ESTCP
Cost and Performance Report

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Loading Rate and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation

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ACRONYMS AND ABBREVIATIONS

AFB	Air Force Base
AFCEE	Air Force Center for Engineering and the Environment
AMIBA	Aqueous and Mineral Intrinsic Bioremediation Assessment
ATK	Alliant Techsystems, Inc.
BUCHLORAC	buffering of dechlorination acidity (geochemical model)
CA	chloroethane
CAH	chlorinated aliphatic hydrocarbon
CCAFS	Cape Canaveral Air Force Station
CEC	cation exchange capacity
CSM	conceptual site model
DCE	dichloroethene
DIC	dissolved inorganic carbon
DNAPL	dense non-aqueous phase liquid
DO	dissolved oxygen
DOC	dissolved organic carbon
DoD	Department of Defense
DoE	Department of Energy
ESTCP	Environmental Security Technology Certification Program
EOS®	Emulsified Oil Substrate®
EVO	emulsified vegetable oil
ft/day	feet per day
HRC®	hydrogen release compound
INEEL	Idaho National Engineering and Environmental Laboratory
ITRC	Interstate Technology and Regulatory Council
MCL	maximum contaminant level
MSDS	material safety data sheet
NAVFAC ESC	Naval Facilities Engineering Command Engineering Service Center
NSWC	Naval Surface Warfare Center
NTC	Naval Training Center
NWIRP	Naval Weapons Industrial Reserve Plant
O&M	operations and maintenance
ORP	oxidation reduction potential
PCE	tetrachloroethene
POC	point of contact

ACRONYMS AND ABBREVIATIONS (continued)

PRG	preliminary remediation goal
redox	oxidation-reduction potential
SABRE	Source Area BioREmediation Project
SERDP	Strategic Environmental Research and Development Program
SOP	standard operating procedure
TCA	trichloroethane
TCE	trichloroethene
TDS	total dissolved solids
TOC	total organic carbon
USACE	U.S. Army Corps of Engineers
USAF	U.S. Air Force
USEPA	U.S. Environmental Protection Agency
VC	vinyl chloride
VFA	volatile fatty acid
VOC	volatile organic compound

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

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1.0 EXECUTIVE SUMMARY

A Technology Demonstration for Loading Rates and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation was conducted to evaluate differing approaches to determining substrate loading rates and the impacts of substrate delivery for enhanced in situ anaerobic bioremediation.

1.1 BACKGROUND

Enhanced in situ anaerobic bioremediation involves the delivery of organic substrates into the subsurface to stimulate anaerobic degradation of contaminants in groundwater. Effective application of the technology depends primarily on the delivery of appropriate levels of organic substrate in the subsurface and the development of optimal geochemical and oxidation-reduction (redox) conditions for anaerobic degradation processes to occur.

Substrate loading rates are defined as the volume, concentration, and frequency of injection of organic substrates for in situ anaerobic bioremediation. Insufficient substrate loading rates or non-uniform delivery and mixing may result in areas of the aquifer that are not sufficiently reducing for complete dechlorination to occur, thereby increasing the potential for accumulation of regulated intermediate degradation products, for example, the potential accumulation of dechlorination products *cis*-1,2-dichloroethene (DCE), vinyl chloride (VC), or chloroethane (CA).

The presence of excessive substrate may result in uncontrolled fermentation reactions (e.g., lowering of pH and formation of undesirable fermentation products (e.g., alcohols and ketones), degradation of secondary water quality (e.g., mobilization of metals), and poor utilization of substrate for anaerobic degradation of the contaminants of concern. The ability for aquifer systems to recover to pre-injection redox conditions and the long-term impacts on groundwater quality after enhanced bioremediation are not well documented.

Given these effects, many enhanced anaerobic bioremediation applications fail to achieve performance expectations or develop unanticipated long-term compliance problems. The cost associated with poor performance (e.g., a need for longer term operation) or with compliance issues such as degradation of secondary water quality (typically requiring additional monitoring or system modifications) may greatly increase the life-cycle costs of full-scale applications. Therefore, determining an appropriate substrate loading rate and an effective distribution method for the various substrate types commonly applied is a critical design and operational objective.

1.2 OBJECTIVES OF THE DEMONSTRATION

The objectives of this study were to:

1) Better understand the effects that substrate amendment loading rates have on substrate distribution and persistence (maintenance of the reaction zone)
2) Determine how control of substrate loading rates affects amendment utilization and development of optimal geochemical and redox conditions

3) Identify substrate loading rates that have adverse impacts on secondary water quality

4) Evaluate the effect that differing substrate types or loading rates may have on hydraulic conductivity based on physical/chemical or biological (biomass) effects of the substrate amendment

5) Develop practical guidelines for designing and optimizing substrate loading rates and injection scenarios for differing substrate types and for differing geochemical and hydrogeologic conditions based on observations from representative case studies.

To achieve these objectives, 15 case studies were evaluated regarding system operation and performance. Quantitative and qualitative performance objectives were developed to evaluate the case studies and to identify limiting factors for enhanced in situ bioremediation. Supporting information for the case studies may be found in the Final Technology Demonstration Report (Parsons, 2010a).

1.3 DEMONSTRATION RESULTS

A number of limiting factors commonly impact the effectiveness of enhanced in situ bioremediation applications. These limiting factors and the best practices to mitigate them include the following:

Insufficient Substrate Distribution. The ability to effectively distribute substrate is often impacted by site-specific lithology (low or high permeability, heterogeneity) and groundwater hydraulics (low or high rates of groundwater flow). In some cases the quantity of substrate that can be injected is limited by a low aquifer buffering capacity and adverse lowering of pH. This reinforces the need to, and benefits of, conducting adequate site characterization prior to design and implementation of substrate addition. In most cases these conditions can be mitigated by modifying the injection mixture and substrate loading rate (for example, more frequent and less concentrated substrate solutions, or adding a buffering amendment) or selecting an appropriate delivery technique (for example, closer spaced injection points and larger injection volumes).

Adverse Geochemical Conditions. The most common geochemical condition was an adverse excursion (lowering) of pH, resulting from a combination of low buffering capacity of the aquifer and high concentrations of dissolved organic carbon (DOC). Control of the substrate loading rate is critical when treating aquifers with low buffering capacity. Mitigation measures include careful screening of the site to determine whether a buffering compound should be added to the injection protocol and selecting substrate delivery techniques that provide for more uniform distribution of substrate without excessive “spikes” in DOC.

Loss of Hydraulic Conductivity or Biofouling of Injection Wells. A decrease in hydraulic conductivity (permeability) may result in bypass of contaminated groundwater around the reaction zone or uneven distribution of substrate during subsequent injections. One option to mitigate the potential for loss of hydraulic conductivity is to conservatively design the reaction zone to extend beyond the limits of contaminated groundwater to be treated. For example, a biobarrier may be installed an additional 20 to 50 feet beyond the edge of the groundwater contaminant plume to avoid potential for bypass around the ends of the reaction zone.

Substrate Persistence and Longevity. Concentrations of DOC typically need to be sustained above 50 to 100 milligrams per liter (mg/L) over the design life of the application for effective treatment of chlorinated aliphatic hydrocarbons (CAH). Buildup and degradation of biomass may sustain the reaction zone and limit the amount of rebound that may occur after the initial substrate is depleted. Rebound of concentrations in the treatment zone will depend on whether a residual source of contaminant mass remains upgradient of the treatment zone, or in low permeability sediments within the treatment zone.

Difficult Hydrogeological Conditions. Rates of groundwater flow less than 0.1 feet per day (ft/day) (37 feet per year [ft/yr]) or greater than 2.7 ft/day (1,000 ft/yr) require special design considerations. Low rates of groundwater flow may require closer injection point spacing because the distribution of organic acids by dispersion will be limited. High rates of groundwater flow will require more frequent and higher concentration injections as the substrate is dispersed over a large volume of the aquifer. In the case of emulsified vegetable oil (EVO) products, the retention of the oil droplets is a critical parameter to sustain adequate substrate concentrations in the reaction zone. Oil retention in coarse-grained sediments, combined with a high rate of groundwater flow, may not be sufficient without additional injections. In addition, as the degree of aquifer heterogeneity increases, so may the need for closer injection well spacing or for “targeted” injections within lower permeability sediments.

A better understanding of site conditions may have limited the impact of these factors for several of the case studies. Development of a conceptual site model (CSM) and an understanding of the natural processes that are being stimulated is useful to guide the site selection and design process (Air Force Center for Engineering and the Environment [AFCEE] et al., 2004). This should include an assessment of site-specific data on native electron donors and electron acceptors, metabolic by-products, geochemical parameters, contaminant trends, and hydrogeology. A CSM also summarizes the fate and transport of contaminants, migration pathways, exposure mechanisms, and potential receptors. Therefore, a CSM provides important information to identify and mitigate the limiting factors described above.

1.4 SUBSTRATE ESTIMATING TOOL

A substrate estimating tool was developed to assist the practitioner in evaluating a site for an enhanced in situ bioremediation application (Appendix B). This tool was used during the case study evaluations to compare the substrate amendment designs and actual quantities used to the substrate requirements calculated by the tool using site-specific electron acceptor demand.

The substrate estimating tool is useful to screen site conditions that will impact substrate delivery and utilization. The tool provides an estimate of *total* substrate required over the design life of the application, given a user specified design factor. The tool calculates a time-weighted average concentration of substrate by dividing the total volume of groundwater treated by the total substrate quantity.

The quantities and time weighted average substrate concentrations can be used for comparison to proposed or planned bioremediation applications as a check on the quantities of substrate being proposed or the performance targets for DOC. This should assist in avoiding application of either too little substrate or generating excessive substrate levels. Design tools are often provided by substrate vendors, and the estimated substrate quantity should always be compared to recommendations by the provider or with case studies in the literature.

1.5 IMPLEMENTATION ISSUES

The primary objective when selecting a substrate loading rate is to achieve a uniform distribution of substrate over time and space. The substrate requirement for each of the case studies was calculated using the substrate estimating tool. Based on these calculations and observations of case study performance, a conservative design factor on the order of three to seven times the estimated substrate requirement should be suitable for limiting the potential for insufficient substrate for slow release substrates injected in a one-time event. For soluble substrates, lower design factors on the order of two to three times the estimated substrate requirement are beneficial to avoid over-stimulating the aquifer and driving pH downward. The delivery methods for soluble substrates should target uniform substrate concentrations without excessive “spikes” in concentration.

Design tools that assist the practitioner with the configuration (well spacing) and injection volumes are being developed and should be incorporated into the design exercise. Examples include the Edible Oil Substrate tool being developed under Environmental Security Technology Certification Program (ESTCP) Project ER-200626 (Borden et al., 2008). The loading rates calculated by this (or any other design tool) should be compared to the recommended guidelines above to ensure that the input parameters to the design tool are producing realistic and appropriate calculations for substrate requirements.

The use of very high substrate concentrations to enhanced dissolution of dense non-aqueous phase liquids (DNAPL) into the aqueous phase is an exception to typical substrate loading rates. These applications require special consideration of the buffering capacity of the aquifer and the system configuration. For example, it may be acceptable to induce adverse geochemical conditions in the source zone to mobilize chlorinated solvent mass if a suitable downgradient reaction zone for biodegradation and geochemical recovery is established.

Six of the 15 case study sites exhibited issues with pH excursion. For all these sites, initial background pH values were below 6.5 and alkalinity was below 150 mg/L. Based on these observations from the case studies, a combination of pH below 6.0 to 6.5 and alkalinity below 300 mg/L indicates that modifications to buffer and control pH will be necessary. Sodium bicarbonate was the most common buffering compound used, typically at concentrations in excess of 10,000 mg/L. Sodium bicarbonate is a relatively weak buffering compound and may be most suitable for applications using frequent injections of soluble substrates. The use of stronger and more persistent buffering compounds (e.g., sodium carbonate or sodium phosphates) may be necessary for applications using slow release substrates, and further research and product development will be beneficial for sites with low buffering capacity.

In practice, the amount of site characterization data that is available or that can be economically obtained is always limited to some extent. It is not unusual to design an enhanced in situ bioremediation application at a "well characterized" site, only to encounter difficult hydrogeological conditions such as low permeability sediments or heterogeneity that limits effective substrate distribution. Therefore, it is useful to consider practices that mitigate the uncertainty associated with subsurface environments.

Soluble substrate systems that use frequent injections have the most flexibility in modifying the injection protocol. When using infrequent applications of slow-release substrates, potential problems such as the need to add a buffering agent should be evaluated prior to substrate addition, and buffer should be added during substrate injection as a precautionary measure when the buffering capacity of the aquifer is in question.

Inadequate or excessive distribution of substrate due to aquifer permeability and/or groundwater flow rates can be adjusted by increasing or decreasing the substrate loading rate, and/or by modifying injection frequency or well spacing. Substrate loading rates may be increased in the event of inhibitory electron acceptor demand (e.g., sulfate over 50 to 100 mg/L).

Finally, incomplete or delayed dechlorination is a common limitation resulting in accumulation of intermediate dechlorination products. Prior to considering bioaugmentation, the system should be evaluated to ensure that the proper geochemical conditions have been achieved and that a sufficient acclimation period has been allowed for ecological succession and development of appropriate microbial consortia. Bioaugmentation with commercially available culture can be implemented if it has been determined that indigenous *Dehalococcoides* species are lacking or do not exhibit the reductase enzymes that indicate a capability for complete dechlorination of VC to ethene (e.g., Steffan et al., 2010).

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2.0 INTRODUCTION

This Cost and Performance Report summarizes a Technology Demonstration for Loading Rates and Impacts of Substrate Delivery for Enhanced Anaerobic Bioremediation that evaluates differing approaches to determining substrate loading rates and the impacts of substrate delivery for enhanced in situ anaerobic bioremediation (Parsons, 2010a). Select case study sites were evaluated regarding 1) the methods that are used to determine substrate loading rates, 2) the results achieved by these approaches, 3) the methods and tools available for determining substrate requirements and substrate loading rates, and 4) an assessment of differing approaches and the cost impact of design modifications. Supporting information for the case studies may be found in the Final Technology Demonstration Report (Parsons, 2010a). Recommendations for determining substrate requirements and for designing substrate amendments were developed based on these evaluations.

2.1 BACKGROUND

Effective application of enhanced in situ anaerobic bioremediation depends primarily on the delivery of appropriate levels of organic substrate in the subsurface and the development of optimal geochemical and redox conditions for anaerobic biodegradation processes to occur. This project specifically addresses anaerobic dechlorination of chlorinated solvents, but the concepts of adequate substrate distribution and achieving optimal geochemical conditions apply to other contaminants amenable to anaerobic degradation processes.

Substrate loading rates are defined as the volume, concentration, and frequency of injection of organic substrates for in situ anaerobic bioremediation. Insufficient substrate loading rates or non-uniform delivery and mixing may result in areas of the aquifer that are not sufficiently reducing for complete dechlorination to occur, thereby increasing the potential for accumulation of regulated intermediate degradation products (e.g., the potential accumulation of DCE, VC, or CA). Little is known regarding the minimum or threshold concentrations of substrates that are required to sustain reductive dechlorination at sites with a history of substrate addition and mature microbial populations.

The presence of excessive substrate may result in uncontrolled fermentation reactions (e.g., lowering of pH and formation of undesirable fermentation products (e.g., alcohols and ketones), degradation of secondary water quality (e.g., mobilization of metals and semi-metals), and poor utilization of substrate for anaerobic degradation of the contaminants of concern. The ability for aquifer systems to recover to pre-injection redox conditions and the long-term impacts on groundwater quality after enhanced bioremediation is not well understood.

Given these effects, many applications fail to achieve performance expectations or develop unanticipated long-term compliance problems. ***The cost associated with poor performance (e.g., a need for longer term operation) or with compliance issues such as degradation of secondary water quality (typically requiring additional monitoring or system modifications) may greatly increase the life-cycle costs of full-scale enhanced bioremediation applications.*** Therefore, determining an appropriate substrate loading rate and an effective distribution method for the various substrate types commonly applied is a critical design and operational objective. This technology demonstration is intended to evaluate various substrate emplacement strategies to optimize the performance of enhanced in situ anaerobic bioremediation applications.

2.2 OBJECTIVES OF THE DEMONSTRATION

This study is intended to supplement guidance developed to date by ESTCP for enhanced in situ anaerobic bioremediation. The objectives of this study are to:

1) Better understand the effects that substrate amendment loading rates (volume, concentration, and frequency of injection) have on substrate distribution (mixing and radius of influence) and persistence (maintenance of the reaction zone)
2) Determine how control of substrate loading rates affects amendment utilization or reactivity and the development of optimal geochemical and redox conditions for anaerobic biodegradation
3) Identify substrate loading rates that have adverse impacts on secondary water quality
4) Evaluate the effect that differing substrate types or loading rates may have on hydraulic conductivity based on physical/chemical or biological (biomass) effects of the substrate amendment
5) Use this information to develop practical guidelines for designing and optimizing substrate loading rates and injection scenarios for differing substrate types and for differing geochemical and hydrogeologic conditions.

This Cost and Performance Report summarizes the results of the case study evaluations and the implications on system design and performance.

2.3 REGULATORY DRIVERS AND STAKEHOLDER BENEFITS

Enhanced in situ anaerobic bioremediation has gained widespread acceptance as a remedy for contaminants in groundwater, including chlorinated solvents and other compounds subject to anaerobic degradation. While it is widely applied, regulatory concerns with performance of the technology persist. A review of state policies on enhanced bioremediation conducted by the Interstate Technology and Regulatory Council (ITRC) (ITRC, 1998) identified generation of VC in the reaction zone as a typical regulatory concern. Degradation of secondary drinking water quality or production of noxious gases are also concerns where potential exposure pathways exist. These issues are typically addressed through additional monitoring, which raises the cost of the remedy. Therefore, any methods that reduce the potential for production of toxic intermediate degradation byproducts, degradation of secondary drinking water quality, or production of noxious gases will lead to increased regulatory confidence and will limit costs associated with additional injections or extended monitoring.

