doi:10.2533/chimia.2014.788 Chimia 68 (2014) 788–792

Isotope Effects as New Proxies for Organic Pollutant Transformation

Thomas B. Hofstetter*[ab], Jakov Bolotin[a], Sarah G. Pati[ab], Marita Skarpeli-Liati[ab], Stephanie Spahr[ac], and Reto S. Wijker[b]

Abstract: Assessing the pathways and rates of organic pollutant transformation in the environment is a major challenge due to co-occurring transport and degradation processes. Measuring changes of stable isotope ratios (*e.g.* $^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{15}N/^{14}N$) in individual organic compounds by compound-specific isotope analysis (CSIA) makes it possible to identify degradation pathways without the explicit need to quantify pollutant concentration dynamics. The so-called isotope fractionation observed in an organic pollutant is related to isotope effects of (bio)chemical reactions and enables one to characterize pollutant degradation even if multiple processes take place simultaneously. Here, we illustrate some principles of CSIA using benzotriazole, a frequently observed aquatic micropollutant, as example. We show subsequently how the combined C and N isotope fractionation analysis of nitroaromatic compounds reveals kinetics and mechanisms of reductive and oxidative reactions as well as their (bio)degradation pathways in the environment.

Keywords: Compound-specific isotope analysis · Degradation mechanism · Kinetic isotope effect · Pollutant transformation

Challenges Associated with Contaminant Transformation

Quantifying extent and rates of organic micropollutant transformation in the environment is a major challenge.[1,2] Numerous synthetic chemicals pollute natural waters, soils, and the atmosphere where they undergo a cascade of transport and transformation processes that determines their concentration dynamics in the respective media.[3] To protect humans and the environment from the adverse effects of chemical pollution and to implement efficient mitigation strategies, a thorough understanding of the (bio)chemical reactions that lead to pollutant degradation is essential. Unfortunately, a number of factors complicate the interpretation of a contaminant's concentration dynamics in the environment: i) Dilution, dispersion, volatilization, as well as sorption to organic and mineral phases need to be distinguished from reactive processes. ii) Many organic micropollutants can be transformed by several chemical, biological, and photochemical pathways simultaneously, sometimes giving rise to products that exceed the toxicity of the parent compounds. iii) Degradation pathways of many pollutants, especially of emerging contaminants, are often not known. iv) Timescales of transformation can be very variable ranging from a few hours to decades.

In our research, we explore new avenues for assessing contaminant transformation with which (some of) the above challenges can be overcome. Our approach is based on the analysis of stable isotope ratios in individual organic compounds and the characterization of (bio)chemical reactions using their kinetic and equilibrium isotope effects. In this contribution, we briefly review the most important analytical and conceptual features of compound-specific isotope analysis (CSIA). We show how changes in contaminant isotope ratios, known as isotope fractionation, can be related to reaction mechanisms and thus contaminant (bio)degradation pathways. Isotope effects of enzymatic and abiotic redox reactions of organic contaminants are still poorly understood and subject to ongoing studies. Here, we also illustrate how the knowledge gained from examining reaction mechanisms in environmental organic chemistry using theory and experiment can be implemented to quantify pathways and rates of pollutant reactions in contaminated environments.[1,4,5]

*Correspondence: PD Dr. T. B. Hofstetter[ab]
Tel.: +41 58 765 50 76
E-mail: thomas.hofstetter@eawag.ch
[a]Eawag, Swiss Federal Institute of Aquatic Science & Technology
Department of Environmental Chemistry
Überlandstr. 133
CH-8600 Dübendorf
[b]Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich
[c]Institute of Environmental Engineering, EPF Lausanne

Stable Isotope Tools/ Instrumentation

Compound-specific isotope analysis of organic contaminants has become popular thanks to commercially available modern analytical instrumentation that makes it possible to separate individual compounds in a sample mixture followed by an online quantification of isotope ratios at natural isotopic abundances (*e.g.* $^{13}C/^{12}C$ of 0.01106, $^{2}H/^{1}H$ of 0.0001557, $^{15}N/^{14}N$ of 0.003663).[4,6,7] This task is achieved by coupling gas chromatography and, much less frequently, liquid chromatography to isotope ratio mass spectrometry (GC/- and LC/IRMS), with which C, H, N, O, S, Cl, and Br isotopes can be measured. A schematic representation of a widely used instrumental setup is shown in Fig. 1a. The key step for the analysis of C, H, N, and O isotopes by GC/IRMS is the conversion of organic compounds into small analyte gases that consist of only few isotopologues of an element. Some of the advantages of this approach are the very high instrumental precision and the possibility to calibrate isotope ratios to an internationally recognized scale. Information on the position of isotopic substitution, however, is lost and isotopomers cannot be distinguished as would be possible by nuclear magnetic resonance. Finally, the versatility of stable isotope analysis by GC/- and LC/IRMS is especially beneficial for the isotopic analysis of organic contaminants given their enormous structural diversity.

