Modeling 3D-CSIA data: Carbon, chlorine, and hydrogen isotope fractionation during reductive dechlorination of TCE to ethene

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\textbf{ABSTRACT}

Reactive transport modeling of multi-element, compound-specific isotope analysis (CSIA) data has great potential to quantify sequential microbial reductive dechlorination (SRD) and alternative pathways such as oxidation, in support of remediation of chlorinated solvents in groundwater. As a key step towards this goal, a model was developed that simulates simultaneous carbon, chlorine, and hydrogen isotope fractionation during SRD of trichloroethene, via cis-1,2-dichloroethene (and trans-DCE as minor pathway), and vinyl chloride to ethene, following Monod kinetics. A simple correction term for individual isotope/isotopologue rates avoided multi-element isotopologue modeling. The model was successfully validated with data from a mixed culture Dehalococcoides microcosm. Simulation of Cl-CSIA required incorporation of secondary kinetic isotope effects (SKIEs). Assuming a limited degree of intramolecular heterogeneity of $\delta^{37}$Cl in TCE decreased the magnitudes of SKIEs required at the non-reacting Cl positions, without compromising the goodness of model fit, whereas a good fit of a model involving intramolecular C–Cl bond competition required an unlikely degree of intramolecular heterogeneity. Simulation of H-CSIA required SKIEs in H atoms originally present in the reacting compounds, especially for TCE, together with imprints of strongly depleted $\delta^2$H during protonation in the products. Scenario modeling illustrates the potential of H-CSIA for source apportionment.

1. Introduction

At many contaminated sites, monitored natural attenuation (MNA) of chlorinated ethenes is the preferred and cost-effective remediation approach (Meckenstock et al., 2015). Microbial sequential reductive dechlorination (SRD) of chlorinated ethenes is usually the main transformation process in MNA. The dechlorination proceeds from the primary contaminants tetrachloroethene (PCE) and/or trichloroethene (TCE), to daughter products cis- and trans-1,2-dichloroethene (cDCE and tDCE), 1,1-dichloroethene (1,1-DCE), vinyl chloride (VC), and finally to nontoxic ethene (ETH). Degradation may, however, also occur via alternative transformation pathways such as (an)aerobic oxidation (Bradley, 2011; Bradley and Chapelle, 2011; Chu et al., 2004; Pooley et al., 2009) and chemical reduction (Damgaard et al., 2013; Darlington et al., 2013; Ferrey et al., 2004; Lee and Batchelor, 2002) of lower and higher chlorinated ethenes, respectively. At field sites, the efficacy of SRD can be verified by quantitation of the degradation products that are pathway-specific. However, assessment of the alternative pathways of chlorinated ethene destruction is more difficult, since the degradation products (Cl\textsuperscript{–}, CO\textsubscript{2}) blend with the natural background levels. Moreover, degradation of SRD products can lead to an underestimation of the SRD performance as it seems that parent compounds are not well degraded because product concentrations are low. Consequently, less sustainable remedies, such as pump and treat, may be instituted or continued unnecessarily.

Compound-specific stable isotope analysis (CSIA) has been applied in contaminant studies, to detect and identify degradation processes (Hunkeler et al., 2008). One complication in interpretation of CSIA data is potential occurrence of sequential/parallel transformations, e.g., SRD followed by aerobic degradation of SRD products (Arp et al., 2001; Mundle et al., 2012). Multi-dimensional CSIA, i.e., CSIA of more than one element (C, Cl, H), as started with 2-D carbon and hydrogen CSIA of MTBE to discriminate anaerobic from aerobic transformation, holds particular promise also for detecting individual degradation pathways of chlorinated solvents. In addition to a growing number of reports on combined C and Cl isotope effects in various chlorinated solvent...
degradation systems (Abe et al., 2009; Audi-Miro et al., 2013; Cretnik et al., 2013; Wiegert et al., 2012) several CSIA applications focus specifically on discrimination of such alternative reaction mechanisms for chlorinated ethenes (Badin et al., 2016; Badin et al., 2014; Dogan-Subasi et al., 2017).

Reactive transport modeling (RTM) could become an essential tool in evaluation of such CSIA data. RTM can simulate sequential/parallel degradation reactions with chains of daughter products in SRD of halogenated hydrocarbons (Atteia et al., 2008; Höhener, 2016; Van Breukelen et al., 2005), and can account for potential relevance of the physical processes DNAPL dissolution (Aeppli et al., 2009; Hwang et al., 2013), sorption (Eckert et al., 2013; Höhener and Yu, 2012; Kopinke et al., 2005; Van Breukelen and Prommer, 2008), and transverse hydrodynamic dispersion (Eckert et al., 2013; Rolle et al., 2010; Van Breukelen and Rolle, 2012; Wanner and Hunkeler, 2015). Isotope effects modeling during SRD of chlorinated ethenes has been performed for lab (Van Breukelen et al., 2005) and field-generated C-CSIA data, but modeling of Cl fractionation has been relatively limited (Höhener, 2016; Palau et al., 2014b; Wiegert et al., 2012) while a model on H-CSIA does not yet exist.

Studies of Cl isotope fractionation in reactions of organochlorine compounds focus on the so-called primary isotope effects, i.e., isotope fractionation of the atoms positioned at the reacting molecular bonding (Paneth, 2006). In accordance with that consensus, the existing models of Cl isotope fractionation were developed for isotope fractionation involving only the primary effects (Hunkeler et al., 2009; Jin et al., 2013). However, it was also suggested that reactions of chlorinated hydrocarbons (Palau et al., 2014a) may result in fractionation of non-reactive Cl atoms (secondary isotope effects) or possibly combine both primary and secondary effects (Abe et al., 2009; Cretnik et al., 2014; Kuder et al., 2013; Palau et al., 2014a). Höhener (2016) recently extended the analytical BIOCHLOR model with calculation of carbon and chlorine stable isotope ratios in chloroethenes and accounting for secondary chlorine isotope effects in the TCE to cDCE step. Likewise, to address the potential contributions from Cl isotope effects occurring at non-reactive positions, the present numerical model considers isotope effects occurring at all Cl positions within the reacting molecules.

Recently, Kuder et al. (2013) presented a dataset on SRD of TCE to ETH by a Dehalococcoides culture, including 3D-CSIA (C, Cl, H) results, at high temporal resolution. This dataset allows for further validation and improvement of the chlorine isotopologue fractionation model developed by Hunkeler et al. (2009) and the development and validation of a hydrogen isotope fractionation model. The objectives of the current study were (i) to extend the current chlorine isotope fractionation model (Hunkeler et al., 2009) as adopted in subsequent studies (Höhener, 2016; Jin et al., 2013; Palau et al., 2014b; Wiegert et al., 2012) for isotope effects occurring at multiple Cl positions of TCE (Höhener, 2016) and of DCE, and for intramolecular heterogeneity in \( ^{37}\)Cl of the source compound (TCE); (ii) to develop a completely novel model of hydrogen isotope fractionation during SRD; and (iii) to validate the models with the experimental data (Kuder et al., 2013). The validated models requiring Monod kinetics are expected to form a template for CSIA interpretation of SRD of halogenated hydrocarbons and contribute towards CSIA-based support of remediation of chlorinated solvent groundwater pollution at field sites.

