

## *In situ* reductive dechlorination of chlorinated ethenes in high nitrate groundwater

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### Abstract

*In situ* bioremediation using carbohydrate was evaluated as an *in situ* treatment alternative for trichloroethene (TCE) and *cis*-1,2-dichloroethene (cDCE) in groundwater containing high nitrate concentrations. Upon addition of carbohydrate to groundwater, sequential reduction of electron acceptors was observed, where nitrate was reduced early in the pilot test, followed by sulfate and TCE. Reduction of cDCE to vinyl chloride and ethene occurred in conjunction with increased iron and manganese, and increased methane concentrations, approximately 7 months into the evaluation period, following depletion of nitrate and sulfate. TCE, cDCE, and vinyl chloride concentrations decreased from approximately 500 to >10 µg/L within 21 months of operation.

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### 1. Introduction

The addition of organic substrates to groundwater containing chlorinated ethenes has proven to be an effective *in situ* remedial strategy at many field sites, where the addition of organic substrates such as carbohydrate have been used to stimulate native microbial populations and promote the *in situ* reduction of trichloroethene (TCE) and *cis*-1,2-dichloroethene (cDCE) by a microbiologically mediated process called reductive dechlorination [1]. This evaluation was completed as a pilot program with the overall objective of identifying alternatives for expediting groundwater cleanup. The 678-day pilot program comprised of three components: (1) a field evaluation of the hydraulic performance of groundwater circulation wells as a delivery system for adding carbohydrate to groundwater, (2) an assessment of carbohydrate loading rate required to sustain reductive dechlorination, and (3) an assessment of the feasibility of using an *in situ* bioremediation approach to remediate chlorinated hydrocarbons (primarily TCE and cDCE) within groundwater containing

relatively high nitrate concentrations. This paper focuses on the third component of the pilot test, where the results of the first and second components have been described in an earlier paper [2].

#### 1.1. Site description

The site is a former semiconductor manufacturing facility located near San Francisco Bay, in Sunnyvale, CA, USA. Coarse-grained alluvial deposits underlie the site, and intermingle with fine-grained over bank deposits. Clayey to poorly graded sand and gravel layers were encountered in five borings drilled within the treatment area between depths of 2.7 and 5.2 m below ground surface, with a cumulative vertical thickness of 0.9–2.1 m. Site groundwater is encountered at a depth of about 3 m below grade and naturally contains sulfate at concentrations of approximately 200–300 mg/L. Initial nitrate concentrations were above 230 mg/L in the treatment zone, where background nitrate concentrations upgradient of the treatment zone averaged >10 mg/L. TCE and cDCE are the primary chemicals of concern that have been detected in site groundwater. Pre-test treatment-zone concentrations of these chlorinated ethenes were approximately 300 and 200 µg/L, respectively. Although close to the former source location, these relatively low concentrations

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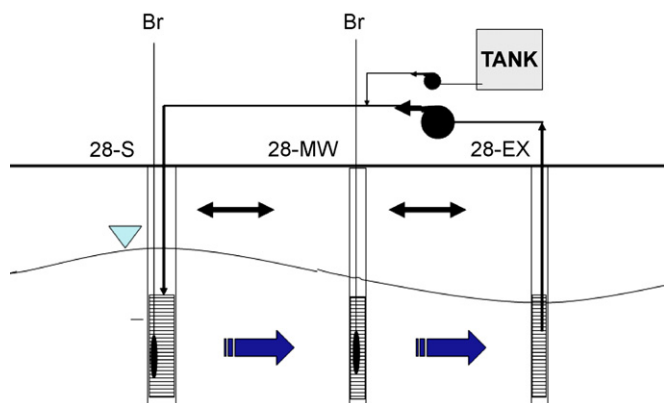
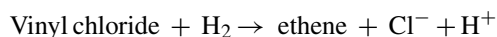


Fig. 1. Schematic of pilot test system. Well 28-S is the injection well, located approximately 2 m from monitoring well 28-MW, and 3.5 m from extraction well 28-EX. The tank contained the carbohydrate solution that was metered into the injection flow with a smaller peristaltic pump.

follow approximately 20 years of pumping and treating site groundwater.