The benefits of the proposed research to stakeholders and end users are performance and financial based. Failure to meet remedial objectives or potential adverse impacts to secondary water quality affect the ability of the Department of Defense (DoD) to protect human health and the environment. By improving remedy performance, the potential exists to save hundreds of thousands to millions of dollars by reducing long-term operational and monitoring costs. ***For many applications, the cost to operate, modify, or monitor system performance over periods of years is often greater than the cost to design and install the system***.

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3.0 TECHNOLOGY

3.1 TECHNOLOGY DESCRIPTION

Enhanced in situ anaerobic bioremediation can be an effective method of degrading various chlorinated solvents dissolved in groundwater. Addition of an organic substrate creates an anaerobic treatment zone within and downgradient of the zone of injection (Figure 1). Creating and sustaining the appropriate anaerobic geochemical conditions is essential to an effective application of the technology.

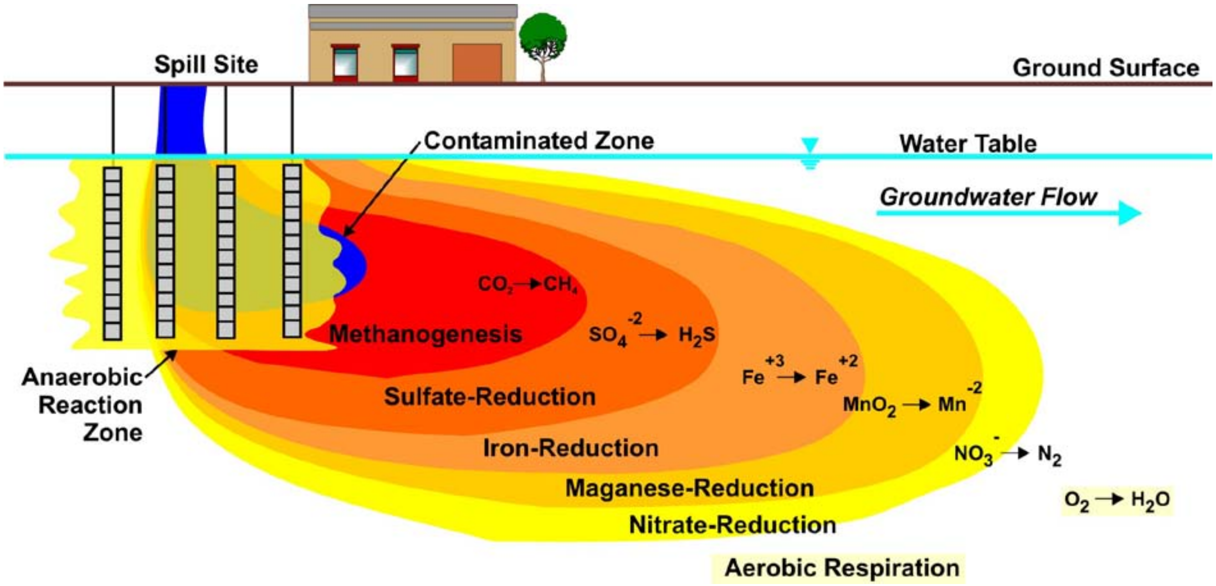

Figure 1. Reducing zones established downgradient of substrate injection.
(from AFCEE et al., 2004)

Many different substrate types have been used for in situ anaerobic bioremediation of chlorinated solvents in groundwater. Common substrate types include soluble substrates (lactate, molasses), slow-release substrates (vegetable oil, EVO, Hydrogen Release Compound [HRC®]), and solid substrates (mulch and compost). Other amendments also may need to be delivered with the substrate, including pH buffering agents, nutrients, or bioaugmentation cultures. The substrates used for enhanced bioremediation each have differing physical, chemical, and biodegradation characteristics. Therefore, the approach used to design an application and to determine substrate requirements varies between the different substrate types.

Determining substrate requirements is not an exact science, and the degree of uncertainty in the methods currently employed is considerable. Two general approaches have been used to estimate substrate requirements and to derive a substrate loading rate. One approach is to target an empirical concentration of substrate in the reaction zone that is based upon previous experience and experimentation at sites with similar hydrogeology, geochemistry, and contaminant distribution. The other approach is to calculate a substrate (electron donor) requirement based on estimates of native and contaminant electron acceptor mass and mass flux though the contaminant treatment zone. The rate at which the substrate is applied (amount and frequency) is equally as important as determining a total substrate requirement. To undertake a calculation of this kind may infer an understanding of the biological and geochemical processes that is greater than the current state of the science. To make the process work a substantial engineering design factor is frequently applied.

Users of soluble substrates typically use an empirical-based approach because they are able to modify the substrate loading rate on a more frequent basis until the desired geochemical conditions are achieved (for example, Suthersan et al., 2002). In these cases the substrate loading rate is commonly based on experience, field observations, and practical engineering considerations. Users or vendors of slow-release substrates (e.g., HRC® and EVO) typically rely on calculated substrate requirements because the product is usually applied in a single event (e.g., see Appendix G of AFCEE, 2007). More recently, users of EVO products have realized that the retention of oil droplets in the aquifer is dependent on the physical and chemical properties of the EVO product (e.g., the ionic strength of the emulsifiers) and of the aquifer matrix (e.g., % clay and organic material in the aquifer matrix). The retention capacity of the aquifer may limit how much oil substrate can be retained within the reaction zone.

Formulating substrate amendments to stimulate biogeochemical transformation processes has not been developed in the literature. However, the stoichiometry for the production of reduced iron mono-sulfides is relatively straightforward (AFCEE, 2002; Kennedy and Everett, 2003). Stimulating the formation of reactive iron sulfides based on native geochemistry and iron and sulfate amendments also requires evaluating substrate requirements.

3.2 ADVANTAGES AND DISADVANTAGES

Enhanced in situ bioremediation may in some cases offer the following advantages:

- **Lower Capital and Maintenance Costs:** Lower capital costs often are realized because substrate addition can be easily accomplished using conventional technologies, and operation and maintenance (O&M) is generally routine.
- **Destruction of Contaminants In Situ*:*** Chlorinated solvents have the potential of being completely mineralized or destroyed. Destruction of contaminants in situ is highly beneficial because contaminant mass is not transferred to another phase, there is no secondary waste stream to treat, potential risks related to exposure during remediation are limited, and there is minimal impact on site infrastructure.
- **Interphase Mass Transfer:** Enhanced anaerobic processes may increase the rate of DNAPL source zone dissolution. This has sparked interest in enhanced bioremediation as a more efficient and expeditious method for remediating chlorinated solvent source areas (ITRC, 2008a and 2008b).
- **Potential Application to a Variety of Contaminants:** In addition to chlorinated solvents, enhanced in situ bioremediation has the potential to treat any contaminant that can be made less toxic or less mobile through reduction reactions.

Injection of an organic substrate causes profound changes to the subsurface environment, and the effectiveness of the technology may be subject to hydrogeological, geochemical, and biological limitations. Some of these limitations also affect other in situ remedial techniques. Potential issues that should be considered when applying enhanced in situ bioremediation include the following:

- **Site-Specific Limitations.** Site-specific limitations may include low permeability or a high degree of heterogeneity that limits the ability to effectively distribute the substrate throughout the aquifer. Other site-specific limitations may include high levels or influx of competing electron acceptors (e.g., sulfate), inhibitory geochemical conditions (e.g., pH), or lack of appropriate microbial species. As a result, degradation may be limited.
- **Time Frame for Remediation.** Enhanced in situ bioremediation is not an instantaneous or rapid process. The time required to develop the appropriate environmental conditions and acclimation of a microbial population capable of complete degradation is on the order of several months to years. Therefore, the technology may require prolonged operation and monitoring.
- **Incomplete Degradation Pathways and Accumulation of *cis*-DCE and VC.** Microbial populations capable of anaerobic dechlorination of the highly chlorinated compounds (e.g., tetrachloroethene [PCE] and trichloroethene [TCE] to *cis*-DCE) are thought to be ubiquitous in the subsurface environment. However, the ability of these dechlorinators to compete with other native microbial populations or to complete the degradation of chlorinated compounds to innocuous end products may be an issue at some sites.
- **Secondary Degradation of Water Quality.** Secondary degradation of groundwater quality may occur under the highly anaerobic conditions resulting from substrate addition. Degradation reactions or excessive changes in groundwater pH and redox conditions may lead to solubilization of metals (e.g., iron, manganese, and potentially arsenic), formation of undesirable fermentation products (e.g., aldehydes and ketones), and other potential impacts to secondary water quality. Many of these changes are not easily reversed and it may take many years for the effects of the substrate addition to diminish.
- **Generation of Volatile Byproducts and Noxious Gases.** Stimulating biodegradation also may generate volatile byproducts and noxious gases (e.g., VC, methane, or hydrogen sulfide) that may degrade groundwater quality and/or accumulate in the vadose zone.

While these concerns and potential limitations should be considered when evaluating enhanced anaerobic bioremediation, many of them can be mitigated or compensated for by understanding the biogeochemical and hydrogeologic conditions of the aquifer system and using an appropriate design and substrate loading strategy.

In summary, determining an optimal substrate loading rate and an effective distribution method are critical design and operational objectives. Guidance is limited for determining optimal substrate amendment strategies. This study 1) compares techniques used to calculate or design substrate loading rates, 2) evaluates performance to determine optimal injection scenarios,

3) provides a substrate estimating tool for site screening, and 4) provides recommendations for design of substrate amendments.

4.0 PERFORMANCE OBJECTIVES

The objectives (Section 1.2) of this study were addressed by comparative evaluations of fifteen (15) case studies, primarily consisting of DoD and Department of Energy (DOE) applications. Additional field sampling and analysis were performed for two sites to support evaluation of the project objectives. Quantitative and qualitative performance objectives developed to evaluate and measure the success of the demonstration sites are listed in Table 1.

The distribution of substrate was evaluated using concentrations of soluble organic carbon measured within the intended reaction zone. Concentrations achieved are compared to target concentrations described in the application design (i.e., work plans). These data are used to better understand the effects that substrate amendment loading rates (volume, concentration, and frequency of injection) have on substrate distribution (mixing and radius of influence).

Achieving optimal geochemical conditions was evaluated by analyzing indicator parameters of anaerobic conditions such as dissolved oxygen (DO), oxidation-reduction potential (ORP), nitrate, manganese, ferrous iron, sulfate, and methane.

Pre- and post-treatment concentrations of contaminants were evaluated to determine the effectiveness of the remedy. Success was evaluated by comparing concentrations to site-specific performance criteria, if established. Otherwise, a reduction in contaminant concentration of 99% or greater, or a reduction in the total molar concentration of CAHs of greater than 90%, was considered successful.

The term “secondary water quality” is used in this document to refer to water quality issues that result from substrate addition, apart from the primary contaminants being treated. Secondary water quality parameters that were evaluated included pH, chloride, total dissolved solids (TDS), sulfide, and dissolved metals or semi-metals (iron, manganese, arsenic, and selenium).

The hydraulic conductivity of the aquifer may be impacted by physical, chemical, or biological processes. Pre- and post-treatment of hydraulic conductivity (typically from slug tests) were evaluated to determine the degree to which hydraulic conductivity within the reaction zone may have been reduced.

Effective enhanced bioremediation applications must sustain the reaction zone over the design life of the application. Substrate persistence and long-term effectiveness were evaluated using concentrations of soluble substrate and contaminants over the design life of the application. A rebound in contaminant concentrations after the remedy was halted was evaluated when possible. At least one year of post-remediation contaminant data from the treatment zone was considered sufficient to evaluate potential rebound.

The cost associated with poor performance or compliance issues may significantly increase the life-cycle costs of full-scale enhanced in situ bioremediation applications. Actual work performed was compared to the application design or work plan to determine whether additional work was required.

Finally, there are limits to the hydrogeological conditions under which enhanced in situ bioremediation may be applied. A qualitative assessment was made to determine whether performance was related to adverse site conditions such as low permeability sediments, a high degree of heterogeneity, or high rates of groundwater flow.

Table 1. Performance objectives.

Performance Objective	Data Requirements	Success Criteria
Quantitative Performance Objectives		
Determine ability to uniformly distribute substrate	Post-injection concentrations of soluble organic carbon in groundwater	Achieving the concentration of substrate targeted in the design at all monitoring locations within the reaction zone is considered successful.
Determine if optimal geochemical conditions were achieved	Pre- and post-injection concentrations of geochemical indicator parameters in groundwater	Achieving highly reducing conditions with ORP less than -200 millivolts (mV) throughout the reaction zone is considered successful.
Determine remediation effectiveness	Pre- and post-treatment contaminant concentrations in groundwater	A greater than 99% reduction in compound-specific concentrations is considered successful. A greater than 90% reduction in total molar concentration of CAHs is considered successful.
Determine impacts to secondary water quality	Post-treatment concentrations of secondary water quality parameters (e.g., dissolved metals such as iron and manganese)	Maintaining concentrations of secondary water quality parameters below applicable regulatory standards downgradient of the reaction zone is considered successful.
Determine impacts on hydraulic conductivity	Pre- and post-treatment measurements of hydraulic conductivity	A less than 50% decrease in hydraulic conductivity is considered successful.
Determine substrate persistence and long-term effectiveness	Post-treatment concentrations of contaminants and soluble organic carbon at the end of the intended design life of the application	A rebound in concentrations of less than 1.0% of the initial contaminant concentration after the application has been completed is considered successful.
Qualitative Performance Objectives		
Determine need for and cost of additional injections or monitoring	Actual work performed is compared to the application design plan. The cost of additional work is calculated when data are available, or a qualitative assessment is made when cost data are not available.	An application that does not require additional injections or monitoring beyond that in the original design is considered successful.
Application in difficult hydrogeological conditions	Site geology (permeability, heterogeneity) and groundwater hydraulics (hydraulic conductivity, hydraulic gradient, and rate of groundwater flow)	An application where permeability, heterogeneity, or the rate of groundwater does not limit effectiveness is considered successful. Guidelines are developed from examples where they impacted the effectiveness of the application

5.0 DEMONSTRATION SITE SELECTION

Fifteen sites were selected for evaluation of soluble, slow-release viscous fluids, and solid phase substrate types to ensure that a representative number of sites was included. Permeable mulch biowalls are included because in many cases they need to be replenished with slow release substrates such as EVO.

5.1 SITE SELECTION CRITERIA

Site screening criteria for this demonstration are summarized in Table 2. Desired site characteristics for the demonstration sites included 1) readily available work plans and results reports, 2) adequate site characterization and monitoring to evaluate system performance, 3) a representative number of sites for each of the substrate categories, 4) a variety of injection methods, and 5) a diversity of hydrogeological and groundwater geochemical conditions. Not all selection criteria could be satisfied for every site. For example, not all substrates have well documented cases studies and some sites had not completed their designed treatment period.

Table 2. Summary of site selection criteria.

Parameter	Preferred Value(s)	Relative Importance	Comment
Design	Final Work Plan	1	Adequate description of design criteria
Monitoring and reporting	Final Report or Current Monitoring Results	2	Adequate documentation
Representative substrates	Minimum of two sites for each substrate type	3	To include vegetable oil, HRC®, molasses, lactate, and mulch biowalls
Point of contact (POC)	DoD POC with access to data and site access	4	Beneficial to obtain work plans and data required for Phase II evaluations
Diverse injection scenarios	Differing injection and installation methods One time and multiple injection events	5	Beneficial to evaluate substrate distribution and persistence between different amendment approaches
Adequate site characterization	Well defined distribution of contaminants	6	Adequate characterization of hydrogeology and groundwater geochemistry also desired
Diverse hydrogeology and geochemistry	Low and high permeability, low and high rates of flow, low and high electron acceptor demand	7	Determination of substrate loading rates that are sensitive to varied site conditions

5.2 DEMONSTRATION SITES

Site summary evaluations are included in Appendix C of the *Final Technology Demonstration Report* (Parsons Infrastructure & Technology Group, Inc. [Parsons], 2010a). The breakdown by substrate type includes three sites using vegetable oil or EVO, two sites using a combination of EVO and sodium lactate, two sites using HRC® or HRC-X™, one site using whey, two sites using molasses, one site using ethanol, two sites using sodium lactate, and two sites using mulch and compost (one of which has been replenished with EVO). Sites selected for the demonstration are listed in Table 3.

Table 3. Summary of sites selected for evaluation of substrate loading rates.

Site/Facility	Agency/Contractor	Notes	Usefulness
Vegetable Oil			
1. Hangar K, Cape Canaveral Air Force Station (CCAFS), FL	Air Force/Parsons	DNAPL source area, neat oil injection, over 5 years of monitoring data	Document long-term impacts of groundwater quality
2. Area C, Alliant Tech Systems (ATK), Elkton, MD	ESTCP/Solutions IES	Perchlorate and 1,1,1-trichloroethane (TCA)	Substrate distribution and depletion, co-contaminants (1,1,1-TCA and perchlorate)
3. SA17, Naval Training Center (NTC) Orlando, FL	Navy/AGVIQ-CH2M Hill, Solutions IES	Temporary recirculation for injection. Low pH, low alkalinity, high sulfate.	Distribution of EVO substrate using recirculation techniques, pH excursion requiring buffering
Vegetable Oil/Lactate Mix			
4. DP98, Elmendorf Air Force Base (AFB), AK	Air Force/Parsons	High iron and manganese, low groundwater temperature, dechlorination stalled at *cis*-DCE.	Used stoichiometric approach in design. Lack of *Dehalococcoides*.
5. Kenney Avenue Plume, Elmendorf AFB, AK	Air Force/Parsons	High iron and manganese, low groundwater temperature, high rate of groundwater flow, dechlorination stalled at *cis*-DCE.	Required modification to the injection amendment to achieve sufficient reducing conditions. Lack of *Dehalococcoides*.
HRC® Products			
6. Contemporary Cleaners, Orlando, FL	Florida Department of Environmental Protection/IT Corporation	Required additional injection, difficult hydrogeology and geochemistry.	Application methodology for HRC® products, revised injection plans.
7. Springdale Cleaners, Portland, OR	Oregon Department of Environmental Quality	Inferred DNAPL site, use of extended release HRC-X™ formulation.	Application methodology for HRC® products
Whey			
8. East Gate Disposal Yard, Fort Lewis Logistics Center, WA	United States Army Corps of Engineers (USACE), ESTCP/ North Wind, CDM	Compared low and high concentration electron donor solutions to enhance DNAPL bioavailability	Application methodology for whey using high electron donor solutions
Molasses			
9. Site 1, Hanscom AFB, MA	Air Force/ARCADIS	Required multiple injections and revisions to injection design.	Application method for molasses.
10. Demonstration Site, Vandenberg AFB, CA	Air Force/ARCADIS	Required multiple injections and revisions to injection design.	Application method for molasses, required buffering for pH control.
Ethanol			
11. Aerojet Facility, CA	Aerojet/ESTCP/ GeoSyntec	Treatment of TCE, 1,2-dichloroethane (1,2-DCA), and perchlorate (Strategic Environmental Research and Development Program [SERDP] ER-1164)	Used stoichiometric calculations for design to limit sulfate reduction and production of dissolved metals.

