ASSESSMENT OF NATURAL ATTENUATION OF HALOGENATED SOLVENTS USING STABLE ISOTOPE TECHNIQUES

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Summer School on Contaminated Soils:
from characterization to remediation
Universite PARIS EST, 25-06-2012

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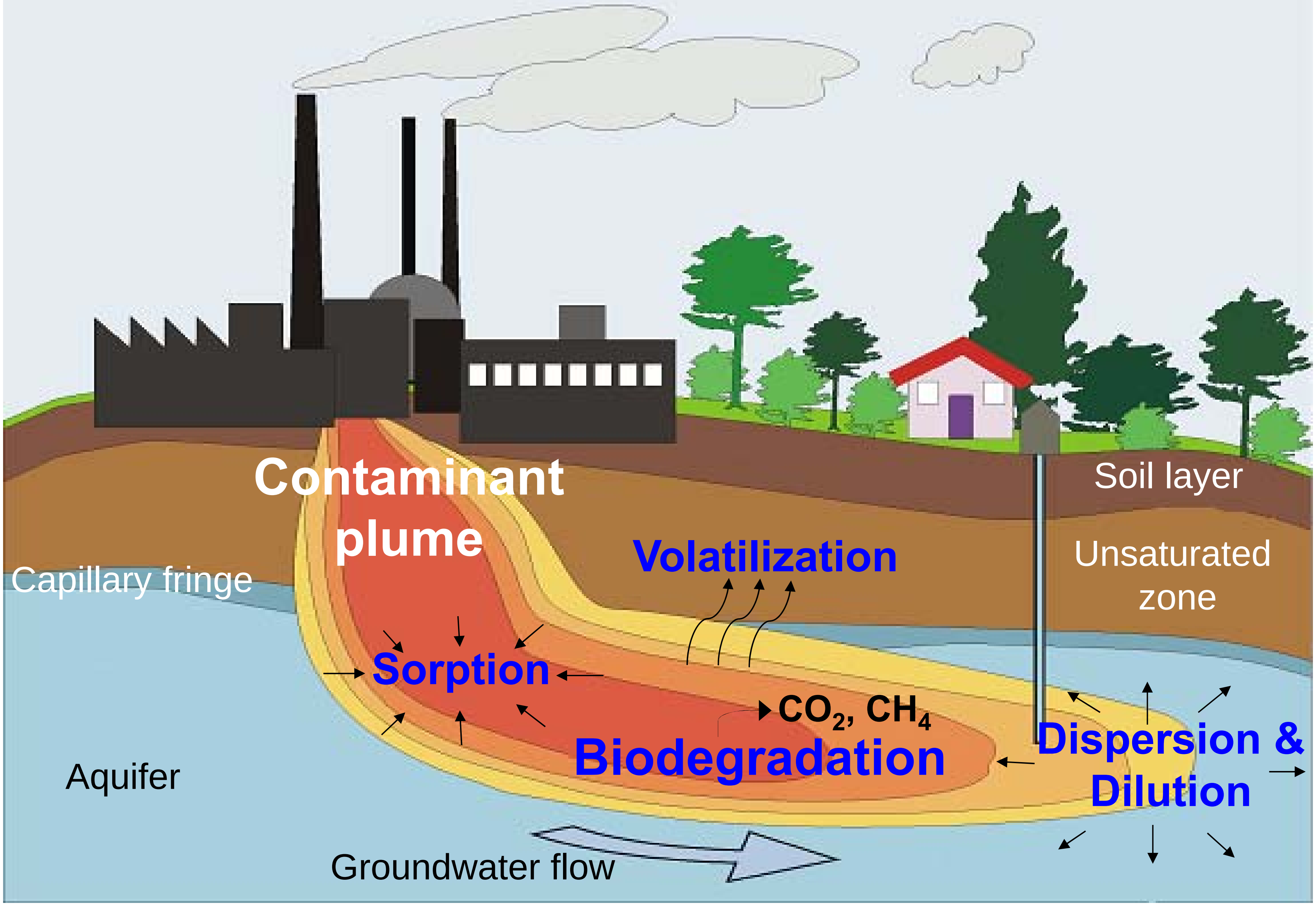
Contaminated site scenario

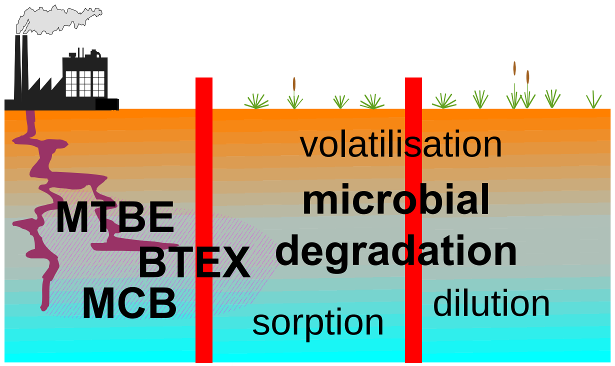


Application of Stable Isotopes for the Assessment of Natural Attenuation (NA)

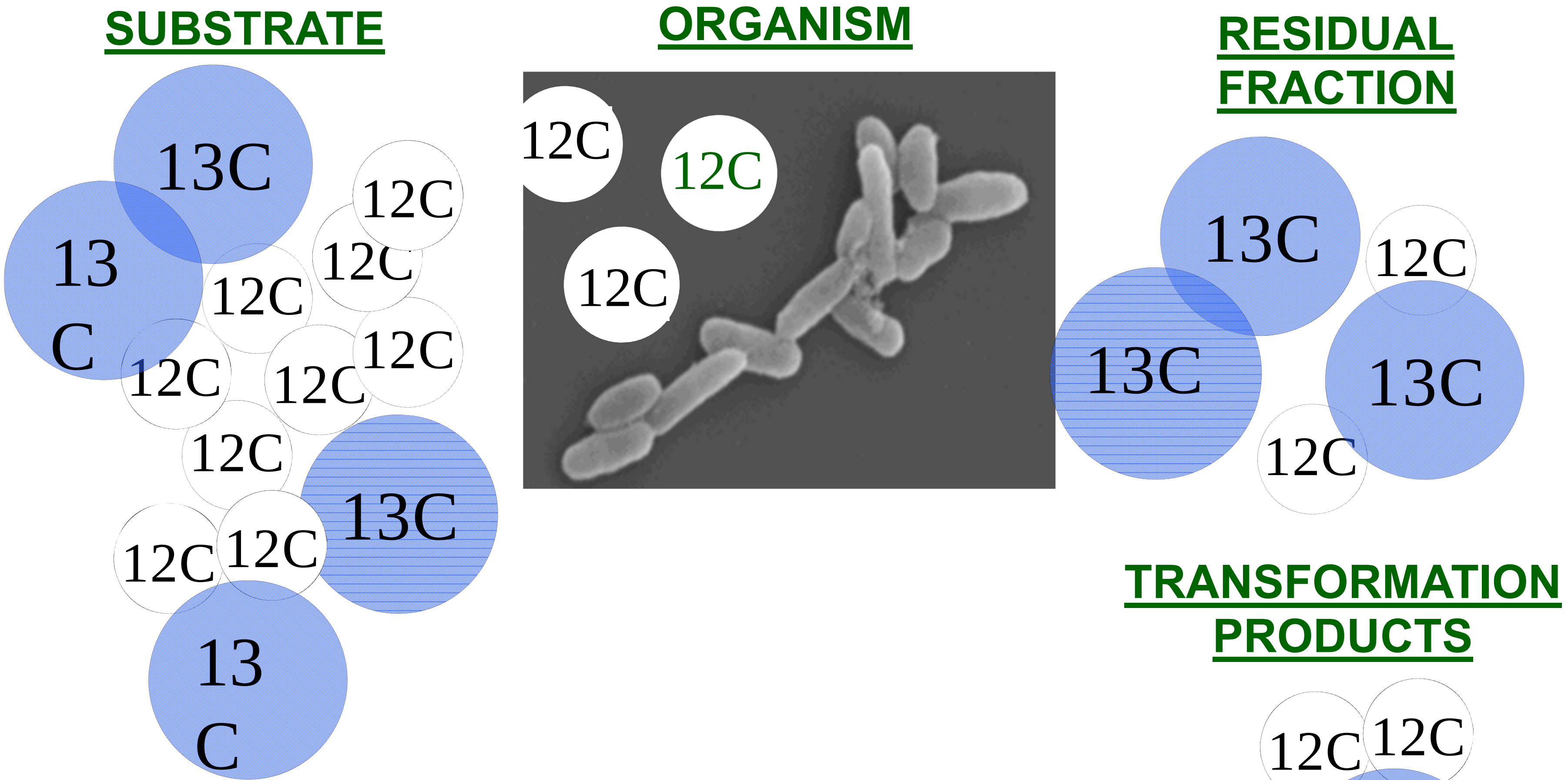
NA (= physical, chemical and **biological** processes)

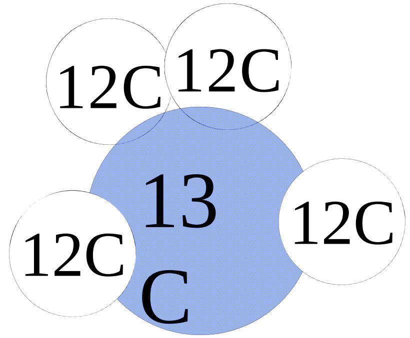
- alternative, cost-efficient in situ remediation approach
- taking advantage of the ability of microorganisms to degrade pollutants
- abiotic processes only lead to a decrease in contaminant concentration
- only in situ biodegradation results in **sustainable** removal of contaminants

→ Proof of in situ biodegradation required!!!