Measurements of sulfur and halogen isotope ratios in organic compounds can be achieved with different instrumental strategies ranging from the use of standard quadrupole mass spectrometers to the use of inductive coupled plasma with multi-collector isotope ratio mass spectrometers.[7] Running such instrumentation is less straightforward and subject to ongoing research[8,9] and explains in part why CSIA of those elements is not carried out as frequently as that of C and H.

Analytical procedures for CSIA of organic contaminants need to be developed on a compound-by-compound basis.[10–12] Enrichment of an analyte from the environmental matrix as well as its conversion to analyte gases may lead to changes in isotope ratios and analytical artifacts. In addition, the efficiency with which different organic compounds are transformed into analyte gases varies widely and is not known *a priori*. Finally, many organic micropollutants of current interest are rather polar compounds and thus not perfectly suited for gas chromatographic analysis. An example of such a compound is the corrosion inhibitor benzotriazole, which is used in dishwashing detergents, aircraft deicing, and cooling and heating fluids. Owing to incomplete removal in sewage treatment plants, benzotriazole is frequently found in Swiss rivers.[13–15] The ability of benzotriazoles to complex metals impedes analysis by conventional GC/IRMS where Cu-containing catalysts are necessary to produce CO_2 and N_2 for C and N isotope analysis, respectively. Only after the substitution of metal parts and Cu in the catalyst did one succeed with determining the C and N isotope ratios accurately.[10] Fig. 1b shows an excerpt of a chromatogram, in which benzotriazole was converted to isotopically light ($^{14}N_2$) and heavy ($^{15}N^{14}N$) molecular nitrogen for N isotope analysis of benzotriazole. A comparison of C and N isotope signatures, $\delta^{13}C$ and $\delta^{15}N$, illustrates that benzotriazoles from different chemical manufacturers exhibit very different N isotope compositions whereas C isotope ratios are identical within uncertainty (Fig. 1c). Such isotopic 'fingerprints' likely originate from the C and N isotope ratios of the raw materials used. In addition, the synthesis process involves several reactions at N atoms, which are sensitive to isotopic substitution and could generate benzotriazoles of different $^{15}N/^{14}N$-ratios. A first comparison of random samples of dishwashing detergents suggests that six of seven samples were of similar $\delta^{13}C$- and $\delta^{15}N$-value and thus likely contained benzotriazole from the same chemical manufacturer. This example illustrates that, like in forensics and food and beverage authentication,[16] organic micropollutants may be related to their producers by comparing their isotope signatures.