### 1.2. *In situ* bioremediation by reductive dechlorination

*In situ* bioremediation of TCE and cDCE by reductive dechlorination involves electron transfer from an electron donor (such as lactate, carbohydrates, alcohols or hydrogen) to an electron acceptor (such as TCE or cDCE). The electron transfer is facilitated by hydrogen ( $H_2$ ) that is produced from the fermentation of organic compounds [3]:



In some cases, electron donor addition results in only partial dechlorination of TCE, where the accumulation of cDCE and or vinyl chloride results, without further transformation to environmentally benign end-products [4]. Reasons for partial dechlorination include (1) the absence of microbes capable of reducing cDCE and vinyl chloride by reductive dechlorination [5], (2) competition from other microbial processes for electron donor [6], and (3) kinetic limitations may preclude complete transformation of vinyl chloride to ethene [7]. Secondary effects on groundwater during *in situ* bioremediation using carbohydrate include increased organic acid content, decreased pH, increased dissolved solids including metals, methane and sulfide production, and a transient increase in vinyl chloride. These effects are expected to be transient and limited to the treatment zone area.

### 1.3. Challenges for *in situ* bioremediation via reductive dechlorination

Two key challenges for enhancing the reductive dechlorination process at this study site were (1) the relatively high nitrate and sulfate concentrations were expected to interfere with the

effectiveness of electron donor (carbohydrate) addition, and (2) the relatively low TCE and cDCE concentrations and associated kinetic limitations which can result in the accumulation and persistence of cDCE and vinyl chloride [7].

## 2. Experimental/materials and methods

The objective of the pilot test was to obtain information that could be used to design and predict the performance of a full-scale system. To accomplish these objectives, a pilot test was designed to allow for the detailed evaluation and control of groundwater flow, chemical concentrations, and amendment (carbohydrate) utilization within a well-defined treatment zone. Groundwater circulation wells were used to create a well-mixed stationary treatment zone for delivering carbohydrate and yeast extract while monitoring the effects on treatment zone chemistry. The pilot test system was operated between December 2002 and October 2004 (approximately 678 days), and utilized a pair of extraction and injection wells for groundwater circulation.

The well pair was composed of a 5 cm (cm) diameter extraction well (28-EX) from which groundwater was pumped into a 10 cm diameter injection well (28-S) installed at a location approximately 3 m, in the pre-test upgradient direction. A 5 cm diameter performance monitoring well (28-MW) was installed between wells 28-EX and 28-S. These three wells were screened over similar depth intervals in the uppermost coarse-grained unit. Fig. 1 shows the layout of the pilot test wells in section view. Carbohydrate injections were completed by metering in carbohydrate solutions (either sucrose or a dextrose/fructose mixture) from a tank (Fig. 1) and completing batch injections of carbohydrate with yeast extract added as a nutrient source. Averaged daily carbohydrate addition rates ranged between 300 and 1500 g/day.

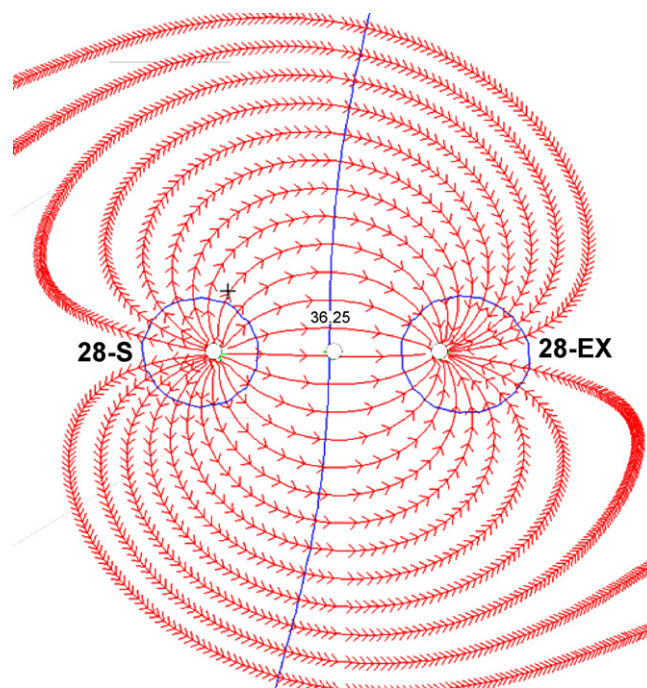


Fig. 2. Simulated groundwater flowpaths for the pilot test system.

