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REDOX GRADIENTS, TCE REDUCTIVE
DECHLORINATION AND Cr(VI) DETOXIFICATION IN
BIOAUGMENTED MODEL AQUIFER SYSTEMS

presented by

Timothy Joseph Mayotte

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**REDOX GRADIENTS, TCE REDUCTIVE DECHLORINATION, AND Cr(VI)
DETOXIFICATION IN BIOAUGMENTED MODEL AQUIFER SYSTEMS**

By

Timothy Joseph Mayotte

A DISSERTATION

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Michigan State University
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ABSTRACT

REDOX GRADIENTS, TCE REDUCTIVE DECHLORINATION, AND Cr(VI) DETOXIFICATION IN BIOAUGMENTED MODEL AQUIFER SYSTEMS

By

Timothy Joseph Mayotte

A mixed microbial culture enriched for tetrachloroethene or trichloroethene (TCE) dehalogenation activity was introduced into and conveyed through granular aquifer materials under an imposed hydraulic gradient. Over time, colonization of the aquifer solids by the enrichment culture resulted in a permeable stationary-phase biologically active zone (BAZ) within which complete reductive dechlorination of TCE occurred when provided organic carbon and nutrients. During a period of acclimation, a unique stationary-phase community profile was expected to result from successive depletion of electron acceptors and consumption and/or production of electron donors in pore fluids passing through the BAZ. It was postulated that the near steady-state community profile within the BAZ would exhibit spatially- and/or temporally discrete intervals of sulfate-reduction, methanogenesis, and halorespiration.

Batch and continuous-flow column systems containing solvent- and hexavalent chromium (Cr{VI})-impacted soil and groundwater from the Schoolcraft, Michigan site were used to transiently and spatially examine: 1) redox gradients; 2) TCE transformation patterns; and 3) successional adaptations of mobile- and stationary-phase microbial communities following inoculation of the aquifer materials with a TCE-dechlorinating enrichment culture from the Bachman Road site in Oscoda, Michigan. Real-Time *SYBR Green* Polymerase Chain Reaction (RT-PCR) analyses were employed to measure the mobile-phase community structure at temporally discrete intervals over the acclimation

period and upon
an attempt to id-
the fate and quan-
treatment, and as

Data from
inoculation, TCI
rate coefficient of
flow column were
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appeared to dec
to inhibit dech
optimized feed
within the BAZ

period and upon attainment of near steady-state treatment conditions across the BAZ. In an attempt to identify the primary zone(s) of halorespiration, RT-PCR was used to track the fate and quantify the mass of *Dehalococcoides ethenogenes* during inoculation, treatment, and as part of the post-treatment BAZ characterization of the aquifer solids.

Data from the semi-batch column experiments indicated that within one week of inoculation, TCE dechlorination to vinyl chloride was rapid with an apparent first-order rate coefficient of 0.6 day^{-1} . TCE concentrations (1-4 mg/L) entering the continuous flow column were reduced by approximately 30-40% within a seven-day interval following each of nine deliveries of lactate and nutrients to the BAZ. Influent Cr(VI) levels (200-250 $\mu\text{g/L}$) consistently decreased by over 90% during these intervals, presumably by reduction to less soluble Cr(III). Following lactate fermentation, iron and sulfate reduction were the predominant redox processes within the BAZ over the duration of the experiment. Reductive dechlorination of TCE was spatially correlated to the occurrence of lactate fermentation. Halorespiration appeared to be the dominant mechanism of reductive dechlorination, based on the presence of *Dehalococcoides sp.*, the corresponding rates of TCE depletion and production of both cis-1,2-dichloroethene and vinyl chloride. However, the rates and completeness of TCE transformation appeared to decrease with an increase in sulfidogenic activity. Methanogenesis appeared to inhibit dechlorination. Fate and transport modeling simulations suggest that an optimized feed delivery scheme may result in greater than 90% TCE treatment efficiency within the BAZ over a one-week period of time.

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2003

To Kathleen

For all her love, sacrifices, and selfless dedication to my goals.

To the
support and guidance

My wife
constant sources
facilitate my education
experience was
have never pursued

Mike D
learning experience
ability to think
to technical problems
addressed over

Commitment
provided valuable
and experience

Haekyu
and generous assistance
quickly to the last

Similarly,
guidance. I have
She has provided

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LIST OF SYMBOLS

α_L	Longitudinal dispersivity, m
b_{DC}	Microbial decay rate, 1/day
C_0	Initial pore volume concentration of TCE within small column, $\mu\text{g/L}$
C_1	Aqueous solute concentration at start of small column exchange, $\mu\text{g/L}$
C_2	Aqueous solute concentration at end of small column exchange, $\mu\text{g/L}$
C_{liquid}	Concentration of TCE within pore volume of small column, $\mu\text{g/L}$
C_{column}	Initial pore volume concentration of TCE within small column, $\mu\text{g/L}$
C_{Br}	Concentration of bromide within pore volume of large column, $\mu\text{g/L}$
C_{TCE}	Concentration of TCE within pore volume of large column, $\mu\text{g/L}$
C_{DCE}	Concentration of DCE within pore volume of large column, $\mu\text{g/L}$
C_{VC}	Concentration of VC within pore volume of large column, $\mu\text{g/L}$
C_{ETH}	Concentration of ethene within pore volume of large column, $\mu\text{g/L}$
C_{H_2}	Concentration of hydrogen within pore volume of large column, $\mu\text{g/L}$
d	Soil particle diameter, cm
D_{dis}	Distribution coefficient, cm^2/day
f	Fraction of exchange sites at equilibrium
K_{at}	Microbial attachment rate, 1/day
K_{de}	Microbial detachment rate, 1/day
K_{TCE}	TCE half-saturation coefficient, mg/L
K_{H_2}	Hydrogen half-saturation coefficient, mg/L
K_d	Distribution coefficient, L/mg
K_d^{TCE}	TCE distribution coefficient, L/mg
K_d^{DCE}	DCE distribution coefficient, L/mg
K_d^{VC}	VC distribution coefficient, L/mg
K_d^{ETH}	Ethene distribution coefficient, L/mg
$K_d^{H_2}$	Hydrogen distribution coefficient, L/mg
k'_{TCE}	TCE reaction rate, $\text{Lmg}^{-1}\text{day}^{-1}$
k'_{DCE}	DCE reaction rate, $\text{Lmg}^{-1}\text{day}^{-1}$
k'_{VC}	VC reaction rate, $\text{Lmg}^{-1}\text{day}^{-1}$
k'_{ETH}	Ethene reaction rate, $\text{Lmg}^{-1}\text{day}^{-1}$
κ_{TCE}	TCE kinetic (de) sorption rate, 1/day
κ_{DCE}	DCE kinetic (de) sorption rate, 1/day
κ_{VC}	VC kinetic (de) sorption rate, 1/day
κ_{ETH}	Ethene kinetic (de) sorption rate, 1/day
L	Characteristic length of a control volume, cm
M	Total mass of solute, g
M_r	Mass of solute retained on solids, g
$M_{dissolved}$	Mass of solute dissolved in pore fluids, g
M_{TCEm}	Mass of TCE within column, g
M_{soil}	Mass of soil within column, g
M_{TCE}	Monod-type saturation term for TCE
M_{H_2}	Monod-type saturation term for hydrogen, g

μ	Bacterial growth coefficient, day ⁻¹
μ_{max}	Maximum specific growth rate, 1/day
P	Model parameter
ΔP	Change in model variable
R_e	Reynolds coefficient
R_p	Dimensionless equilibrium partitioning coefficient
R_t	Retardation factor
R_{TCE}	TCE retardation coefficient
R_{DCE}	DCE retardation coefficient
R_{VC}	VC retardation coefficient
R_{ETH}	Ethene retardation coefficient
R_{H_2}	H ₂ retardation coefficient
ρ_b	Soil bulk density, mg/L
S_{TCE}	Solid-phase TCE concentration, mg/mg
S_{DCE}	Solid-phase DCE concentration, mg/mg
S_{VC}	Solid-phase VC concentration, mg/mg
S_{ETH}	Solid-phase ethene concentration, mg/mg
θ	Sediment porosity
t	Time, days
ϕ	Variation coefficient
V_{column}	Empty bed volume of small column, mL
V_{pore}	Pore volume within small column, mL
V	Superficial velocity of groundwater flow, cm/min
$v_{contam.}$	Average linear velocity of solute transport, cm/hr
v_{eff}	Average linear velocity of groundwater flow, cm/hr
ν	Kinematic viscosity, cm ² /hr
X	Total biomass concentration, mg/L
X_m	Biomass concentration in aqueous phase, mg/L
X_{im}	Biomass concentration on the solid phase, mg/L
$Y_{TCE-DCE}$	TCE/DCE stoichiometric constant
Y_{DCE-VC}	TCE/DCE stoichiometric constant
Y_{VC-ETH}	DCE/ethene stoichiometric constant
y_{TCE-X}	TCE yield
y_{H_2X}	Hydrogen yield
W	Model variable
ΔW	Change in model variable
x	Distance along one-dimensional length of column, cm

Chapter 1

INTRODUCTION

1.1 Introduction

The experimental work summarized herein is directed at understanding relationships within and between, and the extent of biodegradation associated with, mobile- and stationary-phase microbiological communities resulting from bioaugmentation of an aquifer system contaminated with trichloroethene and hexavalent chromium. Knowledge gained through these efforts could be of significant benefit to the design and maintenance of bioremediation strategies for treating chlorinated solvent-impacted groundwater with non-native enrichment cultures. The research has been conducted as part of a comprehensive bioremediation technology development program funded by the State of Michigan, and to partially fulfill the requirements for the degree of Doctor of Philosophy in Environmental Engineering at Michigan State University.

1.2 Background

The Village of Schoolcraft is a rural community of some 1,200 persons located approximately 10 miles south of the City of Kalamazoo in southwestern Michigan (Figure 1.1). The area is underlain by a regionally extensive, glacial outwash plain that forms a large shallow aquifer system. The aquifer represents a valuable regional resource used extensively for domestic, industrial, and agricultural purposes.

Since 1979, Schoolcraft has been the focal point of a series of State-sponsored investigations into the causes and distributions of several occurrences of groundwater

contamination. Through 1995, a total of seven distinct plumes of impacted groundwater had been identified and delineated during these investigations. The plumes have been designated A through G by the State of Michigan (Figure 1.1). Of the seven plumes, five (Plumes A, C, D, E and G) have resulted from releases of chlorinated solvents into the environment. With the exception of Plume A, all of these plumes include trichloroethene (TCE) as the primary contaminant of concern.

TCE is a suspected human carcinogen, based on studies performed with laboratory animals. It has also been linked to mutagenic effects in humans (Mallinckrodt Baker, Inc., 2000). Due to its widespread occurrence as an environmental pollutant and its potential threat to public health, TCE was ranked 15th of the 275 compounds comprising the 2001 CERCLA Priority List of Hazardous Substances (ASTDR, 2001). Consequently, there is a critical need to understand the processes that control the fate of TCE in the environment and to develop effective strategies to manage or remediate TCE-impacted environmental media.

1.2.1 Plume G

Plume G is distinctly the largest and most severe example of TCE-impacted groundwater in Schoolcraft. The plume originated at a now-defunct flexible rubber parts fabricating facility positioned in the center of the Village. From its source, Plume G extends downgradient approximately 1.5 miles, is nearly a quarter mile in width, and in some locales penetrates the entire saturated thickness of the aquifer (e.g., 70 to 90 feet).

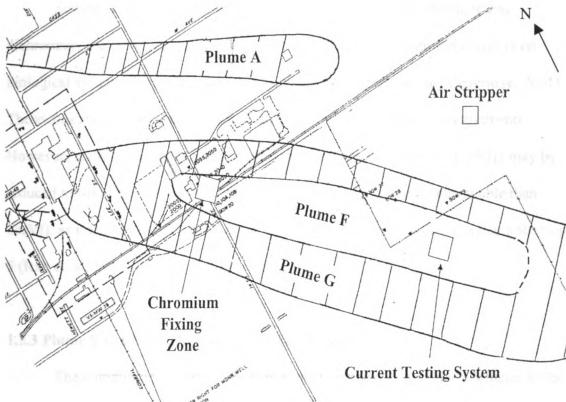


Figure 1.1. Central Schoolcraft, Michigan and groundwater contaminant Plumes F and G.

1.2.2 Plume F

Plume F is the only known occurrence of groundwater contamination by toxic metals in Schoolcraft. Its source is a former wood pressure treatment operation at which extensive quantities of chromated copper arsenate solutions were released to the subsurface, resulting in hexavalent chromium and arsenic contaminated groundwater. The site of the facility at which Plume F originated is located hydraulically downgradient of the source of Plume G. Consequently, Plumes F and G are commingled and are henceforth referred to as Plume F/G.

Soluble ionic species of hexavalent chromium, including chromate and dichromate, are extremely toxic and exhibit mutagenic and carcinogenic effects on biological systems due to their strong oxidizing nature (McLean and Beveridge, 2001). These ions are the dominant species of chromium in most aqueous environments. However, under suitably reducing conditions, hexavalent chromium {Cr(VI)} may be reduced to trivalent chromium {Cr(III)}. Cr(III) is less toxic and bioavailable than Cr(VI), as it readily forms insoluble oxides and hydroxides in aqueous systems above pH 5 (Rai, et. al, 1987).

1.2.3 Plume F/G Bioremediation Research Program

The commingled nature of Plumes F and G represents a unique challenge to the regulators responsible for the management and remediation of these impacts. In fact, since 1990 the State of Michigan has struggled to develop a technically- and cost-effective strategy for the combined clean up of these plumes. Remediation of groundwater impacted with TCE by conventional means, such as through pore fluid extraction and ex-situ treatment by air stripping or activated carbon, in most cases, has proved to be excessively costly and of limited effectiveness. TCE has the tendency to sorb to soil particles owing to its low aqueous solubility and relatively high octanol-water partitioning behavior. Consequently, the ratio of TCE mass per unit volume of groundwater extracted for treatment is inefficiently low. Due to these phenomena, alternative remedial strategies for TCE-impacted groundwater are desired. Favorable alternative remedies include those that emphasize in-place (in-situ) treatment.

To expand the potential for development of an innovative solution, the State has contracted with researchers at MSU to formulate and evaluate bioremediation strategies for Plume F/G. Natural bioattenuation strategies and biological treatment processes such as bioenhancement and bioaugmentation offer the potential for transforming TCE to non-toxic byproducts in-situ, eliminating the need to remove contaminated media from the subsurface for treatment. Accordingly, MSU is engaged in conducting a series of bioenhancement and bioaugmentation experiments designed to completely reductively dehalogenate TCE and create reducing conditions favorable for the in-situ conversion of Cr(VI) to Cr(III).

1.2.4 Previous Bioremediation Research

Research conducted over the past 20 years confirms that a variety of soil microorganisms possess enzyme systems capable of directly metabolizing or co-metabolically dehalogenating chlorinated solvents such as TCE (Lee, et. al, 1998). Vogel and McCarty (1985) performed one of the first studies that revealed the potential for mineralization of perchloroethene (PCE) in soil and aquifer systems under anaerobic conditions. They conducted column experiments using both glass beads and quartzite rock to demonstrate step-wise dechlorination of PCE to TCE, cis-1,2-dichloroethene (cis-DCE), and vinyl chloride (VC), respectively, under methanogenic conditions. VC conversion to CO₂ was also observed, suggesting that complete detoxification of PCE is possible under these conditions. Barrio-Lage, et al., (1986 and 1987), later demonstrated the conversion of TCE to cis-DCE in a variety of sediments maintained under anoxic conditions in batch microcosms. Their study offered clear evidence that soil

microorganisms from a variety of environmental settings may reductively dechlorinate TCE. DeBruin, et. al. (1992) observed complete reductive dechlorination of PCE to ethene and ethane in Rhine River sediments fed anaerobic granular sludge within a flow-through column system. The implication of their work was that mixed cultures of microorganisms could be introduced into non-native sediments to effect in-situ remediation of PCE (e.g., bioaugmentation). Since these studies, work by numerous other researchers has served to establish bioenhancement and bioaugmentation as viable treatment strategies for many sites with solvent-impacted groundwater.

Over the past ten years researchers have demonstrated the ability to use aquifer materials from solvent-contaminated sites to develop enrichment cultures capable of accelerated and/or complete transformation of PCE and TCE. In some cases the strain(s) responsible for the reductive dechlorination have been isolated and characterized. Holliger, et al. (1993) isolated a *Dehalobacter restrictus* species (PER K23) from anaerobic packed-bed column materials that is capable of coupling the reductive dechlorination of TCE to cis-DCE to growth. Hydrogen and formate were the only electron donors that could support growth with TCE as an electron acceptor. Wild, et al. (1997) identified another *D. restrictus* species from a mixed culture that completely reduces chlorinated ethenes to ethene. This organism, strain TEA, also coupled reductive dehalogenation of TCE to cis-DCE to growth using molecular hydrogen as the electron donor. Neumann, et al. (1994) discovered *Dehalospirillum multivorans*, a strictly anaerobic bacterium that is able to dechlorinate PCE to TCE and cis-DCE as part of its energy metabolism. Studies with *D. multivorans* by Miller, et al. (1996) identified a cytoplasm-associated dehalogenase that facilitates the transfer of electrons from

hydrogen or formate to PCE, resulting in a reduction to TCE. Gerriste, et al. (1999) isolated *Desulfitobacterium frappieri* TCE1 from a PCE-dechlorinating soil slurry. Like *D. restrictus* and *D. multivorans*, TCE1 couples the dehalogenation of TCE to cis-DCE to growth. Sung, et al. (2003) describes an acetate-oxidizing, PCE to cis-DCE dechlorinating *Desulfuromonas* species (*Desulfuromonas michiganensis* strain BRS1) that was cultivated from contaminated aquifer materials from eastern Michigan. Maymogatell, et al., (1997) described the isolation of a bacterium, referred to as strain 195, that dechlorinates PCE to ethene. To date, strain 195, a *Dehalococcoides* species, is the only known bacterium that is able to completely dechlorinate PCE and TCE to ethene. *Dehalococcoides* populations that couple hydrogen oxidation with VC respiration have also been identified (He, et al., 2003).

However, in virtually all of these cases, the reductive dechlorination activity expressed by the isolate appears to depend directly or indirectly on mutualistic interactions with other members of the community from which it was isolated (Bouwer, 1995). For example, the PCE transformation activity of strain 195 (*Dehalococcoides ethenogenes*) is inferred to depend on a supply of vitamin B₁₂ in culture media. *D. ethenogenes* does not produce vitamin B₁₂ and, therefore, must depend on other microorganisms for a supply of this co-factor in the environment. A recent study by Drzyzga and Gottschal (2002) revealed that TCE dehalogenation by TCE1 appears to depend on the activity of a sulfate-reducing bacterium. Specifically, sulfate reducers capable of fatty acid fermentation produce hydrogen and grow in syntrophic association with TCE1. This study suggests that bacterial dehalogenation and/or halorespiration of

TCE may be indirectly stimulated by the activity of fermentative sulfate reducers under sulfate-depleted conditions in soil environments.

Recent studies reveal that dehalogenating and halorespiring microorganisms may exist within a variety of soil types and conditions. Hendrickson, et al. (2002) conducted a study on the environmental distribution of the *Dehalococcoides* group of organisms in association with chloroethane-contaminated sites across North America and Europe. They used PCR assays to detect *Dehalococcoides* 16S rRNA gene sequences and demonstrated that members of this group are widely distributed in nature and can be found within a variety of geologic formations and in different climatic zones.

For these reasons, the natural biodegradation of chlorinated solvents, including TCE, is suspected at many sites. In some cases, the rates and completeness of compound transformation is deemed sufficient to allow these natural processes to clean up the site without direct human intervention. Conversely, at other sites, natural degradation is slow and/or incomplete. At such sites, natural attenuation processes are not favorable to remediate chlorinated solvents without anthropogenic enhancement or augmentation. For these sites, bioenhancement or bioaugmentation techniques may be employed to accelerate the natural rates and extent of dehalogenation such that bioremediation may be considered an effective treatment alternative.

Bioenhancement strategies often require a significant investment in time, effort, and associated cost to accelerate treatment rates or enhance the activity of certain microbes and ensure that the pathway of TCE transformation is complete. Alternatively, mixed cultures of native soil microorganisms enriched for dechlorinating activity may be introduced into aquifer systems to augment the native dechlorinating microflora and

thereby promote more rapid and complete treatment. The acclimation of enrichment cultures introduced into non-native sediments during bioaugmentation may be accelerated if environmental conditions within the host medium are first adjusted to favor the growth and activity of the dehalogenators. This may be accomplished through pH-adjustment, or provision of a growth substrate preferred by the enrichment.

Most experimental work to evaluate the dechlorination behavior of mixed and pure cultures in native and non-native aquifer materials has been performed in laboratory microcosms in which environmental and nutritional conditions are strictly controlled. Work by Isalou and Sleep (1998), Harkness, et. al. (1999), and Cirpka, et. al. (1999), examined dehalogenation activities expressed by non-native mixed enrichment cultures and halorespiring isolates in flow-through systems packed with aquifer materials and maintained under natural conditions. These studies emphasized the spatial measurement of electron donors and acceptors, chlorinated solvent dehalogenation and metabolic byproducts associated with the activity of the augmented microbial community. However, with the exception of work done in fractured and granulated basalts by Lehman, et.al. (2001), few studies have attempted to evaluate the transport behavior of inoculates during delivery, or correlate the spatial variability of aquifer geochemistry and contaminant transformation with the structures of mobile- and stationary-phase microbial communities resulting from bioaugmentation. Such evaluations could significantly advance the current understanding of the successional adaptations of in-situ treatment regimes following inoculation of aquifer materials. Further, the knowledge gained from this experimentation could benefit strategies for optimizing biotransformation capacities within bioaugmentation treatment zones.

1.3 Plume F/G Bioaugmentation Studies

The bioaugmentation research being conducted at MSU for the Plume F/G project is focused on assessing the efficacy of introducing a mixed culture of non-native microorganisms enriched for PCE dechlorination activity into site aquifer materials to effect complete dehalogenation of TCE. It is postulated that a concomitant decrease in oxidation-reduction potential will result sufficient to cause the reduction of Cr(VI) to Cr(III). The initial phase of the assessment entailed the completion of small-scale, semi-batch column microcosm studies designed to compare the effects of bioaugmentation against bioenhancement and sorption controls. This phase was followed by experiments using large-scale, continuous-flow columns to examine transient and spatial TCE transformation and successional adaptations of mobile- and stationary-phase microbial communities following inoculation of the Plume F/G aquifer materials.

1.3.1 The BR Enrichment Culture

The enrichment culture used for the Plume F/G experiments was developed using PCE-impacted aquifer materials from the Bachman Road site in Oscoda, Michigan. Like Plume F/G, the Bachman Road site yielded data indicating that natural attenuation of PCE has been occurring, but not at favorable rates. Further, there are disproportionately higher levels of dichloroethene isomers (primarily cis-DCE) than VC or ethene (ethene) concentrations in the groundwater, suggesting that the pathway of PCE transformation by native aquifer flora is incomplete over much of the site. Activity measurements from batch microcosm studies with sediment and groundwater from a variety of locations and

depths within the PCE plume revealed a heterogeneous distribution of dechlorinating populations in the Bachman Road aquifer (Lendvay, et al., 2003).

From a series of experiments in which Bachman Road aquifer materials were exposed to an anaerobic culture medium and elevated concentrations of PCE, cis-DCE and electron donor, the activities of the native dehalogenating microorganisms were greatly enhanced. The resulting mixed culture (“the BR Enrichment”) can completely dechlorinate PCE when fed lactate as a primary carbon source. Recent work to characterize the structure of the microbial community in, and cultivate dechlorinating members from, the mixed culture revealed the presence of *Desulfuromonas* and *Dehalococcoides* species (Sung, et al., 2003; He, et al., 2003), including strain BRS1 and *D. ethenogenes*. Further, it appears that the activity of *D. ethenogenes* is directly, if not exclusively, responsible for PCE dechlorination to ethene in the enrichment. The enrichment has been successfully re-introduced into a portion of the impacted Bachman Road aquifer to accelerate PCE detoxification in those sediments. The BR Enrichment and associated PCE transformation activity has been maintained for over three years within a 75 L chemostat operated by EFX Systems of Lansing, Michigan.

Similar to the Plume F/G site, the Bachman Road sediments consist of glacial and post-glacial alluvium. The hydraulic characteristics of these materials are compatible with those measured in the Schoolcraft aquifer. Sediments sampled at the two sites also exhibit similarities with respect to geochemistry and microbiology. For these reasons, it has been inferred that the BR Enrichment may be effectively introduced into Plume F/G aquifer materials where it will readily acclimate and express similar TCE-transformation characteristics.

In the completely-mixed reactor system used for culture growth, the BR Enrichment is maintained in either municipal water or Plume F/G groundwater. In both instances, water is re-circulated through the reactor so that the residence time of the fluids is extended many-fold. Periodically, PCE and electron donor (lactate) and nutrients (e.g., urea and phosphoric acid) are added to the re-circulating water and the subsequent extent and magnitude of dehalogenation is monitored. It is assumed that dehalogenation is maximized only after culture growth and metabolically induced redox conditions within the re-circulating fluids have reached appropriate levels. As it acclimates to fresh make-up water or following feeding, the enrichment undergoes successional changes during which the activities of certain key community members respond to progressive changes in the concentrations of electron donors and acceptors. For example, when fresh groundwater is added to the reactor, the activities of nitrate reducers and fermenting organisms are expected to be at their maximum. Over time, as a decrease in the levels of nitrate occurs and the levels of dissolved hydrogen increase from fermentation, the activities of iron, manganese and sulfate reducers will dominate. Finally, as the concentrations of the more energetically favorable electron acceptors such as soluble Fe(III) and sulfate begin to diminish, the activity of hydrogen-utilizing methanogens will prevail.

Similarly, the magnitude of dechlorination activity will be time-dependent in response to the addition of fresh water or feed to the reactor. Specifically, incomplete dechlorination activity is expected to predominate amongst sulfate-reducing populations and methanogens utilizing organic carbon for reducing power. At this stage, TCE concentrations will give way to an accumulation of cis-DCE. As the partial pressure of

dissolved hydrogen increases, the activities of H₂-utilizing halorespiring microorganisms, including *D. ethenogenes*, is expected to also increase, resulting in a greater occurrence in the conversion of cis-DCE to ethene.

The metabolic activity and transformation capacities observed over time in the reactor environment are likely to be manifested over space in a flow-through system, such as downgradient of aquifer injection points. This largely is due to the lack of completely mixed conditions between microflora and groundwater. Therefore, in a model aquifer system fed TCE-impacted groundwater, we would expect to observe a spatial-dependence in community structure that generally correlates with concentrations of electron donors and acceptors along the path of groundwater flow. A monitoring system with spatial resolution downgradient of the point of inoculation would provide a unique opportunity to measure TCE transformation activity as well as geochemical conditions and microbiological diversity along the longitudinal path of contaminant transport. The information derived from such observations and measurements could improve the current understanding of how to establish, maintain, and monitor efficient biologically active treatment zones during the implementation of bioaugmentation strategies.

1.3.2 Summary of Preliminary Results

As noted above, an experiment with a series of small-scale column microcosms was completed as part of the MSU bioaugmentation research program. The details associated with this work are presented in the following chapter (Experimental Design).

Comprehensive treatment of the data from the small column experiment is provided in Chapter 3 (Results). General observations of the data set include the following:

- A composite profile of biomass breakthrough resulting from the inoculation of triplicate columns is provided on Figure 3.1. This figure reveals that the inoculum traveled through the subject column systems at a rate very close to the average linear velocity of groundwater flow, as the breakthrough of biomass occurred nearly co-incident with that of a conservative tracer. However, the profile also indicates that a significant fraction (e.g., nearly 50%) of the biomass introduced to the columns was apparently retained in the sediments during the inoculation event.
- As expected, nitrate levels in the Plume F/G groundwater fed to the columns were depleted during the incubation periods following the weekly exchange of pore fluids, suggesting that nitrate reduction by the inoculum was complete during these periods.
- Conversely, sulfate reduction was minimal, suggesting that the duration of pore fluid contact with the inoculated sediments was not sufficient to permit successional development of predominantly sulfate reducing conditions by the microflora in the small-scale columns.
- Organic carbon (e.g., lactate) concentrations fed to the inoculated columns were consistently reduced by approximately 60% to 90% during the incubation periods between pore fluid exchanges. Given that lactate was the only carbon source added

to the feed water, it is inferred that acetate and hydrogen produced through lactate fermentation were also sources of reducing power available to the microflora within the column systems, including BRS1 and *D. ethenogenes*.

- The analyses of headspace gases revealed that hydrogen levels within the atmosphere of the anaerobic glove box in which the experiments were conducted interfered with the accurate measurement of hydrogen partial pressures resulting from bioactivity within the columns. Specifically, hydrogen concentrations measured within control samples of the glove box atmosphere were consistently greater than levels detected within the samples of the head space of the small columns, rendering these results suspect due to the potential for cross-contamination of sample containers stored within the glove box. Other than hydrogen, methane was the only gas detected in head space samples. However, methane was only measurable in samples collected from bioaugmented columns. In general, the relative amounts of methane observed were greatest within the columns that received an undiluted inoculate suspension. These results suggest that methanogenic conditions developed in at least a portion of each of these columns during the incubation periods between exchanges, even though sulfate levels appeared to persist within the pore fluids.
- During a ten-week period immediately following inoculation, TCE transformation to cis-DCE and VC was evident. In the columns that received undiluted inoculum, TCE removal was complete during this period. In these columns, TCE appeared to be converted to cis-DCE (approximately 10%) and VC (approximately 50%), suggesting

that efficient dechlorination was occurring but was incomplete due to insufficient residence times between pore water exchanges within the columns.

- No significant TCE removal was observed in radiation-sterilized and bioenhancement control columns during the period parallel with inoculation and subsequent feeding exchanges.
- Cr(VI) levels in post-incubation period effluent samples were, in general, significantly lower than the concentrations in the Plume F/G groundwater fed to the inoculated controls during each exchange.
- Increases in nutrient and TCE loadings during the late stages of the small-scale column experiment had little to no observable effect on TCE transformation activity within inoculated columns sets.

From these observations, it is inferred that a mixed microbial culture enriched for PCE or TCE dehalogenation activity, such as the BR enrichment, can be introduced into and conveyed through the Plume F/G aquifer materials under an imposed hydraulic gradient. Along the inoculum path of migration, a fraction of the introduced biomass will be retained within the sediment, thereby promoting colonization of aquifer solids. The biomass retained in the aquifer will initially exhibit spatial segregation of key community members owing to differences in physical transport characteristics including sorption, straining and dispersion. Over time, colonization of the aquifer solids by the enrichment

culture is expected to result in the establishment of a permeable stationary-phase biologically active zone (BAZ) across the width of which complete reductive dechlorination of TCE will occur when provided appropriate sources and dosages of organic carbon and nutrients. During a period of acclimation leading to near steady-state treatment conditions within the BAZ, a more pronounced spatial dependency of the stationary-phase community structure is expected to result from successive depletion of electron acceptors and consumption and/or evolution of electron donors in pore fluids passing through the BAZ. It is postulated that the near steady-state community profile within the BAZ will exhibit spatially- or temporally-discrete intervals of sulfate-reduction, methanogenesis, and halorespiration.

Resolution of the spatial relationships between these regions and redox gradients will provide valuable insight into how the microbial community structure within the BAZ can be maintained to optimize TCE dechlorination. For example, there may be a benefit in controlling the influx of lactate conveyed through the BAZ to limit stimulation of non-dehalogenating organisms and maintain dissolved hydrogen concentrations at or near threshold levels associated with TCE halorespiration. Conversely, a higher input of organic carbon could stimulate rapid TCE reduction to cis-DCE through partial dehalogenation by acetate-oxidizers, sulfate reducers and methanogens. The predominance of cis-DCE could serve to increase the rates of transformation to ethene, as downgradient halorespiring organisms, such as *D. ethenogenes*, can dechlorinate cis-DCE to ethene more rapidly after TCE is depleted.

It is further postulated that groundwater passing through the treatment zone will become progressively reduced due to the increase in anaerobic metabolic diversity and

activity. The decrease in oxidation-reduction potential is expected to become sufficient to favor the reduction of Cr(VI) to Cr(III). Due to its insoluble nature, immobilization of Cr(III) will ensue, potentially resulting in a reduction in the concentrations of total dissolved and hexavalent chromium to be below State-mandated cleanup levels.

1.4 Hypotheses and Research Objectives

A goal of the Plume F/G bioaugmentation research is to explore issues such as those summarized above and thereby improve the understanding of the structure of microbial diversity within biological treatment zones resulting from inoculation of aquifer systems with non-native enrichment cultures. Accordingly, through completion of the studies summarized herein, the following hypotheses were tested:

1. The inoculation of the Plume F/G aquifer materials with the BR Enrichment will produce a spatially dependent, stationary-phase microbial community profile.
2. Within the community profile, a biologically active zone (BAZ) consisting of spatially and/or temporally discrete regions of sulfate-reduction, methanogenesis, and halorespiration will exist wherein transformation of TCE to non-toxic end products will occur.
3. Within the BAZ, the groundwater redox chemistry will favor for the reduction of Cr(VI) to and immobilization of Cr(III).

To prove these hypotheses, it was necessary to:

1. Define the general transport characteristics of the BR Enrichment within the Plume F/G aquifer materials.
2. Verify that the completeness of TCE reductive dechlorination associated with inoculation of the Plume F/G aquifer materials is consistent with that achieved by the BR Enrichment under controlled laboratory conditions.
3. Establish a range of substrate and nutrient loadings that are practical for enhancing the reductive dechlorination activity expressed by the BR Enrichment within the Plume F/G aquifer materials.
4. Estimate the dimensions of the BAZ for reductive dechlorination activity established by the BR Enrichment in the Plume F/G aquifer materials under enhanced delivery and maintenance conditions.
5. Characterize the profile of the augmented microbial community structure across the width of the BAZ at specific time intervals leading up to and including the period when near steady-state treatment conditions were established.
6. Identify the portion of the BAZ and associated redox conditions in which halo-respiration predominates.

7. Estimate the rates of reductive dechlorination expressed within the BAZ between feeding events.
8. Identify correlations between: a) nutrient and redox gradients and the extent of reductive dechlorination, and b) the microbial community structure across the steady-state BAZ.
9. Evaluate the occurrence and extent of in-situ chromium detoxification and immobilization associated with the development of reducing conditions within the BAZ.

Chapter 2

EXPERIMENTAL DESIGN

2.1 Introduction

Based on the observations and the objectives summarized in Chapter 1, a research program, consisting of the following experiments and associated tasks, was undertaken:

1. Small-Scale Column Microcosm Experiment

Task 1.1 - Characterization of Flow-Through Properties

Task 1.2 - TCE Mass Loading and Sorption Capacity Evaluation

Task 1.3 - Treatment Strategy Evaluation

Subtask 3.1 - Baseline Maintenance Exchange Events

Subtask 3.2 - Optimization Exchange Events

Task 1.4 - Post-Treatment Characterization

2. Large-Scale Column Experiment

Task 2.1 - TCE Mass Loading and Sorption Capacity Evaluation

Task 2.2 - Inoculation/Characterization of Flow-Through Properties

Task 2.3 - Treatment

Task 2.4 - Post-Treatment Characterization

3. Measurement of TCE Transformation Kinetics

4. TCE Transport Modeling

The small-scale column experiment was conducted between July of 2001 and April of 2002. The Large-scale column experiment began in June of 2002 and concluded in late October of 2002. Measurements of TCE transformation kinetics associated with the BR Enrichment and fate and transport modeling activities were performed concurrent with and subsequent to the large-scale column experiment, respectively. Ms. Leslie Dybas and Baolin Sun performed DNA analyses associated with the small- and large-scale experiments. DNA extraction from environmental samples and all other analyses were performed by the author. Retec Group, Inc. was responsible for completion of the TCE transformation kinetics study. Dr. Mantha Phanikumar assisted with the reactive transport model development.

2.2 Small-Scale Column Microcosm Experiment

Between July of 2001 and April of 2002, a four-phase experiment was performed using small-scale soil column microcosms. The objectives of the experiment were to verify that the BR Enrichment could be effectively delivered through Plume F/G sediments under an imposed hydraulic gradient, and to temporally measure the levels of dechlorination in inoculated columns as well as sterile and non-sterile controls (e.g., Objective #s 1, 2, and 3 from Chapter 1). Further, the fate of Cr(VI) was examined to verify that reduction to Cr(III) was occurring in inoculated columns (Objective #9).

A total of 21 columns were operated during this experiment. These were grouped into seven (7) distinct sets, each consisting of three (3)-columns. The seven column sets were organized into the following categories:

1. Radiation-sterilized Columns (Columns A, B, and C)
2. Natural Attenuation Columns I (Columns D, E, and F)
3. Natural Attenuation Columns II (Columns G, H, and I)
4. Inoculated Columns I (1% inoculum concentration; Columns J, K, and L)
5. Inoculated Columns II (10% inoculum concentration; Columns M, N, and O)
6. Inoculated Columns III (100% inoculum concentration; Columns P, Q, and R)
7. Biostimulation Columns (Columns S, T, and U)

2.2.1 Column Design and Assembly

The design and operation of the small-scale column microcosms were consistent with those described by Dolan and McCarty (1996) and Mayotte, et al. (1996). A schematic of an example column is provided on Figure 2.1. Columns consisted of 85 L borosilicate glass test tubes (Pyrex). Each column was filled partially with site groundwater prior to assembly to ensure that internals (e.g., soil and plumbing) were perturbed under saturated conditions. The base of each column was then packed with a disk of fused silica wool (Restek Corporation Cat. # 20790) over which a disk of glass paper (Whatman Glass MicroFibre) was carefully placed. Plume F/G aquifer material was deposited over the support medium in 1 to 3 cm thick lifts by allowing soil fines to gravity settle through the groundwater. After a lift had settled, the bottom of

each column was tapped on a solid surface to dynamically compact the soil. The final soil bed depth within each column measured approximately 10 cm, resulting in a packed soil volume of about 58-60 mL. Once packed, each column was fitted with internal and external plumbing.

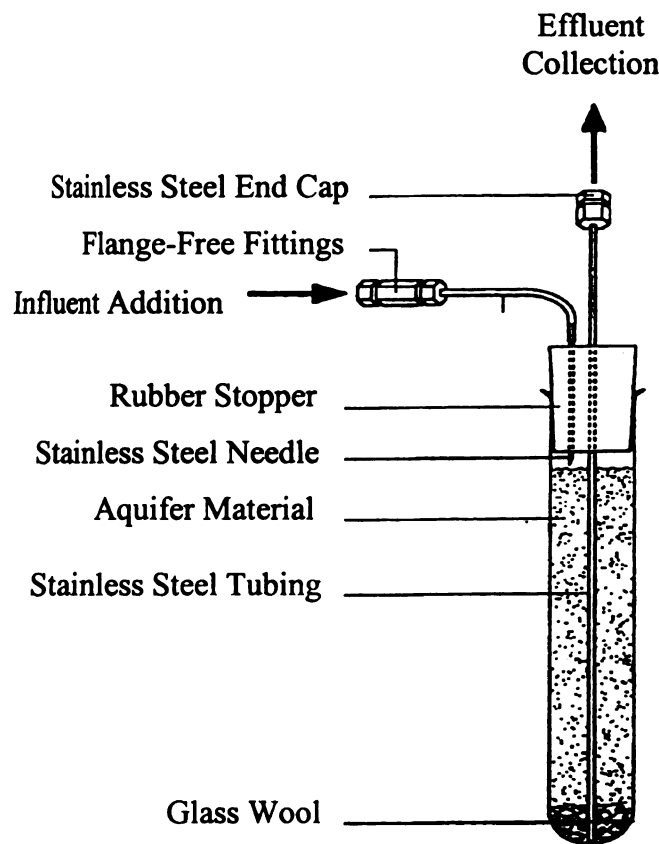


Figure 2.1. Small-Scale Column Microcosm (Dolan and McCarty, 1996).

First, an effluent line (1/16-inch stainless steel tubing, Alltech Associates, Inc.) **ashed through** the soil and into the support medium at the base of each column. A **of 0.027-in. piano wire** (Small Parts, Inc.) inserted inside the tubing prevented soil

coring and plugging of the line during placement. The wire was removed immediately after the terminal end of the tubing had been positioned within the support medium at the base of the column. A butyl-rubber stopper (No. 4) was then used to fix the tubing in-place and seal the contents of the column. To further secure the seal, electrical tape was wrapped around the outside of the stopper and column. To facilitate the delivery of groundwater into a column, a two-inch long, 18-gauge stainless steel needle (VWR Scientific Products) with a luer lock coupling was also inserted through the rubber stopper and positioned at a depth approximately 2 cm above the water level over the soil. Groundwater was delivered at a fixed volumetric flux through 100 mL capacity, gas-tight, glass syringes (SGE, Inc.) and the stainless steel tubing (1/16-inch inside dia.) and the influent needles. The controlled delivery of groundwater was accomplished using Harvard syringes pumps (Harvard Apparatus Model #22), which could be programmed to compress the plungers in the glass syringes at a constant rate. External plumbing connections consisted of leak-tight stainless steel compression fittings (Swagelok). Assembled columns were placed in triplicate sets within customized wood boxes to minimize disturbance of internals during operations.

All column components were autoclaved under 20 lbs/in² (psi) of pressure at 121 °C for a minimum of 25 minutes prior to column assembly. Column assembly activities were performed within an anaerobic glove box (Coy Laboratory Products, Inc.) over an atmosphere of approximately 95% N₂ and 5% H₂ and at a temperature of 18 °C. Control columns A, B, and C were radiation sterilized using a cobalt-60 energy source emitting approximately 1.2 millirads per hour for 1.7 hours. Radiation sterilization was performed at the Phoenix Memorial Laboratory at the University of Michigan in Ann

Arbor. Assembled columns were maintained under anoxic conditions at 18 °C within the glove box throughout the duration of the experiment.

2.2.2 Column Operation and Monitoring

Columns were operated by periodic semi-batch exchange of soil pore fluids.

Specifically, this entailed a series pore fluid exchanges performed once weekly. Each exchange involved a rapid transfer of pore fluids (Plume F/G groundwater spiked with TCE and amendments, as necessary) within each column, followed by a week-long incubation (no flow) period. After incubation, the pore fluids were re-exchanged.

The small-scale column microcosm experiment consisted of four (4) tasks, as described below. In total, approximately 35 weeks of pore-fluid exchanges were performed during this experiment.

Task 1.1 - Characterization of Flow-Through Properties

Task 1.1 entailed determining the flow-through characteristics of the soil within each column. Specifically, the flow-through characteristics were estimated by exchanging groundwater spiked with 50 mg/L of sodium bromide (NaBr) through each column and tracking the effluent concentrations of the tracer by ion chromatography. The breakthrough profile of bromide thus obtained revealed the approximate internal pore volume of the packed soil in each column. Similarly, the breakthrough of TCE conveyed through a subset of columns was used to estimate the “apparent” internal pore volume, which is a crude measure of the magnitude of the combined effects of adsorption and dispersion relative to the transport of the conservative tracer.

Measurement of both parameters was necessary to calculate the volume of TCE-spiked groundwater necessary to ensure complete contaminant breakthrough during an exchange event. In addition to TCE and bromide, the pore fluids at both the onset and conclusion of each exchange were analyzed by ion chromatography to measure acetate, chloride, nitrite, nitrate, phosphate and sulfate levels and thereby establish the baseline geochemical conditions within the columns prior to inoculation with the BR Enrichment.

An additional component of Task 1.3 was the tracking of biomass breakthrough during inoculation. Accordingly, a triplicate set of biomass breakthrough profiles was obtained by measuring the optical density of effluent fractions during the inoculation of a set of three columns with a non-diluted suspension of BR Enrichment. These data were used for estimating the inoculum transport characteristics and quantity of biomass retained within the columns during inoculation. Biomass concentrated from replicate samples were used to track the breakthrough of *D. ethenogenes* using RT-PCR.

Task 1.2 - TCE Mass Loading and Sorption Capacity Evaluation

Task 1.2 included a series of exchange events during which each soil column was loaded with approximately 1,000 $\mu\text{g/L}$ of TCE until solid and aqueous phase concentrations of the solute appeared to be near equilibrium.

Task 1.3 - Treatment Strategy Evaluation

Task 1.3 entailed inoculation and subsequent pore fluid exchanges in BR-enriched control columns to evaluate TCE transformation as well as mobile-phase biomass transient geochemical gradients resulting from the bioaugmentation. During this

phase, the weekly exchange of pore fluids, including the input of TCE (500-1,000 $\mu\text{g/L}$), organic carbon, and nutrients, were performed as follows:

- All columns (A through U) received the specified dosage of TCE on a weekly basis;
- Every two weeks, sterile (e.g., sorption) controls (Columns A, B, and C) received organic carbon and nutrients;
- Inoculated columns (J, K, L, M, N, O, P, Q, R) received organic carbon and nutrients bi-weekly;
- Bioenhancement controls (Columns S, T, and U) received organic carbon and nutrients bi-weekly;

Back-up columns, D, E, F, G, H, and I, received the weekly dosage of TCE only.

Bi-weekly feeding entailed loading the relevant columns with organic carbon (240 M DL-lactic acid), as well as nitrogen ($15.4 \mu\text{M NH}_4\text{Cl}$) and phosphorus ($2 \mu\text{M H}_2\text{PO}_4$).

During the final three weeks of Task 1.3, a series of exchanges were conducted during which the influent concentrations of TCE and feed to the inoculated and control columns were adjusted to evaluate the associated effects on inoculum performance (task 3.2). Specifically, the TCE dosage to columns C, R and U was increased 10-

ld, and the feeding of columns B, Q, and T was increased to weekly. Conversely, no alterations were made to the TCE loading or feeding protocols for columns A, P, and S during this period.

Analytes measured in samples collected during the majority of the exchange events conducted as part of Task 1.3 are summarized on Table 2.1.

Task 1.4 - Post-Treatment Characterization

Task 1.4 entailed the disassembly of the inoculated and biostimulated control sets. During these activities, soil within the drained columns was collected for measurement of biomass levels and characterization of the solid-phase microbial community structure by PicoGreen® DNA Quantification, and T-RFLP and RT-PCR analyses, respectively.

Table 2.1. Summary of Aqueous Sample Analyses – Small-Scale Column Microcosm Experiment.

Aqueous Solutes	Headspace Gasses	Biomass
Gas Chromatography (electron capture detection)/Mass Spectrometry	Reduced Gas Analysis Hydrogen	Optical Density
PCE	Gas Chromatography (flame ion detection)	PicoGreen® dsDNA Quantification
TCE	Methane	Polymerase Chain Reaction (PCR)-based Terminal Restriction Fragment Length Polymorphism (T-RFLP)
cis-DCE	Ethene	
trans-DCE		
DCE		
VC		Real-Time (<i>SYBR Green</i>) PCR
Ion Chromatography		
Acetate		
Chloride		
Nitrite		
Bromide		
Nitrate		
Phosphate		
Sulfate		
Atomic Absorption		
Hexavalent chromium		

Operational and Sampling Procedures

During a pore fluid exchange, 100 mL of amended groundwater was conveyed through each column at a rate of 1.00 mL/min. Effluent samples were collected in 2 mL fractions of the initial 12-16 mL and final 70-100 mL of groundwater exchanged. Triplicate samples from these volumetric ranges were collected in: 1) volatile organic analysis (VOA) vials (40 mL capacity), containing 3 mL of sodium bisulfate (2%) as preservative; and 2) in 15 mL, sterile plastic centrifuge vials (Corning). During exchange events that included feeding amendments, two sets of centrifuge vials were filled. Following sampling, VOA vials were stored at 4°C pending analysis by GC/MS. The contents of the centrifuge vials were filtered through 0.2 µm syringe filters (Titan Filtration Systems) and decanted into 1-mL glass or polyethylene ion chromatography vials (Alcott) and stored at -20°C pending analysis for the major anions listed above. During exchange events that entailed feeding or targeted biomass analyses, the 2 mL volume within the each centrifuge vial was split, with approximately 1 mL of the sample being transferred to a 1.5 mL vial (Eppendorf) for subsequent centrifugation at 1,400 rpm for 20 min (Eppendorf Centrifuge 5415 C) to concentrate planktonic biomass for DNA extraction and the molecular procedures listed above. The remaining sample 1 mL was processed for ion chromatography analysis, as described above. The 2 mL of groundwater collected within the second centrifuge vial was filtered, as previously described. Approximately 1 mL of the filtrate was then transferred to a 1.5 mL glass vial (SUN Catalog #200 000) and sealed with a Teflon-lined butyl rubber septa secured with a snap cap (Alltech). These samples were stored at -20 °C pending analysis for volatile fatty acids by high performance liquid chromatography (HPLC). To facilitate the

Measurement of soluble chromium, the residual 1 mL volume of the filtrate was delivered to a 1.5 mL Eppendorf vial that was pre-preserved with 20 μ L of concentrated nitric acid (Fisher Scientific Products). The preserved samples were stored at 4 °C pending analysis for soluble chromium by atomic adsorption.

Periodically, samples of the headspace gasses in each column were collected by withdrawing triplicate 1 mL aliquots of the atmosphere above the soil within the vessels. These samples were acquired using sterile, 1 mL or 3 mL plastic syringes (Becton Dickinson) with luer tips. By detaching the influent conveyance plumbing from a groundwater delivery syringe, the sampling syringes could be affixed to facilitate the extraction of headspaces gas by suction. Immediately following sampling, the syringes were fit with sterile, 22-gauge disposable needles (Becton Dickinson), which were then inserted into oversized butyl rubber stoppers to seal the contents of each syringe prior to analysis. Headspace samples were immediately analyzed for the presence of hydrogen, methane and ethene, as prescribed above.

Theoretically, the average concentration of the target analytes measured in a triplicate set of groundwater samples obtained from the initial 12-16 mL of groundwater exiting each column represented the solute concentrations in the pore fluids at the conclusion of the week-long incubation period. Conversely, the average solute concentrations measured in triplicate samples of the final 70-100 mL of groundwater exiting each column at the conclusion of an exchange represented the initial conditions of the subsequent incubation period. By comparing the initial conditions of an exchange with the post-incubation concentrations measured during the following exchange, the change in solute mass during the incubation period can be estimated. From these

comparisons over the duration of the small-scale column experiment, mass balances were calculated for select analytes.

Data Reduction

In order to evaluate the fate of TCE within the three inoculated column sets (J-L, M-O, and P-R) and the natural attenuation and stimulated controls (D-I and S-U, respectively), mass balances were developed using effluent concentrations measured during each exchange. The protocol for examining the conservation of the mass of each of these solutes was summarized by Mayotte, et al. (1996). Similar mass balances were established for cis-DCE and VC within Columns P, Q, and R, which were inoculated with non-diluted BR Enrichment.

During the initial exchange of fluids in each column, the breakthrough of both bromide and CT were evaluated. For this first exchange, $C_1 = 0$. Using the bromide data, the porosity, θ , of the aquifer media within each column was calculated by:

$$\theta = \frac{M}{(C_2 - C_1)} \left(\frac{1}{V_{column}} \right) = \frac{V_{pore}}{V_{column}} \quad (2.1)$$

when $C_2 = C_0 = C_{column}$, and $M = C_{column} V_{column} \theta = C_2 V_{pore}$.

Unlike bromide, TCE sorbed to the aquifer solids during exchange. In addition, TCE transport appeared slightly more dispersed. These two mechanisms were reflected in the shape of the TCE breakthrough profile, which, upon inspection, lagged behind and sloped less than the profile for bromide (Figure 3.1).

Mass removal between exchange events was assumed to occur as the result of either sorption or biotransformation. Thus to determine the cumulative mass removed between exchange events it was only necessary to know C_2 resulting at the end of an exchange event (i-1) and C_1 measured at the beginning of the following exchange event (i). These data enabled the estimation of cumulative mass removal using:

$$M_r = V_{column} \theta \sum_{i=1}^n (C_2^{i-1} - C_1^i). \quad (2.2)$$

Prior to inoculation, cumulative mass removal data were used to quantify sorption. Sorption was estimated by comparing the total mass of TCE that had been removed to the liquid phase concentration of TCE present in the pore fluid once the solids were saturated. This ratio is defined as the dimensionless equilibrium partition coefficient, R_p , where:

$$R_p = \frac{M_r}{M_{dissolved}} = \frac{V_{column} \theta \sum_{i=1}^n (C_2^{i-1} - C_1^i)}{V_{column} \theta C_1^i}. \quad (2.3)$$

With knowledge of R_p , the retardation factor, R_t , and distribution coefficient, K_d (cm^3/g), for TCE in the Plume F/G aquifer materials could be estimated from:

$$R_t = \frac{\nu_{eff}}{\nu_{contaminant}} = R_p + 1 \quad (2.4)$$

and

$$K_d = \frac{R_p \theta}{\rho_b} \quad (2.5)$$

where v_{eff} = average linear velocity of groundwater flow through the aquifer solids within the column (cm/hr); $v_{contam.}$ = the average linear velocity of the solute front in the aquifer solids within the column (cm/hr); ρ_b = soil bulk density (g/cm³).

Kinetics of Biotransformation

Assuming a first-order kinetic expression and equilibrium between the sorbed and solid phases, the following mass balance was used to describe TCE removal attributable to transformation by the Bachman Road Enrichment:

$$-\frac{dM_{TCEm}}{dt} = k' C_{liquid} V_{column} \theta = k' \left(\frac{M_{TCE}}{V_{liquid} + K_d M_{soil}} \right) V_{liquid} \quad (2.6)$$

$$\ln \left(\frac{M_{TCEm}^i}{M_{TCEm}^{i-1}} \right) = - \left(\frac{k' V_{column} \theta}{V_{column} \theta + K_d M_{soil}} \right) t \quad (2.7)$$

where k' = first order rate coefficient (day⁻¹); C_{liquid} = concentration of TCE in liquid-filled volume of the column (μg/L); M_{TCEm} = mass of TCE within column at (i - 1) and i (μg); M_{soil} = mass of soil in column (g); t = time interval between (i - 1) and i (days).

2.3 Large-Scale Column Experiments

In contrast to small-scale columns receiving semi-batch pore fluid exchanges, experimentation with large-scale sediment columns delivered continuous flow of groundwater is believed to be more conducive for both the development and examination of a BAZ and associated redox and TCE transformation gradients resulting from bioaugmentation. Therefore, laboratory-scale evaluations of spatial and temporal redox gradients and mobile- and stationary-phase microbial diversity during and subsequent to inoculation of Plume F/G aquifer materials with the BR Enrichment were accomplished using large-scale continuous flow column systems.

Large-scale continuous flow column systems were assembled using Plume F/G sediments and groundwater. The expanded scale of these columns enabled more detailed examination of the transport characteristics of the BR Enrichment and *D. ethenogenes* in site aquifer materials. Further, the larger columns facilitated observations and measurements of mobile and stationary phase contaminant, geochemical, and microbiological profiles both prior to inoculation and during the period leading up to and including steady-state treatment.

Other researchers have performed large-scale column studies in attempts to evaluate the spatial relationships between donor and acceptor consumption and the extent of reductive dechlorination. However, to date no single study has been designed to: 1) measure or characterize the successional adaptations of microbial communities in treated sediments following inoculation; and 2) spatially and temporally correlate the occurrence of near steady-state dechlorination with electron donor and redox gradients, and microbial community structure. Accordingly, for this study an attempt was made to

characterize the solid-phase microbial community within the Plume F/G aquifer solids prior to inoculation of the column systems with the BR Enrichment. The migration of the mobile-phase of the enrichment was then tracked during inoculation. Immediately following inoculation, the spatial distribution of both the mobile-and solid-phase community structure was examined over the length of the column. Post inoculation, the mobile-phase community structure was measured at temporally-discrete time intervals over the period leading up to and including the time at which near steady-state treatment activity was observed. To identify the primary zone(s) of dehalogenation activity, attempts were made to track the transport, fate and growth of *D. ethenogenes* during inoculation, treatment, and as part of the post-treatment characterization effort.

Two columns of a similar scale (designated Columns A and B) were used to complete the first two tasks associated with the experiment. Two columns were deemed necessary to provide spatial resolution and duplicate results of the transport behavior of the BR Enrichment in the Plume F/G sediment, and to facilitate examinations of solid-phase contamination, geochemistry and microbiology immediately following inoculation. This was accomplished by sacrificing Column B for soil sample extraction immediately following inoculation.

With the database produced from the large-scale column experiment, it was intended that the following information would be elucidated to fulfill Objective #s 4, 5, 6, 7, 8, and 9 outlined in Chapter 1:

- Baseline concentrations of VOCs (PCE, TCE, cis-DCE, trans-DCE, DCE, VC) and soluble Cr and Fe in soil and groundwater over the length of the column prior to bioaugmentation.
- Geochemical (redox) gradients in groundwater over the length of the column prior to bioaugmentation.
- The effective porosity (internal pore volume) of the aquifer solids within the two column systems.
- The TCE sorption characteristics of the aquifer solids within both large column systems.
- General structure of the indigenous microbial community within the soil and groundwater over the length of the column prior to bioaugmentation.
- The bulk transport behavior of the BR Enrichment and *D. ethenogenes* in the Plume F/G sediments over the length of the two large column systems.
- Spatial VOC and Cr(VI) mass removal in Column A following bioaugmentation.
- Spatial and temporal redox gradients across the Column A BAZ following bioaugmentation.

- Spatial and temporal shifts in microbial community structure in Column A following bioaugmentation based on suspended-phase biomass measurements.
- The solid-phase microbial community profile and localization of a discrete region(s) of dehalogenation activity within the BAZ after a near steady-state level of TCE dechlorination was established in Column A.

2.3.1 Column Design and Assembly

The two column systems used for this experiment were constructed of glass tubes (2.54 cm inside dia.) encased within glass jackets (5 cm inside dia.) and fit with length-specific sampling ports positioned on 13.97 cm to 15.24 cm centers. The MSU Glass Shop had manufactured these columns at the request of the Environmental Engineering Laboratory for previous applications. Column A measured 1.22 m in length; Column B was 1.83 m long. Schematics of assembled column systems are presented on Figure 2.2. In total, Column A had seven sampling ports. Column B, which was assembled from two, 0.91 m long segments compression (U-clamp) sealed together with a Teflon-lined butyl rubber O-ring, had 10 ports. Each sampling port was plugged with pre-sterilized silica wool and Teflon-faced butyl rubber septa secured in-place with aluminum crimps. The temperature of the contents within each column was controlled by circulating non-contact cooling water through the outer jackets at 15°C, using a combination refrigerated water bath/centrifugal pump (Market Forge Sterilmatic). Cooling water was conveyed to and from the columns with Tygon tubing.

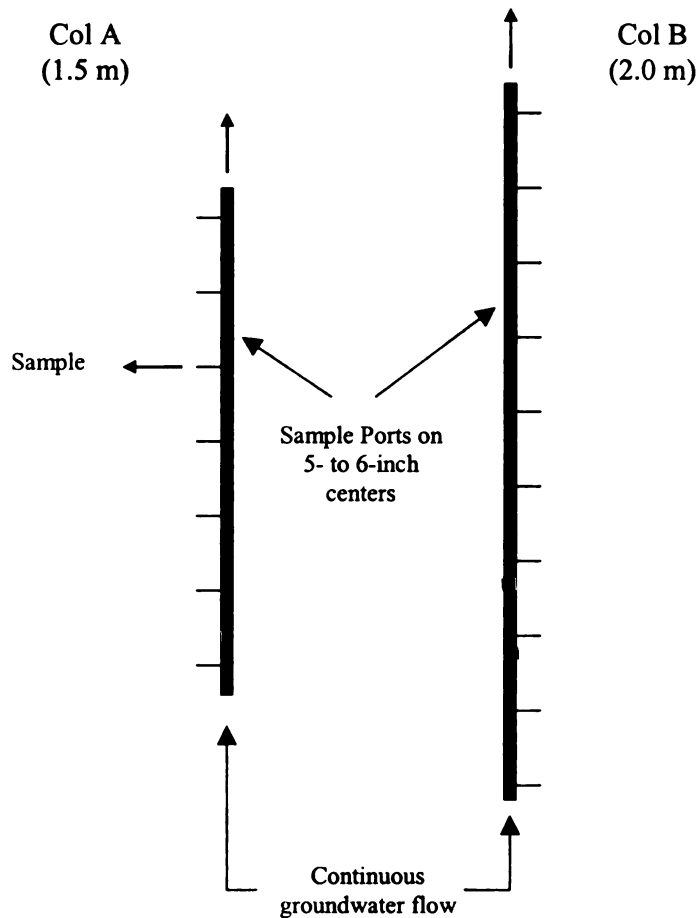


Figure 2.2. Large-Scale Column Design.

A butyl-rubber stopper (No. 4) was affixed to the base (influent) of each column. Two lines of stainless steel tubing (1/16- and 1/8-inch dia.) were inserted through each stopper to facilitate groundwater delivery into the base of the column systems. The stopper/influent tubing assemblies were secured in-place with U-clamps. A wad of sterilized fused-silica wool was placed over the stopper within each column. A layer

(approximately 2.54 m in thickness) of pre-sterilized, 3 mm diameter solid glass beads (Baxter Scientific Products G-6000-1) was placed over the silica wool. The combination of glass beads and silica wool prevented clogging the influent lines with fines from overlaying soil. The stainless steel influent plumbing extended from each column to an anaerobic glove box within which the groundwater and pumping equipment were maintained. The atmosphere of the glove box consisted of 95% N₂/5% H₂ and a temperature of 18 °C. The lengths of tubing extending from the glove box to each column were seamless, thereby preventing leakage of fluids out of or atmospheric oxygen into the lines.

Following assembly, each column system (including influent plumbing) was filled and sanitized with a solution of 200 mg/L sodium hypochlorite for a minimum of eight hours. The sterilization fluid was then drained by suction using pre-sterilized tubing (Tygon #3603) inserted through the top of each column. The columns and associated plumbing were then rinsed by batch cycling a minimum of three empty bed volumes of autoclaved, high purity de-ionized water (16 mega ohm/cm) through the vessels. After draining the final rinse solutions, non-sterile, anoxic Plume F/G groundwater was delivered to each column from gas-tight, glass syringes attached to the influent plumbing. Groundwater delivery was facilitated by Harvard syringe pumps. Syringe pumps and contents were stored within the anaerobic glove box under an atmosphere of approximately 95% N₂ and 5% H₂ and at a temperature of 18 °C. Anoxic conditions were maintained while filling the columns by pumping filtered N₂ through pre-sterilized Teflon tubing inserted through a No. 4 rubber stopper secured within the top of each

vessel. Once filled with groundwater, the columns were packed with Plume F/G aquifer materials.

The placement of Plume F/G soil into the two columns was performed under anoxic conditions by securing a disposable sterile glove bag (Aldrich AtmosBag) over the top of each and providing a continuous feed of N₂ to the bag headspace. Soil and pre-sterilized (autoclaved) tools and funnels were placed inside the glove bag. Soil was then placed into the columns in small lifts (approximately 5 to 8 centimeters deep) by spooning small quantities into the water column and allowing soil particles to settle. While each lift was settling, the column was lightly tapped with a plastic rod or touched with a mechanical vibrator to dynamically compact the soil.

Once filled with soil, the top (effluent) of each column system was sealed with a pre-sterilized butyl rubber stopper (No. 4). Inserted within each stopper were two stainless steel effluent lines. The entry point of one of the lines (1/8-inch inside dia.) was positioned approximately 5 cm above the top of the soil to permit the discharge of groundwater conveyed vertically upward through the column. The exit point of this line was set at an elevation approximately 0.5 m below the entry point to facilitate gravity drainage. The effluent plumbing was physically conformed into a trap to exclude entry of the external atmosphere into the columns during periods when the water level within the vessels dropped below the entry point of the line. The second line (1/16-inch outside dia.) permitted periodic withdrawal of headspace gasses. The entry point of this line was positioned immediately below the bottom of the rubber stopper at the top of each column. The exit was fit with two (2) polycarbonate, two-way valves with luer lock connections (Cole-Parmer) to either seal-off the line or facilitate attachment of 5 mL-capacity, gas-

tight, glass syringes (Popper & Sons, Inc.) for headspace sampling. Upon completion of assembly, Column A was wrapped with aluminum foil to prevent light penetration during the experiment.

2.3.2 Column Operation and Monitoring

The large-scale column experiment consisted of four (4) tasks, as outlined below. Column A was operated and monitored during each of these tasks. As introduced previously, Column B was utilized during Task 2.2 exclusively.

Task 2.1 - TCE Mass Loading and Sorption Capacity Evaluation

Task 2.1 included a series of batch pore fluid exchanges within Column A during which TCE-spiked Plume F/G groundwater was conveyed through the sediments to bring the solid and aqueous phase solute concentrations to near equilibrium levels. As with the small-scale columns, the exchanges entailed the rapid replacement of several pore volumes of groundwater (e.g., 600-800 mL), followed by a period of incubation. The incubation periods were 2-3 days in length to allow time for TCE to sorb to the aquifer solids. GC/MS measurements of TCE concentrations in column effluent samples were performed to verify that equilibrium conditions were approximated.

Task 2.1 also entailed effluent sampling and measurement of acetate, chloride, nitrite, nitrate, phosphorus and sulfate concentrations by ion chromatography to establish the background geochemical conditions within Column A prior to inoculation with the BR Enrichment.

Task 2.2 - Inoculation/Characterization of Flow-Through Properties

Task 2.2 entailed inoculation of Columns A and B with the BR Enrichment and characterizing the transport behavior of the inoculum relative to specific anions and a conservative tracer. This was accomplished by tracking the breakthrough of biomass, nitrate, phosphate, sulfate and NaBr along the length of Column B and within effluent samples of Column A during inoculation. This included measuring the port-specific (Column B) and effluent (Columns A and B) biomass by optical density, PCR-based molecular analyses, and ion chromatography of groundwater samples collected at specific volumetric throughput intervals. Once breakthrough was achieved, length-specific groundwater samples were obtained and processed for the biomass analyses identified above. Also during Task 2.2, Column B was used to obtain soil samples from each sampling port immediately upon the conclusion of inoculation. DNA extracted from the aqueous-phase biomass and soil samples was then analyzed by PCR-based T-RFLP and Real-Time (*CYBR Green*) PCR using primers specific to *D. ethenogenes*.

Task 2.3 - Treatment

Task 2.3 encompassed a four-month period during which maintenance of the inoculum and developing BAZ occurred and the extent of associated aqueous-phase geochemical and microbiological changes and dechlorination activity was monitored. Task 2.3 included two operational phases (A and B) corresponding with two separate inoculations of Column A with the BR Enrichment. A comprehensive summary of the operational history of Task 2.3 is summarized in Appendix A.

Throughout the duration of Task 2.3, groundwater was continuously conveyed through the Column A at a rate of 1.27 mL/hour. The resulting volumetric flux of groundwater corresponds to average linear velocity of groundwater flow at the Plume F/G site (e.g., approximately 15 cm/day). Table 2.2 summarizes the analyses conducted on samples collected daily to weekly from the length-specific ports and effluent headspace of Column A.

Task 2.4 - Post-Treatment Characterization

The fourth and final task of the large-scale column experiment consisted of solid-phase characterization of TCE mass and spatial microbial diversity. This was accomplished by retrieving soil samples from the length-specific sampling ports of Column A using 1 mL sterile syringes. TCE measurements were conducted by GC/MS. Microbial diversity was assayed by: PicoGreen[®] DNA Quantification to estimate biomass concentrations; PCR-based T-RFLP for examination of community structure; and RT-PCR for *D. ethenogenes* quantification.

Table 2.2. Summary of Aqueous Sample Analyses – Large-Scale Column Experiment.

Aqueous Solutes	Headspace Gasses	Biomass
Gas Chromatography/Mass Spectrometry (ion capture detection)	Reduced Gas Analysis Hydrogen	Optical Density
PCE	Gas Chromatography	PicoGreen® dsDNA
TCE	(flame ion detection)	Quantification
cis-DCE	Methane	Polymerase Chain Reaction
trans-DCE	Ethene	(PCR)-based Terminal
DCE		Restriction Fragment Length
VC		Polymorphism (T-RFLP)
Ion Chromatography		Real-Time (<i>SYBR Green</i>) PCR
Acetate		
Chloride		
Nitrite		
Bromide		
Nitrate		
Phosphate		
Sulfate		
High Performance Liquid Chromatography		
Acetate		
Lactate		
Propionate		
Atomic Absorption		
Hexavalent chromium		
Ferrous Iron		

Operational and Sampling Procedures

The operation of the large-scale column systems consisted of a series of batch pore-fluid “acclimation” exchanges followed by an initial inoculation event (Columns A and B). To facilitate post-inoculation soil sampling, Column B was then taken out of service at the conclusion of Task 2.2. For Column A, inoculation was followed by an “incubation” period during which TCE-spiked Plume F/G groundwater was continuously delivered through the system. Typically, the flow of groundwater through Column A was interrupted every seven days to permit the rapid delivery of substrate and nutrients to

feed the BAZ. After each feeding, a new “flow-through” incubation period was initiated. Approximately mid-way through the experiment, Column A was re-inoculated during one of the scheduled “feeding” events. The complete operational history of Column A is summarized in Appendix A. Operational Phase A included all of the initial inoculation and all of the incubation periods leading up to the second inoculation event. The second inoculation represented the start of Operational Phase B.

Inoculation and Feeding

Inoculation and feeding events entailed the rapid delivery (5-10 mL/min) of Plume F/G groundwater amended to contain 100-250 mg/L of DL-lactate, 3.1 mg/L of nitrogen (from urea), and 0.6 mg/L of phosphorus (phosphoric acid). For inoculation, dilute concentrations of the BR Enrichment (10% during Operational Phase A and 20% during Operational Phase B) were also mixed into the groundwater feedstock. The total volume of amended groundwater delivered during individual inoculation and feeding events are summarized in Appendix A. Inoculation of Column A required an exchange of approximately 700 mL of pore fluid. Column B received approximately 1,100 mL of nutrient- and enrichment-amended groundwater during inoculation. Typically, feeding events entailed delivery of 75-100 mL of nutrient amended groundwater.

During the initial inoculation, biomass sampling required extracting 2 mL of groundwater from select ports on Column B and from the effluent of both columns. One milliliter of each sample was extracted from the ports using 3 mL-capacity sterile plastic syringes and decanted into a 1 mL cuvette for immediate analysis of the optical density of the solution. The remaining 1 mL was transferred to a pre-sterilized, 1.5 mL Eppendorf

vial for centrifugation at 1,400 rpm for 20 minutes to concentrate the planktonic biomass. The processed biomass samples were then stored at -20°C pending DNA extraction and the prescribed molecular analyses. Coincident with the biomass sampling activities, 1 mL fractions of groundwater were collected and processed for ion chromatography analysis, as previously described.

