Ground Water Monitoring&Remediation

Oxygen and Ethene Biostimulation for a Persistent Dilute Vinyl Chloride Plume

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Abstract

Contamination of groundwater with chlorinated ethenes is common and represents a threat to drinking water sources. Standard anaerobic bioremediation methods for the highly chlorinated ethenes PCE and TCE are not always effective in promoting complete degradation. In these cases, the target contaminants are degraded to the daughter products DCE and/or vinyl chloride. This creates an additional health risk, as vinyl chloride is even more toxic and carcinogenic than its precursors. New treatment modalities are needed to deal with this widespread environmental problem. We describe successful bioremediation of a large, migrating, dilute vinyl chloride plume in Massachusetts with an aerobic biostimulation treatment approach utilizing both oxygen and ethene. Initial microcosm studies showed that adding ethene under aerobic conditions stimulated the rapid degradation of VC in site groundwater. Deployment of a full-scale treatment system resulted in plume migration cutoff and nearly complete elimination of above-standard VC concentrations.

Introduction

The chlorinated ethene vinyl chloride (VC) is a toxin and human carcinogen (EPA 2000) that has been found at over 37% of National Priority List sites tested by the US Environmental Protection Agency as of 2003 (ATSDR 2006). It is also highly mobile in groundwater, thus posing a threat to drinking water supplies and complicating remediation efforts. VC often persists in groundwater after direct contamination of a site with VC-containing wastes or following in situ generation of VC as a daughter product of the anaerobic degradation of more highly chlorinated ethenes such as tetrachloroethene (PCE), trichloroethene (TCE), and dichloroethene (DCE) (Smith and Dragun 1984; Kielhorn et al. 2000).

A variety of bacteria can attenuate chlorinated ethenes anaerobically using these contaminants as terminal electron acceptors for anaerobic respiration, but only *Dehalococcoides ethogenes* has been shown to completely dehalogenate PCE and TCE to ethene, (Holliger et al. 1993; Maymo-Gatell et al. 1999). At sites contaminated with chlorinated ethenes, the design of remediation systems, generally based on these anaerobic pathways, may involve biostimulation with electron donors and/or bioaugmentation with cultures of anaerobic dehalogenating bacteria.

However, such remediation strategies may not be effective at sites with high concentrations of competing electron acceptors, low concentrations of VC, or conditions that are otherwise not conducive to maintaining the growth and complete dehalogenation activity of *D. ethogenes* (EPA 2000; OPPTD 2006; ITRC 2008). This may lead to a condition often referred to by remediation practitioners as stall. In VC stall, the more highly chlorinated ethenes (PCE, TCE, and DCE) are degraded to VC, but VC reduction slows and concentrations stabilize at levels above the regulatory limit for drinking water sources. This is due to the thermodynamics of reductive dehalogenation (Cupples et al. 2004). Consequently, while one environmental problem has been solved, another one has been created: generation and persistence of the more toxic and carcinogenic daughter product—VC.

Bioremediation strategies that utilize aerobic bacteria may afford a solution to this problem. A variety of bacteria are able to cometabolically degrade VC under aerobic conditions using a wide range of substrates for growth, including alkanes, alkenes, and aromatic hydrocarbons (reviewed in Arp et al. 2001 and Mattes et al. 2010). Even more promising than cometabolism, certain bacteria have been shown to directly degrade VC, using it as a growth substrate (Hartmans and De Bont 1992; Verce et al. 2000; Verce et al. 2001; Coleman and Spain 2003; Singh et al. 2004; Mattes et al. 2005; Jin and Mattes 2008). These bacteria, called ethene-assimilators or ethenotrophs, which can directly metabolize VC through their catabolic pathway for ethene, have been isolated from numerous VC-contaminated

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sites (Hartmans and De Bont 1992; Verce et al. 2000, 2001; Mattes et al. 2005; Hartmans et al. 1985; Coleman et al. 2002; Danko et al. 2004). Isolates that have been described thus far belong to several different phyla, suggesting the possibility that this metabolic activity may be widespread.

