Biotic and abiotic degradation studies of organic contaminants

Manuela Peschka

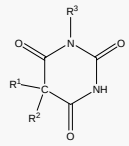
Ankara, 8th October 2007

EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

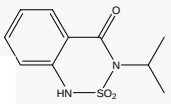
Outline

- **Organic pollutants**
  - *Selected compounds*
- **Simulation of abiotic / biotic degradation**
  - *Fixed bed bioreactor*
  - *Bottle test*
  - *Suntest*
- **Analysis of micopollutants and identification of transformation products**
  - *Sample pretreatment*
  - *Analytical Instrumentation*
- **Results:**
  - *Case study I: barbiturates*
  - *Case study I: photodegradation of bentazone*
  - *Case study II: biodegradation of a new fluorosurfactant*
  - *Case study IV: biotransformation of clotrimazole*

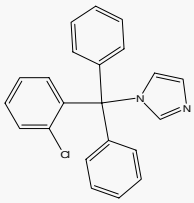
EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES



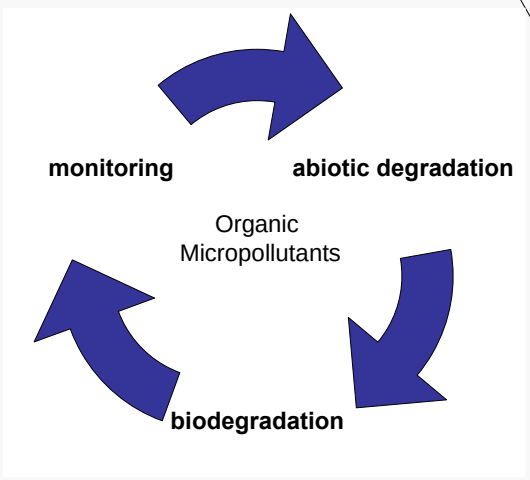
barbiturates

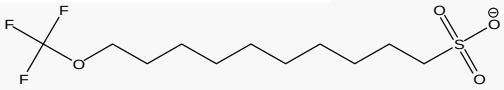


bentazone



clotrimazole



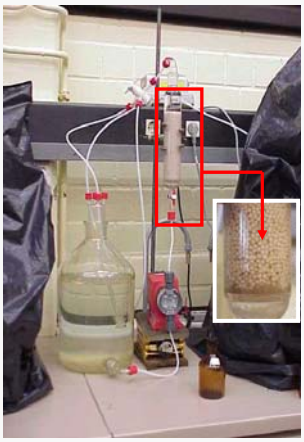


10-(trifluoromethoxy) decane-1-sulfonate

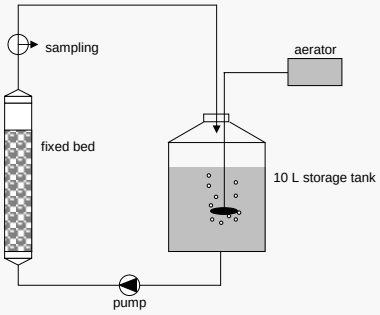
EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Degradation Tests:

EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

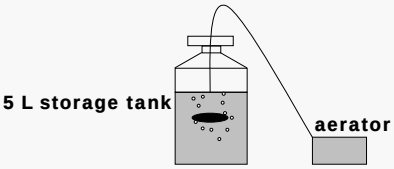


Fixed Bed Bioreaktor



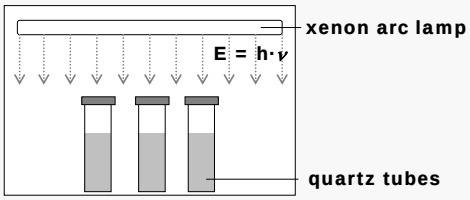
EUROPA FACHHOCHSCHULE
FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Bottle Test



EUROPA FACHHOCHSCHULE
FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

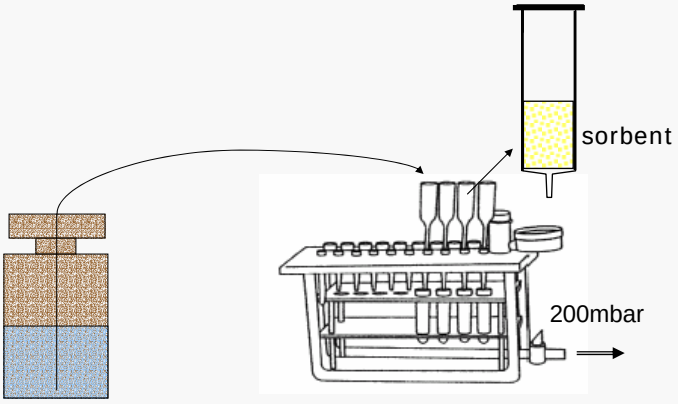
Suntest – simulation of abiotic photodegradation



EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Sample Pretreatment:

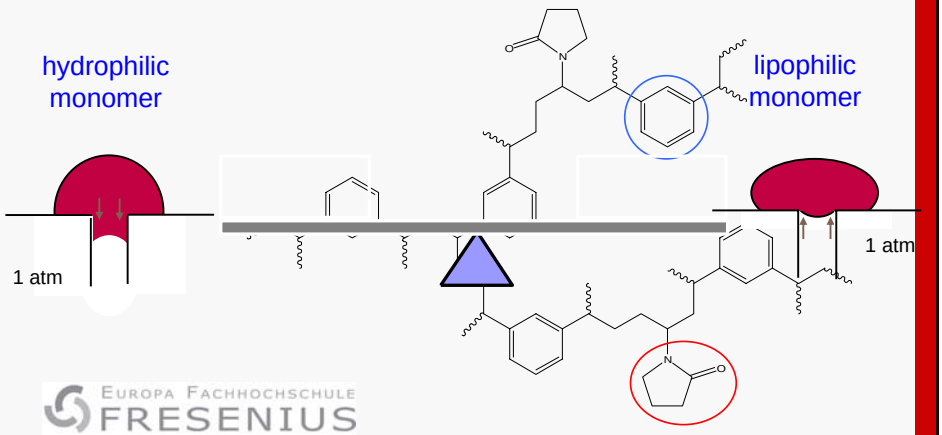
EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES



EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

OAIS® HLB

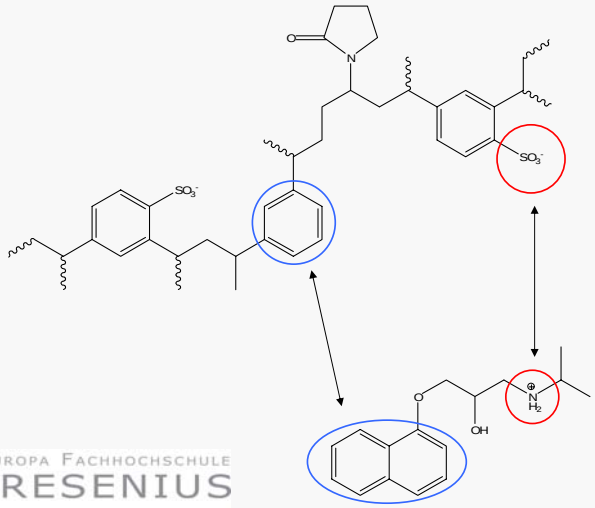
Reversed Phase material with **H**ydrophilic – **L**ipophilic **B**alance
A = 810 m^2/g
pore diameter = 80 Å
V = 1,3 cm^3/g
particle diameter = 60, 30, 25, 15 und 5 µm



EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

OAIS® MCX

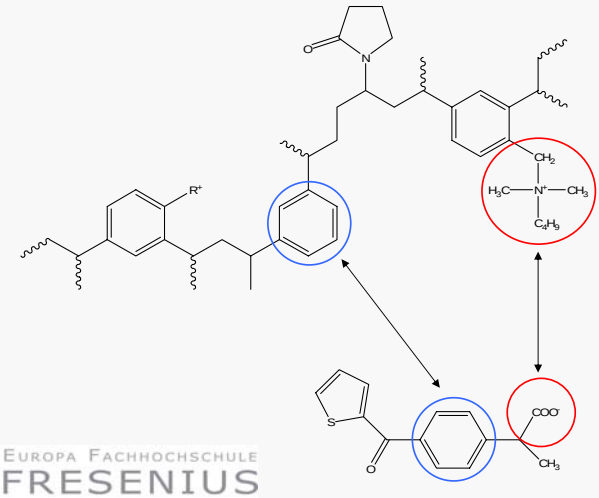
Mixed-Mode **C**ation e**X**change and Reversed Phase
1,0 meq/g SO_3H Ion exchange capacity



EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

OAIS® MAX – Phasenchemie

Mixed-Mode **A**nion e**X**change and Reversed Phase

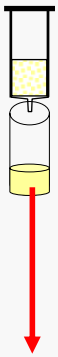


EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Elution (for non polar SPE)

- typical solvents:
  - methanol
    - methanol with acid
    - methanol with base
  - acetonitrile
  - acetic acid
  - THF
  - acetone
  - dichlormethan, chloroform
  - hexane

increasing elution potential



A B

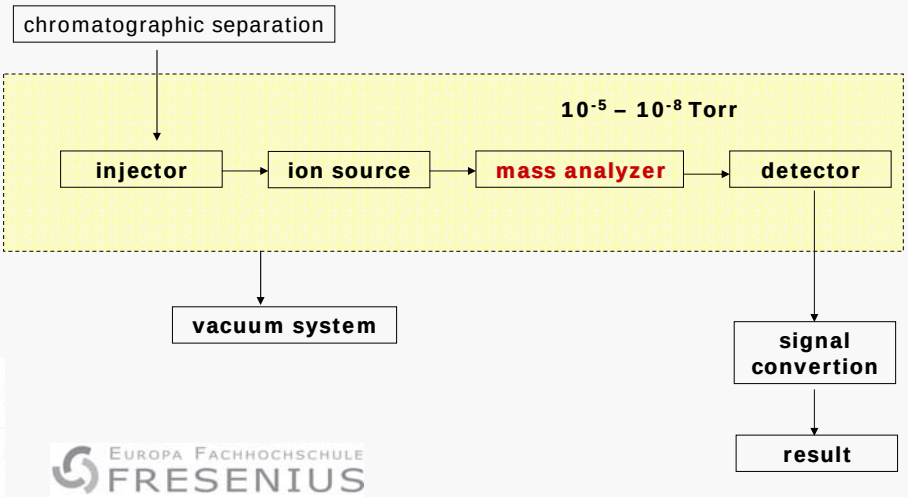
Analysis

EUROPA FACHHOCHSCHULE FRESENIUS UNIVERSITY OF APPLIED SCIENCES

Analytical Instrumentation:

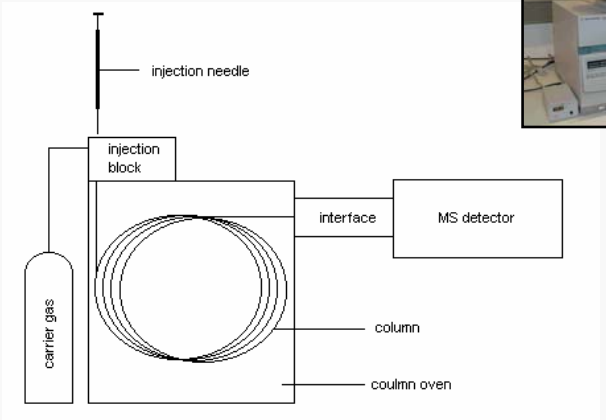
EUROPA FACHHOCHSCHULE FRESENIUS UNIVERSITY OF APPLIED SCIENCES

General setup of a mass spectrometer

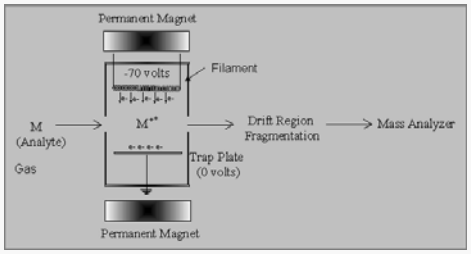


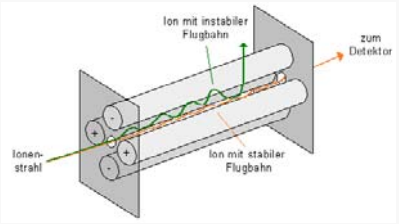
EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

GC-MS



EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES



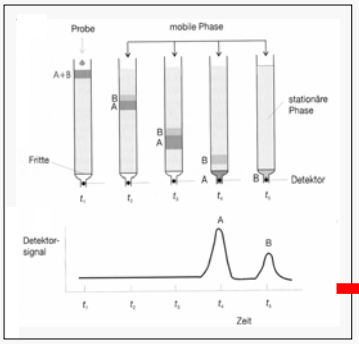


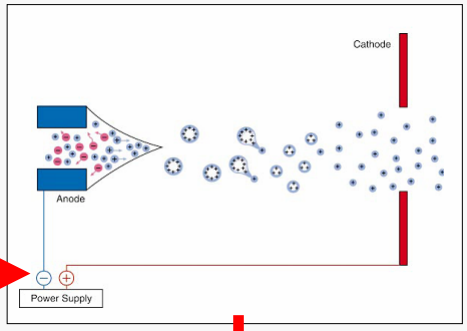
Advantage: utilization of mass spectra libraries
strong fragmention gives structural information

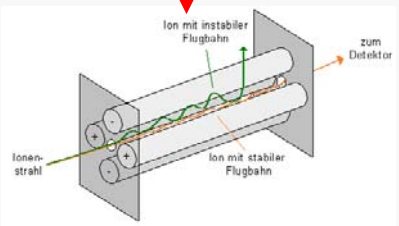
Disadvantage: single MS
EI only in combination with GC

EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

LC-MS







EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Advantage: - analysis of polar, thermolabile compounds
- very robust for routine analysis

