

FINAL REPORT

SERDP Project ER-1369

**Sustainability of Long-Term Abiotic Attenuation of
Chlorinated Ethenes**

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Executive Summary

The goal of our SERDP project (ER-1369) was to identify abiotic degradation mechanisms that may contribute to the attenuation of chlorinated ethene plumes. To this end, we measured the reduction of chlorinated ethenes by a series of chemically and microbially generated reductants under a range of natural conditions. During the the project, we collected, synthesized, and characterized a variety of reductants and measured the extent and rate of PCE, TCE and cDCE reduction in batch reactors. The reductants included, (i) Fe(II) sorbed on Fe oxides, (ii) minerals containing Fe(II), such as iron sulfides (e.g., mackinawite) and green rusts, (iii) precipitates and supernatant collected from dissimilatory iron reducing (DIR) cultures of *Shewanella* species and an sulfate reducing bacterial (SRB) culture of *Desulfovibrio desulfuricans*, and (iii) sorbed and reduced natural organic matter (NOM) and NOM model compounds.

The chlorinated ethenes have been surprisingly stable in the presence Fe(II) sorbed on Fe oxides, as well as magnetite, and reduced and sorbed natural organic matter (NOM). We have repeated these experiments over a range of pH and buffer conditions and conducted several positive controls to make sure that (i) we would observe PCE, TCE, and cDCE transformation and the formation of products if it occurred, and (ii) the reductants have not been comprised and are indeed capable of reducing other oxidized compounds. The lack of reactivity with sorbed Fe(II) and magnetite is contrary to what we expected based on thermodynamic calculations and previous work showing PCE and TCE reduction in the presence of magnetite and Fe(II).

Reduction of TCE was observed with chemically synthesized green rust minerals, as well as the iron sulfide minerals, mackinawite and pyrite. Acetylene was the major product observed for TCE reduction by mackinawite, consistent with the work by Butler and colleagues. TCE was reduced rather slowly by carbonate and sulfate green rust with half-lives on order of about a month. Carbon recoveries based on dechlorinated gas products (Σ ethene, ethane, acetylene) varied from 48 to 89% of the initial TCE added. Data from PCE reduction by green rusts in inconclusive. In one experiment, some products were observed after 2 monthes, but the data was not reproducible and controls without green rust also showed significant loss of PCE.

cDCE was reduced quite rapidly by carbonate and chloride green, however, we suspect that an interaction between the aluminum foil used to cover the septa and the green rust may be playing a role in the rapid reduction of cDCE. At this time, the nature of the interaction is unclear. Given the propensity for aluminum to substitute into Fe minerals, the rapid rate of cDCE reduction by green rusts in the presence of aluminum foil may represent an interesting abiotic reaction pathway to follow up on.

We also investigated the reduction of chlorinated ethenes by these same iron species, i.e., green rusts and mackinawite, formed in the presence of anaerobic bacteria. Specifically, we collected and characterized precipitates formed in the presence of dissimilatory iron reducing and sulfate reducing bacteria (DIRB and SRB). Carbonate green rust was formed as a result of DIR by a diverse range of *Shewanella* sp. and mackinawite was formed in an SRB cultures of *Desulfovibrio desulfuricans*. Whole cultures (cells + precipitates) of both SRB and DIRB could reduce TCE. The kinetics of TCE reduction varied among different cultures and product recoveries as dechlorinated gases varied, but appreciable amounts of acetylene was observed indicating some reduction via elimination.

We expected that the biogenic precipitates, that is, the carbonate green rust and mackinawite, were responsible for the TCE reduction observed in the whole cultures, but

experiments with isolated components from the culture suggested otherwise. Specifically, the washed green rust from the Fe reducing culture was unreactive with TCE, in contrast to the significant reduction we observed with chemically synthesized green rust. Some reduction of TCE with autoclaved cultures indicate that there is an abiotic component, but similar amounts of reduction were also observed in sterile medium controls containing lepidocrocite suggesting that DIR was not necessary for abiotic reduction to occur. At this point, we can only speculate that there is some interaction between a soluble microbial exudate and the green rust surface that is responsible for the TCE reduction observed in the whole cultures.

In the sulfate reducing culture, TCE was reduced to acetylene and mackinawite was identified. It appears, however, that high concentrations of sulfate, as well as soluble microbial exudates inhibit the rate of TCE reduction compared to that of chemically synthesized FeS. Images of chemically synthesized mackinawite and the biogenic FeS show that the biogenic FeS surface is coated with some cellular or precipitate material and is more heterogeneous than the chemically synthesized FeS, suggesting caution when extrapolating reactivity behavior from chemically synthesized minerals to complex biogeochemical environments.

1. Objectives

The goal of this project was to identify processes and mechanisms that could potentially account for the abiotic transformation of chlorinated ethenes in contaminated aquifers, and to assess their likely importance under field conditions. Our focus was on chlorinated ethene reduction by both chemically and microbially generated reactants. More specifically, we:

- (1) measured and compared the rate and extent of chlorinated ethene reduction by a series of reactive precipitates and dissolved species formed via chemical dissolution or microbial activity,
- (2) explored how water chemistry (e.g., pH, bicarbonate, and presence of natural organic matter) affected the rates and extent of chlorinated ethene reduction.

We also proposed a third objective, focused on

- (3) evaluating the sustainability of abiotic transformation of chlorinated ethenes under flow conditions using a recirculating packed-bed reactor.

As discussed in our quarterly progress reports, we did not pursue this third objective because of the lack of reactivity of the chlorinated ethenes with most of the reductants we considered. Instead, we focused on expanding our list of reductants and trying to isolate the reactive component in the microbial cultures.

Specific questions that we addressed as part of this work include:

Chemically synthesized reactants

- (1) Do reduced iron minerals (such as green rust and mackinawite) reduce chlorinated ethenes? If so, how fast and what are the products?
- (2) Does ferrous iron sorbed to minerals reduce chlorinated ethenes? If so, how fast and what are the products?

- (3) How do water chemistry conditions, such as pH, buffer, and natural organic matter affect chlorinated ethene reduction rates and product distributions?
- (4) Is the production of acetylene useful as an indicator of an abiotic degradation mechanism?

Microbially generated reactants

- (5) Do microbially reduced precipitates (i.e., biogenic precipitates) formed in the presence of iron and sulfate reducing bacteria reduce chlorinated ethenes? If so, how fast and what are the products?
- (6) What are the reactive species in biogenic precipitates responsible for chlorinated ethene reduction (e.g. is it bound ferrous iron or sulfide species, reduced organic matter, etc.)?
- (7) Do reduced or sorbed humic substances reduce chlorinated ethenes?

2. Background

Chlorinated solvents, such as tetrachloroethene (PCE) and trichloroethene (TCE), are by far the most prevalent priority pollutants at hundreds of Department of Defense (DoD) sites and remain among the most difficult to remediate despite years of research and development. The relative ineffectiveness and prohibitive costs of conventional pump and treat cleanup practices have spurred a paradigm shift towards in situ cleanup technologies. Among these, monitored natural attenuation (MNA), in conjunction with source control is potentially the most cost-effective risk-management approach.

Monitored natural attenuation (MNA) has recently been identified by the U.S. Environmental Protection Agency as a viable, alternative method for cleanup of contaminated soil and groundwater. Natural attenuation relies on natural chemical and biological processes to transform or immobilize contaminants. Despite the clear interest in the application of MNA to chlorinated solvent plumes, little has been done to assess the role of chemical (or abiotic) transformation pathways. Although there is some evidence that laboratory synthesized iron minerals can transform chlorinated ethenes, it is unclear whether this transformation process is likely to be sufficiently sustainable under field conditions to impact the attenuation of chlorinated ethenes.

The results from this work are directly relevant to SERDP's mission to achieve more efficient and effective environmental remediation of soil and groundwater at DoD and DOE sites. Our goal was to assess the likely importance of abiotic transformation of chlorinated ethenes. Our focus was on chlorinated ethene transformation by both chemically and microbially generated reactants. Understanding the factors that contribute to abiotic transformations is critical to assess the feasibility of natural attenuation and promote the rationale design of strategies for accelerating groundwater remediation of chlorinated ethene plumes.

3. Technical Approach

To identify processes and mechanisms that could potentially account for the abiotic transformation of chlorinated ethenes in contaminated aquifers, we measured the rate and extent of chlorinated ethene reduction by a series of reactive precipitates and dissolved species formed from chemical dissolution or microbial activity. Reactive Fe(II) precipitates included green rusts, mackinawite, and Fe(II) sorbed on minerals. The microbially generated reductants included

precipitates, supernatants, and natural organic matter collected from sulfate and iron reducing microbial cultures.

Both the microbially generated precipitates and chemically synthesized precipitates were identified and characterized by X-ray diffraction (XRD), Mössbauer spectroscopy, and scanning electron microscopy (SEM) with elemental detection by energy dispersive X-ray spectroscopy (EDS). Characterization was done before and, in some cases, after reaction with chlorinated ethenes. XRD provided identification of crystalline phases. Mössbauer spectroscopy provided identification of small, amorphous mineral particles often observed as a result of microbial activity.

The extent and rates of chlorinated ethene reduction were measured in batch reactors containing a suspension of the chemically or microbially generated reductants. All reactors were prepared using strict anoxic protocols and individual reactors were sacrificed periodically for chlorinated ethene and product analyses. The chlorinated ethenes, such as perchlorethene, trichloroethene, dichloroethenes, and vinyl chloride were identified and quantified via gas chromatography with an electron conductivity detector (ELDC). The more volatile, unchlorinated products, such as acetylene, ethene and ethane, were analyzed via gas chromatography with a flame ionization detector (FID).

4. Materials and Methods

Chemicals. The target compounds in this study included perchloroethylene (PCE), trichloroethylene (TCE), and later in the project, we also included the dichloroethylene isomer, cDCE, as well as acetylene. Based on previous results in the literature, a number of potential transformation products were also measured. The majority of these chemicals were obtained as neat liquids, PCE, TCE, cDCE. Vinyl chloride was purchased as a 2000 $\mu\text{g mL}^{-1}$ solution in methanol for use in standard preparation. Other chemicals used include hexane, methanol, ferrous chloride (FeCl_2), sodium sulfide (Na_2S), sodium azide (NaN_3), and chloramphenicol. During these experiments, three buffer compounds were used; N-tris[Hydroxymethyl]methyl-3-aminopropane-sulfonic acid (TAPS buffer), tris(hydroxymethyl)aminomethane (TRIS buffer), and 3-N-morpholino propansulfonic acid (MOPS buffer), as well as a bicarbonate buffer. Gases used in the studies include ethane, ethene, acetylene, nitrogen, and N_2/H_2 (93% / 7% v/v) for use in the anaerobic glovebox. Ferrous iron ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) and potassium bromide were obtained from Fisher (certified).

Chemical Analyses. Stock solutions of PCE, TCE, and cDCE were prepared by adding a gravimetrically determined amount of the neat compound to a known amount of methanol. Concentrated stock solutions were targeted at 20 mM, and subsequent 1 mM stock solutions were made by diluting the concentrated stocks in methanol. Chlorinated ethenes were measured using an Agilent Technologies 6890N gas chromatography detector (GCD) equipped with a DB-5 column (J & W Scientific, 30 m x 0.53 mm i.d. x 5 μm film thickness) and a 5320 O. I. analytical electrolytic conductivity detector (ELCD). Aqueous calibration standards were prepared in 10 mL headspace vials containing 10 mL of hexane and known masses of the target compounds. Preparing standards in hexane allowed them to be directly injected into the GC without the need for an extraction procedure. Analysis showed that multiple compounds could be added to a single standard solution in hexane without compromising calibration curves for individual compounds. At each sampling, a five-point calibration curve was constructed using these standards. Instrument response was plotted versus known standard concentration and fitted with a linear regression. Dimensionless Henry's coefficients were used to determine the total

concentration (C_{tot} , M) from the aqueous concentration (C_{aq} , M) of each compound (equations 1 and 2). All concentrations subsequently are reported as total compound mass (aqueous and gaseous) per liquid volume. Table 1 lists dimensionless Henry's constants and other physical parameters for target compounds.

$$K_H = \frac{C_{\text{gas}}}{C_{\text{aqueous}}} \quad \text{eq. 1}$$

$$C_{\text{tot}} = \frac{C_{\text{aq}}(V_{\text{aq}}) + C_{\text{aq}}(K_H)(V_{\text{gas}})}{V_{\text{aq}}} \quad \text{eq. 2}$$

The non-chlorinated gas products were analyzed on an Agilent Technologies 6890N gas chromatography detector with a GS-Q column (J & W Scientific, 30 m x 0.53 mm i.d.) and a flame ionization detector (FID). Headspace samples (100 μL) were taken from the reactor with a gas-tight syringe and manually injected into the GCD. The samples were compared to standards that were prepared by adding known volumes of gas (ethene, acetylene, and ethane) into 7 ml of DI and using the same 7 ml glass vials used in the experiments. Partitioning was accounted for by using the dimensionless Henry's constants in Table 2.

Dissolved Fe(II) was determined by colorimetric analysis using phenanthroline as the colorimetric reagent. Absorbance was measured at 510 nm on a Spectronic® Genesys™ 5 spectrometer. Aliquot samples were taken from the reactor via syringe, filtered through a 0.2 μm pore size filter, and diluted with deionized deoxygenated water to an Fe(II) concentration between 0 and 50 μM . Concentrated HCl was used to lower the pH and prevent oxidation of Fe(II). Hydroxylamine HCl was used in some samples to reduce Fe(III) to Fe(II) in order to quantify the total Fe. Phenanthroline, followed by the addition of ammonium acetate buffer, was added for color development. Adsorbed Fe(II) was quantified by the difference between the initial aqueous Fe(II) concentration and the aqueous concentration at the time the sample was taken.

Chemically Synthesized Minerals. Mackinawite, $\text{FeS}_{(1-x)}$ hereafter referred to as FeS, was synthesized using a method adapted from Butler and Hayes (1998) by slowly adding 300 mL of 1.1 M Na_2S to 500 mL of 0.57 M FeCl_2 inside an anaerobic glovebox. The resulting slurry was mixed for three days and then decanted into polypropylene centrifuge bottles. These bottles were tightly sealed and centrifuged at 8000 rpm for 10 minutes. The supernatant in the bottles was discarded, and fresh nitrogen-purged deionized water was added. The bottles were then resealed, vortexed to resuspend solids and allowed to equilibrate before recentrifuging. This process was repeated for a total of eight centrifuge cycles. The black precipitate was removed from the centrifuge bottles and freeze-dried under vacuum. The resulting powder was sieved through a 100-mesh brass sieve (150 μm grid size) to ensure a more uniform particle size distribution. This procedure was done in an anaerobic glovebox under 93 % N_2 / 7 % H_2 atmosphere, except for brief periods of centrifugation and transport to freeze-drying in which case sample containers were sealed before removal from the anaerobic glovebox. Pyrite (FeS_2) was obtained from Ward's.

