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Biodegradation of chlorinated solvents in a water unsaturated topsoil

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Abstract

In order to investigate topsoils as potential sinks for chlorinated solvents from the atmosphere, the degradation of trichloromethane ($CHCl_3$), 1,1,1-trichloroethane (CH_3CCl_3), tetrachloromethane (CCl_4), trichloroethene (C_2HCl_3) and tetrachloroethene (C_2Cl_4) was studied in anoxic laboratory experiments designed to simulate denitrifying conditions in water unsaturated topsoil. Active denitrification was demonstrated by measuring the release of ^{15}N in N_2 to the headspace from added ^{15}N labeled nitrate. The degradation of chlorinated aliphatic compounds was followed by measuring their concentrations in the headspace above the soil.

The headspace concentrations of all the chlorinated solvents except CH_3CCl_3 were significantly ($P \leqslant 0.05$) lower after 41 days in biologically active batches as compared to sterile batches. For the compounds with significantly decreasing headspace concentrations, the decline was the least for $CHCl_3$ within the 41 days of incubation. The headspace concentrations of trichloro- and tetrachloroethene decreased more than 50% during the first 20 days with no considerable indication of abiotic transformation. While slow abiotic removal was observed, tetrachloromethane was completely biotransformed after 16 days. Based on the results in this study, we conclude that anaerobic topsoils are potential sinks for these contaminants, and that a natural attenuation potential exists, even in water unsaturated topsoils.

Keywords: Biodegradation; Anaerobic; Denitrification; Volatile organochlorines; Natural attenuation; Agricultural soil; Biotic; Abiotic

1. Introduction

Volatile organic compounds, and in particular the chlorinated aliphatic hydrocarbons (CAH) such as 1,1,1-trichloroethane (CH_3CCl_3), tetrachloromethane (CCl_4), trichloroethene (C_2HCl_3) and tetrachloroethene (C_2Cl_4), are of major environmental concern since these contaminants are often found in the groundwater, the soil and the atmosphere. The degradation of chlorinated aliphatics in the environment is of particular interest, as many of the chlorinated compounds are of public health concern and suspected carcinogens or mutagens,

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potentially toxic to humans and microorganisms (Bouwer and McCarty, 1983b). Furthermore, these compounds were found to contribute to atmospheric photochemical reactions such as stratospheric ozone depletion (Solomon, 1990).

Biodegradation can be an important process affecting the fate of chlorinated aliphatics in aquifers. Degradation of CAH under anaerobic conditions via microbial reductive dechlorination is well documented (Bouwer and McCarty, 1983a; Barrio-Lage et al., 1986; DiStefano et al., 1991) and biodegradation of CCl_4, CH_3CCl_3 and C_2Cl_4 under denitrifying conditions has been described by several authors (Bouwer and McCarty, 1983b; Criddle et al., 1990; Pavlostathis and Zhuang, 1993; Petersen et al., 1994; Sherwood et al., 1998). Most studies have focused on biodegradation of the chlorinated solvents in saturated batches (Bouwer and McCarty, 1983b; Parsons et al., 1984, 1985; Petersen et al., 1994; Gupta et al., 1996; Sherwood et al., 1998). In such studies, water saturated conditions with sufficient substrate added to insure reducing conditions have mostly been employed. Reducing, water saturated conditions are found in the deeper parts of the groundwater zones of many deeper aquifers. However, the conditions in most topsoils are water unsaturated with dominating aerobic conditions although the presence of anaerobic microniches is well known. Partially anaerobic conditions and nitrate reduction have been observed in and close to organic particles of topsoil (Ambus, 1996). These conditions can even occur with a water content in the bulk soil below the water holding capacity of the soils. Also, denitrifying microorganisms are well represented in soils and sediments (Brock et al., 1994).

Knowledge about biodegradation of chlorinated aliphatics in water unsaturated topsoils is limited. No biodegradation rates were reported for water unsaturated soil systems under denitrifying conditions without the supplementary addition of substrate. However, dechlorination of C_2Cl_4 and C_2HCl_3 was observed in a batch system with a ratio of added nutrient solution volume to wet field soil weight of 1:2 yielding close to water unsaturated conditions (Pavlostathis and Zhuang, 1993). A low pseudo-first-order (dechlorination) constant for C_2Cl_4 was found, which they interpreted to be the consequence of depletion of the microcosms of available electron donors (i.e. the primary substrate necessary for the dechlorination to proceed) (Pavlostathis and Zhuang, 1993).

Regardless of the massive number of studies on the biodegradation of individual chlorinated solvents, there is not a lot of information concerning mixtures of these components even though half of all contaminated sites may contain a mixture of pollutants (Westrick et al., 1984). However, Adamson and Parkin (1999, 2000) and Kaseros et al. (2000) have recently focused on this issue. They report that a combination of chlorinated solvents may impact the overall degradation rate due to the production of inhibiting metabolites (i.e. trichloromethane).

