BIOCIDE APPLICATION PREVENTS BIOFOULING OF A CHEMICAL INJECTION/RECIRCULATION WELL

Kelly Millar, Suzanne Lesage, and Susan Brown
(NWRI, Environment Canada, Burlington, Ontario, Canada),
Carol S. Mowder, Tim Llewellyn, Sarah Forman, and Dave Peters
(URS, Linthicum, Maryland, USA),
George DeLong (AIMTech, Oak Ridge, Tennessee, USA),
Donald J. Green and Heather McIntosh
(U.S. Army Garrison, APG, Maryland, USA).

ABSTRACT: A pilot test for *in situ* vitamin B_{12}-catalyzed reductive dechlorination of chlorinated solvents was conducted for 14 weeks in 1999 at Graces Quarters, Aberdeen Proving Ground, Maryland. Groundwater at the site is contaminated with 1,1,2,2-tetrachloroethane (TeCA), carbon tetrachloride (CT), trichloroethene (TCE), tetrachloroethene (PCE), and chloroform (CF). Although analytical groundwater data from the pilot test showed favorable results, biological fouling of the chemical injection/recirculation well and the near-by formation halted pilot test operations. After successful redevelopment of the injection/recirculation well, and several modifications to the chemical treatment mix and injection strategy (aimed at preventing future biofouling) the pilot test was resumed. Modifications included the use of a non-oxidizing biocide, Redux® B-T20, containing tetrakis(hydroxymethyl)phosphonium sulfate (THPS) as the active ingredient. The data show that daily application of B-T20 at a 150 mg/L active concentration for 4 hours prevented biological fouling of the area surrounding the well. The inhibitory effects of B-T20 were limited to the anaerobic treatment zone. Microbial activity was resumed in the aquifer as biocide concentrations decreased. A laboratory microcosm study confirmed the inhibitory effects of B-T20 on the site bacteria.

INTRODUCTION

A two-phase pilot test was conducted at Graces Quarters, APG, to evaluate the use of a concentrated mixture of vitamin B_{12} and Ti(III)-citrate to treat chlorinated volatile organic compounds (VOCs) in groundwater (Lesage et al. 2001). Groundwater was treated *in situ* using a recirculation well designed to optimize contact between the vitamin B_{12}/Ti(III)-citrate mixture and groundwater contaminants, yet minimize contact with the surrounding soil. A slow, continuous injection approach was used during phase one (Fall 1999), to maximize dechlorination reactions in the well. The chemical mix consisted of vitamin B_{12}, Ti(III)-citrate, and a glucose/fructose syrup. Although glucose is not a necessary component of the reactive vitamin B_{12} mixture, it was added as a preferred bacterial substrate over citrate (Millar and Lesage, 1997), to try to increase the longevity of the Ti(III)-citrate complex in the recirculation well and aquifer. Analytical results of the initial phase of treatment were favorable, with complete degradation of CT and 50-80 % removal of TeCA occurring within the recirculation well (Mowder et al. 2000). However, excessive bacterial growth in the vicinity of the recirculation well, made treatment delivery increasingly difficult, finally shutting the system down after 14 weeks of operation.

The purpose of the second phase of the pilot test was to minimize biofouling, using a biocide and a pulsed approach to treatment injection. In addition, the vitamin B_{12}/Ti(III)-citrate mix was injected into deeper regions of the aquifer where residual DNAPL was suspected and at increased pumping rates, to deliver higher concentrations a further distance from the recirculation well.

Most chemical well treatments for biofouling, being highly oxidizing, are incompatible with the reduced redox conditions required for the vitamin B_{12}-catalyzed reaction. Therefore, the non-oxidizing biocide B-T20 (Redux Technology), containing THPS (Fig. 1), was selected. THPS was widely used as a flame retardant in fabrics before its biocidal properties were recognized. Current usage is for controlling bacteria, algae, and fungi in industrial cooling systems, oilfield operations, and papermaking processes. Its benefits include low overall toxicity, low effective treatment concentrations, and no bioaccumulation. In addition, while B-T20 is effective under anaerobic conditions, it degrades quickly under aerobic conditions; therefore its inhibitory effects do not extend outside of the treatment zone.