All ferric oxides were synthesized in accordance to procedures detailed in Iron Oxides in the Laboratory (Schwertmann and Cornell, 1991). Lepidocrocite was synthesized by oxidizing dissolved $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ crystals with air at a controlled pH between 6.7-6.9. Goethite was

prepared from a diluted combination of 5 M KOH and 1 M $\text{Fe}(\text{NO}_3)_3$. The combination formed a ferrihydrite precipitate that was heated at 70 °C for 60 hours transforming it into goethite. Similar to the preparation of goethite, hematite was prepared by the combination 0.2 M $\text{Fe}(\text{NO}_3)_3$ and 1 M KOH to precipitate ferrihydrite. One M NaHCO_3 was added to the solution for buffering. All solutions were preheated to 90 °C for 48 hours to prevent the formation of goethite and promote the formation of hematite. After synthesis, all oxides were washed several times with deionized water and freeze-dried. Each ferric oxide was sieved with a 100-mesh (250 μm) sieve to create a more uniform particle size distribution. Magnetite was obtained commercially from Cerac (5 micron or less, 99%).

Green rust synthesis was accomplished by titration of a ferric/ferrous solution with a 1 M basic solution under an anaerobic atmosphere (93% N_2 /7% H_2). A 26.5 mM ferric solution ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and a 198.8 mM ferrous solution ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ or $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) were raised to pH 7.0 with 1 M Na_2CO_3 or NaOH. The two solutions were combined into one beaker where the pH was then maintained at 8.5 for 1-2 hours until a blue-green precipitate is formed and the pH stabilizes. Chloride green rust used $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, and NaOH. Sulfate green rust used $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and NaOH. Carbonate green rust used $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, and Na_2CO_3 . After synthesis, the green rusts were washed with deionized water, freeze-dried, and sieved (250 μm). In some experiments, an alternate air-oxidation method was used to synthesize sulfate green rust {O'Loughlin, 2003 #14371}. A 1 M ferrous sulfate solution ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) was oxidized by ambient air while being magnetically stirred. The solution was titrated with 1 M NaOH until pH 7.5 was reached. The precipitate was then brought into a glovebox where it was washed and filtered. This green rust was used wet and not freeze-dried. All oxides were verified by X-ray diffraction (XRD) on a Siemens D-5000 Diffractometer with $\text{CuK}\alpha$ radiation. Green rust samples were mixed with glycerol to slow down oxidation during analysis. Specific surface area was determined by five-point BET analysis performed with a Quantachrome NOVA 1200 surface area analyzer. A special cell-seal assembly was used to prevent oxidation of the green rust during analysis.

Sulfate Reducing Bacteria: *Desulfovibrio desulfuricans* subspecies *desulfuricans* (ATCC[®] 13541) was obtained from the American Type Culture Collection (ATCC). The culture was maintained in Modified Baar's Medium for Sulfate Reducers (ATCC #1249) utilizing lactate as the electron donor, and sulfate as the electron acceptor (Table 3). To induce precipitation of FeS, ferrous iron was added as ferrous ammonium sulfate or ferrous chloride to selected cultures. Medium 1249 was prepared as 3 components with pH adjusted to 7.5, autoclaved (121°C, 30 minutes), and recombined under sterile conditions. All media bottles were autoclaved at 121°C prior to use.

Dissimilatory Iron Reducing Bacteria: *Shewanella putrefaciens* CN32, as well as several other *Shewanella* species was obtained from Dr. Ed O'Loughlin at Argonne National Laboratories. Cultures utilized medium described in Ona-Nguema et al. (2002) with lactate as a carbon source / electron donor and lepidocrocite as the electron acceptor. The reduced iron precipitates were characterized with ^{57}Fe Mössbauer spectroscopy, X-ray diffraction (XRD), and Scanning Electron Microscopy (SEM) after about one month.

Microbe – Precipitate Separation and Isolation Techniques. A number of techniques were employed to separate out biological contributions to contaminant reduction from the abiotic ones. These methods encompassed both physical separation (washing and freeze drying) and inactivation of biological components (azide, chloramphenicol, pasteurization), in both cases with emphasis on minimizing any alteration of the precipitated solids. For washing, the reactors

were vortexed to suspend all solids, decanted into centrifuge tubes, and sonicated for 10 minutes. After sonication, the solutions were centrifuged 2000 rpm for 10 minutes. The supernatant was poured off and stored for later use, and the pelleted contents from each bottle were resuspended in 25 mM TAPS buffer solution. The procedure of resuspend / sonicate / centrifuge was carried out a total of 4 times. All above procedures occurred in the glovebox under 93% N₂ / 7% H₂ with the exception of sonication and centrifugation, which were performed in tightly sealed centrifuge bottles to prevent infiltration of oxygen. This procedure was adapted from McCormick et al. (2002), who found via protein assay that 4 successive washes removed biological material below detectable levels. For freeze-drying, pelleted solids were transferred to a freeze drying vessel and freeze dried under vacuum. Freeze dried precipitates were scraped with a spatula, then sieved through a #100 mesh (150 µm) sieve to provide uniform particle size, and sealed in a vial. Pasteurization to stop bioactivity in samples containing microbes and iron sulfide, reactors were pasteurized in a Fisher Scientific muffle furnace at 70°C for 1 hour.

Natural Organic Matter. Throughout the course of this study, three different types of NOM have been used to represent an organic presence in groundwater systems. Surface water was collected from the Little Gratiot River in northern Michigan and concentrated via reverse osmosis to yield a “natural” source of organic matter, which will subsequently be referred to as NOM. Humic acid (Aldrich HA) was purchased in granular form from Aldrich and suspended in water to be used as a commercial source of organic matter. The use of 9, 10-Anthraquinone-2,6-disulfonic acid as a “NOM analogue” is a common procedure in humic research involving humic material, and the sodium salt of this material was purchased from Sigma and used in selected trials. This compound, subsequently referred to as AQDS, has two quinone groups that can be reduced to yield the corresponding hydroquinone, AHDS, and in this way serves to model the electron shuttling capabilities of naturally occurring organic matter. AHDS and the reduced forms of the other two organic materials were created using a Pd-hydrogen catalyst. After removing oxygen from an aqueous sample through N₂-sparging or extended equilibration in an anaerobic glovebox, a few Palladium-coated aluminum pellets were added as a catalyst and the solution was crimp-sealed with a rubber stopper before removal from the glovebox. H₂ gas was bubbled into the solution at a rate of roughly 35 mL/min through a needle piercing the rubber septa, and was continued for at least 5 hours before the sample was returned to the anaerobic glovebox overnight before use.

Batch Reactor Experiments. During initial reactor development, five varieties of rubber septa with aluminum crimp seals were tested, however none of the septa alone performed adequately for retaining volatile organic compounds. Analysis indicated that up to 80% of PCE loss observed was due to sorption onto the rubber septa. Aluminum foil, lead foil, and aluminum lined rubber septa (with screw cap vials) were tested as means to prevent sorption of organics to the septa, with and without a layer of saran wrap to seal out oxygen. While all three adequately inhibited sorption, reactors using saran wrap, lead foil or screw caps had problems with vial sealing, leading to volatilization loss of organics and oxidation of reactor solutions via oxygen infiltration. Flame sealed glass ampoules were also tested, but similar problems with oxygen infiltration occurred during the sealing procedure. Only systems using aluminum foil on a Teflon faced butyl rubber septum were consistently able to retain volatile organic compounds and prevent oxidation of vial contents.

All experiments involving chlorinated ethenes were performed in 6 mL glass headspace vials (Supelco) with 6 mL liquid volume and approximately 2 mL headspace. All reactors were prepared in an anaerobic glovebox to exclude oxygen. For each reactor 6 mL of aqueous solution

was added via pipet, and a known volume of chlorinated ethene stock was added with a glass gas tight syringe. Vials were sealed with a two-layer system of a Teflon faced rubber septum with aluminum foil covering the Teflon face. Initial samples were taken one hour after the addition of target compound to allow partitioning between the liquid and headspace, and reactors were sacrificed for each subsequent sampling. Reactors were stored on their sides on a rotary shaker at 200 rpm for the duration of the experiments, and covered with aluminum foil to prevent photolysis of target chlorinated ethenes. At selected time points, reactors were sacrificed and analyzed for chlorinated ethenes and their reduction products as described above.

Aluminum Foil Interference. Note that later observations prompted us to test the stability of cDCE and acetylene in the presence of aluminum foil alone, as well as aluminum foil and aqueous Fe(II). We found that acetylene was transformed to ethane and ethene in a control experiment containing a small ball of aluminum foil (15 g/L of aluminum foil), and that the addition of 3 mM aqueous Fe(II) increased the rate of transformation (Figure 1). Unfortunately, this complicates our interpretation of product speciation, since we cannot be sure if the aluminum foil was responsible for further transformation of acetylene to ethane/ethene. The concentration of aluminum foil in the control experiments was several orders of magnitude greater than that used for lining the septa, but nonetheless, we have only reported the sum of gas products (i.e., acetylene + ethane + ethene) in any experiments that used aluminum foil lined septa.

A similar set of control experiments with cDCE also showed some reduction to ethane and ethene in the presence of Al foil and aqueous Fe(II), but at much slower rate (even at the much higher 15 g/L aluminum foil concentration). As a precaution, we did a second set of experiments without Al foil using just headspace analysis, and found that in green rust alone did not result in appreciable reduction of cDCE. When a small piece of Al foil was added to the reactor, however, substantial cDCE reduction was observed suggesting that some interaction between the Al foil and green rust may have been responsible for the rapid cDCE reduction.

Both PCE and TCE were stable in the presence of aluminum foil and aqueous Fe(II) and we can therefore still make conclusions regarding their reduction and total carbon recovered. Clearly, we caution that aluminum foil should not be used as a septa-liner when working with chlorinated ethenes, but we also speculate that there may be an interesting pathway involving Al foil, or even Al(III) that is worth further investigation.

5. Results and Accomplishments

Despite recent indication from field data that nonbiological mechanisms play a role in the attenuation of chlorinated solvent plumes (Ferrey et al., 2004), it is still not clear what abiotic reductants are responsible. The goal of our SERDP project (ER-1369) was to identify abiotic degradation mechanisms that may contribute to the attenuation of chlorinated solvent plumes. Our approach was to measure the extent and rate of chlorinated ethene reduction by a series of chemically and microbially generated reductants under a range of natural conditions. During the project, we collected, synthesized, and characterized a variety of reductants and measured the extent and rate of PCE, TCE, cDCE, and acetylene reduction in batch reactors. The reductants included:

- Fe(II) sorbed on iron oxides,
- chemically synthesized green rusts,

- green rusts formed in dissimilatory iron reducing (DIR) cultures of *Shewanella* species,
- iron sulfide minerals,
- iron sulfide minerals formed in sulfate reducing bacterial (SRB) culture of *Desulfovibrio desulfuricans*, and
- reduced and sorbed natural organic matter.

5.1 Fe(II) Sorbed on Fe Oxides

Reduced forms of iron are efficient natural reductants found in soil and sediments. Currently, we know of thirteen types of iron oxides (the term “iron oxides” will include both iron oxyhydroxides and oxides). The eight major types and their chemical composition are listed in Table 1 (Schwertmann and Cornell, 1991). Contaminant reduction is often enhanced in natural systems because of the presence of an electron transfer process that is mediated by the cycling of Fe(II) and Fe(III) (Haderlein and Pecher, 1998). We investigated the reaction of PCE, TCE, cDCE, and acetylene with Fe(II) sorbed on the surface of four of the most common iron oxides: lepidocrocite, goethite, magnetite and hematite.

We observed negligible transformation of PCE and TCE over about a one month period with 1 mM aqueous Fe(II) in the presence of hematite, lepidocrocite, goethite, and magnetite (Figure 2 and Table 4). Additional experiments were done with PCE at pH 7.5 and with TCE at pH 7.5 and 8.4 (Table 4). Negligible reduction and formation of products was observed in all cases, with the exception of magnetite and TCE at pH 7.5 and 8.4, where some trace products (< 10% of the total carbon) were observed.

The lack of PCE and TCE reduction by sorbed Fe(II) is consistent with a previous report of only trace (< 10%) PCE and TCE reduction by Fe(II) sorbed on magnetite over a 100 day period (Lee and Batchelor, 2002a). We conducted additional experiments with TCE at higher aqueous Fe(II) concentrations (20 mM) that were closer to the 42.6 mM concentration used in Lee and Batchelor study, and we still observed negligible TCE reduction (Table 4). To verify our experimental reactors, we ran a positive control with Fe(0) metal and observed almost complete removal of TCE with significant product formation (data not shown). It is important to note that a 42.6 mM Fe(II) concentration, or even a 20 mM Fe(II), is significantly above the solubility limit of ferrous hydroxide and is much higher than what would be expected at a field site. At these higher Fe(II) concentrations, significant phase transformations of the oxides has most likely occurred (e.g., lepidocrocite to magnetite), as well as some precipitation of ferrous hydroxide.

Unlike PCE and TCE, we did observe a small loss of cDCE with some product formation in the presence of oxides with sorbed Fe(II) (Figure 2 and Table 4). Similar amounts of products, however, were formed in the presence of oxides alone (in the absence of ferrous iron), which led us to explore whether there was some other reaction occurring that did not involve the sorbed Fe(II). Control experiments with a small ball of aluminum foil (to give 15 g/L Al foil) revealed appreciable cDCE reduction (57%) over a nineteen day period. We used a much smaller amount of aluminum foil to line the septa, but suspect that the about 10% cDCE reduction observed in the sorbed Fe(II) experiment was due to the presence of aluminum foil. Significant cDCE reduction (about 35%) was previously observed with magnetite and high Fe(II) concentrations (Lee and Batchelor, 2002a), which is not consistent with our results of negligible cDCE reduction over about a month.

Our observations suggest that PCE, TCE, and cDCE are relatively stable in the presence of Fe(II) sorbed on hematite, Goethite, lepidocrocite, and magnetite, as well magnetite alone. When we initially started this project, we were surprised that there were no studies on chlorinated ethenes and sorbed Fe(II) (other than the previously mentioned work with high concentrations of Fe(II) and magnetite), but we have since had several personal communications with researchers who indicated that they did indeed try these experiments and observed negligible reduction of the chlorinated ethenes.

