

Degradation of chlorobenzenes by autochthonous bacteria from a polluted aquifer

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Abstract

Anaerobic groundwaters and sediments of a site contaminated with chloroorganics in Bitterfeld (Germany) were colonized with bacteria down to the maximum drilling depth of 50 meters. In the autochthonous biocenoses denitrifying and iron-reducing bacteria predominated, but sulfate-reducing bacteria were also present. A surprisingly high abundance of aerobic bacteria was found throughout. The main contaminants in the quaternary aquifer were monochloro-, 1,2- and 1,4- dichlorobenzene. These substances were degraded simultaneously by the autochthonous biocenoses after addition of different terminal electron acceptors. Under aerobic conditions monochlorobenzene and 1,4-dichlorobenzene had disappeared completely and 1,2-dichlorobenzene to about 88 % after 20 days. Using nitrate-, sulfate-, iron-, and manganese-reducing conditions all three chlorobenzenes were degraded by about 80 % within 40 days.

Trichloroethane, predominantly occurring in the groundwater of a lower, tertiary aquifer, was not modified by the autochthonous biocenosis of this aquifer under any of the conditions tested.

1. Introduction

The contamination and potential danger to the groundwater in the area of Bitterfeld (Germany) results from leakages during chemical production and dumping of huge amounts of waste products from the chemical industry (mainly chloroorganic compounds) in disused open cast coal mines. With the closure of the open cast mines the groundwater is no longer lowered. A decontamination process in that particular region has been proposed based on the "funnel and gate" principle for the off-flowing anaerobic groundwater which combines physical-chemical techniques for the dehalogenation and microbiological bioremediation (Project SAFIRA).

Because of its importance in bioremediation and environmental protection the microbiological degradation of chloroaromatic compounds has been subject of a number of investigations in the past few years [for reviews see 1 - 3]. Anaerobic degradation is not fully understood yet. Under anaerobic conditions the reductive dehalogenation is the only well known degradation mechanism for higher chlorinated aromatics [4, 5]. Anaerobic

mineralization of halogenated phenols and benzoates using the terminal electron acceptors NO_3^- , SO_4^{2-} or Fe^{3+} has been demonstrated [6,7]. In contrast to benzene chlorobenzenes have not been reported to be degraded through the use of electron acceptors other than oxygen [8]. The aim of this study was to characterize the groundwater biocenoses of two contaminated aquifers and to evaluate the degradation potential of autochthonous bacteria for the primary contaminants monochloro- (MCB), 1,2- dichloro-(1,2-DCB), 1,4- dichlorobenzene (1,4-DCB) and trichloroethane (TCE) under aerobic and anaerobic conditions.

2. Materials and methods

2.1. Enumeration of microorganisms

Colony forming units (CFU) of aerobic bacteria were determined using R2A agar (Difco, Germany) and for CFUs of anaerobes the bacteria were grown on triple sugar iron (TSI) agar (Merck, Germany). Fungi were counted on MYP-agar pH 5.0 (7.0 g malt extract, 0.5 g yeast extract, 1.0 g peptone from soya, and 15 g agar per liter).

Most probable numbers (MPN) were estimated in four replicates using enrichment media for denitrifying bacteria [9], iron-reducing bacteria [10] and sulfate-reducing bacteria [11]. Incubation temperature was 16°C.

2.2. Experimental set-up

Degradation experiments were carried out in 5 ml suspension cultures. Anaerobic cultures were incubated in 11 ml headspace vials with 6 ml N_2/H_2 (95/5, v/v), aerobic cultures in 20 ml vials with 15 ml air. Each of the vials was closed with crimp seals (PTFE-coated butyl rubber).

Chlorobenzenes were added simultaneously to final concentrations of 30 mg MCB, 10 mg 1,2-DCB and 10 mg 1,4-DCB per liter; TCE (trichloroethene) was added to separate cultures to a final concentration of 30 mg per liter.

Cultures contained per liter 250 ml inoculum, 250 ml xenobiotics stock solution in distilled water and a total of 500 ml substrates-, electron acceptors-, and reductants- solutions prepared with sterilized groundwater of the corresponding aquifer.

The influence of auxiliary substrates and macronutrients was investigated by adding per liter medium: 50 mg sodium acetate $\cdot 3 \text{H}_2\text{O}$, 50 mg sodium lactate, 5 mg yeast extract, 25 mg NH_4Cl , and 10 mg $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$. Each liter denitrifying medium contained 0.5 g KNO_3 , sulfidogenic medium contained 0.5 g $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$, 100 mg thioglycollate, 100 mg ascorbic acid, and 20 mg sodium dithionite. The medium for iron-reducing bacteria contained approximately 5 g amorphous FeOOH [preparation according to 12] and 200 mg thioglycollate per liter.