Isotope fractionation process

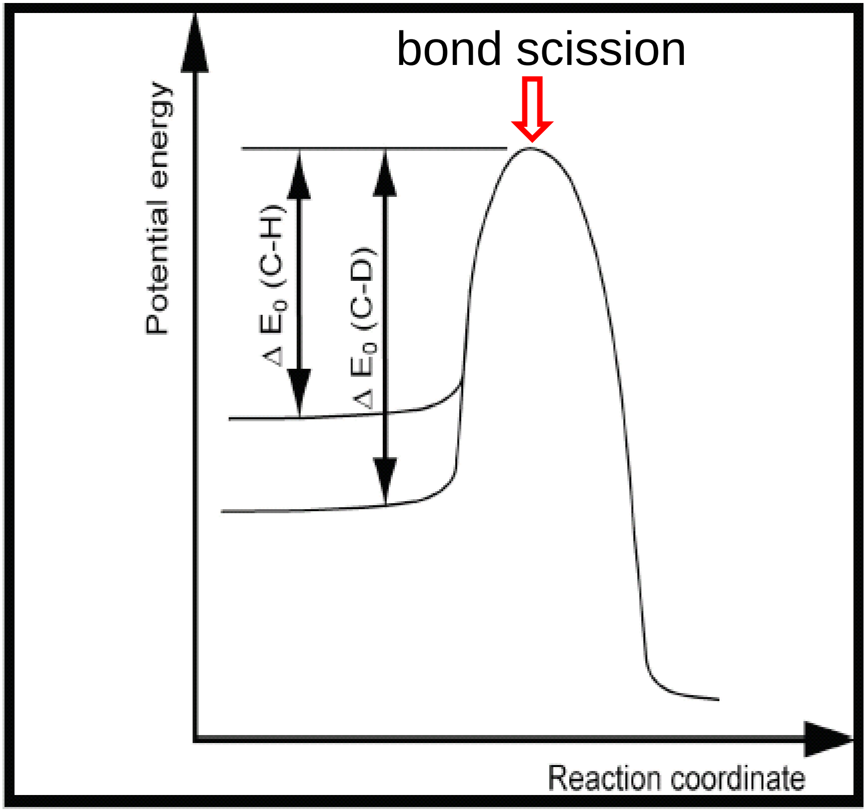


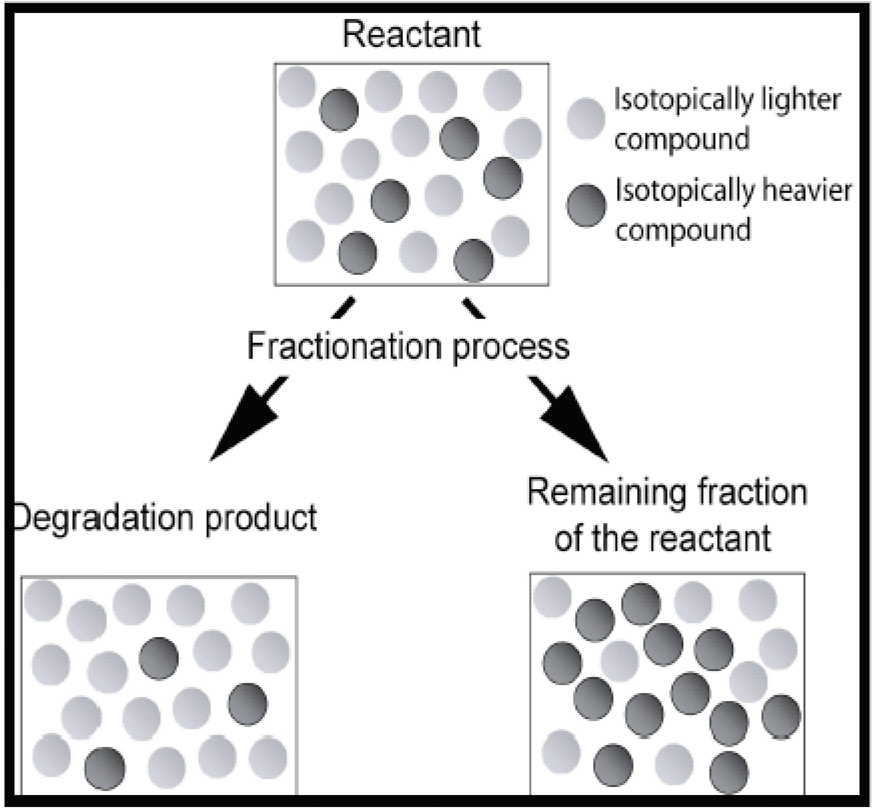


- methanogenesis (CO_2-reduction, acetogenesis)
- methane oxidation
- degradation of contaminants

Kinetic Isotope Effect

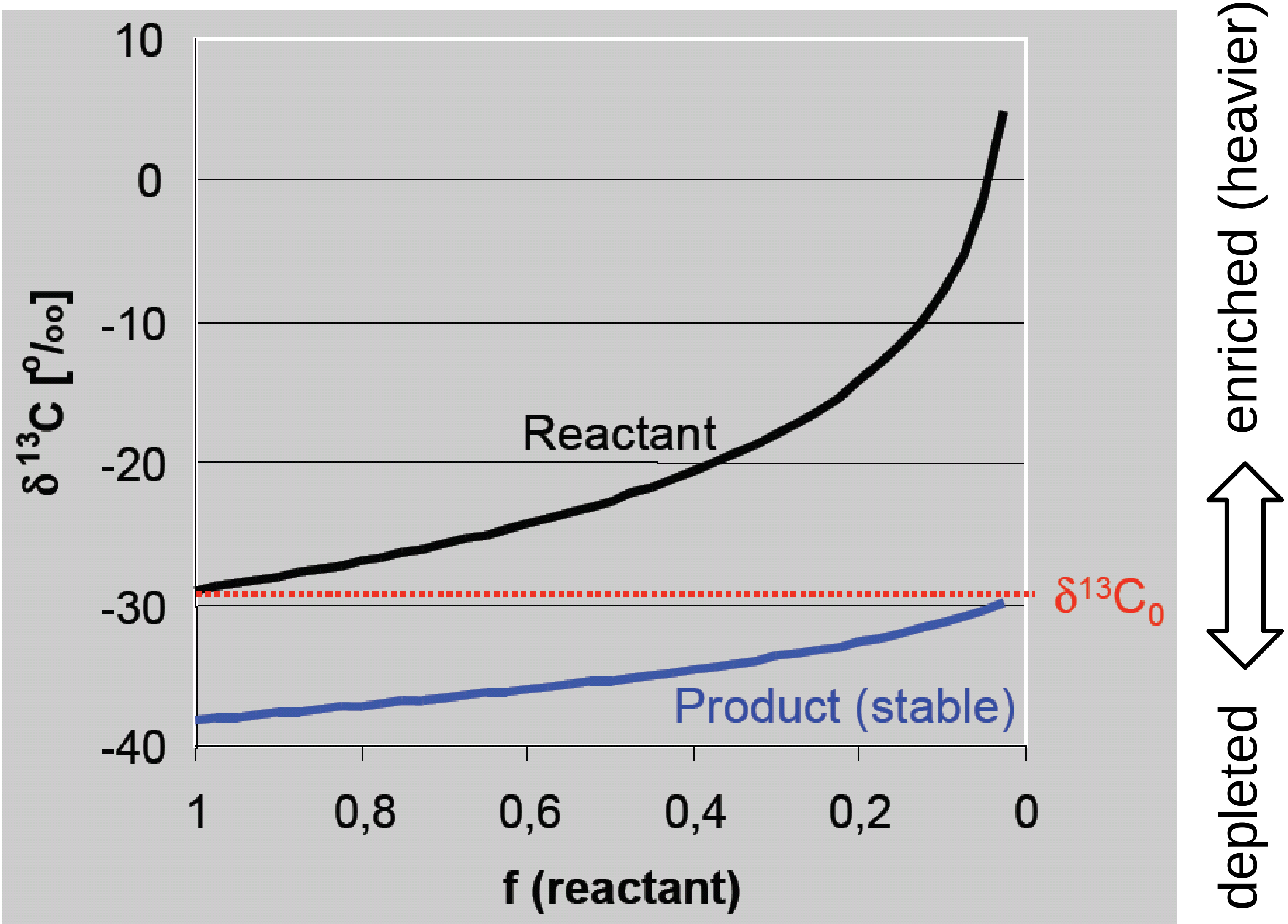
Difference of reaction rates (activation energy) between light and heavy isotopes leads to fractionation



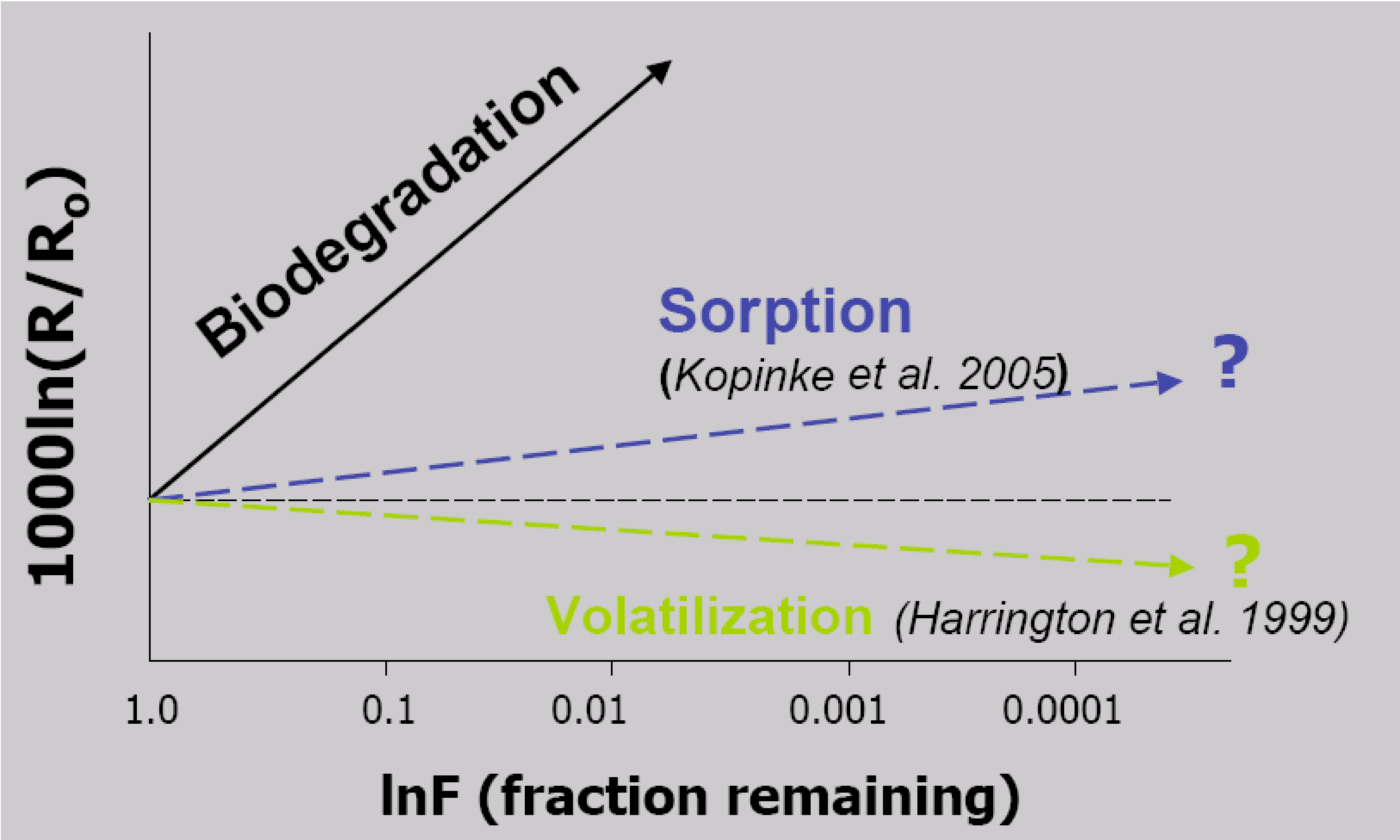


Stefan Harderlein, 2006

Kinetic Isotope Fractionation in a in a Closed System: Generic Example



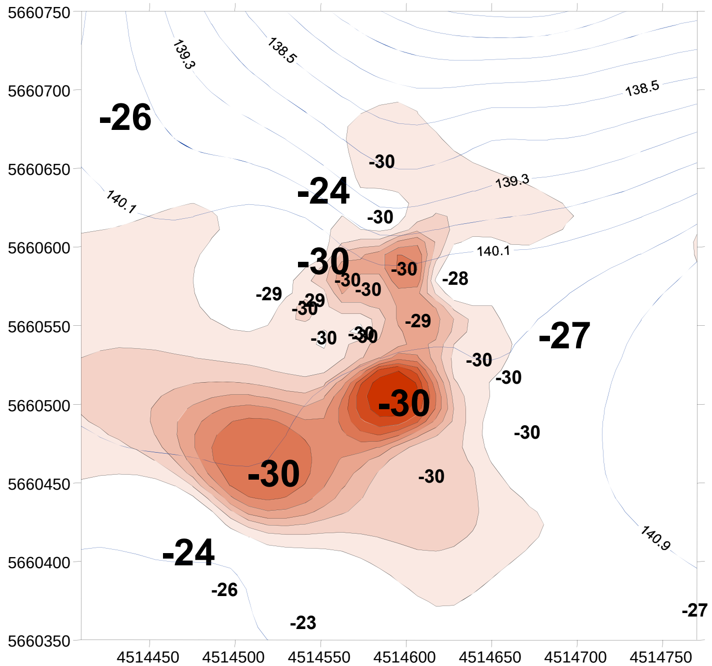
Process identification and quantification based on isotope signatures



