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Use of Electron Shuttles to Biologically Enhance Abiotic Dechlorination

(A Research Study of the Monitored Natural Attenuation/Enhanced Attenuation for Chlorinated Solvents Technology Alternative Project)

September 19, 2006

Washington Savannah River Company
Savannah River Site
Aiken, SC 29808

Prepared for the U.S. Department of Energy Under Contract Number DEAC09-96-SR18500

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SAVANNAH RIVER NATIONAL LABORATORY

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Executive Summary

Biological anaerobic reductive dechlorination is a robust attenuation mechanism for chlorinated solvents and, under appropriate site conditions, is the dominant attenuation process. When conditions do not favor anaerobic biodegradation, other processes need to be assessed and quantified (EPA, 1998). Abiotic reductive dechlorination is one such mechanism that may contribute to attenuation. A team of researchers conducted studies to develop a method to measure acetylene as an indicator of abiotic reductive dechlorination and to develop a method to enhance this process using electron shuttles. The results of their work indicate additional research is needed to understand and measure this mechanism.

Introduction

Over the past three decades, much progress has been made in the remediation of soil and groundwater contaminated by chlorinated solvents. Yet these pervasive contaminants continue to present a significant challenge to the U.S. Department of Energy (DOE), other federal agencies, and other public and private organizations. The physical and chemical properties of chlorinated solvents make it difficult to rapidly reach the low concentrations typically set as regulatory limits. These technical challenges often result in high costs and long remediation time frames. In 2003, the DOE through the Office of Environmental Management funded a science-based technical project that uses the U.S. Environmental Protection Agency's technical protocol (EPA, 1998) and directives (EPA, 1999) on Monitored Natural Attenuation (MNA) as the foundation on which to introduce supporting concepts and new scientific developments that will support remediation of chlorinated solvents based on natural attenuation processes. This project supports the direction in which many site owners want to move to complete the remediation of their site(s), that being to complete the active treatment portion of the remedial effort and transition into MNA.

The overarching objective of the effort was to examine environmental remedies that are based on natural processes – remedies such as Monitored Natural Attenuation (MNA) or Enhanced Attenuation (EA). The research program did identify several specific opportunities for advances based on: 1) mass balance as the central framework for attenuation based remedies, 2) scientific advancements and achievements during the past ten years, 3) regulatory and policy development and real-world experience using MNA, and 4) exploration of various ideas for integrating attenuation remedies into a systematic set of "combined remedies" for contaminated sites. These opportunities are summarized herein and are addressed in more detail in referenced project documents and journal articles, as well as in the technical and regulatory documents being developed within the ITRC.

Three topic areas were identified to facilitate development during this project. Each of these topic areas, 1) mass balance, 2) enhanced attenuation (EA), and 3) innovative characterization and monitoring, was explored in terms of policy, basic and applied research, and the results integrated into a technical approach. Each of these topics is documented in stand alone reports, WSRC-STI-2006-00082, WSRC-STI-2006-00083,

and WSRC-STI-2006-00084, respectively. In brief, the mass balance efforts are examining methods and tools to allow a site to be evaluated in terms of a system where the inputs, or loading, are compared to the attenuation and destruction mechanisms and outputs from the system to assess if a plume is growing, stable or shrinking. A key in the mass balance is accounting for the key attenuation processes in the system and determining their rates. EA is an emerging concept that is recognized as a transition step between traditional treatments and MNA. EA facilitates and enables natural attenuation processes to occur in a sustainable manner to allow transition from the primary treatment to MNA. EA technologies are designed to either boost the level of the natural attenuation processes or decrease the loading of contaminants to the system for a period of time sufficient to allow the remedial goals to be met over the long-term. For characterization and monitoring, a phased approach based on documenting the site specific mass balance was developed. Tools and techniques to support the approach included direct measures of the biological processes and various tools to support cost-effective long-term monitoring of systems where the natural attenuation processes are the main treatment remedies. The effort revealed opportunities for integrating attenuation mechanisms into a systematic set of "combined remedies" for contaminated sites.

An important portion of this project was a suite of 14 research studies that supported the development of the three topic areas. A research study could support one or more of these three topic areas, with one area identified as the primary target. The following report documents the results of research to develop a method to measure acetylene as an indicator of abiotic reductive dechlorination and to develop a method to enhance this process using electron shuttles. This effort was led by Patrick McLoughlin of Microseeps. This study supports the topic area(s) of enhanced attenuation with mass balance being a secondary development area. There were several specific objectives for this study. They were: 1) Investigate indicator parameters. 2) Evaluate potential to enhance the process. Specifically, a) establish, at bench-top scale, a remediation strategy in which rapid abiotic remediation of chlorinated ethenes is brought about through the addition of a carbon source and humic substance to a contaminated aquifer. b) Develop an analytical methodology for measurement of a parameter that can be used to optimize the treatment process. c) Evaluate methods for sampling and preserving the end-product of the abiotic remediation so the progress of the remediation can be traced.

There is a growing body of literature (Lee and Batchelor [2002a, 2002b, 2003, 2004], Ferrey *et al* [2004], Danielsen and Hayes [2004], Cervini-Silva *et al* [2001], and Elsner *et al* [2004]) documenting abiotic reductive dechlorination. However, techniques to measure the process insitu and methods to sustain the process are needed. This research effort was selected for funding, because advancement of remediation activities supporting abiotic reductive dechlorination will meet the preference as stated in EPA documentation (EPA 1998, 1999) for destructive mechanisms and advancement of techniques for measuring the process in situ will provide quantifiable monitoring methods.

The research team worked diligently and creatively to achieve all of their objectives. Although the proposed hypotheses were reasonable and the work was well performed it was not possible to prove the hypotheses valid. The researchers drew several worthwhile conclusions about the value of existing and alternate methods, even though the proposed

techniques associated with acetylene and electron shuttle assays did not fully materialize. Acetylene was proven stable at the pH of a range of preservatives, but it proved to be difficult to measure, presumably because it is not well conserved in the environment. The studies described within did not indicate that electron shuttles resulted in significant increases in abiotic degradation rates. Nor did the bulk electron shuttle capacity assay measurements using chemical reagents prove feasible.

This work should provide some good background information on abiotic processes and monitoring technologies and results.

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Final Project Report: Use of Electron Shuttles to Biologically Enhance Abiotic Dechlorination

Principal Investigators: Patrick W. McLoughlin, John T. Wilson, Robert J. Pirkle and Barbara J. Wilson

Table of Contents

List of Tables

List of Figures

List of Acronyms

Conc.	concentration
AHDS	the reduced form of AQDS
AQDS	anthraquinone-2,6-disulfide
AU	absorbance units
CSIA	compound specific isotope analysis
cVOC	chlorinated volatile organic compound
DI	de-ionized water
EOS	a suspension of edible soybean oil in water – not an acronym
ES	electron shuttle
ICAP	inductively coupled argon plasma
NA	not applicable
ORP	oxidation-reduction potential
RO	reverse osmosis
SRS	Savannah River Site
TES	total electron shuttle
VOA	volatile organic analysis
bgs	below ground surface
C	concentration
C_0	initial concentration
Kg	kilogram
e^-	electron
E	cell half potential
kJ/mol	kilo-joule per mol
ln	natural logarithm
mg/l	milligram per liter
min	minutes
ml	milliliter
mM	millimolar
mMolar	millimolar
mV	millivolt
N	normal
nM	nano-molar
PPB	part per billion (in this report PPB = ug/l)
PPM	part per million by mass (in this report PPM = mg/l)
PPM $CaCO_3$	PPM as calcium carbonate
ug/Kg	microgram per kilogram
ug/l	microgram per liter
V	volts
‘	feet

Chemicals

C	carbon
C_2H_2	acetylene
C_2H_4	ethene
C_2H_6	ethane
CO_2	carbon dioxide
1,1DCE	1,1 dichloro-ethene
cis-DCE	cis-dichloro-ethene
cDCE	cis-dichloro-ethene
tDCE	trans dichloro-ethene
Fe	iron
Fe^{+2}	ferrous iron
Fe^{+3}	ferric iron, iron(III)
$FeCl_2$	ferrous chloride
Fe_2O_3	iron(III) oxide
H_2	molecular hydrogen
H^+	hydrogen ion
HCO_3^-	bicarbonate anion
HCl	hydrochloric acid
H_2O	water
H_2S	hydrogen sulfide
Mg(0)	magnesium metal, not ionized
NaOH	sodium hydroxide
Na_2S	sodium sulfide
$Na_2B_4O_7$	sodium tetra-borate
O_2	molecular oxygen
PCE	tetra-chloro ethene
S^{2-}	sulfide anion
SO_4^{2-}	sulfate anion
TCE	tri-chloro ethene
TSP	tri-sodium phosphate
VC	vinyl chloride
Zn(0)	zinc metal, not ionized

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Introduction

The initial concept was to stimulate reducing conditions and to use electron shuttle compounds to extend the range and extent of iron reduction, creating aqueous ferrous iron that would in turn adsorb to the iron surfaces and stimulate abiotic remediation. This mechanism had numerous advantages, but relied upon a significant advancement in the science of both electron shuttles and abiotic remediation. While the results are suggestive, the ultimate remedial technique was unable to produce convincing remediation. However, many lessons were learned during the course of this study. To share those lessons most informatively, the results of this work will be presented in five separate sections:

- Use of dissolved gases as indicators of abiotic remediation.
- Electron Shuttle Assay
- Abiotic remediation of PCE in sterilized, reduced sediment
- Abiotic remediation of PCE in sediment amended with an organic substrate and electron shuttles
- Abiotic remediation of TCE in sterilized, reduced sediment

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1.0 Use of Dissolved Gases as Indicators of Abiotic Remediation

If abiotic remediation is going to become a useful remedial tool, we must be able to document it. It is hoped that acetylene, typically the first non-chlorinated product of abiotic remediation, can be used as an indicator that would allow us to document abiotic remediation. However, there are significant questions about the chemistry of acetylene, particularly of acetylene hydrolysis, biotic activity and abiotic interactions. The potential for these interactions must be reviewed if dissolved acetylene is to be used as an indicator of abiotic remediation. This would enable practitioners to know *a priori* the conditions under which acetylene is not reliably conserved.

1.1 Hydrolysis

One possible fate of acetylene would be hydrolysis. Hydrolysis rates are often pH dependant. To investigate the stability of acetylene in water at multiple pH's buffered solutions were prepared, boiled and then placed in serum vials with no headspace and crimp-sealed butyl stoppers. An aliquot of the water was then replaced with an acetylene seeded gas mixture. The vials were shaken to allow for equilibration, and then transferred into VOA vials that were closed with gas-impermeable septa. Two of those vials were then sacrificially sampled on day 0 (the day of sample preparation), 1, 2, 4, 7, 11 and 18. The solutions were a pH of 2 (that typical of HCl preservation), 4, 7, 11 (that typical of TSP preservation) and 14 (that typical of NaOH preservation).

The results are displayed in Figures 1 – 5 and summarized in Table 1. As revealed by the data in Table 1, within the error of this experiment, acetylene, ethene and ethane (the later two also in the seed mixture) did not show appreciable loss within 18 days. This implies that on the time scale of typical laboratory turnaround, the hydrolysis of acetylene would not be a problem at any pH.

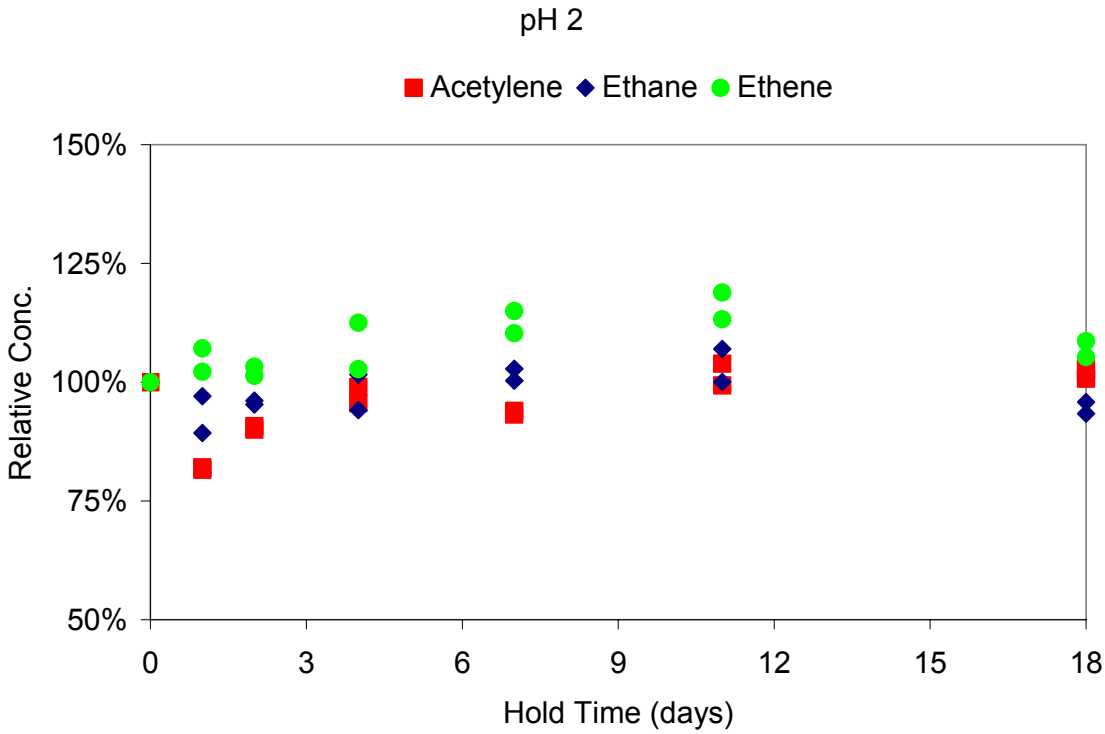

Figure 1. Stability of acetylene in sterile water at pH 2.

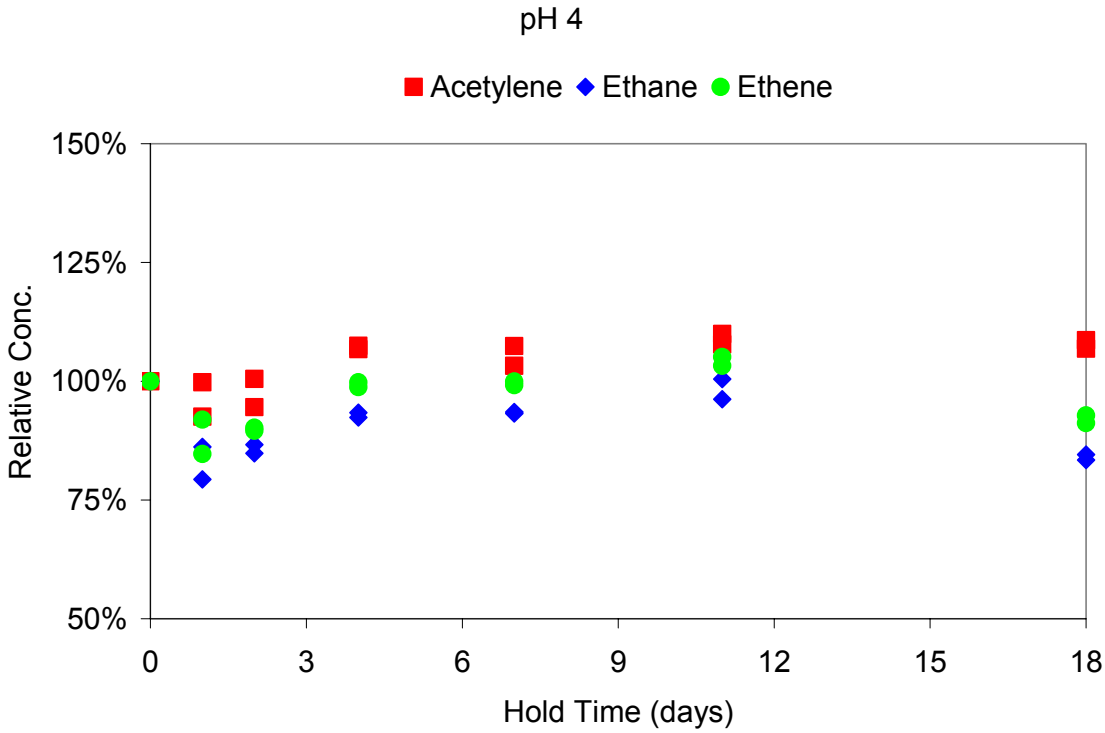

Figure 2. Stability of acetylene in sterile water at pH 4.

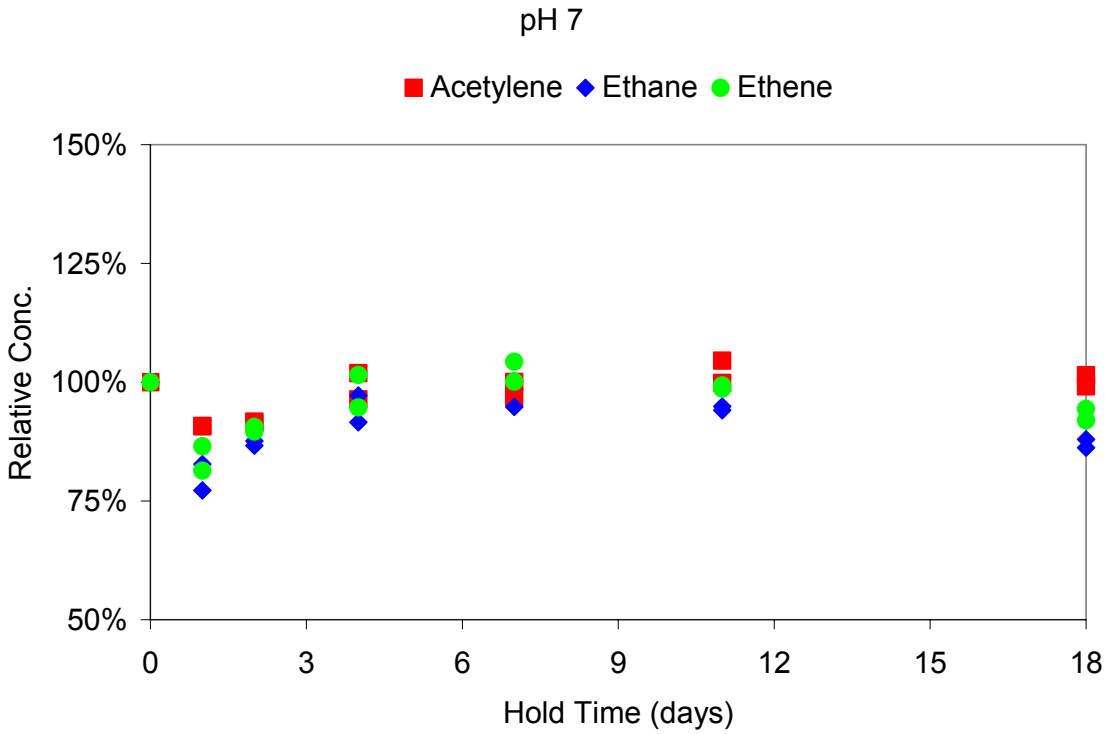

Figure 3. Stability of acetylene in sterile water at pH 7.

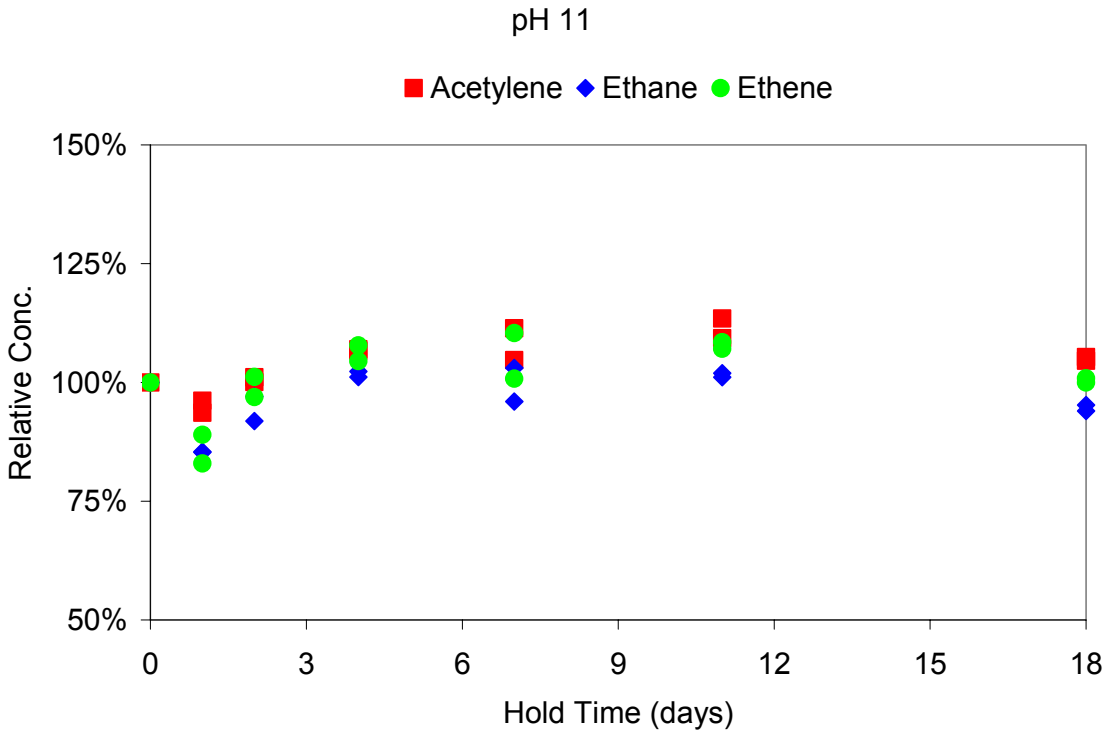

Figure 4. Stability of acetylene in sterile water at pH 11.

