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REVIEW

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Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges

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Abstract Compound-specific stable isotope analysis (CSIA) using gas chromatography-isotope ratio mass spectrometry (GC/IRMS) has developed into a mature analytical method in many application areas over the last decade. This is in particular true for carbon isotope analysis, whereas measurements of the other elements amenable to CSIA (hydrogen, nitrogen, oxygen) are much less routine. In environmental sciences, successful applications to date include (i) the allocation of contaminant sources on a local, regional, and global scale, (ii) the identification and quantification of (bio)transformation reactions on scales ranging from batch experiments to contaminated field sites, and (iii) the characterization of elementary reaction mechanisms that govern product formation. These three application areas are discussed in detail. The investigated spectrum of compounds comprises mainly *n*-alkanes, monoaromatics such as benzene and toluene, methyl *tert*-butyl ether (MTBE), polycyclic aromatic hydrocarbons (PAHs), and chlorinated hydrocarbons such as tetrachloromethane, trichloroethylene, and polychlorinated biphenyls (PCBs). Future research directions are primarily set by the state of the art in analytical instrumentation and method development. Approaches to utilize HPLC separation in CSIA, the enhancement of sensitivity of CSIA to allow field investigations in the $\mu g\,L^{-1}$ range, and the development of methods for CSIA of other elements are reviewed. Furthermore, an alternative scheme to evaluate isotope data is outlined that would enable estimates of position-specific kinetic isotope effects and, thus, allow one to extract mechanistic chemical and biochemical information.

Keywords CSIA · IRMS · Isotope fractionation · Isotope ratio · Isotopic shift · Fingerprinting · Source allocation · Biodegradation · Degradation · Transformation · Weathering · Environmental forensics · Geomicrobiology · Contaminant hydrology · SPME · Purge and trap · ^{13}C · D/H · Primary isotope effect · Secondary isotope effect · Kinetic isotope effect · Rayleigh equation · Chloromethane · Tetrachloromethane · Trichloroethylene · Tetrachloroethylene · Methane · Perylene · Creosote · Sulfate · Chlorinated solvents · PAH · PCB · Aromatic hydrocarbon · Biogenic · Petrogenic · BTEX · VOC

Abbreviations *BTEX* benzene, toluene, ethylbenzene, xylenes · *MTBE* methyl *tert*-butyl ether · *PAHs* polycyclic aromatic hydrocarbons · *VOCs* volatile compounds · *PCBs* polychlorinated biphenyls · *CSIA* compound-specific (stable) isotope (ratio) analysis · *GC-IRMS, GC/IRMS or GCIRMS* gas chromatography-isotope ratio mass spectrometry · *GC-C-IRMS, GC/C/IRMS or GCC-IRMS* gas chromatography-combustion-isotope ratio mass spectrometry · *irmGC/MS* isotope ratio monitoring gas chromatograph-mass spectrometry · *GC/P/IRMS* gas chromatography-pyrolysis-isotope ratio mass spectrometry (used for D/H) · *KIE* kinetic isotope effect · *PSIA* position-specific isotope analysis (for intramolecular isotope distribution) · *SNIF-NMR* site-specific natural isotopic fractionation by nuclear magnetic resonance spectroscopy

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Introduction

The identification and quantification of organic compounds present in the environment is a major application area of

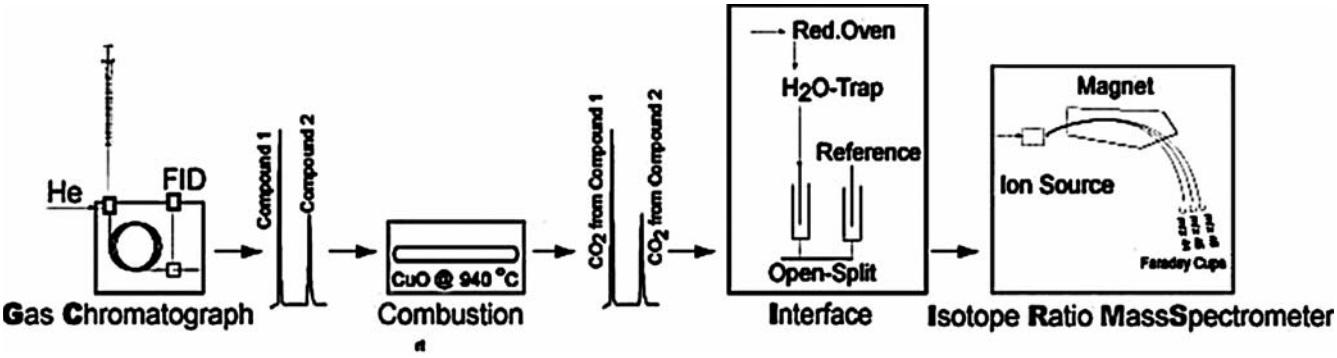

Fig. 1 Schematic set-up of GC/IRMS for $\delta^{13}C$ measurements

modern analytical chemistry. However, it is still hardly recognized that in addition to the chemical identity and the concentration of organic compounds there is more information available to infer sources and fate in the environment, namely the isotopic composition. Compound-specific stable isotope analysis (CSIA) quantifies this isotopic composition and hence provides additional and often unique means to (i) allocate and distinguish sources of organic compounds, and (ii) identify and quantify transformation reactions, sometimes even on a mechanistic level. Illustrative examples of these applications are discussed below. The physicochemical basis for utilizing isotopic composition data is that non-equilibrium phase transfer and, in particular, transformation processes frequently show a kinetic isotope effect (i.e., compounds that have identical chemical structures but differ in their isotope composition react at different rates).

In contrast to a classical dual-inlet isotope-ratio mass spectrometer (IRMS), CSIA is a continuous flow (CF) technique, which utilizes the hyphenation of a separation method (until now solely gas chromatography) via an online combustion/pyrolysis unit with a multicollector mass spectrometer. This method was developed mainly by Hayes and coworkers, resulting in the first paper on CSIA in 1978 [1] and a number of subsequent landmark papers on technical innovations (e.g., refs. [2, 3, 4]). The principal set-up for gas chromatography isotope-ratio mass spectrometry (GC/IRMS) is depicted in Fig. 1. Nowadays, the isotope ratios of five elements can be measured in continuous flow (Table 1). Except for $^{34}S/^{32}S$ (see below), all of these isotope ratios have also been measured with GC/IRMS. The major difference to (high-resolution) GC/MS is the very high precision achieved with GC/IRMS instruments due to the simultaneous measurement of the ions on fixed collectors. Standard deviations are in the order of four to six significant figures [5], which is a prerequisite for the measurement of small changes in isotopic composition at the low natural abundance level of the heavier isotopes. Conversely, due to its lower precision, the use of GC/MS is restricted to experiments with isotopically enriched compounds. A review of CSIA principles and technical aspects has been published by Meier-Augenstein [5]. These aspects are therefore only briefly covered here. An overview of application areas of continuous flow IRMS can be found in a review by Lichtfouse [6].

The aim of this review is (i) to provide an overview of recent applications of CSIA in environmental science that highlights the large potential of this method and (ii) to point out existing limitations and discuss attempts to overcome them. Note that inlet types other than GC interfaces are not covered here, since they are used for bulk phase analysis and are often routine methods in earth science. For these methods excellent textbooks exist that describe both principles and environmental applications of isotope ratio mass spectrometry (e.g., ref. [7]). Another area that is only briefly mentioned in this review is position-specific isotope analysis (PSIA) that determines *intramolecular* isotope patterns in organic molecules and their changes.

Table 1 Elements whose stable isotope ratios can be measured online

Stable isotopes	Natural abundance of the heavier isotope (%)	Conversion gas	Measured *m/z*	Detection limits (nmol element on-column)	Precision (‰)	Commercially available since
D/H	0.015	H_2	2,3	8–10	5	1998
$^{13}C/^{12}C$	1.11	CO_2	44, 45, 46	1	0.2	1988
$^{15}N/^{14}N$	0.366	N_2	28, 29, 30	0.8–1.5	0.5	1989
$^{18}O/^{16}O$	0.204	CO	32, 33, 34	5[b]	0.8	1998
$^{34}S/^{32}S$[a]	4.21	SO_2	64, 66	n.a.	n.a.	–

[a]No GC-IRMS applications published so far
[b]Note that exchange reactions with the reactor wall surface during high-temperature pyrolysis have been observed frequently and may obscure the $\delta^{18}O$ measurements
n.a. no information available

PSIA requires either (i) great amounts of substances in pure form for analysis by site-specific natural isotopic fractionation by nuclear magnetic resonance spectroscopy (SNIF-NMR) [8]), (ii) the use of position-specific isotopically enriched compounds, or (iii) rather sophisticated means that allow the separate isotope ratio MS measurement of various atoms of the same element in a substance (e.g., refs. [9, 10]). Therefore, this technique has hitherto hardly been used in environmental studies. However, new developments in this area might change this situation in the future [11]. Due to the background of the authors, this review mainly comprises work on organic contaminants in soil–water systems, although there are also important contributions of CSIA in atmospheric sciences and biogeochemistry. The latter were reviewed some time ago by Hayes et al. [12].

Definitions and calculations

CSIA yields data of the isotopic composition of a single compound x relative to an international standard that are usually expressed as δ values in per mil (‰) according to Eq. 1.

$$\delta_{\mathrm{x}} = \left(\frac{R_{\mathrm{x}} - R_{\mathrm{reference}}}{R_{\mathrm{reference}}} \right) \times 1000\,[\mathrm{per\ mil}] \tag{1}$$

where R_{x} and $R_{\mathrm{reference}}$ are the ratios of the heavy isotope to the light isotope (e.g., $^{13}C/^{12}C$ or D/H) in compound x and an international standard, respectively. Thus, rather than absolute values, the differences in relative ratios are reported to allow a correction for mass-discriminating effects in a single instrument and to facilitate the comparison of published GC/IRMS data. Only such relative isotope ratios can be determined with the required precision. A $\delta^{13}C$ value of +10‰ then corresponds to a sample with an isotope ratio one percent higher than that of the international standard (usually Vienna Peedee Belemnite, VPDB). For VPDB a ratio of ^{13}C versus ^{12}C of 0.011180 has been reported [13]. The $\delta^{13}C$ value of +10‰ for the sample then corresponds to a ^{13}C-to-^{12}C ratio of 0.011292, which demonstrates the very subtle changes that need to be measured. Details of referencing strategies in IRMS can be found in a review of Werner and Brand [13]. It is important to emphasize that accurate isotopic data for single compounds in a complex matrix/mixture can only be obtained if the corresponding peaks are well resolved.

