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Role of Iron and Vitamin B12 Amendments in Stimulating Reductive Dechlorination of TCE in High Sulfate Groundwater

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ABSTRACT: A column study was performed to test the effectiveness of magnetite powder and emulsified vegetable oil (EVO) for their ability to support the reductive dechlorination of trichloroethylene (TCE) in a high sulfate environment. The study demonstrated that while magnetite was effective at controlling sulfide concentrations within the amended columns, additional nutrients, particularly vitamin B12, were required to promote the complete reductive dechlorination of TCE to ethene. This result was surprising, given that complete reductive dechlorination of TCE to ethene was observed in microcosms which received similar amendments. However, reductive dechlorination in the columns slowed at cis-1,2-dichloroethylene (cDCE) and vinyl chloride (VC) unless vitamin B12 was supplied on a regular basis. This may be due to washout of vitamin B12 from the columns under flow conditions and coprecipitation of the cobalt co-factor due to reaction with sulfide. The latter process may make the cobalt unavailable to the dechlorinating bacteria and limit the bacterial manufacture of enzymes required to reduce VC to ethene. These findings show that both sulfide mitigation and vitamin B12 play a critical role in supporting biological degradation of TCE in high sulfate environments.

INTRODUCTION

High sulfate environments present particular challenges for the application of enhanced in situ bioremediation (EISB) of chlorinated compounds. Sulfate reducers compete with dechlorinating bacteria for electron donor and sulfate is reduced to hydrogen sulfide, high concentrations of which can be inhibitory to biological processes such as donor fermentation and reductive dechlorination. Previous work in this area by the General Electric Company's (GE's) Global Research Center has shown that the addition of iron amendments can effectively suppress sulfide accumulation in high-sulfate environments (Matis et al., 2015). In this situation, the iron is reduced (where an oxidized form is used) and the ferrous iron formed reacts with sulfide to form insoluble iron sulfide precipitates. Microscale magnetite powder was particularly effective at sulfide mitigation in microcosm studies.

A column study was performed to test the effectiveness of magnetite powder and EVO for their ability to support the reductive dechlorination of 200 milligrams per liter (mg/L) of TCE in a high (600-900 mg/L) sulfate environment using crushed gypsum bedrock and groundwater obtained from an industrial site in Upstate New York. The 400-day study was designed to evaluate the effect of magnetite dosage combined with EVO and supplemental nutrients on degradation of TCE under realistic groundwater flow conditions. The study consisted of three columns, two of which were amended with EVO and varying amounts of magnetite powder, while the third was a control that received neither EVO nor magnetite and therefore represented current site conditions (Table 1).

MATERIALS AND METHODS

The column study was performed in three glass chromatography columns (50 millimeters [mm] in diameter by 60 centimeters [cm] in length) fitted with threaded Teflon® endcaps and sampling ports located at equally spaced intervals along the column length

(Figure 1). Each column was packed by gently adding crushed gypsum bedrock to the column in small increments by hand while non-sterile site groundwater was pumped into the bottom of the column using a peristaltic pump. Magnetite powder was distributed throughout Column 2 (12.2 grams) and Column 3 (48.8 grams) during this setup phase and occupied approximately 0.5 and 2% of the available porosity, respectively. About 15 grams of black, fine-grained sediment removed under anaerobic conditions from monitoring wells at the site was also added at four to five points over the length of the columns to provide a source of the native dechlorinating bacteria.

TABLE 1. Column study setup.

