



**Sneller en goedkoper saneren van
vervuilde bodems**

met behulp van

Dehalococcoides ethenogenes

**Thin-section electron micrographs showing
coccoïd and elongated cells**

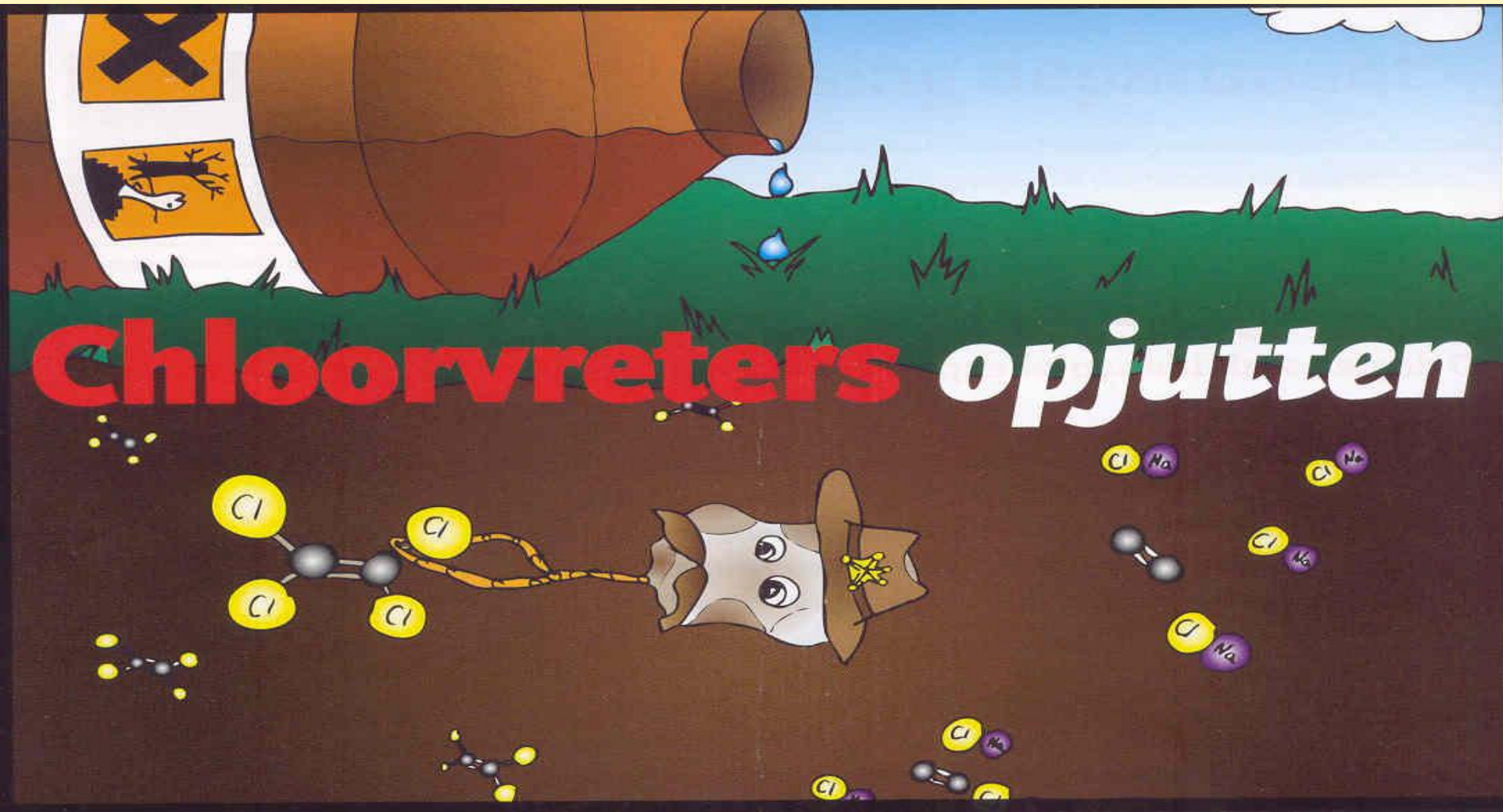


Photos courtesy of Dr. Steve Zinder, Cornell University



Sneller en goedkoper saneren van vervuilde bodems

Bron: Chemisch Weekblad, juli 2009





Chloorvreeters opjutten

Door: poly-lactaat als slurry aan de vervuilde grond toe te voegen!!!

Volgens het Chemisch Weekblad artikel zouden na jaren inwerking door bacteriën op poly-lactaat eerst waterstof vrij komen en daarna lactaat, waarmee Dehalococcoides de chloorverbindingen af zou breken.

Lactaat, waterstof, de tijd en Dehalococcoides zouden dan de vervuilde bodems selectief schoon maken was de boodschap.



Chloorvreeters opjuttten

Deze aanpak was al jaren bekend en werkte niet naar behoren. In principe bracht het Chemisch Weekblad met dit artikel geen nieuws.

Theo Lalleman meldde dat ik met iets revolutionairs zou komen.

Aan mij de plicht om Theo gelijk te laten geven.

Trouwens Theo heeft altijd gelijk,

tot hij ongelijk heeft.



Chloorvreeters opjuttten

PTS staat voor PER en TRI Saneringen.

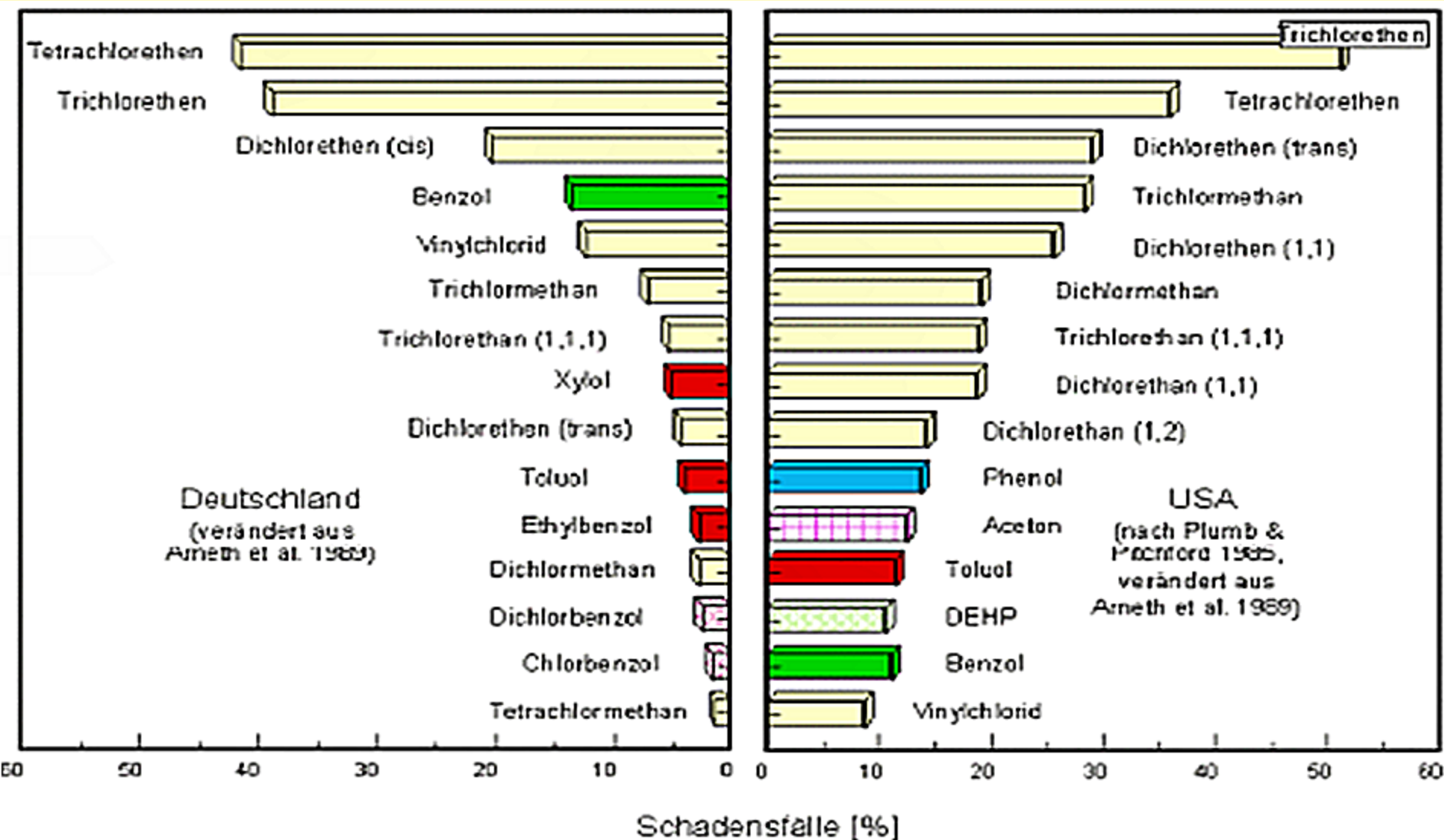
Een milieu-firma in Coevorden die binnenkort ook met mijn methode in Nederland aan de slag zal gaan.

Mijn naam is dr G.J.J. Beukeveld,

o.a. uitvinder en staatshouder.



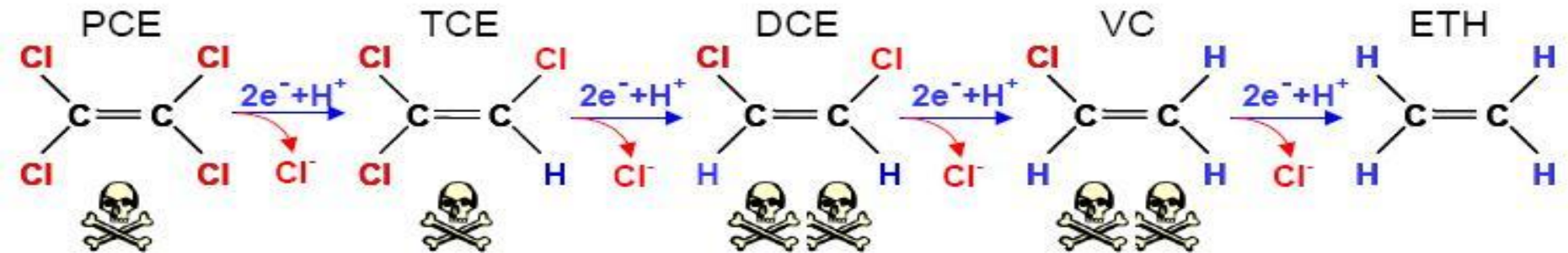
Frequenties van verontreinigingen





Ontchloren met bacteriën?

Anaerobic microbial reductive dechlorination

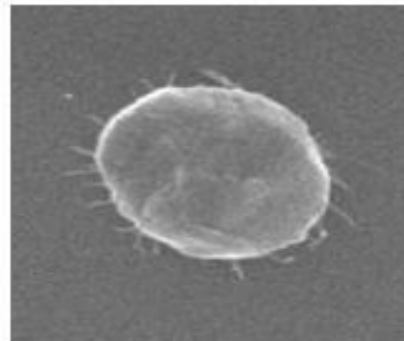


Dehalobacter, Dehalospirillum, Desulfitobacterium, Desulfomonile, Desulfuromonas, Enterobacter

Dehalococcoides

species:

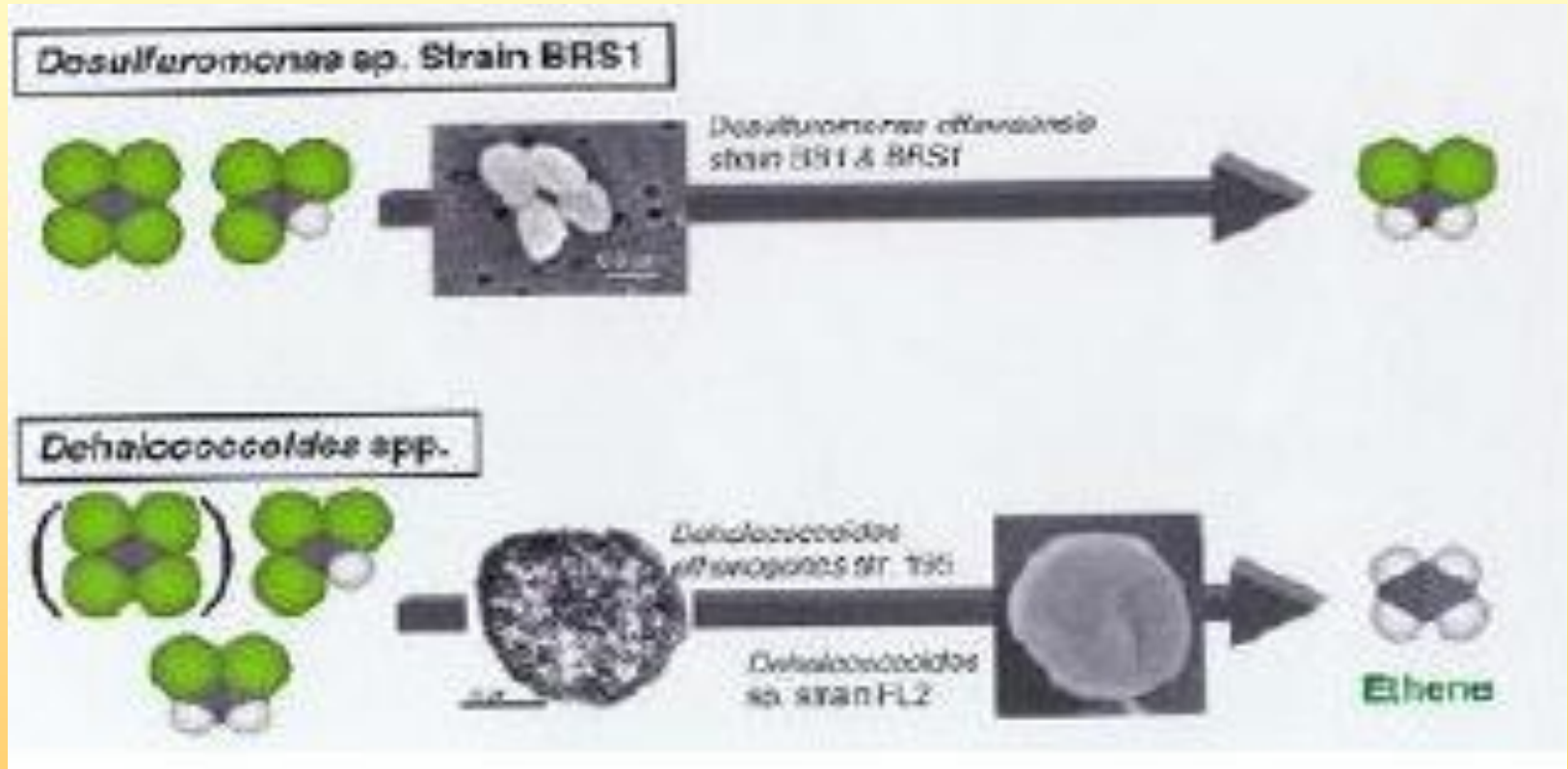
D. ethenogenes 195
BAV1
FL2
VS
CBDB1



He et al., Nature, 2003



Ontchloren door de bacterie te vertroetelen





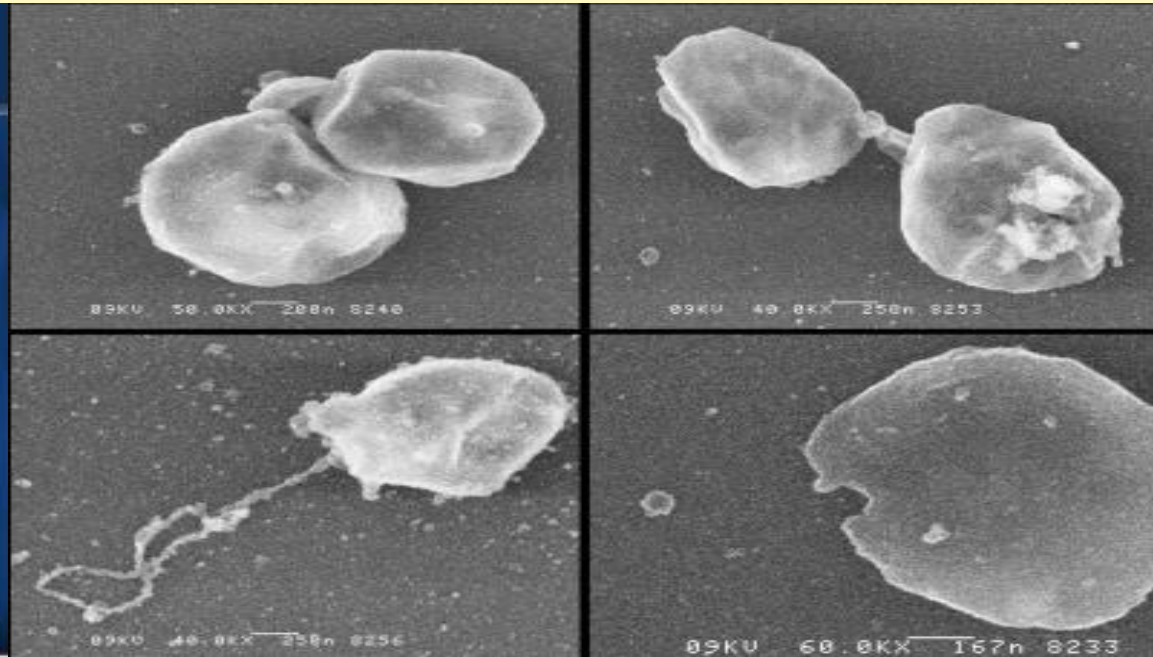
Anaërobe Biostimulatie

Anaerobic Bioaugmentation

- *Dehalococcoides* sp. (DHC) are microorganisms capable of completely dechlorinating PCE and TCE to the innocuous product ethene via halorespiration
- Notably, DCE and VC
- DHC are naturally-occurring, non-pathogenic microorganisms.
- Naturally occurring, non-pathogenic bacterium



