Monitoring of in situ biodegradation of groundwater contaminants using a test system (BACTRAP) with ^{13}C-labelled substrates

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Department of Isotope Biogeochemistry in Leipzig

Assessment of Natural Attenuation as remediation strategy needs the proof of

in situ

Biodegradation

<u>Natural Attenuation</u> processes lead to a removal of contaminants by

Dilution, Sorption, Evaporation, *Biodegradation, …*

- conventional test-systems:
- counting of cell-numbers
- lab-scale microcosm-studies
- metabolites + usage of electron acceptors

- *isotope fractionation*
- *reactive tracer experiments*

disadvantages:

- „*ex-situ*“
- *in-situ* activity unknown
- indirect proof
- time consuming
- cultivability

solution:

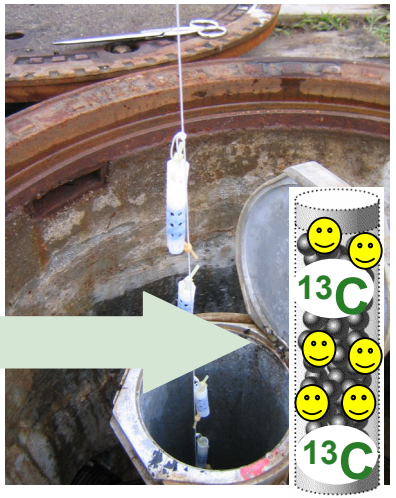
In situ microcosms (BACTRAP) to provide evidence of biodegradation at the field with isotopically labelled tracer substances!

BACTRAP - Concept

1. Adsorption-material + isotopically labelled compounds (e.g. ^{13}C)

BACTRAP + ^{13}C-substrate

2. Exposure in the aquifer (6-8 weeks)



3. Incorporation of the ^{13}C-label in the biomass:

- lipids: fatty acids
- proteins: amino acids
- DNA / RNA
- amino sugars

4. Extraction

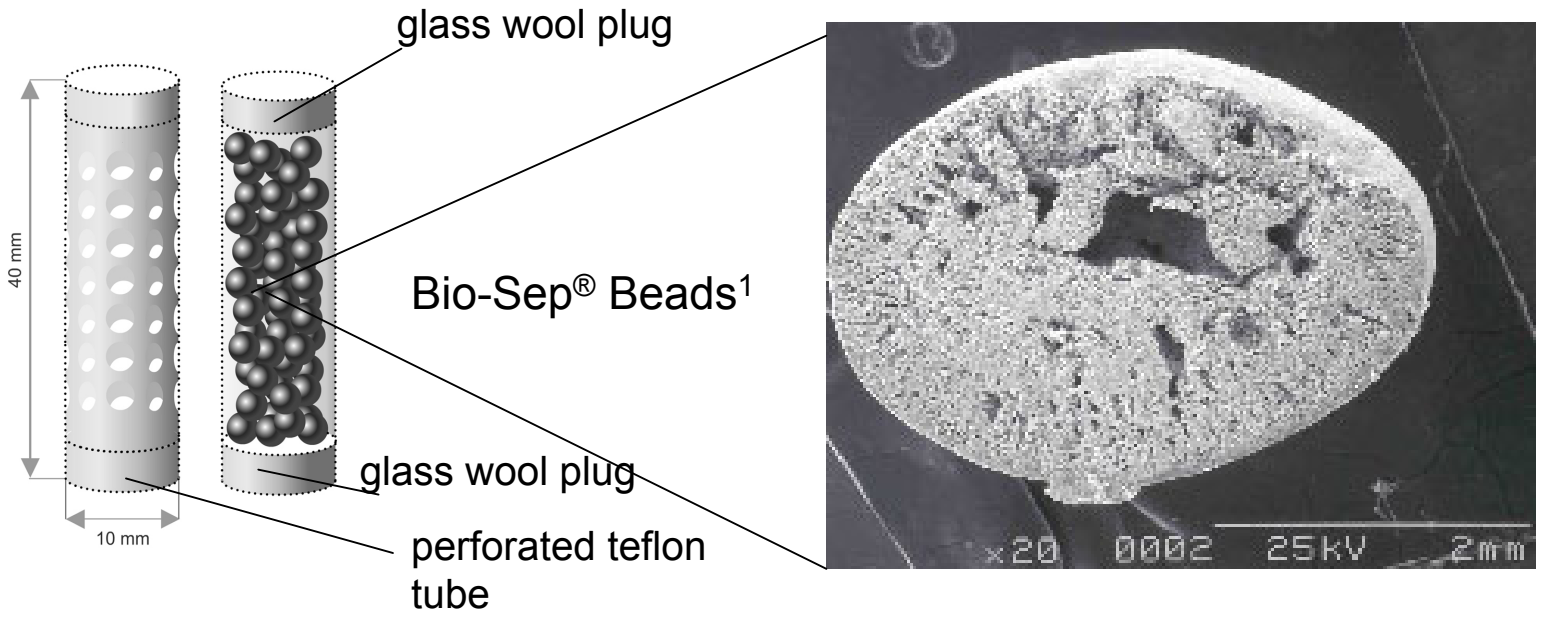
^{13}C in biomarker & ^{13}C in metabolites (activity & process identification)

GC-MS, GC-C-IRMS, EA-IRMS

Outlook: molecular biology RNA / DNA (structure and function)

SIP, PCR, SSCP, DGGE

In situ microcosms (BACTRAP)

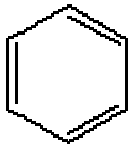


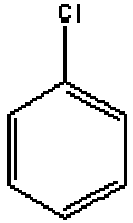
- **∅ 2-3 mm**
- **internal surface 600 m^2 g^{-1}**
- **bulk density 0,16 g cm^{-3}**
- **powdered activated carbon (75 %) in a NOMEX matrix (25 %)**
- **with outer pores < 5 µm**

[1]BIO-SEP® Beads: kindly provided by Kerry L. Sublette, University of Tulsa (USA)

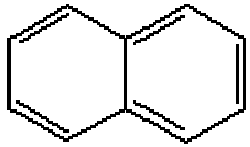
White et al. (2003), Environmental Forensics 4:63-74
Peacock et al. (2004), J. Microbial Ecology 47(3): 284-292

Which contaminants we can test?