Table 3. Summary of sites selected for evaluation of substrate loading rates (continued).

Site/Facility	Agency/Contractor	Notes	Usefulness
Lactate			
12. Test Area North, Idaho National Engineering and Environmental Laboratory (INEEL), ID	DOE/North Wind	Multiple injection scenarios during optimization, DNAPL application	Evaluate multiple injection scenarios and enhanced dissolution of DNAPL
13. Building 1419, Indian Head Naval Surface Warfare Center (NSWC), MD	Army/Shaw Group	Perchlorate application in low pH environment	Required buffering to raise pH for effective degradation of perchlorate
Mulch Biowalls			
14. Area E, S, M, and F, Naval Weapons Industrial Reserve Plant (NWIRP) McGregor, TX	Navy/CH2M Hill	Recharge is part of long-term O&M	Recharge based on substrate depletion and select geochemical indicator parameters
15. Building 301, Offutt AFB, NE	Air Force/Groundwater Services, Inc. (GSI) and URS Corporation	Over 6 years of data for evaluation of long-term sustainability	Sustainability of mulch biowalls

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6.0 CASE STUDY EVALUATIONS

6.1 EVALUATION METHODS

This demonstration consisted of an evaluation of sites based on work plans and results reports, and collection of additional data (field mobilizations) to fill data gaps necessary to evaluate field methods or performance. Case study evaluations consisted of a comparison of substrate loading rates and delivery methods. The objective of this evaluation was to better understand how substrate loading rates are currently estimated and to evaluate how different substrate amendment scenarios affect substrate distribution, geochemical conditions, and groundwater hydraulics. The case study evaluations included the following:

- How substrate loading rates were calculated or designed for each application (work plan review).
- Evaluate distribution (using existing monitoring networks) and trends in concentrations of substrate (soluble organic carbon and volatile fatty acids [VFA]) over time.
- Evaluate what modifications to the substrate amendment protocol were required (e.g., concentration and frequency for soluble substrates, and depletion and additional injection of slow-release substrates such as HRC® and EVO).
- Evaluate the impact on life-cycle cost to implement modifications to injection protocols or for extended monitoring.
- Evaluate efficiency in removal of chlorinated solvents based on trend analysis and geochemical conditions such as redox levels and alternate electron acceptors. Attempt to determine “threshold” concentrations of DOC that represent the minimum levels required to sustain complete reductive dechlorination.
- Note which geochemical or hydrogeological conditions enhanced or inhibited bioremediation performance.
- Evaluate changes in secondary water quality parameters as a function of substrate type, concentration, and availability.
- Evaluate hydraulic data, including potentiometric surface and hydraulic conductivity, before and after injection to determine adverse impacts to groundwater flow.

The performance objectives listed in Table 1 were used to measure the relative success of the demonstration sites. Stoichiometric hydrogen demand was calculated for each site for comparison to guidelines and methods used by various practitioners.

Select field sampling activities were conducted to evaluate the following:

- For EVO, methods to calculate the amount of substrate needed are starting to be based in part on the retention of oil within the soil matrix (Borden et al., 2008). Values used for oil retention are currently based on limited laboratory bench tests conducted by Dr. Robert Borden at North Carolina State University. The calculation method was tested for samples from NTC Orlando.

- Sampling was conducted to determine the persistence of substrate and longevity of an application at the DP98 Site and causes for incomplete dechlorination at the DP98 and Kenney Avenue Plume sites at Elmendorf AFB, AK.

Sampling activities at NTC Orlando are summarized in the case study evaluation in Appendix C of the *Final Technology Demonstration Report* (Parsons, 2010a), while sampling activities and locations for the DP98 and Kenney Avenue Plume sites at Elmendorf AFB, AK, are described in Appendix D.

6.2 SUBSTRATE ESTIMATING TOOL

A substrate estimating tool was developed to assist the practitioner in evaluating a site for an enhanced in situ bioremediation application. A summary of the tool is attached to this report as Appendix B, while a complete description is included in Appendix B of the Final Technology Demonstration Report (Parsons, 2010a). The primary objectives of this tool are to:

- Evaluate the site-specific conditions regarding hydrogeology and geochemistry in regard to the demand exerted by both natural and anthropogenic electron acceptors
- Screen for site conditions that require special consideration, such as excursion of pH outside a range optimal for dechlorinating microorganisms
- Evaluate and compare the concentrations of differing substrate types necessary to meet the electron acceptor demand.

This tool was used during the case study evaluations to compare the substrate amendment designs and actual quantities used to the substrate requirements calculated by the tool using site-specific electron acceptor demand.

7.0 PERFORMANCE ASSESSMENT

7.1 SUMMARY OF CASE STUDY PERFORMANCE CRITERIA

The following evaluation of the demonstration case studies focuses on the performance criteria listed in Table 1. These criteria are intended to identify specific performance issues, including the ability to modify the system design to mitigate the limiting factors. Table 4 presents a summary evaluation of the success of each site to meet each performance criteria. References for the case study sites are listed in Section 11.

7.1.1 Ability to Uniformly Distribute Substrate

The ability to uniformly distribute organic substrate is a primary operational objective when applying enhanced in situ bioremediation. An application is considered successful for this objective when the targeted concentrations of soluble organic carbon are achieved in all monitoring locations within the intended reaction zone. The distribution of substrate was evaluated using concentrations of total organic carbon (TOC), DOC, or VFAs in groundwater. Overall, 10 of the 15 case study sites were able to effectively distribute substrate without modifications to the substrate delivery design (Table 4). The other five sites had limited success due to site conditions, and several required modifications to the injection protocol.

Injection of EVO using a recirculation configuration was used at the SA-17 site at NTC Orlando, Florida. Low yield from the extraction wells and moderate aquifer heterogeneity limited the distribution of substrate (Hudson et al., 2009). Additional injections using direct-push techniques have been used to obtain better substrate distribution.

A commercial EVO product was readily injected at the Kenney Avenue Plume at Elmendorf AFB, AK. However, the coarse-grained nature of the aquifer and a high rate of groundwater flow (over 800 ft/yr) resulted in the substrate being dispersed over a broad area and a highly reducing reaction zone could not be sustained (U.S. Air Force [USAF], 2009). This was corrected by modification of the substrate mixture to a coarse, field-mixed emulsion of larger droplet size. Greater entrapment of the oil droplets in the reaction zone resulted in appropriate reducing conditions being achieved and sustained over a minimum period of 9 months.

Injection of HRC® products in the upper and lower surficial aquifers at the Contemporary Cleaners site in Florida generally resulted in reducing (methanogenic) conditions and degradation of TCE (Kean et al., 2000; 2003). Data for organic acids indicates that the presence and persistence of soluble substrate in both the upper and lower surficial aquifers was highly variable. The slow rate of groundwater flow (estimated to be 16 ft/yr in the upper surficial aquifer and 2.6 ft/yr in the lower surficial aquifer) likely limited the dispersion of organic acids.

The distribution of molasses at Hanscom AFB, MA, and at Vandenberg AFB, CA, was impacted by adverse excursions in pH, where both aquifers exhibit low buffering capacity. The substrate loading for each injection event was modified to limit pH excursion in the injection wells while trying to achieve target concentrations of TOC in downgradient wells.

Limiting the substrate dose, reducing the frequency of injection, and injection of a clean water "push" to disperse the substrate away from the injection wells was initially used to control pH. This revised dosing regimen limited the distribution of TOC. Adding a buffering agent allowed higher strength substrate solutions and distribution of substrate and degradation results improved.

In the cases described above, the ability to effectively distribute substrate was impacted by site-specific lithology (low or high permeability, heterogeneity), groundwater hydraulics (low or high rates of groundwater flow), and geochemistry (low buffering capacity). This reinforces the need for and benefits of conducting adequate site characterization prior to design and implementation of substrate addition.

A better understanding of site conditions may have limited the impact of these factors for several of the case studies. Development of a CSM and an understanding of the natural processes that are being stimulated is useful to guide the site selection and design process (AFCEE et al., 2004). This includes an assessment of site-specific data on native electron donors and electron acceptors, metabolic byproducts, geochemical parameters, contaminant trends, and hydrogeology. A CSM also summarizes the fate and transport of contaminants, migration pathways, exposure mechanisms, and potential receptors. Therefore, a CSM provides important information to identify and mitigate the limiting factors described above. In many cases the adverse conditions observed could be mitigated by modifying the injection mixture (e.g., buffering amendment) or delivery technique (e.g., closer spaced injection points).

7.1.2 Achieving Optimal Geochemical Conditions

Achieving optimal geochemical conditions was evaluated by analyzing indicator parameters of anaerobic conditions, including DO, ORP, nitrate, manganese, ferrous iron, sulfate, methane, pH, and alkalinity. Achieving optimal geochemical conditions was usually defined as when the groundwater environment is highly anaerobic with DO less than 0.5 mg/L, ORP less than -200 mV, sulfate reduced by more than 50% relative to background, and methane greater than 1.0 mg/L (AFCEE et al., 2004). These criteria may not apply in all cases, and several sites attempted to limit sulfate reduction and methanogenesis as processes not necessary for effective treatment; contaminants such as perchlorate require less reducing conditions (Coates and Jackson, 2009).

Reducing conditions suitable for reductive dechlorination of CAHs or for reduction of perchlorate were achieved when sufficient substrate was present. This typically required concentrations of DOC or TOC on the order of 20 to 50 mg/L. Mulch and compost substrates are an exception to a direct correlation between DOC and reducing conditions. Concentrations of DOC measured within the biowalls at the B301 Site at Offutt AFB in August 2006 were less than 5.0 mg/L. But given evidence for sulfate reduction and methanogenesis within the biowalls, it appears that DOC alone is not a good indicator of the degree to which biowalls can sustain anaerobic degradation processes (Parsons, 2010b). Therefore, biowalls require a "multiple lines of evidence approach" to evaluate substrate depletion.

Table 4. Summary of achieving performance objectives.

	Performance Criteria (from Table 1)							
Site	**1. Substrate Distribution**	**2. Optimal Geochemical Conditions**	**3. Remediation Effectiveness**	**4. Impacts on Secondary Water Quality**	**5. Impacts on Hydraulic Conductivity**	**6. Substrate Persistence/ Longevity**	**7. Need for Additional Injections or Monitoring**	**8. Difficult Hydrogeological Conditions**
Hangar K, CCAFS, FL	Successful	Successful	Successful	Potential—elevated levels of manganese and iron	Successful (no apparent impact)	Successful (4 to 5 year lifespan)	Successful (no additional injections)	None encountered
Area C, ATK, Elkton, MD	Successful	Successful	Moderately successful for perchlorate, less successful for chlorinated solvents. Greater removal efficiencies could have been achieved with longer contact time.	Potential—elevated levels of manganese and iron. Fouling of downgradient extraction/air stripping system occurred until substrate levels began to decrease.	Moderately successful. A moderate decrease in hydraulic conductivity was observed but did not impact overall effectiveness.	Successful (2.5 to 3.5 year lifespan)	None for pilot test. Long-term operation of a biobarrier may need additional injections for operation over periods of 3 years or more.	Higher rates of groundwater flow were encountered after injection due to a downgradient extraction trench. This limited the extent of degradation.
SA-17, NTC Orlando, FL	Initial recirculation was limited by low yield and aquifer heterogeneity	Low pH	To be determined—follow up injections performed	To be determined	To be determined	To be determined	Follow up injections using direct-push techniques	Low yield and heterogeneity limited the effectiveness of a recirculation approach
DP98, Elmendorf AFB, AK	Successful	Successful	Limited—incomplete dechlorination to *cis*-DCE (lack of *Dehalococcoides*)	Potential—high levels of manganese and iron, but background above criteria	Moderately successful. A moderate decrease in hydraulic conductivity was observed but did not impact overall effectiveness.	Successful (minimum 3 year lifespan)	Successful (no additional injections)	None encountered
Kenney Avenue Plume, Elmendorf AFB, AK	Successful only after modifications	Successful with modifications	Limited—incomplete dechlorination to *cis*-DCE (lack of *Dehalococcoides*)	Potential—high levels of manganese, but background above criteria	Moderately successful. A moderate decrease in hydraulic conductivity was observed but did not impact overall effectiveness.	Successful only after modifications	Required modifications—last injection met longevity objectives	High rate of groundwater flow limited ability to achieve reducing conditions
Contemporary Cleaners, Orlando, FL	Limited success—uneven distribution of organic acids in space and time	Highly reducing with methanogenesis but low pH (often less than 5.0 su)	Limited—rapid removal of PCE but accumulation of *cis*-DCE in lower aquifer	Potential—high levels of iron but background above criteria	Successful (no observed impact)	Moderately successful—organic acids elevated for periods of 7 to 27 months	Successful (no additional injections)	Low rates of groundwater flow may have limited dispersion of organic acids
Springdale Cleaners Site, Portland, OR	Appears successful—limited monitoring data	Successful	Limited—rapid removal of PCE and TCE but accumulation and limited degradation of *cis*-DCE and VC	Potential—high levels of manganese and iron but background above criteria	Successful (no observed impact)	Successful—effective for minimum 1.5 years in dissolved plume and 3.4 years in source zone	Successful (no additional injections)	None encountered
East Gate Disposal Yard, Fort Lewis Logistics Center, WA	Successful	Successful	Successful	Potential—low pH and elevated ferrous iron	Successful (no observed impact)	Successful (no observed rebound)	Successful (no additional injections)	A moderate degree of heterogeneity and low yield did require re-installation of extraction and injection wells.
Site 1, Hanscom AFB, MA	Limited success—pH excursion limited substrate quantities until a buffer was added	Limited success—only achieved in immediate vicinity of injection wells	Limited success—performance objectives achieved only in immediate vicinity of the injection zone	Potential—high levels of manganese and iron but background above criteria	Successful (no observed impact)	Successful—no rebound observed after 17 months	Additional injections required. Fouling and compromise of injection seal reduced injection rate from 10 gallons per minute (gpm) to 1-2 gpm	Performance objectives could only be achieved after buffering agent added to the injection regimen.
Site 35, Vandenberg AFB, CA	Limited success—pH excursion limited substrate quantities until a buffer was added	Limited—reductions in TCE but *cis*-DCE and VC increased	Limited success—performance objectives achieved only in immediate vicinity of the injection zone	Potential—low pH and elevated sulfide persisted downgradient	Successful (no observed impact)	Successful—no rebound observed	Additional injections required and addition of buffer compound were required	Performance objectives could only be achieved after buffering agent added to the injection regimen

Table 4. Summary of achieving performance objectives (continued).

	Performance Criteria (from Table 1)							
Site	**1. Substrate Distribution**	**2. Optimal Geochemical Conditions**	**3. Remediation Effectiveness**	**4. Impacts on Secondary Water Quality**	**5. Impacts on Hydraulic Conductivity**	**6. Substrate Persistence/ Longevity**	**7. Need for Additional Injections or Monitoring**	**8. Difficult Hydrogeological Conditions**
Area 20, Aerojet Facility, CA	Successful	Successful	Successful	Successful—only limited manganese production	Successful	Not applicable—short-term pilot test	Not applicable—short-term pilot test	None, with the exception of biofouling of the injection well
Test Area North, INEEL, ID	Successful	Successful	Successful		Successful—none observed	Successful	Several successful optimization studies were conducted	None
Building 1419, Indian Head NSWC, MD	Successful	Successful	Successful	Insufficient data reported	Successful—none observed	Not applicable—short-term pilot test	Not applicable—short-term pilot test	None, other than a need to account for moderate aquifer heterogeneity
Areas E,F,M, and S, NWIRP McGregor, TX	Successful	Successful	Successful	Successful—none observed	Successful—none observed	Successful with rejuvenation option	Rejuvenation with EVO is typically required every 3 to 4 years	Periods of high rates of precipitation may increase local rates of groundwater flow, and biowall performance could be impacted during these periods.
Building 301, Offutt AFB, NE	Successful	Successful	Successful	Potential—slightly elevated levels of manganese and iron but background above criteria	Biowall conductivity has decreased to slightly below that of the surrounding formation.	Successful—continues to be effective 5 years after installation	May require replenishment within a couple years	None encountered

The use of recirculation allows the best control of geochemical conditions. Demonstrations at the Area 20 Site at the Aerojet Facility, CA, and the Building 1419 Site at Indian Head NSWC, MD, were both able to induce geochemical conditions suitable for reduction of perchlorate without stimulating a high degree of sulfate reduction and methanogenesis.

