

Stable carbon isotope analysis to distinguish biotic and abiotic degradation of 1,1,1-trichloroethane in groundwater sediments

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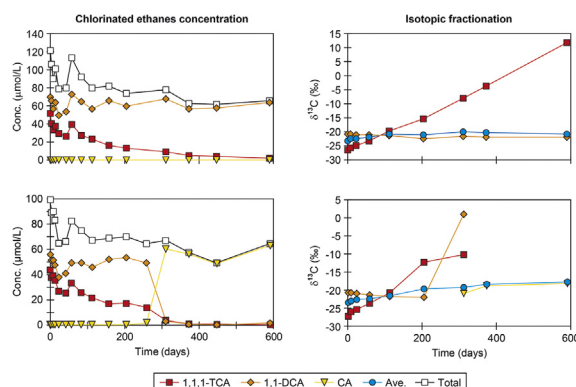
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HIGHLIGHTS

- The ratio of stable carbon isotopes is very sensitive to 1,1,1-TCA degradation.
- Biotic FeS formation mediated abiotic degradation of 1,1,1-TCA.
- CSIA enabled verification of abiotic degradation by a different pathway than RD.
- Field data suggest that abiotic degradation of 1,1,1-TCA is a relevant process also at the field site.

GRAPHICAL ABSTRACT



ABSTRACT

The fate and treatability of 1,1,1-TCA by natural and enhanced reductive dechlorination was studied in laboratory microcosms. The study shows that compound-specific isotope analysis (CSIA) identified an alternative 1,1,1-TCA degradation pathway that cannot be explained by assuming biotic reductive dechlorination. In all biotic microcosms 1,1,1-TCA was degraded with no apparent increase in the biotic degradation product 1,1-DCA. 1,1,1-TCA degradation was documented by a clear enrichment in ¹³C in all biotic microcosms, but not in the abiotic control, which suggests biotic or biotically mediated degradation. Biotic degradation by reductive dechlorination of 1,1-DCA to CA only occurred in bioaugmented microcosms and in donor stimulated microcosms with low initial 1,1,1-TCA or after significant decrease in 1,1,1-TCA concentration (after ~day 200). Hence, the primary degradation pathway for 1,1,1-TCA does not appear to be reductive dechlorination via 1,1-DCA. In the biotic microcosms, the degradation of 1,1,1-TCA occurred under iron and sulfate reducing conditions. Biotic reduction of iron and sulfate likely resulted in formation of FeS, which can abiotically degrade 1,1,1-TCA. Hence, abiotic degradation of 1,1,1-TCA mediated by biotic FeS formation constitute an explanation for the observed 1,1,1-TCA degradation. This is supported by a high 1,1,1-TCA ¹³C enrichment factor consistent with abiotic degradation in biotic microcosms. 1,1-DCA carbon isotope field data suggest that this abiotic degradation of 1,1,1-TCA is a relevant process also at the field site.

Keywords:
Groundwater
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Biodegradation
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1. Introduction

Chlorinated solvents such as 1,1,1-trichloroethane (1,1,1-TCA) are dense non-aqueous phase liquids (DNAPL) and have been widely used as degreasing agents in industry. The complex distribution of DNAPLs in the subsurface make their remediation challenging. Remediation in clayey till and other fractured low permeability media poses a special challenge due to diffusion limitations (Manoli et al., 2012; Hønning et al., 2007; Scheutz et al., 2010). Hence, natural and enhanced degradation of chlorinated ethanes and ethenes is of special interest.

Chlorinated ethanes and ethenes can be degraded by different pathways depending on the environmental conditions. During biotic reductive dechlorination, chlorinated ethanes and ethenes serve as electron acceptors (Holliger et al., 1993; Scheutz et al., 2011). Natural and enhanced reductive dechlorination of chlorinated ethenes has been intensively studied, whereas the literature concerning natural and enhanced 1,1,1-TCA degradation is limited (Scheutz et al., 2011).

Sequential biotic reductive dechlorination of 1,1,1-TCA yields 1,1-dichloroethane (1,1-DCA) and chloroethane (CA). Frequently, 1,1-DCA accumulate as a result of incomplete reductive dechlorination caused by an inadequate redox potential, the lack of available electron donor or absence of specific bacteria able to reductively dechlorinate these compounds (Scheutz et al., 2008). Theoretically, CA may be further reduced to ethane by reductive dechlorination. However, there is no comprehensive experimental evidence for this degradation pathway in the literature (Scheutz et al., 2011). The most likely pathway for CA degradation is abiotic hydrolysis to ethanol or possibly acetate (Scheutz et al., 2011).

1,1,1-TCA may also be degraded abiotically by dehydrochlorination (α -dehaloelimination) to 1,1-DCE, by hydrolysis to acetate (Scheutz et al., 2011) or by the reaction with reduced Fe, Cr or S (Butler and Hayes 2000; Gander et al., 2002; Elsner et al., 2007; Choi et al., 2009). The pathways to 1,1-DCE and acetate run in parallel and result in $\sim 27\%$ of the degradation products as 1,1-DCE (Scheutz et al., 2011). The rate of degradation varies from half-lives in years at 10–25 °C to half-lives in hours at 80 °C+ (Gerken and Franklin, 1989). Abiotic degradation of 1,1,1-TCA by FeS has been investigated in several studies (Butler and Hayes, 2000; Gander et al., 2002; Choi et al., 2009). The initial rate limiting transformation step likely consists of a one electron transfer leading to the cleavage of a C–Cl bond and the formation of a radical (e.g. Elsner and Hofstetter (2011)), which is stabilized by the remaining chlorine atoms. The further transformation can proceed via different routes leading to hydrogenolysis products (1,1-DCA) or α -elimination products (e.g. ethane, ethene, acetaldehyde). In all three studies with FeS reported so far, 1,1-DCA was a minor transformation product (2–4%). Other identified products were 2-butyne (1 study, 4%) and ethene (1 study, 1%). Analysis by Gander et al. (2002) also included but did not detect the following potential degradation products: acetate, 1,1-DCE, acetylene, ethane, acetaldehyde, cis-2-butene, and VC. Gander et al. (2002) suggested ethanol or some volatile sulfur-containing compounds as other possible products not analyzed for. Gander et al. (2002) also studied the degradation of 1,1,1-TCA by a methanogenic consortium alone and combined with FeS and observed synergistic effects. Elsner and Hofstetter (2011) proposed an abiotic pathway for 1,1,1-TCA (via organochloride radical and/or carbanion) with primarily non-chlorinated degradation products (including acetaldehyde and ethane) analogue to the abiotic pathway for carbon tetrachloride (CTET) degradation (to carbon monoxide and formate or carbon disulfide) by pyrite and FeS observed by Kriegman-King and Reinhard (1994) and Devlin and Müller (1999), respectively, and reviewed by Elsner and Hofstetter (2011). In a treatability study for 1,1,1-TCA

