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Environnement Canada

Pilot Scale IN-Situ Remediation of Chlorinated
Solvents in Groundwater using Vitamin B12

By:

S. Lesage, C. Mowder, T. Llewellyn, S. Brown...

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ABSTRACT

This paper describes a series of pilot scale applications of a combined chemical/biological remediation method based on the use of a vitamin B₁₂ and titanium citrate. This technology, which has the ability to degrade mixtures of chlorinated methanes, ethanes and ethenes, was developed by Environment Canada and applied at Graces Quarters, Aberdeen Proving Grounds in Maryland. The major contaminants of concern at the site are carbon tetrachloride, 1,1,2,2-tetrachloroethane (TeCA), and trichloroethene. A 10" RW was used for optimal delivery of the treatment and mixing with the groundwater. The project was conducted in three three-month phases conducted a year apart. In the initial phase (Fall 1999), the emphasis was placed on maximizing the amount of reaction occurring in the well itself while minimizing chemical addition. The purpose of the second phase (Fall 2000) was to deliver a concentrated treatment as directly as possible to areas of higher contaminant concentrations, indicative of a dense non-aqueous phase residual. The treatment mixture was added in weekly concentrated pulses of 20 hours. The pH and Eh, as surrogate parameters for the movement of the active ingredients, were monitored weekly in the 24 monitoring points installed radially up to 80 ft away from the RW. After three months of uninterrupted pulsed treatment the contaminant concentrations decreased to below guidelines in a 20-ft radius around the recirculation well. In the third year, the recirculation well was used without further chemical addition, to bring contaminated groundwater through the bio-active zone that was created by active treatment. This resulted in the concurrent biodegradation of CT, TeCA and TCE without the need for further chemical addition, showing that the vitamin B₁₂ treatment can be effectively delivered to the subsurface and integrated into subsequent bioremediation.

Restauration *in situ* à l'échelle pilote, à l'aide de vitamine B₁₂, d'eaux souterraines contaminées par des solvants chlorés

RÉSUMÉ

La présente communication décrit une série d'applications à l'échelle pilote d'une méthode combinée chimique/biologique, basée sur l'utilisation de la vitamine B₁₂ et de citrate de titane. Cette technologie, qui a la capacité de dégrader des mélanges de méthanes, d'éthanes et d'éthènes chlorés, a été mise au point par Environnement Canada et appliquée à Graces Quarters, au polygone d'essais d'Aberdeen, au Maryland. Les principaux contaminants préoccupants du site étaient le tétrachlorure de carbone, le 1,1,2,2-tétrachloroéthane (TeCA) et le trichloroéthène. Un puits de recirculation (PR) de 10" a été utilisé pour l'application du traitement et le mélange optimisés avec les eaux souterraines. Le projet a été réalisé en trois phases d'une durée de trois mois chacune, à 1 an d'intervalle entre deux phases successives. Dans la phase initiale, (automne 1999), on a surtout mis l'accent sur la maximisation de la quantité de réaction se produisant dans le puits comme tel tout en réduisant au minimum l'addition de produit chimique. Le but de la seconde phase (automne 2000) était d'appliquer un traitement concentré de façon aussi directe que possible dans les zones à fortes concentrations de contaminants, indiquant la présence d'un résidu dense en phase aqueuse. Le mélange du traitement était ajouté par giclées concentrées hebdomadaires d'une durée de 20 heures. Le pH et l'Eh, paramètres substitués pour le déplacement des ingrédients actifs, ont été suivis hebdomadairement aux 24 points de contrôle, installés dans un rayon allant jusqu'à 80 pieds du PR. Après trois mois de traitement ininterrompu par giclées, les concentrations de contaminants ont baissé en-dessous des lignes directrices s'appliquant à un rayon de 20 pieds autour du PR. Lors de la troisième année, le puits de recirculation a été utilisé sans addition de produit chimique, de façon à amener les eaux souterraines contaminées à travers la zone bioactive créée par le traitement actif. Cela a entraîné la biodégradation concurrente du CT, du TeCA et du TCE sans qu'il soit nécessaire d'ajouter des produits chimiques supplémentaires, ce qui montre que le traitement à la vitamine B₁₂ peut être appliqué efficacement sous la surface et intégré à la restauration subséquente.

NWRI RESEARCH SUMMARY

Plain language title

Remediation of Chlorinated Solvents in Groundwater using Vitamin B12

What is the problem and what do scientists already know about it?

Chlorinated solvents used in industry and as dry cleaning fluids can persist for decades in groundwater. Depending on the conditions, sometimes bacteria can biodegrade them, but other times not, because they are toxic at high concentrations. NWRI has developed, in the laboratory, a groundwater remediation method based on the use of vitamin B12 and titanium citrate that can replace bacterial action.

Why did NWRI do this study?

This paper reports a series of field-scale application of the technology at a US Military site where a mixture of solvents that were used more than 40 years ago contaminated an aquifer.

What were the results?

There were three different type of applications done at the site. The most successful one was based on using a recirculation well to deliver a concentrated treatment, in pulsed fashion, once a week.

How will these results be used?

These results will be used to develop a full-scale remediation of the site.

Who were our main partners in the study?

We were partners with URS Corporation, who were the contractors to the US Army.

Sommaire des recherches de l'INRE

Titre en langage clair

Restauration d'eaux souterraines contaminées par des solvants chlorés, à l'aide de la vitamine B12

Quel est le problème et que savent les chercheurs à ce sujet?

Les solvants chlorés utilisés dans l'industrie et comme liquides pour le nettoyage à sec peuvent persister pendant des décennies dans l'eau souterraine. Selon les conditions du site, les bactéries peuvent parfois les dégrader, mais pas toujours en raison de leur forte toxicité à concentration élevée. L'INRE a mis au point en laboratoire une méthode de restauration des eaux souterraines, basée sur l'emploi de la vitamine B12 et du citrate de titane, qui peuvent remplacer l'action bactérienne.

Pourquoi l'INRE a-t-il effectué cette étude?

La présente communication présente une série d'applications de cette technologie, à l'échelle du terrain, à un site militaire aux États-Unis, où un mélange de solvants utilisés il y a plus de 40 ans a contaminé une formation aquifère.

Quels sont les résultats?

Trois différents types d'applications ont été effectuées à ce site. Le plus efficace utilisait un puits de recirculation pour appliquer un traitement concentré, par giclées, à raison d'un par semaine.

Comment ces résultats seront-ils utilisés?

Les résultats serviront à mettre au point une méthode de restauration du site à l'échelle réelle.

Quels étaient nos principaux partenaires dans cette étude?

Nous avons travaillé en partenariat avec l'URS Corporation, l'entrepreneur dont les services ont été retenus par l'armée américaine.

Pilot Scale In-Situ Remediation of Chlorinated Solvents in Groundwater using Vitamin B₁₂

Suzanne Lesage, Carol S. Mowder, Tim Llewellyn, Susan J. Brown, KellyR. Millar, Sarah Forman
George DeLong, Donald J. Green.*

AUTHOR ADDRESS (Word Style "BC_Author_Address"). NWRI, Environment Canada, 867 Lakeshore Rd., Burlington, Ontario, Canada; Arcadis, Linthicum, Maryland, USA, AIMTech, Oak Ridge, Tennessee, USA, U.S. Army Garrison, APG, Maryland, USA.

E-MAIL: Suzanne.Lesage@ec.gc.ca

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CORRESPONDING AUTHOR FOOTNOTE (Word Style "FA_Corresponding_Author_Footnote").

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ABSTRACT (Word Style "BD_Abstract"). This paper describes a series of pilot scale applications of a combined chemical/biological remediation method based on the use of a vitamin B₁₂ and titanium citrate. This technology, which has the ability to degrade mixtures of chlorinated methanes, ethanes and ethenes, was developed by Environment Canada and applied at Graces Quarters, Aberdeen Proving Grounds in Maryland. The major contaminants of concern at the site are carbon tetrachloride, 1,1,2,2-tetrachloroethane (TeCA), and trichloroethene. A 10" RW was used for optimal delivery of the

treatment and mixing with the groundwater. The project was conducted in three three-month phases conducted a year apart. In the initial phase (Fall 1999), the emphasis was placed on maximizing the amount of reaction occurring in the well itself while minimizing chemical addition. The purpose of the second phase (Fall 2000) was to deliver a concentrated treatment as directly as possible to areas of higher contaminant concentrations, indicative of a dense non-aqueous phase residual. The treatment mixture was added in weekly concentrated pulses of 20 hours. The pH and Eh, as surrogate parameters for the movement of the active ingredients, were monitored weekly in the 24 monitoring points installed radially up to 80 ft away from the RW. After three months of uninterrupted pulsed treatment the contaminant concentrations decreased to below guidelines in a 20-ft radius around the recirculation well. In the third year, the recirculation well was used without further chemical addition, to bring contaminated groundwater through the bio-active zone that was created by active treatment. This resulted in the concurrent biodegradation of CT, TeCA and TCE without the need for further chemical addition, showing that the vitamin B12 treatment can be effectively delivered to the subsurface and integrated into subsequent bioremediation.

