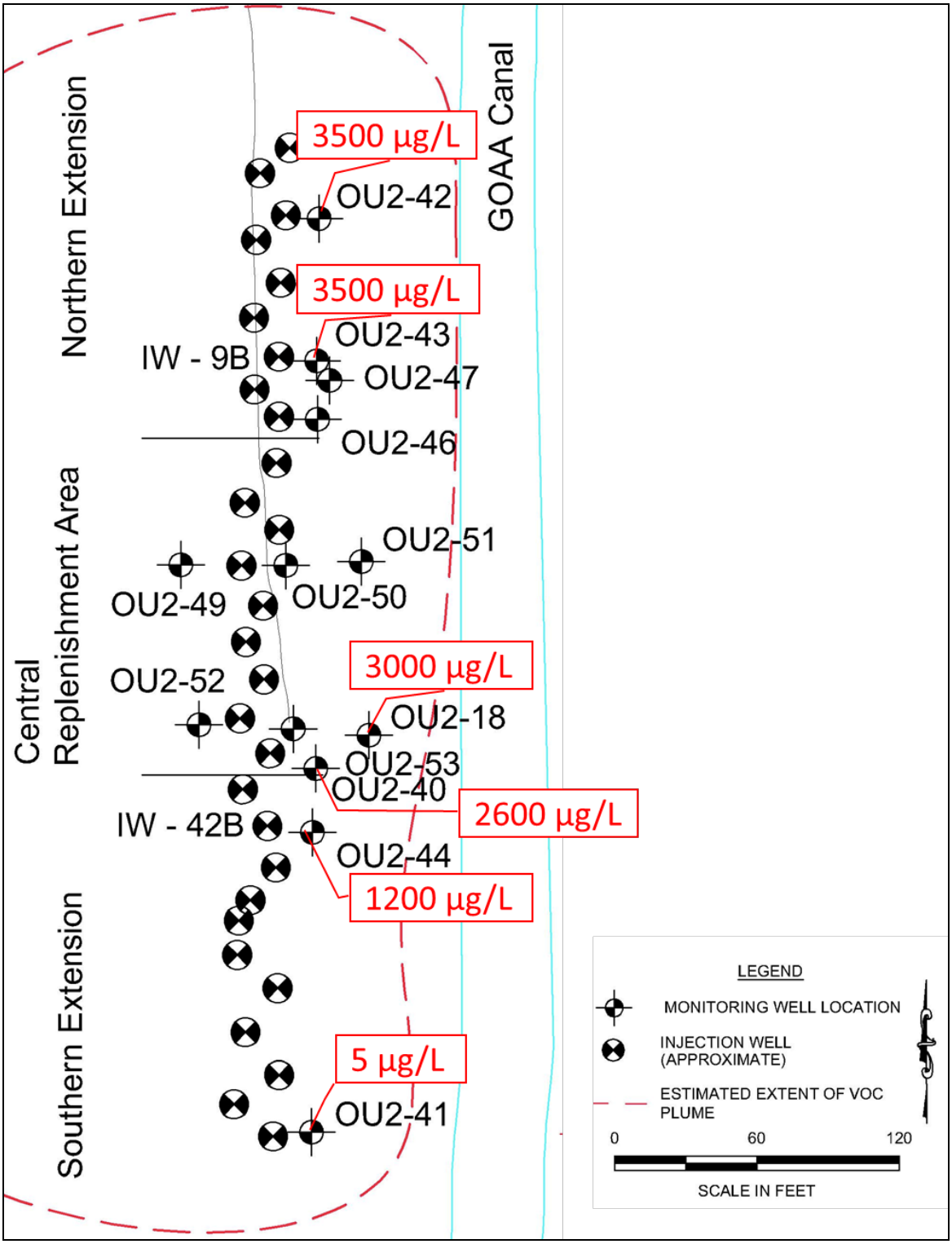


Figure 10. Injection and Monitor Wells Installed at OU2 PRB with Average TCE Concentrations Prior to 2007.

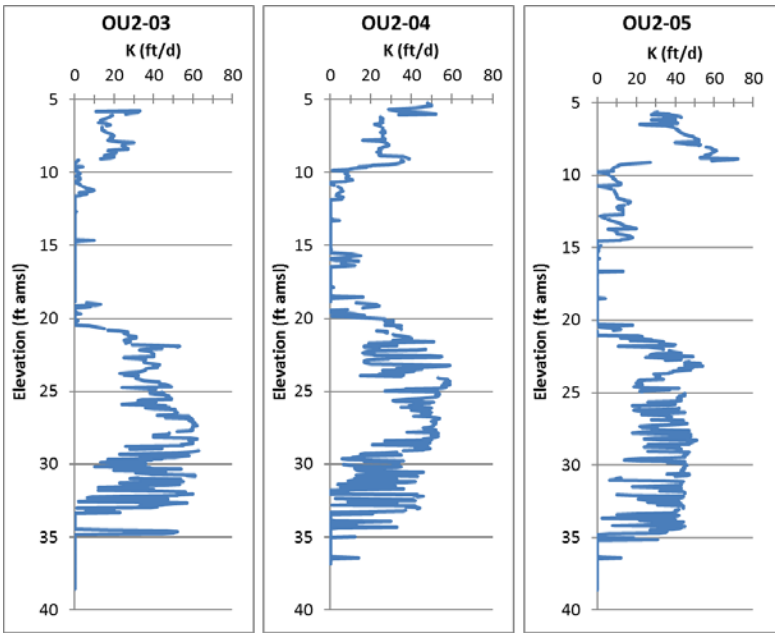


Figure 11. HPT K Profiles in Longitudinal Cross Section Near OU2-43.

The bottom of the GOAA canal typically intersects the water table. Water level data from well pairs installed in Zones A and B indicate that both the unconfined aquifer and GOAA ditch behave as one hydrologic unit with respect to groundwater movement and contaminant distribution. Water levels in the confined, upper Floridian aquifer are about 40 ft bgs indicating a strong downward hydraulic gradient through the Hawthorne confining unit (Tetra Tech, 2001).

The primary COCs in groundwater are benzene, TCE, vinyl chloride, and iron. In the southern portion of the site, a plume with higher concentrations of TCE (> 1,000 µg/L) is present in the B zone with the highest concentrations immediately above the clay confining layer. Contaminated groundwater extends laterally from the western side of the waste boundary at the approximate location of the former landfill trenches and extends beneath the site toward the GOAA canal. **Figure 10** shows the PRB layout and average concentrations of TCE in B zone monitor wells prior to installation of the PRB. Where concentrations are not reported, the wells were not sampled prior to PRB installation.

In summary, there were significant spatial variations in K typical of many aquifers, but no evidence of unusual or excessively complex site conditions. However, the presence high TCE concentrations in the lower K silty sand, will make it more difficult to effectively distribute remediation amendments in the most contaminated intervals.

3.2 REMEDIAL ACTIVITIES

In 2007, CH2M HILL conducted a pilot study to evaluate the feasibility of *in situ* bioremediation using EVO as the electron donor to treat TCE in groundwater (CH2M HILL, 2008). Based on the pilot test results, an EVO PRB was selected as the preferred alternative to intercept the portion of the southern groundwater CVOCs plume entering the GOAA property. The PRB was installed in phases with the central portion of the barrier installed in August 2008 using 11 pairs of 2-inch diameter injection wells (central replenishment area in **Figure 10**). During 15 months

of post-injection monitoring, the concentrations of TCE decreased appreciably in several monitor wells (AGVIQ-CH2M HILL JV-II, 2010). Complete metabolism to VC and ethene was noted in some wells, but low pH was reported and use of a buffer was strongly recommended for future injections at this site; bioaugmentation was also recommended to increase the population of dechlorinating bacteria in the aquifer.

In July 2010, Solutions-IES was contracted by NAVFAC SE to complete installation of the PRB at OU2. Monitoring of several injection wells used in 2008 indicated they were clogged with solidified oil from the 2008 injection and could not be effectively rehabilitated. In February 2012, Solutions-IES installed nine new injection wells north of the previous biobarrier (IW-3B to IW-11B), nine new injection wells south of the previous biobarrier (IW-23B to IW-31B), and an additional eleven replacement injection wells (IW-32B to IW-42B) in the area of the previous PRB (**Figure 10**). The injection wells were installed in two rows perpendicular to groundwater flow, with wells spaced approximately 30 ft on-center in each row. Two emulsified oil products, EOS® 598B42 and AquaBupH™, were diluted with water, and injected to replenish the existing PRB and extend it to the north and south. In April 2012, the injection wells were inoculated with BAC-9™, a microbial consortium containing *Dhc*. *Dhc* is the primary microorganism capable of complete reductive dechlorination of TCE to ethene. Details of the 2008 and 2012 injections are provided in **Appendix C**.

3.3 REMEDIAL SYSTEM PERFORMANCE

3.3.1 Biogeochemistry

Performance of the ERD remediation system was evaluated using historical groundwater monitoring data (CH2M HILL, 2008; Solutions-IES, 2013b) and data collected as part of this project (groundwater monitoring, membrane interface profiles, and soil analysis for VOCs). Groundwater monitoring data included samples collected from monitor and injection wells in the treatment zone and analyzed for VOCs, methane, ethane, and ethene (MEE), nitrate (NO_3), sulfate (SO_4), geochemical indicators (O_2, pH, oxidation-reduction potential (ORP), acidity, alkalinity), major cations and anions (Na, K, Ca, Mg, Fe, Mn, Cl, Br), molecular biological tools (major dechlorinators and genes) and VFAs. As the first step in this process, all available data was compiled into a single master database and reviewed to identify trends in important parameters, important biological and geochemical factors that might influence ERD, and outliers.

Prior to installation of the EVO PRB, the aquifer was reducing, with moderate levels of dissolved iron (1 to 3 mg/L), sulfate (10 to 20 mg/L), and CH_4 (0.3 to 2 mg/L). TOC concentrations varied from 5 to 8 mg/L. The pH in most wells was ~5 with occasional variations between 4.5 and 5.5.

Figure 12 shows methane (CH_4), TOC, and pH in monitor wells located 15 to 30 ft downgradient from the Northern, Central, and Southern portions of the EVO PRB. Pilot test injections were conducted in wells upgradient of OU2-47B and OU2-18B in 2007 and the rest of the central barrier was injected in 2008. In 2012, the Northern and Southern portions were installed and the central portion was reinjected.

In the Central portion, TOC concentrations increased shortly after the 2007-08 injections, and then declined to background levels within a few years. Methane concentrations increased more slowly

and remained high. pH has remained constant at ~5 with no evidence of a significant decrease due to substrate addition or an increase from $Mg(OH)_2$ addition. Shortly after injection, SO_4 concentrations declined below 5 mg/L and remained low (data not shown). In general, these results indicate the EVO injections were effective in generating methanogenic conditions near the injection wells. However, the injected EVO did not reach the downgradient monitor wells. The temporary increase in TOC was likely associated with the soluble substrates in the EVO or an initial release of soluble TOC during the initial hydrolysis of the vegetable oil triglycerides (Long et al., 2006; Hiortdahl and Borden, 2014). There is no evidence of a TOC or pH increase following the 2012 injections, indicating the effective treatment zone did not reach the downgradient monitor wells. However, CH_4 continues to be high indicating fermentable carbon is still present closer to the injection wells. Geochemical trends in the Northern and Southern portions of the EVO PRB followed generally similar trends to the Central portion, although delayed due to the later injection of these areas. In most of the monitor wells, CH_4 is continuing to slowly increase. However, CH_4 increased very rapidly and is now declining in OU2-41 at the southern end of the barrier, suggesting most of the fermentable organic carbon has been depleted.

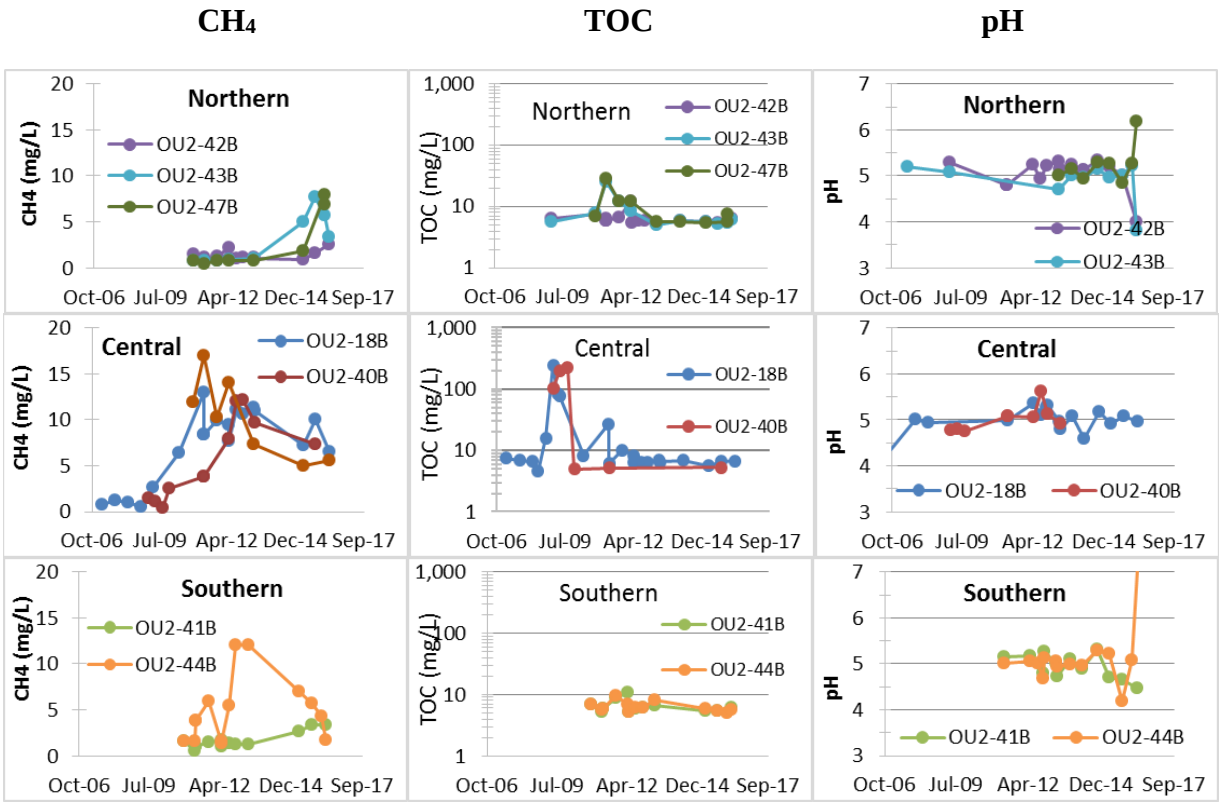


Figure 12. CH4, TOC, and pH in Monitor Wells Downgradient of the Northern, Central, and Southern Portions of the EVO PRB.

3.3.2 Microbiology

Monitoring results for dehalogenating bacteria (*Dhc*, *Dhb*) and functional genes (*tceA*, *bvcA*, *vcrA*) are presented in **Appendix C, Table C-1**. Numbers of dechlorinating microorganisms in the monitor wells were low, but did increase slightly following substrate addition and bioaugmentation. The low dechlorinators numbers are likely due to the low TOC concentrations and low pH which would have limited microbial growth. The low dechlorinators numbers do not necessarily indicate that subsurface microbiology limited remediation, since these wells were located downgradient from the active treatment zone.

3.3.3 Injection Well Sampling

One of the hypotheses evaluated in this project was that EVO and/or pH buffer were not effectively distributed throughout the treatment zone and reagent distribution in the aquifer was limiting treatment. To evaluate this hypothesis, we sampled four injection wells at various locations along the length of the PRB for biogeochemical indicators, ethenes, and dechlorinating microorganisms in October 2015 and February 2016. We assumed that transport through the aquifer would not limit treatment in these wells since both EVO and buffer were injected directly into these wells. Monitoring results are presented in **Appendix C, Table C-2**.

Monitoring results indicate that PCE, TCE and their degradation products were being effectively treated in two of the injection wells (OU2-IW-9B and OU2-IW-10B). In these two wells, pH is greater than 6, TOC concentrations were greater than 30 mg/L, TCE and DCE have been substantially reduced, and relatively high numbers of dechlorinators (*Dhc*) are present with enzymes capable of reducing VC to ethene.

In OU2-IW-42B, ERD is more limited, high concentrations of *c*DCE and VC persist, likely due to the low pH (<5), high acidity, low Mg, and associated low *Dhc* counts. The cause of the low pH is unknown, since records indicate that AquabupH containing $Mg(OH)_2$ was injected into this well. It may be that the hydraulic conductivity near this well is somewhat higher resulting in more rapid washout of added $Mg(OH)_2$.

In OU2-IW-23B, PCE and TCE are below detection. However, significant concentrations of *c*DCE and VC persist. The pH is >6 and TOC> 30 indicating appropriate conditions for ERD. While dechlorinators with enzymes capable of reducing VC to ethene are present, their numbers are relatively low, potentially indicating some other limitation to growth.

3.3.4 Chlorinated Ethenes

Figure 13 shows the variation in PCE, TCE, *c*DCE, VC, and ethene with time in monitor wells downgradient of the EVO PRB. In all the wells, EVO injection resulted in a substantial decline in TCE and increase in *c*DCE and VC a few years after EVO injection.

Figure 14 illustrates the use of ΣCl and Cl# for evaluating ERD progress in OU2-18B. Prior to EVO addition, TCE was the dominant chloroethene, Cl# was ~3 and ΣCl was approximately 3 times the TCE concentration in micro-moles per liter (µM). Following EVO addition in 2008, TCE declined by over 99.9%, with a concurrent drop in Cl# from ~3 to ~1, and ΣCl declined by 50 to 90%. The much more limited decline in ΣCl compared to TCE is due to accumulation of *c*DCE and VC.

Figure 15 shows the variation of ΣCl and Cl# with time in monitor wells downgradient of the EVO PRB. Throughout the barrier, dechlorination has stalled at either *c*DCE (Cl# = 2) or VC (Cl# = 1). Declines in ΣCl vary from minimal in OU2-41B to over 90% in OU2-51B.

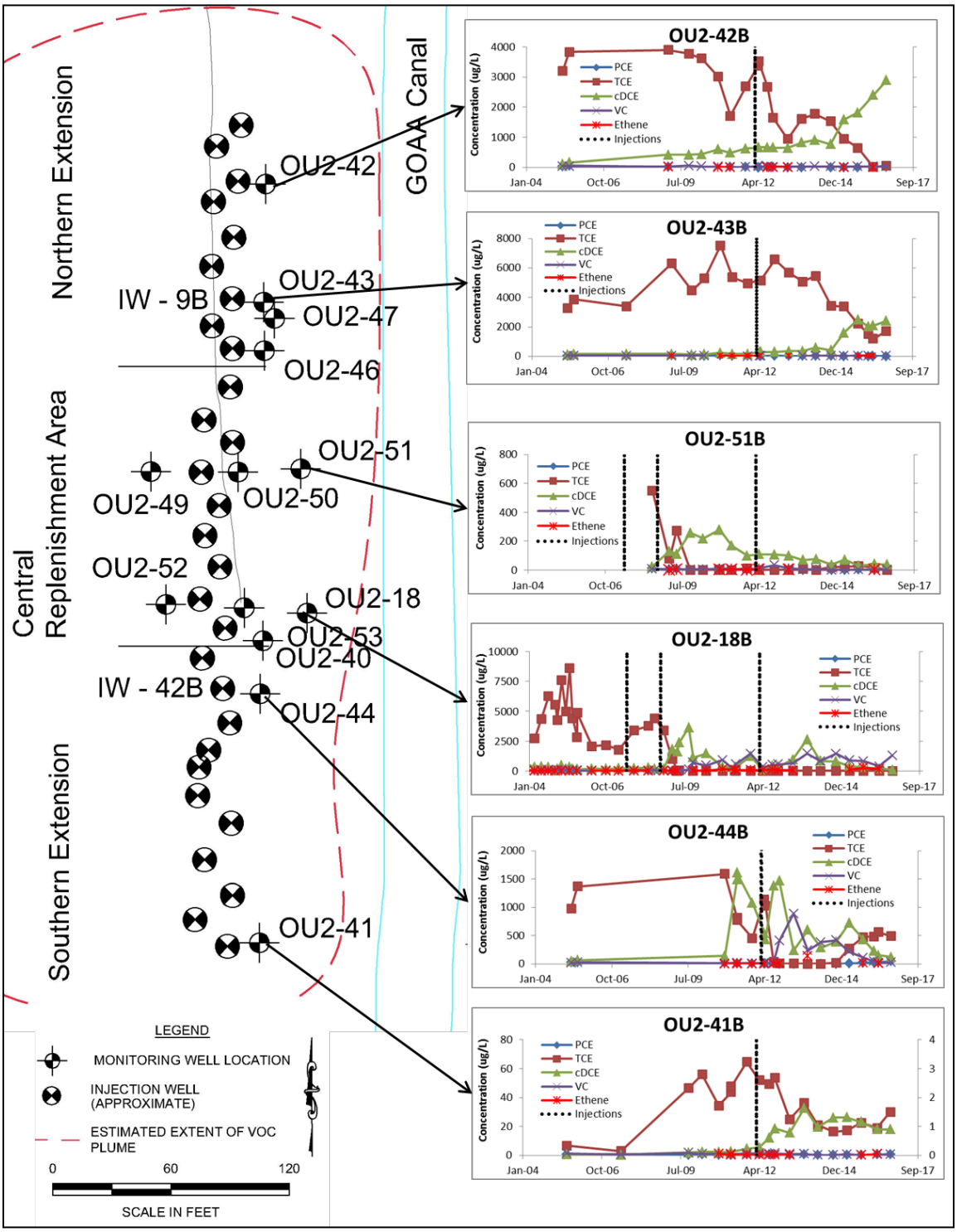


Figure 13. PCE, TCE, *c*DCE, VC, and Ethene in Monitor Wells Downgradient of the Northern, Central, and Southern Portions of the EVO PRB.

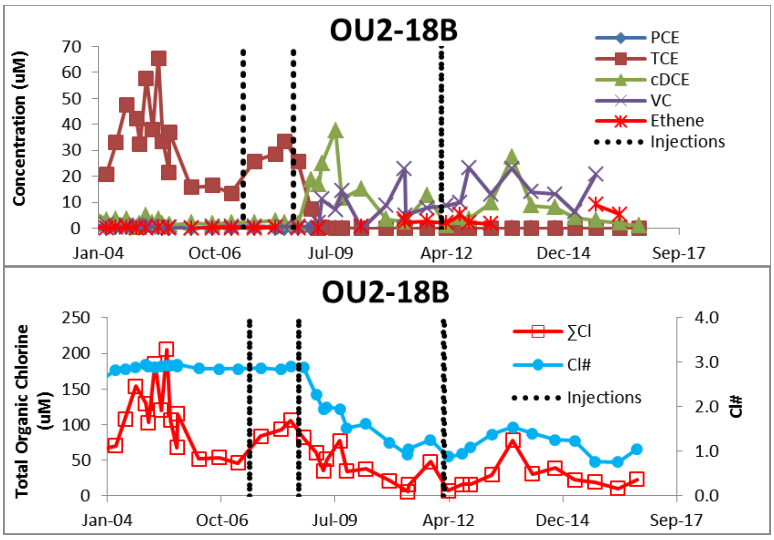


Figure 14. Variation in TCE, cDCE, VC, Ethene, ΣCl, and Cl# versus Time in OU2-18B.

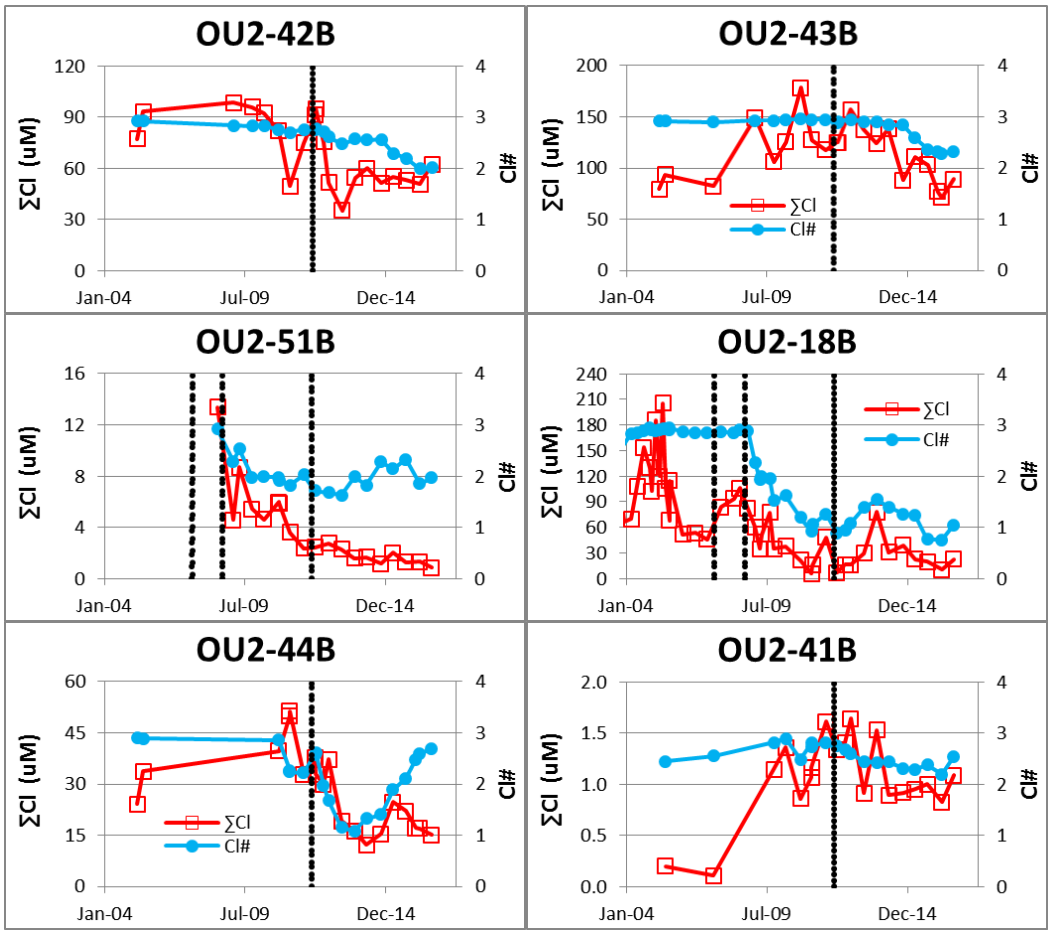


Figure 15. Variation in ΣCl and Cl# in Monitor Wells Downgradient of the Northern (OU2-42B and OU2-43B), Central (OU2-51B and OU2-18B), and Southern (OU2-44B and OU2-41B) Portions of the EVO PRB.

3.3.5 Spatial Distribution of TCE and *c*DCE

To evaluate the potential for back-diffusion of TCE out of the underlying confining unit, a continuous soil core was collected adjoining OU2-43B from 25 to 40 ft bgs. Subsamples were collected and analyzed for CVOCs and soil particle size distribution. **Figure 16** shows the results of this evaluation along with the K profile measured by HPT at this location. TCE concentrations were a maximum 2 ft into the confining layer where the clay+silt content was highest.

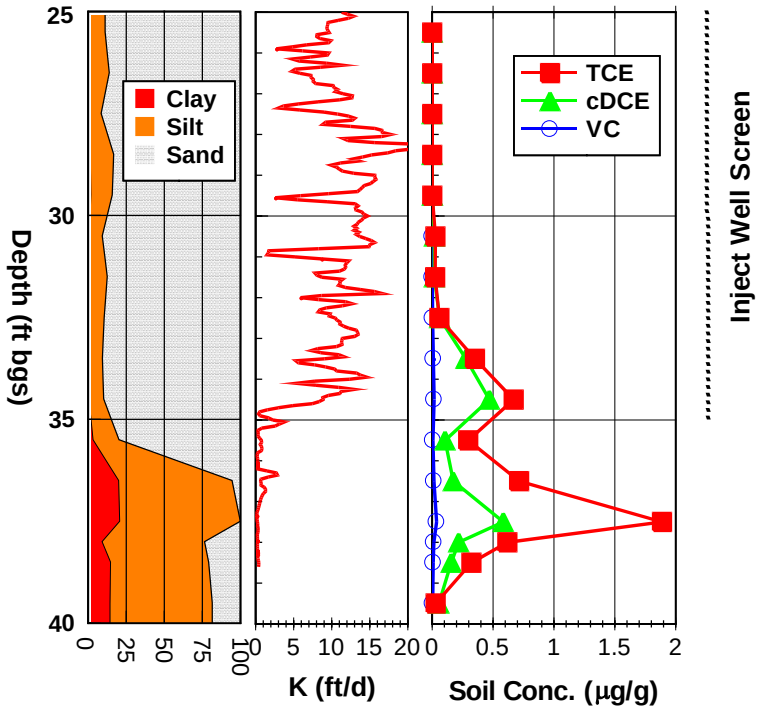


Figure 16. Profiles of Soil Composition, Hydraulic Conductivity, and TCE, *c*DCE and VC Concentrations in Soil Samples Collected from a Boring Adjoining OU2-43B.

3.4 EVALUATION OF FACTORS LIMITING REMEDIAL PERFORMANCE

3.4.1 Back Diffusion

The Dandy-Sale (DS) model (Sale et al., 2008) within the Matrix Diffusion Toolkit (MDT) (Farhat et al., 2012) was used to evaluate TCE diffusion into the clayey silt confining layer and back diffusion over time. Details of the model calibration and mass release calculations are presented in **Appendix C, Section C.4.1.** Overall, the DS model provided a relatively good match to the measured concentrations, matching the depth and maximum concentration observed (**Appendix C, Figure C-14)**. Predicted mass discharge drops rapidly following installation of the EVO PRB in 2012 (**Appendix C, Figure C-15**). However, after 2017, mass discharge declines more slowly as TCE is released from the confining layer by back diffusion downgradient of the PRB and upgradient of the GOAA canal. The total mass released by back diffusion is relatively small due to the short distance from the PRB to the canal (~50 ft).

By 2030, TCE concentrations in a monitor well adjoining the GOAA canal are predicted to drop below 5 µg/L. In summary, back diffusion from the confining layer, downgradient of the PRB, is not expected to substantially limit the effectiveness of the PRB in reducing TCE discharge to the GOAA canal.

3.4.2 Evaluation of EVO Loading and Injection Volumes

The EVO design tool developed under ER-0626 (Borden et al., 2008, Weispfenning and Borden, 2008) was used to evaluate the 2006 and 2012 injections and determine if sufficient EVO and water were injected to generate a permeable reactive barrier with residual oil in close contact with over 80% of the groundwater migrating through the barrier. Details of this evaluation are presented in **Appendix C, Section C.4.2.**

As discussed in **Section 2.4.2**, the increased ionic strength of the groundwater resulting from buffer injection, can reduce the oil droplet-sediment particle repulsion, increasing oil retention by the aquifer material. Sampling of monitoring wells in 2015 showed low ionic strength groundwater with typical average concentrations of 9 mg/L Na, 2 mg/L K, 9 mg/L Ca and 2 mg/L Mg. However, injection wells sampled in 2015 and 2016 (**Table C-2**) had higher concentrations of Ca (average of 46 mg/L) and Mg (average of 49 mg/L) due to injection of $Mg(OH)_2$ to raise aquifer pH. Similar to results from SA17, the zeta potential of aquifer material from OU2 was more strongly negative in DI water than a 200 mg/L $CaCl_2$ solution, indicating that oil droplet-sediment particle repulsion will be greater and oil retention will be lower in DI water (**Appendix C, Table C-3**). Measured oil retention in laboratory columns packed with OU2 sediment and flushed with 200 mg/L $CaCl_2$ was significantly higher than for the same material flushed with DI water consistent with reduced electrical repulsion between the oil droplets and sediment (**Appendix C, Table C-4**).

Table C-5 in **Appendix C** shows the input parameters used in the original design of the 2008 and 2012 EVO injections (CH2M Hill, 2006; Solutions-IES, 2013b) and our current best estimates for these parameters, based on additional site characterization results and laboratory column tests. Evaluation of the 2008 and 2016 injection volumes with the ESTCP EVO Design tool indicates that:

- Based on the original design assumptions in 2008 and 2012, the total volume of diluted EVO and chase water should have been more than sufficient to effectively distribute the oil droplets throughout the treatment zone, achieving EVO contact efficiencies greater than 80%.
- The measured oil retention with DI water was 0.0144 g/g or over ten times the value assumed in the original design. This under-estimate resulted in too little EVO being injected, which would have significantly reduced contact efficiency and treatment.
- Injection of a mixture of EVO and colloidal $Mg(OH)_2$ likely increased ionic strength of the injection solution and oil retention near the injection wells, further reducing EVO contact efficiency.

3.4.3 Hydraulic Design

The groundwater flow model, MODFLOW (Harbaugh et al., 2000) was used to simulate flow patterns during the 2012 injection in the northern study area. The EVO transport and retention was simulated using the reactive transport model RT3D (Clement, 1997) with the reaction module developed to simulate retention of colloidal oil droplets (Coulibaly et al., 2006). Injection volumes and flowrates used in model calibration are presented in **Appendix C, Sections C.2.1 and C.2.2**. A maximum oil retention of 0.0144 g/g was used in all simulations for consistency. Details of the model simulations are presented in **Appendix C, Section C.4.3**.

Figure 17 shows the simulated oil distribution following the 2012 injection at 25, 30, and 35 ft bgs. Oil distribution is reasonably good at the 25 and 30 ft depths forming a continuous barrier. However, at 35 ft bgs where TCE concentrations are highest, oil distribution is more limited due to the preferential injection in the shallower, high permeability zones. Additional simulations with the sequential dechlorination module in RT3D indicated that: (a) TCE removal may be limited by poor EVO distribution at the 35 ft depth where TCE concentrations are highest; and (b) *c*DCE and VC removal rates are very low, consistent with strong inhibition by low pH.

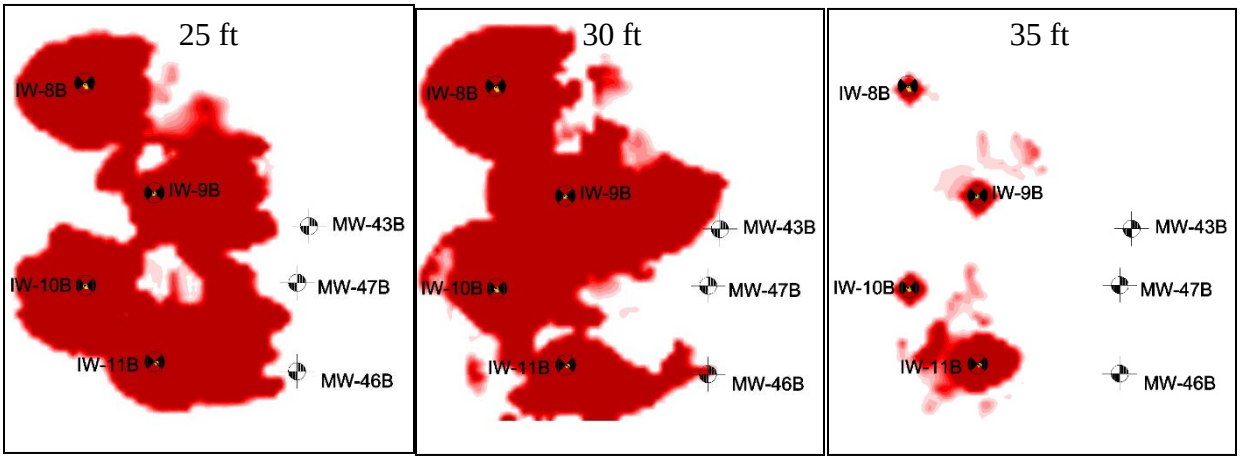


Figure 17. Simulated EVO Distribution at 25, 30, and 35 ft bgs in Northern Study Area at OU2 Following 2012 Injection.

3.4.4 Buffer Design

As part of this project, a MS Excel spreadsheet was developed to estimate the amount of base required to raise the aquifer pH to the desired level, and maintain it at that level for the design treatment period. The theoretical basis of the spreadsheet calculation procedures is presented in **Appendix D**. Details of the actual calculations for the 2012 injections at OU2 are presented in **Appendix C, Table C-7**. Results presented in **Figure 18** indicate that amount of base required is very sensitive to the target pH. For target pH values < 5.4, little or no base is required. Increasing the target pH to 6 or 7, requires greater amounts of base because of the soil acidity and carbonic acid released from substrate fermentation. In 2012, a total of 240 lb of $Mg(OH)_2$ were injected. This small amount of base provided only ~1% of the base required to raise the pH to 7 and maintain it at that level for five years.

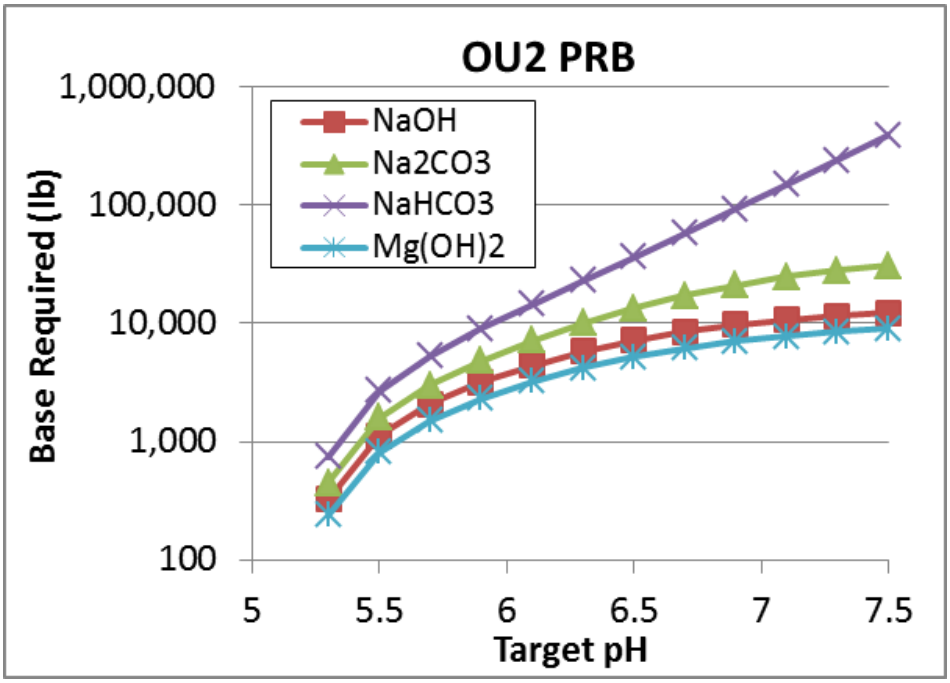


Figure 18. Required Base Addition for 2012 OU2 PRB for Different Target pH Values.

3.5 OVERALL PERFORMANCE EVALUATION

Results of the performance evaluation of OU2 are presented in **Table 3**. In summary, treatment of the TCE plume at OU2 by the EVO barrier did not meet performance criteria. TOC and pH never reached adequate levels in downgradient monitor wells. While it is possible that TOC and pH were adequate in some portions of the PRB treatment zone, the available monitoring data indicate low TOC concentrations and low pH limited efficient dechlorination. As a result, TCE removal was low and *c*DCE and VC accumulated, with low removal of ∑Cl. The relatively poor treatment achieved was likely due to injection of too little EVO and pH buffer. However, spatial variations in K and TCE concentrations appear to have complicated EVO and buffer distribution at some locations.

Table 3. OU2 Performance Evaluation

Performance Criteria	Actual Performance
Substrate levels adequate for complete dechlorination • TOC > 20 mg/L • Total VFAs excluding acetate > 2 mg/L	TOC below 20 mg/L in all downgradient wells for most of the monitoring period. However, TOC remains (high 77 to 222 mg/L) in sampled injection wells 3-4 years after injection. Monitoring data not sufficient to determine if TOC was effectively distributed throughout PRB treatment zone. However, data suggests that TOC levels were not adequate. DID NOT MEET PERFORMANCE CRITERIA
pH appropriate for complete dechlorination (6.0 <pH < 8.0)	pH remained near 5 in all downgradient monitor wells, indicating base addition had no measurable impact on downgradient monitor wells. pH was greater than 6 in three injection wells and less than 5 in one injection well at 3-4 years after injection. Monitoring data not sufficient to determine if pH buffer was effectively distributed throughout PRB treatment zone. However, data suggests that pH was too low for effective treatment of *c*DCE and VC. DID NOT MEET PERFORMANCE CRITERIA
Generate strongly reducing conditions • SO_4 < 10 mg/L • CH_4 > 3 mg/L	Strongly reducing conditions generated in downgradient wells with SO_4 at 1 to 20 mg/L and CH_4 > 3 mg/L in all wells. MET PERFORMANCE CRITERIA
Microbial population adequate for complete dechlorination • *Dhc* counts > 10^4 cells/mL • *bvcA* or *vcrA* > 10^3 cells/mL	Dehalogenating bacteria were low throughout the monitoring period in downgradient wells where biogeochemical conditions (pH~5 and and TOC<10 mg/L) inhibited growth. In some injection wells, *Dhc* > 10^4 and *vcrA* > 10^3 cells/mL was observed 3-4 years after injection, indicating required dechlorinators can survive and degrade TCE to ethene where conditions were appropriate. MET PERFORMANCE CRITERIA
TCE reduced to ethene with little accumulation of *c*DCE or VC • TCE reduced by > 2 OoM • ΣCl reduced by > 2 OoM • Cl# < 0.5	Median TCE removal ~ 0.5 OoM indicating low to moderate parent compound removal. *c*DCE and VC accumulated with Cl# varying between 2 and 3 in most wells. Median ΣCl declined by 0.2 OoM indicating relatively poor removal of all chlorinated ethenes. DID NOT MEET PERFORMANCE CRITERIA
Uniform treatment with >90% of monitor wells meeting performance criteria.	Treatment was highly variable. TCE removal was relatively good in OU2-18, but much lower in other wells. TCE was not effectively reduced to ethene in any monitor well. DID NOT MEET PERFORMANCE CRITERIA

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4.0 COMPARISON WITH OTHER SITES

To provide some reference for comparing treatment performance, we compare Order of Magnitude (OoM) concentration reductions in Zones B and C wells at SA17 and downgradient wells at OU2 with OoM reductions in TCE concentration reported by Tillotson and Borden (2017) for 37 ERD sites containing 184 treatment zone monitor wells and in situ remediation performance monitoring results reported by McGuire et al. (2016). OoM reductions at SA17 were calculated by comparing monitoring results from June 2006 (immediately before injection) to October 2015. OoM reductions at OU2 were calculated by comparing monitoring results from immediately before injection to October 2016. The performance monitoring results reported by McGuire et al. (2016) were sorted to select 19 ERD sites treated with Semi-Soluble / Slow-Release substrates where TCE was the parent compound. The McGuire database provides OoM removal results for both geometric mean concentrations and maximum concentrations before and after treatment.

