

**Reductive Anaerobic Biological In-Situ Treatment
Technology (RABITT) Treatability Test
Interim Report**



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1.0 INTRODUCTION

Chloroethene compounds, such as tetrachloroethene (PCE) and trichloroethene (TCE), have been widely used for a variety of industrial purposes. Past disposal practices and accidental spills have led to widespread contamination at U.S. Department of Defense (DoD) and industrial facilities. Enhanced anaerobic dechlorination is a promising treatment approach for remediating chlorinated ethene-contaminated groundwater in situ. The goal of this effort is to develop and validate a comprehensive approach for conducting a treatability test to determine the potential for applying reductive anaerobic biological in situ treatment technology (RABITT) at any specific site. A treatability protocol has been written (Morse et al., 1998) and is being applied to four DoD chlorinated solvent contamination sites in the United States. The protocol will be revised as needed upon completion of the effort based on lessons learned and field test results.

2.0 BACKGROUND

Because both PCE and TCE are stable compounds that resist aerobic degradation or require the presence of an electron-donating co-contaminant for anaerobic transformation, these compounds tend to persist in the environment. However, in reductive systems, highly oxidized contaminants (e.g., PCE) can be utilized as electron acceptors. RABITT attempts to stimulate this reductive pathway by supplying excess substrate (electron donor) to the native microbial consortium. The presence of the substrate expedites the exhaustion of any naturally occurring electron acceptors. As the natural electron acceptors are depleted, microorganisms capable of discharging electrons to other available electron acceptors, such as oxidized contaminants, gain a selective advantage.

The reductive dechlorination of PCE to ethene proceeds through a series of hydrogenolysis reactions, with each reaction becoming progressively more difficult to carry out. The selection of an appropriate electron donor may be the most important design parameter for developing a healthy population of microorganisms capable of dechlorinating PCE and TCE. Recent studies have indicated a prominent role for molecular hydrogen (H_2) in the reductive dechlorination process (DiStefano et al., 1992; Gossett et al., 1994; Holliger et al., 1993; Maymo-Gatell et al., 1995; Zinder and Gossett, 1995). Most known dechlorinators can use H_2 as an electron donor, and some can only use H_2 . Because more complex electron donors are broken down into metabolites and residual pools of H_2 by other members of the microbial community, they may also be used to support dechlorination (Fennell et al., 1997; DiStefano et al., 1992; Smatlak et al., 1996).

When designing an enhanced bioremediation approach, the rate and quantity of H_2 available to a degrading consortium must be considered so as to limit competition for hydrogen from other microbial groups, such as methanogens and sulfate-reducers. Competition for H_2 by methanogens is a common cause of dechlorination failure in laboratory studies. As the methanogen population increases, the portion of reducing equivalents used for dechlorination quickly drops and methane production increases (Fennell et al., 1997; Gossett et al., 1994). The use of slowly degrading nonmethanogenic substrates could help prevent this type of system shutdown.

Because of the complex microbial processes involved in anaerobic dechlorination, thorough site characterization and laboratory microcosm testing are an important part of the RABITT protocol. The protocol presents a phased or tiered approach to the treatability test, allowing the user to screen out RABITT in the early stages of the process to save time and cost. The protocol guides the user through a decision process in which information is collected and evaluated to determine if the technology should be given further consideration. RABITT would be screened out if it is determined that site-specific characteristics, regulatory constraints, or other logistical problems suggest that the technology will be difficult or impossible to employ or if a competing technology clearly is superior.

3.0 APPROACH

A summary of the protocol approach is presented here. For detailed information on sample collection techniques or analytical methods, please refer to Morse et al. (1998).

The protocol is conducted in four phases. The first phase of the treatability test includes a thorough review of existing site data to develop a conceptual model of the site. The protocol contains a rating system that can be used to assess the suitability of a site for RABITT testing. The rating system is based on an analysis of the contaminant, hydrogeologic, and geochemical profiles of the site. The decision to proceed with the RABITT screening process should be supported by data indicating that the site meets requirements for successful technology application.

The second phase of the approach involves selecting a candidate test plot location within the plume for more detailed site characterization. Characterization activities will examine contaminant, geochemical, and hydrogeologic parameters on a relatively small scale to determine the suitability of the selected location as a RABITT test plot.

Based on the information generated during the characterization of the test plot, a decision is made to proceed to phase three of the treatability study, which consists of conducting laboratory microcosm studies. Soil cores collected from the selected testing location are used to construct microcosms, but prior to microcosm setup, cores are visually examined to assess soil type and stratigraphy. In addition, soil core subsamples are sent to an off-site laboratory and analyzed for volatile organic compounds (VOCs), total organic content (TOC), and total iron. The microcosm studies are conducted to determine what electron donor/nutrient formulation should be field-tested to provide optimum biological degradation performance. Yeast extract, propionate, lactate, butyrate, and lactate/benzoate are evaluated for their effectiveness at stimulating anaerobic dechlorination. If the results from the microcosm testing indicate that reductive dechlorination does not occur in response to the addition of electron donors and/or nutrients, the technology is eliminated from further consideration.

