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Vitamin B12 and Reduced Titanium For the Remediation of Residual Chlorinated Solvents: Field Application

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MANAGEMENT PERSPECTIVE

This work was conducted at the CFB Borden site of the University of Waterloo, Department of Earth Sciences, Groundwater Research Centre with laboratory and technical support from National Water Research Institute. It was funded by both Environment Canada under the Toxics Issue and the University Consortium Solvent-in-Groundwater Research fund.

This is an account of the first field trial for the method and the subsequent laboratory research that was done to solve the problems encountered. A patent has been issued on the process.

Further research is needed to explore the full applicability of the technology. A feasibility study for a potential client will be conducted shortly.

ABSTRACT

A first pilot-scale field experiment using vitamin B12-reduced with titanium citrate to treat a mixture of 1,1,1-trichoroethane and tetrachloroethene was conducted in an *in-situ* vertical circulation column at CFB Borden. The objective of the experiment was to test the applicability of the method for treating aquifers contaminated by a mixture of dense non-aqueous phase liquids (DNAPLs). Vitamin B12 catalyzes the reductive dechlorination of a variety of organic compounds. A highly reducing and slightly alkaline environment (Eh<-480 mV and 7<pH<9) has to be maintained to maximize the rate of degradation. The preparation of titanium citrate was modified for the field application. Injection of the highly reduced remedial solution was feasible in the field. PCE and 1,1,1-TCA degraded to a limited extent under the experimental conditions and 1,1,1-TCA degraded more readily. Indigenous bacteria were found to metabolize citrate, thus causing titanium precipitation and limiting degradation. The addition of glucose at the end of the second field season, was an effective way of limiting citrate disappearance and helped recover the optimal redox potential by keeping the reduced titanium in solution. A post-field laboratory column was used to confirm the field results. The addition of glucose enhanced the dissolution of DNAPL, presumably by the formation of biosurfactants. It also produced a significant biomass, which provided an additional source of organic carbon onto which the solvents sorbed.

Utilisation de la vitamine B12 et de titane réduit pour l'assainissement des solvants chlorés résiduels : applications *in situ*

D. Sorel, S. Lesage, K. Millar et S. Brown

SOMMAIRE À L'INTENTION DE LA DIRECTION

On a effectué cette étude à la BFC de Borden, sur le site du Centre de recherche sur les eaux souterraines (Département de sciences de la terre de l'Université de Waterloo), à l'aide des services de laboratoire et de soutien technique de l'Institut national de recherche sur les eaux. L'étude était financée par Environnement Canada, dans le cadre des études sur la toxicité, ainsi que par le fonds de recherche sur les solvants dans les eaux souterraines de cette université.

Cet article est un compte rendu des premiers essais *in situ* de cette méthode, ainsi que de recherches subséquentes en laboratoire destinées à résoudre les problèmes observés. On a obtenu un brevet pour ce procédé.

Des études supplémentaires sont requises afin de déterminer toute la gamme des applications possibles de cette technologie. On doit effectuer bientôt une étude de faisabilité pour le compte d'un client possible.

RÉSUMÉ

On a effectué un premier essai *in situ* à l'échelle pilote avec de la vitamine B12 réduite à l'aide de citrate de titane pour traiter un mélange de 1,1,1-trichoroéthane et de tétrachloroéthène dans une colonne *in situ* à circulation verticale, à la BFC de Borden. L'objectif de cette expérience était de vérifier les possibilités d'application de la méthode de traitement des aquifères contaminés par un mélange de liquides non aqueux denses (LNAD). La vitamine B12 catalyse la déchloration réductive de divers composés organiques. On doit maintenir un milieu fortement réducteur et légèrement alcalin ($Eh < -480$ mV et $7 < pH < 9$) pour obtenir une vitesse de dégradation maximale. La préparation du citrate de titane était modifiée en fonction de la demande *in situ*. L'injection de la solution d'assainissement fortement réduite était réalisable dans les conditions *in situ*. On observait une dégradation limitée du PCE et du 1,1,1-TCA dans les conditions expérimentales, ainsi qu'une dégradation plus rapide du 1,1,1-TCA. On a constaté que des bactéries indigènes métabolisaient le citrate, ce qui entraînait la précipitation du titane et limitait la dégradation. De plus, on a constaté que l'addition de glucose à la fin de la deuxième saison d'essais *in situ* était une façon efficace de limiter la disparition du citrate, et cela contribuait également à rétablir la valeur optimale de potentiel d'oxydo-réduction en maintenant le titane réduit en solution. On a utilisé une colonne en laboratoire pour confirmer les résultats des essais *in situ*. L'addition de glucose améliorait la dissolution des LNAD, probablement à cause de la formation de biosurfactants. De plus, elle produisait une biomasse significative, qui constituait une source supplémentaire de carbone organique dans laquelle les solvants étaient sorbés.

INTRODUCTION

The limitations of traditional pump-and-treat methods for the restoration of contaminated groundwater sites (1) has provided an incentive to develop alternative technologies. *In-situ* mass destruction technologies (2) are developed to destroy the contaminant mass either biologically, by stimulating the intrinsic biological activity of the aquifer with the injection of nutrients (3) or chemically, by delivering compounds such as potassium permanganate to promote oxidation (4) or reduction of the contaminant, such as the vitamin B_{12}/titanium citrate method discussed here.

Vitamin B_{12} (cyanocobalamin) belongs to a class of compounds called corrinoids, which are synthesized primarily by anaerobic bacteria and by a few species of aerobic microorganisms. When cobalt, located at the center of the vitamin B_{12} corrin ring, is reduced from its Co(III) oxidation state (noted as B_{12a}) to Co(I) (noted B_{12s}), it becomes a very potent nucleophile (5). The dehalogenative capabilities of certain strains of anaerobic bacteria (7-10) has been correlated to their production of corrinoids (11-13). Although microorganisms are now being acclimated to higher concentrations of chlorinated solvents (14), microbial degradation is often limited by toxic levels of pollutants, adverse environmental conditions (pH, temperature, predators, nutrient limitations) and problems with cell membrane permeability for the uptake of certain contaminants (2,15). The use of a cell-free biocatalyst such as vitamin B_{12} was selected as an attractive alternative method for *in-situ* restoration, since it was expected not to have many of the limitations encountered when using biological systems (16). In recent years, those observations have encouraged many researchers to consider the use of vitamin B_{12} to restore waters contaminated by chlorinated solvents (17-25). Few of these studies have examined the vitamin B_{12}-catalyzed dehalogenation reactions in columns of aquifer material and in contact with DNAPL. In addition, no field experiments using vitamin B_{12} have been reported in the literature.

In this study, a field experiment was conducted to identify the processes and limitations of the vitamin B_{12} method when used to restore aquifers impacted by chlorinated contaminants. The degradation of a mixture of tetrachloroethene (PCE) and 1,1,1-

trichloroethane (1,1,1-TCA) was studied. Due to cost and safety considerations, it was not desirable to simply scale-up laboratory methods of preparing the remedial solution (26) for field applications. A new method of preparing titanium (III) citrate from titanium metal was devised (27) and its first application is presented here.