The isotope fractionation between two compounds (e.g., a substrate and its degradation product) can be expressed either with the fractionation factor α or the enrichment factor ε according to Eqs. 2 and 3.

$$\alpha_{\mathrm{p-r}} = \frac{R_{\mathrm{product}}}{R_{\mathrm{reactant}}} = \frac{10^{-3}\delta_{\mathrm{p}} + 1}{10^{-3}\delta_{\mathrm{r}} + 1} \tag{2}$$

and

$$\varepsilon_{\mathrm{p-r}} = \left(\frac{R_{\mathrm{product}}}{R_{\mathrm{reactant}}} - 1 \right) \times 1000 = (\alpha - 1) \times 1000\,[\mathrm{per\ mil}] \tag{3}$$

where subscripts r and p refer to reactant and product, respectively, and R_{reactant} and R_{product} are the ratios of the heavy isotope to the light isotope in the substrate and the degradation product, respectively, that appear in an infinitely short period of time [7, 14]. For small molecules in which all isotopes are located in the same reactive position, α can also be interpreted according to Eq. 4.

$$\alpha = {}^{\mathrm{heavy}}k / {}^{\mathrm{light}}k = \mathrm{KIE}^{-1} \tag{4}$$

where ${}^{\mathrm{heavy}}k$ and ${}^{\mathrm{light}}k$ are the rate constants of compounds containing heavy and light isotopes at the reactive position and KIE = ${}^{\mathrm{light}}k/{}^{\mathrm{heavy}}k$, which is the kinetic isotope effect of the reaction (see Sects. "Insight into reaction mechanisms from isotope fractionation" and "Can we learn more from fractionation data?"). It must be pointed out that sometimes the inverted definitions of α and ε (i.e., the ratio $R_{\mathrm{reactant}}/R_{\mathrm{product}}$, are used), therefore care must be taken in the comparison of values from various references [15, 16]. Since ε values are usually rather small, the per mil notation (as in Eq. 3) is often used. However, ε values are also reported without this notation (i.e., $\varepsilon=\alpha-1$ is used).

The enrichment factor ε or the fractionation factor α is usually determined by using the relationship between substrate concentration change and isotope fractionation given in Eq. 5.

$$\frac{R_t}{R_0} = \left[f \frac{(1+R_0)}{(1+R_t)} \right]^{(\alpha-1)} = \left[f \frac{(1+R_0)}{(1+R_t)} \right]^{\varepsilon} \tag{5}$$

where R_t and R_0 are the ratios of the heavy isotope to the light isotope in the reactant r at time t=0 and t, respectively, and f is the remaining fraction of the reactant at time t according to Eq. 6 [16].

$$f = \frac{L_t + H_t}{L_0 + H_0} = \frac{L_t\,(1+R_t)}{L_0\,(1+R_0)} \tag{6}$$

where L_0 and H_0 are the concentrations of the light isotope and the heavy isotope at time t=0, respectively, and L_t and H_t are the concentrations of the light isotope and the heavy isotope at time t, respectively.

If studies at the low natural abundance level of the heavy isotopes are carried out (i.e., $H+L\approx L$) or the fractionation is very small (i.e., $1+R_t\approx 1+R_0$), Eq. 5 can be approximated by following the classical Rayleigh-type equation originally derived by Lord Rayleigh to describe fractional distillation of mixed liquids:

$$\frac{R_t}{R_0} = f^{(\alpha-1)} \tag{7}$$

After ln transformation and combination with Eq. 3 we obtain:

$$\ln\left(\frac{R_t}{R_0} \right) = (\alpha - 1)\ln f = \frac{\varepsilon}{1000}\ln f \tag{8}$$

which yields:

$$1000 \ln\left(\frac{10^{-3}\delta_{\mathrm{r},t} + 1}{10^{-3}\delta_{\mathrm{r},0} + 1} \right) = \varepsilon \ln f \tag{9}$$

where $\delta_{r,0}$ and $\delta_{r,t}$ are the ratios of the heavy isotope to the light isotope in the reactant r at time t=0 and t, respectively, expressed in the δ notation.

For enrichment factors typically obtained during transformations ($|\varepsilon|<20‰$), $\ln(1+10^{-3}\delta_r) \approx 10^{-3}\delta_r$, and Eq. 9 is often simplified to:

$$\delta_{r,t} - \delta_{r,0} \cong \varepsilon \ln f \quad (10)$$

This equation is the most frequently used in environmental sciences to derive isotope enrichment factors ε or fractionation factors α.

A plot of $\delta_{r,t}$ versus f calculated using Eq. 10 shows an illustrative example of the *changes* in isotopic composition of a reactant r and its degradation product p (Fig. 2). For p, the *changes* in isotopic composition of both the instantaneous product formed and the accumulating product are given. From Eqs. 2 and 3 it follows that the difference between the reactant and the instantaneous product formed always equals ε_{p-r} normalized to δ_r according to:

$$\delta_p - \delta_r = \varepsilon_{p-r}(1+\delta_r/1000)[{}^0/_{000}] \quad (11)$$

It can be easily seen that the extent of fractionation (expressed in δ values) depends on the remaining reactant fraction. Note that this plot is only applicable if several conditions are fulfilled: (i) we have a closed system (i.e., if the reactant pool is limited), (ii) only one product is formed in the transformation, and (iii) the product does not react further (which would change its isotopic composition). The last two conditions, however, are only relevant for the isotope ratio and its change in the product which is rarely measured.

It must be pointed out that the use of the simplified Eq. 10 instead of Eq. 9 might yield slightly different results even in cases where $|\varepsilon|<20‰$. For example, if the original data for laboratory microcosms from ref. [17] is plotted using Eq. 9 a slightly different ε of –9.30‰ instead of –9.16‰ (Eq. 10) is obtained. For the calculation of enrichment factors, we therefore generally recommend the use of Eq. 9.

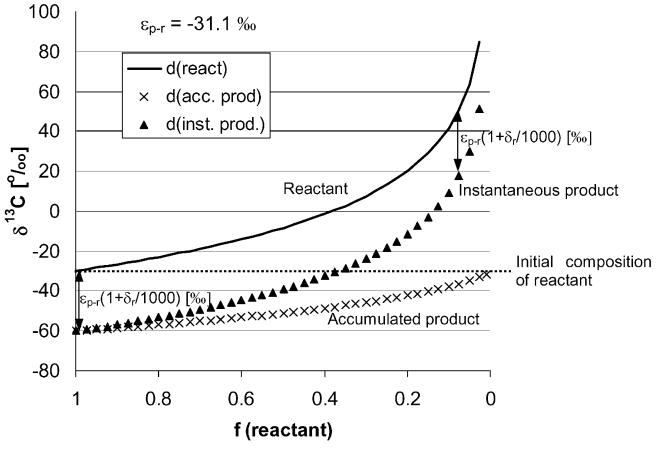

Fig. 2 Plot of $\delta_{x,t}$ versus f calculated using Eq. 10. The exemplary isotope enrichment factor, ε, (–31.1‰) and the estimated initial isotopic composition of the reactant, $\delta^{13}C_0$, (–30‰) were taken from a laboratory study of vinyl chloride degradation to ethene described in ref. [114]

CSIA applications in environmental science

Source apportionment

The molecular isotopic signature of environmental contaminants can often be used to trace their sources on local to global scales. On a local scale it is often necessary to allocate a contamination to a specific source in order to allow appropriate means of risk reduction and/or to identify responsible parties in litigation. In particular, work in the latter area has been termed "environmental forensics" in the US [18]. Traditional approaches in environmental forensics use chemical fingerprinting, biomarker analysis, and chemometrics [19, 20]. However, the potential of isotopic signatures of single contaminants in this area has only recently been explored. Frequently, it is possible to allocate sources of a chemical or to trace the time of contaminant releases because isotopic signatures of chemicals show differences between manufacturers depending on the conditions and the pathways used to synthesize the compound: this has been found for $\delta^{13}C$ in BTEX [21], $\delta^{13}C$ in MTBE [22], $\delta^{13}C$ in PCBs [23, 24], $\delta^{13}C$, $\delta^{2}H$, and $\delta^{37}Cl$ in chlorinated solvents [25], and $\delta^{13}C$ and $\delta^{15}N$ in trinitrotoluene [26]. In contrast, no differences in $\delta^{13}C$ were found in PAHs from two different creosote-contaminated sites [27].

Chemical fingerprinting of the *n*-alkane fraction in crude oils and refined products in combination with isotopic characterization of carbon in the individual homologues has been successfully used to allocate sources of sediment contamination [28] and bird feather oiling [29, 30]. Pond et al. [31] suggest the preferred use of hydrogen isotopic composition of longer chain alkanes (*n*-C19 to *n*-C27) for source identification because the isotopic signature of hydrogen in crude oil components varies much more compared with carbon and is hardly changed during weathering and degradation of crude oil. However, no application to source allocation based on hydrogen isotopic data has been reported so far.

On a regional scale, source apportionment of polycyclic aromatic hydrocarbons (PAHs) both in the atmosphere and in sediment records has been studied intensely utilizing $\delta^{13}C$ analysis. Interestingly, $\delta^{2}H$ analysis of individual PAHs has not been reported to date. With a combination of concentration measurements and $\delta^{13}C$ isotopic analysis of individual PAHs in sediments from Lake Erie it was possible to distinguish three areas of different contamination history. Furthermore, it could be shown that the main emission pathway for PAHs was fluvial input [32]. In a recent study, various sources of PAHs were distinguished in sediments along the St. Lawrence River. For example, very high $\delta^{13}C$ values were found for three ring PAHs originating from aluminum smelting in one area [33]. McRae et al. have shown that it is even possible to relate coal-de-

rived PAHs released during different thermal conversion processes (combustion, pyrolysis, gasification) with the resulting $\delta^{13}C$ values and that these isotopic compositions are conserved in soil [34, 35]. Isotopically extremely light PAHs ($\delta^{13}C$=–31 to –62‰) in lagoon sediments near Ravenna led to the conclusion that emissions were dominated by a former plant that used biogenic methane ($\delta^{13}C$=–69 to –73‰) as feedstock rather than by operating plants using petrogenic feedstocks of much higher ^{13}C content [36]. Wilcke et al. [37] used $\delta^{13}C$ analysis of perylene to substantiate their earlier hypothesis that in tropical environments recent biological sources of PAHs related to termites are important, whereas pyrolytic sources dominate in temperate climate.

