



## **Identifying the concurrent oxidation of toluene and reduction of nitroaromatic contaminants in anoxic environments using compound-specific carbon and nitrogen isotope analysis**

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In anoxic environments, oxidation of organic contaminants such as fuel components by dissimilatory Fe(III) reduction can generate mineral-bound Fe(II) species, which in turn are able to reduce major groundwater pollutants such as chlorinated solvents, nitroaromatic explosives, pesticides or dyes. While there are numerous studies on either dissimilatory Fe(III) reduction or reductive contaminant transformation by mineral-bound Fe(II) species, little is yet known about the coupling of the two processes. Results from our studies in laboratory model systems show that complete oxidation of toluene to CO<sub>2</sub> by *Geobacter metallireducens* in the presence of different Fe(III) bearing minerals leads to the formation of reactive, mineral-bound Fe(II) species capable of reducing nitroaromatic compounds (NACs). This coupling of oxidative and reductive contaminant transformation would therefore allow the concurrent natural attenuation of different pollutant classes and may be ideally suited for sites with multiple contaminations.

An evaluation of this coupled process can only be achieved by analysis of all involved chemical species via labor-intensive mass- and electron-balances, which are feasible in laboratory systems, but not in field settings. In addition, physical processes such as sorption or evaporation often affect pollutant concentrations at contaminated sites, rendering the identification and quantification of concurrent oxidative and reductive transformation impossible. Compound-specific isotope analysis (CSIA), however, offers the possibility to detect such transformation processes even in very complex matrices. It was therefore the goal of this study (i) to determine the magnitude of car-

bon and nitrogen isotope fractionation by the coupled contaminant transformation of toluene and NACs, respectively, and (ii) to verify the applicability of this approach by analyzing the variability of isotope enrichment factors under different experimental conditions.

In a first step, microbial toluene oxidation and abiotic reduction of nitroaromatic compounds (NACs) by mineral-bound Fe(II) species were studied in isolated experimental systems. Carbon isotope enrichment factors for toluene oxidation by *Geobacter metallireducens* with an amorphous Fe(III) mineral as electron acceptor were in the range of -1.2‰ to -1.5‰, corresponding with an apparent carbon kinetic isotope effect ( $AKIE_C$ ) of  $1.010 \pm 0.002$ . These results are the first carbon enrichment factors for toluene oxidation coupled to the reduction of a *solid* Fe(III) phase, and the values are in good agreement with previous results for toluene oxidation by reduction of Fe(III) citrate (1). Reduction of 3-chloronitrobenzene, a model compound for nitroaromatic contaminants, by goethite-bound Fe(II) species yielded nitrogen  $AKIE_{NS}$  of  $1.041 \pm 0.002$  for pHs between 6.8 and 7.5 and different mineral-bound Fe(II) concentrations. These  $AKIE_{NS}$  are significantly higher than values published so far for reductive cleavage of aromatic N-O bonds in abiotic reactions (2). Finally,  $AKIE$ s for toluene oxidation and 3-chloronitrobenzene reduction in a *coupled* experiment with amorphous iron oxide as electron acceptor and goethite as sorbent for microbially generated Fe(II) were almost identical as in experiments of the isolated processes. Therefore, our data suggest that CSIA may be used for the detection and quantification of coupled oxidative and reductive pollutant transformation, albeit further investigations using different Fe(III) phases, broader pH conditions as well as other contaminants are necessary to test the robustness of determined  $AKIE$ s.

(1) Meckenstock, R. U.; Morasch, B.; Warthmann, R.; Schink, B.; Annweiler, E.; Michaelis, W.; Richnow, H. H. *Environmental Microbiology* **1999**, 1, 409-414

(2) Hartenbach, A.; Hofstetter, T. B.; Berg, M.; Bolotin, J.; Schwarzenbach, R. P. *Environ. Sci. Technol.* **2006**, 40, 7710-7716