

OBSTACLES TO COMPLETE PCE DEGRADATION DURING REDUCTIVE DECHLORINATION

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ABSTRACT: Reductive dechlorination is a viable technology for treating chlorinated ethene contaminated groundwater especially when facilitated by the addition of hydrogen releasing compound (HRCTM), molasses, sodium lactate, vegetable oil, or some other organic carbon source. However, no technology works equally well at every site. This paper describes a problem that appears relatively common at sites undergoing enhanced reductive dechlorination, the accumulation of cis-1,2-DCE.

Enhanced reductive dechlorination was accomplished by two HRC applications made at a dry cleaning site in Florida. The first application principally targeted the shallow surficial aquifer with 144 direct injection points and approximately 3300 lbs. of HRC. The second application occurred 18 months later and targeted the lower surficial and intermediate aquifers with 146 delivery points and an additional 3300 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 subsequent HRC delivery was focused on the deeper groundwater; however, the anticipated dechlorination did not occur. In fact, the cis-1,2-DCE concentration increased in the lower surficial aquifer.

Site data have been evaluated in an attempt to understand the behavior of the chlorinated ethenes in the presence of HRC. Results from the first application of HRC in the shallow aquifer clearly indicated the reductive dechlorination of PCE and TCE and to a much lesser extent cis-1,2-DCE and VC dechlorination. Only low concentrations of ethene were observed. This indicated that complete dechlorination was occurring but that the rate was much less than the dechlorination rates for PCE and TCE. Site samples were examined for the presence of *Dehalococcoides ethenogenes*, groundwater geochemistry was critically examined, the concentration of lactic acid and other metabolic acids was determined, and the influence of climatic conditions on groundwater gradients was evaluated. The experience gained at this site should not preclude the application of accelerated reductive dechlorination at other sites; rather, the lessons learned thus far should be used to develop more rigorous site qualification procedures and design criteria.

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 CompoundTM (HRC), Regenesis 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 degrading 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 and they seem less robust in their ability to compete with other microbial populations in the subsurface. *Dehalococcoides ethanogenes* is one species that is known to dechlorinate chlorinated ethenes.

Two applications of HRC have been made at the subject dry cleaner site. 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). The first application of HRC targeted the shallow surficial aquifer with delivery points as shown in Figure 1. The first application in the shallow surficial aquifer included 144 direct injection points and approximately 3300 lbs. of HRC. The second application occurred 18 months later and targeted the lower surficial and intermediate aquifers with 146 delivery points and an additional 3300 lbs. of HRC. The locations of the delivery points for the second application are shown in Figure 2.

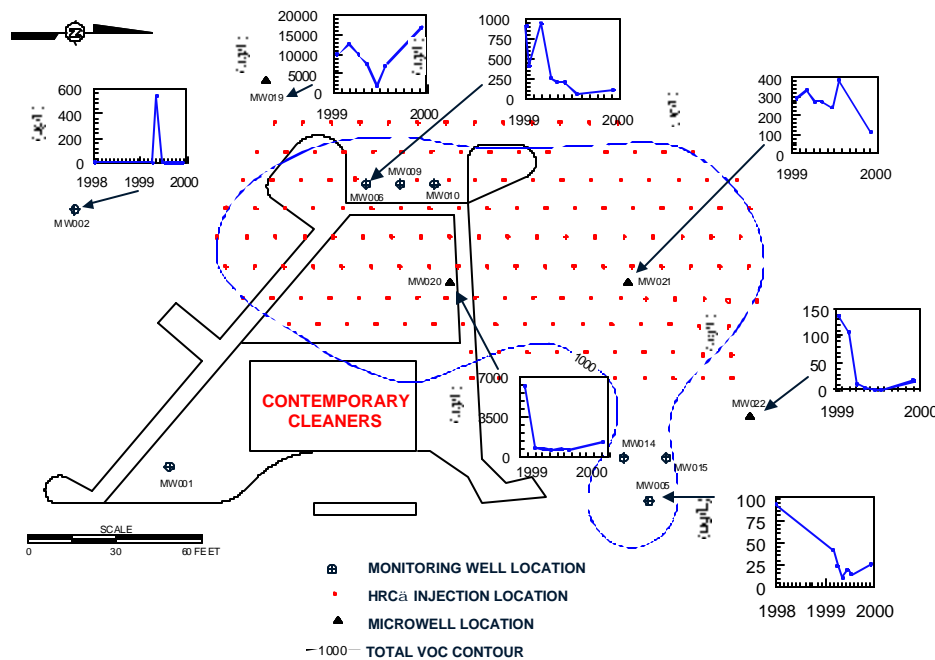


FIGURE 1. Location of initial HRC injection points.

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.