Figure 19 compares OoM removals for the McGuire database (19 site geomeans and maximums), Tillotson and Borden (2017) database for 37 ERD sites containing 184 wells, and individual wells and OU2 and SA17 zones B and C. At OU2, TCE concentrations in individual monitoring wells declined by 0 to 3.2 OoM at OU2 with a median reduction of 0.5 OoM. These OoM removals are lower than values reported by McGuire et al. (2016) and Tillotson and Borden (2017), indicating relatively poor treatment. At SA17, TCE concentrations in individual monitoring wells declined by 2.8 to 4.6 OoM in Zone B and 2.0 to 3.7 OoM in Zone C. These OoM removals are greater than typical values reported by McGuire et al. (2016) and Tillotson and Borden (2017), indicating that parent compound removal at SA17 was good in comparison to other ERD projects.

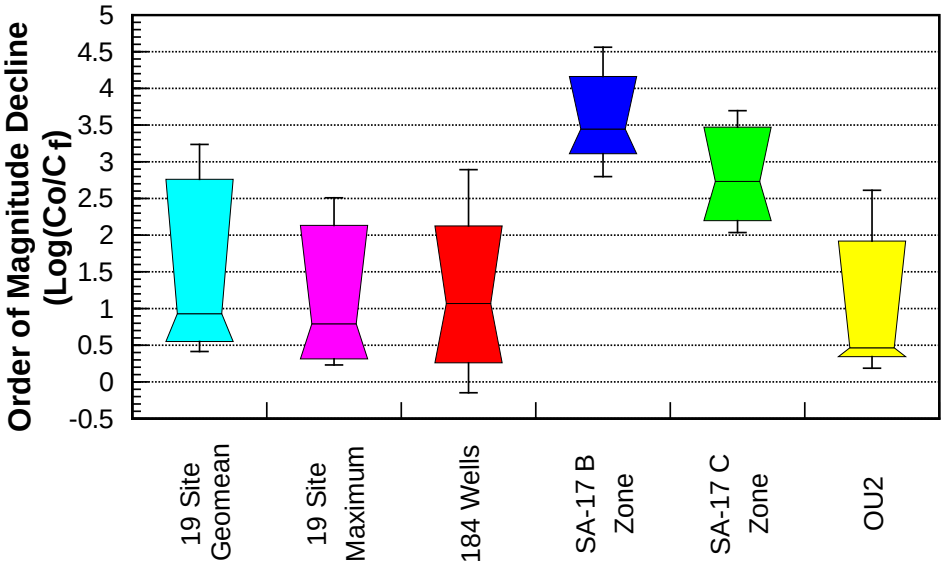

Figure 19. Order of Magnitude (OoM) Concentration Reductions in TCE for 19 Sites (McGuire et al., 2016), 184 Wells (Tillotson and Borden, 2017), SA17 B and C Zones Monitoring Wells, and OU2 Monitoring Wells.

While parent compound removal is an important metric, accumulation of ERD biotransformation products was a significant concern at both SA17 and OU2. **Figure 20** compares OoM reductions

in TCE, *c*DCE, VC, and ΣCl in OU2 and SA17 Zones B and C monitor wells with results from Tillotson and Borden (2017).

TCE removal was below average at OU2. In addition, *c*DCE accumulation was worse at OU2 in comparison to results from Tillotson and Borden (2017). A more balanced measure of treatment performance is OoM removal in ΣCl. Overall, ΣCl removal at OU2 was not as good as at other sites.

TCE removal was very good in both Zones B and C at SA17. However, daughter product accumulation was worse at SA17 in Zone C compared to results from Tillotson and Borden (2017). A more balanced measure of treatment performance is removal of ΣCl. Overall, ΣCl was better than average of other sites in Zone B and fairly typical in zone C.

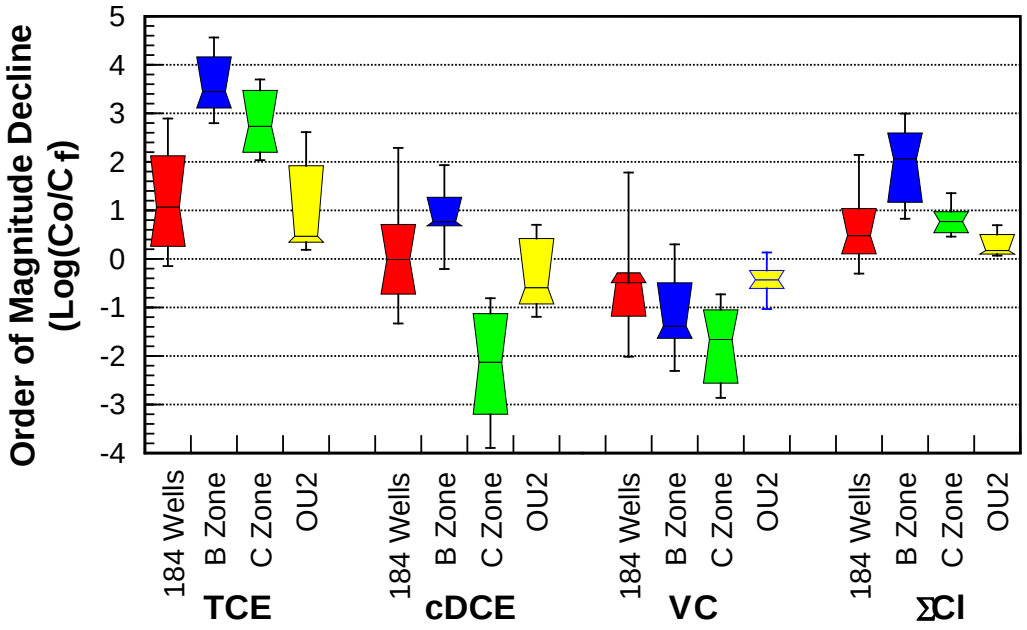

Figure 20. Order of Magnitude (OoM) Decline in TCE, *c*DCE, VC, and ΣCl for 184 Wells (Tillotson and Borden, 2017), SA17 B and C Zones Monitoring Wells, and OU2 Monitoring Wells.

In summary, parent compound (TCE) removal was very good at SA17. However, daughter product accumulation in Zone C was more severe than at some other sites. This was reflected in the lower OoM removal for Zone C. Treatment performance at OU2 was lower than at other ERD sites. Both parent compound removal and daughter product accumulation were below average resulting in lower than average ΣCl removal.

5.0 CONCLUSIONS & LESSONS LEARNED

5.1 CONCLUSIONS

Bioremediation performance in Zone B at SA17 was good with 2.8 to 4.6 OoM reductions in TCE. While *c*DCE and VC removal were lower, ΣCl declined by 0.8 to 3 OoM indicating a substantial portion of the parent compound was reduced to non-toxic end-products. TCE removal was also good in Zone C at SA17. However, higher levels of *c*DCE and VC accumulated with ΣCl declining by only 0.5 to 1.5 OoM. EVO distribution in both Zones B and C at SA17 was limited by: (a) injection of too little EVO; and (b) development of stagnation zones during injection. *c*DCE and VC removal in Zone C was inhibited by the low pH due to injection of too little base to neutralize acidity produced during ERD and the background acidity of the aquifer. While *Dhc* populations were low at many locations, substantial populations of *Dhc* capable of growing on VC developed at locations with sufficient substrate and appropriate pH, indicating ERD was not limited by absence of required microorganisms. There was no evidence of significant lower permeability zones near the target treatment zone that would result in substantial back diffusion of contaminants, limiting treatment. In summary, the primary factors limiting bioremediation performance at SA17 were inadequate levels of fermentable substrate and low pH due to injection of too little substrate, too little base to increase pH, and limited distribution of these materials throughout the target treatment zone.

Bioremediation was less effective in reducing chlorinated solvent concentrations at OU2. TCE concentrations in individual monitoring wells declined by 0 to 3.2 OoM at OU2 (median reduction of 0.5 OoM) with production of large amounts of *c*DCE. ΣCl removal at OU2 varied from 0.1 to 0.7 OoM with a median reduction of 0.2 OoM, which is lower than reported for other ERD projects. Effective distribution of EVO at OU2 was limited by: (a) injection of too little EVO; and (b) the presence of high TCE concentrations with and/or immediately adjoining lower permeability zones. Conversion of *c*DCE to ethene was inhibited by the low pH due to injection of too little base to neutralize acidity produced during ERD and the background acidity of the aquifer. While *Dhc* populations were low at many locations, substantial populations of *Dhc* capable of growing on VC developed at locations with sufficient substrate and appropriate pH, indicating ERD was not limited by absence of required microorganisms. While back diffusion of contaminants out of the underlying low permeability unit does occur downgradient of the OU2 PRB, the short travel distance from the PRB to the discharge point would greatly limit the impact of this process. In summary, the primary factors limiting bioremediation performance at OU2 were inadequate levels of fermentable substrate and low pH. The low substrate concentrations and low pH were due to injection of too little substrate, too little base to increase pH, and challenges in distributing these materials within and adjoining lower permeability units.

5.2 LESSONS LEARNED

1. Parent compound (TCE) removal was relatively good in both Zones B and C at SA17, even though the amount of EVO and base injected was much less than that required for optimum treatment. This indicates that ERD with EVO is a fairly robust technology and good parent compound removal can be achieved with a less-than-perfect design.

2. For the most effective treatment, amendments need to be distributed throughout the entire target treatment zone. If the treatment amendments are not uniformly distributed, CVOCs can persist in untreated zones, increasing the treatment duration.
3. Generating strongly reducing conditions with methane production is a poor indicator of effective substrate distribution. Once produced in an EVO treated zone, methane is relatively unreactive and can be transported away from the residual oil.
4. EVO was not effectively distributed throughout the target treatment zone at both SA17 and OU2. The most likely causes of limited oil distribution include:
    - Common rules of thumb used for designing EVO injections can greatly under-estimate the actual oil requirement. Oil retention tests should be run to generate more accurate estimates of the actual amount of EVO required for effective treatment.
    - Addition of alkaline materials to increase aquifer pH can significantly increase oil retention, reducing contact efficiency. If possible, the alkaline materials should be added after the EVO is injected to reduce these impacts. When groundwater ionic strength is high due to background geochemistry and/or amendment addition, oil retention tests should be run with solutions representative of the groundwater geochemistry.
    - The common practice of simultaneously injecting all wells to reduce injection time can result in stagnation zones, leaving some areas untreated. Injecting every other well in a group, then injecting the remaining wells in a second group can improve amendment distribution.
    - While not directly addressed in this project, recirculation systems can be used to more effectively distribute substrate, pH buffers, microorganisms, and the target contaminants, improving treatment.
5. pH less than 6 can significantly reduce *c*DCE reduction to VC and ethene. Low pH can result from a variety of factors including low background pH, HCl release during dechlorination, and VFA/carbonic acid produced during substrate fermentation.
    - When the aquifer pH is less than 6.3, site characterization should include measurement of inorganic carbon, mineral acidity, and aquifer buffering capacity (pHBC). With this information, designers can generate reasonable estimates of the amount of alkaline material require to maintain pH within a suitable range.
    - In some cases, the amount of base required to maintain an appropriate pH can equal or exceed the amount of organic substrate required.
6. Dechlorinator populations including *Dhc* and *bvcA*/*vcrA* can increase and decrease with time due to temporal variations in amount of organic substrate and/or contaminant concentrations. Low dechlorinators numbers do not necessarily indicate absence of required organisms, but can result from unfavorable geochemical conditions.
7. Remedial performance at SA17 and OU2 has improved over time as the treatment system has been modified based on our improved understanding of site conditions and in situ bioremediation processes. Site managers should recognize that it may not be practical to remediate a contaminated site with a single EVO injection. It may be more efficient and effective to employ an iterative process where a lower cost remedial system is installed, followed by monitoring and site characterization to identify treatment issues, and then the system is modified to improve performance.

6.0 REFERENCES

Adamski, J.C. and German, E.R., 2004. Hydrogeology and quality of ground water in Orange County, Florida. US Geological Survey.

AFCEE, 2007. Protocol for In Situ Bioremediation of Chlorinated Solvents Using Edible Oil. Air Force Center for Engineering and the Environmental, Environmental Science Division, Technology Transfer Outreach Office

AGVIQ-CH2M HILL JV-II, 2006. Remedial Action Work Plan, Injection and Recirculation of Emulsified Oil Substrate (EOS®) at Study Area 17, Former Naval Training Center Orlando, Orlando, Florida. U.S. Naval Facilities Engineering Command Southern Division

AGVIQ-CH2M HILL JV-II, 2010. Construction Completion Report, Phase II Pilot Injections, Biobarrier at Operable Unit 2, Former Naval Training Center Orlando, Orlando, Florida. NAVFAC Southeast

Angelidaki, I., Petersen, S.P. and Ahring, B.K., 1990. Effects of lipids on thermophilic anaerobic digestion and reduction of lipid inhibition upon addition of bentonite. Applied microbiology and biotechnology, 33(4), pp.469-472.

Aulenta, F., Majone, M. and Tandoi, V., 2006. Enhanced anaerobic bioremediation of chlorinated solvents: environmental factors influencing microbial activity and their relevance under field conditions. Journal of Chemical Technology and Biotechnology, 81(9), pp.1463-1474.

BFA (Barnes, Ferland and Associates, Inc.), 2012. September 2012 Semiannual Monitoring Report Study Area 17 - McCoy Annex, Former Naval Training Center Orlando, Florida. Naval Facilities Engineering Command Southeast.

Bolster, C.H., Mills, A.L., Hornberger, G.M. and Herman, J.S., 2001. Effect of surface coatings, grain size, and ionic strength on the maximum attainable coverage of bacteria on sand surfaces. Journal of contaminant hydrology, 50(3), pp.287-305.

Borden, R.C., 2007a. Effective Distribution of Emulsified Edible Oil for Enhanced Anaerobic Bioremediation. Journal of Contaminant Hydrology, 94(1), pp.1-12.

Borden, R.C., 2007b. Anaerobic Bioremediation of Perchlorate and 1,1,1-Trichloroethane in an Emulsified Oil Barrier. Journal of Contaminant Hydrology, 94(1), pp.13-33.

Borden, R.C., 2007c. Engineering Delivery of Insoluble Amendments. Partners in Environmental Technology Symposium & Workshop, SERDP, Washington, DC.

Borden, R.C., Beckwith, W.J., Lieberman, M.T., Akladiss, N. and Hill, S.R., 2007. Enhanced anaerobic bioremediation of a TCE source at the Tarheel Army Missile Plant using EOS. Remediation Journal, 17(3), pp.5-19.

Borden, R.C., Clayton, M., Weispfenning, A.M., Simpkin, T. and Lieberman, M.T., 2008. Technical Report-Design Tool for Planning Emulsified Oil Injection Systems. Environmental Security Technology Certification Program, Arlington, Virginia.

Borden, R.C., Tillotson, J.M., Ng, G.-H.C., Bekins, B.A., Kent, D.B., and Curtis, G.P., 2015. Final Report-Impacts of Enhanced Reductive Bioremediation on Post-Remediation Groundwater

Quality, SERDP Project ER-2131. Strategic Environmental Research and Development Program, Arlington, Virginia.

CH2M HILL, 2006. Technical Memorandum - Biobarrier Injection Pilot Study Work Plan, Operable Unit 2, Naval Training Center Orlando, Florida. NAVFAC Southern Division.

CH2M HILL, 2008. Technical Memorandum - Biobarrier Injection Pilot Study Summary, Operable Unit 2, Naval Training Center Orlando Florida. NAVFAC Southern Division.

CH2M HILL, 2010. Evaluation of Enhanced Reductive Dechlorination Treatment Progress, Study Area 17, Former Naval Training Center Orlando, Florida. NAVFAC Southern Division.

Chapelle, F.H., Haack, S.K., Adriaens, P., Henry, M.A. and Bradley, P.M., 1996. Comparison of E h and H2 Measurements for Delineating Redox Processes in a Contaminated Aquifer. Environmental Science & Technology, 30(12), pp.3565-3569.

Clayton, M.H. and Borden, R.C., 2009. Numerical modeling of emulsified oil distribution in heterogeneous aquifers. Groundwater, 47(2), pp.246-258.

Clement, T.P., 1997. A Modular Computer Code for Simulating Reactive Multi-Species Transport in 3-D Groundwater Systems. PNNL-11720. Pacific Northwest National Laboratory, Richland, WA.

Coulibaly, K.M. and Borden, R.C., 2004. Impact of edible oil injection on the permeability of aquifer sands. Journal of contaminant hydrology, 71(1), pp.219-237.

Coulibaly, K.M., Long, C.M. and Borden, R.C., 2006. Transport of edible oil emulsions in clayey sands: One-dimensional column results and model development. Journal of Hydrologic Engineering, 11(3), pp.230-237.

Duhamel, M., Wehr, S.D., Yu, L., Rizvi, H., Seepersad, D., Dworatzek, S., Cox, E.E. and Edwards, E.A., 2002. Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, cis-dichloroethene and vinyl chloride. Water Research, 36(17), pp.4193-4202.

Eaddy, A., 2008. Scale-Up and Characterization of an Enrichment Culture for Bioaugmentation of the P-Area Chlorinated Ethene Plume at the Savannah River Site. M.S. Thesis, Clemson University.

ESTCP, 2005. Bioaugmentation for remediation of chlorinated solvents: Technology development, status, and research needs. Environmental Security Technology Certification Program, Arlington, VA, USA.

Farhat, S.K., Newell, C.J., Sale, T.C., Dandy, D.S., Wahlberg, J.J., Seyedabbasi, M.A., McDade, J.M., and Mahler, N.T., 2012. Matrix Diffusion Toolkit. Environmental Security Technology Certification Program, Arlington, Virginia.

Harbaugh, A.W., Banta, E.R., Hill, M.C. and McDonald, M.G., 2000. MODFLOW-2000, The U. S. Geological Survey Modular Ground-Water Model-User Guide to Modularization Concepts and the Ground-Water Flow Process. Open-file Report. U. S. Geological Survey, (92), p.134.

Hiortdahl, K.M. and Borden, R.C., 2014. Enhanced reductive dechlorination of tetrachloroethene dense nonaqueous phase liquid with EVO and $Mg(OH)_2$. Environmental science & technology, 48(1), pp.624-631.

He, J., Ritalahti, K.M., Aiello, M.R. and Löffler, F.E., 2003. Complete detoxification of vinyl chloride by an anaerobic enrichment culture and identification of the reductively dechlorinating population as a Dehalococcoides species. Applied and Environmental Microbiology, 69(2), pp.996-1003.

HLA (Harding Lawson and Associates), 1999. Base Realignment and Closure Environmental Site Screening Report, Study Area 17, Naval Training Center Orlando.

Holliger, C., Schraa, G., Stams, A.J. and Zehnder, A.J., 1993. A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. Applied and Environmental Microbiology, 59(9), pp.2991-2997.

Holliger, C., Hahn, D., Harmsen, H., Ludwig, W., Schumacher, W., Tindall, B., Vazquez, F., Weiss, N. and Zehnder, A.J., 1998. Dehalobacter restrictus gen. nov. and sp. nov., a strictly anaerobic bacterium that reductively dechlorinates tetra-and trichloroethene in an anaerobic respiration. Archives of Microbiology, 169(4), pp.313-321.

Hunter, W.J., 2005. Injection of innocuous oils to create reactive barriers for bioremediation: Laboratory studies. Journal of contaminant hydrology, 80(1), pp.31-48.

Jiang, C., 2012. Development of Enrichment Cultures for Anaerobic Reductive Dechlorination of Tetrachloroethene Under Low pH Conditions. M.S. Thesis, Clemson University.

Johnson, P.R. and Elimelech, M., 1995. Dynamics of colloid deposition in porous media: Blocking based on random sequential adsorption. Langmuir, 11(3), pp.801-812.

Jung, Y., Coulibaly, K.M. and Borden, R.C., 2006. Transport of edible oil emulsions in clayey sands: 3D sandbox results and model validation. Journal of Hydrologic Engineering, 11(3), pp.238-244.

Kovacich, M.S., D. Beck, T. Rabideau, K. Smith-Pettypiece, M. Noel, M.J. Zack, and M.T. Cannaert, 2007. Full-Scale Bioaugmentation to Create a Passive Biobarrier to Remediate a TCE Groundwater Plume. In Proceedings: Ninth International In Situ and On-Site Bioremediation Symposium, Baltimore, Maryland, USA.

Krajmalnik-Brown, R., Hölscher, T., Thomson, I.N., Saunders, F.M., Ritalahti, K.M. and Löffler, F.E., 2004. Genetic identification of a putative vinyl chloride reductase in Dehalococcoides sp. strain BAV1. Applied and Environmental Microbiology, 70(10), pp.6347-6351.

Krumholz, L.R., Sharp, R. and Fishbain, S.S., 1996. A freshwater anaerobe coupling acetate oxidation to tetrachloroethylene dehalogenation. Applied and Environmental Microbiology, 62(11), pp.4108-4113.

Kuo, Y.C., Liang, S.H., Wang, S.Y., Chen, S.H. and Kao, C.M., 2013. Application of emulsified substrate biobarrier to remediate TCE-contaminated groundwater: Pilot-scale study. Journal of Hazardous, Toxic, and Radioactive Waste, 18(2).

Li, J.J., 2012. Adaptation of a Dechlorinating Culture, KB-1, to Acidic Environments, M.S. Thesis, University of Toronto.

Liang, S.H., Kuo, Y.C., Chen, S.H., Chen, C.Y. and Kao, C.M., 2013. Development of a slow polycolloid-releasing substrate (SPRS) biobarrier to remediate TCE-contaminated aquifers. Journal of hazardous materials, 254, pp.107-115.

Löffler, F.E., Yan, J., Ritalahti, K.M., Adrian, L., Edwards, E.A., Konstantinidis, K.T., Müller, J.A., Fullerton, H., Zinder, S.H. and Spormann, A.M., 2013. Dehalococcoides mccartyi gen. nov., sp. nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation, belong to a novel bacterial class, Dehalococcoidia classis nov., order Dehalococcoidales ord. nov. and family Dehalococcoidaceae fam. nov., within the phylum Chloroflexi. International journal of systematic and evolutionary microbiology, 63(2), pp.625-635.

Logan, B.E., 1999. Environmental transport processes. John Wiley & Sons, New York.

Long, C.M. and Borden, R.C., 2006. Enhanced reductive dechlorination in columns treated with edible oil emulsion. Journal of contaminant hydrology, 87(1), pp.54-72.

Luijten, M.L., de Weert, J., Smidt, H., Boschker, H.T., de Vos, W.M., Schraa, G. and Stams, A.J., 2003. Description of Sulfurospirillum halorespirans sp. nov., an anaerobic, tetrachloroethene-respiring bacterium, and transfer of Dehalospirillum multivorans to the genus Sulfurospirillum as Sulfurospirillum multivorans comb. nov. International Journal of Systematic and Evolutionary Microbiology, 53(3), pp.787-793.

Maillard, J., Regeard, C. and Holliger, C., 2005. Isolation and characterization of Tn-Dha1, a transposon containing the tetrachloroethene reductive dehalogenase of Desulfitobacterium hafniense strain TCE1. Environmental microbiology, 7(1), pp.107-117.

Maymó-Gatell, X., Nijenhuis, I. and Zinder, S.H., 2001. Reductive dechlorination of cis-1, 2-dichloroethene and vinyl chloride by "Dehalococcoides ethenogenes". Environmental science & technology, 35(3), pp.516-521.

McCarty, P.L., Chu, M.Y. and Kitanidis, P.K., 2007. Electron donor and pH relationships for biologically enhanced dissolution of chlorinated solvent DNAPL in groundwater. European Journal of Soil Biology, 43(5), pp.276-282.

McGuire, T.M., D.T. Adamson, M.S. Burcham, Philip B. Bedient, and C.J. Newell, 2016. Evaluation of Long-Term Performance and Sustained Treatment at Enhanced Anaerobic Bioremediation Sites, Groundwater Monitoring & Remediation, 36(2), pp. 32-44.

Mohn, W.W. and Tiedje, J.M., 1992. Microbial reductive dehalogenation. Microbiological reviews, 56(3), pp.482-507.

Moretti, L., 2005. In situ bioremediation of DNAPL Source Zones. US Environmental Protection Agency, Office of Solid Waste and Emergency Response, Technology Innovation and Field Services Division.

Müller, J.A., Rosner, B.M., Von Abendroth, G., Meshulam-Simon, G., McCarty, P.L. and Spormann, A.M., 2004. Molecular identification of the catabolic vinyl chloride reductase

from Dehalococcoides sp. strain VS and its environmental distribution. Applied and Environmental Microbiology, 70(8), pp.4880-4888.

Resolution (Resolution Consultants), 2013. Final Long Term Monitoring Optimization Report, Study Area 17 (SA17), Former NTC Orlando, Florida.

Riha, B.D., Looney, B.B., Noonkester, J.V., Hyde, K., Walker, R. and Rambo, B., 2009. Treatability Study for Edible Oil Deployment for Enhanced cVOC Attenuation for T-Area, Savannah River Site: Interim Report–Year One. Technical Report SRNL-RP-2009-00539. Savannah River National Laboratory, Aiken, SC.

Rijnaarts, H.H., Norde, W., Bouwer, E.J., Lyklema, J. and Zehnder, A.J., 1996a. Bacterial deposition in porous media related to the clean bed collision efficiency and to substratum blocking by attached cells. Environmental science & technology, 30(10), pp.2869-2876.

Rijnaarts, H.H., Norde, W., Bouwer, E.J., Lyklema, J. and Zehnder, A.J., 1996b. Bacterial deposition in porous media: effects of cell-coating, substratum hydrophobicity, and electrolyte concentration. Environmental science & technology, 30(10), pp.2877-2883.

Ritalahti, K.M., Amos, B.K., Sung, Y., Wu, Q., Koenigsberg, S.S. and Löffler, F.E., 2006. Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple Dehalococcoides strains. Applied and environmental microbiology, 72(4), pp.2765-2774.

Rowlands, D., 2004. Development of optimal pH for degradation of chlorinated solvents by the KB-1™ anaerobic bacterial culture. M.S. Thesis, University of Guelph.

Ryan, J.N. and Elimelech, M., 1996. Colloid mobilization and transport in groundwater. Colloids and surfaces A: Physicochemical and engineering aspects, 107, pp.1-56.

Sale, T.C., Zimbron, J.A. and Dandy, D.S., 2008. Effects of reduced contaminant loading on downgradient water quality in an idealized two-layer granular porous media. Journal of Contaminant Hydrology, 102(1), pp.72-85.

Scheutz, C., Durant, N.D., Dennis, P., Hansen, M.H., Jørgensen, T., Jakobsen, R., Cox, E.E. and Bjerg, P.L., 2008. Concurrent ethene generation and growth of Dehalococcoides containing vinyl chloride reductive dehalogenase genes during an enhanced reductive dechlorination field demonstration. Environmental science & technology, 42(24), pp.9302-9309.

Solutions-IES, 2006. Protocol for Enhanced In Situ Bioremediation Using Emulsified Edible Oil. Environmental Security Technology Certification Program, Arlington, VA, USA.

Solutions-IES, 2011b. Work Plan - In Situ Bioremediation of Chlorinated Solvents in Groundwater at SA-17, Former Naval Training Center Orlando, Orlando, Florida. Naval Facilities Engineering Command Southeast

Solutions-IES, 2013. Construction Completion Report - In Situ Bioremediation of Chlorinated Solvents in Groundwater at SA-17, Former Naval Training Center Orlando, Orlando, Florida. Naval Facilities Engineering Command Southeast

Solutions-IES, 2013b. 14-Month Post-Injection Performance Monitoring Report - In Situ Bioremediation of Chlorinated Solvents in Groundwater at SA-17, Former Naval Training Center Orlando, Orlando, Florida. Naval Facilities Engineering Command Southeast.

Soo, H. and Radke, C.J., 1984. Flow mechanism of dilute, stable emulsions in porous media. Industrial & engineering chemistry fundamentals, 23(3), pp.342-347.

Soo, H. and Radke, C.J., 1986. A filtration model for the flow of dilute, stable emulsions in porous media—I. Theory. Chemical Engineering Science, 41(2), pp.263-272.

Soo, H., Williams, M.C. and Radke, C.J., 1986. A filtration model for the flow of dilute, stable emulsions in porous media—II. Parameter evaluation and estimation. Chemical Engineering Science, 41(2), pp.273-281.

Stroo, H.F., Major, D.W. and Gossett, J.M., 2010. Bioaugmentation for anaerobic bioremediation of chlorinated solvents. In In situ remediation of chlorinated solvent plumes (pp. 425-454). Springer New York.

Stroo, H.F., Major, D.W., Steffan, R.J., Koenigsberg, S.S. and Ward, C.H., 2013. Bioaugmentation with Dehalococcoides: A decision guide. In Bioaugmentation for Groundwater Remediation (pp. 117-140). Springer New York.

Stroo, H.F., Leeson, A. and Ward, C.H. eds., 2012. Bioaugmentation for groundwater remediation (Vol. 5). Springer Science & Business Media.

Stroo, H.F., West, M.R., Kueper, B.H., Borden, R.C., Major, D.W. and Ward, C.H., 2014. In situ bioremediation of chlorinated ethene source zones. In Chlorinated solvent source zone remediation (pp. 395-457). Springer New York.

Sung, Y., Ritalahti, K.M., Sanford, R.A., Urbance, J.W., Flynn, S.J., Tiedje, J.M. and Löffler, F.E., 2003. Characterization of two tetrachloroethene-reducing, acetate-oxidizing anaerobic bacteria and their description as Desulfuromonas michiganensis sp. nov. Applied and Environmental Microbiology, 69(5), pp.2964-2974.

Sung, Y., Ritalahti, K.M., Apkarian, R.P. and Löffler, F.E., 2006. Quantitative PCR confirms purity of strain GT, a novel trichloroethene-to-ethene-respiring Dehalococcoides isolate. Applied and Environmental Microbiology, 72(3), pp.1980-1987.

Suthersan, S.S., Lutes, C.C., Palmer, P.L., Lenzo, F., Payne, F.C., Liles, D.S. and Burdick, J., 2002. Technical protocol for using soluble carbohydrates to enhance reductive dechlorination of chlorinated aliphatic hydrocarbons. AFCEE and ESTCP.

Tang, G., Wu, W.M., Watson, D.B., Parker, J.C., Schadt, C.W., Shi, X. and Brooks, S.C., 2013. U (VI) bioreduction with emulsified vegetable oil as the electron donor–microcosm tests and model development. Environmental science & technology, 47(7), pp.3209-3217.

Tang, G., Watson, D.B., Wu, W.M., Schadt, C.W., Parker, J.C. and Brooks, S.C., 2013. U (VI) bioreduction with emulsified vegetable oil as the electron donor–model application to a field test. Environmental science & technology, 47(7), pp.3218-3225.

Tetra Tech (Tetra Tech NUS), 2001. Remedial Investigation Report for Operable Unit 2, McCoy Annex Landfill, Naval Training Center, Orlando, Florida (Rev. 2). Naval Facilities Engineering Command Southern Division.

Tetra Tech (Tetra Tech NUS), 2003. Final Feasibility Study Report, Operable Unit 2, McCoy Annex Landfill Naval Training Center, Orlando, Florida (Rev. 1). Naval Facilities Engineering Command Southern Division.

Tillotson, J.M. and Borden, R.C., 2017. Rate and Extent of Chlorinated Ethene Removal at 37 ERD Sites. Journal of Environmental Engineering, 143(8), p.04017028.

Vainberg, S., Steffan, R.J., Rogers, R., Ladaa, T., Pohlmann, D. and Leigh, D., 2006. Production and application of large-scale cultures for bioaugmentation. Proc 5th Internat Conf Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, USA. May 22-25. Paper No. A-50.

Vogel, T.M. and McCARTY, P.L., 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. Applied and Environmental Microbiology, 49(5), pp.1080-1083.

Watson, D.B., Wu, W.M., Mehlhorn, T., Tang, G., Earles, J., Lowe, K., Gihring, T.M., Zhang, G., Phillips, J., Boyanov, M.I. and Spalding, B.P., 2013. In situ bioremediation of uranium with emulsified vegetable oil as the electron donor. Environmental science & technology, 47(12), pp.6440-6448.

Weispfenning, A.M. and Borden, R.C., 2008. A design tool for planning emulsified oil-injection systems. Remediation Journal, 18(4), pp.33-47.

Westall, J.C. and Gschwend, P.M., 1993. "Mobilizing and depositing colloids." Manipulation of groundwater colloids for environmental restoration, Lewis, Ann Harbor, Mich.

Yang, Y., 2012. Exploring Anaerobic Reductive Dechlorination at Low pH Environments. M.S. Thesis, University of Tennessee.

Yang, Y., 2016. Bioremediation of Chlorinated Ethenes: pH Effects, Novel Dechlorinators and Decision-Making Tools. Ph.D. Dissertation, University of Tennessee.

Zhong, L., Truex, M.J., Kananizadeh, N., Li, Y., Lea, A.S. and Yan, X., 2015. Delivery of vegetable oil suspensions in a shear thinning fluid for enhanced bioremediation. Journal of contaminant hydrology, 175, pp.17-25.

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APPENDIX A POINTS OF CONTACT

Post-Remediation Evaluation of EVO Treatment - How Can We Improve Performance?

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APPENDIX B NTC ORLANDO SA17

B.1. Site Characteristics

B.1.1. Description and History

Study Area 17 (SA17) occupies approximately 9 acres in the central part of the McCoy Annex at Former Naval Training Center (NTC) Orlando (**Figure B-1**). The site is located near the intersection of Avenue C and Andros Place. A fenced area was used for motor pool storage and maintenance, including a wash rack with drainage to a former leach bed and a building used to store hazardous and flammable materials. Additionally, there was a drum and transformer storage area at the site. All former buildings have been removed from the site.

Previous site activities related to the motor pool area are suspected to have contributed to subsurface soil and groundwater impacts including exceedances of screening criteria for polynuclear aromatic hydrocarbons (PAHs) in soil and chlorinated volatile organic compounds (CVOCs) in groundwater.

The current RAOs state that the remedial goals for SA17 are at or below the Florida Department of Environmental Protection (FDEP) groundwater cleanup target levels (GCTLs) monitored for two consecutive semi-annual sampling events, with a No Further Action (NFA) to be recommended in accordance with Risk Management Option Level 1, as described in Chapter 62-780.680(1) FAC.

Environmental activities conducted at SA17 have included an initial site screening, supplemental site screening, soil interim remedial action (IRA), groundwater IRA, site investigation action and source area investigation. Major site investigation and remedial activities included the following.

- Several underground storage tanks (USTs) and aboveground storage tanks (ASTs) were removed from the site prior to 1994. Subsequent site screening in 1995 and 1996 identified polyaromatic hydrocarbons (PAHs) in soil and CVOCs in groundwater at levels exceeding residential and industrial screening levels. In May 1999, the Environmental Detachment Charleston completed a soil interim remedial action (IRA) to remove PAH contaminated surface soil. The removal action mitigated PAH-contaminated soil to levels compatible with a future non-residential land use (Tetra Tech, 2004).
- Between 2000 to 2002, organic contaminants in saturated soil and groundwater were treated by in situ chemical oxidation (ISCO). This involved the injection of 100,000 lb of hydrogen peroxide (H_2O_2) and trace quantities of metallic salts over a roughly 100 ft by 300 ft area in four injection events. ISCO was effective in reducing dissolved phase TCE concentrations by 88%. However, TCE concentrations rebounded over time. Following the ISCO treatment, a Membrane Interface Probe (MIP) investigation identified areas with residual CVOCs (CH2M HILL, 2006).

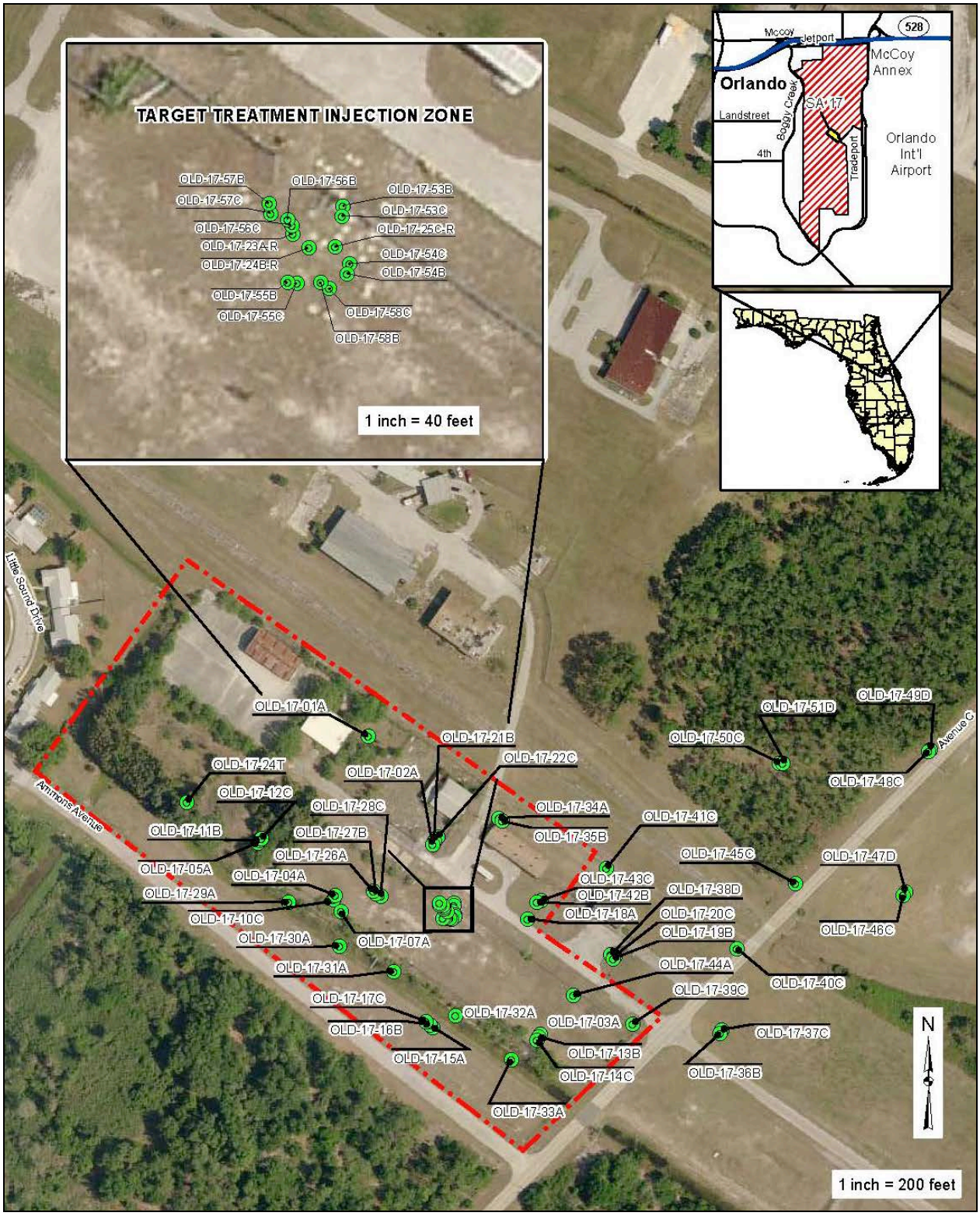

Figure B-1. SA17 Monitor Well Location Map (BFA 2012).

- In 2006, Emulsified Vegetable Oil (EVO) was injected at the site to stimulate Enhanced Reductive Dechlorination (ERD). Based on the results of the MIP survey, the EVO injection targeted a 50 ft x 50 ft area. Two vertical intervals, B zone (15 to 25 ft bgs) and C zone (30 to 40 ft bgs), were treated. Details of the injection system design are presented in the Remedial Action Work Plan (CH2M HILL, 2006). The remedial design was based, in part, on results of an August 16, 2005, constant rate pumping test in which aquifer parameters for B and C zones were measured. Groundwater modeling was then used to evaluate different injection and extraction systems for distributing the EVO. The recommended system included six injection wells surrounding a central extraction well. During the field implementation, the soils within B zone were found to be less permeable than anticipated, and less EOS® was injected than planned. To more effectively treat B zone, CH2M HILL performed a "polishing" injection of EVO in 2008 using direct-push technology in the area around OLD-17-55B and OLD-17-56B. Post-injection monitoring revealed the polishing event had induced ERD and increased the concentrations of daughter products cis-1,2-dichloroethene (*c*DCE) and vinyl chloride (VC).
- On April 18, 2008, the property was transferred to the City of Orlando (City) for commercial or industrial use through the Early Transfer Process. The City agreed to continue implementing the groundwater restrictions already in place at the site
- In February 2012, Solutions-IES (2013a) injected additional EVO and some buffered EVO through the previously installed injection wells in both B and C zones to replenish the previous treatment and raise the aquifer pH to a level appropriate for ERD. Monitoring data collected after the 2012 injections indicated that the additional EVO injections did stimulate ERD in some wells, but pH remained low in much of C zone.
- In 2013, the number of wells routinely monitored was reduced, based on results of a Long Term Monitoring Optimization study and comments from FDEP (Resolution, 2013).

B.1.2. Hydrogeology

The site lies within the mid-peninsular zone geomorphic province, which consists of low relief marine terraces underlain by limestone (Scott et al., 2001). The marine terraces are products of a low energy marine depositional environment, resulting in discontinuous layers of fine sand, sandy silt and silty sand. The topography at the site is relatively flat. A drainage ditch runs from the northwest to the southeast and appears to form a hydraulic barrier, which affects the groundwater flow direction depending upon the amount of precipitation and, correlatively, whether the stream is gaining or losing water to the surficial aquifer.

The water table is typically encountered at 4 to 6 ft bgs. Hydrogeologic units in the area include two aquifer systems; the surficial aquifer and the Floridian aquifer (Adamski and German 2004). The surficial aquifer and the upper Floridian aquifer are separated by the Miocene Hawthorne Group, which is classified as a confining hydrogeologic unit. Cone penetrometer test (CPT) results (HLA, 1999) indicated that soils in the upper 30 feet of the unconfined aquifer consists of fine sand with multiple discontinuous layers of silty sand at intervals ranging from 10 to 20 ft bgs and 20 to 30 ft bgs at thicknesses ranging from less than one foot to greater than five feet. Beneath the lower silty sand intervals lies a layer of fine to coarse grained sand that extends from 30 to 50 ft bgs. The confining unit of the Hawthorne Group occurs at approximately 55 ft bgs and is considered to be the bottom of the surficial aquifer.

The subsurface has been delineated into four different zones designated Zone A (5 to 15 ft bgs), Zone B (15 to 30 ft bgs), Zone C (30 to 50 ft bgs), and Zone D (>50 ft bgs). CH2M HILL (2006) report that, in some locations, a thin layer of lower permeability silty sand separates Zones A and B, and a thin semi-confining silty sand separates Zones B and C. Zone D is the upper Hawthorn Aquifer and is separated from the surficial aquifer by a confining clay.

