

Quantifying biodegradation rate constants of o-xylene by combining compound-specific isotope analysis and groundwater dating

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ABSTRACT

The objective of this study is to estimate hydraulic conductivities and biodegradation rate constants in a coal-tar contaminated aquifer by compound-specific isotope analysis (CSIA) and tracer-based (³H-³He) groundwater dating (TGD). In two observation wells downgradient from the contaminant source in situ biodegradation of o-xylene, toluene and naphthalene under sulfate-reducing redox conditions could be demonstrated using CSIA. Median biodegradation rate constants for o-xylene ranging between 0.08 and 0.22 a⁻¹ were estimated. By using tracer-based groundwater dating in these two wells, hydraulic conductivities could be also estimated, which are in a similar range as k-values derived from sieve analysis, a pumping test and a calibrated groundwater flow model. These results clearly demonstrate the applicability of tracer-based groundwater dating for the determination of in situ hydraulic conductivities in aquifers without pumping contaminated groundwater. Finally, a sensitivity analysis is performed using a Monte Carlo simulation. These results indicate high sensitivities of the assumed effective porosity for the estimation of the hydraulic conductivity and the selected isotope enrichment factor for the biodegradation rate constant, respectively. Conversely, the outcome also evidently demonstrates the main limitations of the novel combined isotope approach for a successful implementation of monitored natural attenuation (MNA) at such field sites.

1. Introduction

The contamination of the subsurface due to leaking point sources from industrial plants is a widespread issue and often requires extensive long term monitoring and remediation (Schwarzenbach et al., 2010). A common example for such incidences is the release of coal tar and creosote constituents into the subsurface. Most of the latter occur as dense non-aqueous phase liquids (DNAPL). Once they enter the subsurface, these liquids typically seep through unsaturated and saturated zones of the aquifer until reaching a natural barrier, such as impermeable or low-permeable soils or geological layers, where they tend to accumulate and migrate in groundwater flow direction (e.g. D'Affonseca et al., 2008a). When it comes to monitoring and remediation, biodegradation is often considered as an appropriate remediation strategy in the form of monitored natural attenuation (MNA) at such sites (e.g. Blum et al., 2007, 2011; Martus and Schaal, 2010). The qualitative and quantitative proof of biodegradation is crucial for a successful implementation of MNA (e.g. Rügner et al., 2006).

During the last decades stable compound-specific isotope analysis (CSIA) has been established as the major method for assessing the occurrence of biodegradation in contaminated aquifers (e.g. Sturchio et al., 1998; Hunkeler et al., 1999a; Sherwood Lollar and Slater, 2001; Richnow et al., 2003a, 2003b; Griebler et al., 2004). It uses the fact that (bio-)chemical reactions degrading organic contaminants are accompanied by isotope fractionation according to the kinetic isotope effect (Elsner, 2010). A large number of studies exist showing the potential of CSIA. Thus, one can assess the fate of organic pollutants (Meckenstock et al., 2004; Braeckevelt et al., 2012), differentiate between multiple source zones (Hunkeler et al., 2004), or reveal transformation mechanisms and pathways via multi isotope analysis (Elsner et al., 2005). However, the concept of CSIA also have some challenges and limitations: It struggles when concentrations of organic contaminants are low and therefore only tap the full potential at highly polluted field sites (Schmidt et al., 2004). Furthermore, field applicability suffers from factors that potentially influence observable isotope shifts, namely limited bioavailability (Braeckevelt et al., 2012), and physical processes,

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such as sorption and diffusion, even though their impact is considered to be minor (Kuntze et al., 2020).

Nevertheless, CSIA has been used for successful assessment of biodegradation in field studies for various pollutants such as mono- (BTEX) and polyaromatic hydrocarbons (e.g. Richnow et al., 2003a; Blum et al., 2009; Dorer et al., 2016;), chlorinated compounds (Hunkeler et al., 1999b; Mundle et al., 2012; Damgaard et al., 2013; Wanner et al., 2016) and most recently for micropollutants such as pesticides and pharmaceuticals (Schürner et al., 2016), and herbicides (Milosevic et al., 2013).

The time scale of bio-degradative processes can be evaluated using tracer-based groundwater dating methods, which are particularly advantageous in more complex geological settings. Amaral et al. (2009) and Aeppli et al. (2010) first combined CSIA with ^3H - ^3He groundwater dating. While Amaral et al. (2009, 2011) were unable to quantify biodegradation rates of the studied poly-nitroaromatic compounds (P-NACs) and chlorinated ethenes (CEs), Aeppli et al. (2010), who also studied the transformation of chlorinated ethenes, could determine pseudo first order biodegradation rates.

