

FINAL

PROTOCOL FOR *IN SITU* BIOREMEDIATION OF  
CHLORINATED SOLVENTS USING EDIBLE OIL



October 2007

Prepared for:

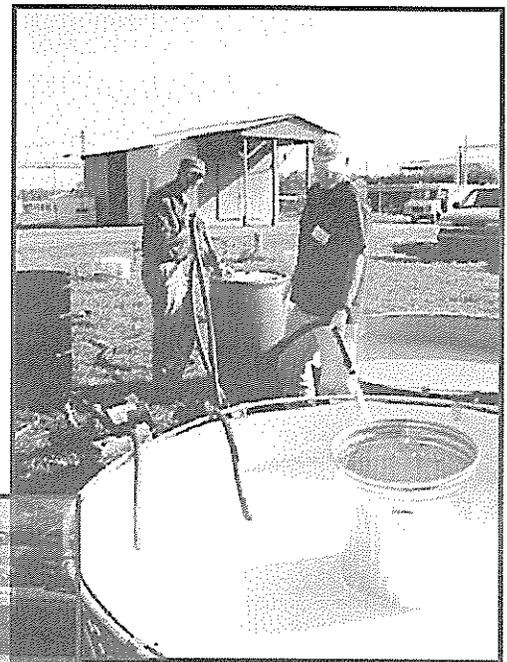
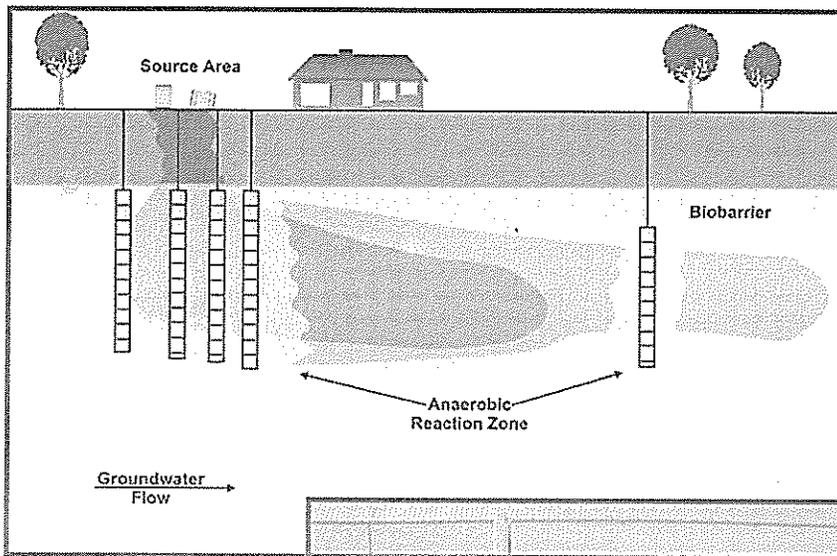
Air Force Center for Engineering and the Environmental  
Environmental Science Division  
Technology Transfer Outreach Office

(AFCEE)



# FINAL

## Protocol for *In Situ* Bioremediation of Chlorinated Solvents Using Edible Oil



U.S. AIR FORCE

October 2007



AFCEE [ Protocol For In Situ Biodegradation Remediation  
of Chlorinated Solvents using  
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## SECTION 6

### DATA EVALUATION AND REPORTING

Several methods are available to assess the effectiveness of using edible oil for enhanced *in situ* anaerobic bioremediation of chlorinated solvents. In all cases, multiple line of converging contaminant, hydrogeologic, geochemical, and microbial data should be used. Among these assessments are changes in contaminant concentration and mass over time, changes in groundwater geochemistry, and an increase in contaminant biodegradation rates. Protocols used to evaluate MNA (e.g., USEPA, 1998; AFCEE, 2000b; AFCEE, 2003) and for enhanced anaerobic bioremediation (AFCEE *et al.*, 2004) provide references for many of these methods and techniques.

#### 6.1 CHANGES IN CONTAMINANT CONCENTRATION AND MASS

The primary objective of enhanced *in situ* anaerobic bioremediation is a decrease in contaminant concentration and mass by anaerobic degradation processes. Measurement of contaminant concentrations in groundwater both before and after edible oil addition that demonstrate a reduction in contaminant mass can be used to show that enhanced bioremediation is an effective remedy. In addition, a change in the molar ratios of parent compounds to dechlorination products can be useful in evaluating the extent to which anaerobic reductive dechlorination is occurring.

Many of these methods have been developed to evaluate MNA. The AFCEE Long Term Monitoring Decision Support Software Package with the Monitoring and Remediation Optimization System (MAROS) (AFCEE, 2003) is a useful example of computational tools used to evaluate contaminant trends and plume attenuation (accessed on the AFCEE web page at <http://www.afcee.brooks.af.mil/products/techtrans/models.asp>). Typically, trends resulting from substrate addition will be readily apparent in the immediate reaction zone. Therefore, some of these tools will more useful for determining the effects of localized treatment (e.g., source reduction or a biobarrier) on greater overall plume dynamics.

It is important when evaluating the attenuation of a contaminant plume that the data demonstrate a clear and meaningful trend in contaminant concentration and/or mass over time at appropriate monitoring locations. The following sections describe some of the common considerations and methods used to determine trends in contaminant concentration and mass when using edible oil for enhanced *in situ* anaerobic bioremediation of chlorinated solvents. Both visual and mathematical methods can be used to evaluate mass reduction and plume attenuation.

Section 3 Pilot Test Planning & Monitoring

Radius of Influence



# Chlorinated Aliphatic Hydrocarbons (CAH)

## 6.1.1 Partitioning of Contaminant Mass into Edible Oil

Interpretation of trends in dissolved CAH concentrations is complicated by the effects of dissolution, sorption/desorption, and partitioning. For example, partitioning of CAH mass from the dissolved phase to the edible oil may cause an initial 'apparent' reduction in dissolved concentrations. The practitioner of enhanced bioremediation using edible oil should be aware of these processes when interpreting groundwater analytical results.

Because chlorinated compounds are hydrophobic, partitioning of these compounds into the edible oil is likely to occur. Table D.1 in Appendix D lists experimental partitioning coefficients of chlorinated ethenes into edible oil. Table 6.1 shows concentrations of chlorinated compounds in edible oil and groundwater in an injection well before and after injection of pure soybean oil for a pilot test site at CCAFS in Florida (Parsons, 2002a; Appendix H). The concentration of TCE in groundwater in the injection well dropped from 100,000 micrograms per liter ( $\mu\text{g/L}$ ) to 84  $\mu\text{g/L}$  within approximately 2 months of oil injection. The concentrations of *cis*-1,2-DCE and VC also decreased rapidly.

**Table 6.1**  
Concentrations of Chlorinated Compounds in Vegetable Oil and Groundwater in an Injection Well at the Hangar K Site, CCAFS, Florida (Parsons, 2002a)

Compound	Date and Concentration ( $\mu\text{g/L}$ )									
	6/8/99 <sup>a/</sup>		8/24/99		10/19/99		11/17/99		12/14/99	
	Oil	Water	Oil	Water	Oil	Water	Oil	Water	Oil	Water
TCE	ND	100,000	44,000	84	99,000	230	47,000	130	68,000	160
<i>cis</i> -1,2-DCE	ND	48,000	15,000	230	29,000	660	13,000	340	22,000	460
VC	ND	330	3,900	<10	<1,000	56	<1,000	<10	<1,000	<10

<sup>a/</sup> Oil injected on June 15, 1999.