EXPERIMENTAL SECTION

The field experiment was conducted at the University of Waterloo experimental field site located on the Canadian Forces Base Borden, Ontario. An In-Situ Vertical Circulation Column (ISVCC) (28, 29) consisting of a 76 cm-diameter and 2.5 m long steel cylinder was driven into the sandy aquifer and keyed into the clay aquitard to isolate a portion of aquifer material and to minimize the impact of the experiment on the surrounding environment. The remedial solution was prepared on-site and circulated through the portion of aquifer contained inside the ISVCC (Figure 1). The ISVCC was equipped with an injection port located at 0.52 m above the clay aquitard and an extraction port located at 2.08 m creating an upward flow through the column. Between the injection and extraction ports, sixteen stainless steel tubes terminated by Mott porous stainless steel cups (40 μm pore size, Gasmac Inc. Guelph, ON) were inserted inside the column on four levels to allow for sampling along various flow paths. The pore volume between the injection and the extraction well was determined to be 220 L, based on the results of a conservative tracer experiment.

Remedial solution preparation. A schematic diagram of the experimental setup is shown on Figure 2. Because the stability of the reduced titanium solution is limited above pH 7, the reduced titanium was kept in a separate drum under acidic conditions and mixed on-line just prior to injection.

The reduced titanium solution was prepared in the field using an excess of titanium metal sponge (1 kg; Aldrich Chemical Co., Milwaukee, WI and Atlantic Equipment Engineers, Bergenfield, NJ) soaked in a 4% solution of oxalic acid (Van Waters & Rogers, Mississauga, ON) in groundwater from the site. The solution was contained in a 220 L polyethylene drum and covered by a loose-fitting lid to let hydrogen, which is generated as titanium is oxidized, escape. After curing for one week, the titanium(III) oxalate

solution had a dark amber color which remained stable for more than six months in the field. Another 220 L polyethylene drum contained filtered groundwater from the site, to which 6 g/L sodium citrate (Van Waters & Rogers, Mississauga, ON) and 10 mg/L vitamin B_{12} were added (Guervy & Berry Co Inc., Toronto, ON). This solution was mixed with the reduced titanium solution at a ratio of 10:1 (v/v), resulting in a pH of approximately 4. The vitamin B_{12} reductive dechlorination reaction requires slightly alkaline conditions. It was therefore necessary to increase and buffer the pH.

Remedial Solution Buffering. The buffering chamber consisted of a 30 L plastic container (Rubbermaid™) filled with layers of 10x150 mesh crushed high calcium limestone (BeachviLime Limited, Ingersoll, ON) and pea size gravel, to increase the hydraulic conductivity of the porous media. The limestone served to both buffer the remedial solution and precipitate oxalate as calcium oxalate. Argon gas was used to strip the CO_2 produced in the limestone and to prevent oxidation of the mixture. The final solution was pumped directly into the ISVCC using a piston metering pump at a rate of 30 mL/min.

Aquifer Reduction. In order to perform the reductive dechlorination reaction *in-situ,* the geochemical conditions of the aquifer have to be adjusted to the optimal Eh and pH. Therefore, before any chlorinated solvent or vitamin B_{12} were introduced into the ISVCC, 12.3 pore volumes of reduced titanium solution were circulated through the aquifer over 72 days to attain an Eh of approximately -400 mV and a pH between 7 and 9.

Injection of Chlorinated Solvents and Remedial Solution Circulation. Two hundred milliliters of an equimolar mixture of PCE and 1,1,1-TCA (Caledon Laboratories, Georgetown, ON) were introduced into the ISVCC through the contaminant injection port located 3 cm above sampling level 3. Because the DNAPL did not migrate downward significantly, most likely because of natural horizontal heterogeneities of the aquifer (30), and in order to increase the contact time between the solvent and the remedial solution, another 50 mL of the same equimolar mixture were added through the middle sampling port on level 1 (1D7), closest to the remedial solution injection port. In both cases, the

DNAPL was slowly pumped into the formation at approximately 1 mL/min using a peristaltic pump with solvent-inert tubing.

After the introduction of chlorinated solvents, 10 mg/L of vitamin B_{12} were added to the reduced titanium solution and delivered for 6.2 pore volumes (55 days). During the winter months (days 127 to 238), no remedial solution was circulated and only monthly monitoring took place. Delivery of the remedial solution was resumed in the spring (day 239) and continued until day 326 (5.7 equivalent pore volumes) . Food grade glucose solids consisting of 15-19% glucose and 11-15% maltose (Grain Process Enterprises Ltd., Scarborough, ON and Traynor's Bakery Wholesale, Hamilton, ON) were added to the remedial solution on day 306 at a 33 mM glucose concentration, as measured using a Glucometer Encore™ meter.

Sampling and Analysis. Eh and pH, chlorinated solvents, chloride and organic acids (citrate, formate, acetate, propionate) were monitored routinely from 17 locations inside the test column (Figure 1). Chlorinated solvents were analysed by purge-and-trap GC/MS using an Envirochem Unacon purge and trap equipped with a Dynatech PTA-30 autosampler interfaced to a Hewlett-Packard 5790 GC/MSD. The acids were measured by ion exclusion chromatography using an IC-PAK column (Waters Chromatography, Milford, MA) and a Waters model 430 conductivity detector. The eluent was 1mM HCl at 1 mL/min.

At the end of the experiment, three 2-inch diameter cores were collected to measure the residual solvent concentration left in the aquifer and the titanium, iron and manganese content of the sand (28).

Laboratory Column. A laboratory column (4.8 cm i.d. X 30 cm length) was packed with aquifer material from the site and placed in an anaerobic chamber. The remedial solution was prepared in the laboratory in a manner similar to that in the field, but scaled-down. The solution was made in batches and kept in Tedlar bags to feed the column. Several pore volumes of the solution were pumped through the column to establish reducing conditions. The column was then inverted, opened and two mL of PCE were

introduced at the bottom. The DNAPL was allowed to sink in for a few minutes before reestablishing the flow. The effluent of the column was collected in a Tedlar bag to capture the gases as well as the liquid. At the end of the experiment (27.6 pore volumes), the sand was extruded out of the glass column in 5 cm sections and extracted in methanol (100 mL, 24 hours, 240 rpm) for the determination of residual PCE and products. To ensure complete extraction of strongly bound residue, the methanol was decanted off the soil extracts, the soil air-dried for 3 days to remove all methanol and then re-extracted with acetone (100 mL) and KOH (10 g) in an ultrasonic bath for 30 minutes.

Biomass Sorption Study. A series of microcosms were established to assess whether the presence of biomass, or the extracellular material produced by the Borden aquifer bacteria, would sorb significant amounts of PCE and its degradation products. Buffered remedial solution was exposed to Borden sand for 3 days to establish a significant biomass. The sand was then allowed to settle and the solution containing biomass was distributed into serum vials with or without additional sand added. The vials were spiked with PCE and TCE for a final concentration of 200 µg/L. PCE and TCE concentrations were monitored over 14 days by headspace analysis. Controls were prepared with identical headspace to liquid ratios in vials containing ultrapure water (Milli-Q, Waters/Millipore, Mississauga, ON).

RESULTS AND DISCUSSION

The purpose of this first field test was to identify the potential problems that would occur in a large scale application and which are difficult to observe in a laboratory experiment. For example, while it was easy to reduce a small sand column in the laboratory in an anaerobic chamber, it was unknown whether it would be possible to reproduce this in the field, where air cannot be totally excluded. Other factors such as the distribution of DNAPL and the effect of having an inactive season during the winter could also only be assessed *in-situ*.