Table B-1 presents a summary of hydraulic conductivity (K) measurements at SA17. In 1999, slug tests were conducted in wells screened in the A, B, and C zones along the perimeter of the site (HLA, 1999). In 2005, CH2M HILL conducted a 8 hour pump test by extracting water from well 51C (screened 42 – 47 ft bgs) at a constant rate of 5 gallon per minute (gpm) while monitoring drawdown in five Zone C wells, nine Zone B wells and two Zone A wells. In B zone, drawdown varied from 0.34 to 0.57 ft at 10 to 20 ft horizontally and 5 to 10 ft vertically from the pumping well. Drawdowns in C zone varied from 1.06 to 1.22 ft at radial distances of 10 to 20 ft from the pumping well. The substantial drawdown in the B zone wells indicate a relatively good hydraulic connection between the Zones B and C. Wells to the northeast showed greater draw down in the B zone and less drawdown in C zone indicating a better hydraulic connection in this area.

In February 2015, K profiles were measured using a hydraulic profiling tool (HPT) at five locations in the northern portion of the ERD treatment area (**Figure B-2**). The K profiles shown in **Figure B-3** are reasonably consistent, showing a more heterogeneous zone extending from the water table (~5.5 ft bgs) to ~15 ft bgs (Zone A) with an average K of 6.3 ft/d, underlain by a more homogeneous, lower K (average K = 3.9 ft/d) region. The average K in the Zone C (=5.8 ft/d) is somewhat higher and reasonably constant with depth. There is no evidence of significant lower K zones in any boring from 5 to 45 ft bgs that would limit vertical water movement.

Table B-1. Summary of Hydraulic Conductivity (K) Measurements.

Zone	Depth Interval (ft bgs)	K (ft/d)		Comments	Source
		Mean	Range		
A	2 - 12	1.4	0.3 - 3.4	slug tests in 4 wells	HLA, 1999
B	15 - 20	0.4	0.2 - 0.6	slug tests in 3 wells	
B	25 - 30	0.5	--	slug test in 1 well	
C	43 - 52	2.0	0.6 - 3.8	slug tests in 4 wells	
A/B	0 - 30	4.7	3.1 - 6.5	8 hr pump test from C zone at 5 gpm	CH2M HILL 2006
C	30 - 50	6.9	6.4 - 8.3		
B	20 - 25	0.5	--	slug tests in 1 well	This project
C	35 - 40	5.8	5.2 - 11.7	slug tests in 3 wells	
A	5 - 15	6.3	0.6 - 20.7	Vertically averaged K from 5 HPT profiles	This project
B	15 - 30	3.9	0.4 - 10.3		
C	30 - 50	5.8	0.2 - 13.8		

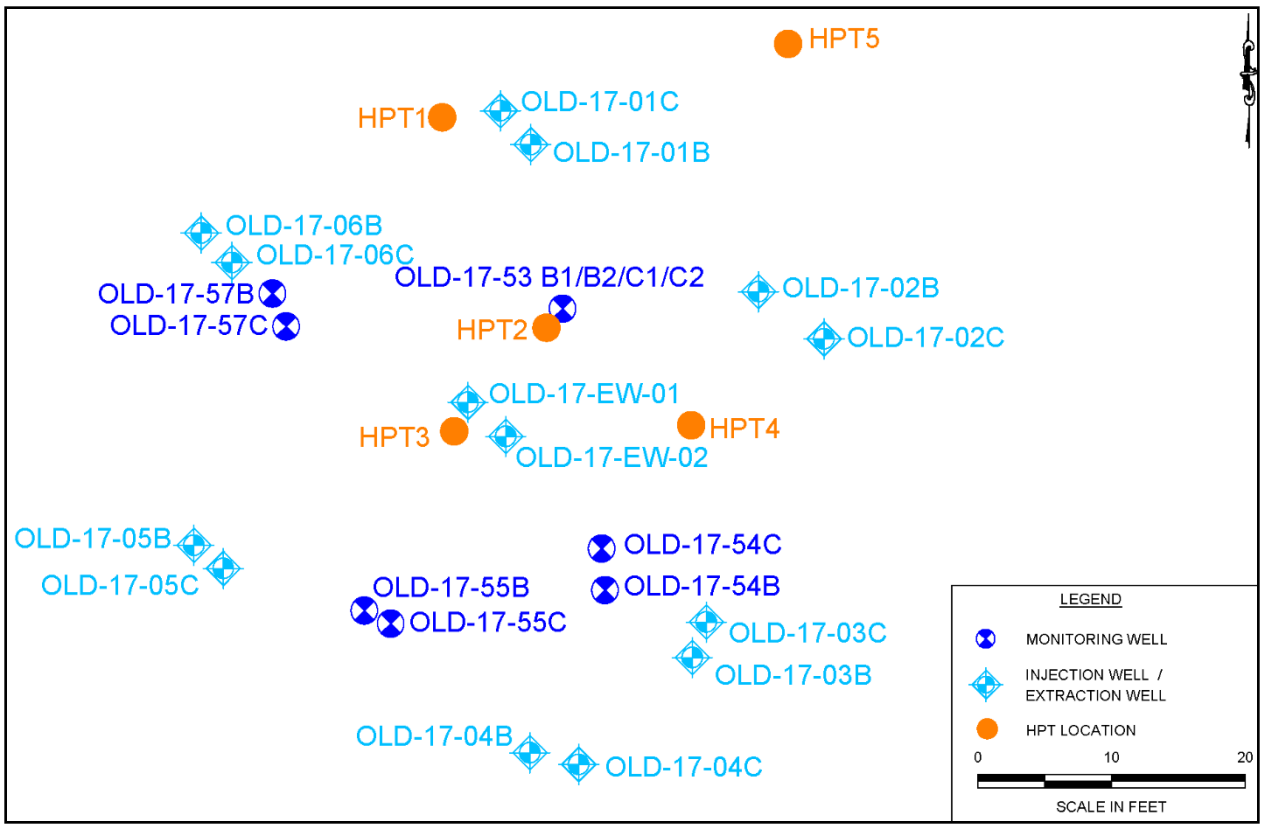

Figure B-2. Location of HPT Profiles, Soil Borings, Injection and Monitor Wells near SA17 Bioremediation Project.

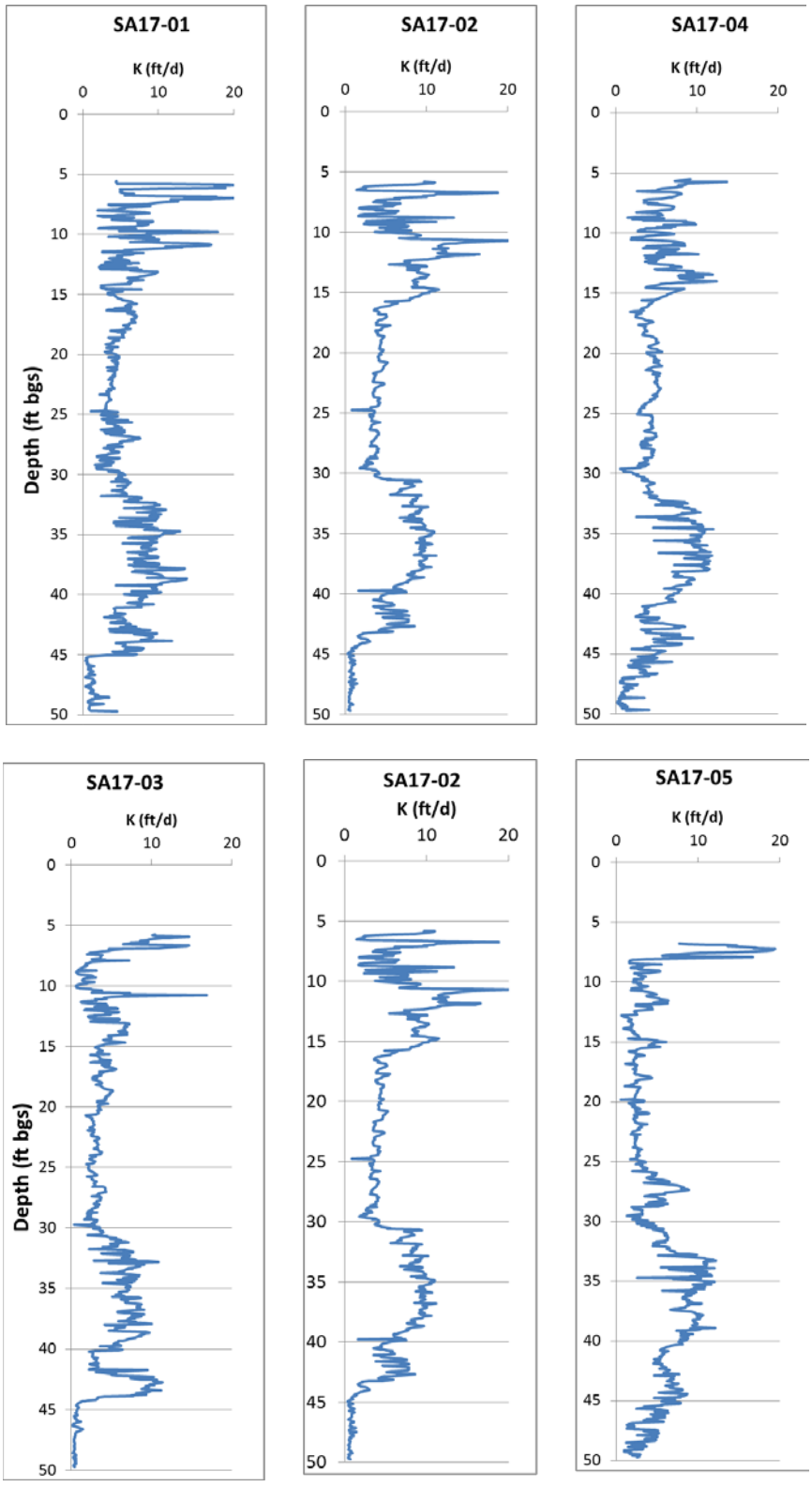

Figure B-3. HPT K Profiles Measured in February 2015.

In the shallow A Zone, groundwater is influence by the large drainage channel to the south and small drainage ditch to the north. During the wet summer season, flow is radially to the north and south towards these drainage features. In the deeper C Zone, flow is to the east. Contaminant migration indicates a northerly component to the deep groundwater flow further east from the site. Groundwater flow in the intermediate B Zone is influenced by both the shallow and deep systems. A downward vertical hydraulic gradient of 0.007 to 0.020 ft/ft exists within the surficial aquifer except near the drainage ditch, where groundwater discharges to the ditch and an upward gradient of approximately 0.25 ft/ft exists. In the C Zone, horizontal gradient ranges from 0.003 to 0.004 ft/ft. Assuming an effective porosity of 0.25, the average seepage velocity in the C zone is about 30 ft/yr.

B.1.3. Contaminant Distribution Prior to ERD

Following the ISCO treatment, a MIP investigation was conducted to identify locations and depths of residual elevated concentrations of CVOCs. Confirmatory laboratory analyses are shown in **Figure B-4** with maximum CVOCs concentrations up to 577,000 µg/L.

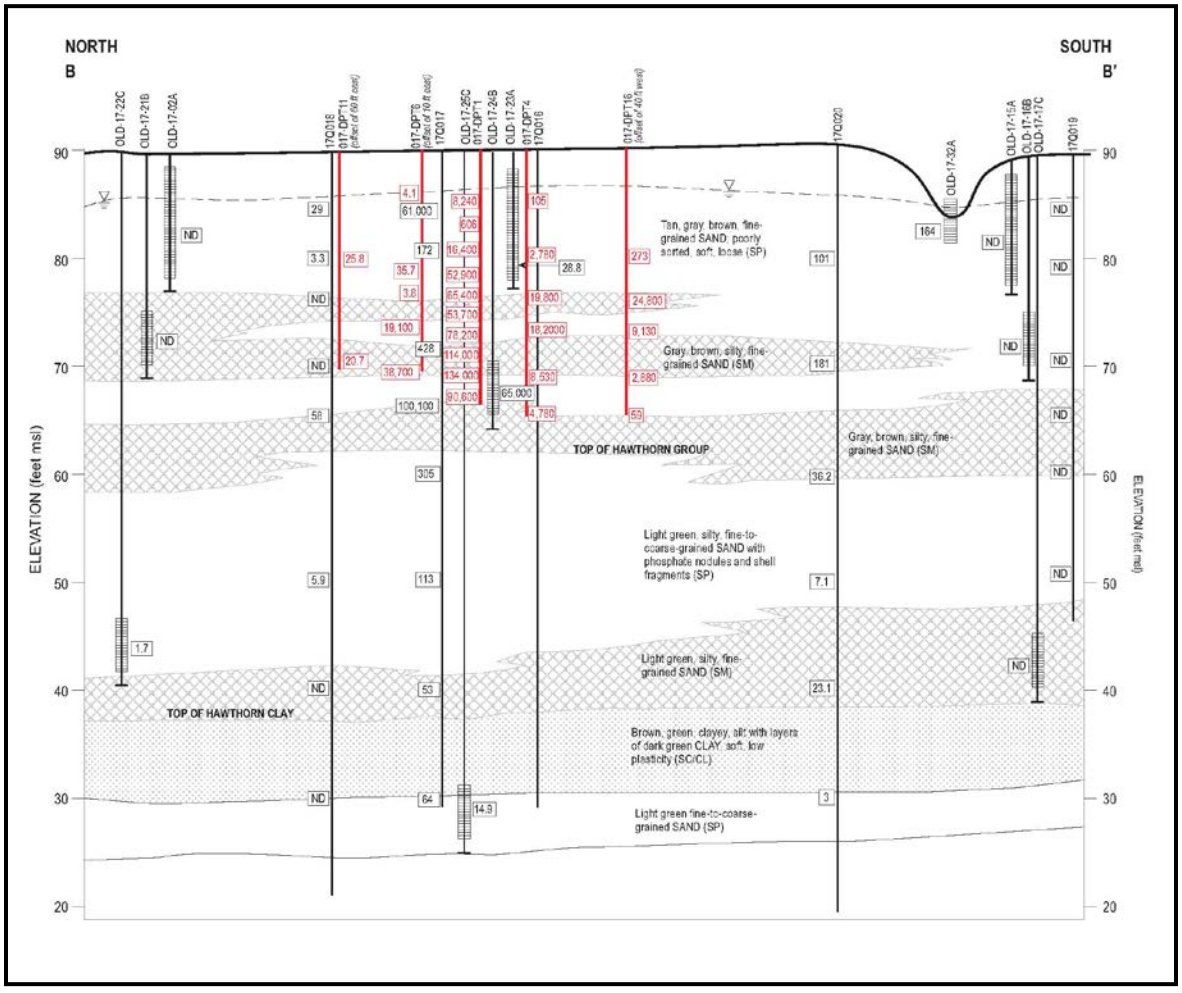

Figure B-4. SA17 Lithologic Cross-section with Results of Confirmatory TCE Measurements Shown in Red (AGVIQ-CH2M HILL JV-II, 2006)

As discussed above, CH2M HILL established the target treatment zone based upon the contaminant distribution determined by the remedial investigation performed prior to the 2006 injection. The results of groundwater sampling and Direct Push Technology (DPT) soil sampling indicated highly localized impacts, specifically near monitoring well OLD-17-25C, with the majority of contaminant mass between 15 and 35 ft bgs, and extending approximately 50 ft laterally. The maximum TCE concentration prior to ERD was 47,300 µg/L in OLD-17-53C1 and the *c*DCE and VC concentrations were much lower with maximum concentrations of 2,500 µg/L *c*DCE in OLD-17-53C2 and 820 µg/L VC in OLD-17-20C.

B.2. Remedial Operations

B.2.1. 2006 Injection

Prior to implementing the ERD project, CH2M HILL (2006) implemented an extensive microbial and geochemical characterization (CVOCs and Monitored Natural Attenuation (MNA) parameters from 50 wells, phospholipid fatty acids (PLFA), and quantitative polymerase chain reaction (qPCR) from 8 wells). Results showed that the effect of the ISCO treatment had dissipated and the aquifer was moderately reducing with some nitrate, some dissolved iron, low to moderate methane levels and 1 to 4 nanomolar (nM) dissolved H_2. Groundwater at SA17 was slightly acidic, with pH ranging from 5.4 to 6.3, and total alkalinity ranging from 8.6 to 111 milligrams per liter (mg/L). *Dehalococcoides spp. (Dhc)* was detected in both the control and baited (lactate) Bio-Traps.

Using the results of the source area delineation, CH2M HILL (2006) developed a detailed design for ERD in the SA17 source area. The target treatment zone is shown in **Figure B-5**.

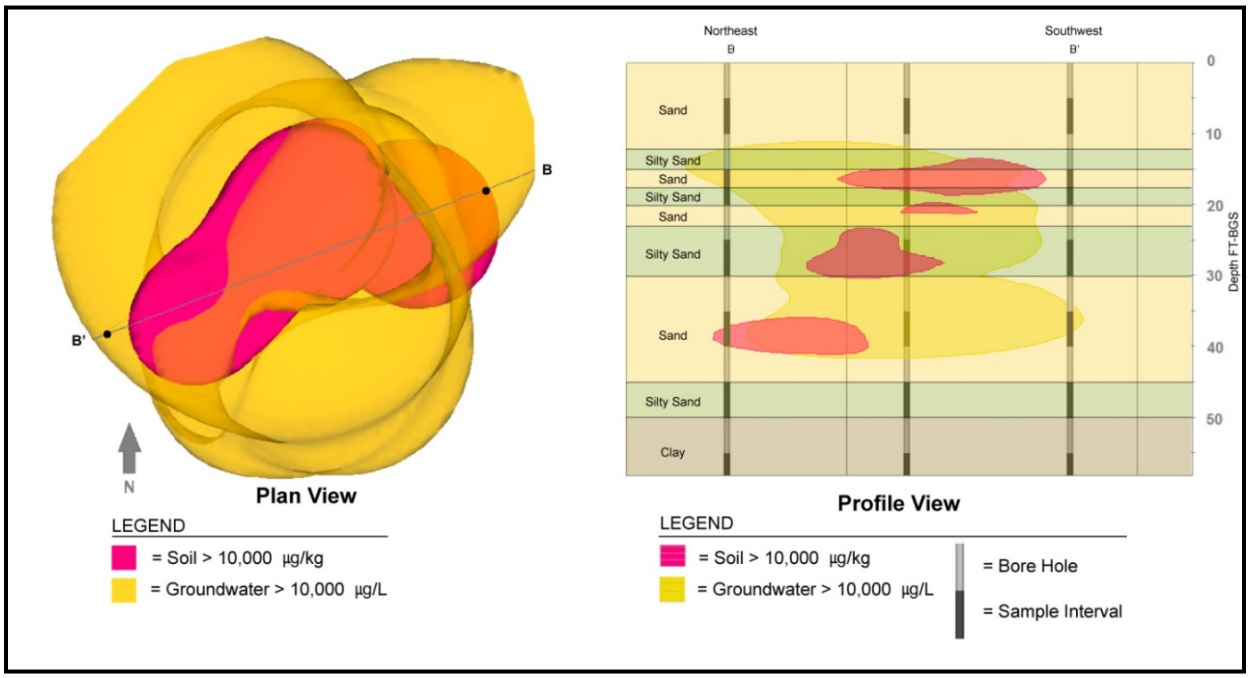

Figure B-5. Target Treatment Zone of SA17 Bioremediation Project (AGVIQ-CH2M HILL JV-II, 2006)

The final design included separate injection and extraction systems in B and C zones. Groundwater was extracted from a central well, amended with EOS 598B42, and distributed between six injection wells located in a rough circle surrounding the center well. Injection wells were screened from 15 to 25 ft (B zone) and 30 to 40 ft bgs (C zone) with a 5-foot gap between the injection intervals. Injection and monitor well locations are shown in **Figure B-2**.

Between June 23 and July 6, 2006, CH2M HILL injected EOS 598B42 diluted with groundwater through 12 injection wells targeting Zones B and C. The EOS® 598B42 contained 60% soybean oil, 4% soluble substrate, 10% emulsifiers and vitamin B-12. Extraction wells OLD-17-EW-1 (B zone) and OLD-17-EW-2 (C zone) were located at the center of the injection array, and were pumped at a rate proportional to the injection rates. The average permeability in B zone was somewhat lower so the flow rates were reduced. Approximately 2412 gallons of an 8% dilution of concentrated EOS 598B42 (1,470 lbs) was injected into B zone, followed by 30,500 gallons of chase water to further distribute the EVO. In C zone, approximately 9,839 gallons of 8% dilution (6,090 lb concentrate) was injected followed by 35,600 gallons of chase water (CH2M HILL, 2008). The injection volumes and injection rates are presented in **Table B-2**.

Table B-2. Injection Volumes and Flow Rates for 2006 Injection Event.

Well	Injection Duration (min)	Amount of EOS Injected (lb)	Total Volume Injected (gal)	Injection Rate (gal/min)	Pore Volumes Injected
B zone					
OLD-17-IW-01B	18,925	353	7,890	0.42	0.15
OLD-17-IW-02B	18,925	158	3,566	0.19	0.07
OLD-17-IW-03B	18,925	263	5,850	0.31	0.11
OLD-17-IW-04B	18,925	158	3,571	0.19	0.07
OLD-17-IW-05B	18,925	203	4,563	0.24	0.09
OLD-17-IW-06B	18,925	338	7,524	0.40	0.14
Total		1,470	32,964	1.74	0.10
C zone					
OLD-17-IW-01C	18,925	773	5,764	0.30	0.11
OLD-17-IW-02C	18,925	834	6,230	0.33	0.12
OLD-17-IW-03C	18,925	1,201	8,977	0.47	0.17
OLD-17-IW-04C	18,925	1,148	8,548	0.45	0.16
OLD-17-IW-05C	18,925	979	7,294	0.39	0.14
OLD-17-IW-06C	18,925	1,155	8,614	0.46	0.16
Total		6,090	45,426	2.40	0.14

Due to the lower permeability of the B zone, the total mass of EOS® and volume of water injected was lower than intended. Post-injection monitoring results indicated that while TCE was being reduced to *c*DCE and VC, complete reduction of TCE to ethene was limited. In 2008, approximately 142 gallons of 6% EOS 598B42 solution was injected at three locations near monitor well OLD-17-55B and three locations near OLD-17-56B using DPT to further stimulate ERD (total of 850 gallons injected). Diluted EVO was distributed from 16-20 ft and 20-24 ft bgs. No extraction and/or recirculation was performed (CH2M HILL, 2010).

B.2.2. 2012 Injection

Monitoring data collected in 2010 and 2011 indicated that TCE concentrations were beginning to rebound in some wells and total organic carbon (TOC) levels in monitor wells were low. pH was also below levels appropriate for reduction of VC to ethene.

Between February 14 -16, 2012, Solutions-IES performed a third injection at the site. Five existing injection wells and one existing extraction well from each of the two target treatment zones (B and C zones) were used for injection (**Figure B-2**). Two six-well groups were manifolded together. While the total injection duration differed for each well, the injection began concurrently for all wells. A total of 4 drums EOS 598B42 (1680 lb) and 1 drum AquaBupH™ (525 lb) were distributed evenly between the six Zone B injection wells. The AquabupH™ contained 39% soybean oil, 4% soluble substrate, 7% emulsifiers and 10% $Mg(OH)_2$. Identical amounts were injected into zone C. Total fluid injection volumes were 47,330 gallons in Zone B and 63,185 gallons in zone C.

B.3. Remedial System Performance

Performance of the ERD remediation system was evaluated using historical groundwater monitoring data (CH2M HILL 2010, BFA 2012, Resolution 2013, Solutions-IES 2013b) and data collected as part of this project (groundwater monitoring, membrane interface profiles, and soil analysis for VOCs). Groundwater monitoring data included samples collected from monitor and injection wells in the treatment zone and analyzed for VOCs, methane, ethane, and ethene (MEE), nitrate (NO_3), sulfate (SO_4), geochemical indicators (O_2, pH, oxidation-reduction potential (ORP), acidity, alkalinity), major cations and anions (Na, K, Ca, Mg, Fe, Mn, Cl, Br), molecular biological tools (major dechlorinators and genes) and volatile fatty acids (VFAs). As the first step in this process, all available data was compiled into a single master database and reviewed to identify trends in important parameters, important biological and geochemical factors that might influence ERD, and outliers.

B.3.1. Biogeochemistry

Prior to implementation of the ERD system in 2006, the aquifer was slightly reducing, with moderate to high dissolved iron (1 to 5 mg/L) and some CH_4 (typically 0.1 to 2 mg/L). Sulfate levels at some locations were above 200 mg/L, likely due to prior injection of sulfuric acid during ISCO. Shortly after EVO injection, SO_4 concentrations declined in all wells and remained between 5 and 20 mg/L for the duration of monitoring. Sulfide levels were occasionally monitored and were consistently below 1 mg/L. There is no indication that elevated levels of sulfate or sulfide inhibited reductive dechlorination.

Shortly after the first EVO injections in 2006, there was a sharp increase in TOC in many of the B and C zone wells (**Figure B-6**). However, in a few wells, TOC never increased above baseline (**Table B-3**) suggesting poor EVO distribution in some areas. In Zone B, TOC declined rapidly in all wells, with the average TOC concentration dropping below 10 mg/L within one year. In zone C, TOC declined somewhat more gradually. However, average TOC in zone C wells was near 10 mg/L at 2 to 3 years after injection. TOC increased somewhat in several of the B and C zones wells following the 2012 reinjection, then quickly declined to near background levels. In summary, TOC levels were below optimum (< 20 mg/L) in most wells for most monitoring events. Low levels of bioavailable substrate likely limited reductive dechlorination.

Methane (CH_4) levels were less variable than TOC, gradually increasing with time in all wells. There was no apparent correlation between average CH_4 and TOC concentrations. CH_4 was elevated in some wells where TOC was low, while CH_4 was lower in some wells with high TOC. This indicates that the presence of methane is not a good indicator of bioavailable carbon. TOC is consumed relatively quickly in the subsurface and only remains elevated close to residual vegetable oil. In contrast, once CH_4 is produced, it is relatively unreactive and can migrate significant distances away from the residual vegetable oil (Borden et al., 2015).

In Zone B, the average pH was 5.8 prior to injection, and then gradually declined to ~ 5.5. After the 2012 AquabupH injections, pH increased in Zone B, remaining at or above 6 through 2016. An important exception to this general trend was well OLD-17-57B, where pH remained low with an average value of 5.7. TOC also remained low in OLD-17-57B, indicating this well was not effectively contacted. In zone C, the initial pH was lower (~5.2), but pH remained constant or increased slightly following the 2006 injection. Average pH increased in zone C following the AquabupH injection reaching a maximum of 5.9 at six months after injection, then gradually declined to near 5 in 2016. While the AquabupH injection did temporarily increase the average pH in zone C, results were variable with pH ranging from 5.0 to 7.3 at six months after injection. Overall, these results indicate that pH was below optimum in all wells from 2006 to 2011. Buffer addition was effective in raising the pH in Zone B and maintaining it at an appropriate level for four years. However, buffer addition was less effective in zone C, and pH declined below appropriate levels within one year after injection.

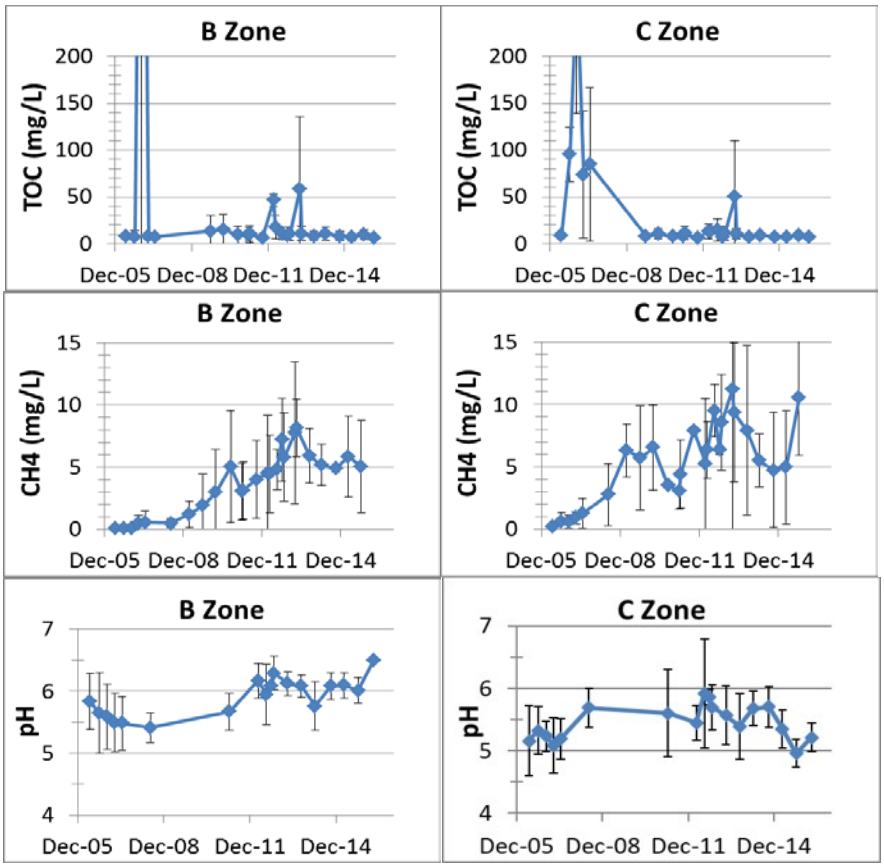

Figure B-6. Average CH_4, TOC, and pH in SA17 B and C Zone Monitor Wells (error bars are ±1 standard deviation).

Table B-3. Average CH_4, TOC, pH, ΣCl removal rate, and OoM ΣCl removal in SA17 monitor wells for 2006 to 2011 and 2012 to 2016.

Well	2006 to 2011					2012 to 2016				
	CH_4 (mg/L)	TOC (mg/L)	pH	ΣCl Rate (yr^{-1})	OoM ΣCl	CH4 (mg/L)	TOC (mg/L)	pH	ΣCl Rate (yr^{-1})	OoM ΣCl
OLD-17-53B1	1.1	8	5.7	1.15	2.07	2.8	8	6.4	1.44	0.62
OLD-17-53B2	1.1	9	5.8	0.91	1.70	3.3	7	6.4	1.34	0.49
OLD-17-54B	0.8	199	5.9	0.59	0.75	4.0	11	6.1	0.29	0.15
OLD-17-55B	2.5	32	5.1	1.20	1.91	9.3	24	6.1	0.79	1.07
OLD-17-56B	3.8	23	5.8	0.58	1.60	8.2	25	6.1	0.04	0.42
OLD-17-57B	0.3	16	5.2	0.32	1.16	4.4	7	5.7	0.88	0.20
OLD-17-58B	0.1	437	5.7	2.25	1.62	6.7	9	6.1	0.17	0.45
B Average	1.9	86	5.6	1.00	1.55	5.4	17	6.1	0.71	0.49
OLD-17-53C1	2.4	11	5.9	0.19	2.73	4.3	9	5.7	-0.09	-0.52
OLD-17-53C2	4.1	22	5.7	0.18	1.35	7.9	9	5.4	-0.34	0.94
OLD-17-54C	5.1	9	5.1	NA	NA	9.3	10	5.4	-0.10	0.34
OLD-17-55C	3.1	71	5.0	0.09	1.02	8.7	15	5.3	-0.02	-0.04
OLD-17-56C	8.4	16	5.2	-0.03	0.47	8.8	12	5.7	0.41	0.55
OLD-17-57C	3.1	76	5.0	-0.24	-0.54	13.5	25	5.6	0.53	1.02
OLD-17-58C	0.4	142	4.9	-0.13	0.56	13.4	10	5.5	0.11	0.16
C Average	3.6	58	5.3	0.01	0.93	7.6	13	5.5	0.07	0.35

B.3.2. Chlorinated Ethenes

In the source area, TCE levels were initially much greater than *c*DCE and VC, indicating limited natural attenuation. At the downgradient edge of the plume (OLD-17-45C), TCE has been consistently below detection with *c*DCE slowly declining from ~200 to below 100 µg/L, with a concurrent increase in VC from less than 1 to over 100 µg/L, indicating reductive transformation of TCE to *c*DCE to VC under ambient conditions (natural attenuation). However, further transformation of VC to ethene is limited by the low pH (~5).

In this work, we examine two different indicators of remediation performance: Sum of Organic Chlorine (ΣCl) and Chlorine Number (Cl#). These parameters are defined below

$$\Sigma Cl = 4^* [PCE] + 3^* [TCE] + 2^* [DCE] + 1^* [VC]$$

$$Cl\# = \Sigma Cl / \{[PCE] + [TCE] + [DCE] + [VC]\}$$

where [ ] indicates the concentration in micro-moles per liter (µM). ΣCl is the total amount of organic chlorine and Cl# is the average number of chlorine atoms per ethene molecule.

Figure B-7 illustrates the use of ΣCl and Cl# for evaluating ERD progress in OLD-17-56B. Prior to substrate EVO addition, TCE was the dominant chloroethene, Cl# was ~3 and ΣCl was approximately 3 times the TCE concentration in micro-moles per liter (µM). Following EVO addition in 2006, TCE declined below detection with a concurrent drop in Cl# from ~3 to ~1, and ΣCl declined from ~ 100 µM to 10 µM, indicating a 90% reduction in the amount of organic chlorine. However, from 2009 to 2015, ERD appeared to stall, with a very limited decline in *c*DCE and small increase in VC and ethene. The apparent stall in ERD is reflected in the near constant values of Cl# and ΣCl. In 2016, *c*DCE levels dropped sharply and ethene increased which is reflected in the decline in Cl# and ΣCl.

In an evaluation of bioremediation performance in 185 wells at 37 ERD projects, Tillotson and Borden (2017) found that 1st order ΣCl removal rates varied from 0.15 to 0.76/yr with a median value of 0.62/yr. The ΣCl removal rate in OLD-17-56B was 0.58/yr from 2006 to 2011, then declined to 0.04/yr from 2012 to 2016 due to the stall in ERD.

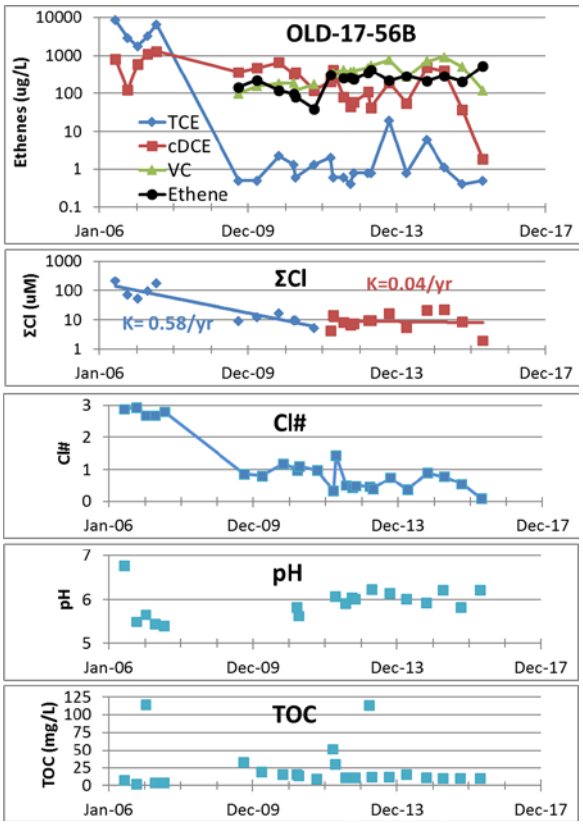

Figure B-7. Variation in TCE, *c*DCE, VC, Ethene, ΣCl, Cl#, pH and TOC versus Time in OLD-17-56B.

Figure B-8 shows the variation of ΣCl, Cl# and pH with time in Zone B and C monitor wells. In the B zone wells, ΣCl declined by one to two orders of magnitude between 2006 and 2009 as the Cl# decreased from 3 (indicating mostly TCE) to between 1 (indicating VC) and 2 (indicating *c*DCE). Between 2006 and 2011, pH was low which likely inhibited conversion of VC to ethene. AquabupH addition in 2012, increased the pH to near 6, followed by gradual declines in both ΣCl and Cl#. 1st order ΣCl removal rates in Zone B varied from 0.32 to 2.25/yr (average = 1.0/yr) for 2006 to 2011 and from 0.04 to 1.44/yr (average = 0.71/yr) for 2012 to 2016 (**Table B-3**). These rates are typical of ERD projects and reflect reasonably good progress towards remediating the B zone.

In the C zone wells, ERD was much less effective. Following the EVO injection in 2006, the Cl# of all the C zone wells declined from 3 (TCE) to near 2 (*c*DCE), then stalled, presumably due to the low pH (average pH in zone C was 5.3). Following AquabupH addition in 2012, there was a temporary increase in pH in most wells, but then pH declined again to below 5.5. 1st order ΣCl removal rates in zone C varied from -0.24 to 0.19/yr (average = 0.01/yr) for 2006 to 2011 and from -0.34 to 0.53/yr (average = 0.07/yr) for 2012 to 2016 (**Table B-3**). These rates are much lower than typical of ERD projects and reflect poor performance towards remediating the C zone.

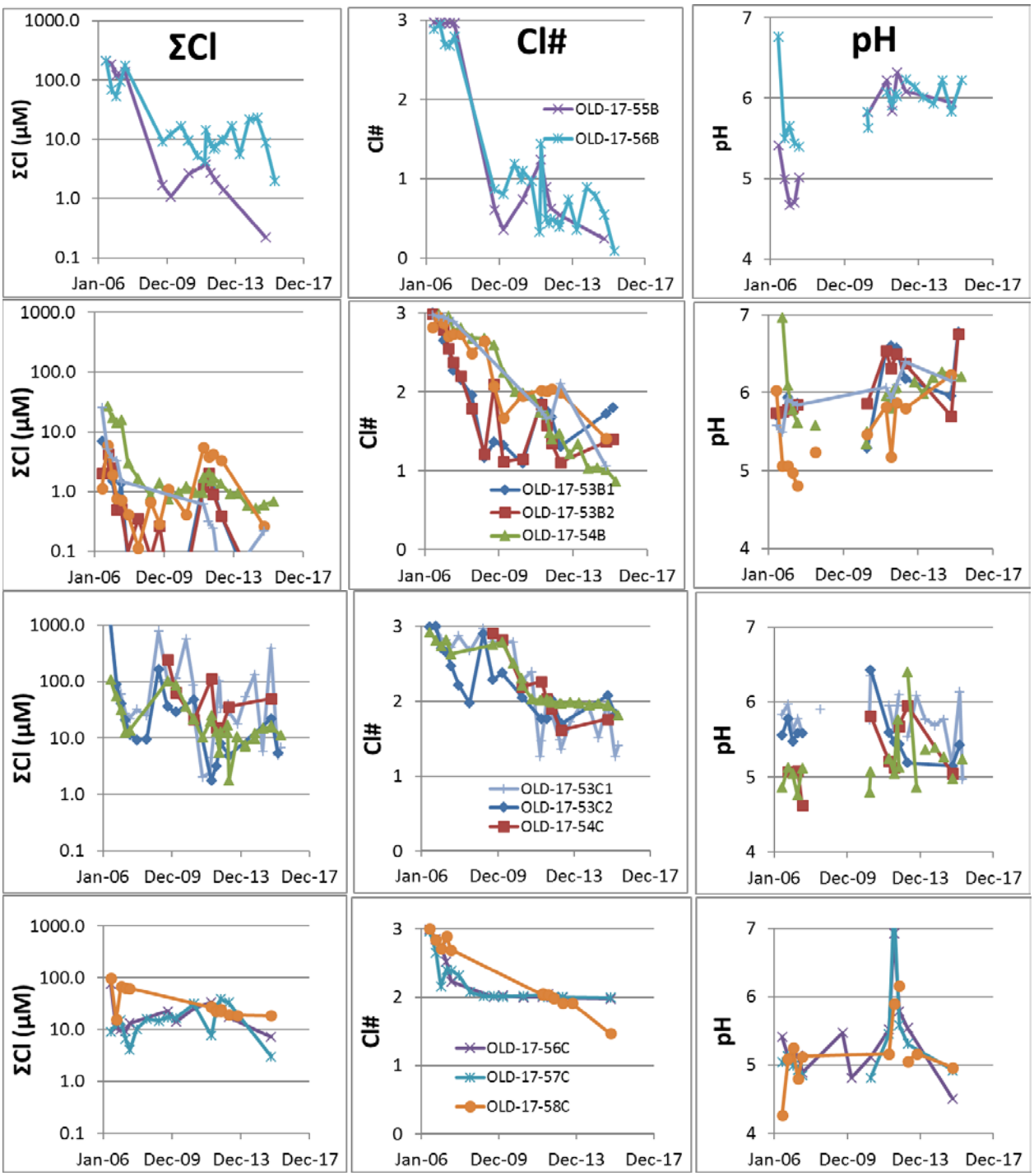

Figure B-8. Variation in ΣCl, Cl# and pH in Zone B and C Monitor Wells.

B.3.3. Microbiology

Monitoring results for dehalogenating bacteria [*Dhc*, *Dehalobacter spp.* (*Dhb*)] and functional genes (*tceA, bvcA, vcrA*) presented in **Table B-4** indicate large spatial and temporal variations in microbial numbers in both the B and C zones. Sampling of lactate baited biotraps in 2005 prior to EVO injection indicated low, but detectable numbers (10^1 to 10^3 *Dhc* cells/biotrap bead, AGVIQ-CH2M HILL JV-II, 2006).

Prior to reinjection with EVO and buffer in 2011, dechlorinator populations were low in OLD-17-54B, OLD-17-54C, OLD-17-55C, and OLD-17-56C. However, *Dhc* counts greater than 10^4 cells/mL and *bvcrAcrA* reductase counts greater than 10^3 cells/mL were observed in OLD-17-55B and OLD-17-56B indicating substantial numbers of *Dhc* with the ability to reduce VC to ethene were present in the B zone. Following EVO and buffer addition, *Dhc* increased to over 10^5 and *bvcrA* and/or *vcrA* increased to over 10^4 cells/mL in OLD-17-55B and OLD-17-56B. However, dechlorinator numbers in these wells began to decline in 2012, even though pH was near 6 and substantial levels of *c*DCE and VC remained. The decline of dechlorinator numbers with time is likely due to depletion of fermentable organic carbon. While 10 to 20 mg/L of total organic carbon (TOC) was present in these wells, propionic and other high molecular weight fatty acids that can be fermented releasing H_2 were below detection (data not shown). *Dhc* numbers remained below 10^3 in OLD-17-54B, presumably due to the rapid depletion of organic carbon in this well. In C zone wells OLD-17-55C and OLD-17-56C, *Dhc* numbers spiked immediately after injection, then declined as pH declined. In contrast, *Dhc* numbers increased over time in OLD-17-54C, reaching over 10^4 cells/mL of *Dhc* and *bvcrA*. The increase in *Dhc* numbers coincided with an increase in pH.

These results indicate chloroethene degradation was not limited by absence of required microorganisms. Instead, microbial growth and chloroethene removal was limited by low levels of fermentable organic carbon and/or low pH.

