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

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Annual Meeting 2005, Jena
October 10-12, 2005
Assessment of Natural Attenuation as remediation strategy needs the proof of

*in situ*

**Biodegradation**

**Natural Attenuation** 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)

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)

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

SIP, PCR, SSCP, DGGE

GC-MS, GC-C-IRMS, EA-IRMS
Bio-Sep® Beads

- Ø 2-3 mm
- internal surface 600 m² g⁻¹
- bulk density 0.16 g cm⁻³
- powdered activated carbon (75 %) in a NOMEX matrix (25 %)
- with outer pores < 5 μm

¹BIO-SEP® Beads: kindly provided by Kerry L. Sublette, University of Tulsa (USA)
White et al. (2003), Environmental Forensics 4:63-74
Proof of biodegradation is only possible if the microorganisms use the $^{13}\text{C}$-labelled substrate as their carbon source for biosynthesis.

Outlook: usage of $^{15}\text{N}$-labelled substrates
Reproducible loading of BIO-SEP® Beads via gas phase

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

Benzene, Toluene, Monochlorobenzene, MTBE

valve open

vacuum pump - on -

substrate (e.g. $^{13}$C-benzene)

p ~ 60mbar

valve close

vacuum pump - off -
Application of BACTRAPs in field experiments

BTEX-concentration in µg/l

Saf Zz 32/02
Saf Zz 23/00
Saf Zz 507/97
Saf Zz 24/02
Application of BACTRAPS in field experiments

Chromatogram (GC-C-IRMS-measurement)

$^{13}$C-Benzene BACTRAP Saf Zz 32/02

Incorporation of the $^{13}$C-label into biomass proves biodegradation!!!
Application of BACTRAPs in field experiments

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

BACTRAP

concentration [mmol L⁻¹]

deepth [m]

concentration [µmol L⁻¹]

deepth [m]

δ¹³C benzene [%]

deepth [m]

δ¹³C benzene [%]

natural variability

isotope signature of the source

stable isotope fractionation

BACTRAP

δ¹³C FA [%]

depth [m]

concentration [mg L⁻¹]

deepth [m]

concentration [µmol L⁻¹]

deepth [m]

δ¹³C benzene [%]

depth [m]
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 $^{13}$C-methyl-toluene

Benzylsuccinate after 92d of incubation with $^{12}$C-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)
Financial support of Nicole Stelzer by the Deutsche Bundesstiftung Umwelt (DBU) is gratefully acknowledged (grant 20004/751).
contaminants

**BTEX**
Benzene, Toluene, Ethylbenzene, Xylene → Hexafluorobenzene

**Chlorinated compounds**
Chlorobenzene → Hexafluorobenzene

**PAH**
Naphthalene, Phenanthrene, Anthracene → Perfluorphenanthrene → Perfluoronapththalene

**MTBE**
BACTRAPs in multilevel experiments

Lovley, 2001 Science

Direction of groundwater flow
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

Stable isotope fractionation

“natural abundance” substrate

$^{12}\text{C}:^{13}\text{C} = 99:1$

microorganisms

substrate + product

$^{13}\text{C}>1\%$ $^{12}\text{C}>99\%$

Stable isotope tracer

$^{13}\text{C}$-enriched substrate

$^{13}\text{C}$-methane

$^{13}\text{C}$-CO$_2$

$^{13}\text{C}$-Metabolite

$^{13}\text{C}$-biomass

$^{13}\text{C}$-PLFA

$^{13}\text{C}$-RNA
### Recent studies

<table>
<thead>
<tr>
<th>Site</th>
<th>Industry</th>
<th>Contaminant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeitz (SAFIRA)</td>
<td>Hydrogenation plant</td>
<td>Benzene, Toluene</td>
</tr>
<tr>
<td>Hanau</td>
<td>Tar oil (creosote)</td>
<td>PAH</td>
</tr>
<tr>
<td>Dortmund</td>
<td>Gas works</td>
<td>Benzene</td>
</tr>
<tr>
<td>Schwedt</td>
<td>Refinery</td>
<td>MTBE</td>
</tr>
<tr>
<td>Leuna (SAFIRA)</td>
<td>Refinery</td>
<td>MTBE, Benzene</td>
</tr>
<tr>
<td>Bitterfeld (SAFIRA)</td>
<td>Chlorine chemistry</td>
<td>Chlorobenzene</td>
</tr>
<tr>
<td>Hamburg Moorfleet</td>
<td>Chlorine chemistry</td>
<td>Chlorobenzene</td>
</tr>
</tbody>
</table>