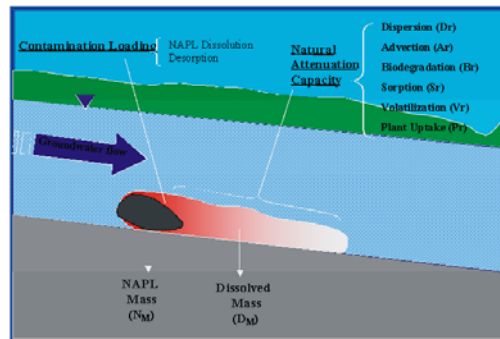# BASELINE NATURAL ATTENUATION PROCESSES:

LINES OF INQUIRY SUPPORTING MONITORED

NATURAL ATTENUATION OF CHLORINATED

SOLVENTS

May 18, 2004

$$\frac{\partial C}{\partial t} = -v\,\frac{\partial C}{\partial x} + D\,\frac{\partial^2 C}{\partial x^2} - \frac{K_d\,\rho_b}{n}\frac{\partial C}{\partial t} - R_{bio} + R_{NAPL}$$

Westinghouse Savannah River Company
Savannah River Site
Aiken, SC 29808

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BASELINE NATURAL ATTENUATION PROCESSES:

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SOLVENTS

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OVERVIEW

The Department of Energy (DOE) is sponsoring an initiative to facilitate efficient, effective and responsible use of Monitored Natural Attenuation (MNA) and Enhanced Passive Remediation (EPR) for chlorinated solvents. This Office of Environmental Management (EM) "Alternative Project," focuses on providing *scientific and policy support for MNA/EPR*. A broadly representative working group of scientists supports the project along with partnerships with regulatory organizations such as the Interstate Technology Regulatory Council (ITRC) and the Environmental Protection Agency (EPA). The initial product of the technical working group was a summary report that articulated the conceptual approach and central scientific tenants of the project, and that identified a prioritized listing of technical targets for field research. This report documented the process in which: 1) scientific ground rules were developed, 2) lines of inquiry were identified and then critically evaluated, 3) promising applied reseach topics were highlighted in the various lines of inquiry, and 4) these were discussed and prioritized. The summary report will serve as a resource to guide management and decisionmaking throughout the period of the subject MNA/EPR Alternative Project. To support and more fully document the information presented in the summary report, we are publishing a series of supplemental documents that present the full texts from the technical analyses within the various lines of inquiry (see listing). The following report – documenting our evaluation of the state of the science of baseline natural attenuation processes-- is one of those supplemental documents.

Summary Report:
Natural and Passive Remediation of Chlorinated Solvents: Critical Evaluation of Science and Technology Targets, WSRC-TR-2003-00238

Supplemental documents:
Baseline Natural Attenuation Processes: Lines of Inquiry Supporting Monitored Natural Attenuation of Chlorinated Solvents, WSRC-TR-2003-00329
Potential Enhancements to Natural Attenuation: Lines of Inquiry Supporting Enhanced Passive Remediation of Chlorinated Solvents, WSRC-TR-2003-00330
Multiple Lines of Evidence Supporting Natural Attenuation: Lines of Inquiry Supporting Monitored Natural Attenuation and Enhanced Passive Remediation of Chlorinated Solvents, WSRC-TR-2003-00331
Potential Enhancements to the Characterization and Monitoring of Natural Attenuation: Lines of Inquiry Supporting Monitored Natural Attenuation and Enhanced Passive Remediation of Chlorinated Solvents, WSRC-TR-2003-00332
Historical and Retrospective Survey of Monitored Natural Attenuation: A Line of Inquiry Supporting Monitored Natural Attenuation and Enhanced Passive Remediation of Chlorinated Solvents, WSRC-TR-2003-00333

Historically, the recognition, evaluation and reliance on natural processes for remediation and final polishing of contaminated sites has been problematical. Over the past fifteen years, however, significant progress has been made due to the efforts of regulatory and federal agencies such as the Environmental Protection Agency (EPA) and the Department of Defense (DOD), and others. This progress has taken the form of regulatory protocols and case studies from attempted implementation. To be successful, the DOE Alternative Project must link to, and build upon, this progress. A key component of responsibly advancing the technical basis for the use of MNA/EPR was documenting the baseline processes that contribute to natural attenuation, the state of the science for each process and areas for additional science. This document discusses the latest research on mechanisms and rates of processes that occur without any enhancement. This includes abiotic degradation, anaerobic biodegradation, aerobic biodegradation, phytoremediation (either in the rhizosphere or through uptake and subsequent processes), sorption and in-stream processes such as volatilization

Natural attenuation can be conceptualized as a balance between the delivery to and removal of contaminants from ground-water systems (Figure O-1). If there is a certain mass of a non-aqueous phase (NAPL) contaminant (N_m) in an aquifer, contaminant mass will dissolve over time and form a contaminant plume (G_r). G_r represents the mass discharge from the residual source and the equivalent mass loading to the plume. Once in solution, this dissolved contaminant mass will be transported by flowing groundwater and be subject to modifying processes (these processes, as well as G_r, are often expressed or converted into rates with units of mass/time). Within any "control" volume, contaminants will be diluted by dispersion (D_r), advection (A_r), destroyed by biodegradation and abiotic degrradation (B_r), slowed by sorption (S_r), and removed by volatilization (V_r), plant transpiration (P_r) and similar processes (Figure O-1). The sum of the processes contributing dissolved contaminant mass to water (NAPL dissolution, desorption, etc) represents the *contaminant loading* part of the mass balance. Similarly, the sum of these removal/dispersive processes (except for advection) can be thought of as the *natural attenuation capacity* part of the mass balance within the control volume. Advection represents plume expansion beyond the control volume. While highly simplified, this conceptualization illustrates how these processes can interact in the subsurface (Figure O-1).

For example, if the contaminant loading to the plume (G_r) is greater then the sum of the removal mechanisms

$$G_r > D_r + B_r + S_r + V_r + P_r \qquad (O1)$$

then the dissolved plume will expand over time (i.e., Advection is carrying contaminants beyond the control volume). Conversely, if contaminant loading is less than the sum of the removal mechanisms

$$G_r < D_r + B_r + S_r + V_r + P_r \qquad (O2)$$

then the dissolved plume will contract over time. Finally, if contaminant loading is equal to the sum of the removal mechanisms

$$G_r = D_r + B_r + S_r + V_r + P_r \qquad (O3)$$

then the dissolved plume will be stable. The regulatory basis for evaluating MNA as a remedial strategy often rests upon whether a contaminant plume is stable, expanding, or contracting.
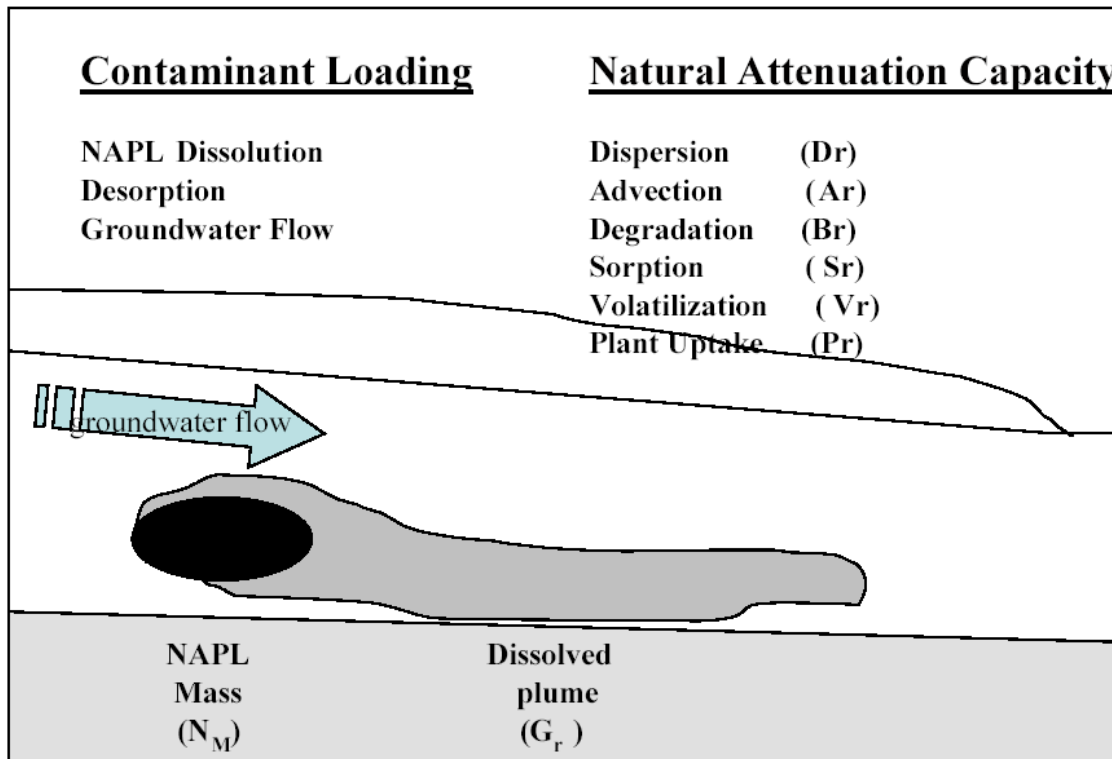

Contaminant Loading

NAPL Dissolution
Desorption
Groundwater Flow

Natural Attenuation Capacity

Dispersion	(Dr)
Advection	(Ar)
Degradation	(Br)
Sorption	(Sr)
Volatilization	(Vr)
Plant Uptake	(Pr)

groundwater flow

NAPL
Mass
(N_M)

Dissolved
plume
(G_r)

Figure O-1   Schematic diagram of contaminant loading and natural attenuation capacity in ground-water systems.

As implied in the conceptual model, the first step in the mass balance approach is developing a clear and agreed definition of the target plume volume and then determining the balance between contaminant loading and the natural attenuation capacity of that given vadose-ground-water system (Figure O-1). Under this scenario, the feasibility of MNA is related to the contaminant loading (G_r) combined with the composite "rate" of the relevent attenuation processes. Similarly, the overall timeframe of remediation is related to the source quantity (N_m) combined with the composite "rate" of the relevent attenuation processes. If the attenuation capacity is too low for plume stability, options for EPR can be examined. There are many complexities in implementing this approach. For example: what is the best method to account for spatial and temporal variation in the

attenuation capacities? and what is the best approach to quantify these rates in a simple fashion for practical use at real-world sites? These topics are the basis of some of the key science and technology targets for advancing MNA and EPR.

This conceptualization also illustrates another characteristic of evaluating natural attenuation. Except in cases where the system itself provides unambiguous information that the plume is stable or shrinking (a direct measure of the balance), then the individual contributors to the balance need to be determined.

> The different components contributing to the mass balance of a given site cannot necessarily be directly measured. Rather the measured components are indirect indicators of the attenuating mechanisms.

Each component of equations O1-O3 is a definable quantity (Figure O-1), but not necessarily one that can be directly measured. Because of this, actual evaluations of natural attenuation must rely on indirect measurements and indicators for each component of the mass balance problem. This is analogous to an artist who must draw a portrait from the shadow of the person being sketched. Drawing an accurate image from a shadow is much more challenging then observing the subject directly. The indirect nature of the evidence used to assess MNA imposes similar difficulties and challenges to the environmental professional. A clear extension of this discussion is the concept that new technologies, which provide more direct measures of the key processes, are potentially beneficial in illuminating the picture.

The indirect nature of the evidence used to assess natural attenuation capacity can be illustrated using biodegradation processes as an example. Biodegradation processes of chlorinated ethenes are highly complex, and consist of reductive, fermentative, oxidative, and cometabolic processes. Furthermore, one or more of these processes may occur simultaneously, sequentially, or not at all as contaminants are transported along the hydrologic gradient. Direct measurements of which processes occur at particular points in a system, and at what rate these processes occur, are not presently technically feasible. Thus, a series of *indirect* indicators are used to assess which biodegradation processes occur and at what rate they occur. The most common of these indirect indicators is concentrations of dissolved oxygen in ground water. If dissolved oxygen concentrations are higher than about 2 mg/L in a system, then reductive dechlorination of TCE will not occur efficiently whereas oxidative degradation of vinyl chloride will be highly efficient. In the same manner, the presence of methane (methanogenesis), sulfate/sulfide (sulfate reduction), ferrous iron (Fe(III) reduction), nitrate/nitrous oxide (nitrate reduction), and concentrations of dissolved hydrogen are indirect indicators of which biodegradation processes (reductive dechlorination, anaerobic oxidation, etc) are occurring, what their spatial distribution is, and how rapidly these processes occur. Thus, assessing how much biodegradation is contributing to the overall natural attenuation capacity is an indirect, highly interpretive process. This is not only true for biodegradation, but also for each individual component of natural attenuation capacity (Figure O-1). The indirect nature of the methods used to assess natural attenuation capacity makes evaluating MNA an inherently complex procedure.

Finally, another source of complexity stems from the heterogeneous nature of groundwater systems. Biodegradation rates, sorptive capacity, and volatilization can differ by orders of magnitude over distances of just a few inches. Rates of ground-water flow can vary over several orders of magnitude in time and space. Due to this inherent, ubiquitous, and unavoidable heterogeneity, it is difficult to obtain representative estimates for each component of natural attenuation capacity at real sites. As a result, monitoring tools that integrate processes over an appropriate scale represent another potentially high priority research and technology development target.

The technical working group strongly supported the conceptualizing of MNA/EPR as a balance between contaminant loading and attenuation capacity. MNA/EPR performance evaluation based on a balance of attenuation capacity and loading is intuitive and powerful. It provides an understandable organizing framework that is compelling and yet flexible enough to handle real world complexities and technically challenging situations. The ideas can be supported by the multiple lines of evidence that are necessarily embodied in MNA and EPR protocols. Importantly, when framed in terms of attenuation capacity and loading, the confusion that is often associated with long lists of required and optional analytes can be overcome. Why are certain parameters collected? How should they be used? The loading-capacity balance concept is also adaptable to each site. At some sites, unambiguous measurement of plume stability or shrinkage provides a field scale verification that attenuation capacity is greater than or equal to loading and little further evidence will be necessary. For sites where there is more uncertainty, more challenges or where enhancements to MNA are being considered, additional data and complexity can be added as needed – always within the framework. The concept is also portable in terms of modeling – simple and complex models can easily generate results that explicitly allow the balance to be calculated. Finally, and most importantly, an approach that balances contaminant loading and attenuation capacity is exactly the type of tool needed to link MNA/EPR into the overall planning necessary at a contaminated site.

Recent scientific studies for source remediation are examining performance in terms of mass discharge from the source to the plume. The mass discharge from the source is the initial contaminant loading to the plume and provides a direct link to support decision making and answering the difficult questions that often arise at chlorinated solvent sites. How much source needs to be removed to integrate with the overall long term goals for the site? When can an active treatment system be turned off? How can the mass balance approach be integrated into an overall strategy to protect receptors and reduce risk (e.g., account for exposures through all routes – indoor air, etc.)? The concept also suggests a few changes in the types of data that would be most useful. In source removal or destruction efforts, for example, a measurement or estimate of the reduction in mass discharge, rather than total mass, would be of value. All of the efforts that are currently being invested in estimating mass discharge from the source can be directly applied to improving environmental decisions. Similarly, understanding the flux or loading in different portions of a plume will provide a powerful tool for determining the size of an

active plume treatment and the appropriate conditions that would justify transition to MNA/EPR.

Complete Text for Baseline Natural Attenuation Processes Writings

(Mechanisms and rates of natural attenuation processes that occur without enhancements)

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Mechanisms and Rates of Natural Attenuation Processes that Occur without Enhancements

The concept of "assimilative capacity" is well known in soil science (Charbeneau and Daniel, 1993, p. 151) and surface water hydrology (Chapra, 1996, p. 11) and refers to the capacity of a system to transform and/or immobilize pollutants to innocuous byproducts. In surface-water systems, assimilative capacity depends upon abiotic (stream flow, mixing, and hydrodynamic dispersion), and biological (biological oxygen demand) factors and is often assessed using analytical or digital water-quality models. By analogy, a "natural attenuation capacity" for ground-water systems may be thought of as the capacity of an aquifer to lower contaminant concentrations as ground water flows downgradient (Chapelle and Bradley, 1998). Natural attenuation capacity (NAC) can be defined as the sum of all physical, biological, and chemical processes serving to disperse, biodegrade, chemically transform, immobilize, or permanently sequester contaminants in a ground-water system (Figure 1). A brief description of the processes contributing to NAC follows.

1.0   Abiotic Processes

1.1   Hydrodynamic Advection and Dispersion

Advection refers to the migration of solutes in the subsurface as they are carried by ground-water flow. In sandy aquifers with relatively high horizontal hydraulic conductivity (K > 10 ft/d), advection is the dominant transport mechanism for relatively soluble contaminants such as chlorinated ethenes. If subsurface sediments were homogeneous, consideration of advective transport alone would account for much of the transport behavior of subsurface contaminants. However, the lithology, and thus the hydraulic conductivity, of ground-water systems are highly heterogeneous. This heterogeneity causes variations in solute transport speed. If it were possible to account for these variations, then advection could be used by itself to describe solute transport. However, because these variations in advective transport are so complex, it is not practical to account for them in a meaningful way.

The traditional method for incorporating the unknowns associated with advective transport is with what is termed hydrodynamic dispersion. Dispersion is a mechanical process that causes a solute to occupy a larger volume of porous media than would be expected by advection alone. In part, dispersion accounts for the tendency of solutes to spread along concentration gradients, thus increasing the volume of the plume. But it is also a mathematical way of accounting for the uncertainty associated with hydraulic conductivity encountered in all ground-water systems. The most widely used form of the coefficient of hydrodynamic dispersion is:

$$D = D_o\tau + \alpha v \qquad\qquad (1)$$
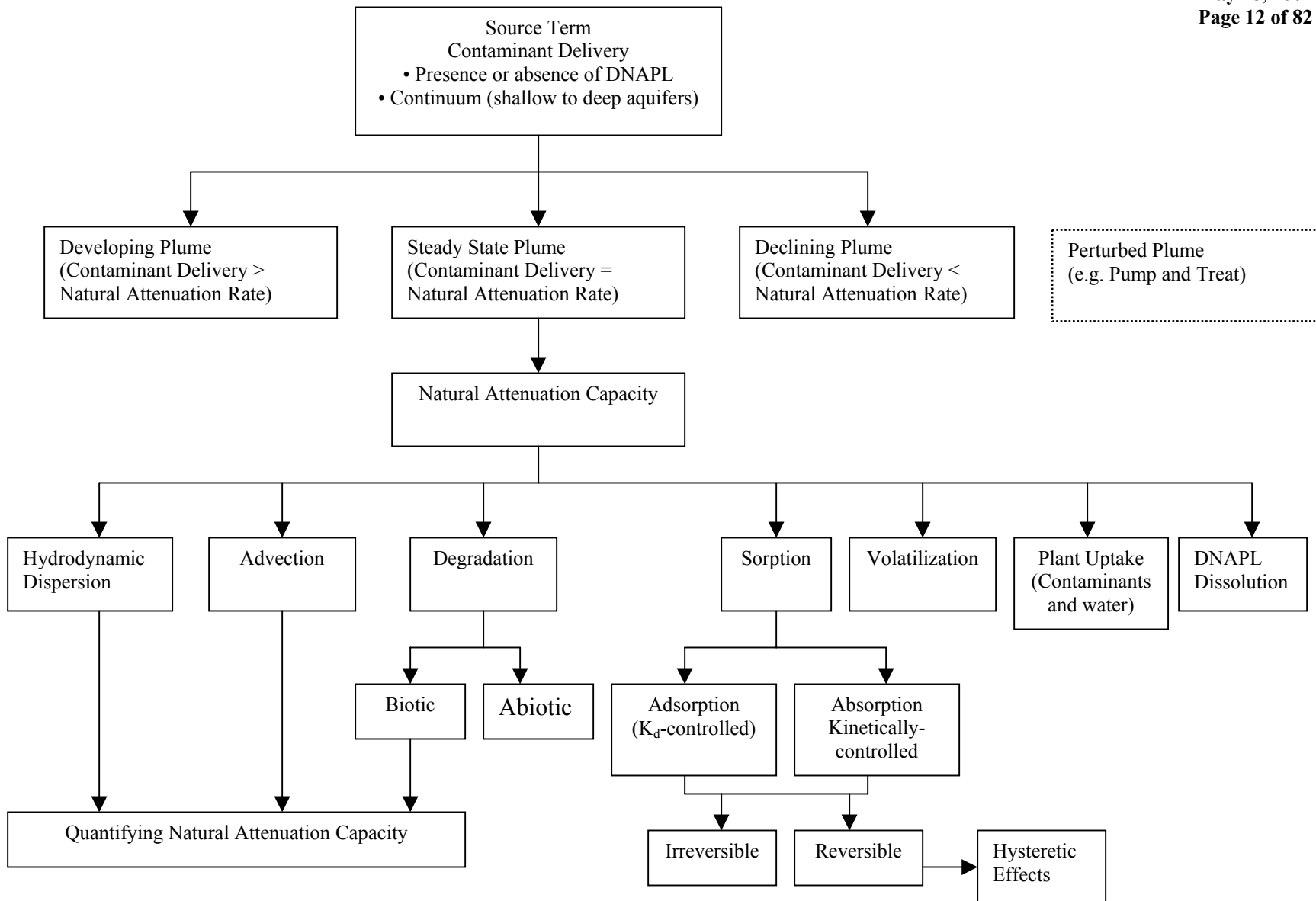
Figure 1. Block diagram of the natural processes that when summed defines Natural Attenuation Capacity

where D_o is the diffusion coefficient $(L^2 T^{-1})$ for the solute in question, τ is a dimensionless tortuosity factor, α is the dispersivity of the porous media (L), and v is pore-water velocity (LT^{-1}). Under conditions where there is significant ground-water flow, the first term of equation 1 (diffusion) is negligible compared to the second term, and it reduces to:

$$D = \alpha v. \tag{2}$$

Thus, the "advective mixing", or dispersion, of a solute is proportional to how fast ground-water is flowing (v) and the ability of the aquifer material to "mix" the water (α). In reality, dispersivity is a representation of uncertainty in the hydraulic conductivity of aquifers, and the uncertainty of ground-water velocity. Because this uncertainty is additive with distance along a given flowpath, dispersivity also increases as the size of a plume increases. This scale-dependent behavior is a characteristic feature of dispersivity (Gelhar and others, 1992).

