Degradation of 1,2-dichloroethane by microbial communities from river sediment at various redox conditions

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\textbf{Abstract}

Insight into the pathways of biodegradation and external factors controlling their activity is essential in adequate environmental risk assessment of chlorinated aliphatic hydrocarbon pollution. This study focuses on biodegradation of 1,2-dichloroethane (1,2-DCA) in microcosms containing sediment sourced from the European rivers Ebro, Elbe and Danube. Biodegradation was studied under different redox conditions. Reductive dechlorination of 1,2-DCA was observed with Ebro and Danube sediment with chloroethane, or ethene, respectively, as the major dechlorination products. Different reductively dehalogenating micro-organisms (\textit{Dehalococcoides} spp., \textit{Dehalobacter} spp., \textit{Desulfitobacterium} spp. and \textit{Sulfurospirillum} spp.) were detected by 16S ribosomal RNA gene-targeted PCR and sequence analyses of 16S rRNA gene clone libraries showed that only 2–5 bacterial orders were represented in the microcosms. With Ebro and Danube sediment, indications for anaerobic oxidation of 1,2-DCA were obtained under denitrifying or iron-reducing conditions. No biodegradation of 1,2-DCA was observed in microcosms with Ebro sediment under the different tested redox conditions. This research shows that 1,2-DCA biodegradation capacity was present in different river sediments, but not in the water phase of the river systems and that biodegradation potential with associated microbial communities in river sediments varies with the geochemical properties of the sediments.

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1. Introduction

Chlorinated aliphatic hydrocarbons (CAHs) like tetrachloroethene (PCE), 1,2-dichloroethane (1,2-DCA) and vinyl chloride (VC), are widely used in industries as solvents or as intermediates in chemical processes. Due to leakage and improper disposal, CAHs have often been detected in the environment (Westrick et al., 1984). Here, they form a long-lasting danger to humans and the environment because of high water solubility, a long environmental half-life and carcinogenic effects (Vogel et al., 1987).

Transformation of CAHs has been studied extensively, with specific attention for the microbiology (Mohn and Tiedje, 1992; Smidt and de Vos, 2004), in situ biodegradation in soil systems (Maes et al., 2006; Suchomel et al., 2007; Takeuchi et al., 2005) and enzymology (Bunge et al., 2007). However,
little information is available with respect to the variation of the microbial degradation potential and activity in river sediment systems (Bradley and Chapelle, 1998). This is in sharp contrast to the demands of regulatory bodies that require specific guidelines based on fundamental knowledge of the biodegradation of pollutants in river systems for adequate river management (Barth et al., 2007; Gerzabek et al., 2007).

Much attention has been given to 1,2-DCA as a model compound (de Wildeman et al., 2003b; Faita et al., 2005; Fishein, 1979; Janssen et al., 1994), since it can biologically be degraded under different geochemical conditions. Moreover, it has been produced in larger quantities than any other CAH (Pankow and Cherry, 1996). Currently, more than 17.5 million tons are produced annually in the United States, Western Europe and Japan (Field and Sierra-Alvarez, 2004). 1,2-DCA is frequently detected in river systems at several tens of micromolars (ATSDR, 1999; Gotz et al., 1998; ICPS, 1995; Yamamoto et al., 1997), which is above the natural background level of 5 μmol in non-industrialized areas (de Rooij et al., 1998). In Europe, the Water Framework Directive (WFD, Directive 2000/60/EC) classified 1,2-DCA as one of the 33 priority pollutants and it has been identified similarly by the U.S. Environmental Protection Agency. The 1,2-DCA pollution in rivers and estuaries is generally considered to be caused to a large extent by anthropogenic sources; however, 1,2-DCA can in low concentrations originate from natural sources (de Rooij et al., 1998).