BRIEFS: Pilot scale demonstration of the delivery of vitamin B12 and titanium citrate through a recirculation well at Aberdeen Proving Grounds in Maryland was successful in treating groundwater contaminated with carbon tetrachloride, 1,1,2,2-tetrachloroethane and trichloroethylene.

Introduction

In-situ bioremediation is now recognized and used frequently to remediate groundwater contaminated with chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethylene (TCE), either naturally or through the addition of carbon sources. However, when the contaminants include chlorinated ethanes and methanes, the success of bioremediation has often been mitigated. A cell-free biocatalyst such as vitamin B12 presents an attractive alternative as an in situ restoration method, because it does not have many of the limitations encountered when using biological systems. Although microorganisms are now

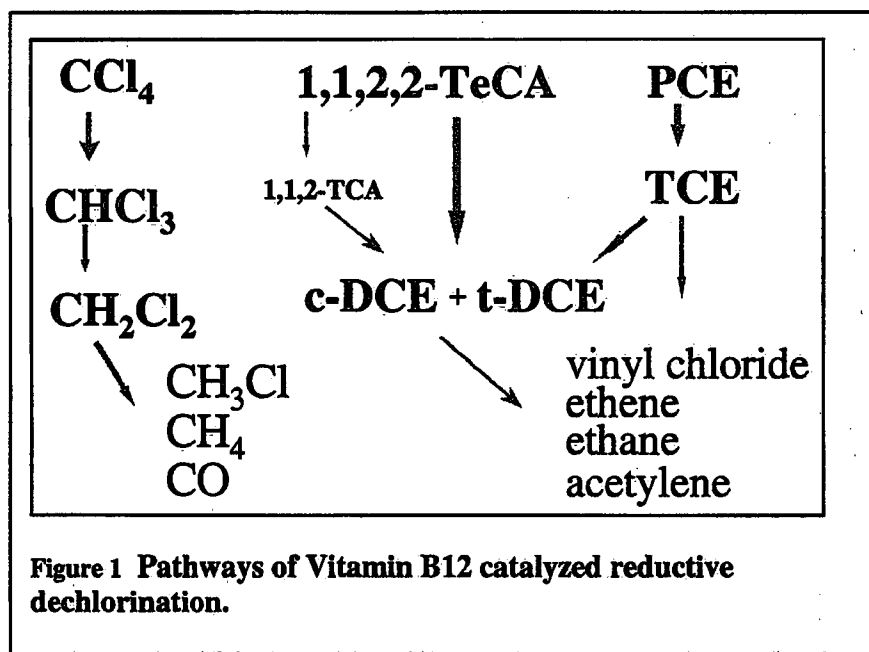
being acclimated to higher concentrations of chlorinated solvents [1] DiStefano et al. 1991), microbial degradation often is limited by toxic levels of pollutants or adverse environmental conditions. The Graces Quarters area at Aberdeen Proving Ground is a site which has presented such a challenge. This site is part of a U.S. Army facility located on the shores of Chesapeake Bay near Edgewood, Maryland, that was used for testing weapons delivery systems and chemical warfare materials between the 1940s and early 1970s. As part of these activities, a decontamination agent, composed primarily of 1,1,2,2-tetrachloroethane (TeCA), was used.

Graces Quarters is located on the Gunpowder Neck Peninsula. The Pilot Test site is located in the Primary Test Area which is approximately 22 acres (0.089 square kilometers (km²)) in size. This area is underlain by an unconfined surficial aquifer, which is separated from an underlying confined aquifer by a silty clay confining unit. This confining unit is absent in some areas allowing the surficial and confined aquifers to form one hydrologic unit at these locations. Groundwater flows south-southwest in the surficial aquifer. Upon encountering the migration pathways in the confining layer, the groundwater flows into the confined aquifer and flows outward in a radial pattern that is superimposed on an overall southward flow field.

The Surficial Aquifer was impacted by the decontaminating agent which resulted in the groundwater to be contaminated with mostly TeCA but also TCE, carbon tetrachloride (CT), and chloroform (CF), which were also present in the commercial product. Generally, volatile hydrocarbon (VOCs) concentration range up to 6,000 µg/L in total, however, TeCA, has been detected at concentrations as high as 181,000 µg/L, using a geoprobe sampler, indicating the likelihood of residual DNAPL being present. In addition, the surficial aquifer was generally aerobic, with pH ranging between 4- 4.5, conditions that are not favourable for anaerobic microbial degradation. These conditions are very different than those prevailing in the wetlands area of Aberdeen Proving Grounds, as reported by Lorah and Olsen [2](1999).

As part of a remedial investigation feasibility study, a laboratory study was conducted first to verify the suitability of using vitamin B12/titanium citrate to degrade the contaminants at the site ([3,4]Millar et al. in submittal; Lesage et al GWMR accepted). Many researchers had suggested that vitamin B12 would potentially be useful to treat waters contaminated by chlorinated solvents (5 Glod et al. 1997; 6 Lesage et al. 1996; 7 Burris et al. 1996; 8 Chiu and Reinhard 1995; 9 Assaf-Anid et al. 1994; 10 Shanke and Wackett 1992; 11 Gantzer and Wackett 1991; 12 Krone et al. 1991; 13 Krone and Thauer 1989), but the technology (14 Lesage 1997) had only been tested once in the field (15,16 Sorel, 1998 and 2001) using an artificial spill of less than 1L of a mixture of PCE and 1,1,1-trichloroethane into in-situ vertical column installed a carbonate sand aquifer (pH 7.8). Because the reaction requires a strong reductant, its interaction with the aquifer material is crucial in determining the amount of reagent required (17 Barcelona 1991; 18. Heron 1994).

Vitamin B12 (cyanocobalamin) belongs to a class of compounds called corrinoids, which are synthesized primarily by anaerobic bacteria. When the cobalt located at the center of the vitamin B12 corrin ring is reduced from its Co(III) oxidation state in the natural environment to Co(I), using a strong



reducing agent such as titanium(III) citrate, it becomes a potent nucleophile (19 Schrauzer) displacing a chlorine atom and forming a carbon-cobalt bond (20 Lesage 1998). The complex thus formed undergoes either homolytic cleavage, or further elimination or reductive dechlorination to produce a series

of dechlorination products. The pathways for TeCA, CT and PCE are shown on Figure 1.

The project was conducted in three three-month phases. In the initial phase (Fall 1999), the emphasis was placed on maximizing the amount of reaction occurring in the recirculation well (RW) itself while minimizing chemical addition. The mixture was supplemented with a glucose-fructose syrup as a preferential carbon source to citrate and as a source of organic acids known to generate H_2 to support dechlorinating bacteria. While this strategy was successful in allowing titanium citrate to be transported at least 7 m from the RW (21 Francis, 1992), it caused excessive bacterial growth resulting in the clogging of the RW and the adjacent aquifer.

After using various methods to clean the well screens, it was decided to change the rate of delivery of the chemical treatment and to add Tolcide™ as a bacteriostatic agent. (22 Forman et al. 2001; 23 Millar et al. 2001). The purpose of the second phase (Fall 2000) was to deliver a concentrated treatment as directly as possible to areas of higher contaminant concentrations, indicative of a dense non-aqueous phase residual. The direction of flow in the RW was reversed and the vitamin B12 concentrate, without the glucose/fructose syrup, was added in weekly concentrated pulses of 20 hours, followed by four hours of Tolcide™.

In the third phase in the fall of 2001, it was decided to turn on the RW pump without any further chemical addition. The purpose of this phase of the experiment was to evaluate the residual biological activity and the extent of bioremediation possible, long after chemical addition had ceased.

Materials and Methods

Chemical Process: The vitamin B₁₂ concentrate was generated on site in weekly batches. This process (14. Lesage et al. 1997) involved preparing titanium oxalate from titanium metal and oxalic acid, followed by the addition of sodium citrate. Titanium oxalate was prepared in 500 gallon batches by adding 50 kg of oxalic acid 500 gallons of water and 10 kg of titanium (sponge grade), letting sit for 1

week The solution was then transferred to a 750 gallon mixing chamber where, sodium citrate, calcium carbonate and sodium carbonate were added in sequence. The oxalate in solution was precipitated as calcium oxalate after the addition of calcium carbonate and calcium citrate. Sodium carbonate was added to increase the pH to above 8.0 and decrease the Eh to approximately -900 millivolts (mV). Vitamin B₁₂ and glucose were then added. The vitamin B₁₂ concentrate was transferred to a collapsible pillow tank (ATL Flex-Tanks, Aero-Tec Laboratories Inc., Ramsey, NJ) to ensure anaerobic conditions were maintained as it was continuously metered into the RW. Approximately 600 gallons of vitamin B₁₂ concentrate were prepared each week. In the first phase, glucose was added as a preferential carbon source to prevent citrate degradation by bacteria, which had been found to result in titanium precipitation (16 Sorel 2001). In the second phase, the same amount of concentrate was prepared, but glucose was omitted.