1.1.1 Importance to NAC

As part of the critical evaluation of MNA, a survey was conducted among industries and agencies that applied MNA as part of their remedial efforts on chlorinated solvent plumes. Disperson was an active process in over 70 percent of the MNA sites; but, was considered important in the overall natural attenuation capacity in only about 5 percent of the sites.

1.2   Sorption

Sorption refers to the partitioning of a solute between the aqueous and solid phases present in aquifers. The solute may be adsorbed onto the surfaces of aquifer materials, or may be absorbed into the interior of the sorbent matrix. As shown in figure 2, sorption may be reversible, irreversible, or permanent.

1.2.1   Reversible Sorption

Sorption can act either to remove solute from solution (adsorption), or to add solute to solution (desorption). These combined adsorption-desorption processes are called *reversible sorption* and can have important effects on the transport of solutes in ground-water systems. The simplest description of reversible sorption is given by a linear isotherm that relates aqueous concentrations of a solute (C) to solutes adsorbed onto solid aquifer materials (S) by a distribution coefficient (K_d) according to the equation:

$$S = K_d C \tag{3}$$

```
┌─────────────────────────────────────────────────────────────────────────┐
│                            Bulk Mobile Phase                              │
│                             (Groundwater)                                 │
└─────────────────────────────────────────────────────────────────────────┘
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Fast Equilibrium Sorption/Desorption K_d Controlled	Time-Dependent Sorption/Desorption Irreversible Sorption Resistant Fraction Formation	Chemical Reaction with Bond Formation Permanent Sequestration
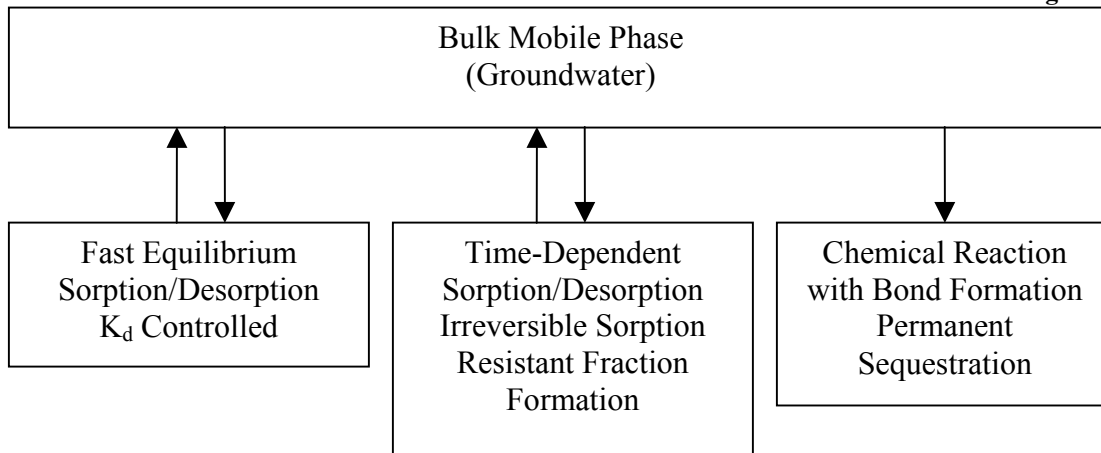
Figure 2. Key Sorption Processes Influencing Contaminant Concentration in Groundwater

In this case, if a sorbing solute C is transported by ground-water flow, it's velocity of transport (v_T) will be retarded relative to the velocity of ground-water flow according to the equation:

$$v_T/v_{gw} = (1 + \rho_b/n) \, K_d \qquad\qquad (4)$$

where ρ_b is the bulk density of the aquifer material, n is the effective porosity, and K_d is defined by equation (3).

When a plume first develops from a new contaminant source, the sorptive capacity of the aquifer sediments is largely unfilled. However, as solutes migrate away from the source, they tend to adhere to aquifer sediments, and gradually saturate the sorptive capacity of the aquifer material. This causes the transport of contaminants to be initially retarded relative to the velocity of ground water. However, as time goes on, the sorptive capacity of the sediments becomes saturated, and the effects of retardation become less and less. The principal effect of reversible sorption on the development of plumes is to retard the spread of contaminants. However, it does not stop contaminant spread. The steady-state configuration of plumes (that is, their extent when contaminant delivery from the source is balanced by the sum of the natural attenuation mechanisms) is independent of the sorption capacity of the aquifer. However, low sorptive capacity aquifers will reach the steady-state configuration sooner than high sorptive capacity aquifers.

Desorption, the processes by which solutes are released from contaminated aquifer sediments, is an important factor in any remediation effort. When aquifer sediments contain large amounts of contaminant mass in the adsorbed phase, they can continue to be sources of contaminants for long periods of time. This contributes to the contaminant "rebound" effect often observed in pump-and-treat remediation systems once the pumps are turned off, and can greatly increase the time needed to effect site cleanup. As a

general rule, plumes develop rapidly in low-sorption capacity aquifers, and can be remediated rapidly as well. Conversely, in high-sorption capacity aquifers, plumes develop more slowly, and take longer to remediate. For these reasons, remediation strategies should always include a quantitative evaluation of aquifer sorption capacity. As solutes diffuse into pore spaces and chemically react with sorption media, a portion of the solutes can become chemically immobilized, and are no longer capable of desorption. This is known as irreversible sorption, and can represent a net sink of chemical contaminants. When irreversible sorption is significant in a system, it can provide significant natural attenuation capacity.

1.2.2   Irreversible Sorption

Prolonged contact of contaminated groundwater with aquifer sediments (i.e., as a contaminant plume migrates in the aquifer) results in contaminant sorption into sediment particles. Contaminants migrate into the meso and micropores and fractures of mineral and organic matter components of sediments. The process (known as aging) is one of time dependent sorption (i.e., kinetic control) and results in sequestration of contaminants in sediments. The nature of the sequestration is dependent on the physical/chemical properties of the contaminant molecule and its surrounding environment in the sediment matrix. For some functionalized contaminants chemical bond formation can occur with the sediment matrix (e.g., humic substances) leading to permanent sequestration of the molecule (i.e., irreversibly bound). The molecule is no longer available for migration in groundwater. Organic compounds such as naphthol and trinitrotoluene have been shown to irreversibly bind to soil humic material (Burgos et al., 1996; Achtnich et al., 2000).

Organic compounds that do not have structural features that permit covalent bond formation but can sorb into the mineral and organic matter components of the sediment matrix include common groundwater contaminants (e.g., petroleum and chlorinated hydrocarbons) associated with Department of Energy (Riley and Zachara 1992), as well as Department of Defense installations (US DOD 1994) and industrial sites (US EPA1990) throughout the United States. Release of neutral hydrocarbon compounds from sediment into groundwater has been demonstrated to be non-linear (Kan et al., 1997; Kan et al., 1998). This behavior, also known as "irreversible sorption" (i.e., sorption/desorption hysteresis occurs)[1] is the result of the behavior of a resistant fraction, and is said to occur because desorption of the resistant fraction takes place from a different molecular environment (i.e., a physical-chemical rearrangement in the solid phase after sorption occurs) than that in which sorption of the neutral hydrocarbon occurred. A neutral hydrocarbon compound released from a resistant fraction exhibits slow release (i.e., the aqueous phase equilibrium concentration for the hydrocarbon is much lower than would be predicted with conventional hydrophobic partitioning theory) (Kan et al, 1998).

---

[1] Irreversible sorption, sorption/desorption hysteresis and non-linear behavior of a contaminant are terms that have been used to describe the same behavior of a neutral hydrocarbon in a resistant fraction.

1.2.2.1 Scientific Evidence for the Presence of Contaminant Resistant Fractions in Soils and Sediments

Evidence for the presence of contaminant resistant fractions in soils and sediments for a wide range of organic contaminants has been discussed in two reviews (Pignatello and Xing 1996; Luthy et al. 1997). The effects of resistant fractions on contaminant bioavailability have been summarized separately (Alexander 2000). Studies have focused on identifying those characteristics that are significant to controlling the slow release of contaminants from soils and sediments. Three polycyclic aromatic hydrocarbons (i.e., phenanthrene, pyrene, and benzo(a)pyrene) were "aged" on soils for approximately one and a half years (525 days). Eighty percent of the phenanthrene, 70% of the pyrene, and only 25% of the benzo(a)pyrene was released from the soils after 350 hrs of aqueous desorption, indicating in all three cases the existence of contaminant resistant fractions (Northcott and Jones 2001). The adsorption and desorption from sediments of seven organic compounds with water solubility ranging from 0.005 to 517 mg/L was recently investigated. In every case, fractions of the amounts of compound present exhibited irreversible sorption behavior, yet the compounds were not covalently bonded to the sediment, indicating the presence of contaminant resistant fractions (Kan et al. 1998). Slow release of chlorinated volatile organic compounds (e.g., CCl_4, $CHCl_3$, perchloroethene, TCE, dichloroethane) has been observed from soils, sediments, soil mineral fractions, and soil or sediment organic matter (SOM) under both unsaturated and saturated conditions (Farrell and Reinhard 1994a; Aochi and Farmer 1997; Werth and Reinhard 1997; Farrell et al. 1999).

1.2.2.2 Factors Influencing Slow Release of Contaminants in Resistant Fractions of Soils and Sediments

Slow release from meso- and micro-pores, areas between mineral layers (e.g., clay layers), and various structures of organic components of soils and sediments has been the basis for numerous studies (Farrell and Reinhard 1994a; Farrell and Reinhard 1994b; Corley et al. 1996; Thibaud-Erkey et al. 1996; Aochi and Farmer 1997; Carmachael et al. 1997; Chang et al. 1997; Cornelissen et al. 1997; Huang and Weber 1997; Werth and Reinhard 1997; Cornelissen et al. 1998; Deitsch et al. 1998; Kan et al. 1998; Farrell et al. 1999; Schlebaum et al. 1999; Deitsch et al. 2000; Northcott and Jones 2001).

Slow release of contaminant from soils and sediments has been shown to be highly dependent on the characteristics of the SOM. Analogies have been drawn between different types of SOM and different types of synthetic organic polymers, suggesting that sorption into rubbery ("soft") polymers is fast while uptake into glassy ("hard") organic polymers is slow (Brusseau et al. 1991; Pignatello and Xing, 1996; Weber and Wang 1996). Soft forms of SOM consist of humic substances that are expanded while hard forms consist of geologically old (mature) organic matter that is condensed (highly cross linked). The two types of SOM have different diagenetic histories and distinct sorption properties (Kleineidam et al. 1999). Hard forms of SOM include soot, charcoal, and coal-like particles. Hard carbon contains highly sorptive surfaces and a meso- or a microporous structure. As little as 3% of the organic carbon in this component can

significantly affect a contaminant's partition coefficient and, thus, the contaminant's potential desorption kinetics (Karapanagioti et al. 2000).

From the above studies, diffusion from the organic components of soils is suggested to dominate slow release of contaminants from resistant fractions (Luthy 1997; Mader et al. 1997; Cornelissen et al. 1998; Nam et al. 1998; Deitsch et al. 2000; Northcott and Jones 2001). However, others suggest that resistant fraction behavior cannot be completely explained based on a diffusion-limited process alone (Kan et al., 1997). The diffusion process may also be moderated by trapping effects caused by the presence of dominant concentrations of organic co-contaminants that result in matrix deformation and increased non-linearity of the solute (Weber et al. 2002; Lu and Pignatello 2002; Washington et al., 2003). It has been suggested that competition between water and contaminant for sorption sites during the aging process limits the overall amount of contaminant incorporated into meso- and micro-pores, thus limiting the amount of contaminant that could undergo slow release from the mineral component of a soil (Chiou et al. 1983; Chiou and Shoup 1985; Lin et al. 1996). Recent studies have suggested there is a limit to the amount of resistant fraction that can be accommodated by a soil or sediment and that it can be represented in a semi-empirical model that is a function of a soil or sediments carbon content and the octanol-water partition coefficient of the neutral hydrocarbon contaminant (Kan et al., 1998).

1.2.2.3 The Influence of Resistant Fraction on the Magnitude of Natural Attenuation Capacity (NAC)

The governing equation for solute transport (equation 5) consists of transport, source and sink terms. A sink term within the sorption process is that for covalent bonding (Figure 2) and represented as the k_3C term in equation 5. Organic compounds with structural functionality (e.g., hydroxyl or nitro groups) can react with the sediment matrix resulting in permanently removing contaminant from further transport. This term has relevance for groundwater plumes containing certain contaminant types (e.g., explosives such as trinitrotoluene). Neutral petroleum and chlorinated hydrocarbons contain no such functionality, therefore, they do not have the potential to covalently bond to aquifer sediment during groundwater transport. The term can be eliminated from equation 5 when applied to these compound types.

$$\frac{\partial C}{\partial t} = D \bullet \frac{\partial^2 C}{\partial \chi^2} - v \bullet \frac{\partial C}{\partial \chi} - \left(\rho_b K_d\right)/n \bullet \frac{\partial C}{\partial \chi} - k_1 C - k_2 C - k_3 C + R^{NAPL} \qquad (5)$$

where:

C = the aqueous concentration of contaminant
D = the coefficient of hydrodynamic dispersion
v = velocity of groundwater flow
ρ_b = bulk density of the aquifer material
K_d = contaminant partition coefficient
n = = effective porosity

k_1 = a first-order biodegradation rate constant
k_2 = a first order abiotic degradation rate constant
k_3 = a rate of formation of irreversibly bound contaminant
R^{NAPL} = addition of contaminant dissolving from NAPL

Following the derivation given by Chapelle and Bradley (1998) and Chapelle et al. (2000), the definition of natural attenuation capacity can be expanded to include the effects of reversible sorption. Under steady-state conditions (i.e., when a plume is not expanding or contracting with time and change in concentration over time is constant), the sorption term becomes small relative to the dispersion and velocity terms. Assuming degradation is predominantly biotic and there is no NAPL in the aquifer, equation 5 simplifies to equation 6.

$$\frac{\partial C}{\partial t} = D \bullet \frac{\partial^2 C}{\partial \chi} - v \bullet \frac{\partial C}{\partial \chi} - k_1 C = 0 \qquad (6)$$

Neutral hydrocarbon transport in groundwater is affected by the partitioning of contaminant between sediment and groundwater (fast equilibrium-Figure 2) and the accumulation and depletion of contaminant in a resistant fraction (Figure 2) during transport. The size and behavior of resistant fractions for neutral hydrocarbons has been reported (Kan et al., 1998; Kan et al., 1997) and it is proposed that its affect on the calculation of natural attenuation capacity can be captured in a modification of the velocity term in equation 6 through the application of equation 7. In this equation, the inverse of a contaminant's retardation factor (1/R) reflects the degree to which a contaminant's relative velocity in groundwater (v_T/v_{gw}) is affected by the magnitude of its partition coefficient.

When a plume first develops from a new contaminant source, some of the contaminant begins to accumulate into the resistant fraction. As the plume reaches maturity, the resistant fraction reaches its maximum size. Under these conditions (i.e., steady-state), the resistant fraction is neither increasing nor decreasing in size. Under these conditions, R in equation 7 would be based on a value of K_d that assumed linear first order kinetics and, ideally, the resistant fraction is having no affect on the magnitude of natural attenuation capacity. When the plume dissipates (e.g., as a result of source remediation), contaminant release from the resistant fraction, now acting as a secondary source, becomes a relevant factor in the natural attenuating capacity of the dissipating plume. The K_d of a contaminant under dissipating plume conditions would be that of the resistant fraction. The K_d value for a contaminant at steady-state versus dissipated plume conditions can differ by more than two orders of magnitude (Kan et al. 1998). Thus the natural attenuation capacity of a plume under dissipating conditions could be significantly higher than under steady state conditions.

$$1/R = v_T/v_{gw} = 1 \div [(1 + \rho_b /n) K_d] \qquad (7)$$

Where:

R = contaminant retardation factor
v_T = contaminant velocity of transport
v_{gw} = pore velocity of groundwater
ρ_b = bulk density of the aquifer material
n = effective porosity
K_d = contaminant partition coefficient

Equation 6 is modified to reflect the elements of equation 7 to give equation 8. The elements of the sorption process serve to reduce the contaminant velocity in groundwater from that of purely advective transport (i.e., $K_d = 0$).

$$\frac{\partial C}{\partial t} = D \bullet \frac{\partial^2 C}{\partial \chi^2} - v\left(v_T / v_{gw}\right) \bullet \frac{\partial C}{\partial \chi} - k_1 C = 0 \qquad (8)$$

For boundary conditions of C= C_0 at x = 0, as x →∞, equation 8 has the particular solution:

$$C(x) = C_0 \exp^{Ax} \qquad (9)$$

Where:

$$A = (-z + [\, z^2 + 4Dk_1]^{1/2} \div 2D) \qquad (10)$$

and

$$z = v\,(v_T/v_{gw}) $$

The slope of the solute concentration is proportional to the value of A which has units of feet^{-1}. This represents the capacity of an aquifer to lower contaminant concentrations as groundwater moves down gradient, and can be referred to as the natural attenuation capacity (NAC) of a groundwater system. Multiplying equation 10 by 100:

$$NAC = A \bullet 100 \qquad (11)$$

Note that lowering the contaminant velocity in groundwater allows more time for reaction by sink terms (e.g., biotic or abiotic degradation). The net affect is higher values of NAC.

1.2.2.4 Irreversible Sorption Capacity

Assessing the potential capacity of aquifer sediment to store a contaminant during plume migration toward compliance boundaries might prove to be useful information in

assessment of natural attenuation at a specific site. The maximum irreversible sorption capacity q_{mas}^{irr} of a sediment is described by the equation (Kan et al., 1998):

$$q_{max}^{irr}(mg/g) =$$
$$a \ x \ OC(C_{aq}^{sat})\exp\{\frac{-\Delta G_{ads}^{irr}(j/A^2)HSA(A^2/molecule}{RT/N_{Av}}\} \tag{12}$$

where:
Δ_{ads}^{irr} = free energy of adsorption per unit area of molecule
a = the area of the adsorbing phases
C_{aq}^{sat} = the aqueous concentration of solute at sorption saturation
HAS = the hydrophobic surface area
R = the Ideal gas constant
T = temperature, in degrees K
N_{av} = Avrogadro's number

Emperically, equation 12 has been found to approach:

$$q_{mas}^{irr} \sim 37765 \ OC \ x \ K_{ow}^{-0.23} \tag{13}$$

for conditions characteristic of groundwater systems where:

q_{mas}^{irr} = maximum concentration of contaminant in resistant fraction in sediment
OC = organic carbon content of the sediment, and
K_{ow} = octanol-water partition coefficient of contaminant of interest.

