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Applying Bioaugmentation to Treat DNAPL Sources in Fractured Rock

ESTCP Project ER-201210

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1.0 INTRODUCTION

1.1 DNAPL in Fractured Rock

Management of fractured rock sites impacted by chlorinated solvents remains one of the top environmental challenges for the Department of Defense (DoD). These chlorinated solvents, such as tetrachloroethene (PCE) and trichloroethene (TCE), are used as industrial degreasers and cleaners, (TCE), and cause many unintended discharges and improper disposal practices that affect fractured rock sites. This has resulted in subsurface impacts that produced regulatory exceedances in both soil and groundwater.

Chlorinated solvents such as PCE and TCE have a density greater than water, and as neat phases are termed dense nonaqueous phase liquids (DNAPLs). The DNAPL density allows downward migration within the saturated zone. Upon reaching bedrock, DNAPL can enter fractures and continue to migrate both laterally and vertically, which ultimately impacts rock aquifers.

Many studies have examined DNAPL sources in unconsolidated subsurface media. These studies have shown that DNAPL typically serves as a long-term contaminant source for groundwater, as the DNAPL slowly dissolves into surrounding groundwater (Bradford et al. 2003; Wilking et al. 2013). Unfortunately, even within unconsolidated media, treatment of DNAPL sources has proven challenging. These challenges have been due to identification and quantification of the DNAPL itself, mass transfer limitations with respect to DNAPL mass removal, concerns regarding uncontrolled DNAPL mobilization, and/or inhibition of complete microbial dechlorination (Adamson et al. 2003; Yang and McCarty 2005; Amos et al. 2008). Thus, for many DNAPL-impacted sites, the DNAPL source area is the focus of site management and remedial efforts.

The challenges associated with DNAPL in fractured rock are similar to those encountered in unconsolidated media. However, these challenges are exacerbated by the complexities associated with the dual porosity nature of fractured rock, as well as the lack of insight into the highly complex DNAPL architecture at the field scale. Bench-scale studies examining DNAPL in single fractures (Dickson and Thomson 2003; Schaefer et al. 2009) and simple fracture networks (Christensen et al. 2015) have highlighted many of these issues. However, while several studies have examined treatment of dissolved PCE and TCE in fractured rock, field-scale studies that carefully assess treatment of DNAPL sources in fractured rock are lacking, and thus key insights and guidance on how to treat DNAPL sources in fractured rock are not readily available.

1.2 Bioaugmentation for Treatment of DNAPL

The use of anaerobic bioremediation as treatment for chlorinated ethenes such as PCE and TCE has been one of the most widely and successfully applied *in situ* treatment technologies for this class of contaminants.

This treatment typically involves either biostimulation (which involves delivery of electron donor and nutrients to the subsurface) or bioaugmentation (which involves delivery of electron donor, nutrients, and dechlorinating bacteria to the subsurface). Biostimulation relies solely on the presence of native bacteria to serve as the catalyst for the microbially driven dechlorination reactions, while bioaugmentation typically provides an initial dose of bacteria that are capable of completely dechlorinating PCE and TCE (Major et al. 2002; Schaefer et al. 2010). Several widely applied bioaugmentation cultures are commercially available for treating chlorinated ethenes; these cultures all contain *Dehalococcoides* sp. (DHC), which are the only known bacteria capable of completely dechlorinating PCE (Maymó-Gatell et al. 1997). PCE and TCE are treated via a sequential reductive dechlorination pathway, with ethene or ethane as innocuous end products.

While bioaugmentation for chlorinated ethenes has been successfully applied in fractured rock *in situ* (Mora et al. 2008; Pérez-de-Mora et al. 2014; Révész et al. 2014), there are no reported studies that assess DNAPL mass removal during bioaugmentation treatment in fractured rock. Such studies have been performed in unconsolidated media (USEPA 2004; ITRC 2007; Hood et al. 2008), but many of the tools and approaches used in these studies are not appropriate for fractured rock systems. The objective of this document is to provide practical guidance and insight into the application of bioaugmentation to treat DNAPL sources in fractured rock. It is noted that the focus herein is on treatment of residual (i.e., non-mobile) DNAPL sources. This guidance is based largely on insights attained through a field demonstration performed in fractured granite at Site 37 at Edwards AFB (ESTCP Project ER-201210). The demonstration location is provided in Figure 1. This ESTCP demonstration was focused in the vicinity of Building 8595, adjacent to the location of a reported surface release of PCE (Earth Tech 2008). The following sections of this document provide: 1) recommended approaches for source area identification and characterization, 2) guidance on amendment delivery and operation, 3) a recommended monitoring approach, 4) a strategy for assessing performance data (including rebound), and 5) a discussion of secondary groundwater impacts and biofouling.

Although every site is unique, both technically and with respect to remedial goals, the intent of this document is to provide basic insights and guidance that will facilitate appropriate remedial design and implementation, and will result in an overall improvement in the management of DNAPL sources in fractured rock.
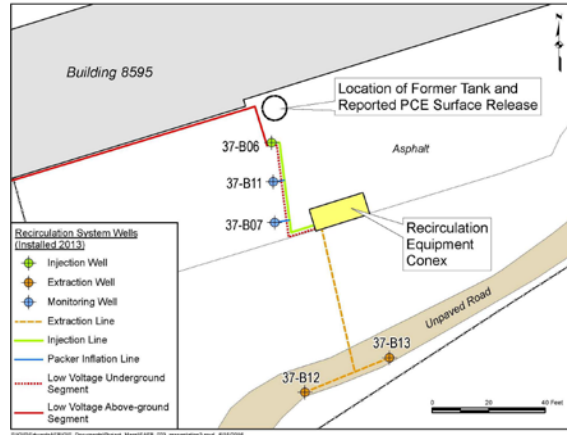
Figure 1. Demonstration Location. ESTCP demonstration (ER-201210) performed at Edwards AFB, adjacent to Building 8595.

2.0 SOURCE AREA IDENTIFICATION AND CHARACTERIZATION

2.1 Potential Locations of DNAPL Sources

Identifying the potential location(s) of DNAPL sources is the first step in characterizing and treating the source area. Information regarding the location of the DNAPL release, DNAPL impacts within the overlying unconsolidated materials, and groundwater concentrations within the fractured rock all can serve as a guide for this initial assessment. This information can serve as a useful guide, but it is important to note that fracture network complexity can result in a convoluted DNAPL architecture, and can cause DNAPL to migrate unpredictably. In addition, the 1% solubility "rule-of-thumb" suggested as a guide for determining if DNAPL potentially is present based on aqueous concentrations (Cohen 1992; USEPA 1992) is not appropriate for fractured rock, as substantial dilution from a heterogeneous fracture flow field may result in lower than expected contaminant concentrations in the presence of residual DNAPL sources. In contrast, lack of dilution along a discrete fracture flow path may cause the DNAPL source area to appear larger than it actually is, with very large zones having dissolved concentrations greater than 1% solubility. Thus, groundwater concentrations should be used cautiously as an initial guide to the potential presence of a DNAPL source.

While identification of product within a well is a clear indicator that DNAPL is present within the fractures, in many cases such a clear indicator of DNAPL is not observed. DNAPL is often present as a residual, and will not be mobile and thus will not enter a well under ambient or pumping conditions. Photoionization detector (PID) screening of collected rock cores, and collection, extraction, and analysis of rock core samples (i.e., samples of the rock matrix directly adjacent to hydraulically conductive fractures), potentially provides indication that DNAPL sources are present. During the initial phase of investigation at the suspected PCE DNAPL source area at Site 37 (Edwards AFB), rock cores were collected from within the suspected source area. Elevated PID and rock core PCE concentrations from extractions were observed adjacent to conductive fractures that were later shown (via partitioning tracer methods discussed in Section 2.3) to contain DNAPL. Similarly, elevated PCE core concentrations were identified in fractured limestone at a former quarry site at Loring AFB (ME) (USEPA 2005), where DNAPL presence was later confirmed via application of a partitioning tracer test. It is important to note that rock core methods are not quantitative for DNAPL assessment since it may not be possible to distinguish between elevated dissolved contaminant concentrations, and the actual presence of DNAPL since both scenarios may produce similar mass in the rock core matrix. Furthermore, to employ these rock core techniques, a high quality core is required, which precludes the use of air or mud rotary drilling techniques. Residual DNAPL also may become dislodged during core collection, and thus may be missed by these core screening techniques. *In situ* techniques to identify and quantify DNAPL sources are therefore preferred, and discussed in the following sections.