The fourth and final phase of the treatability test entails field testing the electron donor/nutrient formulation determined in the laboratory microcosm tests to be most effective for supporting biologically mediated reductive dechlorination. A small test plot is set up for the addition of electron donor to the plot in situ. The plot is then monitored for evidence of effective dechlorination. Groundwater samples are analyzed for the following parameters: dissolved

oxygen, temperature, pH, Fe⁺², conductivity, chloroethenes, dissolved organic carbon, ammonia, CH₄, C₂H₄, C₂H₆, NO₃, NO₂, SO₄, Cl, Br, alkalinity, and total iron. Table 1 presents the performance monitoring parameters and their measurement frequency during field-testing.

Analysis	Method	Testing Location	Frequency of Analysis
Dissolved Oxygen	DO probe	Field	Every 2 weeks
Temperature	Temperature probe	Field	Every 2 weeks
pH	pH probe	Field	Every 2 weeks
Fe ⁺²	Hach test kit	Field	Every 2 weeks
Redox Potential	Redox probe	Field	Every 2 weeks
Chloroethenes	SW 846 Method 8260B	Laboratory	Every 2 weeks
Dissolved Organic Carbon	EPA Method 415.1	Laboratory	Monthly
Volatile Fatty Acids	RSKSOP-177	Laboratory	Monthly
NH ₃	EPA Method 350.2	Laboratory	Monthly
CH ₄ , C ₂ H ₄ , C ₂ H ₆	SW 3810 modified or Kampbell et al., 1989	Laboratory	Monthly
NO ₃ , NO ₂ , SO ₄	EPA Method 300	Laboratory	Monthly
Cl, Br	EPA Method 300	Laboratory	Monthly
Conductivity	EPA Method 120.1	Laboratory	Monthly
Alkalinity	EPA Method 310.1	Laboratory	Monthly
pH	EPA Method 150.1	Laboratory	Monthly

The standard RABITT field treatability test design consists of an extraction/amendment/reinjection system within a small test plot. Contaminated groundwater is extracted near the end of the treatment plot, amended with nutrients and/or electron donor, and then reinjected near the head of the treatment plot. This design creates a hydraulic gradient to direct the flow of groundwater through the treatment plot. Monitoring points are placed within the treatment plot, in between the injection and extraction wells. Groundwater extraction and injection are optimized to achieve approximately a 30-day hydraulic residence time within the treatment plot.

The data from this phased treatability test is evaluated to determine the potential for successfully applying RABITT in the field. The entire project effort consists of developing a draft protocol, applying the protocol at four chlorinated solvent contaminated sites, and then revising the protocol based on the results observed at the four demonstration sites. To date, field treatability tests have been completed at Cape Canaveral Air Station, FL, and Naval Air Station Alameda, CA. Field treatability testing was initiated at Ft Lewis, WA, in August 2000, and microcosm core samples were collected at Marine Corps Base, Camp Lejeune, NC, in November 2000.

4.0 RESULTS

4.1 Site #1: Cape Canaveral Air Station , FL

4.1.1 Site Description

Facility 1381, the Ordnance Support Facility at Cape Canaveral Air Station, contains a shallow, 110-acre VOC plume consisting primarily of TCE, dichloroethylene (DCE), and vinyl chloride (VC). Improper disposal of solvents used for cleaning and degreasing operations contributed to this groundwater contamination plume. Field data suggest that TCE is naturally being dechlorinated to DCE and subsequently to VC. Each of these contaminants has been detected in a surface water body adjacent to the site.

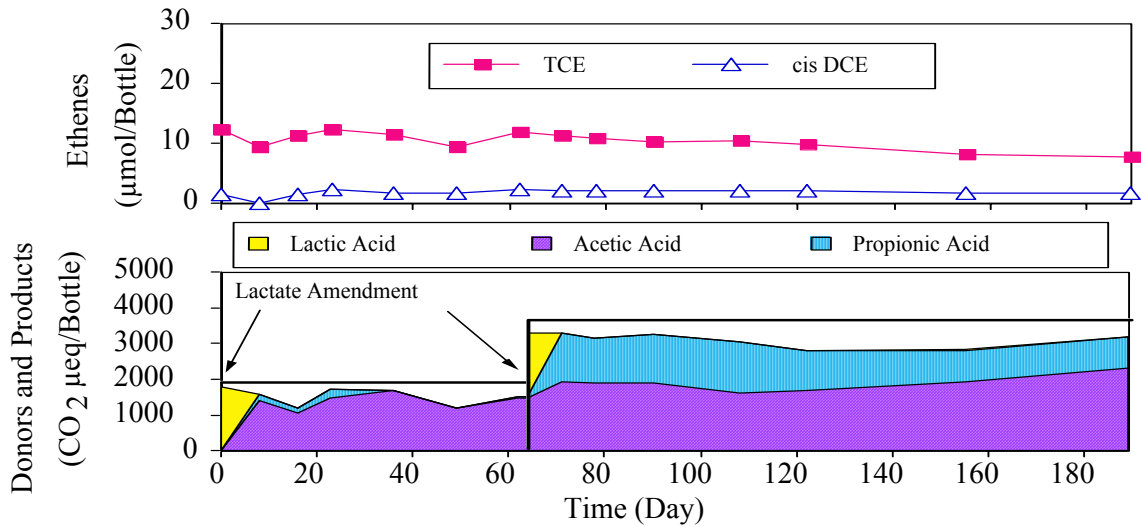
The geology at the site is characterized by poorly sorted coarse to fine sands and shell material from ground surface to approximately 35 ft below ground surface (bgs). From approximately 35 to 50 ft bgs, sands show a decrease in grain size, and the silt and clay content increases. From 48.5 to 51 ft bgs, a continuous clay unit appears to underlie the entire area at Facility 1381. Groundwater at the site is very shallow, generally ranging between 4 and 7 ft bgs. The hydraulic conductivity for the shallow groundwater has been determined to be approximately 88.7 ft/day. The pH of the groundwater ranged from 6.87 to 8.14, and conductivity readings ranged from 464 to 5,550 $\mu\text{mhos/cm}$. The groundwater flow velocity has been calculated to be 0.21 ft/day. The suspected source area contains high levels of TCE (up to 342 mg/L), but TCE concentrations drop off quickly, and only DCE and VC are detected toward the edges of the plume.

