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Influence of Ferric Iron on Complete Dechlorination of Trichloroethylene (TCE) to Ethene: Fe(III) Reduction Does Not Always Inhibit Complete Dechlorination

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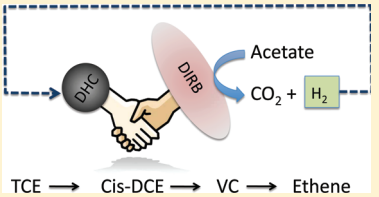
Supporting Information

ABSTRACT: The effects of Fe(III) reduction on TCE, *cis*-DCE, and VC dechlorination were studied in both contaminated aquifer material and enrichment cultures. The results from sediment batch experiments demonstrated that Fe(III) reduction did not inhibit complete dechlorination. TCE was reduced concurrently with Fe(III) in the first 40 days of the incubations. While all incubations (plus and minus Fe(III)) generated approximately the same mass of ethene within the experimental time frame, Fe(III) speciation (ferrihydrite versus Fe(III)-NTA) had an impact on daughter product distribution and dechlorination kinetics. 16S rRNA gene clone library sequencing identified *Dehalococcoides* and Geobacteraceae as dominant populations, which included *G. lovleyi* like organisms. Quantitative PCR targeting 16S rRNA genes and Reductive Dehalogenase genes (*tceA, bvcA, vcrA*) indicated that *Dehalococcoides* and Geobacteraceae were enriched concurrently in the TCE-degrading, Fe(III)-reducing sediments. Enrichment cultures demonstrated that soluble Fe(III) had a greater impact on *cis*-DCE and VC reduction than solid-phase Fe(III). Geobacteraceae and *Dehalococcoides* were also coenriched in the liquid cultures, and the *Dehalococcoides* abundance in the presence of Fe(III) was not significantly different from those in the cultures without Fe(III). Hydrogen reached steady-state concentrations most amenable to complete dechlorination very quickly when Fe(III) was present in the culture, suggesting that Fe(III) reduction may actually help dechlorination. This was contrasted to hydrogen levels in nitrate-amended enrichments, in which hydrogen concentration was too low for any chlororespiration.



INTRODUCTION

Fe(III) has been reported as a strictly competitive electron acceptor with respect to trichloroethylene (TCE), *cis*-dichloroethylene (*cis*-DCE), and vinyl chloride (VC) reduction in aquifer material[1,3,4,6] and liquid cultures.[1−5] Field and laboratory data have been interpreted in a way that suggests that Fe(III)-reducing microorganisms metabolize electron donors to concentrations that inhibit reductive dechlorination, especially for less-chlorinated compounds *cis*-DCE and VC. This has implications for chlorinated solvent bioremediation, in that Fe(III) is viewed as an inhibitor and the Fe(III)-reducing microorganisms are characterized as deleterious, rather than commensal or beneficial, to the remediation efforts. Complete dechlorination has therefore been correlated with "highly reducing" conditions such as sulfate reduction and methanogenesis,[7−11] and typically electron donors are added at high concentrations to compensate for the "electron donor demand" exhibited by Fe(III).

Fe(III)-reducing conditions are prevalent in subsurface environments, but the effect of Fe(III) reduction on complete TCE degradation has often been reported using field data or laboratory data in which understanding the dynamic between these processes was not the goal. Iron-bearing minerals are abundant in natural environments,[12] and Fe(III) compounds are present in aquifer and lake sediments.[13,14] Fe(III) reduction has been documented during natural attenuation of chlorinated ethenes[15] and during TCE bioremediation where high concentrations of electron donor were added to stimulate reductive dechlorination.[16−18] However, previous studies of the effects of alternative electron acceptors on reductive dechlorination have focused on nitrate, sulfate, or carbon dioxide (methanogenesis);[19−25] few, if any, data are available as to the effect of Fe(III) despite its significance.

The effects of Fe(III) on complete TCE dechlorination are complex. Based on the redox potentials and hydrogen threshold concentrations reported (Table S1, Supporting Information), chlororespiration is more thermodynamically favorable than sulfate reduction and methanogenesis and less favorable than nitrate reduction. However, the reduction potential values for chlororespiration and Fe(III) reduction have notable overlaps, and "favorability" of one process versus the other will depend on Fe(III) speciation and concentrations of all substrates and products (Table S1, Supporting Information). In addition, the

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steady-state hydrogen threshold for reduction of the different chlorinated ethenes ranges widely,[5,26,27] suggesting that Fe(III) reduction may interact differently with each chlorinated compound. Therefore, it is necessary to evaluate the effects of Fe(III) reduction on each step of complete TCE dechlorination with respect to different forms of Fe(III). Finally, *cis*-DCE and VC can be oxidized by Fe(III)-reducing bacteria, another pathway for *cis*-DCE and VC at sites where TCE is being reduced.[8,28−30]

Several Fe(III)-reducing bacteria can use chlorinated compounds as terminal electron acceptors. *Desulfuromonas chloroethenica*, *D. michiganensis*, and *Geobacter lovleyi* can use acetate as electron donor to reduce PCE or TCE to *cis*-DCE,[31−33] with concomitant Fe(III) reduction reported in studies with *Desulfuromonas michiganensis* and *Geobacter lovleyi*.[33,34] Fe(III) and TCE can be coreduced, with TCE being reduced to *cis*-DCE, suggesting that Fe(III) reducers play a role in the initial reactions of TCE dechlorination. What remains unknown is the extent to which Fe(III) reduction influences "complete" dechlorination, particularly ethene generation. The objectives of this study were to compare kinetics and daughter products of TCE dechlorination, and microbial community composition, in the presence and absence of active Fe(III) reduction, and to determine how different Fe(III) species influence complete TCE dechlorination.

MATERIALS AND METHODS

Chemicals. TCE, *cis*-DCE, and VC (1000 ppmv in nitrogen) were obtained from Sigma-Aldrich (Milwaukee, WI). Poorly crystalline Fe(III) (hydr)oxide (ferrihydrite) was synthesized from ferric chloride as previously described.[35] Nitrilotriacetic acid-Fe(III) (Fe(III)-NTA) was prepared by combining equimolar concentrations of NTA and Fe(III)-chloride as previously described.[36] All other chemicals used were of reagent grade quality or higher.

Sediment Batch Experiments. TCE-contaminated aquifer material was obtained from Camp, Dresser, and McKee (CDM) from a confidential site. The material had a total iron content of 2.4 μmol/g; it was generally fine silt. TCE was the primary contaminant; daughter products were negligible.

Batch experimental setup has been described previously.[37] The bottles were preincubated for 80−90 days with TCE plus acetate to obtain active TCE degrading material. When ethene was present, the material was homogenized, and 35 mL of the homogenate was redistributed to each serum bottle (nominal volume 60 mL) in an anoxic glovebag with N_2, CO_2, and H_2 (85/10/5%). The bottles were sealed with butyl rubber stoppers and crimped. Once they were removed from the glovebag, the headspaces were flushed with (H_2/CO_2-free) nitrogen gas, which had passed through a heated, reduced copper column to remove trace oxygen. Three treatments were used to investigate the effects of insoluble and soluble forms of Fe(III) on TCE dechlorination: (1) TCE+ferrihydrite (10 mmol/L) + acetate; (2) TCE + Fe(III)-NTA (10 mM) + acetate; (3) TCE + acetate alone (no-Fe(III) control); sterile controls were prepared by autoclaving at 121 °C for one hour per day for three consecutive days. Each treatment was prepared in triplicate.