In the present study we therefore intend to determine hydraulic conductivities and biodegradation rate constants of monoaromatic compounds (BTEX), 1,2,3-trimethylbenzene and naphthalene by applying this combined isotope approach at a coal tar contaminated aquifer in Germany. Hence, a monitoring campaign at nine monitoring wells downstream of the source zone was performed. The sampled groundwater was analyzed amongst others for the following stable isotopes: $\delta^{13}\text{C}$, deuterium (^2H) and tritium (^3H). In addition, a sensitivity analysis using a Monte Carlo simulation was implemented to assess uncertainties and the impact of aquifer characteristics and other parameters on the determination of hydraulic conductivities and biodegradation rate constants.

2. Material and methods

2.1. Study site

The studied field site is located about 40 km southwest of Hamburg, Germany (Fig. 1a). A former wood impregnation plant triggered the seepage of coal tar and creosote constituents, resulting in a significant subsurface impact down to the regional upper aquifer at a depth of about 50 m below surface (Fig. 1b). Glacial sand deposits of 130 m thickness form two aquifers separated by an aquitard. The unconfined upper aquifer is divided into three sand layers with distinct grain sizes (Fig. 1b): layer one comprises medium to coarse sands starting from the surface down to 20 m, layer two consists of medium sands from 20 m to 40 m below surface, and the third layer is composed of coarse to medium sands down to 49 m. The aquitard, however, mainly remains pristine and is not affected by the contamination, due to a low-permeable very silty fine sand layer acting as a natural barrier for vertical contaminant migration. The water table varies between 18 and 19 m below surface and the groundwater flow direction is southeast (Blum et al., 2009).

The DNAPLs are enriched within the coarse to medium sand layer, where they migrated in groundwater flow direction and formed an extensive contaminant plume of more than 450 m length (Blum et al., 2009). In this layer, the average hydraulic conductivity and effective porosity were previously determined to be 4.2×10^{-4} m/s and 21%, respectively (Blum et al., 2007). The mean hydraulic gradient at the site is 1.5×10^{-3} (Blum et al., 2007). The source zone is located about 50 m northwest from well G10m (Fig. 1a) and is well described in a previous study by D'Affonseca et al. (2008a,b). The contaminants at the field site include mono-, polyaromatic hydrocarbons and also NSO heterocycles, representing the main constituents of the coal tar (Blum et al., 2011). Since remediation started in 1986, numerous monitoring wells have been installed around the source zone and downgradient, forming a large monitoring network of more than 40 observation wells (e.g. D'Affonseca et al., 2008a; Blum et al., 2009). Most of the wells are

installed in the lower aquifer layer with screen lengths of 4 to 5 m (Fig. 1b). The upper aquifer has also been well investigated and characterized by reactive transport models of the source and also the contaminant plume (D'Affonseca et al., 2008a, 2008b, 2011). Furthermore, in-situ biodegradation could be qualitatively assessed by redox sensitive tapes and signature metabolite analysis (Blum et al., 2006). Quantitative approaches for biodegradation were later performed using Michaelis-Menten kinetics and CSIA (Blum et al., 2009). With the latter only biodegradation rate constants of o-xylene and naphthalene could be explicitly derived under sulfate-reducing conditions, indicating the limitations of the CSIA. Further information on the main electron acceptors of the aquifer and the stable carbon isotope concentrations of the other sampled compounds are provided by Blum et al. (2009) and in Table 2 for the second sampling campaign.

2.2. Compound-specific stable isotope analysis

Groundwater samples were taken from nine monitoring wells downgradient of the source zone (Fig. 1). Stable carbon and hydrogen isotope ratios of BTEX (benzene, toluene, m-/p-xylene, o-xylene), 1,2,3-trimethylbenzene and naphthalene were determined using gas chromatography on line combustion isotope ratio mass spectrometry (GC-C-IRMS). The contaminants were extracted in n-pentane and introduced into the GC via splitless injection on a capillary column. Organic compounds were separated in the GC and converted to CO_2/H_2 within a combustion reactor. The latter were transferred to the IRMS for gauging corresponding ratios of $^{13}\text{CO}_2/^{12}\text{CO}_2$ and $^2\text{H}/^1\text{H}$. A detailed description of the laboratory procedure is given by Blum et al. (2009). The CSIA results were expressed in the δ -notation as shown below for carbon as example:

$$\delta^{13}\text{C} [\text{‰}] = \left(\frac{R_{\text{Sample}}}{R_{\text{std}}} - 1 \right) \times 1000 \quad (1)$$

with R_{Sample} being the isotope ratio of the sample and R_{std} representing the isotope ratio of a standard. Derived δ -notations of ^{13}C are summarized in Fig. 1b. Of all investigated compounds only carbon isotope ratios of o-xylene showed noticeable shifts downgradient indicating active degradation according to the minimum shift of 2‰ relative to a reference well upgradient of the source (Hunkeler et al., 2009). Thus, carbon isotope shifts of the sections formed by wells G10m – G28u ($\Delta\delta^{13}\text{C} = 2.1\text{‰}$) and wells G10m – G30u ($\Delta\delta^{13}\text{C} = 4.3\text{‰}$) were considered in the following calculations (Fig. 1b). Similar findings were also found in a previous study by Blum et al. (2009).

