Hochschule Zittau/Görlitz
University of Applied Sciences
FG Biotechnologie

Kolloquium 2/2011

Dr. rer. nat. Peter Kuschk

Helmholtz-Zentrum Leipzig (UFZ)
Department Umweltbiotechnologie

zum Thema

„Pflanzen-Kläranlagen"

Zeit: Mittwoch, 06. April 2011, 15.00 Uhr

Ort: Haus IV, Raum 0.04

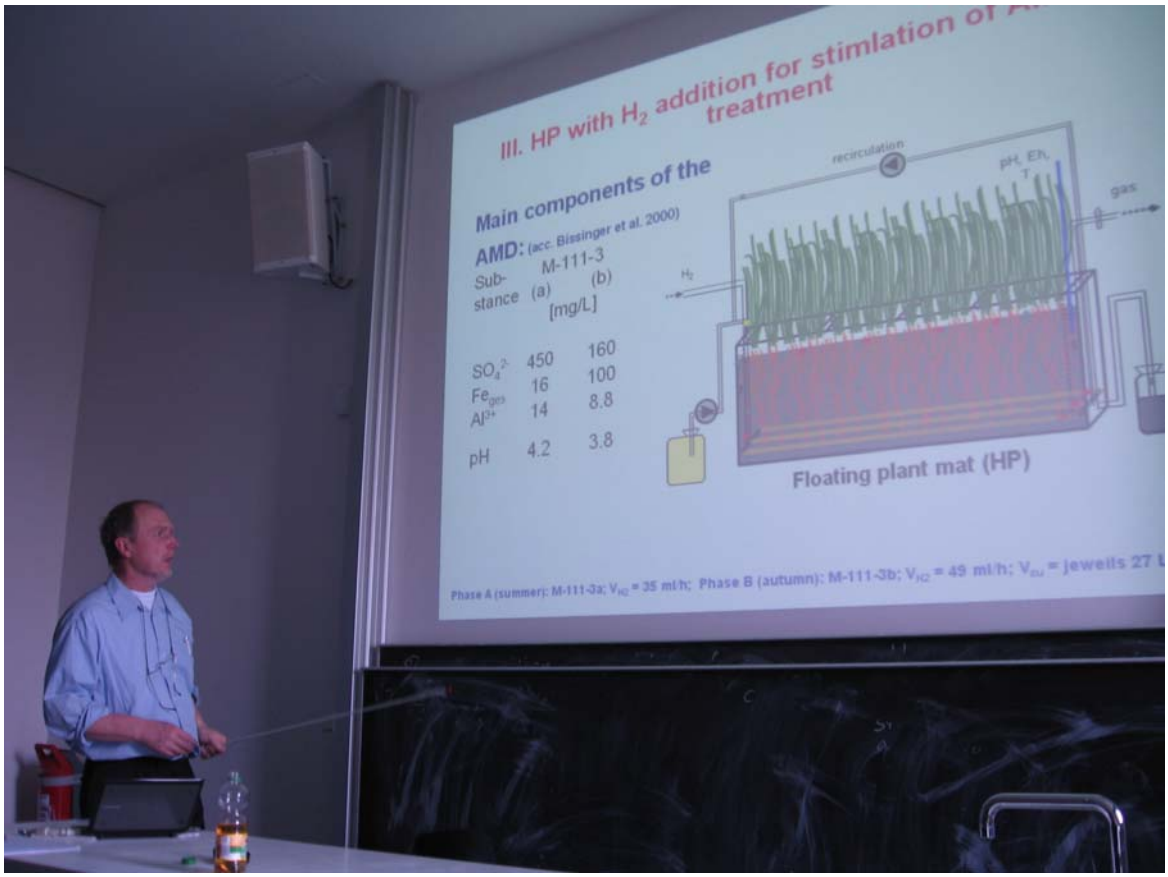
Alle Interessenten, KollegInnen und StudentInnen sind sehr herzlich eingeladen!

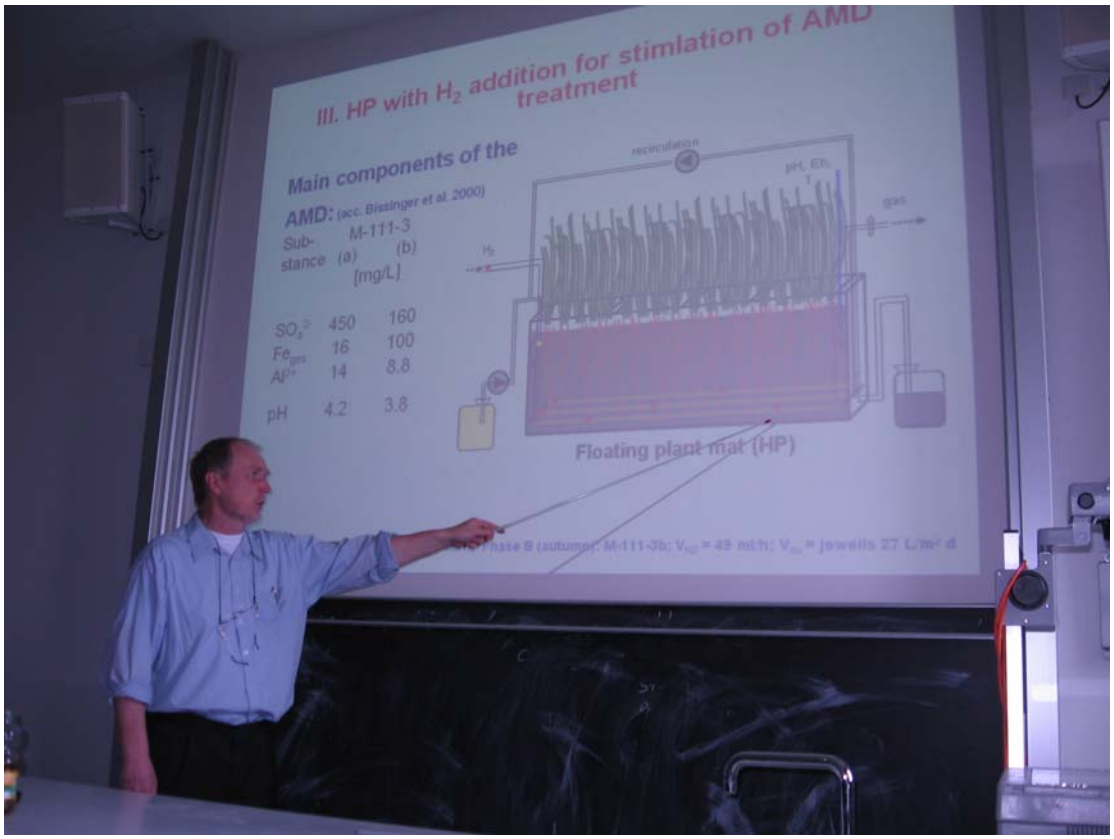
gez.:
Prof. Dr. rer. nat. habil. Manfred Gey

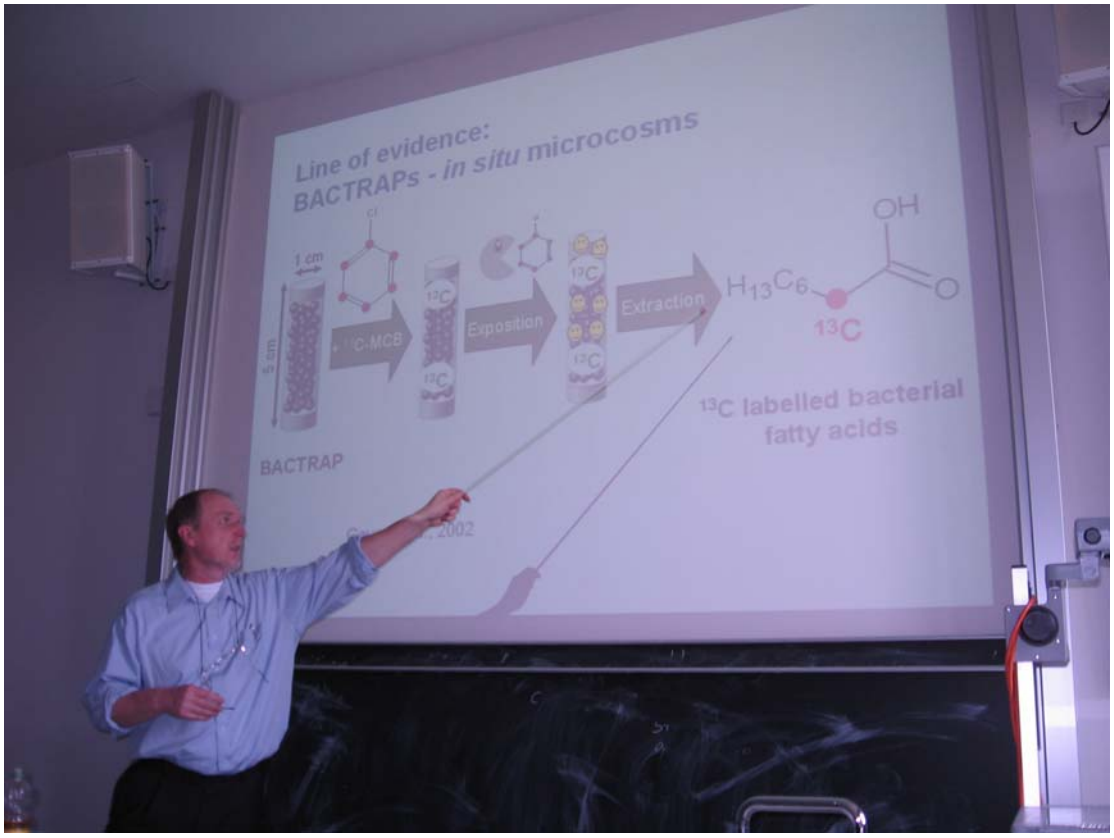
Ps.:
Im Anschluss (ca. ab 16.30 Uhr) findet noch eine Nachsitzung bei Kaffee/Gebäck/Kuchen in der **„Mäuseburg zu Zittau"** statt: **Come as you are!**

Zittau, 06.04.2011

Dr. Peter Kuschk – Pflanzenkläranalagen“




III. HP with H_2 addition for stimlation of AMD treatment
Main components of the
AMD: (acc. Bissinger et al. 2000)
Sub-stance
M-111-3
(a)
(b)
[mg/L]
SO_4^{2-} 450 160
Fe_{ges} 16 100
Al^{3+} 14 8.8
pH 4.2 3.8
recirculation
pH, Eh, T
gas
H_2
Floating plant mat (HP)

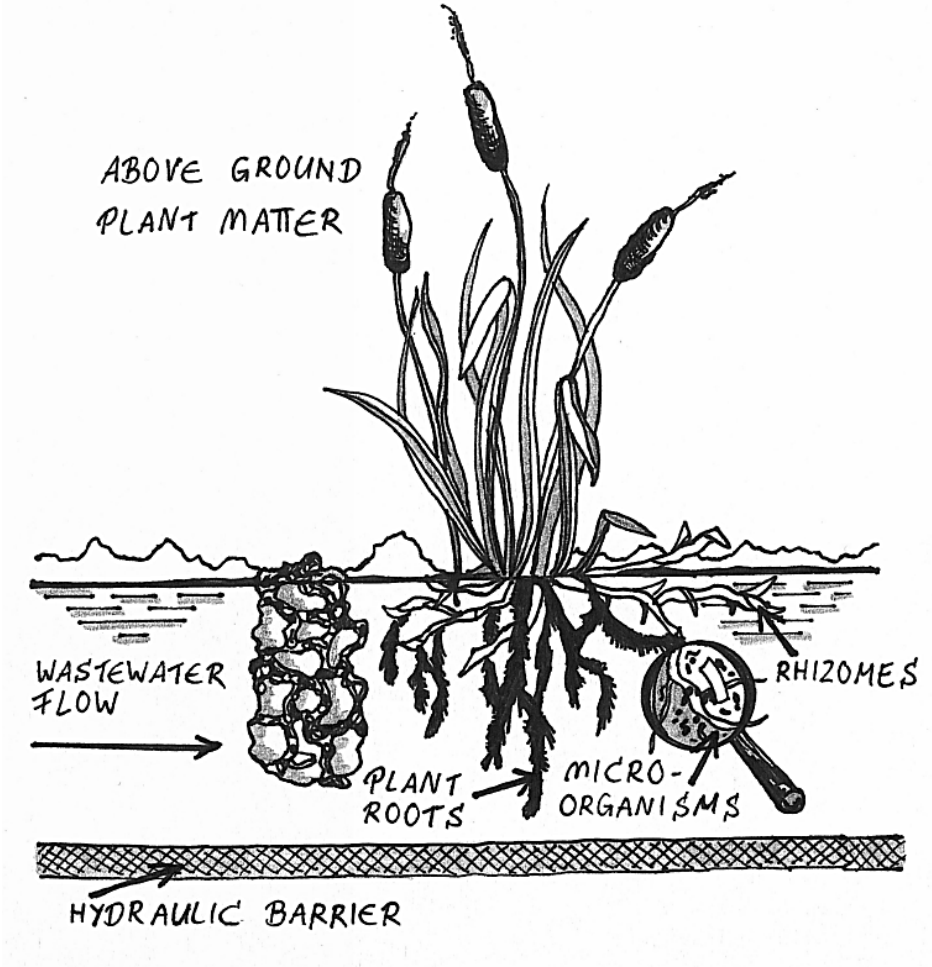

Line of evidence:
BACTRAPs - in situ microcosms
1 cm
5 cm
^{12}C-MCB
Exposition
Extraction
$H_{13}C_6$
^{13}C
OH
O
^{13}C labelled bacterial fatty acids
BACTRAP

Ecological Water Treatment Technologies

Constucted Wetlands

Peter Kuschk, Arndt Wießner, Mareike Braeckevelt, Eva Seeger, Zhongbing Chen, Shubiao Wu, Nam-Hoang Nguyen, Anja Hahn, Uwe Kappelmeyer, Juliane Richter

Contact to: peter.kuschk@ufz.de, www.phyto.ufz.de



Helmholtz Centre for Environmental Research – UFZ

www.ufz.de

Outline:

- The UFZ and research on constructed wetlands (CWs)

- CWs – general aspects, types of pond/wetland systems

- Role of microorganisms and plants in CWs

- About the mosaic structure of aerobic and anaerobic micro-niches in the root zones – experimental results

- Nitrogen and sulphur transformation processes

- Behaviour of heavy metals and comparison of AMD treatment in different types of CWs in laboratory-scale systems

- Experiments on the stimulation of the sulphate reducing bacteria by H_2 addition in a bioreactor and a hydroponic system with a floating plant mat (HP-CW)

- Removal/transformation process of recalcitrant organic compounds

- Outlook

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UFZ – Data and Facts

Personnel 2008

- approx. 910 employees
- 86 % in the scientific-technical domain
- 14 % executive board / staff officers / administration
- approx. 150 postgraduates and post-docs
- approx. 65 guest scientists
- approx. 50 trainees in eight different fields

