NAVFAC

Naval Facilities Engineering Command

ENGINEERING AND EXPEDITIONARY WARFARE CENTER

TECHNICAL REPORT
TR-NAVFAC EXWC-EV-1601

BIOGEOCHEMICAL TRANSFORMATION HANDBOOK

This document was a joint effort of
NAVFAC and AFCEC

Prepared for NAVFAC EXWC under Contract No. N62583-11-D-0515

October 2015

This page intentionally blank.

REPORT DOCUMENTATION PAGE

FORM APPROVED
OMB NO. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From – To)*
01-10-2015	Technical Report	2015-

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
BIOGEOCHEMICAL TRANSFORMATION HANDBOOK	N62583-11-D-0515/TO 64
	5b. GRANT NUMBER
	N/A
	5c. PROGRAM ELEMENT NUMBER
	N/A

6. AUTHOR(S)	5d. PROJECT NUMBER
Ramona Darlington (Battelle)	N/A
Heather Rectanus (Battelle)	**5e. TASK NUMBER**
	N/A
	5f. WORK UNIT NUMBER
	N/A

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
Battelle 505 King Avenue Columbus, OH 43221	N/A

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR / MONITOR'S ACRONYM(S)
NAVFAC EXWC 1000 23rd Ave. Port Hueneme, CA, 93043	NAVFAC EXWC
	11. SPONSOR / MONITOR'S REPORT NUMBER(S)
	TR-NAVFAC EXWC-EV-16010

12. DISTRIBUTION / AVAILABILITY STATEMENT

Distribution Statement A: Approved for public release; Distribution is unlimited

13. SUPPLEMENTARY NOTES

Click here to enter text.

14. ABSTRACT

In situ biogeochemical transformation (ISBGT) processes result in the degradation of contaminants through combined biological, mineral, and chemical pathways. The handbook can serve as a key resource for Remedial Project Managers and stakeholder teams in evaluating, selecting, and/or implementing ISBGT at Navy sites. In this handbook, the fundamentals of ISBGT are presented in a question and answer format. The mechanisms that contribute to ISBGT processes and what contaminants are degraded by ISBGT are explored. Key considerations for enhancing, monitoring, and evaluating ISGBT processes are provided. This handbook also presents the importance of site characterization in recognizing and accounting for the contributions of ISBGT as part of monitored natural attenuation.

15. SUBJECT TERMS

Click here to enter text.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					Tony Nelson
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	U	41	**19b. TELEPHONE NUMBER** *(include area code)*
Click here	Click here	Click here			805-982-2632

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

This page intentionally blank.

Acronyms and Abbreviations

AFCEE	Air Force Center for Engineering and the Environment
AVS	acid volatile sulfide
BB1	Desulfuromonas michiganensis strain BB1
BDI	BioDechlor Inoculum
bgs	below ground surface
C-C	carbon-carbon
CRS	chromium reducible sulfide
DCE	dichloroethene
EDB	ethylene dibromide
EPA	U.S. Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
Fe(II)	reduced ferrous iron
Fe(III)	reduced ferric iron
FeS	mackinawite
FeS_2	pyrite
g/L	gram per liter
ISBGT	in situ biogeochemical transformation
MCL	maximum contaminant level
mg/kg	milligram per kilogram
mg/L	milligram per liter
MNA	monitored natural attenuation
mV	millivolts
ORP	oxidation reduction potential
PCE	tetrachloroethene
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
SEM-EDS	scanning electron microscopy/energy dispersive spectroscopy
Sm	Sulfurospirillum multivorans
TCA	trichloroethane
TCAAP	Twin Cities Army Ammunition Plant
TCE	trichloroethene
TCLP	toxicity characteristic leaching procedure

USGS   United States Geological Survey

VC   vinyl chloride
VOC   volatile organic compound

XAS   X-ray absorption spectroscopy
XANES   X-ray adsorption near edge spectroscopy

ZVI   zero valent iron

Table of Contents

List of Tables

List of Figures

This page intentionally blank.

1.0   INTRODUCTION

This handbook provides an introduction into the physical, chemical, and biological processes that have the potential to transform contaminants (principally chlorinated solvents) in groundwater and introduces an innovative treatment process by which these mechanisms are exploited for treating contaminated groundwater – in situ biogeochemical transformation (ISBGT). The handbook can serve as a key resource for Remedial Project Managers and stakeholder teams in evaluating, selecting, and/or implementing ISBGT at Navy sites. In this handbook, the fundamentals of ISBGT are presented in a question and answer format. A variety of questions such as what mechanisms contribute to ISBGT processes and what contaminants are degraded by ISBGT are explored. Key considerations for enhancing, monitoring, and evaluating ISGBT processes are provided. This handbook also presents the importance of site characterization in recognizing and accounting for the contributions of ISBGT as part of monitored natural attenuation (MNA).

2.0   WHAT IS IN SITU BIOGEOCHEMICAL TRANSFORMATION?

ISBGT is the transformation of contaminants by highly reduced iron minerals that have been formed by microbial activity. ISBGT processes result in the degradation of contaminants through combined biological, mineral, and chemical pathways.

The contaminants can be transformed abiotically through a chemical reaction with reduced iron (Fe[II]) minerals. These minerals can be present naturally as part of the site geology or formed by microbial activity (Environmental Security Technology Certification Program [ESTCP], 2008). Iron sulfides, such as mackinawite (FeS) and pyrite (FeS_2), are the primary minerals formed by microbial activity and are capable of abiotic contaminant transformations.

As shown in Figure 1, the first step to iron sulfide formation is biogenic and occurs when dissolved sulfate is reduced by sulfate-reducing bacteria under anaerobic conditions to form hydrogen sulfide. In Step 2, hydrogen sulfide interacts with the aquifer matrix to precipitate iron sulfides in one of two ways:

- Aqueous (i.e., dissolved) ferrous iron in the groundwater instantaneously reacts with hydrogen sulfide to form iron sulfides. Aqueous Fe(II) is generated when bioavailable Fe(III) is reduced by iron-reducing bacteria. This Fe(III) can be available in situ or provided in several different forms including Fe(III) oxyhydroxide (goethite, [α-FeOOH]), ferrihydrite (Fe(OH), hematite and even magnetite.

- Reductive dissolution of Fe(III) oxyhydroxides by hydrogen sulfide also forms iron sulfides.

In the final and third step of ISBGT, the highly reactive iron sulfides abiotically transform the contaminants. Perchloroethene is the contaminant shown in Figure 1.

Step 1 Biotic	Step 2 Precipitation	Step 3 Abiotic
$SO_4^{2-} + 9H^+ + 8e^- = 8HS^- + 4H_2O$ $SO_4^{2-} + 2CH_2O \rightarrow H_2S + 2HCO_3^-$	$HS^- + Fe^{2+} = FeS + H^+$ $2FeOOH + 3HS^- = 2FeS + S + H_2O + 3OH^-$	$C_2Cl_4 + FeS + 4H_2O = C_2H_2$ $+Fe^{3+} + SO_4^{2-} + 4Cl^- + 6H^+$
Days	Instantaneous	$t_{1/2} = 30 \pm 15$ days

(Courtesy of Battelle)

Figure 1. Mechanism of ISBGT Showing Formation of Iron Sulfides and Their Transformation of Chlorinated Solvents

ISBGT processes were discovered in part through numerous research efforts using zero valent iron (ZVI) for treatment of chlorinated solvents. In laboratory studies, abiotic transformation of contaminants was observed with zero valent metals such as iron and copper (Gillham and O'Hannesin, 1994). As ZVI reduces the contaminant, the iron becomes oxidized and Fe(II) bonds to the surface of ZVI. It was discovered that this surface-bound Fe(II) participates in the transformation of contaminants (U.S. Environmental Protection Agency [EPA], 2009). This discovery initiated new research studies focused on the ability of iron minerals to degrade contaminants (volatile organic compounds [VOCs], pesticides, metals, and munitions constituents). The iron minerals investigated included iron sulfides (Butler and Hayes, 1999 and 2001), magnetite (Lee and Batchelor, 2002a), and green rusts (Lee and Batchelor, 2002b; O'Loughlin and Burris, 2004). These studies revealed that reduced iron minerals can play a significant role in the degradation of contaminants.

Several years later at Altus Air Force Base, iron sulfides were detected in the field where a petroleum hydrocarbon and trichloroethene (TCE) plume intersected. Downgradient of the intersection, TCE concentrations decreased to below maximum contaminant levels (MCLs) without evidence of biodegradation products being formed. Upon evaluation of the site conditions, high levels of sulfate (2,000 mg/L) in groundwater due to gypsum lenses in the site's geology and high Fe(III) levels (>2,000 mg/kg) in the soil chemistry were discovered. This intersection of electron donor (petroleum hydrocarbon), sulfate, and ferric iron minerals provided the conditions needed to form reactive iron sulfide minerals and result in abiotic degradation of TCE (Kennedy et al., 2006). Similarly, iron sulfide formation has been observed at sites where enhanced bioremediation followed in situ chemical oxidation via iron-activated persulfate (Darlington et al., 2010, 2011). These observances of ISBGT occurring naturally and fortuitously led to the advent of enhancing biogeochemical reactions in situ by amending the subsurface to favor the formation of iron sulfide minerals.

3.0   WHAT MINERALS ARE INVOLVED IN ISBGT?

The minerals typically involved in abiotic transformations include iron sulfides (mackinawite and pyrite), iron oxides (magnetite), green rust, and phyllosilicate clays (vermiculite and biotite). Iron sulfides are often found naturally in anaerobic sediments such as wetlands and salt marsh environments. Green rusts are found naturally in soils and sediments in suboxic (low levels of oxygen and sulfur present simultaneously) and anoxic (no oxygen) conditions.

Iron sulfides, magnetite, and green rust have been studied the most since they are the most reactive. Among these three types of iron minerals, iron sulfides are the best understood. Research has shown that reaction rates are several orders of magnitude greater for mackinawite than magnetite, which reacts faster than green rust.

Since the reactions with iron minerals are surface reactions, the greater the mineral surface area, the faster the reaction rate due to the larger cumulative surface area in smaller grain sizes. In addition, studies have shown that the presence of dissolved ferrous iron (Fe[II]]) sorbed to the iron mineral surface enhances reaction rates (EPA, 2009). The results of these iron mineral studies correlate well to past ZVI studies indicating ferrous iron on the surface of ZVI participates in the transformation of contaminants. It should be noted that all reactive iron minerals are interrelated, which makes studying and understanding their reactivity complex. For example, hematite is transformed to magnetite in the presence of Fe(II), and magnetite is transformed to pyrite, while under sulfate-reducing conditions. Each of these reactive species has different reaction rates, and the reaction rates can be dependent on contaminant type, ferrous iron concentration, and surface area. Therefore, maintaining an accurate understanding of the site's geochemistry is a necessity when assessing the impact of ISGBT.

4.0   WHICH CONTAMINANTS CAN BE TRANSFORMED BY ISBGT?

Reactive minerals are able to abiotically transform a variety of contaminant classes. For example, ISBGT processes mediated by iron minerals have been observed for VOCs, pesticides, munitions constituents, and metals in both laboratory and field studies. Among these examples, transformation of chlorinated solvents by iron minerals has been researched the most. Mechanisms of transformation are not fully understood and it is possible that contaminant transformation pathways are different depending on the mineral. Given that the abiotic transformation mechanisms for iron minerals are the best understood, the discussion below provides the current understanding of abiotic processes mediated by iron minerals.

