

ENHANCED REDUCTIVE DECHLORINATION AND THE RELATIONSHIP BETWEEN CIS-1,2-DCE ACCUMULATION AND METHANOGENESIS

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ABSTRACT: Enhanced reductive dechlorination is a viable technology for treating chlorinated ethenes whether implemented through the addition of Hydrogen Releasing Compound (HRC[®]), molasses, sodium lactate, vegetable oil, or other organic carbon sources. However, no technology works equally well at every site, and it is important to understand the complexities of the local microbial community. This paper describes a problem that appears relatively common at sites undergoing enhanced reductive dechlorination, the accumulation of cis-1,2-dichloroethene (cis-1,2-DCE).

Enhanced reductive dechlorination was stimulated by two HRC[®] applications made at a dry cleaning site in Florida. The first application principally targeted the shallow surficial aquifer with 147 direct injection points and approximately 6,000 lbs. of HRC[®]. The second application occurred 18 months later and targeted the lower surficial and intermediate aquifers with 128 delivery points and an additional 3,200 lbs. of HRC[®]. As a consequence of the first application, PCE and TCE were quickly dechlorinated to cis-1,2-DCE and to some vinyl chloride (VC) in the shallow surficial aquifer indicating the occurrence of HRC[®] mediated dechlorination. The limited amount of HRC[®] applied to the lower surficial aquifer during the first application presumably prevented appreciable dechlorination in the deeper groundwater. The latter HRC[®] delivery was focused on the deeper groundwater; however, the anticipated complete dechlorination did not occur, resulting in a dramatic increase in the cis-1,2-DCE concentration in the lower surficial aquifer.

The accumulation of cis-1,2-DCE in a biologically active aquifer provided an opportunity to investigate the reasons for the incomplete reductive dechlorination reaction. Groundwater samples were routinely analyzed for chlorinated ethenes, ethene, ethane, methane, pH, oxidation reduction potential, and dissolved oxygen. In addition to these analyses, soil and groundwater samples were also analyzed for volatile fatty acids (VFA), *Dehalococcoides ethenogenes*, and bioavailable iron. Vertical and horizontal groundwater gradients were examined to evaluate the movement of cis-1,2-DCE between the surficial and intermediate aquifers.

Using the analytical results, a conceptual model of the site emerged to explain the behavior of chlorinated ethenes in the aquifer. *D. ethenogenes* was present, only a small amount of bioavailable iron was present, and HRC[®] released lactic acid that was further degraded to other VFA. The accumulation of cis-1,2-DCE was directly correlated with methanogenesis such that when the electron donor (HRC[®], lactate, and VFA) concentration was high, methanogenesis and partial dechlorination reactions dominated but as the electron donor (and hydrogen concentration) decreased, methanogenesis subsided and cis-1,2-DCE dechlorination began to occur. These observations suggested that complete dechlorination of chlorinated ethenes depended on the abundance of

electron donating compounds. Although this behavior may be specific to this site, the methods and approach used to investigate the competing processes provide a framework to assess sites for the potential for successful reductive dechlorination and to diagnose sites that behave in undesirable or unexpected ways.

INTRODUCTION

The biological reductive dechlorination reaction stimulated by the addition of biodegradable organic carbon depends on two different microbial processes. In this case, Hydrogen Release Compound[®] (HRC[®]), Regensis Bioremediation Products, Inc., was used as the source of organic carbon. The first process is the biodegradation of lactic acid that is slowly released from the HRC[®]. This process generates hydrogen that supports the second reaction, reductive dechlorination. Different types of bacteria catalyze the two processes and both types must be present for reductive dechlorination to occur. Lactic acid fermenting bacteria are extremely common and would never be expected to limit the reaction. However, reductive dechlorinating bacteria (also known as halo-respirers) may not be as widespread (Major, et al., 2002). They may be less robust in their ability to compete with other microbial populations in the subsurface. *Dehalococcoides ethanogenes* is one species known to dechlorinate chlorinated ethenes

The upper portion of the surficial aquifer at the site generally consists of sand and silty sand to a depth of approximately 29 feet below land surface (bls). It is underlain by approximately 3 feet of sandy clay and clay which separates it from the lower portion of the surficial. The average linear groundwater velocity in the upper portion of the surficial aquifer was calculated as 16 feet/year.