2. Methodology

2.1. Experimental data for model validation

Model validation used data from a microcosm experiment on dechlorination of TCE by a Dehalococcoides (Dhc) culture. A detailed description of that experiment is available elsewhere (Kuder et al., 2013). In summary, microcosms were set up with Bio-Dechlor Inoculum (BDI) culture (Amos et al., 2008) a consortium of Dhc strains that is capable of complete dechlorination of PCE via TCE, DCE, and VC to ETH. The microcosms were amended with TCE and lactate electron donor. Concentrations and C, Cl, and H isotope ratios were determined for TCE and the aforementioned reaction products over the course of degradation.

2.2. Nomenclature of the chlorine and hydrogen isotope effects

Parameters describing the magnitude of isotope effects use the rate constants (k) for heavy vs. light isotope species, where: light\(k^\text{light} \neq k^\text{heavy}\) = 1/\(c \in [1, \infty]\). The fractionation factor (\(\epsilon\)) and enrichment factor (\(\delta\)) can describe position-specific or “bulk” effects. The latter, indicated by the “bulk” subscript (e.g., \(\delta_{\text{bulk}}\)) are averaged for all reactive and non-reactive positions of a molecule. KIE (kinetic isotope effect) is the ratio of \(k^\text{light} \neq k^\text{heavy}\) for a specific molecular position and a specific transformation process. In a primary KIE, the isotope substituted-position is at the reaction center. A secondary KIE (SKIE) is the effect of isotope substitution at a position remote from the reaction center (Elsmier et al., 2005).

In the discussion, we refer to position-specific isotope effects, to reflect the different fates of individual atoms of the reacting compounds. We refer to \(\epsilon_{\text{RP}}\) (at “reactive position”, i.e., the Cl position undergoing dechlorination) or \(\epsilon_{\text{NRP}}\) (at “non-reactive position”, i.e., the Cl position not undergoing dechlorination). Isotope effects for the Cl atoms remote from the dechlorination site (e.g., \(\alpha\) and \(\beta\) for TCE in Fig. 1a) are by definition secondary KIEs (SKIEs). Observable isotope effects for the Cl atom undergoing dechlorination may be in fact primary or secondary, depending on the dechlorination process involved. The latter may occur if the initial transformation step does not involve C–Cl cleavage. It was previously proposed that the initial step in abiotic dechlorination of VC and cDCE by cobalamin is nucleophilic addition of cobalamin and the chloroethene species (Glod et al., 1997). A similar process was postulated for biodegradation of the same species by Dhc (Abe et al., 2009; Kuder et al., 2013). It is also possible that the
nucleophilic addition mechanism may apply to biodegradation of TCE (Kuder et al., 2013). In such reactions, the only observable isotope effects may in fact be secondary KIEs of the addition step. For simplicity, the model treats the ε<sub>SNAP</sub> as a primary KIE (see Supplementary Table S2), and, in the narrative, we will refer to the effect at the reactive position as KIEs and the effects at the non-reactive positions as SKIEs; however, the model structure can equally accommodate reaction scenarios with SKIEs at all Cl positions of the parent compound and no observable primary KIEs.

Recently, Cretnik et al. (2014) proposed an alternative model of TCE dechlorination, where cDCE resulted from conversion of tDCE product. In that pathway, both germinal Cl atoms would contribute to cDCE. That mechanism will be discussed in detail in Supplementary data Section S4.

In the case of H isotope fractionation, the change of H isotope ratios of TCE over the progress of reaction is only controlled by the (secondary KIE) ε<sub>SNAP</sub> at the single H atom of that compound. In the remaining reactions, the net changes of isotope ratio of the daughter compounds combine ε<sub>SNAP</sub> (H atoms transferred from the parent compounds) and the isotope composition of the H atom added in protonation (ε<sub>protonation</sub>; Fig. 1b). Protonation is discussed more extensively in Section 3.4.1.

### 2.3. Simulation model

#### 2.3.1. Reaction kinetics

The model was developed with the PHREEQC code (Parkhurst and Appelo, 1999). SRD of TCE to ETH, via DCE and VC, was simulated. cisc-DCE was the main DCE isomer produced in the microcosm, but minor quantities of trans-DCE and 1,1-DCE were detected (Kuder et al., 2013). For model simplicity, the sum of the latter two DCE isomers was explicitly simulated as trans-DCE. Two minor pathways were, therefore, added to the model: TCE to trans-DCE and trans-DCE to VC. Monod kinetics was applied without microbial growth (Bekins et al., 1998) for all reactions:

\[
\text{Rate}_m = -v_m \times \left( \frac{C_m}{K + C_m} \right)
\]

where \(\text{Rate}_m\) is the reaction rate of molecule \(m\) (\(-v_m\) is production rate of its daughter product), \(v_m\) is the substrate utilization rate \((\text{M} \cdot \text{L}^{-3} \cdot \text{T}^{-1})\), \(C_m\) is the concentration of the molecule \((\text{M} \cdot \text{L}^{-3})\), and \(K\) is the half-saturation constant \((\text{M} \cdot \text{L}^{-3})\). Individual lag periods, i.e., the period before the reaction in question began (7), were used for all reactions. The selection of kinetic parameter values is discussed in Section 3.1. A time step of 1 h was taken.

#### 2.3.2. Simulation of carbon isotope fractionation

The “bulk isotope” method was applied (Van Breukelen et al., 2005). In other words, for each compound, the light and the heavy isotope species were defined reflecting the compound’s concentration fraction of the light and heavy isotopes, respectively. The concentration of an isotope species was taken to be equal to its fraction multiplied by the compound’s concentration. Reaction rates were as follows:

\[
\text{Rate}_L = \text{Rate}_m \times \left( \frac{C_L}{C_m} \right)
\]

\[
\text{Rate}_H = \text{Rate}_m \times \left( \frac{C_H}{C_m} \right) \times [c_{\text{bulk}} + 1]
\]

where \(\text{Rate}_L\) and \(\text{Rate}_H\) are the rates of the light and heavy isotopes, respectively, \(C_L\) and \(C_H\) are the concentrations of the light and heavy isotopes, respectively, and \(c_{\text{bulk}}\) is the bulk kinetic isotope enrichment factor of the reaction step. Isotope ratios were calculated from the simulated concentrations of the light and heavy isotopes.