Previous studies have focused on the fate of 1,2-DCA in the environment and showed that it can be transformed through abiotic as well as biotic reactions. Abiotically, 1,2-DCA is transformed to ethylene glycol or VC. However, this is a very slow process (half life >72 years) (Jeffers et al., 1989), and the resulting products may even be more toxic than 1,2-DCA itself (Gallegos et al., 2007). In contrast, micro-organisms can transform 1,2-DCA rapidly to non-toxic end products via different pathways. In the presence of oxygen, 1,2-DCA can completely be oxidized to CO₂, H₂O and Cl⁻ by different bacterial species, including Xanthobacter autotrophicus (Janssen et al., 1985) and Ancyllobacter aquaticus (van den Wijngaard et al., 1992). However, in aquifers and sediments, oxygen is often not available. The oxidation of 1,2-DCA in the absence of oxygen has also been observed with nitrate as alternative electron acceptor (Gerritsen et al., 1999). Recently, this process has been described in more detail by Dinglasan-Panlilio and co-workers (Dinglasan-Panlilio et al., 2006).

Under methanogenic conditions, ethene is usually the main end product of 1,2-DCA dechlorination (Egli et al., 1987) and is formed via either a cometabolic or halorespiration process. Dehalococcoides ethenogenes strain 195 (Maymo-Gatell et al., 1999) and Desulfotobacterium dichloroeliminans strain DCA1 (de Wildeman et al., 2003a) are bacterial isolates that can convert 1,2-DCA anaerobically into ethene by reductive dechlorination. Chloroethane and ethene can also be formed via reductive dechlorination of 1,2-DCA. However, this has scarcely been reported and only some strains of methanogens are known to be responsible for the process (Holliger et al., 1990).

In some environmental studies, a thorough characterization of 1,2-DCA biodegradation potential was done resulting in insight in lag periods of microbial adaptation required for biotransformation of 1,2-DCA (Klecka et al., 1998) and evidence that fermentative as well as anaerobic oxidative pathways can be involved in natural attenuation of 1,2-DCA (Gerritsen et al., 1999). However, an investigation of the local biogeochemical conditions is required before it can be assessed to what extent and via which pathways 1,2-DCA is likely to be degraded in a specific sediment or groundwater system.

The aim of the present study was to assess the potential for biodegradation of 1,2-DCA by microbial communities of sediments originating from the rivers Ebro, Danube and Elbe at different redox conditions, and to identify biodegradation mechanisms and micro-organisms involved. To this end, microcosms and cultivation-independent approaches were combined to obtain insight to what extent the mechanisms and micro-organisms can be related to prevailing redox conditions and characteristics of the tested river inoculum material.

2. Material and methods

2.1. Sampling of river sediments and river water

Sediments were sampled from the rivers Ebro, Elbe and Danube. Ebro sediment was sampled from a rice field in the southern part of the river delta (N: 40°41’11.8”, E: 00°37’31.5”) in July 2004 and had a clay-like structure. The rice field is flooded with water from the river, every year from April to September and has been formed by sedimentation.

Elbe sediment was sampled near Schönberg (N: 52°90’73.2”; E: 11°87’21.9”) at the west bank of the river in October 2004. The sediment was clear and sandy. Artificial reconstruction activities of the river bank were carried out about a year before sampling.

Danube River sediment was sampled at the East bank near Budapest (47°27’25.8”; E: 19°03’46.8”) in April 2005. This sediment had a clayey structure.

Sediment samples were taken as 20 cm long vertical cores, using sterilized PVC tubes (30 cm x 3.2 cm inner diameter). The PVC tube was completely pushed into the sediment and closed with butyl rubber stoppers on both sides when the tube was drawn back. The tubes were stored at 4 °C for maximally 1 month until microcosms were set up.

Water sampling was done using a 1-liter sterile glass jar, which was completely filled under water, closed with a Viton stopper, and stored at 4 °C.

Sediments and river water were characterized by partners in the Aquaterra project: Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, Spain; for Ebro samples; Helmholtz-Zentrum für Umweltforschung (UFZ), Magdeburg, Germany, for Elbe samples; International Commission for the Protection of the Danube River (ICPDR) for Danube samples; and additional analyses on the sediments were performed by Al-West B.V. (Deventer, The Netherlands) (Table 1). The concentration of most CAHs (vinyl chloride, 1-chloroethane, trans-1,1-dichloroethene, cis-1,1-dichloroethene, 1,1-dichloroethane, 1,1,1-trichloroethane, 1,2-dichloroethane, trichloroethene and tetrachloroethene) in river water samples was below detection limit (<1 μg/l). In contrast, 1,2-DCA was detected in the river water of all locations and ranged from 1 to 2 μg/l, which is well above the natural background level.
reported for non-industrialized areas (de Rooij et al., 1998). In Elbe river water, in addition to 1,2-DCA also trichloroethene and tetrachloroethene were present at 2 μg/l.