2.2 Fracture Assessment

Identification of DNAPL sources, as well as determination of DNAPL architecture, in fractured rock requires assessment of the fracture flow paths. Many tools are available

for evaluation of the fracture flows paths and bedrock hydrogeology, including borehole geophysics (e.g., Pehme et al. 2014), discrete interval and aquifer hydraulic testing (Gernand and Heidtman 1997; Quinn 2011), electrical techniques (e.g., Wishart et al. 2008), single- and multi-well tracer testing (Jardine et al. 1999; Jamin et al. 2015), and transmissivity profiling using borehole liners (Broholm et al. 2016). High resolution flux meters (water and contaminant) have been recently developed for fractured rock (Hatfield 2015; Klammler et al., 2016). While a detailed discussion of these various tools and techniques is beyond the scope of this document, it is important to recognize that proper assessment of the fracture flow paths is critical for assessing DNAPL sources and, ultimately, for the efficient and effective application of bioaugmentation for treatment of DNAPL sources in fractured rock. Selection of these tools is dependent upon many site-specific conditions, including basic bedrock and fracture structure and the availability and type of monitoring wells available.

Fracture flow assessment at the Edwards AFB Site 37 ESTCP demonstration began with a combination of borehole geophysics to identify potential locations of transmissive fractures, as well as the orientation of those fractures. Acoustic and optical televiewers, coupled with heat pulse flow meter testing, were employed to identify these zones. Discrete interval draw-down testing using inflatable packers was used to confirm the location of transmissive zones, and semi-quantitatively assess transmissivity. Short-term pump testing was employed in these zones to assess hydraulic connectivity with nearby bedrock wells. It is noted that open borehole wells were used for this initial phase of testing, which allowed for flexibility in assessing fracture flow along a substantial vertical interval of the targeted bedrock zone.

2.3 DNAPL Distribution

Partitioning tracer tests (PTTs), which employ the use of conservative (e.g., bromide) and partitioning (e.g., long-chain alcohols), have been shown to be an effective tool for locating and quantifying residual DNAPL sources along groundwater flow paths in unconsolidated media (Annable et al. 2005; Hartog et al., 2010). It is important to note that PTTs may not identify DNAPL that is present in low permeability zones that have poor mass transfer with solutes along the primary flow paths. However, DNAPL located in such zones may have a relatively small impact on groundwater quality compared to DNAPL sources located along preferential groundwater flow paths.

Once hydraulically conductive fractures that potentially contain DNAPL are identified, PTTs can be applied to fractured rock. Such fractures can be identified by using the approaches described in Section 2.1 and 2.2. PTTs can be applied at a single location using a single well push-pull technique, or performed as an interwell tracer test. The single well push-pull testing may be useful for screening purposes. Interwell PTTs typically will provide more quantitative data and cover a greater areal extent than single well techniques, but also require greater project resources. Parallel testing in a location known to be absent of DNAPL is recommended to properly account for tracer sorption to organic matter or diffusion into the rock matrix; such tracer uptake processes could produce misleading results with respect to the presence and quantity of DNAPL. Two conservative tracers with

differing aqueous diffusivities also can be used to assess any potential impacts of matrix diffusion on tracer results (Jardine et al. 1999).

Tailing or retardation of the partitioning tracers relative to the conservative tracers typically is evidence that DNAPL is present within or in close proximity to hydraulically conductive fracture flow paths, assuming negligible sorption to aquifer solids. Identifying locations and discrete fracture intervals where DNAPL is present might, in some cases, be sufficient for remedial design or overall site management. However, if an estimate of the DNAPL mass along the fracture flow path is required, then a determination of the fracture aperture and groundwater velocity also is required. This information can be obtained via interwell tracer testing (Schaefer et al., 2016); fracture aperture also can be estimated by use of the cubic law applied to permeability data (Quinn et al. 2011).

At Edwards AFB Site 37, push-pull PTTs were initially utilized to confirm the presence of DNAPL. Results from one of these tests, performed as an open borehole test in well B07, are shown in Figure 2 (refer to Figure 1 for well locations). Parallel testing at other locations showed that tracer retardation was negligible. The PTT data in Figure 2 clearly showed retardation and tailing of the more hydrophobic tracers, indicating that DNAPL sources were present along the targeted fracture flow paths associated with this borehole location. Subsequent PTTs were performed as interwell tracer tests, where the tracer mixture was injected in a discrete interval in B06 and monitored in 3 discreet intervals (isolated using straddle packers) in B07. Results of the interwell PTT provided information regarding the fracture flow field, and the DNAPL mass distribution relative to the flow field. For example, tracer elution at the deepest monitored interval in B11, shown in Figure 3, indicated that the DNAPL occupied less than 1% of the fracture volume; this result is significant, as it suggests that only a relatively small DNAPL mass exists within the targeted treatment area. Such small quantities of DNAPL mass within the fractures are likely conducive to *in situ* treatment via bioaugmentation, whereas much larger volume of residual DNAPL (e.g., 30%) may be problematic for *in situ* bioaugmentation due to the time needed for DNAPL removal. Results for this PTT also showed that DNAPL sources were primarily located in low permeability or mass-transfer controlled fracture zones (Schaefer et al., 2016). This quantitative assessment was useful in evaluating bioaugmentation performance, as described in Section 4.3.
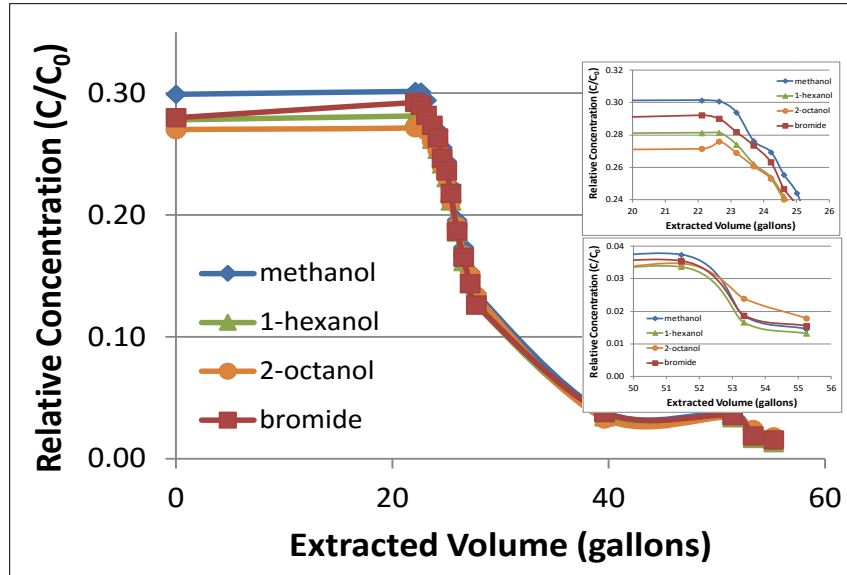
Figure 2. B07 Push-Pull Tracer Test Results. Results from a single well push-pull partitioning tracer test performed in borehole B07 at Edwards AFB as part of ESTCP Project ER-201210.
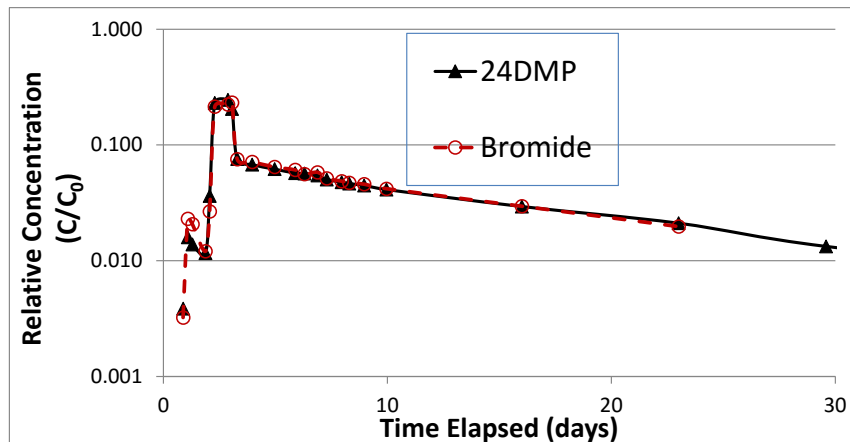
Figure 3. Tracer Elution during the Interwell PPT. Data are from the deep interval at B11 at Edwards AFB, performed as part of ESTCP Project ER-201210. 24DMP = 2,4-dimethyl propanol (partitioning tracer used in this test).