5.2. Green Rusts

Green rusts are a class of layered double hydroxides composed of Fe(II)(OH)₂ and Fe(III)(OH)₂⁺ octahedra layers separated by interlayer water molecules and anions, such as carbonate, sulfate, chloride, and hydroxide (Brindley and Bish, 1976). Green rusts are recognized as intermediate phases formed during abiotic- and microbial-induced corrosion of iron metal, reduction of iron oxides (Ona-Nguema et al., 2004), and oxidation of ferrous hydroxide (Chaudhuri et al., 2001). Thermodynamically, green rusts are capable of reducing a variety of environmentally relevant contaminants, and numerous studies have shown that the kinetics of reduction can be quite rapid. The recent identification of green rusts in anoxic soils (Feder et al., 2005), in combination with the rapid kinetics observed in laboratory studies of contaminant reduction, has led us, as well as many others (Genin et al., 2001; Hansen et al., 1994), to suggest that green rusts may play an important role in redox transformations of contaminants in anoxic, iron-rich environments.

We have used both green rusts chemically synthesized in the laboratory, as well as green rusts formed during reductive microbial Fe respiration. Our goal was to better understand the formation of green rusts in field environments and the biogeochemical factors controlling chlorinated ethene reduction by green rusts.

5.2.1 Chemically Synthesized Green Rusts

We observed little reduction of PCE by 5 g/L of chemically synthesized carbonate and sulfate green rust over about a one month time period (Table 5). Some PCE removal was observed, but it was similar to what was observed in the buffer controls and only trace reduction products were observed. At a higher loading of green rust (7 g/L), negligible reduction was still observed with carbonate and chloride green rust, but about 24% of the initial PCE was recovered as unchlorinated gas products (ethene, ethane, and acetylene) indicating some reduction of PCE by sulfate green rust. The 20 to 40% loss of PCE is consistent with the 40% loss previously reported in the presence of sulfate green rust (Lee and Batchelor, 2002b). In the previous study, the 40% loss was also accompanied by incomplete carbon recovery, similar to what we observed here.

Initial experiments with TCE showed negligible reduction by carbonate and sulfate green rusts over a month time period (data not shown). Additional experiments run for a longer time period and at higher green rust concentrations of 5 and 10 g/L, however, revealed some TCE removal. Dechlorinated gas products (Σ ethene + ethane + acetylene) were observed indicating reduction did take place. Carbon recoveries varied from 48 to 89% (Table 5). TCE half-lives were similar with carbonate and sulfate green rust and were on the order of about 3 to 4 weeks (Figure 3 and 4A), which is reasonably similar to a previous report of TCE reduction by 7 g/L sulfate green rust (Lee and Batchelor, 2002b). Here though, we observed a larger percent removal of TCE and more product recovery than was previously observed, but that is most likely

due to the lower initial TCE concentration used in our study (60 to 80 μM compared to 300 μM in the previous study).

In contrast to PCE and TCE, we observed rapid reduction of cDCE by green rusts, however, we suspect that an interaction between the Al foil (used to line the septa) and the green rust may have played a role. In the presence of carbonate and chloride green rust, cDCE was almost completely removed in less than two days. Carbon recoveries ranged from 28 to 79% as dechlorinated gas products, indicating a significant amount of reduction had occurred (Figure 5). A comparison of the kinetics of cDCE removal is shown in Figure 6. Interestingly, cDCE reduction by sulfate green rust was significantly slower than both chloride and green rust. The half-life of about one month is similar to what was previously observed by Lee and Batchelor for cDCE with sulfate green rust (Lee and Batchelor, 2002b).

A comparison of rates of reduction of TCE, cDCE, and acetylene by carbonate green rust is shown in Figure 8 and highlights the fairly rapid removal of cDCE and acetylene compared to the slower kinetics of TCE reduction.

5.2.2 Formation of Green Rusts in Presence of Dissimilatory Iron Reducing Bacteria

Although several laboratory studies have reported reduction of chlorinated ethenes by reduced iron precipitates, including iron sulfides, sulfate green rust, and adsorbed ferrous species (Butler and Hayes, 1999) (Lee and Batchelor, 2002a; Lee and Batchelor, 2002b), there is little data available on these same iron species formed in the presence of anaerobic bacteria. During this project, we examined the potential for green rust formation resulting from the bioreduction of lepidocrocite ($\gamma\text{-FeOOH}$) by a series of *Shewanella* species and explored whether these “biogenic” green rusts were capable of reducing perchloroethene (PCE) and trichloroethene (TCE).

We examined the potential for green rust formation by a series of *Shewanella* species (*S. alga* BrY, *S. amazonensis* SB2B, *S. baltica* OS155, *S. denitrificans* OS217T, *S. loihica* PV-4, *S. oneidensis* MR-1, *S. putrefaciens* ATCC 8071, *S. putrefaciens* CN32, *S. saccharophila*, and *Shewanella* sp. ANA-3). All *Shewanella* species, with the exception of *S. denitrificans* OS217T, were able to couple the oxidation of formate to the reduction of Fe(III) in lepidocrocite; however there were significant differences among species with respect to the rate and extent of Fe(II) production. Despite these differences, green rust was the only Fe(II)-bearing solid phase formed under our experimental conditions, as indicated by X-ray diffraction, Mössbauer spectroscopy, and scanning electron microscopy. The formation of green rust by *Shewanella* species isolated from a wide range of habitats and possessing varied metabolic capabilities suggests that under favorable conditions biogenic green rusts may be formed by a diverse array of DIRB. This work has been published in *Geomicrobiology Journal* (2007).

5.2.3 Chlorinated Ethene Reduction in Dissimilatory Iron Reducing Bacteria (DIRB) Cultures

We also explored whether reduced iron precipitates formed in the presence of dissimilatory iron reducing bacteria (DIRB) can degrade perchloroethene (PCE) and trichloroethene (TCE). Several cultures of *Shewanella putrefaciens* CN32 were incubated with lepidocrocite as an electron acceptor and lactate as the electron donor following a previously published protocol (Ona-Nguema et al., 2002; Ona-Nguema et al., 2004). The lepidocrocite was completely consumed over about a month time period and resulted in the formation of carbonate green rust as identified by XRD and Mössbauer spectroscopy. The spectra reveal both a ferrous and ferric component with Mössbauer parameters that are consistent with a green rust structure

(Figure 7). XRD patterns of the same precipitates indicate the green rust is the carbonate form (data not shown).

Reduction of TCE was observed in three separate whole cultures of *S. putrefaciens* CN32 (Figure 8). Whole cultures which included cells, media, and the precipitated carbonate green rust. Upwards of 90% of the TCE added to the culture after green rust formation was removed over a one month time period. The major products formed were dechlorinated gas products that accounted for between 20 and 80% of the initial TCE added. No formation of dichlorethenes, or vinyl chloride was observed. Preliminary data with a pasteurized culture (heating at 70°C for one hour) showed similar reduction of TCE and suggest that abiotic processes, such as reduction by the carbonate green rust, may be responsible (data not shown).

In an attempt to separate out contributions to chlorinated ethene reduction from various culture components (*Shewanella putrefaciens* CN32, precipitated green rust, any aqueous materials) we ran two sets of experiments using killed whole cell cultures and various washing and separation procedures (Tables 6 and 7). In the first set, summarized in Table 6 and Figure 9, reactors were designed using whole cell cultures killed with either (i) sodium azide or (ii) chloramphenicol, (iii) washed precipitated green rust, and (iv) washed and (v) washed freeze-dried precipitated carbonate green rust, all resuspended in 25 mM TAPS buffer (pH 7.5). Supernatant (vi) after sonication and centrifugation was also tested without any modification.

Reactors containing inactivated cultures (chloramphenicol, azide) showed no signs of TCE reduction (TCE concentration stayed constant while no detectable quantities of reduction products were generated). TCE concentration also remained constant in reactors containing washed green rust. TCE disappearance was observed in reactors containing freeze-dried green rust (unwashed or washed) and sonicated / centrifuged supernatant (Figure 9). All three reactors that had appreciable TCE loss however had only about 50% conversion to products (48-66%) (Table 6). Supernatant was also unreactive with PCE and cDCE (data not shown). While intended to isolate the contributions of various whole cell culture components to TCE reduction, these experiments actually showed that none of these individual parts is wholly responsible. In fact, it appears that even the sum of the components (green rust and cell culture supernatant) could not account for the capacity for reduction observed in Figure 8. In any reactors where the supernatant was removed (washed solids, freeze dried solids, azide and chloramphenicol), reduction products amounted to no more than 10% of initial TCE (Table 6). Similar amounts of reduction products were detected in reactors containing cell culture supernatant.

Since the first set of experiments showed little reactivity of the washed biogenic green rust with TCE, we conducted a second set of experiments. The goal of these experiments was to evaluate whether there was indeed an abiotic component that was contributing to TCE reduction, and whether our washing procedure was responsible for the lack of green rust reactivity. We tried several different washing procedures, in addition to a “killed” whole culture (autoclave, 2-heltyl-4hydroxyguinoline N-oxide) and several supernatant controls (Table 7). Similar to our first set of experiments, we observed significant TCE reduction with gas products accounting for between 70 and 80% of the TCE added for three different cultures (Table 7 and Figure 10). With the washed green rust, we observed only minor (2 to 14%) formation of TCE reduction products, despite our extensive efforts to preserve the carbonate green rust. Less products were formed in the killed whole cultures (about 50% of the initial TCE), but enough to indicate that there is indeed a significant abiotic component contributing to the TCE reduction. Surprisingly, we also observed 42% product recovery in the sterile medium containing lepidocrocite and the culture media (which includes AQDS). The significant product recovery in

the sterile medium with lepidocrocite and the lack of reactivity of the washed carbonate green rust suggests that the biogenic green rust is not responsible for the observed abiotic component. At this time it is unclear what is responsible for the abiotic component, but interestingly, when the sterile medium is filtered to remove the lepidocrocite, no products are formed, indicating that there is some heterogeneous chemistry at work.

Although most of the experiments with DIRB cultures were conducted with TCE, we did also observe PCE reduction in a whole DIRB culture containing carbonate green rust (Figure 11). The kinetics of PCE reduction were significantly slower than that of TCE, with only about half of the PCE removed after a month. Significant gas products were recovered, but mass balances significantly exceeded 100%, indicating some analytical difficulties.

5.3. Iron Sulfides

5.2.1 Chemically Synthesized Iron Sulfides

Reduction of chlorinated ethenes by iron sulfides, such as mackinawite and pyrite, has been reported in several previous studies (Butler and Hayes, 1999; Butler and Hayes, 2001; Lee and Batchelor, 2002a; Sivavec et al., 1995; Weerasooriya and Dharmasena, 2001). Consistent with these studies, we observed reduction of TCE to acetylene in the presence of mackinawite (Figure 12). For the conditions used here (pH 7.5 and 10 g/L FeS), TCE was completely removed over one month.

5.2.1 Chlorinated Ethene Reduction in Sulfate Reducing Bacteria (SRB) Cultures

Although several laboratory studies have reported reduction of chlorinated ethenes by chemically synthesized iron sulfides (Butler and Hayes, 1999; Butler and Hayes, 2001; Lee and Batchelor, 2002a; Sivavec et al., 1995; Weerasooriya and Dharmasena, 2001), there is little data available on these same iron species formed in the presence of anaerobic bacteria. Abiotic reduction by iron sulfides have been implicated as an important mechanism for reduction of chlorinated aliphatics (Kenneke and Weber, 2003) and recently, the addition of sulfate to stimulate Biogeochemical Reductive Dechlorination (BiRD), has been proposed as an alternative treatment strategy for chlorinated ethenes (Kennedy et al., 2006b). A recent field-scale demonstration of BiRD at Dover Air Force Base confirmed the formation of iron sulfides after injection of sulfate and reported 95% removal of PCE, TCE, and cDCE in less than one year (Kennedy et al., 2006a). Here we explore the mechanism of TCE reduction by iron sulfides formed in the presence of sulfate reducing bacteria.

A pure culture of *Desulfovibrio desulfuricans* was maintained in Modified Baar's Medium for Sulfate Reducers (ATCC #1249) utilizing lactate as the electron donor, and sulfate as the electron acceptor. During the initial set of experiment, 1 g/L ferrous iron was added as ferrous ammonium sulfate (FAS) to induce precipitation of FeS. Lower concentrations of FAS were tried, but would not result in appreciable FeS formation.

Results from ^{57}Fe Mössbauer spectroscopy and X-ray diffraction indicate the presence of both mackinawite (FeS) and an Fe(II)-sulfate phase in precipitates formed when 1.0 g/L of FAS was added to a sulfate reducing culture. All three characterizations (Mössbauer, XRD, SEM) show distinct differences between the solids depending on whether they were formed in the presence or absence of sulfate reducing bacteria. Based on XRD patterns, the FeS formed in the presence of SRB appears more amorphous and has a higher tendency to aggregate than the crystalline chemically synthesized FeS. This is consistent with previous observations of FeS

precipitated in the presence of SRB (Herbert et al., 1998). The solids formed in the presence of SRB also have a higher surface of 35 m²/g compared to about 8 m²/g for chemically synthesized FeS.

Despite these differences, the majority of the Fe(II) in both exists as FeS. Negligible removal, however of both TCE and PCE was observed in the presence of the whole SRB culture (cells, media, and the FeS precipitate formed from the addition of 1 g/L FAS) or washed FeS formed in the SRB culture (shown for TCE in Figure 13). The limited PCE and TCE reduction observed in the presence of the whole SRB culture was rather unexpected based on the results of mineral characterization. The presence of an additional Fe(II) phase, or a lower relative abundance of FeS in the SRB cultures could explain a slower rate of reduction, but is unlikely to cause a complete loss of reactivity. Taking into consideration the specific surface area of biologically produced FeS (35.3 m²/g) relative to the chemically synthesized (8.0 m²/g), this seems especially true. Interestingly, the freeze-dried whole culture did reduce TCE,

A potential explanation for the lack of reactivity of the biogenic FeS is formation of a surface precipitate (such as the Fe sulfate phase observed in XRD) that passivates the FeS surface. If this is the case, it could also explain the increase in specific surface area, and the different appearances of the two iron sulfides under high magnification. Another potential explanation is the distinct differences in the FeS crystallinity in the absence and presence of SRB. This apparent lack of crystal structure, possibly combined with surface accumulation of other Fe(II) species may explain the differences in reactivity observed between the chemically produced and biologically produced iron sulfides. Other possibilities include the accumulation of cellular exudates on the mackinawite surface, which could block reactive sites or some sort of artifact from the batch reactor design and addition of Fe(II) following FeS formation. These compounds should be removed by washing and freeze-drying, however it is possible that some may remain associated with the solids surface. Interestingly, we did observe some removal, but only minor product formation, in the presence of the whole culture once it was freeze-dried (Figure 13).