Remedial solution preparation. Initially, titanium (III) citrate and oxalate were injected directly into the test column. However, after several pore volumes were injected, a significant decrease in hydraulic conductivity was observed. Using laboratory columns

filled with sand from the site, it was found that oxalic acid reacted slowly with calcium present as limestone in the Borden aquifer, to form highly insoluble calcium oxalate salt. To prevent further reduction in conductivity, a crushed limestone buffering chamber was added on-line before injection into the column, in order to simultaneously precipitate the oxalate and buffer the pH. No oxalate was found in the column after pre-treating the remedial solution with limestone. After removal of oxalate, citrate must be available to chelate the titanium and maintain its solubility.

The *in-situ* concentration of titanium (III) could not be evaluated. An indication of the total titanium in solution was obtained by adding hydrogen peroxide to acidified samples collected at the extraction, and monitoring for a characteristic yellow color (31). The redox potential, which indicates the ratio between Ti3+/Ti4+species, was used to determine if Ti3+ was present in solution. In a water-titanium system, the concentrations of Ti3+ and Ti4+ are equal at a potential of approximately -480mV (Eh) (32). Because of the sensitivity of Ti3+ to oxidation, quantitative speciation is difficult. A method for measuring the concentration of Ti3+ *in-situ* remains to be developed.

Geochemical and microbiological aquifer interactions

Table 1 lists the potential interactions between the remedial treatment and a carbonate aquifer. Optimal geochemical conditions were achieved in the field after injecting approximately 13 pore volumes (approximately 2,900L) of titanium (III) citrate solution. The aquifer oxidation capacity of a site should be obtained before selecting vitamin B_{12} as a remediation method (33, 34) to indicate how much of the reducing agent will interact with the aquifer material. If the oxidation capacity of an aquifer is significant, pre-treatment using an inexpensive reductant may be appropriate. In this experiment, assuming that most of the oxidation capacity of the Borden aquifer is born by iron and manganese oxides, the amount of reduced titanium required to reduce the aquifer can be calculated stoichiometrically. Nicholson (35) measured concentrations of leachable iron and manganese in the Borden aquifer to be in the 10-35 mmol/kg and 0.04-0.15 mmol/kg range, respectively. Assuming a pore volume of 220 L and a sand dry bulk density of 1.76 g/cm^3, the test column would contain 4 to 14 moles of leachable iron and 0.02 to

0.06 moles of leachable manganese. With a concentration of reduced titanium in solution of approximately 4 mM, 1,250 to 3,750 L of remedial solution should have been required to reduce the portion of aquifer isolated by the test column. After injecting more than 2,500 L, the reducing potential in the test column was less than -400 mV at sampling level 1 but remained around -300 mV higher up in the column. It therefore seems that the reduced titanium was not the main redox species in solution over the whole duration of the experiment. Based on the above data, the aquifer should have contained 33 μg/g of titanium. The titanium content of the aquifer, as calculated at the end of the experiment, ranged from one to two orders of magnitude higher than this value, a clear indication that precipitation had occurred.

If aquifer minerals are soluble under reducing conditions, the mobility of some metals will be increased during delivery of the remedial treatment. The impact of high metal concentrations in the aquifer can be twofold. First of all, since it impairs the water quality and treatment of the effluent, it may be necessary to remove the metals from solution. Secondly, high concentrations of metal ions can affect the equilibrium of the titanium (III) citrate complex. If some metals have greater affinity for citrate or if their concentrations are high enough in solution, most of the citrate could react with the aquifer metals resulting in the precipitation of titanium. Critical stability constants are not available for Ti^{3+}-citrate (36). Without this information, it is very difficult to determine which metals have greater affinity for citrate. Mn^{2+} and Fe^{2+} have stability constants in the range of log K= 4 and Fe^{3+} has a great affinity for citrate with log K= 11.2 (37).

Smith and Martell (38) report stability constants between trivalent ions of the first transition series and EDTA, suggesting increasing affinity in the following order:

$Ti^{3+}<Sc^{3+}<Cr^{3+}<Fe^{3+}<Mn^{3+}<V^{3+}<Co^{3+}$. To ensure that titanium remains in solution, enough citrate should be present to complex with all available metals.

At the end of the first field season, because of inconsistent Eh conditions, the status of titanium (III) citrate in the column was examined. It became evident that citrate was being biodegraded to acetate, propionate, and to a lesser extent, formate, especially in zones not containing DNAPL (Figure 3a). Zehnder and Whurmann (26) studied the use titanium

(III) citrate as a redox buffer for the culture of obligate anaerobic bacteria and found that none of the three microorganisms studied metabolized citrate significantly. On the other hand, Francis et al. (39) found that metals forming mononuclear bidendate complexes such as calcium, iron (III) and nickel were readily degradable by citrate-degrading bacteria isolated from landfill leachate. The addition of a carbon source that would be preferred to citrate was therefore sought as a possible solution. After glucose was added on day 306, an increase in citrate concentration within the column was observed (Figure 3b). This was coupled with a decrease in the redox potential (Figure 4) stressing the importance of maintaining the integrity of the titanium (III) citrate complex to keep the reduced titanium in solution and preserve the optimal geochemical conditions.

Solvent Degradation. One of the first observations made about the solvent degradation was that 1,1,1-TCA transforms without any vitamin B_{12} present (Figure 5). The solvent was introduced into the column on day 72, vitamin B_{12} delivery started on day 73 at a rate of 25 mL/min and samples were collected on day 75. Based on a calculation using the Ogata-Banks transport equation, the vitamin B_{12} concentration present at sampling level 4 should not have been high enough to promote significant degradation (28). The degradation of 1,1,1-TCA to 1,1-DCE is the result of a dehydrohalogenation reaction occurring in an alkaline environment (40).

The second observation was that almost no dechlorination of PCE was occurring, in contrast with the results in a laboratory column using sand from the same location (18), where more than 70% dechlorination of PCE to TCE had occurred in a period of five hours. The amount of vitamin B_{12} should not have been rate limiting at this point. Competition between PCE and 1,1,1-TCA for vitamin B_{12} may have resulted in decreased dechlorination rates. Titanium precipitation was also causing difficulties in sustaining optimal pH and Eh conditions.

The best conditions were achieved close to the injection point. Figures 6a and b show the chlorinated solvents concentration profiles observed at port 2B2 during the first part of the experiment (days 75 to 127). These profiles show that when redox conditions were

maintained around -500 mV (Figure 6c) and the pH was between 8 and 9, significant concentrations of degradation products, mainly TCE and 1,1-DCA were observed. Because this was a flowing system, degradation products correlate to the aquifer conditions, one level below. The products measured at level 2 were therefore most likely formed close to the injection point and transported.

In the second season, once a citrate-degrading bacterial population was established, significant titanium precipitation occurred. The expected conditions for reductive dechlorination were not achieved until glucose was added to the mixture as stated above. Unfortunately by then, the hydraulic conductivity in the column was already much lower. Also, because the amount of chlorinated solvents measured at the extraction well had declined significantly, it was not possible to assess the potential of the method using the finally optimized conditions. Therefore, it was decided to return to the laboratory column to try to replicate field conditions.