To achieve uniform reducing conditions suitable for anaerobic degradation processes, careful thought should be given to how the substrate will be distributed and how uniform the concentration of DOC can be controlled. In practice, a "range" of concentrations of DOC will occur within the aquifer, with the highest concentrations at the point of injection. Substrate concentrations will decrease with time and distance from the point of injection as the substrate is diluted and utilized for microbial processes. Achieving optimal geochemical conditions will depend on establishing a reaction zone that sustains an appropriate range of substrate concentration. Over-stimulation of the aquifer at the point of injection may cause adverse lowering of pH, while too low a concentration may result in portions of the treatment zone having insufficient substrate for effective treatment.

For slow release substrates, the reaction zone is often most reducing at or near the point of injection (Figure 1). Similarly, the reaction zone for solid substrates is typically most reducing within the biowall or bioreactor. For soluble substrate, the most reducing conditions may occur in a zone downgradient of the point of injection, as illustrated in Figure 2.

Lowering of pH was also a significant limitation at several sites, resulting from a combination of low buffering capacity of the aquifer and high concentrations of DOC. Control of the substrate loading rate is critical when treating aquifers with low buffering capacity. The best practices to mitigate pH excursion include careful screening of the site to determine whether a buffering compound should be added to the injection protocol, and selecting substrate delivery techniques that provide for more uniform distribution of substrate without excessive "spikes" in DOC. More frequent injections or closer spacing of injection points using lower doses (concentration) of substrate may be effective.

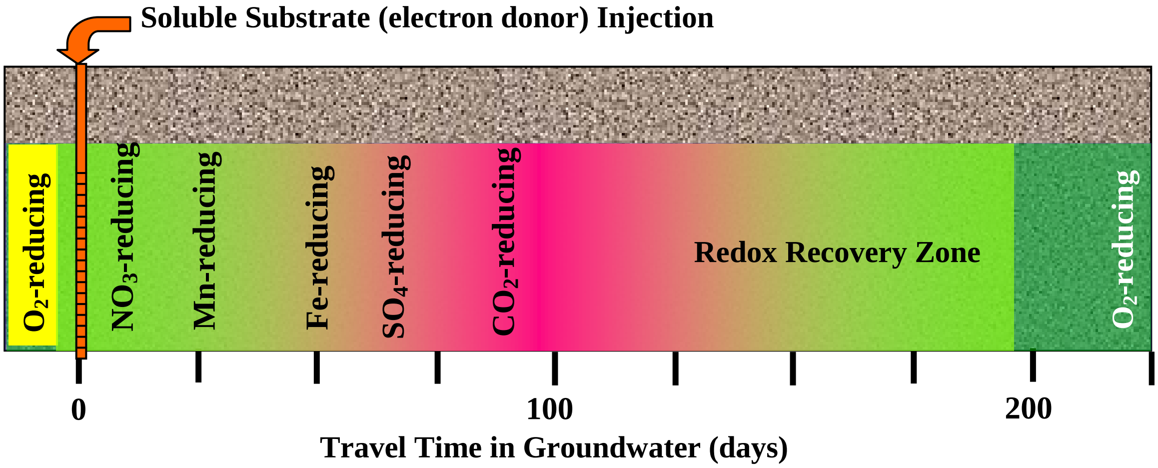

Figure 2. Reducing zones established downgradient of injection in a high-flow aerobic aquifer.
(Suthersan and Payne, 2003)

7.1.3 Remediation Effectiveness

Groundwater analytical data was used to determine whether performance objectives were met. Several sites exhibited incomplete dechlorination of CAHs. The primary factors attributed to incomplete dechlorination of chlorinated solvents included the following:

- Insufficient substrate distribution, either non-uniform distribution or low substrate concentrations
- Inability to induce highly anaerobic conditions due to high rates of groundwater flow (e.g., initial injections at the Kenney Avenue Plume, AK)
- Insufficient residence time due to too small a reaction zone or high rates of groundwater flow (e.g., Area C at the ATK Facility, MD)
- Lack of growth or activity of *Dehalococcoides* species (e.g., DP98 and Kenney Avenue Plume sites at Elmendorf AFB, AK)
- Low pH that inhibits complete dechlorination (Section 8).

Similar to inducing optimal geochemical conditions, the first three factors correspond to adequate substrate distribution at appropriate substrate concentrations. A lack or growth of appropriate strains of *Dehalococcoides* species at the Alaska sites may be due, in part, to low groundwater temperatures.

The best practices to mitigate these factors include the following:

- Selection of delivery techniques (e.g., closer spaced injection points or recirculation) that optimize uniform substrate distribution
- Modifying the injection mixture (substrate type and buffering amendment) to limit adverse excursions in pH
- Bioaugmentation for sites with low populations of *Dehalococcoides* species or with species that lack the ability to transform cis-DCE and VC to ethene.

The Area 20 Site at the Aerojet Facility in California is an example of using recirculation to optimize substrate distribution, combined with bioaugmentation to achieve complete dechlorination of TCE. The careful control of substrate dosing and use of a bioaugmentation culture resulted in effective treatment without inducing highly anaerobic conditions (i.e., sulfate reduction and methanogenesis) and limiting the potential for mobilization of metals.

7.1.4 Impacts to Secondary Water Quality

Creating an anaerobic groundwater environment may lead to degradation of water quality. The term "secondary water quality" is used to refer to water quality issues or concerns, apart from the primary contaminants being treated, that result from substrate addition. Production of regulated intermediate degradation products of the primary contaminant (e.g., production of VC from TCE and DCE) is not considered a secondary water quality issue for this evaluation. Exceeding secondary water quality standards within the reaction zone may be acceptable if water quality downgradient of the reaction zone is maintained. If concentrations of secondary water quality parameters are maintained below regulatory standards downgradient of the reaction zone, then the application is considered successful in limiting or mitigating any potential adverse impacts.

Table 5 lists common parameters monitored during enhanced in situ bioremediation and associated federal drinking water quality standards. This list is not inclusive, as many U.S. Environmental Protection Agency (USEPA) regions and states enforce additional water quality standards. Several USEPA Region 9 preliminary remediation goals (PRGs) are included in Table 5 as examples. Note that these standards may not be applicable if the impacted groundwater is not a drinking water aquifer, or may not be enforced by all regulatory agencies.

Table 5. Secondary water quality parameters subject to regulatory compliance.
(modified from AFCEE et al., 2004)

Compound or Element	Molecular Formula	USEPA MCL (mg/L) [a]	Secondary Standard [b] (mg/L)	Region 9 PRGs for Tap Water [c] (mg/L)
Volatile Organic Compounds				
Acetone	C_3H_6O	--	--	5.5
Carbon disulfide	CS_2	--	--	1.0
Isobutanol	$C_4H_{10}O$	--	--	1.8
Methyl ethyl ketone (2-butanone)	C_4H_8O	--	--	7.0
Total trihalomethanes (includes chloroform)	--	0.080	--	
General Water Quality Parameters				
Nitrate (as nitrogen)	NO_3^-	10	--	10
Nitrite (as nitrogen)	NO_2^-	1.0	--	1.0
Sulfate	SO_4^-	--	250	--
Chloride	Cl^-	--	250	--
pH	--	--	<6.5, >8.5	--
TDS	--	--	500	--
Odor (e.g., sulfide)	--	--	3 threshold odor number	--
Metals/Inorganics				
Arsenic	As	0.01	--	0.045
Selenium	Se	0.05	--	0.18
Iron	Fe	--	0.3	11
Manganese	Mn	--	0.05	0.88

[a] USEPA MCL = USEPA maximum contaminant level; mg/L = milligrams per liter.

[b] USEPA national secondary drinking water regulations are non-enforceable guidelines. However, states may choose to adopt them as enforceable standards.

[c] PRGs are USEPA Region 9 preliminary remediation goals for tap water.

Secondary water quality parameters that were evaluated for this study included volatile organic compounds (VOCs) resulting from fermentation reactions (e.g., acetone and methyl ethyl ketone), sulfate and sulfide, chloride, pH, TDS, and dissolved metals or semi-metals (ferrous iron, manganese, arsenic, and selenium). Not all parameters were measured at each site, and often data is only available for just a few of these parameters. Nonetheless, the evaluation provides some insight into how much of an issue secondary water quality is, and what parameters typically have the most potential to create a secondary water quality issue. Table 6 summarizes this comparison for select case studies with available data.

The most common secondary water quality issues include the following, in order of most common occurrence:

- **Dissolved Manganese.** Manganese oxides are common minerals in many aquifer sediments, and reduction of Mn^{4+} to soluble Mn^{2+} is a common occurrence. Manganese does not precipitate or sorb out of solution as readily as ferrous iron (e.g., with sulfide), and dissolved manganese tends to persist farther downgradient.
- **Dissolved Iron.** Dissolved (ferrous) iron is commonly observed at concentrations above its USEPA secondary water quality standard. However, dissolved iron typically precipitates or sorbs out of solution within a short distance of migrating out of the anaerobic reaction zone.
- **pH.** Lowering of pH to below 6.5 is a common occurrence. While low pH by itself may not by itself present a serious health hazard or nuisance issue, it may create other secondary problems. Low pH may enhance the solubility of metals, enhance the potential for adverse fermentation reactions, and inhibit complete dechlorination.
- **Sulfide.** Hydrogen sulfide produced by sulfate reduction has a low odor threshold and is commonly observed during sampling of anaerobic sites. Sulfide attenuates rapidly downgradient of the anaerobic treatment zone, and rarely persists as substrate is depleted.

Adverse impacts for dissolved arsenic and selenium appear to be less common, perhaps because minerals containing these elements are present at much lower concentrations in most aquifer sediments. However, it is prudent to evaluate whether arsenic or other heavy metals may be prevalent in the aquifer matrix, and what the impact of lowering the pH and redox state of the aquifer may be on their solubility.

Best practices to mitigate these secondary water quality issues include the following:

- Site screening to identify site-specific potential for secondary water issues. Examples may include characterizing the iron, manganese, and heavy metal content of aquifer sediments and evaluating the buffering capacity of the aquifer (pH and alkalinity).
- Establishing natural concentrations of secondary water quality parameters and determining the beneficial use of the impacted groundwater. Groundwater at many sites is not used for drinking water, and secondary water quality criteria may not apply.

Table 6. Summary of secondary water quality issues.

Secondary Water Quality Parameters (Comparison Criteria)								
Site	**pH (<6.5, >8.5)**	**TDS (500 mg/L)**	**Ferrous Iron (0.3 mg/L)**	**Manganese (0.05 mg/L)**	**Arsenic (0.01 mg/L)**	**Selenium (0.05 mg/L)**	**Acetone (5.5 mg/L) / 2-butanone (7.0 mg/L)**	**Notes**
Hangar K, CCAFS, FL	No impact	Not analyzed	Elevated up to 22 mg/L downgradient of reaction zone	Elevated up to 12 mg/L downgradient of reaction zone	No impact	No impact	No impact	Potential impacts only – not a drinking water supply
Area C, ATK, Elkton, MD	No impact	Not analyzed	Elevated up to 37 mg/L downgradient of reaction zone	Elevated up to 46 mg/L downgradient of reaction zone	No impact, only a few isolated detections above the criterion	Not analyzed	Not analyzed	Downgradient extraction system was shut down until substrate levels decreased. Downgradient concentrations of iron and manganese moderated after substrate depletion. Not a drinking water supply.
DP98, Elmendorf AFB, AK	No impact	Not analyzed	Elevated but background above criteria	Elevated up to 44 mg/L downgradient of reactions zone, but background above criteria	Background close to criteria and slightly elevated in treatment zone	No impact	No impact	Potential iron and manganese impacts, but background concentrations above criteria and not a drinking water supply
Kenney Avenue Plume, Elmendorf, AFB, AK	No impact	Not analyzed	Elevated but background above criteria	Elevated up to 32 mg/L downgradient of reactions zone, but background above criteria	No impact	No impact	No impact	Potential manganese impact, but not a drinking water supply
Contemporary Cleaners, Orlando, FL	Low pH, typically below 5.0 after injection	Not analyzed	Elevated but background above criteria	Not analyzed	Not analyzed	Not analyzed	Not analyzed	Potential impacts only – not a drinking water supply
Springdale Cleaners, Portland, OR	Not Reported	Not analyzed	Elevated but background above criteria	Elevated but background above criteria	Not analyzed	Not analyzed	Not analyzed	Potential impacts only – not a drinking water supply
East Gate Disposal Yard, Fort Lewis, WA	Low pH, typically below 6.0 after injection	Not analyzed	Elevated with concentrations > 3.3 in of reaction zone	Not analyzed	Not analyzed	Not analyzed	Not analyzed	Potential impacts only – not a drinking water supply
Site 1, Hanscom AFB, MA	Low pH – did not persist downgradient	Not analyzed	Elevated but background above criteria	Elevated but background above criteria	Not analyzed	Not analyzed	Not analyzed	Sulfide, biological oxygen demand, and chemical oxygen demand all elevated in treatment zone but impacts did not persist downgradient. Not a drinking water supply.
Area 20, Aerojet Facility, CA	No impact	Not analyzed	No impact	Elevated downgradient at 1 to 2 mg/L	No impact	No impact	Not analyzed	The sole groundwater impact appears to be the mobilization and persistence of low levels (1 to 2 mg/L) of dissolved manganese.
Building 301, Offutt AFB, NE	No impact	No impact	Elevated but background above criteria	Slightly elevated downgradient at 0.66 mg/L	No impact	No impact	No impact	Potential manganese impact, but appears to decrease with distance downgradient

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- Providing more a more uniform distribution of substrate without “spikes” of highly concentrated substrate solutions and adding a buffering amendment to control pH.
- Providing for an adequate redox recovery zone downgradient of the treatment zone.

In many cases providing a downgradient redox recovery zone is sufficient for impacts on secondary water quality to diminish. This is readily accomplished at many large DoD facilities, but may be more difficult to incorporate at small industrial or commercial sites.

7.1.5 Impacts on Hydraulic Conductivity

The hydraulic conductivity of the aquifer may be impacted by physical, chemical, or biological processes. For example, the growth of biomass or the presence of non-soluble substrate (e.g., vegetable oil) in the pore space of the aquifer matrix may significantly reduce hydraulic conductivity. Pre- and post-treatment of hydraulic conductivity (typically from slug tests) were evaluated to determine the degree to which hydraulic conductivity within the reaction zone may have been reduced. Because the hydraulic conductivity of most sediments range over several orders of magnitude, a reduction of less than 50% in the average hydraulic conductivity within the reaction zone is considered to be acceptable (Solutions IES, 2006). A reduction in hydraulic conductivity greater than 50% may potentially result in contaminant bypass, unexpected deviation in plume migration patterns, or non-uniform delivery of subsequent substrate injections.

A loss of hydraulic conductivity was observed for only a few sites, primarily based on data collected from single well slug tests. In general, the loss of hydraulic conductivity was 50% or less, and no adverse impact on system performance was observed. Potential impacts on hydraulic conductivity may be analyzed using tracer tests through the reaction zone before and after treatment. Tracer tests are often conducted to characterize groundwater flow, for example, flow through a biowall (Lu et al., 2008) or to optimize groundwater flow in a recirculation system (Cramer et al., 2004). Tracer studies may be repeated to determine whether substrate addition has had an adverse impact on groundwater flow through the reaction zone.

One way to mitigate the potential for loss of hydraulic conductivity is to conservatively design the reaction zone to extend beyond the limits of contaminated groundwater to be treated. For example, a biowall may be installed an additional 20 to 50 ft beyond the edge of the groundwater contaminant plume to avoid potential for bypass around the ends of the biowall.

7.1.6 Substrate Persistence and Long-Term Effectiveness

Substrate persistence and long-term effectiveness were evaluated using concentrations of dissolved substrate and contaminants over the design life of the application. The depletion of organic substrate may result in less effective degradation of the targeted contaminants, and when this is observed an attempt was made to determine an approximate “threshold” concentration of soluble organic carbon that must be maintained for that particular site. A rebound in contaminant concentrations may also occur after the remedy is halted. A rebound in contaminant concentrations of less than applicable regulatory standards, or less than 1.0% of the initial concentration before treatment, is considered a successful endpoint to the remedy.

Approximate threshold concentrations were estimated for the following sites:

- Hangar K, CCAFS, FL. Concentrations of chlorinated ethenes did not exhibit any rebound until TOC dropped below approximately 20 mg/L.
- DP98, Elmendorf AFB, AK. A rebound in TCE concentrations at downgradient wells occurred when TOC dropped from over 90 mg/L to less than 20 mg/L, suggesting that the threshold concentration to sustain dechlorination of TCE is between 20 and 90 mg/L.
- Kenney Avenue Plume, Elmendorf AFB, AK. Initial injections could only sustain concentrations of TOC at 30 to 40 mg/L, with little evidence of dechlorination of TCE. The use of a field-mixed emulsion resulted in concentrations of TOC greater than 100 mg/L being sustained over a 9-month period. During this period methanogenesis was induced and dechlorination of TCE to *cis*-DCE occurred.
- Contemporary Cleaners Site, FL. A rebound in concentrations of CAHs appears to correlate to total organic acid concentrations falling below 30 to 40 mg/L.
- Springdale Cleaners Site, OR. A threshold concentration of 80 to 100 mg/L total organic acids was interpreted to be required for effective dechlorination of CAHs.
- Area S, NWIRP McGregor, TX. The minimum range at which breakthrough of perchlorate occurred appeared to be between 5 and 10 mg/L.

Based on these observations, the minimum threshold concentrations of DOC or TOC for effective treatment range from 5 to 10 mg/L for perchlorate to 20 to 100 mg/L for CAHs. While the threshold concentration to sustain dechlorination of CAHs is site specific, sustaining concentrations of DOC greater than 100 mg/L should be effective for most sites. These minimum threshold concentrations should be used with caution, particularly where degradation of secondary water quality is a concern. For example, concentrations of DOC were limited to less than 50 mg/L to limit the production of soluble manganese and iron for a recirculation system treating perchlorate and chlorinated solvents (GeoSyntec, 2002).

For sites with extended monitoring data, a rebound in concentrations of CAHs or perchlorate was not usually observed. The potential for a rebound in concentrations depends primarily on the presence and persistence of an upgradient source.