- *Chlorinated Solvents*: The majority of chlorinated solvent studies have focused on the reaction of tetrachloroethene (PCE) and TCE with FeS; only limited studies have included *cis*-dichloroethene (DCE) and vinyl chloride (VC) as contaminants. The mechanism of abiotic transformation of chlorinated ethenes is dihaloelimination, where two adjacent chlorines are removed forming an additional carbon-carbon (C-C) bond. For TCE, chloroacetylene is formed via dihaloelimination leading to the formation of acetylene. Conversely, hydrogenolysis, which is the pathway for biotic reductive dechlorination that sequentially removes chlorine atoms, is a minor abiotic transformation pathway. As a result, the pattern for abiotic transformation of chlorinated ethenes is *simultaneous* degradation of all chlorinated ethenes present (as seen in Figure 2) in contrast to *sequential* dechlorination of parent then daughter products as seen during biological degradation.
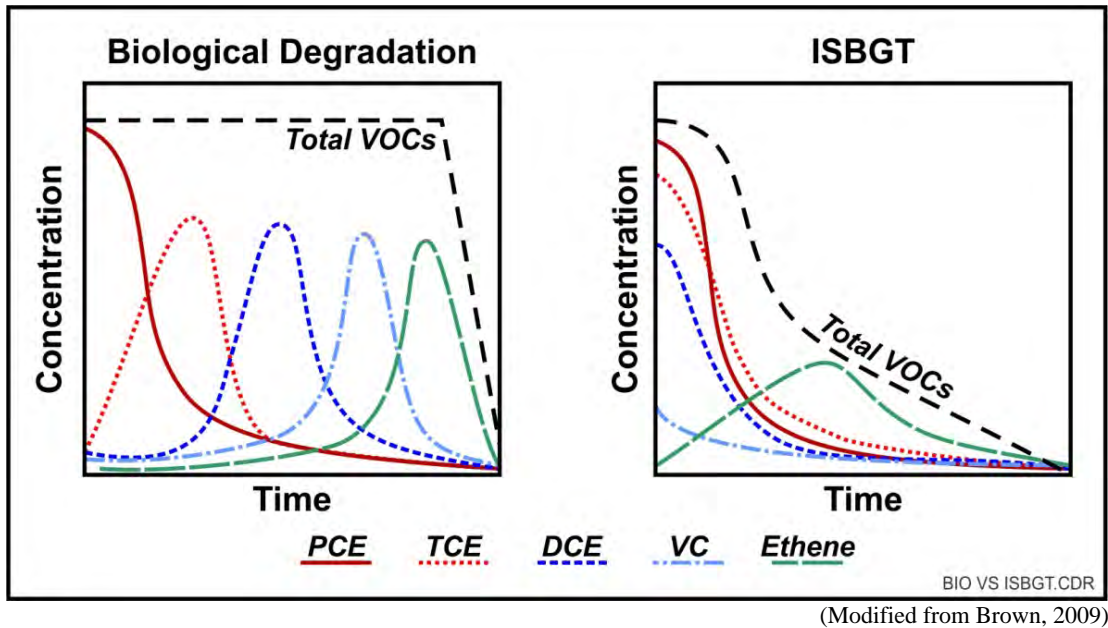
Figure 2. Abiotic versus Biological Degradation Pattern for Chlorinated Solvents

The dominant transformation product of chlorinated ethenes in the presence of iron sulfides is acetylene. For example, as much as 60 to 70% of chlorinated ethenes were transformed in laboratory studies to acetylene by iron sulfides (e.g., FeS, pyrite) via chloroacetylene (EPA, 2009). Acetylene is energetically favorable to support microbial growth and is therefore used quickly once formed. As such, it is difficult to detect acetylene in the field. Similarly, chloroacetylene is difficult to detect in the field. Therefore, abiotic transformation pathways are often characterized as not having observable transformation products.

Figure 3 presents possible abiotic degradation pathways for chlorinated ethenes by FeS and magnetite. Major reaction pathways are represented with black arrows, while minor degradation pathways are shown with orange arrows. For both minerals, rates of reaction for abiotic degradation pathways have been found to be first order or represented as a surface normalized first order reaction rate. Although limited information is available on the degradation of chlorinated solvents with magnetite, several studies have observed a reduction in chlorinated ethanes in the presence of magnetite (Lee and Batchelor, 2002a; Ferrey et al., 2004). However, no degradation products were identified in these studies. Darlington et al. (2008) observed the formation of organic acids in the presence of chlorinated ethenes and iron minerals. Later, these organic acids were postulated as degradation products of abiotic transformation by magnetite (Darlington et al., 2013). Concentrations of Fe(II) sorbed to the surface of magnetite were directly related to the contaminant transformation rate (i.e., as sorbed ferrous iron increased, degradation rates increased).

Limited reaction has been seen with chlorinated ethanes in the presence of iron sulfides. However, as much as 90% removal of chlorinated methanes (specifically, carbon tetrachloride to chloroform, formate carbon disulfide, and carbon dioxide) has been seen to occur (Devlin and Müller, 1999).
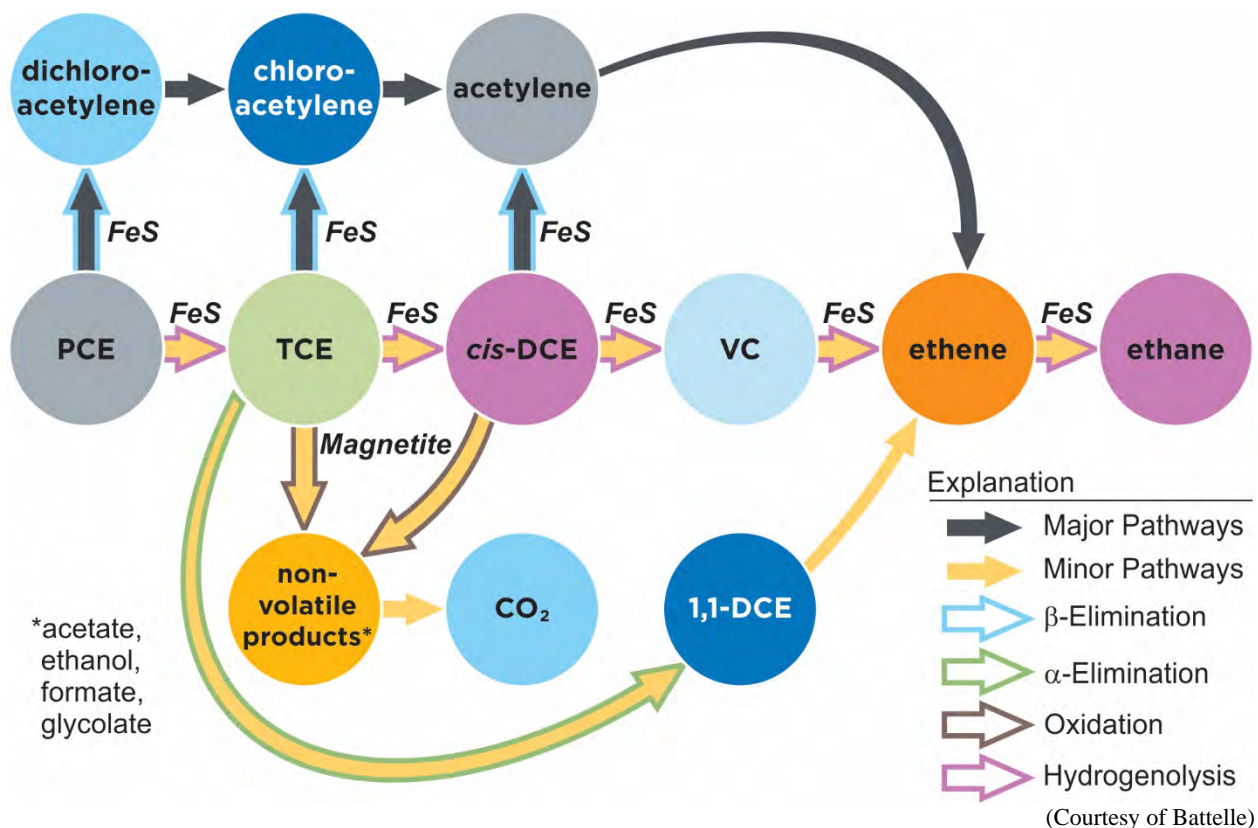
Figure 3. Major and Minor Degradation Products of Chlorinated Ethenes with Different Minerals

- *Pesticides*: Pesticides and insecticides are highly oxidized and polyhalogenated structures have significant potential to benefit from abiotic transformations. Pesticides such as ethylene dibromide (EDB) degrade in the presence of iron sulfide minerals with the major degradation product being ethene (Wilson et al., 2008). The rate of degradation of EDB by FeS follows first order kinetics without a lag. Immediate reaction rates indicate abiotic degradation mechanisms opposed to biotic degradation mechanisms because the reaction is not initially limited or lagging due to a low population of microorganisms. Similarly, the insecticide hexachlorocyclohexane, also known as lindane, can undergo abiotic transformations in the presence of iron monosulfide. The transformation products are less chlorinated aromatics that have shorter environmental half-lives than the parent compound (Liu et al., 2003).

- *Munitions Constituents*: With the slower rates of biodegradation for munitions constituents, research into the potential for faster rates via abiotic transformations has been conducted. Similar to chlorinated solvents, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) appeared to be reduced completely in the presence of ZVI (Oh et al., 2005). In microcosms, rapid degradation of RDX was observed in the presence of iron (hydr)oxides, ferrous iron, and alkaline pH (Boparai et al., 2008). Studies also showed that Fe(II) bound to solid surfaces such as magnetite contributed to degrading RDX (Gregory et al., 2004; Kim and Strathmann, 2007). In these studies, no reaction occurred with Fe(II) alone or with magnetite alone. However, when Fe(II) and magnetite were combined, RDX was abiotically transformed, and

the RDX degradation rates increased as pH increased. A comparison of biotic and abiotic degradation efficiencies in separate treatments in a seven-day bench-scale study indicated that abiotic degradation was 50% more effective at degrading aqueous RDX concentrations (Britto and Nolin, 2012). These mechanisms occur simultaneously in the field. Pilot studies are being conducted using a variety of amendments such as dissolved iron, iron salts, sulfate, organic substrates, and organo-green rust (Britto and Nolin, 2012).

- *Metals:* Stabilization of metals in the form of metal sulfides has been demonstrated both in the laboratory and under field conditions (Zhong et al., 2009; Gong et al., 2012). Metals stabilized as metal sulfides include mercury, lead, and uranium. At a site with lead concentrations as high as 2,400 mg/L, lead was precipitated as lead sulfide by the addition of sulfate and mushroom compost. The compost served as an electron donor for sulfate-reducing bacteria to stimulate sulfide generation and subsequent formation of lead sulfide. The precipitated lead sulfide was stable at multiple pH levels, and toxicity characteristic leaching procedure (TCLP) tests of the soil found that immobilized lead was not toxic. Rather, TCLP lead concentrations were an order of magnitude below the regulatory limit of 5 mg/L. Similarly, mercury was stabilized using iron sulfide nanoparticles in both laboratory and field-scale studies (Zhong et al., 2009; Gong et al., 2012). As with lead, TCLP tests demonstrated that the leachability of the stabilized mercury was reduced two orders of magnitude (Zhong et al., 2009; Gong et al., 2012; Figure 4). Practitioners should be aware that, in some cases, if the geochemical conditions change, the metal sulfide may solubilize and put the metal back in solution.

Although ISBGT is known to be an effective transformation method for a variety of contaminant classes, the bulk of research has been done on chlorinated solvents. Therefore, the remainder of this document refers to abiotic transformation mechanisms with respect to chlorinated solvents.
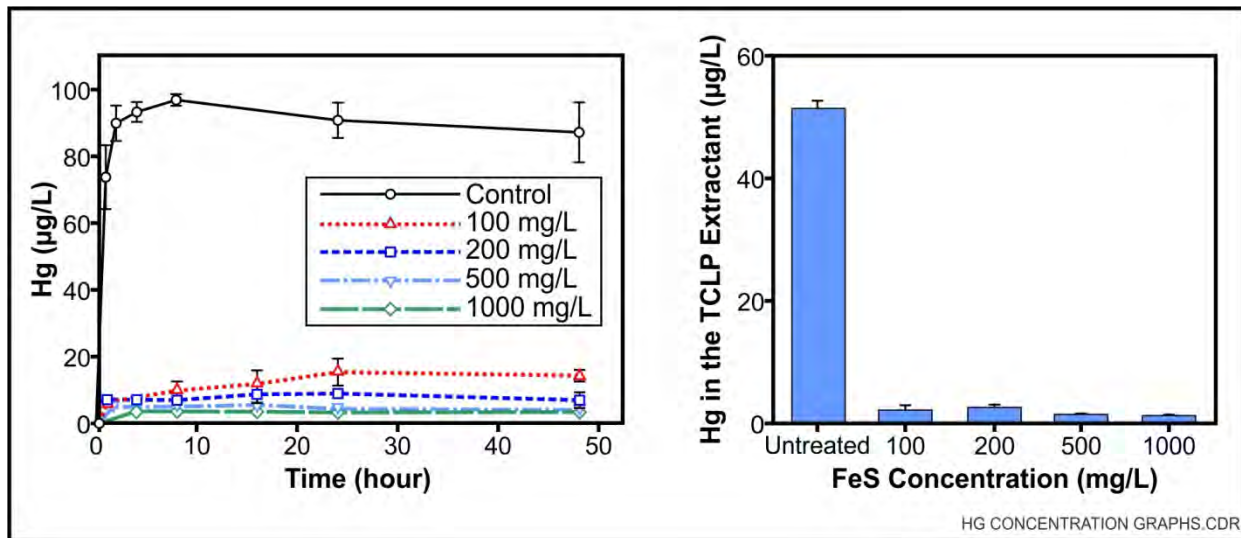
(Modified from Zhong et al., 2009)