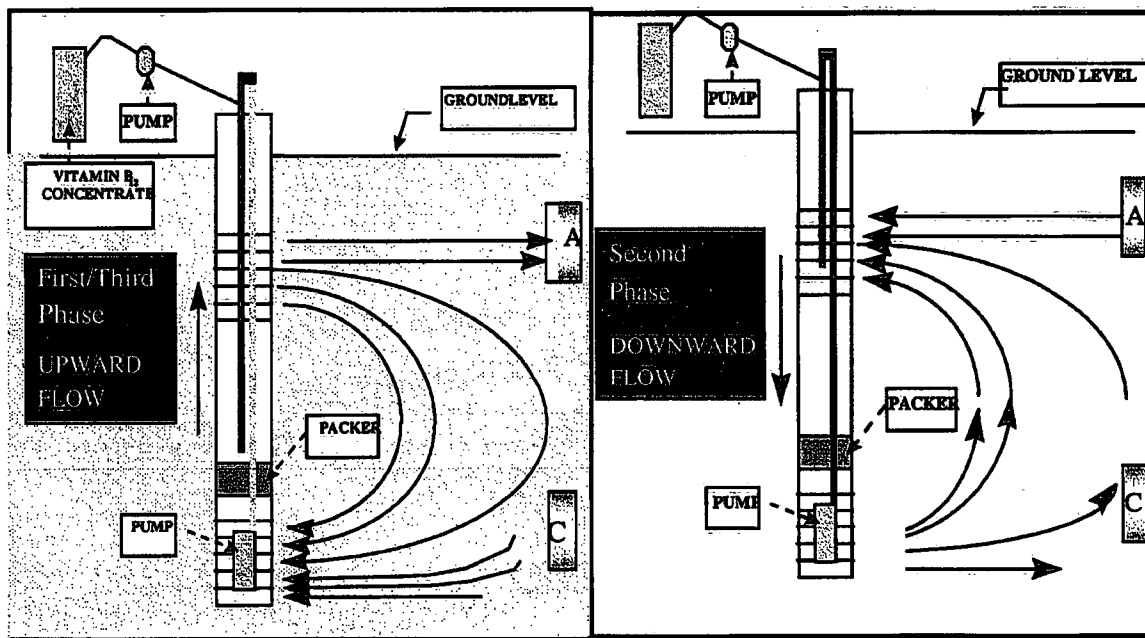


Figure 2 Schematic view of the RW in upward(a) and downward (b) flow configuration. The rectangles marked A and C respectively represent the screened intervals of the monitoring wells.

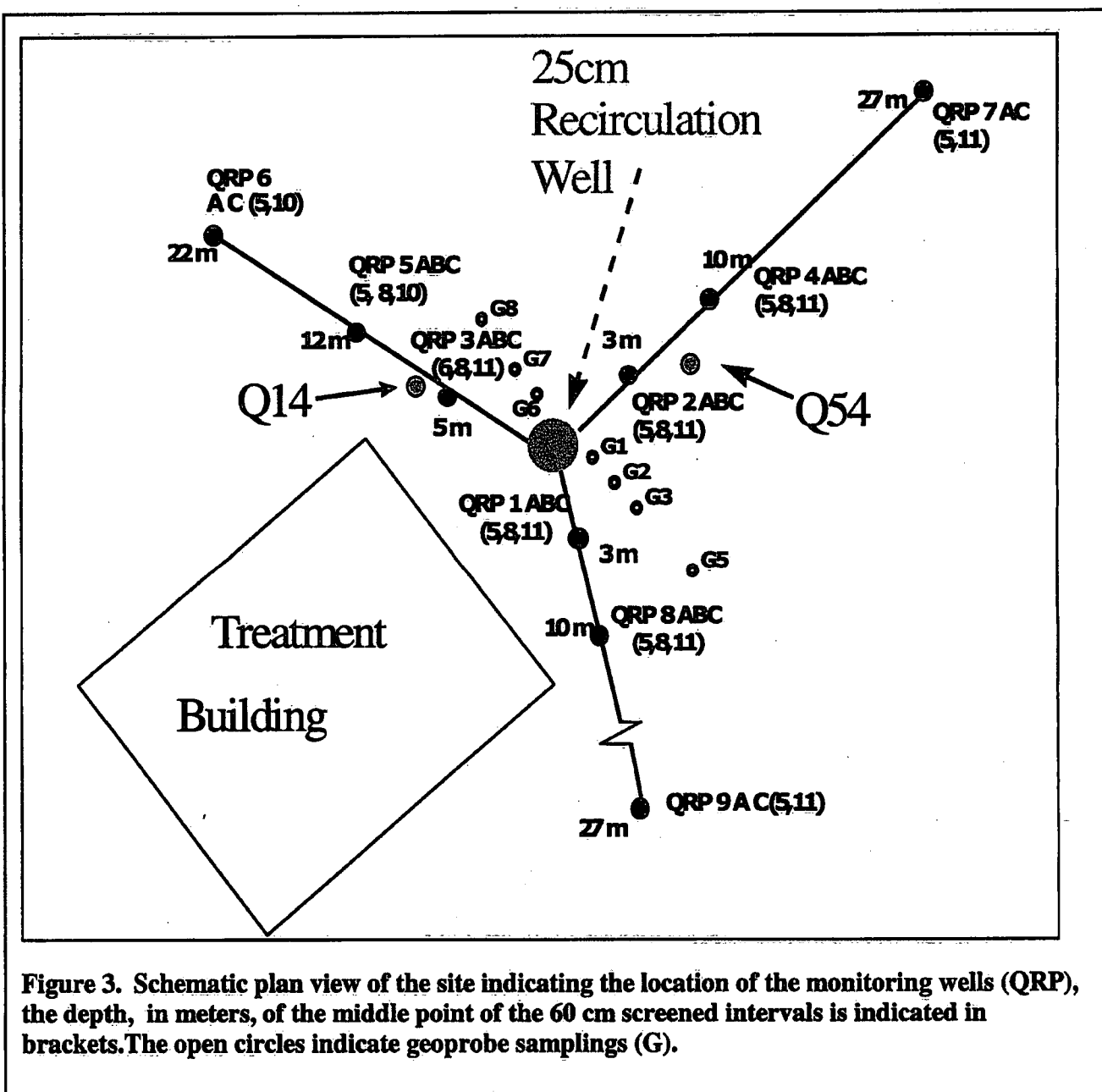
Injection. A RW (RW) was used to facilitate *in situ* mixing of the vitamin B₁₂ concentrate and contaminated groundwater during the pilot test. RWs are large diameter (15 to 25 cm in diameter) with two screened areas separated by an in-well pump that forces flow in either direction. They exist in many

configurations for different applications (24. US EPA), including vacuum systems to strip out volatiles, or air injection to enhance bioremediation. Although its use for adding reagents under anaerobic conditions had never been documented, it was possible to configure it to exclude any air intrusion and tee-in a chemical feed line. A schematic of the RW and vitamin B₁₂ feed system is shown on Figure 2. In the first and third phases (Figure 2a), contaminated groundwater from the site was drawn into the lower well screen of the 10-inch diameter stainless RW and pumped above the inflatable packer which separated the upper and lower well screens. Groundwater was pumped at 2 gallons per minute (gpm) and mixed with the vitamin B₁₂ concentrate, which was added at approximately 0.05 gpm. This system allowed a nominal residence/reaction time of 30 minutes in the well before discharging the groundwater/vitamin B₁₂ concentrate through the upper well screen and into the aquifer. In the second phase (Figure 2b), the direction of flow was reversed and the pumping rate was increased to 4 gpm resulting in a 10 min in-well residence time. The concentrate was added weekly to the well over a 20 hour period, followed by a Tolcide™ (Tetrakis(hydroxymethyl)phosphonium sulfate; supplier Albright and Wilson) concentrate for four hours, at a final concentration of 150 ppm, active ingredient. The four-hour Tolcide™ addition was repeated every day.

Monitoring: The RW and surrounding monitoring wells are shown in plan view in Figure 3. During the first phase, groundwater samples collected every 2 weeks from the lower and upper part of the RW, and the surrounding multiple-depth monitoring wells, were analyzed for VOCs, dissolved gases (methane, ethane, ethene, and acetylene), volatile fatty acids (VFAs - acetate, butyrate, citrate, formate, lactate, and propionate), titanium, iron, manganese, and chloride. All analyses were performed in a fixed-base commercial laboratory using US EPA approved methods. Additionally, Eh and pH were monitored in the field using a flow-through cell. In the second phase, daily sampling of Eh and pH was conducted, then reduced to monthly. The change in Eh (± 100 mV) was used to select the wells for a complete set of analytical parameters.

