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

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ABSTRACT

Semi-batch and continuous-flow column systems containing TCE and Cr(VI)-impacted soil and groundwater were used to examine: 1) redox gradients; 2) TCE transformation patterns; and 3) successional adaptations of mobile- and stationary-phase microbial communities following inoculation with a dechlorinating enrichment culture. Data from the semi-batch experiments revealed that TCE dechlorination to vinyl chloride was rapid with a first-order rate coefficient of approximately 0.6 day^{-1}. Within the continuous flow system, influent TCE concentrations (1-4 mg/L) were reduced 30-40% during 7-day intervals following each of nine feedings with lactate and nutrients. Soluble chromium levels (200-250 μg/L) decreased over 90% following the feedings, presumably by reduction of Cr(VI) to less soluble Cr(III). Following lactate fermentation, iron and sulfate reduction were the predominant redox processes within the biologically active zone of the continuous flow system. Reductive dechlorination of TCE was spatially correlated to 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. TCE transformation rates/completeness appeared to decrease with an increase in sulfidogenic activity. Methanogenesis appeared to inhibit dechlorination.

INTRODUCTION

The Village of Schoolcraft is a rural community located 10 miles of Kalamazoo, Michigan. Since 1979, Schoolcraft has been the focal point of State-sponsored investigations into the causes and distributions of several occurrences of groundwater contamination. A total of seven distinct plumes of impacted groundwater had been identified and delineated through these investigations. The plumes were designated A through G. 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. Plume F consists of hexavalent chromium (Cr(VI)) and arsenic.

Plume G encompasses the largest region of TCE-impacted groundwater in Schoolcraft. From its source (a former auto parts manufacturing facility), 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). Plume F originated at a former wood pressure treatment operation at which chromated copper arsenate solutions were released to the subsurface. The source of Plume F is located downgradient of the source of Plume G. Consequently, Plumes F and G are commingled (Figure 1).

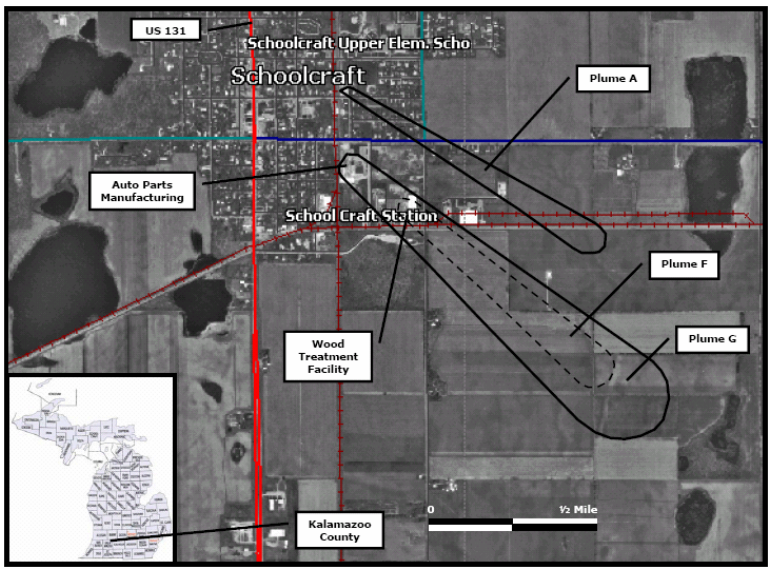


Figure 1 – Schoolcraft, Michigan and Plume F/G.

