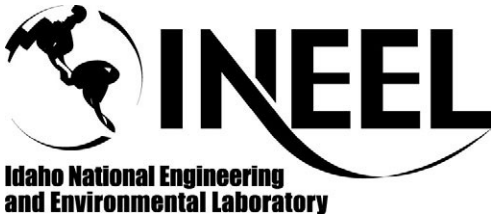

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Mesoscale Tank Experiments for Investigating Carbon Tetrachloride Biodegradation

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ABSTRACT: Mesoscale tank experiments were performed to simulate bioremediation of saturated zone carbon tetrachloride (CCl_4) originating from a vadose zone source. The total volume of the experimental domain was 12 m^3. Vapor-phase CCl_4 partitioned into a water-saturated zone in the lower section of the tank yielding a water concentration of ~600 µg CCl_4/L. Bioremediation was initiated by injecting a CCl_4-degrading microbial population isolated from the Subsurface Disposal Area (SDA) at the Idaho National Laboratory (INL). Stimulation of the microbial population was accomplished by injecting 2.5 L of lactate (1,000 g/L) every 3-5 days; initially, followed by continuous injection during the final month of testing. Analysis of water samples from the saturated zone indicated formation of chloroform after five days of lactate addition. CCl_4 concentrations in the simulated groundwater dropped to near 40 µg/L; while chloroform concentration averaged 44 µg/L. Reduction of CCl_4 past chloroform was not noted during testing. Vadose zone CCl_4 concentrations were above 900 ppmv at the beginning of the experiment and dropped to below 200 ppmv by the end of lactate addition. Results suggest that bioremediation may be a feasible technology for clean up of the CCl_4 plume beneath the SDA at the INL.

INTRODUCTION

The Radioactive Waste Management Complex (RWMC) is on the southwest portion of the INL. Since 1952, transuranic (TRU) and low-level radioactive wastes have been buried in pits, trenches, soil vaults and stored on aboveground pads in the SDA. The waste is also reported to contain other nonradioactive hazardous materials such as mercury, beryllium, asbestos, zirconium fines, solidified acids and bases, solvents and degreasing agents, and sodium and potassium salts. Since burial, a large amount of radioactive and nonradioactive material has migrated to the surficial soils and the vadose zone directly beneath the SDA.

Contaminants of interest to the current study are the volatile organic compounds detected in the SDA-area soil gas. These VOCs include; acetone, benzene, 2-butanone, CCl_4, chloroform, methylene chloride, tetrachloroethylene, toluene, 1,1,1-trichloroethane (1,1,1-TCA) and trichloroethylene (TCE)[1, 2]. The primary contaminants of interest are CCl_4, chloroform and TCE. CCl_4 extends vertically from land surface to the water table and laterally greater than 1 km beyond the SDA boundary. CCl_4concentrations from 3,000 to 5,000 ppmv have been measured in the center of the SDA. Since 1996, 53,000 kg of CCl_4, 13,200 kg of chloroform and TCE, 4,500 kg 1,1,1-TCA and 3,200 kg of PCE have been removed from the subsurface by vacuum vapor extraction units installed as the remedial action for the contamination[3].

Sampling of groundwater beneath and adjacent to the SDA indicates the presence of CCl_4 in the groundwater. Groundwater concentrations have risen steadily since 1995 to

above maximum contaminant levels (MCL) in some areas. Since the source of CCl_4 is still present and vadose zone concentrations of CCl_4 remain high, it is expected that groundwater concentrations would continue to rise. For this reason, technologies need to be developed to treat the CCl_4 after it partitions into the Snake River Aquifer. Reductive dechlorination of the CCl_4 by microbes indigenous to the subsurface beneath the SDA represents one technology that could be developed and deployed to treat groundwater contaminated with CCl_4.

Microbially mediated reductive dechlorination is typically studied in the laboratory using batch or small-scale, continuous-flow, column studies. Typically, these studies are performed in a completely saturated environment where conditions are optimum for reductive dechlorination. While useful for developing kinetics of specific growth conditions, these experiments do not address issues related to larger-scale phenomena. To investigate processes in the vadose and saturated zones simultaneously, highly controlled mesoscale experiments were performed to monitor system dynamics during bioremediation of CCl_4. System dynamics for the purpose of this project included groundwater flow, contaminant transport from the vadose zone, and growth and degradation the CCl_4 by the SDA culture.