#### 2.3.3. Simulation of chlorine isotope fractionation

The isotopologue approach was applied (Hunkeler et al., 2009; see Fig. 2) which considers all isotologues in the reaction network, and, for TCE, also all Cl isotopomers (i.e., isotologues with same number of heavy isotopes but located at different positions). This model developed by Hunkeler et al. (2009) was extended in the current study to also account for isotope effects at the positions away from the dechlorination center (SKIEs). The model also addresses the possibility of intramolecular δ<sup>37</sup>Cl heterogeneity of TCE and the potential of intramolecular C–Cl bond competition (IBC) in TCE degradation (Fig. 2; Supplementary Section S4).

The initial Cl isotopologue/isotopomer concentrations assuming either intramolecular homogeneity or heterogeneity in δ<sup>37</sup>Cl of initial TCE were calculated as described in Supplementary Section S2. Isotope ratios were calculated from the simulated concentrations of the isotologues/isotopomers.

The reaction rate for each isotopologue/isotopomer (\(\text{Rate}_{\text{iso}}\)) was obtained by:
To simulate reactions). Note, the sum of $x$ where

$$\Sigma x = 35\,k/37\,k; \text{ and}$$

$$R_{\Pi(1)} = 1/(\Sigma NRP) = 1/(3\,KIE);$$

$$\delta_{\text{Hi}} = (\Sigma NRP) = 1/(3\,KIE);$$

$$\delta_{\text{Hi}} = (\Sigma NRP) = 1/(3\,KIE);$$

$$\delta_{\text{Hi}} = (\Sigma NRP) = 1/(3\,KIE);$$

$$\delta_{\text{Hi}} = (\Sigma NRP) = 1/(3\,KIE);$$

where $\alpha$ is the bulk isotope fractionation factor in the case of $C$, and the multiplication of $\alpha_{\text{Hi}}$ for $C$ and $H$. Values of correction terms consequently varied per isotope pair and compound. This correction term is in principle similar as the corrections done by Hunkeler et al. (2009) for first-order chlorine isotope fractionation. Note that as the heavy isotopes react a factor $\alpha$ slower, the abundance of the heavy isotopes multiplied with $\alpha$ together with the abundance of the light isotopes (multiplied with 1) $= \alpha_{\text{Hi}} \times \alpha$ represents the extent that the summed isotope/isotopologue network reacts slower than aimed with Eq. (1). Therefore, multiplying the individual isotope/isotopologue rate equations with the inverse of $(\alpha_{\text{Hi}} \times \alpha)$ makes the sum of the individual isotope/isotopologue rate equations to become identical to the intended Monod kinetics rate as defined in Eq. (1).

With this correction, compound concentrations (sum of their isotopes/isotopologues) were identical among $C$, $H$, and $Cl$ isotope networks over the entire course of reaction. Therefore, simulation of multi-element isotope models (e.g., $^{12}\text{C}-^{13}\text{C}-^{35}\text{Cl}-^{37}\text{Cl}-^2\text{H}-^2\text{H}$ as one of the 27 multi-element $C/Cl/H$ DCE isotopologues) as performed for combined $C/-\text{Cl}$ isotopologues of chlorinated ethenes by Jin et al. (2013) albeit an elegant and sophisticated approach, was not required, avoiding the dramatic increase in the number of isotopologues and reactions to be modeled.

2.3.4. Simulation of hydrogen isotope fractionation

The bulk hydrogen isotope ratio of TCE is affected only by the SKIE at the single $H$ atom present (Fig. 1b). The bulk $\delta^H$ of the daughter products is affected predominantly by the isotope signatures of the $H$ atoms incorporated during dechlorination/protonation (Fig. 1b) (Ernl et al., 1998; Shouakar-Stash et al., 2003). Hydrogen isotope ratios were simulated with an extended “bulk isotope” method (see Section 2.3.2). To simulate $\delta^H$ of a daughter product, the model considered (i) isotope fractionation of the $H$ atoms transferred from the parent to daughter product and the subsequent daughter product (Eqs. (2)-(3), where $\epsilon_{\text{Hi}}$ results exclusively of SKIEs ($\epsilon_{\text{Hi}} = \Sigma NRP/\Sigma NRP$), and (ii) the rates of the light, $R_{\text{Hi}}$, and the heavy, $R_{\text{Hi}}$, $H$ isotopes replacing the $C$ atom of the parent compound, i.e., through protonation, at each dechlorination step calculated as the total rate multiplied by the light and heavy $H$ isotopic abundance, respectively:

$$R_{\text{Hi}} = -R_{\text{Hi}} \times (1 + \delta^H_{\text{Hi}} + \epsilon_{\text{Hi}} + 1) \times \text{VSMOW}^{1-1}$$

$$R_{\text{Hi}} = -R_{\text{Hi}} \times (1 + \delta^H_{\text{Hi}} + \epsilon_{\text{Hi}} + 1) \times \text{VSMOW}^{1-1}$$

where $\Delta_{\text{Hi}}$ (Eq. (1)) is the degradation rate of the corresponding parent compound, the terms between parentheses in Eqs. (5)-(6) are the isotopic abundances of light and heavy hydrogen isotopes, respectively, $\epsilon_{\text{Hi}}$ is the overall hydrogen isotopic enrichment factor expressed with respect to $\delta^H$ of water and associated with this reaction step, and VSMOW is the ratio of $^2H$/H of the international standard for the H isotopic composition of water. Note that the values of $\epsilon_{\text{Hi}}$ and $\delta^H_{\text{Hi}}$ as input for Eqs. (5) and (6) are not converted to forming, following the IUPAC recommendation for isotope ratio notation (Coplen, 2011). The rates of $H$ addition through protonation and of $H$ transfer from the parent compound were weighted to account for the different numbers of $H$ atoms involved. For example, for $VC$ with three $H$ atoms, two $H$ atoms are transferred from DCE, whereas one $H$ atom is added via protonation. Consequently, the $H$ transfer flux is multiplied by $\frac{1}{2}$ and the protonation flux by $\frac{1}{2}$. Supplementary Section S7 presents further details on the H-CSIA model.
The relatively high rates occurred after 41 days. The model chlorination of VC to ETH was slow in the subsequent period but high calibration for this reaction step. 1,1-DCE greatly increased the number of model variables that required day 5 well. This may relate to the slight mass balance variations observed, however, not describe the shape of the cDCE concentration peak around the small effect of the transient presence of these minor DCE isomers under the simplification that only tDCE occurred. The model did, however, not describe the shape of the cDCE concentration peak around day 5 well. This may relate to the slight mass balance variations observed during this period. Moreover, co-production of dDCE, tDCE, and 1,1-DCE greatly increased the number of model variables that required calibration for this reaction step.

