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INTRINSIC BIOREMEDIATION OF CHLORINATED SOLVENTS AT THE ST. JOSEPH, MI AQUIFER - LAKE MICHIGAN INTERFACE

Peter Adriaens, Jack Lendvay, Nikolaos Katopodes, and Sean Dean
The University of Michigan, Department of Civil and Environmental Engineering
181 EWRE Building, 1351 Beal St. Ann Arbor MI 48109-2125
(313) 763-1464

and

John Wilson, and Don Kampbell
U.S. EPA Robert S. Kerr Environmental Laboratory
Ada, OK

1. INTRODUCTION

The anaerobic aquifer at the St. Joseph, MI National Priority List (NPL) site has been contaminated with trichloroethylene (TCE) of up to 160 mg/L, which in the last decade has been shown to have dechlorinated to cis- and trans-dichloroethylene (DCE), 1,1-dichloroethylene, vinyl chloride (VC), ethylene, and ethane. Previously, it was established that the occurrence of these products was due to intrinsic bioremediation, based on both laboratory investigations (1,2) and temporal monitoring of the groundwater , which showed significant levels of ethene and methane (3-5).

The flux of all alkyl halides into Lake Michigan is of major public concern due to the suspected carcinogenicity of VC. Moreover, as the plume moves towards and emanates into the aerobic surface water, the dominant redox conditions can be expected to change due to wave action and vertical seepage which promote the interchange of oxygen-rich lake water with the anaerobic groundwater. This phenomenon is likely to have an impact on the microbial community structure, and thus the biodegradative processes. The current presentation provides the results of field investigations at the interface with Lake Michigan, and presents some preliminary results on the fate of chlorinated solvents at a simulated interface between earobic and anaerobic conditions using using both experimental and modeling approaches.

2. METHODOLOGY

Off shore sampling was conducted using a truck-mounted Geoprobe chained on a 80' x 30' barge, which could be anchored using steel spuds (The Probing Times, 1994). The barge was put in place with the help of a tugboat, and an on-shore surveying team. Barge locations were based on a previously determined offshore survey plan, generated based on ground penetrating radar (GPR) and sonar measurements (B. Sauck, Eastern Michigan University). The four sampling points were approximately 100 m off shore at a depth of approx. 15', which corresponds to the expected hydraulic connection between the aquifer and Lake Michigan. A specially designed drill pipe was centered with a two-inch PVC sleeve, which was anchored with a steel plate in the lake sediment surface. A 0.5' vertically slotted screen well point was advanced to depths of up to 21 feet into sand and silt layers in 15-20' of water off shore, at depth intervals of 2-3'. Water samples were collected and analyzed for conductivity, pH, inorganic redox couples, methane, ethane, ethene, and chlorinated volatile hydrocarbons. Sediment samples were collected in the more reduced regions, were saturated with anaerobic groundwater, and shipped to the University of Michigan laboratories, where they were transferred to an anaerobic chamber.

On shore sampling was conducted using a Geoprobe with a 1' screen, which was driven into the beach sands using an 80lb electric hammer to depths of up to 32'. Groundwater samples were collected at 3' depth increments, and sediment samples were collected from the most reduced zones at 17-20'. The locations of the barge and the beachhead sampling points are shown on Figure 1, relative to the five previously developed transects on shore (modified from Wilson et al., 1994).

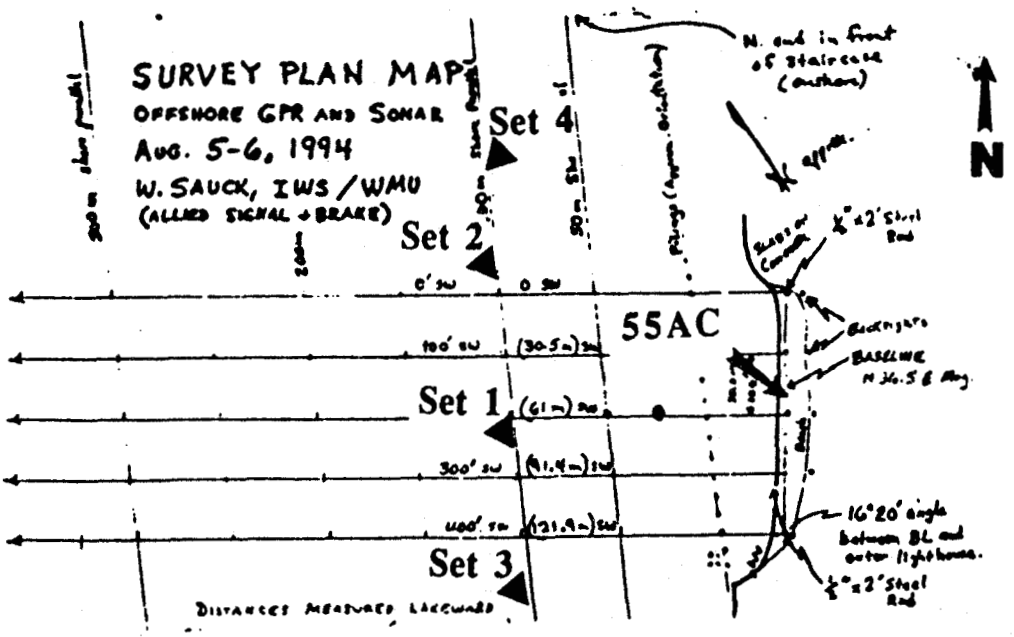

Figure 1. Location of beachhead and offshore sampling points at St. Joseph, MI.