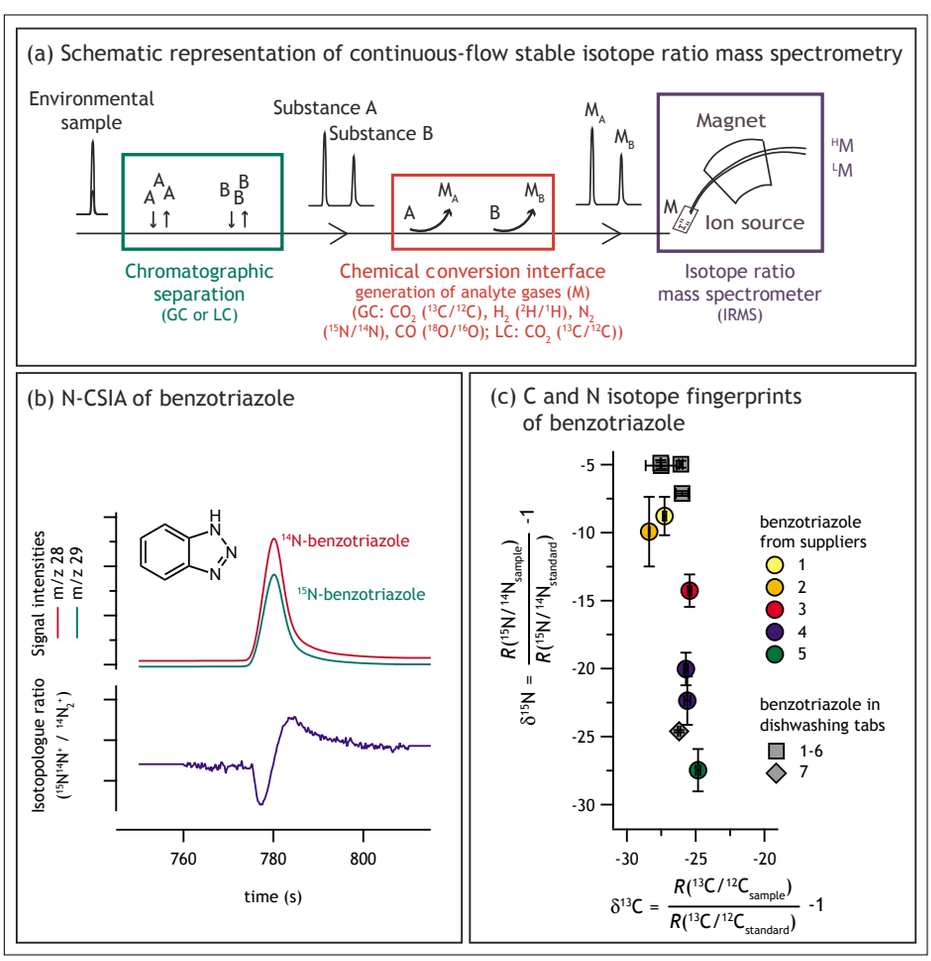

Fig. 1. (a) Schematic representation of continuous-flow isotope ratio mass spectrometry coupled to gas- or liquid-chromatography (GC/- or LC/IRMS, modified from Elsner *et al.*[7]). (b) Excerpt of a GC/IRMS chromatogram, in which heavy and light N-isotopologues of benzotriazole are measured. Note the slightly different chromatographic retention of the two N_2-isotopologues. (c) C and N isotope signatures of pure benzotriazole from different chemical manufacturers and of benzotriazole isolated from commercially available dishwashing taps. The mathematical definition of isotope signatures from isotope ratios is displayed on the x- and y-axes (data from Spahr *et al.*[10]).

Transformation Mechanisms and Their Isotope Effects

Stable isotope ratios in organic contaminants vary systematically as a consequence of chemical, biological or photochemical reactions.[17] The phenomenon of stable isotope fractionation originates largely from kinetic isotope effects at the reacting bonds (*KIE*, Eqn. (1)). *KIEs* result from different reaction rates of light (^{l}k) and heavy (^{h}k) isotopologues of a contaminant.

$$KIE = \frac{^{l}k}{^{h}k} \qquad (1)$$

The magnitude of kinetic isotope effects depends on physico-chemical entities such as changing bond energies and geometries that determine the energy difference between ground and transition state of a reaction.[18] Therefore, *KIEs* can be indicative of a reaction mechanism. The impact of *KIEs* on the isotopic composition of an organic compound is often 'diluted' by the presence of non-reactive atoms of the same element. The isotope fractionation measured in the bulk compound is referred to as isotope enrichment factor, ε. This parameter relates to a *KIE* as shown in Eqn. (2).

$$\varepsilon_E \approx \frac{1}{n} \cdot \left(\frac{1}{KIE} - 1 \right) \qquad (2)$$

where n is the number of atoms of element E. Note that Eqns (1) and (2) refer to the simplified situation where a molecule exhibits only one reactive position, the reaction is not concerted, and secondary KIEs are neglected. For a more detailed discussion of isotope fractionation and isotope effects, readers are referred to the literature.

The isotope fractionation observed during a degradation reaction is usually

larger than that induced by phase-transfer processes (*e.g.* volatilization, sorption) or dispersion.[19–21] Variations of isotope ratios are thus good evidence that contaminant disappearance is due to reaction rather than dilution or sorption. Finally, the extent of isotope fractionation is proportional to the fractional conversion of the contaminant, which – provided that the *KIEs* and ε-values of a reaction are known – enables one to derive the extent of degradation from the comparison of isotope ratios. This relationship is shown in Eqn. (3), which connects the measured changes of contaminant isotope signatures ($\Delta\,\delta^h E$) to ε and thus, indirectly to the *KIE* of a reaction.

$$\frac{c}{c_0} = \left(\frac{\delta^h E_0 + \Delta\delta^h E + 1}{\delta^h E_0 + 1} \right)^{1/\varepsilon_E} \qquad (3)$$

where c/c_0 is the fraction of remaining reactant, $\delta^h E_0$ is the initial isotope signature of element E in the compound of interest, and $\Delta\,\delta^h E$ is its change relative to the initial value.

Understanding stable isotope fractionation of organic contaminants serves two general purposes that are of practical relevance for assessing transformation processes. First, an interpretation of isotope fractionation in terms of *KIEs* enables one to identify the reaction mechanism and kinetics of a degradation process. Studying isotope fractionation associated with the degradation of emerging organic micropollutants is therefore a promising first step in deciphering unknown degradation routes. Second, knowledge of the bulk isotope enrichment factors for organic contaminants and their typical chemical and biological transformation processes can be applied to identify even combinations of degradation pathways in the environment. Especially when isotope fractionation of two or more elements is studied simultaneously, CSIA makes it possible to quantify the extent of reaction without the explicit need to measure contaminant concentrations. Over the last years, our group has pursued both avenues of research in CSIA for several compound classes and their relevant transformation processes. A short survey of some typical chemical, biological, and photochemical transformation processes of organic contaminants, their reaction mechanisms, and range of observed KIEs determined by our research group is compiled in Table 1. In the following chapters, we feature illustrative examples for the contaminant class of nitroaromatic compounds. Note that the principles of CSIA applied for this purpose also apply to most other contaminant classes and types of (bio)degradation processes.