Here we describe the implementation of a full-scale biostimulation treatment for a large dilute groundwater plume with persistent low levels of VC. Oxygen was added to provide aerobic conditions for VC oxidation and ethene was added to stimulate the growth of ethenotrophic bacteria in order to maximize direct aerobic VC degradation. In addition to this direct aerobic pathway where VC serves as a carbon and energy source for bacterial growth, addition of ethene as well as oxygen to groundwater may also stimulate cometabolic degradation of VC (Freedman and Herz 1996; Freedman et al. 2001).

The remediation project described here was implemented in response to a release of PCE at a landfill resulting in a dilute groundwater plume with persistent low levels of VC (2 to 27 μg/L). The PCE leached into groundwater along with unresolved dissolved organic matter that contained electron donors, resulting in anaerobic conditions and reductive dechlorination of PCE to VC. However, there were also significant concentrations of competing electron acceptors for anaerobic respiration and conditions less favorable to anaerobic dechlorination downgradient of the landfill. The plume migrated away from the source and detached following landfill capping. Risk management actions at the site included the implementation of in situ aerobic biobarriers designed to deliver a constant supply of oxygen with initial periodic pulsing of ethene to stimulate the growth of aerobic ethenotrophs in the groundwater. This treatment option was chosen in part because initial VC concentrations were too low to stimulate efficient aerobic degradation using oxygen alone (Verce et al. 2001; Coleman et al. 2002).

Dissolved oxygen and ethene were delivered to groundwater primarily using in situ mass transfer devices deployed in treatment wells arranged in lines in order to cut off plume migration. We used biochemical, genetic, and microbiological tools to establish feasibility and to monitor treatment progress. Together with field data showing diminution of VC concentrations in site groundwater, these results suggest that aerobic biostimulation can provide an efficient remediation strategy for low-level VC plumes where complete anaerobic treatment is not feasible.

Materials and Methods

Site Description

The study site was contaminated by disposal of material containing PCE at a demolition debris landfill in 1986. PCE and other dissolved organic compounds leached into the groundwater where reducing conditions resulted in incomplete reductive dechlorination of PCE to VC. This gave rise to a migrating dilute VC plume in the groundwater.

When the site was assessed in 2002, there was a detached plume of VC at concentrations >2 μg/L extending over 3000 feet. The plume was approximately >400 feet wide and 30 feet thick and located 50 feet below the water table in the downgradient area. The maximum VC concentration measured was 27 μg/L, which is >13 times the applicable Massachusetts standard of 2 μg/L.

The VC plume was found to be migrating at the base of a glacial outwash aquifer in fine sand with silt that includes lenses of coarse sand, gravel, silt and clay. The VC was expected to continue migrating at close to groundwater velocity, which was estimated at 0.5 ft/d.

The groundwater within the core of the plume was characterized by elevated dissolved organic carbon (up to 30 mg/L), sulfate (up to 180 mg/L), dissolved iron (up to 16 mg/L), and manganese (up to 1 mg/L). The initial concentration of methane was around 1 mg/L and ethene was approximately 0.001 mg/L. Mildly reducing conditions were observed in the plume area where dissolved oxygen and oxidation-reduction potential were initially around 0.5 mg/L and −50 mV, respectively. Consequently the methanogenic conditions necessary for reductive dechlorination of VC to ethene were unlikely to be achievable.

Treatment System Design

Baseline site assessment was conducted in 2002 and field treatment pilots, adding oxygen to the aquifer, began in 2003. Microcosm studies evaluating ethene addition were completed in 2004. Because the half-saturation (K_S) constants for direct aerobic metabolism (Verce et al. 2001; Coleman et al. 2002) and aerobic cometabolism of VC (Chang and Alvarez-Cohen 1996), 62 and 56 μg/L, respectively, are higher than the VC concentrations throughout the plume and more than 30-fold higher than the lowest VC concentrations of concern, additional substrate was deemed necessary to accelerate degradation to achieve remediation goals.