4.1.2 Microcosms: Cape Canaveral Air Station, FL

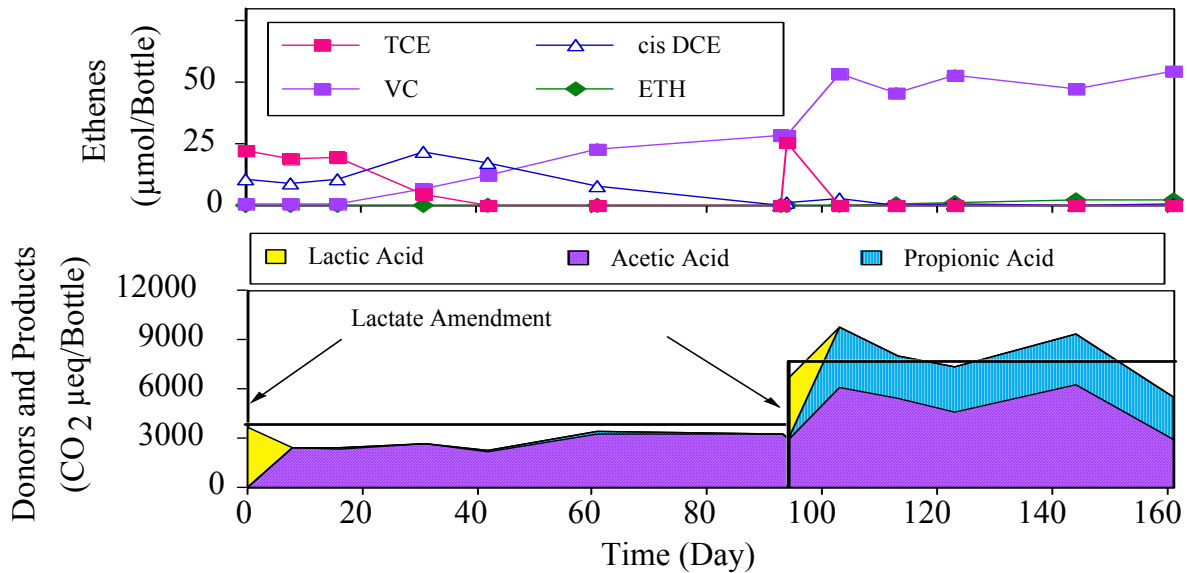
Microcosm studies at Cape Canaveral showed that all organic electron donors evaluated (lactate, butyrate, propionate, benzoate, yeast extract amendment) promoted enhanced dechlorination of the 2 mg/L TCE, 10 mg/L cDCE, and 1.5 mg/L VC present in the site groundwater. Lactate was selected for the electron donor to be used in the field testing.

The graphs that follow illustrate lessons learned from conducting microcosm studies. Upon the addition of lactate and vitamin B₁₂ with no yeast extract, levels of TCE and cDCE show no significant signs of reduction. Alternatively, the addition of yeast extract along with lactate and vitamin B₁₂ facilitated the onset of and completion of the dechlorination process.

