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**DEVELOPMENT OF A LABORATORY-SCALE MODEL AQUIFER SYSTEM TO MONITOR A CARBON TETRACHLORIDE TRANSFORMING ZONE BY *PSEUDOMONAS* SP. STRAIN KC**

presented by

**Michael Erich Witt**

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# DEVELOPMENT OF A LABORATORY-SCALE MODEL AQUIFER SYSTEM TO MONITOR A CARBON TETRACHLORIDE TRANSFORMING ZONE BY *PSEUDOMONAS* SP. STRAIN KC

By

Michael Erich Witt

A THESIS

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## ABSTRACT

### DEVELOPMENT OF A LABORATORY-SCALE MODEL AQUIFER SYSTEM TO MONITOR A CARBON TETRACHLORIDE TRANSFORMING ZONE BY *PSEUDOMONAS* SP. STRAIN KC

**By**

**Michael Erich Witt**

*Pseudomonas* sp. strain KC is a denitrifying bacteria that has the unique ability to transform carbon tetrachloride (CT) to carbon dioxide, formate, and a non-volatile end product(s) without the production of chloroform. This organism appears to be a good candidate for bioaugmentation. It is able to grow and transform CT in diverse environments, provided the pH is adjusted to 7.8-8.2, and it is readily transported through aquifer materials. Bench-scale laboratory experiments were used to evaluate the feasibility of bioaugmentation with strain KC. Two model aquifer columns packed with Ottawa sand were used to simulate aquifer conditions. CT-contaminated groundwater was pumped through both columns at a flow rate of 85 $\mu$L per minute to yield an average linear velocity of 14.8 cm/day. Transformation of CT by strain KC was analyzed by inoculating the column with strain KC along with base, nutrients (acetate and phosphate), and a conservative tracer. The subsequent development of the biologically-active zone, or biofence, was examined by analyzing for acetate, CT, KC cells, nitrate, nitrite, and phosphate. Data obtained from these analyses led to characterization of certain kinetic parameters of CT degradation by strain KC in the biofence.

**For Laurie,**

**whose life adds so much to mine**

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Much of the encouragement and support provided to me during my graduate school experience was unselfishly given to me by my wife, Laurie. Words alone cannot describe the gratitude and thankfulness I possess for having Laurie and the support she so freely offers. I am truly grateful and honored to be her husband.

I thank my parents for being so generous and loving over the past twenty-four years and for challenging me to continue on to graduate school. The guidance and support endowed to me as their son will be carried with me, as someday it will be my turn to enlighten our children. I believe that I speak for all of my siblings when I say that much of our success is a fortunate consequence of growing up as their children. In addition I would like to thank Deb, Laura, Bill, Julie, and Steve for their love and friendship through all these years. Nobody could ask for a better set of brothers and sisters.

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| | |
|---|---|
| $\alpha_l$ | longitudinal dispersivity, cm |
| $b_{kc}$ | endogenous decay coefficient, $days^{-1}$ |
| $C_a$ | rate limiting substrate concentration of acetate, mg/L |
| $C_{ct}$ | concentration of CT, mg/L |
| $D_l$ | dispersion coefficient, $cm^2/day$ |
| $D^*$ | coefficient of molecular diffusion, $cm^2/day$ |
| $D_x$ | hydrodynamic dispersion coefficient, $cm^2/g$ |
| $f_{oc}$ | weight fraction of organic carbon |
| $K_c$ | deposition coefficient, $days^{-1}$ |
| $K_d$ | distribution coefficient, $cm^3/g$ |
| $K_{oc}$ | the partition coefficient of a compound between organic carbon and water, (g solute sorbed/g soil organic carbon)/(g solute/$cm^3$ solution) |
| $K_s$ | half-velocity coefficient, mg/L |
| $K_y$ | entrainment coefficient, $days^{-1}$ |
| l | distance along flow path, cm |
| $n,\theta$ | soil porosity |
| $R_a$ | retardation coefficient for acetate |
| $\rho_b$ | bulk density of soil material, $g/cm^3$ |
| $R_{ct}$ | retardation coefficient for carbon tetrachloride |
| t | time, days |
| v | specific discharge, cm/day |
| V | average linear groundwater velocity, cm/day |

| | |
|---|---|
| $V_x$ | groundwater velocity in the x-direction, cm/day |
| $X_{kc}$ | concentration of KC cells in the liquid phase, mg/L |
| $\bar{X}_{kc}$ | concentration of KC cells associated with solid phase but expressed per volume of pore water, mg/L |
| $Y_{kc}$ | yield coefficient for KC cells, mg cells/mg acetate |

# CHAPTER 1

## INTRODUCTION

A CT-contaminated aquifer near Schoolcraft, Michigan, has been under investigation by the Michigan Department of Natural Resources (MDNR) since 1987. This contaminant plume is believed to have originated during grain fumigation operations at an abandoned grain elevator located near the center of Schoolcraft. The primary contaminant in this plume is carbon tetrachloride, but high levels of nitrate (60 mg/L) are also present, presumably as a result of local farming operations. CT levels up to 400 $\mu$g/L are found in the groundwater near the center of mass of the plume, with most samples showing concentrations ranging from 50 to 150 $\mu$g/L. A plan to remediate this plume was put forth by the MDNR in 1993. The proposed remedial action involves the extraction of the contaminated groundwater using a single recovery well. The extracted groundwater will be treated on-site by air stripping. The estimated duration of this remedial action is approximately 25 years (Halliburton NUS Environmental Corporation).

A less timely and more inexpensive method of remediating this CT-impacted aquifer may be possible by biodegrading the contamination in-situ and transforming the CT to non-harmful byproducts. Essentially, two microbial in-situ biodegradation options exist: biostimulation and bioaugmentation. Biostimulation involves enhancing the growth of indigenous microflora to stimulate degradation of a compound. A disadvantage of biostimulation is that some or all of the stimulated organisms may not be able to degrade the targeted compound. In addition, the degradation pathway may include undesirable end

products. Bioaugmentation involves the introduction of a non-indigenous organism for the purpose of degrading a compound. However, with bioaugmentation exists competition between indigenous microflora and the organisms being introduced. This competition presents a challenge when attempting to initiate degradation of a particular compound when adding a non-indigenous organism to the environment. A method of overcoming this obstacle is to create conditions in the environment that are favorable for the organism being added. This method, environmental (or niche) adjustment, provides conditions that favor the growth of the non-indigenous organism. A niche is defined as a "specialized functional role of an organism within the ecosystem or community" (Atlas and Bartha, 1993). The specialized functional role of interest in this case is iron-scavenging. A need for this role becomes more important at slightly elevated pH (8.0 to 8.2), providing a competitive advantage for organisms that possess that function and can occupy that niche.

Microorganisms may mediate the degradation of compounds in order to obtain energy for growth and to synthesize cell material (metabolism), or may degrade compounds which do not support metabolism (cometabolism). The energy-yielding reactions are generally oxidation-reduction reactions that involve the transfer of electrons from a donor to an acceptor. Reduced compounds, such as acetate, can serve as electron donors for energy. The electrons released during oxidation are transferred to electron acceptors, such as oxygen and nitrate. In the absence of oxygen, the electron acceptor that tends to be used (if present) is nitrate (Criddle *et al.*, 1991).

Many microorganisms typically convert CT to chloroform, a contaminant that is more persistent in the environment than CT. *Pseudomonas* sp. strain KC is a natural isolate derived from an aquifer in Seal Beach, California, and it is able to persist and compete in aquifer materials and soils (Criddle *et al.*, 1990; Lewis and Crawford, 1993; and Tatara *et al.*, 1993). Under denitrifying conditions, strain KC rapidly transforms CT to carbon

dioxide, formate, and an unidentified non-volatile end product without the production of chloroform (Dybas *et al.*, 1995). The conditions required for rapid transformation of CT by strain KC are: an anoxic environment, an electron donor such as acetate, an electron acceptor such as nitrate, and iron-limiting conditions (Criddle *et al.*, 1990; Lewis and Crawford, 1993; and Tatara *et al.*, 1993). Strain KC grows optimally at temperatures that are typical of aquifers (10-20°C).

Numerous experiments have been completed to better understand the mechanism of CT transformation via bioaugmentation with strain KC. The results of these experiments indicate that CT transformation is influenced by the availability of iron. In iron-rich groundwater and soils inoculated with strain KC, CT transformation can be achieved by raising the pH of the groundwater and soil materials to 7.7-8.2, a range where ferric iron solubility is lowest (Stumm and Morgan, 1981). Dybas *et al.* (1995) investigated the complex mechanism responsible for CT transformation and determined that a plausible model involves: (1) production and export of a CT-transforming factor(s) from the KC cell in response to iron limitation, (2) deactivation of the factor(s) upon transformation of CT, and (3) reactivation of the factor(s) at the cell membrane. Identification of the CT-transforming factor(s) remains an uncertainty and investigations are ongoing. Evidence suggests that production and export of this CT-transforming factor from the cell in response to iron-limitation, along with reactivation of the factor by viable cells after transformation of CT, is the mechanism that enables strain KC to degrade CT.

To investigate the possibility of bioaugmentation at a CT-contaminated site, the establishment of a zone in a model aquifer inoculated and colonized by strain KC is proposed. This colonized zone will form a "biofence" of CT transformation activity in a direction normal to the direction of groundwater flow and downgradient from the targeted contamination. CT will be transported to the biologically-active zone by groundwater flow

induced by the natural hydraulic gradient. An upstream injection port will be used for the addition of alkalinity (niche adjustment) and for the addition of organisms and nutrients. The alkalinity-treated groundwater will undergo mixing with background groundwater establishing conditions that are favorable for growth of strain KC. In a field setting, the pH of groundwater may be restored to near background levels by addition of acidity downstream to mimic the dilution with background groundwater, which in a two-dimensional system will titrate alkalinity. As the pH of the groundwater nears background levels, strain KC will be expected to die off gradually (Knoll, 1994). This defines the thickness of the proposed "biofence" technology. The concept of a "biofence" is further discussed in Chapter 6.

In this thesis, two model aquifer columns were constructed to examine the transformation of CT by strain KC. Various flow parameters and CT-sorption characteristics were determined from the data obtained using these columns. The porosity of the Ottawa sand in both model aquifer columns was determined via tracer studies. In addition, a dispersion coefficient for solute flow within the column was also calculated. From a CT-saturation experiment, a retardation coefficient and a distribution coefficient for CT flow through the column were determined. After inoculating one model aquifer column with strain KC and nutrients, a CT removal efficiency of over 98% was achieved in less than 30 days. Formation and maintenance of the biofence in this model aquifer column was carefully followed over the duration of this study. A growth substrate transformation capacity of up to 58 $\mu$g of CT per mg of acetate consumed was achieved. This data shows that, under favorable conditions, *Pseudomonas* sp. strain KC rapidly transforms carbon tetrachloride in a model aquifer column without the production of chloroform.

# CHAPTER 2

## MATERIALS AND METHODS

**Groundwater.** Groundwater from a CT-contaminated aquifer in Schoolcraft, Michigan, was used in all batch and column studies. Groundwater samples were obtained by extracting groundwater from a two-inch steel well screened at thirty feet below the water table with a Teflon™ bailer. Groundwater samples were stored in pre-sterilized sealed Nalgene carboys at 4°C. All groundwater samples were airstripped for at least 48 hours to remove CT and then placed in 1 L Wheaton bottles equipped with Teflon™-lined caps. To each 1 L bottle, three grams of sodium bicarbonate (Aldrich Chemical Company) were added, and the pH adjusted to 7.50 by bubbling in carbon dioxide gas. Samples were deaired by bubbling nitrogen gas rapidly into each bottle for a period of twenty minutes.

**Organisms.** *Pseudomonas* sp. strain KC (DSM deposit number 7136, ATCC deposit number 55595), derived originally from aquifer solids from Seal Beach, California, is routinely maintained in our laboratory on nutrient agar plates.

**Chemicals and radioisotopes.** Carbon tetrachloride (99% purity) was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. $^{3}H$-labeled water (1.0 mCi/g) was provided by DuPont NEN Products. All chemicals used were ACS reagent grade (Aldrich or Sigma Chemical Company). All water used in reagent preparation was deionized 18 megaohm resistance or greater.

**Media.** Medium D contained the following per liter of deionized water: 2.0 g of $KH_2PO_4$, 3.5 g of $K_2HPO_4$, 1.0 g of $(NH_4)_2SO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 3.0 g of sodium acetate, 2.0 g of sodium nitrate, 1 mL of 0.15 M $Ca(NO_3)_2$, and 1 mL of trace nutrient stock TN2. Stock solution TN2 contained the following per liter of deionized water: 1.36 g of $FeSO_4 \cdot 7H_2O$, 0.24 g of $Na_2MoO_4 \cdot 2H_2O$, 0.25 g of $CuSO_4 \cdot 5H_2O$, 0.58 g of $ZnSO_4 \cdot 7H_2O$, 0.29 g of $Co(NO_3)_2 \cdot 6H_2O$, 0.11 g of $NiSO_4 \cdot 6H_2O$, 0.035 g of $Na_2SeO_3$, 0.062 g of $H_3BO_3$, 0.12 g of $NH_4VO_3$, 1.01 g of $MnSO_4 \cdot H_2O$, and 1 mL of $H_2SO_4$ (concentrated). Medium D is adjusted to pH 8.2 using KOH pellets followed by 3 M KOH stock solution.

**Sampling Procedure.** Samples obtained from the model aquifer column for the purposes of CT analysis were withdrawn using a 500 $\mu$L Pressure-Lok™ gastight syringe (Alltech Associates) equipped with a 1.5-inch sideport sampling needle. Both the syringe needle and sampling port septum were sterilized with an ethanol-soaked cotton swab. Each 200 $\mu$L sample was dispensed into separate 2 mL glass vials (Sun Brokers, Inc.) previously sealed with Teflon™-lined crimp tops (Sun Brokers, Inc.). Immediately after dispensing each sample into the glass vial, 20 $\mu$L of 30% hydrogen peroxide solution was added to quench any bioreaction. The gastight syringe was rinsed internally with 0.5 mL methanol, followed by 0.5 mL autoclaved/deionized water between each sampling event.

The ion chromatograph (IC) required 700 $\mu$L samples for analysis. Disposable 1 mL syringes (Becton Dickinson) were used to withdraw the sample from the column and to dispense the sample into 1 mL IC tubes. Each sample was obtained by attaching a 1.5-inch 22 gauge needle (Becton Dickinson PrecisionGlide®) to the 1 mL syringe. Both the syringe needle and sampling port septum were sterilized by an ethanol-soaked cotton swab. The syringe needle was then fully inserted into the sampling port, a 700 $\mu$L sample was withdrawn, and the needle was removed from the sampling port. The syringe needle was

then detached from the 1 mL syringe. A 0.2 $\mu$m filter was attached to the syringe tip, and the contents of the syringe were filtered and dispensed into the IC tube.

When appropriate, samples obtained for CT analysis were also analyzed for $^3H_2O$. After headspace analysis via gas chromatography was completed, the contents of each sealed 2 mL vial were removed using the gastight syringe with a 1.5-inch sampling needle. Each 200 $\mu$L sample was then dispensed into separate scintillation vials (Research Products International Corporation) for $^3H_2O$ analysis.

**Analytical Methods.** Carbon tetrachloride was analyzed by removing samples of headspace gas above liquid samples and detected by gas chromatography as described by Tatara *et al.* (1993). External calibration curves were prepared by addition of a primary standard (8.22 ng of CT per $\mu$L of methanol) to secondary water solutions having the same gas/water ratio, ionic strength, and temperature as the assay samples to generate a 4-point calibration curve which bracketed the concentrations in assay samples. CT was assayed by removing 100 $\mu$L of headspace gas with a 500 $\mu$L Pressure-Lok gastight syringe, closing the syringe valve, inserting the syringe needle through the gas chromatograph (GC) port septum, opening the syringe valve, and injecting the sample into the GC. The GC used was a Perkin-Elmer model 8500 equipped with a 100/120 mesh column (10% Alltech CS-10 on Chromsorb W-AW) and an electron capture detector (ECD) with nitrogen carrier (40 mL/min) and nitrogen makeup (27 mL/min). Both methods 1 and 2 were used to assay CT. Method 1 has a lower oven temperature (60°C) than does method 2 (90°C), enabling the user to identify any chloroform production.

