CL:AIRE

AB 10
(March 2015)

ADVOCATE bulletin

CL:AIRE's ADVOCATE bulletins describe practical aspects of research which have direct application to the characterisation, monitoring or remediation of contaminated soil or groundwater. This bulletin describes isotope analysis to assess the biodegradation of chlorinated ethenes.

Dual C-Cl isotope analysis to distinguish processes affecting chlorinated ethenes at field scale

1. Introduction

Sites contaminated with chlorinated ethenes are numerous in Europe and the USA and are still of concern despite their origins dating back to the 1940s-1970s. The earlier inappropriate disposal and accidental spills of these solvents used by industries for dry-cleaning and metal-degreasing purposes are the cause of groundwater contamination as they infiltrate in the soil and migrate deep into aquifers due to their high density.

Several clean-up approaches exist such as *in situ* remediation methods which include degrading the contaminant biologically or chemically directly in the subsurface. To date, several microorganisms (e.g. *Dehalococcoides*, *Sulfurospirillum*, *Desulfitobacterium*) are known to dechlorinate tetrachloroethene (PCE) according to the following sequence: PCE → TCE (trichloroethene) → cDCE (*cis*-dichloroethene) → VC (vinyl chloride) → non-toxic compounds even though not all of them are able to catalyse the whole chain, while chemical degradation (whether oxidation or reduction) yields virtually only non-toxic compounds (Figure 1 illustrates a biodegradation pathway for PCE).

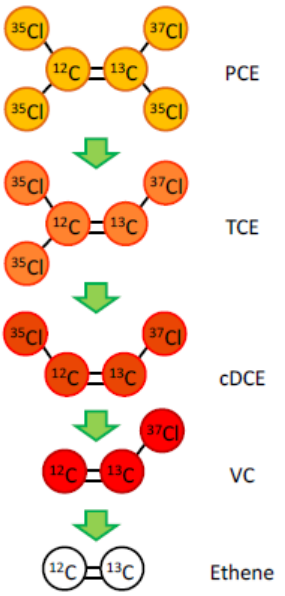

Figure 1. One biodegradation pathway for PCE.

Is the contaminant concentration decrease due to degradation or to dilution? Does the injected chemical degrade the contaminant or is the observed degradation carried out by microorganisms already present in groundwater? These are all pertinent questions that one might be confronted with when dealing with such remediation strategies. More fundamentally, one could also be interested in understanding which mechanism underlies the dechlorination process.

Dual carbon-chlorine (C-Cl) isotope analysis is a promising tool to explore such questions (Meckenstock *et al.*, 2004; Elsner *et al.*, 2005). Through the application of this method to PCE during its dechlorination by two distinct bacterial consortia and in two field sites undergoing PCE dechlorination, we concluded that PCE bacterial dechlorination could be carried out according to two different processes. As a result, dual C-Cl isotope data should be used carefully to distinguish degradation processes in the field as their interpretation is not straightforward.

2. Background to Study Sites

The two study sites, X and Y, were located in Switzerland and exhibited PCE plumes of 800 m and 50 m, respectively. Site X previously used PCE for the processing of slaughterhouse waste while site Y was used for dry-cleaning purposes. PCE concentrations at each site varied from 100 to 1500 ppb, and all metabolites (i.e. TCE, *c*DCE, VC) were measured to a certain extent along the plume, supporting the occurrence of biological dechlorination. On each site, five wells representing various proportions of the contaminants were selected for the study and sampled once during summer 2013.

3. Isotope Analysis

In nature, C and Cl occur as isotopes ^{12}C (98.93%) or ^{13}C (1.07%) and ^{35}Cl (75.76%) or ^{37}Cl (24.24%), respectively. In a compound, the proportion of stable C and Cl isotopes, also denoted as C and Cl isotope ratios $\delta^{13}C$ and $\delta^{37}Cl$, can be measured using Gas-Chromatography coupled to an Isotope Ratio Mass Spectrometer (GC-IRMS) or to a quadrupole Mass Spectrometer (GC-qMS).

Isotope ratios are calculated as follows:

$$\delta = \left(\frac{R}{R_{std}} - 1\right) \cdot 1000\ [‰] \qquad [1]$$

Where R and R_{std} are the ratios between the heavy and the light isotope of one considered element for the measured sample and the standard, respectively.