bioremediation for 3 clay till sites in Denmark by Scheutz et al. (2013) it was inferred from degradation results and mass balance determinations that an alternate biologically mediated 1,1,1-TCA degradation pathway (without accumulation of 1,1-DCA and CA) contributed to 1,1,1-TCA loss in intrinsic controls.

So far compound-specific isotope analysis (CSIA) has mainly focused on chlorinated ethenes and it was demonstrated that reductive dechlorination is associated with significant carbon isotope fractionation, especially for cDCE and VC transformation (Hunkeler et al., 1999). Carbon isotope analysis has been used to demonstrate reductive dechlorination of chlorinated ethenes in groundwater (Hunkeler et al., 1999, 2011; Sherwood Lollar et al., 2001; Imfeld et al., 2008) and streambed sediments (Abe et al., 2009). Sherwood Lollar et al. (2010) studied the isotope fractionation during biodegradation of 1,1,1-TCA and 1,1-DCA by a dehalobacter-containing mixed culture in whole-cell and cell-free extract microcosms. Isotopic fractionation ($\epsilon = -1.8\text{‰}$ for 1,1,1-TCA, $\epsilon = -10.5\text{‰}$ for 1,1-DCA) was small compared to abiotic reduction by metals (Cr(II), Fe(0), Cu/Fe mix) observed by Elsner et al. (2007) ($\epsilon = -15.8\text{‰}$ to -13.7‰). The large kinetic isotope effect expected for C–Cl bond cleavage was almost completely masked by rate limiting steps preceding bond cleavage during biodegradation (Sherwood Lollar et al., 2010). The ^{13}C enrichment of 1,1,1-TCA observed by Elsner et al. (2007) during abiotic degradation by metals indicated the involvement of a single C–Cl bond (likely by single electron transfer forming a dichloroethyl radical) in the initial rate limiting step. The degradation products included 1,1-DCA as well as ethene and ethane. However, apart from this study, there has been little focus on carbon isotope fractionation for chlorinated ethanes, other than CTET and 1,2-DCA. For CTET reduction by iron minerals, Elsner et al. (2004) and Zwank et al. (2005) observed ^{13}C enrichments indicating single C–Cl bond cleavage in the first step of the reaction. Degradation products included chloroform (CF) as well as carbon monoxide (CO) and formate likely formed via the trichloromethyl radicals, CF by reaction with hydrogen radical donors and CO and formate by an alternative pathway likely to involve surface-bound intermediates (Elsner et al., 2004). Product branching ratios may differ greatly depending on the presence of radical scavengers (Elsner and Hofstetter, 2011). ^{13}C enrichments of CTET varied within about a factor of two for different iron (hydr)oxide and iron sulfide minerals (Zwank et al., 2005). For 1,2-DCA, it was demonstrated that two aerobic biodegradation pathways lead to significantly different carbon isotope fractionation (Hunkeler and Aravena, 2000; Hirschorn et al., 2004). Furthermore, Hunkeler et al. (2005) and Hirschorn et al. (2007) demonstrated the use of stable carbon isotope ratios to differentiate chlorinated ethene (TCE, DCE and VC) production by reductive dechlorination of PCE from production by dehydrochlorination (β -dehaloelimination) of 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane and 1,2-dichloroethane, and to differentiate ethane production by reductive dechlorination of VC from dehydrochlorination of 1,2-DCA. Lojkasek-Lima et al. (2012) used CSIA data to verify that biotic degradation of TCE as well as abiotic degradation by Fe(0) occurred at a Fe(0) reactive wall in a TCE plume. CSIA is a strong tool for detection and documentation of compound degradation particularly when processes or degradation products are unknown or may be present from other sources preventing the use of more traditional methods.

The overall scope of this study was to evaluate the fate and treatability of 1,1,1-TCA in clayey till using the Vadsbyvej site, Denmark as an example. Carbon isotope analysis, in addition to 1,1,1-TCA and daughter product concentrations, were used to investigate contaminant degradation in unamended microcosms, microcosms with additional electron donor and bioaugmented microcosms. This approach made it possible to explore condition dependent

pathways governing 1,1,1-TCA degradation in the environment. This paper focuses on the insight gained from the stable carbon isotope data into degradation of 1,1,1-TCA and 1,1-DCA and possible pathways. The study provides the first laboratory (and field) data for carbon isotope fractionation during degradation of chlorinated ethanes in a clayey till.

2. Materials and methods

2.1. Field site

The Vadsbyvej field site is located in Vadsby west of Copenhagen, Denmark. The site has previously served as a solvent distribution facility, where chlorinated as well as non-chlorinated and water miscible solvents were handled and spilled. The dominant contaminants in the source zone located in a fractured clayey till overlying a thin sand aquifer are chlorinated solvents (1,1,1-TCA, TCE and PCE) and their anaerobic metabolites (1,1-DCA, cDCE, VC, ethene, and CA) but water miscible solvents and hydrocarbons have also been detected. The field site investigation is described and evaluated in the supporting information (SI).