PAHs in atmospheric particles resulting from natural burning processes could be distinguished from those stemming from various anthropogenic combustion processes by using fingerprinting and $\delta^{13}C$ analysis of individual compounds [38, 39]. In Chinese urban areas, PAH $\delta^{13}C$ analysis was successfully used to identify either vehicle exhaust or coal combustion as major PAH source [40]. In contrast, PAH fingerprinting did not yield equivalent information.

Many of these studies show the necessity to combine chemical fingerprinting techniques and compound-specific isotope analysis. Often, neither CSIA nor fingerprinting *alone* are conclusive for source apportionment but the information gain from isotopic analysis will certainly make CSIA indispensable in future source allocation investigations. Furthermore, several studies have shown that by determining the isotopic composition of two or more elements (e.g., by combining $\delta^{13}C$ and $\delta^{37}Cl$ [23, 25, 41, 42] or $\delta^{13}C$ and $\delta^{2}H$ [25, 43]), a much better differentiation can be obtained. If the sensitivity of GC/IRMS methods can be further improved to the ng L^{-1} range (see Sect. "Sensitivity of CSIA"), even a distinction of diffuse and point source emissions into groundwater seems feasible.

On a global scale, CSIA measurements can be used as a tool to characterize various sources and sinks of atmospheric gases and to estimate their relative importance. This approach has been used since the early 1980s but only recently GC/IRMS instead of dual-inlet IRMS was applied. The major advantage of GC/IRMS in this area is the much higher sample throughput that allows a higher sampling frequency [44]. Rice et al. [44] presented a thorough method comparison for measuring $\delta^{13}C$ and $\delta^{2}H$ of methane with GC/IRMS and dual-inlet IRMS and found no systematic and only low random deviations between both methods. On a Pacific transect they found $\delta^{13}C$ values ranging from –47.0‰ to –47.3‰ and $\delta^{2}H$ values of –85.8±1.2 and –91.7± 2.0‰ for the southern and northern hemispheres, respectively. Thus, $\delta^{2}H$ analysis was more sensitive to spatial variations than $\delta^{13}C$ analysis. An average global $\delta^{13}C$ for methane of –47.1‰ was found with average variations from northern to southern hemisphere of 0.6‰ and seasonal variations of 0.5‰ (consistent data only in the northern hemisphere) [45]. For chloromethane, Thompson et al. [46] found a global $\delta^{13}C$ average of –36.2‰. Budgeting of atmospheric chloromethane based on isotopic composition of known sources suggests that additional emission sources with an average $\delta^{13}C$ value of –41.9±7.8‰ exist. Harper et al. [47] found very similar values in chloromethane emitted by polypore fungi that was highly depleted in ^{13}C (–43.3‰) compared with the growth substrate, and an even higher depletion in two higher plant species. Recently, the same group found that chloromethane produced by two tropical fern species is significantly depleted ($\delta^{13}C$ –69.3‰ and –72.7‰, respectively) compared to chloromethane resulting from biomass burning and industrial emissions [48].

Identification and quantification of biodegradation processes

The assessment of biodegradation processes of specific contaminants at field sites is very demanding and sometimes impossible. A common problem in the *identification* of degradation processes is the difficulty to analyze for metabolites at trace levels. Such compounds are often quite polar and/or more easily degradable than the parent compound and may also occur as primary contaminants at the site. However, for a number of relevant pollutants degradation pathways and specific intermediates are known and allow at least a qualitative assessment of biodegradation (e.g., ref. [49]). The *quantification* of biodegradation processes is even more difficult because of the need for conclusive mass balances, which are often impossible due to insufficient knowledge about the groundwater flow regime, the limited number of sampling wells, and insufficient observation times. As an example, even at a site with injection of a defined amount of the gasoline oxygenate methyl *tert*-butyl ether (MTBE) into groundwater, well-known hydrogeology, and a dense network of sampling wells, it was not possible to unequivocally determine MTBE biodegradation after a period of eight years [50].

However, especially in the context of natural attenuation, it is essential to estimate the different sinks for organic contaminants such as dilution, sorption, or biodegradation, because the last of these is the only process of contaminant *destruction*. Degradation and in particular biodegradation is frequently accompanied by a substantial kinetic isotope effect (see Fig. 3), whereas many other environmental processes such as dispersion, sorption, or volatilization are not, or only to a much lower extent, subject to isotope fractionation. In such cases, stable isotope analysis provides a complementary opportunity to *identify* degradation processes in situ.

CSIA has been applied successfully as a means to investigate biodegradation in many studies over the last few years. An overview of batch-, column-, and field-scale studies is given in Table 2. Most of the studies report substantial isotope fractionation during microbial degradation of the investigated compounds. In such biodegradation studies, only the isotope ratio of the residual substrate is analyzed and the first enzyme reaction in the degradation pathway has been identified as the fractionating step [51]. Other processes such as uptake of the substrate or diffusion through the aqueous phase to the organisms did not

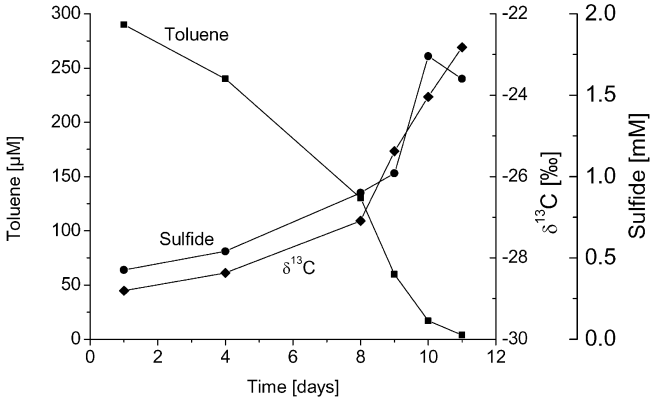

Fig. 3 Growth experiment with the sulfate-reducing strain TRM1. Toluene (■) and sulfide (●) concentrations are measured over time together with the isotope value $\delta^{13}C$ (♦) in the residual toluene fraction. Data are taken from ref. [60]

significantly influence the isotope fractionation. Another important point is that the first enzyme reaction in the degradation of both methylated aromatic hydrocarbons and chlorinated hydrocarbons is an "irreversible" step that does not allow chemical or isotope equilibrium of the substrate with products produced in subsequent enzyme reactions in the pathway. Thus, even if a subsequent reaction would be rate limiting and produce a pronounced isotope fractionation it would not affect the isotope ratio of the substrate.

However, in a few studies microbial degradation without isotopic degradation was reported, for example, see refs. [52, 53, 54]. Morasch et al. [54] have shown that the extent of fractionation may depend on the enzyme mechanism. In this case, degradation of aromatic hydrocarbons by a ring dioxygenase did not yield a significant fractionation in carbon. Several recent studies [43, 54, 55, 56, 57] utilizing both carbon and hydrogen isotope measurements concluded that the frequently much stronger isotope fractionation in hydrogen is a more powerful tool to provide evidence of biodegradation at contaminated field sites, in particular for small extents of biodegradation. However, one should be aware that isotope fractionation for a specific element depends very much on the reaction mechanism in the rate-limiting step of the biochemical reaction (see below and Sect. "Insight into reaction mechanisms from isotope fractionation"). As was found for source apportionment studies (see Sect. "Source apportionment"), the combined use of hydrogen and carbon isotope analysis might improve the assessment of biodegradation.

At a former gas works site, carbon isotope fractionation of BTEX and naphthalenes during anaerobic degradation was observed [58]. The observed fractionation factors for naphthalenes, however, were much smaller than for BTEX. It is not known yet if this is due to different rate-limiting steps during degradation or the larger number of carbon atoms in the naphthalenes that "dilute" the measured isotope fractionation (see Sect. "Can we learn more from fractionation data?").

The *extent* of isotope fractionation for degradation of a chosen contaminant is often first determined in laboratory experiments with pure cultures or enrichments. The results of such experiments are interpreted using the principles described above in Eqs. 1–11, that is, data are plotted in $\ln(R_t/R_0)$ versus $\ln f$ graphs (Eq. 8), and enrichment factors ε or fractionation factors α are obtained from the slope of a linear regression line. Experimental ε values derived in that way are given in Table 2. A *quantitative* comparison of these values even for the same compound is difficult because depending on environmental conditions and the specific biochemical reaction different rate-limiting steps may be dominant, including steps other than the enzymatic reaction (see also Sect. "Insight into reaction mechanisms from isotope fractionation"). However, if α (or ε) values are constant for a specific biochemical reaction at given environmental conditions, and isotope fractionation in the field is governed by the same processes, laboratory-derived α (or ε) values can be used to *quantify* the extent of biodegradation. To this end, the isotope ratios (R_0, R_t) of a pollutant are analyzed from different monitoring wells on the site and the residual substrate concentration (C_t) is calculated based on the initial pollutant concentration (C_0) in the source area of the contamination (usually assumed to be the groundwater well with the highest pollutant concentration). C_t is the expected concentration that should be present if biodegradation were the only process leading to reduction of the pollutant concentration. C_t values can then be compared to measured concentrations on the site. The difference should indicate the contribution of other processes such as dilution or sorption. Field data points that do not follow a linear relationship in $\ln(R_t/R_0)$ versus $\ln f$ graphs might not belong to the same plume [58, 59] or the shift in their isotopic signature is due to a fundamentally different process. In the following, the approach to quantify biodegradation in situ will be exemplified for toluene biodegradation in contaminated aquifers. An extended procedure to quantify degradation is discussed in Sect. "Can we learn more from fractionation data?"