Two applications of HRC[®] have been made at the subject dry cleaner site (Kean et al., 2002). The first application was completed as part of a pilot study for the upper surficial aquifer (6-30' bls) in February 1999. The second application of HRC[®] was completed in August 2000 and was designed to act as part of the total remedial action for groundwater restoration. The second HRC[®] application's primary target was the lower surficial aquifer (30-50' bls).

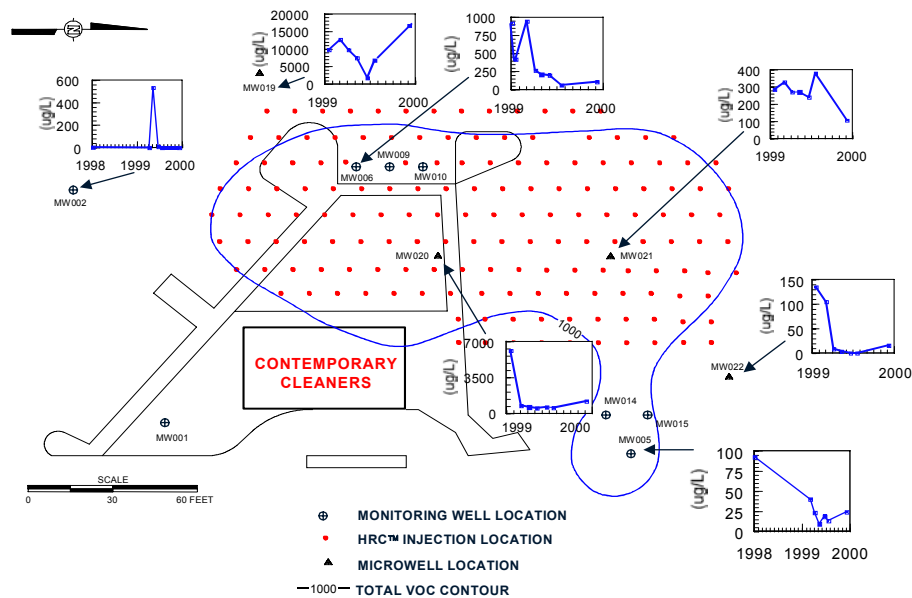


FIGURE 1. Location of initial HRC[®] injection points. Pilot Test.

The first application of HRC[®] targeted the shallow surficial aquifer with 147 delivery points as shown in Figure 1. The proposed amount of HRC[®] to be injected was revised by the manufacture prior to the start of the pilot test from 3,400 pounds to 6,200 pounds. The amount was doubled since HRC[®] was diluted to make it less viscous in order to facilitate injection of the compound. The amount injected varied at each injection point from .5 lb/ft to 2.2 lbs/ ft., and the points were located on 10-foot centers creating a grid of approximately 80 feet by 180 feet.

The second application occurred 18 months later and targeted the lower surficial and intermediate aquifers with 128 delivery points and an additional 3200 lbs. of HRC[®]. The locations of the delivery points for the second application are shown in Figure 2.

The first HRC[®] application resulted in dramatic decreases in PCE and TCE concentration in the shallow surficial aquifer. Based on the success of the first application, a second application was made in the lower surficial aquifer. However, the results were not as favorable. High concentrations of cis-1,2-DCE accumulated and persisted. The reason for the poor performance in the lower surficial aquifer was examined. Specific issues evaluated included:

- The influence of drought conditions causing an increased flux of DCE from the shallow surficial aquifer into the lower surficial aquifer.
- The production and persistence of lactic acid and other metabolic acids.
- The presence of *Dehalococcoides ethenogenes*.
- Geochemical conditions that may interfere with reductive dechlorination.