Acetate, bromide, nitrate, nitrite, and phosphate were assayed by ion chromatography (Dionex model 2000i/SP ion chromatograph with suppressed conductivity detection equipped with a Sarsep™ AN 300 anion exchange column or Dionex Model AS4-A

column and utilizing a 1.8 mM bicarbonate and 1.7 mM carbonate mobile phase at 3 mL/min). External standard calibration curves were prepared by diluting a primary ion standard into secondary water standards having the same ionic composition as the analyzed samples. These calibration curves were used to generate a 5-point calibration curve which bracketed the concentrations in assay samples. Chromatograms were recorded and data integrated using Turbochrom™ 3.0 software (Perkin-Elmer Corporation).

Tritiated water ($^3H_2O$) was detected by liquid scintillation spectroscopy (Packard Tri-Carb® 1500). 200 $\mu$L samples used for headspace analysis were dispensed into scintillation vials containing 10 mL scintillation cocktail (Safety-Solv™) and counted for five minutes.

Measurements of pH were made with a Jenco model 200A pH meter and a Jenco model 6000E pH probe. For small volume measurements (less than 1 mL), a Beckman Instruments model Φ11 pH meter and a Corning model 476540 semi-micro combination probe were used. Immediately prior to analysis, a two-point calibration over the pH range assayed was performed.

**PVC Adsorption Experiment.** An experiment was performed to examine the extent of adsorption of CT onto the sidewall and endcaps of two-inch diameter PVC piping. Holes were drilled into the endcaps of a four-inch piece of PVC piping. Stopcock valves were fit into the holes in each endcap to allow for easy sampling of the internal contents. A 72-hour static leak test was performed on the sealed capsule into which an aqueous CT solution (16 $\mu$g/L) was injected. Three 200-$\mu$L samples were taken at one time period each day for a period of 14 days. The concentration of CT in each sample was determined by analyzing the headspace using gas chromatography. Determination of the amount of CT adsorbed onto the sidewall and endcaps of PVC piping was also investigated by draining the CT-

spiked solution from the sealed capsule and replacing it with distilled, deionized water. The concentration of CT in samples obtained every three days was determined using gas chromatography.

**Column Construction.** One Excelon™ PVC column (183 cm length x 5.2 cm inside diameter) was fabricated and served as a model aquifer system. This column was equipped with 30 injection/extraction ports (1/4-inch NPT-1/4-inch brass Swagelock™ unions fitted with 10/32-inch Thermogreen™ GC septa for sampling) by drilling and threading twenty-three 3/8-inch holes along the length of the column at three-inch spacing (see Figure 1). An additional seven holes were drilled to provide for an injection zone (see Figures 1 and 2). Female endcap fittings were glued onto each end of the 183 cm column using PVC pipe glue. A threaded male endcap was then screwed into each endcap fitting, and silicone sealant (Dow Corning Corporation) was placed in the threads prior to insertion. Each male endcap was drilled to allow a 5/8-inch NPT to 1/4-inch reducing union. A stainless steel screen (40 mesh) was cut and held in place by silicone sealant on the interior portion of each endcap. This screening was provided to prevent sand particles from exiting the model aquifer column. Ottawa sand was obtained from Soiltest, Inc. (Lake Bluff, Illinois), and the column was wet-packed in a vertical position and filled with Schoolcraft groundwater. 200 mL portions of saturated Ottawa sand were poured into the top of the column and allowed to settle. Gentle tapping on the column exterior encouraged more dense packing of the Ottawa sand. The total volume of sand in the column was 3890 mL. The model aquifer temperature was maintained between 15°C and 17°C by running cold tap water through approximately 300 cm of 1/4-inch copper tubing, which was coiled around the entire length of the column. Cooling was enhanced by placing one-inch wide sheet metal strips between the copper coil and the column; this provided a larger surface area for cooling to occur.

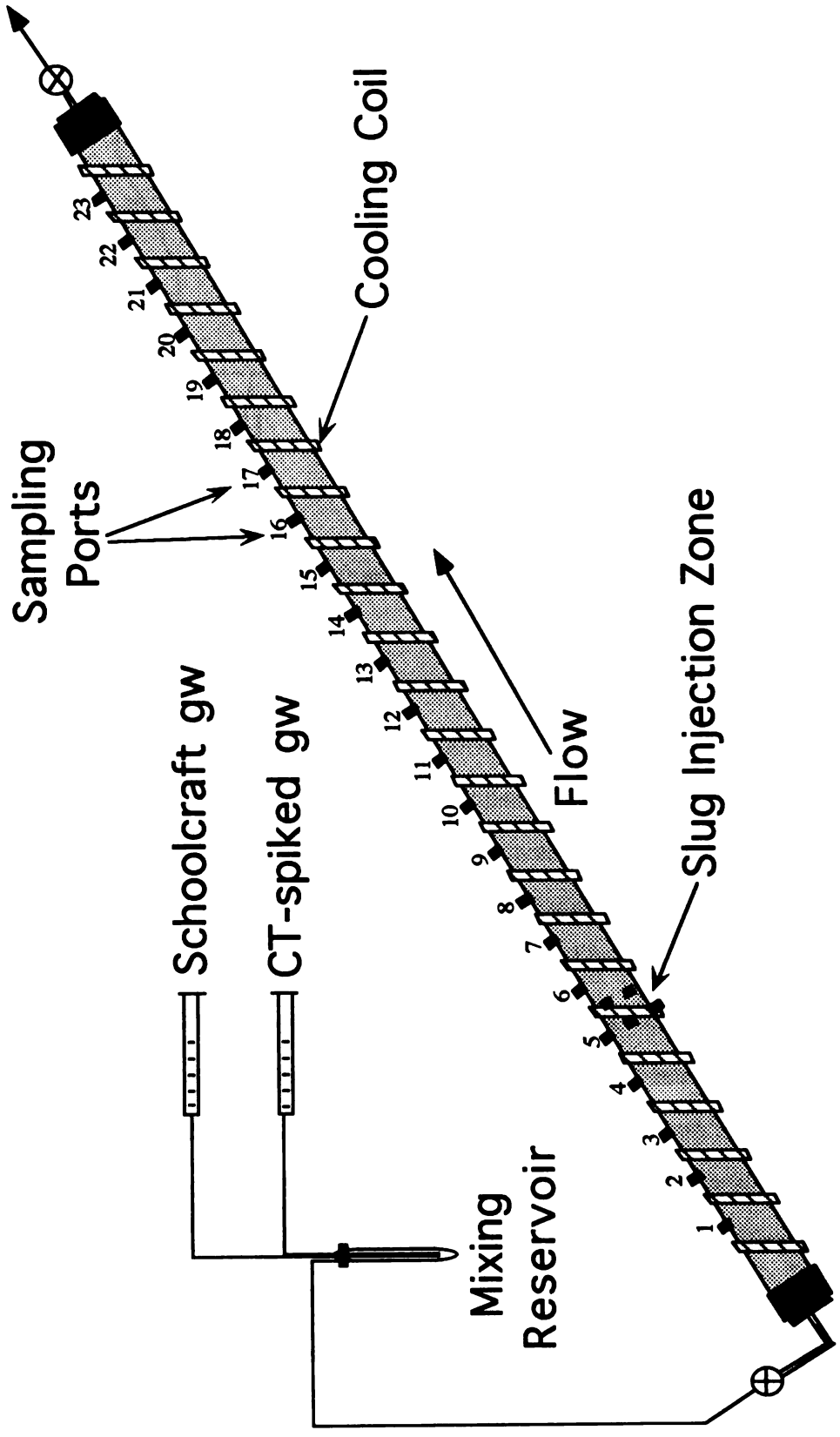



Figure 1. Diagram of model aquifer column.

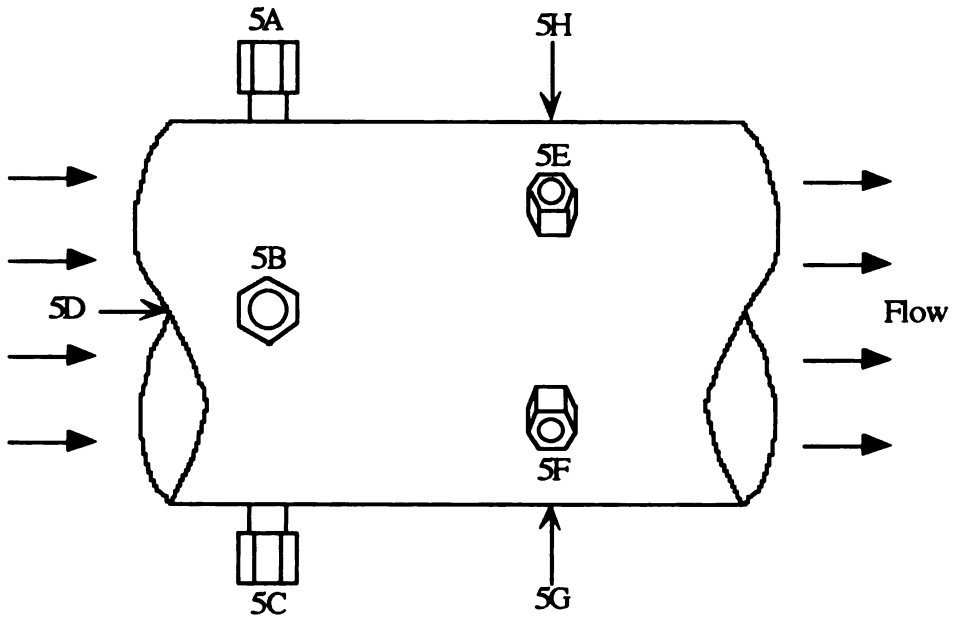



Figure 2. Port locations used for slug injection of KC cells and nutrients.

**Mixing Reservoir Experiment.** A proposal was made to pump Schoolcraft groundwater and CT-spiked Schoolcraft groundwater separately into a mixing reservoir, where then the CT-spiked groundwater would be pumped to the model aquifer column. This proposal was made in light of the fact that carbon tetrachloride is easily adsorbed by various types of soft plastic (polyethylene). The use of a mixing reservoir to deliver the CT-spiked Schoolcraft groundwater was required to avoid having to contain CT-spiked groundwater in plastic 140 mL syringes. The static mixing reservoir consisted of a 28 mL test tube filled three-quarters full with 0.3 cm diameter glass beads, capped using a Teflon™-lined rubber stopper, and secured with an aluminum crimp top. Calculations were made to predict the required flow rates of both the CT-free Schoolcraft groundwater and CT-spiked solution to yield an effluent concentration of 100 $\mu$g/L exiting the mixing reservoir flowing at 85 $\mu$L/min. Four plastic syringes filled with CT-free Schoolcraft groundwater were placed on a syringe pump (Harvard Apparatus 22), and water was pumped to the mixing reservoir at a total flow rate of 76.5 $\mu$L/min. One 100-mL glass

syringe (Unimetrics Corporation), used to contain the CT-spiked groundwater solution (1000 $\mu$g/L), was placed on a separate syringe pump, and its contents were pumped to the mixing reservoir at a rate of 8.5 $\mu$L/min. Two 6-inch veterinary needles were used to penetrate the upper stopper and deliver the groundwater and CT-spiked solutions to the bottom of the static mixing reservoir. The outlet point of the mixing reservoir was located near the top of the test tube just above the layer of glass beads (see Figure 3). It was assumed that as the groundwater and CT-spiked solutions were injected into the mixing reservoir, a dilution of the CT-spiked solution would occur due to the differing flow rates (CT-spiked solution flow rate only one-tenth the total flow rate out of the mixing reservoir). Mixing of the CT-spiked solution with the CT-free Schoolcraft groundwater was encouraged by the tortuous flow pattern of both solutions flowing around the glass beads.

**Convert Column to Denitrifying Conditions.** Inner contents of the model aquifer column were converted to denitrifying conditions by pumping CT-contaminated groundwater to the model aquifer column using two Harvard Apparatus 22™ syringe pumps. One pump contained Schoolcraft groundwater (deaired, nitrogen stripped and adjusted to pH 7.50 by bubbling in $CO_2$ and nitrogen gas) in four 140 mL plastic syringes, and the other pump contained a CT-spiked solution (1000 $\mu$g/L) in a 100 mL glass syringe. The contents of all syringes were pumped into a mixing reservoir (see Figure 3) where the effluent was delivered to the model aquifer column. Teflon™-lined tubing (1/16-inch outside diameter) connected the groundwater syringes to the mixing reservoir. Stainless steel tubing (1/16-inch outside diameter) connected the CT-spiked solution to the mixing reservoir and the mixing reservoir to the model aquifer column. Groundwater flowed through the column at a rate of 85 $\mu$L/min. Both syringe pumps were contained in an enclosed refrigeration system, which kept the contents of all syringes at a temperature of approximately 15°C. Denitrifying conditions were obtained by pumping oxygen-free

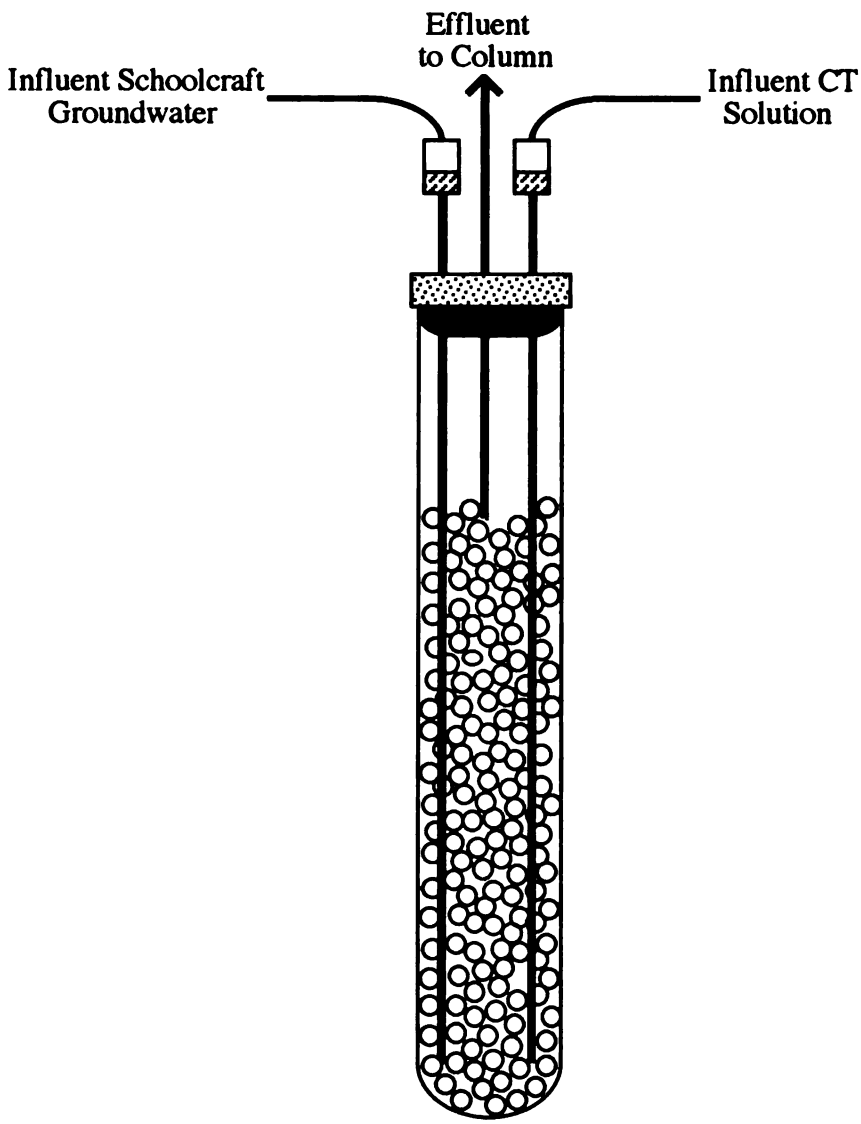



Figure 3. Mixing reservoir used to deliver CT-spiked Schoolcraft groundwater to model aquifer column.