Mass balance. Under varying conditions, it was difficult to assess how much degradation occurred during the total duration of the experiment. To account for the fate of all the products, it is necessary to first evaluate the total mass of solvent that had dissolved. Using the dissolution equation proposed by Banerjee (41), considering that 2,600L of remedial solution were pumped through the test column and assuming that 10% of the remedial solution was in contact with the DNAPL solvents, 0.15 moles of PCE and 0.99 moles of 1,1,1-TCA should have dissolved. The cumulative mass of chlorinated solvents dissolved and pumped out of the column was estimated by multiplying the VOC concentrations measured at the extraction well, with a representative volume of remedial solution pumped between each sampling event. The results are summarized in Table 2. Compounds such as vinyl chloride (VC) and ethene generated from both PCE and 1,1,1-TCA in minimal amounts were not taken into account.

The chloride mass balance was obtained by two different methods: 1) by using an average concentration calculated from all the sampling ports and 2) by using the concentrations at the extraction well. Background chloride concentrations were subtracted from all data. Values of 1.2 ± 0.1 moles and 1.7 ± 0.1 moles were obtained from the average of the

column data and the extraction concentrations, respectively. These results suggest that more degradation had occurred than could be accounted for by the measurement of known reductive dechlorination products.

At the end of the experiment, two 2-inch cores were collected in the test column to determine the mass of DNAPL remaining. By averaging the residual concentrations measured in the core samples and assuming that it was representative of half the volume of aquifer material within the column, 0.03 moles of PCE and 0.013 moles of 1,1,1-TCA remained inside the test column. Adding these results to the VOC mass extracted in solution out of the extraction well, 0.99 moles of PCE and 0.63 moles of 1,1,1-TCA were unaccounted for.

There are significant incongruities in the mass balance. Possibilities for the discrepancies are: 1) some VOC mass was lost through the gas phase; 2) certain degradation products were unaccounted for or; 3) the cores taken at the end of the experiment did not encounter the main residual DNAPL zone.

Towards the end of the second field season, a total of 20 moles of glucose were added to support bacterial growth and limit the consumption of citrate. This potentially produced 120 moles of CH_4 or CO_2 gas. A simple partitioning calculation using Henry's law constants showed that 0.16 moles of PCE and 0.4 moles of 1,1,1-TCA at most could be lost in this manner.

Recently, Burris et al. (19) and Glod et al. (17) demonstrated that vitamin B_{12} and titanium citrate can produce acetylene as a degradation product. An unknown peak was observed in the analysis for methane and ethene in some of the samples collected form the test column, which upon verification was confirmed to be acetylene, but the results were not quantitative.

Finally, 250mL of solvent were introduced in the test column. Although the three cores were taken in the zones which originally contained DNAPL and represent intensive sampling (column diameter of 80 cm), the possibility still exists that the cores collected did not intersect the major DNAPL zones. Assuming that the first 200mL DNAPL

injection expanded laterally around the point of injection and occupied half of the area of the column, the thickness of the DNAPL disk would have been 4.7 cm. It is therefore possible that the samples collected missed the residual DNAPL zone.

Laboratory Column. Because of the difficulties encountered in the field and the recognized fact that optimal conditions had not been achieved for a significant portion of the time, a laboratory column study was initiated to which the best achievable conditions were applied. Also, the column effluent was collected in Tedlar bags to allow for the measurement of all gas phase products formed. At the end of the experiment, the entire column was sectioned and the solids were extracted with methanol, then hydrolysed in KOH. To simplify product analysis and prevent competition between substrates, only PCE was added to the laboratory column.

The residence time in the column was approximately 30 hours, but varied depending on the amount of gas being formed. The degree of dechlorination observed in the column is shown on Figure 7. For the first 7.5 pore volumes, the rate of dechlorination was less than 10%. It was determined that this was due to the low pH of the remedial solution, causing excessive gas production. This was a mixture of CO_2 and methane. It became obvious that CO_2 came not only from the mineralization of glucose, but from the neutralization of the intermediate acids (lactic, acetic and formic) formed during its metabolism. It was found necessary to supplement the action of the limestone buffer by the addition of sodium carbonate. This change resulted in an increase to an average of 25% dechlorination rate, which was sustained throughout most of the experiment. This is much lower than had been reported (18), but it should be recognized that the concentration of PCE in the previous experiment was only 300 µg/L, whereas this column contained pure phase PCE. Indeed, after 24 pore volumes, when the PCE concentration decreased to less than 30 mg/L, the dechlorination rate increased to almost 40%.

The mass balance of the PCE degradation is shown in Table 3. A total of 5 L of solution was passed through the column. Based on the aqueous solubility of PCE and sequential contact with 10 mL portions, a total of 4,220 µmoles should have dissolved, a quarter of the emplaced mass. The volume of 10 mLs was chosen as a representative contact

volume, based on the 5cm diameter of the column, a possible DNAPL zone thickness of 1.5 cm and a porosity of 0.3. Summing all of the measured volatile products, a total of 3,880 µmoles were accounted for, well within experimental uncertainty. To measure the mass of solvent remaining in the column, a methanol extraction of the sand was performed. Only 250 µmoles of PCE were recovered by this method, a fraction of what should have been left (15,360 µmoles). Even accounting for the efficiency of the methanol extraction which was found to be 69% for similar water saturated sand, a major discrepancy exists. Subsequently, an alkaline hydrolysis of the sand in methanol only yielded 1 µmole.

The possibility of sorption onto the biomass was assessed by a series of microcosms containing the nutrient medium, but not reduced, with and without soil. After two weeks, an apparent concentration loss relative to water of 27% (TCE) and 33.9% (PCE) was observed in microcosms containing biomass only, and of 31.2% (TCE) and 43.4% (PCE) in microcosms containing both biomass and soil. No dechlorination products were observed. It is therefore apparent that the biomass and the exopolysaccharides that are produced by these bacteria can be a significant source of organic carbon onto which the solvents can sorb. When applying these correction factors to the above data, a further 2,730 µmoles can be accounted for. This would bring the dissolved total to 6,600 µmoles, which exceeds the amount that was expected to dissolve by 50%. This could be ascribed to the reductive dechlorination reaction acting as a driving force and helping the dissolution or to the effect of the biosurfactants produced by the glucose metabolizing bacteria.

Although ethene and ethane were measured, no acetylene was found in the column effluent,unlike what had been observed sporadically in the field and was seen in solution. This would contradict the mechanism reported by Glod et al. (17), who proposed that ethene was produced from acetylene. It is also possible that other products were formed by interaction of the intermediates with the bacteria or with the aquifer solids. Since free radicals are known to be formed during the reaction with vitamin B12 (42), their reaction with the aquifer material is entirely possible. An alternative pathway for the formation of acetylene from the chloroalkyl cobalamins, which is dependent on the form of titanium

present, and in competition rather than in sequence with the formation of ethene has also been proposed (43).