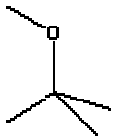


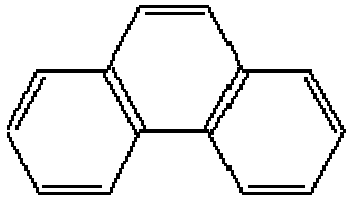


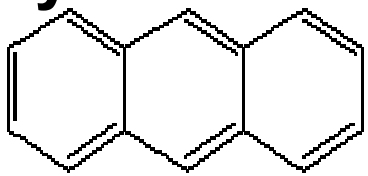
„You are what you eat!“



Proof of biodegradation is only possible if the microorganisms use the ^{13}C-labelled substrate as their carbon source for biosynthesis

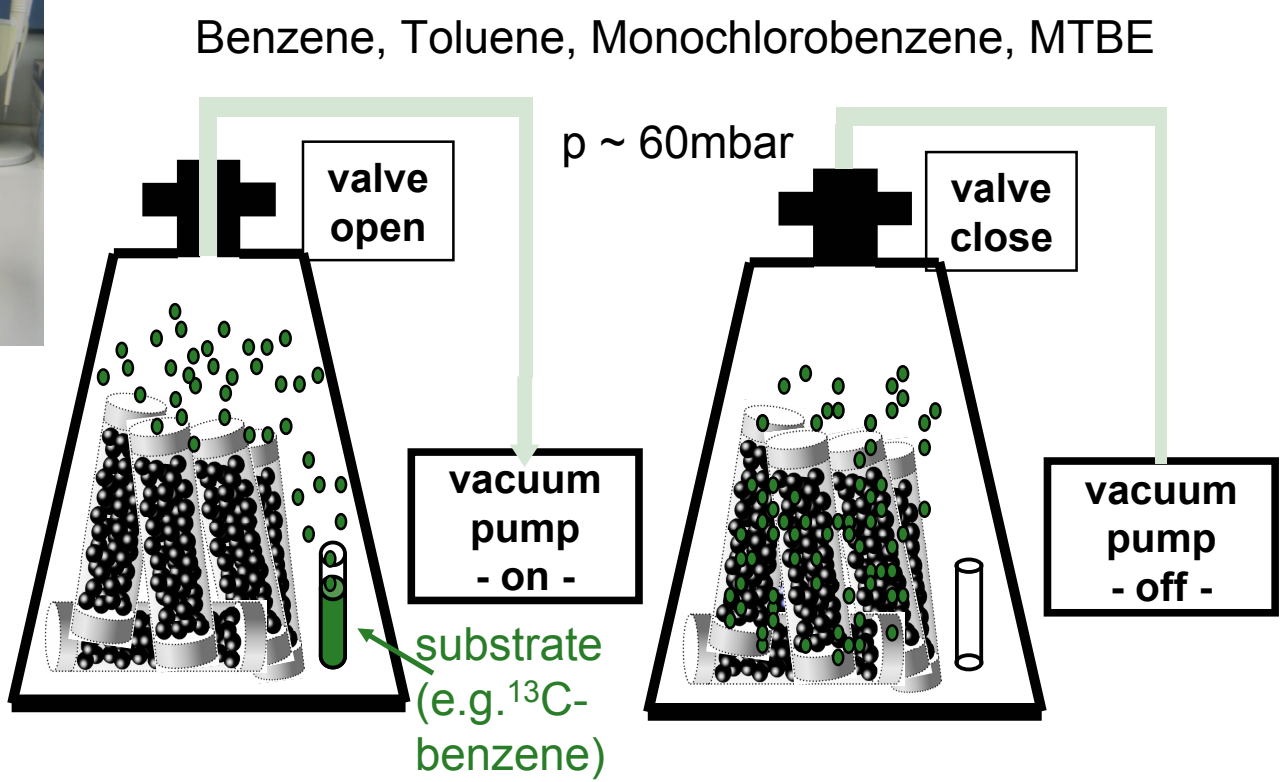






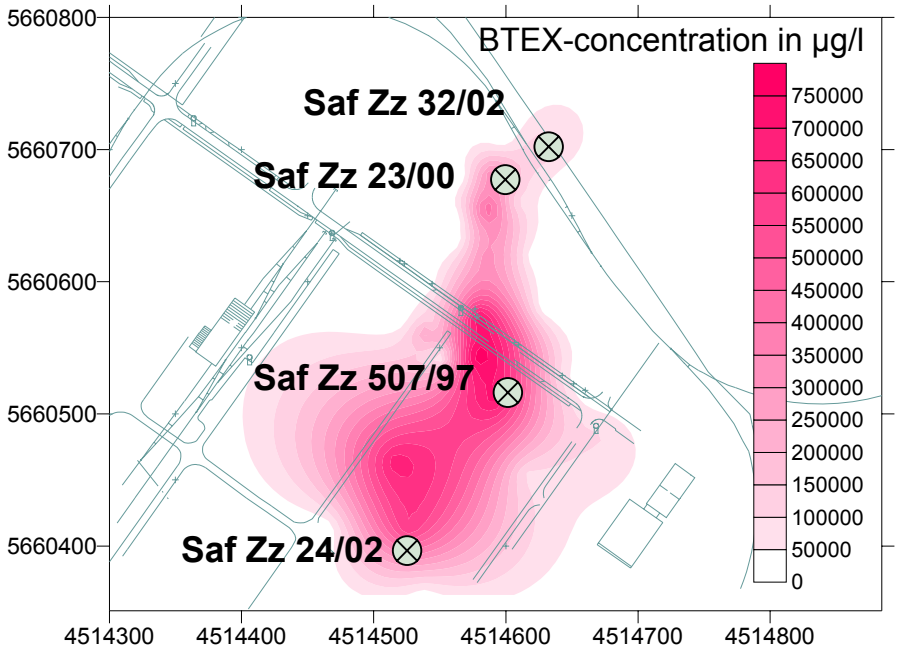
Outlook: usage of ^{15}N-labelled substrates