The objective of the work presented herein was to study the removal of atmospheric $CHCl_3$, CH_3CCl_3, CCl_4, C_2HCl_3 and C_2Cl_4 in unsaturated topsoil incubated under denitrifying conditions.

2. Materials and methods

2.1. Chemicals

Trichloromethane, 1,1,1-trichloroethane, tetrachloromethane, potassium carbonate and potassium chloride of pro analysis quality (purity > 99.9%) were obtained from Merck KGaA (Darmstadt, Germany). Trichloroethene (purity > 99.9%), tetrachloroethene (purity > 99.9%) and sodium azide (purity > 99.5%) were obtained from Fluka Chemie AG (Buchs, Switzerland). Methanol GC-grade (purity > 99.9%) was supplied by J.T. Baker (Deventer, Holland). ^{15}N-labeled nitrate was bought from Campro Scientific (Veenendaal, Holland). Pure water (18 MΩ cm) from a Milli-Q system (Millipore, USA) was nitrogen purged (1 h) for use in this study.

2.2. Set-up of degradation experiments

Soil samples were obtained at Fårevejle, Denmark, from an agricultural field cultivated according to organic farming practices in Denmark. The choice of an organic farming soil was made to prevent any unwanted interferences from e.g. pesticides. The soil samples were collected from a depth of 10–20 cm in December 1998 and stored in closed stainless steel containers at −18 °C until used in the degradation experiments. As batch systems, 120 ml serum vials (crimp-sealed with polytetrafluoroethene (PTFE)-lined septa) were set-up in duplicates. The 120 ml serum vials were baked at 550 °C and the PTFE-lined septa were washed 1 h in methanol to remove organic contaminants prior to use.

To the batch systems, 20 g of air-dried soil (sieved to pass a 2 mm sieve) was added. All batches were purged with nitrogen for 3 min (flow rate 100 ml min^{-1}) before the addition of 5.7 ml test solution. The test solution contained 400 mg of ^{15}N-labeled nitrate-N per kg dry soil (5.4 atom% ^{15}N) and 10 μg each of $CHCl_3$, CH_3CCl_3, CCl_4, C_2HCl_3 and C_2Cl_4 per kg dry soil in Milli-Q water. The chlorinated compounds were first dissolved in methanol and added to the Milli-Q water prior to use (0.01 μl methanol g^{-1} soil). The soil was thus brought to 60% of its water holding capacity.

Sterilized soil controls were prepared by adding 1% (w/v) of sodium azide to the test solution based on results obtained by Kale and Raghu (1982) and Gillham

and O'Hannesin (1994). Sterilization with sodium azide was chosen as the alternative to steam sterilization (autoclaving), due to the fact, that steam sterilization is known to destroy the soil integrity (Kale and Raghu, 1982). Control batch systems without soil were prepared to investigate the amount of sorption/volatilization of the chlorinated solvents.

Denitrification and methane production were investigated in duplicates with an identical set-up to the living soil batches and the sterile control batches. Before the onset of incubation, all microcosms were shaken for 3 min on a Whirlimixer (Fisons, England). All batch experiments were conducted at 21 °C (±3 °C) and the microcosms were incubated in the dark without further agitation. The incubation temperature of 21 °C (±3 °C) was chosen to represent the upper range of typical summer topsoil temperatures in Denmark and many other parts of the temperate zone at similar latitudes.

2.3. Analytical methods

The water holding capacity was determined by adding water to 100 g of dry soil in a Büchner funnel. After total water saturation, the soil was covered with perforated para-film and drained for 24 h followed by measuring the water content gravimetrically (drying 24 h at 105 °C). The pH of the soil was measured by preparing a water suspension of soil and Milli-Q water (ratio 1:2.5) followed by a measurement of the filtrate with a PHM 92 Lab pH-meter (Radiometer, Denmark A/S). Determinations of dry weight and loss on ignition were done by standard procedures: 105 °C overnight and 550 °C for 2 h, respectively (Danish Standard, 1980). Particle density, bulk density and the porosity of the soil were measured by adding 50 ml ethanol to 10 g of soil in a 100 ml measuring flask. The total volume was read after 5 min of mixing. Total carbon determinations were performed on a LECO Carbon Determinator EC-12 (LECO Corporation, USA).

The oxygen concentration in the soil and in the headspace was measured by penetrating the septum of the 120 ml vial with a glass Clark-electrode from Unisense Denmark (Revsbech, 1989). The microelectrode was connected to a picoammeter (Picoammeter PA 2000, Diamond General, USA). Methane in 0.5 ml headspace samples was quantified by gas chromatography on a Hewlett Packard 6890 gas chromatograph equipped with a packed Porapak T (80/100 mesh) column (1.5 m long; 1/8 in. OD; 2 mm ID, Mikrolab, Denmark) and a flame ionization detector (FID). Total flow was 20.9 ml min^{-1} (FID flow: H_2 40 ml min^{-1} and air 450 ml min^{-1}). The oven temperature was held at 31 °C. Methane calibration curves were done by using a 1.7 ppmV methane standard (approximately the same methane concentration as in atmospheric air). Soil NO_3^-- and NH_4^+-concentrations were measured in 2 M KCl-extracts (ratio 1:10 w:v) on an AutoAnalyzer II system (Bran + Luebbe, Germany) with a spectrophotometric detector. Sulfate concentrations were measured in the same 2 M KCl-extracts by a Dionex ion chromatograph 4000I equipped with an IonPac™ AS12A anion-exchange column (Dionex, USA) and detected by a conductivity detector. The eluent was 2.7 mM Na_2CO_3/0.3 mM $NaHCO_3$ and the flow rate was 1.5 ml min^{-1}.