Column	Type	Rock Added (g)	Magnetite Added (g)	Oil Retained (g)
1	Control	1553	None	None
2	Active	1573	12.2	15.9
3	Active	1582	48.8	15.8

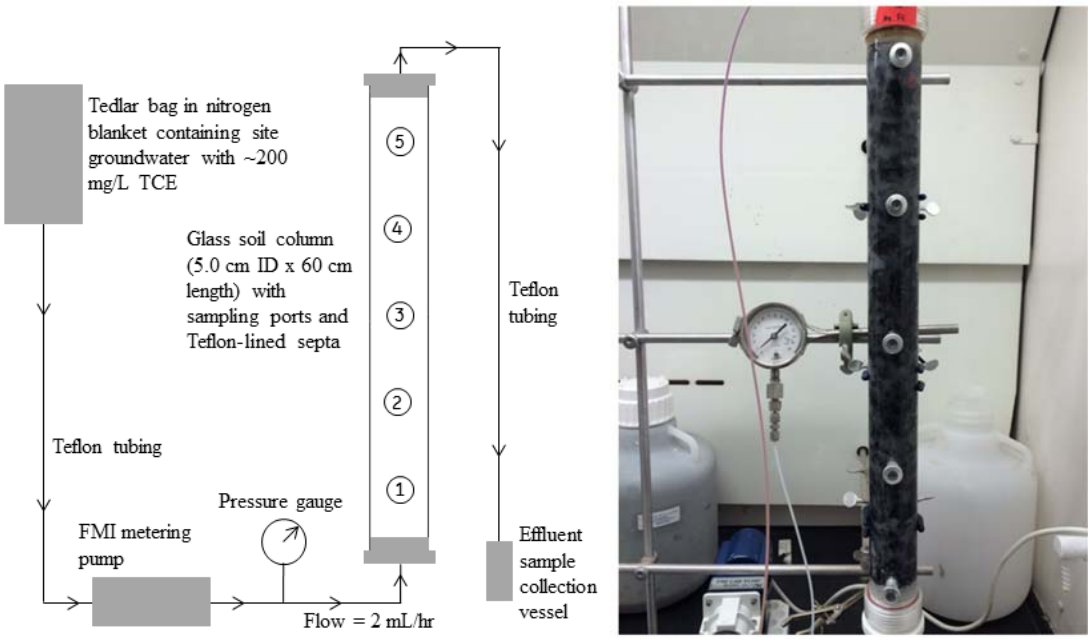

FIGURE 1. Schematic and photo of column set-up, showing numbering of column ports.

Non-sterile site groundwater spiked with ~200 mg/L TCE was stored in a 3-liter Tedlar® bag and pumped through each column using a QG 6 positive displacement metering pump at a nominal flowrate of 2 milliliters per hour (mL/hr). A tracer test using sodium bromide was conducted prior to introduction of the EVO. The total porosity in the columns ranged from 0.456-0.475 and the tracer test yielded mean residence times between 11.0-11.5 days, which was close to the theoretical value. However, the initial breakthrough of bromide was very early in all the columns and the bromide displayed a long, extended tail after the mean

residence time was reached, indicating both the existence of preferential flow paths and the potential for diffusion of bromide into the porous rock matrix.

EVO was added to the active columns as a 5% solution in water. Two-thirds of one pore volume (~0.36 L) of EVO solution containing diammonium phosphate (DAP), lactate, and vitamin B12 was pumped into each active column at 150% of the nominal flowrate. The oil used was EOS®100, produced by EOS Remediation. The DAP and lactate were added at 1% and 2% by weight of the oil loading, respectively. Vitamin B12 was added per the producer's recommendation of 1.15 milliliters (mL) of vitamin B12 solution per 500 mL of EVO concentrate. The amendment solutions were maintained in stirred glass jars during addition. After addition, flow to the columns was suspended for 72 hours to allow the EVO to sorb to the crushed bedrock and fine-grained sediment. The Tedlar® bags were reattached, flow was restarted at the nominal flowrate, and the EVO retention in each column was measured. EVO retention was approximately 93% in each column.

Following the oil loading phase, the columns were operated for approximately 13 months from the start of amendment addition to the end of the study. A wide range of analyses were periodically performed on groundwater samples collected from the columns during this time. TCE and its biodegradation products (i.e., cDCE, VC, ethene), acetylene, methane, pH, total organic carbon (TOC), chloride, sulfate, sulfide, dissolved iron, volatile fatty acids (VFAs), *Dehalococcoides* (Dhc) bacteria and vinyl chloride reductase (vcr-A) counts were measured over time at various locations within the column as well as in the influent and effluent, such that both biological and abiotic degradation processes could be monitored over both time and position within the columns.