Substrate longevity for the slow-release substrates was typically from 1.5 to 3.5 years (Table 4). Exceptions were noted, but longevity less than 1.5 years could be attributed to poor initial distribution of substrate. For the molasses sites, it was noted that dechlorination continued and even improved at some locations after injection ceased. In many cases, decaying biomass may act as a secondary substrate and sustain anaerobic degradation processes for a period of months to perhaps a year or more. The improved performance at the molasses sites (i.e., dechlorination of *cis*-DCE and VC) may be due to moderation of pH as substrate was slowly depleted.

7.1.7 Need for and Cost of Additional Injections or Monitoring

The cost associated with poor performance or compliance issues may significantly increase the life-cycle costs of full-scale enhanced in situ bioremediation applications. For example, poor performance typically results in a need for longer term operation. Compliance issues such as degradation of secondary water quality may require additional monitoring or system modifications, also increasing the cost of the application. Actual work performed for the demonstration studies was compared to the application design or work plan to determine whether additional work was required.

Sites where additional injections were performed or where additional monitoring events were necessary beyond those planned for in the initial design include the following:

- SA-17, NTC Orlando, FL. Recirculation of EVO was less effective in certain areas because rates of groundwater extraction and injection were lower than estimated from aquifer tests (Hudson et al., 2009). Additional testing was conducted and optimization efforts included conducting oil retention tests, targeting the most permeable zones, increasing injection volumes, and using direct-push injection techniques.
- Kenney Avenue Plume, Elmendorf AFB, AK. Greater quantities of substrate and a switch to a field-mixed emulsion were required to achieve appropriate reducing conditions. Additional substrate requirements increased from a proposed cost of $14,900 to a final cost of $62,600, a difference of $47,700.
- Site 1, Hanscom AFB, MA. A total of 32 weekly injections were planned, while a total of 47 injections were conducted over a 2-year period. Cost impacts occurred from increasing the injection frequency from weekly to twice weekly, increased time for each injection due to reduced rates of injection due to biofouling and compromise of the injection well seal, and the cost of additional substrate and buffering amendments.
- Site 35, Vandenberg AFB, CA. A total of 27 weekly injections was initially planned, while a total of 31 injections was conducted over a 27-month period. Additional substrate and a buffering amendment were also required.

Biowall replenishment was anticipated as part of long-term operations at Area S, NWIRP McGregor, TX. Approximately 15 biowall segments were replenished in 2006 and another six biowall segments replenished in 2008. These events occurred on average 4 to 5 years after biowall construction (EnSafe, Inc., 2008), emphasizing the fact that replenishment should be accounted for during selection and design of permeable mulch biowalls.

Several of the activities were anticipated or were conducted for research or optimization purposes and do not necessarily represent a cost impact. But these observations show that modifying the injection loading rate or adding a buffer is often needed to optimize performance. This results in additional monitoring or testing that may increase the cost of the remedy above initial projections.

7.1.8 Application in Difficult Hydrogeological Conditions

There are limits to the hydrogeological conditions under which enhanced in situ bioremediation may be applied effectively. A qualitative assessment was made to determine whether performance is related to adverse site conditions such as low permeability sediments, a high degree of heterogeneity, or high rates of groundwater flow. Demonstration sites that exhibited difficult hydrogeological conditions included the following:

- Variable rates of groundwater flow at Area C, ATK, MD. The highest rate of removal of perchlorate was observed during the first 4 months of operation when concentrations of TOC were high, and during a period between 2 and 3 years post-injection when groundwater flow velocity slowed due to shutdown of a downgradient groundwater extraction system. When the groundwater extraction system was in operation, the contact time in the reaction zone was less than anticipated and resulted in a drop in perchlorate removal efficiency.
- Low yield and heterogeneity at the SA-17 Site at NTC Orlando, FL. Low yield and heterogeneity limited the effectiveness of a recirculation approach at this site, resulting in non-uniform substrate distribution.
- High rate of groundwater flow (>800 ft/yr) at the Kenney Avenue Plume, Elmendorf AFB, AK. The ability to induce highly reducing conditions was limited by high rates of groundwater flow that dispersed the substrate over a broad area at less than suitable concentrations. The rate of groundwater flow is close to an upper limit suitable for applying enhanced in situ bioremediation. The rate of groundwater flow was mitigated by use of a coarse field-mixed emulsion that was better retained within the aquifer matrix.
- Low rates of groundwater flow at the Contemporary Cleaners Site in FL. Variable and non-uniform distribution of substrate is attributed to aquifer heterogeneity and slow rates of groundwater flow (less than 16 ft/yr) that limited dispersion of organic acids released from the HRC® product.
- A moderate degree of heterogeneity at the East Gate Disposal Yard, Fort Lewis Logistics Center, WA, and at the Building 1419 Site at Indian Head NSWC, MD. Injection and extraction wells had to be re-installed to achieve uniform substrate distribution at the East Gate Disposal Yard Site. For the Building 1419 Site, substrate distribution improved over time as substrate dispersed and diffused into lower permeability sediments.

Based on these observations, rates of groundwater flow less than 0.1 ft/day (37 ft/yr) or greater than 2.7 ft/day (1,000 ft/yr) require special design considerations. Low rates of groundwater flow may require closer injection spacing, while high rates of groundwater flow will require more frequent and higher concentration injections. As the degree of aquifer heterogeneity increases, so may the need for closer injection well spacing or for “targeted” injections within lower permeability sediments. In the case of EVO products, the retention of the oil droplets is a critical parameter to sustain adequate substrate concentrations in the reaction zone.

7.2 SUMMARY OF LIMITING FACTORS FOR ENHANCED IN SITU BIOREMEDIATION

A number of limiting factors commonly impact the effectiveness of enhanced in situ bioremediation applications. These limiting factors and the best practices to mitigate them include the following:

Insufficient Substrate Distribution. The ability to effectively distribute substrate is often impacted by site-specific lithology (low or high permeability, heterogeneity) and groundwater hydraulics (low or high rates of groundwater flow). In some cases the quantity of substrate that can be injected is limited by a low aquifer buffering capacity and pH excursion. These observations reinforce the need and benefits of conducting adequate site characterization prior to design and implementation of substrate addition. In most cases these conditions can be mitigated by modifying the injection mixture and substrate loading rate (e.g., more frequent and less concentrated substrate solutions, or adding a buffering amendment) or delivery technique (e.g., closer spaced injection points and larger injection volumes).

Adverse Geochemical Conditions. The most common geochemical problem for the demonstration case studies was an adverse excursion (lowering) of pH, resulting from a combination of low buffering capacity of the aquifer and high concentrations of DOC. Control of the substrate loading rate is critical when treating aquifers with low buffering capacity. Mitigation measures include careful screening of the site to determine whether a buffering compound should be added to the injection protocol, and selecting substrate delivery techniques that provide for more uniform distribution of substrate without excessive “spikes” in DOC.

Loss of Hydraulic Conductivity or Biofouling of Injection Wells. A decrease in hydraulic conductivity (permeability) may result in bypass of contaminated groundwater around the reaction zone or uneven distribution of substrate during subsequent injections. One way to address the potential for loss of hydraulic conductivity is to conservatively design the reaction zone to extend beyond the limits of contaminated groundwater to be treated. For example, a biobarrier or biowall may be installed an additional 20 to 50 ft beyond the edge of the groundwater contaminant plume to avoid potential for bypass around the ends of the reaction zone. It may also be beneficial to provide a degree of overlap (perhaps 20 to 30%) for injection well radius of influence to compensate for reductions in the ability to distribute substrate during subsequent injections.

Substrate Persistence and Longevity. Concentrations of DOC typically need to be sustained above 50 to 100 mg/L for effective treatment of CAHs over the design life of the application. Buildup of biomass may sustain the reaction zone and limit the amount of rebound that may occur after the initial substrate is depleted. Rebound of concentrations in the treatment zone will depend in large part on whether a residual source of contaminant mass remains upgradient of the treatment zone, or in low permeability sediments within the treatment zone.

Difficult Hydrogeological Conditions. Rates of groundwater flow less than 0.1 ft/day (37 ft/yr) or greater than 2.7 ft/day (1000 ft/yr) require special design considerations. Low rates of groundwater flow may require closer injection point spacing, while high rates of groundwater flow will require more frequent and higher concentration injections. In the case of EVO products, the retention of the oil droplets is a critical parameter to sustain adequate substrate concentrations in the reaction zone. Similarly, as the degree of aquifer heterogeneity increases, so may the need for closer injection well spacing or for “targeted” injections within lower permeability sediments.

Development of a CSM and an understanding of the natural processes that are being stimulated is useful to guide the site selection and design process (AFCEE et al., 2004). This should include an assessment of site-specific data on native electron donors and electron acceptors, metabolic byproducts, geochemical parameters, contaminant trends, and hydrogeology. A CSM also summarizes the fate and transport of contaminants, migration pathways, exposure mechanisms, and potential receptors. Therefore, a CSM provides important information to identify and mitigate the limiting factors described above.

The variety of substrates and configurations that can be used for enhanced in situ bioremediation allows the practitioner to design around these limiting factors. Careful site screening and evaluation of each of these limiting factors will lead to higher rates of success and greater effectiveness of the remedy.

8.0 DESIGN OF SUBSTRATE AMENDMENTS

8.1 DETERMINING SUBSTRATE REQUIREMENTS

A spreadsheet tool has been developed to assist the practitioner in determining site-specific electron acceptor demand and to estimate the substrate required to meet that demand over the design life of the application. **This tool to evaluate substrate requirements is not intended to be used as a design tool; rather it is intended only for the purpose of site screening and to evaluate the scientific basis of determining electron acceptor demand and substrate requirements.**

Several providers of organic substrates for enhanced in situ bioremediation provide design tools using similar calculations as the substrate estimating tool. The calculations and assumptions used are not always readily apparent in these design tools. The substrate estimating tool provides information on the reactions, calculations, and assumptions employed in an effort to educate the user on how an estimate of the substrate requirement is determined for a specific site. It is not intended to replace or be used in lieu of a vendor's proprietary design tool.

The technical basis of the substrate estimating tool is described in Appendix B. Substrate loading comparisons for the demonstration sites were conducted (Appendix C of the *Final Technology Demonstration Report*), and summary results are listed in Table 7. The following is a summary of how the tool is useful for site screening and evaluation of enhanced bioremediation designs.

The substrate estimating tool was used to evaluate the electron acceptor demand for each case study. The electron acceptor demand for individual electron accepting processes (assuming they all go to completion) ranged in percent of the total demand as follows:

- Aerobic Respiration: 0.1 to 12.9%, but typically 2% or less
- Nitrate Reduction: <0.1 to 37.4%, but typically 3% or less
- Manganese Reduction: <0.1 to 16.7%
- Iron Reduction: <0.1 to 26.4%
- Sulfate Reduction: 5.6 to 82.7%
- Methanogenesis: 0.2 to 66.7%, but typically greater than 10%
- Contaminant Reduction (CAHs or perchlorate): <0.1% to 75.8%.

The variability in these percentages reflects the wide range of site conditions that may be encountered. Sulfate reduction and methanogenesis have the greatest potential to dominate electron acceptor demand and to increase substrate requirements. This is due to the magnitude of sulfate concentrations that may occur (up to several thousand mg/L), and to the high utilization rate of hydrogen by methanogenesis (1.99 weight of carbon dioxide produced per weight of hydrogen, for example, compared to 11.91 weight of sulfate reduced per weight of hydrogen). In source areas, the electron acceptor demand from CAHs may predominate.

Substrate estimates using the substrate estimating tool with a design factor of one times the electron acceptor demand over the design life of each case study were compared to the total amount of substrate applied in practice to calculate an overall design factor. Design factors ranged from approximately one times the electron acceptor demand to 21 times the electron acceptor demand, a considerable range. A more common range from three to 10 times the estimated electron acceptor demand was observed for six of 11 case studies.

The highest design factor was applied in an early application of neat vegetable oil in 2000 for a potential DNAPL source area at the Hangar K Site at CCAFS, FL (Parsons, 2007). While the use of neat vegetable became less common once emulsified vegetable oil products were available, this case study illustrates that very high substrate loading rates may be considered for DNAPL source area applications. This approach may be beneficial by enhancing the mass transfer of CAHs from a DNAPL or sorbed phase to the dissolved phase where they may be degraded by microbial processes (Macbeth and Sorenson, 2008).

8.2 SUBSTRATE LOADING RATES

8.2.1 Using the Substrate Estimating Tool

The substrate estimating tool is useful to screen site conditions that will impact substrate delivery and utilization. The tool provides an estimate of ***total*** substrate required over the design life of the application given a user-specified design factor. The tool calculates a time-weighted average concentration of substrate by dividing the total volume of groundwater treated by the total substrate quantity.

The substrate estimating tool is also useful to understand how the substrate will be utilized and to screen for potential adverse geochemical conditions. For example, high manganese and iron sites may require monitoring to ensure that secondary water quality is not impacted downgradient of the treatment zone. Alkalinity and pH are included to screen for low buffering capacity.

The quantities and time-weighted average substrate concentrations can be used for comparison to proposed or planned bioremediation applications as a check on the quantities of substrate being proposed or the performance targets for TOC or DOC. This should help avoid application of either too little substrate or generating excessive substrate levels.

While the substrate estimating tool provides a first approximation of total substrate required, it does not provide for any guidance or indication on how the substrates should be applied. Design tools are often provided by substrate vendors, and the estimated substrate quantity should always be compared to recommendations by the provider or with case studies in the literature. Design tools that assist the practitioner with the configuration (well spacing) and injection volumes are being developed and should be incorporated into the design process. Examples include the Edible Oil Substrate tool being developed under ESTCP Project ER-0626 (Borden et al., 2008).

Table 7. Summary of substrate loading calculations.

Site	Design Life (years)	Volume Treated (gallons)	Hydrogen Requirement (lb/gal)	Demand from CAHs/ Perchlorate (percent)	Demand from Aerobic Respiration (percent)	Demand from Nitrate Reduction (Percent)	Demand from Manganese Reduction (percent)	Demand from Iron Reduction (percent)	Demand from Sulfate Reduction (percent)	Demand from Methanogenesis (percent)	Estimated Design Factor	Notes
Hangar K, CCAFS, FL	5	154,877	5.00E-04	75.8	0.1	<0.1	0.9	0.8	5.6	16.8	21	Potential DNAPL source area
Area C, ATK, Elkton, MD	3	814,453	6.62E-05	6.7 (CAHs) 8.8 (Perchlorate)	4.3	11.7	6.9	6.8	29.3	24.4	1+	Higher than anticipated rate of groundwater flow
SA-17, NTC Orlando, FL (Upper Zone/Lower Zone)	5	95,685/ 138,971	2.66E-04/ 2.67E-04	9.9/ 7.5	2.0/ 2.0	3.1/ 3.0	0.6/ 0.6	2.8/ 5.6	56.9/ 65.6	15.8/ 15.7	10/ 7.2	High iron and sulfate site
DP98, Elmendorf AFB, AK	3	78,673	7.32E-05	8.7	1.4	0.1	16.7	6.2	9.6	57.3	14	High iron, high manganese site
Kenney Avenue Plume, Elmendorf AFB, AK	2	3,225,640	3.25E-05	<0.1	1.5	0.5	14.4	0.8	32.4	50.4	20	High manganese, low CAHs, high rate of groundwater flow
Contemporary Cleaners, Orlando, FL (Upper Zone/Lower Zone)	2	794,087/ 10,388	1.04E-04/ 9.43E-05	18.2/ 11.3	2.0/ 1.1	1.2/ 1.3	1.5/ 1.6	2.9/ 3.2	13.5/ 14.9	60.7/ 66.7	4.7/ 9.4	High production of methane, low pH site
Springdale Cleaners Site, Portland, OR (Dissolved-HRC$^{®}$/ Source-HRC-X™)	1.5/ 3.0	151,859/ 102,229	9.92E-05/ 1.57E-04	11.5/ 44.0	1.1/ 0.7	0.1/ 0.1	3.1/ 1.0	11.7/ 8.4	30.4/ 19.2	42.3/ 26.7	7.5/ 2.6	Substrate persisted at target levels over intended design life
East Gate Disposal Yard, Fort Lewis, WA	0.67	484,497	6.03E-05	31.4	2.1	1.5	0.5	0.7	22.1	41.7	NA	High dosage rates were used to enhance DNAPL dissolution
Site 1, Hanscom AFB, MA	2	345,399	3.52E-05	6.3	1.8	<0.1	1.0	26.4	52.5	11.9	2.9	High iron and sulfate site
Site 35, Vandenberg AFB, CA	2	359,360	2.18E-04	0.3	1.4	3.0	0.1	1.4	82.7	11.2	3.0	High sulfate site
Area 20, Aerojet Facility, CA	1	8,349,912	3.27E-05	3.6/ 14.7	12.9	37.4	1.9	0.5	27.9	1.3	3.0	A 1-year design life was used for estimating substrate requirements
Building 1419, Indian Head NWS, MD	0.3	55,690	2.43E-04	48.4 (perchlorate)	0.6	0.7	<0.1	<0.1	50.1	0.2	1.7	Dosing rates were selected to limit sulfate reduction and methanogenesis
Building 301, Offutt AFB, NE	5	4,196,467	4.51E-05	0.9	1.2	4.5	3.4	1.7	41.9	46.4	NA	Mulch substrate

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8.2.2 Recommendations for Design of Substrate Loading Rates

The following recommendations are based on observations from the case studies, including 1) calculation of design factors using the substrate estimating tool, 2) evaluation of overall system performance, and 3) evaluation of limiting factors (Section 7). For slow release substrates injected in a one-time event, a conservative design factor on the order of three to seven times the estimated substrate requirement should be suitable for limiting the potential for insufficient substrate. For soluble substrates, lower design factors on the order of two to three times the estimated substrate requirement are beneficial to avoid over stimulating the aquifer and driving down pH. Substrate quantities can be increased if initial loading rates are insufficient to create suitable reducing conditions throughout the treatment zone. The delivery methods for soluble substrates should target uniform substrate concentrations without excessive “spikes” in concentration.