Figure 4. Mercury Concentration in Soil after Treatment with Different Concentrations of FeS and Mercury Concentrations in TCLP of both Treated and Untreated Soil

5.0   HOW DOES ISBGT CONTRIBUTE TO MNA?

The primary attenuation mechanism for MNA has long been attributed to microbial degradation. However, observations and reports of contaminants such as chlorinated solvents being transformed abiotically have challenged this attribution. To this end, the recently published ESTCP document, ER-201211, "Frequently Asked Questions about Monitored Natural Attenuation in Groundwater" includes a chapter on how reactive minerals contribute to MNA, recognizing that reactive minerals can, and often do, contribute to natural attenuation in groundwater plumes (Adamson and Newell, 2014). Below are three examples where ISBGT was recognized as a primary mechanism for MNA at these sites:

- At the Twin Cities Army Ammunition Plant (TCAAP) in Minnesota, a large dilute TCE and trichloroethane (TCA) plume in iron-reducing and manganese-reducing conditions showed evidence of microbial reduction of TCE to *cis*-DCE. However, VC and ethene daughter product concentrations detected in the groundwater were low and never above 10% of the TCE detected in the groundwater. In addition, as the TCE concentrations decreased, the *cis*-DCE concentrations did not increase stoichiometrically. The simultaneous degradation pattern where both TCE and *cis*-DCE degrade is characteristic to abiotic transformations as is observing low levels of VC and ethane. Given the potential contribution of ISBGT at this site, laboratory studies were conducted to evaluate the ability of reactive minerals in the rock matrix to abiotically degrade TCE and *cis*-DCE. The results of microcosms constructed with site soil and groundwater demonstrated that abiotic degradation could occur at the site by showing the same contaminant transformation patterns in both the live and killed treatments (sodium azide added to kill all bacteria). Mineral analysis reported 0.3 weight percent of magnetite in the soil matrix. With these two lines of evidence for ISGBT, the primary attenuation mechanism at this site was recognized as abiotic degradation by magnetite (Ferrey et al., 2004).

- At an industrial facility in Southern California, field data from a fractured sandstone aquifer contaminated with TCE indicated that TCE was reduced to *cis*-DCE. However, only trace levels of VC and ethene were observed even though the concentration of TCE and *cis*-DCE decreased over time. As in the previous example, microcosm studies were conducted to evaluate the ability of minerals to degrade contaminants. The laboratory study used crushed fractured sandstone from the site and showed degradation of TCE and *cis*-DCE in both the live and killed controls. In the live treatment, TCE was converted biologically to *cis*-DCE. Additionally, acetate, glycolate, formate, and carbon dioxide were detected in the live treatments. In the killed treatment, significant formation of acetate, glycolate, and formate was observed with minimal amounts of *cis*-DCE and carbon dioxide (Darlington et al., 2008). Further analysis of the sandstone from the site indicated the presence of several iron minerals, including magnetite and goethite (Darlington et al., 2013). Similar to the TCAAP in Minnesota, the combination of degradation pattern, microcosms, and mineral analyses demonstrated that ISGBT processes contributed to the attenuation of contaminants at the industrial facility.

- A laboratory experiment was conducted with bedrock cores from the former Naval Air Warfare Center in Trenton, New Jersey. The cores were left intact and TCE was applied to the cores to determine if abiotic reactions could occur in the matrix of cores that had been

exposed to TCE in the field (Schaefer, 2014) and cores that had not been exposed to TCE in the field (Schaefer et al., 2013). In both instances, acetylene, ethane and ethene were the observed degradation products and propane in some instances. The mineralogy of the core samples was analyzed by scanning electron microscopy and measurable levels of Fe(II) were found in the cores. The detection of acetylene and the presence of iron minerals suggest that abiotic mechanisms played a role in the transformation of the contaminant.

6.0   HOW CAN A SITE BE INVESTIGATED FOR ISBGT DURING MNA?

Although there is not currently a protocol that standardizes the approach for assessing abiotic transformation of contaminants within the context of MNA[1], the industry recognizes that sites undergoing abiotic transformations exhibit specific characteristics (Brown et al., 2007). These characteristics are described below and can be used to assess a site for the contributions of ISBGT processes:

- **Abiotic Signature:** The degradation patterns for chlorinated solvents via biotic and abiotic pathways are well understood. Biological degradation leads to the sequential appearance of chlorinated daughter products (e.g., *cis*-DCE and VC), while abiotic degradation leads to the reduction in concentrations of all chlorinated solvents simultaneously (Figure 2). This is observed when monitoring contaminant and daughter product concentration in a single well. The ISBGT abiotic signature is a parallel decline of contaminant and daughter product concentration simultaneously.

- **Plume Degradation Pattern:** Sites where biological degradation of PCE and/or TCE contamination produces *cis*-DCE with little evidence of VC or ethene generation are often labeled as experiencing "*cis*-DCE stall". However, ISBGT processes may be responsible for *cis*-DCE transformation and therefore investigation for ISBGT pathways is warranted. This characteristic loss of parent compound without the appearance of daughter products is characteristic of ISBGT. Plotting molar concentrations versus distance at sites where abiotic transformations are the dominant degradation mechanism shows a parallel decline in concentration. In contrast, when biological degradation occurs, a divergence of concentration with distance occurs. This occurs because when abiotic transformation is the dominant mechanism throughout the plume (especially in the downgradient more dilute plume), the concentration of the daughter products that may be present in the source area decreases throughout the plume simultaneously. When biological degradation occurs, contamination in the source area degrades forming daughter products and daughter products flow downgradient.

- **Mineral Characterization:** The mineral composition in the soil matrix is critical to assess whether abiotic transformations are occurring. Several methods can be used to detect the presence of reactive minerals in the rock matrix and assess the potential for abiotic degradation at the site. Table 1 provides a summary of the methods that can be used; key methods most frequently used are presented below.

---

[1] A protocol is currently in development. Ongoing ESTCP project (ER-201129) titled "Development and Validation of a Quantitative Framework and Management Expectation Tool for the Selection of Bioremediation Approaches (Monitored Natural Attenuation, Biostimulation and/or Bioaugmentation) at Chlorinated Solvent Sites" includes an assessment of abiotic processes.

- **X-Ray Diffraction:** X-ray diffraction is a bulk analysis technique used to determine the different crystalline phases (minerals) in a material. This technique detects minerals present above 1% to 5% by weight of the total soil sample. The detection limit varies depending on the sample matrix and degree of crystallization of the mineral. However, the mineral phases responsible for abiotic reactions are often lower than 1% by weight and may not be detected by x-ray diffraction. Therefore, a pre-concentration step (such as magnetic separation for magnetite or size fractionation for other minerals) should be considered as part of the analysis to improve the detection of the reactive mineral phases responsible for abiotic processes.

- **Magnetic Susceptibility:** Magnetic susceptibility is a method to estimate the concentration of magnetite (a reactive mineral) in the solid phase. This method is favorable because magnetite is the most abundant mineral in natural soils that exhibits a magnetic behavior. Therefore, magnetic susceptibility is a good estimate of the quantity of magnetite in an environmental sample. EPA guidance (2009) provides an equation for determining the magnetite content of a sample from the mass magnetic susceptibility.

- **Scanning Electron Microscopy:** Scanning electron microscopy/energy dispersive spectroscopy (SEM-EDS) can be used to determine the elemental chemistry abundances for the crushed rock core sample. SEM-EDS data provide the atomic percent and weight percent of elements in the sample; this information can be used to semi-quantitatively identify the minerals present in the sample (Whiting et al., 2014).

- **Acid Volatile Sulfides:** Acid volatile sulfides (AVSs) and chromium reducible sulfides (CRSs) are two analytical methods that are used to quantify the sulfide content in the solid phase. AVS is used as a general estimate for FeS, and CRS is used as a general estimate for FeS_2. These analyses are available from commercial laboratories. The main difference between the two methods is that AVS refers to the sulfides that can be extracted with 1M HCl and CRS selects for inorganic sulfide and is easily digestible in 0.5M $CrCl_2$ in 1M HCl. A laboratory study conducted at Oklahoma State University showed that at FeS concentrations above 20 g/L, abiotic transformation is the dominant transformation process (Butler et al., 2013).

- **X-Ray Absorption Spectroscopy:** X-ray absorption spectroscopy (XAS), also referred to as X-ray adsorption near edge spectroscopy (XANES), can be used to determine the oxidation state of iron. When analyzing samples by XAS, a reference compound is also analyzed to confirm the oxidation state detected.

- **Mossbauer Spectroscopy:** Mossbauer spectroscopy is a method that can be used to evaluate the valence state of metals such as iron. This information tells the state that the element is presently in and therefore can be used to evaluate the speciation of iron minerals. Mossbauer spectroscopy has been used to characterize green rusts by tracking the Fe(II)/Fe(III) ratio (EPA, 2009).

Table 1. Methods Used to Detect and Quantify the Mineral Phases Present in a Soil Sample

Test Method	Information Obtained	Commercially Availability
Magnetic Susceptibility	Estimate of magnetite	Yes
X-ray Diffraction	Mineral phases in sample	Yes
Scanning Electron Microcopy/ Energy Dispersive Spectroscopy Electron Microprobe	Elemental analysis of minerals	Yes
Acid Volatile Sulfides and Chromium Reducible Sulfides	Presence of sulfur containing minerals	Limited
X-ray Absorption Spectroscopy	Oxidation state of iron	Limited
Mossbauer Spectroscopy	Oxidation state of iron	Limited

7.0   WHAT SITE CONDITIONS ARE NECESSARY TO ENHANCE ISBGT?

Enhancing ISBGT refers to the injection of amendments into the contaminated zone to promote ideal conditions for the preferred abiotic mechanism. Specifically, engineering ISBGT is focused on the formation of reactive iron minerals. As mentioned previously, most research has been conducted on the transformation potential of iron sulfides. Therefore, engineering ISBGT is accomplished by creating a reactive zone of iron sulfides for treatment of a contaminant plume. When engineering ISBGT, several factors must be considered (Whiting et al., 2014). For example sites that are aerobic may not be good candidate sites for ISBGT or sites that are anaerobic and show evidence of significant biological degradation. Iron monosulfide can form crystals or framboids (microscopic, spherical aggregates), and it has been found that framboidal iron sulfide has a higher surface area and is highly reactive, while crystalline iron sulfides are less reactive to contaminants. As such, the engineered system must be able to generate the more highly reactive, high surface area form of iron monosulfide (Figure 5).
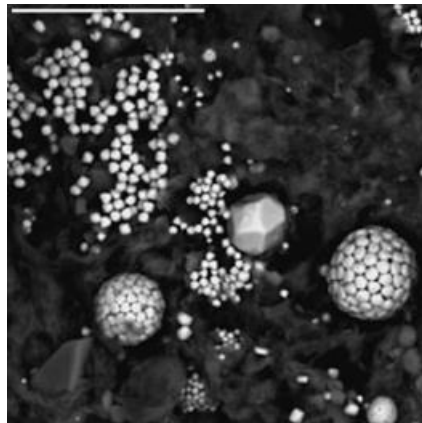

(Courtesy of U.S. Geological Survey [USGS], 2013)

Figure 5. Framboidal Iron Monosulfide

In order to generate the more reactive form of iron sulfides, site conditions must include: a high volumetric sulfate loading rate, sufficient organic carbon, and the availability of iron oxide