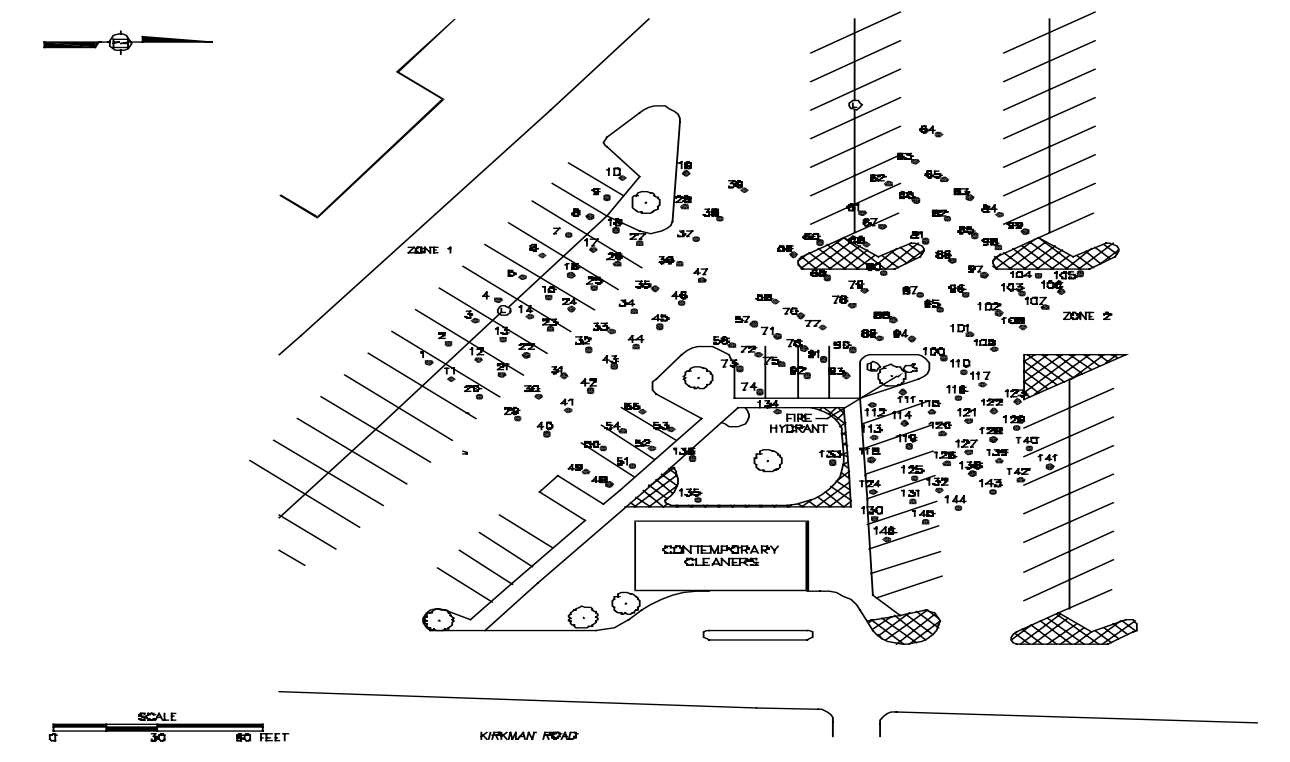


FIGURE 2. Location of injection points for the second HRC[®] application.

MATERIALS AND METHODS

Groundwater was collected from the site using a low flow micropurge method, and soil was collected using a Geoprobe rig. Microbiological, geochemical, and organic acid samples were preserved by chilling to 4°C. Samples for volatile organic analysis were preserved by adjusting the pH to less than 2. All samples were shipped by overnight courier along with a chain of custody.

Samples for microbiological analysis were submitted to DuPont CRG and to Microbial Insights for PCR analysis to detect *D. ethenogenes*. Organic acids were analyzed by gas chromatography. Chlorinated ethenes were analyzed using US EPA SW846 Method 8021, dissolved inorganic ions were analyzed as follows: EPA Method 6010 (manganese, phosphorus, and iron [filtered and un-filtered]), and EPA Method 325.3 (chloride). Total dissolved solids were analyzed by EPA Method 160.1. Methane, ethene, and ethane were analyzed by US EPA draft method RSK175. Total organic carbon and alkalinity were measured using EPA Method 415.1 and EPA Method 310.1, respectively. Dissolved oxygen, pH, ferrous iron, oxidation reduction potential, and depth to water were measured on site using various probes and commercially available test kits.

