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Quantification of biodegradation for *o*-xylene and naphthalene using first order decay models, Michaelis–Menten kinetics and stable carbon isotopes

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A B S T R A C T

At a former wood preservation plant severely contaminated with coal tar oil, *in situ* bulk attenuation and biodegradation rate constants for several monoaromatic (BTEX) and polyaromatic hydrocarbons (PAH) were determined using (1) classical first order decay models, (2) Michaelis–Menten degradation kinetics (MM), and (3) stable carbon isotopes, for *o*-xylene and naphthalene. The first order bulk attenuation rate constant for *o*-xylene was calculated to be 0.0025 d^{-1} and a novel stable isotope-based first order model, which also accounted for the respective redox conditions, resulted in a slightly smaller biodegradation rate constant of 0.0019 d^{-1}. Based on MM-kinetics, the *o*-xylene concentration decreased with a maximum rate of k_{max}=0.1 µg/L/d. The bulk attenuation rate constant of naphthalene retrieved from the classical first order decay model was 0.0038 d^{-1}. The stable isotope-based biodegradation rate constant of 0.0027 d^{-1} was smaller in the reduced zone, while residual naphthalene in the oxic part of the plume further downgradient was degraded at a higher rate of 0.0038 d^{-1}. With MM-kinetics a maximum degradation rate of k_{max}=12 µg/L/d was determined. Although best fits were obtained by MM-kinetics, we consider the carbon stable isotope-based approach more appropriate as it is specific for biodegradation (not overall attenuation) and at the same time accounts for the dominant electron-accepting process. For *o*-xylene a field based isotope enrichment factor ε_{field} of −1.4 could be determined using the Rayleigh model, which closely matched values from laboratory studies of *o*-xylene degradation under sulfate-reducing conditions.

1. Introduction

Active remediation techniques such as pump-and-treat or excavation are economically not feasible for the large number of soil and groundwater contaminations existing worldwide. Monitored natural attenuation (MNA) as a passive remediation strategy is considered a worthwhile alternative. Numerous studies have shown that natural attenuation (NA) processes (e.g. dilution, sorption, dispersion, (bio-) degradation) have the potential to manage groundwater contamination at many sites (e.g. Rice et al., 1995; Mace et al., 1997; Grathwohl et al., 2000), which becomes evident in the observation that many dissolved contaminant plumes are not expanding any more. In recent years, short and steady state contaminant plumes are becoming increasingly tolerated and MNA is implemented as a remediation strategy in the United States and in Europe when no receptor is at risk (NRC, 1999; Rügner et al., 2006). (Bio-)Degradation deserves special attention, because, out of all NA processes in groundwater, it is the only one substantially effective in removing contaminants from the environment for many compound

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classes, e.g. BTEX and PAH. Thus, evidence of biodegradation is mandatory to apply MNA as remediation strategy.

Previous studies have shown that it is often insufficient to measure residual concentrations of organic contaminants in groundwater in order to provide evidence of biodegradation (e.g. Rittmann, 2004; Wilson et al., 2004). However, other standard field techniques to distinguish the biodegradation of contaminants from physico-chemical attenuation processes in the environment are still lacking. Hence, in various studies *in situ* biodegradation of not only PAH, but also of monoaromatic compounds such as toluene and benzene, was approximated using incubation experiments performed in the laboratory (Herbes, 1981; Chapelle et al., 1996; Knightes and Peters, 2003; Morasch et al., 2007). Retrieval of the intrinsic biodegradation potential for single contaminants generally demands elaborate set-ups in conjunction with considerable analytical effort. Even when laboratory conditions are kept close to environmental conditions, the interpretation of field cases based on laboratory findings often is subject to errors and high uncertainties (e.g. Chapelle et al., 1996; Nielsen et al., 1996).

To facilitate a quantitative assessment of *in situ* biodegradation, reaction models following first order decay are feasible and have been used to generate biodegradation rate constants for risk assessment at field sites (e.g. Wiedemeier et al., 1999; Suarez and Rifai, 2004; Bauer et al., 2006). First order decay models, however, are often regarded imprecise or even conceptually incorrect (Beyer et al., 2006; Schäfer et al., 2007). One shortcoming, for example, is that their resolution is not fine enough to reflect the fact that intrinsic biodegradation might be limited to plume fringes where electron acceptors from the surrounding uncontaminated zones mix by dispersion and diffusion (Wilson et al., 2004). A substantial problem is the negligence of various hydrogeological variables and NA processes that may take place in the environment (e.g. dispersive mixing, etc.). Thus, resulting first order rate constants rather describe bulk attenuation than local *in situ* biodegradation kinetics (e.g. Newell et al., 2002).

Another reaction model less frequently used for the *in situ* quantification of biodegradation is Michaelis–Menten (MM) kinetics, also known as Monod, no-growth kinetics (e.g. Simkins and Alexander, 1984; Bekins et al., 1998; Beyer et al., 2006; Schäfer et al., 2007). The MM model originates from biochemistry and is generally applied for enzyme kinetics in the quasi-stationary state. It relates the maximum turnover rate of one specific enzyme to the present substrate concentration (single compound) in the medium. The MM model requires that the amount of enzyme remains constant for the duration of the experiment. When MM kinetics is transferred to a less defined system such as a contaminated aquifer it has to be assumed that (1) the degradation velocity is only related to the contaminant concentration and that (2) degrading microorganisms do not change in number or activity over time (Beyer et al., 2006).

Both the classical first order as well as the MM model are not really process based, as they neglect the manifold dependencies which govern biodegradation in complex systems such as limitation of substrate turnover by the amount of degrading enzymes and electron acceptors, the presence of potentially inhibiting compounds, changes in the number and activities of microorganisms over time, the dependence of biodegradation pathways on local redox conditions, adsorption of non-polar compounds to particle surfaces, and poor bioavailability in generally caused by substrates temporally or physically constrained from potentially degrading microorganisms (e.g. Semple et al., 2004; Thullner et al., 2007). In a numerical experiment, Schäfer et al. (2007) demonstrated that both approaches might be capable of approximating mass and dimensions of contaminant plumes that come from far more complex degradation processes. For a long-term prognosis, both models proved inappropriate, resulting in an underestimation of plume length and contaminant mass.

An effective link between *in situ* biodegradation of organic contaminants and the application of a first order reaction model can be established based on stable isotope fractionation data. It is well known that many biochemical reactions lead to stable isotope fractionation (Bigeleisen and Wolfsberg, 1958; O'Leary, 1980; Grossman, 2002); this means that molecules made up by lighter atoms (^{1}H, ^{12}C) are preferentially degraded and therefore the molecules with isotopically heavier atoms (^{2}H, ^{13}C) become enriched in the residual substrate. Physico-chemical processes such as sorption, diffusion, or phase transfers do not – or just to a minor extent – cause isotope effects (e.g. Harrington et al., 1999; Slater et al., 2000; Kopinke et al., 2005). It has been shown before that the magnitude of isotope shifts is mainly related to the initial mechanism of contaminant degradation and not to various electron acceptors applied or to different bacteria active in degradation (Morasch et al., 2001). As soon as oxic and anoxic zones are known, degradation of environmental contaminants such as aromatic hydrocarbons can be investigated in complex systems using stable isotope-based approaches. In recent years, compound-specific stable isotope analysis (CSIA) has been used increasingly to characterize degradation of single contaminants in the environment and allowed to assess the extent of *in situ* biodegradation (e.g. Sturchio et al., 1998; Hunkeler et al., 1999; Ahad et al., 2000; Sherwood Lollar and Slater, 2001; Richnow et al., 2003a,b; Griebler et al., 2004). Specific isotope fractionation factors have been determined for a multitude of aromatic hydrocarbons, chlorinated solvents, and gasoline additives (for a compilation, see Schmidt et al., 2004; Meckenstock et al., 2004).