Inoculation was performed with fresh groundwater, which had been used to extract bacteria from 100 g wet mixed aquifer sediments per liter for 20 hours in an overhead shaker (1 min^{-1}) under N_2/H_2 (95/5, v/v)-atmosphere and room temperature.

2.3. Chemical analysis

Chloroaromatic compounds were extracted with n-hexane (Merck, Germany) directly from culture vials and detected using a GC/MS system (EM 640, Bruker Franzen-Analytik, Germany).

TCE was measured by headspace-GC after enrichment by solid phase micro extraction (85 μ m polyacrylate, adsorption 20 min at room temperature, desorption 3 min at injection temperature 250°C). The separation was performed with an DB-5 apolar capillary column (30 m \times 0.25 mm) at 40°C, carrier gas hydrogen, FID detection.

3. Results and discussion

3.1. Microbiological characterization of the site

Bacteria were found in the groundwater and sediments throughout the profile down to the maximum drilling depth of 50.5 m below the surface (Figure 1a).

A more comprehensive picture of the physiological diversity of the autochthonous bacterial biocenoses was obtained using the MPN method combined with selective growth conditions for the different physiological groups (Figure 1b). In the autochthonous biocenoses denitrifying and iron-reducing bacteria predominated, but sulfate-reducing bacteria were also present and have been enriched from every sediment sample. Surprisingly a large number of (facultative) aerobes were found. Colony forming units of fungi were found in few samples and only in very low concentrations ($<10^2$ ml⁻¹, data not presented).

3.2. Degradation of chloroorganics

Evaluation of the degradation potential of the different groups of autochthonous bacteria was carried out by selective stimulation through the addition of various electron acceptors and in some cases reductants to the media.

Experimental controls contained autoclaved groundwater/sediment extract instead of inoculum. They showed a decrease in the concentrations of about 20-30 % for MCB and 1,2-DCB and of about 40-50 % for 1,4-DCB during a period of 80 days. This could be due to diffusion through the lids of the vials and/or irreversible adsorption to surfaces.

Under aerobic conditions MCB and 1,4-DCB disappeared completely during the first 10 days of incubation (Figure 2a), while 1,2-dichlorobenzene was degraded at a lower rate than the other chlorobenzenes, but in contrast to investigations of Feidieker et al. [13] it did not persist. The addition of nutrients did not accelerate the degradation of any of the chlorobenzenes, not even under anaerobic conditions. It seemed to have a weak inhibitory effect at least for the first steps of bioconversion.