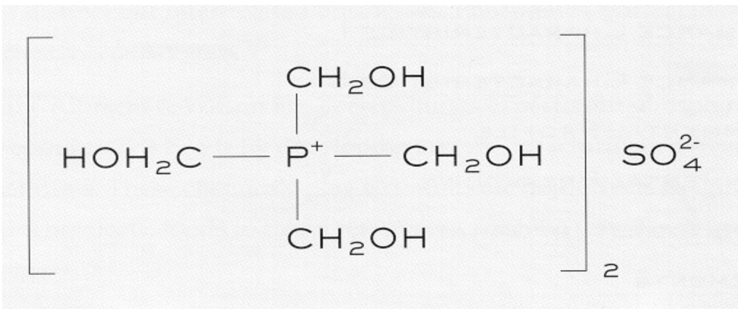

FIGURE 1. The structure of tetrakis(hydroxymethyl)phosphonium sulfate (THPS), the active ingredient in Redux B-T20.

The effect of the dual strategy was evaluated using water level measurements and volatile fatty acid (VFA) analytical data from the recirculation well and surrounding multi-level piezometers and monitoring wells. In addition, the results of a laboratory microcosm study to assess the inhibitory effects of B-T20 using site water collected during the second phase of groundwater treatment, is summarized.

MATERIALS AND METHODS

Pilot Test – Phase Two. Following successful redevelopment of the recirculation well, restoration of aquifer permeability (Forman et al. 2001), and regulatory approval of the use of B-T20 onsite, the pilot test was resumed. On July 28, 2000, system operations began with a 2½-week treatment of B-T20 to inhibit any resident microorganisms. A 2 % active solution of B-T20 was pumped into the recirculation well for 4 hours/day at a rate of 105 mL/min to obtain an active concentration of 150 mg/L within the well. The recirculation well was operated at a flow rate of 4 gallons per minute (gpm) in upward mode during the first week, treating and then exiting the upper well screen, and in downward mode for the final ten days, to treat the lower well screen and surrounding sand pack. For the remainder of the pilot test, the recirculation well was operated in the downward mode, opposite to the flow direction employed in phase one.

Weekly injections of the reformulated vitamin B_{12}/Ti(III)-citrate mix (without glucose) began on August 15, 2000, until November, for a total treatment time of 12 weeks. The mix was injected over a 20-hour period into the recirculation well, once per week. B-T20 was added 4 hours/day every day to achieve a 150 mg/L active concentration within the well. Periodically, the system was backwashed by changing the direction of flow for up to 8 hours.

Water levels in the recirculation well (upper and lower screens) were recorded regularly. Monthly groundwater sampling was conducted for analysis of contaminants, dissolved gases, VFAs, metals, chloride, pH, and Eh. For a plan view of the site see Lesage et al. (2001).

Microcosms. Groundwater from the lower well screen of the recirculation well was collected on October 2, 2000. Microcosms were established in triplicate 60-mL serum vials, in an anaerobic chamber having an atmosphere of 5 % CO_2, 10 % H_2, and 85 % N_2. Four conditions were established, each consisting of site water, inoculum and the following: (A) 10 mM Ti(III)-citrate, 5 mg/L vitamin B_{12}; (B) 150 mg/L (active) B-T20 for 4 hours, then 10 mM Ti(III)-citrate, 5 mg/L vitamin B_{12}, filter-sterilized site water; (C) 150 mg/L (active) B-T20 for 4 hours, then filter-sterilized site water; and (D) 20 mg/L (active) B-T20, 1.9 mM Ti(III)-citrate, 1 mg/L vitamin B_{12}. A fifth microcosm (E) that contained site water alone was established as a control. Inocula were prepared by centrifuging groundwater from the site, then resuspending the bacterial pellets in 3 mL of site water per vial. For (B) and (C), vial contents were re-centrifuged following a 4-hour B-T20 exposure and pellets were resuspended as above. Analyses of VFAs and methane were conducted.

RESULTS AND DISCUSSION

Water levels. For a schematic of the recirculation well and a detailed description of its operation see Mowder et al. (2000). In brief, the 10" diameter recirculation well consists of two screened intervals separated by an inflatable packer. The reference for water level measurements of the upper-screened interval is the top of the well casing while water levels of the lower-screened interval are measured relative to the packer.

Upper and lower well screen water levels during both phases of the pilot test are shown in Figure 2. During the initial phase, the system was operated in upward mode such that groundwater treatment took place in the upper part of the recirculation well. Continuous pumping of glucose-amended chemical mix resulted in significant increases in the water level of the upper well screen as early as 2 weeks into the experiment. By week six, aquifer permeability was limited (Fig. 2A) and biological growth was evident as foul odors and gelatinous masses of material were observed in the lines of the recirculating system. While aquifer conditions were originally unfavorable for bacterial growth, intrinsic microorganisms responded rapidly to the incoming carbon sources, neutral pH, and reduced redox conditions generated by the chemical treatment. Treatment delivery became increasingly difficult with frequent shutdowns due to high water levels and fouled pumps, resulting in the termination of the test after 14 weeks of operation.