Based on stable isotopes and using zero order kinetics, anaerobic degradation of toluene was quantified (k=5.7 µM/d) at a heavily contaminated site (Vieth et al., 2005). Morrill et al. (2005) determined isotope-derived first order rate constants for *cis*-1,2-dichloroethane (cDCE) using minimum and maximum fractionation factors for cDCE reductive dechlorination (0.12 h^{-1} and 0.17 h^{-1}), and compared those to a concentration-derived rate constant (0.4 h^{-1}). This means that the determined isotope-derived rate constants were 2–3 times lower than the concentration-derived rate constants. This discrepancy is due to that fact that concentration-based values incorporate all processes that contributed to a decrease in cDCE be it sorption, dispersion or biodegradation, while the isotope-based rate constants, in contrast, only account for biodegradation. Hence, isotope-derived rate constants can generally be used to distinguish between degradative and non-degradative processes. Thus, the authors concluded that

stable carbon isotopes might be used to calculate degradation rate constants and, thus, provide an advanced means to quantify *in situ* biodegradation. Recently, McKelvie et al. (2007) found a good agreement between isotope-derived and mass flux-based rate constants. In another recent study, Mak et al. (2006) quantified the *in situ* dilution and biodegradation combining a conservative transport model with CSIA. The results of the study indicated that CSIA alone can only be used for the quantification of the *in situ* biodegradation when dilution is negligible. This condition is best fulfilled when the monitoring wells are located close to each other in the plume centre, the source zone is wide, and the studied contaminant is highly degradable. Another study evaluated a possible application of stable carbon isotopes to determine first order rate constants (Abe and Hunkeler, 2006). CSIA based rate constants always were found to lead to an underestimation of biodegradation with higher uncertainty for faster biodegradation. According to this publication, a systematic error in first order rate constants of up to 50% might be expected. This, however, still gives much more reliable estimates than the classical concentration-based first order approaches, where rate constants can vary by factors of up to three, depending on the analytical model used to derive the rate constants (Stenback et al., 2004).

The objective of the present study is the quantification of the *in situ* biodegradation of mono- and polyaromatic contaminants at the site of a former wood preservation plant. For *o*-xylene and naphthalene, process based rate constants were derived from stable carbon isotopes and were compared to classical first order decay models and Michaelis–Menten kinetics. Furthermore, field based isotope enrichment factors could be determined and were compared to laboratory-derived isotope enrichment factors. Qualitative evidence for substantial biodegradation at the respective site had been previously demonstrated using signature metabolite analysis (SMA), redox-sensitive tapes (RST), and CSIA (Blum et al., 2006). *In situ* bulk attenuation and biodegradation rate constants retrieved from these different models are exemplary for BTEX and low molecular weight PAHs. The subsequent evaluation of the different model approaches aims to provide real and new insights into *in situ* processes and to improve our understanding of contaminant fate in aquifers. Finally, biodegradation rate constants determined in this study – even though they are site-specific (e.g. Beyer et al., 2006; Bauer et al., 2006) – might be of practical use for passive remediation strategies at other sites.

2. Methods and theory

2.1. Field site

The field site, which is located in Buchholz, 40 km south of the city of Hamburg (Germany), is a former wood preservation plant (Fig. 1). The production of coal tar and creosote from 1904 to 1986 resulted in a severe contamination of the unsaturated and the saturated zone down to a depth of 50 m. The geology of the site is composed mainly of glacial sand deposits from the Saale ice age (Pleistocene), which have a local thickness of approximately 130 m and are comprised of an upper and a lower aquifer. The regional upper aquifer can be subdivided into four different sediments: (1) medium to coarse sands down to 20 m below the surface (mbs), (2) a layer of about 20 m thickness composed of medium to fine sands, (3) coarse to medium sands from 40 mbs to 49 mbs, and (4) silty fine sands from 49 mbs (Fig. 2). The groundwater

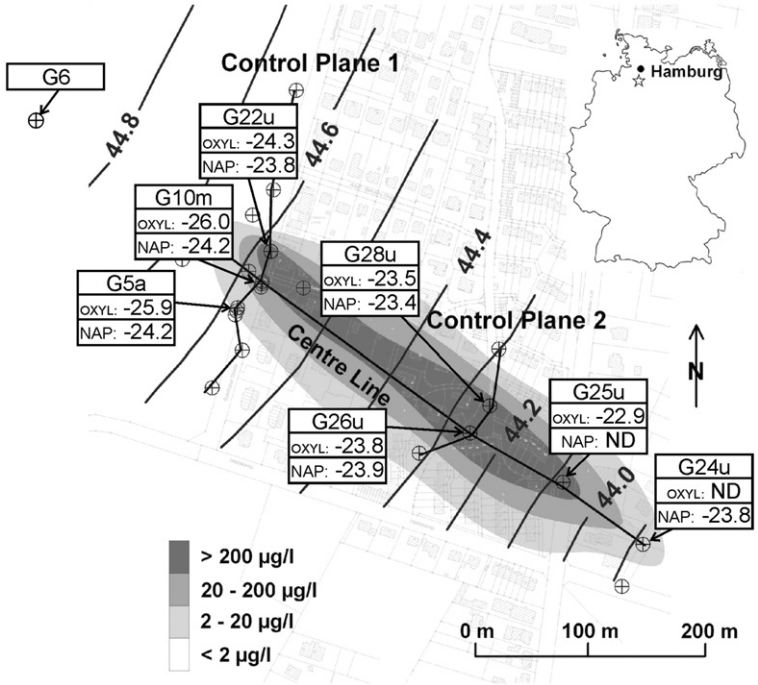

Fig. 1. Location map of the site including all observation wells, groundwater contours, contaminant plume of naphthalene in the lower part of the aquifer and stable carbon isotope concentrations of *o*-xylene (OXYL) and naphthalene (NAP) (ND: not detected).

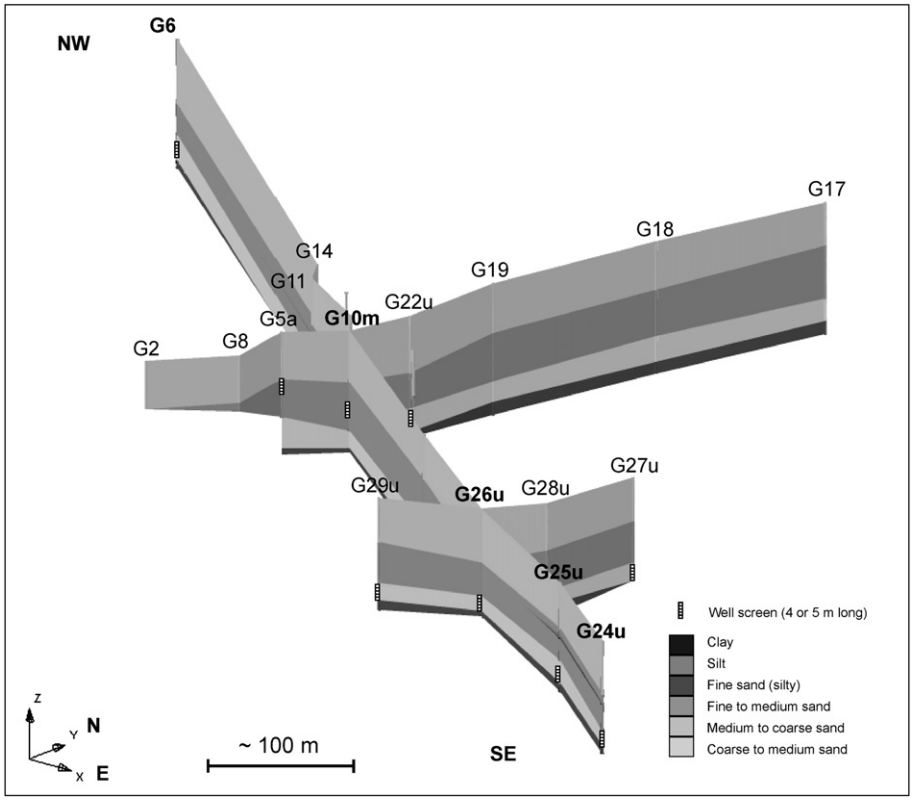

Fig. 2. Schematic three-dimensional geological site model including several well screens.

table of the upper unconfined aquifer is found in a depth of 18 to 19 mbs, which is approximately at the top of the layer of medium to fine sands. The lower confined aquifer, which is not contaminated, is formed mainly by silt and sand deposits.