Fig. 2 illustrates that for o-xylene, 1,2,3-trimethylbenzene (data not shown) and naphthalene (data not shown) based on triple determinations only in 5 out of 9 sampled groundwater wells hydrogen isotope ratios could be detected. Surprisingly, the hydrogen isotope ratios did not show any variation related to potential biodegradation (i.e. no positive isotope shift downgradient of the contaminant source). One explanation, why no hydrogen isotope shift could be observed, might be the difficulties to analyse hydrogen isotopes at low concentrations (i.e. $\leq 15 \mu\text{g/l}$ o-xylene, Fig. 1, Table 2). Thus, in the wells, in which a hydrogen isotope fractionation could occur (G28u and G30u), no hydrogen isotope ratios could be determined due to the detection limit. Hence, with the available data no isotope shift was observed and therefore are not further considered here.

2.3. Tracer-based groundwater dating (^3H - ^3He)

Knowing the age of groundwater can help reconstructing groundwater flow paths and estimating horizontal or vertical flow velocity. Furthermore, the past release of contaminants migrating into the aquifer can be reconstructed. In this study, groundwater ages, i.e. infiltration times, were determined with the ^3H - ^3He method, which uses the radioactive decay of tritium as a tracer. The decay starts with the

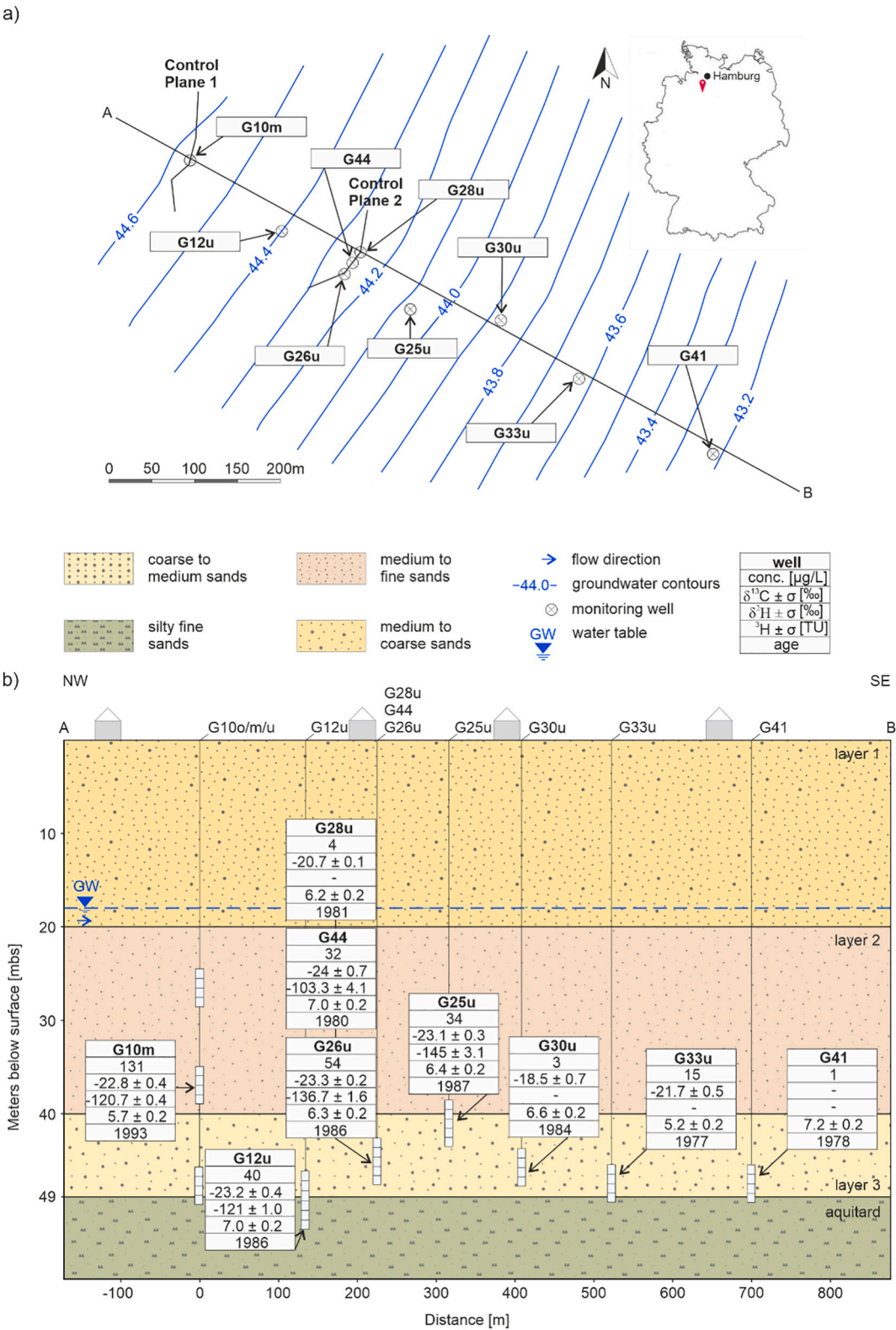


Fig. 1. (a) Location and site plan of the study site showing the sampled monitoring wells and the groundwater contour lines in blue. (b) Simplified geological cross section of the upper aquifer showing the monitoring wells with the well screens from which the groundwater samples were collected. Furthermore, the corresponding data is shown such as *o*-xylene concentrations, $\delta^{13}\text{C}$, $\delta^2\text{H}$ - ^3H data and derived ^3H - ^3He -ages. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

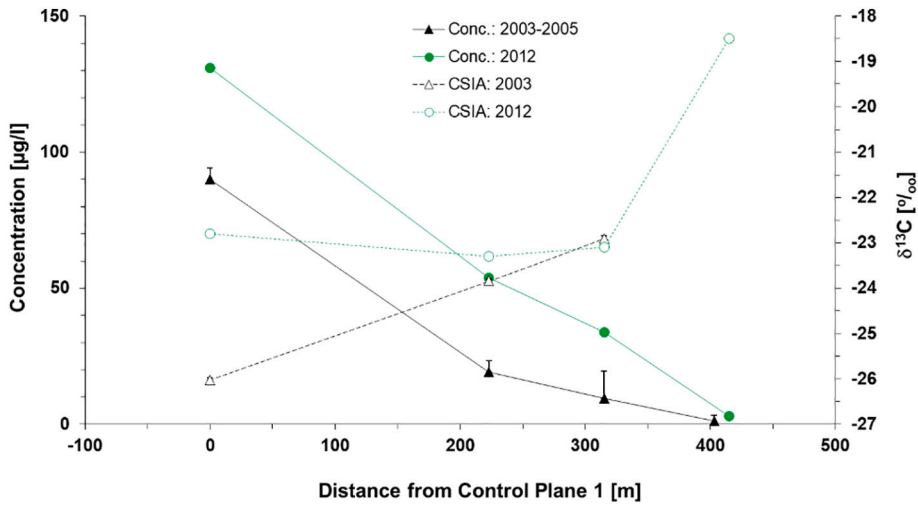