RESULTS AND DISCUSSION

PCE was unintentionally released into the subsurface at this dry cleaner site. HRC[®] was applied to stimulate biodegradation by reductive dechlorination. The initial application made in February 1999 into the shallow surficial aquifer was considered successful based on the rapid removal of PCE. An additional application of HRC[®] was made in the lower surficial and intermediate aquifers in August 2000 and the anticipated dechlorination of PCE was observed; however, the reaction was not complete as indicated by the accumulation and persistence of cis-1,2-DCE (Figures 3, 4). In particular, the cis-1,2-DCE concentration increased in the lower surficial and intermediate aquifers to concentrations higher than anticipated based on the observed PCE concentrations. Investigations were undertaken to determine the cause of the undesirable accumulation of DCE.

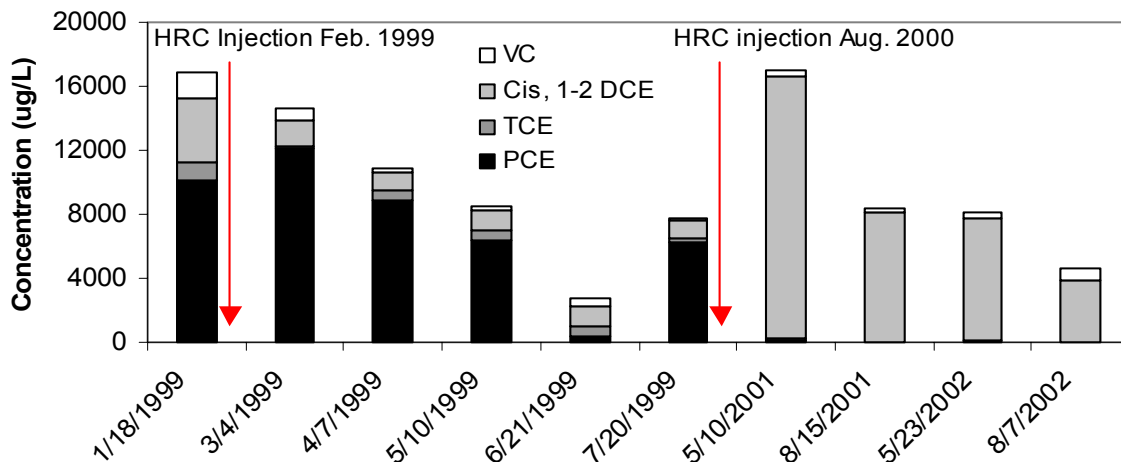


FIGURE 3. Total VOCs in the Upper Aquifer.

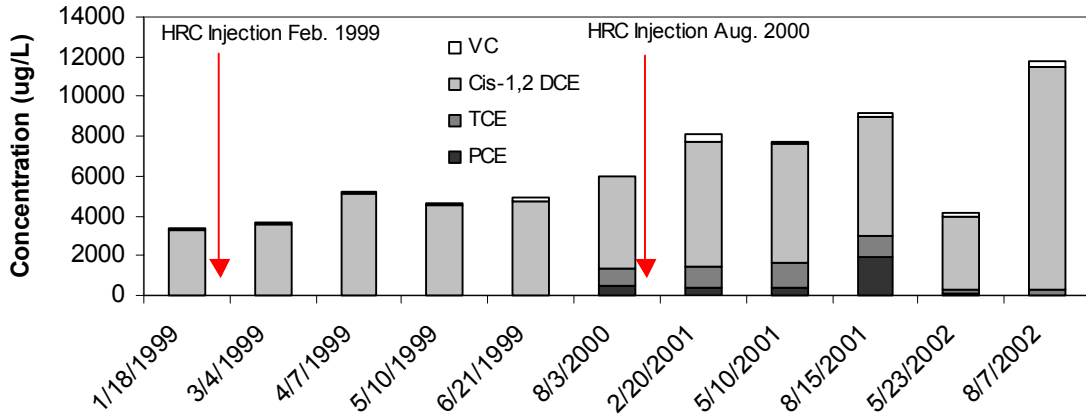


FIGURE 4. Total VOCs in the Lower.