B.3.4. Injection Well Sampling

One of the hypotheses evaluated in this project was that EVO and/or pH buffer was not effectively distributed throughout the treatment zone and was limiting treatment. To evaluate this hypothesis, we sampled three injection wells in the B zone and three injection wells in the C zone for biogeochemical indicators, ethenes, and dechlorinating microorganisms in February 2016. We assumed that transport through the aquifer would not limit treatment in these wells since both EVO and buffer were directly injected into these wells. Monitoring results are presented in **Table B-5**.

Monitoring results indicate that TCE and its degradation products were being effectively treated in three of the injection wells (OLD-17-EW-01, OLD-17-EW-02, and OLD-17-IW-02C). In these three wells, pH is greater than 6, TOC concentrations were greater than 30 mg/L, and TCE and DCE have been substantially reduced. In two of these wells (OLD-17-EW-02 and OLD-17-IW-02C), relatively high numbers of dechlorinators are present. In the third well (OLD-17-EW-01) dechlorinator populations are relatively low, potentially because chlorinated ethene concentrations are too low to support an active population.

In three wells, ERD is more limited and high concentrations of TCE, DCE, and/or VC persist. In two of these wells, ERD is probably limited by pH below optimum levels (pH is 4.9 in OLD-17-IW-01C and 5.7 in OLD-17-IW-02B). However, in OLD-17-IW-01B, pH = 6.1 and TOC = 86 mg/L, while *c*DCE=6,280 g/L with modest levels of ethene and low dechlorinator populations. The reason for the limited degradation in this well is not known.

Table B-4. Dehalogenating Bacteria and Functional Genes in SA17 Monitor Wells.

Well ID	Sample Date	Dehalogenating Bacteria (cells/mL)		Functional Genes (cells/mL)		
		Dhc	***Dhb***	***tceA***	***bvcA***	***vcrA***
OLD-17-54B	4/13/11	1	<1	<1	<1	<1
	4/13/12	360	8,800	<1	3	23
	7/25/12	34	290	2	3	<0.3
	10/30/12	140	34	1	7	<0.3
	4/22/13	650	61	1	200	<0.3
	10/6/15	240	3.4	<0.5	43	<0.5
OLD-17-54C	4/13/11	91	110	<0.5	<0.5	0.2 J
	4/13/12	330	7,200	<1.6	4.1	34
	7/25/12	140	17,000	3	5.7	0.7
	10/30/12	1,700	880	0.5	84	0.4
	4/22/13	30,000	94	<0.3	21,000	210
	10/6/15	7,100	50	<0.5	610	1,500
OLD-17-55B	4/13/11	18,000	160	<0.8	3,470	<0.8
	4/13/12	120,000	2100	<0.9	41,700	32
	7/25/12	9,600	160	2	2,410	0.4 J
	10/30/12	1,200	17	1	750	<0.5
	4/22/13	68	4	1	52	<0.5
	10//6/15	3,500	36	<0.5	990	62
OLD-17-55C	4/13/11	81	335	<0.5	<0.5	<0.5
	4/13/12	3,180	8,100	0.5 J	1.2	19
	7/25/12	63	186	3.8	<0.4	<0.4
	10/30/12	17	159	0.3 J	<0.5	<0.5
	4/22/13	145	20	1.5	1.1	<0.4
	10/6/15	29	24	<0.5	0.1 J	<0.5
OLD-17-56B	4/13/11	38,200	226	<0.5	12	12,000
	4/13/12	670,000	607	<0.9	26,000	260,000
	7/25/12	15,000	121	1.6	1,200	27,000
	10/30/12	14,000	92	0.2 J	2,000	1,100
	4/22/13	1,400	3	<0.4	44	47
	10/6/15	25,000	10	<0.5	2,600	1,500
OLD-17-56C	4/13/11	151	8,600	<0.5	<0.5	3
	4/13/12	4140	1,800	<0.5	53	431
	7/25/12	369	318	1	9	36
	10/30/12	3.9	59	<0.6	<0.6	<0.6
	4/22/13	514	56	26	1	0.2 J
	10/6/15	58	9	<0.5	1.9	<0.5

Table B-5. Monitoring Results for Injection Well Sampling (February 22-23, 2016).

Well ID	Unit	OLD-17-EW-01	OLD-17-EW-02	OLD-17-IW-01B	OLD-17-IW-01C	OLD-17-IW-02B	OLD-17-IW-02C
pH	S.U.	6.2	6.7	6.1	4.9	5.7	6.6
Alkalinity	mg/L	292	598	234	540	154	738
Acidity	mg/L	<2.5	<2.5	27	2.5	59	<2.5
Total Organic Carbon	mg/L	34	79	86	1,620	126	125
Total Inorganic Carbon	mg/L	103	167	100	0	98	188
Nitrate-N	mg/L	<0.05	<0.05	<0.05	<0.25	<0.05	<0.25
Manganese	mg/L	0.27	0.08	0.11	0.19	0.06	0.17
Iron	mg/L	9	32	35	21	24	25
Sulfate	mg/L	0.9	<0.6	<0.6	<3	<0.6	<3
Methane	mg/L	8.7	7.7	4.1	6.8	4.8	8.5
Sodium	mg/L	14	33	10	13	11	17
Potassium	mg/L	4	2	2	2	2	2
Calcium	mg/L	72	91	34	276	31	30
Magnesium	mg/L	23	76	38	148	22	173
Chloride	mg/L	3	10	7	7	8	24
TCE	µg/L	<0.27	<0.27	145	1,440	71.1	7.4
cis-1,2-DCE	µg/L	6.2	4.6	6,280	605	1,950	29.7
VC	µg/L	2.2	153	128	16.3	27	180
Ethene	µg/L	<0.43	5.1	5.8	<0.43	2.6	52
DHC	cells/mL	170	13,000	180	NA	51	1,600,000
tceA	cells/mL	1	1	2	NA	3	160,000
bvcA	cells/mL	<1	2	1	NA	1	4
vcrA	cells/mL	1	4,500	9	NA	3	220,000
ΣEth	µM	0.1	3	68	17	21	5
ΣCl	µM	0.2	3	135	46	42	4
Cl#	µM/ µM	1.65	0.95	1.98	2.61	2.00	0.72

B.3.5. Spatial Distribution of TCE and *cDCE*

As part of the site characterization work for this project, a MIP equipped with a halogen specific detector (XSD) was used to measure vertical profiles of total halogens at the same time the HPT profiler was used to measure hydraulic conductivity. These results indicated that total halogen concentrations were relatively low in four of the five borings. However, in a boring adjoining OLD-17-53, a spike in XSD response was observed at about 30 ft bgs. **Figure B-9** shows results of CVOCs analysis of soil samples collected from a continuous boring installed close to this location. Relatively

high concentrations of TCE and *c*DCE were observed in the interval from 25 to 33 ft bgs. TCE concentrations in this interval varied from 5 to 7.5 µg/g which would result in aqueous concentrations of approximately 40,000 to 60,000 µg/L if 100% of the TCE was dissolved in the pore water. The maximum TCE concentration observed in the closest monitor well screen (OLD-17-53C1 screened from 30 to 35 ft bgs) was 47,200 µg/L in June 2006. Since 2012, TCE concentrations in this well screen have varied from 2.5 to 538 µg/L. However, TCE degradation products were higher with *c*DCE varying between 127 and 18,200 µg/L and VC varying between 117 and 822 µg/L. These concentrations are quite variable with TCE and *c*DCE concentrations declining to low levels, then spiking by two orders of magnitude. The reason for this variability is not known, but is consistent with a residual source in this area.

The continued presence of *c*DCE, VC and smaller amounts of TCE in OLD-17-53C1 appears to be due to an untreated zone with relatively high residual TCE levels in the 25 to 32 ft interval. HPT profiles did not indicate a significant reduction in permeability in this interval, which would have limited reagent distribution. However, the B zone injection wells extended from 15 to 25 ft bgs and the C zone injection wells extended from 30 to 40 ft bgs, so the injection system design could have limited reagent distribution in this interval.

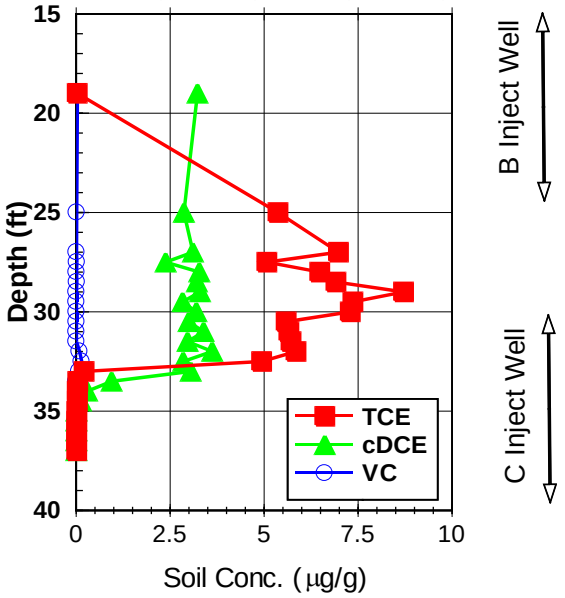

Figure B-9. Concentrations of TCE, *c*DCE and VC in Soil Samples Collected from a Boring Adjoining HPT2 and OLD-17-53.

B.3.6. Assessment of Overall Remedial Performance

To provide some reference for comparing treatment performance, we compare Order of Magnitude (OoM) concentration reductions in B and C zone wells at SA17 with OoM reductions in TCE concentration reported by Tillotson and Borden (2017) for 37 ERD sites containing 184 treatment zone monitor wells and in situ remediation performance monitoring results reported by McGuire et al. (2016). OoM reductions at SA17 were calculated by comparing monitoring results from June 2006 (immediately before injection) to October 2015. The performance

monitoring results reported by McGuire et al. (2016) were sorted to select 19 ERD sites treated with Semi-Soluble / Slow-Release substrates where TCE was the parent compound. The McGuire database provides OoM removal results for both geometric mean concentrations and maximum concentrations before and after treatment.

Figure B-10 compares OoM removals for the McGuire et al. (2016) database (19 site geomeans and maximums), Tillotson and Borden (2017) database for 37 ERD sites containing 184 wells, and individual wells in zones B and C at SA17. Direct comparison of the results of the different studies are not possible because each study used slightly different methods to calculate OoM removal. However, it is obvious from **Figure B-10** that TCE removal in both Zones B and C was very good. Between 2006 and 2015, TCE concentrations in individual monitoring wells declined by 2.8 to 4.6 OoM in Zone B and 2.0 to 3.7 OoM in Zone C. These OoM removals are much greater than typical values reported by McGuire et al. (2016) and Tillotson and Borden (2017), indicating that parent compound removal at SA17 was excellent in comparison to other ERD projects.

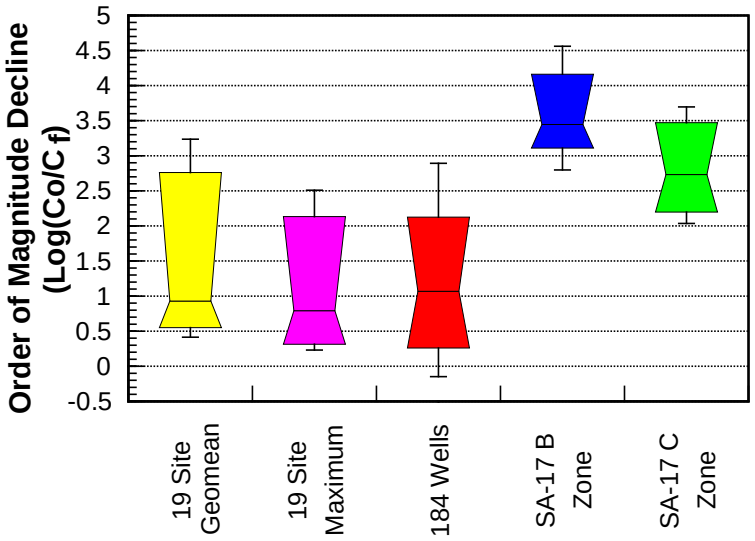

Figure B-10. Order of Magnitude (OoM) Concentration Reductions in TCE for 19 Sites (McGuire et al., 2016), 184 Wells (Tillotson and Borden, 2017), and SA17 B and C Zone Monitoring Wells.

While parent compound removal is an important metric, accumulation of ERD biotransformation products was a significant issue at SA17. **Figure B-11** compares OoM reductions in TCE, *c*DCE, VC, and ΣCl in Zones B and C with results from 184 wells summarized by Tillotson and Borden (2017). As shown above, TCE removal was very good in both zones B and C. Daughter product accumulation in Zone B was limited, with some removal of *c*DCE and limited production of VC, resulting in very good ΣCl removal compared to the 184 wells. However, in zone C, there were relatively large increases in *c*DCE and VC. As a result, ΣCl removal in zone C was similar the median removal in 184 wells.

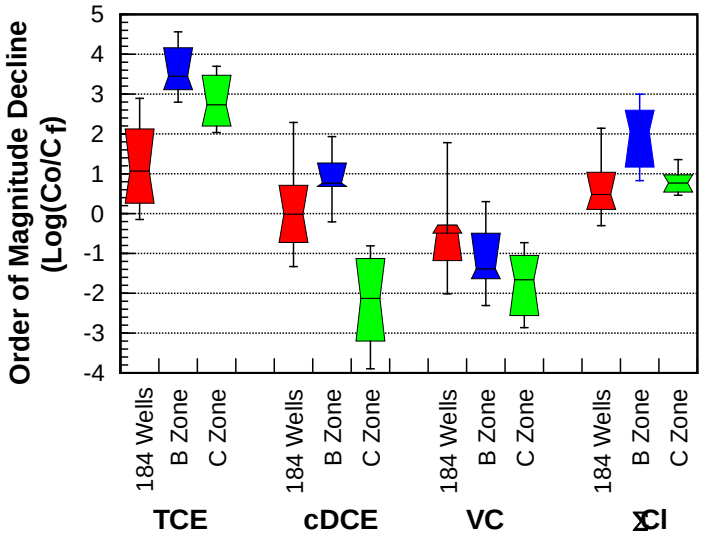

Figure B-11. Order of Magnitude (OoM) Decline in TCE, *c*DCE, VC and ΣCl for 184 Wells Reported by Tillotson and Borden (2017) and for SA17 B and C Zone Monitoring Wells.

Effective 1st order removal rates for ΣCl were also reported by Tillotson and Borden (2017) for their database of 184 wells. ΣCl was observed to be decreasing ($p<0.05$) or probably decreasing ($0.05<p<0.1$) in about 60% of the wells. For those wells with decreasing or probably deceasing, most ΣCl removal rates were between 0.7 and 2/yr. At SA17, about half of the wells had decreasing or probably decreasing rates for ΣCl with rates varying between 0.3 and 2.3/yr (average = 0.5/yr). In Zone B, ΣCl was commonly decreasing or probably decreasing. In zone C, ΣCl was commonly stable or no trend.

In summary, parent compound (TCE) removal was very good at SA17. However, daughter product accumulation in zone C was more severe than at some other sites. This was reflected in the lower OoM removal and 1st order removal rates for ΣCl in zone C.

B.4. Evaluation of Factors Limiting Remedial Performance

B.4.1. Back Diffusion

There is no evidence that the presence of lower permeability zones significantly reduced treatment efficiency or back diffusion of contaminants contributed to contaminant rebound after treatment. HPT profiles shown in **Figure B-3** do not show any indication of substantial lower permeability zones between 15 to 40 ft bgs. Particle size distribution analysis of samples from two soil borings (SB1 and SB2) indicate the material from 15 to 40 ft bgs is predominantly medium sand with 0.5 to 6% silt+clay (**Table B-6**). HPT profiles do show the presence of the Hawthorne confining unit at 45 to 50 ft bgs, which could slowly release dissolved contaminants that had diffused into this lower permeability zone. However, this unit below the target treatment interval and is unlikely to substantially influence treatment performance from 25 to 40 ft bgs.

Table B-6. Percent Clay, Silt and Sand in Borings SB1 and SB2 at SA17.

Boring	Depth (ft bgs)	Clay	Silt	Fine sand	Medium Sand	Coarse Sand
SB1	17-20	2.8	3.2	28.6	63.0	2.5
SB2	22.5-24	0.0	0.5	22.4	75.3	1.8
SB2	24-25	1.0	0.9	13.6	78.6	5.9
SB1	30.5-35	3.0	3.0	21.8	72.1	0.1
SB2	32-34	2.3	3.4	25.9	68.4	0.0
SB2	34-35	2.2	2.2	20.2	74.2	1.2
SB1	41.5-43	1.7	1.2	33.4	63.6	0.0
SB1	43-45	0.0	0.5	61.6	37.9	0.0
SB2	45-48	1.6	0.7	28.9	68.7	0.1
SB2	48-50	1.7	0.8	27.1	70.1	0.2

B.4.2. Evaluation of EVO Loading and Injection Volumes

The EVO design tool developed under ER-0626 (Borden et al., 2008; Weispfenning and Borden, 2008) was used to evaluate the 2006 and 2012 injections and determine if sufficient EVO and water were injected to achieve at least 60% contact efficiency for SA17 (source area treatment). This process was performed using two different sets of design parameters: (a) the literature values used in the original design; and (b) the measured values of these parameters generated in this project.

Prior research has shown that oil droplet retention is influenced by the zeta potential of the oil droplets and the aquifer material (Coulibaly and Borden, 2004). Previous EVO transport experiments were conducted with low ionic strength tap water (Coulibaly et al., 2006) or deionized (DI) water (Borden et al., 2008). Sampling of monitoring wells in both the B and C zones in 2015 showed low ionic strength groundwater with average concentrations of 9 mg/L Na, 2 mg/L K, 19 mg/L Ca and 7 mg/L Mg. However, monitoring of injection wells sampled in 2016 (**Table B-5**) found 10 to 33 mg/L Na, 2 to 4 mg/L K, 30 to 276 mg/L Ca, and 22 to 173 mg/L Mg. To evaluate the potential impact of ionic strength, the zeta potential of EOS 598B42 and aquifer material from SA17 were measured in DI water and a solution of 200 mg/L $CaCl_2$ (approximately 73 mg/L Ca). The zeta potential of all materials was negative in the presence of both DI water and 200 mg/L $CaCl_2$ (**Table B-7**). However, the zeta potential of both the oil droplets and the aquifer material was more strongly negative in DI water than the $CaCl_2$ solution, indicating that oil droplet-sediment particle repulsion will be greater and oil retention will be lower in DI water.

Table B-7. Effect of Solution Composition on Zeta Potential and Oil Retention.

Colloid	Solution	Zeta Potential (mV)	
		Average	Std. Dev.
SA17 Soil 15-23'	DI	-29.4	0.8
	200 mg/L $CaCl_2$	-8.5	0.5
SA17 Soil 30-40'	DI	-22.3	0.9
	200 mg/L $CaCl_2$	-7.5	0.9
EOS 598B42	DI	-43.0	0.7
	200 mg/L $CaCl_2$	-10.3	0.4

Column experiments were conducted in 28 cm x 2.6 cm diameter columns packed with aquifer material collected from 15 to 23 ft bgs (B zone) and 30 to 40 ft bgs (C zone) at SA17 to measure the transport and retention of EVO. The columns were first saturated, then 150 mL (~3 pore volumes [PV]) of a 20% EVO dilution, followed by ~3 PV of chase water. Oil retention was determined by measuring the volatile solids fraction of the aquifer material before and after testing (**Table B-8**). Measured oil retention in the B zone sediment flushed with 200 mg/L $CaCl_2$ was significantly higher than for the same material flushed with DI water consistent with reduced electrical repulsion between the oil droplets and sediment.

Table B-8. Retained Oil Content in Column Tests with D.I. Water and 200 mg/L $CaCl_2$.

	SA17 B Zone		SA17 C Zone
	D.I.	$CaCl_2$	$CaCl_2$
Influent End	0.05%	0.76%	3.79%
Middle	0.18%	1.27%	4.94%
Effluent End	0.57%	1.96%	4.74%
Average	0.27%	1.33%	4.49%

These results demonstrate that ionic strength and concentration of cation can have a major impact on zeta potential and oil retention. The ionic strength of groundwater in the treatment zone varies from low in the monitor wells to high in the injection wells. As a result, oil retention probably varies from high near the injection wells to lower in more distant portions of the aquifer.

Tables B-9 and B-10 shows the input parameters used in the original design of the 2006 and 2012 EVO injections (AGVIQ-CH2M HILL JV-II, 2006; Solutions-IES, 2011b) and our current best estimates for these parameters. Prior to the 2006 and 2012, no column tests of EVO transport were conducted and injection designs were based on assumed parameters obtained from the published literature (Coulibaly and Borden, 2004; Coulibaly et al., 2006). Oil transport and retention column experiments were conducted using either deionized (DI) water or a 200 mg/L $CaCl_2$ solution. The 2012 injections were evaluated with Maximum Oil Retention values measure with DI water (representative of background, low ionic strength groundwater) and 200 mg/L $CaCl_2$ (representative of EVO amended with $Mg(OH)_2$).

Table B-9. Design Tool Evaluation of 2006 and 2012 Injections at SA17 Zone B.

Parameter	Unit	2006		2012		
		Literature Values	Measured DI Water	Literature Values	Measured	
					DI Water	$CaCl_2$
Treatment Area	ft^2	2,500	2,500	2,500	2,500	2,500
Total Thickness	ft	10	10	10	10	10
Maximum Oil Retention	lb/lb	0.0012	0.0027	0.0012	0.0027	0.0133
Maximum Oil Retention	lb	3,600	8,100	3,600	8,100	39,900
Oil Injected	lb	882	882	1,271	1,271	1,271
Injected / Max retention		0.26	0.12	0.38	0.17	0.034
Porosity		0.28	0.28	0.28	0.28	0.28
Effective Pore Volume	ft^3	7,000	7,000	7,000	7,000	7,000
Fluid Vol Injected	ft^3	4,407	4,407	6,328	6,328	6,328
Pore Vol Injected		0.63	0.63	0.91	0.91	0.91
Projected Contact Eff.		34%	20%	45%	28%	<15%

Table B-10. Design Tool Evaluation of 2006 and 2012 injections at SA17 Zone C.

Parameter	Unit	2006		2012		
		Literature Values	Measured DI Water	Literature Values	Measured	
					DI Water	$CaCl_2$
Treatment Area	ft^2	2,500	2,500	2,500	2,500	2,500
Total Thickness	ft	10	10	10	10	10
Maximum Oil Retention	lb/lb	0.0012	0.0027	0.0012	0.0095	0.0449
Maximum Oil Retention	lb	3,360	26,600	3,360	26,600	125,700
Oil Injected	lb	3,654	3,654	1,271	1,271	1,271
Injected / Max retention		1.09	0.14	0.38	0.05	0.01
Porosity		0.28	0.28	0.28	0.28	0.28
Effective Pore Volume	ft^3	7,000	7,000	7,000	7,000	7,000
Fluid Vol Injected	ft^3	6,073	6,073	8,447	8,447	8,447
Pore Vol Injected		0.87	0.87	1.2	1.2	1.2
Projected Contact Eff.		69%	24%	46%	<15%	<15%

Evaluation of the 2006 and 2016 injection volumes with the ESTCP EVO Design tool indicates that:

A. Based on the original design assumptions in 2006 and 2012, the total volume of diluted EVO and chase water should have been more than sufficient to effectively distribute the oil droplets throughout the treatment zone, achieving EVO contact efficiencies greater than 60%.
B. The measured oil retention with DI water was 0.0027 g/g or over twice the literature value assumed in the original design. This under estimate of oil retention resulted in too little EVO being injected, which would have significantly reduced contact efficiency and treatment.
C. Injection of a mixture of EVO and colloidal $Mg(OH)_2$ likely increased the injection solution ionic strength and oil retention near the injection wells, further reducing EVO contact efficiency.

B.4.3. Hydraulic Design

The groundwater flow model, MODFLOW (Harbaugh et al., 2000) was used to simulate flow patterns during the 2006 and 2012 injections. EVO transport and retention was simulated using the reactive transport model RT3D (Clement, 1997) with a reaction module developed to simulate retention of colloidal oil droplets (Coulibaly et al., 2006). Injection volumes and flowrates used in model calibration are presented in **Sections B.2.1** and **B.2.2**. A maximum oil retention of 0.0027 g/g was used in all simulations for consistency.

Figure B-12 shows the simulated oil distribution in the aquifer for three conditions: (a) the 2006 injection; (b) the 2012 injection; and (c) an enhanced design developed to improve EVO distribution. The simulated EVO distribution is shown for three depths: (1) the middle of Zone B (20 ft bgs), (2) midway between the B and C zone well screens (27 ft bgs); and (3) the middle of zone C (35 ft bgs). Following the 2006 injection, EVO distribution was poor with much of the aquifer left untreated due to: (a) the small amount of EVO injected into Zone B; (b) the gap between the Zone B and zone C injection well screens. Contact efficiency was better in zone C due to the larger amount of EVO injected in this zone. However, there are significant gaps between treated zones due to the development of stagnation zones during simultaneous injection of all wells. Simulated EVO distribution was also poor in 2012 due to the small amount of EVO injected.

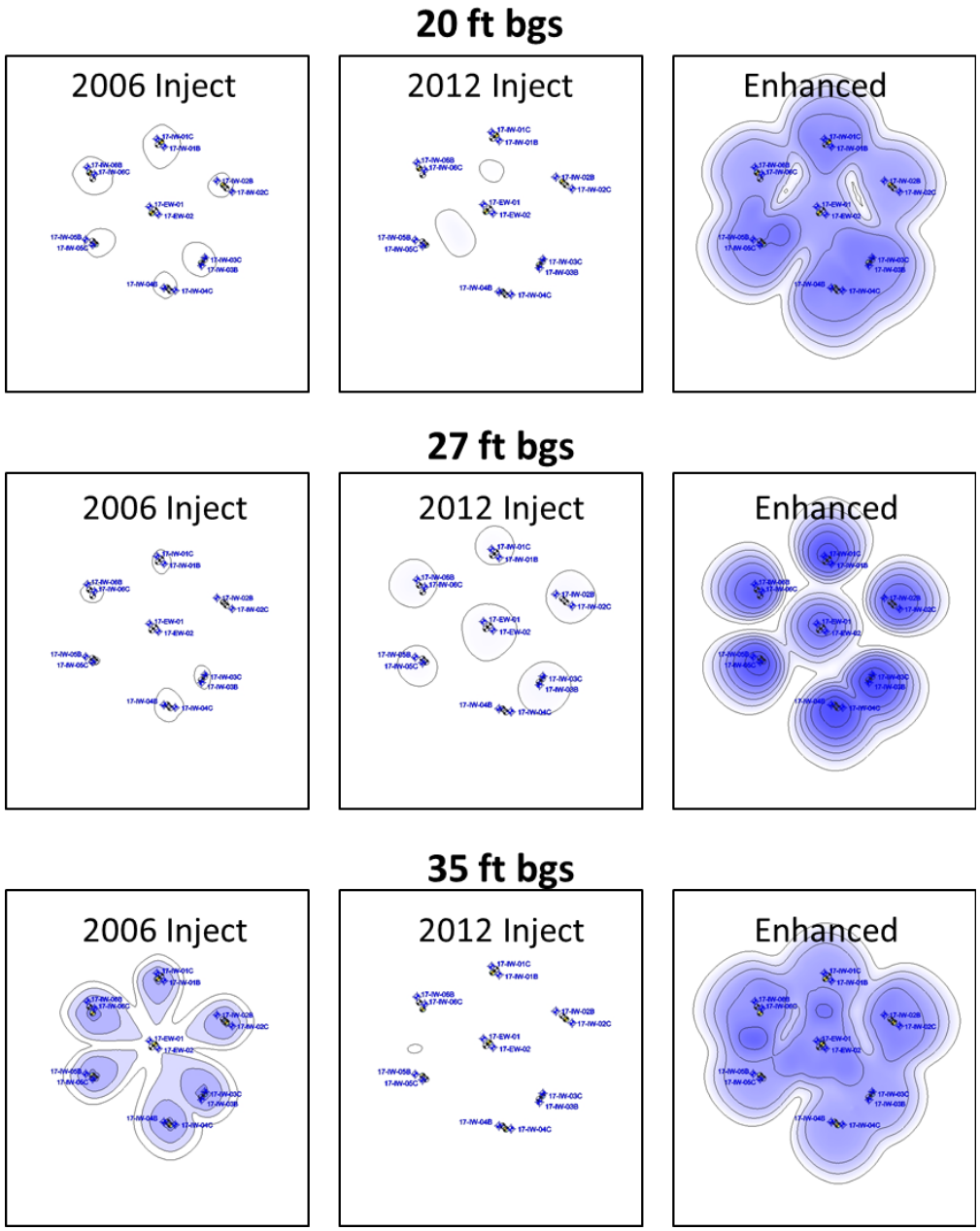

Figure B-12. Simulated EVO Distribution in Middle of B Zone (20 ft bgs), between B and C Zones (27 ft bgs), and Middle of C Zone (35 ft bgs) Following 2006, 2012, and Enhanced Injections.

In the enhanced simulation, the total volume of fluid injected (EVO plus chase water) was similar to the 2012 injection. However, the amount of EVO injected was increased by a factor of 5.3. The injection sequence was also altered so half the wells (every other well) were injected in a group. Then the remaining wells were injected as a group. When injecting the 1st group of wells, EVO was effectively distributed near the injection wells, but stagnation zones with poor EVO distribution occurred midway between the injection wells. Injection of the 2nd group of wells effectively treated the remaining area, since these wells were located in the middle of the former stagnation zone.

Injection of additional EVO combined with the modified injection sequence greatly improved EVO distribution in both zones B and C (**Figure B-12**). Altering the injection sequence was also reasonably effective in pushing EVO into the 5-foot gap between the B and C zone well screens.

B.4.4. Buffer Design

As part of this project, an Excel spreadsheet was developed to estimate the amount of base required to raise the aquifer pH to the desired level, and maintain it at that level for the design treatment period. Details of the spreadsheet development and calculation procedures are provided in Appendix D. This spreadsheet was used to estimate the amount of base required for zones B and C for the 2012 injections. Input parameters and results of this evaluation are summarized in **Table B-11**.

Table B-11. Calculation of Required Base Addition for 2012 SA17 Zones B and C Injections.

Parameter	Unit	Zone B	Zone C
Design Treatment Period	yr	5	5
Mass Soil Treated	lb	2,800,000	2,800,000
Volume Groundwater Treated	gallon	160,000	210,000
Target pH	SU	7.0	7.0
Background pH	SU	5.6	5.3
Total Inorganic Carbon	mg/L	80	80
Background CO_2 Acidity	meq/L	4.4	4.9
Aquifer Buffering Capacity	meq/Kg/pH	2.6	2.6
TCE	Kg	11	11
DCE	Kg	4	4
Vegetable Oil added for ERD	lb	1,271	1,271
Influent Acidity	OH^- eq	2,689	3,898
Base to Raise Starting pH	OH^- eq	4,641	5,636
Acidity from Dechlorination	OH^- eq	325	334
Acidity from Added Substrate	OH^- eq	31,332	31,332
Acidity from e- accept / donors	OH^- eq	-25,123	-25,217
Total Base Demand	OH^- eq	13,864	15,982
Total Base Added	OH^- eq	826	826
Fraction of Base Demand Met		6%	5%

With the exception of the initial pH and mass of EVO injected, geochemical conditions were similar in zones B and C. For both zones B and C, the target treatment volume was 25,000 ft^2.

The volume of groundwater treated in the 5-year design period was somewhat higher in zone C due to the higher K and groundwater velocity of this zone. The acidity of the groundwater entering the treatment zone and soil acidity were significant components of the total base required. However, most of the acidity was generated by the added organic substrate. In both zones B and C, 53 lb of $Mg(OH)_2$ were injected which provided 5 to 6% of the base required to maintain a neutral pH. The base requirements for zones B and C were similar because the amount of EVO injected was identical and geochemical conditions were similar.

To provide guidance on amounts of base required during future injections, the design tool was used to estimate the amount of NaOH, Na_2CO_3, $NaHCO_3$, or $Mg(OH)_2$ required to maintain the pH at different levels following the 2012 injections for both zones B and C. Results presented in **Figure B-13** indicate that amount of base required is very sensitive to the target pH. For target pH less than 6.3, most of the CO_2 produced during ERD remains in the protonated form (H_2CO_3*) and base demand is relatively low. Increasing the target pH to 7, results in the conversion of H_2CO_3* to HCO_3^- and H^+, requiring large amounts of base to neutralize the H^+ released. Further increases in pH above 7 does not require as much base since most of the H^+ has already been released from the H_2CO_3*.

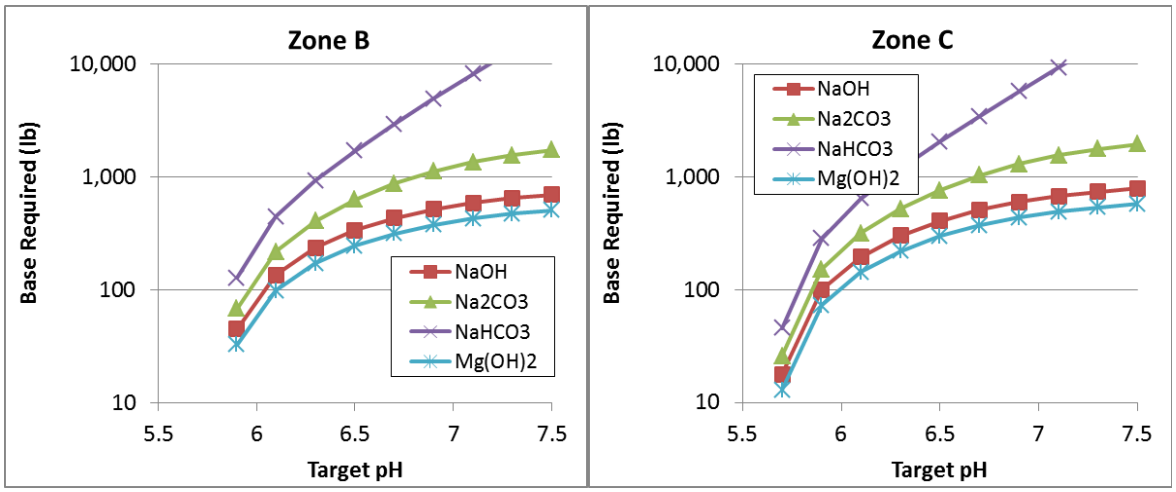

Figure B-13. Required base addition for 2012 SA17 zones B and C for different target pH values.

In the C zone, substantial amounts of base are required to raise the pH to 6 due to the buffering capacity of the aquifer material and the low background pH (~5.3). In the B zone, less base is required to raise the pH to near 6 because the higher background pH (~5.6). Raising the pH to ~7 requires substantial amounts of base because of both the aquifer buffering capacity and conversion of H_2CO_3* to HCO_3^-. In general, it is not practical to raise the pH to near 7 with $NaHCO_3$ due to the small amount of H^+ consumed by this material at near neutral pH. NaOH and Na_2CO_3 can be effective for increasing pH to ~7. However, the high pH associated with these materials can kill bacteria near the injection zone. The total mass of $Mg(OH)_2$ required is less than NaOH, since two moles of OH^- are released per mole of $Mg(OH)_2$. However, $Mg(OH)_2$ has a very low aqueous solubility, so the material must be injected in a colloidal form.

The pH of a pure slurry of $Mg(OH)_2$ is relatively high (~10.3) and can inhibit dechlorinators. However once injected, CO_3^{2-} precipitates on the surface of the $Mg(OH)_2$ particles forming a $MgCO_3$, coating maintaining the aquifer pH between 7 and 8 (Hiortdahl and Borden, 2014).

B.5. Site Summary and Conclusions

At SA17, overall bioremediation performance was good in Zone B, but less than desired in zone C. TCE concentrations were greatly reduced in both B and C zones. However, *c*DCE and VC accumulated in many zone C wells, so removal of total organic chlorine (∑Cl) was similar to other sites. *c*DCE and VC accumulation was due to low levels of fermentable organic carbon and low pH throughout zone C. Several different factors contributed to the less than desired treatment performance.

1) The total amount of EVO injected was significantly less than that required for effective distribution throughout zones B and C.
2) The hydraulic design of the injection system resulted in poor distribution of EVO in substantial portions of the treatment zone, allowing significant mass of TCE to remain untreated. Problems with the hydraulic design included:
   a) Installation of injection well screens from 15 to 25 ft and 30 to 40 ft bgs resulted in bypassing of much of the 25 to 30 ft interval, with poor EVO distribution in this zone.
   b) Simultaneous injection of all wells in 2006 resulted in stagnation zones, with poor EVO distribution in these areas.
3) The total amount of base injected in 2012 was a small fraction of the base required to maintain the pH in a range appropriate for reductive dechlorination of *c*DCE and VC to ethene.

There was no evidence that absence of required microorganisms, back diffusion of contaminants from low K zones, or inadequate chase water volume substantially reduced treatment performance.

B.6. References

Adamski, J.C. and German, E.R., 2004. Hydrogeology and quality of ground water in Orange County, Florida. US Geological Survey.

AGVIQ-CH2M HILL JV-II, 2006. Remedial Action Work Plan, Injection and Recirculation of Emulsified Oil Substrate (EOS®) at Study Area 17, Former Naval Training Center Orlando, Orlando, Florida. U.S. Naval Facilities Engineering Command Southern Division

BFA (Barnes, Ferland and Associates, Inc.), 2012. September 2012 Semiannual Monitoring Report Study Area 17 - McCoy Annex, Former Naval Training Center Orlando, Florida. Naval Facilities Engineering Command Southeast.

Borden, R.C., Clayton, M., Weispfenning, A.M., Simpkin, T. and Lieberman, M.T., 2008. Technical Report-Design Tool for Planning Emulsified Oil Injection Systems. Environmental Security Technology Certification Program, Arlington, Virginia.

Borden, R.C., Tillotson, J.M., Ng, G.-H.C., Bekins, B.A., Kent, D.B., and Curtis, G.P., 2015. Final Report-Impacts of Enhanced Reductive Bioremediation on Post-Remediation

Groundwater Quality, SERDP Project ER-2131. Strategic Environmental Research and Development Program, Arlington, Virginia.

CH2M HILL, 2006. Technical Memorandum - Biobarrier Injection Pilot Study Work Plan, Operable Unit 2, Naval Training Center Orlando, Florida. NAVFAC Southern Division.

CH2M HILL, 2010. Evaluation of Enhanced Reductive Dechlorination Treatment Progress, Study Area 17, Former Naval Training Center Orlando, Florida. NAVFAC Southern Division.

Clement, T.P., 1997. A Modular Computer Code for Simulating Reactive Multi-Species Transport in 3-D Groundwater Systems. PNNL-11720. Pacific Northwest National Laboratory, Richland, WA.

Coulibaly, K.M. and Borden, R.C., 2004. Impact of edible oil injection on the permeability of aquifer sands. Journal of contaminant hydrology, 71(1), pp.219-237.

Coulibaly, K.M., Long, C.M. and Borden, R.C., 2006. Transport of edible oil emulsions in clayey sands: One-dimensional column results and model development. Journal of Hydrologic Engineering, 11(3), pp.230-237.

Harbaugh, A.W., Banta, E.R., Hill, M.C. and McDonald, M.G., 2000. MODFLOW-2000, The U. S. Geological Survey Modular Ground-Water Model-User Guide to Modularization Concepts and the Ground-Water Flow Process. Open-file Report. U. S. Geological Survey, (92), p.134.

Hiortdahl, K.M. and Borden, R.C., 2014. Enhanced reductive dechlorination of tetrachloroethene dense nonaqueous phase liquid with EVO and $Mg(OH)_2$. Environmental science & technology, 48(1), pp.624-631.

HLA (Harding Lawson and Associates), 1999. Base Realignment and Closure Environmental Site Screening Report, Study Area 17, Naval Training Center Orlando.

McGuire, T.M., D.T. Adamson, M.S. Burcham, Philip B. Bedient, and C.J. Newell, 2016. Evaluation of Long-Term Performance and Sustained Treatment at Enhanced Anaerobic Bioremediation Sites. Groundwater Monitoring & Remediation, 36(2), pp. 32-44.

Resolution (Resolution Consultants), 2013, Final Long Term Monitoring Optimization Report, Study Area 17 (SA17), Former NTC Orlando, Florida.

Scott, T.M., Campbell, K.M., Rupert, F.R., Arthur, J.D., Missimer, T.M., Lloyd, J.M., Yon, J.W. and Duncan, J.G., 2001. Geologic map of the State of Florida. Florida Geological Survey.

Solutions-IES, 2011b. Work Plan - In Situ Bioremediation of Chlorinated Solvents in Groundwater at SA-17, Former Naval Training Center Orlando, Orlando, Florida. Naval Facilities Engineering Command Southeast

Solutions-IES, 2013a. Construction Completion Report - In Situ Bioremediation of Chlorinated Solvents in Groundwater at SA-17, Former Naval Training Center Orlando, Orlando, Florida. Naval Facilities Engineering Command Southeast

Solutions-IES, 2013b. 14-Month Post-Injection Performance Monitoring Report - In Situ Bioremediation of Chlorinated Solvents in Groundwater at SA-17, Former Naval Training Center Orlando, Orlando, Florida. Naval Facilities Engineering Command Southeast.

Tetra Tech NUS, 2004. Site Investigation Report for SA17, Former NTC, Orlando, Florida.

Tillotson, J.M. and Borden, R.C., 2017. Rate and Extent of Chlorinated Ethene Removal at 37 ERD Sites. Journal of Environmental Engineering, 143(8), p.04017028.

Weispfenning, A.M. and Borden, R.C., 2008. A design tool for planning emulsified oil-injection systems. Remediation Journal, 18(4), pp.33-47.

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APPENDIX C NTC ORLANDO OU2

C.1. Site Characteristics

C.1.1. Description and History

Operable Unit 2 (OU2) located in the southern portion of the McCoy Annex consists of approximately 114 acres. The western portion of the site was used as a landfill by the Air Force from about 1960 to 1972, while the eastern portion was used as a landfill by the Air Force and the Navy from 1972 until about 1978. Landfill operations consisted of excavating ditches (100 to 200 ft long by 20 to 25 ft wide by 10 to 15 ft deep) and filling with wastes. Occasional burning of the wastes took place in the ditches. Trenches were filled with waste to within 3 or 4 ft of the ground surface and then backfilled with soil and seeded. The estimated volume of waste is more than 1,000,000 cubic yards (yd^3). Landfill waste reportedly included hospital wastes, paint and paint thinner, automobile batteries, airplane parts, low level radioactive waste, and asbestos.