Haderlein 2006

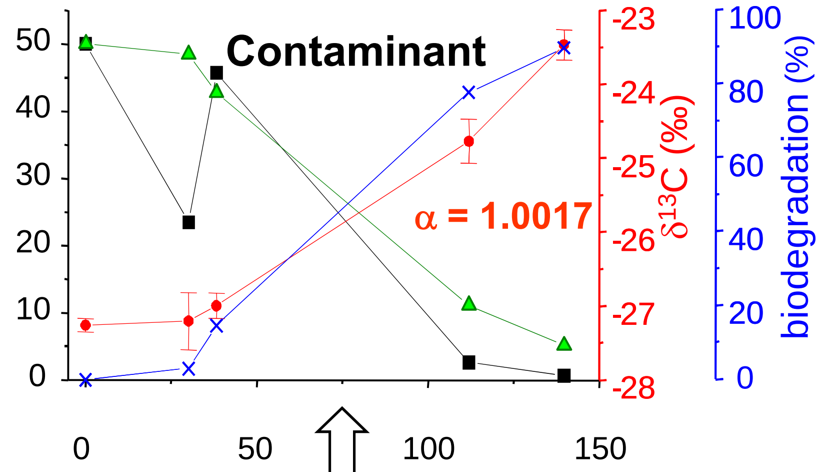
Quantification of microbial in situ degradation

Isotope fractionation by microbial activity

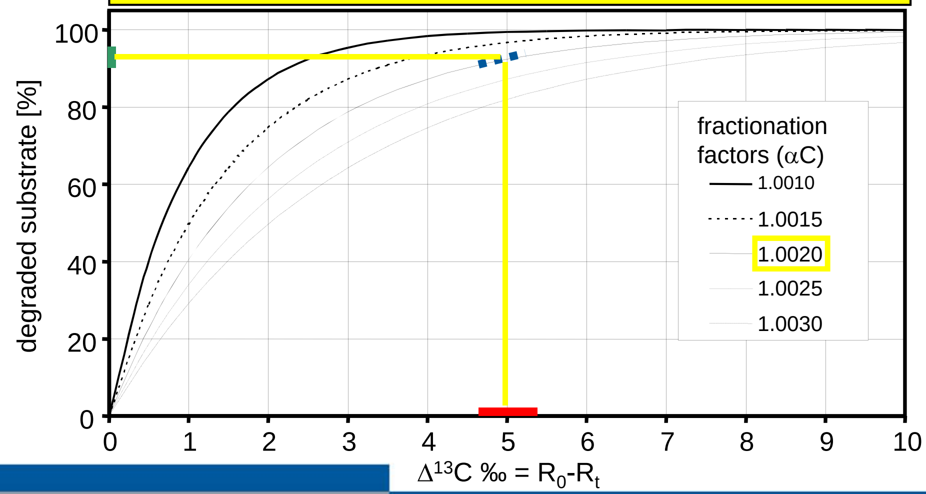


Determination of the compound specific fractionation factor (*αC*) in reference experiments

Quantification of the *in situ*-biodegradation (e.g. on a flow path)

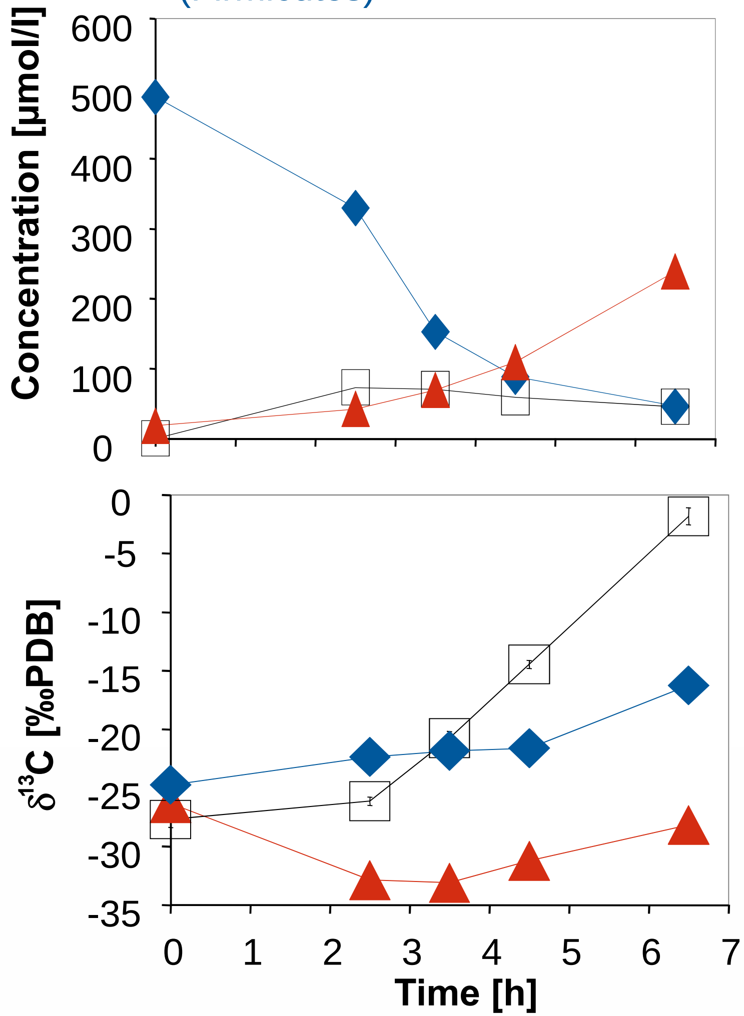


Calculation of biodegradation using the Rayleigh equation



Carbon isotope fractionation of (PCE)

Desulfitobacterium sp.
strain PCE-S
(Firmicutes)



Dehalorespiration

(PCE-> TCE->DCE)

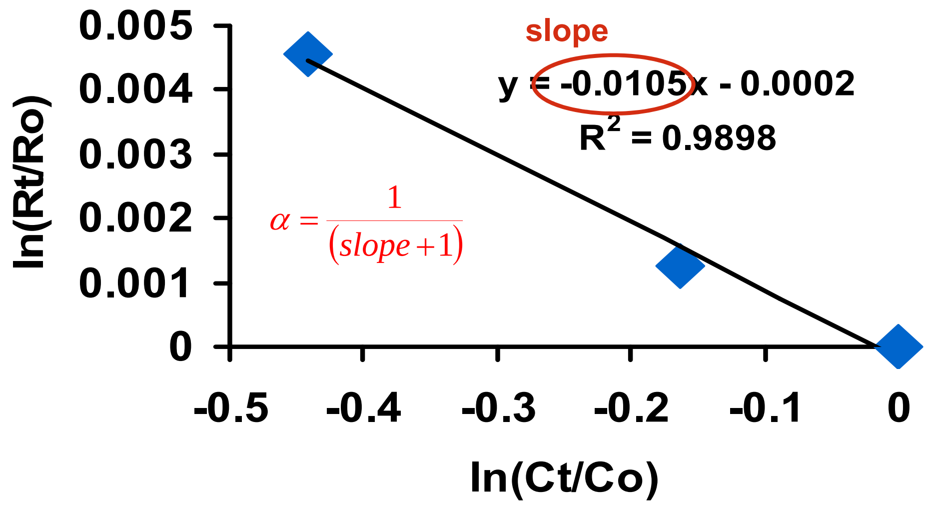
PCE: ♦ TCE: □ *cis*-DCE:▲

Nijenhuis et al. 2005

Quantification of stable isotope fractionation

Rayleigh Equation:

$$\frac{R_t}{R_0} = \left(\frac{C_t}{C_0}\right)^{\left(\frac{1}{\alpha}-1\right)} \longrightarrow \ln\left(\frac{R_t}{R_0}\right) = \left(\frac{1}{\alpha}-1\right) * \ln\left(\frac{C_t}{C_0}\right)$$



C_0 = initial concentration
C_t = concentration at time t
R_0 = initial isotope ratio
R_t = isotope ratio at time t
α = isotope fractionation factor

ε [‰] = slope*1000

Enrichment factors (α) with reference strains

	PCE	TCE	
	ε (‰)	ε (‰)	
S. multivorans	-0.4 ± 0.2	-18.7 ± 4.2	ε-proteobacteria
S. halorespirans	-0.5 ± 0.2	-18.9 ± 1.0	
D. michiganensis	*not sign.*	-3.5 ± 0.2	δ-proteobacteria
G. lovleyi	*not sign.*	-8.5 ± 0.6	
Desulfit. strain PCE-S	-5.2 ± 1.5	-12.2 ± 2.3	Firmicutes
Desulfit. strain Viet1	-16.7 ± 4.5	*does not dechl. TCE*	
*Dehalobacter restrictus**	*not analysed*	-3.3 ± 0.3	
D. ethenogenes strain 195	-6.0 ± 0.7	-13.7 ± 1.8	Chloroflexi
„ by Lee et al		-9.6 ±0.4	
Dehalococcoides strain FL2		-8.0 ±0.4#	

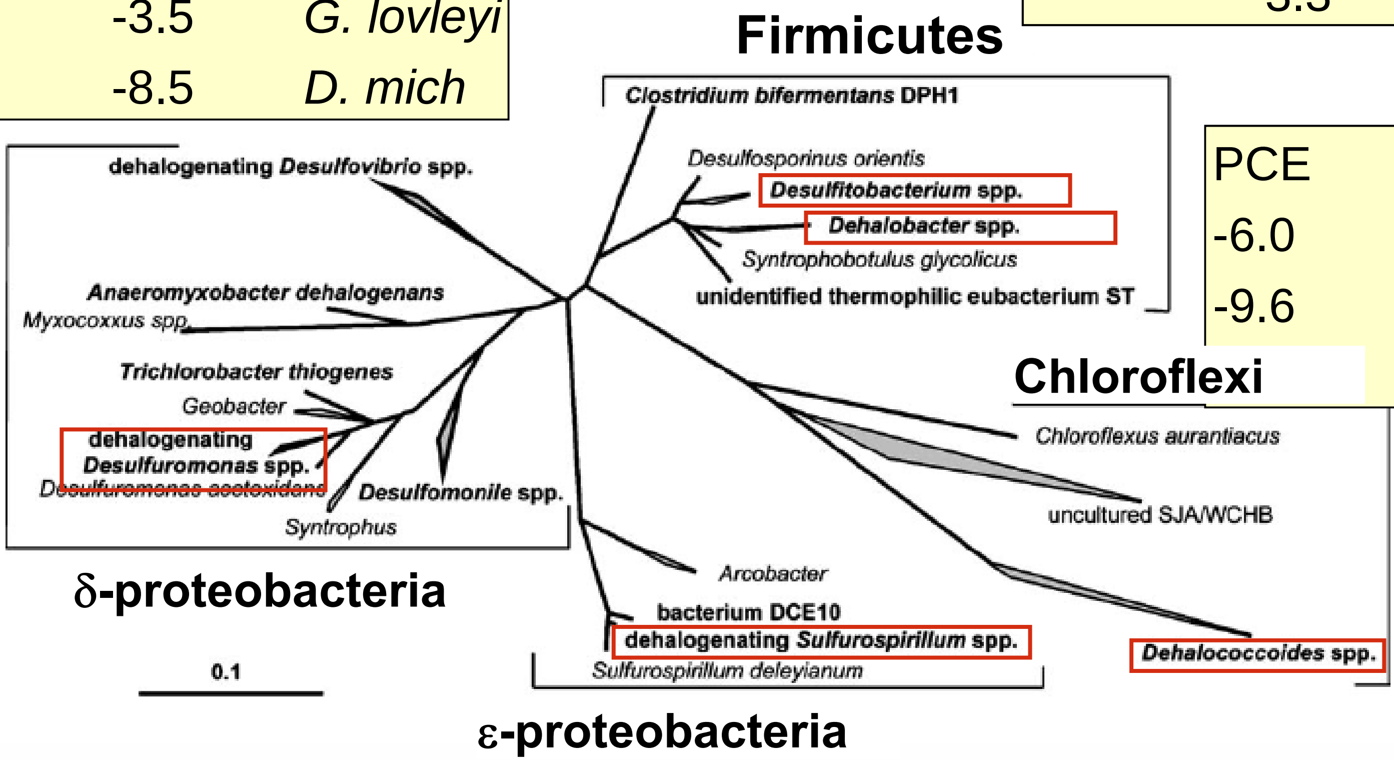
Nijenhuis et al 2005 AEM; Cichocka et al., 2007 FEMS Microbiol Ecol; Cichocka et al., 2008 Chemosphere; *Lee et al., 2007; #unpublised Fletcher et al.