Results and Discussion

First phase: In-well reaction. The system was designed initially to maximize in-well reaction time. The rationale was that, in the absence of aquifer solids, the titanium citrate would preferentially react with the chlorinated compounds in solution. The reaction time of 30-minutes was based on laboratory microcosm data for the reductive dechlorination of TeCA. The degradation of CT was always complete



within the well, without the accumulation of chloroform or dichloromethane, even when the turbidity

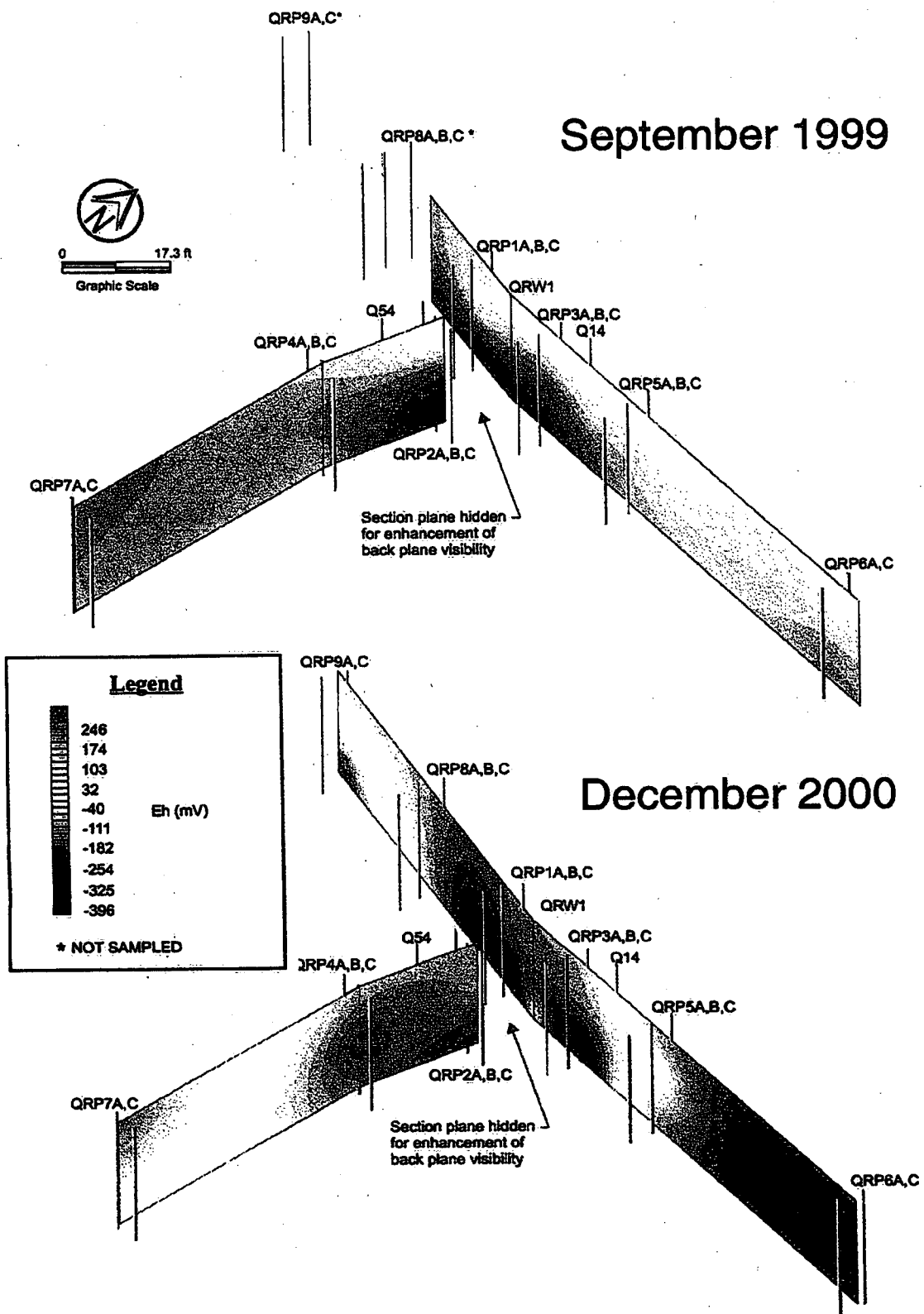
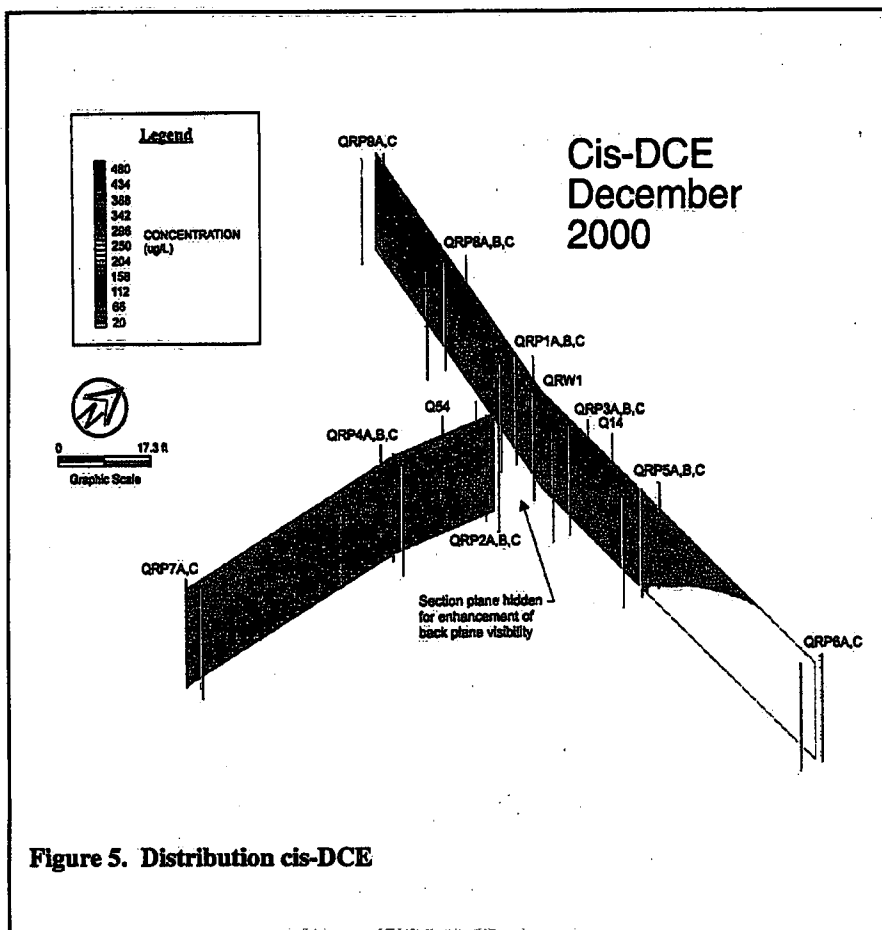


Figure 4 . Eh Distribution

Before treatment, the whole area was essentially aerobic. The Eh decreased to approximately -150 mV at QRP 4A and B, 10 m from the well, and to -100 mV in the east transect at QRP 3A and B, 5 m. No data was available for the west transect past QRP 1A, which was also negative. These results correlated well with the distribution of cis-DCE, a degradation product of TeCA and TCE, which was totally absent from the area prior to treatment (Figure 5). The concentrations of TeCA and CT were also significantly reduced during that period (Figure 6). Although the degradation of TeCA was not complete within the well, the reaction continued in the aquifer where, at 3 m (QRP 1A), the removal ranged from 43 to 99%. In all but one sample, the concentration of TeCA was lower in QRP 1A than in the upper portion of the RW.



The concentration of TCE did not change significantly. This may be because most of the vitamin B₁₂ was reacting with the more abundant and reactive substrates (CT, TeCA, and CF) or because the residual concentration of titanium was not sufficiently high to reduce the vitamin B₁₂ to the lower Eh needed for its degradation. As the vitamin B₁₂ concentrate and treated groundwater extended through the aquifer

with time, a decrease in CT and CF concentrations was also observed in the B level monitoring wells and those further from the RW. Although the conditions were anaerobic, there was no accumulation of vinyl chloride: the highest amount measured was 24.1 $\mu\text{g/L}$ in 4A at Week 8, which had decreased to 5.3 $\mu\text{g/L}$ by Week 18.

Biofouling. The constant addition of glucose-fructose resulted in biological growth that was visible in the water samples taken from the RW and the surrounding wells. This caused enough reduction in permeability that the water levels rose in the RW and caused shutdown. Hydrogen peroxide was added to the RW (without the downhole piping and the packer assembly) to achieve a five percent (5%) solution. The interior of the well was scrubbed with a large brush and water was evacuated to remove the biomass. In addition, the upper screened interval of the well was isolated with a packer and purged.