To quantify the progress of reductive dechlorination in each column, the chloride number was calculated. This composite measure of dechlorination is defined as:

$$\text{Chloride Number} = (\text{moles TCE} \times 3 + \text{moles cDCE} \times 2 + \text{moles VC}) / (\text{moles TCE} + \text{moles cDCE} + \text{moles VC} + \text{moles ethene})$$

where lower chloride numbers indicate greater extent of dechlorination and a chloride number of zero represents complete dechlorination to ethene.

Total chlorine molar balances were also calculated throughout the columns by summing chlorine incorporated into the VOCs as well as free chloride. This was helpful in monitoring the dechlorination process because the chlorine is neither created nor destroyed and provides some particularly useful process insights.

RESULTS AND DISCUSSION

Control Column. The influent VOC concentration was stable in the control column (Column 1) during the study. A limited amount of reductive dechlorination was observed in the control column, such that cDCE was produced from TCE along with much lower amounts of VC (Figure 2a). Most of the dechlorination activity took place very early in the column and was largely complete by Port 2. This dechlorination activity is similar to that seen in the field and was likely supported by residual acetone in the groundwater feed. Chlorine balances in the control column initially showed greater influent than effluent chlorine concentrations, suggesting that VOCs were partitioning into the porous matrix or the organic carbon present in the column. However, the chlorine balance closed by Day 53 and was very good for the rest of the study.

After an initial increase due to sulfate dissolution from the gypsum bedrock, effluent sulfate concentrations from the control column stabilized at approximately the same concentration as the influent. This suggests that sulfate reduction was not significant in the control column. Dhc counts in the effluent of the control column ranged from 1×10^5 to $1 \times$

10^6 cells per liter (cells/L) throughout the study and are considered low to moderate (Figure 3a). Vcr-A counts were at or near the quantification limits for most of the study, consistent with the low levels of VC generated in the column (Figure 3b).

