Sneller en goedkoper saneren van vervuilde bodems

met behulp van

Dehalococcoides ethenogenes

Thin-section electron micrographs showing coccoid and elongated cells

Photos courtesy of Dr. Steve Zinder, Cornell University

Sneller en goedkoper saneren van vervuilde bodems

Bron: Chemisch Weekblad, juli 2009

Chloorvreters 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.

Chloorvreters opjutten

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.

Chloorvreters opjutten

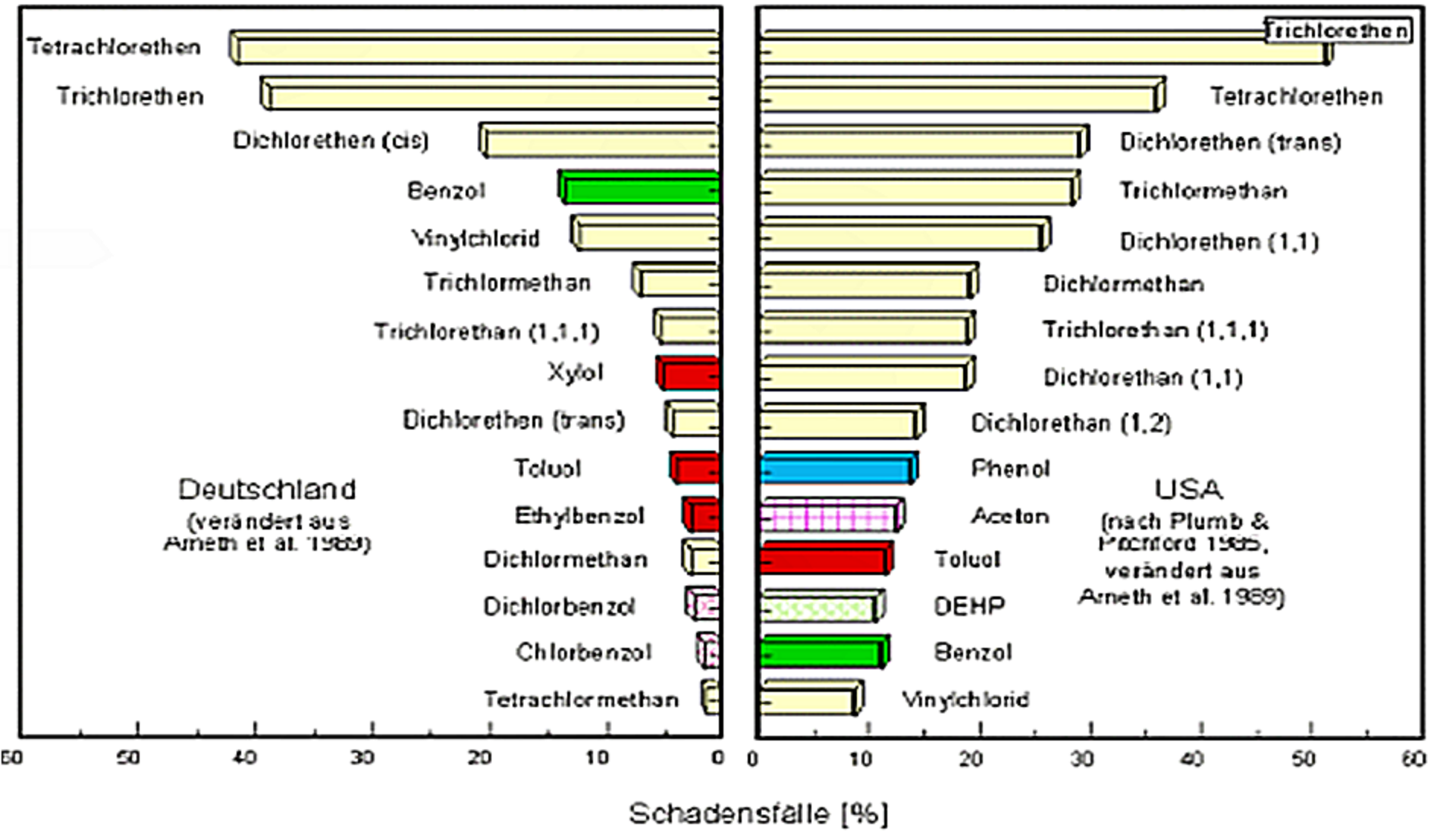
PTS staat voor PER en TRI Saneringen.

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,

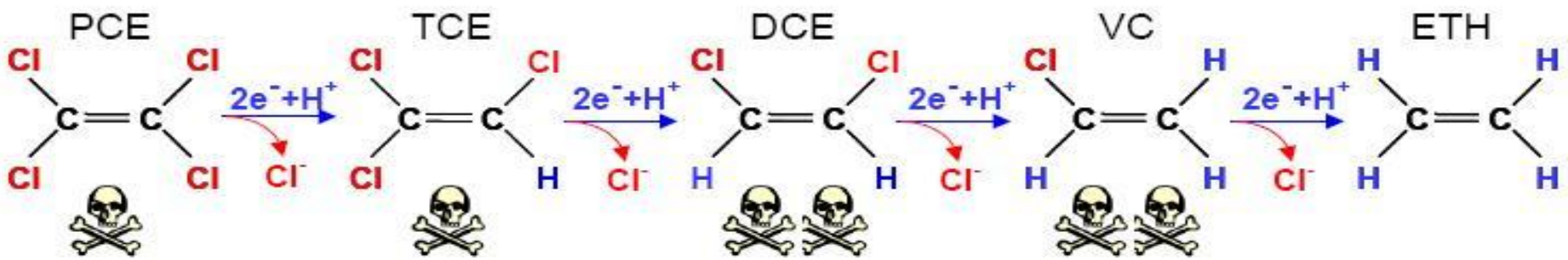
o.a. uitvinder en staatshouder.

Frequenties van verontreinigingen



Ontchloren met bacteriën?

Anaerobic microbial reductive dechlorination

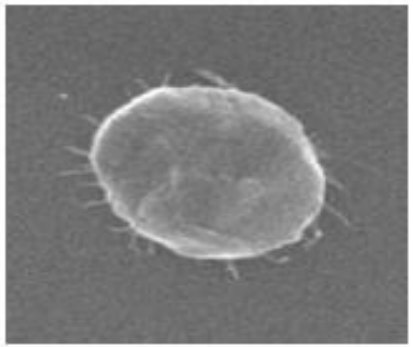


Dehalobacter, Dehalospirillum, Desulfitobacterium, Desulfomonile, Desulfuromonas, Enterobacter

Dehalococcoides

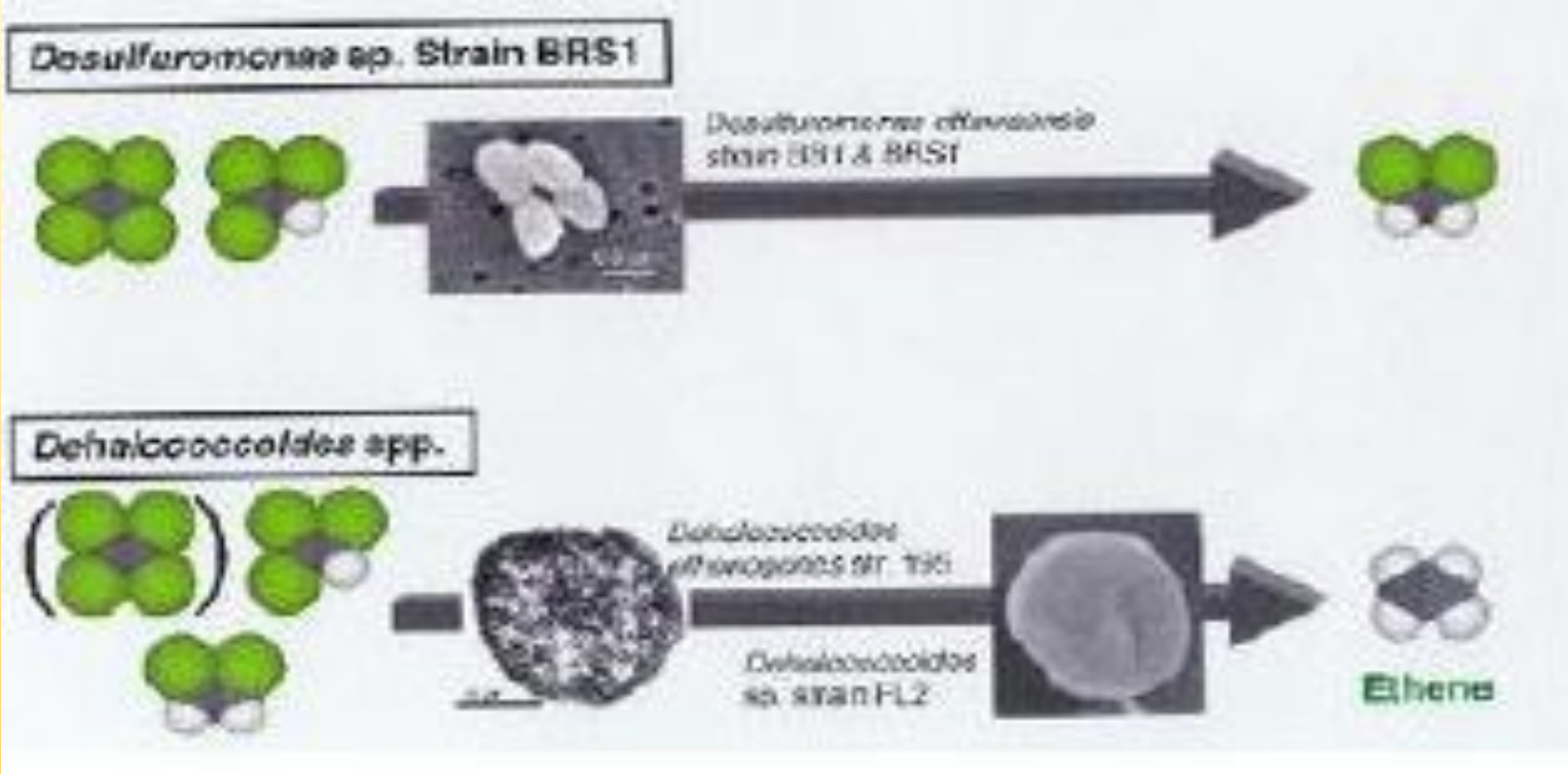
species:

D. ethenogenes 195
BAV1
FL2
VS
CBDB1

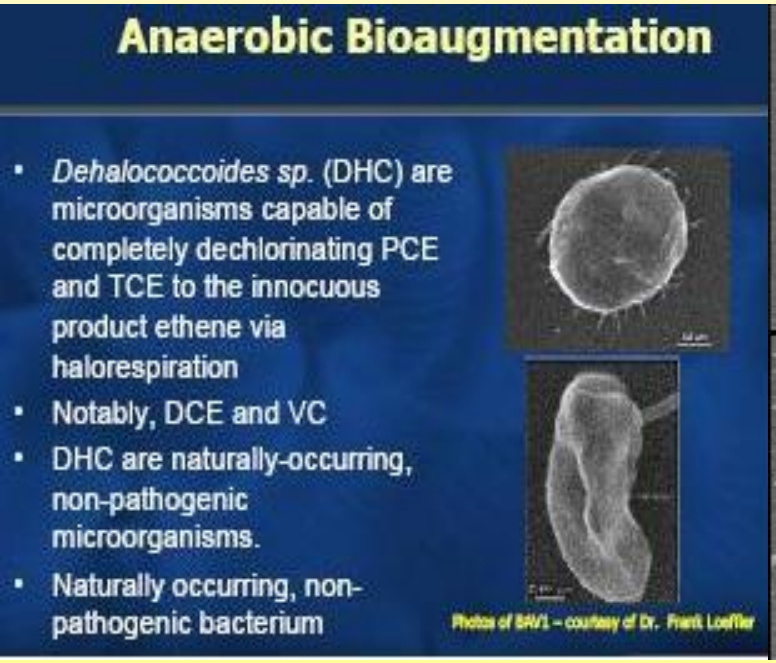


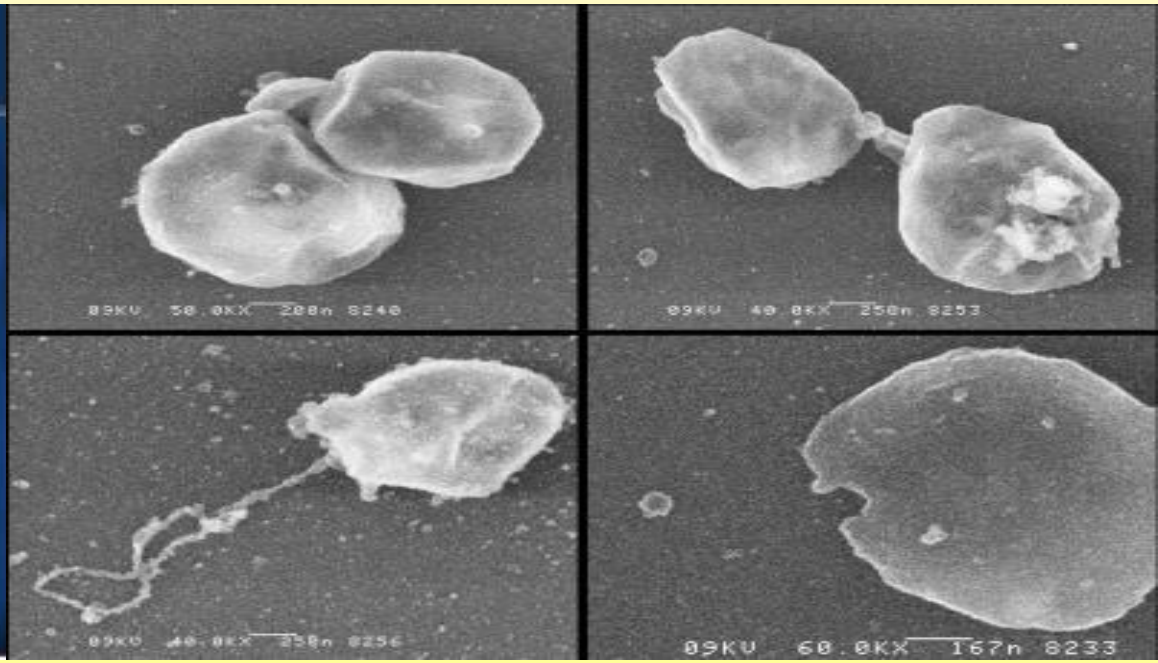
He et al., Nature, 2003

Ontchloren door de bacterie te vertroetelen



Anaërobe Biostimulatie



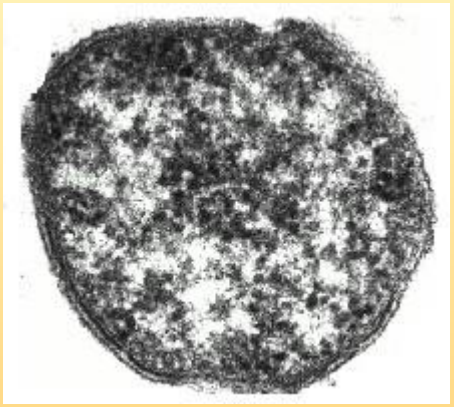


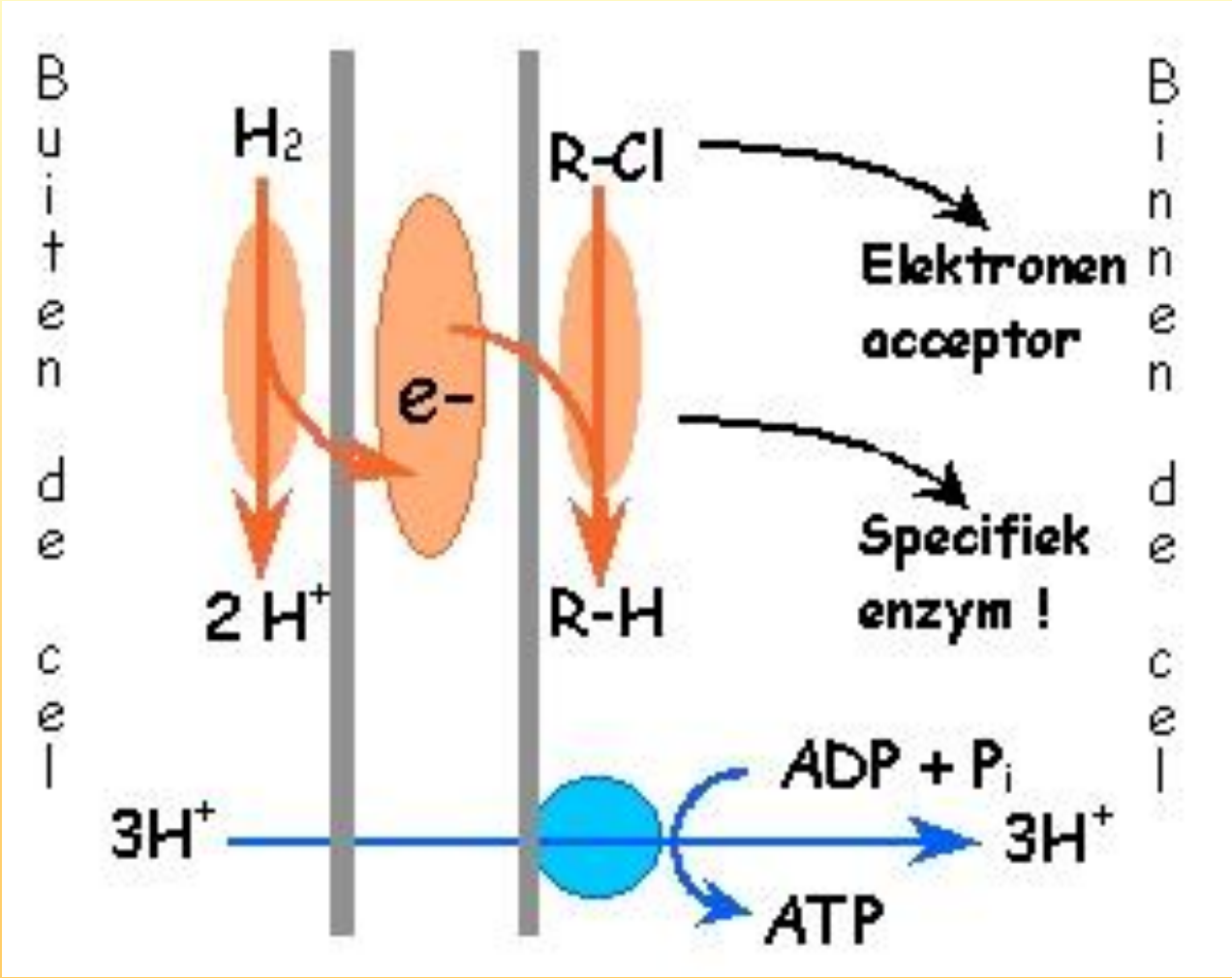
Biostimulatie omstandigheden aanpassen zodat bacteriegroei ontstaat

Bioaugmentatie bacteriën toevoegen zodat extra omzetting ontstaat

Bioremediatie biosysteem herstellen, verbeteren

Ademhaling van onze bacterie





DNA volgorde Dehalococcoides

1,4 miljoen baseparen

bestaande uit A, T, G en C

Drie letters coderen voor aminozuren

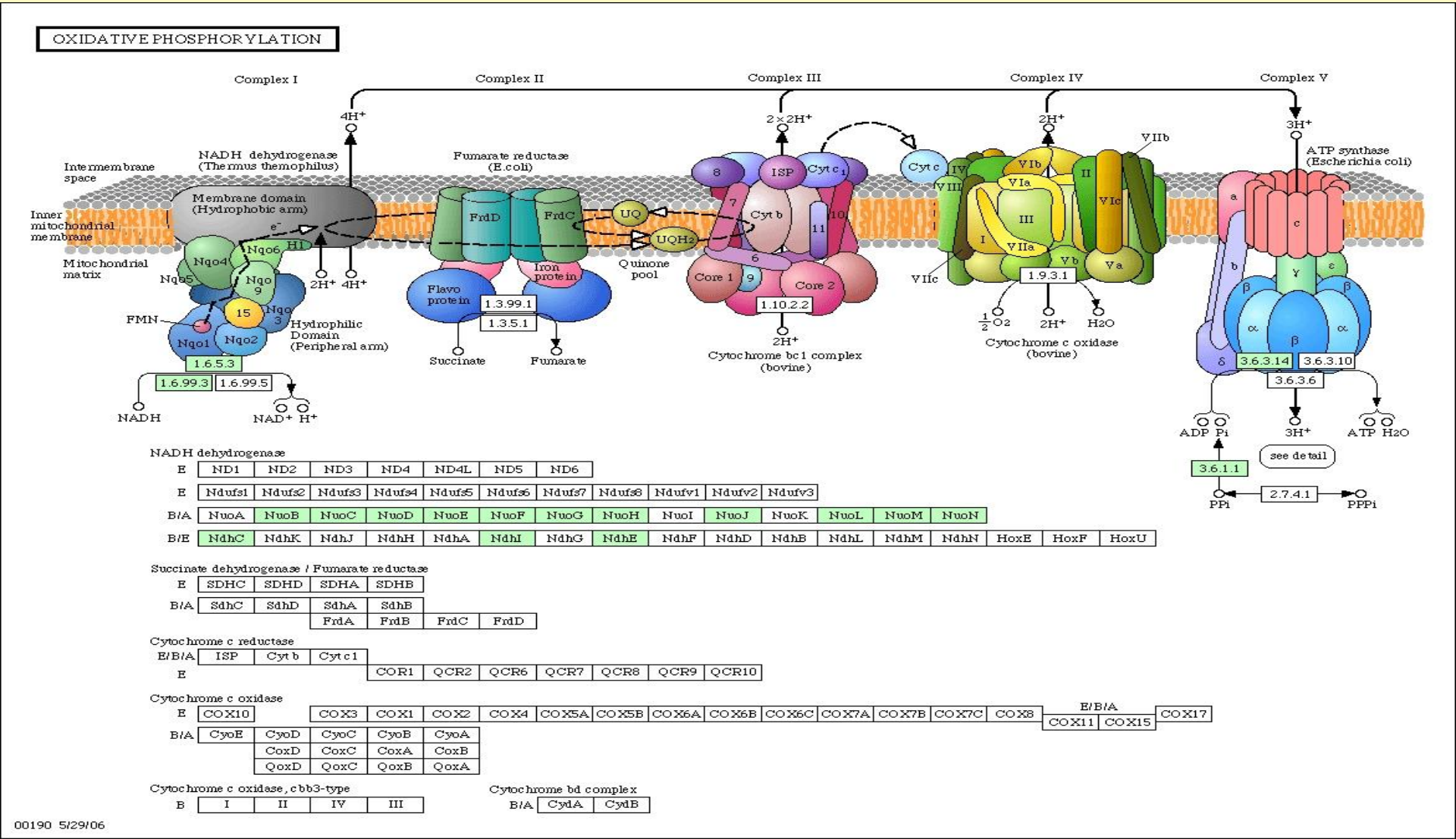
20 aminozuren vormen de eiwitten

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
877642..877717	-	76	57223978		-	DET_tRNA-Arg-3	-	-	Arg tRNA
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 Dehalococcoides