Disadvantage: - no spectral libraries
- matrix effects

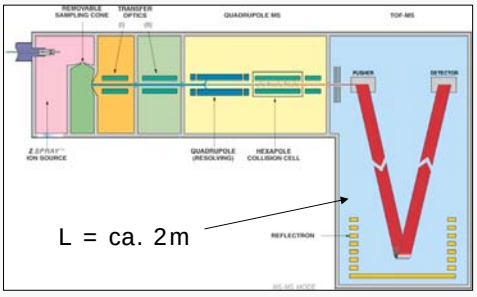
EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Quadrupole Time of Flight Mass Spectrometer

$$E_{kin} = \frac{1}{2} * m * v^2 = z * e * U$$

$$\frac{1}{2} * m * \left(\frac{1}{2}\right)^2 = z * e * U$$

$$\frac{m}{z} = (2 * e * U) * \frac{t^2}{L^2}$$



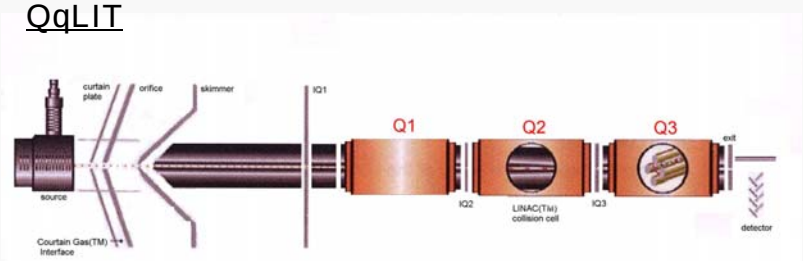
EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Advantage: - high resolution, e.i. determination of up to the fourth dezimal (e.g. 255.0432 amu)
- determination of the elemental composition
- MS²

Disadvantage: - not sensitive

EUROPA FACHHOCHSCHULE FRESENIUS UNIVERSITY OF APPLIED SCIENCES

QqLIT



- user can select various modi, e.g. Q1MI, MRM, product ion scan
- enhanced modi: Q3 acts as trap resulting in higher sensitivity

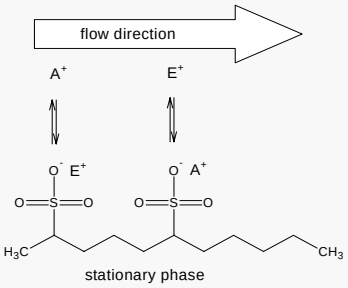
Advantage: - structure elucidation of unknown compounds
- high sensitivity

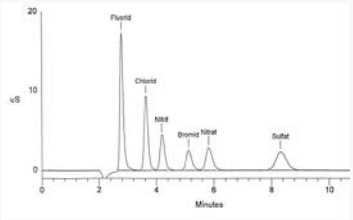
Disadvantage: ?

EUROPA FACHHOCHSCHULE FRESENIUS UNIVERSITY OF APPLIED SCIENCES

Ion chromatography

-determination of inorganic paramter, e.g. SO_4^{2-}, Cl^-, F^-
- mineralization of a molecule





EUROPA FACHHOCHSCHULE FRESENIUS UNIVERSITY OF APPLIED SCIENCES

monitoring

LC-MS

GC-MS

(IC)

structure elucidation

QqLIT

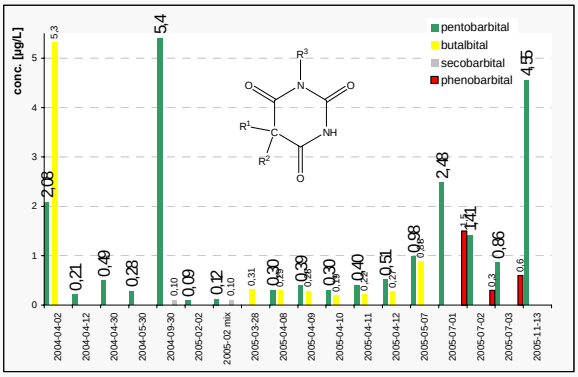
QqTOF

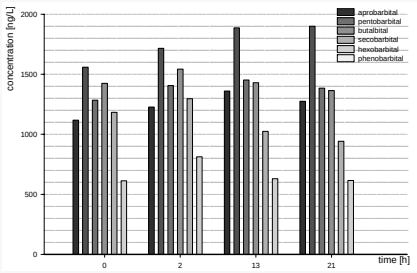
EUROPA FACHHOCHSCHULE FRESENIUS UNIVERSITY OF APPLIED SCIENCES

Case studies:

EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Barbiturates





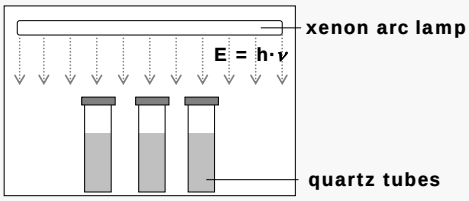
~~Fixed Bed Bioreactor~~
~~Exposion to sunlight~~
~~Hydrolysis (pH 2 – 12)~~

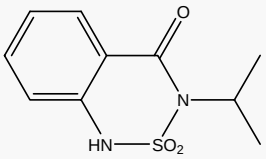
→ groundwater in Berlin

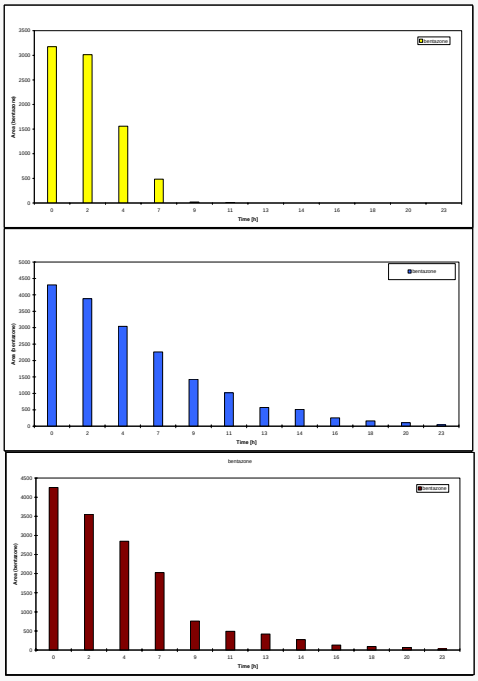
Bentazone

- one of the most applied herbicides in the Ebro river delta
- 31 µg/L in application time from May to August (mean value) (Ebro 0.27 µg/L)
- comparable high concentrations river waters of the Tiber region in Italy

- not biodegradeable in a fixed bed bioreactor
- passes through membrane bioractor
- present in ground- and surface water





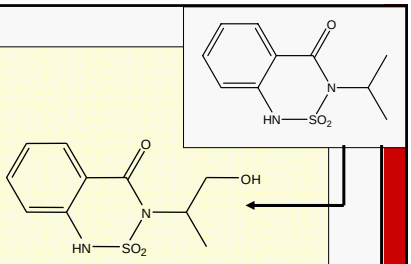


A – distilled water

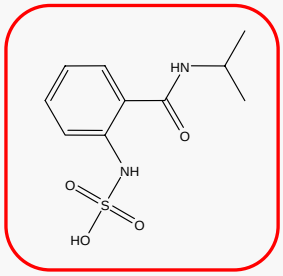
B - moderately hard water

C – moderately hard water
plus 5 µg/mL humic acids

observed mass (m/z)	elemental composition actual mass	Δ mDa
255.0432 ± 0.0013	$C_{10}H_{11}N_2O_4S$ m/z = 255.0440	- 0.8
197.0044	$C_7H_5N_2O_3S$ m/z = 197.0021	2.3
191.0826	$C_{10}H_{11}N_2O_2$ m/z = 191.0821	0.5
132.0320	$C_7H_4N_2O$ m/z = 132.0324	- 0.4