Isotope fractionation for anaerobic toluene degradation was determined in batch experiments with various terminal electron acceptors [60, 61]. Remarkably constant α values were found under different anaerobic redox conditions with various pure cultures. The isotope fractionation factor α obtained from the sulfate-reducing bacterial culture was later on used to predict toluene degradation in a more complex environment, that is, anoxic column experiments with sediments from a contaminated site and sulfate as electron acceptor [61, 62]. Carbon isotope ratios of the remaining toluene fraction in samples taken along the column showed increasing $\delta^{13}C$ values qualitatively indicating biodegradation (Fig. 4a). A calculation of the biodegraded toluene fraction was performed with the measured isotope ratios, the initial toluene concentration at the inlet of the column, and the α value from the batch experiments [60]. It perfectly matched the measured concentration profile along the column [61]. This finding showed that isotope fractionation can be used to quantify biodegradation

Table 2 Biodegradation of organic contaminants investigated by CSIA

Compound (classes)	Scale	Assumed terminal electron acceptors	Isotopes measured	Isotope fractionation[a]	Reference
n-Alkanes, phenanthrene	batch	aerobic	$^{13}C/^{12}C$	not significant	[53]
n-Alkanes	batch	aerobic	D/H	Max. $\Delta(\delta^2H)$=26‰ (C16), lower for longer chain alkanes	[31]
Tetrachloroethene (PCE)	batch, field	methanogenic, sulfate-reducing	$^{13}C/^{12}C$	ε(C)=–2‰ (batch) Max. $\Delta(\delta^{13}C)$=6‰ (field)	[100]
Chlorinated ethenes	batch	methanogenic	$^{13}C/^{12}C$	ε(C)=–2.5/–6.6‰ (TCE) ε(C)=–14.1/–16.1‰ (*cis*-DCE) ε(C)=–21.5/–26.6‰ (VC)	[99]
PCE, TCE	field	anaerobic	$^{13}C/^{12}C$	Max. $\Delta(\delta^{13}C)$=6.4‰ (PCE) Max. $\Delta(\delta^{13}C)$=8‰ (TCE)	[101]
PCE	batch	methanogenic	$^{13}C/^{12}C$	ε(C)=–1.8 to –5.5‰	[102]
Trichloroethylene (TCE)	batch	aerobic	$^{13}C/^{12}C$	ε(C)=–18.2 to –20.7‰	[98]
trans-Dichloroethene (*trans*-DCE)	batch	aerobic	$^{13}C/^{12}C$	ε(C)=–3.5 to –6.7‰	[106]
Toluene	Batch	aerobic, sulfate-, nitrate- and Fe(III)-reducing	$^{13}C/^{12}C$	ε(C)=–1.5 to –2.6‰	[60]
Benzene, styrene, other hydrocarbons	batch, field	aerobic	$^{13}C/^{12}C$	Max. $\Delta(\delta^{13}C)$=2.2‰ (benzene, batch) Max. $\Delta(\delta^{13}C)$=1.7‰ (styrene, batch)	[115]
Toluene	batch	methanogenic, sulfate-reducing	$^{13}C/^{12}C$	ε(C)=–0.5/–0.8‰	[108]
Toluene	batch	methanogenic	D/H	Max. $\Delta(\delta^2H)$=>60‰	[57]
Benzene	batch	aerobic	$^{13}C/^{12}C$, D/H	ε(H)=–11.2/–12.8‰ ε(C)=–1.5/–3.5‰	[55]
Toluene	batch	aerobic, sulfate-, nitrate- and Fe(III)-reducing	D/H	ε(H)=–198 to –730‰ (using deuterium-labeled toluene)	[51]
Benzene, ethylbenzene	field	methanogenic, partly sulfate- and Fe(III)-reducing	$^{13}C/^{12}C$, D/H	Max. $\Delta(\delta^{13}C)$=2‰ Max. $\Delta(\delta^2H)$=27‰ (benzene)	[56]
Toluene	column, field	sulfate-reducing	$^{13}C/^{12}C$	Max. $\Delta(\delta^{13}C)$=3‰ (field)	[61]
Aromatic hydrocarbons	batch	aerobic	$^{13}C/^{12}C$, D/H	ε(C)=–0.1 to –3.3‰ (depending on enzyme mechanism) ε(H)=–16 to –905‰ (using deuterium-labeled substrates)	[54]
Aromatic hydrocarbons, naphthalene	batch, field	sulfate-reducing	$^{13}C/^{12}C$	ε(C)=–1.1‰ (naphthalene, batch) Max. $\Delta(\delta^{13}C)$=3.3‰ (benzene), 8.1‰ (toluene), 3.7‰ (ethylbenzene), 9.5‰ (*o*-xylene), 6.8‰ (*m*-/*p*-xylene), 3.3‰ (naphthalene), 1.4‰ (1-methylnaphthalene), 2.3‰ (1-methylnaphthalene) (all field)	[58]
Benzene	batch	nitrate- and sulfate-reducing, methanogenic	$^{13}C/^{12}C$, D/H	ε(C)=–1.9 to –3.6‰ ε(H)=–29 to –79‰	[63]
Aromatic hydrocarbons, naphthalene	column, field	sulfate-reducing (column), nitrate-reducing, sulfate-reducing	$^{13}C/^{12}C$	ε(C)=–1.1‰ (*o*-xylene, column) ε(C)=–1.5‰ (toluene, column)	[62]
Aromatic hydrocarbons	field	methanogenic, sulfate- and Fe(III)-reducing	$^{13}C/^{12}C$	ε(C)=–1.5‰ (ethylbenzene) ε(C)=–2.1‰ (*m*-/*p*-xylene)	[59]

Table 2 (continued)

Compound (classes)	Scale	Assumed terminal electron acceptors	Isotopes measured	Isotope fractionation[a]	Reference
PAHs	field	aerobic, unsaturated	$^{13}C/^{12}C$	$\Delta(\delta^{13}C)$=2–8‰	[109]
PCBs	batch	anaerobic	$^{13}C/^{12}C$	no significant fractionation	[52]
Phenol, benzoate	batch	aerobic	$^{13}C/^{12}C$	$\Delta(\delta^{13}C)$=2–8‰	[110]
MTBE	batch	aerobic	$^{13}C/^{12}C$	ε(C)=–1.5–2.0‰	[78]
MTBE	batch	aerobic	$^{13}C/^{12}C$, D/H	ε(C)=–1.4–2.4‰ ε(H)=–29 to –66‰	[43]
MTBE	batch, field	methanogenic	$^{13}C/^{12}C$	ε(C)=–9.2‰ (batch) ε(C)=–8.1‰ (field)	[17]

[a] $\Delta(\delta^{13}C)$ and $\Delta(\delta^{2}H)$: reported difference in isotopic composition; max. $\Delta(\delta^{13}C)$: maximum difference in isotopic composition

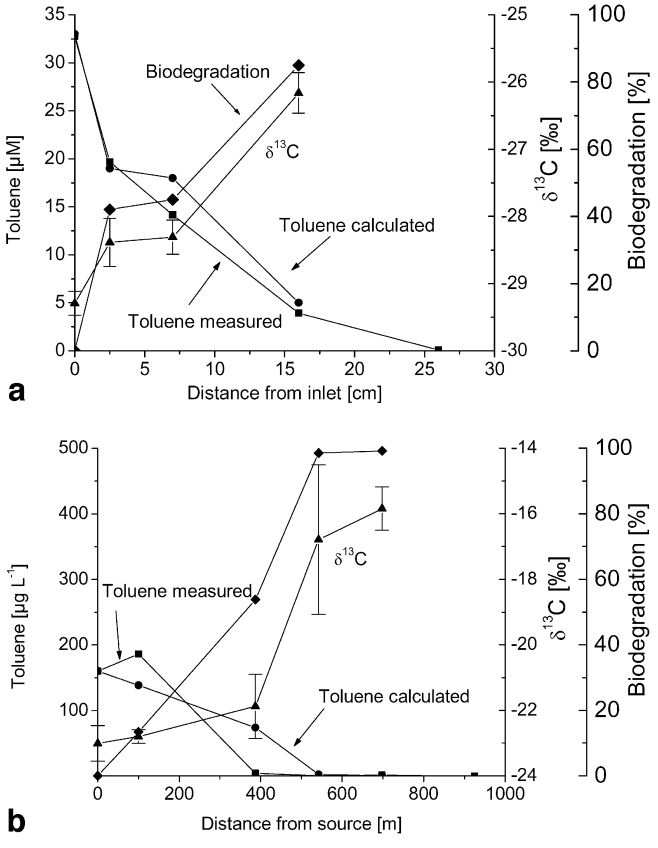

Fig. 4a,b Assessment of toluene degradation with isotope fractionation analysis in (**a**) sediment column experiment with contaminated aquifer material and (**b**) contaminated aquifer in Hamburg, Germany. Toluene (■) is measured in the aqueous phase. The theoretical substrate concentration C_t (●) is calculated with Eq. 8 from the measured toluene stable carbon isotope ratios (▲) at the respective ports of the column, the isotope fractionation factor α for the sulfate-reducing culture TRM1 (α=0.9983; Fig. 3), and the initial toluene concentration at the inlet of the column or the well in the source area. The percentage of biodegradation is calculated with Eq. 12. Data taken from refs. [58, 59, 62]

not only in batch experiments which are closed systems but also in sediment columns (i.e., flow-through systems).

Under the assumption that a contamination plume in an aquifer behaves similar to such sediment column systems, the quantitative isotope fractionation concept has been successfully applied in a number of field cases so far. One of the first was a tar oil-contaminated site where an almost 1,000-m-long hydrocarbon plume was located in an anoxic aquifer [62]. By using carbon isotope fractionation factors for anaerobic degradation of toluene and *o*-xylene, the observed isotope ratios and the initial substrate concentration at the source, the expected residual substrate concentration C_t along the plume was calculated (Fig. 4b). The calculated concentrations could describe the steep concentration gradients along the monitoring transect [62]. Similar results were obtained for sites contaminated with benzene, toluene, ethylbenzene and xylenes (BTEX) from a tanker truck accident [61], in a landfill leachate plume [59], and at a former gas works site [58]. The calculation of the residual substrate concentration along such monitoring transects relies on R_0, the isotope ratio of the substrate in the source. This parent value (usually the most negative) may also be replaced by the isotope ratio of the substrate in the most upstream monitoring well near the source if the source itself is not available without changing the result of the calculation.

A similar approach has been successfully used to estimate the extent of biodegradation for benzene at a contaminated site [56] from laboratory-derived carbon and hydrogen fractionation factors [63] with mostly rather good agreement between findings from the two elements.