Phylogeny

PCE	TCE	
ns	-3.5	*G. lovleyi*
ns	-8.5	*D. mich*

PCE	TCE	
-5.2	-10.6	*Des.*PCE-S
-16.7	-	*Des.* Viet1
-	-3.3	*Dh. restrictus**

PCE	TCE	
-6.0	-13.7	*Dhc* 195
-9.6	„	
		Dhc FL2



PCE	TCE	
-0.4	-18.7	*S.multi*
-0.5	-18.9	*S. halo*

*** Lee et al 2007 ES&T**

Phylogenetic tree from:

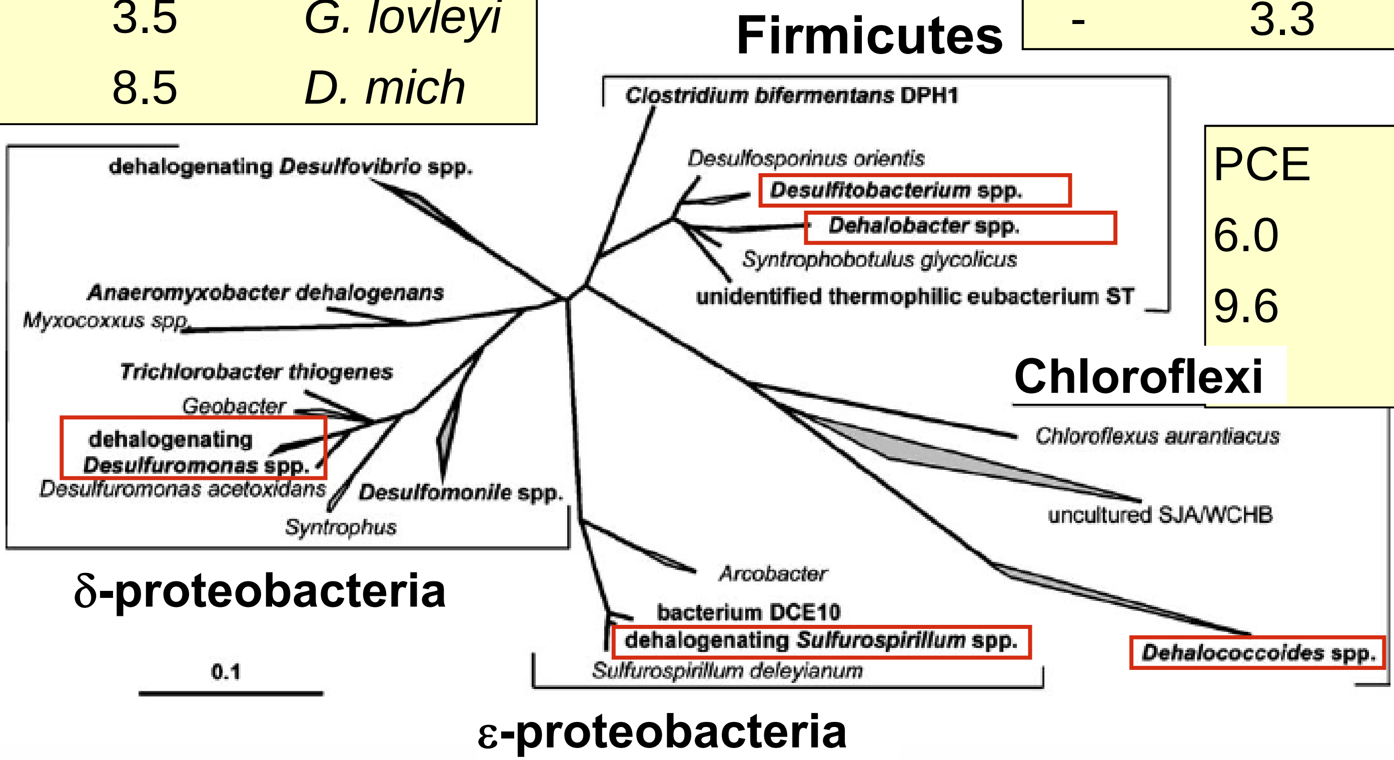
Smidt & De Vos 2004 Annu. Rev. Microbiol.

Can CSIA be used to assess *in situ* chlorinated ethene degradation?

PCE	TCE	
ns	3.5	*G. lovleyi*
ns	8.5	*D. mich*

PCE	TCE	
5.2	10.6	*Des.*PCE-S
16.7	-	*Des.* Viet1
-	3.3	*Dh. restrictus**

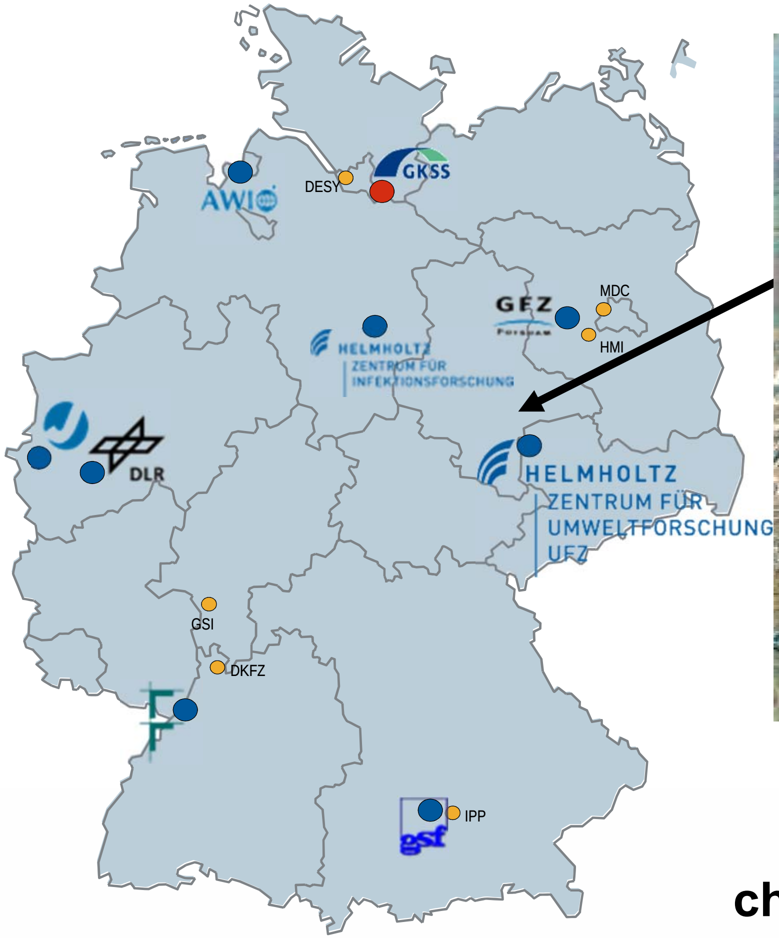
PCE	TCE	
6.0	13.7	*Dhc* 195
9.6	„	
		Dhc FL2



PCE	TCE	
0.4	18.7	*S.multi*
0.5	18.9	*S. halo*

*** Lee et al 2007 ES&T**
Phylogenetic tree from:
Smidt & De Vos 2004 Annu. Rev. Microbiol.

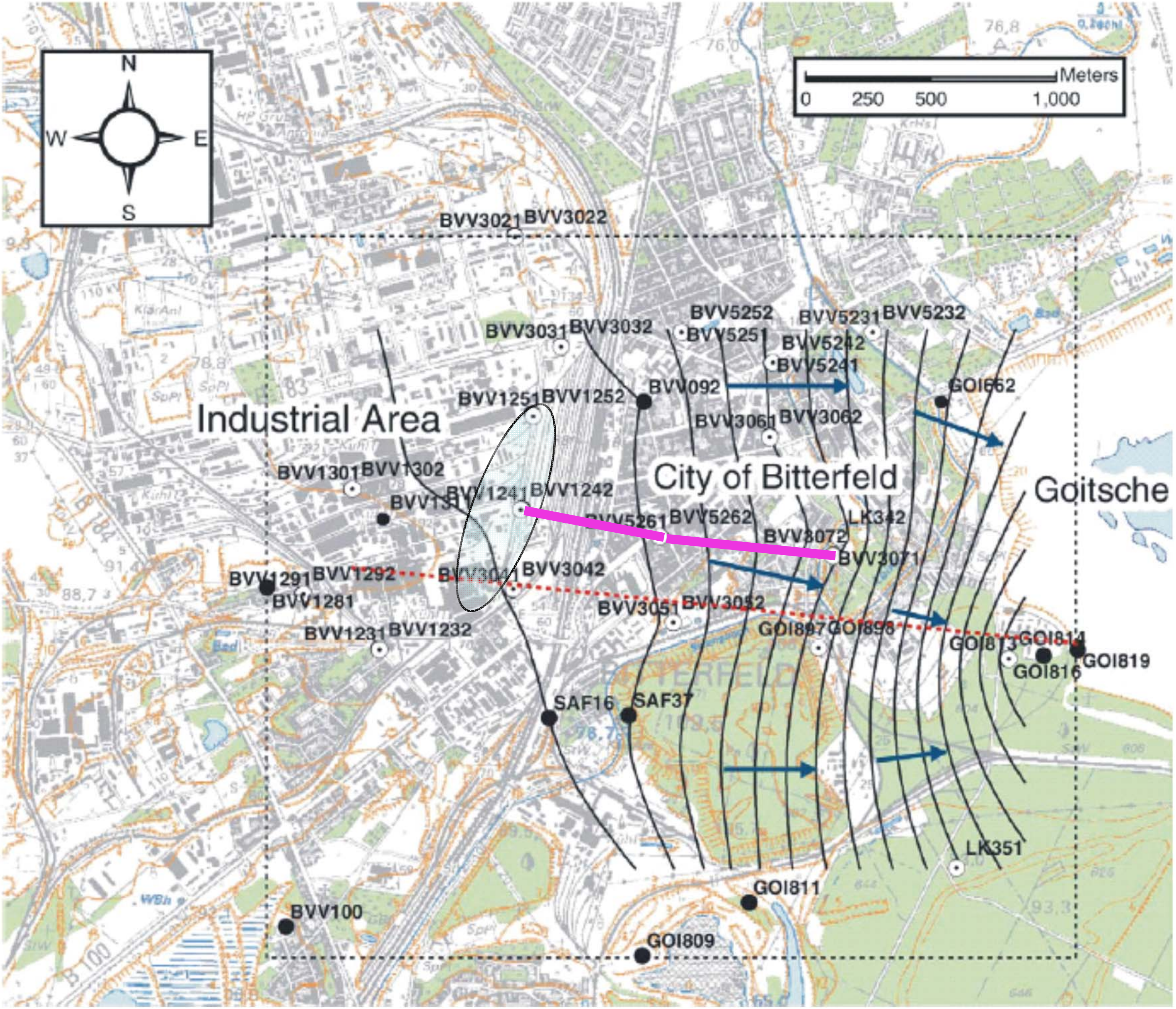
Case study: Bitterfeld



Groundwater contamination

Area ~ 25 km² Volume ~ 200.000.000 m³

chlorinated aliphatics, chlorinated benzenes, BTEX, HCH, DDT and much more

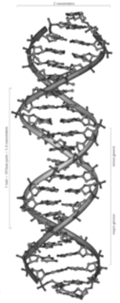

N
W
E
S
Meters
0
250
500
1,000
Industrial Area
City of Bitterfeld
Goitsche
BVV3021
BVV3022
BVV3031
BVV3032
BVV5252
BVV5251
BVV5231
BVV5232
BVV5242
BVV5241
BVV092
GOI662
BVV1251
BVV1252
BVV3061
BVV3062
BVV1301
BVV1302
BVV1241
BVV1242
BVV5262
LK342
BVV3072
BVV3071
BVV1291
BVV1292
BVV3042
BVV1281
BVV3051
BVV3052
GOI897
BVV1231
BVV1232
GOI813
GOI816
GOI819
SAF16
SAF37
LK351
GOI811
BVV100
GOI809