Fig. 2. Concentrations (closed symbols) and the respective $\delta^{13}\text{C}$ -values (open symbols) of o-xylene in groundwater wells along the centre line of the plume sampled in October 2003 and December 2012 including the minimum and maximum concentrations of the four monitoring campaigns (October 2003, June 2004, November 2004, June 2005) indicated by the error bars (data taken from Blum et al., 2009). Error bars by the isotope data are standard deviations of the $\delta^{13}\text{C}$ -values analyzed by CSIA (only October 2003).

infiltration in the subsurface as part of precipitation

$$\tau = t_{1/2} / \ln 2 \times \ln(1 + {}^3\text{He}/{}^3\text{H}) \quad (2)$$

where $t_{1/2}$ represents the half-life of tritium (12.32 ± 0.02 years), and ${}^3\text{He}$ and ${}^3\text{H}$ are the concentrations of the tritiogenic ${}^3\text{He}$ and tritium, respectively. The decay product of ${}^3\text{H}$ is ${}^3\text{He}$, which is an inert gas, accumulates in the saturated zone. Thus, the ratio of ${}^3\text{He}/{}^3\text{H}$ of a groundwater sample enables to calculate a piston flow age (e.g. Schlosser et al., 1988). An advanced mass spectrometric system was used to analyse He isotopes, and neon and tritium by the ingrowth of ${}^3\text{He}$ (Sülfenfuß et al., 2009). Fig. 1b summarizes the resulting groundwater ages at all monitoring wells representing infiltration times from 19 years (G10m) up to 35 years (G33u).

2.4. Determination of hydraulic conductivities

Hydraulic conductivities (k_f) were derived by combining the simple expression $v = s/t$ (v = velocity, s = distance, t = time) with Eq. (3) resulting in Eq. (4).

$$v_a = \frac{v_f}{n_e} = \frac{k_f \times i}{n_e} \quad (3)$$

$$k_f = \frac{s \times n_e}{t \times i} \quad (4)$$

v_a is the seepage velocity, v_f is the apparent velocity of the groundwater flow, n_e is the effective porosity and i represents the hydraulic gradient. Furthermore, t is the time needed for the groundwater to pass a certain distance s . This period of time is determined using the previously described tracer-based groundwater dating.