Routine Groundwater Delivery and Pore Fluid Sampling

During the incubation periods that followed each inoculation and feeding event, TCE-spiked Plume F/G groundwater was continuously fed to Column A at a rate of 1.27 mL/hr. Before use in the columns, the Plume F/G groundwater from storage carboys was transferred to pre-sterilized 1 mL Wheaton bottles and stripped with 80% N_2 /20% CO_2 to remove residual TCE and adjust pH to within the range measured in Plume F/G groundwater (e.g., 7.0 – 7.2). The groundwater was then either amended for periodic feeding purposes or decanted directly into four (4) 100 mL-capacity, gas-tight, glass syringes. After elimination of the headspace within the syringes, each was loaded into a Harvard syringe pump. An additional gas-tight glass syringe (10 mL-capacity) containing pre-stripped site groundwater spiked with 500 mg/L of TCE was loaded onto an adjacent pump and connected to a 1/16-inch stainless steel feed line extending from a header system of tubing connected to groundwater feed syringes. A static in-line mixer positioned immediately downstream of this confluence received the combined discharge the spiked and non-spiked groundwater syringes. The in-line mixer consisted of a 15.24 cm long section of 1.25 cm diameter stainless steel tubing packed with 3-mm diameter glass beads. Stainless steel screen was placed at the ends of the tube to hold the glass

beads in place. The upstream end of the tube was fit with a stainless steel reducing union (1/4-inch to 1/16-inch). The downstream end of the tube was fit with a straight union (1/4-inch to 1/4-inch). As noted previously, 1/4-inch tubing extended from the mixer to the base of each column. The in-line mixer, internals and fittings were heat sterilized prior to being affixed to the groundwater conveyances.

Unless specified otherwise in Appendix A, the duration of each incubation period was seven days. Port-specific sampling of pore fluids occurred prior to and at the conclusion of each inoculation and feeding event. Sampling was also performed at the conclusion of each incubation period. During certain incubation periods, sampling was performed daily.

Sampling of the length-specific ports on the large-scale columns was accomplished using 2.5 mL-capacity, gas-tight, glass syringes (Hamilton) fit by luer connection with 5.08 cm long, 20-gauge, stainless steel, side-port needles (Hamilton). Prior to each use, sampling syringes were cleansed by triplicate flushing with methanol followed by triple rinsing with ultra-high purity de-ionized water. The cleansed syringes were then disinfected by heat drying at 60 °C for at least 24 hours.

During sampling, a sterile sampling syringe was inserted through the septa until the entire length of the needle penetrated to column. Groundwater was then retrieved in 2-2.5 mL volumes and decanted into pre-labeled sample containers. Depending on the objectives for a given sampling event, up to 6 mL of groundwater was extracted from a port in this manner. Samples for GC/MS analysis required 40 mL glass VOA (pre-preserved with 2% sodium sulfate) vials with Teflon-lined septa. Fatty acid samples for HPLC measurement were collected in 2 mL capacity, glass serum vials with Teflon-lined

butyl rubber septa and snap cap. For concentrating planktonic biomass, a 1.5 mL capacity Eppendorf centrifuge vial was filled. Similarly, sample portions used for analyses of anions and fatty acids required a separate Eppendorf vial. A third set of Eppendorf vials was needed for collecting samples for soluble metals.

Typically, column sampling entailed the extraction of 4 mL of pore fluid from select sample ports. 2 mL of sample was transferred to pre-preserved (3 mL of 2% sodium sulfate) 40 mL VOA vials; duplicate 1 mL fractions were dispensed into 1.5 mL Eppendorf vials. Following sampling, VOA vials were stored at 4°C pending analysis by GC/MS. The contents of one of the Eppendorf vials were filtered through 0.2 µm syringe filters and decanted into a secondary centrifuge vial pre-preserved with 20 µL of concentrated nitric acid. The nitric acid preserved samples were stored at 4°C pending analysis for soluble metals by atomic adsorption. The second Eppendorf vial was centrifuged at 1,400 rpm for 20 min to concentrate planktonic biomass for DNA extraction and the molecular procedures listed above. A 0.6 mL fraction of the supernatant of the centrifuged sample was decanted into 1-mL glass or polyethylene ion chromatography vial and stored at -20°C pending analysis for the major anions listed above. 0.1 mL of the remaining supernatant was then transferred to a 2 mL glass vial containing 0.9 mL of de-ionized water and sealed with a Teflon-lined butyl rubber septa secured with a plastic snap cap. These samples were stored at -20 °C pending analysis for volatile fatty acids by high performance liquid chromatography (HPLC).

During certain sampling events, an additional 1-2 mL of pore fluid were withdrawn from select sample ports for analysis of soluble gasses, including hydrogen, methane, ethene. These fractions were transferred to 2 mL glass vials and sealed with

Teflon-lined butyl rubber septa secured with aluminum crimp caps. The samples thus obtained were allowed to sit undisturbed for approximately 1 hour prior to withdrawal of 0.5-1 mL aliquots of the headspace for gas-phase analyses, as listed on Table 2.2.

Post-Treatment Soil Sampling

The length-specific sampling of soil in Columns A and B was accomplished by first removing the septa and wad of silica wool on each sample port using sterile utensils. Then, a 1-3 mL capacity, sterile, pre-trimmed, plastic syringe was inserted into each port to collect soil by coring. The plastic syringes were pre-trimmed using sterile cutting tools. Trimming entailed cutting the syringe shaft just below the tip to enlarge the cross-sectional area for soil coring. Once filled, the syringe contents were ejected with the plunger into a pre-preserved (3 mL of 2% sodium sulfate) 40 mL VOA vial and/or a 20 mL serum bottle. Typically, it was necessary to re-insert and fill the syringe in this manner 3-5 times to obtain a sufficient mass of soil to complete the requisite analyses. Upon retrieval of a sufficient quantity of soil to complete a sample, the partially filled containers were sealed with Teflon-lined septa and air-tight caps.

At the conclusion of Task 2.4, Column A was also opened within the anaerobic glove box to facilitate retrieval of depth-specific segments of the bulk soil under anoxic conditions. The bulk soil samples were obtained for analysis of solid-phase chromium and iron. These segments were transferred directly into 40-ounce jars, packed on ice and shipped to AAC Trinity, Inc. laboratory in Farmington Hills, Michigan. At AAC Trinity, the samples were analyzed for chromium and iron by USEPA Method 6020.

2.4 Soil and Groundwater

Soil for preparation of the small-scale column microcosms was composited from samples collected at several adjacent locations within the Plume F/G field-test plot southeast of Schoolcraft, Michigan (Figure 1.1). The soils were obtained from between 65 feet and 85 feet below grade, which corresponds to the depth interval at which the highest levels of TCE and cis-DCE have been consistently measured during MDEQ-sponsored investigations (HALLIBURTON NUS Environmental Corporation, 1991). Soil samples were collected with a saturated sand (piston core) sampler that had been cleansed by high-pressure steam washing prior to each use. Once retrieved from the subsurface the soil samples were immediately transferred to a field glove box and handled aseptically under an N₂-enriched atmosphere. Select portions of the samples were therein transferred to pre-sterilized aluminum cans. Sterilized can lids were fitted with two canulas, constructed of butyl rubber stoppers and 2.54 m, 20 gauge sterile needles, to facilitate headspace purging with N₂. The samples were then immediately transported to the MSU Engineering Research Laboratory where they were transferred to an anaerobic glove box for storage under an atmosphere of 95% N₂/5% H₂ and a temperature of 18 °C prior to use for column assembly.

Soil used for the large-scale column studies was composited from sediments collected at the Plume F/G test site (core SB-5-15) in January of 2002 from a depth interval of 65 to 66 feet below grade. The sediment sample was obtained by roto-sonic drilling methodologies, which promoted aseptic and anoxic acquisition of soil cores. A segment of the core from the aforementioned depth-interval was isolated and sealed within the rubber sleeve from the roto sonic core. The soil sample was then placed in a

pre-sterilized aluminum paint can within a field glove box fed 100% N₂. The lid of the can was pre-fit with a canula to allow the headspace to be purged with N₂ after being firmly secured in place. The can and contents were then transported on the day of acquisition to the MSU lab and placed into the anaerobic glove box for storage pending column preparation.

Before insertion into either the small- or large-scale columns, source soils were first processed by sieving to remove coarse sands and gravel particles. Soil was passed through No. 8 and No. 5 sieves (Entecotts, Ltd and Bort Longyear), which had been heat-sterilized prior to use. The well sorted, passing soil fraction was then used for column preparation.

Groundwater from the Plume F/G test plot was used for both the small- and large-scale column experiments. Groundwater was routinely obtained from MSU-001, a 5.08 cm diameter well positioned at the southern edge of the test plot. This well is constructed of a 1.52 m long stainless steel well screen (0.025 cm wire-wound slot) attached to a galvanized steel riser. The screen of MSU-001 is positioned from 60 feet to 65 feet below grade (18.3 m to 19.8 m b.g.). Groundwater was retrieved from MSU-001 by extraction with a dedicated electrical submersible pump and conveyed to the ground surface through polyethylene tubing. Typically, groundwater was extracted at a rate of approximately 4 liters per minute. During sampling, groundwater was initially discharged to waste for a period of approximately one (1) hour, or until a volume of water was withdrawn approximately equivalent to the sum of three static water columns within the well. The pH and oxidation-reduction potential of the extracted groundwater were measured using Cole-Parmer DigiSense portable electrodes to evaluate the

stabilization of these general geochemical parameters during this period. Following the wasting period, the extracted groundwater was conveyed into pre-sterilized 20 L Nalgene carboys. The headspace within a sample carboy was maintained anoxic by bubbling N₂ through the water accumulating within the vessel. Once filled, the carboy(s) were capped and transferred to a large, heavy-duty garbage bag. The bag was then purged and filled with 100% N₂ and sealed to prevent leakage. The packaged carboys were then immediately transferred to the MSU laboratory. At the lab, the carboys were either directly transferred to a storage box in which the headspace atmosphere was maintained anoxic with continuous throughout of N₂, or their contents were transferred to pre-sterilized, 1 L Wheaton bottles. Groundwater contained in either carboys or Wheaton bottles was stored anoxically and at 18 °C pending use. Groundwater storage never exceeded one month in duration before use or disposal.

2.5 Analytical Procedures

2.5.1 Aqueous Solutes

Identification and quantification of PCE, TCE, cis-DCE, trans-DCE, 1,1-DCE, and VC in soil and groundwater samples was accomplished using a gas chromatograph/mass spectrometer (GC/MS) equipped with an Agilent 5973 Mass Selective Detector and combined with purge-and-trap system (Tekmar/Dohrmann Precept II and Tekmar/Dohrmann 3100 Sample Concentrator). Compound identification was subsequently confirmed by routine injection of pure material into the GC while analyzing sample degradation. GC calibration was performed by measuring the instrument response to known masses of PCE, TCE, cis-DCE, trans-DCE, 1,1-DCE, and VC added directly to a

40-mL VOA bottle containing 2 mL chilled distilled de-ionized water and 3 mL of 2% sodium bisulfate.

Anions were assayed by ion chromatography using suppressed conductivity detection within a Dionex model 2000i/SP ion chromatograph fitted with a Dionex AS4A IonPac column and utilizing a 1.8 mM biocarbonate-1.7 mM carbonate mobile phase conveyed at 8 mL/min. Chromatograms were recorded and data integrated using Turbochrom R 4 software (Perkin Elmer, Incorporation). Five-point calibration curves were prepared by diluting primary anion standards into secondary de-ionized water standards. A sample volume of 600 μ L was used to accomplish the ion chromatography analyses during both experiments.

Acetic, lactic and propionic acids were measured by high performance liquid chromatography using a Supelco Discovery C8 column (No. 59354-U; 25 cm, 4.6 mm, 5 μ m). Eluent consisted of 3.4 g/L of KH_2PO_4 . Acetonitrile was used to regenerate the column between samples. Compound detection was accomplished through measurement of sample response to ionization with 210 nm of ultraviolet radiation at rate of 0.6 mL/min. Turbochrom R 4 software (Perkin Elmer, Incorporation) was used for chromatographic integration and data processing.

Soluble metals samples (1 mL pre-preserved with 20 μ L of concentrated nitric acid) were analyzed by atomic absorption with a Perkin-Elmer Analyst 800 Atomic Absorption Spectrometer operated with AA WinLab Analyst Software (version 4.1 SP1). Samples were atomized electrothermally through a five step temperature ramp. Radiation sources emitted wavelengths of 357.9 nm and 248.3 nm for detection of soluble chromium and iron, respectively. Quantitation of sample constituents was done through comparison with

standard curves developed from the analyses of standards containing pre-measured solute concentrations. For each set of analysis, a five point calibration curve was established for soluble chromium quantification; for soluble iron samples, a four point calibration curve was used.

2.5.2 Headspace Gasses

Hydrogen levels within the headspace of small-scale columns or in low-volume samples taken from sample ports on Column A were measured using a RGA3 reduction gas analyzer (Trace Analytical, Menlo Park, CA) operated with Turbochrom R4 software and data integration. Headspace samples were injected onto a 1% SP-1000 Carbopack B column through an automated switching valve timed at 2 minutes. Standards were prepared by adding measured volumes of 100 ppmv hydrogen to serum bottles purged with N₂ and crimp capped with butyl rubber septa.

Headspace samples for methane and ethene quantification were measured by gas chromatographic (GC) analysis. 0.5-mL of headspace sample was injected into a Perkin Elmer Autosystem GC equipped with a flame-ionization detector and a 3.2-mm x 2.44-m stainless-steel column packed with 1% SP-1000 on 60/80 Carbopack-B (Supelco, Inc.) maintained isothermally at 50 °C. Compound identification and quantification was accomplished by matching the retention times of authentic material with those of peaks from the headspace samples.

2.5.3 Biomass and DNA

Optical density measurements were performed on 1 mL samples using a Shimadzu UV-160 spectrophotometer measuring the transmittance of energy at a wavelength of 660 nm.

Extraction of total community DNA from cell pellets of planktonic biomass samples was initiated by re-suspending and digesting pellets in a 0.5 mL solution of sucrose-NaCl-Tris (0.05 M Tris-Cl [pH 8], 0.1 M NaCl, and 25% sucrose) and lysozyme (Sigma-Aldrich) (5 mg/mL). Sample digestion occurred under gentle agitation at 30°C for 10 minutes. To facilitate decomposition of extraneous cell components, approximately 0.2 g of sterile zirconium beads (Biospec #1007901Z) were added to each sample. Samples were then shaken at 5,000 rpm for 90 seconds using a Biospec Mini Beadbeater. To decompose fatty acids and other non-genomic macromolecules and improve DNA solubility, 250 μ L of a solution of 0.5 M Tris, 0.05 M EDTA, 1% SDS and 6% Tris-Phenol was then mixed with each sample. Samples were then chilled at 0°C for 20 minutes. Sample contents were mixed with 750 μ L of phenol chloroform isoamyl alcohol (25:24:1) and centrifuged at 1,000 rpm for 10 minutes. After centrifugation, the supernatant was removed aseptically from each sample and replaced with 750 μ L of chloroform isoamyl alcohol (24:1). Samples were again centrifuged at 1,000 rpm for 10 minutes. Approximately 450 μ L of the supernatant of each sample was transferred to a sterile 1.5 mL vial. 45 μ L of 3 M sodium acetate and approximately 1 mL of 96% ethyl alcohol was then added to each sample to precipitate DNA overnight while stored at -20°C. Precipitated samples were centrifuged at 14,000 rpm for 30 minutes. Supernatant was then removed and the pellet washed with 1 mL of chilled 70% ethyl alcohol.

Washed samples were centrifuged again at 14,000 rpm for 10 minutes. Supernatant was then removed and the samples were air dried in a fume hood at room temperature for approximately 2 hours. Dried samples were then stored at -20°C pending analyses.

Extraction of total community genomic DNA from soil samples was accomplished using Ultra-Clean Soil DNA Kits from MoBio Systems, Inc. (Solana Beach, CA) following manufacturer's guidelines.

DNA quantification was accomplished using PicoGreen[®] dsDNA Quantification Kits and reagents (Molecular Probes, Inc., Eugene, OR, Cat. # P-7589). PicoGreen[®] quantification reagent is an ultra sensitive fluorescent nucleic acid stain for quantitating double-stranded DNA (dsDNA) in solutions. Quantification assays were performed using 5-10% dilutions of sample extracts re-suspended in 50 μL of 10 mM TRIS-HCl, [pH 7.5], following manufacturers guidelines. Sample quantitations were accomplished using an MFX Microtiter Plate Fluorometer and integration software.

Verification of the presence of methanogens and sulfate-reducing bacteria (SRBs) in biomass samples was accomplished by PCR amplification and electrophoretic separation of 16S rRNA genes, and comparison to positive and negative controls. Primers selected for these analyses included those specific to archaea and SRBs. A non-specific eubacterial primer set was also used. For PCR, 1 μL of each primer solution was added to each re-suspended sample (containing 10-100 ng of DNA), along with 5 μL of 10X PCR buffer (500 mM Tris [pH 8.3], 20 mM MgCl_2 , 5-10% ficoll, 10 mM Tartrazine), 5 μL of 10X bovine serum albumin (2.5 mg/mL), 5 μL of 10X dNTP (2 mM each), 2 Units of *Taq* DNA polymerase, and sterile deionized water to create a 50 μL sample.

Archean-specific PCR primers included 340f (5'-CCT ACG GGG CGC A(C/G)CA GG(C/G) GC-3') and 915r (5'-GTG CTC CCC CGC CAA TTC CT-3'). Reaction conditions included a denaturing cycle of 3 minutes at 94 °C, followed by 30 cycles, each consisting of 30 seconds of denaturing at 94 °C, annealing for 45 seconds at 60 °C, and extension for 2 minutes and 10 seconds at 72 °C. Final elongation occurred at 72 °C for 6 minutes. Primers for amplification of a 1.9-kb DNA fragment encoding most of the alpha and beta subunits of the dissimilatory reductase (DSR) from all recognized lineages of sulfate-reducing bacteria (SRB) included DSR1F (5'-AC(C/G)CACTGGAAGCACG-3') and DSR4R (5'-GTGTAGCAGTTACCGCA-3') (Baolin, pers. commun.). Reaction conditions entailed denaturing for 15 seconds at 94 °C, 30 denaturation/annealing cycles (94 °C for 15 seconds; 30 seconds at 54 °C; and 1 minute at 72 °C). Final elongation occurred for 1 minute at 72 °C. Eubacterial-specific primers included fD1 (5'-AGA GTT TGA TCC TGG CTC AG-3') and RD1 (5'-AAG GAG GTG ATC CAG CC-3'). Reaction conditions entailed denaturing at 94 °C for 3 minutes followed by 30 cycles of denaturing at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 2 minutes and 10 seconds. Final elongation was at 72 °C for 7 minutes. Negative controls containing no added DNA, as well as positive controls containing pure culture genomic DNA, were included alongside reactions. Aliquots (10 µL) of PCR products were separated by electrophoresis in a 1.5% (wt/vol) agarose gel in 1X TAE buffer. The gel was stained with ethidium bromide (0.5 µg/mL) and visualized by ultraviolet excitation (Helton, 2001).

T-RFLP analysis was performed as previously described by Liu, et al. (1997) and Flynn et al. (2000). PCR was performed with bacterial domain specific small subunit

(SSU) ribosomal ribonucleic acid (rRNA) primers 8-27 forward labeled with hexachlorofluorescence (Hex) at the 5'-end (synthesized by Operon Technologies, Inc., Alameda, CA) and 1510-1492 reverse each having the sequences AGAGTTGATCMTGGCTCAG and RGYTACCTTGTTACGACTT, respectively. In the reverse primer the degeneration positions R and Y employed the pyrimidine and purine derivatives dK and dP, respectively (Glen Research, Sterling, VA). The SSU rRNA genes were amplified using approximately 40 ng of DNA and a Perkin-Elmer 9600 thermocycler (Perkin-Elmer, Norwalk, CT). The PCR reactions were performed under the following conditions: 3 minutes at 95 °C, 1 minute at 55 °C, and 3 minutes at 72 °C, followed by a 7 minute extension period at 72 °C. The resulting PCR products were purified using the Wizard PCR purification kit (Promega, Madison, WI) and digested overnight at 37 °C with 20 units of *HhaI*, *MspI*, or *RsaI* separately (Life Technologies, Gibco BRL, Gaithersburg, MD). The digest fragments were then resolved on an ABI 373A sequencer running in the gene scan mode with 6% urea-containing polyacrylamide gels (PEApplied Biosystems, Foster City, CA). Size calibration was performed with the Tamara 2500 standard (PEApplied Biosystems). The resulting gel patterns were analyzed using the Genescan software, version 2.1 (PEApplied Biosystems).

Real-time PCR procedures employed for this study followed those outlined by Grüntzig, et al. (2001). The reaction mixture for real-time PCR consisted of 1X *TaqMan* Universal Master Mix (containing AmpliTaq Gold DNA polymerase, AmpErase uracil-N-glycosylase, which degrades PCR carryover products from previous reactions, deoxynucleoside triphosphates with dUTP, a passive reference [6carboxy-X-rhodamine], and optimized buffer components) (PE Applied Biosystems), 300 nM forward primer,

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900 nM reverse primer, and 525 nM fluorescent probe. MicroAmp optical caps and tubes were used for the final reactions. PCR conditions were as follows: 2 minutes at 50 °C, 10 minutes at 95 °C, then 40 cycles of 15 seconds at 95 °C and 1 minute at 60 °C. Negative controls with no template DNA or no probe were run in each reaction.

The increase in fluorescence emission, due to the degradation of the probe by the DNA polymerase in each elongation step, was monitored during PCR amplification using the 7700 Sequence Detector (PE Applied Biosystems). The fluorescence signal was normalized by dividing the emission of the reported dye (6-carboxyfluorescein) by the emission of the passive reference dye, 6-carboxy-X-rhodamine. The parameter CT (threshold cycle) is the fractional cycle number at which the fluorescence emission crosses an arbitrarily defined threshold within the logarithmic increase phase (0.1 in our reactions). The higher the amount of initial template DNA, the earlier the fluorescence will cross the threshold and the smaller will be CT. The CT values obtained for each sample were compared with a standard curve to determine the initial copy number of the target gene.

PicoGreen[®], PCR, T-RFLP and RT-PCR procedures were completed by Leslie Dybas. Technical assistance was provided by Baolin Sun.

2.6 Measurement of TCE Transformation Kinetics

Batch microcosm experiments were performed by The Retec Group, Inc. (Retec) of Lansing, Michigan to estimate rates of PCE transformation associated with the BR Enrichment. A summary of the Retec experimental media, protocol, analytical results, and data reduction is provided in Appendix B.

Seven batch microcosms (4 test and 3 control) were prepared to facilitate the experiment. Microcosms were prepared using 250 mL glass serum bottles partially filled with 160 mL of either BR Enrichment (test bottles) or de-ionized water (controls). Each bottle also received: 2 mL of a mineral salt solution (50 g/L NaCl, 25 g/L $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 10 g/L KH_2PO_4 , 15 g/L NH_4Cl , 15 g/L KCl, and 0.75 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$); 0.2 mL of a trace metal solution (10 mg/L HCl, 1,500 mg/L $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 190 mg/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 100 mg/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 70 mg/L ZnCl_2 , 6 mg/L H_3BO_3 , 36 mg/L $\text{Na}_2\text{MnO}_4 \cdot 2\text{H}_2\text{O}$, 24 mg/L $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, and 2 mg/L $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$); 0.2 mL of sodium sulfide (48 g/L stock) to maintain an ORP favorable for dechlorination; 0.2 mL of a 0.1% resazurin solution; 2 mL of phosphate buffer (80% H_3PO_4); 1 mL of 60% DL-lactic acid; and 0.4 μL of neat PCE. The total solution volume within each microcosm at the start of the experiment was 165.6 mL. Microcosms were sealed with Teflon-lined butyl rubber septa secured with aluminum crimp caps. Following preparation, each microcosm was covered in foil to prevent light penetration and stored on an orbital shaker at room temperature pending sampling. Microcosm preparation and sampling was performed aseptically and under anoxic conditions.

Each microcosm was sampled once prior to the addition of PCE, and at approximately 0.5 to 1 day intervals thereafter over a period of approximately 197 hours, or eight days. In total, 13 samples were obtained from each control and test microcosm following the addition of PCE. Dechlorination was measured by following PCE, TCE, cis-, trans- and 1,1-DCE and VC concentrations over the duration of the experiment. Ethene, methane and total suspended solids concentrations were also tracked. Sampling and analyses were performed by Retec.

2.7 Fate and Transport Modeling

A computer model was developed from a system of mass balance equations to simulate TCE movement and transformation during the operational history of Column A. Specifically, one-dimensional, numerical modeling techniques were used to simulate the fate and transport of TCE, dissolve hydrogen, and attached and planktonic *D.*

ethenogenes. The analytical data from the spatial and temporal sampling of the column and empirical TCE transformation kinetic data were used to calibrate the model. The calibrated model was then used to predict and optimize treatment performance within the BAZ under a variety of feeding scenarios. The objectives for the numerical modeling were to verify the transport characteristics of TCE and the BR Enrichment within the Plume F/G aquifer materials, and to simulate a range of substrate and nutrient delivery strategies that could be used to optimize TCE treatment in the Column A BAZ.

A series of mass balance equations describe the changes in solute concentrations associated with TCE transformation by *D. ethenogenes*. The equations presented below were developed from those previously described by Witt et al. (1995), Clement et al. (2000) and Phanikumar et al. (2002). Hydrogen serves as an electron donor in these equations. TCE, cis-DCE, and VC serve as electron acceptors. Modifications by the author for this study included the inclusion of rate-limited (de)sorption to describe the fate of cis-DCE, VC, and ethene.

For this study, it was assumed that TCE is transformed by both attached and planktonic cells and that transformation is first order with respect to TCE concentration and first order with respect to the concentration of *D. ethenogenes* biomass. Further, it

was assumed that partial equilibrium exists between pore fluid and solid-phase TCE concentrations, with desorption limitations on the remaining mass of sorbed TCE.

The following processes were mathematically described and incorporated into the numerical model for Column A: (1) advective transport of tracer, TCE (electron acceptor) and its transformation products and dissolved hydrogen (electron donor), as influenced by hydrodynamic dispersion and sorption; (2) sorption and desorption of TCE, cis-DCE and VC; (3) the biochemical response of *D. ethenogenes* as indicated by its rates of substrate utilization, TCE transformation, growth and decay; and (4) attachment and detachment of *D. ethenogenes*.

Transport of a non-reactive tracer such as bromide is described by:

$$\frac{\partial C_{Br}}{\partial t} = D \frac{\partial^2 C_{Br}}{\partial x^2} - U \frac{\partial C_{Br}}{\partial x}. \quad (2.8)$$

The fate and transport of chlorinated ethene species mediated by dechlorination reactions such as halorespiration may be represented by a set of coupled mathematical expressions. Assuming second-order biodegradation kinetics, transport and transformation of TCE, cis-DCE, VC and ethene, respectively, can be simulated by solving the following set of partial differential equations (Clement et al., 2000, Phanikumar, et al. 2002):

$$\begin{aligned} \left(1 + \frac{\rho_b f K_d^{TCE}}{\theta}\right) \frac{\partial C_{TCE}}{\partial t} = D \frac{\partial^2 C_{TCE}}{\partial x^2} - U \frac{\partial C_{TCE}}{\partial x} - k'_{TCE} C_{TCE} (X_m + X_{im}) \\ - \frac{\rho_b \kappa_{TCE}}{\theta} [(1-f) K_d^{TCE} C_{TCE} - S_{TCE}] - \frac{\mu}{Y_{TCE-X}} (X_m + X_{im}) \end{aligned} \quad (2.9)$$

$$\begin{aligned}
\left(1 + \frac{\rho_b f K_d^{DCE}}{\theta}\right) \frac{\partial C_{DCE}}{\partial t} = D \frac{\partial^2 C_{DCE}}{\partial x^2} - U \frac{\partial C_{DCE}}{\partial x} + Y_{TCE-DCE} k'_{TCE} C_{TCE} (X_m + X_{im}) \\
- Y_{DCE-VC} k'_{DCE} C_{DCE} (X_m + X_{im}) - \frac{\rho_b \kappa_{DCE}}{\theta} [(1-f) K_d^{DCE} C_{DCE} - S_{DCE}] \quad (2.10)
\end{aligned}$$

$$\begin{aligned}
\left(1 + \frac{\rho_b f K_d^{VC}}{\theta}\right) \frac{\partial C_{VC}}{\partial t} = D \frac{\partial^2 C_{VC}}{\partial x^2} - U \frac{\partial C_{VC}}{\partial x} + Y_{DCE-VC} k'_{DCE} C_{DCE} (X_m + X_{im}) \\
- Y_{VC-ETH} k'_{VC} C_{VC} (X_m + X_{im}) - \frac{\rho_b \kappa_{VC}}{\theta} [(1-f) K_d^{VC} C_{VC} - S_{VC}] \quad (2.11)
\end{aligned}$$

$$\begin{aligned}
\left(1 + \frac{\rho_b f K_d^{ETH}}{\theta}\right) \frac{\partial C_{ETH}}{\partial t} = D \frac{\partial^2 C_{ETH}}{\partial x^2} - U \frac{\partial C_{ETH}}{\partial x} + Y_{VC-ETH} k'_{VC} C_{VC} (X_m + X_{im}) \\
- \frac{\rho_b \kappa_{ETH}}{\theta} [(1-f) K_d^{ETH} C_{ETH} - S_{ETH}] \quad (2.12)
\end{aligned}$$

Sorbed-phase TCE is characterized by

$$\frac{\partial S_{TCE}}{\partial t} = \kappa_{TCE} [(1-f) K_d^{TCE} C_{TCE} - S_{TCE}] \quad (2.13)$$

The fate and transport of dissolved hydrogen is described as

$$R_{H_2} \frac{\partial C_{H_2}}{\partial t} = D \frac{\partial^2 C_{H_2}}{\partial x^2} - U \frac{\partial C_{H_2}}{\partial x} - \frac{\mu}{y_{H_2} X} (X_m + X_{im}). \quad (2.14)$$

When microbial growth is limited by a single substrate, mobile microbial production may be described by (Phanikumar, et al, 2002):

$$\frac{\partial X}{\partial t} = (\mu - b - K_{at})X. \quad (2.15)$$

If the rate of growth of *D. ethenogenes* is limited by a single substrate (either hydrogen or TCE), it can be estimated using a Monod kinetic expression. For hydrogen, the expression is

$$\frac{\partial C_{H_2}}{\partial t} = -\frac{\mu_{\max}}{y_{H_2}X} \left(\frac{C_{H_2}}{C_{H_2} + K_{H_2}} \right) X = -\frac{\mu_{\max}}{y_{H_2}X} M_{H_2} X. \quad (2.16)$$

When multiple limiting solutes are present (hydrogen and TCE), the growth rate of *D. ethenogenes*, μ , can be expressed by the following interactive relation (Phanikumar et al. 2002):

$$\mu = \mu_{\max} M_{H_2} M_{TCE} = \mu_{\max} \left(\frac{C_{H_2}}{C_{H_2} + K_{H_2}} \right) \left(\frac{C_{TCE}}{C_{TCE} + K_{TCE}} \right). \quad (2.17)$$

Mobile- and immobile-phase *D. ethenogenes* concentrations may be described by

$$\frac{\partial X_m}{\partial t} = D \frac{\partial^2 X_m}{\partial x^2} - U \frac{\partial X_m}{\partial x} + [\mu - b_{DC}(1 - M_{H_2}) - K_{at}]X_m + K_{de}(1 - M_{H_2})X_{im}, \quad (2.18)$$

and

$$\frac{\partial X_{im}}{\partial t} = [\mu - b_{DC}(1 - M_{H_2}) - K_{de}(1 - M_{H_2})] X_{im} + K_{at} X_m, \quad (2.19)$$

respectively.

In the above equations, C_{Br} , C_{TCE} , C_{DCE} , C_{VC} , C_{ETH} , and C_{H_2} are aqueous concentrations of bromide, TCE, cis-DCE, VC, ethene and hydrogen, respectively; X_{im} and X_m are the immobile and planktonic concentrations of *D. ethenogenes*, respectively; and S_{TCE} is the concentration of sorbed TCE. The coefficients κ_i and f define the kinetic (de)sorption rate (day^{-1}) and fraction of equilibrium sorption sites, respectively. Y_i and y_i are stoichiometric and anaerobic halo-respiration reaction yield coefficients, respectively. Second-order biotransformation reaction rates are defined by k'_i . The coefficient of hydrodynamic dispersion is defined here as $D = \alpha U$, where U is the average linear velocity (cm/day) of groundwater flow through Column A and α is the dispersivity (m) of the aquifer solids within the column. M_i is the Monod-type saturation kinetics term.

The MODFLOW (McDonald and Harbaugh, 1988) and RT3D (Clement et al. 1998) computer codes were used to establish the hydraulic head distribution within Column A and solve the equations describing bioreactive transport, respectively.

Chapter 3

RESULTS AND DISCUSSION

3.1 Characterization of Aquifer Materials

Table 3.1 summarizes select physical and geochemical characteristics of the Plume F/G-impacted soil and groundwater, as determined from analyses performed during this study and previous investigations sponsored by the MDEQ (Mayotte, et al., 1996). These data reveal that the site aquifer materials support a significant microflora (measured in numbers of cell forming units per gram of dry soil or mL of groundwater). In fact, it is probable that the cis-DCE and VC detected in groundwater samples from the Plume F/G test grid have originated from biotransformation of TCE by indigenous microorganisms, since alternative sources of these compounds are unknown and unlikely.

TCE concentrations in Plume F/G groundwater collected from site monitoring well, MSU-1, for both the small- and large-scale column experiments were consistently within the range of 300 µg/L to 500 µg/L.

3.2 Flow-Through Properties

The flow-through properties of the small-scale columns are presented in Table 4.1. A composite profile for bromide transport through Columns A through F is presented on Figure 3.1. As expected, the flow-through characteristics for each of the small-scale columns are similar. In general, the effective porosities of the re-packed aquifer solids within the columns were estimated to range from 25% to 39%, indicating internal pore volumes of 14-22 mL. The average linear velocities, v_{eff} , of groundwater flow during each exchange were determined from the porosity data and the superficial

velocity, v (0.4 cm/min), to range from 1.29-1.74 cm/min. The Reynolds number ($R_e = vd/\nu$) was 0.067, assuming an average soil grain diameter, d , of 0.1 cm, and kinematic viscosity, ν , of 36 cm²/hr at 20°C (Siegrist and McCarty, 1987). A value of R_e less than unity indicates that inertial forces dominate. Therefore, flow was essentially laminar during the exchanges (Freeze and Cherry, 1979).

Table 3.1. Characteristics of Schoolcraft Aquifer Materials.

Parameter	Media	
	Soil	Groundwater
pH	8.8 ¹	7.04-7.5
Alkalinity	-	331 mg/L ¹
Hardness	-	410 mg/L ¹
Fraction of organic carbon	0.1% ¹	-
Iron	37 mg/Kg ¹	11 mg/L
Copper	-	0.03 mg/L ¹
Nitrate	<1 mg/Kg ¹	39 mg/L
Phosphate	-	<1 mg/L
Sulfate	-	25 mg/L
TCE		300 – 500 µg/L
Microbial population (anaerobic)	2.3 x 10 ⁷ CFU/g ¹	1 x 10 ⁶ CFU/mL ¹

¹Brown & Root Environmental
CFU – colony forming unit.

The movement of groundwater through each column deviated slightly from ideal plug flow. The composite breakthrough profile (Figure 3.1) was used to evaluate the dispersion characteristics of the aquifer solids within the columns. The first 12 mL of pore fluids was essentially free of bromide, and a total throughput of 30-32 mL of bromide-spiked groundwater was necessary to achieve complete breakthrough, or saturation. The approximate spreading of the breakthrough front (as defined by the

dimensionless quantity $D_{dis}/v_{eff}L$, where L is the column length) was 0.01, giving a dispersion coefficient, D_{dis} , of approximately $10 \text{ cm}^2/\text{hr}$.

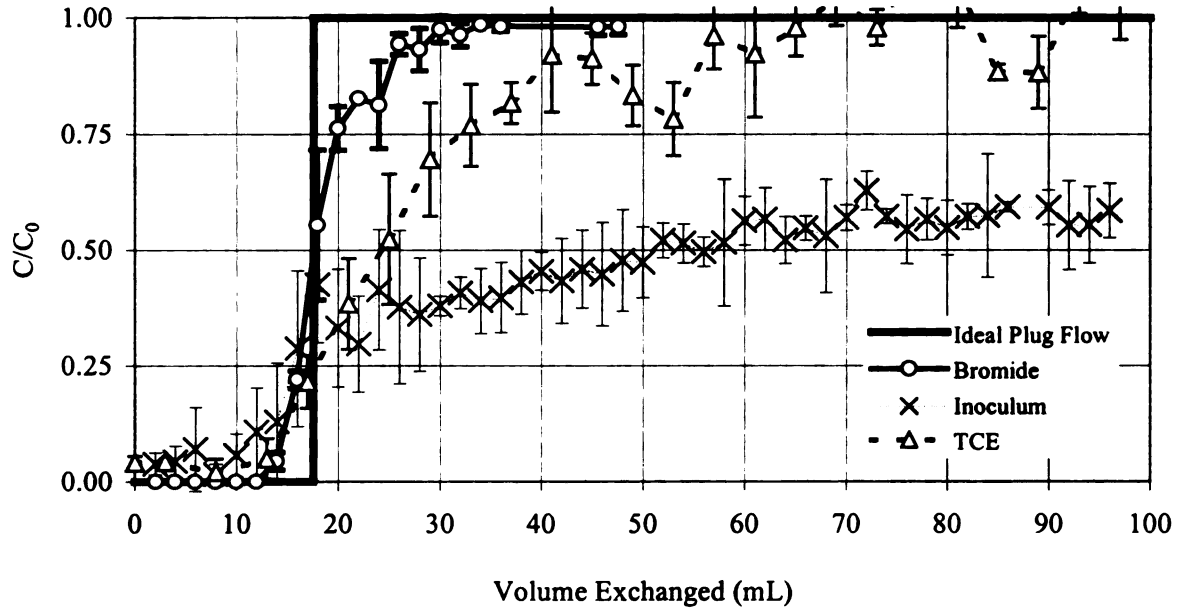


Figure 3.1. Composite breakthrough profiles of bromide, TCE and inoculum through small-scale columns.

Figures 3.2 and 3.3 present bromide breakthrough profiles for large-scale Column A and select length-specific sampling ports of Column B, respectively. In general, these profiles indicate internal pore volumes of 220 mL (Column A) and 280 mL (Column B). The effective porosity, θ , for both columns was calculated to be approximately 39%. The results from numerical modeling simulations performed to simulate the transport of a non-sorptive, non-reactive solute (e.g., bromide) are presented on Figures 3.2 and 3.3. These data reveal that the re-packed Plume F/G aquifer solids within both columns are characterized by a longitudinal dispersivity, α_L , of 0.01 m (1 cm).

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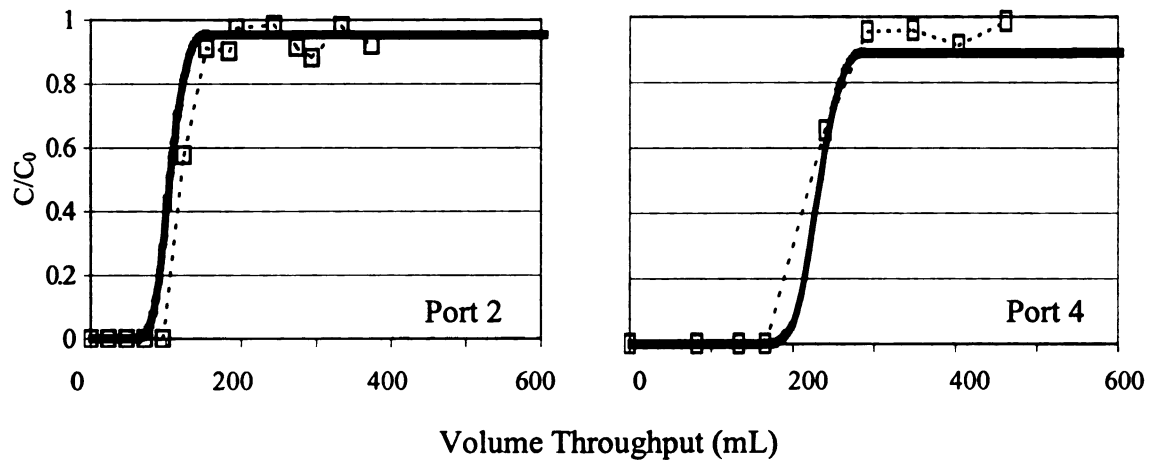


Figure 3.2. Bromide breakthrough profiles at sample Ports 2 and 4 of large-scale Column B. Dashed line with open boxes represent bromide data from each port. Solid line represents numerical simulation.

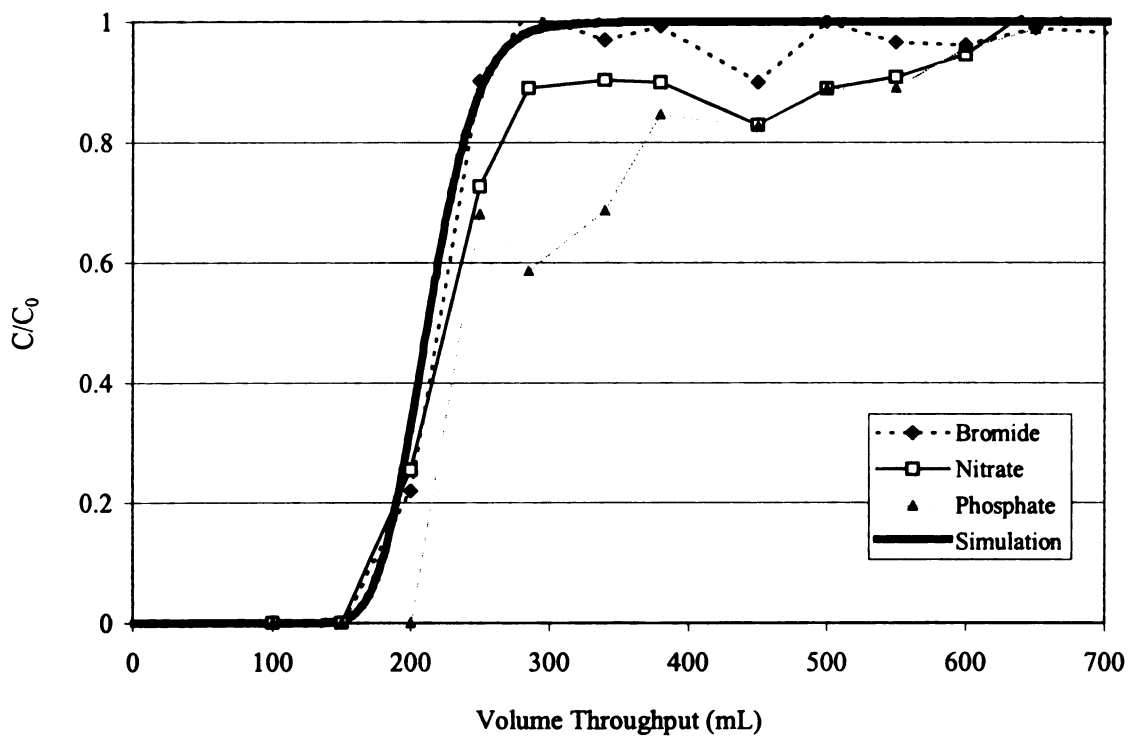


Figure 3.3. Bromide and major anion breakthrough profiles for large scale Column A.

3.3 Sorption Characteristics

Composite breakthrough profiles were developed for TCE and bromide transport through small columns A, B, and C (Figure 3.1). As illustrated on this figure, TCE transport was retarded in comparison to bromide. TCE concentrations were first observed in these columns after approximately 14 mL of pore fluids had been exchanged. TCE breakthrough was complete during these exchange events after throughput of 60-65 mL of spiked groundwater. Therefore, for small column mass balances, TCE concentrations measured in up to three 2 mL samples obtained from the initial 14 mL and the final 30 mL of fluid emerging from each column (e.g., 70-100 mL throughput) during the pore fluid exchanges were averaged to determine C_1 and C_2 , respectively. The profile of TCE breakthrough composited from the Column A, B, and C data is typical of flow through a closed vessel (Levenspiel, 1979) with a dispersion coefficient, D_{dis} of less than $0.1 \text{ cm}^2/\text{hr}$ (Table 4.1).

The ratio of solute mass sorbed to aquifer solids to mass in pore fluids defines the equilibrium partition coefficient, R_p . A summary of the change in magnitude of R_p for columns A, B, and C is presented in Figure 3.4. After five exchanges within these three columns, R_p values stabilized, indicating that sorption was essentially complete. The resulting “equilibrium” R_p values ranged from a low of 4.56 (Column P) to a high of about 5.44 (Column Q) (Table 3.2). These results would suggest that retardation factors, R_t , of 5.56 to 6.44 characterize the movement of TCE relative to the rate of groundwater flow within the repacked aquifer solids. However, from examination of the breakthrough profile for TCE within the small columns (Figure 3.1), it is evident that the values of R_p and R_t are unrealistically high, and most likely reflect excess solute sorption onto the base

of the rubber stoppers that sealed each column. Therefore, transport modeling simulations were performed to simulate TCE breakthrough in these columns and calibrate R_t . The calibrated modeling results indicate that an R_t value of 1.3 more closely characterizes TCE transport in the repacked Plume F/G aquifer materials.

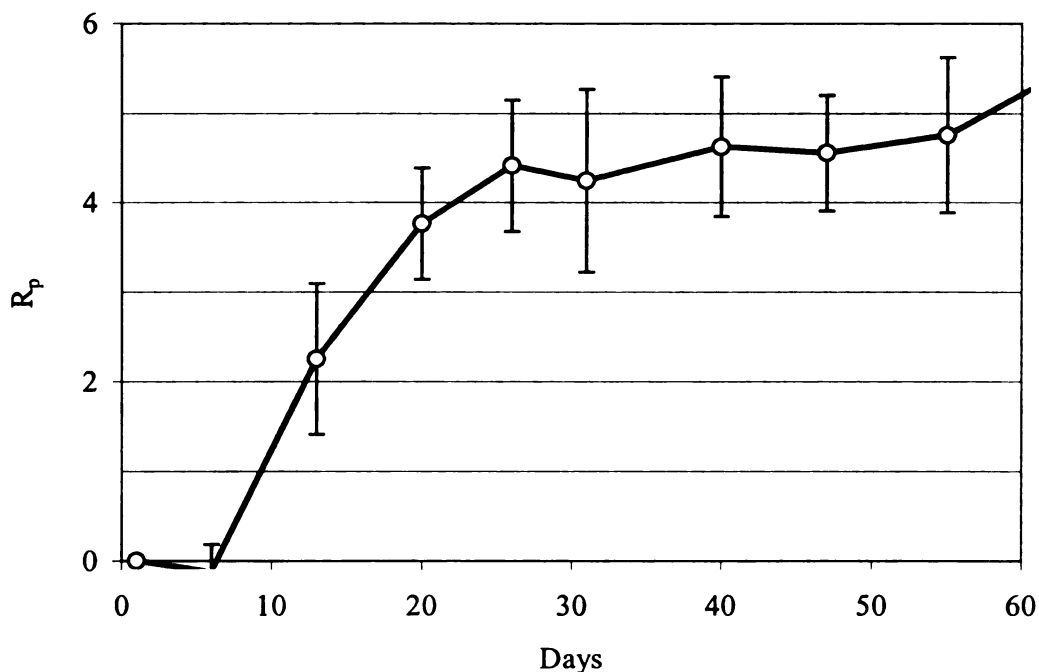


Figure 3.4. Dimensionless equilibrium partitioning coefficients composited from small-scale columns D-U.

From the value R_p reported on Table 3.2 and assuming linear (de)sorption kinetics, a distribution coefficient, K_d of $0.07 \text{ cm}^2/\text{g}$ was calculated. This indicates that approximately 28% of the total TCE mass within the three columns was retained on the solid matrix following the completion of sorption. Given that the source of the soil and processing protocols were consistent between the two experiments, it is assumed that the

distribution coefficient estimated from the small-column data is valid for characterizing sorption within large-scale Column A.

3.4 Inoculum Transport

As illustrated on Figure 3.1, the BR Enrichment was readily transported through the re-packed Plume F/G aquifer solids in small-scale columns A, B, and C. In fact, breakthrough was slightly more rapid than the average linear velocity of the exchange fluids (as defined by bromide breakthrough), probably owing to charge and/or pore size exclusion and the motility of some community members within the inoculum. Only about 60% of the mass of cells introduced exited the column during the breakthrough. The remaining 40% of the inoculum was retained within the column. The mechanism of retention is unknown.

In general, the tracking of optical density in port-specific and effluent samples from the large-scale columns was inconclusive. Plots of these data are provided on Figure 3.5. As seen on the figure, no distinct trend in optical density increase is evident. Further, DNA concentrations from PicoGreen[®] analysis did not indicate trends in increasing biomass levels at Column B sample ports or within Column A effluent during inoculation. These observations were not unexpected, considering that dilute concentrations (e.g., 38 µg/L or 4×10^6 CFU/mL) of the BR Enrichment were used for inoculation of Columns A and B. Consequently, it is likely that much of the biomass introduced during inoculation was retained within the sediment upgradient of the sampling points. *Dehalococcoides sp.* were measured at levels within the 10^2 CFUs/mL range within the inoculum (Table 3.2), but were not detected in any of the aqueous

biomass samples collected from Columns A or B during these initial breakthrough studies.

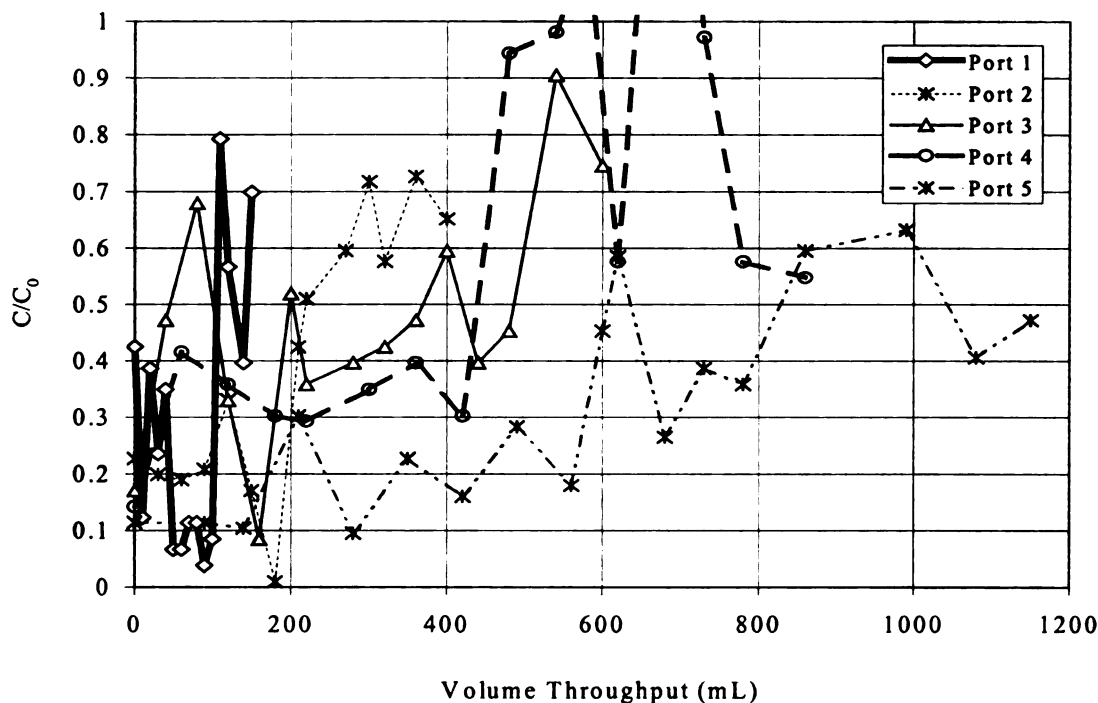


Figure 3.5. Column B port-specific inoculum breakthrough based on optical density (OD_{660}) measurements.

3.5 Biotransformation

Biotransformation activity within both the small- and large-scale column systems was evaluated through an integrated examination of several elements of data. For the small-scale columns, the primary data elements included temporal carbon source (fatty acid), major anion, and TCE transformation data. Limited sets of biomass, headspace gas, and soluble chromium data were also examined. The assessment of biotransformation within large-scale Column A was based on spatial and temporal

biomass, fatty acid, headspace gas (e.g., methane), major anion, soluble chromium and iron, and TCE dechlorination data.

Table 3.3 RT-PCR results for small- and large-scale column samples.

Sample Designation	$\mu\text{g/mL}$	CFU/mL
<i>Source Soil</i>	Not detected	Not detected
<i>EFX Reactor Sample</i>	1.60×10^{-5}	1.70×10^2
<i>Small Column Post-Treatment Soils:</i>		
P (top 1/3)	3.65×10^{-7}	41.30
Q (top 1/3)	4.60×10^{-8}	5.20
U (top 1/3)	1.96×10^{-8}	2.22
<i>Large-Scale Column A:</i>		
Op. Phase B pre-inoc. effluent 1	7.99×10^{-7}	9.04
Op. Phase B inoculation breakthrough	1.19×10^{-3}	1.35×10^4
10/1/02 Port 4 pore water	9.76×10^{-9}	1.10
10/15/02 Port 5 pore water	1.50×10^{-8}	1.71
10/15/02 Port 7 pore water	1.00×10^{-8}	1.13
Post-Treatment Soil – Port 2	1.24×10^{-8}	1.41
Post-Treatment Soil – Port 4	5.74×10^{-8}	6.49
Post-Treatment Soil – Port 6	2.28×10^{-8}	2.57
Post-Treatment Soil – Port 7	1.07×10^{-8}	1.21

Biomass concentrations extrapolated from DNA quantification results assuming 8.84×10^{-15} g of DNA per cell, 2.13 molecules of DNA per cell, and a typical cell mass of 9.5×10^{-13} g.

3.5.1 Small-Scale Column Experiment

Biomass

Biomass measurements during the small column experiment were restricted to the analyses of post-treatment soils from inoculated columns P, Q, and R, and stimulated columns S, T, and U. Curiously, these data indicate that the average solid-phase biomass accumulation within the stimulated columns (7×10^7 CFU/g) was greater than the quantity detected in the bioaugmented columns (4×10^6 CFU/g). Further,

Dehalococcoides sp. were detected in all but one soil sample from both column sets at approximately 10^{-1} CFU/g, which may indicate that these microorganisms are native to the Plume F/G sediment. However, a sample collected from inoculated Column P yielded a concentration of *Dehalococcoides sp.* within a range of 1-100 CFU/g (Table 3.2), possibly indicating a growth dependency on environmental conditions imposed by the BR Enrichment.

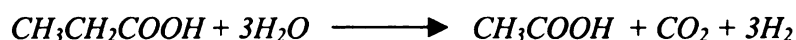
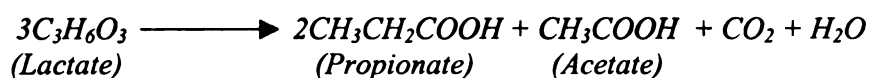
Fatty Acids

Although no specific fatty acid analyses were performed on samples collected from the small-scale column, the results from the IC analysis of samples collected immediately following feeding events, when lactate was the only fatty acid within the column system at detectable concentrations, exhibited organic acid concentrations consistent with those associated with the levels of lactate fed to the columns. Therefore, IC results were used to track fatty acid levels within the small-scale columns. However, interpretation of the IC-generated results required an understanding of the feeding history of each column, as these data indicated the presence of lactate in samples collected immediately following feeding, and total fatty acids in samples obtained at all other times, assuming a certain degree of lactate decomposition post-feeding.

In this context, the anion (IC) data for the inoculated and stimulated small-scale columns indicate that fatty acid levels persisted during the post-inoculation incubation periods (data for column P, Q, and R shown on Figure 3.6). This trend was not evident within the sterile control columns (data not shown). However, the mass of fatty acids remaining with the bioaugmented and biostimulated columns was significantly less than

anticipated, assuming lactate fermentation occurred to completion during each incubation period. The depletion in mass coupled with the presence of methane (see below) may have indicated that acetate was produced through lactate fermentation and that acetate-metabolism, likely by acetotrophic methanogens, had occurred during the incubation periods.

The general mechanisms of lactate decomposition resulting in production of propionate, acetate and molecular hydrogen are stoichiometrically described by:



Molecular hydrogen produced through lactate and propionate fermentation was, therefore, a source of reducing power for hydrogenotrophic microorganisms (including halorespiring bacteria) present in the re-packed Plume F/G sediments. Note that, based on stoichiometry and background concentrations for nitrate (22 mg/L) and sulfate (48 mg/L), an estimated 200 mg/L of 60% DL-lactate needed to be fed to the bioaugmented column systems on a weekly basis in order to completely reduce these solutes and dechlorinate a TCE concentration of 2.5 mg/L.

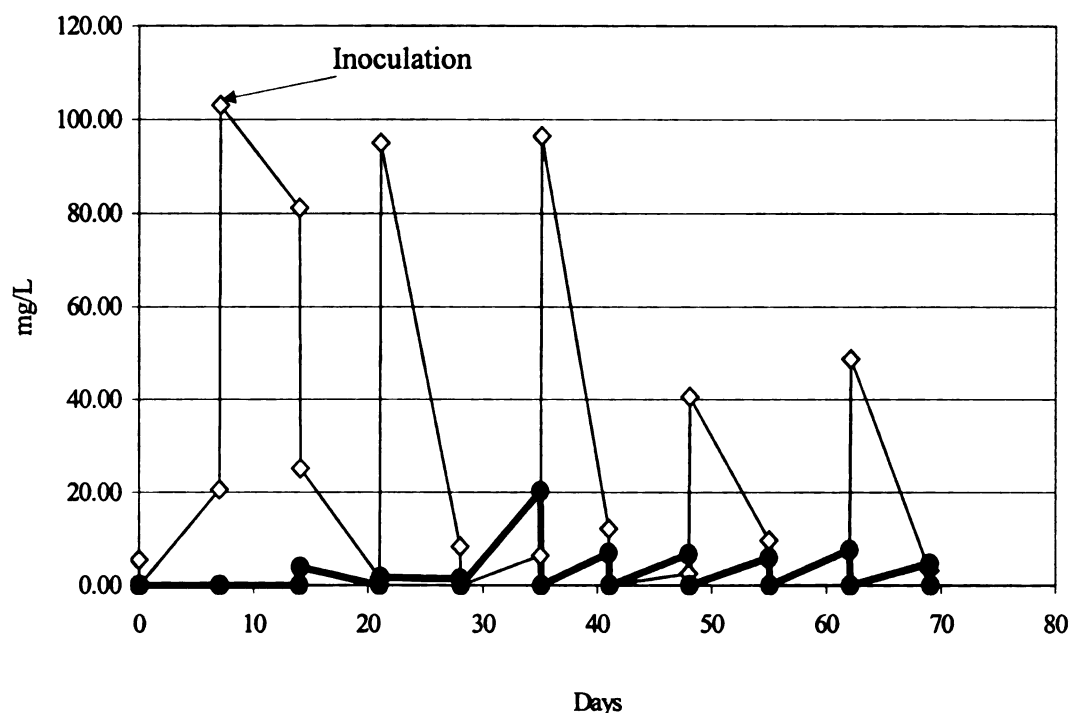


Figure 3.6. Organic acid and phosphate data for Columns P, Q, and R. Organic acid levels are indicated by the open diamonds. Phosphate trends are presented by the closed circles and bold tie-lines.

Headspace Gasses

The analyses of headspace gas data (not shown) revealed that hydrogen levels within the atmosphere (3-5% or approximately 42 μM) of the anaerobic glove box in which the experiments were conducted likely interfered with the measurement of hydrogen partial pressures associated with bioactivity within the columns. Other than hydrogen, methane was the only gas detected in headspace samples. Methane was measurable in samples collected from both the bioaugmented columns (Columns J, K, L, M, N, O, P, Q, and R) and the biostimulation controls (Columns S, T, and U). In general, the occurrence of methane was most significant within Columns P, Q, and R, which were

inoculated with non-dilute BR Enrichment. These qualitative results indicate that methanogenic conditions developed in all of the columns that received bi-weekly dosages of lactate. The depletion of fatty acid levels during the associated incubation periods may indicate that acetate-utilizing methanogens were active. The occurrence of acetotrophic methanogenesis in both bioaugmented and biostimulated columns indicates that these microorganisms were likely associated with the Plume F/G aquifer materials.

Phosphate

During post-inoculation incubation periods, phosphorus levels measured in columns P, Q, and R consistently increased to 5-10 mg/L (Figure 3.6). This trend was not evident in other inoculated or control column sets and indicates that there was a mechanism causing phosphate solubilization that was unique to the columns receiving non-diluted BR Enrichment. The exact mechanism is unknown, but may have included chelation with ferrous iron, the concentration of which was likely increasing due to ferric iron reduction associated with the elevated bioactivity and associated decrease in ORP within these columns.

Nitrate and Sulfate

Influent nitrate levels (15-25 mg/L) within columns P, Q, and R were consistently depleted during the post-inoculation incubation periods. Curiously, this trend was evident within all columns (including controls) throughout the duration of the experiment. Therefore, the mechanism of nitrate removal is unclear. Influent sulfate levels (50-75 mg/L) consistently decreased approximately 50% during the post-

inoculation incubation periods associated with Columns P, Q, and R (Figure 3.7). In general, the magnitude of sulfate reduction was less pronounced in the other two inoculated column sets. No discernable reductions in sulfate levels were evident within the biostimulated or control columns (data not shown). The persistence of sulfate in the inoculated columns suggests that the bulk of the reducing equivalents resulting from lactate fermentation were consumed by other terminal electron accepting processes such as nitrate and iron reduction and halorespiration. The duration of contact of amended pore fluids with the inoculated Plume F/G sediments was apparently not sufficient to permit successional development of predominantly sulfate reducing conditions by the microflora in the columns. It is likely that sulfate-reducing conditions would have developed if a more concentrated biweekly dosage of lactate was fed to the columns to drive the bulk ORP of the system to the level of sulfidogenesis or methanogenesis, or the duration of the incubation periods were increased to produce a similar effect.

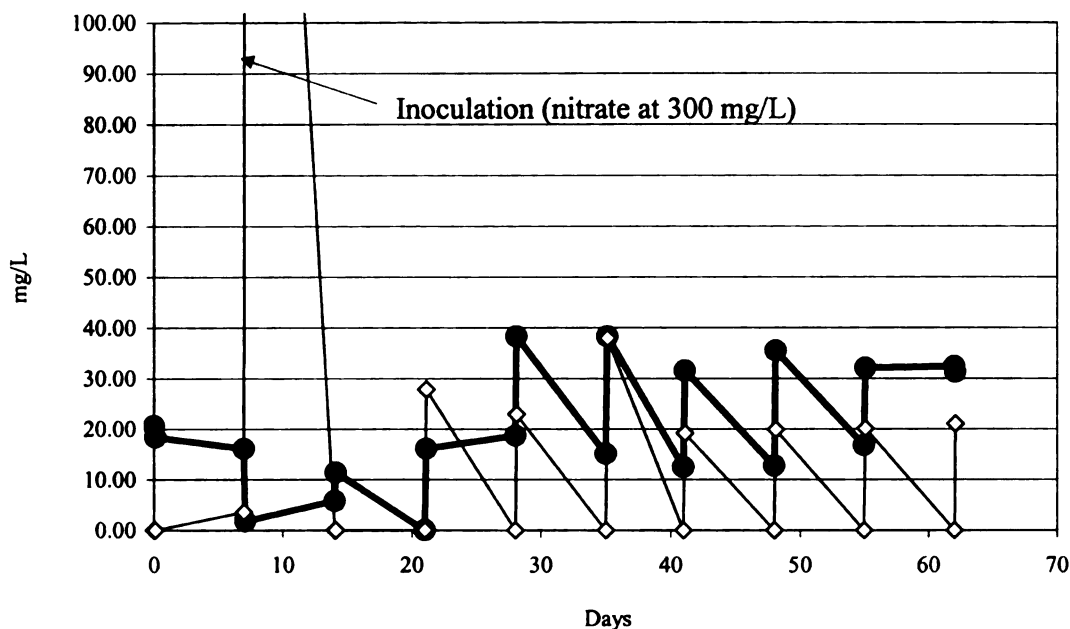


Figure 3.7. Nitrate and sulfate data for Columns P, Q, and R. Nitrate data indicated by open diamonds; sulfate data indicated by closed circles and bold tie-lines.

Soluble Metals

No measurements of soluble iron were made during the small-scale column experiment. However, evidence of sulfate reduction in Column P, Q, and R, albeit limited, suggests that iron reduction had occurred in the sediments that were inoculated with non-diluted BR Enrichment. Cr(VI) levels in post-incubation period effluent samples (<10 mg/L) were, in general, significantly lower than the concentrations in the Plume F/G groundwater fed to the inoculated controls (>200 mg/L) during the exchange events evaluated. These data indicate that the ORP within the inoculated columns was consistently below the level necessary to favor the reduction of chromium.

TCE Transformation

Examination of the small-scale column time-series plots (Figure 3.8) reveals that prior to inoculation, sorption was essentially complete within column sets 2 through 7, as evident by the lack of significant TCE removal between the exchange events during weeks 4 through 6. After inoculation, TCE removal was distinct within the three bioaugmented column sets (Columns J, K, L, M, N, O, P, Q, and R), indicating biotransformation. This trend was more pronounced within Columns P, Q, and R, which were inoculated with undiluted BR Enrichment. Cumulative mass balances for these columns indicate that following inoculation between 50-100% of the TCE mass input into each vessel during an exchange was transformed during the subsequent incubation periods. Greater than 90% of the dechlorination resulted in the production of VC in Columns P, Q, and R (Figure 3.9). The accumulation of VC was significantly less in column set #'s 3 and 4 (Figure 3.9). Less than 10% of TCE dechlorination represented an accumulation of cis-DCE. The preponderance of VC accumulation relative to cis-DCE suggests that reductive dechlorination processes were trending toward completion during the incubation periods.

No significant TCE removal was observed in radiation-sterilized, natural attenuation, and bioenhancement control columns during the period parallel with inoculation and subsequent feeding exchanges. Further, increases in nutrient and TCE loadings during the late stages of the small-scale column studies had little to no observable beneficial effect on TCE transformation activity in inoculated columns.

In summary, the direct comparison of bioaugmentation versus natural attenuation and biostimulation within the small-scale column systems produced strong evidence of a

causal relationship between the dechlorination activity of the in-situ microbial community supplemented with the BR Enrichment and removal of TCE from the Plume F/G aquifer materials.

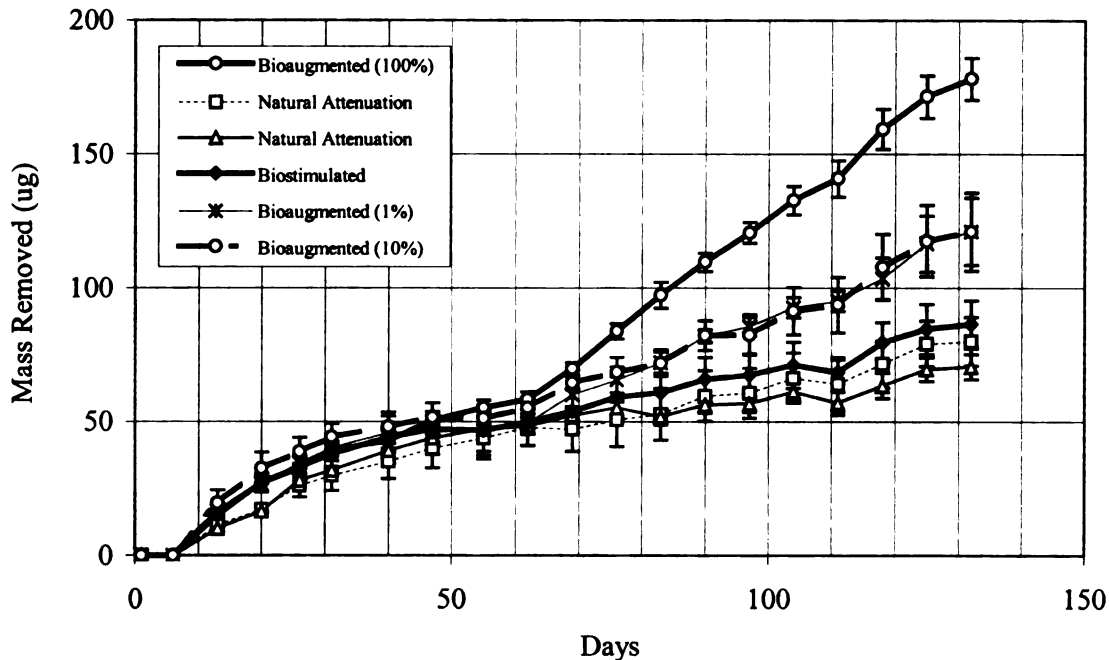


Figure 3.8. Cumulative TCE mass removed in each small-scale column set.

TCE Transformation Kinetics and Mass Removal

Figure 3.8 illustrates the cumulative removal of TCE within each of the six column sets over the duration of the small-scale column experiment. TCE removal increased sharply in column set #6 (columns P, Q, and R) following inoculation with the Bachman Road Enrichment. The increase is progressively less pronounced within column set #5 (e.g., column M, N, O, which received a 10% inoculate strength) and column set #5 (e.g., columns J, K, and L, which received a 1% inoculate strength).