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

E/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

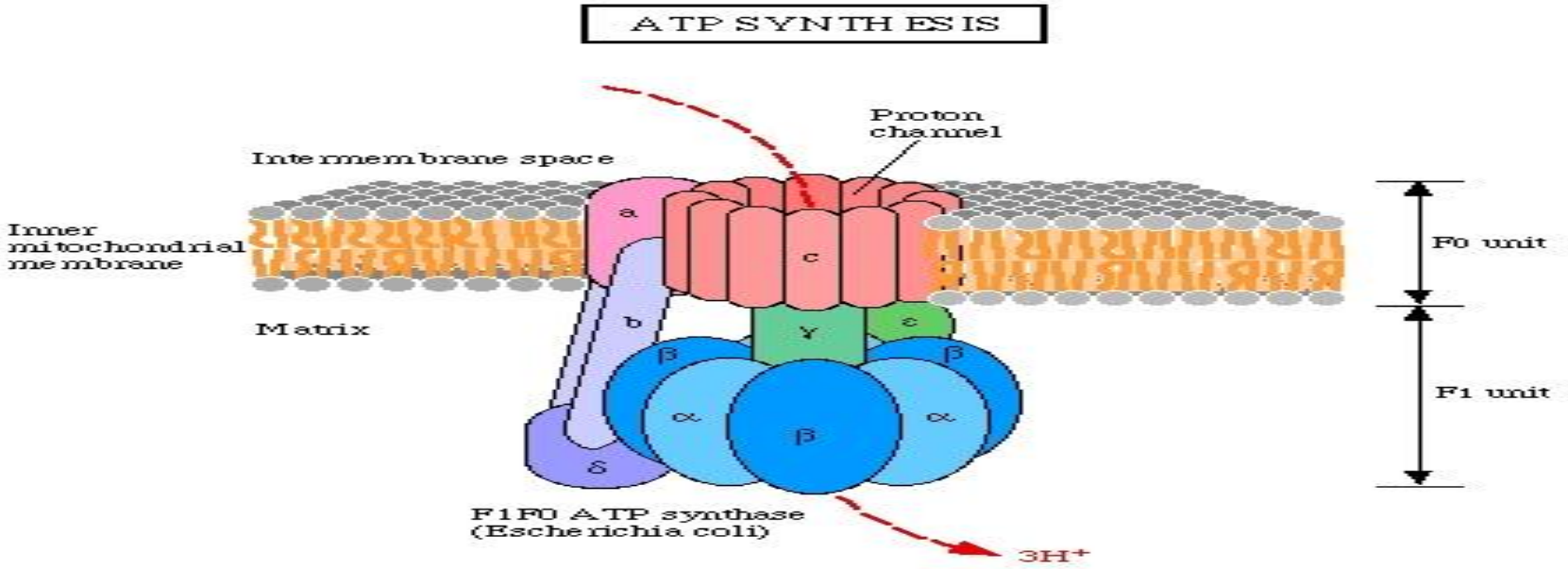
B	I	II	IV	III

Cytochrome bd complex

B/A	CydA	CydB

00190 5/29/06

Synthese van ATP



F-type ATPase (Bacteria)

beta	alpha	gamma	delta	epsilon	c	a	b

F-type ATPase (Eukaryotes)

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

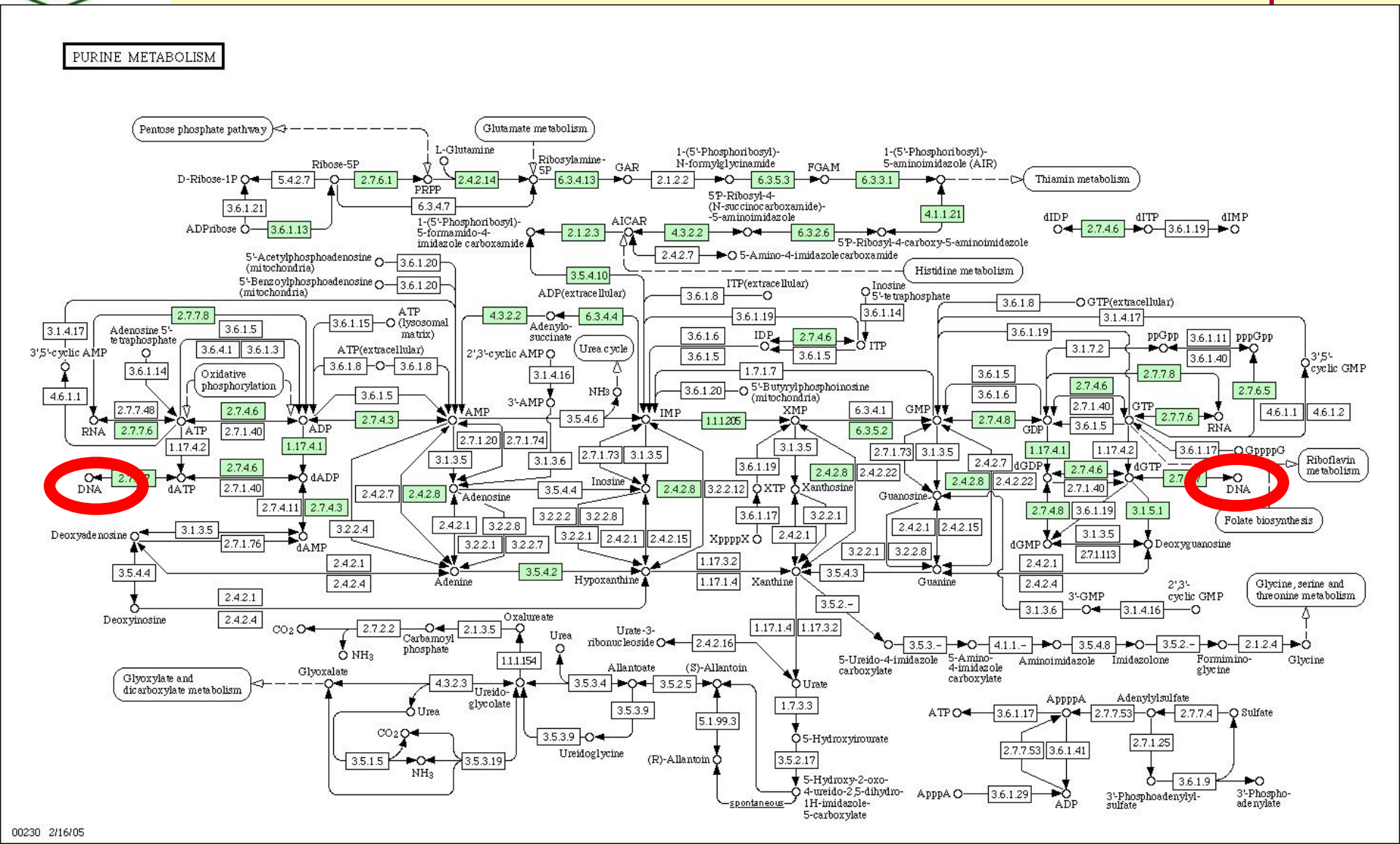
V-type ATPase (Prokaryotes)

A	B	C	D	E	F	I	K

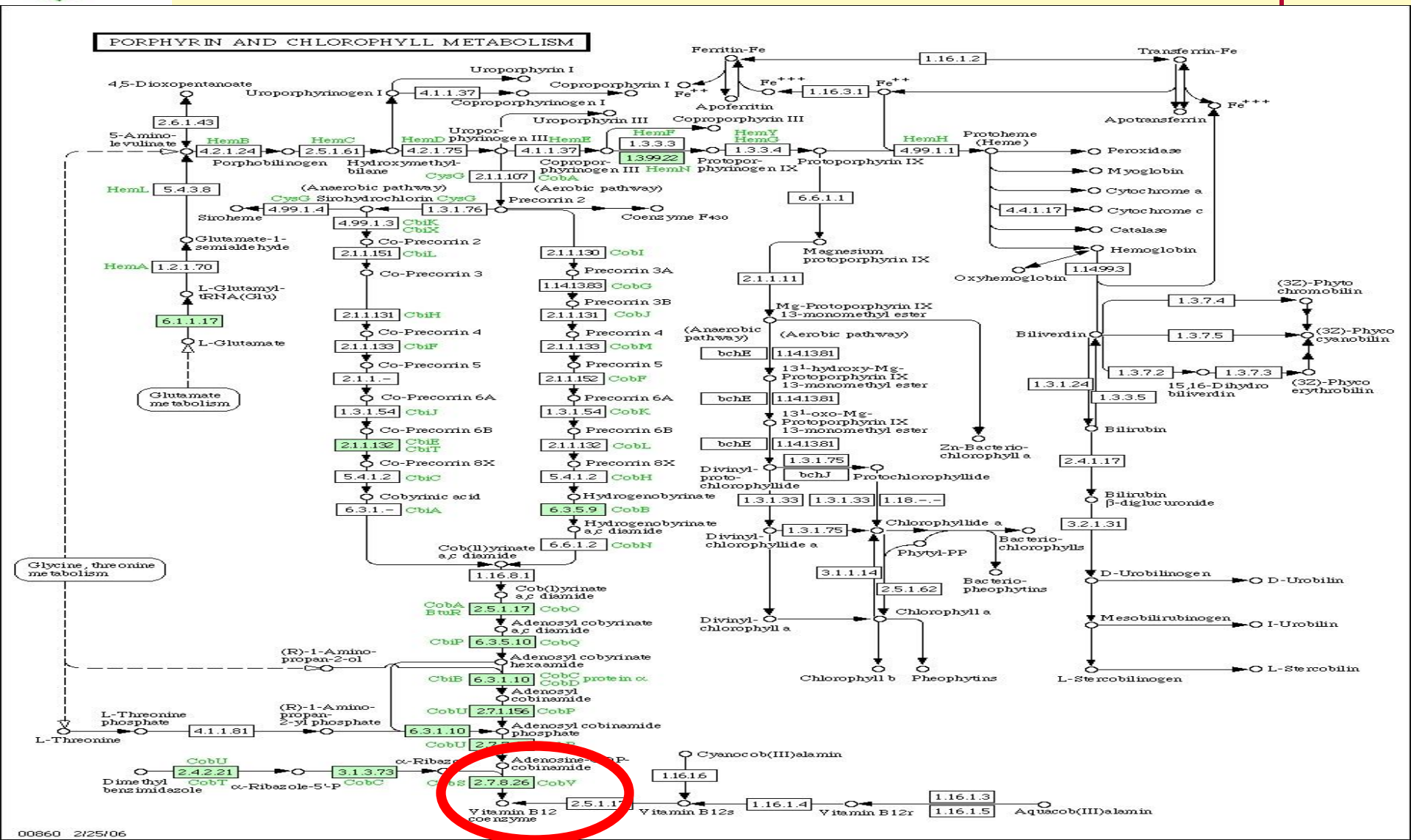
V-type ATPase (Eukaryotes)