Molecular investigations

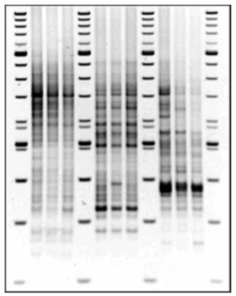
Ground water sampling

Complex bacterial communities

Environmental DNA isolation



Bacterial community structure analysis



PCR-DGGE approach

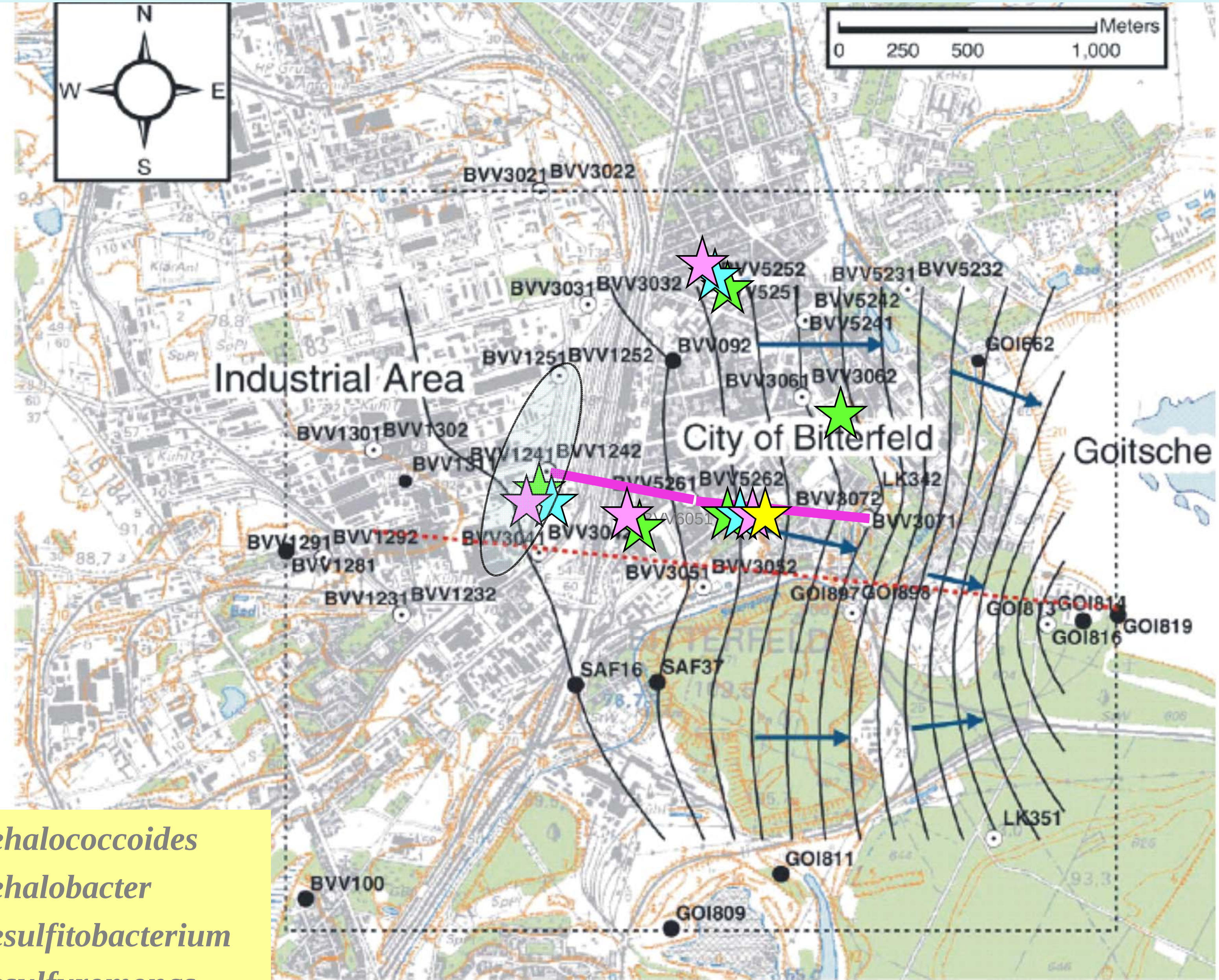
Detection of potential dechlorinating bacteria

Taxon-specific PCR assays

Bacterial composition

16 rRNA genes clone libraries

Detection of microorganisms using taxon specific PCR



Dehalococcoides
Dehalobacter
Desulfitobacterium
Desulfuromonas

Quantification of contaminant biodegradation

Influence of abiotic processes on pollutant concentration

$$B[\%] = \left(1 - \frac{C_t}{C_0}\right) \times 100$$

Rayleigh-Equation approach:

$$B[\%] = \left[1 - \left(\frac{R_t}{R_0}\right)^{\left(\frac{1000}{\varepsilon}\right)}\right] \times 100$$

Substitution of concentrations by isotope ratios using Rayleigh-Equation

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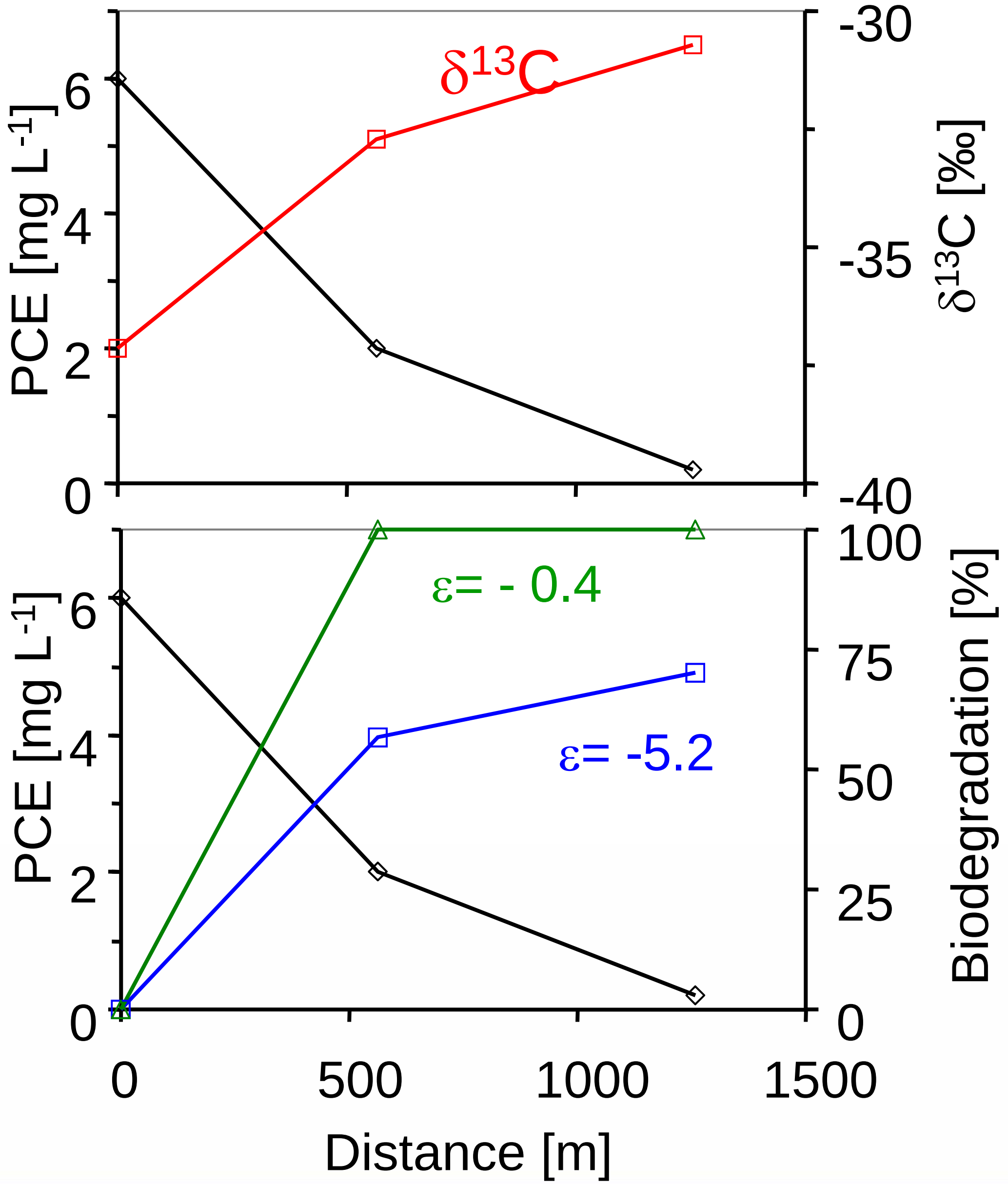
Case study Bitterfeld (PCE)



Estimation of

in situ

biodegradation

ε = > -0.5

Sulfurospirillum spp.