2.2. Microcosm treatability study

The microcosm treatability study for the Vadsbyvej site included 20 microcosms with clayey till and groundwater from a borehole and well situated in the clayey till in the source zone and 14 microcosms with sand and groundwater from a borehole and well situated in the sand aquifer beneath the source zone. It included unamended abiotic controls (autoclaved 3 times), unamended biotic controls, stimulated microcosms (lactate added), and bioaugmented microcosms (KB1® and/or ACTIII® cultures supplied by SiREM as well as lactate added) with addition of 1,1,1-TCA and/or TCE, each in two replicates. No apparent effect was observed on chlorinated ethanes degradation by TCE addition and degradation (data not included in this manuscript). Therefore, TCE data are not discussed any further. An overview of the conditions for the microcosms included in this paper is summarized in Table 1. For all microcosms, sampling and analysis were conducted for chlorinated ethanes, chlorinated ethenes, ethene and ethane in each of the 16 sampling rounds over the duration of 590 d. In some sampling rounds all microcosms were also sampled and analyzed for fatty acids and redox sensitive parameters. For selected conditions one of the replicates was sampled and analyzed for isotope ratios of 1,1,1-TCA, 1,1-DCA and CA in approximately every second sampling round. Only data from microcosms for which carbon isotope ratios were determined (total of 9 microcosms, see Table 1) are reported in this paper. A key for the legend-name used for each microcosm is given in Table 1. Sediment and groundwater

sampling for microcosms and the set-up and sampling of the microcosms is described in SI.

2.3. Chemical analysis

Samples for chlorinated ethanes and ethenes, ethene and ethane, and methane were stored at 4 °C and analyzed within a couple of days. Samples for non volatile organic carbon (NVOC), volatile fatty acids (VFA, including lactate, propionate, acetate and formate), anions and dissolved iron were filtered and preserved with acid or frozen for subsequent analysis. The analytical techniques for these parameters have previously been described by Scheutz et al. (2010) and are enclosed in the SI.

Samples for stable carbon isotope analysis were preserved with acid and stored upside down at 5 °C till analyzed. Carbon isotope ratios of chlorinated ethanes were analyzed by a GC coupled to an isotope-ratio mass spectrometer with a combustion interface (Thermo Finnigan, Germany). The system was equipped with a purge-and-trap concentrator (Velocity XPT, USA) connected to a cryogenic trap. A 25-mL sample was purged for 10 min at a rate of 40 mL min⁻¹ onto a VOCARB3000 trap (Supelco), followed by thermodesorption and transfer to the GC via a cryogenic trap held at -140 °C. Minimum required concentrations for carbon isotope analysis for individual chlorinated ethanes and ethenes were approximately 10 µg L⁻¹. However, high concentrations of some compounds requiring sample dilution in some instances resulted in higher detection limits for other compounds.

2.4. Data treatment

In order to account for the changing aqueous to gas volume ratio resulting from the sampling of the microcosms, the aqueous concentrations, determined on water samples, were corrected based on Henry's law to include the mass of the compounds in the headspace (Henry's law constants are given in SI). No correction was done to account for sorption to or desorption from the sediment. Therefore, some initial decrease in concentration of added compounds and increase in concentration of compounds not added but initially present on the sediment, which occur in abiotic controls as well as the other microcosms, is attributed to sorption/desorption processes. As degradation products sorb less than the mother compounds this may lead to higher total molar concentrations in the aqueous phase as the parent compounds degrade.

Apparent degradation rates (*k*) for 1st order degradation were determined by linear regression of the data using:

$$\ln(c(t)/c(t=0)) = -kt \quad (1)$$

where *c* is concentration (µmol L⁻¹), *t* is time (d) and *k* is degradation rate (d⁻¹).

Table 1
Overview of microcosms for which stable isotope analysis were conducted.

Batch#	Zone, material	Type	1,1,1-TCA conc. ^a (µg L ⁻¹)	TCE conc. ^a (µg L ⁻¹)	CA conc. ^a (µg L ⁻¹)	Lactate ^b added (µL sol.)	KB1 PLUS ^c added (µL)	Legend name (abbreviation)
2	Source, clayey till	Abiotic control	5000	0	0	0	0	CT ac TCA
4		Biotic control	5000	0	0	0	0	CT bc TCA
8		Stimulated	5000	1000	0	100	0	CT s TCA TCE
10		Stimulated	0	1000	0	100	0	CT s TCE
14		Bioaugmented	5000	1000	0	100	300	CT b TCA TCE
20	Plume, sand	Bioaugmented	0	1000	0	100	300	CT b TCE
26		Bioaugmented	1000	1000	0	100	300	S b TCA TCE
28		Abiotic control	0	0	1000	0	0	S ac CA
34		Bioaugmented	0	0	1000	100	300	S b CA

^a Concentration of added compounds aimed for, 1000 µg L⁻¹ is equivalent to 7.5 µmol L⁻¹ for 1,1,1-TCA, 7.6 µmol L⁻¹ for TCE and 15.5 µmol L⁻¹ for CA.

^b 60% Sodium lactate solution.

^c KB1PLUS refers to 50% KB1® and 50% ACTIII® culture.

All isotope ratios are reported relative to a standard (VPDB) using the delta notation:

$$\delta^{13}\text{C} = (R/R_{\text{std}} - 1) \quad (2)$$

where R and R_{std} are the isotope ratio of the sample and the standard, respectively.