CCAS Microcosm 5C-II (GPW09/10) Lactate + B₁₂



CCAS Microcosm 5D-I (GPW06) Lactate +YE + B₁₂



4.1.3 Field Study: Cape Canaveral Air Station, FL

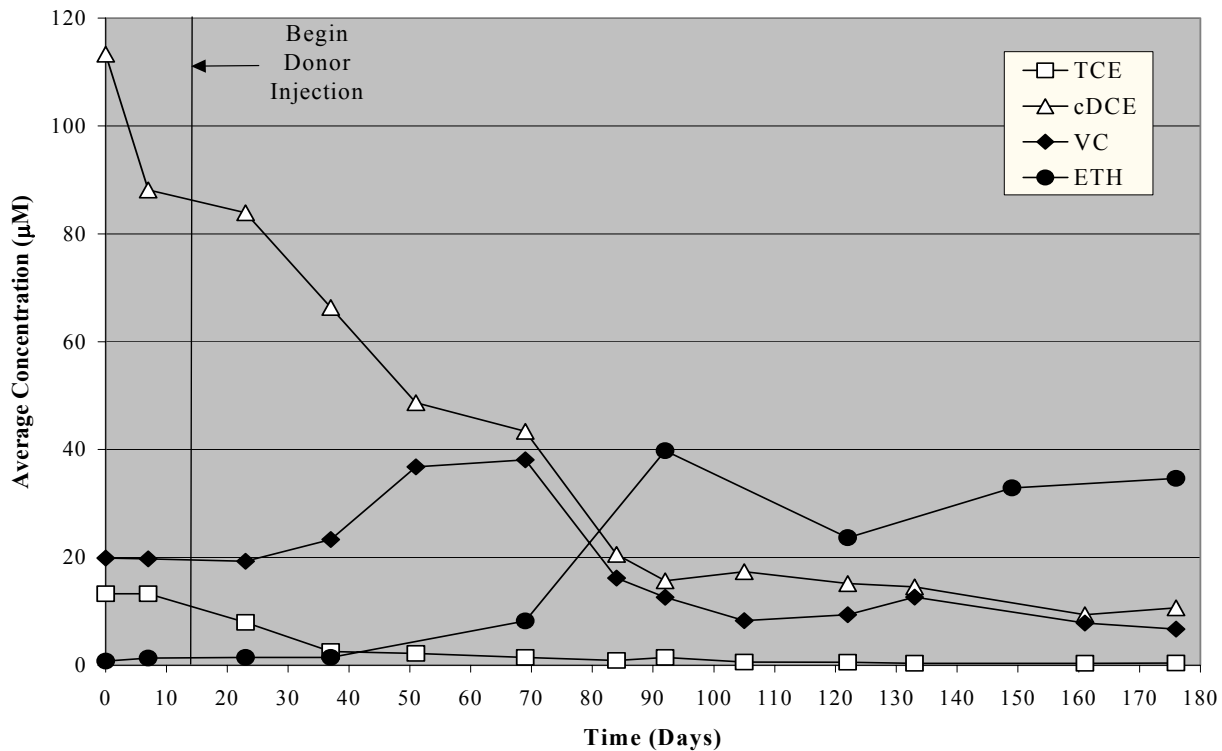
The standard RABITT design was modified for the site at Cape Canaveral Air Station in order to meet the State of Florida Underground Injection Control regulatory requirements. This regulation does not allow for reinjection of contaminated groundwater. The objective of the modified system was to allow for effective delivery and distribution of nutrients and electron donors and to provide for extensive monitoring and hydraulic control, without pumping groundwater aboveground. The modified system was installed at Facility 1381 in March 1999 and operated for six months.

The modified design consisted of two communicating wells, a series of 13 tri-level groundwater monitoring probes, and upgradient and downgradient monitoring wells. The system wells were a

dual screen design, with one operating in an upflow mode and the other in a downflow mode. Each well was screened within two distinct zones (10-12.5 and 17.5-20 ft bgs). The wells were placed close enough to affect each other with the effluent from one well feeding the other. This results in groundwater circulation that can be used to mix and distribute the electron donor/nutrient formulation. Tri-level monitoring points were screened in three zones that covered similar depths and an intermediate zone. The monitoring probes were positioned around the treatment cell to provide three-dimensional data that was required to track the tracer and added electron donor/nutrients, calculate mass reductions during treatment, and evaluate gains and losses from the treatment cell through background groundwater migration. The monitored plot dimensions were 39 ft by 10 ft.

After initial tracer testing established the site hydrological conditions, lactic acid was injected into the communicating well system to maintain an initial groundwater concentration of 3 mM lactate. The total system pumping rate was approximately 2,880 gal/day (7.6 L/ min).

Cape Canaveral Air Station, Facility 1381
TCE and Daughter Products
Average Chloroethenes



Cape Canaveral field-testing showed rapid dechlorination of TCE and cDCE to VC, followed by slower subsequent dechlorination to ethene under the established sulfate reducing to methanogenic conditions. Molecular probing indicated the presence of a dechlorinating organism similar to *Dehalococcoides ethenogenes*, an organism that has been shown to promote

complete dechlorination with slow removal of VC. The treatment demonstrated reduction of TCE, cDCE, and VC by 88.7%, 90.6%, and 66.3%, respectively. The ethene concentration increased significantly to approximately 0.04 mM, but good molar balances were not possible due to diffusion. Overall, there was reasonable agreement between laboratory microcosm and field results.