After the site closure in 1986, several site investigation campaigns were performed. Two control planes, close to the contaminant source (control plane 1, CP1) and halfway between source and the tip of the contaminant plume (CP2) were installed perpendicular to the flow direction (Fig. 1). Based on the extensive groundwater monitoring network, a comprehensive three-dimensional conceptual geological, hydrogeological, and hydrogeochemical site model was developed (Fig. 2). A more detailed two-dimensional geological cross-section of the source zone and control plane 1 is also given by D'Affonseca et al. (2008). The average hydraulic conductivity of the lower part of the upper aquifer (medium to coarse sands between 40 and 49 mbs), in which the contaminant plume is predominantly seated, was determined by hydraulic pump tests and sieve analyses using the method of Beyer (1964), resulting in an average logarithmically distributed hydraulic conductivity of 4.2×10^{-4} m/s. The average seepage velocity in this layer is approximately 90 m/a when using an average observed hydraulic gradient of 0.0015 and an average effective porosity of 21% (Blum et al., 2007).

The projected development of depth and screen length of the observation wells was done by organoleptic tests resulting in screen lengths of 4 or 5 m (Fig. 2). Contaminant odour was not registered in the silty fine sands in the lowest part of the upper aquifer. Hence, a vertical delineation could be achieved, which was later confirmed using redox-sensitive tapes (Blum et al., 2006). The horizontal delineation of the contaminant plume was done by sampling wells of the control planes CP1, CP2, and observation wells along the plume centre line, which resulted in a plume length of 450 m and a maximum width of 150 m. An example for the dissolved contaminant plume of naphthalene in the lower part of the aquifer (medium to coarse sands) is illustrated in Fig. 1.

2.2. Groundwater sampling procedures and sample analysis

Groundwater was sampled from observation wells with screen lengths ≤5 m of high density polyethylene (diameter of 125 mm) using a submersible pump (MP1, Grundfos) placed 1 m below the top of the well screen. Dissolved O_2, pH, electric conductivity, redox potential, and temperature were determined by on-line probes (WTW, Weilheim, Germany). After pumping at a rate of 0.45 L/s for approximately 1 h, on-site parameters remained constant. Then groundwater was sampled and stored at a constant temperature of 4 °C. Nitrate (NO_3^-) and dissolved sulfide (HS^-) concentrations were determined by spectrophotometry; sulfate (SO_4^{2-}), ferrous iron (Fe^{2+}), and manganese (Mn^{2+}) were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Methane (CH_4) concentrations were determined using a headspace gas chromatograph equipped with a flame ionization detector (FID). A gas chromatography-mass spectrometer (GC-MS) equipped with FID was used to determine concentrations of PAHs and BTEX from previously-extracted liquid-liquid groundwater samples using methylene chloride and hexane, respectively, as solvents. Limit for quantification

was at 0.2 µg/L for 16 PAHs according to EPA, and at 0.5 µg/L for BTEX.

2.3. Stable carbon isotope ratios

Stable isotope ratios were determined by compound-specific isotope analysis (CSIA) using a gas chromatography-isotope ratio monitoring mass spectrometer (GC-IRMS; MAT-252, Finnigan, Bremen, Germany). BTEX and naphthalenes were extracted in *n*-pentane and aliquots of 0.2–5 µL were injected splitless on a capillary column (DB-1, 60 m length, 0.32 mm diameter, 1 µm film thickness; J&W Scientific, Folsom, Ca., USA). The temperature program was as follows: 35 °C isothermal for 5 min, heating with 3 °C per minute up to 280 °C, held for 5 min. Organic compounds separated by the GC column were oxidized to CO_2 in a combustion reactor and subsequently transferred to the mass spectrometer where $^{13}CO_2/^{12}CO_2$ was determined. The method detection limit for BTEX and naphthalene was <5 µg/L. Isotope measurements were performed in duplicate. $\delta^{13}C$ values were calculated relative to external CO_2 reference gas and are given in [‰]:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{std}} - 1\right) \times 1000. \quad (1)$$

R_{sample} and R_{std} are the carbon stable isotope ratios of the sample and the standard, respectively. Kinetic isotope fractionation factors α_C are derived from the Rayleigh equation for closed systems (Rayleigh, 1896):

$$\ln\left(\frac{R_t}{R_0}\right) = \ln f \times (\alpha_C - 1). \quad (2)$$

f is the fraction of the substrate remaining in the sample at time t. A convenient expression of stable isotope fractionation is the isotope enrichment factor ε that can be retrieved directly from α_C using Eq. (3) (Clark and Fritz, 1997).

$$\varepsilon = 1000 \times (\alpha_C - 1). \quad (3)$$

A simplified expression of isotope fractionation is obtained by combining Eqs. (2) and (3)

$$\ln\left(\frac{R_t}{R_0}\right) = \ln f \times \frac{\varepsilon}{1000} \quad (4)$$

and by expressing the changes in isotope signatures over time as absolute differences $\Delta\delta^{13}C$

$$\Delta\delta^{13}C = \varepsilon \times \ln f. \quad (5)$$

In this field study, changes in isotope ratios are not regarded over time but over distance. t was approximated via distance and seepage velocity ($t = \Delta x/v_a$).

2.4. Determination of bulk attenuation and biodegradation rate constants

2.4.1. Classical first order decay models

Preconditions for the application of first order decay models are (1) no bacterial growth and (2) initial substrate concentrations that are relatively low compared to a half-saturation constant. This causes the uptake rate per cell to become a linear function of the substrate concentration. Compilations of different first order model approaches to estimate degradation rate constants from centre line data are provided by Beyer et al. (2006) and Bauer et al. (2006). The first standard method applied to determine first order degradation rate constants uses concentration data from several piezometers located along the centre line of the steady state plume (G10m, G26u, G25u, G24u; Figs. 1 and 2):

$$\lambda_1 = -\frac{v_a}{\Delta x} \ln\left(\frac{C_{(x)}}{C_0}\right). \quad (6)$$

The bulk attenuation rate constant λ_1 is obtained by linear regression plotting the logarithmic concentration change $C_{(x)}/C_0$ over the travel time $t = \Delta x/v_a$. Eq. (6) can be amended by measurements of a conservative, i.e. non-degradable tracer $C_{(x)}$*, which is emitted from the same source zone as the considered contaminant (e.g. Wiedemeier et al., 1996):

$$\lambda_2 = -\frac{v_a}{\Delta x} \ln\left(\frac{C_{(x)}}{C_0}\frac{C_0^*}{C_{(x)}^*}\right). \quad (7)$$

This normalization allows a correction of contaminant concentrations with respect to dispersion, dilution, or volatilization and thus yields a bulk biodegradation rate constant λ_2. To quantify the exact contributions of biodegradation to overall attenuation with Eq. (7), 1,2,3-trimethylbenzene (TMB or hemellitol) was taken as tracer for monoaromatic compounds. In general, TMB isomers are fairly recalcitrant to biodegradation under anoxic conditions (e.g. Wiedemeier et al., 1999), which is true for the centre of the studied contaminant plume. 1,2,3,4-tetrahydronaphthalene (tetralin) was chosen as a conservative tracer for PAHs even though a former experimental study demonstrated that it is related to anaerobic degradation of naphthalene and could be degraded in lab studies with sulfate as electron acceptor (Annweiler et al., 2002). However, concentrations of tetralin in groundwater samples of the study site proved tetralin to be the most recalcitrant compound of all PAHs under the prevailing conditions. We found no indications for in situ biodegradation along the centerline between G10m and G25u 315m further downgradient.