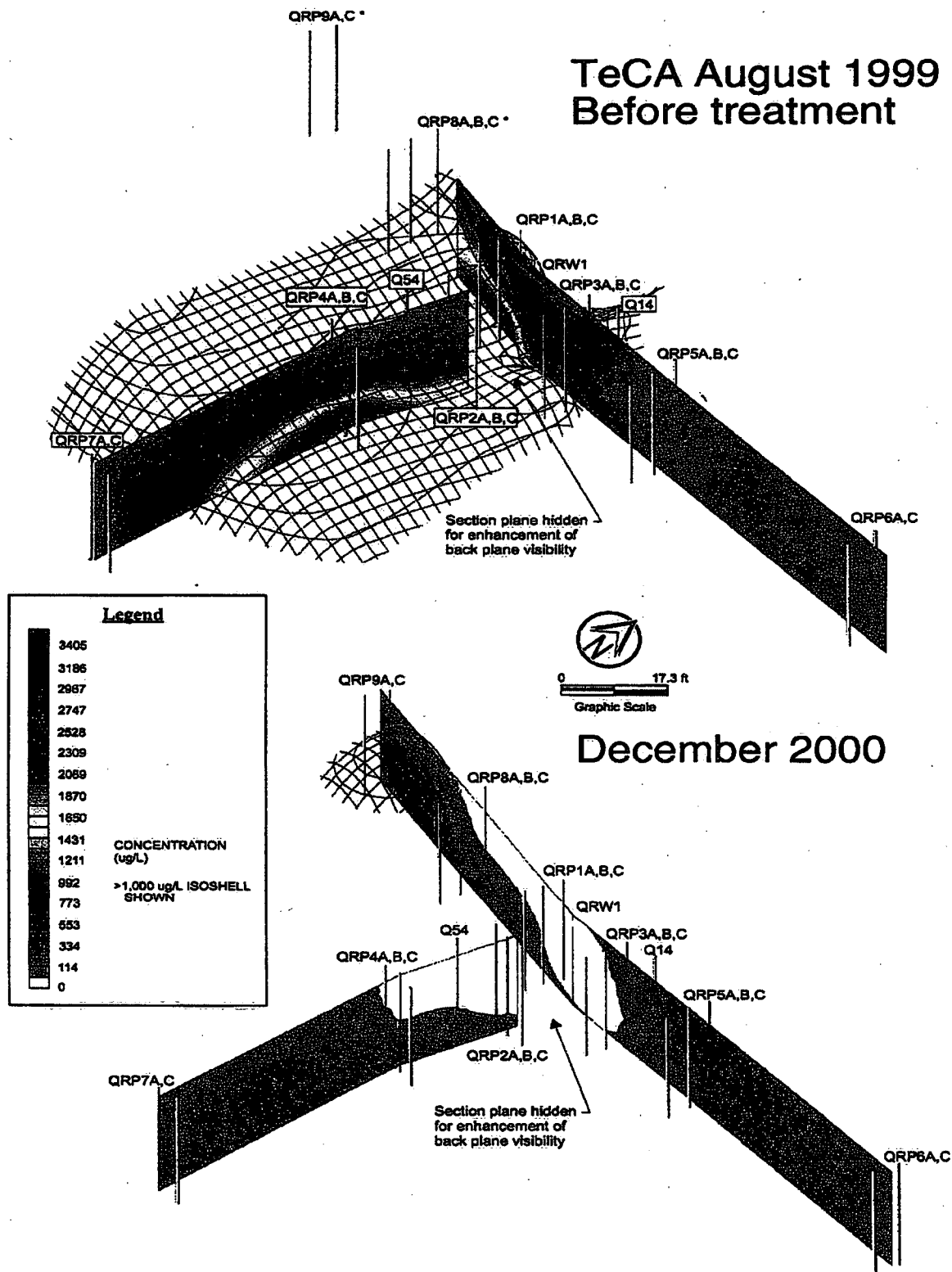


Figure 6. Distribution TeCA

Although this method succeeded in cleaning the bacteria from the inside of the well, it remained difficult to inject groundwater into the upper screen of the RW, indicating that well pack and the surrounding formation were also affected. The lower screen of the well remained relatively unaffected by the biofouling.

In order to select an appropriate remedy, an evaluation of the lateral extent of bacterial growth was done by collecting a series of geoprobe samples of the aquifer matrix at seven locations (Figure 3). Each geoprobe was advanced to a depth of 20 feet (6.10 m) below ground surface (bgs) and soil cores were collected at each location from 10 to 20 feet (3.05 to 6.10m) bgs. Five geoprobe cores were sent to a geotechnical laboratory for permeability testing. It was found that permeability correlated more with lithology than with bacterial density. Geoprobe samples were also analyzed for titanium, iron, and manganese. The samples were analyzed for titanium because bacterial activity is associated with the degradation of citrate and the resulting deposition of titanium dioxide. The metals results indicate that the titanium had moved through the system as far as 20 feet (6.10 m) from the RW. There was no correlation between concentration and distance from the RW. The highest concentrations were associated with the presence of fines. Since Ti concentrations were on the same order of magnitude as Fe concentrations, the redistribution of TiO_2 over the treated area would not cause of a significant reduction in permeability. The manganese concentrations were non-detect to low to within approximately 10 feet (3.05 m) of RW, with higher concentrations seen at the geoprobe collected at a distance of 20 feet (6.10 m) away (DMGP05). This would indicate that the metals were dissolved and redeposited.

Six samples of the aquifer matrix were submitted for analyses of microbial phospholipid fatty acids (PLFA), and deoxyribonucleic acid (DNA) by denaturing gradient gel electrophoresis (DGGE). PLFA analysis indicates the type of microbes present and how they are reacting to environmental factors. The results shown on Figure 7 indicated that area of extensive biological growth was in the shallow zone (3 m) and not further than 3 m radially.

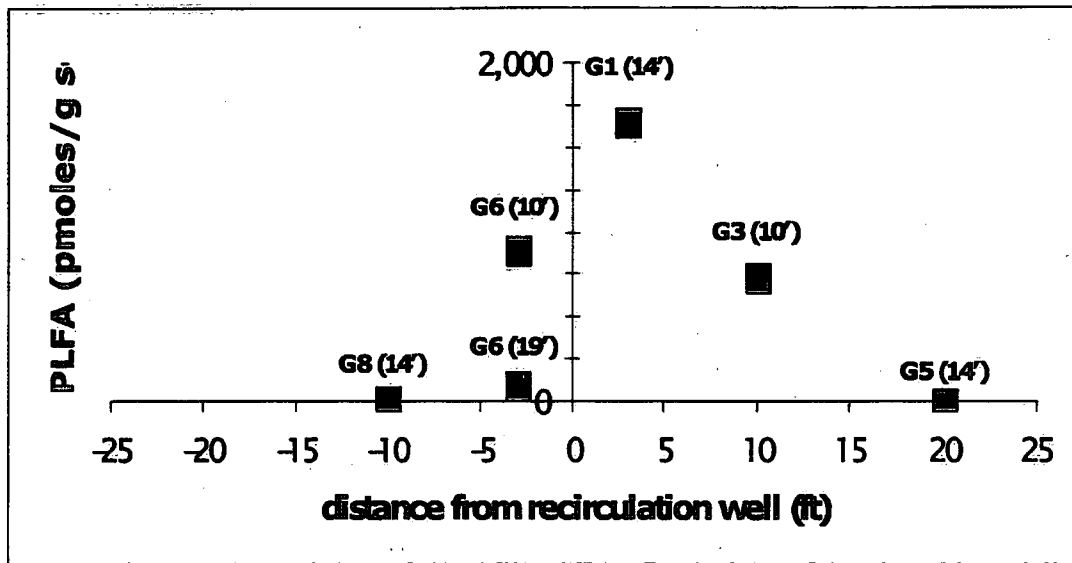
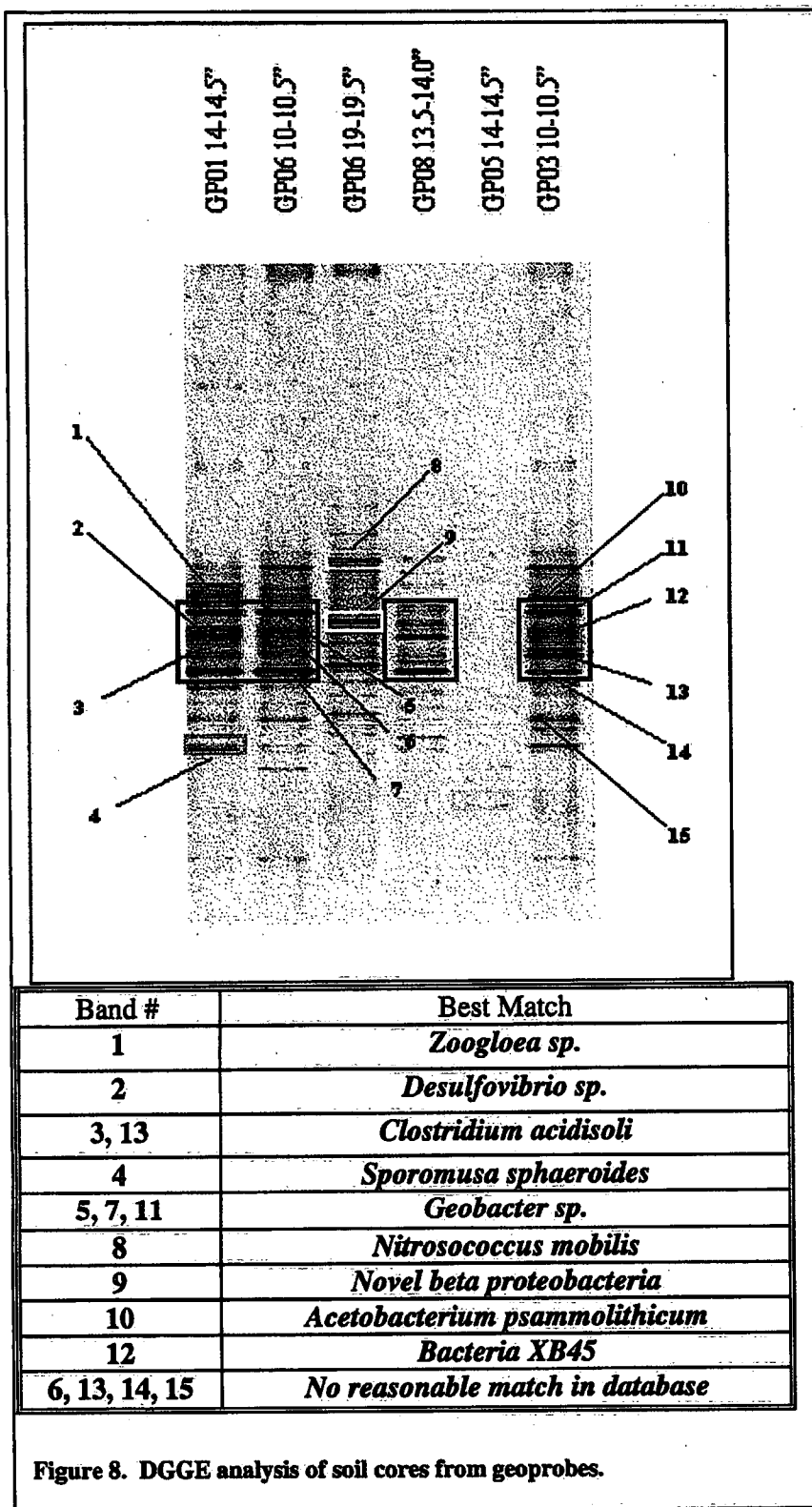