2.2. Microcosm experiments

Microcosms were prepared in an anaerobic glove box with 50 ml local river water, with and without 1–10% (w/w) sediment material and 100 μM 1,2-DCA. For Ebro river microcosms with sediment, artificial river water was used, which consisted of (in mM): Na2HPO4, 0.3; KH2PO4, 0.05; NaCl, 1.7; NaSO4, 0.7; NaHCO3, 1.2; CaCl2·2H2O, 0.44; NH4Cl, 0.9; MgCl2·6H2O, 0.2; KCl, 0.13; NaNO3, 0.06; Na-acetate, 0.06; humic acids (technical mix 50–60% as humic acids, Acros Organics, Geel, Belgium), 5; trace elements solution SL10 (DSMZ, medium 320), 0.1 ml/l and vitamin solution (Heijthuijsen and Hansen, 1986), 0.1 ml/l. The used 120 ml serum bottles were capped with Viton stoppers to prevent evaporation and absorption of 1,2-DCA (Evans and Hardy, 2004).

Biodegradation of 1,2-DCA was tested at the following redox conditions: aerobic (21% (v/v) O2 in the gas phase), nitrate-reducing (2 mM NaNO3 added), iron-reducing (10 mM FeOOH (Brock and O’dea, 1977) added), sulfate-reducing (Na2SO4 added), and methanogenic (1–5 mM fatty acids added). For all conditions, duplicate microcosms were prepared as well as an autoclaved negative control. Elbe samples were not tested for nitrate-reducing, iron-reducing and sulfate-reducing conditions.

All anaerobic microcosms had a nitrogen atmosphere and the volumetric ratio headspace/liquid-sediment phase was 7:5. The pH was neutralized to 7.0 with NaOH. The microcosms were incubated at 30 °C (Ebro) or 20 °C (Danube and Elbe), shaken at 150 rpm, for at least 12 months. The incubation temperature for Ebro microcosms was higher than Elbe and Danube microcosms, corresponding to the natural environmental conditions.

Active, 1,2-DCA dechlorinating microcosms were transferred (10%, v/v) to fresh medium (described above) containing 100–500 μM 1,2-DCA, to enrich the 1,2-DCA dechlorinating consortia. After three transfers, following incubation periods of 2 months and regular screening for 1,2-DCA dechlorination, these microcosms were sampled for DNA-based community analyses. Microcosms with sediment that did not show 1,2-DCA dechlorination, were also sampled for further DNA-based analyses.

2.3. Molecular analyses of microbial communities

2.3.1. DNA extraction

DNA was extracted from 5 ml of suspended microcosm material using the BIO101 fast DNA Kit for Soil (Qbiogene Inc., CA). The only modification to the manufacturer’s protocol was extension of the bead-beat step from 30 to 45 s. Extracted DNA was stored at −20 °C.

2.3.2. Genus- and catabolic gene-specific PCR

Samples from 1,2-DCA dechlorinating microcosms were screened for the presence of known anaerobic dechlorinating bacteria, including the genera Dehalococcoides, Dehalobacter, Desulfitibacterium, Sulfurospirillum, using 16S ribosomal RNA (rRNA) gene-targeted assays, and the genes encoding the 1,2-DCA dechlorinating enzymes DCA reductase A (dcaA) (Marzorati et al., 2007) and haloalkane dehalogenase dhIA (Song et al., 2004) by nested PCR. 16S rRNA gene fragments