Two shallow groundwater plumes covering approximately 23 acres have been identified that appear to be discharging from the former landfill and migrating towards a nearby drainage canal. Constituents of concerns (COCs) in groundwater included benzene, trichloroethene (TCE), vinyl chloride (VC), and iron. TCE and its associated degradation compounds, *cis*-1,2-dichloroethene (*c*DCE) and VC have been detected at concentrations in excess of the FDEP Groundwater Cleanup Target Levels (GCTLs). The plume intersects the adjacent drainage canal, but volatile organic compounds (VOCs) impact to surface water above regulatory standards have not been detected. Installation of an emulsified vegetable oil (EVO) permeable reactive barrier (PRB) was selected to prevent discharge of VOCs to the drainage canal (Tetra Tech, 2003). **Figure C-1** shows the PRB layout and monitor well locations. As part of this project, one area in the northern and one area in the southern portions of the PRB were intensively characterized to better understand the factors controlling PRB performance.

C.1.2. Hydrogeology

The site lies within the Atlantic Coastal Plain physiographic province, Mid-peninsular Zone geomorphic province, which consists of low relief marine terraces underlain by limestone (Scott et al., 2001). The marine terraces are products of a low energy marine depositional environment, resulting in discontinuous layers of fine sand, sandy silt, and silty sand. The topography at the site is relatively flat. A drainage ditch runs from north to south along the eastern side of the site and appears to form a hydraulic barrier. Groundwater at the site flows to the east/southeast.

The soils at the site belong to the Smyrna-Bassinger-St. John's and the Smyrna Urban Land complex, which consist of fine sands that are nearly level to gently sloping, and poorly to moderately-well drained (US Department of Agriculture Web Soil Survey). The sediments across the site consist primarily of quartz sand with varying amounts of silt, clay, and shell fragments that vary both laterally and vertically.

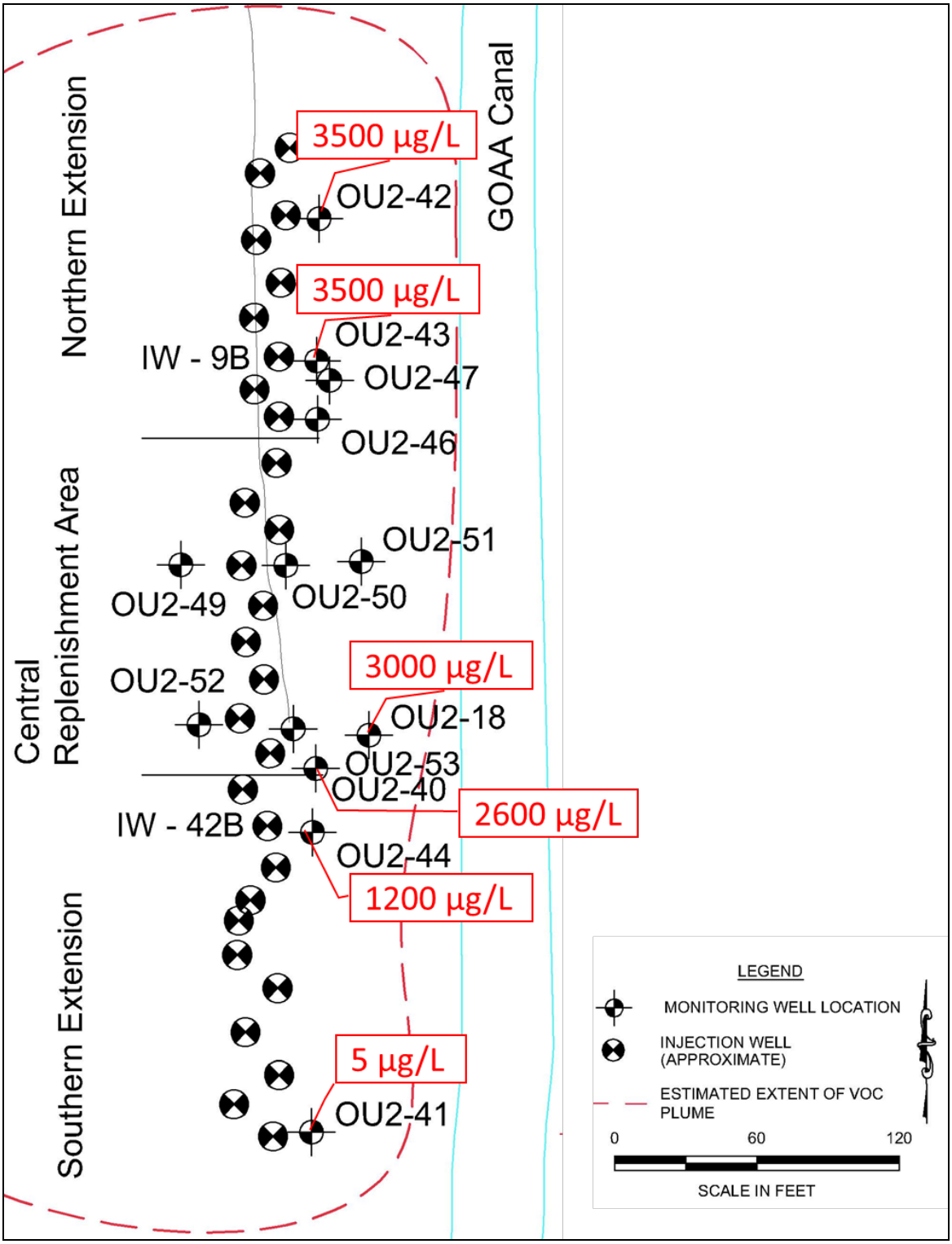

Figure C-1. Injection and Monitor Wells Installed at OU2 PRB with Average TCE Concentrations Prior to 2007.

The surficial aquifer at the site is divided into two sub-units, labeled Zone A (0 to 25 ft bgs) and Zone B (25 to 40 ft bgs), which are reportedly separated by a semi-confining unit. The primary water supply aquifer in the area is the Floridian aquifer which is separated from the surficial aquifer by the Miocene Hawthorne Group, which is classified as a confining hydrogeologic unit. Information obtained from boring logs, indicates that the Hawthorne Group was encountered at ~35 ft bgs at OU2 (Tetra Tech, 2001).

Figure C-2 shows the layout of cone penetration test (CPT) and hydraulic profiling tool (HPT) borings installed near OU2-43B in 2016. **Figure C-3** shows the results of the CPT profiling in a cross-section extending from CPT-1 to CPT-13. A clay unit over 10 ft thick is encountered at an elevation of ~47 ft msl or 35 ft bgs. Overlying this clay is 1 to 2 ft of silty sand followed by interbedded clean sand, sand, and silty sand. In general, silty sand with thin clay layers is more common at 15 to 20 ft bgs with clean sand above and below. While not continuous, layers of clean sand or silty sand can be traced tens of feet horizontally.

Based on a 1998 pumping test, the surficial aquifer has an average estimated transmissivity of 602 ft^2/d, storativity of 0.04, and average hydraulic conductivity (K) of 25 ft/d, based upon a saturated thickness of 24 ft (Tetra Tech, 2001). Slug test results are variable, ranging from 4 to 24.7 ft/d.

In 2016, the HPT was used to measure vertical profiles of K at six locations. Cross-sections of estimated K in the Northern and Southern characterization areas are shown in **Figures C-4** and **C-5,** respectively. In the Northern area (**Figure C-4**), K is relatively low in the Zone A from 10 to 20 ft bgs. From 20 to ~34 ft bgs (Zone B), K is variable ranging from 5 to over 50 ft/d. Between the clay layer at 35 ft and the overlying sand, there is a silty zone roughly 1 to 2 ft thick with K less than 1 ft/d. In the Southern area (**Figure C-5**), K in the Zone A is more variable with a higher K values observed downgradient near the drainage canal. In the Zone B, K is also quite variable with large variations observed over a few inches vertically.

The potentiometric surface of the unconfined (water table) aquifer typically occurs at depths of about 6 to 8 ft bgs. The HPT results indicate K varies from less than 1 to over 50 ft/d in the Zone B. Groundwater flow is generally to the east towards the GOAA drainage canal with an average hydraulic gradient of ~0.002 ft/ft. Assuming an effective porosity of 0.2, groundwater velocity is expected to vary from 4 to over 150 ft/yr.

The bottom of the GOAA canal typically intersects the water table. Water level data from well pairs installed in the Zones A and B indicate that both the unconfined aquifer and GOAA ditch behave as one hydrologic unit with respect to groundwater movement and contaminant distribution. Water levels in the confined, upper Floridian aquifer are about 40 ft bgs indicating a strong downward hydraulic gradient through the Hawthorne confining unit (Tetra Tech, 2001).

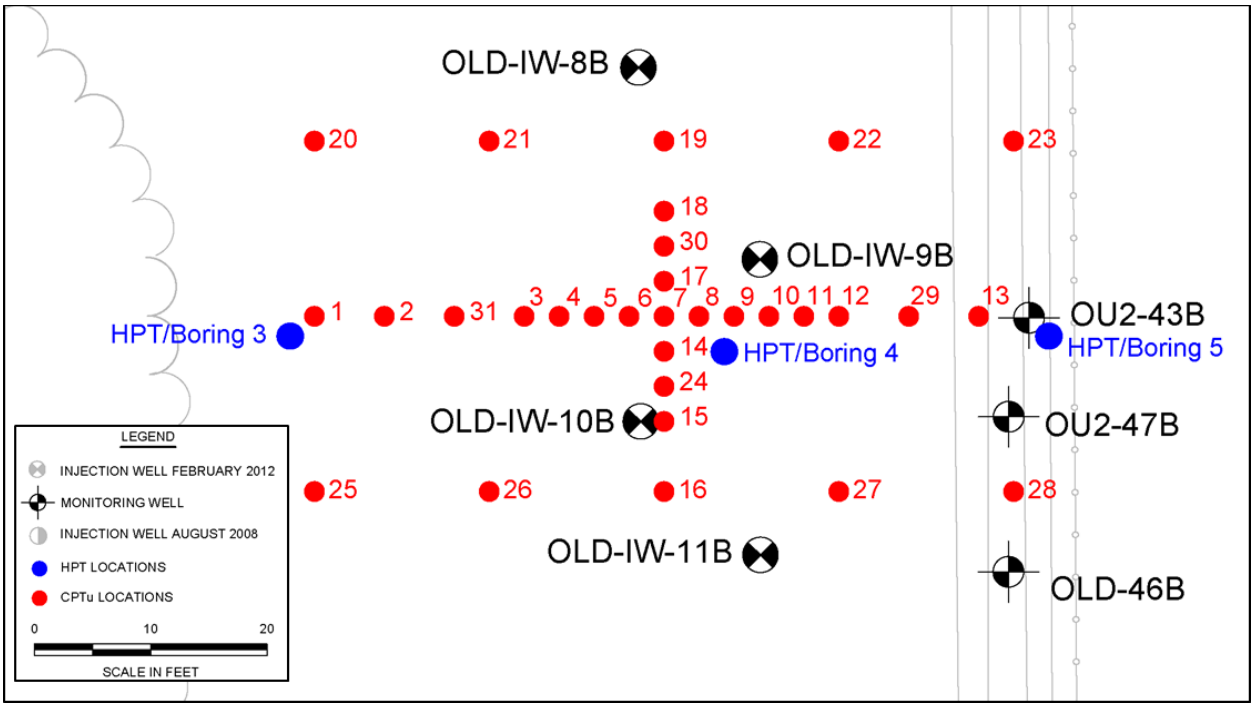

Figure C-2. CPT and HPT Boring Locations near OU2-43B.

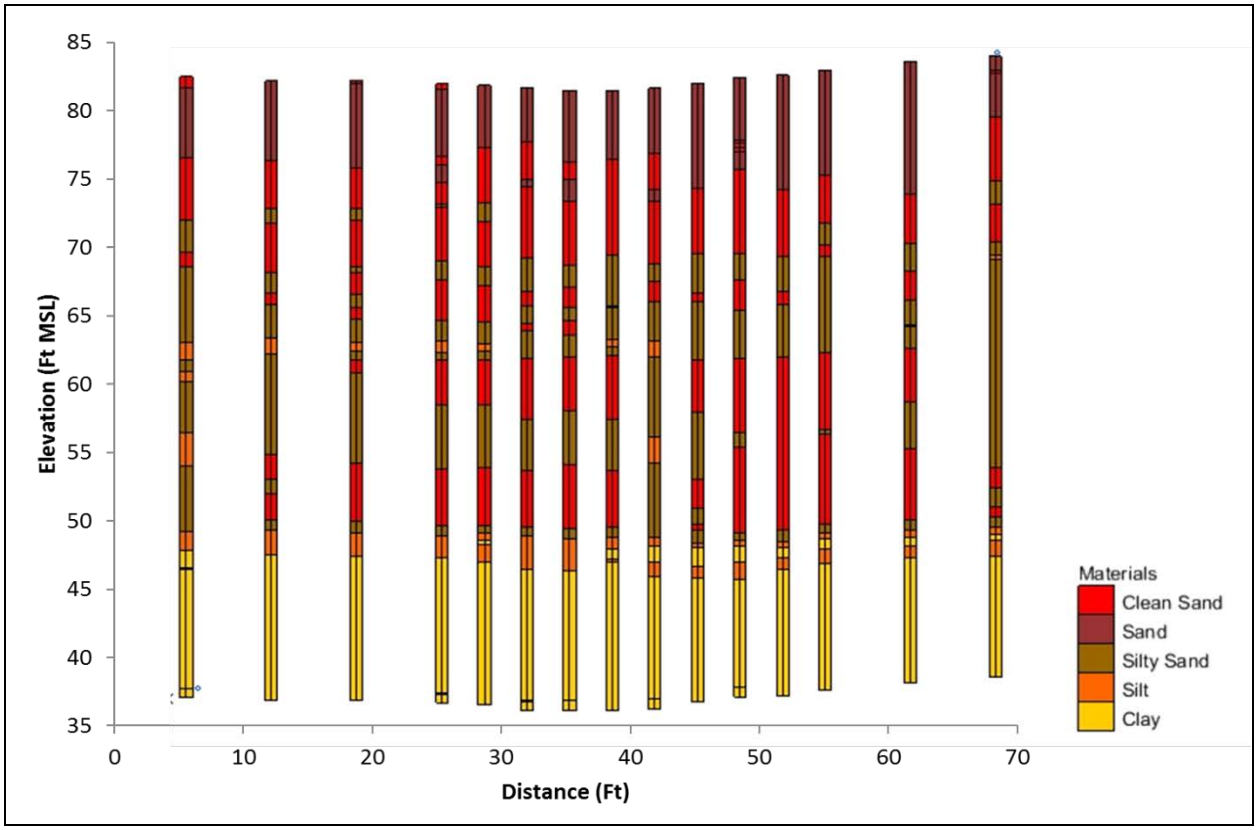

Figure C-3. Soil Classification Results from CPT Borings in Cross Section Extending from CPT 1 to 13.

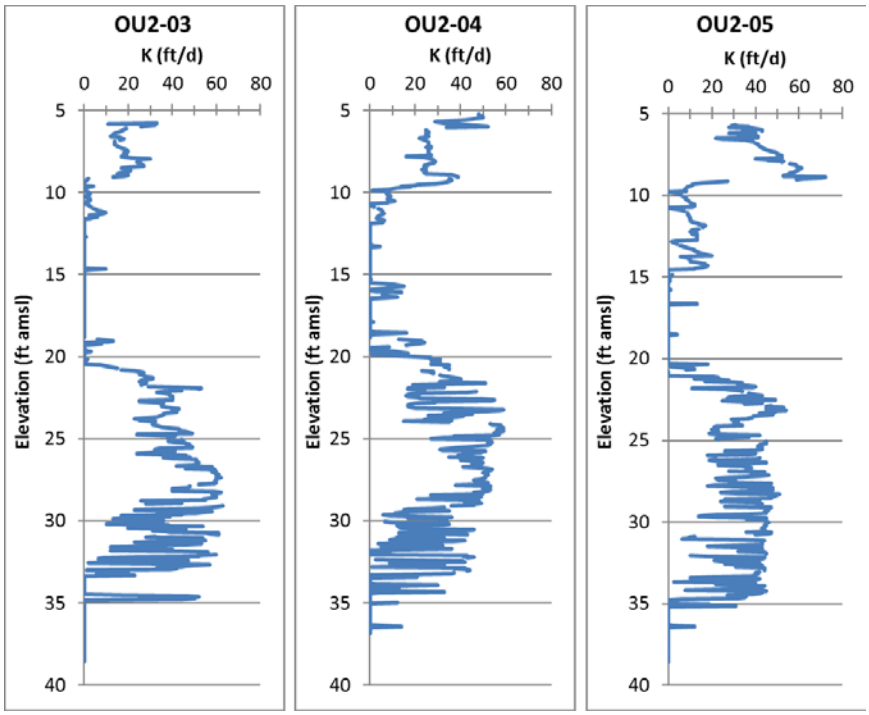

Figure C-4. HPT K Profiles in Longitudinal Cross Section near OU2-43B.

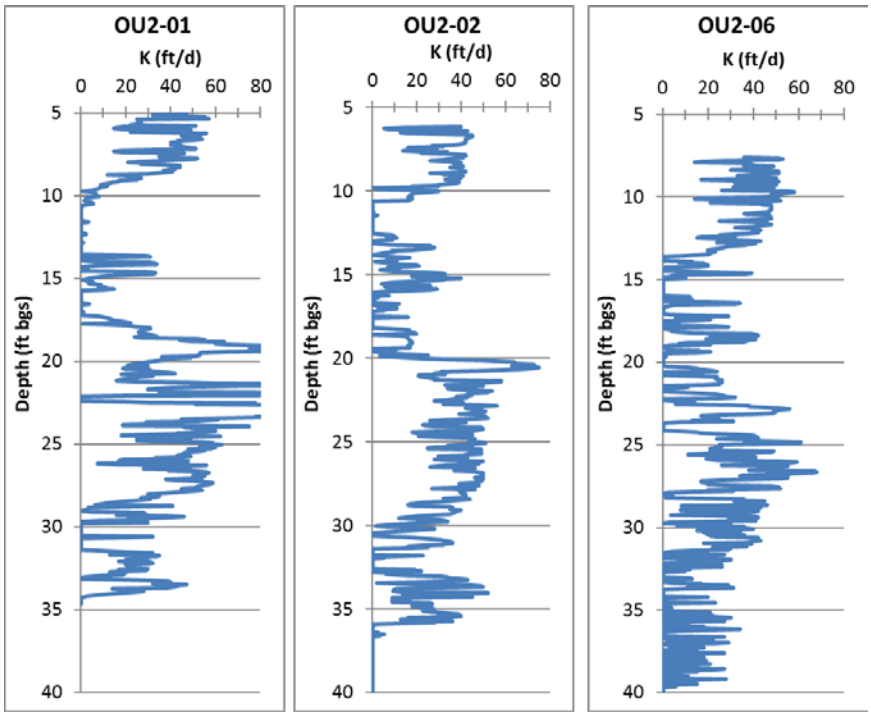

Figure C-5. HPT K Profiles in Longitudinal Cross Section near OU2-18.

C.1.3. Pre-2007 Contaminant Distribution

As discussed in **Section C.1.1**, the site formerly operated as a landfill, which accepted waste that included paint, solvents, airplane parts, transformers, and possibly motor oil. During the remedial investigation (Tetra Tech, 2001), several areas of contamination were identified through soil, groundwater, and soil gas sampling. The primary COCs identified in groundwater were benzene, TCE, VC, and iron. Low levels of tetrachloroethene (PCE), TCE, and reductive dechlorination degradation products were detected in multiple wells east of the former landfill. In the southern portion of the site, a plume with higher concentrations of TCE (> 1,000 µg/L) is present in the B zone with the highest concentrations immediately above the clay confining layer. Contaminated groundwater extends laterally from the western side of the waste boundary at the approximate location of the former landfill trenches and extends beneath the site toward the GOAA canal. **Figure C-1** shows the PRB layout and average concentrations of TCE in B zone monitor wells prior to installation of the PRB. Where concentrations are not reported, the wells were not sampled prior to PRB installation.

Groundwater contamination at the site appears to be limited to the surficial aquifer. As discussed in **Section C.1.2**, the Hawthorn Group confining layer lies approximately 35 ft bgs beneath the site, and separates the plume from the underlying upper Floridian aquifer. The clay and silt encountered at this depth may also serve as an adsorptive layer at the base of the surficial aquifer and may be releasing chlorinated volatile organic compounds (CVOCs) back into the surficial aquifer.

C.2. Remedial Operations

In 2007, CH2M HILL conducted a pilot study to evaluate the feasibility of *in situ* bioremediation using EVO substrate as the electron donor to limit plume migration (CH2M HILL, 2008). During the Phase I pilot study, CH2M HILL injected Emulisified Oil Substrate (EOS® 598B42) and a non-reactive tracer (Br) at two locations: (a) Area 1 – 5 ft upgradient of OU2-47; and (b) Area 2 – 5 ft upgradient of OU2-48. The EOS® 598B42 contained 60% soybean oil, 4% soluble substrate, 10% emulsifiers and vitamin B-12. At both areas, monitor wells were located 5, 10, and 15 ft away from the injection wells and sampled for total organic carbon (TOC) and Br to evaluate reagent distribution in the aquifer.

- At Area 1 in the northern portion of the groundwater plume, the sustainable injection flow rate was 1.25 gpm. At Area 2 in southern portion of the groundwater plume, the sustainable injection flow rate ranged from 2 (deep zone) to 3 gpm (shallow zone).
- Based on the TOC concentrations detected in sentry wells at Areas 1 and 2, the estimated radius of influence (ROI) ranged from 3 ft in Area 1, to 7 to 10 ft in Area 2. Based on the detected sodium bromide concentrations in the sentry wells, the estimated ROI was 15 ft in both areas.
- Groundwater TOC concentrations remained elevated in the injection wells about four months after injections. However, TOC concentrations returned to baseline levels in Zone B wells within four months after injection. It was recommended that additional EOS® be injected to sustain TOC concentrations adequate to foster reductive dechlorination.
- Using the TOC derived ROI, approximately 10,000 to 13,000 gallons of EOS® were estimated to be required for each injection location.

Based on the pilot test results, a PRB was selected as the preferred alternative to intercept the portion of the southern groundwater CVOCs plume entering the GOAA property. The PRB was installed in phases with the central portion of the barrier installed in August 2008 using 11 pairs of 2-inch diameter injection wells **(**central replenishment area in **Figure C-1**). Each injection well pair consisted of a well installed in Zone A (screen interval 15 to 25 ft bgs) and Zone B (screen interval 25 to 35 ft bgs). Injection well pairs were staggered, approximately 30 ft on center. A total of 106,520 gallons of 2% EOS® were injected into the 11 pairs of injection wells.

During 15 months of post-injection monitoring, the concentrations of TCE decreased appreciably in several monitor wells (AGVIQ-CH2M HILL, 2010). Complete metabolism to VC and ethene was noted in some wells, but low pH was reported and use of a buffer was strongly recommended for future injections at this site; bioaugmentation was also recommended to increase the population of dechlorinating bacteria in the aquifer.

In July 2010, Solutions-IES was contracted by NAVFAC SE to complete installation of the PRB at OU2. Monitoring of several injection wells used in 2008 indicated they were clogged with solidified oil from the 2008 injection and could not be effectively rehabilitated. In February 2012, Solutions-IES installed nine new injection wells north of the previous biobarrier (IW-3B to IW-11B), nine new injection wells south of the previous biobarrier (IW-23B to IW-31B), and an additional eleven replacement injection wells (IW-32B to IW-42B) in the area of the previous PRB (**Figure C-1**). The injection wells were installed in two rows perpendicular to groundwater flow, with wells spaced approximately 30 ft on-center in each row. The new injection wells were constructed with 2-inch diameter PVC casing and 10 ft of 0.002-inch (20-slot) screen extending from 25 to 35 ft bgs. Two emulsified oil products, EOS® 598B42 and AquaBu*pH*™, were purchased from EOS Remediation, LLC, diluted with water, and injected to replenish the existing PRB and extend it to the north and south. An average of 10.75 gallons of dilute buffered substrate (AquaBu*pH*™), 144 gallons of dilute substrate (EOS 598B42), and 4600 gallons of water were injected into each well. The AquabupH™ contained 39% soybean oil, 4% soluble substrate, 7% emulsifiers and 10% $Mg(OH)_2$. This resulted in a total of 10.9 lb of $Mg(OH)_2$ and 750 lb of soybean oil injected per well. In April 2012, the injection wells were inoculated with BAC-9™, a microbial consortium containing *Dehalococcoides spp*. (*Dhc*). *Dhc* is the primary microorganism capable of complete reductive dechlorination of TCE to ethene. Additional details of the injection process and the results of the 2-month post-injection monitoring event were provided in Solutions-IES (2013).

C.3. Remedial System Performance

C.3.1. Biogeochemistry

Performance of the Enhanced Reductive Dechlorination (ERD) remediation system was evaluated using historical groundwater monitoring data (CH2M Hill, 2008; Solutions-IES, 2013b) and data collected as part of this project (groundwater monitoring, membrane interface profiles, and soil analysis for VOCs). Groundwater monitoring data included samples collected from monitor and injection wells in the treatment zone and analyzed for VOCs, methane, ethane, and ethene (MEE), nitrate (NO_3), sulfate (SO_4), geochemical indicators (O_2, pH, oxidation-reduction potential (ORP), acidity, alkalinity), major cations and anions (Na, K, Ca, Mg, Fe, Mn, Cl, Br), molecular biological tools (major dechlorinators and genes) and volatile fatty acids (VFAs). As the first step in this process, all available data was compiled into a single master database and reviewed to identify

trends in important parameters, important biological and geochemical factors that might influence ERD, and outliers.

Prior to installation of the EVO PRB, the aquifer was reducing, with moderate levels of dissolved iron (1 to 3 mg/L), sulfate (10 to 20 mg/L), and CH_4 (0.3 to 2 mg/L). TOC concentrations varied from 5 to 8 mg/L. The pH in most wells was ~5 with occasional variations between 4.5 and 5.5.

Figure C-6 shows methane (CH_4), TOC, and pH in monitor wells located 15 to 30 ft downgradient from the Northern, Central, and Southern portions of the EVO PRB. Pilot test injections were conducted in wells upgradient of OU2-47B and OU2-18B in 2007 and the rest of the central barrier was injected in 2008. In 2012, the Northern and Southern portions were installed and the Central portion was reinjected.

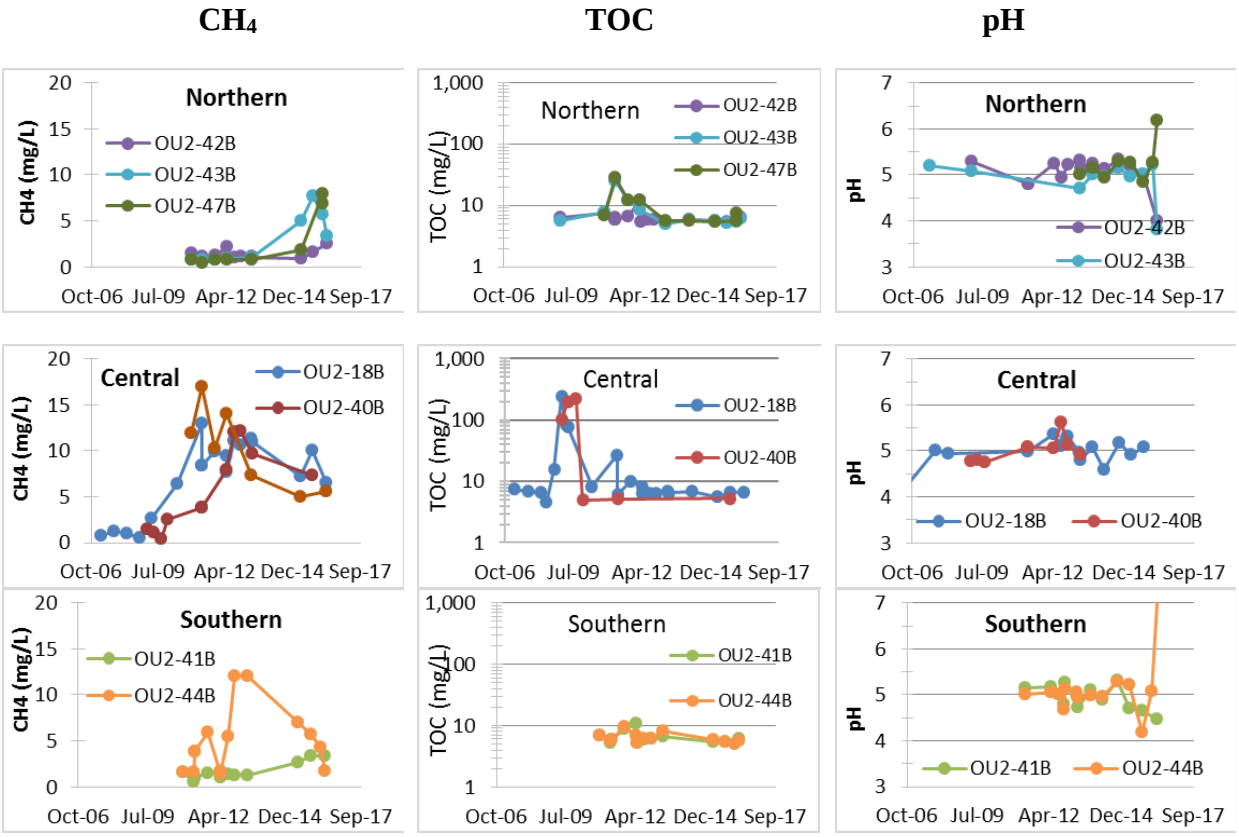

Figure C-6. CH_4, TOC, and pH in Monitor Wells Downgradient of the Northern, Central, and Southern Portions of the EVO PRB.

In the Central portion, TOC concentrations increased shortly after the 2007-2008 injections, and then declined to background levels within a few years. Methane concentrations increased more slowly and remained high. The pH has remained constant at ~5 with no evidence of a significant decrease due to substrate addition or an increase from $Mg(OH)_2$ addition in 2012. Shortly after injection, SO_4 concentrations declined and generally remain below 5 mg/L (data not shown). In general, these results indicate the EVO injections were effective in generating methanogenic conditions near the injection wells. However, the injected EVO did not reach the downgradient

monitor wells. The temporary increase in TOC was likely associated with the soluble substrates in the EVO or an initial release of soluble TOC during the initial hydrolysis of the vegetable oil triglycerides (Long et al., 2006; Hiortdahl and Borden, 2014). There is no evidence of a TOC or pH increase following the 2012 injections, indicating the effective treatment zone did not reach the downgradient monitor wells. However, CH_4 continues to be high indicating fermentable carbon is still present in closer to the injection wells. Geochemical trends in the Northern and Southern portions of the EVO PRB followed generally similar trends to the Central portion, although delayed due to the later injection of these areas. In most of the monitor wells, CH_4 is continuing to slowly increase. However, CH_4 increased very rapidly and is now declining in OU2-41 at the southern end of the barrier, suggesting most of the fermentable organic carbon has been depleted.

C.3.2. Chlorinated Ethenes

Figure C-7 shows the variation in PCE, TCE, *c*DCE, VC, and ethene with time in monitor wells downgradient of the EVO PRB. In all the wells, EVO injection resulted in a substantial decline in TCE and increase in *c*DCE and VC a few years after EVO injection.

In this work, we examine two different indicators of remediation performance: Sum of Organic Chlorine (ΣCl) and Chlorine Number (Cl#) defined as

$$\Sigma Cl = 4^* [PCE] + 3^* [TCE] + 2^* [DCE] + 1^* [VC]$$

$$Cl\# = \Sigma Cl / ([PCE] + [TCE] + [DCE] + [VC])$$

where [ ] indicates the concentration in micro-moles per liter (μM). ΣCl is the total amount of organic chlorine and Cl# is the average number of chlorine atoms per ethene.

Figure C-8 illustrates the use of ΣCl and Cl# for evaluating ERD progress in OU2-18B. Prior to EVO addition, TCE was the dominant chloroethene, Cl# was ~3 and ΣCl was approximately 3 times the TCE concentration. Following EVO addition in 2008, TCE declined by over 99.9%, with a concurrent drop in Cl# from ~3 to ~1, and ΣCl declined by 50 to 90%. The much more limited decline in ΣCl compared to TCE is due to accumulation of *c*DCE and VC.

Figure C-9 shows the variation of ΣCl and Cl# with time in monitor wells downgradient of the EVO PRB. Throughout the barrier, dechlorination has stalled at either *c*DCE (Cl# = 2) or VC (Cl# = 1). Declines in ΣCl vary from minimal in OU2-41B to over 90% in OU2-51B.

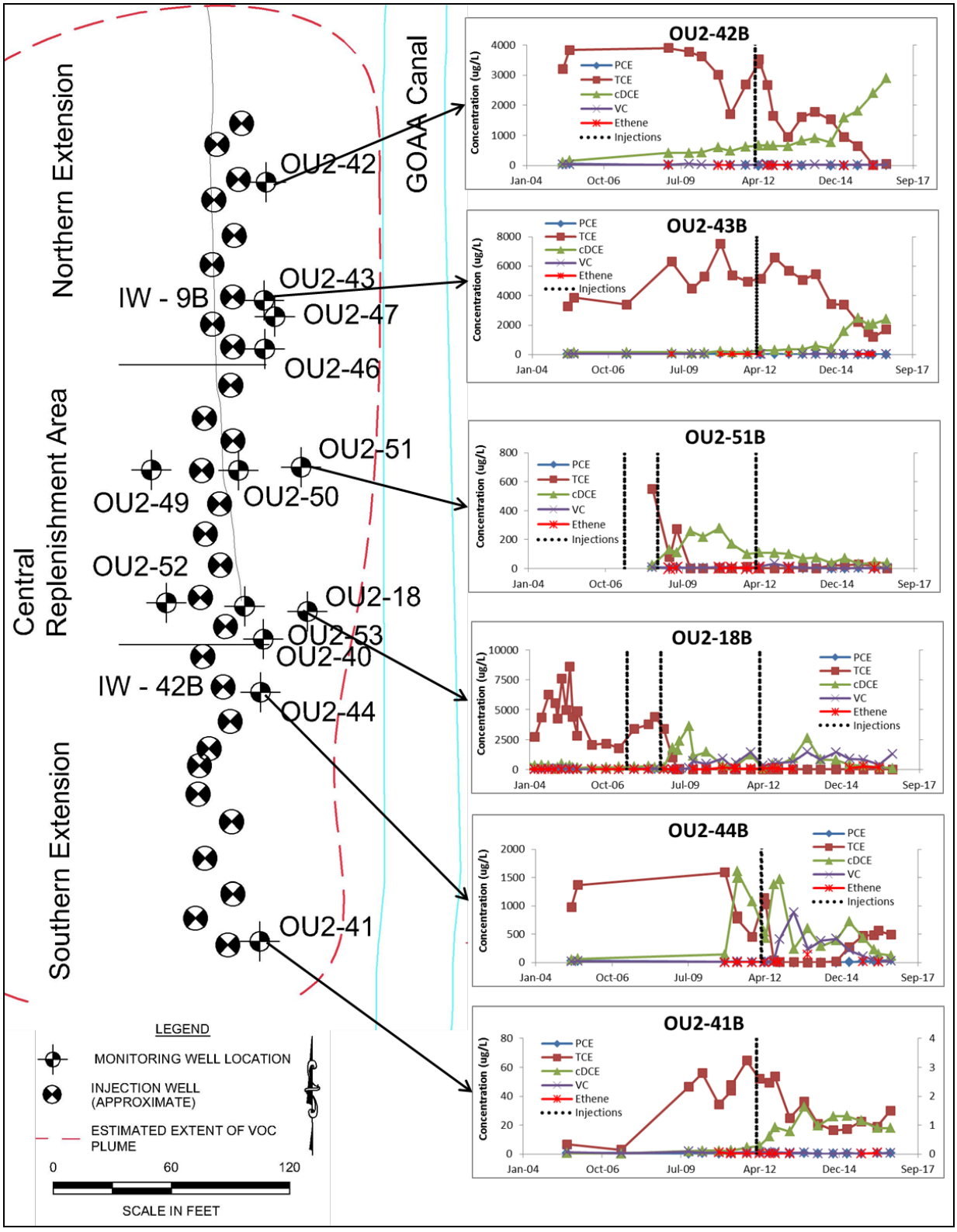

Figure C-7. PCE, TCE, *c*DCE, VC, and Ethene in Monitor Wells Downgradient of the Northern, Central, and Southern Portions of the EVO PRB.

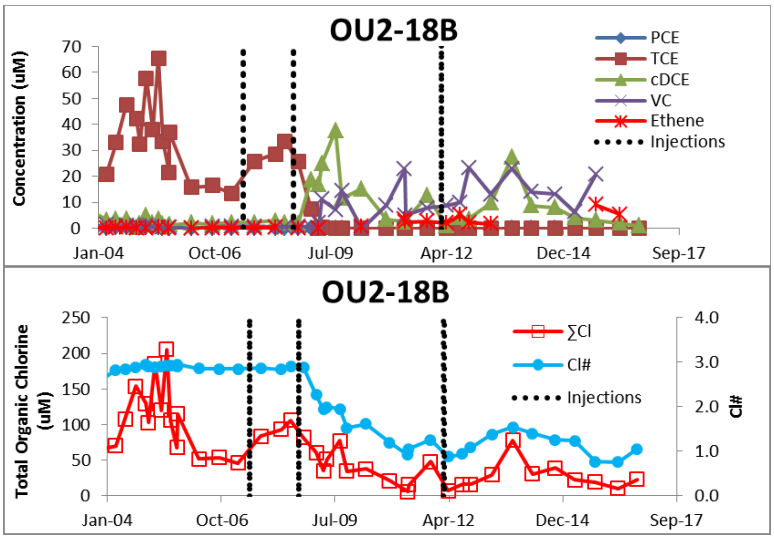

Figure C-8. Variation in TCE, *c*DCE, VC, Ethene, ΣCl, and Cl# versus Time in OU2-18B.

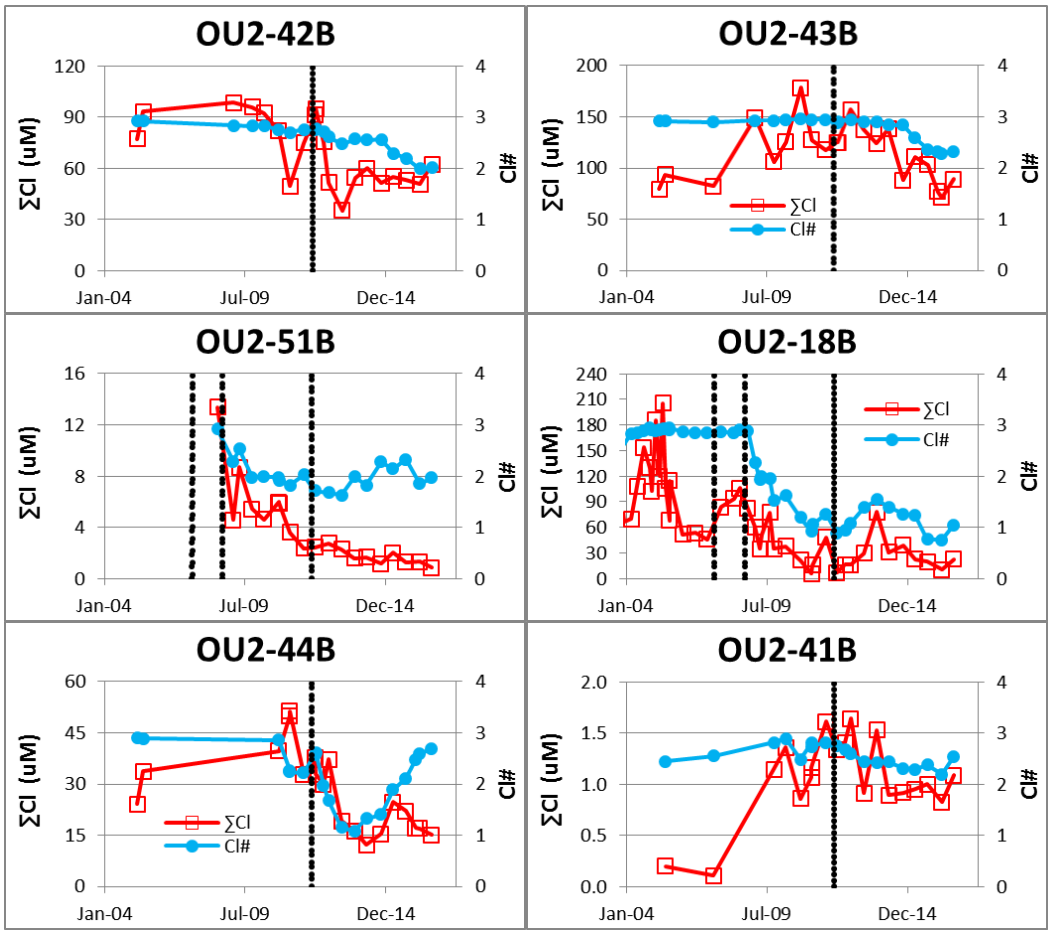

Figure C-9. Variation in ΣCl and Cl# in Monitor Wells Downgradient of the Northern (OU2-42B and OU2-43B), Central (OU2-51B and OU2-18B), and Southern (OU2-44B and OU2-41B) Portions of the EVO PRB.

C.3.3. Microbiology

Monitoring results for dehalogenating bacteria [*Dhc*, *Dehalobacter spp.* (*Dhb*)] and functional genes (*tceA, bvcA, vcrA*) presented in **Table C-1**. Numbers of dechlorinating microorganisms in the monitor wells were low, but did increase slightly following substrate addition and bioaugmentation. The low dechlorinators numbers are likely due to the low TOC concentrations and low pH which would have limited microbial growth.

Table C-1. Dehalogenating Bacteria and Functional Genes in OU2 Monitor Wells.

Well ID	Sample Date	Dehalogenating Bacteria (cells/mL)		Functional Genes (cells/mL)		
		Dhc	***Dhb***	***tceA***	***bvcA***	***vcrA***
OU2-18B	10/08/15	21.3		<0.5	<0.5	<0.5
OU2-39A	04/13/11	8.8	<3	<0.5	<0.5	<0.5
OU2-39B	04/14/11	8.8		<0.5	<0.5	<0.5
OU2-41B	04/13/11	17	<3	<0.5	<0.5	<0.5
	04/13/12	196	31	<0.5	<0.5	18.2
	07/26/12	316	17	1.8	<0.5	<0.5
	10/31/12	3.9	5	0.2 J	<0.4	<0.4
	04/23/13	125	9	0.6	0.9	<0.5
OU2-42B	04/13/11	9.5	23	<0.5	<0.5	<0.5
	04/13/12	234	8	<0.5	<0.5	13
	07/26/12	5	9	0.8	<0.3	<0.3
	10/31/12	<0.2	9	<0.2	<0.2	<0.2
	04/23/13	5.6	3	1	0.9	0.1 J
OU2-43B	06/06/07	314				
	02/25/16	616		<0.5	<0.5	<0.5
OU2-44B	04/13/11	26	38	<0.5	<0.5	<0.5
	04/13/12	468	217	<0.5	<0.5	10.4
	07/26/12	160	207	1.2	<0.5	<0.5
	10/31/12	1.8	28	<0.4	<0.4	<0.4
	04/23/13	168	11	1.1	0.2 J	0.2 J
	02/25/16	8		<1.0	2.7	<1.0
OU2-47B	02/25/16	304		<0.5	<0.5	<0.5
OU2-49B	04/13/11	0.9	75.3	<0.5	<0.5	<0.5

C.3.4. Injection Wells Sampling

One of the hypotheses evaluated in this project was that EVO and/or pH buffer were not effectively distributed throughout the treatment zone and reagent distribution in the aquifer was limiting treatment. To evaluate this hypothesis, we sampled four injection wells at various locations along the length of the PRB for biogeochemical indicators, ethenes, and dechlorinating microorganisms in October 2015 and February 2016. We assumed that transport through the aquifer would not limit treatment in these wells since both EVO and buffer were directly injected into these wells. Monitoring results are presented in **Table C-2**.