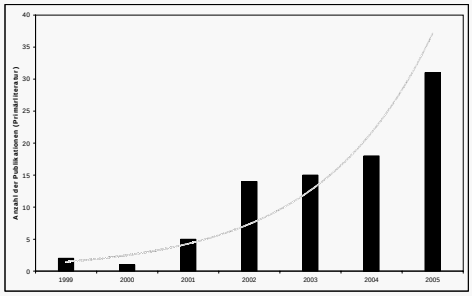


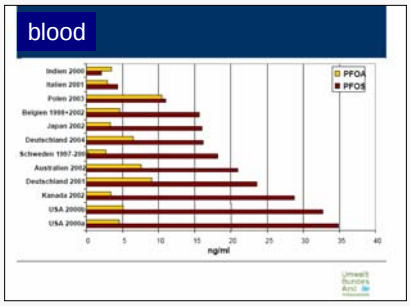
observed mass (m/z)	elemental composition actual mass	Δ mDa
257.0584 ± 0.0034	$C_{10}H_{13}N_2O_4S$ m/z = 257.0596	- 1.2
177.1024 ± 0.0003	$C_{10}H_{13}N_2O$ m/z = 177.1028	- 0.4
92.0497	C_6H_6N m/z = 92.0500	- 0.3



present in drainage channels in the Ebro delta

Fluorosurfactants

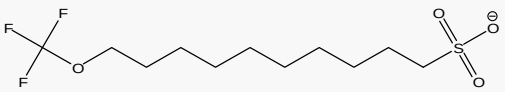




properties:

- persistent to hydrolysis, oxidation, reduction and biodegradation
- accumulate in liver, kidneys, mussel tissue and bind to blood proteins

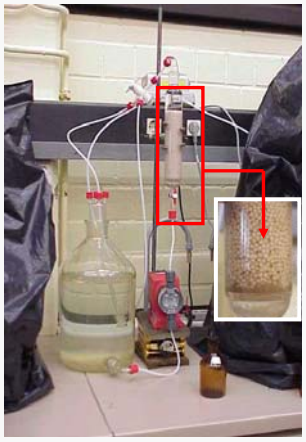
EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES



Biodegradation in a Fixed Bed Bioreaktor

10-(trifluormethoxy)decane-1-sulfonate

β = 100 mg/L
V = 5 L surface water
pH 7
T = room temperature (20°C)

