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CHLOROETHENE BIODEGRADATION POTENTIAL, ADOT/PF PEGER ROAD MAINTENANCE FACILITY, FAIRBANKS, ALASKA

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Prepared in cooperation with the

ALASKA DEPARTMENT OF ENVIRONMENTAL CONSERVATION

U.S. Department of the Interior
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ABBREVIATIONS AND ACRONYMS

Abbreviations	
°C	degrees celsius
g	gram(s)
h	hour(s)
M	mole(s) per liter
mg/L	milligram(s) per liter
mL	milliliter(s)
µCi/µmole	microcuries per micromole
µg/L	microgram(s) per liter
µm	micrometer(s)
µM	micromoles per liter
µmole	micromole
nM	nanomoles per liter
nmole	nanomole
Acronyms	
ANOVA	analysis of variance
DCE	dichloroethene
PCE	perchloroethene
PSI	per square inch
SD	standard deviation
TCE	trichloroethene
VC	vinyl chloride

ABSTRACT

A series of ^{14}C-radiotracer-based microcosm experiments were conducted to assess: 1) the extent, rate and products of microbial dechlorination of trichloroethene (TCE), *cis*-dichloroethene *(cis*-DCE) and vinyl chloride (VC) in sediments at the Peger Road site; 2) the effect of three electron donor amendments (molasses, shrimp and crab chitin, and "Hydrogen Release Compound" (HRC)) on microbial degradation of TCE in three Peger Road sediments; and 3) the potential significance at the site of chloroethene biodegradation processes other than reductive dechlorination.

In these experiments, TCE biodegradation yielded the reduced products, DCE and VC, and the oxidation product CO_2. Biodegradation of DCE and VC involved stoichiometric oxidation to CO_2. Both laboratory microcosm study and field redox assessment results indicated that the predominant terminal electron accepting process in Peger Road plume sediments under anoxic conditions was Mn/Fe-reduction. The rates of chloroethene biodegradation observed in Peger Road sediment microcosms under low temperature conditions (4 °C) were within the range of those observed in sediments from temperate (20 °C) aquifer systems. This result confirmed that biodegradation can be a significant mechanism for *in situ* contaminant remediation even in cold temperature aquifers. The fact that CO_2 was the sole product of *cis*-DCE and VC biodegradation detected in Peger Road sediments indicated that a natural attenuation assessment based on reduced daughter product accumulation may significantly underestimate the potential for DCE and VC biodegradation at the Peger Road.

Neither HRC nor molasses addition stimulated TCE reductive dechlorination. The fact that molasses and HRC amendment did stimulate Mn/Fe-reduction suggests that addition of these electron donors favored microbial Mn/Fe-reduction to the detriment of microbial TCE dechlorinating activity. In contrast, amendment of sediment microcosms with shrimp and crab chitin resulted in the establishment of mixed Mn/Fe-reducing, $SO_4^{=}$-reducing and methanogenic conditions and enhanced TCE biodegradation in two of three Peger Road sediment treatments.

INTRODUCTION

Activities associated with the Alaska Department of Transportation and Public Facilities Peger Road Operations and Maintenance Facility (Peger Road) site have resulted in contamination of the underlying, shallow aquifer with the chloroethene solvent, trichloroethene (TCE). A groundwater TCE contaminant plume appears to originate from the area of the Materials Laboratory and to extend offsite toward the northwest. The northern extent of the plume has not yet been identified, but output from the U.S. Geological Survey/Virginia Tech Natural Attenuation Software package indicates a probable plume extent in excess of 1800 ft (Shannon and Wilson, Inc. 2003).

TCE concentrations in monitoring wells located in the source area and middle portions of the plume appear to be decreasing (Shannon and Wilson, Inc. 2003). TCE natural attenuation is presumed to result primarily from dilution, because *in situ* accumulation of reductive dechlorination products is limited to rare incidences of low concentrations (circa 5 µg/L) of *cis*-DCE in the source area despite the fact that significant portions of the plume are anoxic (Shannon and Wilson 2003). The presence of *cis*-DCE, the apparent lack of *cis*-DCE transport along the groundwater flowpath, the lack of VC accumulation and the apparent predominance of relatively oxidized, Mn/Fe-reducing conditions suggest that the potential for biodegradation of chloroethene contaminants to innocuous, non-diagnostic products (such as CO_2) may be significant at the Peger Road site. The apparent offsite migration of the Peger Road TCE plume, prompted a cooperative investigation between the Alaska Department of Environmental Conservation (ADEC) and the Microbial Studies Group of the U.S.G.S.

Specific investigation objectives were:

- Measure dissolved H_2 concentrations and conduct a field evaluation of the *in situ* redox conditions present in the shallow aquifer at the Peger Road site. Use the results to characterize the predominant redox conditions and evaluate the potential for chloroethene biodegradation under *in situ* conditions.
- Conduct a microcosm investigation using ^{14}C-radiolabeled substrates to evaluate individual rates of biodegradation for TCE, *cis*-DCE, and VC in sediments collected from two locations in the plume area. Such rate data are intended for quantitative modeling of contaminant fate and transport in the TCE plume.
- Conduct a microcosm investigation using ^{14}C-TCE to assess the relative effectiveness of three readily available electron donor amendments for stimulating TCE reductive dechlorination in sediments collected from three locations in the plume.
- Assess the potential for non-reductive biodegradation of chloroethene, groundwater contaminants in the chloroethene contaminant plume. Specifically, evaluate the potential for mineralization of ^{14}C-chloroethene compounds to $^{14}CO_2$ and $^{14}CH_4$.