To evaluate whether the high concentration of FAS added was responsible, we ran a second set of experiments (with a new culture of *Desulfovibrio desulfuricans*) and added ferrous iron as both FeCl₂. With the addition of ferrous iron as FeCl₂, we did observe reduction of TCE to acetylene as shown in Figure 14. As a control, we use the same culture and added FAS and again, observed no reduction of TCE (Figure 15). Characterization of the solids also revealed the presence of some Fe(II) precipitate other than mackinawite when FAS was added and we suspect that this precipitate was responsible for the inhibition. A comparison of the kinetics of TCE removal in the presence of a similar mass loading of chemically synthesized FeS reveals that the biogenic FeS is a little slower. Images of the chemically synthesized FeS and the biogenic FeS are shown in Figures 16 and 17 and it is clear that the biogenic FeS surface coated with material and is more heterogeneous than the chemically synthesized FeS.

A last set of experiments was done to try to determine what part of the SRB culture might be responsible for the slower TCE reduction rate observed in Figure 15. Chemically synthesized FeS was added to the whole SRB culture, as well as isolated components of the SRB culture (Table 8 and Figure 18). Addition of FeS to any component of the SRB culture after incubation resulted in some inhibition, including the filtered supernatant, which suggests that potentially some soluble microbial exudate is responsible. Note that the sterile medium before incubation with SRB completely inhibited the chemical FeS, most likely due to the high sulfate concentrations in the medium.

5.4. Natural Organic Matter

Natural Organic Matter (NOM) has been suggested as a possible reductant for chlorinated organic molecules, serving either as a direct source of electrons or as an indirect source of reducing power via some sort of electron shuttling mechanism. The reactivity of NOM associated with iron oxide surfaces was explored, as well as the interaction between previously reduced NOM and chlorinated ethenes such as PCE, TCE, and c-DCE.

We have observed no reduction of PCE, TCE or cDCE with the Little Gratiot NOM in the absence and presence of goethite and Fe(II). Data for PCE is shown in Figure 19. Additional experiments were conducted with AQDS as a model NOM. Reduction of AQDS to AHDS was monitored with UV/vis spectroscopy, and a noticeable shift could be seen after bubbling hydrogen gas in the presence of our Pd catalyst. As a preliminary test, of the effectiveness of this reduction method, we decided to use our reduced AHDS to replicate previous studies showing the reduction of hexachloroethane (HCA) to PCE in the presence of AHDS (Curtis and Reinhard, 1994). This experiment was conducted in reactors sealed with only Teflon-coated rubber septa, and, as expected we observed the reduction of HCA to PCE (Figure 20). As reported in the previous work, the PCE remains fairly stable, indicating that it is most likely not reactive with AHDS. Follow up experiments with AHDS in the presence of TCE failed to stimulate reduction of TCE. Reduced forms of Aldrich HA and NOM were also exposed to TCE with similar results over the course of three weeks. All three reduced organics were also used as potential reductants in batch reactors with cDCE, and no significant reduction was observed over the course of four weeks. It does not appear that reduced NOM or AHDS are capable of reducing chlorinated ethenes under the conditions we explored in this work. The complexity of NOM, however, makes it difficult to extrapolate this conclusion to all other forms of NOM.

6. Conclusions

Based on the results of this project, we make the following conclusions:

1. Reduction of chlorinated ethenes by magnetite and sorbed Fe(II) are not likely significant abiotic natural attenuation pathways in Fe reducing environments. Rather, we suspect that green rusts, which are often transformed to magnetite are more likely candidate for chlorinated ethene reduction.
2. Reduction of chlorinated ethenes by iron sulfides is indeed rapid and results in the formation of acetylene. The iron sulfides produced in the presence of SRB, however, tend to be coated with cellular or precipitate material and may result in some inhibition. Despite the presence of these coatings, TCE reduction to acetylene was still observed and the recently demonstrated “BiRD” technology is a promising alternative remediation strategy.
3. There appear to be additional abiotic reductive dechlorination pathways beyond Fe(II) driven reduction that may be occurring during dissimilatory iron reduction.
4. “Abiotic” natural attenuation is a complex biogeochemical process and the reactivity of precipitates formed in the presence of bacteria is difficult to estimate based on the reactivity of their chemically synthesized analogs.
5. Despite our current results from this project that shows little reactivity of adsorbed or reduced NOM with chlorinated ethenes, we still suspect that there is some, yet unidentified, redox reactions between NOM and the oxides that may contribute to abiotic

attenuation of chlorinated ethenes.

Table 1. The major iron oxides and their chemical formula.

Mineral	Formula	Mineral	Formula
Goethite	α -FeOOH	Ferrihydrite	$\text{Fe}_5\text{HO}_8 \bullet 4\text{H}_2\text{O}$
Akaganeite	β -FeOOH	Hematite	α -Fe ₂ O ₃
Lepidocrocite	γ -FeOOH	Maghemite	γ -Fe ₂ O ₃
Feroxyhyte	δ' -FeOOH	Magnetite	Fe ₃ O ₄

Source: Schwertmann, U., and Cornell, R.M. (1991) *Iron Oxides in the Laboratory*. Weinheim: VCH.

Table 2. Chemical and physical properties of chlorinated ethenes and reduction products.

Compound	MW (g/mole)	Density (g/mL)	Henry's Coefficient ^{a,b} (mol L ⁻¹ gas / mol L ⁻¹ aqueous)	MCL ^c (mg/L)	K _{oc} ^d (mL/g)	Solubility ^d (S, mg /L)	MDL ^e (μM)
PCE	165.8	1.623	0.545	0.005	364	200	0.02
TCE	131.4	1.462	0.296	0.005	126	1100	0.04
cis-1,2- DCE	96.9	1.284	0.122	0.070	86	3500	0.06
trans-1,2- DCE	96.9	1.257	0.300	0.100	59	6300	0.02
1,1-DCE	96.9	1.213	0.857	0.007	65	3350	N/A
VC	62.5	0.911	0.903	0.002	56	2700	0.16
Acetylene	26.0	--	--	--	--	--	0.85
Ethane	30.1	--	--	--	--	--	1.58
Ethene	28.1	--	--	--	--	--	0.46

^aDimensionless Henry's coefficients are calculated for 20°C

^bSource: {Sander, 2004 #71}

^cSource: {USEPA, 2005 #56}

^dValues for vinyl chloride obtained from {USEPA, 2005 #73}. Other values obtained from {Pankow, 1996 #70}

^eMethod detection limits calculated using USEPA MDL calculation for 7 samples.

Table 3. ATCC medium #1249 (Modified Baar's medium for sulfate reducers)

Compound	Concentration in Stock Reactor (mM)
MgSO ₄	166
sodium citrate	19
CaSO ₄	7
NH ₄ Cl	19
K ₂ HPO ₄	3
sodium lactate	31
yeast extract	1.0 (g/L)

Table 4: Percent Carbon mass recoveries for chlorinated ethene reaction with sorbed Fe(II)

<u>Mineral</u>	<u>Product</u>	<u>PCE</u> ^b	<u>TCE #1</u> ^c	<u>TCE #2</u> ^d	<u>TCE #3</u> ^e	<u>cDCE</u> ^f
Lepidocrocite	PCE	100	–	–	–	–
	TCE	1	105	100	119	–
	cDCE	<1	<1	<1	<1	81
	gas products ^a	<1	<1	1	1	10
	Total	101	105	101	120	91
Goethite	PCE	90	–	–	–	–
	TCE	2	103	86	137	–
	cDCE	<1	<1	<1	<1	77
	gas products ^a	<1	<1	2	1	8
	Total	92	103	88	138	85
Hematite	PCE	108	–	–	–	–
	TCE	–	93	113	107	–
	cDCE	<1	<1	<1	<1	71
	gas products ^a	<1	<1	1	<1	5
	Total	108	93	114	107	76
Magnetite	PCE	101	–	–	–	–
	TCE	<1	108	107	111	–
	cDCE	<1	<1	<1	<1	80
	gas products ^a	<1	1	5	4	15
	Total	102	109	112	115	95

^a Gas products is the sum of observed ethene, acetylene, and ethane. Vinyl chloride is also included, but it was only observed in the samples with cDCE. Gas products cannot be speciated due to reaction of aluminum foil (used to line septa) with acetylene as discussed in Materials & Methods.

^b [PCE]₀ = 0.1mM. [Fe(II)]₀ = 10mM. Solids loading = 5 g/L. pH₀ = 7.5. Buffer: 15mM NaHCO₃

^c [TCE]₀ = 0.1mM. [Fe(II)]₀ = 20mM. Solids loading = 5 g/L. pH₀ = 7.0. Buffer: 20 mM KBr/MOPS

^d [TCE]₀ = 0.1mM. [Fe(II)]₀ = 10mM. Solids loading = 5 g/L. pH₀ = 7.5. Buffer: 20 mM KBr/HEPES

^e [TCE]₀ = 0.1mM. [Fe(II)]₀ = 10mM. Solids loading = 5 g/L. pH₀ = 8.4. Buffer: 20 mM KBr/TAPS

^f [cDCE]₀ = 0.1mM. [Fe(II)]₀ = 10mM. Solids loading = 5 g/L. pH₀ = 7.5. Buffer: 15mM NaHCO₃

Table 5: Percent carbon mass recoveries for chlorinated ethene reaction with green rusts.

Green Rust	Product	PCE #1 ^b	PCE #2 ^c	TCE #1 ^d	TCE #2 ^e	cDCE #1 ^f	cDCE #2 ^g
Sulfate	PCE	62	36	-	-	-	-
	TCE	<1	<1	82	26	-	-
	cDCE	<1	<1	<1	<1	47	-
	Gas Products ^a	2	24	7	51	36	-
	Total	65	60	89	77	83	-
Carbonate	PCE	46	58	-	-	-	-
	TCE	<1	<1	48	24	-	-
	cDCE	<1	<1	<1	<1	12	9
	Gas Products ^a	<1	2	9	24	43	28
	Total	47	61	57	48	55	37
Chloride	PCE		57			-	
	TCE		1			-	
	cDCE		<1			<1	
	Gas Products ^a		4			79	
	Total		61			80	

^aGas products is the sum of observed ethene, acetylene, and ethane. Vinyl chloride is also included, as was only observed in the samples with cDCE. Products cannot be distinguished individually due to problems with Al foil lined septa as discussed in Materials & Methods.

^b[PCE]₀ = 0.1 mM. [GR] = 5 g/L. pH₀ = 8.3. Buffer: 20 mM NaHCO₃. t = 26 dy (SLS)

^c[PCE]₀ = 0.1 mM. [GR] = 7 g/L. pH₀ = 7.0. Buffer: 10 mM NaHCO₃. t = 33 dy (SLS)

^d[TCE]₀ = 0.1 mM. [GR] = 5 g/L. pH₀ = 8.3. Buffer: 20 mM NaHCO₃. t = 26 dy (SLS)

^e[TCE]₀ = 0.1 mM. [GR] = 10 g/L. pH₀ = 8.2. Buffer: 20 mM NaHCO₃. t = 60 dy (HAA)

^f[cDCE]₀ = 0.1 mM. [GR] = 5 g/L. pH₀ = 8.3. Buffer: 20 mM NaHCO₃. t = 26 dy for sulfate and carbonate, 5 days for chloride (SLS)

^g[cDCE]₀ = 0.1 mM. [GR] = 10 g/L. pH₀ = 8.2. Buffer: 20 mM NaHCO₃. t = 11 hr (HAA)

Table 6 Carbon mass balances for TCE reduction by carbonate green rust precipitated in the presence of *Shewanella putrefaciens* CN32 culture components isolated via physical or chemical means. (TSP)

<u>Treatment</u>	<u>Products</u>	<u>Concentration (μM)</u>	<u>Mass Recovery as %</u>
Freeze Dried	TCE Initial	48	-
Unwashed	TCE Final	27	55
	Products ^a	5	12
	Total	-	67
Freeze Dried	TCE Initial	59	-
Washed	TCE Final	24	41
	Products ^a	9	16
	Total	-	57
Washed	TCE Initial	48	-
	TCE Final	41	86
	Products ^a	3	21
	Total	-	107
Azide (Biocide)	TCE Initial	46	-
	TCE Final	41	92
	Products ^a	10	15
	Total	-	107
Chloramphenicol (Biocide)	TCE Initial	39	-
	TCE Final	43	92
	Products ^a	7	15
	Total	-	107
Supernatant	TCE Initial	50	-
	TCE Final	12	25
	Products ^a	10	19
	Total	-	44

^a Products is the sum of observed ethene, acetylene, and ethane. No measurable cDCE was observed.

Table 7 Product formation for TCE reduction by carbonate green rust precipitated in the presence of Fe-reducing bacteria treated with various means. Samples were taken at 50 days, except where noted. Solids were suspended in 25 mM MOPS pH 7.2 when applicable. (CAG)

<u>Experiment</u>	<u>Conditions</u>	<u>Total Products^a</u> (μ M)	<u>Mass Recovery</u> as %
Whole Culture	<i>S. putrefaciens</i> CN32	56	70
	<i>S. oneidensis</i> MR-1 ^b	64	80
	<i>S. alga bry</i> ^c	55	69
	Sterile medium	34	42
Treated Whole Culture	CN32 Autoclaved 1 hr	34	45
	CN32 with HQNO ^d	42	53
	<i>S. alga bry</i> exposed to γ ^e	58	72
Washed Solids ^f	MR-1 washed in MOPS	11	14
	CN32 A in MOPS	0	0
	CN32 B in MOPS	3	4
	CN32 A in DI	6	7
	CN32 B in DI	2	2
Controls	25 mM MOPS	0	0
	Sterile Medium Supernatant	0	0
	CN32 A Supernatant ^c	12	16
	CN32 B Supernatant	15	18
	CN32 A Supernatant Autoclaved ^c	1	1
	CN32 B Supernatant Pasteurized ^g	9	11

^a Products is the sum of observed ethene, acetylene, and ethane. No measurable cDCE was observed.

^b Sample take at day 31

^c Sample take at day 38

^d Culture spiked with 50 μ M HQNO (2-heltyl-4-hydroxyquinoline N-oxide) 2 days prior to starting the experiment.

^e Culture exposed to γ -radiation by ²⁴¹Am (550 mCi) source for 1 hr.

^f Samples washed in MOPS were suspended in 25 mM MOPS, pH 7.2 in each

Table 8 Carbon mass balances for TCE reduction by 10 g/L chemically synthesized FeS in the presence of several biological conditions. pH = 8.3. Final measurements were taken after 79 days.

<u>Solution Conditions</u>	<u>Products</u>	<u>Concentration (μM)</u>	<u>Mass Recovery as %</u>
25 mM MOPS	TCE Initial	62	-
	TCE Final ^b	0	0
	Products ^a	55	89
	Total	-	89
Whole Culture	TCE Initial	48	-
	TCE Final	7	15
	Products ^a	50	104
	Total	-	119
Whole Culture spiked with 0.2 g/L FeSO ₄ prior to addition of chemical FeS	TCE Initial	64	-
	TCE Final	3	5
	Products ^a	49	77
	Total	-	82
Culture Supernatant	TCE Initial	56	-
	TCE Final	4	7
	Products ^a	66	118
	Total	-	125
Sterile Medium	TCE Initial	60	-
	TCE Final	44	73
	Products ^a	15	25
	Total	-	98

^a Products is the sum of observed ethene, acetylene, and ethane. No measurable cDCE was observed.