ND = Not Detected

It is important that this rapid reduction in groundwater contaminant concentration not be attributed entirely to biodegradation. In this case, much of the observed reduction in contaminant concentrations was caused by partitioning of the chlorinated compounds into the edible oil. An evaluation of molar ratios may be required in this case to determine if conversion of higher chlorinated compounds to lower chlorinated compounds is occurring due to anaerobic reductive dechlorination. The following sections provide a brief discussion of several methods that are used to visualize the biological transformations that are occurring in the aquifer.

## 6.1.2 Visual Techniques for Determining Contaminant Trends

There are several ways to present data showing changes in contaminant concentrations and plume configuration over time after edible oil injection. The following subsections describe some of the common techniques available to illustrate contaminant reduction.

### 6.1.2.1 Concentration Isopleth Maps

One method consists of preparing isopleth maps of contaminant concentrations over space and/or time. Note that incomplete site characterization may bias the interpretation of isopleth maps. **Figure 6.1** shows isopleth maps of TCE in groundwater for a field test site at Travis AFB, California (Parsons, 2004b). Note that the multi-year contaminant data were collected during approximately the same seasonal time period. This is important because seasonal variations in aquifer recharge can cause significant changes in contaminant concentrations and groundwater geochemistry. **Figure 6.1** indicates that enhanced biodegradation is effective in removing TCE from the subsurface.

### 6.1.2.2 Concentration Versus Time and Distance Plots

A method that can be used to present data showing changes in contaminant concentrations and plume configuration is to plot contaminant concentrations versus time for individual monitoring wells, or to plot contaminant concentrations versus distance downgradient for several wells along a groundwater flow path over several sampling events. Traditional data presentations show the changes in concentration of each target compound or indicator parameter. After treatment is initiated, plots of individual contaminants including parent compounds and degradation products also can be useful in evaluating the effectiveness of the edible oil application.

Trends in the data can be analyzed by plotting concentration data versus time. Where there are high starting concentrations, data can be plotted on semi-log paper with log concentration being plotted against linear time. Plotting the concentration data on the log scale counters the relatively large changes in concentration data (*e.g.*, a concentration reduction from 1.0 mg/L to 1.0 µg/L represents a 1,000-fold reduction). **Figure 6.2** shows conceptually how concentrations of individual compounds change as sequential anaerobic reductive dechlorination proceeds. It is important to keep in mind that Figure 6.2 is a conceptual model only.

Evaluating the change in the molar concentrations and fractions (or ratios) of parent compounds to dechlorination products also can be very useful in determining the efficacy of biodegradation brought about by edible oil injection. During biodegradation, the molar ratios of the compounds involved in the reaction chain will change. **Looking at molar concentrations is more accurate and informative than evaluating changes in concentration alone for the parent/dechlorination products because of the different molecular weights of the compounds.** **Appendix D** provides the molecular weight of various chlorinated compounds. Calculation of molar concentrations is described in Section 6.3 of AFCEE, *et al.* (2004).

Plotting the molar fraction or ratio over time is often used when there is a constant or continuing source of contaminant mass entering a treatment zone. In this case, the total molar concentration may remain elevated or even increase because of continuing mass inputs, but an increase in the molar ratio of dechlorination products will demonstrate that sequential anaerobic reductive dechlorination is occurring (AFCEE *et al.*, 2004).

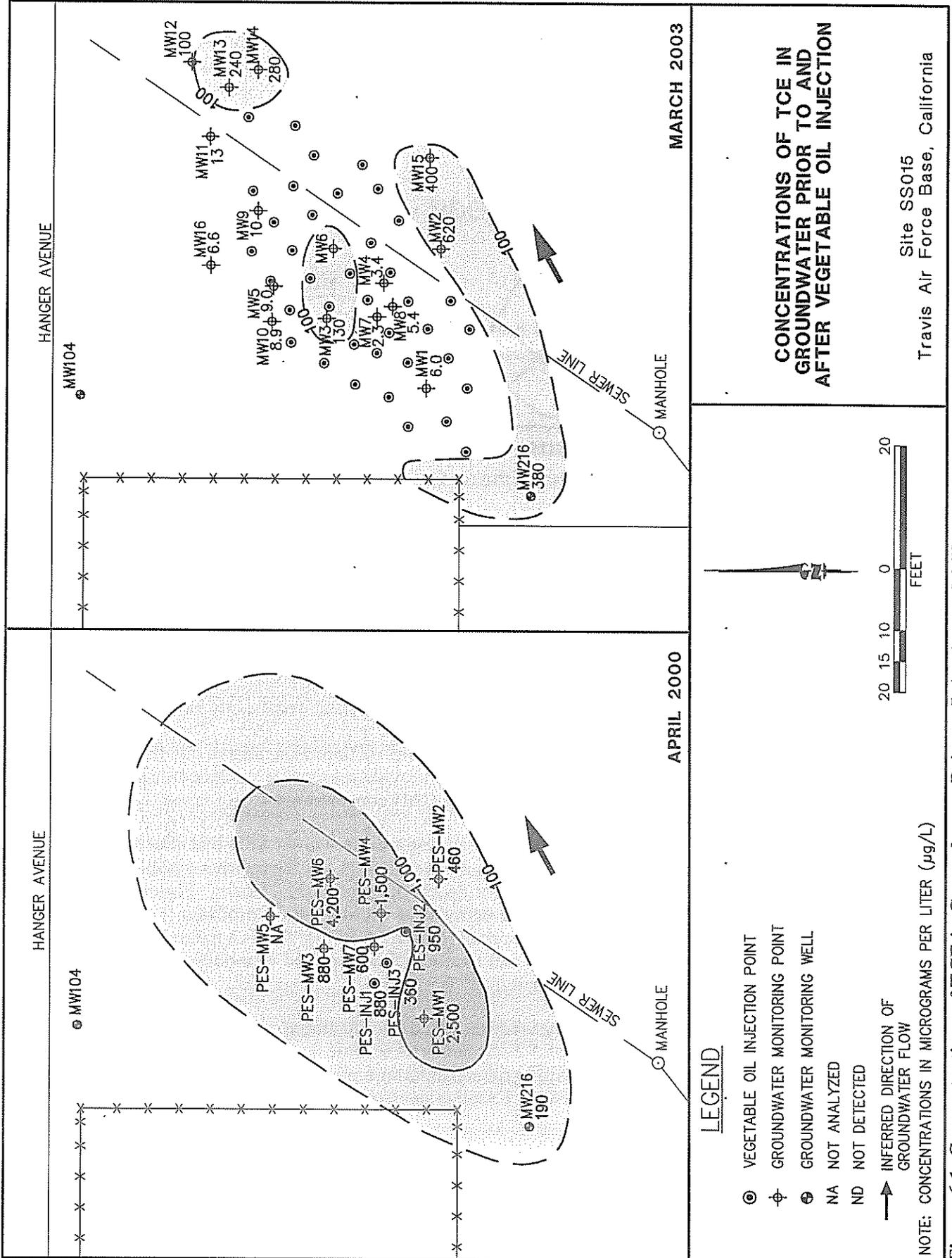
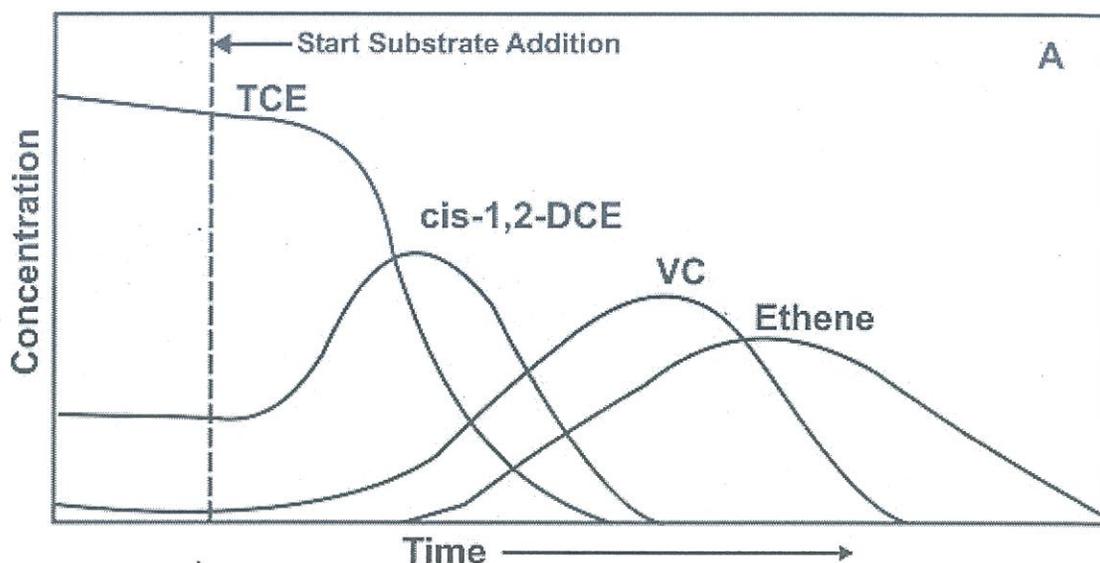