ε = < - 5.2

Dehalococcoides

Dehalobacter

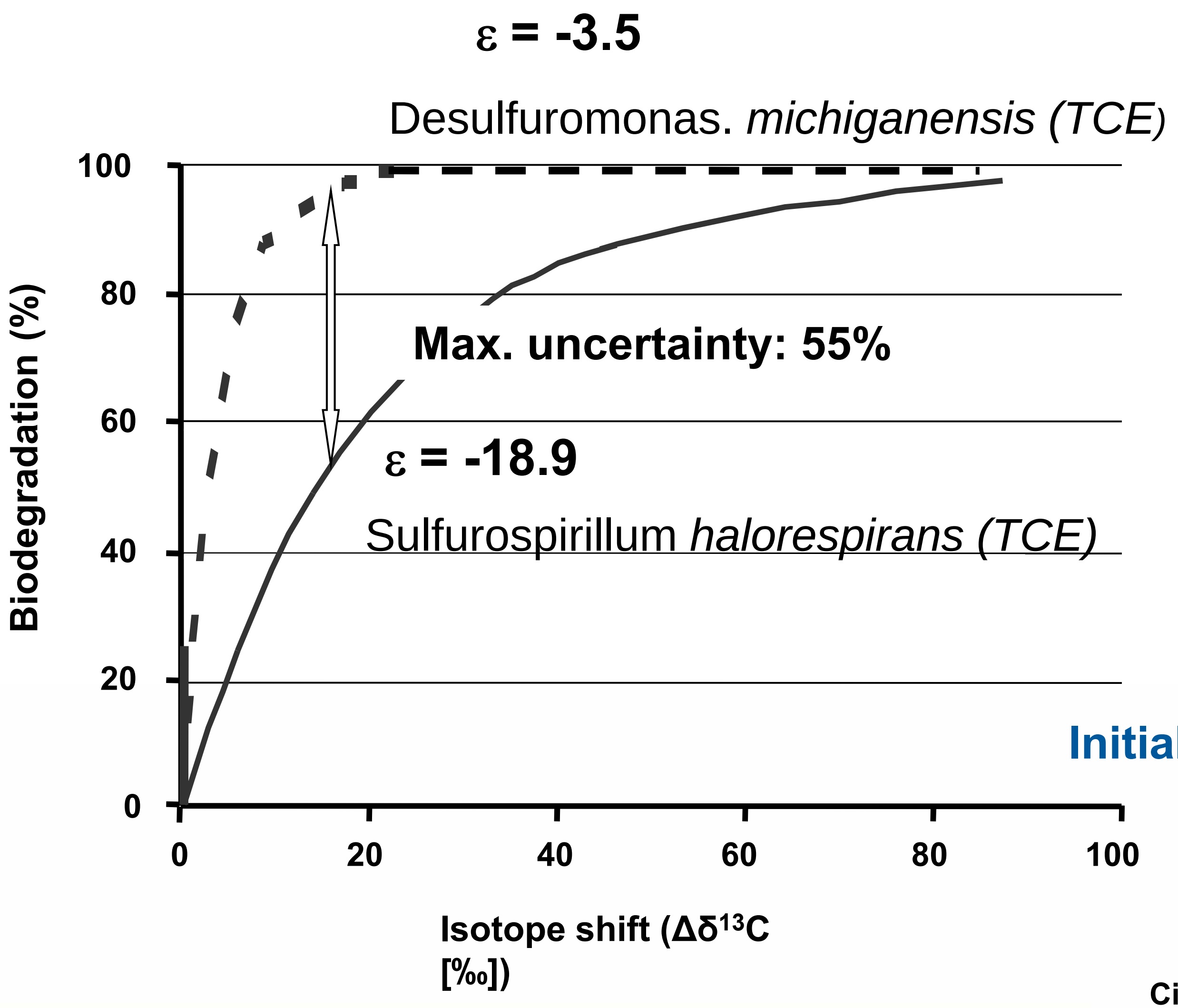
Desulfitobacterium

Desulfuromonas

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH – UFZ

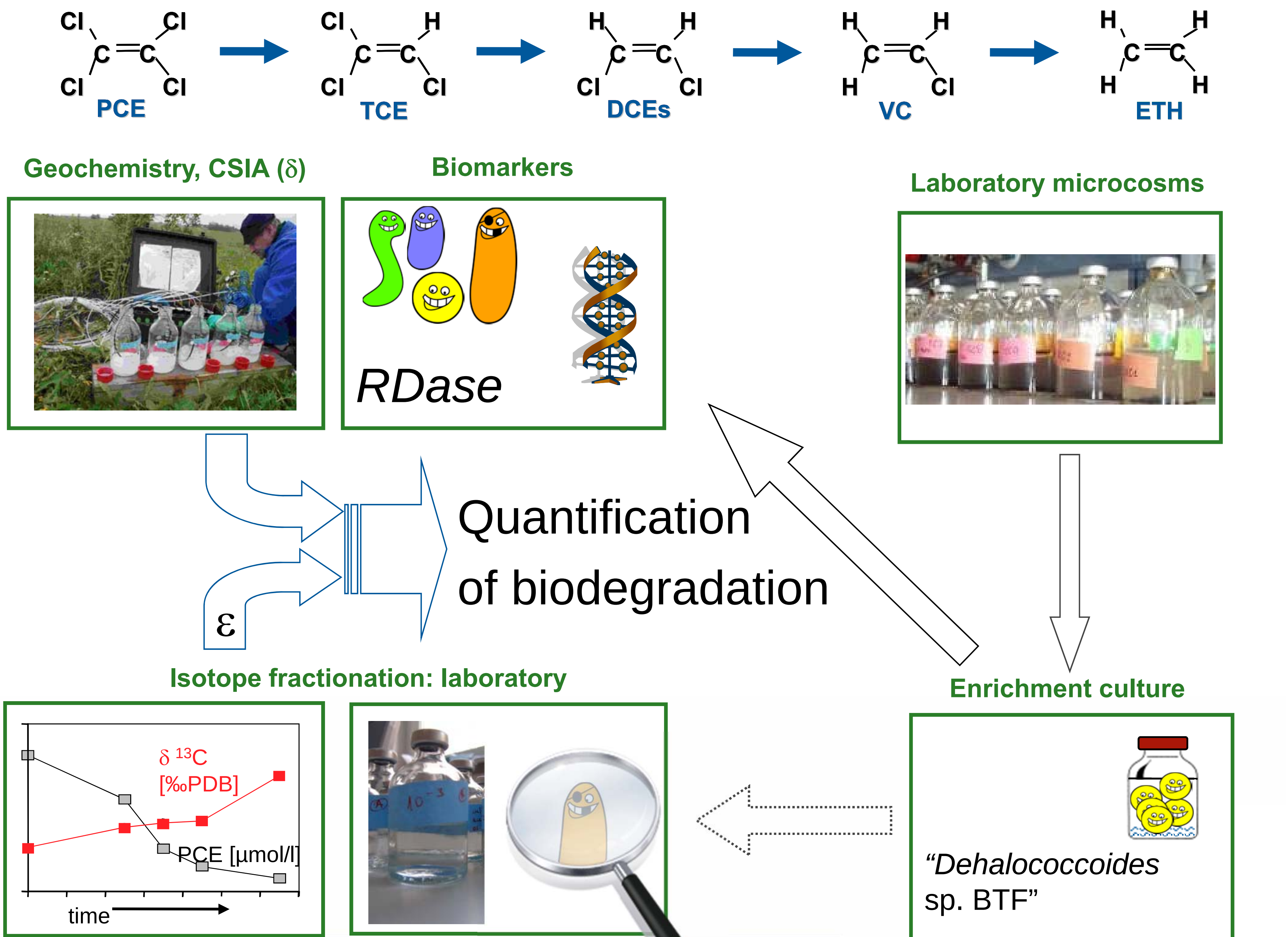
Case study Bitterfeld (TCE degradation)

$$B[\%] = \left[1 - \left(\frac{R_t}{R_0}\right)^{\left(\frac{1000}{\varepsilon}\right)}\right] \times 100$$



Initial $\delta^{13}C_{TCE}$ = -28.0 [‰]

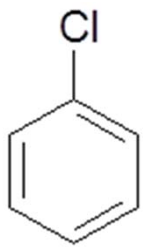
Cichocka et al., 2008 Chemosphere

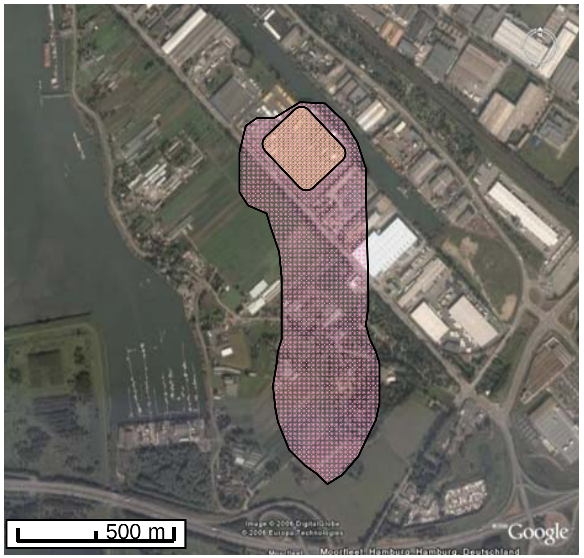


PCE
TCE
DCEs
VC
ETH
Geochemistry, CSIA (δ)
Biomarkers
RDase
Laboratory microcosms
Quantification
of biodegradation
ε
Isotope fractionation: laboratory
Enrichment culture
δ 13C
[‰PDB]
PCE [µmol/l]
time
"Dehalococcoides
sp. BTF"

The field site contaminated with chlorobenzenes (CB)

- former chemical production site in Germany
- main products: pesticides, insecticides
- release of contaminants to the subsurface (chlorinated benzenes, lindane)
- construction of a containment in the source zone to avoid further distribution of pollutants
- anoxic aquifer; potential electron acceptors: Fe(III) and SO_4^{2-}
- MCB plume of about 1 km length down-gradient
- max. MCB concentration of > 1000 µg L^{-1}





Investigation of anaerobic MCB degradation

in situ and *ex situ*

1. Isotope Fractionation

(natural abundance)

2. In situ microcosms

(BACTRAPs)

(^{13}C-labelling)

3. Laboratory microcosms

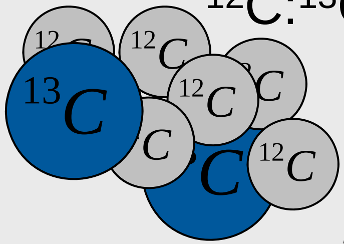
(^{13}C-labelling)

Stable Isotope Tools

Stable Isotope Fractionation

"natural abundance" substrate

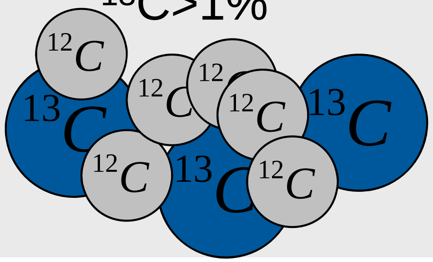
$^{12}C:^{13}C = 99:1$



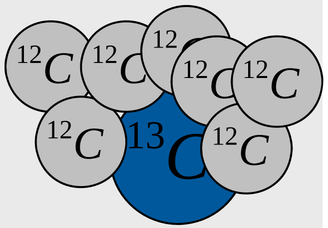
microorganisms

residual substrate + product

$^{13}C > 1\%$



$^{12}C > 99\%$



Stable Isotope Tracer

^{13}C-enriched substrate

microorganisms

^{13}C-methane

^{13}C-CO_2

^{13}C-metabolites

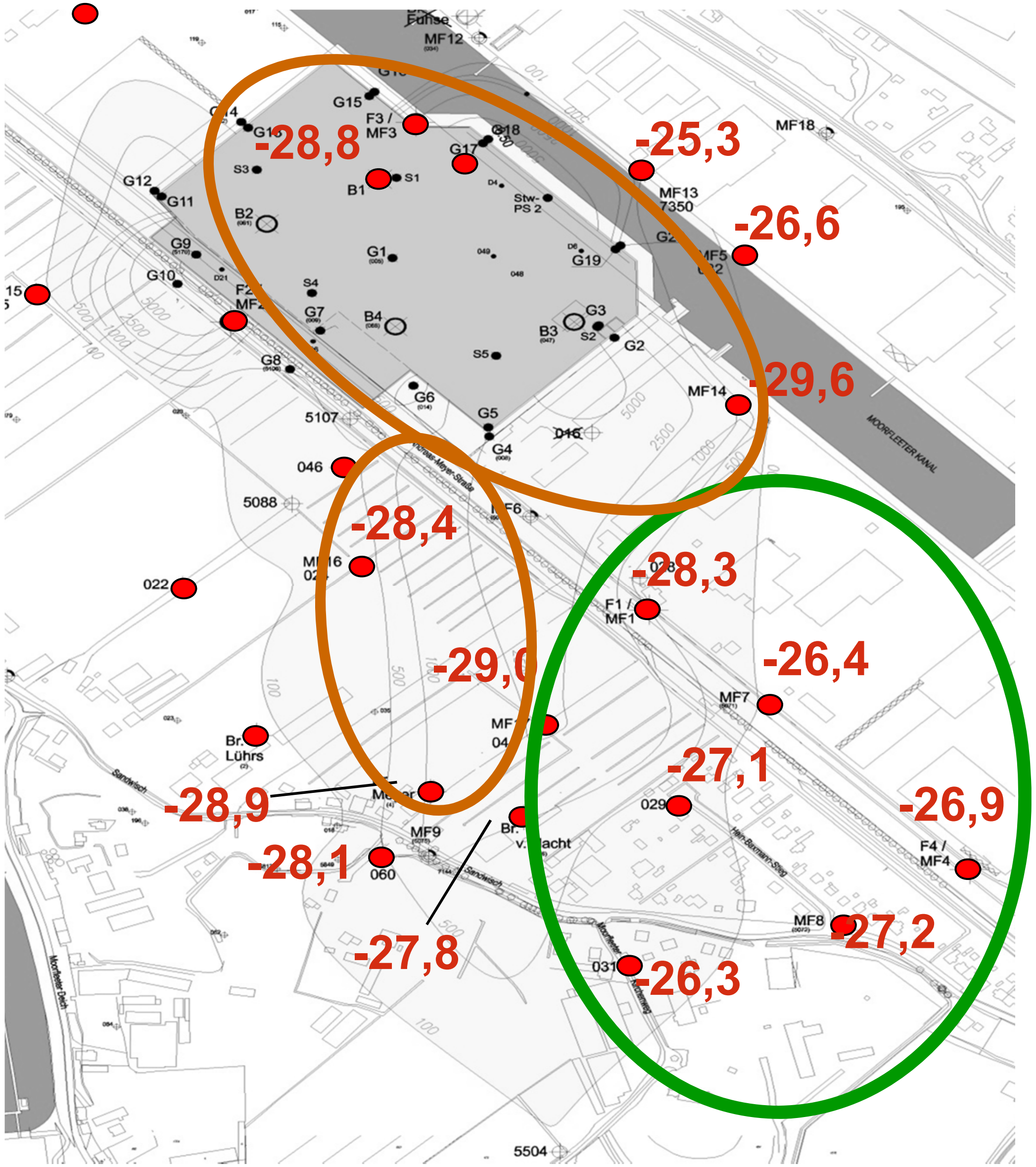
^{13}C-biomass (FA)

