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Title: Continuous-Flow and Batch Column Studies of Anaerobic Carbon Tetrachloride Biotransformation on Hanford Soil.

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Continuous-flow and batch experiments were conducted with a column reactor system containing Hanford aquifer material in order to evaluate the potential of *in-situ* bioremediation of carbon tetrachloride (CT) at Hanford. The effectiveness of benzoate and acetate as primary substrates was considered. Nitrate and sulfate were potential electron acceptors. Transport experiments indicated the following characteristics: porosity, 27%; longitudinal dispersivity, 11 cm; and CT retardation factor, 3.9. Denitrification and CT transformation occurred during periods of benzoate and acetate addition. Chloroform (CF) was detected as a product of CT transformation in all cases. Benzoate generally induced the fastest rates of CT transformation. However, acetate was much better at inducing denitrification. Sulfate reduction was never observed, even during extended absences of nitrate and nitrite. The continuous-flow experiments showed more rapid transformation near the point of injection; however, residence times were not long enough to completely degrade CT. In batch experiments CT transformation appeared to follow pseudo-first-order kinetics, with rates decreasing from the point of injection. Switching from continuous-flow to batch experiments appeared to be an effective means of determining spatial differences in microbial activity within the column. Overall, these results indicate that the microbial population at Hanford is capable of transforming CT in the subsurface. However, methods to control the production of CF may be necessary before this technology can be successfully employed.

Continuous-Flow and Batch Column Studies of Anaerobic Carbon Tetrachloride Biotransformation on Hanford Soil

by

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Continuous-Flow and Batch Column Studies of Anaerobic Carbon Tetrachloride Biotransformation on Hanford Soil

1. INTRODUCTION

Carbon tetrachloride (CT) is a colorless, nonflammable, volatile liquid used primarily as an industrial solvent. Improper disposal, heavy use and particular chemical properties of this compound make it one of the most common organic groundwater contaminants (Westrick *et al.*, 1984). Its relatively high density, solubility and biological recalcitrance, along with it being weakly sorbed to soil, make it able to quickly migrate down to the water table and create large contaminant plumes. CT is also a known carcinogen with a Maximum Contaminant Level (MCL) of only 5 μg/L in drinking water in the United States (Fetter, 1993). Therefore, efforts aimed at remediating groundwater contaminated with CT must be capable of treating to very low levels.

The Hanford Site, located in south central Washington State just north of the city of Richland, is the location of a large CT release. The site has been a defense materials production complex since 1943. CT was used to recover plutonium from side steams using column extraction processes. It was disposed of in three unlined subsurface liquid disposal facilities which permitted direct infiltration of the underlying soil column. It has been estimated that up to 153,000 gallons of CT was discharged to the soil between 1955 and 1973. Despite that less than 2% of this has been estimated to have migrated to the groundwater, about 240 feet below the land surface, a plume of up to 5 square miles has been observed (Last *et al.*, 1991).

The Arid Site Demonstration Program is a project sponsored by the U.S. Department of Energy to consider the possibility of using *in-situ* bioremediation for cleaning up the CT problem at Hanford. However, the class of compounds termed *chlorinated aliphatic hydrocarbons* (CAHs) have been shown to be very resistant to biological degradation (Vogel *et al.*, 1987). This is partly because microorganisms have

not shown the ability to directly metabolize most CAHs, including CT. Instead, they are cometabolized in the presence of a primary substrate.

In addition, biotransformation of a compound may not entirely result in its mineralization, where the contaminant is completely transformed to harmless elemental products (*e.g.* CO_2, HCl and H_2O). For example, CF is often produced during the anaerobic transformation of CT (Semprini *et al.*, 1991). CF has properties similar to CT and is also a known carcinogen. However, the greatest potential for bioremediation of CF appears to be through aerobic cometabolism (Alvarez-Cohen and McCarty, 1991 and Kim and Semprini, in press). Therefore, if significant amounts of CF are formed as a result of anaerobic processes, complete mineralization of CT might be accomplished through the use of a combined anaerobic/aerobic system. There are also many important environmental factors to address when considering *in-situ* bioremediation, such as: availability of nutrients, redox conditions, microbial presence, temperature, pH and hydrogeology.

In support of the Arid Site Demonstration Program, the primary objective of this research was to evaluate the potential for CT biotransformation in the Hanford subsurface under anaerobic conditions. Past research involved with the Hanford demonstration (Skeen *et al.*, 1993, Stensel and DeJong, 1994, Truex *et al.*, 1994 and Petersen *et al.*, 1994) has dealt with microbial cultures, primarily denitrifiers, isolated and grown separately from Hanford soil. However, actual subsurface environments have been shown to be comparatively more unpredictable, due to complex interactions between biological and chemical systems as well transitions between redox conditions (Semprini *et al.*, 1992).

Therefore, in order to more closely simulate conditions expected in the field, a bioreactor system containing actual Hanford aquifer material was considered. To that end, the specific objectives of this study were to:

1. Fabricate and test the bioreactor system; as well as screen for abiotic transformations and losses within the system.
2. Characterize transport through the bioreactor.

3. Determine which electron acceptor, present in Hanford groundwater, is capable of inducing the highest degree of CT transformation.
4. Evaluate the effect of different primary substrates on CT transformation.
5. Observe the extent of CF production as a result of CT biotransformation under the different electron donor/acceptor conditions.
6. Establish simple models to predict the observed degradation kinetics.
7. Observe if the addition of nutrients, such as those present in yeast extract, enhance transformation.
8. Detect possible problems resulting from microbial stimulation, such as flow impediment due to excessive biological growth.
9. Make the system expandable, in order to incorporate the objectives of future research.

2. BACKGROUND

Chemical Properties

CT is a colorless, volatile, nonflammable, heavy liquid with a strong sweetish, ether-like odor. It has been used as follows: preparation of refrigerants, aerosols and propellants; metal degreasing; agricultural fumigant; production of semiconductors; solvent for fats, oils, rubber, etc.; dry cleaning operations; industrial extractant; spot remover; fire extinguisher; anesthetic; and chlorinating organic compounds (Montgomery, 1991).

Some important properties of CT are summarized in Table 2.1. Also shown are: chloroform (CF), a possible intermediate of CT transformation; and 1,1,1-trichloroethane (TCA) and trichloroethylene (TCE), which were used for tracers and internal standards in this study. Each of the properties in Table 2.1 is of particular importance to some facet of this research.

Briefly, the boiling point can be used to determine the order in which compounds elude from the gas chromatography (GC) column (see Appendix I). The relatively low

Table 2.1. Properties of selected groundwater contaminants (Montgomery, 1991).

	CT carbon tetrachloride	CF chloroform	TCA 1,1,1-trichloroethane	TCE trichloroethylene
Formula	CCl_4	$CHCl_3$	CCl_3CH_3	$CHCl=CCl_2$
Boiling Point (°C)	76.5	61.7	74.1	87.2
Aqueous Solubility @ 20°C (mg/L)	800	8000	480-1360	1100
Specific Density @ 20°C	1.594	1.489	1.339	1.464
Henry's Law Constant @ 20°C ($atm \cdot m^3/mol$)	3.02×10^{-2}	3.39×10^{-3}	1.5×10^{-2}	9.9×10^{-3}
Log Octanol/Water Partition Coefficient.	2.73-2.83	1.90-1.97	2.18-2.49	2.29-3.30
U.S. Drinking Water MCL (µg/L)[a]	5	100	200	5

[a](McCarty and Semprini, 1994)

aqueous solubility of CT, in combination with its high specific density, dictate its designation as a Dense Nonaqueous Phase Liquid (DNAPL). Where, in significant quantities (exceeding the solubility) it sinks as a separate phase to the bottom of an aqueous environment. The high Henry's law constant for CT indicates that significant losses can be expected in open systems (see Appendix F). The octanol/water partition coefficient (see Appendix F) indicates some potential to partition onto organic media; and is thus likely to reside on soils with high organic contents. The low drinking water Maximum Contaminant Level (MCL) for CT, in combination with its aqueous solubility, indicates that dangerously high concentrations can be dissolved and transported through aqueous environments.

Solute Transport

The transport of a solute through porous media can be affected by a process called *sorption*. Sorption is a broad term which encompasses all phenomena by which a solute clings to a solid surface. The process by which a contaminant, originally in solution, is distributed between the solid and the solution is called *partitioning*. The extent to which the solute will partition on the solids depends on the properties and concentration of the solute as well as the soil characteristics.

The basic advection-dispersion equation governing 1-dimensional flow in the subsurface, shown in Appendix F, can be modified to incorporate the effect of sorption as shown below (Fetter, 1993),

$$\frac{\partial C}{\partial t} = D_L \frac{\partial^2 C}{\partial x^2} - v_x \frac{\partial C}{\partial x} - \frac{B_d}{n_e} \frac{\partial C^*}{\partial t} \qquad \textbf{Equation 2.1}$$

where: C is the aqueous solute concentration (g/m^3); C^* is the mass sorbed per unit weight of solid (g/m^3); D_L is the longitudinal hydrodynamic dispersion coefficient (m^2/s); v_x is the average linear velocity of the fluid through the porous media (m/s); B_d is the bulk density of aquifer (g/m^3); and n_e is the effective (connected) porosity.

Because C^* is usually difficult to measure in the field, a relationship between C^* and C at equilibrium is often obtained experimentally for a particular soil. This

relationship is called an *equilibrium isotherm*; from which a variety of mathematical models can be used to fit an equation to the data. In the simplest case, the data is approximated by linear model in which,

$$C^* = K_d C \qquad \textbf{Equation 2.2}$$

where, K_d represents the slope of the line through a plot of C^* versus C, and is called the *distribution coefficient*.

Solutes that tend to be strongly sorbed will move considerably slower than the water transporting them. This effect is called *retardation*; and the factor by which the solute velocity is reduced relative to the water is called the *retardation factor*, r_f. The retardation factor can be found from Equation 2.1 by substituting the equation derived from the equilibrium isotherm for C^* and rearranging the equation to be of the form,

$$r_f \frac{\partial C}{\partial t} = D_L \frac{\partial^2 C}{\partial x^2} - v_x \frac{\partial C}{\partial x} \qquad \textbf{Equation 2.3}$$

Thus, for the special case of a linear isotherm, substitution of Equation 2.2 into Equation 2.1 and rearranging yields,

$$r_f = 1 + \frac{B_d}{n_e} K_d \qquad \textbf{Equation 2.4}$$

However, in the case of column breakthrough experiments, the retardation factor can be determined directly from,

$$r_f = \frac{v_x}{v_c} \qquad \textbf{Equation 2.5}$$

where: v_x is average linear velocity of the groundwater and v_c is the average velocity of the solute front where the concentration is one-half of the original.

Sorption of solutes can be complicated due to the time required for equilibrium to be established between phases. If the sorptive process is slow compared to the rate of fluid flow, it is said to be *rate-limited*, and a kinetic model is required to accurately describe the process (Wu and Gschwend, 1986). Due to the mathematical complexity of

these models, van Genuchten (1981) developed a computer program called Cfitm to aid in predicting the effects of rate-limited sorption.

Transformations of Carbon Tetrachloride

The reactions capable of transforming CT are relatively few, the possibilities of which include (Vogel *et al.*, 1987): *substitution* of halides through hydrolysis or other nucleophilic reactions; *oxidation* by hydroxylation; and *reduction* by hydrogenolysis.

Substitution Reactions

The most common type of nucleophilic substitution is hydrolysis (solvolysis) with water, where a hydroxide ion (OH^-) is substituted for a chloride (Cl^-) ion. For CT the reaction is,

$$CCl_4 + H_2O \rightarrow CCl_3OH + HCl \qquad \textbf{Equation 2.6}$$

The product, trichloromethanol, can be subsequently hydrolyzed to dichloromethanoic acid and then to carbon dioxide. Hydrolysis rates have been shown to follow pseudo-first-order kinetics, but tend to slow dramatically as the degree of halogenation increases. Thus, because CT is fully chlorinated its hydrolysis rate is extremely slow, with a half-life of 7000 years at 20°C (Vogel *et al.*, 1987). This long term stability leads researchers to generally disregard the possibility of CT hydrolysis in uncatalyzed systems (Criddle and McCarty, 1991).

Although not commonly found in natural waters, the sulfhydryl group (HS^-) is chemically more reactive than the hydroxyl group (OH^-) and can undergo nucleophilic substitution as shown by (Vogel *et al.*, 1987),

$$CCl_4 + HS^- \rightarrow CCl_3SH + Cl^- \qquad \textbf{Equation 2.7}$$

Kriegman-King and Reinhard (1992 and 1994a) reported a half-life of 2600 days for CT in the presence of a homogeneous solution of 1mM HS^- at 25°C.

Oxidation and Reduction Reactions

Substitution reactions do not change the oxidation state of the compound. However, oxidation and reduction reactions result in the transfer of electrons to and from some agent other than the chlorinated compound itself, consequently changing the net oxidation state.

Oxidation/reduction (*redox*) reactions are compared based on the electrochemical potential that exists between the electron donor and electron acceptor half-reactions. Conventionally, these potentials are measured relative to a standard hydrogen electrode and are reported in the form of reductions (Schwarzenbach *et al.*, 1993). Many environmentally significant half-reactions and their potentials are displayed graphically in Figure 2.1. Compounds with a greater tendency to accept electrons (greater oxidants) are listed higher on the diagram.

The redox condition of a particular environment, whether its reducing or oxidizing, is determined by the electron donor/acceptor couple capable of yielding the largest amount of free energy. In the figure, the couple with the largest difference between them provides the most free energy and controls the redox state. Electrons are transferred from reactions with lower potentials to those with higher potentials. The electron donor reaction is underneath the electron acceptor reaction and proceeds in reverse from that shown in the diagram.

Because the oxygen reduction half-reaction provides a high energy yield when coupled with typical primary substrates, aerobic conditions usually provide the fastest rates of microbial growth (McCarty, 1971). However, under these conditions, CT has been shown to posses little potential for transformation (Bouwer *et al.*, 1981). This stems from the highly oxidized nature of CT; the oxidation state of the carbon atom in CT (+4) is the same as CO_2.

If the environment becomes oxygen depleted, the microbial consortia sequentially turns to the next best electron acceptor (Bouwer, 1994). This results in the environment becoming more reducing. As more reducing conditions prevail, CT becomes increasingly favorable as an electron acceptor, based on theoretical energetics. However, despite the

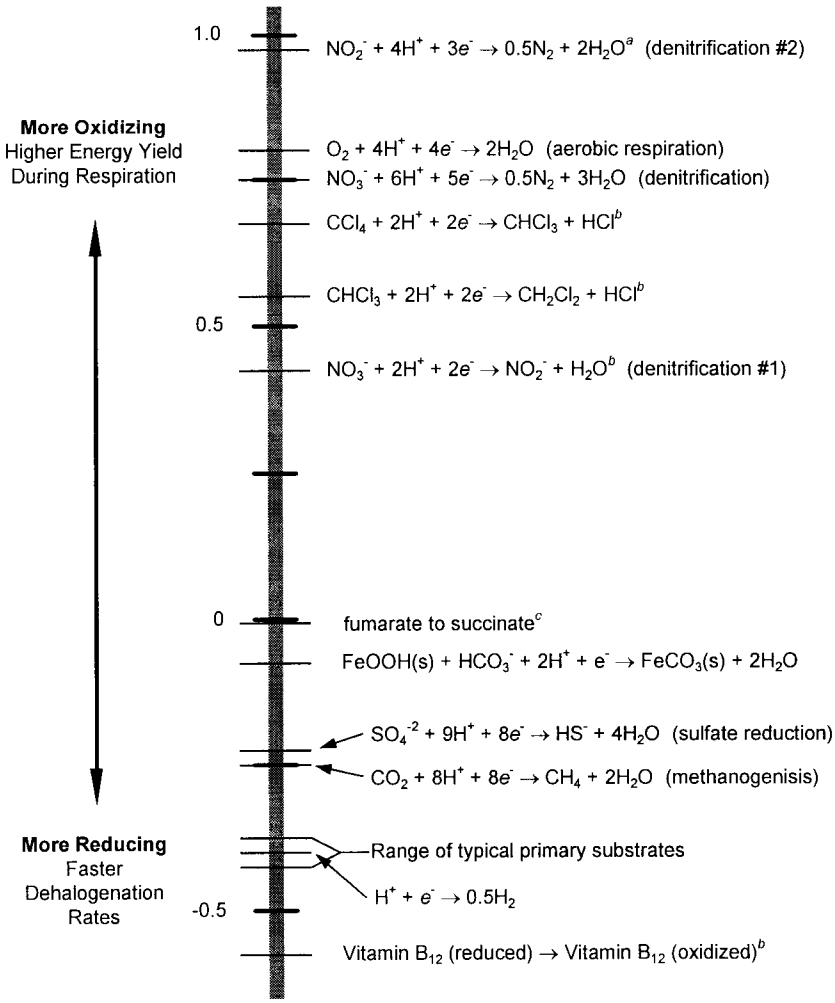

Figure 2.1. Comparison of redox potentials, in Volts, for selected half-reactions, assuming unit activity and corrected for pH = 7. Based on Bouwer (1994), [a]McCarty (1971), [b]Vogel *et al.* (1987) and [c]Criddle *et al.* (1990a).

thermodynamic feasibility, there is no evidence to date that demonstrates that microbes can use CT as an electron acceptor in their metabolic reactions. Instead, studies suggest that CT reduction proceeds through fortuitous utilization of electrons intended for other reactions (Egli *et al.*, 1990). Thus, CT transformation results as an indirect consequence of efforts to respire with typical electron acceptors (*e.g.* nitrate), and proceeds with no apparent advantage to the microorganisms.

Rates of CT transformation have been shown to generally increase as the environment becomes more reducing (Bouwer and Wright, 1988). Although, from the

standpoint of microbial growth, more reducing conditions are less desirable. In reducing environments CT is transformed by hydrogenolysis. In which a chloride ion is removed by a reduced species such as a primary substrate, a reduced transition metal or a transition metal complex, through exchange for an electron as follows (Vogel *et al.*, 1987),

$$CCl_4 + e^- \rightarrow \bullet CCl_3 + Cl^- \qquad \textbf{Equation 2.8}$$

The alkyl radical, formed during the reaction, is very reactive and could result in a variety of compounds depending on the environmental conditions. Criddle and McCarty (1991) summarized the various products reported in the literature, which are displayed graphically in Figure 2.2. The formation of the trichloromethyl radical is shown as pathway 2 of the diagram. Pathway 1 shows the kinetically limited hydrolysis reaction discussed previously.

Pathway 3 indicates the remote possibility of two radicals combining to form hexachloroethane by dimerization. Reduction to CF, pathway 4, is known as *reductive dechlorination* and has been almost always observed to some extent when CT is transformed under anaerobic conditions. Further reductive dechlorination to dichloromethane (DCM) appears to be possible in sufficiently reducing environments (Galli and McCarty, 1989, and Vogel *et al.*, 1987). In the unlikely formation of the trichloromethyl radical in an aerobic environment, it could combine with molecular oxygen as shown in pathway 5. Pathway 6 shows the result of further reduction to a carbenoid. The possibility of the radical covalently binding to cell material is shown in pathway 7. Typically, more than one pathway operate simultaneously and competitively in both microbial and abiotic systems (Criddle and McCarty, 1991).

The production of intermediates, especially CF, from the transformation of CT is undesirable, since one toxic compound is merely converted to another. In addition, CF has been shown to be fairly recalcitrant to sequential reduction under anaerobic conditions (Semprini *et al.*, 1991). Greater potential for CF transformation appears to lie in aerobic cometabolic processes (Kim and Semprini, in press). Therefore, complete mineralization of CT may require an anaerobic/aerobic multi-environmental system, where the bi-products of the anaerobic system are passed on to a subsequent aerobic

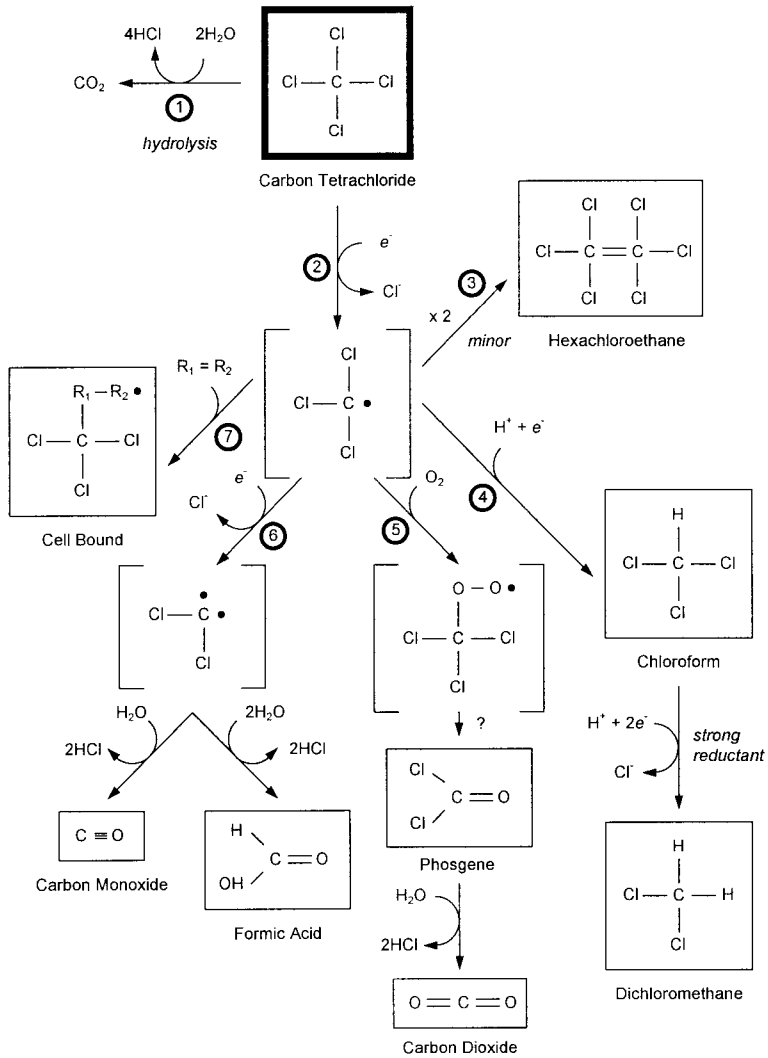

Figure 2.2. Known abiotic and biotic transformations of CT. Boxed compounds indicate products and intermediates that have been reported in the literature. From Criddle and McCarty (1991).

system. For example, methane-utilizing microorganisms have been shown to oxidize CF by hydroxylation as follows (Alvarez-Cohen and McCarty, 1991 Speitel and Leonard, 1992 and Speitel *et al.*, 1993):

$$CHCl_3 + H_2O \rightarrow CCl_3OH + 2H^+ + 2e^- \qquad \textbf{Equation 2.9}$$

Where, further microbial and chemical processes can degrade the resulting trichloromethanol with relative ease.

The ability of CT to corrode metals in the absence of oxygen was noticed as long ago as 1925 (Rhodes and Carty, 1925). This prompted Matheson and Tratnyek (1994) to study the transformability of CT in the presence of iron metal. They observed rapid transformation to CF, on the order of hours. CF reduction to DCM was also observed but proceeded much slower. Rates were found to increase with greater iron contact area.

CT transformation in the presence of iron containing minerals has been observed, under both anaerobic and aerobic conditions, by Kriegman-King and Reinhard (1992, 1994a and 1994b). Degradation kinetics were zero-order with respect to CT concentration. Greater than 90% transformation was observed within 12-36 days, with CF, CS_2, CO_2 and formate, in various proportions, detected as intermediates. Although transformations were less extensive under aerobic conditions, a larger percentage of CT was mineralized under aerobic than anaerobic conditions.

Biological Considerations

Although abiotic chemical transformations of CT, and other CAHs, can be thermodynamically feasible, they may occur at extremely slow rates. Biotic reactions may proceed much faster through the utilization of special proteins, such as *enzymes* and *cofactors*, which serve as catalysts. These compounds significantly lower the activation energy of reactions. The effect can be so profound that rates may increase by a factor of 10^9 or more (Schwarzenbach *et al.*, 1993). However, in spite of the enormous potential of biological systems to degrade environmental contaminants, successful degradation of CAHs can be very complicated.

The difficulty is mainly due to the fact that microorganisms have not been shown to posses the ability to metabolize most CAHs directly. Instead, these compounds are degraded through a process called *cometabolism*. Typically, in a cometabolic process, enzymes and cofactors produced to catalyze metabolic reactions for growth on a particular *primary substrate*, fortuitously transform the contaminant as well (Criddle, 1993). CT does not fall directly under this category because its highly oxidized nature prevents it from acting in place of the primary substrate as an electron donor. However,

CT does appear to have the potential to undergo a similar fortuitous catalytic transformation by intercepting electrons intended for the "primary electron acceptor". Metallo-porphyrin containing compounds, produced within the cell, are suspected of being responsible for catalyzing the electron transfers (Egli *et al.*, 1990).

Therefore, in cometabolic processes, transformation of a contaminant will not proceed for an extended period without the presence of a primary substrate or a suitable electron acceptor. Also, after transformation of the contaminant has been catalyzed, the products are not usually incorporated into the cell but instead are passed out. Subsequent degradation may continue externally by other biological or abiotic processes (McCarty and Semprini, 1994).

Further complications arise from both the primary substrate, or electron acceptor, and the contaminant being transformed by the same enzyme or cofactor. The presence of the primary species can inhibit transformation of the contaminant through competition for the catalytic agent. This effect is called *competitive inhibition*. However, without the primary substrate and electron acceptor present, the microorganisms lose vitality and cease production of the required agents. Therefore, careful control may be required to maintain the appropriate balance conducive to optimal rates of contaminant degradation (Criddle, 1993).

Literature Review

Recently, there has been surge in research dedicated to CT biotransformation. However, knowledge of the processes involved is still limited, particularly in comparison to petroleum hydrocarbons as well as some other CAHs, specifically PCE and TCE. The following discussion will be limited to microbially mediated transformations of CT. However, because the distinction between biological and non-biological (chemical) processes is often unclear, certain abiotic studies were considered that provided significant insight into biological systems.

Batch Studies

Mixed Culture Systems

Bouwer *et al.* (1981) tested the potential of various CAHs to biological degradation under aerobic and methanogenic conditions using mixed microbial populations in batch systems. Successful anaerobic transformations prompted subsequent studies (Bouwer and McCarty, 1983a, 1983b and 1985). Here, the transformability of CT was investigated under methanogenic and denitrifying conditions. The methanogenic cultures, using acetate as a primary substrate, mineralized nearly all of the CT to CO_2 within 3 weeks. The denitrifying cultures, grown on ethanol, removed most all of the CT within 6 weeks. Both CF and CO_2 were identified as products. CF increased initially but then decreased. It was not understood how CO_2 was produced in an environment conducive to reductive dechlorination. The evidence suggested that CT was transformed via two separate pathways: direct mineralization to CO_2; and reduction to CF followed by subsequent oxidation to CO_2.

Pure Culture Systems

Egli *et al.* (1987 and 1988) used pure bacterial strains in batch systems. Their combined work looked at one methanogen (grown on acetate), two sulfate reducers (grown on lactate and acetate), one denitrifier (hydrogen-utilizing, autotroph) and two acetogens (grown on fructose and glucose). One of the sulfate reducers and the denitrifier failed to transform CT. The other sulfate reducer and the methanogen sequentially transformed CT to CF then DCM, with the sulfate reducer exhibiting faster rates. The products of the sequential reductive dechlorinations accounted for all of the CT transformed. The acetogens produced the fastest transformation rates and resulted in the formation of both CO_2 and products derived from reductive dechlorinations. They speculated that the acetogens metabolized CT via two pathways. One being a reductive branch catalyzed by corrinoid enzymes, the other a substitutive branch consisting of unknown reactions.

Galli and McCarty (1989) also showed sequential reductive dehalogenation of CT to CF and DCM with pure methanogenic cultures. Mikesell and Boyd (1990), using a different methanogenic species, showed similar products as a result of CF biotransformation, in addition to a slight amount of CO_2. Of the primary substrates considered (methanol, methylamine, dimethylamine and trimethylamine), methanol induced the highest degree of CF transformation. 7% of the CF transformed was converted to CO_2. The remaining fraction consisted of DCM and another unidentified dechlorination product, possibly chloromethane. Interestingly, no CF was transformed to methane, yet the extent of dehalogenation was proportional to the amount of methane produced.

Egli *et al.*, (1990) studied the ability of pure autotrophic and heterotrophic (grown on lactate and fructose) batch cultures to transform CT. All of the cultures produced CO_2, CF, and DCM in various proportions. Conversion of CT to CO_2 was about 2 times faster than the conversion of CF to CO_2, indicating that sequential conversion of CT to CF then to CO_2 was unlikely. Cell-free extracts were shown to produce similar but attenuated results. Autoclaved cells also induced transformation. The researchers suggested that metallo-porphyrin compounds within the cells were responsible for catalyzing the reductive dehalogenation of CT to CF, as well as the substitutive dehalogenation of CT to CO_2 and the oxidative dehalogenation of CF to CO_2.

Criddle *et al.* (1990a) studied the ability of *Escherichia coli* to transform CT under highly aerobic, micro-aerobic (~1% oxygen), denitrifying, fumarate respiring and fermenting conditions. Glycerol was used as the primary substrate in all cases except fermentation, where glucose was used. No significant transformation was observed under denitrifying or highly aerobic conditions. Slight transformation occurred under micro-aerobic conditions; in which case most of the CT transformed was converted to cell material or CO_2. Fermenting conditions produced more CF and less CO_2 than the fumarate respiring conditions. In general, more reducing conditions were more conducive to transformation by reductive dechlorination.

Criddle *et al.* (1990b) reported the discovery of a strain of denitrifying bacteria capable of completely transforming CT to CO_2 without the production of less chlorinated intermediates. The strain (*Pseudomonas* KC) was isolated from aquifer solids obtained from Orange County, CA, and was grown on acetate as a primary substrate. The mechanism was believed to be either a two electron reduction to form a dichlorocarbene radical, which was thereby hydrolyzed to formate then oxidized to CO_2 (see pathway 6 Figure 2.2), or a direct catalytic hydrolysis reaction yet unknown. Trace levels of metals inhibited transformation, leading to the belief that the transformation may have resulted from the microorganism's process of scavenging for trace metals.

Further experimentation with *Pseudomonas* strain KC by Lewis and Crawford (1993) resulted in the production of CF. They evaluated the cultures ability to transform CT under aerobic and denitrifying conditions with different primary substrates. Of the substrates tested (acetate, glucose, glycerol, and glutamate), acetate yielded the highest rate of CT transformation. CT transformation was observed under aerobic conditions, but rates increased with decreasing oxygen concentration and were fastest under denitrifying conditions.

Picardal *et al.* (1995) studied the ability of *Shewanella putrefaciens* 200 to transform CT in batch systems with a variety of primary substrates, including: lactate, glucose, glycerol, pyruvate and formate. *S. putrefaciens* 200 has the unique ability to respire anaerobically using a variety of electron acceptors. Oxygen, nitrate, oxidized iron (Fe^{+3}), fumarate and trimethylamine oxide were considered. CT transformation was independent of the electron donor present. Dehalogenation did not occur under aerobic conditions. Dehalogenation proceeded rapidly, and at similar rates, with oxidized iron, fumarate, and trimethylamine oxide as electron acceptors. However, equally rapid rates were observed in cultures containing no electron acceptor. The researchers concluded that cellular reserves were sufficient to establish CT transformation. A significant lag phase was observed before transformation proceeded with nitrate as the electron acceptor. The extent of CF resulting from the CT transformations was not reported.

Cell-Free Metallo-Porphyrin Systems

A fair amount of work has been dedicated to the study of CT transformations in metallo-porphyrin containing systems. Although these experiments were performed in the absence of living microorganisms, their relevance to living systems stems from the fact that microorganisms are suspected of using such porphyrins and enzymes to mediate their metabolic reactions (Lesage *et al.*, 1992). Four compounds are suspected to play important roles as catalysts and electron carriers in CT reductive dehalogenation (Gantzer and Wackett, 1991 and Egli *et al.*, 1990).

1. reduced iron (II) porphyrin (hematin), iron center
2. cytochrome P_{450}, iron center
3. vitamin B_{12} (cyanocobalamin), cobalt center
4. coenzyme F_{430}, nickel center

Klecka and Gonsior (1984) explored the potential of cell-free reduced iron (II) porphyrins to transform CT. They found that CT was rapidly degraded to non-detectable levels after about 2 days. Some of the CT transformed could be accounted for as CF. CF was also transformed, but rates were much slower. Krone *et al.* (1989) used corrinoids, derivatives of cyanocobalamin, to dehalogenate CT in the presence of titanium (III) citrate, a strong reductant. CT was rapidly dehalogenated sequentially to methane within hours.

Continuous-Flow Column Studies

Bae and Rittmann (1990) used a continuous-flow column, packed with glass beads, to study the ability of a mixed culture to degrade CT under aerobic and denitrifying conditions using acetate as a primary substrate. The column had a hydraulic residence time of approximately 2 hours. CT was reduced by 20-30% under both aerobic and denitrifying conditions. In both cases 3-13% of the CT transformed resulted in CF. They suggested that reductive dechlorination was responsible for the CF production, even under aerobic conditions. More CT was transformed when nitrate was removed under

anaerobic conditions. Removal of acetate from the feed resulted in a decline in CT transformation.

Cobb and Bouwer (1991) constructed a three-zone continuous-flow column containing bacteria grown from sewage seed. Acetate was used as the primary substrate. The column was packed with glass beads and had a hydraulic residence time of 1.5 hours. The zones developed from preferential utilization of oxygen, nitrate and sulfate as electron acceptors. The aerobic zone developed in the first few centimeters, followed by the denitrifying then the sulfate reducing zone. The fastest rates of CT removal were observed after the sulfate reducing zone had become fully established. CF was formed as a product of transformation but was not quantified.

Studies Involving Systems Containing Aquifer Material

Semprini *et al.* (1991) used aquifer material in batch microcosms in order to determine the ability of a native microbial consortia (Moffett Naval Air Station, Santa Clara Valley, CA) to degrade CT. Growth was induced through the use of acetate as a primary substrate and nitrate and sulfate as possible electron acceptors. CT was transformed to CO_2 (40-60%) and CF (30-40%). Despite that denitrifiers were growing, it appeared that they may not have been responsible for the transformation. Twenty pure cultures of denitrifiers were isolated from the microcosms, but none were found to have the ability to degrade CT. Sulfate reducers may have been responsible, during periods of low nitrate, but this was not confirmed.

Semprini *et al.* (1992) evaluated the potential for *in-situ* transformation of CT in a shallow confined aquifer at Moffett Naval Air Station. Acetate, nitrate, sulfate and CT were injected into the aquifer and their fate was monitored by a sampling well network. A small amount of CF (3-4% of the CT injected) was detected immediately, but rapidly disappeared. Otherwise, no CT transformation was observed for a period of two weeks; which coincided with the time required to establish denitrification. Following denitrification CT levels began to decline. CF was observed as a product of transformation and accounted for approximately 30-60% of the CT transformed.

Several observations indicated that the presence of nitrate had an inhibitory effect on CT transformation. First, the lag time before CT transformation coincided with the time required for denitrification. Second, CT transformation was greater further from the injection well, where denitrification was more complete. Also, after nitrate was intentionally removed from the injected groundwater CT transformation increased near the injection well. The removal of nitrate from the injected water also resulted in a smaller fraction of CT being converted CF. It was suggested that sulfate reducing microorganisms may have been responsible for the increase in CT transformation after nitrate was removed. Other suggestions were that nitrate possibly acted as a competitor for electrons with CT, or that microorganisms living off the decay products of the denitrifiers were responsible for the transformation.

Studies in Support of the Hanford Demonstration

Recently, a large amount of work has been initiated in support of the demonstration at Hanford. Because of the nitrate contamination present in Hanford groundwater the researchers have focused on denitrifying cultures. Stensel and DeJong (1994) inoculated a fluidized-bed reactor with a denitrifying culture from Hanford. Acetate was used as the primary substrate. Higher rates of CT transformation were observed under conditions of high acetate and CT. Removal of nitrate increased CT transformation rates 7-12 times. CF production increased from 10 to 20% of the CT transformed when nitrate was removed.

Truex *et al.* (1994) tested the ability of a denitrifying culture from Hanford to degrade CT using acetate, methanol, ethanol and glycerol as primary substrates in a large batch reactor. Similar pseudo-first-order kinetics were observed for CT transformation with acetate, methanol and ethanol as substrates. Glycerol induced a biphasic degradation pattern, where CT degradation occurred during a lag phase before denitrification and cell growth. It was suggested that this may have been a result of fermentation which occurred previous to denitrification.

Petersen *et al.* (1994) tested the effect of acetate, nitrate and nitrite concentration on CT transformation in batch systems inoculated with a Hanford denitrifier. Nitrite and/or high levels of nitrate inhibited CT degradation and cell growth. Interestingly, no CF was observed as a result of CT transformation under any of the conditions considered.

Summary

Most of the research regarding biotransformation of CT has been at the bench-scale with isolated microorganisms in ideal environments. These types of studies are useful for screening purposes and for evaluating the biological processes responsible for chemical decomposition. However, to directly extrapolate the results of experiments of this type to predict the outcome of an actual *in-situ* demonstration may not be appropriate. As indicated by Semprini *et al.* (1992) real subsurface systems can be considerably more unpredictable due to complex biological and chemical interactions and transitions between redox conditions. Also, a particular environment could be deficient in a particular trace minerals or capable microbial populations.

To date, very little work has been dedicated to field- or bench-scale studies involving actual aquifer materials and groundwater; and no work has been done in this area with Hanford site materials. Therefore, based on the current research needs, it was chosen to construct a continuous-flow laboratory system containing aquifer material from Hanford. A setup of this type could effectively simulate the flow and environmental conditions in the subsurface while providing a reasonable degree of flexibility and control. The results, when compared to those of previous studies, would provide valuable information into the feasibility of the Hanford demonstration.

Also, the results of past research indicate that CF production, resulting from the transformation of CT is very unpredictable. Most cases in the literature reported the production of CF to some degree. However, Petersen *et al.* (1994) had observed CT transformation without the production of CF by Hanford denitrifiers. Thus, it was of interest to observe the extent of CF produced in a complex soil environment similar to that at Hanford. In addition, since soil can posses a large variety of microorganisms, it

was of interest to observe the effect of progression through redox states. The effect of nitrate on CT transformation also needs to be explored further. Stensel and DeJong (1994) and Petersen *et al.* (1994) both reported greatly enhanced transformation when nitrate was removed; despite that their systems contained pure denitrifying cultures.

Finally, past research has not evaluated the potential of benzoate as a primary substrate for inducing transformation of CT. Benzoate has been shown to be an effective substrate for stimulating cometabolic transformation of PCE (Beeman *et al.*, 1994). Conversely, acetate has been used extensively in CT biotransformation studies. Therefore, a comparison between substrates would be interesting because the difference in molecular structure, ring opposed to aliphatic, could result in significant variability.

Overall, these findings and research needs provided the basis of the goals and objectives of this study, as stated in Chapter 1. It was also of primary concern to maintain experimental procedures in support the demonstration at Hanford.

3. MATERIALS AND METHODS

Column Reactor

A column reactor design that has been used successfully for studying biodegradation of chlorophenols (Cole, 1993) was chosen for the preliminary design. Slight modifications were necessary in order to accommodate different external equipment. The resulting design is shown in Figure 3.1. The column was fabricated out of stainless steel due to the high volatility, reactivity and sorptivity of the CAHs used in this study. Throughout the remainder of this section all materials mentioned were stainless steel unless otherwise noted.

The internal dimensions of the column were: length = 30 cm; diameter = 5.4 cm; volume = 690 mL. Both ends were sealed with removable endcaps equipped with dual Viton® O-rings (Parker Seals, Lexington KY). The endcaps were secured in place by three threaded rods running between them; wingnuts on the outside of each endcap were then used to firmly compress the endcaps to the column (not shown). 100 mesh screen material (Baldwin Filter, Albany OR) placed on the inside of the endcaps helped prevent particles from being washed out of the column. Groves were machined on the inside of the endcaps in order to distribute the flow more efficiently throughout the cross-section.

The four sample port lugs consisted of tube socket weld fittings with 1/8 inch NPT female threads (Cajon Co., Macedonia OH), that were silver-brazed to the column at logarithmic intervals (3.5, 7.6, 14.0, and 23.0 cm) from the base (see Appendix D for a consideration of the reaction of CT with the silver brazing material). Also brazed to the lugs were pieces of 1/8 inch tubing that extended to the column center to help prevent the sampling needles from becoming plugged with grit. Ball valves with Swagelock® fittings (Whitey Co., Highland Heights OH) were connected to the lugs using 1/8 inch fractional tube adapters (Cajon Co., Macedonia OH). The same tube adapters were also used to connect the exterior plumbing to the endcaps. A detailed parts description in included in Appendix K.

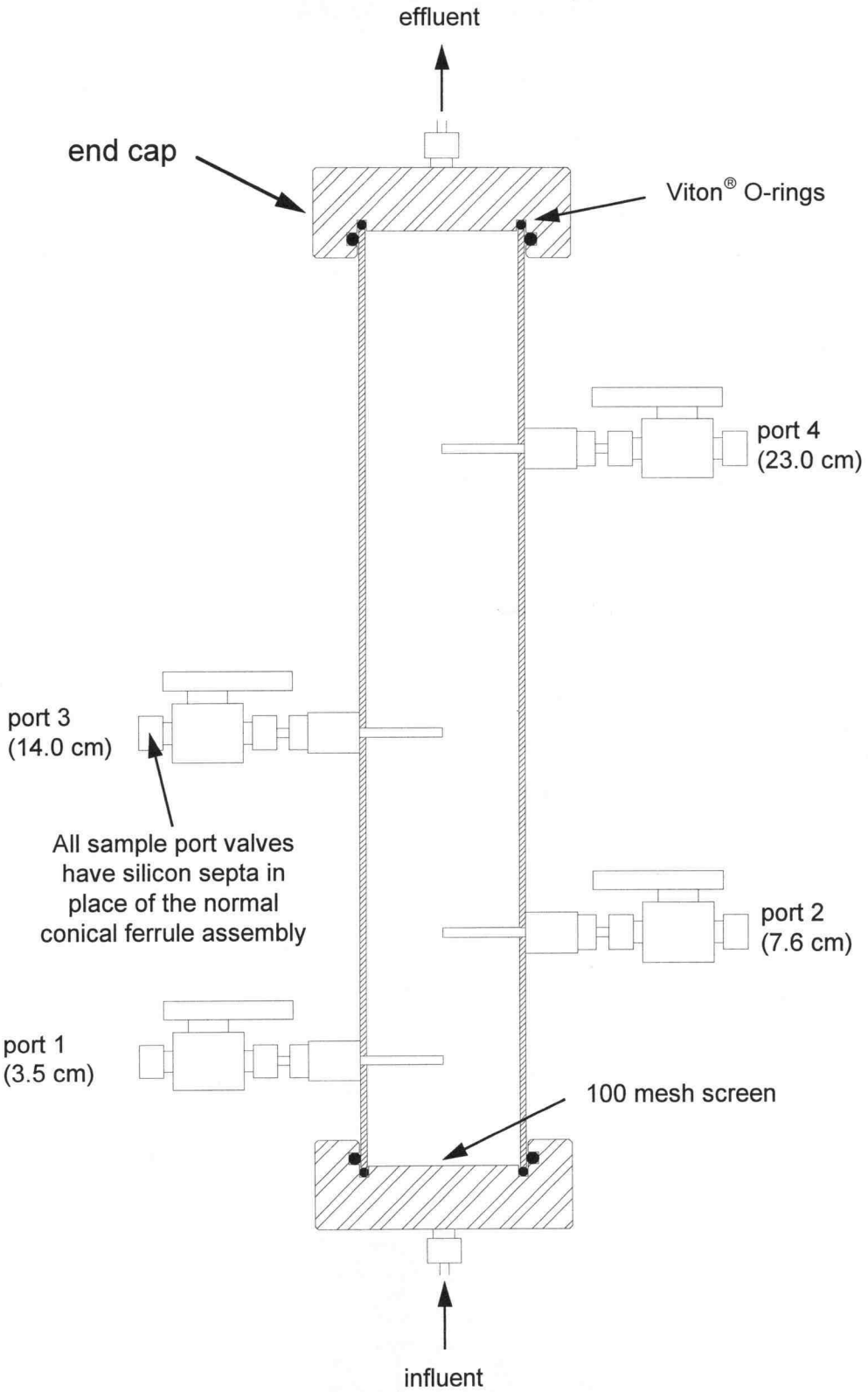

Figure 3.1. Column configuration.