TCE amendment was 4.4 μmol/bottle to deliver 0.14 mM aqueous concentration, and 2 mM acetate was added as electron donor, which was superstoichiometric based on available electron acceptors. The bottles were incubated in the dark at 18 °C without shaking. Dechlorination rates and daughter products were quantified and compared. Fe(III) reduction was quantified for Fe(III) amended incubations. Sediment samples were collected for microbial community analysis; samples were stored at −80 °C until use.

Enrichment Culture Development. Sediment from batch incubations was inoculated (10% m/v) into defined growth medium with a N_2 headspace. The medium was a modified freshwater medium as previously described.[38] HEPES buffer was used, and resazurin was added to indicate trace oxygen. TCE (1:20 diluted in hexadecane) was added at a 0.35 mM aqueous concentration; ferrihydrite (10 mmol/L) was added, and 3 mM acetate was amended as the sole electron donor. The enrichment cultures were incubated at 18 °C in the dark without shaking. TCE reduction and Fe(III) reduction were monitored by measuring headspace and liquid samples, respectively. When TCE and/or Fe(III) were depleted, the cultures were reamended with the appropriate electron acceptor. Cultures were transferred to fresh medium once TCE had been depleted three times. All enrichment culture experiments described herein were performed with liquid cultures that were transferred at least five times (no solids remaining).

Enrichment Culture Experiments. Incubations used the defined medium described above; each 26-mL tube contained 15 mL of medium and a nitrogen headspace (prior to amendments). Approximately 0.2 μL of TCE (~120 μM aq.), 0.2 μL of *cis*-DCE (~120 μM aq.), or 15 mL of VC gas (~22 μM aq.) were added to respective incubations. Triplicate tubes with and without Fe(III) amendment were set up for each of the three chlorinated ethenes. Acetate (2 mM) was added as the sole electron donor and carbon source. Experiments began with a 5% (v/v) inoculum, and tubes were incubated in the dark at 18 °C without shaking.

Two batch experiments with different forms of Fe(III) were conducted: one with ferrihydrite and the other with Fe(III)-NTA. The effect of nitrate amendment on TCE dechlorination and hydrogen steady-state concentration was tested as a control versus Fe(III); 2 mM sodium nitrate was added instead of Fe(III); all other conditions were identical.

Analytical Methods. Headspace samples were collected using an anoxic syringe and needle.[39] Chlorinated ethenes, ethene, ethane, acetylene, and methane were analyzed using a gas chromatograph equipped with a flame ionization detector (Hewlett-Packard Series 6890A); the column was a 30 m by 0.53 mm GS-Q column (J&W Scientific, Germany). H_2 in headspace samples was quantified using a gas chromatograph (Shimadzu GC-14A) with a carbosieve S-II Spherical Carbon Column (Supelco, Bellefonte, PA) equipped with an SRI reduction gas analyzer; results were converted to dissolved H_2 (nM) following a previously described method.[40,41] Aqueous Fe(II) and total bioavailable iron were quantified by the Ferrozine assay.[42] Nitrate and nitrite were quantified by ion chromatography (Dionex, IC-1000, Sunnyvale, CA, USA) with conductivity detection.

DNA Extraction and Clone Library Analysis. Genomic DNA from all samples was extracted using FastDNA SPIN Kits (MP Biomedicals, Solon, OH, USA). Clone libraries of 16S rRNA genes were constructed using previous methods.[37] Details are provided in the Supporting Information.

The sequences reported in this study have been deposited in GenBank under accession numbers JF502573 to JF502595.

Quantitative PCR (qPCR). Analyses were performed with a MiniOpticon qPCR detection system and IQ SYBR Green

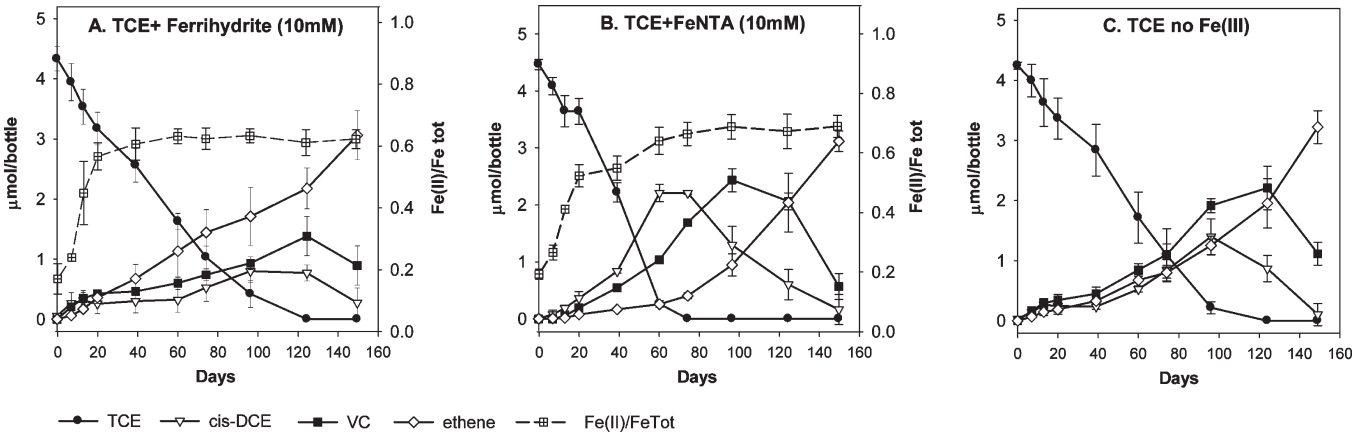

Figure 1. TCE degradation and daughter product transformation in sediment incubations with ferrihydrite (A), Fe(III)-NTA (B), or no Fe(III) amendment (C). Results are the means of triplicate incubations. Bars indicate one standard deviation.