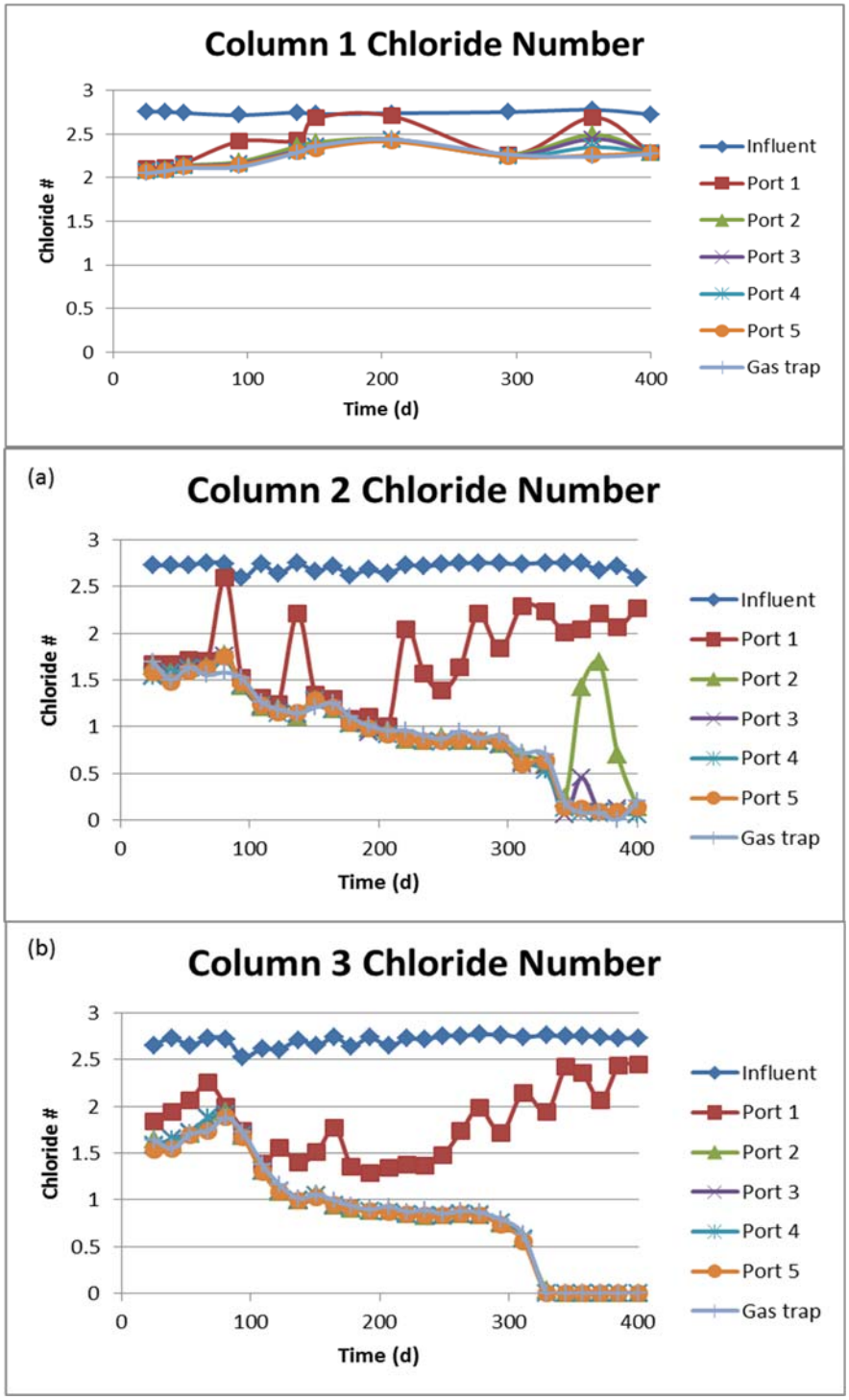

FIGURE 2. Chloride number over time in Columns 1-3.

Active Columns. The influent VOC concentrations were also quite stable in both active columns (Columns 2 and 3). A moderate amount of reductive dechlorination was observed in the columns during the first two months of operation, such that the TCE was almost completely depleted and approximately equimolar amounts of cDCE and VC were being generated (Figures 2b and 2c). Very little ethene was observed at this time. Most of the dechlorination activity took place very early in the columns and was largely complete by Port 2. This was surprising because the EVO was well distributed throughout the column and TOC levels remained elevated in the effluent, suggesting that donor limitation was not the issue. The chloride number varied from 1.5 to 2.0 during this period and was increasing rather than decreasing, while the chlorine balances indicated that VOCs were being sorbed into the bedrock matrix.