Figure 7. Viable biomass from geoprobe samples. Numbers refer to geoprobe location (see Figure 3) and depth of sample in feet.

DNA analysis provides a closer look at the diversity of the microbial populations. The bacterial populations for the shallow samples from GP – 1, 6 and 3, with the highest biomass, were found to be similar to each other in composition, and very different from the samples taken deeper or further away, which are presumed not to have received treatment (Figure 8). The main species identified were from the *Clostridium* and *Desulfovibrio* species, both strict anaerobes, indicating that the changes in redox caused by the addition of titanium citrate promoted a change in bacterial population and generated conditions that are supportive of reductive dechlorination, although these two species haven't been associated with the process (25 Fathepure). Sequences results also showed several bands affiliated with



the genus *Geobacter*. It is suggested that *Geobacter* is a dissimilatory Fe(III)- and sulfur-reducing bacterium (26 Lonergan et al, 1996).

Since the areal extent of reduced permeability was limited, the permeability was restored using the Aqua Freed® well redevelopment process, which consists of the injection of carbon dioxide under pressure. Gaseous carbon dioxide was injected into the upper and lower screened intervals of the RW, as well as several nearby piezometers. A total of 3,000 pounds (1,360 kg) of gaseous carbon dioxide were injected into the ground and 6,500 gallons (24,600 L) of groundwater were evacuated during this process. The packer, pumps, and associated downhole equipment were decontaminated with a

hydrogen peroxide solution prior to reinsertion into the RW.

Hydraulic testing showed that it had been effective. However, in order to prevent further occurrences of the problem, alternatives were sought for the following season. Changes included the addition of a bacteriostatic agent (Tolcide®); the removal of sugar from the concentrate; reversal of flow; and pulse injection to the subsurface. Tolcide® was selected because it is considered a green biocide, has a limited lifetime in groundwater and is not affected by titanium citrate. The second major change was to alter the delivery regime of the concentrate. In order to decide on the optimal schedule, the Tolcide® and vitamin B₁₂ concentrate injection concentrations and delivery scenarios were modeled using a modified version of a previously generated MODFLOW model and the MT3DMS model code. The options of pulsing the mixture into the aquifer in one day or three-day weekly injections were simulated, and relative pulse concentrations and propagation through the aquifer were evaluated. The selected delivery scenario (one day weekly pulses of vitamin B₁₂ with daily biocide injection) controlled the biomass on-site and field data validated the model. Hydrographs and a stable functioning system confirm that the biomass was controlled with the aid of the biocide. The reformulation of the vitamin B₁₂ concentrate aided in the control of the biomass.

Second Phase: Distribution in the aquifer. The interim activities carried out to take care of the excess biological growth in the immediate vicinity of the RW required that a new baseline round of sampling be conducted. The results showed that most of the residual contaminants were at the bottom of the surficial aquifer. Therefore, the flow in the RW was reversed (Figure 2b) and the pumping rate doubled to 4gpm. Modeling results indicated that pulsing the treatment would give an effectively higher concentration at a distance from the well, than continuously injecting a constant but lower concentration. It was also possible to reduce the total amount of vitamin B₁₂ to 100g per batch of 2300 L, which was injected over a 20 hour period and maintain 5.5 mg/L of vitamin B₁₂ in the well during injection. Similarly, the titanium concentration during injection was approximately 10 mM. Monitoring data showed concentrations up to 248 mg/L (5 mM) Ti at 3C. This may not have been the highest

concentration reached at that point, but nonetheless showed significant transport of the titanium compared to the first phase where the titanium concentration at 1A reached only 1 mM.

Eh, pH values and titanium concentrations indicated that the treatment had reached QRP 8C within 2 weeks. The large difference in total VOC concentrations between 1C (700 μ g/L) and 8C (4550 μ g/L) indicates the probable presence of residual contamination in the vicinity of 8C, for which unfortunately no historical data is available. CT decreased from 1312 μ g/L to not detected by the end of 12 weeks (Figure 9), whereas the final TeCA (Figure 6) and TCE (data not shown) concentrations were 20%

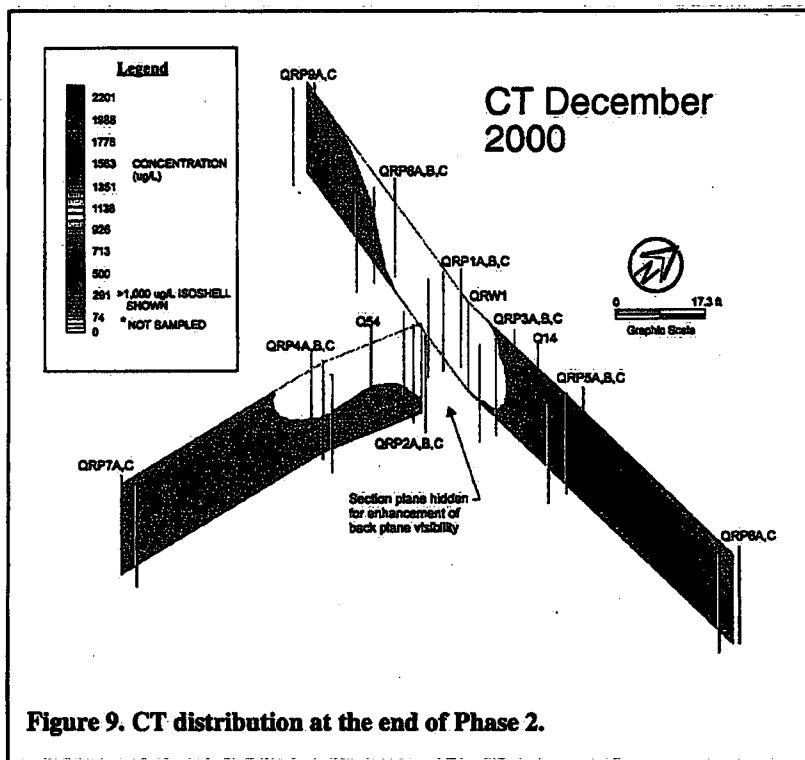


Figure 9. CT distribution at the end of Phase 2.

and 10% of their initial concentrations, respectively. VC did not exceed 5 μ g/L during the entire period. QRP 4C was at the same distance from RW as QRP 8C, but in a lower permeability zone. Even though the treatment did not reach it until the very last week, the CT concentration decreased from 2010 μ g/L to below detection, and TeCA and TCE were reduced to 20 and 16% of their initial concentrations. No evidence of treatment could be seen in QRP 5C. The furthest QRPs 9C, 7C and 6C did not receive any treatment and the concentration of the contaminants increased compared to baseline. In a longer-term treatment, this water would be recaptured and returned to the RW for treatment. Modeling of the RW using MODFlow indicated that this would take approximately 3 years.