Supermix (BioRad, Hercules, California). Each qPCR analysis was conducted in triplicate; calibration curves and no-template controls were also performed for each time point. Specific primers were used to target and quantify copies of *Dehalococcoides*, Geobacteraceae, and universal Eubacterial 16S rRNA genes. In addition, reductive dehalogenase (RDase) functional genes (*tceA, bvcA, and vcrA*) were quantified to differentiate specific *Dehalococcoides* populations. The *Dehalococcoides* and bacterial 16S rRNA gene primers and RDase gene primers have been described previously.[43] The Geobacteraceae-specific primers were Geo494Forward (5′-AGG AAG CAC CGG CTA ACT CC-3′)[44] and Geo825Reverse (5′-TAC CCG CRA CAC CTA GTT CT-3′).[45] Each 25 μL reaction contained 12.5 μL of 2x IQ SYBR Green Supermix (Biorad), 0.3 mM of forward and reverse primers each, 2 μL of template DNA, and sterile, nuclease-free water (Invitrogen). The PCR program for *Dehalococcoides* 16S rRNA gene, *tceA, bvcA, and vcrA* genes was as follows: 50 °C (2 min), 95 °C (10 min), 45 cycles of 95 °C (15 s), and 58 °C (1 min); the annealing temperature was changed to 52 °C for bacterial 16S rRNA gene primers[43] and 56 °C for Geobacteraceae primers. Finally, a melting curve ranging from 55 to 99 °C was performed with steps of 1 °C and a hold of 5 s. Calibration curves were made by serially diluting PCR-amplified DNA from pure cultures (*Dehalococcoides* sp. FL2 for *Dehalococcoides* or *Geobacter metallireducens*) or plasmids carrying a single cloned *Dehalococcoides* target gene (*tceA, vcrA, or bvcA*). The curves describe relationship of the gene copy number versus the cycle number at which the fluorescence intensity reaches a set cycle threshold value (C_T). The amplification efficiencies ranged between 1.85 and 2.05 depending on the primers and methods. Calibration curves had linear range over 7 orders of magnitude, from 10^9 to 10^2 gene copies per μL DNA template for all the primer sets. All calibration curves had R^2 values >0.98. The gene copy numbers of the standard DNA template were calculated based on parameters described previously.[43]

Statistical Analysis. ANOSIM was performed using PRIMER 6 for Windows (PRIMER-E, Plymouth, United Kingdom). The R-values generated by ANOSIM analysis for dechlorination daughter product distribution at each time point during the sediment batch experiments were used to compare the similarity among different conditions. MANOVA, ANOVA with Tukey's test, and *t* tests were additionally performed using the statistical program GNU R.

RESULTS AND DISCUSSION

Sediment Batch Experiments. TCE and Fe(III) were reduced concurrently in the first 40 days of incubation (Figure 1). TCE was reduced in the ferrihydrite-amended incubations at a rate similar to that of TCE-alone (0.036 ± 0.002 μmol/day in TCE + ferrihydrite and 0.037 ± 0.001 μmol/day in TCE-alone incubations) (Figure 1A). In the incubations amended with Fe(III)-NTA, the TCE degradation rate was faster (Figure 1B) (0.065 ± 0.002 μmol/day); TCE was sequentially transformed through *cis*-DCE and VC, but all chlorinated ethenes were eventually transformed to ethene. Methane was at or below 0.1 μmol/bottle in all Fe(III) amended incubations.

The relative abundance of all daughter products was calculated as the mole fraction of all the chlorinated ethenes plus ethene. An analysis of similarity (ANOSIM) was used to examine statistical significance. ANOSIM generates a test statistic R, which indicates the degree of separation among groups of samples, with a value of 1 indicating complete dissimilarity and 0 indicating no difference. The R value between TCE+Fe(III)-NTA incubations and TCE-alone was 0.722, while the R value between TCE + ferrihydrite and TCE-alone was 0.307 (ANOSIM, $P < 0.005$), suggesting a larger dissimilarity between Fe(III)-NTA and TCE-alone than ferrihydrite and TCE-alone.

These results demonstrated that Fe(III) reduction did not inhibit complete dechlorination; all incubations (plus and minus Fe(III)) generated approximately the same mass of ethene within the experimental time frame. Ethene was quantifiable while Fe(III) was being reduced, and the rate of TCE reduction was faster in the presence of Fe(III)-NTA than in Fe(III)-free incubations. The question remained, however, whether Fe(III) amendment influenced the microbial community in terms of enriching dechlorinating microorganisms as well as the abundance of Fe(III)-reducing organisms.

Microbial Community Analyses in Sediment Incubations. Clone libraries were constructed to target specific phylotypes for qPCR analyses. The primary results demonstrated that DIRB and DHC were codominant phylotypes recovered from the material, with DIRB increasing as a function of Fe(III) amendment. Detailed information about the total microbial community is described in the Supporting Information (Table S2).

Quantification of *Dehalococcoides* and DIRB in Sediment Incubations. The bacterial 16S rRNA gene copy number was

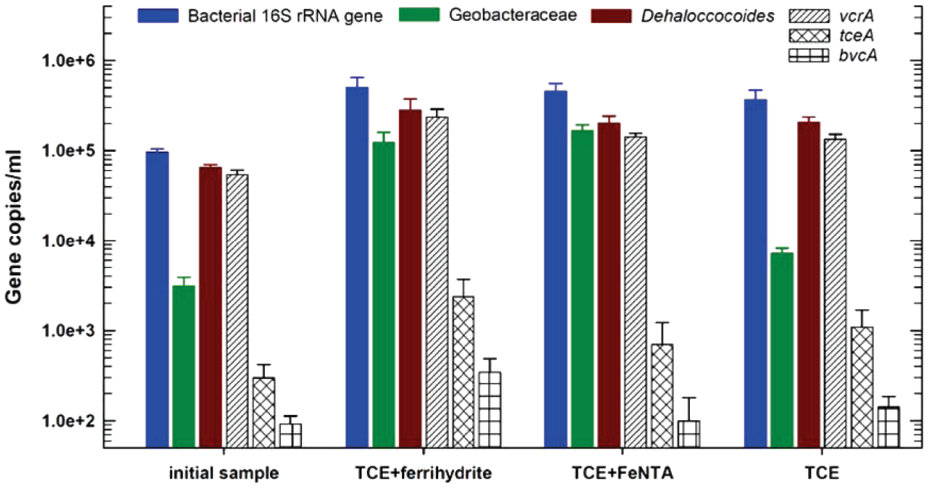

Figure 2. qPCR analyses of samples from the sediment incubations with the specific qPCR primers. The bar graph depicts gene copy numbers in the initial sediment samples, and 98-day samples from the three incubation conditions. Each bar represents the mean of DNA samples from triplicate incubations under each of the conditions tested (ferrihydrite, Fe(III)-NTA, or TCE-alone). Error bars represent one standard deviation. The gene copy numbers are given on a log scale.

$9.6 \times 10^4 \pm 0.82 \times 10^4$ per mL in the initial sample. *Dehalococcoides* were $6.5 \times 10^4 \pm 0.48 \times 10^4$ per mL. Initial Geobacteraceae 16S rRNA genes were much less abundant ($7.1 \times 10^3 \pm 0.99 \times 10^3$ per mL) and represented only 7.4% of the total bacterial 16S rRNA gene copies. 83% of the *Dehalococcoides* organisms were *vcrA*-carrying strains; *tceA* and *bvcA* genes were detected, but the gene copies were low (near or below quantification limit) (Figure 2). The qPCR results were consistent with the clone library data where a dominant clone group was related to *Dehalococcoides* sp. strain GT, which carries the *vcrA* gene.