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

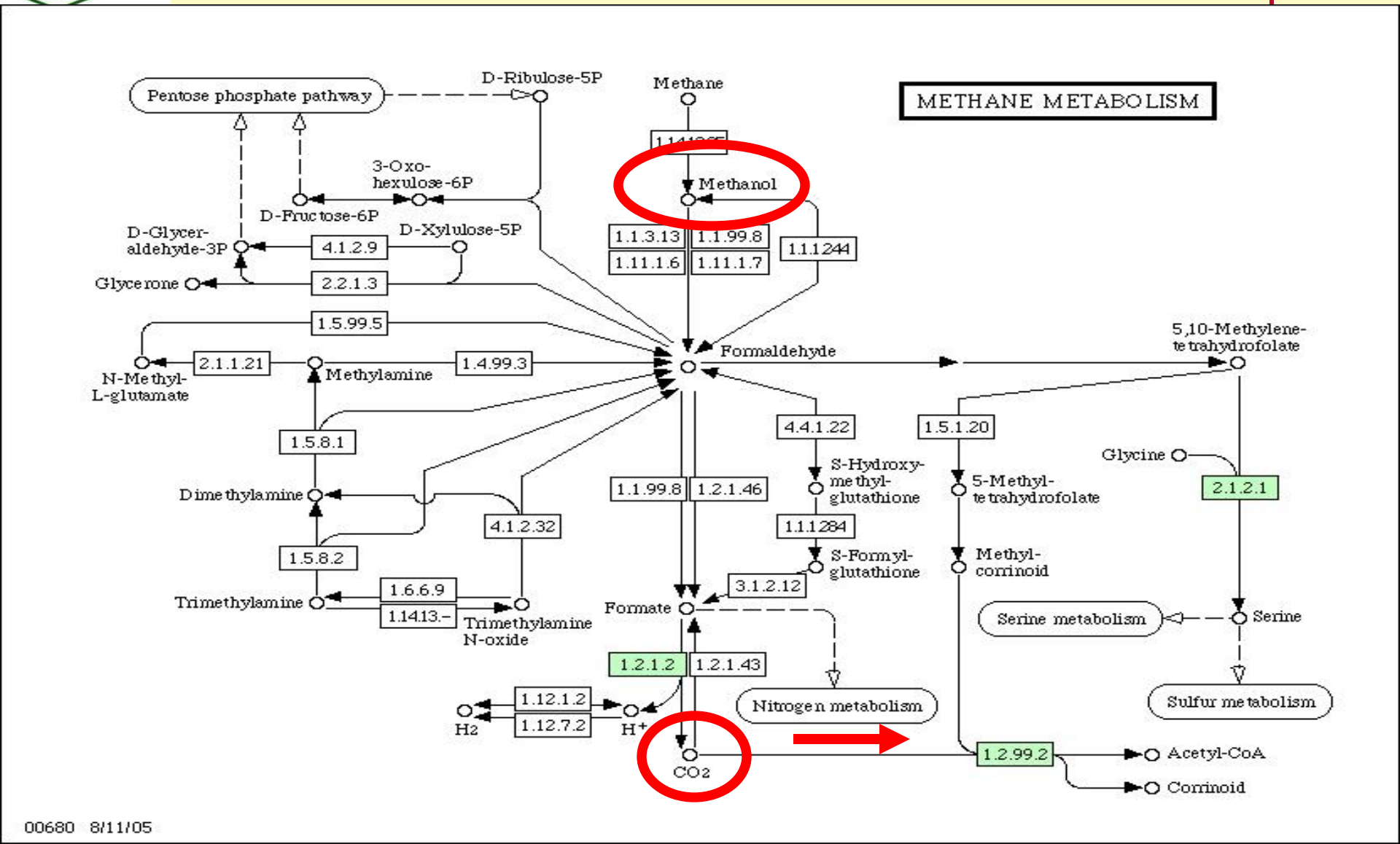
Maakt DNA zelf



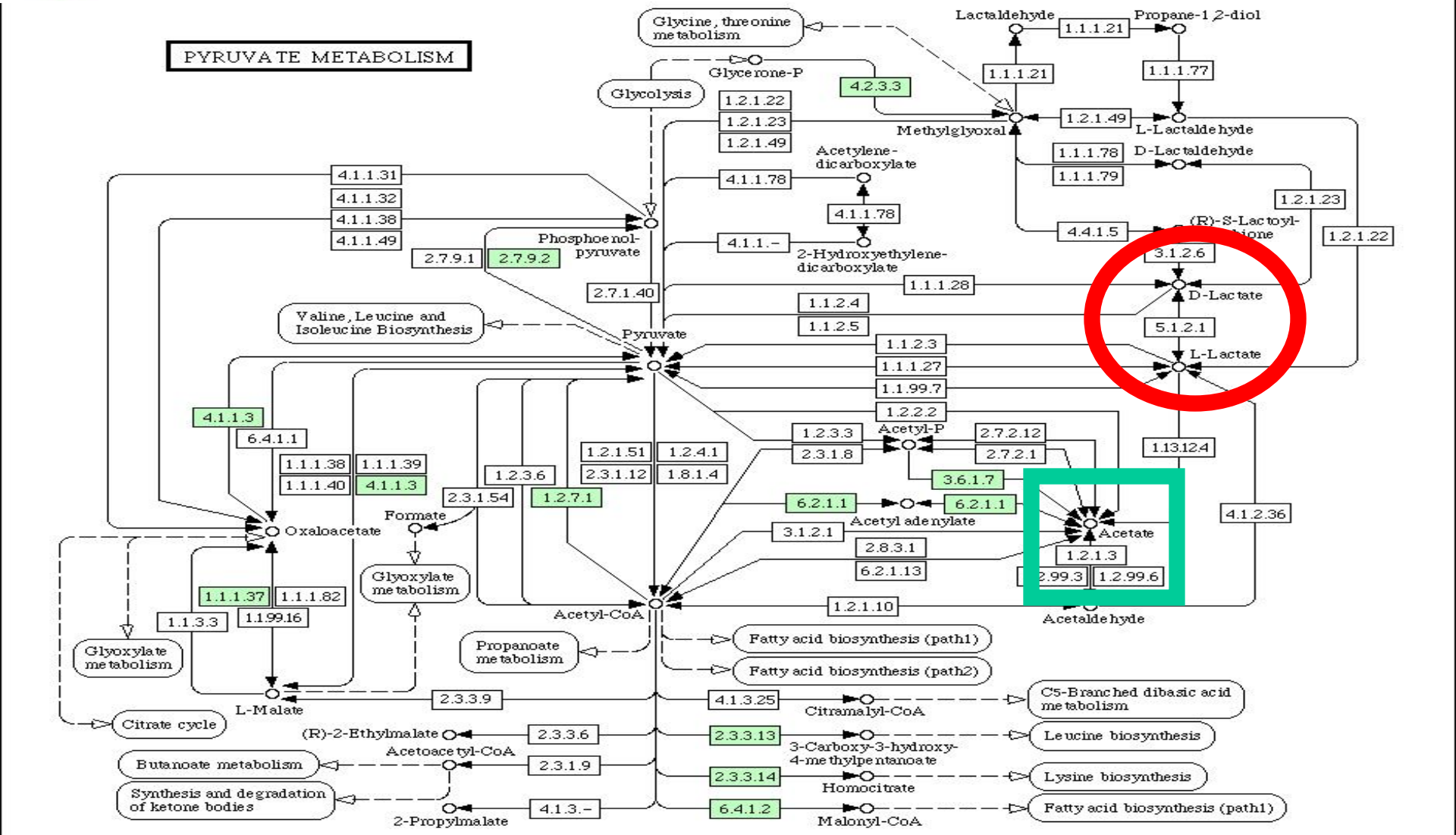
Maakt vitamine B12



Gebruikt geen methanol



Geen lactaat wel acetaat



Twee belangrijke koolstofbronnen

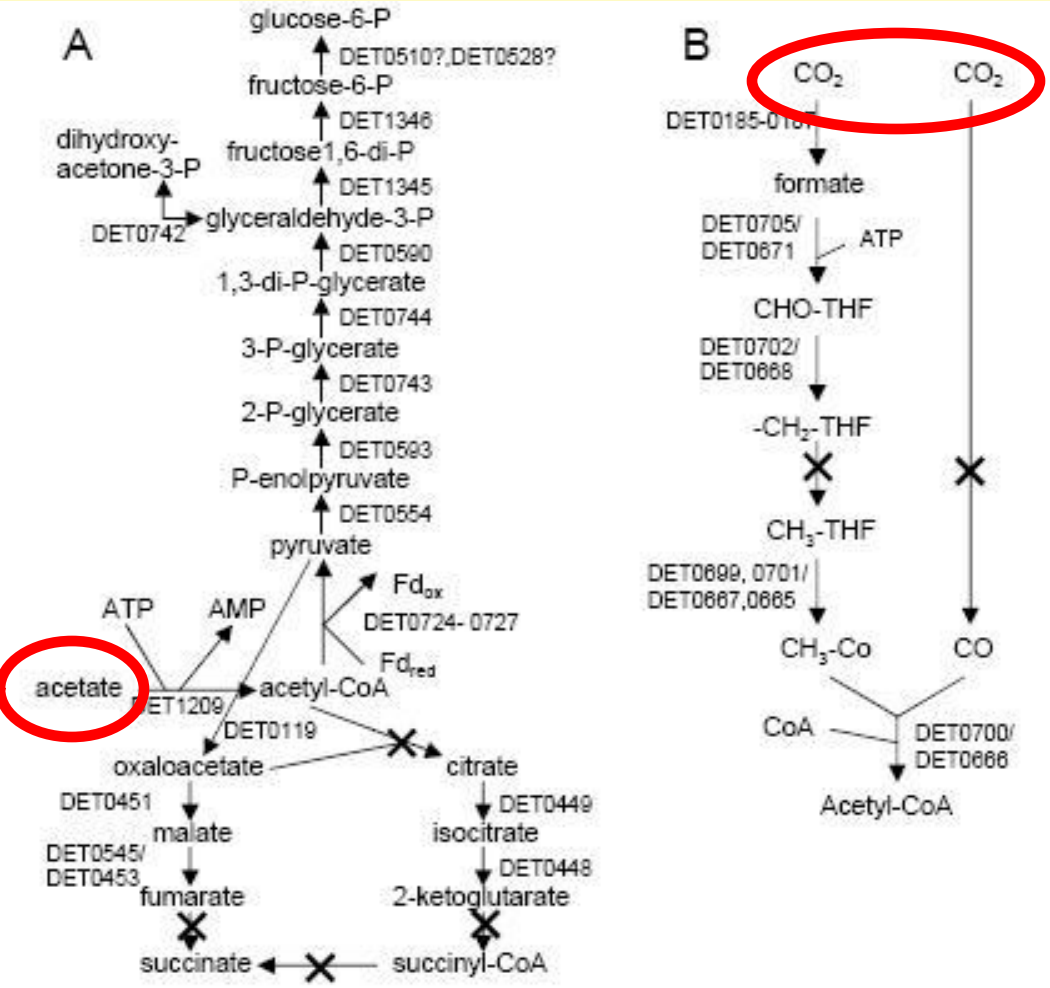
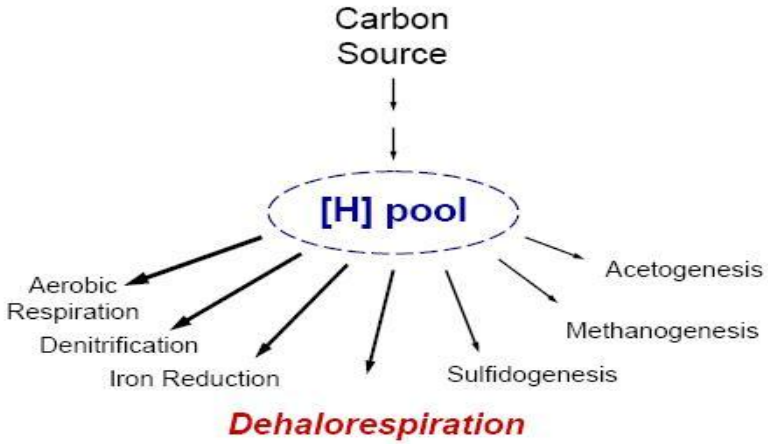


Figure S6. Central carbon metabolism pathways in *D. ethenogenes* predicted from the genome

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 H_2 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
Dehalorespiration	< 0.31
Sulfate reduction	1 - 4
Methanogenesis	>5
Acetogenesis	>336