Figure 6.1 Concentrations of TCE in Groundwater Prior to Injection and 3 Years After Injection at site SS-015, Travis AFB, California

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**Figure 6.2** Changes in Chlorinated Ethenes Over Time Due to Sequential Reductive Dechlorination

By converting concentration to molar concentration and plotting these values versus distance from the treatment zone allows the practitioner to evaluate the effectiveness of the treatment and its influence along the groundwater flow path. Plotting changes to the molar fraction or total molar concentration at one location in the treatment zone is a way of determining the effectiveness of the treatment at that location.

Figure 6.3 presents a plot of total molar concentration of CAHs versus distance along the flow path through an edible oil treatment zone for three sampling events, one before edible oil injection (July 2000) and two after injection (April 2002 and April 2003). The combination of decreasing contaminant concentrations after edible oil injection shown by the plot on Figure 6.3, and the lack of contaminant migration provide reasonable evidence that addition of an edible oil substrate was effective in reducing CAH concentrations.

Figure 6.4 shows data derived from a monitoring well at Travis AFB that illustrate how concentrations of individual compounds changed over time at a location where edible oil was used to stimulate sequential reductive dechlorination of chlorinated ethenes. Figure 6.4 also points out that temporal accumulation of intermediate dechlorination products (*i.e.*, *cis*-1,2-DCE and VC) may be observed. It is important to understand that this is a natural occurrence with sequential reductive dechlorination and that a sufficient period of monitoring time must be allowed for the process to run its course.

Figure 6.5 is an alternative presentation of data illustrating the changes in molar concentration of the target chlorinated compounds over time in a source area. The data, derived from a pilot test using emulsified oil substrate in a pilot test conducted at the Tarheel Army Missile Plant in Burlington, North Carolina averages data from four injection/monitor wells.

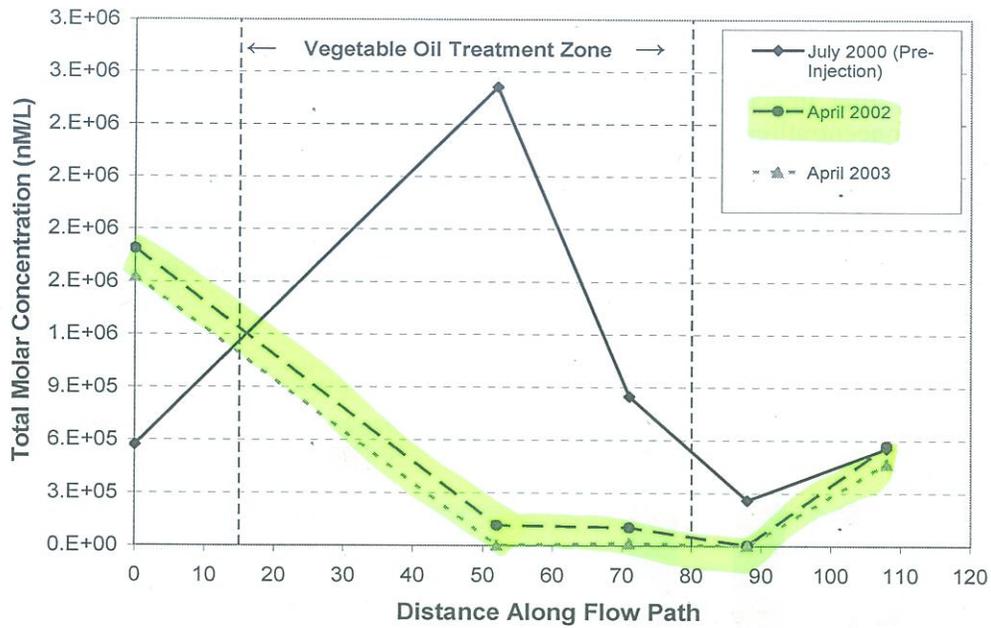


Figure 6.3 Changes in Total Molar Concentration (PCE + TCE + DCE + VC + Ethene) Over Distance along a Central Flow Path through a Treatment Zone at CCAFS, Florida

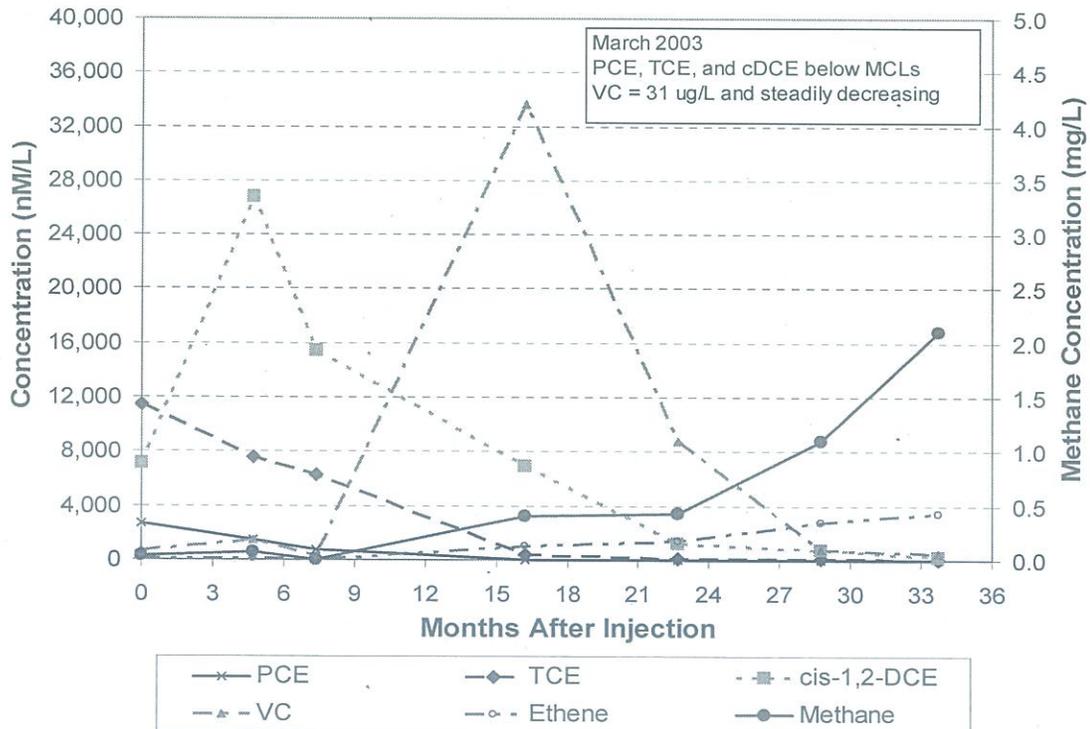


Figure 6.4 Changes in Concentration of Chlorinated Ethenes, Ethene, and Methane Over Time at Well MP04, Travis AFB, California (Parsons, 2004a)