2.4 Method of Moment Analysis to Determine DNAPL Mass

The quantification of unknown NAPL saturation and volume is based on well-characterized linear tracer partitioning relationships between aqueous and NAPL phases:

$$K_{NW} = \frac{C_{NAPL}}{C_w} \hspace{4cm} \text{Eq. 1}$$

where K_{NW} = NAPL-water partition coefficient and C_{NAPL} = tracer concentration in the NAPL phase, and C_w = tracer concentration in the aqueous phase.

Temporal moment analysis is a statistical modeling approach used to infer average NAPL saturations (volume DNAPL/fracture volume) in tracer swept regions following the application of a PTT (Annable et al., 1998; Jin et al., 1995). Residence time statistics are estimated from the concentration-time breakthrough curves from each tracer monitoring location. The mean tracer residence time is determined by:

$$m_n = \int t^n C(x,t)\, dt \qquad \text{Eq. 2}$$

where m_n is the absolute nth-temporal moment of the tracer breakthrough curve at a monitoring well located at a distance, x, from the tracer injection well. The trapezoidal rule is used to approximate the integral at each time step of the concentration-time breakthrough curve. A retardation factor, R, is the ratio of mean resident time of each partitioning tracer to the non-partitioning tracer:

$$R = \frac{\bar{t}_p}{\bar{t}_{np}} \qquad \text{Eq. 3}$$

where $\bar{t}_p$ and $\bar{t}_{np}$ represent the mean residence times for the partitioning and non-partitioning tracer to the monitoring well as determined by the first normalized moment of each tracer with a correction for the duration of tracer pulse injection:

$$\bar{t} = \frac{m_1}{m_0} - \frac{t_p}{2} \qquad \text{Eq. 4}$$

where t_p is the duration of the injected tracer pulse. For the partitioning tracers, the average DNAPL saturation for the tracer swept pore volume is calculated using (Annable et al., 1998):

$$S_n = \frac{R - 1}{R + K_{NW} - 1} \qquad \text{Eq. 5}$$

where the NAPL-water partitioning coefficients, K_{NW}, may be obtained from previous laboratory batch experiment based estimates (Cho and Annable, 2005; Hartog et al., 2010). The volume of NAPL (V_n) can then be determined using (Annable et al., 1998):

$$V_n = \frac{S_n V_e}{1 - S_n} \qquad \text{Eq. 6}$$

$$V_e = Q\bar{t}_{np} \qquad \text{Eq. 7}$$

where V_e is the effective pore volume swept by the injected tracer solution and Q is the average recirculation pumping rate

Mass discharges are calculated using Equation 8 based on average concentrations and pumping rate (Q). The complete dissolution time of the residual NAPL within the fractures is then estimated using Equation 9.

$$M_D = \bar{c}Q \qquad \text{Eq. 8}$$

$$\Delta t = \frac{M_{NAPL}}{M_D}$$
<div align="right">Eq. 9</div>

Breakthrough curves should be exponentially extrapolated to minimize truncation errors when data are sparse or if breakthrough curves are incomplete at later times (Annable et al., 1998; Brooks et al., 2002). Breakthrough curves with extended tailing or multiple peaks are common in fractured rock systems and may require additional analysis. When multiple peaks occur, the separate peaks may represent unique flow zones (e.g. high permeability or low permeability). The breakthrough may be divided into segments representing each unique fracture flow zone to examine the relative contribution of each to the total NAPL saturation at each fracture flow domain. A moment analysis can be performed on each portion of the breakthrough curve representing the unique flow zones (e.g. initial peak, second peak, and tail). Extended tailing may also be characteristic of non-equilibrium transport. In such cases, non-equilibrium inverse modeling approaches may be warranted for determination of DNAPL mass.

2.5 Site-wide Application of PTTs

Application of PTTs during the ESTCP demonstration at Edwards AFB described in Sections 2.3 and 2.4 was limited to a relatively small footprint and depth interval compared to the where elevated (>10% solubility) dissolved concentrations of PCE were observed. For assessing the DNAPL distribution for large source areas, multiple tracer tests likely would be required. Cost restraints might limit DNAPL distribution testing to simply identifying the approximate extents of the DNAPL source zone in order to most effectively target remedial technologies. Other effective strategies might involve a large number of push-pull tests to determine the approximate extent and distribution of DNAPL sources across the site. It is important to couple the design of PTTs with the known fracture flow field; often, the latter might prove to be the harder to define.

3.0 IMPLEMENTING BIOAUGMENTATION IN A FRACTURED ROCK DNAPL SOURCE AREA

3.1 Targeting DNAPL Zones and Overall Strategy

3.1.1 Strategy Based on Remedial Goals

After identifying the DNAPL sources (described in Section 2), the next step is to identify where to implement bioaugmentation. Two factors are important for selecting where to implement bioremediation of DNAPL sources: the fracture flow field and overall remedial goals. The fracture flow field must be considered, because not all zones that contain residual DNAPL will be amenable to biological treatment. For example, if the transmissivity is too low to handle amendment injections, or if the fracture network is not sufficiently connected, treating DNAPL sources via bioaugmentation is unlikely to be effective.

Furthermore, overall remedial goals need to be considered. It is generally recognized that attainment of MCLs in a DNAPL source area is unlikely. Thus, remedial objectives often are based upon some percentage reduction in contaminant concentration, or some other pre-determined contaminant concentration in groundwater. These type of remedial goals will require treatment of DNAPL sources in both high and low transmissivity fracture zones, which might require treating a large volume of the source area. Treating low transmissivity zones might also require additional time and resources due to the difficulties in delivering remedial amendments.

Alternately, remedial objectives that are based on overall mass discharge (with the goal of substantially mitigating the downgradient dissolved contaminant plume) require a different strategy. For mass discharge based remedial objectives, only DNAPL sources present in high transmissivity zones would be targeted. In many cases, this would limit the treatment volume, and eliminate the challenges associated with amendment delivery to low transmissivity zones. This mass discharge based bioaugmentation strategy is currently being applied to treat a fractured rock PCE DNAPL source zone in a former quarry area at former Loring AFB in Limestone, ME.

Selection of targeted zones for bioaugmentation will likely require the use of packers (or other borehole tools) to isolate selected intervals within the fractured rock. This approach was used at Loring AFB, as well as in the ESTCP demonstration at Edwards AFB. Caution should be taken to ensure that a secure seal is attained between the packer and borehole wall, and that no significant leaks exist within the packer and packer connections. It is recommended that the packers are connected to an inert gas tank, and that pressure is checked regularly and/or instrumented with a low pressure alarm. Alternate approaches for isolating bedrock intervals, such as FLUTe™ liners, can be used in place of inflatable packers in some instances. However, installation of flute liners may require substantial project resources, and may prove challenging if well redevelopment is needed and/or if modifications are needed to the targeted treatment intervals.

3.1.2 Implementation Approach

Prior to field-scale implementation of bioaugmentation, it is recommended that treatability testing is performed to verify that complete dechlorination of the PCE or TCE DNAPL source is achievable. This is important because the presence of unknown co-contaminants (organic or inorganic) can inhibit reductive dechlorination, and/or geochemical affects might be observed that require modifications to the amendment delivery strategy (e.g., pH buffering). Treatability study approaches include in-well techniques via the use of Bio-Trap® samplers (Microbial Insights) or bench-scale microcosm studies (e.g., Schaefer et al. 2009b) using site groundwater and aquifer solids. Rock solids can be obtained using cut or crushed rock (<1 cm pieces) obtained from rock core collected adjacent to targeted fracture zones. There are pros and cons to each of these treatability testing approaches; the discussion of which is beyond the scope of this document. It is important to note that the presence of DNAPL within the closed-system microcosms can inhibit biological activity, and give a false-negative result with respect to *in situ* treatment potential (Schaefer et al. 2010b). Thus, bench-scale microcosms should be performed using dissolved PCE/TCE concentrations only.