Furthermore, Eq. (4) was evaluated by performing a Monte Carlo analysis. Table 1 shows the corresponding input parameters and setup for the Monte Carlo simulation. The parameter values for n_e and i were taken from the previous study by Blum et al. (2007). For the distance s between the reference well G10m and G28u/G30u a uniform distribution (U) was set with s_{min} representing the direct horizontal pathway, while s_{max} additionally includes the maximum vertical variation in distance. A normal distribution (N) was defined for groundwater residence times obtained from the ${}^3\text{H}$ - ${}^3\text{He}$ groundwater dating, including standard deviations from Sülfenfuß et al. (2009). The number of iterations for the Monte Carlo simulation was set to 100.000.

2.5. Determination of biodegradation rate constants

Biodegradation (λ) was quantified using Eq. (5) by Blum et al.

Table 1

Input parameters and setup for the determination of hydraulic conductivities and first order biodegradation rate constants including the assumed parameter distributions (U = uniform distribution, N = normal distribution and W = Weibull distribution, numbers in brackets show the necessary input values, i.e. minimum and maximum values for the uniform distribution, mean value and standard deviations for the normal distribution, and scale and shape parameters for the Weibull distribution, respectively).

Output	Name	Parameter	Unit	Distribution (range of assumed input values)
Hydraulic conductivity	Distance	s	m	$U(415, 423)^a$ $U(225, 233)^b$ $N(0.21, 0.09)^c$
	Effective porosity	n_e	–	$N(3.15 \times 10^{-8}, 1.57 \times 10^{-8})^{a, d}$ $N(4.10 \times 10^{-8}, 1.57 \times 10^{-8})^{b, d}$
	Residence time	t	s	$N(1.3 \times 10^{-3}, 1.6 \times 10^{-3})^{c, e}$
First order biodegradation rate constant	Hydraulic gradient	i	–	$W(1.3 \times 10^{-3}, 1.6 \times 10^{-3})^{c, e}$
	Isotope shift	$\delta^{13}\text{C}$	‰	$N(4.3, 0.7)^{a, g}$ $N(2.1, 0.1)^{b, h}$
	Distance	s	m	$U(415, 423)^a$ $U(225, 233)^b$
	Hydraulic gradient	i	–	$W(1.3 \times 10^{-3}, 1.6 \times 10^{-3})^{c, e}$
	Effective porosity	n_e	–	$N(0.21, 0.09)^c$
	Hydraulic conductivity	k_f	m/s	–
	Isotope enrichment factor	ϵ	–	$U(-3.2, -1.1)^e$

^a Wells: G10m – G30u.

^b Wells: G10m – G28u.

^c Blum et al. (2007), truncated to (0.12, 0.30).

^d Sülfenfuß et al. (2009), truncated to (3.0×10^{-8} , 3.3×10^{-8}) for G10m-G30u and (3.9×10^{-8} , 4.3×10^{-8}) for G10m – G28u.

^e e.g. Meckenstock et al. (2004).

^f Truncated to (1.3×10^{-3} , 1.6×10^{-3}).

^g Truncated to (3.8, 4.8).

^h Truncated to (1.6, 2.6).

(2009), which combines the general first order expression with a simplified Rayleigh approach and Eq. (4). The latter was solved for t and inserted in Eq. (5) which then result in Eq. (6):

$$\lambda = -\frac{4\delta^{13}\text{C}}{\epsilon \times t} \quad (5)$$

$$\lambda = -\frac{\Delta\delta^{13}C \times k_f \times i}{\epsilon \times s \times n_e} \quad (6)$$

In line with Chapter 2.4 Eq. (6) is also evaluated using Monte Carlo analysis. Hence, a normal distribution (N) was defined for isotope shifts ($\Delta\delta^{13}C$) accounting for measurement errors. For well G30u, however, no errors could be quantified. Instead, the largest error of all wells (G40) was used for the analysis. Parameters s , i and n_e remained equal and an unmodified set of parameter values resulting from the prior simulation was used for hydraulic conductivities (k_f). Due to the lack of data for isotope enrichment factors (ϵ) under sulfate-reducing conditions (Meckenstock et al., 2004), a uniform distribution (U) was defined.

3. Results and discussion

The results of the combined isotope approach (CSIA and TGD) are exemplarily shown for *o*-xylene in Fig. 2, which is beside toluene with $\Delta\delta^{13}C = 3.8\text{‰}$ between the wells G10m and G33u and naphthalene with $\Delta\delta^{13}C = 0.4\text{‰}$, the only compound that actually indicates a noticeable change in carbon isotope signature. These isotope shifts for *o*-xylene, toluene and naphthalene were already observed in the previous study by Blum et al. (2009). The minor isotope shift for naphthalene was also confirmed with the second sampling campaign (December 2012) resulting in exactly the same isotope shift of 0.4‰ (G10m and G33u). In this previous study in situ first order biodegradation rate constants could therefore be estimated for *o*-xylene and also naphthalene under sulfate-reducing conditions. The latter could be demonstrated by the significant change in sulfate concentrations in the centre of the contaminant plume (Blum et al., 2009), which was also confirmed for the second sampling campaign in December 2012 (Table 2).