The principle contaminant prior to injection of substrate was TCE with some *cis*-1,2-DCE present. Soon after injection, TCE was reduced substantially with production of *cis*-1,2-DCE. By 287 days after injection, much of the *cis*-1,2-DCE was converted to VC. The relative changes in concentrations of chloroethenes in Figure 6.5 are consistent with the conceptual changes associated with sequential reductive dechlorination as illustrated in Figure 6.2. However, the sharp decrease in total molar concentration between 50 and 107 days from injection may also be indicative of an alternate attenuation mechanism (e.g., biogeochemical reduction) because conservation of molar concentrations of TCE and *cis*-1,2-DCE to VC and ethene was not observed.

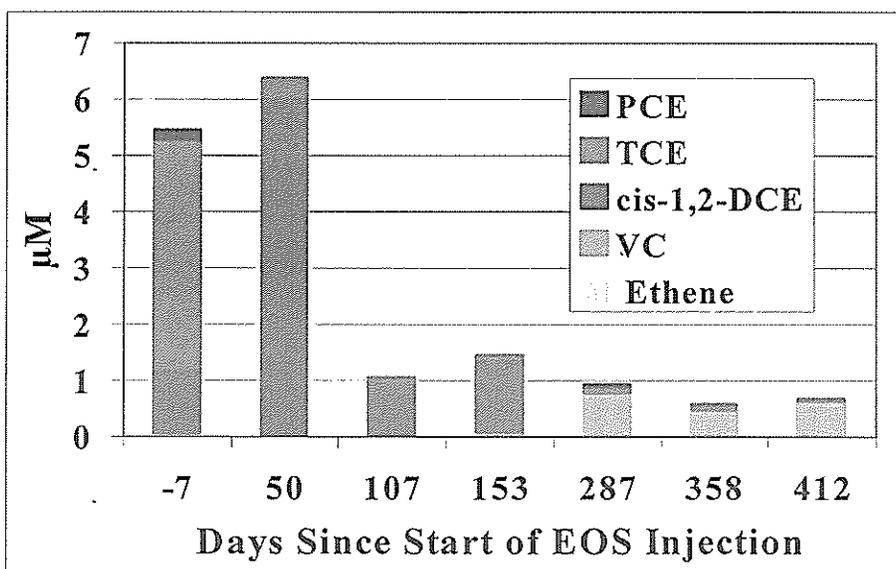


Figure 6.5 Changes to Molar Concentrations of Chlorinated Compounds in Groundwater after Injection of Emulsified Oil Substrate (Solutions-IES, 2005)

### 6.1.3 Chlorine Number Plots

Chlorine number (Cl#) is another approach for evaluating the effect of anaerobic biodegradation processes, particularly the extent to which sequential degradation of PCE or TCE is occurring. Cl# is calculated as:

$$\text{Cl\#} = \frac{4 [\text{PCE}] + 3 [\text{TCE}] + 2 [\text{DCE}] + [\text{VC}]}{[\text{PCE}] + [\text{TCE}] + [\text{DCE}] + [\text{VC}] + [\text{ethene}] + [\text{ethane}] + [\text{acetylene}]}$$

where [ ] indicates concentration in moles per liter. For example, groundwater containing only TCE would have a Cl# = 3.0. However, if half of the TCE is reduced to DCE, the Cl# would decline to 2.5. When calculating Cl#, it is assumed that non-detect measurements are equal to zero and that ethene, ethane, and acetylene (due to abiotic transformation) are stable under reducing conditions. The change in Cl# to <1.0 suggests complete transformation from chlorinated parent molecules to non-chlorinated, non-toxic end products.

**Figure 6.6** shows the variation in Cl# before (November 2001) and 13 months after emulsion injection (January 2003) in a pilot test at Altus AFB Site SS-17. There was a substantial decline in Cl# in all of the injection wells following emulsion injection. In contrast, there was no significant change in Cl# in upgradient monitoring well TS-MW-1.

In the downgradient monitoring wells, the results were more variable. In TS-MW-5, the Cl# dropped from 2.17 prior to injection to 0.63 in January 2003 indicating substantial conversion of TCE to lesser-chlorinated compounds. In downgradient monitoring wells TS-MW-2 and TS-MW-3, there was no substantial change in Cl# with time due. This was due to the very low permeability of the aquifer in this area, which prevented effective distribution of organic substrate to these wells. Over the next year, the Cl# in TS-MW-2 and TS-MW-3 did gradually drop as treated water migrated into this area from the upgradient biobarrier (data not shown in **Figure 6.6**).

#### **6.1.4 Statistical Techniques for Determining Contaminant Trends**

The Principles and Practices document (AFCEE *et al.*, 2004; Section 6) provides an introduction to statistical techniques for determining contaminant trends. These techniques are usually intended for use in determining the effectiveness of MNA, but may be useful for evaluating the impact of a source reduction or biobarrier configuration on overall plume dynamics. The following overview of statistical methods is excerpted from the Principles and Practices document.

First, trends can be analyzed by plotting concentration data versus time, usually on semi-log paper with log concentration being plotted against linear time. Linear regression calculations can then be used to evaluate concentration trend. Discerning trends in the plotted data can be subjective process, particularly if the data do not display a uniform trend, but show some variability over time. In these cases a statistical test such as the Mann-Whitney U Test or Mann-Kendall Test can be useful. As mentioned previously, seasonal effects on contaminant concentrations should be considered in any trend analysis. Statistical methods should not be used to analyze apparent trends across data points that are not comparable. Initial comparative analyses should be conducted using data from similar hydrogeological conditions (such as seasons) and data quality. More detailed presentations of the steps required to use either the Mann-Whitney or the Mann-Kendall Tests are provided in AFCEE *et al.* (2004).

The Mann-Whitney U test (also called the Wilcoxon Rank-Sum Test) is currently being used by the State of New Jersey to determine plume stability (28 N.J.R. 1143). The test is performed using data for every contaminant at every monitoring well at a site where this plume stability test is being applied. The test is nonparametric (Mann and Whitney, 1947), which means that the test does not assume that the data are normally distributed.

