Helmholtz Association Centre for Environmental Research - UFZ

Compound specific isotope analysis (CSIA) to characterize degradation pathway and to quantify in situ degradation of fuel oxygenates and other fuel derived contaminants

Anko Fischer, Monica Rosell[2], Max M. Häggblom[3] and Hans H. Richnow

Dpt. of Isotope Biogeochemistry, UFZ
[2]Dpt. of Environmental Chemistry, IIQAB-CSIC
[3]Dpt of Biochemistry, Microbiology, and Biotechnology, The State University of New Jersey

Out line

- Principles of isotope chemistry
- Analysis of stable isotopes
- Analysis of degradation possesses
- Quantification of in situ degradation

Major elements of organic substances

	natural abundance (atom %)		
Hydrogen	^{1}H 99.9844	^{2}D 0.0156	
Carbon	^{12}C 98.89	^{13}C 1.11	
Nitrogen	^{14}N 99.64	^{15}N 0.36	
Oxygen	^{16}O 99.763	^{17}O 0.0375	^{18}O 0.1995

Hoefs, 1997

Isotope ratio

$$\frac{^{13}C}{^{12}C} = \frac{1{,}11}{98{,}89} = R$$

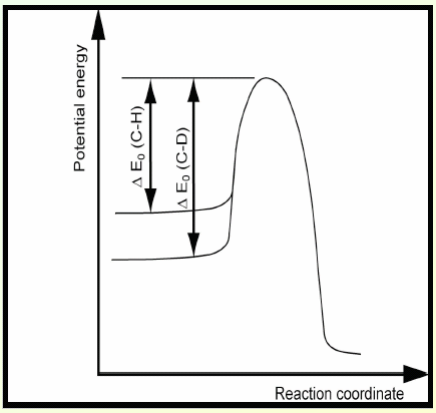
Isotope rations are reported in δ notation relative to an international standard

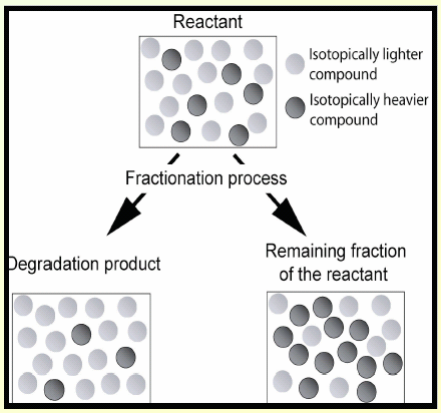
Carbon: in ‰ PDB (Belemnite, Cretaceous Peedee Formation, South Carolina)

$$\delta^{13}C\ (‰) = \left(\frac{^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{standard}}{^{13}C/^{12}C_{standard}} \right) \times 1000$$

Kinetic Isotope Effect (unidirectional reactions)

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





Stefan Harderlein, 2006

Isotope Fractionation

Kinetic isotope effect

- Irreversible/unidirectional reactions
- Isotopes move/react at different rates (smaller isotopes have greater velocity bonds with light isotopes are broken more easily)
- *Can be very substantial*

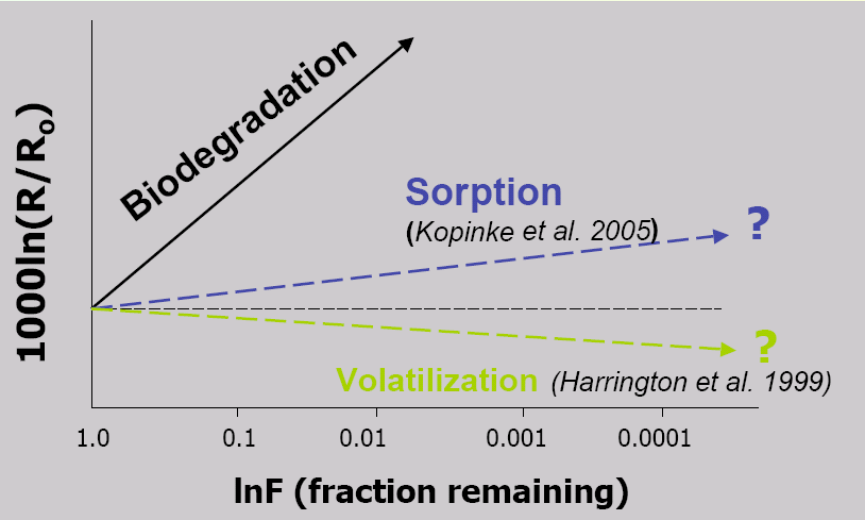
•*Thermodynamic* isotope effect

(also known as equilibrium or exchange effect)

- Reversible reactions *at equilibrium*
- Based on free energy differences (heavier isotope forms stronger bonds / accumulates in phase with stronger interactions)
- For organic molecules *often rather small*