### 2.1. Pilot test flow simulations

The hydraulic performance of the groundwater circulation well pair was simulated using a 2D analytical simulation (TWODAN<sup>®</sup>). The particle-tracking feature of TWODAN<sup>®</sup> was used to evaluate travel times along flowpaths from the injection well toward the extraction well during groundwater recirculation. The simulated flowpaths are shown in Fig. 2, where the distance between each successive arrowhead on a flow line represents a time step of approximately 6 h. The model parameters were obtained from a 24-h pumping test, where hydraulic conductivity and specific yield values of 5.5 m/day and 0.20, respectively, were reported using time drawdown data for well 28-S. The results suggest that the stationary treatment zone created by the circulation wells is approximately circular, with a diameter of about 6.1 m. For a vertical thickness of 1.8 m, and an effective porosity of 0.20, the pore volume for this treatment zone would be approximately 11,000 L.

### 2.2. Groundwater sampling and analysis

Groundwater samples were collected from wells 28-MW and 28-EX throughout the 678-day test period using a peristaltic pump. Because groundwater was continuously pumped from well 28-EX (the extraction well) into well 28-S (the injection well), samples were not collected from well 28-S, as the results should be essentially identical to those for 28-EX.

Analysis of groundwater samples for purgeable halocarbons (including chlorinated ethenes), ethene, methane, nitrate, sulfate, dissolved iron and manganese, and other analytes not discussed in this paper were performed by a professional analytical laboratory under contract using standard methods. Groundwater samples to be analyzed for purgeable halocarbons (including chlorinated ethenes) were transferred to 40 mL glass screw-cap vials with Teflon-lined silicone septa. The vials were filled such that headspace was minimized, and the samples were preserved by acidification with hydrochloric acid to pH < 2, and then placed in an ice-chilled cooler for transport to the contract laboratory. Chlorinated ethenes were analyzed by gas chromatography (GC)/mass spectrometry following sample preparation according to EPA Method 5030B and analysis according to EPA Method 8260B [8]. The same sample collection method was used for dissolved hydrocarbon gases (including ethene and methane), except the samples were not acidified. Methane and ethene samples were analyzed by a GC equipped with a flame ionization detector following Method AM20GAax [9]. Groundwater samples to be analyzed for nitrate and sulfate were transferred to 250 mL screw-cap polyethylene bottles and placed on ice during transport to the laboratory. Nitrate and sulfate were quantified by ion chromatography following EPA Method 300.0 [10]. Groundwater samples to be analyzed for dissolved metals (iron and manganese) were filtered with a 0.45  $\mu\text{m}$  filter, then transferred to 250 mL plastic screw-cap vials and preserved by acidification with nitric acid, and then placed in an ice-chilled cooler for transport to the contract laboratory. Dissolved metals were analyzed by inductively coupled plasma-atomic emission spectroscopy, following sample prepa-

ration according to EPA Method 3010A and analysis according to EPA Method 6010B [8]. Groundwater samples were analyzed for nitrate within 48 h of sample collection; other analytes were quantified within 7 days of sample collection.

### 3. Results

The addition of carbohydrate to treatment zone groundwater resulted in a somewhat sequential reduction in electron acceptors, including TCE, cDCE, and vinyl chloride, as described below. Fig. 3 shows the utilization of carbohydrate by different groups of microbes, apparently the most important of which were nitrate reducers, sulfate reducers, iron reducers and