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References

(1) Mackay, D. M.; Cherry, J. A. *Environ. Sci. Technol.* **1989,** 23, 630-636.
(2) Cherry, J. A.; Feenstra, S.; Mackay, D. M. *Dense Chlorinated Solvents and other DNAPLs in Groundwater;* Waterloo Press, **1996**, 475-506.
(3) Beeman, R. E.; Howell, J. E.; Shoemaker, S. H.; Solazar, E. A.; Buttram, J. R. *Proceedings of the In Situ and On Site Bioremediation Conference*, Battelle, San Diego, **1993**.
(4) Truax, C.L.; Farquhar, G.J.; Schnarr, M.J.; Hood, E.D.; Stickney, B. *Journal of Contaminant Hydrology* **1997**(accepted)
(5) Schrauzer, G.N. ; Deutsh, E. *J. Am. Chem. Soc.*, **1968**, 91, 3341-3350.
(6) Freedman, D.L.; Gossett, J.M. *Applied and Environmental Microbiology*, **1989,** 55, 2144-2151.
(7) Fathepure, B. Z.; Nengu, J.P.; Boyd, S. A. *Appl. Environ. Microbiol.*, **1987,** 53, 2671-2674.
(8) Fathepure, B. Z.; Tiedje, J.M.; Boyd, S.A. *Appl. Environ. Microbiol.*, **1988,** 54, 327-330.
(9) Vogel T.M.; McCarthy, P. L. *Appl. Environ.Microbiol,* **1985,**49, 1080-1083.
(10) Bouwer, E. J.; McCarty, P. L. *Appl. Environ.Microbiol.*, **1983,** 45, 1286-1294.
(11) Holliger, C.; Schraa, G.; Stams, A. J.; Zenhder, A. J. B. *J. Bacteriology,* **1992,** 174, 4427-4434.
(12) Egli C.; Stromeyer, S.; Cook, A. M.; Leisinger, T. *FEMS Microbiol. Let.*, **1990,**68, 207-212.
(13) Stupperich, E. S.; Kraütler, B. *Arch. Microbiol.*, **1988** , 149, 268-271.
(14) DiStefano, T. D.; Gossett, J. M.; Zinder, S. H. *Applied and Environmental Microbiology*, **1991,** 57, 2287-2292.
(15) Nannipieri, P.; Bollag, J.-M. *J. Environ. Qual.*, **1991,** 20, 510-517.
(16) Lesage, S.; Brown, S.. Emerging technologies for Hazardous Waste Management V, Atlanta GA Sept.27-29 . **1993**, 2, 539-543.
(17) Glod, G.; Angst, W.; Holliger, C.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **1997,**31, 253-260.
(18) Lesage, S.; Brown, S.; Millar, K. *Ground Water Monitoring and Remediation.* Fall **1996**, 76-85.
(19) Burris, D.R.; Delcomyn, C.A., Smith, M.H., Roberts, A.L. *Environ. Sci. Technol.* **1996,** 30, 3047-3052.
(20) Chiu, P.-C.; Reinhard, M. *Environ. Sci. Technol.*, **1995,**29, 595-603.
(21) Assaf-Anid, N.; Hayes, K. F.; Vogel, T.M. *Environ. Sci. Technol.*, **1994,** 28, 246-252.
(22) Shanke, C. A.; Wackett, L. P. *Environ. Sci. Technol.* **1992,** 26, 830-833.

(23) Gantzer, C. J.;Wackett, L. P. *Environ. Sci. Technol.* **1991,** 25, 715-722.
(24) Krone, U. E.; Thauer, R. K. *Biochemistry,* **1991,** 30, 2713-2719.
(25) Krone, U. E.; Thauer, R.K.; Hogenkamp, H. P. C. *Biochemistry,* **1989,** 28, 4908-4914.
(26) Zehnder, A. J. B.; Whurmann, K. *Science.* **1976,** 194, 1165-1166.
(27) Lesage, S.; Brown, S. J.; Millar, K.R. **1997.** US Patent 5,645,374, July 8.
(28) Sorel, D. M.Sc. Thesis, Department of Earth Sciences, University of Waterloo. **1996.**
(29) Sorel, D. Cherry, J.A., Lesage, S. *Groundwater Monitoring and Remediation* **1998,** 18, no. 1, _____
(30) Kueper, B.H.; Redman, D.; Starr, R.C.; Reitsma, S.; Mah, M. *Ground Water,* **1993,** 31, 756-766.
(31) Sandell, E. B. *Chemical Analysis* (Vol. III). B. L. Clarke, P. J. Elving and I. M. Kolthoff (Eds.) Interscience Publishers, Inc. New York, **1965**, 868-874.
(32) Pourbaix, M. J. N. Atlas d'équilibres électrochimiques, Gauthiers-Villars & Cie , Eds., Paris, 1963.
(33) Heron, G.; Christensen, T. H.; Tjell, J. C. *Environ. Sci. Technol.* **1994,** 28, 153-158.
(34) Barcelona, M. J.; Holm, T.R. *Environ. Sci. Technol.,* **1991**, 25, 1565-1572.
(35) Nicholson, R. V. ; Cherry, J. A.; Reardon, E. J. *Journal of Hydrology,* **1983**, 63, 131-176.
(36) Martell, A. E.; pers. commun.
(37) Martell, A. E.; Smith, R. M. *Critical Stability Constants.* Volume 5: First Supplement. Plenum Press, New York, London, **1974**.
(38) Smith, R. M.; Martell, A. E. *The Science of the Total Environment,* **1987,** 64, 125-147.
(39) Francis, A. J.; Dodge, C. J.; Gillow, J. B. *Nature.* **1992,** 365, 140-142.
(40) Vogel, T. M.; Criddle, C. S.; McCarthy, P. L. *Environ. Sci Technol.* **1987,** 21, 722-736.
(41) Banerjee, S. *Environ. Sci. Technol.* **1984**, 18, 587-591.
(42) Lesage, S. and S. Brown. 1994. American Chemical Society, 208th National Meeting of the, Washington DC, August 21-25. Environmental Chemistry Extented Abstract, Pages 652-656.
(43) Lesage, S.; Brown, S.; Millar, K. *Environ. Sci Technol* . in submittal.

Table 1: Summary of possible interactions between the remedial treatment and the aquifer