Because ethene is assimilated by the same pathway as VC (Hartmans and De Bont 1992; Verce et al. 2000; Coleman et al. 2002; Coleman and Spain 2003), ethene addition was incorporated into the treatment design to overcome the substrate limitation problem and accelerate degradation. In addition to stimulating the growth of ethenotrophs capable of using ethene as a carbon and energy source (i.e., direct metabolism), ethene can also stimulate aerobic cometabolism of chlorinated ethenes, (Freedman and Herz 1996; Freedman et al. 2001), leading to fortuitous VC degradation.

The pilot scale treatment tested two oxygen delivery systems: bio-sparging and in situ mass transfer of pure oxygen. The oxygen delivery via iSOC in situ gas infusion technology (InVentures Technologies, Fredericton, Canada) was selected for full-scale treatment. This technology provided the flexibility to deliver ethene as well as oxygen and was less likely to be negatively impacted by the presence of impermeable clay layers at the site. The treatment system was designed to promote plume cut-off by stimulating the growth of aerobic, ethenotrophic bacteria at treatment lines. The initial treatment schedule alternated gas supplies with one week of ethene delivery followed by one month of oxygen delivery.

The full-scale treatment system was comprised of the original pilot bio-sparging line plus six iSOC treatment lines. Gas cylinders supplied oxygen and a dilute mixture of ethene and nitrogen gas and no power was required to operate the iSOC systems. The iSOC treatment lines included

four cut-off lines, with treatment wells set across the entire width of the plume, and two shorter lines focused on the plume core. Treatment wells were spaced a distance of 20 to 25 feet cross-gradient and screened along the full 30 feet thickness of the plume (see Figure 1).

Groundwater Sampling and Chemical Analysis

Periodic groundwater monitoring events were used to assess and optimize treatment system performance. The monitoring program included, at minimum, semiannual measurements of field parameters including dissolved oxygen (DO), oxidation-reduction potential (ORP), pH, temperature, specific conductance, and laboratory analyses for volatile organic carbon (VOC), inorganics, and bacteria. Selected wells in the immediate vicinity of the treatment systems were monitored on a more frequent basis to optimize treatment efficiency.

Groundwater samples were obtained using low-flow sampling (EPA 1996) and transported in sterile bottles on ice to commercial laboratories for analysis. All containers, preservatives, and shipping requirements were consistent with Massachusetts DEP Standard References. Appropriate quality control samples were collected and trip blanks accompanied the project samples. Groundwater laboratory test data were reviewed for compliance with the Compendium of Quality Assurance and Quality Control Requirements in accordance with the Massachusetts Contingency Plan.

Microcosm Studies

Equal volumes of groundwater were combined from six monitoring wells and 100 mL of this mixed groundwater sample were added to each of six 160-mL serum bottles, which were sealed with Teflon-lined butyl rubber septa affixed with crimped caps. Amendments were prepared and added to microcosms by syringe from stock solutions or pure compressed gas cylinders. All microcosms were amended with VC to 29 ppb. Ethene gas (Scott Gas) and/or mineral salts medium (2.2 mM K_2HPO_4, 1.7 mM KH_2PO_4, and 0.4 mM KNO_3 plus trace minerals) were added to microcosms as indicated. One milliliter of 100% O_2 was added to all microcosms on day 1. Microcosm bottles were wrapped in aluminum foil and slowly shaken in a rotary shaker. Dissolved gases and VC in the microcosms were analyzed according to EPA Method 5021A.