1. Stable Isotope Fractionation ($\delta^{13}C_{MCB}$ [‰])

Isotope ratios reported in Delta (δ)–notation [‰] relative to an international standard:

$$\delta^{13}C\ [‰] = \left(\frac{(^{13}C/^{12}C)_{Sample}}{(^{13}C/^{12}C)_{Standard}} - 1 \right) \times 1000$$

Carbon: in [‰] PDB
Belemnite, Cretaceous Peedee Formation, South Carolina

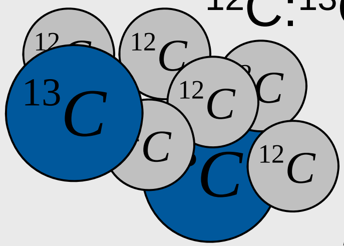


Stable Isotope Tools

Stable Isotope **Fractionation**

"natural abundance" substrate

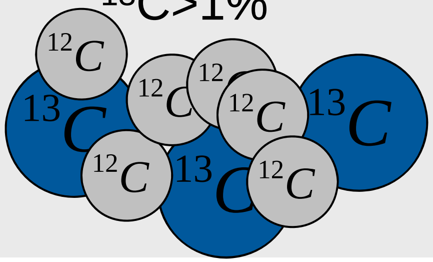
^{12}C:^{13}C = 99:1



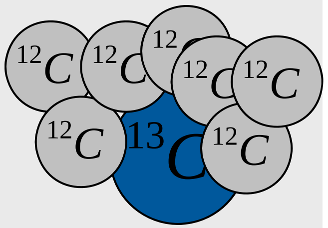
microorganisms

residual substrate + product

^{13}C>1%



^{12}C> 99%



Stable Isotope **Tracer**

^{13}C-enriched substrate

microorganisms

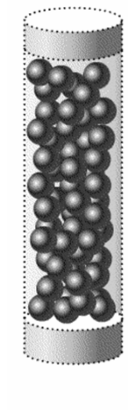
^{13}C-methane

^{13}C-CO_2

^{13}C-metabolites

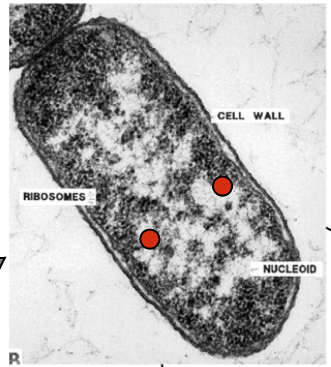
^{13}C-biomass (FA)

2. In situ microcosm analysis (BACTRAP)



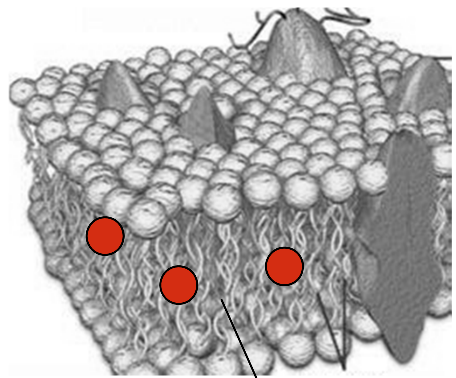
^{13}C substrate is converted into **^{13}C** biomass which can be analysed.

Bacteria consist up to 50 % of carbon!

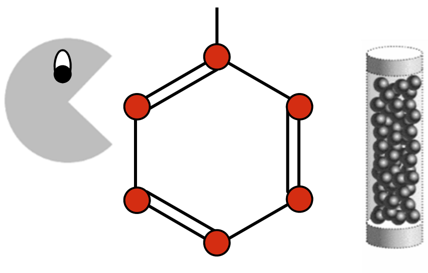


source: www.ucmp.berkeley.edu

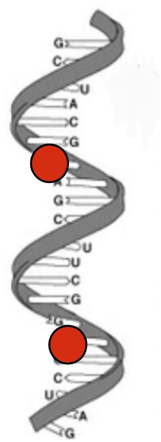
membrane/lipids



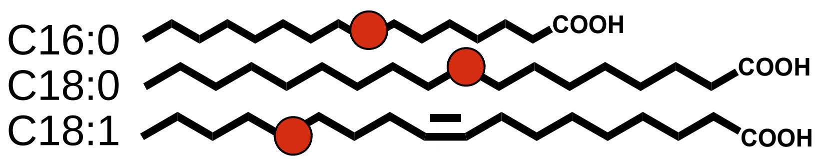
substrate



$^{13}C_6$-MCB

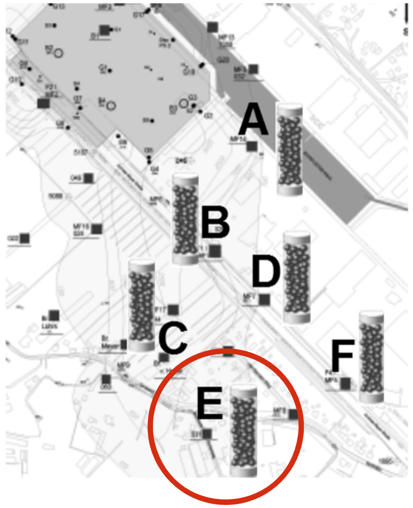


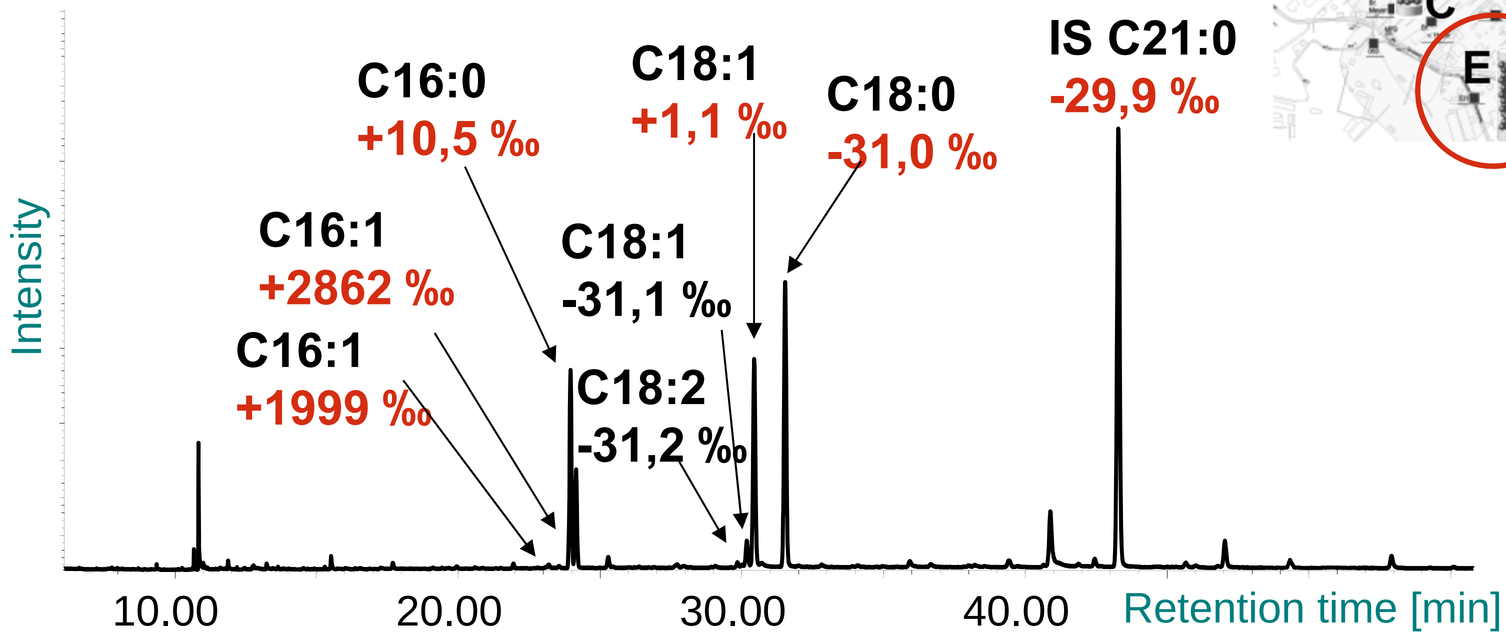
RNA



fatty acids

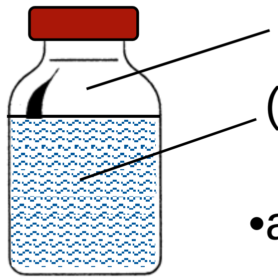
2. BACTRAP results





Activity for anaerobic MCB degradation in all wells!

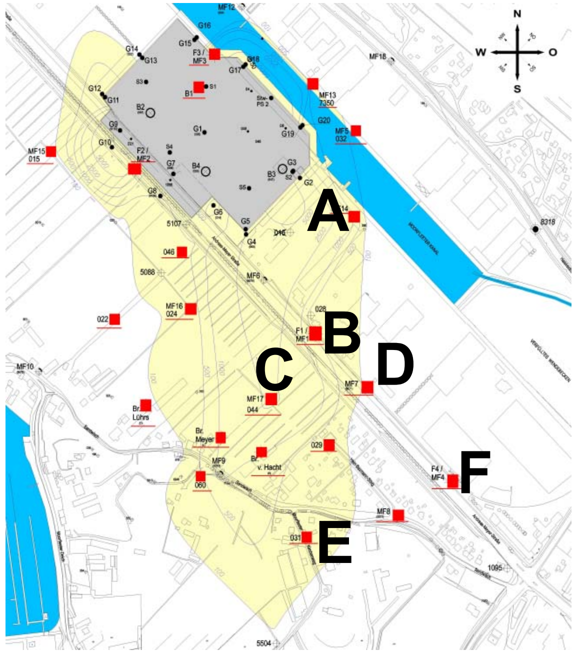
3. Ex situ (laboratory) microcosms



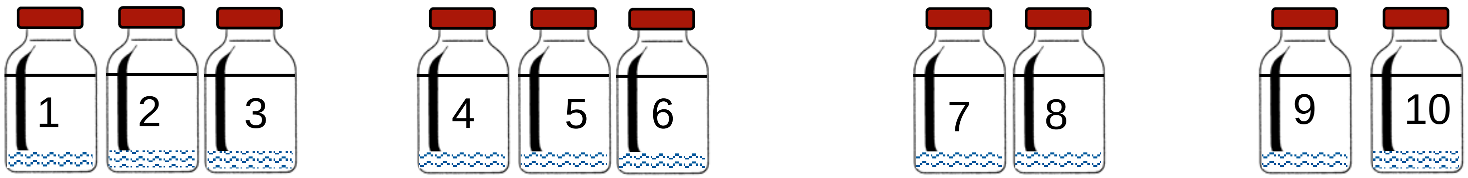
anaerobic headspace (N_2 or N_2/CO_2)