(Figure 6) minerals to ensure constant renewal of iron sulfides and sustain contaminant transformation (Lebrón et al., 2010; Evans et al., 2014; Smith et al., 2014). Continual generation of iron sulfides is the key site condition for supporting ISBGT. If any ingredient (iron, sulfate, or electron donor) becomes limiting, this leads to aging of the iron sulfides and slowing of the reaction, as well as the generation of larger, less reactive iron sulfide particles. As such, enhancing ISBGT requires balancing the supply of iron, sulfate, and/or electron donor based on the site-specific conditions (Figure 6).
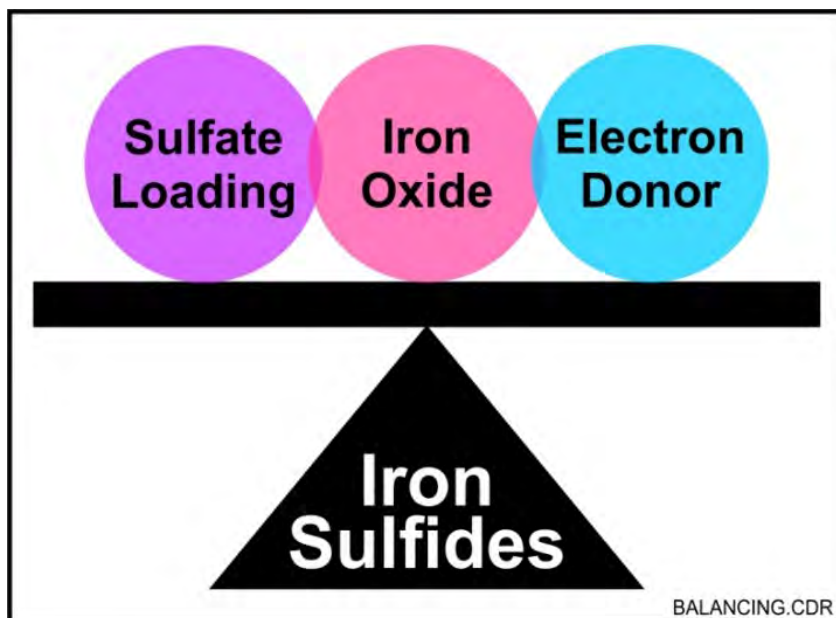

(Courtesy of Battelle)

Figure 6. A Balance between Sulfate Loading, Iron Oxide Availability and Electron Donor is Required to Form the Highly Reactive Framboidal Iron Sulfides

- To obtain a ***high sulfate loading*** rate there should be elevated concentrations of sulfate in the treatment area and sufficient groundwater flow to replenish the sulfate as it is being reduced to sulfide. If sulfate concentrations are allowed to decrease to a point where sulfide production is limited, then the rate of formation of iron sulfides becomes the rate limiting step slowing the rate of abiotic transformation and may lead to methanogenic conditions that favor biological degradation. Although there is no ideal concentration of sulfate, based on sites where ISBGT has been observed, the optimal sulfate concentration is 1,000 to 1,500 mg/L (Evans et al., 2014), but 500 mg/L is sufficient as long as it can be maintained.

- ***Iron*** is one of the most abundant minerals existing in nature. Iron content in native soils can be as high as 10%. It is, however, the bioavailable iron (III) that is capable of transformation to iron sulfides. To provide sufficient ferric iron that can be biologically or chemically reduced to ferrous iron, a site needs (at minimum) a bioavailable iron concentration of at least 1%. If sufficient bioavailable iron is not available, the iron can be added to the treatment area. For example, within a biowall, the Fe(III) provides bioavailable iron for iron sulfide formation and also provides surfaces to which the iron sulfides attach. For enhanced ISBGT

reactors, Butler et al. (2013) reported abiotic transformations being the dominant transformation mechanisms when iron sulfide concentrations were 20 g/L and above.

- Sufficient *organic carbon* is the component necessary to support iron sulfide formation by maintaining sulfate-reducing conditions and subsequent sulfide production. For sulfate reduction and iron sulfide formation, optimal oxidation reduction potential (ORP) values range from 50 mV to -250 mV (Figure 7). Highly anaerobic conditions (i.e., ORP < -250 mV) support methanogenesis and there may be competition between methanogenic and sulfate-reducing bacteria. A site having a high sulfate loading rate and sufficient electron donor will be characterized by sustained sulfate reduction and therefore generation of sulfide for iron sulfide formation. Therefore, redox is an important parameter to monitor when engineering iron and sulfate reduction to support iron sulfide production.
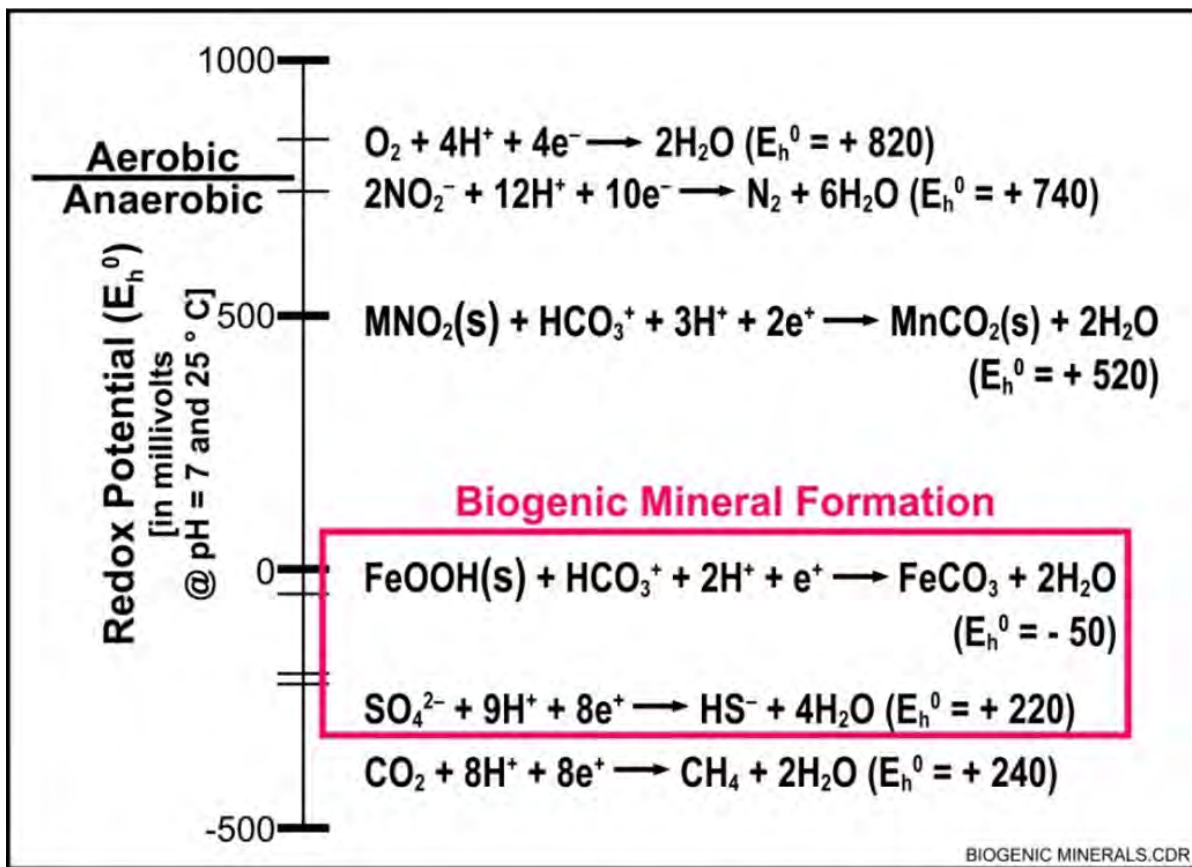
(Modified from Wiedemeir et al., 1999)

Figure 7. Redox Potential Showing Sulfate Reduction and Mineral Formation

In addition to having the required components (iron, sulfate, and electron donor), sufficient *hydraulic residence time* in the target treatment zone is necessary to ensure amendments are provided enough time to form the reactive minerals and subsequently transform the contaminants. Hydraulic retention time is critical during ISBGT because it is a three-step process involving microbiology, mineralogy, and chemistry. Sufficient retention time is required to allow for the formation of the iron sulfides and then the reaction between the iron sulfides and the

contaminants. At sites where the groundwater flow is high, the success of ISBGT is limited because sufficient time is not available for all three steps to take place. A recommended hydraulic retention time is 15 to 30 days (Evans et al., 2014; Kennedy, 2011). It has been observed that once sufficient electron donor is available, sulfate is reduced to sulfide within days as is Fe(III) to Fe(II). Once Fe(II) and sulfide ions are formed and come into contact, FeS is immediately formed. The reaction between FeS and the contaminants takes approximately 15 to 30 days and appears to be the rate-limiting step when in situ conditions are ideal for ISBGT to occur. Therefore, for ISBGT to be successful, there should be sufficient retention time for formation of the iron sulfides and also for the iron sulfides to react with the chlorinated solvent. Note that at high flow rate sites where hydraulic retention may not be sufficient, increasing the size of the reaction zone in the direction of the flow can provide the required hydraulic retention time.

Although the transformation of contaminants by iron minerals is an abiotic process, enhancing the formation of these iron minerals in the treatment area relies on microorganisms to generate the reactive species. ***Iron- and sulfate-reducing bacteria*** are critical to the success of enhanced ISBGT. Fortunately, these microorganisms are ubiquitous in the environment, and bioaugmentation of treatment areas has not been necessary. If bacterial counts are low in the presence of both iron and sulfate ($<10^3$ cells/mL), the addition of an electron donor stimulates their growth to produce optimal iron and sulfate bacterial levels ($>10^6$ cells/mL). Care must be taken to not overstimulate microbial growth so as not to enhance dechlorinating microbes.

Monitoring and maintaining near neutral **pH** of a treatment area is critical during biological degradation to support optimal microorganism growth. However, for abiotic transformation of chlorinated solvents by iron minerals, the reaction rate appears to be dependent on pH. Laboratory studies have shown a noticeable increase in the rate of reaction with increasing pH (EPA, 2009). Figure 8 shows the rate of transformation of chlorinated solvents and EDB at different reaction pH levels based on laboratory studies. Therefore, a desirable pH range for ISBGT would be a neutral to slightly elevated pH to increase reaction rates. The Eh/pH diagram (Figure 9) for an iron sulfur system shows higher stability of iron sulfide minerals at slightly higher pH levels and low redox conditions which provides further evidence for the higher rate of transformation at slightly alkaline pH levels.

(Modified from EPA, 2009)
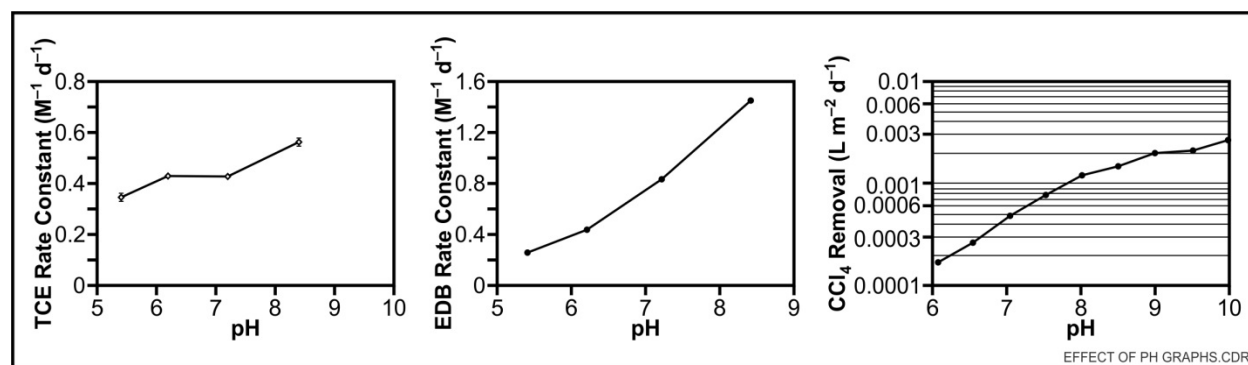
Figure 8. Effect of pH on the Rate of ISBGT for Different Contaminants

13
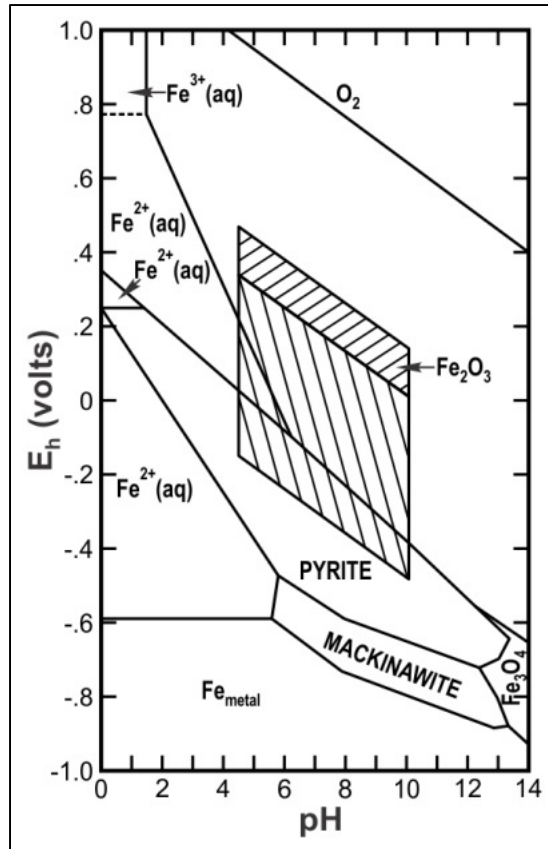
(Obtained from McNeil and Little, 1999)

Figure 9. Eh/pH Diagram for an Iron, Water, Sulfur System

8.0   HOW CAN SYSTEMS BE ENGINEERED FOR ISBGT?

Engineering ISBGT involves the design of a system that will distribute the amendments to the treatment area in the most cost effective and efficient way possible to lead to transformation of the contaminant (Air Force Center for Engineering and the Environment [AFCEE], 2008). ISBGT designs depend primarily on the depth of the contamination, as well as geology and hydrogeology of the contaminated area. For shallow plumes, trench biowalls and bioreactors are used to treat contamination that extends to a maximum of approximately 35 feet below ground surface (bgs). For deeper plumes (>35 feet bgs), injection systems are used. For each site, the geology impacts the types of amendments required, while the hydrogeology controls the engineered hydraulic residence time of the target treatment zone.