Budget 2008

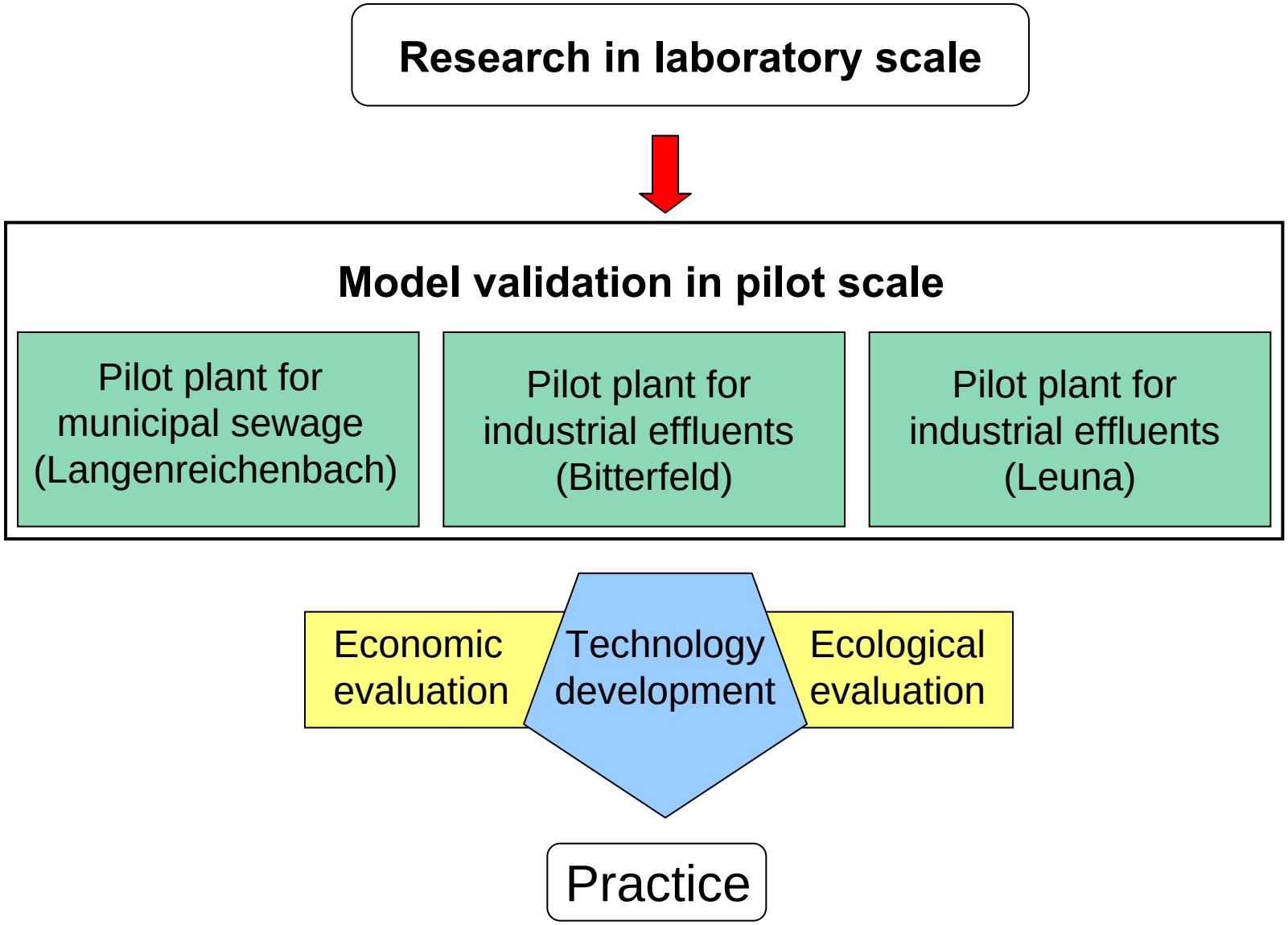
- 60 million EUR (funding Federal Ministry of Education and Research and federal states)
- 14 million EUR (third party funding, e.g. federal government, EU, etc.)

Shortage of (clean) water - What is to do ?

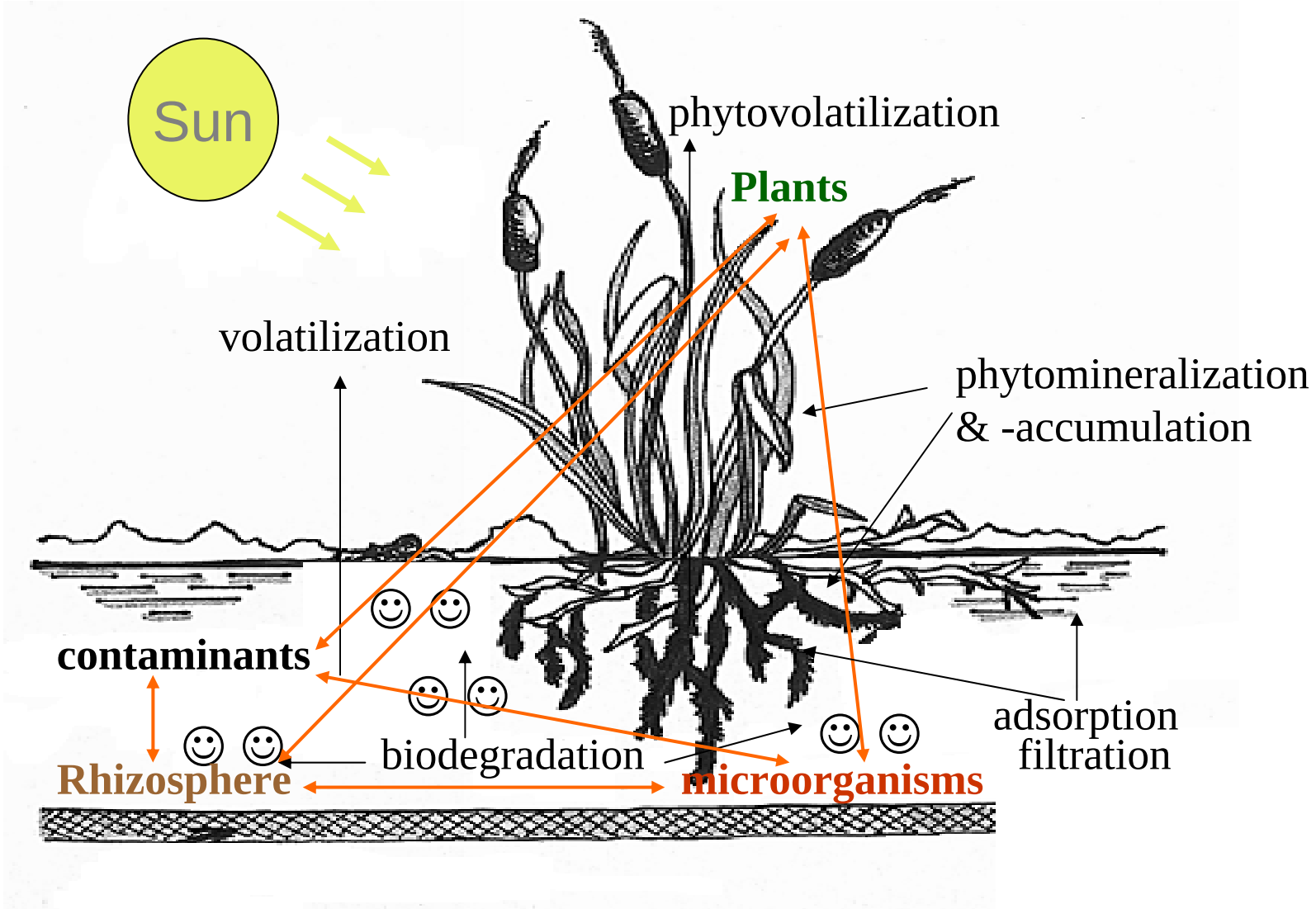
Better Management	Sea Water	Wastewater Treatment and Reuse
Water–pipe system	Desalination	Activated sludge system
Prices		Trickling filter
Education		.
.		.
.		Anaerobic digestion
.		Ponds
		Constructed wetlands

Low Energy Need !

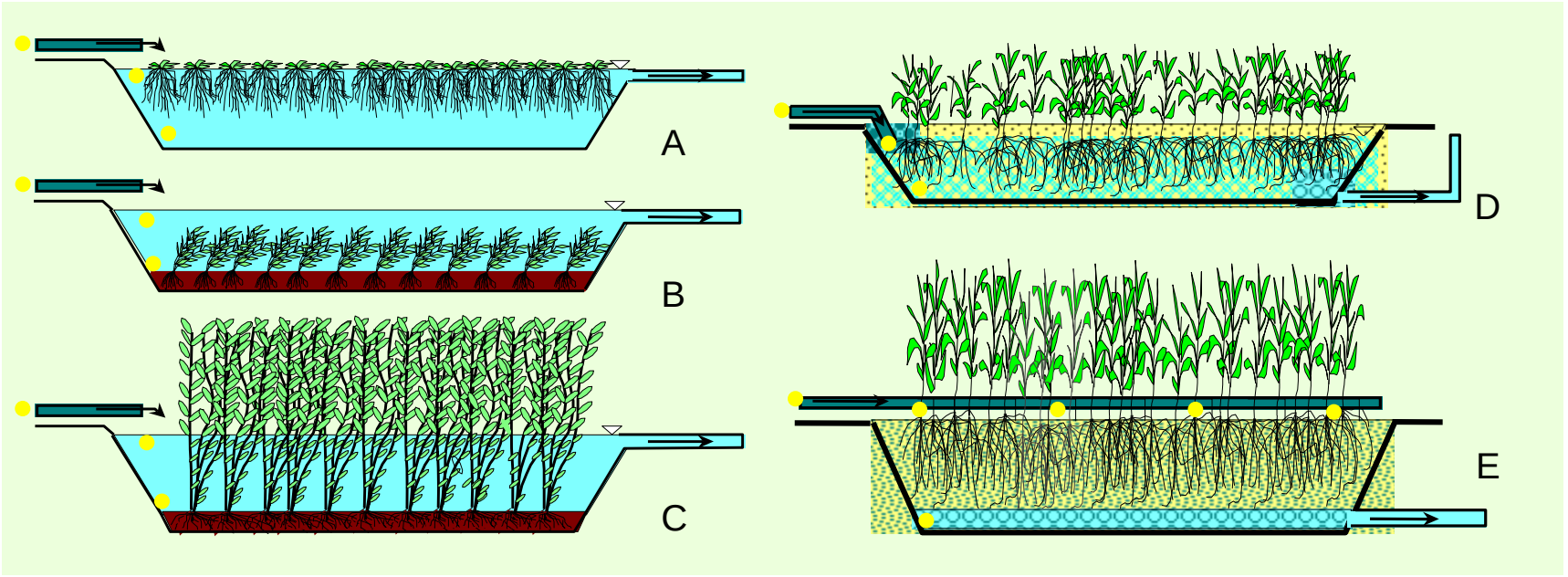
Wetland Research - Levels



The Complexity of Constructed Wetlands



Types of ponds/constructed wetlands:



A: pond with floating plants

B: pond with submersed water plants

C: pond with emersed waster plants

D: CW, horizontal subsurface-flow

E: CW, vertical flow

- different intensities of wastewater to root contact!

- different intensities of toxic effects

Soil matrix-based CW

Construction phases of the first modul

Teneria Europea – Leon, Mexico

Foto: BioPlanta GmbH, Leipzig

Horizontal subsurface-flow CW

CW planted with willow (*Salix* spec.)

CW of a steel mill

Ponds

Eichhornia crassipes

Lemna pond

Nutrient composition of water hyacinths and duckweed grown in wastewater

Constituent	Dry weight, % Water hyacinth	Duckweed
Crude protein	18.1	38.7
Nitrogen (N)	2.9	5.9
Phosporus (P)	0.6	0.6

Source: WPCF (1989).

Soil matrix-free CW

Ponds with floating helophyte mats

CW on a roof (John Deer-Manheim)

CW on a roof (John Deer-Manheim)

Hydroponic culture of *Typha latifolia*

Mobile container in Hirschfeld (BMU)

Mobile container in Hirschfeld (BMU)

Floating reed bed in the experimental station Leuna

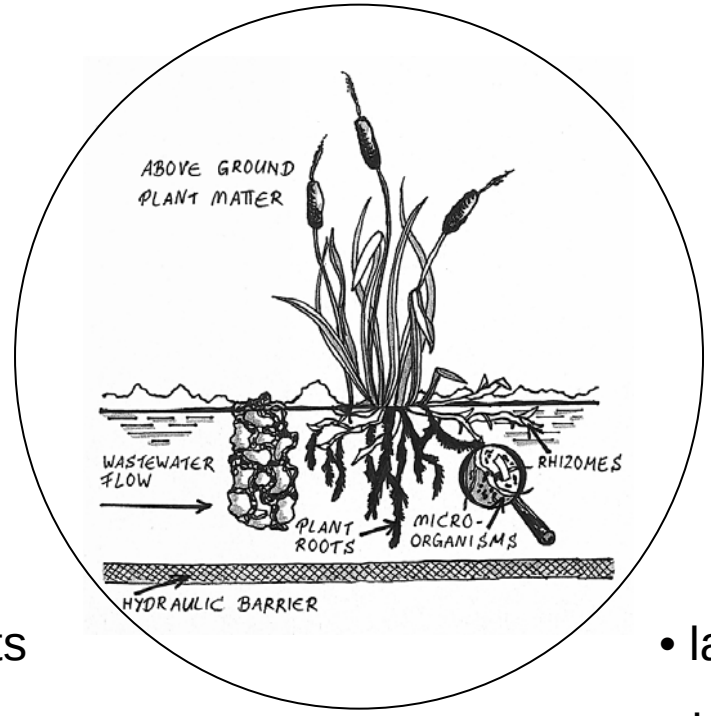
Floating reed bed in the experimental in Leuna

Water treatment by constructed wetlands:

- Domestic sewage
- Industrial effluents
- Leachate
- Agricultural effluents
- Highway runoff water
- Mine drainage
- Sludge treatment

Combinations with other treatment technologies

Wastewater Treatment in Constructed Wetlands



Advantages

- low initial costs
- low running costs
- provision of habitats
- suitable for rural areas
- construction with local materials

Disadvantages

- sensitive system
- high demand on ground
- lack of experience
- need of research
- not suitable for centralized sewage systems

Ornamental plants in CWs

like *Canna, Heliconia, Iris pseudacorus, Lytrum salicaria*, etc.

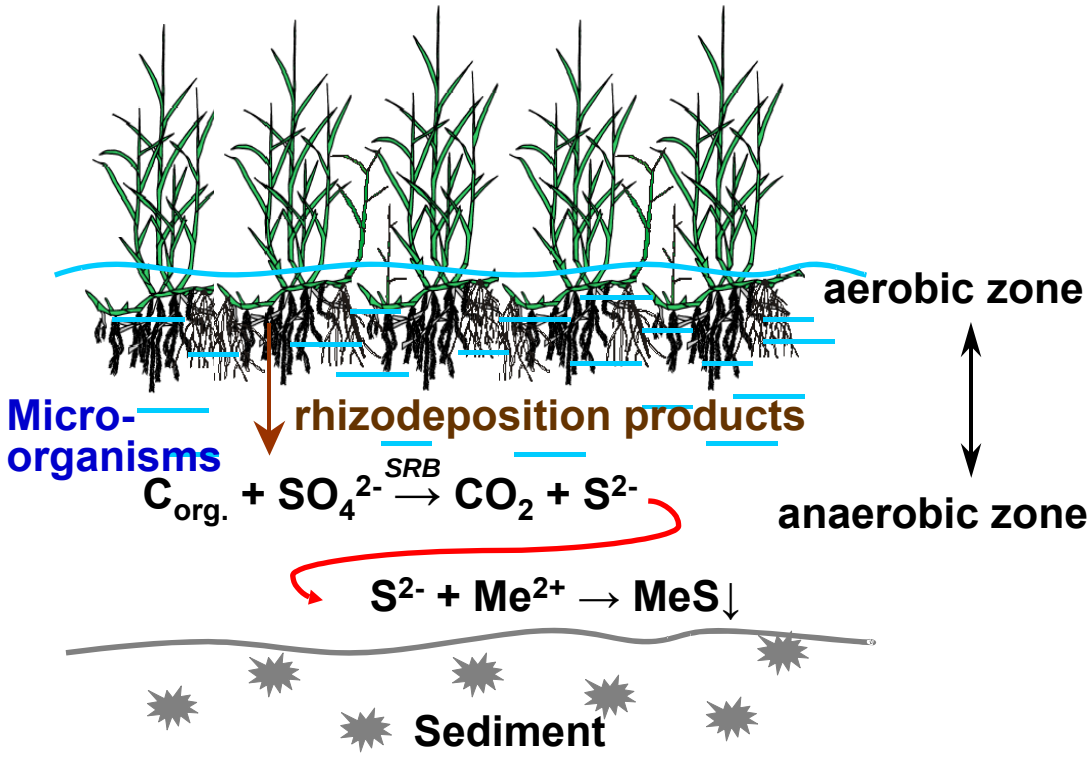
Constructed Wetlands (CWs):

Role of plants

- Input of organ. compounds and O_2 into the rhizosphere
- uptake, assimilation accumulation and metabolisation of inorgan. ions

Role of soil matrix

- adsorption, ion exchange
- filtration, surface for biofilms



Role of microorganisms

- metabolisation, degradation/transformation of organ. and inorgan. compounds
  - **Aerobic microorganisms**
    - e.g. oxidation of Fe(II)
  - **Anaerobic microorganisms**
    - e.g. reduction of sulphate, Fe(III), etc.