From 1999 to 2001, Florida experienced dryer than normal conditions. This resulted in a decrease in the groundwater elevation and an increase in the vertical hydraulic gradient at the site. A site wide correlation between DCE concentration and groundwater elevation was performed using normalized data. As shown in Figure 5, no correlation existed between DCE concentration and groundwater elevation. Therefore, hydraulic gradient changes due to drought conditions were not an apparent cause of the increasingly high DCE concentrations in the lower surficial and intermediate aquifers.

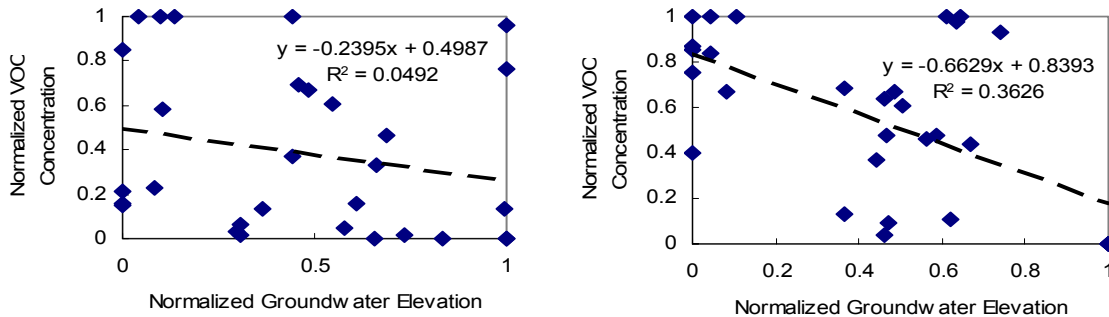


FIGURE 5. Correlation of normalized VOC concentration and normalized groundwater elevation.

The presence and persistence of organic acids to stimulate reductive dechlorination conditions in both the shallow and lower aquifers were examined. Table 1 indicates that while the total organic acid concentration of the groundwater was variable, organic acids were generally available. This observation suggested that reductive dechlorination was probably not stalled due to lack of a biodegradable, hydrogen-generating carbon source.

TABLE 1. Total organic acid concentration in shallow and intermediate surficial aquifers.

Date	Total Organic Acids (mg/L)				
	MW006	Shallow MW019	MW020	Intermediate MW014	MW023
04/07/99	0	0	138	0	0
05/10/99	106	0	180	0	23
06/21/99	108	0	410	8	32
07/20/99	40	0	331	33	8
02/20/01	0	29	0.2	833	0.1
05/10/01	2	4	27	0.3	1
08/15/01	NA	NA	NA	29	69

In addition to having an appropriate carbon source, a competent microbial population is also required. *D. ethenogenes* has been identified as a bacterium capable of completely dechlorinating chlorinated ethenes. The presence of this microbe or very closely related ones is considered highly indicative of the potential for a site to completely dechlorinate PCE.

Groundwater samples were submitted to two different laboratories for the detection of *D. ethenogenes* using PCR targeted to the 16s rRNA of the microbe. The results conflicted (Table 2). One lab detected *D. ethenogenes* and the other did not. Reasons for the discrepancy may include the use of different primer sets for the PCR reaction or lower sensitivity in one lab compared to the other. The positive detection of *D. ethenogenes* in all samples submitted to one of the labs provided sufficiently strong evidence to suggest that an appropriate halo-respiring microbial population was present in the aquifer to facilitate reductive dechlorination.

Aquifer geochemistry plays a significant role in the type of microbial processes that occur. A variable but relatively high iron and sulfide concentration was observed in the groundwater (Table 3). Iron and sulfate respiration are both suspected to be competitively inhibitory to reductive dechlorination, especially for lesser chlorinated compounds such as DCE.

TABLE 2. Analysis of *Dehalococcoides ethenogenes* by PCR using 16s rRNA primers.