Media

Aquifer Material

The core sample, from which all subsurface solids were obtained, was taken from the Hanford field site on October 9, 1993. It was kept in cold storage for approximately 7 months before being opened. The core was originally packed in argon gas. However, samples of the gas prior to unpacking showed that oxygen, at nearly atmospheric concentrations, had diffused into the core.

The core was unpacked inside of an anaerobic glove box under a nitrogen atmosphere. Material was directly transferred to the reactor column, while upward flowing groundwater helped to remove silts and prevent gas from being trapped in the pore spaces. A materials characterization was not done before packing the column because the glove box inferred time constraints and difficulties in handling. The material appeared to consist mostly of fine gravel; however, cobble sized stones were not uncommon. Large particle sizes (>0.25 inches) were excluded from the reactor column. The remainder of the material (20-40%) was made up of coarse sand and silt. See Appendix G for the protocol followed during column packing.

Groundwater

Due to regulatory difficulties, Hanford groundwater was unavailable and had to be synthesized in the laboratory. Previous studies (Last *et al.*, 1991) indicated that the groundwater contained the ionic concentrations shown in Table 3.1 and had a pH between 7.64 and 7.9. The *synthetic groundwater* was initially prepared by combining the mineral salts shown in Table 3.2 in a 2 L volumetric flask. In which case, all ion concentrations were within the ranges observed at Hanford. However, the resulting solution was well buffered at a pH of 8.3. Also, a white precipitate formed as a result of autoclaving. Therefore, bicarbonate was eventually removed from the synthetic groundwater. It was thought that the soil contained in the column may have contained minerals that would raise the alkalinity of the water, possibly compensating for its removal. With the bicarbonate removed, the pH remained near neutrality after deairing with nitrogen gas.

Table 3.1. Concentration ranges of Hanford groundwater constituents (Last *et al.*, 1991).

	mg/L		mmol/L	
	low	**high**	**low**	**high**
K^+	3	6	0.077	0.15
Na^+	21	52	0.91	2.3
Ca^{2+}	17	59	0.42	1.5
Mg^{2+}	6	17	0.25	0.70
Cl^-	4	22	0.11	0.62
NO_3^-	27	162	0.44	2.6
SO_4^{2-}	17	47	0.18	0.49
HCO_3^-	76	155	1.3	2.5

Table 3.2. Synthetic groundwater composition.

salts added	**mg/L**	**mmol/L**
KCl	9.7	0.13
$MgSO_4$	48.2	0.40
$Ca(NO_3)_2$	69.6	0.42
$NaHCO_3$ (later removed)	134.4	1.6
resulting ions	**mg/L**	**mmol/L**
K^+	5.1	0.13
Na^+ (later removed)	36.8	1.6
Ca^{2+}	17.0	0.42
Mg^{2+}	9.7	0.40
Cl^-	4.6	0.13
NO^{3-}	52.6	0.84
$SO4^{2-}$	38.4	0.40
HCO_3^- (later removed)	97.6	1.6

The synthetic groundwater did not contain trace nutrients or growth medium of any kind. The only constituents present in the solution were those listed in Table 3.2, with the exception of bromide and phosphate which were added only briefly (their addition is described in detail where appropriate). Trace nutrients required for growth had to be acquired from the soil. The only other source of components to the influent solution was provided by the amendment stream, described below.

Amendments

Two separate amendment solutions were used to add reactive compounds to the synthetic groundwater shortly before entry into the soil column. One contained CT and TCA, the other benzoate and/or acetate. TCA was selected as a CAH tracer since it had been shown to be resistant to biotransformation in the *in-situ* studies of Semprini *et al.* (1992). For a short period, a third amendment solution, containing yeast extract was employed. In each case, the amendment solutions were prepared from dilutions of stock solutions made from autoclaved deionized water. The dilutions were made in autoclaved 100 mL volumetric flasks containing autoclaved deaired synthetic groundwater. Special considerations were made in order to prepare CAH stock solutions free of organic solvents (*e.g.* methanol) which could possibly act as carbon sources (see Appendix H). The amendment solutions were drawn into separate autoclaved 50 mL gas-tight syringes (SGE, Ringwood Australia) for use in the supply system described below.

Supply System

The column supply network is shown in Figure 3.2. The column was fed in an up-flow fashion by two low-flow pumps. The synthetic groundwater feed pump (Fluid Metering Inc., Oyster Bay NY), supplied about 85% of the combined flow and had stainless steel and ceramic wetted parts. A syringe pump (Orion Research, Boston MA) provided the remainder of the flow along with the synthetic groundwater amendments. After the influent streams were combined, they were mixed in a 125 mL serum bottle placed on a magnetic stir plate.

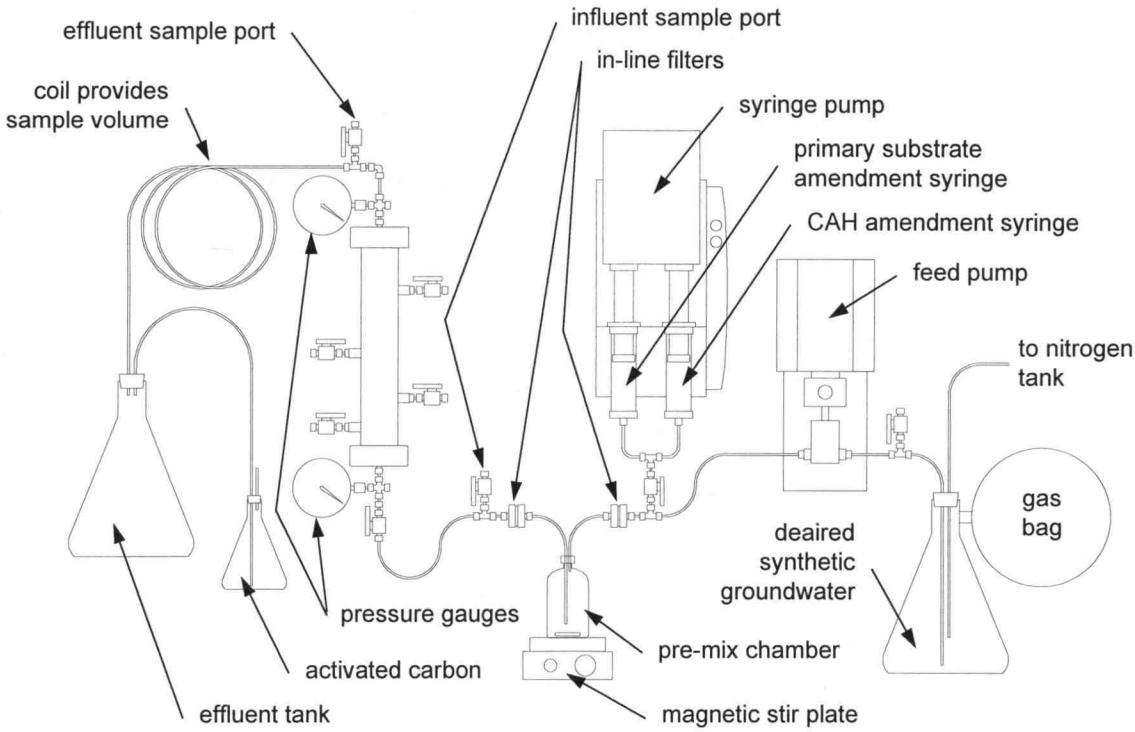

Figure 3.2. Column supply system.

Pressure gauges (Davis Instrument Co., Baltimore MD) on both ends of the column were used to indicate if the column was clogging. The gauges contained stainless steel wetted parts and were capable of measuring between 0 and 15 psi. A coil of stainless steel tubing attached to the effluent end of the column supplied 4 mL of fluid to the column during sampling. The influent and effluent containers consisted of 2 L Erlenmeyer flasks. A rubber gas bag (VWR Scientific, Seattle WA) was attached to the influent flask to maintain a positive pressure of nitrogen. The effluent flask was attached to a small flask containing activated carbon, in order to remove any contaminants that volatilized in the effluent.

Two in-line filters (Gelman Sciences Inc., Ann Arbor MI) were installed, for later experiments, on either side of the 125 mL pre-mix chamber to prevent biological growth in the chamber. The filter body installed upstream of the pre-mix chamber was made of Delrin® plastic. The downstream filter body was stainless steel. Both filters used

Gelman 25 mm FP Vericel® membranes capable of filtering particles over 0.2 μm. The filter membranes were changed periodically (every 1-2 weeks), or when the flow rate began to drop significantly.

All components in contact with fluid in the supply network were either autoclaved, soaked in a bleach solution, soaked in methanol or flamed prior to assembly. A detailed parts list of the components used in the supply system is included in Appendix K.

Experimental Procedure

For all experiments the column reactor system was operated in one of two modes, continuous-flow or batch. In the continuous-flow experiments all components shown in Figure 3.2 were operational. One of two flow conditions was established: high flow ($Q \cong$ 340 mL/day), establishing a hydraulic residence time of 0.5 days; or low flow ($Q \cong 100$ mL/day), with a residence time of 2 days. Where, Q represents the total amended flow rate; which will be subsequently referred to as the influent flow rate. In all cases, it was attempted to keep the concentration of CT and TCA in the influent at approximately 250 μg/L. The entire laboratory system was operated at room temperature (approximately 22°C) throughout the study. There were slight variations in temperature periodically, however, these fluctuations did not occur for extended periods.

In the batch experiments, flow was terminated and the fluid was allowed to remain static within the column for a specified period. This procedure was an effective and simple means of increasing the fluid residence time within the column. Conditions established during continuous-flow operation, just prior to termination, determined the initial conditions of the batch tests. The influent end of the column was sealed by closing the valve just upstream of the influent pressure gauge. The effluent end was sealed by installing a 50 mL gas-tight syringe containing synthetic groundwater amended with CT and TCA at 250 μg/L at the end of the effluent coil. This served as a source of make-up fluid to compensate for volume lost during sampling. To conserve fluid, samples were taken from a single port (port 3, see Figure 3.1) during the batch experiments. To

conclude the batch experiments, samples were taken from all ports to determine the final concentration distribution across the column.

Overall, the study consisted of two phases. The first phase served to characterize transport through the reactor column. During this portion of the research, important hydrodynamic properties were determined through breakthrough experiments with a conservative tracer. Bromide was chosen as the conservative tracer since it had a low background concentration in the synthetic groundwater. Continuous flow tracer studies were performed with a long pulse injection of bromide at 25 mg/L in the column influent. Following the transport experiments bromide was removed from the influent.

Table 3.3. Summary of the various conditions considered.

experiment	conditions
Transport Studies	high flow[a], bromide = 25 mg/L
Transformation Studies	
Pre Substrate	high flow
Benzoate as the Primary Substrate	
First Addition	high flow, benzoate = 20 mg/L
First Batch Test	initial conditions given above
Second Addition	high and low flow[b], benzoate = 20 mg/L, acetate = 0, 20, 32, and 40 mg/L
Second Batch Test	initial conditions given above (acetate was not present)
Acetate as the Primary Substrate	
First Addition	low flow, acetate = 40 and 50 mg/L
Yeast Extract Addition	low flow, acetate = 50 mg/L, yeast extract = 1 and 10 mg/L
Second Addition	low flow, nitrate absent, acetate = 50 mg/L
First Batch Test	initial conditions given above
Third Addition	low flow, acetate = 50 mg/L, nitrate replaced
Second Batch Test	initial conditions given above

[a]high flow = 0.5 day retention time

[b]low flow = 2 day retention time

The second phase of the experiment served to induce and monitor biotransformations of CT within the column. The effectiveness of benzoate and acetate as primary substrates was considered. The target concentration of benzoate and acetate in the influent varied from 20 to 50 mg/L depending on the experiment. For a short period, yeast extract was amended to the influent to provide concentrations of 1 and 10 mg/L. The yeast extract amendment differed from the CAHs and substrates in that, the line from the syringe pump was joined to the main influent line just prior to column entry (not shown in Figure 3.2) at a fitting across from the influent pressure gauge. Table 3.3 summarizes the conditions considered throughout the different phases of the experiment. Appendix A includes a chronological representation of the various events that occurred throughout the study.

Sampling Procedure

All samples were drawn from the various valved sample ports (see Figure 3.1 and Figure 3.2) using gas-tight 1 mL syringes with luer-lock tips (SGE, Ringwood Australia) equipped with 6 in. 20 ga. stainless steel needles with deflected points (Hamilton Co., Reno NV). Each port was additionally sealed by a white silicon No. 2 test tube septa (Oregon State University, Chem Store, Corvallis OR), modified to fit in place of the conical ferrule assembly on the outside of the valve. The purpose of the septa was to prevent oxygen from entering the system while sampling.

Typically, two 1 mL samples were drawn from each port, the first for anion analysis, the second for CAH analysis. During flow experiments column profiles were frequently obtained; in which all four sample ports plus the influent and effluent port were sampled. The volume of the effluent coil allowed four samples to be taken without drawing air into the back of the column. A period of approximately one hour was required to reestablish sufficient volume for a subsequent sampling set. Samples were drawn in the following sequence: effluent, port 4; one hour lapse; port 3, port 2; one hour lapse; port 1, influent. The sampling syringe was rinsed three times in both methanol and

deionized water between sample ports. The samples were further processed as described in the appropriate section below

Analytical

Chemical Sources

HR-GC grade methanol (purity 99.95%) was obtained from EM Science (Gibbstown NJ). Pentane specially purified for analysis of trace trihalomethanes in water, was obtained from Baxter (Muskegon MI). The CAHs were obtained from the following sources: carbon tetrachloride (purity 99.9%), EM Science (Cherry Hill NJ); chloroform (purity 99.9%), Mallinckrodt (Paris KY); 1,1,1-trichloroethane (purity 99.5%), J.T. Baker (Phillipsburg NJ); trichloroethylene (purity > 99.5%), Aldrich (Milwaukee WI).

Sodium benzoate (purity 99%) and sodium acetate (purity > 99%) were obtained from Aldrich and Spectrum (Gardena CA) respectively. Chemicals used to produce the synthetic groundwater were obtained from the chemical stock room in the Oregon State University Environmental Engineering Laboratory. All solutions were made with water obtained from a Culligan Series E Plus™ reverse osmosis unit (Culligan International, Northbrook IL) followed by a Barnstead NANOPure II™ deionizer (Barnstead Co., Newton MA).

CAHs

The method for CAH analysis was adapted from the method for trihalomethane analysis presented in Standard Methods for the Examination of Water and Wastewater (1992). Concentrations (CT, CF, TCA, TCE) were quantified on a Hewlett Packard (Wilmington DE) 5890 gas chromatograph equipped with a 7673A autosampler, a 3393A integrator and a ^{63}Ni electron capture detector. Separations were obtained via a stainless steel packed column (1/8" x 8'; 15% squalene; CPAW-DMCS; 80/100; 5327PC, Alltech, Deerfield IL) operated isothermally at 65°C. An argon/methane (95/5) mixture, at a head

pressure of 41 psi (flow rate = 25 mL/min), was used as the carrier gas. 2 μL injections were used; and injection port and detector temperatures were maintained at 65 and 320°C respectively.

The 1 mL aqueous samples were prepared for analysis through a liquid/liquid extraction technique. The technique consisted of first, dispensing 2 mL of pentane containing an internal standard (100 μg/L TCE) into 16 x 125 mm glass culture tubes with Teflon® lined screw-top caps (VWR Scientific, Seattle WA). Next, the aqueous samples were injected into the pentane and the tubes capped. They were then agitated on a vortex mixer for 30 seconds. Finally, 1.6 mL of the pentane phase was transferred to 2 mL amber glass autosampler vials, with Viton® crimp-top caps (Sun Brokers, Wilmington NC), for use on the autosampler. The prepared samples were usually analyzed immediately; however, on occasion freezer storage overnight was required due to instrument availability. Calibration curves were developed using external standards in methanol (see Appendix I).

Attempts were made to quantify dichloromethane (DCM) and *cis*-1,2-dichloroethylene (c-DCE) using this method. However, these compounds were much less sensitive to detection due to their lesser degree of chlorination, and could not be detected in the concentration range of interest.

Anions

Anion concentrations (nitrate, nitrite, chloride, bromide, sulfate, benzoate and acetate) were quantified on a Dionex (Sunnyvale CA) 4000i ion chromatograph equipped with an autosampler and a 4270 integrator. The instrument contained a Dionex Ionpac AS4A column; and utilized a regenerant containing H_2SO_4, and an eluant consisting of a mixture of Na_2CO_3 and $NaHCO_3$. The autosampler was programmed to deliver an injection volume of 50 μL.

Samples were prepared for analysis by dispensing them into 1.5 mL polypropylene flat top microcentrifuge tubes (Fisher Scientific, Pittsburgh PA) and centrifuging for at least 5 minutes in order to remove any suspended particles. 0.6 mL of

the supernatant was then transferred to Dionex Polyvials™ with filter caps for use on the autosampler. The prepared samples were either frozen or immediately analyzed, depending on the availability of the instrument. Calibration curves were developed using external anion standards (see Appendix J).

Other Analyses

Briefly during the course of the study, methods were used to analyze for total carbon and for total soluble iron. Total carbon was analyzed on a Rosemount Analytical (Santa Clara CA) Dohrmann® DC-190 TOC analyzer. 0.5 mL manual injections were used. Soluble iron was quantified on a Perkin Elmer (Norwalk CT) 360 atomic absorption spectrophotometer. The instrument included a HGA-2100 controller and graphite furnace, as well as a Westinghouse S728226 neon lamp. 50 μL manual injections were used.

4. RESULTS & DISCUSSION

Transport Studies

This phase of the experiment served to characterize transport through the soil column before attempts were made to induce biodegradation. Shortly after the soil column had been installed into the plumbing network, synthetic groundwater amended with bromide and CAHs was pumped through the column at a rate of 340 mL/day. The target influent concentrations (C_0) were 25 mg/L for bromide, and 250 µg/L for CT and TCA. Bromide was assumed to be a non-reactive (conservative) tracer and was added directly to synthetic groundwater solution; as opposed to CT and TCA which were amended to the influent by a separate side-stream (see Figure 3.2). Once pumping was initiated, the concentrations (C) were frequently measured in the influent and effluent. The resulting breakthrough curve for the bromide tracer is shown in Figure 4.1.

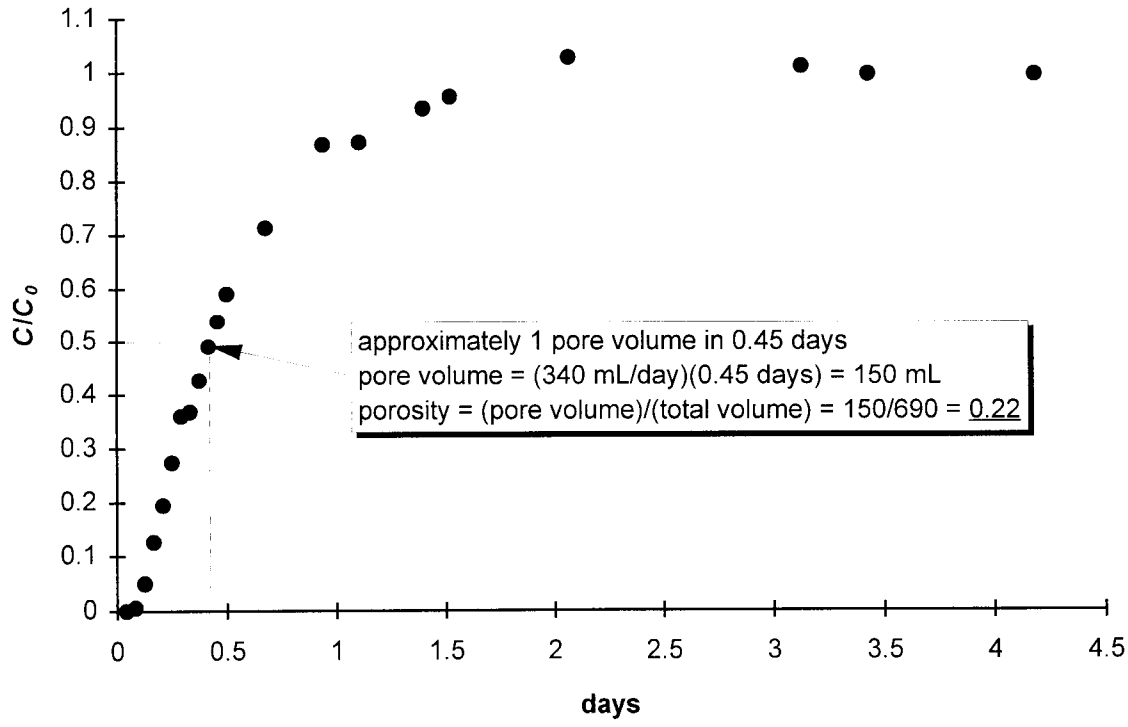

Figure 4.1. Bromide breakthrough data; flow rate = 340 mL/day.

If dispersion is negligible the breakthrough curve will be symmetric about the point where $C/C_0 = 0.5$; where one pore volume of fluid has passed through the column (Domenico and Schwartz, 1990). Thus, knowing the flow rate and the total internal volume, the porosity (n_e) can be estimated as 22% from the breakthrough curve as shown in Figure 4.1. However, the breakthrough curve was not symmetric about $C/C_0 = 0.5$. It tended to bend over more after this point, which is indicative of significant dispersive mixing. Therefore, the value of C/C_0 was greater than 0.5 after one pore volume had passed. In order to obtain a more accurate value for the porosity, it was required to fit the advection-dispersion equation to the data (see Appendix F). However, in addition to the porosity the Peclet number (P_e) was unknown, and thus the equation could not be solved directly.

A 1-D advection-dispersion computer model, developed by van Genuchten (1981) called Cfitm, was used to solve for n_e and P_e. Equilibrium between sorbed and aqueous phases was assumed. Values of n_e were arbitrarily selected until the best value of P_e was determined through a non-linear least squares technique. The combination yielding the smallest standard deviation (0.007) was: $n_e = 27\%$ and $P_e = 2.7$. The longitudinal dispersivity (α_L) was calculated from Equation E.5 to be 11 cm; using: $D_d = 2.01 \times 10^{-5}$ cm^2/s for bromide in water (Domenico & Schwartz, 1990) and $D^* = 0.7D_d$ for uniform sand (Fetter, 1993). These results confirmed that dispersion was significant within the column, which is reasonable considering the heterogeneous nature of the aquifer solids.

In addition to determining values for P_e and n_e, Cfitm also returned a corresponding retardation factor (r_f). For the combination of P_e and n_e above, the respective retardation factor was 1.01; which is supportive of the assumption of bromide being a conservative tracer. The CT and TCA breakthrough data was modeled using the values of P_e and n_e determined from the bromide data. Values of r_f were found to be 3.90 for CT and 2.64 for TCA, with standard deviations of 0.23 and 0.17 respectively. The breakthrough curves of all three compounds as well as their respective model simulations are shown in Figure 4.2.

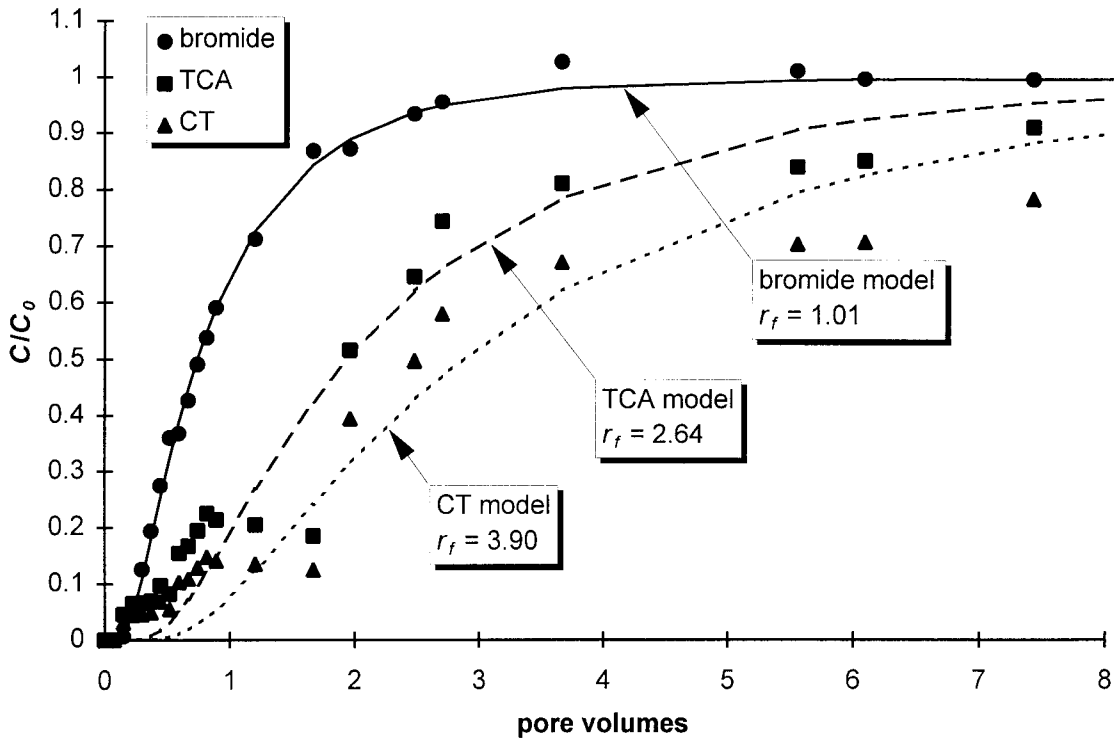

Figure 4.2. Comparison of breakthrough data to equilibrium Cfitm model; flow rate = 340 mL/day.

The model fit the bromide data exceptionally well, and did a reasonable job fitting the CAHs. Some variability in the CT and TCA influent concentrations occurred during the course of the experiment that could not be incorporated into the analytical model. CT and TCA appeared to "tail out" more at later times and breakthrough sooner at earlier times than the equilibrium model. This may be indicative of rate-limited sorption. Therefore, a more complex nonequilibrium model would likely yield better results.

Despite these limitations, the data and modeling results show that transport in the column is dominated by the processes of advection, dispersion and sorption. Significant sorption of CT and TCA occurs on Hanford subsurface solids. The response of the CAHs at the outlet of the column are delayed compared to those of a conservative tracer. TCA is less strongly sorbed and is subsequently less retarded than CT. This observation is consistent with TCA having a lower K_{ow} (Table 2.1). Based on the porosity of 27% and

the flow rate of 340 mL/day, the hydraulic residence time within the column was approximately 0.5 days throughout the transport experiments.

Transformations

Pre-Substrate

The period before, during and shortly after the transport experiments served as a pseudo-control period, to observe if transformations were occurring before the addition of growth substrate. The flow conditions for this period were the same as for the transport experiments: flow rate ≅ 340 mL/day and CT and TCA target influent concentration ≅ 250 μg/L. The first samples of effluent (taken a week after packing) showed a complete absence of nitrate. A surprising result considering nitrate was present at approximately 40 mg/L in the synthetic groundwater used to pack the column. Later samples indicated that nitrate was being transformed to nitrite which was subsequently being degraded

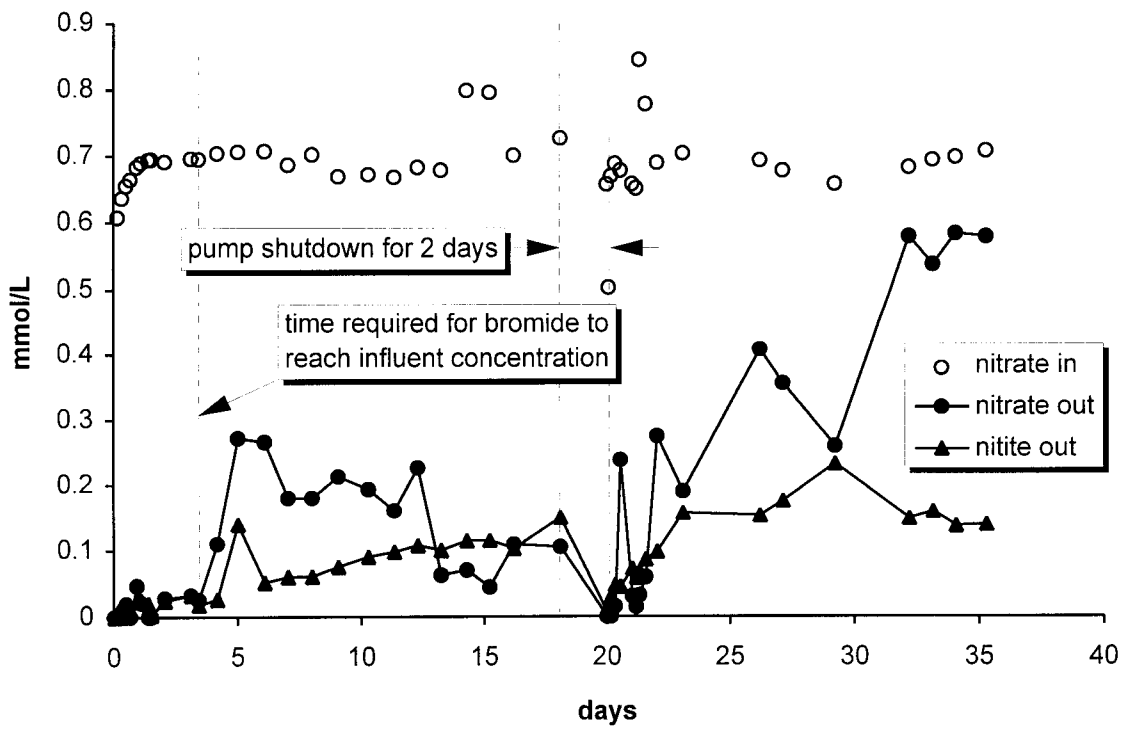

Figure 4.3. Nitrate and nitrite concentrations prior to substrate addition; retention time = 0.5 days.

within the column. The nitrate and nitrite concentrations in the influent and effluent during this period are shown in Figure 4.3. The bromide breakthrough curve (Figure 4.1) shows essentially complete breakthrough after 3 days. The lack of significant nitrate breakthrough after 3 days, indicates the loss of nitrate within the column in the absence of an added growth substrate.

This process slowed with time, as indicated by the increase in nitrate. Nitrate reached a steady concentration after about 1 month, at which time only a small fraction of nitrate was reduced in the column. Between 18 and 20 days, nitrate and nitrite dropped below the detection limit. This was caused by a pump failure which allowed the fluid to remain static in the column for 2 days, thereby effectively increasing the residence time for denitrification.

A total carbon analysis on the influent solution showed that approximately 3 mg/L of organic carbon existed in the deionized water used to prepare the synthetic groundwater; none of which could be removed by a 0.2 μm filter. However, that small of

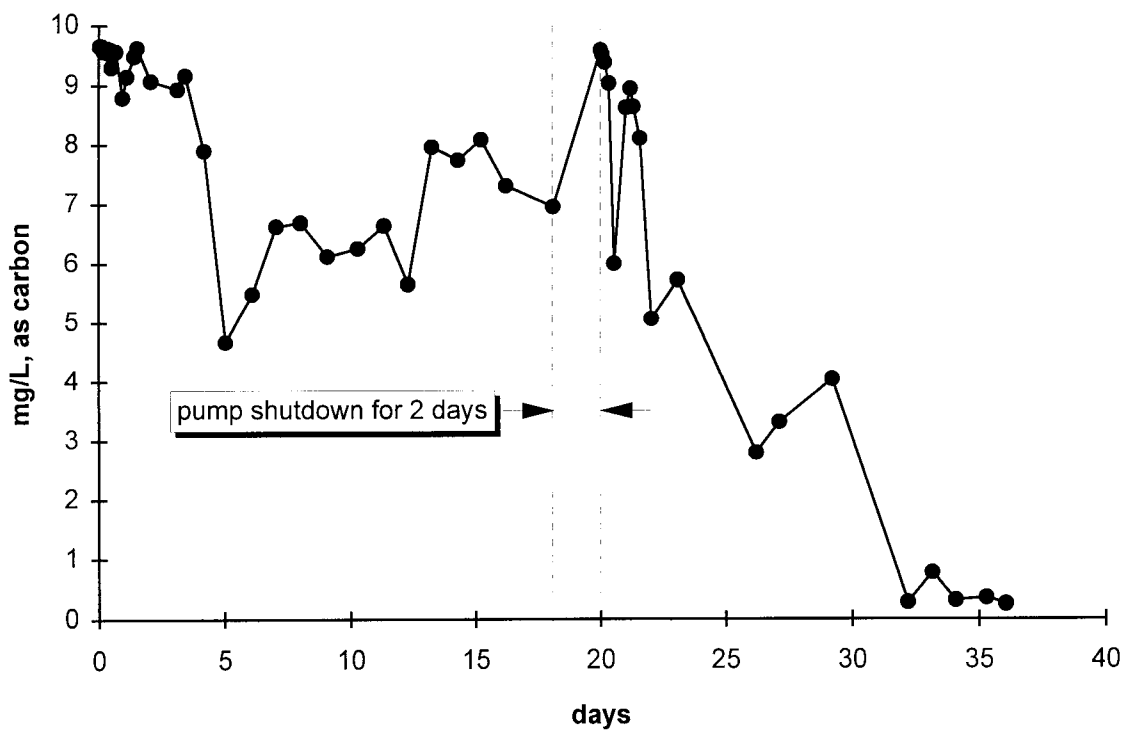

Figure 4.4. Amount of benzoate, expressed as carbon, required to denitrify the amounts shown in Figure 4.3.

an amount of carbon, even if it could all be used by microorganisms, could not be responsible for all of the denitrification observed. Figure 4.4 represents the stoichiometric amount of carbon, on a benzoate basis, required to denitrify the amounts shown in Figure 4.3 (see Appendix E for stoichiometry). The estimate assumes that nitrate is sequentially reduced to nitrite then nitrogen gas. The figure illustrates the decrease in denitrification with time.

The influent and effluent CAH concentrations are shown in Figure 4.5. The data showed a small amount of CT transformation taking place during the first month of this pre-substrate period, indicated by the presence of CF in the effluent. Also, during this period, the ratio of the effluent to the influent concentration was almost always smaller for CT than TCA; indicating that less CT was recovered than TCA. A 125 mL serum bottle, placed on a magnetic mixer, was installed 20 days into the experiment in order to stabilize influent concentrations (see Figure 3.2). As with denitrification, the process slowed with time except for the 2 day period when the pumped stopped.

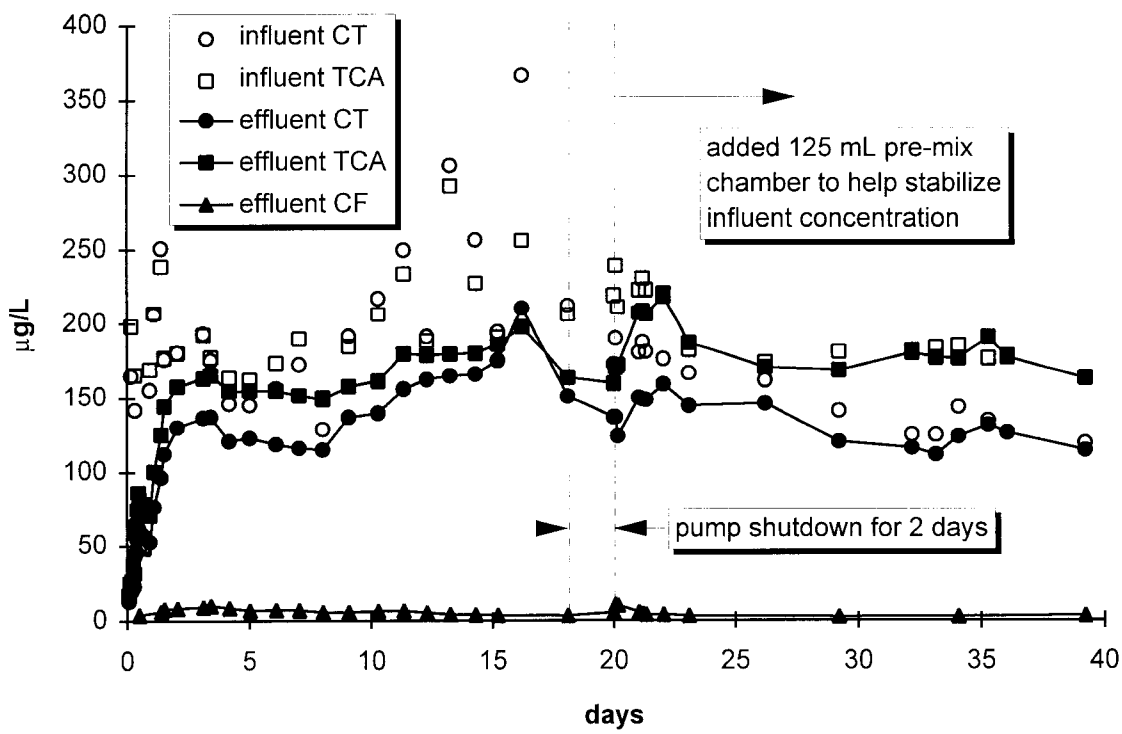

Figure 4.5. Influent and effluent CAH concentrations prior to substrate addition; retention time = 0.5 days.

Profiles of the anions and CAHs taken at days 27 and 29 respectively are shown in Figure 4.6. Nitrate consumption and nitrite production occurred throughout the length of the column. Rates of denitrification appeared to be higher near the influent end of the column. No significant changes were observed in the sulfate and chloride concentrations across the column (data not shown). CT and TCA were reduced by about 15 and 7%, respectively, across the column. CF rapidly increased then gradually decreased after port 1 (3.5 cm).

CT appeared to be transformed most rapidly near the influent end of the column. However, the concentration gradually increased after port 1. The similar trend in TCA concentration is supportive of the influence of non-biological processes. Inconsistent influent CAH concentrations were probably responsible for the majority of the fluctuations in the column profiles. It was found that changes in temperature changed the concentration of the saturated aqueous stock solutions. Also, minor fluctuations in pumping rates could not be avoided. Sorptive processes may also have contributed to the changing conditions.

Assuming that the fluctuation of CT in the influent was proportional to that of TCA and that the sorptive effects of the two compounds were similar, then the non-biological losses in CT across the column could be approximated by normalization to the reduction in TCA (assumed to be biologically recalcitrant) across the column. In that regard, since TCA was reduced by 7%, only 8% of the observed 15% reduction in CT across the column can be attributed to transformation processes. In which case, CF in the effluent would account for 23% of the CT transformed, on a molar basis. Without normalization CF accounted for 14% of the CT transformed.

It is unclear why denitrification and CT transformation occurred in the absence of a primary substrate. It is possible that a carbon source may have been present in the aquifer material initially, and was slowly degraded over time. The observed slowing in transformations over time is supportive of this hypothesis. The fact that denitrification proceeded without a period for growth suggests that a reasonable population of denitrifiers already exists in the Hanford subsurface as a result of the nitrate contamination there.

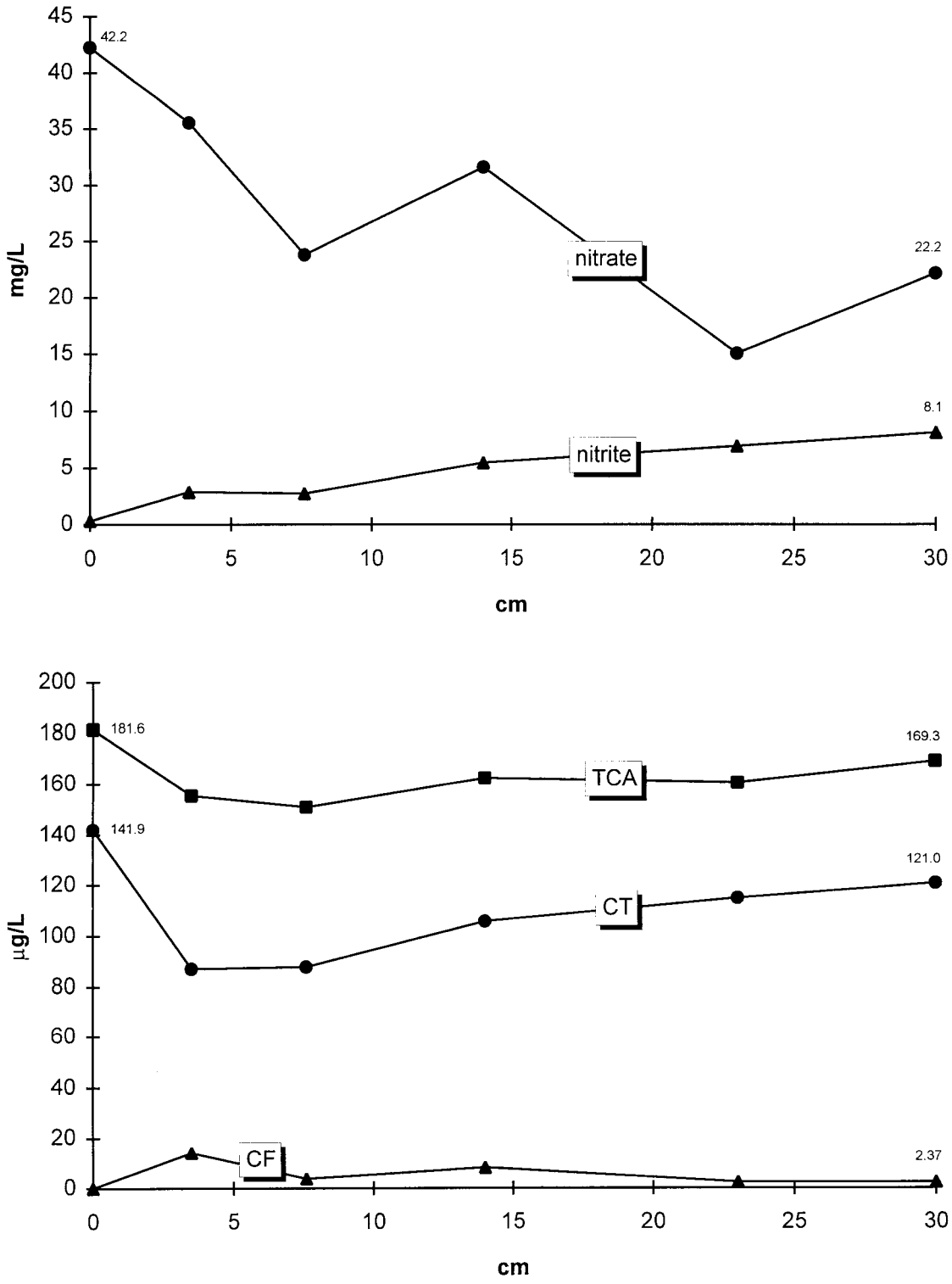

Figure 4.6. Column profiles (day 27 anions and day 29 for CAHs) prior to substrate addition; retention time = 0.5 days.