Dehalococcoides increased in all incubations at 98 days - including those with Fe(III) amendment (Figure 2). Geobacteraceae populations were enriched in Fe(III) amended incubations. Total *Dehalococcoides* cells increased to $2.8 \times 10^5 \pm 0.92 \times 10^5$ per mL (TCE + ferrihydrite), $2.0 \times 10^5 \pm 0.40 \times 10^5$ per mL (TCE+Fe(III)-NTA), and $2.1 \times 10^5 \pm 0.30 \times 10^5$ per mL (TCE only), respectively. The *Dehalococcoides* 16S rRNA gene copy numbers under different incubation conditions were not significantly different from each other (Tukey's Test, $P > 0.05$).

The number of Geobacteraceae in the Fe(III)-amended sediments was 1 order of magnitude higher than that in the incubations without Fe(III). The small increase of Geobacteraceae in TCE-alone incubations was likely due to Fe(III)-reducing cells that use TCE for growth, such as *G. lovleyi*. *Dehalococcoides* and Geobacteraceae were enriched concurrently over the course of the experiments in the TCE-degrading/Fe(III)-reducing sediments, indicating that Fe(III) reduction did not inhibit *Dehalococcoides* growth or complete dechlorination.

The *vcrA*-carrying strains were the dominant *Dehalococcoides* at 98 days, comprising 83% (TCE + ferrihydrite), 71% (TCE + Fe(III)-NTA), and 65% (TCE only) of all *Dehalococcoides* quantified. The *tceA*- and *bvcA*-carrying strains represented <1% of the total *Dehalococcoides*. The *vcrA* gene copies in TCE + ferrihydrite incubations were significantly higher than in the other two incubation conditions (Tukey's test, $P < 0.05$) and represented a larger portion of the total *Dehalococcoides* community (Figure 2), which accounts for the lesser accumulation of *cis*-DCE and VC during TCE degradation as compared to TCE + Fe(III)-NTA incubations, which had fewer *vcrA*-carrying organisms but a higher relative abundance of Geobacteraceae.

Cis-DCE and VC accumulated to a greater extent in the Fe(III)-NTA amended incubations; we suggest two potential mechanisms. First, Fe(III)-NTA enriched DIRB that also reduced TCE to *cis*-DCE at rates faster than *cis*-DCE could be further reduced to VC/ethene. Second, Fe(III)-NTA is soluble and freely bioavailable and may slow dechlorination relative to ferrihydrite, by slightly increasing the competition for acetate. While ethene was produced in all incubations, Fe(III) speciation had an impact on daughter product distribution and reduction kinetics. Starting biomass for both DIRB and DHC were higher in the ferrihydrite-amended incubations, which may also have contributed to these differences. We further tested the effects of the two Fe(III) forms on *cis*-DCE and VC reduction using enrichment cultures, as reported below.

Ferrihydrite versus Fe(III)-NTA in Liquid Enrichment Cultures. 90% of the Fe(III)-NTA was reduced within the first 17 days in all incubations, whether TCE, *cis*-DCE, or VC was the starting chlorinated electron acceptor (Figure 3). Degradation rates for TCE were nearly identical in the presence or absence of Fe(III)-NTA, but dechlorination rates were slower in Fe(III)-NTA amended incubations that had *cis*-DCE or VC as the electron acceptor than in those incubations that lacked Fe(III)-NTA (Figure 3). *cis*-DCE accumulated in the TCE+Fe(III)-NTA incubations, but it did not accumulate in the TCE-alone incubations, which was similar to the sediment incubations. Ethene production rate was slower in Fe(III)-NTA-amended incubations that had VC as the parent electron acceptor, but the extent was the same with or without Fe(III).

Ferrihydrite amended incubations were different. Fe(III) and each chlorinated compound were concurrently reduced, and dechlorination rates were similar irrespective of the presence of Fe(III) (Figure 4). In incubations that started with either TCE or *cis*-DCE as the parent compound, *cis*-DCE and VC were transient intermediates, with ethene produced to a similar extent in the presence or absence of Fe(III). This runs counter to prevailing thought, where Fe(III) is a strictly competitive electron acceptor that will "inhibit" complete dechlorination.