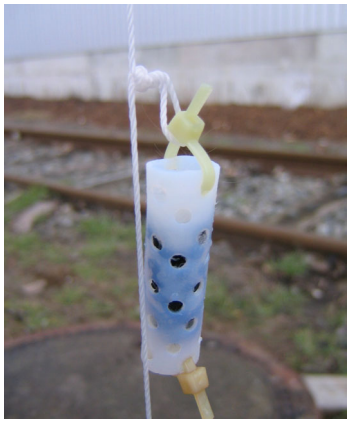
Reproducible loading of BIO-SEP® Beads via gas phase

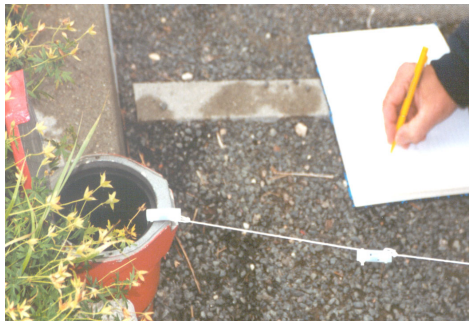


Geyer et al. (2005), ES&T 39: 4983-4989

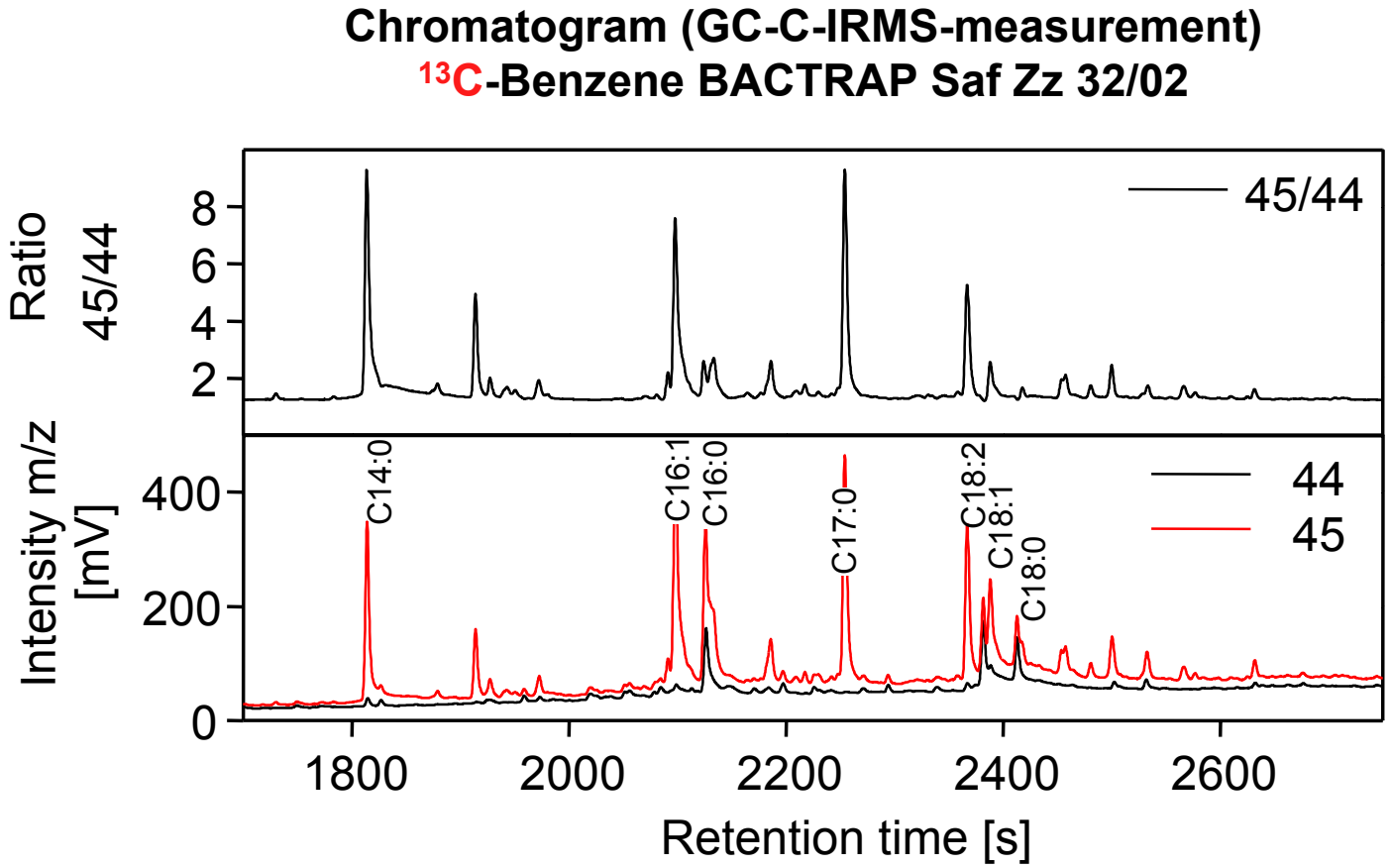
Application of BACTRAPs in field experiments