Schoolcraft groundwater through the column for a period of four weeks. Random samples obtained from various ports along the column were analyzed for dissolved oxygen using a dissolved oxygen electrode (Orion Research model 97-08-00) and millivolt meter (Orion Research model 611).

**CT Saturation Experiment.** Saturation of the model aquifer column with CT was achieved by a continuous pumping of CT-spiked Schoolcraft groundwater at a concentration of approximately 80 $\mu$g/L through the model aquifer column. The total flow rate of groundwater moving through the column was 85 $\mu$L/min (average linear flow velocity of 14.8 cm/day). The duration of this experiment was 120 days.

**Conservative Tracer Studies.** Tracer studies were performed to evaluate flow characteristics within the model aquifer column, specifically effective porosity and dispersion. Tracers used for these analyses were bromide, fluoride, and tritiated water ($^3H_2O$). The syringe containing the CT-spiked solution was alternately spiked with the conservative tracers previously mentioned. Both bromide and fluoride concentrations were analyzed using the appropriate bromide/fluoride probes to measure bromide/fluoride concentrations in 1 mL samples obtained from the column. Tritiated water counts (counts per minute, or CPM) were obtained by placing 200 $\mu$L of sample into a liquid scintillation vial containing 10 mL scintillation cocktail and analyzing on the liquid scintillation counter.

**Slug Injection Experiment.** This experiment was performed to determine the optimum flow rate at which a solution containing microorganisms and nutrients should be injected to achieve a cylindrical slug with constant concentration. Achieving a cylindrical slug with constant concentration is desirable in an attempt to adequately model the movement of nutrients within the model aquifer column. Once the initial conditions are known, modeling can then be used to predict the migration of particular nutrient solutes

through the column. An experimental test cell comprised of an 11 cm section of two-inch PVC piping was wet packed with Ottawa sand and plugged at both ends using large rubber stoppers. Syringes containing deionized water dyed with blue dextran (Sigma Chemical Company #D-5751) were placed on an injection/extraction syringe pump (Harvard Apparatus 22). Four 2-inch needles were pushed through the stoppers on each end of the test cell. The full syringes were attached to the needles using Teflon™-lined tubing and appropriate connections. Four empty syringes were placed on another injection/extraction syringe pump and connected to the four needles at the other end of the test cell. Mixing was achieved by simultaneously injecting with one syringe pump and extracting with the other. Numerous flow rates were evaluated as to the degree of mixing achieved after fifteen minutes time. Samples withdrawn from various locations within the test cell were analyzed for optical density at 660 nm using a Shimadzu UV-160 spectrophotometer.

An additional column identical to the model aquifer column used in this study was constructed in the same fashion as the original column. Figure 2 profiles the injection port region for both model aquifer columns. Ports 5A through 5D are upgradient 1.5 inches and offset 45 degrees from ports 5E through 5H. Flow through the column was stopped by turning the groundwater feed pumps off and closing the valves at both ends of the column. Slug injection experiments were performed using tritiated water as the tracer. Mixing in the injection zone was evaluated by withdrawing 200 $\mu$L samples from all the ports in the injection zone and dispensing each into a scintillation vial containing 10 mL of scintillation cocktail. Each sample was analyzed for $^3H_2O$ by liquid scintillation spectroscopy.

**Inoculation.** Prior to inoculation with strain KC, the pH of samples obtained from ports 1 through 23 were determined by withdrawing 0.5 mL from each port, dispensing the sample into 1 mL plastic tubes, and measuring the pH using the Beckman pH meter and

Corning pH probe. A slug injection of pH-adjusted Schoolcraft groundwater containing sodium hydroxide and tritiated water was then performed. This was done to make certain that the pH in the vicinity of the injection zone was at or near 8.2. The injection scheme used for this slug addition was identical to the slug injection discussed previously in this chapter.

KC cells were obtained from a nutrient agar plate located within the laboratory. A KC culture was started by placing one drop of aerobic medium D onto the agar plate containing strain KC and withdrawing the liquid (containing KC cells) into a 1 mL disposable syringe containing 0.7 mL aerobic medium D. The contents of the syringe were then dispensed into a 28 mL sealed test tube containing an additional 4 mL medium D. The test tube was placed on the shaker and allowed to shake overnight. Approximately 2 mL of the medium D containing KC cells was transferred from the test tube to a larger Erlenmeyer flask containing 330 mL of aerobic medium D. This was again allowed to shake on the shaker overnight. One hundred and sixty mL of KC cells and medium D contained within the erlenmeyer flask were transferred to centrifuge tubes. The cells were spun on a centrifuge (Sorvall Superspeed RC2-B) at 10,000 rpm for a period of ten minutes. After the centrifuge tubes were removed from the centrifuge, the supernatant was removed and the cell pellets were resuspended in a solution of Schoolcraft groundwater containing CT (80 $\mu$g/L), phosphate (20 mg/L), acetate (120 mg/L), and bromide (40 mg/L). The cell mass in the inoculum was determined using the modified Lowry method to assay protein, with bovine serum albumin as the standard (Marxwell *et al.*, 1981). Thirty mL of this solution were then removed and divided equally amongst four 10-mL sterile syringes and placed on an infusion/extraction syringe pump. An additional four syringes were placed on another infusion/extraction syringe pump. All syringes were then connected to the column injection zone using 1/16-inch Teflon™-lined tubing, 1.5-inch 18 gauge needles, and appropriate connections. Flow was initiated at a rate of 5.0 mL/min for each syringe (20 mL/min total

for all four influent syringes). Once the initial injection syringes were nearly empty, the toggle switches (infusion/extraction) on both syringe pumps were switched. This procedure was repeated 25 times.

After the slug injection was performed and the injection system disassembled, 700 μL samples were obtained from ports 4, 5A, 5E, 6, and 7 using 1 mL disposable syringes and 1.5-inch 22 gauge needles. These samples were analyzed for acetate, bromide, nitrate, nitrite, and phosphate in the mixing zone. An additional 1 mL was taken from ports 2, 4, 5, 6, 9, 12, 15, 18, and 21 for use in gene probing and 300 μL were obtained from each port for use in identifying the most probable number (MPN) of KC cells at each location along the column.

**Nutrient Addition.** After the initial inoculation, KC cells were no longer included in the weekly addition of nutrients to the column. The 30 mL nutrient slug that was added each week contained acetate (120 mg/L), phosphate (20 mg/L), and an alternating conservative tracer (40 mg/L bromide or tritiated water). Seven-hundred μL samples were obtained from each port using 1 mL disposable syringes and 1.5-inch 22 gauge needles. Samples were analyzed for acetate, bromide, nitrate, nitrite, and phosphate via ion chromatography on Tuesday, Thursday, and Sunday of each week. Two-hundred μL samples obtained on Monday, Wednesday, and Friday of each week were first used to determine the extent of CT degradation via headspace analysis. After headspace analysis, each sample volume (200 μL) was dispensed into scintillation vials, along with 10 mL scintillation cocktail, and analyzed for $^{3}H_2O$ as previously described. Table 1 describes the daily sampling and analysis routine followed throughout the course of this experiment.

Table 1. Description of daily sampling and analyses routine for model aquifer column.

| Sunday | Monday | Tuesday | Wednesday | Thursday | Friday | Saturday |
|---|---|---|---|---|---|---|
| •Obtain 700 $\mu$L samples from all ports for ion analysis on IC.<br><br>•Refill syringes with Schoolcraft groundwater, if needed.<br><br>•Refill CT-spiked Schoolcraft groundwater syringe, if needed. | •Obtain 200 $\mu$L samples from all ports for CT analysis on GC.<br><br>•Dispense 200 $\mu$L samples into respective scintillation vials for LSC analysis.<br><br>•Refill syringes with Schoolcraft groundwater, if needed.<br><br>•Refill CT-spiked Schoolcraft groundwater syringe, if needed. | •Inject KC cells and/or nutrients into column ports 5A through 5H.<br><br>•Obtain 700 $\mu$L samples from ports 4 through 7 for ion and $^3H_2O$ analysis.<br><br>•Obtain 300 $\mu$L samples from all ports for MPN analysis.<br><br>•Obtain 1 mL samples from all ports for gene probe analysis.<br><br>•Refill syringes with Schoolcraft groundwater, if needed.<br><br>•Refill CT-spiked Schoolcraft groundwater syringe, if needed. | •Obtain 200 $\mu$L samples from all ports for CT analysis on GC.<br><br>•Dispense 200 $\mu$L samples into respective scintillation vials for LSC analysis.<br><br>•Refill syringes with Schoolcraft groundwater, if needed.<br><br>•Refill CT-spiked Schoolcraft groundwater syringe, if needed. | •Obtain 700 $\mu$L samples from all ports for ion analysis on IC.<br><br>•Refill syringes with Schoolcraft groundwater, if needed.<br><br>•Refill CT-spiked Schoolcraft groundwater syringe, if needed. | •Obtain 200 $\mu$L samples from all ports for CT analysis on GC.<br><br>•Dispense 200 $\mu$L samples into respective scintillation vials for LSC analysis.<br><br>•Refill syringes with Schoolcraft groundwater, if needed.<br><br>•Refill CT-spiked Schoolcraft groundwater syringe, if needed. | •Refill syringes with Schoolcraft groundwater, if needed.<br><br>•Refill CT-spiked Schoolcraft groundwater syringe, if needed. |

# CHAPTER 3

## CONSTRUCTION OF MODEL AQUIFER COLUMN

**PVC Adsorption Experiment.** Using a short length of PVC piping (two-inch diameter) and capping both ends with PVC endcaps, a solution of CT-spiked Schoolcraft groundwater was injected into the short capsule. 200 $\mu$L samples were obtained at various times and analyzed for CT via gas chromatography. Figure 4 shows that the CT concentration decrease over time was minor. Using this data, and considering that the residence time of water in the model aquifer column was just more than 12 days, only 3% of the CT in solution would adsorb onto the PVC sidewalls and/or endcaps. After draining the CT-solution from the enclosed PVC capsule, it was filled with CT-free deionized water. Samples obtained over a period of two weeks were analyzed for CT and no amounts were detected.

**Column Construction.** The final design of the model aquifer column was a result of upscaling and altering a similar column previously designed in our laboratory. After the PVC piping was obtained, the holes were tapped and threaded, both of the endcaps were glued on, and all of the brass unions (sampling ports) were screwed into the column. A static leak test was performed for a period of 48 hours. Leakage of water from a port was fixed by tightening the brass union to the column. Leakage was not observed from either of the endcaps. The column was then wet-packed using Ottawa sand. After packing, the column was again placed in a vertical position for a period of 48 hours to test for any

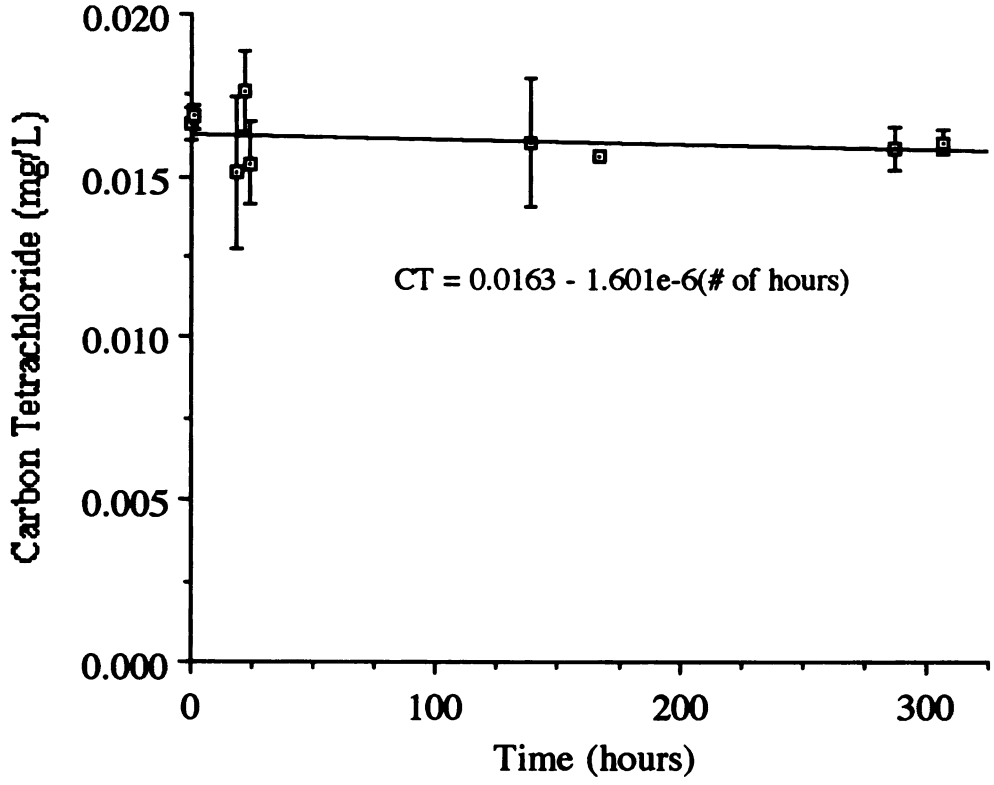



Figure 4. Concentration of CT versus time for PVC adsorption experiment.

leakage. Additional silicone sealant was placed around the endcaps, plus influent and effluent valves were attached to the column.

**Mixing Reservoir Experiment.** CT concentration of the effluent from the mixing reservoir was analyzed via gas chromatography. After the experiment was started, 5 mL samples were collected continuously for a period of four hours on each of two days. Volatilization of CT was avoided by collecting these samples in sealed 28 mL test tubes. Initial results indicated that CT concentration was highly variable from sample to sample. However, as time passed, it was evident that the effluent CT concentration was starting to

equilibrate. After 48 hours, the effluent CT concentration was fairly constant at 80 $\mu$g/L. The calculations performed prior to running this experiment indicated that the effluent CT concentration should have been closer to 100 $\mu$g/L based on the flow rates of the two influent solutions (CT-free Schoolcraft groundwater and CT-spiked Schoolcraft groundwater). Preferential flow paths for each solution and/or incomplete mixing may account for this phenomena.

**Convert Column to Denitrifying Conditions.** Denitrifying conditions were obtained by pumping oxygen-free Schoolcraft groundwater through the column for a period of four weeks. After this time period, random samples collected from various ports on the column contained no detectable amounts of dissolved oxygen (less than 0.1 mg/L).

# CHAPTER 4

## DETERMINATION OF FLOW PARAMETERS IN THE MODEL AQUIFER COLUMN

**CT Saturation Experiment.** Upon completion of the model aquifer column, CT-spiked groundwater was pumped into the column using separate Schoolcraft groundwater syringes and a CT-spiked groundwater syringe. The total flow rate of the effluent from the mixing reservoir was back-calculated after assuming an average linear flow velocity, V. The average linear flow velocity assumed for groundwater flow through the model aquifer column, 15 cm/day, was identical to the average linear flow velocity determined for groundwater flow in the Schoolcraft aquifer (Halliburton NUS Environmental Corporation, 1991). Allowing syringe pumps to pump both Schoolcraft groundwater and CT-spiked Schoolcraft groundwater into the mixing reservoir, the CT concentration of the effluent from the mixing reservoir was analyzed for a period of four days. After this time had elapsed, the concentration of CT in the effluent was maintained at approximately 80 $\mu$g/L (parts per billion, or ppb). The effluent from the mixing reservoir was then pumped to the model aquifer column. As shown in Figure 5, the time required for the CT concentration to become relatively constant (±10%) throughout the column was approximately 90 days.