Photos of BW1 - courtesy of Dr. Frank Loeffler



Biostimulatie

omstandigheden aanpassen zodat bacteriegroei ontstaat

Bioaugmentatie

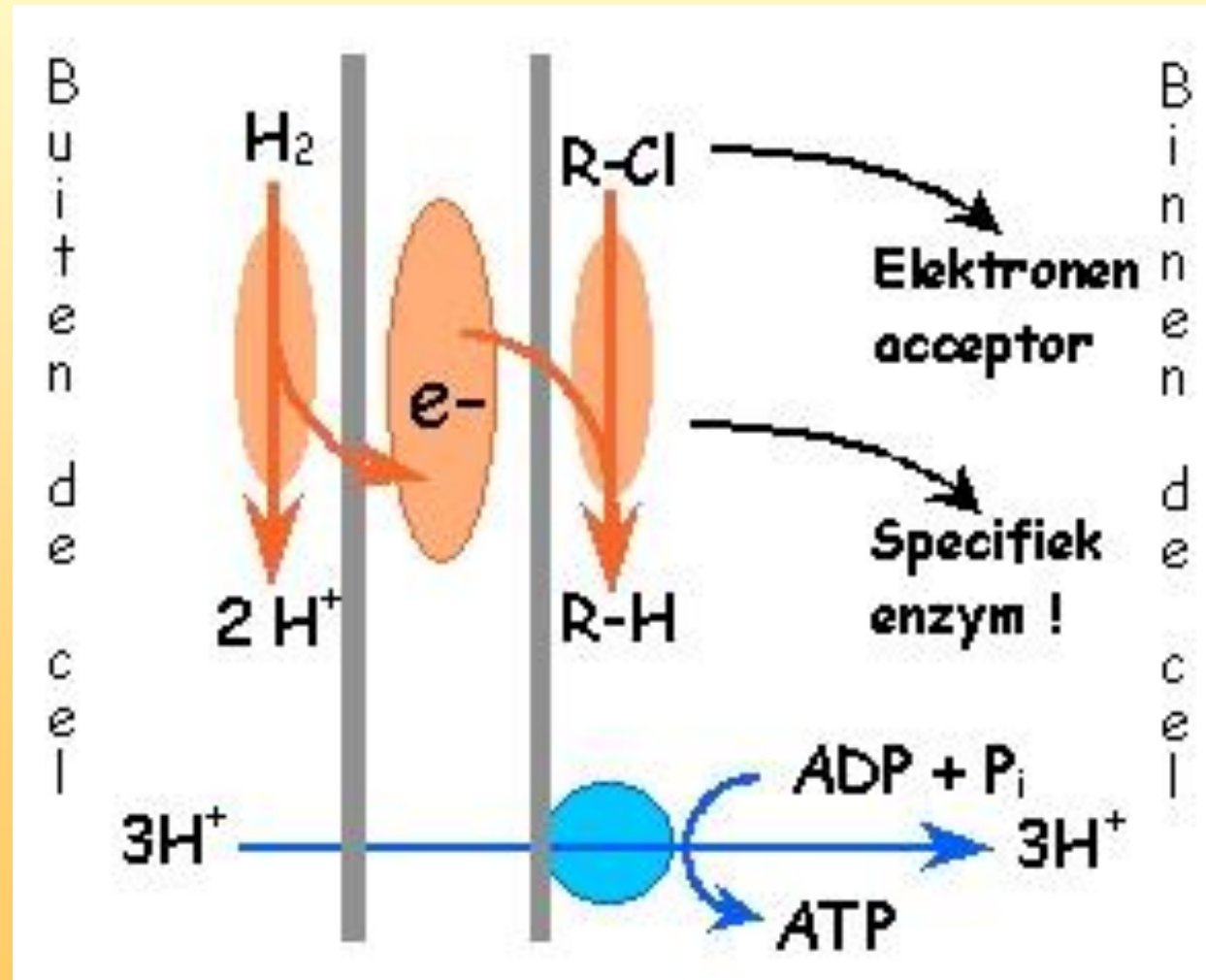
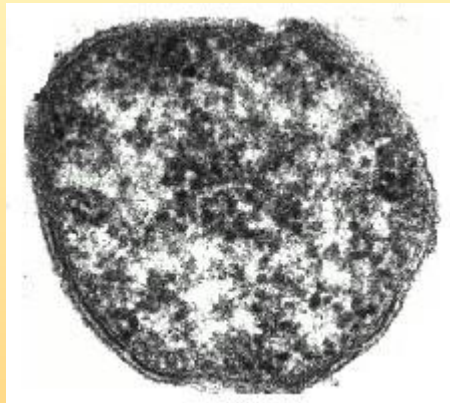
bacteriën toevoegen zodat extra omzetting ontstaat

Bioremediatie

biosysteem herstellen, verbeteren



Ademhaling van onze bacterie





DNA volgorde Dehalococcoides

>gi|57233530|ref|NC_002936.3| Dehalococcoides ethenogenes 195, complete genome

ACAAC TTTA TTTT AATTT ATGC ATCT ATGAG GGGCC GAAAG AGGCT AAAAA CAGAC ATACC AGTG AGATTT
GGAG AGTA AGGG GGTGT CAAGT GACCT TCTAT CCCTG CACAG GCACCT TGCTT GTTGT CAACC GCCATT
GTTACT ATTT ATCTA GTCTTT AACAG ATTGGT ACTTATT CTTTA AGGGG TGGAT CATT TTTCA TGGC AGAG
TGCTT GCCCC CTTG ATTT CAGATA CGGTTT AATTACT GGGAG GGATAT TTTTGT CTGTT CGGCCT GCACA
AGAACT TTTGG GAAAC AGCCCT GGGGG AGATTG AACTT CAGGT AAACA AGCCCA ATTTCC GCACCT GGT T
GCCAAA ACTGT CGGACT TAAGT ACGAC GGCAG TGAGTT TATTAT AGGTG TTCCCA ATA CT TTT CGTAG CCG
AATAT CTGG AGGTCA ATCAG CGTT CGCTG ATTGAAA AACCCCT GATAA ATATT ACCC ACAG CCCC GGTGA
GCTGGG TTTCC AGCTT GTCC AGTCC GCTC AGGTAC AGGCT GCCG CTCCC A AACAG CCAG AGAAAA ACC
GTTTCA CCCTTT AAAC CCCC GTTAC ACCTTT GAATC CTTT ATAGT GGGTT CCTG CAACC GCCTGG GCTCATG
CCGCTT CGCTGG CCGCC ACCCAGA ATCCC GGC AAAGAG CTACA ACCCG CTTT ATATCT ATGCG GCAG CCGG
TTTAGG CAAG ACCCAT CTTT ACAGG CTATCG GGCAT CTAGC AAACTT AAACC GCGC AAGGCC CTGTAT
GTCAG CGGGG AACAG TTTACC ACTGACT TTTAT ATCAT CTATCC GGAAC GGCCAA ACCG AAGAG TTCCGTG
CCC GTTACC GGGATG TAGATTT ATTGCT GCTGG ATGATG TCCAG TTTAT CGGC GGC AAAGG AGCAG ACCGA
AGAAT GCCTG TTTCC ATACCT TTAAT GACCT GCATA ACTCCA ACCGCC AGATAG TCATTT CAGC AGATTCA
CCCC CAAGTCA CTGCC CAGCTGG CCGAA AGACTG CGTTCCC GTTTT GAGTGG GGGCTG ACGATTGAGA
TTGA ACCGCC GGATG AAAAA ACCCG CTTGG AGCTTTT GCAGCTT AAAGCTG AGCAGTCC GGCACC GAACT
TAATAT GGAC ACCCTGG AGTACCT GGCTC AGGAAGTAA AGCATA ATATCC GCGAA CTGGA AGGCAG CCTT
AACC GGGT GCTGG CCTATGCC CGCCTGCTA AGGGCTACC ATTAC CCCGG ACTGG CCGCC AGAGCC CTGT
CGGATAT CGGCTC ACGCCC GGT CAGGG AAAAC AGCCCTCT CCGCCCC GGTA ATATA ATTT CAGC GGTATC
CCAGGTAT TTTCAA ATACCC GCAACC GAACTTTT GGGT GCCG CCGGTG ACA AAGATA ACCGCTCT GGGCCGT
CAGTTT GCCATG TTTATGCT AAAAG CAGCAA AACTCC GCCTCA CTGGT AGAAATAG GCCAGTCC CTGGGGG
GCAGA AGCGCTTCC ACCGT CAGCC ACGCCTG CGATA AAAATCC AGCTGG AGTTGG AAAAC AGCGCCTTTCT
GCGCCTG AAAATGTCC GAGGTTCAA AAC CAGCTTTCCC AGCAG CGCCTTTCCC GCTGATATTAG CCCTTT
TCGCC GCACC GCTTTT CCTTATC CTTATAT TTTTCTTT ATTTT CATTCTGCC AGCAGGGT GCTTTT GCCG
GTTTT CTATTTCTTTT TAGAC AGTCTTTCTATA AACTTTAT TATAT TTTT CGATA ACCGGGCTGA ATTTT C
GATAACTTTGC AGATACTTTGCTTT AAAAATA AAGAC CCGGAGG ACAGGA ATAGAG TTTTACT CGTAAAGC
CTTATTATTAGTATTTCTGATATACTTAAAAATAAAC CAGTTAAAGTAAATGGGATTTTCGACA ACTTAAG
AATTAGATAACTTTT TAGATAAGGTG TTTTATATG TTTGACAGAGTGG AAAATAGG ATTAAGTCCGGAG
ACGGCGGCAG CCGCAAGT TAGTTTCC GCGCG AAAAATTTGTGCCTTACGG CCGGCCAGGGGGTGA
CGGCGGGGACGGGGCAATGTATATCTGGA AGCTGATTCCGGCTTTACAGCCTTTTAAATTTTAAACAT
AAACGGGTGCATAAAGGCGGCCAACGGCGAGAACGGTATGGGCAGCCGCTGTACC GGCCACAACGGGGCTG
ACCTGGTGATAAAAAGTGCCGGTGGGTACGGTTGCTACTATTGTGGAAGAAAACGGCCAGAAAACGGGTTCT
GGCTGATTTGGCCGCTGACGGAGACCGTACCCTGGTAGCCCGCGGCCGGGCAGGGCGGACTGGGCAATACC
CACTTTGTTTCGTCCACCAACCAGGCCCCCATGCTGGCTCAGAAAAGGCCAGCCGGGCGGCGAATATGAGC
TGATACTGGAACTGAAACTGATTGCCGATGTGGCTATTATCGGCTATCCGAATGTGGGCAAACTTTCTGCT

**1,4 miljoen
baseparen**

**bestaande uit A,
T, G en C**

**Drie letters
coderen voor
aminozuren**

**20 aminozuren
vormen de
eiwitten**

**1580 eiwitten
gevonden**



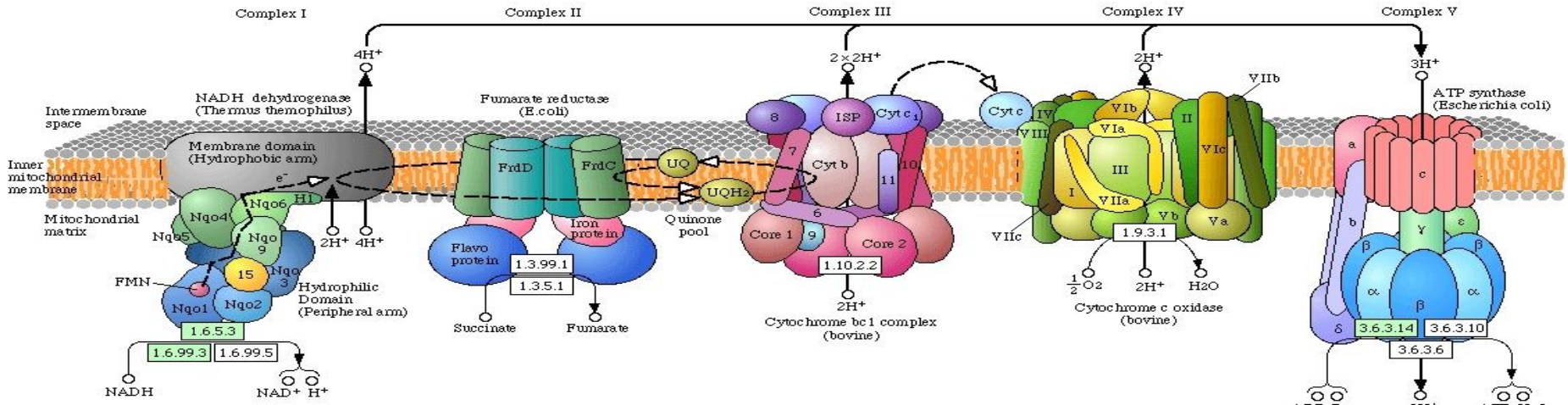
Dehalococcoides ethenogenes 195, complete genome. - 0..1469720

51 RNAs

Location	Strand	Length	PID	Gene	Synonym	Code	COG	Product
47719..50667	+	2949	57223978		-	DET_De23S	-	23S ribosomal RNA
50767..50889	+	123	57223978		-	DET_De5S	-	5S ribosomal RNA
57782..57857	+	76	57223978		-	DET_tRNA-Ala-1	-	Ala tRNA
149037..149112	+	76	57223978		-	DET_tRNA-Val-1	-	Val tRNA
308964..309039	-	76	57223978		-	DET_tRNA-Ala-2	-	Ala tRNA
342947..343207	+	261	57223978		-	DET_DernpB1	-	sRNA
364688..364762	+	75	57223978		-	DET_tRNA-Thr-1	-	Thr tRNA
396447..396522	-	76	57223978		-	DET_tRNA-Met-1	-	Met tRNA
527290..527379	-	90	57223978		-	DET_tRNA-Ser-1	-	Ser tRNA
666312..666401	-	90	57223978		-	DET_tRNA-Ser-2	-	Ser tRNA
666436..666525	-	90	57223978		-	DET_tRNA-Ser-3	-	Ser tRNA
668207..668278	+	72	57223978		-	DET_tRNA-Arg-1	-	Arg tRNA
674395..674472	+	78	57223978		-	DET_tRNA-Pro-1	-	Pro tRNA
676389..676463	-	75	57223978		-	DET_tRNA-Gly-1	-	Gly tRNA
677016..677090	+	75	57223978		-	DET_tRNA-Met-2	-	Met tRNA
689105..689181	+	77	57223978		-	DET_tRNA-Arg-2	-	Arg tRNA
698661..698745	-	85	57223978		-	DET_tRNA-Leu-1	-	Leu tRNA
701192..701268	+	77	57223978		-	DET_tRNA-Pro-2	-	Pro tRNA
740686..740761	+	76	57223978		-	DET_tRNA-Phe-1	-	Phe tRNA
740836..740922	+	87	57223978		-	DET_tRNA-Leu-2	-	Leu tRNA
740952..741025	+	74	57223978		-	DET_tRNA-Gln-1	-	Gln tRNA
741036..741110	+	75	57223978		-	DET_tRNA-Asn-1	-	Asn tRNA
748011..748083	-	73	57223978		-	DET_tRNA-Lys-1	-	Lys tRNA
781966..782040	+	75	57223978		-	DET_tRNA-Gly-2	-	Gly tRNA
832857..832929	-	73	57223978		-	DET_tRNA-Lys-2	-	Lys tRNA
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877734..877809	-	76	57223978		-	DET_tRNA-His-1	-	His tRNA
913341..913416	-	76	57223978		-	DET_tRNA-Thr-2	-	Thr tRNA
913479..913562	-	84	57223978		-	DET_tRNA-Tyr-1	-	Tyr tRNA
913603..913675	-	73	57223978		-	DET_tRNA-Thr-3	-	Thr tRNA
928040..928116	-	77	57223978		-	DET_tRNA-Ile-1	-	Ile tRNA
928439..929873	-	1435	57223978		-	DET_De16S	-	16S ribosomal RNA
930500..930576	-	77	57223978		-	DET_tRNA-Glu-1	-	Glu tRNA

Ademhaling Dehalococcooides

OXIDATIVE PHOSPHORYLATION



NADH dehydrogenase

E	ND1	ND2	ND3	ND4	ND4L	ND5	ND6
---	-----	-----	-----	-----	------	-----	-----

E	Ndufs1	Ndufs2	Ndufs3	Ndufs4	Ndufs5	Ndufs6	Ndufs7	Ndufs8	Ndufv1	Ndufv2	Ndufv3
---	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------

B/A	NuoA	NuoB	NuoC	NuoD	NuoE	NuoF	NuoG	NuoH	NuoI	NuoJ	NuoK	NuoL	NuoM	NuoN
-----	------	------	------	------	------	------	------	------	------	------	------	------	------	------

B/E	NdhC	NdhK	NdhJ	NdhH	NdhA	NdhI	NdhG	NdhE	NdhF	NdhD	NdhB	NdhL	NdhM	NdhN	HoxE	HoxF	HoxU
-----	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------	------

Succinate dehydrogenase / Fumarate reductase

E	SDHC	SDHD	SDHA	SDHB
---	------	------	------	------

B/A	SdhC	SdhD	SdhA	SdhB	FrdA	FrdB	FrdC	FrdD
-----	------	------	------	------	------	------	------	------

Cytochrome c reductase

B/A	ISP	Cyt b	Cyt c1
-----	-----	-------	--------

E	COR1	QCR2	QCR6	QCR7	QCR8	QCR9	QCR10
---	------	------	------	------	------	------	-------

Cytochrome c oxidase

E	COX10	COX3	COX1	COX2	COX4	COX5A	COX5B	COX6A	COX6B	COX6C	COX7A	COX7B	COX7C	COX8	E/B/A	COX11	COX15	COX17
---	-------	------	------	------	------	-------	-------	-------	-------	-------	-------	-------	-------	------	-------	-------	-------	-------