The measured electron acceptors clearly show that sulfate reducing-conditions are combined with stable carbon isotope shifts of *o*-xylene (in wells G28 and G30u). In contrast, in wells with high methane concentrations $\geq 1500 \mu\text{g/l}$ (G12u, G26u, G44, G33u and G41u), where methanogenesis occur simultaneously with iron reduction, no stable carbon isotope shifts are detected. This co-occurrence of iron reduction and methanogenesis pathways were recently also observed in a studied BTEX plume by Teramoto et al. (2020). Hence, the data suggest that if any significant isotope shifts occur, it only takes place for biodegradation of *o*-xylene, toluene and naphthalene under sulfate-reducing conditions.

Table 3 summarizes the derived hydraulic conductivities, in situ first order biodegradation rate constants and half-lives for *o*-xylene. The derived half-lives for *o*-xylene range between 3.0 and 7.9 years and therefore are in good agreement with the previously determined half-lives based on sieve analysis and pumping test data (Blum et al., 2009) and a calibrated numerical flow model at the site (D'Affonseca et al., 2011). Due to the low detection limit, the groundwater dating using the He-ingrowth method was very effective for the determination of a mean effective groundwater velocity (v_a) of the studied aquifer. The main advantage of the combined isotope approach using CSIA and TGD, however, is that no hydraulic tests are necessary and therefore no contaminated groundwater has to be extracted and treated before reinjection. As the combined isotope approach only requires a single sampling and analysis campaign, it is also more cost-efficient. Yet, in the present study this approach was only feasible for *o*-xylene.

Table 2

Main electron acceptors, *o*-xylene and naphthalene concentrations along the centre line of the plume of the second sampling campaign in December 2012.

Compound/well	Unit	G10m	G12u	G28u	G26u	G44	G30u	G33u	G41u
Sulfate	mg/l	46	53	46	13	2	29	44	20
Iron	mg/l	0	16	11	17	7	0	30	< 0,01
Methane	$\mu\text{g/l}$	140	1500	< 2	4900	6200	31	2000	740
<i>o</i> -xylene	$\mu\text{g/l}$	131	40	4	54	32	3	15	1
Naphthalene	$\mu\text{g/l}$	9170	2460	39	1720	236	5	65	37

Table 3

Summary of derived hydraulic conductivities (k_f), groundwater velocities (v_a), first order biodegradation rate constants (λ) and half-lives ($t_{1/2}$) for *o*-xylene based on the stable carbon isotopes, a calibrated numerical flow and groundwater dating using an effective porosity $n = 21\%$ and hydraulic gradient $i = 0.0015$. Results from this study are given as median values and standard deviation.

Method	k_f [m/s]	v_a [m/a]	λ [a^{-1}]	$t_{1/2}$ [a]	Reference
Groundwater dating	$1.9 \pm 0.5 \times 10^{-4}$	43 ± 11	0.22 ± 0.07	3.0	This study (distance between wells G10m and G30u = 410 m)
Groundwater dating	$8.2 \pm 2.0 \times 10^{-5}$	18 ± 5	0.08 ± 0.03	7.9	This study (distance between wells G10m and G28u = 225 m)
Sieve analysis and pumping test	4.2×10^{-4}	95	0.72	1.0	Blum et al. (2009)
Numerical flow model	1.5×10^{-4}	32	0.27	2.6	D'Affonseca et al. (2011)

3.1. Monte Carlo simulation

To further investigate the uncertainty and sensitivity of the presented approach a Monte Carlo simulation was performed using the values and distributions provided in Table 1. The outcome of the simulation is shown in Fig. 3 along with results from previous studies (Table 3). The simulated hydraulic conductivities for the two studied profiles (wells G10m – G28u and G10m – G30u) vary between $8.2 \pm 2.0 \times 10^{-5}$ m/s and $1.9 \pm 0.5 \times 10^{-4}$ m/s, respectively. The latter in particular is in good agreement with the hydraulic conductivity derived from the calibrated numerical groundwater flow model (D'Affonseca et al., 2011), whereas sieve analysis and pumping test data are out of range (Fig. 2a). This general overestimation of hydraulic conductivities derived from grain-sieve analysis was already observed in various previous studies (e.g. Storz et al., 2017). The lower and higher hydraulic conductivities are caused by different evaluated infiltration times. This can be explained by local heterogeneities, which were also observed in the previous study by D'Affonseca et al. (2008a). Furthermore, both distributions are bell curved indicating a normal distribution. In contrast, both determined distributions for the biodegradation rate constants are right skewed indicating a triangular distribution (Fig. 3b). This skewness might be related to the chosen Weibull distribution for the hydraulic gradient, which favors low hydraulic gradient values, and/or the truncated normal distribution of the isotope shift values, which puts more emphasis on higher isotope shift values. The relative contribution of the individual input parameters to the obtained results is further investigated by performing a sensitivity analysis in the following chapter.