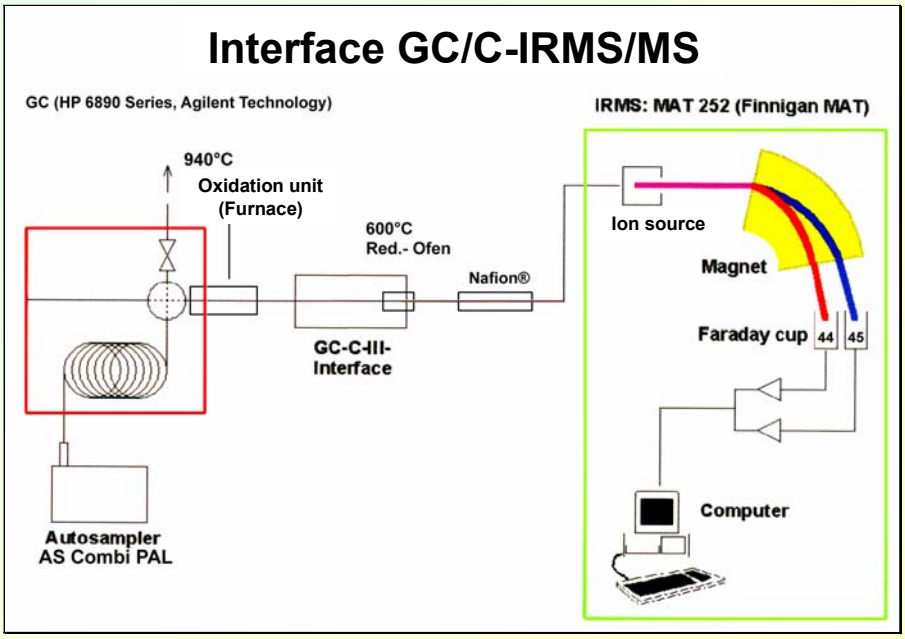
Stefan Harderlein, 2006

Challenge:
Process identification and quantification based on isotope signatures



Haderlein, 2006

Interface GC/C-IRMS/MS

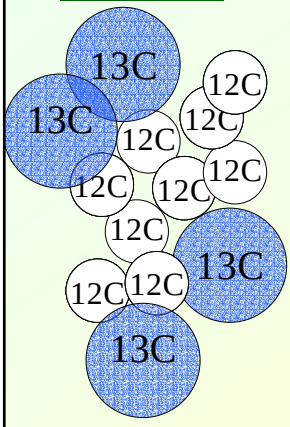


Sensitivity and Precision of GC-IRMS

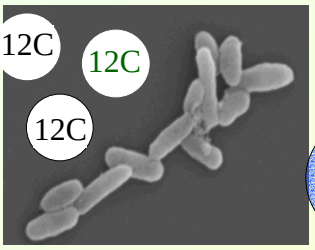
Element	Analyzed gas	[nmoL] On-column	[ng] On-column	Precision
Carbon	CO_2	0.8 nmoL C	10 ng C	0.2 ‰
Nitrogen	N_2	1.5 nmoL N_2	42 ng N	0.5 ‰
Hydrogen	H_2	15 nmoL H_2	30 ng H	3 ‰
Oxygen	CO	80 nmoL O	80 ng O	0.8 ‰

Isotope fractionation process

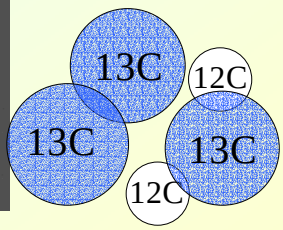
SUBSTRATE



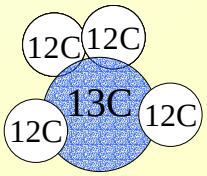
ORGANISM



RESIDUAL FRACTION



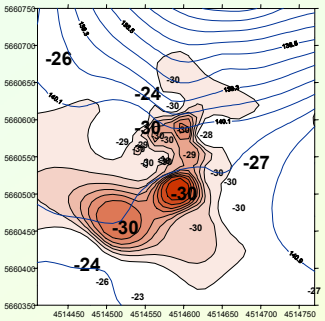
TRANSFORMATION PRODUCTS



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

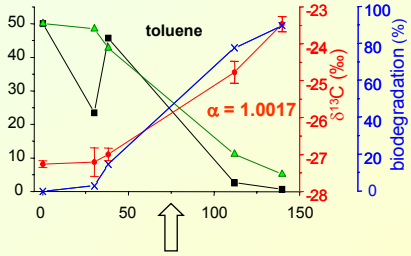
Quantification of microbial activity in aquifers using isotope fractionation

Isotope fractionation by microbial activity

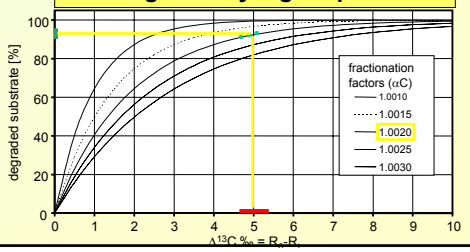


Determination of the compound specific fractionation factor (αC) in reference experiments

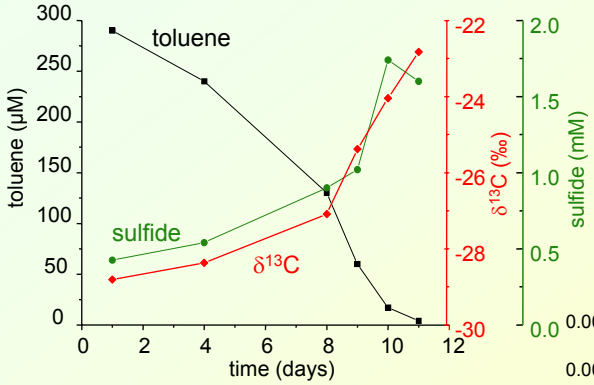
Quantification of the *in situ*-biodegradation (e.g. on a flow path)



Calculation of biodegradation using the Rayleigh equation

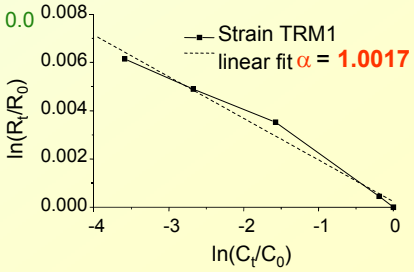


toluene degradation by sulfate-reducing bacteria



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

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

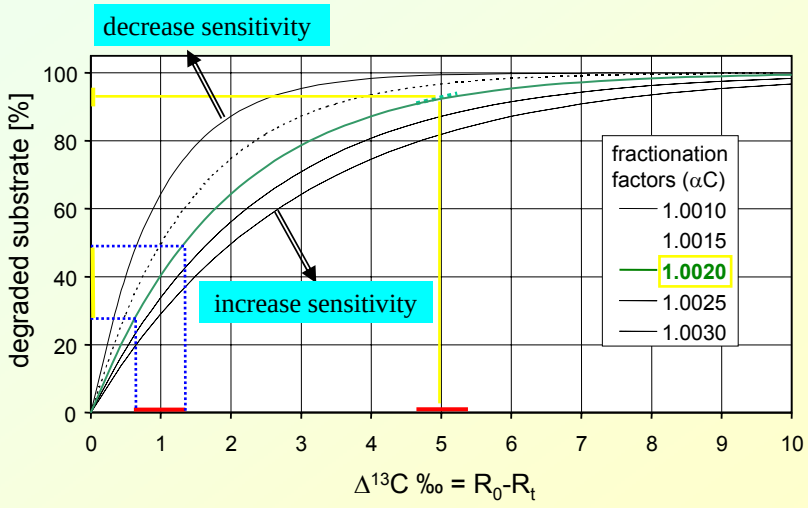


Enrichmentfactor (ε)
ε[‰] =(α-1) *1000

Meckenstock et al., (1999)
***Environmental Microbiology* 1, 409- 414**

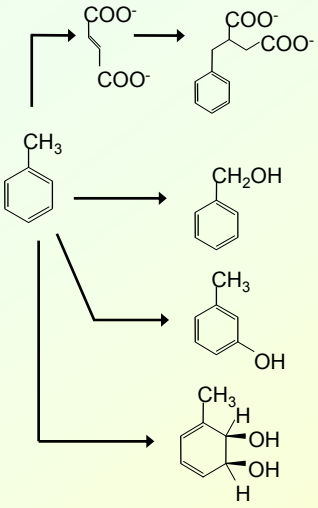
Precision of the calculation

Degradation model with various fractionation factors (αC)