^b All TCE was removed by day 4.

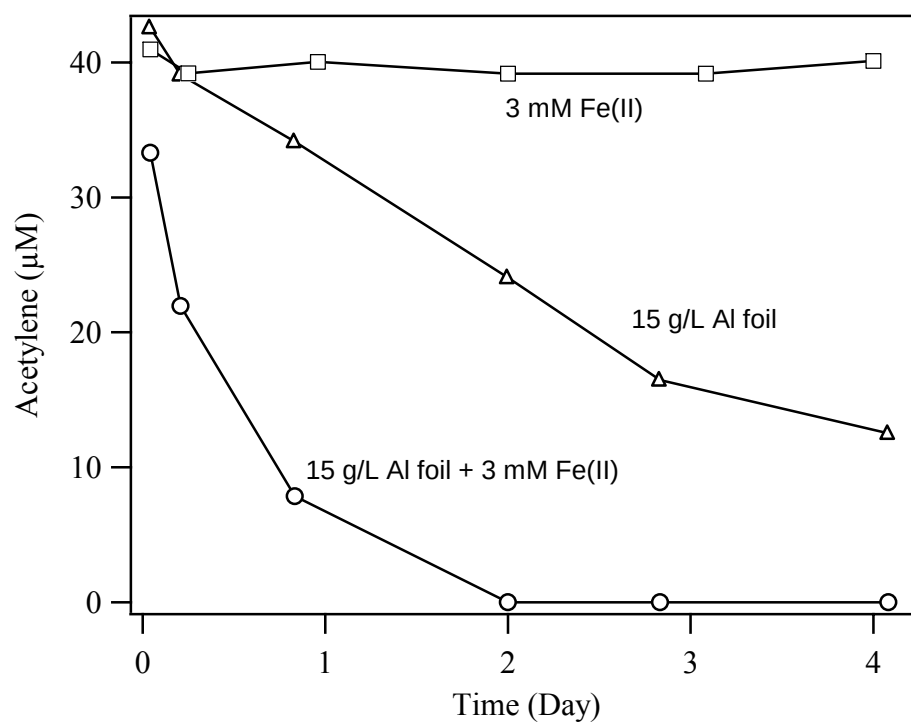


Figure 1. Acetylene disappearance in presence of aluminum foil and aqueous Fe(II). The aluminum foil was added as a ball of aluminum foil (15 g/L). Between 80 and 100% of the acetylene was recovered as ethane and ethene.

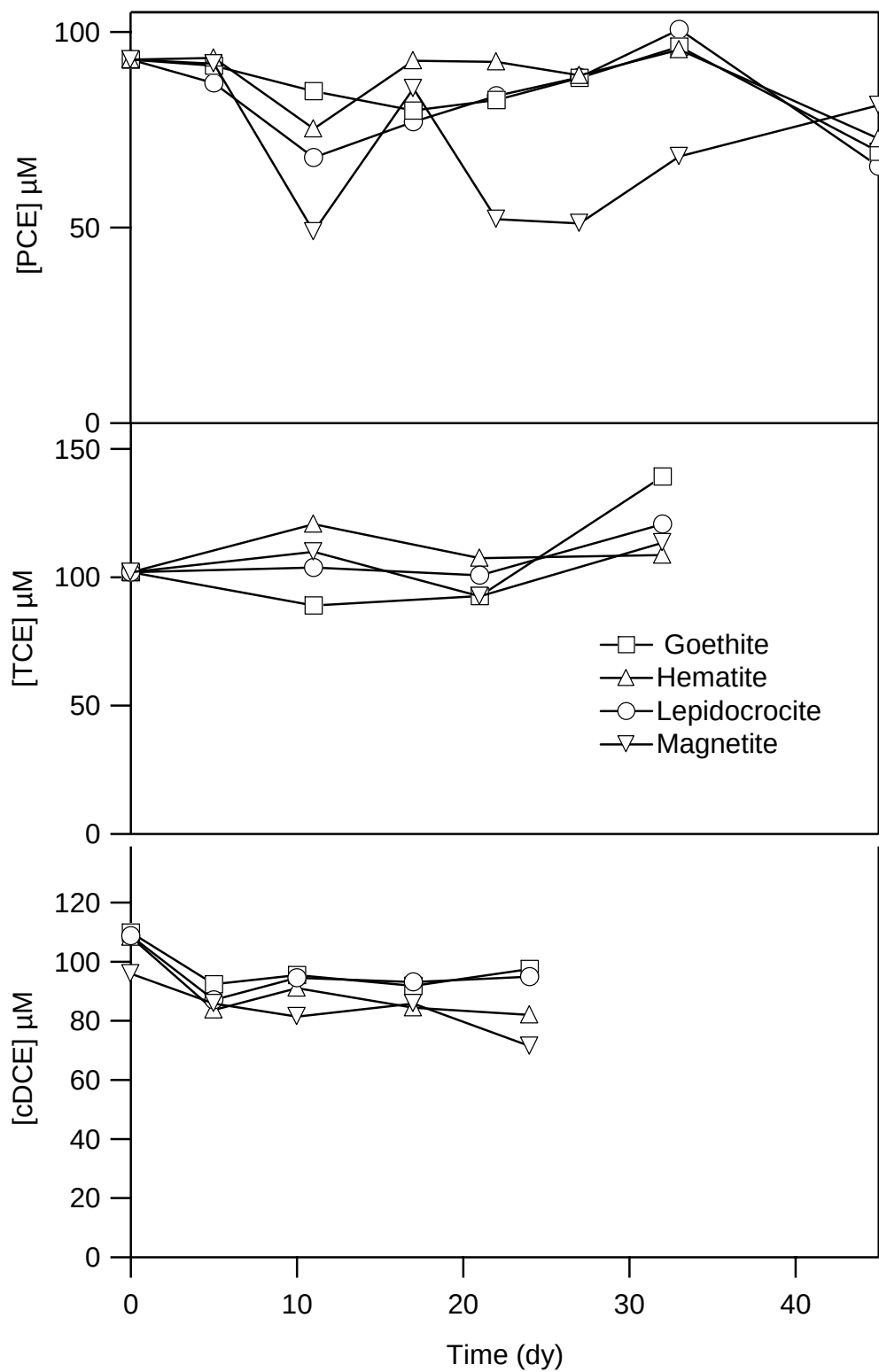


Figure 2. PCE, TCE, and cDCE concentration over time in batch reactors containing 5 g L⁻¹ of iron oxide with 10 mM aqueous Fe(II) added. Experimental details are given in Table 2 for PCE, TCE #3, and cDCE.

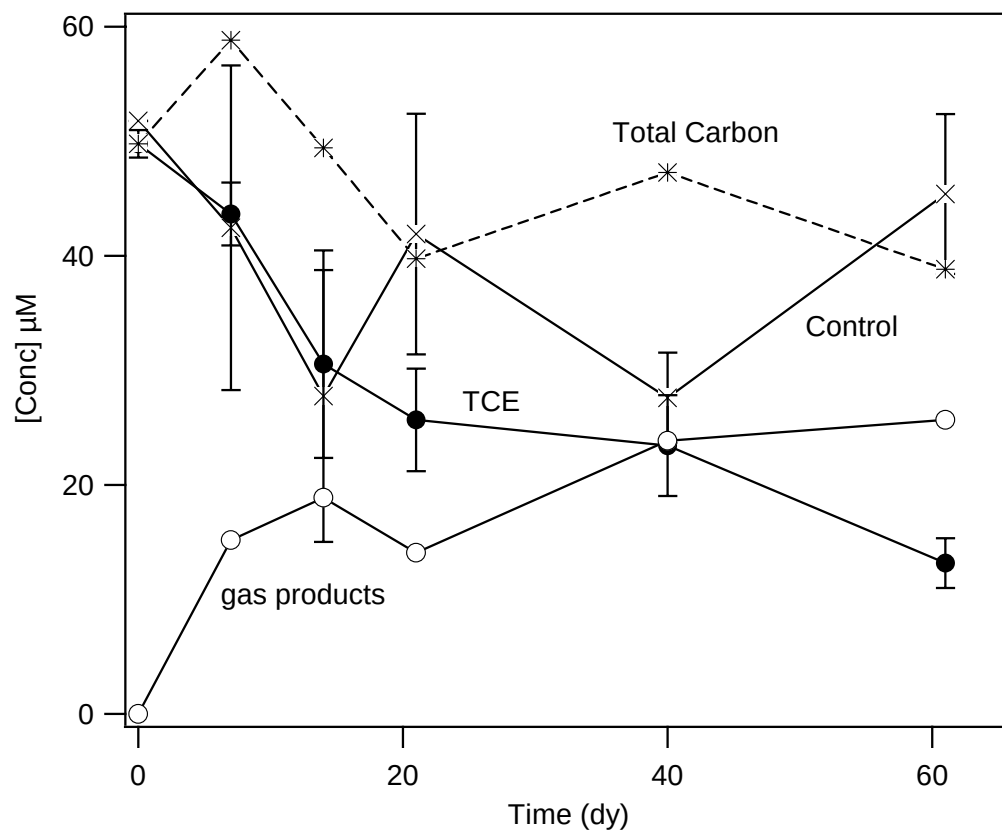


Figure 3. Product formation from TCE reduction in the presence of sulfate green rust. Experimental conditions: $[\text{TCE}]_0 = 50 \mu\text{M}$, $[\text{TCE}]_i = 51 \mu\text{M}$; 10 g/L green rust, 20 mM NaHCO_3 solution, Initial pH = 8.2. (HAA)

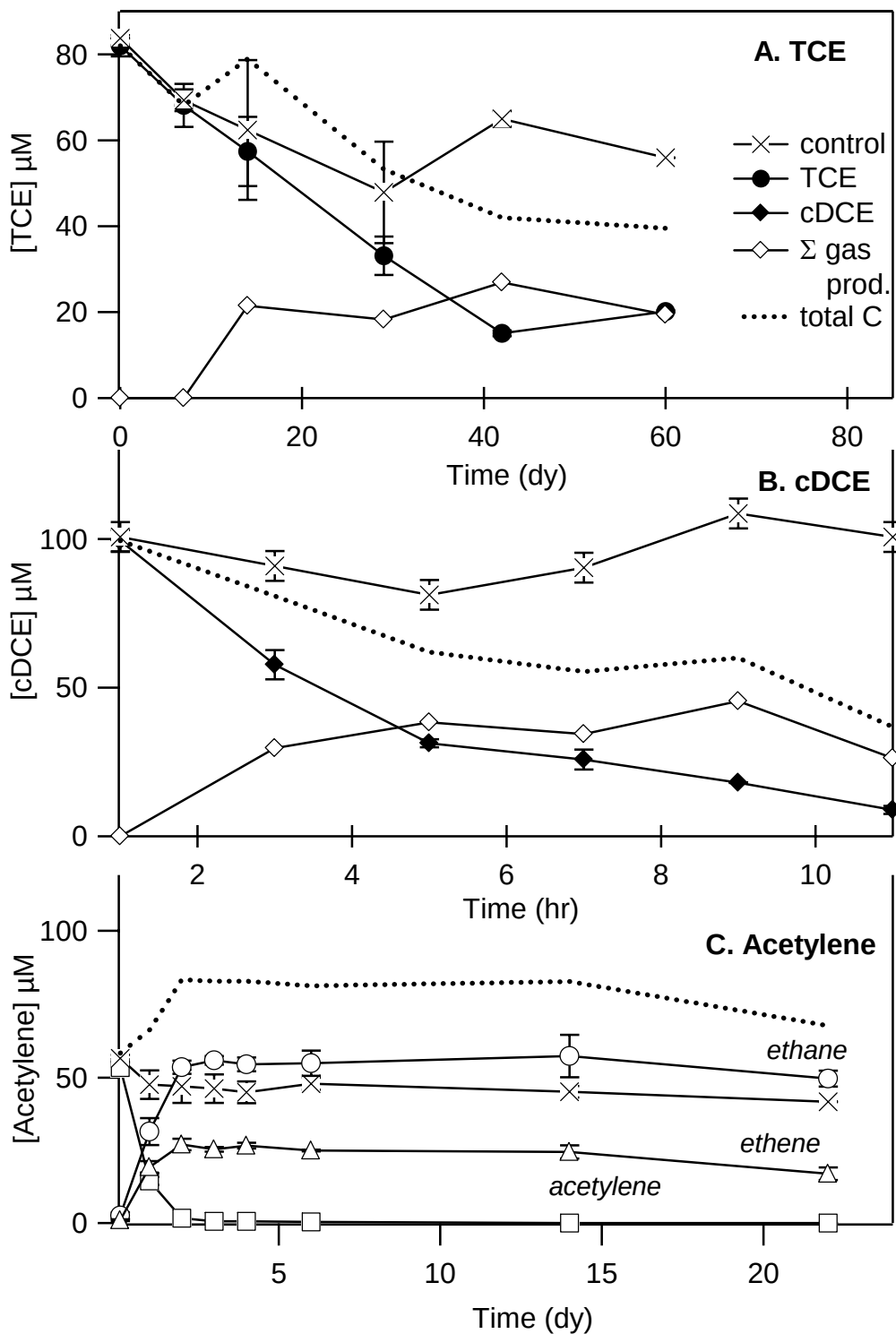


Figure 4. TCE, cDCE and acetylene reduction by carbonate green rust. Experimental conditions: 10 g/L green rust, 20 mM NaHCO₃, at pH 8.2. Note that we suspect that an interaction between the Al foil and the green rust may play a role in the cDCE and acetylene reduction observed here (HAA)