Data for application of the model requires specific knowledge of the total mass of different sediment units that are in contact with the groundwater and their organic carbon contents. The octanol-water partition coefficient is also required for all contaminants of interest. Total capacity of the aquifer sediments for a given contaminant can then be calculated (estimated) and compared to estimated amounts of contaminant disposed in the source term before, during and after remediation. Sorption capacity in conjunction with natural attenuation capacity can be used together in assessing the impact of natural attenuation in site-specific hydrocarbon plume management and remediation and decision-making activities. It should be noted that interpretation of sediment storage capacity could be confounded by the presence of DNAPL in the aquifer. Estimation of possible percent DNAPL saturation in aquifer sediments is a necessary step to address this concern.

1.2.3   Importance to NAC

In the survey, sorption and retardation were active processes in 65 percent of the MNA sites surveyed. However, of that 65 percent, sorption was considered playing only a minor role in the overall NAC at most MNA sites, such that, it represented an important process in less than 5 percent of the sites.

1.3   Volatilization

Volatilization is defined as the loss of a compound in its gaseous phase to the atmosphere from the soil surface. Volatilization is a relatively simple process that is based on straightforward chemical and mass transfer properties and conditions. Volatilization has been evaluated for many purposes and has been studied under wide ranging conditions by scientists and engineers with diverse backgrounds. Nonetheless, when considered as a process supporting natural attenuation, understanding and quantifying its role becomes complex. The nature and significance of volatilization in various segments of the contaminant plume flow system varies substantially. Because of the underlying simplicity of the volatilization process, it provides a good example of the extreme conditional variability that occurs in real world situations for many of the baseline natural attenuation processes that impact chlorinated solvents.

Given a generalized situation where a plume of chlorinated solvents in the vadose zone migrates downward to the groundwater system, the plume trajectory is from the vadose zone (I) to the groundwater system (II) (Figure 3). Once in the groundwater system (II), the plume moves along a curvilinear path, controlled by the large scale sources and sinks of subsurface water and the geological structure, toward the downgradient wetland (III) and stream zones (IV) (Figure 3). Discharge of the plume to the wetland or stream zones is dependent upon the natural attenuation capacity. If the natural attenuation capacity in zones I through III is less than the contaminant delivery rate from the source, the contamination will reach the wetland or stream zones. The location of the source, the relative thickness and geometry of the various zones, and specific geologic features control the nature of the migration of the plume from the subsurface into the surface water system (hyporheic, riparian or fluvial). Several versions of this simple paradigm in which the nature of the source and setting are varied have been examined in developing and prioritizing critical science associated with natural attenuation – all of the versions, however are represented in a general sense by the generic diagram below.

The simple conceptual model described above lends itself to a qualitative examination of volatilization and identification of relevant scientific literature. In particular, there has been substantial study of volatilization of chlorinated solvents from shallow sources to the atmosphere. Study of volatilization in the deeper parts of the system has been more limited but key references provide useful insights. In some cases, such as for volatilization from the source, the literature supports relatively defensible calculation of the amount of volatilization, but the calculation approaches do not necessarily lend themselves to incorporation into the overarching concept of natural attenuation capacity
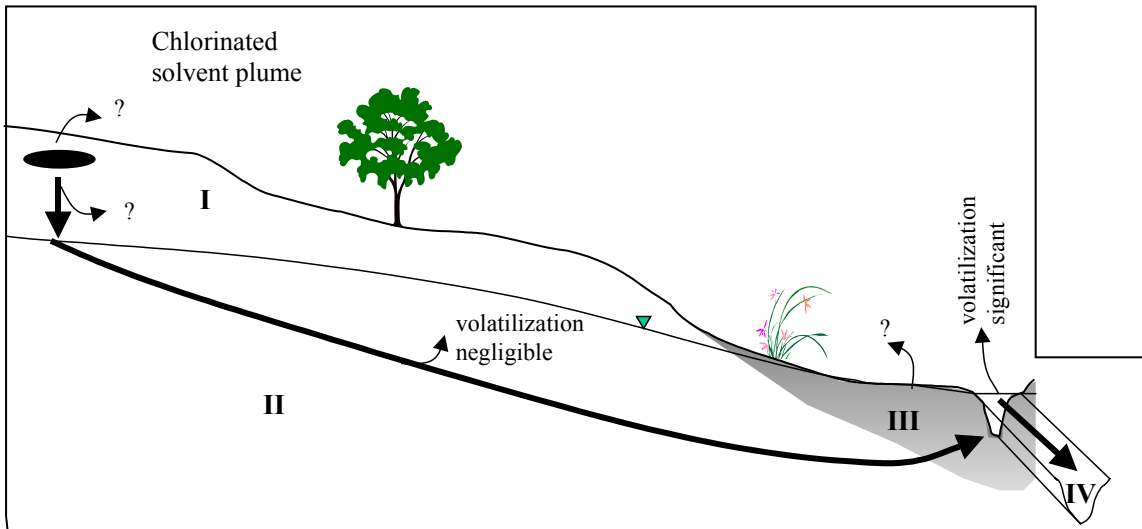
Figure 3. Simplified schematic diagram of the major zones potentially traversed by a chlorinated solvent plume. I = vadose zone, II = groundwater, III = "wetland", IV = stream. Bold arrows indicate overall plume trajectory and geometry. Small arrows examine volatilization.

(in units of length^{-1}). In these instances, direct consideration of volatilization in the summation of natural attenuation capacity in the subject zones along the flow path and direct comparison with other mechanisms represents a challenge. In such cases, more complex modeling approaches may be required. Volatilization in each of the zones along the flow path is briefly discussed below.

1.3.1 Vadoze zone (Zone I)

Transportation of gaseous phases of chlorinated ethenes to the atmosphere from the vadoze zone is through the soil zone and the stagnant air boundary layer at the soil surface by processes of vapor diffusion for low permeability soils and by advection for high permeability soils. In low permeability soils, the rate of movement away from the surface will be proportional to the diffusion coefficient and the vapor density of the chemical at the evaporating surface. However, factors such as wind velocity and surface cover (plants) alter volatilization through their effect on the thickness of the stagnant air layer. In high permeability soils, transport of gaseous phases are affected by advection processes due to changes in barometric pressure, temperature gradients, rainfall or irrigation, or wind. In the survey of MNA sites, the primary NAC processes occurred in the vadose zone only in 2 percent of the sites and capillary fringe in 16 percent of the sites.

An equation was developed by Jury and others (1983) and represented an extended form of Fick's Law. This equation is commonly used in the quantification of vapor diffusion through the vadose zone. The equation computes the vapor flux using the thickness of

the stagnant boundary layer above the soil surface and the chemical gas concentration in the well-stirred air above the boundary layer (these variables equal the effective transfer coefficient - based on molecular diffusion). Diffusion coefficients often are estimated by the Millington-Quirk tortuosity formula (Millington and Quirk, 1961; Jury and others, 1983; Sleep and Sykes, 1989) and generally range from 0.1 to 0.4 cubic meters per day for VOCs (Minnich, 1993). The thickness of the stagnant boundary layer above the soil surface is evaluated by measuring the water evaporation rate from the soil. The other commonly cited equation relates to the vapor- and liquid-phase partition coefficients (Kd` and Kd) derived from the headspace analysis method (Garbarini and Lion, 1986; Ong and Lion, 1991). Falta and others (1989), Massmann and Farrier (1992), and Poulsen and Kueper (1992) describe density gradients, barometric pressure fluctuations, and soil permeability and capillary characteristics, respectively, as important drivers of the gas advection process.

The quantification of the effects of the distribution of the compounds in the subsurface, concentration in the gas phase, adsorption, temperature, and inherent soil properties on volatilization of the chlorinated ethenes in the vadose zone have been attempted by computing a retardation factor. The total retardation factor (R_t) for VOCs can be divided into four terms that represent the influence of the above factors (Kim and others, 2001):

$$R_t = \beta_g + \beta_w + \beta_i + \beta_d \ , \tag{14}$$

where,

β_g is the dimensionless partial retardation factor for the gas phase and is equal to 1;
β_w is the dimensionless partial retardation factor for the water partitioning and is equal to

$$\beta_w = \vartheta_w / [K_H \vartheta_g], \tag{15}$$

where,

ϑ_w and ϑ_g are the volumetric water and gas contents, respectively, in cubic centimeters per cubic centimeters (cm^3/cm^3); and
K_H is the dimensionless form of Henry's law constant.

β_i is the dimensionless partial retardation factor for the air-water interfacial adsorption and is equal to:

$$\beta_i = a_i K_i / \vartheta_g \ , \tag{16}$$

where,

a_i is the specific air-water interfacial area, in cm^2/cm^3,
K_i is the adsorption coefficient at the air-water interface, in cm.

β_d is the dimensionless partial retardation factor for the solid phase and is equal to:

$$\beta_d = \rho K_d / K_H \, \vartheta_g , \qquad\qquad (17)$$

where,

ρ is the bulk density of the medium in grams (g) /cm^3
K_d is the sorption coefficient for the medium, in cm^3 /g.

Henry's law constant can be computed from the ratio of the concentration of the compound in the gas phase (C_G), to the concentration of the compound in the aqueous phase (C_L) expressed in the same units (e.g., moles per liter or moles per cubic centimeter):

$$K_H = C_G / C_L. \qquad\qquad (18)$$

This ratio can be determined experimentally by the method that involves determining the chemical concentrations in air and water which have been mixed for a long enough time to establish an equilibrium distribution (Lincoff and Gossett, 1984; Garbarini and Lion, 1985: Ong and Lion, 1991). Schwarzenbach, and others (1993) provide a comparison of measured and estimated Henry's Law for several chlorinated methane and ethane compounds.

However, Munz and Roberts (1987) report that good agreement exists between experimental and predicted values of Henry's Law constant for TCE and TCA but poor agreement for PCE. Distribution of the chlorinated solvent between the vapor phase and aqueous solution at equilibrium is directly proportional. For dilute solutions, Henry's Law states this relation is linear:

$$P_i = H_i X_i, \qquad\qquad (19)$$

where P_i is the partial pressure of compound *i*, H_i is Henry's Law constant for compound *i* at a given temperature, and X_i is the mole fraction of compound *i*, yielding units of atmospheres – cubic meters per mole (atm-m^3/mol). However, Henry's Law can be applied to systems to the point of saturation for many chemicals (Spencer and Cliath, 1970; Schwartzenbach and others, 1993).

The effect of temperature on Henry's Law constant is of similar magnitude as the temperature effect on the vapor pressure of the aqueous solution (Schwartzenbach and others, 1993). Henry's Law constants have been reported to increase by a factor of approximately 1.6 for every 10 degree Celsius rise in temperature (Munz and Roberts, 1987).

Because of its density and low K_{oc}, chlorinated ethenes ultimately will move downward through the vadose zone until an impermeable barrier is reached. Movement of large concentrations of chlorinated ethenes through the vadose zone can be quite rapid and

restrict removal of the compounds by volatilization as the solvent plume traverses the vadose zone and enters the shallow groundwater. Volatilization from the vadose zone will tend to reduce the concentrations in the plume centerline, but will serve as a source to the surrounding areas and may serve as a residual source of contamination after the plume passes.

1.3.2 Groundwater zone (Zone II to Zone IV)

As shown in Figure 3, typical contaminant plumes migrate along a curvilinear trajectory. As a result, the contaminant is only in contact with the vadose zone (I) beneath and immediately downgradient of the source. While there have been a few studies suggesting volatilization may occur from groundwater (Zone II) to the vadose zone along the flow path (Figure 3), there is little evidence that such a process could significantly contribute to natural attenuation. As the plume migrates laterally through the system, the plume migrates below the water table and is "capped" by "clean" water. The volatilization pathway is cut off. As a result, the volatilization arrow in the generic figure is considered minimal or negligible for this portion of the flow path.

Once the plume enters the distal transport zones and discharges to the wetlands (zone III) and streams (zone IV), contaminated water and air are once again in contact and volatilization is again theoretically possible and quantifiable. In these areas, volatilization, as well as other mechanisms such as uptake by plants, rhizosphere degradation processes, and anaerobic or aerobic degradation appear to be relatively active. For chlorinated solvent plumes, the distal portions of the plume appear to have particular promise in supporting natural attenuation. Our survey of recent natural attenuation experience suggests that the dominant natural attenuation processes occur in these distal zones in approximately 10 % of successful implementations. Several recent field studies (see Conant, 2001; Mukherjee, 2003; and others) have focused on the distal plume zones. These studies confirm the presence of relatively active natural attenuation mechanisms in these zones. Despite these positive measurements, the studies also identify the possibility for limited contaminant breakthrough into surface water. Importantly, the studies document that volatilization from seep areas and streams are measurable and quantifiable.

Mukherjee (2003), working with Alan Fryar and colleagues from University of Kentucky, measured the relative behavior of TCE and propane (constituents that volatilize) and compared the behavior to technitium, bromide and rhodamine (constituents that do not volatilize). This specific type of study of volatilization is typically used to determine reaeration of oxygen into streams and is well documented in the literature (Yotsukura et al.). Such tracer and analog studies, as wells as development of system performance criteria based on such studies, may be justified if volatilization in the distal plume areas is determined to be a viable and accepted natural attenuation mechanism. The example results of Mukherjee (2003) provided compelling evidence that volatilization is a significant natural attenuation mechanism for chlorinated solvents in the vicinity of where these compounds transition into surface water (Minnich, 1993). Investigators quantitatively examined the rate of volatilization using OTIS, a standard

surface water model (Runkel, 1998). Example surface water models that can include volatilization are listed in Table 1 – these particular models are primarily applicable to streams and rivers rather than seeps and wetlands. Interestingly, some of the models incorporate volatilization using relatively complete constitutive relationships (mass transfer coefficients and henry's law driving forces) while other incorporate volatilization as a pseudo 1st order loss process (Smith et al., 1980; Genereaux and Hammond, 1990). In either case, the constitutive formulations are similar to the other baseline processes and are candidates for inclusion in the attenuation capacity for zone IV and, possibly, portions of zone III.

In the case of small quantities of solvents entering a receiving stream (zone IV in Figure 3), there is a significant potential for volatilization to attenuate the solvents and for models to be simplified and framed in terms of natural attenuation capacity compatible with summation with other mechanisms. Incorporation of this process into a natural

Table 1. Example surface water models that incorporate volatilization or that can incorporate volatilization as a 1st order loss mechanism

Model	Sponsor/Developer	Reference
One-dimensional transport with inflow and storage (OTIS)	USGS & University of Colorado	Runkel, 1998
Soil and Water Assessment Tool (SWAT)	USDA Agricultural Research Service	S.L. Neistch et al., 2002
Hydrological Simulation Program—Fortran (HSPF)	EPA & USGS	Bicknell et al, 1997
Simplified Method Program – Variable Complexity Stream Toxics Model (SMPTOX3)	EPA & LTI Limno-Tech, Inc.	US EPA, 1993
A Modular Fate and Effects Model for Aquatic Ecosystems (AQUATOX)	EPA	US EPA, 2001
RIVWQ	Waterborne Environmental, Inc.	http://www.waterborne-env.com/modeling/index.html

attenuation protocol would recognize and exploit a specific fundamental characteristic of chlorinated solvents – their tendency to transfer from water phases to air phases when given an opportunity. While scientifically defensible in cases where the contaminant flux poses no local ecological or human health risk, an approach that includes discharge to surface water and subsequent volatilization would require careful regulatory consideration because it represents a departure from traditional MNA protocols.

1.4   *Abiotic Degradation*

Abiotic degradation mechanisms can affect the fate and transport of chlorinated solvents in groundwater. The most common groups of abiotic reactions affecting these compounds include hydrolysis, dehydrohalogenation, and reduction reactions including hydrogenolysis and reductive elimination. These reactions are discussed in the following sections.

1.4.1   Hydrolysis

Hydrolysis is a substitution reaction in which a compound reacts with water, and a halogen substituent is replaced with a hydroxyl (OH-) group. Hydrolysis of organic compounds illustrated in the following equations frequently results in the formation of alcohols and alkenes (Knox et al., 1993 after Johnson et al., 1989):

$$RX + HOH \Leftrightarrow ROH + HX \tag{20}$$

$$H_3C - CH_2X \Leftrightarrow H_2C = CH_2 + HX \tag{21}$$

Where: X = Cl or Br

In general, the rates of hydrolysis are often quite slow within the range of normal groundwater temperatures, with half-lives of days to centuries (Vogel et al., 1987; Vogel, 1994). Hydrolysis results in reaction products that may be more susceptible to biodegradation, as well as more soluble (Neely, 1985).

The likelihood that a halogenated solvent will undergo hydrolysis depends in part on the number of halogen substituents. More halogen substituents on a compound will decrease the chance for hydrolysis reactions to occur (Vogel et al., 1987), and will therefore decrease the rate of the reaction. In addition, bromine substituents are more susceptible to hydrolysis than chlorine substituents (Vogel et al., 1987); for example, 1,2-dibromoethane is subject to significant hydrolysis reactions under natural conditions. McCarty (1996) lists 1,1,1-TCA as the only major chlorinated solvent that can be transformed chemically through hydrolysis (as well as elimination), leading to the formation of 1, 1-DCE and acetic acid. Based on data from Cline and Delfino (1989) and Haag and Mill (1988), McCarty estimates that 20% of TCA is converted to 1, 1-DCE, while 80% is transformed into acetic acid. The 20% converted to 1, 1-DCE is significant, however, because 1,1-DCE is considered more toxic than TCA.

Locations of the halogen substituent on the carbon chain also may have some effect on the rate of reaction. The rate may increase with increasing pH; however, rate dependence upon pH typically is not observed below a pH of 11 (Mabey and Mill, 1978; Vogel and Reinhard, 1986). Rates of hydrolysis may be increased by the presence of clays, which can act as catalysts (Vogel et al., 1987). Other factors that impact the level of hydrolysis include dissolved organic matter and dissolved metal ions.

Hydrolysis of chlorinated methanes and ethanes has been well demonstrated in the literature. Vogel (1994) reports that monohalogenated alkanes have half-lives on the order of days to months, while polychlorinated methanes and ethanes have half-lives that may range up to thousands of years (e.g., TCE, ethenes). As the number of chlorine atoms increases, dehydrohalogenation may become more important (Jeffers et al., 1989). Butler and Barker (1996) note that chlorinated ethenes do not undergo significant hydrolysis reactions (i.e., the rates are slow). Butler and Barker reported they were unable to find any studies on hydrolysis of vinyl chloride. A listing of half-lives for abiotic hydrolysis and dehydrohalogenation of some chlorinated solvents is presented in Table 2. Note that no distinctions are made in the table as to which mechanism is operating; this is consistent with the references from which the table was derived (Vogel et al., 1987; Washington, 1995; Butler and Barker, 1996).