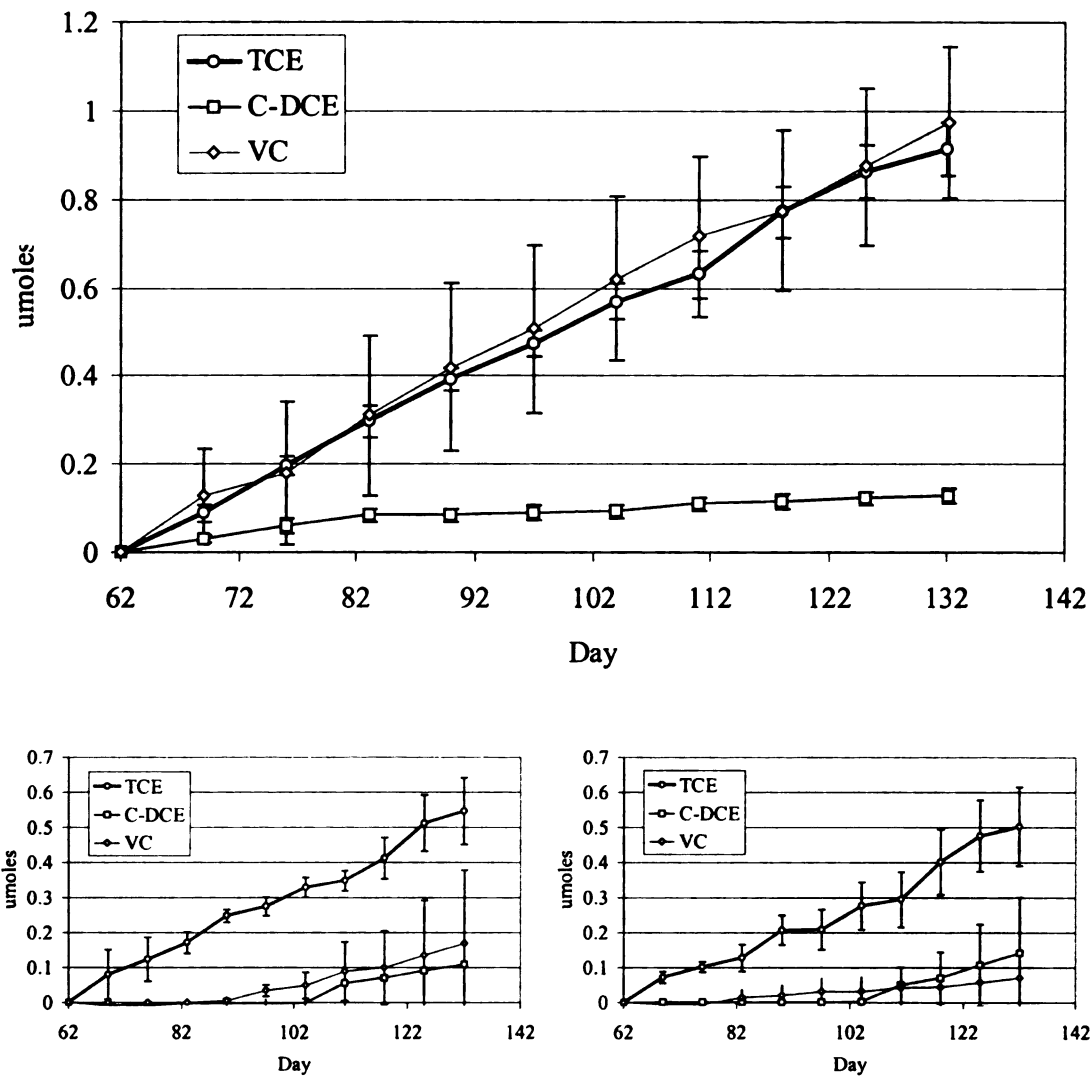


Figure 3.9. TCE removal and cis-DCE and VC accumulation in columns P, Q, R (a), J, K, L (b), and M, N, O (c).

No substantial TCE degradation was observed in the control column sets. For the inoculated column sets, the increase in TCE removal was expected. However, there was also some expectation that feeding of the non-sterilized controls (columns S, T, and U) would result in stimulation of indigenous microorganisms capable of transforming TCE.

Although there was some evidence of limited TCE removal within this column set, the trend was similar to those observed in the data sets for the TCE-fed control (natural attenuation) columns (Columns D, E, F, G, H, and I). This indicates that no significant biostimulation occurred over the duration of the experiment.

Figure 3.8 reveals a trend that was common to columns P, Q, and R. After inoculation, TCE levels steadily decreased at a relatively consistent rate. Further, TCE transformation during this period was equal to or exceeded the mass of solute loaded into the columns during the associated exchange events, indicating removal of sorbed contaminant.

Figure 3.10 illustrates changes in the apparent first-order rate coefficient, k' , of TCE mass removal in columns P, Q, and R during the treatment period (e.g., days 60 through 130). From these data, an average k' of 0.62 day^{-1} was obtained. Laboratory studies have shown that the rate of TCE transformation in batch experiments is a function of culture age; decay of transformation activity occurs as cells enter a stationary phase. The k' value reported herein reflects this loss of activity. Apparent first-order rate coefficients estimated from data from column sets 4 (J, K, L) and 5 (M, N, O) were 0.24 day^{-1} and 0.15 day^{-1} , respectively.

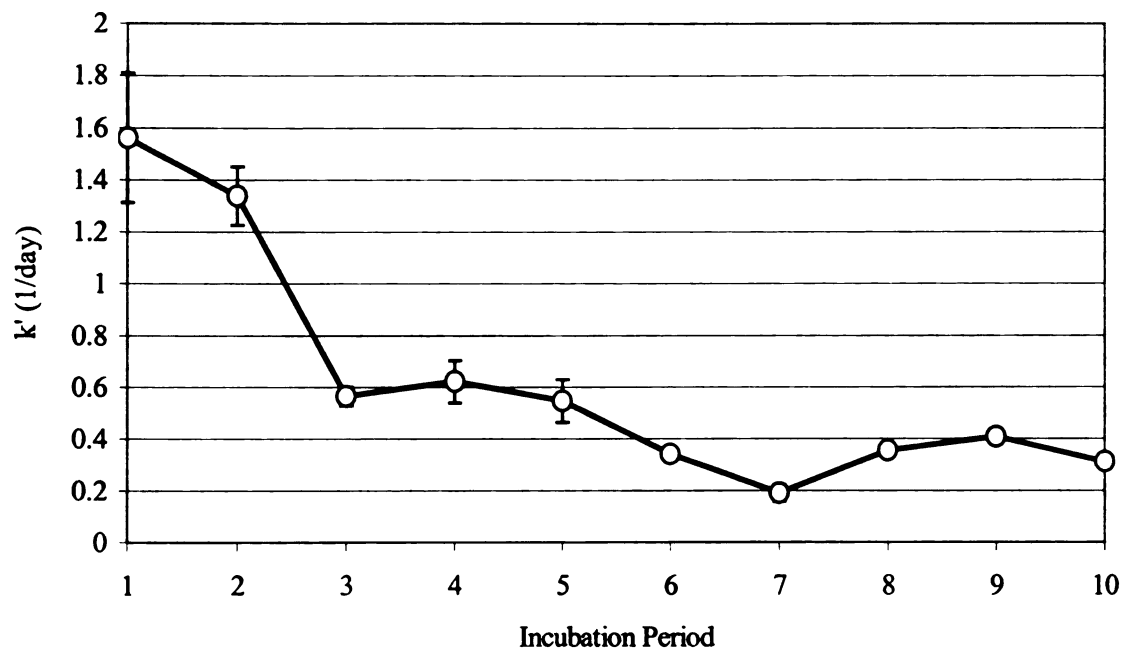


Figure 3.10. Apparent first-order rate coefficient trend, Columns P, Q, and R.

The results from the analyses of the batch microcosm samples are summarized in Appendix B. From these data, a zero-order rate of PCE dechlorination of $29.6 \mu\text{M/g (biomass)}/\text{day}$ was measured. First-order TCE dechlorination rates of 0.58 day^{-1} to 3.54 day^{-1} were also estimated from the PCE transformation data. Therefore, the range of k' estimated from the small column data is consistent with the range associated with the culture maintained under laboratory conditions.

The cumulative mass removal data from the column experiments indicate that bioaugmentation of the Plume F/G aquifer materials with the BR Enrichment will result in TCE transformation. The small-scale column data revealed that VC was the predominant end product resulting after seven days of treatment. The range of apparent

first-order rate coefficients reported here suggests that favorable rates of TCE transformation may be attainable from engineered bioaugmentation of Plume F/G.

3.5.2 Large-Scale Column Experiment

Appendix A summarizes the sampling dates associated with the operational history of Column A, as well as the influent concentrations of TCE, fatty acids, hydrogen, major anions, and soluble chromium and iron in the fluids delivered to the column over the duration of the experiment. As shown on Table 3.5, Column A was inoculated with the Bachman Road enrichment culture on two separate occasions. The periods of operation that followed each inoculation event are referenced as Operational Phases A and B. The duration of Operational Phase A was 9 1/2 weeks and included a total of eight distinct incubation periods that followed the initial inoculation and seven subsequent feeding events. From a preliminary examination of the Column A database at the conclusion of Operational Phase A it was originally inferred that TCE dechlorination was not occurring to the extent anticipated, based on the treatment performance of the BR Enrichment within both the EFX reactor and the small-scale columns. On this basis, re-inoculation of Column A was considered necessary. This second inoculation event and five subsequent incubation periods extending over a duration of six weeks comprised Operational Phase B. Time series plots of sample port-specific solute concentrations (e.g. TCE/cis-DCE/VC, fatty acids, anions, and soluble chromium and iron) measured during each incubation period of Operational Phase A and Operational Phase B are provided in Appendix C and Appendix D, respectively.

Operational Phase A

Biomass

DNA quantification of soil and groundwater samples revealed no distinct trends in spatial or temporal biomass accumulation. Biomass concentrations were extrapolated from the DNA quantification results assuming 8.84×10^{-15} g of DNA per cell, 2.13 molecules of DNA per cell, and a typical cell mass of 9.5×10^{-13} g. In general, post-inoculation aqueous samples exhibited biomass concentrations of 4×10^4 CFU/mL to 5×10^8 CFU/mL, with an average of 3×10^7 CFU/mL. Soils samples from Column B yielded 1×10^4 CFU/g to 3×10^6 CFU/g. *Dehalococcoides sp.* were measured at levels within the 10^2 CFU/mL range (Table 3.2) in inoculum samples, but were not detected in soil or groundwater during Operational Phase A.

Headspace Gases

Throughout the duration of the large-scale column experiment, quantification of hydrogen partial pressures in aqueous samples was not possible apparently due to the limited sample volumes (0.5-1.0 mL) collected. No samples collected during Operational Phase A exhibited detectable levels of hydrogen. Conversely, methane was consistently detected over the entire length of the column. However, due to limited available sample volumes (0.5 – 1.0 mL) methane measurements were marginally above the limits of quantification. Qualitative assessment of peak heights did reveal a slight increase in methane levels over the length of the column, suggesting a progressive increase in methanogenic activity. Ethene peaks were not clearly evident in any of the Column A samples obtained during Operational Phase A, although its presence was suspected. As

with hydrogen, the lack of detectable concentrations of ethene is most likely due to the limited sample volumes collected during the experiment.

Fatty Acids

Examination of the fatty acid data collected during Operational Phase A (Appendix C-1) reveals that lactate rapidly decomposed after inoculation and each subsequent feeding event. During the two-week long incubation period immediately following inoculation (e.g. Incubation Period 1A), lactate fermentation was incomplete with propionate as the exclusive bi-product. During subsequent incubation periods, lactate in feed solutions introduced into the column was completely decomposed. Through Incubation Period 6A, propionate appeared to have been the principal byproduct of lactate fermentation. However, the maximum levels of propionate measured over this time interval were consistently less than 50% of the influent lactate concentrations delivered to the column. Subsequently, significant concentrations (e.g., > 50 mg/L [830 mM]) of acetate were measured during Incubation Periods 7A and 8A, predominantly in samples from the downgradient ports 5 and 7. This suggests that either the rate and completeness of lactate fermentation improved, or that the activity of hydrogenotrophic acetogens increased. In general, this progressive increase in lactate and propionate decomposition indicated temporal acclimation of fermenting microorganisms to the Column A aquifer materials. By Incubation Periods 7A and 8A, fermentation rates appeared to be essentially instantaneous.

With lactate levels delivered to Column A being 100-300 mg/L (1.1-3.3 mM), stoichiometric dissolved hydrogen concentrations in excess of 2.5-7.5 mM were rapidly

and consistently produced as the result of fermentation following inoculation and each feeding event during Operational Phase A.

Phosphate

As shown in Appendix C-2, phosphate was detected only sporadically within any port-specific groundwater samples collected during Operational Phase A. Further, these few detections revealed no distinct spatial or temporal patterns.

Nitrate and Sulfate

Major anion data are presented in Appendix C-2. During the second week of Incubation Period 1A, immediately following inoculation of Column A, the concentrations of major anions such as chloride and sulfate steadily decreased. Nitrate levels were not measurable during this period, with the exception of a single detection (approximately 20 mg/L) in a sample collected from Port 7 at the conclusion of Incubation Period 1A. During the period following the first feeding event (e.g., Incubation Period 2A) nitrate concentrations at Ports 1 and 2 exceeded 10 mg/L. However, over the length of the column, nitrate levels decreased to below detectable levels. Incubation Period 3A revealed that the influent concentrations of nitrate (20 mg/L) were reduced to below detection limits upgradient of Port 1. Port-specific sulfate concentrations appeared to persist at levels between 10-20 mg/L. The time-series and spatial profiles of major anion concentrations during Incubation Period 4A are similar to those associated with Incubation Period 3A. However, the sharp decrease in sulfate observed at Port 7 on August 9, 2002 suggests that sulfate reduction may have

temporarily been the dominant terminal electron-accepting process early during Incubation Period 4A. Similar profiles for nitrate and sulfate are associated with Incubation Periods 5A, 6A and 7A. Incubation Period 8A revealed a much stronger trend toward sulfate reduction, with sulfate concentrations below detectable levels at Ports 5 and 7 at the conclusion of the period. These data suggest that sulfate-reduction occurred during the first three days of the incubation period when acetate concentrations, produced from the rapid fermentation of lactate and propionate characteristic of this stage of Operational Phase A, were maximized.

Soluble Metals

Throughout the duration of Operational Phase A, ferric iron and hexavalent chromium reduction were evident (Appendix C-3). Soluble iron concentrations generally increased over the length of the column during each incubation period comprising Operational Phase A. In some instances, soluble iron levels increased from below 100 $\mu\text{g/L}$ within influent pore fluids to 1,000-2,000 $\mu\text{g/L}$ between Ports 2 and 4. Influent concentrations of Cr(VI) in excess of 200 $\mu\text{g/L}$ were consistently reduced to less than 20 $\mu\text{g/L}$ between Ports 1 and 2 by the time sampling was completed following each feeding. These data suggest that the ORP resulting from inoculation and lactate feeding was sufficiently low for promoting consistent and rapid reduction of hexavalent chromium.

It is not known if the Cr(VI) reduction in Column A was biologically mediated or the result of purely physicochemical processes. However, Cr(VI)-reducing microorganisms are ubiquitous in soil and sediments. Many aerobic and anaerobic microorganisms can reduce Cr(VI) while utilizing a range of substrates at near neutral

pH, and several have been isolated that can use Cr(VI) as a terminal electron acceptor for growth (Criddle and Middleton, 1999). The Plume F/G aquifer materials have been exposed to Cr(VI) for decades and it is likely that the native microflora includes microbial populations capable of dichromate reduction. Alternatively, it is well known that Cr(VI) is readily reduced by ferrous iron. Ferrous iron concentrations in feed groundwater increased as much as an order of magnitude within the column system during Operational Phase A. Therefore, either mechanism for Cr(VI) reduction in Column A is plausible.

TCE Transformation

As described in Chapter 2, the large-scale column experiment was designed to consistently deliver Plume F/G groundwater spiked with approximately 1 mg/L of TCE to Column A. However, technical difficulties associated with stock preparation during the initial inoculation and the subsequent two weeks of groundwater delivery to Column A inadvertently resulted in the throughput of substantially lower TCE concentrations. Specifically, during inoculation, the concentration of TCE in groundwater conveyed to the column was slightly less than 50 µg/L. Groundwater fed to Column A during the first two weeks of Incubation Period A contained between 240-435 µg/L of TCE. The stock preparation difficulties were corrected prior to the initial feeding of Column A, two weeks following inoculation. Consequently, Incubation Period 2A began with the replacement of pore fluids with groundwater containing TCE in excess 2,000 µg/L. Subsequent stock preparations during Operational Phase A resulted in TCE delivery concentrations consistently greater than 1,000 µg/L.

A potential consequence of the early stock preparation difficulties was that the effects of TCE sorption onto the Plume F/G solids could have obscured evidence of biotransformation during the post-inoculation incubation periods. From inspection of the data presented in Appendix C-4, it appears that the effects of sorption following Incubation Period 3A are of minor consequence. TCE concentrations measured at downgradient ports (Ports 4-7) during Incubation Period 4A closely match the concentrations within the groundwater delivered to the column, suggesting that sorption was essentially complete. However, there was evidence of a localized reduction in TCE concentration that appears anomalous. Specifically, TCE concentrations at Port 3 appeared to have decreased from background levels by approximately 30%. Given the progressive increase in cis-DCE levels over the length of the column, this anomaly is believed to represent the first clear evidence of reductive dechlorination during Operational Phase A. A reduction in TCE levels consistent with that observed during Incubation Period 4A was evident within the length of column extending from Port 1 to Port 4 during the four subsequent incubation periods comprising Operational Phase A. Port-specific reductions in TCE concentrations as much as 1,000 $\mu\text{g/L}$ (7.6 μM) were accompanied by a coincident increase of less than 200 $\mu\text{g/L}$ (2 μM) of cis-DCE and little to no accumulation of VC. More specifically, at the conclusion of each incubation period, cis-DCE concentrations were at maximum levels in samples from Ports 6 and 7, while TCE transformation appeared to persist between the column influent and Port 3. This indicated that cis-DCE accumulated early in the incubation period immediately following feeding, when TCE transformation rates were likely maximized. Thereafter, cis-DCE production gradually diminished, but TCE transformation appeared to remain

fairly constant. These observations suggest that the dominant mechanism of reductive dechlorination during these incubation periods was halorespiration by *Dehalococcoides* populations.

Summary – Operational Phase A

When viewed collectively, the anion and soluble chromium and iron monitoring data indicate that anoxic conditions prevailed within Column A during Operational Phase A. Following inoculation and through Incubation Period 2A, nitrate and iron reduction appeared to be the dominant terminal electron-accepting processes. Between Incubation Periods 2A and 4A, iron reduction prevailed. Following Incubation Period 4A, there is evidence that the oxidation-reduction potential (ORP) decreased to the level of sulfidogenesis. Specifically, these data reveal that sulfate was reduced to below detectable levels during the early stages of Incubation Periods 7A and 8A.

With nitrate and iron reduction being essentially complete and sulfate reduction only occurring temporarily, it is inferred that the reducing power that persisted within the column over the duration of each incubation period was sufficient to maintain the system ORP at or just above the level favorable for sulfidogenesis. Fatty acid data corresponding to these periods suggest that this source of reducing power was dissolved hydrogen produced through lactate and propionate fermentation. According the Löffler, et al. (1999), the hydrogen threshold concentrations associated with nitrate and iron reduction, and halorespiration are lower than those associated with sulfate reduction and methanogenesis. Therefore, it is plausible that nitrate- and iron-reduction and

halorespiration could occur through the duration of each incubation period Operational Phase A, without co-incident occurrence of sulfidogenesis.

Dissolved hydrogen is the preferred source of electrons for halorespiration. As previously discussed, direct measurement of dissolved hydrogen concentrations within Column A was not possible due to sample volume constraints. However, if the inferences associated with the anion and fatty acid data are accurate, then the redox conditions favorable for TCE respiration would approach optimal levels after lactate and propionate fermentation was complete. However, acetate produced by these fermentations could have stimulated less efficient halorespiring populations, resulting in an accumulation of cis-DCE. It is probable that efficient halorespiration by *D. ethenogenes* would not predominate until acetate was metabolized within or transported out of the BAZ.

Operational Period B

Biomass

As noted previously, DNA quantification of soil and groundwater samples revealed no distinct trends in spatial or temporal biomass concentrations. Similar to Operational Phase A, aqueous samples yielded average biomass concentrations of 3×10^7 CFU/mL. Soils samples from both Columns A at the conclusion of Operational Phase B exhibited 1×10^6 CFUs/g to 7×10^6 CFU/g. The inoculum used for Operational Phase B contained *Dehalococcoides sp.* within the 10^2 CFU/mL range (Table 3.2). During inoculum breakthrough, *Dehalococcoides sp.* were detected at levels of 9 CFU/mL to 1×10^4 CFU/mL. Only three aqueous samples collected during Operational Phase B,

collected during Incubation Periods 4B and 5B yielded detectable (e.g., 1-2 CFU/mL) quantities of *Dehalococcoides sp.* Conversely, soil samples collected from Ports 2, 4, 6 and 7 of Column A at the conclusion of the experiment exhibited *Dehalococcoides sp.* at levels ranging between 1-7 CFU/g. Peak levels were observed at Port 4 (Table 3.2).

Headspace Gasses

No samples collected during Operational Phase B exhibited detectable levels of hydrogen. However, as shown on the time-series plot provided on Figure 3.11, as much as 6 mM of hydrogen should have been produced as a result of lactate and propionate fermentation. In environments saturated with hydrogen, nitrate and ferric iron are expected to be rapidly reduced. Therefore, it is plausible that hydrogen concentrations were rapidly depleted by nitrate- and ferric iron-reducing microorganisms to below levels quantifiable in the 0.5 – 1.0 mL sample volumes obtained from Column A. Methane was consistently detected over the entire length of the column. However, due to the limited sample volumes obtained, methane levels were only slightly above quantifiable limits. Qualitative assessment of peak heights did reveal a slight increase in methane levels over the length of the column, suggesting a progressive increase in methanogenic activity with distance traveled by pore fluids. Ethene was not detected in any of the Column A samples obtained during Operational Phase B, though its presence was suspected. As with hydrogen, the lack of detectable concentrations of ethene is most likely due to the limited sample volumes collected during the experiment.

Fatty Acids

Column A was re-inoculated on September 3, 2002. Fatty acid data (Appendix D-1) indicate that lactate concentrations were generally equally distributed over the length of the column immediately following re-inoculation. At the conclusion of Incubation Period 1B, lactate levels remained equally distributed but decreased over 50% at each sampling port. However, unlike during the period immediately following the initial inoculation event and subsequent incubation periods, lactate concentrations persisted above 25 mg/L. Propionate and acetate were not measured at significant levels during Incubation Period 1B. During Incubation Period 2B, lactate feed levels decreased nearly 10-fold at Port 1 to below detectable levels at Ports 5 and 7. Correspondingly, acetate concentrations increased inversely over the length of the column to approximately 400 mg/L (7 mM) at Port 7. Minor levels (< 50 mg/L [0.7 mM]) of propionate were observed at Port 5 during Incubation Period 2B.

During Incubation Periods 4B through 6B, time-series and spatial gradients of lactate decomposition and byproduct formation were similar. However, with the temporal resolution of sampling increased to bi-weekly during Incubation Period 4B and daily during Incubation Periods 5B and 6B, it became apparent that lactate and propionate were very rapidly decomposed following feeding, resulting in an immediate accumulation of acetate and, undoubtedly, hydrogen. Generally, the mass of acetate traveling through the column was preserved or slightly reduced, indicating that hydrogenotrophic acetogenesis did not occur to a measurable extent. With a doubling of the lactate input concentration during Incubation Period 6B, greater masses of propionate and acetate were produced.

Phosphate

Phosphate concentrations associated with the second inoculation (200-250 mg/L) decreased to less than 10 mg/L during Incubation Period 1B (Appendix D-2). Subsequently, phosphate levels remained at or below detectable levels at Port 1. However, in contrast to the observations made during Operational Phase A, at the conclusion of each Phase B incubation period, a gradual increase in phosphate levels was evident over the length of Column A. This behavior was consistent with the phenomena observed in small-scale columns P, Q, and R, and suggests that a solubilization mechanism was associated with the elevated bioactivity in the column. Specifically, solids-associated phosphates may have chelated with the soluble ferrous iron produced from ferric iron reduction.

Nitrate and Sulfate

The results from the analyses of anions in Column A samples following the second inoculation (Appendix D-2) and at the conclusion of Incubation Period 1B indicate that nitrate reduction was consistent during these periods. Conversely, sulfate levels persisted at or close to background concentrations. However, as during Incubation Periods 7A and 8A, there was consistent evidence that sulfate reduction occurred temporarily during Incubation Periods 2B through 6B. The refined temporal resolution of Incubation Periods 4B-6B reveal that sulfate levels were reduced to below detectable levels during a finite interval and within a specific segment of Column A. During these periods, sulfate reduction was evident within two days of feeding and occurred most significantly within the column length between Ports 2 and 4 where and when acetate and

hydrogen levels were, presumably, at their maximum. Ultimately, sulfate in this interval was reduced to below detectable levels. Under these conditions, methanogenic activity was likely enhanced. However, after three days, the ORP within this portion of the column apparently increased with the infiltration of non-amended groundwater that followed each feeding. Specifically, sulfate concentrations began to gradually increase at these ports to near background levels (e.g., 50 mg/L) during days 4-7 of each incubation period. The decrease in sulfidogenesis corresponds with a progressive decrease in and competition for reducing power associated with acetate and hydrogen.

Soluble Metals

Soluble chromium and iron data associated with Operational Phase B (Appendix D-3) indicate that redox conditions within Column A were consistently sufficiently low and favorable for Cr(VI) reduction to concentrations below 20 mg/L over the entire length of the column, immediately following feeding and throughout the duration of each incubation period. As during Operational Phase A, iron reduction was also evident. However, iron reduction did not become significant or consistent over the length of Column A until Incubation Period 2B, presumably after nitrate-reduction was complete. The progressive increase in soluble iron concentrations over the column length following Incubation Period 2B suggests that iron reduction was gradual. The observed soluble iron gradients could, in part, have been influenced by precipitation within the upstream portion of the column. Initially, during Incubation Period 1B, this may have been the result of precipitation with phosphate remaining within the column following inoculation. During subsequent incubation periods, the lower levels of soluble iron are likely to have

been partly attributable to ferrous iron precipitation with sulfide resulting from the reduction of sulfate between Ports 2 and 4.

TCE Transformation

TCE monitoring data from Operational Phase B is summarized in Appendix D-4. For a two-week period following the second inoculation of Column A, there was no clear evidence of TCE transformation. By the first week of the two-week-long Incubation Period 3B, evidence of TCE dechlorination re-appeared. By Incubation Period 4B, the zonation and magnitude of TCE transformation was consistent with those observed during the final five weeks of Operational Phase A.

Sampling was performed every 2-3 days during Incubation Period 4B. The increase in temporal resolution revealed that TCE transformation was maximal during the first four days of the period. Further, co-incident cis-DCE production was observed. However, port-specific concentrations of cis-DCE did not account for the mass of TCE transformed. Specifically, a cumulative loss of TCE of approximately 9 μM was observed at Port 7 at the conclusion of Incubation Period 4B. During this same period, a cis-DCE concentration of approximately 2 μM was measured after 4 days (Port 4) of pore fluid travel time. From day 4 onward, the cis-DCE concentrations progressively decreased with no co-incident accumulation of VC. The loss in aqueous cis-DCE mass while TCE transformation continued may indicate a transition to more complete halorespiration downgradient of Port 4.

The behavior revealed during Incubation Period 4B suggested that even tighter temporal and spatial sampling resolution would more accurately reveal the zonation,

magnitude and gradients associated with TCE transformation. Therefore, sampling was conducted daily, and, when possible, at each sampling port during Incubation Periods 5B and 6B. Time series plots of TCE, cis-DCE and VC over Incubation Periods 4B, 5B and 6B are presented in Appendix E. As seen on these figures, the data produced during Incubation Period 5B confirmed that TCE transformation occurred predominantly within the column segment bordered by the influent and Port 4 and that, although transformation appeared to occur during the entire period, k' values were generally at maximum levels through day 3 (Port 3). Approximately 6 μM of TCE transformation within this zone was accompanied by co-incident production of 5 μM of cis-DCE and 1 μM of VC.

During the subsequent and final inoculation period (Incubation Period 6A), the concentration of lactate in the feed solution was doubled to approximately 500 mg/L (5-6 mM). This was done to evaluate if the additional lactate mass would extend the duration or increase the magnitude of TCE transformation. However, the only clearly observable result was a proportionally greater production of cis-DCE and VC through day 2 of this incubation period compared to Incubation Period 5B. By the second day of the period, 8 μM of aqueous TCE was transformed, while 10 μM of cis-DCE and 3 μM of VC were produced. VC levels continued to increase through Day 3. Thereafter, cis-DCE and VC transformation appeared to occur with consistency. Conversely, TCE levels in groundwater actually increased by approximately 2 μM through Day 5, suggesting that TCE desorption rates exceeded the transformation rates between Ports 3 and 5. Sorbed and aqueous TCE levels apparently equilibrated after this time and, between days 5-7, TCE dechlorination rates progressively increased, resulting in a net loss of TCE.

Summary – Operational Phase B

In total, the fatty acid and inorganic data reveal that the ORP within Column A was highest immediately following re-inoculation, with nitrate reduction as the dominant terminal electron accepting process. Within one week of re-inoculation and throughout the remainder of Operational Phase B, iron reduction became consistent, and short-term occurrences of sulfate reduction became evident. Sulfate reduction appeared to occur for 1-2 days within the length of the column bordered by Ports 2 and 4, after which the ORP of the system slowly recovered to background levels with the influx of non-amended groundwater. As during Operational Phase A, Cr(VI) was consistently reduced, resulting in soluble chromium levels dropping from the influent concentration range of 200-250 $\mu\text{g/L}$ to below 20 $\mu\text{g/L}$. Typically, treatment to these levels was complete at a point upgradient of Port 2.

TCE Dechlorination Kinetics and Mass Removal

Assuming first-order kinetics, an examination of TCE, cis-DCE and VC transformation rates was facilitated through performing mass balances on a series of discrete control volumes juxtaposed over the length of Column A. The column length associated with each control volume was defined by the distance traveled by pore fluids over a 1-day period, as determined from the average linear velocity of groundwater flow. Accordingly, a total of seven column segments approximately 15 cm in length were established. Due to the temporal resolution of the associated data sets, TCE results from Incubation Periods 4B-6B were used to complete the analysis of transformation rates.

Port-specific TCE results for each day of sampling during these incubation periods are presented in Appendix F. The results from these analyses are summarized on Table 3.3.

Table 3.3. First order rate coefficients, k' , within control volumes of Column A between October 3 and October 22, 2002.

Date	Influent to Port 1	Port 1 to Port 2	Port 2 to Port 3
October 3, 2002	0.39 day ⁻¹	0.36 day ⁻¹	0.25 day ⁻¹
October 5, 2002	0.45 day ⁻¹	0.04 day ⁻¹	0.05 day ⁻¹
October 8, 2002	0.28 day ⁻¹	0.06 day ⁻¹	0.06 day ⁻¹
October 9, 2002	0.20 day ⁻¹	0.25 day ⁻¹	0.08 day ⁻¹
October 10, 2002	0.27 day ⁻¹	0.07 day ⁻¹	0.00 day ⁻¹
October 11, 2002	0.47 day ⁻¹	0.11 day ⁻¹	0.04 day ⁻¹
October 12, 2002	0.47 day ⁻¹	0.09 day ⁻¹	0.12 day ⁻¹
October 14, 2002	0.23 day ⁻¹	0.07 day ⁻¹	0.01 day ⁻¹
October 15, 2002	0.67 day ⁻¹	0.14 day ⁻¹	0.00 day ⁻¹
October 16, 2002	0.06 day ⁻¹	0.07 day ⁻¹	0.26 day ⁻¹
October 17, 2002	0.53 day ⁻¹	0.37 day ⁻¹	0.36 day ⁻¹
October 18, 2002	0.32 day ⁻¹	0.10 day ⁻¹	0.00 day ⁻¹
October 19, 2002	0.40 day ⁻¹	0.31 day ⁻¹	0.00 day ⁻¹
October 20, 2002	0.37 day ⁻¹	0.15 day ⁻¹	0.00 day ⁻¹
October 21, 2002	0.36 day ⁻¹	0.40 day ⁻¹	0.00 day ⁻¹
October 22, 2002	0.44 day ⁻¹	0.27 day ⁻¹	0.00 day ⁻¹

The rate analyses revealed that TCE transformation was most rapid within the column segment between the influent and Port 1. Here, k' values of 0.2-0.7 days⁻¹ were measured. These rates are well within the range estimated from both the batch microcosm study and the small-scale column experiment. Beyond Port 1, the rates of transformation decrease. Specifically, between Ports 1 and 2, k' values of 0.06 to 0.4 days⁻¹ were observed. As exhibited in Appendix F, cis-DCE and VC accumulated upgradient of Port 4 during Incubation Periods 4B-6B. Beyond Port 4, apparent first-order rate transformation rates for cis-DCE and VC averaged 0.51 day⁻¹ and 0.45 day⁻¹,

respectively. Maximum k' values estimated from the batch microcosm data were 3.04 day⁻¹ (cis-DCE) and 0.49 day⁻¹ (VC).

Whole-column mass balance analyses were conducted to estimate the approximate mass removal efficiency during each 1-2 week incubation period of the large-scale column experiment. The results from these calculations are presented on Table 3.4. These data reveal that, on average, 37% of the TCE mass entering Column A was removed during the eight incubation periods (e.g. Incubation Periods 5A-8A and 3B-6B) during which transformation was most evident. Maximum TCE removal (45%) appeared to occur during Incubation Period 4B. TCE removal was lowest (26%) during Incubation Period 6A. Based on the transformation rate examination, it is inferred that TCE removal is nearly exclusively attributable to microbial dechlorination. From the analyses of transformation rates summarized above, it is evident that the bulk of TCE dechlorination occurred between the column influent and Port 3, where k'' values were most significant and consistent. Downgradient of Port 2, transformation appears to be most significant between days 1 and 4 of each incubation periods. Therefore, the bulk of TCE transformation occurred within 4 days of each feeding event.

Table 3.4. TCE mass treatment rates and efficiency over the length of Column A over the duration of incubation periods 5A-8A and 3B-6B.

Incubation Period	TCE Removal Rate (µg/L)	Mass Removed (µg)	Treatment Efficiency (%)
5A	42	292	36
6A	24	169	26
7A	36	253	30
8A	30	207	30
3B	47	656	43
4B	35	248	42
5B	20	141	29
6B	24	171	36
<i>Average</i>	35	276	37

3.5.3 Redox Gradients and Halorespiration Activity

Within the Column A environment, spatially distinct but not separate zones of fermentation activity, nitrate and ferric iron reduction and sulfidogenesis were evident. Under near steady-state conditions lactate fermentation occurred over less than a 24-hour period upstream of Port 1. Nitrate and ferric iron levels were maintained at low concentrations between the column influent and Port 1 due to biologically-mediated reduction reactions accelerated by hydrogen and acetate produced from lactate and propionate fermentations. However, in contrast to the sources of nitrate and sulfate, aqueous ferric iron concentrations within Column A were probably sustained by the dissolution of oxyhydroxides from the aquifer solids. Consequently, iron reduction was evident over the length of the column. Coincident with the sharp increase in fermentation byproducts, sulfate reduction was the dominant terminal electron accepting process, as sulfidogenesis in Column A was enhanced in the presence of acetate. Under such conditions, a certain level of methanogenesis was also expected, as methanogens are

more efficient utilizers of acetate. For the quantity of lactate typically fed to the system, nitrate- and iron-reduction were sufficient to limit the availability of reducing equivalents for sustained sulfidogenesis and methanogenesis throughout the duration of each incubation period. During Incubation Period 6B, perturbing the system with a pulse of lactate mass in excess of that to which the microflora in the column had become acclimated following previous feeding events appeared to temporarily increase the magnitude and duration of sulfate reduction, as sulfidogenesis was enhanced by the excess acetate produced from fermentation reactions. However, evidence of significant levels of sulfate reduction downgradient of Port 3 was scarce. Conversely, the qualitative assessment of methane data collected during the latter stages of the experiment suggested that methanogenesis gradually increased over the length of the column.

Halo-respiration activity appeared to coincide to some extent with nearly all of these redox processes. However, it was most strongly associated with the fermentation activity and sulfidogenesis. During the large column study, TCE dechlorination was not clearly evident until efficient lactate- and propionate-fermenting conditions prevailed. Such conditions were initially evident during Incubation Periods 4A and 3B, which were also the first instances of distinct TCE transformation during Operational Phases A and B, respectively. However, the completeness of TCE dechlorination increased as acetate concentrations became limited. Specifically, less complete TCE dechlorination was consistently evident immediately behind the pulse of acetate passing through Column A during each incubation period. For example, during Incubation Period 6B, when the lactate loading to the column was increased from approximately 200 mg/L to over 500 mg/L, acetate and propionate concentrations more than doubled over levels measured

during previous incubation periods. The reducing power associated with the excess acetate, and residual propionate, served to locally and temporarily decrease the ORP to the level of the sulfate reduction and methanogenesis as the pulse of these fermentation byproducts traveled through the column. Once sulfate levels became limited, the activity of acetate-oxidizing, halo-respiring populations like *Desulfuromonas* probably increased. In general, wherever sulfate levels were depleted in the presence of acetate, incomplete reductive dechlorination prevailed. The result was an accumulation of cis-DCE. However, downstream of Port 4, evidence of sulfate reduction was scarce and may have been inhibited by an increase in methanogenic activity. Consequently, evidence of TCE transformation from Port 4 to the column effluent is limited.

As acetate and propionate levels decreased due to bulk transport through the column segments in which incomplete reductive dechlorination activity was occurring, respiration of TCE by more efficient hydrogenotrophic dechlorinators such as *Dehalococcoides* became evident and cis-DCE levels began to decline. This was expected, based on the presumption that halo-respiring microorganisms such as *D. ethenogenes* prefer dissolved hydrogen as an electron donor for their energy-deriving TCE dechlorination reactions. An environment possessing limited levels of dissolved hydrogen as the predominant source of reducing power is likely to be selective for *Dehalococcoides sp.* populations over microorganisms capable of partial TCE halo-respiration such as *Desulfuromonas*, which prefer acetate as an electron donor. Concurrently, VC accumulated in response to cis-DCE dechlorination. With continued consumption and transport, hydrogen levels within the column segment between the influent and Port 3 eventually dropped to sub-micromolar concentrations. Under these

conditions, halorespiration activity was apparently exclusively attributable to *Dehalococcoides*, as TCE dechlorination occurred without coincident production of cis-DCE. With time, TCE dechlorination gradationally decreased over the column length in correspondence with the spatial and temporal decline in hydrogen partial pressures.

As previously discussed, a symbiotic relationship appeared to exist between TCE to cis-DCE dechlorinating populations and *Dehalococcoides* during this study.

Specifically, the dechlorination of TCE to cis-DCE was accomplished rapidly by acetate-oxidizing microorganisms such as *Desulfuromonas sp.* Under acetate-limited conditions, the corresponding reduction in TCE levels facilitated cis-DCE and VC dechlorination by *Dehalococcoides sp.* at accelerated rates. However, because of differences in scale and hydrodynamics, the balance of this relationship was markedly different within the small-scale columns than within large-scale Column A.

The incubation periods during the small-scale column experiment were hydraulically stagnant. During these “no-flow” periods, acetate and hydrogen produced from lactate and propionate fermentation were continuously in direct contact with the Plume F/G aquifer materials. Due to this prolonged exposure to the acetate and hydrogen pulses, the efficiency of the symbiotic relationship between the two sets of dechlorinating populations was probably less than within the continuous flow system of Column A. Consequently, evidence of reductive dechlorination exhibited a more pronounced transition from 1) TCE respiration and cis-DCE accumulation to 2) cis-DCE respiration and 3) VC dechlorination. This may, in part, explain the disproportionately greater accumulation of VC at the conclusion of each incubation period of the small-scale

column experiment compared to the minor accumulations of VC observed at any point within Column A over the same interval of time.

It is also plausible that VC dechlorination rates in the small columns were significantly less than those observed in Column A because of the comparatively lower soil mass and, therefore, mass and availability of iron in those systems. The increased availability of ferric iron associated with the scale and flow-through operation of Column A may have served to promote complete TCE dechlorination by creating a hydrogen sink, thereby maintaining H₂ partial pressures at levels that permitted *D. ethenogenes* to out-compete other dechlorinators in the system. Acetate and hydrogen produced through fermentation may have been sufficient to drive ferric iron reduction to near completion near the column influent. However, time-series data reveal that iron reduction continued over the length of the column. Acetate and hydrogen pulses migrating through the column decreased the ORP within the system and promoted solubilization of iron hydroxides off the aquifer solids. This provided a surplus of electron acceptors for hydrogenotrophic iron-reducing bacteria, thereby contributing to consumption of hydrogen reducing equivalents.

Consideration must also be given to the possibility that the elevated hydrogen partial pressures within the spatially constrained small columns resulting from continuous exposure to the anaerobic glove-box environment served to upset the relationship between complete and incomplete dechlorinating populations. Under hydrogen-rich conditions the rates of TCE and cis-DCE respiration and subsequent VC dehalogenation would likely be suppressed as a result of competitive inhibition by other hydrogenotrophic populations, including sulfidogens and methanogens.

From an engineering standpoint, these observations emphasize the need to collect a temporally relevant set of soil and groundwater samples for characterization of major anions and dissolved gasses, and on which to perform biomolecular analyses such as T-RFLP and RT-PCR to assess microbial community structures and identify the presence of dechlorinating populations in solvent-impacted aquifer materials. This information will provide the insight necessary to strategize the magnitude and frequency of hydrogen loadings to promote complete TCE dechlorination. If an organic acid such as lactate is delivered to aquifer materials to release hydrogen for stimulation of dechlorination activity, establishment and acclimation of a BAZ will be most dependent on the colonization of the aquifer materials by fermenting microorganisms.

3.5.4 Hexavalent Chromium Reduction and Immobilization

During both the small- and large-scale column experiments, the decrease in ORP resulting from bioaugmentation and lactate feeding appeared sufficient to consistently provide conditions favorable for Cr(VI) reduction. At the pH range typical of the Plume F/G groundwater (e.g., 6.8 to 7.4), Cr(VI) reduction to Cr(III) is favored when the redox potential of a system is less than 60 mV (Palmer and Wittbrodt, 1991). Eh measurements periodically obtained during the large-scale column experiment revealed that the redox potential of the Column A environment was consistently less than -50 mV, and frequently as low as -200 mV. Therefore, conditions favorable for Cr(VI) reduction prevailed within the bioaugmented Plume F/G aquifer materials throughout the duration of the experiment. Further, soluble chromium concentrations entering the column were

consistently reduced by more than an order of magnitude to less than 20 $\mu\text{g/L}$, indicating fixation of Cr(III) to the aquifer solids.

3.6 Summary

From the interpretations summarized above, the following observations may be made regarding Cr (VI) detoxification, TCE transformation, redox gradients, and microbial community structure within the continuous flow model aquifer system, Column A:

1. The decrease in ORP resulting from inoculation and periodic feeding of Column A resulted in rapid and consistent decreases in soluble chromium levels within pore fluids conveyed through the system. Therefore, bioaugmentation of the Plume F/G aquifer materials with the BR Enrichment appears beneficial for reduction Cr(VI) and immobilization of Cr(III).
2. Substrate and nutrient stock fed to Column A each week was restricted to a volume sufficient to displace the pore fluids in the segment of column between the influent and sampling Port #3. Evidence of lactate fermentation was restricted to this column segment (e.g., “the fermentation zone”).
3. Reductive dechlorination activity observed during the experiment was highly associated with the fermentation zone. Outside this zone, including within column

segments through which residual propionate and acetate were transported, evidence of dechlorination was sparse.

4. The association of reductive dechlorination and fermentation activity may indicate a nutritional dependency of halorespiring populations on other members of the consortia. This distinct spatial relationship also suggests that the bulk of TCE transformation resulted from solids-associated dechlorinators.
5. Stoichiometrically excessive quantities of lactate will result in accumulations of acetate and propionate favorable for the stimulation of sulfidogenesis and methanogenesis. A redox shift to methanogenic conditions appears to inhibit dechlorination activity.
6. It is plausible that excess levels of acetate may create conditions that are selective for acetate-oxidizing halorespiring microorganisms that dechlorinate TCE to cis-DCE, such as *Desulfuromonas sp.*
7. When sulfate reduction was the dominant redox process within the fermentation zone, halorespiration rates were accelerated, but with a greater incidence of cis-DCE accumulation. More specifically, halorespiration appeared to be distinctly bi-phasic, with interspecies transfer of cis-DCE occurring between dechlorinators such as *Desulfuromonas sp.* and population(s) like *Dehalococcoides sp.* This was most evident at the downgradient edge of the fermentation zone and over other portions of

the Column A system temporarily affected by residual acetate levels, elevated hydrogen partial pressures, and increased rates of sulfate reduction.

8. The rates and completeness of TCE dechlorination appeared to increase as residual acetate levels within the fermentation zone diminished due to metabolism by sulfate reducing bacteria (SRB) and flushing. This was likely due to a corresponding decrease in methanogenesis and the low hydrogen thresholds associated with halorespiring microorganisms.
9. Within the upgradient portion of the fermentation zone, following the passage of acetate and propionate pulses, hydrogen partial pressures were at trace levels. Under these conditions, sulfate reduction was limited. Conversely, TCE dechlorination was rapid and complete. Under acetate- and hydrogen-limited conditions, TCE transformation was apparently less dependent on the transfer of cis-DCE between distinct populations of dechlorinating microorganisms, and more a result of halorespiration by microorganisms such as *Dehalococcoides sp.*

10. In summary, TCE dechlorination was spatially associated with the fermentation zone. Temporally within the fermentation zone, three phases of dechlorination activity were evident:

- I. **Fermentation:** At near steady-state conditions, lactate fermentation and nitrate reduction were complete within 24 hours of feeding. During this period, the

apparent rates of reductive dechlorination were comparatively low, and TCE transformation resulted in the accumulation of cis-DCE.

II. Iron and Sulfate Reduction: Following the decomposition of lactate, the activities of iron- and sulfate-reducing bacteria appeared to increase within the fermentation zone. The magnitude and completeness of reductive dechlorination associated with this period appeared to be a function of the extent of sulfate reduction. Specifically, when sulfate reduction was apparently complete, methanogenesis occurred and the rates and completeness of reductive dechlorination were very low, suggesting inhibition. Such conditions occurred when excess lactate concentrations were fed to the column resulting in an increase in acetate levels and apparent stimulation of acetate-oxidizing microorganisms including SRB and methanogens. Conversely, when the ORP of the system was insufficient to drive sulfate reduction to completion, but the activity of SRB appeared consistent, the rates of TCE dechlorination were high, and resulted in a rapid accumulation of cis-DCE. This suggested that TCE dechlorination was attributable to acetate-oxidizing bacteria like *Desulfuromonas sp.* The cis-DCE produced was further dechlorinated, but at comparatively lower rates, resulting in minor and temporary accumulations of VC. cis-DCE dechlorination was apparently associated with the activities of halorespiring microorganisms like *Dehalococcoides sp.*

III. Substrate-Limited Conditions: After a 3 to 4 day period, the time required for the acetate and propionate pulses produced during fermentation to travel through the BAZ, high rates of TCE dechlorination were evident, with little to no evidence of cis-DCE and VC accumulations. During the 3 to 4 day period leading up to the next feeding period, hydrogen partial pressures within the BAZ progressively decreased. This likely created environmental conditions that were selective for TCE halorespiring microorganisms like *Dehalococcoides* sp.

Chapter 4

FATE AND TRANSPORT MODELING

4.1 Introduction

Numerical modeling was performed to quantitatively examine biotransformation processes, and evaluate substrate/nutrient delivery schemes to optimize TCE treatment efficiency within the Column A system. The mathematical bases of the governing hydrodynamic and solute transport and fate processes and the computer codes used to solve the equations describing these processes, are summarized in Chapter 2. The physical, hydraulic and bio-kinetic parameters and associated values provided as input into these codes are presented in Chapter 3 and on Table 4.1. As identified on this table, these data were determined through direct measurement, scientific literature, or model calibration.

The hydraulic model established for this study was calibrated through simulations of tracer and TCE breakthrough, as described in Chapter 3. The numerical transport and fate model was calibrated using solute and biomass monitoring data obtained during Incubation Period 6B, due to the representativeness and abundance of both the spatial and temporal measurements associated with that period.

4.2 Model Calibration

To simulate the transport and transformation of TCE and growth and movement of biomass over the seven day duration of Incubation Period 6B, the eight mass balance equations summarized in Chapter 2 were solved numerically by finite difference

approximations using the RT-3D computer code (Clement, et.al., 2000). The spatial domain of Column A, as defined by the soil mass confined laterally within the 2.54 cm diameter glass tube and bordered longitudinally by the column influent and effluent, was discretized into a grid of 300 rectangular blocks, or cells. Each cell measured 2.25 cm in both height and width, and 0.37 cm in length. The total length of the column domain was 110.49 cm. The simulation period of seven days was discretized into 7,500 time steps, each 4×10^{-4} days in duration. The Euler Forward-Time Center-Space (FTCS) forward-stepping numerical integration method was used to solve the finite difference equations.

Initial and boundary conditions were imposed on each of the eight finite difference equations. The initial conditions specified that the concentrations of each parameter over the length of the column corresponded to direct laboratory measurements in samples collected from Column A on day 0 of Incubation Period 6B. Boundary conditions were specified at the influent and effluent ends of the column. These included constant flow conditions at both ends, and constant TCE flux at the influent. The initial and boundary conditions imposed on the model are summarized in Appendix A.

Assumptions implicit with these conditions include:

- Solid- and aqueous-phase TCE concentrations are in equilibrium.
- *Dehalococcoides sp.* are the exclusive agents of TCE, cis-DCE and VC dechlorination.
- Hydrogen is produced exclusively through lactate fermentation.
- Lactate fermentation is instantaneous.

The computer model simulated biomass growth and mobility, and the transport and utilization/transformation of TCE and hydrogen within Column A under both natural and imposed hydraulic gradients. For calibration and sensitivity analyses, model output, including TCE (both aqueous and sorbed concentrations), cis-DCE, VC and biomass (both attached and unattached) were compared to experimental data collected daily from sampling ports 1-7 during Incubation Period 6B. The model also produced hydrogen and ethene results.

To calibrate model-predicted with measured concentrations and gradients, second-order rate coefficients for TCE, cis-DCE and VC and spatial biomass concentrations (both planktonic and solid) were adjusted within ranges considered plausible from literature-derived values and measured quantities, respectively. Calibrated rates coefficients are presented on Table 4.1. The calibrated biomass distributions are graphically presented in Appendix G. In summary, the second-order rate coefficients calibrated from the modeling efforts were $4 \text{ Lmg}^{-1}\text{day}^{-1}$ for both TCE and cis-DCE transformation and $2 \text{ Lmg}^{-1}\text{day}^{-1}$ for VC dechlorination. As expected, the calibrated biomass distributions reveal significant accumulations of both solid phase and planktonic microbes within and immediately downgradient of the BAZ.

Experimental and predicted concentrations for all eight-model parameters (e.g., TCE, cis-DCE, VC, ethene, sorbed TCE, hydrogen, and attached and detached microbes) are presented in Appendix G-1. Both the discrete concentration data and chemical gradients associated with these constituents compare within a favorable level of tolerance to direct measurements, suggesting that the transport and fate model was well posed. In general, the model accurately predicted the transformation of TCE within the BAZ.

Downgradient of the BAZ, the model appears to underestimate TCE removal. The model results for cis-DCE and VC are also slightly under-predicted compared to measured concentrations. However, the spatial and temporal gradients of cis-DCE and VC production are consistent with measured results.

The model results indicate that as much as 2.5 mg/L of dissolved hydrogen were consumed during the incubation period by the halorespiring microorganisms resident in the column. Further, approximately 16 $\mu\text{g/L}$ of ethene were produced by the dechlorination reactions. Sorbed TCE levels decreased 3-fold within the BAZ during the seven-day simulation.

Predicted immobile biomass concentrations reached a maximum of 1.1×10^{-1} mg/L (approximately 10^6 CFU/mL) three days following feeding. Mobil biomass levels increased to a peak of 1.6×10^{-3} mg/L (approximately 10^4 CFU/mL) on day four of the simulated incubation period. These values appear excessive when compared to measured concentrations of *Dehalococcoides sp.* in Column A (refer to Section 3.5.2, Large-Scale Column Experiment, Operational Phase B, *Biomass*). However, it is likely that these assays were successful in quantifying only a small percentage of the population of halorespiring microorganisms within the column. Future studies should include RT-PCR assays with a broader array of primers to identify and quantify not only *Dehalococcoides sp.*, but also partial TCE dechlorinators such as, but not limited to, *Desulfuromonas sp.*

Table 4.1. Numerical model input parameter values and data sources.

Input Parameter	Definition	Value	Procedure	Source
θ	Sediment porosity	0.39	Measured	This study
α_L	Longitudinal dispersivity, m	0.01	Calibration	This study
K_d	Distribution coefficient, L/mg	7×10^{-8}	Measured	This study
ρ_b	Soil bulk density, mg/L	1.4×10^6	Measured	Mayotte, et al., 1996
f	Fraction of exchange sites at equilibrium	0.437	Literature	Phanikumar, et al., 2002
R_{DCE}	DCE retardation coefficient	1	Measured	This study
R_{VC}	VC retardation coefficient	1	Estimated	This study
R_{ETH}	Ethene retardation coefficient	1	Estimated	This study
R_{H_2}	H ₂ retardation coefficient	1	Estimated	This study
κ_i	Kinetic (de) sorption rate, 1/day	0.3	Literature	Phanikumar, et al., 2002
k'_{TCE}	TCE reaction rate, Lmg ⁻¹ day ⁻¹	4	Calibration	This study
k'_{DCE}	DCE reaction rate, Lmg ⁻¹ day ⁻¹	4	Calibration	This study
k'_{VC}	VC reaction rate, Lmg ⁻¹ day ⁻¹	2	Calibration	This study
k'_{ETH}	Ethene reaction rate, Lmg ⁻¹ day ⁻¹	0	Calibration	This study
μ_{max}	Maximum specific growth rate, 1/day	0.5	Literature	Ballapragada, et al., 1997
K_{TCE}	TCE half-saturation coefficient, mg/L	6.6×10^{-2}	Literature	Haston, McCarty, 1999
K_{H_2}	H ₂ half-saturation coefficient, mg/L	2×10^{-5}	Literature	Ballapragada, et al., 1997
$Y_{TCE-DCE}$	TCE/DCE stoichiometric constant	0.74	Calculation	This study
Y_{DCE-VC}	TCE/DCE stoichiometric constant	0.64	Calculation	This study
Y_{VC-ETH}	TCE/DCE stoichiometric constant	0.45	Calculation	This study
y_{TCE-X}	TCE yield	0.5	Estimated	This study
y_{H_2X}	Hydrogen yield	0.5	Estimated	This study
b_{DC}	Microbial decay rate, 1/day	0.1	Literature	Phanikumar, et al., 2002
K_{at}	Microbial attachment rate, 1/day	0.9	Literature	Phanikumar, et al., 2002
K_{de}	Microbial detachment rate, 1/day	0.018	Literature	Phanikumar, et al., 2002

4.3 Sensitivity Analyses

The sensitivities of variables to key bio-kinetic parameters and conditions were analyzed following model calibration. Specifically, these analyses included evaluating numerical changes in model variables, such as TCE, cis-DCE, VC, ethene, sorbed TCE, hydrogen and solid-phase and planktonic biomass concentrations, to a range of values for parameters such as: TCE, cis-DCE, and VC second-order rate coefficients; TCE and

hydrogen half-saturation and yield coefficients; the maximum specific microbial growth rate; and the endogenous decay rate. Additional sensitivity analyses included performing simulations by excluding or modifying certain terms of the mass balance equations describing the fate of the aforementioned model variables.

4.3.1 Variation Coefficients

The sensitivities of model variables to changes in the input values of the bio-kinetic parameters were evaluated through a series of independent model simulations. For each simulation, the value of a single parameter was increased by 50%. The resulting values for each variable were then systematically compared to calibrated values. The comparison included calculation of variation coefficient, ϕ , using the following relationship (Witt, 1998):

$$\phi = \frac{\Delta W / W}{\Delta P / P} \quad (4.1)$$

where P is a model parameter (k'_{TCE} , k'_{DCE} , k'_{VC} , μ_{max} , K_{TCE} , K_{H2} , y_{TCE-X} , y_{H2X} and b) and W is a variable (C_{TCE} , C_{DCE} , C_{VC} , C_{ETH} , C_{H2} , S_{TCE} , X_{im} and X_m). Variation coefficients associated with each parameter were calculated by first determining the largest change in W over the length of the column resulting from the change in P . This value was then divided by the original variable quantity. As indicated by equation 4.1, the result was further divided by the value of the magnitude of the change in P divided by the unchanged value of P . Since each parameter was increased by 50%, the value of the

denominator in equation 4.1 was fixed at 0.5. Therefore, a variation coefficient of less than unity indicated that the value of a variable changed less than two-fold (e.g., 50%) in response to the 50% increase of the value of a parameter. In general, a value ϕ of less than 0.80 indicated that changes in the magnitudes and distributions of the model variables were insignificant.

Variation coefficients determined through these sensitivity analyses are summarized on Table 4.2. These data indicate that C_{TCE} , C_{DCE} , C_{VC} , C_{ETH} , C_{H2} , S_{TCE} , X_{im} and X_m are all insensitive to changes in half-saturation and yield coefficients (K_{TCE} , K_{H2} and, y_{TCE-X} , y_{H2X} respectively). Only C_{H2} is sensitive to the changes in the decay coefficient, b_{DC} . C_{VC} and C_{ETH} are only slightly sensitive to changes in the second-order rate coefficients for TCE (k'_{TCE}) and DCE (k'_{DCE}); but both variables are significantly sensitive to changes in the maximum specific growth rate, μ_{max} . Similarly, both solid-phase and planktonic biomass concentrations (X_{im} and X_m , respectively) are highly sensitive to a change in μ_{max} .

Table 4.2. Variation coefficients for key model parameters on day 7 of Incubation Period 6B.

	k'_{TCE}	k'_{DCE}	k'_{VC}	μ_{max}	K_{TCE}	K_{H2}	y_{TCE-X}	y_{H2X}	b_{DC}
C_{TCE}	0.56	0.00	0.00	0.58	0.02	0.00	0.03	0.08	0.20
C_{DCE}	0.01	0.13	0.03	0.19	0.01	0.03	0.05	0.22	0.00
C_{VC}	0.79	0.24	0.01	0.50	0.02	0.01	0.02	0.09	0.33
C_{ETH}	0.84	0.92	0.01	1.70	0.05	0.01	0.01	0.22	0.68
C_{H2}	0.84	0.91	1.00	4.05	0.09	0.02	0.02	0.38	0.93
S_{TCE}	0.33	0.01	0.03	0.33	0.02	0.01	0.02	0.04	0.06
X_{im}	0.07	0.01	0.02	12.15	0.18	0.01	0.02	0.34	0.44
X_m	0.07	0.00	0.02	10.70	0.14	0.01	0.02	0.25	0.41

4.3.2 Bio-kinetics and Sorption

The responses of model variables to the exclusion or modification of certain terms in the eight mass balance equations were determined through additional sensitivity simulations. Specifically, these equations were numerically solved using calibrated model input values and initial and boundary conditions, after certain terms were manipulated to impose: 1) hydrogen starvation; 2) the absence of biochemical reactions; and 3) instantaneous TCE sorption/desorption. Time series plots of the results from these simulations are presented in Appendix G-2. As seen on these plots, both hydrogen starvation conditions and the absence of biochemical reactions, as described by the energy terms associated with equations 2.9 through 2.19, have profound effects on C_{TCE} , C_{DCE} , C_{VC} , C_{ETH} , C_{H2} , S_{TCE} , X_{im} and X_m . These results were expected. Specifically, the absence of hydrogen imposes a no-growth condition on biomass within the column, which in turn reduces the utilization of electron acceptor, TCE. Therefore, TCE transformation is severely limited. Similarly, the lack of biochemical reactions in the mass balance equations essentially eliminates all mechanisms of TCE transformation. Therefore, as expected, both the presence of hydrogen and inclusion of energy terms in the balance equations are necessary for providing accurate predictions of the TCE transformation observed in Column A. Conversely, the absence of rate-limited TCE sorption/desorption had a minimal affect on the predicted values of C_{TCE} , C_{DCE} , C_{VC} , C_{ETH} , C_{H2} , S_{TCE} , X_{im} and X_m .

4.3.3 Summary

The results from the sensitivity analyses support the assertion that biochemical reactions are the predominant mechanisms for TCE removal in Column A. Further, the products of these reactions are dependent on the selection of, and highly sensitive to changes in, the value of the maximum specific growth rate, μ_{max} for halorespiring biomass. Further, the reactions affecting ethene and hydrogen concentrations are sensitive to changes in second-order rate coefficients for TCE and cis-DCE and the microbial decay rate. Rate-limited sorption, although important for accurately simulating the rate of TCE transport through the column, appears to have minimal affect on the predictions of TCE transformation or microbial growth.

4.4 Predictions

The calibrated flow and reactive transport models were used to evaluate two (2) distinct substrate and nutrient delivery schemes. These schemes and the rationale associated with each included:

1. Increasing the dimensions of the BAZ through doubling the weekly feed volume conveyed through the column. The resulting expansion of the BAZ is expected to increase contaminant residence time and thereby improve TCE removal efficiency.
2. Using pore water from downgradient of the non-expanded BAZ as make-up water for feed solution mixing and delivery. Doing so may reduce the initial TCE load, and therefore the overall mass removal burden, within the BAZ immediately following

feeding. In effect, this should increase the rate and efficiency of solute treatment under the natural gradient conditions occurring between feeding events.

The model-generated results from simulations of each scenario are presented in Appendix G-3. These results suggest that both substrate/nutrient delivery strategies will enhance TCE treatment. However, although treatment rates are achieved rapidly when the BAZ is fed solutions mixed with BAZ-treated groundwater, the overall efficiency of TCE removal is not greatly improved. Conversely, TCE removal is clearly increased after the BAZ is widened through consistently delivery of an increased quantity of feedstock.

BAZ dimensions required for achieving requisite treatment objectives at field sites can be established and preliminarily evaluated by numerical analyses such as described herein. From the model-generated results presented in Appendix E-3, it appears that a 3-foot wide BAZ will reduce influent TCE levels of 2,500 $\mu\text{g/L}$ in Column A by approximately 90% within a four to seven day period following each feeding event.

Chapter 5

CONCLUSIONS AND RECOMENDATIONS

5.1 Conclusions

From the data presented in Chapter 3 and the results of the reactive transport modeling discussed in Chapter 4, the following conclusions may be made regarding the stated hypotheses (presented in bold text) of this study:

- 1. The inoculation of the Plume F/G aquifer materials with the BR Enrichment will produce a spatially dependent, stationary-phase microbial community profile.**

Due to unexpected analytical difficulties associated with quantifying biomass within the limited discrete quantities of soil obtained during the treatment phase of the study, it was not possible to directly characterize the solid-phase community profile within large-scale Columns A and B. However, dsDNA and RT-PCR analyses revealed that biomass, including *Dehalococcoides sp.*, was approximately equally distributed on both solids and in the liquid phase over the length of Column A. Further, evidence associated with substrate utilization, redox, and TCE transformation gradients indirectly revealed spatial and temporal relationships between key biologically mediated processes, without discretion to phase associations. Specifically, upon development of near steady-state TCE transformation conditions, fermentation was most evident between the column influent and Port 3. Nitrate reduction occurred to completion within the column segment between the influent and Port 1. Iron reduction was consistently evident over the length

of the column and appeared to be the dominant terminal electron accepting process during this period. Sulfate reduction occurred on a temporary basis between Port 1 and Port 4. The duration and magnitude of sulfate reduction was dependent on the mass of lactate delivered to the column. Similarly, methanogenesis was evident on a temporary basis between Port 2 and the column effluent.

- 2. Within the community profile, a biologically active zone (BAZ) consisting of spatially and/or temporally discrete regions of sulfate-reduction, methanogenesis, and halorespiration will exist wherein transformation of TCE to non-toxic end products will occur.**

Substrate and nutrient stock fed to Column A each week was restricted to a volume sufficient to displace the pore fluids in the segment of column between the influent and sampling Port 3. As discussed above, evidence of lactate fermentation was confined to this column segment (e.g., “the fermentation zone”). Reductive dechlorination activity observed during the experiment was highly associated with the fermentation zone. Outside this zone, including within column segments through which residual propionate and acetate were transported, evidence of dechlorination was sparse.

Delivery of a quantity of lactate in excess of that used to establish near steady-state TCE dechlorination activity resulted in accumulations of acetate and propionate favorable for the stimulation of sulfidogenesis and methanogenesis. A redox shift to methanogenic conditions appeared to inhibit dechlorination activity. When sulfate reduction was the dominant redox process within the fermentation zone, halorespiration

rates were accelerated, but with a greater incidence of cis-DCE accumulation. The rates and completeness of TCE dechlorination appeared to increase as residual acetate levels within the fermentation zone diminished due to metabolism by sulfate reducing bacteria (SRB) and flushing. Within the upgradient portion of the fermentation zone, following the passage of acetate and propionate pulses, hydrogen partial pressures were at trace levels, based on modeling results. Under these conditions, sulfate reduction was limited. Conversely, TCE dechlorination was rapid and complete. In summary, three temporally distinct phases of dechlorination activity were evident within the fermentation zone/BAZ:

- I. Fermentation: At near steady-state conditions, lactate fermentation and nitrate reduction were complete within 24 hours of feeding. During this period, the apparent rates of reductive dechlorination were comparatively low, and TCE transformation resulted in the accumulation of cis-DCE.
- II. Iron and Sulfate Reduction: Following the decomposition of lactate, the activities of iron- and sulfate-reducing bacteria appeared to increase within the fermentation zone. The magnitude and completeness of reductive dechlorination associated with this period appeared to be a function of the extent of sulfate reduction. Specifically, when sulfate reduction was complete, methanogenesis occurred and the rates and completeness of reductive dechlorination were low, suggesting inhibition. Such conditions occurred when excess lactate concentrations were fed to the column resulting in an increase in acetate levels and stimulation of acetate-oxidizing

microorganisms including SRB and methanogens. Conversely, when the ORP of the system was insufficient to drive sulfate reduction to completion, but the activity of SRB appeared consistent, the rates of TCE dechlorination were high, and resulted in a rapid accumulation of cis-DCE. This suggested that TCE dechlorination was attributable to acetate-oxidizing bacteria. The cis-DCE produced was further dechlorinated, but at comparatively lower rates, resulting in minor and temporary accumulations of VC.

III. Substrate-Limited Conditions: After a 3 to 4 day period, the time required for the acetate and propionate pulses produced during fermentation to travel through the BAZ, high rates of TCE dechlorination were evident, with little to no evidence of cis-DCE and VC accumulations.

3. Within the BAZ, the groundwater redox chemistry will favor for the reduction of Cr(VI) to and immobilization of Cr(III).

The decrease in ORP resulting from inoculation and periodic feeding of Column A consistently resulted in rapid decreases in soluble chromium levels within pore fluids conveyed through the system. Specifically, soluble chromium levels in excess of 200 µg/L entering the column were consistently reduced to below 20 µg/L before pore fluids traveled to Port 1 of Column A. Therefore, bioaugmentation of the Plume F/G aquifer materials with the BR Enrichment was beneficial for reduction Cr(VI) and immobilization of Cr(III).

To date, most experimental work to evaluate the dechlorination behavior of mixed and pure cultures in native and non-native aquifer materials has been performed in laboratory microcosms in which environmental and nutritional conditions are strictly controlled. Departures from this trend include work by Isalou and Sleep (1998), Harkness, et. al. (1999), and Cirpka, et. al. (1999), which examined dehalogenation activities expressed by non-native mixed enrichment cultures and halorespiring isolates in flow-through systems packed with aquifer materials and maintained under natural conditions. These studies emphasized the spatial measurement of electron donors and acceptors, chlorinated solvent dehalogenation and metabolic byproducts associated with the activity of the augmented microbial community. However, with the exception of work done in fractured and granulated basalts by Lehman, et.al. (2001), few studies have attempted to evaluate the transport behavior of inoculates during delivery, or correlate the spatial and temporal variability of aquifer geochemistry and contaminant transformation with the structures of mobile- and stationary-phase microbial communities resulting from bioaugmentation. The work summarized herein was an attempt to examine such relationships and advance the current understanding of the successional adaptations of in-situ treatment regimes following inoculation of aquifer materials. Specifically, the results produced from this experimentation reveal that TCE transformation by halorespiring microbial populations is closely associated with fermentation activity, and that dechlorination rates and completeness will depend on redox conditions. Evidence associated with substrate utilization, redox, and TCE transformation gradients indirectly revealed spatial and temporal relationships between key biologically mediated processes, without discretion to phase associations. Reductive dechlorination activity observed

during the experiment was highly associated with the fermentation zone. Outside this zone, including within column segments through which residual propionate and acetate were transported, evidence of dechlorination was sparse. A redox shift to methanogenic conditions appeared to inhibit dechlorination activity. When sulfate reduction was the dominant redox process within the fermentation zone, halorespiration rates were accelerated, but with a greater incidence of cis-DCE accumulation. The rates and completeness of TCE dechlorination appeared to increase as residual acetate levels within the fermentation zone diminished due to metabolism by sulfate reducing bacteria (SRB) and flushing. Within the upgradient portion of the fermentation zone, following the passage of acetate and propionate pulses, hydrogen partial pressures were at trace levels, based on modeling results. Under these conditions, sulfate reduction was limited. Conversely, TCE dechlorination was rapid and complete. This knowledge could benefit strategies for optimizing biotransformation capacities within bioaugmentation treatment zones. General recommendations developed from these observations are presented below.

5.2 Recommendations

Engineering considerations associated with the use of the Bachman Road Enrichment for full-scale bioaugmentation of non-native aquifer materials include:

1. Design of full-scale bioaugmentation strategies will depend on the TCE concentrations within target treatment zones, regulatory cleanup criteria, and the viability and rates of TCE dechlorination expressed by the enrichment in site aquifer

materials. Apparent dechlorination rates estimated from small-scale column studies may be used to establish the width of the BAZ (and, therefore, zone of fermentation) necessary to accomplish regulatory cleanup objectives.

2. Any bioaugmentation strategy that requires lactate as a carbon and energy source should include substrate delivery schemes that maximize the production of hydrogen and minimize accumulation of acetate and propionate. Ideally, the feeding scheme should minimize the activity of SRB and promote a swift transition from fermentation to acetate-starvation within the BAZ. In general, this may be accomplished by pulsed injection of a lactate mass that is stoichiometrically less than the mass required to drive sulfidogenesis to completion, but sufficient to provide residual hydrogen at levels above threshold concentrations for halorespiring microorganisms like *Dehalococcoides sp.*
3. Design of full-scale bioaugmentation strategies using the Bachman Road Enrichment should include the following general steps:
 - a. Establish range of TCE dechlorination rates from column microcosm studies using the enrichment and aquifer materials from the treatment site.
 - b. Estimate the width of the fermentation zone (e.g., BAZ) required to accomplish the desired level of treatment, including consideration of an appropriate safety factor.

- c. Develop strategies to deliver inoculum and feed rapidly and uniformly over the desired width of the BAZ. Use of a three well injection/extraction/re-injection strategy is recommended to maximize the uniformity of inoculum and feed delivery.
- d. If practical, assess performance of the BAZ with a multi-well, multi-level monitoring system installed across the width of the BAZ to facilitate sample acquisition at spatial and temporal resolution of the redox and dechlorination dynamics within the zone of fermentation.

Based on the experiences during the experiments summarized herein, the following recommendations should be considered in the design of column studies for research on the relationships between redox gradients, microbial community structure and dechlorination activity:

1. Improve gas-monitoring capabilities to facilitate more accurate measurement of headspace gasses and thereby improve the assessment of hydrogen consumption and completion of TCE transformation mass balances.
2. Improve resolution of longitudinal sampling to improve the tracking of inoculum transport and breakthrough and the understanding of redox zonation within fermentation zones.