Meckenstock et al., JCH, 2005

Isotopic fractionation factors (αC) of anaerobic and aerobic degradation pathways **(Morasch et al. 2001, 20002, 2004)**



Benzylsuccinate synthase pathway (Beller & Spörmann, 1994)

εC = -1.7 to -2.2 **εH = -728**

Tauera aromatica nitrate
Geobacter. Metallireducens Fe(III)
TMR1, D. cetonicum sulfate

Xylene monooxygenase pathway (Shanklin et al., 1994)

εC = -33 **εH = - 144**

(*P. putida* mt2)

Toluene-3-monooxygenase pathway (Olsen et al., 1994)

εC = -1.1 **εH = -7.7**

(*R. pikettii* PK01)

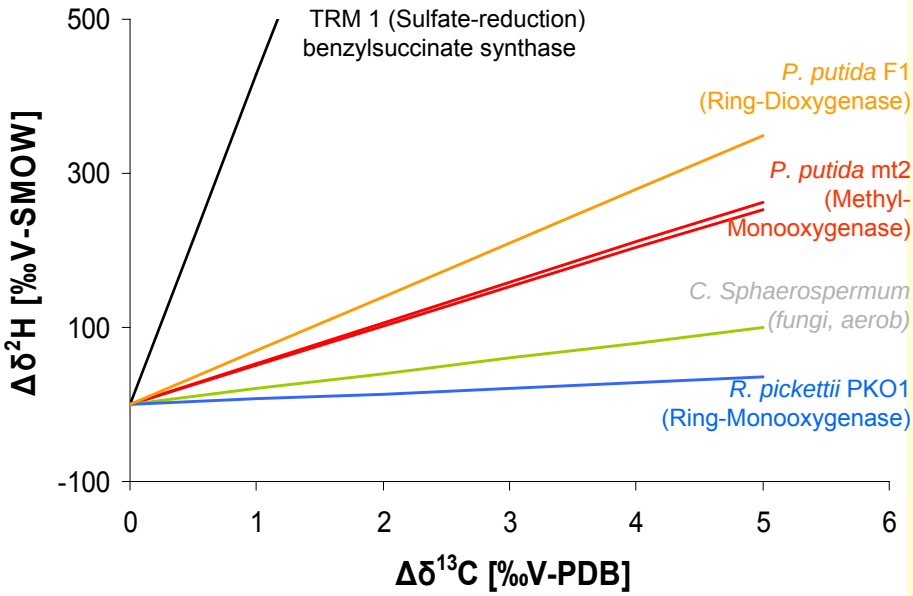
2,3 dioxygenase pathway (Yeh et al., 1977)

εC = -0.4 **εH = -28**

(*P. putida* F2)

Selection of a fractionation factor (α) representing the geochemical degradation conditions is important !

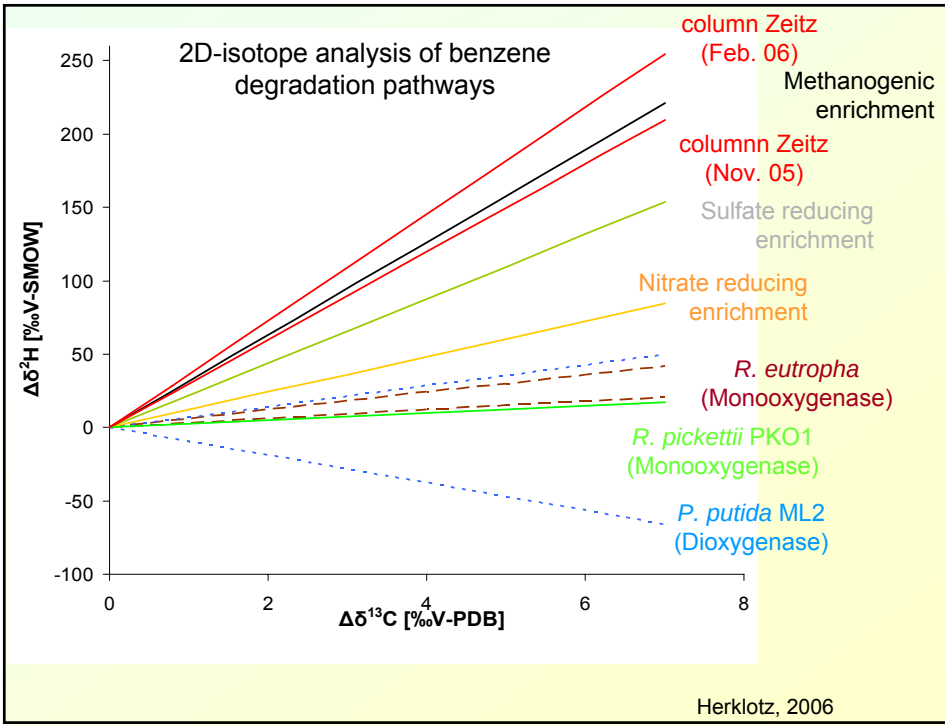
Carbon and hydrogen fractionation to analyze the toluene degradation pathway (2D-isotope analysis)



Mancini et al. 2003; Morsch et al. , 2001,2002; Meckenstock et al. 1999, Herklotz, 2006