Alternative models for determining the first order degradation rate constant λ_3 were developed which are derived from the steady-state solution of the 1D or 2D advection-dispersion-equations (Buscheck and Alcantar, 1995; Zhang and Heathcote, 2003). The Zhang and Heathcote method is given by

$$\lambda_3 = \frac{v_a}{4\alpha_L}\left(1 - 2\alpha_L \frac{\ln(C_{(x)}/(C_0\beta))}{\Delta x}\right)^2 - 1\right) \quad \text{with} \quad \beta = erf\left(\frac{W_S}{4\sqrt{\alpha_T \Delta x}}\right). \quad (8)$$

Longitudinal dispersivity α_L was estimated to be 8.4 m using the empirical relationship of Xu and Eckstein (1995). For the estimation of the horizontal transverse dispersivity α_T the frequently used relationship of $\alpha_T = 0.1 \times \alpha_L$ was applied resulting in 0.84 m. The source width W_S was determined to be approximately 30 m (D'Affonseca et al., 2008). All estimated rate constants were evaluated using a 2D analytical transport model (Domenico, 1987) to calculate $C_{(x)}$ at the four wells located in the centre line.

2.4.2. Michaelis–Menten (MM) degradation kinetics

The MM model is also often termed Monod kinetics without growth, which means that the initial cell number was

much higher than the number of cells that could be produced using the substrate present at time zero (Simkins and Alexander, 1984). The general expression of MM is

$$\frac{dC}{dt} = -k_{\max}\frac{C}{C+M_C} \tag{9}$$

with k_{max} being the maximum degradation rate and M_C the half-saturation concentration. When the integrated form of Eq. (9) is rearranged (assuming $t=\Delta x/v_a$) it yields Eq. (10). This model accounts for advection and degradation by MM kinetics and relates concentrations to travel distances:

$$\frac{\Delta x}{v_a\left(C_0 - C_{(x)}\right)} = \frac{M_C \ln\left(C_0/C_{(x)}\right)}{k_{\max}\left(C_0 - C_{(x)}\right)} + \frac{1}{k_{\max}}. \tag{10}$$

If $\left[\Delta x/v_a\left(C_0 - C_{(x)}\right)\right]$ is plotted over $\left[\ln\left(C_0/C_{(x)}\right)/\left(C_0 - C_{(x)}\right)\right]$ the intercept of a linear data fit is the inverse k_{max}, and M_C is retrieved from the slope of the curve multiplied by k_{max}.

2.4.3. Isotope-derived first order biodegradation rate constants

Biodegradation B [%] was calculated based on the measured ^{13}C content $\delta^{13}C$ of a residual groundwater contaminant plus the isotope enrichment factor ε determined in previous laboratory experiments (e.g. Richnow et al., 2003b; Meckenstock et al., 2004; Griebler et al., 2004):

$$B[\%] = (1-f_t)\times 100 = \left(1 - \left(\frac{R_t}{R_0}\right)^{\left(\frac{1000}{\varepsilon}\right)}\right)\times 100. \tag{11}$$

Finally, first order biodegradation rate constants λ_4 were determined based on stable isotope measurements by combining the general first order expression:

$$C = C_0 \times e^{-\lambda t} \tag{12}$$

with the simplified Rayleigh Eq. (5) to result in Eq. (13)

$$\lambda_4 = -\frac{\Delta\delta^{13}C}{\varepsilon \times t}. \tag{13}$$

To apply first order calculations based on stable isotope data, either isotope enrichment factors ε derived from aerobic or from anaerobic degradation experiments were chosen, according to the respective dominant redox conditions in the groundwater.

Calculated and measured concentrations were used to determine model efficiencies (*EF*) for the different rate constants (Loague and Green, 1991):

$$EF = 1 - \frac{\sum_{i=1}^{n}\left(C'_{(x_i)} - C_{(x_i)}\right)^2}{\sum_{i=1}^{n}\left(C_{(x_i)} - \overline{C}_{(x)}\right)^2} \tag{14}$$

where n is the number of measurements, $C_{(x_i)}$ the measured concentration at piezometer i, $\overline{C}_{(x)}$ the average of all measured concentrations, and $C'_{(x_i)}$ the predicted concentration in piezometer i. The *EF* is defined as the proportion of the total variance of observed data explained by the model, with $EF=1.0$ indicating an exact representation of measured data by the model, whereas $EF<0$ indicate that the modeled data are less accurate than simply using the observed mean.

3. Results and discussion

3.1. Hydrogeochemistry and organic contaminants at the field site

At the Buchholz site, groundwater is depleted in oxygen and nitrate in most parts of the contaminated aquifer (Fig. 3). Both oxygen and nitrate were completely consumed upgradient from control plane 2. In contrast to oxygen, nitrate became available again as an electron acceptor at well G24u attesting at least nitrate reducing conditions. Nevertheless, in the strictly anoxic center of the contaminant plume sulfate served as primary electron acceptor. Locally, conditions in this part of the plume even became methanogenic, e.g. in groundwater of well G26u more than 1000 µg/L of methane were detected. Dissolved Fe^{2+} concentrations of up to 37 mg/L were reported and generally were most elevated in groundwater from G26u and G25u indicating that this section of the plume was anoxic and iron reduction was taking place (e.g. Christensen et al., 2000). Dissolved Mn^{2+} was detected in minor amounts. Only a part of the reduced iron and manganese species presumably originated from biological activities but were above all a product of chemical reduction by HS^-. This explains why HS^- itself was only found in concentrations of around 1 mg/L even though more than 30 mg/L were consumed. Whereas strictly reducing conditions dominated the milieu between well G10m in control plane 1 and G26u, subsequent conditions, further downgradient, became less reducing. The presence of nitrate and oxygen in small quantities in groundwater of G24u and the abrupt rise in sulfate demonstrated that denitrification and aerobic degradation were the predominant processes at the tip of the contaminant plume (G24u). In June 2005, for example, the oxygen concentration in well G25u was 0.5 mg/L but further downgradient in well G24u 1.6 mg/l of O_2 were measured clearly indicating oxic conditions. Hence, the rough horizontal delineation that could be achieved by groundwater sampling exhibited a classical onion-shaped redox zonation (e.g. Wiedemeier et al., 1999).

The spreading of the organic contaminants was mapped 3-dimensionally based on four monitoring campaigns performed at the site between October 2003 and June 2005 (Figs. 1 and 2). The contaminant plume moved down within the first 100 m to the lower part of the upper aquifer. At this depth, the plume had its longest extension, e.g. naphthalene was still present 403 m downgradient from the source in well G24u at a concentration of 7±4 µg/L (mean of all four monitoring campaigns). Subsequent monitoring campaigns revealed that the plume reached its stationary phase, indicating that the contaminant load from the source was equal to the attenuation capacity of the aquifer (Fig. 4).