Organic contaminants transported by groundwater are distributed between three phases: gas phase, solid phase, and solution phase. Henry's Law governs the extent to which an organic compound will exist in the vapor phase. For this column experiment, it was assumed that CT was distributed amongst the solid and solution phases. Extremely small

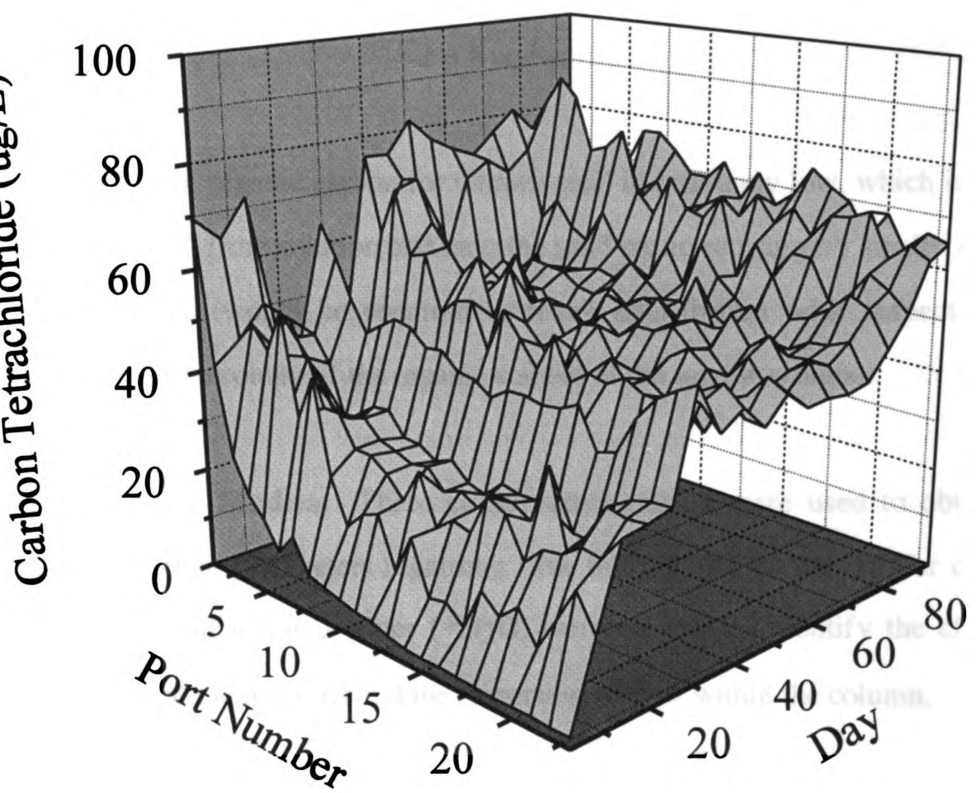



Figure 5. Concentration of CT versus time and port number for CT saturation experiment using model aquifer column.

air pockets may have existed in certain areas of the column, however the effects of such occurrences would be minor. Therefore, migration of CT through the Ottawa sand column was assumed to exist only in the solid and solution phases. Studies (Schwarzenbach *et al.*, 1981) have shown that the degree of adsorption is proportional to a distribution coefficient, $K_d$. The distribution coefficient is defined as the following:

$$K_d = K_{oc} f_{oc}$$

The weight fraction of organic carbon for Ottawa sand is extremely low, which indicates that partitioning of CT from solution and onto the sand particles was very small. A much longer period of time would be required to reach equilibrium with respect to CT concentration within the column if the organic content of the sand was higher.

**Conservative Tracer Studies.** Three conservative tracers were used to obtain the values of two important parameters regarding flow through this model aquifer column. Bromide, fluoride, and tritiated water ($^3H_2O$) were all used to identify the effective porosity of the packed Ottawa sand and the dispersion of flow within the column.

Bromide and fluoride were evaluated as tracers, and bromide/fluoride probes were used to detect conductance changes in 1 mL samples (and subsequently bromide/fluoride concentrations). Difficulty was experienced when using fluoride as the conservative tracer. Concentrations of fluoride were accurately identified using samples from the influent syringes and samples taken from the effluent of the mixing reservoir. However, samples taken from all of the ports within the model aquifer column contained no fluoride according to the results obtained using the fluoride probe. Using high influent fluoride concentrations (greater than 100 mg/L), samples taken from port 1 did not contain fluoride at concentrations over 1 mg/L. Apparently fluoride did not act as a conservative tracer in this

model aquifer column setup. Materials used to construct the column may absorb the fluoride contained in the influent Schoolcraft groundwater. Other problems existed when using a bromide probe to detect bromide in Schoolcraft groundwater samples obtained from the column. Consistent conductance readings were never achieved using this probe, possibly indicating a faulty bromide probe. The ease of using tritiated water as a conservative tracer within the model aquifer column led to confident results for parameter estimation. Samples obtained from the column were injected into liquid scintillation vials containing scintillation cocktail and assayed for $^3H_2O$ on the liquid scintillation analyzer.

The physical processes that control the movement of solutes (or tracer) through porous media are advection and hydrodynamic dispersion. Advection is the component of transport attributable to the flow of groundwater where solutes are transported by the bulk mass of the flowing water. Hydrodynamic dispersion occurs as a result of mechanical mixing and molecular diffusion. The coefficient of hydrodynamic dispersion can be expressed in the following manner:

$$D_l = \alpha_l V + D^*$$

Figure 6 shows the breakthrough of tritiated water in the model aquifer column. Using the method discussed by Freeze and Cherry (1979), the effective porosity of the Ottawa sand packed in this column was determined to be 0.39 (see Appendix B). The bulk of the tritiated water in the Schoolcraft groundwater was carried by the moving groundwater. However, the spreading of the breakthrough curve of tritiated water migrating through the model aquifer column was caused by both mechanical dispersion and molecular diffusion. If hydrodynamic dispersion was close to zero for the flow of tritiated water, the breakthrough curve ($C/C_O$) would show a sharp increase at breakthrough. For the

relatively high groundwater velocity used in this column, molecular diffusion is negligible, and mechanical mixing is the dominant dispersive process.

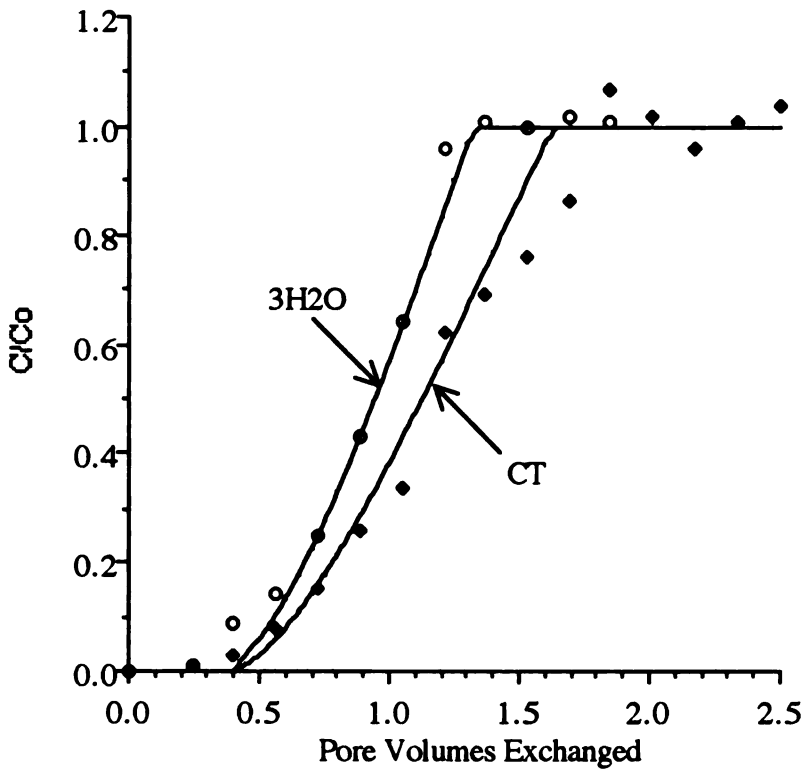



Figure 6. Breakthrough curves for CT-spiked Schoolcraft groundwater and $^3H_2O$.

The dispersion coefficient of tritiated water flowing through this column was calculated to be 217 $cm^2$/day (see Appendix B for calculations). This was calculated using the following equation, applicable for flow through saturated homogeneous porous medium (Ogata, 1970):

$$\frac{C}{C_o} = \frac{1}{2}\left[\operatorname{erfc}\left(\frac{1 - Vt}{2\sqrt{D_l t}}\right) + \exp\left(\frac{Vl}{D_l}\right)\operatorname{erfc}\left(\frac{1 + Vt}{2\sqrt{D_l t}}\right)\right]$$

Using this equation, a dispersion coefficient was also determined using the data obtained for the breakthrough of CT. Table 2 summarizes column specifications, flow parameters, and CT sorption characteristics for the model aquifer column. Longitudinal dispersivities were calculated using the hydrodynamic dispersion equation and assuming that $D^*$ was negligible. Dispersivity ($\alpha_l$), a function of the pore geometry, was calculated to be 14.7 cm for the flow of tritiated water (see Appendix B).

Table 2. Column specifications, flow parameters, and CT sorption characteristics for model aquifer column.

| PARAMETER | VALUE |
|---|---|
| **Column Specifications:** | |
| Length (cm) | 183 |
| Diameter (cm) | 5.2 |
| Empty Volume ($cm^3$) | 3890 |
| **Flow Parameters:** | |
| Porosity | 0.39 |
| Pore Volume ($cm^3$) | 1520 |
| Soil Bulk Density ($g/cm^3$) | 1.6 |
| Specific Discharge (cm/day) | 5.8 |
| Average Linear Velocity (cm/day) | 14.8 |
| Dispersion Coefficient ($cm^2$/day) | 217 |
| Dispersivity (cm) | 14.7 |
| **CT-Sorption Characteristics:** | |
| Retardation Coefficient | 1.2 |
| Distribution Coefficient ($cm^3/g$) | 0.05 |

**Slug Injection Experiment.** The optimum flow rate to achieve a cylindrical slug of nutrients with constant concentration was 20 mL/min. This was achieved by first injecting into ports numbered 5A, 5E, 5C, and 5G and simultaneously withdrawing from ports number 5B, 5F, 5D, and 5H. After the initial volumes were injected/extracted, the toggle switch on the back of each syringe pump was changed to switch each pump from injection to extraction or extraction to injection. This was very convenient because it prevented having to switch syringes and syringe pumps after each injection period. A total of 25

injections/extractions were performed for each slug injection. Assuming a 1:1 dilution, the initial concentration within the slug injection zone was one half of the initial concentration of the contents in the syringes before injecting.

The results of numerous slug injections indicate that 25 injections/extractions yield an adequately mixed slug in the slug injection zone. 200 $\mu$L samples taken from ports 4, 5A, 5C, 5E, 5H, and 6 were dispensed into scintillation vials and analyzed for $^3H_2O$. Both shallow and deep samples were obtained from ports 5A, 5C, 5E, and 5H. One sample was taken at a depth of 0.5 inches, and another sample was taken at a depth of 1.5 inches. The results show that adequate mixing occurred since the total CPM for each sample obtained from ports 5A through 5H was within 10% of half the total CPM for the sample containing the influent solution.

# CHAPTER 5

## INOCULATION AND BIOFENCE FORMATION

**Inoculation.** The pH of the groundwater that flowed through the model aquifer column was examined just prior to the slug injection of the pH-adjusted Schoolcraft groundwater slug (see Appendix C for pH data). The pH of the groundwater gradually increased from 7.5 at port 1 to 8.0 at port 5. From port 5 through port 23, the pH varied from 7.9 to 8.0. This gradual pH increase indicates that the Schoolcraft groundwater flowing through the column is not in equilibrium with the Ottawa sand in the column. Minerals from the sand are most likely adding alkalinity to the groundwater, thus causing an increase in pH along the column (see Appendix D for calculations).

The slug injection of pH 8.2 Schoolcraft groundwater (80 $\mu$g/L CT and spiked with $^3H_2O$) was completed to adjust the pH of injection zone where the inoculation using strain KC would be performed. This pH adjustment provides strain KC with a competitive advantage over the indigenous Schoolcraft flora (Knoll, 1994).

Inoculation of the model aquifer column was performed as described in Chapter 2. The concentrations of acetate, bromide, and phosphate in the injection volume were twice that of what was desired inside the column. This was because the initial injection volume would be diluted 1:1 with the contents of the pore water in the injection zone of the column. The initial concentration of acetate in the injection zone was approximately 55 mg/L, only 5 mg/L less than theoretically calculated. Results from a protein assay and serial

dilution/plate count analysis indicated that the initial cell density in the inoculum was approximately 2 x $10^8$ cells per mL.

Degradation of CT by strain KC was evaluated by analyzing the CT concentration in 200 $\mu$L samples taken from ports 1 through 23. Samples were obtained from the column on every Monday, Wednesday, and Friday starting the day after inoculation. Figure 7 shows a three-dimensional CT concentration profile for the model aquifer column following inoculation (day 0 is the day of inoculation).

Two days after inoculation, the concentration of CT in the slug injection region dropped approximately 40%. As time progressed (in the negative y-direction in Figure 7) the CT concentration in the slug injection zone continued to drop until it reached approximately 10 $\mu$g/L on day 29. This is evidence that only a small biofence thickness is needed to achieve a removal efficiency of over 90%. Most of the transformation of CT by strain KC was occurring in this region near port number 5. Realizing that samples withdrawn from this port were taken from the upgradient boundary of the slug injection region and considering that over 90% CT removal was occurring in this small region indicated that the biofence thickness required for 90% CT removal was approximately 5 cm.

Figure 7 shows that the CT concentration at port 12 was 20 $\mu$g/L on day 2. This was most likely a result of CT transformation in the inoculum and uneven distribution of flow during inoculation. As strain KC was added to the initial slug volume prior to injection, sufficient time existed between making up the solution and the actual injection time for transformation to occur which resulted in a low CT concentration in the inoculum. This was supported by tracer analysis, which showed that the initial inoculum slug had migrated to port 12 by the time this sampling had occurred.

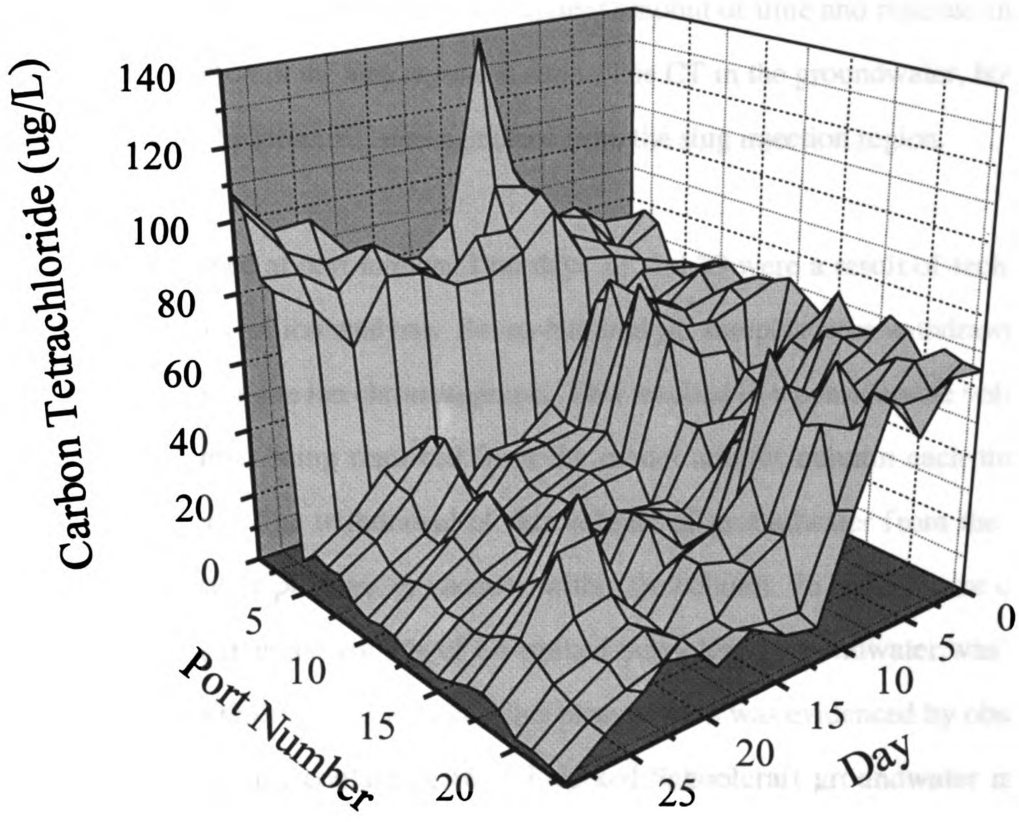



Figure 7. Carbon tetrachloride concentration profile for the model aquifer column following inoculation.