Mosaic structure of aerobic and anaerobic micro-niches in the root zones

Dynamics of carbon- and sulphur transformation processes in the root near environment of wetlands

Example:

- **Apparently contradictory results concerning redox processes in a horizontal subsurface-flow CW**

Horizontal Subsurface-Flow CW

(in Bitterfeld, Germany)

Main pollutants: [mg/L]

- **Ammonium: 40 - 50**
- **MCB: 10 – 12**

(monochlorobenzene)

- **Sulphate: 750 - 810**
- **Sulphide: 1 - 3**

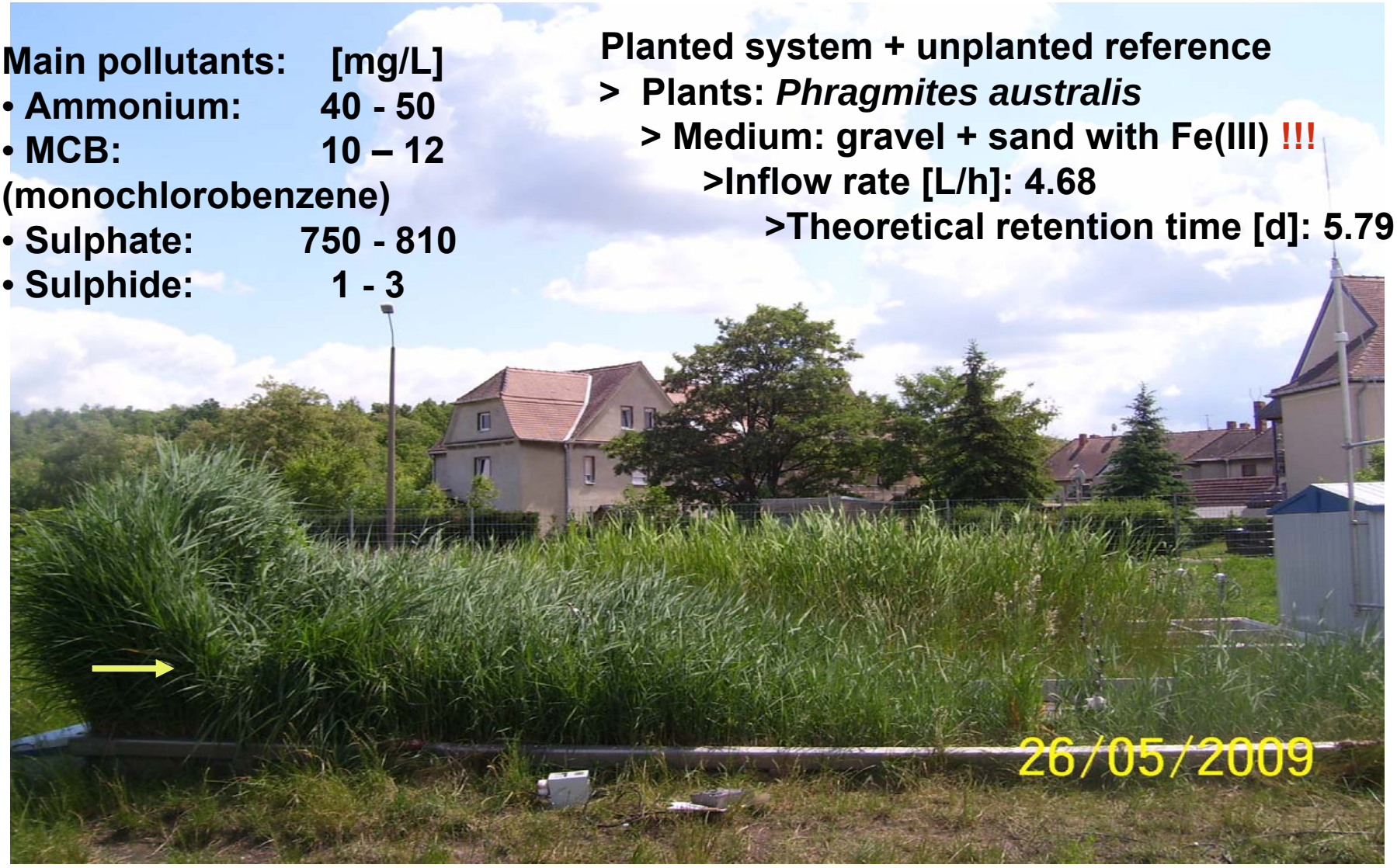
Planted system + unplanted reference

> Plants: *Phragmites australis*

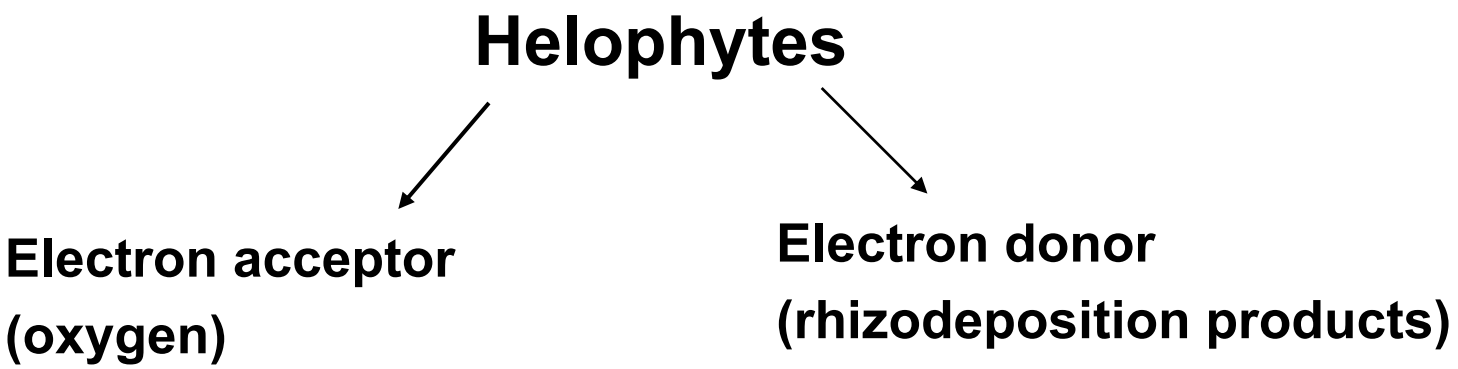
> Medium: gravel + sand with Fe(III) !!!

>Inflow rate [L/h]: 4.68

>Theoretical retention time [d]: 5.79



Mosaic structure of aerobic and anaerobic micro-niches in the root zones (Kickuth 1989)



The actual parameters (rH, oxygen concentration etc.) do not reflect the actual net flow of oxygen and rhizo-deposition products!

Influencing factors:

- Plant species
- Climatic conditions (sun, temperature)
- Water composition (organics, electron acceptors, pH, buffer capacity)
- Hydraulic conditions (stagnant or flowing pore water)

Oxygen input depending on day time

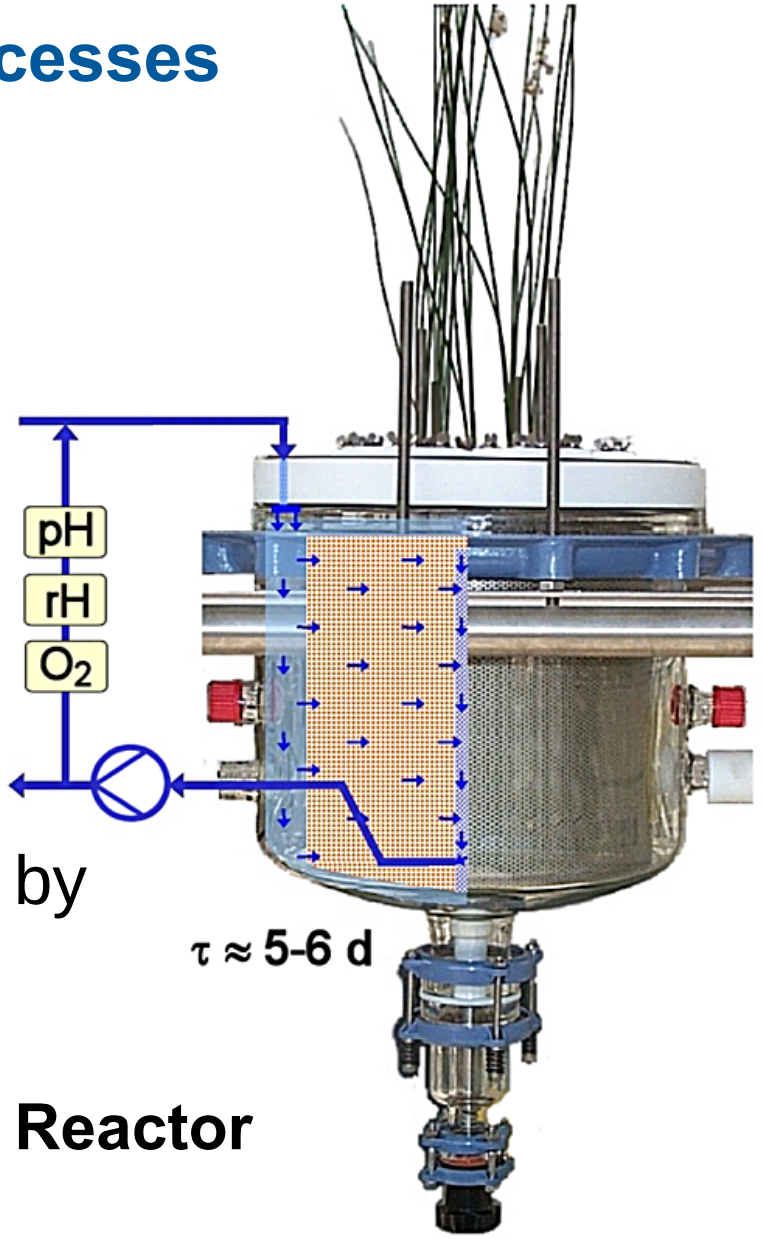
Technical Equipment -Phytotechnicum

Experimental tools: Macro-gradient free test system for simulating root-near processes

Only for basic research!

e.g.:

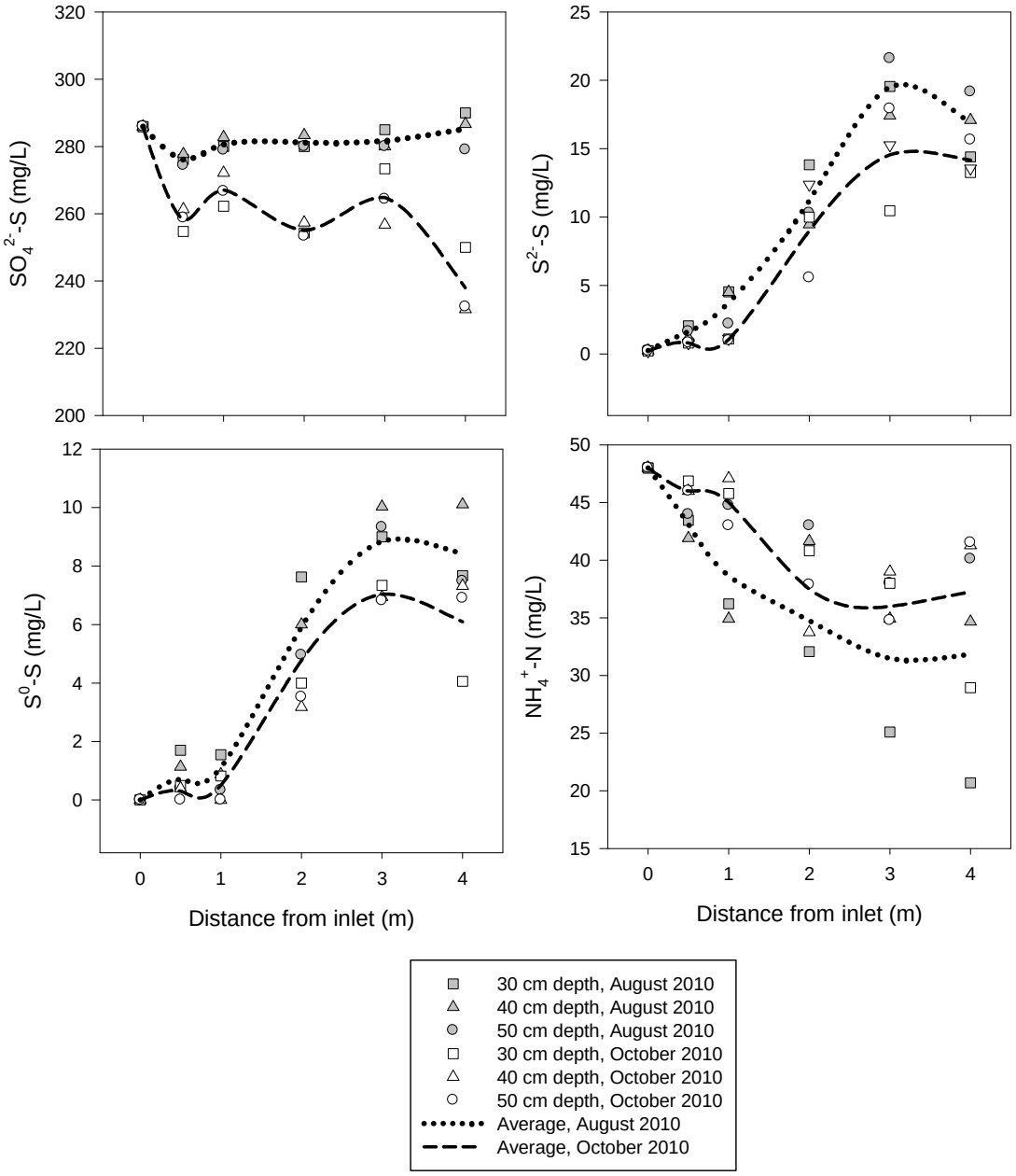
- Routes and interrelations of C, N and S transformations
- Sulphur immobilization, stability
- Toxicity effects (are influenced by hydraulic flow conditions)
- …



Planted Fixed Bed Reactor
(Kappelmeyer et al. 2002)

Nitrogen and Sulphur Transformation Processes

Nitrogen and Sulphur Transformation Processes in a CW



Main pollutants: [mg/L]

Ammonium: 40 - 50

MCB: 10 – 12

(monochlorobenzene)

Sulphate: 750 - 810

Sulphide: 1 - 3

Metals

Coal mine in Ha Long (Vietnam)

Microbially catalyzed pyrite oxidation

$$2\ FeS_2 + 2\ H_2O + 7\ O_2 = 2\ Fe^{2+} + 4\ H^+ + 4\ SO_4^{2-}$$

(Pyrite oxidation)