Application of BACTRAPs in field experiments

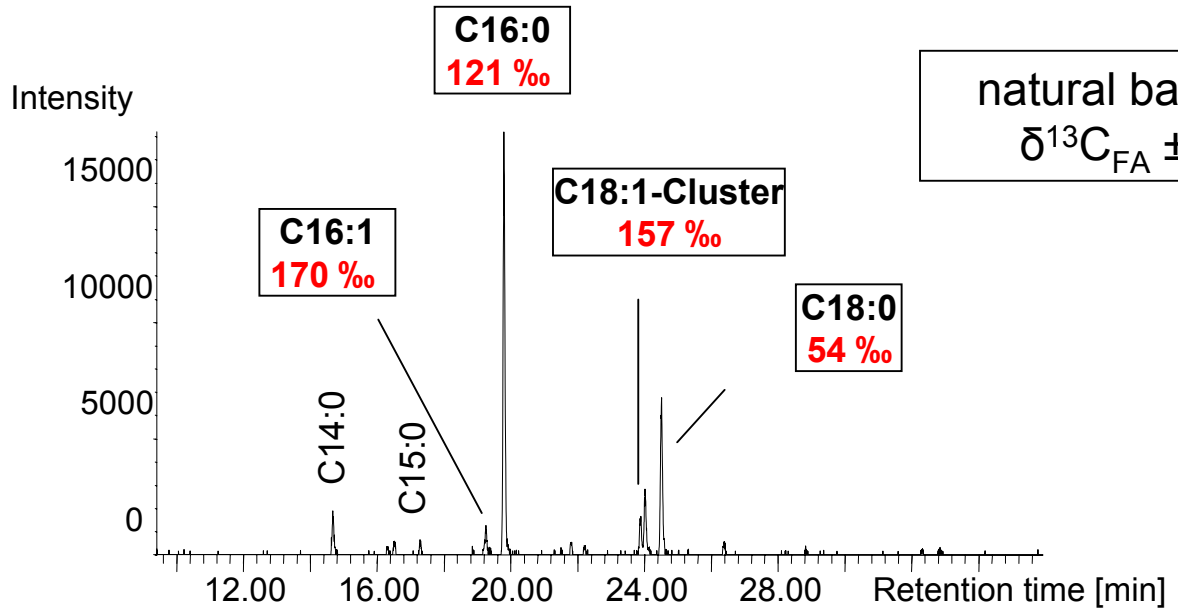


Incorporation of the ^{13}C-label into biomass proves biodegradation!!!

Application of BACTRAPs in field experiments

Chromatogram GC-MS Measurement & $\delta^{13}C$-signature of fatty acids

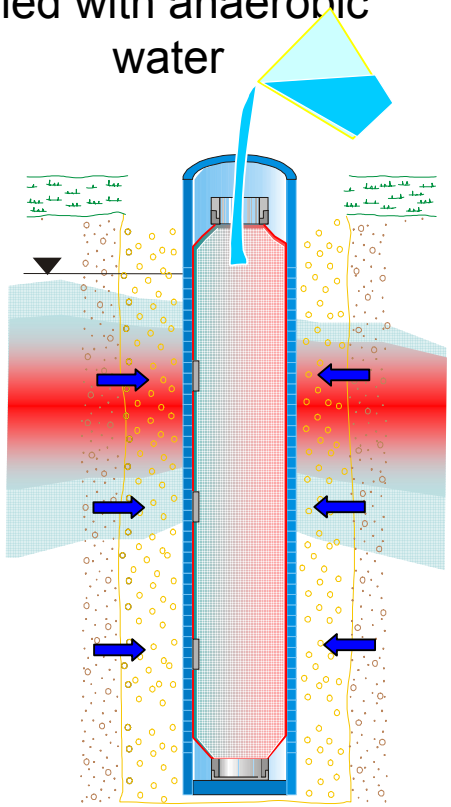
^{13}C-Benzene BACTRAP Saf Zz 507/97

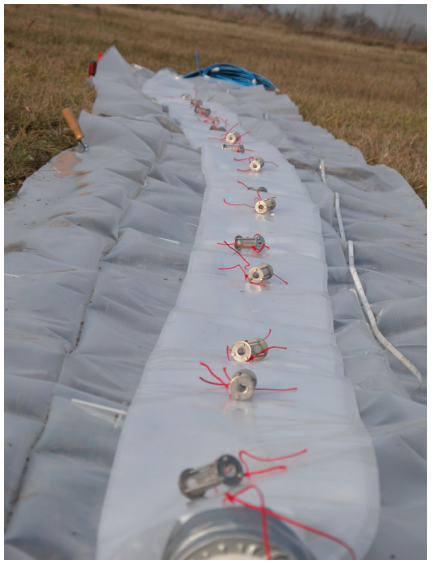


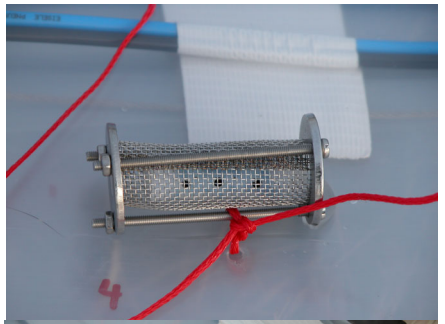
Incorporation of the ^{13}C-label into biomass proves biodegradation!!!

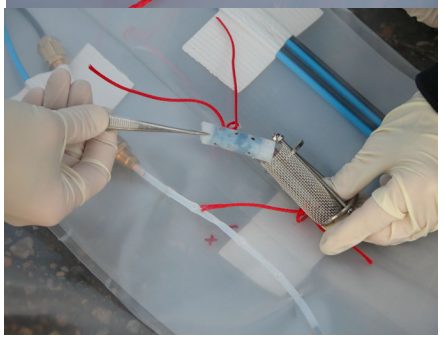
Exposure and sampling of BACTRAPs in Multilevel sampling systems