Investigations of larger areas at several contaminated sites revealed that the spatial distribution of the extent of biodegradation can also be described with the help of isotope fractionation data [58, 62]. At these sites, the monitoring wells were not located along a single groundwater flow path. In the case of a former gas work plant, the calculated residual toluene and xylene concentrations perfectly matched the measured contaminant concentrations [58]. However, it is important to note that the determination of the spatial distribution of biodegradation based on isotope data is only feasible if the area of interest is contaminated by only one source with a defined source isotopic composition of the contaminant, R_0. If multiple plumes

from different sources commingle at a site, this approach is not possible. Especially in such cases, the isotope data have to be tested by a plot of $\ln(R_t/R_0)$ versus $\ln f$ according to Eq. 8. Data points that do not lay on a straight line in this plot might belong to plumes of other sources and may not be taken for a quantitative calculation of biodegradation. Such wells on the contaminated gas work site mentioned above were identified based on the isotope fractionation data. Geochemical parameters such as chloride concentrations were analyzed in parallel and confirmed that the groundwater from the spotted wells was not hydrologically connected to the contaminant source area. Although stable isotope fractionation has obviously a great potential for the assessment of biodegradation, this emphasizes the necessity to put isotope data into the context of geochemical parameters.

The spatial distribution of the monitoring wells on a site that are not located along one monitoring transect or along the same groundwater flow path implies that the water flows through different parts of the heterogeneous aquifer matrix. However, this did not affect the calculation of biodegradation with Eq. 8 at the former gas work site [58]. As only biodegradation influences the isotope ratios, the isotope data were independent on the heterogeneity of the aquifer material and undefined groundwater flow paths. This is especially important with respect to the fact that one will never find a monitoring transect that lays exactly along the groundwater flow path of interest. Aquifers are usually too heterogeneous and most monitoring wells will be more or less off the flow path. However, this does not seem to affect the isotope fractionation data significantly.

A thorough error propagation revealed that the total error of the calculated residual substrate concentration C_t is mainly dependent on the input value of the initial substrate concentration C_0 [58]. Input errors of other parameters in the Rayleigh equation are only of minor relevance to the final result. This clearly shows that the substrate concentrations in monitoring wells of the source area have to be accurately determined keeping in mind that only the aqueous concentration of the contaminant of interest is relevant here.

However, even if no concentration data are available, a semiquantitative description of the microbial degradation activity is possible which was termed percentage of biodegradation B [58, 61, 62]. The percentage of biodegradation B is calculated with Eq. 12.

$$B = 100\left(1-\frac{C_t}{C_0}\right) = \left(1-\left(\frac{R_t}{R_0}\right)^{\frac{1}{\alpha-1}}\right) 100\,[\%] \qquad (12)$$

Thus, B is dimensionless and relies only on the measured isotope ratios (R_0 and R_t) in the monitoring wells and the isotope fractionation factor α. The extent of biodegradation B depicts the extent to which the substrate has been degraded independently from the initial concentration. The contaminant concentration might have been reduced in addition by other sinks such as adsorption or dilution but the remaining substrate in the sample was then degraded to a certain percentage B. The extent of biodegradation B is a useful value to indicate different levels of biodegradation and can easily be converted into a quantitative value if the source concentration for the respective sample is accessible.

Insight into reaction mechanisms from isotope fractionation

In the applications of CSIA discussed so far it was assumed that the isotopic signature develops in a very predictable way when contaminants are transformed in the environment. Source allocation (Sect. "Source apportionment") relies on the assumption that this signature is conservative (=no contaminant reaction), whereas the quantification of in situ (bio)transformation, as discussed in Sect. "Identification and quantification of biodegradation processes", assumes that the change in the average isotopic signature is subject to a very robust fractionation process, as expressed by a constant factor α of the Rayleigh equation (Eq. 7). These assumptions are valid if no transformation occurs or if only one type of transformation is predominant. However, Ward et al. have shown that hydrogen fractionation during anaerobic toluene degradation might be controlled by a non-Rayleigh process indicated by a decreasing fractionation factor α during degradation [57]. In general, isotope fractionation can be highly variable for a contaminant, depending on the kind of transformation reaction. At first sight, this seems to complicate the interpretation of field studies. In many laboratory experiments, however, such variability in isotope fractionation has provided the crucial information that was instrumental in the mechanistic understanding of (bio)chemical reactions. Such experiments were generally performed with substrates labeled at the reactive site, in contrast to CSIA, which measures the change in the average isotopic signature of compounds at natural isotopic abundance. The differences between the outcomes of both kinds of studies (kinetic isotope effects versus fractionation factors α) will be discussed in Sect. "Can we learn more from fractionation data?" This section gives an overview about the factors that can contribute to an observed isotope fractionation and illustrates how measured isotope effects can help to solve the mechanism of (bio)chemical reactions. Because of the wealth of literature in this field, this overview can only discuss exemplary cases and is by no means exhaustive.

Kinetic isotope effects (KIEs) in mechanistic (bio)chemical studies are reported as a ratio of rate constants ${}^{light}k/{}^{heavy}k$ of compounds containing light versus heavy isotopes at a reactive site (see also Eq. 4). One distinguishes between *primary* isotope effects, in which the bond to the isotopic atom being studied is broken (or formed), and *secondary* isotope effects for isotopic atoms that are in immediate proximity to the reactive bond. If the ratio ${}^{light}k/{}^{heavy}k$ is greater than unity (i.e., light isotopes react faster, heavy isotopes become enriched in the substrate) the isotope effect is called *normal*. If the ratio is less than unity (i.e.,

light isotopes become enriched in the substrate) the effect is *inverse*. KIEs reflect changes in bonding between the ground state and the transition state of a reaction. The more pronounced these changes are (in many cases corresponding to a late transition state) the larger the observed isotope effect. If the bonding to the isotopic atom is looser in the transition state, the KIE is normal; if it is tighter in the transition state, the KIE may become inverse. Examples for inverse effects are secondary KIEs for hydrogen atoms that are bound to a trigonal sp^2-hybridized center in the ground state and to a tetragonal sp^3-hybridized center (i.e., a more cramped environment) in the transition state. If the intrinsic isotope effect in the elementary reaction is directly observable (i.e., there are no preceding or consecutive rate-limiting steps) it reflects directly the changes in bonding. Important examples are aliphatic nucleophilic substitutions. In S_N1 reactions the hybridization at the carbon center changes from sp^3 to sp^2 in the trigonal-planar transition state, which leads to small primary carbon isotope effects and large normal secondary hydrogen isotope effects. Conversely, S_N2 reactions proceed via a cramped trigonal-bipyramidal transition state leading to large primary carbon isotope effects and small normal or even inverse secondary hydrogen isotope effects. Because of the contrariness of the effects, isotope fractionation is a powerful method to distinguish between both reactions [64]. However, this example shows how transformations that are very similar and often lead to the same products can give rise to very different isotope fractionation.

Interpretation of isotope effects becomes more complicated, but even more informative if bond breaking/forming is preceded or followed by other slow reaction steps. In order to investigate the mechanisms of such multistep reactions, isotope effects are often measured for more than one element and at several positions of the molecule. If, for example, the isotope fractionation is equally pronounced in two bonds that are both broken/formed in an overall reaction, this can be an indication for a concerted process. Conversely, in a stepwise mechanism the isotope effect would be most pronounced only in the bond that is broken in the slow step. Illustrative examples of such studies investigated dehydrohalogenations (H^+ and X^- abstraction; see refs. [65, 66]) and fragmentations (hydride abstraction and decarboxylation by malic enzyme; see refs. [67, 68]). However, these examples show again how variable isotope fractionation may depend on subtle changes in reaction mechanism.

In enzyme-catalyzed reactions the isotopically sensitive elementary reaction is generally preceded by several enzyme–substrate association/interconversion steps and followed by numerous consecutive steps until product release. If these secondary processes become rate-limiting steps (as in the case of enzymes that have reached catalytic perfection and operate diffusion-controlled) the measured overall isotope effect can become much smaller than the actual effect in the elementary reaction. As first suggested by Northrop [69], this situation can be described in terms of so-called forward and reverse commitment to catalysis, terms that contribute to the masking of intrinsic isotope effects. This mathematical treatment is now commonly accepted. Interpretations, however, may be complicated by the fact that changes in environmental conditions (pH, availability of co-substrates) can slow down the secondary processes, unmask the intrinsic isotope effect again, and may thus increase the observed overall fractionation. Care must thus be taken to recognize such effects in environmental studies. Luckily from an environmental chemist's point of view, catalytic perfection will have generally not yet been reached in the case of microbial contaminant transformation so that the elementary bond cleavage can be expected to be rate-determining in most cases. The intrinsic isotope effect will therefore be at least partially expressed in most cases and can be expected to be rather robust.

Owing to (i) the complexity of the processes that can contribute to an overall observed isotope fractionation and (ii) to the difficulty in converting the parameters α or ε from the Rayleigh equation into KIEs (see Sect. "Can we learn more from fractionation data?"), detailed mechanistic interpretations in terms of ${}^{light}k/{}^{heavy}k$ are still rare in environmental studies. Both aspects are discussed in greater detail in a critical review by Elsner et al. [70]. Hunkeler et al. [71] investigated the microbial transformation of 1,2-dichoroethane and concluded from a very pronounced carbon KIE that the reaction proceeded via a nucleophilic substitution (S_N2 reaction). From reported values for forward and reverse commitment to catalysis they calculated a very high intrinsic KIE in the elementary step of 1.090. Reddy et al. [72] measured the chlorine KIE for dehydrochlorination of DDT in a study that included a careful determination of the intramolecular differences in $\delta^{37}Cl$ between different positions of the molecule. They obtained a value of 1.009, which was consistent with an $E1_{cb}$ mechanism for this reaction. Elsner et al. [73] examined the reductive dehalogenation of tetrachloromethane (CCl_4), where a simultaneous transfer of two electrons and cleavage of two C–Cl bonds in CCl_4 could be expected to completely circumvent production of problematic chloroform. All observed ${}^{12}k/{}^{13}k$ values for different electron transfer reactions (1.023–1.027) differed significantly from calculations for simultaneous cleavage of two C–Cl bonds (${}^{12}k/{}^{13}k$= 1.05) indicating that only one C–Cl bond was broken in the critical first step of the reaction and chloroform formation cannot be excluded a priori.