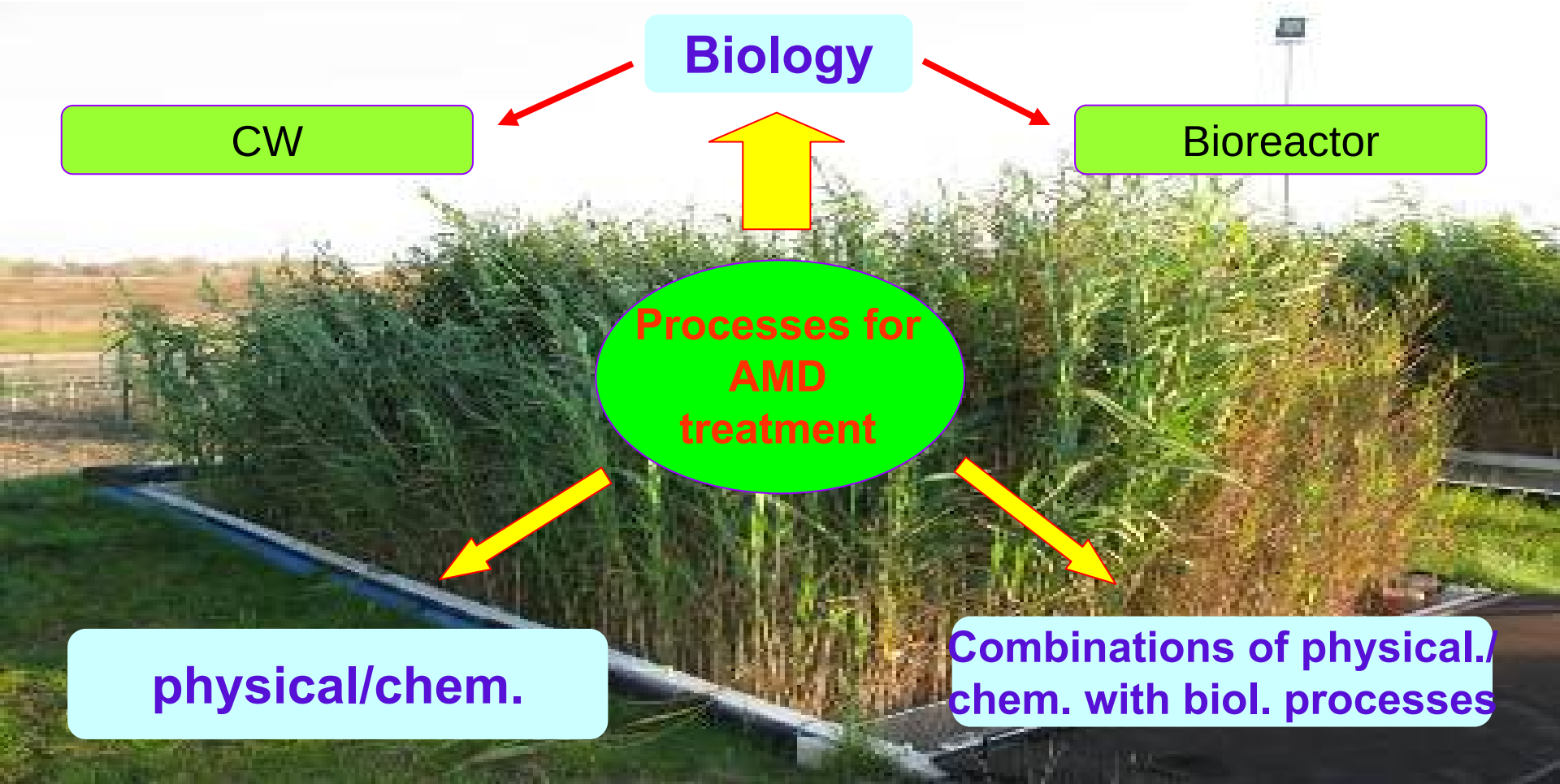
$$4\ Fe^{2+} + O_2 + 4\ H^+ = 4\ Fe^{3+} + 2\ H_2O$$

(Ferrous oxidation)

$$Fe^{3+} + 3\ H_2O = Fe(OH)_3 + 3\ H^+$$

(Ferric hydrolysis)

- Acid mine drainage (AMD) is a global problem
- AMD: - low pH
  - high conc. of heavy metals and sulphate



Mechanisms responsible for heavy metal removal in constructed wetlands:

- Filtration of suspended particles
- Incorporation into the plant matter (**very low!**)
- Adsorption on the soil, microorganisms and plant roots
- Aerobic precipitation of oxyhydroxides and carbonates as a result of the degradation of organic matter to CO_2 and NH_4^+ and the following increase of the pH
- Precipitations in consequence of microbial anaerobic activities (sulphides)
- *Steep concentration gradiants on the root surface because of plant transpiration → precipitations*

Metals as Electron Acceptors for Anaerobic Respiration

Reaction	Microorganisms	Reference
$2\ Fe^{3+} + H_2 \leftrightarrow 2\ Fe^{2+} + 2\ H^+$	*Geobacter metallireducens*	LOVLEY and LONERGAN (1990)
	Pelobacter carbinolicus	LOVLEY et al. (1995)
$Mn^{4+} + H_2 \leftrightarrow Mn^{2+} + 2\ H^+$	*Geobacter metallireducens*	LOVLEY (1991)
	mixed culture	LANGENHOFF et al. (1997)
$2\ Cr^{6+} + 3\ H_2 \leftrightarrow 2\ Cr^{3+} + 6\ H^+$	*Desulfovibrio vulgaris*	LOVLEY and PHILLIPS (1994)
	Bacillus strain QC1-2	CAMPOS et al. (1995)
$Se^{6+} + H_2 \leftrightarrow Se^{4+} + 2\ H^+$	*Thauera selenatis*	MACY et al. (1993)
$Se^{6+} + 3\ H_2 \leftrightarrow Se^{0} + 6\ H^+$	strain SES-1; SES-3	OREMLAND et al. (1989)
$Te^{4+} + 2\ H_2 \leftrightarrow Te^{0} + 4\ H^+$	*Schizosaccharomyces pombe*	SMITH (1974)
$Pb^{2+} + H_2 \leftrightarrow Pb^{0} + 2\ H^+$	*Pseudomonas maltophila*	LOVLEY (1995)
$As^{5+} + H_2 \leftrightarrow As^{3+} + 2\ H^+$	*Geospirillum arsenophilus*	AHMANN et al. (1994)
$Hg^{2+} + H_2 \leftrightarrow Hg^{0} + 2\ H^+$	*Escherichia coli*	ROBINSON and
	Thiobacillus ferrooxidans	TUOVINEN (1984)
$U^{6+} + H_2 \leftrightarrow U^{4+} + 2\ H^+$	*Shewanella putrefaciens*	LOVLEY et al. (1991)

Chemical precipitation of metal hydroxides and the effectiveness of different precipitants (Förstner 1993).

Metal	Start of precip. pH	Re-dissolution pH	Solubility for Metal hydroxide
Iron	2.8	-	$Fe(OH)_3$ $9 * 10^{-38}$
Aluminium	4.3	8.3	$Al(OH)_3$ $2 * 10^{-32}$
Chromium	5.8	9.2	$Cr(OH)_3$ $3 * 10^{-28}$
Copper	5.8	-	$Cu(OH)_2$ $2 * 10^{-19}$
Lead	6.5	-	$Pb(OH)_2$ $ca.10^{-13}$
Zinc	7.6	11.0	$Zn(OH)_2$ $4 * 10^{-17}$
Nickel	7.8	-	$Ni(OH)_2$ $6 * 10^{-15}$
Cadmium	9.1	-	$Cd(OH)_2$ $1 * 10^{-14}$

Effectiveness of different precipitants

Precipitant	High effectiveness	Low effectiveness
Digested sludge (sulphides)	Cu, Pb, Zn, Cd	Ni
Precipitation with Fe-salts	Ag, Cr, Pb, Cu, Hg, Sn	Mn, Co, Sb, Se
Precipitation with chalk hydrate	Ag, Co, Cr, Pb, Ni, Cd	Sb, Se, As
Precipitation with Al-salts	Ag, Be, Hg, Cr, Cd, Pb	Zn, Mn, Ni

Mechanisms and processes of heavy metal removal in the root zone of CWs partially unknown
→ potentials for further developments/improvements

Tasks:

I. Lack of comparable data for different types of CWs
➢ comparison of different types in laboratory-scale CWs

II. Activity of the sulphate reducing bacteria (SRB) in CWs
is limited by the availability of electron donors;
for bioreactors H_2 is known as a relatively low-cost electron donor;
but mainly H_2 + CO_2 is used
➢ that is why H_2 + organic compounds should be tested

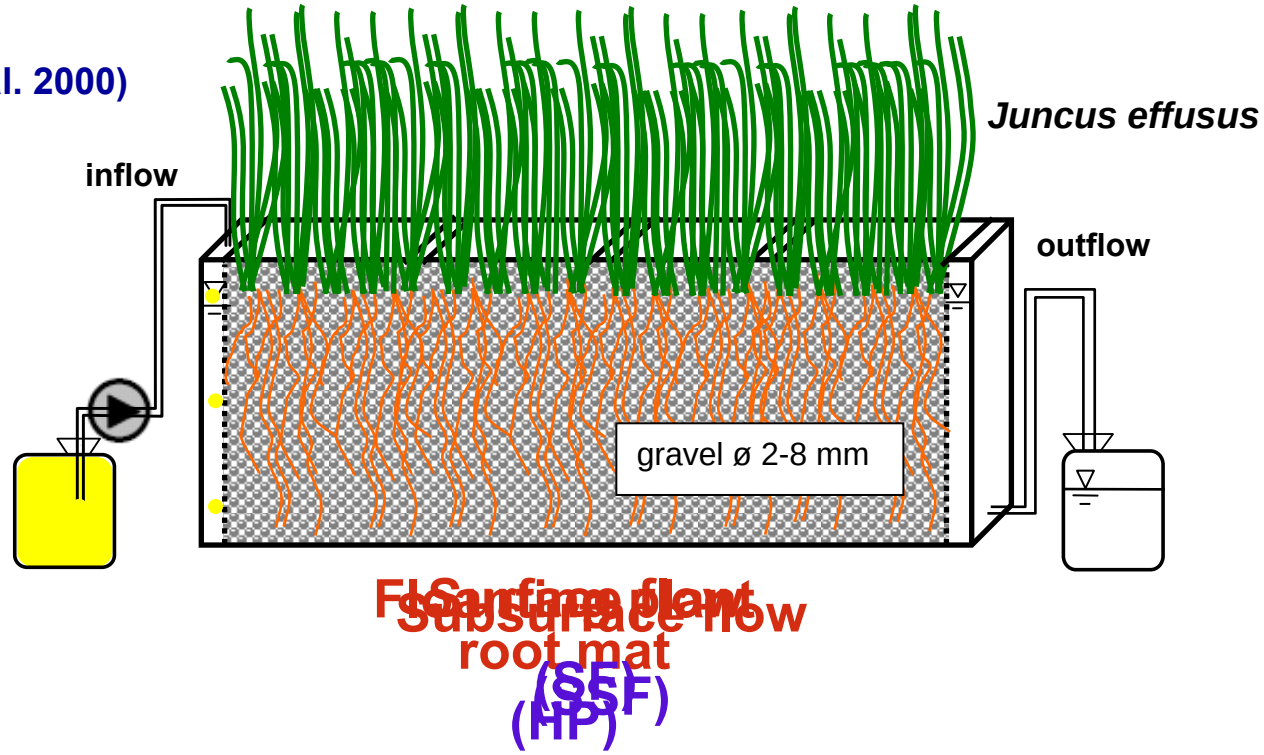
III. **New Strategies?**
➢ experiments on the stimulation of SRB by H_2 addition into a hydroponic-CW (HP)

I. Comparison of AMD treatment in different types of CWs in laboratory-scale systems

I. Laboratory-scale experimental CWs

Main components of the AMD: (nach Bissinger et al. 2000)

Sub-stance	M-111-1 [mg/L]
SO_4^{2-}	1420
$Fe_{ges.}$	116
Al^{3+}	20.1
pH	2.7



(Length: 100 cm, width: 15 cm und hight: 35 cm, pore water volume: 13.5 L)

Phase A (summer): 43 L/m²d; Phase B (autumn): 25 L/m²d

Results

pH:

- **inflow:**

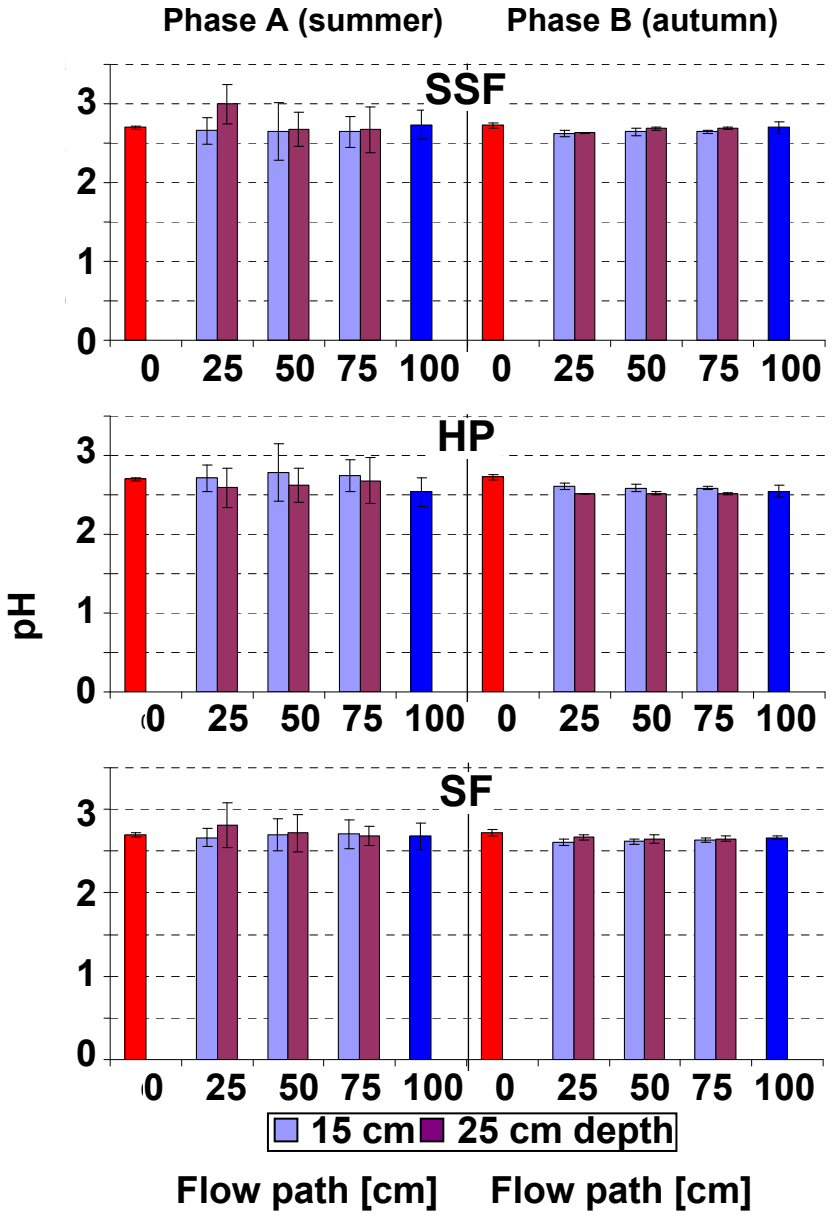
Phase A: 2.69

Phase B: 2.71

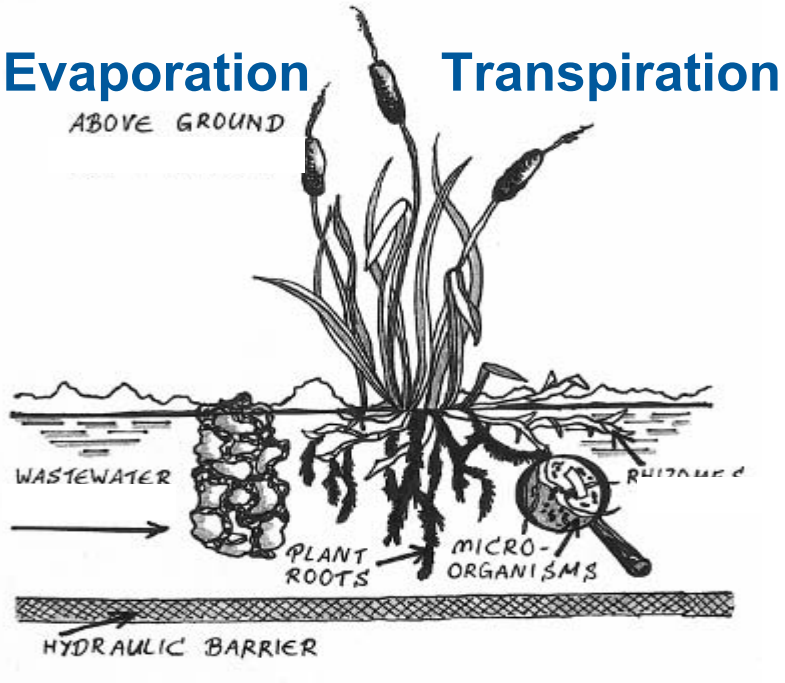
- **outflow:**

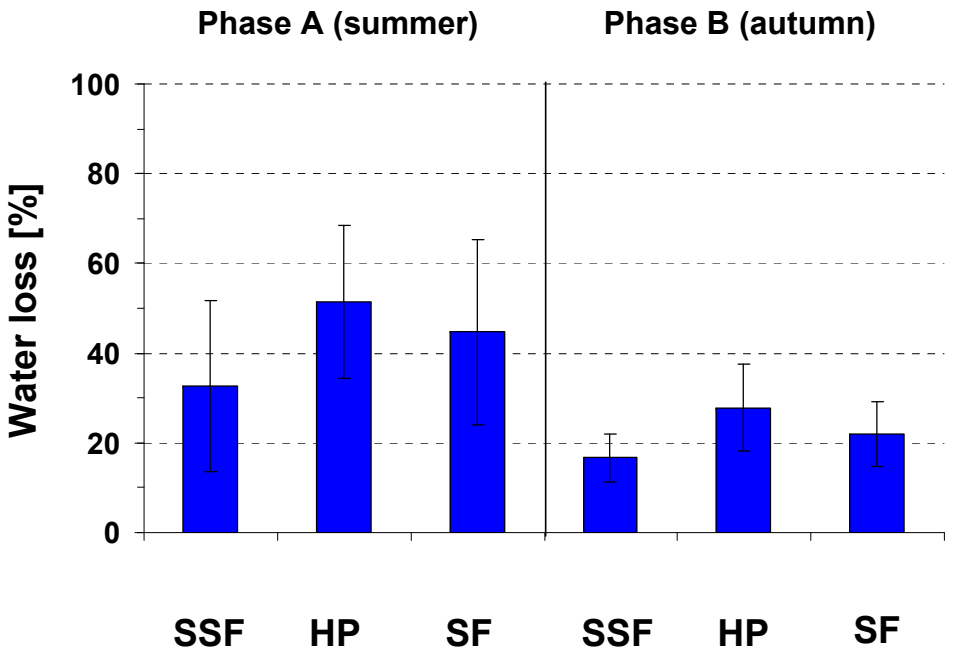
	Phase A	Phase B
SSF:	2.73	2.71
HP:	2.53	2.55
SF:	2.67	2.66

- **no influence of season, flow path and depth**
- **no sulphide formation!**



Water Loss by Evapotranspiration:





- Water loss by EVA
- different uptake rates of H_2O and ions
  → high ion concentrations nearby the root surface
  results of precipitations
- EVA in summer > autumn; im HP > SF > SSF

SSF: (Subsurface flow); HP: (floating plant mat); SF: (Surface flow)

Load of base capacity:

➢ **inflow:**

Phase A: 461 mmol NaOH/m² d

Phase B: 245 mmol NaOH/m² d

➢ **% decrease in the outflow:**

	Phase A	Phase B
SSF:	32	21
HP:	17	15
SF:	25	23

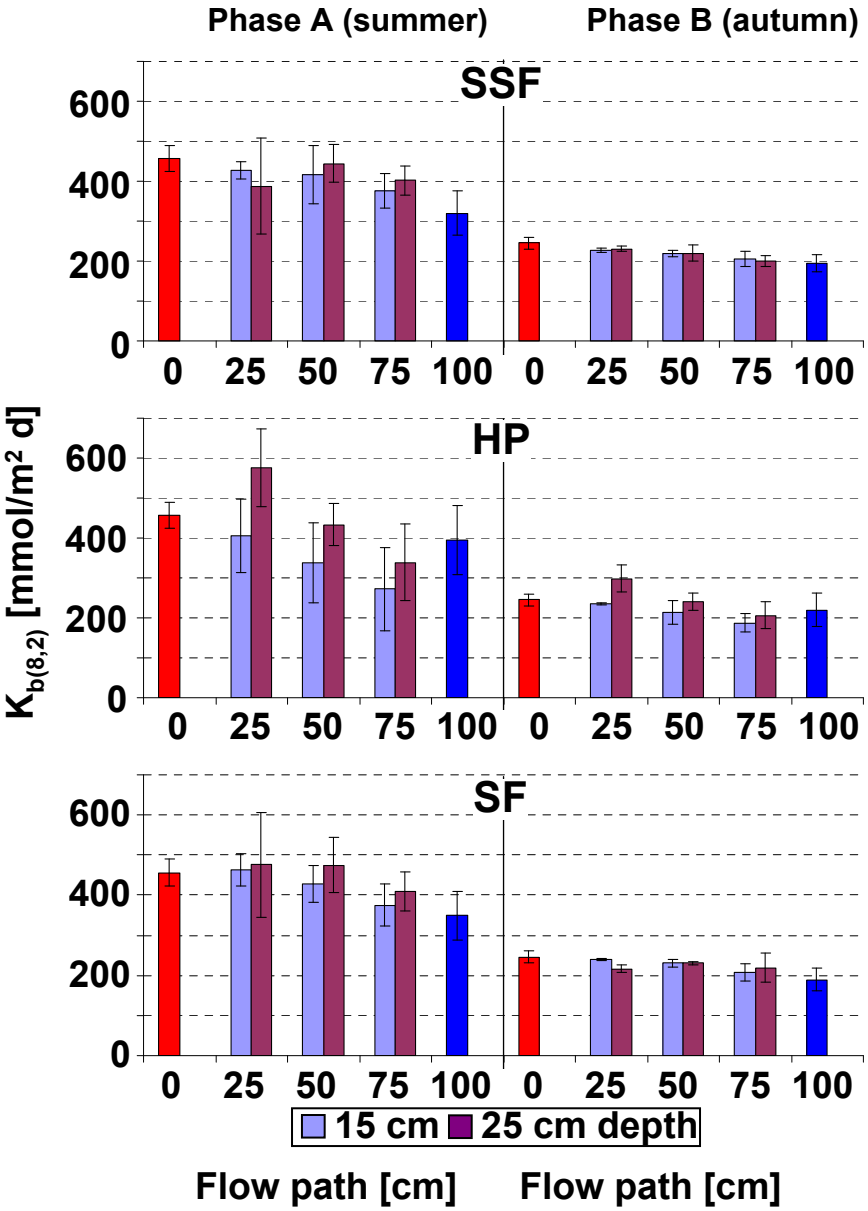
➢ **within the beds:**

- values > of the outflow
- HP > SSF > SF

❖ **in comparison:**

- SSF > SF > HP

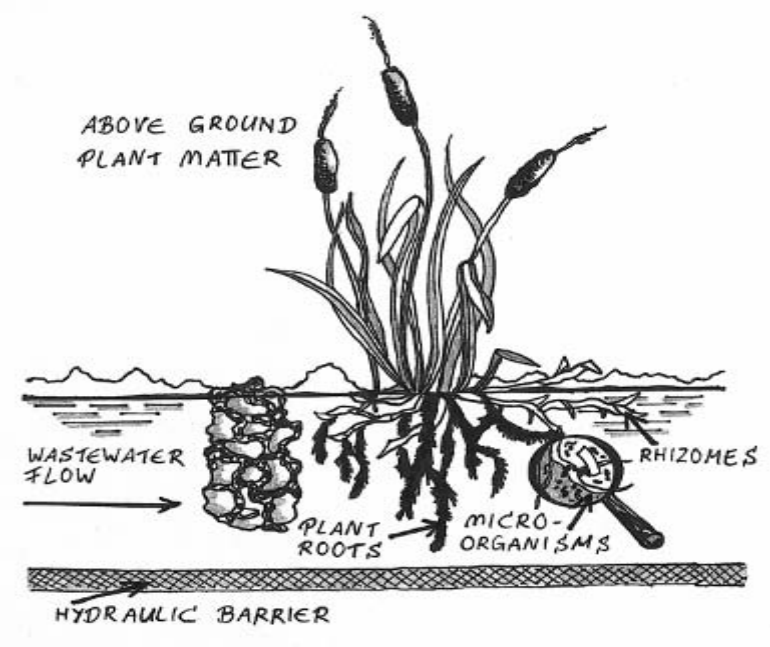
❖ **decrease of base capacity by metal removal**



Decrease of the iron load by formation of minerals like:

- iron hydroxosulphate **$Fe(SO_4)(OH)$**
- iron hydroxide **$Fe(OH)_3$**
- Schwertmannite **$Fe_8O_8(SO_4)(OH)_6$**
- H-Jarosite **$HFe_3(SO_4)_2(OH)_6$**
- K-Karosite **$KFe_3(SO_4)_2(OH)6$**

DOC concentration:



DOC content in the pore water < 5 mg/L

Shortage of $C_{org.}$

Reducing equivalents

Rhizodeposition products

Bacterial growth

- $2\ \{CH_2O\} + SO_4^{2-} + 2\ H^+ \rightarrow H_2S + 2\ CO_2 + 2\ H_2O$
- $\{CH_2O\} + 4\ FeOOH + 8\ H^+ \rightarrow 4\ Fe^{2+} + CO_2 + 7\ H_2O$
- $\{CH_2O\} + 4\ Fe^{3+} + H_2O \rightarrow 4\ Fe^{2+} + CO_2 + 4\ H^+$

Potential further sources of electrondonors for the sulphate reduction:

Source of electron donors	€ /kg SO_4^{2-}	r_{SO4} [mmol/L h]
Hydrogen	**0.14**	**0.9 – 6.0**
Ethanol	0.22	0.1 – 0.12
Sugar	0.38	0.68
Sodium propionate	1.00	1.6
Lactic acid	1.74	0.023-0.05
Dairy wastes, saw dust	-	0.002 –0.01
Wood chips, litter	-	0.015 –0.068

(Preuß, V. 2004, Koch *et al.* 2006 und Kothari *et al.* 2008)

Hydrogen as electron donor

Advantages	Disadvantages
Lower price	Low solubility
High sulphate reduction rate (SRR)	Explosive
Less remaining components in the outflow	

II. Testing of org. compounds + H_2 in bioreactor

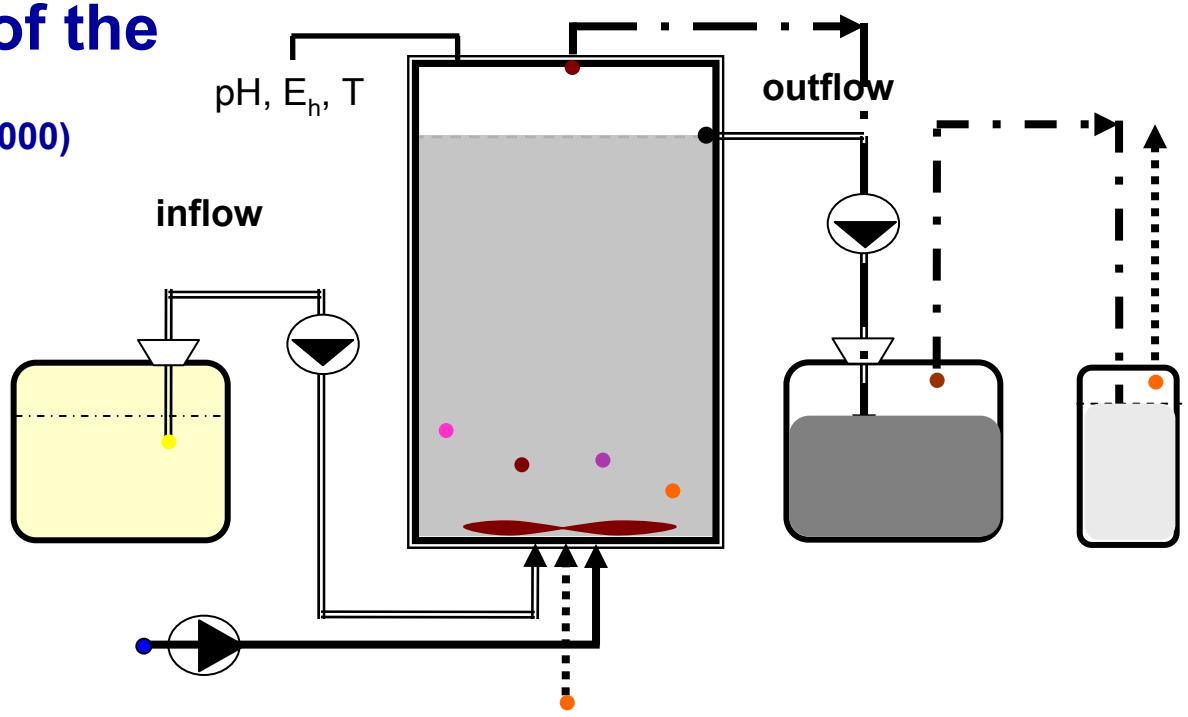
- Potential advantages of H_2 in comparison to other chemicals as electron donor source
- in the past mainly H_2 + CO_2 was tested in bioreactors
- Testing of H_2 + organic compounds for the AMD treatment in bioreactors

II. Laboratory-scale reactor system for SRB

Main components of the AMD: (acc. Bissinger et al. 2000)

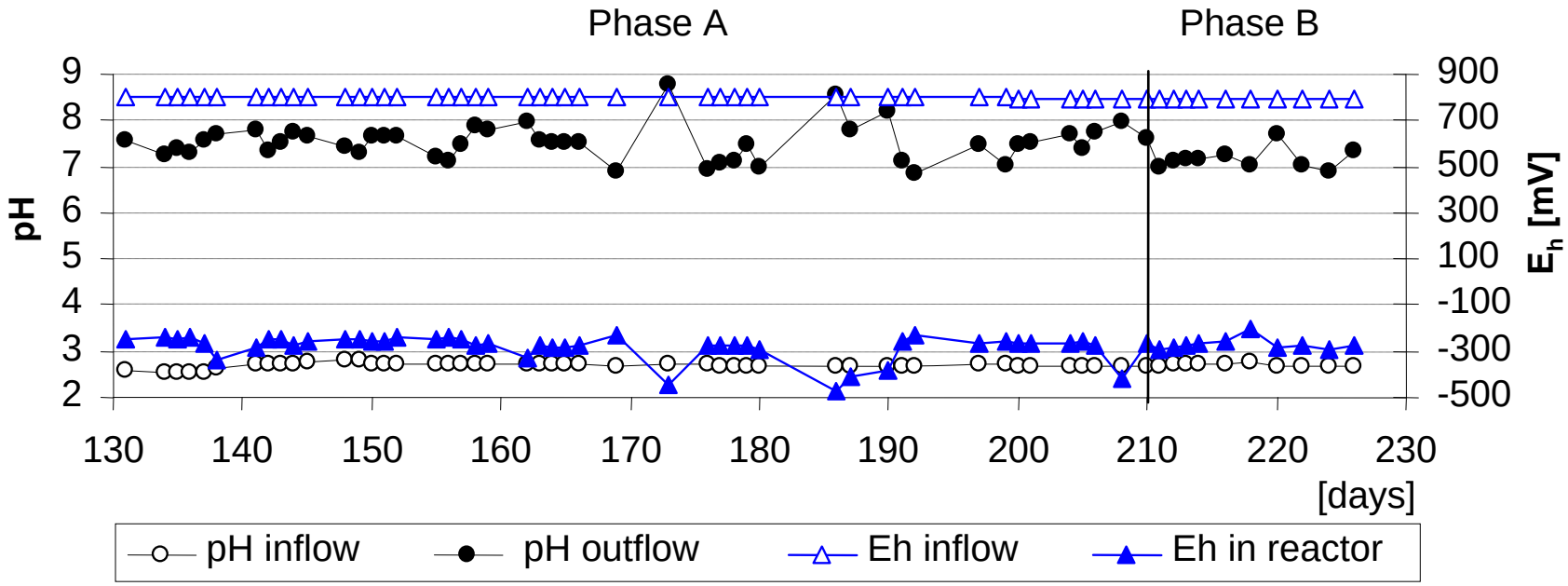
Sub-stanz	M-111-2 [mg/L]
SO_4^{2-}	560
Fe_{ges}	58
Al^{3+}	10
pH	2.7