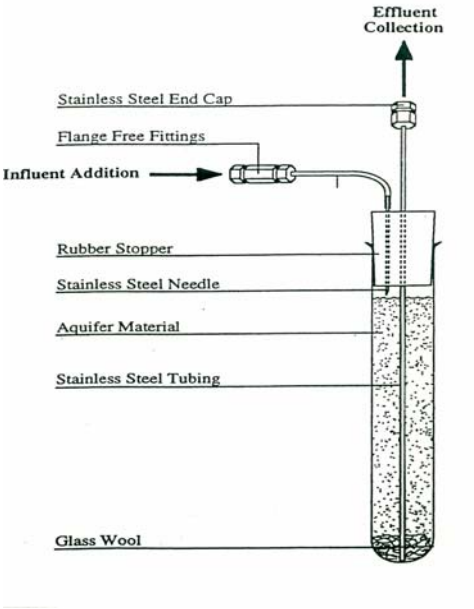


Figure 2 – Small Column Design

Due to the commingled nature of Plumes F and G, the State of Michigan has been challenged to develop a technically- and cost-effective strategy to simultaneously clean up these plumes. Recently, the State engaged researchers at MSU to evaluate bioremediation strategies for Plumes F and G. MSU has conducted bioaugmentation experiments designed to reductively dehalogenate TCE and create conditions favorable for the in-situ reduction of Cr(VI) to Cr(III).

Objectives

The goals of the Plume F/G bioaugmentation research were to:

- Determine if a non-native TCE-dechlorinating enrichment culture could be effectively delivered and distributed through the Plume F/G sediments;
- Determine if the inoculum will increase the rates and completeness of TCE reductive dechlorination; and
- Evaluate if the redox conditions created by inoculation and feeding will promote the reduction of Cr(VI) and immobilization of Cr(III).

Experimental Design

To accomplish these objectives, a research program, consisting of the following experiments and associated tasks, was undertaken: I) Small-Scale Column Microcosm Experiment (including characterization of the flow-through properties of the Plume F/G aquifer materials; determination of the TCE mass loading and sorption capacity of these materials; evaluation of an inoculation and long-term feeding strategy; and post-treatment characterization of the aquifer materials); II) Large-Scale Column Experiment (including a TCE mass loading and sorption capacity evaluation; inoculation and characterization of flow-through properties; long-term treatment; and post-treatment characterization of the aquifer materials); and III) TCE fate and transport modeling. These experiments are described further in the following paragraphs.

Small-Scale Column Microcosm Experiment

A four-phase experiment was performed using small-scale soil column microcosms. The objectives of the experiment were to verify that a TCE 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, sterile and non-sterile control columns. The fate of Cr(VI) was also examined to verify that reduction to Cr(III) occurred within inoculated columns.

A total of 21 columns were operated. These were grouped into seven (7) distinct sets, each consisting of three (3)-columns. The seven column sets were organized as follows:

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

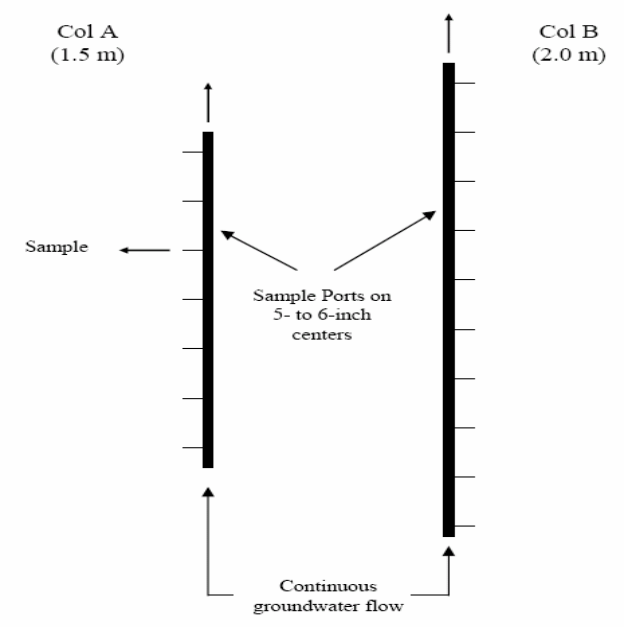
The design and operation of the small-scale column microcosms were consistent with those described by Dolan and McCarty (1) and Mayotte, et al. (2). A schematic of an example column is provided on Figure 2.

Large-Scale Column Experiment

In contrast to small-scale columns receiving semi-batch pore fluid exchanges, experimentation with large-scale sediment columns delivered continuous flow of groundwater was believed to be more conducive for both the development and examination of a biologically active zone (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 were accomplished using large-scale continuous flow column systems.