During degradation by a chemical or a biological process, the initial substrate isotopic composition changes. Since it is easier from an energetic point of view to break bonds between light isotopes than heavy ones, the substrate gets enriched in heavy isotopes over time.

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This results in an increase of $\delta^{13}C$ and $\delta^{37}Cl$ in the substrate during degradation. Based on these principles, compound-specific single-element isotope analysis (usually $\delta^{13}C$) can be applied in order to identify and characterise biodegradation at the field scale (Hunkeler, *et al.*, 2008). More specifically, an enrichment factor, which reflects the extent to which the substrate gets enriched in heavy isotopes of one element during the process of degradation, can be determined based on microcosm studies using the following Rayleigh equation:

$$\ln \frac{\delta_t + 1000}{\delta_0 + 1000} = \varepsilon_E \cdot \ln f \qquad [2]$$

Where ε_E is the enrichment factor of the element E, δ the isotope ratio of this element in the reactant and f the remaining fraction of reactant.

Assuming first order degradation, the enrichment factor ε may further help quantify the extent of biotic degradation in the field. However, choosing the right enrichment factor remains challenging as they cover a relatively important range (Table 1). This also prevents the use of enrichment factors to gain more insight into the degradation process occurring contrary to what is expected with dual isotope analysis. Enrichment factors alone could thus not help differentiate biotic from abiotic degradation in the field either. An increasing interest is therefore devoted to the use of dual isotope analysis as it could help answering the questions mentioned in the introduction.

Table 1: Range of C enrichment factors associated to PCE, TCE, *c*DCE and VC

Compound	ε_C min (‰)	ε_C max (‰)	Reference
PCE	-0.4	-16.4	Nijenhuis *et al.*, 2005, Cichocka *et al.*, 2008
TCE	-2.5	-18.4	Bloom *et al.*, 2000, Cichocka *et al.*, 2007
cDCE	-14.1	-21.1	Bloom *et al.*, 2000, Lee *et al.*, 2007
VC	-19.9	-31.1	Fletcher *et al.*, 2011, Hunkeler *et al.*, 2002

4. Developed Research

Dual C-Cl isotope analysis of the substrate can sometimes help to identify biodegradation more confidently than single-element isotope analysis as a clear linear correlation between $\delta^{13}C$ and $\delta^{37}Cl$ can be observed along biotic degradation. Observing such a correlation along the degradation path in a plume might thus bring an answer to the first question "Is the contaminant concentration decrease due to degradation or to dilution?". While such linear correlation is very likely to reflect the occurrence of a degradation process, it could also be due to the mixing of two end-points having different initial isotopic signatures. The chances that such situation arises are however not high as it would require very specific site conditions. A thorough site characterisation before dual C-Cl isotope analysis will help avoiding misinterpreting a linear correlation between $\delta^{13}C$ and $\delta^{37}Cl$.

Additionally, dual C-Cl isotope analysis of the substrate could help determine which degradation process is affecting the contaminant as C-Cl isotope correlation trends are assumed to differ depending on the chemistry underlying the process responsible for the degradation (Elsner *et al.*, 2005). C-Cl isotope analysis could thus enable biotic degradation processes to be distinguished from abiotic ones. This would be valuable at the field scale as it would allow evaluating whether an applied bioremediation strategy is efficient or not.

Yet, some uncertainties remain regarding the variability of degradation processes involved in PCE biodegradation. If several reaction mechanisms could be involved during PCE reductive dechlorination, two C-Cl isotope trends could reflect the difference both between biotic and abiotic processes and between biotic processes themselves. The variability in biodegradation process in terms of C-Cl isotope patterns should hence be assessed in order to further distinguish biotic from abiotic degradation based on isotope analysis. Our study thus aimed to evaluate whether different C-Cl isotope correlation patterns (or slopes) could be observed depending on the bacteria studied (Badin *et al.*, 2014). PCE biotic reductive dechlorination experiments were performed in the laboratory using two bacterial consortia containing members of the *Sulfurospirillum* genus (consortia A and B).

The evolution of $\delta^{13}C$ and $\delta^{37}Cl$ was followed along time. Additionally, C-Cl isotope analysis was carried out in two sites undergoing PCE reductive dechlorination where groundwater was sampled in five different locations for each site at one time-point during summer 2013. Each sampled well showed different concentrations in PCE and its dechlorination products TCE, *c*DCE and VC.