3. Improve temporal monitoring under near steady-state conditions by sampling daily and at every port. Aqueous analyses of these samples should include soluble metals.
4. Extend the duration of similar experiments to more precisely resolve trends in dechlorination activity, redox gradients, and the occurrence and completeness of halorespiration.
5. Improve liquid and solid microbiological monitoring of communities structure and halorespiring microorganisms to refine interpretations of correlations between redox conditions and halorespiration activity.
6. Utilize molecular techniques such as RT-PCR to identify and quantify the presence of both *Desulfuromonas sp.* and *Dehalococcoides sp.*
7. Comprehensively evaluate the individual sorption characteristics of TCE, cis-DCE and VC within the subject aquifer materials to improve the validation and accuracy of numerical models used to simulate and optimize the performance of in-situ bioaugmentation treatment zones.
8. Measure the yield of subject enrichment cultures when grown in the presence of TCE, cis-DCE and VC to provide plausible input to and accuracy of numerical modeling efforts.

9. Future modeling efforts should endeavor to include consideration of acetate utilization and cis-DCE production by *Desulfuromonas sp.* and hydrogen utilization and cis-DCE respiration by *Dehalococcoides sp.*

APPENDIX A

Large Scale Column A Operational History

COLUMN A OPERATIONAL HISTORY - CONCENTRATIONS

Stress Period	Flow Rate (mL/min)	Duration (min)	Stock Date (feedings in italics)	Influent Solute Concentration (- mg/L)															
				TCE	DCE	VC	LA	AA	PA	H2	Nitrite	Cl	Br	Nitrate	PO4	Sulfate	Cr	Fe	
1	2.0000	200	June 11, 2002	0.34	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
2	0.0000	2780	June 13, 2002	0.33	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
3	8.0000	100		0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
4	0.0000	4220	June 16, 2002	0.33	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
5	8.0000	100		0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
6	0.0000	2780	June 18, 2002	0.33	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
7	8.0000	100		0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
8	0.0000	2780	June 20, 2002	0.29	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
9	8.0000	100		0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
10	0.0000	2780	June 22, 2002	0.38	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
11	8.0000	100		0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
12	0.0000	1340	June 23, 2002	0.41	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
13	8.0000	100		0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	79.00	0.00	20.00	5.00	38.00	0.29	0.04	
14	0.0000	5630	June 27, 2002	0.24	0.03	0.00	466.00	0.00	0.00	20.69	0.00	197.00	0.00	28.00	59.00	55.00	0.95	0.35	
15	10.0000	70		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	32.00	0.00	56.00	1.03	0.03
16	0.0000	60	June 28, 2002	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	98.00	0.00	31.00	18.00	51.00	0.95	0.35	
17	1.2700	2880		0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	78.00	0.00	28.00	0.00	39.00	1.13	0.18
18	1.2700	4320	July 3, 2002	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	32.00	0.00	56.00	1.03	0.03	
19	0.1000	720		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	32.00	0.00	56.00	1.03	0.03
20	0.0000	60	July 3, 2002	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	32.00	0.00	56.00	1.03	0.03	
21	1.2700	1380		0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	77.00	0.00	19.00	0.00	30.00	0.94	0.32
22	1.2700	4320	July 6, 2002	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	0.00	4.00	22.00	1.02	0.11	
23	1.2700	2100		0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	0.00	4.00	22.00	1.02	0.11
24	0.0000	60	July 11, 2002	2.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	0.00	4.00	22.00	1.02	0.11	
25	1.2700	1360		2.11	0.00	0.00	0.00	337.00	0.00	0.00	14.96	0.00	83.00	0.00	18.00	0.00	34.00	0.73	0.03
26	10.0000	20	July 12, 2002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	83.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27	0.0000	60		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	83.00	0.00	0.00	0.00	0.00	0.00	0.00
28	1.2700	4320	July 12, 2002	2.26	0.00	0.00	0.00	0.00	0.00	0.00	21.00	83.00	21.00	35.00	5.00	18.00	1.47	0.05	
29	1.2700	5700		1.29	0.01	0.00	0.00	0.00	0.00	0.00	0.00	21.00	83.00	21.00	35.00	5.00	18.00	0.29	0.04
30	0.0000	60	July 19, 2002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	154.00	0.00	54.00	0.00	146.00	0.66	0.04	
31	1.2700	5760		1.07	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	132.00	0.00	39.00	0.00	82.00	0.77	0.06
32	1.2700	4190	July 23, 2002	1.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	134.00	0.00	40.00	0.00	105.00	0.82	0.01	
33	0.0000	60		2.20	0.00	0.00	0.00	410.00	0.00	0.00	18.21	0.00	134.00	0.00	40.00	0.00	105.00	0.82	0.01
34	10.0000	10	July 26, 2002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	134.00	0.00	40.00	0.00	105.00	0.82	0.01	
35	0.0000	60		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	134.00	0.00	40.00	0.00	105.00	0.82	0.01
36	1.2700	4320	July 26, 2002	2.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	80.00	0.00	17.00	0.00	28.00	0.69	0.00	
37	1.2700	5700		3.67	0.01	0.00	0.00	0.00	7.00	0.00	0.00	0.00	80.00	0.00	17.00	0.00	28.00	0.69	0.00
38	0.0000	60	July 29, 2002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	78.00	0.00	18.00	0.00	28.00	0.71	0.01	
39	10.0000	10		1.88	0.00	0.00	0.00	288.00	0.00	0.00	12.79	0.00	78.00	0.00	18.00	0.00	28.00	0.71	0.01

Stress Period	Flow Rate (mL/min)	Duration (min)	Stock Date (feedings in italics)	Influent Solute Concentration (- mg/L)															
				TCE	DCE	VC	LA	AA	PA	H2	Nitrite	Cl	Br	Nitrate	PO4	Sulfate	Cr	Fe	
40	0.0000	60																	
41	1.2700	5760	August 1, 2002	3.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	78.00	0.00	18.00	0.00	28.00	0.71	0.01	
42	1.2700	4190	August 6, 2002	2.82	0.01	0.00	0.00	0.00	0.00	0.00	0.00	70.00	0.00	71.00	0.00	77.00	0.25	0.19	
43	0.0000	60																	
44	10.0000	10	August 9, 2002	3.59	0.01	0.00	134.00	0.00	2.00	5.95	0.00	35.00	0.00	66.00	0.00	91.00	0.24	0.03	
45	0.0000	60								0.00									
46	1.2700	5760	August 9, 2002	4.89	0.01	0.00	0.00	0.00	0.00	0.00	0.00	35.00	0.00	66.00	0.00	91.00	0.24	0.05	
47	1.2700	4191.8	August 13, 2002	4.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00	99.00	0.00	21.00	0.00	30.00	0.22	0.29	
48	0.0000	60								0.00									
49	10.0000	8.2	August 16, 2002	3.51	0.03	0.00	169.00	0.00	11.00	7.50	0.00	109.00	0.00	1.00	0.00	44.00	0.22	0.03	
50	0.0000	60								0.00									
51	1.2700	5760	August 16, 2002	2.34	0.02	0.00	0.00	0.00	0.00	0.00	0.00	109.00	0.00	1.00	0.00	44.00	0.22	0.03	
52	1.2700	4260	August 20, 2002	3.82	0.01	0.00	0.00	0.00	0.00	0.00	0.00	78.00	0.00	18.00	0.00	31.00	0.26	0.14	
53	0.0000	60								0.00									
54	10.0000	10	August 23, 2002	3.71	0.01	0.00	218.00	0.00	2.00	9.68	0.00	79.00	0.00	18.00	0.00	34.00	0.25	0.00	
55	0.0000	60								0.00									
56	1.2700	4320	August 23, 2002	4.47	0.01	0.00	0.00	15.00	0.00	0.00	0.00	79.00	0.00	18.00	0.00	34.00	0.25	0.00	
57	1.2700	5630	August 26, 2002	3.57	0.01	0.00	0.00	0.00	0.00	0.00	0.00	65.00	0.00	0.00	0.00	21.00	0.22	0.08	
58	0.0000	60								0.00									
59	10.0000	10	August 30, 2002	2.36	0.00	0.00	230.00	0.00	0.50	10.21	0.00	78.00	0.00	19.00	0.00	31.00	0.24	0.00	
60	0.0000	60								0.00									
61	1.2700	5569	August 30, 2002	3.21	0.01	0.00	0.00	0.00	0.00	0.00	0.00	78.00	0.00	19.00	0.00	31.00	0.24	0.00	
62	0.0000	60								0.00									
63	10.0000	71	September 3, 2002	0.07	0.00	0.00	65.00	16.00	0.00	2.89	0.00	150.00	46.00	12.00	237.00	20.00	0.17	0.04	
64	0.0000	60								0.00									
65	1.2700	4320	September 3, 2002	3.33	0.00	0.00	0.00	0.00	0.00	0.00	3.00	97.00	6.00	12.00	62.00	25.00	0.23	0.01	
66	1.2700	5630.4	September 6, 2002	8.14	0.00	0.00	0.00	0.00	0.00	0.00	3.00	18.00	0.00	16.00	0.00	29.00	0.27	0.10	
67	0.0000	60								0.00									
68	10.0000	9.6	September 10, 2002	2.33	0.00	0.00	304.00	0.00	2.00	13.50	3.00	18.00	0.00	16.00	2.00	31.00	0.26	0.04	
69	0.0000	60								0.00									
70	1.2700	2880	September 10, 2002	1.86	0.00	0.00	0.00	29.00	0.00	0.00	3.00	18.00	0.00	17.00	0.00	32.00	0.27	0.04	
71	0.8800	1440	September 10, 2002	1.86	0.00	0.00	0.00	0.00	0.00	0.00	3.00	18.00	0.00	17.00	0.00	32.00	0.27	0.04	
72	1.2700	5630.6	September 13, 2002	3.18	0.00	0.00	0.00	0.00	0.00	0.00	4.00	77.00	0.00	11.00	0.00	31.00	0.27	0.07	
73	0.0000	60								0.00									
74	10.0000	9.4	September 17, 2002	4.62	0.00	0.00	243.00	0.00	0.00	10.79	3.00	75.00	0.00	11.00	1.00	31.00	0.27	0.06	
75	0.0000	60								0.00									
76	1.2700	4320	September 17, 2002	3.86	0.00	0.00	0.00	0.00	0.00	0.00	4.00	77.00	0.00	11.00	0.00	35.00	0.27	0.02	
77	1.2700	5700	September 20, 2002	3.23	0.01	0.00	0.00	0.00	0.00	0.00	4.00	15.00	0.00	13.00	0.00	43.00	0.27	0.05	
78	0.0000	60								0.00									
79	1.2700	4320	September 24, 2002	4.35	0.01	0.00	0.00	0.00	0.00	0.00	3.00	12.00	0.00	0.00	0.00	43.00	0.27	0.05	
80	1.2700	5630.5	September 27, 2002	2.83	0.00	0.00	0.00	0.00	0.00	0.00	2.00	77.00	0.00	13.00	0.00	32.00	0.23	0.06	

Stress Period	Flow Rate (mL/min)	Duration (min)	Stock Date (feedings in italics)	Influent Solute Concentration (- mg/L)															
				TCE	DCE	VC	LA	AA	PA	H2	Nitrite	Cl	Br	Nitrate	PO4	Sulfate	Cr	Fe	
81	0.0000	60	<i>October 1, 2002</i>	2.84	0.00	0.00	152.00	0.00	0.00	0.00	6.75	0.00	80.00	0.00	3.00	0.00	43.00	0.03	0.16
82	10.0000	9.5		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
83	0.0000	60		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
84	1.2700	2820	<i>October 1, 2002</i>	2.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	77.00	0.00	23.00	0.00	31.00	0.22	0.01
85	1.2700	60	<i>October 1, 2002</i>	2.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	77.00	0.00	23.00	0.00	31.00	0.22	0.01
86	1.2700	1440	<i>October 1, 2002</i>	2.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	77.00	0.00	23.00	0.00	31.00	0.22	0.01
87	1.2700	1380	<i>October 4, 2002</i>	2.65	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	76.00	0.00	10.00	0.00	30.00	0.21	0.05
88	1.2700	60	<i>October 4, 2002</i>	2.65	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	76.00	0.00	10.00	0.00	30.00	0.21	0.05
89	1.2700	3600	<i>October 4, 2002</i>	2.65	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	76.00	0.00	10.00	0.00	30.00	0.21	0.05
90	0.0000	60	<i>October 8, 2002</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
91	10.0000	10		0.00	0.00	0.00	229.00	0.00	0.00	0.00	10.17	0.00	78.00	0.00	20.00	0.00	31.00	0.23	0.00
92	0.0000	60		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	78.00	0.00	19.00	0.00	32.00	0.23
93	1.2700	1380	<i>October 8, 2002</i>	2.27	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	78.00	0.00	19.00	0.00	32.00	0.23	0.00
94	1.2700	60	<i>October 8, 2002</i>	2.27	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	78.00	0.00	19.00	0.00	32.00	0.23	0.00
95	1.2700	1380	<i>October 8, 2002</i>	2.27	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	78.00	0.00	19.00	0.00	32.00	0.23	0.00
96	1.2700	60	<i>October 8, 2002</i>	2.27	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	78.00	0.00	19.00	0.00	32.00	0.23	0.00
97	1.2700	1380	<i>October 8, 2002</i>	2.27	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	78.00	0.00	19.00	0.00	32.00	0.23	0.00
98	0.0000	60	<i>October 11, 2002</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
99	1.2700	1380		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	17.00	0.00	28.00	0.24	0.06
100	1.2700	60		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	17.00	0.00	28.00	0.24	0.06
101	1.2700	1380	<i>October 11, 2002</i>	2.25	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	17.00	0.00	28.00	0.24	0.06
102	1.2700	60	<i>October 11, 2002</i>	2.25	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	17.00	0.00	28.00	0.24	0.06
103	1.2700	1380	<i>October 11, 2002</i>	2.25	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	17.00	0.00	28.00	0.24	0.06
104	1.2700	60	<i>October 11, 2002</i>	2.25	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	17.00	0.00	28.00	0.24	0.06
105	1.2700	1311	<i>October 11, 2002</i>	2.25	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	17.00	0.00	28.00	0.24	0.06
106	0.0000	60	<i>October 15, 2002</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
107	10.0000	9		0.00	0.00	0.00	233.00	0.00	0.00	0.00	10.35	0.00	76.00	0.00	19.00	2.00	31.00	0.24	0.01
108	0.0000	60		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
109	1.2700	1380	<i>October 15, 2002</i>	2.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	77.00	0.00	20.00	0.00	30.00	0.24	0.01
110	1.2700	60	<i>October 15, 2002</i>	2.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	77.00	0.00	20.00	0.00	30.00	0.24	0.01
111	1.2700	1380	<i>October 15, 2002</i>	2.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	77.00	0.00	20.00	0.00	30.00	0.24	0.01
112	1.2700	60	<i>October 15, 2002</i>	2.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	77.00	0.00	20.00	0.00	30.00	0.24	0.01
113	1.2700	1380	<i>October 15, 2002</i>	2.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	77.00	0.00	20.00	0.00	30.00	0.24	0.01
114	0.0000	60	<i>October 18, 2002</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
115	1.2700	1380		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	14.00	0.00	24.00	0.24	0.08
116	1.2700	60		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	14.00	0.00	24.00	0.24	0.08
117	1.2700	1380	<i>October 18, 2002</i>	2.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	14.00	0.00	24.00	0.24	0.08
118	1.2700	60	<i>October 18, 2002</i>	2.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	14.00	0.00	24.00	0.24	0.08
119	1.2700	1380	<i>October 18, 2002</i>	2.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	14.00	0.00	24.00	0.24	0.08
120	1.2700	60	<i>October 18, 2002</i>	2.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	14.00	0.00	24.00	0.24	0.08
121	1.2700	1380	<i>October 18, 2002</i>	2.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	74.00	0.00	14.00	0.00	24.00	0.24	0.08

COLUMN A OPERATIONAL HISTORY - FLOW

Stress Period	Flow Rate (mL/min)	Duration (min)	Stock Date (feedings in italics)	Extraction Rate (- mL/min)							Flow	
				Port 1	Port 2	Port 3	Port 4	Port 5	Port 6	Port 7	Effluent	Balance
1	2.0000	200	June 11, 2002								2.0000	0.0000
2	0.0000	2780									0.0000	0.0000
3	8.0000	100	June 13, 2002								8.0000	0.0000
4	0.0000	4220									0.0000	0.0000
5	8.0000	100	June 16, 2002								8.0000	0.0000
6	0.0000	2780									0.0000	0.0000
7	8.0000	100	June 18, 2002								8.0000	0.0000
8	0.0000	2780									0.0000	0.0000
9	8.0000	100	June 20, 2002								8.0000	0.0000
10	0.0000	2780									0.0000	0.0000
11	8.0000	100	June 22, 2002								8.0000	0.0000
12	0.0000	1340									0.0000	0.0000
13	8.0000	100	June 23, 2002								8.0000	0.0000
14	0.0000	5630									0.0000	0.0000
15	10.0000	70	<i>June 27, 2002</i>	0.1000			0.1000			0.1000	10.0000	0.0000
16	0.0000	60									0.0000	-0.3000
17	1.2700	2880	June 28, 2002								1.2699	0.3000
18	1.2700	4320	June 30, 2002								1.2700	0.0000
19	0.1000	720	July 3, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.1000	0.0000	0.0000
20	0.0000	60									0.0000	-0.4662
21	1.2700	1380	July 3, 2002								1.2697	0.4662
22	1.2700	4320	July 6, 2002								1.2700	0.0000
23	1.2700	2100	July 9, 2002								1.2700	0.0000
24	0.0000	60		0.0666			0.0666			0.0666	0.0000	-0.1998
25	1.2700	1360	July 11, 2002								1.2699	0.1998
26	10.0000	20	<i>July 12, 2002</i>	0.0666	0.0666	0.0666					10.0000	0.0000
27	0.0000	60									0.0000	-0.1998
28	1.2700	4320	July 12, 2002								1.2700	0.1998
29	1.2700	5700	July 15, 2002								1.2700	0.0000
30	0.0000	60		0.0666			0.0666			0.0666	0.0000	-0.1998

Stress Period	Flow Rate (mL/min)	Duration (min)	Stock Date (feedings in italics)	Extraction Rate (- mL/min)							Flow Balance	
				Port 1	Port 2	Port 3	Port 4	Port 5	Port 6	Port 7		Effluent
31	1.2700	5760	July 19, 2002								1.2700	0.1998
32	1.2700	4190	July 23, 2002								1.2700	0.0000
33	0.0000	60	July 26, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000	-0.4662
34	10.0000	10		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	9.9534	0.4662
35	0.0000	60	July 26, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000	-0.4662
36	1.2700	4320	July 29, 2002								1.2699	0.4662
37	1.2700	5700	August 1, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	1.2700	0.0000
38	0.0000	60		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000	-0.4662
39	10.0000	10	August 1, 2002								9.9534	0.4662
40	0.0000	60		0.0666		0.0666		0.0666		0.0666	0.0000	-0.2664
41	1.2700	5760	August 1, 2002								1.2700	0.2664
42	1.2700	4190	August 6, 2002								1.2700	0.0000
43	0.0000	60	August 9, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000	-0.4662
44	10.0000	10		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	9.9534	0.4662
45	0.0000	60	August 9, 2002								1.2699	0.4662
46	1.2700	5760		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	1.2700	0.0000
47	1.2700	4191.8	August 13, 2002								0.0000	-0.4662
48	0.0000	60	August 16, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	9.9431	0.4662
49	10.0000	8.2		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000	-0.4662
50	0.0000	60	August 16, 2002								1.2699	0.4662
51	1.2700	5760	August 20, 2002								1.2700	0.0000
52	1.2700	4260	August 23, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000	-0.2664
53	0.0000	60		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	9.9734	0.2664
54	10.0000	10	August 23, 2002								0.0000	-0.4662
55	0.0000	60	August 23, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	1.2699	0.4662
56	1.2700	4320	August 26, 2002								1.2700	0.0000
57	1.2700	5630	August 30, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000	-0.2664
58	0.0000	60		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	9.9734	0.2664
59	10.0000	10	August 30, 2002								0.0000	-0.2664
60	0.0000	60	August 30, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	1.2700	0.2664
61	1.2700	5569		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000	-0.2664
62	0.0000	60	August 30, 2002								1.2700	0.2664
				0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000	-0.2664

Stress Period	Flow Rate (mL/min)	Duration (min)	Stock Date (feedings in italics)	Extraction Rate (- mL/min)							Flow Balance
				Port 1	Port 2	Port 3	Port 4	Port 5	Port 6	Port 7	Effluent
63	10.0000	71	<i>September 3, 2002</i>	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	9.9962
64	0.0000	60									0.0000
65	1.2700	4320	<i>September 3, 2002</i>								1.2699
66	1.2700	5630.4	<i>September 6, 2002</i>								1.2700
67	0.0000	60		0.0666		0.0666		0.0666		0.0666	0.0000
68	10.0000	9.6	<i>September 10, 2002</i>								-0.2664
69	0.0000	60		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	9.9723
70	1.2700	2880	<i>September 10, 2002</i>								0.0000
71	0.8800	1440	<i>September 10, 2002</i>								1.2698
72	1.2700	5630.6	<i>September 13, 2002</i>								0.8800
73	0.0000	60		0.0666		0.0666		0.0666		0.0666	1.2700
74	10.0000	9.4	<i>September 17, 2002</i>								0.0000
75	0.0000	60		0.0666		0.0666		0.0666		0.0666	1.2700
76	1.2700	4320	<i>September 17, 2002</i>								0.0000
77	1.2700	5700	<i>September 20, 2002</i>								1.2699
78	0.0000	60		0.0666		0.0666		0.0666		0.0666	1.2700
79	1.2700	4320	<i>September 24, 2002</i>								0.0000
80	1.2700	5630.5	<i>September 27, 2002</i>								1.2699
81	0.0000	60		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	1.2700
82	10.0000	9.5	<i>October 1, 2002</i>								0.0000
83	0.0000	60		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	1.2698
84	1.2700	2820	<i>October 1, 2002</i>								1.0036
85	1.2700	60	<i>October 1, 2002</i>	0.0666		0.0666		0.0666		0.0666	1.2700
86	1.2700	1440	<i>October 1, 2002</i>								1.2700
87	1.2700	1380	<i>October 4, 2002</i>								1.2700
88	1.2700	60	<i>October 4, 2002</i>	0.0666		0.0666		0.0666		0.0666	1.0036
89	1.2700	3600	<i>October 4, 2002</i>								1.2700
90	0.0000	60		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000
91	10.0000	10	<i>October 8, 2002</i>								0.0000
92	0.0000	60		0.0666		0.0666		0.0666		0.0666	9.9534
93	1.2700	1380	<i>October 8, 2002</i>								0.0000
94	1.2700	60	<i>October 8, 2002</i>	0.0666		0.0666		0.0666		0.0666	1.2698
											1.0036
											0.0000

Stress Period	Flow Rate (mL/min)	Duration (min)	Stock Date (feedings in italics)	Extraction Rate (- mL/min)							Flow Balance		
				Port 1	Port 2	Port 3	Port 4	Port 5	Port 6	Port 7		Effluent	
95	1.2700	1380	October 8, 2002	0.0666		0.0666		0.0666			0.0666	1.2700	0.0000
96	1.2700	60	October 8, 2002	0.0666								1.0036	0.0000
97	1.2700	1380	October 8, 2002	0.0666		0.0666		0.0666		0.0666		1.2700	0.0000
98	0.0000	60		0.0666								0.0000	-0.2664
99	1.2700	1380	October 11, 2002	0.0666		0.0666		0.0666		0.0666		1.2698	0.2664
100	1.2700	60	October 11, 2002	0.0666								1.0036	0.0000
101	1.2700	1380	October 11, 2002	0.0666			0.0666				0.0666	1.2700	0.0000
102	1.2700	60	October 11, 2002	0.0666								1.0702	0.0000
103	1.2700	1380	October 11, 2002	0.0666		0.0666		0.0666		0.0666		1.2700	0.0000
104	1.2700	60	October 11, 2002	0.0666					0.0666		0.0666	1.0036	0.0000
105	1.2700	1311	October 11, 2002	0.0666		0.0666		0.0666		0.0666		1.2700	0.0000
106	0.0000	60		0.0666								0.0000	-0.2664
107	10.0000	9	October 15, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666		9.9704	0.2664
108	0.0000	60		0.0666								0.0000	-0.4662
109	1.2700	1380	October 15, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666		1.2697	0.4662
110	1.2700	60	October 15, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.8038	0.0000
111	1.2700	1380	October 15, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	1.2700	0.0000
112	1.2700	60	October 15, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.8038	0.0000
113	1.2700	1380	October 15, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	1.2700	0.0000
114	0.0000	60		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000	-0.4662
115	1.2700	1380	October 18, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	1.2697	0.4662
116	1.2700	60		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.8038	0.0000
117	1.2700	1380	October 18, 2002	0.0666		0.0666		0.0666		0.0666		1.2700	0.0000
118	1.2700	60	October 18, 2002	0.0666					0.0666		0.0666	1.0036	0.0000
119	1.2700	1380	October 18, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	1.2700	0.0000
120	1.2700	60	October 18, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.8038	0.0000
121	1.2700	1380	October 18, 2002	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	1.2700	0.0000
122	0.0000	60		0.0666	0.0666							0.0000	-0.4662
123	0.4662	60		0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0666	0.0000	0.4662

APPENDIX B

Retec Group, Inc. TCE Transformation Kinetics Study

Schoolcraft kinetic test

Kinetic medium:

Mineral Salt	g/L
NaCl	50
MgCl ₂ · 6H ₂ O	25
KH ₂ PO ₄	10
NH ₄ Cl	15
KCl	15
CaCl ₂ · 2H ₂ O	0.75

Trace Element	mg/L
HCl	10
FeCl ₂ · 4H ₂ O	1500
CoCl ₂ · 6H ₂ O	190
MnCl ₂ · 4H ₂ O	100
ZnCl ₂	70
H ₃ BO ₃	6
Na ₂ MnO ₄ · 2H ₂ O	36
NiCl ₂ · 6H ₂ O	24
CuCl ₂ · 2H ₂ O	2

Na ₂ S Solution	g/L
Na ₂ S	48

Resaruine solution	
Resaruine	0.10%

Schoolcraft Kinetic Protocol

Manager

Jing

Date

12/2/2002

PCE initial concentration

4 mg/L

Kinetic aqueous volume

165.6 ml

PCE density

1.69 g/ml

PCE injection amount in aqueous

0.4 uL

	Control-1	Control-2	Control-3	1	2	3	4
distilled water (ml)	160	160	160				
aqueous from reactor (ml)				160	160	160	160
mineral salt (ml)	2	2	2	2	2	2	2
trace element (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Na ₂ S solution (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
resazurin (0.1 %)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
buffer (ml)	2	2	2	2	2	2	2
lactate (ml)	1	1	1	1	1	1	1
PCE (uL)	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Total (ml)	165.6	165.6	165.6	165.6	165.6	165.6	165.6

Sampling

Time	Control-1	Control-2	Control-3	1	2	3	4
before pce 12/03/02 9:00 am	c 1-1	c 2-1	c 3-1	1-1	2-1	3-1	4-1
after pce 12/03/02 11:00am	c 1-2	c 2-2	c 3-2	1-2	2-2	3-2	4-2
12/03/02 16:00pm	c 1-3	c 2-3	c 3-3	1-3	2-3	3-3	4-3
12/04/02 8:00am	c 1-4	c 2-4	c 3-4	1-4	2-4	3-4	4-4
12/04/02 3:00pm	c 1-5	c 2-5	c 3-5	1-5	2-5	3-5	4-5
12/05/02 9:00am	c 1-6	c 2-6	c 3-6	1-6	2-6	3-6	4-6
12/05/02 4:00 pm	c 1-7	c 2-7	c 3-7	1-7	2-7	3-7	4-7
12/06/02 9:00am	c 1-8	c 2-8	c 3-8	1-8	2-8	3-8	4-8
12/6/02 4:00PM	c 1-9	c 2-9	c 3-9	1-9	2-9	3-9	4-9
12/07/02 5:00pm	c 1-10	c 2-10	c 3-10	1-10	2-10	3-10	4-10
12/08/02 6:00pm	c 1-11	c 2-11	c 3-11	1-11	2-11	3-11	4-11
12/09/02 4:00pm	c 1-12	c 2-12	c 3-12	1-12	2-12	3-12	4-12
12/10/02 3:00pm	c 1-13	c 2-13	c 3-13	1-13	2-13	3-13	4-13
12/11/02 2:00pm	c 1-14	c 2-14	c 3-14	1-14	2-14	3-14	4-14

Schoolcraft Kinetic Protocol

Manager
Date

Jing
12/2/2002

PCE initial concentration	4 mg/L	24.12108786	Kinetic aqueous volume				165.6 ml
PCE density	1.69 mg/ul		PCE injection amount in aqueous				0.4 uL
			PCE MW				165.83
distilled water (ml)		Control-1	Control-2	Control-3	1	2	3
aqueous from reactor (ml)		160	160	160			
mineral salt (ml)		2	2	2	160	160	160
trace element (ml)		0.2	0.2	0.2	2	2	2
Na ₂ S solution (ml)		0.2	0.2	0.2	0.2	0.2	0.2
resazurin (0.1 %)		0.2	0.2	0.2	0.2	0.2	0.2
buffer (ml)		2	2	2	2	2	2
lactate (ml)		1	1	1	1	1	1
PCE (ul)		1	1	1	1	1	1
Total (ml)		165.6	165.6	165.6	165.6	165.6	165.6

Sampling

Time	Acc. Time	Days	Control-1	Control-2	Control-3	PCE	PCE	PCE	Control	Std
before pce 12/03/02 9:00 am	hr.	0	c 1-1	c 2-1	c 3-1	5.5	0	4.5	0.0	0
after pce 12/03/02 11:00am	2	0.1	c 1-2	c 2-2	c 3-2	2698	2380	2144	14.5	2
12/03/02 16:00pm	7	0.3	c 1-3	c 2-3	c 3-3	2317	2668	2643	15.3	1
12/04/02 8:00am	23	1.0	c 1-4	c 2-4	c 3-4	3030	2935	3370	18.8	1
12/04/02 3:00pm	30	1.3	c 1-5	c 2-5	c 3-5	2460	2628	2654	15.6	1
12/05/02 9:00am	48	2.0	c 1-6	c 2-6	c 3-6	2290	2375	2620	14.6	1
12/05/02 4:00 pm	55	2.3	c 1-7	c 2-7	c 3-7	2170	1795	2556	13.1	2
12/06/02 9:00am	72	3.0	c 1-8	c 2-8	c 3-8	2375	2714	3073	16.4	2
12/06/02 4:00PM	79	3.3	c 1-9	c 2-9	c 3-9	2345	2525	2365	14.5	1
12/07/02 5:00pm	104	4.3	c 1-10	c 2-10	c 3-10	3015	2370	2405	15.7	2
12/08/02 6:00pm	129	5.4	c 1-11	c 2-11	c 3-11	2415	2635	2940	16.1	2
12/09/02 4:00pm	151	6.3	c 1-12	c 2-12	c 3-12	3040	3050	2490	17.2	2
12/10/02 3:00pm	174	7.3	c 1-13	c 2-13	c 3-13	3030	3275	3225	19.2	1
12/11/02 2:00pm	197	8.2	c 1-14	c 2-14	c 3-14	2955	2355	2530	15.8	2

Schoolcraft Kinetic Protocol

Manager
Date

Jing
12/2/2002

PCE initial concentration
PCE density

4 mg/L
1.69 g/ml

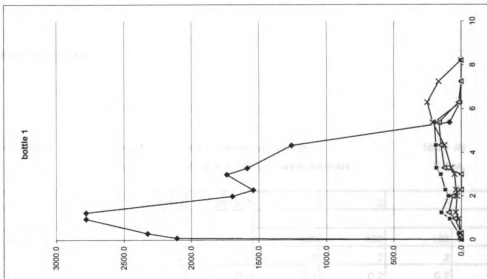
Kinetic aqueous volume
PCE injection amount in aqueous

165.6 ml
0.4 uL

	Control-1	Control-2	Control-3	1	2	3	4
distilled water (ml)	160	160	160				
aqueous from reactor (ml)				160	160	160	160
mineral salt (ml)	2	2	2	2	2	2	2
trace element (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Na2S solution (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
resazurin (0.1 %)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
buffer (ml)	2	2	2	2	2	2	2
lactate (ml)	1	1	1	1	1	1	1
PCE (uL)	1	1	1	1	1	1	1
Total (ml)	165.6	165.6	165.6	165.6	165.6	165.6	165.6

Sampling

Time	Days	Sample Name	PCE	TCE	DCE	VC	eth./meth.
before pce 12/03/02 9:00	0	1-1	0.0	0.0	0.0	0.0	0.0
after pce 12/03/02 11:00	0.1	1-2	2107.0	9.8	4.5	9.0	0.0
12/03/02 16:00pm	0.3	1-3	2325.0	20.0	0.0	4.0	0.0
12/04/02 8:00am	1.0	1-4	2779.0	85.5	47.0	19.0	0.0
12/04/02 3:00pm	1.3	1-5	2779.0	146.0	92.0	41.0	3.0
12/05/02 9:00am	2.0	1-6	1694.0	94.0	62.5	31.0	0.0
12/05/02 4:00 pm	2.3	1-7	1540.0	117.5	0.0	38.0	0.0
12/06/02 9:00am	3.0	1-8	1736.0	150.5	0.0	50.0	0.0
12/06/02 4:00PM	3.3	1-9	1584.0	184.1	125.0	74.5	4.5
12/07/02 5:00pm	4.3	1-10	1258.0	187.0	150.0	123.5	1.0
12/08/02 6:00pm	5.4	1-11	85.0	197.5	164.0	211.0	6.5
12/09/02 4:00pm	6.3	1-12	7.5	4.5	18.5	250.5	0.0
12/10/02 3:00pm	7.3	1-13	0.0	0.0	0.0	166.0	7.0
12/11/02 2:00pm	8.2	1-14	0.0	0.0	0.0	3.5	9.0



Schoolcraft Kinetic Protocol

Manager Jing
Date 12/2/2002

PCE initial concentration 4 mg/L Kinetic aqueous volume 165.6 ml
PCE density 1.69 g/ml PCE injection amount in aqueous 0.4 uL

	Control-1	Control-2	Control-3	1	2	3	4
distilled water (ml)	160	160	160				
aqueous from reactor (ml)				160	160	160	160
mineral salt (ml)	2	2	2	2	2	2	2
trace element (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Na ₂ S solution (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
resazurin (0.1 %)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
buffer (ml)	2	2	2	2	2	2	2
lactate (ml)	1	1	1	1	1	1	1
PCE (uL)	1	1	1	1	1	1	1
Total (ml)	165.6	165.6	165.6	165.6	165.6	165.6	165.6

Sampling

Time	Days	sample Name	PCE	TCE	DCE	VC	eth./meth.
before pce 12/03/02 9:00	0	2-1	8.0	3.5	0.0	0.0	0.0
after pce 12/03/02 11:00	0.1	2-2	2030.0	5.7	0.0	4.5	0.0
12/03/02 16:00pm	0.3	2-3	2048.0	10.0	0.0	0.0	0.0
12/04/02 8:00am	1.0	2-4	2510.0	35.0	23.5	13.0	0.0
12/04/02 3:00pm	1.3	2-5	2815.0	40.0	34.0	34.5	0.0
12/05/02 9:00am	2.0	2-6	2356.0	31.0	26.5	33.0	0.0
12/05/02 4:00 pm	2.3	2-7	2180.0	30.5	0.0	47.0	0.0
12/06/02 9:00am	3.0	2-8	2260.0	33.0	26.0	42.5	0.0
12/6/02 4:00PM	3.3	2-9	2234.0	49.0	33.5	69.0	21.5
12/07/02 5:00pm	4.3	2-10	2265.0	55.5	41.5	67.5	0.8
12/08/02 6:00pm	5.4	2-11	2190.0	70.5	57.0	81.5	4.5
12/09/02 4:00pm	6.3	2-12	1936.5	73.0	53.5	100.5	5.0
12/10/02 3:00pm	7.3	2-13	1684.0	75.0	56.0	135.0	6.0
12/11/02 2:00pm	8.2	2-14	1005.0	50.5	37.5	138.5	4.5

do not count on the final data summary

Schoolcraft Kinetic Protocol

Manager Jing

Date 12/2/2002

PCE initial concentration

4 mg/L

Kinetic aqueous volume

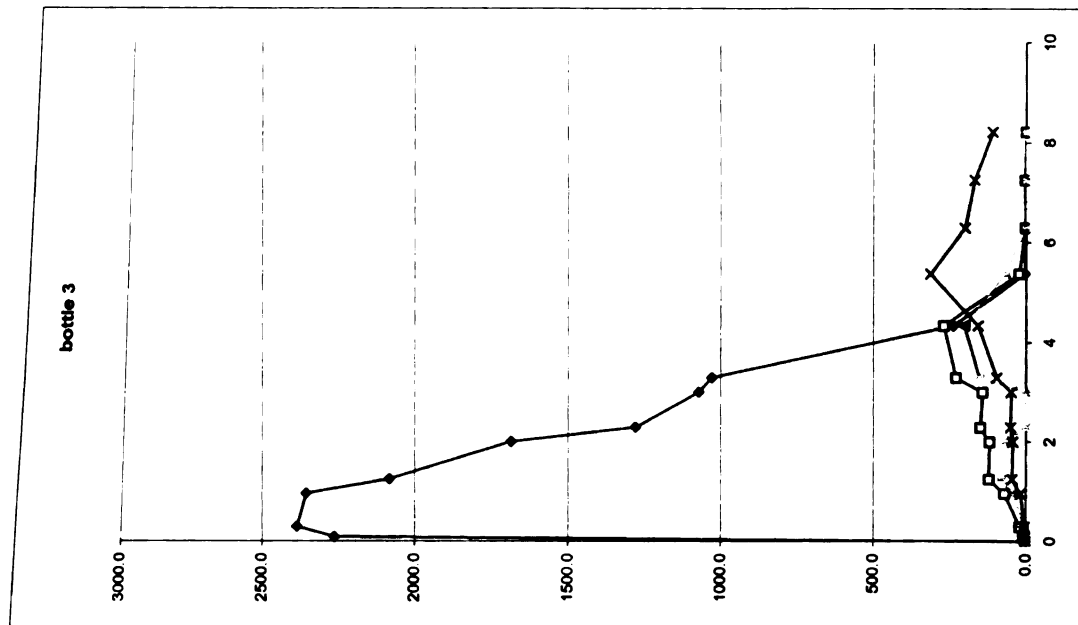
165.8 ml
0.4 uL

PCE density 1.69 g/ml
PCE injection amount in aqueous

	Control-1	Control-2	Control-3	1	2	3	4
distilled water (ml)	160	160	160				
aqueous from reactor (ml)				160	160	160	160
mineral salt (ml)	2	2	2	2	2	2	2
trace element (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Na2S solution (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
resazurin (0.1 %)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
buffer (ml)	2	2	2	2	2	2	2
lactate (ml)	1	1	1	1	1	1	1
PCE (uL)	1	1	1	1	1	1	1
Total (ml)	165.8	165.8	165.8	165.8	165.8	165.8	165.8

Sampling

Time	Days	Sample Name	PCE	TCE	DCE	VC	eth./meth.
before pcs 12/03/02 8:00 am	0	3-1	8.5	3.2	0.0	0.0	0.0
after pcs 12/03/02 11:00am	0.1	3-2	2282.0	8.9	0.0	4.5	0.0
12/03/02 16:00pm	0.3	3-3	2385.0	21.0	8.0	5.5	0.0
12/04/02 8:00am	1.0	3-4	2355.0	71.0	42.5	17.5	0.0
12/04/02 3:00pm	1.3	3-5	2082.0	122.0	82.0	45.0	0.0
12/05/02 9:00am	2.0	3-6	1685.0	120.0	72.5	43.0	0.0
12/05/02 4:00 pm	2.3	3-7	1278.0	150.0	0.0	50.2	0.0
12/06/02 8:00am	3.0	3-8	1071.0	142.0	0.0	48.5	0.0
12/6/02 4:00PM	3.3	3-9	1028.0	229.0	152.5	95.5	0.0
12/07/02 5:00pm	4.3	3-10	238.0	289.5	202.5	157.5	0.0
12/08/02 6:00pm	5.4	3-11	4.5	22.5	68.5	312.5	5.1
12/09/02 4:00pm	6.3	3-12	0.0	2.5	0.0	200.5	5.0
12/10/02 3:00pm	7.3	3-13	0.0	3.5	0.0	189.0	5.0
12/11/02 2:00pm	8.2	3-14	0.0	0.0	0.0	110.0	5.5

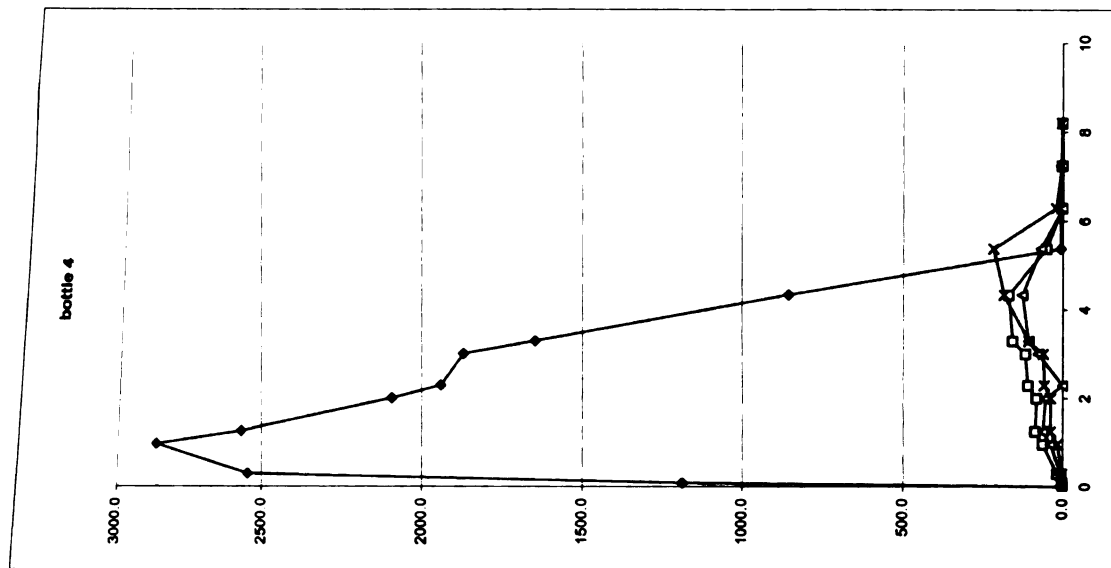


Schockcraft Kinetic Protocol

Manager Jing
 Date 12/2/2002

PCE initial concentration 4 mg/L
 PCE density 1.69 g/ml
 Kinetic aqueous volume 165.6 ml
 PCE injection amount in aqueous 0.4 uL

	Control-1	Control-2	Control-3	1	2	3	4
distilled water (ml)	160	160	160				
aqueous from reactor (ml)				160	160	160	160
mineral salt (ml)	2	2	2	2	2	2	2
trace element (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
NasS solution (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
resazurin (0.1 %)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
buffer (ml)	2	2	2	2	2	2	2
lactate (ml)	1	1	1	1	1	1	1
PCE (uL)	1	1	1	1	1	1	1
Total (ml)	165.6	165.6	165.6	165.6	165.6	165.6	165.6



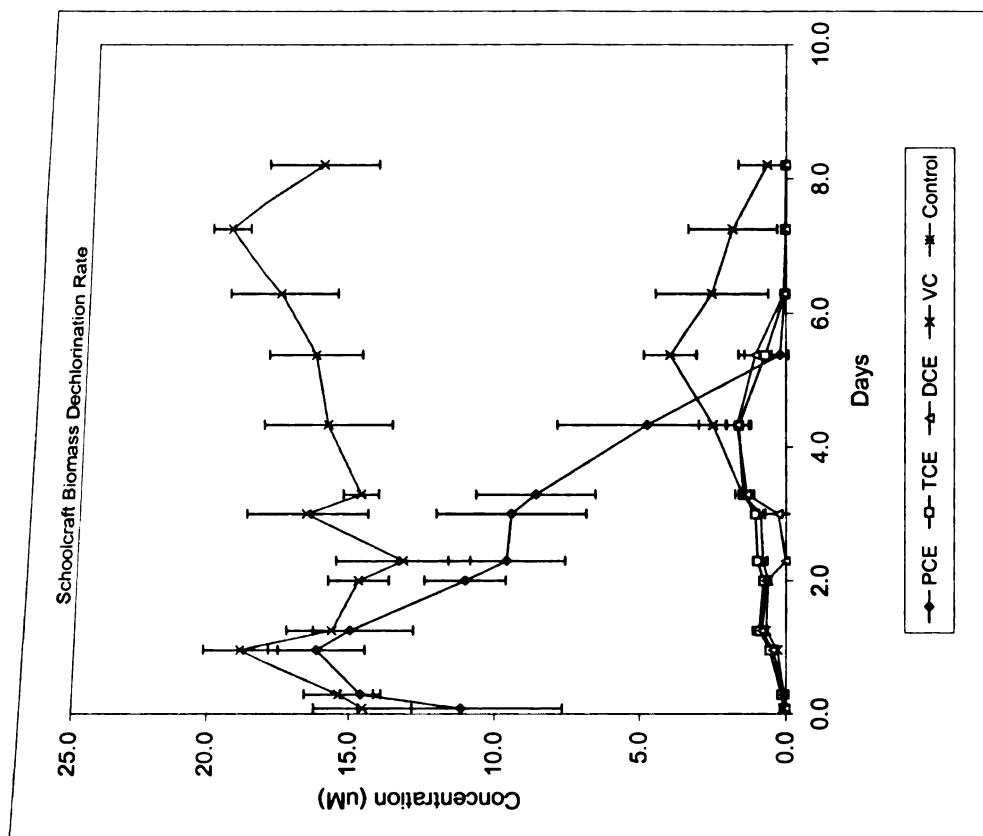
Sampling Time	Days	Sample Name	PCE	TCE	DCE	VC eth./meth.
before pce 12/03/02 9:00 am	0	4-1	8.0	0.0	0.0	0.0
after pce 12/03/02 11:00am	0.1	4-2	1187.0	3.3	0.0	4.0
12/03/02 16:00pm	0.3	4-3	2546.0	17.5	7.0	3.0
12/04/02 8:00am	1.0	4-4	2865.0	62.5	37.5	16.5
12/04/02 3:00pm	1.3	4-5	2569.0	86.0	61.5	38.5
12/05/02 9:00am	2.0	4-6	2081.5	82.0	54.0	38.0
12/05/02 04:00 pm	2.3	4-7	1939.0	109.2	0.0	57.5
12/06/02 9:00am	3.0	4-8	1870.0	117.0	76.0	61.0
12/06/02 4:00PM	3.3	4-9	1647.0	157.0	106.5	105.5
12/07/02 5:00pm	4.3	4-10	855.0	169.0	126.5	183.5
12/08/02 6:00pm	5.4	4-11	6.0	50.5	67.5	216.5
12/09/02 4:00pm	6.3	4-12	7.5	3.5	0.0	21.0
12/10/02 3:00pm	7.3	4-13	4.8	2.5	0.0	3.5
12/11/02 2:00pm	8.2	4-14	0.0	0.0	0.0	3.5

Schoolcraft Kinetic - Average concentration (uM)

mg/L	average	165.83	131.39	96.94	62.5 uM				
Time	SN	PCE	TCE	DCE	VC	PCE	TCE	DCE	Total
0	1	5.5	1.1	0.0	0.0	0.0	0.0	0.0	0.0
0.1	2	1852.0	7.3	1.5	5.8	11.2	0.1	0.0	0.1
0.3	3	2418.7	19.5	5.0	4.2	14.6	0.1	0.1	0.1
1.0	4	2666.3	73.0	42.3	17.7	16.1	0.6	0.4	0.3
1.3	5	2476.7	118.0	78.5	41.5	14.9	0.9	0.8	0.7
2.0	6	1823.5	98.7	63.0	37.3	11.0	0.8	0.6	0.6
2.3	7	1585.7	125.6	0.0	48.6	9.6	1.0	0.0	0.8
3.0	8	1559.0	136.5	25.3	53.2	9.4	1.0	0.3	0.9
3.3	9	1419.7	190.0	128.0	91.8	8.6	1.4	1.3	1.5
4.3	10	783.7	208.5	159.7	154.8	4.7	1.6	1.6	2.5
5.4	11	31.8	90.2	100.0	246.7	0.2	0.7	1.0	3.9
6.3	12	5.0	3.5	6.2	157.3	0.0	0.0	0.1	2.5
7.3	13	1.6	2.0	0.0	112.8	0.0	0.0	0.0	1.8
8.2	14	0.0	0.0	0.0	39.0	0.0	0.0	0.0	0.6

Schoolcraft Kinetic - standard deviation concentration (uM)

mg/L	average	165.83	131.39	96.94	62.5 uM				
Time	SN	PCE	TCE	DCE	VC	PCE	TCE	DCE	VC
0	1	4.8	1.8	0.0	0.0	0.0	0.0	0.0	0.0
0.1	2	581.1	3.5	2.6	2.8	3.5	0.0	0.0	0.0
0.3	3	114.3	1.8	4.4	1.3	0.7	0.0	0.0	0.0
1.0	4	273.0	11.6	4.8	1.3	1.6	0.1	0.0	0.0
1.3	5	357.6	30.2	15.5	3.3	2.2	0.2	0.2	0.1
2.0	6	232.1	19.4	9.3	6.0	1.4	0.1	0.1	0.1
2.3	7	332.9	21.6	0.0	9.9	2.0	0.2	0.0	0.2
3.0	8	427.9	17.4	43.9	6.8	2.6	0.1	0.5	0.1
3.3	9	340.7	36.4	23.1	15.8	2.1	0.3	0.2	0.3
4.3	10	513.7	53.6	38.9	30.1	3.1	0.4	0.4	0.5
5.4	11	46.0	94.0	55.4	57.1	0.3	0.7	0.6	0.9
6.3	12	4.3	1.0	10.7	120.7	0.0	0.0	0.1	1.9
7.3	13	2.8	1.8	0.0	94.7	0.0	0.0	0.0	1.5
8.2	14	0.0	0.0	0.0	61.5	0.0	0.0	0.0	1.0



Schoolcraft Project Dechlorination Kinetic

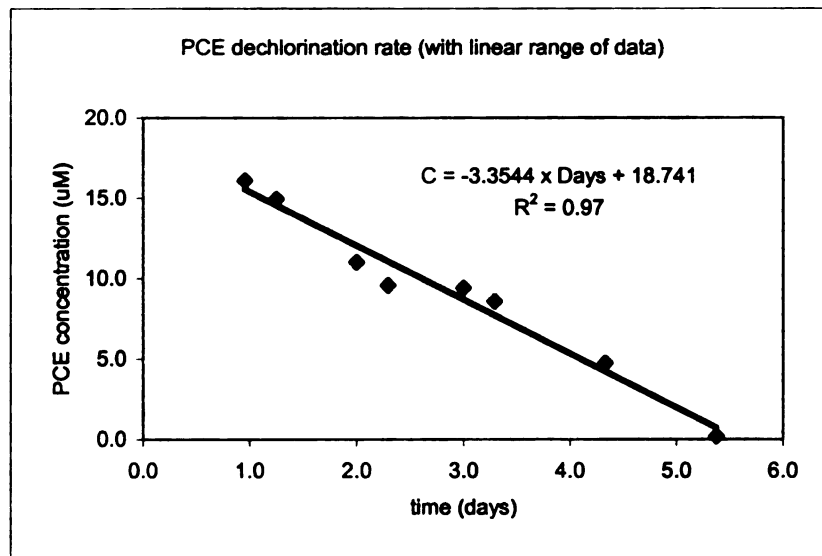
Date 12/16/2002

Zero order of PCE dechlorination rate uM/g (biomass)/day		
		29.60
		with linear range data

TSS	0.15 L		g/L	average	stdev
1	1.2457	1.2616	0.106	0.113	0.009
3	1.2325	1.2511	0.124		
4	1.2436	1.2601	0.110		

Linear fitting

Time days	PCE uM
0.1	11.2
0.3	14.6
1.0	16.1
1.3	14.9
2.0	11.0
2.3	9.6
3.0	9.4
3.3	8.6
4.3	4.7
5.4	0.2
6.3	0.0
7.3	0.0
8.2	0.0



TCE

Time (days)	Cave	Stddev	k' (1/day)
0	208.5	53.58871	
1.041667	90.16667	94.00177	0.804748
1.958333	3.5	1	3.544251
2.916667	2	1.802776	0.583947
3.875	0	0	

C-DCE

Time (days)	Cave	Stddev	k' (1/day)
0	159.6667	38.91122	
1.041667	100	55.42788	0.449201
1.958333	6.166667	10.68098	3.039286
2.916667	0	0	
3.875	0	0	

VC

Time (days)	Cave	Stddev	k' (1/day)
0	154.8333	30.08876	
1.041667	246.6667	57.07962	
1.958333	157.3333	120.6859	0.49055
2.916667	112.8333	94.69732	0.346909
3.875	39	61.4878	

APPENDIX C

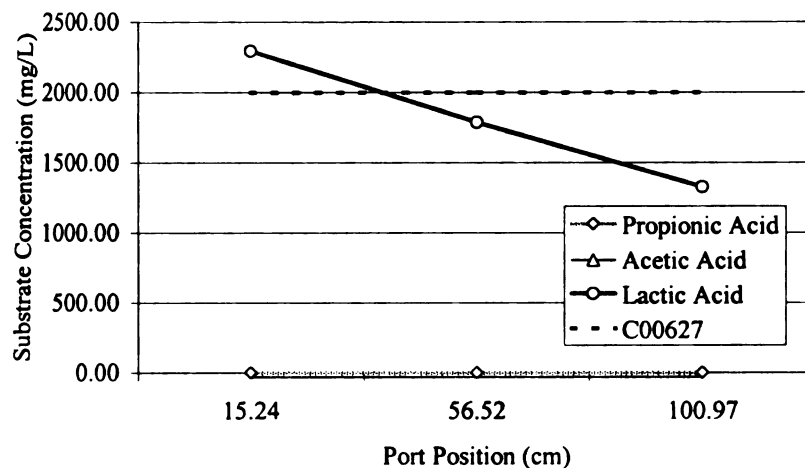
Large Column Study Analytical Results – Operational Phase A

APPENDIX C-1

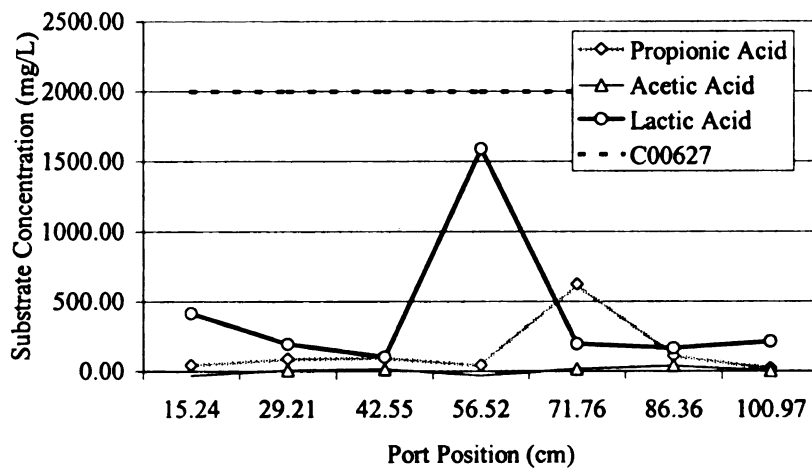
Fatty Acids

**FATTY ACIDS
COLUMN A
Incubation Period 1A
June 28 - July 11, 2002**

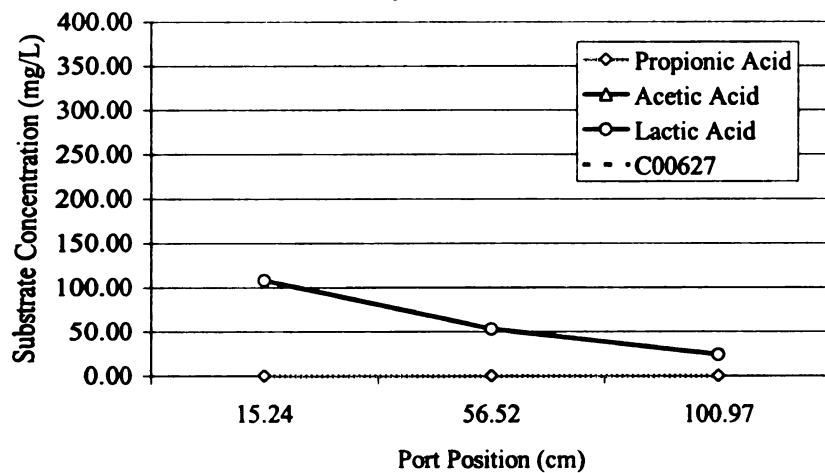
June 28, 2002



July 5, 2002

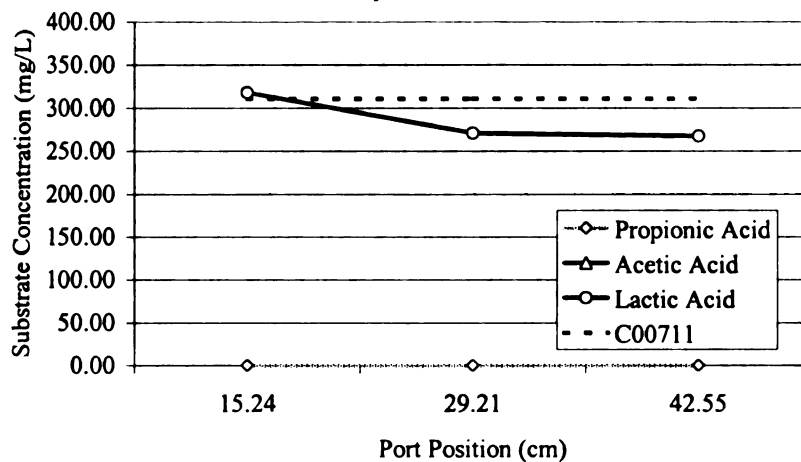


July 11, 2002

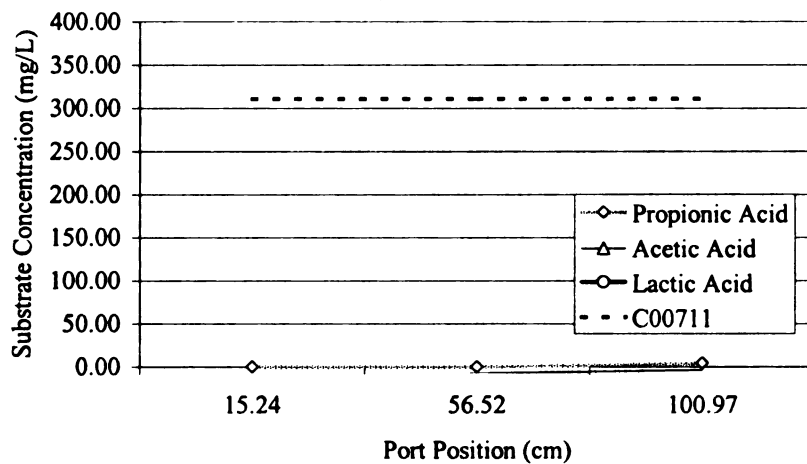


**FATTY ACIDS
COLUMN A
Incubation Period 2A
July 12 - 26, 2002**

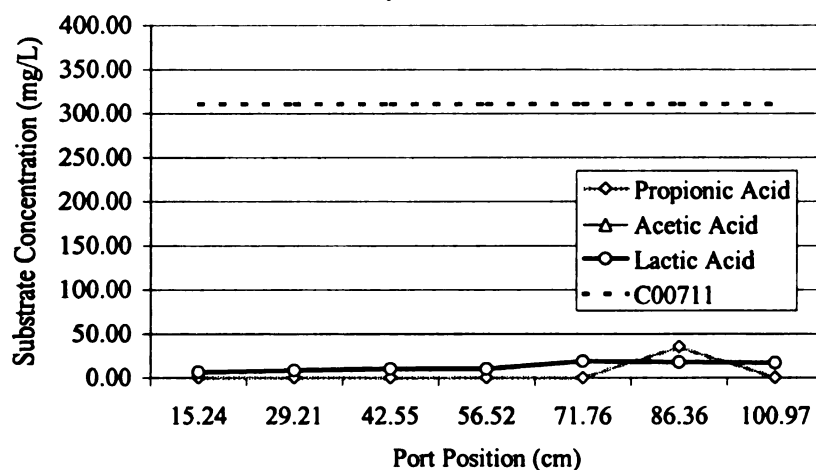
July 12, 2002



July 19, 2002

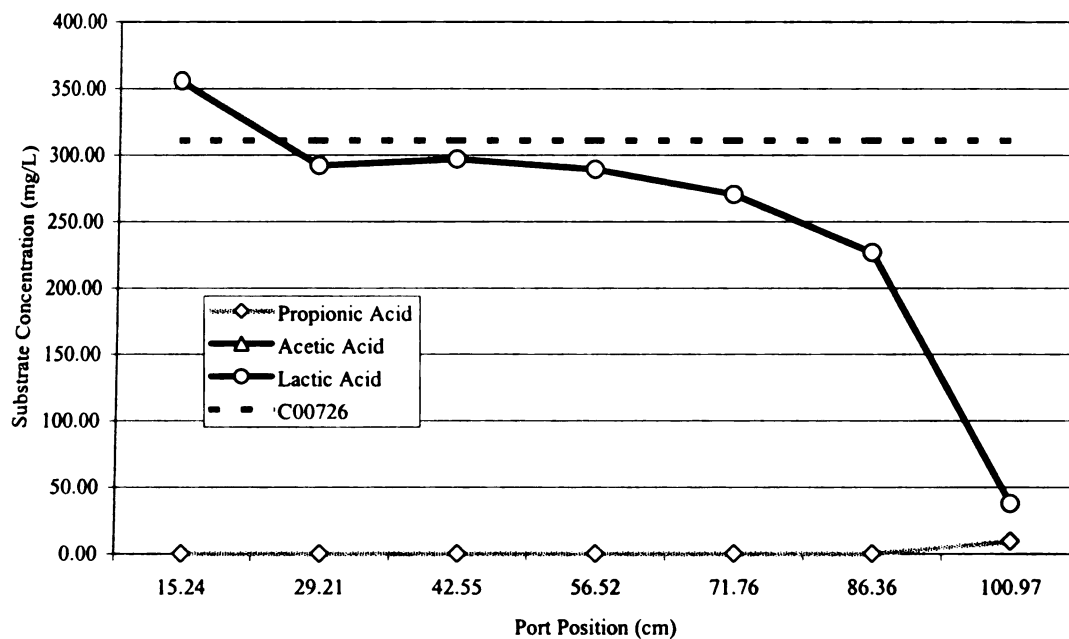


July 26, 2002

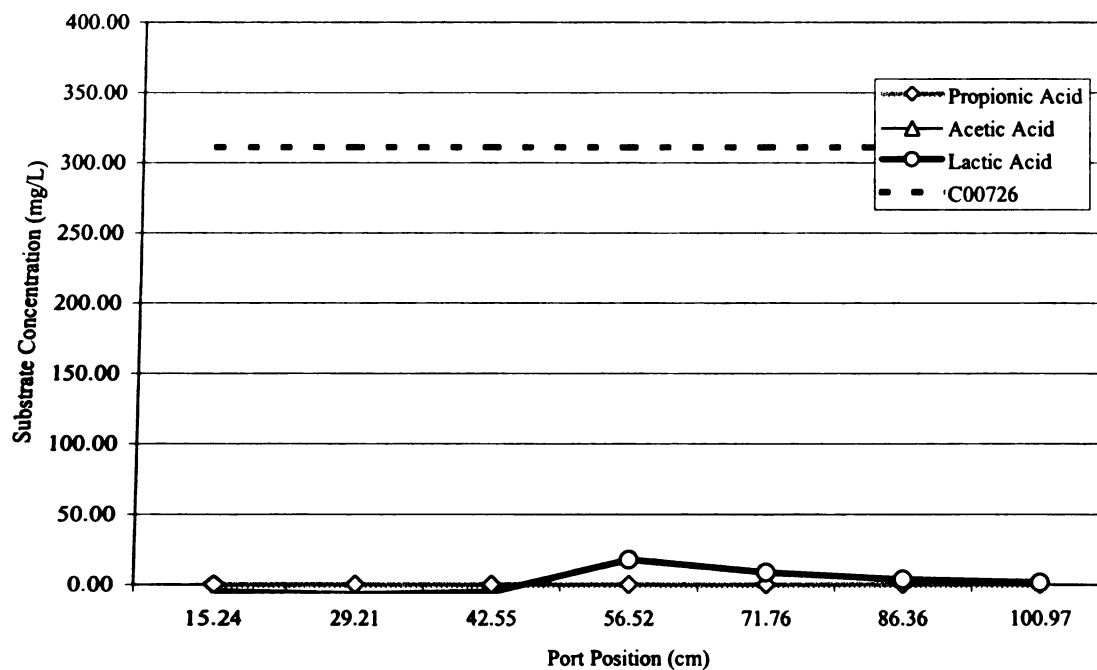


**FATTY ACIDS
COLUMN A
Incubation Period 3A
July 26 - August 2, 2002**

July 26, 2002

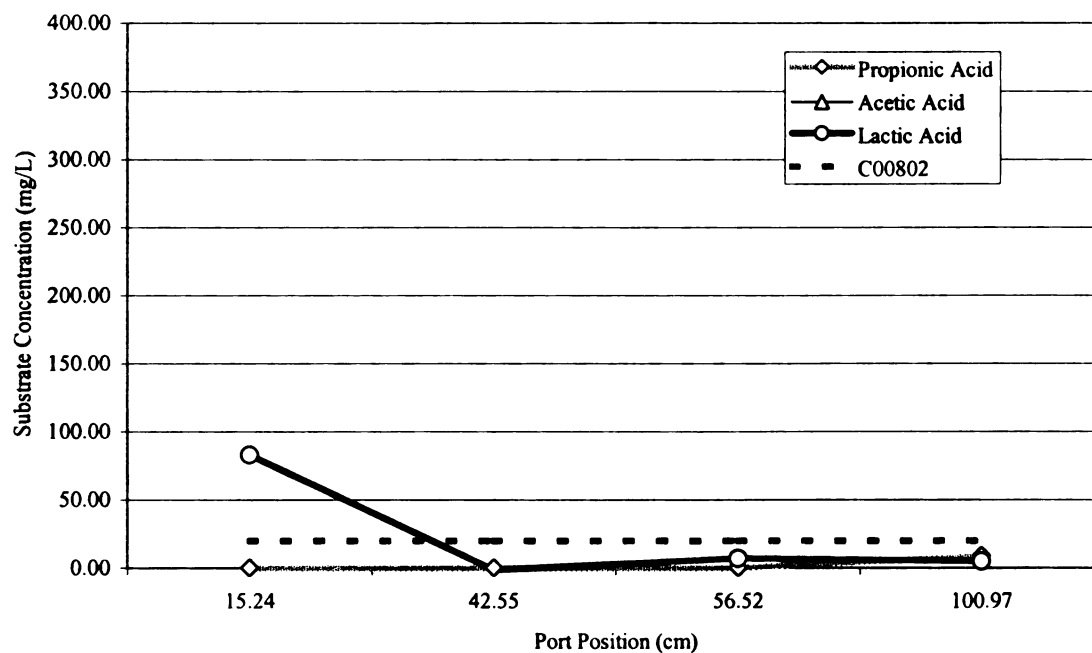


August 2, 2002

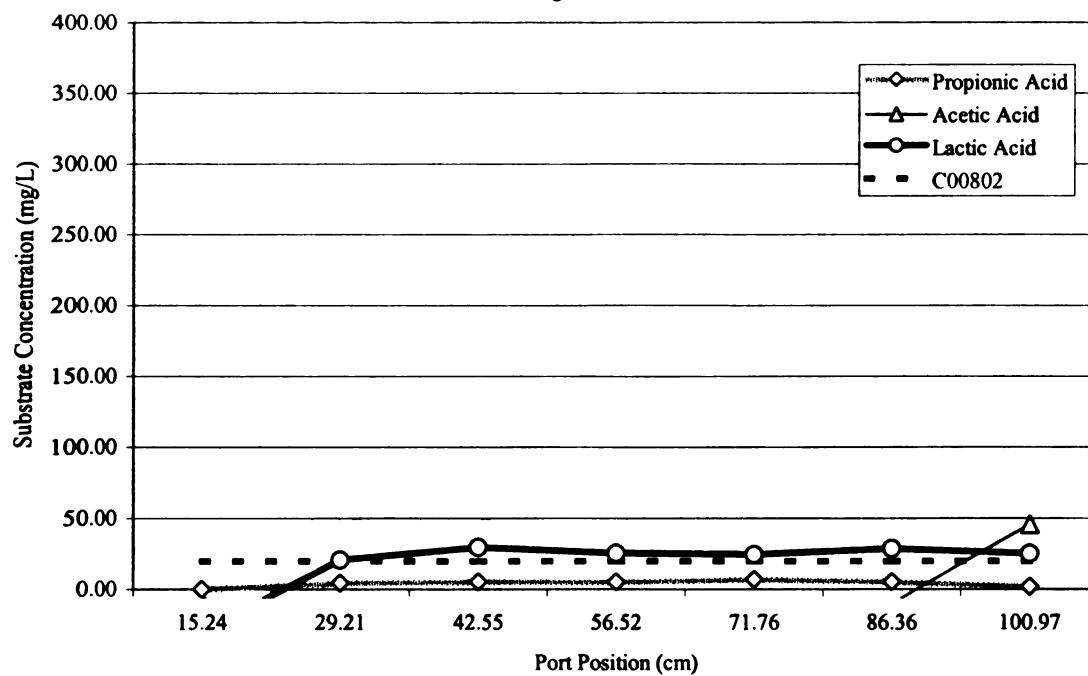


FATTY ACIDS
COLUMN A
Incubation Period 4A
August 2 - 9, 2002

August 2, 2002

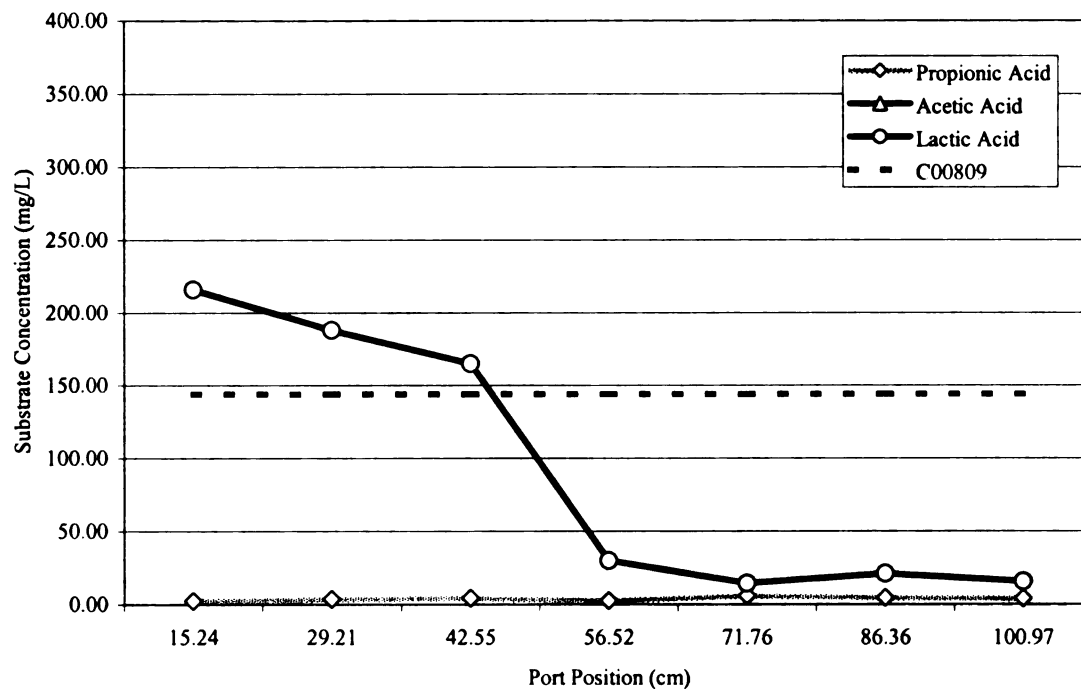


August 9, 2002

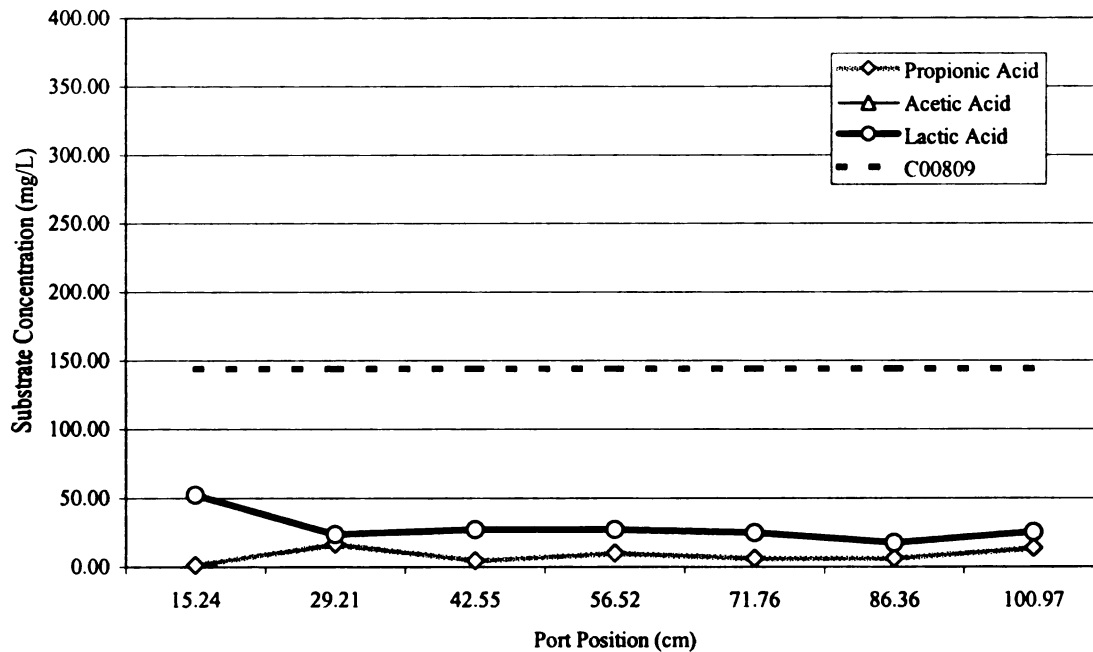


FATTY ACIDS
COLUMN A
Incubation Period 5A
August 9 - 16, 2002

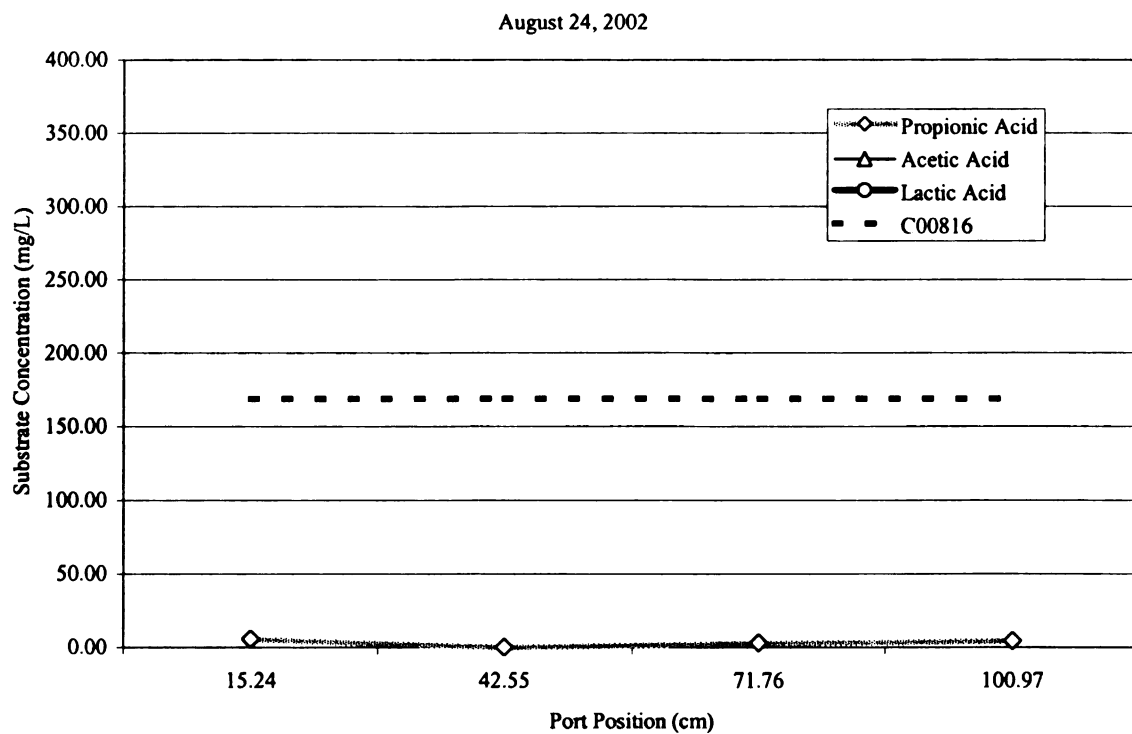
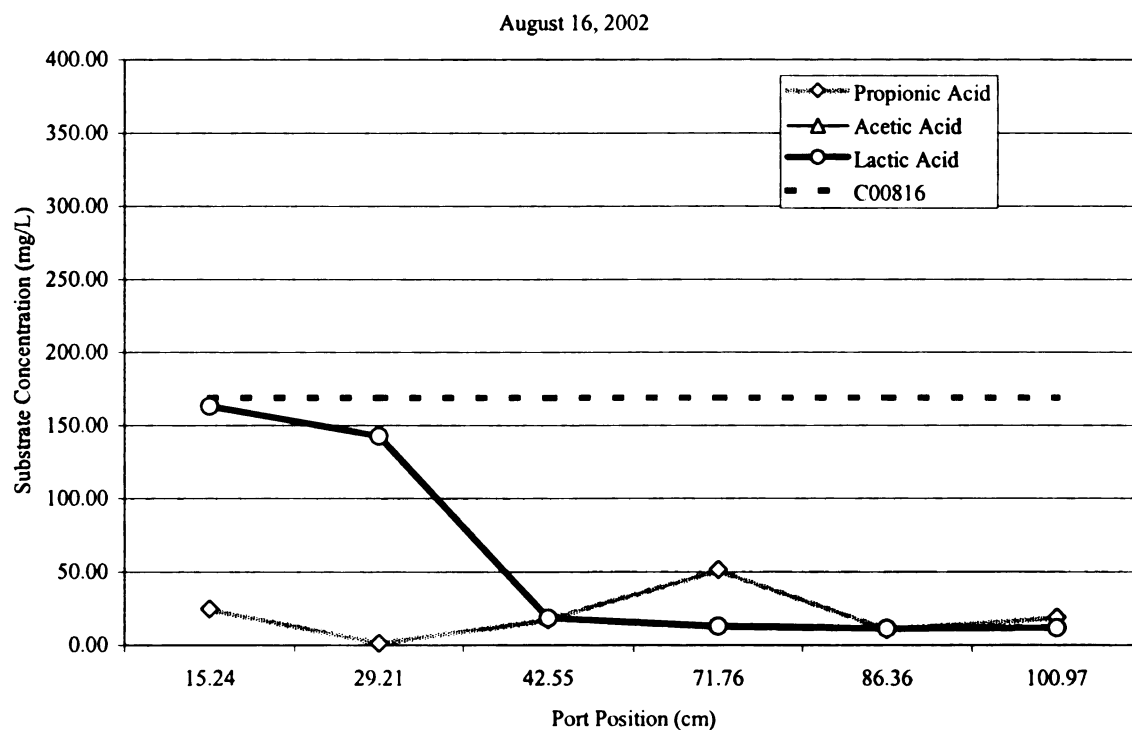
August 9, 2002