Biofilm Assays

Biofilm formation was measured by a modified crystal violet dye-binding assay (O'Toole and Kolter 1998). Briefly, 1 mL samples of groundwater were incubated at room temperature with aeration in 24-well microtiter plates for 1 to 2 weeks. No media or supplements were added. Following incubation, the groundwater was decanted and the wells were washed with sterile distilled water. One milliliter of crystal violet was added to each well and allowed to bind for 5 min. The crystal violet was decanted and the wells were washed three times with water. The bound dye

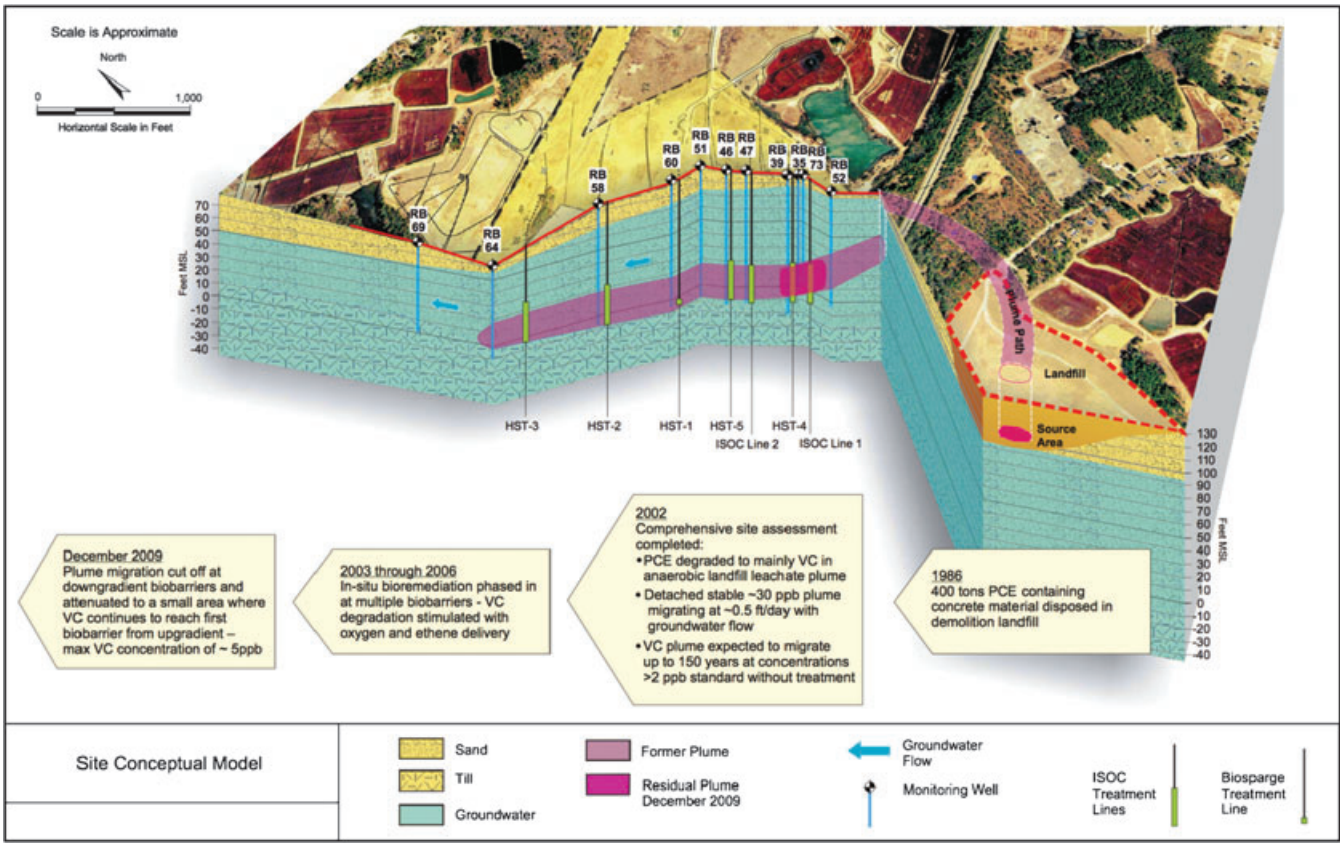

Figure 1. Site model. The light pink shading shows the plume path and the dark pink shading indicates the only remaining area with VC concentrations above 2 μg/L (iSOC treatment lines include iSOC Lines 1 and 2 plus HST-2 through HST-5).