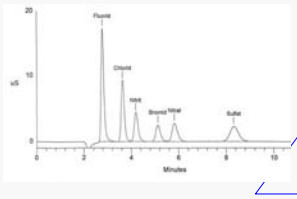


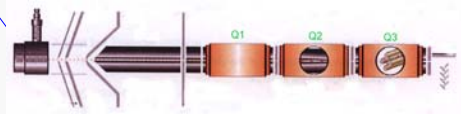
EUROPA FACHHOCHSCHULE FRESENIUS UNIVERSITY OF APPLIED SCIENCES

Analytical methodologies

Q-Trap

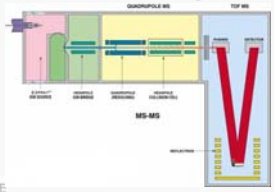
MS³-experiments





Ion Chromatography
F^-, SO_4^{2-}, TOC

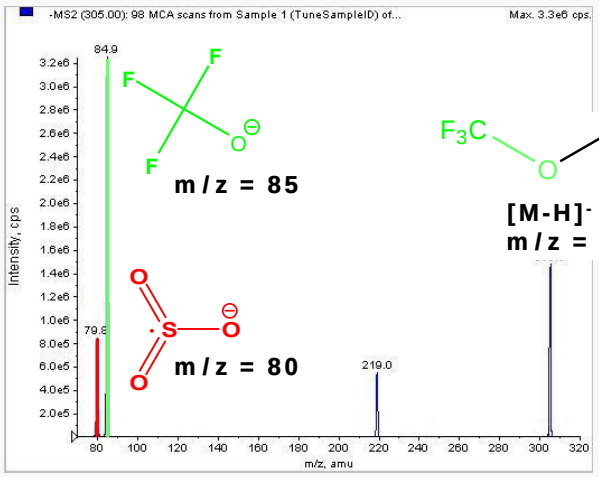
Q-ToF



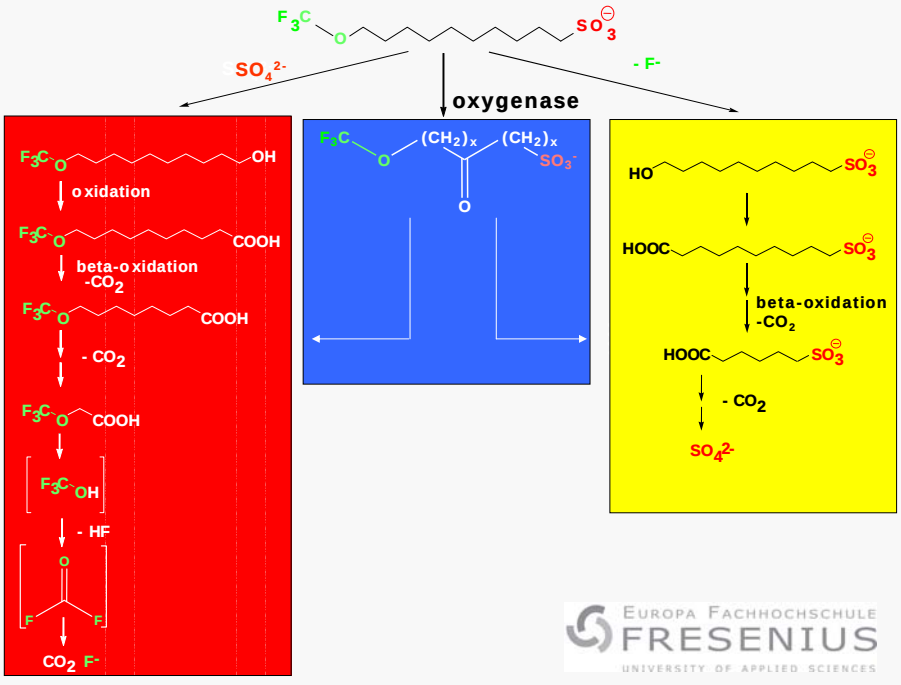
high resolution
mass spectrometry

EUROPA FACHHOCHSCHULE FRESENIUS UNIVERSITY OF APPLIED SCIENCES

mass spectrum of 10-(trifluormethoxy)-decane-1-sulfonate

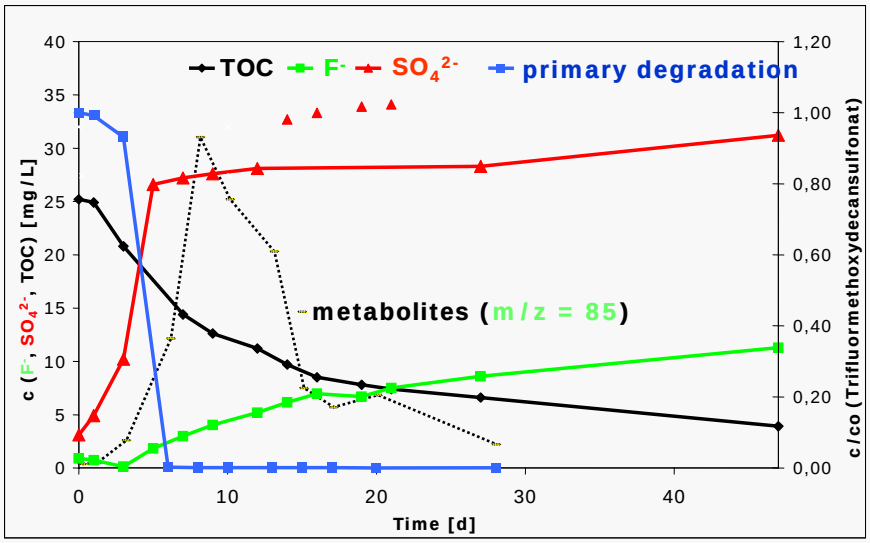


EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

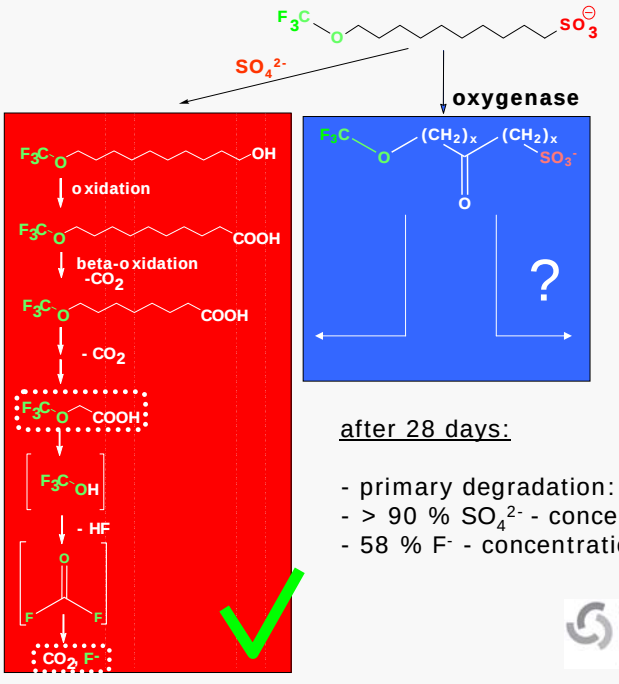


EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Monitoring of TOC, F^- and SO_4^{2-}



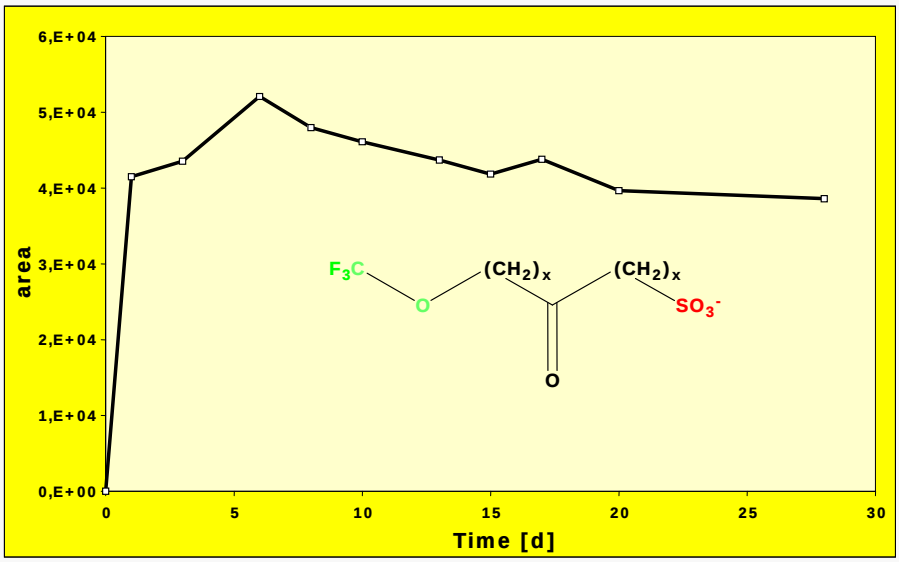
Europa Fachhochschule FRESENIUS
University of Applied Sciences



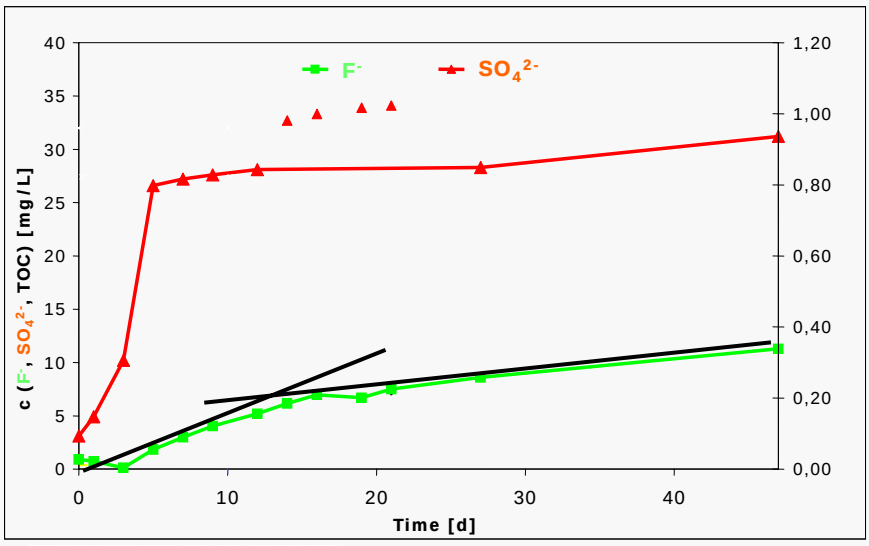
after 28 days:

- primary degradation: 100%
- > 90 % SO_4^{2-} - concentration$_{theoret.}$
- 58 % F^- - concentration$_{theoret.}$

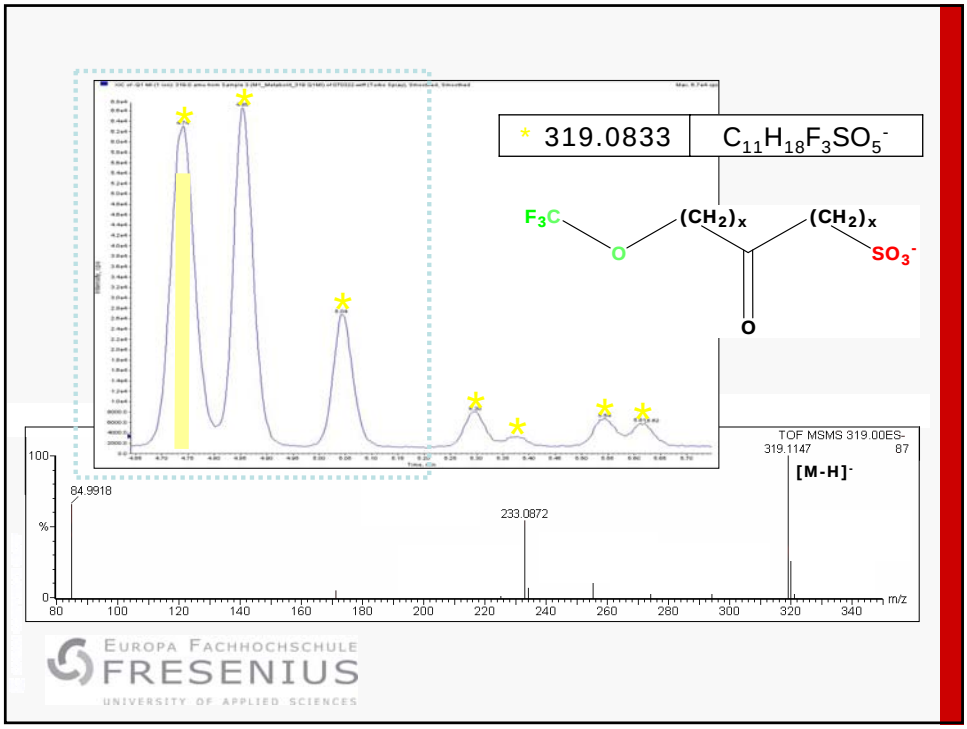
Europa Fachhochschule FRESENIUS
University of Applied Sciences



EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

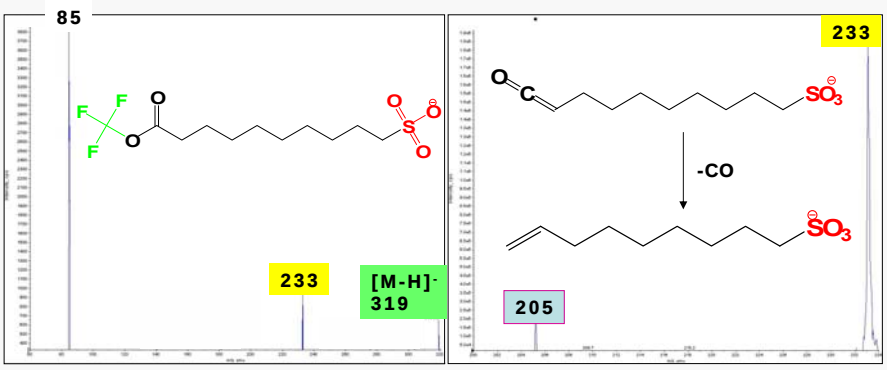


EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES



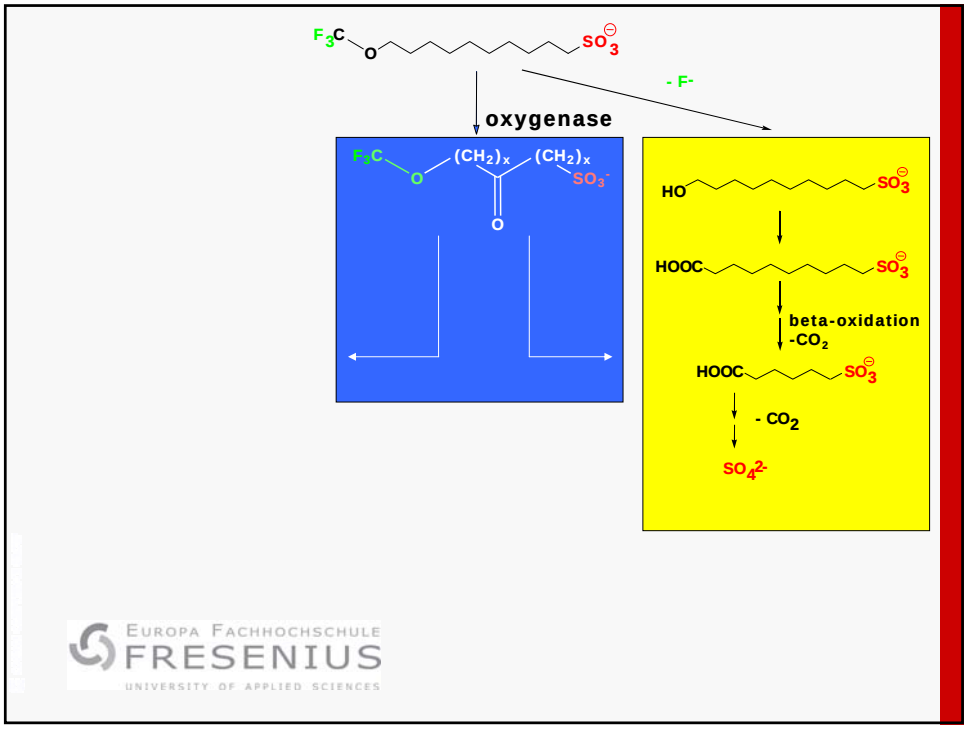
EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