Conversion to VC was complete after 8 days. Reductive dechlorination of VC to ETH was slow in the subsequent period but high rates occurred after 41 days. The model fitted VC and ETH concentrations well. The relatively high $K_s$ compared to the previous reaction steps (200 $\mu$M versus 8–33 $\mu$M; Table 1) resulted in pseudo first-order degradation kinetics of VC. The concentration and C isotope mass balances indicated that no further conversion of ETH occurred.

### 3. Results and discussion

#### 3.1. Reaction kinetics

Using the model described, the substrate utilization rate, $v_m$, the half-saturation constant, $K_s$, and the lag period were determined for each pathway of the SRD reaction network by manually fitting the model to the concentration data. Table 1 lists all kinetic parameter values. A reasonably good agreement was obtained for all concentration observations (Fig. 3a). As metrics of goodness of model fit, the root mean squared weighted error (RMSWE) was computed for all simulations and for each parameter (see Table S7).

The transformation of TCE to DCE started almost immediately. The overall linear concentration decline reflects zero-order kinetics, which follows from Monod kinetics with a low $K_s$ relative to the TCE concentration. However, the initial decay rate was very slow and could not be simulated with this kinetic model. The lag period had to be set longer (2.4 days) than actually observed. Consequently, the first $\delta^{13}$Cl-cDCE observation after 1.1 days was not simulated.

TCE dechlorination produced mostly cDCE but minor quantities of tDCE and 1,1-DCE were determined and were part of the total mass balance. Their summed molar concentration ranged between 4 and 9% of tDCE and 1,1-DCE were determined and were part of the total mass balance. Their summed molar concentration ranged between 4 and 9% of.

### 3.2. Carbon isotope fractionation

The model fitted C-CSIA of all compounds very well (Fig. 3b). The C

where the accuracy is taken as the measurement error of the CSIA data (C-CSIA: 0.5%; CI-CSIA: 0.8%; TCE and VC; 1.0%; cDCE; H-CSIA: 20%) or the observed concentration value multiplied by the measurement error (CEs: 7%; ETH: 15%). Thus observations with higher accuracy get a higher weight and vice versa. Note that the value 1.96 in Eq. (9) is the approximate value of the 97.5 percentile point of the normal distribution, i.e., the 95% confidence interval lies within roughly 1.96 standard deviations of the mean. Consequently, a RMSWE of 1.96 means that the model on average deviates from the observations with a value equal to the accuracy of the observations.

![Fig. 3. Evolution of a) concentrations, b) C-CSIA, c) Cl-CSIA, and d) H-CSIA during complete reductive dechlorination of TCE to ETH in a microcosm experiment. Modeled (lines) and measured data (symbols) are shown for comparison. The time period between 10 and 35 days is condensed as VC degradation was slow during this time interval. Error bars are shown for all observations but are in some cases smaller than the symbol size.](image)
3.3. Chlorine isotope fractionation

3.3.1. Secondary KIEs in chlorine isotope fractionation

In the early stage of transformation, the Cl isotope ratios showed clear offsets of $^{37}$Cl among the chlorinated ethenes (Fig. 3c). Instantaneously produced cDCE and VC were thus more depleted in $^{37}$Cl than their precursors. In SRD, as illustrated in Fig. 1a, Cl atoms at the C–Cl bonds undergoing dechlorination are split off the ethene skeleton of the reacting molecule, while the non-reactive Cl atoms are transferred to the daughter compounds. Therefore, if the bulk chlorine isotope effect consists exclusively of a primary KIE (at the reactive position), then isotope fractionation does not affect the Cl atoms transferred to the daughter compounds. However, in the case where the bulk chlorine isotope effect also includes contributions from SKIEs, the $^{37}$Cl value of the daughter chlorinated ethene compound should differ from that of the precursor, and the difference should be equal the average SKIEs of the reaction, as postulated by Hunkeler et al. (2009). Indeed, inclusion of SKIEs in the model was required to adequately simulate the evolution of $^{37}$Cl values of all reaction products during SRD, as explained below. An alternative explanation for the TCE to cDCE $^{37}$Cl offset might be intramolecular heterogeneity (IH) in $^{37}$Cl of initial TCE (discussed in Section S4).

The position-specific isotope effects and bulk enrichment factors of all reaction steps are listed in Table 2 (determined experimentally in the microcosm study by Kuder et al. (2013) with the exception of the $\alpha$ and $\beta$ isotope effect in the transformation of TCE to tDCE). The isotope effects are explained as follows. First, in the transformation of TCE to cDCE, the mean of the SKIEs (type $\alpha$ and $\beta$; $\epsilon_{\text{SKIE (MEAN)}} = -3.3\%\text{e}$) was calculated from the difference between $^{37}$Cl-TCE and initial $^{37}$Cl-cDCE observed (Kuder et al., 2013); since only the average of the $\alpha$ and $\beta$ effects could be determined experimentally, they were considered as equal in the model. This was done for the sake of model simplicity even though SKIEs in the $\beta$ position tend to be smaller than those in the $\alpha$ position (Elmer, 2010; Elmer et al., 2005); the $\beta$ effect (in the TCE to tDCE step) was assumed to be equal in magnitude ($\epsilon_{\text{SKIE (t)}} = -3.3\%\text{e}$). Second, the reactive position effect in the TCE to cDCE step ($\epsilon_{\text{RP (c)}}$) followed from $3 \times \epsilon_{\text{SKIE (t)}} - 2 \times \epsilon_{\text{SKIE (MEAN)}}$, where $\epsilon_{\text{SKIE (t)}}$ was $-3.6 \pm 0.3\%\text{e}$ (Kuder et al., 2013). Third, the SKIE in the cDCE to VC step ($\epsilon_{\text{SKIE (c)}} = -1.7\%\text{e}$) followed from the difference between $^{37}$Cl-cDCE and initial $^{37}$Cl-VC observed (Kuder et al., 2013). Fourth, the reactive position effect in the cDCE to VC step ($\epsilon_{\text{RP (c)}} - 4.5\%\text{e}$) followed from $-2 \times (^{37}\text{Cl-VC}_{\text{final}} - ^{37}\text{Cl-VC}_{\text{initial}}) + \epsilon_{\text{SKIE (c)}}$ (cf. Eq. S1 in Kuder et al. (2013)). The $\beta$ SKIE ($\epsilon_{\text{SKIE (c)}}$) of the tDCE to VC step was assumed equal to the $\beta$ SKIE ($\epsilon_{\text{SKIE (t)}}$) of the cDCE to VC step. Fifth, as only a $\epsilon_{\text{RP}}$ occurred in the VC to ETH step, its value followed directly from fitting the Rayleigh equation to the observations ($\epsilon_{\text{RP}} = \epsilon_{\text{bulk}}, -2.7 \pm 0.4\%\text{e}$; (Kuder et al., 2013)).