After 34 days the effluent CT concentrations nearly equaled the influent, indicating that losses across the column were slight. An average of the 3 influent and effluent data samples after this point show that CT was reduced by only 2% across the column. Also CF in the effluent was very low at this time; being below the detection limit for most samples. Figure 4.4 indicates that denitrification had basically stopped at this point as well. The low levels of transformation would appear to indicate that microbial activity had slowed to a minimal level, and that CT could pass relatively unabated by abiotic reactions through the soil column at the particular flow conditions considered.

Benzoate as the Primary Substrate

First Benzoate Addition

Benzoate was amended to the influent solution 56 days after initiation of the transport experiments. The target influent benzoate concentration was 20 mg/L, slightly more than the amount required for stoichiometric denitrification. The flow conditions were the same as for the transport experiments: flow rate ≅ 340 mL/day, CT and TCA target influent concentration ≅ 250 μg/L. Unfortunately, due to problems with the ion chromatograph, most of the data for the anions were unusable for this portion of the experiment. However, reliable data indicated that after one week of addition, benzoate was reduced by only 36% across the column. Also, significant amounts of nitrate and nitrite remained in the effluent, meaning that denitrification was incomplete.

Analysis of the CAHs showed similar limited transformation. A 12% reduction in CT was observed across the column. However, a 7% reduction in TCA resulted in CT being reduced by only 5% after normalization. In which case, CF present in the effluent (~2 μg/L), would represent about 24% of the CT transformed (16% if not normalized). This removal was similar to that observed at later times in the pre-substrate experiments (Figure 4.6).

First Batch Test with Benzoate

One week after starting the benzoate addition a batch experiment was initiated. This provided a simple means of screening for the potential of benzoate to induce CT degradation by increasing the retention time and allowing time for acclimation. After 12 days, samples were taken from ports 1 and 4 and compared to the influent and effluent at the time of shutdown. The results of the experiment are shown in Table 4.1.

Benzoate, nitrate, and nitrite were essentially removed from the column; sulfate and chloride (chloride data not shown) varied insignificantly. The majority of CT was removed as well, with 84 and 72% being removed from ports 1 and 4 respectively. TCA was reduced by 20% at port 1 and 23% at port 4. The CF produced accounted for 20 and 14% of the CT transformed at ports 1 and 4 respectively. More complete removal of CT at port 1 than port 4 was consistent with the greater loss of benzoate in the front of the

Table 4.1. Comparison of the transformations during the first batch experiment with benzoate to those prior.

	just prior to shutdown			12 days after shutdown			
	influent	effluent		port 1		port 4	
	conc. µg/L	conc. µg/L	% reduction	conc. µg/L	% reduction	conc. µg/L	% reduction
CT	215	190	12 (5)[a]	35	84 (64)	61	72 (49)
CF	∅	2	-16[b] (-24)	27	-20 (-25)	17	-14 (-21)
TCA	215	200	7	173	20	166	23
	mg/L	mg/L		mg/L		mg/L	
benzoate	25	16	36	2	92	0	100
nitrate	50	45	10	0	100	1	98
nitrite	∅	4	NA	2	NA	2	NA
sulfate	38	38	0	39	0	39	0

[a]Percentages in parenthesis represent reductions normalized to percent change in TCA.

[b]CF % reduction represents the percent of CT transformed to CF on a molar basis, and is of negative sign since CF was produced as a result of CT transformation.

column during the flow test. This is supportive of microbial, as opposed to abiotic, transformation being responsible for the majority of the CT reduction.

The fact that approximately the same amount of TCA was lost at both ports 1 and 4 (with even a slightly higher loss at port 4) would seem to indicate that the TCA reduction was not the result of biological processes. Assuming that the non-biological losses of CT and TCA from the column were approximately the same, then the loss of CT as a result of biological processes (normalized reduction) was 64% from port 1 and 49% from port 4. In which case, CF accounted for 25 and 21% of the CT transformed at ports 1 and 4 respectively.

Part of the CT loss could have resulted from sorptive uptake onto the aquifer solids. CT sorbs more strongly than TCA, based on the breakthrough curves shown in Figure 4.2. However, if equilibrium between the aqueous and sorbed phases is assumed prior to initiation of the batch experiment, CT could have possibly desorbed from the aquifer solids as the aqueous concentration dropped. Thus, possibly increasing the amount of CT in the aqueous phase and the mass of CT transformed.

Second Benzoate Addition (with and without Nitrate)

Because of the success of the batch test it was decided to reestablish a flow of benzoate to the column. One week after completion of the batch experiment, benzoate injection was initiated under the same conditions considered previously (flow rate $\cong$ 340 mL/day; target influent concentrations: benzoate $\cong$ 20 mg/L; CT and TCA $\cong$ 250 μg/L). After 3 days it became apparent that no significant improvement in benzoate utilization, denitrification or CT transformation was being achieved over that of the previous benzoate flow condition.

After 2 weeks the flow was lowered to 100 mL/day to establish a retention time of approximately 2 days within the column. This was about 4 times less than the original flow. Representative column profiles for the reduced flow condition, taken 6 days after the reduction, are shown in Figure 4.7.

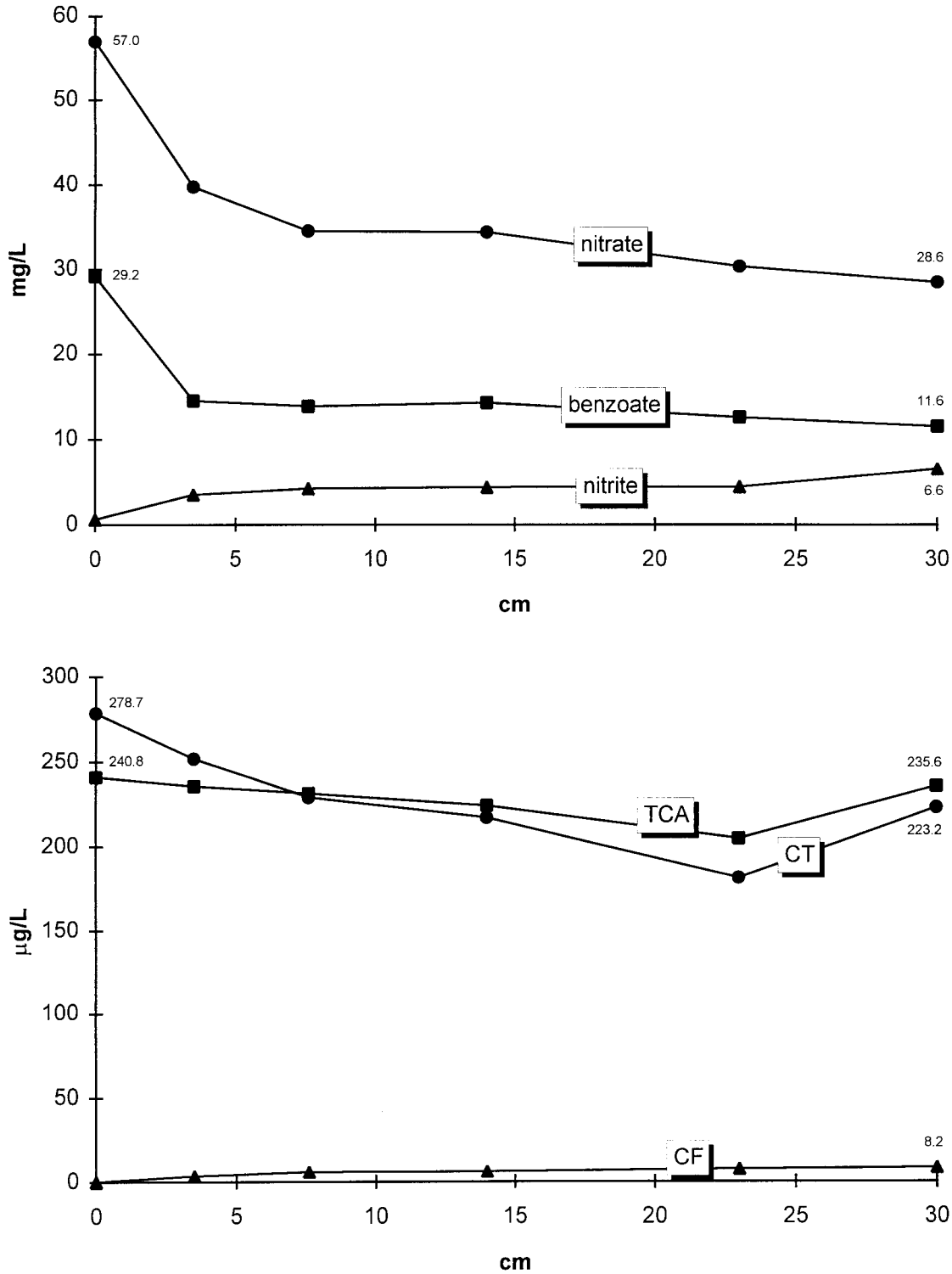

Figure 4.7. Column profiles 6 days after flow reduction; primary substrate = benzoate, retention time = 2 days.

The profiles showed increased transformations compared to the higher flow conditions. However, benzoate consumption and denitrification were still only partially complete. CT transformation was also limited. CT was reduced by approximately 20% across the column, while benzoate was reduced by about 60%. Again, sulfate and chloride remained constant across the column (data not shown). More CF existed in the effluent than the previous cases. Also, the CF showed an increasing trend along the entire column, and represented about 21% of the CT transformed across the column. The gradual decrease to port 4, then sudden rise in CT and TCA is probably indicative of difficulties maintaining consistent influent concentrations. However, the net change in TCA across the column was negligible (2%), therefore CT normalization was not performed in this case.

It was considered that the sluggishness of denitrification may have been due to a phosphate limitation, since no phosphate was present in the synthetic groundwater. Therefore, phosphate was added to the synthetic groundwater so that the concentration in the influent solution was 0.5 mg/L. However, no improvement in benzoate utilization or CT transformation was observed, and the phosphate addition was discontinued.

2 weeks after the flow reduction it was decided to add acetate at 20 mg/L along with benzoate (20 mg/L). The effects were noticed almost immediately. 1 day after the addition was initiated, slightly more denitrification was observed. After 3 days denitrification was almost complete. Despite the improved denitrification, no simultaneous improvement in CT transformation was observed.

At no time was acetate detected at any of the sampling ports, including the influent port. Samples of the fluid inside of the pre-mix chamber indicated that acetate was totally utilized within it (see Figure 3.2), producing an approximately stoichiometric amount of denitrification. Apparently, microorganisms rapidly growing on acetate, had contaminated the pre-mix chamber.

It was decided to take advantage of the situation at hand before making changes that would allow acetate to enter the column. The acetate concentration was gradually increased to 40 mg/L, which produced a situation where denitrification was complete and acetate was removed prior to entry into the soil column. Thus, it could be observed if CT

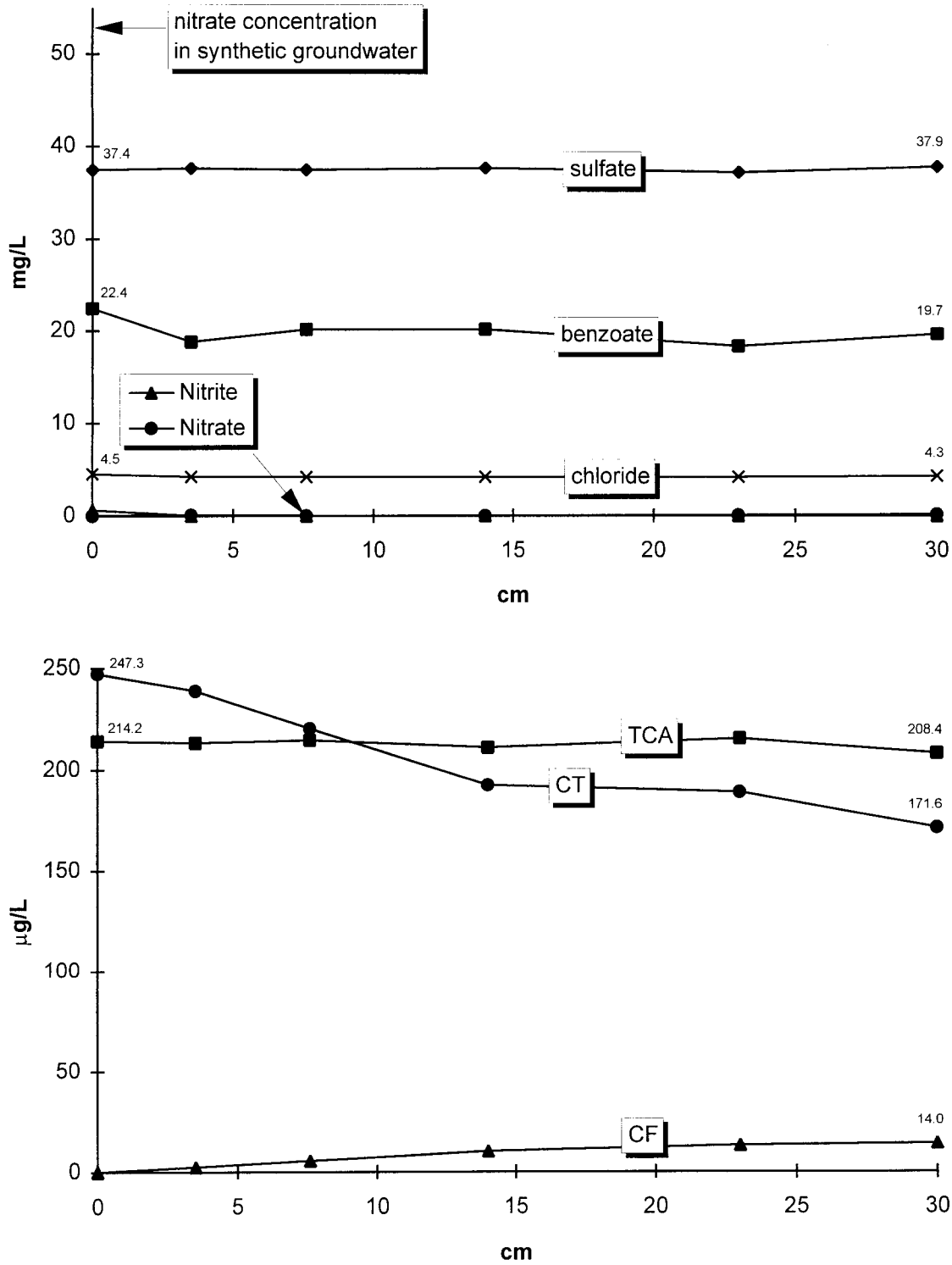

Figure 4.8. Column profiles 1 week after acetate was added at 40 mg/L to complete denitrification within the pre-mix chamber; primary substrate (within soil column) = benzoate, retention time = 2 days.

transformation in the column was enhanced in the absence of nitrate or nitrite. This would be possible because benzoate was passing, relatively unabated, through the pre-mix chamber.

Anion and CAH profiles taken 1 week into the 40 mg/L acetate addition are shown in Figure 4.8. The CAH profile indicated more CT reduction and CF production than any previous profile taken during flow conditions. The anion profile revealed that the benzoate concentration was only slightly reduced. CT was reduced by approximately 30% across the column, where benzoate was reduced less than 10%.

The level profile of TCA was indicative of consistent operating conditions. Normalization of the CT reduction was not necessary because TCA was negligibly reduced across the column (3%). CF accounted for 24% of the CT transformed. More complete reduction of CT in the absence of denitrifying conditions seems to indicate that the presence of nitrate and/or nitrite inhibited CT reduction, which is supportive of Semprini *et al.* (1992), Petersen *et al.* (1993) and Stensel and DeJong (1994). However, denitrification with acetate in the pre-mix chamber may have produced soluble products which passed through the in-line filter and influenced transformations within the column.

Again, sulfate and chloride remained constant across the column. It is interesting that excess benzoate did not bring about sulfate reduction in absence of nitrate and nitrite. However, insufficient time may have been allowed to induce sulfate reducing conditions. It is possible that iron reducing bacteria may have been stimulated. Quantitative analysis for the presence of reduced iron in the effluent was not performed. The effluent possessed a slightly orange tint, possibly indicative of the reduction of ferric (Fe^{+3}) to ferrous (Fe^{+2}) iron in the column, with reoxidation of the iron when the effluent water became exposed to oxygen.

Second Batch Test with Benzoate (without Nitrate)

A second batch experiment was conducted to further observe CT transformation in the absence of nitrate and nitrite. In this experiment, samples were taken not only at

the beginning and end of the experiment, but throughout. Thus, a better understanding of the kinetics of CT transformation could be established.

The experiment was initiated approximately 3 weeks after acetate was added in combination with benzoate. At this point in time: acetate was totally utilized in the pre-mix chamber; denitrification was essentially complete before entering the column; and benzoate into the column remained at approximately 20 mg/L. Figure 4.8 (shown previously) represent the conditions which existed throughout the column just prior to initialization of the experiment.

The batch experiment was conducted over a period of 3 weeks. All samples were drawn from port 3 on the column, which is essentially at the center of the column length. A 50 mL gas-tight syringe, attached to the stainless steel effluent tubing coil, provided synthetic groundwater amended with 250 μg/L of CT and TCA, to make up for volume lost during sampling.

Figure 4.9 shows the anion and CAH concentrations throughout the batch experiment. Time zero on the figure represents the concentrations at port 3 just before the batch experiment was initiated. Benzoate was reduced by about 50% over a two week period, based on the concentration in the influent during flow conditions just prior to termination. No significant change in chloride or sulfate was observed. It is possible that some of the benzoate reduction was due to dilution effects, since the 50 mL make-up syringe did not contain benzoate. The lack of anion data for the final week of the experiment resulted from problems with the IC analysis at that time.

Based on influent concentrations prior to shutdown, CT and TCA were reduced by approximately 97 and 14%, respectively, over the entire 3 week test. Normalization to losses in TCA resulted in a 83% reduction in CT. CF accounted for 43% of the CT transformed; 50% in the normalized case. It is possible that more CT was transformed than indicated above, as a result of CT desorbing from the aquifer solids as the concentration decreased. In addition, CF would be expected to be less strongly sorbed than CT based its smaller octanol/water partition coefficient (see Table 2.1)

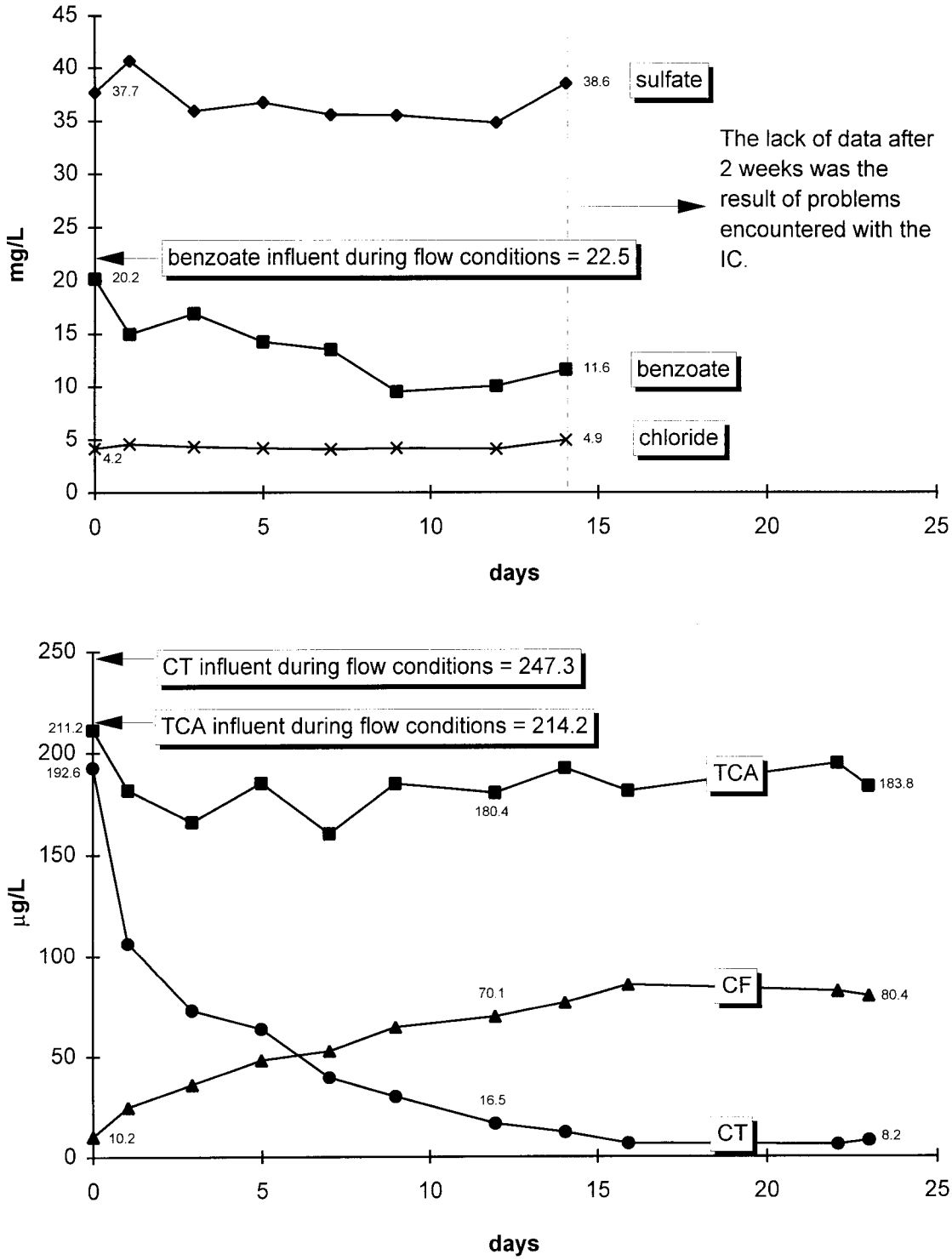

Figure 4.9. Concentrations at port 3 during the second batch experiment with benzoate, acetate was used to remove nitrate prior to entry into soil column.

Rapid transformation of CT and production of CF was observed at early time, but the rates slowed over time. This trend indicated that the transformations could possibly be modeled as first-order process(es). Therefore, assuming that the cell concentration was constant (steady-state), a plot of $-\ln(C / C_0)$ vs. time would yield a straight line, where the slope of the line is the first-order rate constant (k). Figure 4.10 shows the data plotted in this fashion. C_0, in the equation, represents the concentration at the port of interest (port 3 in this case) at the time the batch experiment was initiated, not the influent concentration.

In the figure, CF is plotted with CT and TCA for convenience. Actually, it is of opposite sign since it is being produced, not degraded in the column. CT was the only CAH that followed a linear trend. Linear regression of the data yielded a first-order rate constant (k) of about 0.21 day^{-1} with an R^2 of 0.97. The last two data points were not included in the regression because the semi-log plot dramatized the error in quantifying

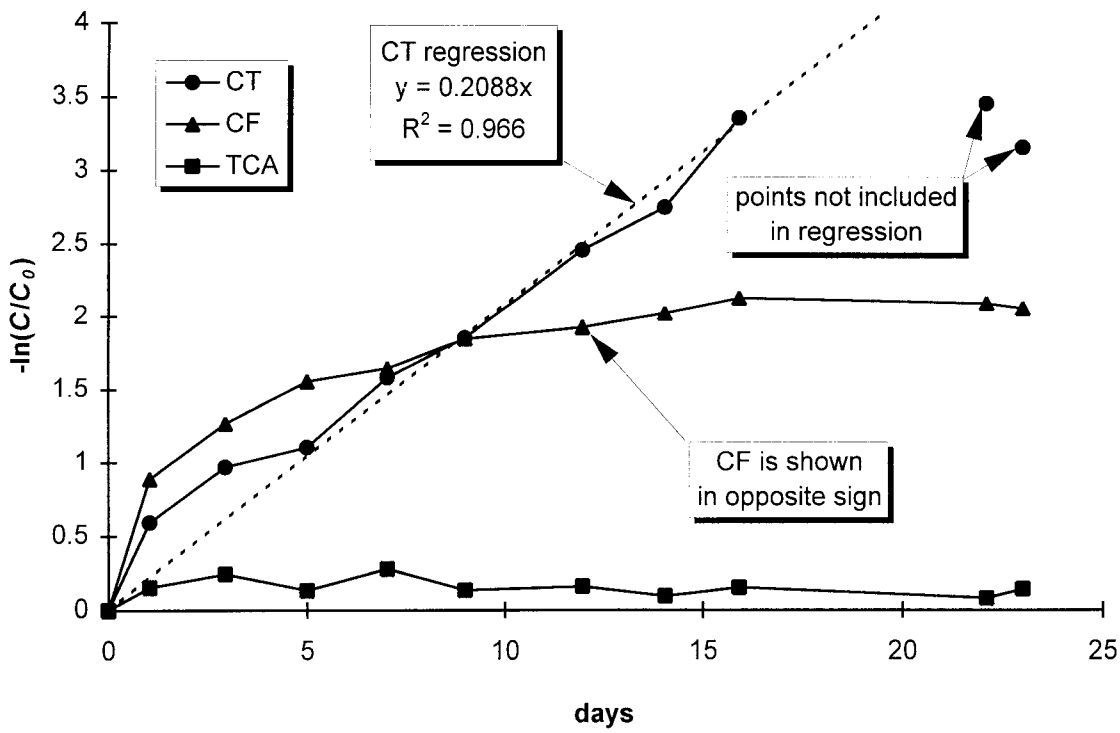

Figure 4.10. First-order check on CAHs for the second batch experiment with benzoate.

CT concentrations near the detection limit. CF did not follow a first-order trend since it is both formed and possibly degraded in the column. Also, the ratio of the amount of CF formed to the amount of CT degraded was not necessarily constant during the test.

A comparison of the data for CT and CF, at day 12 from Figure 4.9, to the first batch test (12 days in length) is shown in Table 4.2. It is reasonable to assume that the concentration at port 3, for the first test, would fall between that for ports 1 and 4. In which case, significantly less CT remained in the column after the second batch test than the first. Much more TCA was lost in the first test than the second. Also, the amount of CF present after the second test is much higher than the first.

The first batch test differed from the second in that significant amounts of nitrate and nitrite were present at the initiation of the experiment. The presence of nitrate and/or nitrite likely inhibited CT transformation. However, the fraction of CF observed in the second test suggests that the absence of nitrate and nitrite can result in higher residual CF concentrations. This could mean that less CF is formed under denitrifying conditions, however CT is degraded at a slower rate. More complete removal of benzoate in the first batch test is a result of the demand on benzoate to complete denitrification.

Table 4.2. Comparison of residual concentrations and % reductions after 12 days, between the first and second batch test with benzoate.

	first batch test influent flow concentrations CT = 215 μg/L, TCA = 215 μg/L				second batch test influent flow concentrations CT = 247 μg/L, TCA = 214 μg/L	
	port 1		port 4		port 3	
	μg/L	% red.	μg/L	% red.	μg/L	% red.
TCA	173	20	166	23	195	9
CT	35	84 (64)[a]	61	72 (49)	16	94 (85)
CF	27	-20[b] (-25)	17	-14 (-21)	70	-39 (-43)

[a]Percentages in parenthesis represent reductions normalized to percent change in TCA.

[b]CF % reduction represents the percent of CT transformed to CF on a molar basis, and is of negative sign since CF was produced as a result of CT transformation.

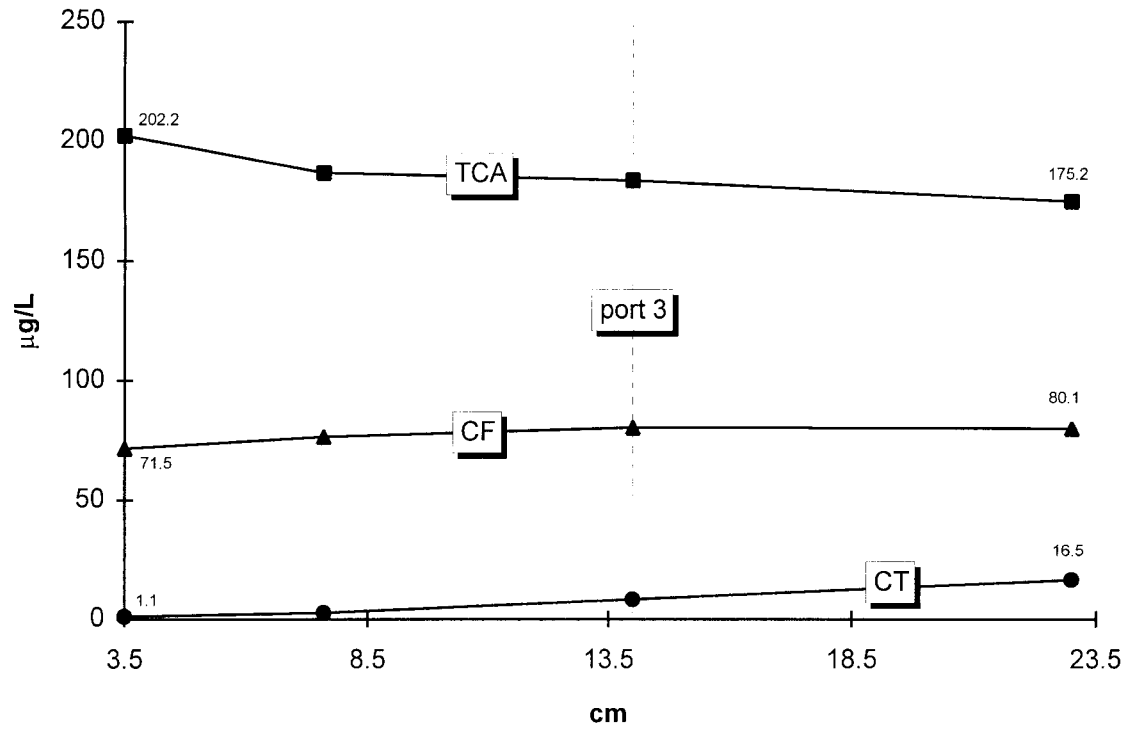

Figure 4.11. CAH concentrations throughout the column at the end of the second batch experiment with benzoate.

Upon completion of the second batch experiment, samples were taken at all ports along the length of the column. The results, shown in Figure 4.11, indicate that CT transformation was more complete near the front of the column. Subsequently, it would seem reasonable that more CF would have accumulated there as a result. However, CF was slightly lower near the front of the column, indicating that CF production was less or CF transformation was occurring there. Overall, these effects would seem to indicate that a larger biological mass existed near the front of the column, despite that excess benzoate existed throughout. It is also possible that soluble products from denitrification with acetate in the pre-mix chamber may have influenced CT transformations.

The lower CT concentration at the influent end of the column may also be indicative of different rates of transformation across the column. First-order rate constants throughout the batch test, at ports 1-4, were approximated using the column profile during flow conditions (Figure 4.8) and Figure 4.11. The calculated constants for

Table 4.3. First-order rate constants at ports along the column length at the end of the second batch experiment with benzoate.

port	1 (3.5 cm)	2 (7.6 cm)	3 (14.0 cm)	4 (23.0 cm)
initial CT concentration (μg/L)	238.9	220.4	192.6	189.2
final CT concentration (μg/L)	1.1	2.6	8.2	16.4
k (day^{-1})	0.23	0.19	0.14[a]	0.11

[a]The value calculated from Figure 4.16 is different because it was calculated from the regression of a series of data.

the 23 day period are shown in Table 4.3; efforts were not made to normalize to changes in TCA. The table shows that rates were approximately a factor of 2 higher at port 1 than port 4. This trend seems to support that a larger biological mass existed near the influent end.

Acetate as the Primary Substrate

First Acetate Addition

Although acetate had been amended to the influent solution previously it had not been exposed to the soil column for any significant length of time. It had already been shown that acetate was able to complete denitrification much faster then benzoate. Therefore, in order to determine if acetate could also increase the rate of CT transformation, benzoate was removed from the influent solution and acetate was added to the column at 40 mg/L. The influent lines were resterilized and in-line (0.2 μm) filters were installed on both sides of the pre-mix chamber to prevent biological growth within it (see Figure 3.2). The addition was started after flushing the column with synthetic groundwater (flow rate ≅ 100 mL/day; and CT and TCA target influent concentration ≅ 250 μg/L) for 17 days following the second batch test with benzoate.

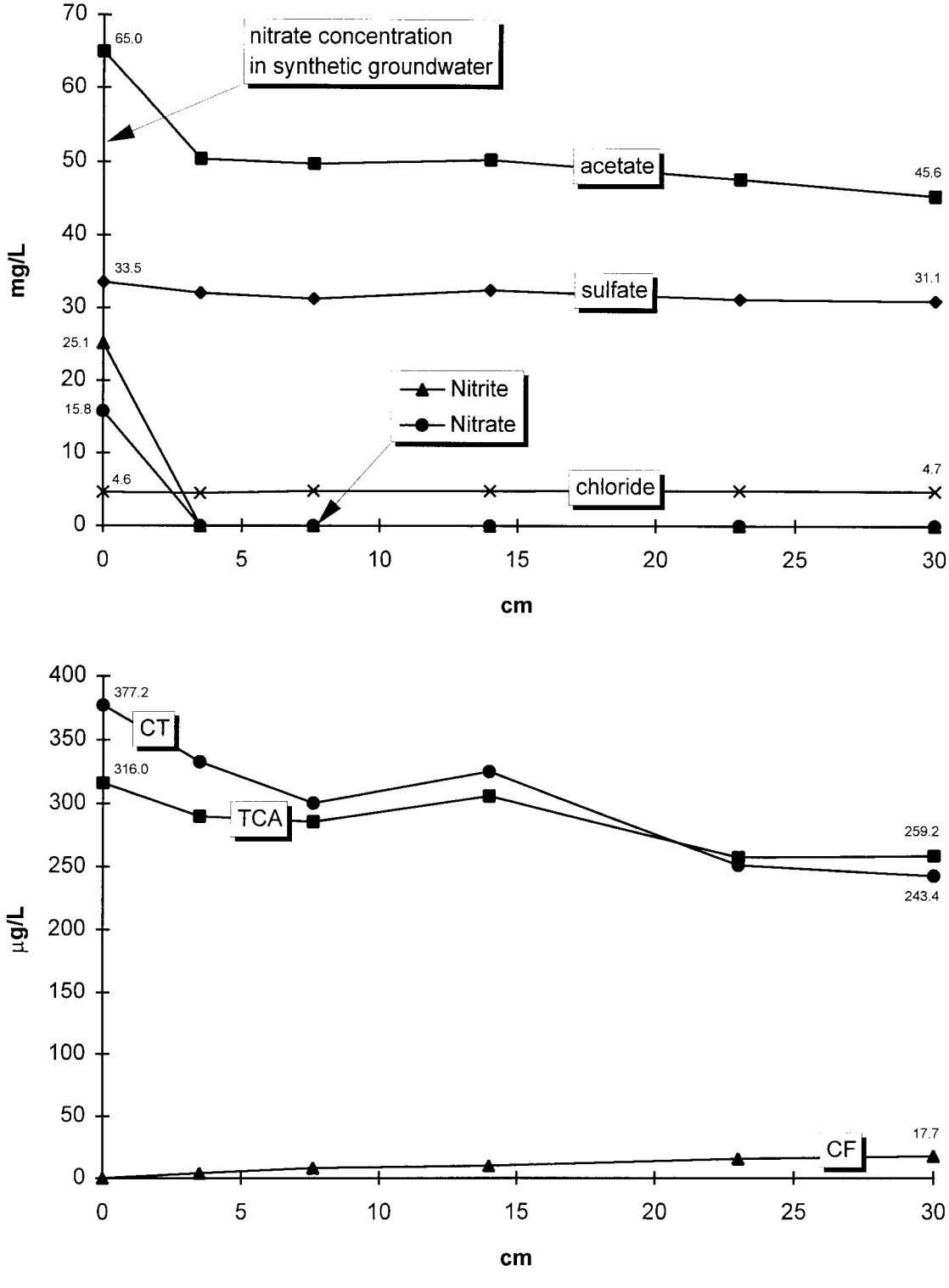

Figure 4.12. Column profiles 4 weeks after the addition of acetate to soil column; retention time = 2 days.

After 1 week of acetate addition at 40 mg/L under the flow conditions stated above, denitrification was still incomplete within the column while acetate was completely consumed. Also, CT transformation at this time was small (15% or less). Therefore, the acetate concentration was raised to 50 mg/L. Shortly following the increase in acetate concentration, nitrate and nitrite were no longer detected in the effluent, indicating that denitrification was complete within the column. In addition, following the change in acetate concentration, a gradual increase in CT reduction and CF production was observed until steady-state conditions were established after 3 weeks.

The corresponding anion and CAH profiles are shown in Figure 4.12. The CAH profile shows a loss in CT of about 36% across the column. However the TCA concentration shows a reduction of approximately 16%. This effect was due to bio-clogging of the in-line filters, resulting in gradually increasing influent concentrations (which eventually reached concentrations significantly higher than the previous experiments). If the CT reduction is normalized to losses in TCA to help correct for this effect, then the resulting CT transformation was 20%. This is similar to what was observed in the benzoate experiment with nitrate present in the influent. The CF produced accounted for 17% of the CT transformed (30% based on normalized CT reduction), which is higher than observed in any of the benzoate flow experiments.

Analysis for soluble iron indicated that less than 50 μg/L of total iron existed in samples of the effluent. However, despite the lack of significant iron in solution, it is possible that biologically mediated transformation of CT was occurring simultaneously with iron reduction on mineral surfaces.

Yeast Extract Addition with Acetate

Yeast extract was amended to the influent solution 2 months after acetate had been established as the primary substrate. The flow conditions were the same as those previously: flow rate ≅ 100 mL/day; and CT and TCA target influent concentration ≅ 250 μg/L. The yeast extract was intended to provide a rich mixture of vitamins and nutrients

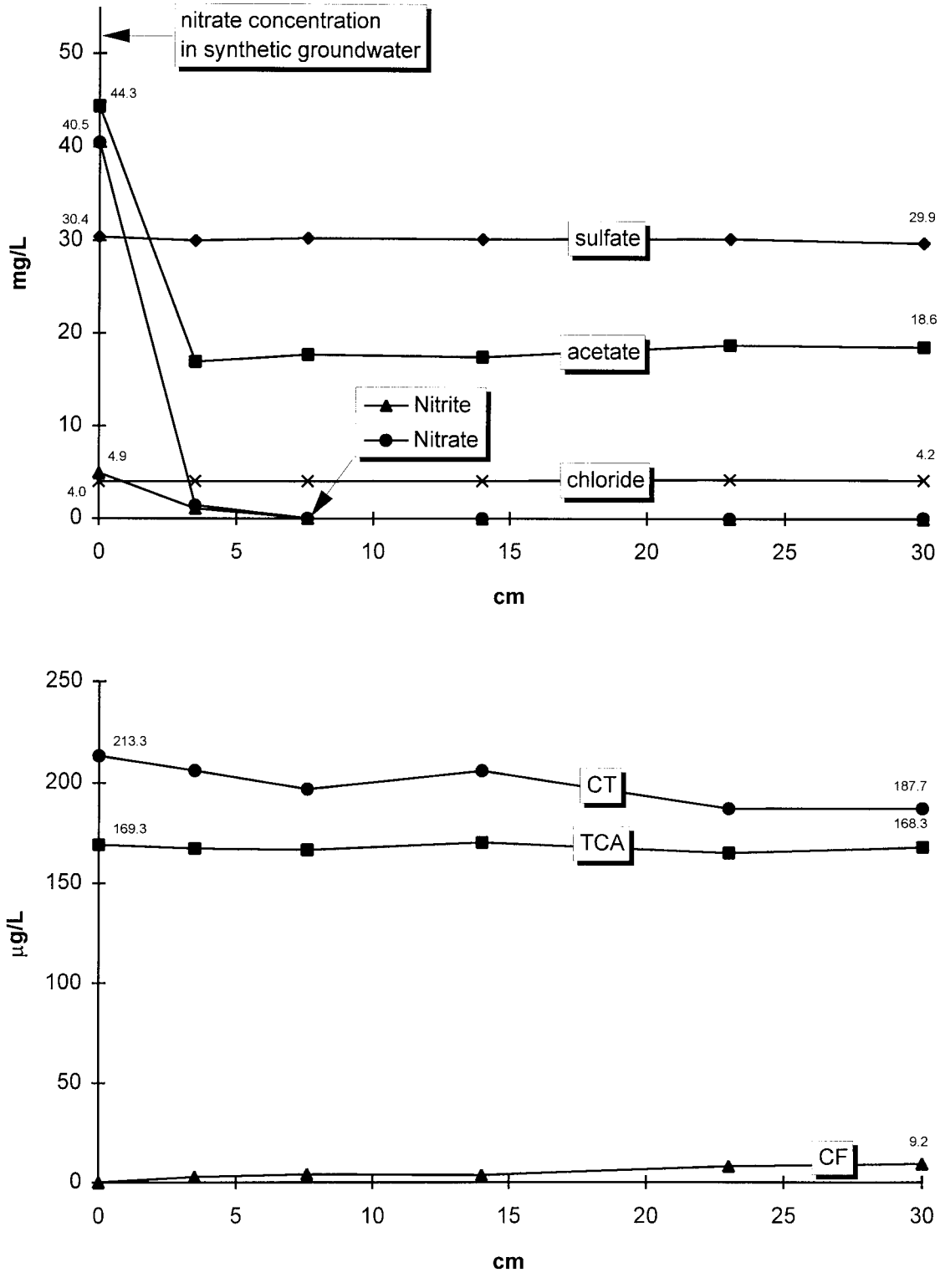

Figure 4.13. Column profiles 4 days after the addition of yeast extract at 10 mg/L to soil column (following a 3 week addition at 1 mg/L); primary substrate = acetate, retention time = 2 days.

in order to determine if the deficiency of these compounds in the synthetic groundwater may have been responsible for limiting CT transformation.

Initially, the concentration of yeast extract in the influent was approximately 1 mg/L. This addition was continued for 3 weeks. However, no significant changes were observed in CT transformation, acetate utilization or denitrification. Therefore, the yeast extract concentration was increased to 10 mg/L. Addition at the higher concentration was continued for approximately 1 week.

Column profiles 4 days after the increase to 10 mg/L are show in Figure 4.13. Acetate was reduced by 58% across the column. No significant changes were observed in sulfate or chloride. Interestingly, denitrification within the pre-mix chamber slowed during this period. The anion profile prior to the addition of yeast extract (Figure 4.12) showed almost complete denitrification prior to entry into the soil column. However, Figure 4.13 shows that only a small amount of the nitrate in the synthetic groundwater (~50 mg/L, initially) had been reduced through the pre-mix chamber.

CT was reduced by only 12% across the column. Normalization to the change in TCA was not required because the loss in TCA across the column was negligible (< 1%). The CF produced accounted for 46% of the CT transformed. It is unclear why the extent of CT transformation decreased and the relative CF production increased, following the addition of yeast extract (from 20 and 30% respectively, in the prior situation). Due to the lack of enhanced transformation of CT, the addition of yeast extract was discontinued approximately 1 week following its addition at 10 mg/L.

Second Acetate Addition (without Nitrate)

Nitrate was removed from the synthetic groundwater by discontinuing the addition of $Ca(NO_3)_2$ one month after the experiment with yeast extract was initiated. The addition of yeast extract to the influent solution was discontinued at this time as well. The flow conditions were the same as those previously: flow rate ≅ 100 mL/day; and CT and TCA target influent concentration ≅ 250 μg/L. The removal of nitrate from the synthetic groundwater was considered to be a simple means of evaluating if it was

inhibiting CT transformation. Also, its removal provided an interesting comparison to the situation where benzoate was the primary substrate and nitrate and nitrite were degraded prior to entry into the soil column.