Further examination of the data presented in Figure 7 shows that multiple spikes of CT were detected on day 22. It is believed that these spikes are a result of micropore formation and channeling within the model aquifer column. Small volumes of groundwater containing elevated levels of CT most likely flowed through the biologically-active zone through channels. By flowing through these channels, the CT in the groundwater was not available to the strain KC organisms for a sufficient amount of time and resulted in a low degree of transformation in the slug injection zone. The CT in the groundwater, however, was further degraded by strain KC downgradient from the slug injection region.

The CT spikes observed at port number 1 on days 13 and 29 were a result of techniques used to obtain samples for ion analysis. Seven-hundred $\mu$L samples were withdrawn from each port for analysis on the ion chromatograph. This resulted in a considerable volume of groundwater (16.1 mL) being removed from the model aquifer column each time ion analysis is performed. The withdrawal of this volume of groundwater from the model aquifer created a negative pressure, or vacuum, within the column. In order for the column to relieve this stress, an equal volume of CT-spiked Schoolcraft groundwater was drawn into the static mixing reservoir by suction. This phenomenon was evidenced by observing the plunger on the syringe containing the CT-spiked Schoolcraft groundwater moving ahead of the drive plate on the syringe pump. The total volume at which the plunger had moved ahead of the drive plate was approximately 16 mL. A favorable result of this phenomenon is assurance that the model aquifer column was air-tight, as air was unable to be withdrawn into the column to relieve the stress incurred by sampling techniques.

**Nutrient Addition.** Nutrient slug addition was performed exactly one week after the inoculation of the column. Subsequent nutrient additions occurred at this same interval. The method of injecting the nutrient slugs was identical to the method used for inoculation, except strain KC was not added. Analysis of samples obtained from ports 1 through 23 on

every Monday, Wednesday, and Friday showed that CT concentrations continued to decrease through day 29. Figure 7 shows a three-dimensional CT concentration profile for the column from day 0 through day 29. By day 29, effluent CT concentration decreased to approximately 1 $\mu$g/L, a CT removal efficiency of over 98%.

From a kinetics of transformation point of view, it is important to examine the mass of CT transformed for each unit mass of acetate consumed. The importance of this relates to the feeding frequency and concentration of acetate required to achieve a certain removal efficiency of CT. The average influent CT concentration for the first week of this experiment was 78 $\mu$g/L, and the average effluent CT concentration was approximately 59 $\mu$g/L. Since the flow rate of groundwater through the column was 85 $\mu$L/min, a total of 67 $\mu$g of CT entered the column and a total of 51 $\mu$g of CT exited the column. Therefore, 16 $\mu$g of CT were removed from the groundwater due to the transformation capabilities of strain KC. The mass of acetate injected into the column during inoculation was approximately 1.6 mg. Effluent acetate concentrations were low and very close to be zero for the duration of this experiment (see Figure 8). Relating the mass of CT transformed to the mass of acetate consumed yields 10 $\mu$g of CT transformed per mg of acetate consumed. Similar calculations were performed for the duration of this experiment and the results are shown in Table 3 (see Appendix E for calculations). Increasing amounts of CT were transformed per mass of acetate consumed as time increased. This was evidence that the biofence was forming and gradually moving towards a steady-state condition.

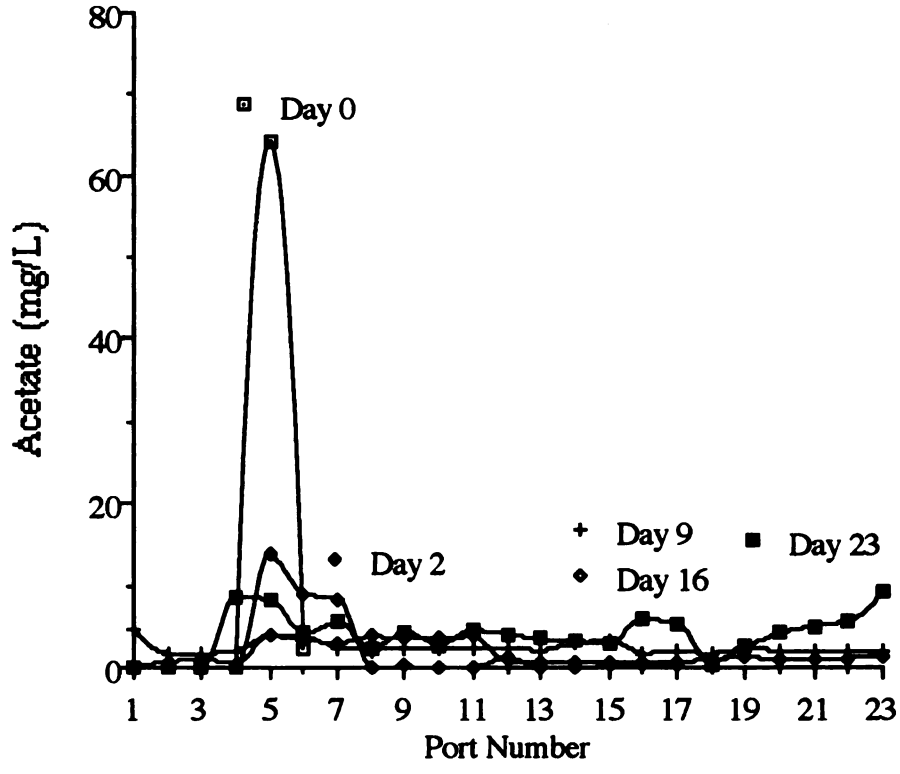



Figure 8. Acetate concentration profile in model aquifer column following inoculation by strain KC.

Table 3. Determination of the number of $\mu$g of CT transformed per mg of acetate consumed after inoculation with strain KC.

| Week | Mass CT Input ($\mu$g) | Mass CT Output ($\mu$g) | Mass Acetate Input (mg) | Mass Acetate Output (mg) | Mass CT Transformed per Mass Acetate Consumed ($\mu$g/mg) |
|---|---|---|---|---|---|
| 1 | 67 | 51 | 1.6 | ~0 | 10 |
| 2 | 90 | 33 | 1.8 | ~0 | 32 |
| 3 | 70 | 13 | 1.8 | ~0 | 32 |
| 4 | 79 | 14 | 1.8 | ~0 | 36 |
| 5 | 95 | 2 | 1.6 | ~0 | 58 |

Examination of nitrate consumption data (see Appendix G) shows that all the nitrate in the Schoolcraft groundwater was being consumed by strain KC by day 23 (see Figure 9). This is evidence that strain KC was clearly nitrate-limited as it transformed CT in the model aquifer column. Furthermore, no nitrite production was detected.

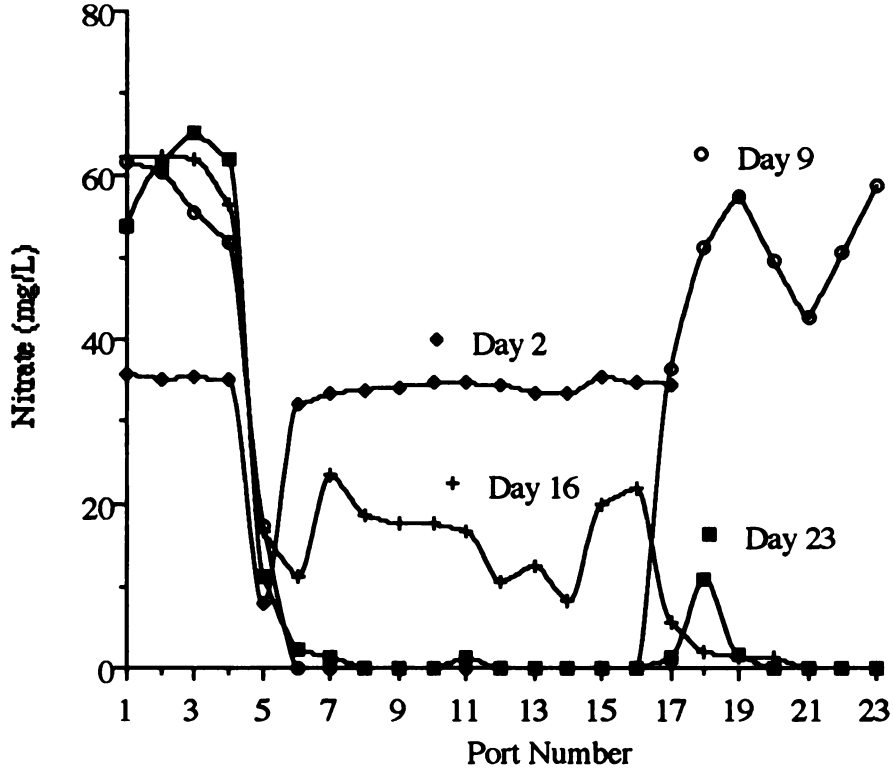



Figure 9. Nitrate concentration profile in model aquifer column following inoculation by strain KC.

# CHAPTER 6

## ENGINEERING APPLICATION

A variety of methods can be used to contain CT-contaminated groundwater and prevent off-site migration. One of the more widely used methods involves hydraulic containment coupled with product recovery, i.e., pump and treat systems. Migration of contaminants is controlled by intercepting groundwater using one or more recovery wells. Since this process relies on pumping groundwater to achieve hydraulic containment, it requires that considerable volumes of groundwater be treated on the surface. This method of pumping and treating the contaminated groundwater ex-situ is both expensive and labor-intensive. Physical barriers, such as slurry walls, have also been used to contain subsurface contamination. However, to maximize efficiency, pumping wells must also be installed inside the barrier wall to insure the direction of groundwater flow is into, rather than out of, the region. This process also requires the ex-situ treatment of large quantities of water.

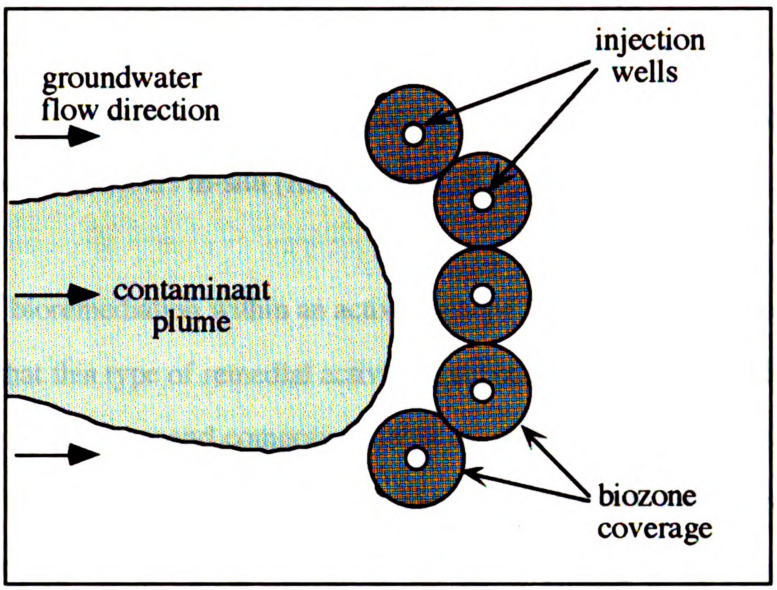
In-situ bioremediation is an alternative approach to these more traditional methods. The major advantage of employing such a method to control off-site migration is that bioremediation attenuates the contaminant in-situ. The physical removal of contaminated soils and pollutants is eliminated. Because this is a passive system, there is a reduced pumping requirement. In addition, in-situ bioremediation doesn't generate waste solids for disposal. Use of strain KC to transform CT in groundwater is potentially useful because of these attractive advantages that in-situ bioremediation offers.

The formation of a biologically active zone, or biofence, may be accomplished in many ways. Microorganisms capable of degrading target contaminants can be delivered into the subsurface by suspending the cells in an aqueous medium and pumping the cells into the contaminated aquifer via injection wells. If suitable conditions exist for the microorganisms, colonization and subsequent degradation of contaminants will occur.

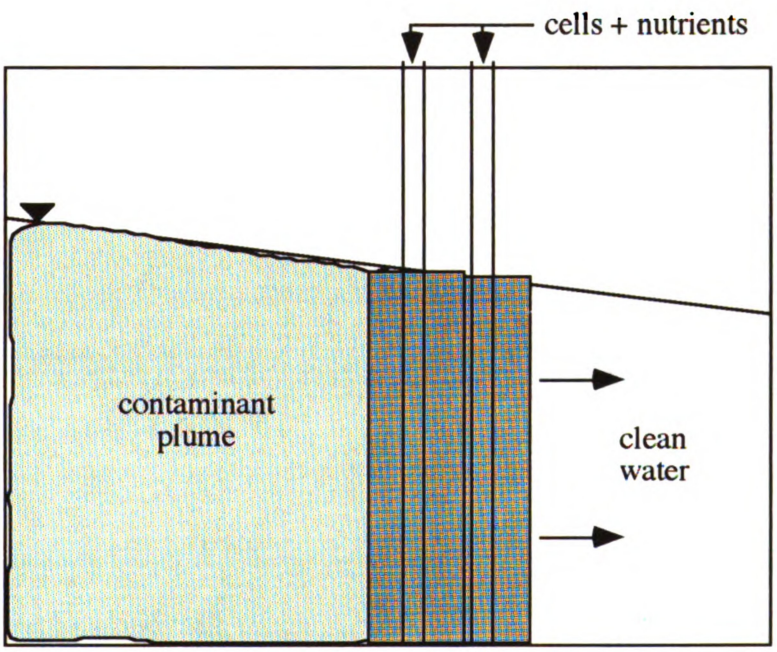
In cases of extremely low hydraulic conductivity (e.g. clayey soils), hydraulic fracturing may be required to provide an area of coverage large enough to intercept the flowing contaminated groundwater. This method is achieved by pumping cells and nutrients into the subsurface at an extremely high flow rate and pressure. The high pressure actually "fractures" the porous media and provides flow paths for the cells and nutrients. Conversely, in situations where the groundwater velocities are high, the cells and nutrients must be injected at high flow rates to insure adequate coverage between each injection well.

Figure 10 illustrates this concept of biofence formation using injection wells to deliver cells and nutrients to the subsurface. The funnel and gate system proposed by Starr and Cherry (1994) is another method which utilizes the capability of microorganisms to degrade groundwater contaminants. This method proposes the use of low hydraulic conductivity cutoff walls to direct contaminated groundwater through biologically active zones that degrade the target contaminants. These low hydraulic conductivity cutoff walls may consist of frozen ground barriers, sheet piling, or slurry walls.

The ability of naturally occurring microorganisms to degrade organic chemicals is termed natural biological attenuation. Attenuation of the groundwater contaminants downgradient of the source is often due to the natural bioremediation processes occurring within the aquifer. Once favorable conditions (e.g. presence of suitable electron acceptor and




Plan




Profile

Figure 10. Plan and profile views illustrating formation of biofence using injection wells for delivery of cells and nutrients.

nutrients) are available to the microorganisms, attenuation of the contaminants will occur naturally at a rate that far exceeds that of a system where only adsorption occurs, along with the added benefit that contaminants are actually destroyed instead of simply retarded. Several studies have demonstrated that natural biological attenuation of groundwater contaminants actually occurs in-situ (Klecka *et al.*, 1990; and Barker *et al.*, 1987).

The process of bioremediation within an active biozone has many applications. It has been demonstrated that this type of remedial activity is successful in treating spills consisting of spent halogenated solvents and compounds from the manufacture of chlorinated aliphatic hydrocarbons; wastes from the use and manufacture of chlorinated phenols, benzenes and their derivatives; spent non-halogenated solvents; metal plating and cleaning wastes; and petrochemical products and wastes (Bourquin, 1989).