Denitrification in the vials was followed by analysis of ^{15}N in the headspace N_2 on a Finnigan MAT Delta Plus isotope ratio mass spectrometer (IRMS). The IRMS was coupled in continuous flow mode to an elemental analyzer Carlo Erba EA 1110 equipped with a port for manual injections. The carrier gas was helium (99.9995%) at a flow rate of 90 ml min^{-1}. Headspace samples of 100 μl were injected in the carrier stream using a 1000 μl Hamilton gas tight syringe, which then passed a reduction tube (Cu at 650 °C) to reduce nitrogen oxides, and finally a magnesium perchlorate water trap prior to separation on a Porapak PQS gas column (length 2 m; ID 4 mm, Carlo Erba, Italy). The carrier stream was entering the IRMS *via* ConFlo II interface, which permitted repeated measurements of reference N_2. The intensities at m/z 28, 29 and 30 were measured simultaneously by using a multiple Faraday cup collector system and the atom% ^{15}N (AP) calculated according to $AP = 100 \times {}^{29}R/(2 + {}^{29}R)$, where $^{29}R = {}^{29}N_2/{}^{28}N_2$ (Mulvaney, 1993).

The chlorinated solvents in the batch experiment were analyzed in duplicates using a dynamic headspace technique on a custom made manual sampling system as described previously (Haselmann et al., 2000); see Fig. 1. The 120 ml glass (borosilicate) serum vials were purged for 15 min with helium at a flow rate of 70 ml min^{-1}. At ambient temperature, the helium stream from the vial passed through a u-shaped glass drying tube filled with potassium carbonate to remove water vapor followed by an adsorbent tube made of glass and filled with a non-polar adsorbent material (200 mg HayeSep D, 80/100 mesh, Supelco, USA). After purging, the adsorbent tube was thermally desorbed at 210 °C for 10 min at a helium flow rate of 60 ml min^{-1} and the chlorinated compounds were cryo-trapped (u-shaped steel-tube with an outer diameter of 1/16 in. and filled with small glass beads). The cryo-trap was cooled by liquid nitrogen. The compounds were transferred onto a capillary column (Hewlett Packard HP-624, 75 m long, 0.53 mm internal diameter, 3 μm film thickness) after cryo-trapping by heating the cryo-trap with boiling water. The gas chromatograph was a Hewlett Packard 5890 II equipped with a ^{63}Ni electron capture detector (ECD). An OSS-2 variable outlet splitter (SGE, USA) was installed between the column and the detector to prevent detector overloading by decreasing the ECD inlet concentration approximately 100 times. The column head pressure was

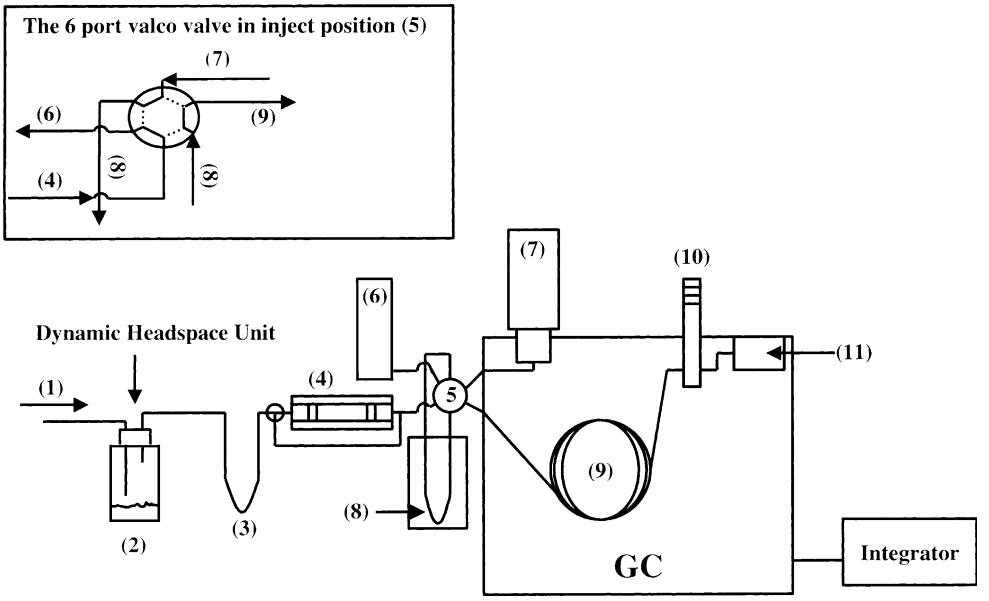

Fig. 1. Scheme of the dynamic headspace system. (1) Helium flow direction, (2) serum vial (microcosm), (3) drying tube, (4) adsorbent tube with thermal desorption unit, (5) 6-port valco valve, (6) flow-meter, (7) split/splitless injector, (8) cryo-trap, (9) column, (10) OSS-2 outlet splitter and (11) the ECD.