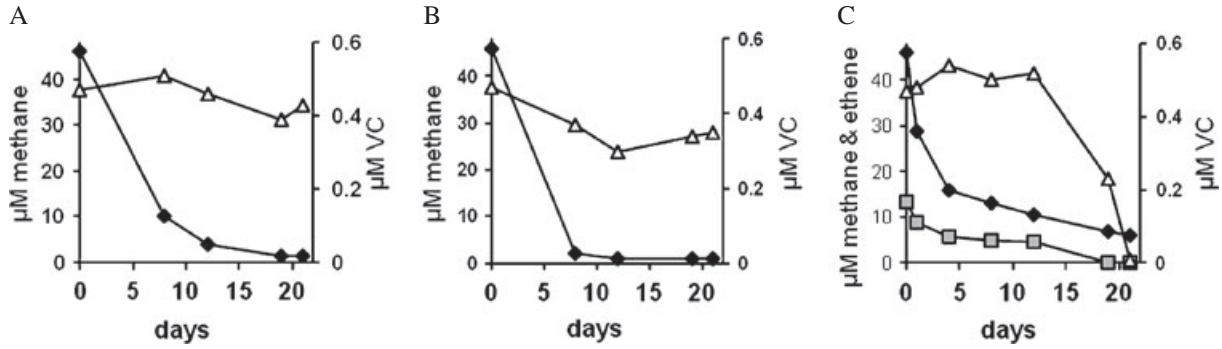

Figure 2. (A) Microcosm with no amendments; (B) microcosm with added mineral nutrients; (C) microcosm with added mineral nutrients and ethene. (♦) VC; (Δ) methane; (□) ethene.

was eluted with 1 mL of 95% ethanol and the absorbance at 600 nm was measured in a spectrophotometer.

Microscopy

Microscopic observations of live biofilm formation were performed using 5 mL of groundwater incubated with aeration in sterile petri plates. No media or supplements were added. The plates were marked so as to allow the same field of view to be observed at each time point. Images were captured under phase contrast with an inverted microscope at 800×.

PCR Analysis

Colonies grown from groundwater on 1:10 R2A medium were screened for the epoxyalkane coenzyme M transferase (EaCoMT) gene. A 431 bp fragment of EaCoMT was amplified by polymerase chain reaction using degenerate primers based on published EaCoMT sequences as previously described (Begley et al. 2009).

Results and Discussion

Laboratory Scale Biodegradation

In order to establish whether aerobic biostimulation might be feasible at the study site, a mixed site groundwater sample was used to generate a series of microcosms to test for aerobic VC degrading activity. Unamended groundwater showed rapid degradation of methane consistent with the activities of native methane-oxidizing bacteria, but only 9% of the VC was degraded after 21 days (Figure 2A). Groundwater amended with mineral nitrogen, phosphorus, and trace elements showed rapid degradation of methane, but only slow degradation of VC, amounting to 25% after 21 days (Figure 2B). However, when groundwater was amended with minerals and 13 μM ethene, degradation of VC was complete in 21 days (Figure 2C). The concentration of VC in the ethene-supplemented microcosm remained unchanged for the first 12 days, but was degraded to nondetectable levels by day 21, suggesting that VC degradation occurred after ethene consumption. No change in the concentration of VC and ethene occurred over a period of 21 days in the autoclaved control microcosm (not shown). The microcosm studies indicated that aerobic microbial VC-degrading activity was present in site groundwater and that it could be stimulated by ethene. Although cometabolic degradation of VC can occur under methanotrophic conditions (Fogel et al. 1986), the metabolism observed in site groundwater, which contains methane, was limited. In contrast complete degradation of VC was observed after addition of ethene to microcosms. So although methane is present, adding oxygen or oxygen and nutrients alone to the microcosms did not stimulate methanotrophic bacteria to degrade VC. These results suggested that methane-stimulated cometabolism would not be the optimal treatment design for this site.