MATERIALS AND METHODS

Mesoscale Cell Design. Experiments were performed in a stainless steel mesoscale cell 3-m long by 2-m wide by 2-m high. The cell was packed with sand and clay to yield a sloped, 25-cm thick, fabricated fracture layer between two layers of unconsolidated sediments (sand). The fracture layer was fabricated by inserting 635 stainless steel tubes, with inside diameters ranging from 2 to 28 mm through a clay mixture, consistent with a random fracture distribution with a correlation length of 50 cm. The total thickness of the packed porous media in the mesoscale cell was approximately 1.8 m, which consisted of 16 tons of sand and 1 ton of clay. The cell was designed so that simulated groundwater flowed along the 3-m length in a controlled manner using constant-head reservoirs. Groundwater exiting the cell was purged to remove residual CCl_4 and associated breakdown products prior to re-injecting into the cell.

Experimental Procedure. Vapor-phase CCl_4 was produced from volatilization of 500 ml of neat CCl_4, which was added to a glass beaker that was buried 10 cm below the upper sand surface. Over the course of two months, the vadose zone concentration of CCl_4 stabilized to near 1,000 ppmv, while groundwater concentrations were near 500 μg CCl_4/L.

Once CCl_4 equilibrium had occurred, the reactor was bioaugmented with a CCl_4-degrading culture enriched from the SDA at the INL. The culture was grown to a cell density of ~ 1.0×10^8 cells/ml and 4 L was injected into the center of the tank approximately 10 cm below the water table using a stainless steel tube connected to a sampling port located at the inlet end of the cell. Following injection of the culture, 2.5 L of lactate (1,000 g/L), nitrogen, and phosphorus were added to the reactor. Thereafter, an additional 2.5 L of lactate was injected on days 5, 7, 10, 13, 18, 20, 22, 27, 29 and 32 after the bioaugmentation. On day 37, a continuous supply of lactate (1,000 g/L) was pumped into the reactor at an average rate of 5 mL/min.

Analytical. Concentrations of CCl_4 and associated breakdown products (e.g., chloroform, dichloromethane, chloromethane and methane) in the unsaturated zone were measured using hydrophobic, hollow fiber membrane (HFM) samplers and SilcoSteel tubes designed and fabricated by Dakota Technologies (Fargo, ND). The internal diameter of the HFMs and tubing was 0.53 mm. Using compressed helium, the gas inside the HFM sampler and tubing was flushed into an HP 5890 Series II gas chromatograph (GC) equipped with an electron capture detector (ECD). The sampling system was computer-automated. There were 28 HFM samplers in the upper and lower unconsolidated sediment layers. Two additional HFMs were used for calibration purposes and were placed into glass 1-gallon jars with known masses of CCl_4.

Water samples were taken from 36 sampling ports that were distributed over 4 levels within the saturated zone of the cell. Stainless steel tubes with an inside diameter of 2.2 mm, which were connected to the sampling ports, allowed water samples to be collected from the internal regions of the tank. Samples were also taken from the inlet and outlet reservoirs to determine the amount of CCl_4 entering and exiting the cell. Samples were taken using a syringe and placed in headspace vials for analysis after 10 ml of water was withdrawn from the sampling tubes. Analysis for chlorinated methanes was accomplished using an HP 5890 Series II GC equipped with a flame ionization detector (FID). Headspace samples were taken using a solid phase microextraction (SPME) technique. Chlorinated methane concentrations were determined using standards prepared in headspace vials containing similar volumes to the groundwater samples from the cell.

Samples were also taken for analysis of volatile organic acids (VOA) and cell density. VOA analysis was performed using high-pressure liquid chromatography (HPLC). Samples were prepared by acidification with 4 N H_2SO_4 followed by filtration to remove any particulate material. Samples were eluted using 0.01 N H_2SO_4 at a flow rate of 0.3 ml/min. The volatile fatty acids were separated using a Brownlee, Polypore H, 10 μ, 220 mm × 4.6 mm column. Cell densities were determined using acridine orange direct counts of liquid samples that had been taken from the reactor. Samples were serially diluted as necessary, stained with acridine orange and filtered onto black 0.2 μm polycarbonate filters. The cells were counted using a Nikon Eclipse E600 microscope.

RESULTS AND DISCUSSION

A mesoscale experiment was conducted to investigate the fate of vapor and water-phase CCl_4 during bioaugmentation and stimulation with lactate as the carbon and energy source. The vapor phase represented the contaminant source for the simulated groundwater during the experiment.