B/A	CyoE	CyoD	CyoC	CyoB	CyoA	CoxD	CoxC	CoxA	CoxB	QoxD	QoxC	QoxB	QoxA
-----	------	------	------	------	------	------	------	------	------	------	------	------	------

Cytochrome c oxidase, cbb3-type

B	I	II	IV	III
---	---	----	----	-----

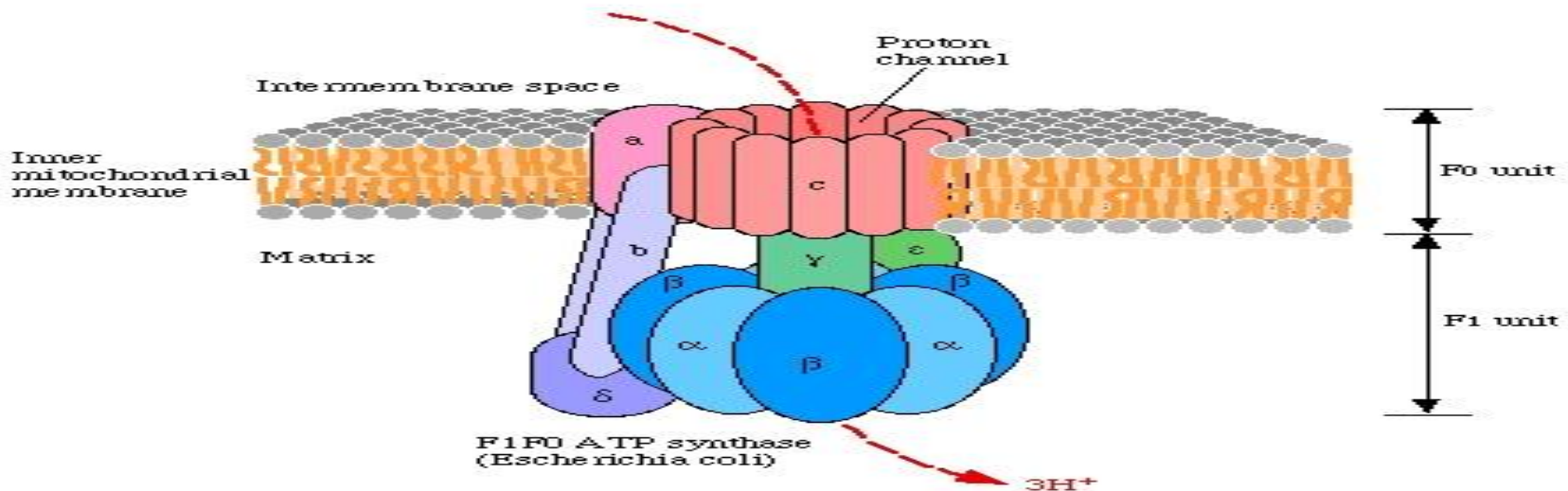
Cytochrome bd complex

B/A	CydA	CydB
-----	------	------



Synthese van ATP

ATP SYNTHESIS



F-type ATPase (Bacteria)

beta	alpha	gamma	delta	epsilon	c	a	b
------	-------	-------	-------	---------	---	---	---

F-type ATPase (Eukaryotes)

beta	alpha	gamma	O SCP	delta	epsilon	c	a
b	e	f6	f	8			
d	f	h	j	k	g		

V-type ATPase (Prokaryotes)

A	B	C	D	E	F	I	K
---	---	---	---	---	---	---	---

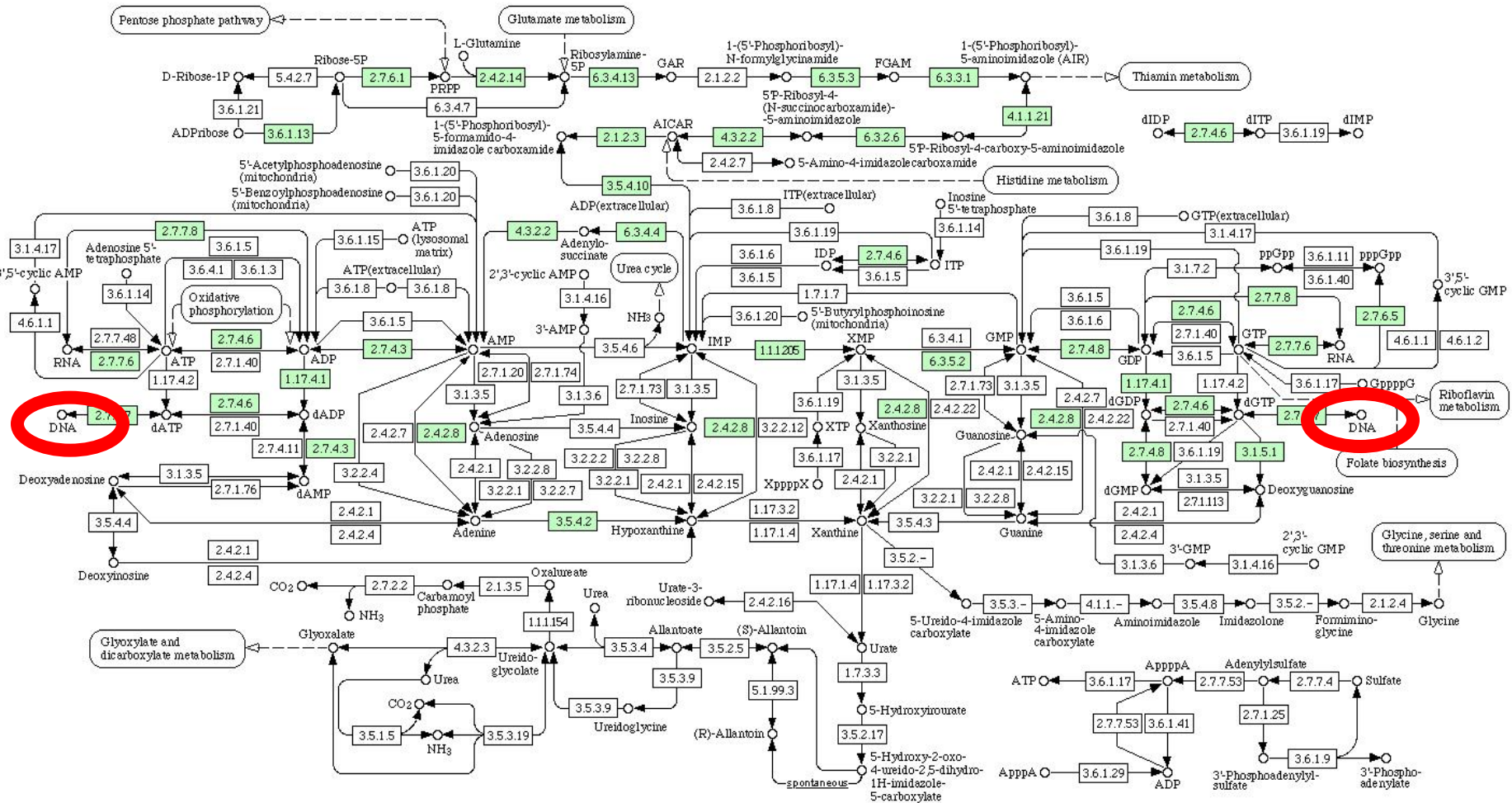
V-type ATPase (Eukaryotes)