Multiport sock sampler filled with anaerobic water





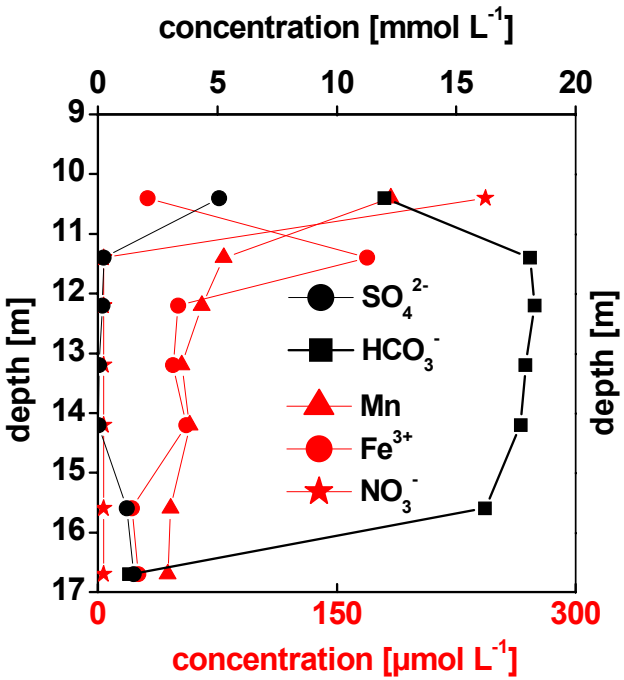




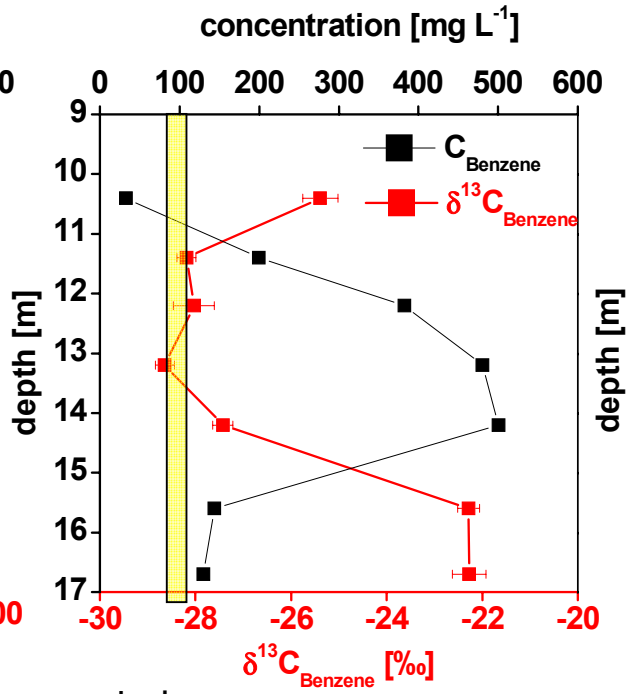
Schirmer et al. (1995), J. Hydrology 171(3-4)

BACTRAPs in Multilevel systems: Results

geochemical conditions

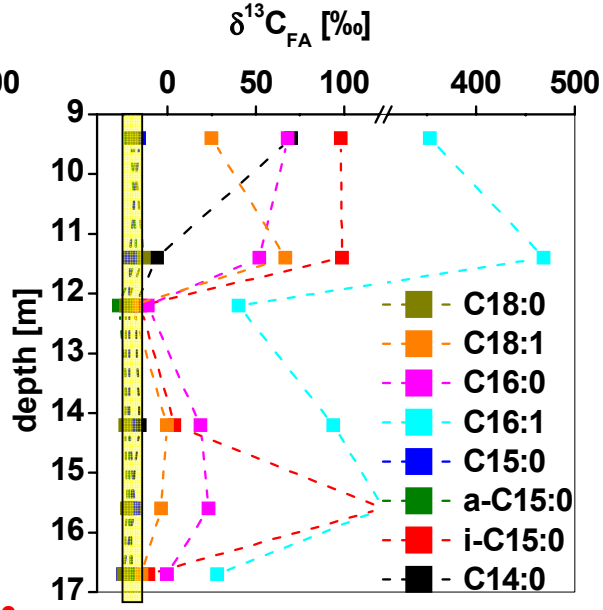


stable isotope fractionation



isotope signature of the source

BACTRAP



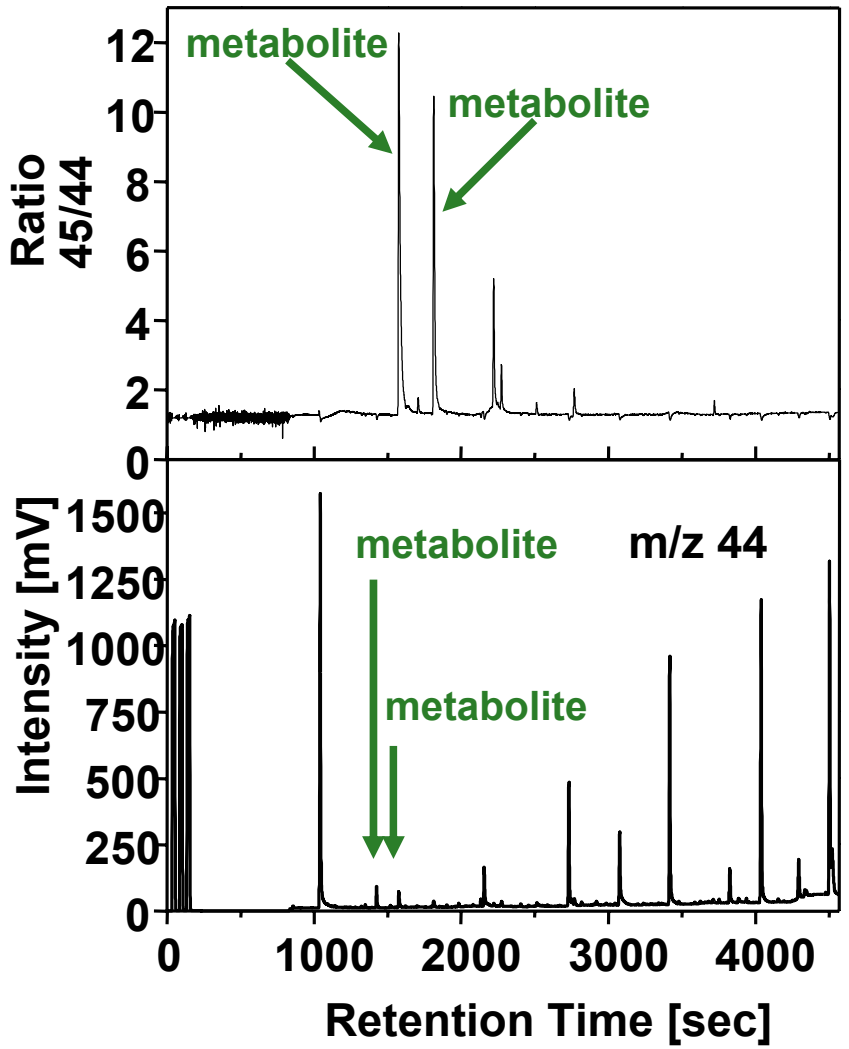
natural variability

Reconstruction of metabolic pathways (I)