| Table 1 – Overview of river water and sediment characteristics of the sampled locations in Ebro, Elbe and Danube river. |
|-----------------|---------|---------|----------------|---------|---------|----------------|---------|---------|----------------|---------|
|                  | Ebro    | Elbe    | Danube         |        |        |                |        |        |                |        |
|                  | Water   | Sediment| Water          | Sediment| Water  | Sediment       |        |        |                |        |
| Physical parameters |        |         |                |        |        |                |        |        |                |        |
| Temperature       | 23.4    | 15.0    | 14.1           |        |        |                |        |        |                |        |
| pH                | 8.2     | 7.9     | 8.2            |        |        |                |        |        |                |        |
| Redox potential   | mV      |          |                |        |        |                |        |        |                |        |
| Conductivity      | μS/cm   |          |                |        |        |                |        |        |                |        |
| Oxygen            | mg/l    |          |                |        |        |                |        |        |                |        |
| Dry matter (dm)   | % (w/w) |          |                |        |        |                |        |        |                |        |
| Total organic carbon | % dm   |          |                |        |        |                |        |        |                |        |
| Anions            |         |          |                |        |        |                |        |        |                |        |
| Chloride          | mg/l    | 63      | 153            | 18     |        |                |        |        |                |        |
| Nitrite           | mg/l    | <3      | <3             | 5      |        |                |        |        |                |        |
| Bromide           | mg/l    | <3      | <3             | <3     |        |                |        |        |                |        |
| Nitrate           | mg/l    | <3      | 13             | 5      |        |                |        |        |                |        |
| Phosphate         | mg/l    | <3      | <3             | <3     |        |                |        |        |                |        |
| Sulfate           | mg/l    | 115     | 111            | 29     |        |                |        |        |                |        |
| Cations           |         |          |                |        |        |                |        |        |                |        |
| Lithium           | mg/l    | <3      | <3             | <3     |        |                |        |        |                |        |
| Sodium            | mg/l    | 58      | 102            | 30     |        |                |        |        |                |        |
| Ammonium          | mg/l    | <3      | <3             | <3     |        |                |        |        |                |        |
| Potassium         | mg/l    | 2       | 11             | 3      |        |                |        |        |                |        |
| Magnesium         | mg/l    | 21      | 18             | 13     |        |                |        |        |                |        |
| Calcium           | mg/l    | 84      | 11             | 54     |        |                |        |        |                |        |
were amplified on an ICycler PCR system (Bio-Rad, Veenendaal, The Netherlands) with 6 μM bacterial primer mixture (fd1/fd2 and rp1/rp2) (Weisburg et al., 1991) using the following cycling program: 94 °C for 3 min, 43 cycles 94 °C for 30 s, 65 °C for 30 s, 72 °C for 1 min, with a temperature decrease of 1 °C for the annealing phase of the first 10 cycles and a time increment of 10 s added to the elongation phase of the last 33 cycles. The resulting PCR product was purified with the Qiaquick PCR Purification Kit (Qiagen, Hilden, Germany) and used as template for subsequent nested PCR. Total DNA isolated from Desulfitobacterium strain DCA1 and Xanthobacter autotrophicus strain GJ10 were used as positive control for dcaA and dhaA gene detection, respectively. Primers targeting the genera Dehalobacter, Desulfitobacterium, genera Dehalococcoides, and Sulfitospirillum were described before (Smits et al., 2004; Ballerstedt et al., 2004). Amplification was achieved in a real-time PCR system (IQ5, Bio-Rad Laboratories B.V., Veenendaal, The Netherlands) using 6 μM primer and 3 μl template DNA added to IQ SYBR Green Supermix (Bio-Rad Laboratories B.V., Veenendaal, The Netherlands) with the following cycling program: 94 °C for 3 min, 35 cycles 94 °C for 30 s, 55 °C or 58 °C (55 °C for Dehalococcoides spp., 58 °C for Dehalobacter spp., Desulfitobacterium spp., and Sulfitospirillum spp.) for 20 s, 72 °C for 30 s and a final extension at 72 °C for 5 min. Relative abundance of bacteria from the different genera was determined by normalizing the quantitative results of the specific abundance of bacteria from the different genera was determined by normalizing the quantitative results of the specific sequences was initially classified using the tools available at Greenegenes (http://greengenes.lbl.gov) (DeSantis et al., 2006). Sequences obtained (MWG-Biotech, Germany) and the obtained sequences were initially classified using the tools available at Greengenes (http://greengenes.lbl.gov) (DeSantis et al., 2006). Sequences obtained (MWG-Biotech, Germany) and the obtained sequences were initially classified using the tools available at Greengenes (http://greengenes.lbl.gov) (DeSantis et al., 2006). Sequences obtained in this study were aligned using the SINA aligner at http://www.silva-silva.de, and the alignment was manually refined using the ARB-editor and appropriate reference sequences. Tree reconstruction was performed using the maximum parsimony method as implemented in ARB (Ludwig et al., 2004). **2.4. Analytical procedures**