80 kPa. Oven temperature program was 40 °C for 2 min, ramp 5 °C min^{-1} to 140 °C, and hold for 1 min, ramp 15 °C min^{-1} to 230 °C, and held for 5 min. The total run-time was 34 min. Quantification of the compounds was achieved by external calibration standards of authentic compounds in methanol and multipoint standard calibration curves.

Analytical parameters (limit of detection (LOD), recovery and purge efficiency) determined for the dynamic headspace system are given in Table 1. The detection limits were calculated as three times the standard deviation on the intercept ($S_{y/x}$) of duplicates of 6 vials filled with 1 μl of methanol standard containing the different compounds of interest (duplicates containing 25, 50 and 100 pg, respectively) into empty 120 ml vials. The recoveries were evaluated by analyzing vials with 100 ng standard dissolved in 1 μl of methanol of each compound investigated. Purge efficiencies were determined by purging a 120 ml vial containing a 50 ng standard dissolved in 1 μl of methanol three consecutive times until blank levels were reached.

3. Results

3.1. Soil properties

The concentration of nitrate, ammonium, sulfate, carbon and soil parameters (water holding capacity, densities, porosity, loss on ignition) and pH were measured in air-dried soil after sampling from the field site (Table 2). Background concentrations of $CHCl_3$ (720 pg vial^{-1}), CH_3CCl_3 (36 pg vial^{-1}), CCl_4 (60 pg vial^{-1}), C_2HCl_3 (16 pg vial^{-1}) and C_2Cl_4 (13 pg vial^{-1}) were measured after 3 h of equilibration in a 120 ml serum vial containing 20 g of air-dried agricultural soil. The analysis showed a higher concentration of $CHCl_3$ compared to the other compounds, probably caused by a high $CHCl_3$ concentration in the air of the laboratory where the soils were air-dried (2–16 times higher compared to the other compounds investigated). All compounds except for $CHCl_3$ were present in the soil at concentrations well below 1% of the amount added in the degradation experiments.

Table 1
Limit of detection (LOD), recoveries (including observed range of measurements) and purge efficiencies of the compounds investigated

	$CHCl_3$	CH_3CCl_3	CCl_4	C_2HCl_3	C_2Cl_4
LOD (nmol), ($n = 6$)	0.05	0.03	0.05	0.03	0.03
Recovery (%), ($n = 2$)	102.8 (102–103)	96.5 (95–98)	113.3 (111–115)	95.4 (94–96)	99.4 (98–100)
Purge-efficiency (%), ($n = 3$)	100.0	100.0	99.6	100.0	100.0

Table 2
Soil characterization of the homogenized air-dried soil from the field site

Water holding capacity	495 g water kg^{-1}
Bulk density	1.0 g cm^{-3}
Particle density	2.2 g cm^{-3}
Porosity	0.6
Nitrate-N	1.7 mg kg^{-1}
Ammonium-N	8.5 mg kg^{-1}
Sulfate	<0.5 mg kg^{-1}
Loss on ignition	164 g kg^{-1}
Total carbon	79 g kg^{-1}
pH	7.9

3.2. Experimental system properties

The sterile control batches without soil addition showed minor losses (sorption/volatilization) of $CHCl_3$ ($22 \pm 4\%$), CH_3CCl_3 ($16 \pm 6\%$), CCl_4 ($4 \pm 7\%$), C_2HCl_3 ($11 \pm 4\%$) and C_2Cl_4 ($21 \pm 7\%$) based on duplicates measured at day 1 and 42. As observed in most spiking experiments, initial increase in headspace concentrations were observed due to the slow equilibration between the aqueous solution (added), soil and headspace.

