



## Integrative approach to delineate natural attenuation of chlorinated benzenes in anoxic aquifers

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*Lines of evidence for in situ biodegradation of chlorinated benzenes in an anoxic aquifer by combining hydrogeochemical and stable isotope data with multivariate statistics.*

### ARTICLE INFO

#### Article history:

Received 14 August 2008

Received in revised form

26 January 2009

Accepted 27 January 2009

#### Keywords:

Monochlorobenzene (MCB)

Dichlorobenzene (DCB)

Compound-specific isotope analysis (CSIA)

Anaerobic degradation

Natural attenuation (NA)

### ABSTRACT

Biodegradation of chlorobenzenes was assessed at an anoxic aquifer by combining hydrogeochemistry and stable isotope analyses. In situ microcosm analysis evidenced microbial assimilation of chlorobenzene (MCB) derived carbon and laboratory investigations asserted mineralization of MCB at low rates. Sequential dehalogenation of chlorinated benzenes may affect the isotope signature of single chlorobenzene species due to simultaneous depletion and enrichment of <sup>13</sup>C, which complicates the evaluation of degradation. Therefore, the compound-specific isotope analysis was interpreted based on an isotope balance. The enrichment of the cumulative isotope composition of all chlorobenzenes indicated in situ biodegradation. Additionally, the relationship between hydrogeochemistry and degradation activity was investigated by principal component analysis underlining variable hydrogeochemical conditions associated with degradation activity at the plume scale. Although the complexity of the field site did not allow straightforward assessment of natural attenuation processes, the application of an integrative approach appeared relevant to characterize the in situ biodegradation potential.

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### 1. Introduction

Chlorinated aromatic compounds are intensively used worldwide to synthesize pesticides and other chemicals leading to ubiquitous distribution in the environment (Field and Sierra-Alvarez, 2008). Chlorinated benzenes (CBs) can also be formed during anaerobic microbial transformation of hexachlorocyclohexanes, of which the  $\gamma$ -isomer (Lindane) is worldwide used as pesticide (Van Agteren et al., 1998; Phillips et al., 2005). Due to their toxicity, persistence and accumulation in the food chain CBs are of great environmental concern.

CBs may be subjected to both aerobic and anaerobic microbial degradation (Van Agteren et al., 1998; Adrian and Gorisch, 2002; Field and Sierra-Alvarez, 2008). With increasing chlorination CBs tendency for mineralization by oxidative processes decreases but the potential for preferential transformation by reductive dehalogenation increases (Van Agteren et al., 1998; Adrian and Gorisch,

2002). The occurrence of reductive dehalogenation under methanogenic and sulfate reducing conditions has been observed in a variety of anaerobic mixed cultures (Van Agteren et al., 1998; Adrian and Gorisch, 2002), but only one bacterial strain (*Dehalococcoides* strain CBDB1) capable to couple energy conservation with reductive dehalogenation of CBs ( $\geq 3$  chlorine substituents) has been isolated so far (Adrian et al., 2000; Jayachandran et al., 2003). Reductive dehalogenation generally results in the transformation of higher chlorinated CBs but may lead to an accumulation of lower chlorinated CBs such as monochlorobenzene (MCB) under strongly reducing conditions. Preliminary indications for reductive dehalogenation of MCB have been presented (Nowak et al., 1996). Kaschl et al. (2005) suggested that anaerobic MCB degradation may lead to significant stable carbon isotope fractionation and applied compound-specific isotope analysis (CSIA) to further indicate in situ biodegradation of MCB in an anoxic aquifer. Recently, complete mineralization of MCB under anaerobic conditions has been shown using stable isotope tracer techniques, which enabled very sensitive detection of low rate degradation processes (Nijenhuis et al., 2007b).