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

concurenten

The Ubiquity of Hydrogenase

H_2 →

CO_2 —Methanogens→ CH_4

CO_2 ←Acetogens— Acetate

SO_4^{-2} —Sulfur Reducers→ H_2S

Fe^{3+} —Iron Reducers→ Fe^{2+}

NO^{3-} —Denitrification→ N_2

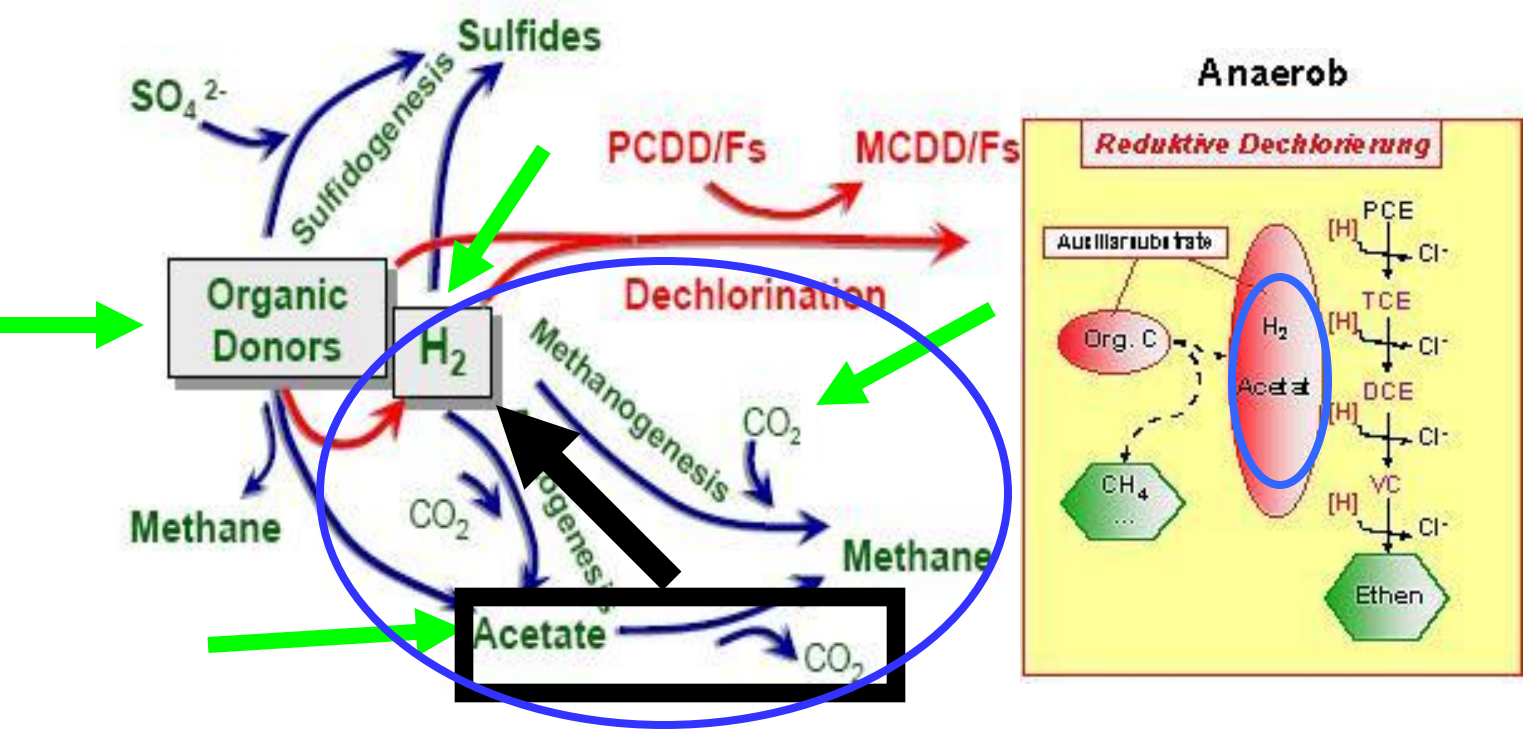
N_2 —Nitrogen fixation→ NH_2^-

CO_2 —Photosynthesis→ Sugars

Chemautotrophy

Anaërobe 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

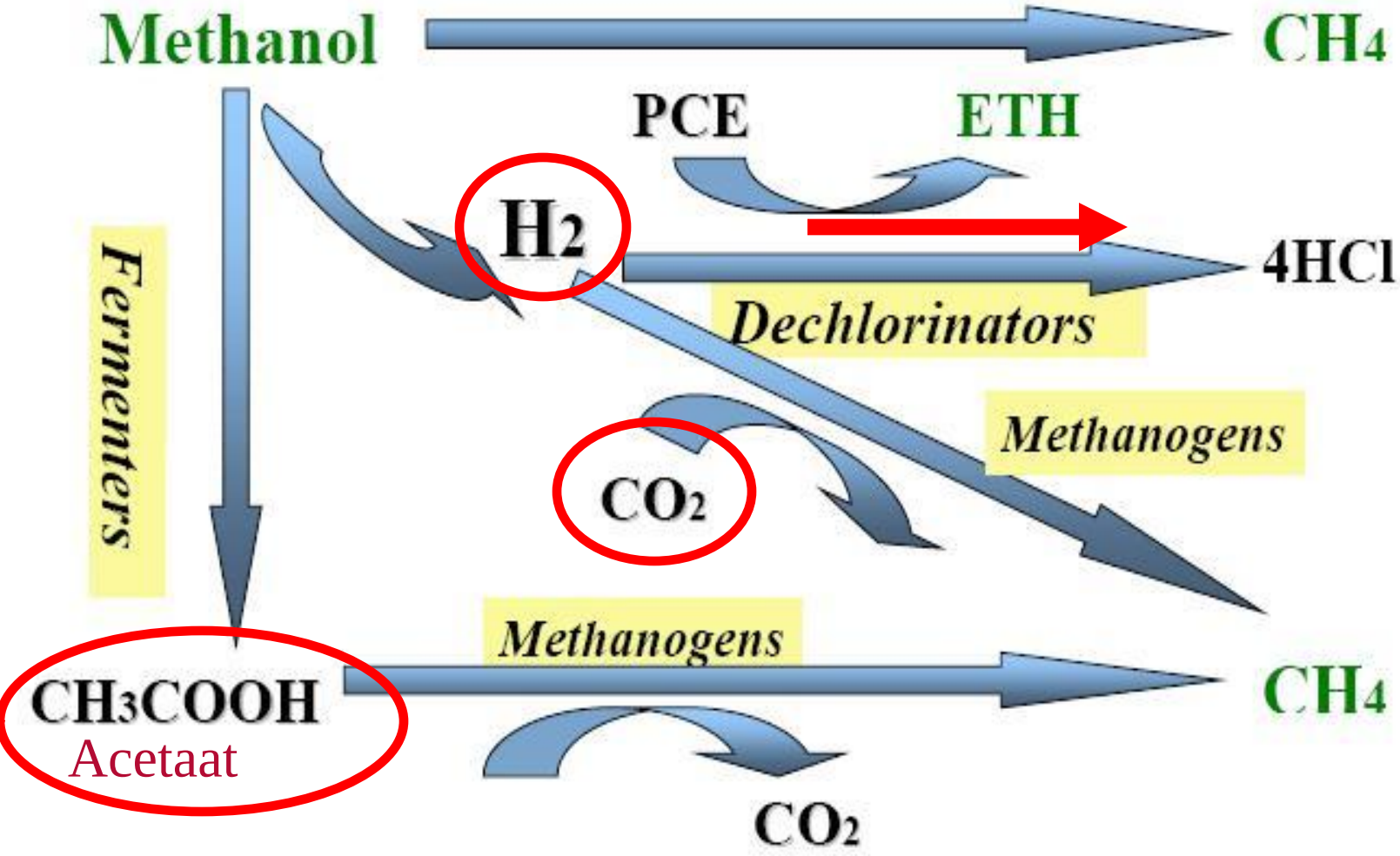
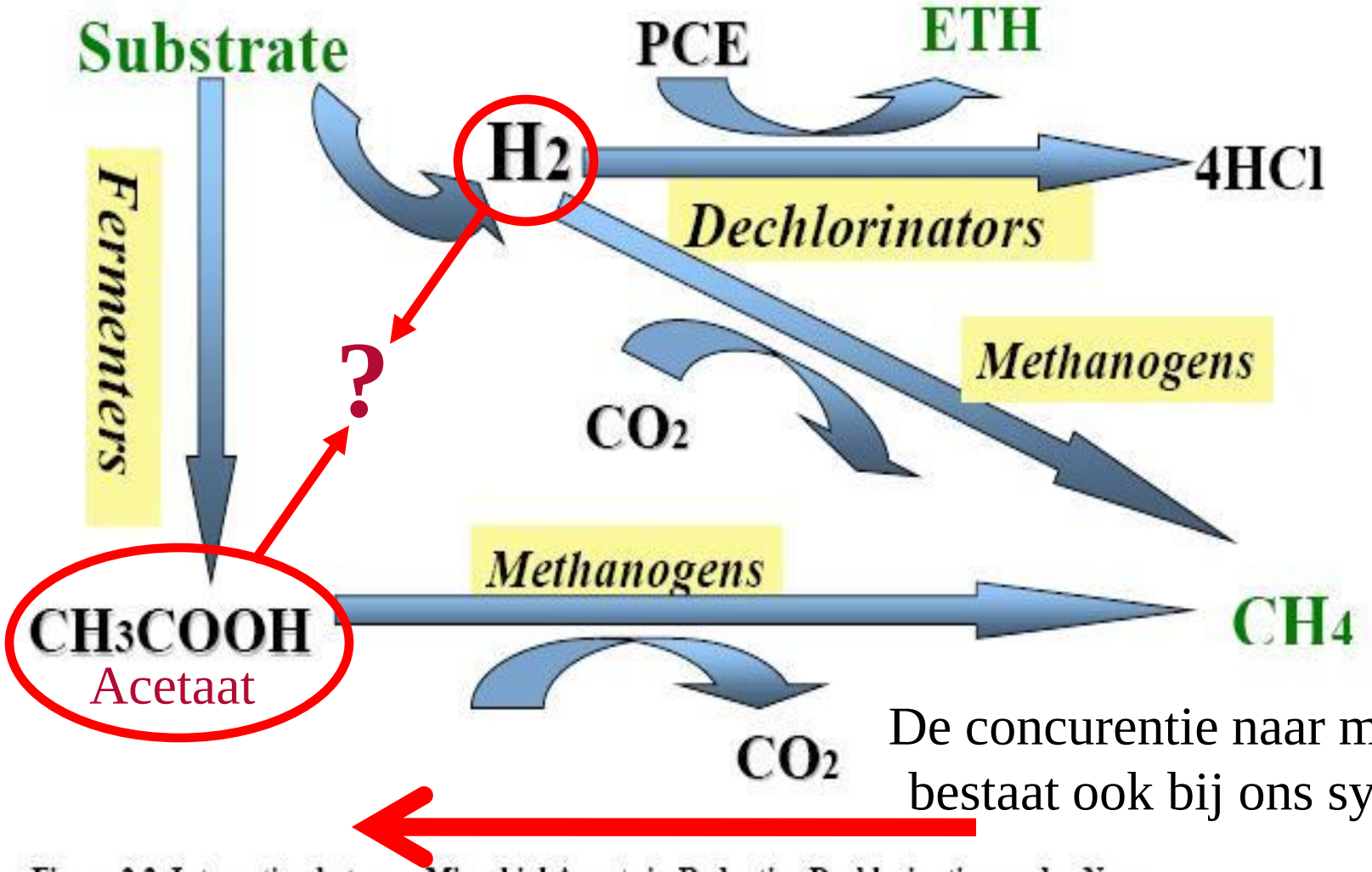


Figure 2.1 Interaction between Microbial Agents in Reductive Dechlorination using Methanol as Electron Donor (Non-Inhibitory PCE concentrations)

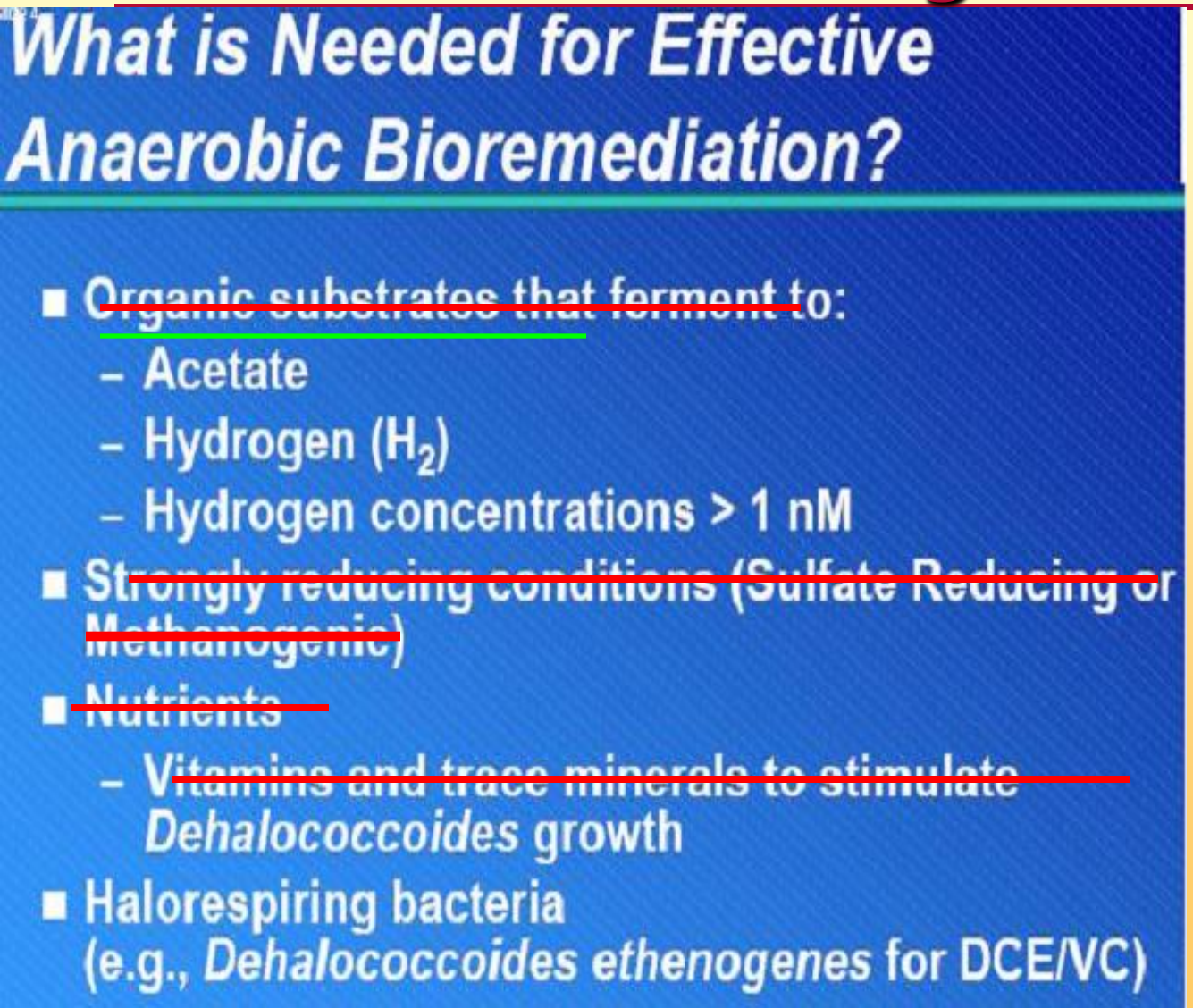
Elk substraat is indirect en niet trefzeker



De concurentie naar methaan bestaat ook bij ons systeem

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

Wat is voor een snelle dechlorering nodig?