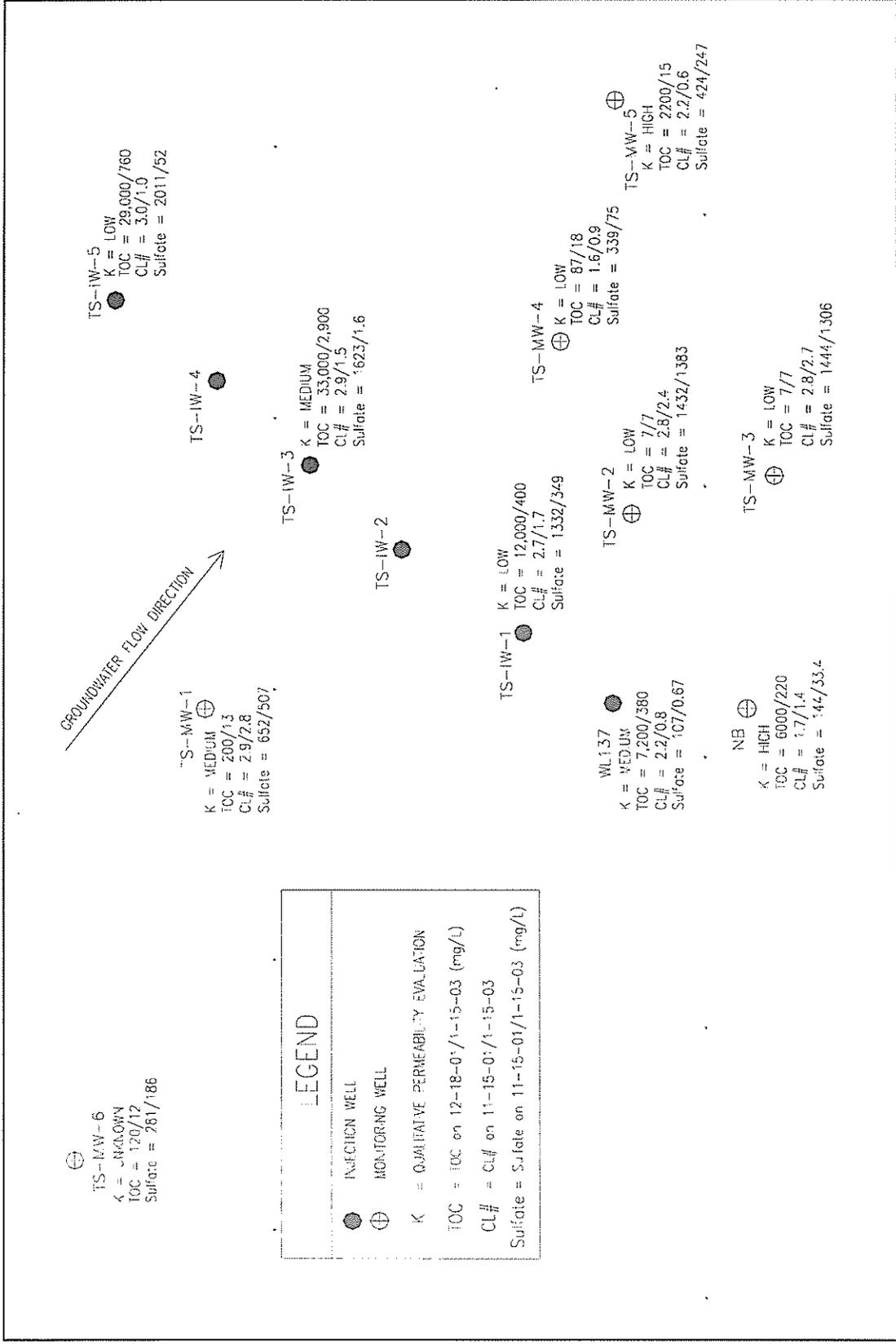


Figure 6.6 Relative Hydraulic Conductivity, TOC, Sulfate, and Chlorine Numbers throughout Altus AFB Pilot Test Plot

The Mann-Kendall Test is another nonparametric test (Gilbert, 1987) that can be used to define the stability of a solute plume (*i.e.*, stable, diminishing, or expanding) based on concentration trends at individual wells. To evaluate plume stability or attenuation, four or more independent sampling events are required. As with the Mann-Whitney test, the Mann-Kendall test is applied to data for each contaminant of interest at each monitoring well located in plume area. This approach has limitations, as data sets can show a tremendous amount of scatter but still return the conclusion that the plume is stable (*i.e.*, no significant trend could be established statistically). To counter this problem, one can apply a more sophisticated analysis using Mann-Kendall by comparing the Mann-Kendall S statistic, a calculated confidence level, and the coefficient of variance for the sample data (Gilbert, 1987).

## 6.2 CHANGES IN GROUNDWATER GEOCHEMISTRY

Assessing biological activity at a field site based on monitoring data can be difficult. However, there are a number of monitoring parameters that can be indicative of anaerobic biodegradation processes. First, the presence of methane in the groundwater indicates that fermentation is occurring and that the potential for reductive dechlorination exists. Second, the transformation of PCE and TCE has been studied intensely and many researchers report that of the three possible DCE isomers, 1,1-DCE is the least significant intermediate and that *cis*-1,2-DCE predominates over *trans*-1,2-DCE (Barrio-Lage *et al.*, 1987, Parsons *et al.*, 1984; Parsons *et al.*, 1985). If *cis*-1,2-DCE comprises more than 80 percent of the total mass of the DCE isomers then the DCE is likely the result of biodegradation (USEPA, 1998).

This is in line with statements made by the Remediation Technologies Development Forum (RTDF) research consortium (RTDF, 1997) who reported that TCE biodegradation yields greater than 80% of the *cis*-1,2-DCE isomer (*trans*-1,2-DCE may be produced as well), while manufactured DCE is typically only 10 to 20% *cis*-1,2-DCE. Note that if 1,1,1-TCA is present at the site, then dehydrochlorination of 1,1,1-TCA will produce 1,1-DCE, which may interfere with this type of analysis. Third, because chlorinated ethenes are 55 to 85 percent chlorine by mass, the degradation of these compounds releases a large mass of chloride. Therefore, elevated chloride concentrations are also indicative of reductive dechlorination.

To summarize, the following rules-of-thumb indicate that site conditions are suitable for anaerobic reductive dechlorination to occur:

1. DO concentrations are low (less than 0.5 mg/L) and ORP is low (less than 0.0 mV),
2. Fe(II) is being produced and sulfate is depleted,
3. Methane is being produced, and
4. Hydrogen concentrations are greater than 1 nanomolar or nanomoles per liter (1 nM or nmol/L).

The following are site conditions that indicate anaerobic reductive dechlorination is occurring:

1. Dechlorination products are being produced (such as *cis*-1,2-DCE or VC),
2. Ethene and ethane are being produced (even low concentrations are indicative of biodegradation), and
3. Chloride concentrations are elevated.

Table 6.2 summarizes the trends in various analyte concentrations during biodegradation. Figure 6.7 illustrates conceptually how select geochemical parameters typically change over time after edible oil injection. The variability associated with collecting groundwater samples makes precise definitions of reactions or zones of differing oxidation-reduction potential difficult, and the various types of evidence should be weighed together to determine if edible oil addition has stimulated reductive dechlorination.