MATERIAL AND METHODS

Field Redox Assessment. Ground-water samples for measurement of redox-sensitive parameters were collected in September 2003 from selected groundwater monitoring wells at the Peger Road site (Appendix A). Dissolved hydrogen (H_2) was measured using the gas-stripping procedure described in Chapelle et al. (1997). Concentrations of dissolved Mn(II), Fe(II), sulfide (Hach Co., Loveland, Colorado), and oxygen (Chemetrics Inc., Calverton, Virginia) were measured in the field using colorimetric methods. The dissolved concentration of major anions, including the potential terminal electron accepting compounds, nitrate and sulfate, were determined using anion exchange chromatography with conductivity detection (EPA 300.0). Samples for the analysis of methane were collected by filtering 2 mL of groundwater (0.2 m filters) into sealed 10 mL serum vials. In the laboratory, headspace methane concentrations were measured using gas chromatography with thermal conductivity detection. The amount of methane in groundwater was then calculated from the headspace methane concentration using the Henry's law partition coefficient. Determination of predominant terminal electron-accepting processes was based on consumption of electron acceptors such as dissolved oxygen and sulfate, production of final products such as Fe(II), sulfide, and methane, and concentrations of the intermediate product hydrogen as described in detail elsewhere (Chapelle et al.. 1995).

Radiochemicals. The potential for chloroethene biodegradation at the Peger Road site was investigated using uniformly labeled [1,2-^{14}C] TCE (5.4 µCi/µmole; Sigma Biochemicals, St. Louis, Missouri), [1,2-^{14}C] *cis*-DCE (4 µCi/µmole; Moravek Biochemicals, Brea, California), and [1,2-^{14}C] VC (1.6 µCi/µmole; Perkin Elmer Life Sciences, Boston, Massachusetts). The radiochemical purity of the [1,2-^{14}C] chloroethene stocks was evaluated in our lab by direct injection radiometric detection gas chromatography (GC/RD) and found to be greater than 98% pure. Authentic $H^{14}CO_3^-$ (Sigma Biochemicals, St. Louis, Missouri) and $^{14}CH_4$ (Perkin Elmer Life Sciences, Boston, Massachusetts) were used as radiolabeled standards for calibration and methods development. All had radiochemical purities > 98%.

Microcosm Studies. All sediment handling and microcosm preparation activities were conducted in a glove box under a nitrogen atmosphere. In general, sediment microcosms were composed of 10 mL serum vials with 10±0.5 g of saturated sediment and an atmosphere of nitrogen. Quadruplicate experimental treatments were prepared for each sediment. Triplicate autoclaved control microcosms and a single sediment free control microcosm were prepared for each sediment treatment and autoclaved twice for 1 h at 15 PSI and 121 °C. All microcosms were preincubated in the dark at 4 °C for five days prior to the addition of ^{14}C-substrates and electron donor amendments.

For the dechlorination rate study, anoxic microcosms were prepared with MW9810 and MWN4 sediments as described above and amended with [1,2-^{14}C] TCE, [1,2-^{14}C] DCE or [1,2-^{14}C] VC to yield initial dissolved substrate concentrations of 2, 2 and 0.6 mg/L TCE, *cis*-DCE and VC, respectively. Microcosms were incubated in the dark at 4 °C for 200 days.

For the electron donor study, anoxic microcosms were prepared with MW9820, MW9810 and MWN4 sediments as described above and amended with [1,2-^{14}C] TCE to yield an initial dissolved TCE concentration of approximately 500 µg/L. The effect of three electron donor treatments consisting of 3:1 by volume aqueous emulsions of molasses, ground shrimp and crab chitin, or "Hydrogen Release Compound," (HRC) and a distilled water control on ^{14}C-TCE reductive dechlorination was assessed in microcosms incubated in the dark at 4 °C for 180 days.

Analytical Methods. Headspace concentrations of CH_4, $^{14}CH_4$, CO_2, $^{14}CO_2$, ethene, ^{14}C-ethene, ethane, ^{14}C-ethane, VC, and ^{14}C-VC were monitored by analyzing 0.5 mL of headspace using packed column gas chromatography with radiometric detection and thermal conductivity detection. Headspace concentrations of ^{14}C-TCE, ^{14}C-DCE and ^{14}C-VC were monitored by analyzing 0.5 mL of headspace by capillary gas chromatography with radiometric detection. The headspace sample volumes were replaced with nitrogen. Dissolved phase concentrations of ^{14}C-analytes were estimated based on experimentally determined Henry's partition coefficients. Because inorganic carbon is present as dissolved CO_2 and HCO_3^- at the pH values observed in the collected sediments (pH = 6.3-6.8), a dimension-less partition coefficient for the distribution of inorganic carbon between the headspace and the dissolved phase was determined by injecting $H^{14}CO_3^-$ into the dissolved phase of triplicate autoclaved sediment microcosms (prepared as described for the degradation study), allowing the microcosms to equilibrate for 24 h, and measuring the $^{14}CO_2$ radioactivity in the headspace using radiometric detection gas chromatography. The radiometric detector was calibrated by liquid scintillation counting using $H^{14}CO_3^-$.

Dissolved concentrations of NO_3 and SO_4 were determined by ion chromatography. Concentrations of dissolved Mn(II) and Fe(II) were determined colorimetrically using commercially available test kits (Hach Co., Loveland, Colorado). Estimates of bioavailable, reducible Mn and Fe were made by chemical reduction and extraction with 0.5 M hydroxylamine solution in 0.25 M HCl followed by colorimetric analysis of resultant Mn(II) and Fe(II) using commercially available test kits (Hach Co., Loveland, Colorado).

RESULTS AND DISCUSSION

Implications of *In Situ* Redox Conditions. The results of the September 2003 field redox assessment conducted by the USGS are presented in Appendix A. Based on these data, a number of conclusions can be made regarding the potential for efficient chloroethene biodegradation in the chloroethene contaminant plume at the Peger Road site.

- **(MW206)** The background conditions at the site appeared to be oxidized as indicated by dissolved O_2 concentrations of 2 mg/L in this background well. Methane was omnipresent at the site and appeared to be independent of contamination. Background methane is not uncommon in permafrost areas.
- **(MW9810 and MW9817)** MW 9810 and MW 9817 exhibited background level oxygen concentrations. Possible explanations for the presence of oxygen in the otherwise anoxic plume beneath an asphalt cap included the following. 1) Oxygenated groundwater was transported from the grassy area immediately to the

south, near the Main Office building. This hypothesis was undermined by the apparent lack of oxygen in the immediately up-gradient well MW 9816. 2) MW 9810 and MW 9817 were screened across the water table. 3) These flush-mounted wells were contaminated with oxygenated surface water during rainfall or snowfall events.