Client Sample ID	Microbial Insights Primer Set Band Intensity Score*	DuPont Primer Set Band Intensity Score*
MW009/GW329	-	++
MW023/GW330	-	++
MW014/DW331	-	++
Ref. Standard	+++	+++
Neg. Control	-	-

* +++ = high band intensity, ++ = moderate band intensity, + = low band intensity, - = no band

TABLE 3. Selected monitor well geochemical characteristics of groundwater within the treated area.

Date	Nitrate (mg/L)	Sulfate (mg/L)	Hydrogen Sulfide (mg/L)	Filtered Iron (mg/L)	Total Iron (mg/L)	Ethane (mg/L)	Ethene (mg/L)	Methane (mg/L)
MDL	0.05	1	0.05	0.005	0.005	0.001	0.001	0.001
1/19/99	0.6	BDL	0.08	3.49	13	BDL	BDL	4.7
3/4/99	1.5	BDL	3.2	4.53	4.8	BDL	BDL	7.7
4/7/99	0.93	BDL	5	3.64	4.6	BDL	BDL	7.5
5/10/99	0.69	BDL	1.5	4.91	5.3	BDL	BDL	12
6/21/99	0.55	BDL	>5.0	18.6	21	BDL	BDL	13
7/20/99	0.62	3	1.8	20.6	21	BDL	BDL	21
8/3/00	BDL	BDL	>5.0	2.8	-	BDL	BDL	8.7
2/20/01	0.36	3	-	2.38	2.7	BDL	BDL	125
5/10/01	0.65	2.1	5	0.98	1.9	BDL	BDL	54
8/15/01	BDL	BDL	3	1.98	2.1	BDL	0.001	12
5/23/02	-	-	0.8	-	3.9	BDL	BDL	2.14

BDL=Not detected above the Method Detection Limit for the analyte.
mg/L-milligrams per liter

Methane was present in high concentrations in several wells (Table 3). This observation along with the almost total absence of ethene and ethane, suggests that methanogens may also be out competing the halo-respiring bacteria. Nitrate and manganese were detected, however, the concentrations were low enough that these compounds were not suspected to be involved in the DCE accumulation observed on site, especially considering that PCE dechlorination had occurred under the same conditions. Other geochemical parameters such as dissolved oxygen, oxidation reduction potential, alkalinity, total organic carbon, chloride, and pH were consistent with an anaerobic aquifer having conditions suitable for reductive dechlorination.

CONCLUSIONS

From the analytical results, a conceptual model of the site emerged to explain the behavior of chlorinated ethenes in the aquifer. *D. ethenogenes* was present, only a small amount of bioavailable iron was present, HRC[®] released lactic acid that was further degraded to other VFA, and high concentrations of methane accumulated in groundwater following HRC[®] applications. The accumulation of cis-1,2-DCE was positively correlated with methanogenesis such that when the electron donor (HRC[®], lactate, and VFA) concentration was high, methanogenesis and partial dechlorination reactions dominated but as the electron donor (and presumably hydrogen concentration) decreased, methanogenesis subsided and cis-1,2-DCE dechlorination began to occur (Figure 6, 7).