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

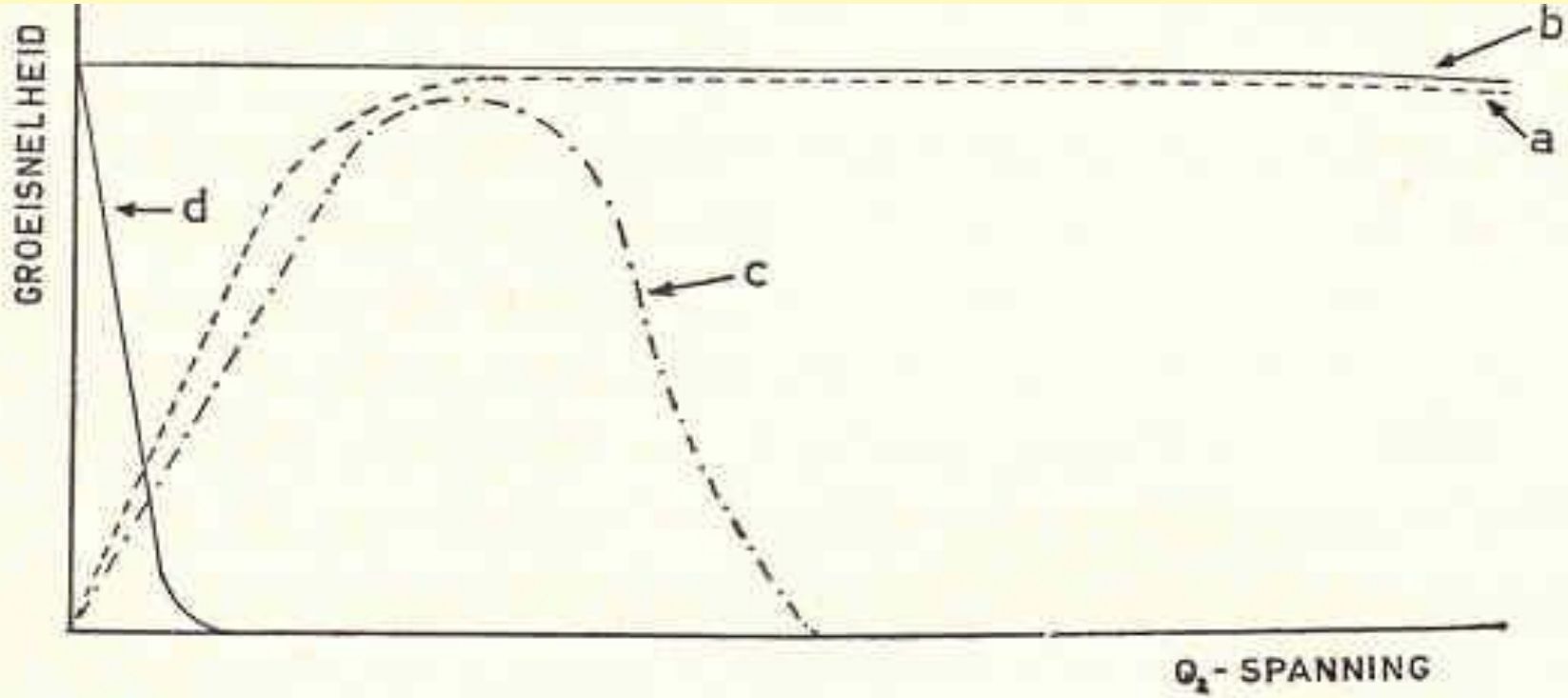
- Soluble – lactate, molasses, ethanol
- Viscous Fluids – HRC®, HRC-X, neat vegetable oil
- Low Viscosity Fluids – vegetable oil microemulsions, cheese whey
- Solids Substrates – mulch, compost, chitin
- Experimental – hydrogen gas, humic acids

- Soluble, mobile
  - alcohols, sugars, lactate, acetate, citrate
- Semi-soluble or emulsions, less mobile
  - oleate, sterate, 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	10[illegible] to 500 mg/L (TOC)

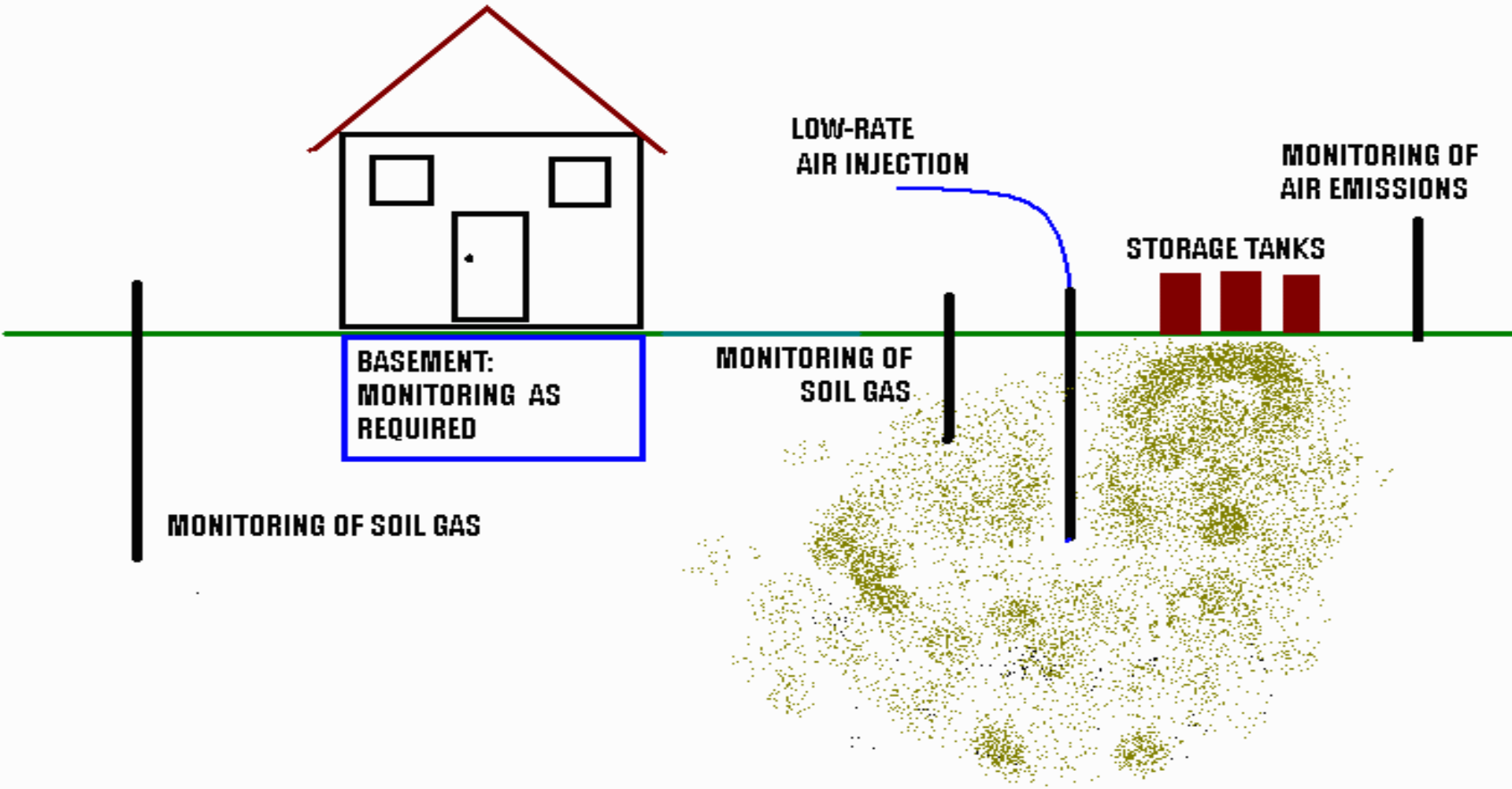
Zuurstof is dodelijk



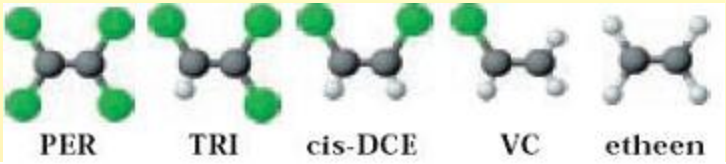
Verband tussen groeisnelheid en zuurstofspanning bij verschillende microben

Curve a: obligaat aerobe microben (verzadigingscurve)
Curve b: facultatief anaerobe microben
Curve c: microaerofiele microben (optimumcurve)
Curve d: obligaat anerobe microben

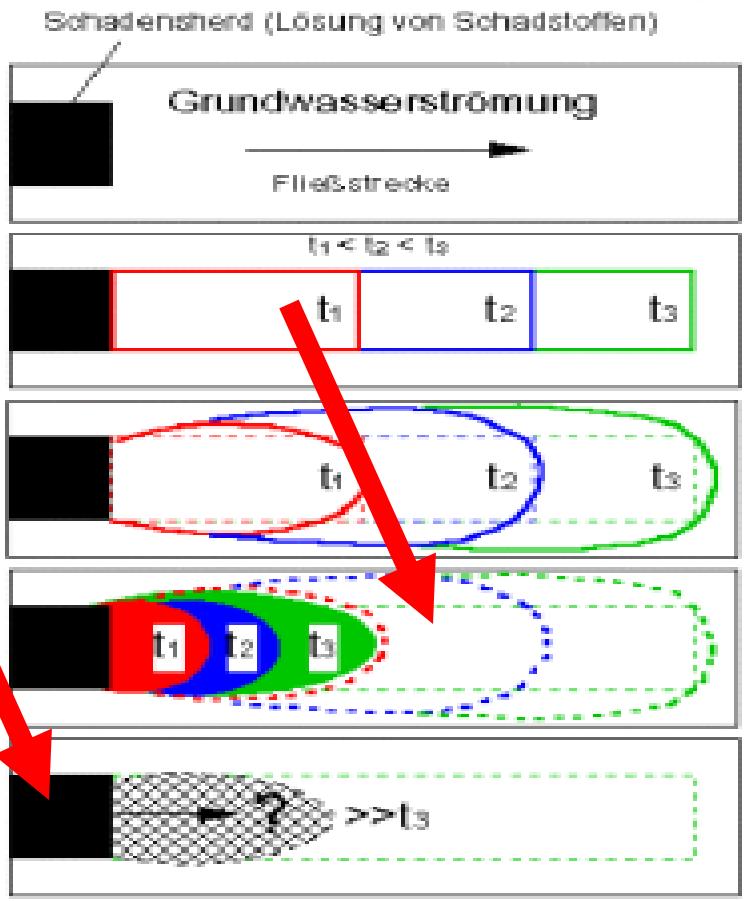
Voorbeeld waarbij Dehalococcoides dood werd gemaakt



Het diffusieprobleem



Beispiel



Schadstofftransport gesteuert durch:

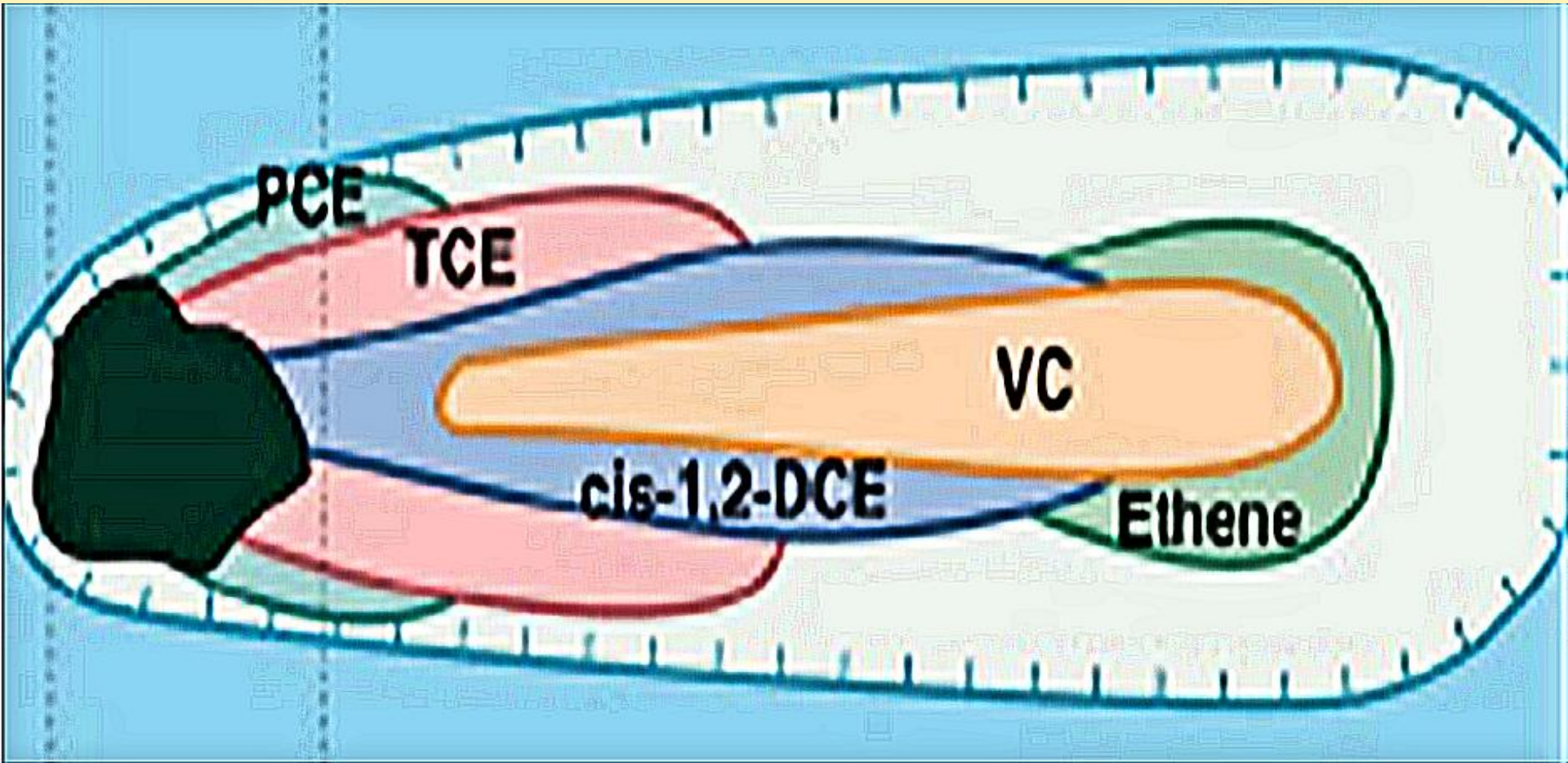
Advektion

Advektion + Dispersion/Diffusion

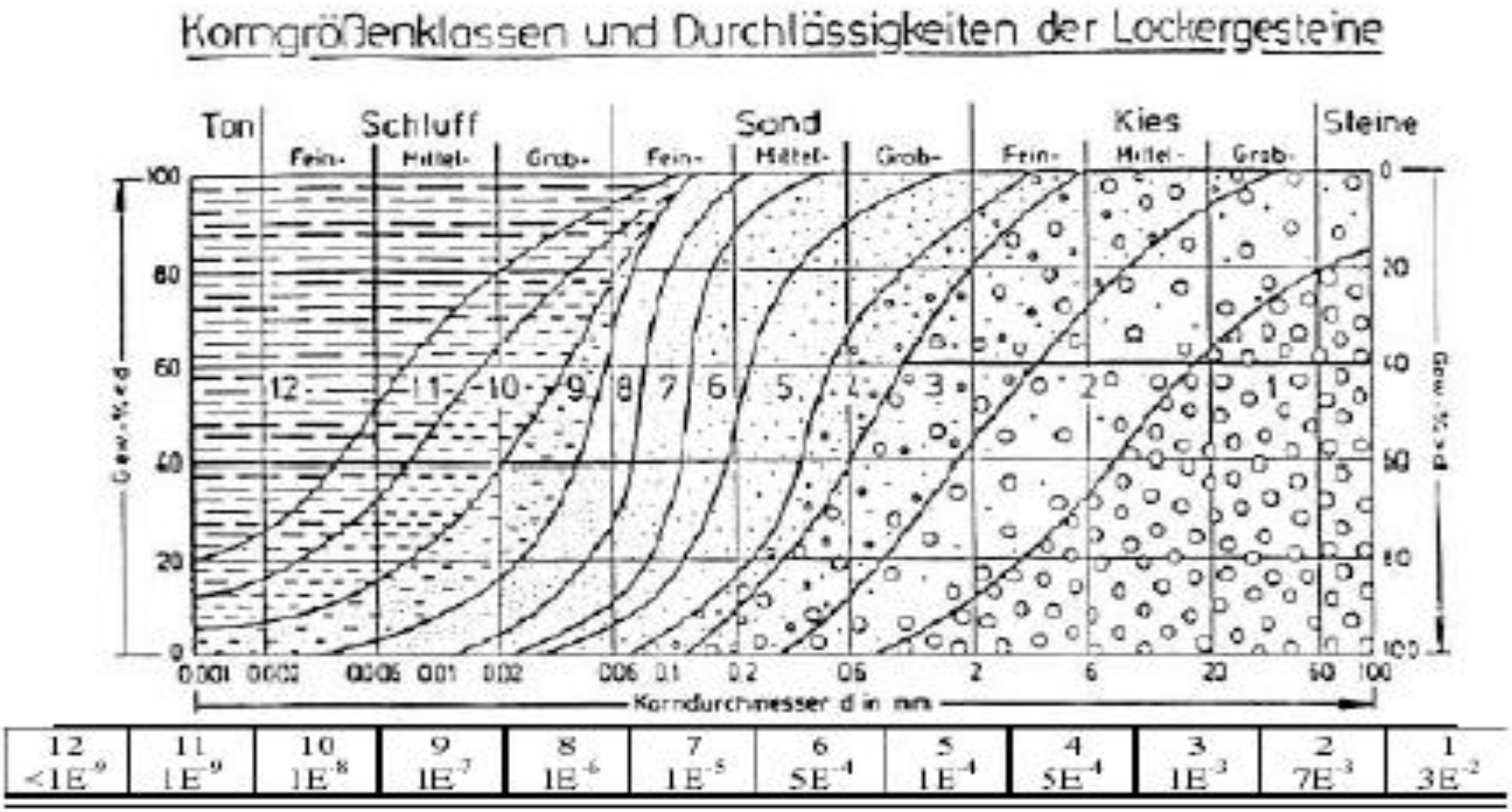
Advektion + Dispersion/Diffusion
+ Sorption/Retardation