A 4-L aliquot of a CCl_4-degrading culture enriched from the SDA at the INL was added to the saturated zone of the mesoscale cell after the tank had remained at equilibrium conditions for approximately 1.5 months. During this time, the CCl_4 concentration remained between 1,000 and 1,200 ppmv. Concentrations of vadose zone CCl_4 are presented as upper and lower unconsolidated zone fractions. Analytical results from 10 HFM were averaged to generate the values for the upper unconsolidated zone, while results from 17 HFM were averaged for the lower unconsolidated zone (Figure 1). No CCl_4 measurements were taken in the fracture layer. The results show that CCl_4 concentrations first began to decrease in the upper zone, yielding higher CCl_4 concentrations in the lower zone than in the upper zone. CCl_4 concentrations began decreasing in the upper

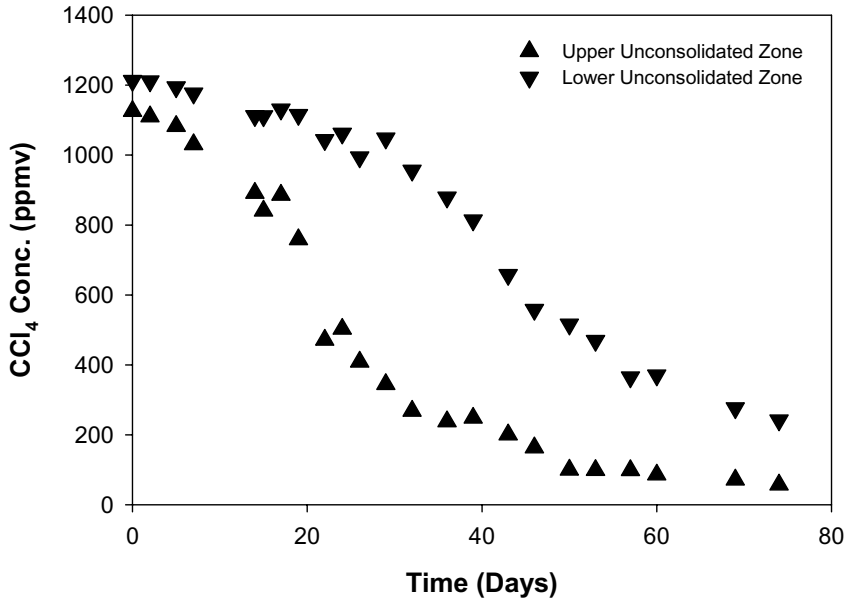

FIGURE 1. Vapor phase CCl_4 concentrations during bioaugmentation and lactate amendment of saturated zone in mesoscale cell. Data points represent average concentrations for all HFM samplers in either the upper or lower unconsolidated zones.

unconsolidated zone between days 7 and 14. The largest decrease in CCl_4 concentrations in the upper unconsolidated zone occurred between days 17 and 36. By the end of the experiment CCl_4 concentrations in the upper sediments were below 100 ppmv.

CCl_4 levels in the lower unconsolidated zone began to decrease approximately 29 days after the culture was added and lactate amendment began, and dropped to near 200 ppmv by the conclusion of the experiment. Interestingly, no chloroform was detected in the unsaturated regions of either unconsolidated zone.

Results for transformation of CCl_4 to chloroform can be seen in Figure 2. Results shown represent sampling ports from the center of the cell where Figure 2A represent samples near the top (closest to vadose zone) of the groundwater column and Figure 2D represents samples deeper in the groundwater column furthest from the vadose zone. While chlorinated volatile organic carbon concentrations (CVOC) were quite variable, all graphs in Figure 2 show that the initial CCl_4 concentration was near 600 µg/L. CCl_4 concentrations in the groundwater decreased steadily following addition of the SDA culture and lactate amendment, with the lowest concentrations occurring near day 30 of the experiment. Chloroform production was first evident after three days of lactate amendment and concentrations increased to near 100 µg/L during the experiment. While no evidence of reductive dechlorination of chloroform was demonstrated, microbial activity toward chloroform was suspected because chloroform levels stabilized rather than continuously increasing with time. CCl_4 concentrations increased in samples taken from all levels of the saturated zone toward the end of the experiment.