Field simulation experiments are conducted in two separate column configurations, to allow for both spatial and temporal differentiation. The sequential column configuration (Figure 2, A) consist of two water-jacketed columns (20 °C) packed with approx. 250 g of saturated aquifer material, in which the first column is maintained methanogenic, and the second methanotrophic. This setup emulats the movement of groundwater from the anaerobic zone to the aerobic zone, as water moves toward Lake Michigan. The second configuration (Figure 2, B) consists of one column in which the redox conditions are temporally switched (every 12 hours) between anaerobic (terminal electron acceptors, HCO_3^- and SO_4^{2-}) and aerobic methanotrophic media. This emulates the zone of the aquifer that is impacted on a temporal basis between aerobic and anaerobic conditions as the wave action of the lake changes. A syringe pump

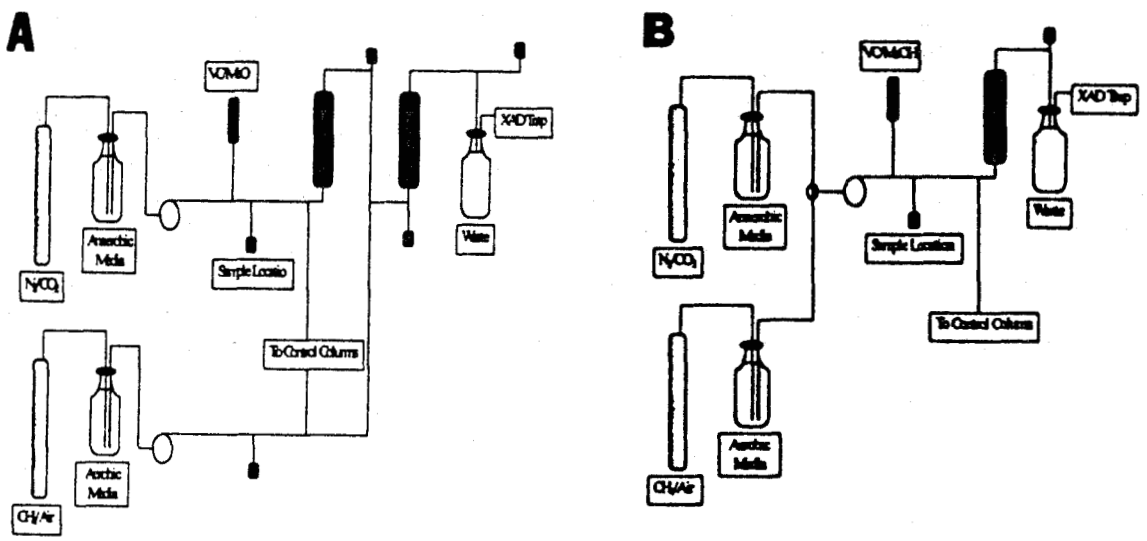

Figure 2. Configuration of the sequential (A) and temporally switched column (B).

located in the media supply line contains vinyl chloride in water (500 mg/L), which will provide solvent pulses no higher than 10 mg/L in the medium. Identical inactive column configurations are monitored along with the active columns.

Laboratory microcosms are established in 125-mL serum bottles, which are inoculated with 50 grams of wet sediment and filled with media (no headspace) specific to the redox conditions of interest. Based on field and laboratory evidence, methanogenic, sulfate-reducing, Fe^{2+}-reducing, and aerobic methanotrophic microcosms have been developed. Some vials, selected at random, are then injected with 10 ppm of vinyl chloride to measure transformation rates.

A numerical model is being developed which will simulate the hydrodynamic, physico-chemical and biological processes that take place at the aquifer-lake interface. Although the hydrodynamic processes are three-dimensional, most of the phenomena of interest can be modeled by a two-dimensional model on the vertical plane. The phenomena of interest include the migration of TCE, DCE, and VC into the lake and transfer of dissolved oxygen into the aquifer from water infiltrating through the surf region. Due to the significant time-scale difference between the nearshore circulation, wave run-up and breaking in the lake compared to the flow in the aquifer (estimated at 7.5 m/day), two separate models are being constructed for the corresponding hydrodynamic phenomena. The resulting flow fields are then integrated in a single mass transport and contaminant fate model. All three components of the model are two-dimensional in space, covering a vertical plane extending from a location on land where uniform flow and mass flux are observed in the aquifer, to a distance inside the lake where most nearshore current activity has diminished.