Table 1. Survey of approximate ranges of ^{2}H-, ^{13}C-, ^{15}N-, and ^{37}Cl-KIEs of different chemical, biological, and photochemical transformation processes of organic contaminants determined in our work.

Transformation Type & Mechanisms	Reactive bond(s)	Isotope system	Range of observed KIEs[a]	Ref.
Reductions				
Aromatic NO_2-groups	(ar-C)–N–O	^{2}H/^{1}H ^{13}C/^{12}C ^{15}N/^{14}N	^{2}H-KIE: 1.010 ^{13}C-KIE: 1.001 ^{15}N-KIE: 1.03–1.04	[22–28]
Reductive dichloro-elimination of chlorohydrocarbons	$C(–Cl)_2$ Cl–C–C–Cl	^{13}C/^{12}C	^{13}C-KIE: 1.02–1.03 ^{37}Cl-KIE: 1.01	[5,29]
Hydrogenolysis of chlorohydrocarbons	C–Cl	^{13}C/^{12}C	^{13}C-KIE: 1.02–1.03	[5,30–32]
Oxidations				
Dioxygenation of aromatic ring	C(H)=C(H)	^{2}H/^{1}H ^{13}C/^{12}C ^{15}N/^{14}N	^{2}H-KIE: 0.94–1.03 ^{13}C-KIE: 1.01–1.03 ^{15}N-KIE: 1.001–1.002	[23,28, 33,34]
Mono-oxygenation of aromatic ring	ar-C–H	^{13}C/^{12}C ^{15}N/^{14}N	^{13}C-KIE: 1.001–1.011 ^{15}N-KIE: 1.001–1.002	[35]
Aromatic CH_3-group oxidation	ar-C–H	^{2}H/^{1}H ^{13}C/^{12}C ^{15}N/^{14}N	^{2}H-KIE: 1.04–4.6 ^{13}C-KIE: 1.01–1.03 ^{15}N-KIE: 1.001–1.002	[34,36]
Aromatic amine oxidation	ar-NH_2 ar-NR_1R_2	^{13}C/^{12}C ^{15}N/^{14}N	^{13}C-KIE: 1.001–1.02 ^{15}N-KIE: 0.987–1.000	[37,38]
Oxidative N-dealkylation	ar-NR-CH_3	^{2}H/^{1}H ^{13}C/^{12}C ^{15}N/^{14}N	^{2}H-KIE: 1.1–3.0 ^{13}C-KIE: 1.001–1.015 ^{15}N-KIE: 0.990–1.002	[39,40]
Eliminations				
Dehydrochlorination	H–C–C–Cl	^{13}C/^{12}C ^{37}Cl/^{35}Cl	^{13}C-KIE: 1.001 ^{37}Cl-KIE: 1.005	[29]
Photochemical transformations				
Photolysis of triazines[b]	triazines	^{2}H/^{1}H ^{13}C/^{12}C ^{15}N/^{14}N	^{2}H-KIE: 1.00 ^{13}C-KIE: 0.988 ^{15}N-KIE: 0.991	[40]

[a]Precision of reported KIE ranges depends on the reaction and element considered; [b]Magnetic isotope effects tentatively assigned as weighted average of all atoms in the triazine ring

Mechanisms and Isotope Effects of Nitroaromatic Compound Reduction and Oxidation

Nitroaromatic compounds (NACs) are toxic and frequent contaminants of soil and water due to their use as explosives, dyes, herbicides, and feedstock in chemical industry.[41–43] Because contamination of the subsurface occurred over decades, microbes have evolved that can use NACs as source of C, N, and energy. Biodegradation follows both reductive and oxidative pathways to generate intermediates that are utilized, for example, in energy metabolism. In addition to biological reactions, NACs can also be reduced by a large number of naturally occurring reductants such as Fe-bearing minerals (*e.g.* magnetite, Fe-containing phyllosilicates) and reduced quinone moieties in natural organic matter.