An isotope balance was calculated as a weighted average isotopic fractionation ($\delta^{13}\text{C}_{\text{ave}}$) by:

$$\delta^{13}\text{C}_{\text{ave}} = (c_{\text{TCA}}\delta^{13}\text{C}_{\text{TCA}} + c_{\text{DCA}}\delta^{13}\text{C}_{\text{DCA}} + c_{\text{CA}}\delta^{13}\text{C}_{\text{CA}})/c_{\text{total}} \quad (3)$$

If the isotopic fractionation of the mother product and all the degradation products is known, and 1,1,1-TCA is the only initial product, then:

$$\delta^{13}\text{C}_{\text{ave}} = \text{constant} = \delta^{13}\text{C}_{\text{TCA}}(t = 0) \quad (4)$$

Isotope enrichment factors are quantified from isotope data by the Rayleigh Equation (Hunkeler et al., 2008) using linear regression:

$$\ln[(\delta^{13}\text{C}(t) + 1000)/(\delta^{13}\text{C}(t = 0) + 1000)] = \varepsilon \ln(c(t)/c(t = 0)) \quad (5)$$

where ε is the isotope enrichment factor which relate to the isotope fractionation factor α by:

$$\varepsilon = (\alpha - 1) * 1000\text{‰} \quad (6)$$

3. Results and discussion

3.1. Evolution of fermentation and redox conditions in microcosms

The lactate added to all stimulated and bioaugmented microcosms as electron donor was practically completely fermented to propionate, acetate (and H_2) within 1–2 months (individual compound data not shown). The resulting total fatty acids (VFA), Fig. 1a, are unchanged, but a brief drop in VFA was observed in most microcosms (day 8), likely caused by lactate degrading via an intermediate not included in the VFA analysis (possibly butyrate). Further fermentation of propionate (via acetate) and acetate, observed as decreasing VFA (Fig. 1a), accompanied by methane production (Fig. 1b) started after more than 450 d in microcosms with high initial 1,1,1-TCA concentration for both clayey till and sand. In contrast, this process started after only 180 d in microcosms with lower initial 1,1,1-TCA concentration and even earlier in microcosms with no initial 1,1,1-TCA (Fig. 1a and b). This suggests that 1,1,1-TCA inhibited fermentative/methanogenic bacteria, as may be expected (Scheutz et al., 2011) at high 1,1,1-TCA concentrations.

Dissolved iron increased to a stable level and sulfate decreased to below 1 mg L^{-1} (from 1.5 to $2 \text{ mg L}^{-1} \text{ SO}_4^{2-} - \text{S}$) within a month in all biotic microcosms (including biotic controls), whereas no sulfate reduction was observed in abiotic controls (Fig. 1c). Reduction of iron and sulfate is likely to result in formation of FeS ($\sim 2.5 \text{ mg FeS L}^{-1}$, based on $\text{SO}_4^{2-} - \text{S}$ decrease). Methane production remained low until significant acetate fermentation occurred as described above, Fig. 1b.

3.2. Chlorinated ethane degradation and carbon isotope evolution in clayey till microcosms

In Fig. 2, the evolution of chlorinated ethane concentrations (Fig. 2a1–a3) and stable carbon isotope ratios (Fig. 2b1–b3) are illustrated for microcosms with clayey till. Very high 1,1-DCA concentrations in the sampled clayey till sediment from the site resulted in higher initial concentration of this compound than of 1,1,1-TCA added to the microcosms. Due to the high solubility

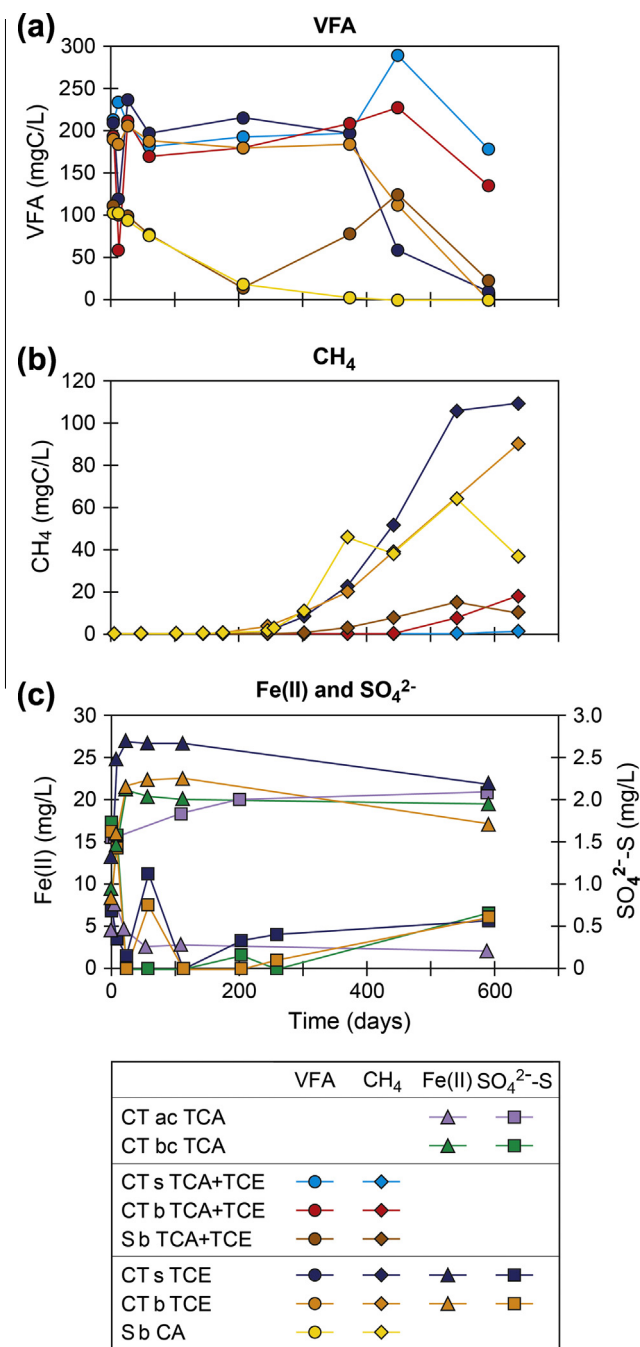


Fig. 1. Donor consumption illustrated by the fermentation products: (a) volatile fatty acids (VFA, sum of lactate, propionate, acetate and formate) concentration decrease and (b) methane production. Evolution in redox conditions illustrated by: (c) the dissolved iron (Fe(II)) and sulfate ($\text{SO}_4^{2-} - \text{S}$) data. For legend name key for microcosms, see Table 1.