# CHAPTER 7

## CONCLUSIONS

1. Degradation of CT by strain KC can be closely monitored using a prototype model aquifer column packed with Ottawa sand.

2. An optimum nutrient pulsing rate of 20 mL/min was determined to yield a slug of nutrients with isoconcentrations in the column.

3. Addition of nutrients (acetate and phosphate) to the column on a weekly basis provides KC cells with sufficient nutrients to reduce the effluent CT concentration up to 98%.

4. Biofence thickness required to achieve a CT removal efficiency of greater than 90% was on the order of 5 cm.

5. Approximately 58 $\mu$g of CT can be transformed for every mg of acetate consumed by strain KC.

## FUTURE WORK RECOMMENDATIONS

1. Construct model aquifer column packed with Schoolcraft aquifer material and perform identical experiments to examine CT transformation.

2. Establish the minimum acetate feeding frequency and concentration required to sustain the required level of CT transformation.

3. Investigate different pulsing strategies for maintenance of the biofence.

4. Construct model aquifer column packed with Schoolcraft aquifer material to evaluate competition between strain KC and indigenous Schoolcraft flora.

5. Construct a model to solve the set of equations relating CT transformation, acetate consumption, and cell growth.

6. Incorporate appropriate phenomenological rate laws to describe detachment and deposition of strain KC.

# APPENDIX A

## APPENDIX A

## NUMERICAL MODEL EQUATIONS

The bioaugmentation system proposed in this thesis involves the transport of four solutes (acetate, CT, nitrate, and nitrite) in addition to strain KC. All of these transported agents are kinetically interacting and will be dispersed by groundwater movement. Successful bioaugmentation and development of a CT-transforming "biofence" will require detailed modeling of the interaction between CT, strain KC, the other solutes, and their transport by groundwater. Once a model is established and verified by the bench-scale column studies, it can be used to optimize spatial and temporal placement of the constituents and maximize the activity of strain KC in a field experiment.

A code is being developed to solve a four-equation model which predicts the movement of CT, KC, and acetate in Schoolcraft groundwater.

$$\frac{\partial C_{ct}}{\partial t} = \frac{D_x}{R_{ct}}\frac{\partial^2 C_{ct}}{\partial x^2} - \frac{V_x}{R_{ct}}\frac{\partial C_{ct}}{\partial x} - \frac{q_{ct}}{R_{ct}}\left(X_{kc} + \bar{X}_{kc}\right)$$

$$\frac{\partial X_{kc}}{\partial t} = D_x\frac{\partial^2 X_{kc}}{\partial x^2} - V_x\frac{\partial X_{kc}}{\partial x} + Y_{kc}(q_a)_{kc}X_{kc} - b_{kc}X_{kc} - K_cX_{kc} + K_y\bar{X}_{kc}$$

$$\frac{\partial C_a}{\partial t} = \frac{D_x}{R_a}\frac{\partial^2 C_a}{\partial x^2} - \frac{V_x}{R_a}\frac{\partial C_a}{\partial x} - \frac{(q_a)_{kc}}{R_a}\left(X_{kc} + \bar{X}_{kc}\right)$$

$$\frac{\partial \bar{X}_{kc}}{\partial t} = Y_{kc}(q_a)_{kc}\bar{X}_{kc} - b_{kc}\bar{X}_{kc} - K_c X_{kc} + K_y \bar{X}_{kc}$$

When solved, this code will assist in the evaluation of chemical delivery strategies and will optimize spatial and temporal placement of wells required for development of a CT-transforming region. Both solid and aqueous phase organisms and CT will be considered. The model will be calibrated using independently obtained kinetic parameters, and its predictive capabilities will be evaluated in laboratory experiments with bench-scale model aquifers.

The processes simulated in this model include growth, decay, advection, dispersion, deposition, and detachment of strain KC; growth and decay of indigenous organisms; advection, dispersion, and consumption of the electron donor (acetate) and electron acceptor (nitrate); and advection, dispersion, retardation, and transformation of CT. Although one-dimensional assumptions will be used in the model, the results will provide useful understanding of the interrelated processes in the CT-transforming zone. This understanding will be used to assist in the design and operation of a full-scale remediation field experiment.

# APPENDIX B

## APPENDIX B

## ORIGINAL DATA AND CALCULATIONS

## FOR FLOW PARAMETERS IN MODEL AQUIFER COLUMN

Table B-1. Original data used for determination of porosity in model aquifer column.

| Time (hours) | $C/C_o$ |
|---|---|
| 0 | 0 |
| 72 | 0.01 |
| 120 | 0.09 |
| 168 | 0.14 |
| 216 | 0.25 |
| 264 | 0.43 |
| 312 | 0.64 |

$C_O$ = 11,000 CPM for tritiated water
Interpolation yields $C/C_O$ = 0.50 at 280 hours for samples taken from port 23
Length from influent to port 23 = 175 cm
Cross-sectional area of column = $\pi/4(5.2\ cm)^2 = 21.2\ cm^2$
Total volume = 175 cm x 21.2 $cm^2$ = 3710 $cm^3$
Volume input at 280 hours = 0.085 $cm^3$/min x 60 min/hr x 280 hours = 1430 $cm^3$
Porosity = 1430 $cm^3$/3710 $cm^3$ = **0.39**

Table B-2. Original data used for determination of dispersion coefficient in model aquifer column.

| Time (hours) | $C/C_o$ |
|---|---|
| 0 | 0 |
| 72 | 0.01 |
| 120 | 0.09 |
| 168 | 0.14 |
| 216 | 0.25 |
| 264 | 0.43 |
| 312 | 0.64 |
| 360 | 0.96 |
| 408 | 1.01 |
| 456 | 1.00 |
| 504 | 1.02 |
| 552 | 1.01 |

$C_O$ = 11,000 CPM for tritiated water
Length from influent to port 23 = 175 cm
V = 14.8 cm/day

$$\frac{C}{C_o} = \frac{1}{2}\left[\operatorname{erfc}\left(\frac{l - Vt}{2\sqrt{D_l t}}\right) + \exp\left(\frac{Vl}{D_l}\right)\operatorname{erfc}\left(\frac{l + Vt}{2\sqrt{D_l t}}\right)\right]$$

where l, V, t, and $D_l$ have been previously defined
For this high flow rate, the exponential part of the right side of the equation goes to zero.
For $C/C_O$ = 0.43, t = 264 hours or 11 days

$$0.43 = \frac{1}{2}\left[\operatorname{erfc}\left(\frac{175 - 14.8(11)}{2\sqrt{D_l(11)}}\right)\right]$$

Solving for $D_l$: $D_l$ = **217 cm$^2$/day**

When $D^*$ is negligible, $\alpha_l = D_l/V$ = (217 cm$^2$/day)/14.8 cm/day = **14.7 cm**

Table B-3. Original data used for determination of average linear velocity, distribution coefficient, and retardation coefficient for CT flow in model aquifer column.

| **Time (hours)** | **$C/C_o$** |
|---|---|
| 0 | 0 |
| 72 | 0.01 |
| 120 | 0.03 |
| 168 | 0.08 |
| 216 | 0.15 |
| 264 | 0.26 |
| 312 | 0.34 |
| 360 | 0.62 |
| 408 | 0.69 |
| 456 | 0.76 |
| 504 | 0.86 |
| 552 | 1.07 |

$C_o = 80\ \mu g$ CT/L
By interpolation, $C/C_o = 0.50$ at 339 hours or 14.1 days
$V_{ct}$ = 175 cm/14.1 days = **12.4 cm/day**
$R = V_{gw}/V_{ct}$ = (14.8 cm/day)/(12.4 cm/day) = **1.20**
n = 0.39
$\rho_s = 2.65\ g/cm^3$ (from Freeze and Cherry, 1979)
$\rho_b = (1-n)\rho_s = (1-0.39)(2.65\ g/cm^3) = 1.62\ g/cm^3$

$$R = 1 + \frac{\rho_b K_d}{n}$$

Solving for $K_d$: $K_d$ = **0.05 $cm^3/g$**

# APPENDIX C

# APPENDIX C

## ORIGINAL DATA FROM pH MEASUREMENTS

Table C-1. Original data used from pH measurements in model aquifer column on day 0.

| Port Number | pH |
|---|---|
| 1 | 7.55 |
| 2 | 7.69 |
| 3 | 7.68 |
| 4 | 7.86 |
| 5 | 7.99 |
| 6 | 7.91 |
| 7 | 7.87 |
| 8 | 7.88 |
| 9 | 7.89 |
| 10 | 7.89 |
| 11 | 7.89 |
| 12 | 7.81 |
| 13 | 7.90 |
| 14 | 7.90 |
| 15 | 7.84 |
| 16 | 7.83 |
| 17 | 7.87 |
| 18 | 7.87 |
| 19 | 7.96 |
| 20 | 7.97 |
| 21 | 8.00 |
| 22 | 8.00 |
| 23 | 7.96 |

# APPENDIX D

## APPENDIX D

## DETERMINATION OF INFLUENT AND EFFLUENT ALKALINITY

Table D-1. Original data used for determination of influent alkalinity (mg/L as $CaCO_3$) in model aquifer column.

Determination of alkalinity using titration method (Greenberg *et al.*, 1992).

| mL Acid | pH |
|---|---|
| 0 | 7.58 |
| 2.0 | 7.43 |
| 3.0 | 7.25 |
| 4.0 | 7.05 |
| 4.5 | 6.95 |
| 5.0 | 6.85 |
| 6.0 | 6.67 |
| 7.0 | 6.40 |
| 9.0 | 5.86 |
| 10.2 | 4.50 |

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times N \times 50{,}000}{\text{mL sample}}$$

where A = mL standard acid used ($H_2SO_4$)
N = normality of standard acid (0.02 N)

$$\text{Alkalinity} = \frac{10.2 \text{ mL} \times 0.02 \text{ N} \times 50{,}000}{50 \text{ mL sample}} = \mathbf{204 \text{ mg/L as } CaCO_3}$$

Table D-2. Original data used for determination of effluent alkalinity (mg/L as $CaCO_3$) in model aquifer column.

Determination of alkalinity using titration method (Greenberg *et al.*, 1992).

| mL Acid | pH |
|---|---|
| 0 | 8.15 |
| 1.0 | 7.54 |
| 3.0 | 7.07 |
| 4.0 | 6.92 |
| 5.0 | 6.79 |
| 6.0 | 6.67 |
| 7.0 | 6.59 |
| 8.0 | 6.35 |
| 9.0 | 6.15 |
| 10.0 | 5.86 |
| 11.0 | 5.36 |
| 11.5 | 4.50 |

$$\text{Alkalinity, mg } CaCO_3/L = \frac{A \times N \times 50{,}000}{\text{mL sample}}$$

where A = mL standard acid used ($H_2SO_4$)
N = normality of standard acid (0.02 N)

$$\text{Alkalinity} = \frac{11.5 \text{ mL} \times 0.02 \text{ N} \times 50{,}000}{50 \text{ mL sample}} = \mathbf{230 \text{ mg/L as } CaCO_3}$$

# APPENDIX E

# APPENDIX E

## ORIGINAL DATA AND CALCULATIONS

## FOR CT TRANSFORMATION AND ACETATE CONSUMPTION

Table E-1. Original data used for determination of $\mu g$ of CT transformed in model aquifer column.

**Experiment started on 10/4/94**

| **Day 2 - 10/6/94** | | method 2 | |
|---|---|---|---|
| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
| 1 | 0.2 | 447.07 | 74.62 |
| 2 | 0.2 | 404.89 | 67.72 |
| 3 | 0.2 | 415.05 | 69.38 |
| 4 | 0.2 | 401.03 | 67.09 |
| 5 | 0.2 | 243.83 | 41.36 |
| 6 | 0.2 | 403.92 | 67.56 |
| 7 | 0.2 | 393.61 | 65.87 |
| 8 | 0.2 | 421.34 | 70.41 |
| 9 | 0.2 | 385.77 | 64.59 |
| 10 | 0.2 | 410.36 | 68.61 |
| 11 | 0.2 | 393.63 | 65.87 |
| 12 | 0.2 | 118.23 | 20.80 |
| 13 | 0.2 | 304.12 | 51.22 |
| 14 | 0.2 | 353.34 | 59.28 |
| 15 | 0.2 | 343.72 | 57.71 |
| 16 | 0.2 | 394.50 | 66.02 |
| 17 | 0.2 | 393.21 | 65.81 |
| 18 | 0.2 | 383.57 | 64.23 |
| 19 | 0.2 | 366.61 | 61.45 |
| 20 | 0.2 | 392.57 | 65.70 |
| 21 | 0.2 | 398.33 | 66.64 |
| 22 | 0.2 | 354.33 | 59.44 |
| 23 | 0.2 | 394.15 | 65.96 |

| **Day 5 - 10/9/94** | | method 2 | |
|---|---|---|---|
| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
| 1 | 0.2 | 702.03 | 81.46 |
| 2 | 0.2 | 687.91 | 79.83 |
| 3 | 0.2 | 632.23 | 73.41 |

| | | | |
|---|---|---|---|
| 4 | 0.2 | 609.18 | 70.75 |
| 5 | 0.2 | 284.48 | 33.29 |
| 6 | 0.2 | 595.81 | 69.21 |
| 7 | 0.2 | 531.13 | 61.74 |
| 8 | 0.2 | 540.55 | 62.83 |
| 9 | 0.2 | 533.65 | 62.04 |
| 10 | 0.2 | 483.53 | 56.25 |
| 11 | 0.2 | 537.52 | 62.48 |
| 12 | 0.2 | 474.15 | 55.17 |
| 13 | 0.2 | 439.86 | 51.22 |
| 14 | 0.2 | 418.15 | 48.71 |
| 15 | 0.2 | 359.04 | 41.89 |
| 16 | 0.2 | 365.72 | 42.66 |
| 17 | 0.2 | 420.64 | 49.00 |
| 18 | 0.2 | 515.06 | 59.89 |
| 19 | 0.2 | 488.65 | 56.84 |
| 20 | 0.2 | 480.62 | 55.92 |
| 21 | 0.2 | 479.89 | 55.83 |
| 22 | 0.2 | 511.49 | 59.48 |
| 23 | 0.2 | 450.55 | 52.45 |

**Day 8 - 10/12/94** method 2

| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
|---|---|---|---|
| 1 | 0.2 | 714.26 | 78.92 |
| 2 | 0.2 | 746.46 | 82.51 |
| 3 | 0.2 | 706.42 | 78.05 |
| 4 | 0.2 | 654.41 | 72.26 |
| 5 | 0.2 | 219.44 | 23.85 |
| 6 | 0.2 | 554.09 | 61.09 |
| 7 | 0.2 | 594.85 | 65.63 |
| 8 | 0.2 | 608.39 | 67.14 |
| 9 | 0.2 | 613.22 | 67.68 |
| 10 | 0.2 | 513.33 | 56.56 |
| 11 | 0.2 | 576.01 | 63.53 |
| 12 | 0.2 | 513.35 | 56.56 |
| 13 | 0.2 | 320.33 | 35.08 |
| 14 | 0.2 | 379.83 | 41.70 |
| 15 | 0.2 | 403.87 | 44.37 |
| 16 | 0.2 | 397.88 | 43.71 |
| 17 | 0.2 | 435.74 | 47.92 |
| 18 | 0.2 | 475.02 | 52.29 |
| 19 | 0.2 | 532.80 | 58.72 |
| 20 | 0.2 | 641.67 | 70.84 |
| 21 | 0.2 | 488.49 | 53.79 |
| 22 | 0.2 | 599.47 | 66.14 |
| 23 | 0.2 | 587.44 | 64.81 |