The nearshore/free surface flow simulation is based on a numerical solution of the Navier-Stokes equations by means of the finite element method. The model can predict the vertical structure of the flow from the seepage face between the aquifer and the lake to the free surface. Wave action is incorporated and special attention is focused on wave run up and breaking. The beach is assumed to be a porous bed so that water from the surf-and-break region is allowed to infiltrate and reach the aquifer.

3. RESULTS AND CONCLUSIONS

Vertical profiles of contaminant and inorganic solute concentrations, as well as measured redox potential values are shown in Figure 3, as a function of depth for sample location AC on the beach.

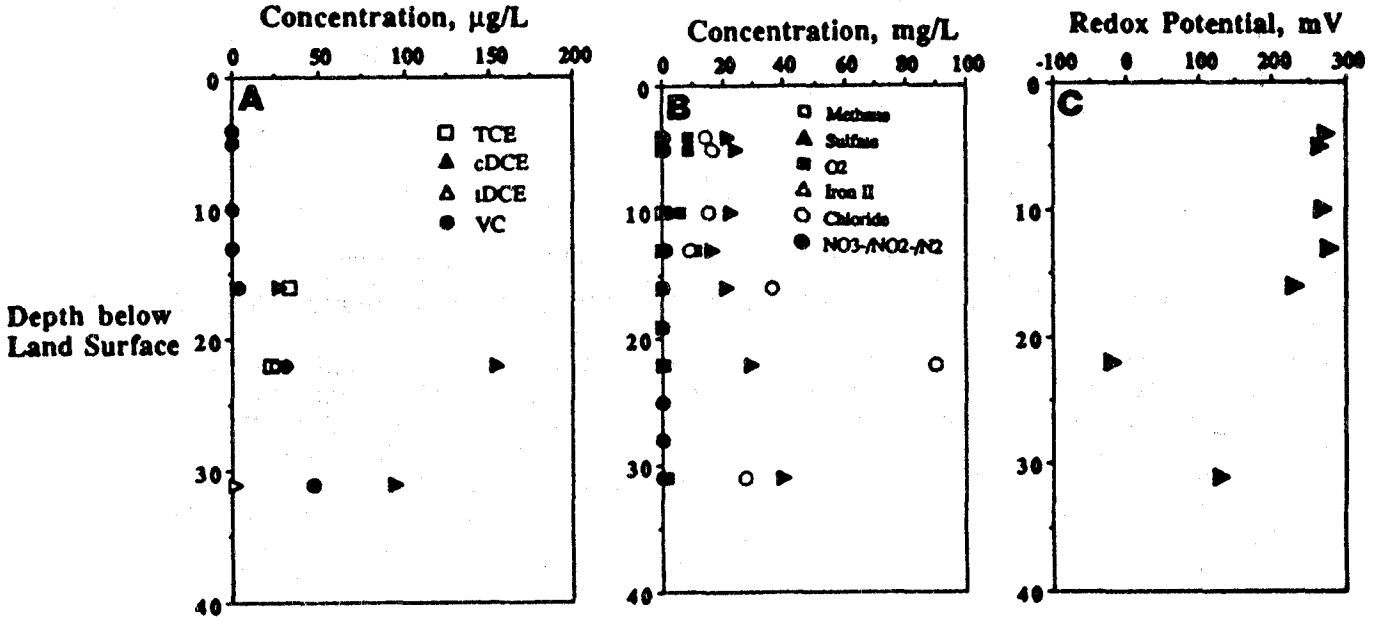

Figure 3. Vertical Profiles of Contaminants (A), Electron Acceptors (B), and Redox Potentials (C) at Beachhead Sampling Location AC.

It can be observed that DCE concentrations peak at a depth between 20-30 feet, while VC concentrations increase up to 32 feet. This region corresponds with the highest inorganic chloride concentrations (up to 90 mg/L), and the lowest redox potential values. However, the measured field potentials are too high for effective methanogenesis to occur, and coincide with high sulfate levels. Sulfide levels were not measured in the water samples, thus no conclusive evidence for sulfidogenic activity was evident, less its importance in dechlorination reactions. Though soluble iron (Fe2+) was only sporadically detected and its concentration was low (< 0.05 - 0.2 mg/L), indirect evidence of active iron reducing activity was based on the observation of magnetite crystals in the water and sediment samples. This informatrion is contrary to the data from well upstream in the aquifer, where reductive dechlorination resulting in ethene and ethane was strongly correlated to methanogenic activity (3).