and/or relatively slow desorption it was not possible to remove 1,1-DCA prior to the start of the experiment. An initial decrease in 1,1,1-TCA concentrations (Fig. 2a1) in abiotic as well as biotic microcosms after addition of 1,1,1-TCA was ascribed to sorption to the clayey till. For all biotic microcosms the 1,1,1-TCA concentration continued to decrease over time, whereas the concentration in the abiotic control remained constant (Fig. 2a1). In microcosms with low initial 1,1,1-TCA concentration (no 1,1,1-TCA added) it was depleted in about one month (Fig. 2a1). In microcosms with high initial 1,1,1-TCA concentration (1,1,1-TCA added) it slowly decreased (Fig. 2a1). Once 1,1,1-TCA had reached a relatively low

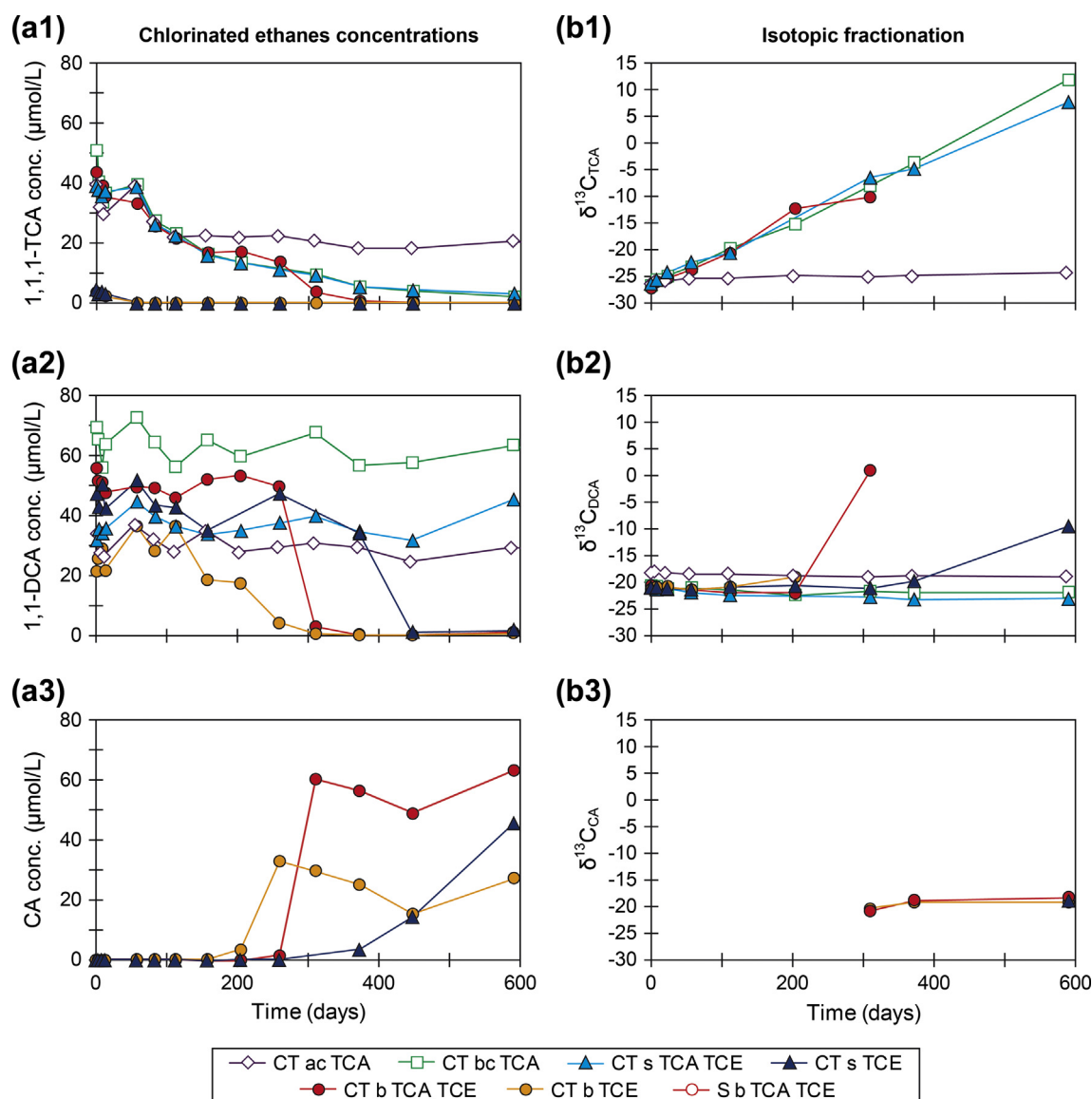


Fig. 2. Degradation of chlorinated ethanes in clayey till microcosms illustrated by evolution in (a1–a3) concentration and (b1–b3) isotopic fractionation over time for: (a1–b1) 1,1,1-TCA, (a2–b2) 1,1-DCA and (a3–b3) CA.

level (day 250) the apparent rate of 1,1,1-TCA concentration reduction increased in the bioaugmented microcosms (Fig. 2a1).

The stable isotope ratio for 1,1,1-TCA (Fig. 2b1) showed a significant enrichment in $^{13}\text{C}_{\text{TCA}}$ in all biotic microcosms but not in the abiotic control. A $^{13}\text{C}_{\text{TCA}}$ -enrichment was observed from day 1 (no lag-phase) and continued until 1,1,1-TCA concentrations decreased to below the detection level for stable isotope analysis. This clearly documented 1,1,1-TCA degradation in all biotic microcosms contrary to the abiotic control. It suggests that the degradation was biotic or biotically mediated. The degradation product 1,1-DCA expected in biotic reductive dechlorination was present in the microcosms from the start, but no apparent increase in 1,1-DCA was observed in any microcosms (Fig. 2a2) during degradation of 1,1,1-TCA. The stable carbon isotope ratio of 1,1-DCA (Fig. 2b2) did not show any depletion in $^{13}\text{C}_{\text{DCA}}$ compared to the initial value, which would be expected if 1,1-DCA was produced by 1,1,1-TCA degradation (see SI Section 5 for a quantitative discussion). This shows, that the degradation pathway for 1,1,1-TCA was not reductive dechlorination to 1,1-DCA.