CVOC concentrations in the level closest to the vadose zone can be seen in Figure 2A. Results are shown in this figure for a couple of weeks after the lactate amendment was stopped after day 65. While this level of sampling ports was closest to the vadose zone and would have been nearer any source of oxygen; transformation of CCl_4 to

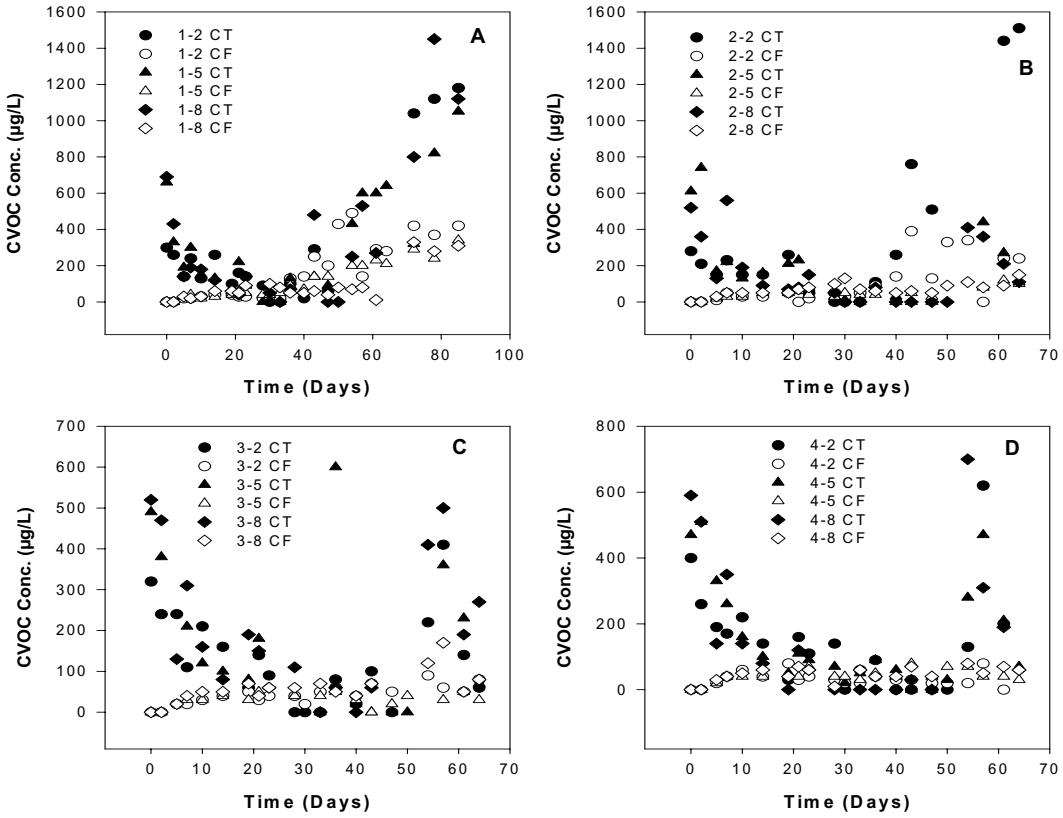

FIGURE 2. Chlorinated volatile organic carbon concentration in the simulated groundwater from the mesoscale cell. (A) level 1 sampling ports; (B) level 2 sampling ports; (C) level 3 sampling ports; and (D) level 4 sampling ports. The number 2 sampling port is near the inlet and represents the lactate injection port. The number 5 sampling port is in the middle of the groundwater zone and number 8 is closer to the outlet of the cell.

chloroform was still demonstrated. Continuous addition of lactate was initiated on day 37 of the experiment. Concentrations of CCl_4 seen at this level began to rebound to values higher than those seen initially prior to batch lactate amendment; between days 50 and 65 CCl_4 concentrations above 2,000 µg/L were seen (data not shown). Chloroform concentration also increased following continuous lactate addition. Increased lactate concentration in the groundwater may have increased the solubility of the CCl_4. Increased chloroform concentrations after lactate injection was stopped may also be an indication that there was microbial activity toward chloroform, but as previously stated no direct evidence of this conversion was demonstrated.

Figure 2B shows CVOC concentrations at the second level of sampling ports (10 cm below the first level), which was also the level at which the lactate was injected into the cell (Port 2-2). As with samples taken from level 1, CCl_4 concentrations decreased steadily following inoculation and lactate amendment to a low point near day 30 of the experiment. Similar increases in CCl_4 concentration were seen after initiation of continuous lactate addition on day 37; however, concentrations demonstrated at this level were not as

high as at level 1. Chloroform concentrations increased near the lactate injection point, but a similar increase was not seen at the midpoint (Port 5) and end of the cell (Port 8).