In our initial work on CSIA of organic contaminants, we found that the reduction of aromatic NO_2 groups is accompanied by substantial N isotope fractionation.[26,27] As shown in Fig. 2a for the reduction of a substituted nitrobenzene to the corresponding substituted aniline, ^{15}N/^{14}N ratios (denoted as N isotope signatures, δ^{15}N) increase strongly in the remaining reactant with reaction progress (*i.e.* $c/c_0 \rightarrow 0$). ^{13}C/^{12}C ratios, in contrast, vary only by a few per mil because the aromatic C atoms are not involved in the reduction reaction. While rates of reduction are very sensitive to type and position of aromatic substitution,[3] N isotope fractionation is not. Through a series of experimental and theoretical investigations,[22-27,44,45] we found that the ^{15}N-*KIE* of NAC reduction amounts to 1.03 to 1.04 (Table 1) regardless whether the reduction was carried out

by dissolved or mineral-bound reductants. The large ^{15}N-*KIE* implied that a bond to N is cleaved in the rate-limiting step. Density functional theory calculations revealed that indeed the transition state for the dehydration of the *N,N*-dihydroxy-aniline (compound **2** in Fig. 3a) to nitrosobenzene (**3**) was responsible for the substantial N isotope fractionation due to the rate-limiting cleavage of an N–O bond.[24,25] Electron- and proton transfer reactions preceding the bond cleavage are the reason for substituent effects on rates of NAC reduction (Fig. 3a, **1** → **2**). N isotope effects of electron and proton transfers are, however, negligible compared to the one associated with N–O bond cleavage.[25]

The detailed characterization of mechanisms and kinetics of NAC reduction suggested that there is an indicative correlation of C and N isotope fractionation for this reaction, which is independent of the reductant. The slopes of such trendlines were proportional to the ratio of primary ^{15}N-*KIEs* and secondary ^{13}C-*KIEs* of NAC reduction. Indeed, as shown in Fig. 2b, the combined C and N isotope fractionation for nitrobenzene reduction at the surface of an Fe(II) bearing mineral (blue line and markers) and by a microorganism (*Pseudomonas pseudoalcaligenes* strain JS45, green line and markers) exhibit identical slopes.[23–25,27] This observation perfectly follows the fact that isotope fractionation arises from the *KIEs* of a chemical elementary reaction step (*i.e.* N–O bond cleavage) regardless whether NAC reduction is enzyme- or mineral-catalyzed.

The so-called multi-element isotope fractionation analysis is a key application of CSIA for both elucidation of reaction mechanisms in the lab and for identifying reactive processes in the environment (see below). The correlation of isotope fractionation trends of multiple elements are a robust indicator for the occurring degradation processes even if a bond-cleavage step is not fully rate-limiting. Such a situation may be encountered in enzyme-catalyzed degradation reactions such as the dioxygenation of NACs to the corresponding catechols by nitroarene dioxygenases. Here, C isotope fractionation is due to a primary ^{13}C-*KIE* associated with the formation of a *cis*-dihydrodiol (**6**, Fig. 3b), whereas N isotope fractionation is only secondary, that is the N atom is not part of the reacting bond(s). Consequently, the C *vs.* N isotope fractionation trendline in Fig. 2b is almost orthogonal to that of reductive transformation. The dioxygenation reaction is the initial step of biodegradation of many persistent aromatic contaminants.[38,46,47] Prior to the addition of oxygen atoms and formation of the *cis*-dihydrodiol (**6**), substrate uptake and O_2-activation take place and can, in principle, be at least partly rate-limiting.[48] We are currently exploring the magnitude and origin of isotope fractionation of such reactions with an investigation of NAC dioxygenation in different biological model systems ranging from pure cultures of wild-type bacteria capable of biodegrading NACs to pure enzymes of Rieske non-heme Fe dioxygenases.[33] Our most recent results show that ^{13}C-*KIEs* for dioxygenations of nitroarenes can be as high as 1.024 (*i.e.* for reaction (iii) in Fig. 3b).[49]