3.3. Redox conditions in batches

No oxygen (detection limit 0.1 μM) was observed in duplicates of control and live batches after 24 h and 30 days of incubation. Thus, the environment in the batches was demonstrated to be anaerobic. The reduced amount of nitrate-N and the following ^{15}N recovery in headspace throughout the degradation experiment (41 days) for the soil and the control (sterilized soil) are given in Fig. 2. A close relation between the decrease in soil nitrate concentrations and the release of ^{15}N-labeled dinitrogen to the headspace was observed, demonstrating that denitrifying conditions prevailed during the degradation experiment. The low observed deviation within the batch duplicates indicates good biological and physical/chemical reproducibility. There was no consumption of nitrate and no production of ^{15}N-labeled dinitrogen in the sterile control soil, indicating an absence of denitrification and thus complete sterility. No sulfate was detected in the soil indicating that sulfate reducing conditions would not be likely to occur. The minor increase of CH_4 (≈0.004 μmol over 42 days as compared to 502 μmol of nitrate reduced over the same period) in both sterile control batches and the active batches (Fig. 3) demonstrate that any methanogenesis was occurring at less than 0.001% of the nitrate

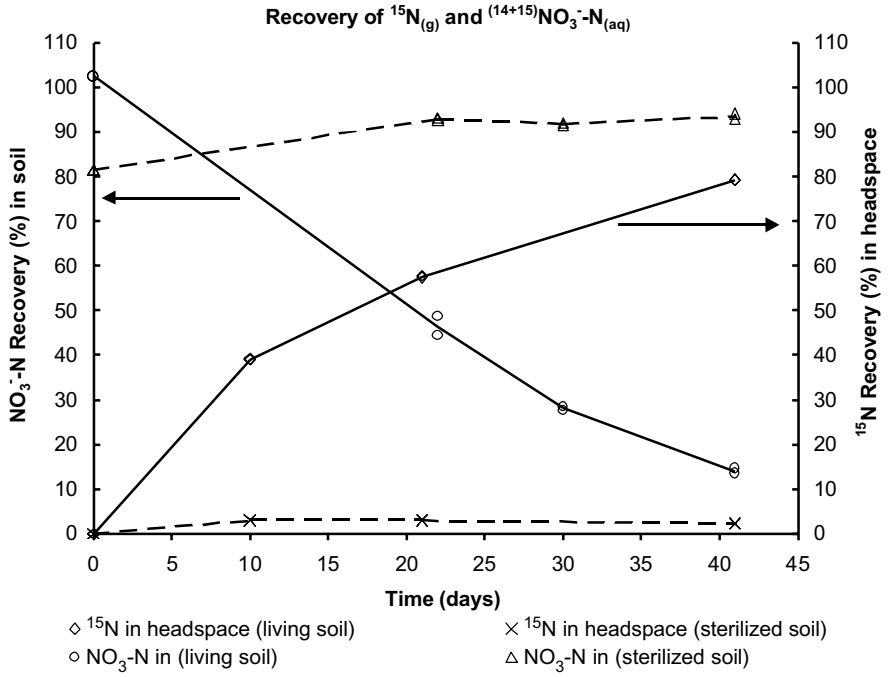

Fig. 2. Recovery (%) of the added nitrate (with and without azide) in soil extracts on the primary y-axis ($n = 2$). Recovery (%) of ^{15}N from the utilized (isotope labeled) nitrate measured by headspace analysis (EA-IRMS) on the secondary y-axis. Range of measurements ($n = 2$) with line through average.

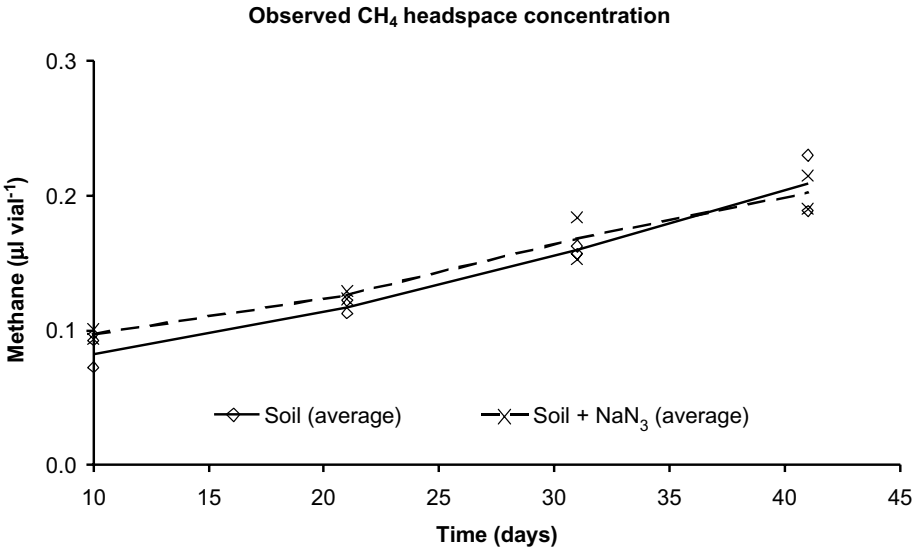

Fig. 3. Methane concentration (µl vial^{-1}) determined by on-column injection of 0.5 ml headspace volume from the microcosms with and without added azide. Range of measurements ($n = 2$) with line through average.

reduction rate. Therefore, neither sulfate reduction, nor methanogenesis was observed to occur in the batches.