A	B	C	D	E	F	G	H
I	AC39	54kD	S1	lipid			



Maakt DNA zelf

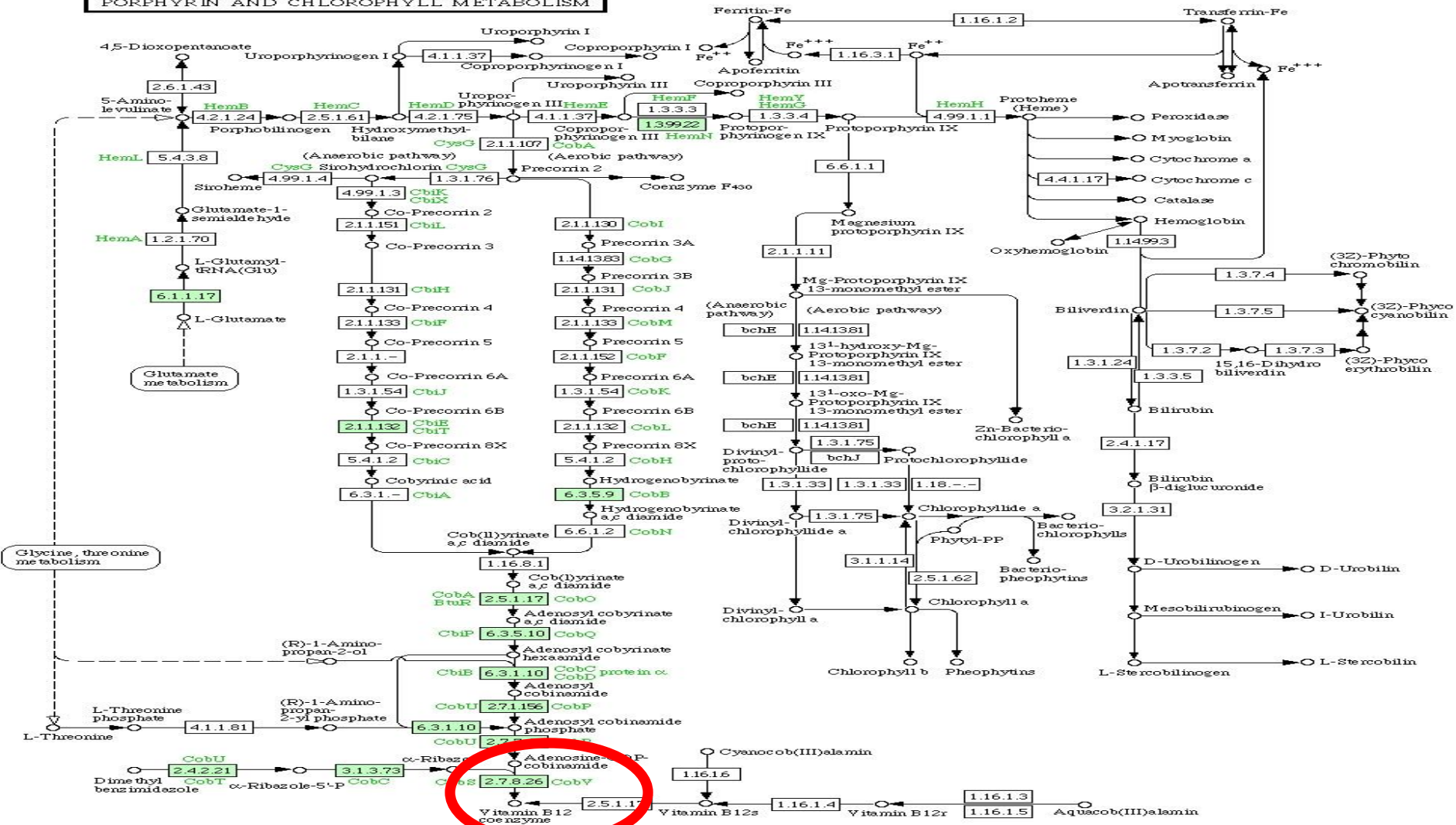
PURINE METABOLISM





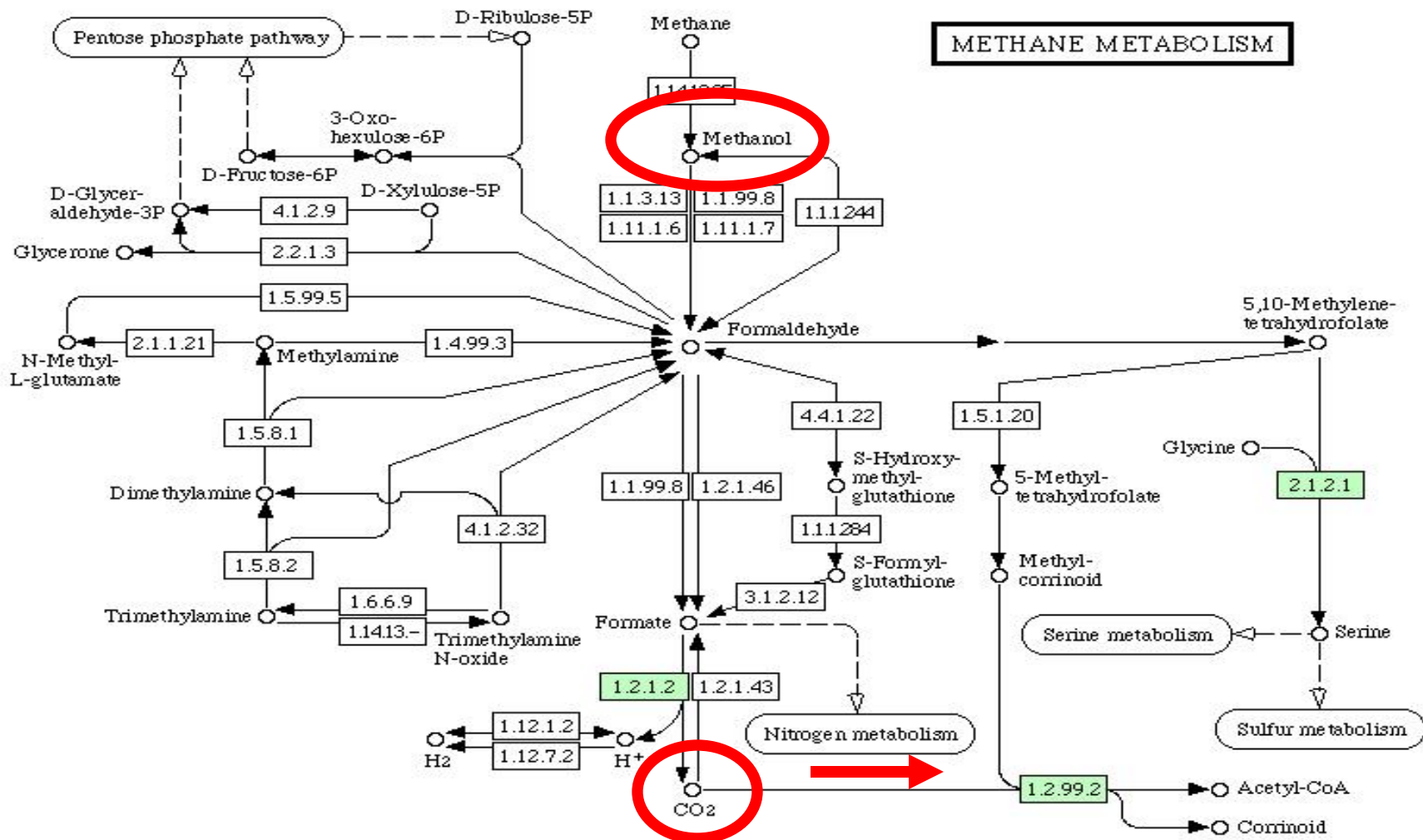
Maakt vitamine B12

PORPHYRIN AND CHLOROPHYLL METABOLISM





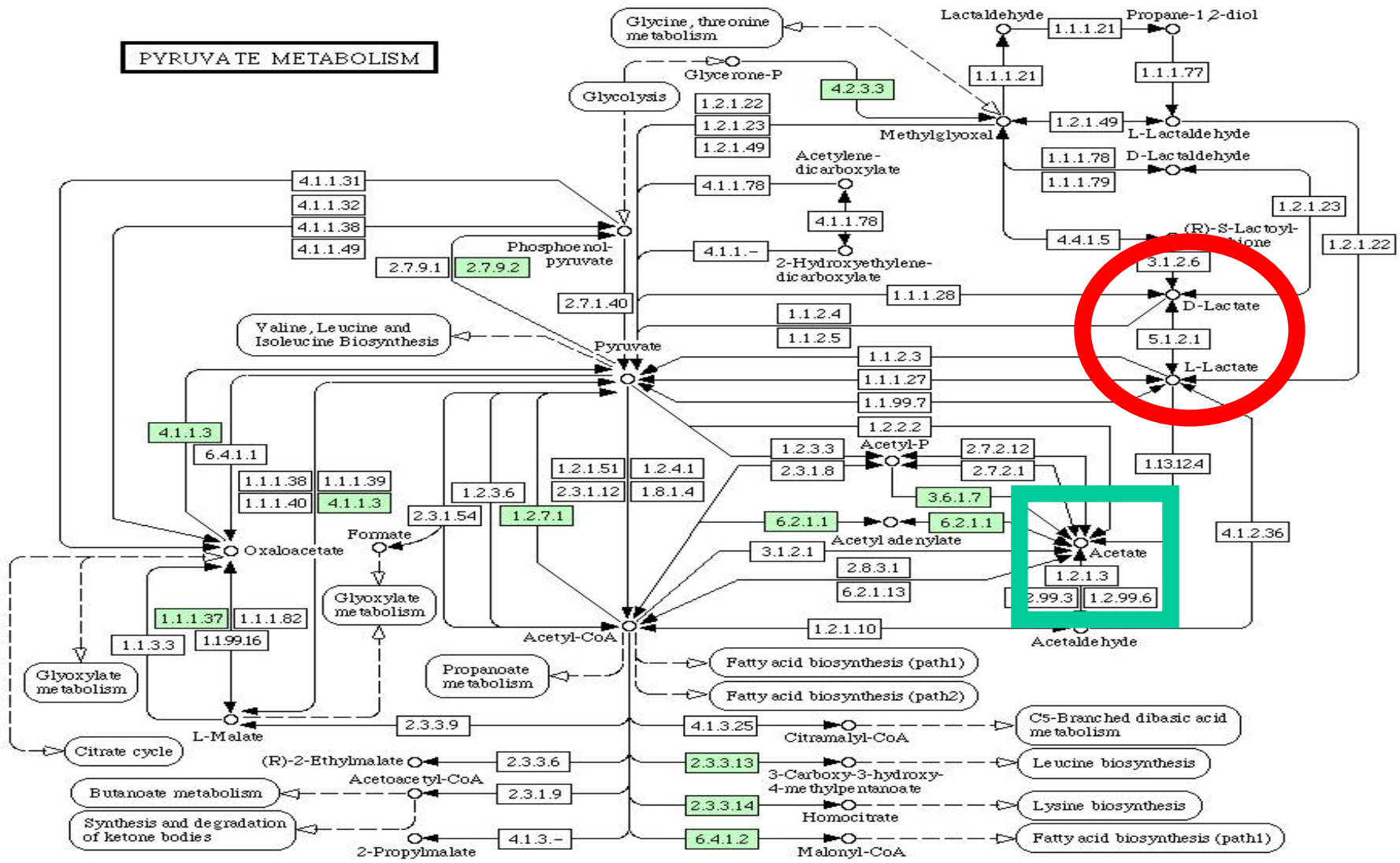
Gebruikt geen methanol





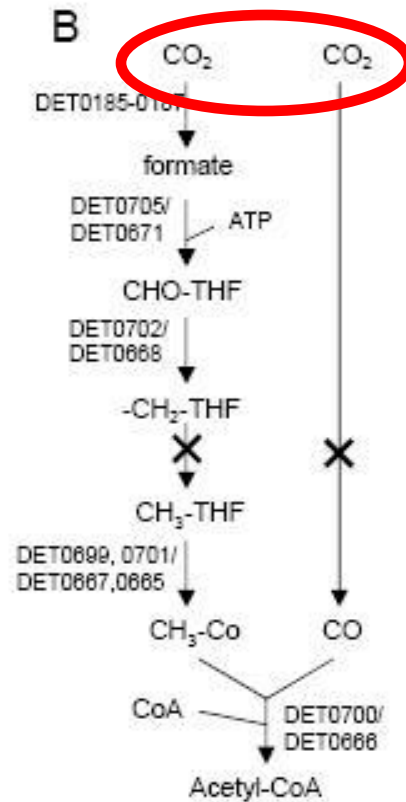
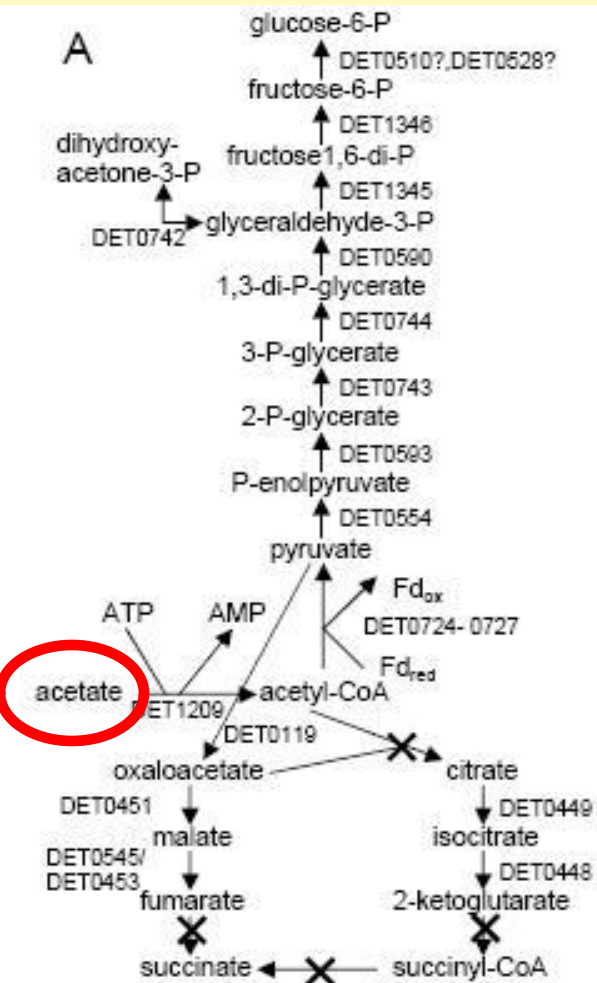
Geen lactaat wel acetaat

PYRUVATE METABOLISM





Twee belangrijke koolstofbronnen

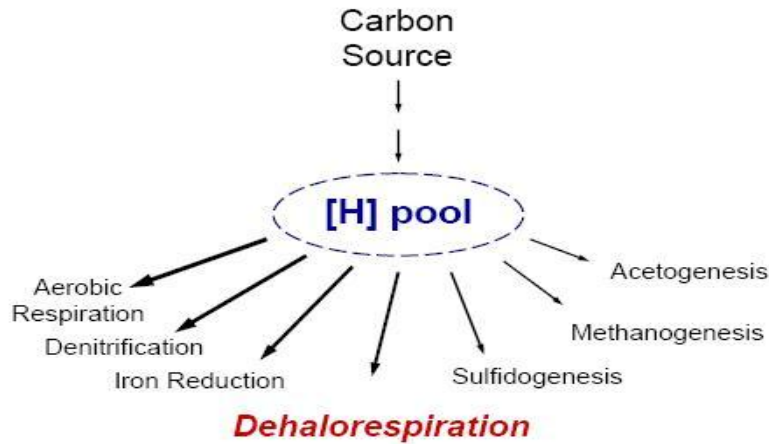


Uit de metabole pathways is op te maken dat Dehalococcoides geen acetaat kan maken en CO₂ gebruikt.

Deze twee stoffen moet de bacterie voor groei vanuit zijn omgeving zien te krijgen.



De centrale rol van waterstof



Affinity for H₂ determines predominant electron accepting processes

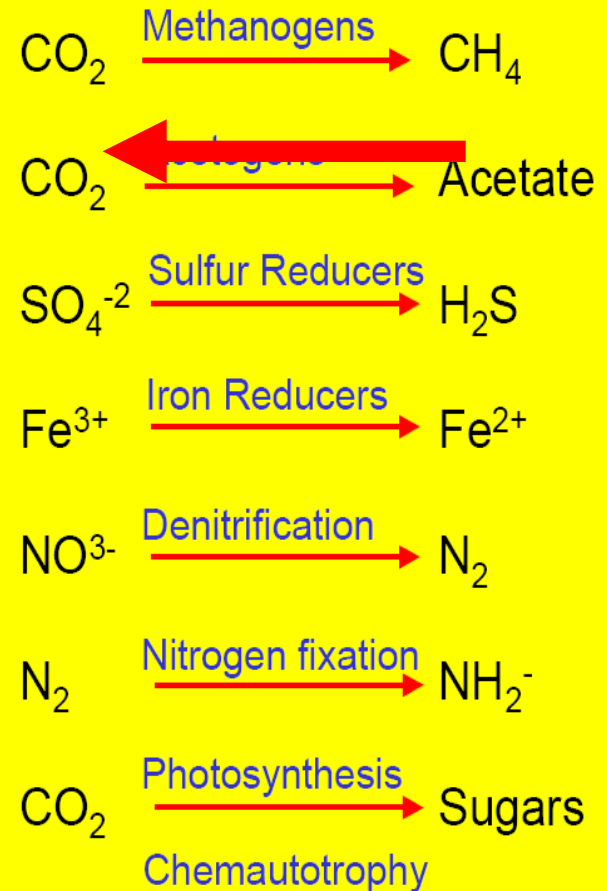
Electron Acceptor Process	Hydrogen Concentration (nM)
Aerobic (O ₂) respiration	<0.1
Denitrification	<0.1
Iron(III) reduction	0.2 - 0.6
<i>Dehalorespiration</i>	<i>< 0.31</i>
Sulfate reduction	1 - 4
Methanogenesis	>5
Acetogenesis	>336

concurrenten

Bottom Line: Dehalorespiring bacteria can out-compete methanogens for hydrogen

The Ubiquity of Hydrogenase

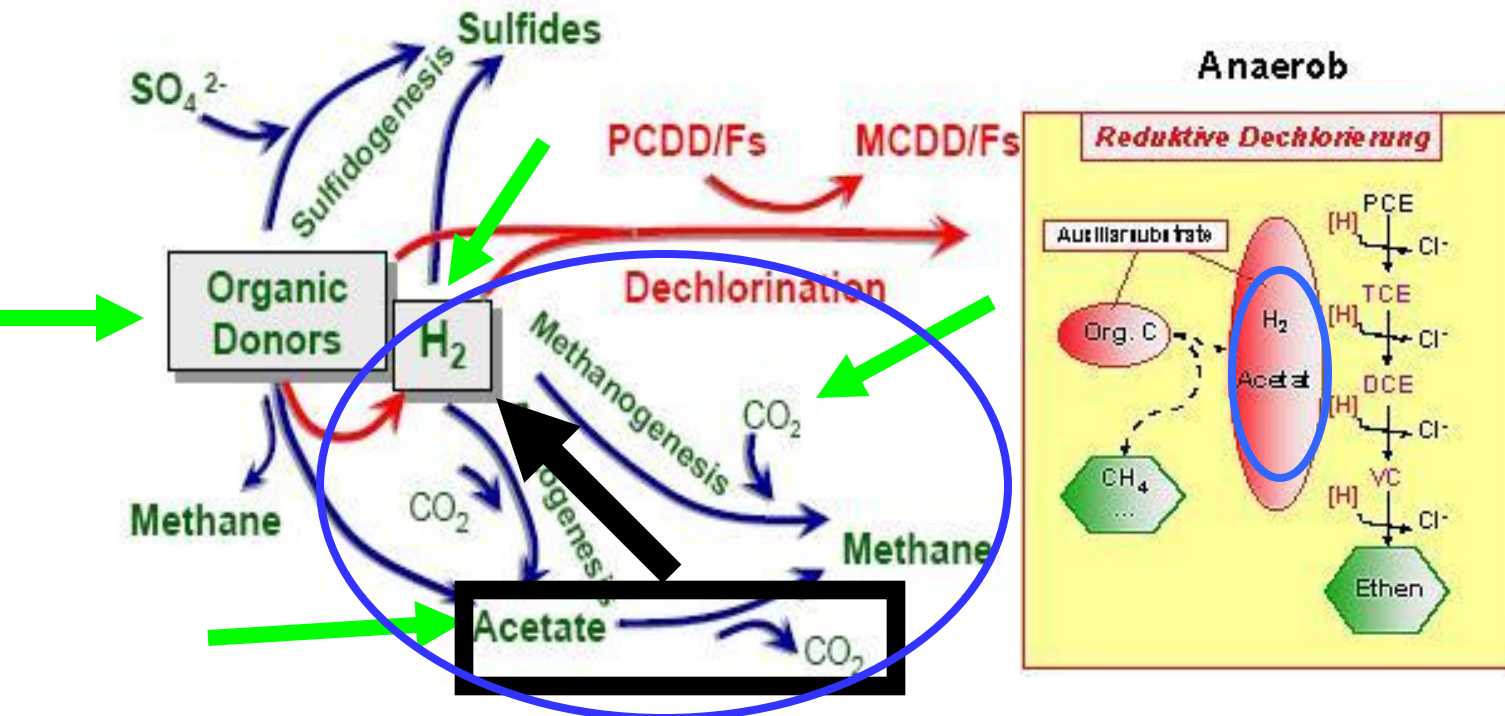
H₂ →





Anaerobe Biostimulatie

Dechlorination in anaerobic food-webs



Competing electron flow pathways in anaerobic sediments

Patent pending: PCT/NL2006/000124 - NL 1028531 (13-03-2005)



Methanol geen goed substraat

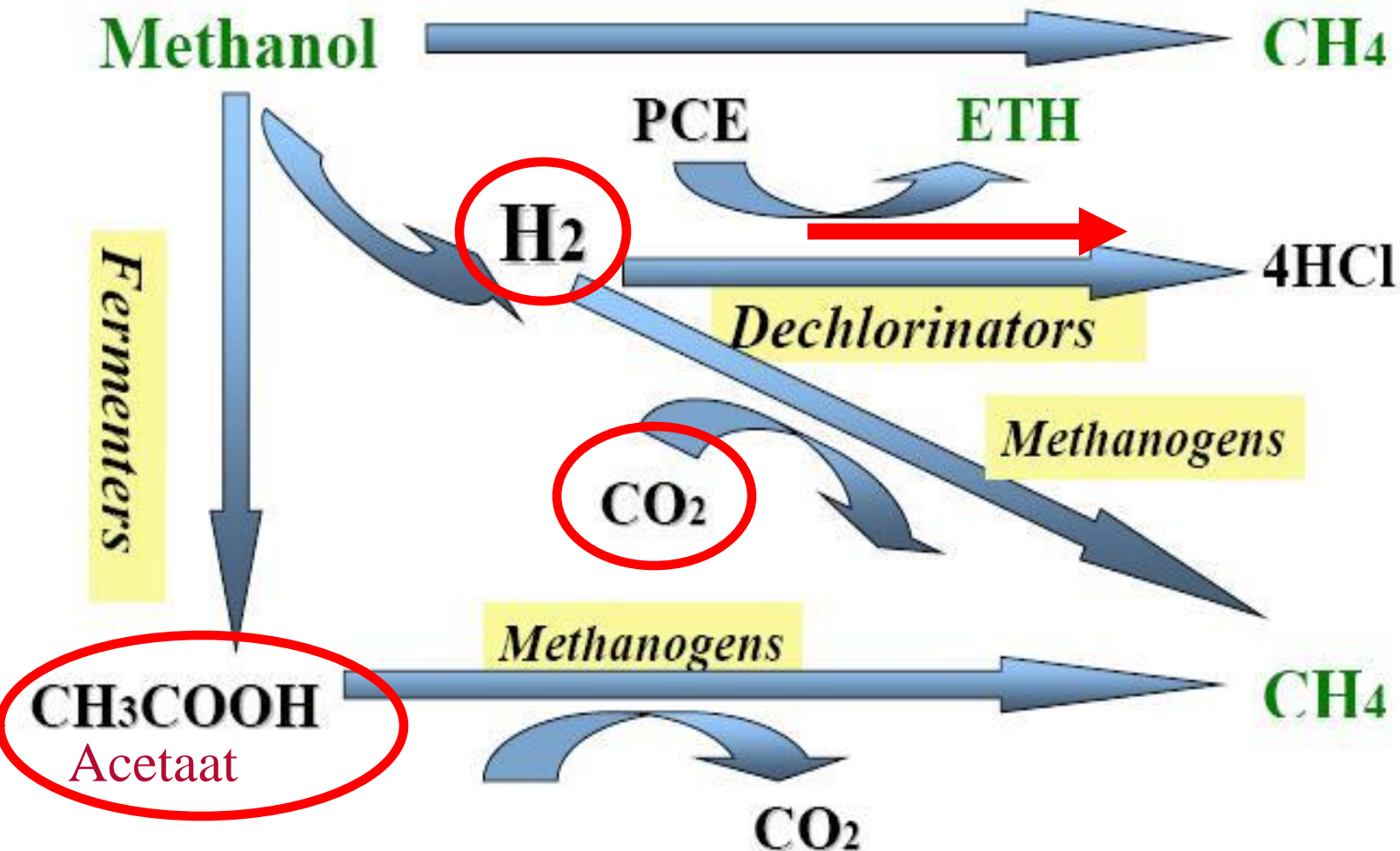


Figure 2.1 Interaction between Microbial Agents in Reductive Dechlorination using Methanol as Electron Donor (Non-Inhibitory PCE concentrations)



Elk substraat is indirect en niet trefzeker

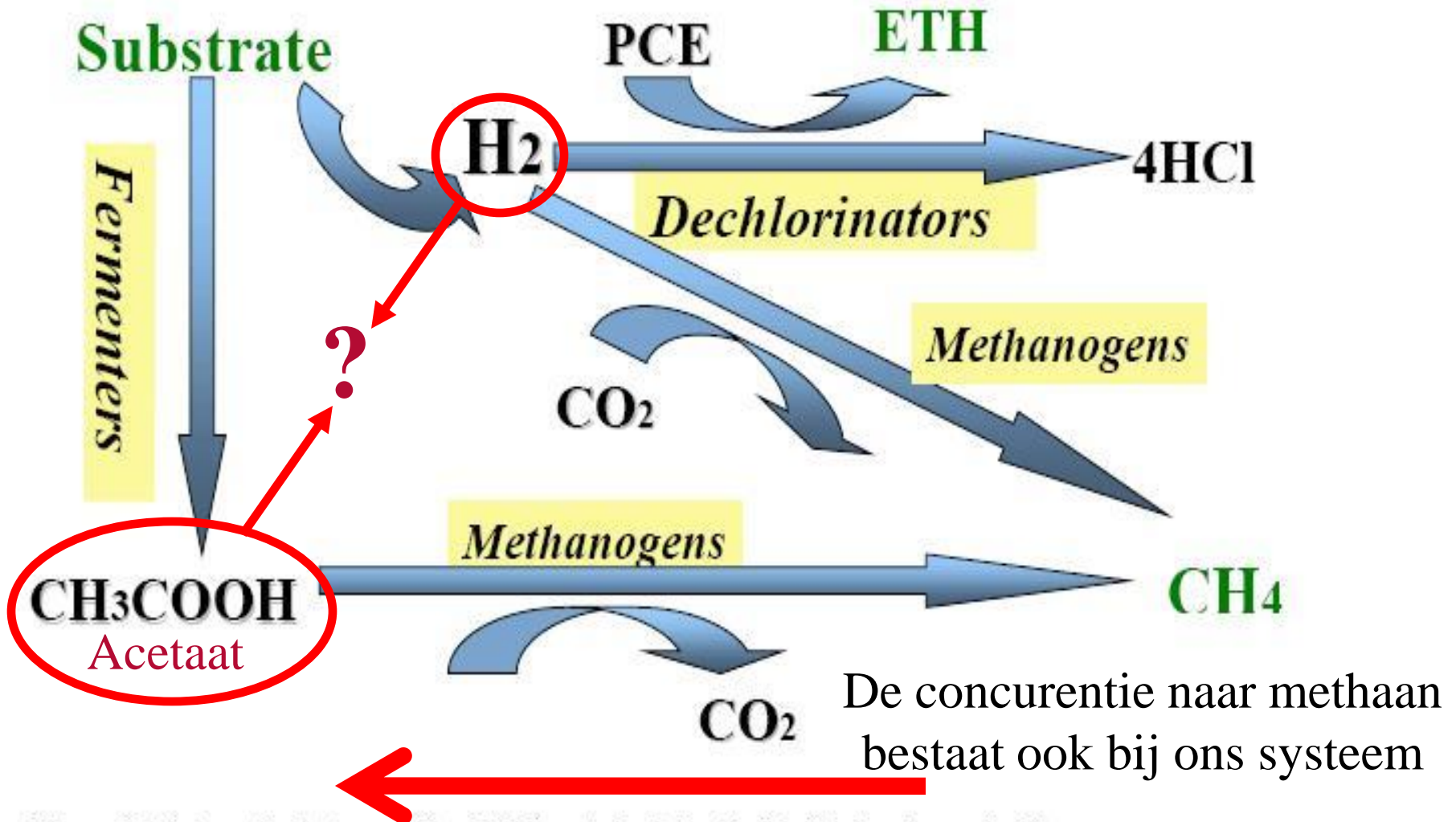


Figure 2.2 Interaction between Microbial Agents in Reductive Dechlorination under Non-Inhibitory PCE concentrations



Wat is voor een snelle dechlorering nodig?