One common chlorinated solvent for which abiotic transformations have been well-studied is 1,1,1-TCA. This compound may be abiotically transformed to acetic acid through a series of substitution reactions, including hydrolysis. In addition, 1,1,1-TCA may be reductively dehalogenated to form 1,1- DCA and then chloroethane (CA), which is then hydrolyzed to ethanol (Vogel and McCarty, 1987) or dehydrohalogenated to vinyl chloride (Jeffers et al., 1989). As summarized in Table 2, several parties have studied these reaction rates.

1.4.2 Dehydrohalogenation

Dehydrohalogenation is an elimination reaction involving halogenated alkanes in which a halogen is removed from one carbon atom, followed by the subsequent removal of a hydrogen atom from an adjacent carbon atom. In this two-step reaction, an alkene is produced. Although the oxidation state of the compound decreases due to the removal of a halogen; the loss of a hydrogen atom increases it. This results in no external electron transfer, and there is no net change in the oxidation state of the reacting molecule (Vogel et al., 1987). Contrary to the patterns observed for hydrolysis, the likelihood that dehydrohalogenation will occur increases with the number of halogen substituents. It has been suggested that under normal environmental conditions, monohalogenated aliphatics apparently do not undergo dehydrohalogenation, and these reactions are apparently not likely to occur (March, 1985; Vogel et al., 1987). However, Jeffers et al. (1989) report on the dehydrohalogenation of CA to vinyl chloride. Polychlorinated alkanes have been observed to undergo dehydrohalogenation under normal conditions and extremely basic conditions (Vogel et al., 1987). As with hydrolysis, bromine substituents are more reactive with respect to dehydrohalogenation.

Dehydrohalogenation rates may be approximated using pseudo-first-order kinetics. Once again, this is not truly a first-order reaction, but such approximations have been used in the literature to quantify the reaction rates. The rates will depend not only upon the number and types of halogen substituent, but also upon the hydroxide ion concentration.

Table 2. Approximate Half-Lives of Abiotic Hydrolysis and Dehydrohalogenation
Reactions involving Chlorinated Solvents at 25 degrees Celsius.

Compound	Half-Life (years)	Products
Chloromethane	no data	
Methylene Chloride (Dichloromethane)	$704^{a/}$	
Trichloromethane (Chloroform)	$3500^{a/}$, $1800^{b/}$	
Carbon Tetrachloride	$41^{b/}$	
Chloroethane	$0.12^{c/}$	ethanol
1,1-Dichloroethane	$61^{b/}$	
1,2-Dichloroethane	$72^{b/}$	
1,1,1-Trichloroethane	1.7^{a}, $1.1^{b/}$ $2.5^{d/}$	acetic acid 1,1-DCE
1,1,2-Trichloroethane	140^{b}, $170^{a/}$	1,1-DCE
1,1,1,2-Tetrachloroethane	47^{b}, $380^{a/}$	TCE
1,1,2,2-Tetrachloroethane	0.3 0.4^{b}, 0.8^{a}	1,1,2-TCA TCE
Tetrachloroethene	$1.3 \times 10^{6 \ b/}$	
Trichloroethene	$1.3 \times 10^{6 \ b/}$	
1,1-Dichloroethene	$1.2 \times 10^{8 \ b/}$	
1,2-Dichloroethene	$2.1 \times 10^{10 \ b/}$	

[a/] From Mabey and Mill, 1978
[b/] From Jeffers *et al*., 1989
[c/] From Vogel *et al*., 1987
[d/] From Vogel and McCarty, 1987
[e/] From Dilling *et al*., 1975

Under normal pH conditions (i.e., near a pH of 7), interaction with water (acting as a
weak base) may become more important (Vogel et al., 1987). Transformation rates for
dehydrohalogenation reactions are presented in Table 2.

The organic compound 1,1,1-TCA is also known to undergo dehydrohalogenation (Vogel
and McCarty, 1987). In this case, TCA is transformed to 1,1-DCE, which is then
reductively dehalogenated to vinyl chloride. The VC is then either reductively
dehalogenated to ethene, or consumed as a substrate in an aerobic reaction and converted
to carbon dioxide. In a laboratory study, Vogel and McCarty (1987) reported that the
abiotic conversion of 1,1,1-TCA to 1,1-DCE has a rate constant of about 0.04 $year^{-1}$. It
was noted that this result was longer than indicated in previous studies, but that
experimental methods differed. Jeffers et al. (1989) reported on several other
dehydrohalogenation reactions; in addition to 1,1,1-TCA and 1,1,2-TCA both degrading
to 1,1-DCE, the tetrachloroethanes and pentachloroethanes degrade to TCE and PCE,
respectively. As noted previously, Jeffers et al. (1989) also report that TCA may degrade
to vinyl chloride, but no information on rates was reported in the general literature.

1.4.3   Reduction Reactions

Reductive dehalogenation reactions are generally divided into two categories:
hydrogenolysis and reductive elimination (Figure 4). Hydrogenolysis is the simple
replacement of a chlorine atom (or other halogen) by a hydrogen atom. This is a
common biologically-mediated reaction but recently this reaction has been observed to
occur abiotically. Reductive elimination results in the loss of chlorine atoms, the
formation of a carbon bond, and possibly the addition of hydrogen atoms.
Dihaloelimination is the removal of two chlorines (or other halogens) accompanied by
the formation of a double carbon-carbon bond. Butler and Barker (1996) reviewed work
by Jafvert and Wolfe (1987), Reinhard et al. (1990), and Acton (1990). This review
suggests that while these reactions are thermodynamically possible under reducing
conditions, they often do not take place in the absence of biological activity, even if such
activity is only indirectly responsible for the reaction.

Microbes may produce reductants that, when combined with minerals in the aquifer
matrix, may transform chlorinated compounds (Gander et al., 2002). These biotic/abiotic
interactions have been suggested by work utilizing aquifer material from the Borden test
site (Reinhard et al., 1990). Moreover, the reducing conditions necessary to produce such
reactions most often are created as a result of microbial activity. It is therefore not clear
if some of these reactions are truly abiotic, or if, because of their reliance on microbial
activity to produce reducing conditions or reactants, they are similar to cometabolic
processes.

In some cases, truly abiotic reductive dechlorination has been observed; however, the
conditions that favor such reactions may not occur naturally. For example, Gillham and
O'Hannesin (1994) describe reductive dehalogenation of chlorinated aliphatics using
zero-valent iron, in which the iron serves as an electron donor in an electrochemical
reaction. However, this is not a natural process. Wang and Tan (1990) reported
reduction of TCE to ethene and carbon tetrachloride to methane during a platinum-
catalyzed reaction between elemental magnesium and water. Given the metals involved
in these reactions are unlikely to occur naturally in the reduced forms used in the
aforementioned work, such processes are not likely to contribute to natural attenuation of
chlorinated solvents.

1.4.4   Abiotic Reductive Dechlorination of Chlorinated Compounds by Iron-Bearing
Soil Minerals

In recent years it has become apparent that minerals found in the shallow subsurface can
cause the abiotic degradation of chlorinated solvents. According to Lee and Batchelor
(2003), some of the most abundant natural reductants are soil minerals that contain
reduced forms of iron and sulfur. Such soil minerals can significantly increase the
transformation rate of chlorinated organics in heterogeneous suspension with HS and
Fe(II) and can also reductively transform organic and inorganic contaminants by
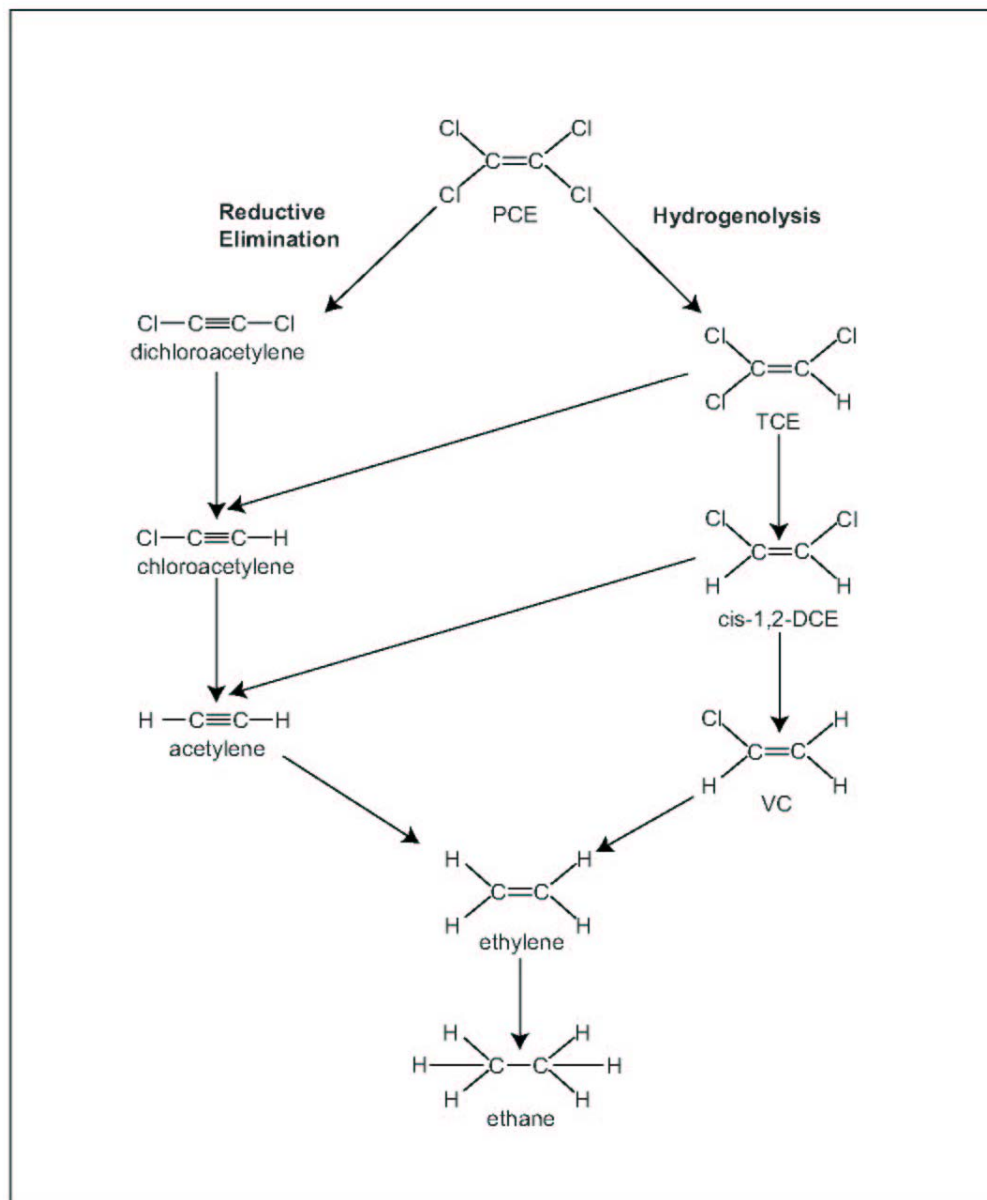themselves (Lee and Batchelor, 2003).

Figure 4. Possible Pathways for Reductive Dehalogenation Reactions.
Reductive Dechlorination of Chlorinated Ethylenes by Pyrite used as an
example. Modified from Lee and Batchelor (2002a).

Reductive capacities of soil minerals and soil for chlorinated ethylenes have been measured and characterized by Lee and Batchelor (2002a, 2002b, and 2003). These researchers found the reductive capacities of iron-bearing sulfide (pyrite), hydroxide (green rust; GR_{SO4}), and oxide (magnetite) minerals for tetrachloroethylene (PCE) were 1-3 orders of magnitude greater than those of iron-bearing phyllosilicates such as biotite, vermiculite, and montmorillonite (Lee and Batchelor, 2003). GR_{SO4} was shown to have the greatest reductive capacity for PCE followed by magnetite, pyrite, biotite, montmorillonite, and vermiculite. Lee and Batchelor (2003) show the reductive capacities of pyrite and GR_{SO4} for chlorinated ethylenes decreased in the order: TCE > PCE > cis-1,2-DCE > VC. They also found that the Fe(II) content in soil minerals was directly proportional to the reductive capacity of soil minerals for PCE, suggesting that Fe(II) content is an important factor that significantly affects reductive transformations of target contaminants in natural systems. Lee and Batchelor (2002a, 2002b, and 2003) did not investigate the reduction of chlorinated compounds by iron sulfide reduction. That process is discussed below in section 1.1.4.4.3.

1.4.4.1 Pyrite and Magnetite

Abiotic reductive dechlorination of chlorinated ethylenes (tetrachloroethylene (PCE), trichloroethylene (TCE), cis-1,2-dichloroethylene (cis-DCE), and vinyl chloride (VC)) by pyrite and magnetite was characterized by Lee and Batchelor (2002a) in a batch reactor system. Surface area-normalized pseudo-first-order initial rate constants for target organics by pyrite were found to be 23.5 to 40.3 times greater than for those target organics by magnetite.

Target organics were mainly transformed to acetylene and small amounts of chlorinated intermediates, which suggests that β-elimination was the main dechlorination pathway. The dechlorination of VC followed a hydrogenolysis pathway to produce ethylene and ethane. The results obtained in this research provide basic knowledge to better predict the fate of chlorinated ethylenes and to understand the potential of abiotic processes in natural attenuation.

1.4.4.1.1   Pyrite

Lee and Batchelor (2002a) found the rate constants for reductive dechlorination caused by pyrite were greatest for VC and decreased in the following order VC (1.71 day^{-1}) > TCE (1.6 day^{-1}) > PCE (1.01 day^{-1}) > cis-1,2-DCE (0.98 day^{-1}). Note the rates are very similar and differ by a factor of only 1.7.

The degradation of PCE by pyrite resulted in the production of the following as a percentage of the removed PCE:
        acetylene - 71.1%
        ethylene - 13.3%
        TCE - 14.9%
        cis-1,2-DCE and VC were not detected.

The degradation of TCE by pyrite resulted in the production of the following as a percentage of the removed TCE:
    acetylene - 86%
    ethylene – 4.4%
    cis-1,2-DCE – 6.6%
    VC was not detected.

The degradation of cis-1,2-DCE by pyrite resulted in the production of the following as a percentage of the removed TCE:
    acetylene – 44.3%
    ethylene – 17.5%
    Neither ethane or VC were detected.

The degradation of VC by pyrite resulted in the production of the following as a percentage of the removed TCE:
    ethylene – 77.3%
    ethane – 11.4%
    Acetylene was not detected.

Based on the discussion presented above, Figure 4 illustrates possible pathways for the reductive dechlorination of chlorinated ethenes by pyrite.

1.4.4.1.2   Magnetite

Lee and Batchelor (2002a) found the rate constants for reductive dechlorination caused by magnetitewas greatest for VC and decreased in the following order VC (0.35 day^{-1}) > TCE (0.25 day^{-1}) > cis-1,2-DCE (0.28 day^{-1}) > PCE (0.2 day^{-1}). The addition of Fe(ll) increased the dechlorination rate of cis-DCE and VC in magnetite suspension by nearly a factor of 10.

1.4.4.2 Green Rust

Green rust is is a mixed Fe(II)-Fe(III) hydroxide that has been shown to degrade carbon tetrachloride (CT), hexa- and pentachloroethane, and the chlorinated ethenes. Abiotic reductive dechlorination of chlorinated ethylenes by the sulfate form of green rust GR_{SO4} was examined in batch reactors by Lee and Batchelor (2002b) (Figure 5). Surface area-normalized pseudo-first-order initial rate constants for chlorinated ethylenes by GR_{SO4} were 3.4 to 8.2 times greater than those by pyrite. As with most of the other abiotic reduction reactions, PCE and TCE were mainly transformed to acetylene, and no detectable amounts of chlorinated intermediates were observed. Figure 5 shows the distribution of target organics resulting from the reductive dechlorination of chlorinated ethylenes caused by GR_{SO4} reduction. These researchers postulate that the main pathway for the transformation of PCE, TCE, and cis-1,2-DCE by GR_{SO4} is probably the reductive elimination pathway with further reduction to ethylene. Lee and Batchelor suggest the following reactions:
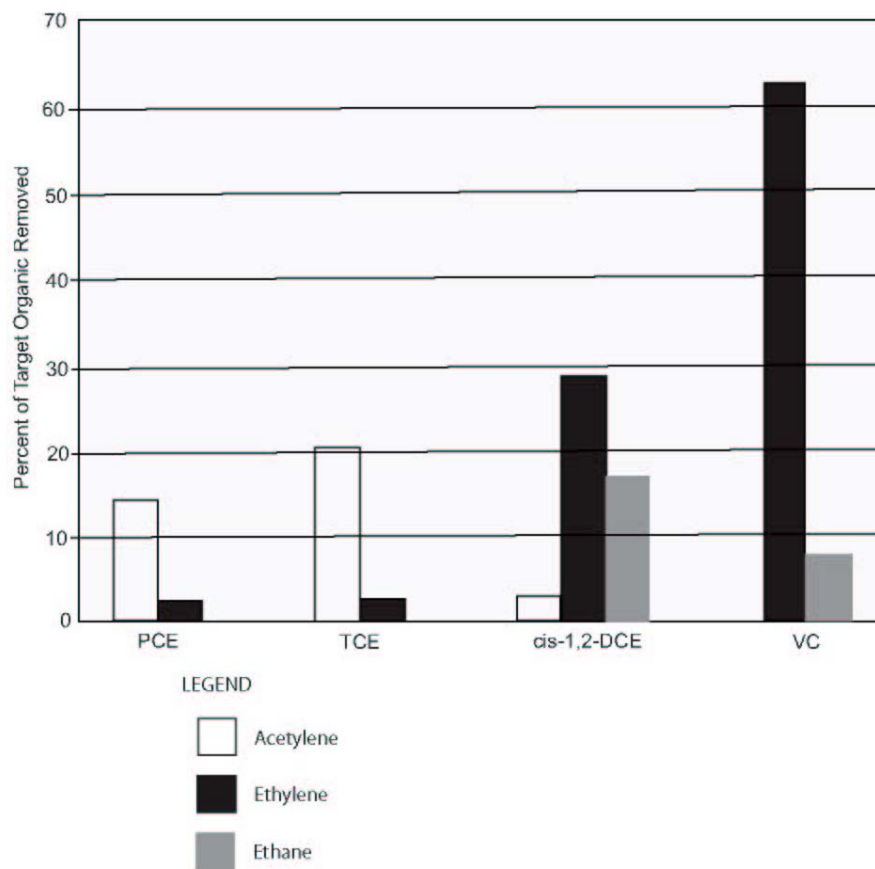
Figure 5. The Product Distribution for the Reductive Dechlorination of Target Analytes in GR_{SO4} Suspension (0.007 g/g). Modified from Lee and Batchelor (2002b).

PCE → dichloroacetylene → chloroacetylene → acetylene → ethylene
TCE → chloroacetylene → acetylene → ethylene
cis-1,2-DCE → chloroacetylene → acetylene → ethylene

The reaction pathway for VC being reduced by GR_{SO4} is probably as follows and occurs via hydrogenolysis.

VC → ethylene → ethane

Lee and Batchelor (2002b) found the rate constants for the reductive dechlorination of trichloroethylene (TCE) increased as pH increased (6.8 to 10.1) but were independent of solid concentration and initial TCE concentration. Magnetite and/ or maghemite were produced by the oxidation of GR_{SO4} by TCE. Lee and Batchelor (2002b) found the rate constants for reductive dechlorination were greatest for PCE and decreased in the following order PCE > VC > TCE > cis-1,2-DCE. According to Lee and Batchelor (2002b and 2003), the relatively slow rate of cis-1,2-DCE degradation is similar to the observed degradation rates for chlorinated ethylenes by pyrite and magnetite. These findings are important for understanding the role of abiotic reductive dechlorination during natural attenuation in environments that contain GR_{SO4}.

1.4.5   Transformation of Chlorinated Solvents by Iron Sulfide

Butler and Hayes (1999) studied the transformation of PCE, TCE, and 1,1-dichloroethylene (1,1-DCE) by FeS in aqueous solution at pH 8.3 in batch experiments. PCE and TCE were transformed by FeS with pseudo-first-order rate constants (Figure 6). A 17% decrease in the concentration of 1, 1-DCE was observed over 120 days; however, no reaction products were detected. TCE and PCE transformation data were fit to a rate law assuming transformation of TCE via parallel reaction pathways to acetylene via reductive elimination and cis-1,2-dichloroethylene (cis-1,2-DCE) via hydrogenolysis and transformation of PCE via parallel reaction pathways to acetylene and TCE. Acetylene was the major reaction product for both TCE and PCE. Determination of rate constants for each reaction pathway indicated that TCE was transformed to acetylene 11.8 ± 1.1 times faster than to cis-DCE and that PCE was transformed to acetylene 8.2 ± 1.8 times faster than to TCE. Additional minor reaction products were vinyl chloride VC for TCE and cis-1,2-DCE for PCE. Detection of acetylene as the major product of both TCE and PCE transformation by FeS contrasts with the sequential hydrogenolysis products typically observed in the microbial transformation of these compounds, making acetylene a potential indicator of abiotic transformation of PCE and TCE by FeS in natural systems. Figure 6 shows hypothesized reaction pathways for transformation of PCE and TCE by FeS.

Although FeS is likely present in the subsurface at many sites where chlorinated compounds are being degraded, its production relies on the occurrence of iron reduction and sulfate reduction, both of which are microbially-mediated reactions. Thus, even though degradation of solvents can occur abiotically, several biological reactions still are necessary to form the correct environment. The production of FeS in the terrestrial
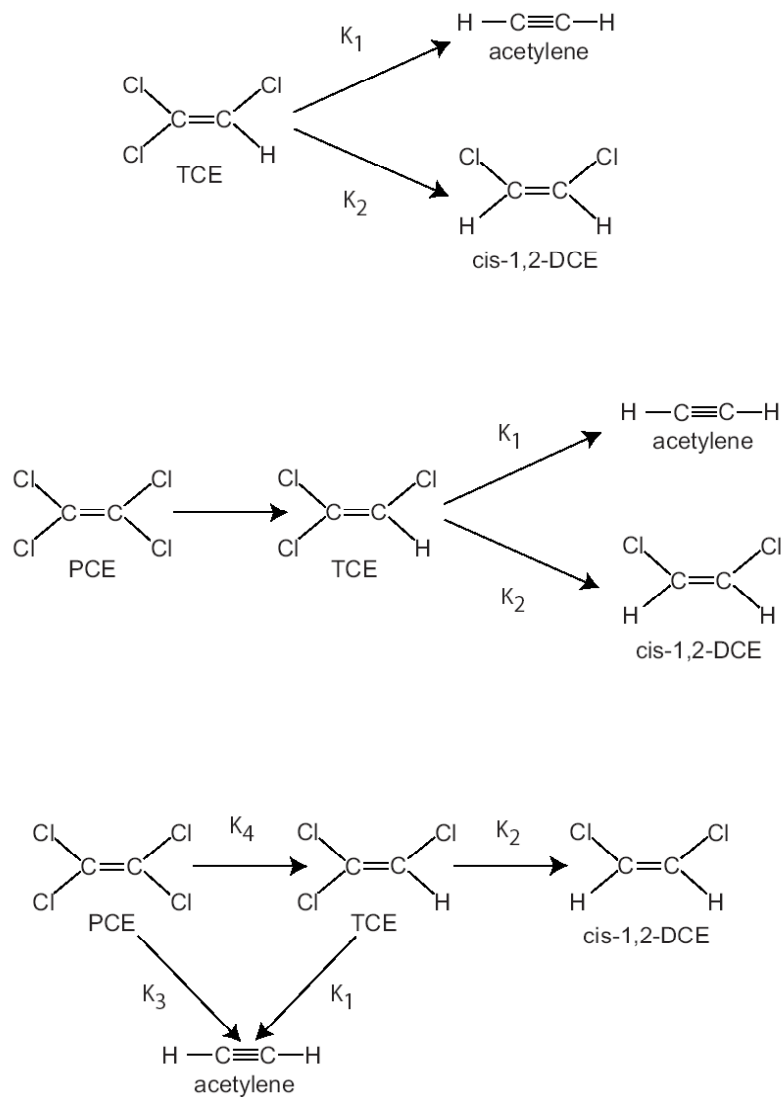
Figure 6. Hypothesized Reaction Pathways for Transformation of TCE and PCE by FeS. Modified from Butler and Hayes (1999).

subsurface results from the production of Fe^{2+} during dissimilatory iron reduction and the production of H_2S from sulfate reduction, both of which occur under reducing conditions caused by microbial degradation of organic carbon.

The following equation describes the overall stoichiometry of ferric iron reduction resulting in the production of Fe2+ using organic carbon generalized as CH is given by:

$$10H^+ + 5Fe(OH)_3 + CH \rightarrow CO_2 + 5Fe^{2+} + 13H_2O$$
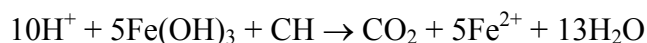
The following equation describes the overall stoichiometry of sulfate reduction resulting in the production of H_2S using organic carbon generalized as CH is given by:

$$1.25H^+ + 0.625SO_4^{2-} + CH \rightarrow CO_2 + 0.625H_2S + 0.5H_2O$$
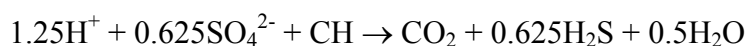
If the system is sulfate reducing or methanogenic and there is reduced iron in the system, then iron sulfide will be produced by the following reaction:

$$H_2S + Fe^{2+} \rightarrow FeS + 2H^+$$
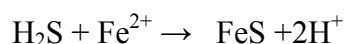
1.4.6   Importance to NAC

In the survey of MNA sites, hydrolysis was an active NAC process in about 8 percent of the sites surveyed and the other abiotic reactions also were active in 9 percent. However, abiotic reactions, with the exception of hydrolysis, were not considered important NAC processes. Hydrolysis was considered important in about 5 percent of the sites.

2.0   *Biodegradation*

Biodegradation processes are an important component of the natural attenuation capacity of chlorinated ethenes. This is because biodegradation can transform chlorinated ethenes to environmentally innocuous compounds such as carbon dioxide, ethene, and chloride. However, because some transformation products, such as vinyl chloride, can increase the toxicity of chlorinated solvents, biodegradation processes must be carefully assessed in applications of MNA.

Chlorinated ethenes are subject to a variety of microbial degradation processes that include reductive dechlorination (Barrio-Lage et al. 1987, 1990; Bouwer 1994; McCarty and Semprini, 1994; Odum et al. 1995; Vogel, 1994; Vogel and McCarty,1985), aerobic oxidation (Hartmans et al. 1985; Davis and Carpenter, 1990; Phelps et al. 1991; Bradley and Chapelle 1996, 1998a, 1998b; Bradley et al. 1998;), anaerobic oxidation (Bradley and Chapelle,1996; Bradley and Chapelle, 1998b; Bradley et al., 1998c), anaerobic fermentation (Bradley and Chapelle, 2000b), and aerobic cometabolism (Wilson and Wilson, 1985; McCarty and Semprini, 1994; Semprini, 1995; Semprini et al. 1990, 1991). Thus, assessing if a particular degradation process is active in a hydrologic system is often complex. However, because the conditions under which these processes occur are

known, it is possible to identify the presence or absence of particular degradation processes at a given site.