As mentioned previously, the use of very high substrate concentrations to enhanced dissolution of DNAPL into the aqueous phase represents an exception to typical substrate loading rates. These applications require special consideration of the buffering capacity of the aquifer and the system configuration. For example, it may be acceptable to induce adverse geochemical conditions in the source zone to mobilize CAH mass if a suitable downgradient reaction zone for geochemical recovery is established.

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9.0 TEMPERATURE, PH, AND BUFFERING AMENDMENTS

Several factors affect the rate at which biological and chemical reactions occur in the subsurface, including temperature and pH. Temperature and pH have an impact on the growth and activity of dechlorinating bacteria, particularly *Dehalococcoides* species. Control of or maintaining pH is a primary geochemical objective for enhanced anaerobic degradation processes.

9.1 IMPACTS OF TEMPERATURE

Most biological and chemical reactions that occur in the subsurface are time-dependent or kinetic reactions. For example, groundwater temperatures may have a significant impact on the rate of growth of microbial species in the subsurface. This may affect both the rate of utilization (e.g., fermentation) of substrate and the rate of activity and growth of dechlorinating bacteria such as *Dehalococcoides* species.

The impact of temperature on native dechlorinating species is often overlooked in bioremediation studies, particularly for *Dehalococcoides* species (Friis et al., 2007). Bradley et al. (2005) report that TCE was dechlorinated to cis-DCE and VC in microcosms constructed with soil and groundwater collected from two sites in Alaska. The microcosms were incubated at 4°C and spiked with radio-labeled (carbon 14) TCE, *cis*-DCE, and VC. However, dechlorination to ethene or ethane was not observed, and reductions in *cis*-DCE and VC (ranging from 25 to 70%) were attributed to anaerobic oxidation based on an accumulation of radio-labeled carbon dioxide.

Bradley et al. (2005) concluded that assumptions regarding low to insignificant microbial activity at water temperatures below 5°C do not consider the presence of cold-adapted (psychrotolerant and psychrophilic) microorganisms. However, it is not clear from this study which microorganisms are facilitating the dechlorination reactions and whether anaerobic oxidation will occur at rates sufficient to limit the accumulation of *cis*-DCE and VC.

Data collected at the DP98 Site (USAF, 2007) and the Kenney Avenue Plume (USAF, 2009) at Elmendorf AFB, AK, showed near molar conversion of TCE to *cis*-DCE, with limited dechlorination to VC. Evidence of further dechlorination to ethene or ethane was not observed. Data collected under this demonstration (Parsons, 2009) indicate that the growth of native *Dehalococcoides* species was limited (if it occurred at all) under ambient groundwater conditions of 7 to 8°C. A microcosm study (GeoSyntec, 2007) for the Kenney Avenue Plume Treatability Study further indicated that the growth of a *Dehalococcoides* mixed bioaugmentation culture (KB-1®) could not be sustained at a laboratory controlled temperature of 10°C.

In 2002, the KB-1® bioaugmentation culture was used to bioaugment a cold temperature site contaminated with PCE at the River Terrace Site in Soldotna, AK (Oasis Environmental, 2006). Prior to bioaugmentation, PCE was converted through TCE and stalled at *cis*-DCE. VC concentrations peaked 1 to 2 years after bioaugmentation, and some ethene production was observed approximately 2.5 years after bioaugmentation. This suggests that while *Dehalococcoides* may be able to grow at low groundwater temperatures, the rate of growth and dechlorination activity will be slow. These studies indicate that dechlorination of TCE to *cis*-DCE and VC may be stimulated in cold water environments. However, the ability to stimulate the growth of native *Dehalococcoides* species to further dechlorinate *cis*-DCE and VC to ethene appears to be a significant limitation.

With the exception of Alaska, groundwater temperatures in the United States are typically above 10°C and temperature is generally not an issue for most DoD sites. However, the impact of temperature may need to be considered for application of enhanced anaerobic bioremediation in northern latitude countries or at high altitude sites.

9.2 IMPACTS OF PH

Anaerobic biodegradation processes are primarily acid producing, and the lowering of pH may result in slow or incomplete dechlorination of chlorinated solvents. Acidification caused by addition of an organic substrate has been observed to inhibit dechlorination in laboratory studies (Cirpka, et al., 1999; Carr and Hughes, 1998; Aulenta et al., 2006; McCarty et al., 2007) and in field studies (Volkering and Pils, 2004). Processes that affect pH during enhanced bioremediation include the following:

- Biological reductive dechlorination produces acidity. During biological reductive dechlorination, molecular hydrogen (H_2) is utilized as an electron donor where a chloride ion (Cl^-) is replaced by a hydrogen ion (H^+). This results in release of both a chloride ion and a hydrogen ion into solution. The increase in hydrogen ion lowers pH.
- Fermentation of organic substrates produce metabolic acids (e.g., butyric, propionic, and acetic), which further degrade to form carbonic acid (H_2CO_3). The production of carbon dioxide (CO_2) gas further depresses the pH equilibrium.
- Terminal electron accepting processes such as sulfate reduction produce alkalinity by production of hydroxide (OH^-).

The ability of the aquifer matrix to buffer the addition of acids produced during enhanced bioremediation is dependent on 1) concentrations of inorganic species in groundwater that potentially neutralize acid (primarily alkalinity), 2) concentrations of aquifer minerals that dissolve to neutralize acid added by biological processes, and 3) proton exchange with charged clay particles. Groundwater pH is strongly controlled by dissolved inorganic carbon, particularly the equilibrium of carbonate species. A source of carbonate minerals in the aquifer matrix may help to further buffer pH.

Dechlorinating species such as *Dehalococcoides* are selective in regard to the range of pH at which they are active. In a literature review of several dechlorinating species (*Dehalobactor restrictus, Dehalospirillum multivorans, Desulfitobacterium,* and *Desulfuromonas chloroethenica*), Middeldorp et al. (1999) report that the optimum range of pH for dechlorinating activity was from 6.8 to 7.8 standard pH units.

Zhuang and Pavlostathis (1995) evaluated the effect of temperature, pH, and electron donor on microbial reductive dechlorination of PCE using acetate-fed methanogenic cultures developed from a contaminated field site. They evaluated PCE dechlorination at pH of 4, 6, 7, 8, and 9.5, and reported that optimum conditions for reductive dechlorination were achieved at a temperature of 35°C and a pH of 7. The rate of dechlorination of PCE dramatically declined below a pH of 6 and above a pH of 8. In addition, production of VC was observed at a pH of 7, but not at a pH of 6 or lower or at a pH of 8 or higher. This resulted in an accumulation of *cis*-DCE at the lower and higher pH ranges.

Shaw Environmental, Inc. (Vainberg et al., 2006; Steffan et al., 2008) conducted a laboratory microcosm study to evaluate the activity of the SDC-9 *Dehalococcoides* mixed culture over a range of pH values. The rate of dechlorination of PCE for this culture drops off dramatically below a pH of approximately 6.0, or above a pH of approximately 7.5. Dechlorination of *cis*-DCE and VC by *Dehalococcoides* may be even more sensitive to pH excursion (Christ et al., 2005). For example, Rosner et al. (1997) investigated the effect of pH on vinyl chloride dechlorination by a mixed anaerobic culture derived from a site in Victoria, TX. The optimum pH for VC dechlorination was 8.5 with only 1% relative activity at a pH of 5.0, 50% relative activity at a pH of 7.0, and 50% relative activity at a pH of 10.0.

9.3 PH AND ALKALINITY IN AQUIFER SYSTEMS

The reactivity of the hydrogen ion (H^+) is an important variable in groundwater geochemistry because the hydrogen ion participates in most of the chemical reactions that affect water composition. The measured pH does not by itself provide any information on the capacity of the aquifer system to maintain (buffer) pH as an acid or base is added by biological or chemical processes. Inorganic carbon species are often the dominant anion in groundwater systems, and they can take up or release hydrogen ions as part of their speciation reactions. Therefore, they provide much of the buffering capacity in natural groundwater systems. The strongest buffering occurs when concentrations of the dissolved inorganic carbon (DIC) constituents is high. The most common measure of carbonate and bicarbonate in groundwater is alkalinity. Therefore, alkalinity is a key groundwater parameter in evaluating the pH and buffering capacity of groundwater.

In the subsurface, the acid neutralizing capacity of the system must also consider the minerals in the aquifer matrix that may also react with acid added to the system, which causes weathering of the minerals. Weathering of silicate minerals is a relatively slow process compared to carbonate minerals, so in most cases the acid neutralizing potential of the aquifer matrix is due to the carbonate minerals present. Therefore, characterization of both groundwater and the aquifer matrix is required when evaluating the potential for buffering of pH from the acid-producing processes of enhanced in situ bioremediation.

9.4 DESIGN TOOLS FOR DETERMINING BUFFERING REQUIREMENTS

Two design tools have been recently developed for evaluating buffering requirements to maintain pH at optimal levels for anaerobic dechlorination of chlorinated solvents. The first tool, BUCHLORAC (BUffering of deCHLORination ACidity), has been developed by the Source Area BioREmediation (SABRE) project. The second tool is being developed by EOS Remediation and North Carolina State University under the direction of Dr. Robert Borden. The EOS design tool is based on an Excel spreadsheet and is used to determine the amount of a substrate/buffering product (AquaBuf*pH*™) to apply based on site-specific conditions.

The two tools differ in the input parameters required to determine buffering requirements. In general, the BUCHLORAC model uses speciation of anions and cations in groundwater and the amount of carbonate and iron oxide minerals in the aquifer matrix as input to a geochemical equilibrium model, while the EOS tool uses direct measurements of soil and groundwater acidity as input to the spreadsheet tool.

9.4.1 BUCHLORAC Model

BUCHLORAC is a geochemical model program in the public domain developed by the SABRE project team (Robinson and Barry, 2009a, 2009b; Robinson et al., 2009). The model was initially implemented through the geochemical program PHREEQC, with modifications to estimate the amount of acid that is produced by anaerobic dechlorination of chlorinated ethenes and biodegradation of organic substrates. After studying the results of detailed modeling exercises, the developers have released a simplified version of the model for preliminary estimates of bicarbonate buffering requirements.

In essence, the BUCHLORAC model is designed to predict the amount of bicarbonate required to maintain a suitable (minimum) pH for dechlorinating bacteria. The model accounts for the amount of chlorinated compounds degraded, site groundwater chemistry, type of substrate applied, alternative terminal electron accepting processes, gas (carbon dioxide) release, and soil mineralogy. The developers indicate that bicarbonate requirements are strongly dependent on the substrate (electron donor) used and the availability of native electron acceptors (particularly ferric iron and sulfate). As the program has only recently been released, there is currently a lack of case studies to document how effective the model is at estimating appropriate buffering quantities.

9.4.2 EOS Design Tool for AquaBuf*pH*™

The EOS spreadsheet model is not in the public domain but is available for use or review by contacting EOS Remediation (www.eosremediation.com). Input parameters for the EOS design tool include the acidity of the aquifer matrix (sediment) and the acidity of the groundwater. Other input parameters include hydraulic properties, size of the treatment zone, concentrations of dissolved chlorinated compounds, and concentrations of common native electron acceptors.

The primary difference between the EOS design tool and the BUCHLORAC model is that the EOS design tool uses values of soil and groundwater acidity instead of modeling the geochemical equilibrium of anions/cations in groundwater and with the aquifer matrix. This is a more simplistic approach, although data for groundwater and soil acidity must still be collected. The tool is limited to the application of the vendors AquaBuf*pH*™ product. The buffering requirements are listed in OH^- equivalents, and the product uses magnesium hydroxide ($Mg(OH)_2$) as the primary buffering compound.

9.5 EVALUATING PH AND BUFFERING REQUIREMENTS

The minimum data that should be collected at a bioremediation site to evaluate pH and the buffering capacity of an aquifer system includes groundwater pH, alkalinity, and acidity; and soil pH and acidity. Other useful parameters may include DIC for groundwater and cation exchange capacity (CEC) for soil. Table 8 and Table 9 list soil and groundwater geochemical parameters and analytical methods that are recommended for evaluating pH and buffering requirements.

Recommended soil analyses include pH, soil acidity, and major cations and anions. Neutralization potential and CEC are optional analyses that may be used to determine the amount of buffering compound required. Recommended groundwater analyses include temperature, pH, alkalinity, acidity, and major anions and cations. These data are useful to evaluate the primary electron-accepting processes that will occur, and may be used for geochemical modeling (BUCHLORAC model).

Table 8. Soil analytical protocol for evaluating pH and buffering requirements.

Analyte	Example Methods	Data Use	Recommendations
Soil			
pH	USEPA SW9045	Measurement of natural soil pH	Recommended
Soil acidity	SM2310	Can be used to calculate amount of buffering agent required to neutralize soil acidity.	Recommended
Major Anions – Cl^-, NO_3^-, CO_3^{-2}, HCO_3 , and SO_4^{-2}	USEPA SW9056 or E300 series	Used for geochemical modeling of buffering requirements (BUCHLORAC model)	Recommended
Major cations—Ca^{+2}, Fe^{+2}, Fe^{+3}, K^+, Mg^{+2}, Mn^{+2}, and Na^+	USEPA SW6010B	Used for geochemical modeling of buffering requirements (BUCHLORAC model)	Recommended
Soil mineralogy (calcite, gypsum, goethite, ferrihydrite)	Laboratory-specific standard operating procedures (SOP) such as x-ray diffraction	Useful for geochemical modeling or to evaluate potential buffering capacity of site sediments.	Optional
Neutralization potential	Laboratory-specific SOP	Measurement of buffering capacity of sediments	Optional method
CEC	Agricultural methods – Laboratory-specific SOP	Indication of potential buffering capacity of sediments	Optional method

Table 9. Groundwater analytical protocol for evaluating pH and buffering requirements.

Analyte	Example Methods	Data Use	Recommendations
Groundwater			
Temperature	Direct-reading meter	Qualitative evaluation of substrate utilization rate	Recommended
pH	Direct-reading meter; SM4500B or Hach Method 8156	Indication of suitability of site groundwater to support dechlorination reactions	Recommended
Alkalinity	EPA 151.1, SM2320B, or Hach Method 8203 or 8221	Indication of potential buffering capacity of native groundwater	Recommended

Table 9. Groundwater analytical protocol for evaluating pH and buffering requirements (continued).

Analyte	Example Methods	Data Use	Recommendations
Acidity	SM2310B, Hach Method 8010, Hach Method 8201, or Hach Method 8202	Indication of amount of buffer needed to neutralize pH	Recommended
Anion suite—Cl^-, NO_3^-, NO_2^-, PO_4^{-3}, HS^-, S^-, and SO_4^{-2}	USEPA E300 series, E365.3, or SM4500 for PO_4^{-3}	Used for geochemical modeling of buffering requirements (BUCHLORAC model)	Recommended for major anions (Cl^-, NO_3^-, NO_2^-, and SO_4^{-2})
Cation suite—Ag^+, Al^{+3}, Ca^{+2}, Cd^{+2}, Cu^{+2}, Fe^{+2}, Fe^{+3}, K^+, Mg^{+2}, Mn^{+2}, NH_4^+, Na^+, Ni^{+2}, Pb^{+2}, and Zn^{+2}	USEPA SW6010B, SM4500 for NH_4^+	Used for geochemical modeling of buffering requirements (BUCHLORAC model)	Recommended for major cations (Ca^{+2}, Fe^{+2}, Fe^{+3}, K^+, Mg^{+2}, Mn^{+2}, NH_4^+, and Na^+)
Dissolved inorganic carbon	Laboratory SOP	Indication of potential buffering capacity of native groundwater	Optional

9.6 CASE STUDY EVALUATIONS OF PH

Of the 15 case studies, six sites exhibited adverse pH excursion that impacted performance (Table 10), with three sites requiring modifications to account for adverse pH excursions. For all these sites, initial background pH values were typically below 6.5 and alkalinity was below 150 mg/L. For screening purposes, a combination of pH below 6.0 to 6.5 and alkalinity below 300 mg/L should indicate that modifications to buffer and control pH excursion will be necessary.

Table 10. Summary of adverse pH impacts on case study sites.

Site Identification	Background pH	Background Alkalinity (mg/L)	Reaction Zone pH	Impacts on Performance	pH or Buffering Amendment Strategy
SA-17, NTC Orlando, FL (EVO)	5.4 to 6.3	8.6 to 111	NA	Low pH was due in large part to a previous chemical oxidation application.	Follow-up injections planned using a buffered EVO product.
Contemporary Cleaners, Orlando, FL (HRC®)	5.0 to 6.0	NA	4.15 to 5.97 (Typically below 5.0)	Accumulation of *cis*-DCE in the lower surficial aquifer was attributed to competition from methanogenesis (Kean et al., 2003). However, the impacts of low pH were not evaluated by the authors.	This site may have benefitted from use of amendments to control pH.

Table 10. Summary of adverse pH impacts on case study sites (continued).

Site Identification	Background pH	Background Alkalinity (mg/L)	Reaction Zone pH	Impacts on Performance	pH or Buffering Amendment Strategy
East Gate Disposal Yard, Fort Lewis, WA (Whey)	6.1 to 6.4	66 to 78	4.6 to 5.9	Initially limited dechlorination with accumulation of *cis*-DCE and VC. Eventually the aquifer was able to buffer the change in pH and complete dechlorination to ethene proceeded.	None. The slow dissolution of the aquifer matrix resulted in greater buffering capacity of groundwater over time, and pH was neutralized to the point that dechlorination proceeded to completion.
Site 1, Hanscom AFB, MA (Molasses)	5.8 to 6.3	Not analyzed during baseline sampling	Close to 4.0 in the injection well	Without a buffer amendment, the amount of substrate that could be injected was limited, which reduced the size of the effective treatment zone.	Sodium bicarbonate was added to the injection regimen.
Site 35, Vandenberg AFB, CA	6.2 to 6.6	96 to 143	4.3 to 5.6	Without a buffer amendment, the amount of substrate that could be injected was limited, which resulted in poor performance during initial injections.	Sodium bicarbonate was added to the injection regimen 20 months after initial injection.
Building 1419, Indian Head NWS, MD	3.8 to 6.0	<2.0 to 92	Buffered to above 7.0	It was known from microcosm studies that perchlorate reduction would not occur below a pH of 4.0.	The injection mixture was amended with a stock solution of sodium carbonate and sodium bicarbonate to maintain pH above 7.0 SU.