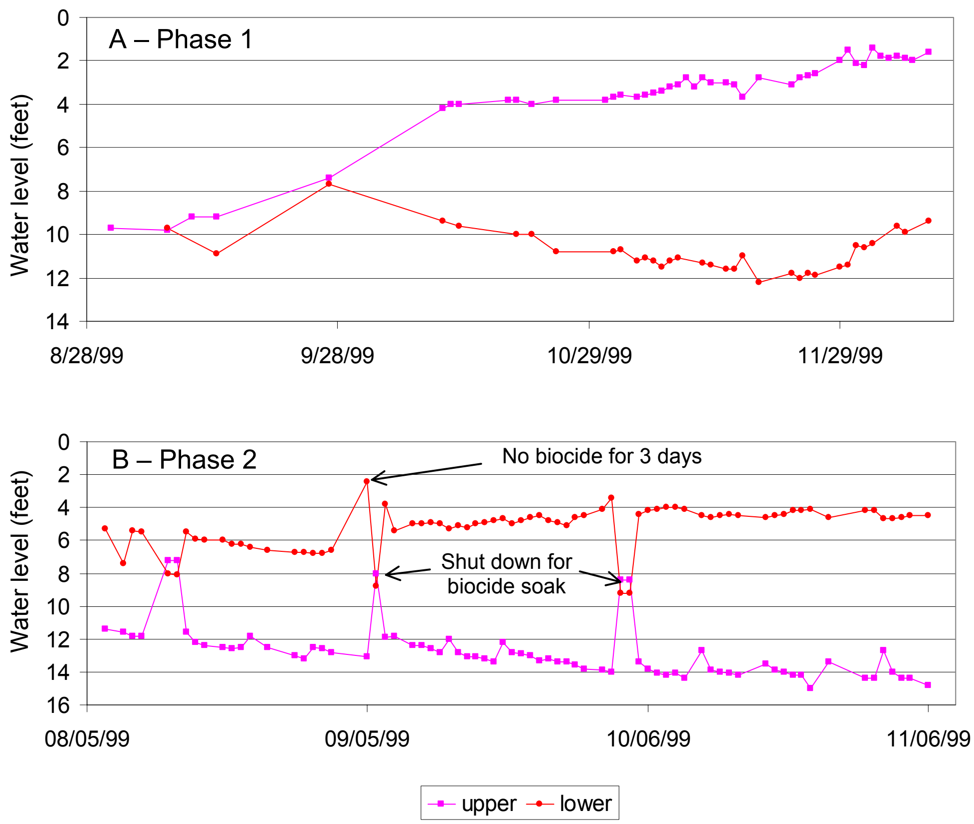

FIGURE 2. Water levels of the upper and lower well screens of the recirculation well during phase one and two of the pilot test.

During the second phase of the pilot, flow was reversed and the lower part of the aquifer received chemical treatment. Daily application of B-T20 at 150 mg/L active concentration was effective in minimizing biological growth in the immediate vicinity of the recirculation well as was evident by the stability of the water levels of the lower well screen (Fig. 2B). The effect of B-T20 on the bacterial community was bacteriostatic such that biological growth was inhibited, as opposed to bactericidal, which would have resulted in cell death. The resilience of the population was evident on September 5th, 2000. The B-T20 injection pump had failed the previous Friday, leaving the aquifer untreated for three days. This resulted in a 4-foot rise in the water level of the lower well screen. To try to restore conditions, the system was backwashed for 8 hours, by reversing flow, then shutting the system down to allow an overnight biocide soak. From then on, water levels began to creep up and another flow reversal/biocide soak was conducted just after the halfway point of the test. It was then decided that a more rigorous approach to controlling biological growth needed to be taken. As such, flow reversals and B-T20 soaks were conducted weekly, the day prior to the 20-hour injection of the Vitamin B_{12}/Ti(III)-citrate mix. This proved effective, with no further increase in water levels for the remainder of the test. In contrast to the initial phase of the pilot test, with reduced aquifer permeability resulting in an 8.5 foot increase in the water level of the upper well, through regular use of B-T20, water levels in the lower well screen rose an average of only one foot, over the course of the 12-week treatment.