- **(TCE Plume)** With the exception of MW 9810 and MW 9817, the TCE plume was anoxic. Water chemistry data indicated that the anoxic plume was characterized by Mn/Fe-reduction. The coincidence of significant dissolved $SO_4^{=}$ concentrations and H_2 concentrations in the 1-3 nM range indicated that, in addition to Mn/Fe-reduction, some potential for sulfate reduction existed at N4B, MW202A and MW304A. In contrast, the remainder of the sampled TCE plume wells exhibited H_2 concentrations consistent with NO_3^--reducing and Mn/Fe-reducing activity. However, the lack of NO_3^- at concentrations deemed necessary to support respiratory activity and growth (approximately 20 micromolar or 1.3 mg/L NO3) suggested that NO_3^--reduction was not generally important within the anoxic TCE plume. Detectable concentrations of Mn(II) were present throughout the site, but remained low and did not trend along the groundwater flowpath. In contrast, dissolved Fe(II) concentrations generally increased along the groundwater flowpath. This observation combined with the H_2 data suggested that Fe(III)-reducing conditions predominated within the anoxic plume at Peger Road. Note: methane was omnipresent at the site and appeared to be clearly independent of contamination. The H_2 data and the lack of increasing methane concentrations along the flowpath were inconsistent with active methanogenesis at Peger Road. Background methane is not uncommon in permafrost areas.
- Reductive dechlorination of TCE can be significant under Fe(III)-reducing conditions (see Bradley 2003, Appendix B). However, H_2 competition between Fe(III)-reducers and chlororespirers is expected to be extensive under Fe(III)-reducing conditions. Thus, under the borderline oxic/metals-reducing conditions that appear to predominate at the Peger Road site, reductive dechlorination of TCE is likely to be limited by microbial H_2/electron donor competition. This conclusion indicates that increased reductive dechlorination of TCE may be achievable with electron donor amendment.
- The metals-reducing conditions present in the anoxic TCE plume at the Peger Road site suggest the potential for significant anaerobic oxidation of VC and DCE.

Dechlorination Rate Study. When practicable, kinetic descriptors of chloroethene biodegradation are best derived from field data, as these data reflect microbial activity under *in situ* conditions (Chapelle and Bradley 1998). This approach is particularly appropriate when estimating the *in situ* biodegradation rate of the parent compound, because the apparent, non-conservative rate of contaminant decay between consecutive wells is a straight-forward estimate of *in situ* biodegradation. In contrast, estimation of the *in situ* biodegradation rate of daughter products is often problematic because the observed concentrations of daughter compounds reflects a balance between *in situ* production and *in situ* degradation. Likewise, such an approach

is problematic if daughter product accumulation is not apparent and concern exists that *in situ* chloroethene biodegradation processes may yield innocuous, non-diagnostic products such as CO_2. When *in situ* estimates prove problematic, laboratory rate estimates can be an useful alternative for modeling the fate and transport of chloroethene reduction products along the groundwater flowpath.

In general, biological processes follow saturation kinetics (Michaelis-Menten), in which first-order, concentration dependent kinetics are observed at substrate concentrations below saturation and zero-order, concentration-independent kinetics are observed at substrate concentrations above saturation. In lieu of a detailed, resource intensive kinetic investigation, rates of biodegradation at low mg/L substrate concentrations are often assumed to follow first-order kinetics. As a minimum, however, rate studies should be conducted at substrate concentrations that are relevant to *in situ* conditions and the results tested for conformity to a first-order kinetic expression. In this study, headspace concentration changes were monitored over time and the rate of change of substrate concentrations was determined to conform to first-order degradation kinetics at the substrate concentrations evaluated in this study (2 mg/L TCE, 2 mg/L *cis*-DCE, and 0.6 mg/L VC). Caution should be taken in applying these first-order degradation rate estimates to substrate concentrations above those tested in this investigation.

Microbial metals-reduction predominated in MW9810 and MWN4 sediment microcosms incubated at 4 °C under anoxic conditions. The lack of significant dissolved O_2 ($[O_2] < 2$ µM) and NO_3 ($[NO_3^-] < 1.0$ µM), the lack of significant change in dissolved $SO_4^=$ concentrations, the lack of significant production of dissolved sulfide (not detected, $[HS^-] < 0.2$ µM) or CH_4 (not detected, $[CH_4] < 1$ µmole/liter headspace), and the significant accumulation of dissolved Mn(II) (56±22 and 11±7 nmoles, respectively) and dissolved Fe(II) (87±33 and 11±3 nmoles, respectively) indicated that Mn/Fe-reducing conditions predominated in MW9810 and MWN4 sediment microcosms under the rate study conditions.

Minimal biodegradation of ^{14}C-TCE was observed in MW9810 and MWN4 sediment microcosms under the dechlorination rate study conditions (Table 1). The mean loss of [1,2-^{14}C] TCE was less than 3 %. The fact that total daughter product production, primarily $^{14}CO_2$, was less than 7% in both sediment treatments confirmed that the rate of [1,2-^{14}C] TCE biodegradation was slow (generally 0.0001 d^{-1} or less; Table 2). The [1,2-^{14}C] TCE degradation observed in this study was attributable to biological activity, because no significant ^{14}C-TCE loss or ^{14}C-product accumulation was observed in autoclaved control or sediment-free control microcosms. The apparent formation of trace amounts of ^{14}C-*cis*-DCE and ^{14}C-VC in MW9810 microcosms is consistent with field observations of low (circa 5 µg/L) *cis*-DCE concentrations in some source area wells (Shannon and Wilson 2003). The predominance of $^{14}CO_2$ as a product of [1,2-^{14}C] TCE biodegradation suggests that ^{14}C-*cis*-DCE and ^{14}C-VC are oxidized to $^{14}CO_2$ under Mn/Fe-reducing conditions. The low rate of [1,2-^{14}C] TCE biodegradation observed in this study is consistent with the slow attenuation of TCE observed in the TCE plume at the Peger Road site (Shannon and Wilson, Inc. 2003). These results suggest that TCE biodegradation may contribute

TABLE 1. Final percentage distribution of ^{14}C-radiolabel in Peger Road sediment microcosms incubated in the dark at 4 °C for 200 days. Initial substrate concentrations were 2, 2 and 0.6 mg/L for ^{14}C-TCE, ^{14}C-DCE and ^{14}C-VC, respectively. Data are means±SD for triplicate experimental microcosms and duplicate autoclaved control microcosms. Only ^{14}C associated with the initial substrate was observed in control microcosms.