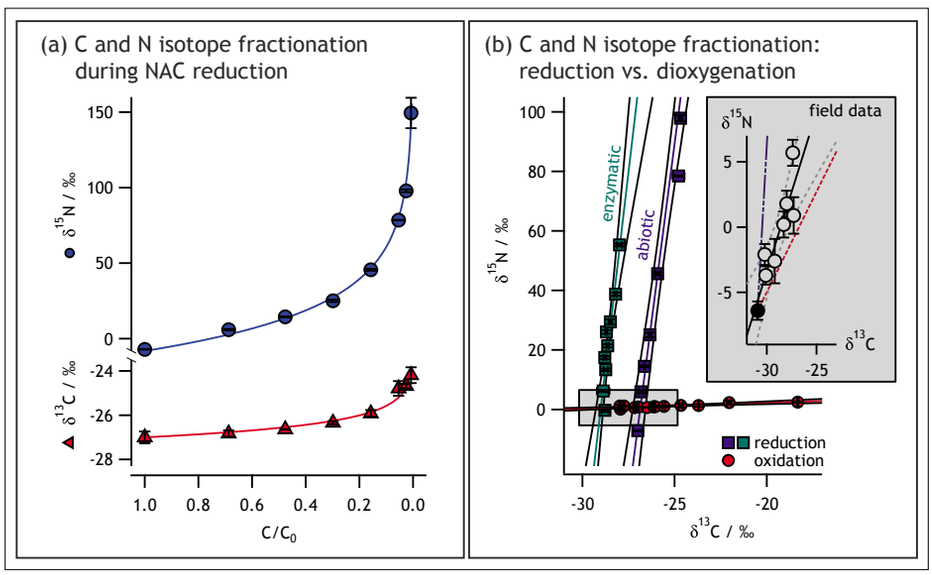

Fig. 2. (a) C and N isotope enrichment associated with the reduction of a substituted nitrobenzene to the corresponding substituted aniline.[27] The reaction scheme is shown in Fig. 3a. (b) Correlation of C and N isotope fractionation during reductive and oxidative NAC transformation.[23] The inset shows the corresponding data for 2,4-dinitrotoluene in soil samples from a contaminated site (data from Wijker *et al.*[28]). The blue and red lines denote the expected C and N isotope fractionation if the transformation occurred purely by reduction and dioxygenation, respectively.

Using Multi-Element Isotope Fractionation Trends to Assess Contaminant Degradation in the Field

Insights obtained from the study of isotope fractionation and magnitude of *KIEs* for multiple elements offer promising perspectives for assessing organic contaminant transformation in the field. In many cases natural attenuation processes take place over time periods of decades thus making it impractical to monitor concen-

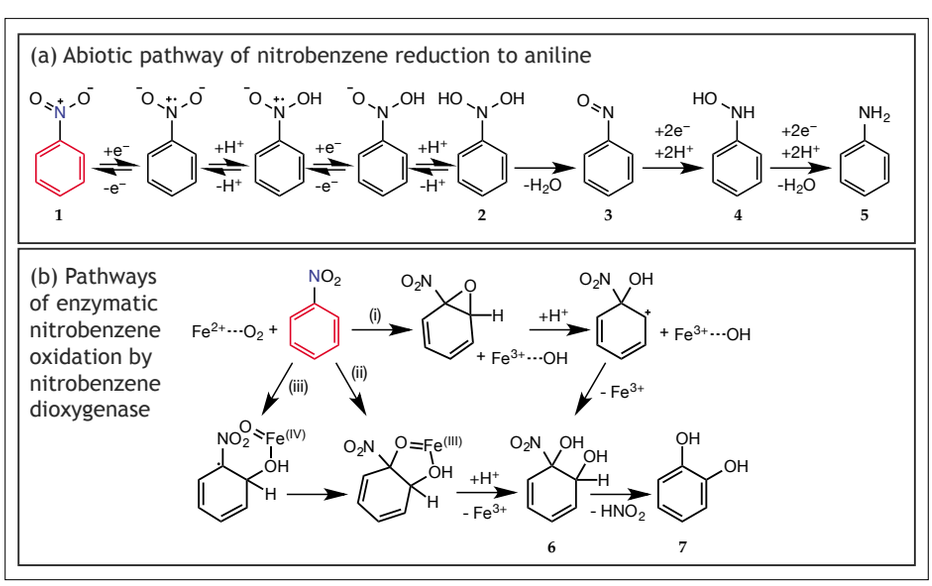

Fig. 3. (a) Reaction schemes of nitrobenzene (**1**) reduction to aniline (**5**) involving the transfer of 6 e^- and 6 H^+.[25] Rate-limiting step of the reduction is the dehydration of *N,N*-dihydroxyaniline (**2**) to nitrosobenzene (**3**). (b) Three possible pathways of enzymatic nitrobenzene dioxygenation to catechol through concerted and stepwise addition of dioxygen.[33]

tration changes systematically. An application of CSIA in the environment is shown in the inset of Fig. 2b. C and N isotope signatures of 2,4-dinitrotoluene measured in a soil profile at a field site, where contamination likely occurred over more than 50 years, show a correlation of C and N isotope fractionation.[28] This trend was interpreted as linear combination of oxidative and reductive transformation processes. Based on the *KIEs* determined for the reactions of 2,4-DNT in laboratory model systems, we proposed that more than 85% of contaminant disappearance was due to dioxygenation, a reaction that initiates mineralization of the contaminant. Many similar applications have been made in the recent years, especially for subsurface contamination with chlorohydrocarbons and fuel components and additives.[50] These examples illustrate the benefits of CSIA, in particular for quantifying natural attenuation processes over timescales of years to decades. Advances in analytical capabilities as well as the understanding of kinetic and equilibrium isotope effects of organic contaminant transformations warrants further development of CSIA for a more widespread application to organic micropollutants and their transformation processes in the environment.