Isotope enrichment = (GC-IRMS)

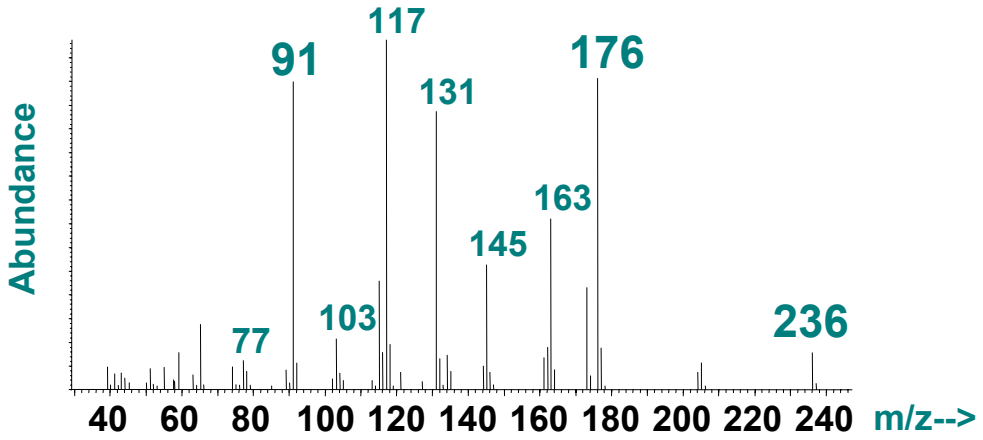
Screening of metabolites for the reconstruction of metabolic pathways applying compound-specific stable isotope analysis (CSIA) techniques

Concentration = (GC-IRMS)

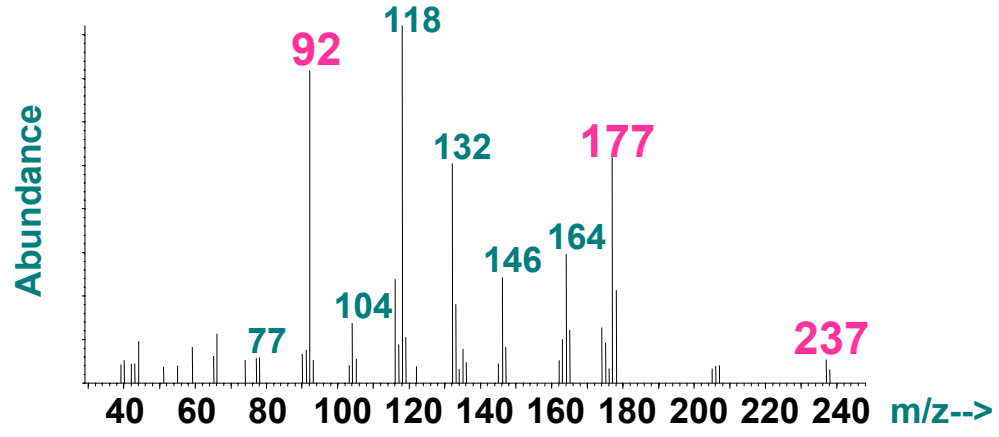


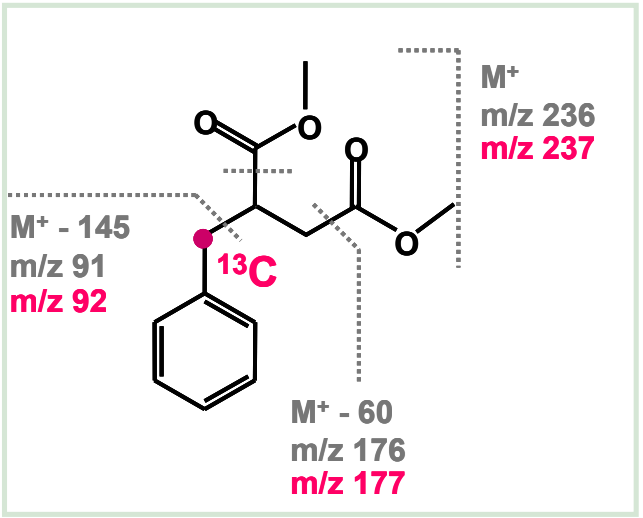
Reconstruction of metabolic pathways (II)

Benzylsuccinate after 92d of incubation with ^{12}C-toluene

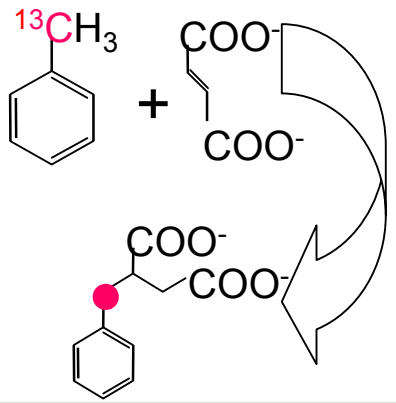


Benzylsuccinate after 92d of incubation with [^{13}C- methyl]-toluene





Benzylsuccinate pathway



Conclusion & Perspectives

- development of a test system to prove in situ biodegradation
- identification of in situ metabolic pathways
- provide a “line of evidence” for the implementation of Natural Attenuation strategies

- **Outlook / Vision :**
- Quantification of degradation processes
- Analysis of the structure and function of the in situ microbial communities (SIP)

Acknowledgment

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FAL - Braunschweig

Innovative Messtechnik Weiß

DMT Deutsche Montan Technologie (Essen)

Thank you for your attention!