The mechanisms by which chlorinated ethenes are transformed depend on its chemical composition and whether it will serve as an electron donor, electron acceptor, or another biologically relevant compound such as a macronutrient. Compounds that serve as electron donors or electron acceptors are readily degraded because oxidation/reduction (redox) reactions are predominant metabolic reactions within microbial populations and among the various microbial communities present in all environments, whether natural or artificial (Chapelle and Lovley 1990; Anderson and Lovley 1997). Microorganisms produce energy in the form of adenosine triphosphate (ATP) via intracellular or extracellular redox reactions. All energy-yielding metabolism depends on these processes, therefore numerous inorganic and organic compounds participate in microbial redox transformations. Compounds that are highly electronegative (electron withdrawing) are electron acceptors; compounds that are highly electropositive (electron donating) are electron donors (Alexander 1994; Brock, Madigan et al. 1994). Some compounds can serve as either electron donors or electron acceptors dependent upon the surrounding chemistry and microbial ecology, because their relative electronegativity is an intermediate between the two extremes (Vogel, Criddle et al. 1987). Redox transformations govern the prevailing geochemistry of sedimentary and subsurface environments, and therefore influence contaminant degradation; these processes will be discussed in greater detail below.

2.1   *Reductive Dechlorination*

Chlorinated ethenes are relatively oxidized compounds due to the presence of electronegative chlorine atoms, and can act as electron acceptors in microbial metabolism (Vogel et al. 1987). In the presence of a suitable electron donor and catalyst, hydrogen can replace a chlorine on a chlorinated ethene molecule. This microbially catalyzed process is called *reductive dechlorination.* A number of reports have described the accumulation of perchloroethene (PCE) and trichloroethene (TCE) transformation products in anaerobic groundwater systems and attributed this phenomenon to microbial reductive dechlorination (Barrio-Lage et al. 1987, 1990; Bouwer 1994, McCarty and Semprini 1994; Odum et al. 1995; Vogel 1994; Vogel and McCarty, 1985). The consensus today is that microbial reductive dechlorination is ubiquitous in anaerobic, chloroethene-contaminated aquifers, but that the extent of dechlorination is highly variable from site to site (Chapelle, 1996; Bouwer,1994; Gossett and Zinder, 1996; McCarty,1996; McCarty and Semprini, 1994; Vogel,1994).

The tendency of chlorinated ethenes to undergo reductive dechlorination decreases with decreasing number of chlorine substituents (Bouwer 1994; McCarty and Semprini 1994; Vogel 1994; Vogel et al. 1987). PCE, with its four chlorine atoms, is a stronger oxidant than all of the naturally occurring electron-accepting species found in groundwater systems, with the notable exception of O_2 (Vogel et al. 1987). Thus PCE readily undergoes reductive dechlorination to TCE except in aerobic aquifers. Reductive dechlorination of TCE to *cis*-dichloroethene (*cis*-DCE) occurs under Fe(III)-reducing

conditions and in more strongly reducing environments. Reductive dechlorination of *cis*-DCE to yield vinyl chloride (VC) apparently requires at least SO_4-reducing conditions (Chapelle 1996; Vogel et al. 1987), but proceeds more readily in methanogenic environments. VC, on the other hand, is the least oxidized of the chloroethenes. Consequently, reductive dechlorination of VC to the nonchlorinated product, ethene, is characteristically slow and significant only under highly reducing, methanogenic conditions (Ballapragada et al. 1995; Barrio-Lage et al. 1987, 1990; Bouwer, 1994; De Bruin et al. 1992; DiStefano et al. 1991; Fennell et al. 1995; Freedman and Gossett 1989; Maymo-Gatell et al. 1995; Odum et al. 1995; Vogel and McCarty 1985; Wu et al. 1995). As a result of this decreasing reductive potential with decreasing number of chlorine substituents, reductive dechlorination of chloroethene contaminants is often incomplete in groundwater systems and frequently leads to the accumulation of *cis*-DCE and VC (Haston et al., 1994; Kitanidis et al., 1993; Major et al., 1991; McCarty and Reinhard, 1993; Weaver et al., 1995; Wilson et al., 1995).

The growing recognition that microbial reductive dechlorination is common in anaerobic aquifers but rarely results in complete reduction to non-chlorinated products undermined early efforts to exploit intrinsic microbial activity for remediation of chloroethene-contaminated ground water. Because VC is a USEPA priority pollutant, its observed production and accumulation in anaerobic aquifers represents a potential increase in risk to human health and the environment. Consequently, in situ production of VC by indigenous microorganisms increased regulatory concern over chloroethene-contamination and prompted widespread application of physical remediation methods like pump and treat in an effort to remediate contaminated groundwater. Unfortunately, because groundwater systems are often hydrogeologically complex, pump and treat systems are generally slow and inefficient (Bouwer, 1994). The lack of an effective alternative combined with subsequent advances in the understanding of microbial mechanisms for degrading chloroethenes has, in recent years, led to a reexamination of bioremediation in chloroethene-contaminated aquifers.

An appreciation for the potential contribution of microbial reductive dechlorination to chloroethene remediation in groundwater systems was delayed by the widely held perception that this process is essentially accidental and of no benefit to the microorganisms carrying out the processes. Furthermore, because the molar concentration of chloroethenes in groundwater systems is generally low in all but the most contaminated source areas, it is generally thought that these compounds are not sufficiently concentrated to serve as primary substrates in microbial metabolism. In addition, the first pure cultures determined to be capable of reductive dechlorination were methanogens that did not grow on chloroethenes and apparently did not gain energy from the reaction. These organisms mediated slow and partial reductive dechlorination of PCE and TCE to yield DCE. Based on these observations, reductive dechlorination has often been viewed as an anaerobic cometabolism brought about by the accidental interaction of chloroethenes with enzymes and co-factors produced by the microorganisms for other metabolic purposes (McCarty and Semprini, 1994). This type of cometabolic dechlorinating process was considered ubiquitous in anaerobic systems but generally incapable of mediating complete reduction to non-toxic products like ethene.

This thinking began to change in 1993, when a number of investigations demonstrated that reductive dechlorination can be carried out by microorganisms which use chloroethene compounds as terminal electron acceptors for energy production. These microorganisms, collectively termed halorespirers, are able to grow using chloroethenes as sole terminal electron acceptors and are capable of much higher rates of reductive dechlorination (Table 3). Although halorespirers capable of reducing PCE or TCE to DCE are relatively common (Holliger et al. 1993; Krumholz et al. 1996; Maymo-Gatell et al. 1997; Sharma et al. 1996), to date only one, *Dehalococcus ethenogenes*, has been shown to completely degrade PCE to ethene (Maymo-Gatell et al. 1997). If microorganisms like *D. ethenogenes* are present in groundwater systems in significant numbers then complete reductive dechlorination to ethene may be a significant mechanism for chloroethene bioremediation in anaerobic aquifers.

Dehalococcoides ethenogenes strain 195, has been reported to completely dechlorinate cis-DCE via vinyl chloride (VC) to ethene. This is supported by various studies that show that cultures containing or isolates of *Desulfitobacterium*, *Dehalobacter restrictus*, *Desulfuromonas*, *Dehalospirillum multivorans,* and *Dehalococcoides ethenogenes* are capable of dechlorinating PCE and TCE to cis-DCE (Sholz-Muramatsu et al. 1995; Gerritse et al. 1996; Krumholz 1997; Maymo-Gatell et al. 1997; Holliger et al. 1998; Loffler et al. 2000; Finneran, 2002). However, only cultures that contain *Dehalococcoides* have been reported to dechlorinate cis-DCE and vinyl chloride and ethene (Adamson and Parkin, 2000; Cupples et al., 2003; Dennis et al., 2003; Duhamel et al., 2003, Ellis et al., 2000, Maymo-Gatell et al. 1997a,b; Fennell et al. 2001; He et al., 2003; Lendvay et al. 2003). Using sensitive molecular analyses, a variety of investigations have observed that the presence of *Dehalococcoides* is a critical pre-requisite to complete dechlorination in situ (Fennell et al. 2001; Hendrickson et al. 2002).

 Fennell et al. (2001) demonstrated a correlation between the presence of *Dehalococcoides* and the extent of dechlorination. They constructed microcosms using materials from two locations, one testing positive and the other testing negative for the presence of *Dehalococcoides* spp, along with sterile controls. Microcosms constructed using materials that were positive for the presence of *Dehalococcoides* reduced TCE to vinyl chloride and ethene, and continued to test positive for the presence of *Dehalococcoides* following dechlorination. In contrast, microcosms constructed using material that was initially negative for the presence of *Dehalococcoides* did completely dechlorinate TCE, and continued to test negative for *Dehalococcoides* at the end of the study. *Dehalococcoides* was not detected in the autoclaved controls. Hendrickson et al. (2002) evaluated the microbiology of chlorinated solvent plumes at 24 sites, and demonstrated that *Dehalococcoides* spp were only detected in samples from sites where PCE or TCE was being completely reduced to ethene. At sites where *Dehalococcoides* were not detected and dechlorination stalled at cis-DCE, the addition of mixed cultures containing *Dehalococcoides* led to complete dechlorination to ethene and the establishment of *Dehalococcoides* organisms in the bioaugmented aquifer (Ellis et al. 2000; Major et al. 2002). Lendvay et al., 2003, demonstrated that bioaugmentation

Table 3. Isolated Chloroethene-Respiring Bacteria (From Major et al., 2003)

Isolate	Closest Phylogenetic Affiliation	Dechlorination Steps Performed	Reference
Dehalobacter restrictus	Low G+C Gram positive bacteria	PCE to cDCE	(Holliger *et al.*, 1993)
Dehalospirillum multivorans	Proteobacteria, ε subdivision	PCE to cDCE	(Scholz-Muramatsu et al., 1995)
Desulfitobacterium Strain PCE1	*Desulfitobacterium* Gram positive	PCE to TCE	(Gerritse *et al.*, 1996)
Desulfuromonas chloroethenica	*Geobacter*	PCE to cDCE	(Krumholz *et al.*, 1996)
Strain MS-1	*Enterobacteriaceae*	PCE to cDCE	(Sharma and McCarty, 1996)
Strain TEA	Low G+C Gram positive bacteria	PCE to cDCE	(Wild et al., 1996)
Desulfitobacterium sp. strain PCE-S	*Desulfitobacterium* Gram positive	PCE to cDCE	(Miller et al., 1997)
Dehalococcoides ethenogenes Strain 195	Green, nonsulfur bacteria	PCE to ethene	(Maymo-Gatell *et al.*, 1997a)
Desulfitobacterium frappieri TCE1.	*Desulfitobacterium* Gram positive	PCE to cDCE	(Gerritse *et al.*, 1999)
Clostridium bifermentans Strain DPH-1	*Clostridium*	PCE to cDCE	(Chang et al., 2000)
Chlorobenzene-degrading Strain CBDB1	*D. ethenogenes*	PCE to tDCE	(Adrian *et al.*, 2000)
Desulfitobacterium sp. Strain Y51	*Desulfitobacterium* Gram positive	PCE to cDCE	(Suyama et al., 2002)
Desulfitobacterium metallireducens	*Desulfitobacterium* Gram positive	PCE to cDCE	(Finneran et al., 2003)
Desulfuromonas michiganenis	*Geobacter*	PCE to cDCE	(Sung et al., 2003)
Dehalococcoides ethenogenes Strain BAV1	Green, nonsulfur bacteria	DCE isomers to ethene	He et al., 2003

shortens the lag time for complete dechlorination to ethene even in aquifers that contain *Dehalococcoides* spp.

Hendrickson et al. (2002) have reported that *Dehalococcoides* species comprise metabolically and phylogenetically distinct subgroups. For example, *Dehalococcoides* strain 195 (grouped with the Cornell strain) conserves energy for growth by reducing cis-DCE to VC, but does not conserve energy by reducing VC to ethene, which is a slow process (Maymo-Gatell et al. 1999, 2001). However, this strain can also use 1,1-dichloroethene (1,1-DCE) and 1,2-dichloroethane (1,2-DCA). In contrast, other *Dehalococcoides* spp sustain dechlorination of VC to ethene, when supplied with only VC as electron acceptor (Duhamel *et al*., 2002, He, 2003) indicating VC reduction will sustain microbial growth by itself. Cupples et al., 2003 reported that Dehalococcoides spp obtain energy from VC through molecular monitoring of *Dehalococcoides* growth kinetics. Other *Dehalococcoides* strains, such as CDBD1, which is part of the Pinellas cluster of *Dehalococcoides*, reduces 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,4-TCB, 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB), 1,2,3,5-TeCB, or 1,2,4,5-TeCB to dichlorobenzenes or 1,3,5-TCB, and polychlorinated dibenzodioxins but cannot dechlorinate PCE or TCE to cis-DCE, VC or ethene (Adrian et al., 2000, Bunge et al., 2003). Wu et al, 2002, reported that more distant relatives of Dehalococcoides reduced PCBs. Therefore, the presence of *Dehalococcoides* does not necessarily mean that cis-DCE will be reduced to ethene. However, all studies to date have found that the converse is true: if *Dehalococcoides* are absent, then dechlorination past cis-DCE (to VC and ethene) was not detected.

At present, the general consensus is that although complete microbial reductive dechlorination is possible in anaerobic aquifers, complete reductive dechlorination to ethene is rarely observed due to low electron donor availability, unfavorable redox conditions, or the absence of appropriate microorganisms. Thus reductive dechlorination alone does not usually remediate chloroethene contamination in groundwater systems. However, in combination with aerobic and anaerobic mechanisms for microbial oxidation of DCE and VC, the process of reductive dechlorination can be an important component of the natural attenuation of chlorinated ethenes in groundwater systems.

2.2   *Aerobic Oxidation*

Although the tendency of chloroethenes to undergo reductive dechlorination decreases as the number of chlorine substituents decreases, the tendency to undergo oxidation increases with decreasing number of chlorine substituents (Vogel et al. 1987). As the least chlorinated of the chloroethenes, VC has the greatest tendency to undergo oxidation. Rapid microbial degradation of vinyl chloride, including mineralization, has been observed in laboratory cultures and aquifer samples under aerobic conditions (Bradley and Chapelle 1996, 1998a, 1998b; Bradley et al. 1998b; Hartmans et al. 1985; Davis and Carpenter, 1990; Phelps et al. 1991). Moreover, under aerobic conditions, VC can be used as a sole carbon source for growth and metabolism (Hartmans et al. 1985; Hartmans and de Bont, 1992). These observations indicate that, in addition to incidental oxidation

via cometabolic mechanisms, VC can be utilized as a primary substrate for microbial oxidation under aerobic conditions.

The co-metabolic oxidation of DCE under aerobic conditions has been demonstrated for a variety of co-substrates including methane, phenol, toluene, and propane (McCarty and Semprini, 1994). In addition, several recent studies indicate that microbial oxidation of DCE to CO_2 can also occur aerobically in the absence of detectable co-substrates (Bradley and Chapelle 1998b; Bradley et al. 1998b, 1998c). Mineralization of DCE without addition of a carbon co-substrate has been documented in aquifer sediments (Bradley and Chapelle 1998b; Bradley et al. 1998b, 1998c). The fact that DCE mineralization activity was observed in liquid cultures (10^6 final dilution) containing DCE as sole carbon source and oxygen as the terminal electron acceptor (Bradley and Chapelle, 2000a) indicates that DCE can be a primary substrate without the need for a carbon co-substrate. Microbial growth with oxygen as an electron acceptor and DCE as sole carbon source has only recently been reported.

The microbial oxidation of DCE and VC under aerobic conditions is of limited relevance in most ground-water systems. In the rare event that VC is directly released to aerobic aquifers (a possibility at PVC manufacturing sites, Hartmans, 1995), rapid aerobic mineralization of VC by aquifer microorganisms can be expected. For the overwhelming majority of sites, however, the presence of DCE and VC in groundwater is attributable to the existence of anaerobic conditions and the activity of reductive dechlorinating microorganisms. Addition of oxygen to groundwater to stimulate aerobic contaminant biodegradation in an otherwise anaerobic aquifer system is expensive and in most cases impractical. Consequently, aerobic biodegradation of chloroethene contaminants in groundwater systems is probably limited to the edge of the contaminant plume where dissolved oxygen has not been depleted by microbial respiration (Bradley et al., 1998b). However, as discussed earlier, production and accumulation of VC requires the reducing conditions associated with SO_4-reduction and methanogenesis. Thus, because the co-occurrence of VC and aerobic conditions is rare in groundwater systems, aerobic oxidation of DCE and VC as primary substrates for microbial metabolism is not considered to be an important mechanism for chloroethene biodegradation. As was the case for cometabolic oxidation, it is worth noting that microbial oxidation of DCE and VC under aerobic conditions can be significant at the interface of groundwater and surfacewater systems (Bradley and Chapelle 1998a, 1998b).

2.3   *Anaerobic Oxidation*

By 1995, efforts to utilize in situ-bioremediation in chloroethene-contaminated aquifers had reached an impasse. By this time, microbial reductive dechlorination of PCE and TCE was considered pervasive in anaerobic aquifers, but, in most systems, reductive dechlorination appeared to stop with DCE and VC. Likewise, the characteristic association of DCE and VC accumulation with anaerobic conditions disqualified aerobic microbial metabolism as a significant mechanism for bioremediation of these compounds under in situ conditions. Without a demonstrable mechanism for in situ biodegradation of chloroethenes to non-toxic products, state and federal regulators were understandably

reluctant to accept in situ biodegradation as a legitimate mechanism for remediation of chloroethenes in ground water.

The discovery in 1996 that microorganisms can oxidize VC to CO_2 under anaerobic conditions paved the way for regulatory acceptance of in situ bioremediation as a defensible mechanism for remediation of chloroethene-contaminated aquifers (Bradley and Chapelle 1996). Oxidation of vinyl chloride can occur under anaerobic conditions, if a sufficiently strong oxidant is available to drive microbial degradation. Fe(III)-oxides are strong oxidants that are ubiquitous in groundwater systems. In an experiment conducted with sediment from a Fe(III)-reducing aquifer, addition of Fe(III) to anaerobic microcosms resulted in VC mineralization rates comparable to those observed under aerobic conditions. Low but significant VC mineralization was also observed in anaerobic microcosms under ambient Fe(III) conditions. The results indicated that vinyl chloride could be mineralized under anaerobic, Fe(III)-reducing conditions, and that the bioavailability of Fe(III) was an important factor affecting the rates of mineralization. More importantly, microbial oxidation of VC under Fe(III)-reducing conditions provided a potential anaerobic alternative to the slow and inefficient reductive dechlorination of VC to ethene.