The profiles for electron acceptors, chloride and redox potential in Lake Michigan sediments are exemplified for Barge Set 4 (for location please see figure 1), as a function of depth below lake surface (Figure 4). Note that the lake bed is at approximately 15' depth. A very strong correlation can be noted between the chloride peak (18-25' below surface) and the reduced environmental conditions (> -100 mV), signifying high microbial activity. Again, methane concentrations were extremely low, but the chloride peak and redox potential minimum appear to coincide with a decrease in sulfate concentrations. Sulfide concentrations measured were very low (< 0.1 mg/L). This observation does not preclude the absence of sulfate reducing activity, as the sulfide produced may have precipitated as iron sulfide (FeS) (note the low remaining Fe (II) concentrations (0.1 - 0.2 mg/L). Contaminants were not observed, as their concentrations were probably below the detection limit.

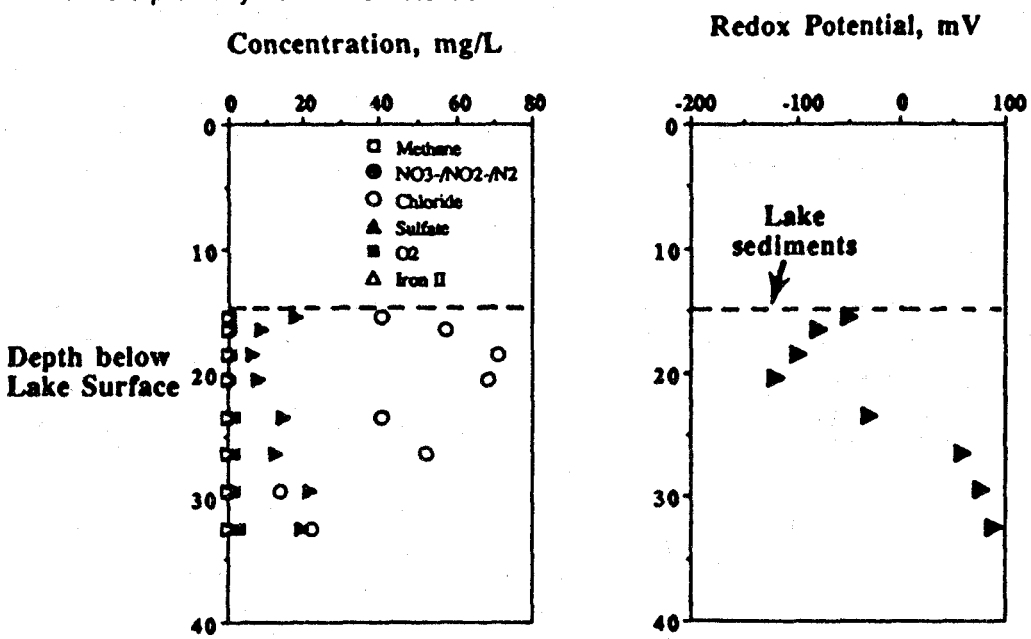

Figure 4. Vertical Profiles of Electron Acceptors (A) and Redox Potentials (B) at Barge Set 4.

The field data suggested that under anaerobic conditions, methanogens, sulfate-reducers and Fe-reducers may play a role at the interface, as well as methanotrophic and aerobic heterotrophs. Thus far, we have stimulated methanogenic and Fe-reducing activity in aquifer material obtained from the beach borings. Microcosms incubated under methanogenic conditions, and spiked with 10 mg/L of VC showed a slight decrease of the VC concentrations after three months of incubation, relative to the inactive controls. Similarly, we have established methanotrophic and methanogenic activity in the sequential soil columns. Microbial characterization of methanotrophic column effluent has resulted in the detection on a single dominant heterotrophic microorganism, as well as two distinct methanotrophic populations. Dechlorination studies have recently been initiated.

A three dimensional regional groundwater flow model based on MODFLOW is being developed. This regional model will be used for parameter estimation as well as to validate the groundwater flow model that

has been developed for this site previously. A computational grid reflecting the actual conditions at the St. Joseph site is currently being implemented for the groundwater model. Following validation of the groundwater model, the nearshore/free-surface flow simulation will be adapted from a code that has already been completed.

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MONITORING AND CONTROL OF *PSEUDOMONAS* SP. STRAIN KC FOR *IN-SITU* BIOAUGMENTATION OF CARBON TETRACHLORIDE CONTAMINATED SITES

Craig S. Criddle, Center for Microbial Ecology and Department of Civil and Environmental Engineering, Engineering Research Complex A126, Michigan State University, East Lansing, MI, 48824, (517) 355-8241

Michael J. Dybas, Center for Microbial Ecology, Engineering Research Complex A119, Michigan State University, East Lansing, MI, 48824, (517) 353-9059

Gregory M. Tatara, Center for Microbial Ecology and the Department of Microbiology, Engineering Research Complex A135, Michigan State University, East Lansing, MI, 48824, (517) 353-9059

David C. Wiggert, Department of Civil and Environmental Engineering, Engineering Research Complex A120, Michigan State University, East Lansing, MI, 48824, (517) 355-5155