The extended model using parameters derived from direct observations fitted the microcosm CI-CSIA data very well. Note that the addition of SKIEs to the model of Hunkeler et al. (2009) implied that isotope fractionation occurred for nearly all chlorine isotopologue reactions except for those that only contained light chlorine atoms (Table S2). This confirmed the conclusion from Kuder et al. (2013) where the SKIEs (in a nucleophilic addition reaction) were postulated for all SRD reactions.

3.3.2. Effect of intramolecular heterogeneity on chlorine isotope patterns

Intramolecular heterogeneity (IH) in $^{37}$Cl of TCE could be an alternative to SKIEs in explaining the initial offset between $^{37}$Cl-TCE and $^{37}$Cl-cDCE. In manufacturing of TCE, chlorinated organic compounds of dissimilar chlorine isotope signatures may be combined, resulting in different position-specific chlorine isotope ratios (Kuder et al., 2013). In the event that the non-reactive chlorine positions in the TCE to cDCE reaction have a lower average $^{37}$Cl versus the $^{37}$Cl-TCE, cDCE produced at the outset of the transformation will be $^{37}$Cl-depleted relatively to the TCE. Note that IH cannot account for the offset between $^{37}$Cl-cDCE and $^{37}$Cl-VC since the transformation of DCE to VC is not Cl position-specific.

Fig. 4 (and also Fig. S1) present various simulations to illustrate the effect of IH and the assumed absence or presence of SKIEs. Table 3 (and also Table S3) presents an overview of the main characteristics of these simulations. A baseline model incorporating only primary KIEs (Table 3) does not describe the observations well, and results in $^{37}$Cl values of daughter products always equal to or higher than the initial value of TCE (Fig. 4a). Note that the two non-reactive chlorine atoms of TCE become part of cDCE and, therefore, in the absence of SKIEs, instantaneously produced cDCE always has the same $^{37}$Cl as source TCE. Similarly, unless the SKIE is present, the initially produced VC cannot have a lower $^{37}$Cl than the cDCE precursor.

The model including IH, but no SKIEs, reproduces the first few $^{37}$Cl-cDCE observations but does not fit the last two $^{37}$Cl-cDCE data points and all $^{37}$Cl-VC data (Fig. 4b). Furthermore, this model requires the assumption of a wide difference in the isotopic ratios for different positions, i.e., a TCE molecule in which the reacting position had a $^{37}$Cl of 9.8% and the two non-reacting positions had $^{37}$Cl values of $-0.1\%\text{e}$ (Fig. 4b; Table 3). As discussed by Kuder et al. (2013) such a large IH is unlikely because the required $^{37}$Cl value of +9.8% at the reactive position is unreasonably large in comparison with the isotope ratios of chloride evaporates used as industrial chlorine sources and the range of $^{37}$Cl reported for synthetic organochlorine compounds ($\sim 5$

Table 2
Calibrated isotope enrichment factors (%).

| Reaction | Carbon | Chlorine | Hydrogen |
|----------|--------|----------|----------|
|          | $\epsilon_{\text{bulk}}$ | $\epsilon_{\text{RP}}$ | $\epsilon_{\text{SKIE (c)}}$ | $\epsilon_{\text{SKIE (t)}}$ | $\epsilon_{\text{SKIE (MEAN)}}$ | $\epsilon_{\text{Bulk}}$ | $\epsilon_{\text{RP (MEAN)}}$ | $\epsilon_{\text{H (DEFINITION)}}$ |
| TCE $\rightarrow$ cDCE | $-16.4 \pm 0.4^a$ | $-4.2^b$ | $-3.3^c$ | $-3.3^c$ | $\text{na}$ | $-3.3^c$ | $-3.6 \pm 0.3^c$ | $+34 \pm 11^f$ | $-170^g$ |
| TCE $\rightarrow$ tDCE | $-16.4$ | $-4.2$ | $-3.3$ | $\text{na}$ | $-3.3$ | $-3.3$ | $-3.6$ | $10^f$ | $-170^g$ |
| cDCE $\rightarrow$ VC | $-26.8$ | $-4.5^f$ | $\text{na}$ | $-1.7^f$ | $-1.7^f$ | $-3.1$ | $10^f$ | $-580^g$ |
| tDCE $\rightarrow$ VC | $-30.3^c$ | $-4.5$ | $\text{na}$ | $-1.7$ | $-3.1$ | $22^c$ | $-580^g$ |
| VC $\rightarrow$ Ethene | $-26.7 \pm 1.9^a$ | $-2.7$ | $\text{na}$ | $\text{na}$ | $\text{na}$ | $-2.7 \pm 0.4^c$ | $15^c$ | $-740^g$ |

* na = not applicable.

$^a$ Results of regression analysis (with ±95% confidence interval) directly used in model and not further calibrated.

$^b$ $\epsilon_{\text{RP}}$ of the TCE to cDCE step follows from $3 \times \epsilon_{\text{SKIE (t)}} - 2 \times \epsilon_{\text{SKIE (MEAN)}}$.

$^c$ Only the average of $\epsilon_{\text{SKIE (c)}}$ and $\epsilon_{\text{RP (c)}}$ can be determined and should equal $\epsilon_{\text{SKIE (MEAN)}}$.

$^d$ Taken as the observed difference between the parent and initial daughter isotope ratios.

$^e$ Carbon and chlorine isotope effects were taken the same for the dominant TCE to cDCE and cDCE to VC steps, respectively. However, the $\epsilon_{\text{RP}}$ for the latter step was taken equal to the one found by Hunkeler et al. (2002).

$^f$ $\epsilon_{\text{RP}}$ of cDCE to VC follows from $-2 \times (^{37}\text{Cl-VC}_{\text{final}} - ^{37}\text{Cl-VC}_{\text{initial}}) + \epsilon_{\text{SKIE (c)}}$ (cf. Eq. S1 in Kuder et al. (2013)).

$^g$ See Section 3.4.2, Section S5, and Table S6 for a detailed explanation of the determination of $\epsilon_{\text{SKIE (MEAN)}}$ Values.

$^h$ $\epsilon_{\text{H (DEFINITION)}}$ Values were manually calibrated by trial-and-error by visually comparing the model-data fit. See Section 3.4.1. for further clarification.
+ 6‰; (Hoefs, 2009)).

The occurrence of SKIEs in RD of TCE and of cDCE was required to simulate the $^{37}$Cl-cDCE and $^{37}$Cl-VC observations (Fig. 4c–d). The final calibrated model assumed absence of IH for the sake of model simplicity (Fig. 4c; Table 3), whereas the alternative model assumed a mild degree of IH and as result a larger degree of IH and as result a larger di

The goodness of fit of these two models was similar in terms of RMSWEs (final versus alternative: 2.4 vs. 2.0 ($^{37}$Cl-cDCE), 0.6 vs. 0.9 ($^{37}$Cl-VC); Table S7). Therefore, absence of IH cannot be ascertained but the occurrence of SKIEs is required to provide a good model fit, particularly with respect to the initial depletion of VC relative to cDCE. Noteworthily, the alternative model assuming a mild degree of IH led to a perhaps more plausible larger difference in the deduced magnitudes of the isotope effects at the reacting and non-reacting positions.