4.2 Site #2: Naval Air Station Alameda, CA

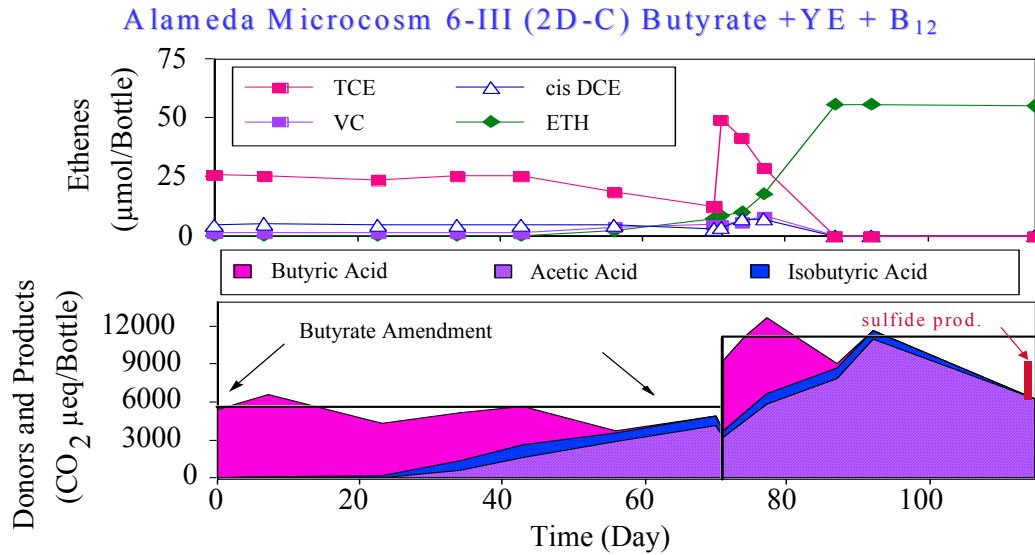
4.2.1 Site Description

Building 360 (Site #4) at Naval Air Station Alameda was selected for the second demonstration. This building has been used as an aircraft engine repair and testing facility and consisted of former machine shops and cleaning areas, as well as plating and welding shops and parts assembly areas. Solvents used in the cleaning shop of Building 360 have included a mixture of 55% PCE and other chemicals such as dichlorobenzene, methylene chloride, toluene, and 30 to 70% solutions of sodium hydroxide. Site characterization activities performed by the facility revealed elevated levels of chlorinated solvents, primarily TCE (24 mg/L), DCE (8.6 mg/L), and VC (2.2 mg/L), between 5.5 and 15.5 feet bgs.

Depth to groundwater in the Building 360 area ranges between 4.4 feet and 6.5 feet bgs. Aquifer testing yielded hydraulic conductivity values from 1.22×10^{-3} to 3.86×10^{-3} cm/sec. The estimated groundwater flow is very low at only 1.1×10^{-5} cm/sec or 11.4 ft/year. It appears that groundwater in this area is very nearly stagnant.

4.2.2 Microcosms: Naval Air Station Alameda, CA

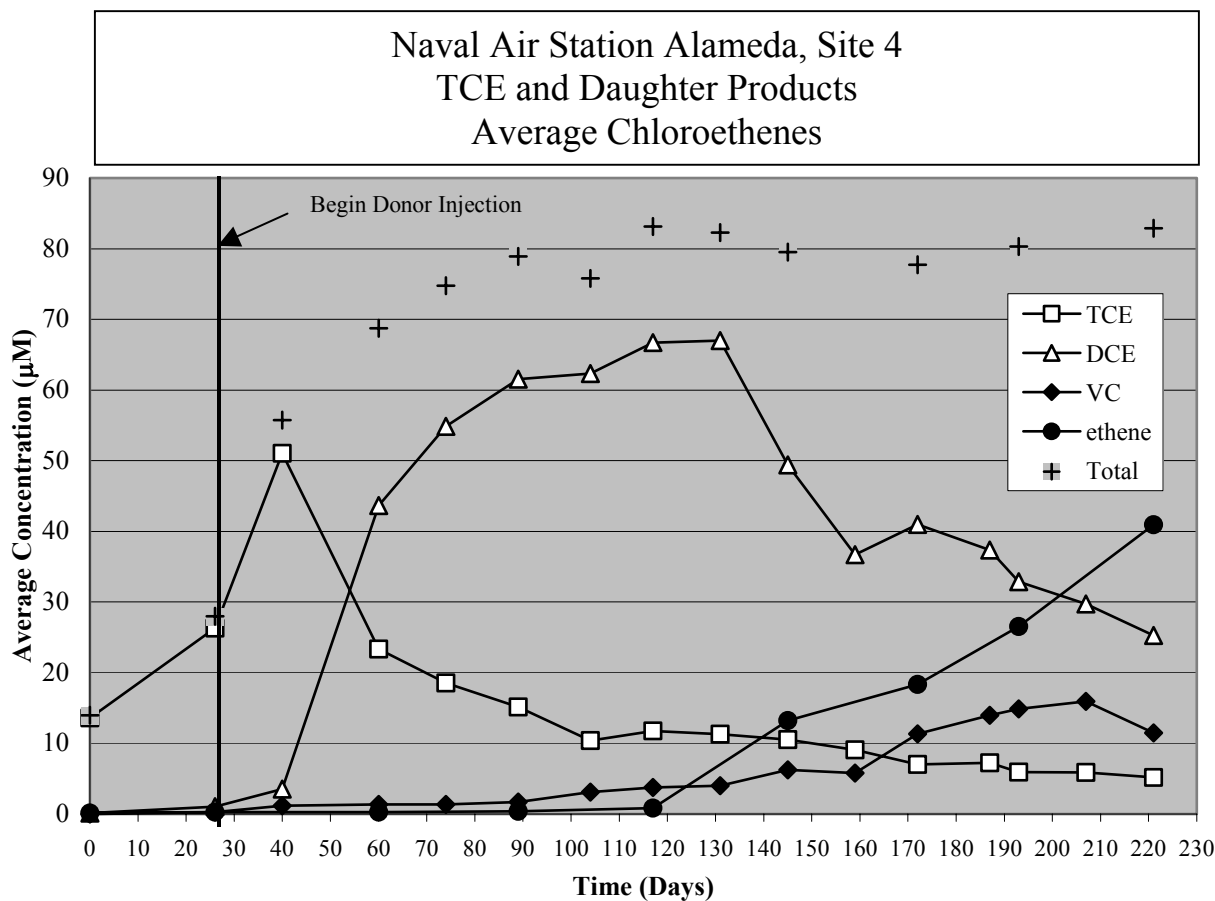
Microcosms showed that all electron donors tested except benzoate promoted enhanced dechlorination of TCE. Butyrate was chosen for field injection because of a shorter lag time associated with stimulating dechlorinating activity. TCE was rapidly dechlorinated to ethene under the established sulfate reducing to methanogenic conditions when supplied with a constant 3 mM supply of butyrate in the injected groundwater obtained from the supply well. Molecular probing to date has been negative for *D. ethenogenes*; however, recent data indicates that a closely related species may be present at the site.