Amendments. For an ISBGT application to be successful, there must be a balance between iron, sulfate, and electron donor in the system (Figure 6). Depending on the site conditions (determined by mineralogical studies and geochemical data), one or more of the three amendments may be required for the site. When using bioreactors and trench biowalls, solid amendments are typically emplaced. For injection approaches, liquid amendments are used. Table 2 lists typical amendments that have been used while engineering ISBGT.

Table 2. Typical Amendments Used in ISBGT Engineered Systems

Component	Bioreactors and Trench Biowalls	Liquid Injections
Iron	Sand with natural high iron content Iron oxide (hematite, magnetite)	Iron sulfate Iron chloride(causes decrease in pH) Ferrous lactate
Sulfate	Calcium sulfate (gypsum)	Iron sulfate Magnesium sulfate (Epsom salts) Sodium sulfate Calcium sulfate
Electron Donor	Tree mulch Cotton gin compost Mulch coated with vegetable oil	Sodium lactate Emulsified vegetable oil Lecithin Soybean oil
Additional Amendments	Sand and/or gravel for permeability Buffer (limestone)	Buffer (Sodium bicarbonate)

A *trench biowall* is a trench dug typically perpendicular to the groundwater flow to intersect the plume (Figure 10). Trench biowalls, as when used for bioremediation, can prevent the plume from migrating offsite or limit plume migration by detaching the plume from the source area. Trench biowalls are typically a depth of no more than 35 feet with widths ranging from 1 to 6 feet. The length of a trench biowall is limited primarily by site conditions, such as underground structures at the site. The width of the trench is based on the groundwater velocity to ensure that there is sufficient hydraulic retention in the biowall for the formation of iron sulfide, as well as the reaction between the iron sulfides and the contaminants. One benefit of a trench biowall is that it can be filled with large quantities of long-lived, reactive amendments.

When applied to ISBGT, trench biowalls are effective for a shallow plume because the trench can be designed with the amendments to promote formation of iron sulfides. The width of the trench and the amendments can be designed to obtain the hydraulic retention necessary to ensure that the transformation of the contaminants takes place and the iron sulfide formed have plenty of surfaces to precipitate onto. Monitoring wells can be located within the trench, as well as upgradient and downgradient of the trench. Deeper monitoring wells screened below the deepest depth of the trench can be installed to monitor the effect of the trench amendment on the groundwater quality.

(Modified from AFCEE, 2008)
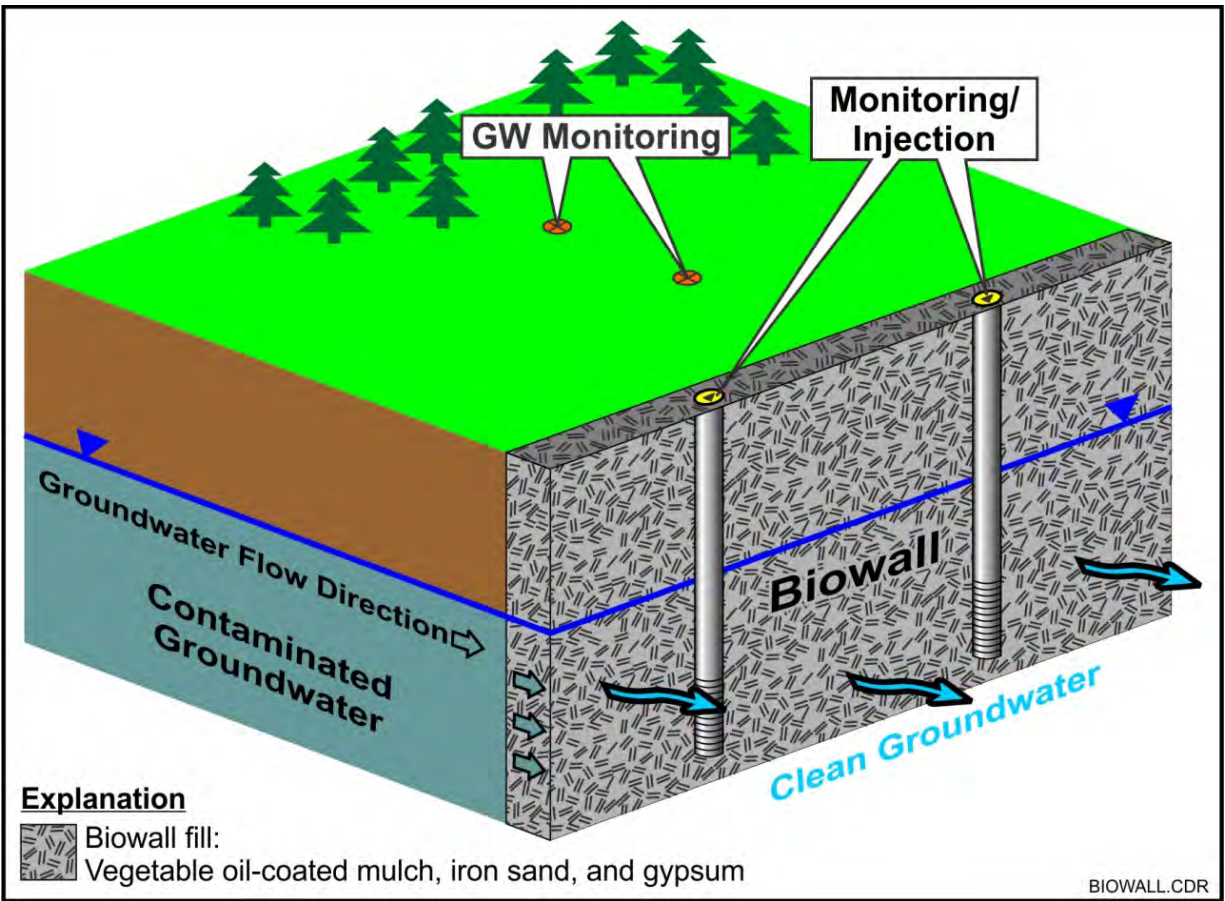
Figure 10. Trench Biowall Configuration for Engineering ISBGT

A *bioreactor* is similar to a trench in that it is limited to a depth of approximately 35 feet bgs. A bioreactor, however, tends to have a smaller footprint than a trench and is installed to treat a source area (Figure 11). After excavation of the highest concentrations, the former source area can be filled with reactive material. Bioreactors are best used at sites with large open areas and limited underground structures.

To enhance ISBGT by a bioreactor, the area of excavation is filled with solid amendments such as mulch, gypsum, and hematite to provide the sulfate, iron, and electron donor necessary to form the reactive iron sulfides. The bioreactor impacts the downgradient plume as the amendments solubilize and are transported with the groundwater. To enhance the effect of the bioreactor, downgradient groundwater can be extracted and recirculated through the bioreactor. This allows treatment of the downgradient plume and reduces the source area and plume size, as well as maximizes treatment. Bioreactors and trenches may need to be rejuvenated by removing the solid amendments and replacing them with fresh amendments.

(Modified from AFCEE, 2008)
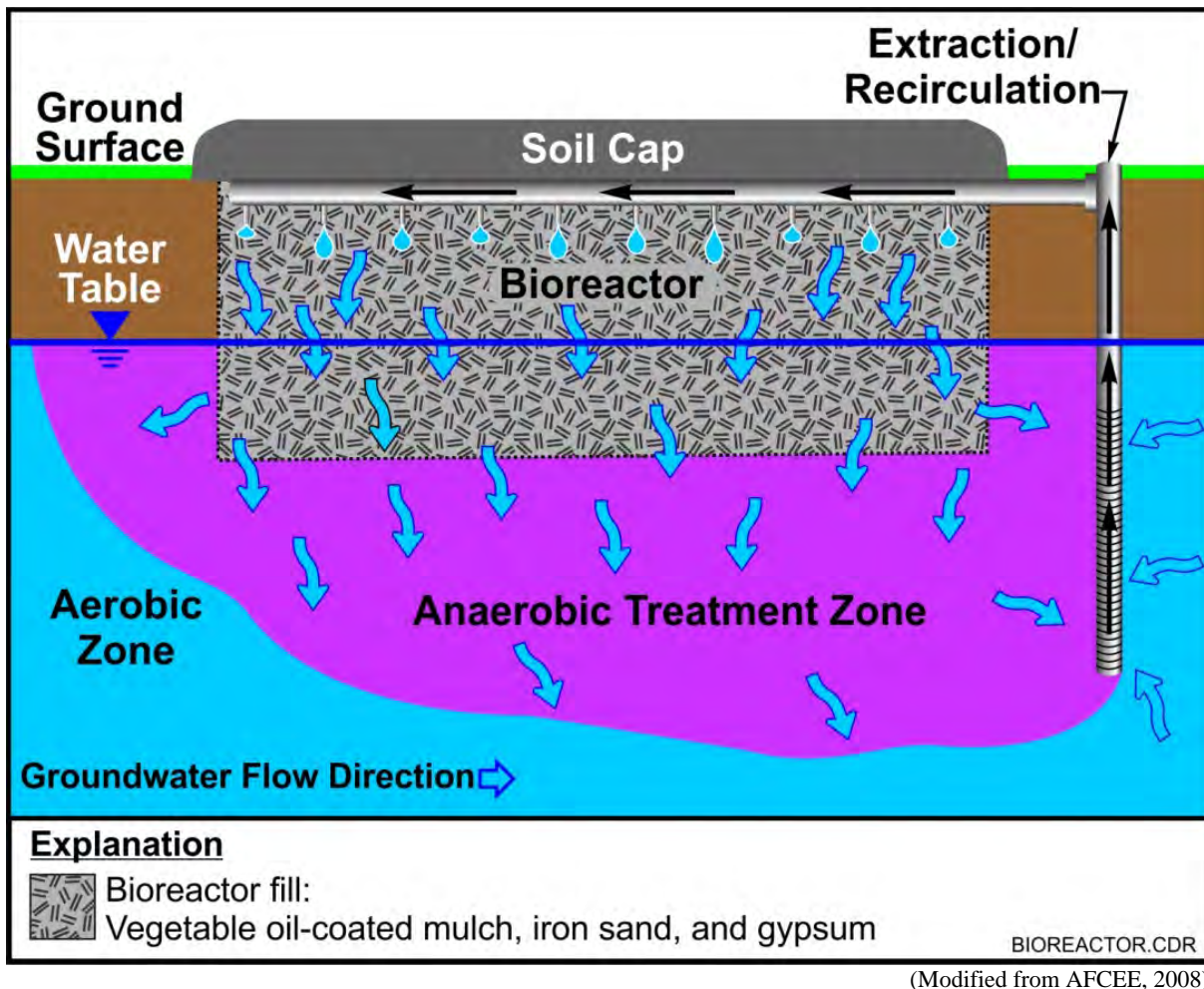
Figure 11. Bioreactor Configuration for Engineering ISBGT

Injection systems (Figure 12) are used for plumes deeper than 35 feet or for areas where subsurface structures are present and a trench or bioreactor is not feasible. For injection systems, liquid amendments are injected into permanent wells or direct push points (Payne et al., 2013; Kennedy et al., 2006). Multiple liquid amendments are injected and it is important that the amendments injected have similar retardation factors in situ so that they can be combined when injected. If the retardation factors vary significantly, the amendments can be injected separately. ISBGT appears to be most successful when designed as a recirculating injection system. Recirculation involves installing injection wells for adding amendments and using extraction wells/points to obtain groundwater that is mixed with the amendments and reinjected into the injection wells. During recirculation, groundwater is extracted simultaneously as the amendments are being injected and once the amendments have been injected, the recirculation continues. This sets up a mixing zone in the treatment area that allows the contaminant to contact the reactive amendments. Recirculation works best for groundwater plumes with a high hydraulic conductivity and when the geology is unconsolidated. High flow rates would limit hydraulic retention time and hinder the reaction from taking place. In this instance, the recirculation would help to generate the retention time required.

