Use of C–Cl CSIA to elucidate origin and fate of DCM in complex contaminated field sites

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Abstract. We used C-Cl dual isotope analysis and microcosm studies for elucidating the origin and fate of the common groundwater pollutant dichloromethane (DCM) in two different multi-contaminant field sites in Catalonia, Spain; where DCM contamination could be the result of direct solvent releases and/or chloroform (CF) transformation. Known commercial solvents isotopic compositions as well as characteristic C-Cl dual isotope slopes from our anaerobic enrichment culture containing Dehalobacterium sp., capable of fermenting DCM, and other bacteria from the literature were used for field data interpretation.

1 Introduction

Dichloromethane (DCM) can be naturally released; however, it is often detected in subsurface waters as a result of its extensive use and improper handling in industrial facilities, causing severe aquifer contamination [1]. Besides its direct anthropogenic release, DCM can also appear as a by-product in the transformation reactions of trichloromethane (usually referred as chloroform, CF), another contaminant frequently detected in groundwater [2].

As DCM is often found diluted in groundwater or accumulated as DNAPL in the deeper parts of aquifers, biodegradation under anoxic conditions is one of the feasible alternatives for its elimination. In absence of oxygen, several bacteria have been reported to

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successfully degrade DCM via the fermentative pathway, such as Dehalobacterium formicoaceticum [3], several mixed bacteria cultures containing Dehalobacter sp. and/or Dehalobacterium sp. [4–6], and a new bacterium identified as Candidatus Dichloromethanomonas elyunquensis [7,8].

Compound specific isotope analysis (CSIA) quantifies the abundance ratio of specific stable isotopes (e.g. $^{13}$C/$^{12}$C, $^{37}$Cl/$^{35}$Cl) in molecules of contaminants relative to an international standard [9]. This technique bears the potential for both contaminant source apportionment as well as monitoring of compound transformation processes [10]. Moreover, isotopic analysis of two elements from the same compound (e.g. C and Cl) allowed more precise identification of pollutant’s origin (related to commercial solvents or its potential parental compound). Dual isotope slopes ($\Lambda$) derived from combination of C and Cl isotope data from field samples reflect ongoing degradation mechanisms and can be compared with characteristic $\Lambda$ values from laboratory studied reactions [11,12].

Previously, we reported carbon isotopic fractionation of DCM ($\varepsilon_{C} = -27 \pm 2\%$) of an anaerobic enrichment culture containing Dehalobacter sp. during DCM fermentation and differing from Dehalobacter and methylotrophic organisms [4]. Recently, several laboratory studies have applied C–Cl dual isotope analysis to characterize DCM biodegradation by different bacteria [13,14]. To compare Dehalobacterium sp. culture mechanisms for the elimination of DCM and to evaluate the use of 2D-CSIA as a tool for assessing DCM origin and transformation reactions in contaminated sites, carbon and chlorine stable isotopes of laboratory and field data have been analysed.

2 Materials and methods

2.1 Groundwater sampling

After measuring in situ the principal hydrogeochemical parameters, groundwater samples for chemical and isotopic characterization were collected with a peristaltic pump or bailer and immediately preserved with HNO$_3$ at pH~2 to stop any biodegradation processes from target boreholes at two different multi-contaminant field sites in Catalonia, Spain. In both sites, DCM contamination could be the result of direct solvent releases and/or CF transformation. In addition, groundwater samples with fine sediments were collected from the bottom of the boreholes to prepare the microcosm experiments in the laboratory.

2.2 Establishment of microcosms

To investigate whether exists an intrinsic biodegradation potential of CF and DCM or demonstrates inhibition issues due to the presence of other co-contaminants in any of the studied sites, three different microcosm experiments were prepared in triplicate containing: (1) only groundwater as a natural attenuation control, (2) groundwater biostimulated with sodium lactate, and (3) groundwater inoculated with a commercial bacterial consortium, capable of CF and DCM degradation, and sodium lactate.

2.3 Analytical methods

For all samples, concentrations of target contaminants were quantified by analysing bottles headspace as indicated elsewhere [15]. Lactate and other short fatty-acids were monitored by HPLC [16].

Stable carbon isotopes composition ($\delta^{13}$C) of target contaminants in the field samples were analysed in duplicate by headspace solid-phase microextraction (HS-SPME) [15]. The
\( \delta^{13} \)C of the pure in-house standards measured along with the samples had been determined previously using six international reference materials with respect to the VPDB standard [17].