3.2. Stable isotope-based first order decay model

In October 2003, groundwater was sampled for CSIA from wells located along the centre line of the contaminant plume and stable carbon isotope shifts in residual one-, two-, and three-ring aromatic hydrocarbons were analyzed. Among BTEX, only *o*-xylene and toluene showed measurable $^{13}C/^{12}C$ isotope shifts of $\Delta^{13}C = 3.1‰$ and 1.6‰ along the groundwater flow path when 89% of the initial 90 µg/L or 94% of 157 µg/L, respectively, were degraded (Fig. 4). These results concur with

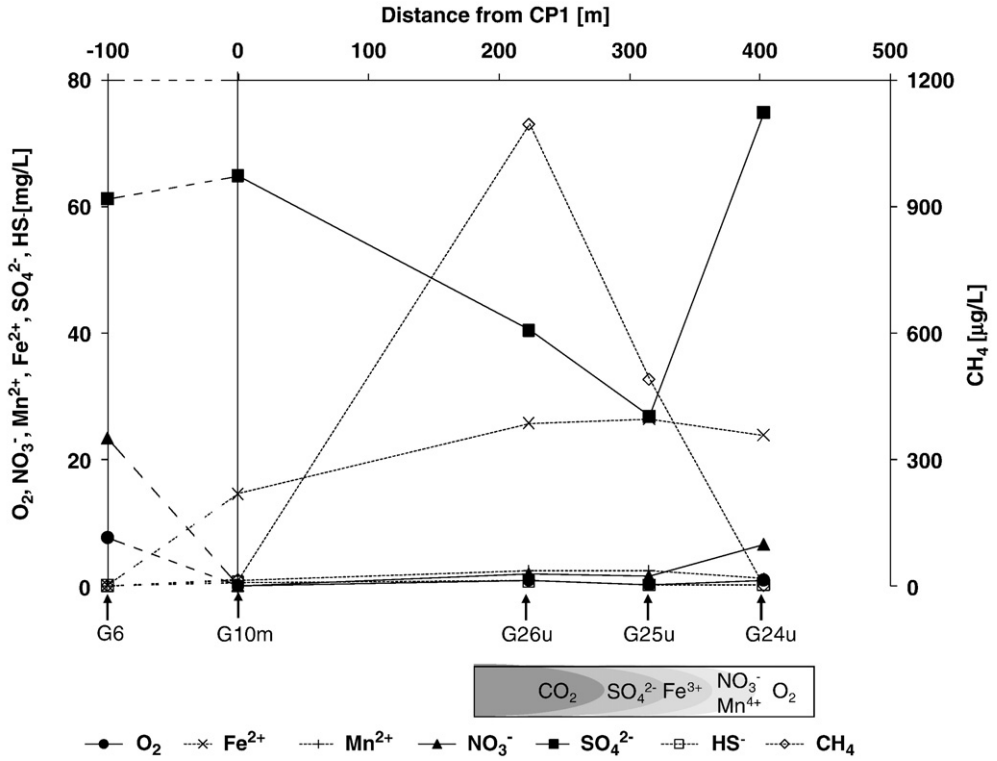

Fig. 3. Average concentrations of the electron acceptors sulfate, nitrate, and oxygen and the products sulfide, iron(II), manganese(II), and methane along the centre line of the plume from the four sampling campaigns in October 2003, June 2004, November 2004, and June 2005. CP1 (G10m) is shown by the horizontal line, G6 is the reference well upgradient from the contamination. Below, a schematic of the putative redox zonation is displayed.

another field study of a tar-oil contaminated aquifer in South Germany that also observed the strongest ^{13}C enrichment for *o*-xylene (Griebler et al., 2004). However, in the latter case, the detected isotope shift for *o*-xylene under sulfate-reducing conditions was more than three times larger with $\Delta^{13}C = 10.7‰$ during degradation of around 95% of about 660 µg/L initially.

Of all PAHs studied (naphthalene, 1- methylnaphthalene, 2-methylnaphthalene, acenaphthene and fluorene), only 2-methylnaphthalene and naphthalene showed minor isotope shifts. The attenuation of 57% of the initial 811 µg/L 2-methylnaphthalene was related to an enrichment of $\Delta^{13}C = 1.6‰$ whereas 79% decrease of the very high 12,020 µg/L of naphthalene only led to $\Delta^{13}C = 0.4‰$ (Figs. 1 and 4). The absence of isotope shifts for 1-methylnaphthalene and three-ring PAHs might be due to insignificant biodegradation under anoxic conditions or to very small isotope effects during the degradation of these compounds (Elsner et al., 2005).

Based on the stable carbon isotope ratios from groundwater wells along the centre line, first order biodegradation rate constants were determined using Eq. (13). The model also accounted for predominant redox conditions, more precisely, for the electron acceptors employed to degrade aromatic hydrocarbons. *o*-Xylene was almost entirely degraded over the first 315 m in the anoxic part of the contaminant plume where sulfate was the dominant electron acceptor (Fig. 3). The rate constant obtained for anaerobic degradation was $\lambda_4 = 0.0019\ d^{-1}$ (Table 1; Fig. 5) using an average isotope enrichment factor for sulfate-reducing conditions of −1.3 (Richnow et al., 2003b; Morasch et al., 2004). In contrast to BTEX, the naphthalene contamination spread over the whole length of the contaminant plume. Consequently, two different rate constants were determined for the strictly anoxic (primarily sulfate- and iron-reducing) part between G10m and G25u and for the oxic to nitrate-reduced section from G25u to G24u. Using an isotope fractionation factor for anaerobic naphthalene degradation of −1.1 (Griebler et al., 2004), the rate constant was calculated as $\lambda_4 = 0.0004\ d^{-1}$ (Table 1; Fig. 5). After 315 m at well G24u, nitrate and minor amounts of oxygen became available again and the decrease in naphthalene was accelerated (Fig. 3). The second rate constant for the predominant aerobic degradation of naphthalene using an isotope fractionation factor of −0.1 (Morasch et al., 2002) was determined as $\lambda_4 = 0.0027\ d^{-1}$ (Table 1). The isotope-based first order rate constants indicated that, firstly, rate constants decreased with increasing number of aromatic rings and, secondly, that biodegradation in absence of oxygen was decelerated considerably. A related observation has been previously reported for benzene (Vieth et al., 2005) in which a pronounced local enrichment of ^{13}C in residual benzene was detected at the margins of a contaminant plume and led to the conclusion that biodegradation is predominantly fringe-controlled.

As the enrichment of the heavier carbon species ^{13}C along the groundwater flow path was (almost) exclusively and, in particular for *o*-xylene, due to contaminant degradation, this model demonstrated its capacity to distinguish biodegradation from the other NA processes such as dispersion and dilution.