17
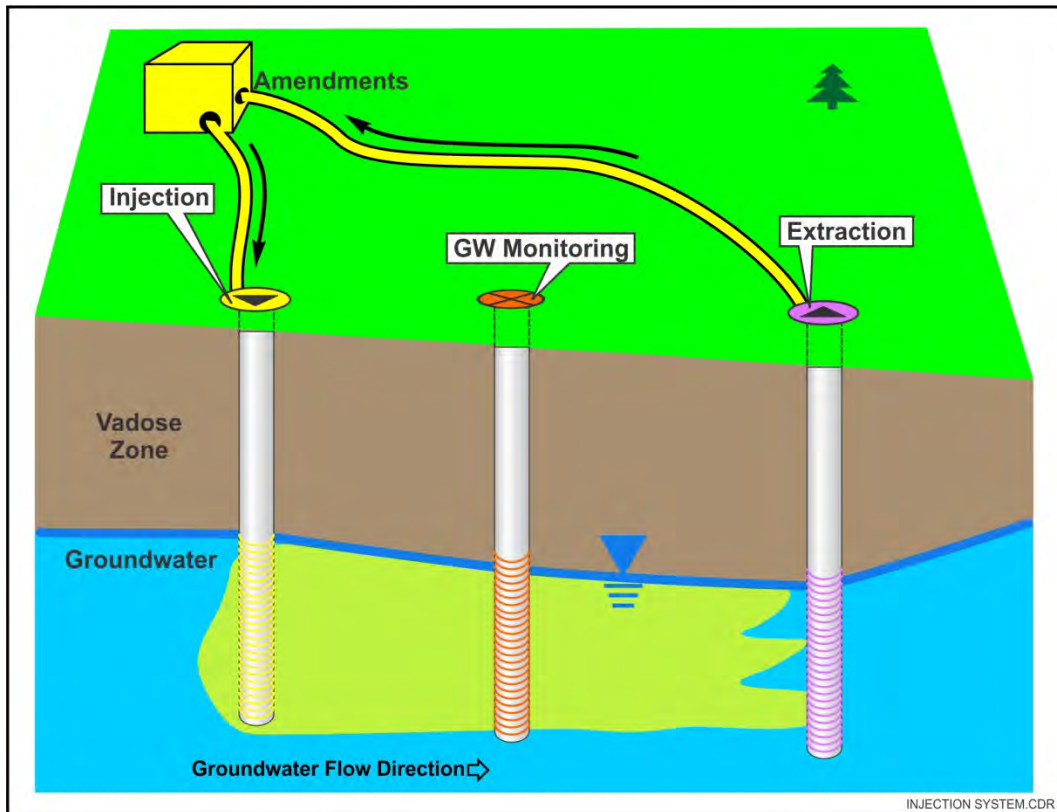
Figure 12. Recirculation System Configuration for Engineering ISBGT

9.0   HOW IS PERFORMANCE MONITORING FOR ISBGT CONDUCTED?

A multidisciplinary performance monitoring program is necessary for evaluating ISBGT. This approach provides the framework for evaluating remedy performance, establishing metrics to evaluate the efficacy of the ISBGT applications, and gathering the necessary data to further optimize the remedy. Specifically, the performance monitoring program should prescribe the following:

- Parameters to evaluate.

- Metrics for parameter evaluation.

- Specific criteria that define the endpoint for ISBGT.

- Applicable milestones (e.g., mass or concentration removal targets, transition from active to passive treatment, transition from ISBGT remediation to MNA or confirmation monitoring).

- Contingency triggers (i.e., additional applications, revised dosing strategy, alternate technology [treatment trains]) in the event that milestones are not being achieved.

- How the monitoring program should be optimized when milestones are achieved, and how the number of monitoring points, sampling frequency, and/or analytical parameters will be reduced at such milestones.

The *monitoring plan* should include two distinct categories of monitoring: process monitoring and performance monitoring. Process monitoring includes monitoring those parameters that provide information on the application of ISBGT during implementation. Performance monitoring provides information on the efficacy of the remedy to achieve remedial goals for ISBGT. This document presents performance monitoring specific to ISBGT; process monitoring during injection of amendment and installation of trenches and bioreactors are typically standard measurements.

During engineering of ISBGT, the *performance monitoring* that should be conducted includes analyzing the contaminant and known degradation product concentrations to determine if the contaminant is being degraded. In addition, groundwater geochemical and water quality parameters should be monitored to determine the redox potential of the treatment area in response to the amendments. The redox is important for microbial activity, formation of the iron sulfides, and transformation of the contaminants by the iron sulfides. Mineral analysis should be done to determine the reactive mineral phases formed. Microbial analysis should be done to understand the change in microbial community as a result of the amendments. These parameters should be monitored prior to adding the amendments and after emplacement of the amendments in the treatment area. Table 3 provides groundwater geochemistry and water quality parameters to be monitored. The purpose for each of the measurements is also provided.

Table 3. Water Quality and Geochemical Parameters Monitored after Injection of Amendment for ISBGT

| Parameters	Purpose
Dissolved oxygen, nitrate, ammonia, Fe(II), total iron, sulfate, sulfide, methane	- To determine dominant terminal electron accepting processes - Groundwater chemistry should be conducive for iron- and sulfate-reducing conditions - Volumetric sulfate loading rate is an important factor in determining whether sufficient sulfate loading is adequate to promote rapid and ongoing formation of fresh iron sulfide phases
Electron donor: volatile fatty acids, total organic carbon, dissolved organic carbon, chemical oxidant demand	Determines whether sufficient organic carbon is present to promote anaerobic conditions and active and sustained sulfate reduction
pH, ORP, temperature, turbidity	- Further confirmation of redox conditions - Slightly alkaline pH increases rate of ISBGT - Biological activity is temperature dependent and slows as groundwater temperature decreases reduction - Turbidity could indicate presence of amendments in the monitoring wells and also presence of the iron sulfide particles
Alkalinity	Analyzed during baseline sampling to determine if the site has sufficient buffering capacity as some amendments may lower the pH of the groundwater, degradation of the contaminant may generate acidic conditions

Solid Phase Monitoring. Collecting soil core samples is recommended as a part of the monitoring program after ISBGT has been enhanced at a site especially if the system is not operating as expected. Soil core samples are beneficial because iron sulfide minerals often precipitate or attach themselves to mineral surfaces. These samples should be taken within the treatment area. The analyses that should be done on the soil core samples are described in Table 4.

Table 4. Mineral Analysis of Cores during ISBGT

Parameters	Method of Analysis	Purpose
Sulfide minerals: AVS and CRS	Laboratory test	Both tests quantify the iron sulfide minerals present. AVS is commonly used as a general estimate for FeS and CRS is an estimate for FeS_2.
Bioavailable and total iron	Laboratory test	This should be done during baseline sampling to determine the concentration of bioavailable iron present. This bioavailable iron will be used by iron-reducing microorganisms to produce Fe(II) which forms reactive iron minerals by further reactions. A site where bioavailable iron is limited can be amended with iron.
Mineral surface area Grain size Mineral compositions	Electron microprobe analysis	Determines the grain size and morphology of the iron sulfide (granular versus framboidal), framboidal is preferred for better abiotic transformation rates; semi-quantitative indication of surface area. Knowing the surface area of the iron minerals aids in calculating transformation rates as ISBGT occurs on the surface and rates are often normalized to the surface area.
Mineral compositions Morphology	Scanning electron microscopy, energy dispersive scatter	The analysis is used to determine elemental chemistry abundances in the sample. The data also present the atomic percent and weight percent of elements in the sample; magnified views of the mineral surfaces show morphology.

Geochemical Modeling. In addition to analyzing site samples for the presence of the minerals in the soil matrix, geochemical modeling can also be done to predict the minerals that may be facilitating abiotic transformation. Geochemical modeling uses groundwater parameters and not soil data. Geochemical modeling does not identify the minerals directly but uses water chemistry at the site to predict the mineralogical composition and constrain the mineral phases present.

Geochemical modeling is beneficial because it provides the saturation level of the system and therefore provides guidance on whether the system should be modified. Soil sample collection may be compromised by being exposed to the atmosphere, making direct identification of reactive minerals in raw samples from the field a difficult task. Thus, geochemical modeling is advantageous because reactive minerals can be unstable in the presence of oxygen in the atmosphere, and they may be present in aquifer sediments at low concentrations. Geochemical modeling, however, assumes that the system is in equilibrium and real-world systems are rarely in equilibrium. Geochemical modeling data should not be isolated from other methods, but should be used as a supporting line of evidence. Commercial software packages for geochemical modeling are readily available. Inputs to the model include pH, and the concentrations of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), chloride (Cl), sulfate (SO_4^{2-}), hydrogen sulfide (H_2S)(aq), and bicarbonate (HCO^{3-}). Outputs to the model include saturation index of minerals such as pyrite, FeS and siderite. Site redox values are also predicted through the model. Figure 13 shows the results of geochemical modeling at a permeable reactive barrier at Altus Air Force Base. From the figure it can be seen that in a carbonate system sulfide minerals are limited and the minerals are dominated by Fe(III) containing minerals such as goethite and magnetite. With the sulfidic system, which the Altus biowall fell into, the minerals that dominated were reduced sulfide minerals such as pyrite. Minerals such as magnetite are present when sulfide is low and Eh is elevated. In addition to Altus Air Force Base geochemical modeling has been conducted at Dover Air Force Base, Seneca Army Depot and Dugway Proving Ground (Lebrón et al., 2010).
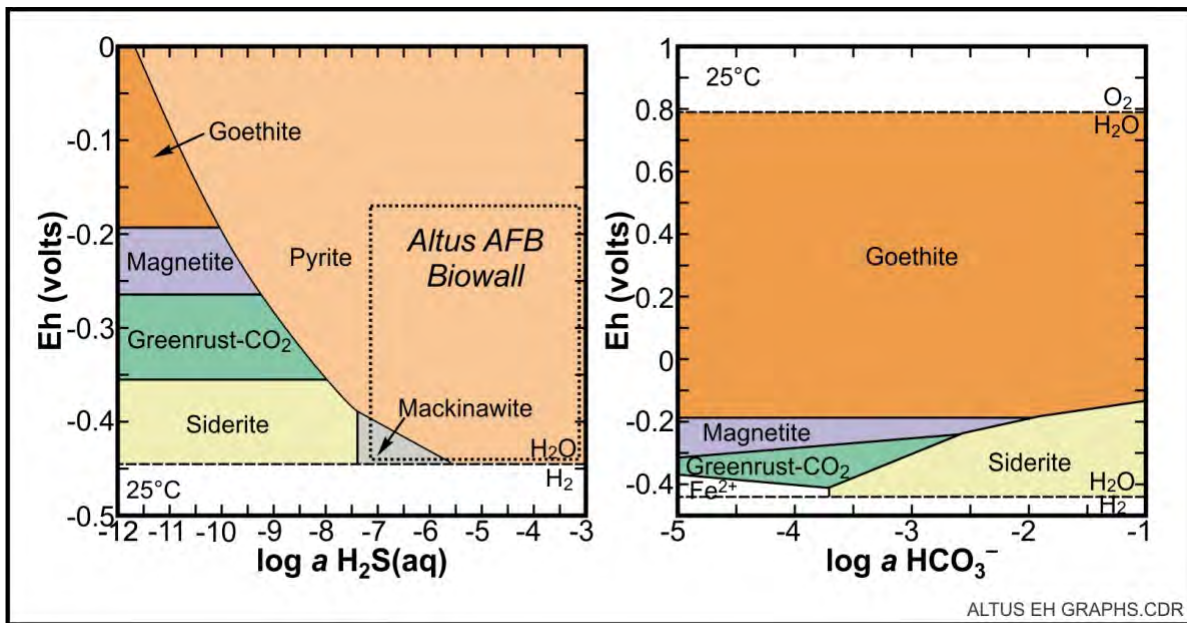
(Modified from EPA, 2009)

Figure 13. Geochemical Modeling at Altus Air Force Base to Identify Minerals Present

Microbial Analysis. Although during ISBGT the contaminant is ultimately degraded by an abiotic process, microorganisms play a role in generating the elements that form the minerals. Knowledge of the microorganisms present and their abundance provides information to make an

informed decision about the success of the amendments in enhancing ISBGT. Quantitative polymerase chain reaction is a method that can be used to quantify sulfate- and iron-reducing bacteria. Sustained bacterial counts of $>10^5$ cells/mL indicate that the microbial population is thriving. Microbial analysis should be conducted for iron- and sulfate-reducing bacteria, as well as microorganisms known to degrade the contaminants of concern. For instance, for chlorinated ethenes, microbial analysis would be done for *Dehalococcoides* sp. and other species known to degrade different end products. It is also recommended that total bacterial counts are taken to understand what is occurring with the whole community of microbes. If sulfate- and iron-reducing microbial counts are decreasing, but the bacterial community counts are elevated, the assumption can be made that different and possibly competing microorganisms are thriving.