CVOC concentration profiles were similar at the level 3 sampling ports (10 cm below level 2) near the beginning of the experiment; i.e., CCl_4 concentrations decreased steadily to a low point near day 30 and chloroform concentrations increased (Figure 2C). One of the primary differences between groundwater taken from this level compared to levels 1 and 2 was that the increase in CCl_4 concentration following continuous lactate addition did not occur until approximately day 55. CCl_4 concentrations upon rebound remained below 800 µg/L. With the exception of a couple of sampling points between days 55 and 60 from sampling port 3-8, chloroform levels remained below 100 µg/L.

Results from the level 4 sampling ports (10 cm below level 3) were nearly identical to samples taken from level 3 sampling ports for the CVOCs in the groundwater (Figure 2D). Chloroform concentrations seen at level 4 sampling ports were lower than at the other levels tested regardless of whether lactate was being fed in a semi-continuous or continuous mode.

Figure 3 shows that cell density increased one order of magnitude following injection of lactate. Initial cell densities in the tank were near 1×10^6 cells/ml, which is presumed to be from microbes on the sand. Cell density in the injection (2-2) and middle (2-5) ports was similar over the duration of the experiment. Between days 10 and 35, there was a gradual increase in cell density and then numbers stabilized between 1.5×10^7 and 2.0×10^7 cells/ml. Cell density as measured at the sampling port (2-8) near the outlet of the cell lagged behind cell density for the other two ports until the end of the experiment. Cell numbers in samples taken from sample port 2-2 began to decrease following initiation of continuous flow lactate. It is not known whether the cells were inhibited due to high lactate concentrations or whether the numbers were diluted because extra liquid was

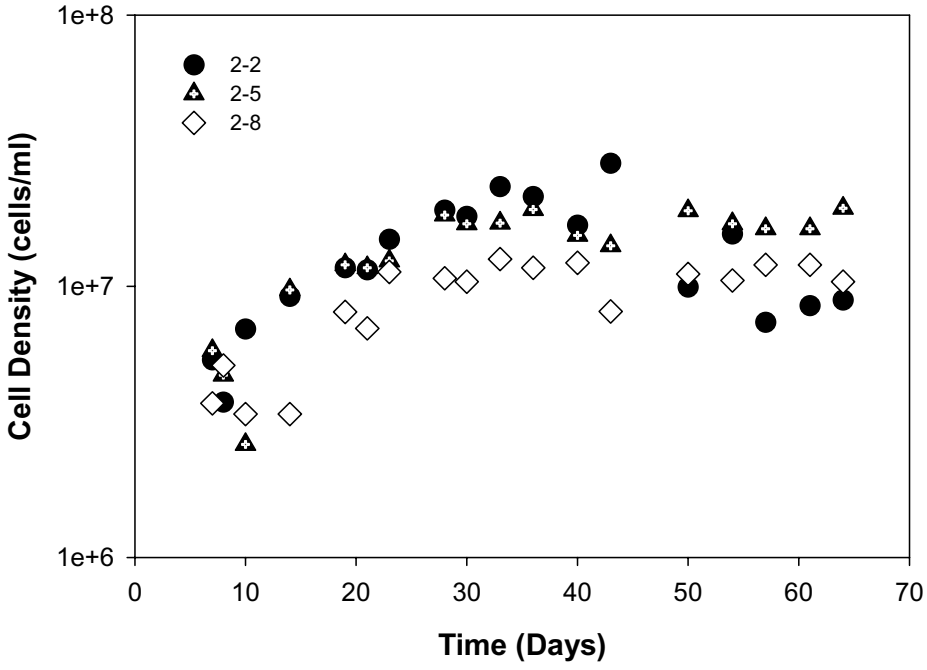

FIGURE 3. Cell density of liquid samples taken from sample ports during bioaugmentation and lactate amendment of mesoscale cell.

being pumped into this port. Due to the design of the experiment, there was no way to determine the cell density of microbes attached to the sand (i.e., sand samples could not be collected during the experiments). Due to the nature of the original culture and production of abundant extracellular material, the amount of attached biomass could be substantial.