Figure 4.14 shows the anion and CAH profiles 3 weeks after nitrate was removed from the synthetic groundwater. No significant changes were observed in any of the anions across the column. The lack of acetate reduction most likely resulted from the absence of a suitable electron acceptor. Apparently, sulfate was unable to act as an electron acceptor in the place of nitrate.

CT was reduced by only 5% across the column. This degree of reduction could be considered negligible. However, the presence of significant CF in the effluent indicates that transformation was occurring. CF was slightly higher in the effluent than was observed in the previous flow experiment with nitrate and yeast extract present (Figure 4.13). The raw data from Figure 4.14 would indicate that the CF in the effluent accounted for 134% of the CT transformed across the column. TCA increased slightly by 11% across the column. This illustrates that relatively minor fluctuations in flow conditions can result in significant error when transformations are slight. Through normalization to the percent change in TCA the data appears to be more reasonable. After normalization, the percent change in CT would be 16%. In which case the CF produced would account for 45% of the CT transformed. This was a small CT reduction and a large CF production, relative to the normalized data of previous experiments with 2 day retention times within the soil column.

Another unique characteristic of this experiment was that the CF profile increased rapidly until port 1 (3.5 cm) and slowed considerably thereafter. This trend was not as dramatic in previous profiles. This seems to suggest that most all of the CT transformation was occurring in the first few centimeters of the column. Since it is suspected that the majority of biological mass existed in that area, and the population had not been respiring with nitrate for an extended period, it may be that the transformation resulted from the utilization of residual cellular reserves.

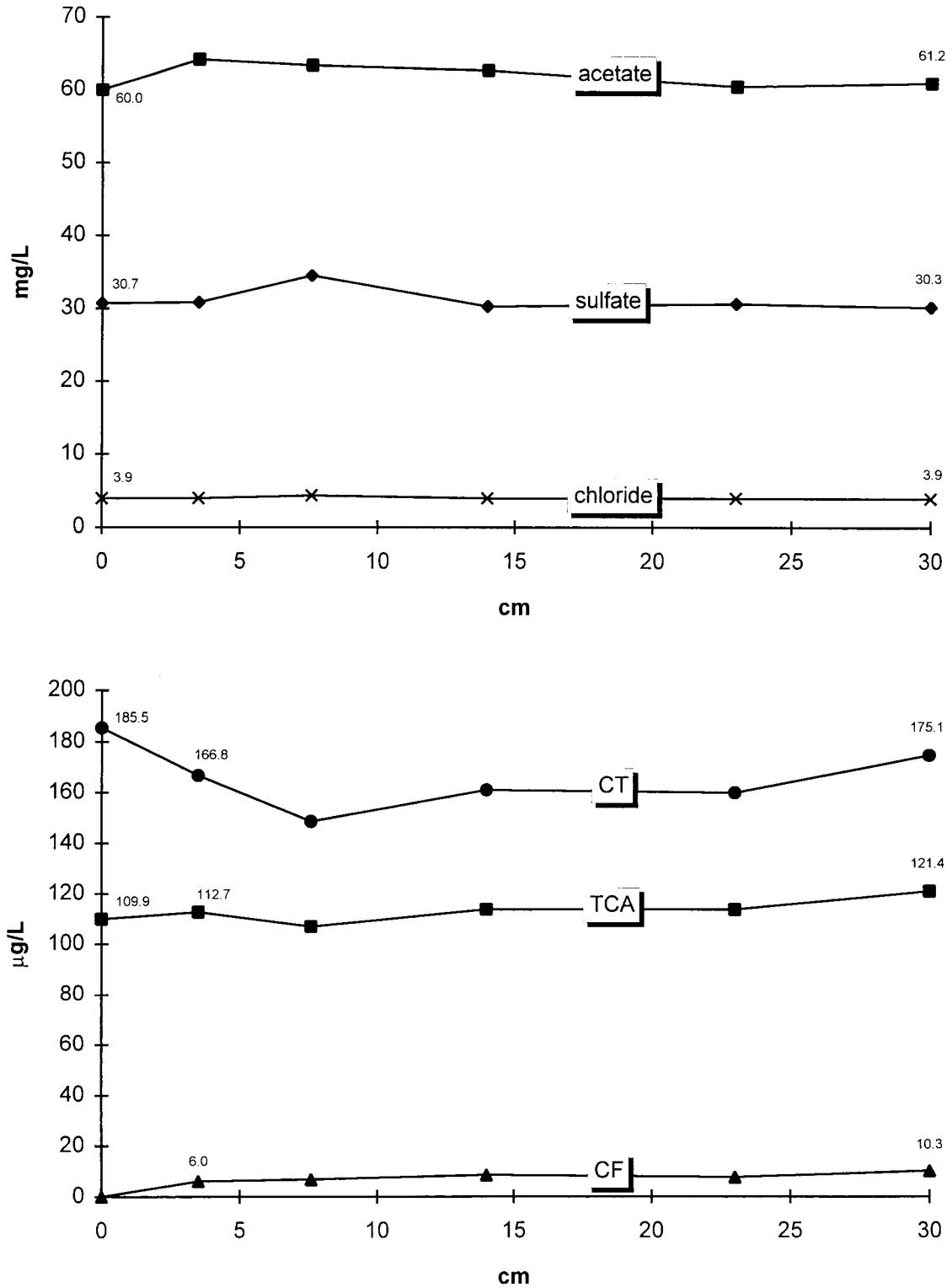

Figure 4.14. Column profiles 3 weeks after the yeast extract experiment and removal of nitrate from the synthetic groundwater; primary substrate = acetate, retention time = 2 days.

First Batch Test with Acetate (without Nitrate)

Three weeks following the removal of nitrate from the synthetic groundwater a batch experiment with acetate as the primary substrate was initiated. The batch experiment was performed for comparison to the second batch experiment with benzoate, where nitrate was not present within the column. In that regard, the methodology of the two experiments were identical. Figure 4.15 shows the concentration history at port 3 throughout the three week experiment. Time zero on the figure represents the conditions just prior to initiation of the batch experiment (see Figure 4.14). After 12 days TCA was virtually unchanged (the concentration actually increased slightly by 3%). CT was reduced by 59% relative to the influent concentration at the time of shutdown. CF represented 41% of the CT transformed at that time. Throughout the test CF consistently accounted for approximately 45% of the CT transformed, ranging from 41-53%.

Over the entire batch experiment (3 weeks) TCA continued to increase, and by the end of the experiment had increased 10% over its initial value. It is unclear why TCA gradually increased in this batch test but decreased slightly in the previous batch test with benzoate (Figure 4.9). One possibility is that TCA was slowly desorbing from the aquifer solids, because difficulties in maintaining a constant TCA stock solution concentration resulted in influent concentrations less than those established for previous experiments. Another possibility is that more concentrated solution was drawn into the effluent end of the column, from the make-up syringe, throughout the experiment. Although the target concentrations of CT and TCA in the make-up syringe were 250 μg/L, the actual concentrations may have differed significantly (just as the influent concentrations during flow conditions) due to inconsistencies in the saturated aqueous stock solutions. This may explain why the make-up fluid did not raise TCA during the previous batch experiment with benzoate if the make-up syringe concentration was not greater than the influent.

It would be incorrect to normalize the CT data to the increase in TCA if its increase was due to desorption from the aquifer solids. However, it would also be a mistake not to normalize the CT reduction if the increase in TCA was caused by mixing.

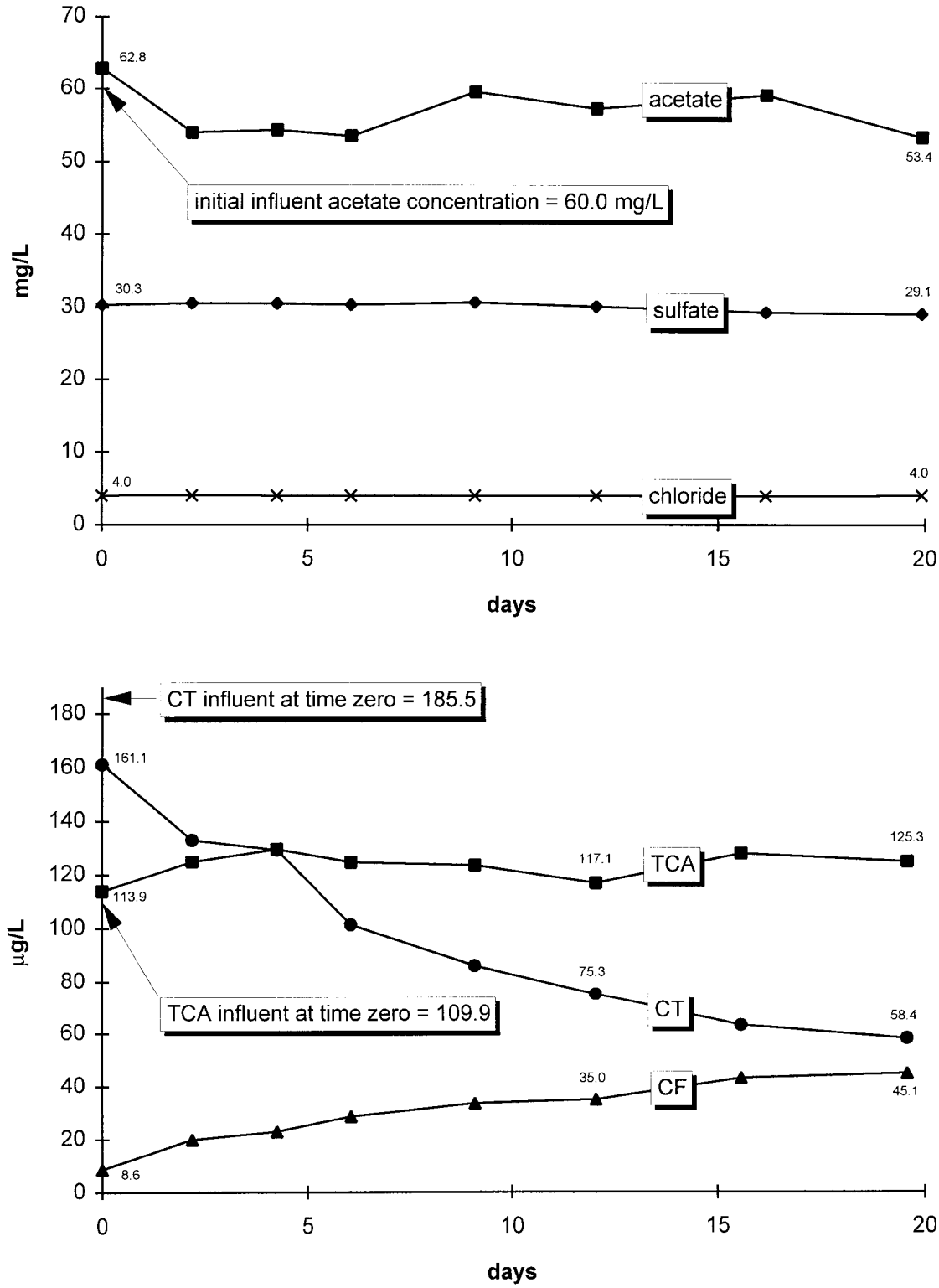

Figure 4.15. Concentrations at port 3 during the first batch experiment with acetate. Nitrate was removed from the synthetic groundwater 3 weeks prior to initialization.

Therefore, both the normalized and unnormalized values are presented for comparison. In which case, CT was reduced by 69% and 79% for the unnormalized and normalized cases, respectively. CF accounted for 46% of the CT transformed in the unnormalized case, and 40% if normalized.

The anions did not change appreciably over the course of the experiment. Interestingly, sulfate reduction could not be induced after 3 weeks in the absence of nitrate. It is unclear why the acetate concentration fluctuated considerably more than the other anions. Perhaps another compound resulting from metabolic processes was coeluding with acetate in the IC analysis.

As with the second batch test with benzoate it appeared that the CT transformation could be modeled as a first order process. Figure 4.16 shows a plot of $-\ln(C/C_0)$ vs. time for the CAHs. C_0 represents the concentration of each of the CAHs at port 3 at the initialization of the batch test. The CT data was reasonably linear; with an

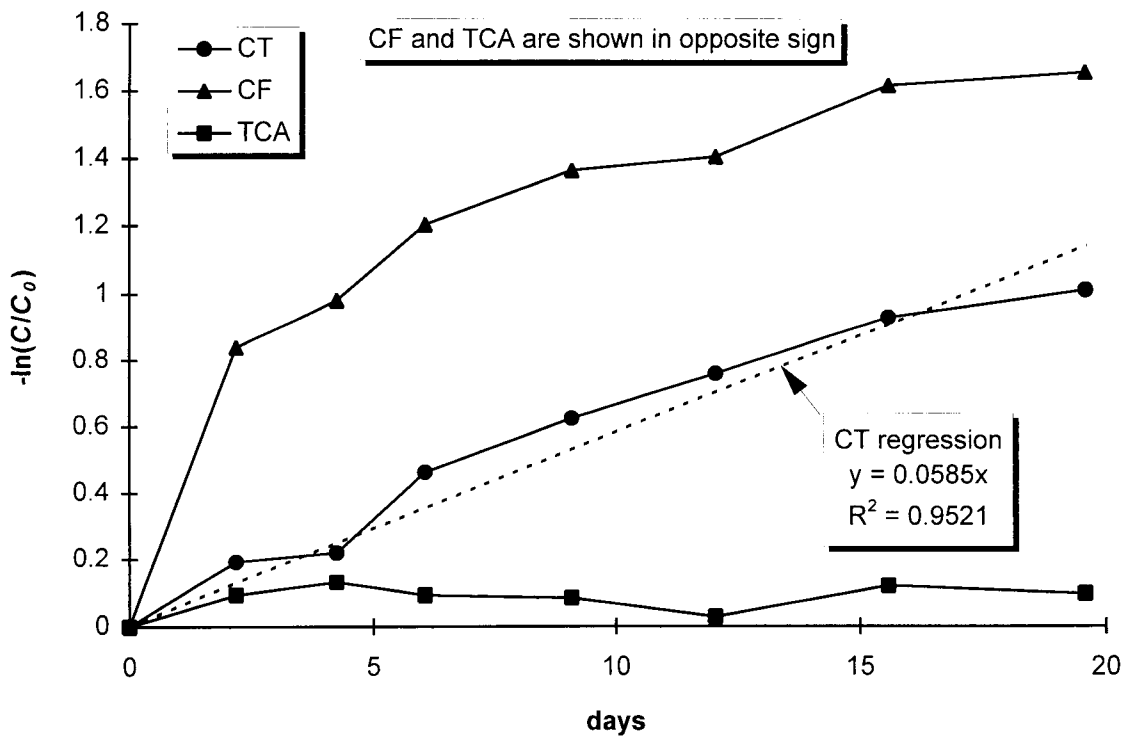

Figure 4.16. First-order check on CAHs for the first batch experiment with acetate (without acetate).

R^2 of 0.95 and a first-order rate constant (k) of 0.059 day^{-1}. CF and TCA could not be linearly approximated, and were plotted of opposite sign for comparison with CT. However, with the exception of the first data point, the CF curve paralleled that of CT. This is consistent with the percent of CF resulting from CT transformation being fairly constant. This also seems to indicate that CF was not being subsequently transformed within the column.

Figure 4.17 shows the concentration of anions and CAHs along the column length at the end of the batch experiment. Sulfate and chloride remained unchanged. However, acetate decreased across the column, with concentrations existing near the influent end greater than those present in the influent during flow conditions. This is supportive of the production of a metabolic bi-product which coeluded with acetate.

TCA showed a gradual increase across the column length. This effect may have been caused by the reasons discussed previously to explain the gradual increase in TCA at port 3. Again, the corresponding sampling sequence for the previous batch test with benzoate showed the opposite trend (Figure 4.11). Another possibility for the lower TCA concentrations near the influent end of the column in this case is that conditions may have existed that were conducive to slight TCA transformation in that area. However, for this to be the case, desorption from the aquifer solids would of had to occurred simultaneously since the TCA concentration was higher throughout the column than in the original influent (~110 μg/L).

CT also increased across the column, however, the effect was more dramatic than for TCA. CF decreased along the column length. Together these effects, along with the corresponding acetate data, would seem to indicate that faster rates of transformation existed near the influent end of the column. This is consistent with more CF being formed in the areas of greatest CT transformation.

First-order rate constants throughout the batch test, at ports 1-4, were approximated using the column profile during flow conditions (Figure 4.14) and Figure 4.17. The calculated constants for the 20 day period are shown in Table 4.4; efforts were not made to normalize to changes in TCA. The table shows that the rates were almost a

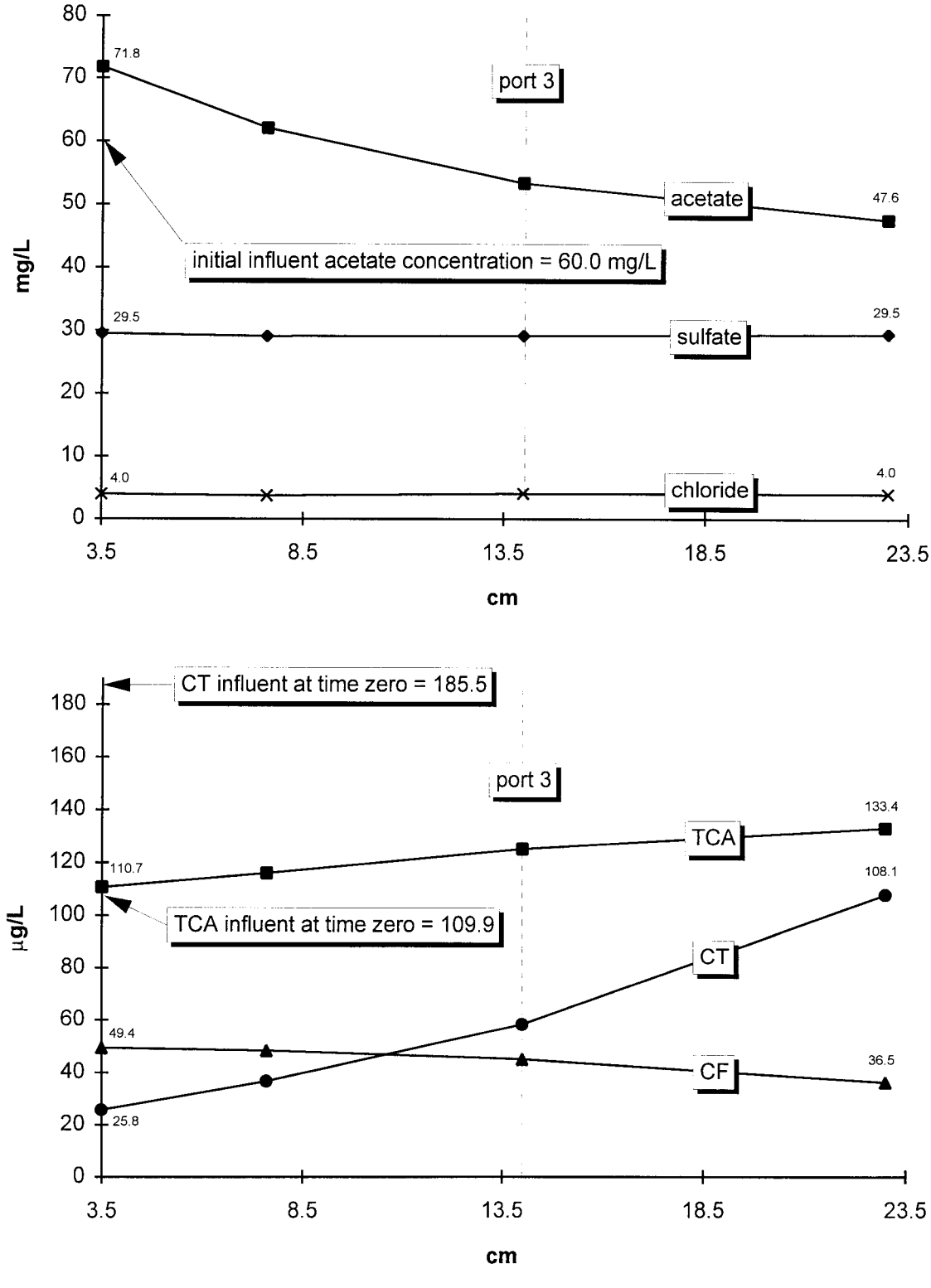

Figure 4.17. Anion and CAH concentrations throughout the column at the end of the first batch test with acetate.

Table 4.4. First-order rate constants at ports along the column length.

port	1 (3.5 cm)	2 (7.6 cm)	3 (14.0 cm)	4 (23.0 cm)
initial CT concentration (μg/L)	166.8	148.7	161.1	160.2
final CT concentration (μg/L)	25.8	36.8	58.4	108.1
k (day^{-1})	0.093	0.070	0.051[a]	0.020

[a]The first-order rate constant shown in Figure 4.16 (0.059 day^{-1}) differs because it was calculated from the regression of a series of data.

factor of 5 higher at port 1 than port 4. This effect was more pronounced than for the previous batch test with benzoate (factor of 2 difference). However, CT was reduced to much lower levels in that test, which may have resulted in larger errors being associated with the rates in that case. The much higher rates observed at the influent end of the column in this experiment is indicative of a larger cell mass in that area. This, combined with the lack of acetate utilization, seems to indicate that residual cellular reserves or fermentation reactions were important in the transformation of CT in this case.

Third Acetate Addition (Nitrate Replaced)

Immediately following the batch experiment, nitrate was reestablished in the synthetic groundwater at its original concentration (see Table 3.2) and flow conditions were reinitialized the same as considered previously: flow rate ≅ 100 mL/day; and CT and TCA target influent concentration ≅ 250 μg/L. Acetate was amended to influent stream in order to obtain an influent concentration equal to those prior (~50 mg/L). During the batch experiment all components of influent supply network were autoclaved and new in-line filters were installed. The intention was to prevent denitrification in the influent system prior to the soil column. Thus, once steady-state conditions were established, a batch experiment with nitrate could be initialized for comparison to the earlier batch tests. Similar flow conditions had been considered previously, however, a

batch experiment was not conducted following the establishment of the prior conditions. By inducing flow conditions similar to those considered previously, it would be interesting to observe if the results could be reproduced.

Nineteen days after reestablishing flow to the column, column profiles indicated that reasonably steady-state conditions existed. The profiles at this time are shown in Figure 4.18. The anion profile showed that despite the precautionary measures, some denitrification was proceeding prior to the soil column. However, significant amounts of nitrate and nitrite were still present in the column influent. Acetate was reduced by 59% across the column; sulfate and chloride were unchanged.

CT was reduced by 19% across the column, where TCA was reduced by only 7%. Normalization of the CT reduced to the percent change in TCA would result in a 12% reduction in CT. CF accounted for 35 and 54% of the CT transformed on a normalized and unnormalized basis, respectively. The extent of CT transformation was not as extensive in this case as it was during the first acetate addition (with nitrate). In that case, CT was reduced 36% without normalization and 20% with normalization. Also, less CF resulted from the transformation of CT in the previous case (17% unnormalized and 30% normalized). The anion profiles, on the other hand, appeared to be fairly similar between the two experiments (see Figure 4.12 for comparison).

It is unclear why the extent of CT transformation differed between the two experiments. In the first case, a significantly higher concentration of CT existed in the influent (377 vs. 180 μg/L). The first experiment also took place relatively soon after the benzoate experiments. It is possible that residual effects from the addition of benzoate were affecting the results of the first test. Also, since the experiments occurred approximately four months apart, the environmental conditions may have changed in the soil column. In addition, in this experiment, the CF profile increased more rapidly near the influent end of the column. This may indicate that the biological mass was less distributed throughout the column in this case.

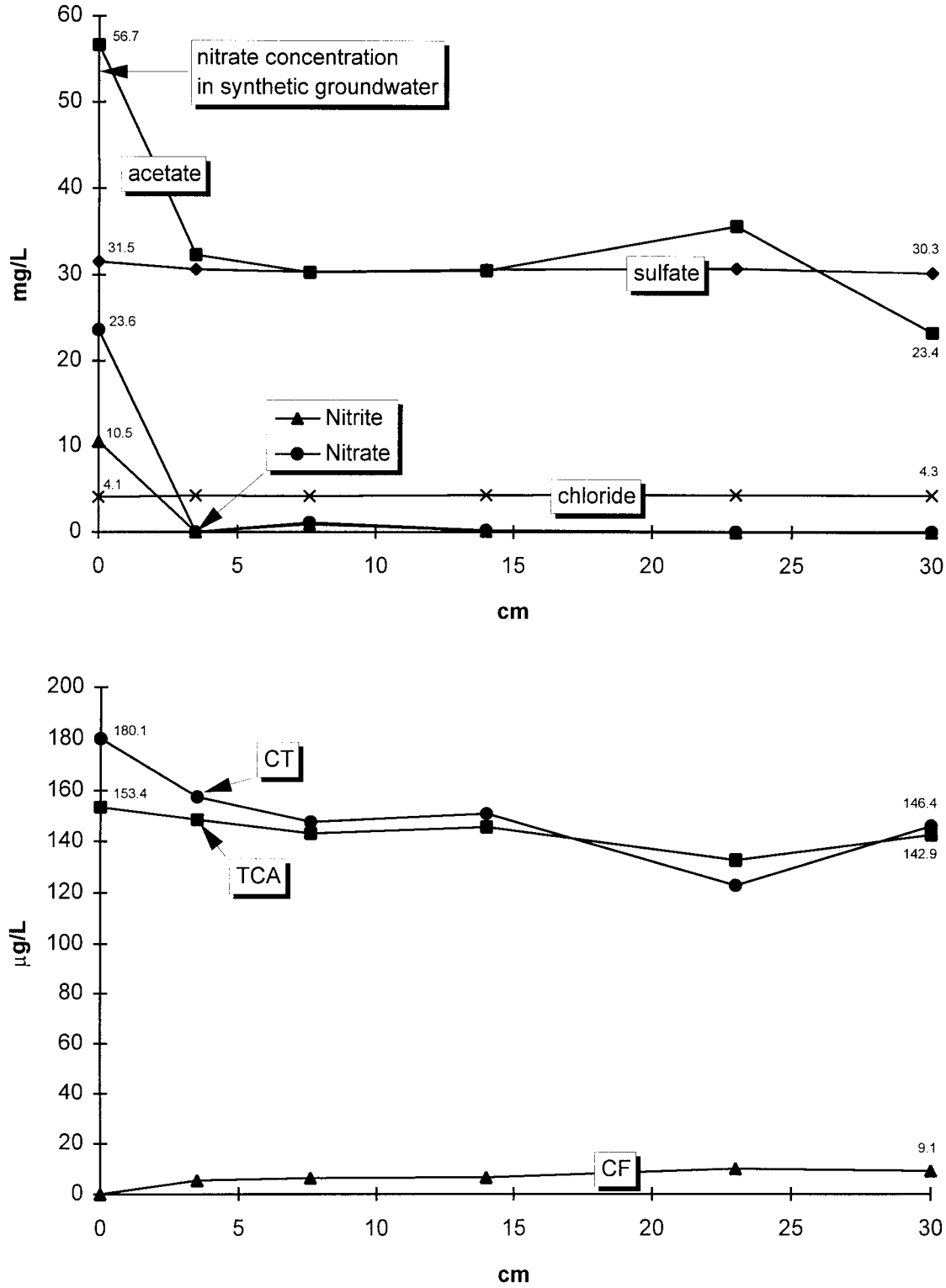

Figure 4.18. Column profiles 19 days after reestablishing nitrate in the synthetic groundwater; primary substrate = acetate, retention time = 2 days.

Second Batch Test with Acetate (with Nitrate)

The previous profiles (Figure 4.18) show the conditions at the initiation of a second batch experiment with acetate. This experiment was performed to compare the effects of nitrate, present in the influent solution, on the extent of CT transformation. The methodology of this batch experiment was similar to the previous two, except that data was collected over a slightly longer period (23 vs. 21 days). Figure 4.19 shows the concentration histories at port 3 over that period. The anion data shows that acetate may have decreased slightly during the first 20 days, then dropped rapidly. Nitrate and nitrite were not detected or at very low concentrations throughout the experiment, with the exception of the data at 23 days. Aside from that data point, this was to be expected since Figure 4.18 showed that denitrification was complete by port 3 during flow conditions. Sulfate and chloride remained unchanged over the experiment.

After 10 days CT was reduced 56% from the concentration in the influent during flow conditions. TCA was reduced by 14% over the same period. Thus after normalization to the change in TCA, the reduction in CT was 42%. CF accounted for 42% of the CT transformed in the normalized case, 42% if normalization is not considered.

Over the entire 23 day experiment CT was reduced by 61% from the influent concentration during flow conditions. However, a 15% reduction in TCA was also observed. Therefore, following normalization to the change in TCA, the reduction in CT would be 46%. CF accounted for 41 and 46% of the CT transformed on a unnormalized and normalized basis, respectively.

Figure 4.20 shows a plot of $-\ln(C / C_0)$ vs. time for the CAHs. C_0 represents the concentration of CAHs at port 3 at the initialization of the batch test. The CT data was reasonably linear (excluding the last data point); with an R^2 of 0.93 and a first-order rate constant (k) of 0.057 day^{-1}. CF and TCA were plotted of opposite sign for comparison with CT on the same chart. This rate constant was approximately the same as the previous batch test with acetate (without nitrate), which was 0.059 day^{-1}. This differs from the observations of the benzoate batch experiments, where the test without nitrate

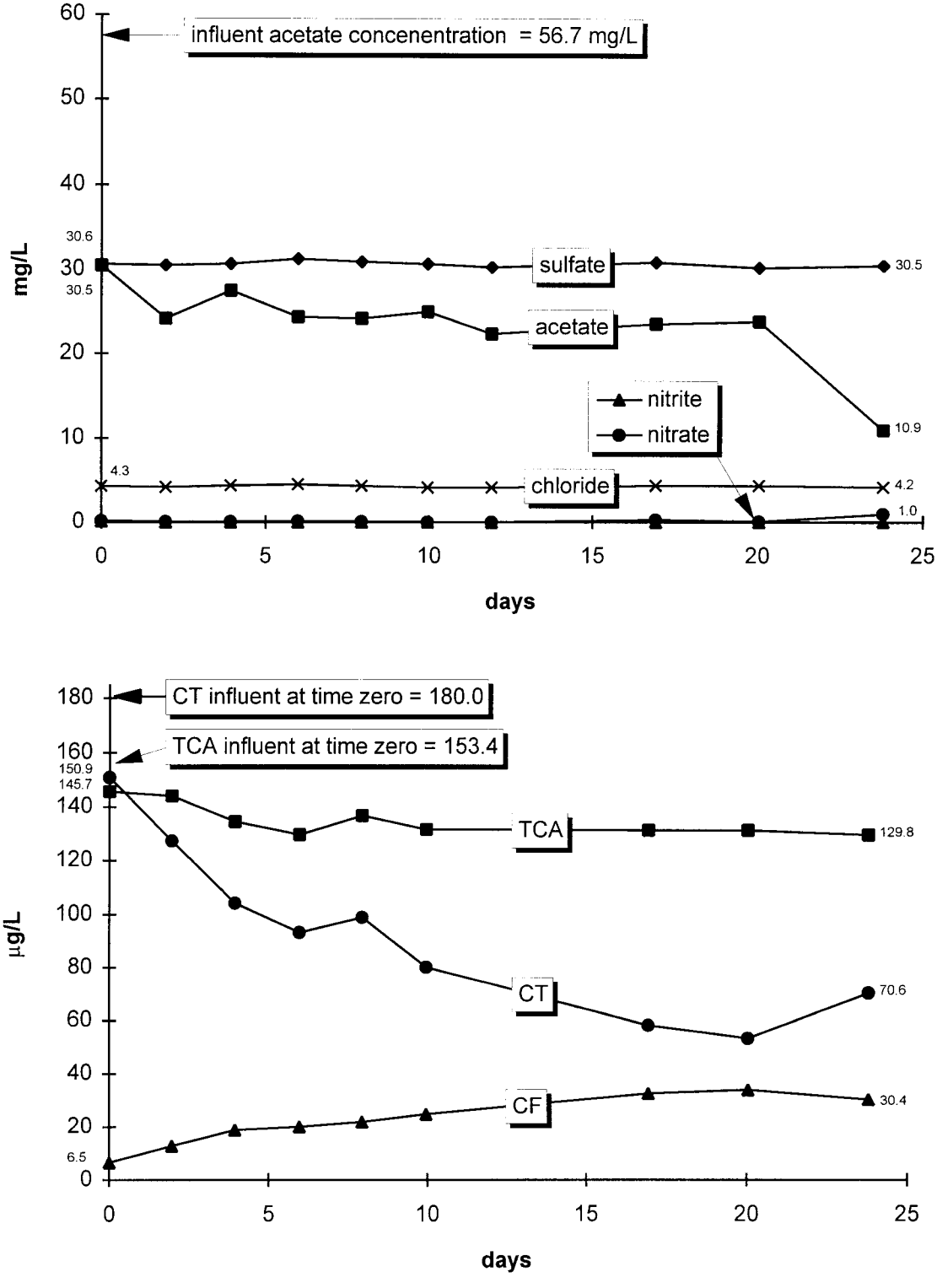

Figure 4.19. Concentrations at port 3 during the second batch experiment with acetate. Nitrate was replaced in the synthetic groundwater 19 days prior to the start of the batch test.

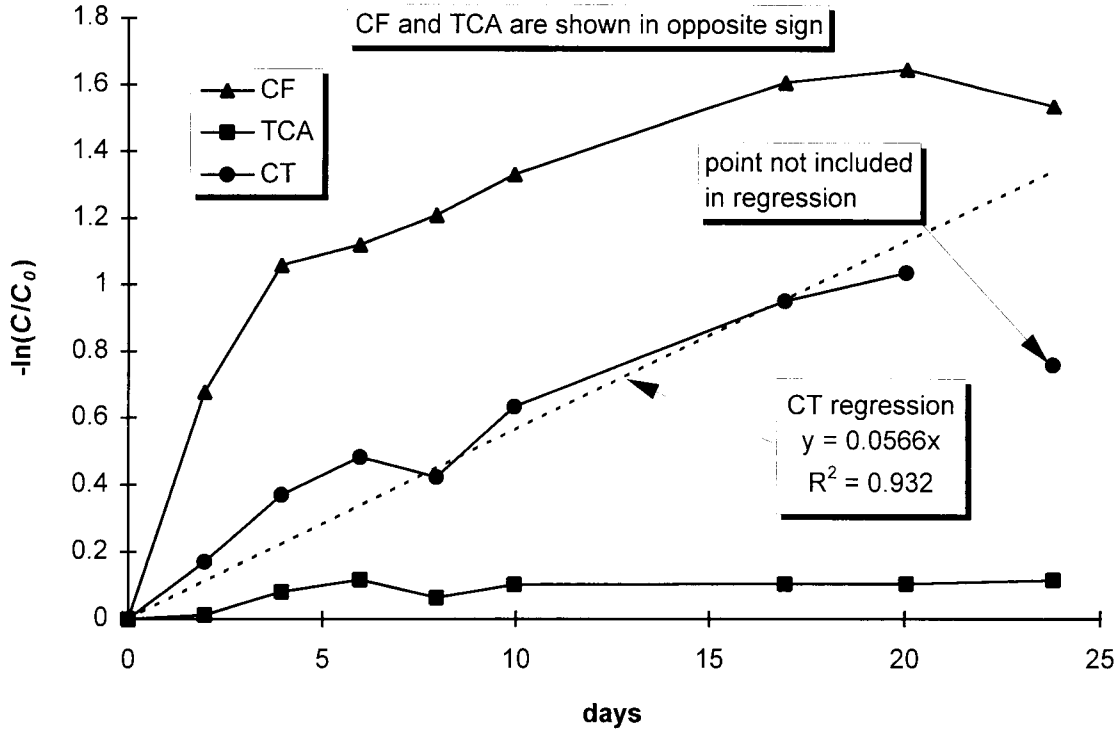

Figure 4.20. First-order check on CAHs for the second batch experiment with acetate (with nitrate).

exhibited a higher rate. The lack of an increased rate in the batch test with acetate in the absence of nitrate may have been the result of the lack of respiration with nitrate for an extended period, as discussed earlier. The rates for all of the experiments are further compared in the following section.

Figure 4.21 shows the concentration of anions and CAHs along the column length at the end of the batch experiment. Sulfate and chloride remained unchanged. Acetate decreased along the column length as in the previous batch test with acetate (without nitrate). Also, acetate again was detected at higher concentrations than present in the influent at port 1 during flow conditions, possibly indicating the production of a metabolic bi-product coeluding with acetate in this test as well. Nitrate, and to a slight extent nitrite, increased along the column despite their absence from that portion of the column during flow conditions (see Figure 4.18). This coupled with the data from port 3 (Figure 4.19), which shows an abrupt decrease in acetate and increase in nitrate and

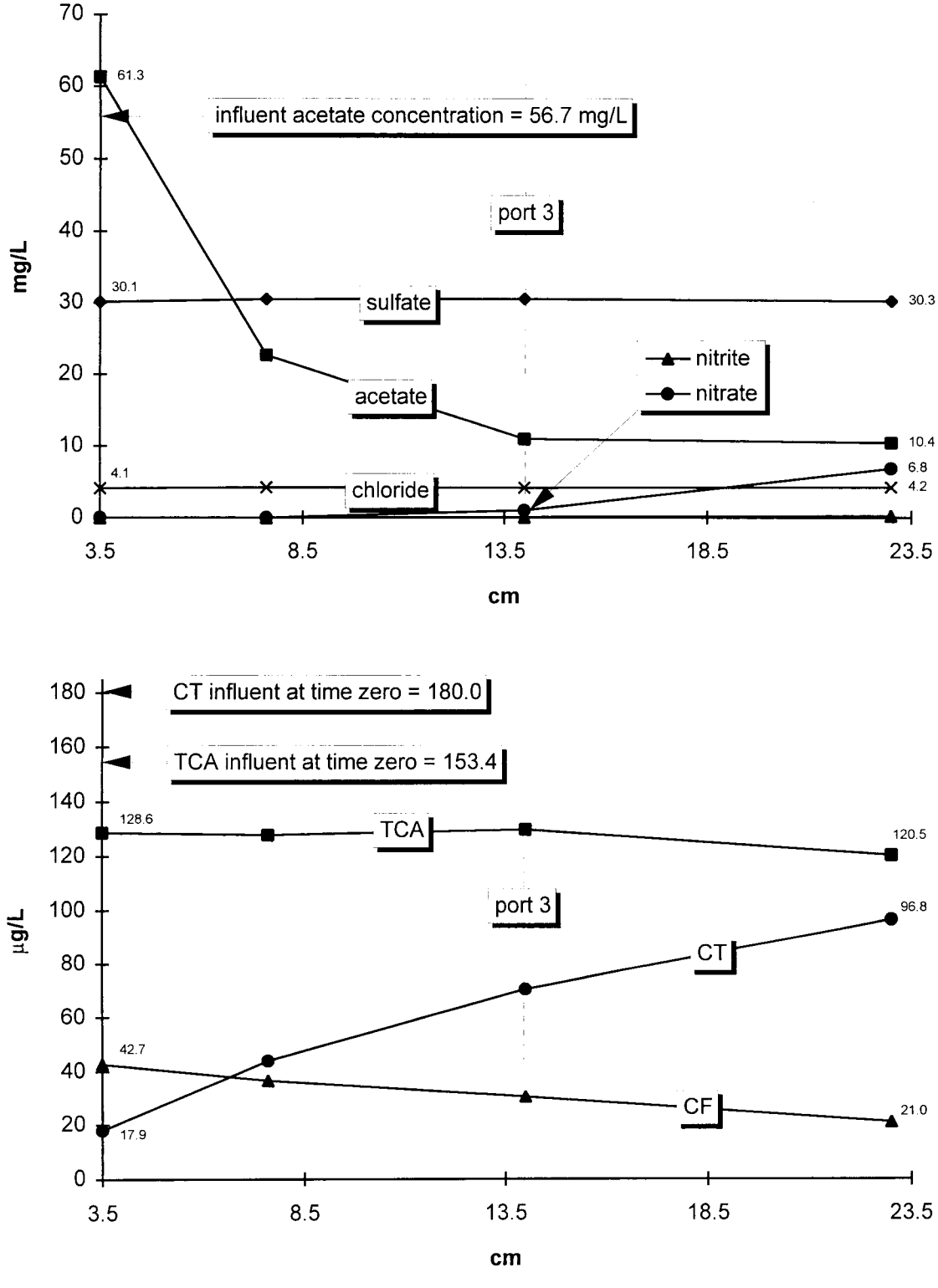

Figure 4.21. Anion and CAH concentrations throughout the column at the end of the second batch test with acetate.

nitrite after day 20, seems to indicate that mixing of synthetic groundwater (containing nitrate) from the make-up syringe was occurring at this time with denitrification ensuing as a result.

TCA decreased slightly across the column by 6% following the batch test as shown in Figure 4.21. This differs from the previous batch test in which TCA increased slightly. The TCA concentration may have been affected by mixing of fluid from the make-up syringe if it was of slightly lower concentration than present in the influent during flow conditions. This corresponds to Figure 4.19 which shows a slight decrease in TCA at port 3 after day 20. However, CT increases significantly at port 3 at the same time, while CF decreases. This could be supportive of mixing of make-up fluid if the CT concentration was higher in the syringe than the original influent. The CF dilution would be expected since CF was not present in the make-up solution.

Similarly to the previous batch experiment with acetate (without nitrate), Figure 4.21 shows that CT increased along the column length, while CF decreased. Which is again supportive of greater microbial activity near the front of the column, since greater CF production would be expected in the areas of the most CT transformation. However, if mixing was significant near the effluent end of the column this effect would appear more pronounced than actually the case.

Table 4.5 shows first-order rate constants for CT calculated from the data following the batch experiment, day 23 (Figure 4.21), and the column profile during flow conditions (Figure 4.18); in addition, a 20 day constant at port 3 was calculated from Figure 4.19. The constants were not normalized to losses in TCA. As with the previous batch experiments, the rates were higher at the influent end of the column. However, in this case, the difference was more dramatic, with the rates differing by almost a factor of 10. Compared to the previous batch test with acetate (without nitrate) the rates calculated from the data at 23 days were similar at port 1, but were increasingly slower toward the effluent side. However, the data at port 3 after 20 days is very similar to the previous test. This may further indicate that mixing of CT from the make-up fluid influenced the process at later times.

Table 4.5. First-order rate constants at ports along the column length.

port	1 (3.5 cm)	2 (7.6 cm)	3 (14.0 cm)		4 (23.0 cm)
days	23	23	20	23	23
initial CT concentration (μg/L)	157.4	147.7	150.9	150.9	123.2
final CT concentration (μg/L)	17.9	43.9	53.5	70.6	96.8
k (day^{-1})	0.095	0.053	0.052[a]	0.033	0.010

[a]The first-order rate constant shown in Figure 4.20 (0.057 day^{-1}) differs because it was calculated from the regression of a series of data.

5. SUMMARY AND CONCLUSIONS

Summary

A summary of the extents of CT transformation observed under the different conditions considered in this study is shown in Table 5.1. The table also compares the percentages of CF produced as a result of the CT reductions. CF was produced as an intermediate of CT transformation in all cases, ranging from 14-45% (based on both raw and normalized CT reductions) of the CT transformed. This is contrary to the results of Petersen *et al.* (1994), who reported that a denitrifying culture isolated from Hanford solids transformed CT without CF production. However, in most all other cases reported in the literature, CF production was observed to some extent.

In all experiments with benzoate as the primary substrate, the presence of nitrate in the influent groundwater resulted in less extensive transformations than a comparative case without nitrate. Which is consistent with the findings of Semprini *et al.* (1992), Stensel and DeJong (1994) and Petersen *et al.* (1994) among others. However, when acetate was the primary substrate, equal or slightly greater CT transformation was observed in the presence of nitrate. This may have resulted from the total absence of nitrate for 3 weeks prior to the acetate batch test without nitrate. Thus, the microbial population may have possibly been weakened from the extended absence of nitrate for respiration.