4.2.3 Field Study: NAS Alameda

After baseline sampling and tracer testing, injection of butyric acid began in June 1999 using a flow through system. The field test involved an upgradient injection well and downgradient extraction well with aboveground recirculation. The injection well was supplemented with TCE-contaminated groundwater from a separate supply well outside the influence of the 3-ft by 15-ft monitored plot. The injection, extraction, and nine monitoring wells were all screened between 24 and 27 ft bgs. The total pumping rate for the system was 236 gal/day (0.62 L/min). Butyric acid and yeast extract were added to maintain initial in situ concentrations of 3mM and 20 mg/L respectively.

Injected groundwater contained average TCE, cDCE, and VC concentrations of 81.7 μM, 7.0 μM, and 3.4 μM respectively. By the end of the demonstration the average TCE concentration observed in the treatment zone had been reduced by 94% despite the continuing input of TCE. In addition, both cDCE and VC were on the decline; ethene levels were steadily increasing and accounted for approximately half of the total chloroethene concentration. On average, 87% of injected chloroethenes could be accounted for during sampling events. Good agreement between microcosm and field results was also observed for the Alameda site.



4.3 Site #3: Fort Lewis, WA

4.3.1 Site Description

The East Gate Disposal Yard (EGDY) covers approximately 29 acres at Ft Lewis, WA. Aerial photographs indicate that between 1940 and 1971 the EGDY was used as a storage and disposal site for various solid and liquid wastes. The photographic evidence shows that the wastes were disposed of in large trenches and pits and that, on occasion, the waste materials were burned. Waste materials disposed of at the EGDY include TCE and petroleum, oil, and lubricant wastes from equipment cleaning and degreasing activities conducted at the Fort Lewis Logistics Center.

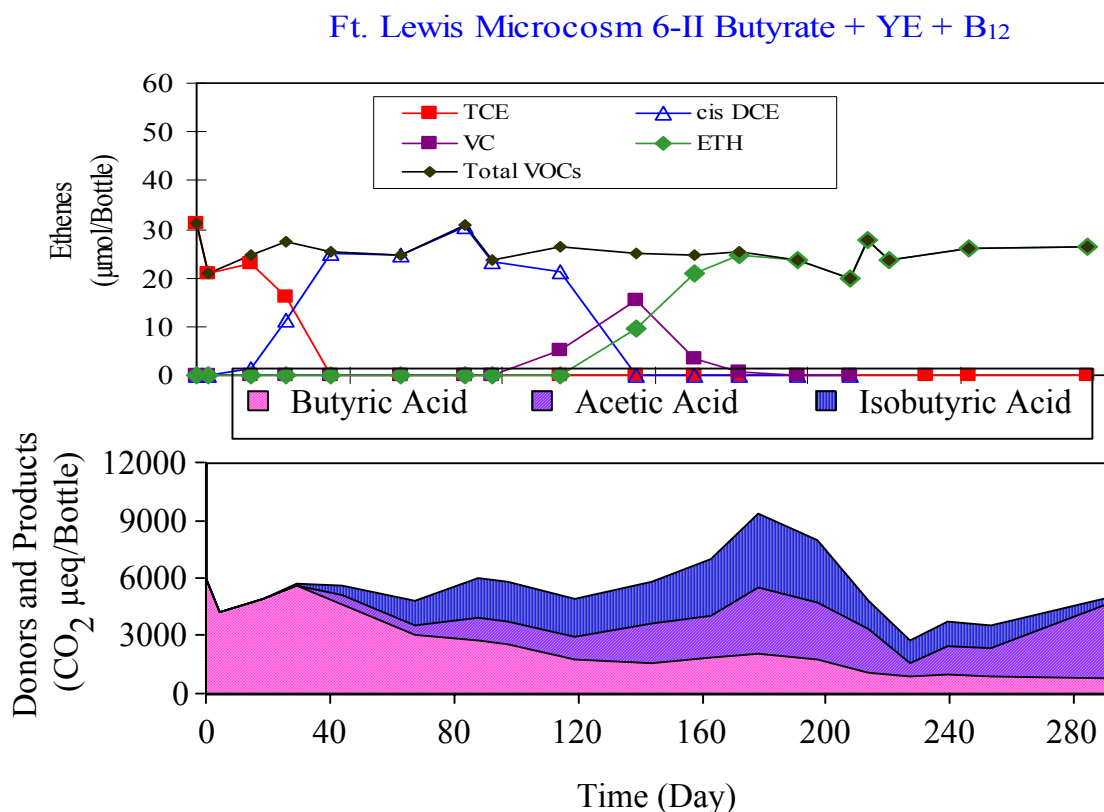
The depth to groundwater at the EGDY Site is approximately 10 feet bgs. Background groundwater velocities across the EGDY are in the range of 0.25 to 0.75 feet per day in the field test location. TCE, cDCE, VC, and BTEX constituents have been detected in groundwater samples from the EGDY Site. Of these, TCE and cDCE are most prevalent. Data from a previous investigation indicated that reductive dechlorination may be occurring in the area but that the process is held up at cDCE.