Titanium and other metals

The addition of titanium (III) citrate was expected to have a marked effect on the geochemistry of the aquifer at the site. Because titanium (III) is a very strong reductant, it would reduce all the available mineral oxides. Therefore, as expected, as the treatment reached a monitoring well, it caused an increase in dissolved Fe, Mn and As. Citrate is also a very good chelating agent and any excess present in the treatment concentrate could bind these metals and increase their mobility. Fortunately, metal citrate complex are biodegradable (21. Francis) and the effect was transient. Similarly the oxidation capacity of the aquifer was such that the metals were redeposited. An example of the fluctuations in metal concentrations during the addition of treatment concentrate in Phase 2 is shown on Figure 10. The data, taken from the first row of monitoring wells, demonstrate that an increase in dissolved titanium concentration yields a concurrent increase in Fe and Mn, and that the total metal concentrations drop in unison. Arsenic concentrations were also measured but are not shown on the graph because of the scale. Concentrations rose to 312 and 300 $\mu\text{g/L}$ in September 2000 in QRP 2C and Q54 respectively, but by December, the concentration at these monitoring wells had dropped to 8.7 and 42.5 $\mu\text{g/L}$. During the long term monitoring (December 2002), most monitoring wells were in compliance for As except QRP4C (109 $\mu\text{g/L}$) and QRP8C (165 $\mu\text{g/L}$). These high concentrations correlated with higher dissolved Fe, Na and alkalinity associated with the treatment.

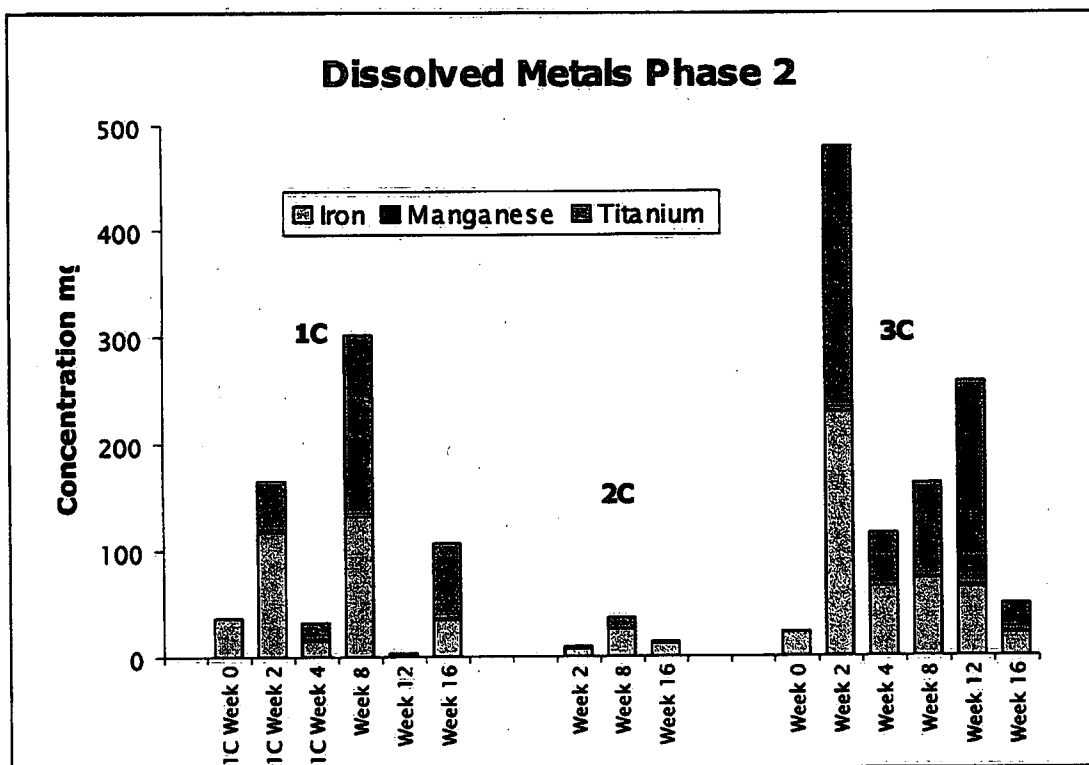


Figure 11. Distribution of volatile fatty acids during the no-treatment phase. The data is presented in the direction of the flow during pumping from August to December 2001. Note that citrate had totally been degraded by then.

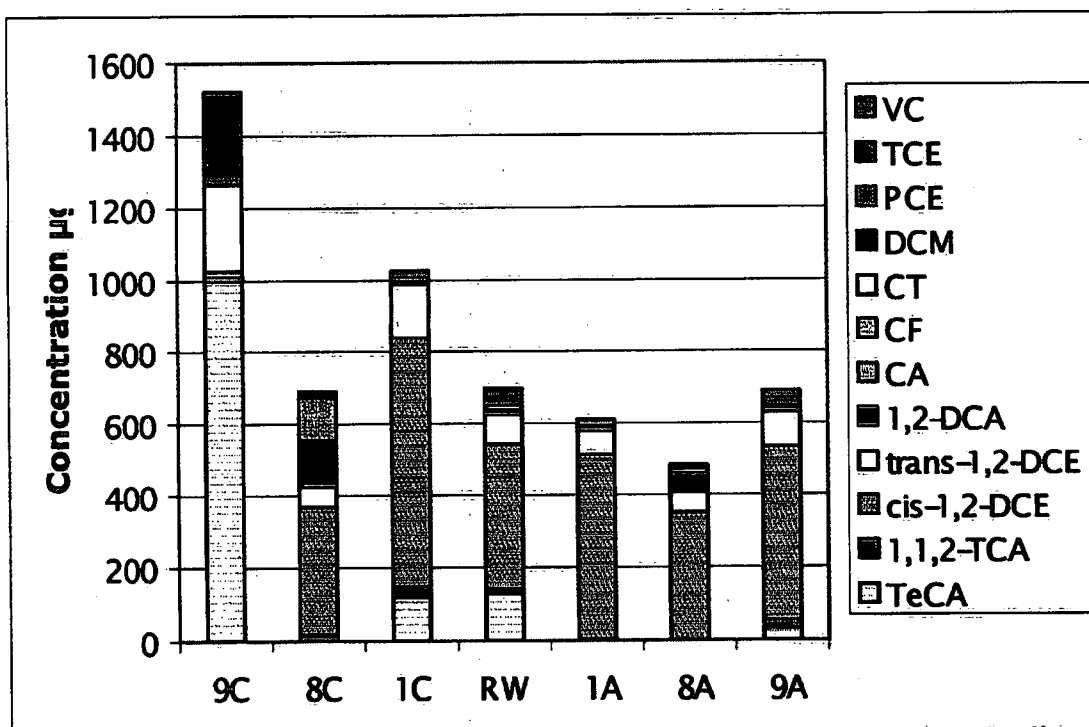


Figure 12. Final results of the post-treatment biological attenuation. The monitoring wells are shown in the order of the flowpath.

Post-treatment biological attenuation.

At the end of the second pilot test in December 2000, the area under the influence of the RW had been almost completely restored in a radius of at least 10 m (Figures 6 and 9). The monitoring wells furthest from the RW were still showing relatively high concentrations of parent compounds (TECA, CT, TCE). The pump was turned back on, in upflow direction (Figure 2b), to bring back some of the contaminated water that was beyond the 10 m radius and determine whether the contaminants could be biodegraded by the newly established bacteria. For this phase, sampling was concentrated on one of the transects only. A new set of samples were taken just prior to the initiation of pumping as a new baseline. The major contaminants at 9C were almost unchanged between the end of December 2000 and the new baseline. There were no biological degradation products and the total VOC concentration was close to 2000 $\mu\text{g/L}$. This dropped to approximately 1500 $\mu\text{g/L}$ during the test, but the relative proportion of the contaminants remained the same, indicating that there was no biodegradation occurring. This area can be considered a source area for the test.