Directions of future research

Hyphenation to separation methods

Similar to the situation in organic mass spectrometry 15–20 years ago, commercial instruments for CSIA are nowadays hyphenated exclusively to gas chromatography (GC). This limits the application range of CSIA to compounds that are sufficiently volatile and thermally stable to be separated by GC. Non-volatile compounds of interest need to be derivatized prior to isotopic analysis. Although this step has been thoroughly investigated, in particular for the

analysis of amino acids, it implies two major drawbacks: extraneous carbon is introduced in the compound, which causes a shift of the isotopic composition and has to be corrected for in later calculations, and the derivatization reaction is usually characterized by a kinetic isotope fractionation. Complete transformation of the reactant and strict control of reaction parameters are therefore necessary.

The reason for the incompatibility of CSIA and liquid chromatography (LC) is that the combustion unit cannot deal with the high amounts of solvent inherently present in chromatographic methods using liquids as the mobile phase. It is therefore of no surprise that attempts to couple LC online to IRMS resemble the early LC/MS interfaces using a moving belt or moving wire [74, 75]. A complementary approach is the use of a thermospray/particle beam (TSP/PB) interface [76, 77]. With both systems solvent removal was sufficient to allow subsequent high-precision isotope ratio measurements. Both approaches have shown a good precision for the $\delta^{13}C$ measurement of various analyte classes, including carbohydrates, chlorophyll, and proteins. However, applications in environmental sciences have not yet been reported and both approaches may still lack the sensitivity required for analysis of environmental samples. Furthermore, the moving wire approach has shown considerable bias at low analyte load. The TSP/PB interface has not yet been combined with commercial IRMS combustion units but rather with a microwave-induced plasma for oxidation of the analytes. The possibility of incomplete oxidation of analytes and nonlinear isotope effects in the plasma therefore have to be considered. Despite the successful application of online LC/IRMS in the groups of Brenna and Abramson [74, 75, 76, 77], there has not yet been a commercialized instrument, which implies that additional fundamental work is needed in this area. Future work will certainly benefit from the latest developments in LC/MS interfaces.

Sensitivity of CSIA

A major drawback of compound-specific stable isotope analysis in environmental applications is its rather poor sensitivity. For a precise isotopic measurement, at least 1 nmol carbon or 8 nmol hydrogen of a given compound is needed for commercially available GC/IRMS instruments, provided that maximum chromatographic resolution and peak sharpness is achieved. If we take the groundwater contaminant trichloroethylene (TCE) as an example, a calculated minimum concentration of 66 mg L^{-1} ($^{13}C/^{12}C$) and of 1,100 mg L^{-1} (D/H) (injection volume 1 µL) would therefore be required for direct injection. Yet, the aqueous concentrations of a large number of groundwater contaminants are below 1 mg L^{-1} and are even regulated at the µg L^{-1} range in most OECD countries. This shows that the application of CSIA in contaminant hydrology (e.g., assessment of in situ degradation or source apportionment) is limited to highly polluted field sites. To overcome this limitation, many studies investigating isotope fractionation of contaminants have therefore been conducted in laboratory experiments, where analyte concentrations can be adjusted to meet the instrumental sensitivity of the GC/IRMS without prior enrichment [43, 51, 78].

Sensitivity is a less limiting factor for apolar and nonvolatile compounds such as, for example, PAHs, long-chain alkanes, or petroleum hydrocarbons that are predominantly sorbed to solid phases. For such compounds, liquid extraction of sediment samples followed by cleanup of the extracts is the method of choice [28, 30, 32, 79, 80]. As for all sample pretreatment and enrichment steps, the isotope fractionation of the analytical procedure needs to be carefully evaluated. However, detection limits for sediment extraction have rarely been reported. To obtain a reliable $\delta^{13}C$ isotopic fingerprint, O'Malley and co-workers [79] required 10 nmol of the most abundant PAH in 1 µL of extract. They have also shown that Soxhlet extraction did not cause a significant shift in the isotopic composition of PAHs [79]. Compound-specific determination of oxygen and nitrogen stable isotope signatures has been successfully applied to determine the authenticity of vanillin [81], 2,6-dimethylaniline, quinoline, nicotine, *p*-nitrobenzylalcohol, and caffeine [82], but no method detection limits have been indicated. Groundwater contaminants are often characterized by a high aqueous solubility and are often quite volatile, so that the abovementioned sample concentration and preparation techniques are not applicable. The various injection and enrichment techniques employed for CSIA of volatile groundwater contaminants are summarized in Table 3 together with reported detection limits and corresponding references. The higher sensitivity for larger molecules (e.g., toluene) compared to small ones (e.g., $CHCl_3$) is due to the higher number of carbon or hydrogen atoms in the molecule. Probing and injection of the supernatant gas phase of an aqueous sample (headspace injection) is a suitable technique for volatile analytes in the concentration range of 100–5,000 µg L^{-1} (see Table 3). For the $\delta^{13}C$ analysis of groundwater contaminants in the low µg L^{-1} range, solid-phase microextraction (SPME) and purge and trap (P&T) enrichment have been applied. The combination of these techniques with isotope ratio mass spectrometry has been thoroughly evaluated by Zwank et al. [83] (Table 3). The SPME technique consists in the exposition of a polymer-coated fiber to a sample either by the direct immersion of the fiber in the liquid sample or into the headspace above the sample. Headspace SPME has been used for the determination of $\delta^{13}C$ [43, 78] and $\delta^{2}H$ [43] of MTBE in aqueous samples, reaching detection limits of 11 µg L^{-1} [78] in the carbon mode. By exposing the SPME fiber directly to the aqueous phase, detection limits for a broad range of volatile compounds range between 9 µg L^{-1} (toluene) and 170 µg L^{-1} ($CHCl_3$) in $\delta^{13}C$ analysis [83]. The $\delta^{13}C$ values measured by headspace SPME or direct immersion SPME did not deviate significantly from those of the pure phase analytes. However, the application of SPME in GC/IRMS to analyze multicomponent mixtures may be compromised by competition among the analytes for sorption sites on the extracting polymer phase, in particular when using a phase that shows adsorption as well as partitioning [83, 84]. For ex-

Table 3 CSIA detection limits reported for volatile groundwater contaminants

Compound	Detection limit (µg L^{-1})		Injection/enrich-ment technique	Isotopic fractionation during analysis	Reference
	$\delta^{13}C$	$\delta^{2}H$			
MTBE	24,000	–	liquid injection[a]	OC[b]<0.3‰; SL[c] ≈1‰	[83]
	5,000	50,000	headspace injection	n.r.[d]	[43]
	350	1,000	headspace SPME	n.r.	[43]
	11	–	headspace SPME	n.r.	[78]
	16	–	direct immersion SPME	Reproducible fractionation (<0.5‰), but presence of BTEX concentrations >3 mg L^{-1} caused 2‰ deviation	[83]
	15	–	P&T	Significant but reproducible shift of $\delta^{13}C$ values (+0.66‰)	[22]
	5	–	P&T	n.r.	[17]
	0.63	–	P&T	n.s.f.[e]	[83]
Benzene	19,000	–	liquid injection	n.s.f.	[83]
	500	–	headspace injection	n.r.	[63]
	22	–	direct immersion SPME	n.s.f.	[83]
	0.30	–	P&T	n.s.f.	[83]
Toluene	9500	–	liquid injectiona	OC n.s.f. SL≈–1‰	[83]
	–	2,000	headspace injection	no deviation from pentane injection of standards	[57]
	100	–	headspace injection	n.s.f.	[111]
	45	–	direct immersion SPME	n.s.f.	[112]
	9	–	direct immersion SPME	n.s.f.	[83]
	0.25	–	P&T	n.s.f.	[83]
Chlorinated methanes	170,000–220,000	–	liquid injection	$CHCl_3$, ≈–1.5‰ CCl_4, OC –3.31±0.34‰	[83]
	170	–	direct immersion SPME	$CHCl_3$, –1.8±0.28‰	[83]
	280			CCl_4, –7.3±0.2.2‰	
	100 (conc. in the headspace)	–	headspace injection	CH_3Cl, n.s.f.	[47]
	≤5.0	–	P&T	$CHCl_3$ and CCl_4, n.s.f.	[83]
Chlorinated ethylenes	71,000–84,000	–	liquid injection	Small but significant fractionation observed for TCE and *cis*-DCE	[83]
	400	–	headspace injection	TCE, n.s.f.	[111]
	130	–	direct immersion SPME	n.s.f.	[113]
	66–130	–	direct immersion SPME	Small (≈1‰) but significant fractionation observed for *cis*-DCE only	[83]
	5	–	P&T	n.s.f.	[86]
	1.4	–	P&T	Small (≈0.7‰) but significant fractiona-tion observed for *cis*-DCE only	[83]

[a] Analyte dissolved in solvent
[b] On column injection
[c] Splitless injection
[d] Not reported in reference
[e] No significant fractionation (<0.5‰) observed

ample, benzene concentrations above 3 mg L^{-1} were strongly hampering the SPME extraction efficiency of MTBE which resulted in a deviation of 2‰ from its true $\delta^{13}C$ value. Significantly lower MDLs can be obtained for volatile organic compounds with P&T than with SPME due to higher sample volumes as well as the higher sorption capacities of the enrichment traps. As can be seen in Table 3, online P&T-GC/IRMS requires only a few µg L^{-1} of volatile organic compounds for reliable $\delta^{13}C$ measurements [83]. So far, the online coupling of P&T with GC/IRMS has rarely been reported [17, 22, 42, 83, 85, 86] but allows the lowest detection limits achieved in CSIA to date (e.g., 2.2 µg L^{-1} PER, 0.25 µg L^{-1} toluene, 0.3 µg L^{-1} benzene, and 0.63 µg L^{-1} MTBE) [83]. Since the P&T procedure includes various extraction steps that may shift the isotopic signature of the analytes (evaporation, sorption, condensation), Zwank et al. [83] evaluated the effects of the P&T method parameters such as purge time, desorption time, and injection temperature on the determination of the $\delta^{13}C$ values of the analytes. They have studied the fractionation potential of these extraction steps for the priority groundwater pollutants methyl *tert*-butyl ether (MTBE), chloroform, tetra-