+ propionate



(reactor volume 2.5 L, water volume 2.0 L, V_{H2} = 1L/h)

Redox potential and pH:



- **Good conditions for microbial diss. sulphate reduction**
  - **suitable pH for the SRB**
  - **suitable E_h for the SRB**

Phase A: HDT = 0.47 days, C-conc.$_{zu}$ = 3.7 mmol/L ; Phase B: HDT = 0.33 days, C-conc.$_{zu}$ = 5.4 mmol/L; V_{H2} = 1L/h

III. New Strategies?

Investigations on the stimulation of the SRB by H_2 addition in a HP-CW

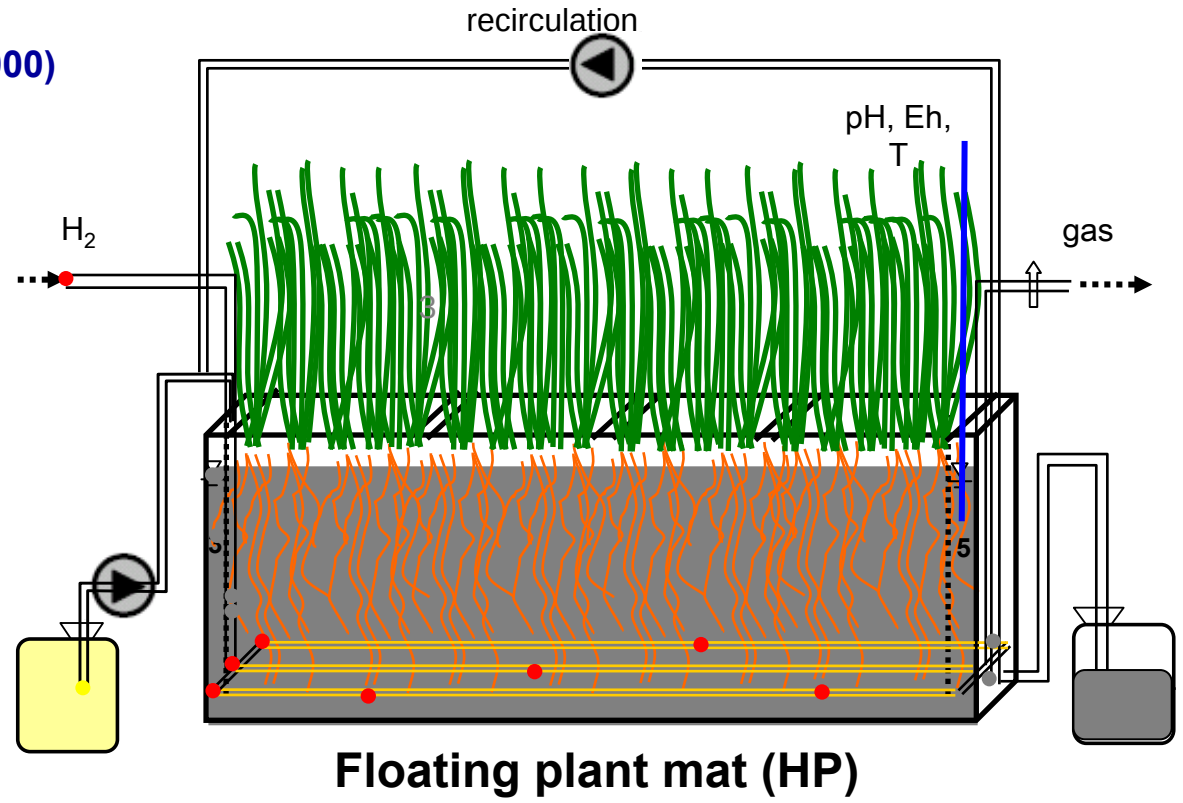
- By the addition of H_2 + org. comound was realised a high turn over rate of sulphate in the bioreactor
- It is possible to stimulate the SRB by addition of H_2 in combination with the rhizodeposition products in CWs?

III. HP with H_2 addition for stimlation of AMD treatment

Main components of the

AMD: (acc. Bissinger et al. 2000)

Sub-stance	M-111-3 (a)	(b)
	[mg/L]	
SO_4^{2-}	450	160
Fe_{ges}	16	100
Al^{3+}	14	8.8
pH	4.2	3.8

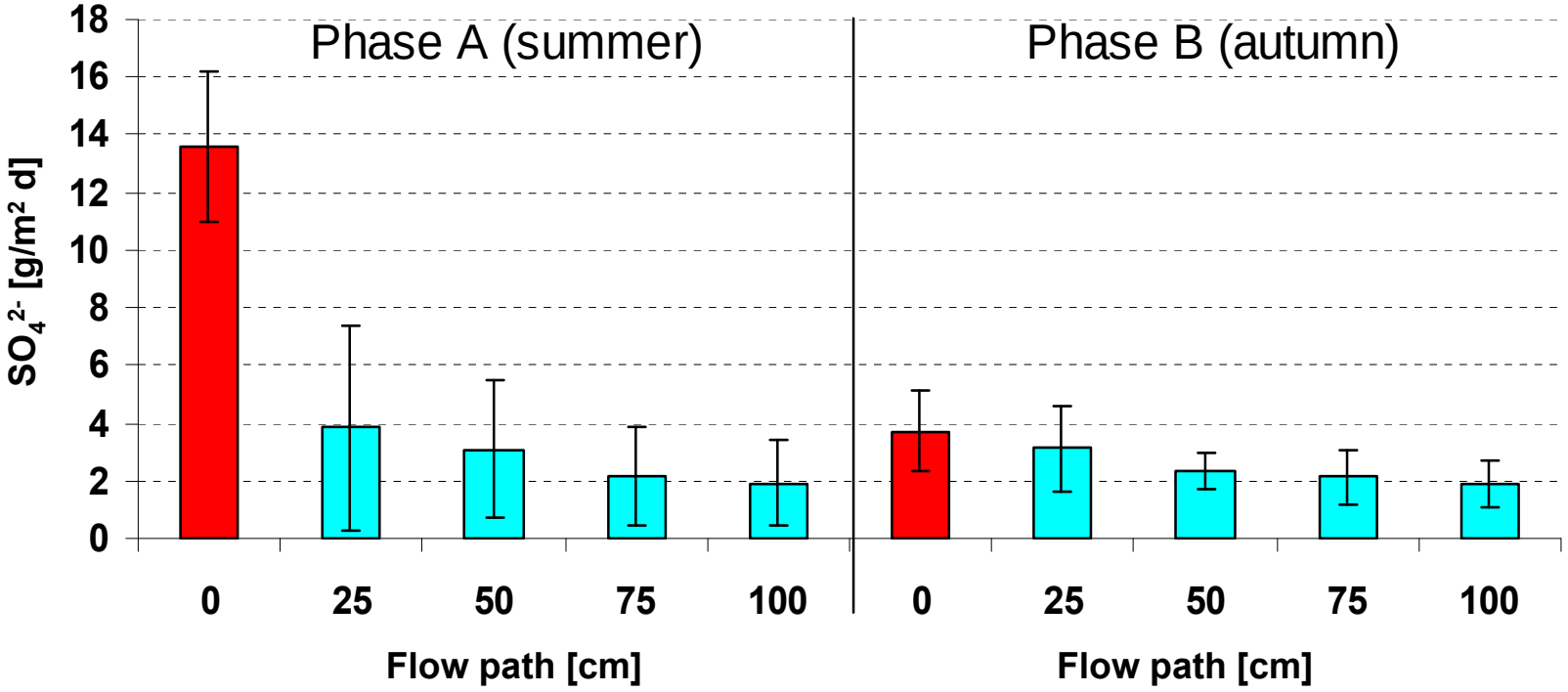


Floating plant mat (HP)

Phase A (summer): M-111-3a; V_{H2} = 35 ml/h; Phase B (autumn): M-111-3b; V_{H2} = 49 ml/h; V_{zu} = jeweils 27 L/m² d

Stimulation of dissimilatory sulphate reduction by addition of electron donors (electrolyt. H_2, biomass)

Sulphate load:

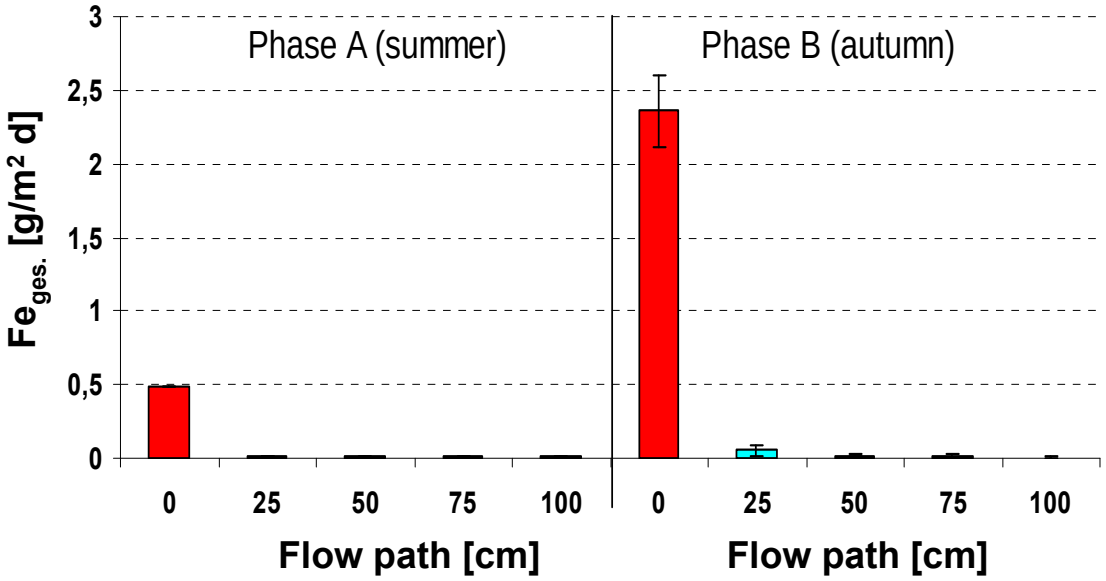


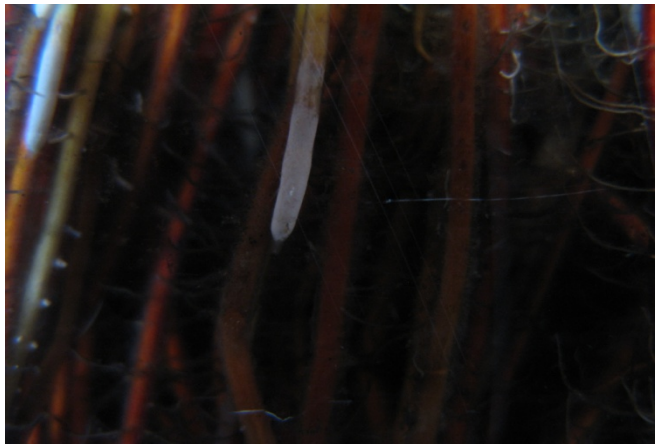
- Decrease of the sulphate load along the flow path
- Dependence of the inflow sulphate concentration

Decrease of the sulphate load by the microbial sulphate reduction,

→adsorption, precipitation etc.

Total iron load:





❖ In both phases A und B:

- sufficient formation of sulphide → almost complete removal of iron
- dependency on S^{2-} formation
- independend on initial concentration and season of the year

❖ Mechanisms of the metals:

- ➢ precipitation, co-precipitation, adsorption

Summary

I. Treatment of the AMD by the 3 different types of CWs:

- only little change of pH! but:
- decrease of base capacity
- removal of heavy metals
- removal of sulphate
- treatment efficiency: SSF > SF > HP ; in summer > in autumn

Reasons and Mechanisms:

- not enough $C_{org.}$ for microbial diss. sulphate reduction
- formation and precipitation of Fe-hydroxosulphate and hydroxides

  → adsorption further heavy metals and sulphate

Summary

II. AMD treatment in the bioreactor

- **Combination of H_2 with org. compound (propionate) for diss. sulphate reduction in the bioreactor resulted a high efficiency:**
  - ✓ **pH**
  - ✓ **SRR**
  - ✓ **Y_{H2}**
  - ✓ **Heavy metal removal: almost complete**

Idea: Application of H_2 for the stimulation of the SRB in CWs

Summary

III. Addition of hydrogen gas into a hydroponic system

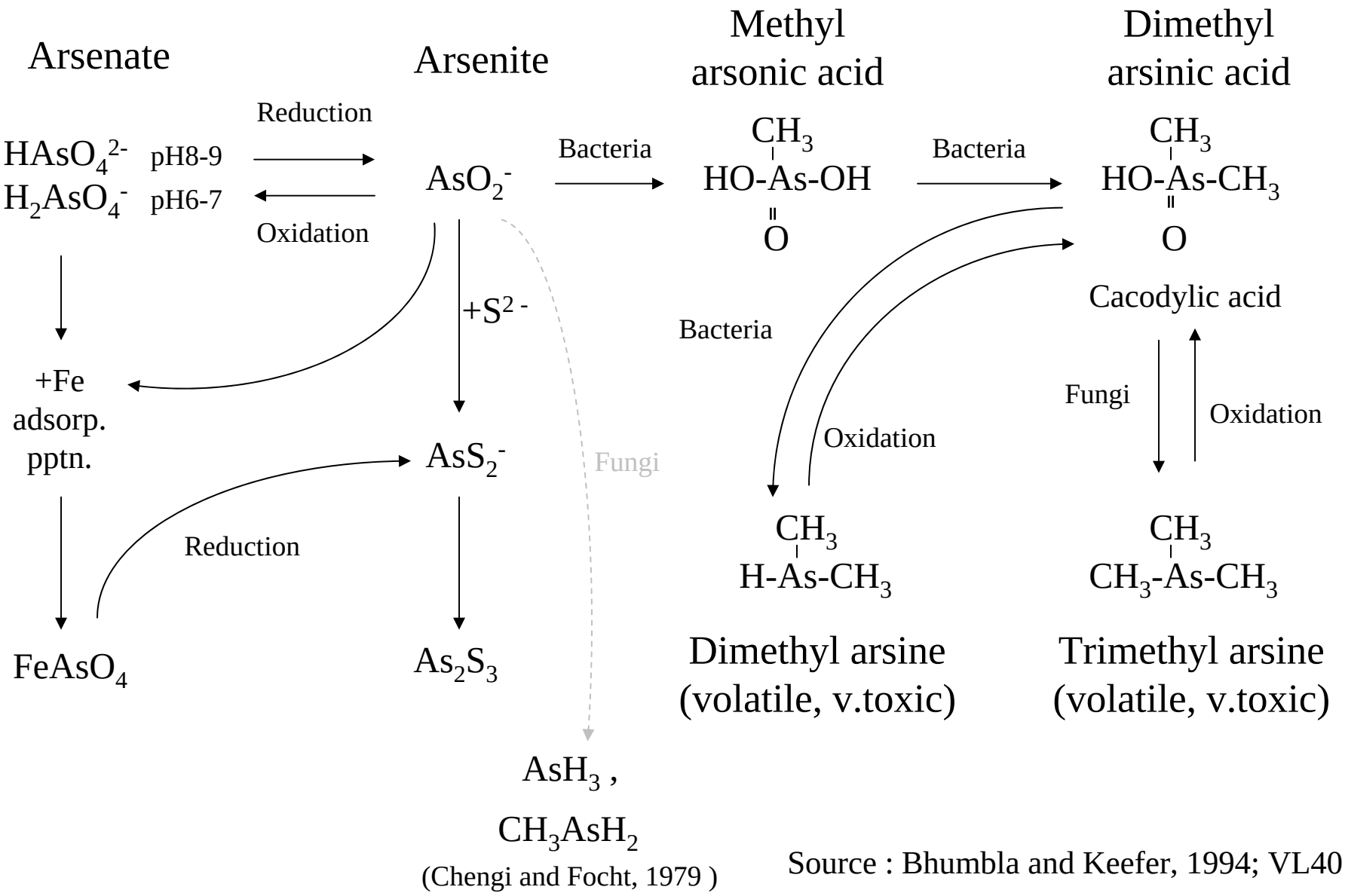
- Good conditions for microbial diss. sulphate reduction:
  - appropriate redox potential
  - sufficient C for growth of bacterial biomass
  - enough electron donors (H_2)
  - Sulphate removal
  - pH
  - Removal of heavy metals: almost complete
- Intensity: summer > autumn

Outlook - Future

- use of the plant biomass in separate bioreactors as substrate for dissimilatory sulphate reduction

- further process optimisation of the hydrogen input into HP
  - increase of the H_2 input?
  - improvement of the process stability?
  - Increase of the yield?