Carbon and hydrogen fractionation of **benzene** degradation pathways

Organisms	Condition	Pathway	εC	εH	Reference
P. Putida	aerob	Dioxygenase	<-0.6	<-0.7	Herklotz 2006
R. Picketii PK01	aerob	Monoxoygenase	-1.8	-4.7	Herklotz 2006
R. Eutropha	aerob	Monooxygenase	-4.3	-19.7	Herklotz 2006
Enrichment	nitrate reducing	??	**-2.4**	**-29**	Mancini et al. 2003
Enrichment	sulphate reducing	??	**-3.6**	**-79**	Mancini et al., 2003
Enrichment	methanogenic	??	**-1.9**	**-60**	Mancini et al., 2003
Sand column	sulphate reducing	??	**-1.5**	**-45.8**	Fischer et al., 2006
Sand column	sulphate reducing	??	**-1.9**	**-70.2**	Fischer et al., 2006



Herklotz, 2006

Field study in Zeitz

- former hydrogenation plant (Zeitz, Germany)
- highly BTEX contaminated aquifer

Microbial degradation processes:

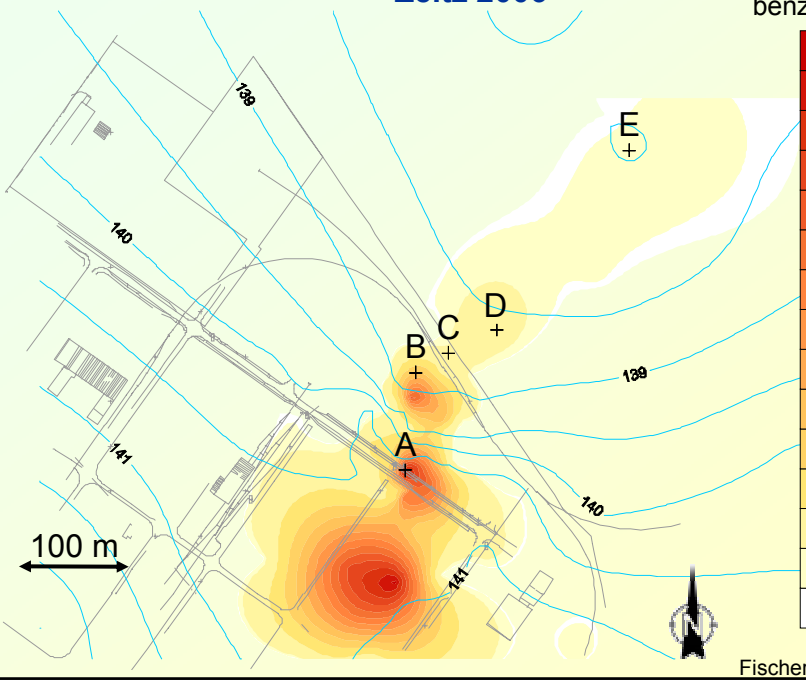
sulfate reduction

> methanogenesis

> denitrification

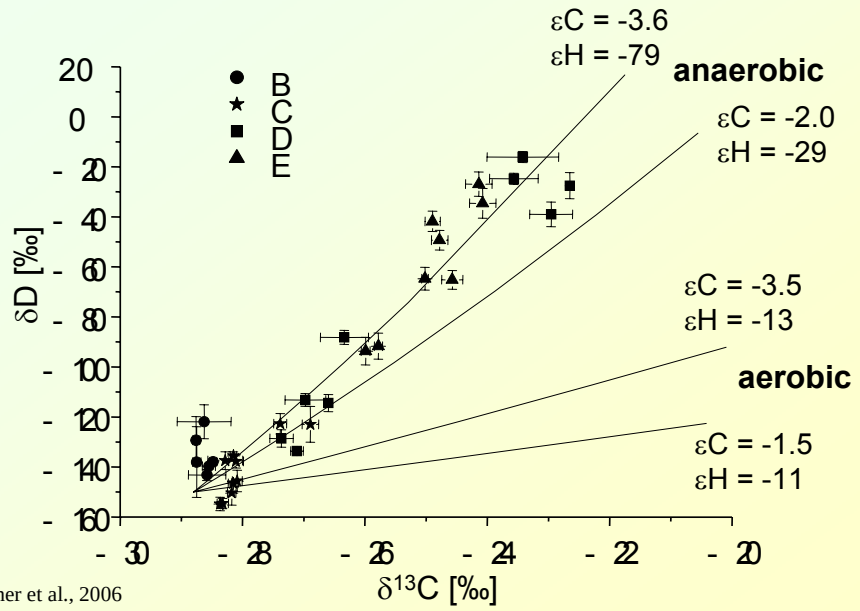
> iron reduction

Zeitz 2006



Fischer et al., 2006

Isotope fractionation of benzene along a water flow path in Zeitz



Fischer et al., 2006

Quantitative assessment using the Rayleigh Equation

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

"percentage of biodegradation" can be used as an index to characterise zones of active in-situ biodegradation

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

calculation of theoretical local concentrations is possible if a representative C_0 entering a flow path and the hydrology is known

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

calculation of theoretical concentrations entering a flow path (C_0) using measured concentration (C_t) and the isotope compositions

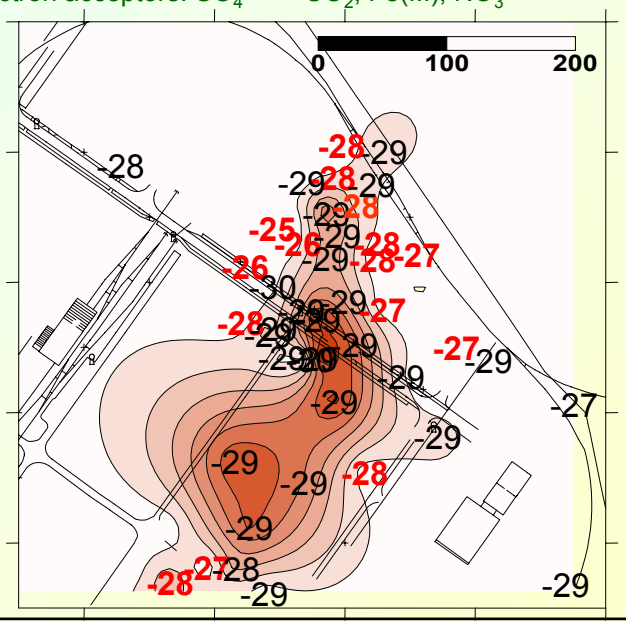
$$C_0 = \frac{C_t}{\left(\frac{R_t}{R_0}\right)^{\left(\frac{1}{\frac{1}{\alpha}-1}\right)}}$$