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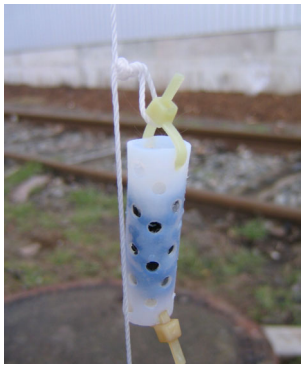
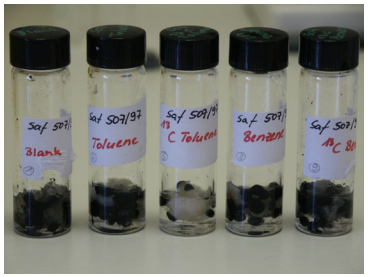



Saf 507
Blank
Saf 507/97
Toluene
Saf 507/
C Toluene
Saf 507
Benzene

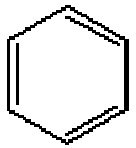

23100

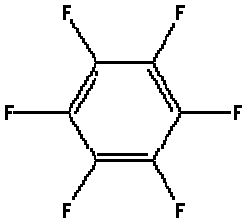

MF1

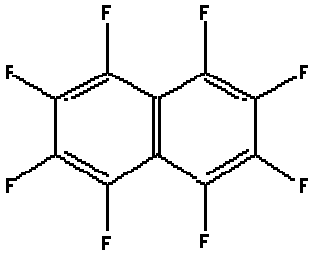
contaminants

BTEX

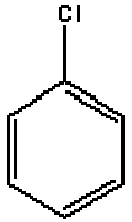
Benzene, **T**oluene,
Ethylbenzene, **X**ylene
→Hexafluorobenzene



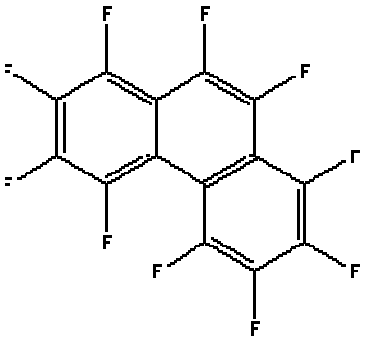




Chlorinated compounds

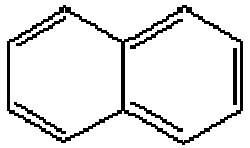


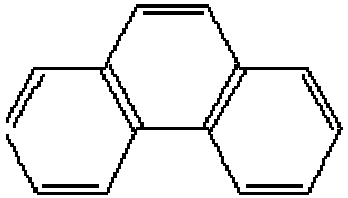
Chlorbenzene
→ Hexafluorobenzene

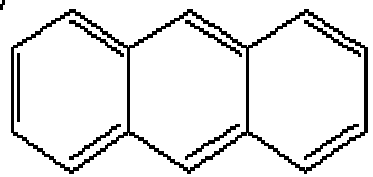


PAH

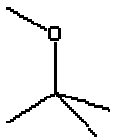
Naphthalene, Phenanthrene, Anthracene
→Perfluorphenanthrene
→Perfluoronaphthalene