4.3.2 Microcosms: Ft Lewis, WA

Dechlorination in the Ft. Lewis microcosms was markedly slower than anticipated based on previous results with samples collected from Alameda Point and Cape Canaveral. The initial dose of TCE was removed from all of the amended, biotic reactors; however, formation of VC and complete conversion to ethene occurred in only a few bottles after 292 days of monitoring.

The two amendments that did result in complete conversion to ethene in two of the three replicates were butyrate and high concentrations of yeast extract. Because the observed degradation of butyrate is slow, it appears to provide a relatively steady, long-term supply of electron equivalents for use.

The single, most important factor influencing dechlorination—both lag and extent—was probably the low native levels of TCE in the materials from which these microcosm sets were created. In the majority of bottles, there was a consistently long lag time observed prior to the initiation of cDCE dechlorination. However, once cDCE dechlorination activity began, it was generally followed by concurrent transformation of VC to ethene. These patterns suggest that the transformation of TCE was mediated by different organisms than those responsible for cDCE and VC dechlorination. This type of pattern is consistent with the presence of a dechlorinating population in which *Dehalococcoides ethenogenes* is not the dominant member.



4.3.3 Field Study: Ft Lewis, WA

A conventional RABITT test system (shown in Figure 6.1 on page 54 of the draft RABITT protocol) was installed at Fort Lewis, with the exception that the gradient well was removed from the design based on the results of the tracer test and the measured gradient in the selected area. The three injection wells were spaced approximately 2 feet apart and the distances between the injection wells and each row of monitoring wells were 10 feet for a plot dimension of approximately 4 feet by 30 feet. A background monitoring well was installed upgradient of the plot to monitor any naturally occurring changes in background contaminant and geochemical profiles. An existing well in a contaminated area was used to provide the required supply of

contaminated groundwater for injection into the test plot. The injected fluid imparts a gradient, which drives the flow of groundwater through the system.

Initial TCE concentrations ranged across the test plot from 11.0 to 47.9 μM (1,450 to 6,300 ppb). Injected groundwater initially contained moderately higher levels that tended to increase over the first 13 weeks of the demonstration from a low of 39.6 μM (5,200 ppb) at system startup to 148 μM (19,400 ppb) at 13 weeks. TCE concentrations remained within this range until Week 24 when concentrations spiked dramatically to 1,286 μM (169,000 ppb). Concerns that TCE levels of this magnitude would prove toxic to the microorganisms catalyzing the dechlorination reaction proved unwarranted as the conversion of TCE to cDCE continued unimpeded.

After 8 weeks of electron donor injection, the influent concentration of TCE was reduced 99.94% from 65 μM (8,500 ppb) to an average concentration of 0.04 μM (5 ppb) by the time it reached the first row of monitoring wells approximately 50 hours later. Assuming pseudo-first order kinetics apply, this rate of TCE removal translates into a half-life of 4.7 hours ($k = -0.1488 \text{ h}^{-1}$). This rate of removal remained constant when the influent concentration of TCE increased to 1,286 μM (169,000 ppb) during Week 24. The concentration in the injected water once it reached nearby MW-3 was only 0.53 μM (69.4 ppb), which translates into a half-life of 4.4 hours ($k = -0.155 \text{ h}^{-1}$).

The dramatic reduction in TCE concentrations contributed to the accumulation of cDCE during the demonstration. Increases in vinyl chloride levels suggest that cDCE was being dechlorinated, but at a significantly slower rate than TCE. The maximum VC concentration was only 3.5 μM (217 ppb) and comprised a very small percentage of the overall chloroethene mass. Ethene and ethane concentration remained at or near detection limits throughout most of the demonstration.