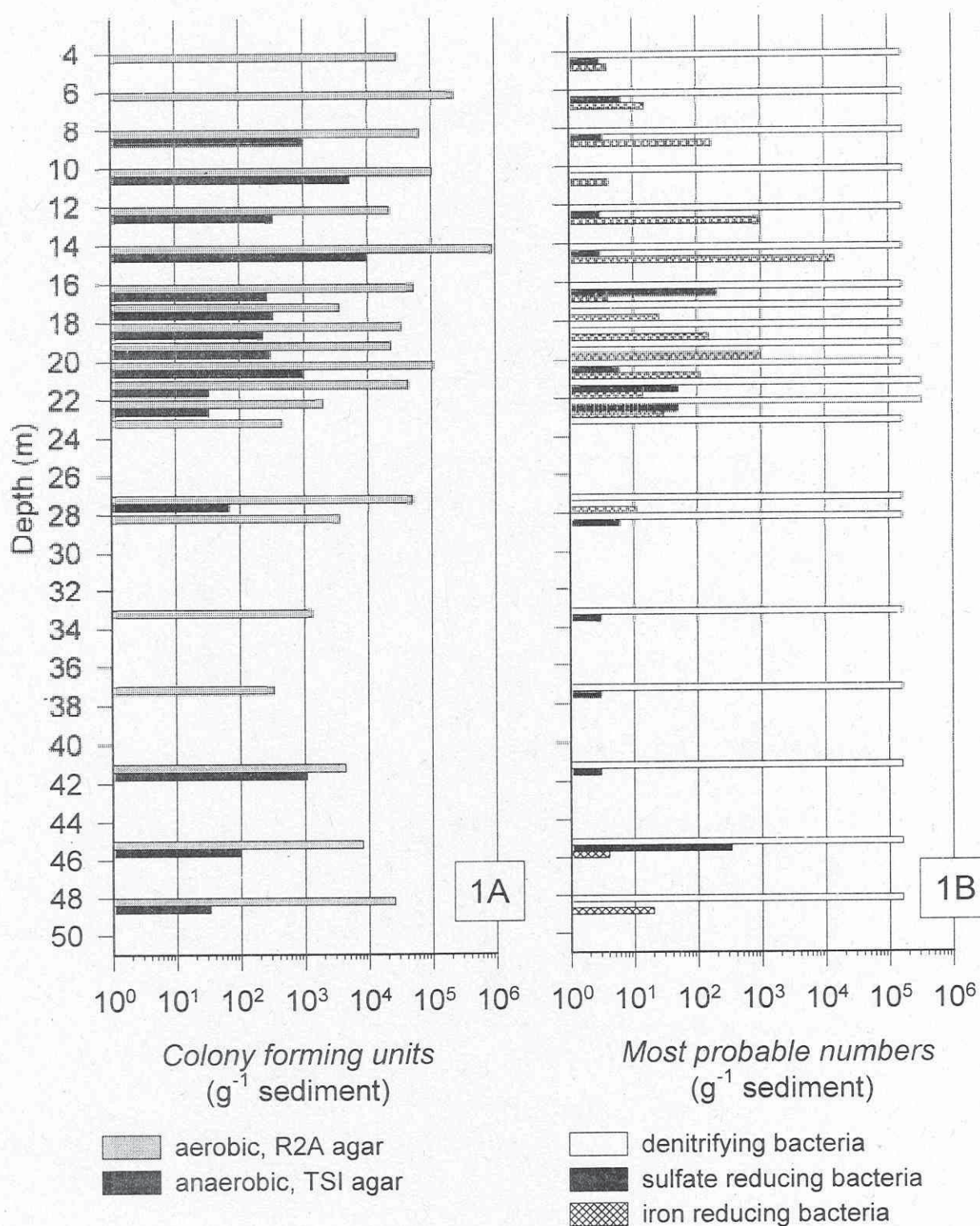


Fig. 1. Abundance of selected groups of bacteria in quaternary and tertiary sediments of a contaminated site (Bitterfeld, Germany)