Received: August 27, 2014

[1] R. P. Schwarzenbach, T. Egli, T. B. Hofstetter, U. von Gunten, B. Wehrli, *Annu. Rev. Environ. Resour.* **2010**, *35*, 109.

[2] R. P. Schwarzenbach, B. I. Escher, K. Fenner, T. B. Hofstetter, C. A. Johnson, U. von Gunten, B. Wehrli, *Science* **2006**, *313*, 1072.

[3] R. P. Schwarzenbach, P. M. Gschwend, D. M. Imboden, 'Environmental Organic Chemistry', John Wiley & Sons, New York, **2003**.

[4] T. B. Hofstetter, M. Berg, *TrAC Trends Anal. Chem.* **2011**, *30*, 618.

[5] M. Elsner, T. B. Hofstetter, in 'Aquatic Redox Chemistry', Eds. American Chemical Society, **2011**, 407.

[6] A. L. Sessions, *J. Sep. Sci.* **2006**, *29*, 1946.

[7] M. Elsner, M. A. Jochmann, T. B. Hofstetter, D. Hunkeler, A. Bernstein, T. C. Schmidt, A. Schimmelmann, *Anal. Bioanal. Chem.* **2012**, *403*, 2471.

[8] J. M. Eiler, B. Bergquist, I. Bourg, P. Cartigny, J. Farquhar, A. Gagnon, W. F. Guo, I. Halevy, A. Hofmann, T. E. Larson, N. Levin, E. A. Schauble, D. Stolper, *Chem. Geol.* **2014**, *372*, 119.

[9] D. A. Stolper, A. L. Sessions, A. A. Ferreira, E. V. Santos Neto, A. Schimmelmann, S. S. Shusta, D. L. Valentine, J. M. Eiler, *Geochim. Cosmochim. Acta* **2014**, *126*, 169.

[10] S. Spahr, S. Huntscha, J. Bolotin, M. P. Maier, M. Elsner, J. Hollender, T. B. Hofstetter, *Anal. Bioanal. Chem.* **2013**, *405*, 2843.

[11] M. Skarpeli-Liati, A. Turgeon, A. N. Garr, W. A. Arnold, C. J. Cramer, T. B. Hofstetter, *Anal. Chem.* **2011**, *83*, 1641.

[12] M. Berg, J. Bolotin, T. B. Hofstetter, *Anal. Chem.* **2007**, *79*, 2386.

[13] S. Huntscha, T. B. Hofstetter, E. L. Schymanski, S. Spahr, J. Hollender, *Environ. Sci. Technol.* **2014**, *48*, 4435.

[14] J. Hollender, S. G. Zimmermann, S. Koepke, M. Krauss, C. S. McArdell, C. Ort, H. Singer, U. von Gunten, H. Siegrist, *Environ. Sci. Technol.* **2009**, *43*, 7862.

[15] W. Giger, C. Schaffner, H. P. E. Kohler, *Environ. Sci. Technol.* **2006**, *40*, 7186.

[16] M. A. Jochmann, T. C. Schmidt, 'Compound-specific Stable Isotope Analysis', The Royal Society of Chemistry, **2012**.

[17] L. Melander, W. H. Saunders, 'Reaction Rates of Isotopic Molecules', John Wiley & Sons, New York, **1980**.

[18] M. Wolfsberg, A. van Hook, P. Paneth, L. P. N. Rebelo, 'Isotope Effects in Chemical, Geological, and Bio Sciences', Springer, Heidelberg, **2010**.

[19] D. Eckert, S. Qiu, M. Elsner, O. A. Cirpka, *Environ. Sci. Technol.* **2013**, *47*, 6900.

[20] D. Eckert, M. Rolle, O. A. Cirpka, *J. Contam. Hydrol.* **2012**, *140*, 95.

[21] S. Qiu, D. Eckert, O. A. Cirpka, M. Huenniger, P. Knappett, P. Maloszewski, R. U. Meckenstock, C. Griebler, M. Elsner, *Environ. Sci. Technol.* **2013**, *47*, 6892.

[22] C. A. Gorski, J. T. Nurmi, P. G. Tratnyek, T. B. Hofstetter, M. M. Scherer, *Environ. Sci. Technol.* **2010**, *44*, 55.

[23] T. B. Hofstetter, J. C. Spain, S. F. Nishino, J. Bolotin, R. P. Schwarzenbach, *Environ. Sci. Technol.* **2008**, *42*, 4764.

[24] T. B. Hofstetter, A. Neumann, W. A. Arnold, A. E. Hartenbach, J. Bolotin, C. J. Cramer, R. P. Schwarzenbach, *Environ. Sci. Technol.* **2008**, *42*, 1997.