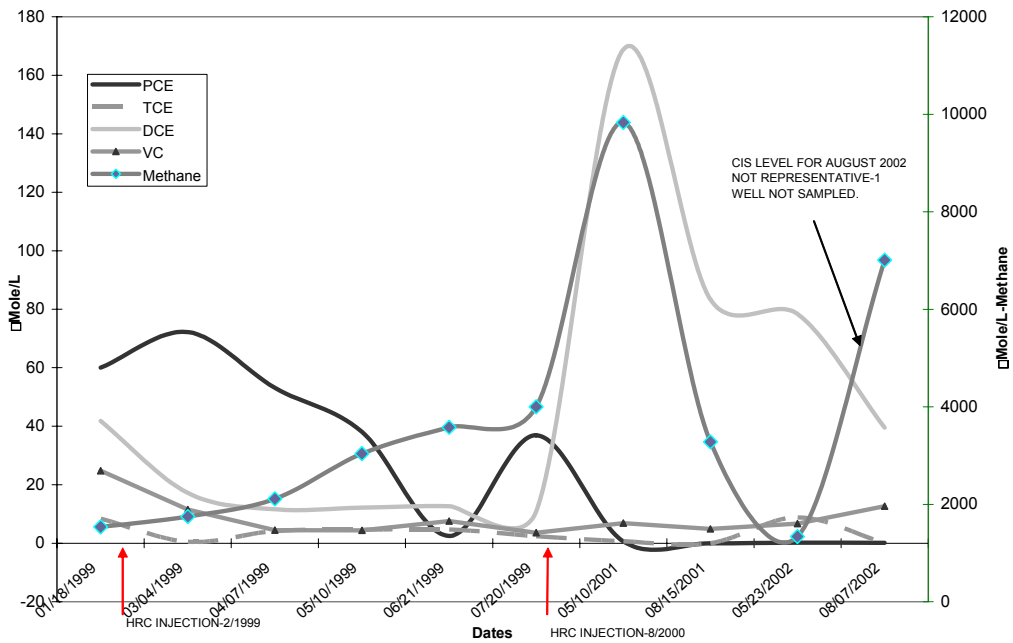


FIGURE 6. Correlation between the accumulation of cis-1,2-DCE and methane in the upper surficial aquifer.

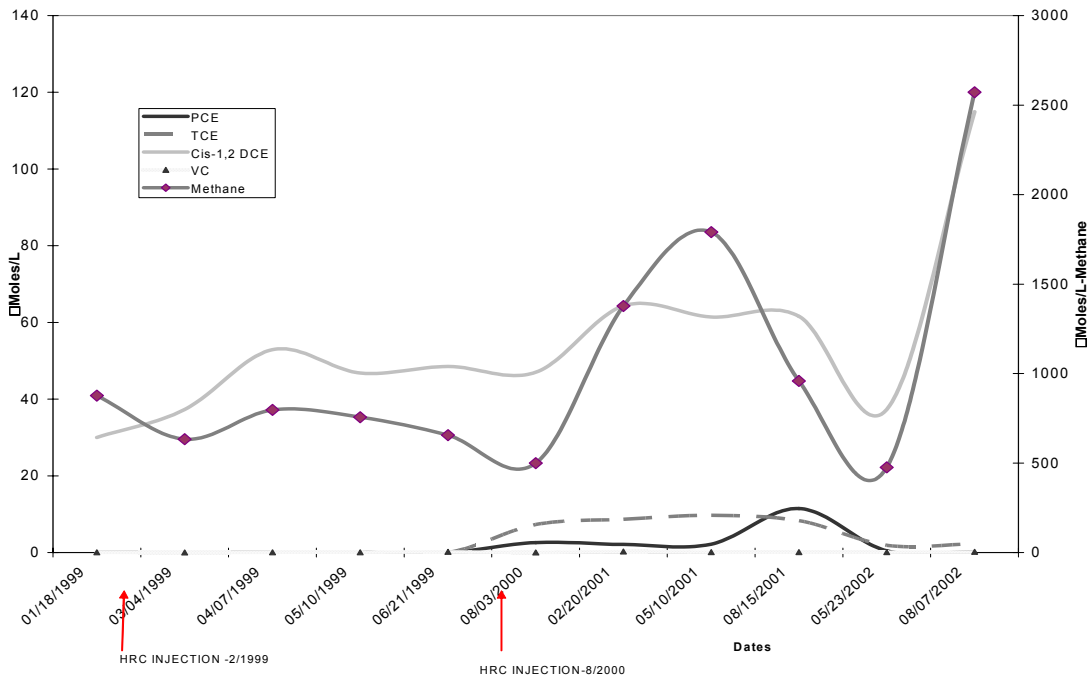


FIGURE 7. Correlation between the accumulation of cis-1,2-DCE and methane in the lower surficial aquifer.

Microbial terminal electron-accepting processes (Fe III, sulfate, methanogenesis) control the concentrations of H in groundwater systems. Significantly, each has a different affinity for H uptake. Iron and sulfate respiration are both suspected to be competitively inhibitory to reductive dechlorination, especially lesser chlorinated compounds such as DCE. Methane was present in high concentrations in several wells after the second injection of HRC[®]. This observation along with the absence of ethene and ethane, suggests that methanogens were out-competing the halo-respiring bacteria.