4.4 Site #4: Marine Corps Base Camp Lejeune, NC

4.4.1 Site Description

The contamination at Site 88, Marine Corps Base Camp Lejeune, occurred as a result of past operating procedures at the Base Dry Cleaners as well as leaking underground storage tanks at the site. The surficial aquifer was encountered at depths of 6 to 15 feet bgs. The aquifer consists of a series of sediments, primarily sand and clay, which commonly extend to depths of 75 feet. The principal water supply for the base is found in the series of sand and limestone beds that occur between 50 and 300 feet bgs. This series of sediments generally is known as the Castle Hayne Formation, associated with the Castle Hayne Aquifer. The top of the Castle Hayne Aquifer was found at a depth of 40 to 60 feet bgs. Clay layers occur in both of the aquifers. However, the layers are thin and discontinuous in most of the area, and no continuous clay layer separates the surficial aquifer from the Castle Hayne Aquifer. Thin, discontinuous layers and lenses of silt, clay, and/or peat were scattered throughout the sand. The hydraulic conductivity values estimated for the upper portion of the surficial aquifer ranged from 0.4 feet/day to 29.7 feet/day. The hydraulic conductivity values estimated for the lower portion of the surficial aquifer ranged from 56.4 feet/day to 85.5 feet/day.

4.4.2 Microcosms: Camp Lejeune, NC

Core samples were taken from Camp Lejeune for microbial analysis in November 2000. Because previous site characterization indicated varying contaminant and geochemical profiles at increasing depths, two distinct microcosm sets were constructed. The first set was assembled using core material and groundwater from 15 to 19 feet bgs, while the second used core material and groundwater from 45 to 49 feet bgs. The construction of two microcosm sets was undertaken to assess more fully the potential for stimulating dechlorinating activity in the area. Transferring material between two microcosms from different depths should provide information about potential inhibitory conditions at the site, as well as an indication of the promise of implementing a recirculating system in the field study. Monitoring of the Camp Lejeune microcosms is currently in progress.

4.4.3 Field Study: Camp Lejeune, NC

A conventional RABITT test system consisting of 3 injection wells and an array of 9 monitoring wells was installed at Camp Lejeune in April 2001. The wells were installed to a depth of 48 ft bgs and covered an area approximately 4 ft wide by 30 ft long. Existing wells are being used to monitor background groundwater characteristics and supply groundwater for the demonstration. Injection of contaminated groundwater amended with butyric acid and yeast extract commenced in June 2001, with a total system pumping rate of approximately 0.65 L/min. The expected completion date for the field demonstration is December 2001.

Preliminary results show groundwater at the site is anaerobic and highly reduced (-200 mV), and PCE concentrations within the testing zone average about 33 μM (5,500 ppb). Other chloroethenes are present, but at considerably lower concentrations. The average concentrations of TCE, cDCE, and VC within the testing zone are 2.9 μM , 0.8 μM , and <0.1 μM , respectively.

5.0 SUMMARY

To date RABITT demonstrations have been completed at three Department of Defense facilities- Cape Canaveral Air Station, FL; Alameda Point, CA; and Ft. Lewis, WA. The fourth and final facility, Camp Lejeune, NC, has been initiated and will be completed by the end of the year.

Microcosm studies were conducted at each demonstration site to gauge the probability of enhancing reductive dechlorination and to examine a suite of electron donors for efficacy. In all four cases, the electron donor butyric acid demonstrated results equal or superior to all other donors tested. This assessment is based on the percentage of reducing equivalents used for dechlorination and on the rate and degree of dechlorination.

The design and operation of each RABITT field demonstration system was tailored to site-specific characteristics. The site's hydrogeology, regulatory environment, and results from microcosm testing all influenced the design and operation of the system. An overview of each system's design is outlined in Table 2. Despite differences in site characteristics and system design, each of the three completed demonstrations showed rapid reduction of TCE to cDCE. At Cape Canaveral and Alameda this reduction proceeded to ethene. The demonstration at Ft. Lewis was exposed to extraordinarily high concentrations of TCE (169,000 ppb), but no slowdown in microbial activity was observed, and dechlorination continued at a remarkable pace. The high-rate dechlorination observed at Ft. Lewis did lead to an accumulation of cDCE,

but increases in the VC concentration suggest that dechlorination was proceeding past cDCE. Results from field demonstrations were generally in agreement with microcosm test results. Results from Camp Lejeune are still pending.

Table 2. Overview of RABITT System Design at Each of the Four Demonstration Sites

Design Parameter	Units	Cape Canaveral	Alameda Point	Ft. Lewis	Camp Lejeune
System flowrate	L/min	7.5	0.6	1.5	0.6
Flow pattern	NA	Circulation	Linear flow-through	Linear flow-through	Radial flow-through
Surface dimensions	ft.	10 x 34	3 x 15	4 x 30	4 x 30
Depth	ft. bgs	10-20	24-27	26-29	45-48
Sampling locations	number	49	12	12	12
Demonstration duration	Days	169	194	179	ongoing
Electron donor	NA	Lactic acid	Butyric acid	Butyric acid	Butyric acid
Yeast extract ^a	mg/L	None	20	20	20
Sodium bicarbonate ^a	mg/L	None	None	279	None

NA – not applicable.

a – target in situ concentration.

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