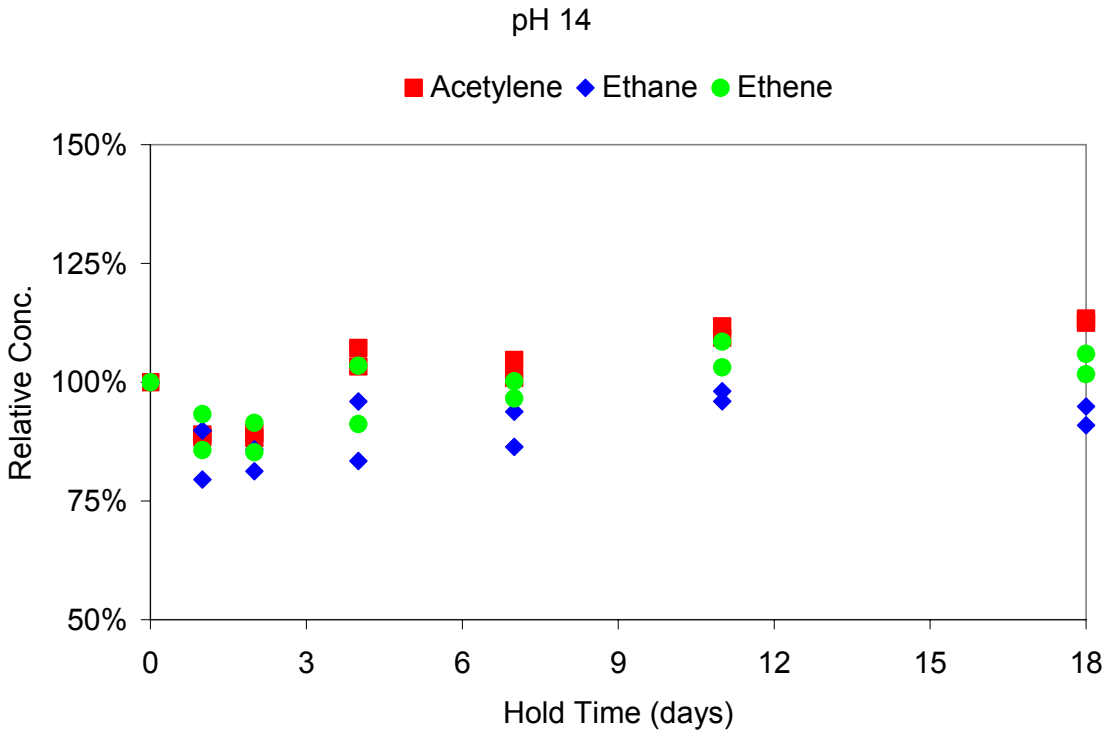

Figure 5. Stability of acetylene in sterile water at pH 14.

Table 1. Averaged recoveries at each pH.

	pH 2	pH 4	pH 7	pH 11	pH 14
Acetylene	94 ± 7	104 ± 6	97 ± 5	104 ± 6	101 ± 10
Ethane	98 ± 5	90 ± 6	90 ± 7	96 ± 6	90 ± 6
Ethene	108 ± 6	96 ± 6	94 ± 7	101 ± 8	97 ± 8

As this data shows, there is no reason to suspect that chemical reactivity would be a problem in a pH adjusted sample collected for dissolved acetylene analysis.

1.2 Biotic Stability

Many examples of acetylene disappearance and/or utilization were found in the literature. The following are summaries of some of the literature articles that indicates how ubiquitous, under all conditions, acetylene consumption is.

- Watanabe and deGuzman (1980) found rapid disappearance of C_2H_2 from soil samples taken from the anaerobic layer of a planted rice paddy. Acetylene disappeared quickly after a 1-day lag.
- Culbertson et al. (1981) found that C_2H_2 disappeared and CO_2 concurrently increased in anaerobically incubated estuarine sediment slurries. Acetylene loss was inhibited by chloramphenicol, air, and autoclaving.

• Kanner and Bartha (1979, 1982) reported that *Nocordia rhodochourus* utilized C_2H_2 under aerobic conditions … They reported that *N. rhodochourus* was capable of utilizing C_2H_2 as its sole carbon source. They found evidence that C_2H_2 is catabolized via acetaldehyde to acetate.

This indicates that there is reason to assume acetylene is a poorly conserved tracer under all conditions in a non-sterile environment.

1.3 Abiotic Interaction

Some reports talked about the interaction of acetylene with soils. Braga and Parkin (1997) stated "Yeomans and Beauchamp (1982) observed C_2H_2 loss within soil slurries over longer incubation periods under low organic C conditions but were unable to conclude that C_2H_2 was being used as a C source. Observing no loss of C_2H_2 when organic C was added, they hypothesized that under low organic C conditions, C_2H_2 may absorb to soil particles…". When they perform flow-rate tests with acetylene and a bromide tracer they note that the peak in the breakthrough curve for C_2H_2 lagged that of the bromide tracer by an average of 16%. They give no explanation for that lag. But clearly there are a number of interactions in the subsurface retarding the acetylene and they could affect not only the transport but also the fate of acetylene.

While this evidence is convincing, it is admittedly limited. To help overcome that lack, an investigation of the thermodynamics of acetylene stability versus both reduction and mineralization was calculated under conditions conducive to iron reduction, sulfate reduction and methanogenesis. The results are displayed in Table 2. For comparison purposes, the same calculations were repeated for ethene. Those results are displayed in Table 3.

Comparing Tables 2 and 3, it can be seen that all fates of acetylene and ethene that were investigated are much more energetically favorable as fates of acetylene. Thermodynamics only predicts the energetics of processes, not the rate of those processes, but this investigation shows that if there is a way to affect acetylene reduction or mineralization under anoxic conditions, there is a tremendous energy yield to doing so. This further supports the suggestion that dissolved acetylene can not be relied on as a conservative indicator.

While the stability of dissolved acetylene in the absence of sediment and under sterile conditions is encouraging, from acetylene's ubiquitous consumption by bacteria, acetylene's uncertain loss in soil and the huge thermodynamic drives behind acetylene's destruction under anoxic conditions, it is clear that the absence of acetylene is not indicative of an absence of abiotic remediation.

Table 2. Gibbs free energies of the reduction and mineralization of acetylene under selected dominant TEAP conditions.

Dominant TEAP	Reduction	Mineralization	Conditions
Iron Reduction	-102 kJ/mol $C_2H_2 + H_2 \Longrightarrow C_2H_4$	-209 kJ/mol $C_2H_2 + 6H_2O \Longrightarrow 2HCO_3^- + 2H^+ + 5H_2$	H_2 – 0.5 nM pH - 7 C_2H_2 – 0.2 PPB C_2H_4 – 0.1 PPB Alkalinity – 300 PPM $CaCO_3$
Sulfate Reduction	-106 kJ/mol $C_2H_2 + H_2 \Longrightarrow C_2H_4$	-223 kJ/mol $C_2H_2 + SO_4^{2-} + 2H_2O \Longrightarrow 2HCO_3^- + H_2S + H_2$	H_2 – 2 nM C_2H_2 – 0.2 PPB C_2H_4 – 0.1 PPB SO_4^{2-} - 200 PPM Alkalinity – 300 PPM $CaCO_3$ H_2S – 2 PPM
Methano-genesis	-110 kJ/mol $C_2H_2 + H_2 \Longrightarrow C_2H_4$	-172 kJ/mol $C_2H_2 + 6H_2O \Longrightarrow 2HCO_3^- + 2H^+ + 5H_2$	H_2 – 10 nM pH - 7 C_2H_2 – 0.2 PPB C_2H_4 – 0.1 PPB Alkalinity – 300 PPM $CaCO_3$

Table 3. Gibbs free energies of the reduction and mineralization of ethene under selected dominant TEAP conditions.

Dominant TEAP	Reduction	Mineralization	Conditions
Iron Reduction	-63.4 kJ/mol $C_2H_4 + H_2 \Longrightarrow C_2H_6$	-105 kJ/mol $C_2H_4 + 6H_2O \Longrightarrow 2HCO_3^- + 2H^+ + 6H_2$	H_2 – 0.5 nM pH - 7 C_2H_4 – 0.1 PPB C_2H_6 – 0.1 PPB Alkalinity – 300 PPM $CaCO_3$
Sulfate Reduction	-66.8 kJ/mol $C_2H_4 + H_2 \Longrightarrow C_2H_6$	-117 kJ/mol $C_2H_4 + SO_4^{2-} + 2H_2O \Longrightarrow 2HCO_3^- + H_2S + 2H_2$	H_2 – 2 nM C_2H_4 – 0.1 PPB C_2H_6 – 0.1 PPB SO_4^{2-} - 200 PPM Alkalinity – 300 PPM $CaCO_3$ H_2S – 2 PPM
Methano-genesis	-70.8 kJ/mol $C_2H_4 + H_2 \Longrightarrow C_2H_6$	-60.6 kJ/mol $C_2H_4 + 6H_2O \Longrightarrow 2HCO_3^- + 2H^+ + 6H_2$	H_2 – 10 nM pH - 7 C_2H_2 – 0.2 PPB C_2H_4 – 0.1 PPB Alkalinity – 300 PPM $CaCO_3$

2.0 Electron Shuttle Assay

Electron shuttles (ES's) are potentially powerful tools to effect remediation. Indeed, the hypothesis of this work was that electron shuttles can help accelerate abiotic remediation. However, it is difficult to unravel the science of a technique that utilizes electron shuttle concentrations when they can not be meaningfully measured. Without that ability to measure, it still may be possible to unravel the bench scale science, but at field scale it is impossible to engineer such a technique without reliable measurements. The efforts of this portion of the research were focused on developing a broad, inexpensive means of measuring all water-borne organic electron shuttles.

By definition, an electron shuttle is a substance which can be cyclically oxidized and reduced. Cyclic voltammetry can be used to perform repeated cycles of oxidation and reduction, so it seemed a natural choice. Unfortunately, literature reports (Nurmi and Tratnyack, 2002a; Nurmi and Tratnyack, 2002b) have indicated that technical practicalities make the measurement of ground water borne electron shuttles through cyclic voltammetry considerably more laborious than one would estimate based upon conceptual considerations. However, the cycles imposed by cyclic voltammetry can also be imposed by various chemical treatments. Products of those cycles can be collected and quantified. Their measurement is a measurement of the electron shuttle concentration.

Since there are already a number of ways to assess redox conditions (USEPA, 2002), it was decided that the ES assay did not need to address the speciation of the shuttles, but measure the total concentration of both the reduced and oxidized forms of the ES. This greatly simplified sampling, handling, storage and analysis issues.

Simple aeration during sample preparation would convert most electron shuttles to their oxidized form. To cycle the shuttles, they would then need to be reduced. While there are a number of ways to accomplish that reduction, the choices were limited by one requirement: the unreacted portion of the chosen reducing agent must be completely removed from solution, preferably not by precipitation.

One of the reducing agents that met this criterion was sodium sulfide. It was hypothesized that the protonated anion of sodium sulfide could be removed by vacuum, leaving behind only the inactive sodium cation and the anion of the acid that was used to reduce the pH and protonate the sulfide.

To test sodium sulfide's effectiveness as a reducing agent at various pH's, the following test was conducted. In 40 ml clear glass vials 1 ml of 100 mMolar (mM) Na_2S was mixed with 10 ml of 10 mM anthraquinone-2,6-disulfide (AQDS), with the AQDS solution made up in various pH buffers. The vials were closed with Microseeps' dissolved gas septa (those septa are impermeable to oxygen). The vials were then mixed and the results are presented in Table 4.

Table 4. Observed changes upon mixture of 10 mM AQDS upon reaction.

Vial	Buffer	Reducing Agent	Color change
A	pH = 1 (0.1 Molar HCl)	10 mM Na_2S	None
B	pH = 4.01	10 mM Na_2S	None
C	pH = 6.86	10 mM Na_2S	started yellow, went to orange
D	pH = 1 (0.1 Molar HCl)	Zn(0)[i]	started yellow, turned dark green quickly, went to brownish orange
E	pH = 4.01	Zn(0)[i]	started yellow, turned green, then dark green, then brownish orange
F	pH = 6.86	Zn(0)[i]	started yellow, went to orange
G	pH = 1 (0.1 Molar HCl)	Mg(0)[ii]	started yellow, formed pale orange precipitate in vial and on ribbon
H	pH = 4.01	Mg(0)[ii]	started yellow, formed dark orange solution with no apparent precipitate
I	pH = 6.86	Mg(0)[ii]	started yellow, went to pale orange with no apparent precipitate but very slow reaction

The first dissociation constant for hydrogen sulfide (H_2S) is 5.7 X 10^{-8}. That means that in water at pHs above 7.2 the majority of the Na_2S will be present as HS^-, while at pHs below 7.24 the majority of the H_2S will be present as H_2S. Less of the anion is present as the pH is lowered. The lack of color change observed at low pHs implies that the anion must be present in substantial portions for the reduction to occur. This is further illustrated by the published half-cell potential for the reduction of sulfide:

$S + 2H^+ + 2e^- \Leftrightarrow H_2S(g)$ E = +0.14 V

$S + 2e^- \Leftrightarrow S^{2-}$ E = -0.48 V

From this it can be seen that the anion is a much stronger reducing agent than the protonated acid, so the reduction favors high pH's (i.e. basic conditions).

The vials from the above experiment were prepared with considerable headspace. The vials were prepared under ambient conditions, so that headspace contained oxygen. When vial C was shaken, the color temporarily reverted to yellow. We hypothesized that this was evidence of the oxygen in the vial oxidizing the reduced AQDS. This was a clear indication of the reversibility of the reaction. It later became apparent that the observed effect of in-vial oxidation was also clear evidence of the sensitivity of the system to oxygen.

Another reducing agent where the un-reacted portion can easily be removed from solution is zinc metal. To test this 3-40 ml clear glass vials were made up with ~5g 20 mesh zinc in each and 10 ml of AQDS, again in various buffers. The results of this experiment are also listed in Table 4. The ready reversibility and the dramatic effect of low amounts of oxygen contamination could be seen by shaking vial F and watching the color temporarily change back to yellow. (That effect could not be seen in the other vials because it was too hard to see through them.)

Forty hours after the preparation of vials D, E and F an orange precipitate could be seen in all vials, with large amounts in vial D. This led to the conclusion that zinc made a poor reducing agent for an electron shuttle test because it formed precipitates with the reduced ES's too easily. Perhaps the concentration of AQDS used in this study (10 mMolar) is higher than will typically be found in field samples, but it was chosen to pursue other alternatives at this juncture.

Because of this precipitate problem 3 more vials were prepared as before but using strips of magnesium ribbon as the reducing agent. The results are presented in Table 4. The lack of a discernable pattern following the varying pH was somewhat unsettling, but the results from vial H were quite promising.

To further explore the use of magnesium ribbon as a reducing agent 1 ml of 10 mM AQDS in 4.01 buffer was mixed into 39 ml of 4.01 buffer and a 3" length of Mg ribbon was added. At first a small headspace was left (~1 ml) and the vial was capped. Within several minutes the color went from pale yellow to pale green and there was an obvious evolution of gas. It was clear that the 1 ml headspace would not be large enough, so half of the volume was removed from the vial and the reaction was allowed to continue. In the end, it produced a colorless solution and there was an orange coating on the ribbon. Apparently there is a problem between the reduced ES and the Mg ribbon that leads to a coating of the ribbon. Perhaps it was some sort of fouling from this process that led to the observations in vial G that were not seen in vial H. This has led to the conclusion that Mg ribbon, while effective, is probably also a poor choice for a quantitative analysis, especially at ES concentrations as high as 10 mMolar.

The reducing agent chosen was sodium sulfide, Na_2S. It was clear from the previous experiments that the reduction would be affected by sodium sulfide. The issue was removing it from the solution. It was shown above that higher pH's were required to drive the reduction, but those high pH's also meant that very small amounts of the sulfide would be present as the acid. An experiment would need to be constructed to determine whether the pressure of that acid was large enough to lead to removal of the sulfide by vacuum extraction and whether that removal could be carried out at a reasonable rate.

To do this we needed to measure sulfide concentrations very sensitively and with minimal sample consumption, but only in a semi-quantitative fashion. Spot-tests allow for the mixing of one drop of test sample with 1-2 drops of reagents. Typically, these tests produce a precipitate if positive. The concentration can be judged semi-quantitatively by the amount of precipitate that is formed. Because of the low solubility of zinc sulfide, zinc acetate is often

used to cause sulfide to precipitate. Since the reduction of the AQDS was to be carried out in a pH = 9.18 buffer solution, a test solution of 20 mMolar Na_2S in 54 mMolar 9.18 buffer and a blank of 60 mMolar 9.18 buffer were prepared, as was a 200 mMolar solution of zinc acetate. When a drop of the blank was mixed with the zinc acetate, a white precipitate was observed. This was likely the insoluble zinc hydroxide. When a drop of the zinc sulfide test solution was tested with zinc acetate, a white precipitate was also formed. It could not be concluded whether the precipitate was zinc hydroxide or zinc sulfide. If the spot test were to be diagnostic, it was clear that the metal-sulfide precipitate would need to be distinctly different from white.

Lead sulfide gives a black precipitate. Since the volume of lead solution consumed in a test would be approximately 0.05 ml, the lead consumption would be minimal and would not produce a significant waste stream concern. The zinc acetate solution was replaced with a lead acetate solution. The spot test was repeated. Using the lead acetate as an indicator, there was a very obvious difference between the blank and the solution that contained sodium sulfide.

With an applicable spot test developed, it was attempted to test the ability to remove the unreacted sodium sulfide from the test solution by applying a vacuum to the test solution. Since it had been found that higher pH's were required to affect the reduction of the electron shuttles, the reduction was to be accomplished in a pH = 9.18 buffer. The test solution was prepared by mixing 54 ml of 60 mMolar 9.18 buffer and 6 ml of a 20 mMolar sodium sulfide solution. That solution was placed under a vacuum and periodically spot-checked for sulfide. The results are presented in Table 5.

Table 5. Sulfide spot-test results from sulfide removal experiment.

Time under vacuum (min)	drops glacial acetic acid	test result	Comment
0	0	+	spot test is dark
15	0	+	spot test is dark
25	0	+	spot test is dark
60	0	+	spot test is dark
60	9	NA	pH was ~10 before, ~8 after
67	9	+	spot less dark
74	9	+	spot less dark
81	9	+	spot less dark
88	9	+	spot less dark
97	9	~	presence of precipitate questionable, slight odor still present.

From Table 5 it is clear that the pH must be lowered to affect the removal of sulfide in a time practical for a routine analysis. To accommodate this, a sodium tetraborate ($Na_2B_4O_7$) buffer of pH = 10 was used rather than a 9.18 buffer. A test solution was constructed to be 10

mMolar $Na_2B_4O_7$ and 10 mMolar Na_2S. This mixture had a pH of ~10. A 20 mL aliquot of that sample was placed into a vacuum chamber and 2 drops of glacial acetic acid were added, bringing the solution pH to ~7. Vacuum was applied to the system and the spot test was used to monitor after 0, 5, 10, 15, 20 and 30 minutes of vacuum. As expected, the initial spot test detected a large presence of sulfide. In the 5, 10 and 15 minute tests, the concentration of the precipitate steadily decreased. In the 20 minute test, no precipitate could be observed, though there was still a discernable sulfide odor emanating from the test solution. After 30 minutes of vacuum the spot test was negative and there was only a very faint sulfide odor emanating from the test solution.