Interaction	Reaction Products	Adverse Effects	Controls on AdverseEffects
1- Incomplete oxalate reaction with excess limestone (step2) will precipitate calcium oxalate in the calcite aquifer $2COO^- + Ca^{2+} \Leftrightarrow Ca(COO)_{2(s)}$	Calcium Oxalate $Ca(COO)_{2(s)}$	• Precipitate reduces hydraulic conductivity	• Increase contact time in the limestone mixing chamber or •Use an acid less reactive with the aquifer minerals to dissolve the titanium metal (formic acid?)
2a- Reducing conditions promotes dissolution of minerals such as Iron oxides and Manganese oxides $Fe(OH)_3 + Ti^{3+} \Leftrightarrow Fe^{2+} + Ti^{4+} + 3\ OH^-$ 2b- Ti^{4+} precipitates as titanium oxide or ilmenite (?)	$Fe^{2+}_{(aq)}$, $Mn^{2+}_{(aq)}$ Titanium Oxide(?) $TiO_2 \cdot H_2O_{(s)}$ Ilmenite (?) $FeTiO_{3\,(s)}$	• Need reducing conditions to remediate the aquifer by reductive dechlorination : vit B_{12} technique may not be appropriate if aquifers contains very high amounts of oxides wich make the aquifer reduction economically impractical • Precipitate reduces hydraulic conductivity	• Manipulate the effluent's Eh and pH to precipitate and separate the aquifer metals in solution before recirculation •Inject an excess of chelating agent which will prevent precipitation of titanium in the aquifer
3-Dissolved minerals compete with titanium for citrate to cause ion exchange(?) $Ti(C_6H_5O_7) + Fe^{3+} \Leftrightarrow Fe(C_6H_5O_7) + Ti^{3+}$ $Ti^{3+} + 3H_2O \Leftrightarrow Ti(OH)_3 + 3H^+$	Titanium Hydroxide ? $Ti(OH)_{3\,(s)}$	• Uncomplexed titanium precipitates • Difficult to reduce the B_{12} and promote reductive dechlorination • Precipitate reduces hydraulic conductivity	•Inject enough chelating agent to complex with titanium and the competing metals
4- Indigenous microorganisms metabolize citrate ($C_6H_5O_7$) titanium citrate $Ti(C_6H_5O_7)$ degradation products titanium (III) Ti^{3+} propionate $C_3H_5O_2^-$ formate CHO_2^- methane CH_4 carbon dioxide CO_2 $Ti^{3+} + 3\ H_2O \Leftrightarrow Ti(OH)_3 + 3H^+$	Methane $CH_{4(g)}$ Carbon Dioxide $CO_{2(g)}$ Titanium Hydroxide $Ti(OH)_{3(s)}$	• Uncomplexed titanium precipitates • Difficult to reduce the B_{12} and promote reductive dechlorination • Gas and precipitate reduce hydraulic conductivity	•Add a bacterial inhibitor to the remedial solution or •Use a less biodegradable chelating agent or •Introduce a favored substrate over citrate to sustain bacterial growth and limit the interference with the remedial treatment

Table 2 Summary of mass balance calculation (field)

	PCE (mole)			1,1,1-TCA (mole)		
	Dissolved	**Degraded**	**Remaining**	**Dissolved**	**Degraded**	**Remaining**
Calculated from Extraction	**0.25 ±0.01**	**0.0032 ± 0.0002 (1%)**	**1.02 ±0.01**	**0.63 ±0.01**	**0.20 ±0.01 (32%)**	**0.64 ±0.01**
Calculated from Cores	**1.24**	**0**	**0.03**	**1.26**	**0.007 (52%)**	**0.013**
Chloride produced from degradation (moles)	**1.2-1.7 ±0.1** 0.01 mole of PCE 0.5 mole of TCA assuming TCA degrades 30 times more than PCE					

Table 3. Recovery of PCE and products for the laboratory column experiment

	PCE	TCE	*cis*-DCE	*trans*-DCE	1,1-DCE	VC	ethene	ethane
aqueous phase	2310	672	3.1	0.8	0.4			
gas phase	794	99.4	0.2	0.1	0.3	0.02	2.4	0.4
methanol extractions (column sand)	200	50.9						
non-extractable[a]	89.9	22.9						
non-measurable-aqueous[b]	1770	305						
non-measurable-gas[b]	609	45.1						

Notes:

[a]Recovery efficiency of methanol extractions was 69%.

[b]Non-measurable TCE and PCE for aqueous and gas phases calculated at 31.2% and 43.4%, respectively.

Figure Captions

Figure 1. Wells and sampling ports layout in the *In-situ* Vertical Circulation Column

Figure 2. Remedial solution circulation system

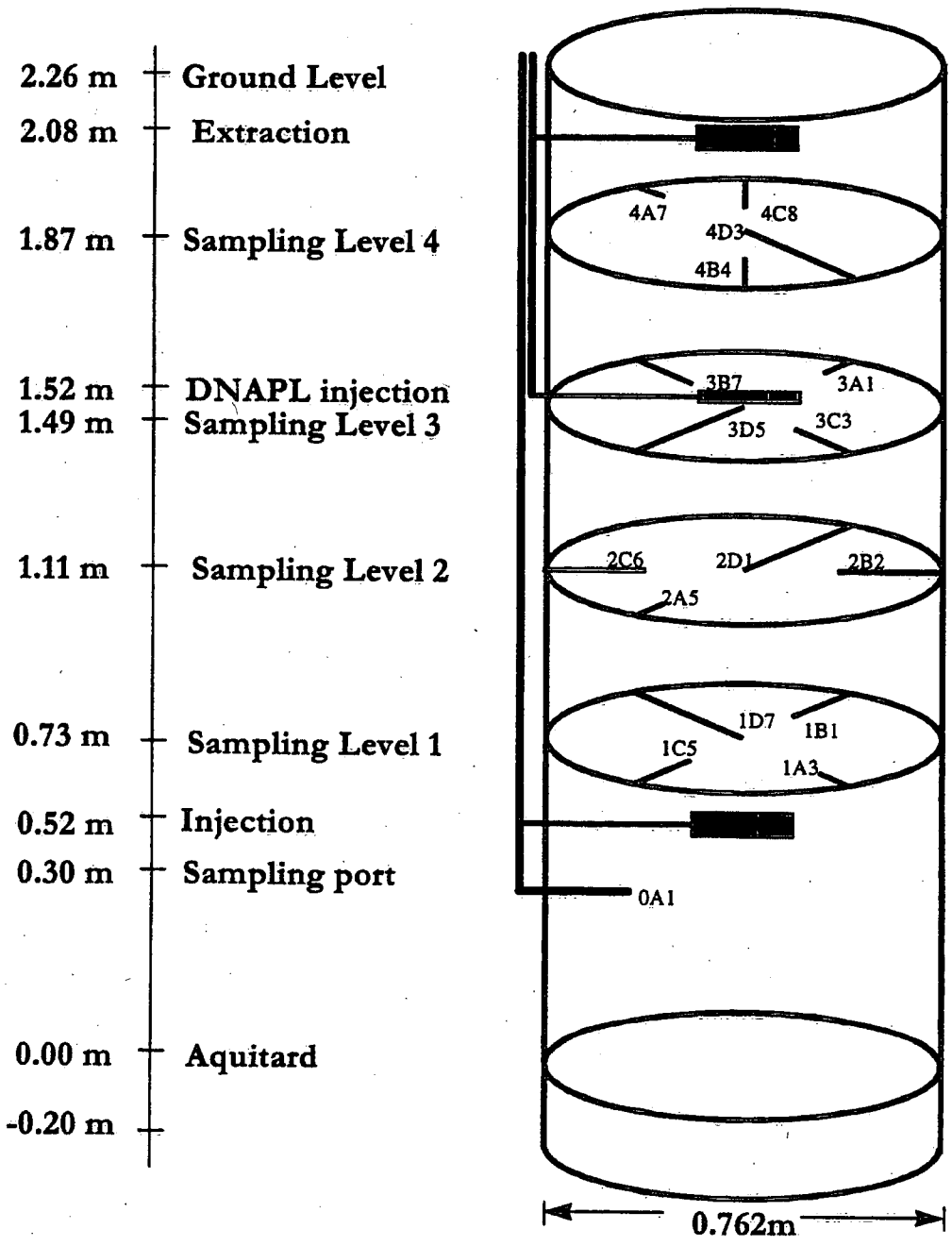
Figure 3. (a) Degradation of citrate on level 2 and above level 3 at the end of the first field season. (b) Increase in citrate concentration after addition of glucose during the second field season.