For groundwater contaminants such as petroleum hydrocarbons that serve as electron donors during biodegradation, the availability of competing terminal electron acceptors affects the efficiency of contaminant biodegradation (Borden 1994, Chapelle et al. 1996). Oxygen-reduction is the most thermodynamically favored and, typically, the most efficient metabolic mechanism for biodegradation of reduced groundwater contaminants. Among the remaining terminal electron acceptors that are commonly observed in ground-water systems, the oxidation potential decreases in the order of $NO_3 > Fe(III) > SO_4 > CO_2$. Thus, in general, the potential for biodegradation of highly reduced groundwater contaminants is greatest under aerobic conditions and least under CO_2-reducing (methanogenic) conditions. Because DCE and VC are relatively reduced compounds, a similar pattern of decreasing oxidation potential under increasingly reducing conditions would be expected (Vogel et al. 1987).

Although these mechanisms have been somewhat overlooked when compared to reductive dechlorination it is likely that anaerobic oxidation can be a significant attenuation pathway for VC and a partial attenuation pathway for cis-DCE in certain sedimentary and subsurface environments. Cis-DCE tends to accumulate during TCE reduction; this may be a function of electron donor concentration, presence or absence of the appropriate microbial community or population, or competing processes. VC has been reported to accumulate as well, indicating that cis-DCE and VC reduction kinetics are slower than TCE reduction, and that cis-DCE and VC reduction is more specialized than TCE reduction (Vogel, Criddle et al. 1987; Maymo-Gatell, Anguish et al. 1999). These compounds may accumulate directly within Fe(III), Mn(IV), and humic substance rich environments; previous reports indicate that known Fe(III)-reducing microorganisms reduce TCE to cis-DCE (Finneran, Forbush et al. 2002). These compounds may also migrate into Fe(III), Mn(IV), and humic substance rich zones after reductive dechlorination has produced them upgradient (Bradley 2000). When cis-DCE and VC

accumulate in the presence of these electron acceptors anaerobic oxidation is the dominant selective pressure, not reductive dechlorination. All electron donors will be favored by Fe(III), Mn(IV), and humics reducers rather than halorespiring microorganisms because they are more energetically favorable processes. Therefore VC is apt to become an electron donor in these circumstances. Cis-DCE will degrade by two competing pathways – reductive and oxidative. The extent of anaerobic cis-DCE oxidation will depend on the concentration of cis-DCE versus Fe(III), Mn(IV), or humic substances, and the presence of other electron donors (notably hydrogen). If cis-DCE concentration is low and the corresponding metals or humic substances are high concentration, then it is likely that cis-DCE will degrade more readily via the oxidative pathway. If cis-DCE concentration is high and corresponding metal or humic substances are low concentration, then it is likely that cis-DCE will be reduced to VC, which will subsequently be oxidized to CO_2.

The effect of redox conditions on DCE and VC oxidation was investigated in aquifer and bed-sediment microcosms (Bradley and Chapelle 1998b). In that study, mineralization of DCE and VC to CO_2 decreased under increasingly reducing conditions, but significant mineralization was observed for both sediments under anaerobic conditions. VC mineralization decreased in the order of aerobic > Fe(III)-reducing > SO_4-reducing > methanogenic conditions. As one would expect given their difference in chlorine number, microbial oxidation of VC was greater than microbial oxidation of DCE for each electron-accepting condition. For both sediments, microbial mineralization of DCE under aerobic conditions was at least twice that observed under anaerobic conditions. It is interesting to note that the rate and magnitude of DCE oxidation did not differ significantly between Fe(III)-reducing, SO_4-reducing and methanogenic conditions. Based on this and other observations, it was concluded that microbial oxidation of DCE under these redox conditions involved an initial rate-limiting reduction to VC which was subsequently oxidized to CO_2. This in turn suggested that direct oxidation of DCE requires a more powerful oxidant than Fe(III)-oxides. A subsequent investigation demonstrated that aquifer microorganisms can anaerobically oxidize DCE to CO_2 under Mn(IV)-reducing conditions without an initial reduction to VC (Bradley et al. 1998c). Because Mn(IV) oxides are common in alluvial and glacial aquifer sediments, microbial oxidation of DCE may be significant in some anaerobic groundwater systems.

The ability of aquifer microorganisms to efficiently oxidize DCE and VC to non-toxic products under anaerobic conditions has important implications for the potential utilization of intrinsic biodegradation in groundwater systems. These results indicate that in anaerobic aquifers, highly oxidized chlorinated chloroethenes, like PCE and TCE, can be readily transformed to DCE and VC which are susceptible to microbial oxidation under a number of anaerobic redox conditions. Thus, the combination of reductive dechlorination of PCE and TCE under anaerobic conditions followed by anaerobic microbial oxidation of DCE and VC provides a possible microbial pathway for complete degradation of chloroethene contaminants in ground-water systems.

2.4   Aerobic Cometabolism

Another potential process for microbial degradation of chloroethenes in groundwater systems is cometabolic oxidation. In 1985, Wilson and Wilson reported that methanotrophic bacteria were capable of oxidizing TCE to CO_2 under aerobic conditions. Since then, a wide variety of aerobic microorganisms, which are able to oxidize TCE, DCE, and VC to CO_2 without accumulation of toxic intermediates, have been identified. These include methane, propane, ethene, aromatic compound, ammonium, isoprene, and vinyl chloride oxidizers (see McCarty and Semprini, 1994). Cometabolic oxidation of chloroethenes does not supply energy for microbial growth or metabolism. Rather, for each of these processes, the responsible microorganisms contain non-specific oxygenases that fortuitously oxidize chloroethenes to CO_2. Consequently, aerobic cometabolism of chloroethenes requires the presence of oxygen and a primary substrate to initiate the production of a suitable oxygenase.

Cometabolic oxidation is not considered to be a significant mechanism for intrinsic biodegradation of chloroethene contaminants in groundwater. Cometabolic oxidation of chloroethenes by subsurface microorganisms has been successfully exploited for engineered remediation of chloroethene-contaminants in groundwater (McCarty and Semprini, 1994; Semprini, 1995; Semprini et al., 1990, 1991). TCE contamination in aerobic aquifers can be biodegraded by methanotrophic microorganisms when methane is supplied to the subsurface in sufficient quantity to stimulate and support methanotrophic activity. Because methane and oxygen do not typically co-occur in groundwater systems, however, methanotrophic oxidation of chloroethenes is unlikely under non-engineered circumstances. A number of ex situ investigations have demonstrated that toluene oxidizers can cometabolize chloroethenes under aerobic conditions (Fan and Scow, 1993; Fuller et al., 1995; Hopkins and McCarty 1995; Mars et al. 1996). Moreover, many groundwater contaminant plumes are the result of mixed waste releases and, as a consequence, contain chloroethenes and aromatic compounds like toluene. Unfortunately, contaminant plumes, which contain aromatic compounds in sufficient concentration to support cometabolic oxidation of chloroethenes, are characteristically anaerobic as a result of oxygen depletion by aerobic respiration. Consequently, significant cometabolic oxidation of chloroethenes in such systems would be, at most, a transient phenomenon. Because circumstances suitable for cometabolic oxidation of chloroethenes are rarely observed in groundwater systems, this process is generally not considered to be an important pathway for microbial degradation of chloroethene contaminants.

However, the interface between groundwater and surfacewater is a non-engineered system in which cometabolic oxidation could potentially be a significant mechanism for biodegradation of chloroethene contaminants. Because groundwater contamination occurs most commonly in local flow systems (that is, flow systems hydrologically connected to land surface), plumes of contaminated groundwater frequently discharge to nearby surface water bodies. The stream-bed sediments of these surfacewater systems are often characterized by geochemical heterogeneity and remarkably active microbial communities. Because methanotrophic activity is commonplace in organic-rich, stream-

bed sediments, discharge of chloroethene contaminated groundwater can reasonably be expected to support significant cometabolic oxidation of chloroethenes. In fact, complete oxidation of DCE and VC has been reported in stream-bed sediments characterized by mixed aerobic/methanogenic conditions (Bradley and Chapelle 1997). These results suggest that cometabolic oxidation of chloroethene contaminants may be significant in stream- and lake-bed sediments.

2.5 Redox Conditions and the Biodegradation of Chlorinated Ethenes in Ground-Water Systems

Although the microbial degradation processes of chlorinated ethenes are complex, they are predictable. Under some redox conditions, all chlorinated ethenes can serve as electron acceptors in microbial metabolism. In this case, microorganisms are respiring the chlorinated ethenes by transferring electrons to them. This process is also called reductive dechlorination. Under other redox conditions, microorganism can use lightly chlorinated ethenes such as DCE and VC as electron donors. In this case, microorganisms oxidize the chlorinated ethenes and use them as a source of energy in the form of electrons.

Under anoxic conditions, highly chlorinated ethenes such as PCE and TCE are subject to reductive dechlorination according to the sequence:

$$\text{PCE} \mid \text{TCE} + \text{Cl} \mid \text{DCE} + 2\text{Cl} \mid \text{VC} + 3\text{Cl} \mid \text{ethylene} + 4\text{Cl} \tag{22}$$
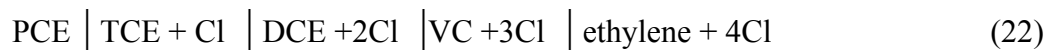
However, the *efficiency* of dechlorination differs depending on ambient redox conditions and/or the availability of dechlorinating microorganisms. Dechlorination of PCE and TCE to DCE occurs under mildly reducing conditions such as nitrate- or Fe(III)-reduction whereas the transformation of DCE to VC, or the transformation from VC to ethene requires the more strongly reducing conditions of methanogenesis or sulfate reduction (Vogel and others, 1987). It has been shown (DiStefano and others, 1991) that reductive dechlorination is driven by molecular hydrogen (H_2). It follows, therefore, that the efficiency of reductive dechlorination is directly related to the availability of H_2.

Concentrations of H_2 in groundwater systems are controlled by ambient microbial terminal electron-accepting processes (TEAPs). Under anaerobic conditions H_2 is continuously produced by fermentative microorganisms metabolizing natural or anthropogenic organic matter. This H_2 is then utilized by respirative microorganisms that most commonly use Fe(III), sulfate, or CO_2 as terminal electron acceptors. Significantly, each of these TEAPs has a different affinity for H_2 uptake (Lovley and Goodwin, 1988). CO_2 reduction (methanogenesis) has the lowest H_2 affinity and steady-state H_2 concentrations in methanogenic aquifers are therefore relatively high (~10 nanomoles per liter (nM)). Sulfate reduction has a slightly greater affinity for H_2 than methanogenesis, and is characterized by slightly lower H_2 concentrations (1-4 nM). Fe(III) reduction (0.2-0.8 nM) and nitrate reduction (<0.10 nM) have even greater affinities for H_2 and are characterized by progressively lower steady-state H_2 concentrations (Figure 7). Thus, the

PCE   TCE   Chloride

$$\underset{Cl}{\overset{Cl}{}}C=C\underset{Cl}{\overset{Cl}{}} \quad\quad \underset{Cl}{\overset{Cl}{}}C=C\underset{H}{\overset{Cl}{}} \quad + \; Cl^-$$

electron flow e^-

H_2 H^+

molecular hydrogen   hydrogen ion
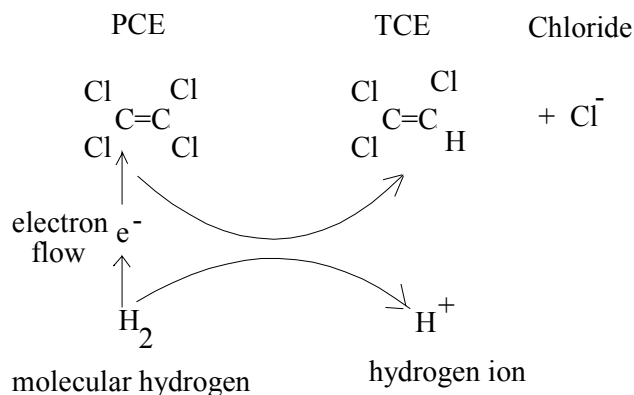
Figure 7. Diagram showing electron flow with hydrogen as an electron donor and PCE as an electron acceptor.

observation that reductive dechlorination is more efficient under methanogenic or sulfate-reducing conditions than under Fe(III)- or nitrate-reducing conditions reflects the greater availability of H_2 for reductive dechlorination, not the activity of methanogenic or sulfate-reducing microorganisms (Smatlack et al., 1996).

The lightly chlorinated ethenes DCE and VC can be oxidized to CO_2 under oxic or Fe(III)-reducing conditions:

<u>DCE</u>

$$Cl_2C_2H_2 + 2O_2 \mid 2CO_2 + 2H^+ + 2Cl^- \tag{23}$$

<u>VC</u>

$$ClC_2H_3 + 5/2\ O_2 \mid 2CO_2 + H_2O + H^+ + Cl^- \tag{24}$$
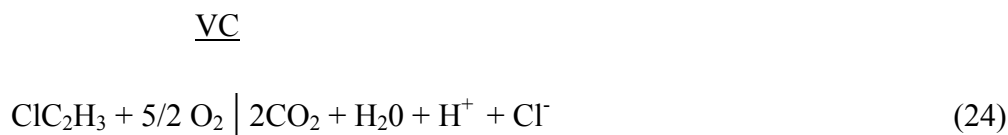
But because DCE and VC are usually degradation products of reductive dechlorination, complete degradation of chlorinated ethenes is favored by sequential anoxic-oxic conditions. First, under reducing conditions, PCE and TCE are transformed to DCE and VC. Then, as groundwater migrates to more oxic conditions, DCE and VC can be oxidized to carbon dioxide:

| anoxic (methanogenic, sulfate-reducing, | | oxic, Fe(III)-reducing |
| or Fe(III)-reducing conditions) | | conditions |

$$\text{PCE, TCE} \mid \text{DCE and VC} \qquad\qquad \text{DCE, VC} \mid 2CO_2 \text{ and } Cl^-$$

Therefore, the efficiency with which chlorinated ethenes are completely degraded is directly related to redox conditions present in an aquifer, and to the succession of redox processes that chlorinated ethenes are exposed to along particular groundwater flowpaths. Thus, an important component to assessing the natural attenuation of chlorinated ethenes is to accurately delineate redox conditions in groundwater systems.

2.6   Delineating the Distribution of Redox Processes in Groundwater Systems

The most common electron-accepting processes in groundwater systems are oxygen-, nitrate-, Fe(III)-, sulfate-, and carbon dioxide-reduction (methanogenesis). Microorganisms that naturally inhabit groundwater systems couple the oxidation of an electron donor (usually organic carbon) with the reduction of electron acceptors. In doing so, microorganisms use the most energetically efficient electron acceptor available. The stoichiometry and Gibbs free energy (ΔG^o in units of kcal/equivalent at pH 7.0, Stumm and Morgan, 1981, p. 453) of these processes can be represented by the generalized equations:

$$O_2 + CH_2O \mid CO_2 + H_2O, \quad \Delta G^o = -29.0 \tag{25}$$

$$4NO_3 + 4H^+ + 5CH_2O \mid 4NO_2 \mid 2N_2 + 5CO_2 + 7H_2O \quad \Delta G^o = -19.6 \tag{26}$$

$$4Fe(OH)_3 + CH_2O + 8H^+ \mid 4Fe^{2+} + CO_2 + 11H_2O \quad \Delta G^o = -10.0 \tag{27}$$

$$2CH_2O + SO_4^{2+} + H^+ \mid 2CO_2 + HS^- + 2H_2O \quad \Delta G^o = -5.9 \tag{28}$$

$$2CH_2O \mid CH_4 + CO_2 \quad \Delta G^o = -5.6 \tag{29}$$
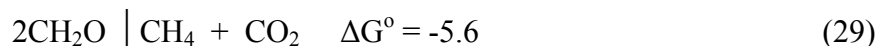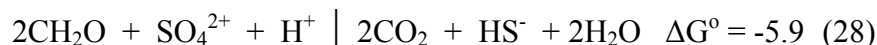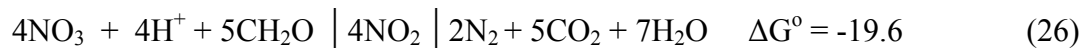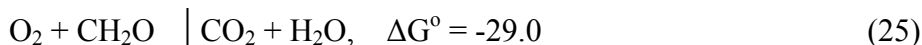
Because oxygen is the most efficient oxidant (that is, ΔG^o is the most negative), microorganisms will use oxygen preferentially to either nitrate, Fe(III), sulfate, or CO_2. If oxygen is not available, however, microorganisms will preferentially use nitrate over Fe(III), sulfate, or CO_2. If oxygen or nitrate is not available, microorganisms will use Fe(III) over sulfate or CO_2. Finally, if oxygen, nitrate, and Fe(III) are not available, microorganisms will use sulfate preferentially to CO_2. Because CO_2-reducers are the least energetically favorable microorganisms, they can only become predominant in the absence of other more efficient electron acceptors. This observed behavior is commonly referred to as the *ecological succession* of terminal electron-accepting processes.

Because of ecological succession, microbial processes tend to segregate into distinct zones dominated by oxygen-, nitrate-, Fe(III)-, sulfate-, or CO_2 reduction. Furthermore, given the different electron acceptors consumed (left side of equations 25-29) and final products produced (right side of equations 25-29), it is possible to logically deduce the zonation of microbial processes in ground-water systems.

If dissolved oxygen is present in groundwater at concentrations greater than 0.5 mg/L, then oxygen reduction (equation 25) will be the predominant microbial process. If dissolved oxygen concentrations are less than 0.5 mg/L, but nitrate is present at concentrations greater than 1 mg/L, nitrate reduction (equation 26) will be the predominant microbial process. Because nitrite (NO_2) is an unstable intermediate product of nitrate reduction (equation 26), the presence of measurable NO_2 concentrations is indicative of nitrate reduction. If groundwater lacks dissolved oxygen, nitrate, or nitrite, but concentrations of Fe^{2+} are greater than 0.5 mg/L, Fe(III) reduction (equation 27) will be the predominant process. If groundwater lacks Fe^{2+} but contains concentrations of sulfate greater than 1 mg/L and hydrogen sulfide greater than 0.05 mg/L, then sulfate reduction (equation 28) will be the predominant process. Finally, if the groundwater lacks dissolved oxygen, nitrate, Fe^{2+}, sulfate, and hydrogen sulfide, but contains concentrations of methane greater than 0.2 mg/L, then methanogenesis (equation 29) will be the predominant process. The logic of deducing predominant electron-accepting processes using observed changes of electron acceptor and final product concentrations in groundwater is summarized in Figure 8 (Chapelle et al. 1995).

In practice, this methodology often encounters uncertainties. Many products of microbial metabolism (such as Fe^{2+}, H_2S, and methane) can be transported by groundwater flow. In some cases where such transport is significant, it is difficult to determine the exact redox zonation with this water-chemistry information. In these cases, direct measurement of dissolved H_2 can be used to help identify ambient redox processes (Figure 8).

Based on this methodology, the following data are required to identify microbial redox processes in groundwater systems (Table 4).

Table 4. Ground-water chemistry parameters used to identify microbial redox processes in ground-water systems, and common methods of analysis.

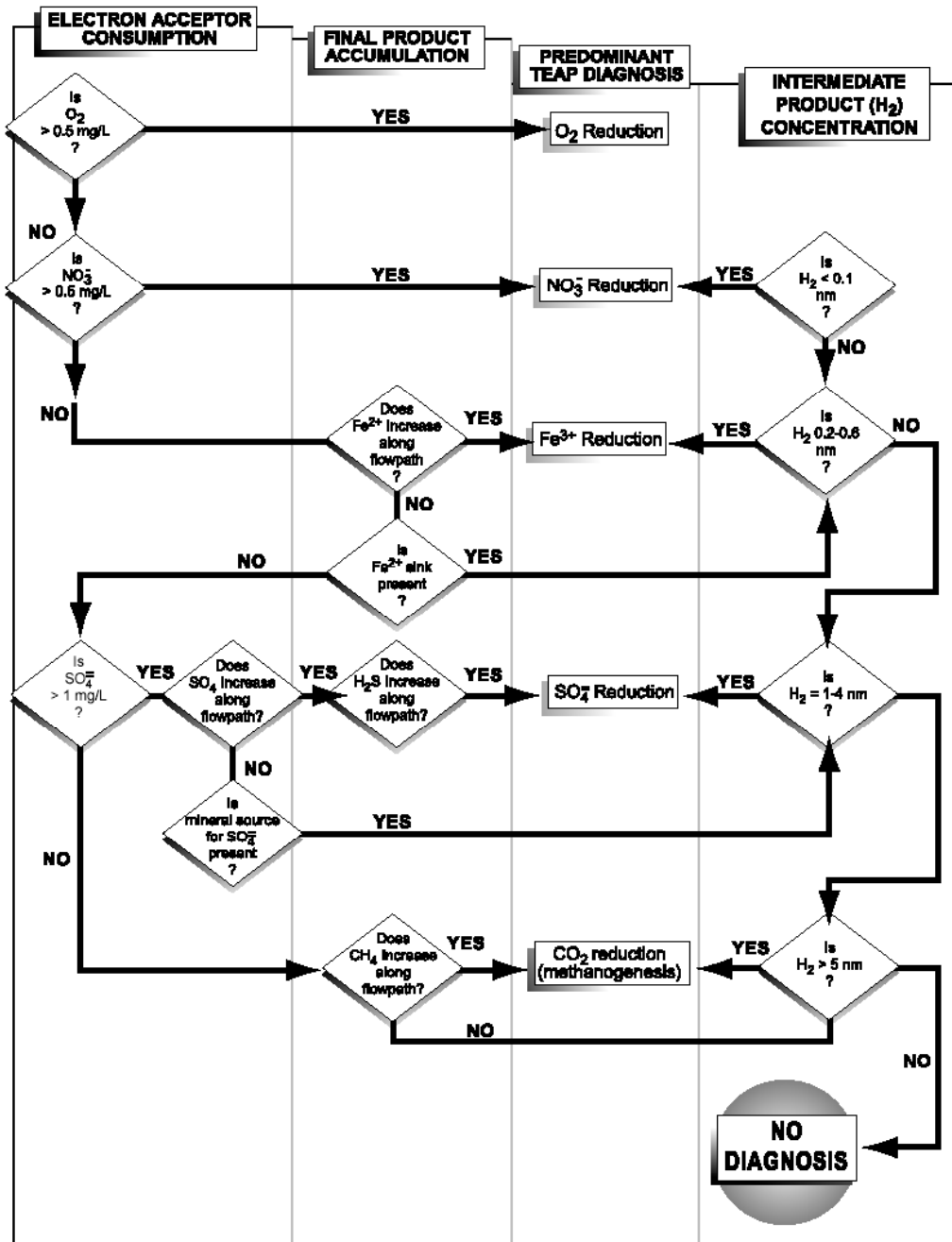| Analytical Parameter	Method of analysis
Dissolved oxygen (DO)	field kit
Nitrate (NO_3)	Ion Chromatography
Nitrite (NO_2)	Ion Chromatography
Dissolved ferrous iron (Fe^{2+})	Field kit
Sulfate (SO_4)	Ion Chromatography
Hydrogen sulfide (H_2S)	Field kit
Dissolved Methane (CH_4)	GC FID
pH (units)	meter
Dissolved Hydrogen (H_2)	gas chromatography

Figure 8. Flowchart showing the logic of deducing terminal electron accepting processes in ground-water systems.