Having developed a spot-test for the presence of sulfide and a way to remove unreacted sulfide from the solution, a quantitative conversion of AQDS to AHDS to dissolved iron (*i.e.* an electron shuttle measurement) was attempted. Solutions of 2, 1, 0.5, 0.1 and 0.05 mMolar AQDS concentration were prepared, each in a 10 mMolar $Na_2B_4O_7$ buffer solution. We put the solutions into a glove bag. The glove bag was repeatedly flushed with 99.9998% nitrogen and the nitrogen purge was continually passed through the glove bag throughout the test. All samples showed a color change indicating reduction. However, the manipulation of the sulfide extraction caused a reversal of the color change. Efforts to refine the glove bag procedure so as to eliminate inadvertent oxygen introduction did not eliminate the problem. Despite this observation, excess iron(III) oxide was put into each vial after sulfide removal. It was allowed to incubate for ~ 36 hours. The solutions were then filtered through a 0.45 micron filter and the filtrate was collected into a vessel containing 1 drop 1:1 nitric acid. The acidified filtrate was then analyzed on an ICAP as per SW846-6010 and the iron concentrations observed are given in Table 6.

Table 6. Dissolved iron concentrations generated from test of electron shuttle measurement.

AQDS conc. (mM)	Iron conc. (ppm)
2.00	+0.0016
1.00	-0.0007
0.500	+0.0096
0.100	-0.0021
0.0500	-0.0013
blank	-0.0005

While the 0.500 mMolar AQDS solution produced some iron (the reporting limit for this test is 0.010 ppm), there is no discernable pattern to the observed iron concentrations, so it could not be said that the test was quantitative. This was consistent with the reversal of the color of the vacuum treated solution. Observing this, it was decided that any further work had to be done in a carefully controlled, oxygen free environment in a glove-box.
To further justify the "proof in concept" behind the postulated procedure and to support the acquisition of the glove-box, it was decided to perform a "proof in concept" test utilizing zinc iron as the reducing agent. Because of the previously observed precipitation problems zinc

caused, it was not anticipated that this test would be quantitative. However, it was hoped that it would provide a reduced solution of AQDS.

In each of two clear 40 mL vials, 20 ml of 2 mM AQDS was combined with 20 ml of 20 mM $Na_2B_4O_7$ and ~5 gr. zinc filings. The solutions were then capped with gas impermeable caps and mixed. The color change indicative of AQDS reduction was apparent in both vials. In a glove bag, the solutions were then filtered through a 0.45 micron filter. For each, the filtrate was collected into another 40 ml vial. An excess of iron(III) oxide (Fe_2O_3) was placed in one vial. The vials incubated for approximately 24 hours. Over that time the color change was lost in both vials. However, if the rate of iron(III) oxide reduction by reduced AQDS was faster than the rate of oxidation of reduced AQDS by diffusion of oxygen into the vial, ferrous iron would have been present in the system. Using a 0.45 micron filter the iron(III) oxide was removed from the solution and the filtrate was collected in a vessel that contained 1:1 nitric acid. That filtrate was analyzed for iron via ICAP according to SW846-6010 (USEPA, 1996). The solution which did not have iron(III) oxide in it gave an iron concentration of -0.0006 ppm, The solution that did have a chance to react with iron(III) oxide had an iron concentration of 0.0036 ppm.

That result was clearly higher than the solution which had no iron and was clearly higher then all but one of the AQDS solutions produced in the experiments that produced the data in Table 6. This suggests that a solution of AQDS can be reduced and then, after removal of any unreacted reducing agent, be used to reduce iron(III) oxide. Further, this demonstrates that extreme measures must be taken to insure there is no inadvertent oxygenation of the reduced sample during reduction, removal of the excess reducing agent, or incubation of the reduced sample with iron(III) oxide.

The use of sodium sulfide to reduce AQDS is similar to the use of hydroxyl amine hydrochloride to reduce iron(III) in the total iron test described in SM 3500 FE (USEPA, 1996). In that test, the hydroxyl amine is used in at least an eight fold excess. This is considerably stronger then was used in the experiment described to date. If the reducing conditions we generated were only sufficient to reduce ~10% of the AQDS, it would be much easier to understand how errant oxygen could easily reverse the color changes we produced. Unfortunately, if such an excess of Na_2S was required, it would imply that chemical reduction via this approach was impractical.

To test how much excess Na_2S was required, two solutions were made up in 1 cm. cuvettes. The solution contained 8.5 ml of 200 PPM Na_2S and one or two drops of an 10 mM AQDS solution such that the concentration of AQDS (or reduced AQDS) was 0.042 mM in cuvette A and 0.085 mM in cuvette B. Using a “Spec 20” the absorbance was measured at 450 nm in each cuvette. The results were 0.651 AU in cuvette A and 1.319 AU in B. These absorbances were considerably higher than would have been expected given the extinction coefficient and spectroscopy reported by Cervantes et al (2000). Oxidation of a similarly prepared solution showed a yellow precipitate, presumably sulfur produced by the oxidation of the sulfide that accompanies the reduction of the AQDS. This precipitate was only partially removed by filtration with a 0.45 micron filter. However, it was clear that as the reaction progressed, the absorbance would continue to increase, and we simply needed to

assess what amount of excess Na_2S was required to go to completion, so absorbance spectroscopy was still an appropriate tool for the diagnosis.

Four additional solutions were made up, this time with a constant amount of AQDS but steadily decreasing Na_2S doses. The compositions are given in Table 7. In that table, the additions are specified in micromoles or umoles.

Table 7. Compositions of test of Na_2S required to reduce 50 umoles of AQDS.

Solution ID	AQDS (umoles)	Theoretical Na_2S (umoles)	Added Na_2S (umoles)
3	50	50	1000
4	50	50	600
5	50	50	400
6	50	50	200

Solutions were prepared in water with a final volume of 50 ml. Following mixing, the solutions were allowed to react overnight. If reduced AQDS were responsible for the color, and the reaction went to completion in all cases, the solutions should all appear the same. In fact, there was a visibly noticeable gradation in color intensity with 3 > 4 > 5> 6. If that pattern were produced by variations in the concentration of reduced AQDS even with an extended reaction period, a very large excess of Na_2S was required to maximize the absorbance. If that pattern were produced by variations in the content of sulfur in the samples, the inadvertent oxidation of Na_2S by very low quantities of stray oxygen was a significant sink to the Na_2S and, despite the quantity of Na_2S added, an excess could not be assured.

At this point the following drawbacks to this experimental approach had been realized:

1) Sodium sulfide was found to react with some electron shuttles.
In reports by Perlinger, Angst and Schwarzenbach (1996) and Perlinger et al (2002), juglone, a prevalent electron shuttling compound, was found to undergo Michael addition when reacted with H_2S. Na_2S, which rapidly forms H_2S when dissolved in water, was used.

2) pH adjustments were necessary to reduce AQDS and again to remove H_2S.
These pH adjustments could readily be achieved through the addition of acids and bases. However, each such addition increases the ionic strength of the solution. Since the ES's are sparingly soluble molecules that are mostly hydrophobic, their solubility tends to decrease as the ionic strength is increased. No quantitative experiments were done to prove this, but the formation of precipitates could easily be observed as the solution ionic strength was increased.

3) Generation of H_2S a hazard.
While this is a readily controlled issue and this analysis would be conducted in a glove-box, it must be remembered that an analysis of electron shuttling capacity could readily become commercialized. This means frequent repetition of the analysis and potential execution of the analysis by technicians without the broad knowledge of chemistry that enables the method developers to recognize such hazards and avoid exacerbating them. These potential hazards are magnified by the requirement to use a large excess of Na_2S to quantitatively reduce all of the ES's. This potential hazard makes this analysis scheme considerably less appealing.

4) Removal requires gas supply with << 1 PPM O_2.
Gases mixtures that are commercially available have a maximum oxygen free capacity of "< 1 PPM O_2". Reduced AQDS would readily oxidize in such an environment. This means that the atmosphere in which this analysis is conducted either needs to be closed or requires significant purification.

5) Clean gas must flow into chamber at the exact same rate dirty gas exits.
To minimize the potential for build-up of H_2S, it is desirable to continuously flush the atmosphere. Further, to reduce stray oxygen it is required to use dry gas, and the gas flushed through the reaction chambers and the H_2S scrubbers is moisture saturated, so replacing that gas is desirable. Since the glove-box can tolerate neither negative nor positive pressure, flushing the glove-box requires an exact balance of inlet and outlet gas flow. This is very difficult to maintain in a low flow environment that can not tolerate any back-streaming of the ambient atmosphere into the glove-box.

6) H_2S removal must be complete – presence of H_2S during final measurement limits sensitivity.
The ultimate measure is the quantity of iron that is reduced by the reduced solution. If any of the H_2S remains in the measured solution, it, not an ES, can reduce iron. It would be assumed that this reduction was due to ES's, and the produced concentration would be biased high. This requirement becomes that much more difficult when considering using large excesses of H_2S to quantitatively reduce the ES's.

7) Questionable availability of Fe_2O_3 to react with ES.
The hypothesis of this test was that the ES, once reduced, would react with solid phase Fe_2O_3. While such a reaction is thermodynamically favored the hypothesized analytical procedure would only work if there were quantitative reaction of all of the reduced ES with the solid phase Fe_2O_3. Since a thermodynamically favored reaction is not necessarily a kinetically favored reaction, this hypothesis had to be tested. The work of this project focused on the production of reduced ES, and the reaction of reduced ES with solid phase Fe_2O_3 was not tested.

These drawbacks suggested that this analytical procedure was not worth further pursuit. Rather, a reduction of ES with reducing bacteria would be worth investigating for the purpose of electron shuttle measurements.

3.0 Abiotic Remediation of PCE in Sterilized, Reduced Sediment

Abiotic remediation is an effective tool at some sites, and it makes little or no contribution to natural attenuation at other sites. Until it can be initiated at sites where its effect is, as yet, minimal and until such an initiation is proven tenable, it remains a site-specific artifact. In an attempt to initiate abiotic remediation in a documented fashion, a microcosm study was undertaken in which a soil was:
1) collected
2) amended with a carbon substrate and a pH buffer
3) allowed to incubate until reducing conditions were established
4) sterilized
5) dosed with ferrous iron salts and PCE
The concentration of PCE and several daughter products in the pore water was monitored over time. The following sections report on that study.

3.1 Method

The sediment was collected by roto-sonic drilling from P-area of the Savannah River Site (SRS). Cores were taken throughout the 54'-60' bgs. and 74'–80' bgs. intervals. The cores showed sections of gravely-sand that were 1-2' thick between dense clays layers of a similar thickness. Sections of the core which yielded to deformation upon squeezing with one hand were chosen because it seemed that they would yield to water flow most readily. In addition, core sections were favored in which a strong red (presumably from iron hydroxides) could be observed. Core samples of the soils showed there were some benzene, PCE, TCE and cis-DCE in the soil, but those concentrations rarely exceeded 10 ug/Kg. There were two exceptions: the TCE at 78' was 354 ug/Kg and the TCE at 80' was 341 ug/Kg. The sediment was collected in 1 liter canning jars which were topped off with either bore-hole water or DI water then sealed and shipped to the R. S. Kerr Environmental Research Center in Ada, OK.

At the laboratory in Ada the sediments were placed into an "anaerobic" glove-box where they were combined and mixed in a large, stainless steel bowl. A 1 mL portion of an EOS solution, diluted by 10 in RO water, was titrated to a pH of 7 with 1N sodium bicarbonate. This titration was used to assess the quantity of bicarbonate that was required to neutralize the EOS, and a larger solution of EOS was prepared accordingly. Thirty (30) mL of that solution was added to four jars of sediment, approximately 6 Kg. The sediment was then mixed and placed into six jars, which were allowed to incubate in the glove-box. After two months, the jar contents were recombined and returned to the six jars. Boiled RO water was added to each jar and allowed to stand overnight. Ferrous iron concentrations and oxidation-reduction potentials (ORP's) were measured in each jar and are reported in Table 8. This data suggested that reducing conditions had been established in the sediment and that added ferrous would not simply oxidize but would have a chance to interact with the sediment.

The data presented in Table 8 indicated that reducing conditions had been established in the soil. At that point approximately 33 g of sediment were placed into each of 30 labeled 20 ml serum vials. Each of the vials was sealed with a Teflon lined butyl rubber stopper type septum, but that septum was not crimped into place. Each microcosm was then weighed and

Table 8. Ferrous Iron concentrations and ORP's for each of the jars used in the effect of ferrous added study.

Jar #	Ferrous Iron (mg/l)	ORP (mV)
1	1.20	-579.0
2	1.55	-574.3
3	1.10	-584.2
4	2.00	-580.5
5	1.35	-575.0
6	1.90	-574.4

aluminum foil was wrapped around it to hold the stopper in place. The vials were then autoclaved at 250º F for 10 hours. After cooling the foil was removed from the vials and they were placed in an anaerobic chamber. Approximately 3 ml of sterile RO water was then added to each microcosm (more was added to those microcosms that had lost more material.

The source of ferrous iron used was ferrous chloride ($FeCl_2$). Three solutions of $FeCl_2$ were prepared in 165 ml serum vials. The first contained 1760 mg $FeCl_2$ and was filled with RO water. The second and third solutions were prepared identically but contained 535 mg and 176 mg of $FeCl_2$, respectively. The PCE dose solution was prepared by dissolving 33 mg PCE into a 165 ml serum vial. Besides the three levels of ferrous iron additions that were investigated (treatments 1Fe-3Fe), there was a control with sediment but no ferrous iron addition (treatment 4Fe) and a container control that contained no sediment (treatment 5Fe). The dose for that container control contained PCE, TCE and cDCE. It was labeled as the cVOC dose and was prepared by dilutions into 165 ml RO water. The $FeCl_2$, PCE and cVOC doses and RO water additions were made as outlined in Table 9. RO water was then added so that there was 5 ml of standing water in each sediment-containing microcosm. The microcosm were then sealed with Teflon lined butyl rubber stoppers and crimped.

Table 9. Microcosm make-up for effect of ferrous additions study (all additions in ml).

Treatment	sediment	$FeCl_2$	PCE	cVOC	Water
1Fe	yes	1 - 1760 solution (300 mg/l)	1	0	0
2Fe	yes	1 – 535 solution (100 mg/l)	1	0	0
3Fe	yes	1 – 176 solution (30 mg/l)	1	0	0
4Fe	yes	0	1	0	1
5Fe	no	0	0	1	filled

3.2 Sampling

Microcosms were sampled on days 2, 7, 14, 21, 28, 41, 54 and 84. All microcosms were sampled in triplicate under anoxic conditions using aseptic techniques.

Prior to sampling, each microcosm was vortex-mixed. After vortex-mixing, nominally one ml was collected from the microcosms and diluted to 40 ml for volatiles analysis (PCE, TCE, cDCE, 1,1DCE, tDCE and VC). A second 1 ml aliquot was diluted into 40 ml and that was used for the analysis of dissolved gasses (acetylene, methane, ethane, ethene, propane, propene, n-butane and iso-butane). A third 1 ml aliquot was collected and diluted to 5 ml for the analysis of ferrous iron. The standing water was then replaced with RO water, the microcosm again was sealed and capped. It was allowed to sit overnight, vortex-mixed, unsealed and sampled for anions (nitrate, nitrite, sulfate and chloride). The remaining solid was then analyzed for weak acid soluble iron. The samples were analyzed at Microseeps Laboratories, using their 2005 Standard Operating Procedures, (i.e. SW846-8260 for the volatiles analysis, PM01/AM20Gax for the dissolved gas analyses, SW846-9056 for the anion analyses, a modified version of SW846 7199 for the ferrous iron analysis and WC43 for the weak acid soluble iron analysis).

3.3 Results and Discussion

The detailed results of the analyses carried out on the microcosms are presented in the Appendix, Table A3 – A15. Figure 6 shows the PCE concentrations versus incubation time for treatments 1Fe-3Fe. Note that the treatments are labeled according to the pore water concentration of ferrous iron added to the microcosms. The concentration versus time profile of the container control for those microcosms is shown in Figure 7. It does not seem that there is significant degradation of the PCE. If the PCE concentrations recorded in treatments 1Fe, 2 Fe and 3Fe are normalized by the average of the first PCE concentrations measured for those treatments and the results are plotted as PCE (C/C_0) vs. t as in Figure 8, it becomes very clear that from these experiments there is no significant degradation of PCE.

However, there must have been some degradation to produce TCE. In Figure 9 the TCE concentration is plotted for the treatment with 300 mg/l of Fe^{+2} added to each microcosm. The appearance of TCE and its rapid degradation are consistent. Again, from Figure 9 we see that some cDCE was formed, but that was very minor. Similar patterns were observed in treatment 2Fe which had 100 mg/l $Fe+^{2}$ added. Indeed, ln(TCE) vs. t from those two treatments is plotted in Figure 10, as well as ln(TCE) for the container control. It can be seen that in 1Fe and 2Fe the degradation is significant.

While it was not clear why TCE was so much more reactive than PCE, the results were quite compelling. It was decided to halt further sampling of the microcosms and to re-spike them with ferrous iron and TCE so that the stimulated abiotic remediation of TCE could be investigated further.

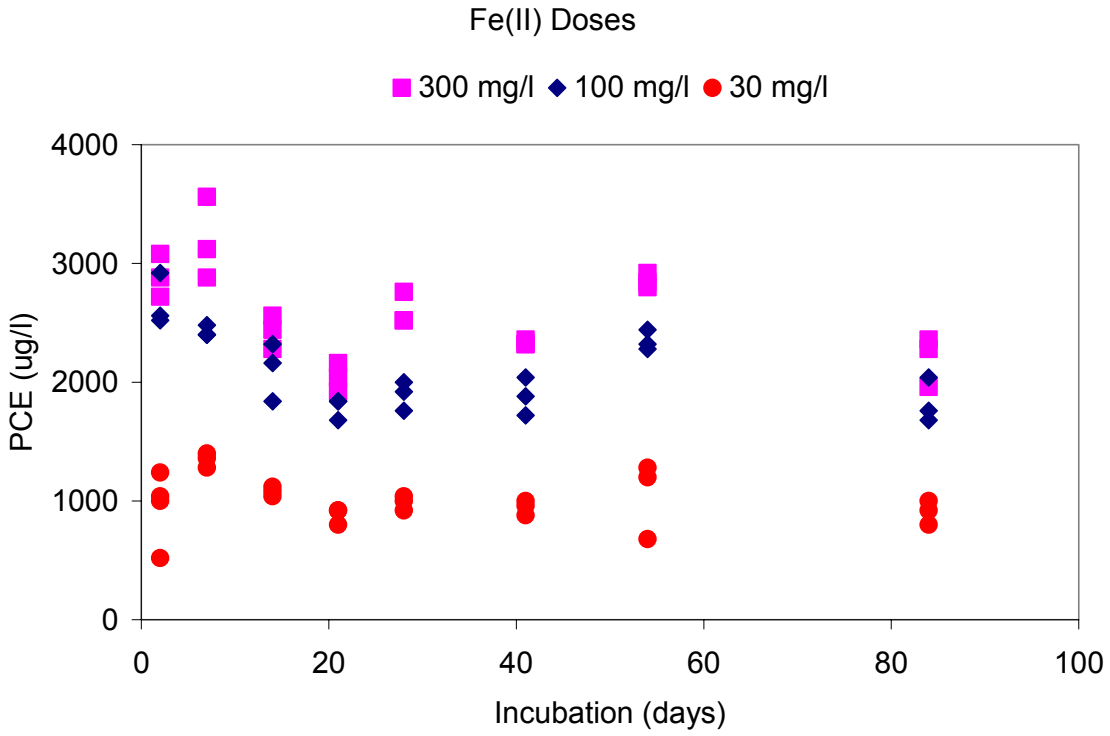

Figure 6. Concentration versus time for the PCE in treatments 1Fe, 2Fe and 3Fe.