Sediment	Substrate	Experimental					Control
		^{14}C-TCE %	^{14}C-DCE %	^{14}C-VC %	$^{14}CO_2$ %	^{14}C-Total %	^{14}C-Total %
MW9810	**^{14}C-TCE**	98±3	1±1	1±2	4±2	104±2	100±2
	^{14}C-DCE	-----	76±9	**ND**[a]	26±6	102±4	98±1
	^{14}C-VC	-----	-----	28±18	70±10	98±8	103±1
MWN4	**^{14}C-TCE**	99±8	**ND**	**ND**	3±2	102±6	97±12
	^{14}C-DCE	-----	73±6	**ND**	31±4	104±4	95±1
	^{14}C-VC	-----	-----	81±7	21±5	102±6	97±4

[a] Not Detected. MDL = 2% and 1% for ^{14}C-DCE and ^{14}C-VC, respectively.

to the slow decline in dissolved TCE concentrations that has been reported in the source area and middle portions of the TCE plume at the Peger Road site (Shannon and Wilson 2003). Consistent with the [1,2-^{14}C] TCE treatment results, significant biodegradation of [1,2-^{14}C] *cis*-DCE and [1,2-^{14}C] VC was observed in MW9810 and MWN4 sediment microcosms under the

TABLE 2. First-order rate estimates for chloroethene dechlorination in Peger Road sediment microcosms incubated in the dark at 4 °C for 200 days. Reported rates were estimated based on either substrate loss (SL) or daughter product production (DPP). Initial substrate concentrations were 2, 2 and 0.6 mg/L for ^{14}C-TCE, ^{14}C-DCE and ^{14}C-VC, respectively. The products of ^{14}C-TCE dechlorination were ^{14}C-DCE and ^{14}C-VC. The product of ^{14}C-DCE and ^{14}C-VC dechlorination was $^{14}CO_2$. Data are means±SD for triplicate experimental microcosms. No significant degradation was observed in autoclaved control microcosms.

Sediment	Method	Dechlorination Rate (d^{-1})		
		^{14}C-TCE	^{14}C-DCE	^{14}C-VC
MW9810	**SL**	0.00010±0.00015[a]	0.0013±0.0004	0.0037±0.0009
	DPP	0.00015±0.00006	0.0013±0.0003	0.0036±0.0005
MWN4	**SL**	0.00005±0.00040[a]	0.0014±0.0003	0.0010±0.0004
	DPP	0.00015±0.00008	0.0016±0.0002	0.0011±0.0003

[a] Not statistically significant. Final percentage recovery of ^{14}C-TCE shown in Table 1 was not statistically different from 100% ($p < 0.05$; ANOVA).

dechlorination rate study conditions (Table 1). Approximately 25% and 70% removal of [1,2-^{14}C] *cis*-DCE and [1,2-^{14}C] VC, respectively, was observed in MW9810 sediment microcosms. The first-order degradation rate estimates for *cis*-DCE and VC biodegradation in MW9810 sediment microcosms under these study conditions were 0.0013±0.0004 d^{-1} and 0.0037±0.0009 d^{-1}, respectively (Table 2). Approximately 30% and 20% removal of [1,2-^{14}C] *cis*-DCE and [1,2-^{14}C] VC, respectively, was observed in MWN4 sediment microcosms. The first-order degradation rate estimates for *cis*-DCE and VC biodegradation in MWN4 sediment microcosms under these study conditions were 0.0014±0.0003 d^{-1} and 0.0010±0.0004 d^{-1}, respectively (Table 2). In all cases, the sole product of [1,2-^{14}C] *cis*-DCE and [1,2-^{14}C] VC biodegradation was $^{14}CO_2$. The degradation of [1,2-^{14}C] *cis*-DCE and [1,2-^{14}C] VC observed in this study was entirely attributable to biological activity because no significant ^{14}C-substrate loss or ^{14}C-product accumulation was observed in autoclaved control or sediment-free control microcosms. The net anaerobic oxidation of DCE and VC observed in this study is consistent with the apparent predominance of Mn/Fe-reduction in MW9810 and MWN4 sediments and demonstrates that a significant potential for anaerobic oxidation of *cis*-DCE and VC exists in the plume at the Peger Road site.

The results of the dechlorination rate study suggest that:

- The rates of ^{14}C-chloroethene biodegradation observed in Peger Road sediments at 4 °C are within the range of rates observed in more temperate aquifer systems (Weidemeier et al. 1998). The rates of [1,2-^{14}C] TCE biodegradation observed in Peger Road sediment microcosms was at the low end of the range of TCE biodegradation rates reported for temperate aquifer systems (Weidemeier et al. 1999). This result confirms that biodegradation can be a significant mechanism for *in situ* chloroethene contaminant remediation even in cold temperature aquifers.
- The rate study results are consistent with the field redox assessment and indicate that, in the absence of electron donor amendment, Mn/Fe-reduction is the predominant terminal electron accepting process in the Peger Road sediments under anoxic conditions.
- The low rate of [1,2-^{14}C] TCE biodegradation observed in this study under Mn/Fe-reducing conditions is consistent with the slow attenuation of TCE observed in the TCE plume at the Peger Road site. These results suggest that TCE biodegradation may contribute to the slow decline in dissolved TCE concentrations that has been reported in the source area and middle portions of the TCE plume at the Peger Road site.
- The fact that the rates of [1,2-^{14}C] *cis*-DCE and [1,2-^{14}C] VC biodegradation were greater than the [1,2-^{14}C] TCE biodegradation rates and the fact that [1,2-^{14}C] *cis*-DCE and [1,2-^{14}C] VC biodegradation was stoichiometric to $^{14}CO_2$ indicate that significant accumulation of *cis*-DCE and VC is not favored in the TCE plume at the Peger Road site.
- The fact that $^{14}CO_2$ was the sole product of [1,2-^{14}C] *cis*-DCE and [1,2-^{14}C] VC biodegradation detected in either sediment indicates that a natural attenuation assessment based on reduced daughter product accumulation may significantly underestimate the potential for DCE and VC biodegradation at the Peger Road site.