What is Needed for Effective Anaerobic Bioremediation?

- ~~Organic substrates that ferment to:~~
 - Acetate
 - Hydrogen (H₂)
 - Hydrogen concentrations > 1 nM
- ~~Strongly reducing conditions (Sulfate Reducing or Methanogenic)~~
- ~~Nutrients~~
 - ~~Vitamins and trace minerals to stimulate~~
Dehalococcoides growth
- Halorespiring bacteria
(e.g., *Dehalococcoides ethenogenes* for DCE/VC)

Acetaat, waterstof,
koolzuur,
chloorethenen én de
bacterie

Dehalococcoides

Geen vitamine B12,
of reducerende
omstandigheden en
spoorelementen

De bacterie is overal
waar geen zuurstof
is.



Voorbeelden van beperkt succes

- Soluble – lactate, molasses, ethanol
- Viscous Fluids – HRC[®], HRC-X, neat vegetable oil
- Low Viscosity Fluids – vegetable oil microemulsions, cheese whey
- Solids Substrates – mulch, compost, chitin
- Experimental – hydrogen gas, humic acids
- Soluble, mobile
 - alcohols, sugars, lactate, acetate, citrate
- Semi-soluble or emulsions, less mobile
 - oleate, stearate, emulsified vegetable oil
- Solid, slow release compounds
 - Chitin, HRC
- Microorganisms (bioaugmentation)
 - Dehalococcoides (DHE)
- Nano-scale particles

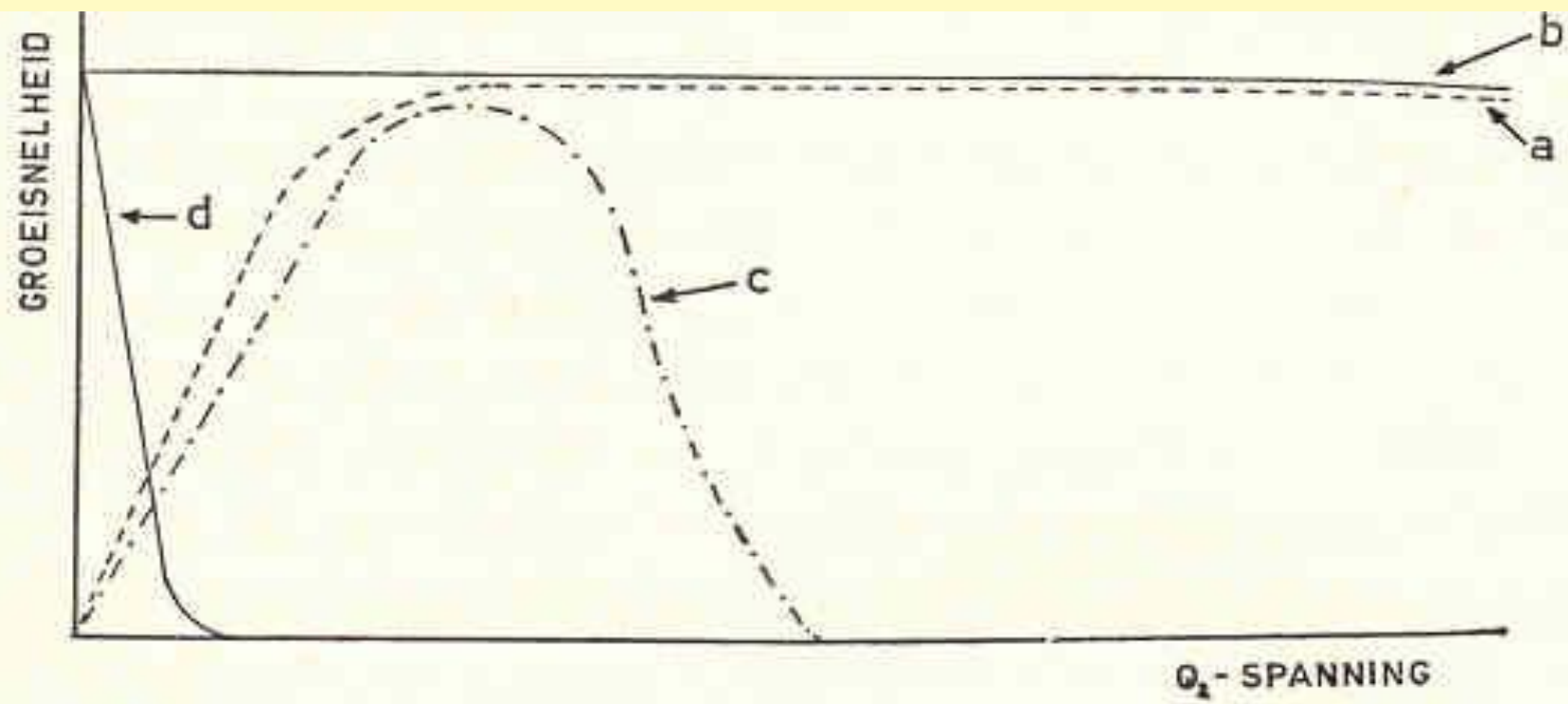


Voorbeelden gebruikte substraten

Substrate	Injected Form and Concentration	Target Concentration in the Formation
Sodium Lactate, Lactic Acid	Diluted 3 to 30 percent by weight	50 to 300 mg/L
Ethanol, Methanol	Diluted 3 to 30 percent by weight	50 to 300 mg/L
Molasses, Fructose Corn Syrup	Diluted 1 to 10 percent by weight	50 to 500 mg/L
Hydrogen Release Compound (HRC®)	Pure product injected at 4 to 12 pounds per foot	100 to 500 mg/L (lactic acid)
Vegetable Oil	Oil-in-water emulsion	100 to 500 mg/L (TOC)



Zuurstof is dodelijk



Verband tussen groeisnelheid en zuurstofspanning bij verschillende microben

Curve a: obligaat aerobe microben (verzadigingscurve)

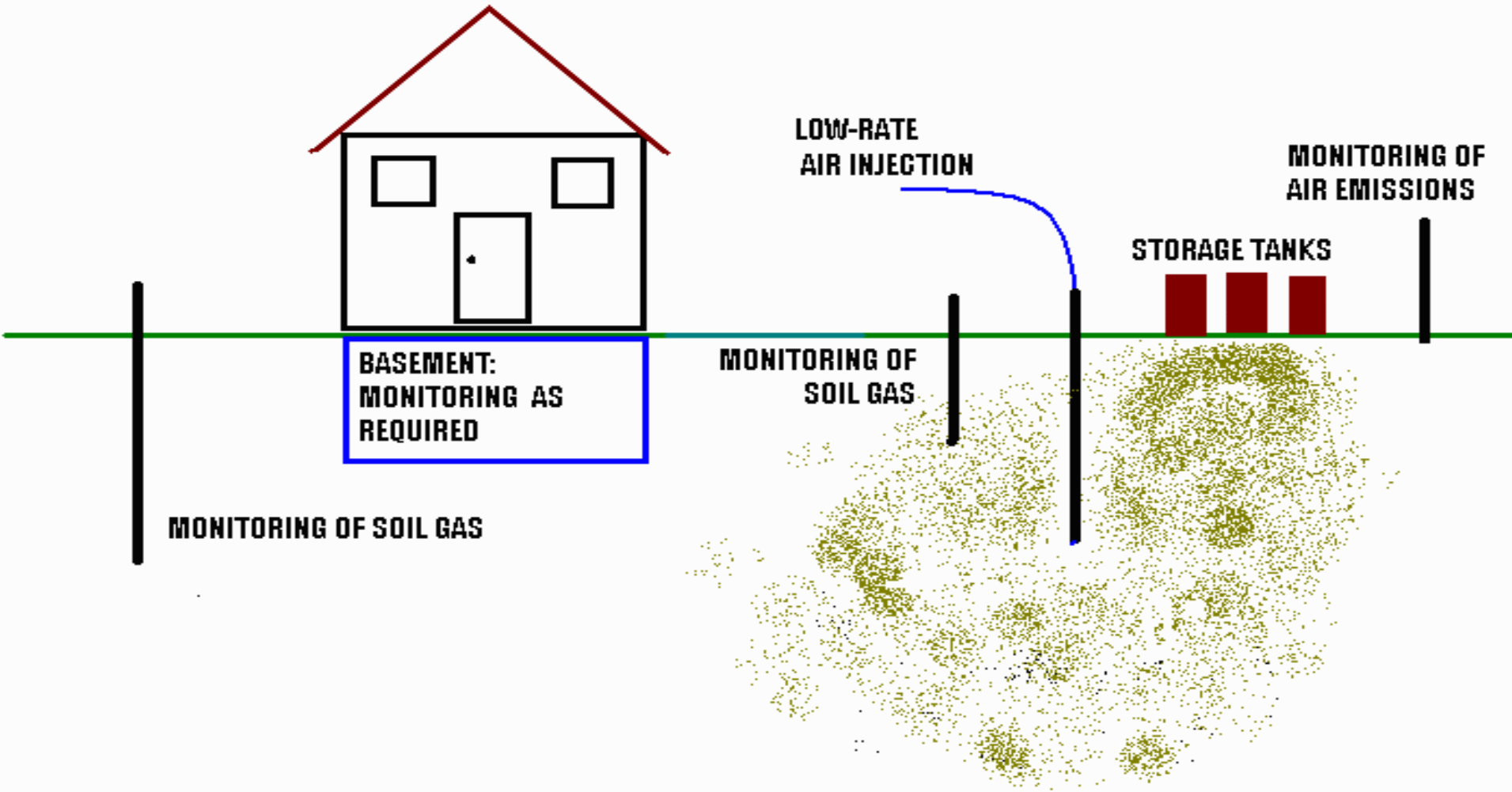
Curve b: facultatief anaerobe microben

Curve c: microaerofiele microben (optimumcurve)

Curve d: obligaat anerobe microben

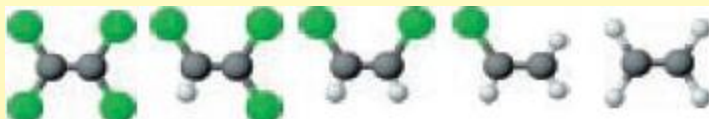


Voorbeeld waarbij Dehalococoides dood werd gemaakt





Het diffusieprobleem

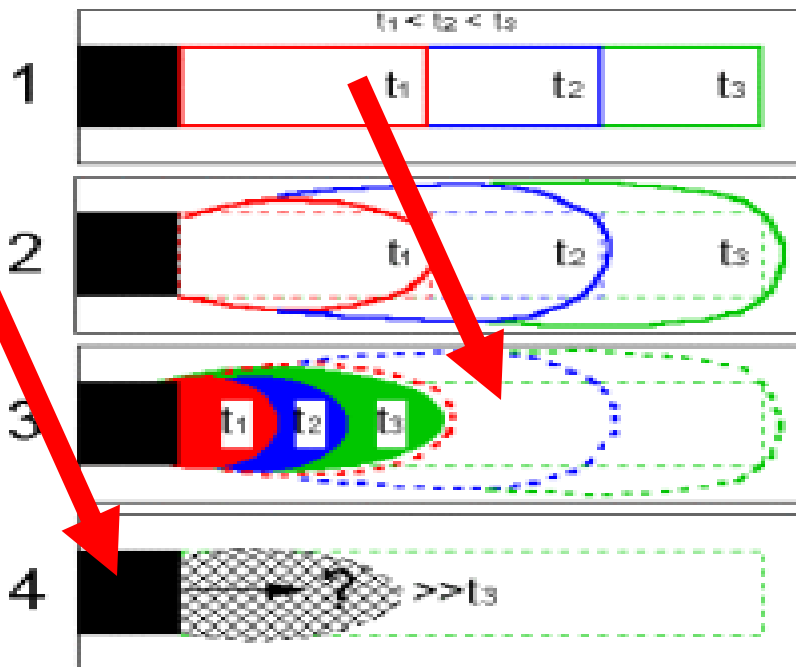


PER TRI cis-DCE VC etheen

Schadensherd (Lösung von Schadstoffen)



Beispiel



Schadstofftransport
gesteuert durch:

Advektion

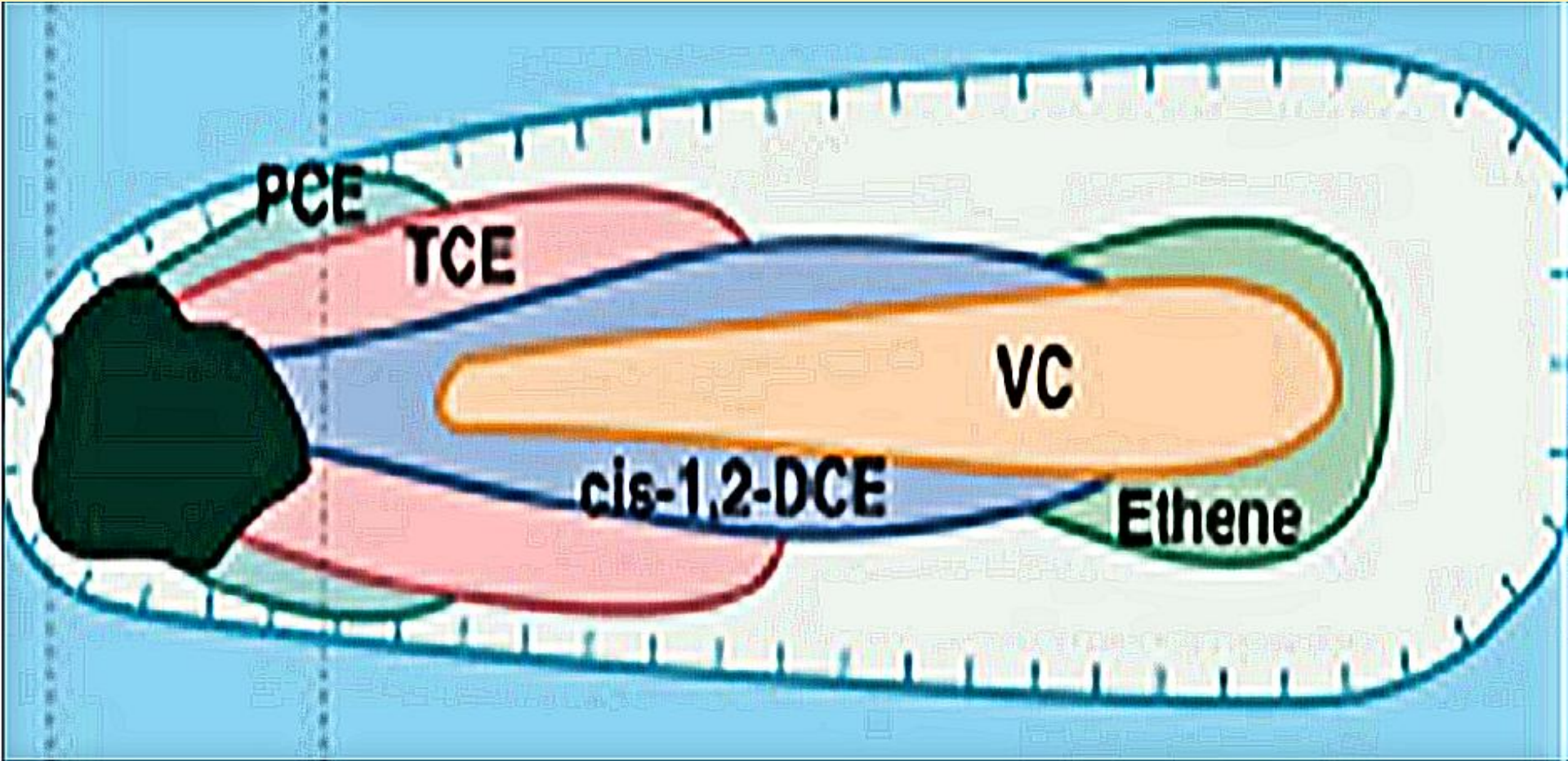
Advektion + Dispersion/Diffusion

Advektion + Dispersion/Diffusion
+ Sorption/Retardation

Advektion + Dispersion/Diffusion
+ Sorption/Retardation
+ **Abbau**



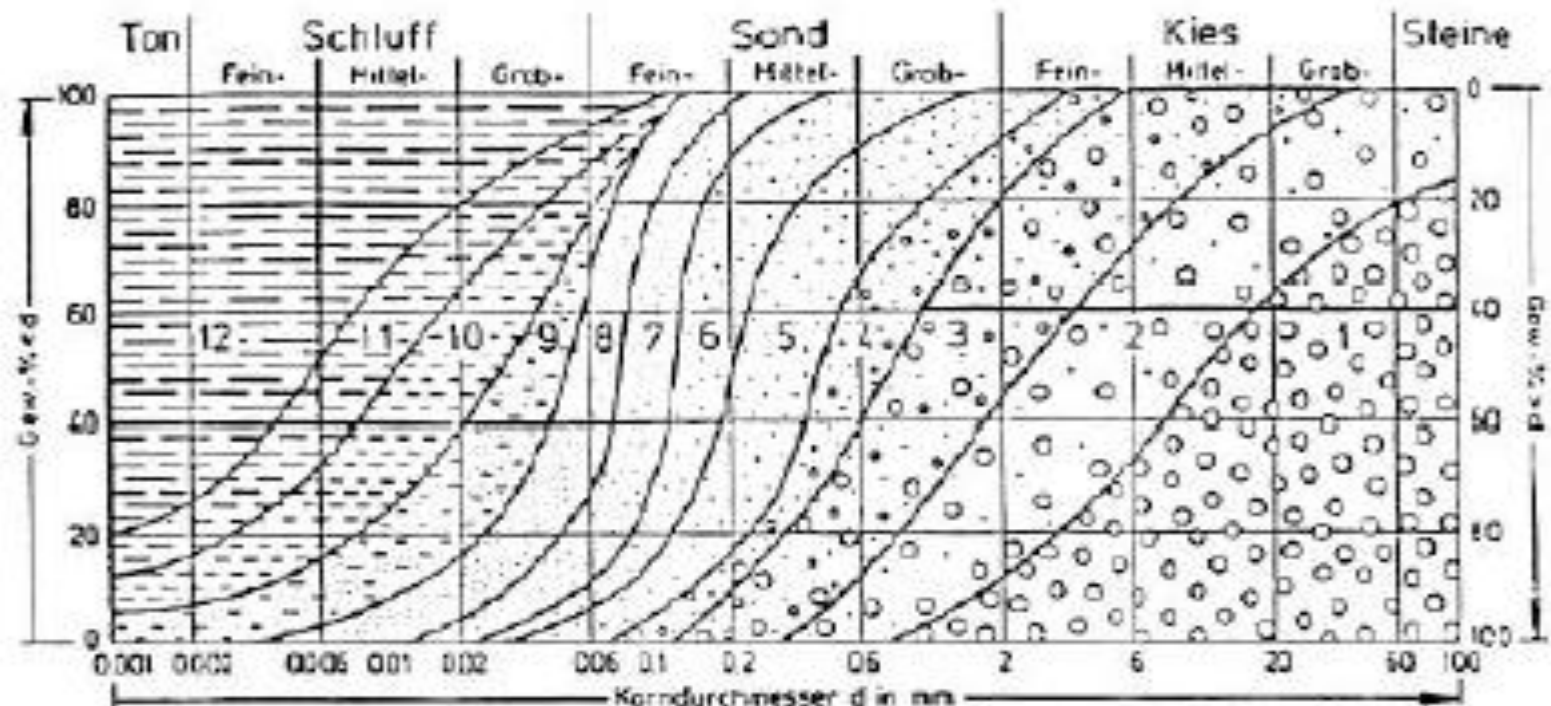
Diffusie van boven bekeken





Doorlaadbaarheid per grondsoort

Korngrößenklassen und Durchlässigkeiten der Lockergesteine



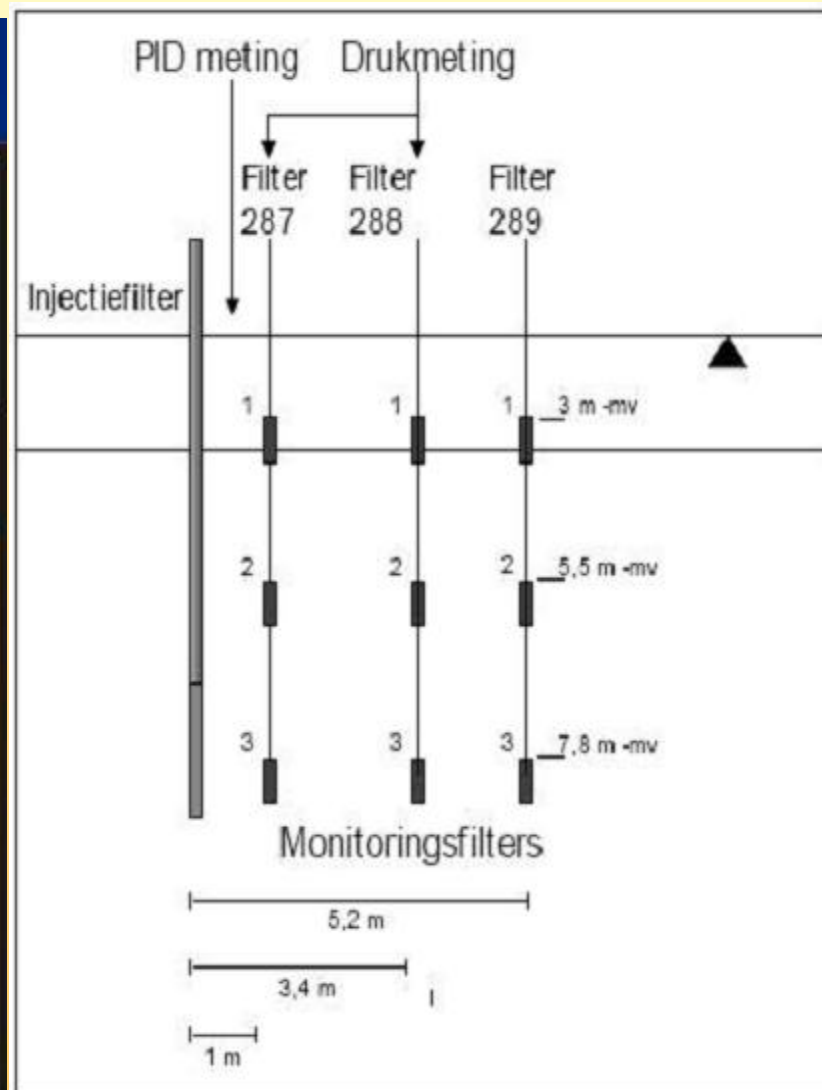
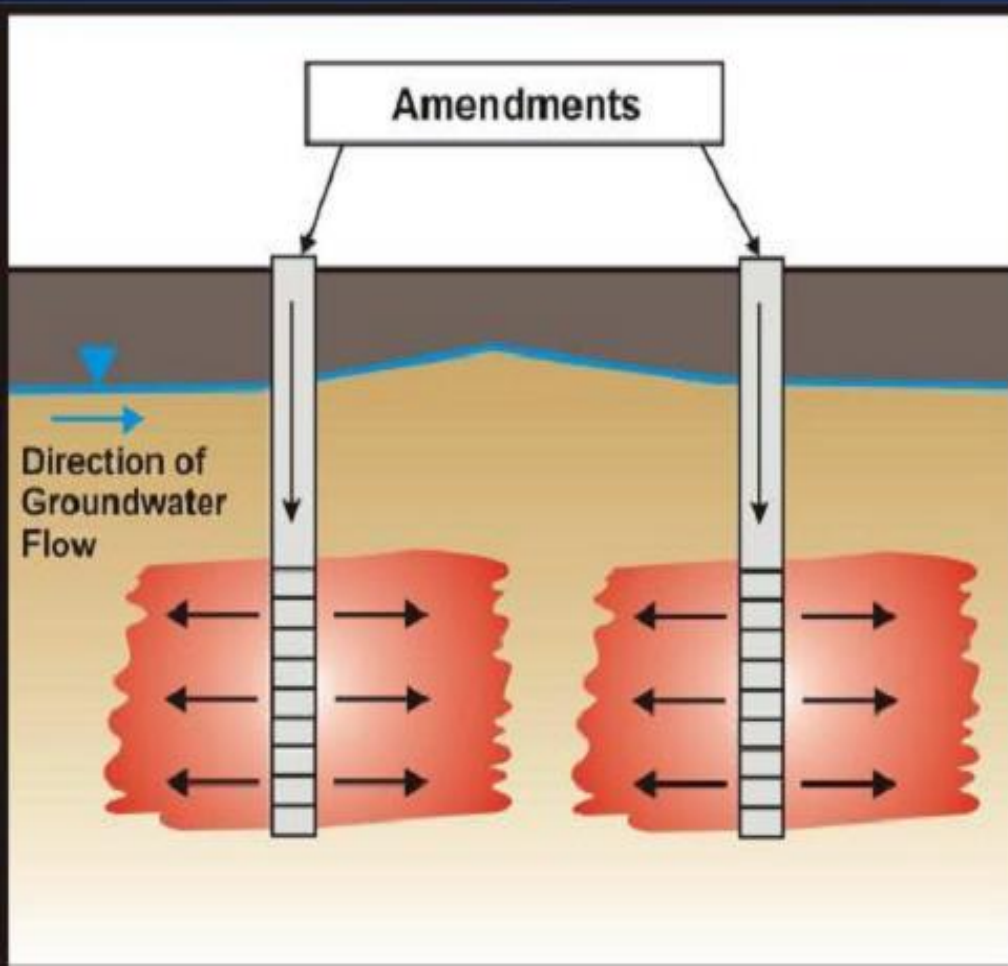
12	11	10	9	8	7	6	5	4	3	2	1
$<1E^{-9}$	$1E^{-9}$	$1E^{-8}$	$1E^{-7}$	$1E^{-6}$	$1E^{-5}$	$5E^{-4}$	$1E^{-4}$	$5E^{-4}$	$1E^{-3}$	$7E^{-3}$	$3E^{-2}$

Substratklassen in Abhängigkeit der Korngröße
 Größenordnung des Durchlässigkeits-Koeffizienten (k_f) in m/s



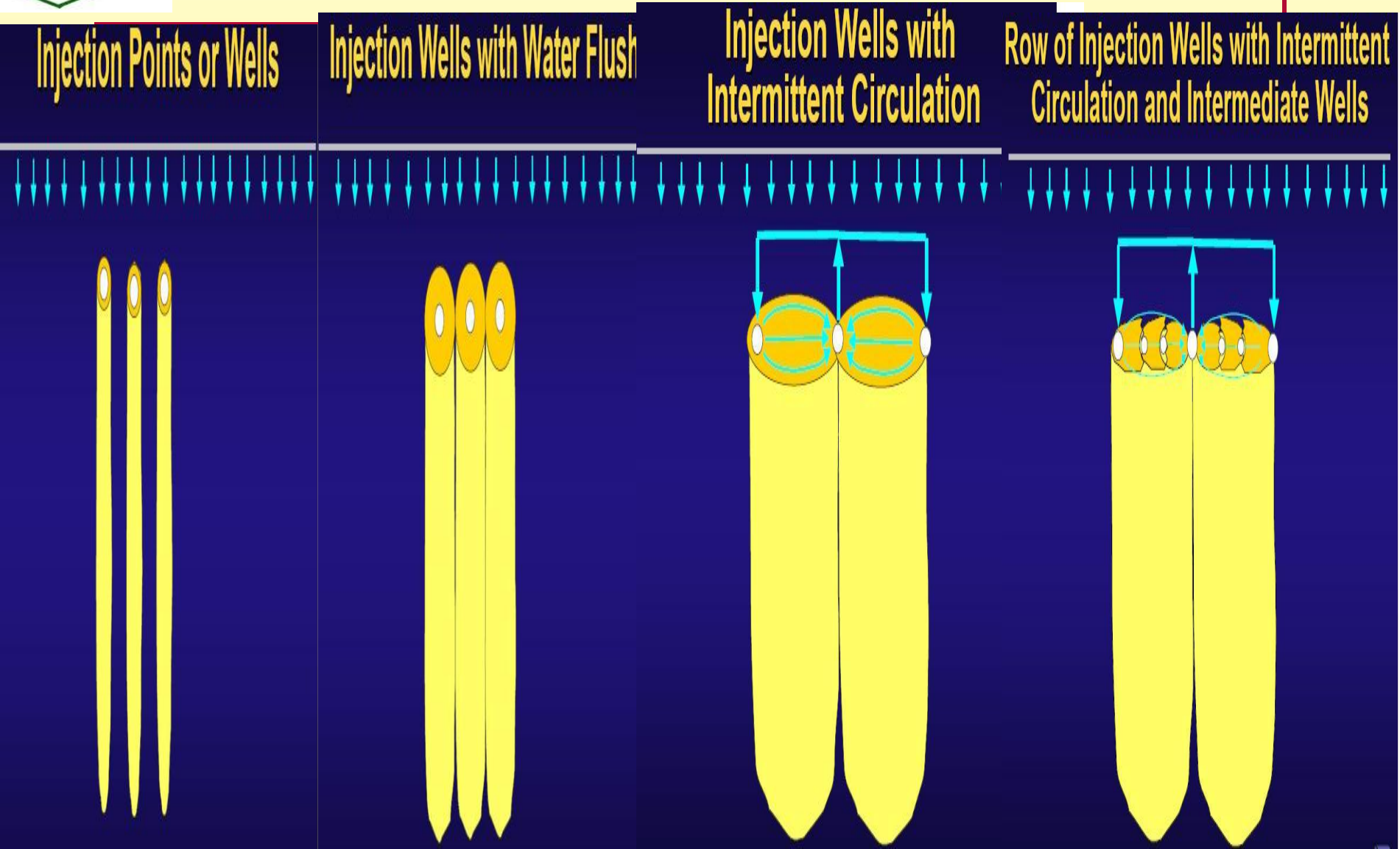
injectievoorbeelden

Direct Injection





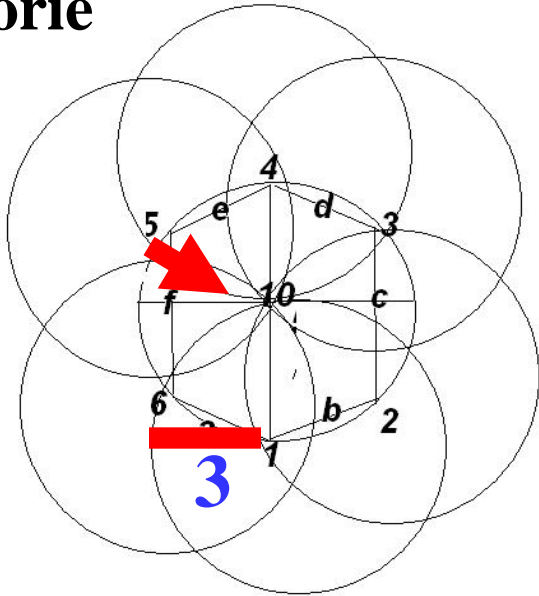
Passieve en actieve injecties





Diffusie en afbraak meten

Theorie



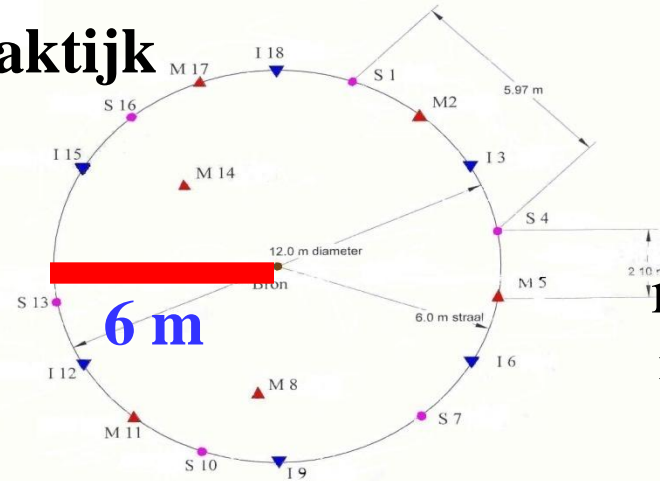
a t/m e acetaatinjecties

1 t/m 6 waterstof/CO₂/N₂

Recirculatie vanuit 10 naar elk acetaatpunt een paar uur per dag

Alle 13 punten zouden meetpunten zijn

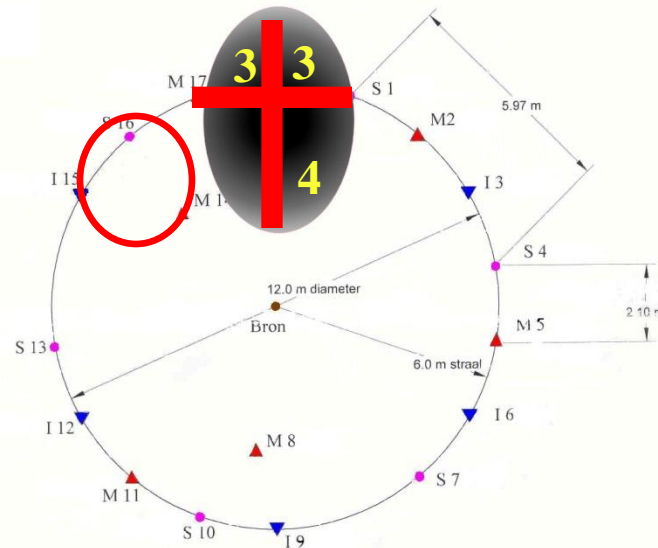
Praktijk



● gassen

● meetpunt
is ertussen
gekomen

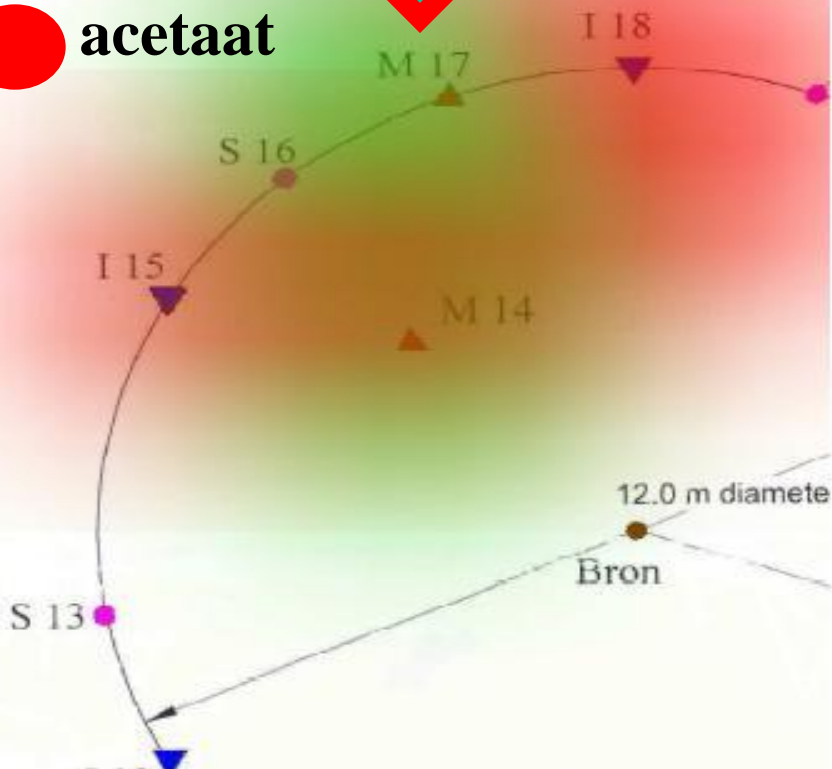
● acetaat





Resultaten Berk

- gassen
- acetaat



Voor: (eenheden ug/l)