Chemical forms of As and their transformations



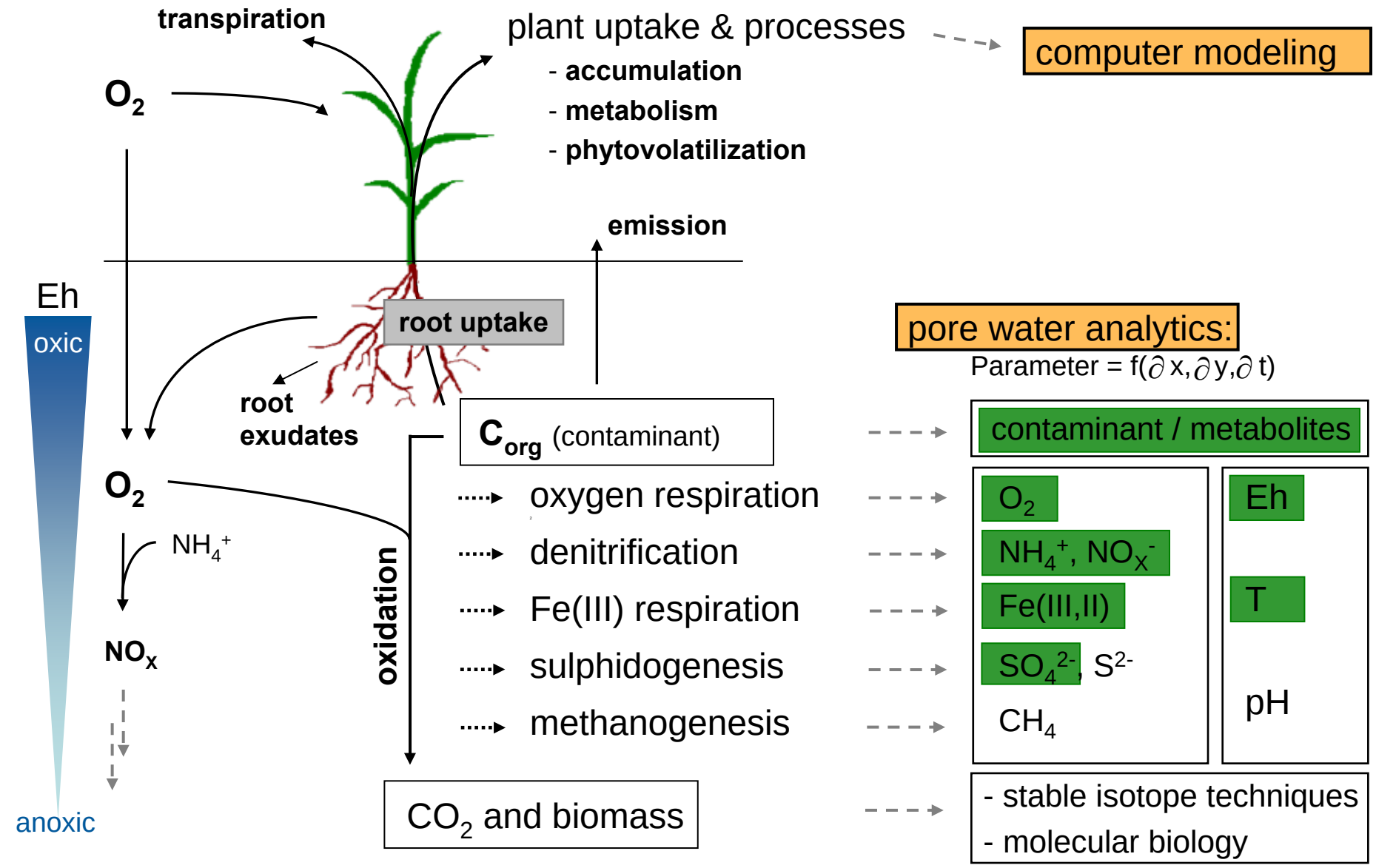
Source : Bhumbla and Keefer, 1994; VL40

Recalcitrant Organic Compounds

Potential role of plants for the degradation of recalcitrant organic compounds

log K_{OW}	Mechanism
< 1.0	Possible Uptake & Transformation
1.0 - 3.5	Uptake, Transformation, Volatilization
> 3.5	Rhizosphere bioremediation or Phytostabilization

Modeling

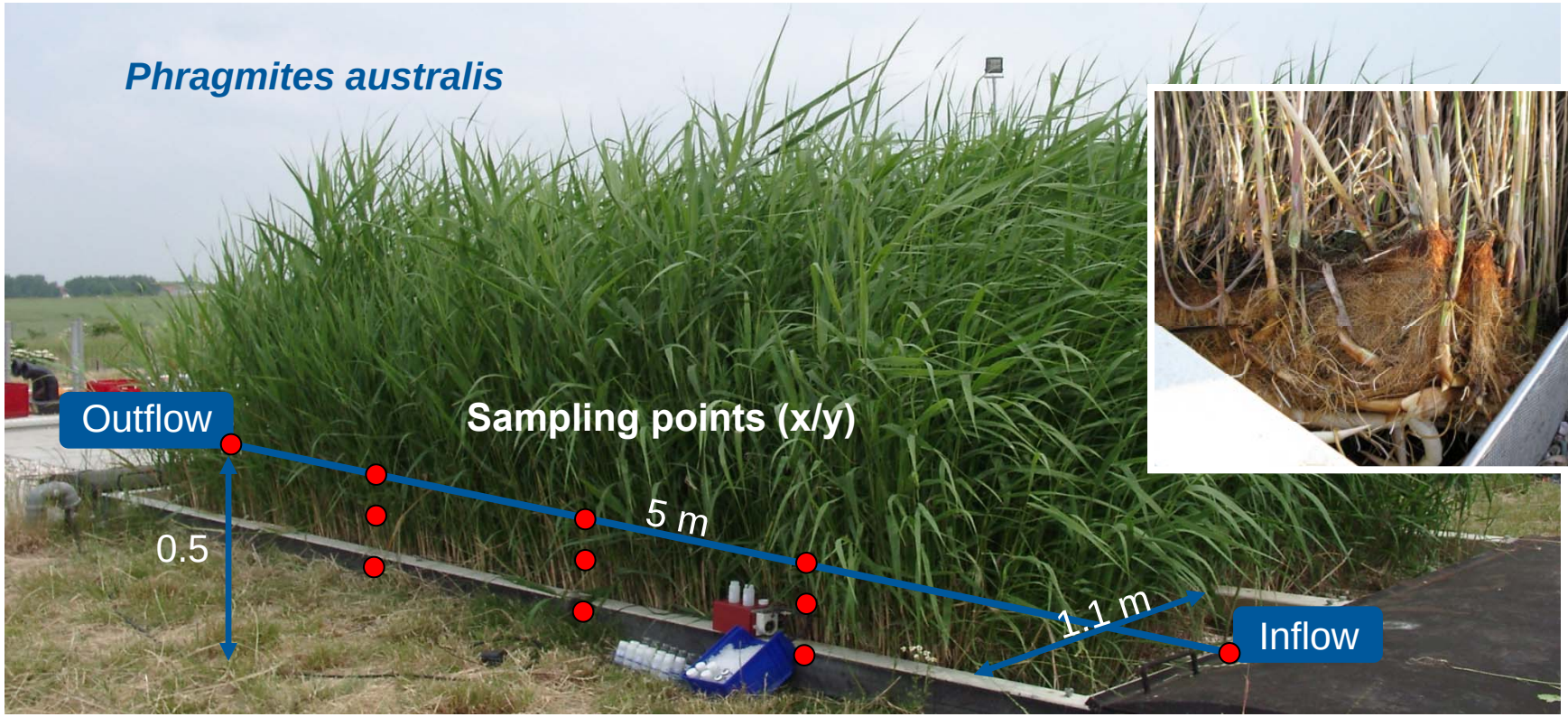

transpiration
plant uptake & processes
- accumulation
- metabolism
- phytovolatilization
computer modeling
O2
emission
Eh
oxic
root uptake
pore water analytics:
Parameter = f(∂ x,∂ y,∂ t)
root exudates
Corg (contaminant)
contaminant / metabolites
O2
oxygen respiration
NH4+
denitrification
oxidation
Fe(III) respiration
NOx
sulphidogenesis
methanogenesis
O2
NH4+, NOX-
Fe(III,II)
SO42-, S2-
CH4
Eh
T
pH
anoxic
CO2 and biomass
- stable isotope techniques
- molecular biology

Experimental Station SAFIRA II in Leuna

Foto: M. Kästner

Experimental Station SAFIRA II in Leuna

Constructed Wetland (CW) at Leuna site



Wetland characteristics:

Filter material

- gravel
- gravel + activated carbon
- gravel + Fe(III)
- plant mat

Contaminant concentration

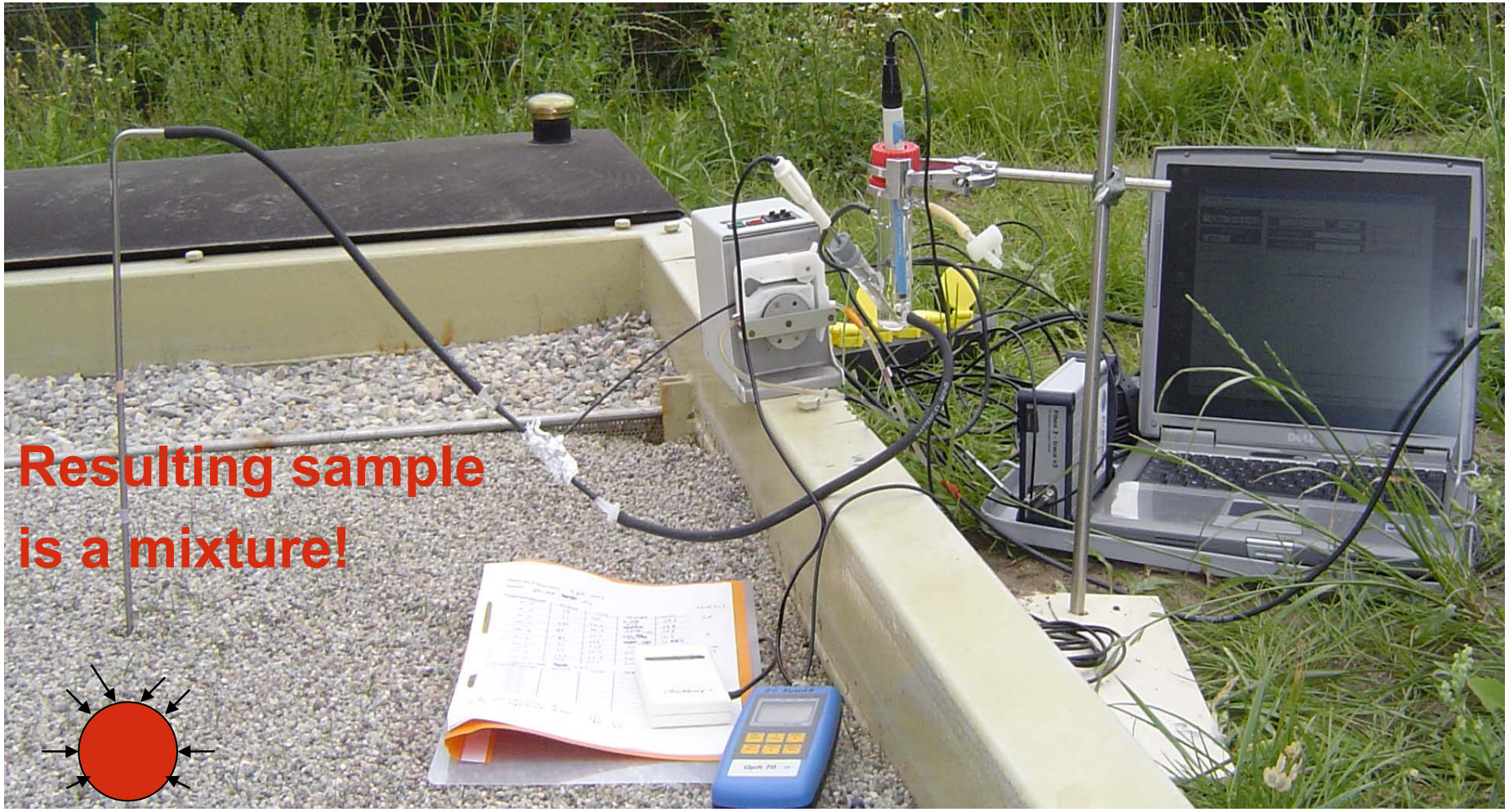
$c_{contaminant,\ inflow}$ [mg/L]:

Benzene	18.6
MTBE	3.7
NH_4^+	57.9

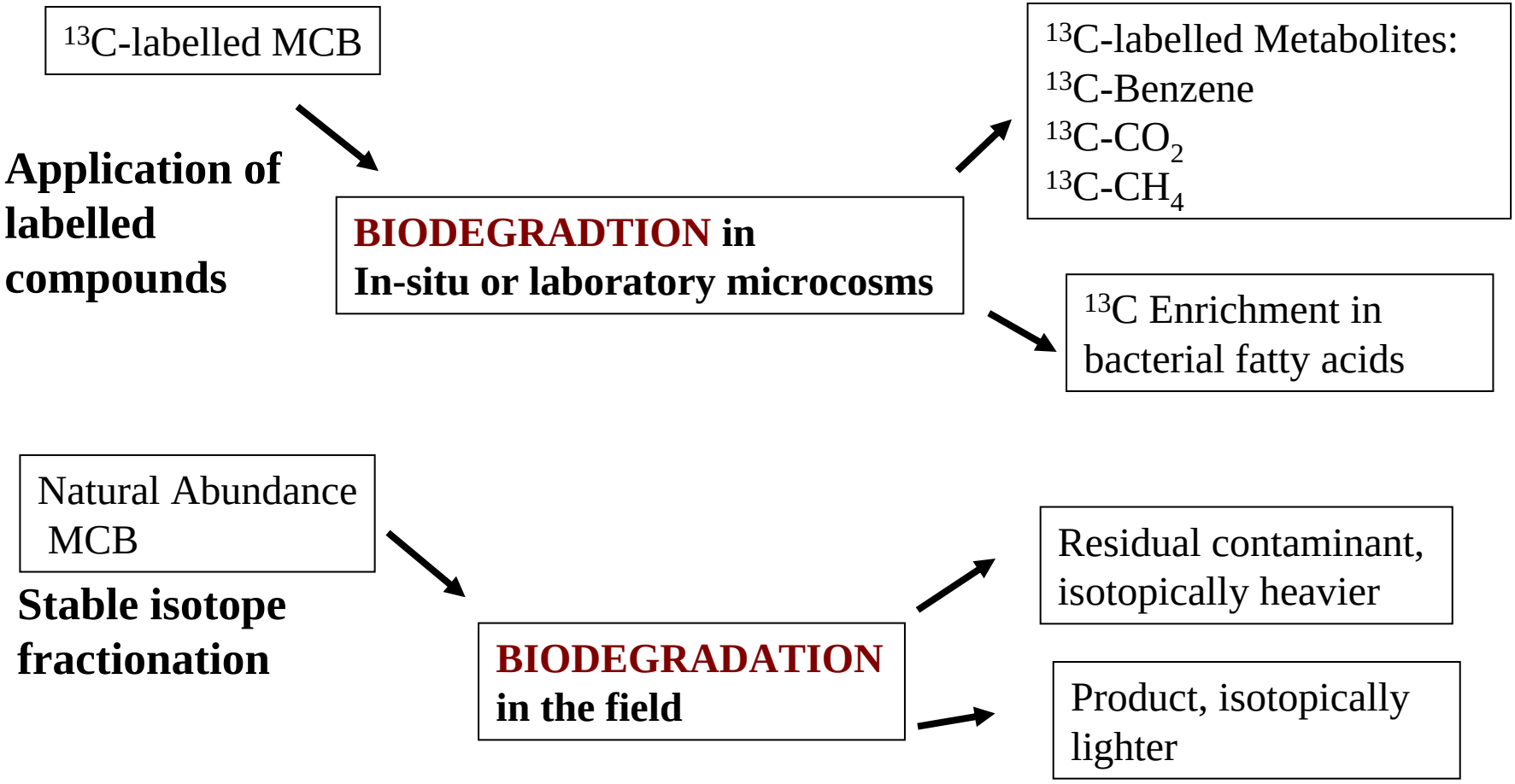
Operation parameter

- inflow velocity [L/h]: 6.0
- $\tau_{theo.}$ (with filter) [d]: 6.0

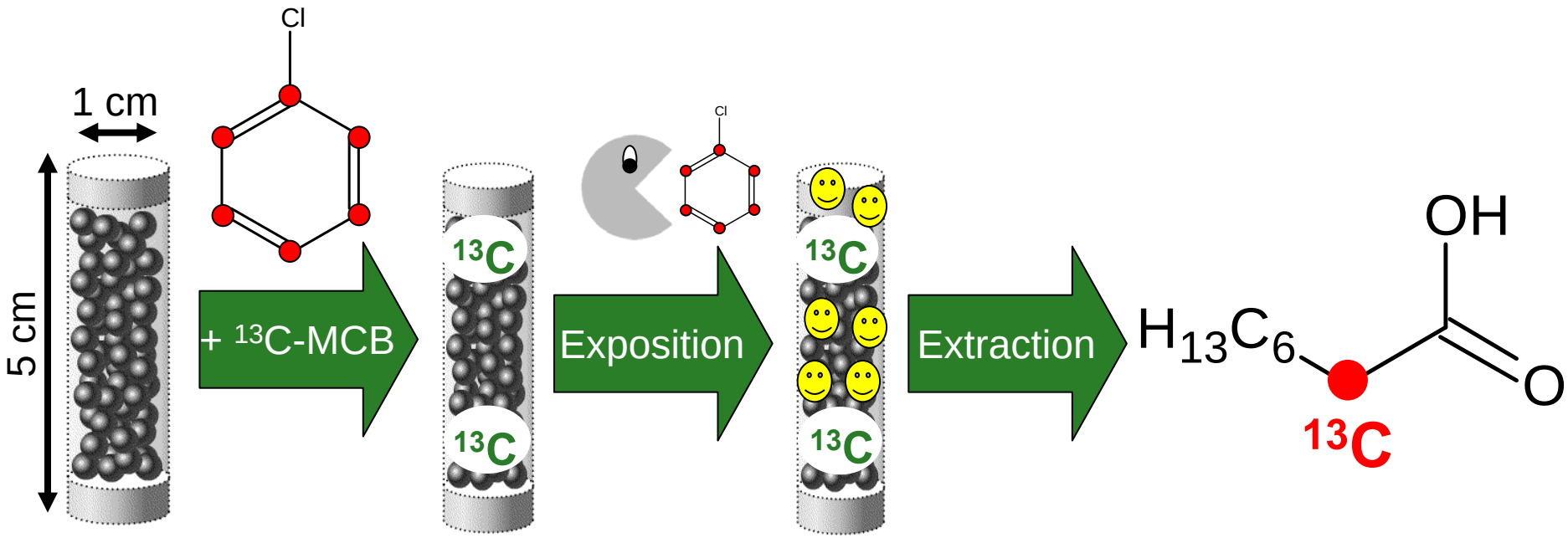
Procedure of pore water sampling



Principles of Stable Isotope Techniques



Line of evidence:
BACTRAPs - *in situ* microcosms



BACTRAP

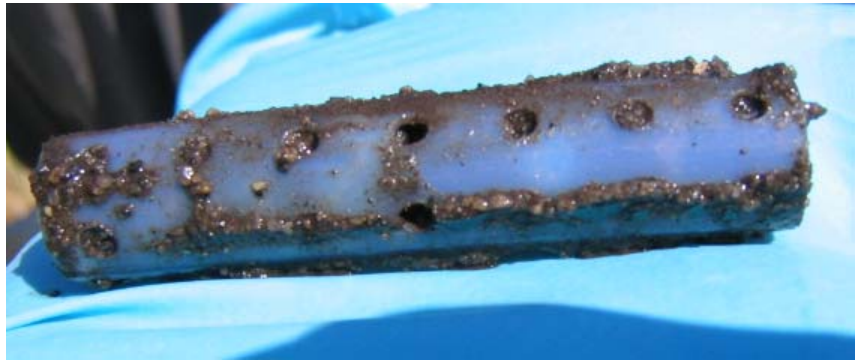
^{13}C labelled bacterial fatty acids

Geyer et al., 2002

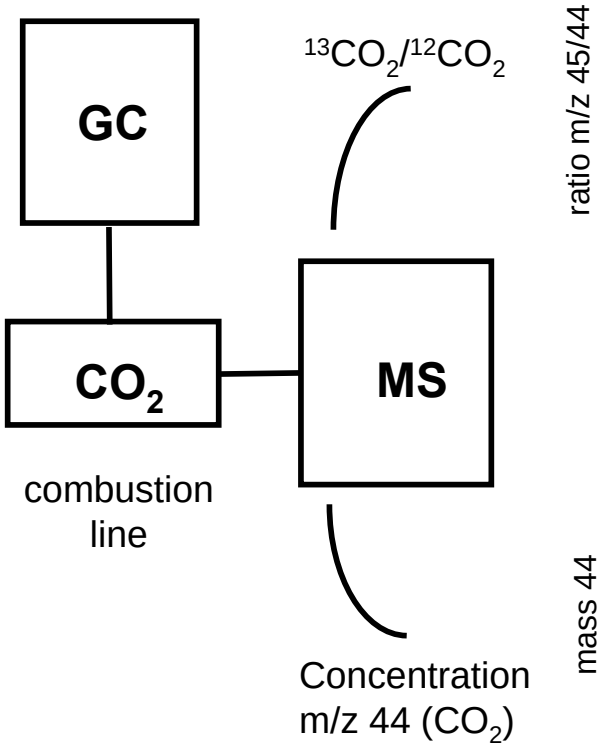
Sampling in the soil filter

Source: Stelzer 2004

Exposition in the field site (6 weeks)



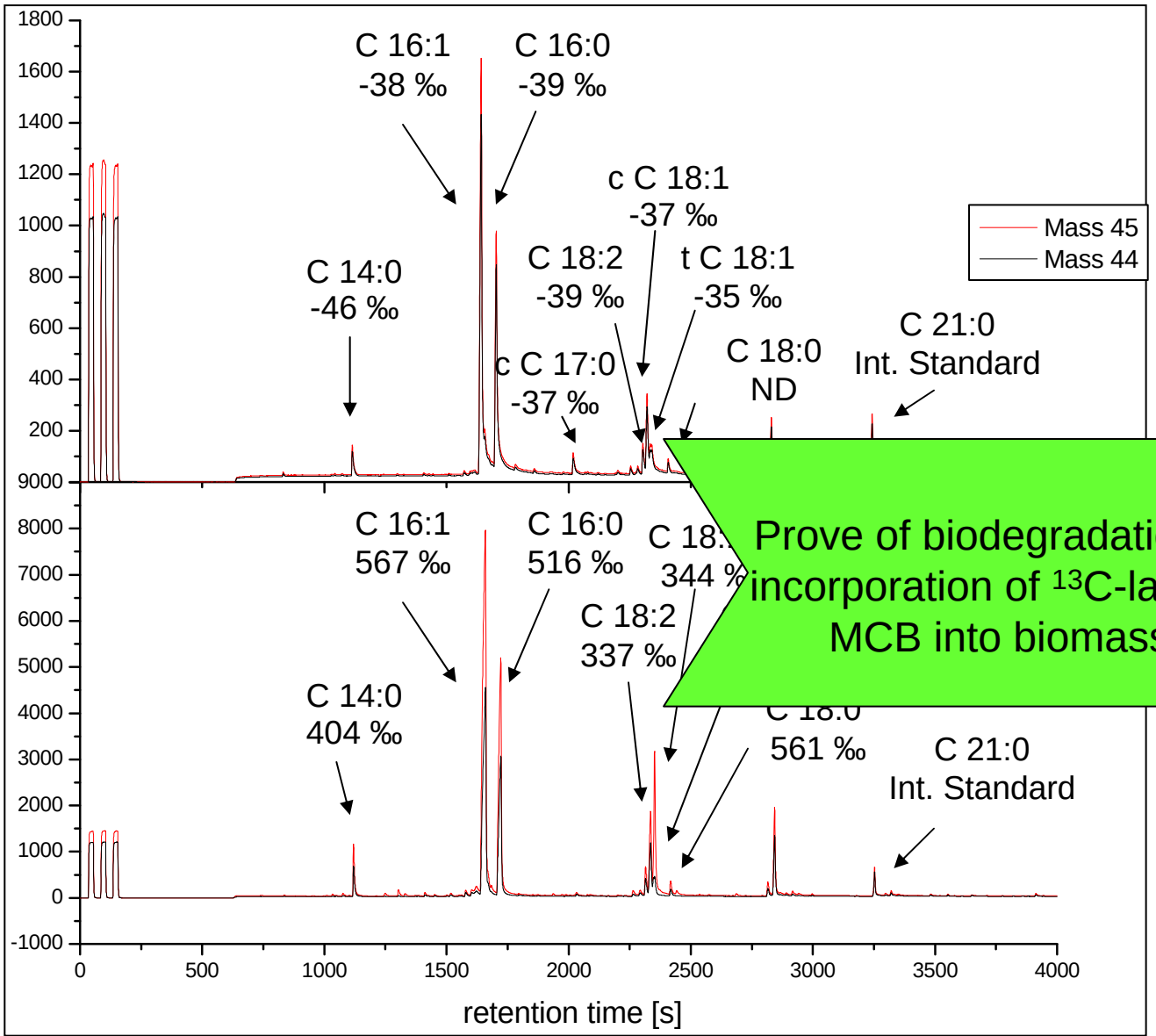
Isotope-Ratio-Monitoring-GC-MS:



Chromatogram of
Isotope-Ratio-Monitoring-GC

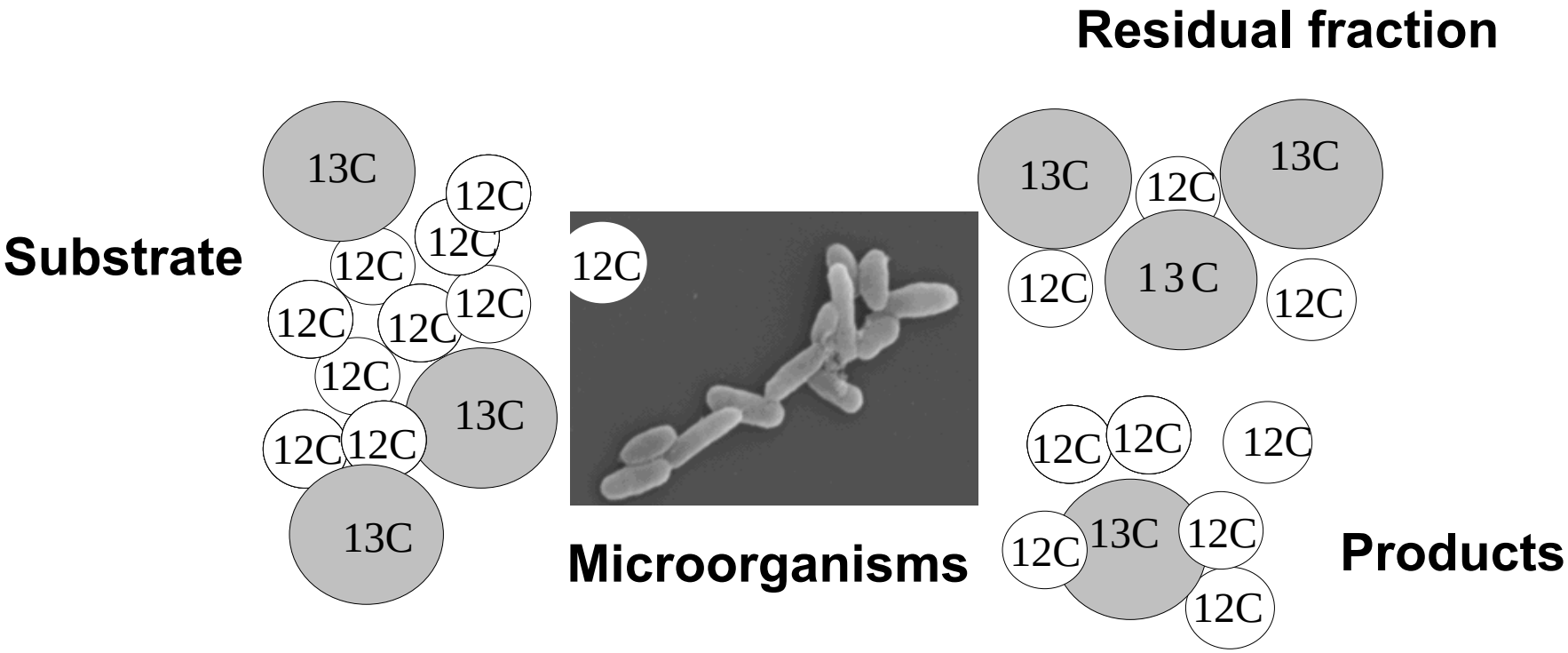
Activated carbon BACTRAP free water ^{12}C

Activated carbon BACTRAP free water ^{13}C



Prove of biodegradation by incorporation of ^{13}C-labelled MCB into biomass!

Carbon isotope fractionation analysis



Carbon isotope ratios are reported in δ ‰ relative to an international standard, the PDB (Belemnite, Cretaceous Peedee Formation, South Carolina)

Future

Landfill leachate, manure treatment
(Optimisation of N-elimination, ANAMMOX)

Removal of recalcitrant organic compounds
(Pharmaceuticals, chlorinated compounds, …)

Mine drainages
(Different strategies for the distinct metals like Zn, Cr, Cd, As …)

Wastewater hygienisation for field irrigation in aride zones
(Specif. role of phages, biolyt. bacteria and protozoa)

Realisation of close nutrient cycles; coupling with an-aerobic digestion (economic and ecological studies)

Thank you for the attention!

www.phyto.ufz.de

peter.kuschk@ufz.de