Bioaugmentation approaches for chlorinated solvents fall into two general categories: passive and active. Hybrid, or semi-passive approaches, also can be employed. Passive approaches typically require an initial injection (or, injections) to distribute amendments (electron donor, nutrients, and bacteria) into the subsurface, followed by monitoring (typically years) as *in situ* mixing and treatment occurs under ambient flow conditions (USEPA 2013). An insoluble long-term electron donor source, such as vegetable oil, is typically used in passive approaches. When treating a DNAPL source area in fractured rock, there are several concerns with a passive treatment approach. First, delivery of oil-based electron donors could result in the mobilization and spread of DNAPL sources (especially if emulsifiers are used), which may be problematic. Second, because of the large reservoir of contaminant mass present in the DNAPL itself, nutrient limitations may quickly become an issue, requiring repeated injections of amendments such as diammonium phosphate and/or micronutrients (see Section 3.2). Finally, the overall rate of DNAPL dissolution typically is controlled by mass transfer. In many fractured rock systems, ambient flow may be very low, thereby limiting the DNAPL dissolution process. For these reasons, an active remediation approach may be preferred for DNAPL sources. Active bioaugmentation approaches typically require continuous (or, intermittent, if a semi passive approach is employed) injection of amendments with groundwater recirculation (USEPA 2013). A soluble electron donor, such as lactate or molasses, is typically used. While active approaches overcome the limitations associated with passive approaches, significant project resources are typically needed to manage and maintain groundwater recirculation systems compared to passive amendment designs. Issues related to biofouling (discussed in Section 6.1) often provide significant challenges when implementing this approach. Efforts to treat DNAPL sources in fractured rock during the ESTCP demonstration at Edwards AFB, as well as ongoing treatment at Loring AFB, have employed active approaches, which we also focus on in subsequent sections of this document. It should be noted, however, that more detailed and controlled comparisons (e.g., at same site) of active versus passive approaches for treatment of DNAPL sources in fractured rock are required to fully discern the relative advantages and disadvantages.

3.2 Remedial Amendments

Remedial amendments for bioaugmentation typically consist of electron donor, nutrients, bacteria (DHC), and (if needed) pH buffer. As mentioned in the previous section, for active bioremediation approaches, a soluble electron donor such as lactate is preferred. Nutrients typically include nitrogen and phosphorous, which can be readily delivered in the form of diammonium phosphate. Micronutrients also may be needed, which can be supplied by amending with yeast extract (Bach et al. 2005). Additional co-factors, such as vitamin B12, also may be beneficial (Reinhold et al. 2012). If the pH of site groundwater is less than 6, or if bench-scale testing indicates that the fermentation associated with electron donor addition cause the pH to decrease below 6, use of a pH buffer should also be considered.

While the DHC dosage required for bioaugmentation has been studied for unconsolidated materials (e.g., Schaefer et al. 2010), the DHC dosage requirements for fractured rock have not been evaluated. Bench scale studies using rock blocks with single fractures (Figure 4) suggest that a DHC dosage, defined as injected DHC per targeted fracture treatment volume, of approximately 5×10^4 DHC/mL is sufficient (Schaefer et al., 2010b). For the ESTCP demonstration at Edwards AFB, assuming a targeted radius-of-influence of 30 feet and based on the fracture volume determined from tracer testing (Schaefer et al., 2016), a DHC dosage of approximately 10^5 DHC/mL was employed. However, as discussed in Section 2, the total treatment volume was difficult to estimate due to the complexity of the fracture flow paths. While these DHC dosages serve as a guide for field applications, it is recognized that determination of the most efficient DHC dosage for fractured rock systems has yet to be determined. For practical considerations, the cost associated with DHC delivery is typically negligible compared to overall project costs, so a robust injection of DHC is recommended.
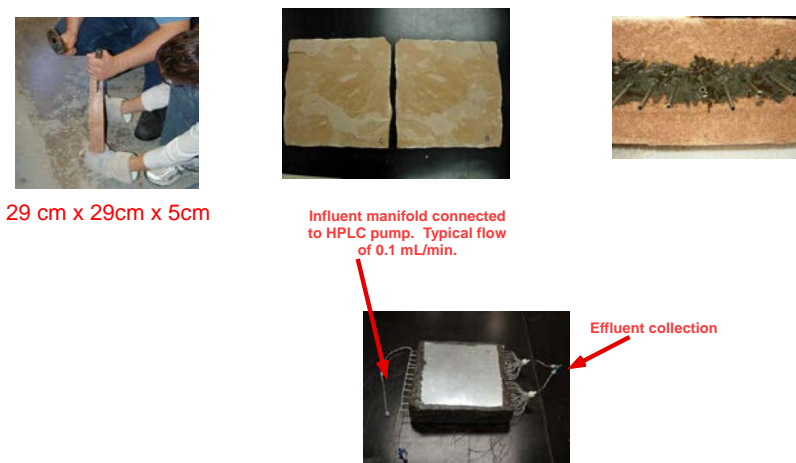
Figure 4. Bench Scale Studies using Rock Blocks with Single Fractures. Construction of single fracture systems in sandstone blocks used to evaluate bioaugmentation of PCE DNAPL sources (Schaefer et al., 2009, 2010).

3.3 Injection and Sampling Methodology

3.3.1 Delivery of Amendments and DHC

Delivery of amendments during active bioremediation approaches typically consists of an initial batch injection to promote anaerobic conditions both within the injection well and the aquifer immediately adjacent to the injection well. This is typically followed by continuous or cycled injection during on-going groundwater recirculation. This approach is expected to promote DHC growth and distribution. Amendment concentrations of 1,000 to 2,000 mg/L of lactate, 100 mg/L diammonium phosphate, and 100 mg/L yeast extract are appropriate for most sites. However, preliminary bench-scale testing is recommended to verify amendment dosages, and to ensure that pH adjustment is not required. A pH between 6 and 8 is ideal. A number of vendors now sell lactate with nutrients already added, which eliminates the need to add them separately in the field.

Upon injection of the initial batch of amendments, recirculation of groundwater should continue for active approaches, with subsequent amendment delivery designed such that the concentrations attained in the recirculation water process stream prior to re-injection are similar to those stated above. The amendments are typically mixed in a holding tank, where (for example) the typically supplied 60% sodium lactate solution is diluted by half to 30% for ease of mixing and injection. A chemical feed pump can be used to inject the amendment solution into the recirculation process stream through non-return (check) injection and anti-siphon valves. A siphon break can also be used on the system manifold piping, if appropriate, though care should be taken to limit air/oxygen from entering the system. Amendments can be delivered continuously during recirculation; however, a pulsed or cycled injection scheme is preferred, allowing delivery of sufficient amendments, while flushing the injection well periodically with un-amended water in an attempt to limit biofouling in or near the injection borehole.

Once recirculation with amendment delivery has been initiated, a DHC-containing consortium can be added to the treatment zone. Several DHC-containing cultures sold through many distributors are commercially available. The DHC-containing consortium can be added directly into the injection borehole, or added to the recirculation process stream, as the system configuration dictates.

3.3.2 Groundwater Sampling

Monitoring wells in fractured bedrock systems are often constructed as open-boreholes, and monitoring of these wells should target the appropriate fracture zones identified during initial site characterization (see Section 2). This can be accomplished through the implementation of a straddle-packer setup, with a top and bottom packer straddling the appropriate vertical monitoring zone containing the fractures being treated. For the ESTCP demonstration at Edwards AFB, groundwater sampling pumps (i.e., bladder pumps) were suspended from the bottom of the top packers, enabling extraction of groundwater from target intervals. Packers should be designed and constructed such that an appropriate number and sizes of pass-through holes (air tight tubes) are available for the down-hole monitoring equipment. This generally includes pass-throughs for nitrogen supply lines for both the bladder pump and inflation of the bottom packer, pump water

discharge line, and water level monitoring access. An example of a straddle packer setup with bladder pump for sampling the intended fracture zone is presented as Figure 5.

Figure 5. Typical Straddle-Packer Setup. Includes a bladder pump suspended between the packers to sample the target fracture zone interval. Tubing associated with bladder pump operation and inflation of the bottom packer, are passed through the top packer using air-tight stainless steel tubes designed and manufactured into the packer.