Redux® B-T20 effects in the aquifer. From baseline sampling in May 2000, it was evident that biological activity in the aquifer had increased since the termination of the initial pilot test. Abiotic *cis*-DCE to *trans*-DCE ratios of 2:1, observed during chemical treatment in phase 1, had increased to ratios characteristic of biological dichloroelimination of TeCA (3:1, Lorah and Olsen, 1999). Methane also began to appear at a number of the monitoring wells. Methane generation, in part, may have been stimulated by well/aquifer restorative procedures during the interim. In particular, residual CO_2 from the Aqua Freed® process (Forman et al. 2001) may have stimulated methanogenic populations that had survived the redevelopment process. Biological dechlorination was apparent at piezometer 1C (10 feet from the recirculation well) by reductions in TCE (655 µg/L to 112 µg/L) and CT (622 µg/L to 37 µg/L) that could not be accounted for by dilution, from incoming A-level waters, alone. In addition, biodegradation of CT had resulted in CF concentrations increasing from 19 µg/L in January to 40 µg/L in May 2000.

A good indicator of the distribution and effectiveness of B-T20 is the persistence of the Ti(III)-citrate complex in the aquifer. During the second phase of treatment, piezometer 1C saw concentrations of citrate and titanium as high as 9.6 mM and 3.5 mM, respectively (Fig. 3A). MT3DMS model simulations predicted maximum titanium concentrations of 4.5 mM at 10 feet, under conditions of no degradation/precipitation. Observed fluctuations in titanium and citrate concentrations at 1C are a result of the pulsed injection and the timing of sample collection. The week 8 (Oct. 6) sampling, which occurred 2 days after injection, was the only sampling event that could have captured the concentrate. It is difficult to determine whether the peak concentrations of citrate and titanium were obtained during this event. However, acetate concentrations did not increase during the active pumping period, suggesting that citrate degradation was minimal. In contrast, piezometer 8C, 30 feet away from the recirculation well, saw minimal concentrations of citrate, indicating that it was being metabolized along the flow path, with acetate accumulating at 8C (Fig. 3B). The increase in acetate at 1C, week 16 (Dec. 4 - four weeks post shutdown) indicates that the inhibitory effects of B-T20 were temporary, and that microbial populations can reestablish upon its disappearance or removal from the system. In addition to acetogenic bacteria, B-T20, though not reportedly tested on methanogens, appeared to exhibit a bacteriostatic effect on methanogens, with little increase in 1C methane concentrations, compared to that observed at 8C. While biological degradation of *cis*- and *trans*-DCE had occurred at 1C,