Table C-2. Monitoring Results for OU2 Injection Well Sampling.

Well ID	Unit	OU2-IW-9B		OU2-IW-10B	OU2-IW-23B	OU2-IW-42B	
Date		10/8/15	2/25/16	2/25/16	2/25/16	10/8/15	2/25/16
pH	S.U.	6.11	6.29	6.32	6.15	3.95	4.61
Alkalinity	mg/L	454	477	481	287	<2.5	<2.5
Acidity	mg/L	256	<2.5	<0.25	<2.5	164	80.9
Organic Carbon	mg/L	128	125	86	77	222	194
Inorganic Carbon	mg/L	153	150	138	112	95	86
Manganese	mg/L	0.2	0.2	0.4	0.068	0.07	0.07
Iron	mg/L	2.3	3.2	3.2	2.1	1.5	1.7
Sulfate	mg/L	<0.6	<0.6	<0.60	13	1.5	<0.6
Methane	mg/L	7.6	8.5	8.4	11.9	7.0	6.9
Sodium	mg/L	11	12	11	12	13	12
Potassium	mg/L	3.4	3.7	2.9	2.7	3.3	3.3
Calcium	mg/L	42	47	92	47	25	24
Magnesium	mg/L	90	88	58	44	6	5
Chloride	mg/L	11	12	11	8	11	9
PCE	mg/L	<0.3	<0.3	<0.3	<0.3	<0.6	<0.6
TCE	µg/L	<0.2	<0.3	4	<0.3	2.5	4
cis-1,2-DCE	µg/L	0.2	3.7	3.5	22	154	177
VC	µg/L	1.7	4.8	2.1	25	77	96
Ethene	µg/L	34	26	4	9	24	19
Dhc	cells/mL		37,000	12,000	6,150	NA*	224
tceA	cells/mL		3,140	1,200	614	NA	32.2
bvcA	cells/mL		<1.6	<1.8	1.3	NA	<0.7
vcrA	cells/mL		6,890	1960	928	NA	15.5
ΣCl	µM	0.04	0.16	0.20	0.87	4.48	5.30
Cl#	µM/ µM	0.03	0.16	0.82	0.92	1.21	1.30

*NA – not analyzed

Monitoring results indicate that PCE, TCE and their degradation products were being effectively treated in two of the injection wells (OU2-IW-9B and OU2-IW-10B). In these two wells, pH is greater than 6, TOC concentrations were greater than 30 mg/L, TCE and *c*DCE have been substantially reduced, and relatively high numbers of dechlorinators (*Dhc*) are present with enzymes capable of reducing VC to ethene.

In OU2-IW-42B, ERD is more limited, high concentrations of *c*DCE and VC persist, likely due to the low pH (<5), high acidity, low Mg, and associated low *Dhc* counts. The cause of the low pH is unknown, since records indicate that AquabupH containing $Mg(OH)_2$ was injected into this well. It may be that the K near this well is somewhat higher resulting in more rapid washout of added $Mg(OH)_2$.

In OU2-IW-23B, PCE and TCE are below detection. However, significant concentrations of *c*DCE and VC persist. The pH is >6 and TOC> 30 indicating appropriate conditions for ERD. While dechlorinators with enzymes capable of reducing VC to ethene are present, their numbers are relatively low, potentially indicating some other limitation to growth.

C.3.5. Spatial Distribution of TCE and *c*DCE

As part of the site characterization work for this project, direct push groundwater samples were collected at four depths (25, 30, 35, and 40 ft bgs) immediately upgradient of the EVO barrier, within the EVO injection zone, and downgradient near OU2-43B in May 2016. These results are compared with K profiles measure by HPT in **Figure C-10**. Upgradient of the PRB, TCE is the dominant chloroethene with the highest concentrations observed immediately adjoining the clayey silt confining layer and decrease rapidly with distance. Within the EVO injection zone, TCE has been extensively converted to *c*DCE. Downgradient, TCE concentrations have rebounded somewhat.

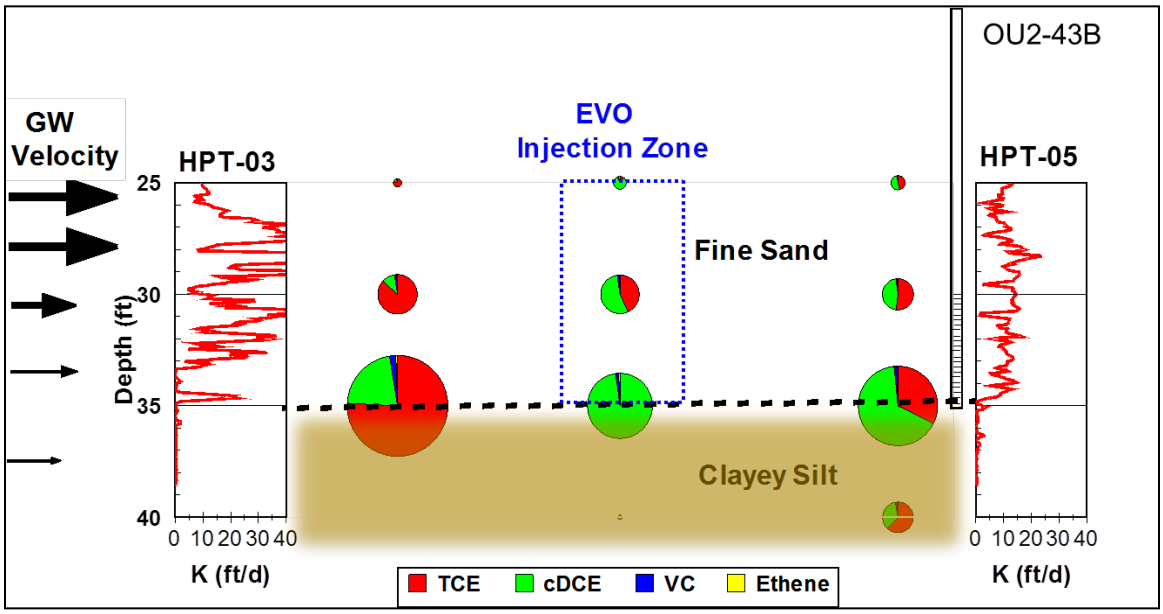

Figure C-10. TCE, *c*DCE, VC, and Ethene at Varying Depths Upgradient, within, and Downgradient of the EVO Injection Zone.

Area in pie chart is proportional to concentration.

To evaluate the potential for back-diffusion of TCE out of the underlying confining unit, a continuous soil core was collected adjoining OU2-43B from 25 to 40 ft bgs. Subsamples were collected and analyzed for CVOCs and soil particle size distribution. **Figure C-11** shows the results of this evaluation along with the K profile measured by HPT at this location. TCE concentrations were a maximum 2 ft into the confining layer where the clay+silt content was highest. This information will be used in **Section C.4.1** to determine if back-diffusion from the confining layer is contributing to slower than desired treatment of the aquifer downgradient of the PRB.

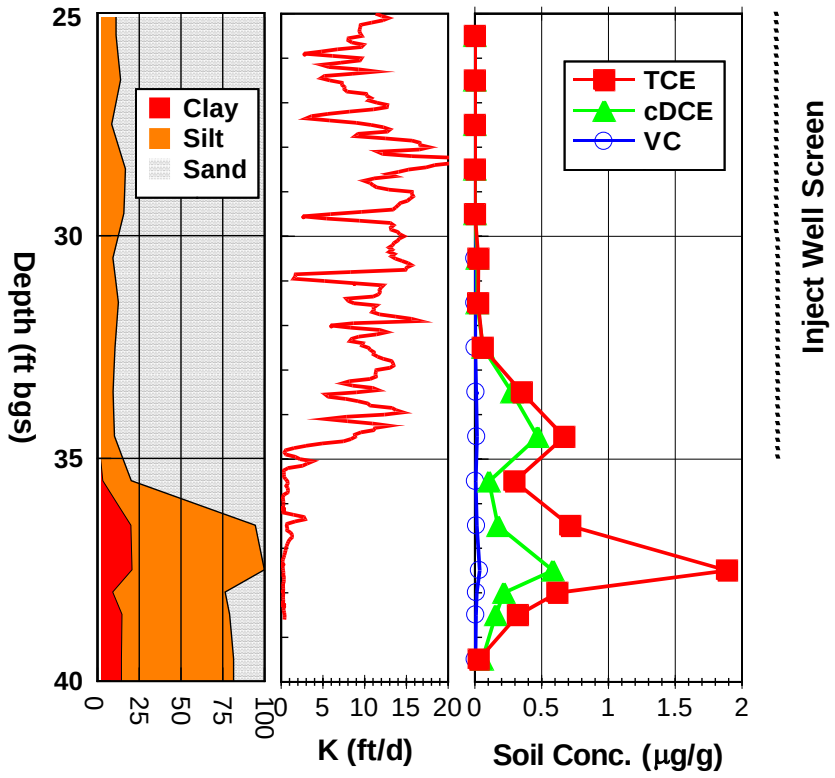

Figure C-11. Profiles of Soil Composition, K, and TCE, *c*DCE and VC Concentrations in Soil Samples Collected from a Boring Adjoining OU2-43B.

C.3.6. Assessment of Overall Remedial Performance

To provide some reference for comparing treatment performance, we compare Order of Magnitude (OoM) concentration reductions at OU2 with OoM reductions in TCE concentration reported by Tillotson and Borden (2017) for 37 ERD sites containing 184 treatment zone monitor wells and in situ remediation performance monitoring results reported by McGuire et al. (2016). OoM reductions at OU2 were calculated by comparing monitoring results from immediately before injection to October 2016. The performance monitoring results reported by McGuire et al. (2016) was sorted to select 19 ERD sites treated with Semi-Soluble / Slow-Release substrates where TCE was the parent compound. The McGuire database provides OoM removal results for both geometric mean concentrations and maximum concentrations before and after treatment.

Figure C-12 compares OoM removals for the McGuire database (19 site geomeans and maximums), Tillotson and Borden (2017) data base for 37 ERD sites containing 184 wells, and individual wells at OU2. Direct comparison of the results of the different studies are not possible because each study used slightly different methods to calculate OoM removal. TCE concentrations in individual monitoring wells declined by 0 to 3.2 OoM at OU2 which is somewhat lower than values reported by McGuire et al. (2016) and Tillotson and Borden (2017).

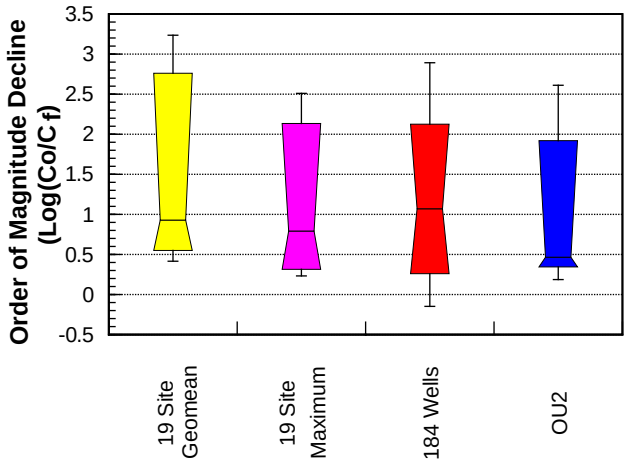

Figure C-12. Order of Magnitude (OoM) Concentration Reductions in TCE for 19 Sites (McGuire et al., 2016), 184 Wells (Tillotson and Borden, 2017), and OU2 Monitoring Wells.

While parent compound removal is an important metric, accumulation of ERD biotransformation products was a significant issue at OU2. **Figure C-13** compares OoM reductions in TCE, *c*DCE, VC, and ΣCl in at OU2 with results from Tillotson and Borden (2017). As shown above, TCE removal was below average at OU2. In addition, *c*DCE accumulation was worse at OU2 in comparison to results from Tillotson and Borden (2017). A more balanced measure of treatment performance is OoM removal in ΣCl. Overall, ΣCl removal at OU2 was not as good as at other sites.

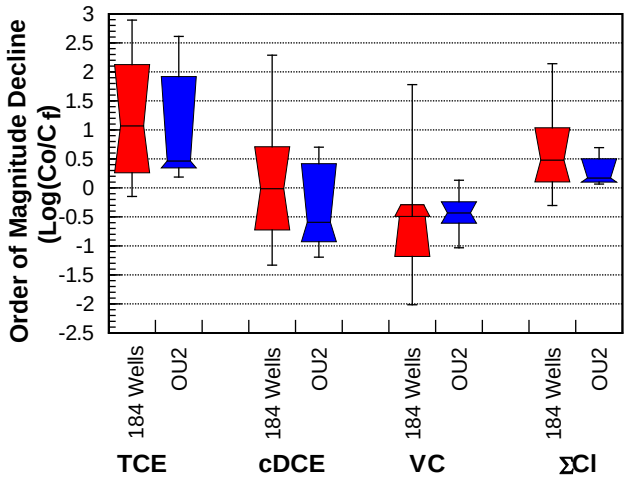

Figure C-13. Order of Magnitude (OoM) Decline in TCE, *c*DCE, VC, and ΣCl for 184 Wells Reported by Tillotson and Borden (2017) and OU2 Monitoring Wells.

In summary, treatment performance at OU2 was lower than at other ERD sites. Both parent compound removal and daughter product accumulation were below average resulting in lower than average ΣCl removal.

C.4. Evaluation of Factors Limiting Remedial Performance

C.4.1. Back Diffusion

The Dandy-Sale (DS) model (Sale et al., 2008) within the Matrix Diffusion Toolkit (MDT) (Farhat et al., 2012) was used to evaluate TCE diffusion into the clayey silt confining layer and back diffusion over time. The TCE release was assumed to occur in 1978 with treatment beginning in 2012 after installation of the EVO PRB. The low K soil organic carbon content was assumed equal to 0.0005 g/g resulting in a TCE retardation factor of 1.07 in the low K zone. Default values from Farhat et al. (2012) were used for all other model parameters. **Figure C-14** shows the simulated vertical profile of TCE in the confining layer at 10 m downgradient of the EVO PRB compared to measured concentrations in 2016. The DS model provided a relatively good match to the measured concentrations matching the depth and maximum concentration observed.

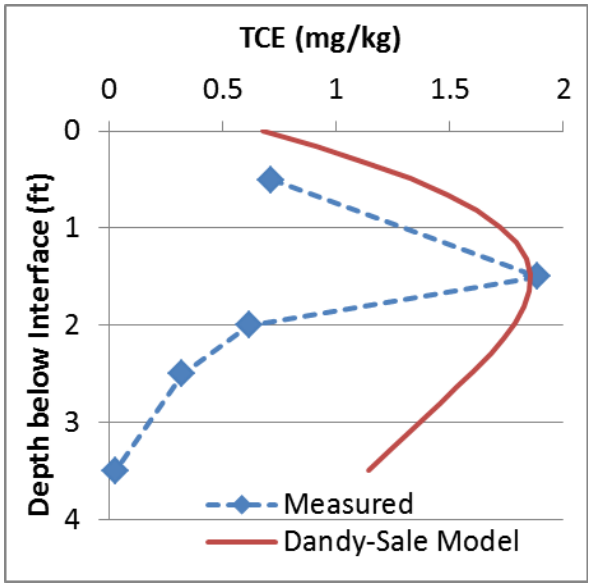

Figure C-14. Comparison of Measured Concentrations versus Simulated TCE Profile Generated with Dandy-Sale Model (Sale et al., 2008).

Figure C-15 shows the simulated mass of TCE entering the GOAA canal over time following installation of the EVO PRB in 2012. For comparison, the second y-axis on **Figure C-15** shows average TCE concentrations in a monitor well (10-ft screen) installed immediately above the confining layer and adjoining the GOAA canal. Mass discharge drops rapidly following installation of the EVO PRB in 2012. However, after 2017, mass discharge declines more slowly as TCE is released from the confining layer by back diffusion downgradient of the PRB and upgradient of the GOAA canal. The total mass released by back diffusion is relatively small due to the short distance from the PRB to the canal (~50 ft). By 2030, TCE concentrations in a monitor well adjoining the GOAA canal are predicted to drop below 5 µg/L.

In summary, back diffusion from the confining layer, downgradient of the PRB, is not expected to substantially limit the effectiveness of the PRB in reducing TCE discharge to the GOAA canal.

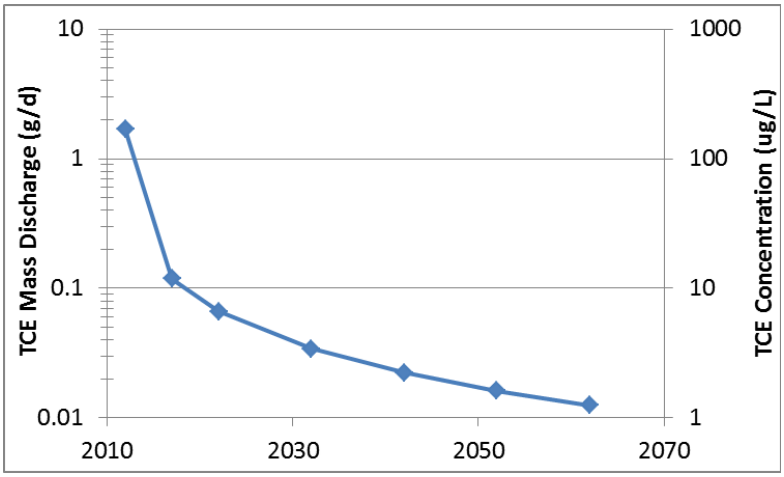

Figure C-15. Predicted Mass Discharge and Average TCE Concentrations in a Monitor Well (10-ft screen) Installed Immediately Above the Confining Layer and Adjoining the GOAA Canal.

C.4.2. Evaluation of EVO Loading and Injection Volumes

The EVO design tool developed under ER-0626 (Borden et al., 2008; Weispfenning and Borden, 2008) was used to evaluate the 2008 and 2012 injections and determine if sufficient EVO and water were injected to generate a PRB with residual oil in close contact with over 80% of the groundwater migrating through the barrier. This process was performed using two different sets of design parameters: (a) the literature values used in the original design; and (b) the measured values of these parameters generated in this project.

Prior research has shown that oil droplet retention is influenced by the zeta potential of the oil droplets and the aquifer material (Coulibaly and Borden, 2004). Previous EVO transport experiments were conducted with low ionic strength tap water (Coulibaly et al., 2006) or deionized (DI) water (Borden et al., 2008). Sampling of monitoring wells in 2015 showed low ionic strength groundwater with typical average concentrations of 9 mg/L Na, 2 mg/L K, 9 mg/L Ca, and 2 mg/L Mg. However, injection wells sampled in 2015 and 2016 (**Table C-2**) had higher concentrations of Ca (average of 46 mg/L) and Mg (average of 49 mg/L) due to injection of $Mg(OH)_2$ to raise aquifer pH. To evaluate the potential impact of ionic strength, the zeta potential of EOS 598B42 and aquifer material from OU2 were measured in DI water and a solution of 200 mg/L $CaCl_2$ (approximately 73 mg/L Ca). The zeta potential of all materials was negative in the presence of both DI water and 200 mg/L $CaCl_2$ (**Table C-3**). However, the zeta potential of both the oil droplets and the aquifer material was more strongly negative in DI water than the $CaCl_2$ solution, indicating that oil droplet-sediment particle repulsion will be greater and oil retention will be lower in DI water.

Table C-3. Effect of Solution Composition on Zeta Potential and Oil Retention.

Colloid	Solution	Zeta Potential (mV)	
		Average	Std. Dev.
OU2 Soil 37-40'	DI	-19.9	0.5
	200 mg/L $CaCl_2$	-12.2	0.9
EOS 598B42	DI	-43.0	0.7
	200 mg/L $CaCl_2$	-10.3	0.4

Column experiments were conducted in 28 cm x 2.6 cm diameter columns packed with aquifer material collected from OU2 to measure the transport and retention of EVO. The columns were first saturated, then 150 mL (~3 pore volumes [PV]) of a 20% EVO dilution, followed by ~3 PV of chase water. Oil retention was determined by measuring the volatile solids fraction of the aquifer material before and after testing (**Table C-4**). Measured oil retention in the OU2 sediment flushed with 200 mg/L $CaCl_2$ was significantly higher than for the same material flushed with DI water consistent with reduced electrical repulsion between the oil droplets and sediment.

Table C-4. Retained Oil Content in Column Tests with DI Water and 200 mg/L $CaCl_2$.

	OU2	
	DI	$CaCl_2$
Influent End	1.36%	2.50%
Middle	1.64%	4.50%
Effluent End	1.31%	4.43%
Average	1.44%	3.81%

These results demonstrate that ionic strength and concentration of divalent cations (Ca^{2+}, Mg^{2+} and Fe^{2+}) can have a major impact on zeta potential and oil retention. The ionic strength of groundwater in the treatment zone varies from low in the monitor wells to high in the injection wells. As a result, oil retention probably varies from high near the injection wells to low in more distant portions of the aquifer.

Table C-5 shows the input parameters used in the original design of the 2008 and 2012 EVO injections (CH2M Hill, 2006; Solutions-IES, 2013b) and our current best estimates for these parameters. Prior to the 2008 and 2012, no column tests of EVO transport were conducted, so the injection designs were based on assumed parameters obtained from the published literature (Coulibaly and Borden, 2004; Coulibaly et al., 2006). Oil transport and retention column experiments were conducted using either DI water or a 200 mg/L $CaCl_2$ solution. The 2012 injections were evaluated with Maximum Oil Retention values measure with DI water (representative of background, low ionic strength groundwater) and 200 mg/L $CaCl_2$ (representative of EVO amended with $Mg(OH)_2$).

Table C-5. Design Tool Evaluation of 2008 and 2012 Injections at OU2.

Parameter	Unit	2008 Central PRB	2012 Entire PRB

		Literature Values	DI Water	Literature Values	DI Water	$CaCl_2$
Treatment Width	ft^2	140	140	435	435	435
Treatment Zone Thickness	ft	20	20	10	10	10
Maximum Oil Retention	lb/lb	0.0012	0.0144	0.0012	0.0144	0.0381
Oil Injected	lb	10,661	10,661	20,224	20,224	20,224
Injected / Max retention		1.05	0.09	1.37	0.11	0.04
Fluid Vol Injected	ft^3	106,520	106,520	133,400	133,400	133,400
Pore Vol Injected		0.67	0.67	1.16	1.16	1.16
Projected Contact Eff.		94%	20%	95%	28%	~15%

Evaluation of the 2008 and 2012 injection volumes with the ESTCP EVO Design tool indicates that:

A. Based on the original design assumptions in 2008 and 2012, the total volume of diluted EVO and chase water should have been more than sufficient to effectively distribute the oil droplets throughout the treatment zone, achieving EVO contact efficiencies greater than 80%.

B. The measured oil retention with DI water was 0.0144 g/g or over ten times the value assumed in the original design. This under-estimate resulted in too little EVO being injected, which would have reduced contact efficiency and treatment.

C. Injection of a mixture of EVO and colloidal $Mg(OH)_2$ likely increased ionic strength of the injection solution and oil retention near the injection wells, further reducing EVO contact efficiency.

C.4.3. Hydraulic Design

The groundwater flow model, MODFLOW (Harbaugh et al., 2000) was used to simulate flow patterns during the 2012 injection in the northern study area. The EVO transport and retention was simulated using the reactive transport model RT3D (Clement, 1997) with the reaction module developed to simulate retention of colloidal oil droplets (Coulibaly et al., 2006). Injection volumes and flowrates used in model calibration are presented in **Sections C.2.1 and C.2.2**. A maximum oil retention of 0.0144 g/g was used in all simulations for consistency. The K distribution was generated with the CPT and HPT results shown in **Figures C-3**, **C-4**, and **C-5**.

Figure C-16 shows the simulated oil distribution following the 2012 injection at 25, 30, and 35 ft bgs. Oil distribution is reasonably good at the 25 and 30 ft depths forming a continuous barrier. However, at 35 ft bgs where TCE concentrations are highest, oil distribution is more limited due to the preferential injection in the shallower, high permeability zones.

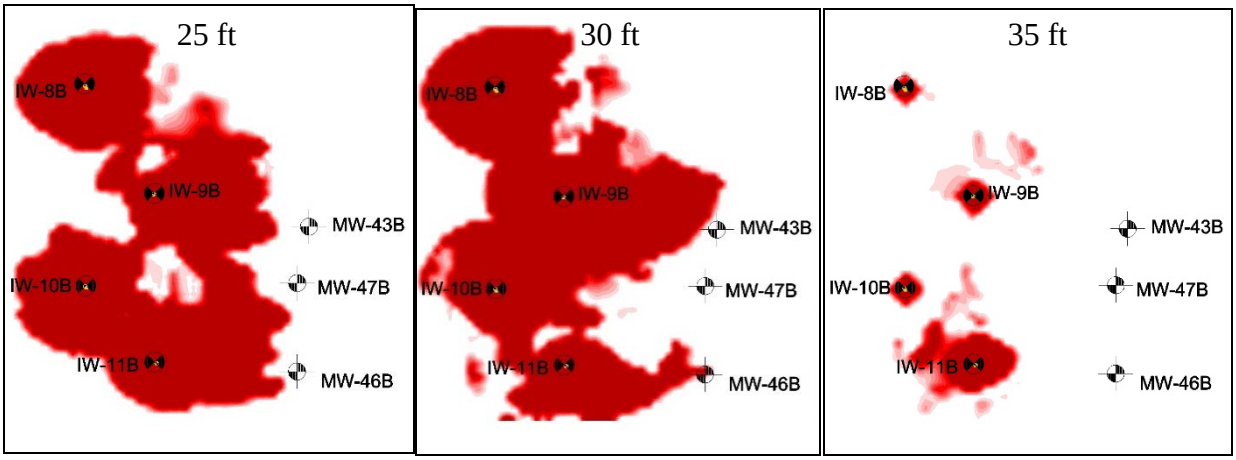

Figure C-16. Simulated EVO distribution at 25, 30, and 35 ft bgs in northern study area following 2012 injection.

To evaluate whether oil distribution was a significant factor limiting treatment performance, biotransformation of TCE, *c*DCE, and VC were simulated using the sequential dechlorination module in RT3D. Following the approach used by Borden (2007), effective 1st order degradation rates were assumed to be linearly proportional to residual oil concentrations on the soil. Two set of rates were used (**Table C-6**). For the first set of simulations (base condition), rates were initially assumed equal to values estimated by Borden (2007). For the second set of simulations (inhibited condition), the TCE transformation rate was set equal to 1% of the rate reported by Borden (2007) and the *c*DCE and VC transformation rates were set to zero.

Table C-6. Chlorinated ethene transformation rates for OU2 treatment evaluation.

Compound	Base Condition (rates from Borden 2007)		Inhibited Condition	
	2nd Order rate (g sed/g oil–d)	Apparent 1st order rates (1/d)	2nd Order rate (g sed/g oil–d)	Apparent 1st order rates (1/d)
TCE	225	0 – 3.24	2.25	0 - 0.0324
*c*DCE	43	0 – 0.62	0	0
VC	65	0 – 0.94	0	0

Figure C-17 shows simulated TCE, *c*DCE, and VC concentrations for both the base condition and inhibited condition compared to measured concentrations at OU2-43B and OU2-47B. For the base condition, TCE was reduced by about 80% with little or no *c*DCE or VC accumulation. TCE removal was less than 100% because of the limited EVO distribution at the 35 ft depth where TCE concentrations are highest. Reducing the *c*DCE and VC rates to zero provided a better match with the field data, consistent with strong inhibition at the low ambient pH.

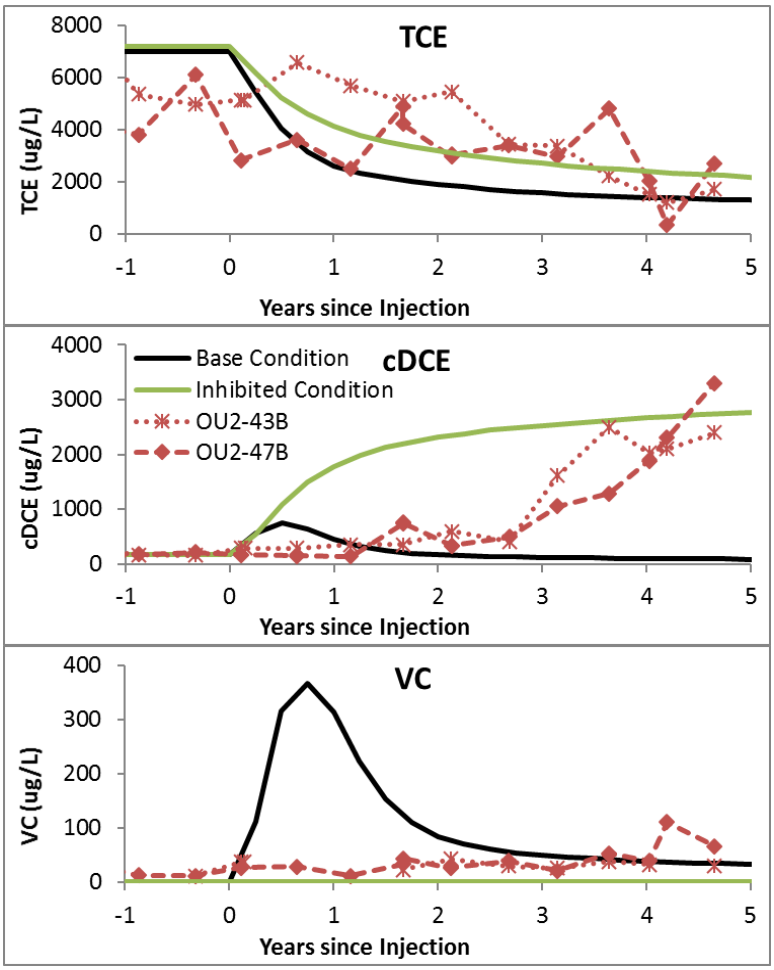

Figure C-17. Comparison of measured and simulated TCE, *c*DCE, and VC concentrations in the northern study area.

C.4.4. Buffer Design

As part of this project, a MS Excel spreadsheet was developed to estimate the base addition required to raise the aquifer pH to the desired level, and maintain it at that level for the design treatment period. Details of the spreadsheet development and calculation procedures are provided in **Appendix D**. This spreadsheet was used to estimate the amount of base required for the 2012 injection assuming a five-year design life. Input parameters and results of this evaluation are summarized in **Table C-7**.

Table C-7. Calculation of Required Base Addition for 2012 Injections.

Parameter	Unit	2012 Injection
Design Treatment Period	yr	5
Barrier width	ft	435
Volume groundwater treated	gallons	3,200,000
Target pH	SU	7.0
Background pH	SU	5.0
Total Inorganic Carbon	mg/L	12
Background CO_2 Acidity	meq/L	0.8
Aquifer Buffering Capacity	meq/Kg/pH	8.0
TCE	Kg	61
Vegetable Oil added for ERD	lb	20,224
Influent Acidity	OH^- eq	10,850
Base to raise starting pH	OH^- eq	143,195
Acidity from Dechlorination	OH^- eq	1,390
Acidity from Added Substrate	OH^- eq	568,050
Acidity from e^- accept / donors	OH^- eq	-415,481
Total Base Demand	OH^- eq	308,004
Total Base Added	OH^- eq	3,742
Fraction of Base Demand Met		1%

The large majority of the base requirement was associated with the added organic substrate and soil acidity. A total of 240 lb of $Mg(OH)_2$ were injected which provided ~1% of the base required to raise the pH to 7 and maintain it at that level for five years.

To provide guidance on amounts of base required during future injections, the design tool was used to estimate the amount of NaOH, Na_2CO_3, $NaHCO_3$, and $Mg(OH)_2$ required to maintain the pH at different levels following the 2012 injections. Results presented in **Figure C-18** indicate that the amount of base required is very sensitive to the target pH. For target pH values < 5.4, little or no base is required. Increasing the target pH to 6 or 7, requires progressively greater amounts of base because of the soil acidity and carbonic acid released from substrate fermentation.

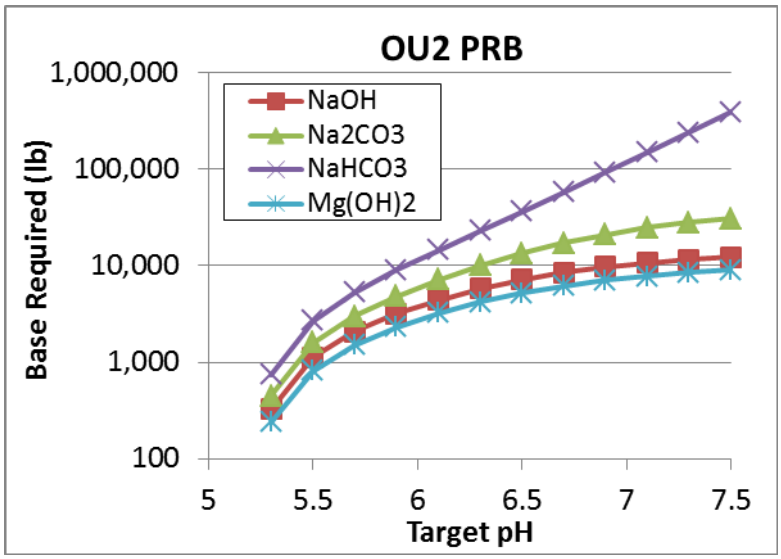

Figure C-18. Required Base Addition for 2012 OU2 PRB for Different Target pH Values.

To achieve a pH~7, 10,200 lb of NaOH or 7,400 lb of $Mg(OH)_2$ are required, which is equivalent to 50% and 37% of the vegetable oil injected. Multiple NaOH injections would be required since a single injection would result in an excessively high initial pH and the base would migrate out of the treatment zone over time with flowing groundwater. The total mass of $Mg(OH)_2$ required is less than NaOH, since two moles of OH^- are released per mole of $Mg(OH)_2$. However, $Mg(OH)_2$ has a very low aqueous solubility, so the material must be injected in a colloidal form. Since the material is a solid and would not migrate downgradient 100% of the required $Mg(OH)_2$ could be injected simultaneously with the EVO. The pH of a pure slurry of $Mg(OH)_2$ is relatively high (~10.3). However once injected, CO_3^{2-} precipitates on the surface of the $Mg(OH)_2$ particles forming an $MgCO_3$, coating maintaining the aquifer pH between 7 and 8 (Hiortdahl and Borden, 2014). In general, it is not practical to raise the pH to near 7 with $NaHCO_3$ due to the small amount of H^+ consumed by this material at near neutral pH. Na_2CO_3 could be effective, but would require multiple injections due to the high pH (~11.7) and downgradient migration with groundwater flow.

C.5. Site Summary and Conclusions

Overall bioremediation performance at of the OU2 PRB was less than desired. While the barrier is somewhat effective at reducing TCE concentrations in downgradient wells, *c*DCE and VC accumulated in many wells, so removal of total organic chlorine ($\sum$Cl) was below average. The *c*DCE and VC accumulation was due to limited distribution of fermentable organic carbon and low pH throughout much of the target treatment zone. Several different factors contributed to the less than desired treatment performance.

1) The total amount of EVO injected was significantly less than that required for high contact efficiency and treatment.
2) The total amount of base injected in 2012 was a small fraction of the base required to maintain the pH in a range appropriate for reductive dechlorination of *c*DCE and VC to ethene.

3) The highest TCE concentrations were present within and adjoining a lower K silty sand at the base of the aquifer, making it more difficult to effectively distribute remediation amendments in the most contaminated interval.

There was no evidence that absence of required microorganisms, back diffusion of contaminants from low K zones, inadequate chase water volume, or the hydraulic design substantially reduced treatment performance.

C.6. References

AGVIQ-CH2M HILL JV-II, 2010. Construction Completion Report, Phase II Pilot Injections, Biobarrier at Operable Unit 2, Former Naval Training Center Orlando, Orlando, Florida. NAVFAC Southeast

Borden, R.C., 2007. Concurrent bioremediation of perchlorate and 1, 1, 1-trichloroethane in an emulsified oil barrier. Journal of Contaminant Hydrology, 94(1), pp.13-33.

Borden, R.C., Clayton, M., Weispfenning, A.M., Simpkin, T. and Lieberman, M.T., 2008. Technical Report-Design Tool for Planning Emulsified Oil Injection Systems. Environmental Security Technology Certification Program, Arlington, Virginia.

CH2M HILL, 2006. Technical Memorandum - Biobarrier Injection Pilot Study Work Plan, Operable Unit 2, Naval Training Center Orlando, Florida. NAVFAC Southern Division.

CH2M HILL, 2008. Technical Memorandum - Biobarrier Injection Pilot Study Summary, Operable Unit 2, Naval Training Center Orlando Florida. NAVFAC Southern Division.

Clement, T.P., 1997. A Modular Computer Code for Simulating Reactive Multi-Species Transport in 3-D Groundwater Systems. PNNL-11720. Pacific Northwest National Laboratory, Richland, WA.

Coulibaly, K.M. and Borden, R.C., 2004. Impact of edible oil injection on the permeability of aquifer sands. Journal of contaminant hydrology, 71(1), pp.219-237.

Coulibaly, K.M., Long, C.M. and Borden, R.C., 2006. Transport of edible oil emulsions in clayey sands: One-dimensional column results and model development. Journal of Hydrologic Engineering, 11(3), pp.230-237.

Farhat, S.K., Newell, C.J., Sale, T.C., Dandy, D.S., Wahlberg, J.J., Seyedabbasi, M.A., McDade, J.M., and Mahler, N.T., 2012. Matrix Diffusion Toolkit. Environmental Security Technology Certification Program, Arlington, Virginia.

Harbaugh, A.W., Banta, E.R., Hill, M.C. and McDonald, M.G., 2000. MODFLOW-2000, The U. S. Geological Survey Modular Ground-Water Model-User Guide to Modularization Concepts and the Ground-Water Flow Process. Open-file Report. U. S. Geological Survey, (92), p.134.

Hiortdahl, K.M. and Borden, R.C., 2014. Enhanced reductive dechlorination of tetrachloroethene dense nonaqueous phase liquid with EVO and $Mg(OH)_2$. Environmental science & technology, 48(1), pp.624-631.

Long, C.M. and Borden, R.C., 2006. Enhanced reductive dechlorination in columns treated with edible oil emulsion. Journal of contaminant hydrology, 87(1), pp.54-72.

McGuire, T.M., D.T. Adamson, M.S. Burcham, Philip B. Bedient, and C.J. Newell, 2016. Evaluation of Long-Term Performance and Sustained Treatment at Enhanced Anaerobic Bioremediation Sites, Groundwater Monitoring & Remediation, 36(2), pp. 32-44.

Sale, T.C., Zimbron, J.A. and Dandy, D.S., 2008. Effects of reduced contaminant loading on downgradient water quality in an idealized two-layer granular porous media. Journal of Contaminant Hydrology, 102(1), pp.72-85.

Scott, T.M., Campbell, K.M., Rupert, F.R., Arthur, J.D., Missimer, T.M., Lloyd, J.M., Yon, J.W. and Duncan, J.G., 2001. Geologic map of the State of Florida. Florida Geological Survey.

Solutions-IES, 2013. Construction Completion Report - In Situ Bioremediation of Chlorinated Solvents in Groundwater at SA-17, Former Naval Training Center Orlando, Orlando, Florida. Naval Facilities Engineering Command Southeast

Solutions-IES, 2013b. 14-Month Post-Injection Performance Monitoring Report - In Situ Bioremediation of Chlorinated Solvents in Groundwater at SA-17, Former Naval Training Center Orlando, Orlando, Florida. Naval Facilities Engineering Command Southeast.

Tetra Tech (Tetra Tech NUS), 2001. Remedial Investigation Report for Operable Unit 2, McCoy Annex Landfill, Naval Training Center, Orlando, Florida (Rev. 2). Naval Facilities Engineering Command Southern Division.

Tetra Tech (Tetra Tech NUS), 2003. Final Feasibility Study Report, Operable Unit 2, McCoy Annex Landfill Naval Training Center, Orlando, Florida (Rev. 1). Naval Facilities Engineering Command Southern Division.

Tillotson, J.M. and Borden, R.C., 2017. Rate and Extent of Chlorinated Ethene Removal at 37 ERD Sites. Journal of Environmental Engineering, 143(8), p.04017028.

Weispfenning, A.M. and Borden, R.C., 2008. A design tool for planning emulsified oil-injection systems. Remediation Journal, 18(4), pp.33-47.

APPENDIX D ESTIMATING BASE REQUIREMENT

Enhanced reductive dechlorination (ERD) is commonly used to treat chlorinated solvents and related contaminants in groundwater by providing a fermentable organic substrate as electron donor and carbon source to stimulate microbially mediated reductive dechlorination (AFCEE, NFESC, and ESTCP 2004; ITRC, 2008; Stroo et al., 2014). During ERD, chlorine atoms are replaced with hydrogen, releasing hydrochloric acid (HCl) (Vogel and McCarty, 1985; Mohn and Tiedje, 1992). However, dechlorinating bacteria appear to be particularly sensitive to pH with dechlorination of *c*DCE and VC to ethene inhibited below a pH of 6 (Rowlands, 2004; Vainberg et al., 2006; Eaddy, 2008) resulting in a significant decline in degradation rates (Duhamel et al., 2002; McCarty et al., 2007). In this Appendix, the various factors controlling aquifer pH are reviewed and a simplified approach is presented for estimating the amount of base required to raise the aquifer pH to an appropriate level and maintain it at that level for the duration of active treatment. Our approach requires simplification of several important processes controlling subsurface pH, and is only appropriate for developing initial estimates of the amount of base required. In practice, aquifer pH should be monitored and periodically adjusted to maintain conditions for optimal microbial growth and contaminant degradation.

D.1. Effect of pH on Microbial Processes

Many biological processes are sensitive to pH. Most microorganisms function most efficiently in near neutral conditions (Lowe et al., 1993), low pH can interfere with pH homeostasis or increase the solubility of toxic metals (Slonczewski, 2009). Microorganisms can expend cellular energy to maintain homeostasis or cytoplasmic conditions may change in response to external changes in pH (Foster, 1999). Some anaerobes have adapted to low pH conditions through alterations in carbon and electron flow, cellular morphology, membrane structure, and protein synthesis (Lowe et al., 1993).