Large-scale continuous flow column systems were assembled using Plume F/G sediments and groundwater, as described by Mayotte, 2003. The expanded scale of these columns enabled more detailed examination of the transport characteristics of the TCE dehalogenating enrichment and *Dehalococcoides sp.* 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.

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 TCE 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. Schematics of the assembled columns are presented on Figure 3.



- Col A - used for assessment of treatment dynamics.
- Col B - used for inoculums transport evaluations.
- Groundwater continuously fed to Col A at 15 cm/day.
- Sampling performed daily to weekly.
- Columns sacrificed to facilitate soil sampling at end of use.
- Col temp controlled at 15^{o}C

Figure 3 – Large Scale Column Systems

The large-scale column experiment consisted of four (4) tasks, as outlined above. Column A was operated and monitored during each of these tasks. Column B was utilized during Task 2.2 exclusively. Operation of the column systems was performed as described in Mayotte (3).

The first task of the large-scale column experiment 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. This task 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.

The second task entailed inoculation of Columns A and B with the TCE 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 task 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.

The third task 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. This task included two operational phases (A and B) corresponding with two separate inoculations of Column A with the TCE enrichment. A comprehensive summary of the operational history of Column A is summarized in Mayotte (3).

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® dsDNA Quantification to estimate biomass concentrations and Real Time-PCR for *Dehalococcoides sp.* quantification.

TCE Transport Modeling

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 Mayotte (3). The physical, hydraulic and bio-kinetic parameters and associated values provided as input into these codes are also presented in Mayotte (3). The hydraulic model established for this study was calibrated through simulations of tracer and TCE breakthrough. The numerical transport and fate model was calibrated using solute and biomass monitoring data obtained during the large scale column experiment.

RESULTS

Cumulative mass removal data from the small column experiments indicate that the bioaugmentation of the Plume F/G aquifer materials resulted in TCE transformation (Figure 4). The range of first order rate coefficients, *k'*, estimated from the small column data confirmed that transformation rates expressed by the TCE enrichment were consistent with the range associated with the culture maintained under laboratory conditions (Figure 5). Further, the range of apparent first-order rate coefficients (0.4 – 0.6 1/day) reported here suggests that favorable rates of TCE transformation can be achieved through engineered bioaugmentation of Plume F/G. dsDNA and RT-PCR data confirmed the presence of *Dehalococcoides sp*. within inoculated columns. *Dehalococcoides sp.* were not detected within the non-inoculated (natural attenuation) columns. These observations suggest a causal relationship between the increased rates of TCE removal and the presence of *Dehalococcoides sp.*