The degradation pathway for 1,1,1-TCA was not dehydrochlorination (to 1,1-DCE), as no increase in 1,1-DCE was observed in any

microcosms during 1,1,1-TCA degradation (trace initial 1,1-DCE ($\sim 0.2 \mu\text{mol L}^{-1}$), lag time of 110–260 d for biotic DCE (cis-, trans-, 1,1-) degradation).

The degradation of 1,1,1-TCA occurred under iron and sulfate reducing conditions, likely resulting in biotic FeS formation, in the biotic microcosms (Fig. 1b). FeS can abiotically degrade 1,1,1-TCA by reduction (Butler and Hayes 2000; Gander et al., 2002; Choi et al., 2009; Elsner and Hofstetter 2011). Hence, the biotic formation of FeS can mediate abiotic degradation of 1,1,1-TCA. 1,1-DCA was a product of the abiotic degradation of 1,1,1-TCA by FeS, but only accounted for 2–4% of the 1,1,1-TCA degraded, in the studies by Choi et al. (2009), Gander et al. (2002), and Butler and Hayes (2000). The main degradation product of abiotic degradation of 1,1,1-TCA by FeS is unknown but likely non-chlorinated. The abiotic pathway for 1,1,1-TCA (via organochloride radical) proposed by Elsner and Hofstetter (2011) leads to formation of acetaldehyde and ethane as well as 1,1-DCA, where the ratio between the non-chlorinated products and 1,1-DCA was likely to depend on the presence of (hydrogen) radical scavengers. The FeS concentrations used in the above mentioned publications were 3–4 orders of magnitude higher than the estimate of freshly formed FeS in

microcosms in this study, whereas initial 1,1,1-TCA concentrations were of the same or about 1–2 orders of magnitude higher. Gander et al. (2002) observed a linear relationship between degradation rate and FeS surface area. If the relationship is extrapolated to the estimated freshly formed FeS in this study, degradation rates about 1–2 orders of magnitude lower than the observed are predicted. However, this assumes comparable surface area to weight ratio for FeS and linearity of the relationship over 3–4 orders of magnitude. In summary, biotically mediated abiotic degradation appears to be a possible explanation for the observed 1,1,1-TCA degradation.

No degradation of 1,1-DCA (Fig. 2a2) or formation of CA (data not included in Fig. 2) was observed in biotic and abiotic controls or in stimulated microcosms with high initial 1,1,1-TCA. In stimulated microcosms with low initial 1,1,1-TCA concentration and in bioaugmented microcosms with low and high initial 1,1,1-TCA concentration, 1,1-DCA (Fig. 2a2) was degraded to CA (Fig. 2a3) after apparent lag-phases of 260–370 d, 110–160 d, and 200–260 d, respectively. In these microcosms the degradation of 1,1-DCA was documented by enrichment of ^{13}C in 1,1-DCA (Fig. 2b2) as well as by the formation of the degradation product CA (Fig. 2a3). CA accumulates and $\delta^{13}\text{C}_{\text{CA}}$ approaches the $\delta^{13}\text{C}_0$ for 1,1-DCA (Fig. 3b3) as the initially dominating chlorinated ethane. This CA isotope trend fits well with complete degradation of 1,1-DCA to CA without further degradation of CA.

Comparison of the evolution in 1,1,1-TCA, 1,1-DCA and CA concentrations and their stable isotope ratios in the individual microcosms exemplified by the abiotic control and one of the bioaugmented microcosm in Fig. SI2 reveal that: In the biotic control only 1,1,1-TCA is degraded (Fig. SI2a1) and the resulting significant and constant increase in ^{13}C over time (Fig. SI2b1) strongly indicate abiotic degradation. Whereas, in the bioaugmented microcosm with high initial 1,1,1-TCA at about 260 d the concentration curve for 1,1,1-TCA becomes steeper at the same time as 1,1-DCA degrades to CA (Fig. SI3a2). The $\delta^{13}\text{C}$ increase for 1,1,1-TCA appear much smaller after day 260 (Fig. SI3b2, based on 1 point only) and at the same time a dramatic increase is observed in $\delta^{13}\text{C}$ for 1,1-DCA (Fig. SI3b2). This suggests that the degradation process for 1,1,1-TCA has changed and that reductive dechlorination of 1,1,1-TCA (via 1,1-DCA) as well as of 1,1-DCA to CA occurs in the bioaugmented microcosm (but not in the biotic control). The same trend can be seen for the bioaugmented and the stimulated microcosms with low initial 1,1,1-TCA (Fig. 2).

The on-set of methane production from acetate fermentation in the stimulated microcosm and bioaugmented microcosms (Fig. 1a and c) occurs after 1,1-DCA degradation, which suggests that biotic

degradation of 1,1,1-TCA and 1,1-DCA was inhibited by 1,1,1-TCA rather than by insufficiently reducing conditions. Grostern and Edwards (2006) observed complete inhibition of 1,1,1-TCA degradation via 1,1-DCA to CA in a mixed culture for concentrations of 1,1,1-TCA exceeding 2.2 mmol L^{-1} , and at concentrations between 1.5 and 2.2 mmol L^{-1} 1,1,1-TCA the degradation of 1,1,1-TCA proceeded only as far as 1,1-DCA in two months. In the current study, biotic 1,1-DCA (and 1,1,1-TCA) degradation appears inhibited at much lower levels ($10\text{--}20 \mu\text{mol L}^{-1}$) and comparable to 1,1,1-TCA inhibition of cDCE and VC degradation reported by Duhamel et al. (2002) at $5.2 \mu\text{mol L}^{-1}$.