1,2-DCA and its potential dechlorination products chloroethane, vinyl chloride, ethene, ethane, and methane were identified and quantified in 500 μl headspace samples from microcosms, obtained by using a 1 ml Pressure-Lock gas syringe (Alltech, Breda, The Netherlands) with a sterile needle. The samples were injected onto a Varian 9001 gas chromatographic system (GC) equipped with a flame ionization detector (FID) and a Porabond-Q column (0.32 mm × 25 m) (Varian, Middelburg, The Netherlands). GC settings were: injector temperature 200 °C, detector temperature 300 °C; oven temperature 3 min at 50 °C, followed by an increase with 10 °C/min to 250 °C; carrier gas helium with a flow rate of 2 ml/min. External standards at five different concentrations were used for calibration. The lower detection limit was 1 μM for all analyzed compounds, except for 1,2-DCA, which had a detection limit of 10 μM.

CO2 was analyzed on an Interscience GC-8000 gas chromatograph with a thermal conductivity detector (TCD) and a Poraplot Q column (Varian, Middelburg, the Netherlands). The injector temperature was set at 100 °C and the detector temperature at 200 °C, with a constant oven temperature of 90 °C. Helium was used as carrier gas at a flow rate of 30 ml/min. External standards were used for calibration.

Liquid phase samples (5 ml) were taken for ion analyses on a Dionex DX-100 ion-chromatograph with Dionex IONPAC AS9-SC ion-column (Dionex Benelux B.V., Amsterdam, The Netherlands).

Fe2+ and Fe3+ concentrations in the iron-reducing microcosms were measured by the spectrophotometric method described before (Viollier et al., 2000). Half-life times (t1/2) were calculated for transformation of 1,2-DCA in the microcosms according to

\[
t_{1/2} = \ln 2/k,
\]

where \( k = (1 - c_1)/c_2 \) (day\(^{-1} \)). \( c_1 \) is the concentration of 1,2-DCA at time \( t_1 \) and \( c_2 \) is the concentration of 1,2-DCA at time \( t_2 \), where \( t_2 > t_1 \) and \( c_1 > c_2 \).

**3. Results**

**3.1. Degradation of 1,2-DCA in microcosms**

Transformation of 1,2-DCA was observed in microcosms prepared with sediment, but not in microcosms with river water without sediment or in sterilized negative controls. This indicated that the observed transformation was biologically derived. Additionally, the results showed that the observed biodegradation capacity for 1,2-DCA of the different river systems was related to the sediment and not to the water phase. Therefore, the study further focused on sediment-inoculated microcosms.

**3.1.1. Ebro River sediment**

Four microcosms with Ebro sediment, incubated under methanogenic conditions, transformed 100 μM 1,2-DCA completely within 100 days. In these microcosms acetate or lactate was supplied as electron donor. Initially, dechlorination of 1,2-DCA yielded chloroethane as the major dechlorination product (80–90% of 1,2-DCA removed), and little lactate was supplied as electron donor. Initially, dechlorination of 1,2-DCA yielded chloroethane as the major dechlorination product (80–90% of 1,2-DCA removed), and little
of chloroethane were detected (<2%) (Fig. 1B). The dechlorination rate of 1,2-DCA in the methanogenic microcosms did not change with the transfers and corresponded to a half-life of 46/14 days.

Under nitrate-reducing conditions, 100 mM 1,2-DCA was degraded within 90 days in microcosms with Ebro sediment, in parallel with the removal of 1.9 mM nitrate. Per mol of 1,2-DCA degraded, 1.9 mol of chloride ions were produced. No other degradation products of a reductive dechlorination pathway were observed. Half-lives for 1,2-DCA in these denitrifying microcosms were 12/6 days, about 4 times shorter than under methanogenic conditions.

At the other tested redox conditions, 1,2-DCA was not transformed in Ebro sediment microcosms within the incubation period of 12 months, although the electron acceptors present (sulfate, iron or oxygen) were consumed over time.