**Table 6.2**  
**Trends in Contaminant, Electron Acceptor, Metabolic Byproduct, and Total Alkalinity Concentration during Biodegradation**

Analyte	Terminal Electron Accepting Process	Trend in Analyte Concentration During Biodegradation
Fuel Hydrocarbons	Aerobic Respiration, Denitrification, Manganese (IV) Reduction, Iron (III) Reduction, Methanogenesis	Decreases
Highly Chlorinated Solvents and Daughter Products	Reductive Dechlorination	Parent Compounds Decrease, Daughter Products Increase Initially and then may Decrease
Lightly Chlorinated Solvents	Aerobic Respiration, Denitrification, Manganese (IV) Reduction, Iron (III) Reduction (Direct Oxidation)	Compound Concentration Decreases
Isotopic enrichment of parent compounds and dechlorination products.	Reductive Dechlorination	Changes in stable carbon isotope fractionation indicate degradation is occurring, versus a change in contaminant concentration due to non-destructive processes such as partitioning or dilution.
Changes in density of dechlorinating population density (e.g., Dehalococcoides)	Reductive Dechlorination	Increase in density (cells per liter) of dechlorinating populations.
Dissolved Oxygen	Aerobic Respiration	Decreases
Nitrate	Denitrification	Decreases
Manganese (II)	Manganese (IV) Reduction	Increases
Iron (II)	Iron (III) Reduction	Increases
Sulfate	Sulfate Reduction	Decreases
Methane	Methanogenesis	Increases
Chloride	Reductive Dechlorination or Direct Oxidation of Chlorinated Compound	Increases
ORP	Aerobic Respiration, Denitrification, Manganese (IV) Reduction, Iron (III) Reduction, Methanogenesis	Decreases
Alkalinity	Aerobic Respiration, Denitrification, Iron (III) Reduction, and Sulfate Reduction	Increases

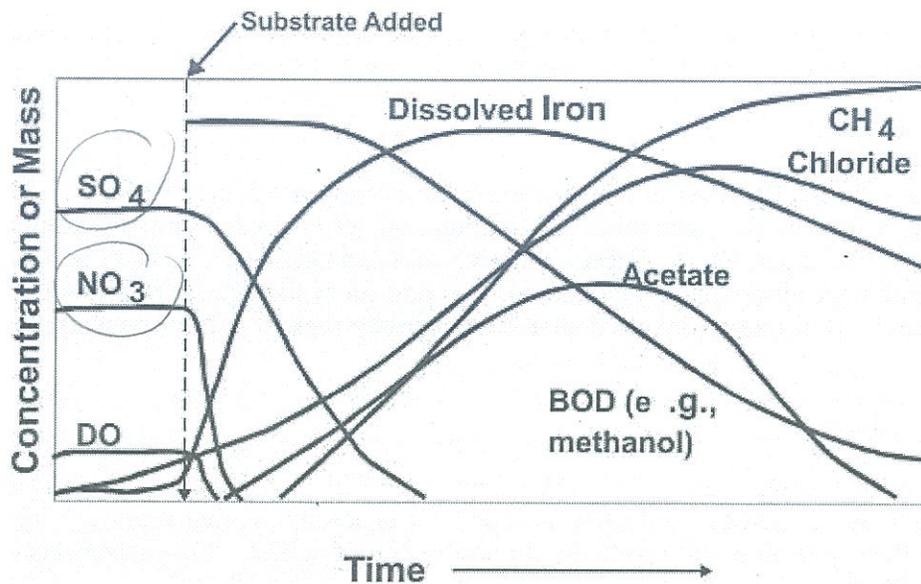


Figure 6.7 Changes in Select Geochemical Indicator Parameters Over Time Due to Anaerobic Biodegradation of Organic Carbon

### 6.2.1 Native Electron Acceptors

Native electron acceptors potentially compete for substrate (*e.g.*, hydrogen) at the expense of anaerobic reductive dechlorination of CAHs. After depletion of DO, anaerobic microbes will use nitrate as an electron acceptor, followed by manganese IV [Mn(IV)], ferric iron [Fe(III)], then sulfate, and finally carbon dioxide (methanogenesis). Each sequential reaction drives the redox potential of the groundwater downward into the range within which reductive dechlorination can occur most efficiently. Some electron acceptors (DO, nitrate, and sulfate) can be measured directly in groundwater. It is easier to evaluate the use of manganese Mn(IV) and Fe(III) based on an measurement of their reduced forms, or the use of carbon dioxide by measuring the production of methane, (Section 6.2.2.2).

#### 6.2.1.1 Dissolved Oxygen

DO is the most thermodynamically favored electron acceptor used by microbes for the biodegradation of organic carbon, whether natural or anthropogenic. Anaerobic bacteria generally cannot function at DO concentrations greater than about 0.5 mg/L, and hence anaerobic reductive dechlorination will not occur. It is important to have a source of organic carbon such as edible oil in the aquifer that can be used by aerobic microorganisms as a primary substrate. During aerobic respiration, DO is depleted and concentrations will decrease.

Trends in concentrations of DO can be used to assess the effectiveness of the edible oil process, especially in areas that previously were aerobic. Depending on the amount of fermentable organic carbon already present in the aquifer, DO levels may already be depleted and addition of an organic substrate may not result in a significant change in DO concentrations across the site. Note that from a practical perspective, systematic problems with the sampling and analysis of DO are common, mainly because even a minor exposure of sampled ground water to atmospheric oxygen can cause a high bias. Conflicting data, like

What analytical Method?

\*

\* elevated DO, should be viewed as primarily a sampling problem when the preponderance of other geochemical parameters suggests that anaerobic conditions exist.

### 6.2.1.2 Nitrate

After DO has been depleted in the microbiological treatment zone, nitrate may be used as an electron acceptor for anaerobic biodegradation of organic carbon, primarily via denitrification. In order for anaerobic reductive dechlorination of CAHs to be an efficient process, nitrate concentrations in the contaminated portion of the aquifer must be less than 1.0 mg/L. However, if nitrate is already depleted or naturally present at low concentrations, there may be little change in nitrate concentrations.

### 6.2.1.3 Sulfate

After dissolved oxygen and nitrate have been depleted in the microbiological treatment zone, sulfate may be used as an electron acceptor for anaerobic biodegradation. This process is termed sulfate reduction and results in the production of sulfide. Concentrations of sulfate greater than 20 mg/L may cause a substantial amount of substrate (e.g., hydrogen) to be used for sulfate reduction. Sulfate must be reduced in order to reach methanogenic conditions, and high sulfate levels may lower the efficiency at which substrate is utilized for reductive dechlorination of CAHs.

However, in many plumes with high concentrations of sulfate, reductive dechlorination of CAHs can still occur. Complete reductive dechlorination has been stimulated at several high-sulfate Air Force sites using edible oil including sites at Altus AFB, Oklahoma (sulfate up to 2,200 mg/L) (Appendix H) and at Travis AFB, California (sulfate up to 5,400 mg/L). It should be recognized that multiple processes may occur (e.g., biogeochemical reduction) within any enhanced bioremediation system, and that the presence of high sulfate concentrations does not necessarily preclude effective application of the edible oil process. Excessive levels of sulfides produced by reduction of sulfate can be inhibitory to microorganisms that facilitate reductive dechlorination of CAHs. This can be the case at high sulfate/low iron sites where there is insufficient iron to precipitate sulfides out of solution.

## 6.2.2 Metabolic Byproducts and Oxidation-Reduction Potential

Depending on initial groundwater geochemistry, metabolic byproducts of biodegradation can include ferrous iron (Fe(II)), manganese II (Mn(II)), methane, ethane, ethene, increased alkalinity, chloride, reduced redox potential, dissolved hydrogen, and VFAs.

### 6.2.2.1 Iron (II) and Manganese (II)

\* In some cases, solid-phase bioavailable Fe(III) and Mn(IV) are used as electron acceptors during anaerobic biodegradation of organic carbon. During this process, Fe(III) is reduced to Fe(II), which is soluble in most groundwater systems. Similarly, Mn(IV) is reduced to soluble Mn(II). Therefore, concentrations of Fe(II) and Mn(II) in groundwater can be used as indicators of anaerobic biodegradation. Care must be taken when interpreting Fe(II) concentrations because they may be biased low by re-precipitation with sulfides.