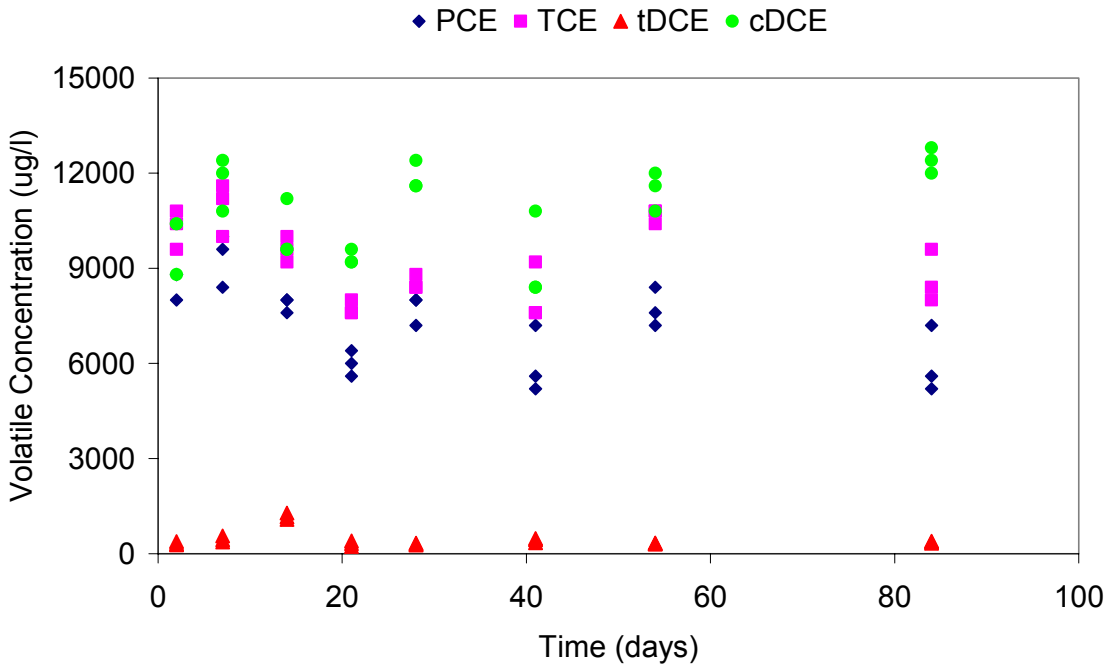

Figure 7. Concentration versus time for the PCE in treatment 5Fe, the container control for treatments 1Fe, 2Fe and 3Fe.

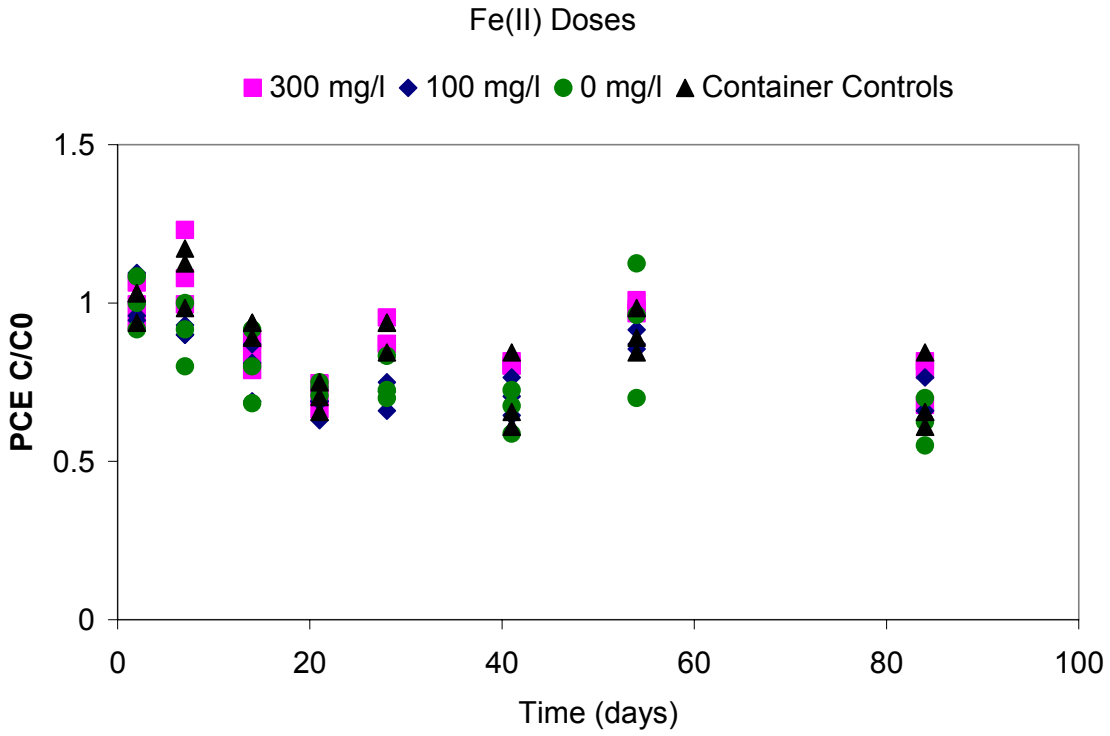

Figure 8. Normalized PCE concentrations vs. time for treatments 1Fe, 2Fe and 4Fe.

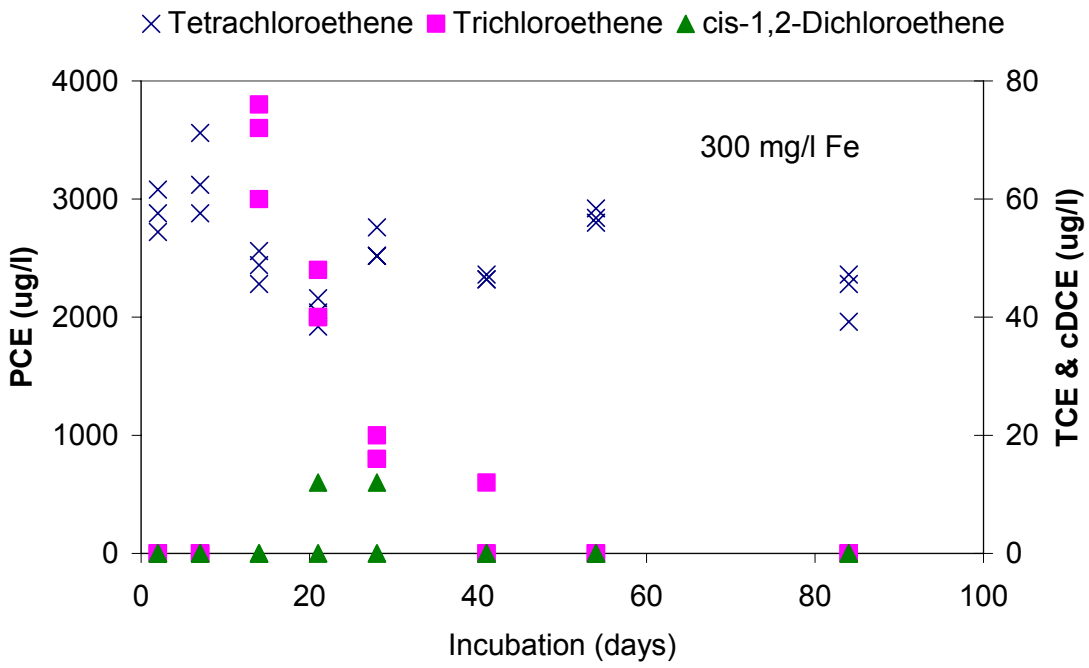

Figure 9. Concentrations of PCE, TCE and cDCE for treatment 1Fe.

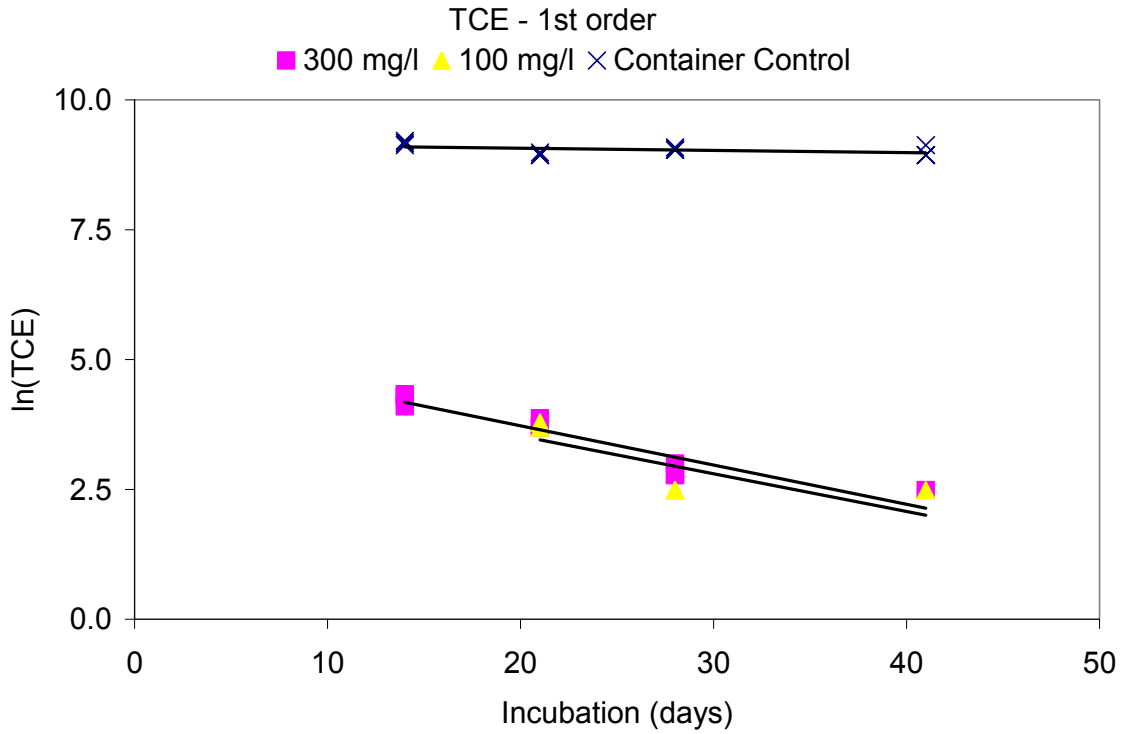

Figure 10. Plots of ln(TCE) vs. time for treatments 1Fe, 2Fe and 5Fe.

4.0 Abiotic Remediation of PCE in Sediment Amended with an Organic Substrate and Electron Shuttles

Since it is not possible to establish abiotic conditions by sterilizing a site, any real world strategy to effect abiotic remediation must also work with and account for biological processes occurring alongside abiotic processes. In recognition of this it was decided not just to let the two work side by side, but rather to make it possible to let biological processes fuel abiotic dechlorination. Such a process moves the restriction upon the participating microbes from one that requires the microbes be capable of remediating chlorinated ethenes to one that simply requires they foster a reducing environment through the anaerobic consumption of a carbon substrate.

4.1 Method

The soil was collected with the soil used in the previous experiment and the microcosms were prepared in Ada, OK. EOS was diluted by ten in RO water and titrated to pH 7 with bicarbonate. Following neutralization 0.2 ml was added to each sediment containing microcosm such that the EOS was present at approximately 1000 mg/L. The PCE dose solution was prepared by dissolving 33 mg PCE into 165 ml of RO water. Two electron shuttles were investigated: AQDS (9,10-anthraquinone-2,6-disulfonic acid disodium salt) and lignite. To dose AQDS at 1 and 5 mM in the microcosms, 10 and 50 mM solutions were made by dissolving 680 mg and 3400 mg AQDS in 165 ml vials of RO water in 165 ml serum vials that contained magnetic stir bars. The vials were then sealed with Teflon-lined butyl rubber stoppers, crimped and mixed. Approximately 33 g of sediment was placed into each of the 20 ml serum vials. Two levels of lignite addition (treatments 1ES and 2ES) were investigated as well as two levels of AQDS addition (treatments 3ES and 4ES) and a control with no electron shuttle addition (treatment 5ES). A container control with only PCE and RO water was also performed (treatment 6ES). The preparation of each treatment is detailed in Table 10.

Table 10. Microcosm make-up for effect of electron shuttles additions study.

Treatment	sediment	AQDS (ml)	lignite	RO Water
1ES	yes	0	0.1 g/10 ml pore water	1 ml
2ES	yes	0	0.01 g/10 ml Pore water	1 ml
3ES	yes	1 – 10 mM solution	0	0 ml
4ES	yes	1 – 50 mM solution	0	0 ml
5ES	yes	0	0	1 ml
6ES	no	0	0	filled

4.2 Sampling

Microcosms were sampled on days 0, 32, 60, 95, 125, 158 and 200. All microcosms were sampled in triplicate under anoxic conditions using aseptic techniques.

Prior to sampling, each microcosm was vortex-mixed. After vortex-mixing, nominally one ml was collected from the microcosms and diluted to 40 ml for volatiles analysis (PCE, TCE, cDCE, 1,1DCE, tDCE and VC). A second 1 ml aliquot was diluted into 40 ml and that was used for the analysis of dissolved gasses (acetylene, methane, ethane, ethene, propane, propene, n-butane and iso-butane). A third 1 ml aliquot was collected and diluted to 5 ml for the analysis of ferrous iron. The standing water was then replaced with RO water, the microcosm again was sealed and capped. It was allowed to sit overnight, vortex-mixed, unsealed and sampled for anions (nitrate, nitrite, sulfate and chloride). The remaining solid was then analyzed for weak acid soluble iron. The samples were analyzed at Microseeps Laboratories, using their 2005 Standard Operating Procedures, (i.e. SW846-8260 for the volatiles analysis, PM01/AM20Gax for the dissolved gas analyses, SW846-9056 for the anion analyses, a modified version of SW846 7199 for the ferrous iron analysis and WC43 for the weak acid soluble iron analysis).

4.3 Results and Discussion

The detailed results of the analyses carried out on the microcosms are presented in the Appendix, Table A16 – A35. In Figure 11 the concentration of PCE is plotted for the experiments where AQDS was added (treatments 3ES and 4ES). Similarly, in Figure 12 the concentration of PCE is plotted for the experiments where lignite was added (treatments 1ES and 2ES) with the TCE. There appears to be some order to the PCE profiles in the AQDS experiments and the rate of decay seems to scale with the AQDS amendment, but it is difficult to find any order in the lignite experiments.

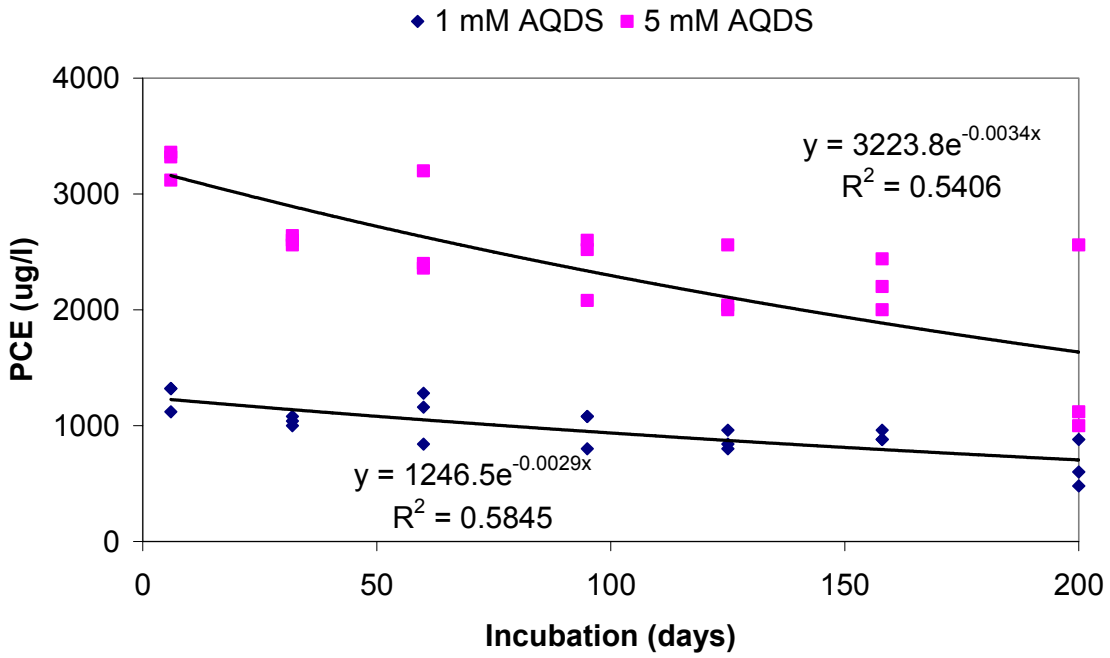

Figure 11. Plot of PCE conc. for treatments 3ES (1 mM AQDS) and 4ES (5mM AQDS).

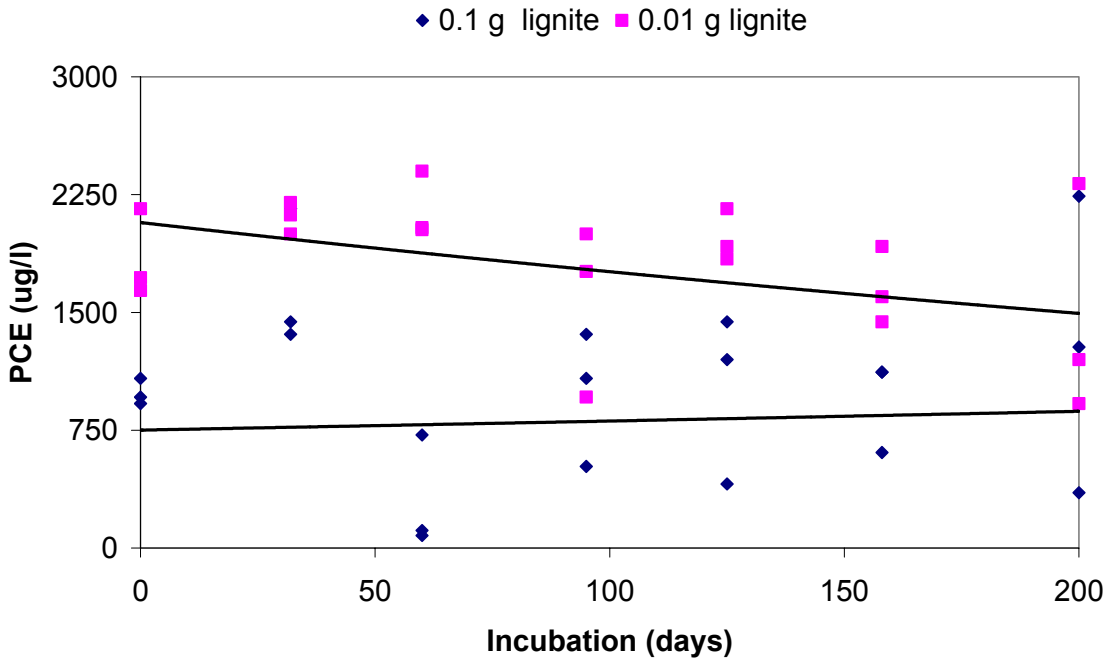

Figure 12. Plot of PCE conc. for treatments 1ES (0.1 g lignite) and 2ES (0.01g lignite).

From the data presented in Figure 11 it is unclear what mechanism drives the loss. The PCE, TCE and DCE concentration profiles for the 1 mMol AQDS experiment (treatment 3ES) are presented in Figure 13. Similarly, the PCE, TCE and DCE concentration profiles for the 5 mMol AQDS experiment (treatment 4ES) are presented in Figure 14.

The lack of TCE initially combined with the presence of TCE later implies that the TCE is produced by at least one of the mechanisms responsible for the PCE loss. However, the fact that the TCE does not accumulate implies that there is a loss mechanism for the TCE as well. As can be seen, cDCE was not observed. The data is not shown, but neither tDCE, 1,1DCE, VC, ethene or acetylene were observed either. This implies that the mechanism responsible for the TCE loss may not go through sequential reductive dechlorination. Abiotic remediation does not go through sequential reductive dechlorination.

While the appearance of TCE suggests that the observed loss is, at least in part, due to transformation, adsorption can not be ruled out. Unfortunately, both the samples with no electron shuttle amendments (treatment 5ES) and the container control for the electron shuttles experiments (treatment 6ES) exhibited excessive noise, presumably because of a failure in the auto-pipette used for dosing the PCE. The PCE concentration profile for treatment 5ES is shown in Figure 15, and that for treatment 6ES is shown in Figure 16.