3.4. Biodegradation of chlorinated compounds

The results from the degradation experiment in the topsoil are given in Fig. 4(a)–(e). The initial concentrations (C_0) for $CHCl_3$, ($CHCl_3 + NaN_3$), CH_3CCl_3, ($CH_3CCl_3 + NaN_3$), CCl_4, ($CCl_4 + NaN_3$), C_2HCl_3, ($C_2HCl_3 + NaN_3$) and C_2Cl_4, ($C_2Cl_4 + NaN_3$) were 0.96, (1.04), 0.83, (0.94), 1.01, (1.22), 0.72, (0.81) and 0.49, (0.57) nmol, respectively. The standard deviations for all data points were in the range from 0.0001 nmol (C_2HCl_3) to 0.16 nmol ($CHCl_3 + NaN_3$).

A one sided paired t-test of the mean C/C_0-values for the headspace concentrations showed that there was a statistically significant ($P < 0.05$) lower ratio in the biological active batches for all chlorinated solvents (except CH_3CCl_3) as compared to the sterile batches (P values are given in Table 3). The results suggest an apparent biodegradation of CCl_4, C_2HCl_3 and C_2Cl_4 in the water unsaturated agricultural soil under denitrifying conditions. The amount of removal was in the order $CCl_4 \gg C_2HCl_3 \approx C_2Cl_4 \gg CHCl_3 > CH_3CCl_3$, where the removal of CH_3CCl_3 was not statistically significant ($P = 0.06$). The removal of $CHCl_3$ and CH_3CCl_3 was clearly lower than observed for the other compounds.

Tetrachloromethane concentrations decreased in both sterile and active batches. No removal of $CHCl_3$, CH_3CCl_3, and C_2Cl_4 was observed under abiotic conditions. It is uncertain whether or not C_2HCl_3 was degraded to a smaller extent abiotically. Table 3 summarizes the results for the five compounds tested including the time for removal of 50% of the initial headspace concentration (t_{50}).

4. Discussion

No major degradation of $CHCl_3$ was found in the unsaturated soil, which is in agreement with previously conducted research in a saturated batch system under denitrifying conditions (Bouwer and McCarty, 1983b). On the other hand, a production of $CHCl_3$ as a result of reductive dechlorination of CCl_4 was not observed in this study. Recently, research suggested that denitrifying bacteria could degrade CCl_4 by two competitive pathways: the formation of either $CHCl_3$ or CO_2 as the primary end product (Criddle et al., 1990; Klecka et al., 1990). Results reported by Sherwood et al. (1996) showed that the production of $CHCl_3$ dominated when the amount of nitrate was limited, whereas the pathway producing CO_2 and only negligible amounts of $CHCl_3$ dominated when an excess of nitrate or nitrite was available. These results correspond well with the observed constant headspace concentrations of $CHCl_3$ in Fig. 4(a), as nitrate was present in surplus in our batches. However, we have no evidence for mineralization in our batch systems.

Apparently, no biodegradation of CH_3CCl_3 occurred in the unsaturated soil (Table 3 and Fig. 4(b)). This is in agreement with results obtained in experiments conducted by Bouwer and McCarty (1983b), Klecka et al. (1990) and Ahlert and Enzminger (1992) in batch slurries under denitrifying conditions.