Of these parameters, the most important--and often the most difficult measurement to make accurately--is dissolved oxygen. This is a very skill-intensive parameter to measure in the field, and extreme care must be taken to avoid oxygenation of the water sample prior to analysis. Dissolved oxygen meters are convenient, but do not reliably measure low (<0.5 mg/L) concentrations of oxygen. Dissolved oxygen kits, on the other hand, can accurately distinguish between truly anoxic groundwater and low concentrations of dissolved oxygen (Chemetrics, Inc, 1984). Concentrations of nitrate, nitrite, and sulfate are most accurately measured in the laboratory using ion chromatography. Hydrogen sulfide and Fe^{2+} are most efficiently measured in the field with appropriate field kits (Table 4). Samples for methane analysis can be collected in the field and analyzed in the laboratory.   Dissolved hydrogen measurements are most reliable when made in the field (Chapelle et al., 1996b; Chapelle et al., 1997).

2.7   Data Gaps in the Current Knowledge

Chlorinated ethene biodegradation remains an actively researched topic because of the extent of contamination in sedimentary and subsurface environments, and the questions that have arisen as more data has been generated. Reductive dechlorination, anaerobic oxidation, and aerobic co-metabolism will likely influence a particular environment's natural attenuation capacity, and more data is necessary to best define the conditions under which these processes are most favorable and what is the greatest rate that can be expected given prevailing geochemical conditions and associated microbiology.

General knowledge regarding reductive dechlorination has increased in the past several years; however, numerous questions are still outstanding. The primary question is whether organisms within the genus Dehalococcoides are absolutely necessary for complete dechlorination to ethene? Recent data indicate that Dehalococcoides-like microorganisms are detected in aquifer material that naturally reduces TCE to ethene, but not in aquifer material in which TCE reduction stalls at cis-DCE, as briefly discussed above (Hendrickson, Payne et al. 2002). To date, Dehalococcoides-like microorganisms are the only pure cultures reported to catalyze complete dechlorination in laboratory incubations (Maymo-Gatell, Anguish et al. 1999). Finally, all completely dechlorinating enrichment cultures contain at least one strain of Dehalococcoides-like microorganism (Hendrickson, Payne et al. 2002). The data generated to date clearly demonstrate that Dehalococcoides-like microorganisms are significant for reductive dechlorination; however – are they the only significant microorganisms? Published data and experience (among numerous microbiological disciplines) indicate that single genera of microorganisms are rarely responsible for a widely reported biologically catalyzed reaction. Basic research to investigate other possible microorganisms involved in complete reductive dechlorination is necessary.

Basic metabolism of the Dehalococcoides-like microorganisms also requires more complete characterization using the isolated pure cultures. To date, Dehalococcoides-like microorganisms are reported to grow using a non-specified medium composed of mixed culture supernatant with various chlorinated organic compounds as the sole electron acceptor. Alternate electron acceptors may be reduced and these acceptors would

influence growth and proliferation of Dehalococcoides like microorganisms within the subsurface. In addition, new methods to quantify growth (e.g., direct counting microscopy) must be developed for this organism. Finally, recent data indicate functional genes including the VC reductase have been identified. These gene sequences must be used to develop molecular probes for micro arrays and quantitative PCR assays to characterize activity in sedimentary and subsurface environments.

The total microbial ecology of contaminated sedimentary and subsurface environments also warrants further investigation; particularly the relevance of various organic compounds as electron donors and the concentration at which they most efficiently promote complete dechlorination. Based on most reviews to date, the general dogma regarding complete dechlorination revolves around hydrogen as the key electron donor. In fact, all reports speculate that any fermentable substrate must first be fermented to less complex organic material and hydrogen prior to utilization in halorespiration (Fennell, Gossett et al. 1997; Carr and Hughes 1998; Yang and McCarty 1998; Haston and McCarty 1999; Boopathy and Peters 2001), regardless of whether the initial substrate is an electron donor directly (e.g., lactate). Several organic acids including lactate, propionate, and butyrate are respiratory electron donors and fermentable substrates. The relative proportion of each mechanism during dechlorination is unknown, although most studies treat the latter as the dominant pathway (Yang and McCarty 1998). Acetate may also serve as a sole electron donor for halorespiration, but it is generally reported as an electron donor for associated microbial populations, but not halorespiring microorganisms directly (Major, McMaster et al. 2002). Hydrogen is a significant electron donor for all anaerobic, microbial processes (Lovley and Goodwin 1988). However, microorganisms that utilize hydrogen are also reported to utilize fermentable substrates directly in respiratory pathways (Lovley, Phillips et al. 1989; Lovley 1991). Also, the concentration of any substrate required to promote complete dechlorination is unknown. Several reports indicate more electron donor equals faster dechlorination; other reports indicate lower concentrations of key donors increases the rate and extent of complete dechlorination. Although this may be a site-specific question, further investigation is required.

Anaerobic cis-DCE and VC oxidation is a relatively recent discovery (Bradley and Chapelle 1996), but it may be significant in sedimentary and subsurface environments in which less-chlorinated ethenes commingle with Mn(IV), Fe(III), humic substance, and possibly sulfate. Mass balance estimates for reductive dechlorination are often incomplete because cis-DCE and VC will disappear without concomitant increases in ethene (Bradley 2000). While site heterogeneity and hydrogeology may contribute to the imbalance; anaerobic oxidation likely accounts for significant cis-DCE and VC removal once it is produced by other mechanisms. Several reports indicate a range of 40% to 100% of VC can be degraded under Mn(IV)-, Fe(III)-, or humics-reducing conditions in freshwater sediment, shallow aquifer sediment, and deep aquifer sediment (Bradley 2000). Cis-DCE is not oxidized to the same extent as VC, but cis-DCE is more oxidized and reductive dechlorination is a significant competing mechanism [ref]. However, cis-DCE is oxidized directly to CO_2 along with VC (Bradley 2000). More research is necessary to determine the relative contribution of anaerobic oxidation in a site's natural

attenuation capacity. In addition, anaerobic oxidation data should be re-applied to sites that could not quantify a mass balance for TCE biodegradation; the mass balance will likely be more complete once it is included in the overall equation.

Compound specific stable carbon isotope analysis has recently been applied to TCE biodegradation and transformation of the TCE metabolites cis-DCE and VC (Sherwood-Lollar, Slater et al. 2001; Slater, Sherwood-Lollar et al. 2001; Mancini, Couloume et al. 2002; Song, Conrad et al. 2002). This technique is based on the biological phenomenon of lighter carbon isotopes entering biochemical pathways more preferentially; thereby leaving residual contamination that is isotopically enriched (heavy). The ^{13}C in the residual contamination can be quantified as a direct measure of biodegradation; no other process is reported to enrich carbon compounds in the same manner (Sherwood-Lollar, Slater et al. 2001). Laboratory and Site data have been reported, but this technique needs to be refined because it is an extremely powerful tool to identify biodegradation and eventually quantify biodegradation rates. This technique is especially relevant to assess natural attenuation capacity because it directly identifies the extent of biodegradation versus all other attenuation processes. As mentioned, it is a new strategy for bioremediation and much more research is necessary to make it practical for a variety of site conditions.

2.8 Conclusions

All sedimentary and subsurface environments will have a natural attenuation capacity for chlorinated ethenes, and biodegradation may be a small or large component of that capacity. In the survey, anaerobic biodegradation was an active NAC process in over 90 percent and the most important NAC process in over 70 percent. Aerobic biodegradation was not as active as anaerobic or physical processes at most of the sites, being listed as an active NAC process in about 33 percent of the sites. Aerobic biodegradation was not considered an important NAC process at any of the MNA sites.

As has been demonstrated in laboratory and field investigations PCE, TCE, cis-DCE, and VC will all biodegrade, and biodegradation can proceed thorough any of several pathways. The rate and extent of biodegradation will be the limiting factor in situ. Engineered or enhanced bioremediation strategies increase the rate and extent by providing a key missing nutrient, microorganism, electron donor, or electron acceptor. Natural attenuation, however, is reliant upon the prevailing geochemistry and microbial ecology. Although natural attenuation may not meet regulatory standards as a sole cleanup strategy it is worth greater understanding because it is the only strategy restoring contaminated environments that are not actively being treated, or that have yet to be discovered.

The *capacity* for a particular environment to transform contaminants will be a function of the interactions between all microbial communities, electron donors and acceptors, and nutrients. Competition among the various populations is the primary interaction influencing the natural attenuation capacity within sedimentary or subsurface environments; although cooperative interactions, such as interspecies hydrogen transfer,

facilitate contaminant degradation. Competition for available electron donors and electron acceptors will influence microbial community composition and the relative activity of one mechanism versus another. Specific processes will dominate on a spatial and/or temporal scale, but the system as a whole is dynamic and one processes dominance changes as the process itself alters the environment. The data presented above may be useful to predict when specific processes will dominate, when mechanisms will change, and when multiple processes may function simultaneously.

3.0   *Phytoremediation*

3.1   *Mechanisms of plant uptake*

Certain plants can remove, transfer, stabilize and destroy contaminants in soil, sediment, groundwater, surfacewater, and leachate through several processes (Schnoor 2002; Pivetz, 2001; Newman and others, 1997; Miller, 1996; Schnoor and others, 1995; Anderson and others, 1993; Walton and Anderson, 1990). A prerequisite for plant uptake is that the contaminants are nontoxic to the plant. However, research has shown that plants have the capacity to withstand relatively high organic and inorganic contaminant concentrations without toxic effects (Burken and Schnoor, 1998; Briggs et al, 1982).

This section describes a compilation of information provided by several agencies and publications on the state of phytoremediation technology. Nellessen and Fletcher (1993), Pivetz (2001), and Schnoor (2002) published a comprehensive review of the literature pertaining to uptake, accumulation, translocation and biotransformation of organic chemicals by plants. Technology reports on phytoremediation are available from the Ground-Water Remediation Technologies Analysis Center at http://www.gwratc.org. An updated bibliography with abstracts is available from the Remediation Technologies Development Forum established by USEPA at http://www.rtdf.org/public/phyto/bib/print.cfm?abstract=1.

The shift in perception of plant uptake of contamination as an ecological exposure risk to treatment technology called phytoremediation is a relatively recent occurrence (Schnoor, 2002; Landmeyer, 2001). Phytoremediation has been described as a continuum of processes that occur at differing degrees and tend to overlap (Pivetz, 2001). Organic chemicals, such as chlorinated ethenes, undergo processes including root sorption, uptake, translocation, metabolic transformation, and/or volatilization that ultimately lead to one of three natural attenuation pathways: contaminant degradation, removal (through accumulation or dissipation), or immobilization (Schnoor, 2002; Pivetz, 2001).

The mechanisms of plant uptake in phytoremediation are grouped into 6 categories: rhizodegradation, rhizofiltration, phytodegradation, phytovolatilization, phytoextraction (also called phyto-accumulation), and phytostabilization (including hydraulic control) (U.S. Environmental Protection Agency, 1999d; Miller, 1996; Cunningham et al, 1995).

3.1.1 Degradation Pathway.

This attenuation pathway results in the destruction or alteration of the organic contaminant in the root zone or plant by the release of exudates and enzymes that stimulate microbial activity and biochemical transformations.

3.1.1.1 Rhizodegradation.

 The plant assists the microbial community (bacteria and fungi) in the rhizosphere with contaminant degradation or immobilization by providing habitat and natural substances such as sugars, alcohols, and organic acids that provide organic carbon sources for the microbes. Hiltner (1904) described the rhizosphere as a zone of unique and dynamic interaction that occurs between plant roots and soil microorganisms (Curl and Truelove, 1986). This specialized region is characterized by enhanced microbial biomass and activity, where the numbers of microorganisms in the rhizosphere are typically an order of magnitude higher than in bulk soils.   In agricultural and natural soils inhabited by plants, microbial activity in the rhizosphere is known to affect the fate of added organic chemicals (Hsu and Bartha, 1979; Knaebel and Vestal, 1992; Walton and Anderson., 1990).   In addition to sustaining higher microbial densities as compared to those in non-vegetated soils, the plant root zone provides a niche that supports a diverse population of microorganisms and facilitates cometabolic transformations (Hsu and Bartha, 1979). Evidence from degradation studies in a rhizosphere system suggests that a diverse and synergistic microbial community, rather than a single microorganism, is involved in the enhanced degradation of these xenobiotics (Anderson et al., 1995).

3.1.1.2 Phytodegradation

Uptake of chlorinated ethenes or ethanes by certain plants can result in subsequent degradation or alteration by metabolic activity of the plants (Orchard and others, 2000b; Newman and others, 1997) or breakdown of contaminants external to the plant by enzymatic activity (U.S. Environmental Protection Agency, 1999). One or more phases of the transformation process include (I) conversion by oxidation-reduction or hydrolysis processes; (II) conjugation with glutathione, sugars, or amino acids; (III) compartmentation whereby conjugates are converted to other conjugates and are covalently bound to cell walls or in vacuoles (Sandermann, 1994; Ohkawa and others, 1999). In conversion phase, the metabolic by-products of TCE through oxidative pathways in hybrid populars are reported as trichloroethanol, trichloroacetic acid, and dichloroacetic acid (Newman and others, 1997; Dietz and Schnoor, 2001a). However, other processes or further transformations are suspected to occur because the overall mass balances for the above pathways are poor (Schnoor, 2002). For this application to be deemed environmentally friendly, it is very important that the breakdown products or metabolites which accumulate or form within the plant tissue be non- toxic, or less toxic that the parent compounds (Schnoor, 1997). Select plants including hybrid poplars are capable of TCE metabolism and transformation (Newman et al., 1997; Schnabel et al., 1997). These transformation products can be measured and quantified in plant tissues (Orchard et al., 2000a & 2000b).

3.1.2 Accumulation Pathway

This attenuation pathway results in containment or removal of contaminants. However, this pathway relates dominantly to metal contaminants.

3.1.2.1 Phytoextraction

The uptake of metals from the soil by plant roots is termed phytoextraction or phytoaccumulation. These metals are stored in aboveground portions of the plant. Certain plants absorb large amounts of metals in comparison to other plants (hyperaccumulators).

3.1.2.2. Rhizofiltration

Rhizofiltration is the adsorption or precipitation of contaminants onto plant roots or the uptake of contaminants into plant roots. This approach is typically used with metal contaminated water such as wastewater from sewage treatment facilities.

3.1.3 Dissipation Pathway

This attenuation capacity pathway removes the organic contaminants by volatilization into the atmosphere.

3.1.3.1 Phytovolatilization

When chlorinated ethenes are not transformed significantly when taken up and translocated by plants, they may be transpired as gases through stomata in leaf tissues (Hong and others, 2001; Trapp and McFarlane, 1994) or diffused through stem tissues and volatilize to the atmosphere (Davis and others, 1999) via the normal plant physiological process of transpiration (Ma and Burken, 2003; Newman et al., 1997). An investigation using tree cores to measure VOCs demonstrated concentration gradients of TCE and cDCE decreasing with elevation (Vroblesky, Nietch, and Morris, 1999). This work demonstrates potential volatilization through the tree tissues.

3.1.4   Immobilization Pathway

This attenuation capacity pathway contains the organic contaminant, preventing further migration.

3.1.4.1 Hydraulic control

Deep-rooted plants, called phreatophytes, are used as pumps to create a cone of depression in the saturated zone and prevent the migration of contaminants. Phreatophytic trees include poplar, cottonwood, and willows and have root systems that can reach shallow ground-water tables as deep as 5 meters (Schnoor, 2002).

3.1.4.2 Phytostabilization

Plants at contaminated sites can immobilize soil and groundwater contaminants. Immobilization is accomplished by one or more processes: absorption and accumulation by roots, adsorption onto roots or precipitation within the root zone, or physical stabilization of the soil that reduces contaminant transport via wind and erosion or leaching into groundwater.

The likelihood of the organic contaminant to undergo these processes depends on its hydrophobicity, solubility, polarity, and molecular weight (Briggs and others, 1982; Schnoor, 1997) which affect whether the contaminant is taken up and translocated or sorbed. When plant roots come in contact with organic contaminants in the soil or shallow groundwater, the contaminants can be sorbed or bound to the root structure and cell walls. Specifically, sorption of hydrophobic (log octanol-water partitioning coefficient [K_{ow}] > 3.5) organic chemicals occurs in the cell wall and the lipid bilayer that contain hemicellulose. This sorption generally is reversible and measurable using standard sorption isotherms (Schnoor, 2002). Hydrophylic and moderately hydrophobic organic chemicals ($K_{ow} = 1.0 – 3.5$), such as chlorinated ethenes, are taken into the roots and translocated to the stems and leaves of the plant through the xylem. An equation to compute the direct uptake of a chemical that has not undergone any biochemical reactions at the root zone into rooted vascular plants was provided by Burken and Schnoor (1998):