3.3.3. Evaluation of intramolecular C–Cl bond competition

We tested whether the mechanism of intramolecular C–Cl bond competition (IBC) postulated by Cretnik et al. (2014) is consistent with our experimental data set. The model was extended as explained in the Supplementary Section S4 to describe the reaction of TCE to trans-DCE with selective interconversion of trans-DCE to cDCE. We assumed the rates of this route and of the normal TCE to cDCE route as equal. Cretnik et al. proposed IBC to explain an unexpectedly high level of position specific Cl isotope effects without invoking significant SKIEs at those positions. We observed that fitting the IBC mechanism to our data is indeed possible, but only for improbably high extent of IH (Supplementary Section S4). Our alternative model Fig. 4d (cf. Table 3) enables reduction of the δNRP (from −3.3‰ down to −1.7‰) under the assumption of half to a quarter of the degree of IH needed for the IBC model (see Supplementary Section S4, Table SS), and therefore seems more probable.

3.4. Hydrogen isotope fractionation

3.4.1. Effects of protonation

As SRD of TCE progressed, a ‘stepped’ H isotope fractionation pattern was observed as the most striking feature, whereby each subsequent daughter product was more depleted in 2H than its predecessor, with ETH reaching 85‰ less than TCE (+ 530‰), whereas initial 85‰ of TCE was + 530‰ (Fig. 3d). Furthermore, in the case of TCE and less for cDCE, 85H continued to decline as the reaction progressed, and related to inverse secondary isotope effects (discussed later). A good agreement was achieved between the modeled and measured 85H patterns (Fig. 3d).

The stepped decrease in 85H values observed in the order TCE, cDCE, VC, and ETH is the result of the addition of a H atom at each dechlorination step that, on average, is strongly depleted in 2H relative to both water (−42‰) and the original TCE (+ 530‰) (Kuder et al., 2005).

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**Table 3**

Model parameter values (%o) of simulations shown in Fig. 4.

| Simulation | Explanation | Initial $^{37}$Cl-TCE | TCE to DCE | DCE to VC |
|------------|-------------|------------------------|------------|-----------|
|            |             | $^{37}$Cl rp | $^{37}$Cl nRP | $^{3}_{\text{rp}}$ | $^{3}_{\text{nRP}}$ | $^{3}_{\text{MEAN}}$ | $^{3}_{\text{rp}}$ | $^{3}_{\text{nRP}}$ |
| Fig. 4a    | Absence of intramolecular heterogeneity (IH), none SKIEs: only isotope effects at reacting positions assumed | + 3.2 | + 3.2 | −10.8 | 0 | −6.2 | 0 |
| Fig. 4b    | IH only, absence of SKIEs | + 9.8 | −0.1 | −10.8 | 0 | −6.2 | 0 |
| Fig. 4c    | SKIEs included, none IH: final calibrated model | + 3.2 | + 3.2 | −4.2 | −3.3 | −4.5 | −1.7 |
| Fig. 4d    | Mild degree of IH, SKIEs optimized: alternative model | + 6.4 | + 1.6 | −7.4 | −1.7 | −6.9 | −1.7 |

* rp = reactive position, nRP = non reactive position, both for TCE to cDCE step.
2013). These results agree with two earlier studies. Shouakar-Stash et al. (2003) measured lumped δ²H values of TCE and byproducts between −352 and −320‰ (δ²H-H₂O was −206‰) for PCE dechlorination by Fe⁶. Ertl et al. (1998) measured δ²H-DCE at −220‰ and +50‰, for biological RD from PCE and TCE (δ²H-TCE was +530‰), respectively, with sucrose and cane-sugar as electron-donor (δ²H-H₂O was −60‰). The current study showed that the overall fractionation effects with respect to water, ε₂Hprotonation, increased with the extent of dechlorination (TCE → DCEs: −170‰; DCEs → VC: −580‰; VC → ETH: −740‰). These values were obtained by manual calibration of the model as those obtained by Kuder et al. (2013) except for the TCE to DCE step (TCE → DCEs: −130‰; DCEs → VC: −590‰; VC → ETH: −750‰) who applied the following equation
\[ \delta²H_{\text{addition}} = n \times \delta²H_{\text{daughter--bulk}} - (n - 1) \times \delta²H_{\text{parent--bulk}} \] (10)

where δ²H of the newly added hydrogen atom in a reaction step, n, is the number of hydrogen atoms in a given daughter product, “bulk” refers to the average δ²H of the daughter and parent compounds, e.g., cDCE and TCE. Subsequently, ε₂Hprotonation follows from δ²H_{addition} − δ²H_{water}. We ascribe the relatively large underestimation by Eq. (10) for ε₂Hprotonation of the TCE to DCE step to the large inverse hydrogen isotope effect of TCE SRD resulting in a much reduced offset between δ²H-TCE and δ²H-cDCE. Whereas Eq. (10) ignores this additional isotope effect, the model accounts for it and enables accurate quantification of protonation effects.

The δ²H of H added during protonation is the outcome of a complex series of fractionation processes. Dhc species used in the experiment require molecular hydrogen as the immediate electron donor (Kuder et al., 2013). Consequently, the hydrogen atom replacing the Cl atom during RD derives from H₂ produced by the fermentation of lactate. The δ²H of produced H₂ should, therefore, depend on the δ²H of lactate and isotopic fractionation effects during fermentation, both of which were unknown. In isotopic equilibrium, δ²H of produced H₂ is strongly depleted with respect to δ²H-H₂O. Its equilibrium value in the current experiment would be -757‰ (α = (D/H)_{lactate}/(D/H)_{H₂} = 3.95 at 20°C (Horibe and Craig, 1995). In biological systems, isotopic equilibration is fast, provided hydrogenases are present (Campbell et al., 2009; Valentine et al., 2004). As an illustration, results from experiments in which D. autotrophicum grew on formate and where δ²H-H₂O and δ²H-formate were varied independently showed that δ²H of the cells fatty acids was entirely controlled by δ²H-H₂O (Campbell et al., 2009). The strongly depleted δ²H of the H atom inserted into SRD product (ε₂Hprotonation) appears to mainly reflect the depleted δ²H of the H₂. Therefore, we normalized ε₂Hprotonation with respect to ambient water.