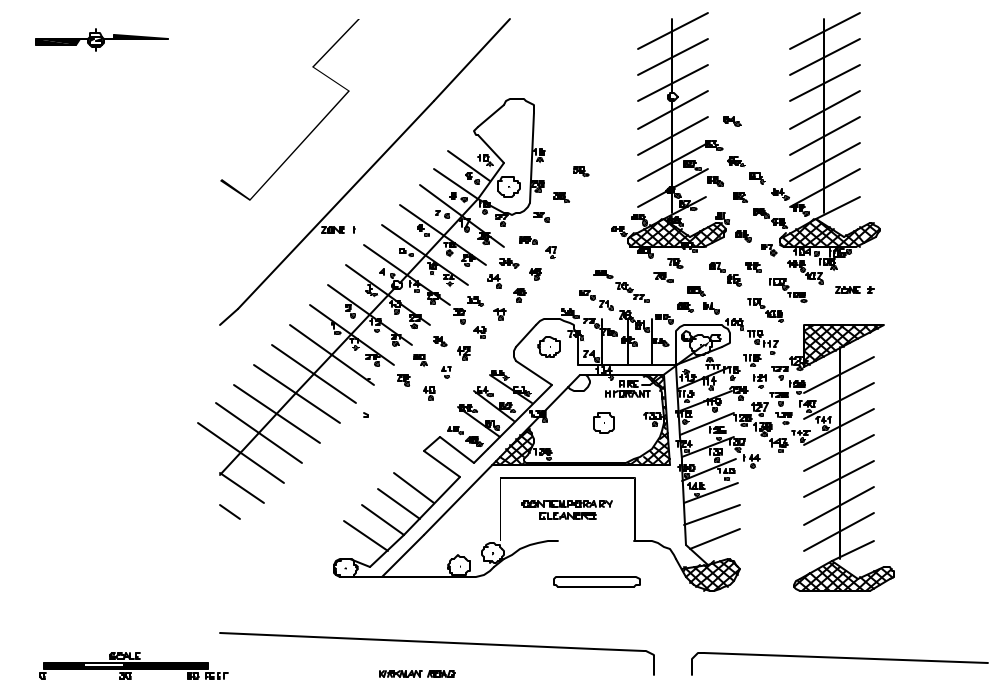


FIGURE 2. Location of injection points for the second HRC application.

MATERIALS AND METHODS

Groundwater was collected from the site using a low flow micropurge method. 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 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 (Figure 1). An additional application of HRC was made in the lower surficial and intermediate aquifers in August 2000 (Figure 2). 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 (Figure 3). 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