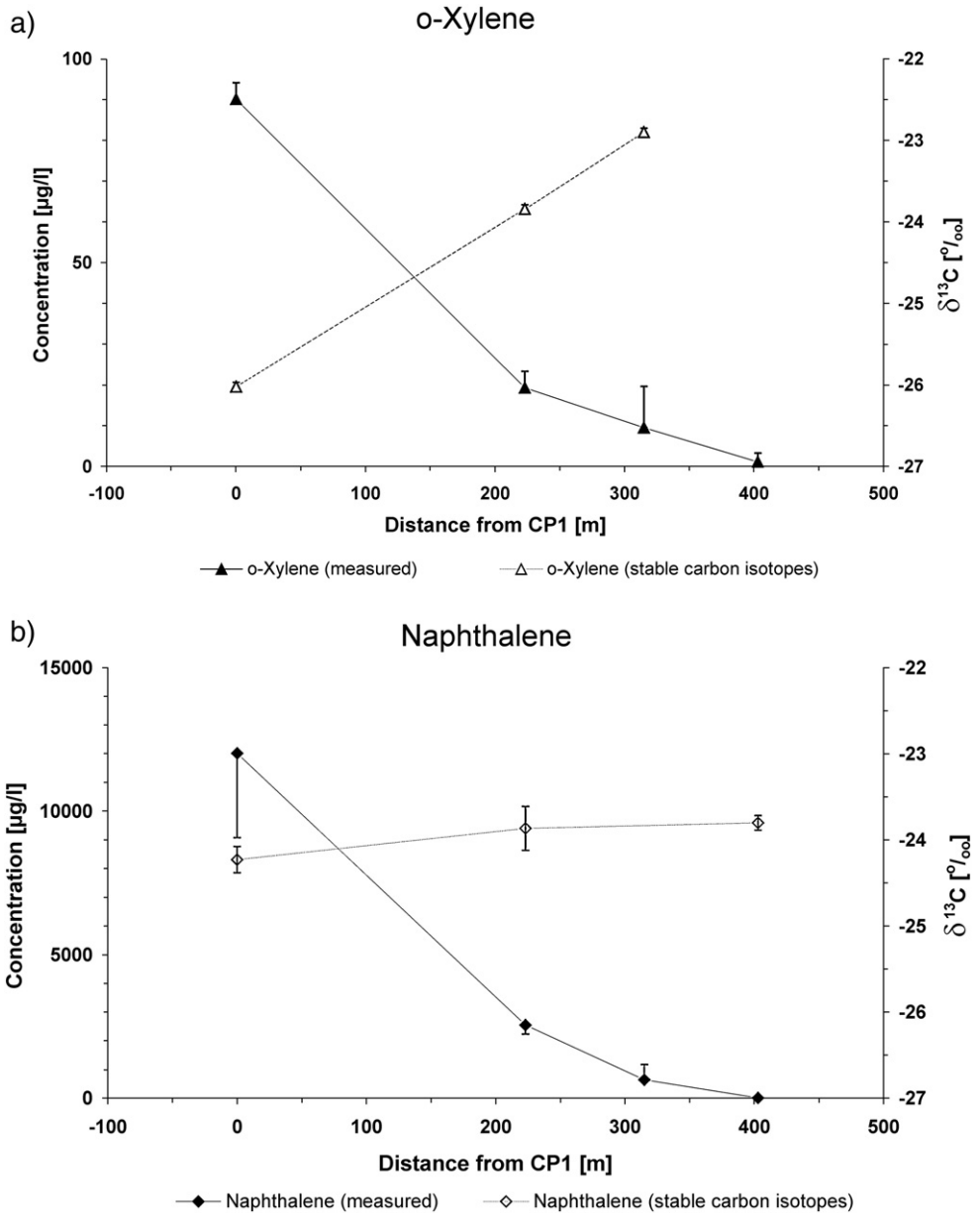

Fig. 4. Concentrations (closed symbols) and the respective δ^{13}C-values (open symbols) of *o*-xylene and naphthalene in groundwater wells along the centre line of the plume sampled in October 2003 including the minimum and maximum concentrations of the four monitoring campaigns (October 2003, June 2004, November 2004 and June 2005) indicated by the error bars. Error bars by the isotope data are standard deviations of the δ^{13}C-values analyzed by CSIA.

The model was no longer based on concentration measurements at different points along the centre line, but instead on stable isotope ratios and on empirical isotope enrichment factors determined beforehand in the laboratory under corresponding redox conditions. This means that λ_4 values were overall or bulk biodegradation rate constants.

Furthermore, using concentrations and the corresponding carbon isotope shifts determined from groundwater samples, we attempted to retrieve field isotope enrichment factors (ε_{field}) according to the Rayleigh model (Eq. (2)). For *o*-xylene a very good linear regression ($R^2 = 1.00$) was obtained and $\varepsilon_{field} = -1.4$ closely matched literature values from laboratory studies for biodegradation under sulfate-reducing conditions (Table 2; Fig. 6). This result concurs with the field study of Fischer et al. (2006), who reported on the applicability of the Rayleigh model to quantify BTEX biodegradation in complex systems. In that study, deuterated toluene was injected as a tracer to quantify *in situ* biodegradation using stable isotope analysis and the Rayleigh model.

The decrease in naphthalene concentrations and corresponding $^{13}C/^{12}C$ carbon isotope did not result in a linear relationship ($\varepsilon_{field} = -0.05$; $R^2 = 0.37$). This result indicated that biodegradation was not the only process that led to a decrease of naphthalene in groundwater along the centre line and that it might be problematic to define field enrichment factors for compounds that are biodegraded under oxic and under

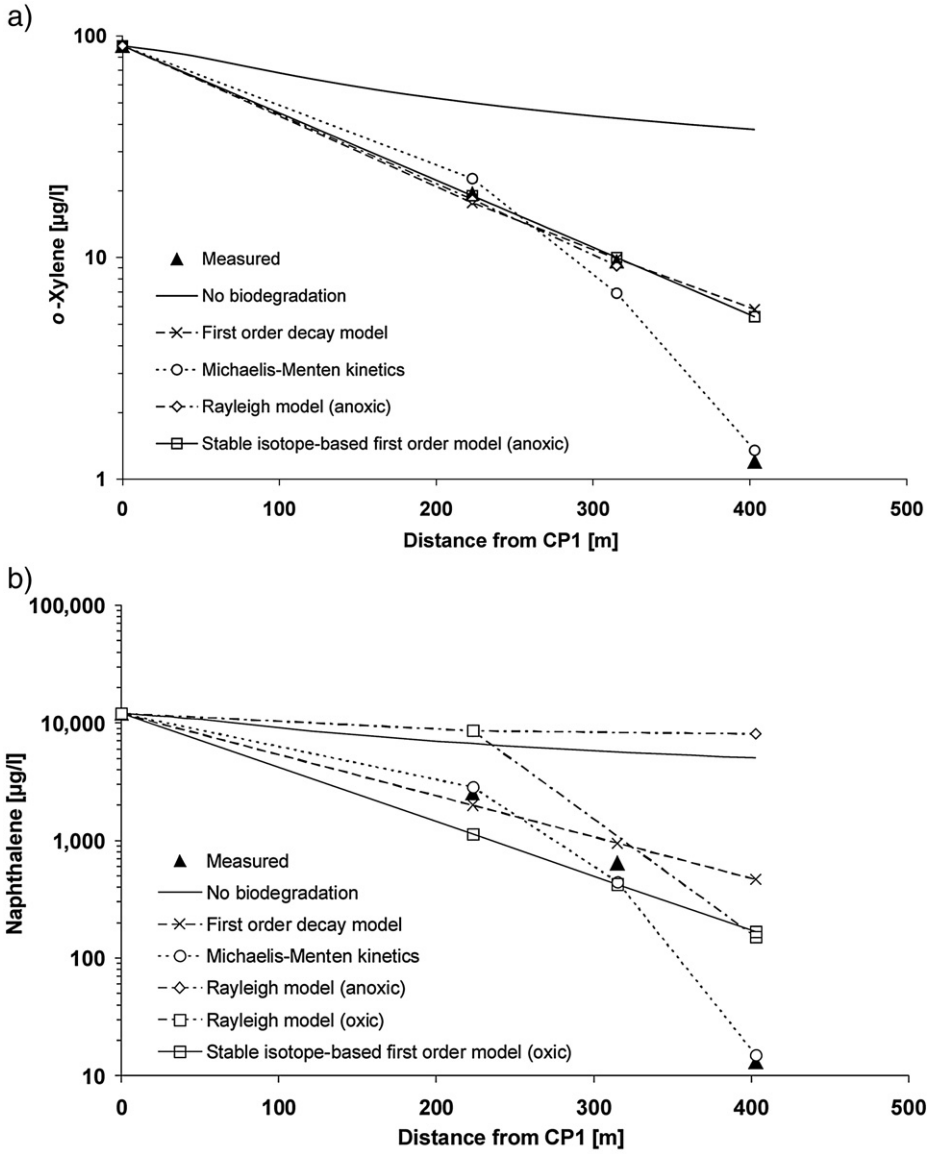

Fig. 5. Summary of the various applied models for the quantification of the *in situ* biodegradation of *o*-xylene (a) and naphthalene (b) along the centre line of plume including the concentrations without biodegradation and the stable isotope-based first order model.

anoxic conditions. Anaerobic naphthalene degradation is accompanied by significant isotope enrichments (ε=−1.1) whereas aerobic degradation only causes a weak isotope effect (ε=−0.1) (Meckenstock et al., 2004). Under varying *in situ* conditions, the resulting isotope enrichment therefore depends on the contributions of anaerobic and aerobic biodegradation. Furthermore, Vieth et al. (2005) determined a field fractionation factor for benzene (α_f=1.0004). They concluded that this field-derived fractionation factor is not only dependent on biodegradation, but also on processes such as sorption and dilution. Thus, this field fractionation factor is always lower than the fractionation factors derived from laboratory experiments, which was also observed in the current study for naphthalene.