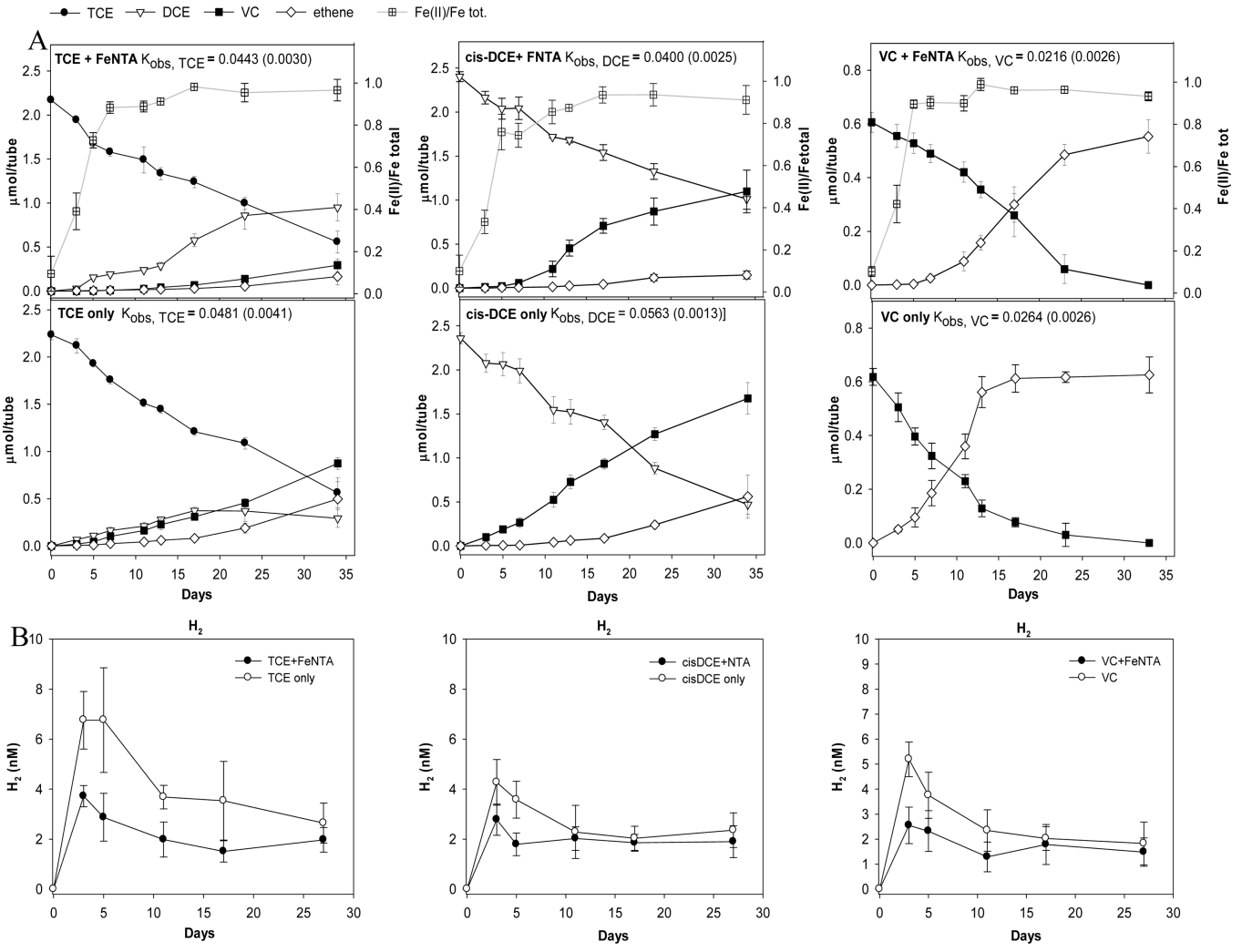

Figure 3. A. Reductive dechlorination with or without Fe(III)-NTA reduction in the enrichment cultures degrading TCE, *cis*-DCE, or VC. The degradation rates (μmol/day) of each chlorinated ethene under each condition are shown; numbers in parentheses indicate standard deviations. B. H2 concentration change in the dechlorinating enrichment cultures; each plot compares the incubations with and without Fe(III)-NTA. Results are the means of triplicate incubations. Bars indicate one standard deviation.

There was a difference based on Fe(III) speciation, which was expected — Fe(III)-NTA, being a soluble electron acceptor, is much more available to cells. In fact, it is possible that much of the reported "inhibition" may be due to the choice of Fe(III) for laboratory experiments — many times soluble Fe(III) forms such as Fe(III)-NTA or ferric citrate are used because they are operationally simpler than ferrihydrite. But, changing the form of Fe(III) does impact the results in terms of daughter product accumulation/degradation over the course of an incubation. Ultimately all of the TCE/cis-DCE/VC was reduced to ethene even in Fe(III)-NTA amended incubations, but using the environmentally relevant form (ferrihydrite) demonstrated that the electron acceptors can be reduced simultaneously.

Dehalococcoides and Geobacteraceae were directly quantified using q-PCR with primer pairs specific for each group. If *Dehalococcoides* were inhibited by Fe(III) reducers, this would be evident in terms of the total number of cells enriched in the presence or absence of Fe(III). A *t* test was used at each time point to determine if the presence of Fe(III)-NTA or ferrihydrite influenced the *Dehalococcoides* abundance. In the TCE or *cis*-DCE degrading cultures, the abundance of *Dehalococcoides* under conditions with and without Fe(III)-NTA was not significantly different at day 18 or day 35 (Figure 5). In the VC degrading cultures, the Fe(III)-NTA amended incubations had slightly (not significantly) lower abundance of *Dehalococcoides* on day 18 than the incubations without Fe(III)-NTA. However, at day 35 the abundance of *Dehalococcoides* was higher in the Fe(III)-NTA amended tubes, while the copy number decreased in VC only cultures. VC was almost depleted within 18 days in the VC only tubes, and the drop in *Dehalococcoides* might be due to the decay of cells as there was no VC to support growth.

The abundance of *Dehalococcoides* increased in all ferrihydrite amended incubations. In TCE or *cis*-DCE reducing enrichments, the *Dehalococcoides* abundance in the presence of ferrihydrite was not significantly different from those in the tubes without ferrihydrite (Figure 6). Although increases in *Dehalococcoides* in VC + ferrihydrite incubations was slightly slower before day 20 relative to VC-alone incubations, the results were not significantly different, and both had statistically the same number of *Dehalococcoides* by day 39 (Figure 6).

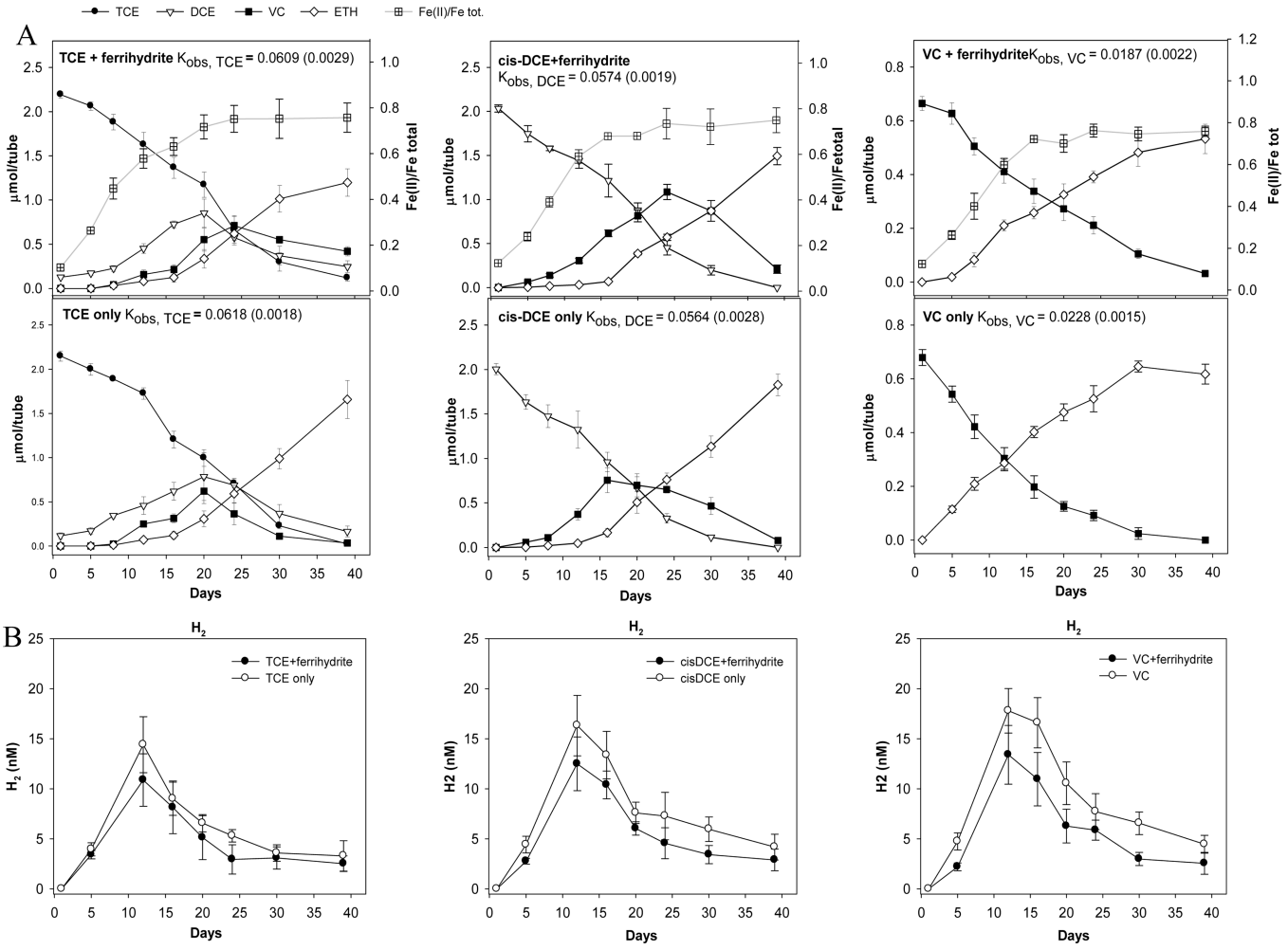

Figure 4. A. Reductive dechlorination with or without ferrihydrite reduction in the enrichment cultures degrading TCE, *cis*-DCE, or VC. The degradation rates (μmol/day) of each chlorinated ethene under each condition are shown; numbers in parentheses indicate standard deviations. B. H2 concentration change in the dechlorinating enrichment cultures; each plot compares the incubations with and without ferrihydrite. Results are the means of triplicate incubations. Bars indicate one standard deviation.