3.2. Sensitivity analysis

Spearman's rank correlation coefficients (SRCC) were determined and are illustrated in Fig. 4. The evaluated hydraulic conductivity is highly sensitive to the effective porosity with a correlation coefficient (γ) of >0.95 , whereas other parameters show a negligible range of values for γ between -0.25 and 0.05 (Fig. 3a). The results clearly demonstrate the importance of the chosen value of effective porosity for a realistic estimate of hydraulic conductivity.

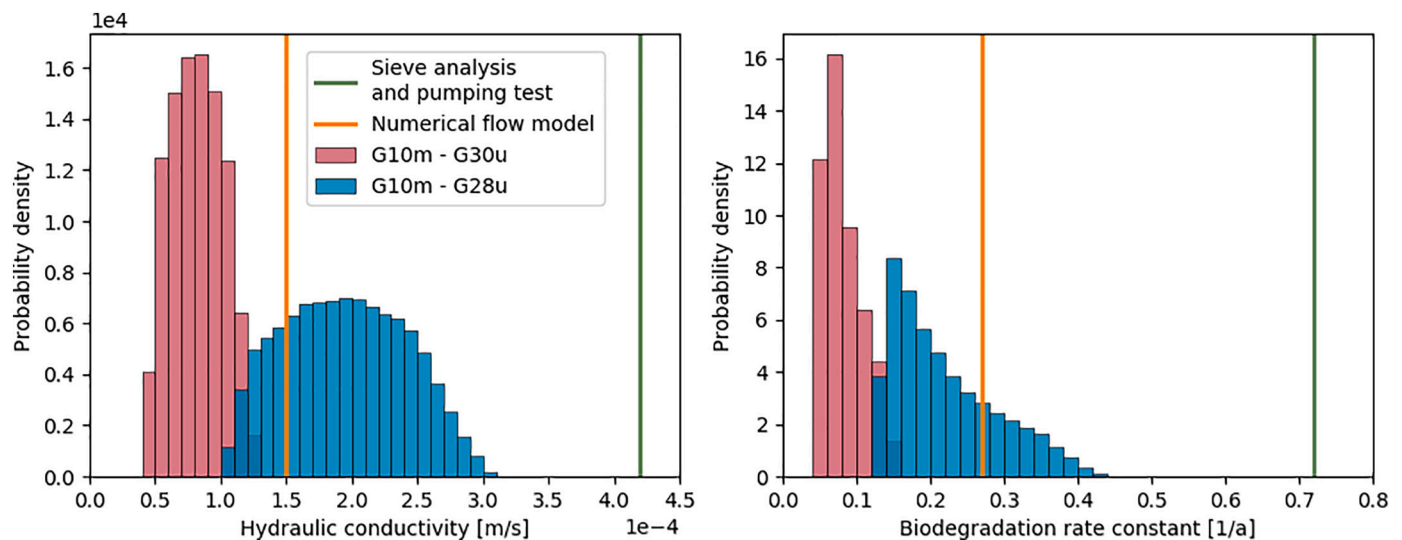


Fig. 3. Results of the Monte Carlo simulation presented in probability distributions for (a) hydraulic conductivity and (b) first order biodegradation rate constant. The continuous vertical lines illustrate mean values obtained from sieve analysis and pumping test (green) and calibrated numerical flow model (yellow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