Semprini *et al.* (1992) suggested that the inhibitory effect of nitrate could be caused by: (1) CT and nitrate competing as electron acceptors; (2) another microbial population living off the decay products of the denitrifiers; (3) the presence of nitrate prevented the stimulation of sulfate reducers. However, here, sulfate reduction was never observed, even when nitrate was absent for extended periods. Another possibility, in this case, would be that iron reducing microorganisms were using iron contained in the aquifer solids to cometabolically transform CT.

Table 5.1. Comparison of CT reduction and CF production (as a percentage of CT reduction) for the conditions considered.

conditions	substrate			
	benzoate		acetate	
	CT reduction	CF produced	CT reduction	CF produced
Flow (w/ nitrate) 0.5 day ret. time	12% (5%)[a] (Table 4.1)	16% (24%)	not determined	not determined
Flow (w/ nitrate) 2 day ret. time	20% (NA) (Figure 4.7)	21% (NA)	19-36%[b] (12-20%) (Figure 4.12) & (Figure 4.18)	17-35% (30-54%)
Flow (w/o nitrate) 2 day ret. time	30% (NA) (Figure 4.8)	24% (NA)	5% (16%) (Figure 4.14)	134% (45%)
Batch (w/ nitrate) 12 day period[c]	72-84% (49-64%) (Table 4.1)	14-20% (21-25%)	56% (42%) (Figure 4.19)	32% (42%)
Batch (w/o nitrate) 12 day period	94% (85%) (Table 4.2)	39% (43%)	59% (NA) (Figure 4.15)	41% (NA)

[a]Percentages in parenthesis represent reductions normalized to percent change in TCA, where applicable.

[b]Based on the results of duplicate experiments performed approximately 4 months apart.

[c]The percentages for the batch test with acetate were calculated from data over a 10 day period.

Comparison of Primary Substrates

In Table 5.1, CT transformation in the presence and absence of nitrate are compared for both primary substrates. However, care should be taken when comparing the different cases between substrates. Experiments were categorized "with nitrate" if nitrate was present in the synthetic groundwater. Acetate, however, was far more effective at inducing denitrification, and nitrate was often removed from the influent solution prior to entry into the soil column. For example, the continuous-flow experiments (2 day retention time) with acetate in the presence of nitrate and benzoate in

the absence of nitrate (the shaded cells in Table 5.1) are not placed in the same row of the table. However, since acetate had induced rapid denitrification, even prior to entry into the soil column (Figure 4.12), it can be argued that these two flow conditions can be compared directly.

The category "without nitrate" was defined more loosely. In the situation with benzoate as the primary substrate, acetate was used to denitrify the influent before the column. Where, with acetate, nitrate was removed from the synthetic groundwater during preparation. Considerable differences may exist between the two situations because: (1) in the benzoate situation, soluble metabolic products may have passed into the soil column which influenced CT transformation; and (2) in the acetate situation, the extended lack of nitrate for respiration may have significantly lowered the cells activity. However, if these concerns are kept in mind, interesting comparisons can still be made.

Under flow conditions with nitrate, both benzoate and acetate induced similar degrees of CT transformation. Perhaps the best transformation during flow conditions occurred with benzoate in the absence of nitrate. Although, in one case, acetate in the presence of nitrate induced slightly higher transformation before normalization, it was significantly less after normalization. The higher level of transformation in the benzoate situation may also have been enhanced by metabolic bi-products resulting from the denitrification with acetate in the pre-mix chamber. Acetate in the absence of nitrate faired poorly in comparison to the other flow situations. However, in this case, the microorganisms had not been able to respire with nitrate for an extended period prior.

Acetate was far more efficient at inducing denitrification. With acetate as the primary substrate, denitrification was usually complete 3.5 cm into the soil column; with the denitrification often occurring before the influent solution reached the soil column (see Figure 4.12). Denitrification was never complete across the column with benzoate as the primary substrate. Also, with benzoate, no significant denitrification occurred prior to the soil column. In the continuous-flow experiments, transformations with acetate as the primary substrate resulted in slightly higher percentages of CF production.

The batch tests showed that CT was almost completely transformed with residence times on the order of weeks, with benzoate inducing faster rates. Table 5.2

Table 5.2. Comparison of the batch experiments after 12 days at port 3. (CF % production is expressed as % of CT transformed on a molar basis).

	benzoate				acetate			
	with nitrate (first batch test)		without nitrate (second batch test)		with nitrate[c] (second batch test)		without nitrate (first batch test)	
	% red.	k (day^{-1})	% red.	k (day^{-1})	% red.	k (day^{-1})	% red.	k (day^{-1})
TCA	20-23		9		14		negligible	
CT	72-84 (49-64)[a]	0.10-0.15[b]	94 (85)	0.21	56 (42)	0.085	59	0.059
	% prod.		% prod.		% prod.		% prod.	
CF	14-20 (21-25)		39 (43)		32 (42)		41	

[a]Percentages in parenthesis represent reductions normalized to percent change in TCA, where applicable.

[b]Estimated from data in Table 4.1.

[c]The data for this batch test was obtained at 10 days.

compares the results of the batch experiments at port 3, 12 days after shutdown. Samples were not taken throughout the first batch test with benzoate (without nitrate), however, CT degradation was shown to follow pseudo-first-order kinetics during the other batch experiments. Therefore, assuming that the first batch test followed first-order kinetics as well, a rate constant was estimated from the initial and final conditions (see Table 4.1). All rate constants were calculated with the assumption that the cell concentration was at steady-state.

Some interesting observations can be made between the rate constants calculated from the initial and final CT concentrations at all 4 ports along the column length for each of the batch experiments where final samples were obtained along the column length. Figure 5.1 shows a plot of the rates at each of the ports along the column length for the respective batch tests. The figure shows that the rates of CT transformation were higher near the influent end of the column. Also, the rates appear to decrease in a fairly linear fashion with respect to location along the column.

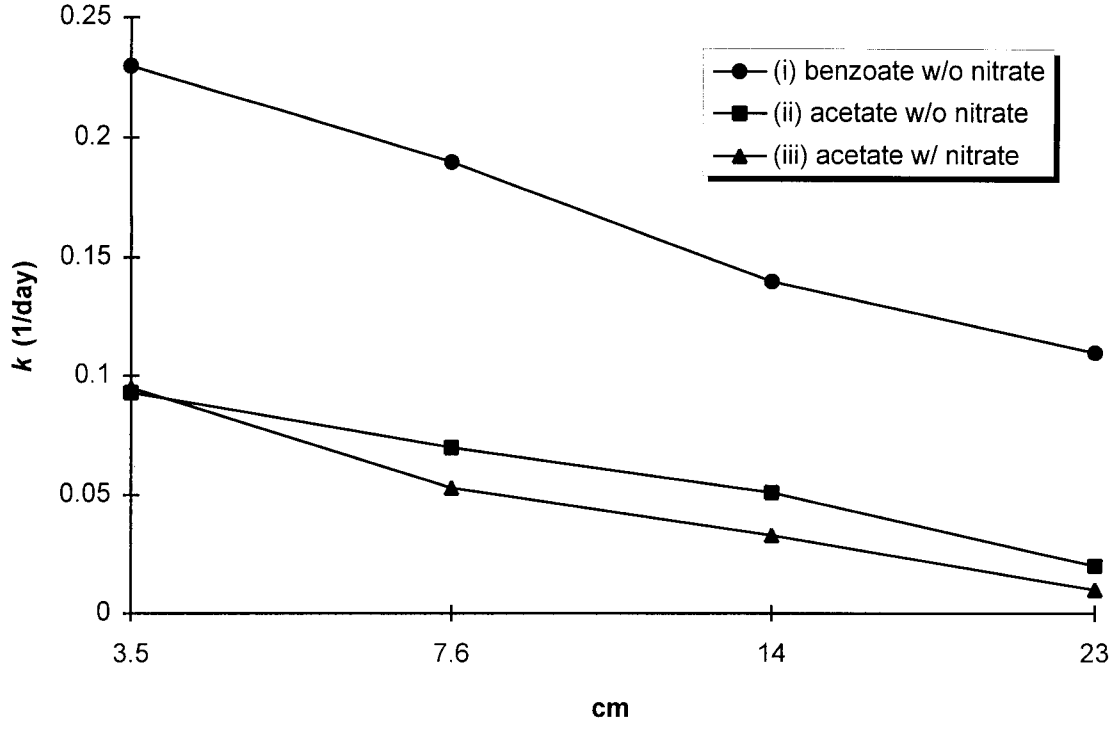

Figure 5.1. First-order rate constants throughout the column for batch experiments.

Additionally, the ratios between the rates at each port generally increase with distance from the influent end of the column, as shown in Table 5.3. This effect is most pronounced for ratios based on data from the last batch test (acetate with nitrate), where a fair degree of mixing was suspected with fluid from the make-up syringe near the effluent end. However, if only the first row of ratios are considered (k_i/k_{ii}), which neglects this test, the ratios are fairly constant over the first three ports. The discrepancy in the ratio at port 4, in this instance, may be related to mixing effects with the make-up fluid as well. Although more data would be required to determine the extent of mixing that occurred, this could suggest that the decrease in rates along the column for the various batch experiments may have been approximately proportional.

If this was the case, it could possibly indicate that the microbial population was proportionally smaller in the acetate situation. However, since rapid microbial growth was observed during periods of denitrification with acetate, prior to the batch experiment,

Table 5.3. Comparison of the ratio between rate constants throughout the column for batch experiments.

port	1 (3.5 cm)	2 (7.6 cm)	3 (14.0 cm)	4 (23.0 cm)
k_i (day^{-1}) benzoate without nitrate	0.23	0.19	0.14	0.11
k_{ii} (day^{-1}) acetate without nitrate	0.093	0.070	0.051	0.020
k_{iii} (day^{-1}) acetate with nitrate	0.095	0.053	0.033	0.010
ratio: k_i/k_{ii}	2.5	2.7	2.7	5.5
ratio: k_i/k_{iii}	2.4	3.6	4.2	11
ratio: k_{ii}/k_{iii}	1.0	1.3	1.5	2.0

it may also indicate that a larger or equal population was transforming CT at a proportionally slower rate.

Truex *et al.* (1994) were able to use a first-order process to model CT transformation by Hanford denitrifiers. Their reported rate constants were not independent of cell concentration and can not be compared directly. However, data from an experiment using glycerol as a primary substrate (Figure 1 of their report) shows that the cell concentration was fairly constant during the first half of the test. If a rate constant is calculated from their data during this period, from the relationship $\ln(C/C_0) = -kt$, a value of approximately 0.24 day^{-1} is obtained. This value is similar to the highest rate constants observed in this study.

An interesting scenario arose from the use of the pre-mix chamber during the flow experiments. Where, the pre-mix chamber served as a pseudo-CSTR reactor in series with, and prior to, the soil column. Under flow rates providing a 0.5 day hydraulic

residence time through the soil column, the 125 mL volume of the pre-mix chamber corresponded to a residence time of approximately 1 day within it. Although, substantial denitrification and biological growth was occurring within the pre-mix chamber during periods of acetate addition, no CF production was observed in the fluid leaving the chamber. Thus, for the conditions existing within the pre-mix chamber, denitrifiers were not transforming CT. If they were, CF was not produced as a transformation product.

At no time during the flow experiments did the addition of benzoate or acetate result in the stimulation of a microbial mass dense enough to impede flow through the column. Pressure gauges on both ends of the column were constantly monitored, and the pressure drop never exceeded 0.4 psi. However, slight turbidity was visible in the pre-mix chamber and in samples removed from the soil column during periods of acetate addition. Also, in-line 0.2 μm filter membranes needed to be replaced periodically due to clogging under periods of acetate addition.

Comparison of Continuous-Flow to Batch Experiments

Under the flow conditions considered in this study, complete removal of CT was not established. The hydraulic residence time in the reactors could not be increased further due practical and physical limitations imposed by the system hardware and analytical techniques. However, the flow experiments were useful for determining how the transformations varied spatially from the point of injection. The batch experiments in turn provided a simple method of increasing the hydraulic residence time within the column. The longer residence times were useful in establishing if CT could be completely transformed as well as determining the kinetics of the transformation.

It is likely that the longer residence times of the batch experiments, combined with the reduction in CT, may have resulted in significant quantities of CT desorbing from the aquifer solids. This would result in more CT being transformed that predicted. Also, since CF can be expected to be less strongly sorbed than CT based on the octanol/water partition coefficients shown in Table 2.1, the fraction of CF resulting from CT transformation may not be as high as expected.

Overall, the CAH profiles showed that CT transformation was greater near the influent end of the column. This was mainly indicated by all of the profiles showing a faster rise in CF concentration at the influent end. Also, following the batch tests, samples along the column length showed more extensive and rapid transformations near the influent end (Figures 4.11, 4.17, 4.21 and Table 5.3). Therefore, it is likely that the results of the batch experiments were influenced by conditions that existed during the continuous-flow regimes.

Conclusions

Considering the overall findings of this study the following conclusions can be drawn:

1. The Hanford subsurface possesses a microbial population able to transform CT and remove nitrate from the groundwater under anaerobic conditions.
2. Anywhere between 14 and 54% of the CT reduced can be expected to be transformed to CF.
3. The transformation of CT appeared to be inhibited by the presence of nitrate and/or nitrite. However, following extended absences of nitrate, the transformations seemed to slow significantly.
4. Both benzoate and acetate are capable of inducing denitrification and CT transformation. However, denitrification is much faster with acetate as the primary substrate; while benzoate generally produced more rapid and slightly more efficient transformation of CT.
5. Sulfate reducers, if present on the Hanford subsurface solids, were not easily stimulated.
6. Significant sorption of CT occurred on the aquifer solids. Results indicated that the retardation factor was 3.9 compared to the bulk flow.

These observations have implications on the use of anaerobic processes for *in-situ* CT remediation at Hanford. Stimulation of an anaerobic population to degrade CT is possible through primary substrate addition. Over time scales of weeks to months complete CT transformation can be achieved. CF will likely be formed as a transformation product, with up to 50% of the CT transformed to CF. It is possible that

CF may be further transformed with longer residence times in the subsurface, but no evidence was observed for CF transformation in these studies.

Benzoate may be the preferred substrate since its slow utilization would permit greater amounts of aquifer to be treated. However, it may be advantageous to use both substrates in combination at Hanford. In which case, the acetate would rapidly remove nitrate from the groundwater near the injection point, while simultaneously transforming slight amounts of CT. The benzoate, reacting much slower, could travel a greater distance through the subsurface resulting in remediation of a larger area. In addition, the lower nitrate concentrations from denitrification with acetate, may provide conditions conducive to faster CT transformation with benzoate.

Future Research

Because of the complexity of systems using microbial processes to transform CT in soil environments, significant possibilities remain for future research. Analytical methods to quantify all of the products resulting from CT transformation would provide valuable insight. This may be obtained through the use of radio-labeled CT and/or a Hall Detector on the gas chromatograph to detect less chlorinated intermediates. Further kinetic modeling, similar to the work of Semprini *et al.* 1991, would be useful. Studies exploring the fate of CF (without CT present), in similar situations as those considered here, would be valuable in determining the appropriate conditions to employ at the Hanford demonstration.

Also, future research may be able to combine the advantages of both the batch and the continuous-flow systems by recycling a portion of the effluent. This would not only increase the overall residence time but provide multiple passes through the most active zone in the column. One scenario would be to modify the original column supply system (refer to Figure 3.2) as shown in Figure 5.2. In this system, the syringe pump, capable of much lower flow rates, would determine the rate at which fluid leaves the system. The pump, previously used to provide the main feed stream, could be used to recycle a portion

of fluid back through the column. The combined flows would determine a hydraulic residence time for a single pass.

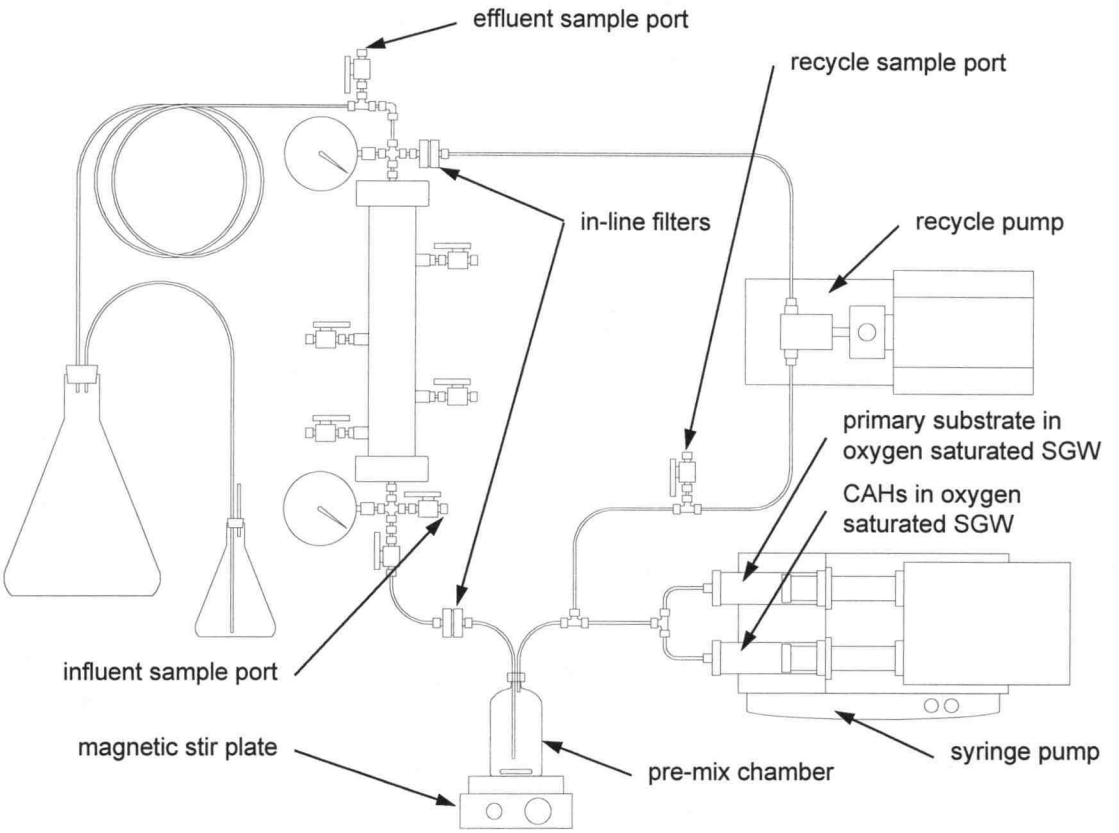

Figure 5.2. Column supply network incorporating recycle

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APPENDICES

APPENDIX A

Timing of Experimental Events

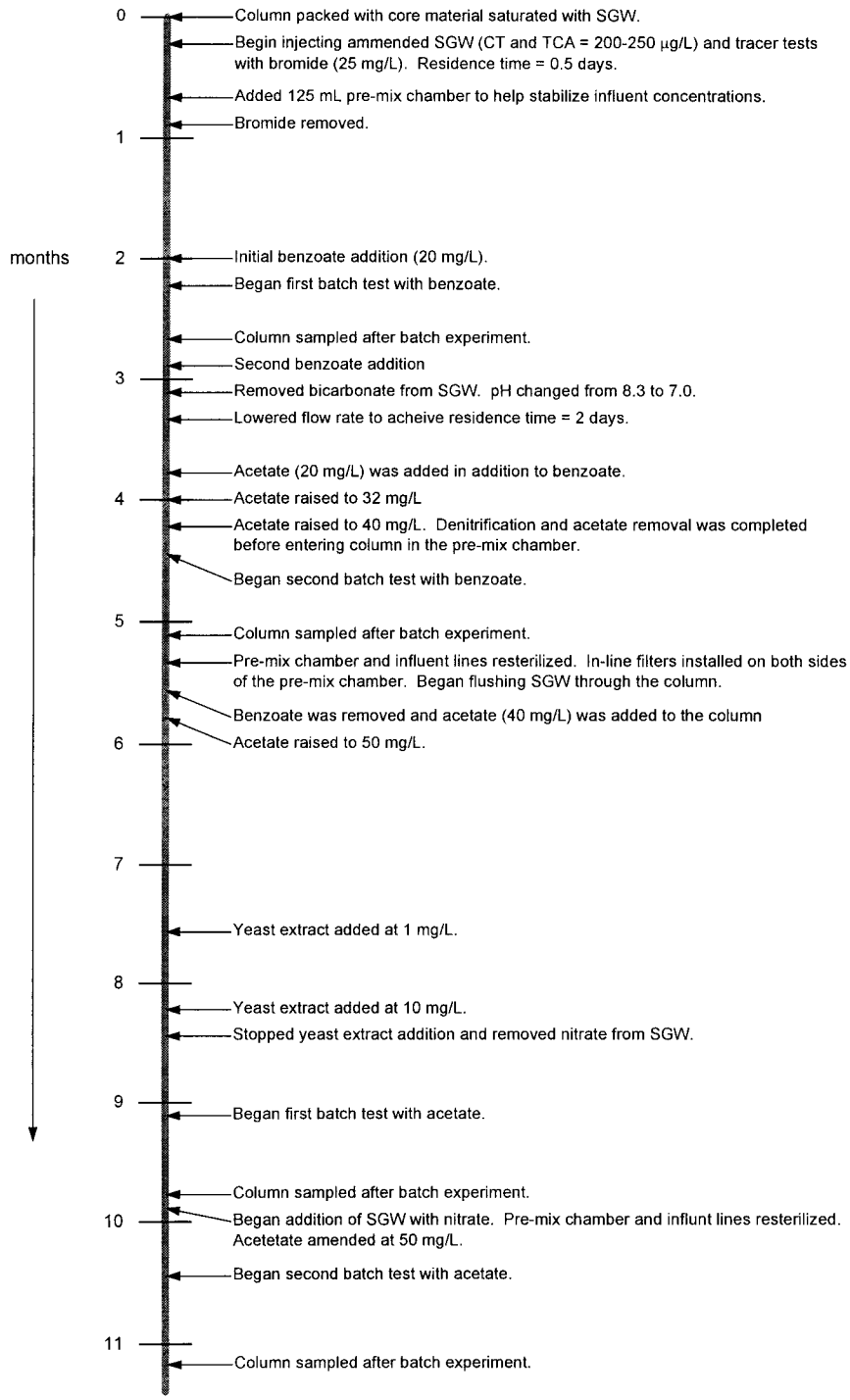


Figure A.1. Experimental Timeline.

APPENDIX B

Summary of Physical Parameters

Reactor Column

- **Design:** upflow
- **Material:** stainless steel
- **Length:** 30 cm
- **Internal diameter:** 5.4 cm
- **Total internal volume:** 690 mL
- [1]**Pore volume:** 190 mL
- **Sample port locations:** port 1 = 3.5; port 2 = 7.6; port 3 = 14.0; port 4 = 23.0 cm
- **Temperature:** ~22°C

Aquifer Material

- **Visual characterization:** heterogeneous, poorly sorted, gravel to silt
- [1]**Porosity:** 27%
- [1]**Peclet number:** 2.7
- [1]**Longitudinal dispersivity:** 11 cm

Synthetic Groundwater (with Amendments)

- **pH:** 8.3 initially, lowered to 7.0 after bicarbonate was removed
- **High flow condition:** 340 mL/day (0.5 day retention time)
- **Low flow condition:** 100 mL/day (2 day retention time)

[1]Determined from the results of the breakthrough experiments.

APPENDIX C

Cumulative Data Summary

Table C.1. Overall CT data.

		concentration @ port/cm						
		influent	1	2	3	4	effluent	
date time	sample #'s	0	3.5	7.6	14	23	30	
6/9/94 9:00	1	1691.98						Began breakthrough experiments, residence time = 0.5 days
6/9/94 10:00	2						0.00	
6/9/94 11:00	3						13.01	
6/9/94 12:00	4						18.91	
6/9/94 13:00	5-6	164.74					19.25	
6/9/94 14:00	7						20.46	
6/9/94 15:00	8						29.03	
6/9/94 16:00	9						22.93	
6/9/94 17:00	10-11	141.94					43.33	
6/9/94 18:00	12						45.90	
6/9/94 19:00	13						54.16	
6/9/94 20:00	14						62.14	
6/9/94 21:00	15-16	47.88					59.32	
6/10/94 1:15	17-18	48.96					56.87	
6/10/94 7:30	19-20	155.34					52.51	
6/10/94 11:30	21-22	206.34					76.44	
6/10/94 18:30	23-24	250.85					96.38	
6/10/94 21:30	25-26	175.87					112.38	
6/11/94 10:30	27-28	180.31					130.28	
6/12/94 12:00	29-30	193.09					136.54	
6/12/94 19:15	31-32	175.14					137.24	
6/13/94 13:20	33-34	146.00					121.04	
6/14/94 9:45	35-36	145.24					123.06	
6/15/94 11:20	37-38	156.21					118.94	
6/16/94 10:15	39-40	172.52					116.18	
6/17/94 9:20	41-42	129.03					115.22	
6/18/94 11:00	43-44	191.61					137.08	
6/19/94 16:00	45-46	217.25					139.89	
6/20/94 17:15	47-48	249.71					156.03	
6/21/94 16:15	49-50	191.38					162.51	
6/22/94 15:00	51-52	306.24					164.95	
6/23/94 16:15	53-54	256.55					165.94	
6/24/94 14:30	55-56	194.33					175.03	
6/25/94 14:20	57-62	352.64	175.70	161.85	178.25	160.66	202.71	
6/27/94 11:30	63-64	212.59					151.02	Pre-mix chamber installed
6/29/94 9:00	65-66	172.54					137.02	
6/29/94 10:45	67						137.02	
6/29/94 13:00	68-69	190.24					124.18	
6/29/94 15:00	70	169.89						
6/30/94 9:45	75-76	180.81					150.27	
6/30/94 13:45	77-78	187.72					149.20	
6/30/94 16:45	79-80	181.09					148.81	
7/1/94 10:00	83-84	176.15					159.21	
7/2/94 11:15	85-86	166.68					144.74	
7/5/94 14:00	87-88	161.88					146.40	
7/6/94 12:00	89-94	119.71	133.95	132.99	137.43	123.99	126.98	
7/8/94 14:15	95-100	141.85	87.10	87.87	105.82	115.06	120.98	
7/11/94 13:45	101-102	125.32					116.27	
7/12/94 13:00	103-104	125.04					111.68	
7/13/94 11:00	105-106	143.99					123.95	
7/14/94 16:00	107-108	134.88					131.79	
7/15/94 10:30	109-114	126.43	107.89	120.41	119.92	105.93	126.36	
7/18/94 14:30	115-116	119.70					114.72	
7/20/94 15:45	117-118	142.79					118.98	
7/22/94 11:15	119-120	137.05					127.20	
7/26/94 15:45	121-122	127.91					117.41	
8/2/94 11:15	123-124	102.62					108.40	
8/4/94 9:00	125	198.75						Began benzoate addition
8/4/94 11:00	126-127	234.04					111.91	
8/4/94 12:50	128-129	230.55					141.69	
8/4/94 14:50	130-131	237.96					160.63	

8/4/94 18:00	132-133	198.14					219.19	
8/4/94 22:00	134-135	202.09					191.39	
8/5/94 0:15	136-137	213.06					180.97	
8/5/94 10:15	138-139	214.76					185.74	
8/5/94 16:30	140-141	204.29					192.54	
8/6/94 12:30	142-143	210.53					195.05	
8/7/94 14:15	144-145	222.38					193.80	
8/8/94 12:45	146-147	212.08					190.83	
8/10/94 12:30	148-149	202.06					175.93	Batch test initialized
8/22/94 12:00	150-151		34.73			61.38		Batch test completed
8/29/94 12:45	152			corrupted data				Restarted continuous-flow, residence time = 0.5 days
8/29/94 14:00	153-154	168.45					44.11	
8/29/94 15:00	155-156	169.52					51.30	
8/29/94 17:00	157-158	168.26					70.13	
8/29/94 21:45	159-160	165.15					101.65	
8/30/94 10:10	161-166	166.25	93.46	139.72	138.75	126.79	133.27	
8/30/94 15:00	167-168	172.62					127.38	
8/31/94 12:00	169-174	168.54	107.83	135.96	142.18	149.51	143.42	
9/1/94 13:15	175-176	173.47					149.24	
9/2/94 11:15	177-178	174.87					145.87	
9/5/94 11:15	179-184	187.32	183.49	108.27	173.70	163.61	170.79	
9/6/94 13:45	185-186	182.64					164.91	
9/8/94 11:00	187-188	174.02					159.53	
9/12/94 10:30	189-190	169.95					160.22	
9/13/94 10:15	191-192	208.59					169.63	Lowered flow-rate, residence time = 2 days
9/14/94 11:30	193-194	205.63					166.65	
9/15/94 11:15	197-202	229.81	198.50	177.64	151.02	183.08	165.47	
9/16/94 11:00	203-204	260.48					193.43	
9/19/94 10:00	205-210	278.71	251.63	229.02	217.15	181.67	223.15	
9/21/94 12:15	211-216	207.33	190.03	158.37	187.41	185.09	197.30	
9/23/94 12:00	217-222	203.53	190.11	188.74	178.88	152.38	174.33	
9/26/94 10:15	223-228	179.91	175.63	141.14	167.65	171.51	171.97	
9/27/94 10:45	229-234	208.15	182.54	164.75	187.09	179.84	179.08	Began acetate addition
9/29/94 10:15	235-240	266.85	231.59	236.11	238.54	216.36	199.41	
10/3/94 10:15	241-246	237.68	226.87	217.17	208.89	192.12	198.46	Increased acetate concentration
10/10/94 11:00	247-252	188.48	173.17	144.94	169.85	150.83	146.78	Increased acetate concentration
10/13/94 11:15	253-258	189.62	168.05	133.58	140.31	97.34	128.47	
10/17/94 11:15	259-264	247.34	238.87	220.41	192.65	189.21	171.63	Batch test initialized
10/20/94 12:45	265				106.61			
10/22/94 10:30	266				73.14			
10/24/94 11:45	267				63.93			
10/26/94 12:15	268				39.56			
10/28/94 11:30	269				30.09			
10/31/94 11:00	270				16.50			
11/2/94 13:00	271				12.27			
11/4/94 10:00	272				6.71			
11/10/94 14:15	273				6.08			
11/11/94 9:30	274-277		1.14	2.58	8.23	16.46		Batch test completed
11/15/94 14:00	278-279	398.13					260.11	Restarted continuous-flow, residence time = 2 days
11/21/94 11:00	280-281	328.56					284.77	
11/22/94 10:30	282-283	323.27					287.50	
11/28/94 12:45	284-285	222.53					246.59	
11/29/94 12:15	286-291	262.33	288.78	271.47	276.84	286.01	256.34	
11/30/94 11:15	292-297	286.44	281.78	277.94	264.25	256.03	247.30	
12/2/94 9:00	298-303	257.81	341.56	341.70	345.18	300.05	275.77	
12/5/94 10:00	304-309	295.30	332.23	325.67	346.95	337.38	324.24	
12/8/94 9:45	310-315	168.31	261.84	247.11	253.36	262.29	257.89	Increased acetate concentration
12/12/94 10:45	316-321	117.33	276.13	268.59	241.16	228.95	204.50	
12/15/94 11:00	322-327	503.66	464.04	395.80	354.19	307.40	277.36	
12/21/94 10:00	328-333	307.77	305.20	289.46	270.16	260.59	236.70	
12/29/94 10:15	334-339	377.19	332.54	300.42	325.09	251.59	243.40	
1/5/95 10:00	340-345	483.76	458.47	388.06	378.17	308.98	276.22	
1/11/95 11:00	346-351	454.91	392.71	341.24	344.27	291.42	276.18	
1/18/95 11:00	352-357	232.25	221.91	201.86	198.42	200.61	200.76	
1/24/95 9:30	358-363	208.94	184.18	169.12	171.56	167.58	160.69	
2/1/95 10:30	364-369	75.71	63.78	57.42	59.33	66.40	66.57	Began yeast extract addition
2/7/95 9:45	370-376	97.94	111.57	105.08	107.84	100.25	102.16	
2/13/95 10:30	377-382	161.46	153.24	134.98	150.65	124.00	127.97	
2/20/95 9:30	383-388	226.08	223.27	212.70	218.59	195.40	157.49	
2/24/95 10:15	389-394	213.26	205.93	196.99	205.93	187.53	187.69	Yeast extract and nitrate removed
3/3/95 10:30	395-400	169.41	209.04	185.23	193.19	189.10	173.17	
3/10/95 12:20	401-406	255.71	246.12	248.90	255.81	235.54	236.80	
3/16/95 12:30	407-412	174.76	178.66	179.60	160.29	116.92	163.15	
3/22/95 10:15	413-418	185.47	166.76	148.73	161.12	160.22	175.08	Batch test initialized
3/24/95 14:45	420				132.98			
3/26/95 16:15	421				129.35			
3/28/95 11:45	422				101.44			
3/31/95 12:30	423				86.26			
4/3/95 11:30	424				75.30			
4/7/95 14:00	425				63.39			
4/11/95 8:00	426-429		25.81	36.81	58.40	108.14		Batch test completed

4/17/95 12:45	430-431	145.23					122.77	Nitrate reestablished in influent
4/24/95 10:00	432-433	185.22					132.05	
4/30/95 12:30	434-439	180.07	157.43	147.67	150.90	123.16	146.37	Batch test initialized
5/2/95 11:30	440				127.30			
5/4/95 11:00	441				104.25			
5/6/95 11:30	442				93.27			
5/8/95 11:00	443				98.93			
5/10/95 11:30	444				80.15			
5/12/95 10:30	445				115.84			
5/17/95 11:00	446				58.24			
5/20/95 13:30	447				53.46			
5/24/95 7:30	448-451		17.88	43.91	70.60	96.78		Batch test completed

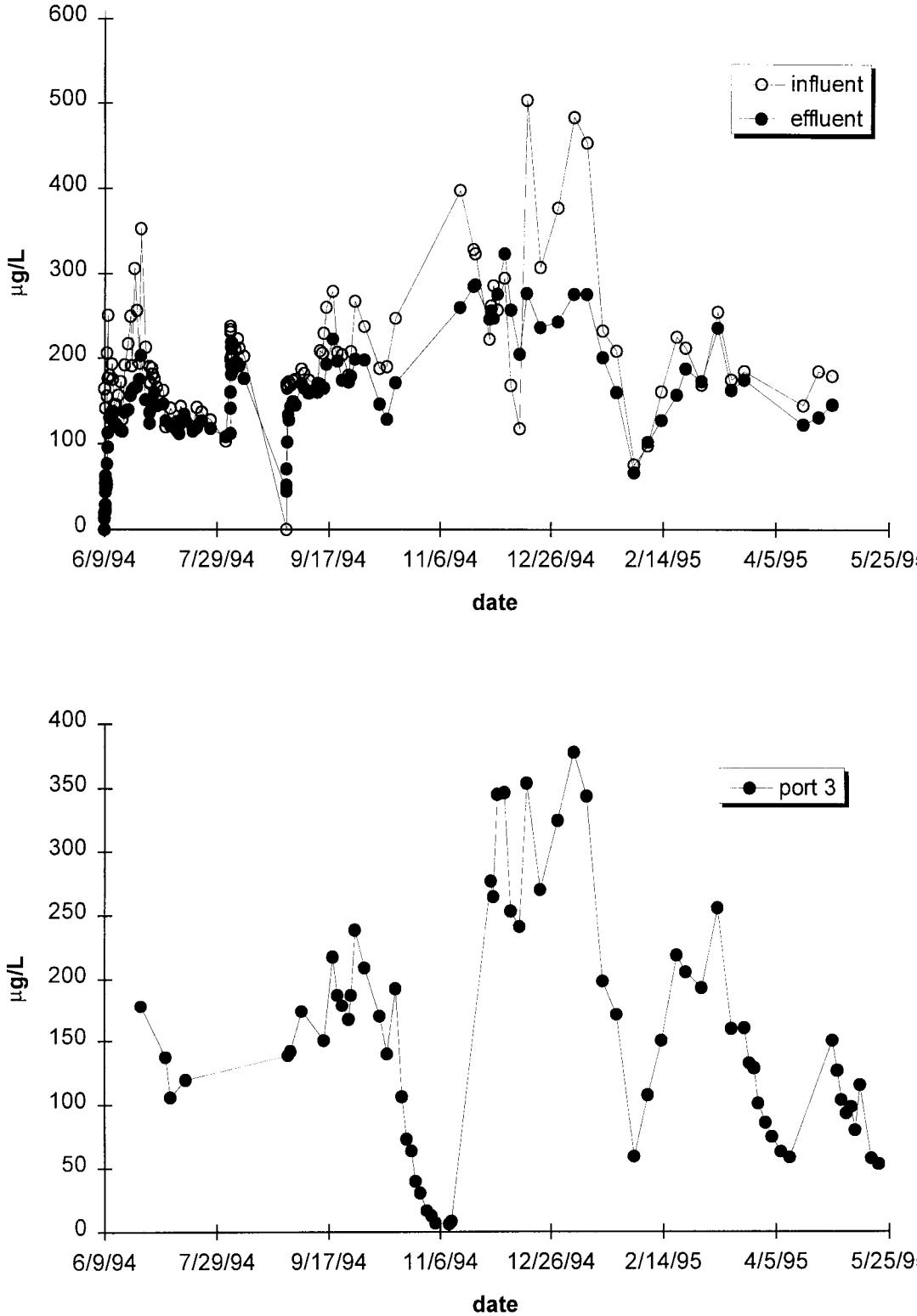


Figure C.1. Plots of overall CT data at the influent (0 cm) and effluent (30 cm) ports and at port 3 (14 cm).