**Day 10 - 10/14/94** method 1

| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1 | 0.2 | 1696.00 | 97.19 |
| 2 | 0.2 | 1634.00 | 93.72 |
| 3 | 0.2 | 1633.00 | 93.66 |
| 4 | 0.2 | 1559.00 | 89.52 |
| 5 | 0.2 | 433.00 | 26.48 |
| 6 | 0.2 | 910.00 | 53.18 |
| 7 | 0.2 | 1151.00 | 66.68 |
| 8 | 0.2 | 1298.00 | 74.91 |
| 9 | 0.2 | 1074.00 | 62.37 |
| 10 | 0.2 | 1338.00 | 77.15 |
| 11 | 0.2 | 1225.00 | 70.82 |
| 12 | 0.2 | 1117.00 | 64.77 |
| 13 | 0.2 | 716.00 | 42.32 |
| 14 | 0.2 | 723.00 | 42.71 |
| 15 | 0.2 | 534.00 | 32.13 |
| 16 | 0.2 | 707.00 | 41.82 |
| 17 | 0.2 | 909.00 | 53.13 |
| 18 | 0.2 | 1267.00 | 73.17 |
| 19 | 0.2 | 1124.00 | 65.16 |
| 20 | 0.2 | 960.00 | 55.98 |
| 21 | 0.2 | 1005.00 | 58.50 |
| 22 | 0.2 | 619.00 | 36.89 |
| 23 | 0.2 | 661.00 | 39.24 |

**Day 13 - 10/17/94** method 2

| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
|---|---|---|---|
| 1 | 0.2 | 1246.00 | 138.10 |
| 2 | 0.2 | 867.00 | 95.92 |
| 3 | 0.2 | 761.00 | 84.12 |
| 4 | 0.2 | 718.00 | 79.34 |
| 5 | 0.2 | 165.00 | 17.79 |
| 6 | 0.2 | 325.00 | 35.60 |
| 7 | 0.2 | 299.00 | 32.70 |
| 8 | 0.2 | 296.00 | 32.37 |
| 9 | 0.2 | 310.00 | 33.93 |
| 10 | 0.2 | 325.00 | 35.60 |
| 11 | 0.2 | 265.00 | 28.92 |
| 12 | 0.2 | 267.00 | 29.14 |
| 13 | 0.2 | 184.00 | 19.90 |
| 14 | 0.2 | 220.00 | 23.91 |
| 15 | 0.2 | 214.00 | 23.24 |
| 16 | 0.2 | 197.00 | 21.35 |
| 17 | 0.2 | 210.00 | 22.80 |
| 18 | 0.2 | 253.00 | 27.58 |
| 19 | 0.2 | 391.00 | 42.94 |
| 20 | 0.2 | 273.00 | 29.81 |
| 21 | 0.2 | 284.00 | 31.03 |
| 22 | 0.2 | 112.00 | 11.89 |
| 23 | 0.2 | 95.00 | 10.00 |

**Day 15 - 10/19/94** method 1

| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
|---|---|---|---|
| 1 | 0.2 | 1597.00 | 85.87 |
| 2 | 0.2 | 1104.00 | 59.77 |
| 3 | 0.2 | 1575.00 | 84.70 |
| 4 | 0.2 | 1378.00 | 74.28 |
| 5 | 0.2 | 300.00 | 17.22 |
| 6 | 0.2 | 493.00 | 27.44 |
| 7 | 0.2 | 547.00 | 30.29 |
| 8 | 0.2 | 559.00 | 30.93 |
| 9 | 0.2 | 497.00 | 27.65 |
| 10 | 0.2 | 500.00 | 27.81 |
| 11 | 0.2 | 524.00 | 29.08 |
| 12 | 0.2 | 506.00 | 28.12 |
| 13 | 0.2 | 360.00 | 20.40 |
| 14 | 0.2 | 448.00 | 25.05 |
| 15 | 0.2 | 496.00 | 27.59 |
| 16 | 0.2 | 489.00 | 27.22 |
| 17 | 0.2 | 373.00 | 21.08 |
| 18 | 0.2 | 472.00 | 26.32 |
| 19 | 0.2 | 577.00 | 31.88 |
| 20 | 0.2 | 448.00 | 25.05 |
| 21 | 0.2 | 460.00 | 25.69 |
| 22 | 0.2 | 223.00 | 13.15 |
| 23 | 0.2 | 208.00 | 12.35 |

**Day 17 - 10/21/94** method 1

| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
|---|---|---|---|
| 1 | 0.2 | 1643.11 | 77.69 |
| 2 | 0.2 | 1606.84 | 75.95 |
| 3 | 0.2 | 1549.04 | 73.19 |
| 4 | 0.2 | 1575.94 | 74.47 |
| 5 | 0.2 | 89.85 | 15.81 |
| 6 | 0.2 | 252.62 | 27.25 |
| 7 | 0.2 | 248.65 | 26.97 |
| 8 | 0.2 | 298.88 | 30.50 |
| 9 | 0.2 | 212.76 | 24.45 |
| 10 | 0.2 | 196.78 | 23.32 |
| 11 | 0.2 | 223.28 | 25.19 |
| 12 | 0.2 | 225.97 | 25.38 |
| 13 | 0.2 | 168.83 | 21.36 |
| 14 | 0.2 | 174.48 | 21.76 |
| 15 | 0.2 | 301.57 | 30.69 |
| 16 | 0.2 | 328.54 | 32.58 |
| 17 | 0.2 | 322.87 | 32.18 |
| 18 | 0.2 | 174.01 | 21.72 |
| 19 | 0.2 | 264.46 | 28.08 |
| 20 | 0.2 | 266.72 | 28.24 |

| | | | |
|---|---|---|---|
| 21 | 0.2 | 84.50 | 15.43 |
| 22 | 0.2 | 84.52 | 15.44 |
| 23 | 0.2 | 127.50 | 18.46 |

**Day 20 - 10/24/94** method 1

| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
|---|---|---|---|
| 1 | 0.2 | 1664.06 | 78.69 |
| 2 | 0.2 | 1670.72 | 79.01 |
| 3 | 0.2 | 1732.32 | 81.95 |
| 4 | 0.2 | 1699.52 | 80.38 |
| 5 | 0.2 | 139.33 | 5.80 |
| 6 | 0.2 | 338.53 | 15.32 |
| 7 | 0.2 | 385.26 | 17.55 |
| 8 | 0.2 | 392.35 | 17.89 |
| 9 | 0.2 | 392.63 | 17.90 |
| 10 | 0.2 | 409.08 | 18.69 |
| 11 | 0.2 | 365.04 | 16.59 |
| 12 | 0.2 | 318.36 | 14.35 |
| 13 | 0.2 | 364.34 | 16.55 |
| 14 | 0.2 | 369.75 | 16.81 |
| 15 | 0.2 | 321.22 | 14.49 |
| 16 | 0.2 | 300.85 | 13.52 |
| 17 | 0.2 | 321.62 | 14.51 |
| 18 | 0.2 | 307.08 | 13.81 |
| 19 | 0.2 | 321.72 | 14.51 |
| 20 | 0.2 | 296.03 | 13.29 |
| 21 | 0.2 | 315.56 | 14.22 |
| 22 | 0.2 | 291.47 | 13.07 |
| 23 | 0.2 | 282.75 | 12.65 |

**Day 22 - 10/26/94** method 2

| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
|---|---|---|---|
| 1 | 0.2 | 664.29 | 84.22 |
| 2 | 0.2 | 698.22 | 87.25 |
| 3 | 0.2 | 725.19 | 89.57 |
| 4 | 0.2 | 640.87 | 82.06 |
| 5 | 0.2 | 85.08 | 14.04 |
| 6 | 0.2 | 240.57 | 36.31 |
| 7 | 0.2 | 276.88 | 41.15 |
| 8 | 0.2 | 287.66 | 42.56 |
| 9 | 0.2 | 230.11 | 34.89 |
| 10 | 0.2 | 191.70 | 29.58 |
| 11 | 0.2 | 224.92 | 34.18 |
| 12 | 0.2 | 194.87 | 30.02 |
| 13 | 0.2 | 144.26 | 22.81 |
| 14 | 0.2 | 156.32 | 24.55 |
| 15 | 0.2 | 225.99 | 34.33 |
| 16 | 0.2 | 273.68 | 40.73 |
| 17 | 0.2 | 328.67 | 47.81 |

| | | | |
|---|---|---|---|
| 18 | 0.2 | 262.85 | 39.29 |
| 19 | 0.2 | 164.18 | 25.68 |
| 20 | 0.2 | 128.08 | 20.45 |
| 21 | 0.2 | 136.16 | 21.63 |
| 22 | 0.2 | 95.37 | 15.59 |
| 23 | 0.2 | 158.44 | 24.86 |

**Day 24 - 10/28/94** method 2

| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
|---|---|---|---|
| 1 | 0.2 | 501.79 | 95.81 |
| 2 | 0.2 | 442.73 | 85.08 |
| 3 | 0.2 | 407.24 | 78.63 |
| 4 | 0.2 | 328.44 | 64.30 |
| 5 | 0.2 | 40.84 | 12.03 |
| 6 | 0.2 | 153.16 | 32.45 |
| 7 | 0.2 | 140.07 | 30.07 |
| 8 | 0.2 | 136.92 | 29.49 |
| 9 | 0.2 | 87.16 | 20.45 |
| 10 | 0.2 | 83.65 | 19.81 |
| 11 | 0.2 | 110.29 | 24.65 |
| 12 | 0.2 | 95.36 | 21.94 |
| 13 | 0.2 | 77.02 | 18.61 |
| 14 | 0.2 | 77.47 | 18.69 |
| 15 | 0.2 | 80.19 | 19.18 |
| 16 | 0.2 | 107.28 | 24.11 |
| 17 | 0.2 | 133.51 | 28.87 |
| 18 | 0.2 | 185.48 | 38.32 |
| 19 | 0.2 | 103.29 | 23.38 |
| 20 | 0.2 | 62.86 | 16.03 |
| 21 | 0.2 | 66.77 | 16.74 |
| 22 | 0.2 | 44.33 | 12.67 |
| 23 | 0.2 | 58.53 | 15.25 |

**Day 27 - 10/31/94** method 2

| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
|---|---|---|---|
| 1 | 0.2 | 847.98 | 95.06 |
| 2 | 0.2 | 808.66 | 90.89 |
| 3 | 0.2 | 765.44 | 86.31 |
| 4 | 0.2 | 689.04 | 78.22 |
| 5 | 0.2 | 56.83 | 11.27 |
| 6 | 0.2 | 148.07 | 20.93 |
| 7 | 0.2 | 139.61 | 20.03 |
| 8 | 0.2 | 135.40 | 19.59 |
| 9 | 0.2 | 90.73 | 14.86 |
| 10 | 0.2 | 76.87 | 13.39 |
| 11 | 0.2 | 94.28 | 15.23 |
| 12 | 0.2 | 100.82 | 15.92 |
| 13 | 0.2 | 69.04 | 12.56 |
| 14 | 0.2 | 74.93 | 13.18 |

| | | | |
|---|---|---|---|
| 15 | 0.2 | 66.31 | 12.27 |
| 16 | 0.2 | 80.59 | 13.78 |
| 17 | 0.2 | 111.59 | 17.07 |
| 18 | 0.2 | 130.60 | 19.08 |
| 19 | 0.2 | 104.44 | 16.31 |
| 20 | 0.2 | 70.72 | 12.74 |
| 21 | 0.2 | 53.41 | 10.90 |
| 22 | 0.2 | 35.99 | 9.06 |
| 23 | 0.2 | 33.66 | 8.81 |

**Day 29 - 11/2/94** method 1

| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
|---|---|---|---|
| 1 | 0.2 | 1927.73 | 107.90 |
| 2 | 0.2 | 1826.49 | 104.31 |
| 3 | 0.2 | 1418.73 | 87.50 |
| 4 | 0.2 | 1381.43 | 85.78 |
| 5 | 0.2 | 131.43 | 9.64 |
| 6 | 0.2 | 194.33 | 14.32 |
| 7 | 0.2 | 176.36 | 12.99 |
| 8 | 0.2 | 180.39 | 13.29 |
| 9 | 0.2 | 109.69 | 8.00 |
| 10 | 0.2 | 84.05 | 6.05 |
| 11 | 0.2 | 97.03 | 7.04 |
| 12 | 0.2 | 93.27 | 6.75 |
| 13 | 0.2 | 59.48 | 4.17 |
| 14 | 0.2 | 66.58 | 4.72 |
| 15 | 0.2 | 56.98 | 3.98 |
| 16 | 0.2 | 79.39 | 5.70 |
| 17 | 0.2 | 105.98 | 7.72 |
| 18 | 0.2 | 120.58 | 8.82 |
| 19 | 0.2 | 101.16 | 7.35 |
| 20 | 0.2 | 41.19 | 2.76 |
| 21 | 0.2 | 44.31 | 3.01 |
| 22 | 0.2 | 30.49 | 1.94 |
| 23 | 0.2 | 20.45 | 1.16 |

**Day 31 - 11/4/94** method 2

| Sample Port # | Volume (mL) | Area Units | CT (ug/L) |
|---|---|---|---|
| 1 | 0.2 | 1082.60 | 111.08 |
| 2 | 0.2 | 1100.26 | 111.92 |
| 3 | 0.2 | 893.65 | 100.19 |
| 4 | 0.2 | 793.87 | 92.99 |
| 5 | 0.2 | 100.91 | 15.20 |
| 6 | 0.2 | 120.85 | 18.12 |
| 7 | 0.2 | 124.45 | 18.64 |
| 8 | 0.2 | 99.43 | 14.98 |
| 9 | 0.2 | 54.63 | 8.28 |
| 10 | 0.2 | 38.39 | 5.80 |
| 11 | 0.2 | 47.84 | 7.24 |

| | | | |
|---|---|---|---|
| 12 | 0.2 | 45.70 | 6.92 |
| 13 | 0.2 | 42.86 | 6.48 |
| 14 | 0.2 | 25.20 | 3.76 |
| 15 | 0.2 | 36.61 | 5.52 |
| 16 | 0.2 | 135.87 | 20.29 |
| 17 | 0.2 | 63.43 | 9.61 |
| 18 | 0.2 | 56.95 | 8.63 |
| 19 | 0.2 | 67.15 | 10.17 |
| 20 | 0.2 | 32.62 | 4.91 |
| 21 | 0.2 | 21.43 | 3.18 |
| 22 | 0.2 | 17.02 | 2.49 |
| 23 | 0.2 | 28.74 | 4.31 |

Calculations of $\mu$g CT transformed for weeks 1 through 5.

Week 1: 10/4/94-10/10/94

Total flow rate = 85 $\mu$L/min = 0.86L/week
Average influent CT ($\mu$g/L) = (75+81)/2 = 78
Mass CT input ($\mu$g) = 78 $\mu$g/L x 0.86L/week = 67
Average effluent CT ($\mu$g/L) = (66+52)/2 = 59
Mass CT output ($\mu$g) = 59 $\mu$g/L x 0.86L/week = 51

Week 2: 10/11/94-10/17/94

Total flow rate = 85 $\mu$L/min = 0.86L/week
Average influent CT ($\mu$g/L) = (79+97+138)/3 = 105
Mass CT input ($\mu$g) = 105 $\mu$g/L x 0.86L/week = 90
Average effluent CT ($\mu$g/L) = (65+39+10)/3 = 38
Mass CT output ($\mu$g) = 38 $\mu$g/L x 0.86L/week = 33

Week 3: 10/18/94-10/24/94

Total flow rate = 85 $\mu$L/min = 0.86L/week
Average influent CT ($\mu$g/L) = (86+78+79)/3 = 81
Mass CT input ($\mu$g) = 81 $\mu$g/L x 0.86L/week = 70
Average effluent CT ($\mu$g/L) = (12+19+13)/3 = 15
Mass CT output ($\mu$g) = 15 $\mu$g/L x 0.86L/week = 13

Week 4: 10/25/94-10/31/94

Total flow rate = 85 $\mu$L/min = 0.86L/week
Average influent CT ($\mu$g/L) = (84+96+95)/3 = 92
Mass CT input ($\mu$g) = 92 $\mu$g/L x 0.86L/week = 79
Average effluent CT ($\mu$g/L) = (25+15+8)/3 = 16
Mass CT output ($\mu$g) = 16 $\mu$g/L x 0.86L/week = 14

Week 5: 11/1/94-11/7/94

Total flow rate = 85 $\mu$L/min = 0.86L/week
Average influent CT ($\mu$g/L) = (108+111)/2 = 110
Mass CT input ($\mu$g) = 110 $\mu$g/L x 0.86L/week = 95
Average effluent CT ($\mu$g/L) = (1+4)/2 = 2
Mass CT output ($\mu$g) = 2 $\mu$g/L x 0.86L/week = 2

Table E-2. Original data used for determination of mg of acetate consumed in model aquifer column.