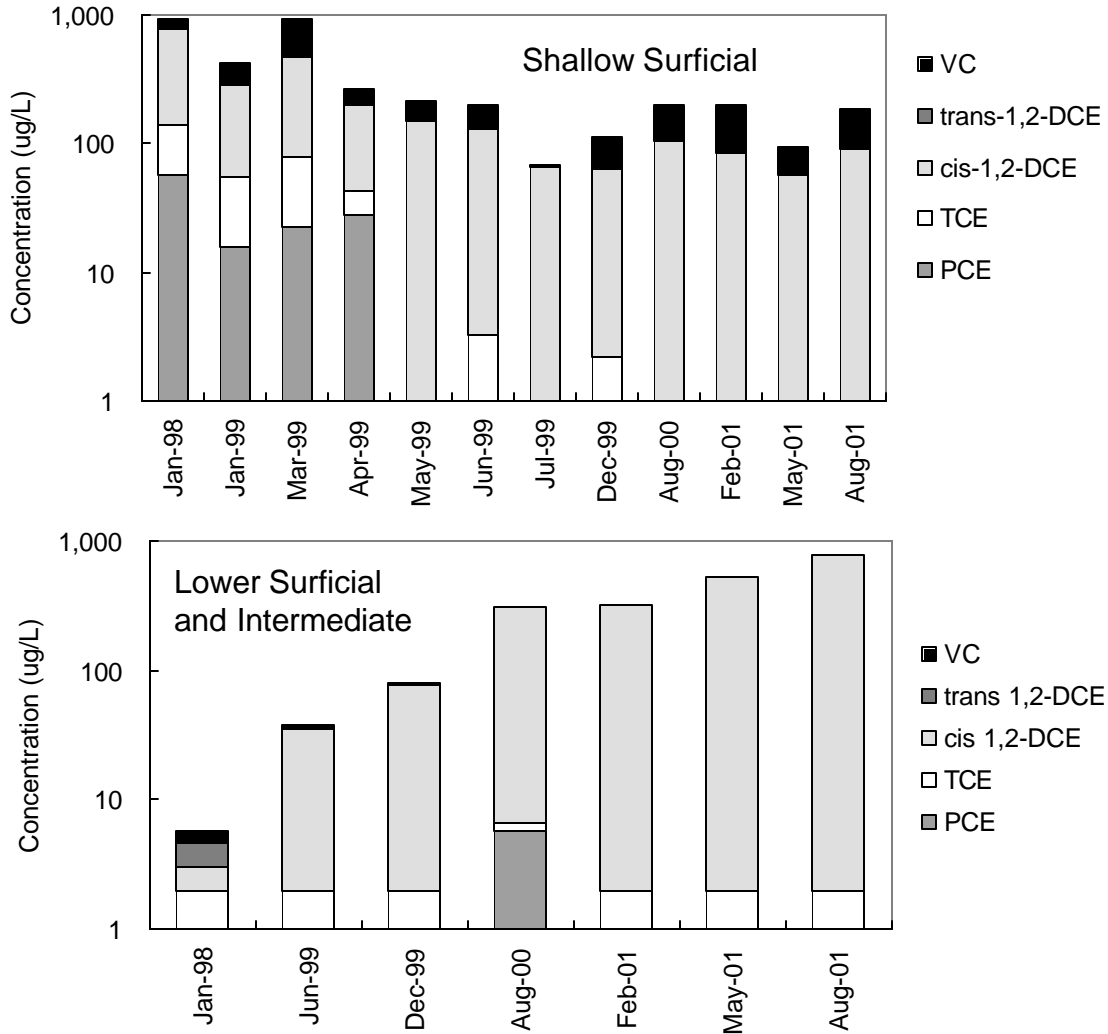


FIGURE 3. Representative chlorinated ethene concentration in shallow surficial and lower surficial and intermediate aquifers.

concentrations. Investigations were undertaken to determine the cause of the undesirable accumulation of DCE.

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 4, 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.

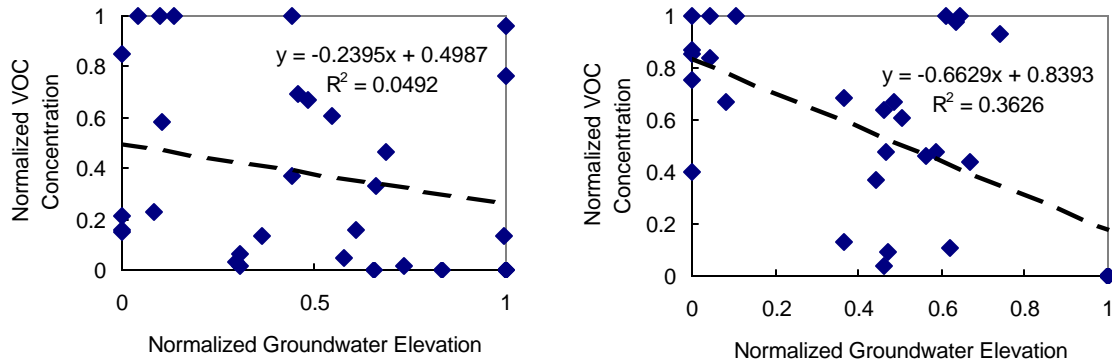


FIGURE 4. Correlation of normalized VOC concentration and normalized groundwater elevation.

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.

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

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. Methane was present in high concentration in several wells (Table 3). This observation along with the absence of ethene and ethane, suggests that methanogens may also be out competing the halo-respiring bacteria. Low concentrations of nitrate and manganese were detected; however, the concentrations were low enough that these compounds were not suspected to be causing 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.

TABLE 3. Selected 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)
Method Detection Limit	0.05	1	0.05	0.005	0.005	0.001	0.001	0.001
01/19/99	0.60	BDL	0.08	3.49	13	BDL	BDL	4.7
03/04/99	1.5	BDL	3.2	4.53	4.8	BDL	BDL	7.7
04/07/99	0.93	BDL	5.0	3.64	4.6	BDL	BDL	7.5
05/10/99	0.69	BDL	1.5	4.91	5.3	BDL	BDL	12
06/21/99	0.55	BDL	>5.0	18.6	21	BDL	BDL	13
07/20/99	0.62	3	1.8	20.6	21.	BDL	BDL	21
08/03/00	BDL	BDL	>5.0	2.80	-	BDL	BDL	8.7
02/20/01	0.36	3	-	2.38	2.7	BDL	BDL	12
05/10/01	0.65	2.1	5.0	0.980	1.9	BDL	BDL	54
08/15/01	BDL	BDL	3.0	1.98	2.1	BDL	0.001	12

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

The results from this evaluation of a site where DCE accumulated and apparently failed to be further dechlorinated suggests that geochemical conditions are the dominant factor influencing the progression of the dechlorination process. Groundwater conditions suggest that iron reduction, sulfate reduction, or methanogenesis could all be influencing the accumulation and persistence of DCE. The specific contribution of each microbial process in the interference with DCE dechlorination can not be determined from this work. However, the presence of *D. ethenogenes*, the presence of seemingly adequate concentrations of biodegradable organic carbon (organic acids), the lack of correlation between DCE concentrations and groundwater elevation lend support to a conclusion that geochemical conditions are the primary influence for the persistence of DCE observed in the aquifer.

Competition between reductive dechlorination and iron and sulfate reduction may be managed by simply allowing the aquifer microbiota to continue reacting until ferric iron and sulfate are expended. Competition between methanogens and halo-respirers may be more difficult to manage. The type and availability of biodegradable organic carbon or the residual hydrogen concentration may be the primary influencing factor controlling the balance between methanogenesis and reductive dechlorination. Adjusting these parameters may require a more aggressive approach than continued incubation under existing conditions.