August 16, 2002

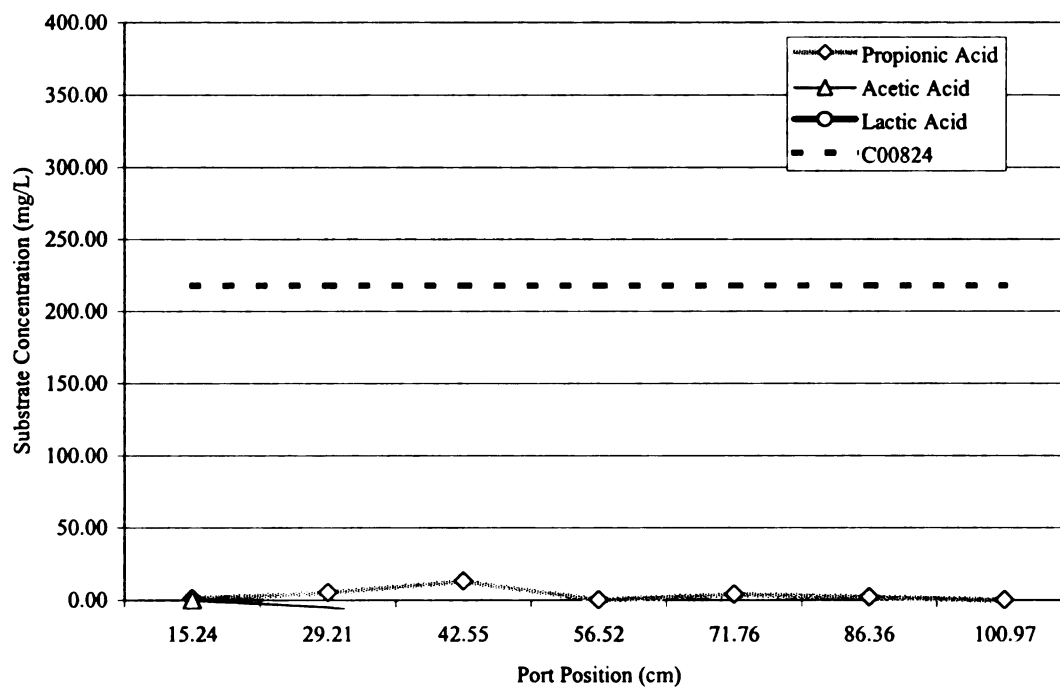


**FATTY ACIDS
COLUMN A
Incubation Period 6A
August 16 - 24, 2002**

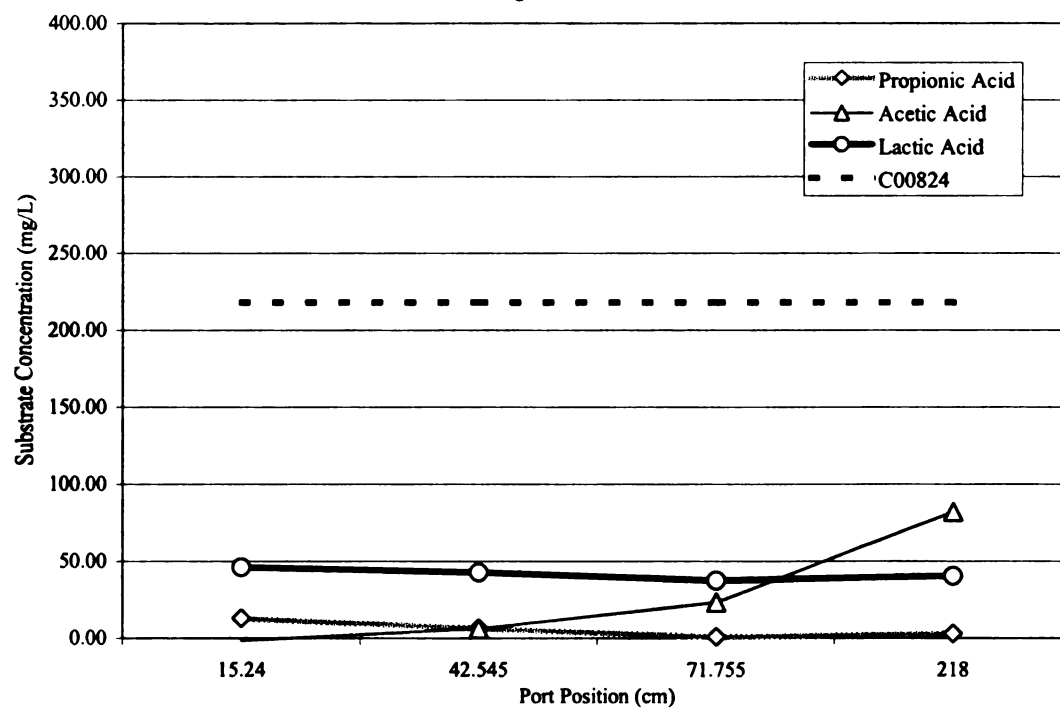


FATTY ACIDS
COLUMN A
Incubation Period 7A
August 24 - 30, 2002

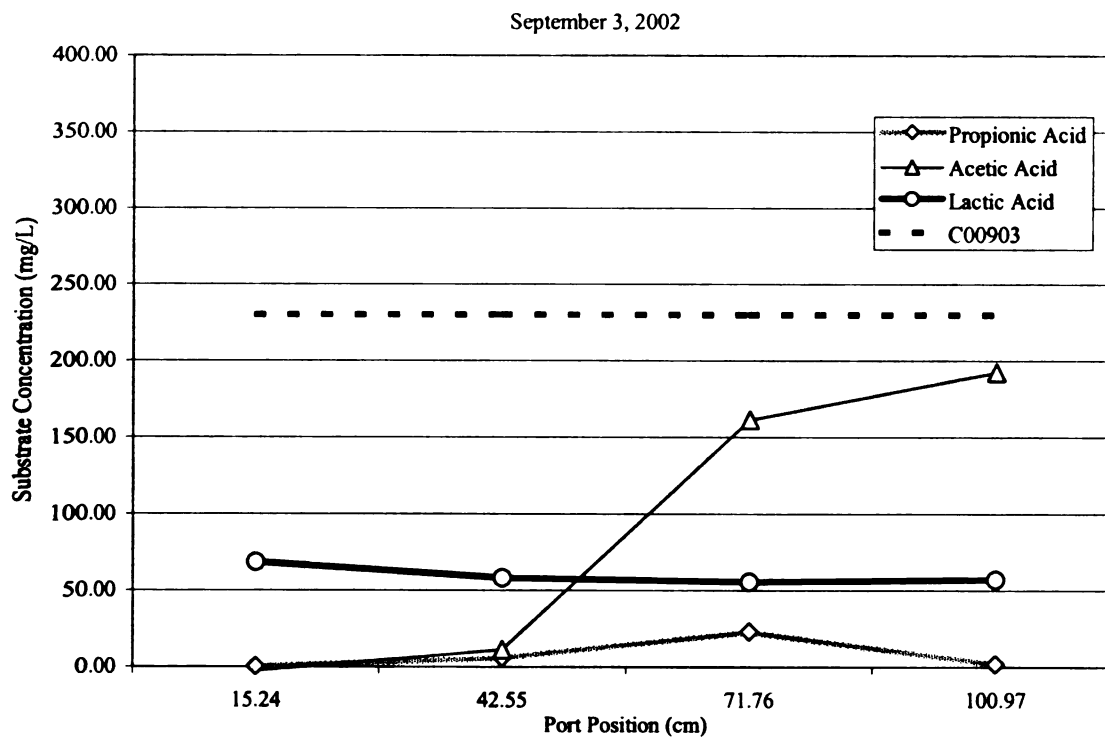
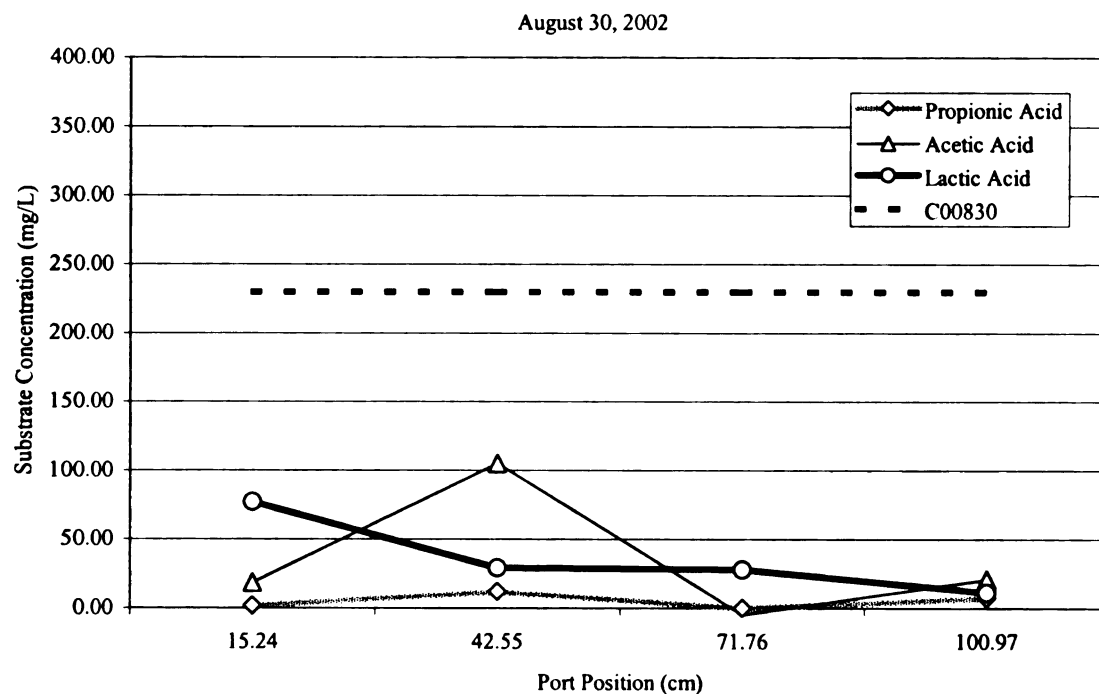
August 24, 2002



August 30, 2002



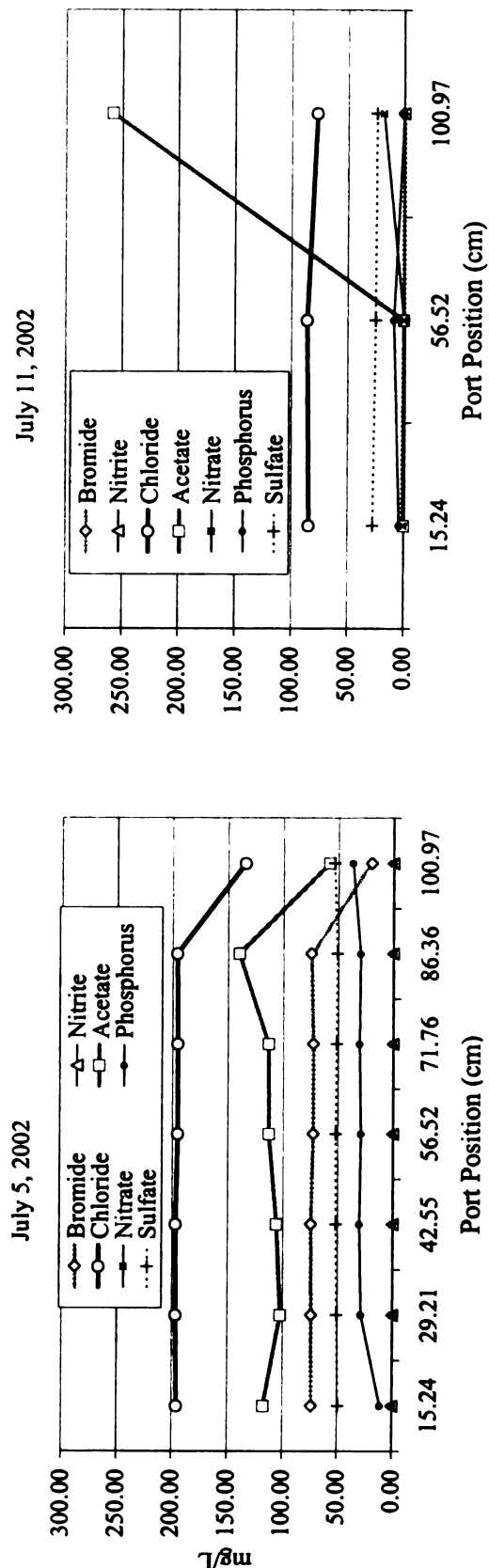
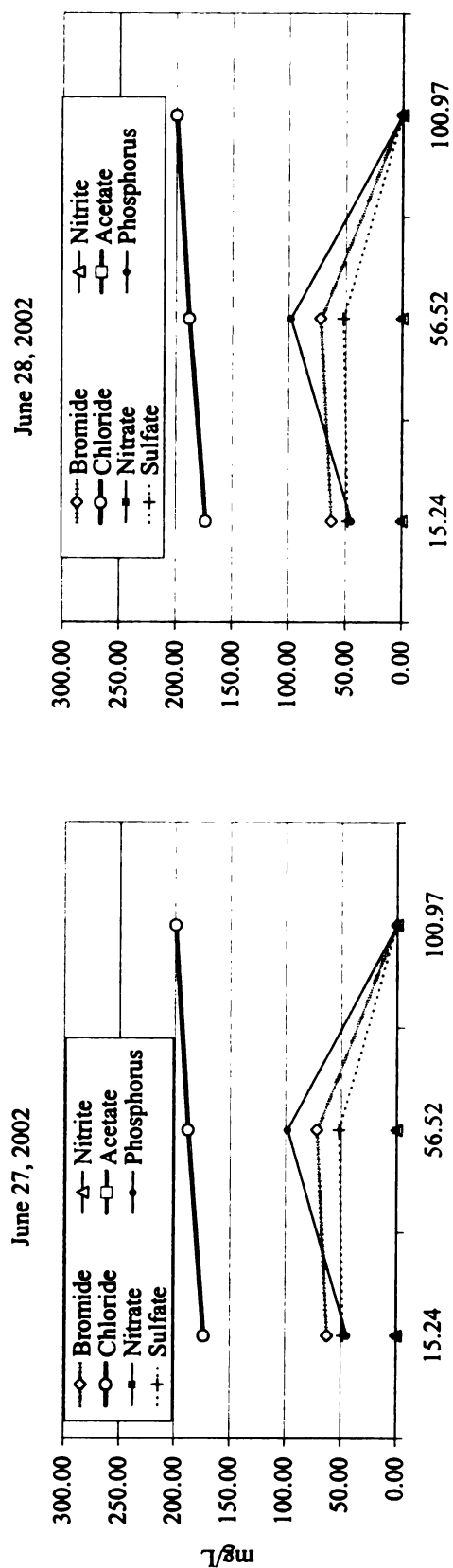
FATTY ACIDS
COLUMN A
Incubation Period 8A
August 30 - September 3, 2002



APPENDIX C-2

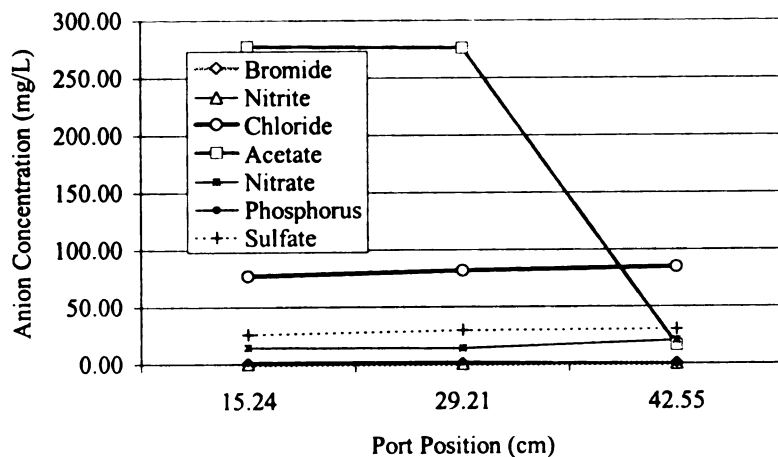
Major Anions

ANIONS
COLUMN A - Incubation Period 1A - June 27 - July 11, 2002

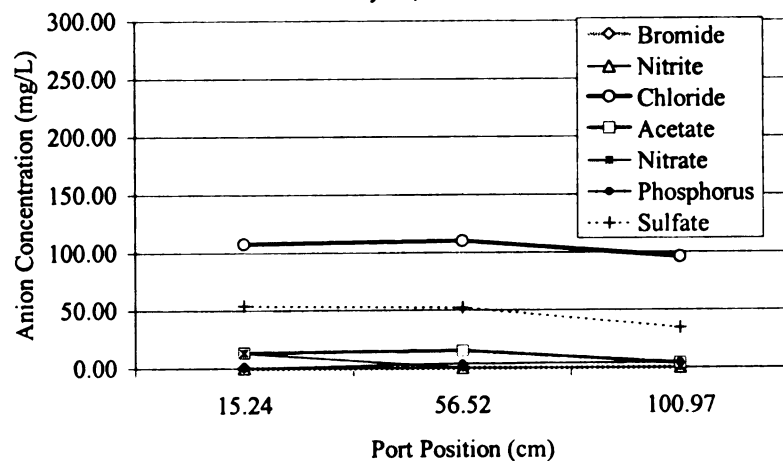


**ANIONS
COLUMN A
Incubation Period 2A
July 11 - 26, 2002**

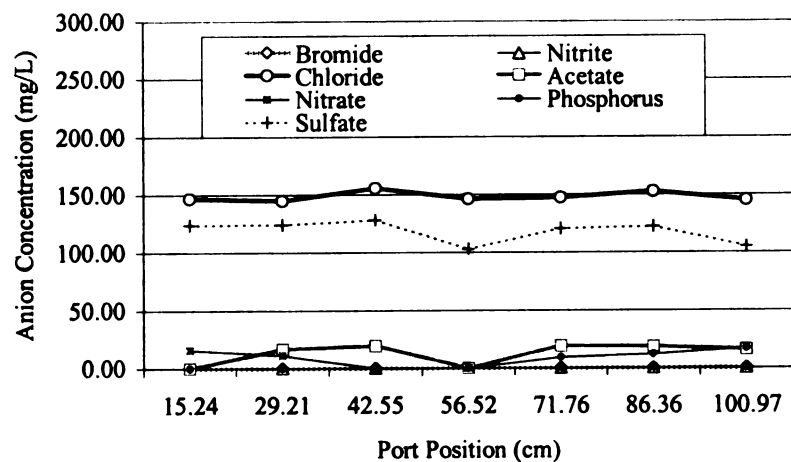
July 11, 2002



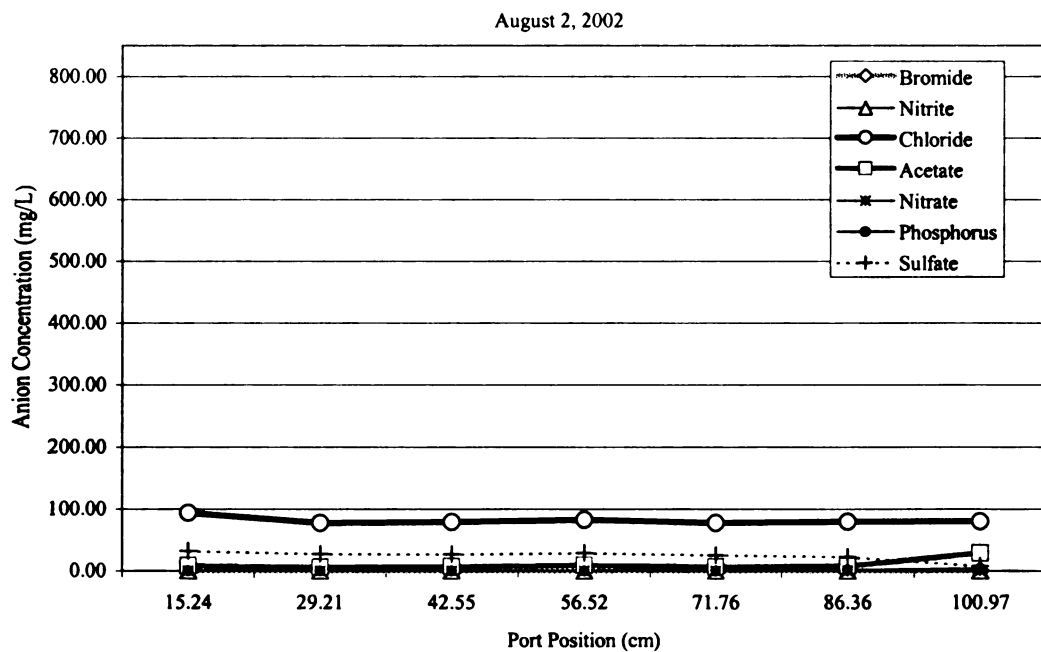
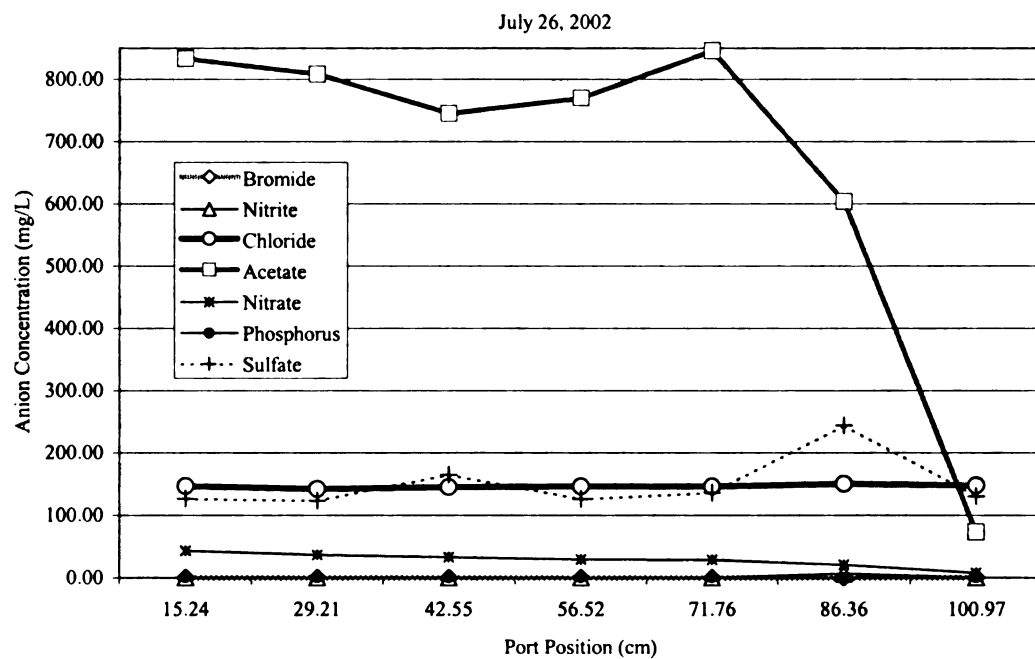
July 19, 2002



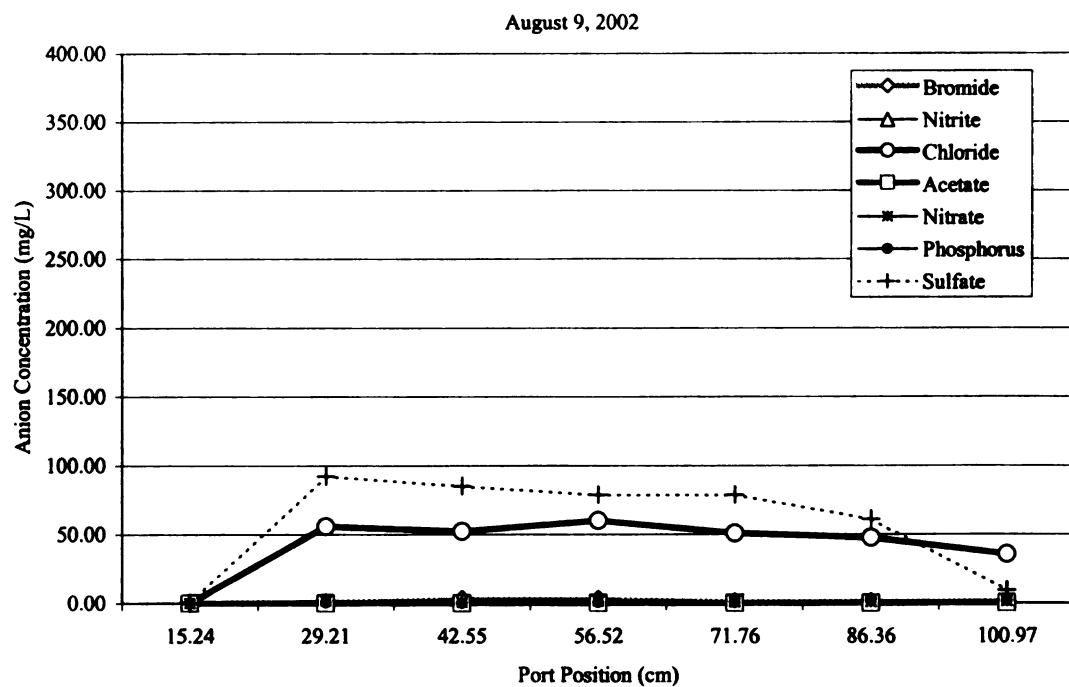
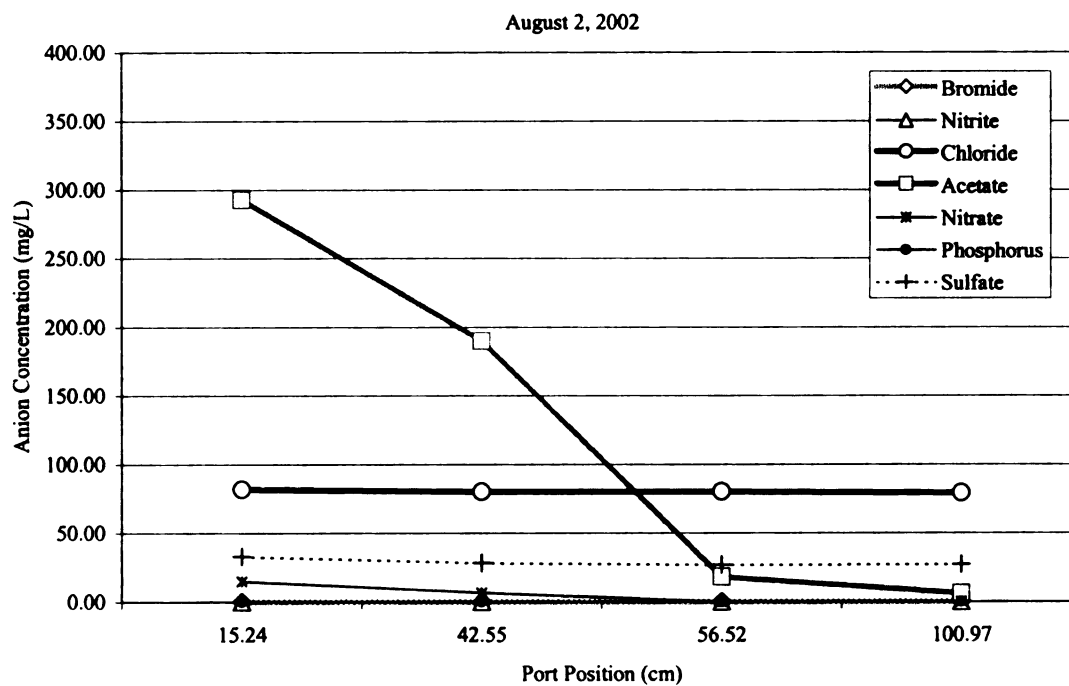
July 26, 2002



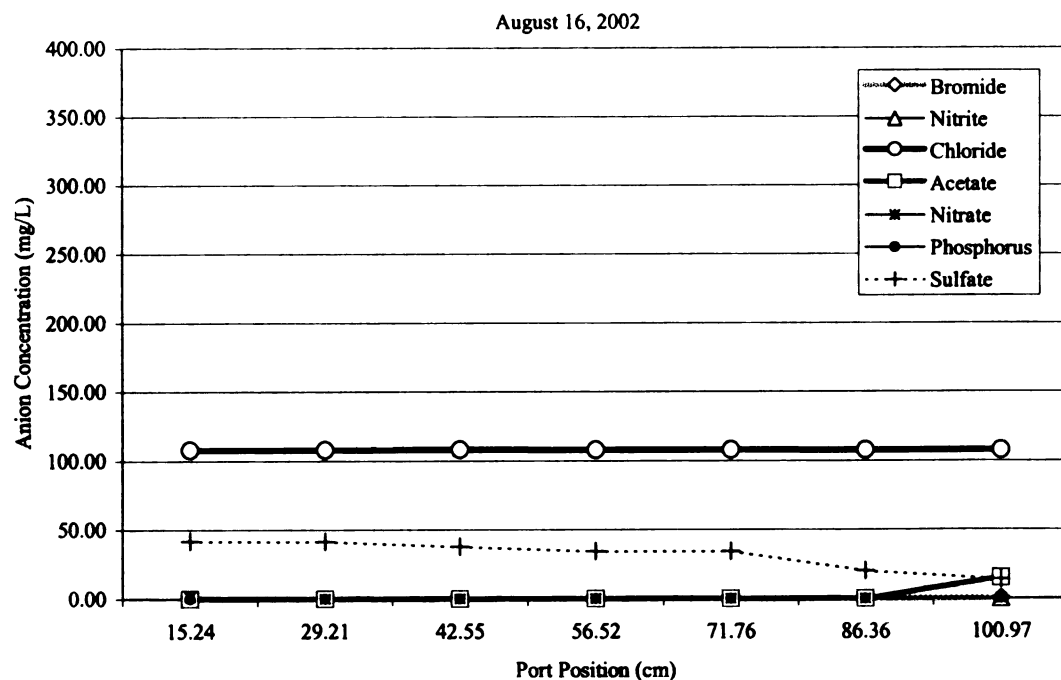
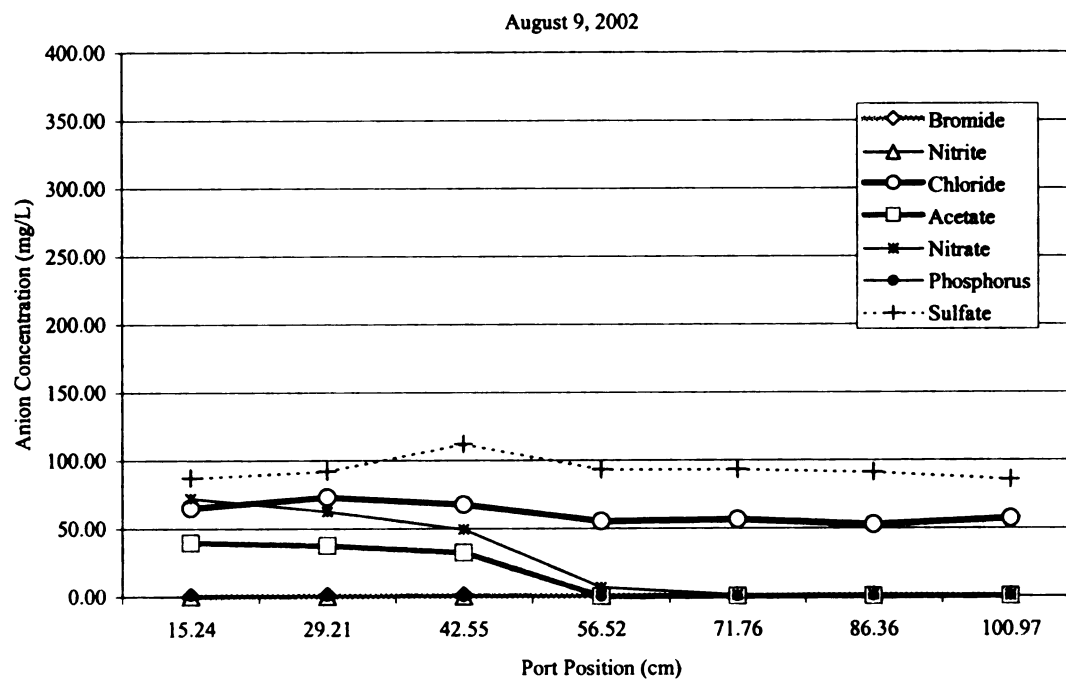
ANIONS
COLUMN A
Incubation Period 3A
July 26 - August 2, 2002



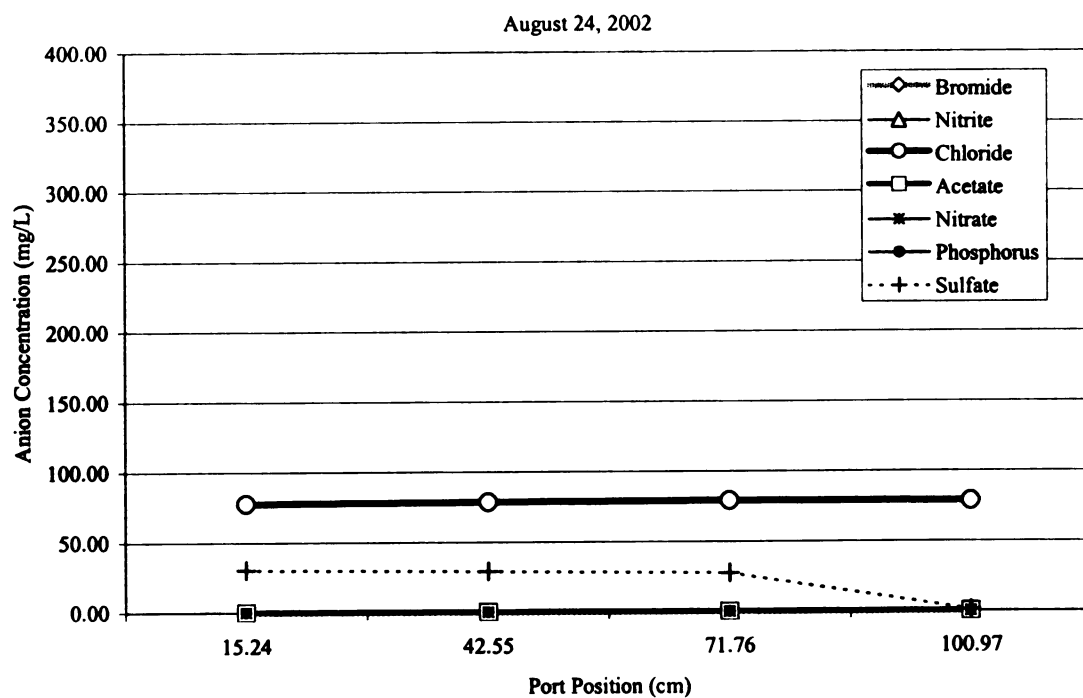
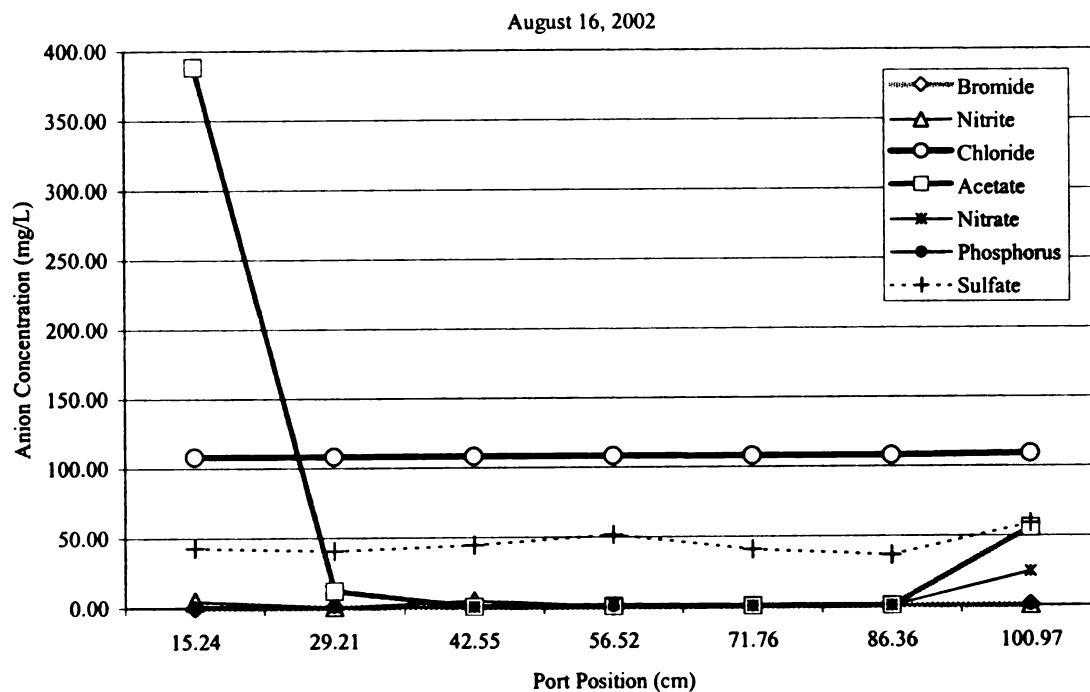
**ANIONS
COLUMN A
Incubation Period 4A
August 2 - 9, 2002**



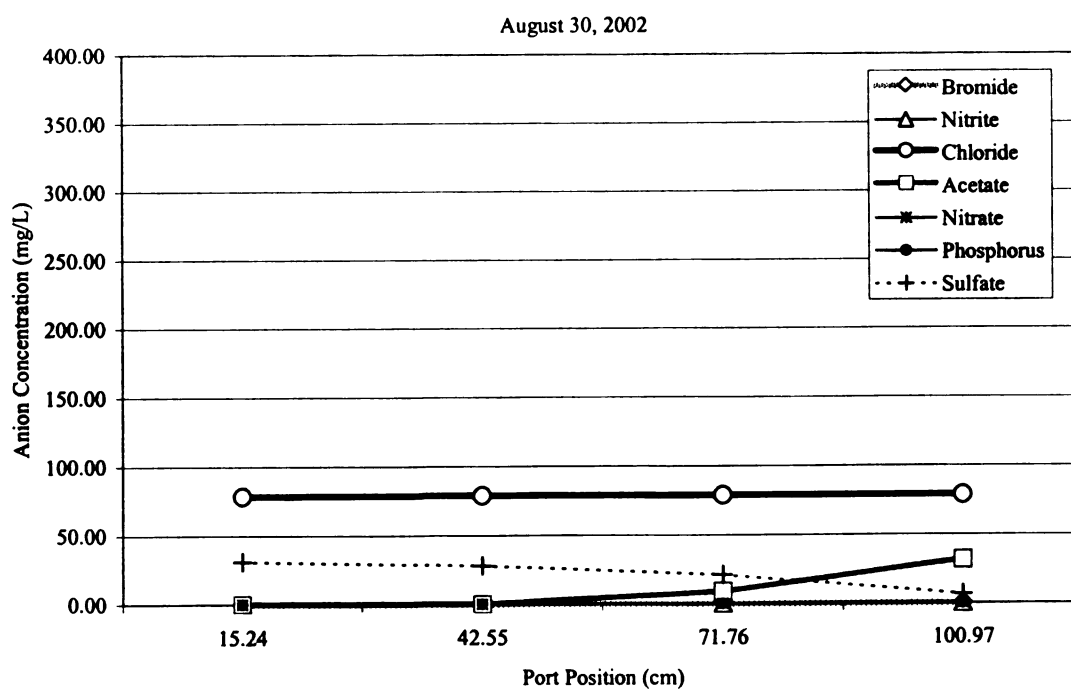
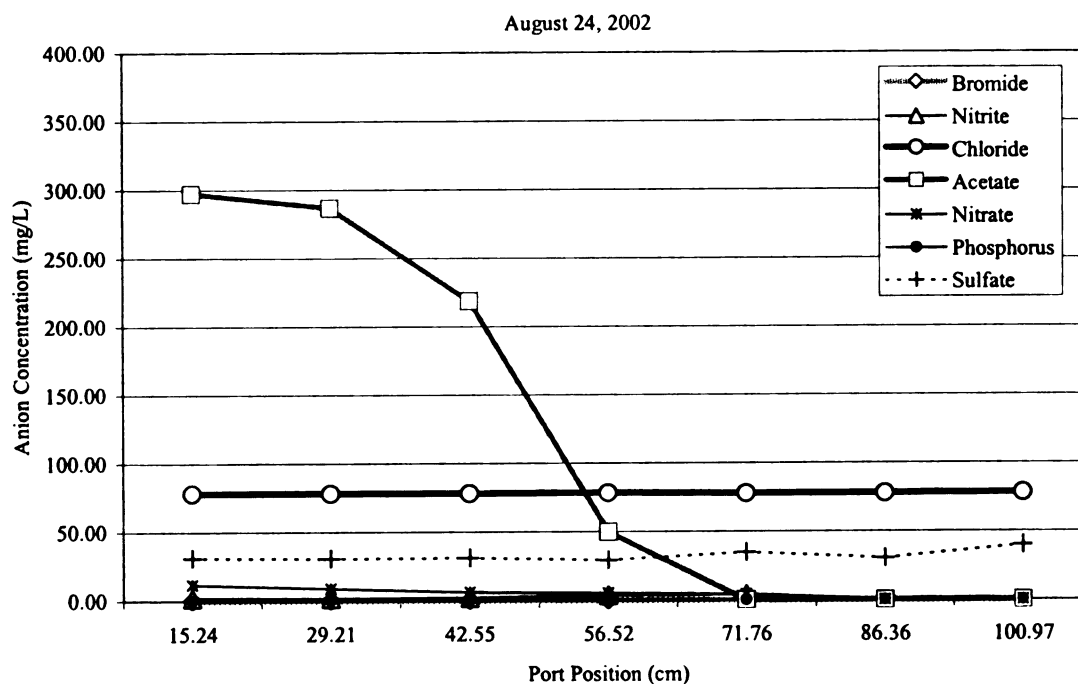
**ANIONS
COLUMN A
Incubation Period 5A
August 9 - 16, 2002**



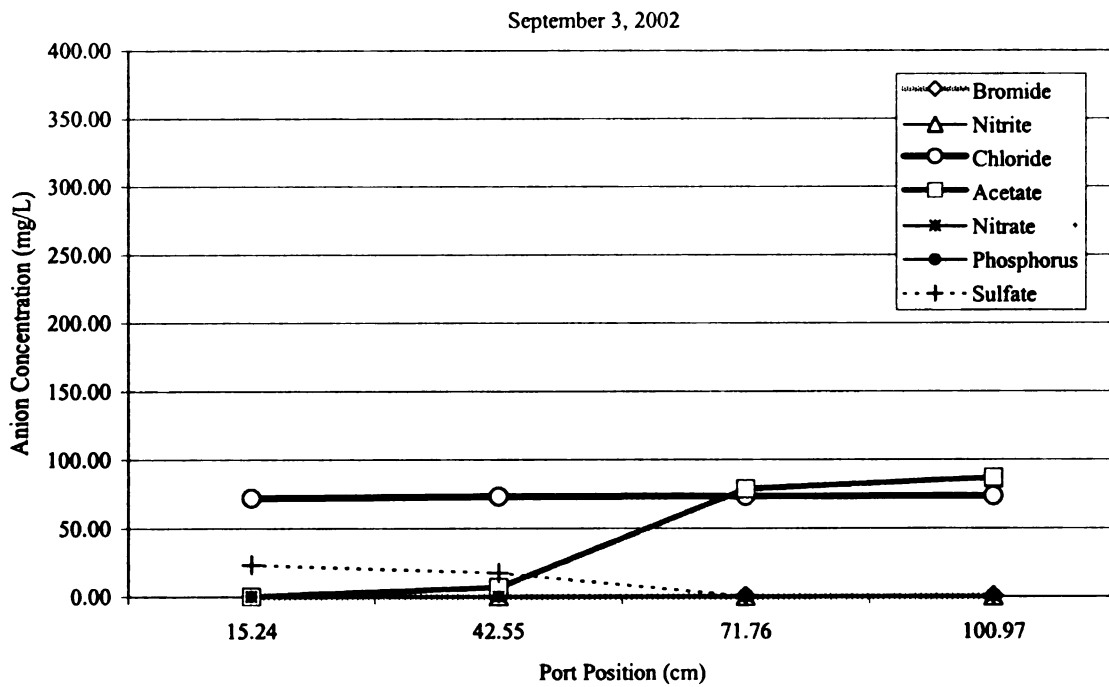
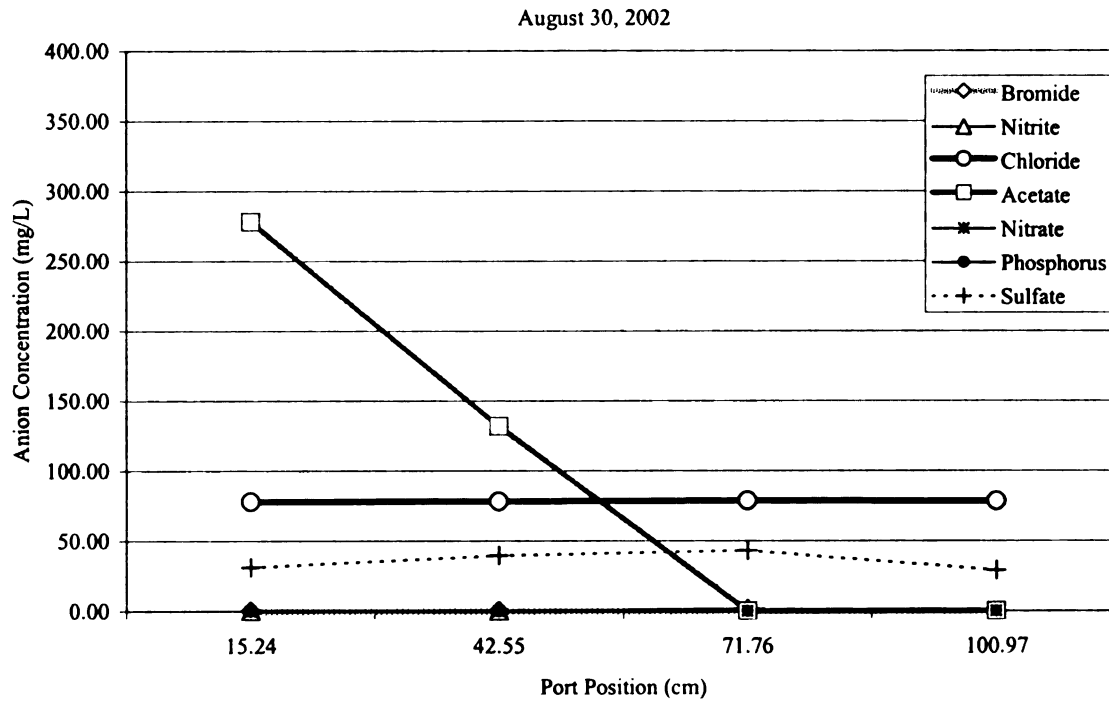
**ANIONS
COLUMN A
Incubation Period 6A
August 16 - 24, 2002**



ANIONS
COLUMN A
Incubation Period 7A
August 24 - 30, 2002



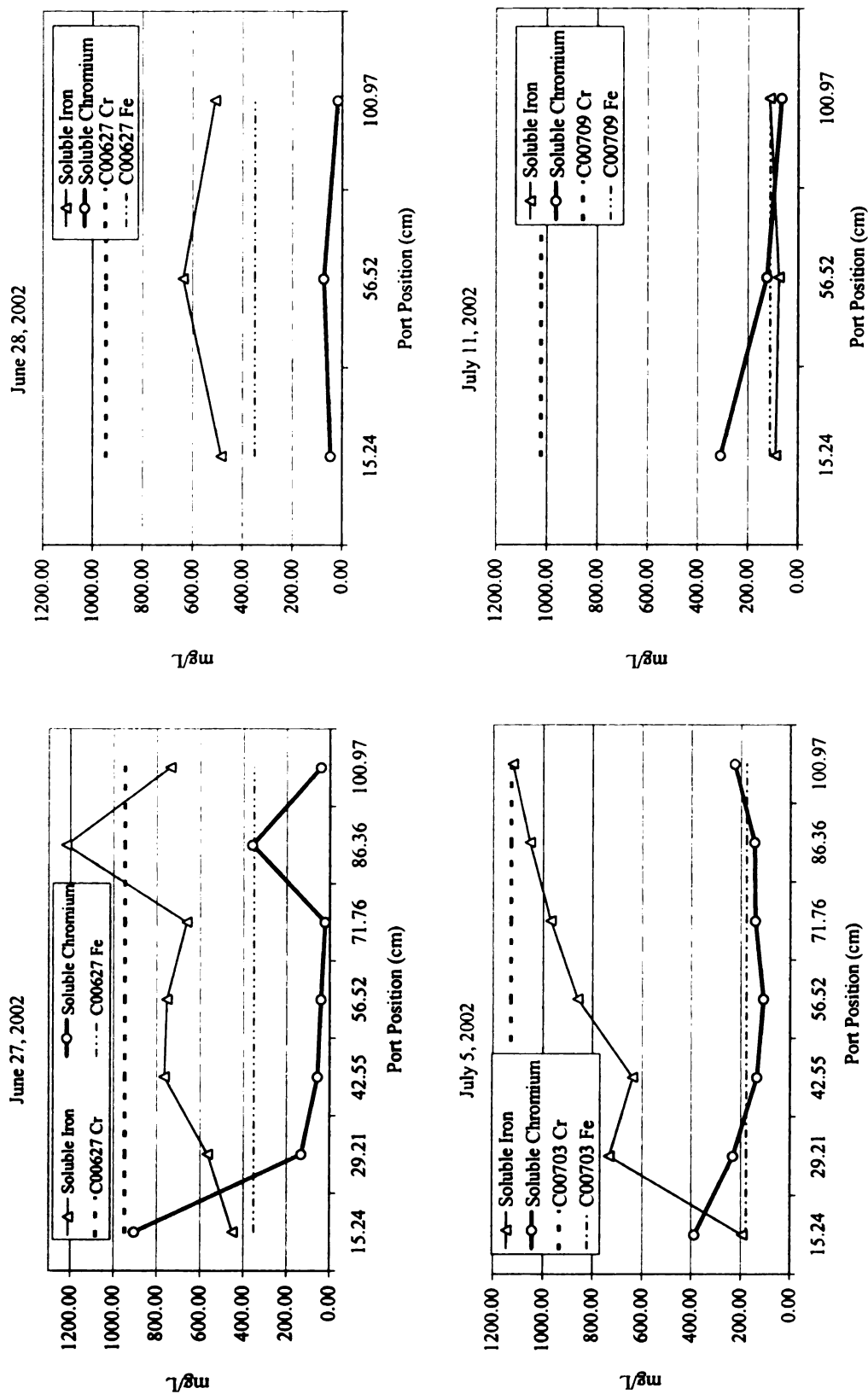
ANIONS
COLUMN A
Incubation Period 8A
August 30 - September 3, 2002



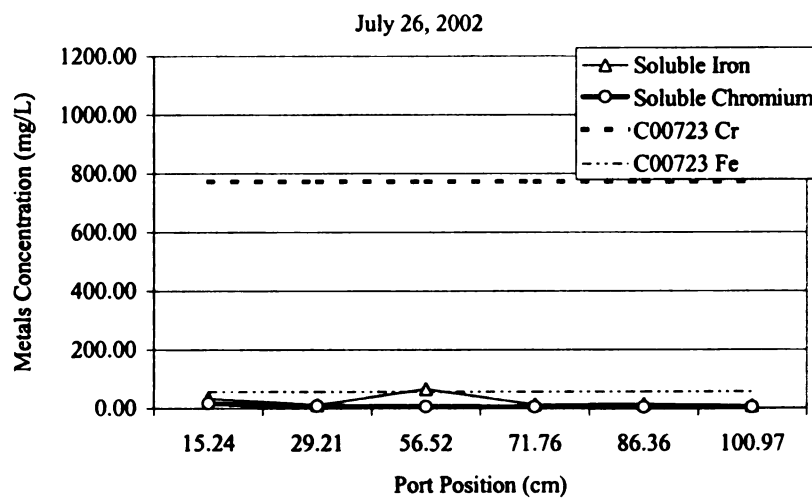
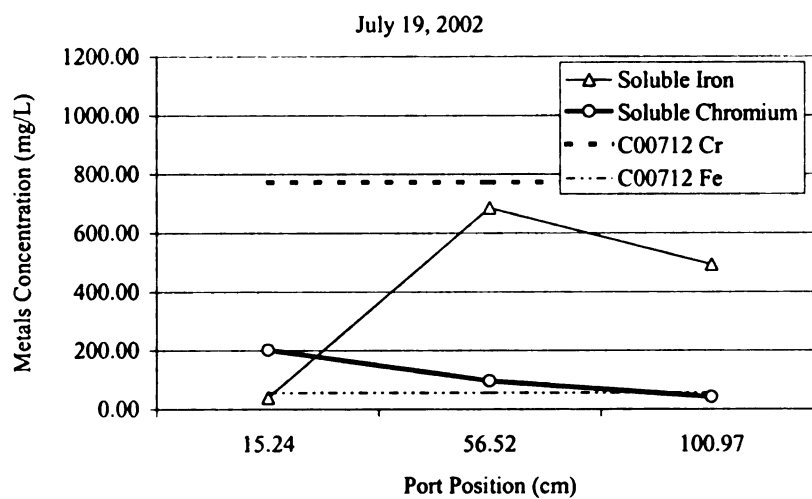
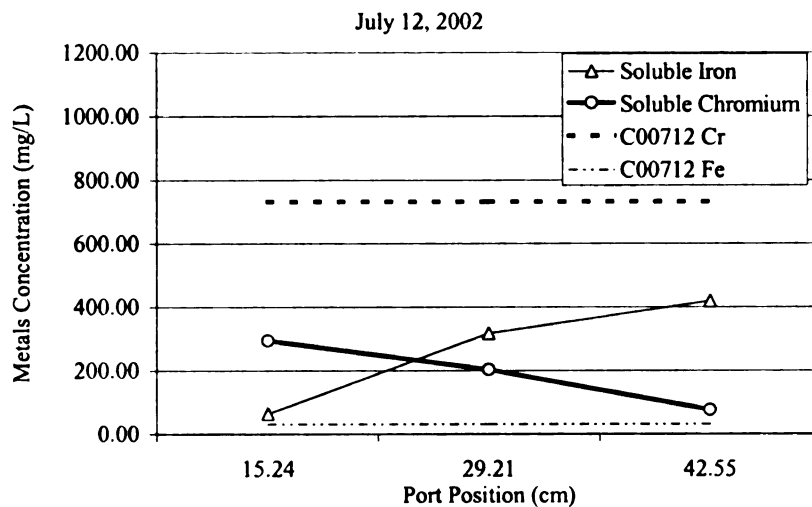
APPENDIX C-3

Soluble Metals

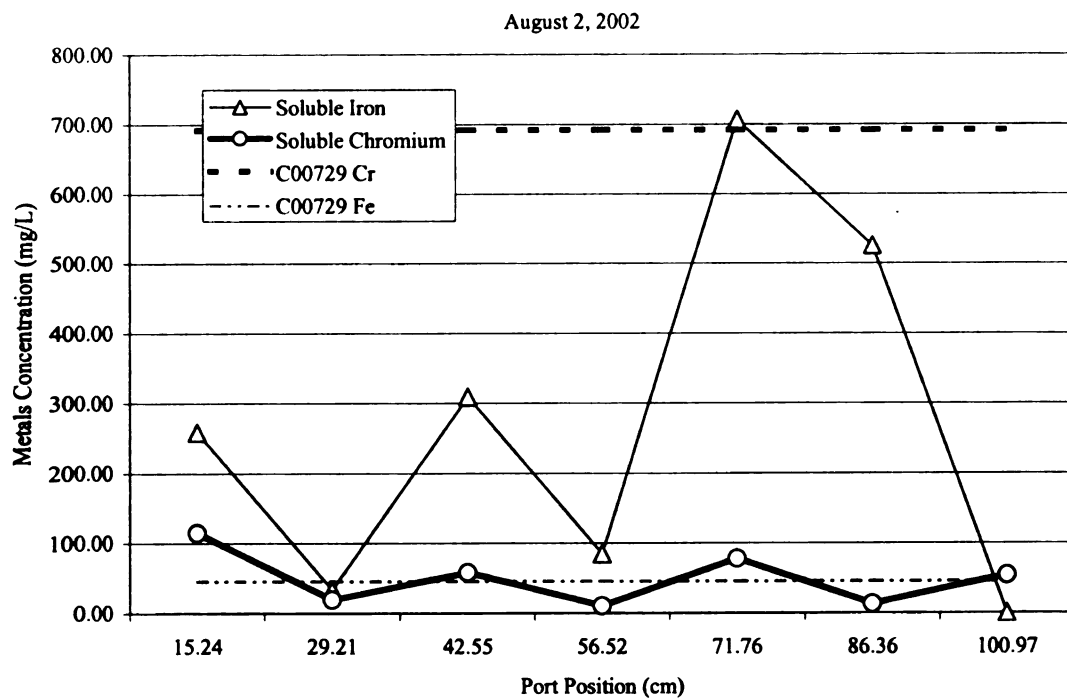
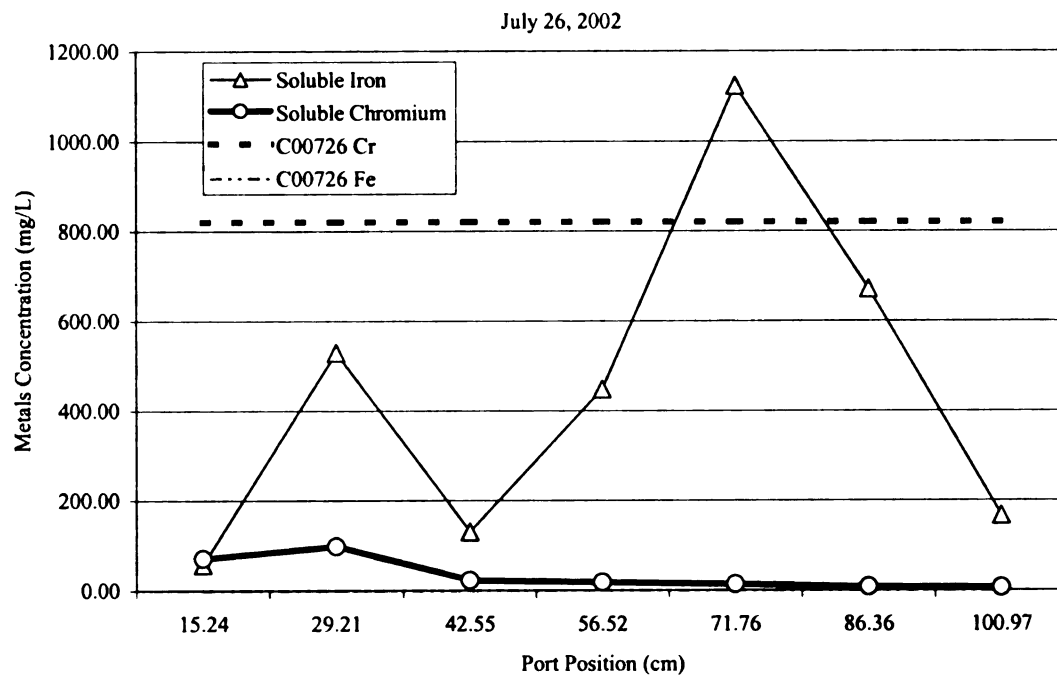
SOLUBLE METALS **COLUMN A** **Incubation Period 1A - June 27 - July 11, 2002**