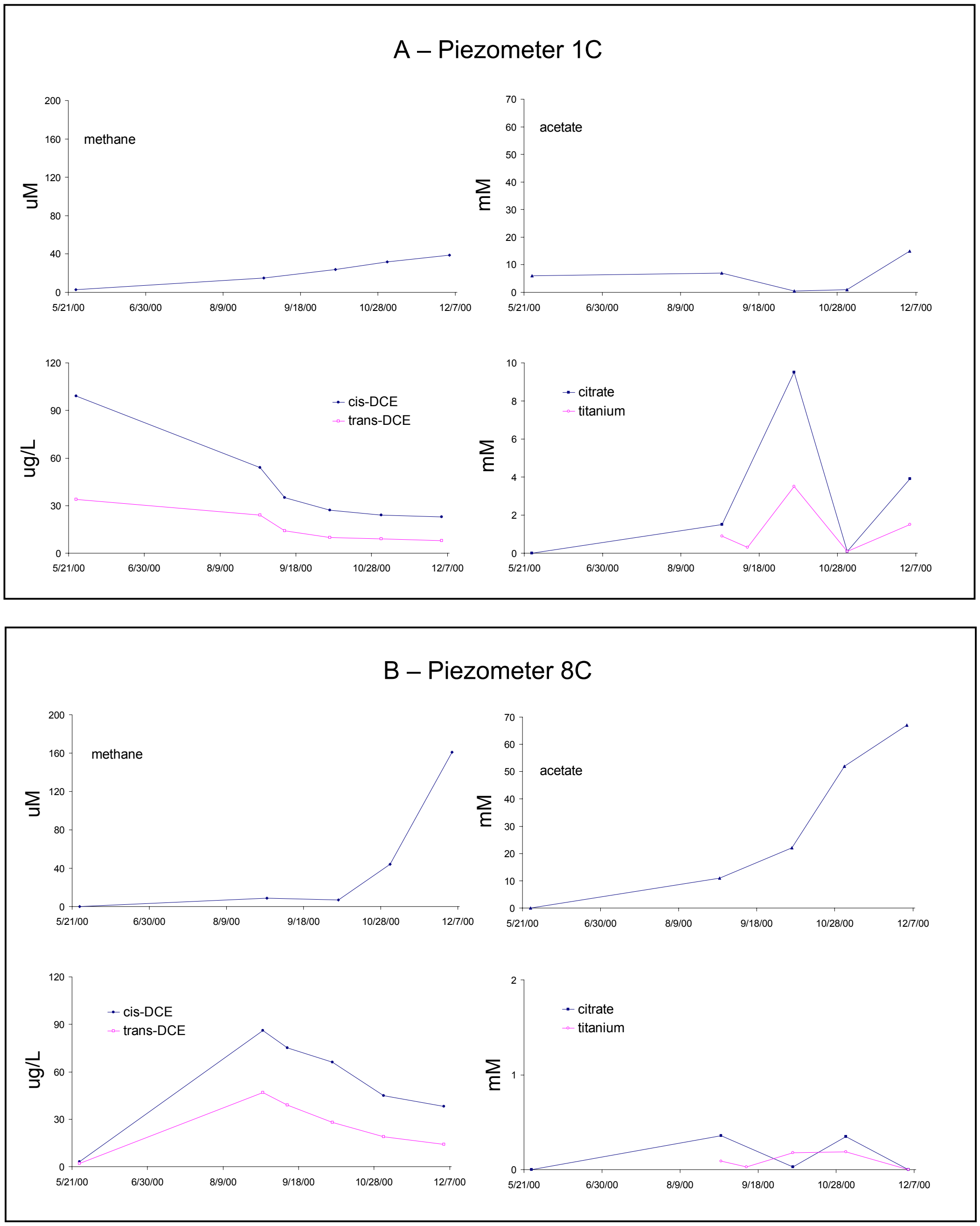

FIGURE 3. Titanium, VFA's, methane, and cis- and trans-DCE at piezometers 1C and 8C.

between baseline and week 2 (Aug. 30) sampling, further degradation appeared to be inhibited upon repeated applications of biocide. At 8C, however, where B-T20 effects were negligible, biodegradation of *cis*- and *trans*-DCE appeared to be continuous.

Microcosms. A series of microcosms were established to assess microbial activity in the aquifer under conditions of the pilot test: (A) Vitamin B_{12} concentrate injection, (B) changeover from biocide to vitamin B_{12}/Ti(III)-citrate mix, (C) 4-hour biocide injection, (D) low biocide, as seen 10 ft away, and (E) site water control. The effect of B-T20 on acetogenic bacteria is presented in Table 1. In treatment (A), citrate utilization forming acetate was variable, from non-measurable (A1), to partial (A2), and complete (A3), within 47 days. Variability between replicates, and the delay in citrate utilization, was likely due to the inoculum, which, despite efforts to concentrate it by centrifugation, was very low. Citrate was also degraded in 2 of the 3 low biocide treatments (D) indicating that 20 mg/L active, would not be an effective inhibitory concentration. The 4-hour, 150 mg/L B-T20 exposure (B) was effective at preventing citrate degradation. The effect of B-T20 on methanogens showed a similar pattern. Methane generation was greatest under conditions of the vitamin B_{12}/Ti(III)-citrate injection and lowest in treatments receiving 150 mg/L active B-T20 for 4 hours (data not shown). Based on the results of the study, it appeared that methanogens were more resistant to B-T20 than acetogenic strains. However, because of the dependence of methanogens on fermentative bacteria to generate H_2, formate, and/or acetate, for growth, inhibition is increased indirectly. This was apparent upon exchanging the anaerobic chamber gas mix with N_2, 7 days into the experiment. Without an external source of CO_2 and H_2, methanogens were dependent upon the activities of the slow-growing fermentative bacteria, and a subsequent drop in the rate of methane production was observed.

TABLE 1. The effects of B-T20 on citrate utilization

	citrate (mM)		acetate (mM)	
	day 0	day 47	day 0	day 47
A1	13.0	12.9	1.2	9.0
A2		4.8		20.4
A3		0.0		28.2
B1	12.9	14.3	1.1	0.9
B2		12.3		0.8
B3		12.8		0.7
C1	0.0	0.0	0.6	0.6
C2		0.0		0.6
C3		0.0		0.6
D1	1.3	0.0	0.7	3.3
D2		1.2		0.8
D3		0.0		3.5
E1	0.0	0.0	0.9	0.3
E2		0.0		0.7
E3		0.0		0.5

CONCLUSIONS

Daily application of Redux® B-T20 at a 150 mg/L active concentration, in combination with pulsed injection of the vitamin B_{12}/Ti(III)-citrate mix was effective for the prevention of biological fouling over a 12-week remediation treatment. Bacteriostatic activity was limited to the anaerobic treatment zone and did not interfere with ongoing biological degradation outside this region. B-T20 was effective in preventing premature citrate utilization and in extending the area of treatment.