Changes in pH have a major influence on the transport and toxicity of major soil components and heavy metals. Aluminum is a major component of soil and the dissolved form is toxic to bacteria (Piña and Cervantes, 1996). However, aluminum is not typically toxic at neutral pH due to its low solubility and sorption to mineral surfaces. Many heavy metals (Cd, Cu, Hg, Ni, Pb, Zn) are more soluble at low pH and are released to solution as dissolved cations. These metals can be strongly retained by surface complexation on Fe and Al oxyhydroxides (Kent et al., 2000; 2007; Parkhurst et al., 2003). However, the surface charge of many hydroxide minerals varies as a function of pH. At a pH below the Point of Zero Charge (PZC), the mineral surfaces have a net positive charge and cation sorption is weak. As the pH rises, the concentration of protons (H^+) on mineral surfaces declines and these surfaces take on a net negative charge, enhancing cation retention (Bradbury and Baeyans, 1997; Dixit and Hering, 2003). As a result, increasing aquifer pH can greatly reduce heavy metals mobility and toxicity in the subsurface (Bethke, 2006; Truex et al., 2011).

There is some evidence that dechlorinating bacteria may be particularly sensitive to changes in pH. Pure cultures and consortia of dechlorinating microorganisms show highest dechlorination rates at circumneutral pH (Yang, 2012). **Table D-1** shows the range and optimum pH for growth of microorganisms that reduce PCE to TCE and *c*DCE. Zhuang and Pavlostathis (1995) found that neutral pH was optimum for reductive dechlorination by a methanogenic mixed culture capable of dechlorinating PCE to VC. Vainberg et al. (2006) reported an optimum pH of 6.0-6.8 for dechlorination of PCE by the SDC-9™ bioaugmentation culture. Dechlorination of PCE and TCE to *c*DCE can occur at pH down to 5.5 (Vainberg et al., 2009).

Table D-1. Range and Optimum pH for Growth of Pure Cultures Reducing PCE (adapted from Damborský, 1999).

Organism	pH Range	pH Optimum	Reference
Dehalobacter restrictus PER- K23	6.5 to 8.0	6.8 to 7.6	Holliger et al., 1998
Dehalosprillium multivorans	5.5 to 8.0	7.0 to 7.5	Neumann et al., 1994; Scholz-Muramatsu et al., 1995; Yang, 2012
Desulfitobacterium dehalogenans JW/IU-DC-1	6.0 to 9.0	7.5	Utkin et al., 1994
Desulfitobacterium sp. PCE-1	7.2 to 7.8	7.2	Gerritse et al., 1996
Desulfitobacterium dichloroeliminans DCA1		7.2 to 7.8	Fogel et al., 2009
Desulfuromonus chloroethenica TT4B	6.5 to 7.4	7.4	Krumholz el al., 1996; Krumholz, 1997
Desulfomonile tiedjei DCB-1	6.5 to 7.8	6.8 to 7.0	DeWeerd et al., 1990
Geobacter lovleyi SZ		6.5 to 7.2	Sung et al., 2006

Multiple researchers have attempted to develop enrichment cultures capable of complete dechlorination of PCE and TCE to ethene at low pH. However, to date, these attempts have had only modest success.

Rowlands (2004) reported that the KB-1™ bioaugmentation culture has an optimal range of 6.0-8.3 and is completely inhibited below pH~5.0. Li (2012) found that rates of VC reduction to ethene by a KB-1 culture declined by a factor of two as the pH was reduced from 7 to 6. Ethene production rates declined slowly over time and multiple degradation cycles. The decline in ethene production rates was accompanied by a decline in *Dhc* numbers, increase in methanogens, and a shift in total electron equivalents from dechlorination towards methanogenesis. In methanol fed pH 6 cultures, ethene production rates slowly increased over hundreds of days, with one culture showing sustained VC dechlorination at pH 5.7.

Using a bioaugmentation culture enriched from Savannah River Site aquifer material, Eaddy (2008) found that dechlorination of PCE and TCE slowed at a pH of 6.0 with increased accumulation of *c*DCE and VC. At pH 5.5, reduction of *c*DCE to VC and VC to ethene was completely inhibited. In subsequent work, Jiang (2012) showed that an enrichment culture was capable of dechlorinating PCE to ethene and/or ethane at pH 5.5.

Yang (2012) found that various dechlorinating pure cultures and the BDI consortium showed the highest dechlorination rates at circumneutral pH. *Sulfurospirillum multivorans* was the only organisms identified that dechlorinated PCE to *c*DCE at pH 5.5. In subsequent work, Yang (2016) found that only one enrichment culture containing *Desulfovibrio, Sulfurospirillum,* and *Megasphaera* showed dechlorination of PCE to *c*DCE after repeated transfers at pH 5.5. Long-term exposure to low pH reduced dechlorination activity by *Dhc*, with strains carrying the *vcrA* gene least tolerant to low pH.

Overall, these results indicate that: (a) dechlorination rates are highest at circumneutral pH; (b) certain microorganisms can grow while dechlorinating PCE and TCE at pH values down to 5.5; (c) a low pH enrichment of the KB-1 culture remained active at pH 5.7 and continued to dechlorinate VC to ethene; and d) active enrichment cultures can continue to dechlorinate *c*DCE and VC to ethene during short term exposure to pH 5.5. However, at pH 5.5, the cultures gradually lose their ability to dechlorinate VC to ethene. In addition, several groups are continuing work on development of improved enrichment cultures that are effective at low pH.

D.2. Factors Influencing pH

Aquifer pH is a function of a variety of factors including natural buffering processes, initial aquifer pH, acids and bases produced during in situ remediation processes, and base or buffer addition.

D.2.1. Background Aquifer pH

In humid areas, leaching by rainfall combined with carbonic acid produced in the soil leaches out base cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) gradually acidifying the soil. **Figure D-1** shows soil pH.

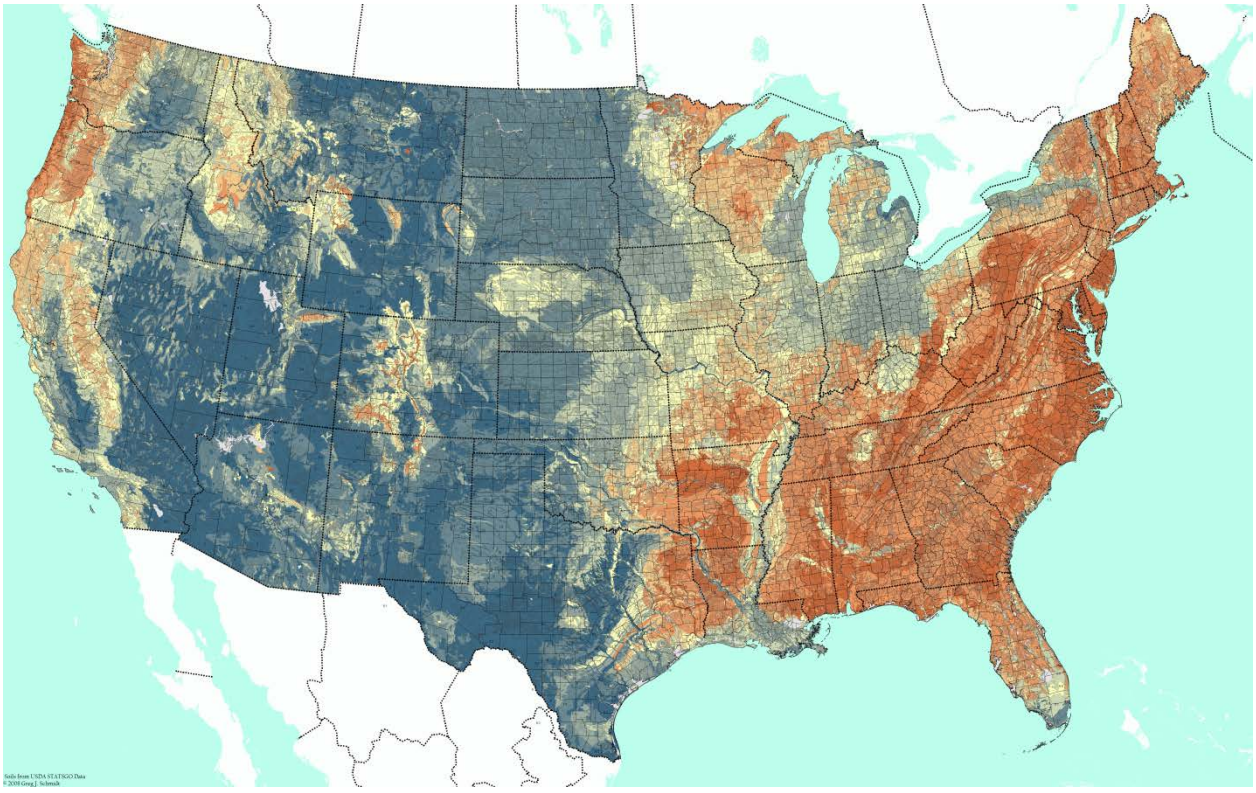

Figure D-1. Soil pH (http://www.bonap.org/2008_Soil/pH20110321.png).

Alkaline soils (pH>7.4) are shown in blue. Acidic soils (pH<6.1) are shown in brown.

Ground water pH is influenced by soil pH, but can vary somewhat. As acidic water infiltrates through the soil profile, some of the acidity may be neutralized by dissolution of soil and aquifer minerals. Silicate minerals including feldspars and micas can hydrolyze over time, consuming acid, and releasing dissolved cations (Na, K, Ca, Mg). However, these weathering reactions are slow and are often not sufficient to prevent pH declines. **Figure D-2** shows a cumulative frequency distribution of groundwater pH at an industrial site in eastern North Carolina. Average soil pH as this site is ~5. The vadose zone and surficial aquifer at the site are predominantly quartz sand and weathered clays, so weathering processes provide minimal buffering capacity. 90% of the groundwater pH measurements were between 4.5 and 6.0 indicating low soil pH at this site was a reasonable predictor of acidic groundwater. At other sites containing carbonate minerals or relatively young rocks, mineral dissolution often results in greater buffering.

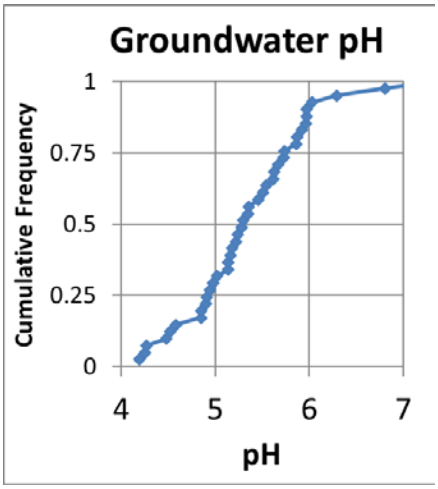

Figure D-2. Cumulative Frequency Distribution of Groundwater pH Measurements at an Industrial Site in Eastern North Carolina.

D.2.2. Aquifer Buffering Capacity

The resistance to pH change or aquifer buffering capacity is primarily due to two processes: (1) buffering by dissolved and solid carbonates; and (2) surface complexation and/or ion exchange reactions on mineral surfaces. While mineral weathering processes do influence long-term pH changes, weathering reactions are often too slow to prevent pH declines due to HCl and CO_2 production during ERD.

D.2.2.1. Buffering by Dissolved and Solid Carbonates

In natural aqueous systems, pH buffers are predominantly weak acid anions that easily bind and release hydrogen ions. The most common are the weak acid anions produced by dissolved CO_2 (Langmuir, 1997; Drever, 1997). When CO_2 dissolves in water, some CO_2 combines with H_2O forming carbonic acid (H_2CO_3). For convenience, the sum of dissolved CO_2 and H_2CO_3 is often written as H_2CO_3*. H_2CO_3*can then disassociate releasing bicarbonate ion (HCO_3^-) and one H^+ or carbonate ion (CO_3^{2-}) and two H^+ by the following reactions.

$$H_2CO_3^* \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2\ H^+ + CO_3^{2-}$$

Figure D-3 shows the relative distribution of these solutes as a function of pH. The reactions are reversible so that (a) an influx of acid will cause the HCO_3^- and CO_3^{2-} ions to protonate, consuming the acid, or (b) an influx of base will cause dissociation (deprotonation) of H_2CO_3* and the bicarbonate ion to consume the base. Maximum resistance to pH change (buffering capacity) occurs when pH equals the dissociation constants of carbonic acid (pH = 6.3 @ 25°C) or bicarbonate ion (pH = 10.3 @ 25°C). Near the water table, CO_2 can degas, essentially stripping acid out of the groundwater.

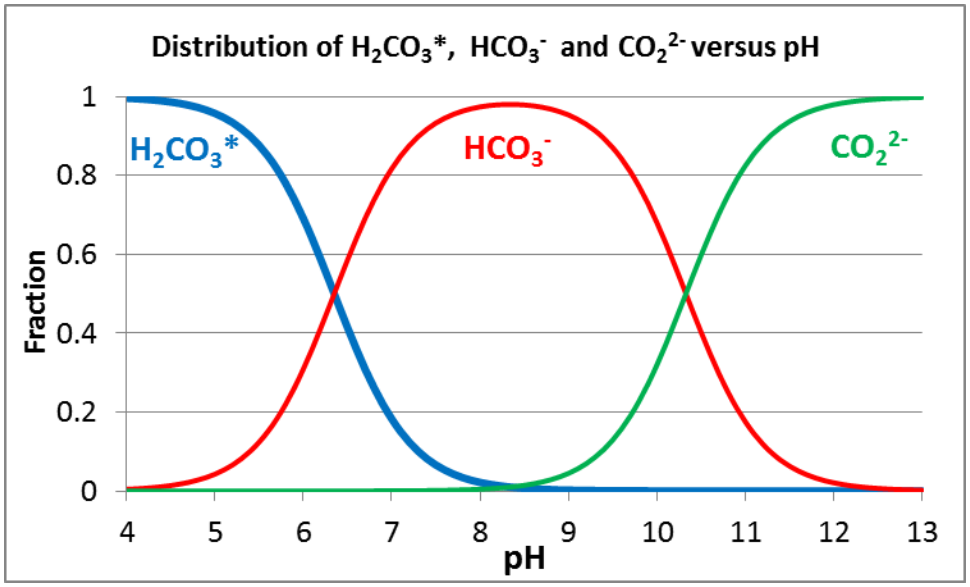

Figure D-3. Distribution of H_2CO_3*, HCO_3^- and CO_3^{2-} as a Function of pH.

For a system open to a large reservoir of carbon dioxide, the buffering capacity (assuming no soluble minerals are present) is a function of the partial pressure of carbon dioxide in the reservoir. In a closed system, the buffering capacity is a function of the total dissolved carbonate (H_2CO_3*, HCO_3^-, and CO_3^{-2}). The buffering capacity of groundwater is measured with an alkalinity titration (USGS, 2015; APWA et al., 2016). The units of alkalinity are often reported as mg $CaCO_3$/L (milligrams of $CaCO_3$per liter):

$$\text{Alkalinity (mg } CaCO_3\text{/L)} = \text{Alkalinity (milliequivalents/L)} \times 100$$

When solid carbonate minerals are present ($CaCO_3$, $MgCO_3$, $CaMg(CO_3)_2$, $FeCO_3$), carbonate mineral dissolution can limit pH declines caused by strong acids (e.g. HCl). For example, in an aquifer containing solid $CaCO_3$, the ambient groundwater is typically saturated with $CaCO_3$(s) and the following reaction is at equilibrium.

$$CaCO_3(s) \leftrightarrow Ca^{2+} + CO_3^{2-}$$

When HCl is produced, groundwater pH declines and the concentration of dissolved CO_3^{2-} declines with a proportionate increase in HCO_3^- and/or H_2CO_3* (see **Figure D-3**). The groundwater is now under-saturated and $CaCO_3$ will dissolve consuming H^+ by the following reaction.

$$CaCO_3(s) + H^+ \rightarrow Ca^{2+} + CO_3^{2-} + H^+ \rightarrow Ca^{2+} + HCO_3^-$$

However, the solubility of carbonate minerals is relatively low, so solid carbonates are much less effective in limiting pH declines due to CO_2 production in the saturated zone. Below the water table, CO_2 may not degas, causing a buildup of HCO_3^- and CO_3^{2-}. Geochemical modeling by Robinson et al. (2009) indicates that the aquifer can become supersaturated with $CaCO_3$ during ERD causing carbonate dissolution to stop. As additional CO_2 is produced, $CaCO_3(s)$ can precipitate with accumulation of H^+ by the following reaction

$$H_2CO_3^* + Ca^{2+} + CO_3^{2-} \rightarrow 2H^+ + Ca^{2+} + 2CO_3^{2-} \rightarrow H^+ + HCO_3^- + CaCO_3(s)$$

D.2.2.2. Surface Complexation and Ion Exchange Reactions

H^+ sorption to Fe and Al oxyhydroxides and clay minerals through surface complexation and ion exchange reactions can have a major impact on pH. H^+ sorbs strongly to some surfaces, absorbing large amounts of H^+ as the solution pH declines and releasing the H^+ back to solution as the pH rises (Davis and Kent, 1990; Davis et al., 1998). This strong buffer can reduce the pH decline in many systems, but can also greatly increase the amount of base required to increase aquifer pH.

Figure D-4 illustrates how mineral surfaces can reversibly bind and release hydrogen ions as pH changes. Broken bonds at mineral surfaces leave oxygens that are not fully bonded and act as weak acid anions that adsorb hydrogen ions (and other cations). Acidity produced during ERD can be neutralized by adsorbing H^+ on negatively charged surface sites and OH^- added to increase pH can be neutralized by adsorbing to positively charged sites. The PZC is the pH value at which the net surface charge density is zero. The buffering capacity for acid or base depends on how far the pH of the system is above or below the PZC. The PZC is different for each mineral and lists are available from many sources (Drever 1997).

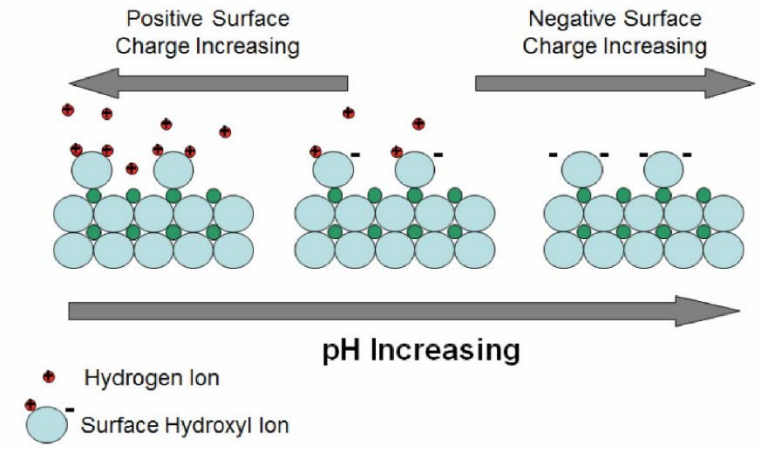

Figure D-4. Diagram of Mineral Surface Exchanging Hydrogen Ions with Varying pH (ITRC, 2010).

D.2.2.3. Estimating Aquifer Buffering Capacity

When carbonate minerals are present, geochemical models (Robinson et al. 2009) are needed to determine the amount of buffering provided by carbonate mineral dissolution. However, in many naturally low pH aquifers, carbonate minerals are absent and the extent of buffering can be estimated by adding a strong base to the aquifer solids, equilibrating for several days, and measuring pH. These buffering curves are typically linear in the pH range of 4.5 to 6.5 (Magdoff and Bartlett, 1985; Liu et al., 2005). The procedure first used in this project involved adding varying amounts of base (0.02N NaOH) and DI water (total fluid volume = 16 mL) to homogenized, dried 5 g samples of aquifer material, equilibrating for 4 days, and then measuring pH. **Figure D-5** shows the measured pH versus meq of OH^- added per Kg dry aquifer material.

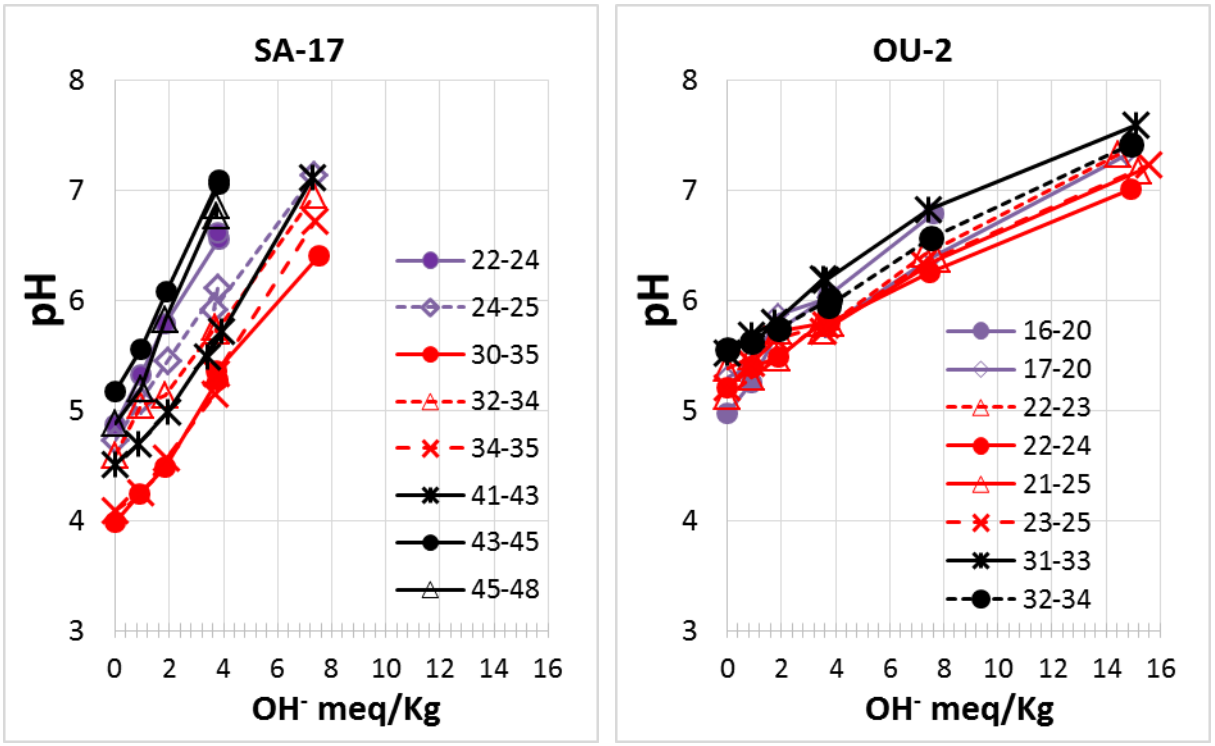

Figure D-5. Buffering Capacity of Aquifer Solids from SA-17 and OU-2 Measured in DI Water.

As seen in **Figure D-5**, incremental base addition results in a nearly linear increase in pH. The slope of these curves was estimated by linear regression. pH buffer capacity (pHBC) was calculated as the inverse of the slope and was reported as meq/Kg per pH unit. **Figure D-6** shows cumulative frequency distributions for pHBC in 6 different contaminated aquifers in the eastern U.S. Within a single unit, pHBC values are reasonably constant, with the middle 50% of measurements varying by a factor of two. However, median pHBC values can vary by a factor of ten between sites and between sandy vs clayey units on the same site. At NTC Orlando, pHBC values were consistently higher at OU-2 than SA-17.

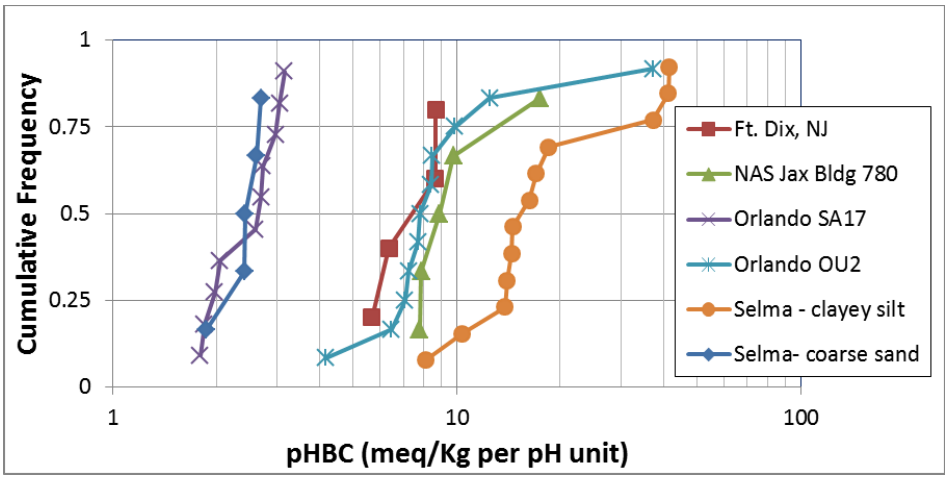

Figure D-6. Buffering Capacity of Aquifer Solids from Multiple Sites Measured in DI Water.

Over the course of this project, we became aware of a problem with the pHBC data was being collected. When running the pHBC measurements in DI water, the pH measurements were often inconsistent. Aitken and Moody (1994) recommend carrying out the titrations in a uniform ionic strength solution, most commonly $CaCl_2$. To determine if this change would significantly influence the measured pHBC values, we ran duplicate measurements of pHBC for samples from OU-2, SA-17 and Selma – clay in DI water and 0.01 M $CaCl_2$. Overall, results were similar (**Figure D-7**). However, the initial pH measurements were about 0.5 pH units lower and pHBC was about 10% higher in the $CaCl_2$ solution. We recommend that a 0.01 M $CaCl_2$ solution be used in all future work.

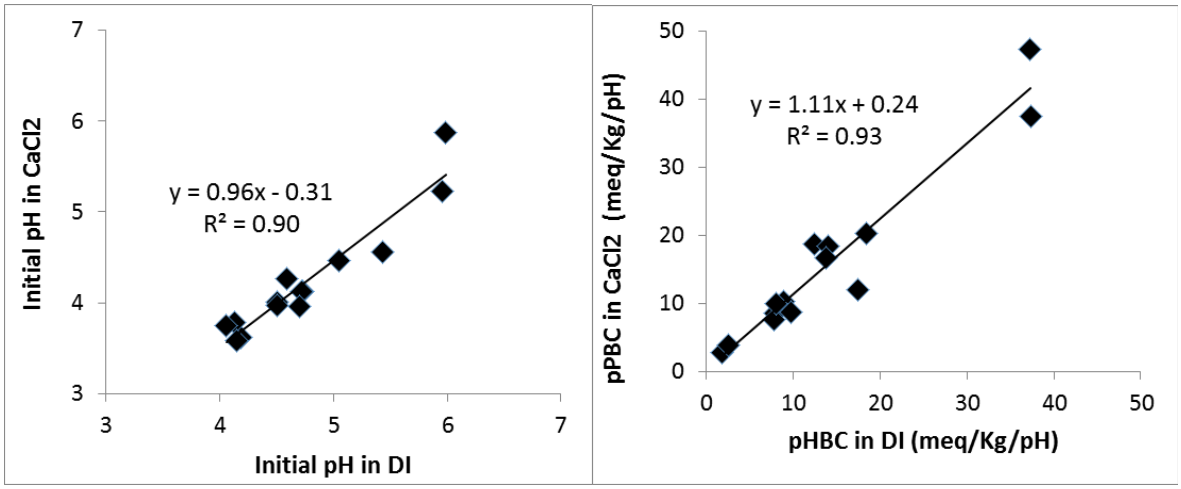

Figure D-7. Comparison of Initial pH and pHBC Measurements in DI Water and 0.01 M $CaCl_2$.

D.2.3. Acid and Base Production

There are a number of different processes that can produce or consume acidity during ERD including redox reaction, precipitation, and hydrolysis. **Table D-2** shows the amount of H^+ released from important redox reactions.

During reductive dechlorination of PCE (C_2Cl_4) to TCE (C_2HCl_3), a chlorine atom (Cl) is replaced with hydrogen (H_2) releasing one proton (H^+) and one chloride (Cl^-).

$$C_2Cl_4 + H_2 \rightarrow C_2HCl_3 + H^+ + Cl^-$$

As TCE is further dechlorinated to *c*DCE, VC and ethene, an additional proton is released for each chlorine removed. As a result, ERD can release large amounts of acid. For example, complete reduction of one kilogram of PCE to ethene produces 0.9 kilograms of HCl.

Organic substrates added as electron donors ferment releasing H_2 and acetic acid. Acetic acid rarely accumulates during ERD, because it can be used by some organisms for reduction of PCE and TCE, for reduction of background electron acceptors, or fermented to methane (CH_4). Consumption of acetic acid by all of these processes produces H_2CO_3*. The amount of H_2CO_3* produced during fermentation of different substrates can be estimated with the following formula,

$$C_\alpha H_\beta O_\gamma + (3\alpha - \gamma)\, H_2O \rightarrow \alpha\, H_2CO_3^* + (2\alpha + \beta/2 - \gamma)\, H_2$$

where α is the number of carbon atoms per mole of substrate, β is the number of hydrogen atoms per mole of substrate, and γ is the number of oxygen atoms per mole of substrate.

As shown in **Figure D-3**, CO_3^{2-} is only present in significant concentrations at pH > 8. For pH ranges relevant for ERD (5>pH>8), H^+ release from H_2CO_3* can be represented by the following reaction.

$$H_2CO_3^* \rightarrow \alpha H_2CO_3^* + (1-\alpha)H^+ + (1-\alpha)HCO_3^-$$

where $\alpha = (1 + 10^{-6.352}/[H^+])^{-1}$. **Figure D-8** shows the variation in α as a function of pH. At pH 6, α = 0.69, so 0.31 moles of H^+ are released for every mole of CO_2 produced. However at pH 7, α = 0.18, so 0.82 moles of H^+ are released for every mole of CO_2 produced.

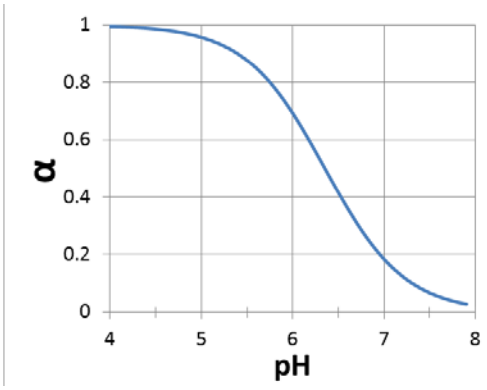

Figure D-8. Variation in Alpha (α) with pH.

Reduction of some electron acceptors produces OH^-, counter-acting acid produced from dechlorination and CO_2 production. Representative reactions for reduction of nitrate (NO_3^-), iron oxides, sulfate (SO_4^{2-}) and bicarbonate (HCO_3^-) are shown in **Table D-2**. Goethite [FeO(OH)] is used as a typical iron oxide for these calculations.

Table D-2. Net Acid Production from Important Redox Reactions.

e^- Acceptor	e^- Donor	Product	Reaction	H^+ Produced
PCE	H_2	TCE	$C_2Cl_4 + H_2 \rightarrow C_2H_3Cl_3 + H^+ + Cl^-$	1
TCE	H_2	*c*DCE	$C_2HCl_3 + H_2 \rightarrow C_2H_2Cl_2 + H^+ + Cl^-$	1
DCE	H_2	VC	$C_2H_2Cl_2 + H_2 \rightarrow C_2H_3Cl + H^+ + Cl^-$	1
VC	H_2	Ethene	$C_2H_3Cl + H_2 \rightarrow C_2H_4 + H^+ + Cl^-$	1
H_2O	Acetic Acid	H_2, HCO_3^-	$C_2H_4O_2 + 4\ H_2O \rightarrow 2\ H_2CO_3^* + 4\ H_2$	2(1-α)
H_2O	Lactic Acid	H_2, HCO_3^-	$C_3H_6O_3 + 3\ H_2O \rightarrow 3\ H_2CO_3^* + 6\ H_2$	3(1-α)
H_2O	Glucose	H_2, HCO_3^-	$C_6H_{12}O_6 + 12\ H_2O \rightarrow 6\ H_2CO_3^* + 12\ H_2$	6(1-α)
H_2O	Soybean Oil	H_2, HCO_3^-	$C_{56}H_{100}O_6 + 162 H_2O \rightarrow 56 H_2CO_3^* + 156 H_2$	56(1-α)
Oxygen	H_2		$O_2 + 2\ H_2 \rightarrow 2\ H_2O$	0
Nitrate	H_2	N_2	$NO_3^- + 2½\ H_2 \rightarrow 2\ H_2O + ½\ N_2 + OH^-$	-1
Goethite	H_2	Fe^{2+}	$FeO(OH) + ½\ H_2 \rightarrow Fe^{2+} + 2\ OH^-$	-2
Sulfate	H_2	HS^-	$SO_4^{2-} + 4\ H_2 + Fe^{2+} \rightarrow FeS + 4\ H_2O$	0
$H_2CO_3^*$	H_2	CH_4	$H_2CO_3^* + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$	α-1

D.3. Base Addition

A variety of different bases have been used to raise the aquifer pH to stimulate ERD including soluble and solid carbonates, soluble and solid hydroxides, phosphates and silicate minerals. The carbonates and hydroxides are used most commonly because of the relatively low cost and easy availability. **Table D-3** summarizes the physical properties of common basic salts used to raise pH.

Table D-3. Physical properties of common basic salts used for pH control.

Base	Formula	Mol. Weight (g/mole)	OH^- per mole	OH^- per Kg	Solubility g/L	Saturated solution pH
Caustic Soda	NaOH	40.0	1	25.0	1,100	>14
Caustic Potash	KOH	56.1	1	17.8	1,200	>14
Soda Ash	Na_2CO_3	106	1+ α	11.2	300	~11.7
Baking Soda	$NaHCO_3$	84	α	2.2	78	~8.3
Hydrated Lime	$Ca(OH)_2$	74.1	2	27.0	1.85	~12.4
Magnesium Hydroxide	$Mg(OH)_2$	58.3	2	34.3	<0.01	~10.3

NaOH and KOH provide a large number of OH^- eq per Kg and are very soluble so only small volumes of base are required to raise the aquifer pH. However, concentrated solutions of NaOH and KOH have pH > 14 which is inhibitory to bacteria, would expose workers to safety hazards, and can partially dissolve aluminosilicates.

As described above, solid carbonate minerals are often not effective in raising pH during ERD because of their low aqueous solubility. Na_2CO_3 and $NaHCO_3$ are much more soluble and can be effective in raising aquifer pH. The amount of H^+ consumed per mole varies as a function of pH between pH 5 and 8 (Stumm and Morgan, 1970). For closed conditions (below water table where CO_2 cannot degas) and pH < 8, $NaHCO_3$ and Na_2CO_3 disassociate to $H_2CO_3^*$ and HCO_3^-, consuming H^+ by the following reactions.

$$NaHCO_3 + \alpha H^+ \rightarrow Na^+ + \alpha H_2CO_3^* + (1-\alpha)HCO_3^-$$

$$Na_2CO_3 + (1+\alpha)H^+ \rightarrow 2Na^+ + \alpha H_2CO_3^* + (1-\alpha)HCO_3^-$$

At pH = 6, $\alpha = 0.69$ so 0.69 moles of H^+ are consumed per mole of $NaHCO_3$ and 1.69 moles of H^+ consumed per mole of Na_2CO_3 (**Figure D-8**). However at pH = 7, $\alpha = 0.18$ so only 0.18 moles of H^+ are consumed per mole of $NaHCO_3$ and 1.18 moles of H^+ consumed per mole of Na_2CO_3. As a result, bicarbonates and carbonates are relatively effective at raising the pH to 6. However these materials provide less alkalinity per unit mass at pH =7, increasing the amount of material required.

$Ca(OH)_2$ and $Mg(OH)_2$ provide large amounts of OH^- per Kg. However, these materials have a low aqueous solubility, make them more difficult to distribute in the subsurface. Borden et al. (2016) describe the use of a colloidal form of $Mg(OH)_2$ with improved transport properties.

D.4. Proposed Approach

An MS Excel based design tool was developed to aid in estimating the amount of base required to maintain a neutral pH during ERD. The design tool approach and calculations were presented in the previous sections. The design tool is specifically focused on pH adjustment for ERD and calculation procedures include the following assumptions.

1. The pH is between 5 and 8.
2. The carbonate system is the primary aqueous pH buffer.
3. Any H_2 or acetate produced by substrate fermentation that is not consumed through reduction of chlorinated solvents and background electron acceptors (O_2, NO_3, iron oxides, and SO_4) is consumed by methanogenesis.

Design tool users must enter the following information.

1. Treatment zone dimensions and design period for this phase of remediation.
2. Site characteristics including average K, porosity, hydraulic gradient, contaminant concentrations in aquifer material and groundwater, and amount of electron acceptors produced or consumed (i.e. O_2, NO_3, Fe, SO_4, CH_4).

3. Background pH, total inorganic carbon, mineral acidity, and pH buffering capacity (pHBC). A database of pHBC measurements is provided to aid users in selecting design values when laboratory measurements are not available.
4. Mass of organic substrate and base to be injected.
5. When vegetable oil is used as a substrate, the fraction of injected oil that is consumed during the design period.
6. Target pH

The design tool calculates the amount of base required to: a) raise the pH of the aquifer material and influent groundwater, and b) neutralize acidity produced during reductive dechlorination and substrate fermentation. The planned amount of base added is compared to the calculated requirement. Users should be aware that results are very sensitive to the target pH since the number of H^+ released from H_2CO_3* and consumed by $NaHCO_3$ and Na_2CO_3 is a function of pH.

D.5. References

AFCEE, NFESC, and ESTCP., 2004. Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents. Air Force Center for Engineering and the Environment, Naval Facilities Engineering Service Center, and Environmental Security Technology Certification Program.

Aitken, R.L. and Moody, P.W., 1994. The effect of valence and ionic-strength on the measurement of pH buffer capacity. Soil Research, 32(5), pp.975-984.

APWA, AWWA, and WEF (American Public Health Association, American Water Works Association and Water Environment Federation), 2016. Standard Method 2320 Alkalinity, Standard methods for the examination of water and wastewater.

Bethke, C.M., 2006. In situ treatment process to remove metal contamination from groundwater. U.S. Patent 7141173.

Borden, R.C., Lai, Y.S., Overmeyer, J., Yuncu, B. and Allen, J.P., 2016. In situ pH adjustment with colloidal $Mg(OH)_2$. Environmental Engineer and Scientist: Applied Research and Practice, accepted for publication, Vol 17, pp.28-33.

Bradbury, M.H. and Baeyens, B., 1997. A mechanistic description of Ni and Zn sorption on Na-montmorillonite Part II: modelling. Journal of Contaminant Hydrology, 27(3-4), pp.223-248.

Damborský, J., 1999. Tetrachloroethene-dehalogenating bacteria. Folia microbiologica, 44(3), pp.247-262.

Davis, J.A., Coston, J.A., Kent, D.B. and Fuller, C.C., 1998. Application of the surface complexation concept to complex mineral assemblages. Environmental Science & Technology, 32(19), pp.2820-2828.

Davis, J.A. and Kent, D.B., 1990. Surface complexation modeling in aqueous geochemistry. Reviews in Mineralogy and Geochemistry, 23(1), pp.177-260.

DeWeerd, K.A., Mandelco, L., Tanner, R.S., Woese, C.R. and Suflita, J.M., 1990. Desulfomonile tiedjei gen. nov. and sp. nov., a novel anaerobic, dehalogenating, sulfate-reducing bacterium. Archives of Microbiology, 154(1), pp.23-30.

Dixit, S. and Hering, J.G., 2003. Comparison of arsenic (V) and arsenic (III) sorption onto iron oxide minerals: implications for arsenic mobility. Environmental science & technology, 37(18), pp.4182-4189.

Drever, J.I., 1997. The Geochemistry of Natural Waters: Surface and Groundwater Environments. Prentice-Hall, Inc.

Duhamel, M., Wehr, S.D., Yu, L., Rizvi, H., Seepersad, D., Dworatzek, S., Cox, E.E. and Edwards, E.A., 2002. Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, cis-dichloroethene and vinyl chloride. Water Research, *36*(17), pp.4193-4202.

Eaddy, A., 2008. Scale-Up and Characterization of an Enrichment Culture for Bioaugmentation of the P-Area Chlorinated Ethene Plume at the Savannah River Site. M.S. Thesis, Clemson University, Clemson, SC, USA.

Fogel, S., Findlay, M., Folsom, S. and Kozar, M., 2009. The importance of pH in reductive dechlorination of chlorinated solvents. In Proceedings Tenth International In Situ and On-Site Bioremediation Symposium, Baltimore, MD, USA, May (pp. 5-8).

Foster, J.W., 1999. When protons attack: microbial strategies of acid adaptation. Current opinion in microbiology, 2(2), pp.170-174.

Gerritse, J., Renard, V., Gomes, T.P., Lawson, P.A., Collins, M.D. and Gottschal, J.C., 1996. Desulfitobacterium sp. strain PCE1, an anaerobic bacterium that can grow by reductive dechlorination of tetrachloroethene or ortho-chlorinated phenols. Archives of microbiology, 165(2), pp.132-140.

Holliger, C., Hahn, D., Harmsen, H., Ludwig, W., Schumacher, W., Tindall, B., Vazquez, F., Weiss, N. and Zehnder, A.J., 1998. Dehalobacter restrictus gen. nov. and sp. nov., a strictly anaerobic bacterium that reductively dechlorinates tetra-and trichloroethene in an anaerobic respiration. Archives of Microbiology, 169(4), pp.313-321.

ITRC, 2008. Enhanced Attenuation: Chlorinated Solvents, Technical and Regulatory Guidance, Interstate Technology and Regulatory Council.

ITRC, 2010. A Decision Framework for Applying Monitored Natural Attenuation Processes to Metals and Radionuclides, Interstate Technology and Regulatory Council, Technical/Regulatory Guidance AMPR-1.

Jiang, C., 2012. Development of Enrichment Cultures for Anaerobic Reductive Dechlorination of Tetrachloroethene Under Low pH Conditions. M.S. Thesis Clemson University, Clemson, SC, USA.

Kent, D.B., Abrams, R.H., Davis, J.A., Coston, J.A. and LeBlanc, D.R., 2000. Modeling the influence of variable pH on the transport of zinc in a contaminated aquifer using semiempirical surface complexation models. Water resources research, 36(12), pp.3411-3425.

Kent, D.B., Wilkie, J.A. and Davis, J.A., 2007. Modeling the movement of a pH perturbation and its impact on adsorbed zinc and phosphate in a wastewater‐contaminated aquifer. Water Resources Research, 43(7).

Krumholz, L.R., Sharp, R. and Fishbain, S.S., 1996. A freshwater anaerobe coupling acetate oxidation to tetrachloroethylene dehalogenation. Applied and Environmental Microbiology, 62(11), pp.4108-4113.