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

Concentration and isotope composition of benzene

Hydrogenation plant, Zeitz

electron acceptors: SO_4^{2-} >> CO_2, Fe(III), NO_3^-

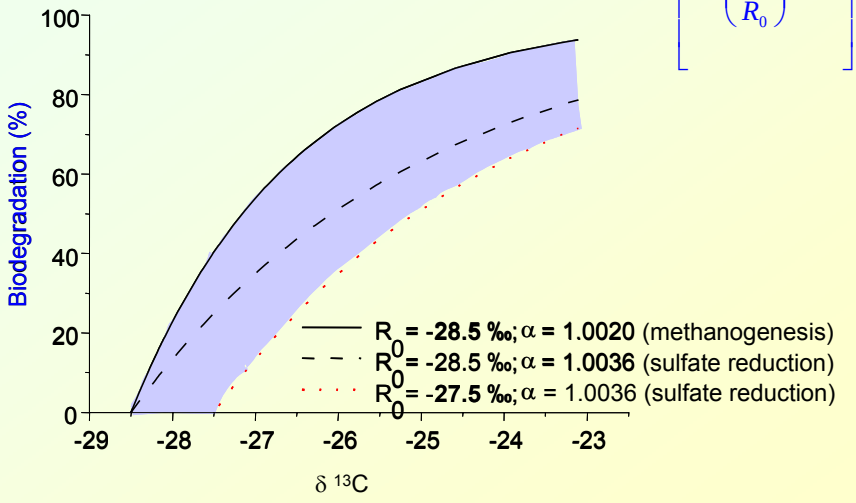


Vieth et al. 2005, ET&C

Uncertainty of *in situ* biodegradation taking into account

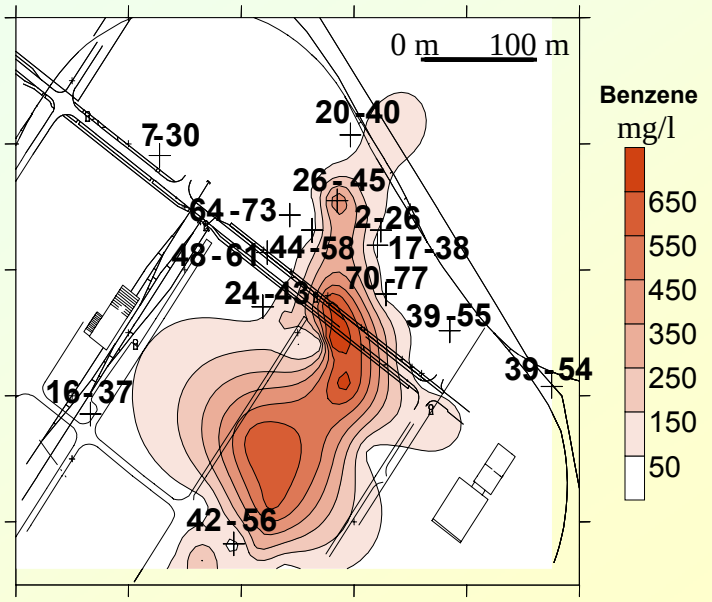
- isotope variability of the source ($\mathbf{R_0}$)
- variability of isotope fractionation factor (α)

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



Vieth et al. 2005, ETC

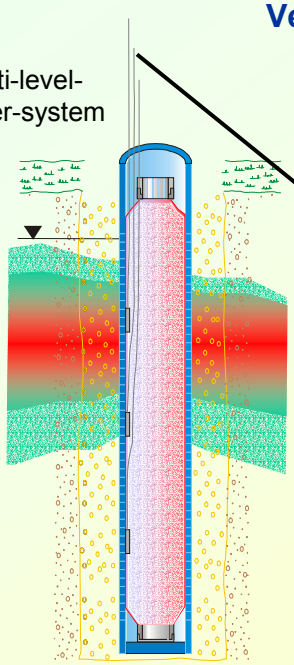
Biodegradation [%] of benzene in sulfidogenic aquifer (Zeitz)