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CSIA has been established for monitoring the biodegradation of pollutants at contaminated field sites (Meckenstock et al., 2004a). This method was successfully applied for monoaromatic hydrocarbons (Mancini et al., 2002; Griebler et al., 2004b; Vieth et al., 2005; Fischer et al., 2007), fuel oxygenates (Kuder et al., 2005; Zwank et al., 2005) and chlorinated ethenes (Hunkeler et al., 1999a; Sherwood Lollar and Slater, 2001; Nijenhuis et al., 2005, 2007a; Imfeld et al., 2008) but, to our knowledge, beside the work of Kaschl et al. (2005) no further field studies were performed for CBs. Except a few studies on chlorinated ethenes, most publications deal with in situ biodegradation of single contaminants instead of addressing complex scenarios such as sequential degradation mechanisms, multiple contaminants and/or various degradation pathways.

Microbial degradation of organic contaminants is associated with isotope fractionation, leading to an enrichment of heavier isotopes in the residual non-degraded fraction. The magnitude of isotope fractionation mainly depends on the reaction mechanism. As shown for reductive dehalogenation of chlorinated ethenes (Nijenhuis et al., 2005; Cichocka et al., 2008) and trichlorobenzenes (TCB) (Griebler et al., 2004a), degradation of CBs should also be associated with significant isotope fractionation, if the reduction of a C–Cl-bond is involved. Similar to anaerobic degradation of monoaromatic hydrocarbons, the anaerobic oxidation of the benzene ring, for which cleavage of a C–H-bond is expected, should lead to isotope fractionation (Mancini et al., 2003; Fischer et al., 2008). However, if the fate of a contaminant is simultaneously controlled by its production (associated with depletion in  $^{13}\text{C}$ ) and further degradation (leading to enrichment in  $^{13}\text{C}$ ), as expected from sequential dechlorination, changes in the isotope signature of a contaminant cannot be attributed to a single process (i.e. biodegradation) which is limiting the use of the Rayleigh approach (Hunkeler et al., 1999a; Nijenhuis et al., 2005).

At the study site, release of CBs resulted in a severe contamination of the groundwater. It is hypothesized that reductive dehalogenation and/or anaerobic oxidation may govern the removal of CBs under anoxic conditions in the aquifer. Because active remediation technologies were recognized as technically and economically not feasible (Thullner and Schäfer, 1999), this study aimed at evaluating the efficacy of natural attenuation (NA) processes as remedial option with an emphasis on most persistent MCB, since its accumulation seems to be the limiting factor to apply NA.

For this purpose, following the US-EPA directive for monitored NA approaches (US-EPA, 1999), we developed an integrative approach which includes the assessment and monitoring of chlorobenzene degradation by means of CSIA and tracer studies under field and laboratory conditions and a parallel delineation of major hydrogeochemical trends associated with observed in situ biodegradation activity. To overcome complications associated with the Rayleigh concept for interpretation of the isotope data, an isotope balance was calculated to assess biodegradation of CBs.

## 2. Material and methods

### 2.1. Study site and sampling

The field site is located at a former chemical plant where the production of mostly pesticides caused a release of a large volume of chlorinated organic contaminants to the subsurface. The direct source zone characterized by high loads of contaminants and dense nonaqueous phase liquid (DNAPL) was completely encapsulated in 1998 and hydraulically disconnected from the surrounding natural groundwater system to prevent further release of contaminants. The initial contamination was dominated by tri- (TCB), tetra- (TeCB) and pentachlorobenzenes (PeCB) and is preserved inside the containment whereas the resulting plume mainly consists of MCB and dichlorobenzene (DCB) with total concentrations up to  $2840 \mu\text{g L}^{-1}$ . A detailed site description is given in supporting information (SI) (Fig. S2, Section 1.1).

Groundwater samples for hydrogeochemical and isotope analyses were taken in 2005 and 2006 from 22 monitoring wells (Fig. 1). The sampling, extraction procedure and analytical methods are described in SI. A table summarizing the geochemical data collected during sampling in 2005 is given in SI (Table S2).