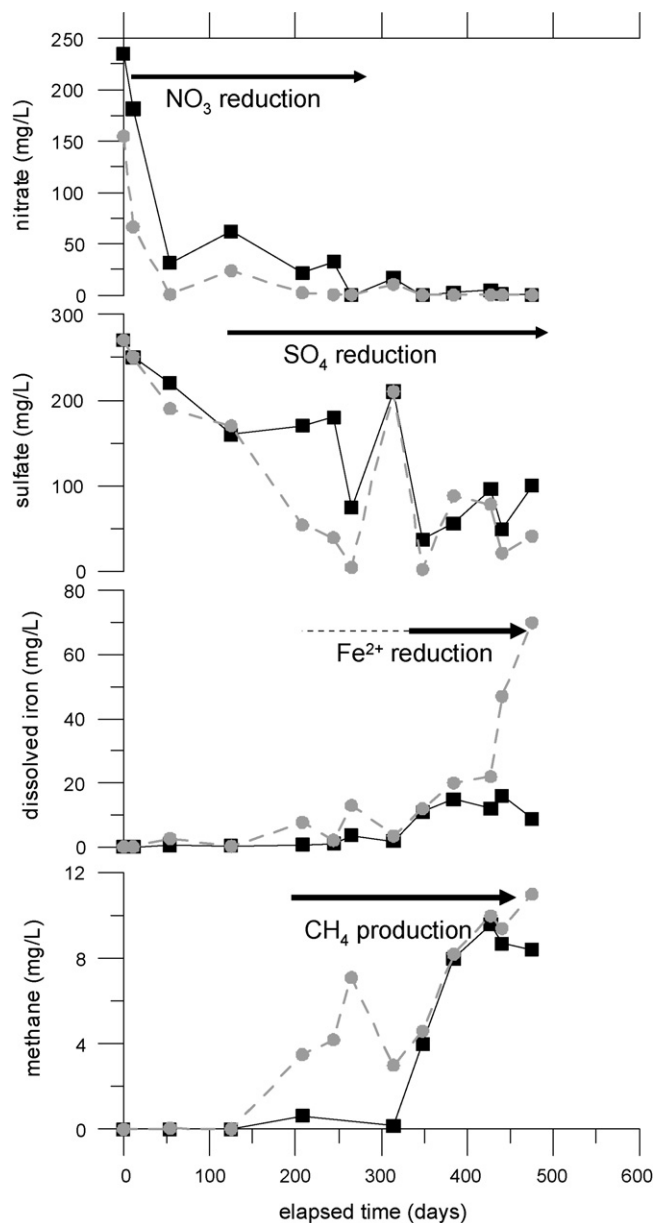


Fig. 3. Changes in the concentration of nitrate, sulfate, dissolved iron, and methane, in groundwater samples from wells 28-EX (black squares) and 28-MW (grey dots) over a 475-day sampling interval since the start of carbohydrate addition at day 0.