	VC	DCE	TRI	PER
M14		1944	139	7689
M17		23	9	46

Na twee maanden:

M14	131	1194	23	755
M17	95	1030	85	6

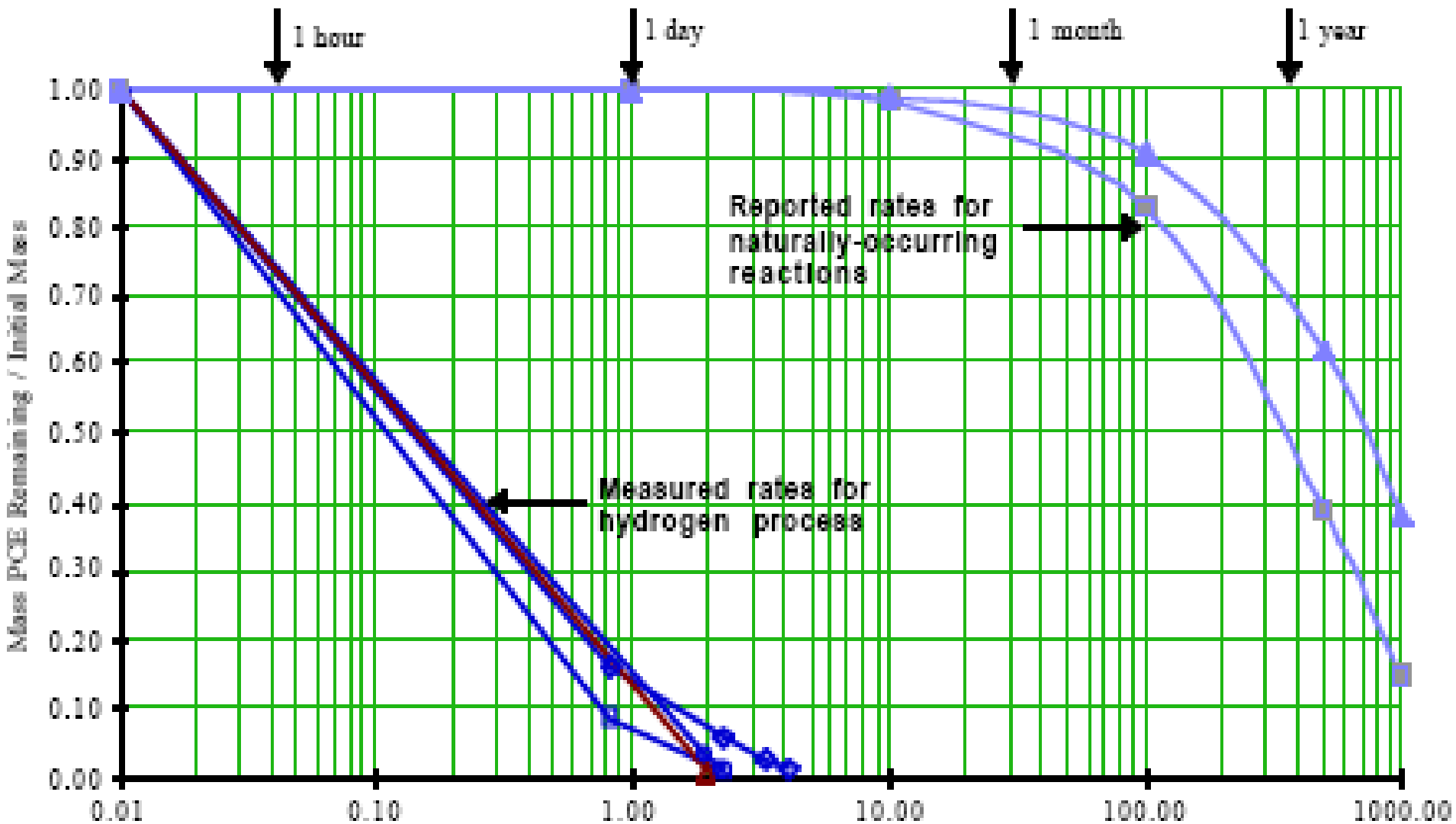
In M14 is 90% reductie van PER en 82% van alle DCE opgetreden, naast een toename van VC

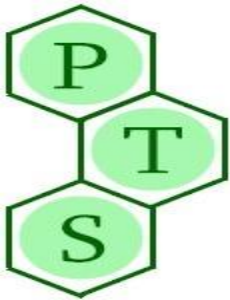
In M17 nemen PER en TRI niet af en is door substraattekort een toename van DCE en VC.

De diffusie van 3 meter in deze bodem is voor acetaat te ver.



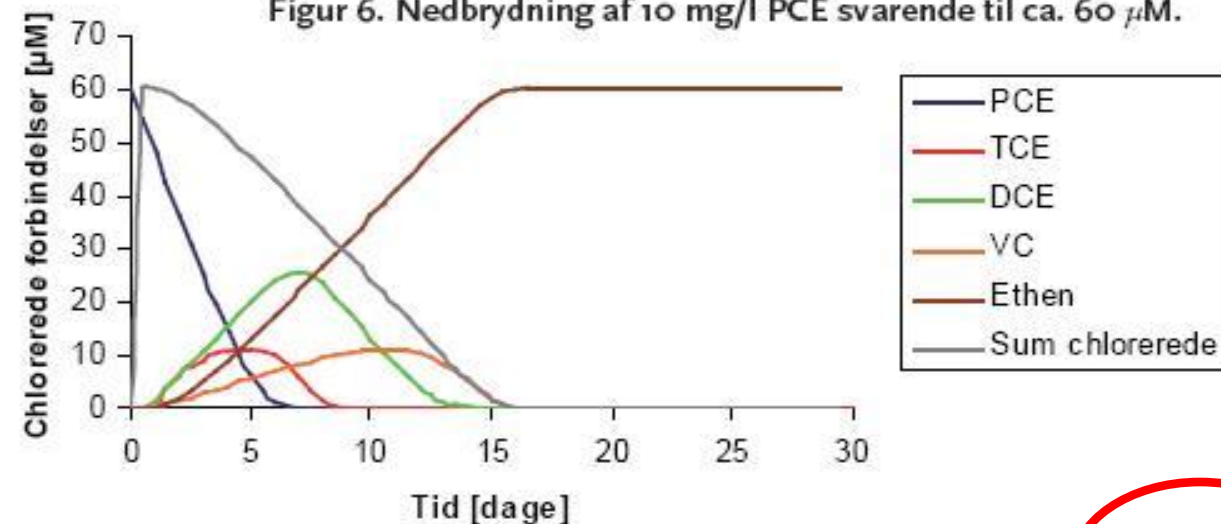
Snelle en langzame omzetting



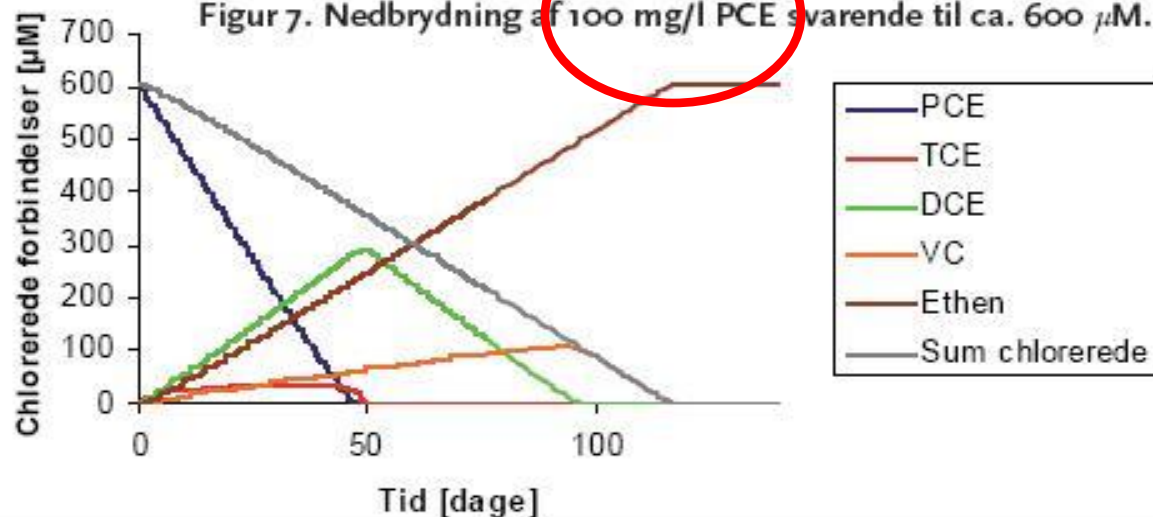


Snelle omzetting in lab

Figur 6. Nedbrydning af 10 mg/l PCE svarende til ca. 60 μM .



Figur 7. Nedbrydning af 100 mg/l PCE svarende til ca. 600 μM .





De snelste ontchloring

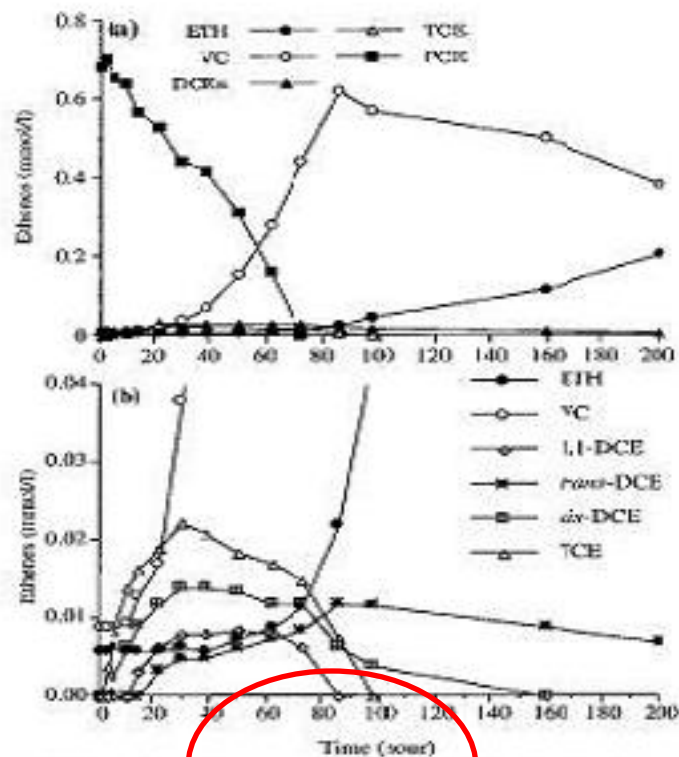


FIG. 2. (a) Product formation by PCE-grown strain 195 inoculated into medium to which one dose of PCE (0.7 mmol/liter) was added. (b) Plot with an expanded scale, showing intermediates in PCE metabolism, including individual DCE isomers.

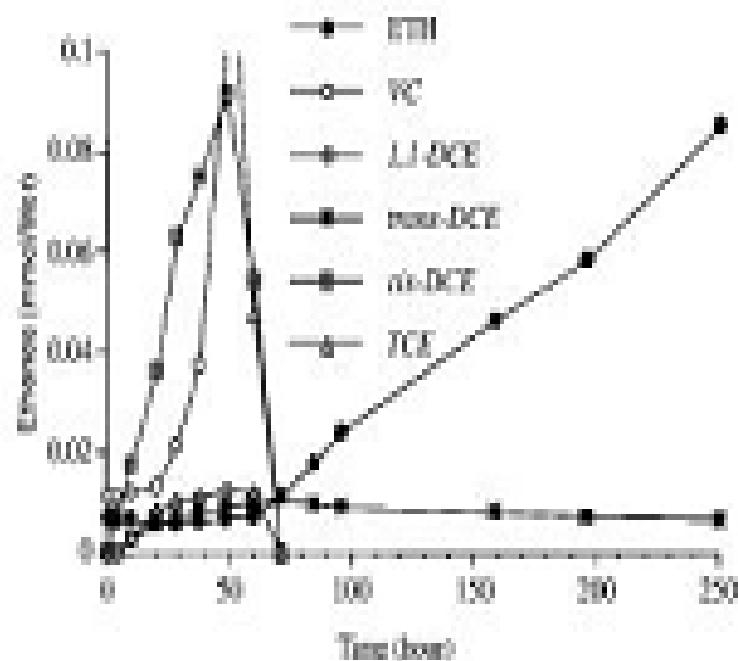
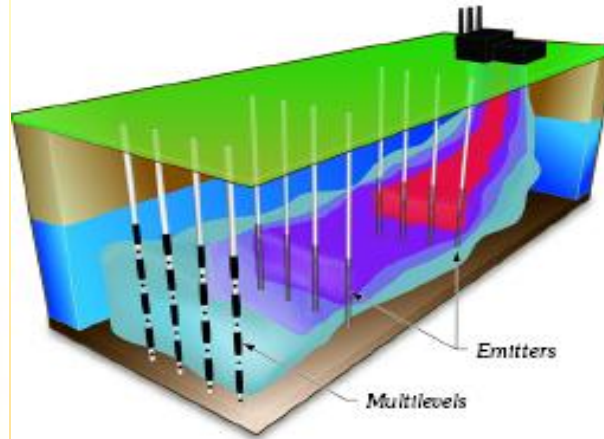


FIG. 3. Intermediate formation by TCE-grown strain 195 inoculated into medium to which a single dose consisting of 0.35 mmol of TCE per liter was added.

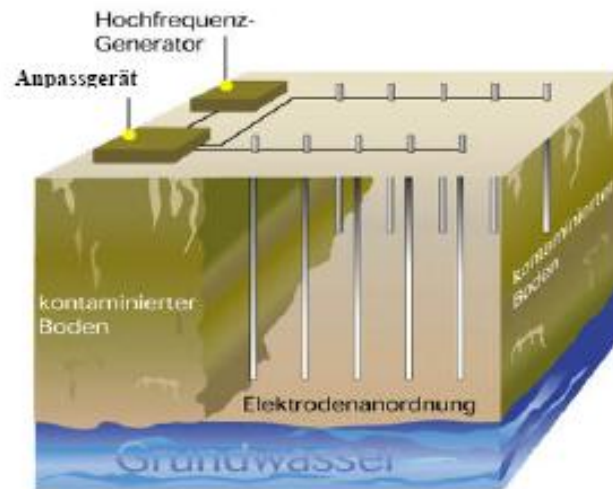
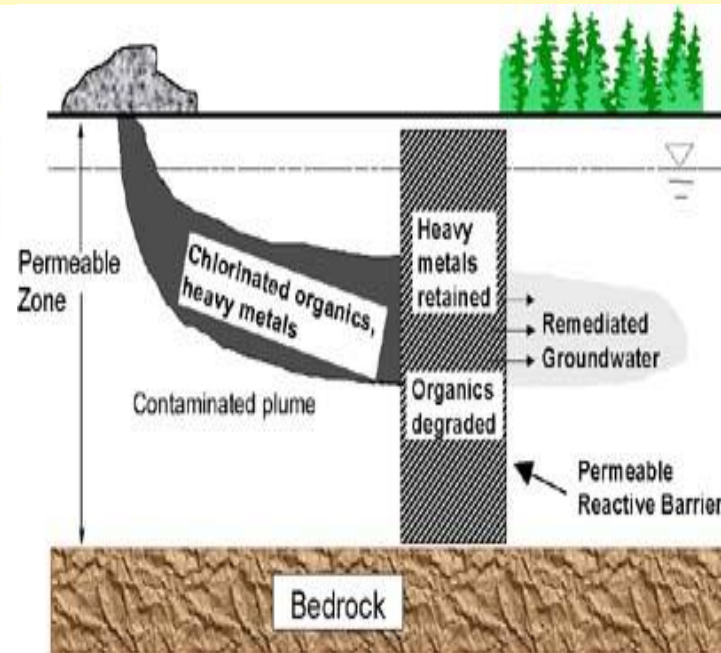
0,7 mmol/l PCE = 110.000 ug/l



Afgraven en alleen in de pluim



Schematic showing staged plume remediation using Waterloo Emitters, monitored by a transect of CMT or Waterloo Multilevel Systems