Geobacteraceae increased in all incubations with Fe(III), whether it was Fe(III)-NTA or ferrihydrite (Figure 5 and Figure 6). TCE-alone also promoted a small increase in Geobacteraceae (Figure 5 and Figure 6), most likely due to cells using TCE → *cis*-DCE for energy and growth. The critical data within these q-PCR experiments is that *Dehalococcoides* and Geobacteraceae (used as a surrogate for total DIRB) increased concurrently over the course of the experiment. When taken with chlorinated solvent and total iron data, this suggests that DIRB do not inhibit *Dehalococcoides*, but in fact they can be coenriched if conditions are appropriate for their growth. These enrichment data reinforce the sediment data, in which Geobacteraceae and *Dehalococcoides* were also coenriched. Previous assertions that Fe(III) reduction will outcompete complete dechlorination may be due to Fe(III) speciation (as described above)[46] or electron donor amendment strategies that are not compatible with coenrichment (this encompasses both electron donor type and concentration). Based on these data we propose another mechanism whereby Fe(III) reduction actually assists complete dechlorination based on steady-state hydrogen concentration, below.

Hydrogen Steady-State Concentration. Molecular H_2 is considered the primary electron donor for complete dechlorination. Acetate for complete TCE dechlorination was reported before though the process was slow and not complete.[27] Therefore a possible mechanism in these experiments is a syntrophic metabolism in which acetate was oxidized to CO_2 and H_2, while at the same time chlororespiring microorganisms and/or DIRB consumed the H_2 produced. Some Fe(III) reducers can use acetate to produce hydrogen in syntrophy with hydrogen oxidizing organisms,[47] or generate H_2 via fermentation, so it is also likely that Fe(III) reducers were involved in producing hydrogen. The hydrogen concentration initially increased and then decreased to a steady-state level (Figures 3 and 4) that is within the ideal range for complete dechlorination (Table S1, Supporting Information). Alternate metabolic processes may move the hydrogen concentration outside of this optimal range, [41,48] but Fe(III) reducers poise the hydrogen concentration at a steady-state that has been reported as ideal for reduction of TCE, *cis*-DCE, and VC. [5,41,48,49]

H_2 concentration decreased more rapidly in the presence of either Fe(III)-NTA or ferrihydrite to a steady-state level between 2 nM and 4 nM, above the threshold values for chlororespiration reported in pure culture studies and within the optimal range of the values reported in mixed cultures (Table S1, Supporting Information) but below levels where

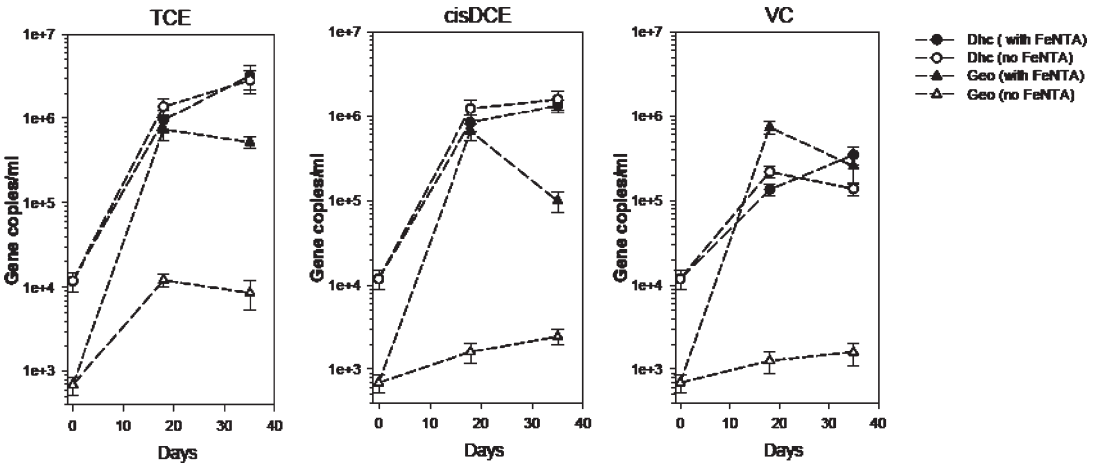

Figure 5. 16S rRNA gene copy numbers of *Dehalococcoides* and Geobacteraceae organisms in the cultures that degraded TCE, *cis*-DCE, or VC, under conditions with or without Fe(III)-NTA. Each point represents the mean of DNA samples from triplicate incubations under each of the conditions tested (ferrihydrite, Fe(III)-NTA, or TCE-alone). Error bars represent one standard deviation. The gene copy numbers are given on a log scale.

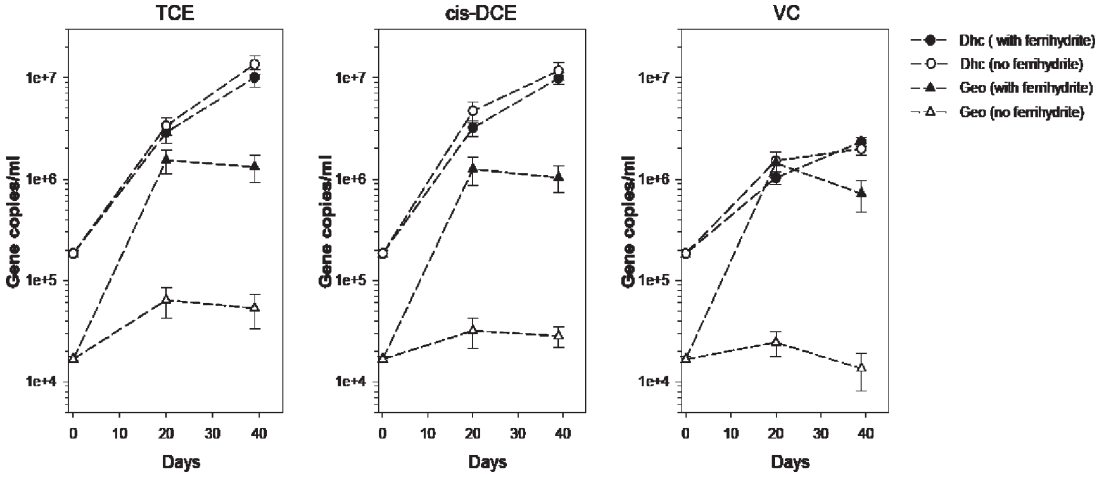

Figure 6. 16S rRNA gene copy numbers of *Dehalococcoides* and Geobacteraceae organisms in the cultures that degraded TCE, *cis*-DCE, or VC, under conditions with or without ferrihydrite. Each point represents the mean of DNA samples from triplicate incubations under each of the conditions tested (ferrihydrite, Fe(III)-NTA, or TCE-alone). Error bars represent one standard deviation. The gene copy numbers are given on a log scale.

other organisms (e.g., methanogens) proliferate.[48] Fe(III) reduction may have actually helped complete dechlorination by bringing hydrogen to an appropriate steady-state more quickly than the chlorinated electron acceptors alone. These data were contrasted to that in the nitrate-amended incubations, where H_2 was undetectable before most nitrate was reduced and TCE degradation was completely inhibited (further discussed in Figure S1 and related text, Supporting Information).