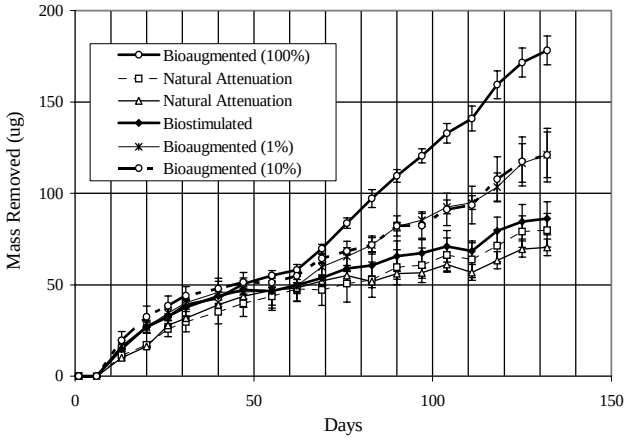


Figure 4 – TCE Mass Removal in Small Columns

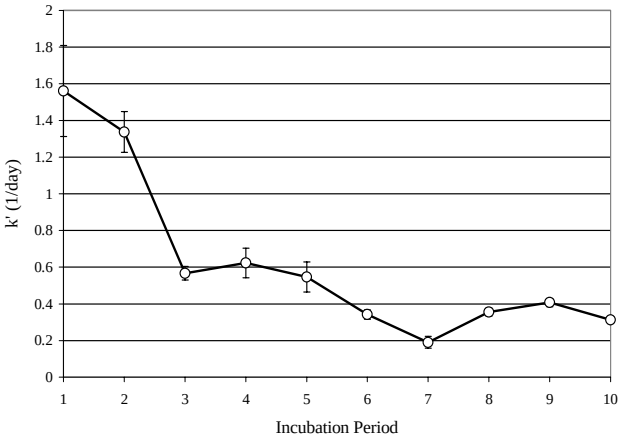


Figure 5 – First Order Rate Coefficients in Small Columns

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 throughout the large-scale column experiment. Evidence associated with substrate utilization, redox, and TCE transformation gradients indirectly revealed spatial and temporal relationships between key biologically mediated processes (Figure 6). 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.

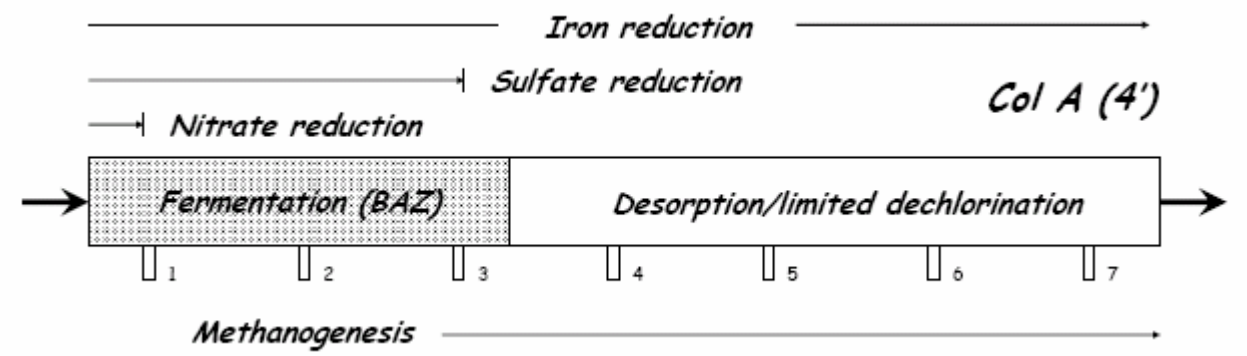


Figure 6 – Redox Zonation within Large-Scale Column A.

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 (Figure 7).

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-1,2-dichloroethene (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: 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 an 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 vinyl chloride (VC) (Figure 7).

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 accumulation of cis-DCE and VC (Figure 7). During this period, halorespiration of TCE by *Dehalococcoides sp*. was likely maximized.

The decrease in ORP from over 100 millivolts (mV) to -100 to -300 mV 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 TCE enrichment was beneficial for reduction Cr(VI) and immobilization of Cr(III).

TCE removal during each of the eight incubation periods comprising the large-scale column experiment is summarized in Table 1. These data reveal that the weekly experimental treatment protocol was sufficient to achieve TCE removal efficiencies averaging 37%. The bulk of TCE transformation occurred within the first four days of each incubation period. Effluent levels of cis-DCE and VC were below target cleanup levels at conclusion of these periods.

The results from the sensitivity analyses of the numerical fate and transport model support the assertion that biochemical reactions are the predominant mechanisms for TCE removal in Column A. Model-generated design-optimization simulations indicate that TCE removal increases significantly when the BAZ is widened through consistent delivery of a quantity of feedstock sufficient to displace an appropriate volume of pore fluids. By increasing the width of the BAZ to approximately 3 feet by doubling the volume of biweekly feedstock injections, TCE levels of 2,500 μg/L in Column A are reduced by approximately 90% within a four to seven day period following each feeding event.