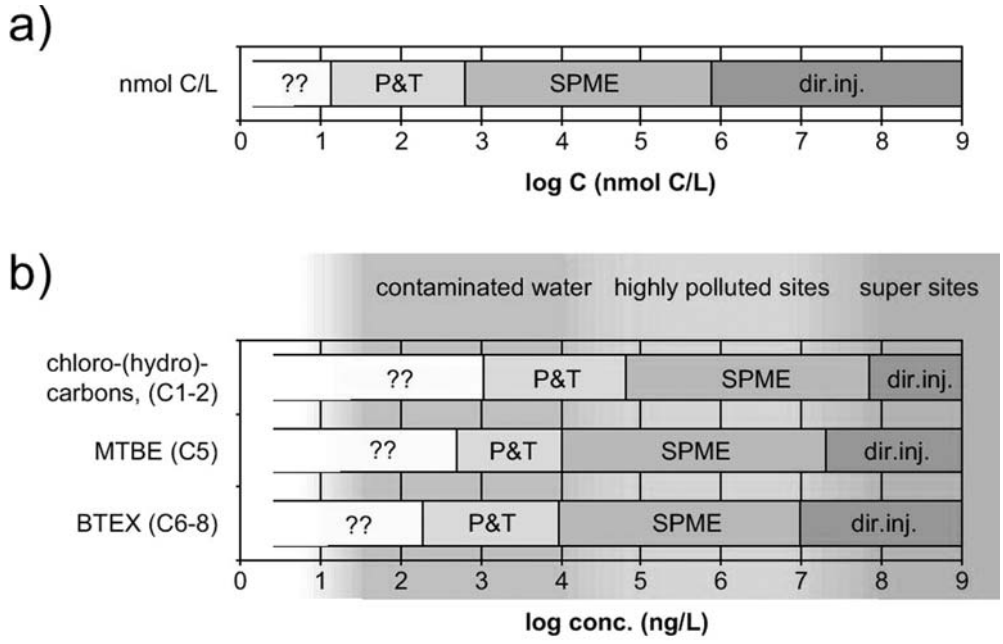

Fig. 5 **a** Detection limits in CSIA and suitable ranges shown in nmol C L^{-1}. **b** Concentration ranges and lowest detection limits achieved in CSIA with direct injection, SPME, and P&T, illustrated for aqueous concentrations found at contaminated sites. *Concentration axes* are plotted in logarithmic scale

chloromethane, chlorinated ethylenes, benzene, and toluene. For all investigated compounds highly reproducible compound-specific isotopic enrichments (0.2–0.9‰) were found. The authors conclude that the isotopic shifts caused by P&T enrichment can therefore be corrected for. However, due to the fact that these shifts are compound-specific, the use of external standards (e.g., standards of known isotopic composition treated identically to the samples) is highly recommended. It is also important to emphasize that the isotopic signature of external standards should preferably be determined in the pure analyte with an elemental analyzer coupled to an IRMS. Otherwise, a fractionation caused by injection and/or chromatographic separation with GC-IRMS cannot be accounted for. Figure 5a illustrates MDL estimations for any compound with the concentration scale in nmol C L^{-1}. In Fig. 5b, concentration ranges and CSIA detection limits (ng L^{-1}) for a selection of priority groundwater contaminants together with concentration ranges found in polluted water are given.

In summary, SPME and especially P&T enrichment are very promising techniques for CSIA of groundwater pollutants, with the latter allowing $\delta^{13}C$ determinations in aqueous samples at very low µg L^{-1} level [83]. Nevertheless, the growing interest to study the isotope fractionation of trace level contaminants (≤500 ng L^{-1}) in natural environments calls for sensitivity improvements of GC/IRMS instruments and further enhanced enrichment techniques.

CSIA for other elements (S, Cl, Br)

Continuous flow (CF) measurements of $\delta^{34}S$ using an elemental analyzer (EA) have been reported as early as 1988 [87]. Since then, CF-IRMS has become the routine method for measurement of $\delta^{34}S$ in sulfate in geochemistry [88], atmospheric science [89], and geomicrobiology [90] because it is often less tedious than the classical dual-inlet method. Applications for $\delta^{34}S$ in organic material and soil have also been reported [91]. In contrast, no compound-specific measurement of $\delta^{34}S$ using GC/IRMS has been reported so far. The major obstacle seems to be the typically low abundance of sulfur in organic compounds leading to a limited sensitivity for this element. However, for organic compounds with a rather high S-to-C molar ratio, such measurements should be feasible and might be a useful tool to study sources and the fate of the many organosulfur compounds of environmental interest such as thiols and dialkylsulfides.

Chlorine is another element of high environmental relevance that has two stable isotopes. Chlorinated hydrocarbons are among the most abundant environmental pollutants, and it is therefore of no surprise that chlorine isotope measurements are frequently pursued. However, no continuous flow measurements have been reported yet because the conversion of chlorine into a measurable gas is rather complex and has not been successfully carried out online. Dual inlet measurements of $\delta^{37}Cl$ values for pure chlorinated solvents [25, 41, 92, 93], PCB mixtures [94], aroclor mixtures, and various pesticides [23] have been reported. The range of $\delta^{37}Cl$ values versus the international standard SMOC (standard mean ocean chloride) in these compounds was –3.31 to –2.11‰ for PCBs [94], –5.10 to 1.22‰ for chlorinated pesticides [23], and –3.54 to 4.08‰ in chlorinated solvents [25]. Furthermore, $\delta^{37}Cl$ analysis has been used for the investigation of reductive dechlorination of chlorinated ethylenes [95, 96, 97]. Numata et al. reported fractionation factors α for the anaerobic degradation of PCE (0.987–0.991) and TCE (0.9943–0.9945) with various anaerobic bacteria [95]. Even smaller isotope effects were reported for the aerobic degradation of dichloromethane (α=0.9962) [97] and TCE (α=0.999475) [96]. Measured $\delta^{37}Cl$ values in all reports were in the

range –2.3 to 2.0‰. Sturchio et al. [96] reported a reliable $\delta^{37}Cl$ measurement for TCE in a field sample with a TCE concentration as low as 4 µg L^{-1} and were able to confirm degradation of TCE in the field using $\delta^{37}Cl$ analysis. Reddy et al. [94] measured a significant chlorine isotope effect during the transformation of PCBs in sediments. However, since only bulk analysis was possible, it was difficult to distinguish a true isotope effect from changes in the isotopic mass balance due to congener-specific $\delta^{37}Cl$ values. Thus, these authors and Yanik et al. [24] concluded that a congener-specific approach utilizing $\delta^{13}C$ and $\delta^{37}Cl$ would be extremely useful for source allocation and tracing studies. An online conversion of chlorine after GC separation of the congeners would be the most efficient means to achieve this. The availability of online $\delta^{37}Cl$ measurements would also be an asset in the numerous studies of chlorinated solvent degradation (see Sect. "Identification and quantification of biodegradation processes") that, with the few exceptions mentioned above, are nowadays limited to online $\delta^{13}C$ measurements (e.g., see refs. [98, 99, 100, 101, 102]).

The development of appropriate means to convert chlorine online into methyl chloride or another species suitable for subsequent isotope analysis therefore remains one of the most challenging research areas in CSIA. A successful approach would certainly be adopted worldwide and commercialized in a short time.

Possibly due to the limited number of environmentally relevant organobromine compounds there has not yet been any work reported for the measurement of the $^{81}Br/^{79}Br$ ratio. However, the environmental behavior of brominated diphenyl ethers used as flame retardants has recently gained considerable interest and CSIA of $\delta^{81}Br$ could again be a useful tool to characterize sources and fate of such compounds. However, the relative mass difference of the two isotopes is rather small and kinetic isotope effects should also be smaller than for the other elements discussed so far. For continuous flow measurements, the same limitations apply as described above for chlorine.

Can we learn more from fractionation data?

The ultimate goal of isotope fractionation measurements in the field is not only to *detect* but also to *quantify* in situ transformation at contaminated sites. As exemplified in Sect. "Identification and quantification of biodegradation processes", enrichment cultures, isolated microorganisms, or abiotic reactants are therefore often taken as model reactants, and the extent of isotope fractionation for a chosen contaminant is first determined in laboratory experiments (as an average over all atoms in a contaminant, measured by GC/IRMS). The results of such experiments are generally interpreted using the principles described above in Eqs. 1–11, that is, data are plotted in $\ln(R_t/R_0)$ versus $\ln f$ graphs (Eq. 8), and satisfactory regression statistics are usually obtained, which is taken as evidence that the underlying physical model is valid and the evaluation procedure correct. The extent of isotope fractionation in such studies is then reported as values of ε (enrichment factors) or values of α ("fractionation factors"). Under the assumption that fractionation in the field follows the same behavior, the ε (or α) values from laboratory experiments are finally used to quantify in situ transformation at contaminated sites. This approach, sketched in the upper part of Fig. 6, has been very successful at many field sites (see Sect. "Identification and quantification of biodegradation processes"). However, there are several aspects that remain unsatisfactory:

(1) Values of ε (or α) must be determined all over again for every new contaminant, which entails a lot of laboratory work. So far, no procedure has been proposed to derive these values from existing data of similar compounds in similar reactions.

(2) What is the physical meaning of ε (or α)? Although these values express to a certain degree the extent of isotope fractionation, they also depend on the molecular size of the investigated contaminant (the larger the contaminant molecule (i.e., the more atoms of one element), the smaller the isotope fractionation of this element as expressed by ε or α!).

(3) As discussed in Sect. "Insight into reaction mechanisms from isotope fractionation", numerous experimental data has been published on kinetic isotope effects $^{light}k/^{heavy}k$, where $^{light}k$ and $^{heavy}k$ are the rate constants involving reactive sites with lighter and heavier isotopes, respectively. Although this database could be highly useful for an understanding of isotope fractionation in nature, interpretation of environmental studies in terms of $^{light}k/^{heavy}k$ has only been attempted in a few cases.

Values of ε (or α) are often found to be highly variable, for example in aerobic/anaerobic biodegradation of MTBE [17, 43]. From such isotopic data alone it is difficult to decide whether fractionation in this reaction varies unpredictably or is manifested in different values owing to different mechanisms, such as described in Sects. "Identification and quantification of biodegradation processes" and "Insight into reaction mechanisms from isotope fractionation". Under such circumstances, quantification of natural degradation at contaminated sites is difficult.