An initial purge volume with an isolated packer interval, pump bladder, and sample tubing should be performed using standard low-flow groundwater sampling techniques. Groundwater sampling for site-related laboratory analytical parameters should include the collection of purge parameters (e.g., pH, ORP DO, conductivity, turbidity) using a flow through cell and YSI 6920 (or equivalent) multi-parameter water quality meter. For very low transmissivity sampling locations, modified sampling approaches that do not employ the use of low-flow sampling techniques, but still include the purging of the water volume associated with the isolated borehole interval, pump bladder, and sample tubing, can be employed.

As previously noted, FLUTe™ liners are a potential alternative to the sampling methodology described above. One major advantage of the FLUTe™ approach is that the borehole volume is minimized. The need to maintain packers also is eliminated. These advantages need to be considered and weighed against the cost differential and difficulties associated with the potential need to modify sampling intervals.

4.0 PERFORMANCE MONITORING AND TREATMENT CONSIDERATIONS

4.1 Monitoring Parameters

Initial groundwater monitoring should be performed (prior to any groundwater recirculation for active bioremediation approaches) to clearly establish natural gradient baseline conditions. This monitoring should focus on both contaminant and daughter product (including ethene/ethane, *cis*-1,2-dichlorethene (DCE), and vinyl chloride (VC)) concentrations, dissolved metals (Fe, Mn, and As), and DHC levels). Such information is needed to properly assess conditions upon cessation of groundwater recirculation and post-treatment monitoring under natural gradient conditions (Section 5). It is important to note that these parameters may change significantly once groundwater recirculation is initiated, even before any amendments are added (Figure 6). Thus, it is important to establish two sets of "baseline" conditions prior to amendment addition: one under ambient flow conditions, and one under imposed groundwater recirculation conditions. Significant differences were observed during the ESTCP demonstration at Edwards AFB under each of these conditions. Having these baseline data facilitated evaluation of treatment performance both during and after active bioremediation.
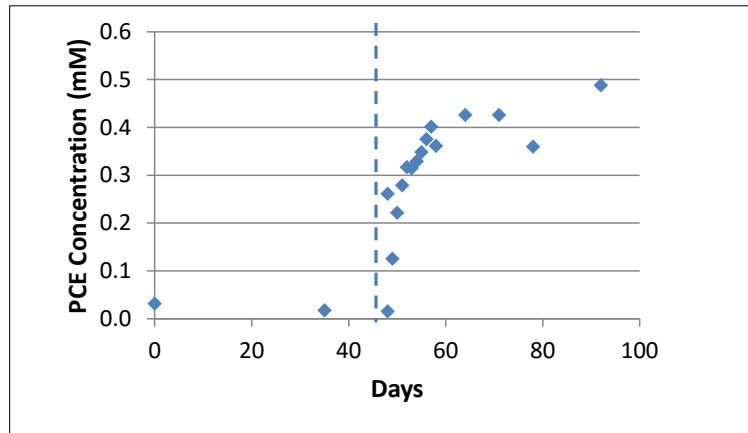
Figure 6. PCE Concentration in Bedrock Monitoring Interval B11. Increase in groundwater PCE concentrations at the shallow monitoring interval at B11 during the ESTCP demonstration at Edwards AFB. The vertical dashed line indicated when groundwater recirculation, extracting from wells B12 and B13 and injecting into B06 (Figure 1), was initiated. These results are prior to any bioremediation amendment addition.

The recommended parameters for monitoring bioaugmentation performance in fractured rock are similar to those that are typically monitored for unconsolidated systems. For active systems, injection pressures and groundwater flows should be monitored. Water elevations in the injection wells also should be continuously monitored. Use of an automated system with proper controls to shut off recirculation if water levels in the injection well get too high, or if sufficient water is not present in extraction wells, is recommended for active systems.

Table 1 summarizes the list of recommended groundwater parameters that should be monitored during bioaugmentation for DNAPL sources in fractured rock. This table serves as a guide for performance monitoring. Site-specific issues or regulatory requirements may require additional monitoring parameters. In general, after attaining the required baseline conditions (under both ambient and groundwater recirculation conditions, if active treatment is performed), initial rounds of monitoring should focus on ensuring that sufficient amendment distribution is occurring. It is noted that the sodium or potassium cations associated with lactate injection can also be used as a tracer, so monitoring for these compounds may be useful. Monitoring during the early stages of treatment also should be performed more frequently, and focus on verifying that the expected biogeochemical reactions are occurring, such as fermentation of the lactate, nitrate and iron reduction, sulfate reduction, and generation of hydrogen. It is noted that accurate *in situ* detection of hydrogen may be problematic due to either sampling issues or *in situ* consumption of hydrogen to trace levels; despite effective biological treatment during the ESTCP demonstration at Edwards AFB, hydrogen detections were sporadic.

Table 1. Recommended Monitoring Parameters.

| Analyte	Comments
Volatile Organic Compounds (VOCs)	Focus on PCE, TCE, DCE, and VC
Reduced Gases	Focus on methane (indicator of methanogenesis), and ethene & ethane (expected final dechlorination daughter products under anaerobic conditions)
Anions	Monitor for nitrate and sulfate reduction, and chloride generation
Dehalococcoides **sp. (DHC)**	qPCR should be employed for this analysis
Volatile Fatty Acids (VFAs)	Monitor for VFAs if lactate is used as the electron donor. The presence of lactate and fermentation daughter products (e.g., acetate) should be evaluated.
Total Organic Carbon (TOC)	TOC can be used in addition to VFA analysis. TOC analysis also can be used if other types of electron donor (e.g., molasses, emulsified vegetable oil) are used.
Hydrogen	The generation of hydrogen can be used to verify fermentation of electron donor.
Metals (Fe, Mn, As)	Increase in Fe and Mn are indicators of reducing conditions. These metals also may be important with respect to groundwater quality.
Cations	Na or K associated with lactate delivery can serve as tracers, although sorption of these cations to aquifer solids can occur.
pH	pH should be monitored to ensure that the pH remains above 6 and below 8.

Monitoring during the early stages of treatment following amendment addition should also carefully focus on changes in pH. The effectiveness of bioaugmentation for PCE and TCE begins to rapidly decrease once the pH deceases below 6 (Vainberg et al., 2009). It is important to note problematic decreases in pH before the pH becomes too low. As discussed in Section 4.5, addition of a pH buffer can be used to remedy this problem.

After this initial phase of monitoring to ensure amendment distribution, biologically-enhanced reducing conditions, and sufficient pH conditions, the subsequent phases of monitoring during active treatment should focus on contaminant dechlorination and DNAPL dissolution (although all parameters should be monitored throughout to ensure no unexpected changes occur). Often, the monitoring frequency can decrease over time, as changes are expected to occur less rapidly. Monitoring for daughter product generation and overall PCE/TCE dechlorination is described in the following section.

4.2 Evaluating Daughter Product Generation

As noted in Table 1 and in the previous section, quantifying daughter product generation is a key component of the bioaugmentation monitoring program. This monitoring is needed to 1) verify that reductive dechlorination of PCE/TCE is occurring, 2) verify that complete dechlorination, evidenced by ethene/ethane generation, is occurring, and 3) ultimately determine the effectiveness of bioaugmentation and the extent of DNAPL removal (discussed in greater detail in Section 4.3).