(composite) groundwater sample + resazurin

- anoxic preparation (glovebox)
- regular analysis of carbon isotope signature of CO_2 and CH_4
- incubation at 20°C since 05/2005



Experimental setup:



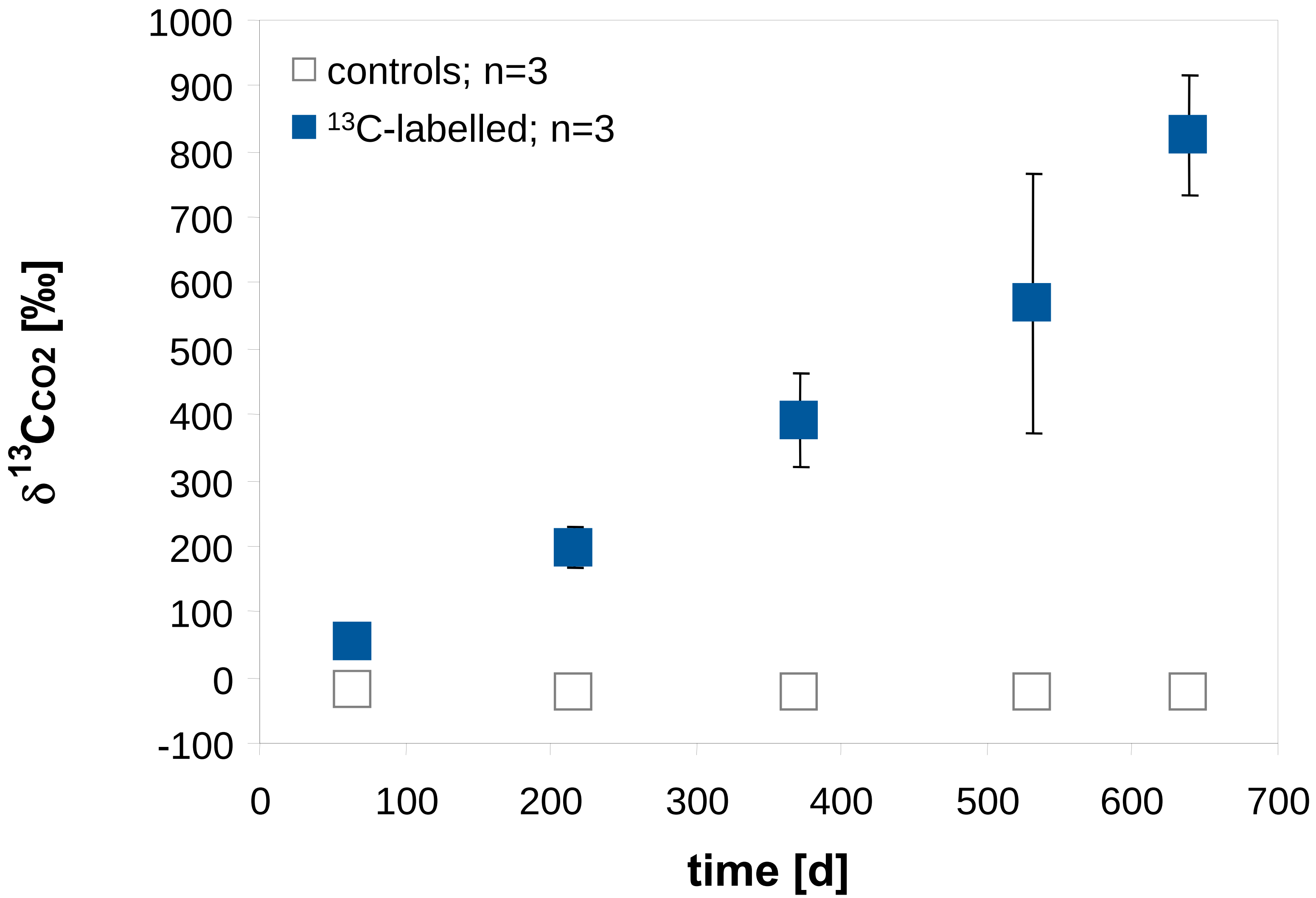
natural abundance + 1 µl ^{12}C-MCB

enriched + 1 µl ^{13}C-MCB

sterile controls + 1 µl ^{12}C-MCB

negative live control no addition

3. Ex situ microcosms results



Summary

- Application of stable isotopes for **assessment of Natural Attenuation**
- **Monitoring of in situ biodegradation** at contaminated field sites
- **Proof of anaerobic MCB degradation** by multiple lines of evidence:
  1. Stable Isotope Fractionation Analysis
  2. *In situ* Microcosm System (BACTRAP)
  3. *Ex situ* (laboratory) Microcosm Studies

⇨ NA concepts are under discussion

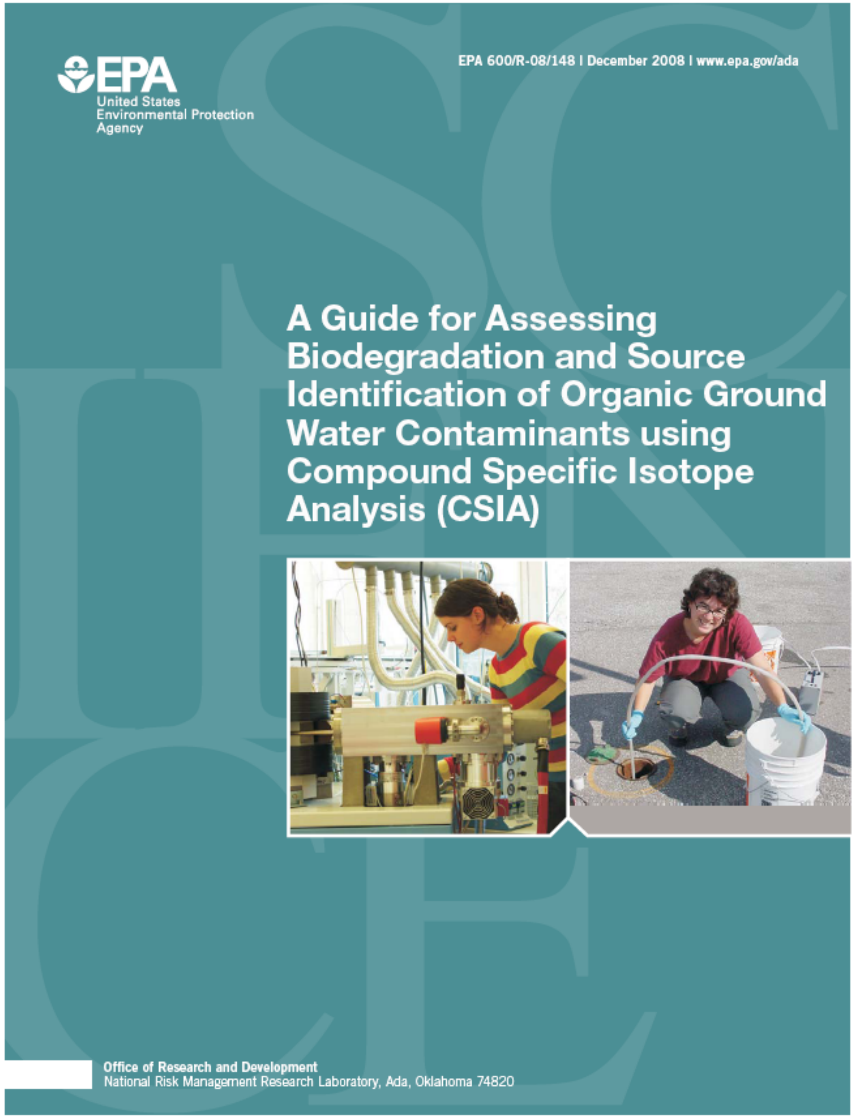
- Stable isotope tools can be applied for various contaminants

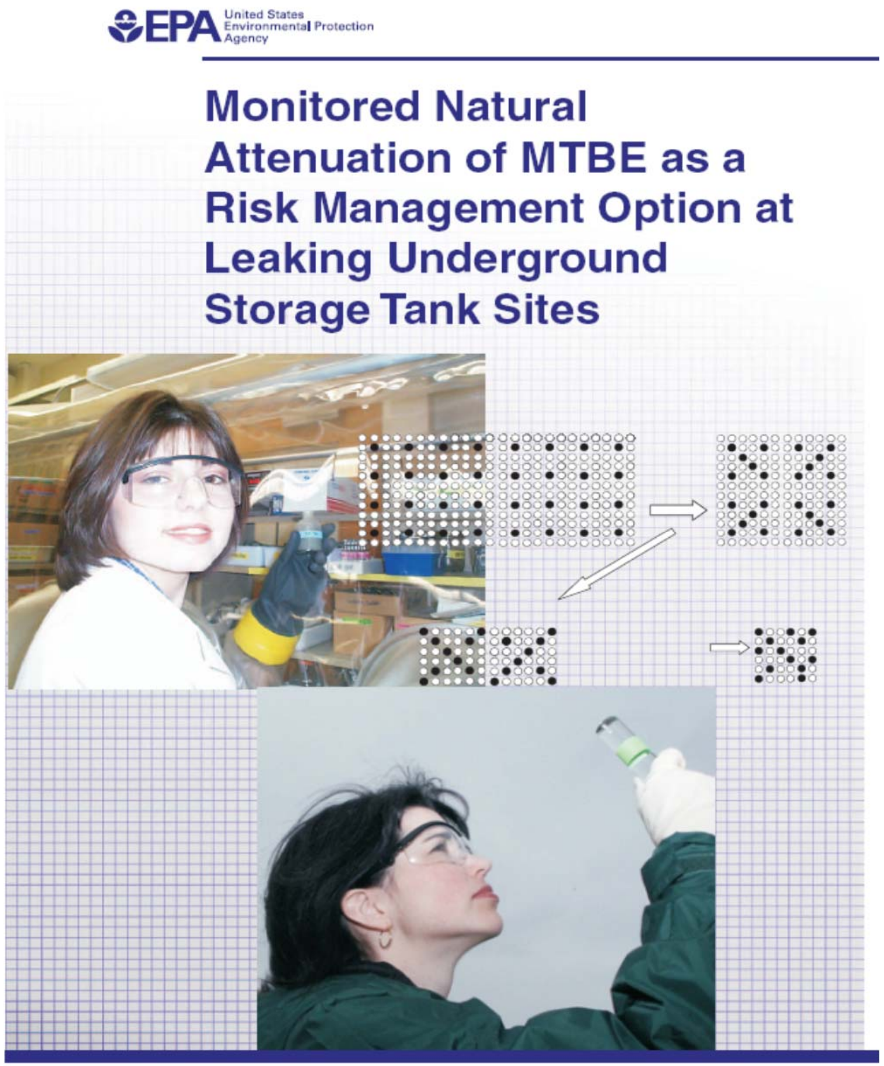
Out line

Pollutants group	**BTEX, PAH, CKW, fuel additives**
Compound	benzene, toluene, ethylbenzene, xylenes, cresol naphthalene, 2-methylnaphtalene, *n*-alkanes, PCE, TCE, DCE, VC, di-chloromethane, di/tri-chloroethane, trichlorobenzene, Lindane (HCHs) MTBE, ETBE, TBA
Enrichment factors	$^{13}C/^{12}C$ (78), D/H (13), $^{37}Cl/^{35}Cl$
Redox conditions	oxic/anoxic; nitrate/sulfate/iron(III)-reducing; methanogenic; dehalogenating
Reference	more than 30 references (PDF-files available)
Field Studies	more than 15 references (PDF-files available)

Data base of fractionation factors: www.isodetect.de

US - EPA Guidelines for the application of CSIA in remediation studies (Office of Research & Development)





Thank you for your attention!