The percentage of biodegradation *B* [%] for *o*-xylene and naphthalene was assessed using Eq. (11) (Table 2). After a flow path of 315 m, 91% from initial 90.2 µg/L of *o*-xylene was removed by anaerobic biodegradation. In contrast, only 29% of initial 12,020 µg/L of naphthalene were found to be biodegraded under anoxic conditions between well G10m and G26u. Further downgradient, up to 99% of naphthalene decreased due to aerobic degradation or other NA processes. The residual concentration of naphthalene was calculated to be 150 µg/L in groundwater from well G24u; it was assumed that biodegradation was the only process leading to substrate removal. Concentration analysis, however, resulted in 13 µg/L. This discrepancy proved that other NA processes apart from biodegradation led to a decrease of naphthalene along the groundwater flow path. This discrepancy may be partly also due to the slight systematic underestimation of *in situ* biodegradation by stable isotope approaches (Abe and Hunkeler, 2006; Van Breukelen, 2007).

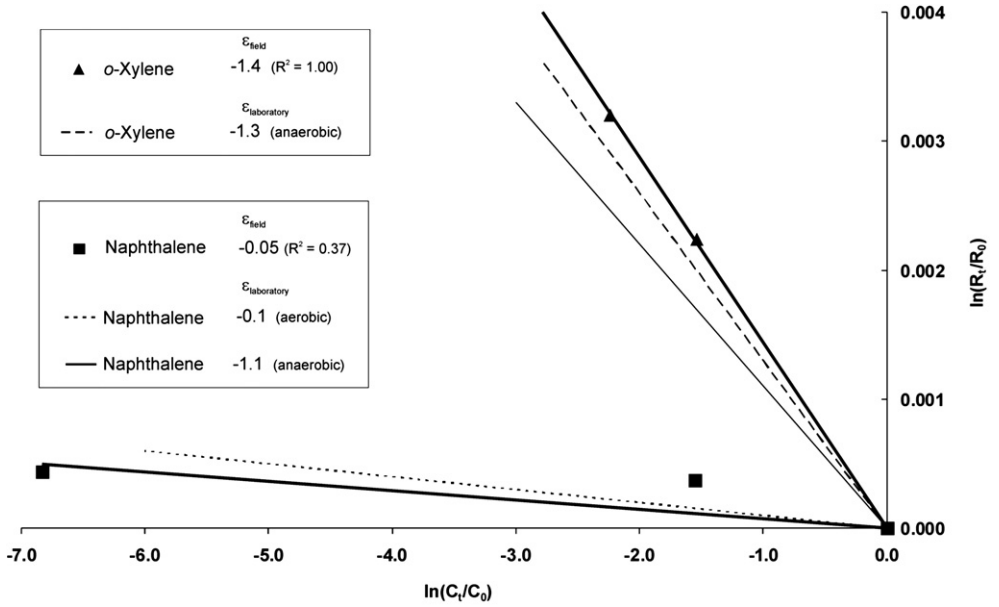

Fig. 6. Isotope enrichment factors for *o*-xylene and naphthalene based on concentrations and isotope data determined from groundwater samples along the centre line of the contaminant plume (ε_{field}). For comparison, enrichment factors determined in laboratory experiments are added in dashed lines.

3.3. First order decay models

In addition, different rate constants λ obtained by the three classical first order decay reaction models (Eqs. (6)–(8)) are presented in Table 1 together with their corresponding model efficiencies *EF* (Eq. (14)). For *o*-xylene all first order degradation rate constants (λ_1–λ_3), including the biodegradation rate constants based on the stable carbon isotopes (λ_4), are in very good agreement. For the other compound the agreement is roughly within a factor of two, which is acceptable for a field study. Highest rate constants were obtained from the simple advection-reaction model (Eq. (6)) that corrected neither for biodegradation using a conservative tracer (Eq. (7)) nor for longitudinal and transversal dispersions (Eq. (8)). Results retrieved from the third first order model, however, crucially depend on a correct parameterization of dispersivities (Bauer et al., 2006). The examples of naphthalene and 2-methylnaphthalene indicated that the frequently assumed relationship of $\alpha_T = 0.1\ \alpha_L$ could not be taken as a reliable estimate of the actual transverse dispersivity at the Buchholz field site. Therefore these degradation rate constant estimates (λ_3) should be taken with caution, as underestimation of α_T results in an overestimation of degradation rate constants (e.g. Stenback et al., 2004; Bauer et al., 2006).

A previously performed sensitivity analysis for the first order decay models showed that the seepage velocity has the greatest influence on first order degradation rate constants with the highest sensitivity to the hydraulic conductivity with a correlation coefficient (γ) of 0.9 followed by the effective porosity with γ=−0.4. All other considered input parameters, such as source concentration, source width, longitudinal and transversal dispersions, only indicated minor influence with correlation coefficients between −0.2 and 0.1. For more details, please see Blum et al. (2007).

Regarding the model efficiencies, the first (Eq. (6)) and the third model (Eq. (8)) generally yielded unsatisfying correspondence with measured concentrations. For both, *o*-xylene and naphthalene, the *EF* values were found to be rather low

Table 1
Summary of the first order degradation rate constants (λ_1–λ_3) including the biodegradation rate constants based on the stable carbon isotopes (λ_4), and Michaelis–Menten parameters (MM) for various organic compounds using the centre line method

Method	Eq.	Parameter		*o*-Xylene	Benzene	Toluene	Ethylbenzene	Naphthalene	2-Methylnap.	Acenaphthene
First order decay models	(6)	λ_1 [d^{-1}]		0.0025 (−0.3)	0.0020 (−0.3)	0.0034 (0.5)	0.0021 (−0.7)	0.0038 (−0.5)	0.0048 (0.3)	0.0022 (−0.4)
	(7)	λ_2 [d^{-1}]		0.0012 (0.9)	0.0008 (−2.3)	0.0022 (0.0)	0.0009 (0.7)	0.0017 (0.7)	0.0029 (0.4)	0.0001 (−8.7)
	(8)	λ_3 [d^{-1}]		0.0021 (0.2)	0.0016 (−0.5)	0.0031 (0.1)	0.0017 (−0.9)	0.0036 (−0.6)	0.0049 (0.2)	0.0017 (0.2)
CSIA	(13)	λ_4 [d^{-1}]	Anoxic	0.0019 (0.9)	–	–	–	0.0004 (−51.9)	–	–
			Oxic	–	–	–	–	0.0027 (0.4)	–	–
MM	(10)	k_{max} [µg/l/d]		0.11 (0.9)	0.07 (−0.01)	0.45 (0.6)	0.15 (0.5)	12.17 (1.0)	1.65 (1.0)	0.50 (0.8)
		M_C [µg/l]		19.4	20.7	100.9	19.8	1129.3	217.6	90.6

Numbers in brackets are calculated model efficiencies *EF* according to Loague and Green (1991).