Michael Witt, Department of Civil and Environmental Engineering, Engineering Research Complex A121, Michigan State University, East Lansing, MI 48824, (517) 353-9059

James M. Tiedje, Center for Microbial Ecology and Departments of Crop and Soil Science and Microbiology, Plant and Soil Science 540, Michigan State University, East Lansing, MI, 48824, (517) 353-9021

Serguei Bezborodnikov, Center for Microbial Ecology, Plant and Soil Science 540, Michigan State University, East Lansing, MI, 48824, (517) 353-9021

David Lubman, Department of Chemistry, 4545 Chemistry Building, The University of Michigan, Ann Arbor, MI, 48109, (313) 764-1669

Scott Fogler, Department of Chemical Engineering, The University of Michigan, Ann Arbor, MI, 48109, (313) 763-0459

INTRODUCTION

Pseudomonas sp. strain KC converts carbon tetrachloride (CT) to carbon dioxide, formate, and non-volatile product(s) without the production of chloroform under denitrifying conditions [1,2]. Most other organisms that transform CT under such conditions do so slowly and/or produce chloroform, making strain KC an attractive candidate for bioaugmentation applications. For strain KC, the mechanism of CT transformation involves secretion of a low molecular weight factor under iron-limiting conditions [3]. The secreted factor fortuitously transforms CT and is activated for transformation by live whole cells [3,4].

To determine whether the capabilities of strain KC can be applied for in-situ remediation of CT contamination, the Michigan Department of Natural Resources is supporting a field experiment in a CT-contaminated aquifer at Schoolcraft, MI. The present report describes laboratory work funded by the Great Lakes and Mid-Atlantic Hazardous Substance Research Center in support of the field project. These laboratory efforts have focused on: (1) isolation and identification of the secreted factor, (2) development and testing of a laboratory-scale model aquifer and numerical simulator to describe establishment and maintenance of a CT-transforming zone, (3) development and testing of a gene probe for detection and monitoring of strain KC, (4) identification of characteristic biochemical markers for strain KC, and (5) identification of key transport and retention mechanisms for strain KC.

METHODOLOGY

Organisms and culture conditions. *Pseudomonas* sp. strain KC (DSM deposit no. 7136, ATTC deposit no. 55595), derived originally from aquifer solids from Seal Beach, CA, (1) is routinely maintained in our laboratories on nutrient agar plates. *P. fluorescens* (ATTC deposit no. 13525) was obtained from the culture collection of the Department of Microbiology at Michigan State University. Medium D was prepared as described elsewhere [1].

Fractionation of CT transformation activity. To localize the CT transformation activity of strain KC, actively transforming cultures were fractionated by centrifugation and ultrafiltration. Cells from 300 ml of actively transforming culture were harvested by centrifugation, and the supernatant filtered through a 0.2 μm filter. In general, cell-free supernatant and its filtered fractions (10,000 and 500 MW filtrate) did not mediate appreciable CT transformation alone. Rapid transformation was usually obtained only when these fractions were combined with viable whole cells. Filtrate and retentate from the 10,000 and 500 MW filters were combined with washed cells of strain KC before being assayed for CT transformation or they were tested in the *P. fluorescens* bioassay.

Bioassay for the secreted factor using *Pseudomonas fluorescens*. Tatara et al. (4) discovered that rapid CT degradation occurs when the secreted factor generated by strain KC is combined with diverse cell types, such as cells of *Pseudomonas fluorescens*. This finding enabled the development of a bioassay for the secreted factor. *P. fluorescens* cells were harvested by centrifugation and resuspended to one tenth the original culture volume in medium D at a cell density of approximately 2×10^9 cfu/ml. Five hundred microliters of the resulting 10 X concentrated cell suspension was added to 4.5 ml samples generated during the fractionation procedure. The samples were rendered anoxic, sealed under N_2 headspace in 28 ml Balch tubes, and spiked with CT. Levels of CT were followed by headspace gas chromatography as previously described [5].

Purification of secreted factor. Filtrate passed through an Amicon 500 MW filter was lyophilized to dryness. The lyophilized filtrate was suspended in approximately 5% of the original filtrate volume (254 mg lyophilized filtrate/ ml) in ultra pure deionized water. The sample was then transferred to a 28 ml test tube and 9 ml of HPLC grade acetone were added Samples were stored at 4°C for 2 hours to allow precipitation. A visible precipitate formed during the first minute after acetone addition. The supernatant was decanted and filtered through a 0.45 μM PTFE filter to remove any particulate matter. The acetone water phase was evaporated to dryness under nitrogen at room temperature. Approximately 80% of the bioassay activity was recovered in the dried acetone fraction. The yield was approximately 7 μg partially purified material per mg dry 500 MW filtrate precipitated. The secreted factor recovered in the acetone extract was further purified by reverse phase HPLC using a methanol-water mobile phase and a C18 column. Samples collected in the HPLC effluent were evaluated for the presence of secreted factor using the *P. fluorescens* bioassay.