Stable chlorine isotope composition (\( \delta^{37} \)Cl) from field and laboratory samples were performed at Isotope Tracer Technologies Inc., Waterloo (ON, Canada). Target contaminants were extracted from diluted aqueous samples by HS-SPME and analysed in duplicate. The \( \delta^{37} \)Cl of pure in-house standards was calibrated to SMOC using an offline methodology [18,19].

A simplified version of the Rayleigh equation in the logarithmic form (Eq. 1) was used to quantify the chlorine isotopic fractionation (\( \varepsilon \text{Cl} \)) [10] of DCM during its degradation by an enrichment culture containing *Dehalobacterium* sp.,

\[
\ln \left( \frac{R_t}{R_0} \right) = \varepsilon \cdot \ln \left( f \right) \tag{1}
\]

where \( R_t/R_0 \) can be described as \( (\delta^{37}\text{Cl}_t + 1) / (\delta^{37}\text{Cl}_0 + 1) \) according to \( \delta^{37} \)Cl definition. Laboratory isotope data was processed and plotted according to Eq. (1), and the slope of the linear regression was used to determine \( \varepsilon \text{Cl} \) with a 95% confidence interval uncertainty.

Changes in carbon isotope signatures (\( \delta^{13} \)C) were plotted against changes in chlorine isotope signatures (\( \delta^{37} \)Cl) to determine the lambda value (\( \Lambda^{C/Cl} \), Eq. 2) of DCM during its transformation by the investigated culture, which derives from the slope of the linear regression of the combined data [10,20].

\[
\Lambda^{C/Cl} = \frac{\Delta \delta^{13} C}{\Delta \delta^{37} Cl} \tag{2}
\]

\( \Lambda^{C/Cl} \) uncertainty was calculated with a 95% confidence interval.

### 3 Preliminary results and discussion

#### 3.1 \( \varepsilon \text{Cl} \) and \( \Lambda^{C/Cl} \) for the DCM-degrading enriched culture

The \( \delta^{37} \)Cl increased significantly during DCM fermentation by the stable DCM-degrading culture containing *Dehalobacterium* sp., reaching values \( > +5\% \). The correlation coefficient of the linear regression (\( R^2=0.95 \)) following equation (2) suggests that such degradation can be well described by the Rayleigh model (Fig. 1A), leading to an \( \varepsilon \text{Cl} \) value of \(-3.7 \pm 0.6\%\).

The linear regression (\( R^2=0.98 \)) of the \( \delta^{13} \)C versus \( \delta^{37} \)Cl plot for DCM degradation by the enriched culture yielded a \( \Lambda^{C/Cl} \) value \( (7.0 \pm 0.7) \) that is higher than that reported previously for *Ca. Dichloromethanomonas elyunquensis* \( (3.40 \pm 0.03) \) but similar to that of *D. formicoaceticum* \( (7.89 \pm 0.12) \) [13].
Fig. 1. (A) Double logarithmic Rayleigh plot of the chlorine isotope ratio versus the residual concentration of DCM (○) during degradation by a culture containing *Dehalobacterium* sp. (B) Dual C-Cl isotope plot for the anaerobic degradation of DCM (i) by the *Dehalobacterium* culture and (ii) by *D. formicoaceticum* and by *Ca. Dichloromethanomonas elyunquensis* from a previous study [13]. Solid lines for the *Dehalobacterium* culture in each panel depict the corresponding linear regression and dashed lines represent the associated 95% confidence intervals. Data points show the error bars related to duplicate measurements.

3.2 Fate of DCM and CF in the field

Most of the measured values of $\delta^{13}C_{CF}$ and $\delta^{37}Cl_{CF}$ (for CF) in both field sites were within or very near the isotopic range of known commercial CF and they were not significantly different across the studied boreholes to clearly conclude that CF biodegradation was occurring [21]. Moreover, for one of the field sites, even the microcosms prepared with the commercial bacterial consortium showed no CF biodegradation, evidencing inhibition issues and supporting the same conclusion.

To determine DCM origin has been struggling, but $\delta^{13}C_{DCM}$ and $\delta^{37}Cl_{DCM}$ values across the studied boreholes showed significant differences and they were, in both fields, outside of the known DCM commercial standards, which would suggest that, to some extent, biodegradation could be feasible. Furthermore, microcosms of one of the sites supported both CF and DCM biodegradation even at natural conditions, since their elimination was observed.

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