3.1.2. Danube River sediment

Microcosms with sediment from Danube river dechlorinated 1,2-DCA completely to ethene under methanogenic conditions. In contrast to microcosms with Ebro sediment, chloroethane or ethane formation was not observed. The half-lives of 1,2-DCA under methanogenic conditions were 38 ± 6 days, which was similar to those observed in Ebro microcosms.

Danube sediment microcosms, incubated under iron-reducing conditions transformed 1,2-DCA over time (Fig 2), with a half-life of 15 ± 3 days. This degradation continued in transfers (10% v/v) into artificial river water with 10 mM FeOOH and 1,2-DCA concentrations of 100 µM or 500 µM (not shown), respectively. Per mol of 1,2-DCA degraded, 12 mol of Fe²⁺ was produced. Further enrichment of the iron-reducing culture was done by re-addition of 1,2-DCA. This did not result in higher degradation rates, suggesting that the process may have been limited by the availability of iron, supplied as amorphous iron oxide (data not shown).

Under aerobic, sulfate-reducing and denitrifying conditions, the concentration of the respective electron acceptors decreased over time in Danube sediment microcosms, but 1,2-DCA transformation was not observed.

3.1.3. Elbe River sediment

No 1,2-DCA transformation was observed in microcosms incubated with Elbe sediment at any of the tested redox conditions.

3.2. Molecular identification of (dechlorinating) microbial populations

Molecular detection by PCR amplification showed that 16S rRNA genes from known reductive dehalogenating microorganisms were present in Ebro and Danube microcosms (Table 2). In all microcosms, the dcaA and dhlA genes were below detection limit (100 gene copies/ml) and in microcosms with Elbe sediment, none of the tested micro-organisms could be detected. Although dehalogenating micro-organisms were detected in both Ebro and Danube river sediment, differences between the river sediments and the different redox conditions used for incubation were observed.

Dehalobacter spp. could not be detected in Danube microcosms, indicating that those micro-organisms were absent in the tested sediment. In contrast, all tested dechlorinating species (Dehalococcoides spp., Dehalobacter spp., Desulfotobacterium spp. and Sulfitospiillum spp.) were present in Ebro sediment, albeit at largely varying relative abundance depending...
on the conditions (Table 2). At methanogenic conditions, *Dehalococcoides* spp. were present in relatively high concentrations, indicating enrichment of members of this genus. This was also observed for Ebro microcosms that were initially incubated aerobically. However, oxygen in these microcosms was depleted during the incubation period, after which 1,2-DCA was transformed to ethene (data not shown). At denitrifying conditions, none of the dechlorinating bacteria could be detected in active and transferred microcosms. In 1,2-DCA transforming microcosms at iron-reducing conditions, only *Dehalococcoides* spp. 16S rRNA genes were detected, but at concentrations that were not higher than in denitrifying and sulfate-reducing microcosms with the same sediment that did not show 1,2-DCA degradation.

A taxonomic classification of sequenced clones (deposited under GenBank accession numbers FJ802135–FJ802386), derived from 1,2-DCA transforming microcosms incubated under methanogenic, denitrifying and iron-reducing conditions, is presented in Table 3. All clones were positioned in a phylogenetic tree of bacterial 16S rRNA sequences, using the ARB software environment (Ludwig et al., 2004; Pruesse et al., 2007) (Supplementary Figure S1).

In the dominant bacterial communities of the three enriched microcosms, only a few Phyla were represented.

### Table 2 – Dechlorination and relative abundance (\(\%\) of total bacterial 16S rRNA genes) of 16S rRNA genes of known reductive dechlorinating bacteria in microcosms. Presence or absence of *Sulfurospirillum* spp. 16S rRNA genes is indicated with “+” (=detected) or “−” (=not detected), respectively. < < indicates that values were below detection limit (\(1 \times 10^{-6}\)).