The effects of Fe(III) on chlorinated ethene reduction are more complex than previously thought. Although there may be competition for electron donors, dechlorinators and DIRB were both enriched concurrently, which is different than a commonly held perception that DIRB will completely outcompete dechlorinators. Soluble Fe(III) did have a greater impact on reduction of *cis*-DCE and VC than solid-phase Fe(III). In addition, Fe(III) promoted growth of DIRB which can also reduce TCE to *cis*-DCE, further contributing to TCE degradation.

These results suggest that adding excessive electron donors in bioremediation applications to bypass the assumed *inhibition* from Fe(III) reduction and Fe(III) reducers may not be necessary and that the presence of Fe(III) compounds does not hinder transformation of chlorinated ethenes to ethene. Based on hydrogen partial pressure data and given that DIRB exist that reduce TCE → *cis*-DCE, it is plausible to suggest that Fe(III) reduction may actually help remediation efforts for TCE.

■ ASSOCIATED CONTENT

Supporting Information. Additional figures/tables, methods descriptions and discussion. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Zaa, C. L. Y.; McLean, J. E.; Dupont, R. R.; Norton, J. M.; Sorensen, D. L. Dechlorinating and Iron Reducing Bacteria Distribution in a TCE-Contaminated Aquifer. *Ground Water Monit. Rem.* **2010**, *30*, 46–57.

(2) McCarty, P. L. Microbiology - Breathing with chlorinated solvents. *Science* **1997**, *276*, 1521–1522.

(3) McLean, J. E.; Dupont, R. R.; Sorensen, D. L. Iron and arsenic release from aquifer solids in response to biostimulation. *J. Environ. Qual.* **2006**, *35*, 1193–1203.

(4) Evans, P. J.; Koenigsberg, S. S. A bioavailable ferric iron assay and relevance to reductive dechlorination. In *Sixth International In Situ and On-Site Bioremediation Symposium*: San Diego, CA, 2001.

(5) Lu, X. X.; Tao, S.; Bosma, T.; Gerritse, J. Characteristic hydrogen concentrations for various redox processes in batch study. *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **2001**, *36*, 1725–1734.

(6) Kouznetsova, I.; Mao, X. M.; Robinson, C.; Barry, D. A.; Gerhard, J. I.; McCarty, P. L. Biological reduction of chlorinated solvents: Batch-scale geochemical modeling. *Adv. Water Resour.* **2010**, *33*, 969-986.

(7) Bennett, P.; Gandhi, D.; Warner, S.; Bussey, J. In situ reductive dechlorination of chlorinated ethenes in high nitrate groundwater. *J. Hazard. Mater.* **2007**, *149*, 568–573.

(8) Chapelle, F. H.; Bradley, P. M. Redox conditions and the efficiency of chlorinated ethene biodegradation: Field studies. *Abstracts of Papers of the American Chemical Society* **2000**, *220*, 122-ENVR.

(9) Cabirol, N.; Jacob, F.; Perrier, J.; Fouillet, B.; Chambon, P. Interaction between methanogenic and sulfate-reducing microorganisms during dechlorination of a high concentration of tetrachloroethylene. *J. Gen. Appl. Microbiol.* **1998**, *44*, 297–301.

(10) Cabirol, N.; Jacob, F.; Perrier, J.; Fouillet, B.; Chambon, P. Complete degradation of high concentrations of tetrachloroethylene by a methanogenic consortium in a fixed-bed reactor. *J. Biotechnol.* **1998**, *62*, 133–141.

(11) Kennes, C.; Veiga, M. C.; Bhatnagar, L. Methanogenic and perchloroethylene-dechlorinating activity of anaerobic granular sludge. *Appl. Microbiol. Biotechnol.* **1998**, *50*, 484–488.

(12) Cornell, R. M.; U, S. *The iron oxides-structure, properties, reactions, occurrence and uses*; VCH: Weinheim, Germany, 1996.

(13) Lovley, D. R. Microbial Fe(III) reduction in subsurface environments. *FEMS Microbiol. Rev.* **1997**, *20*, 305–313.

(14) Thamdrup, B. Bacterial Manganese and Iron Reduction in Aquatic Sediments. *Adv. Microb. Ecol.* **2000**, *16*, 41–84.

(15) Lee, M. D.; Odom, J. M.; Buchanan, R. J. New perspectives on microbial dehalogenation of chlorinated solvents: Insights from the field. *Annu. Rev. Microbiol.* **1998**, *52*, 423–452.

(16) Snoeyenbos-West, O. L.; Nevin, K. P.; Anderson, R. T.; Lovley, D. R. Enrichment of Geobacter species in response to stimulation of Fe(III) reduction in sandy aquifer sediments. *Microb. Ecol.* **2000**, *39*, 153–167.

(17) Lovley, D. R. Chapter 7: Reduction of Iron and Humics in Subsurface Environments. *Subsurf. Microbiol. Biogeochem.* **2001**, 193-217.

(18) He, Y. T.; Wilson, J. T.; Wilkin, R. T. Transformation of reactive iron minerals in a permeable reactive barrier (biowall) used to treat TCE in groundwater. *Environ. Sci. Technol.* **2008**, *42*, 6690–6696.

(19) Vogel, T. M.; Criddle, C. S.; McCarty, P. L. Transformations of Halogenated Aliphatic-Compounds. *Environ. Sci. Technol.* **1987**, *21*, 722–736.

(20) Mohn, W. W.; Tiedje, J. M. Microbial Reductive Dehalogenation. *Microbiol. Rev.* **1992**, *56*, 482–507.

(21) Townsend, G. T.; Suflita, J. M. Influence of sulfur oxyanions on reductive dehalogenation activities in Desulfomonile tiedjei. *Appl. Environ. Microbiol.* **1997**, *63*, 3594–3599.

(22) Gerritse, J.; Drzyzga, O.; Kloetstra, G.; Keijmel, M.; Wiersum, L. P.; Hutson, R.; Collins, M. D.; Gottschal, J. C. Influence of different electron donors and acceptors on dehalorespiration of tetrachloroethene by Desulfitobacterium frappieri TCE1. *Appl. Environ. Microbiol.* **1999**, *65*, 5212–5221.