### 6.2.2.2 Methane, Ethane, Ethene ✓

\* Methanogenesis is characterized by coupled enzymatic reactions. Excess hydrogen released during substrate fermentation can be used to reduce carbon dioxide to methane. In addition, acetate may also be fermented forming carbon dioxide and methane. Methanogenesis generally occurs after oxygen, nitrate, Fe(III), and sulfate have been depleted in the treatment zone. The presence of methane in groundwater is indicative of strongly reducing conditions because methanogenic bacteria are obligate anaerobes. The presence of methane above background concentrations in areas with chlorinated solvents is an indication that the groundwater geochemical conditions are favorable for anaerobic reductive dechlorination. The fastest and most complete reductive dechlorination of CAHs typically occurs under methanogenic conditions.

Ethane and ethene are the desired end-products of the reductive dechlorination process for chlorinated ethenes and chlorinated ethanes. These light hydrocarbon gasses can be detected in groundwater at low concentrations, but tend not to accumulate as a result of diffusion into a volatile phase (gas) or as a result of further biodegradation to carbon dioxide (CO<sub>2</sub>) and water. The presence of elevated levels of ethene and ethane are favorable indicators that the biodegradation pathway for sequential reductive dechlorination is complete.

### 6.2.2.3 pH and Alkalinity ✓

\* There is often a positive correlation between zones of microbial activity and increased alkalinity. Increases in alkalinity result from dissolution of carbonate mineral by carbon dioxide produced during substrate biodegradation and by reduction of iron oxide minerals. Alkalinity is important in the maintenance of groundwater pH because it buffers the groundwater system against acids generated during both aerobic and anaerobic biodegradation. A pH close to neutral (i.e., 6 to 8) is the most conducive to the proliferation of healthy, diverse microbial populations. Low pH conditions (<5) are detrimental to sulfate-reducing and methanogenic bacteria. Fermentative organisms that favor lower pH conditions will compete with both sulfate reducing and methanogenic bacteria in this environment. This can result in the formation of undesirable low-molecular-weight organic byproducts of fermentation, such as ketones, alcohols, and aldehydes. Lowering of pH is more prevalent where excessive amounts of organic substrate have been applied.

\* Aquifer systems with lower buffering capacities are more susceptible to a decrease in pH due to biological activity. Alkalinity is an indicator of the buffering capacity of an aquifer system. From a practical standpoint, alkalinity greater than 300 mg/L are generally sufficient to buffer against adverse pH changes. Alkalinity less than 100 to 200 mg/L is a cause for concern, and pH should be monitored carefully. In such cases, pH buffering may be used during implementation to raise and/or neutralize pH against further decreases.

\* Buffering may be implemented using compounds such as sodium bicarbonate (NaHCO<sub>3</sub>), quicklime (CaO), dolomitic quicklime (CaO/MgO), caustic hydroxide (NaOH), and magnesium hydroxide (Mg(OH)). It is more cost effective to add these materials during the initial injection, rather than re-mobilizing to the site for an additional injection of buffering agent. Commercial emulsified oil products are now available in buffering formulations or with buffering mixtures that can be added when diluting and mixing the concentrated product. ✓

#### 6.2.2.4 Chloride

Chloride is released to groundwater during biodegradation of CAHs. This results in chloride concentrations in the contaminant plume that are elevated relative to background concentrations. As a consequence, elevated chloride concentrations can serve as a useful indicator of reductive dechlorination. However, if substantial levels of chloride are present in the background groundwater, it may be difficult to detect small increases in chloride concentration.

Chloride ions generally do not enter into oxidation-reduction reactions, do not form important solute complexes with other ions unless the chloride concentration is extremely high, do not form salts of low solubility, are not significantly adsorbed on mineral surfaces, and play few vital biochemical roles (Hem, 1985). Thus, physical processes control the migration of chloride ions in the subsurface and chloride behaves as a conservative indicator of biological activity.

#### 6.2.2.5 Oxidation-Reduction Potential

The ORP of groundwater is a measure of electron activity (Eh) and is an indicator of the relative tendency of a solution to accept or transfer electrons. The redox of groundwater generally ranges from -400 mV to +800 mV. Oxidation-reduction reactions in groundwater containing organic compounds (natural or anthropogenic) are usually biologically mediated, and therefore, the redox of a groundwater system depends upon and influences rates of biodegradation. Knowledge of the redox of groundwater is important because some biological processes operate only within a prescribed range of redox conditions. Redox measurements can be used to provide real-time data on the location of areas undergoing anaerobic biodegradation. Due to instability, redox measurements should be taken in the field during well purging and immediately before sample acquisition using a direct-reading meter.

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#### 6.2.2.6 Dissolved Hydrogen

Concentrations of dissolved hydrogen have been used to evaluate redox processes, and thus the efficiency of reductive dechlorination, in groundwater systems (Lovley and Goodwin, 1988; Lovley *et al.*, 1994; Chapelle *et al.*, 1995). The concentration of hydrogen can be used to identify groundwater where reductive dechlorination may be occurring. Hydrogen is continuously produced in anoxic groundwater systems by fermentative microorganisms that decompose natural and anthropogenic organic matter. This hydrogen is then consumed by respiratory microorganisms that use nitrate, Fe(III), sulfate, or CO<sub>2</sub> as terminal electron acceptors. This continuous cycling of hydrogen is called interspecies hydrogen transfer.

Significantly, nitrate-, Fe(III)-, sulfate- and CO<sub>2</sub>-reducing (methanogenic) microorganisms exhibit different efficiencies in utilizing the hydrogen that is being continually produced. Nitrate reducers are highly efficient users of hydrogen and maintain very low steady-state hydrogen concentrations. Fe(III) reducers are slightly less efficient and thus maintain somewhat higher hydrogen concentrations. Sulfate reducers and methanogenic bacteria are progressively less efficient and maintain even higher hydrogen concentrations. Because each TEAP has a characteristic hydrogen concentration associated with it, hydrogen concentrations can be an indicator of predominant redox processes. These characteristic ranges are given in Table 6.3. If hydrogen concentrations are very low, reductive dechlorination is not efficient

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and Type III behavior is indicated. If hydrogen concentrations are greater than approximately 1.0 nM, rates of reductive dechlorination should have environmental significance.

**Table 6.3**  
**Range of Hydrogen Concentrations for a Given Terminal Electron-Accepting Process**

TERMINAL ELECTRON-ACCEPTING PROCESS	DISSOLVED HYDROGEN CONCENTRATION		
	(nmol/L)	(atm)*	(µg/L)
Denitrification and Manganese Reduction	< 0.1	$< 1.3 \times 10^{-7}$	$< 2.0 \times 10^{-4}$
Iron (III) Reduction	0.2 to 0.8	$0.26 - 1.0 \times 10^{-6}$	$0.4 - 1.6 \times 10^{-3}$
Sulfate Reduction	1 to 4	$1.3 - 5.0 \times 10^{-6}$	$2.0 - 8.0 \times 10^{-3}$
Methanogenesis	5 to 20	$63 - 250 \times 10^{-6}$	$1.0 - 4.0 \times 10^{-2}$
<b>Optimum for Anaerobic Reductive Dechlorination</b>	<b>2 to 11</b>	<b><math>2.6 - 125 \times 10^{-6}</math></b>	<b><math>4.0 \times 10^{-3} - 2.2 \times 10^{-2}</math></b>

Adapted from Lovley et al., 1994; Chapelle et al., 1995; and Yang and McCarty, 1998 per AFCEE et al., 2004

\* In gas phase in equilibrium with water containing dissolved hydrogen.

Redox measurements are based on the concept of thermodynamic equilibrium and, within the constraints of that assumption, can be used to evaluate redox processes in groundwater systems. The hydrogen method is based on the ecological concept of interspecies hydrogen transfer by microorganisms and, within the constraints of that assumption, can also be used to evaluate redox processes. These methods, therefore, are fundamentally different.