Electron Donor Amendment Study. For convenience, the results of the control treatments (DIW-amended sediment microcosms) are discussed separately to provide a basis for interpretation of the laboratory electron donor amendment results.

The predominant terminal electron accepting processes observed in DIW-amended, Peger Road sediment microcosms were Mn/Fe-reduction (Table 3 and 4). The lack of significant dissolved O_2 ($[O_2] < 2$ µM) and NO_3 ($[NO_3^-] < 1.0$ µM), the lack of significant change in dissolved $SO_4^=$ concentrations, the lack of significant production of dissolved sulfide (not detected, $[HS^-] < 0.2$µM) or CH_4 (not detected, $[CH_4] < 1$ µmole/liter headspace), and the significant

TABLE 3. Production of dissolved Mn(II) (µmoles) in experimental (EXP) and autoclaved control (AC) microcosms containing Peger Road sediment and in sediment free control (SFC) microcosms after 180 days incubation in the dark at 4 °C.[a]

Sediment	Amendment	Dissolved Mn(II) Production (µmoles)			Reducible Mn[b] (µmoles)
		EXP	AC	SFC	
9820	DIW	0.02±0.01	**NS**[c]	**NS**	5.0±0.17
	MOLASSES	1.0±0.17	0.38±0.05	**NS**	
	CRUST	0.06±0.00	0.01±0.00	**NS**	
	HRC	1.1±0.09	**NS**	**NS**	
9810	DIW	0.05±0.02	0.01±0.01	**NS**	34±0.71
	MOLASSES	4.7±0.90	2.4±0.01	**NS**	
	CRUST	0.08±0.00	**NS**	**NS**	
	HRC	5.6±0.30	**NS**	**NS**	
N4	DIW	0.01±0.01	**NS**	**NS**	22±1.4
	MOLASSES	3.7±1.4	**NS**	**NS**	
	CRUST	0.30±0.05	0.02±0.00	**NS**	
	HRC	6.3±0.31	**NS**	**NS**	

[a]Calculated as the difference in dissolved Mn(II) content measured in microcosms sacrificed at the beginning and end of the study. Except where noted, experimental data for each treatment are means±SD for quadruplicate microcosms. Control data are from triplicate autoclaved microcosms (means±SD) and a single sediment free microcosm.
[b]Estimate of bioavailable, reducible Mn (µmoles) present in sediment microcosm prior to incubation as estimated by hydroxylamine/HCl extractions of triplicate sediment samples. Sediment amendments did not contain significant reducible Mn.
[c]NS: Differences between initial and final dissolved Mn(II) contents not significant ($p < 0.05$; Kruskal-Wallis one-way analysis of variance on ranks).

accumulationof dissolved Mn(II) (21±9, 49±20 and 10±8 nmoles, respectively) and dissolved Fe(II) (49±8, 80±30 and 10±3 nmoles, respectively) indicated that Mn/Fe-reducing conditions predominated in MW9820, MW9810 and MWN4 sediment microcosms under DIW-amended, electron donor study conditions.

Consistent with the [1,2-^{14}C] TCE dechlorination rate study results, minimal biodegradation of ^{14}C-TCE was observed in MW9820, MW9810 and MWN4 sediment microcosms under DIW-amended, electron donor study conditions (Table 5). The mean loss of [1,2-^{14}C] TCE was less than 6 % for each sediment. The [1,2-^{14}C] TCE degradation observed in this study was

TABLE 4. Production of dissolved Fe(II) (µmoles) in experimental (EXP) and autoclaved control (AC) microcosms containing Peger Road sediment and in sediment free control (SFC) microcosms after 180 days incubation in the dark at 4 °C.[a]

Sediment	Amendment	Dissolved Fe(II) Production (µmoles)			Reducible Fe[b] (µmoles)
		EXP	AC	SFC	
9820	DIW	0.05±0.01	0.01±0.01	**NS**[c]	140±22
	MOLASSES	18±3.8	**NS**	**NS**	
	CRUST	0.03±0.01	**NS**	**NS**	
	HRC	29±1.2	**NS**	**NS**	
9810	DIW	0.08±0.03	0.02±0.01	**NS**	710±13
	MOLASSES	41±19	**NS**	**NS**	
	CRUST	1.0±0.05	**NS**	**NS**	
	HRC	46±2.4	**NS**	**NS**	
N4	DIW	0.01±0.00	**NS**	**NS**	430±28
	MOLASSES	7.5±2.6	0.2±0.3	**NS**	
	CRUST	0.14±0.01	**NS**	**NS**	
	HRC	3.7±0.26	**NS**	**NS**	

[a]Calculated as the difference in dissolved Fe(II) content measured in microcosms sacrificed at the beginning and end of the study. Except where noted, experimental data for each treatment are means±SD for quadruplicate microcosms. Control data are from triplicate autoclaved microcosms (means±SD) and a single sediment free microcosm.
[b]Estimate of bioavailable, reducible Fe (µmoles) present in sediment microcosms prior to incubation as estimated by hydroxylamine/HCl extractions of triplicate sediment samples. Sediment amendments did not contain significant reducible Fe.
[c]NS: Differences between initial and final dissolved Fe(II) contents not significant ($p < 0.05$; Kruskal-Wallis one-way analysis of variance on ranks).

attributable to biological activity, because no significant ^{14}C-TCE loss or ^{14}C-product accumulation was observed in autoclaved control or sediment-free control microcosms. The apparent formation of trace amounts of ^{14}C-reduction products in MW9820 and MW9810 microcosms was consistent with field observations of low (circa 5 µg/L) *cis*-DCE concentrations in some source area wells (Shannon and Wilson, Inc. 2003). The predominance of $^{14}CO_2$ as a product [1,2-^{14}C] TCE biodegradation suggested that ^{14}C-*cis*-DCE and ^{14}C-VC were oxidized to $^{14}CO_2$ under Mn/Fe-reducing conditions.