Contaminant concentrations and known degradation products should be monitored in the groundwater after adding amendments to promote ISBGT. For chlorinated solvents, the degradation products would consist of *cis*-DCE, VC, and the dissolved gases ethene, ethane, and carbon dioxide.

Isotope Analysis. Isotope fractionation has also been used to track degradation of contaminants and to identify transformation mechanisms. Measuring the change in the ratio of the carbon isotopes ^{12}C and ^{13}C in the contaminants of concern before and after treatment provides information on the degree of transformation and even the transformation mechanism. During degradation, the bonds attached to the lighter isotope ^{12}C break easier so there is enrichment of the heavier carbon isotope ^{13}C. Isotope enrichment factors have been measured for both microbial degradation and abiotic degradation of contaminants such as chlorinated ethenes (Butler et al., 2009). It has been observed that enrichment of the heavier isotope is greater during abiotic mechanisms (Table 5). This may occur because abiotic mechanisms specifically target the bonds to the lighter isotope, whereas microbial degradation breaks a mix of bonds to lighter and heavier isotopes. Caution must be taken when interpreting abiotic enrichment factors. Detailed analysis of a larger data set of abiotic and microbial isotope enrichment factors show an overlap in the enrichment factors obtained (Slater et al., 2001, 2002; VanStone et al., 2004, 2008; Liang et al., 2007) (Figure 14). Isotope fractionation can therefore be used as a supporting line of evidence for abiotic mechanisms, but should not be used alone. Keep in mind that in systems where contact with the reactive mineral is fast and effective, little fractionation is seen because all contaminants are rapidly degraded. This is because field conditions such as advection and dispersion that may limit contact between the contaminant and the mineral surface may be the rate controlling step.

Table 5. Typical Isotope Enrichment Factors

Contaminant	Mineral	Mineral Degradation	Microbial Degradation
Carbon Tetrachloride	Magnetite	-29 ± 3‰ (C-Cl bond)	
Carbon Tetrachloride	FeS	-15.9 ± 0.3‰	
PCE	FeS	-30.2 ± 4.3‰ (pH 7) -29.54 ± 0.83‰ (pH 8) -24.6 ± 1.1‰ (pH 9)	-1.39 ± 0.21‰ (BB1) -1.33 ± 0.13‰ (Sm) -7.12 ± 0.72‰ (BDI) -5.2
TCE	Green Rust	-23.00 $\pm$ 1.8‰	
TCE	Magnetite	-39 ‰ $\pm$ 12 ‰	
TCE	FeS	-33.4 ± 1.5‰ (pH 8) -27.9 ± 1.3‰ (pH 9)	-4.07 ± 0.48‰ (BB1) -12.8 ± 1.6‰ (Sm) -15.27 ± 0.79‰ (BDI)
TCE	Chloride green rust	-23 ± 1.8‰	
cis-DCE	Magnetite	-0.60 ‰	-20.4
EDB	FeS_2	-20.2 $\pm$ 2.23‰	-5.7‰

Desulfuromonas michiganensis strain BB1 (BB) and Sulfurospirillum multivorans (Sm) and a bacterial consortium (BioDechlor Inoculum [BDI]).
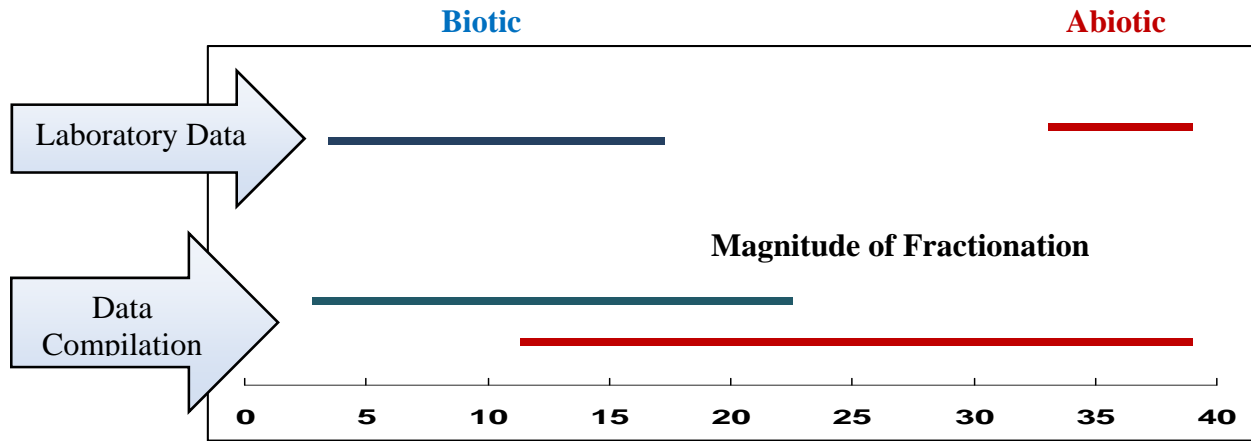
(Modified from Liang et al., 2007)

Figure 14. Comparison of Enrichment Factors for Biotic and Abiotic Mechanisms in Field and Laboratory Studies

10.0   WHAT GUIDELINES ARE USED FOR ISBGT SAMPLING?

Sampling for ISBGT should follow a temporal and spatial design. A temporal design refers to taking samples at time intervals to enable site decision making. Because ISBGT takes place rapidly, requiring a hydraulic retention time of only 15 to 30 days, groundwater samples should be collected within a month after each emplacement of amendments. After collecting the first round of samples within one month of the ISBGT application, groundwater monitoring can be conducted quarterly. Each site is unique and a temporal sampling design should be developed for the specific site. Baseline sampling of groundwater parameters should be conducted.

A spatial sampling design refers to sampling at different locations throughout the plume. Groundwater samples should be taken upgradient of the treatment area, within the treatment area, and downgradient of the treatment area. For trenches, samples should also be taken below the trench. To fully evaluate ISBGT, soil core samples should be collected in addition to groundwater samples. It is often possible to see the presence of iron sulfides in the groundwater. The time point at which these soil core samples should be taken should be decided based on the groundwater sampling results. At this point, core samples can be taken from the treatment area to analyze the precipitated minerals.

Groundwater sampling methods include standard low-flow sampling using monitoring wells or hydropunch samples. Soil sampling methods can be performed using direct push probes and auger drilling techniques. Soil samples potentially containing reduced iron and sulfide minerals are vulnerable to oxidation by exposure to the atmosphere and should be maintained anaerobically. Specialized direct push methods (the frozen core method [Wilkin, 2007]) can be used to collect the intact core samples.

11.0   HOW ARE ISBGT DATA INTERPRETED?

Data obtained from groundwater and soil samples are usually evaluated using the methods listed below:

- PHREEQC is a geochemical model that stands for PH (pH), RE (Redox), EQ (equilibrium) C (model written in C programming language by the USGS). This model can be used to determine the mineral phases that are present based on the geochemistry of the groundwater. The model can also identify the presence of sulfate sources within the upgradient aquifer. While this is an equilibrium model and can indicate which minerals can be expected to form in solution (the saturated and supersaturated phases), the model does not account for any kinetic limitations to mineral formation. Kinetic limitations are important to understand as the specific mineral types and forms present directly control the rate of contaminant destruction.

- Statistical analyses such as mean, median, standard deviation, and coefficient of variance can be used to identify variability in the data and provide a convenient means of comparing sample values.

- Trend plots of concentration data including contaminants of concern and transformation products (e.g., methane, ethene, ethane, acetylene) are used to determine biotic versus abiotic transformation characteristics. The dominant abiotic transformation product of chlorinated

ethenes is acetylene. This degradation product is difficult to measure in the field so abiotic degradation of chlorinated solvents may be characterized by a decrease in contaminant concentration without the stoichiometric increase in daughter products. A stoichiometric increase in daughter product indicates biological degradation.

12.0 CONCLUSIONS

ISBGT is considered a promising technology for remediation strategies employing both MNA and/or enhanced in situ treatment of contaminants. This technology is advantageous because it capitalizes on both abiotic and biological degradation processes understood to occur simultaneously even in systems where only one mechanism is being monitored or enhanced. This is especially beneficial during MNA as accounting for abiotic processes in addition to biological degradation processes during MNA may result in reduced timeframes for reaching the MCL and a greater chance of regulatory acceptance of MNA. The key considerations about ISBGT are:

- The transformation of contaminants by iron sulfides is best understood and their formation can be enhanced in situ.

- Acetylene is the dominant degradation product of chlorinated ethene ISBGT, but this is difficult to measure in the field.

- Enhancing ISBGT is a balance between sulfate loading, iron oxide presence, and electron donor levels.

A few data gaps remain in the understanding of ISBGT. FeS is the only mineral that is well understood, and further research is needed to understand the abiotic mechanisms of other minerals such as magnetite.

With no standardized guidance currently available for enhancing and monitoring for ISBGT, concurrence on documentation of abiotic degradation in the field remains a challenge. Thus, recognition and inclusion of the abiotic pathway as a natural or enhanced attenuation mechanism is inconsistent at different sites.

This handbook has been prepared to help to further the understanding and recognition of the occurrence of ISBGT processes at Navy environmental restoration sites. The mechanisms that contribute to ISBGT processes were explained and important parameters were reviewed for documenting ISBGT as part of MNA and/or for enhancing ISBGT as part of an active remedy.

Glossary

Abiotic: activity in the absence of microorganisms.

Bioaugmentation: the practice of adding actively growing, specialized microbial strains into a microbial community in an effort to enhance the ability of the microbial community to respond to process fluctuations or to degrade certain compounds, resulting in improved treatment.

Biogenic: produced by living organisms or biological processes.

Biogeochemical reductive dechlorination: a process involving both biological and chemical reactions to affect the abiotic reduction of chlorinated solvents, such as trichloroethene and tetrachloroethene. Indigenous sulfate-reducing bacteria are stimulated through the addition of a labile organic and sulfate, if not already present at high concentrations. The stimulated bacteria produce reductants that react in conjunction with minerals in the aquifer matrix. Moreover, the reducing conditions necessary to produce such reactions most often are created as a result of microbial activity.

Bioremediation: engineered treatment processes that use microorganisms to biodegrade contaminants.

Biotic: of, relating to, or resulting from living things, especially in their ecological relations.

Daughter products: decay product resulting from the breakdown of a parent product.

Emulsified zero valent iron: consists of emulsion droplets containing iron particles in water surrounded by an oil-liquid membrane. Composed of food-grade surfactant, biodegradable vegetable oil, water, and zero valent iron.

Framboidal: structure comprising roughly spherical aggregates of discrete equi-regular euhedral microcrystallites of around 0.5 μm in diameter, with the average aggregate size ranging from 5 to 20 μm.

Geochemical modeling: the practice of using chemical thermodynamics, chemical kinetics, or both, to analyze the chemical reactions that affect geologic systems, commonly with the aid of a computer.

In situ: literally meaning "in place," refers to treating a compound where it is rather than first mechanically removing it (by excavation, pumping, venting, etc.) and then treating it.

In situ biogeochemical transformation: in place processes where contaminants are degraded by abiotic reactions with naturally occurring and biogenically formed minerals in the subsurface.

Monitored natural attenuation: naturally occurring processes in soil and groundwater environments that act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in those media.

Permeable reactive barrier: subsurface walls composed of reactive materials that will either degrade or alter the stat of contaminant when that contaminant in a groundwater plume passing through the wall.

Plume: a zone of dissolved contaminants. A plume usually originates from a source and extends in the direction of groundwater flow.