A direct comparison of these methods (Chapelle et al., 1995) has shown that redox measurements were effective in delineating oxic from anoxic groundwater, but that redox measurements could not distinguish between nitrate-reducing, Fe(III)-reducing, sulfate-reducing, or methanogenic zones in an aquifer. In contrast, the hydrogen method could readily distinguish between different anaerobic zones. For those sites where distinguishing between different anaerobic processes is important information, hydrogen measurements are an available technology for making such distinctions. At sites where concentrations of redox sensitive parameters such as dissolved oxygen, Fe(II), sulfide, and methane are sufficient to identify operative redox processes, hydrogen concentrations are not always required to identify redox zones.

In practice, it is preferable to interpret hydrogen concentrations in the context of electron acceptor (oxygen, nitrate, Fe(III), sulfate) availability and the presence of the final products (Fe(II), hydrogen sulfide, methane) of microbial metabolism (Chapelle et al., 1995). For example, if sulfate concentrations in groundwater are less than 0.5 mg/L, methane concentrations are greater than 0.5 mg/L, and hydrogen concentrations are in the 5-20 nM range, it can be concluded with a high degree of certainty that methanogenesis is the predominant redox process in the aquifer. Similar logic can be applied to identifying denitrification (presence of nitrate, hydrogen <0.1 nM); Fe(III) reduction (production of Fe(II), hydrogen 0.2 to 0.8 nM); and sulfate reduction (presence of sulfate, production of sulfide, hydrogen 1-4 nM).

Methods for measuring hydrogen in groundwater are commercially available. Hydrogen measurements can be useful, especially on sites where other parameters may not offer clear-cut indications of conditions favorable for enhanced reductive dechlorination. However, their use is limited, often due to cost of sample collection and analysis.

### 6.3 BIODEGRADATION RATE CONSTANT CALCULATIONS

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If biodegradation has been stimulated by addition of edible oil, then an increase in biodegradation rates should be observed. Biodegradation rate constants should be estimated prior to substrate addition (if possible) and during performance monitoring. Biodegradation rate constant estimates can be calculated by many methods. The reader is referred to such documents as USEPA (1998) and Newell *et al.* (2003) for a detailed discussion of biodegradation rate constant estimation.

While monitoring contaminant biodegradation rates can be very useful, accurate estimation of biodegradation rate constants can be difficult due to partitioning of chlorinated solvents between the sediment, injected oil and aqueous phases. Monitoring well sample protocols tend to preferentially extract sample volume from zones of higher permeability. However, groundwater sampling results will also be influenced by the slow diffusion of contaminants out of lower permeability zones.

\* In contaminant source areas, there are no generally accepted methods for estimating overall average contaminant biodegradation rates. The point decay approach described by Newell *et al.* (2003) can be used to estimate rates of contaminant decline in individual monitor wells. However, these rates may not be representative of the entire treatment zone. Pure edible oil and oil-in-water emulsions are preferentially transported through the higher permeability (K) zones. As a consequence, biodegradation rates may be greater in the higher K zones than low K zones. Contaminant concentrations often decline very rapidly in monitor wells (which preferentially sample the high K zones), even though some contaminants remain in the lower permeability layers.

For a strongly heterogeneous site in the North Carolina Piedmont, Solutions-IES (2005) reported that TCE was reduced from approximately 1,000 µg/L to below detection within 50 days of emulsion injection. However, *cis*-1,2-DCE, VC, and ethene continued to be produced for over 12 twelve months indicating additional TCE was slowly diffusing out of lower permeability zones and being degraded. Slow diffusion of contaminants out of low K zones is not a problem as long as some oil remains to support contaminant biodegradation. However, if the oil is depleted before both the high and low K zones are remediated, additional oil injections may be necessary to maintain biodegradation rates.

≈ 2 mo.

In barrier systems, mass transfer between high and low K zones is less of an issue, and degradation rates can be calculated once geochemical and microbiological conditions stabilize. To be considered 'stable', important indicators of biogeochemical conditions (pH, ORP, DO, sulfate, methane) and contaminant biodegradation (contaminant molar ratios, Cl#) should be reasonably constant over three or more sampling events. Once conditions stabilize, degradation rates can be estimated by adjusting the rate constants in (BIOCHLOR) (Aziz *et al.*, 2000; 2002) until model simulations approximately match average concentrations (after conditions stabilize) in monitor wells at various locations upgradient and downgradient of the barrier. Typically, degradation rates are assumed equal to background conditions, except in areas directly impacted by edible oil (indicated by DOC > 20 mg/L). Accurate estimates of

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hydraulic gradient, permeability and effective porosity will also be required for calibration of BIOCHLOR. Once accurate estimates of degradation rates are available, BIOCHLOR can be used to determine the required barrier width. Typically, a range of groundwater velocities is used in this analysis to account for seasonal variations in groundwater flow.

User's of this protocol should be aware that it may take several years after oil injection for biogeochemical and microbiological conditions to stabilize and to collect sufficient data for accurate estimation of degradation rates. If this extended data collection period is not practical, preliminary degradation rate estimates can be developed using monitoring data collected before conditions stabilize. However, these preliminary rate estimates may be lower than actual long-term degradation rates. Monitoring results from multiple sites treated with edible oil indicates that degradation rates can slowly increase over several years as the anaerobic microbial community gradually grows and adapts to the increased level of organic substrate and native electron acceptors are depleted.

#### 6.4 PROJECT REPORTING

A detailed report should be prepared summarizing and submitting all relevant site data collected during the field test. The report should reiterate the objectives and goals of the field test and to what extent they were achieved, and whether system expansion or a full-scale application is feasible.

Figure 6.8 provides an example outline for reporting the results of an enhanced *in situ* anaerobic bioremediation field test using edible oil. Included in this outline are an introduction and technology description, a site-specific data review, a description of system installation and oil injection, monitoring protocols and data interpretation, and conclusions and recommendations. Specific items in the report should include, but are not limited to, the following:

*Field Test Objectives:*

- Field test and data quality objectives.

*System Installation and Operation:*

- Injection system performance and any operational or safety issues of concern.
- Delivery system efficiency including flow rates, injection pressures, volumes, concentrations, and suppliers of injected reagents.
- Extent and uniformity of reagent distribution and radius of influence.
- As-built drawings and specifications.
- Cost summary.

\* *System Performance:* \*

- Organic carbon released to the aquifer system.

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- Electron donor utilization rates and the efficiency of electron donor utilization for reductive dechlorination as compared to alternate biodegradation processes (e.g., methane production).
- Loss of electron donor and tracer compounds, effective radii of influence, and apparent electron donor requirements.
- Electron acceptor reduction and prevailing electron accepting processes.
- Extent of anaerobic reductive dechlorination of contaminant mass, including actual/significant changes in contaminant concentrations and mass considering volatilization, dilution, degradation, and dechlorination product formation and persistence.
- Reaction kinetics and estimated biodegradation rates.
- Contributions or effects of any additional amendments added to the system (e.g., secondary substrates, microbial augmentation, nutrients, or vitamins/cofactors).

***Secondary Issues:***

- Secondary impacts to water quality.
- Gas accumulation in the unsaturated zone.
- Impacts on site infrastructure and operations.

***Recommendations:***

- Feasibility and relative cost-effectiveness of the edible oil process to meet full-scale remedial objectives.
- Scale-up issues, design considerations, and mitigation or contingency measures.

Based on this information, the report should detail the overall effectiveness of the edible oil injection system and make objective recommendations regarding continued application of enhanced anaerobic bioremediation using edible oil.

Figure 6.8 Example Table of Contents

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