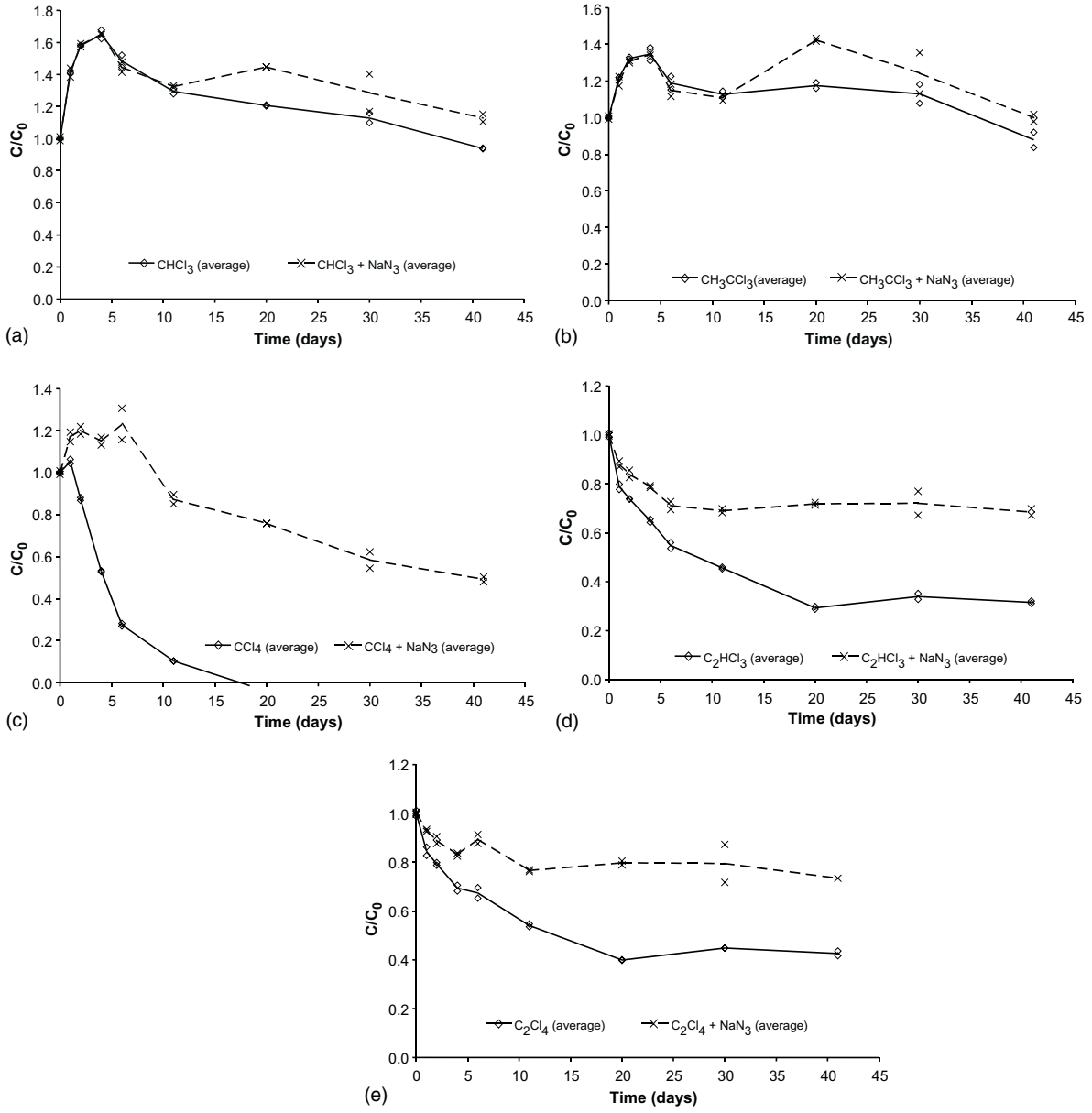

Fig. 4. (a–e) Headspace concentration (C) divided by the headspace concentration at day 0 (C_0) as a function of time. Range of measurements ($n = 2$) with line through average.

Tetrachloromethane was degraded 100% after 16 days in the living soil, whereas approximately 80% of this compound still remained in the sterilized soil after 16 days. This means that CCl_4 was observed to degrade under abiotic conditions but less than under biotic conditions (Fig. 4(c)). Biodegradation of CCl_4 under denitrifying conditions in saturated reactor systems has been reported earlier (Criddle et al., 1990; Lewis and Crawford, 1993; Tatara et al., 1993), and abiotic dechlorination of CCl_4 (and C_2HCl_3) with zero-valent iron (Gillham and O'Hannesin, 1994) and with iron(II) iron(III) hydroxide sulfate (Erbs et al., 1999) were documented and may be the reason for the observed degradation in the sterile soil (Fig. 4(c)).

Trichloro- and tetrachloroethene were degraded more than 50% during the first 20 days of incubation with no indication of substantial abiotic degradation (Table 3, Fig. 4(d) and (e)). Production of *cis*-dichloroethene (*cis*-$C_2H_2Cl_2$) and trace amounts of *trans*-dichloroethene and 1,1-dichloroethene was observed in

Table 3
Time (days) for 50% removal (t_{50}) and P values for paired t-test of the headspace concentration in the biotic samples being significantly lower at the 95% confidence level (except CH_3CCl_3) than in the abiotic controls, and pseudo-first-order removal rate constants (k) observed for CCl_4 including the corresponding correlation coefficient [r^2]

Conditions	$CHCl_3$ t_{50} (P value)[a]	CH_3CCl_3 t_{50} (P value)[a]	CCl_4 t_{50} (P value)[a]	C_2HCl_3 t_{50} (P value)[a]	C_2Cl_4 t_{50} (P value)[a]	CCl_4 k (day^{-1}) [r^2]
Biotic	[b] ($P = 0.01$)	[b] ($P = 0.06$)	4.0 ($P = 10^{-6}$)	8.5 ($P = 10^{-5}$)	13.5 ($P = 10^{-3}$)	0.23 [0.98]
Abiotic	[b]	[b]	41.0	[b]	[b]	0.04 [0.94]

[a] P values are based on an one sided paired t-test of the mean C/C_0-values plotted in Fig. 4(a)–(e).
[b] 50% removal not observed.