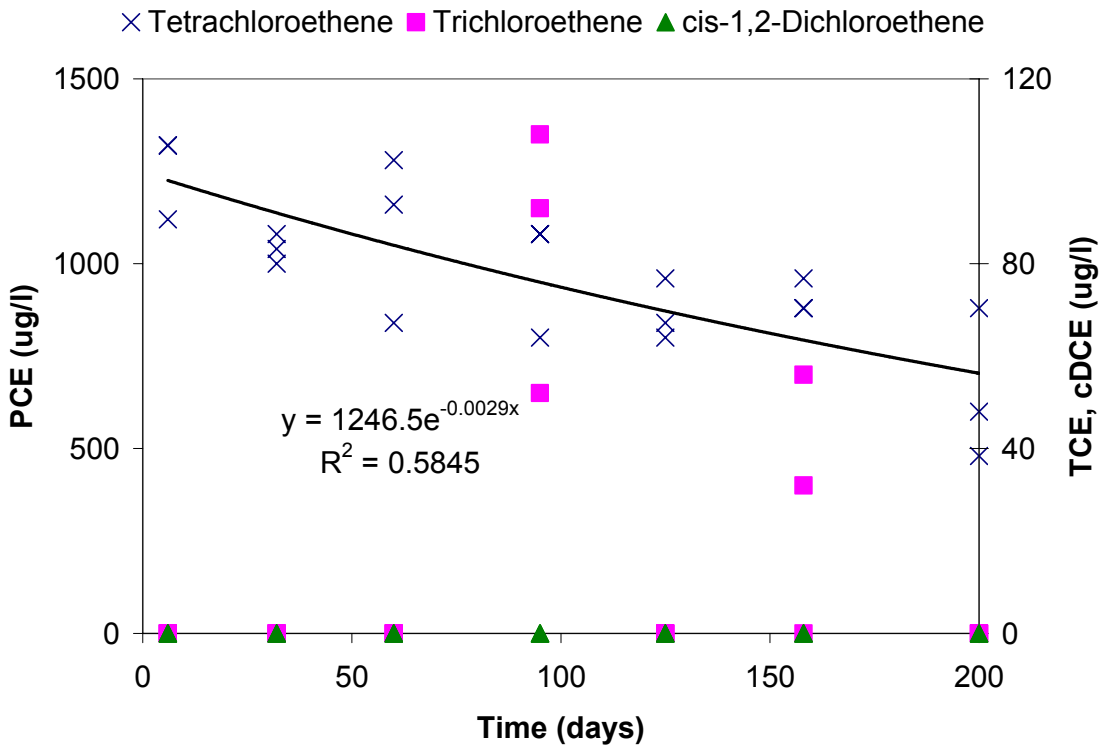

Figure 13. The PCE, TCE and DCE concentration profiles for 3ES.

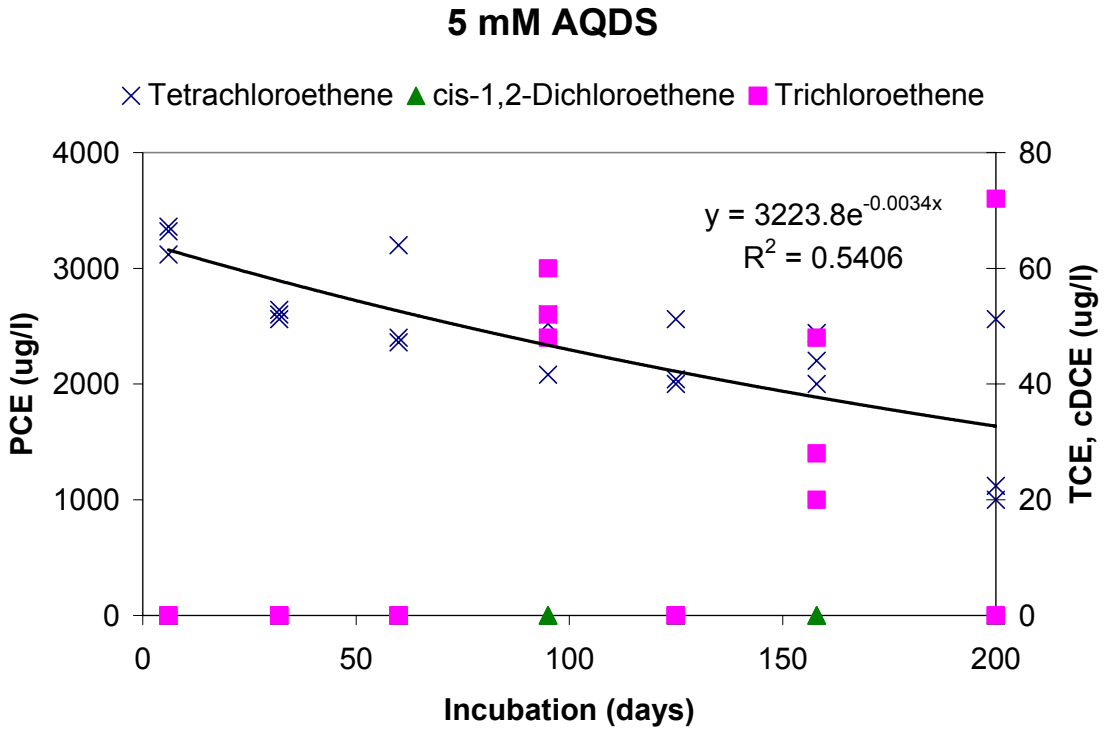

Figure 14. The PCE, TCE and DCE concentration profiles for 4ES.

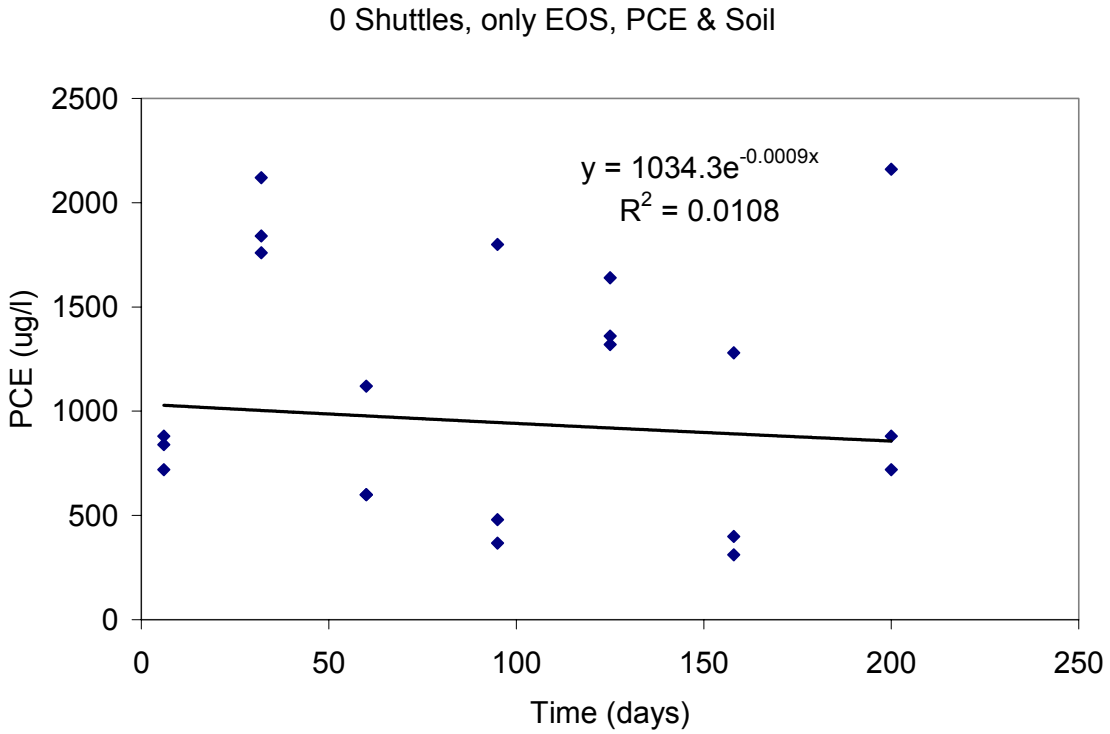

Figure 15. PCE concentration profile for 5ES.

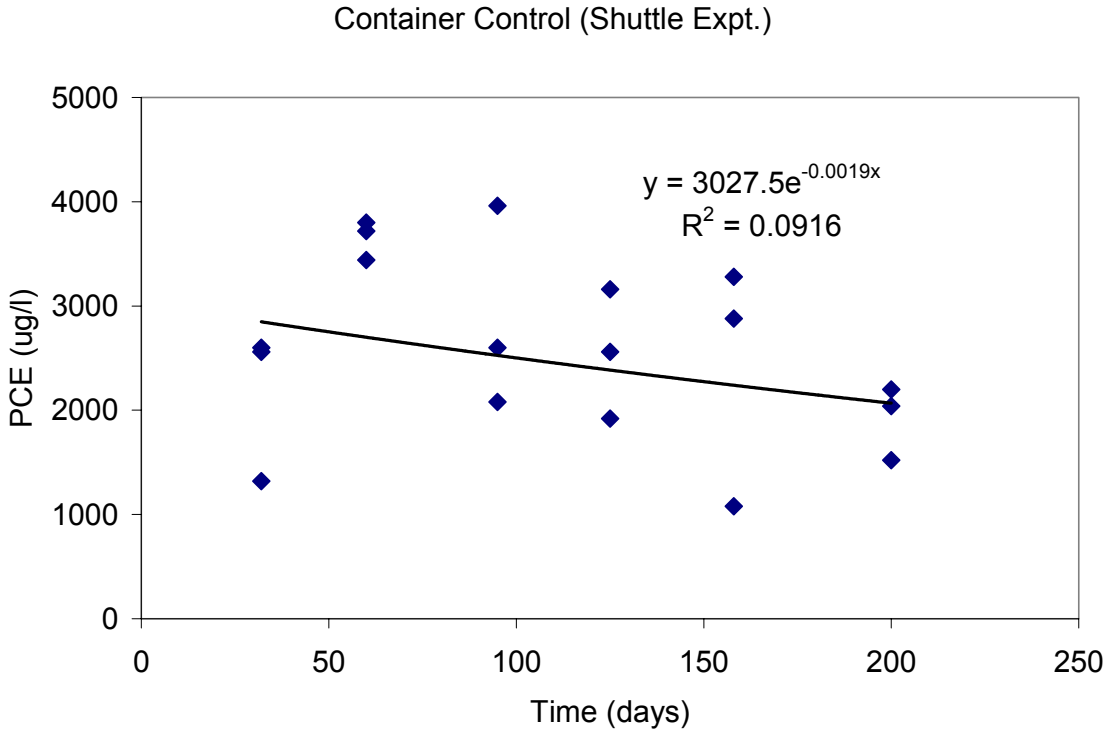

Figure 16. PCE concentration profile for 6ES.

Despite the scatter in the data, it appears that there is a downward concentration trend in the PCE in the microcosms where AQDS was not added (treatment 5ES, Figure 15) and in the container controls (treatment 6ES, Figure 16). While the least-squares fit yields rates of loss in both the zero AQDS added microcosms (treatment 5ES) and the container controls (treatment 6ES) that are less than the rate of loss seen in the 1mMol AQDS microcosms (treatment 3ES) and 5 mMol AQDS microcosms (treatment 4ES), the noise in the zero AQDS microcosms and in the container controls makes that conclusion statistically inappropriate. The results summarizing the statistical analysis are presented in Table 11.

Table 11. Comparison of statistical loss rates in treatments 3ES – 6ES.

Treatment	Mean Loss Rate (per year)	Standard Deviation	Lower 90% (per year)	Upper 90% (per year)
3ES	1.0	21%	-	0.69
4ES	1.2	19%	-	0.79
5ES	0.34	220%	1.6	-
6ES	0.70	79%	1.7	-

In Table 11 a regression analysis was performed assuming a two-tailed error distribution and a 90% confidence limit. This seemed appropriate since the lower boundary of a two-tailed error distribution at 90% confidence is equal to that of a single-tailed error distribution with a 95% confidence interval. To be conservative, it was assumed that the loss rate in the test experiments (3ES and 4ES) could only be slower than the mean loss rate and the loss rate in the controls (5ES and 6ES) could only be faster than the mean loss rate. With a 95% confidence, the loss rates in 3ES and 4ES could be explained by the loss rates in controls. However, this does not invalidate the hypothesis that the loss rate in 3ES and 4ES is greater than that in the controls, it simply says that this data set (i.e. the noisy controls) can not be used to support that hypothesis.

Interpretation is further complicated by the possible occurrence of both biological reductive dechlorination and abiotic dechlorination. Given the noise in the controls, it was decided to halt these experiments. Before they are resumed the tools should be better developed to measure abiotic processes and there also needs to be a way to measure the capacity of ES's. In addition, it was felt that this interpretation required a better understanding of stimulated abiotic reduction in sterilized sediment.

5.0 Abiotic Remediation of TCE in Sterilized, Reduced Sediment

While it is very difficult to detect any degradation of the PCE, the data in Figure 9 makes it clear that there is some TCE produced. Further, that data suggests that the TCE degrades very rapidly. As is evident in the data presented in Figure 10, degradation occurs in both 1Fe and 2Fe, but not in 5Fe. Further, Figure 10 suggests that the rate of disappearance appears to be independent of the ferrous concentration, but clearly requires the sediment to be present.

With these observations it was decided to re-spike several microcosms with $FeCl_2$ and TCE, hoping to observe the degradation of TCE more systematically.

5.1 Method

Fifteen microcosms from treatment 1Fe, fifteen from treatment 2Fe and 8 from treatment 4Fe were sterilized and then amended with $FeCl_2$ and TCE according to Table 12. Fifteen more microcosms were made up with just water and they served as container controls. The $FeCl_2$ dose solutions were prepared as before. The TCE dose solution was prepared just as the PCE dose was prepared in the experiments detailed in the previous sections.

Table 12. Microcosm make-up for abiotic TCE degradation study.

Treatment	Sediment	$FeCl_2$	TCE	Previous PCE
1Fe	Yes	1 ml - 1760 solution (300 mg/l)	1 ml	Yes
2Fe	Yes	1 ml – 535 solution (100 mg/l)	1 ml	Yes
4Fe	Yes	0	1 ml	Yes
6Fe[†]	No	0	1 ml	No

[†]The microcosms for this treatment were freshly constructed at the time this experiment was prepared.

5.2 Sampling

Samples were collected on days 0, 7, 14, 21, 28 and 35. For the first three samplings, three microcosms were sampled from each treatment. In the fourth and fifth samplings, three microcosms from treatments 1Fe, 2Fe and 6Fe were sampled, but only one microcosm was sampled from 4Fe due to limited sample. The microcosms were sampled for TCE, cDCE and VC under aseptic conditions. Prior to sampling, the microcosms were each vortex mixed and then centrifuged to facilitate sampling. For the samples collected on days 0, 7, 14, 21 and 28 the sampling immediately followed the centrifuging process. For the samples collected on day 35, the samples were vortex mixed, then centrifuged, then allowed to set overnight and sampled in the morning.

5.3 Results and Discussion

The detailed results of the analyses carried out on the microcosms are presented in the Appendix, Table A36 – A39. The concentration profile of the data is shown in Figure 17.

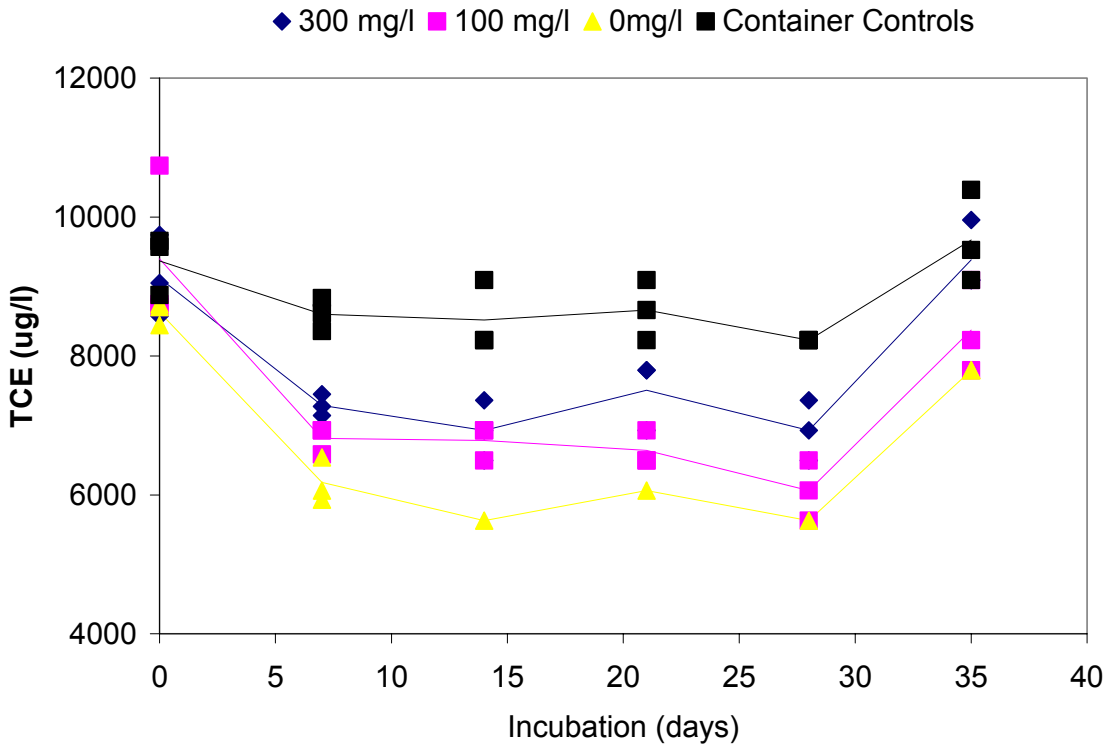

Figure 17. The concentration profile of the TCE in the sterilized sediment.

From day 0 to 28, there appears to be some transformation in the test samples that is not matched in the container controls. When this was realized it was also realized that for the microcosm sampled for the first 28 days, sampling was performed immediately after vortex mixing and centrifuging, but for the samples collected on day 35 approximately sixteen hours elapsed between centrifuging and sampling. This apparently has a significant effect on the TCE concentration. For similarity purposes let us examine the concentration profiles without day 35. Those profiles are presented in Figure 18.

Looking at Figure 17 it appears that there is a loss of the TCE, and that loss becomes more obvious looking at Figure 18. The extent of that loss is inverse to the quantity of ferrous iron in the microcosms. This is apparent in the data presented in Table 13.

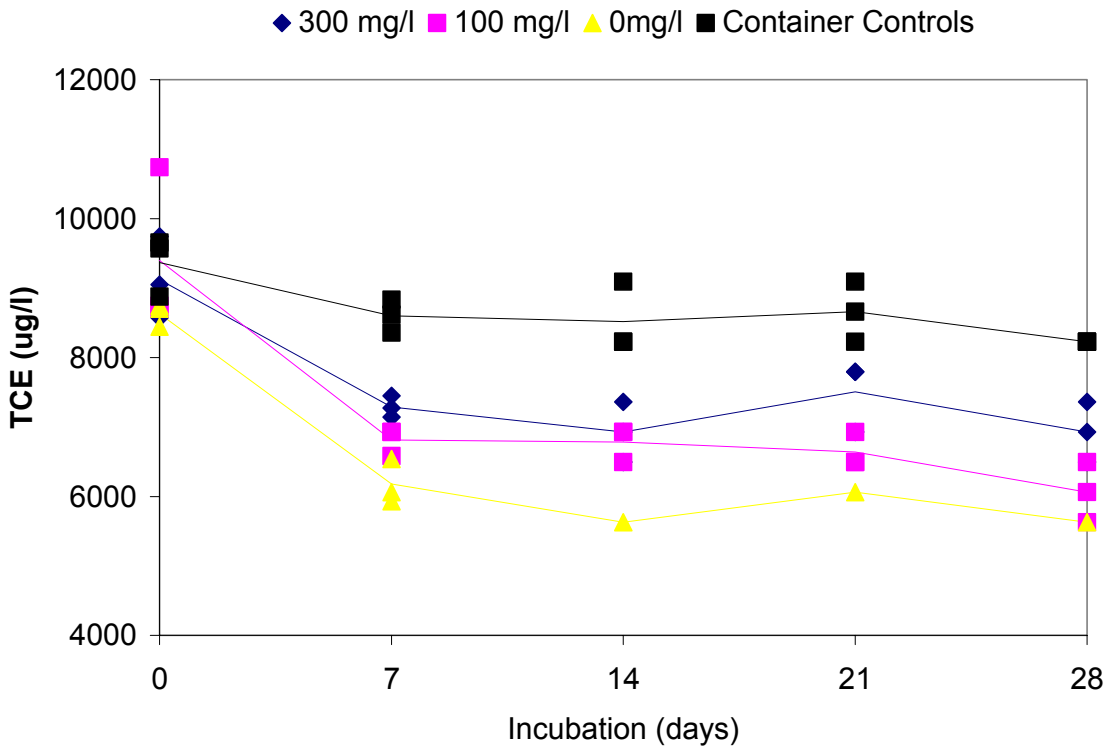

Figure 18. The concentration profile for days 0 through 28 of the TCE in the sterilized sediment.

Table 13. TCE loss between day 0 and day 7.

Treatment	Pore Water Fe(II) (mg/l)	Average % loss	Loss rate (per year)
1Fe	300	20.1	11.7
2Fe	100	27.5	16.8
4Fe	0	28.4	17.4
6Fe	Container Control	8.2	4.4

There are two possible explanations for the loss of the TCE. One possible explanation is that the initial biological reduction of the EOS added to the sediment served provided reducing power to the sediment. Between the reduction of the PCE added in the first experiment and then the reduction of the TCE added in this experiment, it appears that some time prior to day 7 the reductive power had been consumed. This observation would be much like those made by Szecsody et al (2004). This indicates that coupling the abiotic remediation to simple biological reduction of some organic substrate might be an appealing way to utilize abiotic reduction to avoid the production of unwanted daughter products and the need for specialized microorganisms. The ferrous iron may have simply occupied active surface sites, thus reducing both the capacity and rate of the degradation. This would explain why the extent of

loss was greatest in the microcosm to which $FeCl_2$ was not added, and why the other microcosms did not achieve the same loss even at a slower rate (Roden and Urrutia, 2002).