3.3. Chlorinated ethane degradation and isotope fractionation evolution in sand microcosms

The degradation of chlorinated ethanes in the bioaugmented microcosm with sand is illustrated in Fig. 3. For 1,1,1-TCA a lag phase of 57 d indicated by the stable isotope data is followed by a slow decrease in 1,1,1-TCA concentration due to degradation as documented by the ^{13}C enrichment between day 57 and day 204. No production of 1,1-DCA is observed before day 204. These observations are consistent with the observed degradation in biotic clayey till microcosms (Section 3.2). After day 204 faster degradation of 1,1,1-TCA and concurrent 1,1-DCA production was observed indicating biotic degradation by reductive dechlorination. When 1,1-DCA is biotically degraded to CA (day 447–590), the 1,1-DCA concentration becomes too low for determination of the isotope ratio.

No apparent degradation of CA was observed by the evolution in CA concentrations and the persistence of CA was confirmed by the lack of any significant enrichment in ^{13}C (Fig. SI3). CA has been reported as the terminal dechlorination product of 1,1,1-TCA by 1,1,1-TCA degrading cultures in laboratory microcosm studies by Grostern and Edwards (2006) and others (referenced in Scheut et al., 2011).

3.4. Degradation rates and enrichment factors

Degradation rates and enrichment factors are summarized in Table 2. These were estimated by linear regression of concentration and stable isotope data (Fig. SI4 (clayey till) and Fig. SI5 (sand)).

Degradation of 1,1,1-TCA in all biotic microcosms including the biotic control has been documented by the isotope data, and mass and isotope balance have confirmed that 1,1-DCA is not a

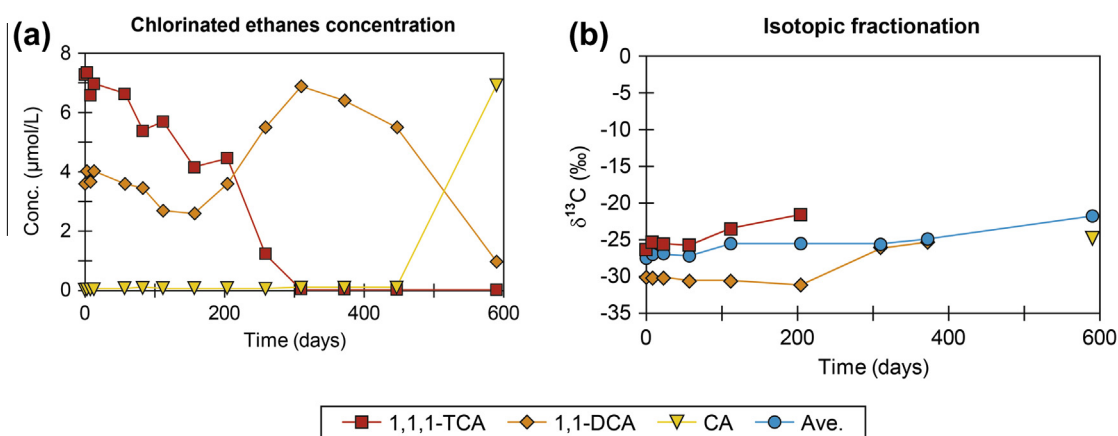


Fig. 3. Degradation of chlorinated ethanes in bioaugmented microcosm with sand illustrated by evolution in (a) concentrations and (b) isotopic fractionation. Ave.: Average $\delta^{13}\text{C}$, see Eq. (4).

Table 2

Degradation rates and enrichment factors deduced from the microcosm study. Equations for the calculations of degradation rates and enrichment factors are given in SI. Individual R^2 values for regressions (range 0.8–0.98) are available in SI, for enrichment factors the number of datapoints in the regressions were limited.

Batch#	Zone, material	Type	Time inter-val (d)	1,1,1-TCA degr. rate (d ⁻¹)	1,1,1-TCA enrichment factor (‰)	Time inter-val (d)	1,1-DCA degr. rate (d ⁻¹)	1,1-DCA enrichment factor (‰)	Legend name (abbreviation)
2	Source, clayey till	Abiotic control		0	–		0	–	CT ac TCA
4		Biotic control	0–590	0.0056	–12.3		0	–	CT bc TCA
8		Stimulated	0–693	0.0048	–13.7		0	–	CT s TCA TCE
10		Stimulated		–	–	0–372	0	–	CT s TCE
						372–447	0.046	(–8.0)	
14		Bioaugmented	0–259	0.0041	–14.0	0–259	0	–	CT b TCA TCE
			259–447	0.019	(–1.4)	259–372	0.041	(–3.5)	
20		Bioaugmented		–	–	0–204	0	–	CT b TCE
						204–447	0.020	–	
26	Plume, sand	Bioaugmented	0–204	0.0023	–10.3	0–447	0	–	S b CA
			204–372	0.035	–	447–590	0.012	–	
Sherwood Lollar et al. (2010)	Biotic			–1.8				–10.5	
Elsner et al. (2007)	Abiotic with metals			–13.6 to –15.8					

–: Not determined/relevant, (–): approximate numbers, the quantification was based on regression of only 2 points and is very uncertain. However, the change in degradation type for 1,1,1-TCA is reflected, and inclusion allows for comparison with the literature values.

significant product of the 1,1,1-TCA degradation (SI Section 5 with Fig. SI6).