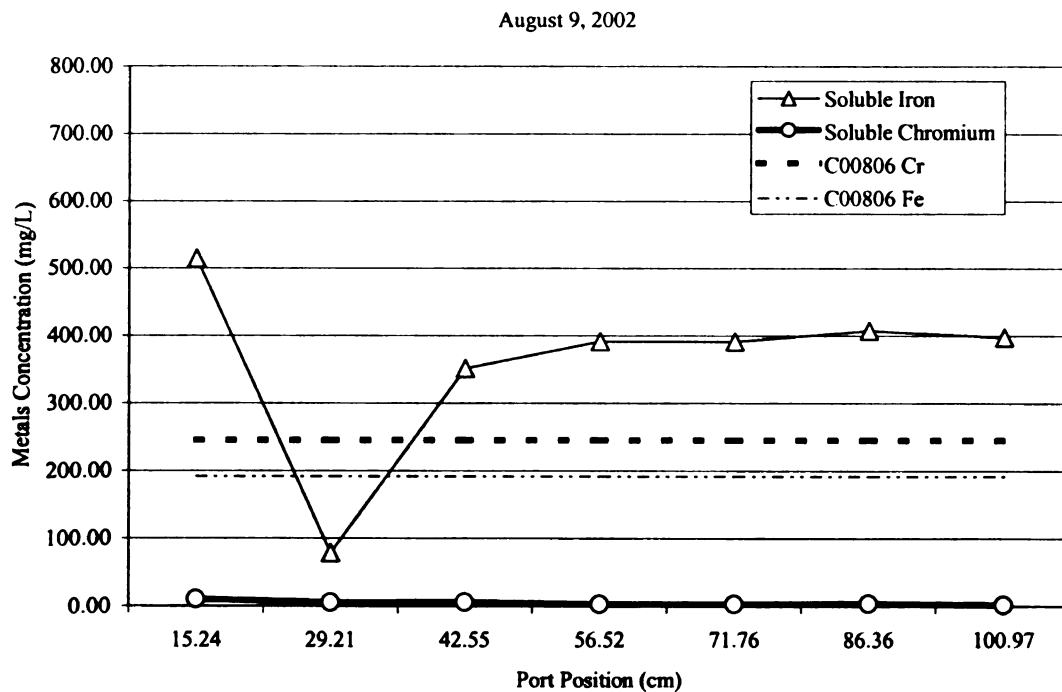
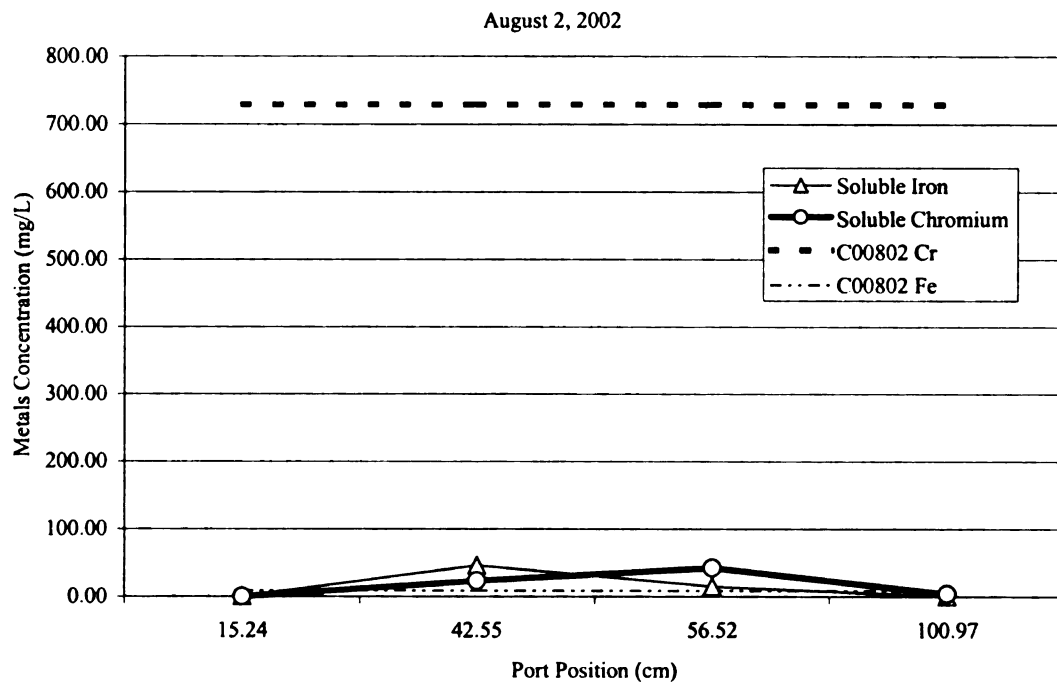
**SOLUBLE METALS
COLUMN A
Incubation Period 2A
July 11 - July 26, 2002**



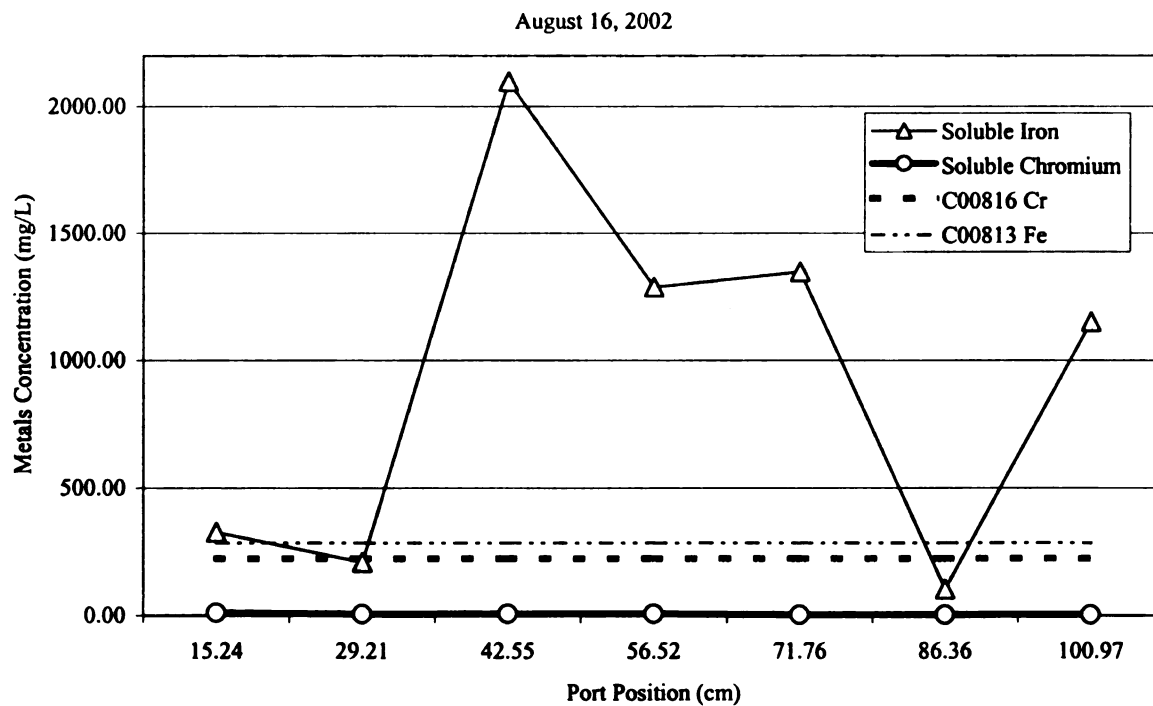
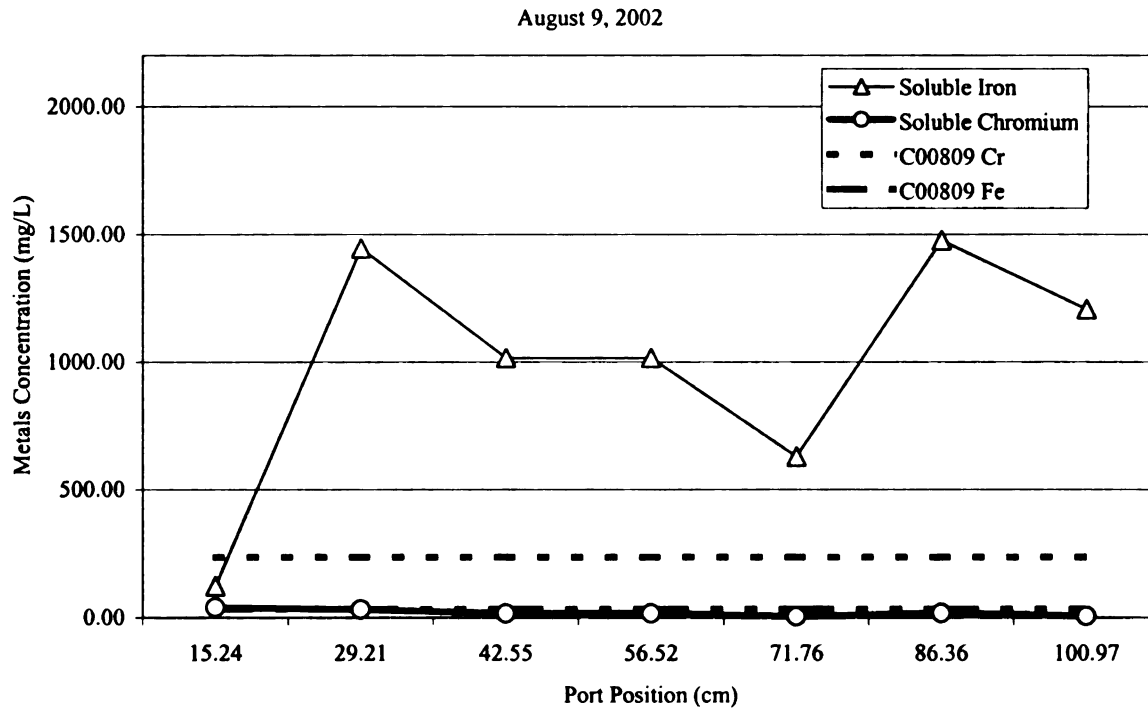
**SOLUBLE METALS
COLUMN A
Incubation Period 3A
July 26 - August 2, 2002**



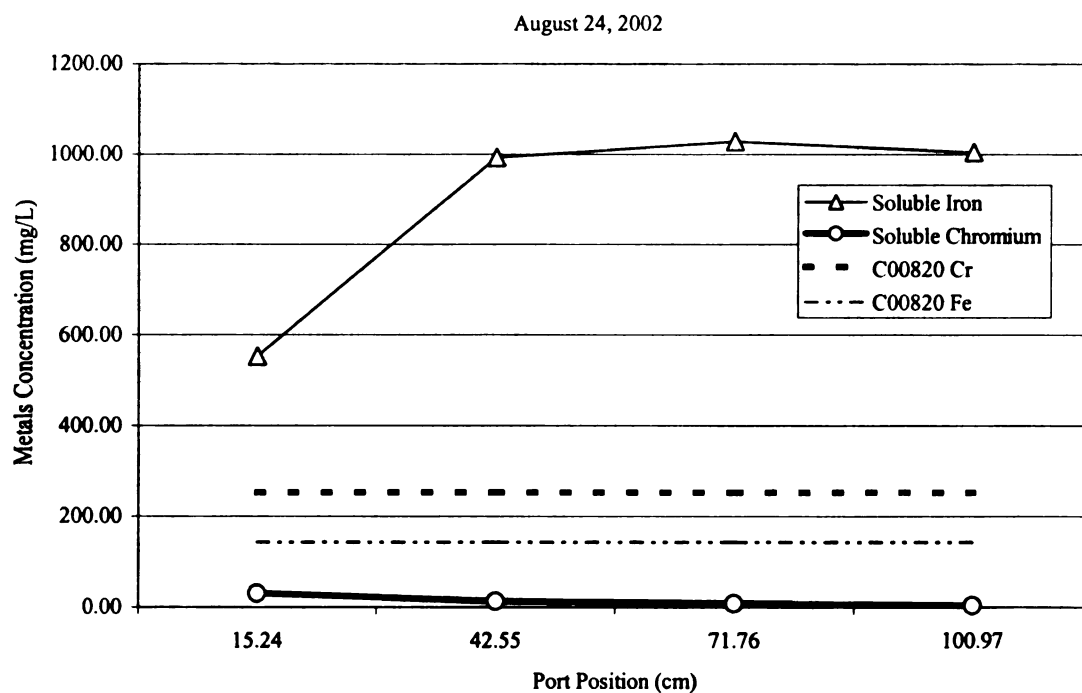
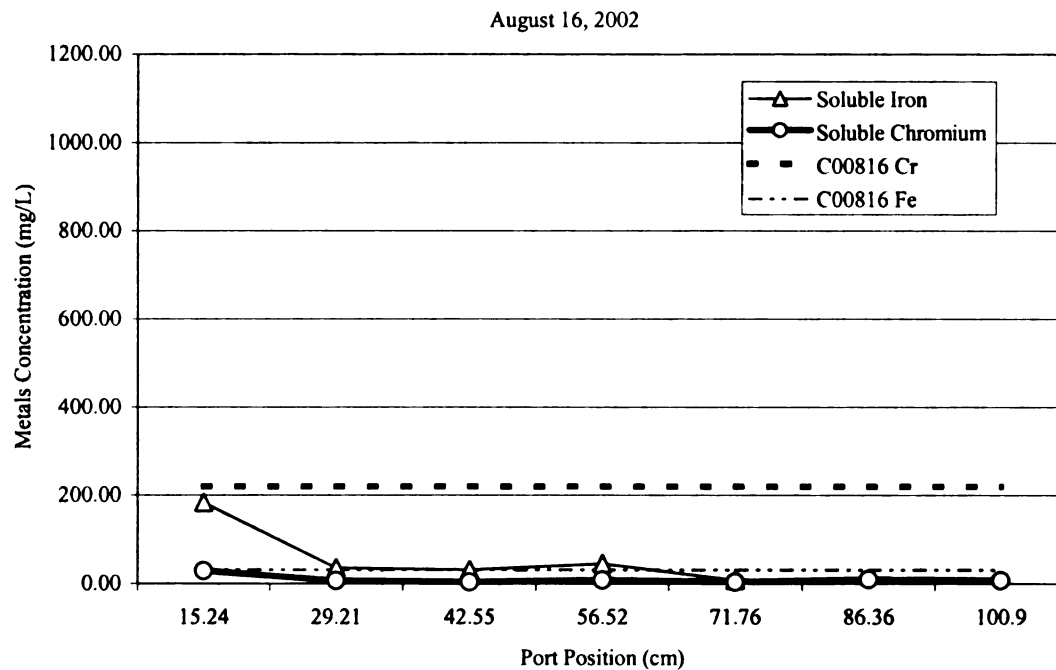
**SOLUBLE METALS
COLUMN A
Incubation Period 4A
August 2 - 9, 2002**



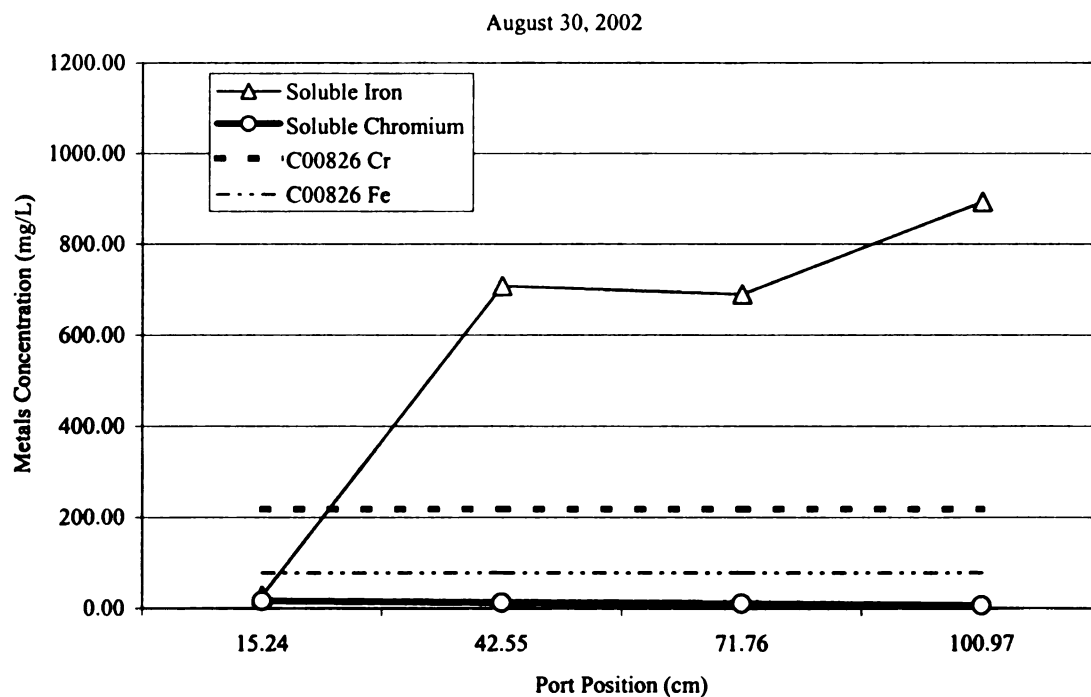
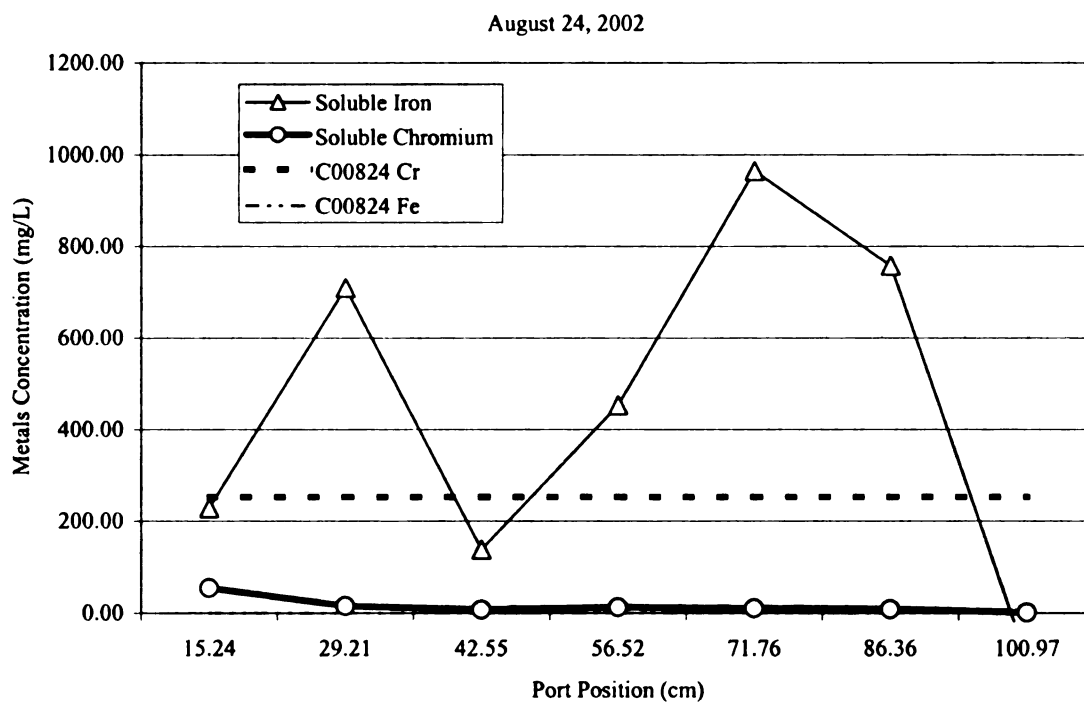
SOLUBLE METALS
COLUMN A
Incubation 5A
August 9 - 16, 2002



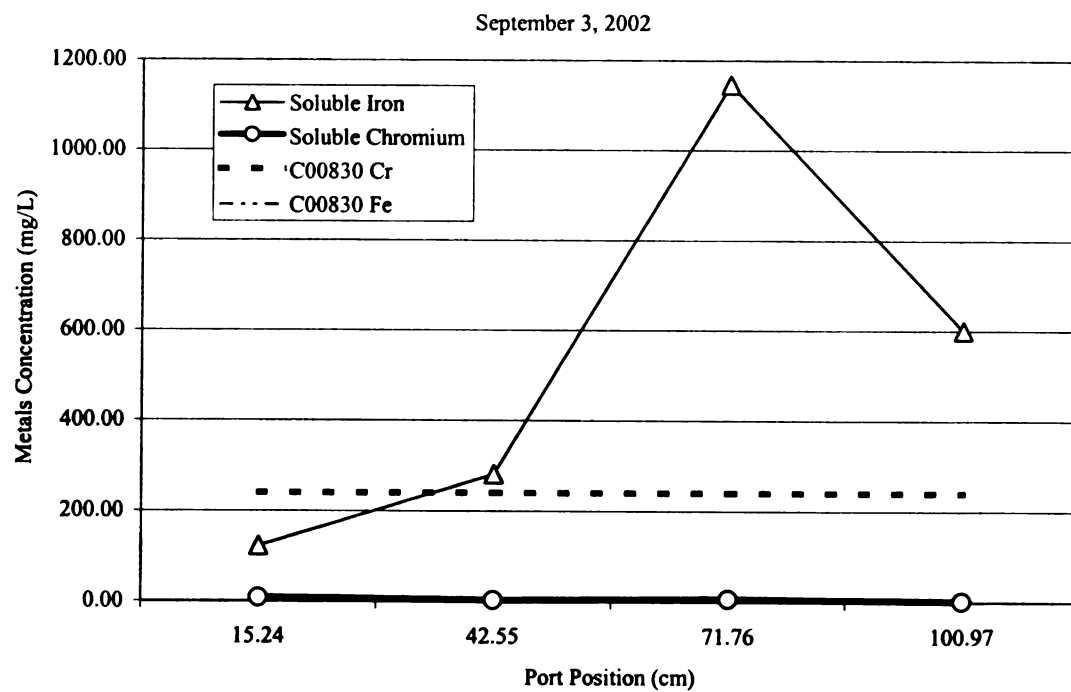
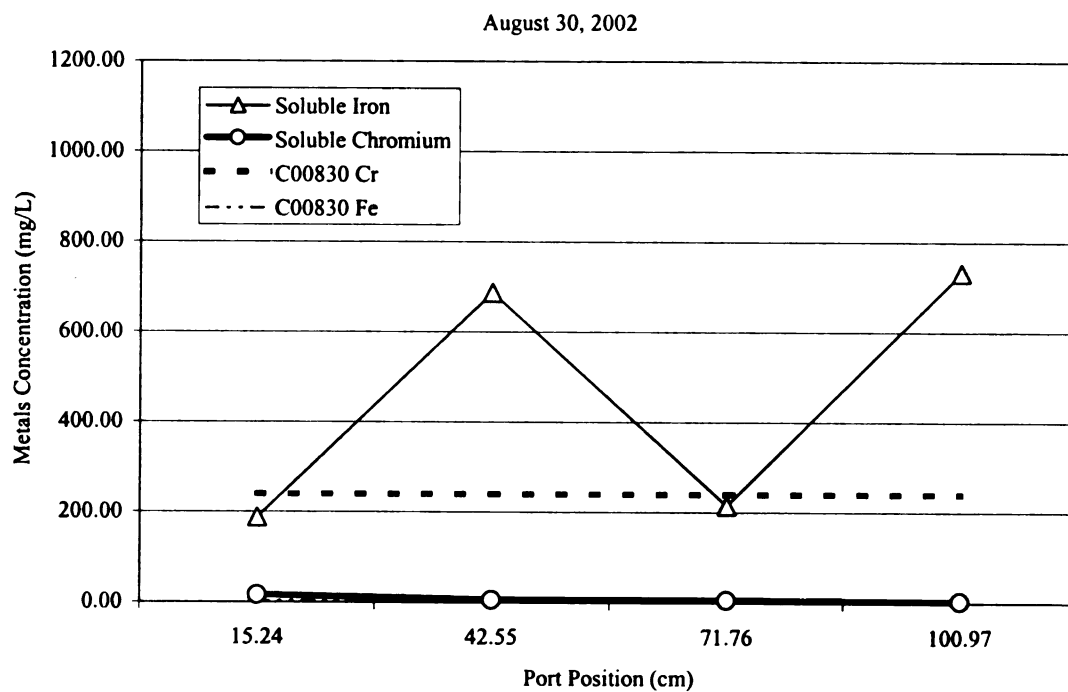
**SOLUBLE METALS
COLUMN A
Incubation Period 6A
August 16 - 24, 2002**



**SOLUBLE METALS
COLUMN A
Incubation Period 7A
August 24 - 30, 2002**



SOLUBLE METALS
COLUMN A
Incubation Period 8A
August 30 - Septmeber 3, 2002

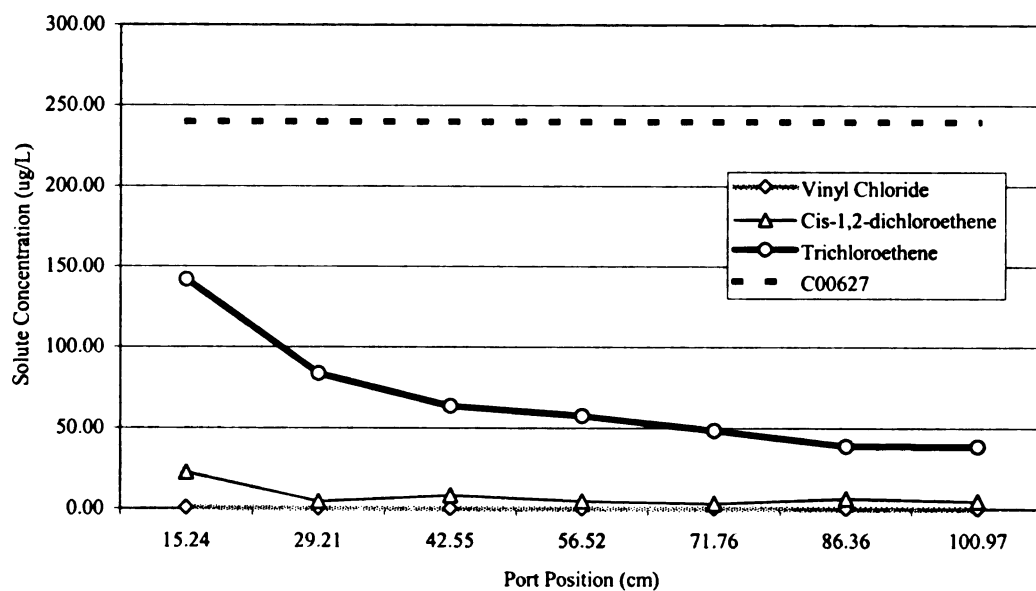


APPENDIX C-4

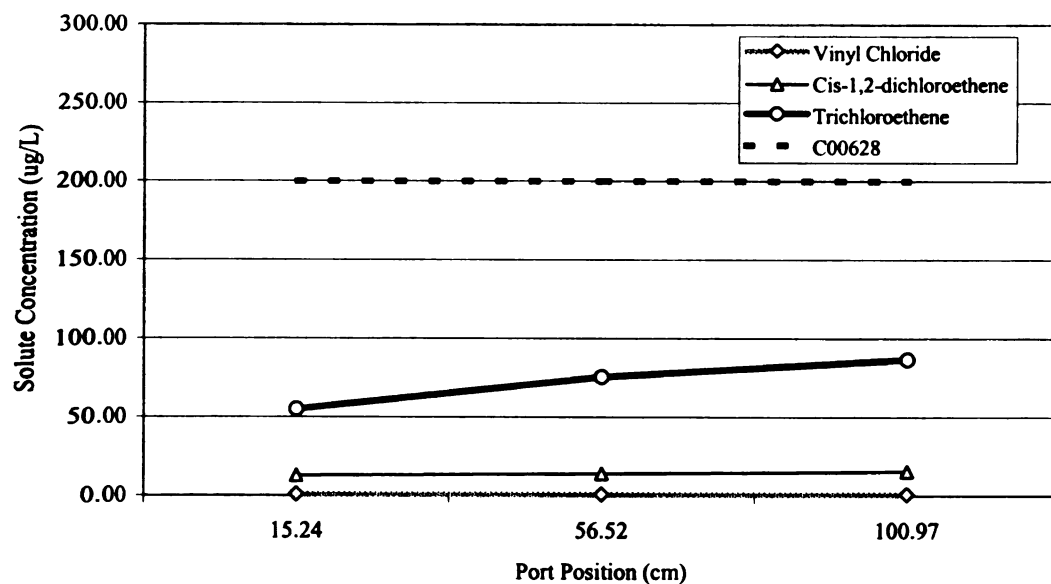
Chlorinated Aliphatics

**CHLORINATED VOCs
COLUMN A
June 27 - 28, 2002
(Pre-Inoculation)**

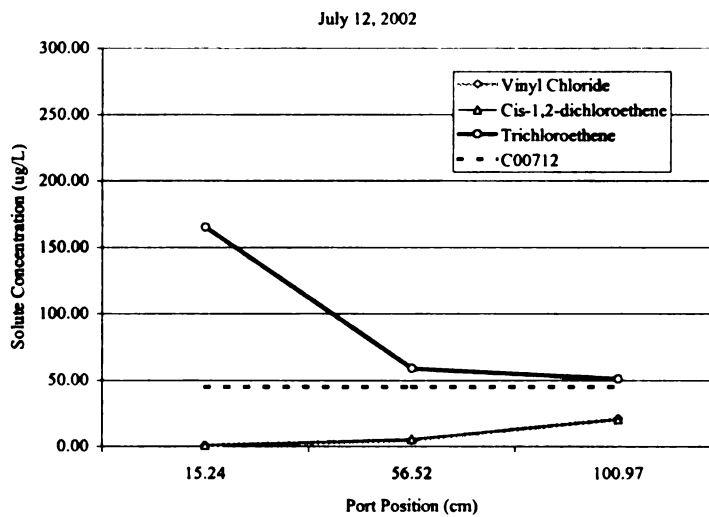
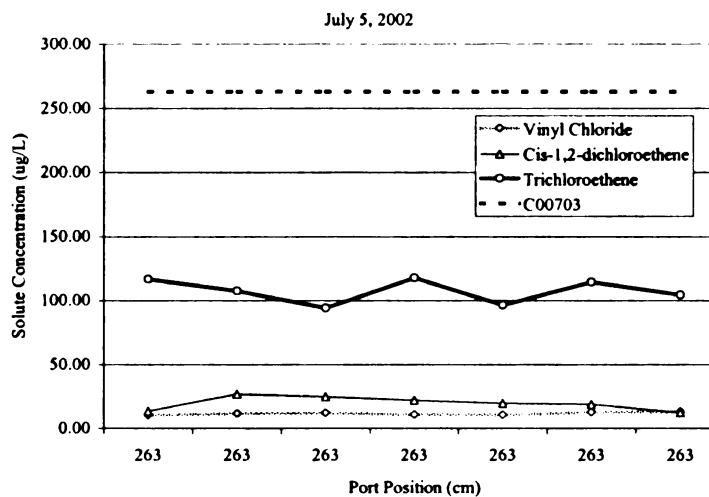
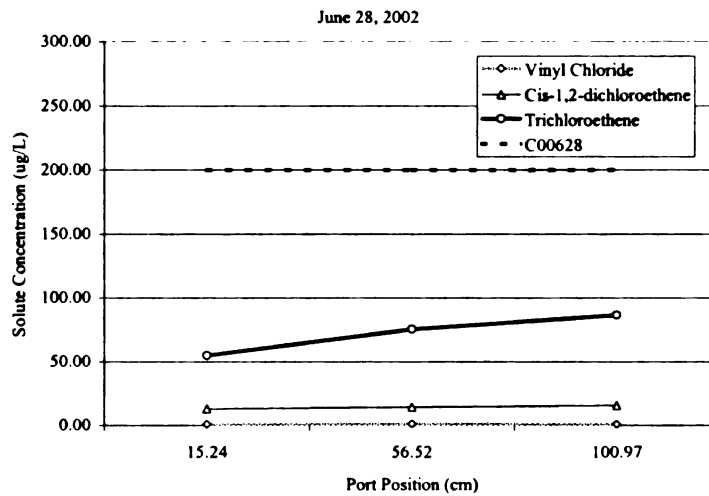
June 27, 2002



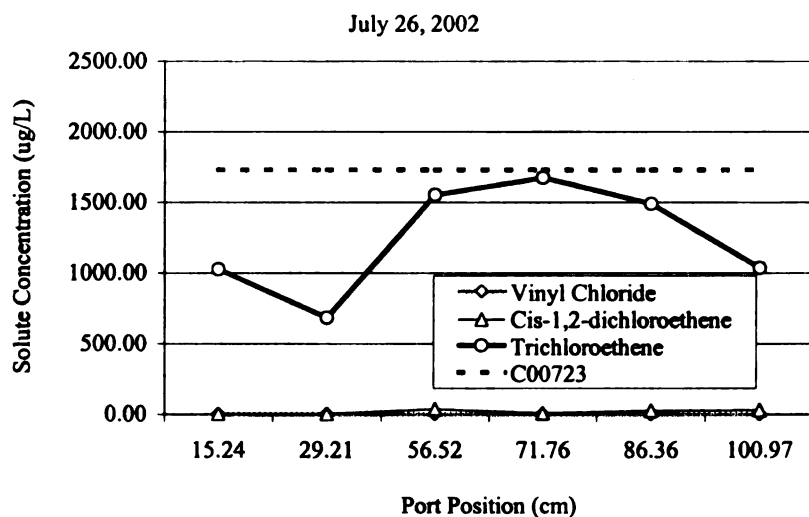
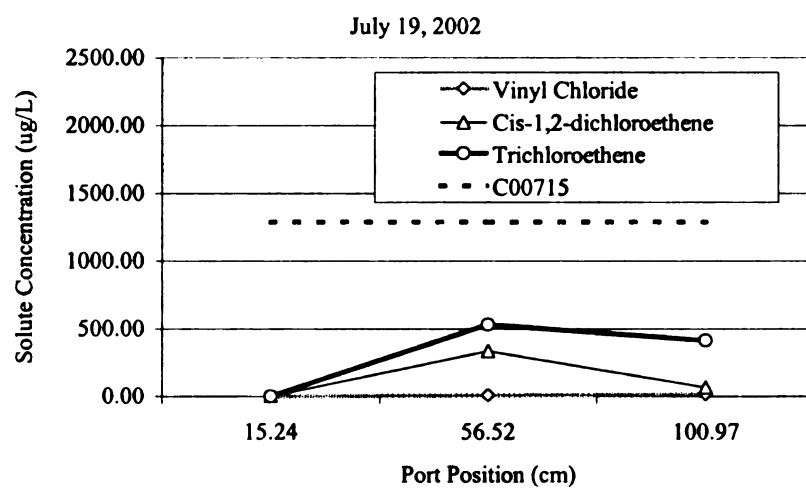
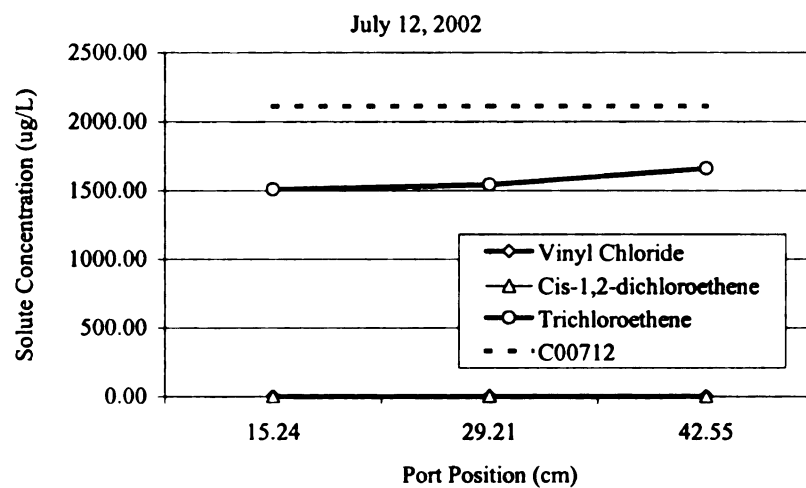
June 28, 2002



**CHLORINATED VOCs
COLUMN A
June 28 - July 11, 2002
(Incubation Period 1A)**

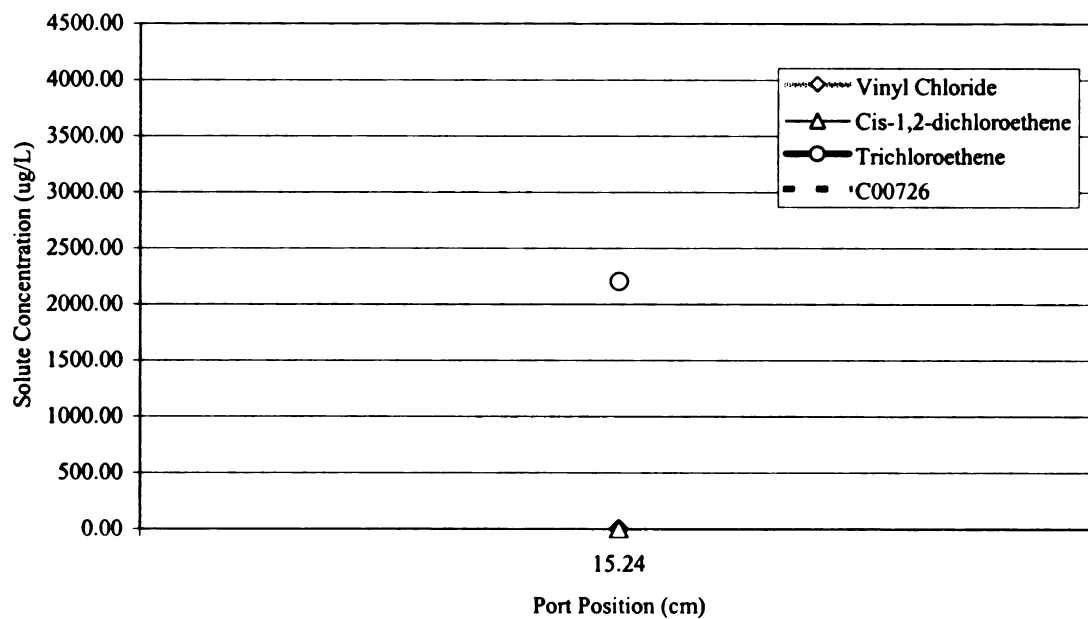


**CHLORINATED VOCs
COLUMN A
July 12 - 26, 2002
(Incubation Period 2A)**

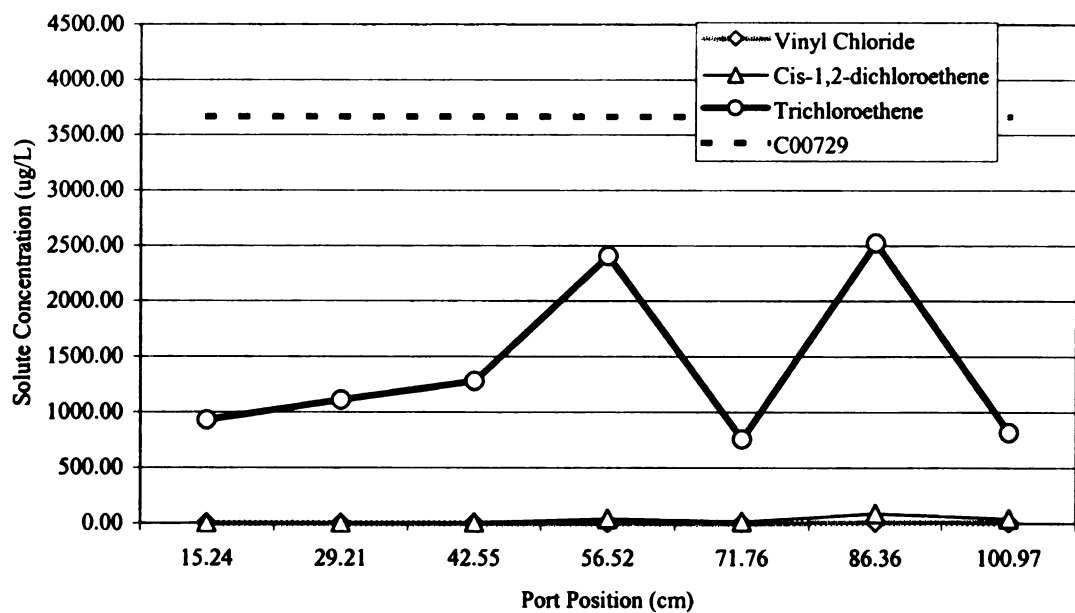


**CHLORINATED VOCs
COLUMN A
July 26 - August 2, 2002
(Incubation Period 3A)**

July 26, 2002

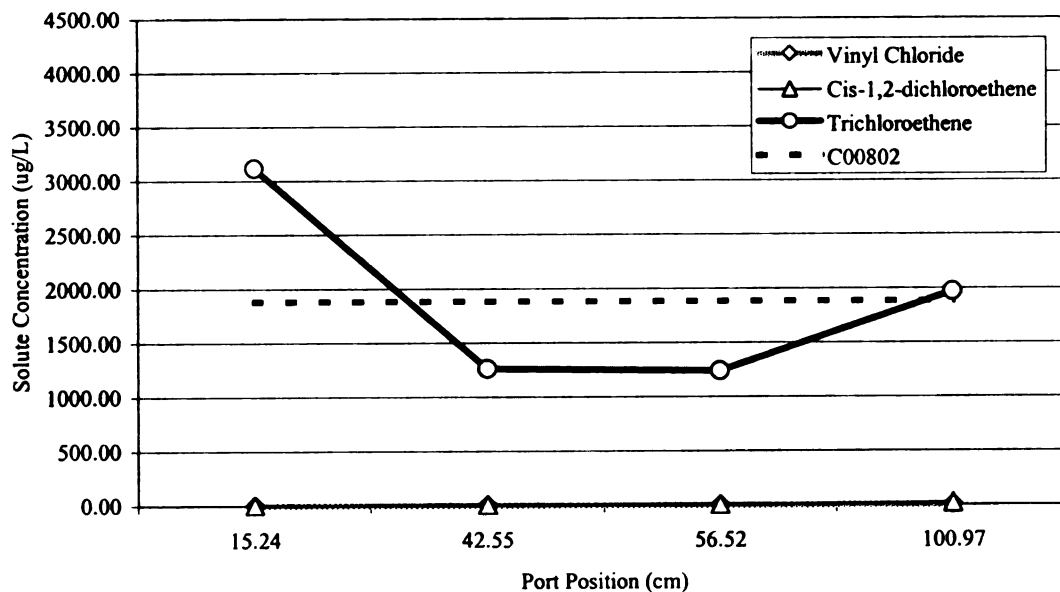


August 2, 2002

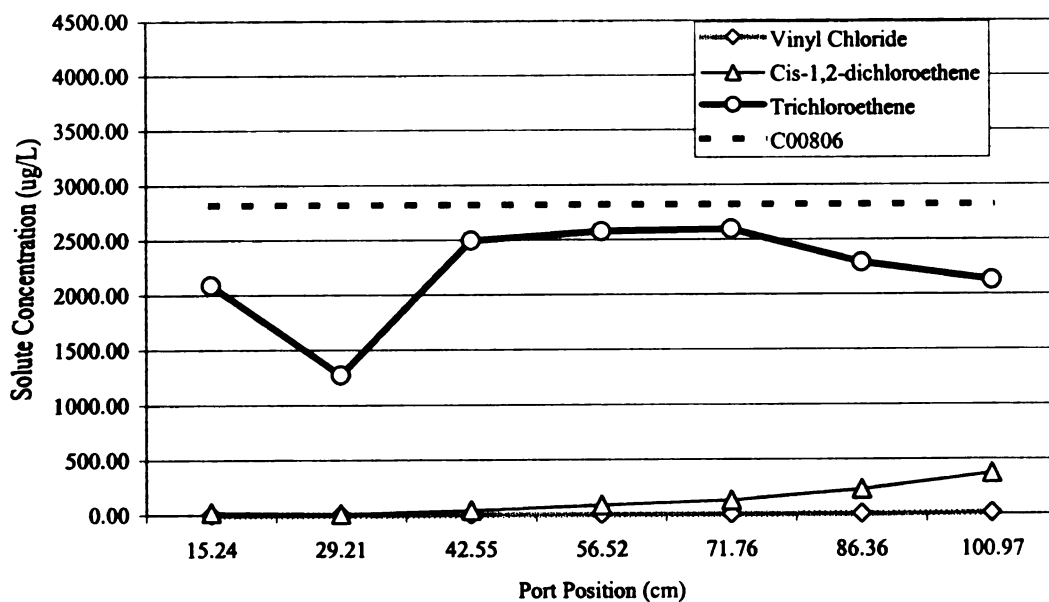


CHLORINATED VOCs
COLUMN A
August 2 - 9, 2002
(Incubation Period 4A)

August 2, 2002

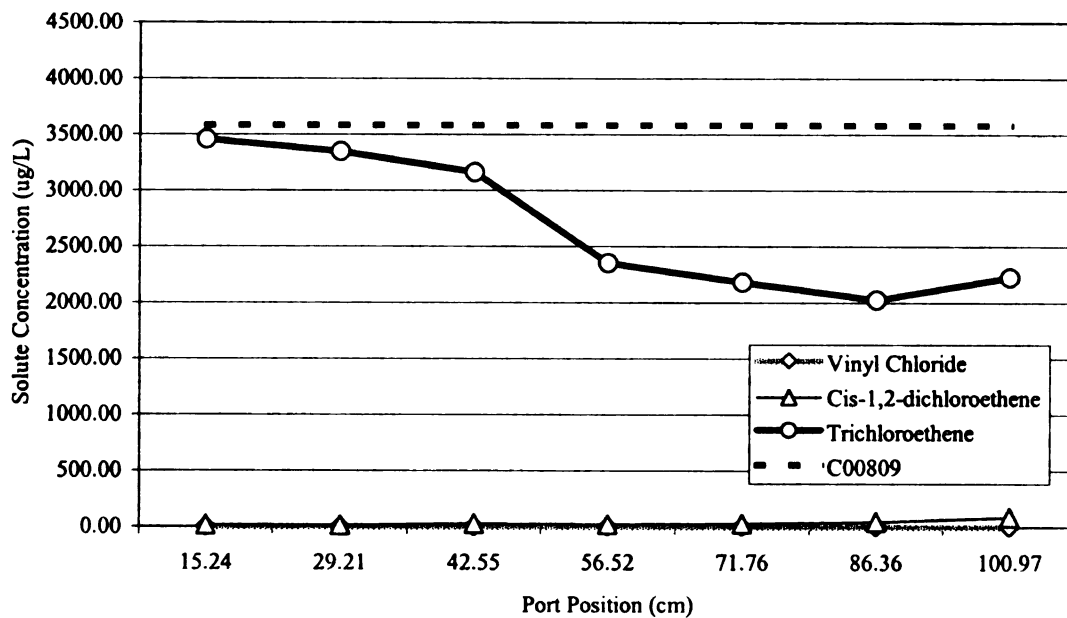


August 9, 2002

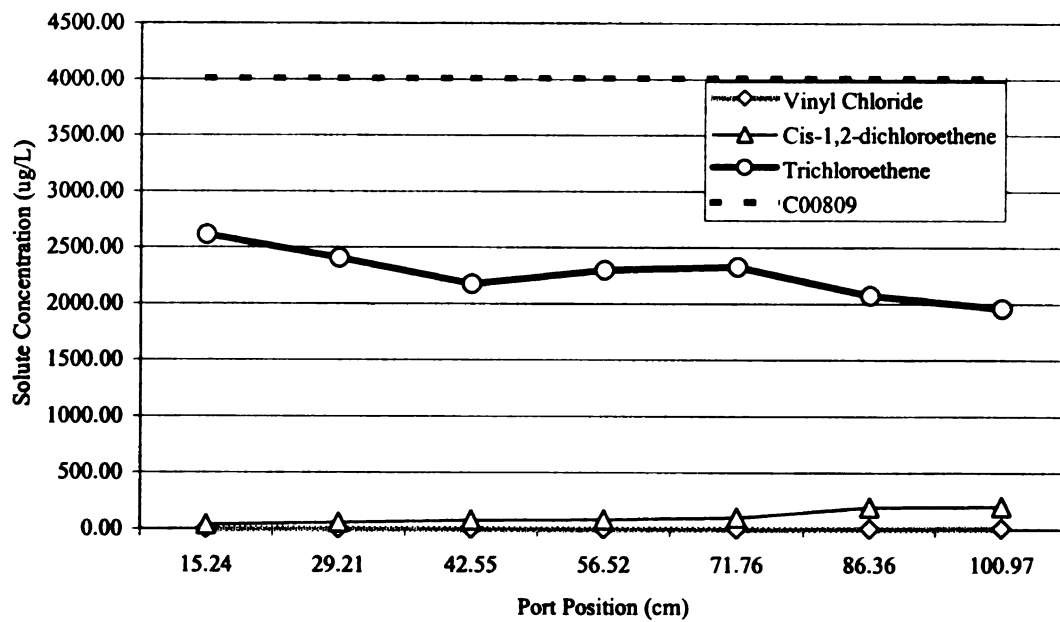


**CHLORINATED VOCs
COLUMN A
August 9 - 16, 2002
(Incubation Period 5A)**

August 9, 2002

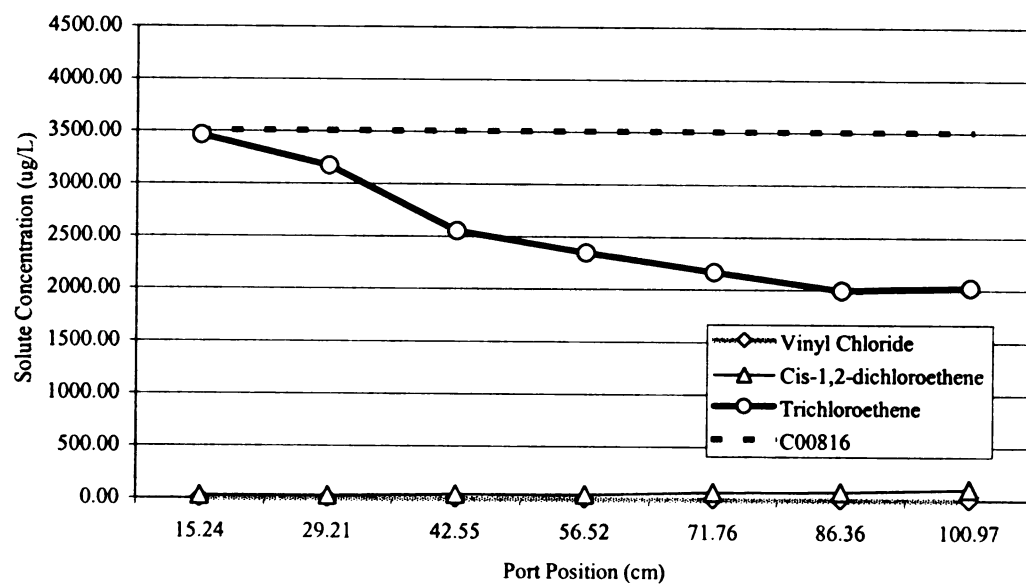


August 16, 2002

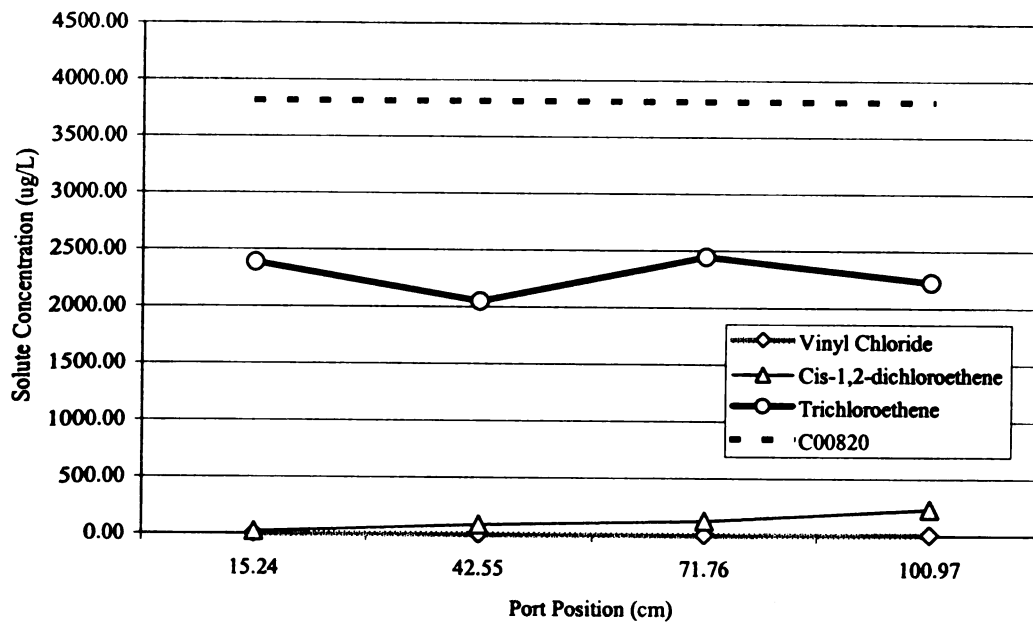


**CHLORINATED VOCs
COLUMN A
August 16 - 24, 2002
(Incubation Period 6A)**

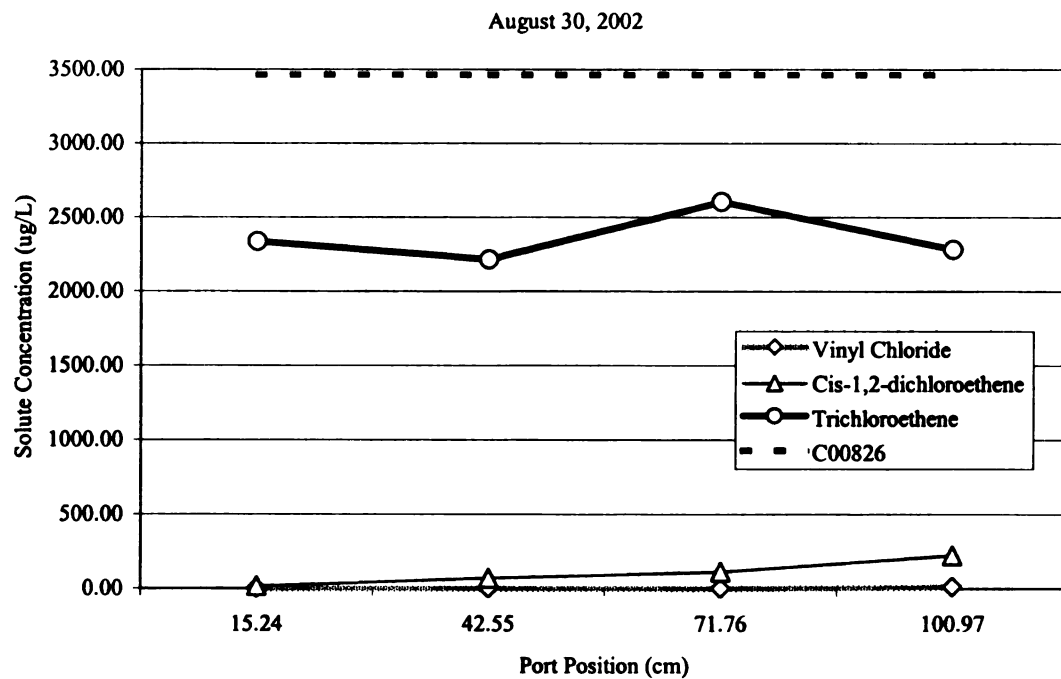
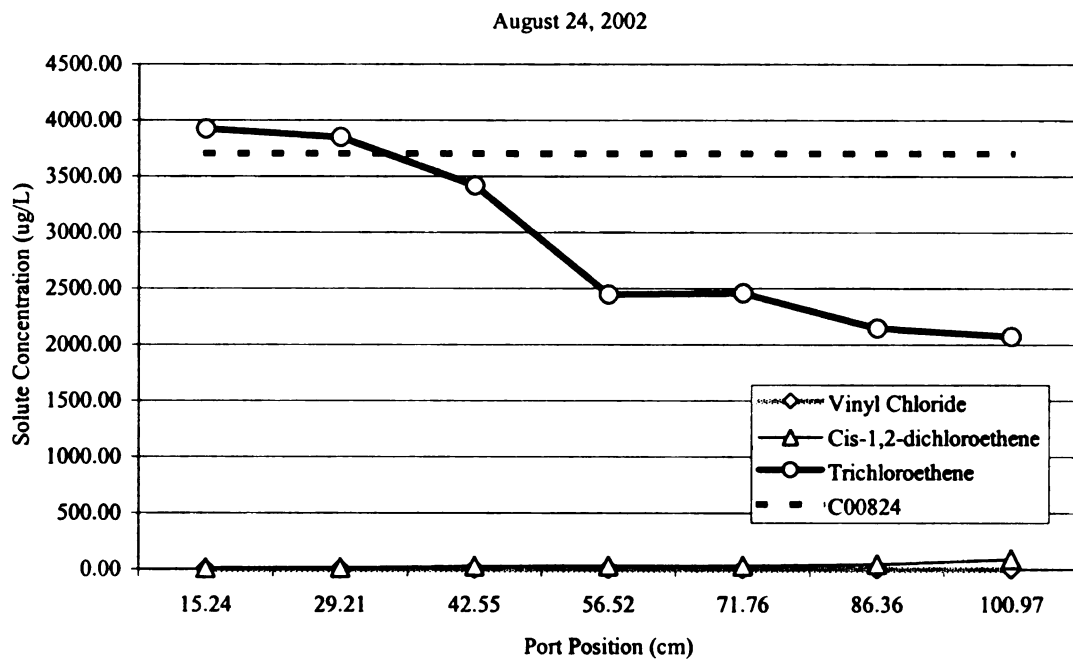
August 16, 2002



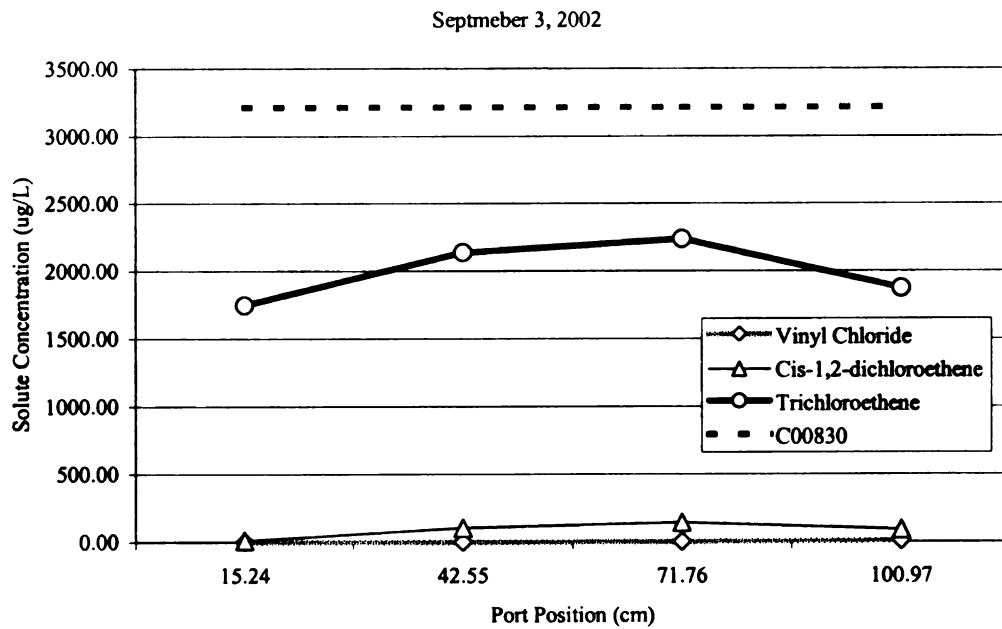
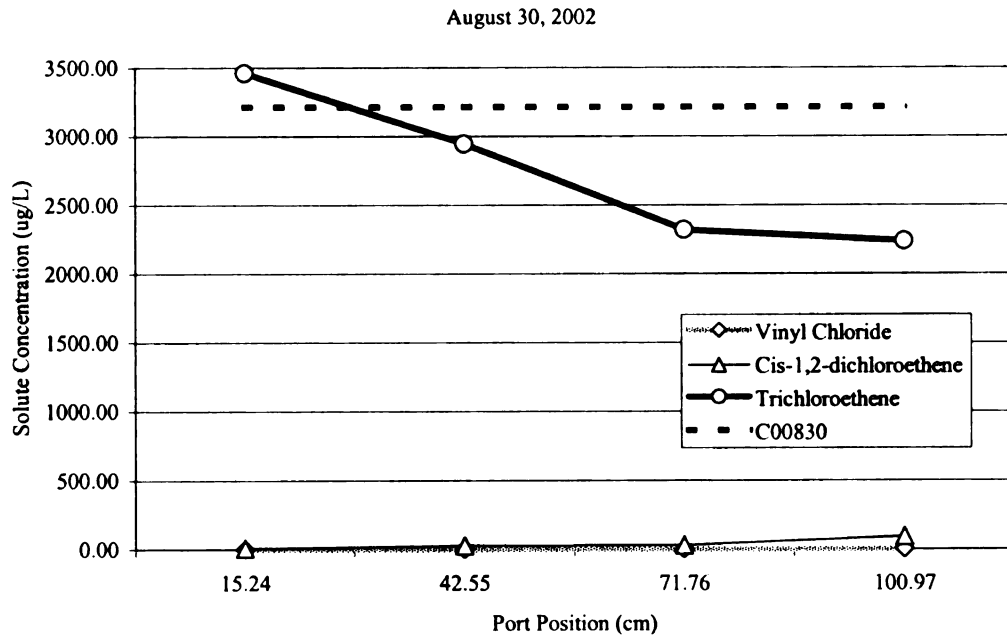
August 24, 2002



CHLORINATED VOCs
COLUMN A
August 24 - 30, 2002
(Incubation Period 7A)



CHLORINATED VOCs
COLUMN A
August 30 - September 1, 2002
(Incubation Period 8A)



APPENDIX D

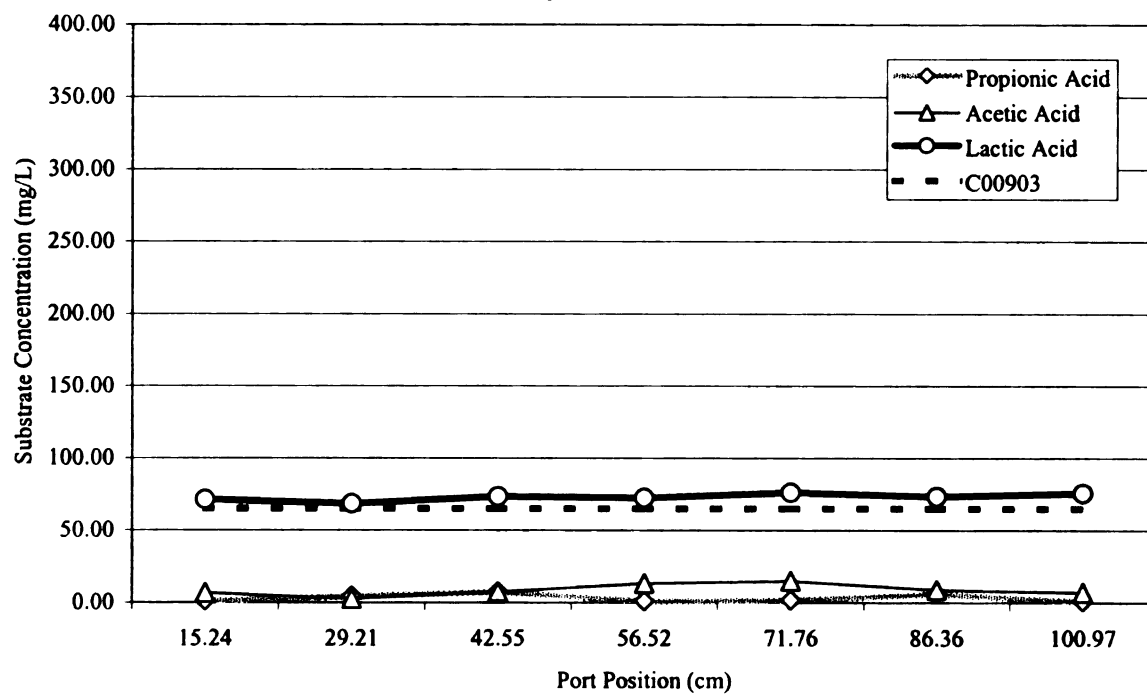
Large Column Study Analytical Results – Operational Phase A

APPENDIX D-1

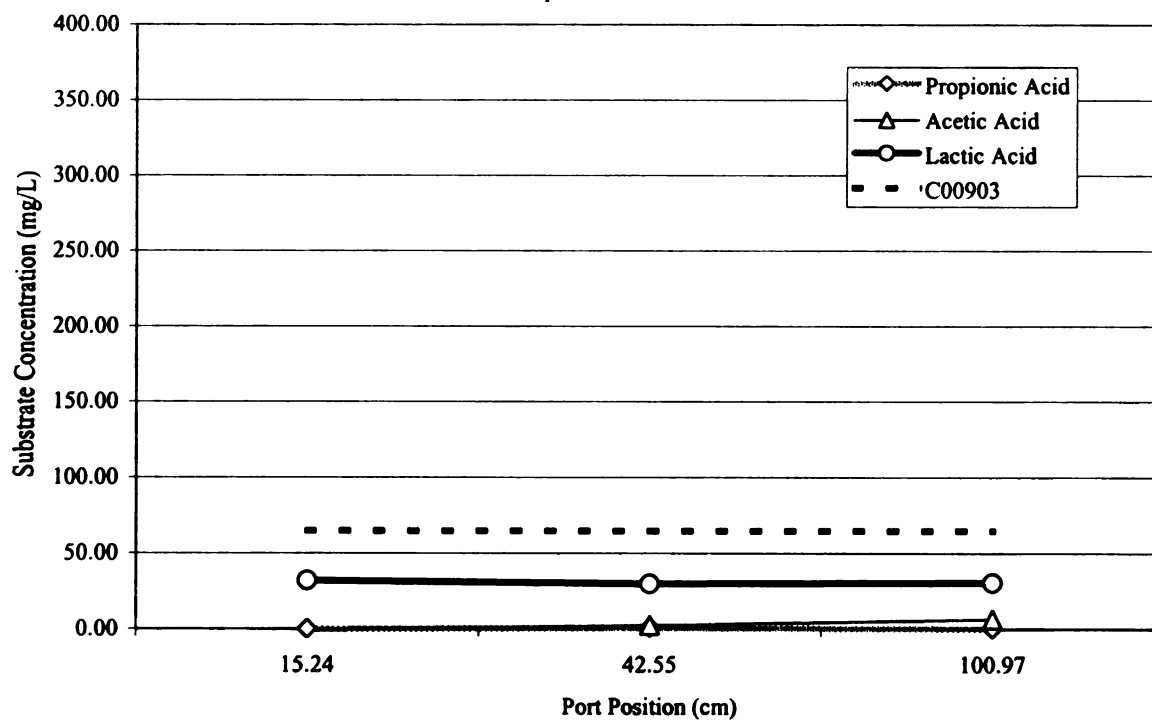
Fatty Acids

**FATTY ACIDS
COLUMN A
Incubation Period 1B
September 3 - 10, 2002**

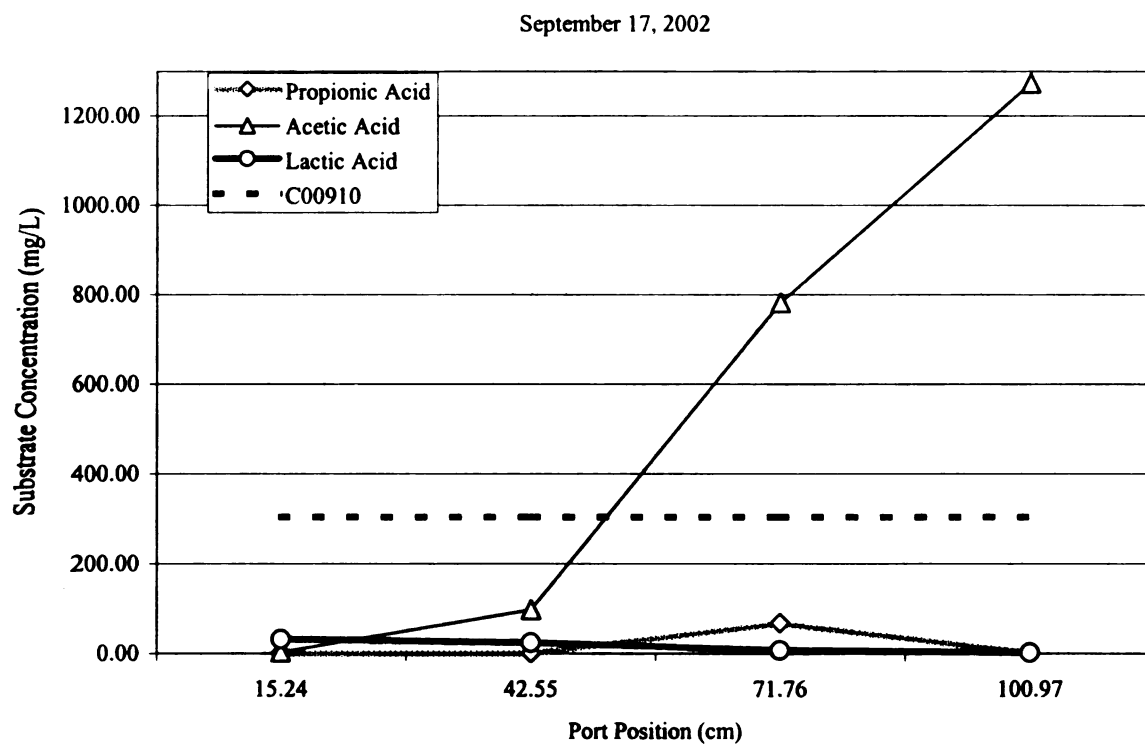
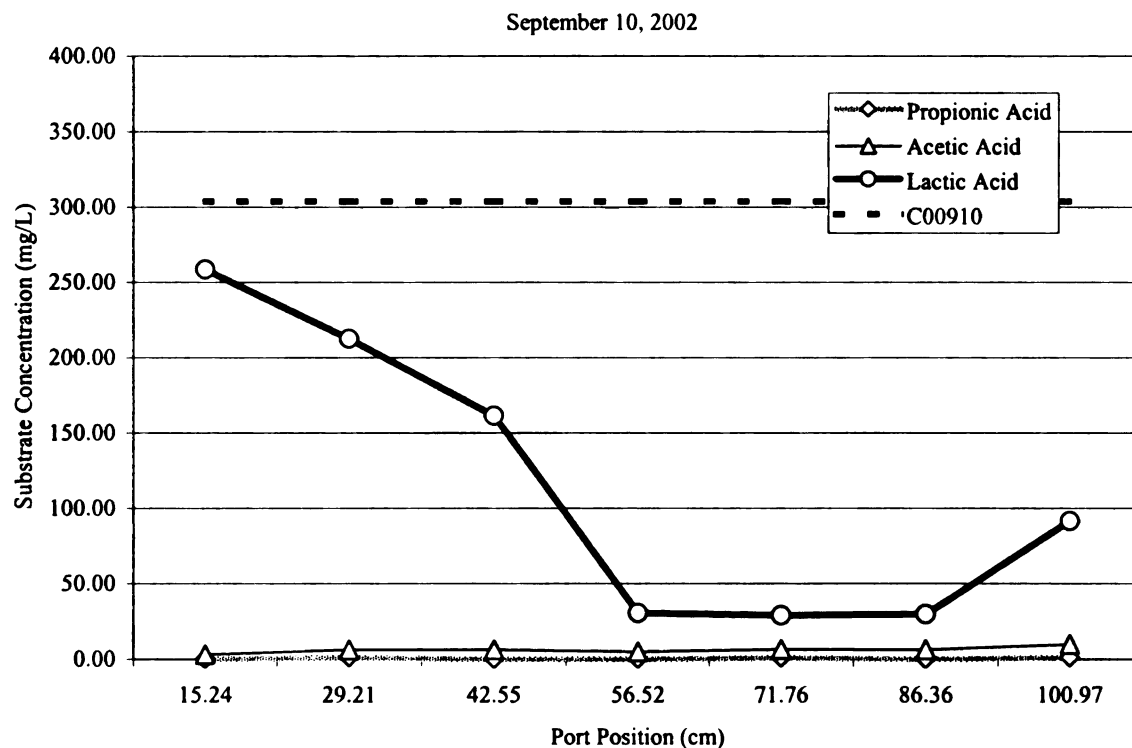
September 3, 2002