Effect of Biostimulation Treatment on Biofilm Forming Ability

Oxygen treatment of anoxic groundwater should stimulate the growth of aerobic bacteria. Because bacteria primarily function in the environment in surface-attached communities, we focused our growth studies on biofilm formation (Peacock et al. 2004). We obtained groundwater samples from study site wells that were either within or outside of the predicted treatment zones and compared growth in a dye binding biofilm assay. This assay, which did not involve any growth media or addition of supplements, indicated approximately 50% greater biofilm formation, on average, in groundwater from treatment zone wells compared to groundwater sampled outside of the treatment zone (Figure 3).

We also directly visualized surface-associated growth in groundwater from a control well and a treatment zone well over a three-week period under aerobic conditions by incubating 5-mL groundwater samples in petri plates and monitoring

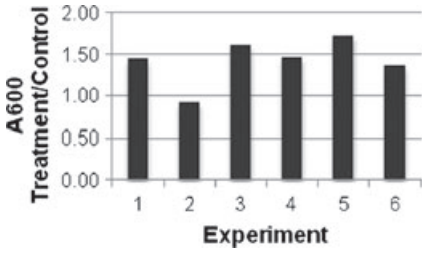

Figure 3. Groundwater samples were taken from a well inside the predicted treatment zone (treatment) and a well outside the predicted treatment zone (control). Biofilm assay data are shown as average growth (A_{600}) of the treatment zone sample relative to average growth of the control sample.

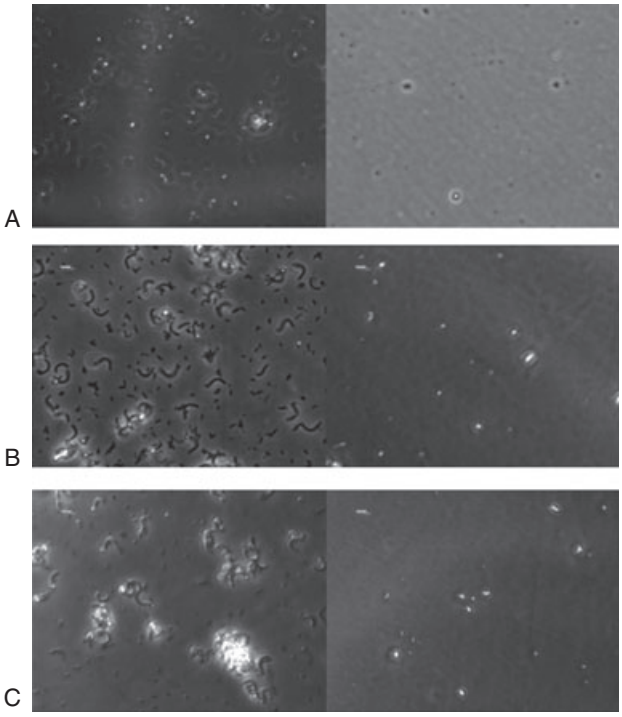

Figure 4. Micrographs of surface-associated growth in groundwater taken from a well within the predicted treatment zone (left) and outside of the treatment zone (right), incubated for one (A), two (B), or three (C) weeks.

growth with a phase contrast inverted microscope. We observed greater biomass in the groundwater from the treatment zone as well as a greater tendency to form surface associated microcolonies (Figure 4). A dye binding assay performed on these samples after the observed incubation period yielded absorbances of 0.167 for the treatment sample and 0.076 for the control sample, consistent with the biofilm assay data.

These results suggest that the remediation treatment employed at the study site stimulates growth of biofilm-forming aerobic bacteria, as predicted. However, biomass measurements are insufficient to demonstrate stimulation of the targeted aerobic VC degradation pathway.