Krumholz, L.R., 1997. Desulfuromonas chloroethenica sp. nov. uses tetrachloroethylene and trichloroethylene as electron acceptors. International Journal of Systematic and Evolutionary Microbiology, 47(4), pp.1262-1263.

Langmuir, D., 1997. Aqueous Environmental Geochemistry. Prentice-Hall, Inc. Upper Saddle River, NJ.

Li, J.J., 2012. Adaptation of a Dechlorinating Culture, KB-1, to Acidic Environments. M.S. Thesis, University of Toronto, Toronto, ON, Canada.

Liu, M., Kissel, D.E., Cabrera, M.L. and Vendrell, P.F., 2005. Soil lime requirement by direct titration with a single addition of calcium hydroxide. Soil Science Society of America Journal, 69(2), pp.522-530.

Lowe, S.E., Jain, M.K. and Zeikus, J.G., 1993. Biology, ecology, and biotechnological applications of anaerobic bacteria adapted to environmental stresses in temperature, pH, salinity, or substrates. Microbiological reviews, 57(2), pp.451-509.

Magdoff, F.R. and Bartlett, R.J., 1985. Soil pH buffering revisited. Soil Science Society of America Journal, 49(1), pp.145-148.

McCarty, P.L., Chu, M.Y. and Kitanidis, P.K., 2007. Electron donor and pH relationships for biologically enhanced dissolution of chlorinated solvent DNAPL in groundwater. European Journal of Soil Biology, 43(5), pp.276-282.

Mohn, W.W. and Tiedje, J.M., 1992. Microbial reductive dehalogenation. Microbiological reviews, 56(3), pp.482-507.

Neumann, A., Scholz-Muramatsu, H. and Diekert, G., 1994. Tetrachloroethene metabolism of Dehalospirillum multivorans. Archives of Microbiology, 162(4), pp.295-301.

Parkhurst, D.L., Stollenwerk, K.G. and Colman, J.A., 2003. Reactive-transport simulation of phosphorus in the sewage plume at the Massachusetts Military Reservation, Cape Cod, Massachusetts. US Department of the Interior, US Geological Survey.

Piña, R.G. and Cervantes, C., 1996. Microbial interactions with aluminium. Biometals, 9(3), pp.311-316.

Robinson, C., Barry, D.A., McCarty, P.L., Gerhard, J.I. and Kouznetsova, I., 2009. pH control for enhanced reductive bioremediation of chlorinated solvent source zones. Science of the total environment, 407(16), pp.4560-4573.

Rowlands, D., 2004. Development of optimal pH for degradation of chlorinated solvents by the KB-1™ anaerobic bacterial culture. M.S. Thesis, University of Guelph, Guelph, ON, Canada.

Scholz-Muramatsu, H., Neumann, A., Meßmer, M., Moore, E. and Diekert, G., 1995. Isolation and characterization of Dehalospirillum multivorans gen. nov., sp. nov., a tetrachloroethene-utilizing, strictly anaerobic bacterium. Archives of Microbiology, 163(1), pp.48-56.

Slonczewski, J.L., 2009 Stress Responses: pH, In Encyclopedia of Microbiology (3rd Ed.), Editor-in-Chief: Schaechter, M., Academic Press: Oxford Vol 5. pp. 477-484.

Stroo, H.F., West, M.R., Kueper, B.H., Borden, R.C., Major, D.W. and Ward, C.H., 2014. In situ bioremediation of chlorinated ethene source zones. In Chlorinated solvent source zone remediation (pp. 395-457). Springer New York.

Stumm, W. and Morgan, J.J., 1970. Aquatic chemistry; an introduction emphasizing chemical equilibria in natural waters.

Sung, Y., Fletcher, K.E., Ritalahti, K.M., Apkarian, R.P., Ramos-Hernández, N., Sanford, R.A., Mesbah, N.M. and Löffler, F.E., 2006. Geobacter lovleyi sp. nov. strain SZ, a novel metal-reducing and tetrachloroethene-dechlorinating bacterium. Applied and Environmental Microbiology, 72(4), pp.2775-2782.

Truex, M., Brady, P., Newell, C., Rysz, M., Denham, M. and Vangelas, K., 2011. The Scenarios Approach to Attenuation-Based Remedies for Inorganic and Radionuclide Contaminants. Savannah River National Laboratory, SRNL-STI-2011-00459.

Utkin, I., Woese, C. and Wiegel, J., 1994. Isolation and characterization of Desulfitobacterium dehalogenans gen. nov., sp. nov., an anaerobic bacterium which reductively dechlorinates chlorophenolic compounds. International Journal of Systematic and Evolutionary Microbiology, 44(4), pp.612-619.

USGS (United States Geological Survey), 2015. National field manual for the collection of water-quality field data, Alkalinity and acid neutralizing capacity. US Geological Survey Techniques of Water-Resources Investigations, Book 9, Chap. A6., Section 6.6.

Vainberg, S., Steffan, R.J., Rogers, R., Ladaa, T., Pohlmann, D. and Leigh, D., 2006. Production and application of large-scale cultures for bioaugmentation. Proc 5th Internat Conf Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, USA. May 22-25. Paper No. A-50.

Vainberg, S., Condee, C.W. and Steffan, R.J., 2009. Large-scale production of bacterial consortia for remediation of chlorinated solvent-contaminated groundwater. Journal of Industrial Microbiology & Biotechnology, 36(9), pp.1189-1197.

Vogel, T.M. and McCARTY, P.L., 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. Applied and Environmental Microbiology, 49(5), pp.1080-1083.

Yang, Y., 2012. Exploring Anaerobic Reductive Dechlorination at Low pH Environments. M.S. Thesis, University of Tennessee, Knoxville, TN, USA.

Yang, Y., 2016. Bioremediation of Chlorinated Ethenes: pH Effects, Novel Dechlorinators and Decision-Making Tools. Ph.D. Dissertation, University of Tennessee, Knoxville, TN, USA.

Zhuang, P, Pavlostathis, S. G., 1995. Effect of temperature, pH and electron-donor on the microbial reductive dechlorination of chloroalkenes. Chemosphere, 31: 3537-3548.

APPENDIX E SOIL BUFFER CAPACITY MEASUREMENT

Objective: Determine soil pH and the equivalents of base required to raise the pH of aquifer material to stimulate anaerobic bioremediation.

Procedure:

1. **Sampling**: Collect soil cores in plastic sleeves from at least 3 locations in the general area of the proposed remediation system. If possible, collect the soil samples near existing monitor wells. At each boring location, collect one core from each major zone. Label cores, seal with packing tape and transport to lab.
2. **Homogenization and drying**: In lab fume hood, cut plastic sleeve open as needed to remove about 500 g of soil from 4 or more depths in the range of interest, and add to labeled 1 L plastic container until nearly full. Include sub-samples from any visually distinct layers. Mix the moist soil in the container to homogenize the sample. Collect triplicate subsamples of soil and analyze for moisture content. Transfer soil to plastic lined tray, spreading the sample evenly and breaking any clumps whenever possible. Leave open trays in fume hood for at least 3 days (stirring daily) to allow VOCs to volatilize. Return air dried samples to plastic containers and seal lids tightly. Retain for further analyses.
3. **Oven drying sub-sample**: Transfer approximately 100 g air dry soil to a clean ceramic bowl and dry at 105°C for at least 24 hours. Homogenize sample with a mortar and pestle, then place about 5 g dry soil in each of nine 20 mL vials. Record mass of each dry soil sample.
4. **Soil pH and Buffering Capacity**: Add 0.01 M CaCl2 solution (555 mg/L) to the 9 vials in the amounts of 16, 15.5, 15, 14 (2 vials), 12, 8, 4 and 0 mL. Then add 0.02N NaOH (800 mg/L) with 0.01 M CaCl2 to the vials in the amounts (taken in the same order) of 0, 0.5, 1, 2 (2 vials), 4, 8, 12 and 16 mL. Cap vials tightly and shake daily for at least 4 days. Allow solids to settle for one day. Do not shake. Measure and record pH of each vial, using gentle agitation to minimize re-suspension of soil particles, and avoiding contact of pH probe with settled soil layer of sample. If none of the vials have pH $\geq$ 8, repeat steps 4 & 5 with a stronger NaOH solution. Report soil pH (0 mL NaOH vial) and OH- milli-equivalents (meq) required per kilogram dry soil.
5. **Calculations and Reporting**: Prepare x-y graph with final pH on x-axis and OH- meq/Kg dry soil on y-axis. Calculate slope of line from initial pH up to pH=8 by linear regression. Slope of line = pH buffer capacity (pHBC) in units of OH- meq/Kg per pH unit.

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APPENDIX F CONCEPTUAL MODEL OF ERD TREATMENT WITH EVO

ERD with EVO and pH buffer can be effective for in situ treatment of chlorinated solvents and related contaminants. However, effective treatment requires that EVO and buffer be distributed throughout the target treatment zone to optimize microbial growth and contaminant degradation. The overall objective of ERD is to adjust biogeochemical conditions in the immediate vicinity of the contaminant to ensure the following:

a. Sufficient levels of fermentable organic substrates are present to support microbial growth and contaminant biodegradation.
b. The aquifer pH is appropriate for microbial growth and contaminant biodegradation.
c. Critical microorganisms are present in sufficient numbers with the required genetic capability to degrade the pollutants to non-toxic end products.

In this section, information generated in this project and prior research is integrated to provide a general conceptual model of the major processes controlling treatment performance of ERD with EVO.

F.1 ERD MICROBIOLOGY AND ORGANOHALIDE RESPIRATION

During ERD of chlorinated ethenes, PCE is reduced to TCE, which in turn is reduced to dichloroethene (primarily the *cis*-1,2 DCE isomer), which then is reduced to VC and finally to ethene (Vogel and McCarty, 1985; Mohn and Tiedje, 1992) (**Figure F-1**). This process is most efficient when microorganisms gain metabolic energy and grow through reduction of PCE and TCE to ethene through a process referred to as organohalide respiration (Löffler et al., 2013). Several different organisms capable of growing on the reduction of PCE to *c*DCE have been identified including *Dehalobacter restrictus* (Holliger et al., 1993; Holliger et al., 1998), *Desulfuromonas* (Krumholz et al., 1996; Sung et al., 2003), *Geobacter lovleyi* (Sung et al., 2006), *Sulfurospirillum multivorans* (Luitjen et al., 2003) and *Desulfitobacterium* (Maillard et al., 2005). Currently, the only bacteria known to grow on the reduction of DCE to VC to ethene are members of the genus *Dehalococcoides* (*Dhc*) (Maymó-Gatell et al., 2001; He et al., 2003; Löffler et al., 2013). However, not all *Dhc* strains can grow using VC as an electron acceptor. The first identified strain of *Dhc* (strain 195) grows with PCE, TCE, *cis*-DCE and 1,1-DCE as electron acceptors, but only slowly dechlorinates VC by a cometabolic process (Maymó-Gatell et al., 2001). More recent studies have identified other *Dhc* strains that rapidly reduce DCE to VC to ethene (He et al., 2003; Sung et al., 2006; Müller et al., 2004).

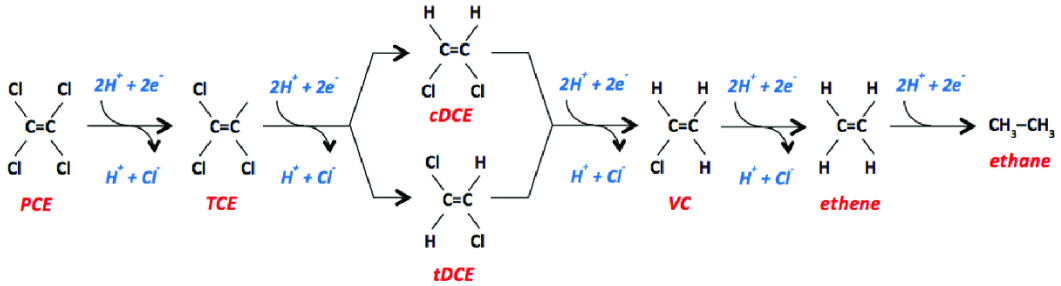

Figure F-1. Stepwise Reduction of PCE and TCE to ethene and ethane (courtesy of D. Freedman).

F.2 ENVIRONMENTAL REQUIREMENTS FOR EFFICIENT DECHLORINATION

A wide variety of organic substrates have been used as electron donors for ERD including soluble materials (lactate, benzoate, molasses, whey, glycerin) and lower solubility materials (vegetable oil, emulsified vegetable oils, Hydrogen Release Compound®, mulch, compost). Once introduced into the subsurface, these materials are used to consume background electron acceptors (oxygen, nitrate, manganese, iron, and sulfate) and are fermented to hydrogen (H_2) and acetate. PCE and TCE can be reduced to cDCE by a variety of microorganisms using H_2 and/or acetate as electron donors (Aulenta et al., 2006). However, *Dhc* requires H_2 as an electron donor and a reduced organic compound such as acetate as a carbon source for reduction of DCE to VC and ethene (Löffler et al., 2013).

Organohalide respiring bacteria are sensitive to changes in pH (**Appendix D**). Pure cultures and consortia of dechlorinating microorganisms show highest dechlorination rates at circumneutral pH (Yang, 2012). The KB-1™ bioaugmentation culture has an optimal range of 6.0-8.3 (Rowlands, 2004). VC reduction to ethene by a KB-1 culture declined by a factor of two as the pH was reduced 7 to 6 (Li, 2012). Using a bioaugmentation culture enriched from Savannah River Site aquifer material, Eaddy (2008) found that dechlorination of PCE and TCE slowed at a pH of 6.0 with increased accumulation of *c*DCE and VC. At pH 5.5, reduction of *c*DCE to VC and VC to ethene was completely inhibited. Yang (2012) found that various dechlorinating pure cultures and the BDI consortium showed highest dechlorination rates and extent at circumneutral pH. In subsequent work, Yang (2016) and Yang et al. (2017) found that only one enrichment culture containing *Desulfovibrio, Sulfurospirillum,* and *Megasphaera* showed dechlorination of PCE to *c*DCE after repeated transfers at pH 5.5. Long term exposure to low pH reduced dechlorination activity by *Dhc*, with strains carrying the *vcrA* gene least tolerant to low pH (Yang, 2016). Overall, these results indicate that: (a) dechlorination rates are highest at circumneutral pH; (b) certain microorganisms can grow while dechlorinating PCE and TCE at pH values down to 5.5; and (c) dechlorination of *c*DCE and VC is generally inhibited below pH of 6.0. However, several groups are working to develop improved enrichment cultures that can reduce VC below a pH of 6.0 (Li. 2012; Jiang, 2012; Yang, 2012; Yang, 2016).

F.3 EVO PROPERTIES, TRANSPORT AND RETENTION IN THE SUBSURFACE

EVO is most commonly purchased from a commercial supplier and shipped to the site as a concentrated emulsion containing 45 to 60% vegetable oil. Soybean oil is commonly used because of its availability, good handling characteristics, and relatively low cost. The oil provides a slow release organic substrate to support long-term anaerobic activity. The remainder of the EVO formulation consists of: (a) more readily fermentable soluble substrates (e.g. fatty acids or alcohols); (b) surfactants to reduce oil droplet interfacial tension, stabilize the emulsion and reduce oil droplet flocculation; and (c) water. The soluble substrates generate rapid, initial growth of the required bacteria. In some cases, additional nutrients are added to enhance growth of *Dhc* including nitrogen, phosphorus, yeast extract and vitamin B_{12}. Median oil droplet size is commonly in the range of 0.5 to 2.0 µm to improve emulsion stability during shipping and improve transport through typical aquifer materials. These small droplet emulsions are commonly prepared using high-pressure homogenizers.

Once injected, the oil droplets are transported through the aquifer pore spaces by flowing groundwater. Experimental and mathematical modeling studies by Soo and Radke (1984; 1986) and Soo et al. (1986) have shown that oil droplets larger than the sediment pores are rapidly removed by straining with a large, permanent permeability loss. The median pore size of sand aquifers is typically over 200 μm (Coulibaly and Borden, 2004) which is orders of magnitude greater than the oil droplet diameter (< 2 μm), so physical straining is not a significant retention mechanism.

Oil droplets are retained by aquifer material when they collide with sediment surfaces and stick (referred to as interception). Oil droplet retention by aquifer material can be described by deep-bed filtration theory (Ryan and Elimelech, 1996; Logan, 1999; Coulibaly et al., 2006) where droplet capture by the sediment surfaces is a function of: (1) the frequency that droplets collide with sediment surfaces; and (2) the collision efficiency which is the fraction of droplets colliding with the sediment surfaces that are actually retained (Westall and Gschwend, 1993; Ryan and Elimelech, 1996; Logan, 1999). The collision frequency between oil droplets and sediment surfaces depends on groundwater flow velocity (advection), Brownian motion (diffusion), and gravitational settling or floatation. Very small droplets vibrate rapidly due to Brownian motion, resulting in frequent collisions with particle surfaces and rapid removal. Large droplets float, colliding the roof of the sediment pores, increasing removal. For vegetable oil emulsions at typical groundwater velocities, the lowest collision frequency occurs at 0.5 to 2 μm (Borden, 2007b).

Collision efficiency varies due to a variety of factors including pH, droplet and sand grain surface coatings, ionic strength, double layer thickness, surface roughness, sediment surface charge heterogeneity, and blocking of the sediment surface with previously retained droplets (Bolster et al., 2001; Johnson and Elimelech, 1995; Rijnaarts et al., 1996a; 1996b). Oil droplets and sediment particles typically take on an electrical charge and are surrounded by a double layer of charged ions. When the both the oil droplets and sediment surfaces have a negative charge, the oil droplets tend to be repelled by the sediments reducing collision efficiency. When the oil droplets are negatively charged and the sediments are positive or neutral, the oil droplets are more likely to stick and be retained by the sediment.

As dilute emulsion containing millions of negatively charged oil droplets migrates through the aquifer pore spaces, they encounter some positively charged locations. If the oil droplet 'bumps into the sediment' at that location, the droplet will likely stick and fill up that site. Additional oil droplets will be repelled by the attached droplet and migrate further through the aquifer, gradually filling up the available attachment sites. In this way, the emulsion gradually saturates the available attachment sites and continues to migrate with the flowing groundwater. The maximum amount of oil that can be retained by an aquifer is a function of the oil droplet properties (diameter, surface charge), chemical characteristics of the sediment surface (presence of organic or iron oxide coatings), and surface area available for droplet attachment. Sediments with a high clay content are expected to have a higher maximum oil retention because of the greater surface area and number of sites available for oil droplet attachment.

A common measure of suspension or emulsion stability is zeta potential which is the potential difference between the bulk fluid and the stationary fluid layer attached to the particle surface.

Particles that have a highly negative (or highly positive) zeta potential will not flocculate. However, when zeta potential is close to zero, attractive forces may exceed this repulsion and the emulsion may break and flocculate. Typical rules of thumb for negatively charged emulsions (zeta potential<0) are (https://en.wikipedia.org/wiki/Zeta_potential):

- rapid flocculation 0 mV < zeta potential < -5 mV
- incipient instability -10 mV < zeta potential < -30 mV
- moderate stability -30 mV < zeta potential < -40 mV
- good stability -40 mV < zeta potential < -60 mV
- excellent stability zeta potential < -61 mV

In studies conducted as part of this project, zeta potential and maximum oil retention were measured on sediments from the SA17 Zone B and OU2 with DI water and a solution of 200 mg/L $CaCl_2$. In DI water, the zeta potential of the EVO (EOS 598B42) was -43 mV indicating moderate stability, while the zeta potential of the soil varied from -20 to -30 mV indicating incipient instability (**Table F-1**). However, in the $CaCl_2$ solution, zeta potential was much closer to zero indicating rapid flocculation. The much weaker repulsion of the oil droplets by the sediment particles in the $CaCl_2$ solution resulted in a large increase in maximum oil retention (**Table F-2**). These results demonstrate that dissolved cation concentration (Na^+, K^+, Ca^{+2}, Mg^{+2}, Mn^{+2}, Fe^{+2}) can have a major impact on zeta potential and oil retention. High dissolved cations will occur naturally in aquifers with high total dissolved solids (Na^+, K^+) or with carbonate minerals (Ca^{+2}, Mg^{+2}). In situ bioremediation can increase cation concentration by release of dissolved Mn^{+2} or Fe^{+2} and addition of alkaline materials ($NaHCO_3$, $Mg(OH)_2$) to raise pH.

Table F-1. Effect of Solution Composition on Zeta Potential

Colloid	Average Zeta Potential (mV) (standard deviation)	
	DI Water	200 mg/L $CaCl_2$
SA17 Soil 15-23'	-29.4 (0.8)	-8.5 (0.5)
SA17 Soil 30-40'	-22.3 (0.9)	-7.5 (0.9)
OU2 Soil 37-40'	-19.9 (0.5)	-12.2 (0.9)
EOS 598B42	-43.0 (0.7)	-10.3 (0.4)

Table F-2. Oil Retention in Laboratory Columns Flushed with EOS598B42 and either D.I. Water or 200 mg/L $CaCl_2$.

Aquifer Material	Average Oil Retention (g oil / g sediment) (standard deviation)	
	D.I. Water	$CaCl_2$
SA17 Zone B	0.0027 (0.0027)	0.0133 (0.0060)
OU2	0.0144 (0.0018)	0.0381 (0.0114)

Detailed laboratory column, sandbox, and field studies have shown that EVO can be transported substantial distances through fine silty or clayey sand and fractured rock (Coulibaly et al., 2006;

Jung et al., 2006; Borden, 2007a; Borden et al., 2007; Riha et al., 2009; Kovacich et al., 2007; Watson et al., 2013). However, once oil droplets attach, they are strongly retained and do not migrate further. Much effort has focused on developing EVO formulations with low retention to reduce the amount of oil required to treat a given volume of aquifer. However, in some cases, higher oil retention is required to treat very high permeability gravels or fractured rock. In these cases, EVO with large oil droplets can be used. The large droplets increase oil retention by straining and by oil droplet buoyancy which causes the large droplets to float and collide with the roof of the sediment pores.

F.4 EVO CONSUMPTION DURING ERD

Shortly after injection, most oil droplets are immobilized on sediment surfaces. The soluble substrates are rapidly consumed during reduction of background electron acceptors (O_2, NO_3, Mn(III/IV), Fe(III), and SO_4). The oil (triglyceride) is fermented to hydrogen and acetate through a two-step process where the ester linkages between the glycerol (an alcohol) and the long-chain fatty acids (LCFAs) are hydrolyzed releasing free fatty acids and glycerol to solution. Glycerol is very soluble and relatively easy to biodegrade, so this material is quickly consumed releasing 1,3-propanediol and then H_2 and acetate. The LCFAs undergo further breakdown by *beta*-oxidation releasing hydrogen (H_2), one molecule of acetate ($C_2H_3O_2^-$), and the original molecule of acid appears as a new acid derivative with two less carbon atoms (Sawyer et al., 1994).

$$C_nH_{2n}O_2 + 2H_2O \rightarrow 2H_2 + C_2H_3O_2^- + H^+ + C_{n-2}H_{2n-4}O_2$$

By successive oxidation at the *beta* carbon atom, long-chain fatty acids are whittled into short chain fatty acids (SCFAs) and acetic acid. Four hydrogen atoms are released from saturated fatty acids for each acetic acid unit produced (Sawyer et al., 1994). Unsaturated fatty acids undergo the same general process, but release two atoms of hydrogen for each acetic acid unit.

Microcosm, modeling, and field studies by Tang et al. (2013a; 2013b) and Watson et al. (2013) indicates that the LCFA consumption rate, and associated H_2 and acetate production rate, is controlled by LCFA solubility. LCFAs have a relatively low aqueous solubility and will precipitate in the presence of divalent cations (Ca^{+2}, Mg^{+2}, Mn^{+2}, Fe^{+2}) or sorb to clay (Angelidaki et al., 1990), reducing their bioavailability and fermentation rate. Since LCFA precipitation/sorption is an equilibrium process, a portion of LCFA will be in aqueous phase and available for fermentation. The SCFAs are much more soluble and sorption/precipitation of SCFA is not a significant factor.

Immediately adjoining the precipitated LCFAs, H_2 and acetate will be produced, and aquifer redox conditions will be sulfate reducing to methanogenic with H_2 varying between 1 and 10 nM (Chapelle et al., 1996) and acetate varying between 10^5 to 10^7 nM (6 to 600 mg/L). H_2 concentrations are maintained at low levels, by rapid consumption of background electron acceptors or chlorinated solvents. If the chlorinated solvents are depleted in the area immediately adjoining the LCFAs, H_2 will be fermented to CH_4 and will no longer be available for ERD. In contrast, acetate turnover is much slower, and dissolved acetate can migrate with flowing groundwater, eventually reaching contaminated portions of the aquifer, stimulating the reduction of PCE, TCE and other more highly chlorinated compounds. However, *c*DCE and VC reduction requires H_2. Elevated H_2 levels only occur near where LCFAs are being fermented, so *c*DCE and VC will only be reduced to ethene in close proximity to the precipitated LCFAs.

Given that the contaminant distribution in the aquifer is almost never known, the best approach is to uniformly distribute EVO throughout the target treatment zone.

F.5 AQUIFER PH AND BUFFERING

During ERD of chlorinated ethenes, PCE is sequentially reduced to TCE, *c*DCE, VC, and finally ethene, by removing one chlorine atom at a time, replacing it with a hydrogen atom, and in the process releasing HCl to solution (Vogel and McCarty, 1985; Mohn and Tiedje, 1992). Organic substrates added as electron donors ferment releasing H_2 and acetic acid. Acetic acid rarely accumulates during ERD, but is consumed releasing carbonic acid, resulting in further pH declines. Other important processes include buffering by carbonate minerals, proton (H^+) exchange on aquifer solids, and use of different alkaline materials to raise pH. Additional information on these processes is presented in **Appendix D** along with an Excel based design tool to assist in determining the amount of buffer or base required to maintain circum-neutral pH.

F.6 INJECTION SYSTEM DESIGN

There are a variety of different approaches that can be used to distribute emulsions in the subsurface including: (a) injection only using grids of temporary or permanent wells; (b) recirculation using systems of injection and pumping wells; and (c) barriers. Each of these approaches has advantages and disadvantages with the 'best' approach dependent on site-specific conditions. For each approach, cost and effectiveness are a function of the well layout and injection sequence.

Projects involving injection of oil emulsions typically, but not always, involve the following steps: (1) installation of injection wells and associated equipment; (2) preparation or purchase of a concentrated emulsion; and (3) dilution of the concentrated emulsion with water and injection. Emulsions can be injected through the end of direct push rods, through temporary 1-inch direct-push wells, or through permanent 2-inch or 4-inch conventionally-drilled wells. The selection of the most appropriate method for installing injection points depends on site-specific conditions including drilling costs, flow rate per well, and volume of fluid that must be injected.

Using properly prepared emulsions, it is possible to move injected emulsions 10, 20, or 50 ft away from the injection point. However, achieving effective distribution of the emulsified oil often requires injecting large volumes of water. Depending on the injection well layout and formation permeability, emulsion injection can require an hour to several days per well. As a consequence, several wells may be injected at one time using a simple injection system manifold.

Modeling studies by Clayton and Borden (2009) showed that EVO distribution throughout a target treatment zone is controlled by: (1) injection point spacing; (2) mass of oil injected relative to the maximum oil retention capacity of the treatment zone (Mass Scaling Factor, SF_M); (3) pore volumes of dilute emulsion and/or chase water injected to distribute the emulsion (Volume Scaling Factor, SF_V); and (4) timing of injection into different wells. If too little oil is injected or too little fluid is injected, the oil will be retained by the sediment close to the injection wells and large portions of the aquifer will remain untreated. **Figure F-2** shows the effect of SF_M and SF_V on volume contact efficiency (fraction of treatment zone contacted) for a moderately heterogeneous aquifer treated with a uniform grid of injection wells. For $SF_M > 0.4$ and $SF_V > 0.4$, contact efficiencies greater than 50% can be achieved. However, contact efficiencies greater than 70% are very difficult to achieve due to the spatial variations in permeability common to most aquifers.

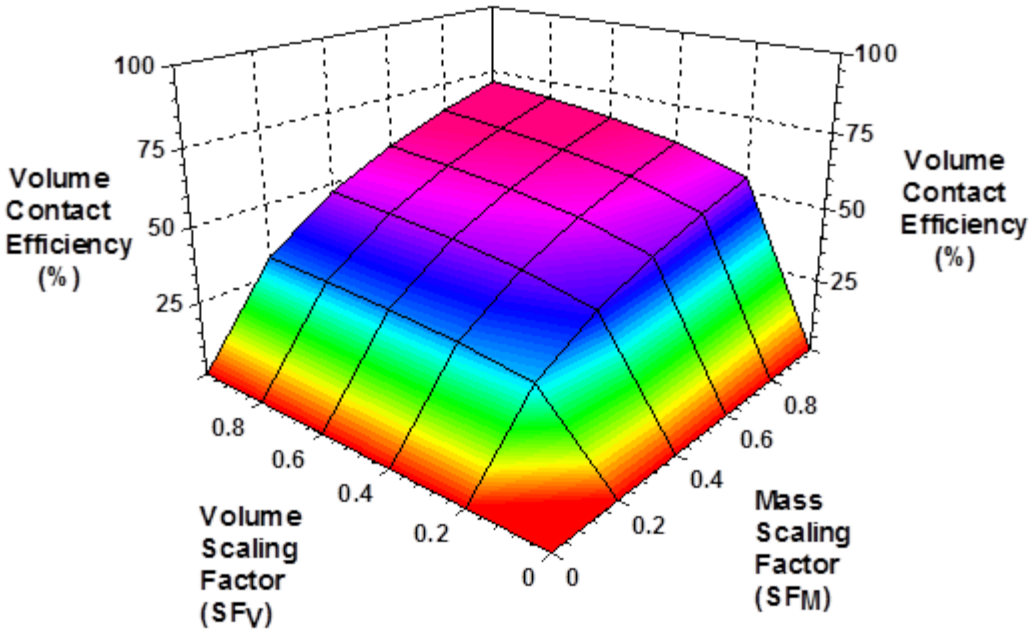

Figure F-2. Effect of Volume Scaling Factor (SF_V) and Mass Scaling Factor (SF_M) on Volume Contact Efficiency for a Moderately Heterogeneous Aquifer with Well Spacing Approximately Equal to Row Spacing (Borden et al., 2008).

Designing an effective and efficient injection system is challenging due to the trade-offs between cost and performance. In general, closer well spacing with more oil and more distribution water will improve contact efficiency, but also increase costs. There are also trade-offs between costs for injection point installation and labor for fluid injection. Increasing the separation between injection wells will reduce the number of wells, reducing drilling costs. However, a larger well spacing can also increase the time required for injection, increasing labor costs. An Excel spreadsheet based design tool is available to assist to assist in developing efficient and effect injection systems (Borden et al., 2008).

Once the well spacing and injection volumes are determined, there are two basic approaches to injecting emulsions: (a) injection of a small volume of more concentrated emulsion (typically 10 to 20% oil by volume) followed by additional chase water to distribute the emulsion throughout the formation; or (b) continuous injection of a more dilute emulsion (typically 0.5 to 2% oil by volume). Numerical modeling results indicate that the two approaches are both effective in distributing emulsion (Borden, 2007a) and the choice should be based on personal preferences and site logistics. In all cases, the concentrated emulsion should be diluted with enough water to reduce the viscosity to near that of water, reducing injection pressures. After emulsion injection is complete, clean water should be injected at the end to push to mobile oil out away from the injection point to reduce fouling with bacteria and oil.

F.7 REFERENCES

Angelidaki, I., Petersen, S.P. and Ahring, B.K., 1990. Effects of lipids on thermophilic anaerobic digestion and reduction of lipid inhibition upon addition of bentonite. Applied microbiology and biotechnology, 33(4), pp.469-472.

Aulenta, F., Majone, M. and Tandoi, V., 2006. Enhanced anaerobic bioremediation of chlorinated solvents: environmental factors influencing microbial activity and their relevance under field conditions. Journal of Chemical Technology and Biotechnology, 81(9), pp.1463-1474.

Bolster, C.H., Mills, A.L., Hornberger, G.M. and Herman, J.S., 2001. Effect of surface coatings, grain size, and ionic strength on the maximum attainable coverage of bacteria on sand surfaces. Journal of contaminant hydrology, 50(3), pp.287-305.

Borden, R.C., 2007a. Effective Distribution of Emulsified Edible Oil for Enhanced Anaerobic Bioremediation. Journal of Contaminant Hydrology, 94(1), pp.1-12.

Borden, R.C., 2007b. Engineering Delivery of Insoluble Amendments. Partners in Environmental Technology Symposium & Workshop, SERDP, Washington, DC.

Borden, R.C., Beckwith, W.J., Lieberman, M.T., Akladiss, N. and Hill, S.R., 2007. Enhanced anaerobic bioremediation of a TCE source at the Tarheel Army Missile Plant using EOS. Remediation Journal, 17(3), pp.5-19.

Borden, R.C., Clayton, M., Weispfenning, A.M., Simpkin, T. and Lieberman, M.T., 2008. Technical Report-Design Tool for Planning Emulsified Oil Injection Systems. Environmental Security Technology Certification Program, Arlington, Virginia.

Chapelle, F.H., Haack, S.K., Adriaens, P., Henry, M.A. and Bradley, P.M., 1996. Comparison of Eh and H2 Measurements for Delineating Redox Processes in a Contaminated Aquifer. Environmental Science & Technology, 30(12), pp.3565-3569.

Clayton, M.H. and Borden, R.C., 2009. Numerical modeling of emulsified oil distribution in heterogeneous aquifers. Groundwater, 47(2), pp.246-258.

Coulibaly, K.M. and Borden, R.C., 2004. Impact of edible oil injection on the permeability of aquifer sands. Journal of contaminant hydrology, 71(1), pp.219-237.

Coulibaly, K.M., Long, C.M. and Borden, R.C., 2006. Transport of edible oil emulsions in clayey sands: One-dimensional column results and model development. Journal of Hydrologic Engineering, 11(3), pp.230-237.

Eaddy, A., 2008. Scale-Up and Characterization of an Enrichment Culture for Bioaugmentation of the P-Area Chlorinated Ethene Plume at the Savannah River Site. M.S. Thesis, Clemson University.

He, J., Ritalahti, K.M., Aiello, M.R. and Löffler, F.E., 2003. Complete detoxification of vinyl chloride by an anaerobic enrichment culture and identification of the reductively dechlorinating population as a Dehalococcoides species. Applied and Environmental Microbiology, 69(2), pp.996-1003.

Holliger, C., Schraa, G., Stams, A.J. and Zehnder, A.J., 1993. A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. Applied and Environmental Microbiology, 59(9), pp.2991-2997.

Holliger, C., Hahn, D., Harmsen, H., Ludwig, W., Schumacher, W., Tindall, B., Vazquez, F., Weiss, N. and Zehnder, A.J., 1998. Dehalobacter restrictus gen. nov. and sp. nov., a strictly anaerobic bacterium that reductively dechlorinates tetra-and trichloroethene in an anaerobic respiration. Archives of Microbiology, 169(4), pp.313-321.

Jiang, C., 2012. Development of Enrichment Cultures for Anaerobic Reductive Dechlorination of Tetrachloroethene Under Low pH Conditions. M.S. Thesis, Clemson University.

Johnson, P.R. and Elimelech, M., 1995. Dynamics of colloid deposition in porous media: Blocking based on random sequential adsorption. Langmuir, 11(3), pp.801-812.

Jung, Y., Coulibaly, K.M. and Borden, R.C., 2006. Transport of edible oil emulsions in clayey sands: 3D sandbox results and model validation. Journal of Hydrologic Engineering, 11(3), pp.238-244.

Kovacich, M.S., D. Beck, T. Rabideau, K. Smith-Pettypiece, M. Noel, M.J. Zack, and M.T. Cannaert, 2007. Full-Scale Bioaugmentation to Create a Passive Biobarrier to Remediate a TCE Groundwater Plume. In Proceedings: Ninth International In Situ and On-Site Bioremediation Symposium, Baltimore, Maryland, USA.

Krumholz, L.R., Sharp, R. and Fishbain, S.S., 1996. A freshwater anaerobe coupling acetate oxidation to tetrachloroethylene dehalogenation. Applied and Environmental Microbiology, 62(11), pp.4108-4113.

Li, J.J., 2012. Adaptation of a Dechlorinating Culture, KB-1, to Acidic Environments, M.S. Thesis, University of Toronto.

Löffler, F.E., Yan, J., Ritalahti, K.M., Adrian, L., Edwards, E.A., Konstantinidis, K.T., Müller, J.A., Fullerton, H., Zinder, S.H. and Spormann, A.M., 2013. Dehalococcoides mccartyi gen. nov., sp. nov., obligately organohalide-respiring anaerobic bacteria relevant to halogen cycling and bioremediation, belong to a novel bacterial class, Dehalococcoidia classis nov., order Dehalococcoidales ord. nov. and family Dehalococcoidaceae fam. nov., within the phylum Chloroflexi. International journal of systematic and evolutionary microbiology, 63(2), pp.625-635.

Logan, B.E., 1999. Environmental transport processes. John Wiley & Sons, New York.

Luijten, M.L., de Weert, J., Smidt, H., Boschker, H.T., de Vos, W.M., Schraa, G. and Stams, A.J., 2003. Description of Sulfurospirillum halorespirans sp. nov., an anaerobic, tetrachloroethene-respiring bacterium, and transfer of Dehalospirillum multivorans to the genus Sulfurospirillum as Sulfurospirillum multivorans comb. nov. International Journal of Systematic and Evolutionary Microbiology, 53(3), pp.787-793.

Maillard, J., Regeard, C. and Holliger, C., 2005. Isolation and characterization of Tn-Dha1, a transposon containing the tetrachloroethene reductive dehalogenase of Desulfitobacterium hafniense strain TCE1. Environmental microbiology, 7(1), pp.107-117.

Maymó-Gatell, X., Nijenhuis, I. and Zinder, S.H., 2001. Reductive dechlorination of cis-1, 2-dichloroethene and vinyl chloride by "Dehalococcoides ethenogenes". Environmental science & technology, 35(3), pp.516-521.

Mohn, W.W. and Tiedje, J.M., 1992. Microbial reductive dehalogenation. Microbiological reviews, 56(3), pp.482-507.

Müller, J.A., Rosner, B.M., Von Abendroth, G., Meshulam-Simon, G., McCarty, P.L. and Spormann, A.M., 2004. Molecular identification of the catabolic vinyl chloride reductase from Dehalococcoides sp. strain VS and its environmental distribution. Applied and Environmental Microbiology, 70(8), pp.4880-4888.

Riha, B.D., Looney, B.B., Noonkester, J.V., Hyde, K., Walker, R. and Rambo, B., 2009. Treatability Study for Edible Oil Deployment for Enhanced cVOC Attenuation for T-Area, Savannah River Site: Interim Report–Year One. Technical Report SRNL-RP-2009-00539. Savannah River National Laboratory, Aiken, SC.

Rijnaarts, H.H., Norde, W., Bouwer, E.J., Lyklema, J. and Zehnder, A.J., 1996a. Bacterial deposition in porous media related to the clean bed collision efficiency and to substratum blocking by attached cells. Environmental science & technology, 30(10), pp.2869-2876.

Rijnaarts, H.H., Norde, W., Bouwer, E.J., Lyklema, J. and Zehnder, A.J., 1996b. Bacterial deposition in porous media: effects of cell-coating, substratum hydrophobicity, and electrolyte concentration. Environmental science & technology, 30(10), pp.2877-2883.

Rowlands, D., 2004. Development of optimal pH for degradation of chlorinated solvents by the KB-1™ anaerobic bacterial culture. M.S. Thesis, University of Guelph.

Ryan, J.N. and Elimelech, M., 1996. Colloid mobilization and transport in groundwater. Colloids and surfaces A: Physicochemical and engineering aspects, 107, pp.1-56.

Sawyer, C.N., P.L. McCarty, and G.F. Parkin. 1994. Chemistry for Environmental Engineering. McGraw-Hill Inc.

Soo, H. and Radke, C.J., 1984. Flow mechanism of dilute, stable emulsions in porous media. Industrial & engineering chemistry fundamentals, 23(3), pp.342-347.

Soo, H. and Radke, C.J., 1986. A filtration model for the flow of dilute, stable emulsions in porous media—I. Theory. Chemical Engineering Science, 41(2), pp.263-272.

Soo, H., Williams, M.C. and Radke, C.J., 1986. A filtration model for the flow of dilute, stable emulsions in porous media—II. Parameter evaluation and estimation. Chemical Engineering Science, 41(2), pp.273-281.

Sung, Y., Ritalahti, K.M., Sanford, R.A., Urbance, J.W., Flynn, S.J., Tiedje, J.M. and Löffler, F.E., 2003. Characterization of two tetrachloroethene-reducing, acetate-oxidizing anaerobic bacteria and their description as Desulfuromonas michiganensis sp. nov. Applied and Environmental Microbiology, 69(5), pp.2964-2974.

Sung, Y., Ritalahti, K.M., Apkarian, R.P. and Löffler, F.E., 2006. Quantitative PCR confirms purity of strain GT, a novel trichloroethene-to-ethene-respiring Dehalococcoides isolate. Applied and Environmental Microbiology, 72(3), pp.1980-1987.

Tang, G., Wu, W.M., Watson, D.B., Parker, J.C., Schadt, C.W., Shi, X. and Brooks, S.C., 2013. U (VI) bioreduction with emulsified vegetable oil as the electron donor–microcosm tests and model development. Environmental science & technology, 47(7), pp.3209-3217.

Tang, G., Watson, D.B., Wu, W.M., Schadt, C.W., Parker, J.C. and Brooks, S.C., 2013. U (VI) bioreduction with emulsified vegetable oil as the electron donor–model application to a field test. Environmental science & technology, 47(7), pp.3218-3225.

Vogel, T.M. and McCarty, P.L., 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. Applied and Environmental Microbiology, 49(5), pp.1080-1083.

Watson, D.B., Wu, W.M., Mehlhorn, T., Tang, G., Earles, J., Lowe, K., Gihring, T.M., Zhang, G., Phillips, J., Boyanov, M.I. and Spalding, B.P., 2013. In situ bioremediation of uranium with emulsified vegetable oil as the electron donor. Environmental science & technology, 47(12), pp.6440-6448.

Westall, J.C. and Gschwend, P.M., 1993. "Mobilizing and depositing colloids." Manipulation of groundwater colloids for environmental restoration, Lewis, Ann Harbor, Mich.

Yang, Y., 2012. Exploring Anaerobic Reductive Dechlorination at Low pH Environments. M.S. Thesis, University of Tennessee.

Yang, Y., 2016. Bioremediation of Chlorinated Ethenes: pH Effects, Novel Dechlorinators and Decision-Making Tools. Ph.D. Dissertation, University of Tennessee.

Yang, Y., Cápiro, N. L., Marcet, T. F., Yan, J., Pennell, K. D. and Löffler, F. E., 2017. Organohalide respiration with chlorinated ethenes under low pH conditions. Environmental Science & Technology, 51(15), pp.8579-8588.