While ε₂Hprotonation is likely a composite parameter (combining the depleted δ²H of the H₂ and possibly kinetic effects or effects associated with hydrogen insertion), model results showed we could simplify this complex series of fractionation steps with a single and constant process-specific overall ‘protonation’ fractionation effect, ε₂Hprotonation-

3.4.2. Secondary kinetic isotope effects in hydrogen isotope fractionation

Imprinted on the main pattern of decreasing δ²H of subsequent reaction products, a clear and surprisingly large inverse isotope effect (+34 ± 11‰) (Kuder et al., 2013) was observed to occur during the transformation of TCE decreasing instead of increasing its δ²H (Fig. 3d). Since primary KIEs do not occur for H during SRD, the inverse isotope effect must therefore be an SKIE of type βc that occurs for the TCE to cDCE step (Fig. 1b). Inverse effects are typical for H atoms bonded to the C adjacent to the site of nucleophilic addition (Elsner et al., 2005), as in the proposed TCE reaction with cobalamin for the current experiment (Kuder et al., 2013). SKIES of the other dechlorination steps could be kept to zero to obtain a good model fit (Fig. S4: simulation S4a).

However, assuming occurrence of SKIES in the cDCE to VC step (ε₂Hprotonation = +10‰), average of SKIEs types α and β improved the fit for the last δ²H-cDCE observation (RMSWE 0.9 vs. 1.4) without changing the results for VC and ETH much (Fig. S4: simulation S4b). For the sake of consistency, the final calibrated model (Figs. 3d; S4) applied the same values for these three SKIEs for each reaction step (see Table S6). For example, an average SKIE (ε₂Hprotonation = + 14.7‰) of types α, β (from cDCE), and βc (from TCE) was applied for the VC to ETH step. It must be stated that considering the analytical error of H-CSIA of ±20‰ (Kuder et al., 2013), it cannot be determined with certainty whether the SKIEs related to the cDCE and VC dechlorination steps truly deviate from zero. Note that the drop in δ²H-VC values around day 5 was captured well by the model (Fig. S4).

It remains to be seen how well the model is able to reproduce data from field sites where ε₂Hprotonation values may be less consistent than observed during the present lab experiment. Relatively stable and depleted δ²H-cDCE field values (−211 ± 20‰, n = 10, one outlier excluded) pointing to RD of PCE seem promising in that respect (Audimiro et al., 2015). Further experimental studies are needed to test how ε₂Hprotonation varies with microbial culture, reaction rate, and temperature in order to obtain further mechanistic understanding of the magnitudes of these prime parameters affecting the δ²H offsets of chlorinated solvents and reaction products.

3.5. Exploring potential H-CSIA patterns in aquifers by means of scenario modeling

In order to explore the potential use of H-CSIA in source apportionment of TCE versus PCE source zones, we extended the H-CSIA model with the PCE to TCE step to assess the δ²H values of TCE and daughter products in scenarios of mixed and pure PCE and TCE sources (see Supplementary Fig. S5).

3.5.1. Model extension

The PCE to TCE step only involves the simulation of protonation of TCE. PCE was added as a molecule to the model and its degradation rate linked to the production and thus protonation rate of TCE. The extended PHREEQC model (see Fig. S5) was used in 1-D advection/dispersion transport mode during complete reductive dechlorination. The summed concentration of PCE and TCE in the source was 1 mmol/L in all simulations. The groundwater flow velocity was 20 m per year, the longitudinal dispersivity coefficient was 1 m, and the duration of simulations was 15 years.

Table 4 shows the applied input parameter values selected for the degradation and H isotope fractionation processes. First-order kinetics was assumed with a set of degradation rate constants in line with previous modeling studies to SRD (Hunkeler et al., 2009; Van Breukelen et al., 2005). The values on hydrogen isotope fractionation were adopted from simulation S4a; Thus ε₂Hprotonation values were zero except for TCE (+34‰). The value of ε₂Hprotonation was not known for the PCE to TCE step and was taken equal to the TCE to DCE step. δ²H-H₂O was taken as −42‰. δ²H-TCE was taken as +500‰, within the range (+467‰ to +682‰) of published values for manufactured TCE (Kuder and Philp, 2013).

| Parameter | PCE | TCE | DCE | VC | ETH |
|-----------|-----|-----|-----|----|-----|
| k₀d (per year) | 1.5 | 1 | 0.5 | 0.5 | 0 |
| ε₂Hprotonation (%) | na | +34 | 0 | 0 | na |
| NRP(MEAN) (%) | −170 | −170 | −580 | −740 | na |

ma = not applicable; k₀d (per year) = first-order RD rate constant per year; ε₂Hprotonation (%) = hydrogen bulk isotope enrichment factor (SKIEs) of hydrogen atoms transferred to daughter product; ε₂Hprotonation = overall hydrogen isotopic enrichment factor expressed with respect to δ²Hwater during protonation.
3.5.2. Model results

Fig. 5 presents the 1-D model simulation results representing concentrations and H isotope patterns for complete dechlorination of PCE and/or TCE to ETH along the simulated flow path. Note that the sum of the CEs and ETH concentrations decreases beyond 250 m downgradient because of longitudinal dispersion with the displaced clean background groundwater.

The parent compound TCE becomes depleted in $\delta^2$H (Fig. 5: left; solid lines) during reductive dechlorination due to the inverse isotope fractionation as observed for this reaction step in this study. Daughter products are increasingly depleted the less they are chlorinated. During protonation strongly depleted hydrogen atoms replace the Cl atoms resulting in strong depletion of the final metabolite, ETH.

Note PCE does not contain H atoms and consequently H isotope ratios are not shown for PCE. In this case (Fig. 5: middle), strongly depleted $\delta^2$H-TCE is produced, about 550–700‰ more depleted than the source TCE of the previous simulation (Fig. 5: left). Correspondingly, the other daughter products are also considerably more depleted than in the TCE as parent compound scenario. Note that the difference in $\delta^2$H between the two simulations (PCE versus TCE) decreases the less chlorinated the compound is. In this scenario of a PCE source, $\delta^2$H-DCE exceeds $\delta^2$H-TCE because of (i) the inverse isotope effect during the TCE to DCE step; and (ii) the inverse fractionation effects associated with protonation and additivity are assumed similar for both the PCE to TCE and the TCE to DCE steps. As a result, both $\delta^2$H values intermediate of these pure source compound values. Likewise, $\delta^2$H-TCE, also $\delta^2$H of lower chlorinated daughter products and ETH become enriched compared to the case of an inverse isotope effect in TCE RD (Fig. 5: left and middle; dashed versus solid lines), whereas final $\delta^2$H-ETH remains identical irrespective of the SKIE values of the preceding steps because of reasons of isotope mass balance. Thus, provided source TCE is strongly enriched, H isotope analysis could be useful to distinguish among source TCE and TCE produced through PCE reductive dechlorination. Besides $\delta^2$H-TCE, also $\delta^2$H of lower chlorinated daughter products and ETH could be informative about their source compound (PCE or TCE) as their $\delta^2$H is strongly different between the two scenarios (about 200‰ or more, which is ten times or more the uncertainty of H-CSIA ($\pm$20‰)).