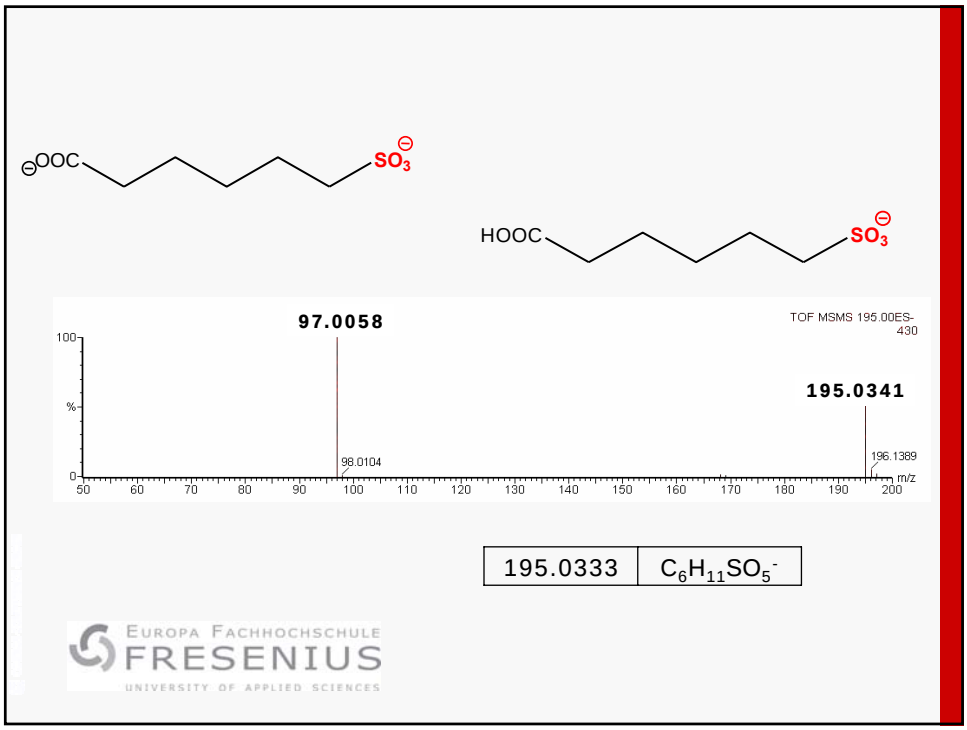
MS² **MS³**



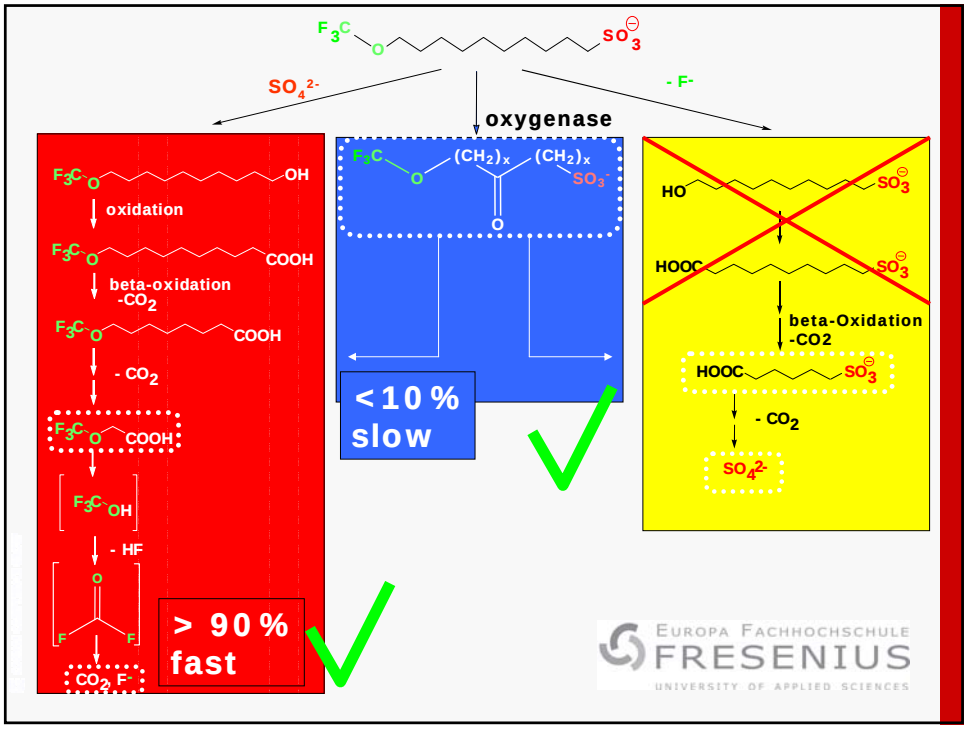
oxidation in omega-position!

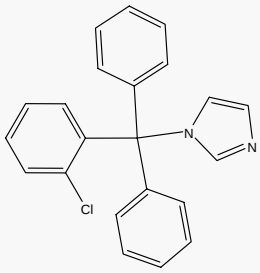
EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

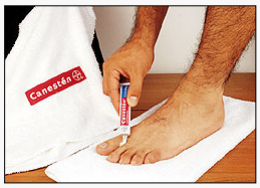




195.0333	$C_6H_{11}SO_5^-$





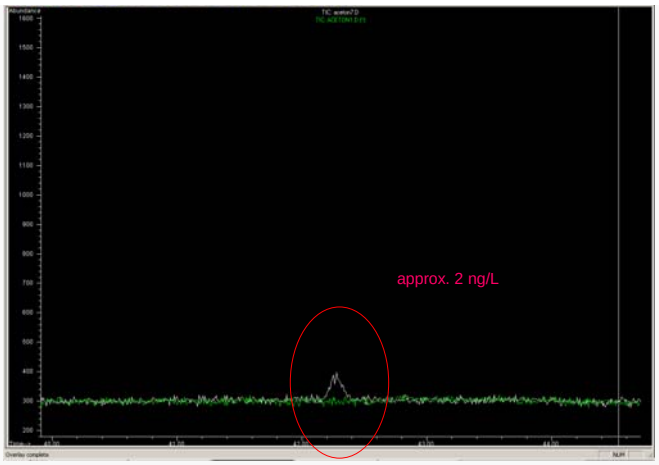


Mode of action:
Inhibitor of ergosterol synthesis resulting in the damage of cell membrane.

MW [g/mol]	344.84
$logK_{OW}$	4.1
S_{water} [mg/L]	0.49
Vp [Pa]	$3.31*10^{-7}$
mp [°C]	141 – 145
bp [°C]	494

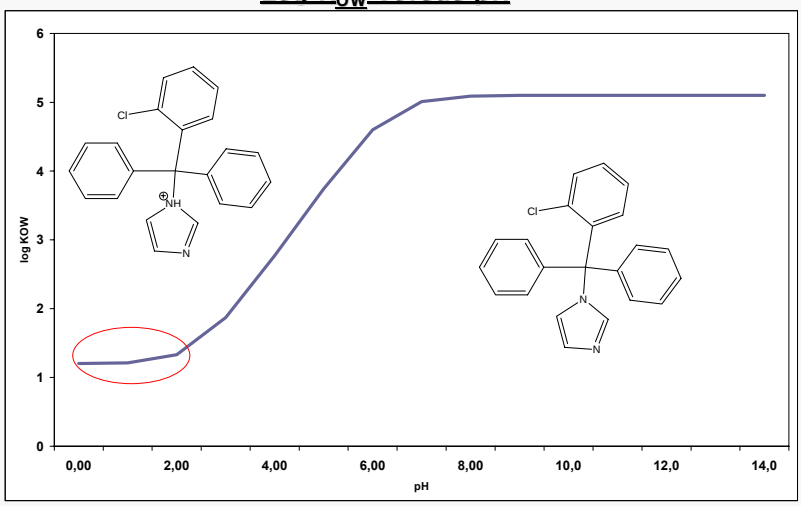
EUROPA FACHHOCHSCHULE
FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

False positive signal in next four measured samples (solvent - acetone)!



EUROPA FACHHOCHSCHULE
FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Log K_{OW} versus pH



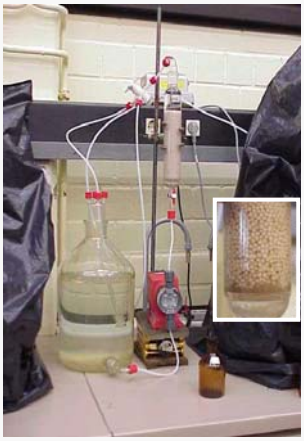
EUROPA FACHHOCHSCHULE
FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

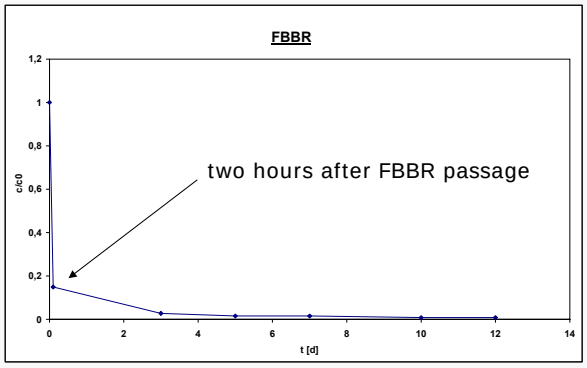
adapted SPE-Method

- Acidification to **pH 2** (stirring 30 min) of samples before filtration
- SPE-Material Oasis HLB 3cc (60 mg), Waters
- conditioning: 1 x 2 mL n-hexan, 3 x 2 mL methanol
  5 x 2 mL groundwater (**pH 2**)
- SPE: 100 ng Atrazin D5 (internal standard)
  flow approx. 20 mL/min
- drying: 45 min (N_2)
- elution: 4 x 1 mL acetone
- evaporation: to 150 µL
- external standard: 100 ng Fluazifop-buthyl (ESTD) and fill up to 200µL final volume with acetone