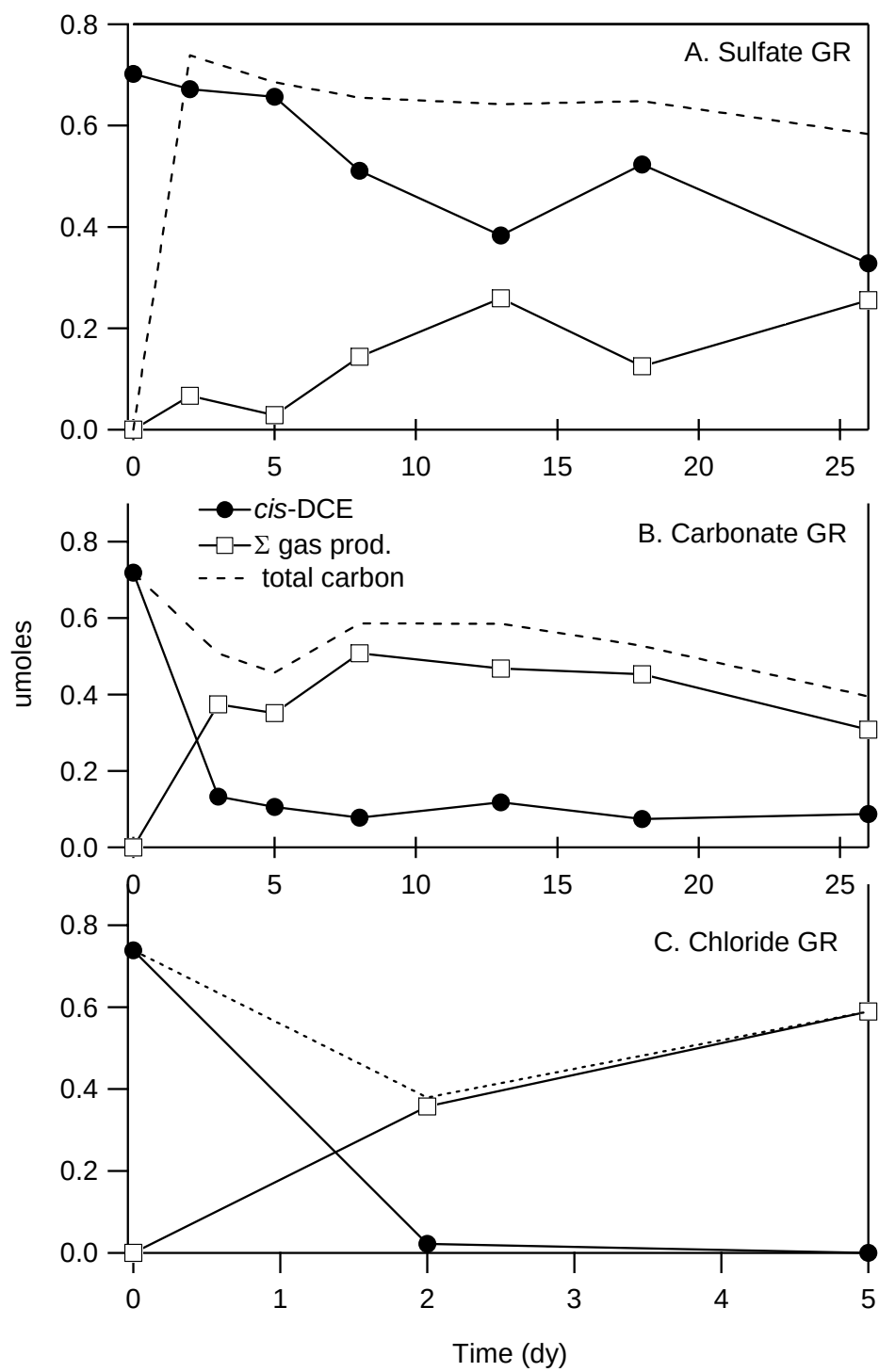


Figure 5. cDCE reduction by 5 g L⁻¹ green rust. Reactors contained 20 mM NaHCO₃ at pH 8.3. Note that we suspect that an interaction between the Al foil and the green rust may play a role in the cDCE reduction observed here (SLS)

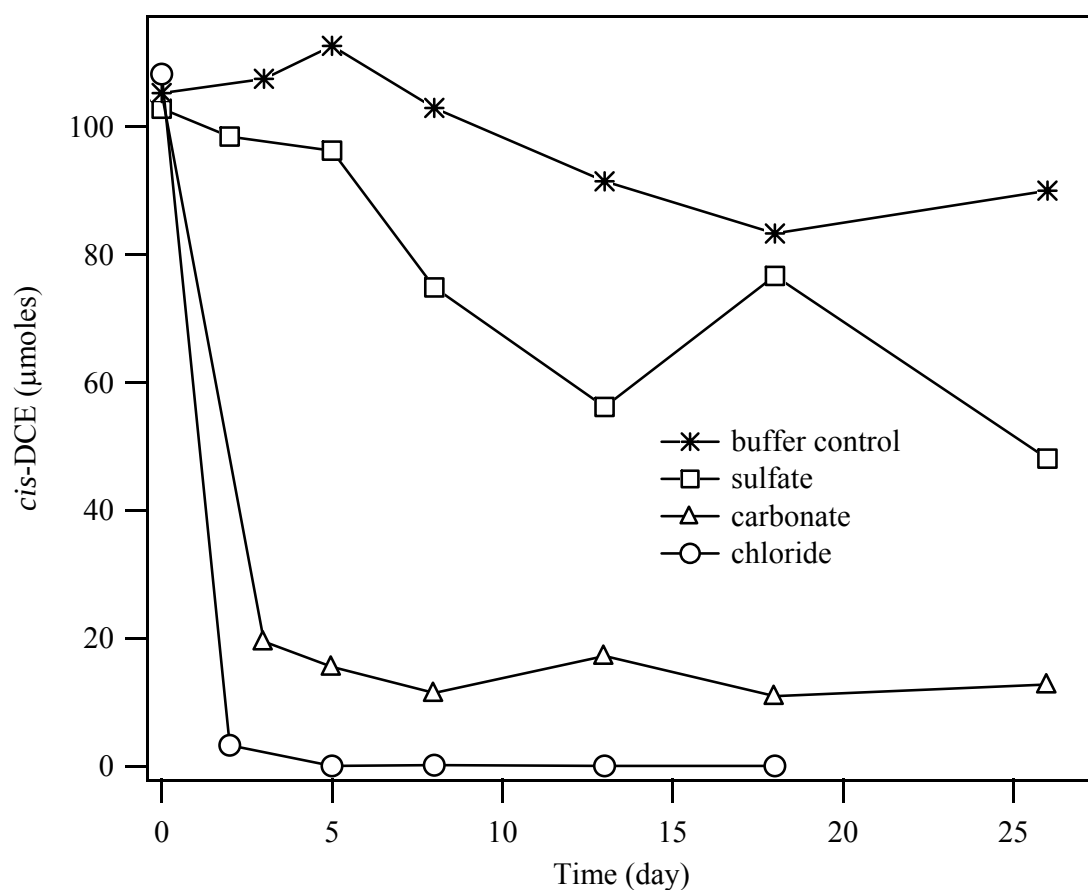


Figure 6. Comparison of cDCE disappearance in the presence of sulfate, carbonate, and chloride green rust in reactors containing Al foil lined septa. Note that we suspect that an interaction between the Al foil and the green rust may play a role in the cDCE reduction observed here. Experimental conditions: [cDCE]₀ = 0.1 mM, 5 g L⁻¹ green rust and 20 mM NaHCO₃ solution at initial pH 8.3. (SLS)

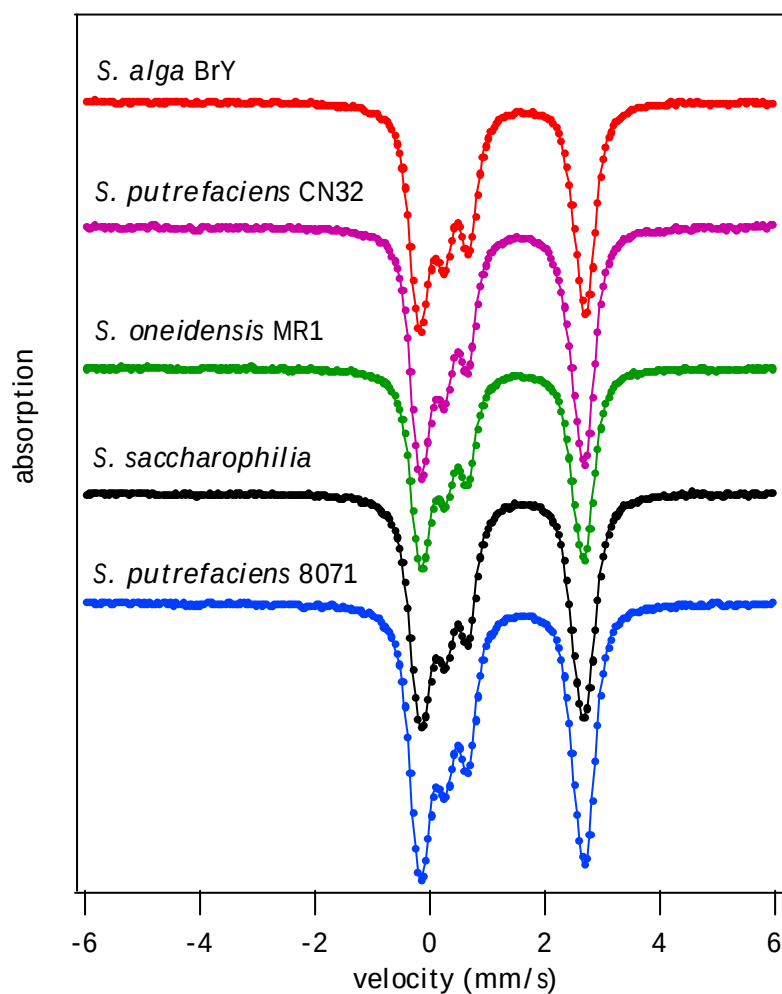


Figure 7. Mössbauer spectra from five different cultures of DIRB species provided with lepidocrocite as the electron acceptor and lactate as the carbon source and electron donor. Culture media was similar to that used by Ona-Nguema et al. 2002 (Ona-Nguema et al., 2002).

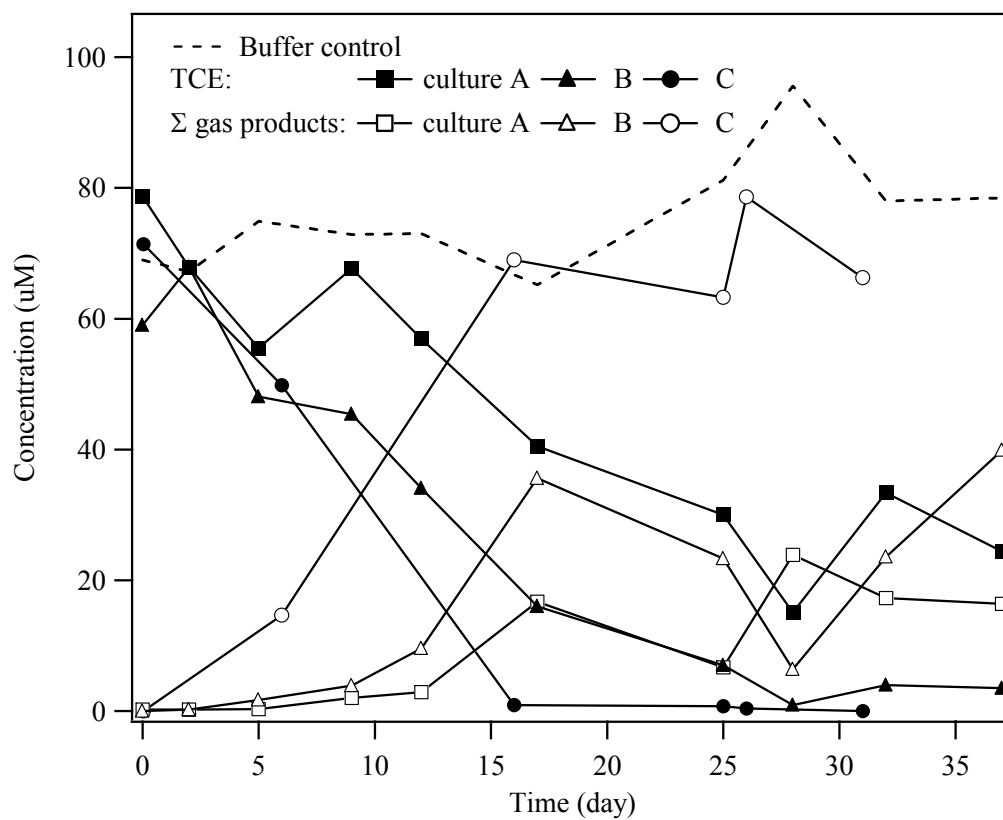


Figure 8. Reduction of TCE and gas product formation in the presence of dissimilatory iron reducing culture containing *S. putrefaciens* CN32 and carbonate green rust. (A and B – CAG, C-TSP).

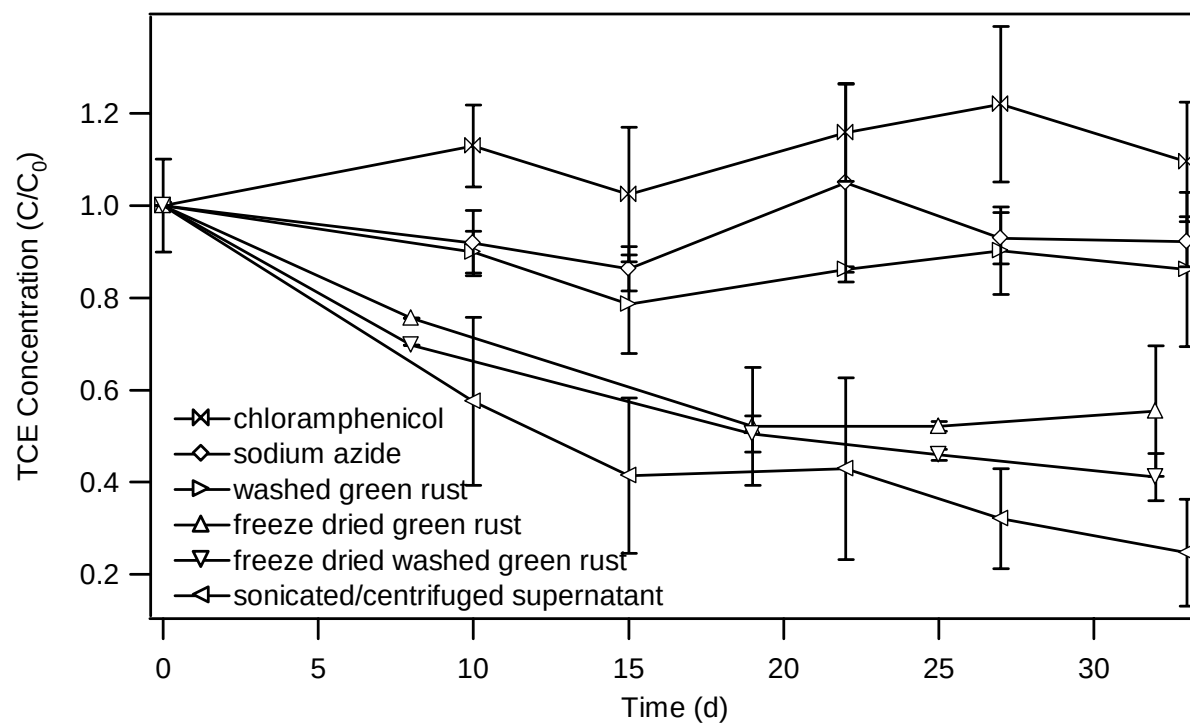


Figure 9: Reduction of TCE by various whole iron reducing culture (Batch B) components. Reactors contained inactivated (azide / chloramphenicol) or removed (freeze-dry / wash procedures) cell culture components, or whole cell culture without precipitated green rust (supernatant). All reactors contained 25 mM TAPS buffer (pH 7.5) except the sonicated/centrifuged supernatant, which contained medium as described in Ona-Nguema et al. (2002).