Sodium bicarbonate was the most common buffering compound used, typically at concentrations in excess of 10,000 mg/L. Sodium bicarbonate is a relative weak buffering compound and may be limited to applications using frequent injections of soluble substrates. The use of stronger and more persistent buffering compounds such as sodium carbonate and sodium phosphates may be necessary for some applications (e.g., Cramer et al., 2004 and Steffan et al., 2010). Further research and investigation into potential buffering compounds and buffer amendment strategies will be beneficial for sites with low buffering capacity.

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10.0 COST ASSESSMENT

10.1 ENHANCED IN SITU BIOREMEDIATION COST ASSESSMENT

Actual cost data for the demonstration sites were often not available. However, a qualitative assessment of cost impacts for the case studies was made by comparing the system design to actual operations. Table 11 identifies the causes of cost impacts for the case studies. The table identifies cases where additional costs are associated with 1) costs for additional substrate injections beyond that specified in project designs or work plan documents, 2) the need to modify the injection protocol to include additional substrate or for unanticipated amendments such as pH buffering compounds, and 3) extended monitoring beyond the system design life.

Table 11. Summary of modifications to case study applications.

Site/Facility	Additional Injections?	Injection Modifications?	Cost Impacts (actual or potential)
Vegetable Oil Substrates			
Hangar K, CCAFS, FL	No	None	None, although an extended monitoring event for research purposes was conducted in 2006.
Area C, ATK Facility, Elkton, MD	No	None	The costs associated with an optional extended monitoring period for research purposes were $12,000 for four additional sampling events ($48,000 total).
SA17, NTC Orlando, FL	Yes—direct-push injections to provide adequate substrate distribution	Use of buffered EVO product	Primarily associated with secondary injections.
DP98, Elmendorf AFB, AK	No	None	Enhanced in situ bioremediation may not be appropriate for this site.
Kenney Avenue Plume, Elmendorf AFB, AK	No, three injections were planned	Yes – greater quantities of substrate and a switch to a field-mixed emulsion were required to achieve reducing conditions.	Additional substrate requirements increased from a proposed cost of $14,900 to a final cost of $62,600, a difference of $47,700.
HRC® Products			
Contemporary Cleaners, Orlando, FL	No	None	It is likely that additional injections (including a buffering compound and/or bioaugmentation) would be needed to meet performance objectives.
Springdale Cleaners, Portland, OR	No	None	It is likely additional injections would be required to meet performance objectives.

Table 11. Summary of modifications to case study applications (continued).

Site/Facility	Additional Injections?	Injection Modifications?	Cost Impacts (actual or potential)
Whey			
East Gate Disposal Yard, Fort Lewis Logistics Center, WA	No	None	Other than having to re-install the extraction and injection wells, no significant modifications were required.
Molasses			
Site 1, Hanscom AFB, MA	Yes—32 weekly injections were planned, while a total of 47 injections were conducted over 2-year period	Yes—addition of buffering amendment and water chase	Increased time for each injection due to rates of injection that were reduced from approximately 10 gpm 1 to 2 gpm due to biofouling and compromise of the injection well seal.
Demonstration Site, Vandenberg AFB, CA	Yes—27 weekly injections were initially planned, while a total of 31 injections were conducted over a 27-month period	Yes—addition of buffering amendment and water chase	Additional monitoring associated with extended operations.
Ethanol			
Aerojet Facility, CA	No	None	Biofouling control would be required for long-term operations.
Lactate			
Test Area North, INEEL, ID	Required for optimization and long-term operation	Yes—for optimization purposes	Modifications were primarily for optimization of the system and do not represent a cost impact.
Building 1419, Indian Head NSWC, MD	No	None	The pilot test was conducted as planned.
Mulch Biowalls			
Area E, S, M, and F, NWIRP McGregor, TX	Yes—required to sustain biowall performance	No—additional injections have been performed as planned.	Biowall replenishment was anticipated as part of long-term operations and has been performed as anticipated.
Building 301, Offutt AFB, NE	No	No	Substrate replenishment may be beneficial in the near future.

For sites using vegetable oil substrates, the SA-17 Site and the Kenny Avenue Plume Site had operational cost impacts associated with additional injections or the need for greater quantities of substrate. For the two molasses sites, it was anticipated that the dosing rate and frequency would be adjusted based on field observations, and determining the optimal strength and frequency of reagent delivery was a primary objective of the demonstration. However, the number of injections and period of operation for both sites were significantly greater than planned.

It is anticipated that biowall and bioreactor applications may require substrate replenishment on the frequency of every 3 to 5 years (AFCEE, 2008). While this does not necessarily represent an unanticipated cost impact, these costs should be considered during technology screening and selection. An example of comparing the long-term cost of a biowall application relative to other enhanced in situ bioremediation techniques can be found in GSI (2008) and in Krug et al. (2009). Even if a biowall system is designed with replenishment in mind, the economics of long-term operation are typically favorable relative to many other in situ bioremediation techniques.

More difficult to evaluate than cost impacts alone is the impact of failing to meet performance objectives. Several of the case studies did not meet performance expectations, and follow-up with modified bioremediation techniques was not conducted. In the case of the Elmendorf AFB sites, it may simply be that enhanced in situ bioremediation is not an appropriate technology based on site-specific conditions. For other sites, it is unknown whether the technology applied could be modified to successfully meet performance objectives. The lack of successful initial results may lead to a lack of confidence in the technology, perhaps leading to more expensive alternative remedial technologies.

10.2 SITE-SPECIFIC COST ASSESSMENTS

Cost data were tracked to evaluate the differing approaches to implementing enhanced in situ anaerobic bioremediation (Appendix C of the *Final Technology Demonstration Report*). A typical cost model breaks down costs into elements for system design and engineering, installation, baseline characterization, waste disposal (if applicable), system operation, and long-term monitoring. Long-term monitoring may be significant, and often exceeds the capital cost of installation over the life of the application. For example, the cost to design, install, and conduct baseline characterization and to prepare a construction report for the Hangar K application at CCAFS totaled $97,000, while the cost to conduct six monitoring events over a period of approximately 5 years was approximately $144,000, greater than the initial cost to implement the remedy.

10.3 SUMMARY OF COST DRIVERS

Cost drivers identified for the demonstration case studies include costs for O&M, and costs for unanticipated injections and additional monitoring. More specifically, cost drivers for enhanced in situ bioremediation include the following (with examples):

- Cost for modifying frequent injections of soluble substrates, including more frequent injections than designed and changes to the substrate amendment (e.g., higher substrate quantities, buffering agents). Examples are sites at Hanscom AFB and Vandenberg AFB.
- Cost for additional injections of slow-release substrates, or for modification of the injection protocol. Examples include the SA-17 Site at NTC Orlando and the Kenney Avenue Plume at Elmendorf AFB.
- Cost to replenish permeable mulch biowalls with EVO (NWIRP McGregor).
- Cost associated with buffering or bioaugmentation for sites with incomplete dechlorination. Examples requiring addition of buffering compounds include applications at Hanscom AFB and Vandenberg AFB.

- Costs for biofouling control (well redevelopment, chemical treatments, and operations down time). An example is the Area 20 application at the Aerojet Facility.
- Cost for additional monitoring and reporting beyond the projected design.

The primary benefit of identifying these cost drivers is to limit or mitigate the potential for additional injections and monitoring during design and operations. For frequent injections of soluble substrate, it should be anticipated that modifications and additional injections may be needed, with contingencies built into the design. It is also useful to identify the buffering capacity of the aquifer to be treated. Incorporating a buffering amendment into the design for sites with low buffering capacity is a relatively inexpensive strategy to mitigate the need for more costly and difficult to implement modifications to control pH once treatment has been initiated. This reinforces the benefits of adequate site screening and using the best available practices when designing and implementing enhanced in situ bioremediation applications.

11.0 IMPLEMENTATION ISSUES

11.1 SITE CHARACTERIZATION FOR DESIGN OF ENHANCED IN SITU BIOREMEDIATION SYSTEMS

Prior to designing an enhanced in situ bioremediation system, thorough site characterization and screening are required to develop a strategy to mitigate any site-specific limiting factors. Characterization for adequate site screening should include the following:

- *Hydrogeology*. Quantify hydraulic conductivity and rate of groundwater flow, and evaluate the degree of heterogeneity. Sites with high or low rates of groundwater flow or with a moderate or high degree of heterogeneity require special consideration.
- *Contaminant Distribution*. The type, magnitude of concentration, and distribution of contaminants is necessary to determine an appropriate treatment configuration.
- *Distribution of Native Electron Acceptors*. The distribution of native electron acceptors directly correlates to substrate requirements. Sulfate often dominates the electron acceptor demand. In addition, it is difficult to predict the degree of iron and manganese reduction that may occur, and it is desirable to collect soil samples whenever possible for analysis of total and bioavailable iron and manganese.
- *Microbiology*. Many sites exhibit incomplete dechlorination which may be due to a lack of, or slow growth of, *Dehalococcoides* species. Molecular screening techniques with low detection limits are readily available to screen for the presence of *Dehalococcoides* and reductase enzymes that indicate the strain is capable of complete dechlorination.
- *pH and Alkalinity*. Both groundwater pH and alkalinity should be known. For sites with pH below 6.5 or alkalinity below 300 mg/L, soil samples should be collected for analysis of soil pH and some type of titration analysis be performed to estimate the buffering capacity of the aquifer. Alternately, groundwater geochemical data may be entered into a geochemical model (e.g., the BUCHLORAC model) to evaluate buffering requirements.

11.2 EVALUATION OF LIMITING FACTORS

An evaluation of limiting factors for the demonstration case studies reinforces the need for adequate site characterization. The most common limiting factors include the following:

Insufficient Substrate Distribution. The ability to effectively distribute substrate is often impacted by site-specific lithology (low or high permeability, heterogeneity) and groundwater hydraulics (low or high rates of groundwater flow). In some cases the quantity of substrate that can be injected is limited by a low aquifer buffering capacity and adverse lowering of pH. In most cases these conditions can be mitigated by modifying the injection mixture and substrate loading rate (for example, more frequent and less concentrated substrate solutions, or adding a buffering amendment) or selecting an appropriate delivery technique (for example, closer spaced injection points and larger injection volumes).

Adverse Geochemical Conditions. The most common geochemical condition was an adverse excursion (lowering) of pH, resulting from a combination of low buffering capacity of the aquifer and high concentrations of DOC. Control of the substrate loading rate is critical when treating aquifers with low buffering capacity. Mitigation includes careful screening to determine whether a buffering compound should be added to the injection protocol, and selecting substrate delivery techniques that provide for more uniform distribution of substrate without excessive "spikes" in DOC.

Loss of Hydraulic Conductivity or Biofouling of Injection Wells. A decrease in hydraulic conductivity (permeability) may result in bypass of contaminated groundwater around the reaction zone or uneven distribution of substrate during subsequent injections. One option to mitigate the potential for loss of hydraulic conductivity is to conservatively design the reaction zone to extend beyond the limits of contaminated groundwater to be treated.

Substrate Persistence and Longevity. Based on observations from the demonstration case studies, the minimum threshold concentrations of DOC range from 5 to 10 mg/L for perchlorate to 20 to 100 mg/L for CAHs. While the threshold concentrations are highly site-specific, sustaining concentrations of DOC greater than 50 to 100 mg/L should be effective for most CAH sites. Concentrations of DOC to sustain perchlorate reduction are typically lower because sulfate reducing and methanogenic conditions are not necessary (or desirable). Buildup of biomass may sustain the reaction zone and limit the amount of rebound that may occur after the initial substrate is depleted. Rebound of concentrations in the treatment zone will depend on whether a residual source of contaminant mass remains upgradient of the treatment zone or with low permeability sediments within the treatment zone.

Difficult Hydrogeological Conditions. Rates of groundwater flow less than 0.1 ft/day (37 ft/yr) or greater than 2.7 ft/day (1000 ft/yr) require special design considerations. Low rates of groundwater flow may require closer injection point spacing because distribution of soluble substrate or organic acids from slow release substrates by dispersion will be limited. High rates of groundwater flow will require more frequent and higher concentration injections. As the degree of aquifer heterogeneity increases, so may the need for closer injection well spacing or for "targeted" injections within lower permeability sediments.

The variety of substrates and configurations that can be used for enhanced in situ bioremediation allows the practitioner to design around these limiting factors. Careful site screening and evaluation of each of these limiting factors will lead to higher rates of success and greater effectiveness of the remedy.

11.3 DETERMINING SUBSTRATE REQUIREMENTS

The substrate estimating tool in Appendix B is useful to screen site conditions that will impact substrate delivery and reactivity. The tool provides an estimate of ***total*** substrate required over the design life of the application given a user specified design factor. The tool also provides a time-weighted average concentration of substrate for the total volume of groundwater treated. The quantities and time-weighted average substrate concentrations can be used for comparison to proposed or planned bioremediation applications as a check on the quantities of substrate being proposed and the performance targets for DOC. This should help to avoid application of either too little substrate or generating excessive substrate levels.

While the substrate estimating tool provides a first approximation of total substrate required, it does not provide any guidance or indication on how the substrates should be applied. Design tools are often provided by substrate vendors, and the estimated substrate quantity should always be compared to recommendations by the provider or with case studies in the literature.

The primary objective when selecting a substrate loading rate is to achieve a uniform distribution of substrate over time and space. Design tools that assist the practitioner with the configuration (well spacing) and injection volumes are being developed and should be incorporated into the design process. Examples include the edible oil substrate tool being developed under ESTCP Project ER-0626 (Borden et al., 2008).

For slow release substrates injected in a one-time event, a conservative design factor on the order of three to seven times the estimated substrate requirement should be suitable for limiting the potential for insufficient substrate. For soluble substrates, lower design factors on the order of two to three times the estimated substrate requirement are beneficial to avoid overstimulating the aquifer and driving pH downward. Substrate quantities can be increased if initial loading rates are insufficient to create suitable reducing conditions throughout the treatment zone. The delivery methods for soluble substrates should target uniform substrate concentrations without excessive “spikes” in concentrations of DOC.

The use of very high substrate concentrations to enhanced dissolution of DNAPL into the aqueous phase is an exception to typical substrate loading rates. Solutions with concentrations of lactate as high as 6% by weight, whey as high as 10% by weight, and molasses as high as 1 to 2% by weight have been used for this purpose. These applications require special consideration of the buffering capacity of the aquifer and the system configuration. For example, it may be acceptable to induce adverse geochemical conditions in the source zone to mobilize CAH mass, if a suitable downgradient reaction zone for biodegradation and geochemical recovery is established. In most cases, these injections are performed in pulses every 4 to 12 weeks to allow the aquifer geochemistry to stabilize between injections.

11.4 DESIGNING FOR UNCERTAINTY

In practice, the amount of site characterization data that is available or that can be economically obtained is always limited to some extent. Therefore, it is useful to consider practices that mitigate the uncertainty associated with subsurface environments. Examples of system modifications are listed in Table 12.

Soluble substrate systems that use frequent injections have the most flexibility in modifying injection scenarios. When using infrequent applications of slow-release substrates, potential problems such as the need to add a buffering agent should be evaluated prior to substrate addition, and buffer should be added during substrate injection as a precautionary measure when in doubt.

Table 12. Example of enhanced bioremediation system modifications.

Potential Condition	Modification
Low pH or low buffering capacity	Addition of a buffering compound Use of water push for soluble substrates Use of slower release substrates
Low permeability/groundwater velocity	Closely spaced injection points Targeted injections into low permeability horizons
High permeability/groundwater velocity	Higher substrate loading rates More frequent injections Multiple rows of injection wells or biowalls High retention (coarse droplet) EVO products
Incomplete dechlorination	Microbial characterization Allow for longer lag times Lower the redox environment Bioaugmentation

Modified from AFCEE et al., 2004 and Suthersan et al., 2002.

Sodium bicarbonate was the most common buffering compound used, typically at concentrations in excess of 10,000 mg/L. Sodium bicarbonate is a relatively weak buffering compound and may be most suitable for applications using frequent injections of soluble substrates. The use of stronger and more persistent buffering compounds (e.g., sodium carbonate or sodium phosphates) may be necessary for applications using slow release substrates, and further research and product development will be beneficial for sites with low buffering capacity.

Inadequate or excessive distribution of substrate due to aquifer permeability and/or groundwater flow rates can be adjusted by increasing or decreasing the substrate loading rate or by modifying injection frequency or well spacing. Substrate loading rates may be increased in the event of inhibitory electron acceptor demand (e.g., sulfate over 50 to 100 mg/L).

Finally, incomplete or delayed dechlorination is a common limitation resulting in accumulation of intermediate dechlorination products. Prior to considering bioaugmentation, the system should be evaluated to ensure that the proper geochemical conditions have been achieved and that a sufficient acclimation period has been allowed for ecological succession and development of appropriate microbial consortia. Bioaugmentation with commercially available cultures can be implemented if it has been determined that indigenous *Dehalococcoides* species are lacking or do not exhibit the reductase enzymes for complete dechlorination of VC to ethene (e.g., Steffan et al., 2010).