Table C.2. Overall CF data.

		concentration @ port/cm						
date time	sample #'s	influent 0	1 3.5	2 7.6	3 14	4 23	effluent 30	
6/9/94 9:00	1							Began breakthrough experiments, residence time = 0.5 days
6/9/94 10:00	2							
6/9/94 11:00	3							
6/9/94 12:00	4							
6/9/94 13:00	5-6							
6/9/94 14:00	7							
6/9/94 15:00	8							
6/9/94 16:00	9							
6/9/94 17:00	10-11							
6/9/94 18:00	12							
6/9/94 19:00	13							
6/9/94 20:00	14							
6/9/94 21:00	15-16						3.83	
6/10/94 1:15	17-18							
6/10/94 7:30	19-20							
6/10/94 11:30	21-22							
6/10/94 18:30	23-24						5.99	
6/10/94 21:30	25-26						7.80	
6/11/94 10:30	27-28						8.19	
6/12/94 12:00	29-30						9.20	
6/12/94 19:15	31-32						9.90	
6/13/94 13:20	33-34						8.40	
6/14/94 9:45	35-36						6.64	
6/15/94 11:20	37-38						7.06	
6/16/94 10:15	39-40						6.88	
6/17/94 9:20	41-42						5.70	
6/18/94 11:00	43-44						5.67	
6/19/94 16:00	45-46						6.37	
6/20/94 17:15	47-48						6.40	
6/21/94 16:15	49-50						5.16	
6/22/94 15:00	51-52						4.03	
6/23/94 16:15	53-54						3.86	
6/24/94 14:30	55-56						3.49	
6/25/94 14:20	57-62							
6/27/94 11:30	63-64						3.19	Pre-mix chamber installed
6/29/94 9:00	65-66						5.26	
6/29/94 10:45	67						10.50	
6/29/94 13:00	68-69						9.97	
6/29/94 15:00	70							
6/30/94 9:45	75-76						5.31	
6/30/94 13:45	77-78						4.62	
6/30/94 16:45	79-80						3.83	
7/1/94 10:00	83-84						3.84	
7/2/94 11:15	85-86						3.05	
7/5/94 14:00	87-88						0.00	
7/6/94 12:00	89-94							
7/8/94 14:15	95-100	0.00	14.11	3.82	8.23	2.34	2.37	
7/11/94 13:45	101-102						0.00	
7/12/94 13:00	103-104						0.00	
7/13/94 11:00	105-106						2.63	
7/14/94 16:00	107-108						0.00	
7/15/94 10:30	109-114	0.00	5.87	1.97	2.03	2.30	0.00	
7/18/94 14:30	115-116						3.34	
7/20/94 15:45	117-118						0.00	
7/22/94 11:15	119-120						0.00	
7/26/94 15:45	121-122						1.84	
8/2/94 11:15	123-124						3.64	
8/4/94 9:00	125							Began benzoate addition
8/4/94 11:00	126-127						2.24	
8/4/94 12:50	128-129						5.28	
8/4/94 14:50	130-131						33.37	
8/4/94 18:00	132-133						0.00	
8/4/94 22:00	134-135						2.37	
8/5/94 0:15	136-137						2.43	
8/5/94 10:15	138-139						2.13	
8/5/94 16:30	140-141						2.33	
8/6/94 12:30	142-143						1.93	
8/7/94 14:15	144-145						2.07	
8/8/94 12:45	146-147						2.34	
8/10/94 12:30	148-149						2.37	Batch test initialized
8/22/94 12:00	150-151		27.21			17.28		Batch test completed
8/29/94 12:45	152	corrupted data						Restarted continuous-flow, residence time = 0.5 days
8/29/94 14:00	153-154						26.91	

8/29/94 15:00	155-156						24.02	
8/29/94 17:00	157-158						16.67	
8/29/94 21:45	159-160						10.32	
8/30/94 10:10	161-166		13.42	4.00	6.26	8.71	5.67	
8/30/94 15:00	167-168						5.19	
8/31/94 12:00	169-174		7.04	3.28	3.25	6.20	4.69	
9/1/94 13:15	175-176						4.19	
9/2/94 11:15	177-178						4.24	
9/5/94 11:15	179-184		1.66	4.43	2.22	3.00	2.67	
9/6/94 13:45	185-186						2.65	
9/8/94 11:00	187-188						6.15	
9/12/94 10:30	189-190						4.18	
9/13/94 10:15	191-192						5.37	Lowered flow-rate, residence time = 2 days
9/14/94 11:30	193-194						8.43	
9/15/94 11:15	197-202		3.72	8.56	8.22	10.36	9.49	
9/16/94 11:00	203-204						9.07	
9/19/94 10:00	205-210		3.45	5.89	6.01	7.55	8.22	
9/21/94 12:15	211-216		2.87	4.41	5.48	7.70	7.35	
9/23/94 12:00	217-222		3.24	5.09	4.95	8.44	6.77	
9/26/94 10:15	223-228		2.35	3.66	4.03	6.63	5.66	
9/27/94 10:45	229-234		2.87	3.78	4.07	7.42	6.48	Began acetate addition
9/29/94 10:15	235-240		2.17	2.99	4.30	6.63	6.22	
10/3/94 10:15	241-246		1.72	3.20	3.38	5.43	6.00	Increased acetate concentration
10/10/94 11:00	247-252		1.97	4.24	5.27	11.54	11.08	Increased acetate concentration
10/13/94 11:15	253-258		2.22	5.03	8.55	10.99	12.04	
10/17/94 11:15	259-264		2.45	5.54	10.20	12.94	13.95	Batch test initialized
10/20/94 12:45	265				24.77			
10/22/94 10:30	266				36.13			
10/24/94 11:45	267				48.31			
10/26/94 12:15	268				52.85			
10/28/94 11:30	269				64.78			
10/31/94 11:00	270				70.12			
11/2/94 13:00	271				77.10			
11/4/94 10:00	272				85.89			
11/10/94 14:15	273				82.68			
11/11/94 9:30	274-277		71.52	76.47	80.40	80.12		Batch test completed
11/15/94 14:00	278-279						17.25	Restarted continuous-flow, residence time = 2 days
11/21/94 11:00	280-281						13.71	
11/22/94 10:30	282-283						12.45	
11/28/94 12:45	284-285						8.23	
11/29/94 12:15	286-291		3.86	4.15	4.29	7.27	8.01	
11/30/94 11:15	292-297		3.14	4.24	4.06	6.75	7.03	
12/2/94 9:00	298-303		2.76	3.21	3.53	6.45	6.49	
12/5/94 10:00	304-309			2.98	3.37	4.99	4.85	
12/8/94 9:45	310-315			2.84	3.33	4.59	4.90	Increased acetate concentration
12/12/94 10:45	316-321		3.05	4.54	5.77	7.91	9.11	
12/15/94 11:00	322-327		4.29	7.90	8.72	10.79	10.97	
12/21/94 10:00	328-333		3.55	6.70	10.80	14.55	15.95	
12/29/94 10:15	334-339		3.93	8.11	9.87	15.46	17.67	
1/5/95 10:00	340-345		5.36	12.54	12.59	20.21	22.92	
1/11/95 11:00	346-351		8.25	13.90	16.27	23.06	24.70	
1/18/95 11:00	352-357		2.26	5.31	5.95	10.32	11.64	
1/24/95 9:30	358-363		2.96	5.55	6.85	9.70	11.16	
2/1/95 10:30	364-369		2.47	4.09	5.73	6.42	5.57	Began yeast extract addition
2/7/95 9:45	370-376			2.63	2.44	4.36	4.61	
2/13/95 10:30	377-382		2.50	2.52	2.73	5.60	5.89	
2/20/95 9:30	383-388		3.43	5.50	5.48	8.07	8.20	
2/24/95 10:15	389-394		2.71	4.00	3.69	8.05	9.25	Yeast extract and nitrate removed
3/3/95 10:30	395-400		4.16	5.51	5.94	6.88	6.62	
3/10/95 12:20	401-406		6.73	7.38	8.32	10.25	10.93	
3/16/95 12:30	407-412		10.40	10.82	11.04	10.25	9.84	
3/22/95 10:15	413-418		6.05	6.80	8.59	7.75	10.30	Batch test initialized
3/24/95 14:45	420				19.89			
3/26/95 16:15	421				22.97			
3/28/95 11:45	422				28.66			
3/31/95 12:30	423				33.65			
4/3/95 11:30	424				35.05			
4/7/95 14:00	425				43.29			
4/11/95 8:00	426-429		49.36	48.36	45.10	36.47		Batch test completed
4/17/95 12:45	430-431						11.50	Nitrate reestablished in influent
4/24/95 10:00	432-433						9.80	
4/30/95 12:30	434-439		5.36	6.28	6.54	10.09	9.15	Batch test initialized
5/2/95 11:30	440				12.87			
5/4/95 11:00	441				18.86			
5/6/95 11:30	442				20.06			
5/8/95 11:00	443				21.94			
5/10/95 11:30	444				24.79			
5/12/95 10:30	445				36.36			
5/17/95 11:00	446				32.68			
5/20/95 13:30	447				34.03			
5/24/95 7:30	448-451		42.69	36.55	30.44	21.01		Batch test completed

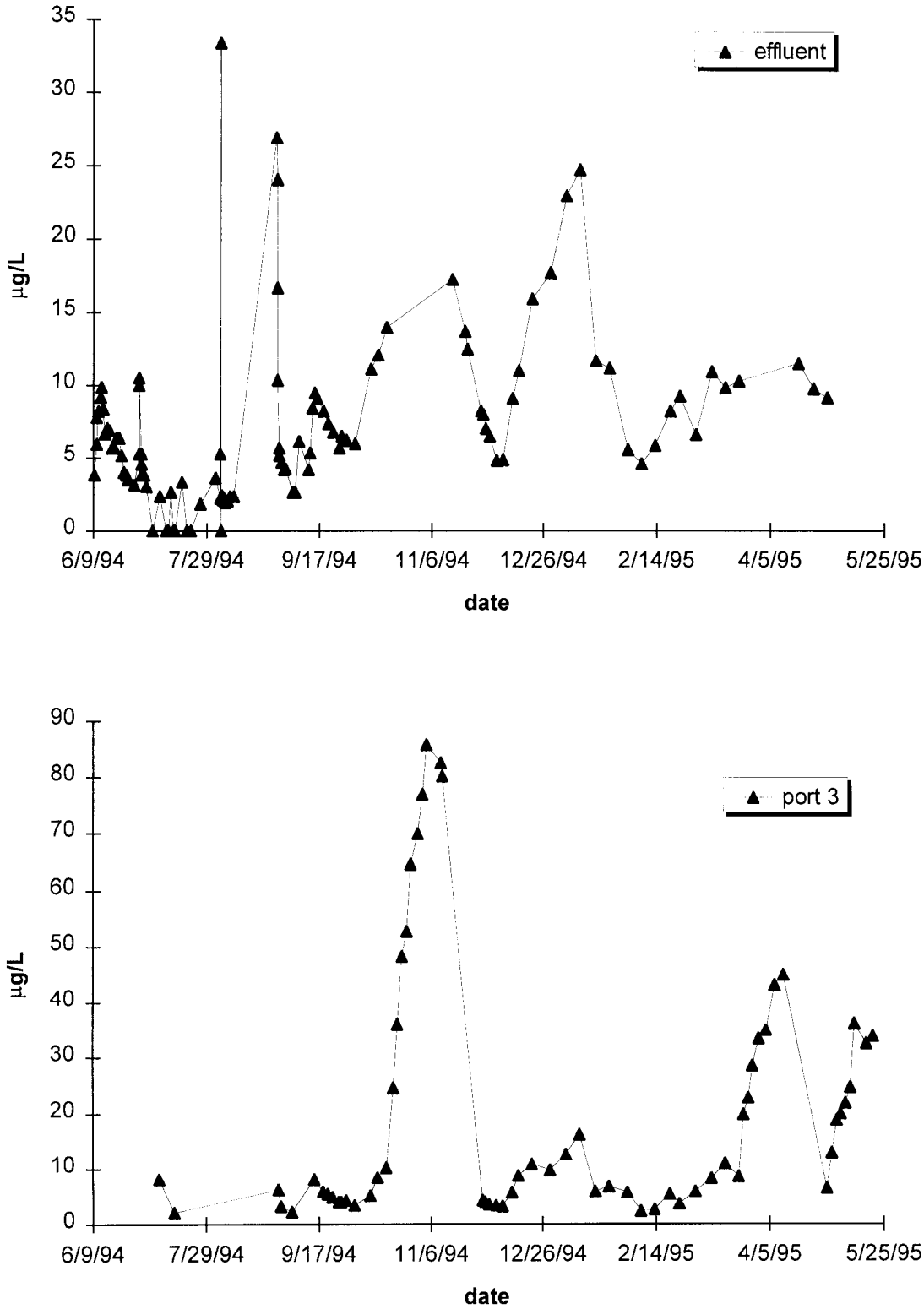


Figure C.2. Plots of overall CF data at the effluent port (30 cm) and at port 3 (14 cm).

Table C.3. Overall TCA data.

		concentration @ port/cm						
date time	sample #'s	influent 0	1 3.5	2 7.6	3 14	4 23	effluent 30	
6/9/94 9:00	1	1436.56						Began breakthrough experiments, residence time = 0.5 days
6/9/94 10:00	2						0.00	
6/9/94 11:00	3						17.31	
6/9/94 12:00	4						24.88	
6/9/94 13:00	5-6	198.27					25.21	
6/9/94 14:00	7						26.56	
6/9/94 15:00	8						37.08	
6/9/94 16:00	9						31.47	
6/9/94 17:00	10-11	165.38					59.06	
6/9/94 18:00	12						63.92	
6/9/94 19:00	13						74.35	
6/9/94 20:00	14						85.83	
6/9/94 21:00	15-16	57.55					81.55	
6/10/94 1:15	17-18	48.19					78.38	
6/10/94 7:30	19-20	169.06					70.62	
6/10/94 11:30	21-22	207.30					100.13	
6/10/94 18:30	23-24	238.77					125.41	
6/10/94 21:30	25-26	176.77					144.49	
6/11/94 10:30	27-28	179.98					157.47	
6/12/94 12:00	29-30	192.22					163.16	
6/12/94 19:15	31-32	177.09					165.29	
6/13/94 13:20	33-34	163.62					154.48	
6/14/94 9:45	35-36	162.31					154.94	
6/15/94 11:20	37-38	173.45					154.86	
6/16/94 10:15	39-40	189.82					151.77	
6/17/94 9:20	41-42	150.27					149.09	
6/18/94 11:00	43-44	184.70					157.73	
6/19/94 16:00	45-46	206.47					161.40	
6/20/94 17:15	47-48	233.73					179.70	
6/21/94 16:15	49-50	188.37					178.55	
6/22/94 15:00	51-52	292.40					179.53	
6/23/94 16:15	53-54	227.52					179.83	
6/24/94 14:30	55-56	190.75					186.02	
6/25/94 14:20	57-62	245.47	182.07	170.21	181.30	185.81	189.82	
6/27/94 11:30	63-64	206.37					163.67	Pre-mix chamber installed
6/29/94 9:00	65-66	219.24					159.83	
6/29/94 10:45	67						170.66	
6/29/94 13:00	68-69	239.32					172.06	
6/29/94 15:00	70	211.44						
6/30/94 9:45	75-76	222.98					207.29	
6/30/94 13:45	77-78	230.84					208.98	
6/30/94 16:45	79-80	223.26					206.44	
7/1/94 10:00	83-84	220.55					218.86	
7/2/94 11:15	85-86	182.16					187.06	
7/5/94 14:00	87-88	173.87					170.56	
7/6/94 12:00	89-94	177.28	179.89	188.71	187.87	175.84	166.87	
7/8/94 14:15	95-100	181.58	155.49	150.93	162.39	160.70	169.31	
7/11/94 13:45	101-102	182.10					180.00	
7/12/94 13:00	103-104	184.04					177.08	
7/13/94 11:00	105-106	185.32					176.54	
7/14/94 16:00	107-108	176.66					190.59	
7/15/94 10:30	109-114	177.90	174.49	175.93	175.06	168.69	176.92	
7/18/94 14:30	115-116	163.38					163.19	
7/20/94 15:45	117-118	182.87					165.81	
7/22/94 11:15	119-120	173.73					173.70	
7/26/94 15:45	121-122	167.53					163.70	
8/2/94 11:15	123-124	134.55					146.94	
8/4/94 9:00	125	199.49						Began benzoate addition
8/4/94 11:00	126-127	226.53					152.04	
8/4/94 12:50	128-129	233.93					178.62	
8/4/94 14:50	130-131	231.48					216.84	
8/4/94 18:00	132-133	213.19					208.34	
8/4/94 22:00	134-135	200.08					200.60	
8/5/94 0:15	136-137	209.64					195.77	
8/5/94 10:15	138-139	209.96					196.74	
8/5/94 16:30	140-141	201.53					203.69	
8/6/94 12:30	142-143	204.43					198.49	
8/7/94 14:15	144-145	214.38					199.66	
8/8/94 12:45	146-147	230.00					207.78	
8/10/94 12:30	148-149	220.78					199.26	Batch test initialized
8/22/94 12:00	150-151		173.42			166.18		Batch test completed
8/29/94 12:45	152	corrupted data						Restarted continuous-flow, residence time = 0.5 days
8/29/94 14:00	153-154	243.51					156.92	

8/29/94 15:00	155-156	243.24					167.48	
8/29/94 17:00	157-158	243.94					182.90	
8/29/94 21:45	159-160	239.46					209.04	
8/30/94 10:10	161-166	241.99	205.23	231.38	234.26	228.69	225.35	
8/30/94 15:00	167-168	246.79					218.37	
8/31/94 12:00	169-174	242.28	214.23	224.31	229.72	248.24	232.16	
9/1/94 13:15	175-176	250.27					237.44	
9/2/94 11:15	177-178	250.07					227.93	
9/5/94 11:15	179-184	232.75	233.14	190.62	227.68	221.59	242.42	
9/6/94 13:45	185-186	226.82					223.86	
9/8/94 11:00	187-188	218.22					216.21	
9/12/94 10:30	189-190	221.97					223.84	
9/13/94 10:15	191-192	262.33					240.07	Lowered flow-rate, residence time = 2 days
9/14/94 11:30	193-194	221.19					238.54	
9/15/94 11:15	197-202	218.97	216.58	227.53	197.75	237.92	224.58	
9/16/94 11:00	203-204	235.98					229.32	
9/19/94 10:00	205-210	240.80	235.41	231.17	224.04	204.88	235.57	
9/21/94 12:15	211-216	203.16	198.21	181.78	205.10	211.03	217.57	
9/23/94 12:00	217-222	201.57	200.36	209.38	199.70	196.32	203.63	
9/26/94 10:15	223-228	181.71	186.81	164.74	188.53	200.23	194.80	
9/27/94 10:45	229-234	199.10	191.57	185.99	204.02	210.92	208.22	Began acetate addition
9/29/94 10:15	235-240	234.19	220.64	229.90	238.07	229.50	219.55	
10/3/94 10:15	241-246	212.18	210.33	212.45	209.73	205.21	210.37	Increased acetate concentration
10/10/94 11:00	247-252	197.03	187.34	180.81	196.13	206.43	202.67	Increased acetate concentration
10/13/94 11:15	253-258	190.36	186.76	168.34	184.69	149.04	181.79	
10/17/94 11:15	259-264	214.18	213.38	214.68	211.19	215.60	208.39	Batch test initialized
10/20/94 12:45	265				181.68			
10/22/94 10:30	266				166.08			
10/24/94 11:45	267				185.10			
10/26/94 12:15	268				160.42			
10/28/94 11:30	269				184.94			
10/31/94 11:00	270				180.40			
11/2/94 13:00	271				192.66			
11/4/94 10:00	272				181.43			
11/10/94 14:15	273				195.08			
11/11/94 9:30	274-277		202.17	186.75	183.77	175.21		Batch test completed
11/15/94 14:00	278-279	335.52					273.34	Restarted continuous-flow, residence time = 2 days
11/21/94 11:00	280-281	298.84					306.57	
11/22/94 10:30	282-283	297.44					305.31	
11/28/94 12:45	284-285	188.39					220.00	
11/29/94 12:15	286-291	204.51	229.07	219.87	222.21	236.88	224.33	
11/30/94 11:15	292-297	211.59	218.45	225.58	212.90	220.80	215.05	
12/2/94 9:00	298-303	184.32	231.59	240.29	240.13	238.68	224.30	
12/5/94 10:00	304-309	218.95	220.21	223.93	230.95	239.21	231.15	
12/8/94 9:45	310-315	198.08	275.35	275.04	277.44	274.79	268.27	Increased acetate concentration
12/12/94 10:45	316-321	173.75	315.13	312.55	293.33	284.48	268.77	
12/15/94 11:00	322-327	354.86	360.14	351.06	337.55	330.16	317.50	
12/21/94 10:00	328-333	207.22	218.22	220.92	225.38	244.98	239.77	
12/29/94 10:15	334-339	313.89	289.85	285.85	305.96	257.83	259.21	
1/5/95 10:00	340-345	362.86	357.74	339.78	331.25	316.03	304.17	
1/11/95 11:00	346-351	343.35	331.51	313.38	321.81	307.06	300.84	
1/18/95 11:00	352-357	209.33	205.85	207.74	211.26	213.28	217.15	
1/24/95 9:30	358-363	202.90	207.43	182.78	186.14	180.32	180.58	
2/1/95 10:30	364-369	76.84	69.88	70.74	74.58	84.35	82.42	Began yeast extract addition
2/7/95 9:45	370-376	98.03	105.78	106.53	107.71	108.68	111.51	
2/13/95 10:30	377-382	133.49	128.17	118.15	126.73	114.93	119.41	
2/20/95 9:30	383-388	167.12	165.59	163.21	167.17	162.06	141.68	
2/24/95 10:15	389-394	169.26	167.44	166.72	170.47	165.41	168.33	Yeast extract and nitrate removed
3/3/95 10:30	395-400	132.84	152.54	147.42	150.08	150.79	143.19	
3/10/95 12:20	401-406	165.51	170.32	170.35	171.89	169.50	170.44	
3/16/95 12:30	407-412	116.35	138.36	138.35	129.85	107.64	126.68	
3/22/95 10:15	413-418	109.92	112.67	107.01	113.89	114.00	121.36	Batch test initialized
3/24/95 14:45	420				124.90			
3/26/95 16:15	421				129.64			
3/28/95 11:45	422				124.81			
3/31/95 12:30	423				123.73			
4/3/95 11:30	424				117.14			
4/7/95 14:00	425				128.24			
4/11/95 8:00	426-429		110.90	116.03	125.31	133.38		Batch test completed
4/17/95 12:45	430-431	35.86					47.39	Nitrate reestablished in influent
4/24/95 10:00	432-433	116.71					96.06	
4/30/95 12:30	434-439	153.39	148.53	143.17	145.72	132.95	142.87	Batch test initialized
5/2/95 11:30	440				144.08			
5/4/95 11:00	441				134.49			
5/6/95 11:30	442				129.68			
5/8/95 11:00	443				136.80			
5/10/95 11:30	444				131.56			
5/12/95 10:30	445				191.48			
5/17/95 11:00	446				131.35			
5/20/95 13:30	447				131.40			
5/24/95 7:30	448-451		128.62	127.82	129.78	120.47		Batch test completed

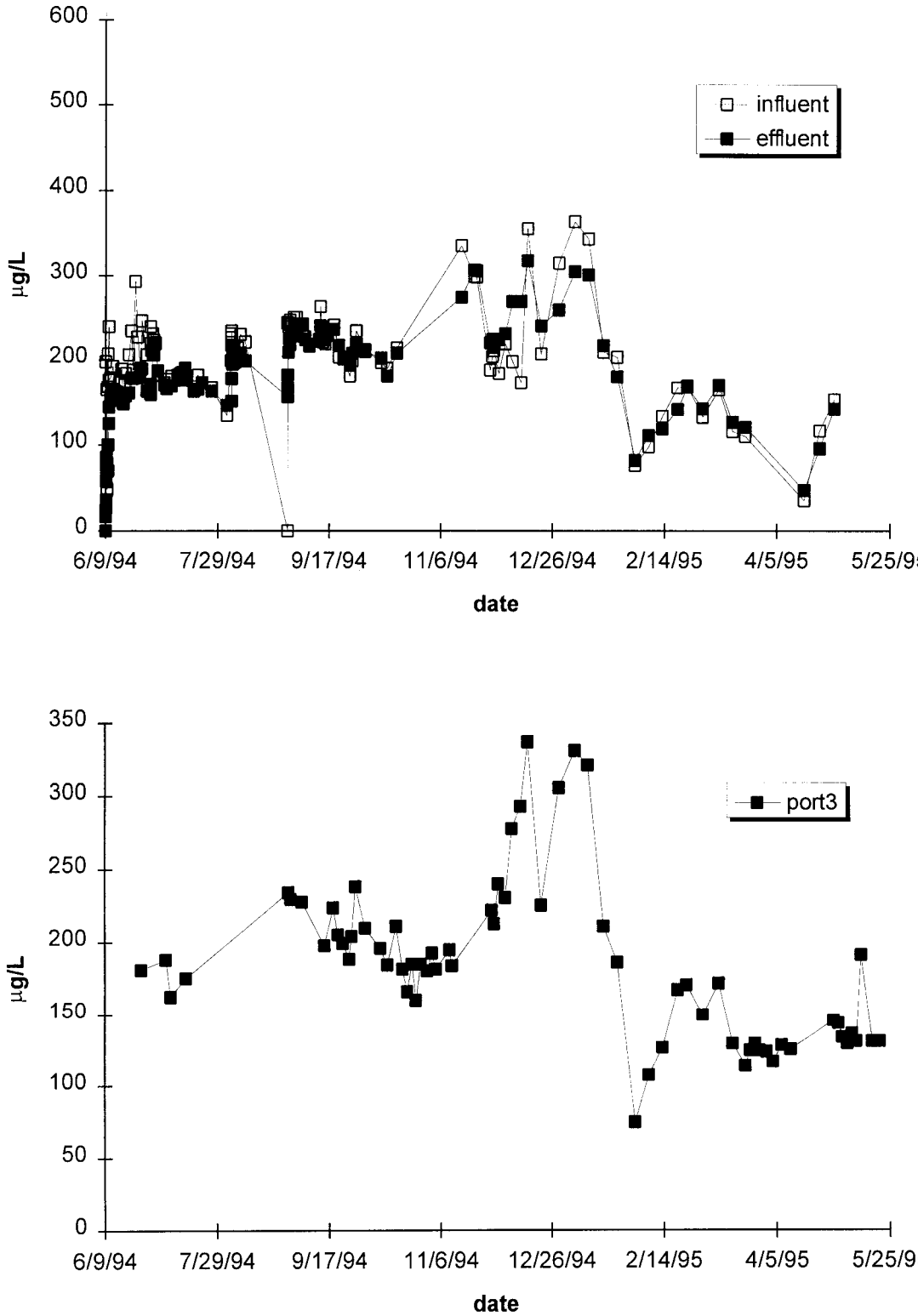


Figure C.3. Plots of overall TCA data at the influent (0 cm) and effluent (30 cm) ports and at port 3 (14 cm).

Table C.4. Overall chloride data.

		concentration @ port/cm						
date time	sample #'s	influent 0	1 3.5	2 7.6	3 14	4 23	effluent 30	
6/9/94 9:00	1	5.92						Began breakthrough experiments, residence time = 0.5 days
6/9/94 10:00	2						6.77	
6/9/94 11:00	3						6.74	
6/9/94 12:00	4						6.72	
6/9/94 13:00	5-6	5.69					6.49	
6/9/94 14:00	7						6.30	
6/9/94 15:00	8						5.96	
6/9/94 16:00	9						6.21	
6/9/94 17:00	10-11	5.69					6.19	
6/9/94 18:00	12						6.22	
6/9/94 19:00	13						6.27	
6/9/94 20:00	14						6.03	
6/9/94 21:00	15-16	5.69					6.66	
6/10/94 1:15	17-18	5.69					6.39	
6/10/94 7:30	19-20	5.82					5.66	
6/10/94 11:30	21-22	5.75					5.83	
6/10/94 18:30	23-24	5.62					5.68	
6/10/94 21:30	25-26	5.33					5.66	
6/11/94 10:30	27-28	5.65					5.58	
6/12/94 12:00	29-30	5.68					5.21	
6/12/94 19:15	31-32	5.69					5.62	
6/13/94 13:20	33-34	5.80					5.56	
6/14/94 9:45	35-36	5.60					5.58	
6/15/94 11:20	37-38	5.69					5.52	
6/16/94 10:15	39-40	5.75					5.59	
6/17/94 9:20	41-42	5.50					5.39	
6/18/94 11:00	43-44	5.62					5.26	
6/19/94 16:00	45-46	5.52					5.39	
6/20/94 17:15	47-48	5.55					5.61	
6/21/94 16:15	49-50	5.59					5.47	
6/22/94 15:00	51-52	5.53					5.43	
6/23/94 16:15	53-54	5.51					5.84	
6/24/94 14:30	55-56	5.97					5.68	
6/25/94 14:20	57-62	6.38	5.85	6.96	6.30	6.08	5.95	
6/27/94 11:30	63-64	6.08					6.09	Pre-mix chamber installed
6/29/94 9:00	65-66	5.46					5.70	
6/29/94 10:45	67						5.94	
6/29/94 13:00	68-69	5.49					6.02	
6/29/94 15:00	70	5.81						
6/29/94 17:00	71-72	5.39					5.62	
6/29/94 22:15	73-74	5.62					5.39	
6/30/94 9:45	75-76	5.70					6.98	
6/30/94 13:45	77-78	5.64					5.47	
6/30/94 16:45	79-80	6.02					5.38	
6/30/94 23:00	81-82	5.87					5.32	
7/1/94 10:00	83-84	5.80					5.82	
7/2/94 11:15	85-86	5.70					5.63	
7/5/94 14:00	87-88	5.65					5.90	
7/6/94 12:00	89-94	5.72	5.76	5.85	5.43	5.67	5.46	
7/8/94 14:15	95-100	6.10					5.84	
7/11/94 13:45	101-102	5.99					6.09	
7/12/94 13:00	103-104	5.92					5.85	
7/13/94 11:00	105-106	5.48					5.31	
7/14/94 16:00	107-108	5.52					6.66	
7/15/94 10:30	109-114		5.82	5.11	5.10	5.60	5.74	
7/18/94 14:30	115-116	2.67					2.67	
7/20/94 15:45	117-118	2.67					2.67	
7/22/94 11:15	119-120	2.67					2.67	
7/26/94 15:45	121-122	2.67					2.67	
8/2/94 11:15	123-124	2.67					2.67	
8/4/94 9:00	125	2.67						Began benzoate addition
8/4/94 11:00	126-127	2.67					2.67	
8/4/94 12:50	128-129	2.67					2.67	
8/4/94 14:50	130-131	2.67					2.67	
8/4/94 18:00	132-133	2.67					2.67	
8/4/94 22:00	134-135	2.67					2.67	
8/5/94 0:15	136-137	2.67					2.67	
8/5/94 10:15	138-139	2.67					2.67	
8/5/94 16:30	140-141	2.67					2.67	
8/6/94 12:30	142-143	2.67					2.67	
8/7/94 14:15	144-145	2.67					2.67	
8/8/94 12:45	146-147	4.37					4.27	

8/10/94 12:30	148-149	5.00					4.34	Batch test initialized
8/22/94 12:00	150-151		5.78			4.09		Batch test completed
8/29/94 12:45	152	4.52						Restarted continuous-flow, residence time = 0.5 days
8/29/94 14:00	153-154	4.70					4.62	
8/29/94 15:00	155-156	4.89					4.61	
8/29/94 17:00	157-158	4.37					3.99	
8/29/94 21:45	159-160	3.86					4.80	
8/30/94 10:10	161-166	3.31	1.54	2.63	3.24	2.46	1.91	
8/30/94 15:00	167-168	3.60					4.11	
8/31/94 12:00	169-174		4.55	4.65	4.77	4.99	4.47	
9/1/94 13:15	175-176	4.62					4.40	
9/2/94 11:15	177-178	4.31					4.68	
9/5/94 11:15	179-184	11.71	9.69	11.65	10.95	9.75	12.07	
9/6/94 13:45	185-186	11.82					11.48	
9/8/94 11:00	187-188	10.26					9.98	
9/12/94 10:30	189-190	7.72					5.00	
9/13/94 10:15	191-192	5.26					4.72	Lowered flow-rate, residence time = 2 days
9/14/94 11:30	193-194	4.91						
9/14/94 13:30	195-196	4.30					4.68	
9/15/94 11:15	197-202	4.89	4.37	4.52	5.14	5.25	4.97	
9/16/94 11:00	203-204	4.66					4.93	
9/19/94 10:00	205-210	4.35	4.07	4.16	4.04	4.47	4.69	
9/21/94 12:15	211-216	4.37	3.96	4.47	4.07	4.71	4.50	
9/23/94 12:00	217-222	4.51	3.84	4.52	4.01	4.20	4.41	
9/26/94 10:15	223-228	4.42	4.75	4.87	4.03	4.09	4.39	
9/27/94 10:45	229-234	4.33	4.47	4.29	3.91	3.86	4.25	Began acetate addition
9/29/94 10:15	235-240	4.24	3.98	4.06	3.98	4.09	4.55	
10/3/94 10:15	241-246	4.03	4.09	4.15	4.18	4.17	5.33	Increased acetate concentration
10/10/94 11:00	247-252							Increased acetate concentration
10/13/94 11:15	253-258	4.29	4.28	4.31	4.35	4.28	4.20	
10/17/94 11:15	259-264	4.48	4.19	4.17	4.16	4.18	4.30	Batch test initialized
10/20/94 12:45	265				4.57			
10/22/94 10:30	266				4.31			
10/24/94 11:45	267				4.17			
10/26/94 12:15	268				4.08			
10/28/94 11:30	269				4.18			
10/31/94 11:00	270				4.09			
11/2/94 13:00	271				4.95			
11/4/94 10:00	272				4.37			
11/10/94 14:15	273				4.46			Batch test completed 11/11
11/29/94 12:15	286-291	4.63	4.40	4.73	4.71	4.52	4.54	Restarted continuous-flow 11/15, residence time = 2 days
11/30/94 11:15	292-297	4.52	4.60	4.60	4.57	4.55	4.52	
12/2/94 9:00	298-303	4.56	4.63	4.52	4.49	4.51	4.48	
12/5/94 10:00	304-309	4.69	4.82	5.06	4.95	4.82	4.89	
12/8/94 9:45	310-315	4.71	4.79	4.79	4.59	4.88	4.69	Increased acetate concentration
12/12/94 10:45	316-321	4.74	4.81	4.95	4.73	4.88	4.84	
12/15/94 11:00	322-327	4.76	4.97	4.82	4.77	4.69	4.64	
12/21/94 10:00	328-333	4.73	5.05	4.84	4.83	4.65	4.72	
12/29/94 10:15	334-339	4.59	4.44	4.76	4.77	4.78	4.74	
1/5/95 10:00	340-345	4.45	4.45	4.48	4.52	4.44	4.46	
1/11/95 11:00	346-351	4.30	4.34	4.35	4.36	4.31	4.28	
1/18/95 11:00	352-357	4.33	4.36	4.36	4.43	4.38	4.49	
1/24/95 9:30	358-363	4.75	4.48	4.39	4.44	4.46	4.38	
2/1/95 10:30	364-369	4.25	4.11	4.21	4.35	4.40	4.15	Began yeast extract addition
2/7/95 9:45	370-376	4.02	4.15	4.23	4.21	4.22	4.11	
2/13/95 10:30	377-382	4.03	4.08	4.20	4.10	4.04	4.02	
2/20/95 9:30	383-388	3.97	4.13	4.03	4.03	4.18	4.18	
2/24/95 10:15	389-394	3.99	3.99	4.01	4.02	4.22	4.18	Yeast extract and nitrate removed
3/3/95 10:30	395-400	3.84	3.90	3.88	3.97	3.93	3.75	
3/10/95 12:20	401-406	3.87	3.78	3.93	3.95	3.85	3.76	
3/16/95 12:30	407-412	3.82	3.87	3.99	3.86	4.31	3.75	
3/22/95 10:15	413-418	3.93	3.98	4.35	3.98	3.98	3.92	Batch test initialized
3/24/95 14:45	420				3.99		0.00	
3/26/95 16:15	421				3.95		0.00	
3/28/95 11:45	422				3.95		0.00	
3/31/95 12:30	423				3.97		0.00	
4/3/95 11:30	424				3.92		0.00	
4/7/95 14:00	425				3.90		0.00	
4/11/95 8:00	426-429		3.95	3.66	4.02	4.02	0.00	Batch test completed
4/17/95 12:45	430-431	3.70					4.23	Nitrate reestablished in influent
4/24/95 10:00	432-433	3.94					4.21	
4/30/95 12:30	434-439	4.08	4.22	4.16	4.28	4.33	4.28	Batch test initialized
5/2/95 11:30	440			0.00	4.18		0.00	
5/4/95 11:00	441			0.00	4.38		0.00	
5/6/95 11:30	442			0.00	4.48		0.00	
5/8/95 11:00	443			0.00	4.29		0.00	
5/10/95 11:30	444			0.00	4.11		0.00	
5/12/95 10:30	445			0.00	4.09		0.00	
5/17/95 11:00	446			0.00	4.32		0.00	
5/20/95 13:30	447			0.00	4.32		0.00	
5/24/95 7:30			4.13	4.21	4.16	4.23	0.00	Batch test completed

Table C.5. Overall bromide data.

		concentration @ port/cm						
date time	sample #'s	influent 0	1 3.5	2 7.6	3 14	4 23	effluent 30	
6/9/94 9:00	1	26.00						Began breakthrough experiments, residence time = 0.5 days
6/9/94 10:00	2						0.00	
6/9/94 11:00	3						0.16	
6/9/94 12:00	4						1.29	
6/9/94 13:00	5-6	22.62					3.27	
6/9/94 14:00	7						5.06	
6/9/94 15:00	8						7.13	
6/9/94 16:00	9						9.35	
6/9/94 17:00	10-11	23.74					9.56	
6/9/94 18:00	12						11.09	
6/9/94 19:00	13						12.76	
6/9/94 20:00	14						13.99	
6/9/94 21:00	15-16	24.41					15.36	
6/10/94 1:15	17-18	24.75					18.54	
6/10/94 7:30	19-20	25.48					22.57	
6/10/94 11:30	21-22	25.69					22.67	
6/10/94 18:30	23-24	25.88					24.29	
6/10/94 21:30	25-26	25.89					24.86	
6/11/94 10:30	27-28	25.76					26.75	
6/12/94 12:00	29-30	25.94					26.35	
6/12/94 19:15	31-32	25.91					25.96	
6/13/94 13:20	33-34	26.24					25.96	
6/14/94 9:45	35-36	26.32					25.96	
6/15/94 11:20	37-38	26.36					25.96	
6/16/94 10:15	39-40	25.61					25.96	
6/17/94 9:20	41-42	26.41					26.29	
6/18/94 11:00	43-44	25.91					25.31	
6/19/94 16:00	45-46	25.97					26.59	
6/20/94 17:15	47-48	25.90					26.73	
6/21/94 16:15	49-50	26.36					26.53	
6/22/94 15:00	51-52	26.14					25.22	
6/23/94 16:15	53-54	26.11					26.66	
6/24/94 14:30	55-56	25.98					25.92	
6/25/94 14:20	57-62	26.08					25.64	
6/27/94 11:30	63-64	26.01					26.06	Pre-mix chamber installed
6/29/94 9:00	65-66						25.76	
6/29/94 10:45	67						25.23	
6/29/94 13:00	68-69						20.46	
6/29/94 15:00	70							
6/29/94 17:00	71-72						13.23	
6/29/94 22:15	73-74						5.31	
6/30/94 9:45	75-76						3.80	
6/30/94 13:45	77-78						3.54	
6/30/94 16:45	79-80						2.55	
6/30/94 23:00	81-82						1.28	
7/1/94 10:00	83-84						0.47	
7/2/94 11:15	85-86						0.00	

Table C.6. Overall nitrite data.

		concentration @ port/cm						
		influent	1	2	3	4	effluent	
date time	sample #'s	0	3.5	7.6	14	23	30	
6/9/94 9:00	1	0.00						Began breakthrough experiments, residence time = 0.5 days
6/9/94 10:00	2						0.00	
6/9/94 11:00	3						0.00	
6/9/94 12:00	4						0.00	
6/9/94 13:00	5-6	1.60					0.34	
6/9/94 14:00	7						0.45	
6/9/94 15:00	8						0.31	
6/9/94 16:00	9						0.46	
6/9/94 17:00	10-11	1.58					0.55	
6/9/94 18:00	12						0.23	
6/9/94 19:00	13						0.44	
6/9/94 20:00	14						0.31	
6/9/94 21:00	15-16	0.72					0.45	
6/10/94 1:15	17-18	0.80					0.49	
6/10/94 7:30	19-20	0.00					1.11	
6/10/94 11:30	21-22	0.00					1.14	
6/10/94 18:30	23-24	0.00					0.91	
6/10/94 21:30	25-26	0.00					0.17	
6/11/94 10:30	27-28	0.00					1.12	
6/12/94 12:00	29-30	0.00					1.49	
6/12/94 19:15	31-32	0.00					0.80	
6/13/94 13:20	33-34	0.00					1.22	
6/14/94 9:45	35-36	0.00					6.42	
6/15/94 11:20	37-38	0.00					2.36	
6/16/94 10:15	39-40	0.00					2.74	
6/17/94 9:20	41-42	0.00					2.77	
6/18/94 11:00	43-44	0.38					3.45	
6/19/94 16:00	45-46	0.00					4.14	
6/20/94 17:15	47-48	0.00					4.49	
6/21/94 16:15	49-50	0.00					4.92	
6/22/94 15:00	51-52	0.00					4.60	
6/23/94 16:15	53-54	0.00					5.26	
6/24/94 14:30	55-56	0.00					5.28	
6/25/94 14:20	57-62	0.48					4.71	
6/27/94 11:30	63-64	0.20					6.90	Pre-mix chamber installed
6/29/94 9:00	65-66	0.00					0.33	
6/29/94 10:45	67						0.55	
6/29/94 13:00	68-69	0.32					1.42	
6/29/94 15:00	70	0.19						
6/29/94 17:00	71-72	0.13					2.18	
6/29/94 22:15	73-74	0.00					2.08	
6/30/94 9:45	75-76	0.00					3.31	
6/30/94 13:45	77-78	0.24					2.70	
6/30/94 16:45	79-80	0.23					3.10	
6/30/94 23:00	81-82	0.48					3.96	
7/1/94 10:00	83-84	0.36					4.51	
7/2/94 11:15	85-86	0.16					7.23	
7/5/94 14:00	87-88	0.08					7.05	
7/6/94 12:00	89-94	0.32					8.09	
7/8/94 14:15	95-100	1.32					10.73	
7/11/94 13:45	101-102	0.38					6.85	
7/12/94 13:00	103-104	0.00					7.33	
7/13/94 11:00	105-106	0.35					6.33	
7/14/94 16:00	107-108	0.19					6.41	
7/15/94 10:30	109-114						5.01	
7/18/94 14:30	115-116	0.04					4.75	
7/20/94 15:45	117-118	0.19					5.77	
7/22/94 11:15	119-120	0.16					5.12	
7/26/94 15:45	121-122	0.12					3.84	
8/2/94 11:15	123-124	0.19					3.39	
8/4/94 9:00	125	0.22						Began benzoate addition
8/4/94 11:00	126-127	1.48					9.71	
8/4/94 12:50	128-129	0.29					7.01	
8/4/94 14:50	130-131	0.18					5.09	
8/4/94 18:00	132-133	0.21					3.64	
8/4/94 22:00	134-135	0.98					3.95	
8/5/94 0:15	136-137	0.46					4.12	
8/5/94 10:15	138-139	0.08					3.60	
8/5/94 16:30	140-141	0.47					2.50	
8/6/94 12:30	142-143	1.43					2.62	
8/7/94 14:15	144-145	0.20					3.04	
8/8/94 12:45	146-147	2.30					4.61	

8/10/94 12:30	148-149	2.16					4.05	Batch test initialized
8/22/94 12:00	150-151		2.01			2.04		Batch test completed
8/29/94 12:45	152	2.17						Restarted continuous-flow, residence time = 0.5 days
8/29/94 14:00	153-154	2.29					2.99	
8/29/94 15:00	155-156	2.34					5.78	
8/29/94 17:00	157-158	2.29					11.67	
8/29/94 21:45	159-160	2.27					8.03	
8/30/94 10:10	161-166	1.54	1.50	3.30	3.87	2.22	2.39	
8/30/94 15:00	167-168	0.74					6.00	
8/31/94 12:00	169-174		1.95	2.23	2.33	4.15	4.27	
9/1/94 13:15	175-176	0.86					4.54	
9/2/94 11:15	177-178	0.62					3.59	
9/5/94 11:15	179-184	0.05	0.73	1.19	1.33	1.89	2.25	
9/6/94 13:45	185-186	0.58					1.58	
9/8/94 11:00	187-188	0.63					2.20	
9/12/94 10:30	189-190	0.01					1.46	
9/13/94 10:15	191-192	0.01					4.03	Lowered flow-rate, residence time = 2 days
9/14/94 11:30	193-194	0.82						
9/14/94 13:30	195-196	0.67					6.45	
9/15/94 11:15	197-202	0.76	3.26	3.75	5.23	4.97	6.59	
9/16/94 11:00	203-204	0.77					6.35	
9/19/94 10:00	205-210	0.61	3.51	4.21	4.38	4.46	6.56	
9/21/94 12:15	211-216	0.71	3.29	4.32	4.47	4.39	6.87	
9/23/94 12:00	217-222	0.71	3.66	6.27	4.37	4.66	7.35	
9/26/94 10:15	223-228	0.90	5.30	6.41	5.10	4.93	7.30	
9/27/94 10:45	229-234	4.39	8.97	8.90	7.49	6.70	8.81	Began acetate addition
9/29/94 10:15	235-240	20.12	15.60	12.58	11.51	8.70	10.75	
10/3/94 10:15	241-246	16.62	8.95	6.16	6.27	3.77	5.31	Increased acetate concentration
10/10/94 11:00	247-252							Increased acetate concentration
10/13/94 11:15	253-258	4.31	0.12	0.00	0.00	0.00	0.00	
10/17/94 11:15	259-264	0.62	0.00	0.00	0.00	0.00	0.00	Batch test initialized
10/20/94 12:45	265				0.00			
10/22/94 10:30	266				0.00			
10/24/94 11:45	267				0.00			
10/26/94 12:15	268				0.00			
10/28/94 11:30	269				0.00			
10/31/94 11:00	270				0.00			
11/2/94 13:00	271				0.00			
11/4/94 10:00	272				0.00			
11/10/94 14:15	273				0.00			Batch test completed 11/11
11/29/94 12:15	286-291	0.07	3.18	3.11	3.25	3.03	2.66	Restarted continuous-flow 11/15, residence time = 2 days
11/30/94 11:15	292-297	0.56	5.33	7.10	7.10	5.14	5.00	
12/2/94 9:00	298-303	2.07	4.68	5.12	5.27	4.99	4.76	
12/5/94 10:00	304-309	3.36	4.63	3.42	3.16	2.58	2.62	
12/8/94 9:45	310-315	3.81	0.47	0.00	0.00	0.11	0.00	Increased acetate concentration
12/12/94 10:45	316-321	8.25	0.04	0.00	0.00	0.00	0.00	
12/15/94 11:00	322-327	7.48	0.57	0.00	0.00	0.00	0.00	
12/21/94 10:00	328-333	18.41	0.06	0.00	0.00	0.00	0.00	
12/29/94 10:15	334-339	25.13	0.00	0.00	0.00	0.00	0.00	
1/5/95 10:00	340-345	27.64	0.00	0.00	0.00	0.00	0.00	
1/11/95 11:00	346-351	30.68	1.05	0.00	0.00	0.00	0.00	
1/18/95 11:00	352-357	5.92	0.46	0.11	0.05	0.00	0.00	
1/24/95 9:30	358-363	4.69	0.14	0.03	0.00	0.00	0.00	
2/1/95 10:30	364-369	3.95	0.02	0.00	0.00	0.00	0.00	Began yeast extract addition
2/7/95 9:45	370-376	1.66	0.32	0.21	0.12	0.03	0.21	
2/13/95 10:30	377-382	3.03	0.58	0.27	0.07	0.04	0.04	
2/20/95 9:30	383-388	4.68	1.94	0.08	0.00	0.02	0.00	
2/24/95 10:15	389-394	4.90	1.07	0.00	0.00	0.00	0.00	Yeast extract and nitrate removed
3/3/95 10:30	395-400	0.26	0.05	0.00	0.00	0.00	0.00	
3/10/95 12:20	401-406	0.00	0.00	0.00	0.00	0.00	0.00	
3/16/95 12:30	407-412	0.00	0.00	0.00	0.00	0.00	0.00	
3/22/95 10:15	413-418	0.00	0.00	0.00	0.00	0.00	0.00	Batch test initialized
3/24/95 14:45	420							
3/26/95 16:15	421							
3/28/95 11:45	422							
3/31/95 12:30	423							
4/3/95 11:30	424							
4/7/95 14:00	425							
4/11/95 8:00	426-429		0.00	0.00	0.00	0.00		Batch test completed
4/17/95 12:45	430-431	0.48					0.00	Nitrate reestablished in influent
4/24/95 10:00	432-433	7.74					0.00	
4/30/95 12:30	434-439	10.55	0.00	0.89	0.14	0.00	0.00	Batch test initialized
5/2/95 11:30	440				0.00			
5/4/95 11:00	441				0.00			
5/6/95 11:30	442				0.00			
5/8/95 11:00	443				0.00			
5/10/95 11:30	444				0.00			
5/12/95 10:30	445				0.00			
5/17/95 11:00	446				0.00			
5/20/95 13:30	447				0.00			
5/24/95 7:30	448-451		0.00	0.00	0.06	0.23		Batch test completed