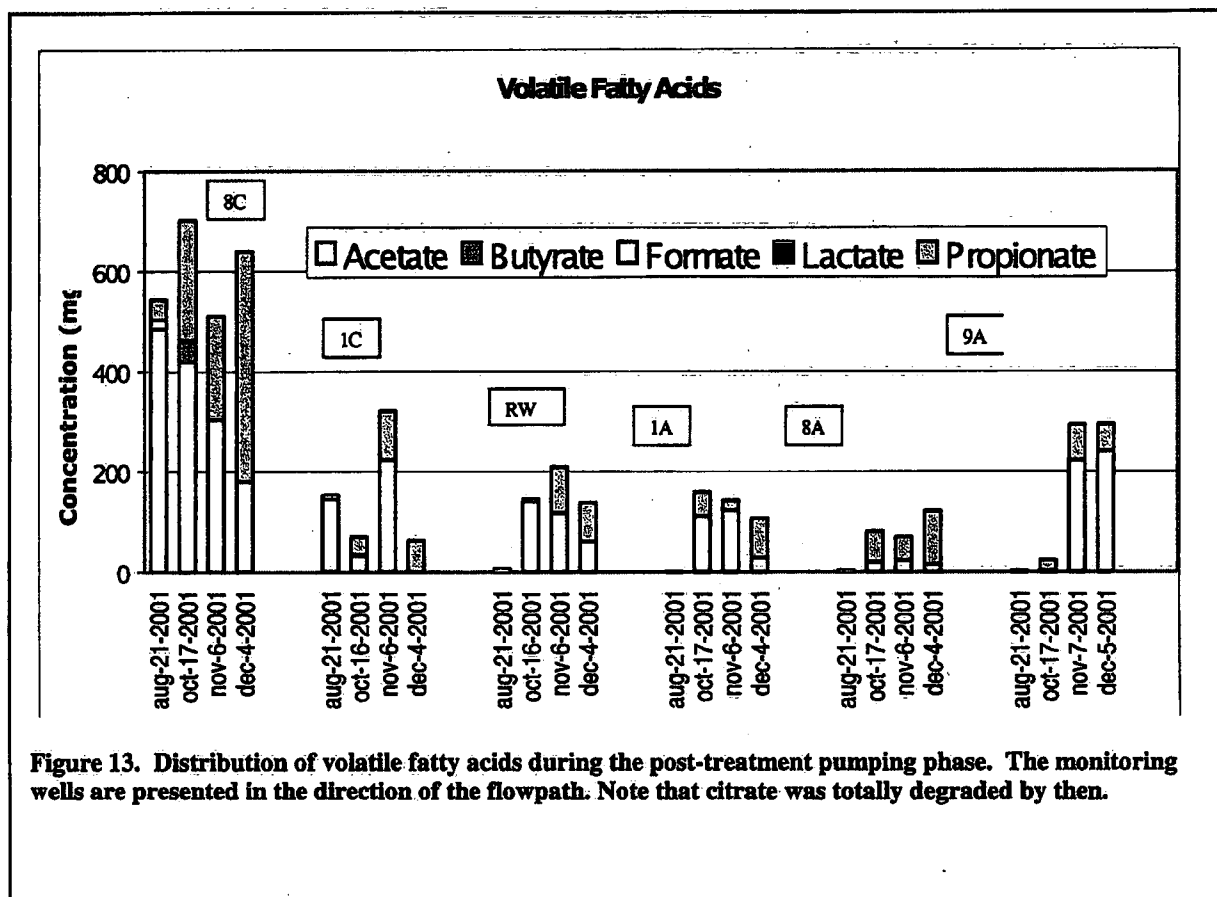
At QRP 8C, the total VOCs the TeCA concentration at the new baseline was 72 $\mu\text{g/L}$. The ratio of *cis/trans* DCE went from 2.5 to 7.3 between December 2000 and August 2001. The concentration of degradation products were: 157 $\mu\text{g/L}$ of *cis*-DCE, 52 $\mu\text{g/L}$ of 1,2-DCA and 37 $\mu\text{g/L}$ of 1,1,2-TCA. The change in the *cis/trans* DCE ratio, as well as the appearance of many other intermediates such as 1,1,2-DCA, 1,2-DCA, and chloroethane are indicative of the biodegradation of TeCA during the inactive period. The purpose of restarting the pump was to determine whether the bacterial population that had grown around the RW would be able to degrade the mixture of TeCA, CT and TCE coming from the outlying areas. Immediately after pump start-up the concentration of TeCA increased at 8C, as the untreated water coming from the direction of 9C arrived. After three months, most of the VOCs were daughter products, predominantly *cis*-DCE, and this, with a constant influx of parent compounds being

drawn in from 9C. The CT concentrations remained non-detectable throughout the test at 8C, and some CF (average 8 $\mu\text{g/L}$) and DCM (average 4 $\mu\text{g/L}$) indicate that it was also being biodegraded. The average CT concentration at 9C remained at 250 $\mu\text{g/L}$ throughout the period. The final results after 12 weeks of pumping are shown on Figure 13.

Further along the flow path, at 1C, there were no detectable VOCs at the new baseline. After pump start-up, the concentration of VOCs increased. These were almost entirely degradation products. *Cis*-DCE had the highest concentration, reaching 860 $\mu\text{g/L}$. This concentration decreased only slightly with time. The RW received water not only from the 1C transect, but from all directions. It is interesting to note that there was little change in the composition of the mixture. This shows that degradation occurred almost equally in all directions. There was a decrease in *cis*-DCE to an average of 400 $\mu\text{g/L}$, some CF (average 10 $\mu\text{g/L}$), and a small increase in TCE (average 50 $\mu\text{g/L}$). The next monitoring point along the flow path was 1A. This well was also devoid of VOCs at the end of December 2000, but after pump start-up, the concentrations increased. By the time the water arrived at 1A, TeCA and TCE had already decreased, leaving only *cis* and *trans*-DCE and less than 20 $\mu\text{g/L}$ each of 1,2-DCA and VC. The ratio of *cis/trans*-DCE was approximately 7.

The concentration of *cis*-DCE decreased from 500 $\mu\text{g/L}$ at 1A to 350 $\mu\text{g/L}$ at 8A while the concentration of VC decreased to less than 10 $\mu\text{g/L}$. The last well along the flow path, 9A contained very little biodegradation products until the last two sampling periods, where the concentration of *cis* and *trans*-DCE were similar to those at 8A a month earlier. Some TeCA was also found in the well, probably from the source area at 9C.

Bacterial processes. Overall at the site, the ratio of *cis/trans* DCE ranged from 2.65 to 3.5 at the end of the active vitamin B12 treatment in December 2000. In this phase, the ratio was much higher, ranging from 4 to 7, indicating a totally different reaction mechanism. All the sequential products of reductive dechlorination of TeCA, 1,1,2-TCA, 1,2-DCA and chloroethane, could be measured at any one point.



This high ratio has never been reported before and contrasts with the results from wetland areas at Aberdeen Proving Grounds, where biodegradation has occurred [2](Lorah and Olsen, 1999) and where the concentration of *trans*-DCE exceeded that of *cis*-DCE.

The other very encouraging and unusual feature of this system was the ability of the bacterial consortium to degrade CT rapidly and completely, without the accumulation of CF or DCM. Indeed CT and CF have been found to inhibit the reductive dechlorination of chloroethenes (27 Bagley et al. 2000; 28. Kaseros et al. 2000) and its presence in the system led to the failure of bioremediation attempts with

samples from the site (29. APG 1999). While some VC was also formed, its concentration was not alarmingly high, and, based on laboratory evidence, was likely to degrade with time.

While it is quite evident that the changes observed post-vitamin B12 treatment were biologically mediated, it is also known that most of these are co-metabolic processes. During the first phase of treatment, glucose had been added as a preferential carbon source to citrate. In the fall of 2000, Tolcide® had been used as a bacteriostatic agent to prevent rapid citrate utilisation. At the end of the treatment, citrate (970 mg/L) and acetate were present in 1C, and 4050 mg/L of acetate was found in 8C. By August, all citrate had been degraded and the concentration of acetate decreased to 485 mg/L and 180 mg/L in 8C and 1C respectively. The fate of the volatile fatty acids during the post-treatment pumping is shown on Figure 13. The concentration of acetate decreased throughout the period, but was accompanied by a significant increase in propionate. Citrate and lactate were now totally absent in the area. The total VFA concentration also decreased along the flow path, correlating with the reductive dechlorination activity described above.

Long term monitoring.

At the end of the post-treatment pumping test, there was more than 500 µg/L *cis*-DCE left in the area surrounding the RW. The prognosis was that, now that a viable consortium had been established, biodegradation would continue as long as a carbon source was available. As part of the long term plume surveillance, a series of samples were taken at all the C level monitoring wells in December 2002, after a year without pumping. In the interim, the concentration of *cis*-DCE had decreased to <5 µg/L in QRP 1C, 2C, 3C and 8C, and was approximately 30 µg/L in 4C and 5C. Surprisingly, *trans*-DCE was more persistent, with as much as 110 µg/L in 8C and 150 µg/L in 1C. The concentration of VC was <5 µg/L in all monitoring wells except 4C (16 µg/L), 1C (5.1 µg/L), Q14 (11 µg/L) and Q54 (15 µg/L). There was no evidence of rebound of the initial contaminants in the RW zone.

The addition of vitamin B12 and titanium citrate through a RW has generated a biologically active zone capable of biodegrading a mixture of TeCA,CT and TCE. The products formed were different than with the vitamin B12 process and went through sequential reductive dechlorination. Degradation of CT was complete, without the accumulation of CF or DCM. While the most abundant and longer lived degradation product was *cis*-DCE, its concentration declined with time. There was some VC formed in the process, but its concentration continued to decline. These results indicate that continuous treatment with vitamin B12 is not necessary and that alternating chemical addition with periods of pumping alone may be a cost-effective strategy for *in-situ* remediation of mixtures of chlorinated solvents.

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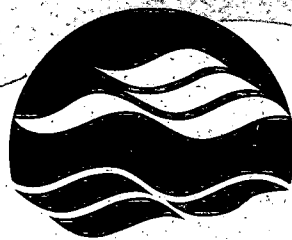
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