Model aquifer column. A PVC column (183 cm length x 5.2 cm i.d.) was fabricated and served as a model aquifer system. The column was equipped with 30 injection/extraction ports along its length at 76-mm intervals, with seven additional holes located between ports 5 and 6 for an injection zone. The column was wet packed with Ottawa sand using groundwater obtained from the Schoolcraft site (containing 60 mg/L nitrate). The column was operated at a bulk flow rate of 90 mL/min (a linear velocity of 150 mm/d), with an inflow CT concentration of 80 μg/L.

To evaluate the feeding pattern planned for the field experiment, pulse injections of nutrients and organisms were simulated using slugs of tritiated water (^3H_2O) followed by inoculation with *Pseudomonas* strain KC (at approximately ~2×10^8 cells/mL), acetate (60 mg/L), phosphate (10 μg/L), and bromide (20 mg/L). Acetate was added in weekly pulses thereafter. Strain KC was monitored using an MPN (Most Probable Number) method, in which growth in medium D and CT transformation were used to indicate presence of strain KC. The presence of strain KC was also confirmed by gene probe. CT was analyzed by headspace sampling and gas chromatography as described elsewhere [5]. Acetate, bromide, nitrate, and nitrite were analyzed by ion chromatography. To simulate column results, we

developed a one-dimensional code for coupled transport of five constituents (acetate, nitrate, CT, attached and suspended organisms) in the 2 m long laboratory columns. We have tested the code for numerical stability and accuracy.

Development of a gene probe for strain KC. Twenty random clones of approximately 1 kb from the *Pseudomonas* KC chromosome were screened against 200 isolates from Schoolcraft groundwater to determine their specificity for KC. Five fragments showing no non-specific hybridization were selected for further evaluation. Fragment #40 was selected as superior for specificity and reliable PCR-amplification. Two sets of primers of 20-22 mer (P1F, P2F and P2R-P1R) have been selected and tested against aquifer isolates, aquifer groundwater and Genbank and found to be specific for strain KC. The primers have been used with PCR amplification to test the movement of strain KC after injection into the test column of aquifer material, described in the previous section. One milliliter water samples were taken from sampling ports along the model aquifer column for DNA extraction using SDS/proteinase K followed by CTAB extraction and propanol precipitation of the DNA. PCR amplification with this template DNA was performed after adding 50 pmol of the two primers and Ampli Tag polymerase. Any PCR products were separated by electrophoresis in 1% agarose gels and stained with ethidium bromide. If any target (KC) DNA is present, a band of 1.35 kb should be observed.

RESULTS

Separation of the secreted factor. As illustrated in the chromatogram of Figure 1, the acetone extraction and HPLC separation procedure was successful, and the secreted factor (as indicated by a strong positive bioassay response) was recovered in a single, separated peak at a retention time of 14 minutes.

We are currently purifying sufficient secreted factor for identification preparative HPLC. The identification step will be conducted by mass spectrometry, using other analytical techniques as needed. Identification will be conducted in collaboration with Dr. David Lubman (UM, Chemistry) and Dr. Douglas Gage (MSU, Biochemistry).

Model aquifer column. As shown in Figure 2, a biologically active region of CT removal was sustained by weekly feeding pulses. By day 29, effluent CT concentration had fallen to approximately 1 µg/L, a CT removal efficiency of 98%. Experimental results from the physical model were compared with numerical simulations to confirm the numerical model's applicability or suggest the need for refinement. The numerical model was capable of simulating the activity of strain KC.

MPN data were consistent with information obtained from the gene probe. Samples taken from all 23 ports before KC injection showed no DNA bands. After injection at port 5, strain KC DNA was detected in ports 4-12 only. Sampling over each of the next 4 weeks showed strain KC movement to ports 14, 15, 18 and 22. This progressive spread of KC with the water flow path over time is consistent with the movement of strain KC as indicated by MPN results.

Factors affecting cell attachment and detachment. In order to assess the initial attachment of KC cells on subsurface solids during the injection stage, we measured the zeta potential of KC cells over the pH range expected in the field experiment (7.5-9). The results indicate a high net negative charge (-49 mV at pH 7.5 increasing to -61 mV at pH 9). Assuming that subsurface minerals will present a negative charge, this suggests that electrostatic repulsive forces will be important in predicting cell retention and placement in the Schoolcraft aquifer. However, over the anticipated operating pH range of 7-10, changes in cell surface charge are small. Therefore, pH fluctuations in the Schoolcraft aquifer will not significantly affect cell retention to subsurface materials.

CONCLUSIONS

1. A bioassay for the secreted factor was successfully developed and tested. The assay consisting of combining the sample to be assayed with *P. fluorescens* cells and with CT and monitoring the rate of CT disappearance.

2. Purification of the low molecular weight factor secreted by strain KC was accomplished by: filtration of supernatant through 0.2 μm, 10,000 MW, and 500 MW filters; acetone extraction; and HPLC separation.