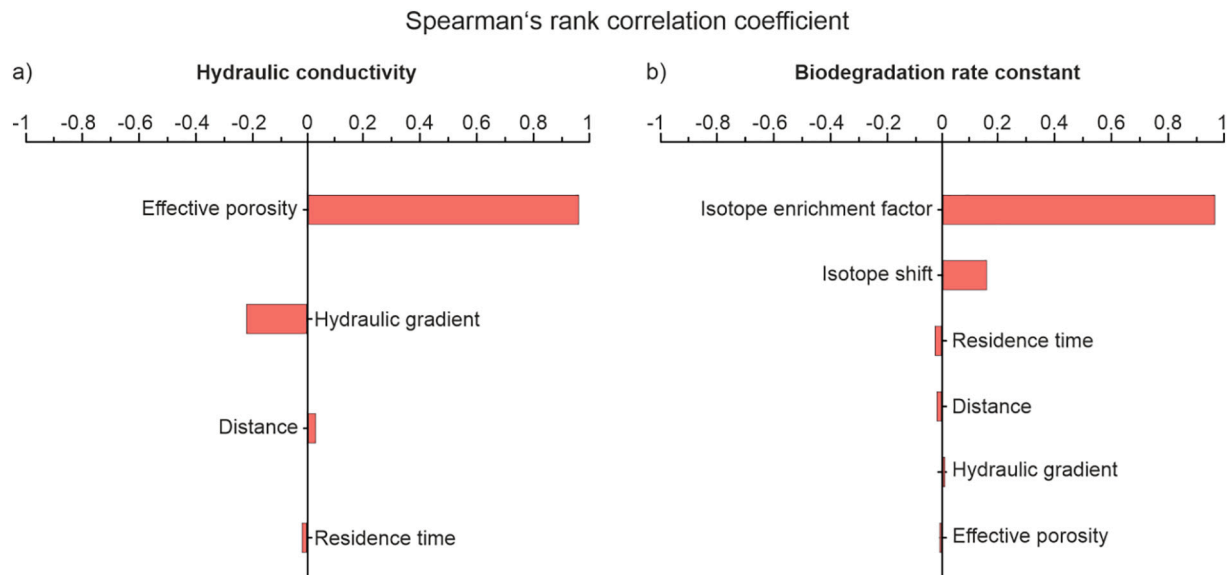


Fig. 4. Spearman's rank correlation coefficients of (a) hydraulic conductivity, (b) first order biodegradation rate constants, and associated parameters for each model.

For biodegradation rate constants, the isotope enrichment factor shows a dominating correlation coefficient of 0.98. Hence, high uncertainties for derived biodegradation rates mainly depend on this unknown quantity. Uncertainties associated with the enrichment factor are due to the applied large range of reported laboratory-derived enrichment factors ranging in case of o-xylene for sulfate-reducing conditions between -3.2 and -1.1 (e.g. Meckenstock et al., 2004; Morasch et al., 2004; Herrmann et al., 2009; Vogt et al., 2016, 2018). The latter can be reduced by determining field-derived isotope enrichment factor, which for the studied site is -1.4 (Blum et al., 2009). Using the site-specific isotope enrichment factor of 1.4 while keeping the remaining parameter uncertainties, yields biodegradation rate constant values of $0.31 \pm 0.02 \text{ a}^{-1}$ (G10m-G30u) and $0.12 \pm 0.01 \text{ a}^{-1}$ (G10m-G28u), respectively, indicating a slight shift into higher median values and a significant reduction in the variance of the results.

Nevertheless, the combined isotope approach of CSIA and ^3H - ^3He -groundwater dating helped addressing these uncertainties and clearly

showed that accurate flow velocity estimates are crucial for evaluating appropriate degradation rate constants owing to aquifer heterogeneity. Höyng et al. (2015) already stated the importance of implementing tracer-based methods in heterogeneous aquifers, when it comes to the evaluation of biodegradation rate constants. The possible overestimation of degradation rates when dealing with heterogeneous flow velocities presented in this work was also observed in previous studies (Bauer et al., 2006; Beyer et al., 2007). They concluded that this would result in imprecise estimates for the overall cleanup time and therefore also influence the risk assessment at contaminant sites.

4. Conclusion

The combination of CSIA and ^3H - ^3He -groundwater dating (TGD) was used to determine hydraulic conductivities and biodegradation rates of o-xylene at a coal tar contaminated aquifer. Hence, isotope data from a monitoring campaign was also evaluated using a Monte Carlo

simulation. The following major findings could be drawn from our results:

- The multi-element CSIA approach of carbon and hydrogen stable isotopes has limited applicability at the studied site. Of all studied compounds only carbon isotopes of *o*-xylene, toluene and naphthalene showed noticeable shifts at two monitoring wells downgradient of the source zone, whereas hydrogen isotope analysis could not be linked to in situ biodegradation.
- Using the tracer-based groundwater dating, effective hydraulic conductivities and groundwater velocities could be appropriately determined, which are in good agreement with results from a calibrated groundwater flow model.
- The combined isotope approach of CSIA and TGD enabled the quantification of bio-degradation rate constants of *o*-xylene under sulfate-reducing conditions.
- The chosen isotope enrichment factor has a major influence on the computed biodegradation rate constants as enrichment factors can be variable due to different biochemical activation reactions leading to different enrichment factors, and the possible masking of isotope fractionation due to mass transfer-related effects.

Finally, we can conclude that tracer-based groundwater dating itself can be used to estimate effective in situ hydraulic properties such as hydraulic conductivities and groundwater velocities. Furthermore, the combined CSIA and TGD approach enables the estimation of realistic in situ biodegradation rate constants. Presently, the main disadvantage of this novel approach is its limitation to only few compounds. Hence, future studies have to prove its reliability for other compounds and contaminated aquifers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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