All electron-donor amendments examined in this study significantly effected the microcosm redox conditions (Tables 3 and 4), but only CRUST-amendment resulted in the establishment of conditions more reducing than Mn/Fe-reducing. The lack of significant dissolved O_2 ([O_2] < 2 µM) and NO_3 ([NO_3^-] < 1.0 µM), the lack of significant change in dissolved $SO_4^=$ concentrations, the lack of significant production of dissolved sulfide (not detected, [HS^-] < 0.2 µM) or CH_4 (not detected, [CH_4] < 1 µmole/liter headspace), and the significant accumulation of dissolved Mn(II)

TABLE 5. Final percentage distribution of ^{14}C-TCE radiolabel in Peger Road sediment microcosms amended with deionized water (DIW), molasses, crustacean pureé (CRUST), or HRC. Microcosms were incubated in the dark at 4 °C for 180 days. The initial dissolved TCE concentration was approximately 500 µg/L. Data are means±SD for quadruplicate experimental and triplicate autoclaved control microcosms. Only ^{14}C-TCE was observed in control microcosms.

Sediment	Electron Donor	Experimental					Control
		^{14}C-TCE %	^{14}C-DCE %	^{14}C-VC %	$^{14}CO_2$ %	^{14}C-Total %	^{14}C-TCE %
MW9820	**DIW**	96±6	**ND**	2±3	3±4	101±5	104±8
	Molasses	96±9	**ND**[a]	**ND**	**ND**	96±9	110±4
	CRUST	95±13	2±3	3±5	**ND**	100±9	101±4
	HRC	100±13	**ND**	**ND**	**ND**	100±13	99±7
MW9810	**DIW**	95±7	1±2	4±5	6±2	106±6	98±13
	Molasses	99±7	**ND**	**ND**	**ND**	99±7	97±9
	CRUST	44±34	57±35	4±4	**ND**	105±7	94±5
	HRC	97±4	1±2	2±4	**ND**	100±4	100±8
MWN4	**DIW**	100±1	**ND**	**ND**	5±1	105±1	99±8
	Molasses	96±8	**ND**	**ND**	**ND**	96±8	101±8
	CRUST	84±2	8±2	13±9	**ND**	105±5	105±2
	HRC	99±7	**ND**	**ND**	**ND**	99±7	105±5

[a] Not Detected. MDL = 2% and 1% for ^{14}C-DCE and ^{14}C-VC, respectively.

and dissolved Fe(II) indicated that Mn/Fe-reducing conditions predominated in all sediment microcosms under molasses-amended or HRC-amended conditions. Amendment with molasses or HRC resulted in at least a 10^2 increase in the apparent production of dissolved Mn(II) and dissolved Fe(II) over that observed under DIW-amended, control conditions (Tables 3 and 4). Nevertheless, the final recovery of Mn(II) and Fe(II) in the dissolved phase represented less than 20% of the estimated bioavailable, reducible Mn and Fe present in the Peger Road microcosms. Decreasing concentrations of dissolved $SO_4^=$ (approximately 50% reduction in all cases), significant accumulation of dissolved sulfide (greater than 10 µmole/L increase in all cases), CH_4 (9200±2800, 5300±3400 and 68±25 nmoles, respectively), dissolved Mn(II) (60±4, 82±4 and 300±52 nmoles, respectively) and dissolved Fe(II) (30±9, 1000±53 and 140±12 nmoles, respectively) and the lack of significant dissolved O_2 ($[O_2] < 2$ µM) and NO_3 ($[NO_3^-] < 1.0$ µM) indicated that mixed Mn/Fe-reducing, $SO_4^=$-reducing and methanogenic conditions existed in MW9820, MW9810 and MWN4 sediment microcosms under CRUST-amended conditions.

No significant [1,2-^{14}C] TCE loss or ^{14}C-degradation product accumulation was observed in molasses-amended or HRC-amended microcosms (Tables 5 and 6). This result indicated that molasses- and HRC-amendments were ineffective in promoting TCE biodegradation in the Peger Road sediments. On the contrary, Molasses- and HRC-amendment effectively eliminated the low levels of [1,2-^{14}C] TCE biodegradation and $^{14}CO_2$ accumulation observed in Peger Road sediment microcosms under DIW-amended control conditions. This response illustrated a potential risk of electron-donor-amendment-based biostimulation efforts: the potential sacrifice of non-reductive biodegradation pathways. This result also indicated that, electron donor supply is not the only factor limiting [1,2-^{14}C] TCE biodegradation in Peger Road plume sediments. The substantial increase in dissolved Mn(II) and Fe(II) concentrations in all three sediment treatments in response to molasses and HRC amendment indicated that microbial Mn/Fe-reductions were limited by the electron donor supply. Thus, the apparent inhibition of [1,2-^{14}C] TCE reductive dechlorination in response to molasses and HRC amendment in this study indicated a stimulation of the activity of

TABLE 6. Final percentage accumulation of ^{14}C-TCE biodegradation products in microcosms containing aquifer sediments from the Peger Road site. Microcosms were incubated 180 days in the dark at 4 °C. Data are means±SD for quadruplicate experimental microcosms. Effect of electron donor amendment (molasses, crustacean pureé (CRUST) or Hydrogen Release Compound (HRC)) is shown for each sediment. As seen in Table 2, no significant loss of ^{14}C-TCE was observed in autoclaved control microcosms.