September 10, 2002

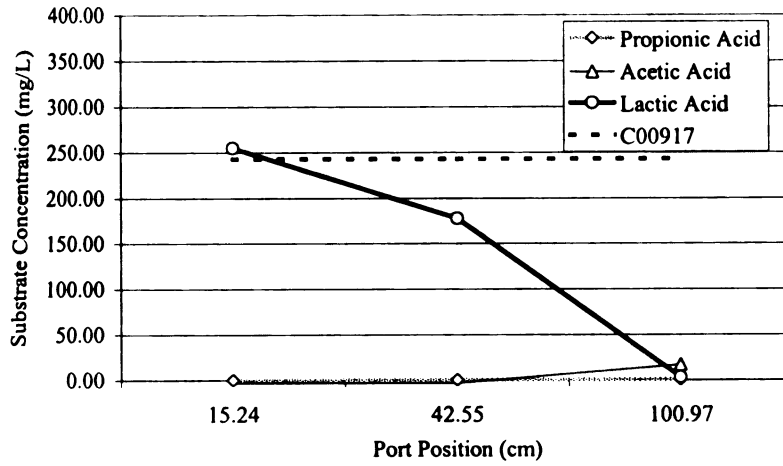


FATTY ACIDS
COLUMN A
Incubation Period 2B
Septmeber 10 - 17, 2002

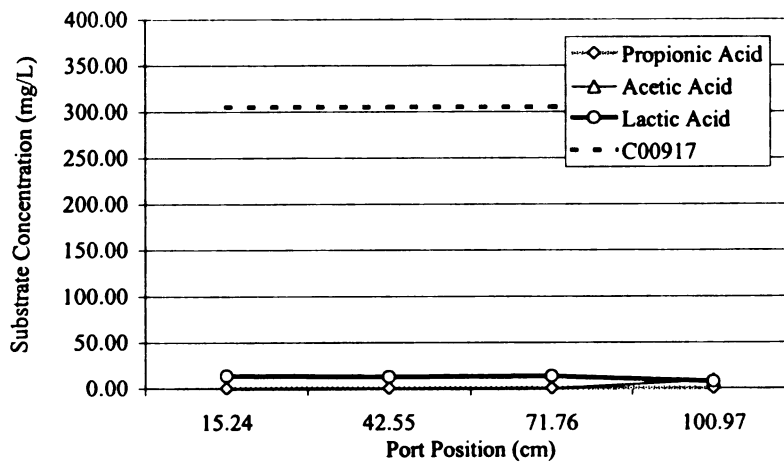


**FATTY ACIDS
COLUMN A
Incubation Period 3B
September 17 - October 1, 2002**

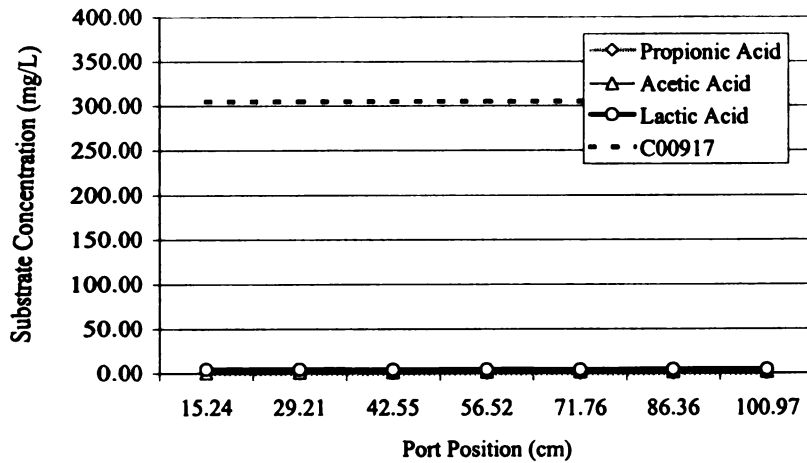
September 17, 2002



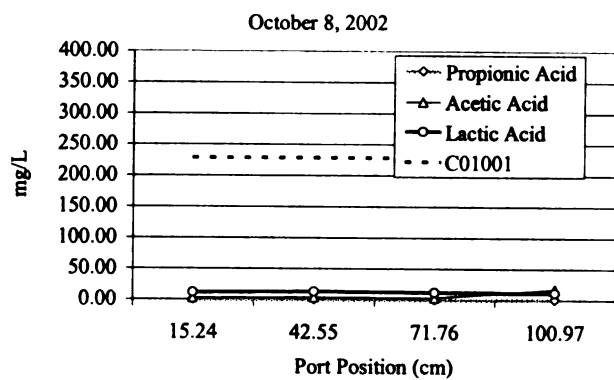
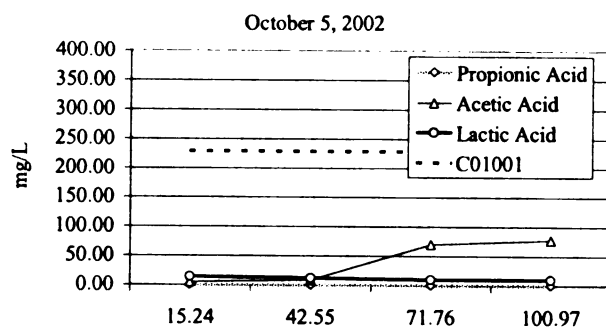
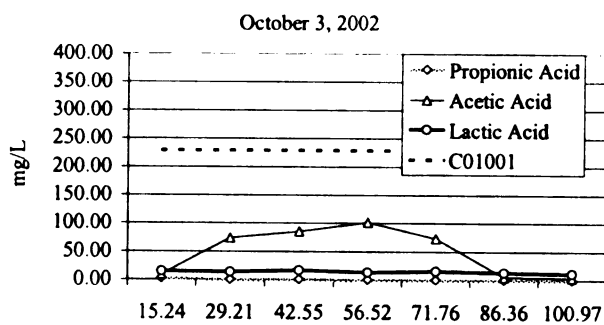
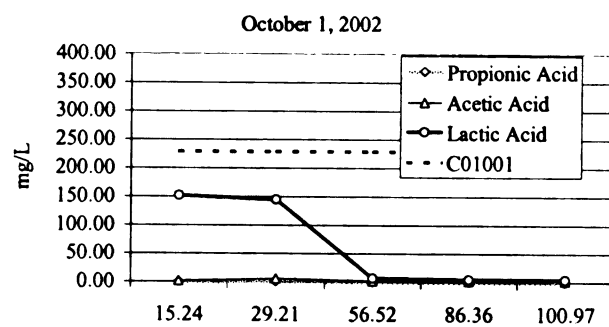
September 24, 2002



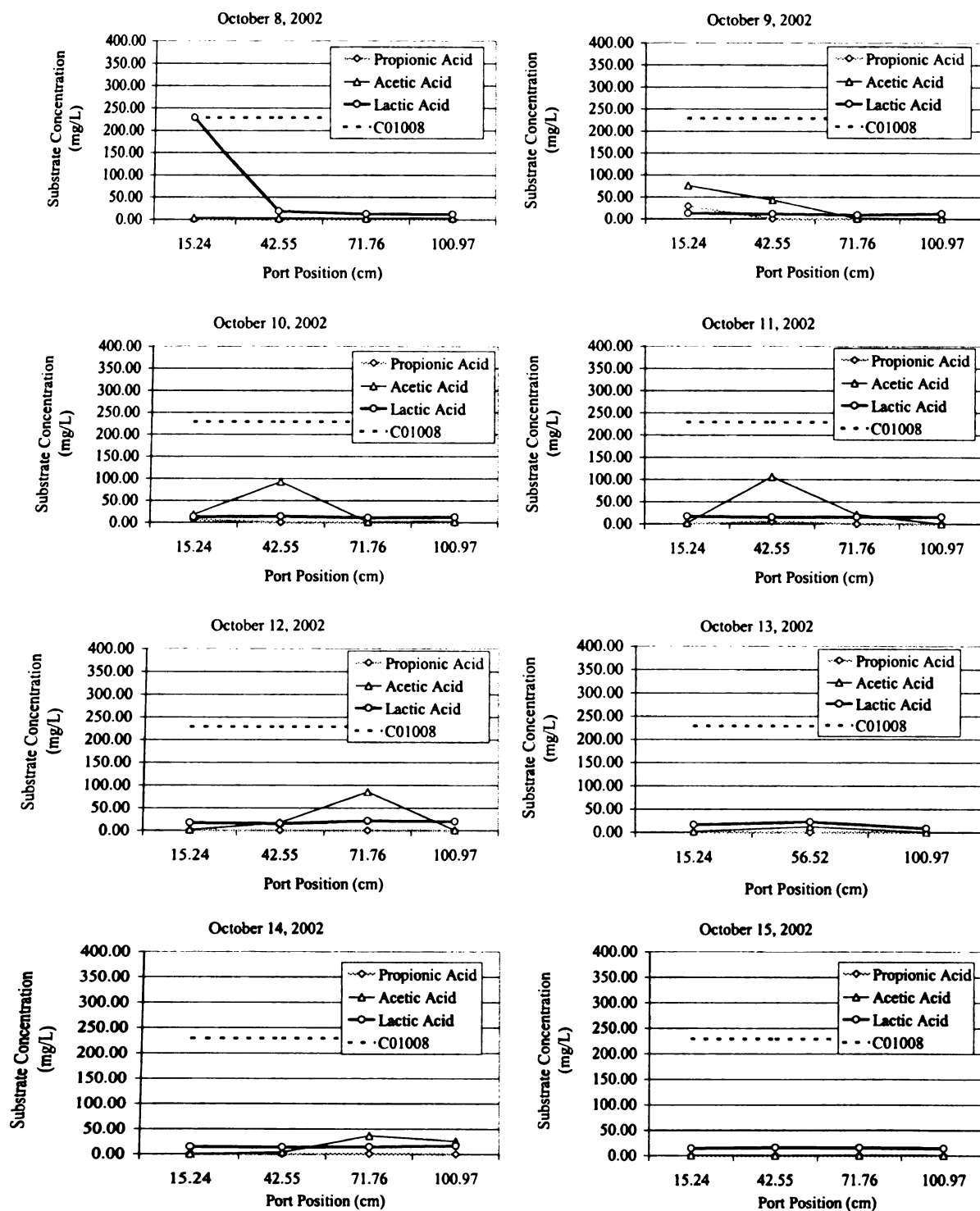
October 1, 2002



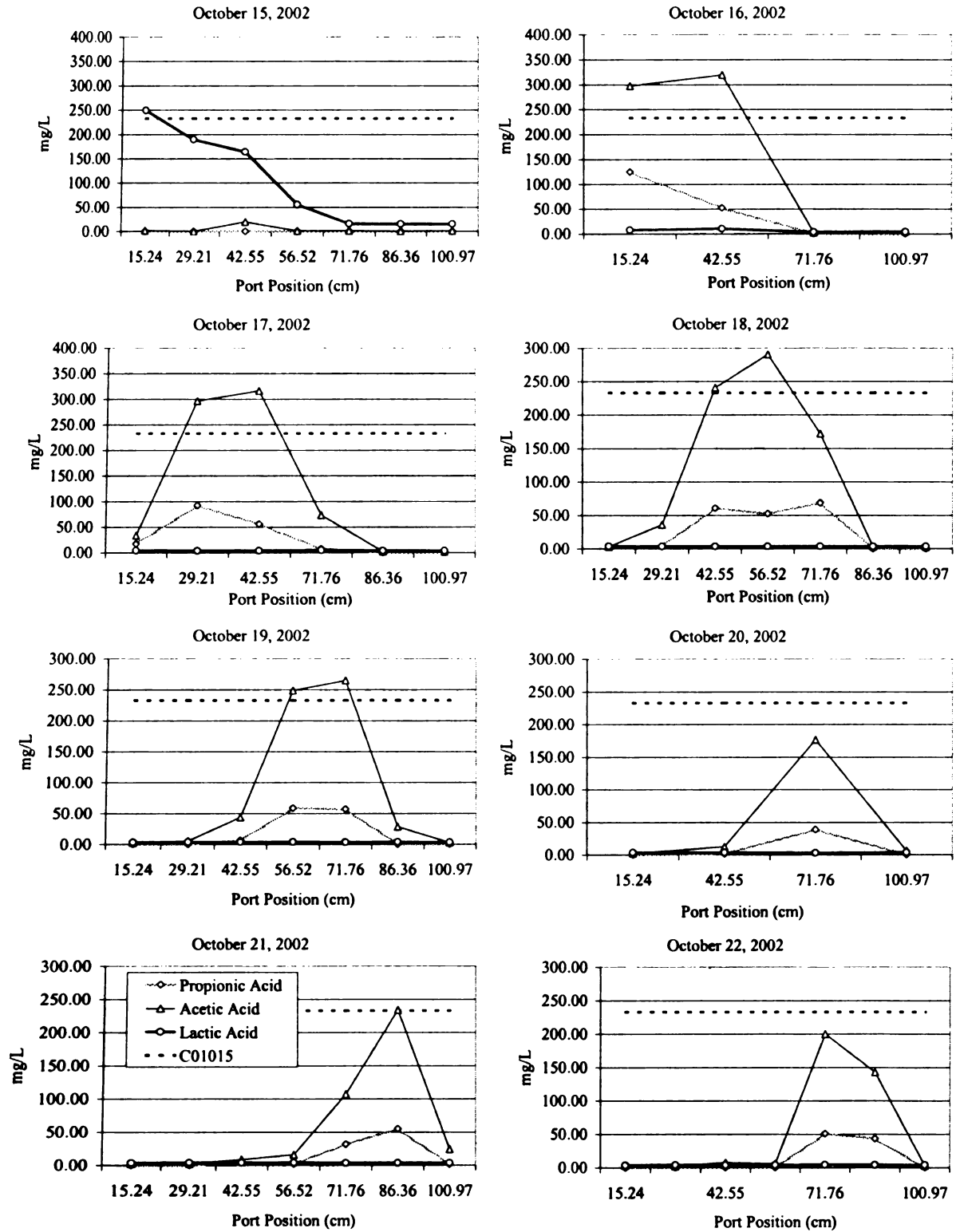
**FATTY ACIDS
COLUMN A
Incubation Period 4B
October 1 - 8, 2002**



**FATTY ACIDS
COLUMN A
Incubation Period 5B
October 8 - 15, 2002**



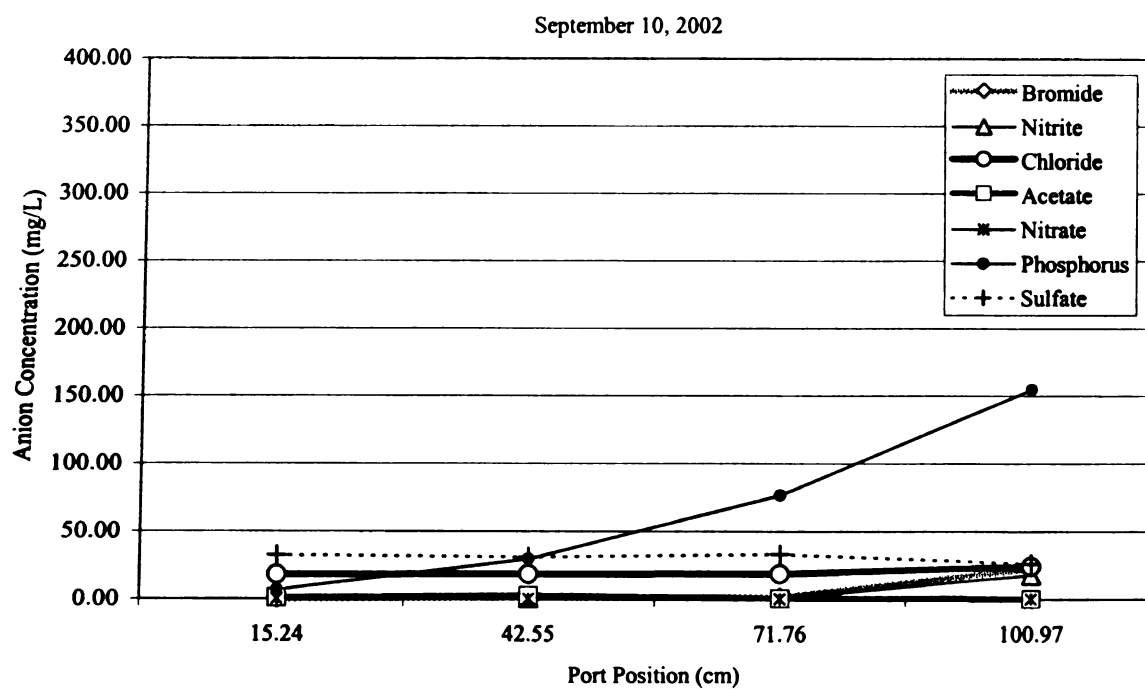
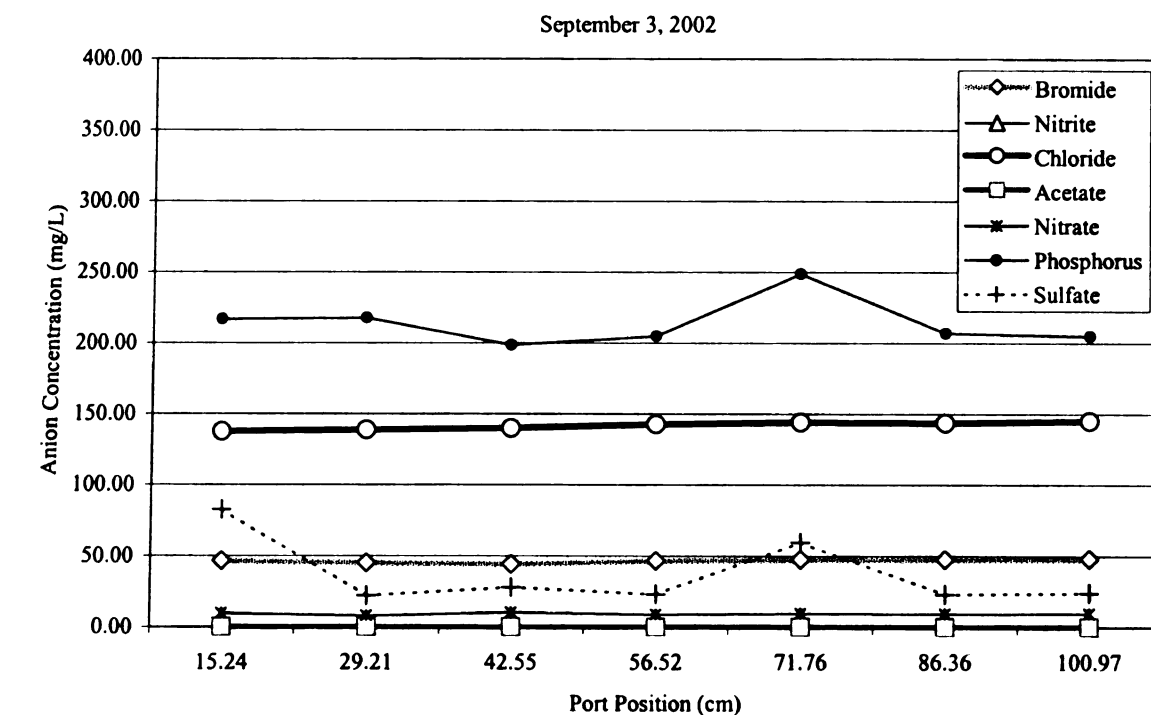
**FATTY ACIDS
COLUMN A
Incubation Period 6B
October 15 - 22, 2002**



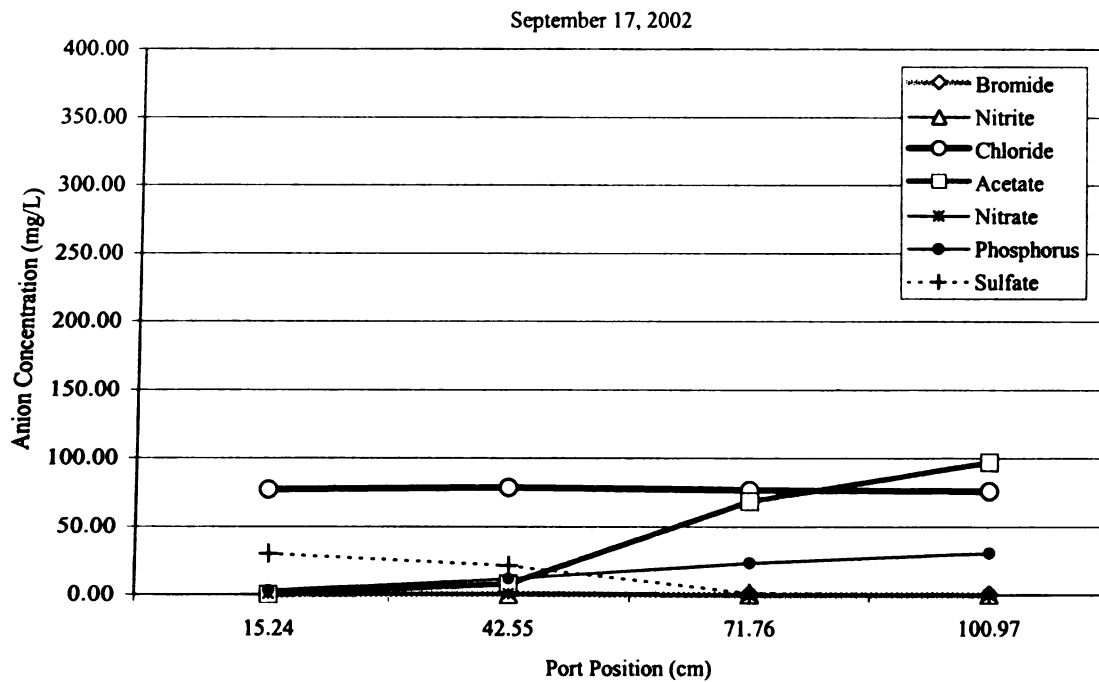
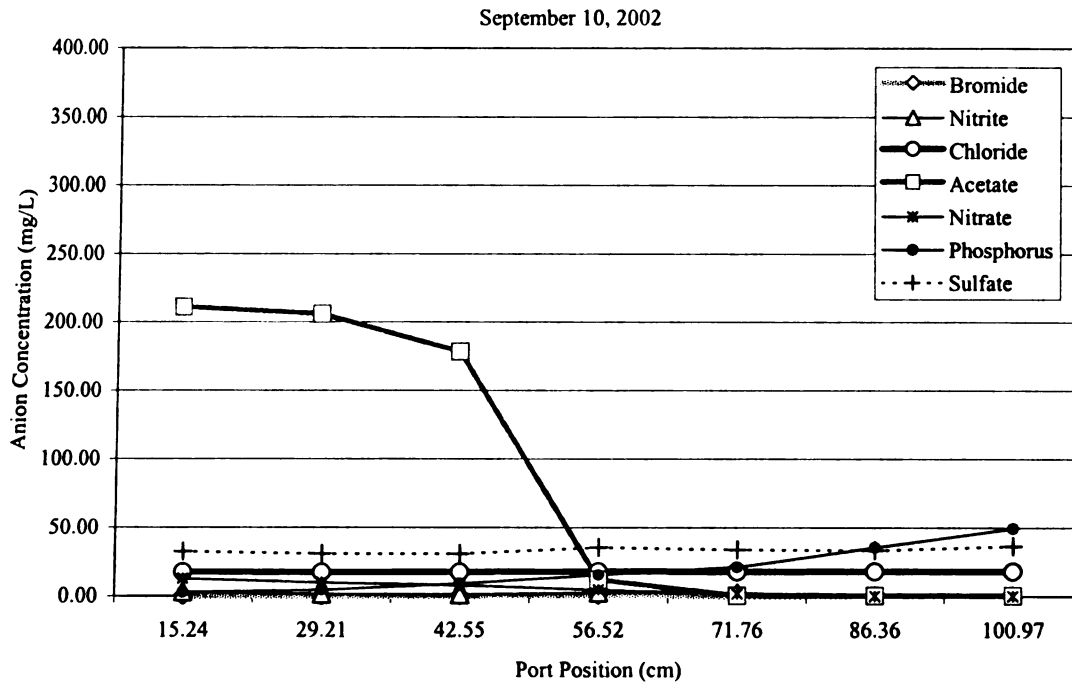
APPENDIX D-2

Major Anions

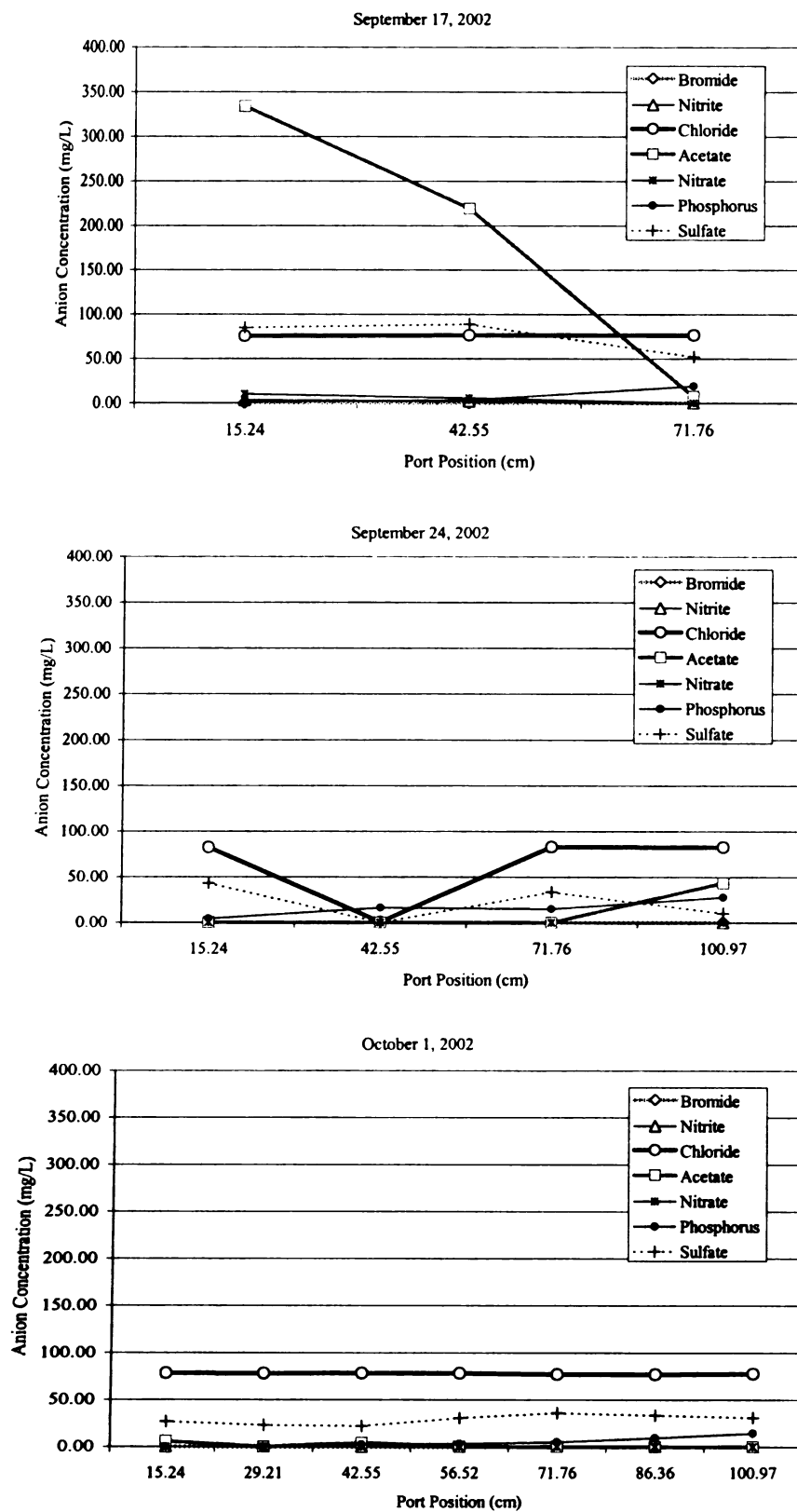
ANIONS
COLUMN A
Incubation Period 1B
September 3 - 10, 2002



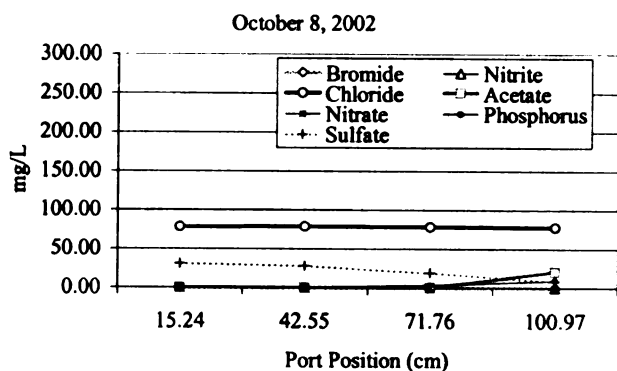
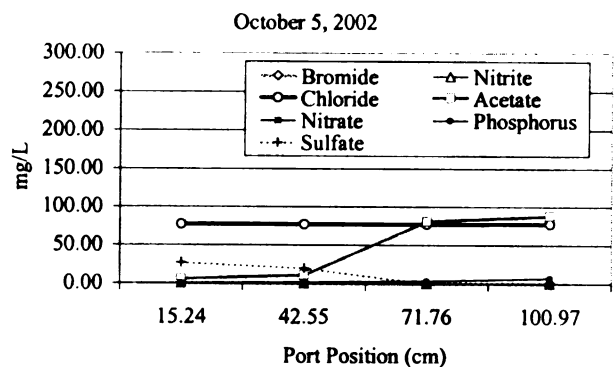
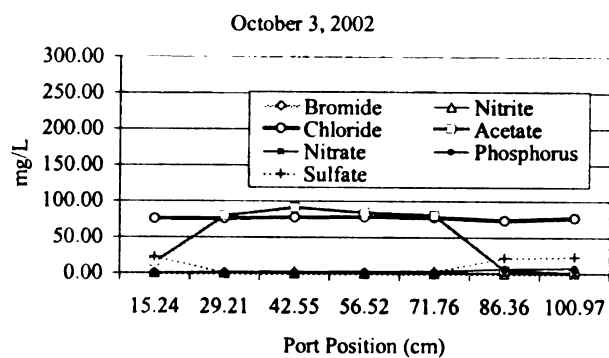
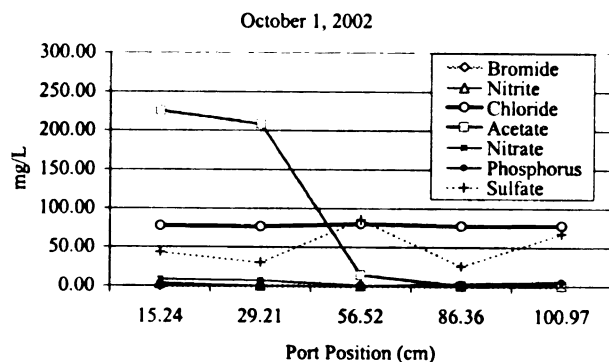
**ANIONS
COLUMN A
Incubation Period 2B
September 10 -17, 2002**



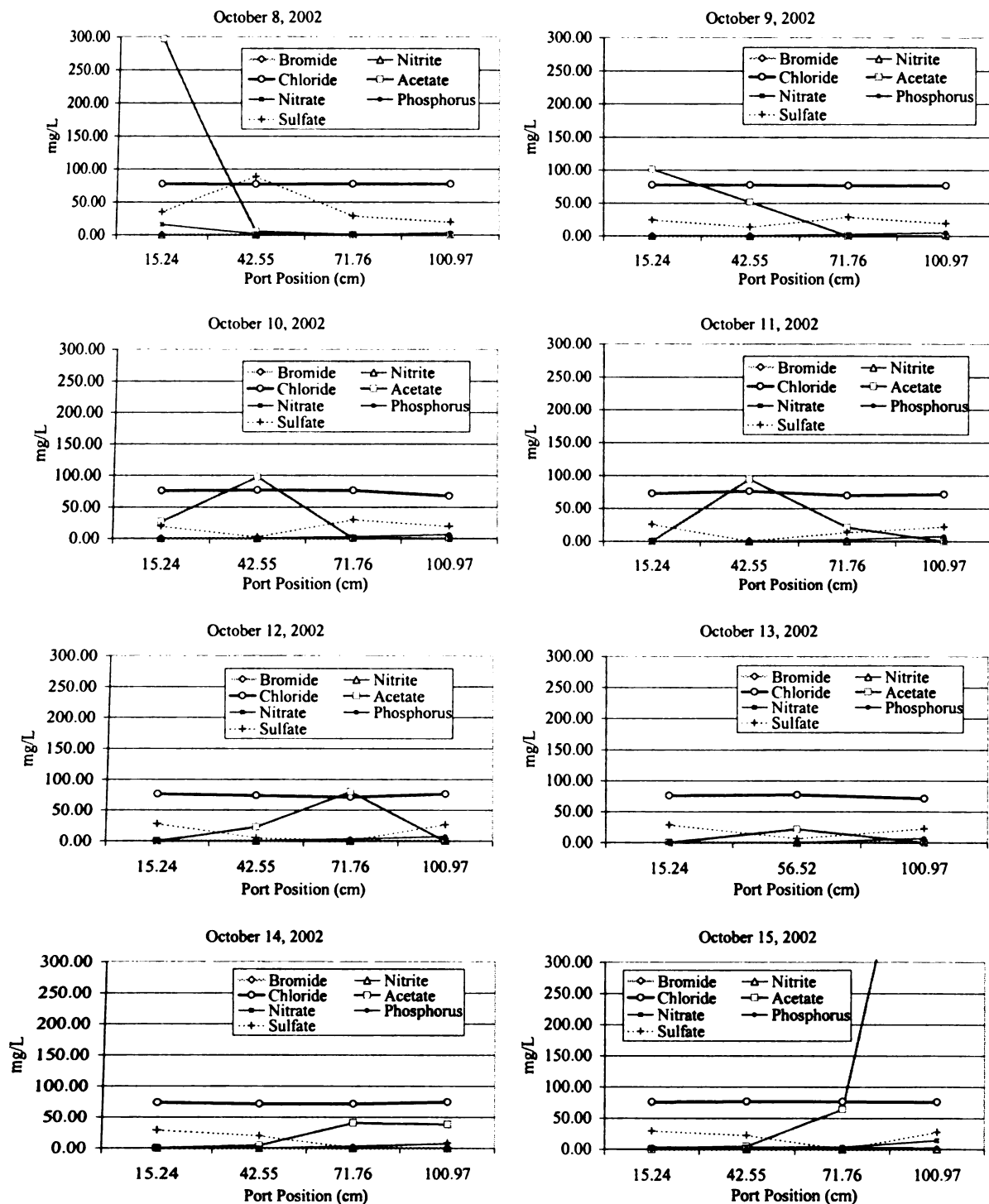
**ANIONS
COLUMN A
Incubation Period 3B - September 17 - October 1, 2002**



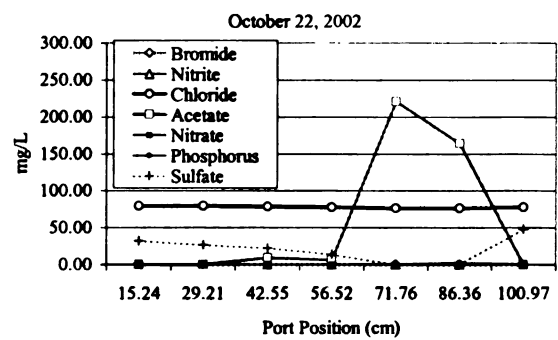
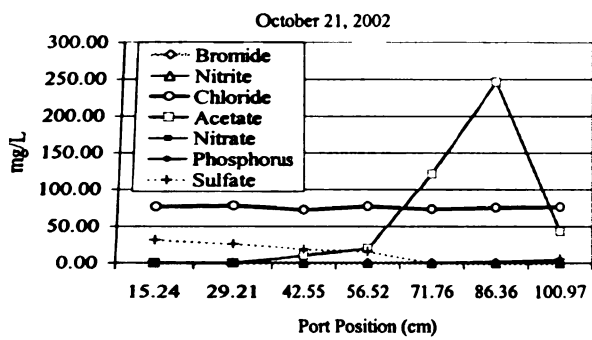
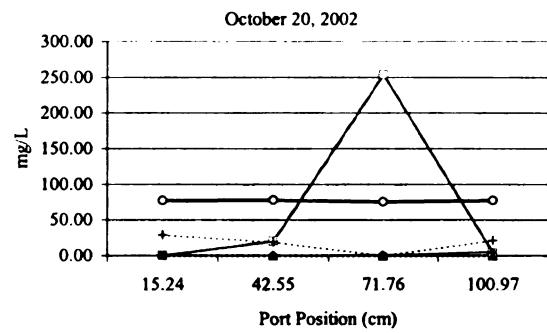
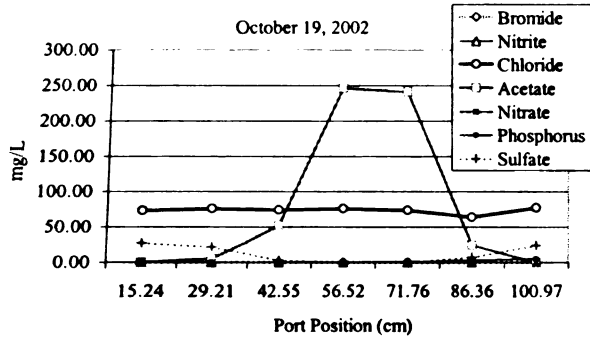
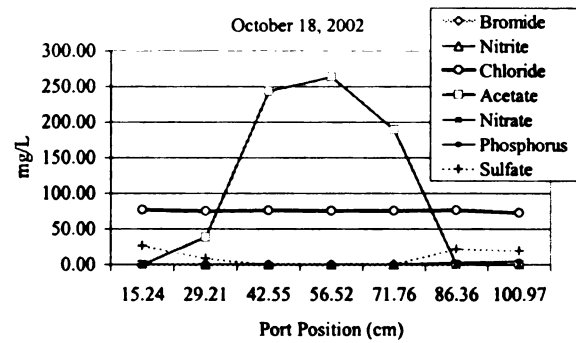
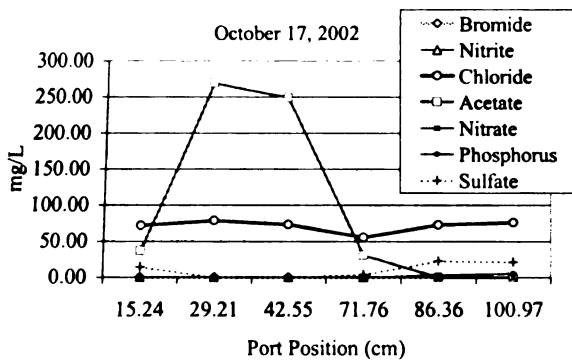
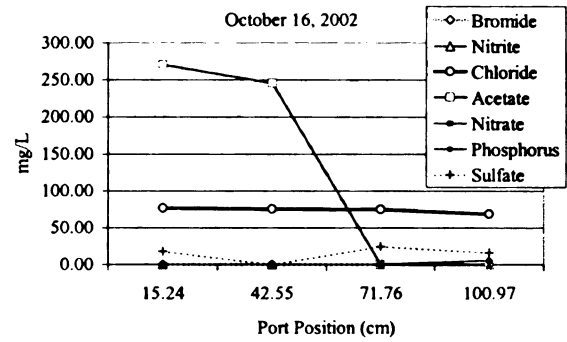
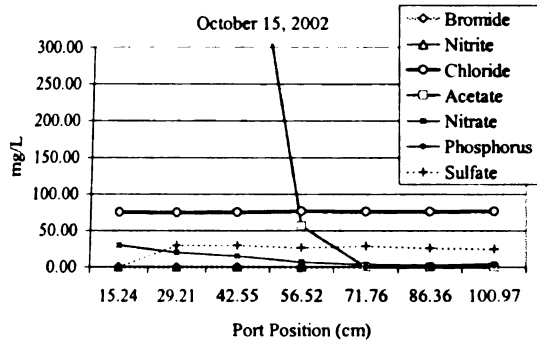
**ANIONS
COLUMN A
Incubation Period 4B - October - 8, 2002**



**ANIONS
COLUMN A
Incubation Period 5B
October 8 - 15, 2002**



**ANIONS
COLUMN A
Incubation Period 6B
October 15 - 22, 2002**

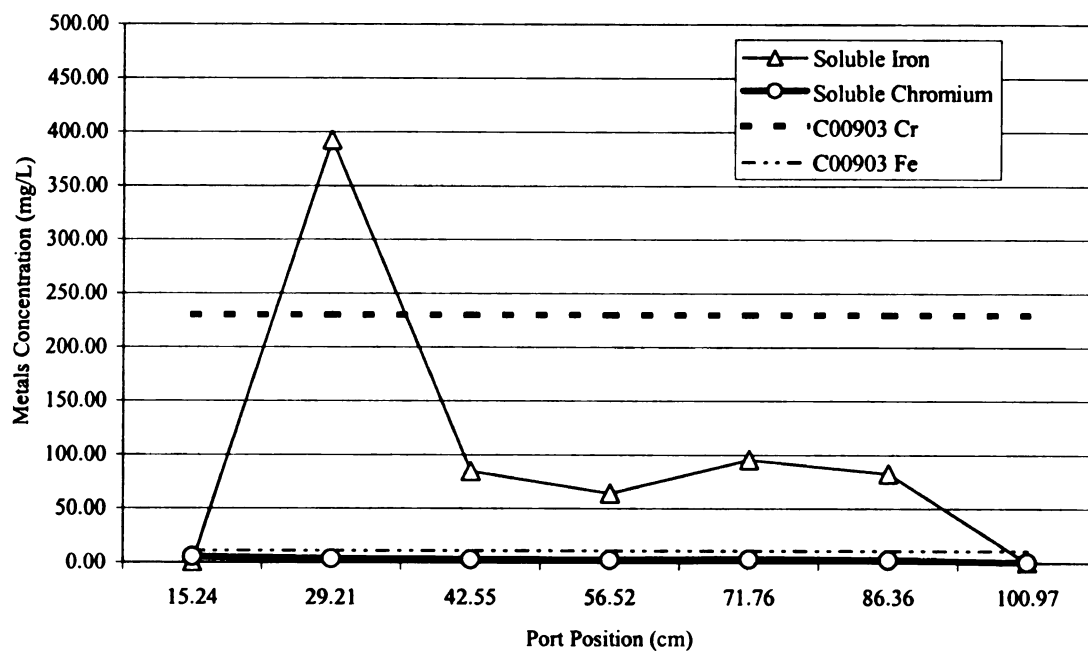


APPENDIX D-3

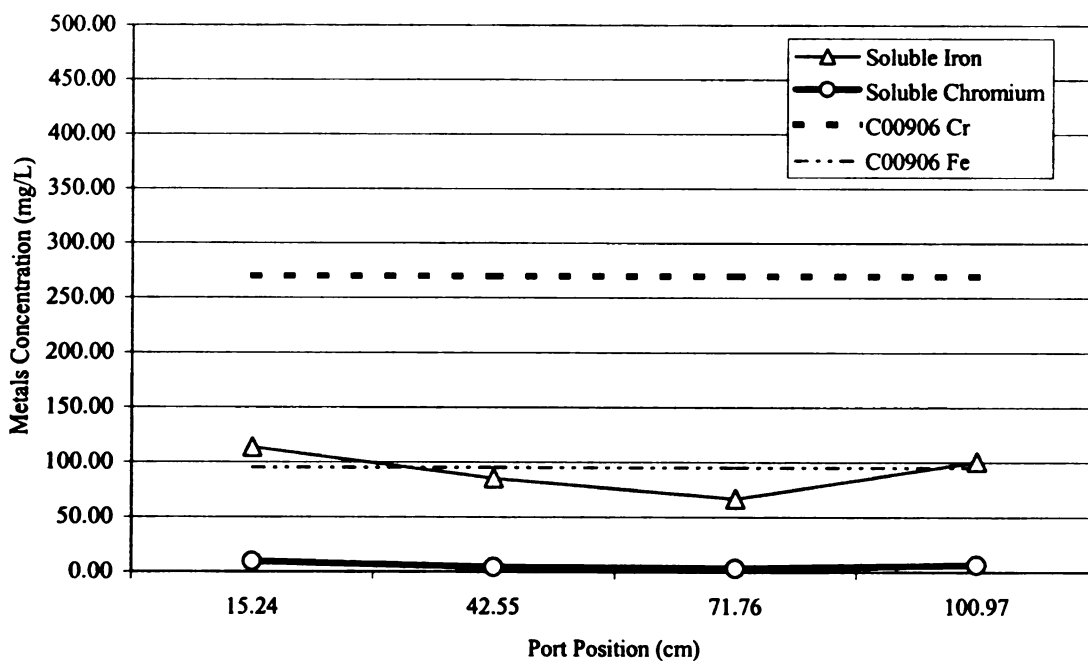
Soluble Metals

SOLUBLE METALS
COLUMN A
Incubation Period 1B
September 3 - 10, 2002

September 3, 2002

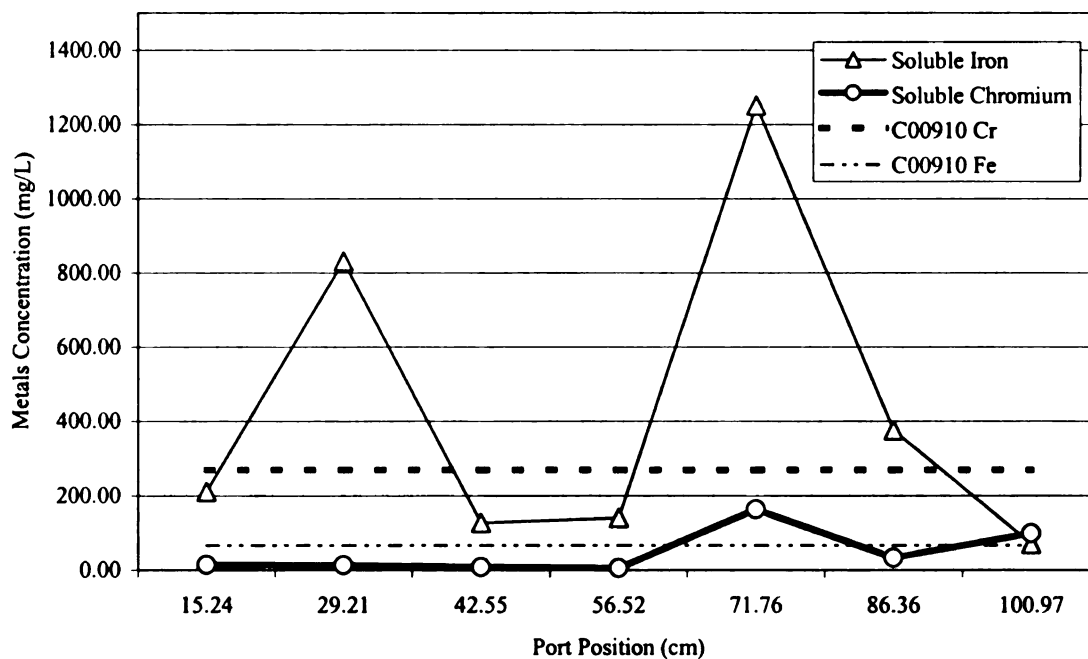


September 10, 2002

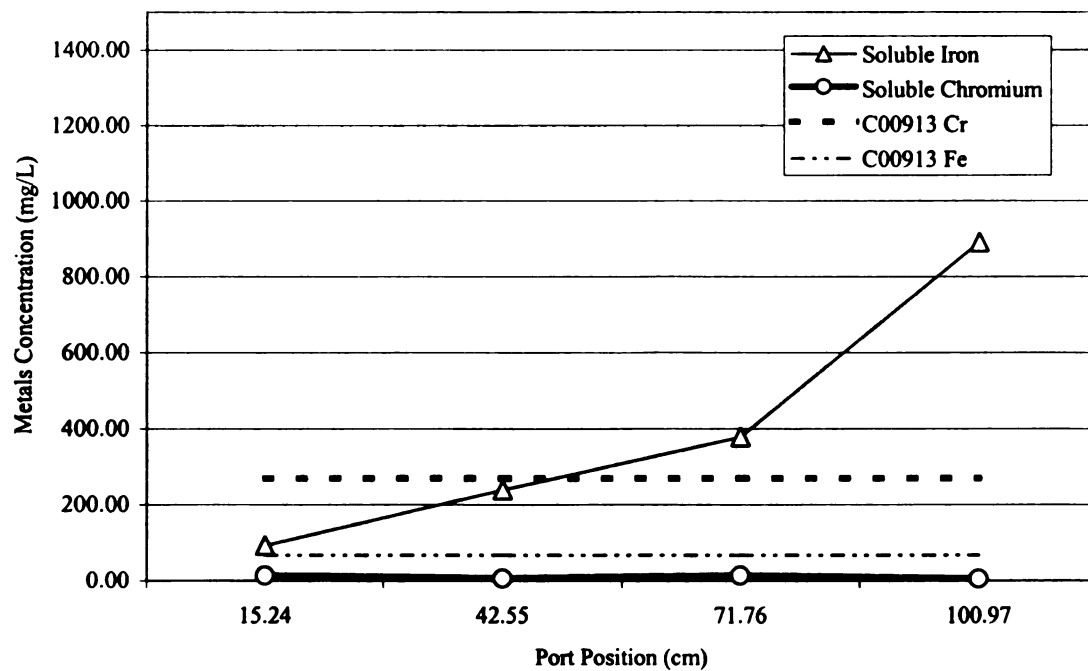


**SOLUBLE METALS
COLUMN A
Incubation Period 2B
September 10 - 17, 2002**

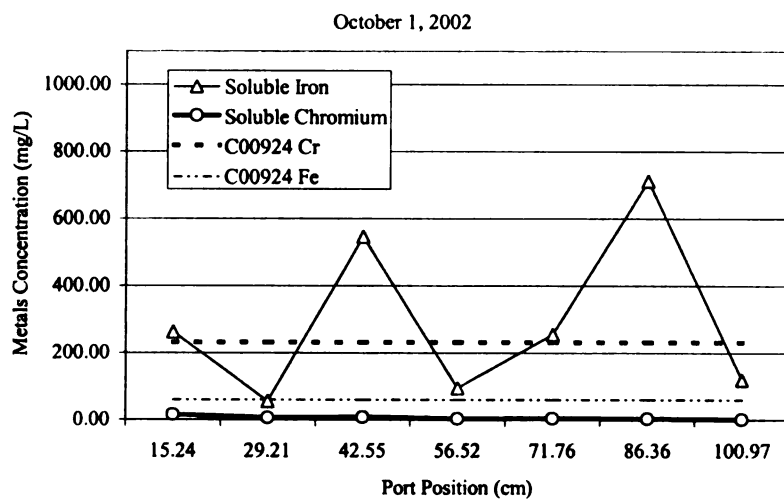
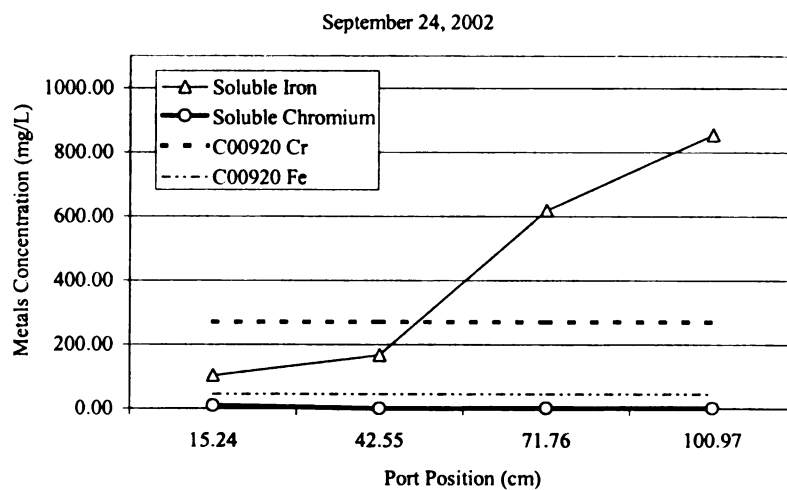
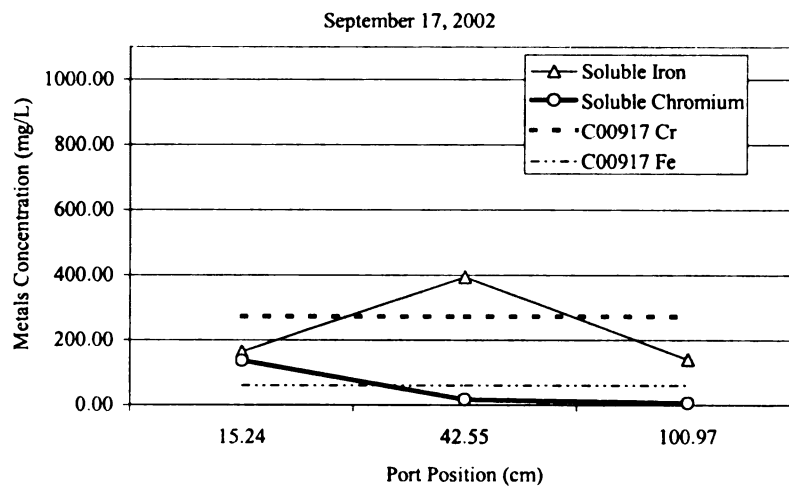
September 10, 2002



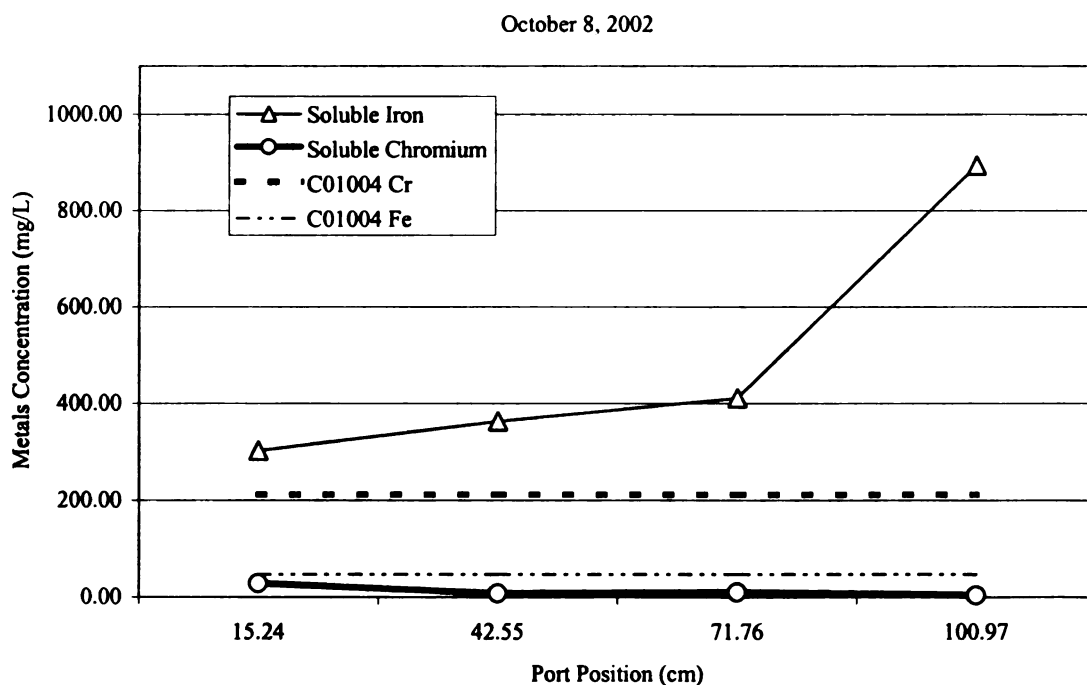
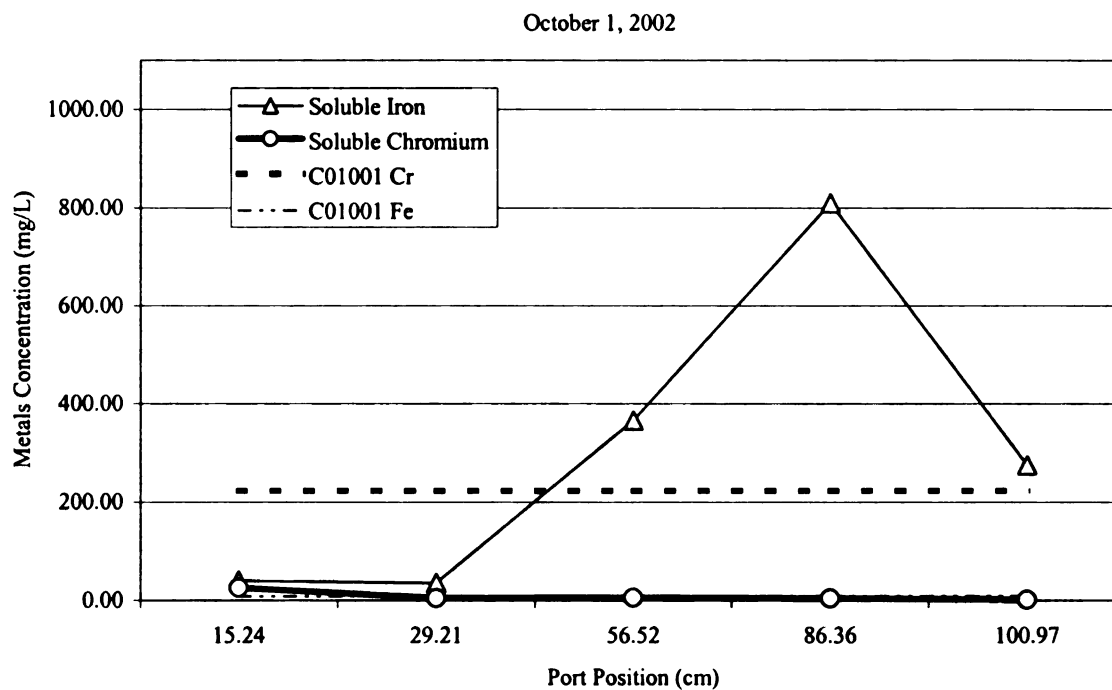
September 17, 2002



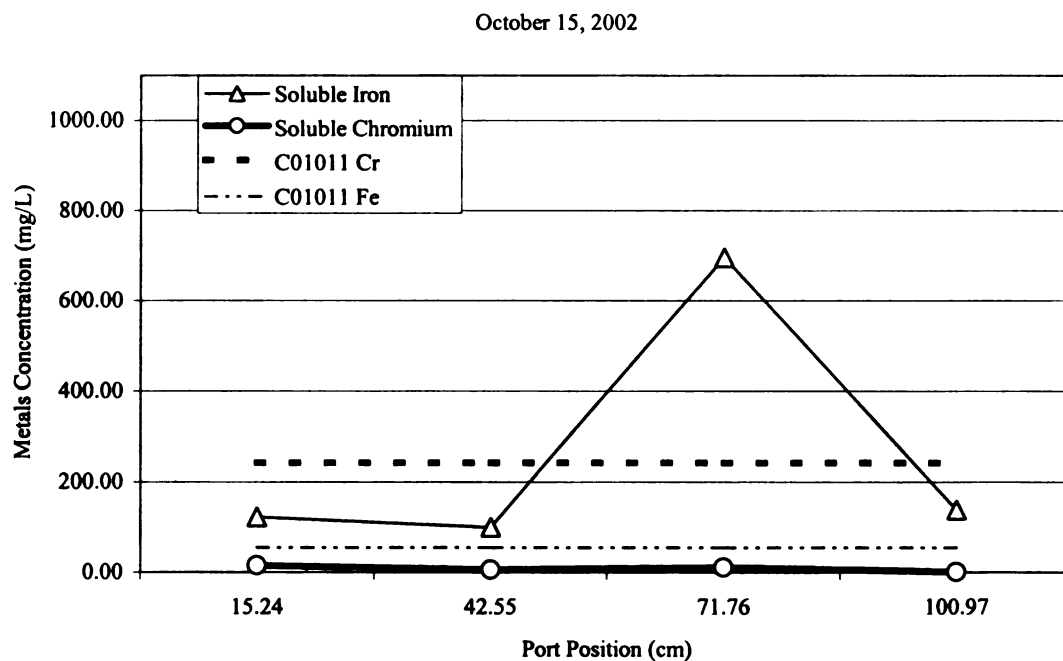
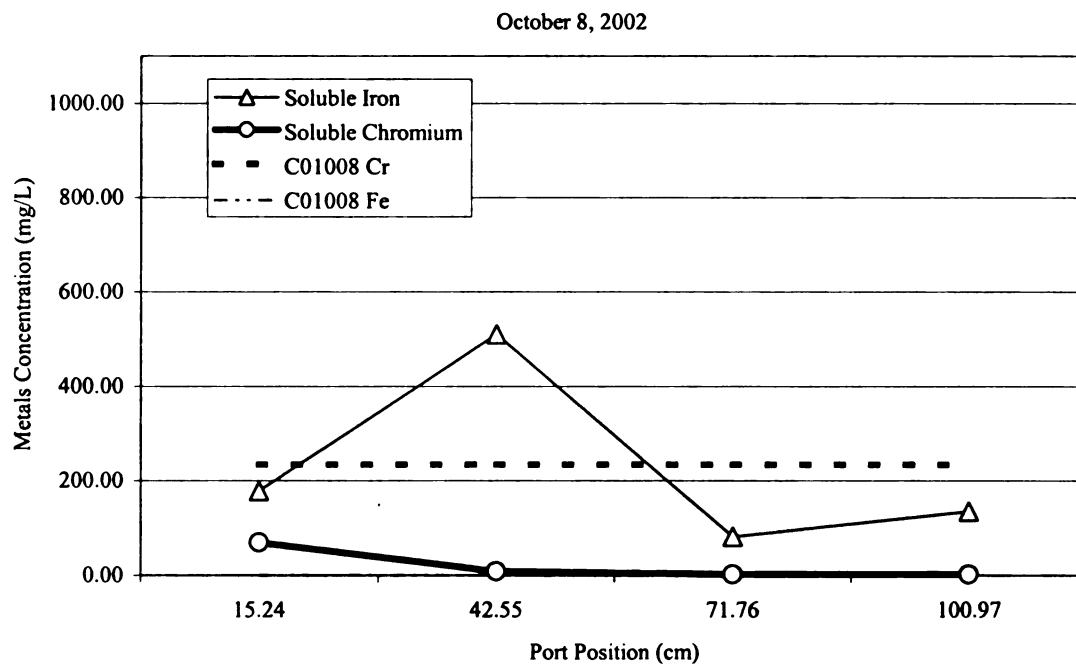
SOLUBLE METALS
COLUMN A
Incubation Period 3B
September 17 - August 1, 2002



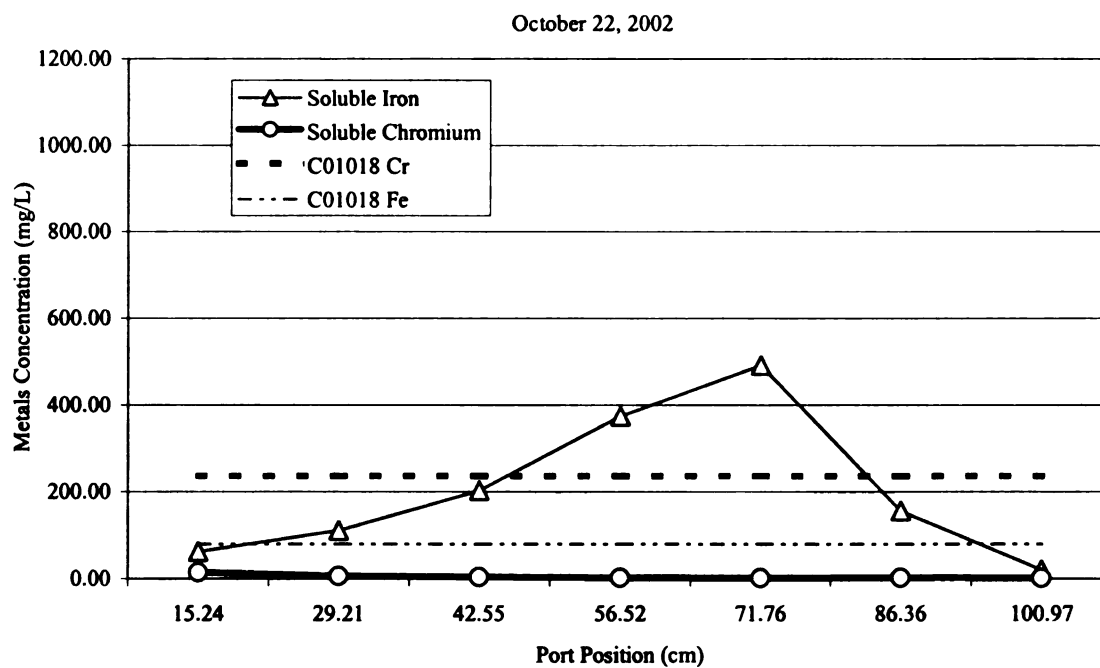
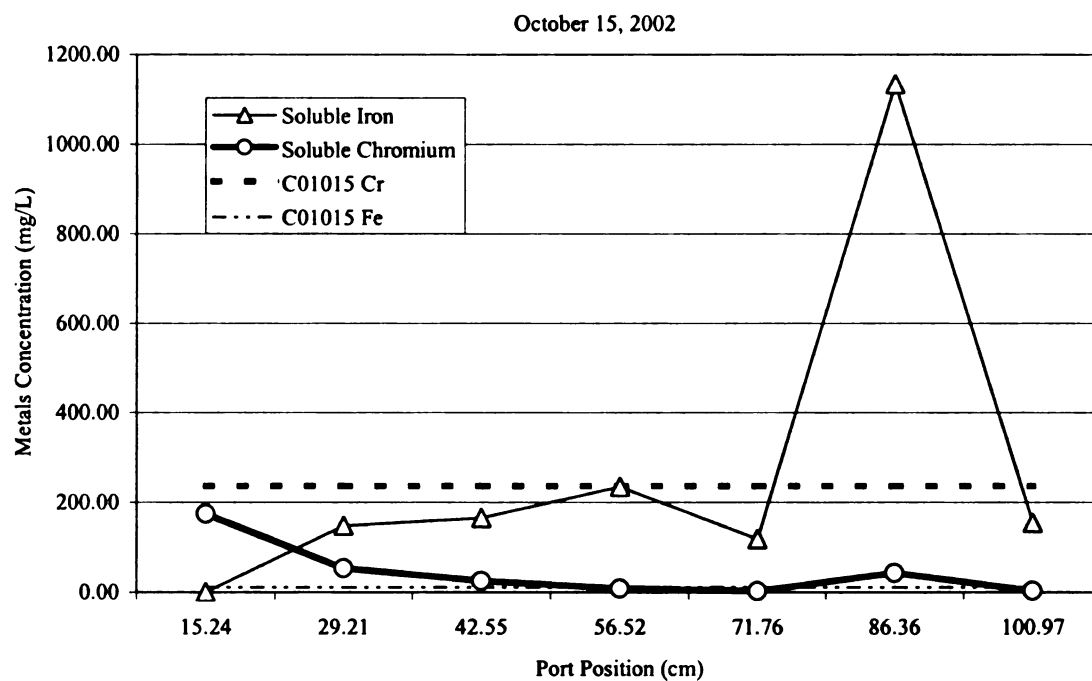
**SOLUBLE METALS
COLUMN A
Incubation Period 4B
October 1 - 8, 2002**



**SOLUBLE METALS
COLUMN A
Incubation Period 5B
October 8 - 15, 2002**



**SOLUBLE METALS
COLUMN A
Incubation Period 6B
October 15 - 22, 2002**

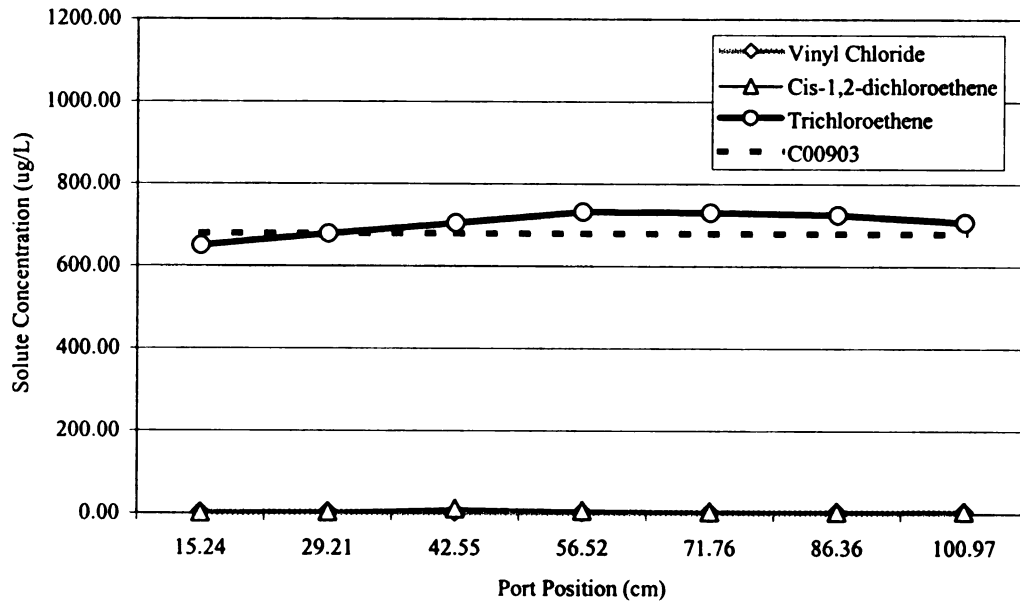


APPENDIX D-4

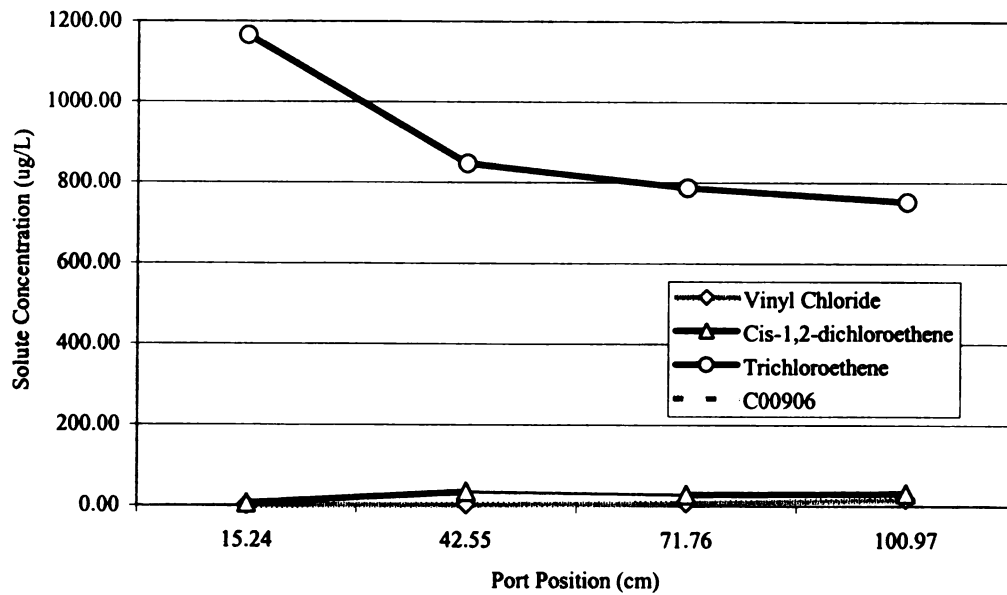
Chlorinated Aliphatics

CHLORINATED VOCs
COLUMN A
September 3 - 10, 2002
(Incubation Period 1B)