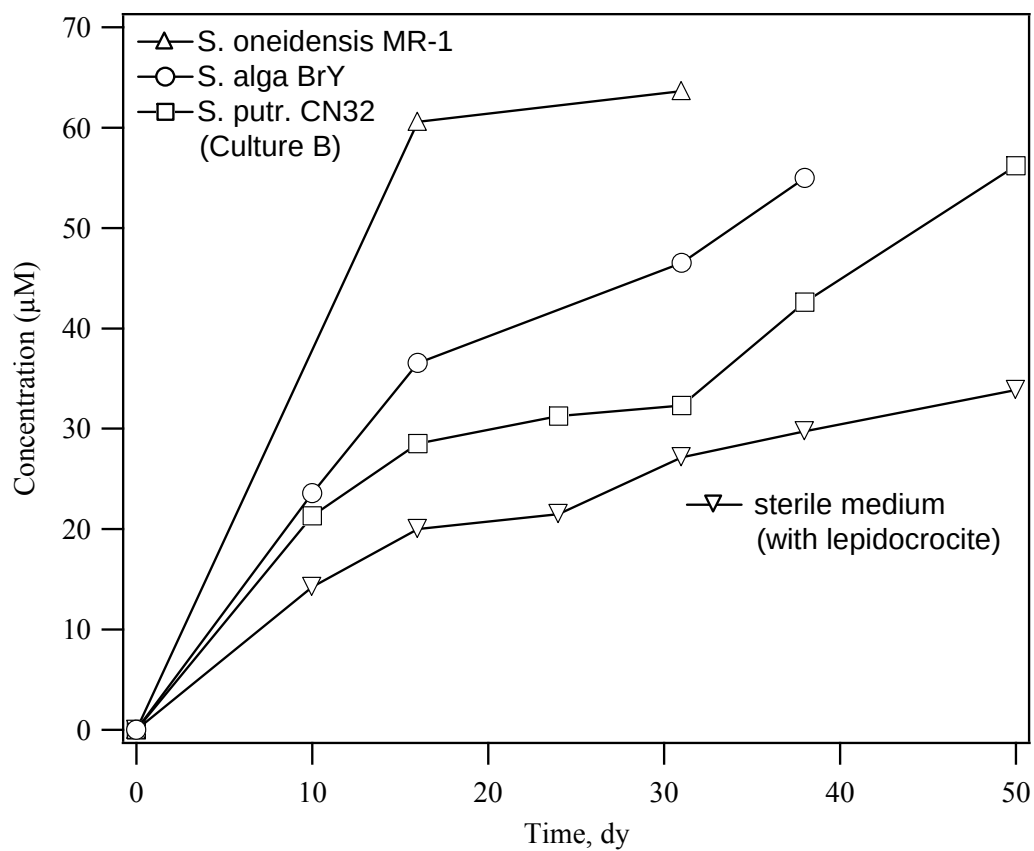


Figure 10. Comparison of gas product formation (ethane + ethene + acetylene) from TCE reduction in the presence of different dissimilatory iron reducing cultures and carbonate green rust. (CAG)

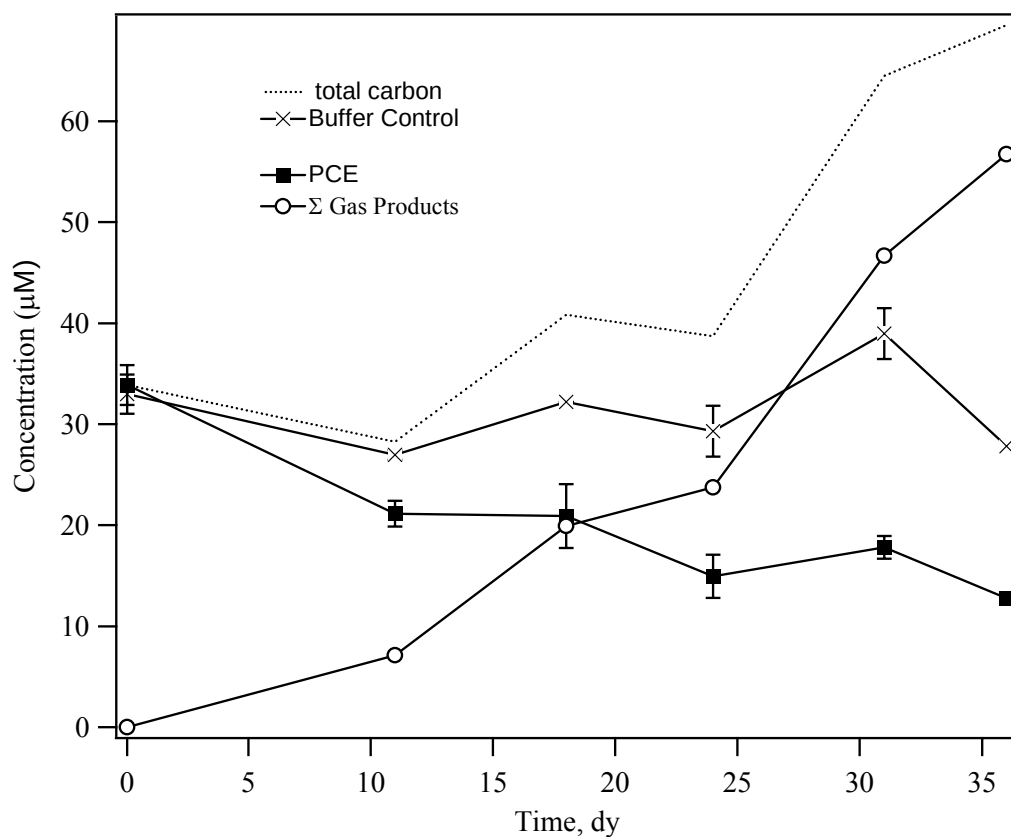


Figure 11. Reduction of PCE and product formation in the presence of dissimilatory iron reducing culture and carbonate green rust (Batch B). *Shewanella putrefaciens* CN32 grew in medium described in Ona-Nguema et al. (2002) containing lepidocrocite. Culture was unmodified when spiked with PCE. (TSP Fig. 4-3)

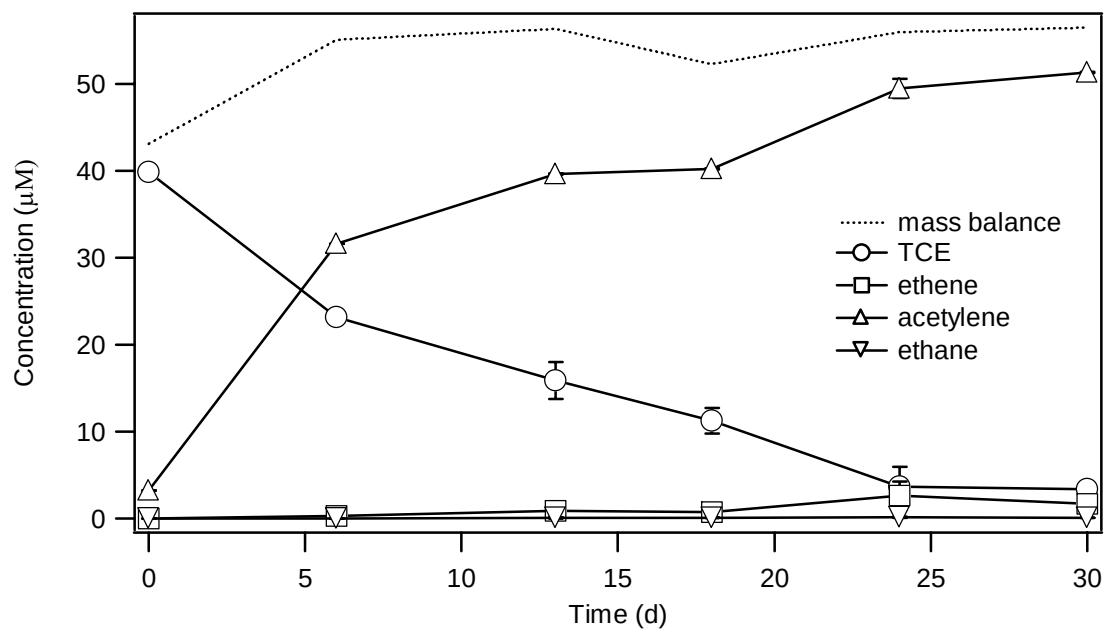


Figure 12. TCE reduction in the presence of 10 g/L freeze-dried chemically synthesized FeS suspended in 25 mM TAPS buffer (pH 7.5) (TP)

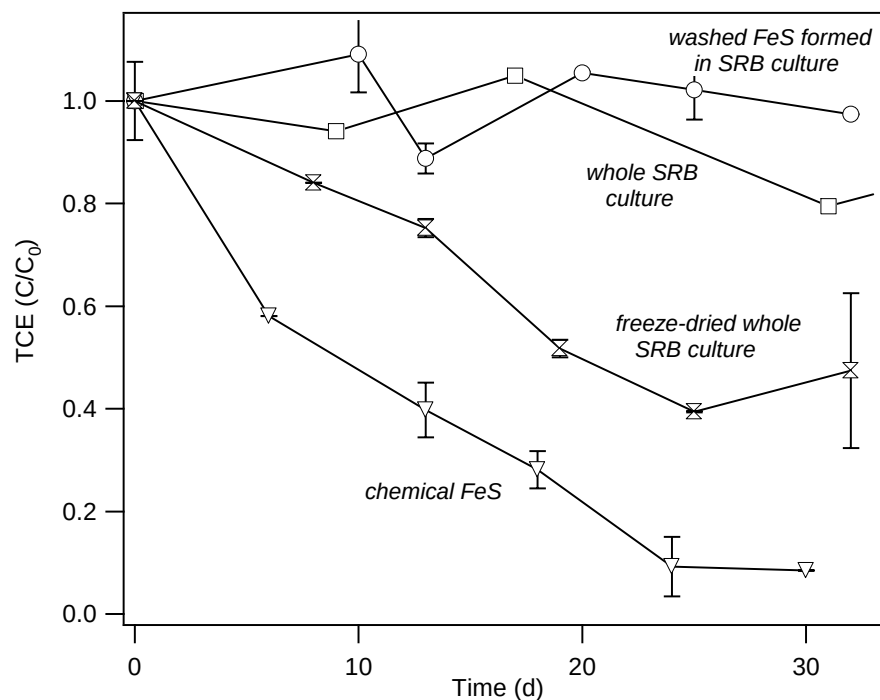


Figure 13. Disappearance of TCE over time in the presence of various iron sulfides precipitated in the absence and presence of sulfate reducing bacteria (SRB). 1 g/L FeSO_4 was added to induce FeS precipitation in the SRB cultures. All reactors with the exception of whole SRB culture contain 25 mM TAPS buffer at pH 7.5. Mineral concentrations were 5 g/L freeze-dried FeS formed in the SRB culture, (10 g/L) freeze-dried chemical FeS, and unknown in the whole SRB culture and the washed FeS formed in SRB culture (TP).

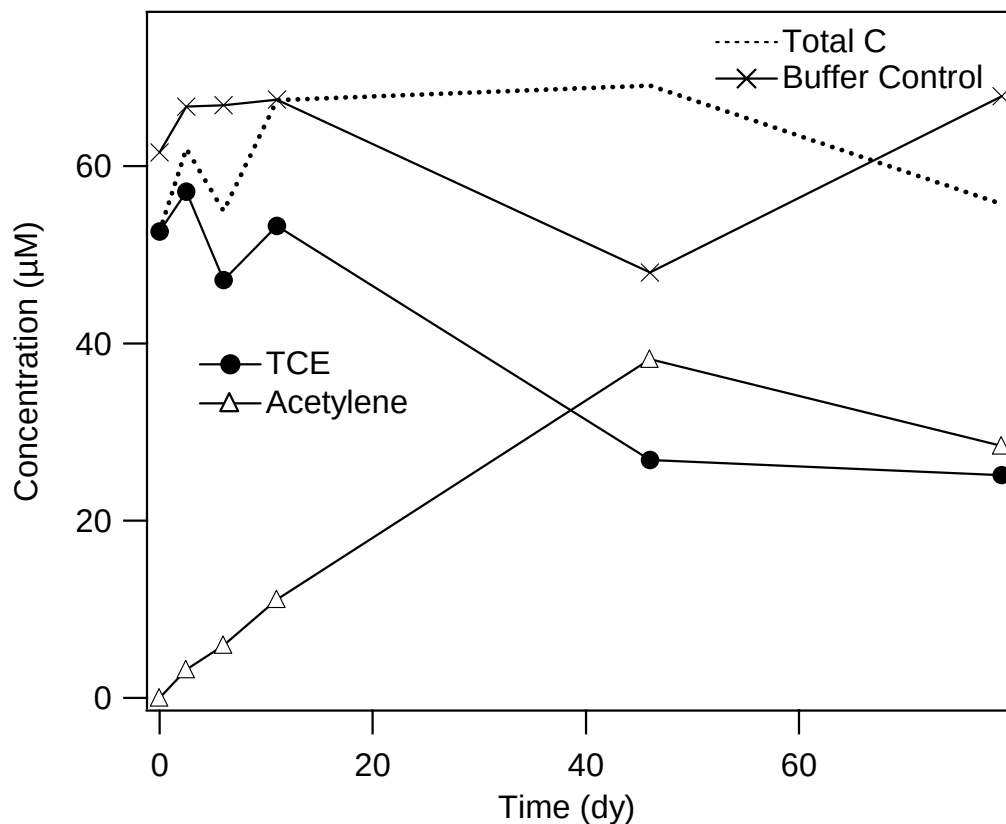


Figure 14. Disappearance of TCE over time in the presence iron sulfides precipitated in the presence of sulfate reducing bacteria (SRB). 1 g/L $\text{FeCl}_2\text{-Fe}$ was added to induce FeS precipitation in the SRB cultures. All reactors with the exception of whole SRB culture contain 25 mM MOPS buffer at pH 8.3 (CAG).