It therefore becomes apparent that without a physicochemical interpretation of the obtained data, ε or α are merely descriptive parameters. The mechanistic information that they contain usually remains hidden and, most importantly, an a priori *prediction* of isotope fractionation that would be highly useful in field studies is restricted to cases of very robust fractionation.

The underlying assumption of the Rayleigh equation is a simple first-order rate law: the amount of isotopes that react at a given time is supposed to be proportional to the amount of isotopes that still remain. If heavy isotopes react more slowly, their rate constant ($^{heavy}k$) will be smaller than that of light isotopes ($^{light}k$); they become enriched in the remaining substrate, and fractionation takes place. This fractionation, however, will manifest itself *only at the reactive site of a molecule* (primary isotope effect) or, less

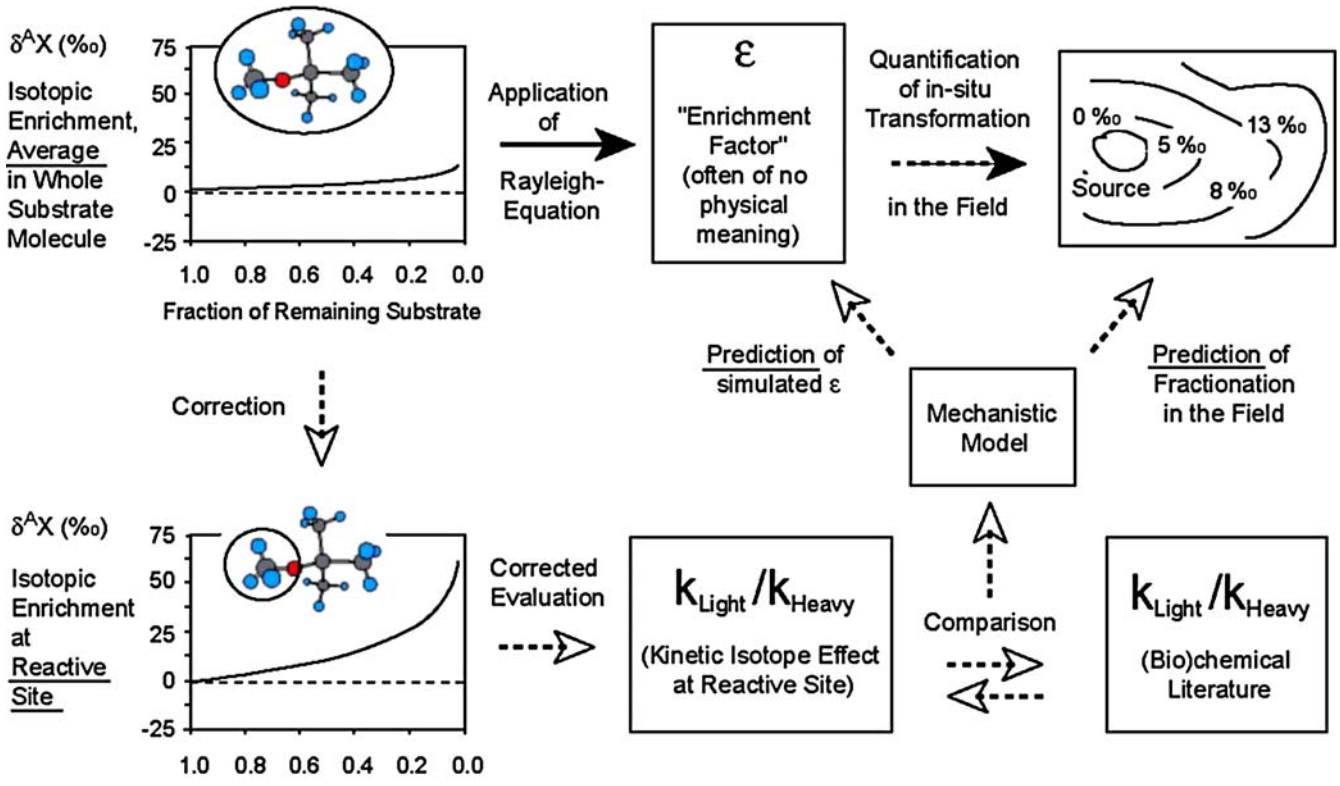

Fig. 6 Current (*solid arrows*) and proposed (*dashed arrows*) evaluation procedure for data from isotope fractionation studies. If isotope effects are calculated based on the enrichment at the reactive site rather than the average value measured by GC/IRMS, mechanistic information can be derived that may even lead to the prediction of isotope fractionation in organic compounds [70]

pronounced, in the direct neighborhood (secondary isotope effect). The isotope ratio at distant non-reactive sites will remain unchanged during the reaction so that the *average* isotopic ratio, as measured by GC/IRMS, changes much less than the isotopic ratio *at the reactive site*. Such a procedure leads to the result that for the very same reaction of, for example, homologues, the magnitude of determined enrichment factors ε depends on the size of the molecules, and that such values of ε "hide" their underlying physical meaning. The frequently cited relationship ${}^{heavy}k/{}^{light}k=\alpha=\varepsilon+1$ (Eq. 4) holds then only for small molecules where all isotopes under investigation are located at the same reactive position, such as in CH_4/CCl_4 (carbon isotope fractionation) or NO_3^- (nitrogen isotope fractionation).

For the case of larger molecules, Elsner et al. [70] propose a modified evaluation procedure that is sketched in the lower part of Fig. 6. The *average* isotopic enrichment as measured by GC/IRMS must first be converted into values for the isotopic enrichment *at the reactive site*. Such a correction is rather straightforward. It relies, however, on several important presuppositions: (i) a mechanistic hypothesis about the type of transformation is needed (e.g., oxidation, hydrolysis) so that the reactive position can be identified; (ii) in a first approximation, secondary isotope effects must be negligible (they are usually by an order of magnitude smaller than primary effects); (iii) the position(or site)-specific isotope ratios within the molecule must be known so that R/R_0 can be calculated at the reactive position.

In the case of hydrogen, position-specific ratios can in principle be measured by SNIF-NMR [8]; however, only in pure substances (e.g., in substrates before they are transformed in laboratory experiments). This method cannot be applied with low contaminant concentrations or complex compound mixtures such as encountered in field situations. An alternative approach to obtaining position-specific isotope ratios is a selective decomposition of substances into smaller fragments that are representative of different positions and can be analyzed separately [68]. However, such a procedure is rather tedious and has only been reported in exceptional cases [72].

In the absence of information about position-specific isotope ratios, it must therefore be assumed as a working hypothesis that the isotope ratio at time 0 does not differ greatly between different sites in a molecule. Available information indicates that this approximation is fairly good for carbon, where the isotope ratios within a compound vary by generally only up to ±50‰ (±5%) from the mean (in most cases less). Conversely, hydrogen is subject to much larger fractionation and can display differences of up to ±100%. This general statement is, for example, illustrated by case studies of vanillin cited in ref. [8]. A discussion by Elsner et al. [70] shows that errors of ±5% in R also result in a *relative* error in estimates for ε of roughly ±5%, which is in most cases tolerable (the maximum error in an estimated enrichment factor of –20‰ would then be ±1‰). Elsner et al. further demonstrate that a non-statistical isotope distribution within a compound causes an in-

trinsic error in ε regardless of whether the evaluation is performed according to the "classical" Rayleigh equation or the proposed alternative evaluation scheme. Nevertheless, the precision of estimated values would profit greatly from knowledge of position-specific isotope ratios at time 0 (R_0).

Once a (more or less accurate) estimate of R/R_0 is calculated for the *reactive position*, the Rayleigh equation can be applied to the corrected data. In a further step of this advanced evaluation scheme, the effect of *intramolecular* isotopic competition is taken into account [103], which is important if different isotopes are present in chemically equivalent positions of a molecule where they can all react (e.g., one ^{2}H and two ^{1}H in a methyl group that is oxidized). With this approach, values for $^{light}k/^{heavy}k$ of the KIE *at the reactive site* can finally be obtained, which are now directly comparable to data from published (bio)chemical studies. Because the magnitudes for such expected isotope effects are already quite well known for different types of reactions (see Sect. "Insight in reaction mechanisms from isotope fractionation"), the overall procedure can effectively be used to *test* different mechanistic models and, in comparison with literature values, identify possible reaction mechanisms for contaminant transformation. This leads to new, exciting possibilities in the interpretation of environmental isotope fractionation studies, as sketched in the right part of Fig. 6: i) mechanistic information about transformations may be derived that can otherwise be very difficult to obtain, ii) such information may be instrumental in identifying hitherto unknown degradation products, and iii) isotope fractionation might be *predicted*, even for new compounds for which isotope fractionation studies have not yet been conducted. The information obtained from isotope fractionation measurements can thus be increased enormously, and quantification of contaminant degradation in the field may be greatly facilitated.

Conclusions

Most CSIA measurements to date have been conducted on volatile organic compounds such as BTEX, chlorinated ethylenes, and MTBE, all of which are notorious groundwater contaminants. In addition, PAHs have been intensively studied, notably to elucidate and distinguish their sources. A large range of compounds, however, has not yet been addressed although there is a multitude of possible applications. Examples include investigations of the proposed covalent bonding of anilines with carbonyl moieties in humic substances [104], and the stepwise reduction of nitroaromatic compounds [105], both of which might show pronounced $^{15}N/^{14}N$ fractionation.

Regarding the measured elements the vast majority of investigations have been published on $^{13}C/^{12}C$. Since the commercial introduction of D/H measurements in single compounds by CSIA in 1998, there has also been considerable interest in this element. $^{15}N/^{14}N$ and $^{18}O/^{16}O$ measurements are to date hardly reported in environmental science although many environmental contaminants contain nitrogen (see above) or oxygen. It is not known yet for which compound classes and types of reaction a significant isotope fractionation occurs. $^{18}O/^{16}O$, though commercialized, is still in its infancy and not yet possible on a routine base.

With regard to environmental contaminants, the development of an online method for the determination of $^{37}Cl/^{35}Cl$ and $^{81}Br/^{79}Br$ would expand substantially the application range of CSIA.

Further methodological developments, more widespread instrument availability, and an increasing number of publications showing the broad applicability and sometimes unique information offered by CSIA will certainly make CSIA one of the key analytical methods in environmental chemistry and microbiology in the future. So far, we have only scratched the surface with this approach in environmental studies.

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