The key monitoring parameters for daughter products include chloride, TCE, DCE, VC, and ethene/ethane. While background chloride levels in groundwater typically preclude its use in evaluating dechlorination of chlorinated solvents in the aqueous phase, the chloride equivalents present in DNAPL sources often result in chloride generation that significantly exceeds groundwater background levels (Figure 7). The use of chloride as a metric for DNAPL mass removal is preferred over chlorinated ethene and ethene daughter products for estimating DNAPL mass removal. This is because chlorinated ethene daughter products can back-partition into the DNAPL or other organic phases, resulting in an under-prediction of DNAPL mass removal (Schaefer et al. 2010b; Torlapati et al. 2012). In addition, the presence of low levels of dissolved oxygen may be responsible for the dechlorination of vinyl chloride via an oxidative pathway, thus resulting in an underestimate of the extent of DNAPL removal based on ethene molar balance (Gossett, 2010). Also, anaerobic oxidation of ethene also has been observed under sulfate-reducing conditions, which also could impact DNAPL mass balance assessments based on accumulation of ethene (Fullerton et al. 2013).
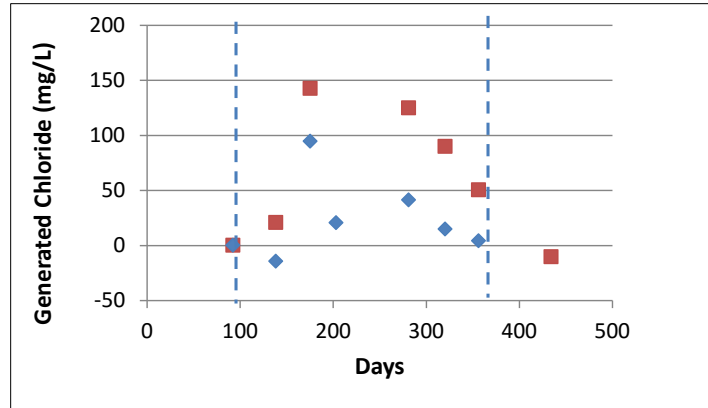
Figure 7. Generated Chloride at Bedrock Monitoring Intervals B11s and B11d.
Generated chloride (above background chloride levels) at B11s (■) and B11d (◆) at Edwards AFB. Vertical dashed lines represent the initiation of bioremediation amendment addition and the cessation of groundwater recirculation.

Several studies have shown that, due to the elevated PCE/TCE concentrations associated with a DNAPL source area, dechlorination will effectively stall at DCE (Adamson et al. 2003; Yang and McCarty 2005; Amos et al. 2008). Thus, bioaugmentation within the DNAPL source area may not exhibit complete dechlorination (at least until the DNAPL mass is partially depleted). The impacts of this on DNAPL dissolution enhancement are discussed in Section 4.3. However, if bioaugmentation is applied immediately downgradient of the DNAPL source area, further dechlorination of the DCE is expected. For the ESTCP demonstration at Edwards AFB, DCE was the primary dechlorination daughter product observed during most of the active treatment phase (i.e., during groundwater recirculation and amendment addition). Complete dechlorination to ethene was not observed until the later stages of the field demonstration (Figures 8 and 9).
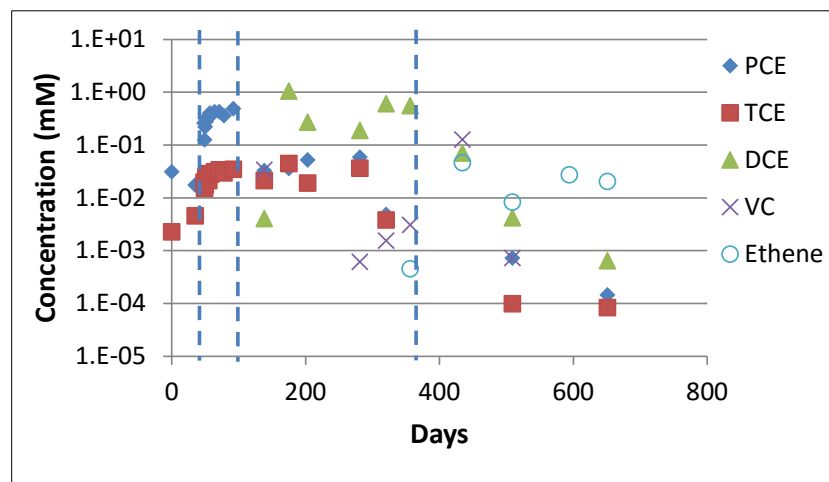
Figure 8. Ethene and Ethane Concentrations at Bedrock Monitoring Interval B11s.
Chlorinated ethene and ethene concentrations at the shallow interval in B11. Only detections of chlorinated ethenes and ethene are shown. Vertical dashed lines represent the start of groundwater recirculation, the initiation of bioremediation amendment addition, and the cessation of groundwater recirculation.
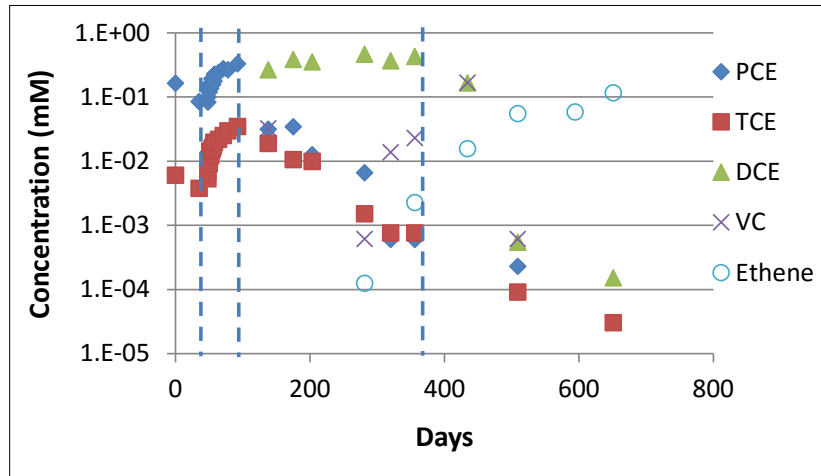
Figure 9. Ethene and Ethane Concentrations at Bedrock Monitoring Interval B11d.
Chlorinated ethene and ethene concentrations at the deep interval in B11. Only detections of chlorinated ethenes and ethene are shown. Vertical dashed lines represent the start of groundwater recirculation, the initiation of bioremediation amendment addition, and the cessation of groundwater recirculation.

4.3 Dissolution Enhancement and DNAPL Removal

There are three key quantitative questions that are critical for assessing bioaugmentation performance in a DNAPL source area:

1) To what extent is bioaugmentation enhancing DNAPL removal?

2) What fraction of the DNAPL mass (or, at least the mass of DNAPL that is along the groundwater flow path as determined by PTTs) has been removed?

3) What is the impact of this DNAPL mass removal on groundwater quality?

The first question above can be addressed by determining the DNAPL dissolution enhancement factor. The dissolution enhancement factor is defined, based on approaches developed by others (Seagren et al. 1994; Schaefer et al. 2010b), as follows:

$$E = \frac{\frac{\left(Cl^*\right)}{Cl^*_{theoretical}}\{[PCE]+[TCE]+[DCE]+[VC]+[ethene]\}_{after\ bioaug}}{\{[PCE]+[TCE]+[DCE]+[VC]+[ethene]\}_{before\ bioaug}} \qquad \text{Eq. 10}$$

where the bracketed terms for PCE, TCE, DCE, VC and ethene are the aqueous molar concentrations, Cl^* is the molar *increase* in chloride following bioaugmentation and $Cl^*_{theoretical}$ is the theoretical *increase* in molar chloride following bioaugmentation based on the amount of chlorinated ethene + ethene daughter products generated. Molar concentrations before and after implementing bioaugmentation are considered in Eq. 10. For the ESTCP demonstration at Edwards AFB, the dissolution enhancement factor (E) was approximately 5, which is in reasonable agreement with bench-scale studies in both

unconsolidated and fractured rock media (Glover 2007; Amos 2009; Schaefer et al. 2010). This result means that application of bioaugmentation increased the rate of DNAPL dissolution 5-times over that which would have been observed if DNAPL dissolution without biological reaction was occurring. It is important to note that this bioaugmentation likely is more effective for PCE than TCE because PCE is substantially less soluble than TCE or DCE. Thus, the dechlorination process increases the effective solubility.