Another possible explanation is that the loss is due to adsorption of the TCE to the EOS and the sediment, both of which are not present in the container controls. Indeed, the data from day 35 might suggest that the loss is reversible, but without similarly collected data on the other days, that can not be examined with this data set. If the Fe(II) adsorbed to sites to which the TCE would otherwise adsorb, the extent of loss would be greatest when no $FeCl_2$ were added and would decrease as the Fe^{+2} concentration increased.

6.0 Summary

The literature study and the experiments done for acetylene indicate that it is stable in carefully prepared abiotic water from a pH of 0-14, but there are so many other potential sinks of it, and both its oxidation and its reduction are so exoergic under a range of conditions, that acetylene, though stable in laboratory prepared, sterile aqueous solutions, is very unlikely to be stable in the environment. Further, the inability to observe acetylene in a system should not be construed as evidence that it is not being produced.

The ultimate outcome of the electron shuttle work was to indicate that this important assay should proceed through a biological reduction and not a chemical one. While this work did not culminate in a workable analytical method it did stress the importance of such a measurement and suggest a pathway for it.

On the timescales examined in the experiments conducted herein, there was no evidence of the abiotic remediation of PCE in sterilized, reduced sediment. However, by some process a small amount of PCE was transformed to TCE in both treatments 1FE and 2Fe, and the loss of that TCE was very rapid, suggesting the biotic remediation of TCE in sterilized, reduced sediment experiments.

The abiotic remediation of PCE in sediment amended with an organic substrate and electron shuttles experiment suggested a loss rate of 1.7 per year with 5 mM AQDS. However, experimental problems occurred with the preparation of the control microcosms that make that conclusion statistically invalid. In addition, it is unclear what mechanism drives that loss. Adsorption is unlikely because that should level off, but a steady loss was seen in this experiment. However, this could be absolutely ruled out through the use of a killed control. The loss could also be driven by biological sequential reductive dechlorination, by biological sequential reductive dechlorination followed by oxidation of DCE (Bradley, Chapelle and Lovely, 1998) or by abiotic remediation or by some combination of all three. The only daughter product observed was TCE, and it did not accumulate. Another tool for unraveling degradation mechanisms has become available and that is compound specific isotopic analysis (CSIA). While it was not used in this experiment, it would be of great value if this experiment were repeated.

The experiments conducted so far are promising, but similar experiments, with additional controls and with use of CSIA, would need to be conducted. There is no need to test multiple electron shuttles in those experiments. The TES analysis should be developed first and it should be used to find appropriate test candidates.

The abiotic remediation of TCE in sterilized, reduced sediment experiments can not be used to discern between adsorption, a potentially reversible mechanism, and contaminant destruction via abiotic remediation. The suggestion that abiotic remediation can be stimulated through the addition of organic substrates is appealing because it minimizes potential formation of toxic intermediate products and eliminates the need for specialized microorganisms. However, it would take another experiment to definitively prove it. In that experiment there should be a control sediment that was spiked with EOS and then

immediately killed. Since it was shown that the loss was not driven by the addition of $FeCl_2$ but instead possibly by the reducing power of the sediment, there is no need to spike $FeCl_2$ at all or to prepare different microcosms where it is spiked at different levels. Instead three sets of test microcosms should be prepared, each of which were allowed to incubate for different times between EOS addition and sterilization.

The promise of abiotic remediation is strong, and there seems to be potential for stimulating it through the addition of electron shuttles and a long acting substrate such as EOS. To make this technology employable at field scale a TES measurement must be developed, and this work suggested how that might be done. This work also studied acetylene and found it to be an unreliable indicator of the presence of abiotic remediation. It is hoped that CSIA provides a more useful tool for indicating abiotic remediation.

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Appendix

Table A1. Data for study of stability of LHC gases in sample vials.

Analyte(s)	Units	Sampling Day					
		1	3	10	15	21	28
HYDROGEN-A	PPMV	9.435	10.596	7.958	9.046	10.157	9.341
HYDROGEN-B	PPMV	9.071	10.018	7.897	9.271	10.222	9.746
HYDROGEN-C	PPMV	10.096	9.683	7.951	9.090	9.599	9.785
METHANE-A	PPMV	122.785	123.595	123.160	126.655	128.884	126.916
METHANE-B	PPMV	122.413	123.359	123.264	128.380	127.588	130.181
METHANE-C	PPMV	123.846	122.287	123.050	126.137	127.052	127.057
ETHANE-A	PPMV	39.465	40.451	38.710	38.285	39.358	37.250
ETHANE-B	PPMV	39.352	40.050	38.484	38.579	39.212	38.671
ETHANE-C	PPMV	39.786	39.808	38.711	38.198	39.020	37.937
ETHENE-A	PPMV	39.364	40.395	38.519	38.124	39.281	37.096
ETHENE-B	PPMV	39.233	39.973	38.313	38.353	39.148	38.376
ETHENE-C	PPMV	39.747	39.769	38.542	38.003	38.904	37.681
PROPANE-A	PPMV	38.826	39.376	35.881	35.909	36.581	33.650
PROPANE-B	PPMV	38.621	38.944	35.610	36.148	36.627	35.514
PROPANE-C	PPMV	39.019	38.760	36.013	35.520	36.374	34.756
PROPENE-A	PPMV	38.429	38.828	34.040	33.793	34.821	30.758
PROPENE-B	PPMV	38.116	38.400	33.660	34.217	34.898	32.856
PROPENE-C	PPMV	38.632	38.101	34.301	33.460	34.578	32.057
ISO-BUTANE-A	PPMV	38.724	39.211	35.592	35.647	36.567	33.417
ISO-BUTANE-B	PPMV	39.606	38.792	35.336	36.014	36.695	35.418
ISO-BUTANE-C	PPMV	38.772	38.597	35.900	35.342	36.397	34.503
N-BUTANE-A	PPMV	38.266	38.584	34.418	34.298	34.495	29.760
N-BUTANE-B	PPMV	37.802	37.924	33.395	34.424	34.844	33.328
N-BUTANE-C	PPMV	38.489	37.863	34.492	33.765	34.397	32.313

Table A2. Data for study of stability of LHC gases in sample vials (continued).

Analyte(s)	Units	Sampling Day				
		35	42	46	51	60
HYDROGEN-A	PPMV	9.764	9.877	10.589	11.256	11.203
HYDROGEN-B	PPMV	9.680	9.643	10.736	10.748	11.489
HYDROGEN-C	PPMV	9.422	9.940	10.502	10.985	10.788
METHANE-A	PPMV	126.033	124.898	125.554	121.373	124.675
METHANE-B	PPMV	126.835	122.412	125.805	119.321	125.036
METHANE-C	PPMV	126.639	125.257	119.453	116.938	123.749
ETHANE-A	PPMV	37.329	38.567	37.986	36.643	37.697
ETHANE-B	PPMV	37.793	37.102	38.214	35.813	37.323
ETHANE-C	PPMV	37.851	38.216	35.536	35.257	37.277
ETHENE-A	PPMV	37.253	38.348	37.895	36.472	37.635
ETHENE-B	PPMV	37.605	37.032	38.092	35.773	37.451
ETHENE-C	PPMV	37.689	38.193	35.585	35.080	37.265
PROPANE-A	PPMV	33.020	34.013	34.976	33.124	34.850
PROPANE-B	PPMV	34.049	32.138	35.210	31.712	33.645
PROPANE-C	PPMV	34.229	33.133	31.919	31.668	34.395
PROPENE-A	PPMV	30.306	31.159	32.796	30.803	32.892
PROPENE-B	PPMV	31.604	29.136	33.072	29.111	31.363
PROPENE-C	PPMV	31.858	30.166	29.729	29.335	32.534
ISO-BUTANE-A	PPMV	32.686	33.956	34.963	33.138	34.807
ISO-BUTANE-B	PPMV	34.056	31.873	35.239	31.631	33.578
ISO-BUTANE-C	PPMV	34.209	32.853	31.608	31.635	34.458
N-BUTANE-A	PPMV	29.167	31.090	32.122	30.005	31.556
N-BUTANE-B	PPMV	31.602	28.037	32.629	27.032	28.286
N-BUTANE-C	PPMV	31.764	28.596	27.804	28.633	31.161

Table A.3 General data for treatment 1Fe microcosms and Ferrous concentrations.

Sample ID	incubation (days)	lab project	Ferrous Iron (mg/l)	Solids (%)
SRS 342	2	P0507166	70	53
SRS 343	2	P0507166	60	50
SRS 348	2	P0507166	60	44
SRS 307	7	P0507294	55	22
SRS 315	7	P0507294	49.5	41
SRS 320	7	P0507294	60	46
SRS 321	14	P0507424	55	50
SRS 326	14	P0507424	50	38
SRS 341	14	P0507424	55	43
SRS 333	21	P0508157	49	40
SRS 340	21	P0508157	55	39
SRS 346	21	P0508157	55	46
SRS 304	28	P0508189	55	45
SRS 317	28	P0508189	55	44
SRS 334	28	P0508189	55	41
SRS 331	41	P0508393	63	35
SRS 335	41	P0508393	48	45
SRS 347	41	P0508393	60	46
SRS 332	54	P0509094	60	47
SRS 337	54	P0509094	55	48
SRS 338	54	P0509094	55	58
SRS 302	84	P0510066	60	48
SRS 322	84	P0510066	60	50
SRS 330	84	P0510066	60	51

Table A.4 Dissolved gas concentrations (ug/l) for Treatment 1Fe microcosms.

Sample ID	Acetylene	Ethane	Ethene	iso-Butane	Methane	n-Butane	Propane	Propene
SRS 342	0	10.8	1.24	3.72	48	6.4	1.76	2.96
SRS 343	0	64	0	0	18.8	0	0	0
SRS 348	0	68	0	0	18.4	0	0	0
SRS 307	0	12	0	0	11.2	0	0	0
SRS 315	0	16.4	0.44	0	14.4	0	0	0
SRS 320	0	22	0.44	0	14.8	0	0	0
SRS 321	0	72	0.56	1	25.2	2.32	1.16	1.32
SRS 326	0	72	0.24	0	18.4	0.92	0.52	0
SRS 341	0	80	0	0	19.2	0	0.56	0
SRS 333	0	0	0	0	0	0	0	0
SRS 340	0	8	0.52	0	7.6	0	0	0
SRS 346	0	9.6	0.56	0	8.8	0	0	0
SRS 304	0	18.8	2.16	0	40	0	0	0
SRS 317	0	19.2	1.36	0	30.4	0	0	0
SRS 334	0	20	1.24	0	29.6	0	0	0
SRS 331	0	6	14	1.24	88	3.72	2	2.48
SRS 335	0	37.6	3.32	0	18.4	0	0	0
SRS 347	0	35.2	3.04	0	20.4	0	0	0
SRS 332	0	13.6	4.4	1.84	104	4.4	2.44	2.12
SRS 337	0	16.4	1.68	0	36	0.68	0	0.4
SRS 338	0	15.6	2.52	0	48	0.8	0.6	0.72
SRS 302	0	4.8	0	0	3.52	0	0	0
SRS 322	0	5.6	0	0	4	0	0	0
SRS 330	0	6.4	0.24	0	4.8	0	0	0

Table A.5 Chlorinated ethene (ug/l) and common ion concentrations (mg/) for Treatment 1Fe microcosms.

Sample ID	11DCE	cDCE	PCE	tDCE	TCE	VC	Chloride	Nitrate	Nitrite	Sulfate
SRS 342	0	0	3080	0	0	0	150	0	0	15
SRS 343	0	0	2880	0	0	0	160	0	0	16
SRS 348	0	0	2720	0	0	0	160	0	0	16
SRS 307	0	0	2880	0	0	0	150	4.9	0	16
SRS 315	0	0	3560	0	0	0	150	0	0	16
SRS 320	0	0	3120	0	0	0	120	0	0	17
SRS 321	0	0	2560	0	76	0	150	0	0	17
SRS 326	0	0	2440	0	72	0	140	5.6	0	29
SRS 341	0	0	2280	0	60	0	160	0	0	17
SRS 333	0	12	2160	0	48	0	120	1.1	0	4.2
SRS 340	0	0	2040	0	40	0	110	1	0	3
SRS 346	0	0	1920	0	40	0	130	0	0	5
SRS 304	0	12	2760	0	20	0	110	0	0	0
SRS 317	0	0	2520	0	16	0	120	0	0	1.2
SRS 334	0	0	2520	0	16	0	110	0.09	0	0
SRS 331	0	0	2320	0	12	0	99	0	0	14
SRS 335	0	0	2320	0	0	0	130	0	0	14
SRS 347	0	0	2360	0	0	0	130	9.8	0	17
SRS 332	0	0	2840	0	0	0	150	4.8	3.1	0
SRS 337	0	0	2800	0	0	0	130	5.2	3.6	15
SRS 338	0	0	2920	0	0	0	160	0	3.1	0
SRS 302	0	0	2360	0	0	0	88	0	0	0
SRS 322	0	0	2280	0	0	0	82	9.4	0	0
SRS 330	0	0	1960	0	0	0	95	0	0	29

Table A.6 General data for treatment 2Fe microcosms and Ferrous concentrations.

Sample ID	incubation (days)	lab project	Ferrous Iron (mg/l)	Solids (%)
SRS 352	2	P0507166	8.5	49
SRS 359	2	P0507166	8	34
SRS 385	2	P0507166	8.5	49
SRS 358	7	P0507294	8.5	51
SRS 371	7	P0507294	8	51
SRS 373	7	P0507294	4	56
SRS 362	14	P0507424	8	38
SRS 370	14	P0507424	7	47
SRS 393	14	P0507424	6	47
SRS 350	21	P0508157	8	39
SRS 354	21	P0508157	7.5	50
SRS 375	21	P0508157	7	48
SRS 363	28	P0508189	7.5	39
SRS 387	28	P0508189	6.5	40
SRS 392	28	P0508189	5.5	51
SRS 351	41	P0508393	8	42
SRS 379	41	P0508393	4	37
SRS 388	41	P0508393	8.5	39
SRS 361	54	P0509094	8	43
SRS 372	54	P0509094	9	58
SRS 378	54	P0509094	11	46
SRS 369	84	P0510066	7	47
SRS 390	84	P0510066	5	49
SRS 394	84	P0510066	7	52

Table A.7 Dissolved gas concentrations (ug/l) for Treatment 2Fe microcosms.

Sample ID	Acetylene	Ethane	Ethene	iso-Butane	Methane	n-Butane	Propane	Propene
SRS 352	0	72	0.24	0	18.4	0	0	0
SRS 359	0	76	0	0	21.2	0	0	0
SRS 385	0	72	0	0	19.6	0	0	0
SRS 358	0	11.6	0.4	0	8	0	0	0
SRS 371	0	14.8	0.32	0	10.4	0	0	0
SRS 373	0	19.2	0.36	0	12.8	0	0	0
SRS 362	0	23.2	0.32	0	8.4	0	0	0.52
SRS 370	0	25.2	0.28	0	8.4	0	0	0
SRS 393	0	28	0.24	0	8.8	0	0	0
SRS 350	0	8.4	0.36	0	6.8	0	0	0
SRS 354	0	10	0.32	0	8	0	0	0
SRS 375	0	11.2	0.36	0	7.6	0	0	0
SRS 363	0	20.4	0.96	0	25.6	0	0	0
SRS 387	0	25.6	0.92	0	25.6	0	0	0
SRS 392	0	27.6	0.88	0	25.2	0	0	0
SRS 351	0	38	3	0	17.6	0	0.48	0
SRS 379	0	80	6.4	0	34.4	0	0	0
SRS 388	0	40	2.72	0	17.2	0	0	0
SRS 361	0	20	1.56	0	30	0	0.52	0
SRS 372	0	17.6	1.2	0	20.8	0	0	0
SRS 378	0	24	1.4	0	29.6	0	0	0
SRS 369	0	2.52	0.24	0	5.6	0	0	0
SRS 390	0	7.2	7.6	2.76	44	8	3.6	4.4
SRS 394	0	6.4	2.64	0.92	11.2	1.04	0.84	1.04

Table A.8 Chlorinated ethene (ug/l) and common ion concentrations (mg/l) for Treatment 2Fe microcosms.

Sample ID	11DCE	cDCE	PCE	tDCE	TCE	VC	Chloride	Nitrate	Nitrite	Sulfate
SRS 352	0	0	2920	0	0	0	72	0	0	0
SRS 359	0	0	2560	0	0	0	72	0	0	0
SRS 385	0	0	2520	0	0	0	72	0	0	0
SRS 358	0	0	2400	0	0	0	50	5	0	15
SRS 371	0	0	2400	0	0	0	44	0	0	15
SRS 373	0	0	2480	0	0	0	48	0	0	15
SRS 362	0	0	2320	0	0	0	49	5.5	3.1	15
SRS 370	0	0	2160	0	0	0	55	5.4	3.2	16
SRS 393	0	0	1840	0	0	0	51	4.9	0	15
SRS 350	0	0	1840	0	44	0	36	0	0	0
SRS 354	0	0	1840	0	44	0	34	1	0	0
SRS 375	0	0	1680	0	40	0	35	1	0	0
SRS 363	0	0	1760	0	12	0	34	0	0	0
SRS 387	0	0	1920	0	12	0	31	0	0	0
SRS 392	0	0	2000	0	12	0	31	0	0	0
SRS 351	0	0	1720	0	0	0	49	0	0	14
SRS 379	0	0	2040	0	0	0	33	0	0	14
SRS 388	0	0	1880	0	12	0	33	0	0	14
SRS 361	0	0	2280	0	0	0	61	5.2	4.2	15
SRS 372	0	0	2440	0	0	0	96	5.5	4.1	19
SRS 378	0	0	2320	0	0	0	17	5.1	3	15
SRS 369	0	0	2040	0	0	0	50	0	0	0
SRS 390	0	0	1760	0	0	0	54	0	0	0
SRS 394	0	0	1680	0	0	0	50	0	0	0

Table A.9 General data for treatment 3Fe microcosms and Ferrous concentrations.

Sample ID	incubation (days)	lab project	Ferrous Iron (mg/l)	Solids (%)
SRS 405	2	P0507166	1	36
SRS 411	2	P0507166	0	48
SRS 448	2	P0507166	0	43
SRS 416	7	P0507294	3.5	47
SRS 417	7	P0507294	3	51
SRS 437	7	P0507294	1.5	54
SRS 414	14	P0507424	1	50
SRS 434	14	P0507424	5	51
SRS 442	14	P0507424	0	48
SRS 401	21	P0508157	1.5	57
SRS 402	21	P0508157	11	52
SRS 420	21	P0508157	3.5	47
SRS 424	28	P0508189	2	47
SRS 425	28	P0508189	1	46
SRS 445	28	P0508189	0	51
SRS 406	41	P0508393	1.5	33
SRS 408	41	P0508393	1.5	39
SRS 439	41	P0508393	2	44
SRS 404	54	P0509094	2.5	55
SRS 430	54	P0509094	3	49
SRS 436	54	P0509094	2	51
SRS 415	84	P0510066	2.5	53
SRS 428	84	P0510066	1	52
SRS 443	84	P0510066	0	49

Table A.10 Dissolved gas concentrations (ug/l) for Treatment 3Fe microcosms.

Sample ID	Acetylene	Ethane	Ethene	iso-Butane	Methane	n-Butane	Propane	Propene
SRS 405	0	72	0.24	0	19.6	0	0	0
SRS 411	0	68	0.24	0	19.2	0	0	0
SRS 448	0	72	0.24	0	20	0	0	0
SRS 416	0	30.4	0.28	0	16	0	0	0
SRS 417	0	33.2	0.48	0	17.2	0	0	0
SRS 437	0	25.2	0.4	0	13.6	0	0	0
SRS 414	0	31.6	0	0	9.6	0	0	0
SRS 434	0	20.4	0	0	8	0	0	0
SRS 442	0	22	0	0	7.2	0	0	0
SRS 401	0	11.6	0.48	0	8	0	0	0
SRS 402	0	12.8	0.44	0	6.8	0	0	0
SRS 420	0	14	0.36	0	7.6	0	0	0
SRS 424	0	26.8	1.08	0	30.8	0	0	0
SRS 425	0	28	0.88	0	24	0	0	0
SRS 445	0	27.6	0.72	0	20	0	0	0
SRS 406	0	39.6	2.88	0	17.2	0	0	0
SRS 408	0	44	2.48	0	15.6	0	0	0
SRS 439	0	52	2.72	0	17.2	0	0	0
SRS 404	0	16.4	1.48	0	22	0	0	0
SRS 430	0	17.2	1.92	0	28	0	0	0
SRS 436	0	18.8	1	0	18	0	0.4	0
SRS 415	0	7.2	2.16	0	10.8	0.76	0.68	0
SRS 428	0	5.6	1.92	0	10	0	0.4	0
SRS 443	0	6	1.76	0	9.6	0	0.44	0

Table A.11 Chlorinated ethene (ug/l) and common ion (mg/l) concentrations for Treatment 3Fe microcosms.