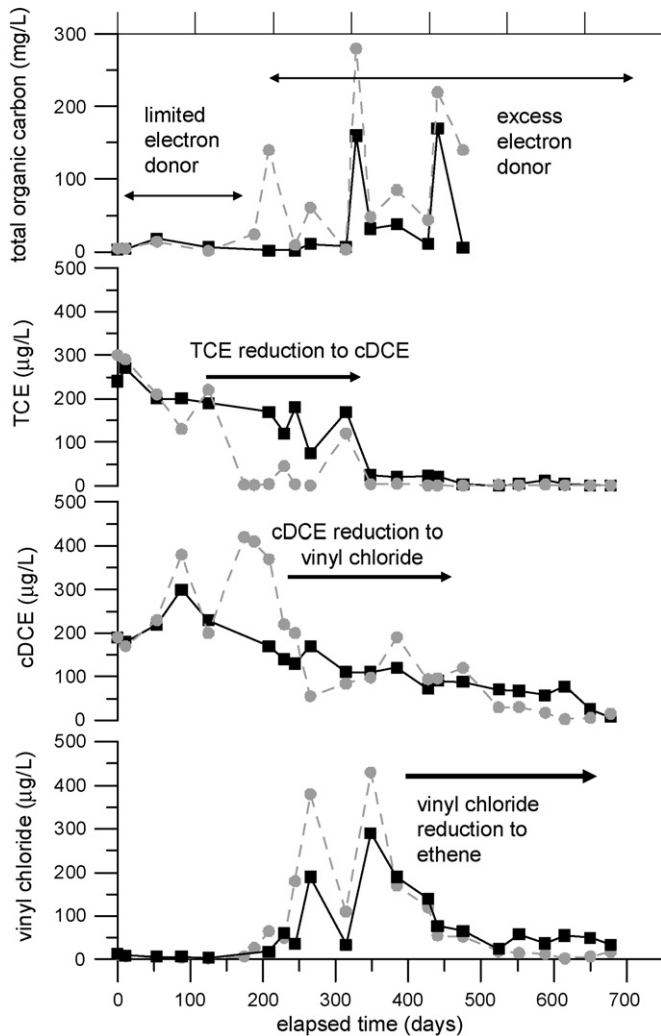


Fig. 4. Changes in the concentration of total organic carbon, TCE, cDCE, and vinyl chloride, in groundwater samples from wells 28-EX (black squares) and 28-MW (grey dots) over a 678-day sampling interval since the start of carbohydrate addition at day 0.

methanogens. Fig. 4 shows the sequential reduction of TCE to less chlorinated ethenes (cDCE and vinyl chloride), followed by the apparent *in situ* destruction of vinyl chloride, by reduction to ethene, and also possibly by anaerobic oxidation. The implications of the results shown in Figs. 3 and 4 are discussed below.

### 3.1. Nitrate reduction

Nitrate was present in shallow site groundwater at relatively high concentrations, potentially due to prior use at the site, or past agricultural use in the area. The addition of carbohydrate to groundwater containing nitrate was expected to promote nitrate reduction, as oxygen was already depleted. Nitrate reduction occurred during the earliest stages of the pilot test, presumably promoted by the addition of carbohydrate. Within the first 54 days of carbohydrate addition, nitrate concentrations decreased from 155 to <1 mg/L in samples from well 28-MW, indicating that rapid nitrate reduction was occurring between the injec-

tion well (28-S) and monitoring well 28-MW, located >2 m downgradient. Over the same time period, nitrate concentrations decreased from 234 to 31 mg/L in samples from well 28-EX (which are representative of both the extraction well and injection well concentrations because of the groundwater circulation loop). Nitrate concentrations did not decrease to non-detect in samples from well 28-EX until day 265 of the test. This apparent time lag can be explained by considering the simulated flowpaths for the pilot test system shown in Fig. 2. Although it may take only about 1 day of travel time on average for a conservative solute to reach well 28-EX after being injected into 28-S, the travel times along longer and less direct flowpaths can be on the order of several days or weeks. Under this scenario, the extracted groundwater at 28-EX represents a mixture of nitrate-depleted groundwater from the area affected by carbohydrate addition, most likely the area near the injection well, and nitrate-rich water from areas of the treatment zone that had not yet been affected by the carbohydrate addition. A relatively narrow zone of nitrate reduction likely initially developed, centered on the injection well, and extending primarily towards 28-MW. This may have been the case at day 54. Over time, the treatment zone likely expanded to encompass a larger volume of the treatment zone subsurface, including the pore space around well 28-EX, apparently before day 265 (when nitrate was not detected in the sample from well 28-EX). The apparent complete removal of nitrate by carbohydrate addition was likely a major factor that promoted the complete reduction of TCE and cDCE at later stages of the test.

### 3.2. Sulfate reduction

Sulfate is naturally occurring in shallow groundwater in the vicinity of the site. The addition of carbohydrate to groundwater containing sulfate was expected to promote sulfate reduction after nitrate was depleted. Sulfate reduction appeared to begin during the earlier stages of the pilot test, but became more substantial between sampling events on days 125 and 208, well after nitrate reduction had been occurring. The concentration of total sulfides was measured at several sampling events but concentrations were between non-detect and 3 mg/L (data not shown), suggesting that sulfide produced may have been in an insoluble form and not detectable by groundwater sampling methods.

### 3.3. Iron and manganese reduction

Iron and manganese are naturally occurring in aquifer solids in the vicinity of the site, and were reported at concentrations as high as 22,000 and 260 mg/kg in soil samples from the treatment zone (prior to adding carbohydrate), respectively. Although the site soils are rich in iron and manganese, not all of the iron and manganese was likely available in a form that could have been reduced by carbohydrate. The effects of iron reduction (and manganese reduction, data not shown), are apparent with the increase in dissolved iron concentrations in the sample from 28-MW collected on day 208. Iron reduction may have occurred before this time, however, the effects may not have been observed if insol-



Table 1  
Chlorinated ethenes and ethene concentrations pre-, during, and post-pilot test