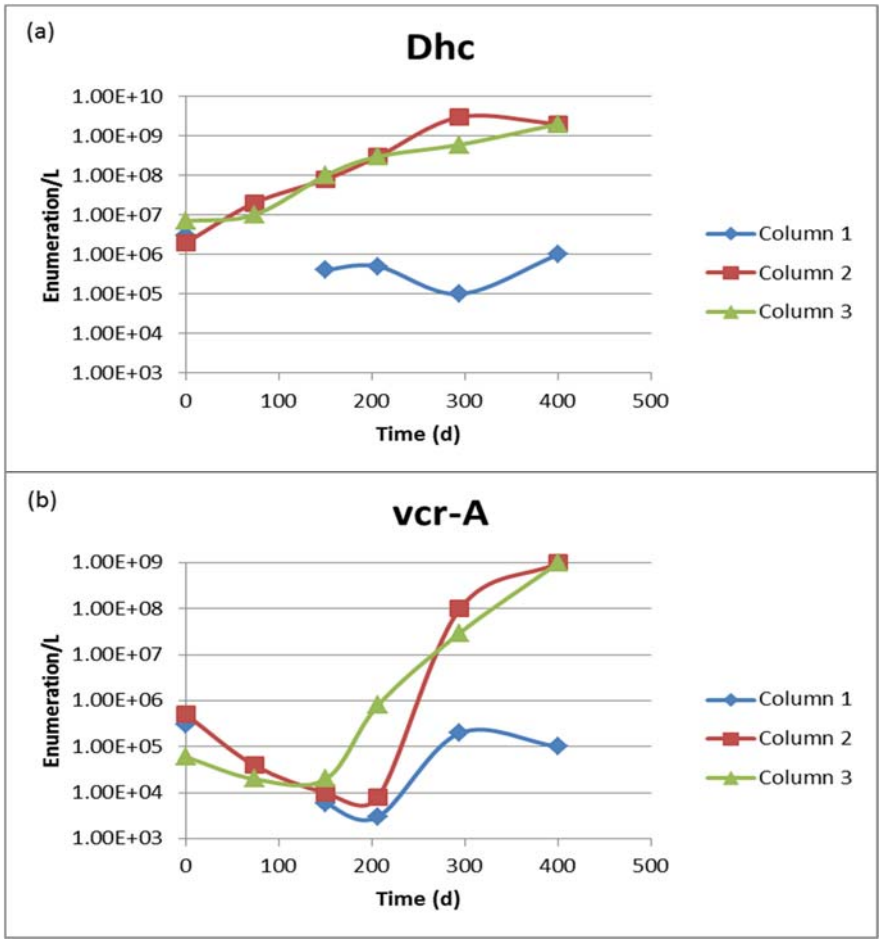

FIGURE 3. Dhc and vcr-A levels over time in the control and active columns.

Sulfate in both the active columns was rapidly eliminated during this period. Sulfate reduction primarily occurred within the early portion of the columns (up to Port 2). Although sulfide was formed in this process, significant levels of ferrous iron were also generated by the reduction of the magnetite, so that sulfide levels in the columns were initially low. Measurements of Dhc and vcr-A in the effluent of the active columns indicated that the population of Dhc bacteria was increasing during this period, but vcr-A was low and appeared to be declining (Figures 3a and 3b).

These VOC results were significantly different than what had been observed in microcosm studies performed with similar amendments (Matis et al., 2015). The largest single difference between the microcosm bottles and the active columns was the potential impact of nutrients. Nutrients in the form of vitamin B12 and DAP were added to both the bottles and the active columns, but would have stayed in the bottles versus being washed out of the columns under flow conditions. This suggested that lack of nutrients could be contributing to the lower dechlorination activity observed in the active columns.

Additional nutrients were introduced into the two active columns on Days 77 and 94 of the study. This was achieved by adding vitamin B12 to the influent bags at a concentration of approximately 22 micrograms per liter (µg/L) and injecting vitamin B12, DAP, and yeast extract (YE) into Port 2 of the columns (25 microliters [µL] vitamin B12 solution, 0.75 mL 1 molar [M] DAP solution, and 0.625 mL 10 grams per liter [g/L] YE solution). The DAP and YE were not added to the influent bags to prevent the stimulation of reductive dechlorination in the feed. Periodic addition of nutrients was continued throughout the remainder of the study at a frequency of once every two to four weeks, particularly when the ammonia concentration in the effluent decreased below approximately 5 mg/L.

The nutrient addition had a rapid and significant impact on the rate of dechlorination in the two active columns (Figures 2a and 2b). The trend in chloride number was reversed, as any residual TCE in the columns was degraded to cDCE, which in turn was degraded to VC, until dechlorination progressed to the point where VC was the primary constituent in each column and ethene generation began to increase. The total VOC concentrations in the columns also increased, as the faster rate of TCE dechlorination produced higher levels of daughter products in the aqueous phase. In addition, the chlorine balances also reversed and showed excess chlorine exiting the columns, presumably due to VOCs partitioning back out of the bedrock matrix. The chlorine balance eventually reached equilibrium around Day 178. Dhc counts increased throughout this period, but vcr-A counts did not respond immediately.