Patterns of lactate utilization in the saturated zone of the mesoscale cell can be seen in Figure 4. Only lactate, acetate and propionate were followed during this phase of the research. Levels of all organic acids remained below 200 mg/L for most of the experiment at all three sample ports tested with the exception of days 30, 36, 48 and 51, when the lactate concentration in sample port 2-2 climbed above 10,000 mg/L (data not shown). These results would be expected on days 48 and 51 since continuous injection was occurring, but is surprising on days 30 and 36 because this was prior to continuous injection of lactate. The cause of the elevated lactate on days 30 and 36 is unknown, but coincides when the cell density measured at this port decreased. Lactate levels measured at sample port 2-2 had dropped to near 100 mg/L by the end of the experiment. While lactate concentrations were elevated at sample port 2-2, the concentration measured at ports 2-5 and 2-8 remained near zero. These results would indicate that there might have been inhibition of microbes near port 2-2 due to elevated lactate concentration. During the first 20 days of batch lactate amendment; very low levels of all organic acids measured were seen in all three sampling ports. On approximately day 20, the propionate concentration began to increase, while acetate levels dropped slightly. Propionate and acetate levels remained fairly uniform at all sampling ports for the remainder of the experiment; even after continuous addition of lactate was initiated.

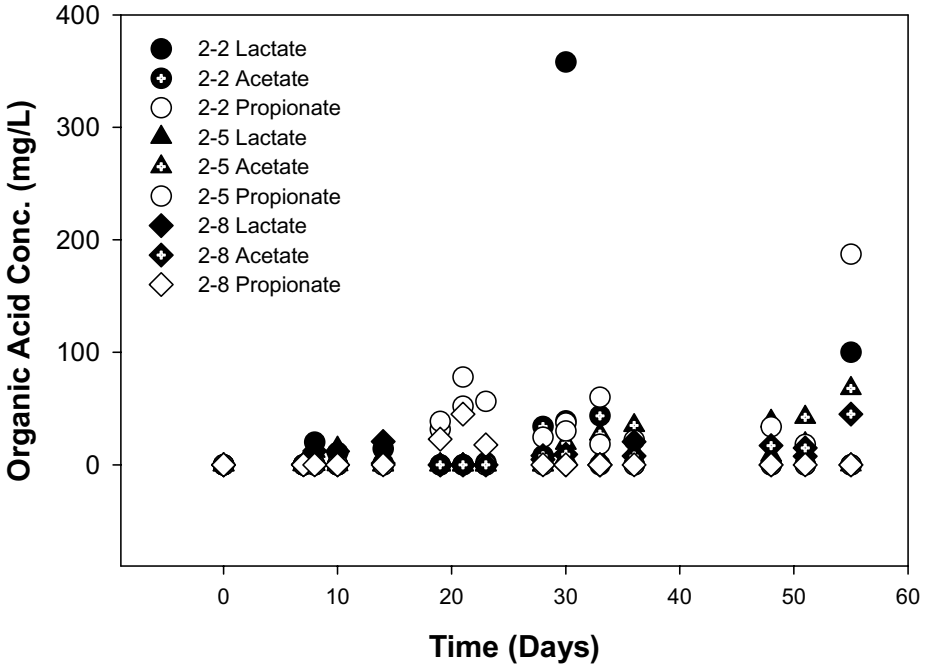

FIGURE 4. Concentration of organic acids in groundwater during bioaugmentation and lactate amendment to stimulate biotransformation of CCl_4.

CONCLUSIONS

Mesoscale experiments indicated that bioaugmentation of groundwater appeared to be a feasible method to remove vapor phase CCl_4 from a simulated vadose zone. From the research results generated, it appeared that removal of vapor phase CCl_4 was driven by the density of the vapor since CCl_4 concentrations in the upper unconsolidated zone began to decrease prior to concentrations in the lower unconsolidated zone. As CCl_4 partitioned into the liquid phase; transformation of CCl_4 to chloroform was evident following initiation of the lactate amendment phase. The microbial population at sample port 2-2 appeared to be adversely affected when lactate concentrations were high during continuous injection. During this time, higher than normal concentrations of CCl_4 were noted compared to earlier measurements. In addition, cell density at this port dropped following continuous addition of lactate; indicating inhibition by the lactate. This is also supported by the fact that cell density remained stable at sample ports 2-5 and 2-8 where the lactate concentration was more dilute. Research results also showed that transformation of CCl_4 in samples taken from the bottom two levels of sampling ports lasted longer than those closer to the vadose zone.

Additional research will be required to optimize the system for complete transformation of the chloroform formed to methane or carbon dioxide. Transformation of the chloroform may have been occurring, but the analytical methods employed were not sufficient for any breakdown products to be detected.

ACKNOWLEDGMENT

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