For both consortia, the C and Cl isotope ratios followed a typical Rayleigh trend (Figure 2) and the enrichment factors obtained are within the range found in the literature. C enrichment factors determined based on Rayleigh fractionation of -3.6 ± 0.2 ‰ and -0.7 ± 0.1 ‰ and chlorine enrichment factors of -1.2 ± 0.1 ‰ and -0.9 ± 0.1 ‰ were obtained for consortium B and A, respectively. Two different dual C-Cl isotope slopes of 2.7 ± 0.3 and 0.7 ± 0.2 were observed for consortium B and A, respectively (Figure 3). At the field scale, two different dual isotope slopes of 3.5 ± 1.6 and 0.7 ± 0.3 were also observed for site Y and site X, respectively. Each slope obtained in the field is statistically similar to one of the two laboratory determined slopes. When taking into consideration the unique other dual C-Cl isotope slope of 2.5 ± 0.8 associated with PCE reductive dechlorination (Wiegert *et al.*, 2013), two distinct slope groups can be identified (i.e. slopes of 2.5-2.7 and 0.7). To

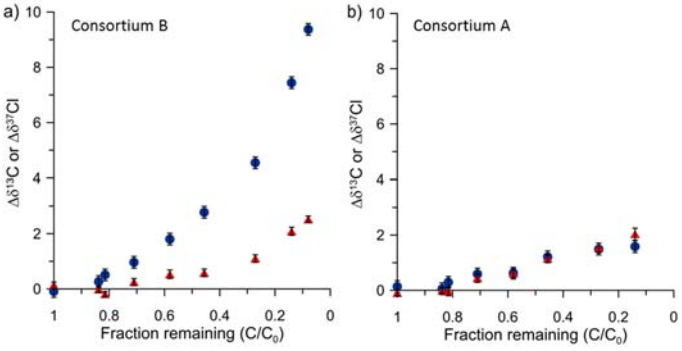

Figure 2. Evolution of C and Cl isotope ratios with decreasing PCE remaining fraction for consortia A and B. Modified from Badin *et al.* (2014). Red triangles represent Cl isotopic ratios while blue circles represent C isotopic ratios.

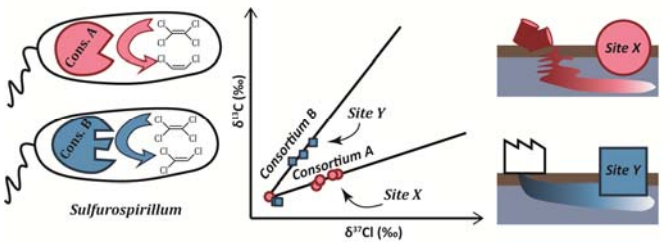

Figure 3. Schematic summary of the results - Each "*Sulfurospirillum*-containing consortium" yields a distinct dual C-Cl isotope slope associated with PCE reductive dechlorination. Similar dual isotope slopes are observed for PCE reductive dechlorination in two different sites. Modified from Badin *et al.*, 2014

date, three reaction mechanisms have been suggested for the reductive dechlorination of PCE (Kliegman and McNeill, 2008). Furthermore, it is believed that the dual C-Cl isotope approach might reflect the reaction mechanism involved in the process of reductive dechlorination (Elsner *et al.*, 2005; Abe *et al.*, 2009). Thus, finding two groups of dual C-Cl isotope slopes suggests that PCE can be dechlorinated under reductive conditions according to two different reaction mechanisms. This results in a less straightforward application of the dual C-Cl approach to differentiate biotic from abiotic degradation as more than one dual C-Cl isotope slope could be attributed to biotic reductive dechlorination. However, systematic dual C-Cl isotope studies during PCE reductive dechlorination might allow the ranges of enrichment factors to be constrained based on their corresponding dual isotope slope group. A resulting narrower range of enrichment factors would allow a more accurate estimation of PCE fate.

5. Acknowledgements

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013 under grant agreement n°265063). This work was supervised by Prof. Daniel Hunkeler from the Centre for Hydrogeology and Geothermics of the University of Neuchâtel and was carried out in collaboration with Géraldine Buttet, Dr. Julien Maillard and Prof. Christof Holliger from the laboratory of environmental biotechnology of the Ecole Polytechnique Fédérale de Lausanne. Géraldine Buttet is financed by the Swiss National Science Foundation grant 31003A_138114. The Federal Office for the Environment is also acknowledged for its support and help in linking research teams.

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