Eventually the extent of dechlorination observed in the active columns again appeared to slow down. This time the residual VOCs were VC and ethene. This slowdown was readily observed in the chloride number plots, where the rate of change over time flattened between 150 and 225 days (Figures 2a and 2b).

Based on these data, the two active columns were bioaugmented with SDC-9™ culture on Day 238 to determine if the dechlorination rates was limited by the bacterial population. SDC-9™ is a commercial bioaugmentation culture used in some of the microcosm work conducted in advance of this column study. The culture was added to Ports 1 and 2 (2 mL into each) of both active columns. However, this bioaugmentation did not result in a significant change in the chloride numbers, suggesting that bioaugmentation did not have a significant effect on VOC concentrations or degradation progress.

In response, the dose of vitamin B12 spiked into the influent bags was quadrupled to 88 µg/L on Day 295, approximately two months after bioaugmentation. The increase in the amount of vitamin B12 added to the active columns resulted in a notable decrease in VC concentrations with concurrent increases in ethene production in both active columns within two weeks, and by one month after the vitamin B12 increase the VC in Column 3 was completely converted to ethene. Column 2 showed about 90 molar percent conversion of VC to ethene but did not progress further. At this point in time sulfide concentrations in Column 2 were increasing and persisting further along the column than in Column 3 (Figures 4a and 4b). This is most likely due to depletion of the magnetite or a decrease in the bioavailability of iron from magnetite. Localized sulfide toxicity may therefore have slowed or inhibited the complete degradation of VC in Column 2.

Bioassays now showed increasing populations of both Dhc and vcr-A (Figure 3a and 3b). Because levels of Dhc in both active columns and vcr-A in Column 3 were already

trending upwards after the increase in nutrient addition and prior to bioaugmentation, it is unclear whether these subsequent increases in Dhc and vcr-A were due to the bioaugmentation with SDC-9™ or to the additional vitamin B12.