To address the second question, an estimate of the DNAPL mass within the treatment zone prior to initiating bioaugmentation is required (as described in Section 2 and by Schaefer et al., 2016). The mass of DNAPL removed during bioaugmentation treatment can then be estimated as follows (Schaefer et al. 2010b; Torlapati et al. 2012):

$$M_D^* = \frac{Cl^*}{2} V_f MW_{PCE}$$

Eq. 11

where M_D^* is the mass of PCE DNAPL removed during bioaugmentation, Cl^* is the average molar increase in chloride observed at the monitoring well due to the reductive dechlorination of PCE to DCE from DNAPL sources, V_f is the swept volume of water based on the partitioning tracer testing described in Section 2, and MW_{PCE} is the molecular weight of PCE. Equation 11 assumes that PCE is transformed primarily to DCE during DNAPL source area treatment, which is consistent with both laboratory (Schaefer et al., 2010b) and field (Edwards AFB and the Quarry area at Loring AFB) results for bioaugmentation of DNAPL sources. Any dissolved PCE extracted from the extraction wells and re-injected into the injection well must be accounted for in the mass balance so that Cl^* is attributable to DNAPL dissolution only. It is noted that the radial flow assumption used to determine the initial DNAPL mass using PTTs (described in Section 2, and by Schaefer et al. 2016) would also be employed in the calculated value of M_D^* in Eq. 11. Thus, the *fraction* of DNAPL mass removed during bioaugmentation treatment becomes independent of the idealized radial flow assumption, thus Eq. 11 is not dependent upon such idealized flow assumptions for determining fractional DNAPL mass removal.

For the ESTCP project at Edwards AFB, the fractional DNAPL mass removal was determined for both the shallow fracture zone and deep fracture zones between B06 (the injection well) and B11 (a multi-level monitoring location approximately 15 feet downgradient of B06 (Figure 1). After 8 months of active treatment, approximately 100% of the DNAPL was removed from the shallow zone, while only 45% of the DNAPL was removed from the deep zone. Thus, the analysis described in Eq. 11 can be used to track remedial performance with time. The difference in performance between the shallow and deep zones was not solely attributed to DNAPL mass (only 27% more DNAPL mass was present in the deep fracture zone compared to the shallow fracture zone), but also to the DNAPL architecture. Much of the DNAPL mass present in the deep zone was located in the "tailing" portion of the tracer curve shown in Figure 10, suggesting that much of the DNAPL resided in a mass-transfer controlled zone, but still in sufficiently close proximity to the groundwater flow path to be detected using the partitioning tracer test. Thus, insight into the DNAPL architecture can be used to qualitatively predict treatment performance.
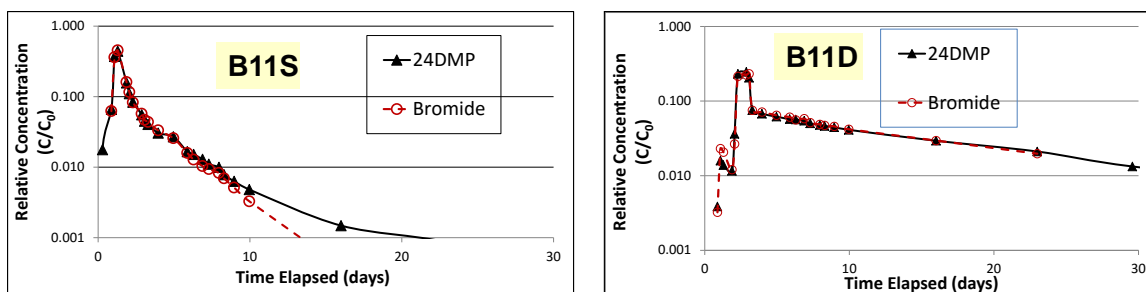
Figure 10. PTT Comparison at Bedrock Monitoring Intervals B11s and B11d. Comparison of PTT for B11 shallow (left) and deep (right) zones during the ESTCP demonstration at Edwards AFB. The mass-transfer controlled tailing zone in B11d (right) corresponded to a lower DNAPL mass removal (45%) compared to that which was observed in the absence of a mass-transfer controlled tailing zone (100% removal for the shallow zone).

Finally, the third key question is related to the impact of DNAPL mass removal (partial or total) on groundwater quality. The impact of partial or complete DNAPL removal on groundwater quality will be dependent upon the fracture flow field, the DNAPL distribution, and matrix back diffusion. For unconsolidated media, models have been developed to predict the impact of partial DNAPL mass removal on groundwater quality (e.g., Falta et al. 2005). These models are dependent upon either an empirical fitting parameter, or a detailed knowledge of the DNAPL distribution relative to the flow field. Even with performance of the partitioning tracer tests described in Section 2, obtaining the information needed to effectively apply these models to fractured bedrock will be very challenging. However, such information is important, as bench scale studies in single blocks containing single fractures showed that removal of only a small fraction of the DNAPL mass can result in a large improvement in groundwater quality (Schaefer et al. 2010b). Thus, depending on the remedial goals, only partial DNAPL removal may be needed.

Remedial progress should be carefully evaluated to determine potential changes in groundwater quality with DNAPL mass removal. During treatment, the molar sum of chlorinated ethene and ethene should be considered, as microbially-enhanced dechlorination may be "masking" the PCE or TCE concentrations that would be present in absence of bioremediation. Pilot tests that focus on only a small portion of the DNAPL source area can also be employed to empirically estimate the impacts of partial DNAPL mass removal on groundwater quality.

4.4 DHC Migration and Growth

Distribution and growth of the bioaugmented DHC is essential for the success of *in situ* treatment. For unconsolidated media, planktonic DHC levels in groundwater on the order of 10^4 DHC/mL typically suggest that the DHC levels are sufficient for dechlorination. However, bench-scale fractured rock studies have suggested that planktonic DHC alone may not be a good indicator of dechlorination activity due to the role of biofilms (Schaefer et al. 2010b). Results from the ESTCP demonstration at Edwards

AFB (Figures 11 and 12) suggest that groundwater sampling confirmed both the migration and growth of DHC 15 feet away from the injection well. Several details are of note:

- DHC levels are similar for the deep and shallow intervals of B11, despite differences in the fracture flow field (Figure 9). This suggests that DHC distribution was not substantially impacted by the lower transmissivity zones associated with B11d.

- While DHC growth is clearly observed in the aqueous phase, the DHC levels observed in the shallow zone of B11 (Figure 11) between 100 and 200 days are not indicative of the extensive dechlorination that is occurring (compare to Figures 7 and 8). This suggests that DHC in biofilms, consistent with previous bench-sale studies (Schaefer et al. 2010b), may be playing an important role and DHC in the aqueous phase may not be indicative of microbial dechlorinating activity.

- DHC levels remain elevated after delivery of biological amendments and groundwater recirculation ceased. However, as discussed in Section 5, the presence of these DHC during the post-treatment phase does not necessarily indicate that dechlorination is continuing.
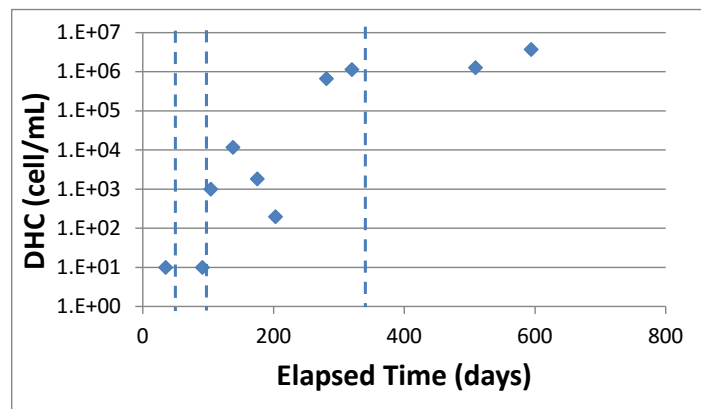
Figure 11. DHC Levels at Bedrock Monitoring Interval B11s. DHC levels measured in groundwater samples measured in the shallow interval of B11 at Edwards AFB. The vertical dashed lines (left to right) represent the initiation of groundwater recirculation, bioaugmentation, and cessation of amendment delivery and groundwater recirculation.
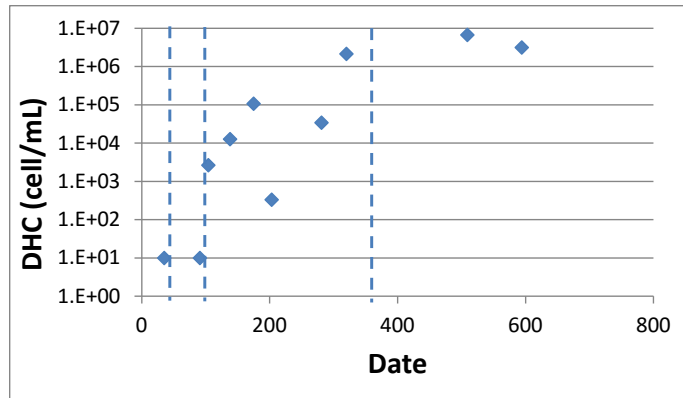
Figure 12. DHC Levels at Bedrock Monitoring Interval B11d. DHC levels measured in groundwater samples measured in the deep interval of B11 at Edwards AFB. The vertical dashed lines (left to right) represent the initiation of groundwater recirculation, bioaugmentation, and cessation of amendment delivery and groundwater recirculation.