3. A zone of CT transformation was sustained in the model aquifer by weekly pulsing of acetate. The bulk of the CT transformation occurs over a narrow region, close to the inoculation site.

4. The gene probe appears to sensitive, specific, and efficient for tracking strain KC in aquifer samples, and thus is ready for use in the field.

5. Strain KC was readily transported through the aquifer materials, glass beads, and sands tested to date. This is apparently due to repulsive forces between the cells and the solids. Strain KC cells have a high net negative charge.

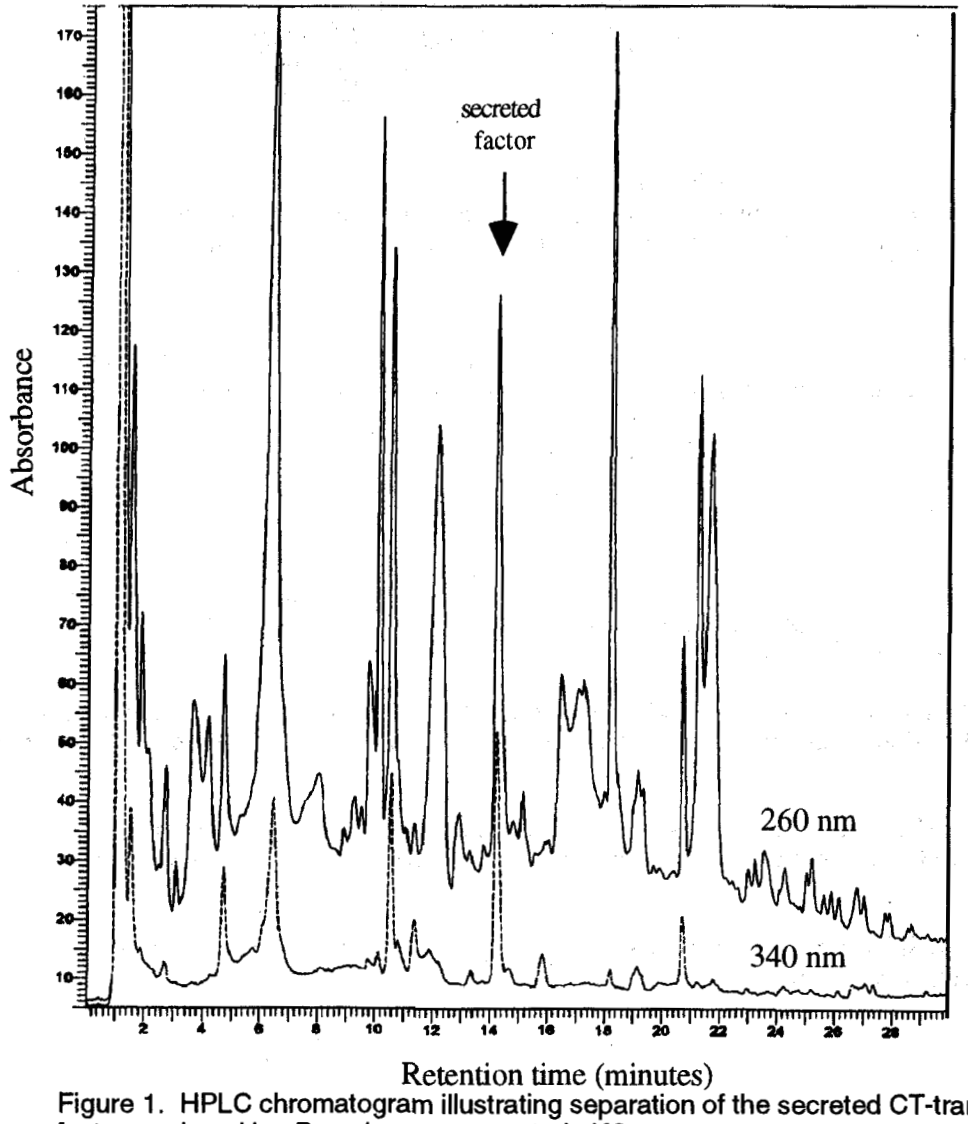

Figure 1. HPLC chromatogram illustrating separation of the secreted CT-transforming factor produced by *Pseudomonas* sp. strain KC.