### 2.2. Chemicals

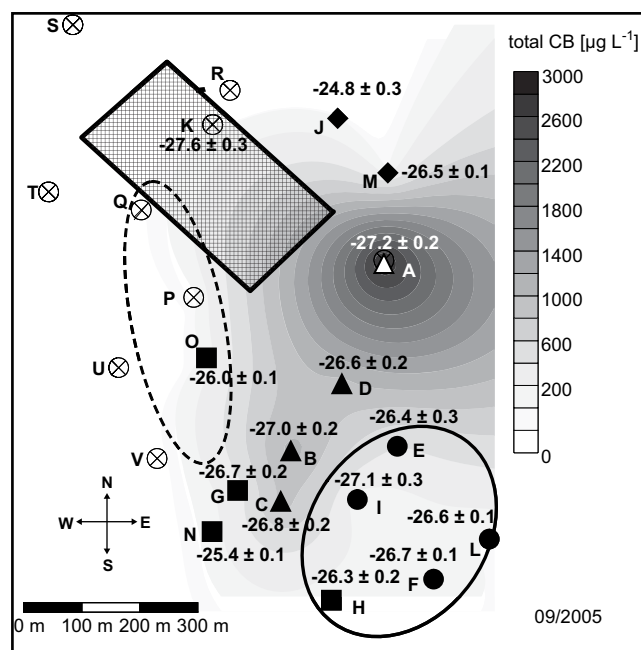
The  $^{13}\text{C}_6$  MCB was purchased from Chemtrade Leipzig (Germany) with chemical and isotopic purity of >99%. All other chemicals used were obtained in p.a. quality or higher.

### 2.3. Field study with in situ microcosms

In February 2005 a field experiment was performed using an in situ microcosm test system as described elsewhere (Peacock et al., 2004; Geyer et al., 2005; Kästner et al., 2006; Stelzer et al., 2006a; Nijenhuis et al., 2007b). Briefly, Bio-Sep® beads were loaded with  $^{13}\text{C}_6$  MCB to a concentration of about  $100 \text{ mg g}^{-1}$  beads. The in situ microcosms were deployed in 6 different wells at the low (H, L), medium (D, E) and high contaminated (A, B) area of the plume in the lower strictly anoxic part of the aquifer (Fig. 1 and Fig. S1). After 72 days in situ microcosms were removed from the aquifer and the carbon isotope signature of total lipid fatty acids (TLFA) was analyzed. Detailed information about the extraction procedure and analytical methods can be found in SI (Section 1.4).

### 2.4. Laboratory study with enrichment cultures

Material of in situ microcosms amended with natural abundance MCB as described above was used as inoculum for the anaerobic laboratory enrichment cultures which were prepared under strict anoxic conditions (see SI). From each selected well (A, B, D, E, H, L) four enrichment cultures were prepared in 38 mL vials. Each vial finally contained approx. 27 mL of groundwater and 4–6 Bio-Sep® beads. MCB was added to the vials: always two cultures were amended with  $1 \mu\text{L}$  unlabeled MCB and another two with  $^{13}\text{C}_6$  MCB. Cultures were incubated stationary at  $20^\circ\text{C}$  in the dark and were sampled at regular time intervals to determine the carbon isotope signature of possible mineralization products ( $\text{CO}_2$ ,  $\text{CH}_4$ ). A more detailed description of the methods is given in SI (Section 1.5).



**Fig. 1.** Concentrations [ $\mu\text{g L}^{-1}$ ] and carbon isotope signatures [‰] of total chlorinated benzenes (numbers) at the study site for sampling in 2005. The letters indicate the name of the wells and the rectangle in diagonal crosses surrounds the area of the containment, which is hydraulically disconnected from the plume. Additionally the dashed (---) black line shows the area where the ratio between MCB and DCB is  $<0.3$  and the solid (—) line indicates where the ratio between MCB and DCB is  $>0.9$ . The symbols indicate the samples which belong to a common cluster as obtained by PCA: ▲ – cluster I (A, B, C, D); ■ – cluster II (G, H, N, O); ● – cluster III (E, F, I, J); ◆ – cluster IV (J, M) (see Fig. 3). Wells which were not considered for PCA are indicated by ⊗.

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