the chromatograms as expected for degradation of C_2HCl_3 and C_2Cl_4 due to reductive dechlorination (data not shown). The intermediates were not accumulated in the biotic microcosms, indicating either mineralization or more likely transformation to metabolites that were not measured in our system (e.g. vinyl chloride (C_2H_3Cl)). Adamson and Parkin (2000) observed that degradation under methanogenic conditions of C_2Cl_4 in the presence of CCl_4 produced less C_2HCl_3 and *cis*-$C_2H_2Cl_2$ but more C_2H_3Cl as compared to treatments where CCl_4 was omitted. However, it is uncertain if the presence of CCl_4 suppresses the production of C_2HCl_3 and *cis*-$C_2H_2Cl_2$ under denitrifying conditions. Pavlostathis and Zhuang (1993) reported that reductive dechlorination of C_2HCl_3 and C_2Cl_4 under nitrate reduction conditions was only observed when substrates such as ethanol and acetate were added to the soil. However, they still perceive removal of C_2Cl_4 with a small non-stoichiometric production of C_2HCl_3 in similar soil-batch systems without the addition of substrate. Results obtained in this study are consistent with Pavlostathis and Zhuang (1993). To specify the exact degradation pathway and to quantify all metabolites produced, an extensive single (and mixture) component study with use of ^{14}C-labeled CAH's is necessary and this has not yet been done for water unsaturated soils.

Overall, the observed degradation pattern of chlorinated solvents in this study conducted with a topsoil under water unsaturated conditions agrees with previously reported biotransformation of chlorinated alkanes and alkenes investigated in batch slurries or columns under saturated conditions (Bouwer and McCarty, 1983b; Parsons et al., 1984, 1985; Vogel and McCarty, 1985; Barrio-Lage et al., 1986; Bouwer et al., 1986; Freedman and Gossett, 1989; Bagley and Gossett, 1990; DiStefano et al., 1991; Pavlostathis and Zhuang, 1993; Petersen et al., 1994; Doong et al., 1997; Lee et al., 1997; DeWeerd et al., 1998).

In general, investigations of reductive dechlorination in batch slurries have shown that the most halogenated compound is also degraded fastest (Vogel et al., 1987). That is (to a certain extent) in agreement with this study, where the following order of degradation was observed: $CCl_4 \gg C_2HCl_3 > C_2Cl_4$ ($\gg CHCl_3 > CH_3CCl_3$) based on t_{50} (Table 3). It should be remembered here, that the degradation rate in a soil–water system depends upon both the reactivity and the availability of a compound. A lower bioavailability of C_2Cl_4 due to a higher K_{oc} when compared to C_2HCl_3 may be the reason for the observed slower degradation rate of C_2Cl_4 in unsaturated soil as compared to batch slurries.

The observed degradation of CCl_4 was found to fit a pseudo-first-order degradation pattern (Table 3). However when rate constants for C_2HCl_3 and C_2Cl_4 were calculated over the entire time period only poor regression ($r^2 \approx 0.75$) was obtained. If on the other hand, rate constants for C_2HCl_3 and C_2Cl_4 were calculated for the first 20 days, a first-order fit was obtained under biotic conditions ($k = 0.03$ and 0.02 day^{-1}, respectively and $r^2 = 0.95$ for both compounds). Conversely, the sterilized controls did not follow pseudo-first-order kinetics for these two compounds ($r^2 = 0.54$ and 0.59, respectively). The modeled rate constants correlated well with the observed t_{50} values.

In the biologically active soil, the degradation of C_2HCl_3 and C_2Cl_4 ended after approximately 20 days, whereas CCl_4 was completely removed during this period. If the microorganisms responsible for biotic degradation of C_2HCl_3 and C_2Cl_4 depend upon the presence of readily degradable soil organic matter (SOM) as their primary carbon source, the slowing down of the degradation process after 20 days may be caused by exhaustion of the pool of available SOM. Alternatively, the decrease in the biodegradation rate of C_2HCl_3 and C_2Cl_4 could be caused by a decreasing bioavailability of these contaminants. Gao et al. (1997) investigated the effects of various electron donors on the dechlorination of C_2Cl_4 in anaerobic soil microcosms and found several orders of magnitude difference for the dechlorination rate depending on the electron donors available. This supports that the decline in degradation rates after 20 days in this study could be explained by the disruption

of the normal turn over processes of the SOM. This disruption may have been the result of the continued complete lack of oxygen in our experimental set-up, causing depletion of degradable SOM. Under natural conditions, only parts of the soil would be anaerobic and only in the more humid periods. Therefore, replenishment of degradable SOM would be expected in the field in periods with sufficient oxygen present in the soil due to the periods with aerobic soil respiration.

Previously, it was assumed that only aerobically degradable compounds would be biodegraded in water unsaturated topsoils and that most chlorinated solvents would consequently be naturally degraded only in the reducing parts of paddy fields, aquatic sediments, peat bogs and deep aquifers. The demonstration of a potential for degradation of CCl_4, C_2HCl_3 and C_2Cl_4 in water unsaturated topsoils in our batch experiments challenges this assumption.

The environmental significance is that in the future we will have to consider a potential for natural attenuation of chlorinated solvents in unsaturated topsoils, investigating sites contaminated with these compounds. Furthermore, the inclusion of water unsaturated topsoils as an additional sink for atmospheric contamination with these contaminants might be necessary in accounting for their atmospheric budgets. Still, further studies are required to investigate the processes behind the observed degradation, the prevalence of reducing conditions in topsoils and quantification of kinetics and fluxes of the degradation.

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