Table C.7. Overall nitrate data.

		concentration @ port/cm						
date time	sample #'s	influent 0	1 3.5	2 7.6	3 14	4 23	effluent 30	
6/9/94 9:00	1	42.07						Began breakthrough experiments, residence time = 0.5 days
6/9/94 10:00	2						0.00	
6/9/94 11:00	3						0.00	
6/9/94 12:00	4						0.00	
6/9/94 13:00	5-6	37.65					0.00	
6/9/94 14:00	7						0.00	
6/9/94 15:00	8						0.00	
6/9/94 16:00	9						0.00	
6/9/94 17:00	10-11	39.45					0.00	
6/9/94 18:00	12						0.00	
6/9/94 19:00	13						0.00	
6/9/94 20:00	14						0.00	
6/9/94 21:00	15-16	40.59					1.20	
6/10/94 1:15	17-18	41.16					0.00	
6/10/94 7:30	19-20	42.37					2.90	
6/10/94 11:30	21-22	42.72					1.32	
6/10/94 18:30	23-24	43.04					0.00	
6/10/94 21:30	25-26	43.05					0.00	
6/11/94 10:30	27-28	42.83					1.69	
6/12/94 12:00	29-30	43.14					1.98	
6/12/94 19:15	31-32	43.09					1.53	
6/13/94 13:20	33-34	43.64					6.78	
6/14/94 9:45	35-36	43.77					16.88	
6/15/94 11:20	37-38	43.84					16.52	
6/16/94 10:15	39-40	42.58					11.17	
6/17/94 9:20	41-42	43.53					11.16	
6/18/94 11:00	43-44	41.48					13.19	
6/19/94 16:00	45-46	41.66					11.99	
6/20/94 17:15	47-48	41.41					9.94	
6/21/94 16:15	49-50	42.35					14.04	
6/22/94 15:00	51-52	42.09					3.85	
6/23/94 16:15	53-54	49.56					4.32	
6/24/94 14:30	55-56	49.37					2.72	
6/25/94 14:20	57-62	43.49					6.72	
6/27/94 11:30	63-64	45.10					6.51	Pre-mix chamber installed
6/29/94 9:00	65-66	40.77					0.00	
6/29/94 10:45	67						0.12	
6/29/94 13:00	68-69	31.25					0.00	
6/29/94 15:00	70	41.54						
6/29/94 17:00	71-72	42.72					0.95	
6/29/94 22:15	73-74	42.09					14.82	
6/30/94 9:45	75-76	40.80					1.88	
6/30/94 13:45	77-78	40.41					0.91	
6/30/94 16:45	79-80	52.44					1.96	
6/30/94 23:00	81-82	48.31					3.68	
7/1/94 10:00	83-84	42.81					17.09	
7/2/94 11:15	85-86	43.68					11.79	
7/5/94 14:00	87-88	43.06					25.31	
7/6/94 12:00	89-94	42.09					22.08	
7/8/94 14:15	95-100	40.85					16.13	
7/11/94 13:45	101-102	42.42					35.98	
7/12/94 13:00	103-104	43.10					33.41	
7/13/94 11:00	105-106	43.39					36.25	
7/14/94 16:00	107-108	43.96					35.99	
7/15/94 10:30	109-114						37.59	
7/18/94 14:30	115-116	46.19					38.35	
7/20/94 15:45	117-118	51.71					38.44	
7/22/94 11:15	119-120	51.05					39.75	
7/26/94 15:45	121-122	50.72					42.33	
8/2/94 11:15	123-124	51.37					44.10	
8/4/94 9:00	125	49.81						Began benzoate addition
8/4/94 11:00	126-127	44.80					29.07	
8/4/94 12:50	128-129	50.92					31.58	
8/4/94 14:50	130-131	50.29					36.58	
8/4/94 18:00	132-133	50.35					38.68	
8/4/94 22:00	134-135	46.42					38.60	
8/5/94 0:15	136-137	47.30					38.76	
8/5/94 10:15	138-139	43.42					35.17	
8/5/94 16:30	140-141	48.43					35.96	
8/6/94 12:30	142-143	34.42					36.48	
8/7/94 14:15	144-145	48.63					42.03	
8/8/94 12:45	146-147	53.24					45.94	

8/10/94 12:30	148-149	50.49					45.10	Batch test initialized
8/22/94 12:00	150-151		0.36			1.25		Batch test completed
8/29/94 12:45	152	24.29						Restarted continuous-flow, residence time = 0.5 days
8/29/94 14:00	153-154	49.54					1.51	
8/29/94 15:00	155-156	49.16					1.27	
8/29/94 17:00	157-158	49.31					0.83	
8/29/94 21:45	159-160	44.91					25.28	
8/30/94 10:10	161-166	41.92	17.27	26.50	33.36	16.10	14.85	
8/30/94 15:00	167-168	46.92					35.20	
8/31/94 12:00	169-174		50.50	32.28	47.26	41.60	45.78	
9/1/94 13:15	175-176	58.81					45.56	
9/2/94 11:15	177-178	58.98					51.01	
9/5/94 11:15	179-184	57.57	45.89	52.61	51.50	45.17	53.60	
9/6/94 13:45	185-186	59.62					50.08	
9/8/94 11:00	187-188	55.41					44.91	
9/12/94 10:30	189-190	61.12					53.68	
9/13/94 10:15	191-192	61.44					47.94	Lowered flow-rate, residence time = 2 days
9/14/94 11:30	193-194	58.10						
9/14/94 13:30	195-196	59.14					33.57	
9/15/94 11:15	197-202	57.45	45.35	40.89	38.26	34.33	31.46	
9/16/94 11:00	203-204	58.33					30.81	
9/19/94 10:00	205-210	56.96	39.80	34.53	34.46	30.46	28.63	
9/21/94 12:15	211-216	55.62	40.79	33.75	34.18	27.04	27.52	
9/23/94 12:00	217-222	56.63	34.99	29.33	28.79	23.47	21.92	
9/26/94 10:15	223-228	55.35	39.80	32.62	29.85	21.36	21.99	
9/27/94 10:45	229-234	47.23	23.49	20.41	20.40	15.97	15.84	Began acetate addition
9/29/94 10:15	235-240	10.51	2.71	1.60	1.34	0.58	0.25	
10/3/94 10:15	241-246	0.09	0.07	0.04	0.08	0.09	0.17	Increased acetate concentration
10/10/94 11:00	247-252							Increased acetate concentration
10/13/94 11:15	253-258	0.06	0.08	0.00	0.00	0.14	0.07	
10/17/94 11:15	259-264	0.00	0.08	0.00	0.00	0.06	0.14	Batch test initialized
10/20/94 12:45	265				0.08			
10/22/94 10:30	266				0.00			
10/24/94 11:45	267				0.00			
10/26/94 12:15	268				0.08			
10/28/94 11:30	269				0.06			
10/31/94 11:00	270				0.05			
11/2/94 13:00	271				0.13			
11/4/94 10:00	272				0.08			
11/10/94 14:15	273				0.09			Batch test completed 11/11
11/29/94 12:15	286-291	52.08	3.18	35.74	36.32	34.64	31.37	Restarted continuous-flow 11/15, residence time = 2 days
11/30/94 11:15	292-297	51.08	5.33	2.53	5.81	18.48	16.40	
12/2/94 9:00	298-303	47.95	4.68	0.00	0.00	3.38	3.89	
12/5/94 10:00	304-309	45.68	4.63	1.09	0.59	0.82	0.82	
12/8/94 9:45	310-315	46.01	0.47	0.00	0.00	0.08	0.00	Increased acetate concentration
12/12/94 10:45	316-321	39.63	0.04	0.00	0.00	0.00	0.00	
12/15/94 11:00	322-327	39.41	0.57	0.00	0.00	0.00	0.00	
12/21/94 10:00	328-333	22.15	0.06	0.00	0.00	0.00	0.00	
12/29/94 10:15	334-339	15.78	0.00	0.00	0.00	0.00	0.00	
1/5/95 10:00	340-345	11.21	0.00	0.00	0.00	0.00	0.00	
1/11/95 11:00	346-351	5.27	1.05	0.00	0.00	0.00	0.00	
1/18/95 11:00	352-357	39.14	0.46	2.80	2.13	0.06	0.00	
1/24/95 9:30	358-363	44.59	0.14	0.18	0.00	0.04	0.05	
2/1/95 10:30	364-369	42.43	0.02	0.00	0.07	0.09	0.04	Began yeast extract addition
2/7/95 9:45	370-376	47.25	0.32	3.86	4.47	2.43	1.51	
2/13/95 10:30	377-382	44.00	0.58	2.41	4.29	0.29	0.18	
2/20/95 9:30	383-388	37.32	1.94	0.02	0.06	0.00	0.03	
2/24/95 10:15	389-394	40.45	1.07	0.00	0.00	0.00	0.08	Yeast extract and nitrate removed
3/3/95 10:30	395-400	0.37	0.05	0.05	0.07	0.03	0.00	
3/10/95 12:20	401-406	0.11	0.00	0.09	0.05	0.02	0.03	
3/16/95 12:30	407-412	0.06	0.00	0.41	0.00	0.03	0.04	
3/22/95 10:15	413-418	0.00	0.00	0.00	0.00	0.00	0.00	Batch test initialized
3/24/95 14:45	420				0.00			
3/26/95 16:15	421				0.00			
3/28/95 11:45	422				0.00			
3/31/95 12:30	423				0.00			
4/3/95 11:30	424				0.00			
4/7/95 14:00	425				0.00			
4/11/95 8:00	426-429		0.00	0.00	0.00	0.00		Batch test completed
4/17/95 12:45	430-431	48.37					0.00	Nitrate reestablished in influent
4/24/95 10:00	432-433	36.93					0.03	
4/30/95 12:30	434-439	23.60	0.00	1.10	0.21	0.04	0.08	Batch test initialized
5/2/95 11:30	440				0.05			
5/4/95 11:00	441				0.05			
5/6/95 11:30	442				0.13			
5/8/95 11:00	443				0.07			
5/10/95 11:30	444				0.00			
5/12/95 10:30	445				0.00			
5/17/95 11:00	446				0.25			
5/20/95 13:30	447				0.05			
5/24/95 7:30			0.00	0.00	0.97	6.83		Batch test completed

Table C.8. Overall acetate data.

		concentration @ port/cm						
date time	sample #'s	influent 0	1 3.5	2 7.6	3 14	4 23	effluent 30	
11/29/94 12:15	286-291	0.00	8.19	5.21	3.56	4.03	3.28	Restarted continuous-flow 11/15, residence time = 2 days
11/30/94 11:15	292-297	36.90	6.72	5.10	4.16	4.95	0.00	
12/2/94 9:00	298-303	33.48	5.75	5.01	3.69	4.35	3.07	
12/5/94 10:00	304-309	37.76	5.07	4.49	5.26	4.81	3.33	
12/8/94 9:45	310-315	48.78	13.85	12.53	6.34	4.47	8.06	Increased acetate concentration
12/12/94 10:45	316-321	50.86	21.90	22.55	21.11	17.46	17.26	
12/15/94 11:00	322-327	55.63	28.61	29.14	27.50	24.06	23.40	
12/21/94 10:00	328-333	55.23	35.83	39.19	37.32	31.19	30.25	
12/29/94 10:15	334-339	64.97	50.41	49.76	50.32	47.73	45.55	
1/5/95 10:00	340-345	79.96	63.57	65.05	65.86	61.48	58.64	
1/11/95 11:00	346-351	72.56	59.60	60.50	59.49	58.89	57.10	
1/18/95 11:00	352-357	44.13	18.46	15.58	14.37	15.77	15.99	
1/24/95 9:30	358-363	49.57	18.72	15.09	15.23	15.44	14.43	
2/1/95 10:30	364-369	40.52	8.10	12.92	24.88	28.34	23.84	Began yeast extract addition
2/7/95 9:45	370-376	34.51	17.12	0.00	0.00	0.00	0.00	
2/13/95 10:30	377-382	47.52	11.77	11.20	13.81	0.00	0.00	
2/20/95 9:30	383-388	40.03	19.73	12.31	14.02	13.71	11.67	
2/24/95 10:15	389-394	44.33	16.93	17.71	17.44	18.78	18.62	Yeast extract and nitrate removed
3/3/95 10:30	395-400	64.60	68.43	66.89	66.63	65.12	60.88	
3/10/95 12:20	401-406	67.50	70.62	68.69	71.82	68.35	66.96	
3/16/95 12:30	407-412	59.01	58.89	59.24	59.54	67.18	57.17	
3/22/95 10:15	413-418	60.02	64.25	63.45	62.83	60.59	61.18	Batch test initialized
3/24/95 14:45	420				54.00			
3/26/95 16:15	421				54.36			
3/28/95 11:45	422				53.53			
3/31/95 12:30	423				59.58			
4/3/95 11:30	424				57.30			
4/7/95 14:00	425				59.18			
4/11/95 8:00	426-429		71.76	62.08	53.43	47.62		Batch test completed
4/17/95 12:45	430-431	60.80					20.82	Nitrate reestablished in influent
4/24/95 10:00	432-433	43.46					22.09	
4/30/95 12:30	434-439	56.67	32.31	30.28	30.52	35.70	23.35	Batch test initialized
5/2/95 11:30	440				24.17			
5/4/95 11:00	441				27.50			
5/6/95 11:30	442				24.35			
5/8/95 11:00	443				24.16			
5/10/95 11:30	444				24.97			
5/12/95 10:30	445				22.32			
5/17/95 11:00	446				23.51			
5/20/95 13:30	447				23.79			
5/24/95 7:30			61.31	22.70	10.94	10.40		Batch test completed

Table C.9. Overall benzoate data.

		concentration @ port/cm						
		influent	1	2	3	4	effluent	
date time	sample #'s	0	3.5	7.6	14	23	30	
8/4/94 9:00	125	15.77						Began benzoate addition
8/4/94 11:00	126-127	35.54					3.96	
8/4/94 12:50	128-129	15.16					7.64	
8/4/94 14:50	130-131	16.05					12.05	
8/4/94 18:00	132-133	17.74					12.02	
8/4/94 22:00	134-135	16.74					12.96	
8/5/94 0:15	136-137	17.30					15.44	
8/5/94 10:15	138-139	16.21					11.54	
8/5/94 16:30	140-141	18.58					12.75	
8/6/94 12:30	142-143	11.77					13.90	
8/7/94 14:15	144-145	17.66					16.05	
8/8/94 12:45	146-147	29.91					16.63	
8/10/94 12:30	148-149	25.25					16.34	Batch test initialized
8/22/94 12:00	150-151		2.52			0.00		Batch test completed
8/29/94 12:45	152	9.65						Restarted continuous-flow, residence time = 0.5 days
8/29/94 14:00	153-154	23.14					3.71	
8/29/94 15:00	155-156	22.83					6.35	
8/29/94 17:00	157-158	23.38					7.77	
8/29/94 21:45	159-160	23.20					12.67	
8/30/94 10:10	161-166	15.86	6.66	11.10	15.31	7.14	6.45	
8/30/94 15:00	167-168	15.89					14.62	
8/31/94 12:00	169-174		17.68	13.27	16.14	14.18	14.78	
9/1/94 13:15	175-176	26.23					14.83	
9/2/94 11:15	177-178	24.17					15.57	
9/5/94 11:15	179-184	18.01	15.56	17.03	17.36	15.23	14.66	
9/6/94 13:45	185-186	24.90					16.29	
9/8/94 11:00	187-188	18.20					13.93	
9/12/94 10:30	189-190	19.21					17.16	
9/13/94 10:15	191-192	21.77					14.60	Lowered flow-rate, residence time = 2 days
9/14/94 11:30	193-194	27.46						
9/14/94 13:30	195-196	29.72					12.09	
9/15/94 11:15	197-202	28.61	15.09	13.22	13.05	11.28	11.91	
9/16/94 11:00	203-204	28.94					12.47	
9/19/94 10:00	205-210	29.18	14.47	13.86	14.28	12.60	11.56	
9/21/94 12:15	211-216	28.53	14.26	13.82	14.28	11.75	11.99	
9/23/94 12:00	217-222	29.07	13.55	12.30	12.46	10.65	9.47	
9/26/94 10:15	223-228	28.73	14.13	13.31	12.89	10.04	9.60	
9/27/94 10:45	229-234	15.98	15.74	12.02	11.82	9.39	8.68	Began acetate addition
9/29/94 10:15	235-240	18.91	16.44	14.31	13.42	11.40	11.24	
10/3/94 10:15	241-246	32.65	15.59	14.49	15.03	12.57	12.39	Increased acetate concentration
10/10/94 11:00	247-252							Increased acetate concentration
10/13/94 11:15	253-258	18.02	18.46	17.98	17.79	16.17	16.39	
10/17/94 11:15	259-264	22.43	18.82	20.15	20.17	18.36	19.72	Batch test initialized
10/20/94 12:45	265				14.96			
10/22/94 10:30	266				16.92			
10/24/94 11:45	267				14.24			
10/26/94 12:15	268				13.51			
10/28/94 11:30	269				9.50			
10/31/94 11:00	270				10.07			
11/2/94 13:00	271				11.63			
11/4/94 10:00	272				10.77			
11/10/94 14:15	273				10.59			Batch test completed 11/11

Table C.10. Overall sulfate data.

		concentration @ port/cm						
		influent	1	2	3	4	effluent	
date time	sample #'s	0	3.5	7.6	14	23	30	
6/9/94 9:00	1	26.64						Began breakthrough experiments, residence time = 0.5 days
6/9/94 10:00	2	0.00					27.25	
6/9/94 11:00	3	0.00					27.48	
6/9/94 12:00	4	0.00					27.47	
6/9/94 13:00	5-6	27.79					27.70	
6/9/94 14:00	7	0.00					29.19	
6/9/94 15:00	8	0.00					31.56	
6/9/94 16:00	9	0.00					29.60	
6/9/94 17:00	10-11	27.83					30.61	
6/9/94 18:00	12	0.00					35.08	
6/9/94 19:00	13	0.00					30.21	
6/9/94 20:00	14	0.00					30.29	
6/9/94 21:00	15-16	31.06					34.83	
6/10/94 1:15	17-18	30.74					31.78	
6/10/94 7:30	19-20	32.19					33.74	
6/10/94 11:30	21-22	34.61					31.77	
6/10/94 18:30	23-24	30.18					31.88	
6/10/94 21:30	25-26	30.69					31.56	
6/11/94 10:30	27-28	32.08					30.77	
6/12/94 12:00	29-30	32.51					31.28	
6/12/94 19:15	31-32	31.04					32.02	
6/13/94 13:20	33-34	32.05					30.88	
6/14/94 9:45	35-36	32.28					32.95	
6/15/94 11:20	37-38	31.36					31.36	
6/16/94 10:15	39-40	33.82					31.92	
6/17/94 9:20	41-42	31.84					32.88	
6/18/94 11:00	43-44	35.01					25.61	
6/19/94 16:00	45-46	34.81					34.23	
6/20/94 17:15	47-48	35.27					32.30	
6/21/94 16:15	49-50	34.12					34.60	
6/22/94 15:00	51-52	33.40					29.65	
6/23/94 16:15	53-54	22.42					34.64	
6/24/94 14:30	55-56	22.76					31.83	
6/25/94 14:20	57-62	30.96					31.29	
6/27/94 11:30	63-64	28.87					26.62	Pre-mix chamber installed
6/29/94 9:00	65-66	34.61					35.45	
6/29/94 10:45	67						36.35	
6/29/94 13:00	68-69	31.56					36.44	
6/29/94 15:00	70	33.41						
6/29/94 17:00	71-72	31.42					36.75	
6/29/94 22:15	73-74	32.16					31.21	
6/30/94 9:45	75-76	36.49					17.07	
6/30/94 13:45	77-78	35.42					26.53	
6/30/94 16:45	79-80	19.79					34.43	
6/30/94 23:00	81-82	25.64					34.79	
7/1/94 10:00	83-84	33.52					20.86	
7/2/94 11:15	85-86	32.58					32.90	
7/5/94 14:00	87-88	33.30					34.35	
7/6/94 12:00	89-94	34.76					33.72	
7/8/94 14:15	95-100	34.51					33.92	
7/11/94 13:45	101-102	34.94					34.88	
7/12/94 13:00	103-104	34.15					31.14	
7/13/94 11:00	105-106	33.51					31.13	
7/14/94 16:00	107-108	33.08					31.87	
7/15/94 10:30	109-114						31.08	
7/18/94 14:30	115-116	29.08					30.83	
7/20/94 15:45	117-118	32.26					31.99	
7/22/94 11:15	119-120	31.71					31.56	
7/26/94 15:45	121-122	31.75					32.00	
8/2/94 11:15	123-124	32.02					32.01	
8/4/94 9:00	125	31.13						Began benzoate addition
8/4/94 11:00	126-127	28.31					31.06	
8/4/94 12:50	128-129	31.16					29.11	
8/4/94 14:50	130-131	31.46					29.77	
8/4/94 18:00	132-133	31.83					29.96	
8/4/94 22:00	134-135	30.65					29.64	
8/5/94 0:15	136-137	30.57					29.84	
8/5/94 10:15	138-139	28.12					27.28	
8/5/94 16:30	140-141	31.04					26.75	
8/6/94 12:30	142-143	24.40					27.07	
8/7/94 14:15	144-145	31.39					31.43	
8/8/94 12:45	146-147	39.35					38.53	

8/10/94 12:30	148-149	37.78					38.31	Batch test initialized
8/22/94 12:00	150-151		39.30			39.18		Batch test completed
8/29/94 12:45	152	39.69						Restarted continuous-flow, residence time = 0.5 days
8/29/94 14:00	153-154	40.86					39.37	
8/29/94 15:00	155-156	42.17					39.47	
8/29/94 17:00	157-158	39.39					34.65	
8/29/94 21:45	159-160	33.77					40.98	
8/30/94 10:10	161-166	28.52	13.44	24.10	29.85	22.76	14.40	
8/30/94 15:00	167-168	29.64					32.68	
8/31/94 12:00	169-174		35.25	33.58	36.76	35.36	36.28	
9/1/94 13:15	175-176	36.74					35.69	
9/2/94 11:15	177-178	37.63					38.71	
9/5/94 11:15	179-184	34.76	30.54	34.21	35.30	32.91	38.22	
9/6/94 13:45	185-186	36.40					35.34	
9/8/94 11:00	187-188	35.51					32.05	
9/12/94 10:30	189-190	38.24					38.61	
9/13/94 10:15	191-192	38.60					38.01	Lowered flow-rate, residence time = 2 days
9/14/94 11:30	193-194	35.63						
9/14/94 13:30	195-196	36.11					37.13	
9/15/94 11:15	197-202	34.88	36.59	35.23	36.51	35.10	35.08	
9/16/94 11:00	203-204	35.31					35.10	
9/19/94 10:00	205-210	34.36	33.92	34.25	34.44	35.12	34.46	
9/21/94 12:15	211-216	33.45	33.93	33.92	35.26	34.82	34.89	
9/23/94 12:00	217-222	34.31	33.77	34.48	34.41	34.39	34.69	
9/26/94 10:15	223-228	34.76	35.69	35.49	36.65	34.32	34.75	
9/27/94 10:45	229-234	35.31	35.92	32.74	34.71	34.17	34.41	Began acetate addition
9/29/94 10:15	235-240	34.96	35.36	35.31	35.41	35.09	35.44	
10/3/94 10:15	241-246	36.33	36.40	36.27	36.20	35.65	35.96	Increased acetate concentration
10/10/94 11:00	247-252							Increased acetate concentration
10/13/94 11:15	253-258	36.77	38.42	38.09	38.12	36.63	36.96	
10/17/94 11:15	259-264	37.44	37.62	37.47	37.66	37.25	37.92	Batch test initialized
10/20/94 12:45	265				40.67			
10/22/94 10:30	266				35.94			
10/24/94 11:45	267				36.75			
10/26/94 12:15	268				35.60			
10/28/94 11:30	269				35.53			
10/31/94 11:00	270				34.86			
11/2/94 13:00	271				38.55			
11/4/94 10:00	272				30.68			
11/10/94 14:15	273				30.94			Batch test completed 11/11
11/29/94 12:15	286-291	31.62	30.80	32.21	31.65	31.35	30.95	Restarted continuous-flow 11/15, residence time = 2 days
11/30/94 11:15	292-297	31.70	32.23	31.99	31.64	31.36	31.59	
12/2/94 9:00	298-303	31.78	32.09	32.14	31.77	31.55	31.67	
12/5/94 10:00	304-309	32.25	32.79	34.34	32.29	32.18	32.97	
12/8/94 9:45	310-315	32.32	32.47	32.99	31.87	32.07	32.47	Increased acetate concentration
12/12/94 10:45	316-321	32.71	33.05	32.52	31.84	32.26	32.57	
12/15/94 11:00	322-327	32.60	33.61	32.92	32.13	31.48	31.27	
12/21/94 10:00	328-333	31.98	32.10	31.94	31.94	31.43	31.57	
12/29/94 10:15	334-339	33.53	32.04	31.23	32.46	31.24	31.11	
1/5/95 10:00	340-345	32.82	32.43	32.88	32.85	32.20	32.32	
1/11/95 11:00	346-351	31.94	31.76	31.94	32.00	31.36	31.20	
1/18/95 11:00	352-357	32.20	32.21	32.28	32.49	31.90	32.17	
1/24/95 9:30	358-363	32.48	32.85	32.50	32.75	32.79	32.03	
2/1/95 10:30	364-369	31.18	30.11	30.82	30.76	32.11	30.24	Began yeast extract addition
2/7/95 9:45	370-376	30.90	30.24	31.11	30.95	30.65	30.85	
2/13/95 10:30	377-382	30.73	29.90	30.66	29.94	30.40	30.39	
2/20/95 9:30	383-388	30.19	29.91	30.24	30.00	30.18	30.04	
2/24/95 10:15	389-394	30.39	29.99	30.26	30.17	30.28	29.90	Yeast extract and nitrate removed
3/3/95 10:30	395-400	30.16	30.42	30.41	30.44	30.52	28.85	
3/10/95 12:20	401-406	30.33	29.27	30.45	30.60	30.24	29.52	
3/16/95 12:30	407-412	29.87	30.06	30.05	30.23	31.25	29.34	
3/22/95 10:15	413-418	30.68	30.86	34.50	30.32	30.70	30.27	Batch test initialized
3/24/95 14:45	420				30.56			
3/26/95 16:15	421				30.52			
3/28/95 11:45	422				30.37			
3/31/95 12:30	423				30.68			
4/3/95 11:30	424				30.12			
4/7/95 14:00	425				29.33			
4/11/95 8:00	426-429		29.51	29.05	29.15	29.49		Batch test completed
4/17/95 12:45	430-431	28.90					29.64	Nitrate reestablished in influent
4/24/95 10:00	432-433	30.75					30.12	
4/30/95 12:30	434-439	31.53	30.63	30.35	30.63	30.81	30.32	Batch test initialized
5/2/95 11:30	440				30.51			
5/4/95 11:00	441				30.67			
5/6/95 11:30	442				31.27			
5/8/95 11:00	443				30.92			
5/10/95 11:30	444				30.65			
5/12/95 10:30	445				30.26			
5/17/95 11:00	446				30.83			
5/20/95 13:30	447				30.23			
5/24/95 7:30			30.11	30.50	30.53	30.26		Batch test completed

APPENDIX D

Preliminary Control Experiments

Reaction with Silver

Concern was expressed over the possibility of CT to act as an oxidant and react with the silver solder used braze the 4 sample port weld fittings to the column. The reactions of concern are shown below,

$$2Ag \rightarrow 2Ag^{+} + 2e^{-} \qquad \textbf{Equation D.1}$$

$$CCl_4 + 2e^{-} + 2H^{+} \rightarrow CHCl_3 + HCl \qquad \textbf{Equation D.2}$$

In combination, these represent a corrosion reaction where silver metal is oxidized and CT is reduced to CF. Rapid corrosion of iron metal in the presence of CT has been noted by Matheson and Tratnyek (1994).

An experiment was conducted with small samples of the silver solder in an aqueous solution of CT. The experimental procedure was as follows:

1. A 500 µg/L aqueous solution of CT was prepared from dilution of a stock solution of CT dissolved in methanol.
2. 10 mL of aqueous solution was added to four 16 x 125 mm glass culture tubes.
3. Small pieces (~ 0.25 in.) of the soldering rod were added to two of the tubes, the remaining two tubes were used as controls.
4. Teflon® lined screw-top caps were firmly secured and the reactors were allowed to stand 2 weeks before analysis.

Analysis of the reactor fluid at that time showed that the concentration in all four reactors was approximately equal (standard deviation of 2.2%). Therefore, it was concluded that the reaction of CT with the silver solder was not of concern during the expected residence times of this study.

System Losses

In order to determine the uncontrolled losses of CAHs that could be expected in the column supply system, a series of control experiments were performed. Of main concern were losses in the tubing, pressure gauges, valves, pump, and syringes. Thus, a system was constructed similar to the one depicted in Figure 5.2 but with the following exceptions: (1) the effluent line was removed at the point of the effluent sample port and the opening was capped; (2) the column was removed and replaced with a piece of stainless tubing, since only losses in the supply system were considered in this test.

The resulting system would allow for continuous fluid recirculation, driven by the feed pump. Make-up fluid for periodic samples was provided by the syringes on the syringe pump (idle for this test). Also, loss from a capped 50 mL gastight syringe, identical to those used on the syringe pump, was examined separately in order to determine the proportion of loss from the syringes in the system.

The experimental procedure for this experiment was as follows:

1. The feed pump was adjusted to deliver a flow of 2 mL/min.
2. The system was assembled, making sure all fittings were secure.
3. The system was purged with pressurized CO_2 and closed with a positive pressure.
4. The approximate internal volume of the system was calculated to be 30 mL using the ideal gas law. This was accomplished by noting the change in pressure (from the installed pressure gauges) due to a corresponding change in volume (from depression of the syringe pump plunger).
5. A dilute bleach solution was prepared from water that was deaired by boiling for 10 minutes.
6. The bleach solution was added to the system and allowed to circulate overnight.
7. 1 L of boiled, deaired water was flushed through the system to remove bleach.
8. Based on the internal volume of the system and the volume to be contained in the syringes (initially 30 mL each), the syringes were filled with the appropriate concentration of CT, and TCA (made from stocks in methanol diluted into boiled, deaired water) to achieve a final concentration of approximately 250 μg/L once combined with the system fluid.
9. The system was allowed to thoroughly mix by exchanging fluid between syringes and allowing the feed pump to recycle fluid overnight.

10. Samples were taken periodically thereafter to monitor for losses in CAH concentration.

Concentrations within the system, as well as in an isolated 50 mL syringe, were monitored for a period of 2 months. The data from these tests are shown in Figure D.1 and Figure D.2.

The data from the external syringe test indicates that the loss rate of both CT and TCA is constant with time and independent of concentration. Loss from the syringe appears to be about 50 µg/L/month. For the system, the data seems to indicate slightly higher losses. However, during this test the pump failed 2 days into the experiment. For the following month, while repairs were underway, the pump was removed from the system and the lines capped.

Once the pump was reinstalled the concentrations rapidly dropped. Indicating that there may have been pockets of low concentration that developed during the period when the fluid was static, and once pumping was reinitiated the pockets were mixed with the

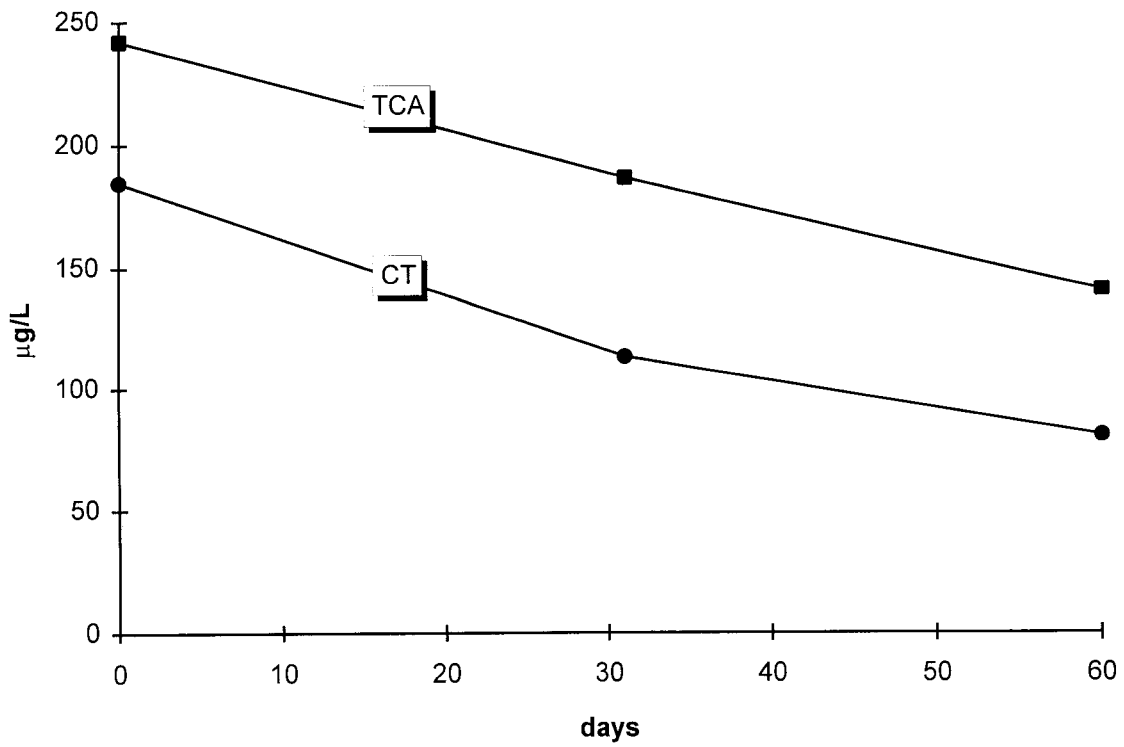


Figure D.1. Losses from a 50 mL gastight syringe.

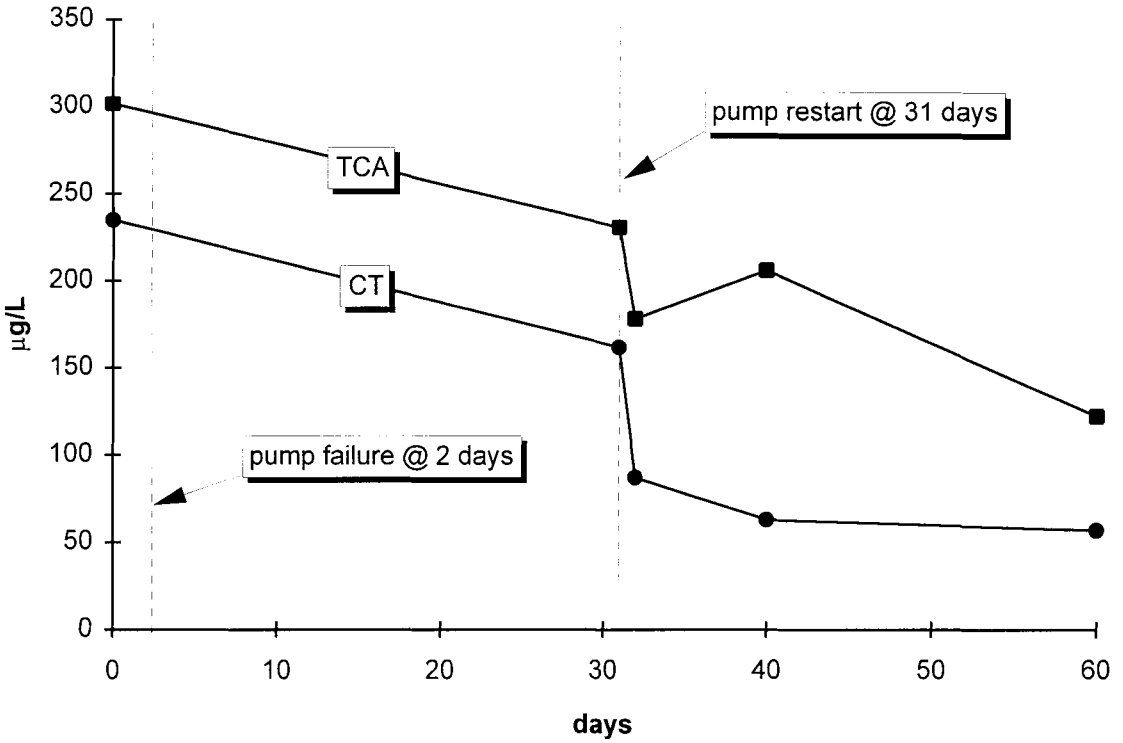


Figure D.2. Losses from the column supply system.

bulk fluid. At later times, after 40 days, it appeared that a fairly steady state condition existed for CT (and possibly TCA if the point at 40 days is neglected). Overall, the results from these tests indicate that losses from the system, in the time frame of concern for the biotransformation experiments (on the order of days), are fairly insignificant.

APPENDIX E

Stoichiometric Balances

Denitrification

Assuming that denitrification is a two-step process where nitrate (NO_3^-) is first reduced to nitrite (NO_2^-) then to nitrogen gas (N_2), the appropriate half-reactions based on 1 mole of electrons transferred are:

$$\frac{1}{2}NO_3^- + H^+ + e^- \rightarrow \frac{1}{2}H_2O + \frac{1}{2}NO_2^- \qquad \textbf{Equation E.1}$$

$$\frac{1}{3}NO_2^- + \frac{4}{3}H^+ + e^- \rightarrow \frac{2}{3}H_2O + \frac{1}{6}N_2 \qquad \textbf{Equation E.2}$$

Thus, if we define $C_1^{NO_3^-}$, $C_2^{NO_3^-}$, $C_1^{NO_2^-}$ and $C_2^{NO_2^-}$ as the influent and effluent concentrations of nitrate and nitrite respectively, then the concentrations of nitrate and nitrite that were reduced according to the previous equations are:

$$\Delta C^{NO_3^-} = C_1^{NO_3^-} - C_2^{NO_3^-} \qquad \textbf{Equation E.3}$$

$$\Delta C^{NO_2^-} = \Delta C^{NO_3^-} + C_1^{NO_2^-} - C_2^{NO_2^-} \qquad \textbf{Equation E.4}$$

Substrates

For the substrates, benzoate and acetate, the half-reactions for the transfer of 1 mole of electrons are respectively:

$$\frac{1}{30}C_6H_5COO^- + \frac{12}{30}H_2O \rightarrow \frac{7}{30}CO_2 + \frac{29}{30}H^+ + e^- \qquad \textbf{Equation E.5}$$

$$\frac{1}{8}CH_3COO^- + \frac{2}{8}H_2O \rightarrow \frac{2}{8}CO_2 + \frac{7}{8}H^+ + e^- \qquad \textbf{Equation E.6}$$

Then, assuming that all of the substrate is used for denitrification (none used for cell growth or maintenance) an electron balance for each substrate with the denitrification equations yields:

$$\Delta C^{C_6H_5COO^-} = \frac{2}{30}\Delta C^{NO_3^-} + \frac{3}{30}\Delta C^{NO_2^-} \qquad \textbf{Equation E.7}$$

$$\Delta C^{CH_3COO^-} = \frac{2}{8}\Delta C^{NO_3^-} + \frac{3}{8}\Delta C^{NO_2^-} \qquad \textbf{Equation E.8}$$

Thus, given the molar concentrations of nitrate and nitrite in the influent and effluent, these equations represent the minimal amount of benzoate or acetate, that is required to produce the observed denitrification.

APPENDIX F

Basic Concepts

Transport Through Porous Media

One of the most important and basic mathematical relationships governing fluid flow through porous media is Darcy's Law. In words, Darcy's Law simply states: the velocity of fluid flow is proportional to the hydraulic gradient. This is shown mathematically as,

$$v_x = -\frac{K}{n_e}\frac{\partial h}{\partial x} \qquad \textbf{Equation F.1}$$

where: v_x is the average linear velocity of the fluid through the porous media (m/s), which is equal to the volumetric flow rate per unit area of connected pore space; K is a proportionality constant called the hydraulic conductivity (m/s); n_e is the effective (connected) porosity; and $\partial h/\partial x$ is the hydraulic gradient. The hydraulic conductivity of a particular media is dependent on the mean particle size, generally increasing with increasing particle size. Some representative values are shown in Table F.1.

When solutes are transported along with the flowing groundwater according to Darcy's law they are said to be transported by *advection.* If a solute is transported one-dimensionally by advection alone its concentration profile will be given by (Fetter, 1993),

$$\frac{\partial C}{\partial t} = -v_x \frac{\partial C}{\partial x} \qquad \textbf{Equation F.2}$$

where: $\partial C/\partial t$ is the change in concentration with time ($g/m^3/s$) and $\partial C/\partial x$ is the change in concentration with position ($g/m^3/m$). The one-dimensional mass flux is given by,

$$F_x = v_x n_e C \qquad \textbf{Equation F.3}$$

where: F_x is the mass flux ($g/m^2/s$) and C is the concentration of solute (g/m^3).

Table F.1. Representative values of hydraulic conductivity for various sedimentary media (Domenico & Schwartz, 1990).

Material	Hydraulic conductivity (m/s)
gravel	3×10^{-4} to 3×10^{-2}
coarse sand	9×10^{-7} to 6×10^{-3}
medium sand	9×10^{-7} to 5×10^{-4}
fine sand	2×10^{-7} to 2×10^{-4}
silt	1×10^{-9} to 2×10^{-5}

Solution to Equation F.2 yields a sharp concentration front. On the upstream side of the front, the solute concentration is equal to that of the source, whereas on the downstream side it is unchanged from the background value. This condition of pure advection is known as *plug flow*. However, in most cases, solutes do not simply move with the groundwater flow. They also diffuse away from areas of high concentration and are dispersed by the indirect flow paths existent in porous media.

The process of solute transport caused by a concentration gradient is called *molecular diffusion*. As long as the source concentration remains constant, the mass of solute diffusing will be proportional to the concentration gradient. This is known as Fick's first law, which for one dimension is,

$$F_x = -D^* \frac{dC}{dx} \qquad \textbf{Equation F.4}$$

where: D^* is the effective diffusion coefficient (m^2/s), which accounts for the longer flow paths in porous media than in plain water and dC/dx is the concentration gradient ($g/m^3/m$).

Because water flowing through porous media can travel a multitude of different paths, and friction with solid surfaces can result in local changes in velocity, a great deal of mixing can take place. This mixing is called *mechanical dispersion*, which results in a spreading of the solute about the advancing front. Since this study was concerned with one-dimensional flow through a column, only mixing that occurs along the direction of

flow was considered. This type of mixing is called *longitudinal dispersion.* The process of molecular diffusion cannot be separated from mechanical dispersion in flowing groundwater. Therefore, the two are combined to define a parameter called the *hydrodynamic dispersion coefficient.* Which for longitudinal dispersion is represented by,

$$D_L = \alpha_L v_x + D^* \qquad \textbf{Equation F.5}$$

where: D_L is the longitudinal hydrodynamic dispersion coefficient (m^2/s) and α_L is the longitudinal dynamic dispersivity(m), a property of the medium.