Water table: the surface where the water pressure head is equal to the atmospheric pressure.

Zero valent iron: strong reducing agent; accepted technology for degradation of chlorinated VOCs, PCE, TCE, metals and pesticides.

13.0 REFERENCES

Adamson, D.T. and C.J. Newell. 2014. Frequently Asked Questions about Monitored Natural Attenuation in Groundwater. ESTCP Project ER-201211. Environmental Security and Technology Certification Program, Arlington, Virginia.

Air Force Center for Engineering and the Environment. 2008. Final Technical Protocol for Enhanced Anaerobic Bioremediation Using Permeable Mulch Biowalls and Bioreactors. May.

Boparai, H.K., S. Comfort, P.J. Shea, and J.E. Szecsody. 2008. "Remediating Explosive-Contaminated Groundwater by in situ Redox Manipulation (ISRM) of Aquifer Sediments," *Chemosphere*, 71, pp. 933-941.

Britto, R. and J. Nolin. 2012. Biogeochemical Treatment of Organic and Inorganic Contaminants in Soil and Groundwater. Remediation Technologies Symposium.

Brown, R. 2009. State of Practice in In Situ Biogeochemical Reduction Transformations. Biogeochemical Workgroup meeting.

Brown, R., J. Wilson, and M. Ferrey. 2007. "Monitored Natural Attenuation Forum: The Case for Abiotic MNA," *Remediation*, 17(2), pp. 127-137.

Butler, E.C. and K.F. Hayes. 1999. "Kinetics of the Transformation of Trichloroethylene and Tetrachloroethylene by Iron Sulfide," *Environ. Sci. Technol.*, 33, pp. 2021-2027.

Butler, E.C. and K.F. Hayes. 2001. "Factors Influencing Rates and Products in the Transformation of Trichloroethylene by Iron Sulfide and Iron Metal," *Environ. Sci. Technol*. 35, pp. 3884-3891.

Butler, E., L. Chen, and R. Darlington. 2013. "Transformation of Trichloroethylene to Predominantly Non-regulated Products under Stimulated Sulfate Reducing Conditions," *Groundwater Monitoring & Remediation*, 33(3), pp. 52-60.

Butler, E., Y. Dong, X. Liang, T. Kuder, R. Philp, and L. Krumholz. 2009. "Abiotic Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene in Anaerobic Environments," SERDP Project ER-1368.

Darlington, R., L.G. Lehmicke, R.G. Andrachek and D.L. Freedman. 2013. "Anaerobic Abiotic Transformations of *cis*-1,2-dichloroethene in Fractured Sandstone," *Chemosphere*, 90(8), pp. 2226-2232.

Darlington, R., S. Rosansky, H. Rectanus, D. Nair, B. Smith, and L. Battaglia. 2011. "Optimization of In-Situ Chemical Oxidation and Enhanced In-Situ Bioremediation to Treat a Dilute Chlorinated Solvent Plume," *First International Symposium on Bioremediation and Sustainable Environmental Technologies*. Reno, Nevada, June 27-30.

Darlington, R., S. Rosansky, H. Rectanus, T. Ford, P. Tamashiro, and A. Gavaskar. 2010. "Evidence of Biogeochemical Transformation Processes in an EVO Biobarrier," *The Seventh International Conference on Remediation of Chlorinated and Recalcitrant Compounds*, Monterey, California, May 24-27.

Darlington, R., L. Lehmicke, R.G. Andrachek, and D. Freedman. 2008. "Biotic and Abiotic Anaerobic Transformations of Trichloroethene and *cis*-1,2-Dichloroethene in Fractured Sandstone," *Environ. Sci. Technol.*, 4(12), pp. 4323-4330.

Devlin, J.F. and D. Müller. 1999. "Field and Laboratory Studies of Carbon Tetrachloride Transformation in a Sandy Aquifer under Sulfate Reducing Conditions," *Environmental Science & Technology*, 33, pp. 1021-1027.

Environmental Protection Agency. . 2009. Identification and Characterization Methods for Reactive Minerals Responsible for Natural Attenuation of Chlorinated Organic Compounds in Ground Water. EPA 600/R-09/115.

Environmental Security Technology Certification Program. 2008. Workshop on In Situ Biogeochemical Transformation of Chlorinated Solvents. February. Retrieved from www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA501302.

Evans, P.J., D. Nguyen, R.W. Chappell, K. Whiting, J. Gillette, A. Bodour, and J.T. Wilson. 2014. "Factors Controlling In Situ Biogeochemical Transformation of Trichloroethene: Column Study," *Groundwater Monitoring and Remediation*, 34(3), pp.65-78

Ferrey, M.L., R.T. Wilkin, R.G. Ford and J.T. Wilson. 2004. "Non-biological Removal of *cis*-Dichloroethylene and 1,1-Dichloroethylene in Aquifer Sediment Containing Magnetite," *Environmental Science & Technology*, 38, pp. 1746-1752.

Gillham, R.W. and S.F. O'Hannesin. 1994. "Enhanced Degradation of Halogenated Aliphatics by Zero-valent Iron," *Ground Water*, 32, pp. 958-967.

Gong, Y., Y. Liu, Z. Xiong, D. Kaback and D. Zhao. 2012. "Immobilization of Mercury in Field Soil and Sediment Using Carboxymethyl Cellulose Stabilized Iron Sulfide Nanoparticles," *Nanotechnology*, 23, pp. 294007.

Gregory, K.B., P.L. Casanova, G.F. Parkin, and M.M. Scherer. 2004. Abiotic Transformation of Hexahydro-1,3,5-trinitro-1,3,5-triazine by Fe(II) Bound to Magnetite," *Environmental Science and Technology*, 38(5), pp. 1408-14.

Kennedy, L., J. Everett, and J. Gonzales. 2006. "Assessment of Biogeochemical Natural Attenuation and Treatment of Chlorinated Solvents, Altus Air Force Base, Altus, Oklahoma," *Journal of Contaminant Hydrology*, 83, pp. 221-236.

Kennedy, L. 2011. "Theory and Practice of Biogeochemical Reductive Dechlorination via Iron Sulfide Reduction," *37th Annual International Conference on Soils, Sediments, Water, and Energy*, October.

Kim, D. and T.J. Strathmann. 2007. "Role of Organically Complexed Iron(II) Species in the Reductive Transformation of RDX in Anoxic Environments." *Environmental Science & Technology,* 41(4), pp. 1257-1264.

Lebrón, C., P. Evans, K. Whiting, J. Wilson, E. Becvar, and B. Henry. 2010. *In Situ* Biogeochemical Transformation of Chlorinated Ethenes Using Engineered Treatment Systems. ESTCP Project ER-0316. February.

Lee, W. and B. Batchelor. 2002a. "Abiotic Reductive Dechlorination of Chlorinated Ethylenes by Iron-bearing Soil Minerals. 2. Green Rust," *Environ. Sci. Technol.*, 36(24), pp. 5348-5354.

Lee, W. and B. Batchelor. 2002b. "Abiotic Reductive Dechlorination of Chlorinated Ethylenes by Iron-bearing Soil Minerals. 1. Pyrite and Magnetite," *Environ. Sci. Technol.*, 36(23), pp. 5147-5154.

Liang, X., Y. Dong, T. Kuder, L.R. Krumholz, R.P. Philp and E.C. Butler. 2007. "Distinguishing Abiotic and Biotic Transformation of Tetrachloroethylene and Trichloroethylene by Stable Carbon Isotope Fractionation," *Environmental Science and Technol*ogy, 41(20), pp. 7094-7100.

Liu, X.M., P. Peng, J.M. Fu and W.L. Huang. 2003. "Effects of FeS on the Transformation Kinetics of γ-hexachlorocyclohexane," *Environmental Science & Technology*, 37, pp. 1822-1828.

McNeil, M.B. and B.J. Little. 1999. "The Use of Mineralogical Data in Interpretation of Long-Term Microbiological Corrosion Processes: Sulfiding Reactions," *JAIC*, 38(2), Article 6, pp. 186 to 199.

Oh, S.Y., D.K. Cha, B.J. Kim, and P.C. Chiu. 2005. "Transformation of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and Methylenedinitramine (MDNA) with Elemental Iron," *Environmental Toxicology & Chemistry*, 24(11), pp. 2812-2819.

O'Loughlin, E.J. and D.R. Burris. 2004. "Reduction of Halogenated Ethanes by Green Rust," *Environmental Toxicology and Chemistry*, 23, pp. 41-48.

Payne, D., R. Darlington, J. Gilette, and A. Bodour. 2013. "Enhanced In Situ Biogeochemical Pilot Study of a Chlorinated Ethene Plume," *Second International Symposium on Bioremediation and Sustainable Environmental Technologies*. Jacksonville, Florida. June 10-13.

Schaefer, C.E., R.M. Towne, D.R. Lippincott, V. Lazouskaya, T.B. Fischer, M.E. Bishop, and H. Dong. 2013. "Coupled Diffusion and Abiotic Reaction of Trichlorethene in Minimally Disturbed Rock Matrices," *Environ. Sci. Technol.*, 2013, 47(9), pp 4291-4298.

Schaefer, C.E., R.M. Towne, D.R. Lippincott, P.J. Lacombe, M.E. Bishop, and H. Dong. 2014. "Abiotic Dechlorination in Rock Matrices Impacted by Long-term Exposure to TCE," *Chemosphere*, 119, pp. 744-749.

Slater, G.F., B.S. Lollar, B.E. Sleep, and E.A. Edwards. 2001. "Variability in Carbon Isotopic Fractionation during Biodegradation of Chlorinated Ethenes: Implications for Field Applications," *Environ. Sci. Technol.*, 35(5), pp. 901-907.

Slater, G.F., B. Sherwood Lollar, R. Allen-King, and S. O'Hannesin. 2002. "Isotopic Fractionation during Reductive Dechlorination of TCE by Zero Valent Iron: Influence of Surface Treatment," *Chemosphere*, Vol. 49, pp. 587-596.

Smith, J., E. Evans, R. Wymore, B. Henry, and J. Hall. 2014. Pilot-Scale Demonstration of In Situ Biogeochemical Transformation for Treatment of Chlorinated Solvents. Ninth International Conference – Monterey, California, May.

United States Geological Survey. 2013. Impacts of Sulfate Contamination on the Florida Everglades Ecosystem. May. Retrieved from http://pubs.usgs.gov/fs/fs109-03/fs109-03.html.

United States Geological Survey. 2015. PHREEQC (Version 3)--A Computer Program for Speciation, Batch-Reaction, One-Dimensional Transport, and Inverse Geochemical Calculations Retrieved from http://wwwbrr.cr.usgs.gov/projects/GWC_coupled/phreeqc/.

VanStone, N.A., R. Focht, S. Mabury, and B. Sherwood Lollar. 2004. "Effect of Iron Type on Kinetics and Carbon Isotopic Enrichment of Chlorinated Ethylenes during Abiotic Reduction on Fe0," *Ground Water*, 42(2), pp. 268-276.

VanStone, N., M. Elsner, G. Lacrampe-Couloume, S. Mabury, and B. Sherwood Lollar. 2008. "Potential for Identifying Abiotic Chloroalkane Degradation Mechanisms Using Carbon Isotope Fractionation," *Environ. Sci. & Technol.*, 42(1), pp. 126-132.

Wiedemeier, T.H., H.S. Rifai, C.J. Newell, and J.T. Wilson. 1999. *Natural Attenuation of Fuels and Chlorinated Solvents in the Subsurface*. John Wiley & Sons. New York.

Wilkin, R.T. 2007. Mineralogical Preservation of Solid Samples Collected from Anoxic Subsurface Environments. *Groundwater Issue,* USEPA.

Wilson, J.T., K. Banks, R.C. Earle, Y.T. He, T. Kuder and C. Adair. 2008. Natural Attenuation of the Lead Scavengers 1,2-Dibromoethane (EDB) and 1,2-Dichloroethane (1,2-DCA) at Motor Fuel Release Sites and Implications for Risk Management. USEPA, EPA 600/R-08/107, Ada, OK.

Whiting, K., P.J. Evans, C. Lebrón, B. Henry, J.T. Wilson, and E. Becvar. 2014. Factors Controlling In Situ Biogeochemical Transformation of Trichloroethene: Field Survey. Groundwater Monitoring & Remediation 10.1111/gwmr.12068.

Zhong, X., F. He, D. Zhao, M.O. Barnett. 2009. "Immobilization of Mercury in Sediment Using Stabilized Iron Sulfide Nanoparticles," *Water Research*, 43, pp. 5171-5179.