Sample ID	11DCE	cDCE	PCE	tDCE	TCE	VC	Chloride	Nitrate	Nitrite	Sulfate
SRS 405	0	0	1240	0	0	0	30	5.3	3.7	16
SRS 411	0	0	1040	0	0	0	60	0	0	0
SRS 448	0	0	1000	0	0	0	30	0	0	15
SRS 416	0	0	1360	0	0	0	27	4.9	0	15
SRS 417	0	0	1280	0	0	0	25	0	0	15
SRS 437	0	0	1400	0	0	0	30	0	0	0
SRS 414	0	0	1120	0	0	0	29	5.7	3.1	15
SRS 434	0	0	1080	0	0	0	28	5.4	3.2	15
SRS 442	0	0	1040	0	0	0	28	5.3	3.6	15
SRS 401	0	0	920	0	36	0	10	0	0	0
SRS 402	0	0	920	0	32	0	12	0	0	0
SRS 420	0	0	800	0	32	0	13	0	0	0
SRS 424	0	0	920	0	0	0	12	0	0	0
SRS 425	0	0	1040	0	12	0	11	0	0	0
SRS 445	0	0	1000	0	0	0	10	0	0	0
SRS 406	0	0	960	0	0	0	25	9.7	5.2	15
SRS 408	0	0	880	0	0	0	20	0	0	14
SRS 439	0	0	1000	0	0	0	23	0	0	14
SRS 404	0	0	1200	0	0	0	33	5	3.3	16
SRS 430	0	0	680	0	0	0	28	5.1	4.3	16
SRS 436	0	0	1280	0	0	0	30	5.1	4.4	16
SRS 415	0	0	920	0	0	0	39	9.4	0	0
SRS 428	0	0	1000	0	0	0	38	0	0	0
SRS 443	0	0	800	0	0	0	36	0	0	29

Table A.12 General data for treatment 4Fe microcosms and Ferrous concentrations.

Sample ID	incubation (days)	lab project	Ferrous Iron (mg/l)	Solids (%)
SRS 462	2	P0507166	3	48
SRS 467	2	P0507166	9.5	56
SRS 496	2	P0507166	6	49
SRS 450	7	P0507294	1.5	50
SRS 455	7	P0507294	4	51
SRS 463	7	P0507294	7.5	51
SRS 468	14	P0507424	1.5	47
SRS 472	14	P0507424	0	39
SRS 476	14	P0507424	6	54
SRS 465	21	P0508157	0	47
SRS 495	21	P0508157	0	47
SRS 497	21	P0508157	22	46
SRS 453	28	P0508189	2	56
SRS 493	28	P0508189	0	44
SRS 499	28	P0508189	0	50
SRS 460	41	P0508393	4	41
SRS 470	41	P0508393	16	41
SRS 479	41	P0508393	5	51
SRS 459	54	P0509094	11	48
SRS 461	54	P0509094	0	46
SRS 486	54	P0509094	0	51
SRS 456	84	P0510066	10	48
SRS 457	84	P0510066	0	48
SRS 474	84	P0510066	2	51

Table A.13 Dissolved gas concentrations (ug/l) for Treatment 4Fe microcosms.

Sample ID	Acetylene	Ethane	Ethene	iso-Butane	Methane	n-Butane	Propane	Propene
SRS 462	0	84	0	0	19.6	0	0	0
SRS 467	0	80	0.32	0	18.8	0	0	0
SRS 496	0	76	0.32	0	19.6	0	0	0
SRS 450	0	17.2	0.52	0	12	0	0	0
SRS 455	0	27.2	0	0	15.2	0	0	0
SRS 463	0	30	0	0	15.6	0	0	0
SRS 468	0	26.4	0	0	7.6	0	0	0
SRS 472	0	26.8	0.28	0	8	0	0	0
SRS 476	0	27.2	0	0	8.4	0	0	0
SRS 465	0	73.6	0.8	0	19.2	0	0	0
SRS 495	0	77.6	0.8	0	20	0	0	0
SRS 497	0	80	0.8	0	22.4	0	0	0
SRS 453	0	26	0.84	0	20	0	0	0
SRS 493	0	50.4	1.36	0	36	0	0	0
SRS 499	0	28.4	0.64	0	17.6	0	0	0
SRS 460	0	96	4.8	0	26.4	0	0	0
SRS 470	0	96	4.4	0	25.6	0	0	0
SRS 479	0	96	4.08	0	24	0	0	0
SRS 459	0	32.8	2	0	32	0	0	0
SRS 461	0	17.6	1.12	0	15.2	0	0.4	0
SRS 486	0	35.2	1.84	0	28.8	0	0	0
SRS 456	0	10.4	3.2	0	15.2	0	0	0
SRS 457	0	10	2.16	0	7.6	0	0	0
SRS 474	0	9.6	1.72	0	8.8	0	0.48	0

Table A.14 Chlorinated ethene (ug/l) and common ion (mg/l) concentrations for Treatment 4Fe microcosms.

Sample ID	11DCE	cDCE	PCE	tDCE	TCE	VC	Chloride	Nitrate	Nitrite	Sulfate
SRS 462	0	0	520	0	0	0	0	0	0	0
SRS 467	0	0	480	0	0	0	0	11	8.1	31
SRS 496	0	0	440	0	0	0	35	0	0	0
SRS 450	0	0	440	0	0	0	19	5.7	4.1	15
SRS 455	0	0	480	0	0	0	18	5.4	4.1	15
SRS 463	0	0	384	0	0	0	19	5.5	4.1	15
SRS 468	0	0	328	0	0	0	18	5.7	4.2	15
SRS 472	0	0	440	0	0	0	18	5.4	3.7	15
SRS 476	0	0	384	0	0	0	18	5.4	3.8	15
SRS 465	0	0	360	0	12	0	3.4	1.2	0	0
SRS 495	0	0	340	0	0	0	3	1.2	0	0.9
SRS 497	0	0	352	0	0	0	5.3	1.5	0	1.4
SRS 453	0	0	2320	0	16	0	3.4	2	0	6.9
SRS 493	0	0	400	0	0	0	1.5	1.6	2.7	3.9
SRS 499	0	0	2240	0	0	0	2.5	1.7	2.9	0
SRS 460	0	0	2320	0	0	0	18	0	0	14
SRS 470	0	0	1880	0	0	0	17	0	0	14
SRS 479	0	0	2160	0	0	0	17	0	0	0
SRS 459	0	0	2240	0	0	0	20	6.8	5.4	17
SRS 461	36	400	3600	0	208	0	19	6.3	4.8	16
SRS 486	20	136	3080	0	72	0	20	5.8	4.6	16
SRS 456	0	0	2000	0	0	0	30	0	0	29
SRS 457	0	0	2240	0	0	0	31	0	0	29
SRS 474	0	0	1760	0	0	0	30	9.4	0	29

Table A.15 General data for treatment 5Fe microcosms and Ferrous concentrations.

Sample ID	incubation (days)	lab project	Ferrous Iron (mg/l)
SRS 513	2	P0507166	0
SRS 530	2	P0507166	0
SRS 534	2	P0507166	0
SRS 509	7	P0507294	0
SRS 523	7	P0507294	0
SRS 533	7	P0507294	0
SRS 512	14	P0507424	0
SRS 520	14	P0507424	0
SRS 522	14	P0507424	0
SRS 504	21	P0508157	0
SRS 508	21	P0508157	0
SRS 510	21	P0508157	0
SRS 502	28	P0508189	0
SRS 503	28	P0508189	0
SRS 507	28	P0508189	0
SRS 526	41	P0508393	0
SRS 531	41	P0508393	0
SRS 537	41	P0508393	0
SRS 500	54	P0509094	0
SRS 501	54	P0509094	0
SRS 506	54	P0509094	0
SRS 515	84	P0510066	0
SRS 521	84	P0510066	0
SRS 529	84	P0510066	0

Table A.16 Dissolved gas concentrations (ug/l) for Treatment 5Fe microcosms.

Sample ID	Acetylene	Ethane	Ethene	iso-Butane	Methane	n-Butane	Propane	Propene
SRS 513	0	76	0	0	15.6	0	0	0
SRS 530	0	84	0	0	17.2	0	0	0
SRS 534	0	84	0	0	17.6	0	0	0
SRS 509	0	33.2	4.4	0	18.4	0	0	0
SRS 523	0	34.4	1.52	0	15.2	0	0	0
SRS 533	0	36.8	5.6	0	20	0	0	0
SRS 512	0	29.6	0	0	7.6	0	0	0
SRS 520	0	30.8	0	0	8.4	0	0	0
SRS 522	0	36.8	0	0	9.2	0	0	0
SRS 504	0	36.4	0.4	0	9.6	0	0	0
SRS 508	0	48	0.44	0	11.2	0	0	0
SRS 510	0	44	0.44	0	10.8	0	0	0
SRS 502	0	40	0.72	0	20	0	0	0
SRS 503	0	12.4	0.6	0	18	0	0	0
SRS 507	0	16	0.72	0	18.4	0	0	0
SRS 526	0	48	1.84	0	11.2	0	0	0
SRS 531	0	52	1.92	0	11.2	0	0	0
SRS 537	0	52	1.92	0	11.2	0	0	0
SRS 500	0	28	1	0	14.4	0	0	0
SRS 501	0	29.2	0.96	0	14.4	0	0	0
SRS 506	0	37.6	0.88	0	13.6	0	0	0
SRS 515	0	12	1.88	0	6.8	0	0	0
SRS 521	0	37.2	1.48	0	7.6	0	0	0
SRS 529	0	29.6	1.08	0	8	0	0	0

Table A.17 Chlorinated ethene (ug/l)and common ion concentrations (mg/l) for Treatment 5Fe microcosms.

Sample ID	11DCE	cDCE	PCE	tDCE	TCE	VC	Chloride	Nitrate	Nitrite	Sulfate
SRS 513	0	10400	8800	280	10800	0	0	0	0	0
SRS 530	0	10400	8800	312	10400	0	0	0	0	2.9
SRS 534	0	8800	8000	384	9600	0	0	0	0	2.9
SRS 509	0	12400	10000	560	11600	0	0	0	0	0
SRS 523	0	12000	9600	368	11200	0	0	0	0	0
SRS 533	0	10800	8400	440	10000	0	0	0	0	0
SRS 512	0	11200	8000	1160	10000	0	0	0	0	0
SRS 520	0	9600	7600	1080	9600	0	0	0	0	0
SRS 522	0	9600	8000	1280	9200	0	0	0	0	15
SRS 504	0	9200	5600	400	7600	0	0	0	0	0
SRS 508	0	9200	6000	232	7600	0	0	0	0	0
SRS 510	0	9600	6400	320	8000	0	0	0	0	0
SRS 502	0	11600	7200	276	8800	0	0	0	0	0
SRS 503	0	12400	8000	332	8400	0	0	0	0	0
SRS 507	0	11600	8000	308	8400	0	0	0	0	0
SRS 526	0	10800	7200	480	9200	0	1.7	0	0	1.4
SRS 531	0	8400	5600	440	7600	0	1.6	0	0	1.4
SRS 537	0	8400	5200	344	7600	0	1.6	0	0	1.4
SRS 500	0	12000	8400	308	10400	0	3.5	0	0	3.1
SRS 501	0	11600	7600	340	10800	0	3.5	0	0	3.2
SRS 506	0	10800	7200	312	10800	0	3.5	0	0	3.1
SRS 515	0	12400	5200	368	8000	0	3.2	0	0	0
SRS 521	0	12000	5600	332	8400	0	2.9	0	0	3
SRS 529	0	12800	7200	392	9600	0	2.9	0	0	2.9

Table A.18 General data for treatment 1ES microcosms and Ferrous Iron concentrations.

Sample ID	incubation (days)	lab project	Ferrous Iron (mg/l)	Solids (%)
EOS 1-1	0	P0505024	4	32
EOS 3-1	0	P0505024	0	40
EOS 4-1	0	P0505024	0	33
EOS 36-1	32	P0505433	28	42
EOS 39-1	32	P0505433	20	48
EOS 40-1	32	P0505433	116	43
EOS 14-1	60	P0506417		46
EOS 6-1	60	P0506417		29
EOS 8-1	60	P0506417		48
EOS 20-1	95	P0508021	26.5	49
EOS 45-1	95	P0508021	40.5	49
EOS 5-1	95	P0508021	13	42
EOS 12-1	125	P0509028	38	53
EOS 17-1	125	P0509028	68	46
EOS 2-1	125	P0509028	76	48
EOS 26-1	158	P0510066	47	57
EOS 44-1	158	P0510066	37	32
EOS 7-1	158	P0510066	49	54
EOS 13-1	200	P0511158	45	63
EOS 29-1	200	P0511158	19	63
EOS 38-1	200	P0511158	40	65

Table A.19 Dissolved gas concentrations (ug/l) for Treatment 1ES microcosms.

Sample ID	incubation (days)	Acetylene	Ethane	Ethene	iso-Butane	Methane	n-Butane
EOS 1-1	0	0	6.4	2.6	2.88	76	8.4
EOS 3-1	0	0	6.4	1.2	0	56	1.32
EOS 4-1	0	0	6.4	1.04	0	56	0
EOS 36-1	32	0	1.76	1.2	0	304	0
EOS 39-1	32	0	3.24	0.84	0	140	0
EOS 40-1	32	0	3.16	0.88	0	112	0
EOS 14-1	60	0	48	0.6	0	48	0
EOS 6-1	60	0	48	0.6	0	52	0
EOS 8-1	60	0	48	0.64	0	52	0
EOS 20-1	95	0	36.8	0.72	1.68	17.6	2.48
EOS 45-1	95	0	42.4	0.64	0	16.8	1.44
EOS 5-1	95	0	30.4	2.24	4.08	35.2	8
EOS 12-1	125	0	13.6	1.2	0	17.6	0
EOS 17-1	125	0	15.2	1.2	0	15.2	0
EOS 2-1	125	0	9.6	1.28	0	12.8	0
EOS 26-1	158	0	112	1.12	0	19.2	0
EOS 44-1	158	0	96	1.12	0	15.2	0
EOS 7-1	158	0	96	0.8	0	15.2	0
EOS 13-1	200	0	11.2	0	0	4.56	0
EOS 29-1	200	0	23.2	0.56	0	7.2	0
EOS 38-1	200	0	20	0.56	0	6.08	0

Table A.20 Chlorinated ethene (ug/l)and common ion concentrations (mg/l) for Treatment 1ES microcosms.

Sample ID	incubation (days)	cDCE	PCE	tDCE	TCE	VC	Chloride	Nitrate	Nitrite	Sulfate
EOS 1-1	0	84	920	0	18.4	120	8.6	5.1	0	87
EOS 3-1	0	68	1080	0	0	112	9.9	0	0	81
EOS 4-1	0	52	960	0	0	72	9	0	0	97
EOS 36-1	32	0	1440	0	0	0	31	11	7.5	90
EOS 39-1	32	0	1360	0	0	0	30	11	6.5	100
EOS 40-1	32	0	2160	0	0	0	30	11	7.2	77
EOS 14-1	60	0	720	0	0	0	31	0	0	82
EOS 6-1	60	0	80	0	0	0	31	0	0	100
EOS 8-1	60	0	112	0	0	0	17	0	0	82
EOS 20-1	95	16	1080	0	284	0	19	5.4	0	52
EOS 45-1	95	20	1360	0	208	0	20	5.4	0	74
EOS 5-1	95	52	520	0	480	0	20	5.5	0	68
EOS 12-1	125	0	1200	0	0	0	32	0	0	100
EOS 17-1	125	0	408	0	0	0	32	0	0	99
EOS 2-1	125	0	1440	0	32	0	32	9.7	0	97
EOS 26-1	158	0	1120	0	64	0	31	9.4	0	51
EOS 44-1	158	0	1120	0	48	0	31	0	0	65
EOS 7-1	158	0	608	0	64	0	46	0	0	58
EOS 13-1	200	0	352	0	0	0	29	9.7	0	41
EOS 29-1	200	0	1280	0	0	0	29	9.9	0	39
EOS 38-1	200	0	2240	0	0	0	30	10	0	39

Table A.21 General data for treatment 2ES microcosms and Ferrous Iron concentrations.

Sample ID	incubation (days)	lab project	Ferrous Iron (mg/l)	Solids (%)
EOS 46-2	0	P0505024	0	41
EOS 47-2	0	P0505024	0	52
EOS 55-2	0	P0505024	0	40
EOS 48-2	32	P0505433	89	47
EOS 53-2	32	P0505433	29	48
EOS 60-2	32	P0505433	28	55
EOS 49-2	60	P0506417		46
EOS 57-2	60	P0506417		47
EOS 62-2	60	P0506417		43
EOS 73-2	95	P0508021	13	
EOS 76-2	95	P0508021	17.5	
EOS 87-2	95	P0508021	9.5	
EOS 68-2	125	P0509028	19	57
EOS 77-2	125	P0509028	14	48
EOS 82-2	125	P0509028	30	56
EOS 51-2	158	P0510066	31	57
EOS 61-2	158	P0510066	14	41
EOS 83-2	158	P0510066	10	50
EOS 88-2	200	P0511158	9	62
EOS 89-2	200	P0511158	8.5	67
EOS-75-2	200	P0511158	9	61

Table A.22 Dissolved gas concentrations (ug/l) for Treatment 2ES microcosms.

Sample ID	incubation (days)	Acetylene	Ethane	Ethene	iso-Butane	Methane	n-Butane
EOS 46-2	0	0	6	0.92	0	56	0
EOS 47-2	0	0	6	0.88	0	52	0
EOS 55-2	0	0	6	0.88	0	56	0
EOS 48-2	32	0	4	0.92	0	80	0
EOS 53-2	32	0	2.6	0.68	0	88	0
EOS 60-2	32	0	4	0.6	0	48	0
EOS 49-2	60	0	48	0.24	0	52	0
EOS 57-2	60	0	52	0.28	0	52	0
EOS 62-2	60	0	48	0.28	0	64	0
EOS 73-2	95	0	44	0	0	20	0
EOS 76-2	95	0	60.8	0	0	19.2	0
EOS 87-2	95	0	26	0	0	10.4	0
EOS 68-2	125	0	15.2	0.88	0	24.8	0
EOS 77-2	125	0	16	0.72	0	176	0
EOS 82-2	125	0	18.4	0.88	0	11.2	0
EOS 51-2	158	0	104	0.72	0	16	0
EOS 61-2	158	0	104	0.8	0	28.8	0
EOS 83-2	158	0	48	0.36	0	44	0
EOS 88-2	200	0	18.4	0.64	0	76	0
EOS 89-2	200	0	22.4	0.64	0	160	0
EOS-75-2	200	0	24.8	0	0	6.96	0

Table A.23 Chlorinated ethene (ug/l)and common ion concentrations (mg/l) for Treatment 2ES microcosms.

Sample ID	incubation (days)	cDCE	PCE	tDCE	TCE	VC	Chloride	Nitrate	Nitrite	Sulfate
EOS 46-2	0	52	1640	0	0	30	16	0	0	0
EOS 47-2	0	36	1720	0	0	33.2	14	0	0	0
EOS 55-2	0	0	2160	0	0	0	10	5.3	0	4.5
EOS 48-2	32	0	2000	0	0	0	30	11	7	27
EOS 53-2	32	0	2120	0	0	0	30	11	7	27
EOS 60-2	32	0	2200	0	0	0	30	11	7.6	28
EOS 49-2	60	0	2400	0	0	0	18	0	0	14
EOS 57-2	60	0	2040	0	0	0	17	0	0	0
EOS 62-2	60	0	2027	0	0	0	0	0	0	0
EOS 73-2	95	16	1760	0	168	0	19	0	0	15
EOS 76-2	95	16	2000	0	144	0	19	0	0	15
EOS 87-2	95	0	960	0	120	0	19	0	0	15
EOS 68-2	125	0	2160	0	0	0	32	9.7	0	29
EOS 77-2	125	0	1920	0	0	0	32	9.6	3.9	29
EOS 82-2	125	0	1840	0	0	0	33	11	4	30
EOS 51-2	158	0	1600	0	64	0	31	0	0	30
EOS 61-2	158	0	1440	0	64	0	31	9.4	0	29
EOS 83-2	158	0	1920	0	56	0	31	9.4	0	30
EOS 88-2	200	0	1200	0	0	0	29	9.7	0	29
EOS 89-2	200	0	920	0	0	0	28	9.5	0	30
EOS-75-2	200	0	2320	0	0	0	28	9.4	0	29

Table A.24 General data for treatment 3ES microcosms and Ferrous Iron concentrations.