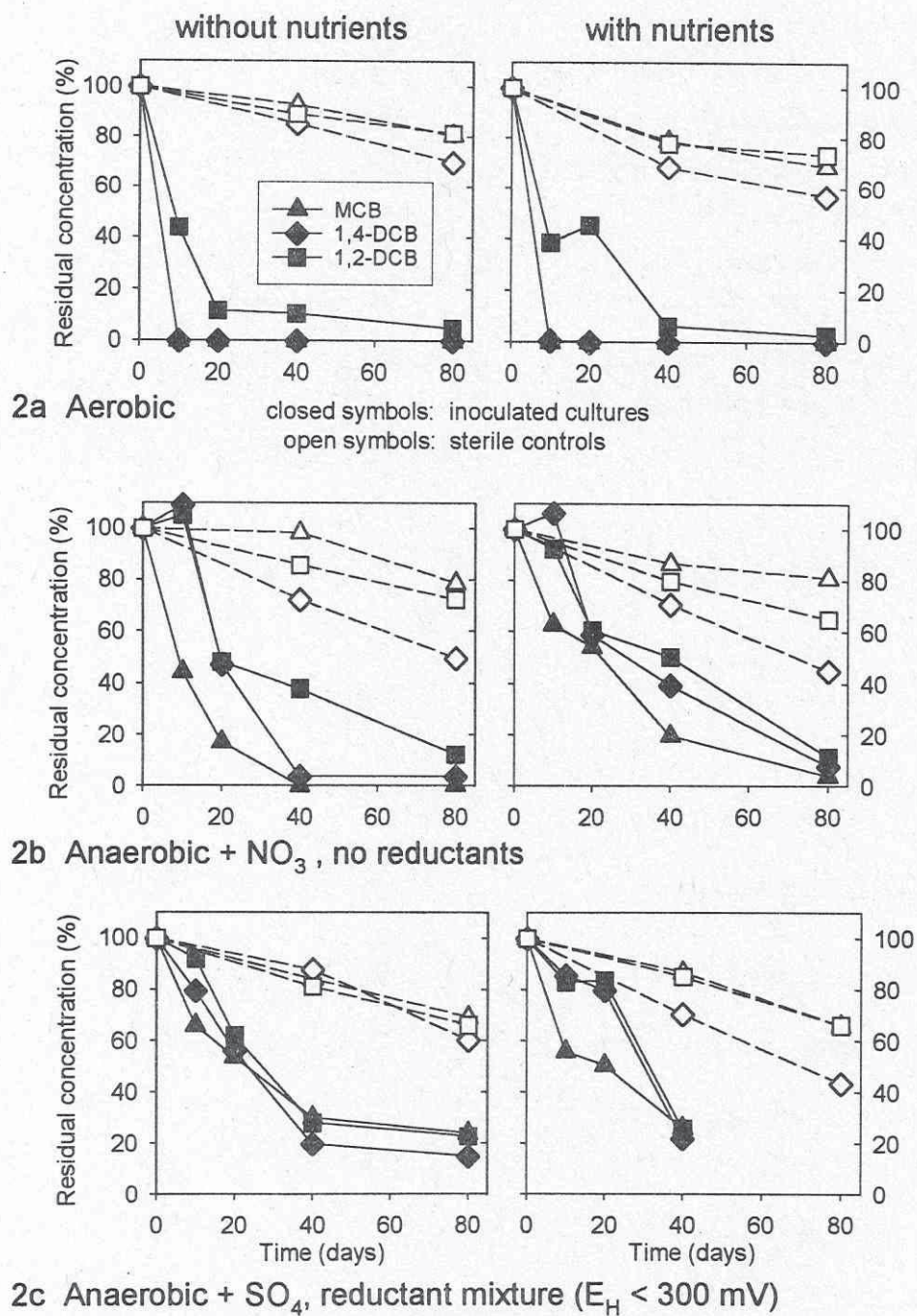


Fig. 2a-c. Simultaneous degradation of mono- and dichlorobenzenes under different culture conditions in groundwater medium at 16°C. All points represent the average of 2 - 3 replicate cultures (5 ml). For legends see figure 2a