Sample date	Day	TCE ( $\mu\text{g/L}$ )	cDCE ( $\mu\text{g/L}$ )	Vinyl chloride ( $\mu\text{g/L}$ )	Ethene ( $\mu\text{g/L}$ )	Chlorinated ethenes $\mu\text{mol/L}$	Ethene $\mu\text{mol/L}$	% dechlorination
12/20/2002	0	200	190	11	0.13	3.7	0.005	0.1
4/8/2004	475	0.5	120	52	6.5	2.1	0.23	10.1
8/26/2004	615	1.3	2.2	2.5	NA	NA	NA	NA
12/6/2005	1082	10	26	28	33	0.8	1.2	59.8

uble ferrous iron sulfides were forming by reaction with sulfide produced during sulfate reduction.

### 3.4. Methanogenesis

Methanogenesis was expected to occur after nitrate and sulfate were depleted, and iron is less available. Increased methane concentrations were observed between days 125 and 208 (28-MW), and concurrent with the previously discussed increase in dissolved iron concentrations. Methane concentrations greatly increased in samples from 28-EX between days 314 and 348, suggesting that methanogenesis was a major process throughout the entire treatment zone, and therefore most of the available naturally occurring electron acceptors had been depleted. The presence of electron acceptors such as sulfate in samples where methane is also present at relatively high concentrations suggests that the treatment zone was not uniform in terms of a single dominant terminal electron accepting process, and there probably remained some areas dominated by sulfate reducers, where other areas may have been dominated by methanogens, iron reducers, etc.

### 3.5. Reductive dechlorination

Fig. 4 shows the total organic carbon (TOC) concentrations (a surrogate for carbohydrate and/or electron donor availability), in conjunction with TCE, cDCE, and vinyl chloride concentrations. Electron donor (as TOC) was relatively limited between days 0 and 125, probably because of utilization due to nitrate reduction. Reductive dechlorination of TCE and cDCE did not appear to be a major process over this time period. Reduction of TCE to cDCE occurred largely between days 125 and 265, followed by an increase in vinyl chloride concentrations. Vinyl chloride concentrations were highest in groundwater samples collected from both 28-MW and 28-EX on day 348 (430 and 290  $\mu\text{g/L}$ , respectively), then decreased steadily, where concentrations of TCE, cDCE, and vinyl chloride reported for the sample from well 28-MW on day 615 were 0.5, 5.1, and 2.5  $\mu\text{g/L}$ , respectively. The excess electron donor and lack of competing electron acceptors likely promoted the *in situ* reduction of chlorinated ethenes to non-toxic end products (e.g. ethene, chloride, bicarbonate).

Ethene concentrations were higher towards the end of the pilot test (Table 1), however, on day 475, the concentration of ethene could only account for 10% of the total ethenes (chlorinated ethenes plus ethene). It could not be determined whether vinyl chloride or ethene, or both, were oxidized by an anaerobic process, which could explain the lower than expected

concentrations of ethene. A groundwater sample was collected from well 28-MW approximately 3 years (1082 days) after carbohydrate injection began, and approximately 1 year (404 days) after carbohydrate injection and groundwater circulation ended. As shown in Table 1, chlorinated ethene concentrations, expressed as micromoles per liter ( $\mu\text{mol/L}$ ), were lower than the ethene concentration, indicating complete reductive dechlorination was a viable process approximately 1 year after the end of carbohydrate addition. It is interesting that the total chlorinated ethene concentration on day 1082 was approximately 0.8  $\mu\text{mol/L}$ , which is similar to the reported 0.7  $\mu\text{mol/L}$  threshold minimum concentration required to sustain a viable population of dechlorinating bacteria [7].

## 4. Summary and conclusions

Carbohydrate addition caused the sequential reduction of electron acceptors, starting with nitrate, and then sulfate and iron, where methanogenic conditions eventually prevailed in conjunction with reductive dechlorination. The reductive dechlorination of TCE and cDCE to ethene was observed, via vinyl chloride, resulting in significant *in situ* destruction of these groundwater contaminants. Carbohydrate addition has been implemented as a full-scale groundwater cleanup technology at the site, based on the results of this pilot test.

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