∗ α = 1,0036 (Mancini et al., 2003) source (R_0) = -27,5 to -28,5 ‰

Vertical plume analysis

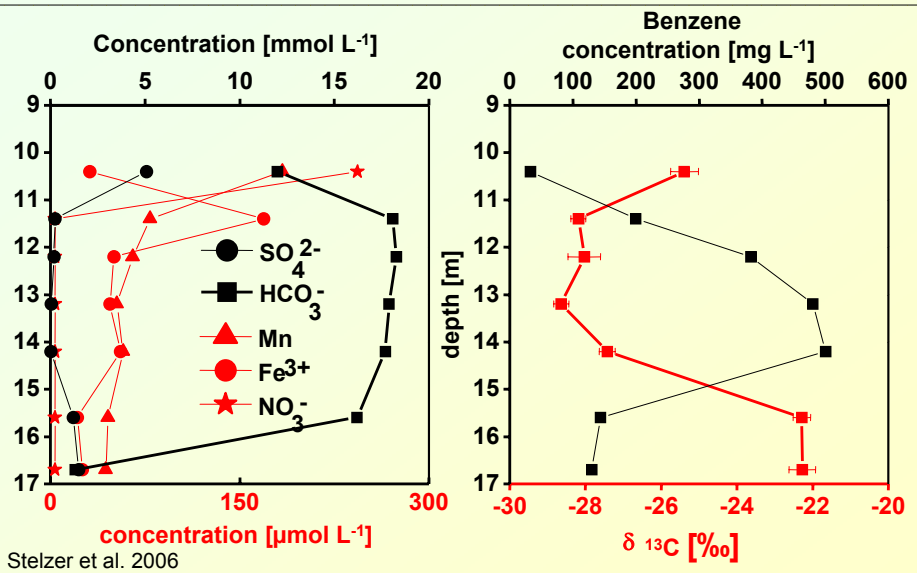
Multi-level-packer-system



Schirmer et al., 1995

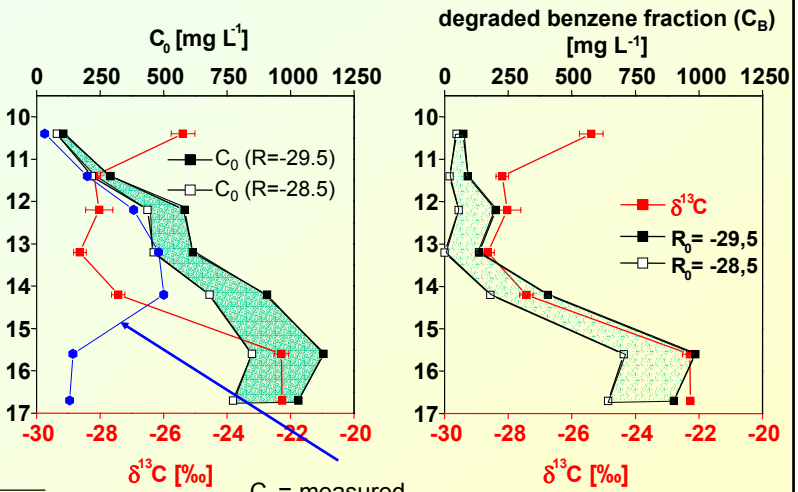
Geochemical conditions, concentrations and isotope composition of benzene

Multi level well 23/00



Stelzer et al. 2006

Calculation of the source concentration **(C_0)** using measured concentrations (C_t) and the isotope composition (R_0 and R_t) + isotope fractionation factor (α)



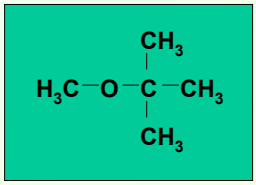
$$C_0 = \frac{C_t}{\left(\frac{R_t}{R_0}\right)^{\left(\frac{1}{\frac{1}{\alpha}-1}\right)}}$$

C_t = measured concentration

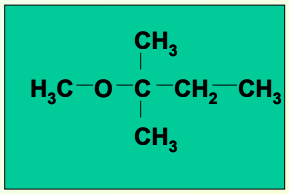
$$C_B = C_0 - C_t$$

α = **1.0036** for sulfate reducing conditions
(Mancini et al. 2003)

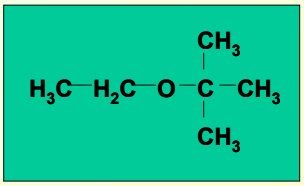
Fuel oxygenates tested for biodegradation and isotope fractionation under aerobic anaerobic biodegradation



methyl *t* -butyl ether (MTBE)



t -amyl methyl ether (TAME)

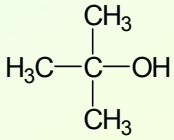


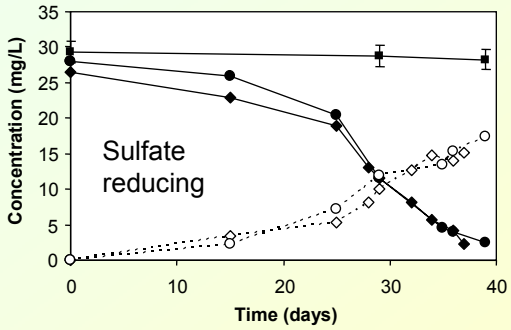
ethyl *t*-butyl ether (ETBE)

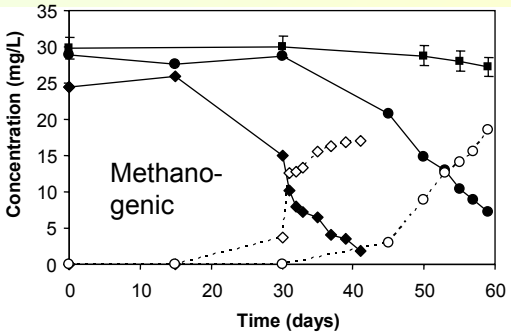
Ether type fuel oxygenates are relatively recalcitrant under both aerobic and anaerobic conditions

- alkyl ethers are relatively stable
- the tertiary carbon structure is relatively resistance to microbial attack

Stoichiometric formation of TBA indicate an ether cleavage during MTBE degradation



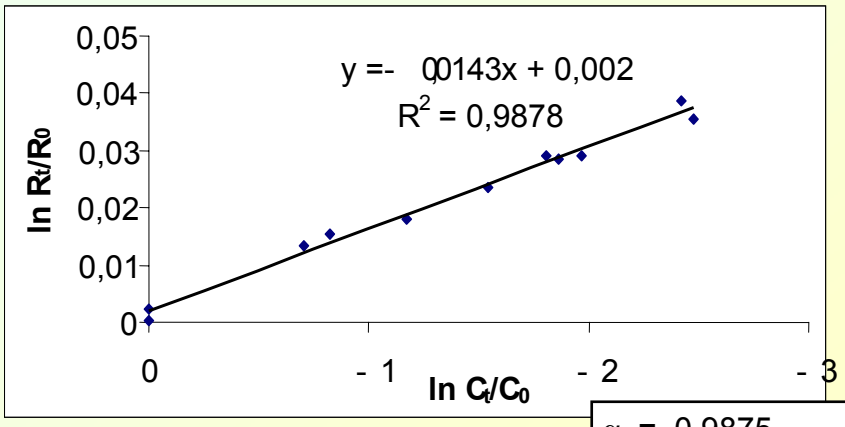




Somsamak et al. 2005

$^{13}C/^{12}C$ Fractionation of sulfidogenic MTBE Degradation

- sulfidogenic enrichment cultures derived from Coronado located on the San Diego Bay in California (USA)