$$U = (TSCF) (T) (C), \tag{31}$$

where,

U is the rate of chemical uptake by the plant, in milligrams per day (mg/d);
TSCF, or Transpiration Stream Concentration Factor, is the dimensionless ratio of the chemical concentration in the transpiration stream of the plant to the chemical concentration in the soil;
T is the transpiration rate, in liters per day (l/d); and
C is the soil concentration of the chemical, in milligrams per day.

Transpiration rate, T, is the key factor is determining chemical uptake and depends on plant type, leaf area, nutrient availability, soil moisture, temperature, wind conditions, and relative humidity. The efficiency of the plant uptake is represented by the transpiration stream concentration factor (TSCF), such that a TSCF of zero represent no uptake and 1.0 represents complete uptake. The TSCF depends on the characteristics of the plant and the properties of the chemical contaminant. For example, the TSCF for hybrid populars and TCE (log K_{ow} of 2.33) is 0.75 (Burken and Schnoor, 1998; Dietz and Schnoor, 2001a), based on the equation: $TSCF = 0.784 \exp[-(\log K_{ow} – 1.78)^2/2.44]$ (Burken and Schnoor, 1998).

Organic chemicals that have greater hydrophobicity (log K_{ow} > 3.0) have a greater tendency for sorption on the plant roots (Briggs and others, 1982). Linear sorption isotherms have been developed to measure the Root Concentration Factor (RCF) of a contaminant, which is the ratio of the organic chemical sorbed on the root to that in solution, with units of liters per kilogram (Briggs and others, 1982; Burken and Schnoor, 1998). The RCF for TCE and hybrid populars is relatively low (3).

Plants also provide a carbon source, particularly in wetland systems that are essential for microbial communities. Plants can subsequently be harvested or grazed under controlled conditions. Tolerance mechanisms include accumulation in the cell walls, chelation with a plant constituent such as citric acid or malic acid, and binding to specific proteins, phytochelatins. This capacity can also be utilized in phytoremediation in that the plant may be able to selectively metabolize the organic contaminant thus rendering it nontoxic and reducing the potential environmental threat. These capacities are both constitutive as well as inducible.

Many factors can limit the applicability of phytoremediation to a contaminated site by influencing the ability of the plant to uptake the contaminant (Interstate Technology and Regulatory Cooperation Work Group, 1999; Boyajian and Devedjian,1997; Miller, 1996). These factors are listed below:

> 3. Limited to sites with lower contaminant concentrations and shallow soils, streams, and groundwater (deeper contamination can be accessed if phreatophytic trees are used) and large surface area of land.
> b. High concentrations of contaminants can be toxic to plants.
> c. Mass transfer limitations as seen with other biotreatments.
> d. Seasonality and other climatic factors can influence the success of phytoremediation.
> 3. Transfer of contamination across media, e.g., from soil to air.
> 3. Not effective for strongly sorbed (e.g., PCBs) and weakly sorbed contaminants.
> 3. Toxicity and bioavailability of biodegradation products are not always known.
> 3. Degradation products may be mobilized into groundwater or bioaccumulated in animals.
> j. Still in the demonstration stage and somewhat unfamiliar to regulators.

3.2 *Scientific evidence for the role of plant uptake in natural attenuation*

Plant-based remediation has evolved as a viable method because of new technology and the need for more cost-effective approaches. The use of plants to remediate contaminants is not new but has been used for the last 300 years to treat wastewater (Hartmans, 1975). Phytoremediation is most applicable to sites where contamination of organic, nutrient or metallic pollutants are shallow and are amenable to one of the five applications: phytotransformation or phytodegradation, phytoextraction, rhizofiltration, phytovolatization, and phytostabilization. Phytoextraction, rhizofiltration, and phytostabilization are more relevant to the phytoremediation of metals and radionuclides.

Phytodegradation, rhizodegradation, and phytovolatilization have been shown experimentally or in remediation demonstrations to be effective approaches for the removal or degradation of VOC's such as TCE.

Biodegradation experiments have usually been conducted in a soil matrix in the absence of plants. However, biodegradation studies involving a plant/soil rhizosphere system where microbial activity, diversity and biomass are greater, offers tremendous untapped possibilities of enhanced microbial remediation of contaminants. Walton and Anderson (1990) observed enhanced degradation of 1,1,2-trichloroethylene (TCE) in slurries of rhizosphere soils and mineralization of ^{14}C-TCE in rhizosphere soils with a variety of different plants. Additional studies showed that a diverse and synergistic microbial community was responsible for TCE degradation (Anderson et al., 1995). These studies also raised the possibility of ectomycorhizal fungi acting as degraders - enhanced mineralization of ^{14}C-TCE in soil containing loblolly pine (*Pinus taeda*) seedlings (Walton and Anderson, 1990).

Sorption of a xenobiotic to soil particles or plant surfaces and uptake by the plant are factors that can influence the effect of rhizosphere microbes on degradation. A review of sorption, binding processes and subsequent desorption discusses the resultant effects on bioavailability for subsequent degradation. Brigmon et al. (1998) showed that sorption was the dominant factor in reduction of TCE residues in seepline soils. Aerobic and anaerobic degradation contributed little to the decrease in TCE residues. Adsorption to the plant root cell wall may decrease xenobiotic availability to the remainder of the rhizosphere. Shann (1995) showed a decrease in mineralization of 2,4-D in a manner consistent with the amount of wheat cell wall extract added. However, the potential for degradation by the cell wall extracts was not examined. Plant uptake would be a more permanent removal of the xenobiotic from the soil. The absorbed molecule would then be subject to translocation, sequestration, conjugation or degradation. The plant can determine xenobiotic availability to rhizosphere microorganisms and subsequent degradation. Reviews of xenobiotic uptake by plants has been presented elsewhere (Ryan et al., 1988; Paterson et al., 1990). This feature of plant influence on xenobiotic levels in soils is the basis of the use of plants in phytoremediation experiments; i.e., the use of plants to remediate contaminated sites.

Previous and ongoing research at the Savannah River Site (SRS) has demonstrated the phytoremediation of chlorinated ethenes. A recent investigation of a shallow VOC contaminant plume at the SRS TNX flood plain demonstrated that bald cypress (*Taxodium distichum*), tupelo (*Nyssa aquatica*) and loblolly pine (*Pinus taeda)* contained significantly higher levels of chlorinated ethenes than adjacent oak (*Quercus* spp.) and sweet gum (*Liquidambar stryaciflua*) trees in the same area (Vroblesky, Nietch, and Morris, 1999). However, Punshon and others (2003) reported the uptake of TCE of native vegetation was minimal, passive, and sporadic. Punshon and others (2003) also reported that trichloroacetic acid (TCAA) was detected within leaf tissues in sweet gum, but not in vascular or root tissue, suggesting metabolic degradation of atmospheric TCE by leaf tissue. Walton and Anderson (1990) previously observed accelerated microbial degradation of TCE in slurries of rhizosphere soil and mineralization of TCE in whole

plant systems collected from samples at a former SRS solvent disposal site, the Miscellaneous Chemical Basin (MCB). Two species where enhanced microbial degradation of TCE was observed at the SRS were a legume, *Lespedeza cuneata* and loblolly pine, *Pinus taeda* (Anderson and Walton, 1995).

It has been suggested that a possible mechanism for the enhanced microbial mineralization of TCE in the pine rhizosphere is excretion of phenolic compounds in root exudates. Since phenol is a known inducer of toluene mono-oxygenase, an enzyme responsible for degradation of TCE, the natural plant exudates could play a role in biodegradation of TCE in the rhizosphere (Anderson et al. 1993). Select plants, including hybrid poplars, are capable of TCE metabolism and transformation (Newman et al. 1997, Schnabel et al. 1997). The two tree species selected for this study based on their phytoremediation potential were the loblolly pine*, Pinus taeda*, and a hybrid poplar, *Trichocarpa* X *deltoides*. In this project, CE attenuation by both soil rhizosphere and vegetation interactions was monitored.

One of the primary functions of root exudates is to mobilize inorganic nutrients in the rhizosphere (Fletcher and Hedge 1995). Exudates also contain natural chelating agents (citric, acetic, and other organic acids) that increase the soil mobility of both nutrients and contaminants. Exudates may also contain enzymes including dehalogenases (Hedge and Fletcher 1996). These enzymes have important natural functions and may also degrade organic contaminants (Fliermans et al. 1988). Some rhizosphere microorganisms secrete hormones that stimulate root growth, thereby increasing the secretion of root exudates that contain metabolites, including proteins and carbohydrates also used by the bacteria (Shann 1995). Exudation of organics by plant roots and turnover of organic root biomass have been found to increase the TCE sorption capacity of soil (Schnabel et al. 1997). There are knowledge gaps as to which mechanism provides the higher degree of VOC removal in phytoremediation systems, the plants or the associated rhizospheric bacteria (Orchard et al. 2000a). The microbial ecology of soils associated with bioremediation in mycorrhizal roots such as pine has not been well characterized even though this environment forms a large habitat and provides extensive surface area for bacterial colonization. It was previously observed that the rhizosphere soils in the SRS Miscellaneous Chemical Basin (MCB) contained higher quantities of potential TCE-degrading bacteria than did SRS soils not exposed to TCE (Brigmon et al. 1999). Nichols et al. (1997) demonstrated higher microbial densities in organic-contaminated rhizosphere soils than in similar but non-contaminated soils. The microbial analysis from this year confirms the bioremediation potential of the seepline soils and provides tools for more focused field biodegradation work.

Increased microbial activity is evident in outcropping zones where available organic carbon in soils and groundwater can stimulate microbial action and lower redox potential (WSRC 2002). This plume outcrop area may support microbial activity to degrade TCE degradation products such cis-1, 2-dichloroethylene (c-DCE) or vinyl chloride (VC), but the rates could be seasonally or nutrient dependent. Contaminants in soil and groundwater must be bioavailable to be remediated (absorbed, modified, degraded, transformed, sequestered, etc.) by either plants or microorganisms (Shimp et al. 1993).

Thus, groundwater movement and nutrient availability in the rhizosphere is a critical factor for phytoremediation. Plants take up more water than is needed for growth. This excess water is transpired through the leaves as the final step in plant metabolism. Transpiration stream concentration factors (TSCFs) are important for predicting the plant uptake of TCE-contaminated groundwater (Orchard et al. 2000b). The groundwater and dissolved contaminants move through the rhizosphere, where they are subjected to bioremediation by microorganisms and soil interactions before entering plant roots. In some instances, the magnitude of microbial transformation of TCE can be significantly larger than plant influence (Anderson and Walton 1995) although this depends on the site and plants used (Nichols et al. 1997 and Schnabel et al. 1997).

It has long been known that PCE and TCE are toxic and that the daughter product VC is a carcinogen (Dougherty 2000). Recent investigations focusing on the kinetics, metabolism, and toxicology of two metabolites of TCE, dichloroacetate (DCA), and chloral hydrate (CH) have proven these compounds to be potential endocrine disruptors (Cornett et al. 1999). The studies are focusing on *in vivo* kinetics and biotransformation of CH, and the influence of CH and DCA on metabolism and toxicity (James et al. 1997). Both CH and DCA can be potential byproducts of microbial degradation of TCE (Brigmon 2001). The risk grows as more of this material moves to surface waters and increases exposure to these chemicals.

3.3 Critical needs in the application of phytoremediation to natural attenuation capacity

Phytoremediation has much potential for future decontamination of soils and waters, especially when the pollutant is near the surface, relatively immobile in the soil or sediment, and poses little imminent health risk. The use of phytoremediation for environmental cleanup of organic contaminants has attracted considerable interest because of its potentially lower cost and its suitability for applications that require longevity and very low manpower maintenance (U.S. Environmental Protection Agency, 1999).

A better understanding of the mechanisms that enhance CE biodegradation in the rhizosphere and the interaction between plants, microorganisms, and contaminants can be useful in phytoremediation deployments (Nelson et al. 1988). This information can lead to improved practices for phytoremediation deployments, including plant selection, soil amendments, and irrigation systems. Future work can be used to determine phytoremediation deployments and strategies in response to TCE/PCE-contaminated groundwater movement. Techniques described here, in conjunction with other applications, provides tools for screening plant species and soils for phytoremediation and MNA activity. Phytoremediation applications have proven advantageous over conventional remediation techniques for CE-contaminated groundwater at Argonne National Laboratory East (Quinn et al. 2001). The metabolic actions of the plants and soils in combination with physical reductions of VOCs by volatilization and dilution enables active remediation at the rhizosphere of the seepline.

A better understanding of the potential mechanisms involved for enhanced biodegradation in the root zone and the interaction between plants, microorganisms, and contaminants can be useful in phytoremediation applications by helping in the plant selection process. This information could lead to improved land management practices for phytoremediation applications including selection of plants, soil amendments, and irrigation systems. Future work could determine optimal phytoremediation deployments and strategies in response to TCE/PCE-contaminated groundwater movement. Application of phytoremediation should provide significant advantages over conventional remediation techniques for chlorinated ethene-contaminated groundwater. The metabolic actions of the plants and soils in combination with physical reductions of VOCs by volatilization and dilution will enable active remediation.

The extent to which rhizosphere VOC remediation varies among sites is uncertain. However, a better understanding of such variability is necessary since rhizosphere responses to seasonal changes including plant succession, rainfall, and temperature can significantly influence potential VOC bioremediation. A microcosm study was undertaken to estimate the potential of SRS Southern Sector rhizosphere soils along the seepline to naturally attenuate TCE. This study demonstrated that sorption to soil was the dominant mechanism removing as much as 90% TCE (Brigmon et al., 1998). A limited amount of TCE aerobic biodegradation and anaerobic reductive dechlorination was observed through the appearance of *cis*-1, 2-dichloroethylene (c-DCE), and *trans*-1, 2-dichloroethylene (t-DCE) in microcosm tests. Soils from vegetated areas mineralized TCE several times greater than soils from adjacent non-vegetated areas (Walton and Anderson, 1990). It has been suggested that a possible mechanism for the enhanced microbial mineralization of TCE in the *L. cuneata* rhizosphere soil is excretion of phenolic compounds in root exudates. Since phenol is a known inducer of toluene monooxygenase, an enzyme responsible for degradation of TCE, the natural plant exudates could play a role in biodegradation of TCE in the rhizosphere (Anderson, Guthrie, and Walton, 1993). Selection of the appropriate plant species can be critical to the success of phytoremediation technology.

The results of recent microbial and microcosm studies at SRS prove there is an active community at the Southern Sector seepline capable of biodegrading TCE (WRSC 2002). Microbial characterization confirmed the presence of potential TCE-degrading sulfate-reducing bacteria (SRBs) in seepline sediments. The occurrence of these bacteria *in situ* indicates favorable bioremediation potential. Questions remain as to how active these SRBs and other CE-degrading species are in the seepline. The focus of the microcosm studies employed in this study proved the effectiveness of MNA and carbon-source/nutrient additions to stimulate *in situ* microbial degradation of TCE. The seepline is saturated in many areas and has associated reducing conditions and evidence of reductive dechlorination (cDCE). The majority of molecular microbiological examinations of reductive dechlorination of ethenes have relied on the PCR primer pairs described by Löeffler et al. (2000) for specific detection of key CE degrading genera, including *Dehalococcoides* (PCE/TCE $\rightarrow$ ethene), *Desulfuromonas* and *Dehalobacter* (both convert PCE $\rightarrow$ c-DCE) species. Molecular detection of these taxa has been demonstrated in PCE/TCE-contaminated aquifers, soils and sediments, and batch reactors

having dechlorination activities (Hendrickson et al. 2002; Löffler et al. 2000). Likewise, DNA extracts from potential TCE-reducing groundwater within the SRS Southern Sector, including seepline influenced groundwater, demonstrated the presence of these species. Field studies have only detected *Dehalococcoides ethenogenes* in samples supporting complete dechlorination reactions, consistent with its described physiology. Sites like the Southern Sector seepline reveal intermediates including c-DCE of partial dechlorination (WSRC 2000a). Biodegradation of these compounds may be kinetically driven. PCE/TCE are often preferred terminal electron acceptors, but may also be catalyzed by specific microbial populations (Brigmon et al. 2002). A microbial community analysis is needed to characterize known halorespiring species that may also play a significant remedial role as well as other dehalogenating species that may involve synergistic interactions. This analysis would include other halorespiring microbes that may facilitate turnover/removal of less chlorinated products in groundwater supplying the plants at the seepline.

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