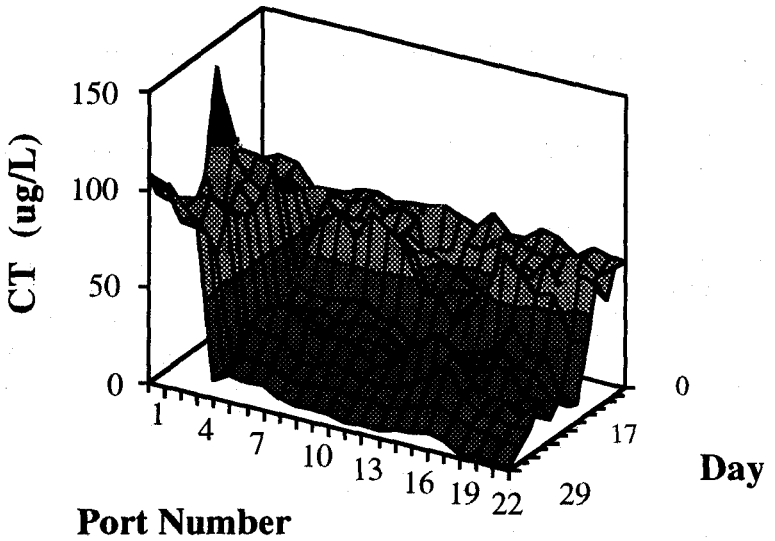

Figure 2. Carbon tetrachloride concentration profile for the model aquifer following inoculation. Acetate is provided once a week as a single pulse.

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FOR MORE INFORMATION: contact Dale Manty, Program Director, Hazardous Substance Research Centers Program, Office of Exploratory Research 8703.

REMEDIATING MUNITIONS CONTAMINATED SOILS

Patrick J. Shea and S.D. Comfort
Department of Agronomy
University of Nebraska
Lincoln, NE 68583-0915
402-472-1502

INTRODUCTION

The former Nebraska Ordnance Plant (NOP) at Mead, NE was a military loading, assembling, and packing facility that produced bombs, boosters and shells during World War II and the Korean War (1942-1945, 1950-1956). Ordnances were loaded with 2,4,6-trinitrotoluene (TNT), amatol (TNT and NH_4NO_3), tritonal (TNT and Al) and Composition B (hexahydro-1,3,5-trinitro-1,3,5-triazine [RDX], and TNT). Process waste waters were discharged into wash pits and drainage ditches. Soils within and surrounding these areas are contaminated with TNT, RDX and related compounds. A continuous core to 300 cm depth obtained from an NOP drainage ditch revealed high concentrations of TNT in the soil profile and substantial amounts of monoamino reduction products, 4-amino-2,6-dinitrotoluene (4ADNT) and 2-amino-4,6-dinitrotoluene (2ADNT). Surface soil contained TNT in excess of 5000 mg kg^{-1} and is believed to contain solid phase TNT. This is supported by measuring soil solution concentrations at various soil to solution ratios (1:2 to 1:9) and obtaining similar TNT concentrations (43 and 80 mg L^{-1}).

Remediating munitions-contaminated soil at the NOP and elsewhere is of vital interest since many of the contaminants are carcinogenic, mutagenic or otherwise toxic to humans and the environment. Incineration, the most demonstrated remediation technology for munitions-containing soils, is costly and often unacceptable to the public. Chemical and biological remediation offer potentially cost-effective and more environmentally acceptable alternatives. Our research objectives are to: (a) characterize the processes affecting the transport and fate of munitions in highly contaminated soil; (b) identify effective chemical and biological treatments to degrade and detoxify residues; and (c) integrate these approaches for effective and practical remediation of soil contaminated with TNT, RDX, and other munitions residues.

METHODOLOGY

Transport and Sorption. Transport experiments were conducted with repacked, unsaturated soil columns comprised of either uncontaminated soil or a contaminated soil layer over uncontaminated soil. A constant pore water velocity was established in the columns and used to pass multiple pore volumes of a solution pulse containing 6.3 or 70 mg TNT L^{-1} in 3 m*M* $CaCl_2$ spiked with ^3H_2O. After conditioning, multiple pore volumes of TNT were pulsed onto the top of the columns by a syringe pump and displaced through the soil with 3 m*M* $CaCl_2$. After the TNT pulse was eluted from the columns, the experiment was terminated and collection tubes were weighed to determine the volume collected as a function of time and pore volumes. Effluent was analyzed for TNT, 2ADNT, and 4ADNT by HPLC. Relative concentrations (C/C_o) were calculated from the ^3H_2O and TNT concentrations (C) in the effluent fractions and the initial concentrations (C_o) of the TNT pulse.

TNT sorption to soil was determined by batch equilibration using 5 g of Sharpsburg silty clay loam soil and 15 mL TNT solution (1:3 soil:solution ratio) at initial concentrations ranging from 3.53 to 61.81 mg L^{-1} in 3 m*M* $CaCl_2$. Solutions were shaken for 24 h in 35-mL teflon centrifuge tubes and centrifuged (3450 *x g*) at room temperature for 30 min. Supernatant was filtered and analyzed by HPLC. The difference between soil-free blanks and equilibrium solutions was assumed to be the result of adsorption. Adsorption was described by a linear isotherm and by the Freundlich equation ($Q = KC^{1/n}$, where Q = amount adsorbed per gram adsorbent, C is the equilibrium TNT concentration, K is the distribution coefficient and 1/n is a constant).