Sample ID	incubation (days)	lab project	Ferrous Iron (mg/l)	Solids (%)
EOS 106-3	6	P0505024	21.5	38
EOS 107-3	6	P0505024	12.5	37
EOS 124-3	6	P0505024	3.5	30
EOS 105-3	32	P0505433	5.5	46
EOS 129-3	32	P0505433	9	49
EOS 91-3	32	P0505433	0	48
EOS 111-3	60	P0506417		53
EOS 116-3	60	P0506417		47
EOS 121-3	60	P0506417		49
EOS 112-3	95	P0508021	26	52
EOS 132-3	95	P0508021	26.5	46
EOS 134-3	95	P0508021	47.5	51
EOS 109-3	125	P0509028	77	56
EOS 110-3	125	P0509028	0	53
EOS 137-3	125	P0509028	0	60
EOS 104-3	158	P0510066	22	
EOS 127-3	158	P0510066	48	49
EOS 130-3	158	P0510066	45	44
EOS 117-3	200	P0511158	27.5	66
EOS 118-3	200	P0511158	30.5	63
EOS 139-3	200	P0511158	25.5	58

Table A.25 Dissolved gas concentrations (ug/l) for Treatment 3ES microcosms.

Sample ID	incubation (days)	Acetylene	Ethane	Ethene	iso-Butane	Methane	n-Butane
EOS 106-3	6	0	10	0.6	0	112	0
EOS 107-3	6	0	9.6	0.72	0	104	0
EOS 124-3	6	0	12	0.76	0	108	0
EOS 105-3	32	0	5.2	0.4	0	72	0
EOS 129-3	32	0	6	0.92	0	84	0
EOS 91-3	32	0	4.4	0.6	0	52	0
EOS 111-3	60	0	48	0	0	48	0
EOS 116-3	60	0	48	0	0	92	0
EOS 121-3	60	0	48	0	0	68	0
EOS 112-3	95	0	38.8	0.24	0	8	0
EOS 132-3	95	0	72.8	0	0	38.4	0
EOS 134-3	95	0	44	0	0	32.4	0
EOS 109-3	125	0	24.8	0.64	0	54.4	0
EOS 110-3	125	0	16	0.88	0	20	0
EOS 137-3	125	0	20	0.96	0	36.8	0
EOS 104-3	158	0	96	0.8	0	80	0
EOS 127-3	158	0	104	0.96	0	88	0
EOS 130-3	158	0	104	0.64	0	36	0
EOS 117-3	200	0	22.4	0.48	0	49.6	0
EOS 118-3	200	0	20.8	0	0	1040	0
EOS 139-3	200	0	24.8	0	0	176	0

Table A.26 Chlorinated ethene (ug/l)and common ion concentrations (mg/l) for Treatment 3ES microcosms.

Sample ID	incubation (days)	cDCE	PCE	tDCE	TCE	VC	Chloride	Nitrate	Nitrite	Sulfate
EOS 106-3	6	0	1120	0	0	0	8.6	0	0	16
EOS 107-3	6	0	1320	0	0	0	13	0	0	20
EOS 124-3	6	0	1320	0	0	0	8.9	0	0	17
EOS 105-3	32	0	1080	0	0	0	30	10	7	27
EOS 129-3	32	0	1040	0	0	0	30	11	7.1	27
EOS 91-3	32	0	1000	0	0	0	29	11	7.7	27
EOS 111-3	60	0	1280	0	0	0	0	0	0	0
EOS 116-3	60	0	1160	0	0	0	0	0	0	0
EOS 121-3	60	0	840	0	0	0	0	0	0	13
EOS 112-3	95	0	1080	0	108	0	19	0	0	0
EOS 132-3	95	0	800	0	92	0	19	0	0	15
EOS 134-3	95	0	1080	0	52	0	19	0	0	15
EOS 109-3	125	0	840	0	0	0	32	9.6	0	29
EOS 110-3	125	0	960	0	0	0	31	0	0	29
EOS 137-3	125	0	800	0	0	0	31	0	0	29
EOS 104-3	158	0	960	0	32	0				
EOS 127-3	158	0	880	0	0	0	30	0	0	29
EOS 130-3	158	0	880	0	56	0	36	0	0	0
EOS 117-3	200	0	880	0	0	0	29	9.8	0	30
EOS 118-3	200	0	480	0	0	0	28	0	0	29
EOS 139-3	200	0	600	0	0	0	29	9.6	0	30

Table A.27 General data for treatment 4ES microcosms and Ferrous Iron concentrations.

Sample ID	incubation (days)	lab project	Ferrous Iron (mg/l)	Solids (%)
EOS 141-4	6	P0505024	0	39
EOS 142-4	6	P0505024	0	43
EOS 154-4	6	P0505024	0	42
EOS 144-4	32	P0505433	7	46
EOS 163-4	32	P0505433	12.5	51
EOS 168-4	32	P0505433	11	42
EOS 152-4	60	P0506417		49
EOS 164-4	60	P0506417		47
EOS 167-4	60	P0506417		49
EOS 159-4	95	P0508021	39.5	48
EOS 182-4	95	P0508021	29.5	40
EOS 188-4	95	P0508021	25	40
EOS 173-4	125	P0509028	93	51
EOS 180-4	125	P0509028	200	56
EOS 189-4	125	P0509028	29	41
EOS 157-4	158	P0510066	6	49
EOS 181-4	158	P0510066	33	59
EOS 186-4	158	P0510066	20	53
EOS 148-4	200	P0511158	80	63
EOS 176-4	200	P0511158	120	66
EOS 179-4	200	P0511158	24	63

Table A.28 Dissolved gas concentrations (ug/l) for Treatment 4ES microcosms.

Sample ID	incubation (days)	Acetylene	Ethane	Ethene	iso-Butane	Methane	n-Butane
EOS 141-4	6	0	12.4	0.72	0	104	0
EOS 142-4	6	0	13.2	1.04	0	104	0
EOS 154-4	6	0	12.4	0.92	0	112	0
EOS 144-4	32	0	8.4	0.44	0	35.6	0
EOS 163-4	32	0	9.2	0.64	0	52	0
EOS 168-4	32	0	9.2	0.96	0	60	0
EOS 152-4	60	0	48	0.32	0	52	0
EOS 164-4	60	0	52	0.36	0	52	0
EOS 167-4	60	0	48	0.44	0	52	0
EOS 159-4	95	0	88	0	0	16	0
EOS 182-4	95	0	36.8	0	0	28	0
EOS 188-4	95	0	37.2	0	0	7.6	0
EOS 173-4	125	0	23.2	1.28	0	12	0
EOS 180-4	125	0	23.2	0.88	0	61.6	0
EOS 189-4	125	0	31.2	0.96	0	9.6	0
EOS 157-4	158	0	48	0.36	0	8	0
EOS 181-4	158	0	104	0.64	0	16	0
EOS 186-4	158						
EOS 148-4	200	0	43.2	0.56	0	56	0
EOS 176-4	200	0	32.8	0	0	9.6	0
EOS 179-4	200	0	33.6	0	0	8	0

Table A.29 Chlorinated ethene (ug/l)and common ion concentrations (mg/l) for Treatment 4ES microcosms.

Sample ID	incubation (days)	cDCE	PCE	tDCE	TCE	VC	Chloride	Nitrate	Nitrite	Sulfate
EOS 141-4	6	0	3320	0	0	0	8.4	0	0	18
EOS 142-4	6	0	3360	0	0	0	7.8	0	0	14
EOS 154-4	6	0	3120	0	0	0	8.3	0	0	16
EOS 144-4	32	0	2600	0	0	0	30	10	7.1	28
EOS 163-4	32	0	2640	0	0	0	29	11	7.8	27
EOS 168-4	32	0	2560	0	0	0	30	10	7.2	27
EOS 152-4	60	0	3200	0	0	0	0	0	0	0
EOS 164-4	60	0	2360	0	0	0	17	0	0	13
EOS 167-4	60	0	2400	0	0	0	17	0	0	13
EOS 159-4	95	0	2600	0	48	0	19	0	0	16
EOS 182-4	95	0	2080	0	60	0	35	0	0	30
EOS 188-4	95	0	2520	0	52	0	35	0	0	30
EOS 173-4	125	0	2000	0	0	0	31	9.5	3.8	29
EOS 180-4	125	0	2040	0	0	0	31	9.5	0	29
EOS 189-4	125	0	2560	0	0	0	31	9.7	3.9	29
EOS 157-4	158	0	2000	0	48	0	31	9.7	0	30
EOS 181-4	158	0	2200	0	20	0	31	0	0	29
EOS 186-4	158	0	2440	0	28	0	31	0	0	29
EOS 148-4	200	0	1120	0	0	0	28	9.6	0	30
EOS 176-4	200	0	1000	0	0	0	28	9.4	0	29
EOS 179-4	200	0	2560	0	72	0	28	9.6	0	30

Table A.30 General data for treatment 5ES microcosms and Ferrous Iron concentrations.

Sample ID	incubation (days)	lab project	Ferrous Iron (mg/l)	Solids (%)
EOS 194-5	6	P0505024	0	36
EOS 233-5	6	P0505024	0	35
EOS 235-5	6	P0505024	1.5	
EOS 200-5	32	P0505433	10.5	42
EOS 208-5	32	P0505433	13	52
EOS 227-5	32	P0505433	12	48
EOS 209-5	60	P0506417		38
EOS 219-5	60	P0506417		45
EOS 238-5	60	P0506417		42
EOS 203-5	95	P0508021	19	41
EOS 204-5	95	P0508021	16	42
EOS 234-5	95	P0508021	7	47
EOS 211-5	125	P0509028	22	47
EOS 212-5	125	P0509028	8	56
EOS 221-5	125	P0509028	10	59
EOS 214-5	158	P0510066	8	48
EOS 223-5	158	P0510066	10	54
EOS 236-5	158	P0510066	7	52
EOS 196-5	200	P0511158	8	74
EOS 230-5	200	P0511158	10	57
EOS 237-5	200	P0511158	17	60

Table A.31 Dissolved gas concentrations (ug/l) for Treatment 5ES microcosms.

Sample ID	incubation (days)	Acetylene	Ethane	Ethene	iso-Butane	Methane	n-Butane
EOS 194-5	6	0	12	0.76	0	108	0
EOS 233-5	6	0	12.4	0.68	0	108	0
EOS 235-5	6	0	11.2	0.4	0	92	0
EOS 200-5	32	0	10.8	1.72	6.4	92	0
EOS 208-5	32	0	10	0.68	0	40	0
EOS 227-5	32	0	11.6	0.64	0	39.6	0
EOS 209-5	60	0	48	0.24	0	56	0
EOS 219-5	60	0	52	0.32	0	56	0
EOS 238-5	60	0	52	0.24	0	60	0
EOS 203-5	95	0	44	0	0	20	0
EOS 204-5	95	0	48	0	0	19.2	0
EOS 234-5	95	0	96	0	0	15.2	0
EOS 211-5	125	0	96	0.88	0	62.4	0
EOS 212-5	125	0	96	0.96	0	76	0
EOS 221-5	125	0	96	0.8	0	104	0
EOS 214-5	158						
EOS 223-5	158	0	36.8	0.72	0	69.6	0
EOS 236-5	158	0	35.2	0.72	0	58.4	0
EOS 196-5	200	0	16.8	0	0	33.2	0
EOS 230-5	200	0	88	0	0	12	0
EOS 237-5	200	0	88	0.48	0	60.8	0

Table A.32 Chlorinated ethene (ug/l)and common ion concentrations (mg/l) for Treatment 5ES microcosms.

Sample ID	incubation (days)	cDCE	PCE	tDCE	TCE	VC	Chloride	Nitrate	Nitrite	Sulfate
EOS 194-5	6	0	880	0	0	0	16	6.2	0	26
EOS 233-5	6	0	840	0	0	0	13	0	0	14
EOS 235-5	6	0	720	0	0	0				
EOS 200-5	32	0	1760	0	0	0	30	11	7.9	27
EOS 208-5	32	0	1840	0	0	0	29	11	6.7	27
EOS 227-5	32	0	2120	0	0	0	30	11	7.1	28
EOS 209-5	60	0	600	0	0	0	17	0	0	14
EOS 219-5	60	0	1120	0	0	0	17	0	0	14
EOS 238-5	60	0	600	0	0	0	17	0	0	0
EOS 203-5	95	0	368	0	56	0	34	0	0	0
EOS 204-5	95	0	1800	0	56	0	35	0	0	30
EOS 234-5	95	0	480	0	44	0	35	0	0	31
EOS 211-5	125	0	1640	0	0	0	32	9.6	0	29
EOS 212-5	125	0	1360	0	0	0	32	9.8	3.8	29
EOS 221-5	125	0	1320	0	0	0	32	0	0	29
EOS 214-5	158	0	1280	0	24	0	31	0	0	29
EOS 223-5	158	0	312	0	0	0	39	0	0	31
EOS 236-5	158	0	400	0	20	0	33	0	0	29
EOS 196-5	200	0	880	0	24	0	29	9.6	0	29
EOS 230-5	200	0	2160	0	0	0	28	9.7	0	29
EOS 237-5	200	0	720	0	0	0	28	0	0	29

Table A.33 General data for treatment 6ES microcosms and Ferrous Iron concentrations.

Sample ID	incubation (days)	lab project	Ferrous Iron (mg/l)	Solids (%)
EOS 247-6	32	P0505433	0	106
EOS 248-6	32	P0505433	0	107
EOS 289-6	32	P0505433	0	108
EOS 251-6	60	P0506417	0	109
EOS 262-6	60	P0506417	0	110
EOS 270-6	60	P0506417	0	111
EOS 258-6	95	P0508021		112
EOS 269-6	95	P0508021		113
EOS 276-6	95	P0508021		114
EOS 255-6	125	P0509028	0	115
EOS 256-6	125	P0509028	0	116
EOS 264-6	125	P0509028	0	117
EOS 244-6	158	P0510066	0	118
EOS 273-6	158	P0510066	0	119
EOS 290-6	158	P0510066	0	120
EOS 265-6	200	P0511158	0	121
EOS 271-6	200	P0511158	0	122
EOS 280-6	200	P0511158	0	123

Table A.34 Dissolved gas concentrations (ug/l) for Treatment 6ES microcosms.

Sample ID	incubation (days)	Acetylene	Ethane	Ethene	iso-Butane	Methane	n-Butane
EOS 247-6	32	0	16.4	0.4	0	33.2	0
EOS 248-6	32	0	13.2	0.48	0	32	0
EOS 289-6	32	0	12	0.32	0	29.2	0
EOS 251-6	60	0	52	0	0	52	0
EOS 262-6	60	0	52	0	0	52	0
EOS 270-6	60	0	52	0.36	0	52	0
EOS 258-6	95	0	100	0	0	17.6	0
EOS 269-6	95	0	76	0	0	14.8	0
EOS 276-6	95	0	112	0	0	30	0
EOS 255-6	125	0	56	0.36	0	8.4	0
EOS 256-6	125	0	56	0.32	0	8.8	0
EOS 264-6	125	0	56	0.28	0	8	0
EOS 244-6	158	0	10.8	0.28	0	5.6	0
EOS 273-6	158	0	10.4	0.36	0	5.6	0
EOS 290-6	158	0	9.6	0.32	0	5.2	0
EOS 265-6	200	0	44	0	0	6.8	0
EOS 271-6	200						
EOS 280-6	200	0	44	0	0	6.4	0

Table A.35 Chlorinated ethene (ug/l)and common ion concentrations (mg/l) for Treatment 6ES microcosms.

Sample ID	incubation (days)	cDCE	PCE	tDCE	TCE	VC	Chloride	Nitrate	Nitrite	Sulfate
EOS 247-6	32	0	2560	0	0	0	28	0	0	0
EOS 248-6	32	0	2600	0	0	0	28	0	0	27
EOS 289-6	32	0	1320	0	0	0	28	0	0	0
EOS 251-6	60	0	3720	0	0	0	2.8	0	0	2.7
EOS 262-6	60	0	3800	0	0	0	2.9	0	0	2.7
EOS 270-6	60	0	3440	0	0	0	2.8	0	0	2.7
EOS 258-6	95	0	3960	0	44	0	0	0	0	0
EOS 269-6	95	0	2600	0	12	0	0	0	0	3
EOS 276-6	95	0	2080	0	0	0	0	0	0	3
EOS 255-6	125	0	1920	0	0	0	2.9	0	0	2.9
EOS 256-6	125	0	3160	0	0	0	2.9	1	0	2.9
EOS 264-6	125	0	2560	0	0	0	2.9	1	0	2.9
EOS 244-6	158	0	1080	0	0	0	3	0	0	3
EOS 273-6	158	0	2880	0	0	0	2.9	0	0	2.9
EOS 290-6	158	0	3280	0	0	0	2.9	0	0	0
EOS 265-6	200	0	2040	0	0	0	2.8	0	0	3
EOS 271-6	200	0	2200	0	0	0	2.8	0	0	3
EOS 280-6	200	0	1520	0	0	0	2.9	0	0	3.1

Table A36. Data from the TCE re-spike experiment for treatment 1Fe.

Sample ID	Incubation	cDCE	TCE	VC	acetylene
314	0	<1	8593	<1	<0.5
316	0	<1	9071	<1	<0.5
336	0	<1	9765	<1	<0.5
306	7	<1	7291	<1	<0.5
318	7	<1	7465	<1	<0.5
324	7	<1	7161	<1	<0.5
300	15	<1	6944	<1	<0.5
305	15	<1	6510	<1	<0.5
327	15	<1	7378	<1	<0.5
313	21	<1	7812	<1	<0.5
325	21	<1	6944	<1	
345	21	<1	7812	<1	
308	28	<1	7378	<1	<0.5
310	28	<1	6510	<1	<0.5
339	28	<1	6928	<1	<0.5
312	35	<1	9093	<1	<0.5
323	35	<1	9093	<1	<0.5
349	35	<1	9959	<1	<0.5

Table A37. Data from the TCE re-spike experiment for treatment 2Fe.

Sample ID	Incubation	cDCE	TCE	VC	acetylene
356	0	<1	8767	<1	<0.5
389	0	<1	8723	<1	<0.5
395	0	<1	10763	<1	<0.5
360	7	<1	6597	<1	<0.5
365	7	<1	6944	<1	<0.5
384	7	<1	6944	<1	<0.5
353	15	<1	6944	<1	<0.5
357	15	<1	6944	<1	<0.5
398	15	<1	6510	<1	<0.5
364	21	<1	6510	<1	<0.5
366	21	<1	6944	<1	<0.5
396	21	<1	6510	<1	<0.5
377	28	<1	6062	<1	<0.5
382	28	<1	6495	<1	<0.5
383	28	<1	5629	<1	<0.5
355	35	<1	9093	<1	<0.5
374	35	<1	7794	<1	<0.5
391	35	<1	8227	<1	<0.5

Table A38. Data from the TCE re-spike experiment for treatment 4Fe.

Sample ID	Incubation	cDCE	TCE	VC	acetylene
487	0	<1	8463	<1	<0.5
491	0	<1	8723	<1	<0.5
498	0	<1	8767	<1	<0.5
466	7	<1	6076	<1	<0.5
468	7	<1	6553	<1	<0.5
494	7	<1	5946	<1	<0.5
471	15	<1	5642	<1	<0.5
458	21	<1	6076	<1	<0.5
464	28	<1	5629	<1	<0.5
478	35	<1	7794	<1	<0.5
490	35	<1	7794	<1	<0.5

Table A38. Data from the TCE re-spike experiment for treatment 6Fe.

Sample ID	Incubation	cDCE	TCE	VC	acetylene
600	0	<1	9591	<1	<0.5
601	0	<1	8897	<1	<0.5
602	0	<1	9678	<1	<0.5
618	7	<1	8637	<1	<0.5
619	7	<1	8854	<1	<0.5
620	7	<1	8376	<1	<0.5
613	15	<1	8246	<1	<0.5
614	15	<1	9114	<1	<0.5
615	15	<1	8246	<1	<0.5
616	21	<1	8680	<1	<0.5
617	21	<1	9114	<1	<0.5
622	21	<1	8246	<1	<0.5
611	28	<1	8227	<1	<0.5
612	28	<1	8227	<1	<0.5
621	28	<1	8227	<1	<0.5
603	35	<1	10392	<1	<0.5
607	35	<1	9093	<1	<0.5
608	35	<1	9526	<1	<0.5

[i] Zero valent zinc, 20 mesh, approximately 5 grams.
[ii] Zero valent magnesium, ribbon form, ~ 3.5" strips, ~ 0.15 grams.