4.5 Contingencies

Modifications or additions to the biological amendments, whether employing an active or passive remedial approach, may be needed based on groundwater results obtained during performance monitoring. Such contingencies are similar to those typically considered for bioaugmentation in unconsolidated systems. For example, pH buffer may be required if the pH begins to decrease below 6. Sodium bicarbonate was used for several weeks during the Edwards AFB demonstration to ensure the pH remained >6. Electron donor (e.g., lactate) levels also can be increased during the demonstration if there is an absence of volatile fatty acids present (including fermentation daughter products). Electron donor levels may be increased if sufficiently reducing conditions, as indicated by the absence of sulfate or iron reduction, is not obtained. When treating a DNAPL source area, it is also important to consider that nutrient depletion may become an issue due to the relatively large contaminant mass. Thus, if dechlorination rates begin to stall, delivery of nutrients (including micronutrient sources such as yeast extract and vitamin B12) should be considered.

5.0 POST TREATMENT

Evaluating remedial impacts to groundwater quality during active treatment often is impeded due to mixing effects and on-going dechlorination reactions. Thus, post-treatment monitoring, after cessation of amendment delivery and groundwater recirculation, is typically needed to fully assess remedial impacts on groundwater quality. For bioaugmentation, post-treatment assessment is complicated by the fact that the microbial dechlorination reactions may persist for months (or perhaps years) after active treatment is completed. This sustained reaction requires careful evaluation of the post-treatment, or "rebound", data to properly assess overall impacts to groundwater.

Post-treatment monitoring was performed during the ESTCP demonstration at Edwards AFB over a 238 day duration, as shown in Figures 7 and 8. After cessation of the groundwater recirculation and amendment delivery at 356 days, DCE concentrations in both the shallow and deep intervals of B11 began to rapidly decrease, with a transient increase in VC and increased ethene generation. Initially, for both the shallow and deep intervals, there was a molar decrease in the sum of chlorinated ethenes + ethene during the beginning stage of the post-treatment period. The decrease in the molar balance after rebound is explained by 1) eliminating the re-injection of high concentration PCE groundwater from the extraction wells as ambient flow conditions were resumed and elevated ethene levels were "flushed" out of the treatment zone, and 2) VC and ethene diffusive uptake into the rock matrix. Assessment of elevated sodium levels (from addition of sodium lactate during the recirculation phase) showed that approximately half of the sodium was depleted by 8 months into the rebound period in both the shallow and deep zones. Thus, the decrease in the elevated chlorinated ethenes + ethene is only partially explained by dilution due to ambient groundwater flow. Comparison of the relative importance of matrix diffusion effects on solute transport has been previously performed for both the shallow and deep fracture zones during groundwater recirculation (Schaefer et al. 2016). Using this approach, but applying a mean residence time of 8 months for ambient flow conditions, dimensional analysis suggests that matrix diffusion effects in the shallow zone will be significant. Due to the complex flow paths present in the deep zone (Figure 9), a similar assessment is not possible, but the mass transfer limitations observed during the tracer test in the deep zone suggests diffusive controls on solute migration are likely. Thus, dissipation of the generated VC and ethene in both the shallow and deep zones near the beginning of the rebound period is likely due to both ambient groundwater dilution and diffusion into low flow (e.g., rock matrix) zones.

Following the initial decrease in molar concentrations observed at the beginning of the post-treatment rebound period, an increasing trend in total molar chlorinated ethene + ethene was observed for B11d, while no increasing trend was observed for B11s. Total molar chlorinated ethene + ethene concentrations more than double over the last 3 sampling events for B11d. Sulfate levels remained low and ferrous iron levels remained elevated at B11d; volatile fatty acid levels also remained elevated. These data indicate that strongly reducing conditions favorable for the complete dechlorination of PCE were maintained at B11d throughout the rebound period, and that the increasing trend in ethene over the last few monitoring events would likely have been PCE in the absence of this sustained biotic dechlorination reaction (i.e., the PCE that was rebounding was being converted to ethene);

this result is consistent with the fact that DNAPL sources remained in the deep fracture zone (Section 4.6 and Figure 9). In contrast, by 5 months into the rebound period at B11s, volatile fatty acids became depleted, sulfate levels had increased from non-detect levels, and dissolved iron levels decreased, which together suggest that strongly reducing conditions were not maintained throughout the rebound period at this location and reductive dechlorination to ethene was likely no longer occurring. Despite this lack of dechlorination activity, no rebound in any of the chlorinated ethenes or ethene was observed; this result is consistent with the removal of DNAPL sources in the shallow fracture zone, as discussed in Section 4.6.

The assessment presented in the previous paragraphs provides an example of how post-treatment monitoring can be used to evaluate remedial performance. While a 238-day rebound period was performed, a longer rebound assessment (1 to 2 years) would have been preferred. The Edwards AFB study highlights the importance of careful post-treatment monitoring, and the need to perform this monitoring over an extended period.

6.0 OTHER CONSIDERATIONS

6.1 Fouling of Injection Wells

Biofouling may occur within the injection well(s) if significant changes in geochemical conditions are induced by the added amendments or if oxygen enters the system during operation. Preventative measures can be taken to reduce the likelihood of biofouling, and corrective measures can be taken to reduce effects if it occurs. Preventative measures include the periodic (rather than continuous) introduction of amendments to the system (as discussed previously). The injection of slightly elevated amendment concentrations, which suppresses biological growth within the injection wellbore, also discourages biofouling. Treatment options if biofouling occurs include the redevelopment of the wellbore or the use of approved chemicals and/or biocides (ideally NSF-certified for potable water wells). Low concentration hydrogen peroxide has proven effective in reducing biofouling, though effective removal of the hydrogen peroxide after use is required.

6.2 Secondary Groundwater Impacts

The changes in geochemical conditions induced by bioaugmentation can result in secondary groundwater impacts. Typically, the generation of dissolved metals, including iron, manganese, and arsenic, is of greatest concern. While increases in dissolved iron and arsenic were observed during treatment at Edwards AFB, concentrations of both began to decrease during the post-treatment period in the shallow zone; dissolved concentrations of both iron and manganese remained elevated in the deep zone where reducing conditions persisted. Thus, while transient impacts of dissolved metals are likely during treatment, adverse impacts will be rapidly mitigated once ambient geochemical conditions are restored, thereby limiting impacts both spatially and temporally.

7.0 SUMMARY

While applying bioaugmentation to treat chlorinated solvent DNAPL sources in fractured rock continues to be a challenge, tools and approaches have been developed to mitigate the difficulty associated with these sites. Highlights from this guidance, based in large part on recent SERDP and ESTCP bedrock projects, include:

- Determination of DNAPL architecture is important for proper management of DNAPL sources in fractured rock;

- Partitioning tracer testing can be a useful tool for locating and quantifying DNAPL sources in fractured rock. These partitioning tracer tests can be applied as push-pull tests, or as interwell tracer tests;

- The effectiveness of bioaugmentation with respect to DNAPL source treatment is dependent upon the DNAPL mass present, and the location of the DNAPL sources relative to the fracture flow field. DNAPL present in lower transmissivity fracture zones, as identified through partitioning tracer testing, may be more challenging to treat;

- DCE accumulation may occur within the DNAPL source area until PCE/TCE levels begin to decrease;

- Chloride generation is a useful metric for assessing DNAPL removal, as DNAPL mass removal estimates based on chlorinated ethene and ethene/ethane transformation products alone may significantly underestimate DNAPL mass removal;

- Aqueous phase DHC may be a poor metric for assessing dechlorination activity during bioaugmentation, as immobile DHC (e.g., biofilms) may play a dominant role in the observed treatment; and

- Post-treatment monitoring, accounting for on-going dechlorination reactions, can provide valuable insight with respect to source mass removal and long term groundwater quality.

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