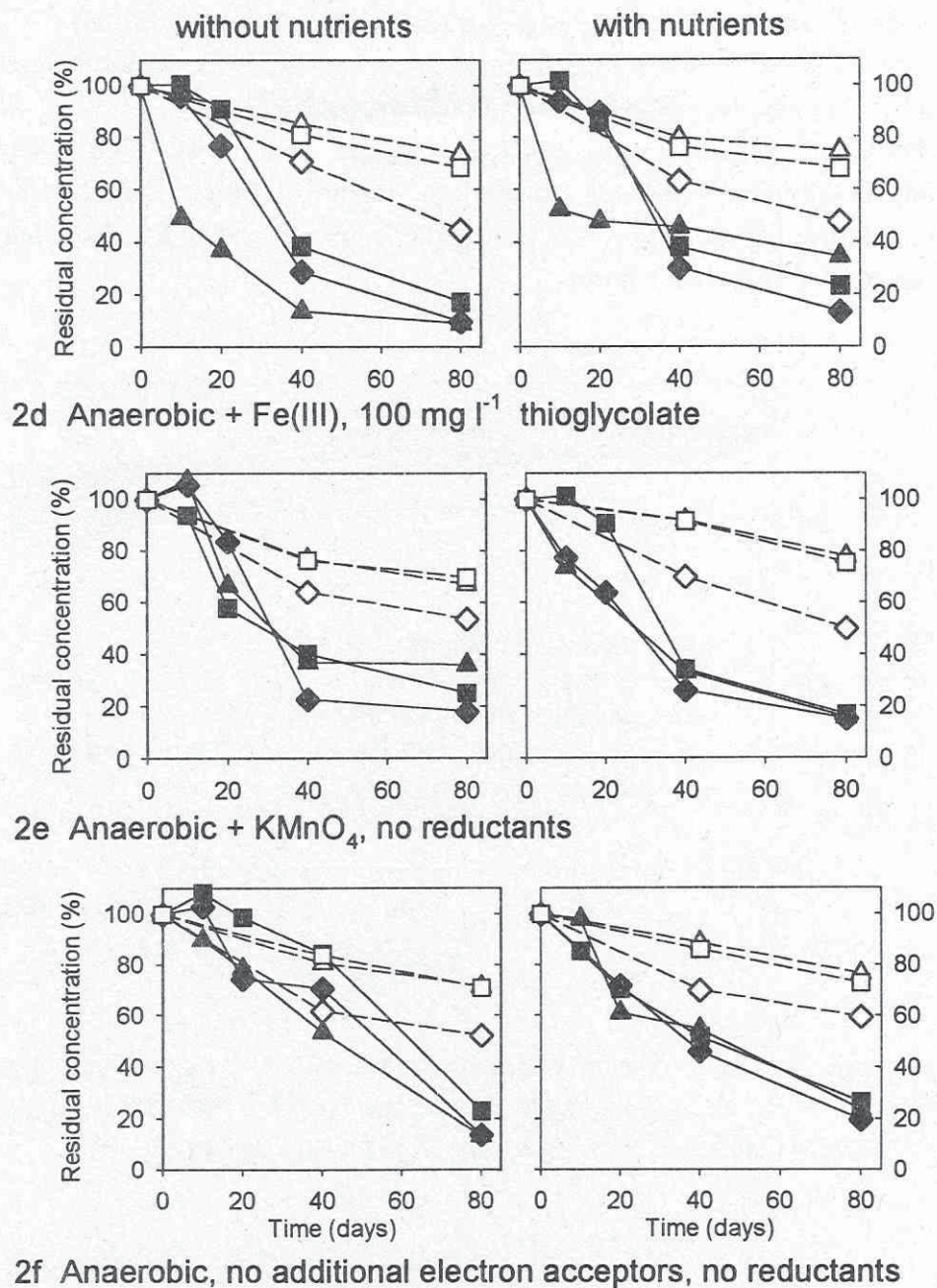


Fig. 2d-f. Simultaneous degradation of mono- and dichlorobenzenes under different culture conditions in groundwater medium at 16°C. All points represent the average of 2 - 3 replicate cultures (5 ml). For legends see figure 2a

Under nitrate-, sulfate-, manganese- and iron- reducing conditions (Figures 2b - e) the 3 chlorobenzenes under investigation disappeared to at least 60 % after 40 days of incubation,

when no other nutrients were added. MCB and 1,4-DCB were degraded in most cases (with the exception of sulfate reducing conditions) at a higher rate than 1,2-DCB. There was no evidence for a long-term persistence of 1,4-DCB which was found by Barber [14]. If no electron acceptors and reductant were added to anaerobic cultures (Figure 2f) the degradation was slowest of all, possibly indicating unsuitable redox conditions for sulfate reduction (sulfate concentrations in the aquifer range from 600 - 1000 mg per liter).

Degradation of TCE by autochthonous bacteria was investigated under identical experimental conditions. It was not modified or degraded neither under aerobic nor any anaerobic conditions. Figure 3 shows data for aerobic, nitrate- and sulfate reducing conditions which are representative for all cultures.

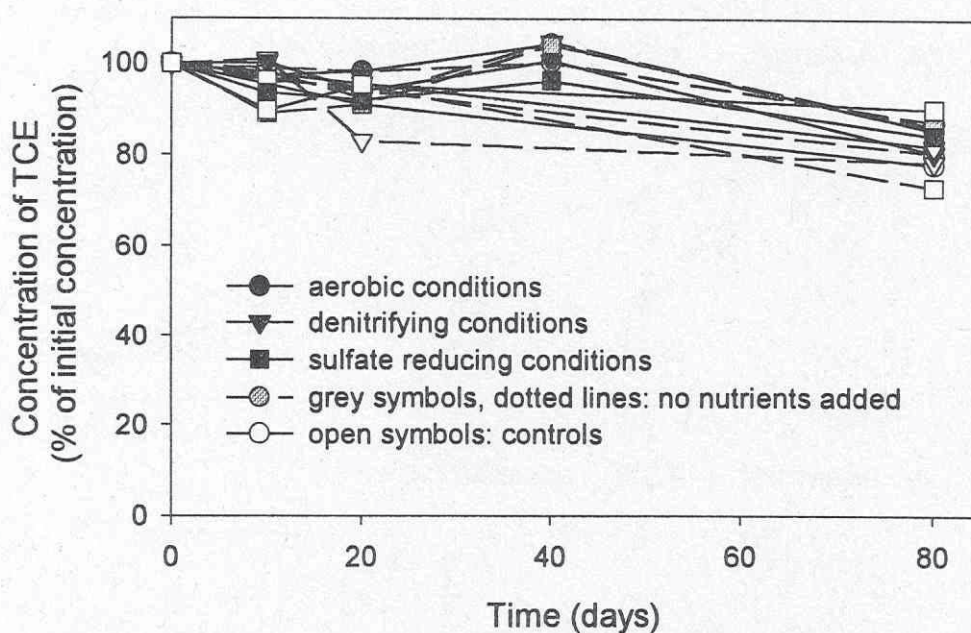


Fig. 3. Persistence of trichloroethene (TCE) under different culture conditions in groundwater medium at 16°C. Each point represents the mean of 2-3 replicate cultures (headspace GC vials with 5 ml medium); sterile controls are single determinations.

The results of this study show that the autochthonous groups of anaerobic/facultatively anaerobic bacteria of this contaminated anoxic aquifer have the potential to degrade the main chloroaromatic contaminants under anaerobic conditions. Identification of metabolites and final products is in progress. In contrast to chlorobenzenes TCE persists under all conditions tested which might indicate *in situ* persistence. This problem needs further attention including the evaluation of methanogenic conditions.

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