SITE	% ACCUMULATION OF ^{14}C-TCE DEGRADATION PRODUCTS			
	DIW	MOLASSES	CRUST	HRC
MW9820	5±4	**NS[a]**	4±9[b]	**NS**
MW9810	11±5	**NS**	61±35	**NS**
MWN4	5±1	**NS**	21±7	**NS**

[a] Not Significantly different from zero ($p < 0.05$; ANOVA).
[b]Degradation products observed in only one of four microcosms.

competitor microbial populations (ex. Mn/Fe-reducers) to the detriment of TCE dechlorinating microorganisms.

In contrast to the molasses- and HRC-amendment results, CRUST-amendment did enhance [1,2-^{14}C] TCE biodegradation in two of the three sediment treatments examined in this study (Tables 5 and 6). For MW9820 sediment microcosms, the extent of [1,2-^{14}C] TCE biodegradation observed under CRUST-amended conditions did not differ significantly from that observed in the DIW-amended control treatment, despite the existence of substantial Mn/Fe-reduction, $SO_4^=$-reduction and methanogenesis in the CRUST-amended treatment. CRUST-amendment did result in a substantial increase in the final percentage recovery of [1,2-^{14}C] TCE degradation products from 11±5% and 5±1% to 61±35% and 21±7% in MW9810 and MWN4 microcosms, respectively. The [1,2-^{14}C] TCE degradation observed in Crust-amended sediment microcosms was attributable to biological activity, because no significant ^{14}C-TCE loss or ^{14}C-product accumulation was observed in autoclaved control or sediment-free control microcosms. CRUST-amendment, while generally stimulating [1,2-^{14}C] TCE biodegradation, also completely inhibited mineralization to $^{14}CO_2$ in all three sediment treatments.

The results of the electron donor amendment study suggest that:

- Electron donor addition appears to be an effective means of stimulation TCE biodegradation in the TCE plume at the Peger Road site.
- However, the fact that electron donor amendment effectively inhibited mineralization of ^{14}C-chloroethenes to $^{14}CO_2$ suggests that electron donor-based biostimulation may lead to increased persistence of DCE and VC at the site. This possibility indicates that restriction of electron donor addition activities to the source areas is appropriate to avoid offsite transport of reduction daughter products such as VC.
- Of the electron donors examined in this study, the ground shrimp and crab chitin was the most effective in stimulating TCE reductive dechlorination in Peger Road sediments.
- Amendment of sediment microcosms with molasses or HRC stimulated microbial Mn/Fe-reduction but did not promote TCE biodegradation in this study. Although an eventual enhancement in TCE biodegradation in response to molasses- or HRC-amendment cannot be ruled out, the presence of high concentrations of bioavailable, reducible Mn (greater than 0.5 µmole/g wet sediment) and Fe (greater than 10 µmoles/g wet sediment) in the Peger Road sediments indicates that any shift toward efficient choroethene reductive dechlorination in response to electron donor amendment is likely to follow an extended lag period (see River Terrace experience with HRC injection).
- Addition of molasses or HRC to Peger Road sediment microcosms appeared to promote microbial Mn/Fe-reducing activity to the detriment of microbial TCE dechlorinating activity.

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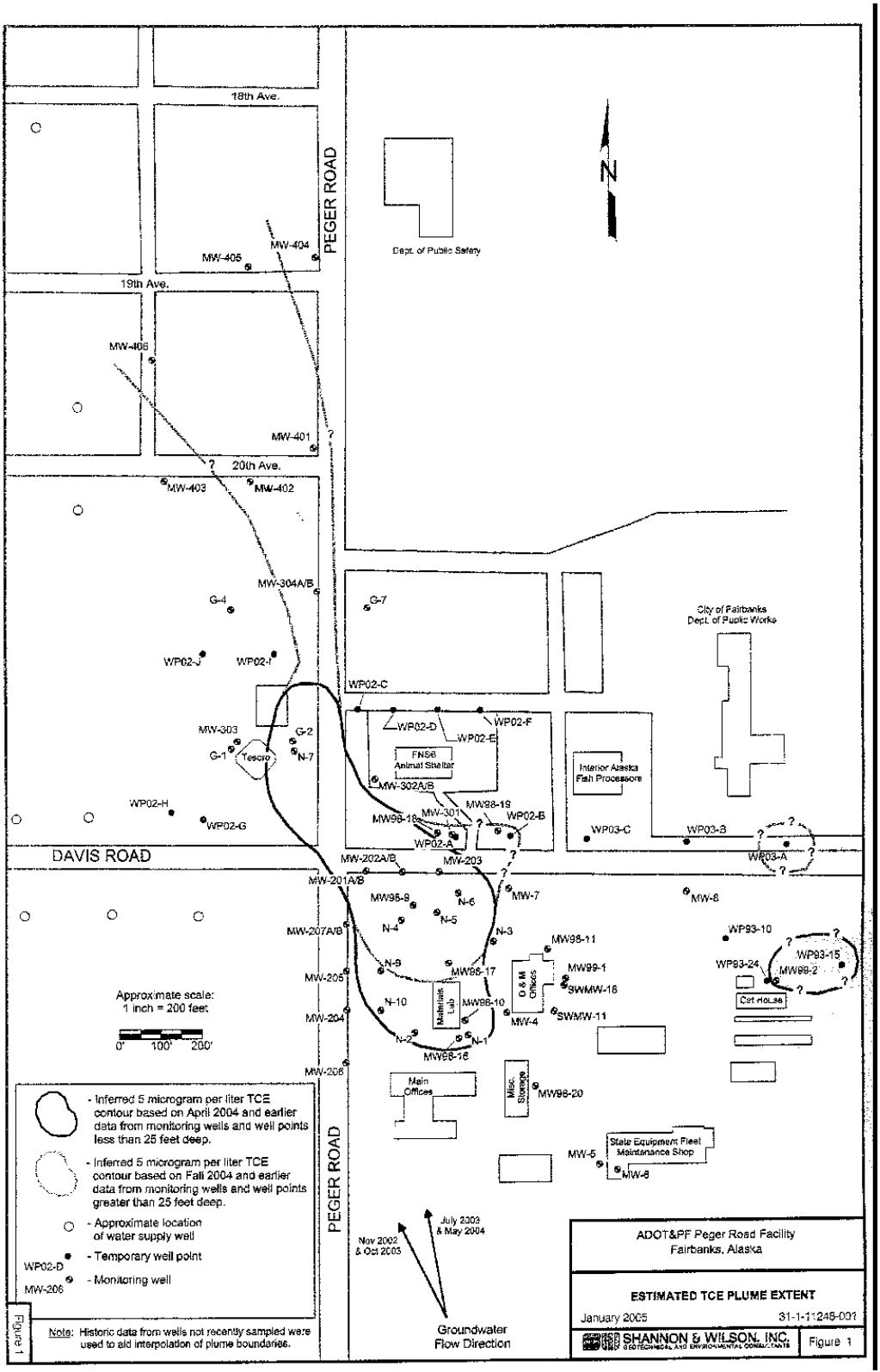
APPENDIX A.