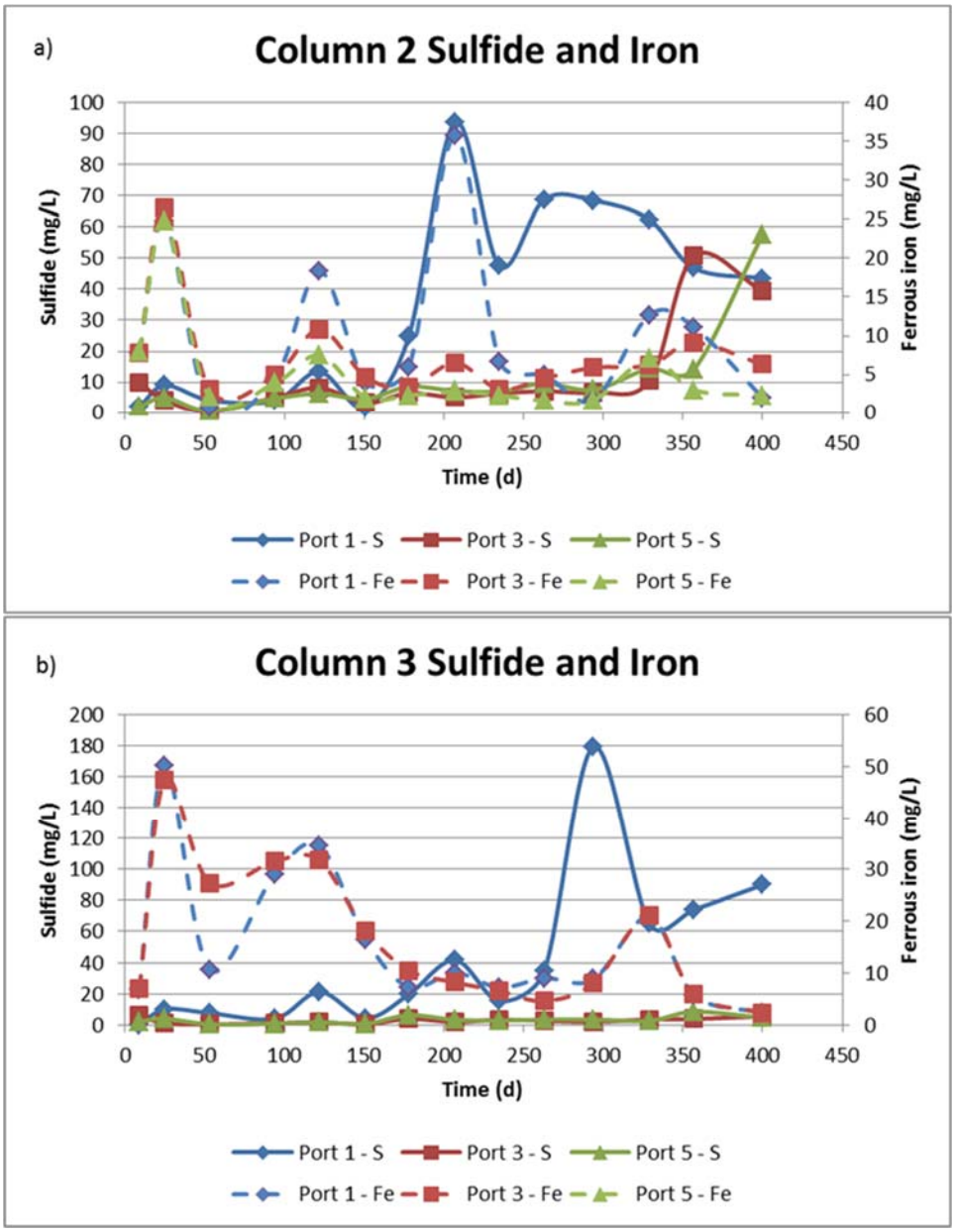

FIGURE 4. Hydrogen sulfide and dissolved iron in Ports 1, 3, and 5 of Columns 2 and 3.

Cobalt is present in vitamin B12 and is known to be an essential element for growth of Dhc bacteria and proper function of the vcr-A gene (Maymo-Gatell et al., 1995; He et al., 2007). The sulfide generated in the columns not only reacts rapidly with ferrous iron, but also with many other metal species in solution, including cobalt (Vaughan and Craig, 1978; Morse and Arakaki, 1993). Although vitamin B12 was added to the influent bags and lower ports of the columns, it is hypothesized here that the overall amount of cobalt was originally

not sufficient either because it was washing out of the columns or was reacting with the elevated sulfide present and precipitating in a non-bioavailable form. Increasing the amount of vitamin B12 during the column study produced much better dechlorination results.
These data indicate that availability of cobalt through the vitamin B12 was a critical factor in producing the complete reductive dechlorination of TCE to ethane in this study.

Finally, iron sulfide minerals were produced in this study and are known to be capable of supporting the abiotic reduction of TCE and cDCE (Butler and Hayes, 1999; Butler and Hayes, 2001). The addition of cobalt as a co-precipitating metal has also been shown to enhance iron sulfide reactivity (Jeong and Hayes, 2007). However, a distinguishing characteristic of this abiotic degradation pathway is that daughter products associated with biotic reductive dechlorination (e.g., cDCE and VC) are not produced. In contrast, TCE is typically reduced to acetylene, which is labile and rapidly degraded to carbon dioxide in the environment. Samples of the gas headspace in the effluent collection vessels were analyzed for acetylene several times during the column study, but no acetylene was detected. In addition, the production of daughter products and relatively good VOC molar balances between TCE, cDCE, VC, and ethene were observed throughout the columns. While these data cannot exclude the possibility that some abiotic degradation occurred, they suggest that iron sulfide-mediated abiotic processes were not the major contributor to the VOC degradation observed in this study.

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