α_C= 0.9875

ε_C= -14.3 ± 1.1 ‰

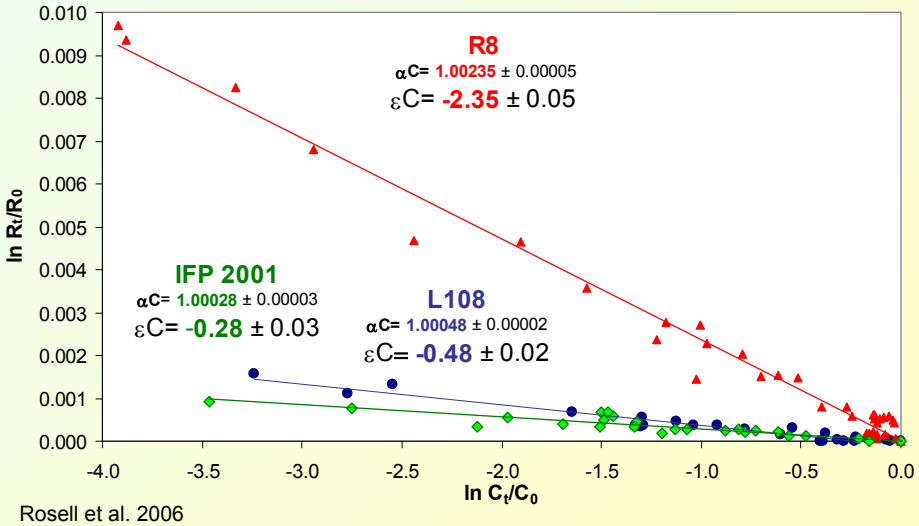
Somsamak et al. 2005

Isotopic enrichment factors (ε) for anaerobic biodegradation of MTBE.

Source	Condition	ε (‰)	R2	n	References
Arthur Kill	Sulfate reducing,	-14.5 ± 2.5	0.9783	7	
	duplicates	-13.9 ± 5.6	0.8900	7	
Coronado Cays	Sulfate reducing,	-14.4 ± 1.5	0.9814	6	
	two enrichments	-14.0 ± 1.5	0.9914	8	
Coronado Cays	methanogenic,	-14.4 ± 3.6	0.9948	5	Somsamak et al. 2005
	two enrichments	-13.7 ± 1.5	0.9925	7	
Arthur Kill	methanogenic	-15.6 ± 4.1	0.9662	6	Somsamak et al. 2005
	with inhibitor of	-14.6 ± 5.2	0.8634	9	
	methanogenesis				
All data, ε (‰) ± 95% Confidence Interval		**-14.4 ± 0.7**	**0.9690**	**55**	
anaerobic laboratory microcosms		-9.16 ± 5.0	0.728		Kolhatkar et al. 2002
anaerobic field		-8.10 ± 0.9	0.946		Kolhatkar et al. 2002
anaerobic laboratory enrichment		-13.0 ± 1.1			Kuder et al. 2005

Somsamak et al. AEM 2006.

MTBE carbon isotope fractionation under aerobic conditions



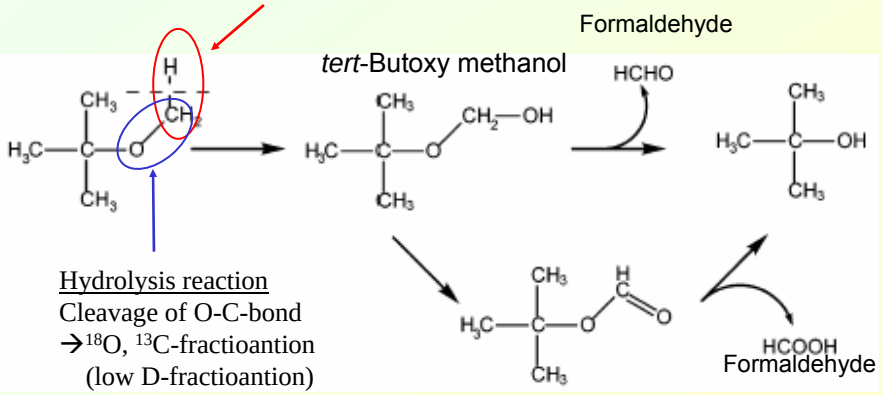
Rosell et al. 2006

MTBE

Culture	εC[‰]	εH [‰]	Reference
Enrichment culture (Borden aquifer, Canada)	-1.52 to -1.97	na	Hunkeler et al. 2001
VAFB mixed consortium, CA	-1.5 to -1.8	-29 to -66	Gray et al. 2002
strain PM1, Los Angeles	-2.0 to -2.4	-33 to -37	Gray et al. 2002
strain R8	-2.35	-40	Rossel et al. 2006
strain L108	-0.48	No enrichment (-0.2)	Rossel et al.2006
strain IFP 2001 (resting cells)	-0.28	No enrichment (+5)	Rossel et al. 2006
anaerobic microcosm	-8.6	-16	Kuder et al., 2005
methanogenic/ sulfate recucing enrichment cultures	-14.6	n.d.	Somasmak et al., 2005/06

Aerobic pathways for initial attack on MTBE

Monooxygenase reaction
Cleavage of H-C-bond → D, ^{13}C-fractioantion



tert-Butyl formate
(TBF)

Hunkeler et al., 2001; Zwang et al., 2005

In situ stable isotope fractionation at a refinery site

Analysis of 21 wells and 1 ML well

MTBE conc. 0.29 to 7200 mg/L

Constant carbon and hydrogen isotop signatures close to the source:

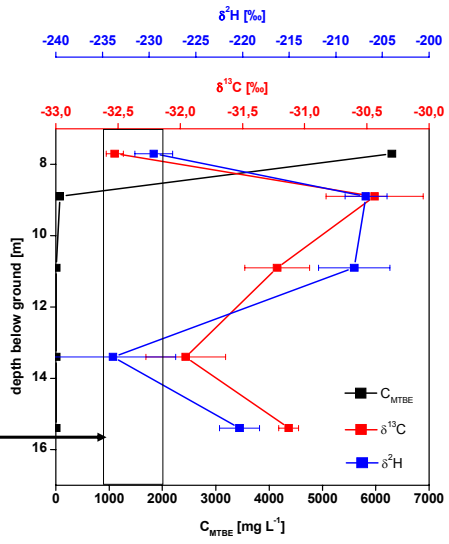
$\delta^{13}C$ = -32.7 ± 0.2 ‰

$\delta^{2}H$ = -238.5 ± 3.1 ‰

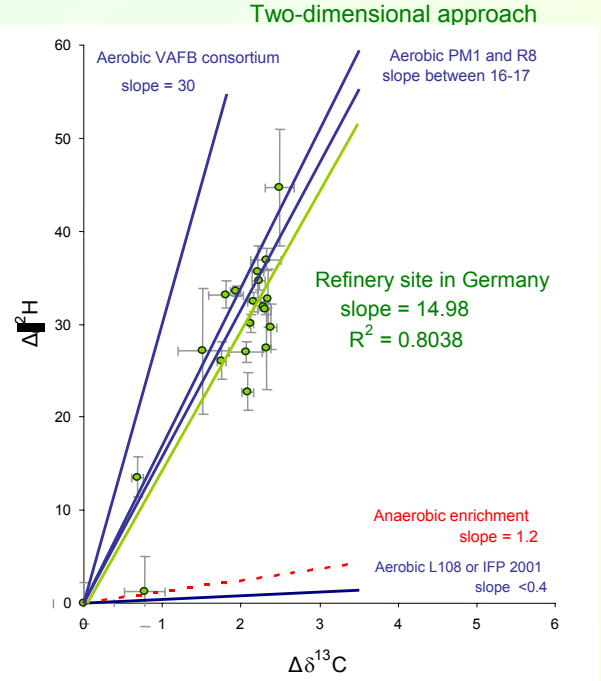
Only fractionation in well P25 (0.29 mg/l

$\delta^{13}C$ = -30.98 ± 0.60 ‰

Multilevel well P26



Two-dimensional approach



Rossel, 2006 unpublished

Isotope fractionation has been applied to evaluate ground water contamination by

- fuel related compounds (BTEX and MTBE)
- chlorinated solvents
- halogenated aromatic compounds

(see Meckenstock et al. 2004, JCH, for an recent overview)

Uncertainties associated to assess in situ biodegradation in the course of a contamination plume

- analytical uncertainty determining the isotope ratio (R_t; R_0) and concentration (C_t;C_0)
- selection of a representative fractionation factor (α)
- determination of a representative source concentration (C_0)
- variability of the isotope composition of the source (R_0)
- aquifer inhomogeneities and associated mixing processes

Acknowledgements

Funding:

DBU

Virtual Institute „VIBE“

HELMHOLTZ GEMEINSCHAFT

Many cooperatives and partner !

AXIOM

MARIE CURIE FELLOWSHIPS Marie Curie Fellowships

Marie Curie
host development fellowship

Isotope Biogeochemistry

DFG and BMBF

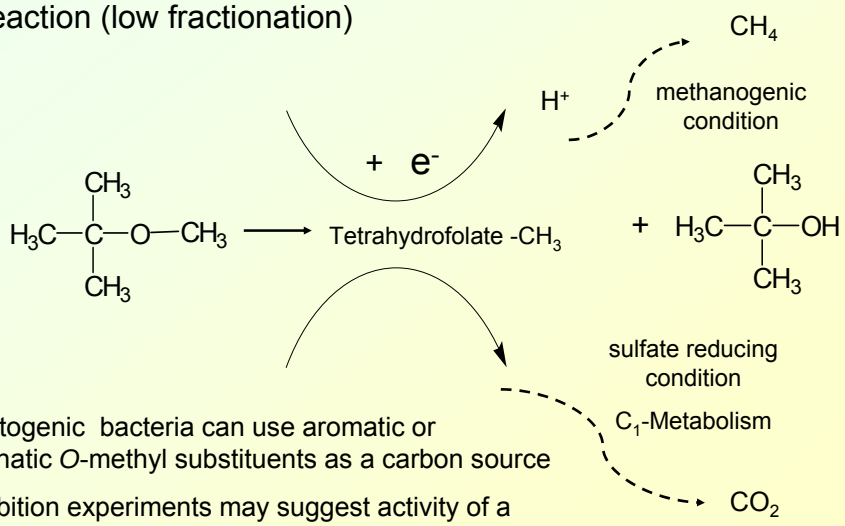
Clients from the industry:

DMT; Roth & Partner;
GICON; HPC and others

Reductive Ether Cleavage ???

- relatively high εC = 8.6 to 16 relatively low εH = 16

→ Hydrogen is not directly involved in the kinetic control of reaction (low fractionation)



- Acetogenic bacteria can use aromatic or aliphatic *O*-methyl substituents as a carbon source
- Inhibition experiments may suggest activity of a synthropic microbial consortia

Summary and Perspective

Isotope fractionation processes (αC, αD) can be used to characterise anaerobic and aerobic BTEX and MTBE degradation processes in the field

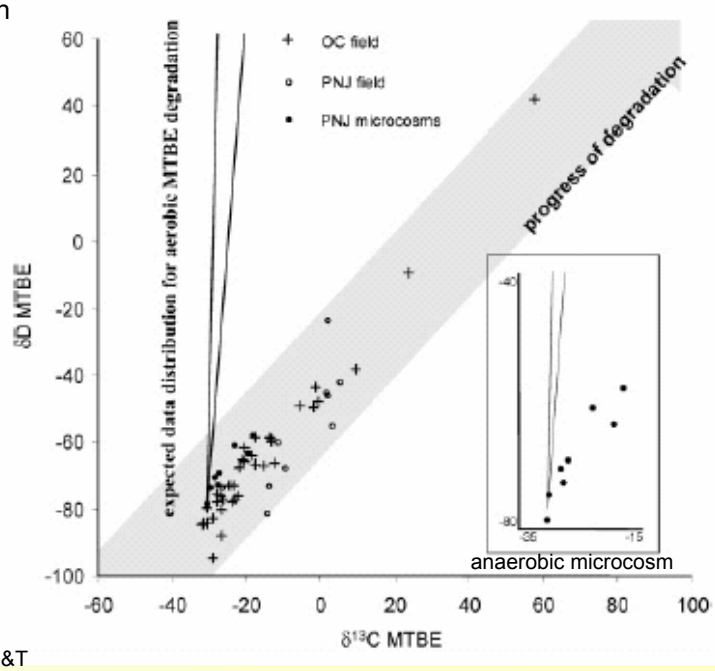
Potential for monitoring operation and quantification of in situ biodegradation

More systematic work is needed on isotope fractionation of fuel oxygenates to obtain fractionation factors (αC, αD)

We need to understand degradation pathways and the physiology of anaerobic microbial in situ communities

Indication for anaerobic biodegradation by carbon and hydrogen isotope fractionation

Evaluation of 3 anaerobic field sites in the US



Kuder et al., 2005 ES&T