**Day 0 - 10/4/94**

| Sample Port # | Sample Volume (mL) | Area Units | Acetate (mg/L) |
|---|---|---|---|
| 4 | 0.7 | 17336 | 0 |
| 5A | 0.7 | 1159412 | 51.61 |
| 5B | 0.7 | 1334041 | 64.27 |
| 6 | 0.7 | LOST ON IC | - |
| 7 | 0.7 | 128508 | 2.22 |

**Day 7 - 10/11/94**
Samples lost on IC

**Day 14 - 10/18/94**
Samples lost on IC

**Day 21 - 10/25/94**
Samples lost on IC

**Day 28 - 11/1/94**

| Sample Port # | Sample Volume (mL) | Area Units | Acetate (mg/L) |
|---|---|---|---|
| 4 | 0.7 | 95140 | 3.78 |
| 5A | 0.7 | 861208 | 62.37 |
| 5B | 0.7 | 638771 | 45.36 |
| 6 | 0.7 | 139662 | 7.19 |
| 7 | 0.7 | 109864 | 4.91 |

Calculations of mg acetate consumed for weeks 1 through 5.

Week 1: 10/4/94-10/10/94
Total slug volume = 0.03 L/week
Influent acetate (mg/L) = 54
Mass acetate input (mg) = 54 mg/L x 0.03L/week = 1.6
Effluent acetate (mg/L) = 0 (assumed)
Mass acetate output (mg) = 0 mg/L x 0.86L/week = 0

Week 2: 10/11/94-10/17/94
Total slug volume = 0.03 L/week
Influent acetate (mg/L) = 60 (assumed)
Mass acetate input (mg) = 60 mg/L x 0.03L/week = 1.8
Effluent acetate (mg/L) = 0 (assumed)

Week 3: 10/18/94-10/24/94
Total slug volume = 0.03 L/week
Influent acetate (mg/L) = 60 (assumed)
Mass acetate input (mg) = 60 mg/L x 0.03L/week = 1.8
Effluent acetate (mg/L) = 0 (assumed)

Week 4: 10/25/94-10/31/94

Total slug volume = 0.03 L/week
Influent acetate (mg/L) = 60 (assumed)
Mass acetate input (mg) = 60 mg/L x 0.03L/week = 1.8
Effluent acetate (mg/L) = 0 (assumed)

Week 5: 11/1/94-11/7/94

Total slug volume = 0.03 L/week
Influent acetate (mg/L) = 54
Mass acetate input (mg) = 54 mg/L x 0.03L/week = 1.6
Effluent acetate (mg/L) = 0 (assumed)

# APPENDIX F

# APPENDIX F

## ORIGINAL DATA FOR DETERMINING ACETATE CONSUMPTION

Table F-1. Original data used for determination of acetate consumption in model aquifer column.

**Experiment started on 10/4/94**

| Day 0 - 10/4/94 Sample Port # | Sample Volume (mL) | Area Units | Acetate (mg/L) |
|---|---|---|---|
| 4 | 0.7 | 17336 | 0 |
| 5A | 0.7 | 1159412 | 51.61 |
| 5B | 0.7 | 1334041 | 64.27 |
| 7 | 0.7 | 128508 | 2.22 |

| Day 2 - 10/6/94 Sample Port # | Sample Volume (mL) | Area Units | Acetate (mg/L) |
|---|---|---|---|
| 1 | 0.7 | 161900 | 0 |
| 2 | 0.7 | 212035 | 0.09 |
| 3 | 0.7 | 168584 | 0 |
| 4 | 0.7 | 125089 | 0 |
| 5 | 0.7 | 1622697 | 13.84 |
| 6 | 0.7 | 1239922 | 8.94 |
| 7 | 0.7 | 1184870 | 8.31 |
| 8 | 0.7 | 206145 | 0.05 |
| 9 | 0.7 | 228943 | 0.18 |
| 10 | 0.7 | 167846 | 0 |
| 11 | 0.7 | 127996 | 0 |
| 12 | 0.7 | 394622 | 1.20 |
| 13 | 0.7 | 164408 | 0 |
| 14 | 0.7 | 206758 | 0.06 |
| 15 | 0.7 | 231735 | 0.20 |
| 16 | 0.7 | 240687 | 0.25 |
| 17 | 0.7 | 239586 | 0.24 |
| 18 | 0.7 | LOST ON IC | - |
| 19 | 0.7 | LOST ON IC | - |
| 20 | 0.7 | LOST ON IC | - |
| 21 | 0.7 | LOST ON IC | - |
| 22 | 0.7 | LOST ON IC | - |
| 23 | 0.7 | LOST ON IC | - |

| Day 9 - 10/13/94 Sample Port # | Sample Volume (mL) | Area Units | Acetate (mg/L) |
|---|---|---|---|
| 1 | 0.7 | 2125931 | 4.55 |
| 2 | 0.7 | 712612 | 1.81 |
| 3 | 0.7 | 649626 | 1.73 |
| 4 | 0.7 | 903779 | 2.09 |
| 5 | 0.7 | 1935382 | 4.09 |
| 6 | 0.7 | 1819390 | 3.82 |
| 7 | 0.7 | 1138327 | 2.46 |
| 8 | 0.7 | 920211 | 2.11 |
| 9 | 0.7 | 1019796 | 2.27 |
| 10 | 0.7 | 1067433 | 2.34 |
| 11 | 0.7 | 1082721 | 2.37 |
| 12 | 0.7 | 1099922 | 2.40 |
| 13 | 0.7 | 881936 | 2.05 |
| 14 | 0.7 | 1377317 | 2.89 |
| 15 | 0.7 | 1555779 | 3.25 |
| 16 | 0.7 | 709995 | 1.81 |
| 17 | 0.7 | 790490 | 1.92 |
| 18 | 0.7 | 666716 | 1.75 |
| 19 | 0.7 | 1034335 | 2.29 |
| 20 | 0.7 | 894296 | 2.07 |
| 21 | 0.7 | 854314 | 2.01 |
| 22 | 0.7 | 755846 | 1.87 |
| 23 | 0.7 | 915017 | 2.10 |

| Day 16 - 10/20/94 Sample Port # | Sample Volume (mL) | Area Units | Acetate (mg/L) |
|---|---|---|---|
| 1 | 0.7 | 33342 | 0.12 |
| 2 | 0.7 | 61114 | 0.70 |
| 3 | 0.7 | 74500 | 0.99 |
| 4 | 0.7 | 51203 | 0.49 |
| 5 | 0.7 | 194372 | 3.97 |
| 6 | 0.7 | 171228 | 3.34 |
| 7 | 0.7 | 158067 | 2.99 |
| 8 | 0.7 | 190032 | 3.85 |
| 9 | 0.7 | 181105 | 3.61 |
| 10 | 0.7 | 177699 | 3.52 |
| 11 | 0.7 | 189653 | 3.84 |
| 12 | 0.7 | 67210 | 0.83 |
| 13 | 0.7 | 62452 | 0.72 |
| 14 | 0.7 | 63292 | 0.74 |
| 15 | 0.7 | 56316 | 0.59 |
| 16 | 0.7 | 63047 | 0.74 |
| 17 | 0.7 | 56855 | 0.60 |
| 18 | 0.7 | 77394 | 1.05 |
| 19 | 0.7 | 83779 | 1.19 |
| 20 | 0.7 | 79297 | 1.09 |
| 21 | 0.7 | 77363 | 1.05 |
| 22 | 0.7 | 82073 | 1.15 |
| 23 | 0.7 | 83282 | 1.18 |

| Day 23 - 10/27/94 Sample Port # | Sample Volume (mL) | Area Units | Acetate (mg/L) |
|---|---|---|---|
| 1 | 0.7 | 41504 | 0 |
| 2 | 0.7 | 37204 | 0 |
| 3 | 0.7 | 35866 | 0 |
| 4 | 0.7 | 194060 | 8.71 |
| 5 | 0.7 | 185785 | 8.22 |
| 6 | 0.7 | 115724 | 4.12 |
| 7 | 0.7 | 139624 | 5.52 |
| 8 | 0.7 | 85289 | 2.34 |
| 9 | 0.7 | 119679 | 4.35 |
| 10 | 0.7 | 92485 | 2.76 |
| 11 | 0.7 | 124017 | 4.61 |
| 12 | 0.7 | 114624 | 4.06 |
| 13 | 0.7 | 104704 | 3.48 |
| 14 | 0.7 | 100567 | 3.24 |
| 15 | 0.7 | 94240 | 2.87 |
| 16 | 0.7 | 147670 | 5.99 |
| 17 | 0.7 | 137100 | 5.37 |
| 18 | 0.7 | 52699 | 0.43 |
| 19 | 0.7 | 87971 | 2.50 |
| 20 | 0.7 | 116591 | 4.17 |
| 21 | 0.7 | 130502 | 4.99 |
| 22 | 0.7 | 141841 | 5.65 |
| 23 | 0.7 | 205319 | 9.37 |

| Day 28 - 11/1/94 Sample Port # | Sample Volume (mL) | Area Units | Acetate (mg/L) |
|---|---|---|---|
| 4 | 0.7 | 95140 | 3.78 |
| 5A | 0.7 | 861208 | 62.37 |
| 5B | 0.7 | 638771 | 45.36 |
| 6 | 0.7 | 139662 | 7.19 |
| 7 | 0.7 | 109864 | 4.91 |

# APPENDIX G

# APPENDIX G

## ORIGINAL DATA FOR DETERMINING NITRATE CONSUMPTION

Table G-1. Original data used for determination of nitrate consumption in model aquifer column.

**Experiment started on 10/4/94**

**Day 0 - 10/4/94**

| Sample Port # | Volume (mL) | Area Units | Nitrate (mg/L) |
|---|---|---|---|
| 4 | 0.7 | 8537002 | 60.27 |
| 5A | 0.7 | 438715 | 4.12 |
| 5B | 0.7 | 265683 | 2.92 |
| 7 | 0.7 | 7893764 | 55.81 |

**Day 2 - 10/6/94**

| Sample Port # | Volume (mL) | Area Units | Nitrate (mg/L) |
|---|---|---|---|
| 1 | 0.7 | 15356646 | 35.84 |
| 2 | 0.7 | 15191321 | 35.19 |
| 3 | 0.7 | 15227480 | 35.33 |
| 4 | 0.7 | 15149244 | 35.03 |
| 5 | 0.7 | 5623551 | 7.71 |
| 6 | 0.7 | 14343176 | 31.97 |
| 7 | 0.7 | 14718747 | 33.38 |
| 8 | 0.7 | 14845242 | 33.86 |
| 9 | 0.7 | 14939823 | 34.22 |
| 10 | 0.7 | 15046214 | 34.63 |
| 11 | 0.7 | 15039740 | 34.61 |
| 12 | 0.7 | 15002588 | 34.46 |
| 13 | 0.7 | 14762065 | 33.54 |
| 14 | 0.7 | 14708659 | 33.34 |
| 15 | 0.7 | 15247293 | 35.41 |
| 16 | 0.7 | 15075331 | 34.74 |
| 17 | 0.7 | 15033535 | 34.58 |
| 18 | 0.7 | LOST ON IC | ? |
| 19 | 0.7 | LOST ON IC | ? |
| 20 | 0.7 | LOST ON IC | ? |
| 21 | 0.7 | LOST ON IC | ? |
| 22 | 0.7 | LOST ON IC | ? |
| 23 | 0.7 | LOST ON IC | ? |

**Day 9 - 10/13/94**

| Sample Port # | Volume (mL) | Area Units | Nitrate (mg/L) |
|---|---|---|---|
| 1 | 0.7 | 24433744 | 61.55 |
| 2 | 0.7 | 24265501 | 60.45 |
| 3 | 0.7 | 23481934 | 55.45 |
| 4 | 0.7 | 22866911 | 51.67 |
| 5 | 0.7 | 15892944 | 17.54 |
| 6 | 0.7 | 5522571 | 0 |
| 7 | 0.7 | 3435622 | 0 |
| 8 | 0.7 | 1142630 | 0 |
| 9 | 0.7 | 251947 | 0 |
| 10 | 0.7 | 178276 | 0 |
| 11 | 0.7 | 616336 | 0 |
| 12 | 0.7 | 164880 | 0 |
| 13 | 0.7 | 5144 | 0 |
| 14 | 0.7 | 67559 | 0 |
| 15 | 0.7 | ? | ? |
| 16 | 0.7 | 1999181 | 0 |
| 17 | 0.7 | 20137912 | 36.40 |
| 18 | 0.7 | 22768090 | 51.07 |
| 19 | 0.7 | 23768836 | 57.26 |
| 20 | 0.7 | 22489692 | 49.41 |
| 21 | 0.7 | 21274217 | 42.46 |
| 22 | 0.7 | 22643987 | 50.33 |
| 23 | 0.7 | 23978749 | 58.60 |

**Day 16 - 10/20/94**

| Sample Port # | Volume (mL) | Area Units | Nitrate (mg/L) |
|---|---|---|---|
| 1 | 0.7 | 8963020 | 62.28 |
| 2 | 0.7 | 8955856 | 62.23 |
| 3 | 0.7 | 8932372 | 62.07 |
| 4 | 0.7 | 8074734 | 56.23 |
| 5 | 0.7 | 2261601 | 16.60 |
| 6 | 0.7 | 1462645 | 11.16 |
| 7 | 0.7 | 3283032 | 23.57 |
| 8 | 0.7 | 2571772 | 18.72 |
| 9 | 0.7 | 2414016 | 17.64 |
| 10 | 0.7 | 2407643 | 17.60 |
| 11 | 0.7 | 2275434 | 16.70 |
| 12 | 0.7 | 1372199 | 10.54 |
| 13 | 0.7 | 1655367 | 12.47 |
| 14 | 0.7 | 1018072 | 8.13 |
| 15 | 0.7 | 2763308 | 20.02 |
| 16 | 0.7 | 3056334 | 22.02 |
| 17 | 0.7 | 659990 | 5.69 |
| 18 | 0.7 | 123180 | 2.03 |
| 19 | 0.7 | 29695 | 1.39 |
| 20 | 0.7 | 6176 | 1.23 |
| 21 | 0.7 | ? | ? |
| 22 | 0.7 | ? | ? |
| 23 | 0.7 | ? | ? |

**Day 23 - 10/27/94**

| Sample Port # | Volume (mL) | Area Units | Nitrate (mg/L) |
|---|---|---|---|
| 1 | 0.7 | 7723529 | 53.83 |
| 2 | 0.7 | 8877348 | 61.70 |
| 3 | 0.7 | 9384082 | 65.15 |
| 4 | 0.7 | 8911763 | 61.93 |
| 5 | 0.7 | 1457216 | 11.12 |
| 6 | 0.7 | 157750 | 2.27 |
| 7 | 0.7 | 19160 | 1.32 |
| 8 | 0.7 | ? | ? |
| 9 | 0.7 | ? | ? |
| 10 | 0.7 | ? | ? |
| 11 | 0.7 | 9956 | 1.26 |
| 12 | 0.7 | ? | ? |
| 13 | 0.7 | ? | ? |
| 14 | 0.7 | ? | ? |
| 15 | 0.7 | ? | ? |
| 16 | 0.7 | ? | ? |
| 17 | 0.7 | 5104 | 1.22 |
| 18 | 0.7 | 1417219 | 10.85 |
| 19 | 0.7 | 55424 | 1.57 |
| 20 | 0.7 | ? | ? |
| 21 | 0.7 | ? | ? |
| 22 | 0.7 | ? | ? |
| 23 | 0.7 | ? | ? |

? = no peak detected

# LIST OF REFERENCES

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