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APPENDIX A

POINTS OF CONTACT

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APPENDIX B

SUBSTRATE ESTIMATING TOOL

DETERMINING SUBSTRATE REQUIREMENTS
(VERSION 1.2 – NOVEMBER 2010)

B.1 SUBSTRATE REQUIREMENTS FOR ENHANCED IN SITU BIOREMEDIATION

To stimulate in situ anaerobic reductive dechlorination of chlorinated solvents in groundwater, a sufficient mass of organic substrate (electron donor) is required to satisfy both native (inorganic) and chlorinated solvent (organic) electron acceptor demand in the reactive treatment zone. To evaluate substrate requirements, a spreadsheet tool has been developed to assist the practitioner in determining site-specific electron acceptor demand and to estimate the substrate required to meet that demand over the design life of the application (available at http://serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/ER-200627/). ***This tool to evaluate substrate requirements is not intended to be used as a design tool; rather, it is intended only for the purpose of site screening and to evaluate the scientific basis of determining electron acceptor demand and substrate requirements.***

Several providers of organic substrates for enhanced in situ bioremediation provide design tools using similar calculations as the substrate estimating tool. The calculations and assumptions used are not always readily apparent in these design tools. This substrate estimating tool provides information on the reactions, calculations, and assumptions employed in an effort to educate the user on how an estimate of the substrate requirement is determined for a specific site. It is not intended to replace or be used in lieu of a vendors proprietary design tool.

B.2 USING THE SUBSTRATE ESTIMATING TOOL

An input table (Table S.1, attached) is used to enter site-specific data for an evaluation of substrate requirements, and a series of calculations are carried out to 1) calculate the rate of groundwater flow and volume of groundwater to be treated over the specified design life, 2) calculate the electron acceptor demand in hydrogen equivalents, and 3) calculate the substrate requirement in hydrogen equivalents with estimates of some common substrates to meet that requirement. The basic treatment zone properties, hydrogen equivalents, relative distribution of electron accepting processes, and estimates of different substrates to meet the electron acceptor demand are summarized in Table S.5. The following subsections describe the input requirements, calculations, and tool output.

B.2.1 Input to the Substrate Estimating Tool (Table S.1)

Treatment zone dimensions and hydrogeological data are entered in Table S.1 to determine the pore volume of the treatment zone and the volume of groundwater flowing through the treatment zone over the design life of the application. The total volume treated is calculated as the initial pore volume and the volume of flow into the treatment zone over time.

Background concentrations of native electron acceptors and contaminants are entered to calculate the hydrogen equivalents required to completely reduce the electron acceptor demand. ***It is recommended that average background concentrations be entered.*** This may overestimate the hydrogen equivalents needed if all electron acceptors are not reduced (e.g., sulfate is not completely reduced). But in general, the objective is to reduce all competing native electron acceptors to achieve a high efficiency of dechlorination of chlorinated aliphatic hydrocarbons (CAHs).

The electron acceptor demand from manganese, iron, and methanogenesis are calculated from the anticipated concentrations of soluble ferrous iron (Fe^{2+}), manganese (Mn^{4+}), and methane produced. This creates some uncertainty in the calculations, and conservative values should be considered. It may be useful to review case studies of enhanced in situ bioremediation in aquifers with similar lithology (e.g., sand and gravel versus silty clay) and groundwater geochemistry (e.g., naturally aerobic versus naturally anaerobic) to estimate the amount of iron, manganese, and methane that may be produced.

Aquifer geochemical parameters may also be entered as an option but are not required for calculation of hydrogen equivalents. These parameters are intended to provide additional information that may impact bioremediation performance. For example, pH values less than 6.0 or an alkalinity concentration less than 300 milligrams per liter (mg/L) are an indication that pH excursion may be an issue that could result in poor dechlorination efficiency and/or accumulation of intermediate dechlorination products. Cautionary notes are provided when the input values are outside of optimal conditions for enhanced in situ bioremediation of chlorinated solvents.

B.2.2 Substrate Estimating Tool Calculations (Tables S.2 through S.4)

Table S.2 is used to calculate the electron and molecular hydrogen equivalents necessary to meet the total electron acceptor demand over the design life of the application. No additional input is required for these calculations.

The pore volume of the treatment zone is simply calculated as the total volume of the treatment zone times the estimated porosity. The rate of groundwater flow through a treatment zone may be calculated based on site-specific hydrogeologic properties of the aquifer. A simplistic approach using Darcy's Law is used. Darcy's Law states that the volumetric flow rate (Q) through a pipe filled with sand can be calculated as follows:

$$Q = -KA(dh/dl) \tag{B-1}$$

where

K = proportionality constant (length divided by time [L/T])
A = the cross sectional area of the pipe (L^2)
dh/dl = the horizontal hydraulic gradient (unitless)

More simply stated, Equation B-1 can be solved to yield the Darcy velocity or specific discharge. As defined, the specific discharge (q) is a volumetric flow rate per unit surface area of porous media:

$$q = Q/A = - K(dh/dl) \qquad \text{(B-2)}$$

This equation is useful because the water balance through a treatment zone can be assumed to be approximately the volumetric flow of water through the aquifer, where values for the proportionality constant are measured as hydraulic conductivity (K). Both K and the horizontal hydraulic gradient (dh/dl) are commonly known from site investigation activities.

Because water moves only through the interconnected pore openings of an aquifer, Darcy's q is a superficial or apparent velocity, that is, q represents the velocity at which water would flow if the aquifer were an open conduit but does not account for the fact that only a portion of the aquifer volume consists of voids that cause water to flow through different pore spaces at different rates along individual flow paths that vary in length**.** The velocity of water through the aquifer pore spaces over distance is termed the average linear or seepage velocity where:

$$\mathrm{v = - K(dh/dl) / ne} \qquad \text{(B-3)}$$

where

v = pore water (seepage) velocity (L/T)
n_e = effective porosity of the aquifer matrix (unit less)

Typical groundwater seepage velocities for enhanced anaerobic bioremediation applications range from 30 to 1000 ft/yr. Seepage velocities outside of this range require special consideration.

Table S.3 lists some common substrates and the weight of molecular hydrogen produced per unit weight of pure substrate (grams per gram). This is perhaps the most subjective aspect of the substrate estimating tool. Therefore, the user is allowed to modify the moles of molecular hydrogen produced per mole of substrate. In the authors judgment, the values listed are conservative and may lead to higher than required substrate estimates in some cases.

Table S.4 then calculates the amount of pure substrate and bulk substrate product based on the hydrogen produced per unit weight of substrate in Table S.3. This is a simple calculation where the hydrogen demand (pounds) is divided by the ratio of hydrogen produced per unit weight of substrate. For the example site in Section B.3, 58.4 pounds of molecular hydrogen divided by a ratio of hydrogen produced per unit weight of ethanol of 0.0875 produces an ethanol equivalent of 668 lb. Multiplying by a design factor of three times results in an ethanol equivalent of 2003 lb of pure ethanol, or 2504 lb of ethanol product at 80% ethanol.

B.2.3 Substrate Estimating Tool Output (Table S.5)

Output from the substrate estimating tool is summarized in Table S.5. This table summarizes the treatment zone physical dimensions, hydrogeological properties, the hydrogen requirement for each electron accepting process, and a plot of the relative distribution of the electron acceptor demand for each electron accepting process. Table S.5 also calculates the hydrogen requirement in pounds per gallon or grams per liter. This information is useful when comparing multiple sites with varying geochemical and contaminant conditions.

Finally, Table S.5 summarizes the substrate requirements for some common substrate types based on the design factor that is input in Table S.1. This information is provided in pounds of the substrate product (assuming the % of active ingredient in each product), the quantity of product for those typically sold by the gallon, and the effective time-weighted average concentration of substrate for the total volume of water treated.

This information may be used to evaluate the design of different enhanced bioremediation applications. It is a useful first approximation of substrate requirements, but professional experience and judgment should always be applied on a site by site basis when designing an enhanced in situ bioremediation application.

B.3 EXAMPLE OF ESTIMATING SUBSTRATE REQUIREMENTS BASED ON HYDROGEN EQUIVALENTS

Practitioners have attempted to calculate substrate requirements based on hydrogen equivalents in coupled redox and fermentation reactions. This method calculates the mass of molecular hydrogen required to satisfy native and CAH electron acceptor demands. These computations assume that a limited, known set of stoichiometric degradation reactions occurs and should be considered order-of-magnitude estimates only. The following is an example of estimating substrate requirements based on calculations of hydrogen equivalents.

B.3.1 Hypothetical Site Conditions

Tables S.1 through S.5 (attached) are for a hypothetical example site used to illustrate the calculation of substrate requirements. Table S.1 contains the input used for the sample site. Table S.2 illustrates the calculation of total electron acceptor demand in terms of hydrogen equivalents. Table S.3 lists the molecular formula, molecular weight, and potential hydrogen production rates for some common substrates based on fermentation or oxidation-reduction reactions. These data are used in Table S.4 to calculate the amount of substrate required to meet the hydrogen requirement estimated in Table S.1. Finally, Table S.5 is a summary table of the electron acceptor and substrate requirement calculations, including the distribution of differing electron acceptors.

The characteristics of the example site and system design are as follows:

- The treatment zone is a barrier configuration of 200 ft in length (perpendicular to groundwater flow) and 20 ft in width (parallel to groundwater flow), with a saturated thickness of 10 ft.
- The design period for the substrate calculations is 1 year.
- The groundwater potentiometric surface slopes uniformly in one direction with an average horizontal gradient of 0.01 foot per foot (ft/ft).
- The total porosity, effective porosity, and hydraulic conductivity of the aquifer matrix are assumed to be 25%, 20%, and 10 ft/day, respectively.
- The soil bulk density and fraction organic carbon of the aquifer matrix are assumed to be 1.7 grams per cubic centimeter (gm/cm^3) and 0.05%, respectively.
- Contaminant concentrations are uniform throughout the treatment zone. Aqueous phase contaminant concentrations are 10,000 micrograms per liter (µg/L) PCE and 1000 µg/L of TCE.
- The existing groundwater geochemistry is relatively aerobic, with an average DO concentration of 5.0 mg/L, average nitrate concentration of 5.0 mg/L, and average sulfate concentration of 50 mg/L. Anaerobic processes utilizing carbon dioxide as an electron acceptor are expected to generate a concentration of 10 mg/L of methane.
- Anaerobic processes utilizing solid-phase electron acceptors are expected to generate a concentration of 5.0 mg/L manganese (Mn^{2+}) and 25 mg/L ferrous iron (Fe^{2+}) for a single pore volume.

The hypothetical site conditions listed above constitute a basic conceptual site model (CSM). Application of a substrate for enhanced bioremediation can take many forms in regard to substrate type, injection configuration, and injection frequency. ***For the purposes of this example, the following discussion describes the calculation of the total hydrogen requirement and substrate requirements for a 1-year design life.***

B.3.2 Calculation of Hydrogen Requirement

In this example, the total treatment zone volume is 40,000 cubic feet (ft^3) (Table S.2). Given an effective porosity of 20%, a pore volume for effective groundwater flow is equivalent to approximately 59,856 gallons. The application of Darcy's Law (calculation not shown) yields a groundwater seepage velocity of 0.5 ft/day, or 182.5 ft/yr. Based upon an effective porosity of 20% (the volume of interconnected porosity through which groundwater will flow), the volumetric groundwater flow rate (discharge) through the treatment zone is equivalent to approximately 546,186 gallons per year.

The mass of hydrogen required to theoretically reduce the mass of each native electron acceptor species and each CAH species is calculated in Steps 3 and 4 in Table S.2. For example, the hydrogen requirement for aqueous native electron acceptor mass in the initial pore volume of the treatment zone is 5.12 lbs of molecular hydrogen (Step 3A in Table S.2).

The total hydrogen requirement required for the 1-year design life is calculated by summing the hydrogen requirements for initial aqueous and solid-phase native electron acceptors, initial aqueous and sorbed phase CAH electron acceptors, and the soluble native and CAH electron acceptor mass flux over time. Based upon these calculations, the total electron acceptor demand (in pounds of hydrogen equivalents) for the example site can be summarized as follows:

Initial aqueous native electron acceptor demand in treatment zone:	5.12 lbs
Solid-phase native electron acceptor demand in treatment zone:	3.21 lbs
Initial soluble CAH electron acceptor demand in treatment zone:	0.27 lbs
Sorbed CAH electron acceptor demand in treatment zone:	0.28 lbs
Soluble native electron acceptor mass loading (1 year):	47.1 lbs
Soluble CAH electron acceptor mass loading (1 year):	2.43 lbs
Total Hydrogen Requirement for 1-Year Design Life:	**58.4 lbs**

The design factor typically used by practitioners (to account for microbial efficiency and uncertainty in electron acceptor demand) using this method is typically between two and 10 times the calculated total hydrogen requirement of the system. For this example, if a design factor of three times was used it would yield a total hydrogen requirement of 175.3 pounds of molecular hydrogen over 1 year.

B.3.3 Calculation of Substrate Requirements

The mass of a particular organic substrate required to meet the total estimated hydrogen requirement can be calculated by dividing the total hydrogen requirement (including the design factor) of the system by the hydrogen production potential associated with the particular substrate of interest. Table S.4 lists the estimated mass of selected substrates that would be required to meet the hydrogen requirement calculated in Table S.2 for a design factor of 1 times the calculated hydrogen demand. For example, the mass of ethanol required to meet the example hydrogen requirement with a design factor of three times (Table S.4) is approximately 2003 lb (175.3 lb of hydrogen divided by 0.0875) of pure ethanol, or 2504 lb of ethanol product, assuming the product is 80% ethanol and 20% water.

The substrate requirements listed in Table S.4 are first listed for 100% pure product. When estimating required substrate mass, it is important to account for the fact that most commercial organic substrate products are less than 100% pure product, and some are mixtures of different organic substrates. For example, HRC® is a complex molecule containing lactate and glycerol, and commercial emulsified vegetable oil products are mixtures of soybean oil, sodium lactate, emulsifiers, and water. Therefore, when estimating substrate requirements for purchase of substrate products, the composition of a substrate mixture should be known with a reasonable degree of certainty. Values for the amount of substrate product in Table S.4 assume that each product is a certain percentage of active (pure) ingredient.

As an example, the material safety data sheet (MSDS) for HRC® lists the product as ranging from 52.5 to 65.0% glycerol tripolylactate and from 35.0 to 47.5% glycerol. For practical purposes, one could consider the product 60% glycerol tripolylactate and 40% glycerol by weight. It is not known by the authors how much of the 60% glycerol tripolylactate yields lactic acid or how much is inactive polymer material. Raymond et al. (2003) writes the formula for HRC® as $C_{39}H_{56}O_{39}$. If 40% of this compound were lactic acid ($C_3H_6O_3$) and 40% were glycerol ($C_3H_8O_3$), you could conceivably end up with the same amount of hydrogen ions per mole of substrate. For the substrate estimating tool, it is assume that 22 moles of molecular hydrogen are produced per mole of HRC® assumed to be equivalent to $C_{39}H_{56}O_{39}$. This value yields comparable substrate quantities when evaluating a similar example site between the substrate estimating tool and the Regenesis design software. ***The practitioner should always refer to the manufacturer's software or recommendations for design purposes. The substrate estimating tool is intended only for screening or evaluating the electron accepting processes as a site***.

As mentioned previously, it is a good practice to compare substrate loading estimates using the hydrogen equivalent method with empirical estimates. As an example, consider the 2003 lb of pure ethanol estimated for the example case. Given an effective pore volume of approximately 59,856 gallons, a groundwater flux of approximately 546,186 gallons per year (Table S.5), and assuming the 2003 lb of pure ethanol is uniformly distributed in space and in time over the entire design life, the ***average time-weighted dissolved concentration*** of ethanol would be approximately 396 mg/L. In practice ethanol would be injected in multiple, frequent events (perhaps daily) at higher concentrations to achieve the overall, long-term target concentration.

This concentration of ethanol is slightly higher than the range typically targeted for ethanol of 50 to 300 mg/L. In this case, a design factor of three or more times may err on the high side due to conservative assumptions in the substrate estimating tool, and perhaps a design factor of one and a half to two times may be more suitable. In practice, design factors for soluble substrate are almost always less than slow-release substrates because greater control of average substrate concentrations over time can be achieved with the multiple injections of soluble substrate. While there are many uncertainties in estimating substrate loading rates using either empirical or a stoichiometric approach, the use of a stoichiometric approach can provide a reasonable first estimate.

B.4 SUMMARY

Practitioners using the methods described in this appendix should recognize the degree of uncertainty involved. One concern is that an inadequate substrate loading rate may lead to reducing conditions that are insufficient for complete dechlorination, with the potential for accumulation of intermediate dechlorination products. Conversely, excessive levels of organic substrate may lead to an adverse excursion in pH affecting dechlorination efficiency, high levels of methanogenesis with low utilization of substrate for anaerobic dechlorination, and potential for adverse impacts to secondary groundwater quality.

While the scientific basis for determining substrate requirements remains an area of uncertainty, the practitioner of enhanced in situ bioremediation must still design or evaluate a substrate loading rate with the methods currently available. The two approaches most commonly employed are to either: 1) target an empirical range of substrate concentration in the reaction zone that is based upon previous experience and experimentation or 2) calculate a substrate (electron donor) requirement based on estimates of the native and CAH electron acceptor mass and mass flux. ***In practice, both methods should be performed and used as a check against the other that the substrate loading rate applied is within practical limits used in other successful bioremediation applications.***

Given the state of knowledge and practice, pilot testing and experimentation may be the best way to optimize substrate loading rates for anaerobic dechlorination of chlorinated solvents. As the level of uncertainty increases, the practitioner may also want to consider designs and techniques that provide for more latitude in modifying substrate loading rates (e.g., recirculation designs). It is anticipated that continued implementation and documentation of enhanced in situ bioremediation will lead to an improved understanding and less uncertainty in the design of substrate loading rates.

B.5 REFERENCES

Air Force Center for Environmental Excellence (AFCEE), Naval Facilities Engineering Command/Engineering Service Center (NAVFAC ESC), and the Environmental Security Technology Certification Program (ESTCP). 2004. *Principles and Practices of Enhanced Anaerobic Bioremediation of Chlorinated Solvents*. Prepared by the Parsons Corporation, Denver, Colorado. August.

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