<table>
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<td></td>
<td></td>
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<td>Methanogenic</td>
<td>+</td>
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<td>(4.0 \times 10^{-4})</td>
<td>(5.5 \times 10^{-4})</td>
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<tr>
<td>Denitrifying</td>
<td>+</td>
<td>&lt; &lt;</td>
<td>&lt; &lt;</td>
<td>&lt; &lt;</td>
<td>−</td>
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<td>Iron-reducing</td>
<td>−</td>
<td>&lt; &lt;</td>
<td>3.0 (\times 10^{-2})</td>
<td>(4.8 \times 10^{-5})</td>
<td>+</td>
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<tr>
<td>Aerobic</td>
<td>−</td>
<td>(1.4 \times 10^{-3})</td>
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<td>+</td>
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<td>&lt; &lt;</td>
<td>(1.7 \times 10^{-3})</td>
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### Table 3 – Phylum- and order-level classification of isolated 16S rRNA gene clones from 1,2-DCA transforming microcosms at methanogenic, denitrifying and iron-reducing conditions. Numbers indicate relative abundance of clones grouped by order in % of the total number of clones.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Order</th>
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<th>Iron-reducing</th>
<th>Denitrifying</th>
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*Supplementary Figure S1.* Positioning in a bacterial phylogenetic tree of the 16S rDNA clones obtained from three microcosms. Clones from the methanogenic, denitrifying and iron-reducing microcosms are indicated in bold by “MET”, “NO₃” or “Fe”, respectively. Numbers in brackets indicate the amount of clones at that position in the tree.
Characteristic for the methanogenic microcosm is the high number of sequences that were most closely related to bacteria within the Syntrophobacteria, including the genera Pelotomaculum, Smithella and Synthrophus. These organisms are often involved in syntrophic consortia that degrade organic compounds. Sequences affiliated with the genus Dehalococcoides were also found at high relative abundance (6%) in the methanogenic microcosm. The sequence identity of these 16S rRNA genes with known Dehalococcoides spp., was relatively low (91%), and clustered separately from the known Dehalococcoides spp., indicating a specific phylogenetic position (Fig S1).

In the iron-reducing microcosm only two Phyla were represented in the corresponding clone library. Most of the detected sequences were closely related to Burkholderiales spp. and Clostridiales spp., which includes the genera Thiobacillus, Acidovorax or Clostridium (Fig S1). For those taxa, one or more iron-reducing species have previously been described (Finneran et al., 2003; Johnson et al., 2001; Lin et al., 2007; Ottow, 1971; Sugio et al., 1985; Vu et al., 2004).

In the denitrifying microcosm a relatively high abundance of Rhodocyclales spp. was found, including the denitrifying genera Azoarcus and Thauera (Fig S1). These organisms are known to degrade aromatic pollutants. In the study of Dinglasan-Panlilio et al. (2006), Thauera-like bacteria were found to be responsible for 1,2-DCA degradation under denitrifying conditions. A total of 4% of the clones shared highest similarity with sulfate-reducing Desulfuromonas spp. or Desulfovibrio spp., some of which are known as dehalogenating bacteria (Smidt and de Vos, 2004).

4. Discussion

The aim of this study was to assess the potential for biodegradation of 1,2-dichloroethane by microbial communities of sediments originating from European rivers at different redox conditions, and to identify biodegradation mechanisms and micro-organisms involved.

1,2-DCA was detected in the water phase of all sampled rivers (Ebro, Danube and Elbe). However, biodegradation capacity of 1,2-DCA was only detected in the sediments, whereas it appeared not present in the water phase. This indicates that the relevant microbial community is present in the sediments, which therefore act as an important sink of 1,2-DCA from the water phase.

Although 1,2-DCA can in principle biologically be degraded under aerobic conditions (Janssen et al., 1985; van den Wijngaard et al., 1992), the present microcosm study showed that in the river systems, transformation of 1,2-DCA occurred only under anaerobic conditions. Anaerobic 1,2-DCA transformation was observed under three different redox conditions: methanogenic, denitrifying and iron-reducing. Under methanogenic conditions, the major process of 1,2-DCA transformation was reductive dechlorination. Initially, the reductively dechlorination products chloroethane and ethene (Holliger and Zehnder, 1996) were detected. The observed half-lives of about 7 weeks were relatively fast compared to half-lives of 1,2-DCA in the environment, estimated at 64–165 days (Belay and Daniels, 1987; Egli et al., 1987) or 1–30 years (Bosma et al., 1998), but lower than those previously reported from highly enriched cultures (1.2 days) (Gerritse et al., 1999).