Geen opties

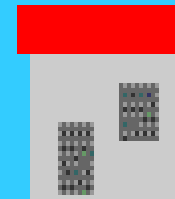


Bioscherm in de pluim



Situatie blijft
zo jaren
voortduren

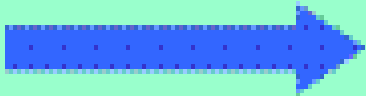
Bioscherm



PER

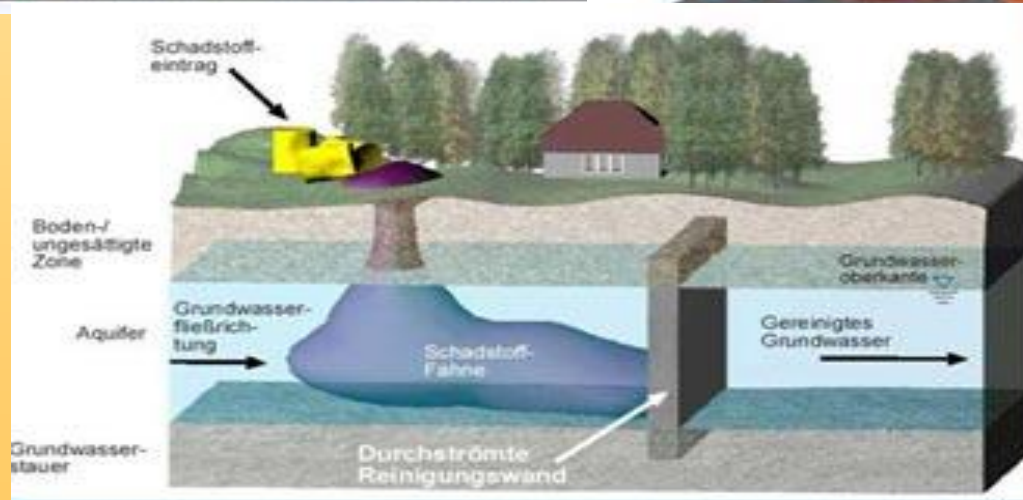
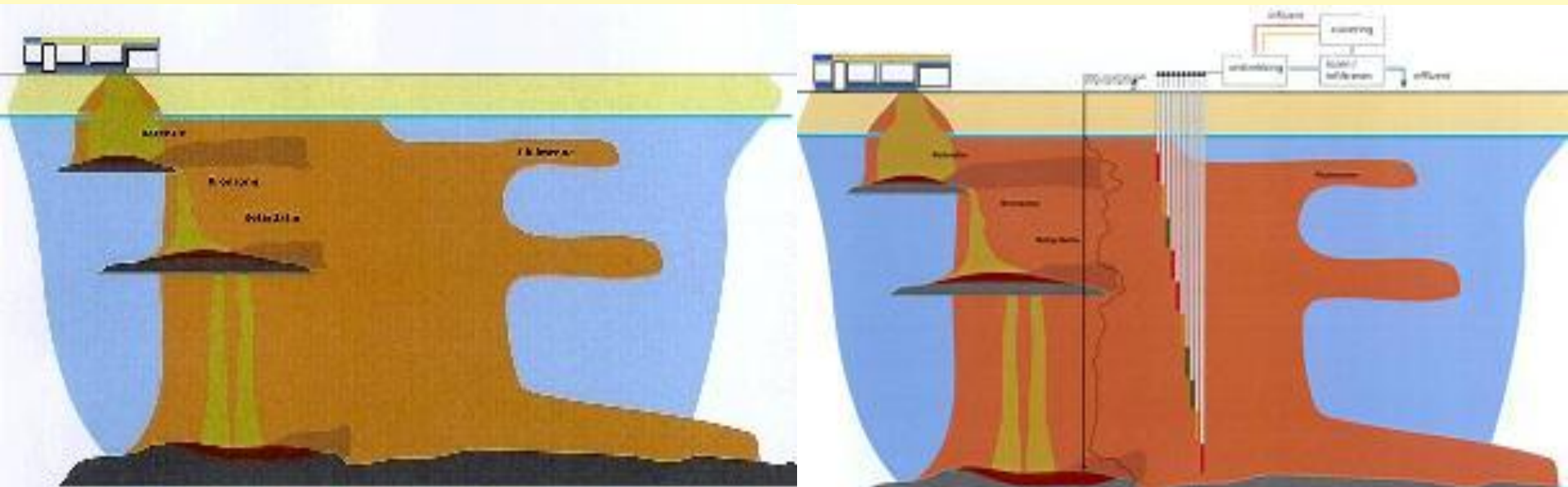
cDCE

Etheen



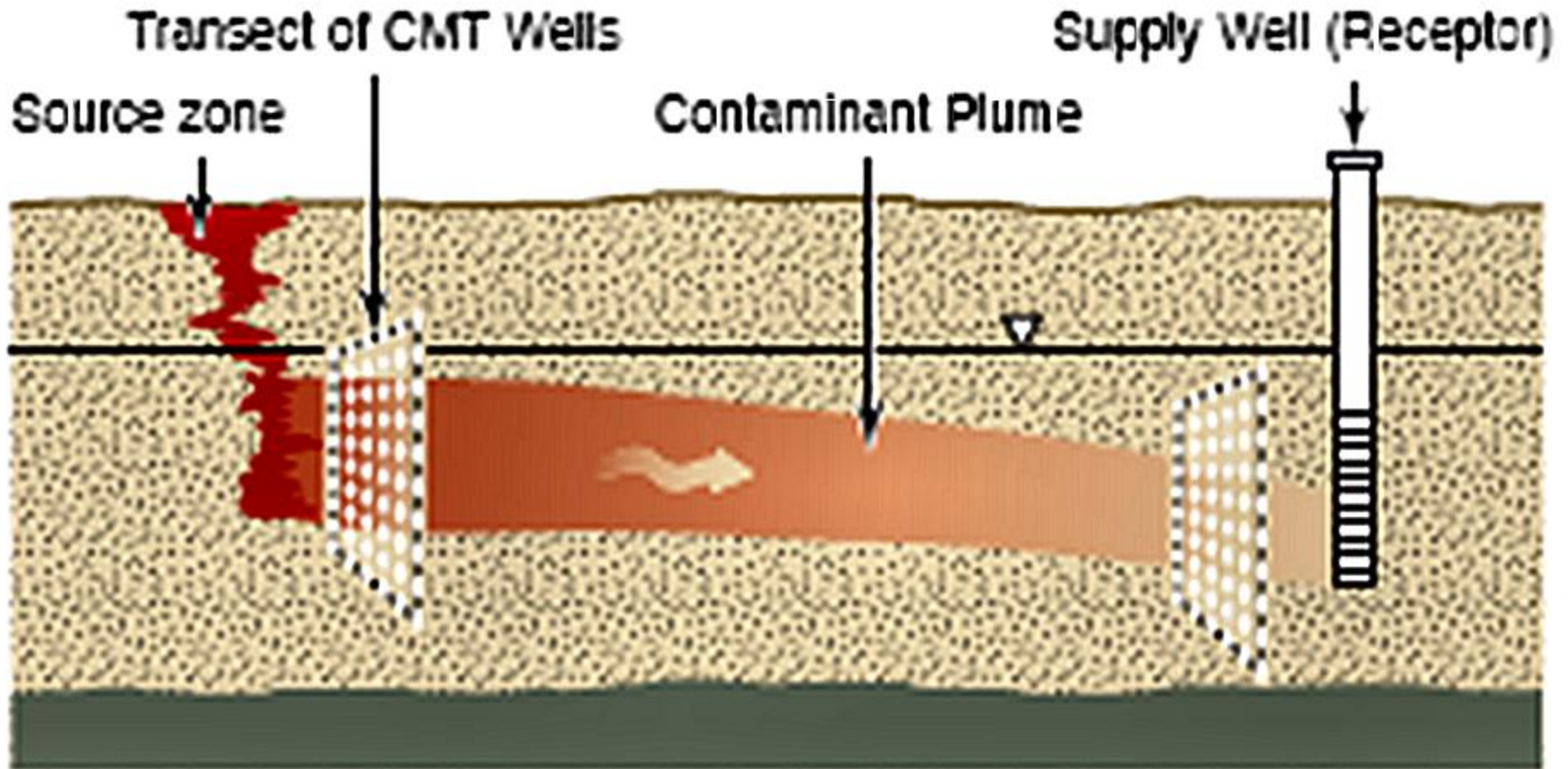


Alleen behandeling van de pluim





Weer niet de bron maar de pluim

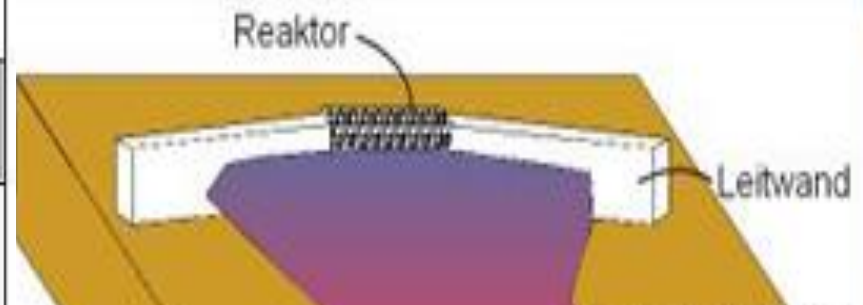
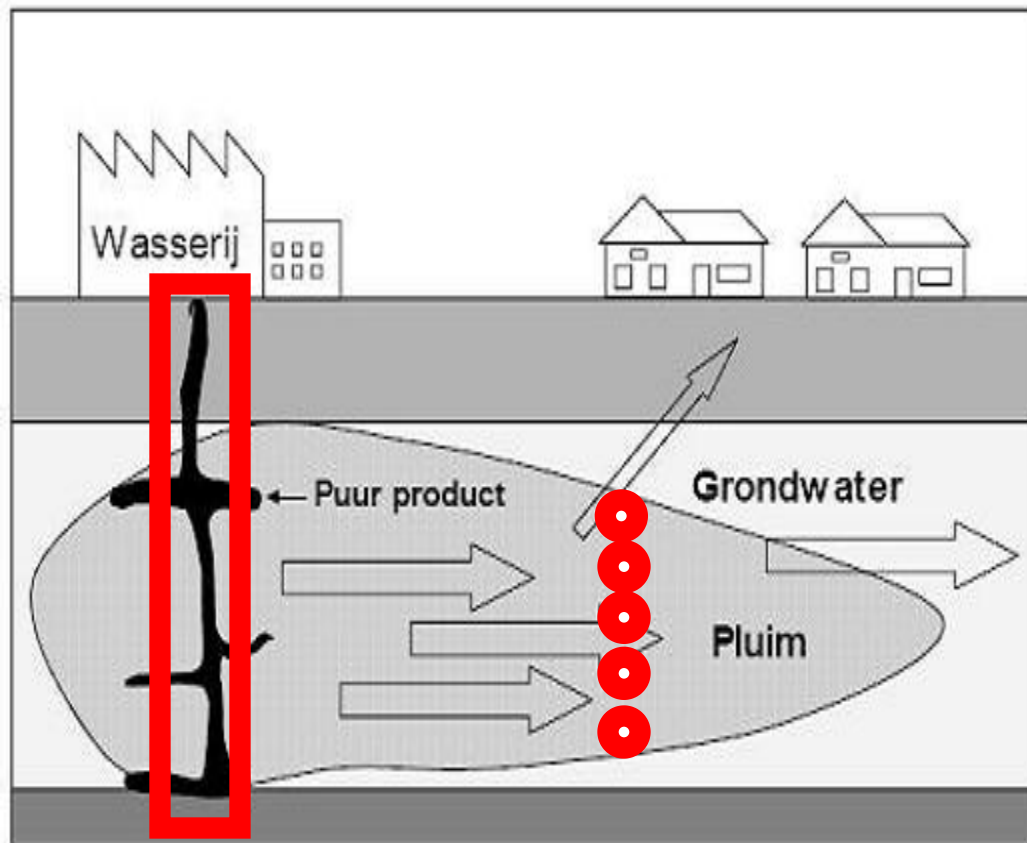


CMT Transects for Mass Flux Assessment



Voorstel tot saneren

Geen duur scherm die de situatie jarenlang in standhoud omdat de bron weg is



Grundwasser-
fließrichtung

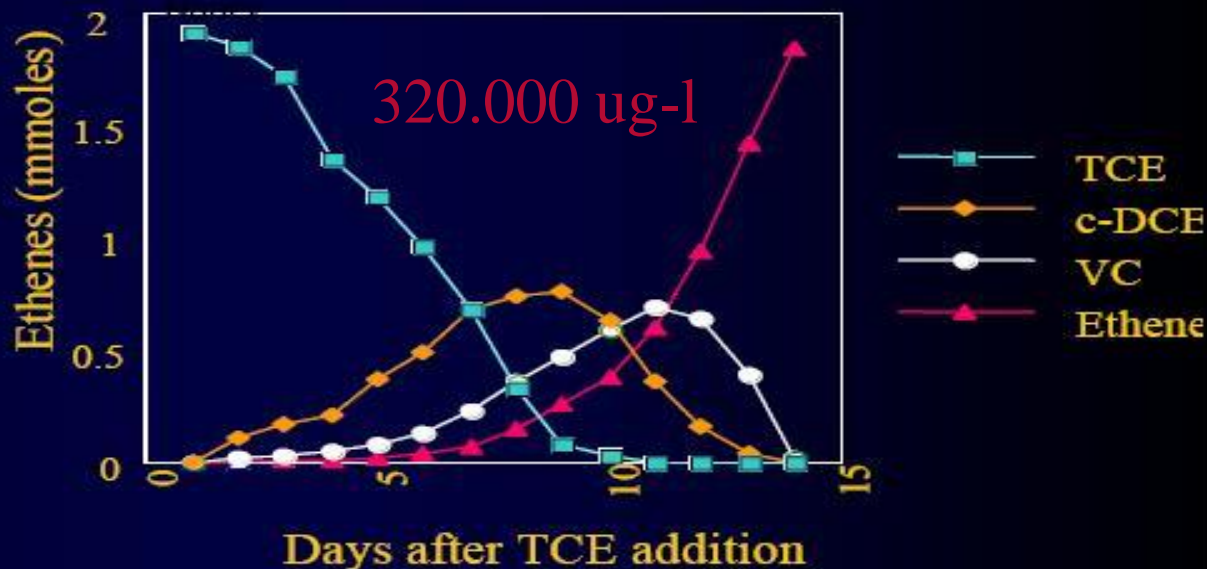
Fig. 1. Verspreiding verontreinigd grondwater.



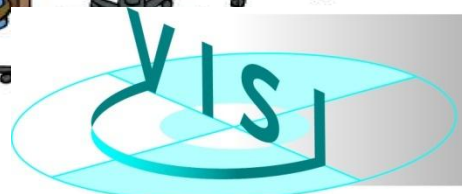
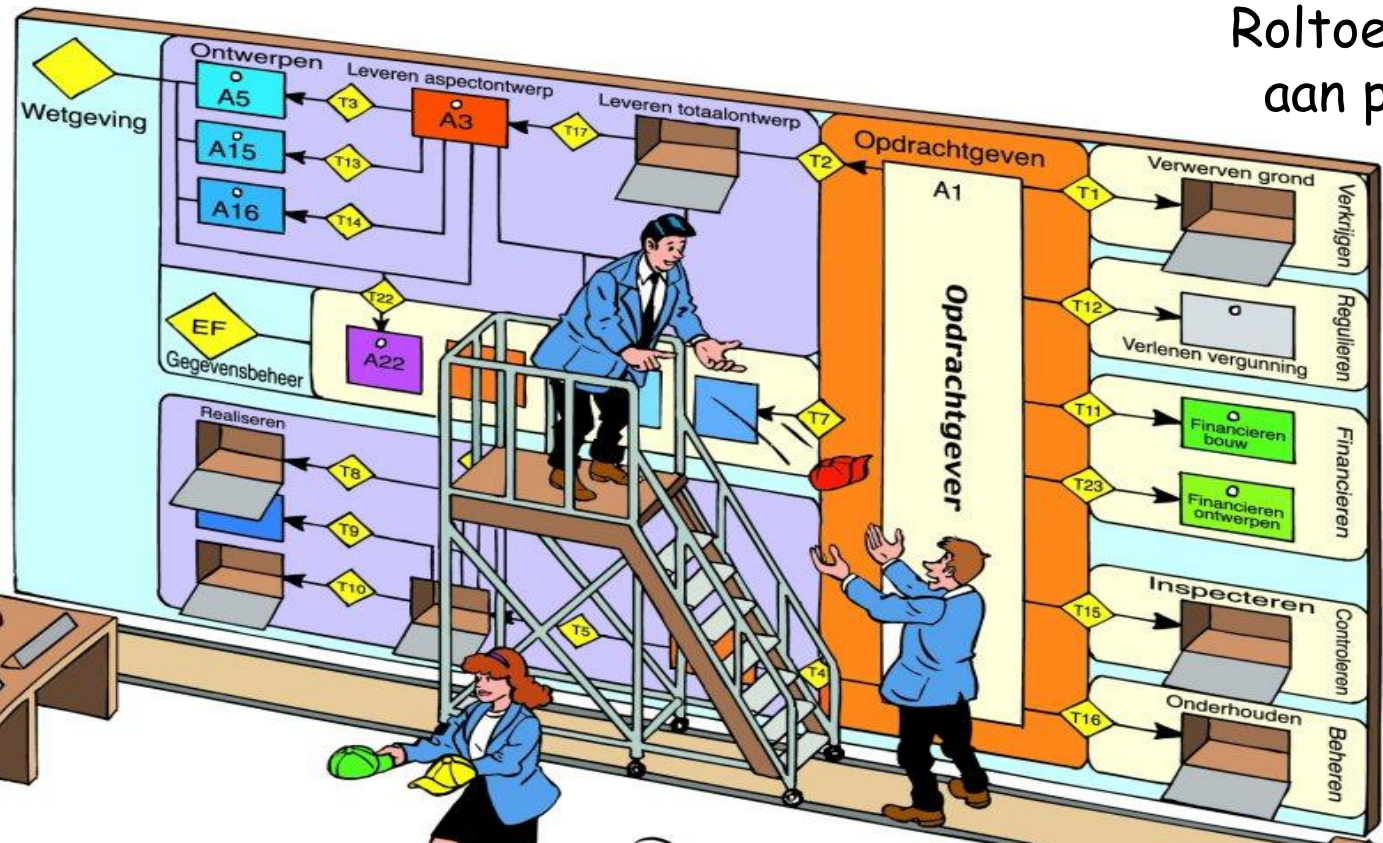
Geen lactaat maar acetaat en het gaat nog sneller

Dehalococcoides culture

- *Dehalococcoides* breathes TCE under strictly anaerobic conditions, using hydrogen for food.
- Microbial consortia ferment lactate to hydrogen, providing *Dehalococcoides* food to degrade TCE to ethene.
- When *Dehalococcoides* is absent, degradation stalls at DCE or VC



Roltoewijzing aan partijen





EuroStaete



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