September 3, 2002

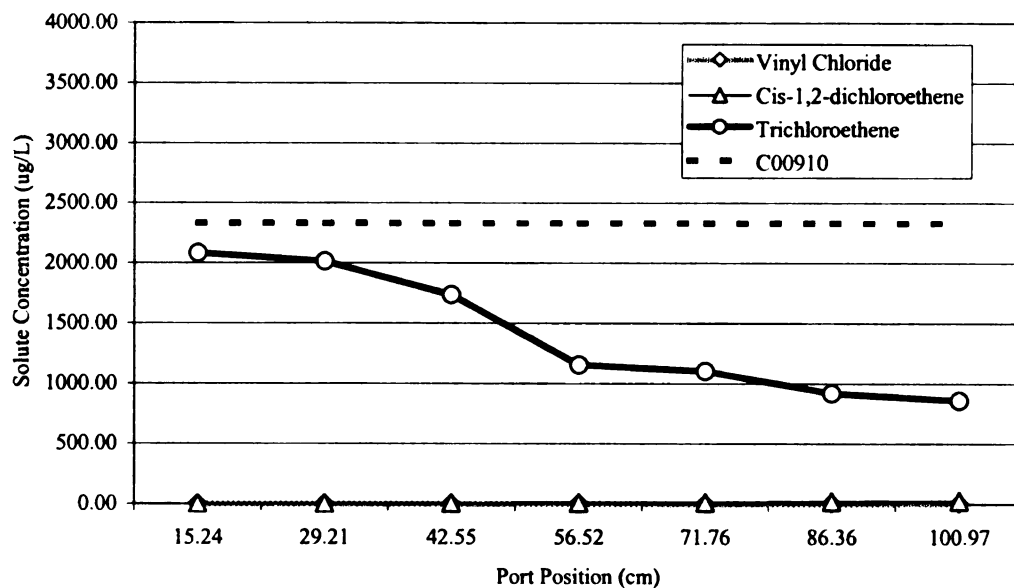


September 10, 2002

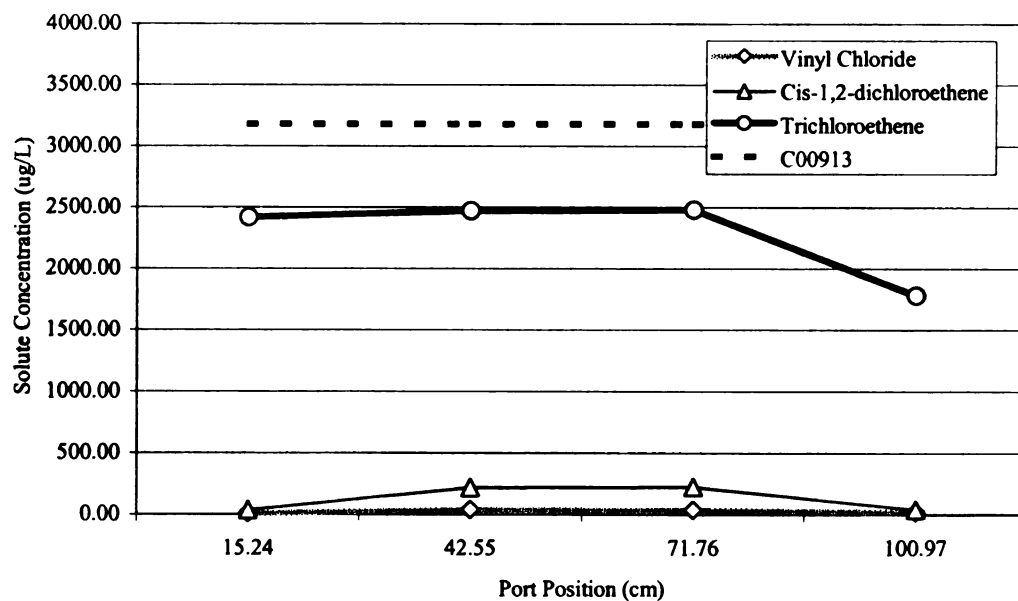


**CHLORINATED VOCs
COLUMN A
September 10 - 17, 2002
(Incubation Period 2B)**

September 10, 2002

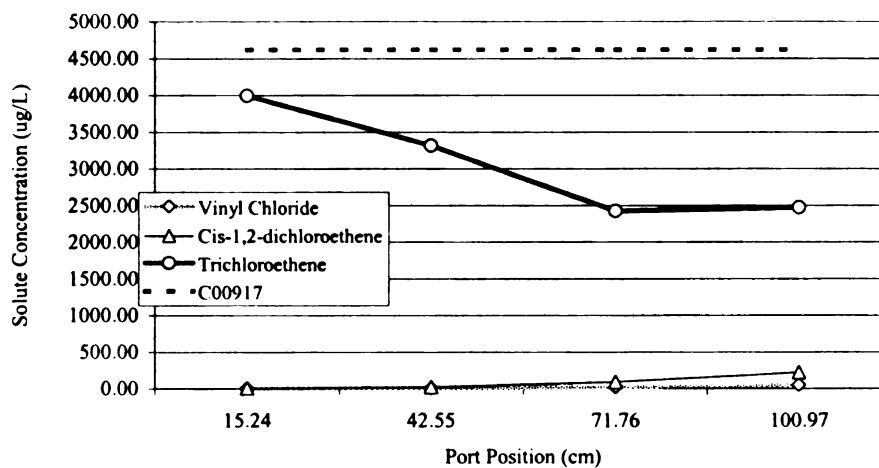


September 17, 2002

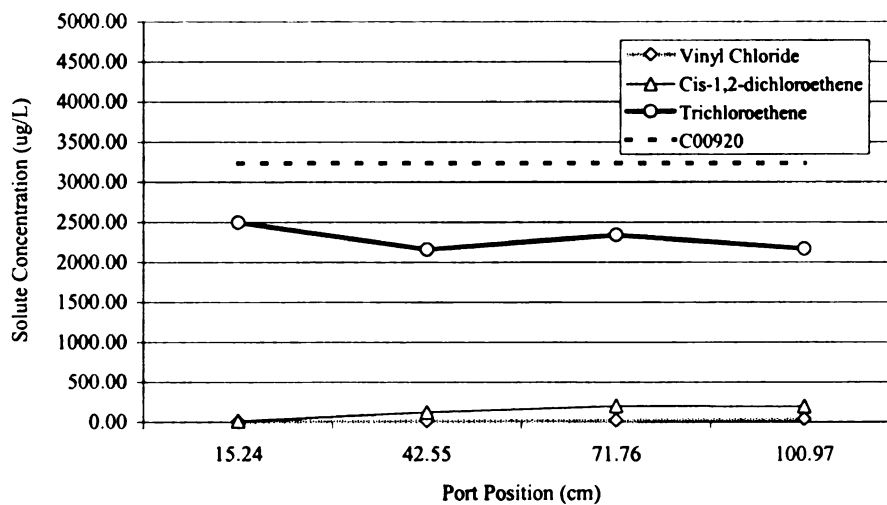


**CHLORINATED VOCs
COLUMN A
September 17 - October 1, 2002
(Incubation Period 3B)**

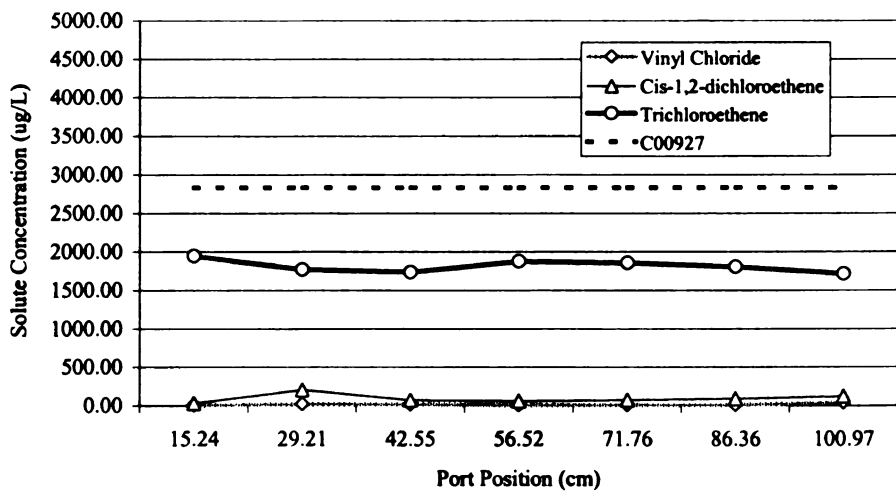
September 17, 2002



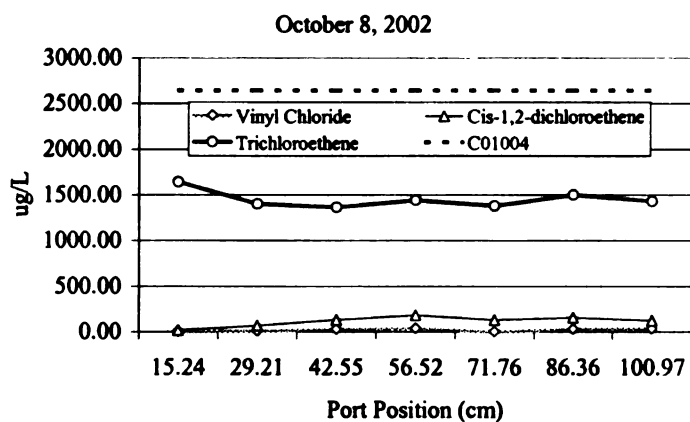
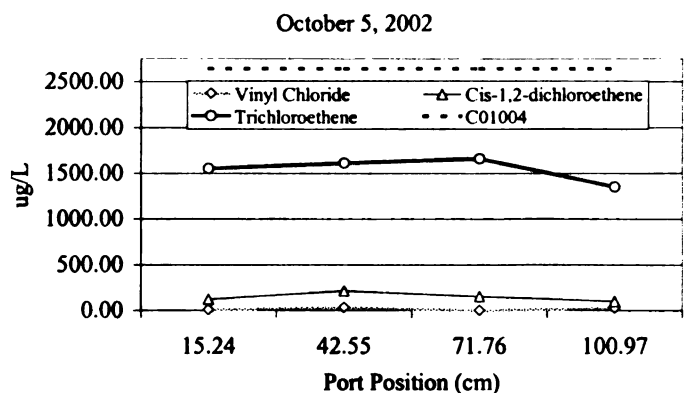
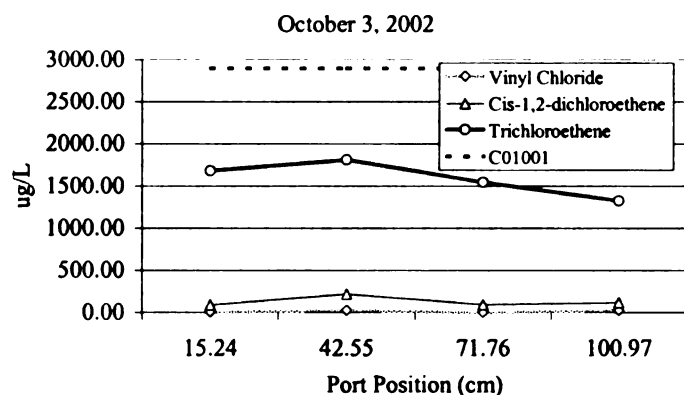
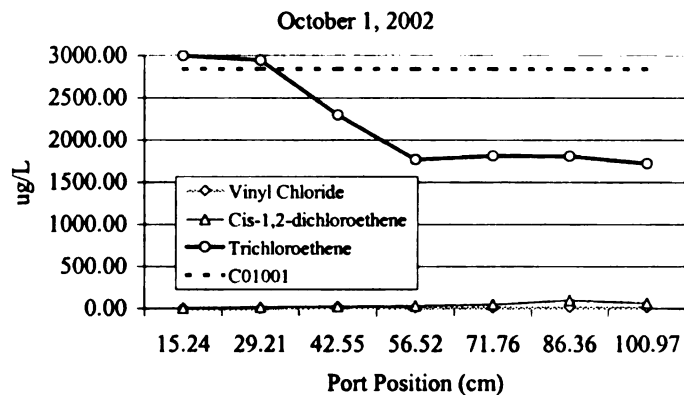
September 24, 2002



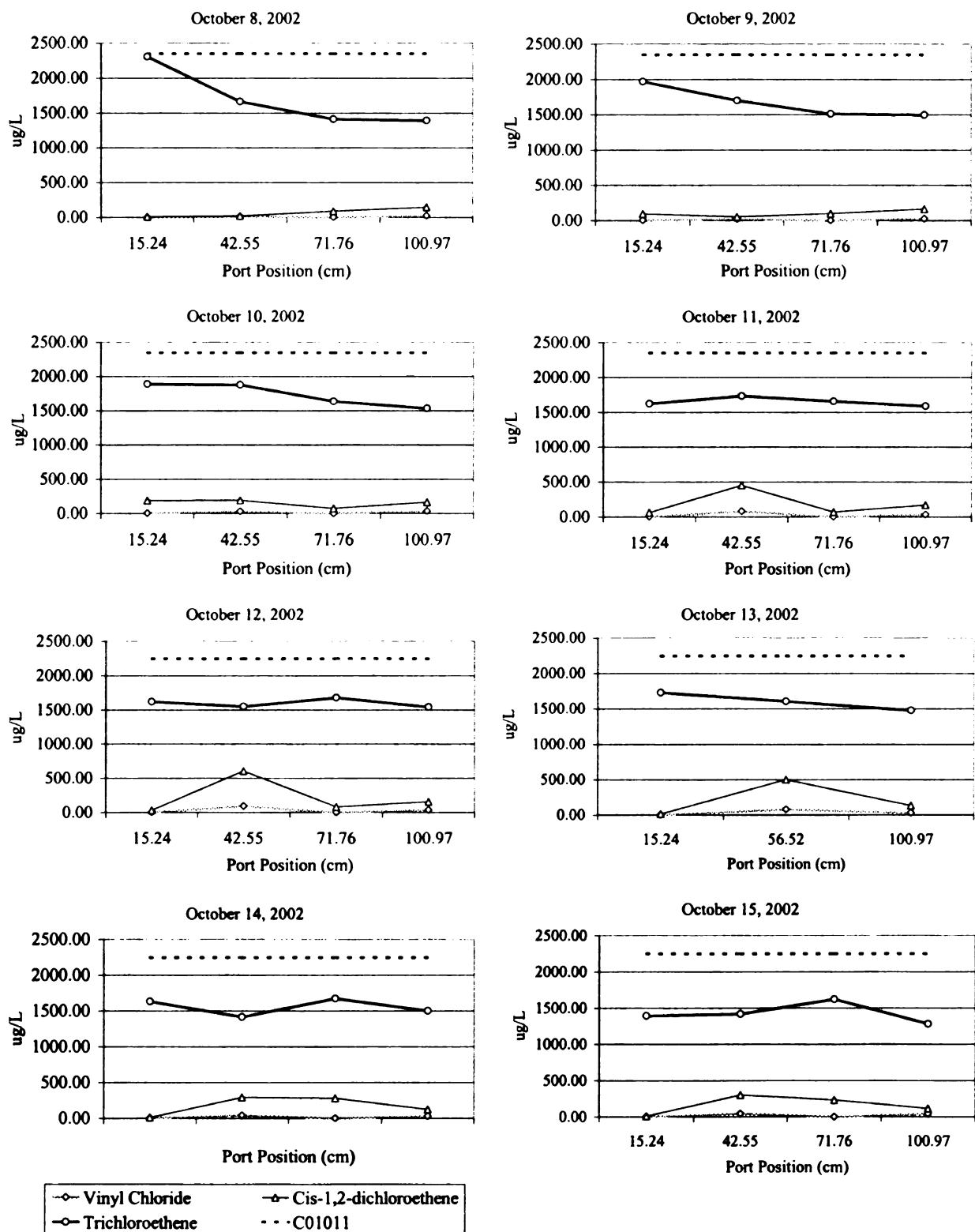
October 1, 2002



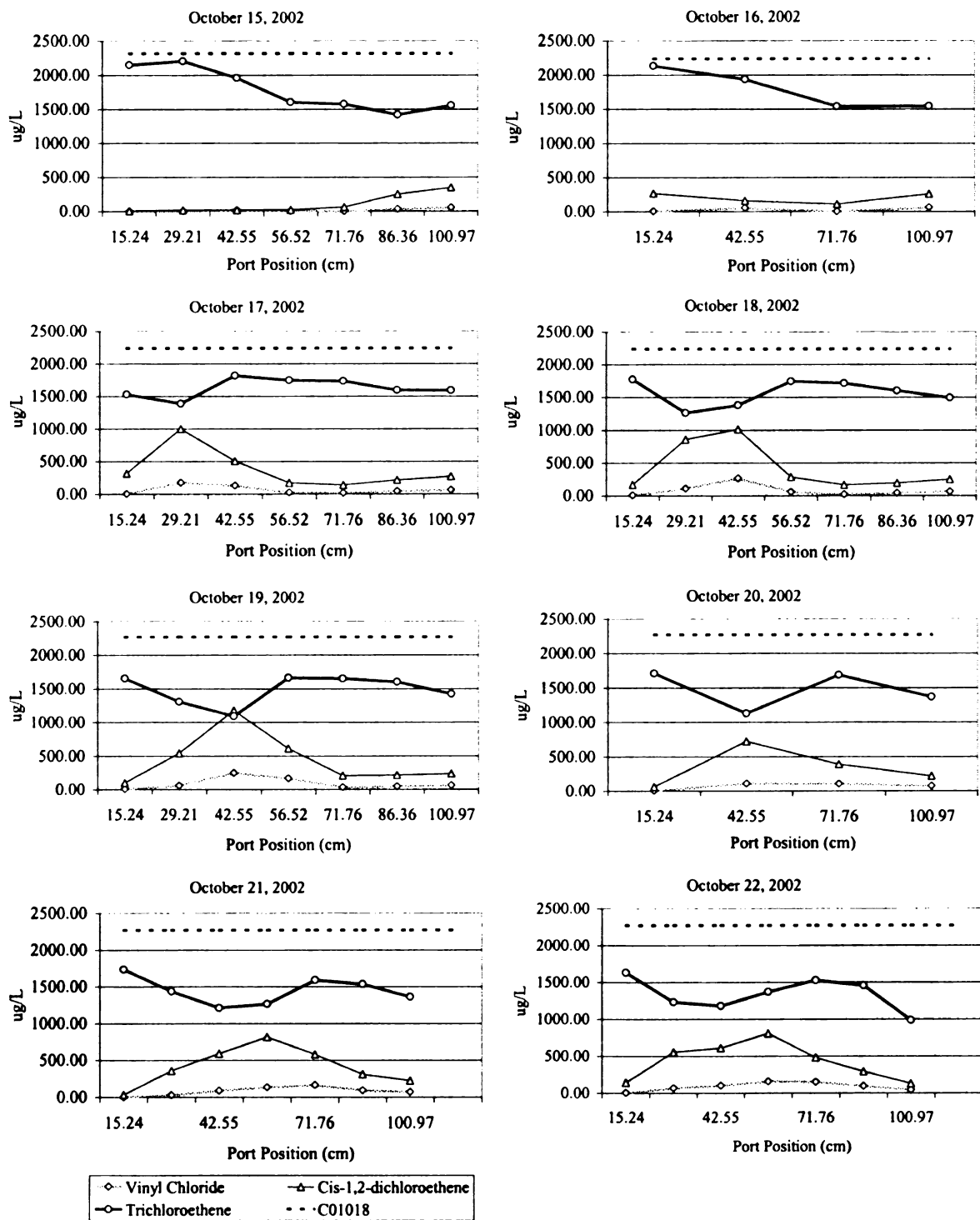
**CHLORINATED VOCs
COLUMN A
October 1 - 8, 2002 (Incubation Period 4B)**



**CHLORINATED VOCs
COLUMN A
October 8 - 15, 2002
(Incubation Period 5B)**



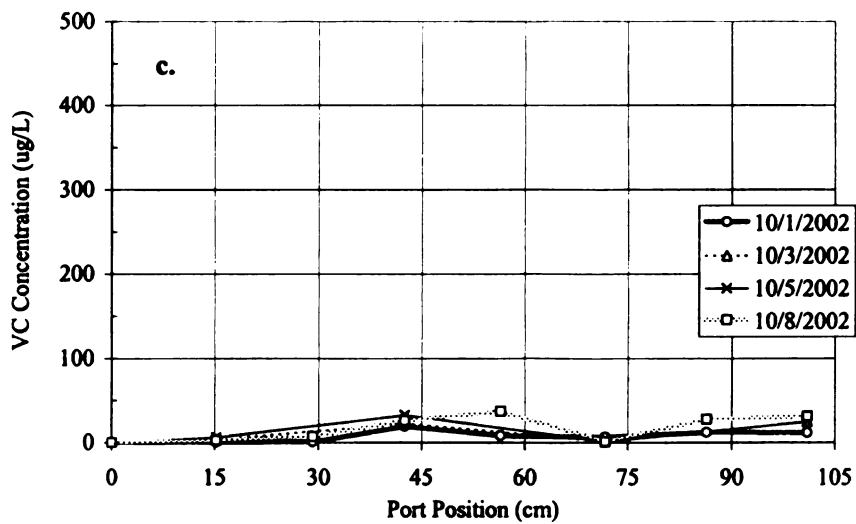
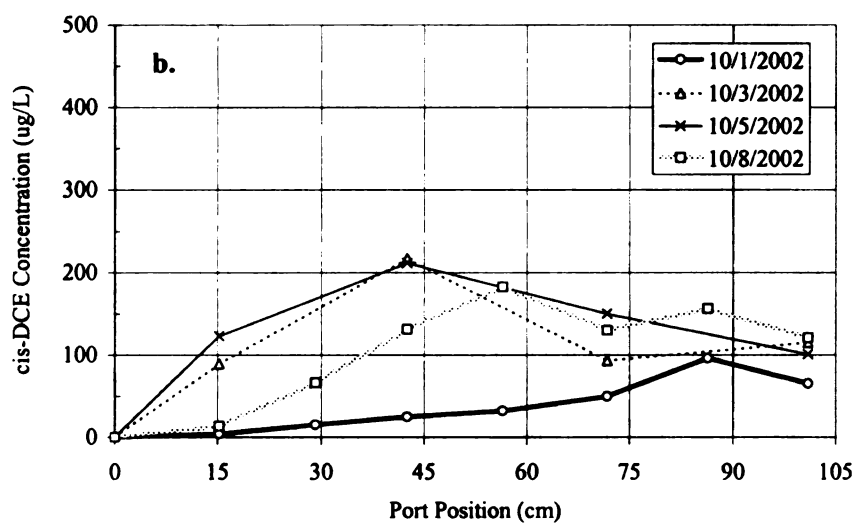
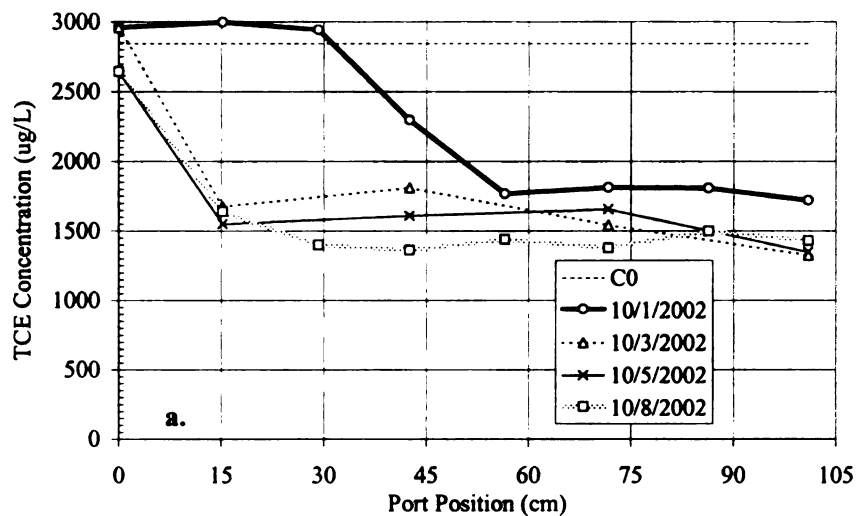
**CHLORINATED VOCs
COLUMN A
October 15 - 22, 2002
(Incubation Period 6B)**



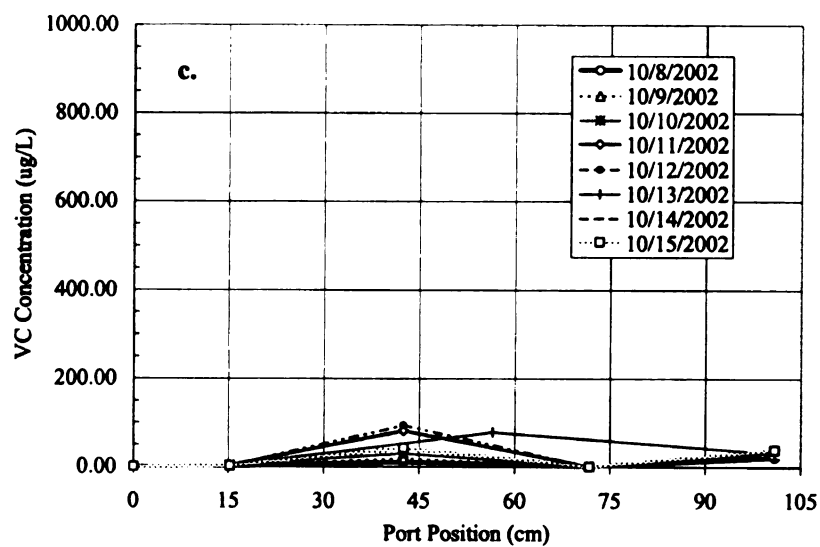
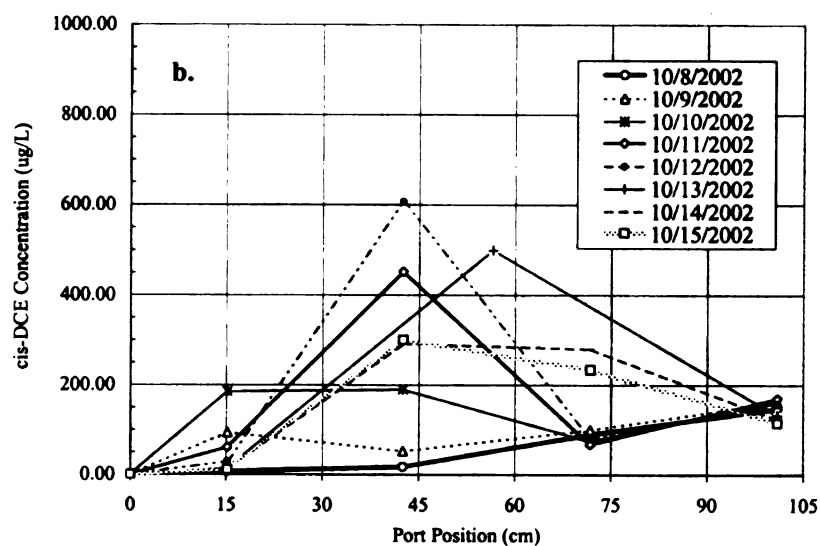
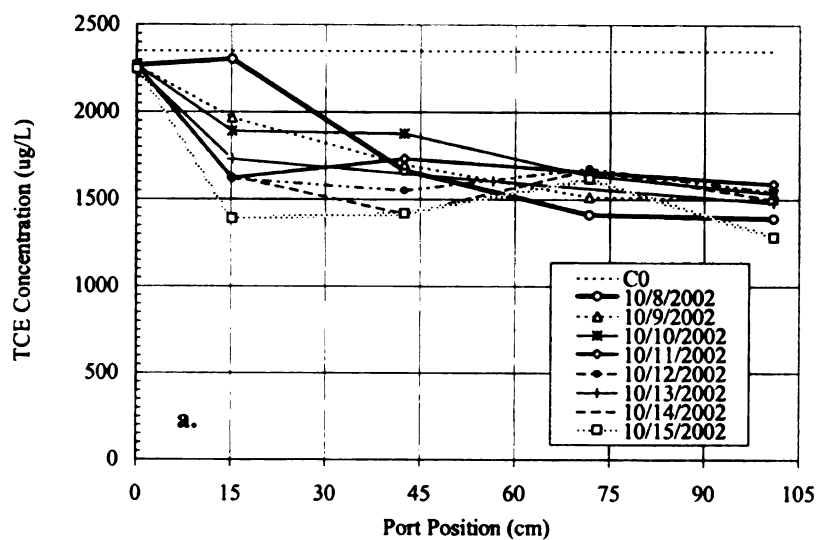
APPENDIX E

**Daily Solute Concentrations within a Control Volume
Conveyed through Column A
Incubation Periods 4B-6B**

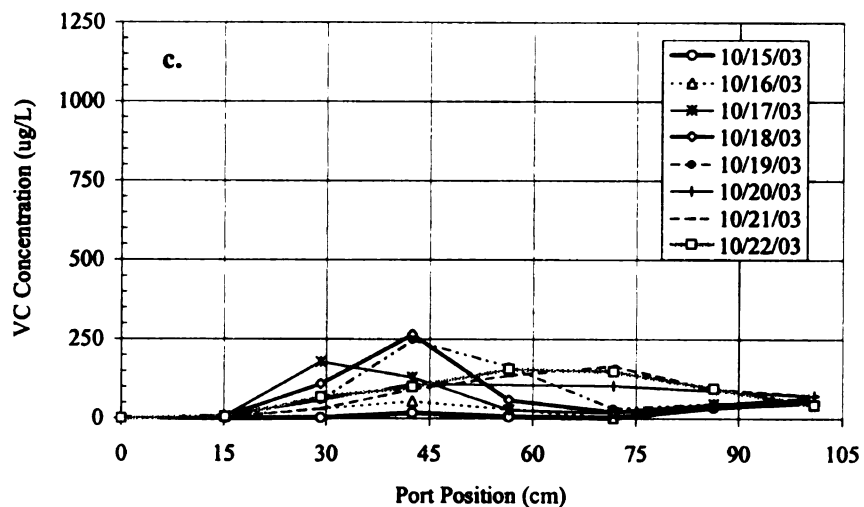
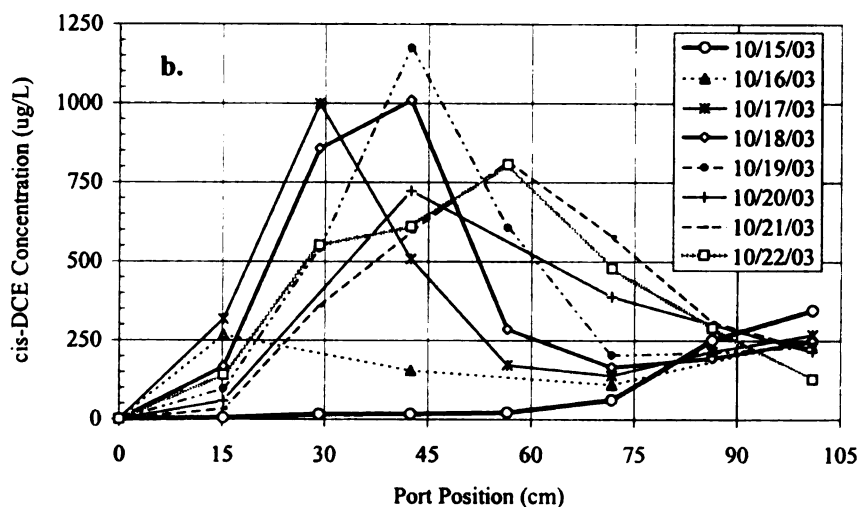
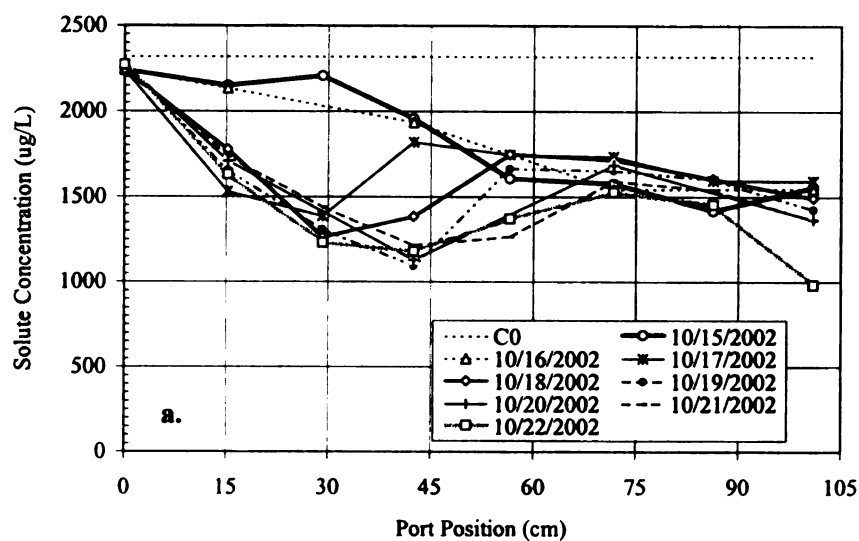
**TCE (a), cis-DCE (b), and VC (c) profiles
over the length of Column A during Incubation Period 4B**



TCE (a), cis-DCE (b), and VC (c) profiles over the length of Column A during Incubation Period 5B



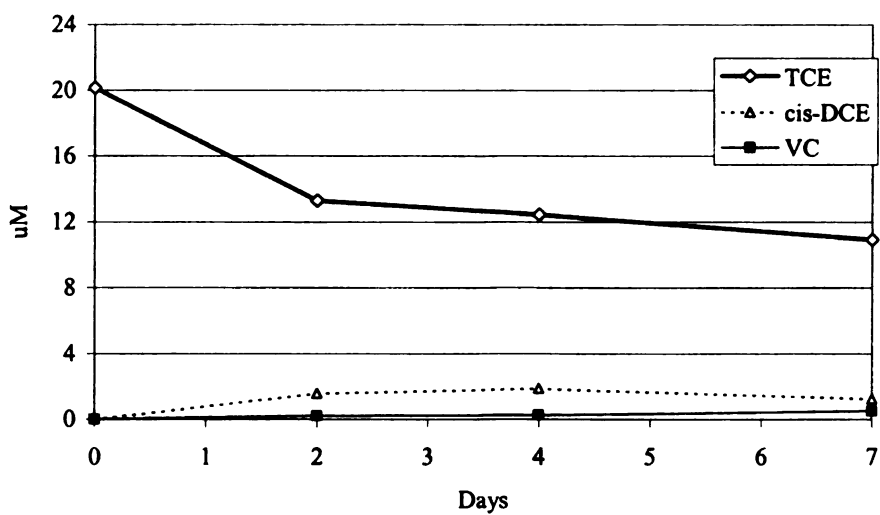
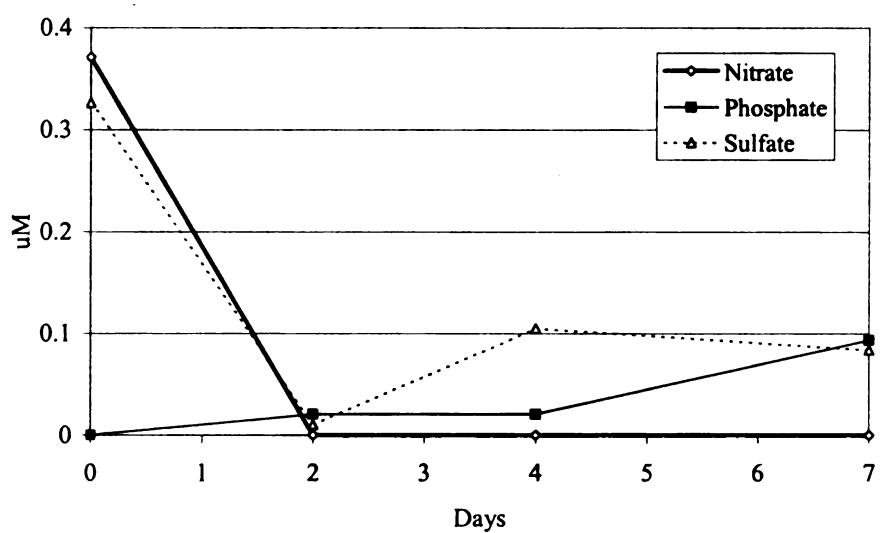
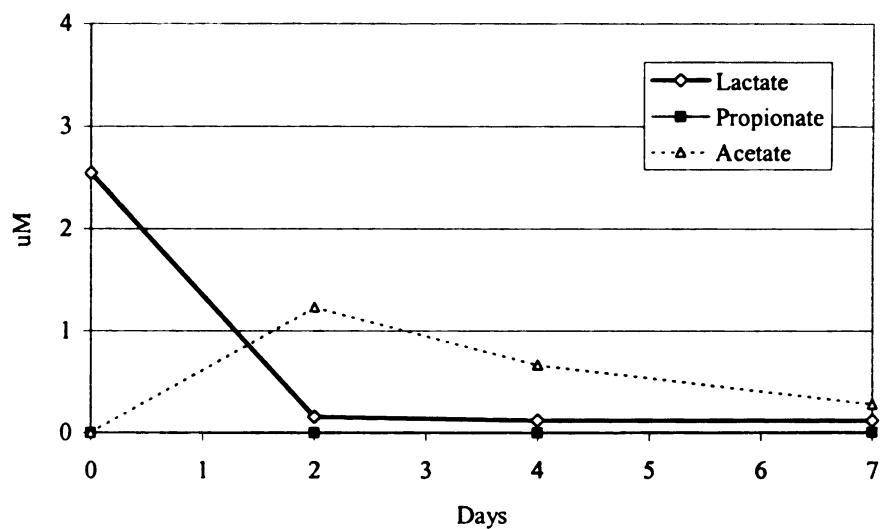
TCE (a), cis-DCE (b) and VC (c) profiles over the length of Column A during Incubation Period 6B



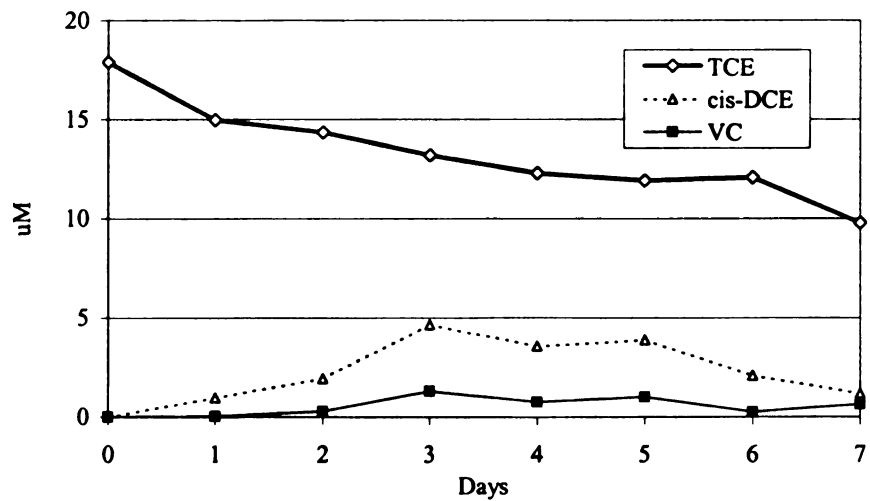
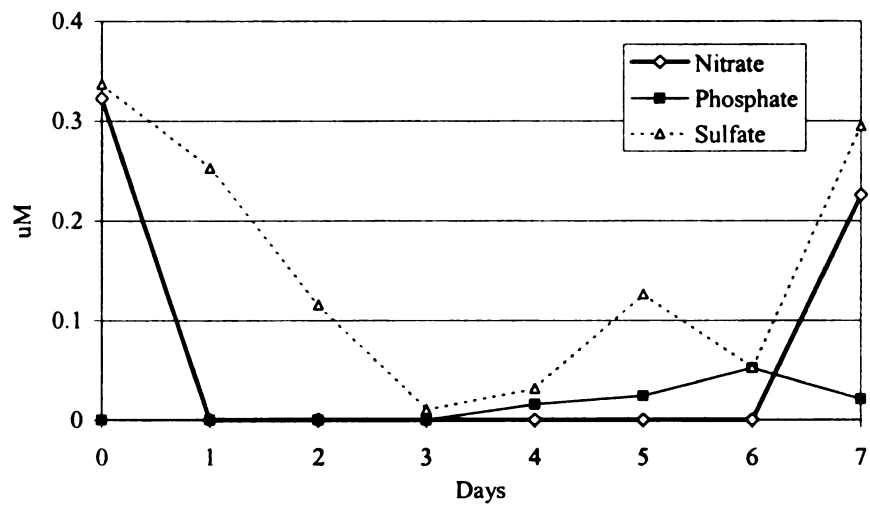
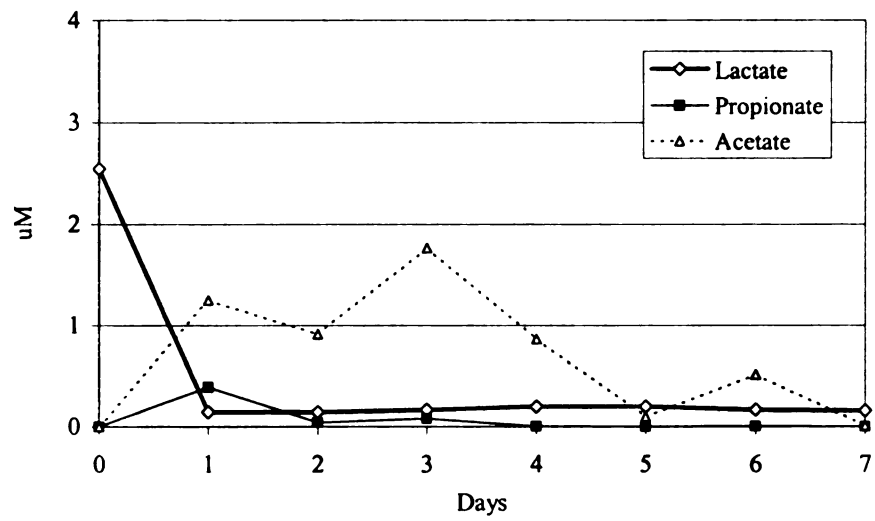
APPENDIX F

Daily Solute Concentration Profiles Over Column A Incubation Periods 4B-6B

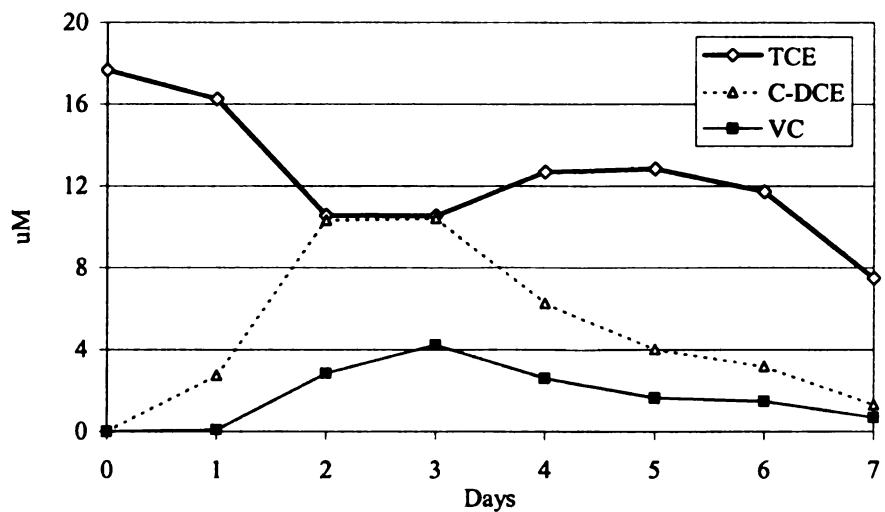
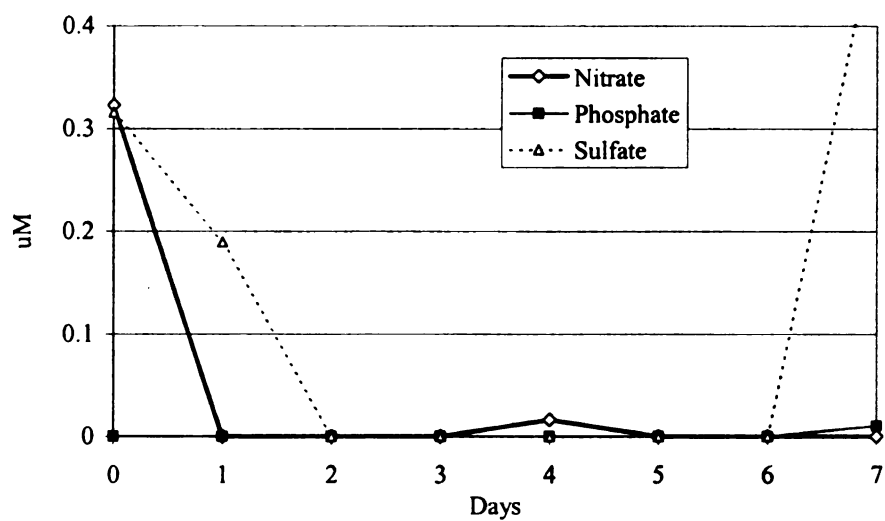
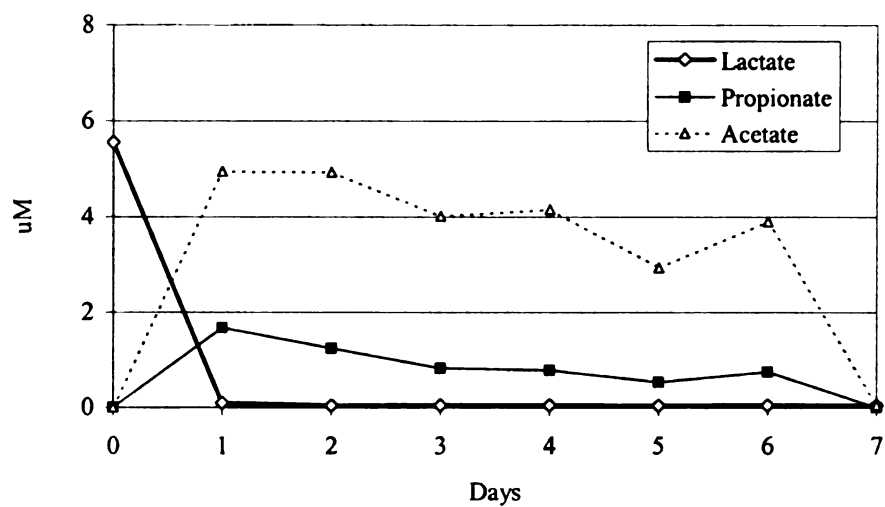
Pore fluid concentrations of fatty acids, major anions, and VOCs
in pore fluids passing through Column A during Incubation Period 4B



Pore fluid concentrations of fatty acids, major anions, and VOCs
in pore fluids passing through Column A during Incubation Period 5B



Pore fluid concentrations of fatty acids, major anions, and VOCs
in pore fluids passing through Column A during Incubation Period 6B



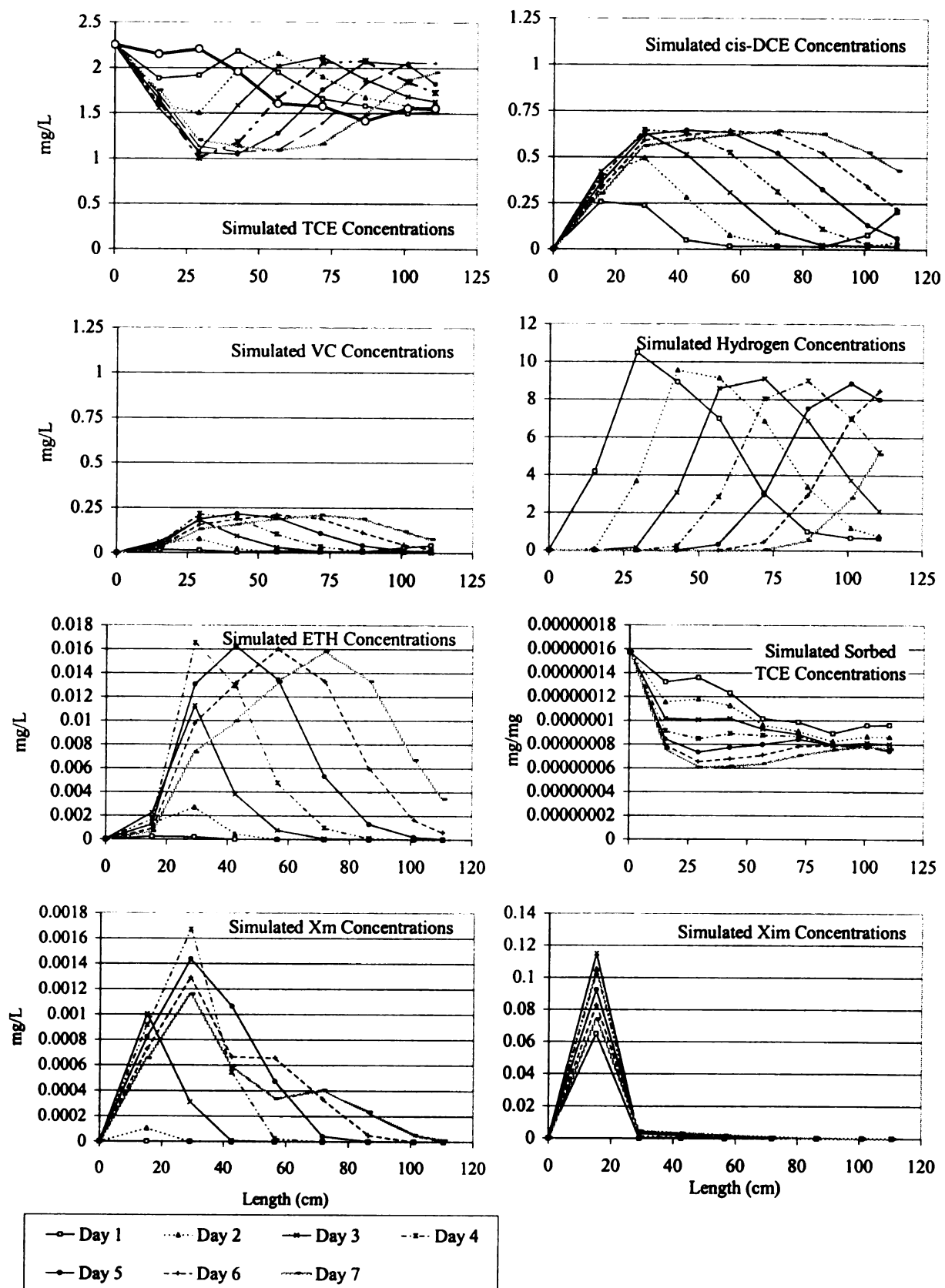
APPENDIX G

Reactive Transport Modeling Results

APPENDIX G-1

Calibration

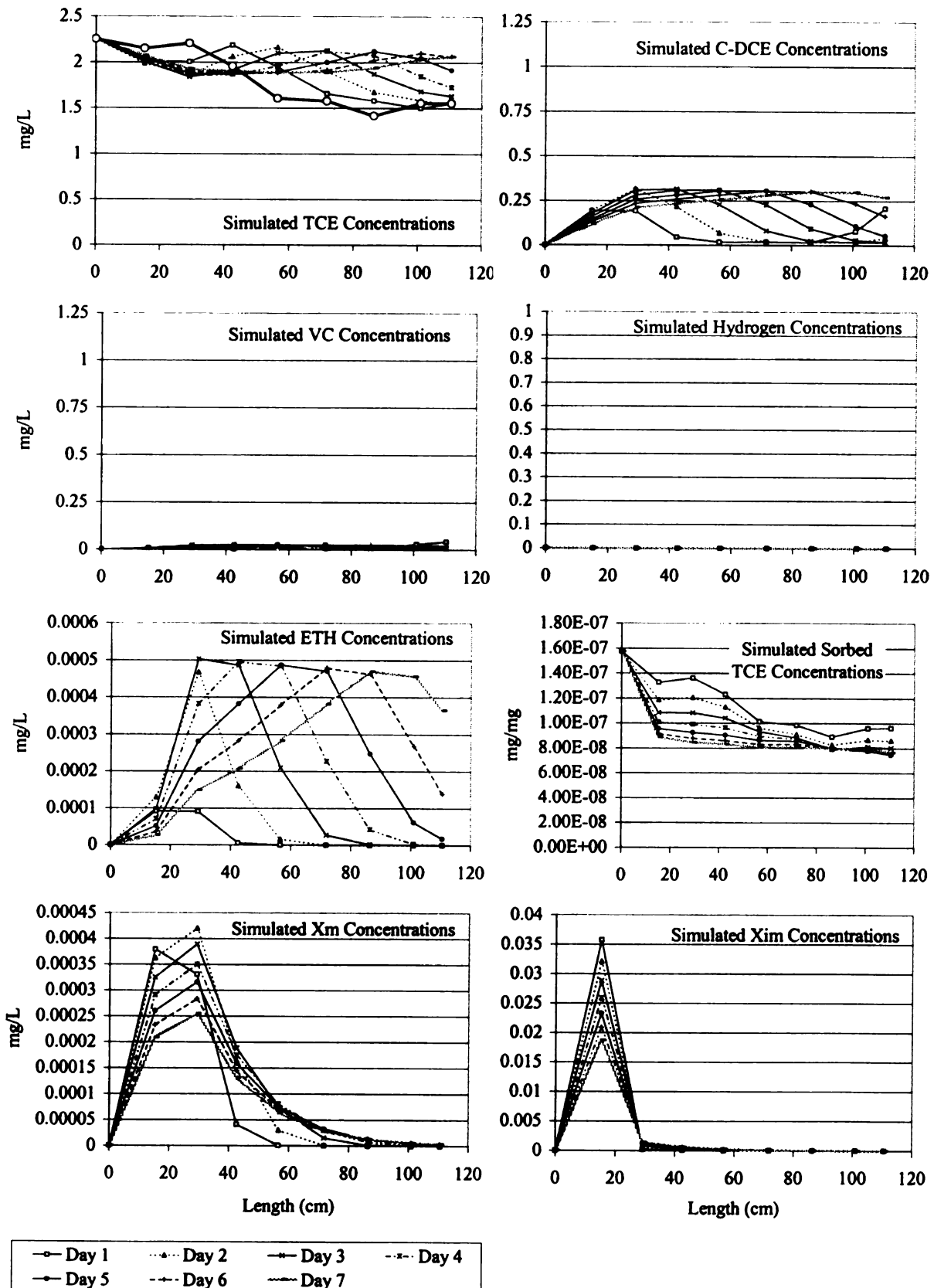
CALIBRATED MODEL RESULTS



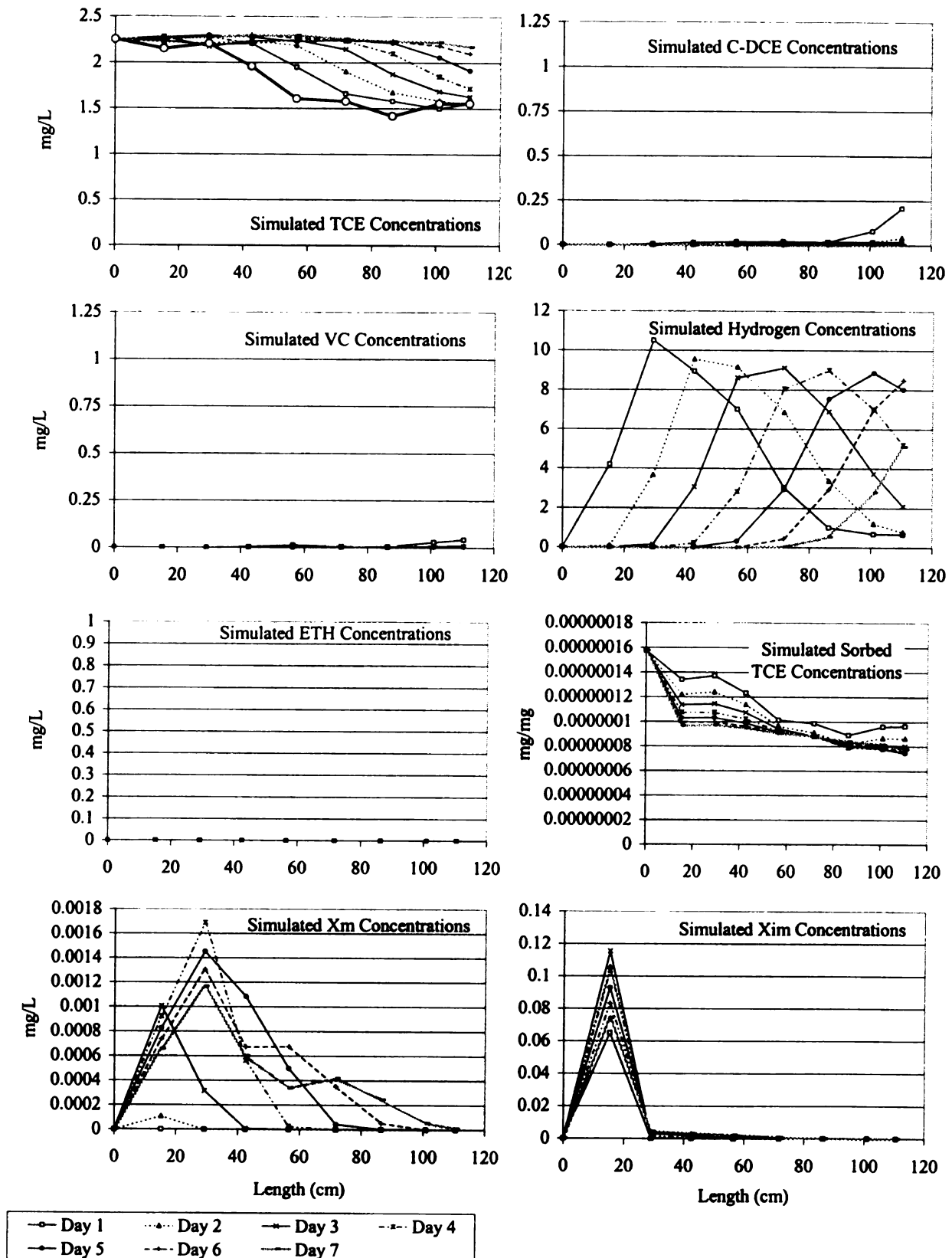
APPENDIX G-2

Sensitivity Analyses

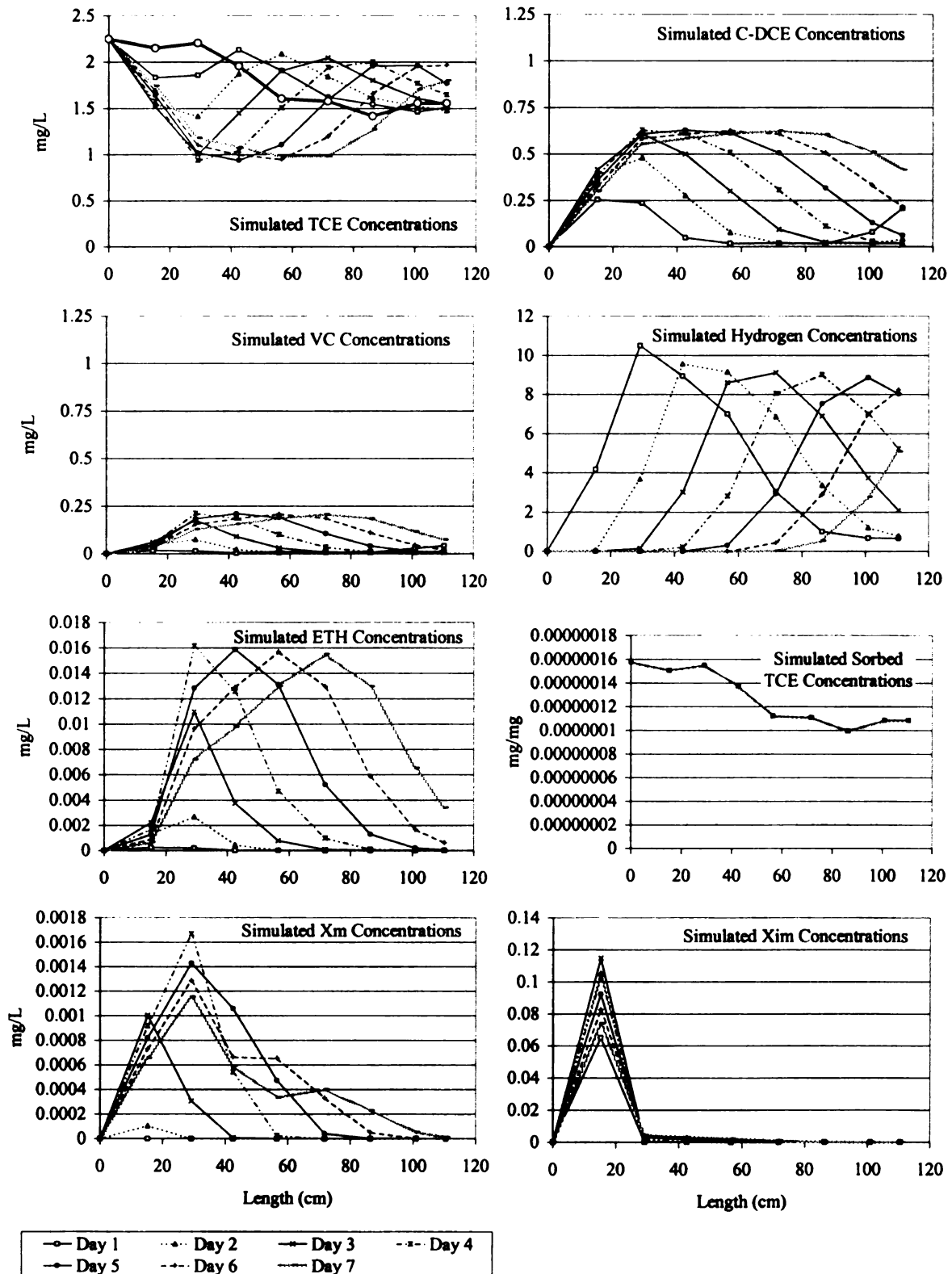
**SENSITIVITY ANALYSIS
NO HYDROGEN IN SYSTEM**



**SENSITIVITY ANALYSIS
NO REACTION TERMS**



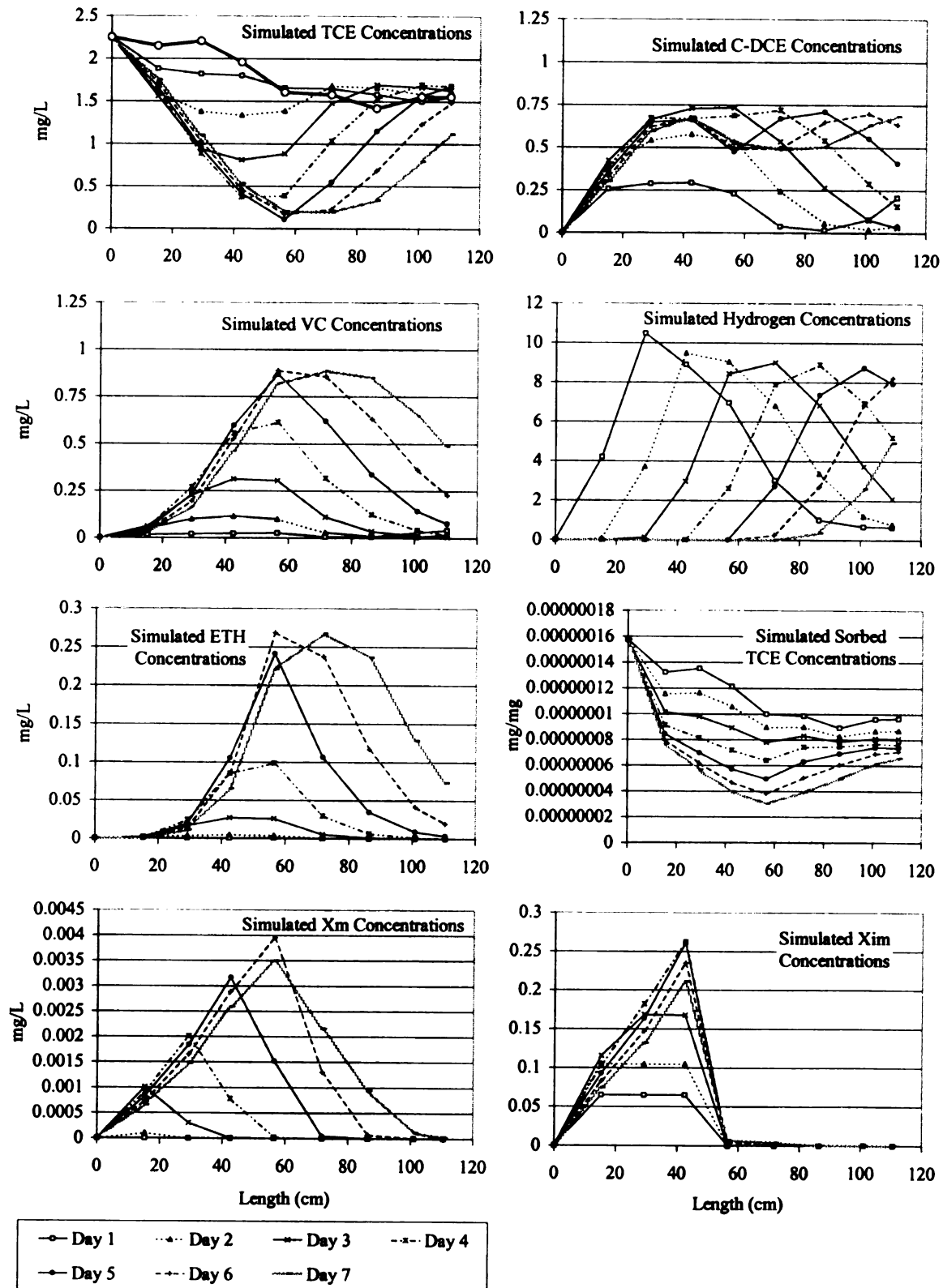
**SENSITIVITY ANALYSIS
NON-RATE LIMITED SORPTION CONDITIONS**



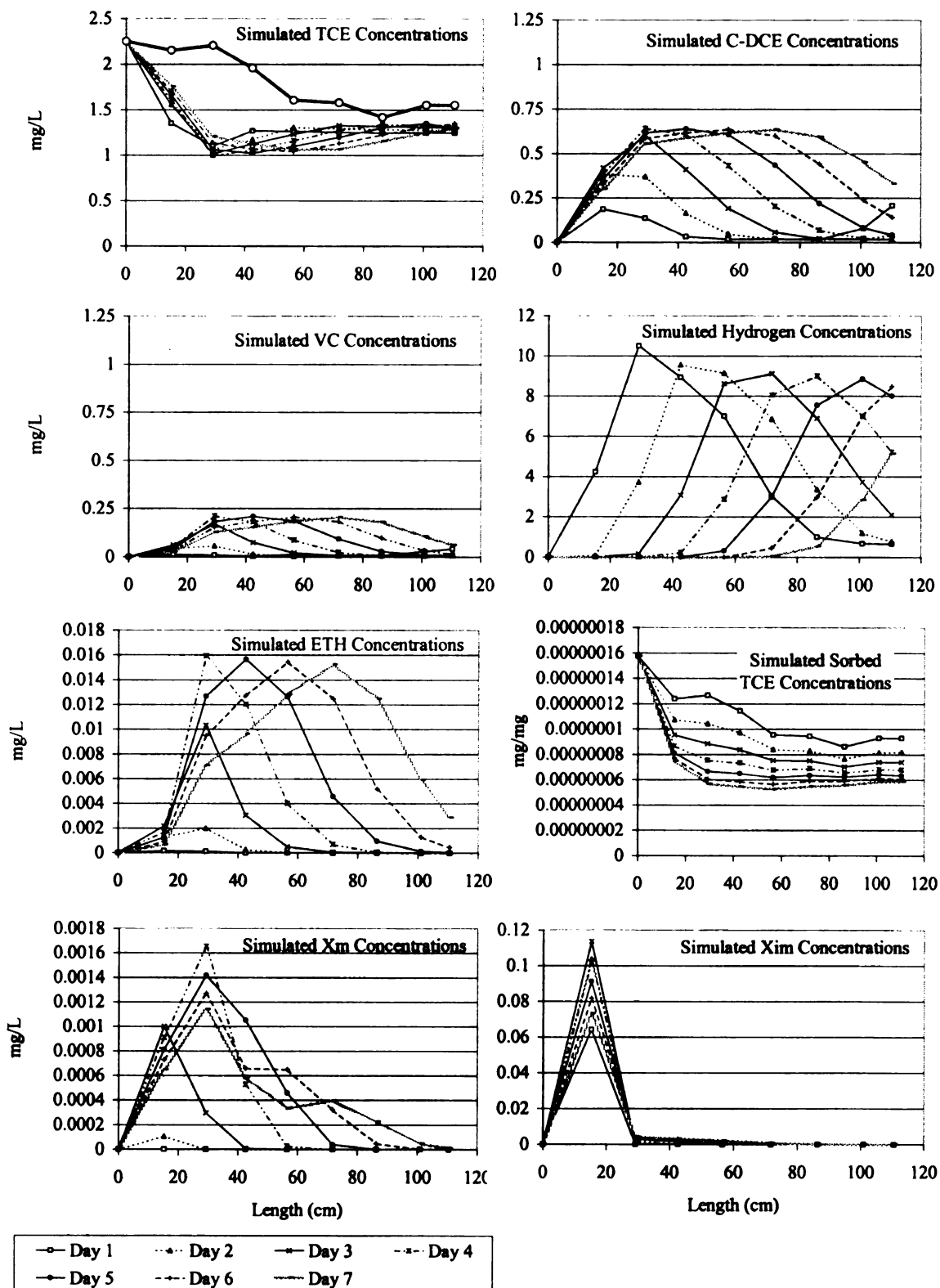
APPENDIX G-3

Predictions

**MODEL PREDICTION
DOUBLE LENGTH OF BAZ**



MODEL PREDICTION
USE OF BAZ TREATED GROUNDWATER FOR FEED MAKE-UP (Recycle)



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