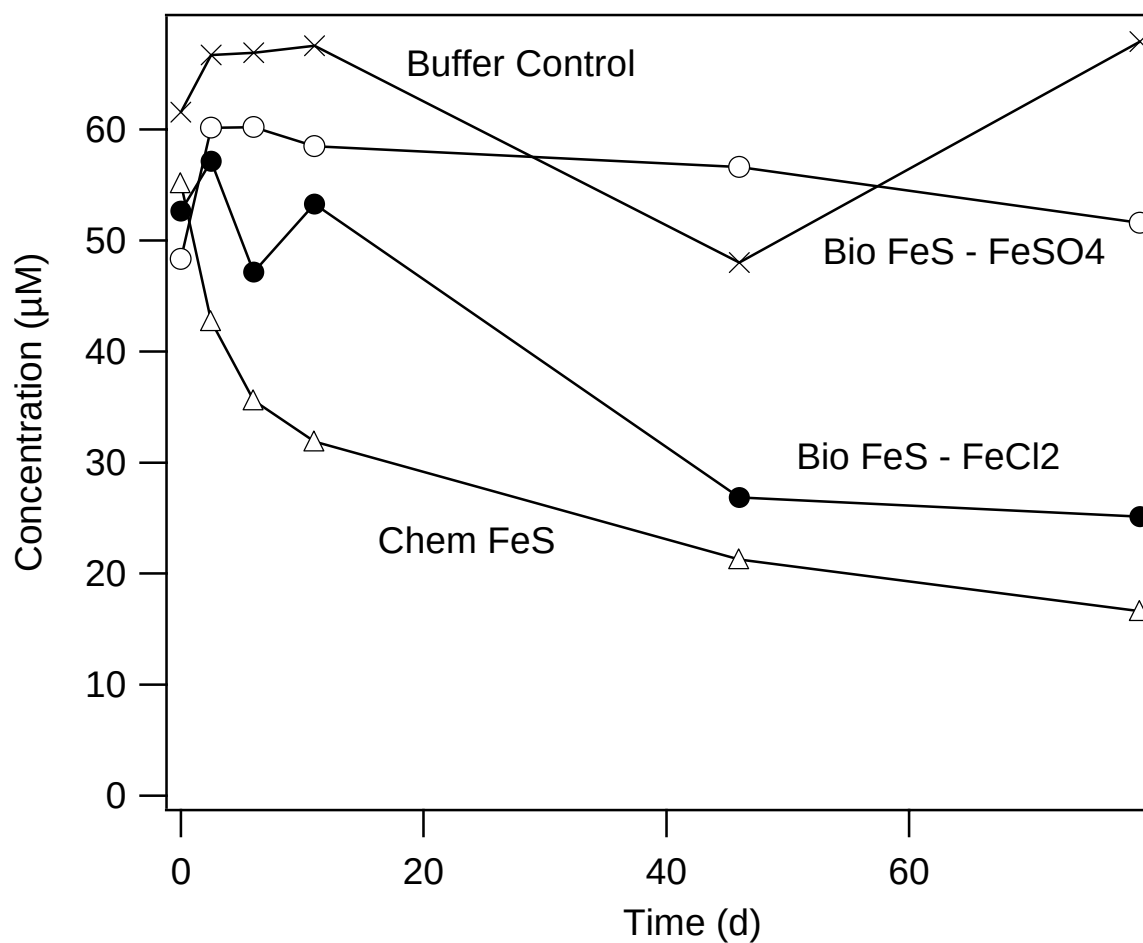


Figure 15. Disappearance of TCE over time in the presence iron sulfides precipitated in the presence of sulfate reducing bacteria (SRB). 1 g/L of $\text{FeCl}_2\text{-Fe}$ and $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2\text{-Fe}$ were added to induce FeS precipitation in the SRB cultures. The chemical FeS reactor contained 25 mM MOPS buffer at pH 8.3. Biological reactors were titrated to pH 8.3 prior to the addition of TCE. Mineral concentrations were 1 g/L(CAG).

Figure 16. SEM Image of freeze-dried FeS after resuspension in 25mM MOPS buffer at pH 8.3, equilibrated 24 hr before filtration and drying.

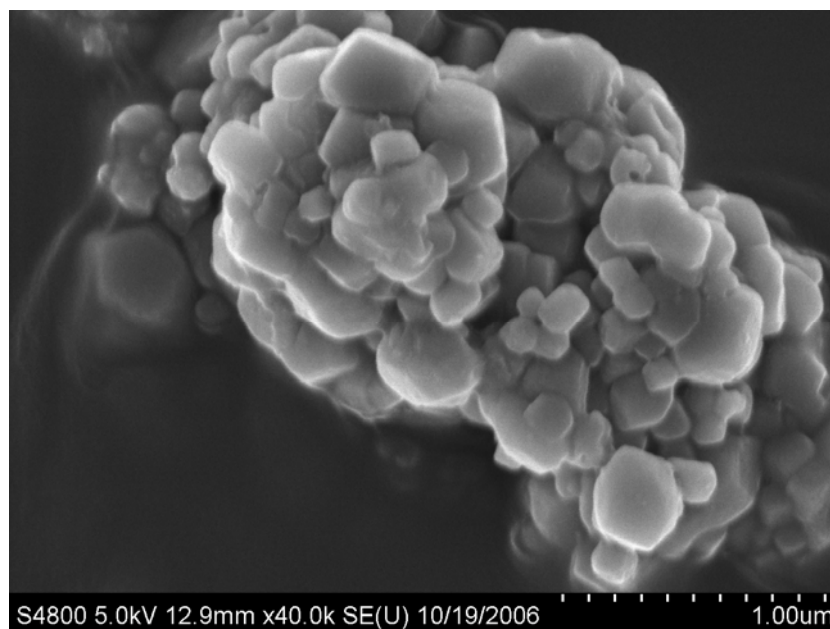
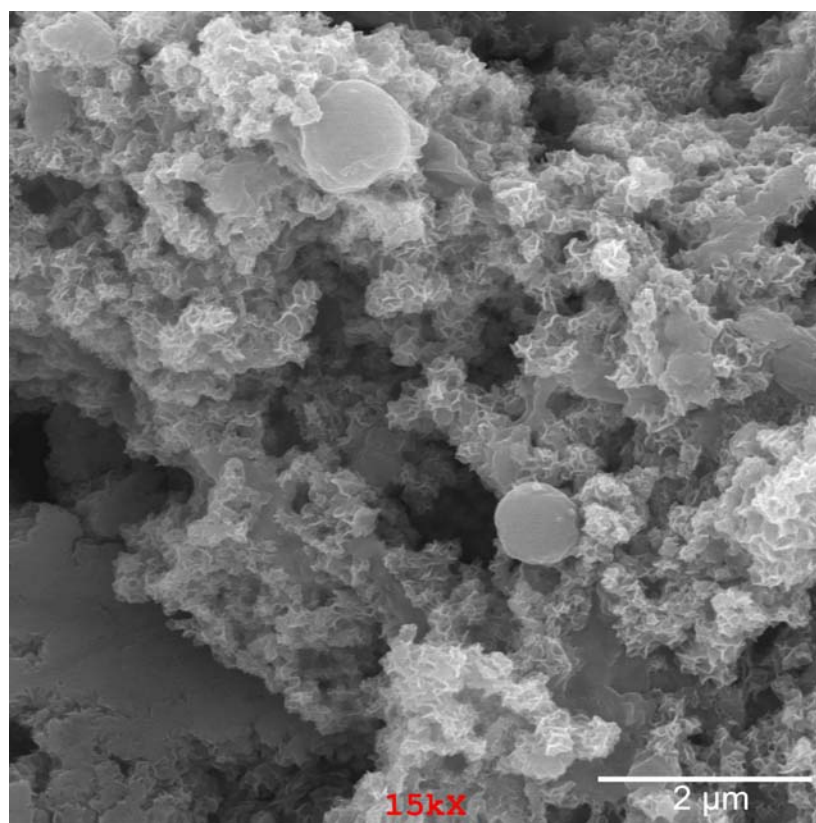


Figure 17. SEM image of freeze-dried solids collected from SRB culture amended with $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$ to form FeS.



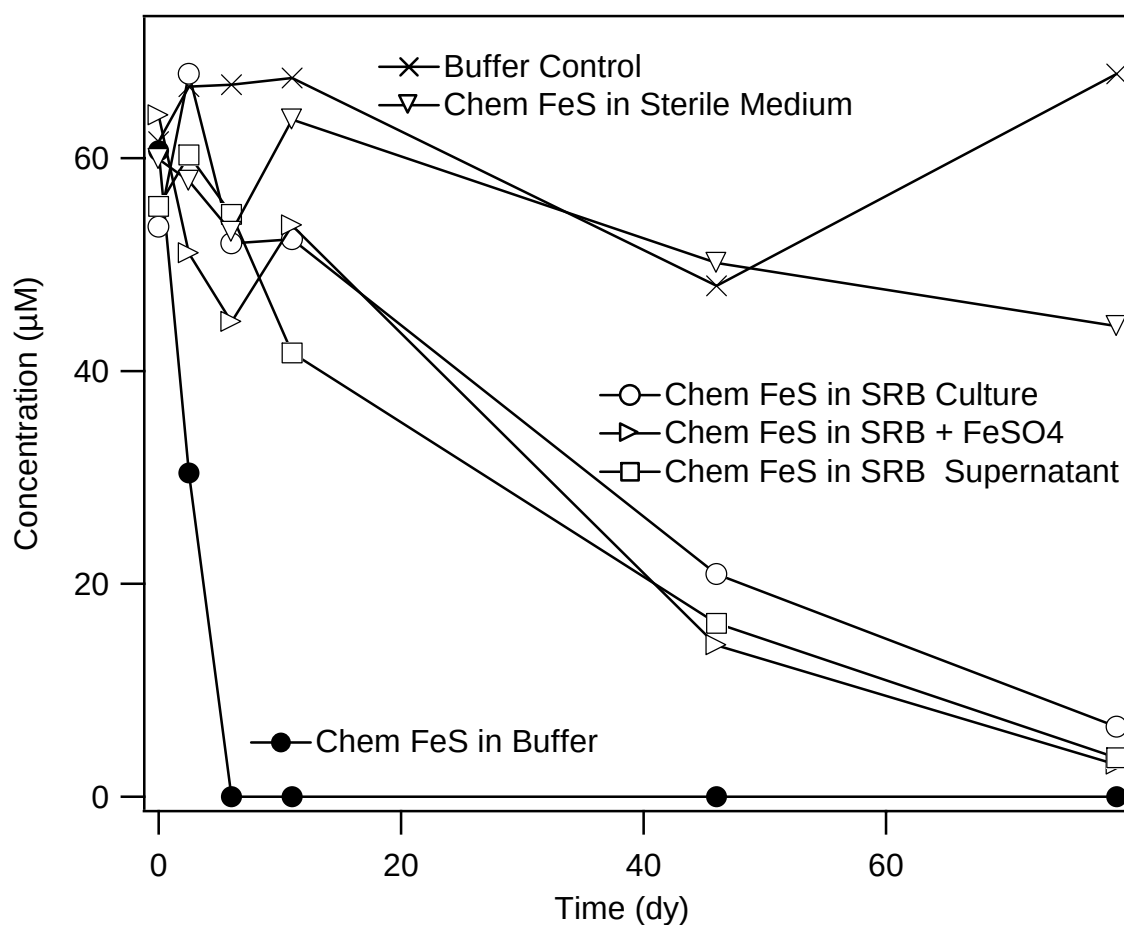


Figure 18. Disappearance of TCE over time in the presence of chemically synthesized iron sulfide added to different components of an SRB culture. 10 g/L of chemically synthesized FeS was used. The buffer used for the chemical FeS was 25 mM MOPS buffer at pH 8.3. Biological reactors were titrated with NaOH to reach pH 8.3 prior to the addition of TCE.

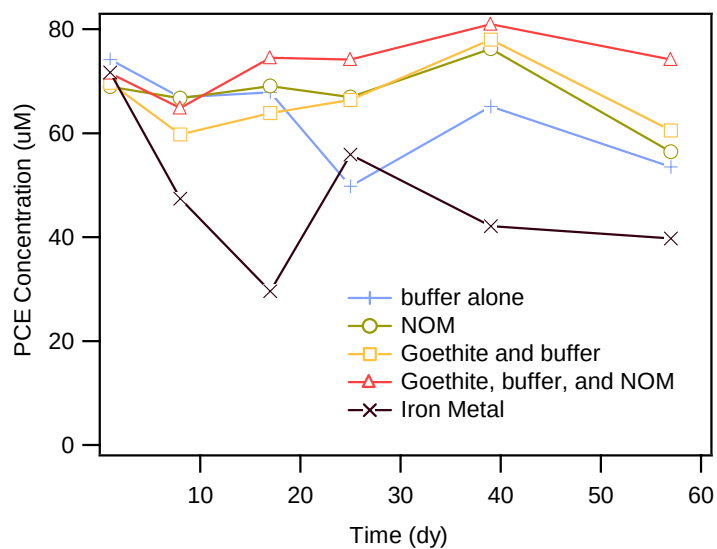


Figure 19. PCE removal in the presence of untreated natural organic matter (NOM) in the aqueous phase and adsorbed on Goethite. The effect of Fe(II) was also investigated. A positive control was run with iron metal to ensure that we could observe PCE reduction. Experiments were conducted in batch reactors with pH 7.5 MOPS.

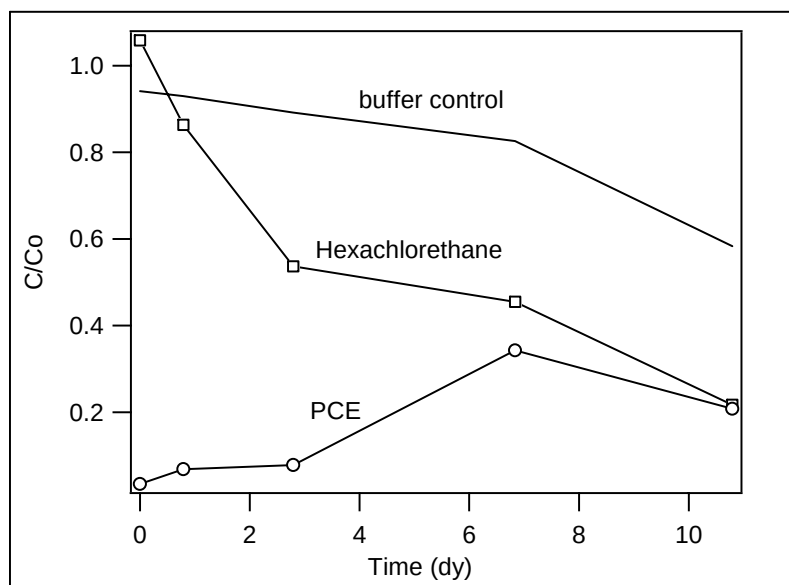


Figure 20. Hexachloroethane removal and PCE formation in the presence of AHDS.

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