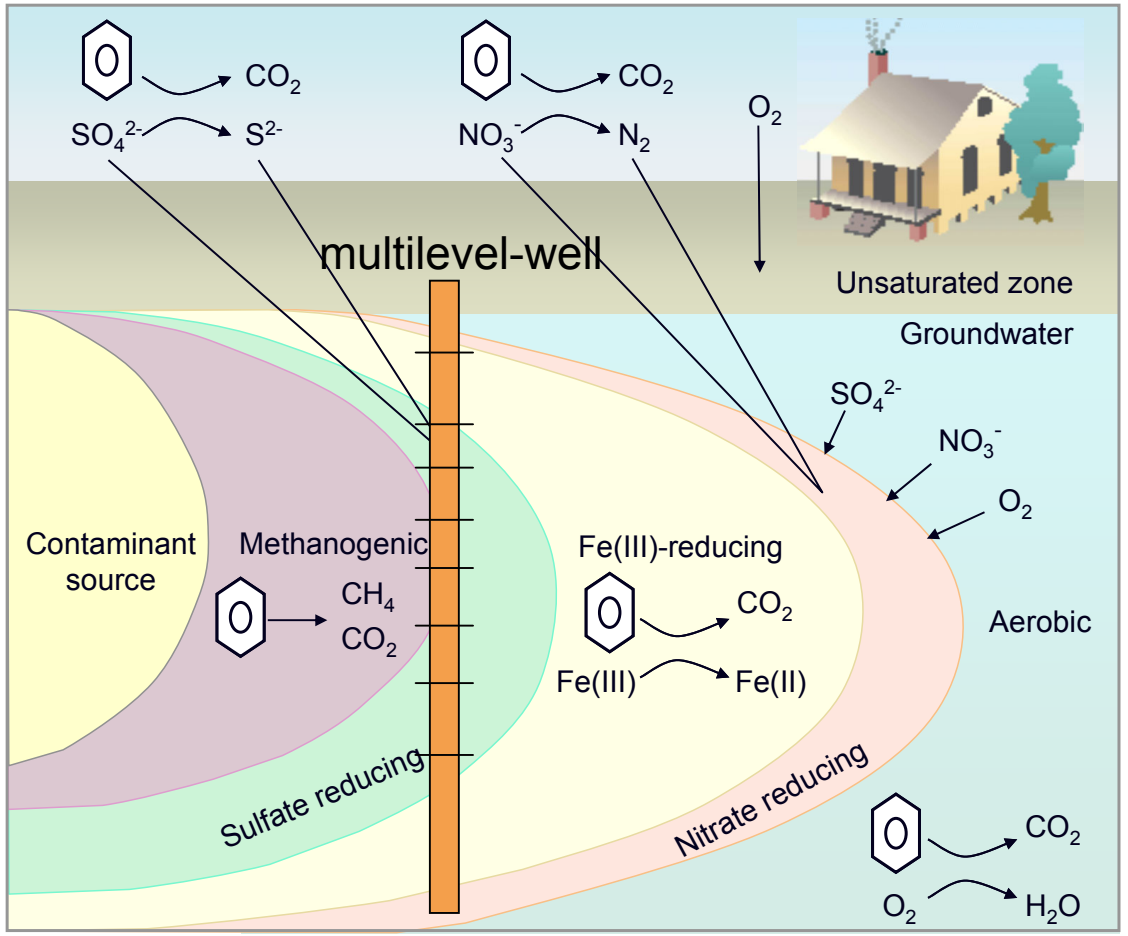




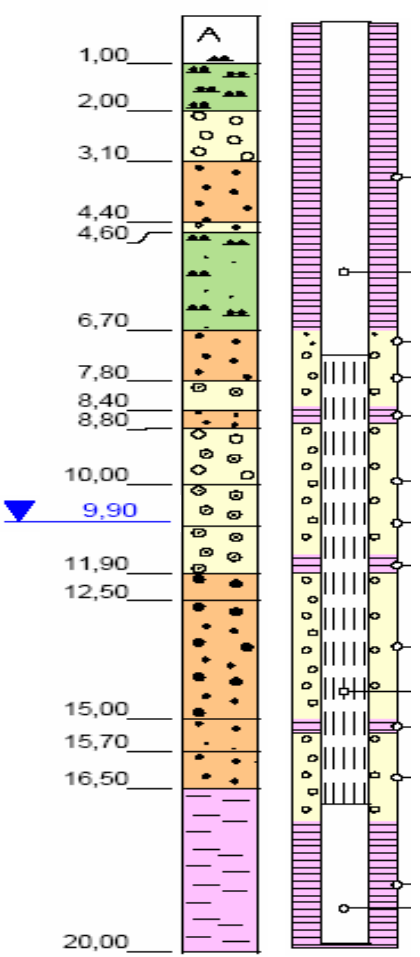
MTBE



BACTRAPs in multilevel experiments



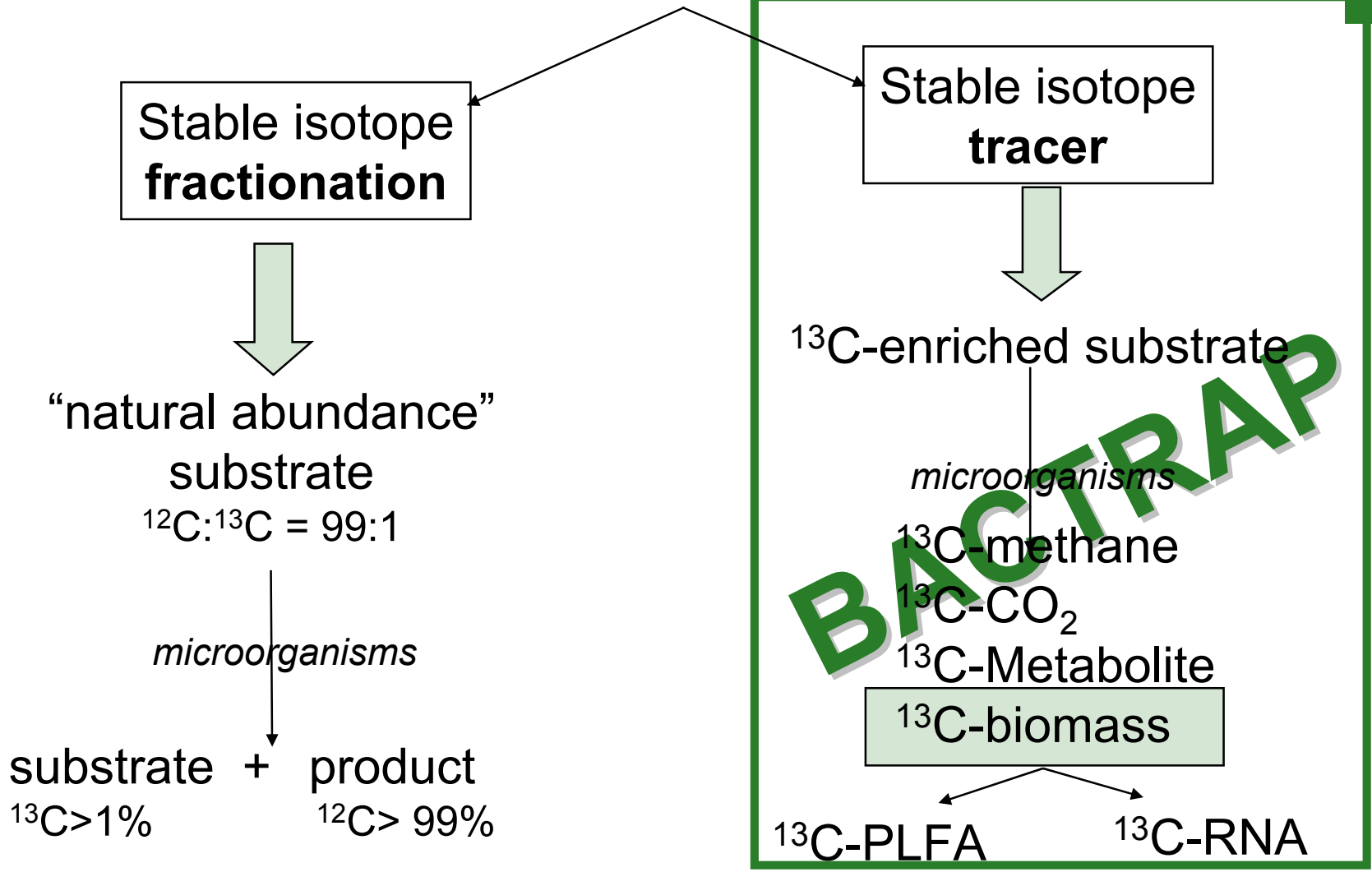
Lovley, 2001 Science



Outline

- What are BACTRAPs?
- Why do we need BACTRAPs?
- How do we use them?
- Proof of biodegradation
- Identification of metabolic pathways
- Outlook

Stable isotope tools



Recent studies

site	industry	contaminant
Zeitz (SAFIRA)	hydrogenation plant	Benzene, Toluene
Hanau	Tar oil (creosote)	PAH
Dortmund	gas works	Benzene
Schwedt	refinery	MTBE
Leuna (SAFIRA)	refinery	MTBE, Benzene
Bitterfeld (SAFIRA)	chlorine chemistry	Chlorobenzene
Hamburg Moorfleet	chlorine chemistry	Chlorobenzene