18th Ave.
19th Ave.
20th Ave.
PEGER ROAD
DAVIS ROAD
Dept. of Public Safety
City of Fairbanks Dept. of Public Works
FNSB Animal Shelter
Interior Alaska Fish Processors
Tesoro
Materials Lab
O & M Offices
Main Offices
Misc. Storage
Cat House
State Equipment Fleet Maintenance Shop
MW-401
MW-402
MW-403
MW-404
MW-405
MW-406
MW-201A/B
MW-202A/B
MW-203
MW-204
MW-205
MW-206
MW-207A/B
MW-301
MW-302A/B
MW-303
MW-304A/B
MW-4
MW-5
MW-6
MW-7
MW-8
MW98-8
MW98-10
MW98-11
MW98-16
MW98-17
MW98-18
MW98-19
MW98-20
MW99-1
MW99-2
SWMW-11
SWMW-16
WP02-A
WP02-B
WP02-C
WP02-D
WP02-E
WP02-F
WP02-G
WP02-H
WP02-I
WP02-J
WP03-A
WP03-B
WP03-C
WP93-10
WP93-15
WP93-24
G-1
G-2
G-4
G-7
N-1
N-2
N-3
N-4
N-5
N-6
N-7
N-9
N-10
Approximate scale:
1 inch = 200 feet
0'
100'
200'
- Inferred 5 microgram per liter TCE contour based on April 2004 and earlier data from monitoring wells and well points less than 25 feet deep.
- Inferred 5 microgram per liter TCE contour based on Fall 2004 and earlier data from monitoring wells and well points greater than 25 feet deep.
- Approximate location of water supply well
- Temporary well point
WP02-D
- Monitoring well
MW-206
Nov 2002 & Oct 2003
July 2003 & May 2004
Groundwater Flow Direction
Note: Historic data from wells not recently sampled were used to aid interpolation of plume boundaries.
Figure 1
ADOT&PF Peger Road Facility
Fairbanks, Alaska
ESTIMATED TCE PLUME EXTENT
January 2005
31-1-11248-001
SHANNON & WILSON, INC.
GEOTECHNICAL AND ENVIRONMENTAL CONSULTANTS
Figure 1

APPENDIX B.

REDOX PARAMETERS: ADOT/PF PEGER ROAD MAINTAINENCE FACILITY, FAIRBANKS, ALASKA

SAMPLING EVENT: SEPTEMBER 16, 2003

SAMPLE	[Cl] ppm	[Br] ppm	[NO3] ppm	[PO4] ppm	[SO4] ppm	[DIC] ppm	[CH4] ppm	[DO] ppm	[Mn(II)] ppm	[Fe(II)] ppm	[Fe]tot ppm	[S] ppm	[H2] nM	pH	T C	COND µS/cm	TURB ntu
PR MW9820	10.0	ND	0.7	ND	68.6	1087.2	3.0	0.2	1.7	15	14	0.010	0.26	6.74	4.0	1673	4.5
PR MW206	8.5	ND	4.2	ND	33.6	921.3	3.4	2.0	0.27	1.1	1.5	ND	0.25	6.72	4.9	988	5.8
PR MW9816	8.1	ND	ND	ND	24.7	524.4	3.6	0.2	0.69	0.02	0.03	0.007	0.53	6.76	5.3	651	0.9
PR MW9810	14.5	ND	ND	ND	39.9	687.6	3.7	3.0	0.26	0.02	0.02	0.003	0.65	6.79	6.5	852	8.4
PR MW9817	11.1	ND	0.9	ND	84.7	560.3	3.3	2.0	0.78	3.4	3.3	0.002	0.26	6.79	5.4	796	2.6
PR N4A	13.6	ND	0.3	ND	36.6	537.5	3.1	0.1	0.40	5.5	5.8	0.002	0.73	6.77	3.9	734	4.1
PR N4B	11.0	ND	ND	ND	22.9	384.9	4.2	ND	1.0	12	13	0.004	2.8	6.35	1.4	613	5.0
PR MW202A	14.4	ND	ND	ND	19.7	498.3	3.5	ND	1.2	13	17	ND	1.7	7.13	1.8	673	18.5
PR MW201A	10.7	ND	ND	ND	22.7	498.2	3.1	0.1	1.0	12	13	ND	0.21	7.20	1.5	656	17.8
PR MW302A	109.7	ND	ND	ND	9.1	314.0	3.6	ND	0.62	38	47	0.034	0.27	6.77	1.1	698	18.0
PR MW302AR	108.0	ND	ND	ND	8.5	298.5	4.1	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------
PR MW304A	45.8	ND	ND	ND	26.4	599.5	4.0	0.1	1.2	21	25	ND	1.2	7.08	3.9	820	4.3

Bromide: ND < 0.01 ppm
Nitrate: ND < 0.01 ppm
Phosphate (as orthophosphate): ND < 0.02 ppm
Dissolved Oxygen: ND < 0.05 ppm
Dissolved Sulfide: ND < 0.002 ppm
Temperature in equilibration cell. Not groundwater temperature.
Use caution in interpreting metals and sulfide numbers when turbidity > 20 ntu.
Background wells (note: MW9820 has petroleum?)
Shallow well