The degradation rates for 1,1,1-TCA in biotic control and stimulated microcosms and initially in bioaugmented microcosms (0.0023–0.0056 d⁻¹) are relatively low compared to biodegradation rates (Scheutz et al., 2013). However, significant carbon isotope enrichment factors (–10‰ to –14‰) are observed. These practically correspond to the ϵ -values reported for abiotic degradation of 1,1,1-TCA by zero-valent metals reported by Elsner et al. (2007). They are consistent with theoretical enrichment for cleavage of a chlorine bond, i.e. formation of the organochloride radical in the first step as proposed by Elsner and Hofstetter (2011). Experimentally determined enrichment factors are not available for FeS catalyzed degradation of 1,1,1-TCA, but the above indicate that degradation of 1,1,1-TCA by ZVI and FeS likely share a common first rate limiting step and hence show a similar enrichment factor. For the analogue first rate limiting step of abiotic CTET degradation (Elsner and Hofstetter 2011) the variation in ¹³C enrichments of CTET was within a factor of two for iron (hydr)oxide and iron sulfide minerals studied by Zwank et al. (2005). This is much less than the order of magnitude difference to that of biotic degradation observed by Sherwood Lollar et al. (2010). In the bioaugmented microcosms the degradation rates later increased (to ~0.019–0.035 d⁻¹) and degradation of 1,1-DCA (rates 0.012–0.046 d⁻¹) was observed at the same time. At this time the enrichment factor for 1,1,1-TCA became much lower (about –1.4‰ but based on just 2 data points) and corresponded quite well with the biotic enrichment factor determined for 1,1,1-TCA in a dehalobacter containing mixed culture during whole cell degradation by Sherwood Lollar et al. (2010). The 1,1-DCA enrichment factors (about –3‰ to –8‰ and each only based on two data points), also corresponded reasonably with the enrichment factor determined by Sherwood Lollar et al. (2010). Though the quantification of the enrichment factors during biotic degradation are uncertain and only a single culture was represented in the study by Sherwood Lollar et al. (2010), these findings support the indicated biotically induced abiotic degradation of 1,1,1-TCA by FeS in biotic microcosms.

3.5. Field site evaluation

At the field site, 1,1-DCA was the dominant compound suggesting that biotic reductive dechlorination played a role. Except for

one location (B106), CA concentrations were low indicating that no further transformation of 1,1-DCA occurred at most locations. If complete biotic reductive dechlorination of 1,1,1-TCA to 1,1-DCA occurred, the formed 1,1-DCA should have the same carbon isotopic composition as the initial 1,1,1-TCA i.e. the $\delta^{13}\text{C}$ of 1,1-DCA should be the same at all locations where 1,1,1-TCA is absent or present at only a very low fraction. However, at several locations (B205, B2, B102, B101, B106), the 1,1-DCA is enriched in ¹³C compared to other locations (B301, B3) – see Fig. SI1d. This pattern could be due to abiotic 1,1,1-TCA transformation before the onset of biotic reductive dechlorination as observed in bioaugmented microcosms (“CT b TCA TCE”, Fig. 1a1 and 1b1). Hence, biotic reductive dechlorination of 1,1,1-TCA would start from an enriched carbon isotope signature that is inherited to 1,1-DCA as reductive dechlorination reaches completion. Given an enrichment of 1,1-DCA by 6.6–9.3‰ and an average isotope enrichment factor for abiotic 1,1,1-TCA of –12.5‰, about 42–53% of abiotic 1,1,1-TCA degradation would be necessary to explain the 1,1-DCA carbon isotope data. This estimation suggests that abiotic degradation of 1,1,1-TCA is a relevant process also at the field site.

4. Conclusions

In all biotic microcosms 1,1,1-TCA was degraded with no apparent increase in the biotic degradation product 1,1-DCA. 1,1,1-TCA degradation was documented by a clear enrichment in ¹³C in all biotic microcosms, but not in the abiotic control, which suggests biotic or biotically mediated degradation. Biotic degradation by reductive dechlorination of 1,1-DCA to CA only occurred in bioaugmented microcosms and in donor stimulated microcosms with low initial 1,1,1-TCA or after significant decrease in 1,1,1-TCA concentration (after ~ day 200). Hence, the primary degradation pathway for 1,1,1-TCA does not appear to be reductive dechlorination via 1,1-DCA. In the biotic microcosms the degradation of 1,1,1-TCA occurred under iron and sulfate reducing conditions. Biotic reduction of iron and sulfate likely resulted in formation of FeS, which can abiotically degrade 1,1,1-TCA. Hence, abiotic degradation of 1,1,1-TCA mediated by biotic FeS formation constitute an explanation for the observed 1,1,1-TCA degradation. This is supported by a high 1,1,1-TCA ¹³C enrichment factor consistent with abiotic degradation in biotic microcosms. The documentation of 1,1,1-TCA degradation by stable carbon isotope ratios was critical to distinguishing

degradation of 1,1,1-TCA from sorption to the sediment. Without isotopic fractionation data for both 1,1,1-TCA and 1,1-DCA the apparent degradation of 1,1,1-TCA by a different degradation pathway than reductive dechlorination to 1,1-DCA would have been speculative only. The high enrichment observed compared to the enrichment expected for biodegradation based on Sherwood Lollar et al. (2010) further supports the 1,1,1-TCA degradation occurred by an abiotic process involving cleavage of a single C–Cl bond by single electron transfer forming a dichloroethyl radical in the initial rate limiting step, as proposed by Elsner and Hofstetter (2011). The findings show that CSIA identified an alternative 1,1,1-TCA degradation pathway that cannot be explained by assuming biotic reductive dechlorination. 1,1-DCA carbon isotope field data suggest that this abiotic degradation of 1,1,1-TCA is a relevant process also at the field site.

5. Implications/future perspectives

The demonstration of degradation of 1,1,1-TCA by an abiotic pathway, which in contrast to biotic reductive dechlorination does not require dechlorinating bacterial activity and is not inhibited by high 1,1,1-TCA concentrations, may be part of the explanation for relatively rare findings of 1,1,1-TCA at field sites. The potential for pathway distinction and high sensitivity of isotopic fractionation to degradation of the compounds holds good promise for the use of isotopic fractionation as a monitoring and documentation method and it is considered a valuable tool in documentation of degradation and evaluation of pathways in complex contamination scenarios.

For sites contaminated by 1,1,1-TCA, where limited or no degradation to 1,1-DCA has occurred naturally, stimulation of biotically mediated abiotic degradation (e.g. sulfate and donor addition), as previously suggested for chlorinated ethenes by Kennedy et al. (2006), or of direct abiotic degradation (FeS addition) may be a viable option e.g. prior to stimulation/bioaugmentation for enhanced reductive dechlorination of chlorinated ethenes. However, the possibilities for controlling/limiting the partial degradation to 1,1-DCA by the abiotic pathway need to be investigated.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2014.01.051>.

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