***In situ* microcosms and compound-specific stable isotope analysis (CSIA) for evaluating biodegradation of ethyl *tert*-butyl ether (ETBE) at a fuel-contaminated field site**

Petra Bombach1,2, Norbert Nägele3, Mònica Rosell1,4, Hans H. Richnow1, Anko Fischer2

1 Helmholtz Centre for Environmental Research - UFZ, Department of Isotope Biogeochemistry, Permoserstrasse 15, 04318 Leipzig, Germany, hans.richnow@ufz.de

2 Isodetect GmbH, Deutscher Platz 5b, 04103 Leipzig, Germany, petra.bomach@isodetect.de, fischer@isodetect.de

3 Kuvier the Biotech Company S.L., Ctra. N-I, p.k. 234 – P.E. INBISA 23ª, 09001 Burgos, Spain, Norbertnagele@kepler.es

4 Current address: Grup de Mineralogia Aplicada i Geoquímica de Fluids, Departament de Cristal lografia, Mineralogia i Dipòsits Minerals, Facultat de Geologia, Universitat de Barcelona (UB), C/Martí i Franquès s/n, 08028 Barcelona, Spain, monica.rosell@ub.edu

Ethyl *tert*-butyl ether (ETBE) is an increasingly used fuel additive in Europe. In contrast to methyl *tert*-butyl ether (MTBE), it can be produced from isobutene and bioethanol which is accounted as a biofuel and is partly promoted by tax incentives. As a result, ETBE is is frequently detected in soil, surface and groundwater. Due to its low odor and flavor thresholds in water (1-2 µg L-1), there is a risk that ETBE makes drinking water resources easily unpalatable. Up to now, limited knowledge exists on sinks of ETBE in the environment. In general, biodegradation of ETBE has been demonstrated by few microorganisms. However, the role of *in situ* biodegradation of ETBE at contaminated field sites has only scarcely been investigated. In order to increase knowledge on the fate of ETBE after an contamination event, biodegradation of ETBE at a fuel-contaminated field site was assessed using: i) compound-specific stable isotope analysis (CSIA) and ii) *in situ* microcosms in combination with total lipid fatty acid (TLFA)-stable isotope probing (SIP).

CSIA provides an appropriate tool to assess *in situ* degradation of contaminants both qualitatively and quantitatively (Meckenstock et al. 2004, Thullner et al. 2010). It is based on the principle that molecules with heavier isotopes in their reactive position(s) (e.g., 13C, 2H) are generally slower degraded than those with lighter isotopes (e.g., 12C, 1H) leading to a shift in the isotope ratio (e.g., 13C/12C, 2H/1H) as the remaining contaminant fractions becomes progressively enriched in heavier isotopes (e.g., 13C, 2H). Within the contaminated aquifer of the investigated field site, CSIA exhibited insignificant changes in carbon isotope ratios (13C/12C) but low hydrogen isotope fractionation (up to +14 ‰ difference in 2H/1H ratios) of ETBE, indicating biodegradation of ETBE along the prevailing anoxic contaminant plume.

In order to stimulate the biodegradation of petroleum hydrocarbons (PH) at the field site, oxygen was injected ten months after the CSIA monitoring campaign. During this remediation measure, the biodegradation of ETBE was assessed using *in situ* microcosms (BACTRAP®s) in combination with TLFA-SIP (for review see Bombach et al. 2010 and for more details about this study Bombach et al. 2015). BACTRAP®s loaded with 13C-labelled ETBE were exposed for 119 days in selected groundwater wells with the following conditions: (i) ETBE as main contaminant; (ii) ETBE as main contaminant subjected to oxygen injection; (iii) ETBE plus other petroleum hydrocarbons (PH); (iv) ETBE plus other PH subjected to oxygen injection. Under all conditions, fatty acids extracted from BACTRAP®s were significantly enriched in 13C, providing clear evidence of ETBE biodegradation at the field site. Since oxic and anoxic conditions were present at the wells of the BACTRAP-study, aerobic and anaerobic degradation of ETBE could be expected at the field site.

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