(23) Hinchee, R.; Wilson, J.; Downey, D. *Intrinsic Bioremediation*; Battelle: Columbus, OH, 1995.

(24) Doong, R.; Wu, S.; Chen, T. Anaerobic Biotransformation of Polychlorinated Methane and Ethene under Various Redox Conditions. *Chemosphere* **1996**, *32*, 377–390.

(25) Smidt, H.; Akkermans, A. D. L.; van der Oost, J.; de Vos, W. M. Halorespiring bacteria-molecular characterization and detection. *Enzyme Microb. Technol.* **2000**, *27*, 812–820.

(26) Yang, Y. R.; McCarty, P. L. Competition for hydrogen within a chlorinated solvent dehalogenating anaerobic mixed culture. *Environ. Sci. Technol.* **1998**, *32*, 3591–3597.

(27) He, J. Z.; Sung, Y. B.; Dollhopf, M. E.; Fathepure, B. Z.; Tiedje, J. M.; Loffier, F. E. Acetate versus hydrogen as direct electron donors to stimulate the microbial reductive dechlorination process at chloroethene-contaminated sites. *Environ. Sci. Technol.* **2003**, *37*, 816-816.

(28) Bradley, P. M.; Chapelle, F. H. Kinetics of DCE and VC mineralization under methanogenic and Fe(III)-reducing conditions. *Environ. Sci. Technol.* **1997**, *31*, 2692–2696.

(29) Bradley, P. M.; Chapelle, F. H.; Lovley, D. R. Humic acids as electron acceptors for anaerobic microbial oxidation of vinyl chloride and dichloroethene. *Appl. Environ. Microbiol.* **1998**, *64*, 3102–3105.

(30) Bradley, P. M.; Chapelle, F. H.; Wilson, J. T. Field and laboratory evidence for intrinsic biodegradation of vinyl chloride contamination in a Fe(III)-reducing aquifer. *J. Contam. Hydrol.* **1998**, *31*, 111–127.

(31) Krumholz, L. R.; Sharp, R.; Fishbain, S. S. A freshwater anaerobe coupling acetate oxidation to tetrachloroethylene dehalogenation. *Appl. Environ. Microbiol.* **1996**, *62*, 4108–4113.

(32) Krumholz, L. R. Desulfuromonas chloroethenica sp. nov. uses tetrachloroethylene and trichloroethylene as electron acceptors. *Int. J. Syst. Bacteriol.* **1997**, *47*, 1262–1263.

(33) Sung, Y.; Ritalahti, K. M.; Sanford, R. A.; Urbance, J. W.; Flynn, S. J.; Tiedje, J. M.; Loffler, F. E. Characterization of two tetrachloroethene-reducing, acetate-oxidizing anaerobic bacteria and their description as Desulfuromonas michiganensis sp nov. *Appl. Environ. Microbiol.* **2003**, *69*, 2964–2974.

(34) Sung, Y.; Fletcher, K. F.; Ritalaliti, K. M.; Apkarian, R. P.; Ramos-Hernandez, N.; Sanford, R. A.; Mesbah, N. M.; Loffler, F. E. Geobacter lovleyi sp nov strain SZ, a novel metal-reducing and tetrachloroethene-dechlorinating bacterium. *Appl. Environ. Microbiol.* **2006**, *72*, 2775–2782.

(35) Lovley, D. R.; Phillips., E. J. P. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl. Environ. Microbiol.* **1986**, *51*, 683–689.

(36) Roden, E. E.; Lovley, D. R. Dissimilatory Fe(Iii) Reduction by the Marine Microorganism Desulfuromonas-Acetoxidans. *Appl. Environ. Microbiol.* **1993**, *59*, 734–742.

(37) Wei, N.; Finneran, K. T. Microbial community analyses of three distinct, liquid cultures that degrade methyl tert-butyl ether using anaerobic metabolism. *Biodegradation* **2009**, *20*, 695–707.

(38) Lovley, D. R.; Giovannoni, S. J.; White, D. C.; Champine, J. E.; Phillips, E. J. P.; Gorby, Y. A.; Goodwin, S. Geobacter-Metallireducens Gen-Nov Sp-Nov, a Microorganism Capable of Coupling the Complete Oxidation of Organic-Compounds to the Reduction of Iron and Other Metals. *Arch. Microbiol.* **1993**, *159*, 336–344.

(39) Finneran, K. T.; Lovley, D. R. Anaerobic degradation of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA). *Environ. Sci. Technol.* **2001**, *35*, 1785–1790.

(40) Wilhelm, E.; Battino, R.; Wilcock, R. J. Low-Pressure Solubility of Gases in Liquid Water. *Chem. Rev.* **1977**, *77*, 219–262.

(41) Loffler, F. E.; Tiedje, J. M.; Sanford, R. A. Fraction of electrons consumed in electron acceptor reduction and hydrogen thresholds as indicators of halorespiratory physiology. *Appl. Environ. Microbiol.* **1999**, *65*, 4049–4056.

(42) Lovley, D. R.; Phillips, E. J. P. Rapid Assay for Microbially Reducible Ferric Iron in Aquatic Sediments. *Appl. Environ. Microbiol.* **1987**, *53*, 1536–1540.

(43) Ritalahti, K. M.; Amos, B. K.; Sung, Y.; Wu, Q. Z.; Koenigsberg, S. S.; Loffler, F. E. Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple Dehalococcoides strains. *Appl. Environ. Microbiol.* **2006**, *72*, 2765–2774.

(44) Holmes, D. E.; Finneran, K. T.; O'Neil, R. A.; Lovley, D. R. Enrichment of members of the family Geobacteraceae associated with stimulation of dissimilatory metal reduction in uranium-contaminated aquifer sediments. *Appl. Environ. Microbiol.* **2002**, *68*, 2300–2306.

(45) Stults, J. R.; Snoeyenbos-West, O.; Methe, B.; Lovley, D. R.; Chandler, D. P. Application of the 5′ fluorogenic exonuclease assay (TaqMan) for quantitative ribosomal DNA and rRNA analysis in sediments. *Appl. Environ. Microbiol.* **2001**, *67*, 2781–2789.

(46) Okutman Tas, D.; Pavlostathis, S. G. The influence of iron reduction on the reductive biotransformation of pentachloronitrobenzene. *Eur. J. Soil Biol.* **2007**, *43*, 264.

(47) Cord-Ruwisch, R.; Lovley, D. R.; Schink, B. Growth of Geobacter sulfurreducens with acetate in syntrophic cooperation with hydrogen-oxidizing anaerobic partners. *Appl. Environ. Microbiol.* **1998**, *64*, 2232–2236.

(48) Lovley, D. R.; Goodwin, S. Hydrogen Concentrations as an Indicator of the Predominant Terminal Electron-Accepting Reactions in Aquatic Sediments. *Geochim. Cosmochim. Acta* **1988**, *52*, 2993–3003.

(49) Chapelle, F. H.; Haack, S. K. Comparison of Eh and H2 measurements for delineating redox processes in a contaminated aquifer. *Environ. Sci. Technol.* **1996**, *30*, 3565.