The previous equations along with the principal of mass conservation can be used to derive the *advection-dispersion equation.* Assuming that the media is homogeneous, isotropic and saturated, then for one-dimensional flow the advection-dispersion equation is (Fetter, 1993),

$$\frac{\partial C}{\partial t} = D_L \frac{\partial^2 C}{\partial x^2} - v_x \frac{\partial C}{\partial x} \qquad \textbf{Equation F.6}$$

For the column experiments of this study it was assumed that the solutes were applied as fixed-step functions. In which case, the initial solute concentration in the column was zero, the concentration of the solute solution was C_0 and the solute solution was instantly applied to the column at time t for an indefinite period. For these conditions, the solution to Equation F.6 is (Fetter, 1993),

$$C = \frac{C_0}{2}\left[\operatorname{erfc}\left(\frac{L - v_x t}{2\sqrt{D_L t}}\right) + \exp\left(\frac{v_x L}{D_L}\right)\operatorname{erfc}\left(\frac{L + v_x t}{2\sqrt{D_L t}}\right)\right] \qquad \textbf{Equation F.7}$$

where: C represents the concentration at a particular reference length, L, and time, t.

If we define $P_e = v_x L/D_L$, $t_R = v_x t/L$ and $C_R = C/C_0$ then the advection-dispersion equation can be written in dimensionless form as,

$$C_R(t_R, P_e) = \frac{1}{2}\left\{\operatorname{erfc}\left[\left(\frac{P_e}{4t_R}\right)^{\frac{1}{2}}(1 - t_R)\right] + \exp(P_e)\operatorname{erfc}\left[\left(\frac{P_e}{4t_R}\right)^{\frac{1}{2}}(1 + t_R)\right]\right\} \qquad \textbf{Equation F.8}$$

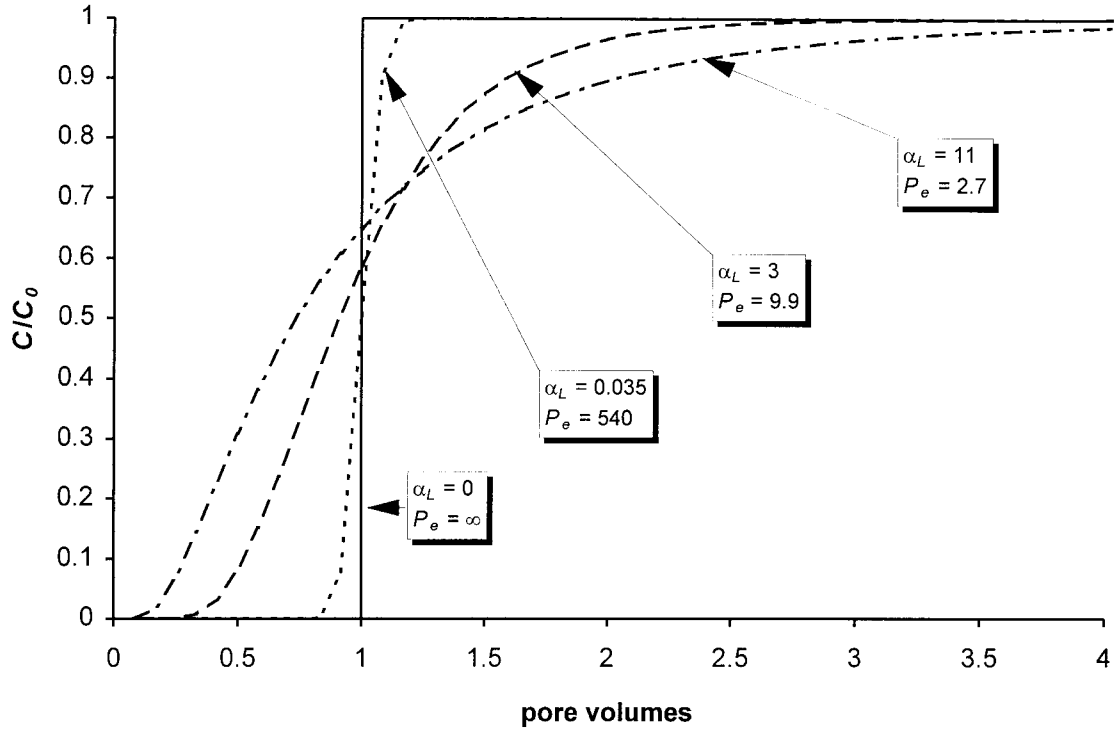


Figure F.1. Breakthrough curves for various dispersivities. The curves were calculated from Equation F.8 with: L = 30 cm; v_x = 60 cm/s; D_d = 2.01 x 10^{-5} cm^2/s (Domenico & Schwartz, 1990) and $D^* = 0.7D_d$ (Fetter, 1993).

where, P_e is a dimensionless quantity called the Peclet number. It relates the effectiveness of mass transport by advection to mass transport by either dispersion or diffusion. The operator, erfc, represents the complimentary error function.

The advection-dispersion equation is shown graphically in Figure F.1 for various dispersivities. The resulting curves are called breakthrough curves, because they show the concentration breaking through the column as a function of volumetric flow. Notice the sharp concentration front of the plug flow condition, where advection is the only source of solute transport ($P_e = \infty$ and $\alpha_L = 0$). As mixing by diffusion and dispersion increases the curves begin to round out. The solute begins to break through sooner and more time is required before a steady-state concentration is established.

Henry's Law

The Henry's law constant is a parameter which describes how a compound will partition between air and water at equilibrium; the relationship is shown by (Schwarzenbach *et al.*, 1993),

$$K_H = \frac{C_a}{C_w}\frac{1}{RT} \qquad \textbf{Equation F.9}$$

where: K_H is the Henry's law constant (atm·m^3/mol); C_a is the solute concentration in air (mol/L); C_w is the aqueous solute concentration (mol/L); R is the ideal gas constant (8.20575 x 10^{-5} atm·m^3/mol·K); and T is the absolute temperature of the water (K).

Octanol/Water Partition Coefficient

The log octanol/water partition coefficient, logK_{ow}, is defined as the $\log_{10}$ of the ratio of the solute concentration in a water-saturated *n*-octanol phase to the solute concentration in a *n*-octanol-saturated water phase. The parameter is a unitless representation of a compound's affinity to reside in an organic phase relative to water. Thus, CT can be said to be approximately $10^{2.8}$ or 631 times more prevalent in *n*-octanol than water. It has also been shown that the logK_{ow} is related to a compounds potential to sorb to soil. A higher logK_{ow} would mean a compound is more strongly sorbed; therefore retarded with respect to normal groundwater movement as well as less available to microorganisms.

APPENDIX G

Protocol for Anaerobic Column Packing

Materials

- duct tape and silicone sealant (for leak repair)
- glove box
- nitrogen gas supply
- methanol
- synthetic groundwater
- mason jars and lids
- utility knife
- rubber tray (for soil)
- rubber tray (for clean tools)
- long, small bladed scooper
- short, wide bladed scooper
- scrapers
- hacksaw
- column materials
- tray for debris

Procedure

1. Seal all holes and potential leaks in glove box, with the exception of the single outlet port.
2. Wash glove box with soap and water and then wipe thoroughly with methanol.
3. Autoclave all tools and materials that can be autoclaved, wipe the remaining items with methanol.
4. Supply deaired synthetic groundwater, by gravity feed, to the column inside of the glove box.
5. Remove the top endcap from the column and allow synthetic groundwater to flow in through the bottom end cap and spill over the top.
6. Make sure all items are inside the glove box and organized for easy access.
7. Seal glove box and purge with nitrogen gas.
8. Regulate the nitrogen gas pressure such that a positive pressure is maintained inside at all times.
9. Open core casing and place the core material in an empty tray.
10. Separate out the larger rocks (> 0.25 inches).

11. Spoon material into the column while the flowing water helps to remove some silts and prevent gas from being trapped in the pore spaces.
12. When full, replace the top endcap and place the remaining core material in mason jars.

APPENDIX H

Protocol for Preparing Saturated Aqueous Stock Solutions

Materials

- 125 mL serum bottle, including crimp-top cap with septa
- small beaker
- CAHs of interest

Procedure

1. Fill beaker and serum bottle with RO/DI water.
2. Autoclave the beaker, serum bottle and cap.
3. Top-off bottle with autoclaved water.
4. Add about 4 times the volume of chemical required to saturate 125 mL of water. The volume can be found as follows.

$$V = \frac{4(0.125)S_W \mathrm{MV}}{\mathrm{MW}} \qquad \textbf{Equation H.1}$$

where: V = volume (µg/L); S_W = aqueous solubility (mg/L); MV = molecular volume (mL/mole); and MW = molecular weight (g/mole). For CT, CF, 1,1,1-TCA and TCE these parameters and the resulting volume is summarized in Table H.1.

5. Cap bottle, label and date.
6. Agitate prior to use.
7. Allow to settle.

Table H.1. Resulting volume and parameters used for its calculation from Equation H.1

	S_W, mg/L	MV, mL/mole	MW, g/mole	V, µL
CT	1160 @25°C	94.25	153.82	350
CF	9300 @25°C	80.60	119.38	3140
1,1,1-TCA	1550 @20°C	99.60	133.42	580
TCE	1080 @20°C	89.70	131.4	370

APPENDIX I

GC Standard Curve Protocol for CAHs

Materials

- high grade CAHs
- 3 10 mL volumetric flasks
- 5 100 mL volumetric flask
- 5 μL microsyringe
- 10 μL microsyringe
- 100 μL microsyringe
- 2 mL volumetric pipette
- automatic Pasteur pipettor with pipettes
- 5 16 x 125 mm glass culture tubes with Teflon® lined screw-top caps
- 5 2 mL amber glass GC autosampler vials and with Viton® caps
- cap crimper
- vortex mixer

Stock Solution Preparation (Standard Methods, 1992)

1. Fill a 10 mL volumetric flask nearly to the meniscus with methanol.
2. Place the uncapped flask on balance, including the stopper, and allow 10 minutes for the methanol on the sides of the meniscus to dry.
3. Add 10 drops of solvent from a 100 microsyringe to flask, being careful to prevent the drops from touching the sides of the flask.
4. Cap the flask and record the added weight.
5. Top the flask and mix the solution.
6. Calculate concentration and label the flask accordingly.
7. Store in refrigerator for later use. Discard stocks after 1 month.

Aqueous Standards Preparation

1. Fill the 5 100 mL volumetric flasks with RO/DI water.
2. Add appropriate amounts of stocks, using the appropriate microsyringe, to obtain combined concentrations of 5, 10, 25, 100, 250 and 500 μg/L in the flasks.
3. Cap and shake.
4. Number and record concentrations.

Extraction Into Pentane

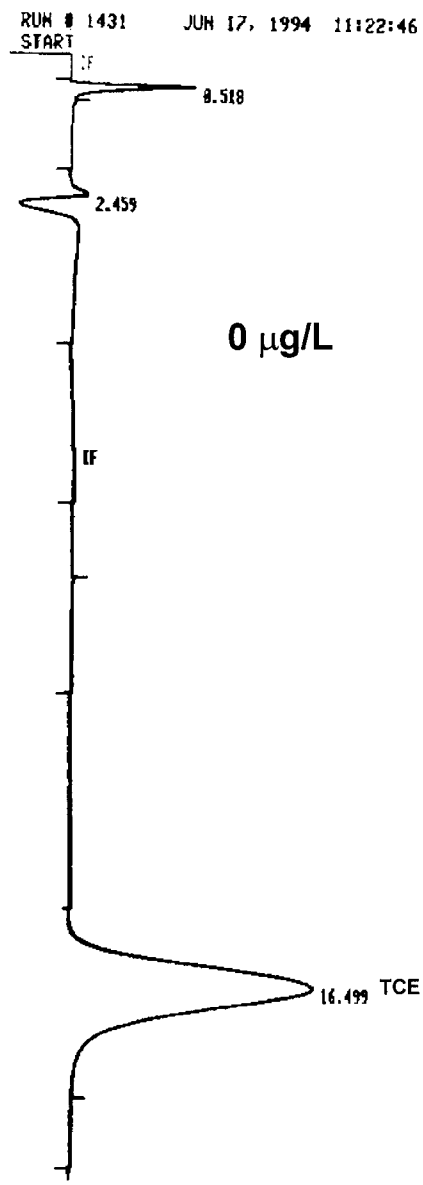
1. Prepare spiked pentane with 100 μg/L of TCE internal standard.
2. Add 2 mL of spiked pentane to each culture tube using the volumetric pipette.
3. Cap the tubes and place on mixer for 30 seconds each.
4. Allow the phases to separate for 10 minutes.
5. Extract 1.6 mL of the pentane phase using the Pasteur pipettor, and place into GC vials.
6. Fill one GC vial with spiked pentane for use as a control.
7. Cap the vials.

Analysis

1. Run samples as described in Materials and Methods.

Sample Chromatograms

A set of chromatograms for a typical standard curve are included below. The compounds eluded from the column in order of increasing boiling point (see Table 2.1). Standard curves were obtained by plotting the signal output (area) vs. concentration for each compound and fitting an equation to the data. Over the range considered (5-500 μg/L) the area was not linear with concentration, with the exception of CF. Reasonable modeling of the data ($R^2 > 0.99$) was obtained by fitting to polynomial equations using Microsoft® Excel 5.0.



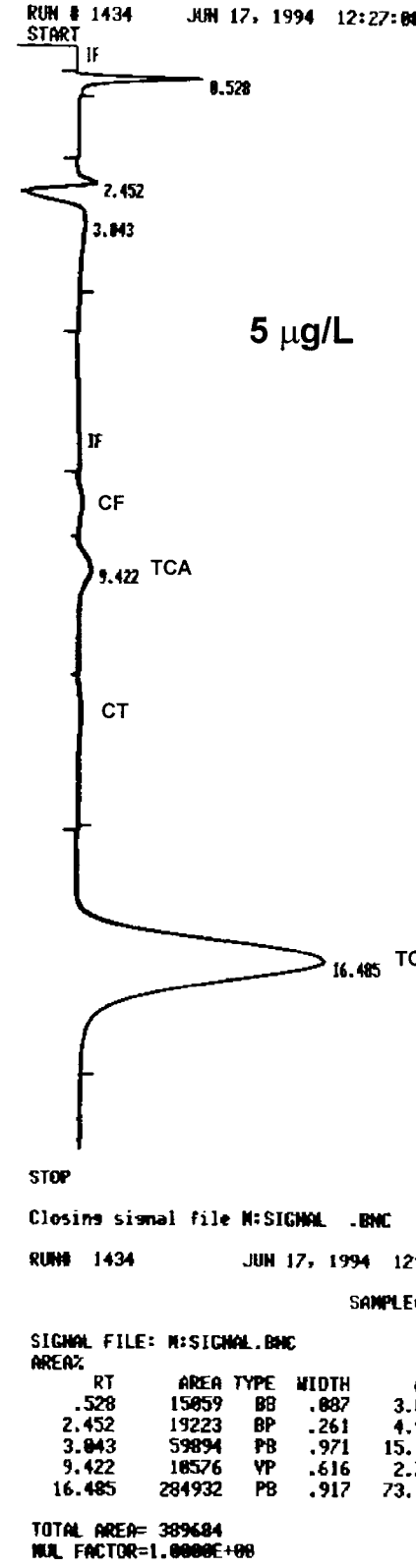


Figure I.1. GC chromatograms for CF, TCA and CT at 0 and 5 µg/L; TCE int. std. = 100 µg/L.

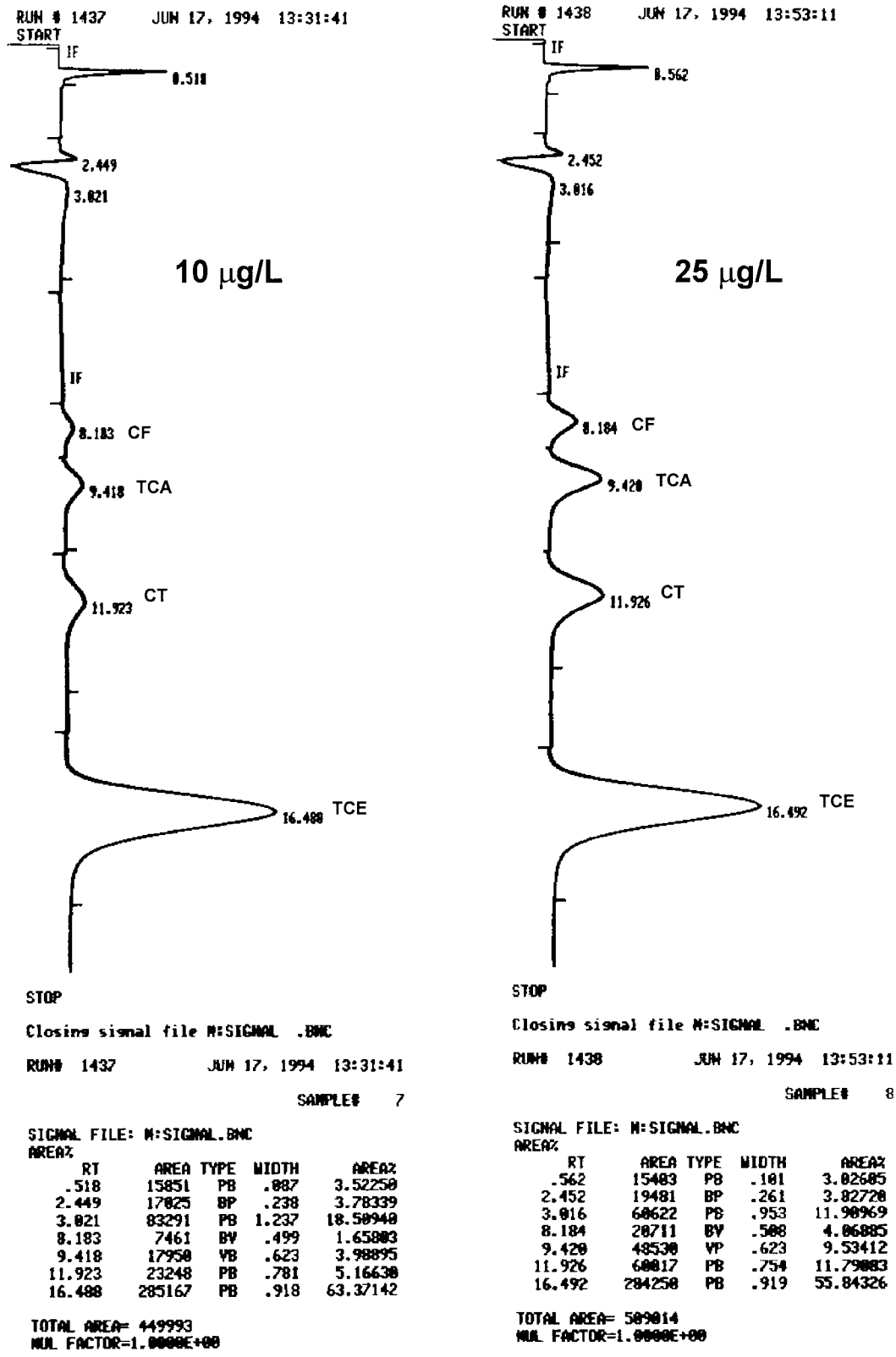


Figure I.2. GC chromatograms for CF, TCA and CT at 10 and 25 µg/L; TCE int. std. = 100 µg/L.

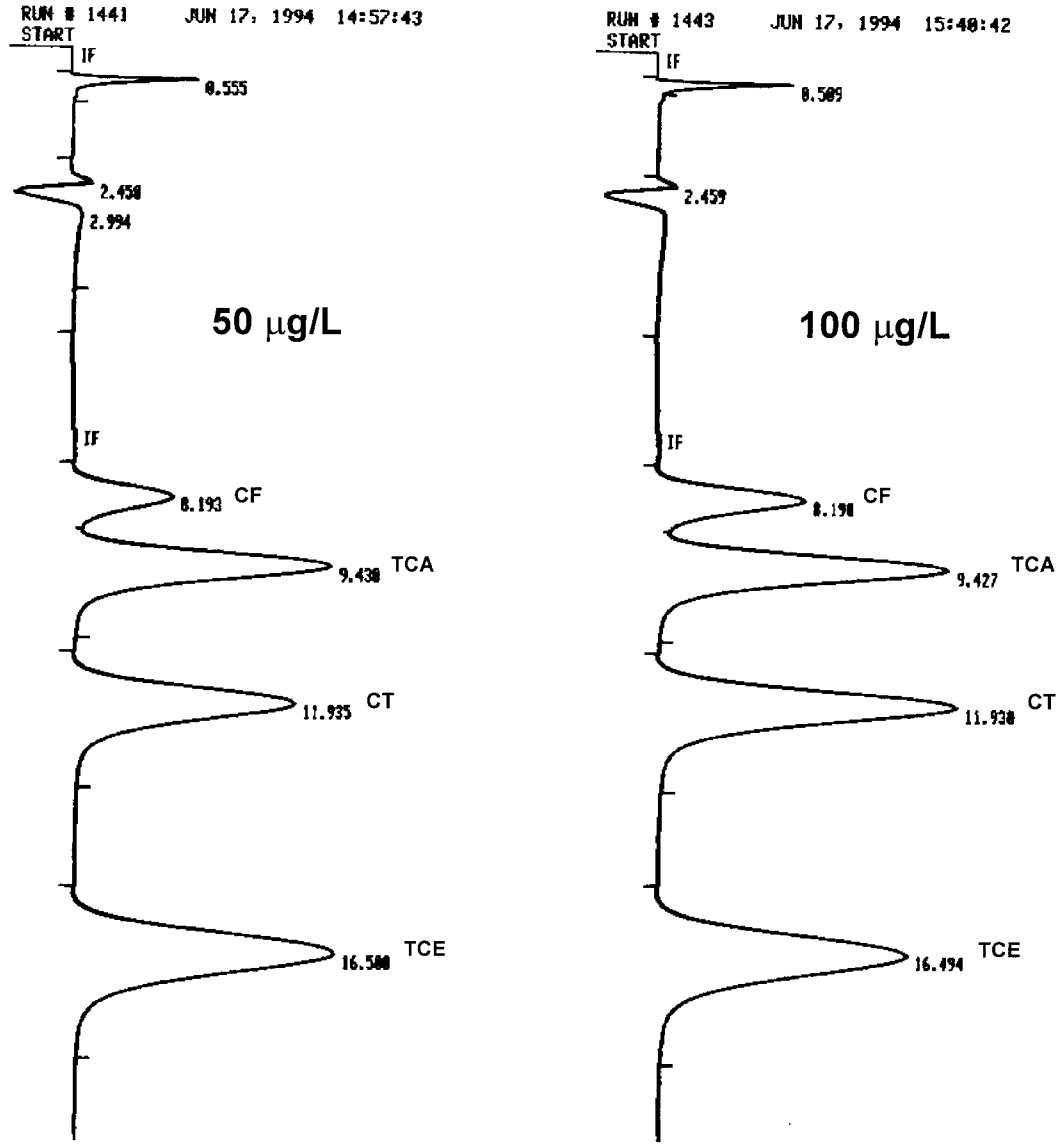
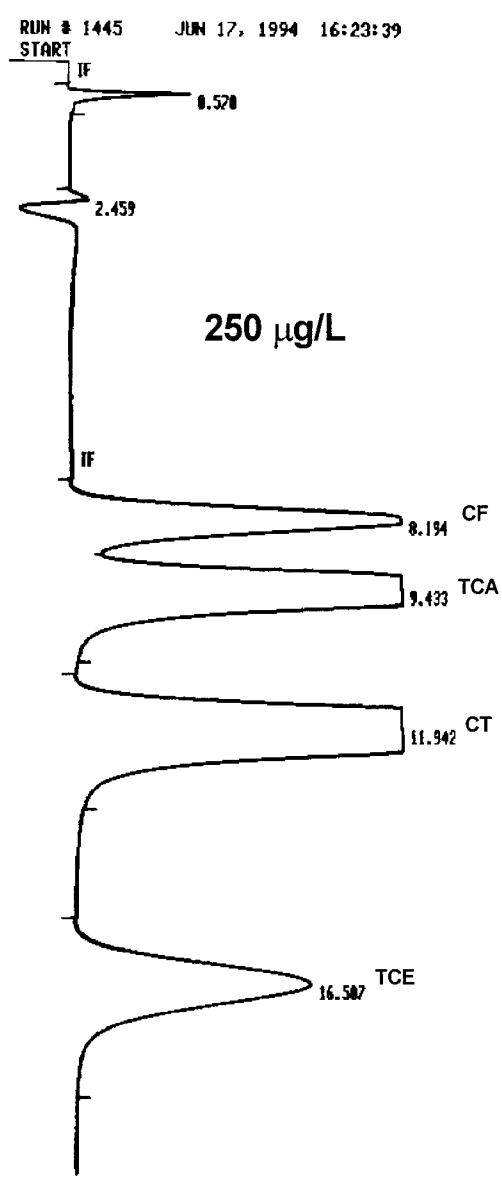


Figure I.3. GC chromatograms for CF, TCA and CT at 50 and 100 µg/L; TCE int. std. = 100 µg/L.



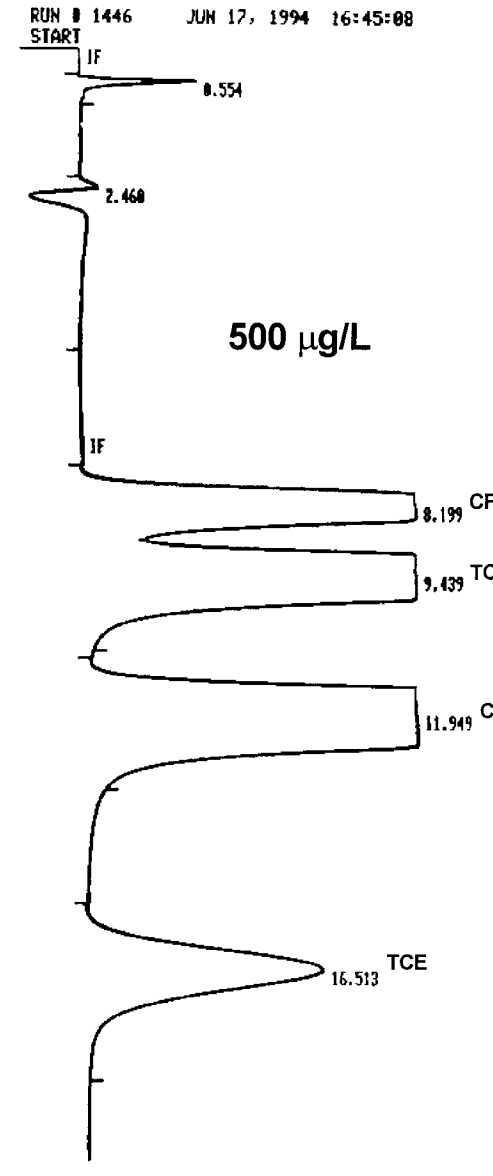


Figure I.4. GC chromatograms for CF, TCA and CT at 250 and 500 µg/L; TCE int. std. = 100 µg/L.

APPENDIX J

IC Standard Curve Protocol for Anions

Materials

- high grade mineral salts containing the anions of interest
- 1 500 mL volumetric flask
- 5 100 mL volumetric flasks
- 1 0-5 mL automatic pipettor with tips
- 6 Dionex Polyvials™ with filter caps

Stock Solution Preparation

1. Calculate the amount of each salt required to obtain a concentration of 1 g/L of the respective anions in 500 mL.
2. Add the calculated amounts of salts to the 500 mL flask.
3. Fill flask with deionized water
4. Shake thoroughly and label.

Aqueous Standards Preparation

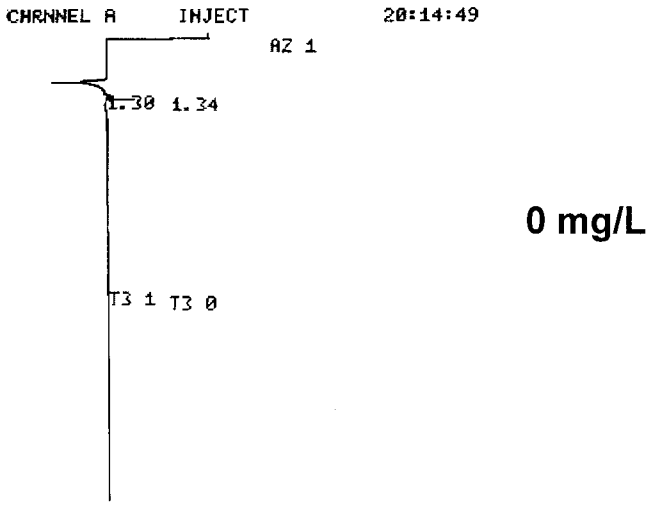
1. Make dilutions of the stock solution in the 100 mL flasks to obtain concentrations of 5, 10, 20, 35 and 50 mg/L. The volume of stock for these dilutions is 0.5, 1.0, 2.0, 3.5 and 5.0 mL, respectively.
2. Shake thoroughly and label.
3. Add 1.7 mL of each standard to a labeled Polyvials™ and cap.
4. Fill one Polyvial™ with deionized water for a blank.

Analysis

1. Run samples as described in Materials and Methods, with the blank first in the sequence.

Sample Chromatograms

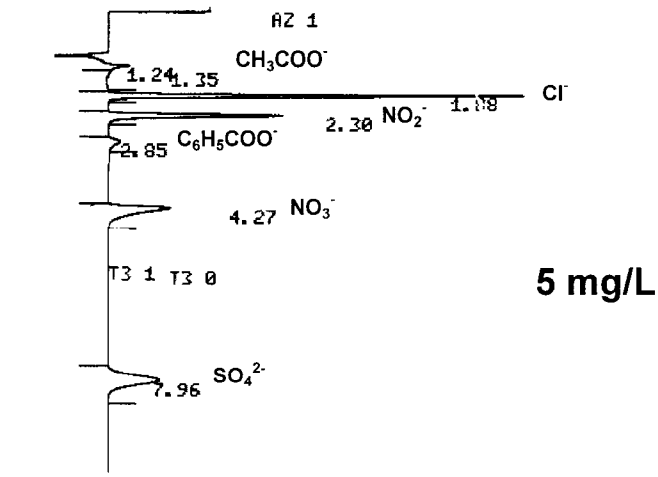
A set of chromatograms for a typical standard curve is included below. Bromide is not included in these examples, however, it eluded from the column between benzoate and nitrate. Standard curves were obtained by plotting the signal output (area) vs. concentration for each compound. Over the range considered (0-50 mg/L) the area was linear with concentration for all anions. Reasonable modeling of the data ($R^2 > 0.99$) was obtained by linear regression using Microsoft® Excel 5.0.



ANIONANAL YSIS 20:14:49 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN 2 INDEX 2

NAME	CONC	RT	AREA BC	RF
1	0.	1.3	159017 02	
2	0.	1.34	40934 03	
TOTALS	0.		199951	

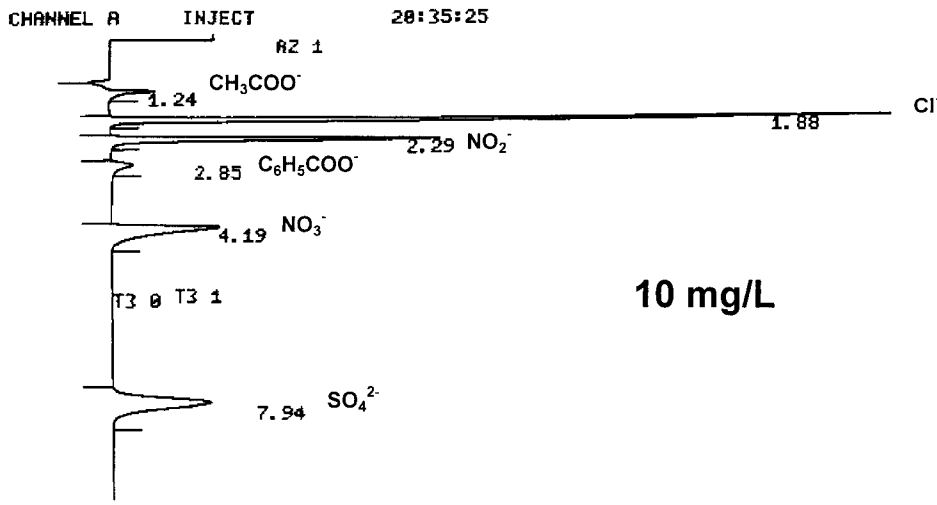


ANIONANAL YSIS 0:25:07 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN 3 INDEX 3

NAME	CONC	RT	AREA BC	RF
1	0.	1.24	349257 02	
2	0.	1.35	207823 03	
3	0.	1.[illegible]	1687446 01	
4	0.	2.3	925672 01	
5	0.	2.85	106110 01	
6	0.	4.27	743273 01	
7	0.	7.96	980771 01	
TOTALS	0.		4998352	

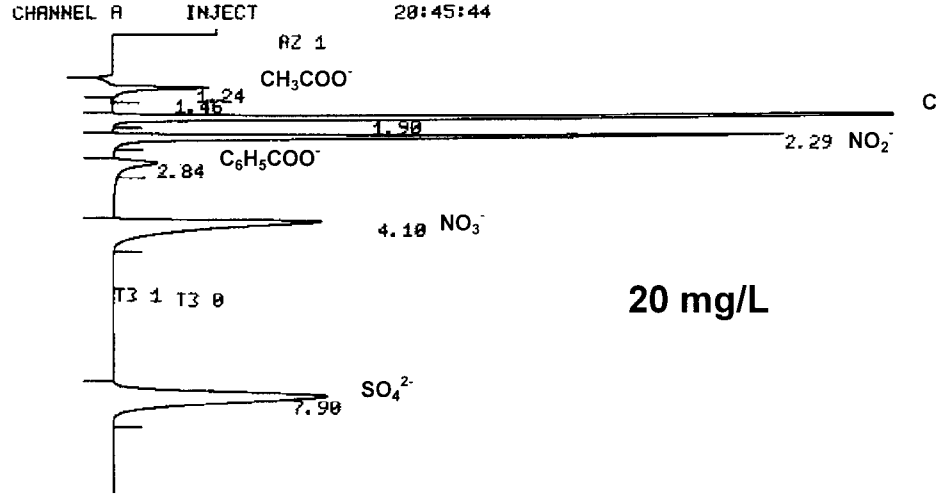
Figure J.1. IC chromatograms, 0 and 5 mg/L.



ANIONANAL YSIS 20:35:25 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN 4 INDEX 4

NAME	CONC	RT	AREA BC	RF
1	0.	1.24	416815 01	
2	0.	1.88	3240829 01	
3	0.	2.29	1734234 01	
4	0.	2.85	202519 01	
5	0.	4.19	1399943 01	
6	0.	7.94	1816979 01	
TOTALS	0.		8811319	

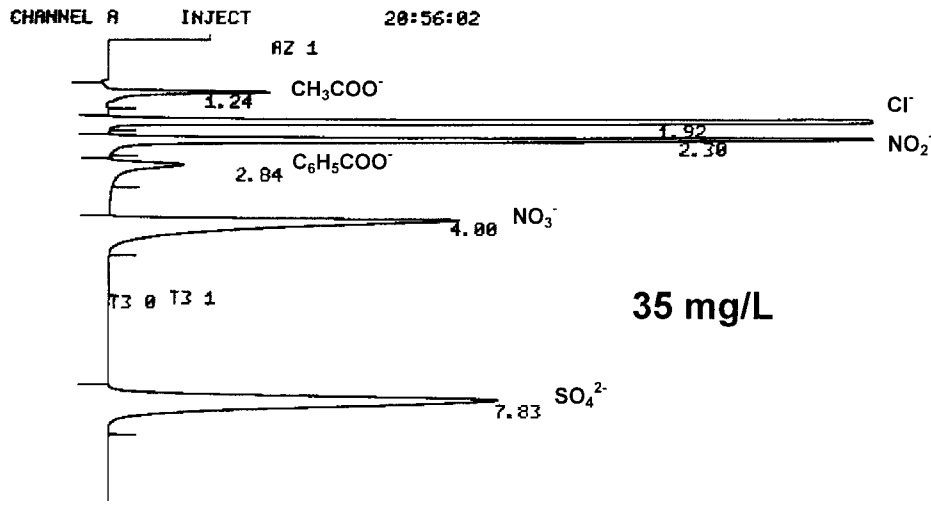


ANIONANAL YSIS 20:45:44 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN 5 INDEX 5

NAME	CONC	RT	AREA BC	RF
1	0.	1.24	723950 02	
2	0.	1.46	16721 03	
3	0.	1.9	6790761 01	
4	0.	2.29	3678666 01	
5	0.	2.84	446984 01	
6	0.	4.1	2975061 01	
7	0.	7.9	3965969 01	

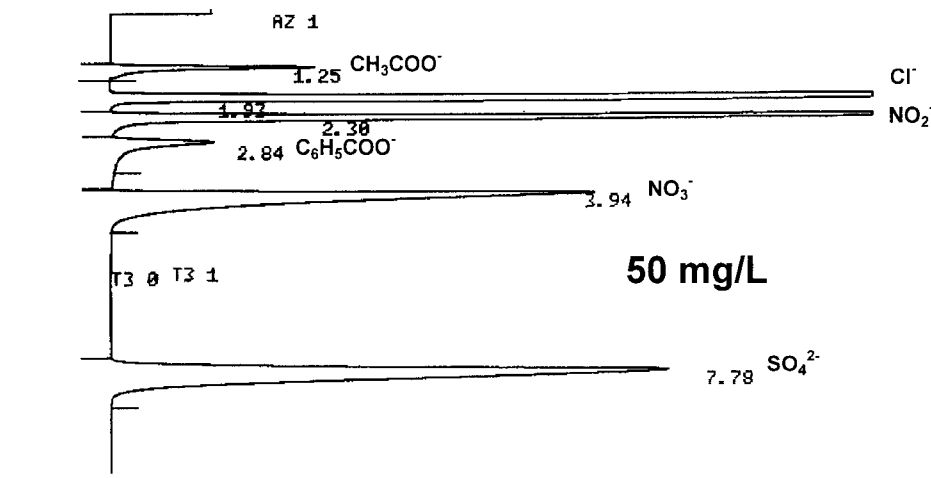
Figure J.2. IC chromatograms, 10 and 20 mg/L.



ANIONANAL YSIS 20:56:02 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN 6 INDEX 6

NAME	CONC	RT	AREA	BC	RF
1	0.	1.24	1060089	01	
2	0.	1.92	11169316	01	
3	0.	2.3	6539820	01	
4	0.	2.84	868979	01	
5	0.	4.	5582617	01	
6	0.	7.83	7534268	01	
TOTALS	0.		32755089		



ANIONANAL YSIS 21:06:20 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN 7 INDEX 7

NAME	CONC	RT	AREA	BC	RF
1	0.	1.25	1311551	01	
2	0.	1.93	14092827	02	
3	0.	2.3	9109178	08	
4	0.	2.84	1281981	05	
5	0.	3.94	8131246	01	
6	0.	7.78	11078345	01	

Figure J.3. IC chromatograms, 35 and 50 mg/L.

APPENDIX K

Column and Supply Network Parts List

Table K.1. Laboratory column parts list.

No.	Part Description	Part no.	Distributor	Tot. price
2	304 solid stainless endcap material (3 1/2" dia. x 1 3/8" long)		Alaskan Copper and Brass Portland, OR (503)238-7171	40.40
1	304 stainless tubing (2 1/4" o.d. x 1/16" wall x 12" long)		Kilsby Roberts Portland, OR (503)283-2251	72.00
1	stainless tubing (11 ga. = approx. 1/8 o.d. x 0.013" wall x 6" long)	21011	Hamilton Reno, NV (702)786-7077	donation
4	Viton O-rings (2 1/4" i.d. x 2 5/8" o.d. x 3/16" w)	2-331 V884-75	Air-Oil Products Eugene, OR (503)485-2022	20.32
4	Viton O-rings (2 1/8" i.d. x 2 3/8" o.d. x 1/8" w)	2-227 V884-75	same as above	10.04
11.5	labor hours		Oregon State Univ. Machine Shop	299.00
3	threaded rod, washers, and wing-nuts		same as above	5.00
4	stainless tube socket weld fitting w/ female connector (1/8" NPT)	SS-2-TSW-7-2	Eugene Valve and Fitting Eugene, Or (503)744-2032	25.20
6	stainless fractional tube adapter to male NPT (1/4" NPT to 1/8" tube)	SS-2-TA-1-2	same as above	40.20
4	Whitey stainless on-off ball valve (1/8" swagelock)	SS-41S2	same as above	215.60
1	stainless steel screen (100 mesh x 2 sq. ft)		Baldwin Filter Albany, OR (503)967-7949	55.00
			subtotal:	782.76

Table K.2. Supply network parts list.

No.	Part Description	Part no.	Distributor	Tot. price
4	304 stainless tubing (1/8" o.d. x 0.02 wall x 5' long)		Kilsby Roberts Portland, OR (503)283-2251	102.00
6	stainless syringe needles with metal hub (No. N711), point style #3, 11ga., 2 in. length	91011	Hamilton Reno, NV (702)786-7077	38.00
2	stainless pressure gauge, all stainless wetted parts, 0-15 psi range	AS109201	Davis Instrument Baltimore, MD 1-800-368-2516	140.00
4	Whitey stainless on-off ball valve (1/8" swagelock)	SS-41S2	Eugene Valve and Fitting Eugene, OR (503)744-2032	215.60
2	stainless male connector (1/4" NPT to 1/8" swagelock)	SS-200-1-4	same as above	12.00
2	stainless union cross (1/8" swagelock)	SS-200-4	same as above	62.40
12	stainless port connector (1/8")	SS-201-PC	same as above	64.80
3	stainless union tee (1/8" swagelock)	SS-200-3	same as above	52.20
2	stainless "C" series poppett check valves, 1/3 psi cracking pressure (1/8" swagelock)		same as above	79.60
2	stainless female connector (1/4" NPT to 1/8" swagelock)	SS-200-7-4	same as above	
1	low flow Q-head pump	QG6-1CSC	Fluid Metering Inc. Oyster Bay, NY 1-800-223-3388	540.00
1	Orion Sage syringe pump	M361	Curtin Matheson	925.00
1	1/8" x 10' stainless tubing		Alltech Assoc. Deerfield, IL 1-800-255-8324	40.00
1	rubber gas bag w/o stopcock	32310-215	VWR Scientific Seattle, WA	21.71
1	stainless 25 mm in-line filter holder	1209	Gelman Sciences Inc. Ann Arbor, MI	163.00
6	Delrin® 25 mm in-line filter holders	1109	same as above	57.95
100	FP Vericel® 0.2μm x 25mm filter membranes	FP-200	same as above	57.00
			subtotal:	2571.26

Table K.3. Miscellaneous parts list.

No.	Part Description	Part no.	Distributor	Tot. price
6	stainless syringe needles with metal hub, deflected point, 20 ga., 6 in. length	7186	O.S.U Chem. Store Part. MSN226	
2	stainless union (1/8" swagelock)		same as above	23.36
	stainless plugs (for 1/8" swagelock)	SS-200-P	same as above	21.84
1	3-prong utility clamp		same as above	14.20
2	stainless male run tee (1/8" NPT and 1/8" swagelock)	SS-200-3TMT	Eugene Valve and Fitting Eugene, OR (503)744-2032	34.80
1	stainless fractional tube adapter to female NPT (1/2" NPT to 1/8" tube)	SS-2-TA-7-2	same as above	8.50
2	stainless male connector (1/2" NPT to 1/8" swagelock)	SS-200-1-8	same as above	20.20
6	stainless plugs (for 1/8" swagelock)	SS-200-P	same as above	29.40
20	stainless tube ferrules (1/8")		same as above	33.60
3	50 mL SGE # 0115341 gas-tight syringe	85096	Alltech Deerfield, IL 1-800-255-8324	345.00
2	5 mL SGE # 008760 gas-tight syringe	85088	same as above	100.00
			subtotal:	630.90
			total:	3984.92