[25] A. E. Hartenbach, T. B. Hofstetter, M. Aeschbacher, M. Sander, D. Kim, T. J. Strathmann, W. A. Arnold, C. J. Cramer, R. P. Schwarzenbach, *Environ. Sci. Technol.* **2008**, *42*, 8352.

[26] N. B. Tobler, T. B. Hofstetter, R. P. Schwarzenbach, *Environ. Sci. Technol.* **2007**, *41*, 7773.

[27] A. Hartenbach, T. B. Hofstetter, M. Berg, J. Bolotin, R. P. Schwarzenbach, *Environ. Sci. Technol.* **2006**, *40*, 7710.

[28] R. S. Wijker, J. Bolotin, S. F. Nishino, J. C. Spain, T. B. Hofstetter, *Environ. Sci. Technol.* **2013**, *47*, 6872.

[29] T. B. Hofstetter, C. M. Reddy, L. J. Heraty, M. Berg, N. C. Sturchio, *Environ. Sci. Technol.* **2007**, *41*, 4662.

[30] C. Aeppli, M. Berg, O. A. Cirpka, C. Holliger, R. P. Schwarzenbach, T. B. Hofstetter, *Environ. Sci. Technol.* **2009**, *43*, 8813.

[31] C. Aeppli, T. B. Hofstetter, H. I. F. Amaral, R. Kipfer, R. P. Schwarzenbach, M. Berg, *Environ. Sci. Technol.* **2010**, *44*, 3705.

[32] A. Neumann, T. B. Hofstetter, M. Skarpeli-Liati, R. P. Schwarzenbach, *Environ. Sci. Technol.* **2009**, *43*, 4082.

[33] S. G. Pati, H.-P. E. Kohler, J. Bolotin, R. E. Parales, T. B. Hofstetter, *Environ. Sci. Technol.* **2014**, *33*, 10750.

[34] R. S. Wijker, P. Adamczyk, J. Bolotin, P. Paneth, T. B. Hofstetter, *Environ. Sci. Technol.* **2013**, *47*, 13459.

[35] R. S. Wijker, Z. Kurt, J. C. Spain, J. Bolotin, J. Zeyer, T. B. Hofstetter, *Environ. Sci. Technol.* **2013**, *47*, 14185.

[36] N. B. Tobler, T. B. Hofstetter, R. P. Schwarzenbach, *Environ. Sci. Technol.* **2008**, *42*, 7786.

[37] M. Skarpeli-Liati, M. Jiskra, A. Turgeon, A. N. Garr, W. A. Arnold, C. J. Cramer, R. P. Schwarzenbach, T. B. Hofstetter, *Environ. Sci. Technol.* **2011**, *45*, 5596.

[38] S. G. Pati, K. Shin, M. Skarpeli-Liati, J. Bolotin, S. N. Eustis, J. C. Spain, T. B. Hofstetter, *Environ. Sci. Technol.* **2012**, *46*, 11844.

[39] M. Skarpeli-Liati, S. G. Pati, J. Bolotin, S. N. Eustis, T. B. Hofstetter, *Environ. Sci. Technol.* **2012**, *46*, 7189.

[40] A. E. Hartenbach, T. B. Hofstetter, P. R. Tentscher, S. Canonica, M. Berg, R. P. Schwarzenbach, *Environ. Sci. Technol.* **2008**, *42*, 7751.

[41] J. C. Spain, J. B. Hughes, H.-J. Knackmuss, 'Biodegradation of Nitroaromatic Compounds and Explosives', Lewis Publishers, Boca Raton, **2000**.

[42] D. Kalderis, A. L. Juhasz, R. Boopathy, S. Comfort, *Pure Appl. Chem.* **2011**, *83*, 1407.

[43] K. S. Ju, R. E. Parales, *Microbiol. Mol. Biol. Rev.* **2010**, *74*, 250.

[44] T. B. Hofstetter, C. G. Heijman, S. B. Haderlein, C. Holliger, R. P. Schwarzenbach, *Environ. Sci. Technol.* **1999**, *33*, 1479.

[45] A. Neumann, T. B. Hofstetter, M. Lüssi, O. A. Cirpka, S. Petit, R. P. Schwarzenbach, *Environ. Sci. Technol.* **2008**, *42*, 8381.

[46] D. T. Gibson, R. E. Parales, *Curr. Opin. Biotech.* **2000**, *11*, 236.

[47] D. R. Boyd, T. D. H. Bugg, *Org. Biomol. Chem.* **2006**, *4*, 181.

[48] T. D. H. Bugg, S. Ramaswamy, *Curr. Opin. Chem. Biol.* **2008**, *12*, 134.

[49] A. Pabis, I. Geronimo, P. Paneth, *J. Phys. Chem. B* **2014**, *118*, 3245.

[50] M. Thullner, F. Centler, H.-H. Richnow, A. Fischer, *Org. Geochem.* **2012**, *42*, 1440.