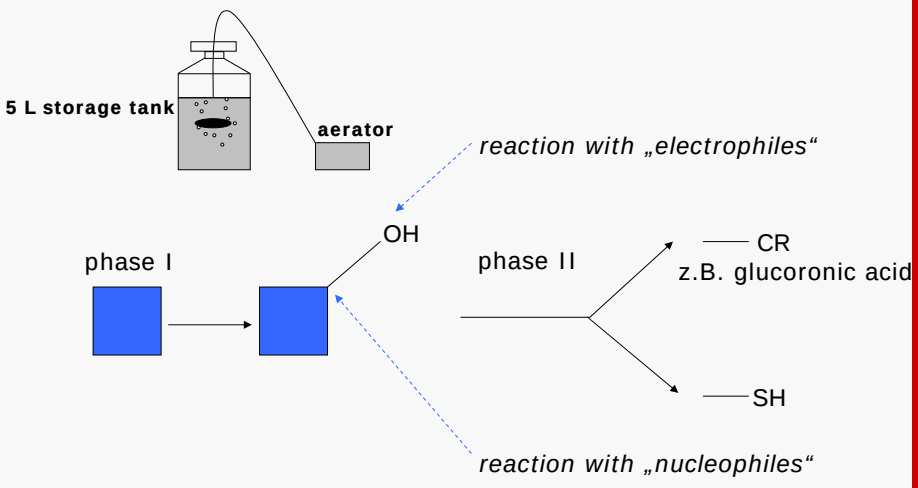
EUROPA FACHHOCHSCHULE FRESENIUS UNIVERSITY OF APPLIED SCIENCES

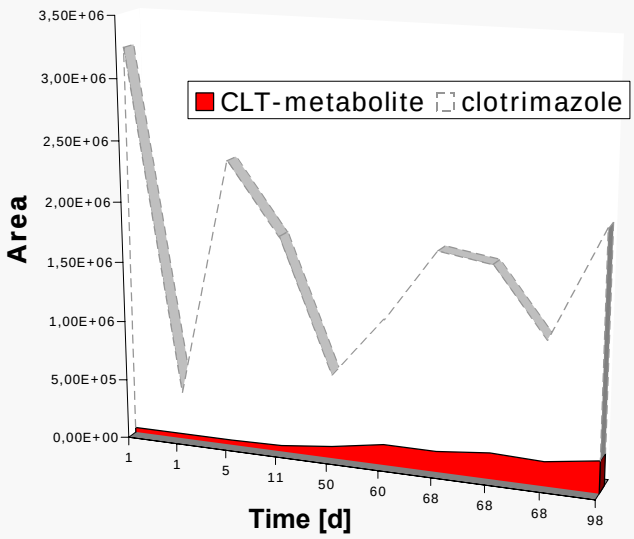
Adsorption hinders standardized biodegradation tests

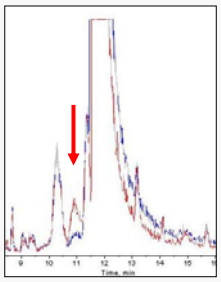


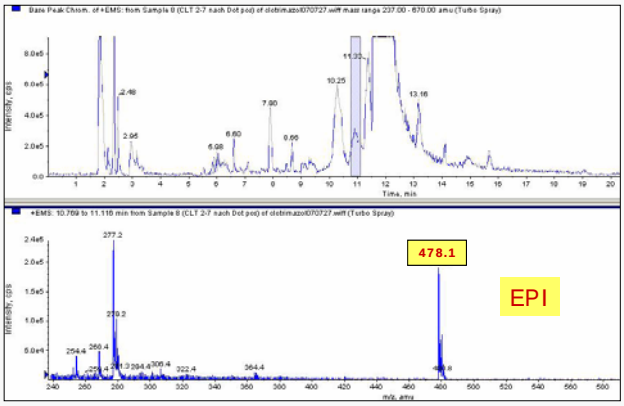


EUROPA FACHHOCHSCHULE FRESENIUS UNIVERSITY OF APPLIED SCIENCES

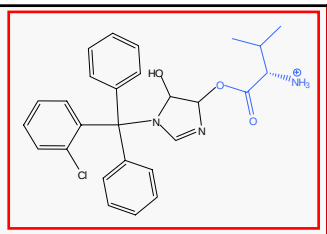

5 L storage tank
aerator
reaction with „electrophiles“
OH
phase I
phase II
CR
z.B. glucoronic acid
SH
reaction with „nucleophiles“

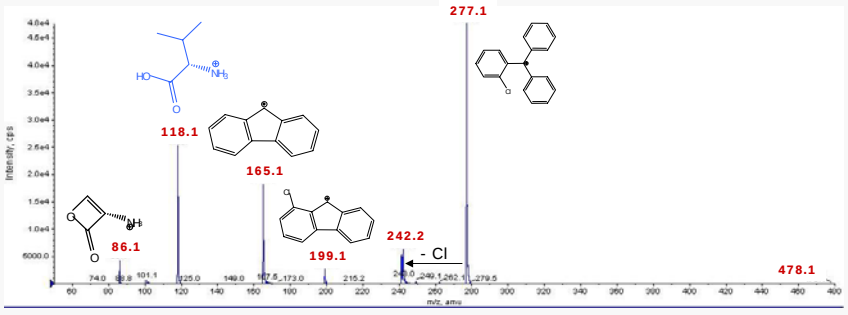

3,50E+06
3,00E+06
2,50E+06
2,00E+06
1,50E+06
1,00E+06
5,00E+05
0,00E+00
Area
CLT-metabolite
clotrimazole
1
1
5
11
50
60
68
68
68
98
Time [d]





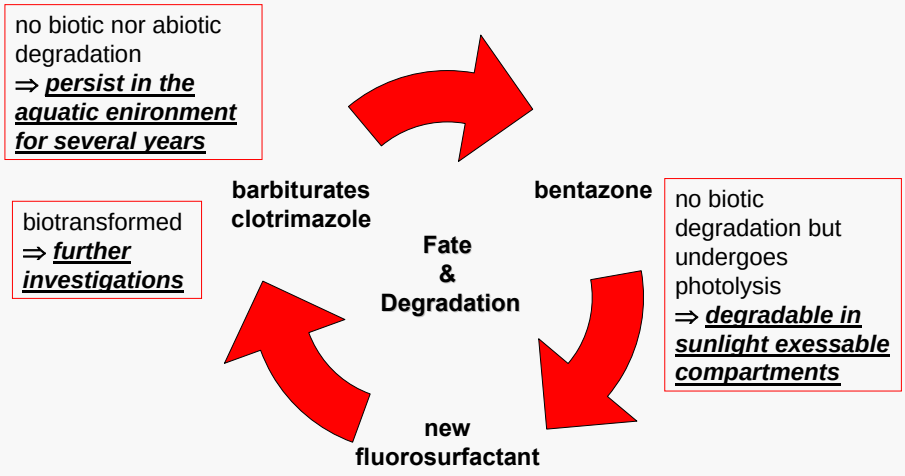
EUROPA FACHHOCHSCHULE
FRESENIUS
UNIVERSITY OF APPLIED SCIENCES





EUROPA FACHHOCHSCHULE
FRESENIUS
UNIVERSITY OF APPLIED SCIENCES

Summary:



Acknowledgement:

- AQUATERRA (Project number 505428 GOCE)
- Spanish Ministry of Education and Science (Project number CTM2005-25168-E)
- Merck KGaA

Thank you for your attention!

EUROPA FACHHOCHSCHULE FRESENIUS
UNIVERSITY OF APPLIED SCIENCES