As noted in Figure 8, a conceptual relationship emerges between Cis-1,2-DCE accumulation and methanogenesis. HRC[®] provides lactate to the groundwater and even though it is slowly released, the overall lactate concentration can raise shortly after the application. This was confirmed by the potential of HRC[®] to dramatically depress the pH shortly after application. Lactate is fermented to other simpler volatile fatty acids and eventually to acetate with the production of hydrogen. Due to the high hydrogen and acetate concentration, methanogens easily compete with *Dehalococcoides* for hydrogen and the degradation of cis-1,2-DCE is arrested. After the lactate and acetate concentrations decline, methanogens become less active, less hydrogen is available, *Dehalococcoides* regains its competitive advantage and the dechlorination of cis-1,2 DCE resumes as indicated by the decrease in DCE concentration and the production of VC and ethene. There is always stiff competition in an aquifer for available hydrogen and carbon. Whichever microbes are best at scavenging the carbon and hydrogen are the ones that dominate the overall microbial population. The dominant population changes depending on the availability of electron acceptors, electron donors, and hydrogen.

These observations suggested that complete dechlorination of chlorinated ethenes depended on the abundance of electron donating compounds. Although this behavior may be specific to this site, the methods and approach used to investigate the competing processes provide a framework to assess sites for the potential for successful reductive dechlorination and to diagnose sites that behave in undesirable or unexpected ways. The working hypothesis of competitive inhibition of complete dechlorination due to an excess of hydrogen that favors methanogenesis has the potential to be a factor at many sites. Our observations from this site suggest that the analysis of the native microbiological population for *Dehalococcoides* sp. and microcosm testing to confirm complete dechlorination and to assess the effect of electron donor concentrations can provide valuable design information that could minimize the potential of add too much electron donor as seemed to be the case at this site. During the remediation process, the VFA and hydrogen content of the groundwater, the presence of ethene and ethane, and the production of respiratory byproducts such as methane provide insights into the progress of the remediation effort.

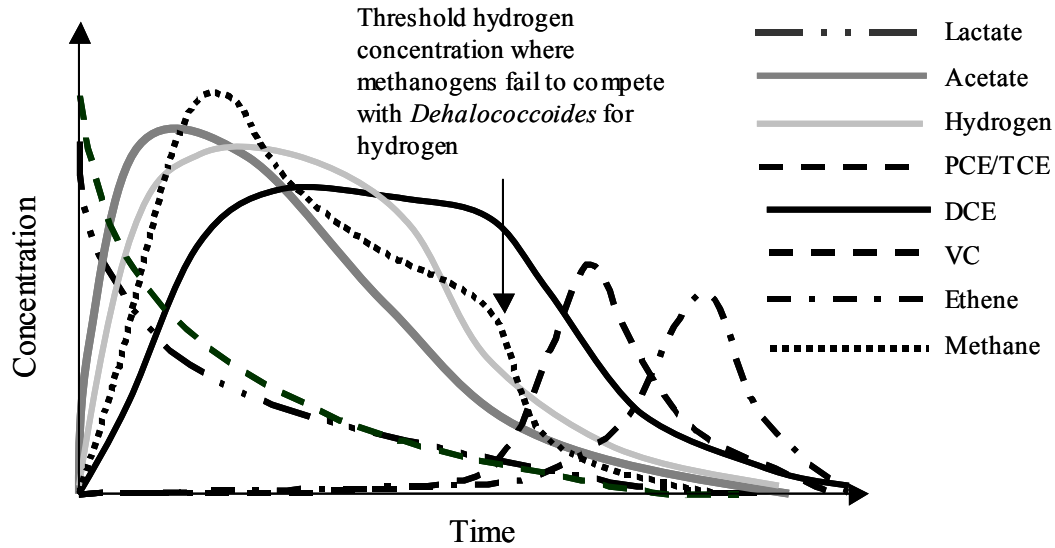


FIGURE 8. Conceptual relationship between cis-1,2-DCE accumulation and methanogenesis.

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