(Table 1). In contrast, the second model (Eq. (7)) resulted in acceptable fits of the measured concentrations for naphthalene and *o*-xylene showing positive and high values for the *EF* of 0.7 and 0.9, respectively. λ_2 generally yielded the highest *EF*, also for other contaminants, and demonstrated good fits for ethylbenzene and 2-methylnaphthalene, while λ_1 and λ_3 were not able to reproduce the measured concentrations. This indicates that, among those three classical applied methods, only the first order decay model that distinguished biodegradation from other NA processes (Eq. (7)) resulted mostly in acceptable fits of calculated to measured concentrations in piezometers along the centre line of the plume (Fig. 5).

Nevertheless, all estimated degradation rate constants given in Table 1 are within the range of other reported values (Aronson and Howard, 1997; Rifai and Newell, 2002; Rogers et al., 2007). In the literature, reports on degradation rate constants for PAH are generally rare, in contrast to reports of rate constants of BTEX. Some values, however, have been reported based on field studies. For acenaphthene, for example, site specific degradation rate constants of 0.0014 d^{-1} and 0.0049 d^{-1} were determined by Stenback et al. (2004) using the centre line approach and a two-dimensional analytical model which accounts for transverse and longitudinal dispersivities. In another study by Godsy et al. (1992) attenuation of acenaphthene over distance was compared to 3,5-dimethylphenol as conservative tracer and was found to be proportional. The authors concluded that acenaphthene was not degraded during downgradient travel in that section of the aquifer.

3.4. Michaelis–Menten kinetics

Finally, minimum and maximum degradation rates k_{max} obtained for BTEX and low molecular weight PAHs were found to range from 0.07 µg/L/d (benzene) to 12.2 µg/L/d (naphthalene). The corresponding half-saturation constants M_C also varied over two orders of magnitude (Table 1), e.g. 20.7 µg/L for benzene and 1129 µg/L for naphthalene. For every compound, the requirement $C_0 > M_C$, was met and allowed the calculation of MM parameters with Eq. (10). The sensitivity of the MM model was studied using the mean and the standard deviation of the hydraulic conductivity, which was determined in a previous study by Blum et al. (2007) resulting in a logarithmically distributed seepage velocity with $v_a = 0.25 \pm 0.18$ m/d. The resulting degradation rates k_{max} range between 3.19 and 21.15 µg/L/d for naphthalene and between 0.03 and 0.18 µg/L/d for *o*-xylene, once more indicating the large influence of the seepage velocity. The determined half-saturation concentrations, however, remain constant and demonstrate no influence of the seepage velocity.

The half-saturation concentrations for BTEX compounds were found to be more or less in the order of those compiled by Mohammed and Allayla (1997) and Bekins et al. (1998). From a mechanistic point of view, M_C in complex systems does not describe half-saturation of one specific enzyme but is rather a fitting parameter composed of any process that influences *in situ* degradation. Nevertheless, MM models are increasingly applied beyond classical enzyme studies to describe biodegradation in rather heterogeneous systems. Very recently, a MM model was used to characterize benzene degradation in large-scale sediment columns under sulfate-reducing conditions; although it could be demonstrated that benzene biodegradation was taking place, rates were about 70 times lower than under oxic conditions (Gödeke et al., 2008). One disadvantage of the double reciprocal solutions for k_{max} and M_C is the emphasis on small concentrations that are more likely subject to errors than medium concentrations. Nevertheless, for both, *o*-xylene and naphthalene, the MM model yielded an excellent fit of the measured concentrations (Fig. 4) with *EF* values of 0.9 and 1.0, respectively. The MM model yielded the highest *EF* also for the other contaminants, which for these contaminants at least, illustrated a better realization of measured values by the MM model than for all considered first order models (Table 1).

Table 2
Comparison between the isotope enrichment factors of *o*-xylene and naphthalene based on laboratory experiments and field data

Compound	Redox conditions	$\varepsilon_{laboratory}$	ε_{field}	*B* [%]
o-Xylene	Sulfate-reducing	−1.3 [a, b]	−1.4	91
Naphthalene	Sulfate-reducing	−1.1 [a]	−0.05	29
	Oxic	−0.1 [a]	−0.05	99

[a] Enrichment factors taken from Meckenstock et al. (2004).
[b] Mean value from two laboratory experiments under sulfate-reducing conditions (Richnow et al., 2003b; Morasch et al., 2004).

4. Conclusions

Classical first order decay models and Michaelis–Menten (MM) degradation kinetics were examined and compared to a novel stable isotope-based first order model using observed and simulated contaminant concentrations along the centre line of a coal tar contaminated aquifer. The major findings of the study can be summarised as follows:

- Using the hydrogeochemical data it was possible to horizontally delineate a classical onion-shaped redox zonation in the studied aquifer. Thereby acquired data on the redox conditions in the contaminant plume were used in the stable isotope-based first order model, which is presently able to account for some predominant redox conditions;
- The studied classical first order decay and also MM models are not process based. Hence, these rather simple approaches are inappropriate to describe and quantify the actual *in situ* biodegradation processes, and can be rather used to fit residual contaminant concentrations over distance. Thus, classical first order models only yield bulk attenuation rate constants, which account for all natural attenuation processes;
- Nevertheless, these classical models provide bulk attenuation rate constants for all measured organic contaminants;
- Although in this study the best fit was achieved with the MM degradation model, the evaluated degradation rates and half-saturation concentrations are pure fitting parameters, which should be used carefully and cannot be transferred to other sites;
- The stable-isotope based first order model relies on compound-specific stable isotope analysis, which could be performed for BTEX and some PAHs. Further studies therefore need to demonstrate the robustness of this model;
- Based on stable isotope ratios from groundwater wells and using the stable isotope-based first order model, it was possible to determine *in situ* first order biodegradation rates

for *o*-xylene and naphthalene. For the latter, two different rate constants could be identified for the more anoxic and oxic part of the contaminated aquifer;
- Furthermore, field-derived isotope enrichment factors (ε_{field}) could be determined for naphthalene and *o*-xylene, which for the latter closely matches laboratory-derived isotope enrichment factors ($\varepsilon_{laboratory}$) for biodegration under sulfate-reducing conditions indicating a predominant core-controlled biodegradation of *o*-xylene;
- When a compound is degraded in oxic and anoxic parts of the aquifer, a field enrichment factor reflects the respective contributions of aerobic and anaerobic biodegradation. For naphthalene, it was shown that concentrations did not only decrease due to anaerobic biodegradation and calculated field enrichment factors are therefore prone to error. Even though it is difficult to calculate ε_{field} for naphthalene (and other PAHs), we strongly recommend applying compound-specific isotope analysis (CSIA) for more-ring compounds, since mere concentration-based rate constants analysis might result in an overestimation of PAH degradation.

Until now, quantification of biodegradation in complex systems remains difficult as it is dependent on a large variety of variables, some of which are potentially still unknown. Nevertheless, a lot of effort is being made to better adjust degradation models to particular contaminants and field sites. We think the stable carbon isotope-based first order approach is an important advancement in understanding of biodegradation in complex environments. Under consideration of hydrological and geochemical conditions, stable isotope-based models can be well adapted and might be useful also for risk assessment and the prediction of long-term development of a contaminant plume. For a successful implementation of MNA at field sites, the stable isotope-based first order model is therefore a valuable tool that helps to determine biodegradation rate constants under specific redox conditions and enables one to better quantify the contribution of biodegradation to contaminant decrease in complex systems.

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