Detection of a Gene Involved in Aerobic Ethene Assimilation

Stimulation of the growth of aerobic bacteria in situ would not be sufficient to affect VC removal if aerobes capable of VC assimilation were not represented in the aerobic community, consequently biomass measures alone may be misleading. Our initial microcosm studies demonstrated ethene-stimulated VC-metabolizing activity in mixed site groundwater. We also used a semiquantitative whole-cell PCR assay (Begley et al. 2009) to detect the presence of the epoxyalkane coenzyme M transferase gene, EaCoMT, which has been implicated in aerobic growth on both ethene and VC (Coleman and Spain 2003). The PCR test was applied to groundwater samples obtained from the site in June of 2007 and 2008, as previously reported (Begley et al. 2009). EaCoMT positive colonies were found in each well, with wells undergoing active treatment having higher numbers of positive colonies, consistent with microcosm and biomass results. Screening of the same wells the following year revealed an interesting pattern: the percentage of EaCoMT positive colonies appeared to track the stage of treatment in these three wells sampled over a three-year period. The wells were at different stages of treatment, based on treatment line startup times and distances downgradient from treatment (Figure 5). This suggests a pattern in which ethenotrophs represent a small, but measurable percentage of the culturable bacteria before treatment, increase to a peak after approximately three years in treatment, and then decline toward the background level. VC concentrations also tended to drop off to low or nondetectable levels over the same time frame.

Groundwater Monitoring

The results of field measurements indicate changes in groundwater chemistry and biological activity resulting from oxygen addition, as well as associated reductions in VC and methane concentrations. Figure 6 shows VC and methane data for three representative monitoring wells within the plume. Well 39I (panel A) is located in the most upgradient plume area immediately downgradient of the first treatment lines and had the highest initial VC concentration before treatment began. Methane concentrations were initially elevated but have increased or pulsed to higher concentrations over time. Well 47D (panel B) is in the mid-plume area and had intermediate initial VC and high methane concentrations. Well 58I (panel C) is in the downgradient plume area and had lower initial VC and methane concentrations. VC concentrations have been reduced to below the regulatory standard of 2 µg/L at wells 58I and 47D and the concentration at 39I has been reduced ~85%. In general monitoring wells located downgradient of treatment lines closer to the source of groundwater contamination showed little change in dissolved oxygen concentration. Increases in dissolved oxygen were noted in the more downgradient part of the plume where monitoring at well 58I showed an increase from less than 0.5 mg/L to up to 3 mg/L. Small transient increases in ethene concentration were noted, increasing from less than 0.001 mg/L to approximately 0.010 mg/L at several locations. The most upgradient treatment area of this detached

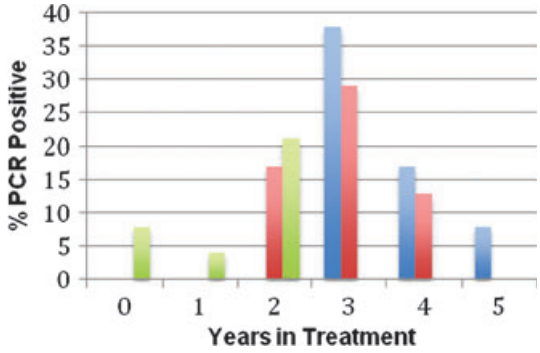

Figure 5. Groundwater was sampled from three wells (green, 51I; red, 58I; blue, 47D) over the same three-year period and colonies were screened for the EaCoMT gene. The percentage of colonies testing positive for the EaCoMT gene is shown for each well relative to the predicted time that the well had been within the treatment zone.