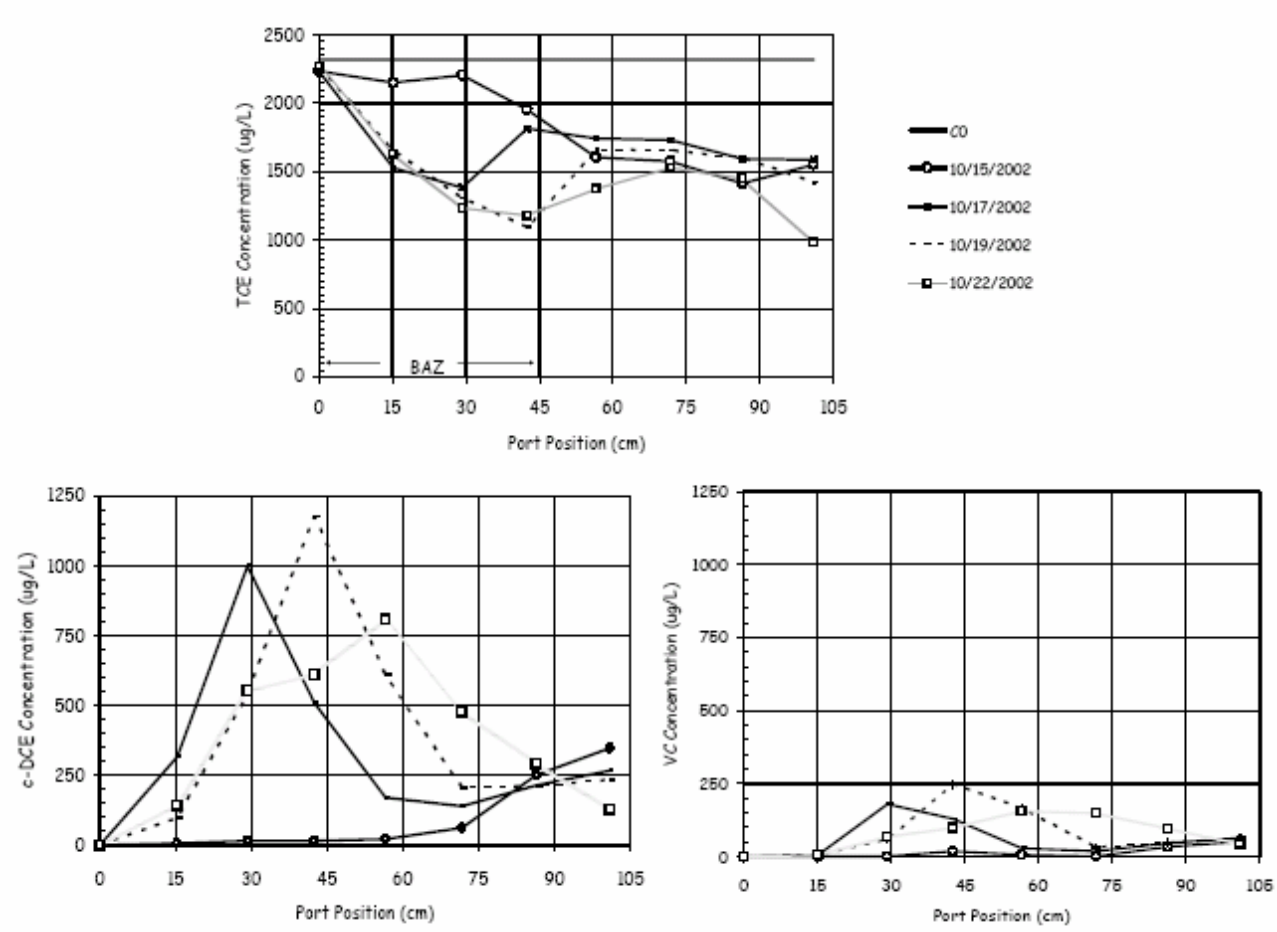


Figure 7 – TCE transformation gradients over length of Column A during an Incubation Period.

Incubation Period	Mass Removed (µg)	Treatment Efficiency (%)
5A	292	36
6A	169	26
7A	253	30
8A	207	30
3B	656	43
4B	248	42
5B	141	29
6B	171	36
Average	*276*	*37*

Table 1 - TCE mass treatment efficiencies over the length of Column A during each 7-day incubation period.

REFERENCES

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(3) Mayotte, T.J., *Ph.D. Dissertation*; Michigan State University, 2003.