Figure 4. Effect of glucose addition on citrate concentrations and reducing potential in sampling port 4D3.

Figure 5. 1,1,1-TCA concentration profile on day 75, three days after introducing the solvent in the test column.

Figure 6. Concentration of parent and degradation products at port 2B2 a)TCA, DCA and DCE and b) PCE and TCE, with respect to c) Eh conditions at levels 1 and 2.

Figure 7. Concentrations of PCE and TCE in laboratory column over 27.6 pore volumes. ◊ represents % dechlorination over the course of the experiment.



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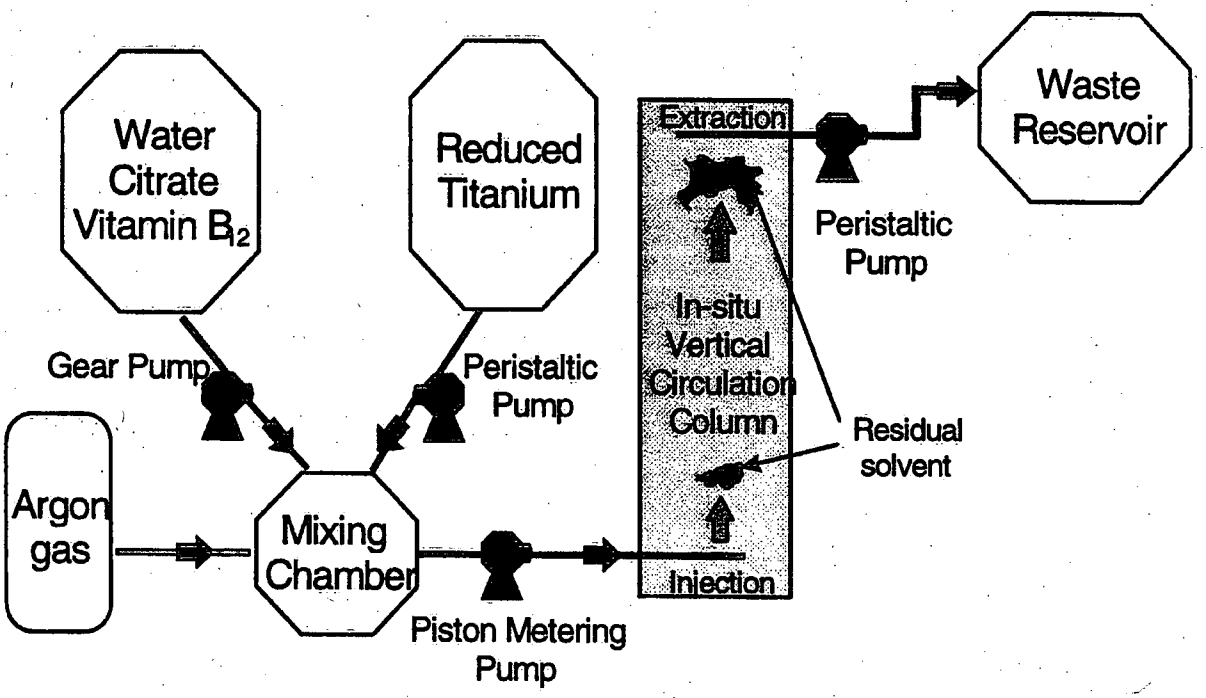
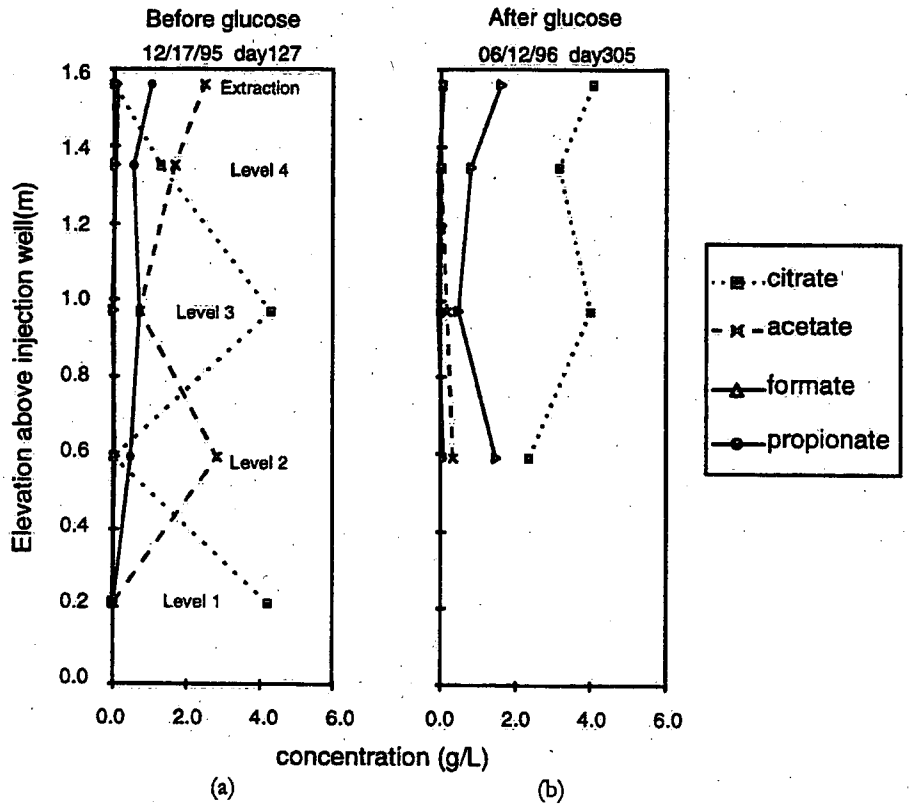


Figure 2. Remedial solution circulation system



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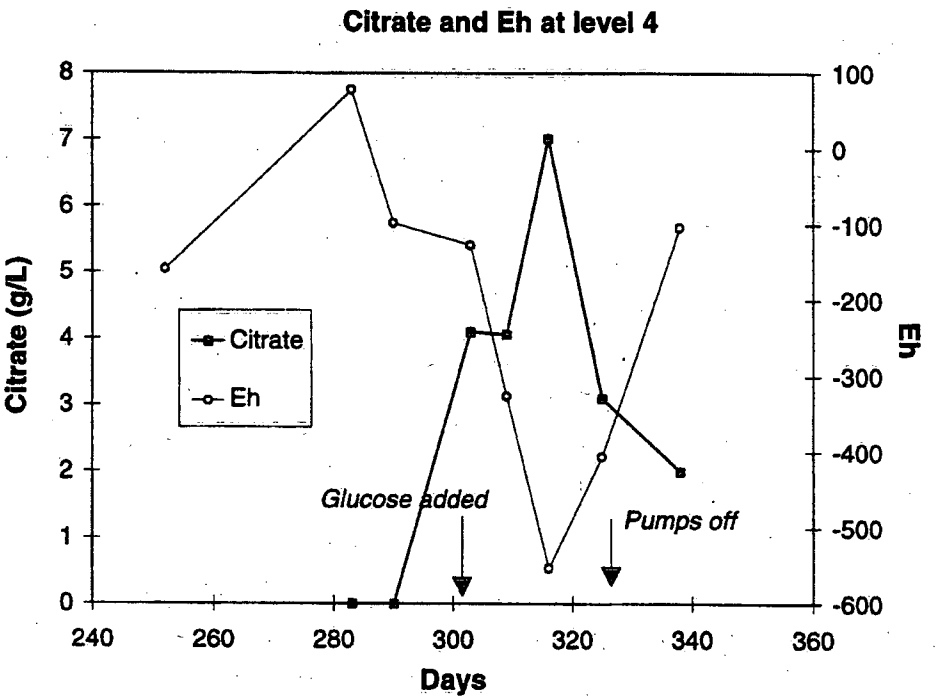


Figure 4. Effect of glucose addition on citrate concentrations and reducing potential at sampling port 4B4.