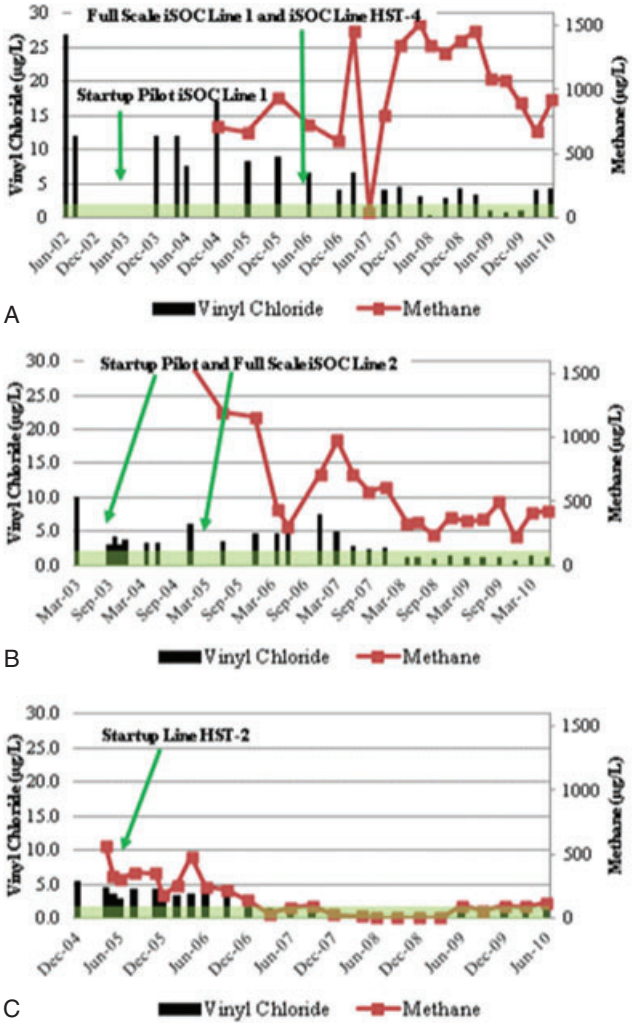

Figure 6. Vinyl chloride and methane concentrations in groundwater from selected monitoring wells. Panels A to C show monitoring data from wells where VC concentrations were initially higher (well 39I), moderate (well 47D), and low (well 58I), respectively. Monitoring wells 47D and 58I are now below the GW-1 groundwater standard for VC (shown in green).

plume near well 39I is approximately 2000 feet downgradient of the original source and contaminated groundwater continues to migrate into the treatment area. Methane is more persistent at higher concentration at well 39I indicating significant oxygen demand in arriving groundwater. It appears that groundwater at treatment lines further downgradient (e.g., well 58I) had lower initial oxygen demand allowing for faster attainment of remediation goals.

Treatment Outcomes and General Applicability

At the time of the initial site assessment, modeling suggested that the plume would continue to migrate passing through areas with private drinking water wells. The plume was projected to discharge to downgradient surface waters in approximately 50 years at VC levels still above the regulatory standard. The bioremediation treatment reported here resulted in cut-off of plume migration and full attenuation of most of the plume only three to four years after full-scale implementation. Three downgradient treatment lines have now been discontinued and the area is undergoing continued monitoring. Residual VC greater than the 2 µg/L GW-1 standard continues to migrate into the area of the first line of treatment from an upgradient source. Groundwater treatment is continuing in this area while the persistent source is the subject of further investigation.

In summary, we have used aerobic biostimulation with oxygen and ethene to treat groundwater at a PCE release site where low-level vinyl chloride has been persistent. In situ delivery of oxygen and ethene gas to the groundwater has resulted in cessation of plume migration, decreased vinyl chloride levels at all monitoring wells, and reduced contaminant concentrations to below the regulatory target throughout most of the plume. Given the low initial VC concentrations, the observed aerobic VC degradation in microcosms supplemented with ethene and detection of temporal changes in a gene involved in ethene/VC degradation suggest that ethene-stimulated aerobic VC degradation led to VC removal observed in the treatment area. At contaminated sites where VC concentrations are higher, oxygen alone may stimulate effective biodegradation, however microcosm studies are recommended to assess the utility of added substrates, as site-specific conditions will vary.

This example of a successful full-scale aerobic treatment of a dilute vinyl chloride plume with oxygen and ethene suggests the possibility of rapid, efficient containment and enhanced attenuation of VC at similar sites where conditions preclude complete anaerobic treatment.

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