Finally, Fig. 5 (right) shows the simulation results of a mixed PCE/TCE source (1:1 molar ratio). PCE reductive dechlorination produces strongly depleted $\delta^2$H-TCE which mixes with the pool of strongly enriched source TCE. As a result $\delta^2$H-TCE values decrease rapidly away from the source and become intermediate of the pure PCE and TCE source scenario values. Likewise, $\delta^2$H values intermediate of these pure source scenarios are simulated for the degradation products. Note that in the case of pure sources (PCE or TCE) and SRD as sole reaction pathway, the $\delta^2$H values of the chlorinated ethenes and ETH are relatively constant in the flow direction. However, in the case of a mixed PCE/TCE source, strong decreases in $\delta^2$H are simulated especially near the source area (Fig. 5: right).

Summarizing, the model scenarios indicate that PCE daughter products are considerably more depleted than those produced from a pure (high $\delta^2$H) TCE source. Predicted $\delta^2$H values of specific daughter products remain fairly constant along flow suggesting the potential of H-CSIA for source apportionment.

4. Conclusions and outlook

The developed numerical model serves as a template model to interpret C, H, and Cl CSIA data in SRD of halogenated hydrocarbons in
general, with the aim of investigating (S)KIs, intramolecular halogen isotope ratio heterogeneity, and protonation effects. Furthermore, this model has great promise in application to CSIA-based DNA of sites polluted with chlorinated solvents to demonstrate and clarify the mechanisms of contaminant destruction. Extending the model to include the PCE to TCE step in SRD and alternative one-step degradation pathways such as chemical reduction and biological oxidation is straightforward. 3-D simulations using this PHREEQC model with either PHAST (Parkhurst et al., 2010) or PHT3D (Prommer and Post, 2010) are possible (Kuder et al., 2014) but would require long calculation times. Alternatively, the recently developed analytical BIO-CHLOR-ISO model (Höhener, 2016) enables rapid 3-D simulations of concentrations and carbon and chlorine isotope ratios but has limitations: it cannot cope with heterogeneous conditions, Monod kinetics, multiple DCE isomers, and does not consider the possibility of intramolecular heterogeneity of $^{35}$Cl in TCE.

The main limitations of model application are besides uncertainty on intramolecular heterogeneity of TCE, probably the current limited sets of fractionation factors, particularly those of hydrogen, which are still uncertain under field conditions for SRD and completely unknown for oxidation. The model may, however, obtain such fractionation factors via model validation provided the level of field site complexity is low and data coverage is high. Finally, the modeling of H-CSIA data may improve source apportionment of daughter products deriving from TCE or PCE since those derived of PCE should be considerably more depleted, provided that the source TCE is strongly enriched as reported for the majority of modern TCE products (Kuder and Philip, 2013; Shouakar-Stash et al., 2003).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jconhyd.2017.07.003.

References

Abe, Y., Aravena, R., Zopfi, J., Shouakar-Stash, O., Cox, E., Roberts, J.D., Hunker, D., 2009. Carbon and chlorine isotope fractionation during aerobic oxidation and reductive dechlorination of vinyl chloride and cis-1,2-dichloroethene. Environ. Sci. Technol. 43 (1), 101–107.
Aeppli, C., Berg, M., Cirpka, O.A., Holliger, C., Schwarzenbach, R.P., Hofstetter, T.B., 2011. Guidelines and recommended terms for expression of stable-isotope fractionation o...
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Journal of Contaminant Hydrology 204 (2017) 79–89

875–891.

Parkhurst, D.L., Appelo, C.A.J., 1999. User’s guide to PHREEQC (version 2): a computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations. In: Water-resources Investigations Report 99–4259. U.S. Geol. Survey, U.S.

Parkhurst, D.K., Kipp, K.L., Charlton, S.R., 2010. PHAST version 2—A program for simulating groundwater flow, solute transport, and multicomponent geochemical reactions. In: U.S. Geological Survey Techniques and Methods 6–A3. USGS.

Pooley, K.E., Blessing, M., Schmidt, T.C., Haderlein, S.B., Macquarrie, K.T.B., Prommer, H., 2009. Aerobic biodegradation of chlorinated ethenes in a fractured bedrock aquifer: quantitative assessment by compound-specific isotope analysis (CSIA) and reactive transport modeling. Environ. Sci. Technol. 43 (19), 7458–7464.

Prommer, H., Post, V., 2010. PHT3D: A Reactive Multicomponent Transport Model for Saturated Porous Media. User's Manual v2.10.

Rolle, M., Chiogna, G., Bauer, R., Griebler, C., Grathwohl, P., 2010. Isotopic fractionation by transverse dispersion: Flow-through microcosms and reactive transport modeling study. Environ. Sci. Technol. 44 (16), 6167–6173.

Shouakar-Stash, O., Frate, S.K., Drinnin, R.J., 2003. Stable hydrogen, carbon and chlorine isotope measurements of selected chlorinated organic solvents. J. Contam. Hydrol. 60 (3–4), 211–228.

Valentine, D.L., Sessions, A.L., Tyler, S.C., Chidthaisong, A., 2004. Hydrogen isotope fractionation during H2/O2 acetogenesis: hydrogen utilization efficiency and the origin of lipid-bound hydrogen. Geobiology 2 (3), 179–188.

Van Breukelen, B.M., Hunkerle, D., Volkering, F., 2005. Quantification of sequential chlorinated ethene degradation by use of a reactive transport model incorporating isotope fractionation. Environ. Sci. Technol. 39 (11), 4189–4197.

Van Breukelen, B.M., Prommer, H., 2008. Beyond the Rayleigh equation: reactive transport modeling of isotope fractionation effects to improve quantification of biodegradation. Environ. Sci. Technol. 42 (7), 2463–2469.

Van Breukelen, B.M., Rolle, M., 2012. Transverse hydrodynamic dispersion effects on isotope signals in groundwater chlorinated solvents' plumes. Environ. Sci. Technol. 46 (14), 7700–7708.

Wanner, P., Hunkerle, D., 2015. Carbon and chlorine isotopologue fractionation of chlorinated hydrocarbons during diffusion in water and low permeability sediments. Geochim. Cosmochim. Acta 157, 198–212.

Wiebert, C., Aeppli, C., Knowles, T., Holmstrand, H., Evershed, R., Pancost, R.D., Machackova, J., Gustafsson, O., 2012. Dual carbon-chlorine stable isotope investigation of sources and fate of chlorinated ethenes in contaminated groundwater. Environ. Sci. Technol. 46 (20), 10918–10925.