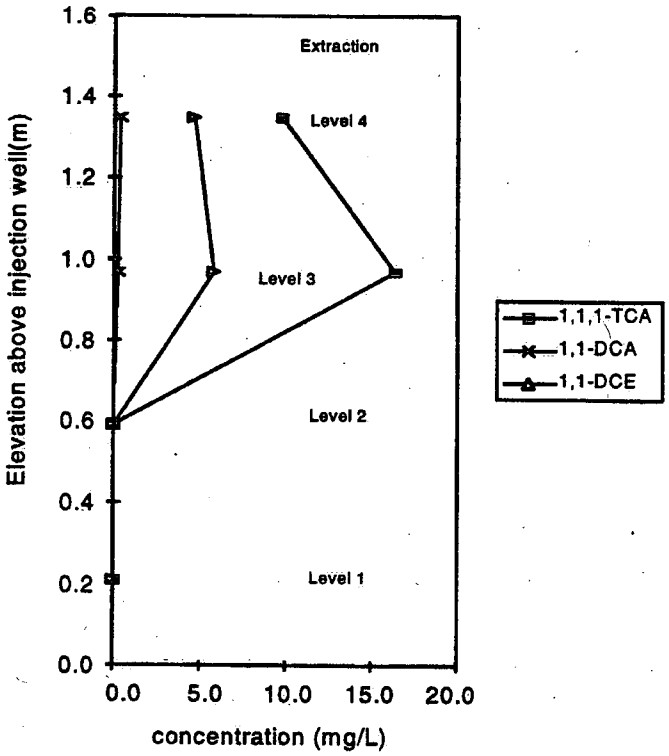
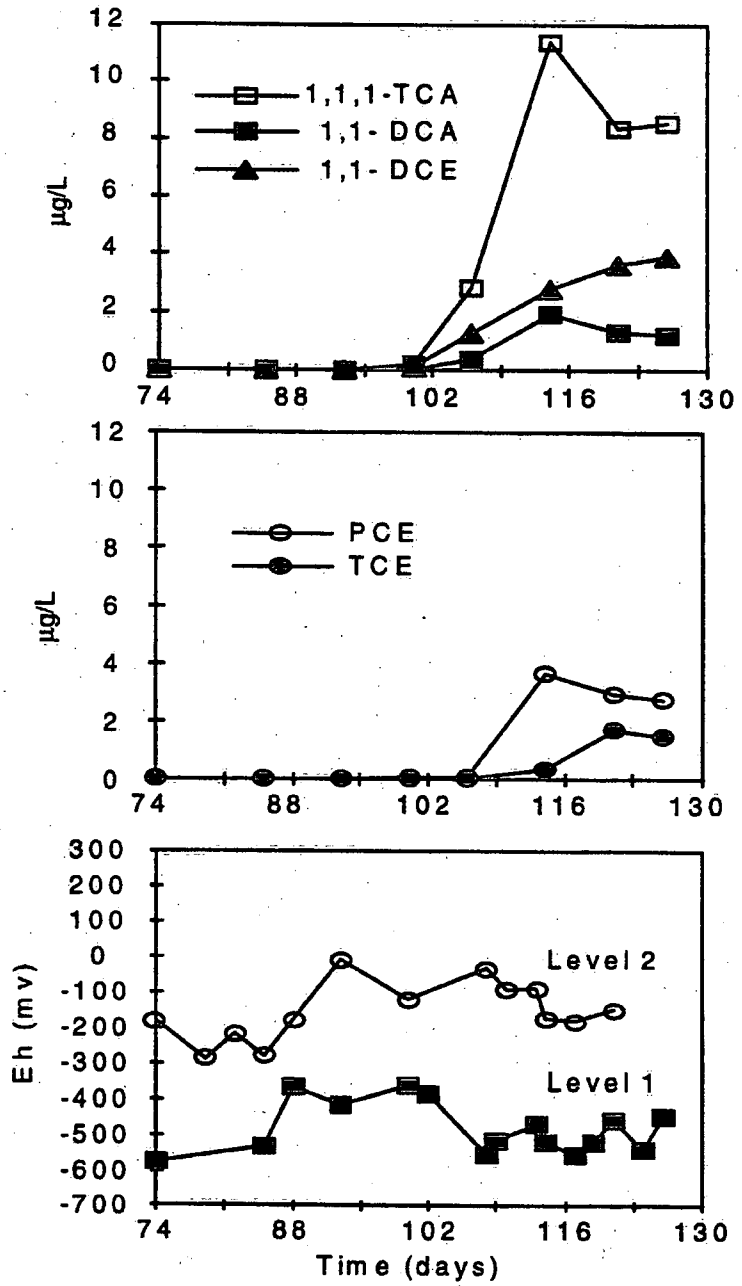


Figure 5. 1,1,1-TCA concentration profile on day 75, three days after introducing the solvent in the test column.



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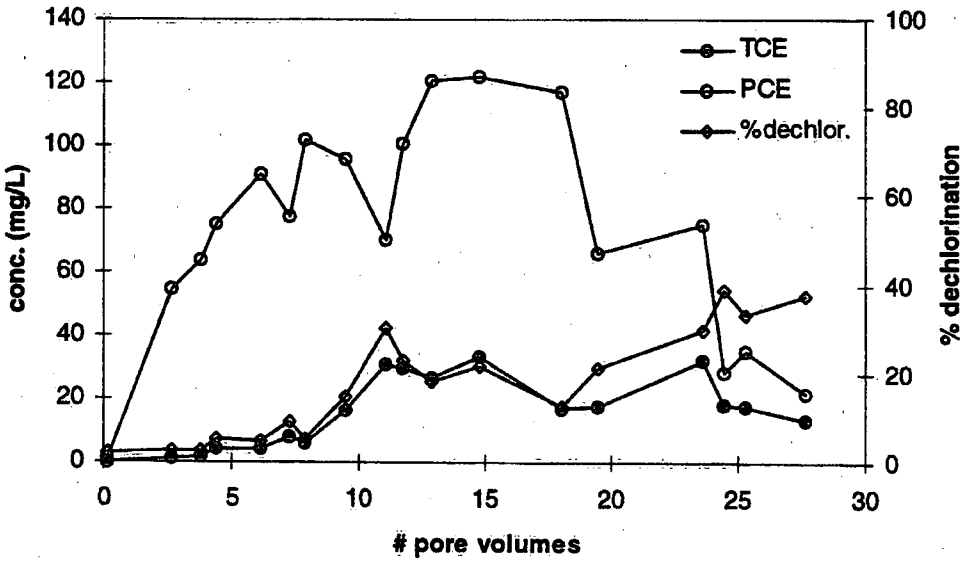

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