Specialized reductive dehalogenating bacteria, such as Dehalococcoides spp., were enriched in the methanogenic microcosms and identified as one of the relatively dominant populations in the clone library, albeit at only moderate 16S rRNA gene sequence similarity with known Dehalococcoides spp. (Fig S1). As the observed high relative abundance of Dehalococcoides spp. based on clone library analysis was in disagreement with that based on real time PCR, we tested and found that the cloned Dehalococcoides spp.-like 16S rRNA gene sequences were inefficient amplified (recovery of 0.01–0.1%) by the method used in this study and by many others. This indicates that the diversity of dehalogenating Dehalococcoides-like bacteria is broader than initially anticipated and is in line with previous reports of dehalogenating populations within the Chloroflexi spp., which are only distantly related to the genus Dehalococcoides, including the DF-1/o-17 clade of marine PCB-dehalogenating bacteria, as well as the Lahn- and Tidal Flat-clusters of PCE-degrading bacteria (Fagerovd et al., 2007; Kittelmann and Friedrich, 2008a,b; Watts et al., 2005).

Transformation of 1,2-DCA under denitrifying or iron-reducing conditions has been reported rarely (Cox et al., 2000; Dijk et al., 2005; Dinglasan-Panlilio et al., 2006; Gerritse et al., 1999). Although it could not be unequivocally concluded, which bacteria or degradation pathways were responsible for the observed 1,2-DCA transformation, this study provided further evidence for nitrate-coupled oxidation of 1,2-DCA. Transformation of 1,2-DCA did not proceed via a reductive dechlorination pathway, since no dechlorination products or reductively dechlorinating micro-organisms were detected. The observed 1,2-DCA dechlorination, nitrate reduction and chloride formation were in accordance with the stoichiometry of nitrate-coupled 1,2-DCA oxidation as previously proposed by Dijk et al. (2005): C2H4Cl2 + 2NO3 → 2CO2 + 2Cl− → N2 + 2 H2O, whereby it is assumed that some NO3 was reduced with other electron-donors in the microcosm.

For iron-reducing conditions, also no evidence for a reductive pathway was obtained. An iron-coupled anaerobic 1,2-DCA oxidative process is likely, since 1,2-DCA was biologically transformed in parallel with reduction of a stoichiometric amount of iron required for complete oxidation of 1,2-DCA: C2H4Cl2 + 10Fe(OH)3 + 18H+ → 2CO2 + 2Cl− + 10Fe2+ + 26H2O. Of the clones from this microcosm, 40% clustered with taxa for which one or more iron-reducing species have been described, while these organisms were not detected in the clone libraries from the methanogenic or denitrifying microcosm.

In contrast to Ebro and Danube sediment, no degradation of 1,2-DCA was observed in microcosms with Elbe river sediment. The apparent absence of 1,2-DCA in Elbe sediment may be caused by a higher oxygen concentration in the water phase and a much lower amount of organic material compared to the other two river sediments (Table 1). These conditions may have constrained the development of anaerobic niches for 1,2-DCA dechlorinating bacteria, as they were not detected in the sediment. The artificial reconstruction activities of the Elbe river bank one year before the sampling campaign may have disturbed the 1,2-DCA degradation capacity. Thus a significant period of time may be required to re-establish an anaerobic dechlorinating potential after
physical perturbations of river sediment. More generally, enrichment of different microbial populations can be linked to different origin, geochemical conditions or pollution. This may explain the observed variation in degradation capacity in microcosms derived from different rivers under the selected redox conditions.

In conclusion, this study showed that biodegradation capacity of 1,2-DCA was present in the sediments, but not in the water phase, indicating that the sediments act as a sink for 1,2-DCA. Under methanogenic conditions, 1,2-DCA can be reductively dechlorinated to chloroethene and ethene in sediment from Ebro river, or to ethene in sediment from Danube river. Under denitrifying or iron-reducing conditions, 1,2-DCA can be degraded by oxidation in sediment from Ebro river or Danube river, respectively. For further description, modeling and forecasting pollutant biodegradation in river systems, the variability in microbial communities, geochemical conditions and associated oxidative and reductive bioconversion processes need to be taken into account.

Acknowledgments

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Appendix A. Supplemental material

Supplementary information for this manuscript can be downloaded at doi: 10.1016/j.watres.2009.04.042.

REFERENCES


http://www.arb-silva.de.