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

Diffusie van boven bekeken

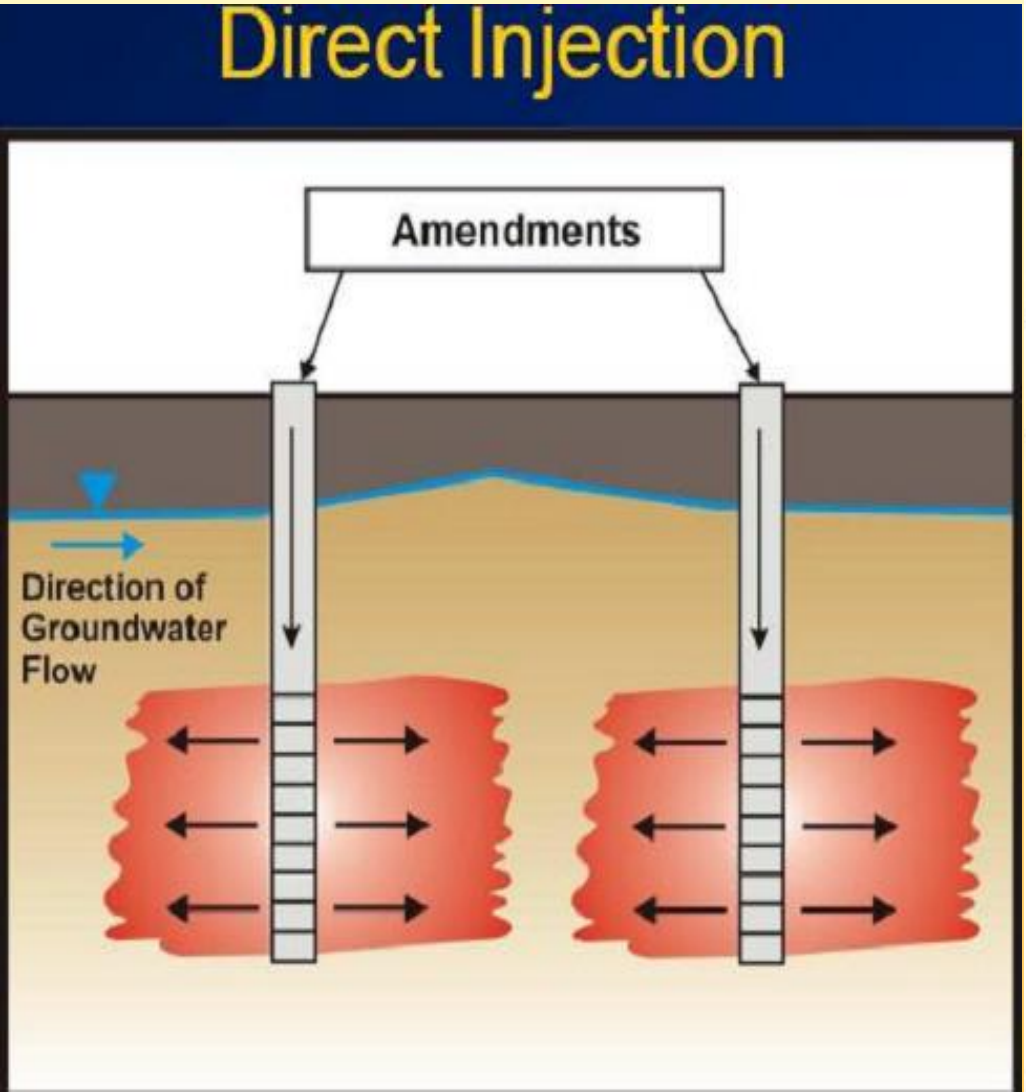


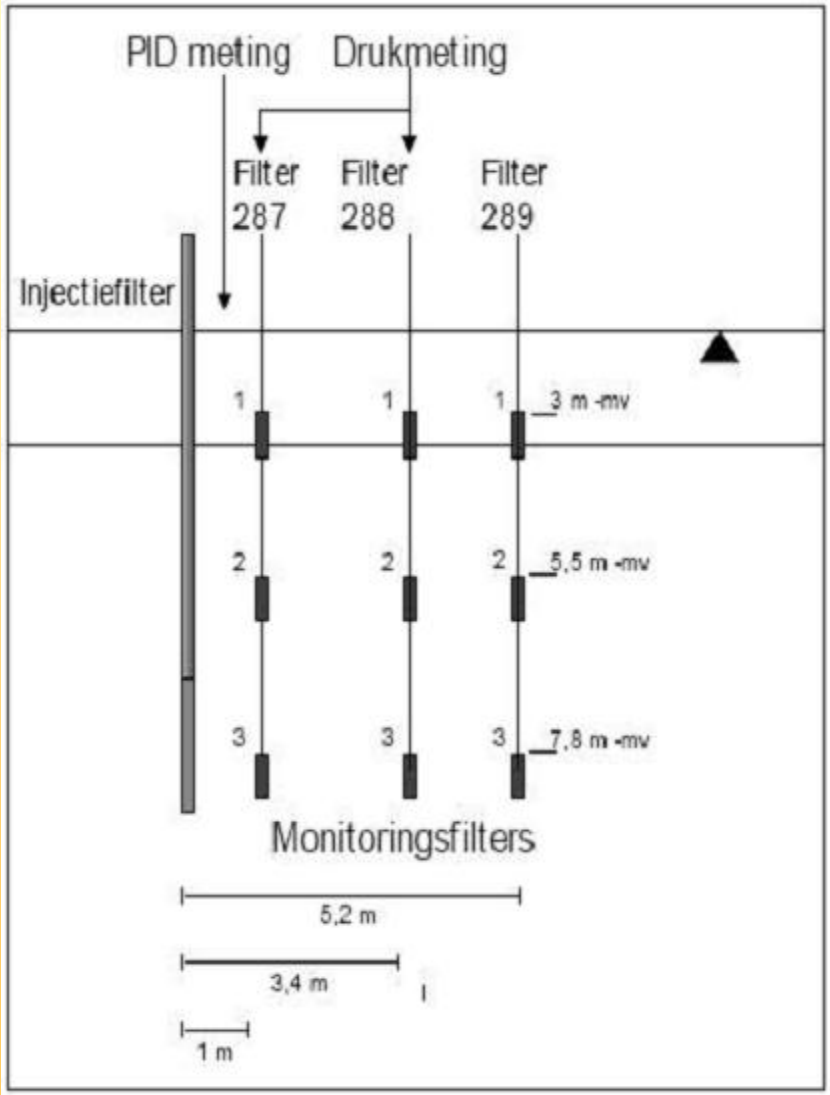
Doorlaadbaarheid per grondsoort



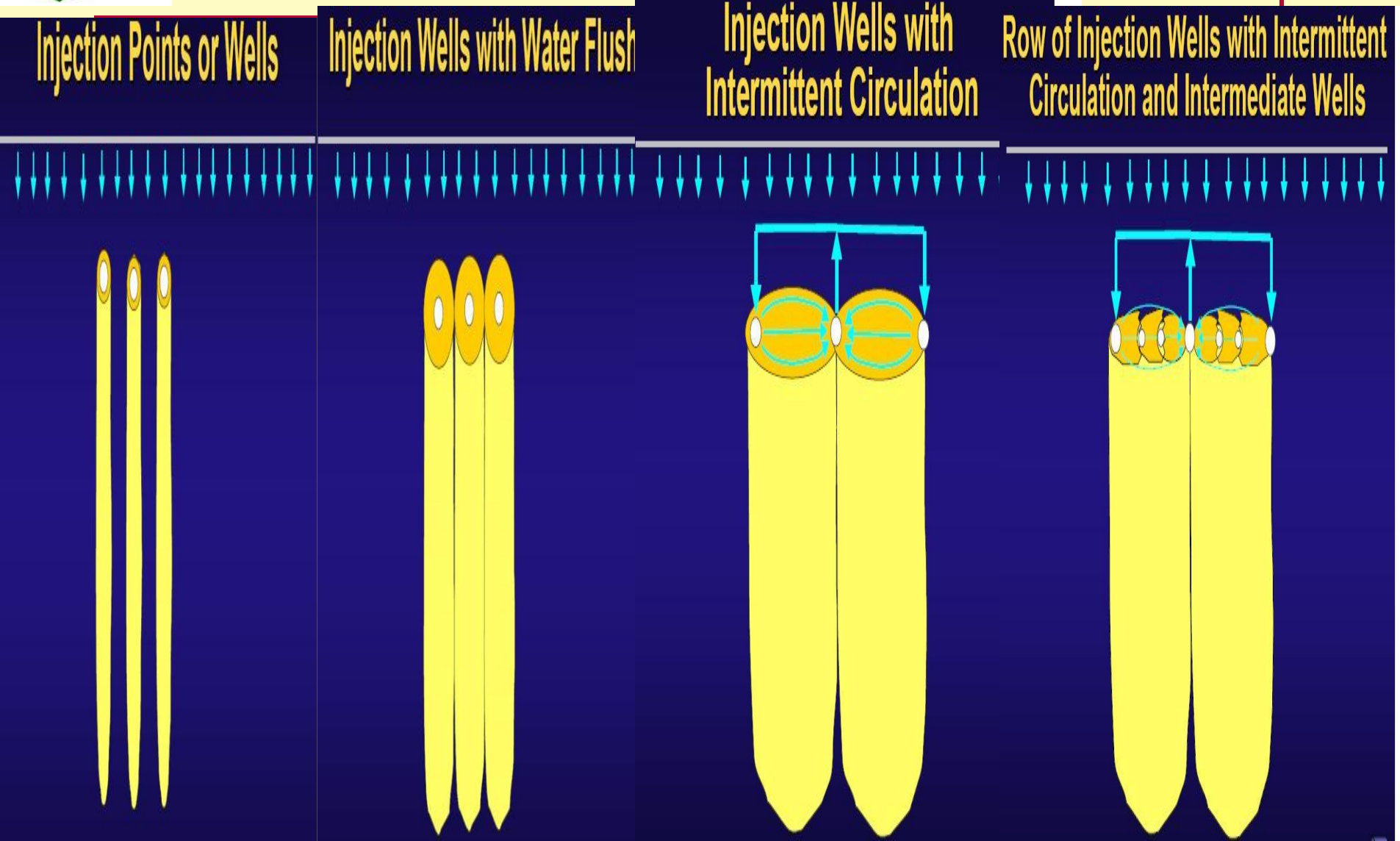
Substratklassen in Abhängigkeit der Korngröße
Größenordnung des Durchlässigkeits-Koeffizienten (k_f) im m/s

injectievoorbeelden



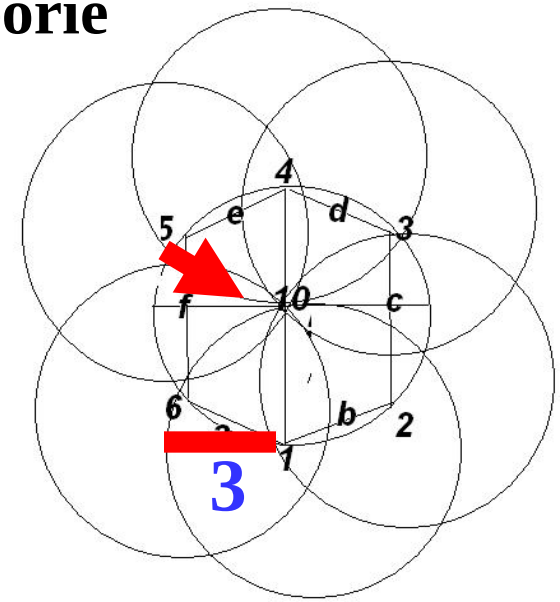


Passieve en actieve injecties

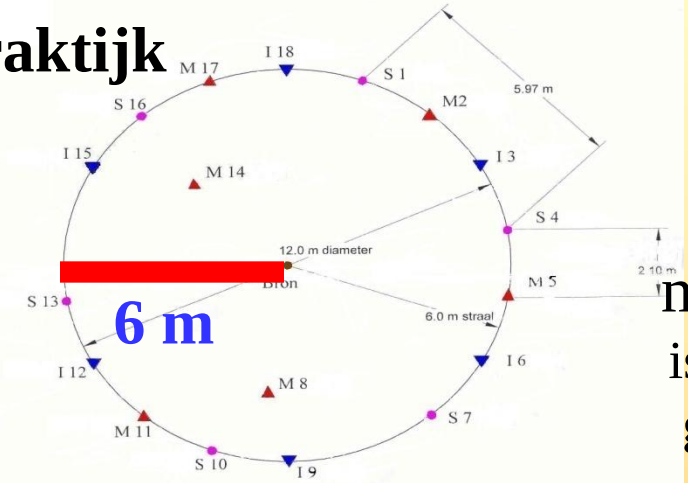


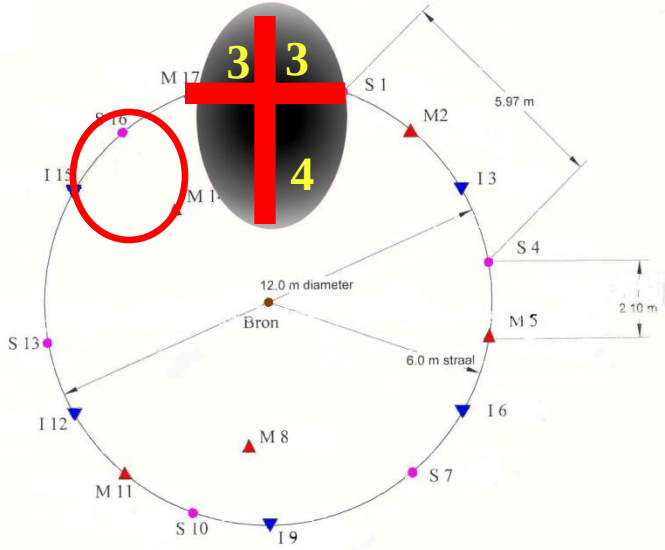
Diffusie en afbraak meten

Theorie



Praktijk





gassen

meetpunt is ertussen gekomen

acetaat

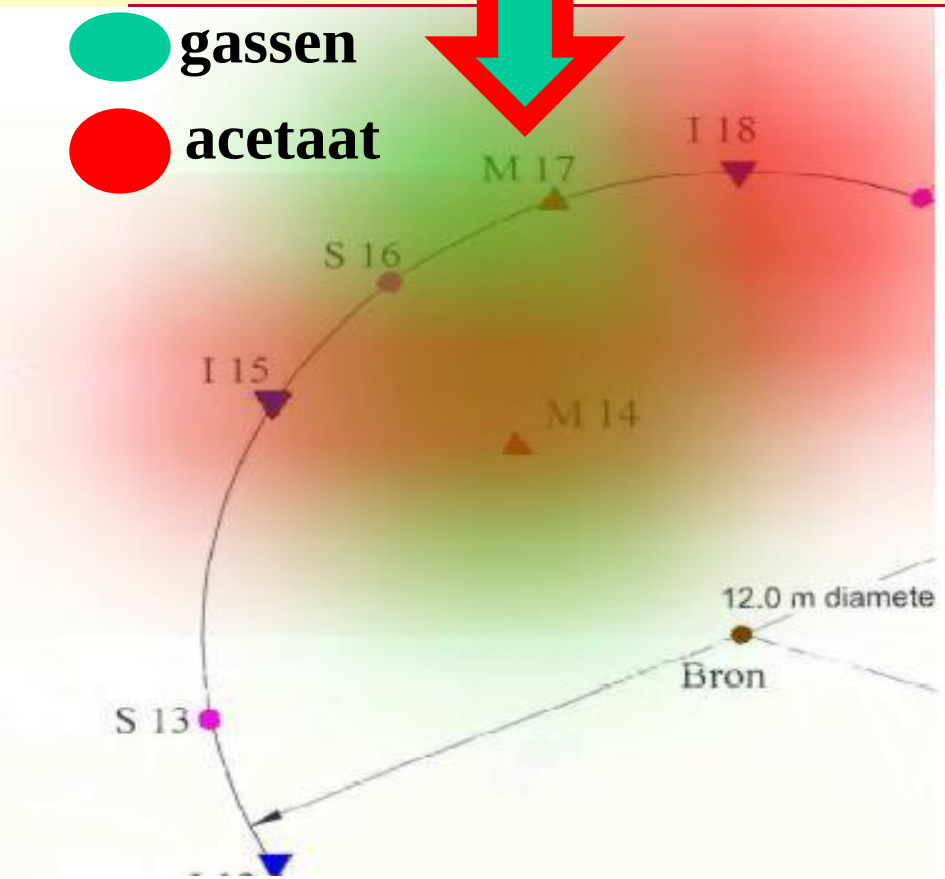
a t/m e acetaatinjecties

1 t/m 6 waterstof/CO2/N2

Recirculatie vanuit 10 naar elk acetaatpunt een paar uur per dag

Alle 13 punten zouden meetpunten zijn

Resultaten Berk



Voor: (eenheden ug/l)

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

Na twee maanden:

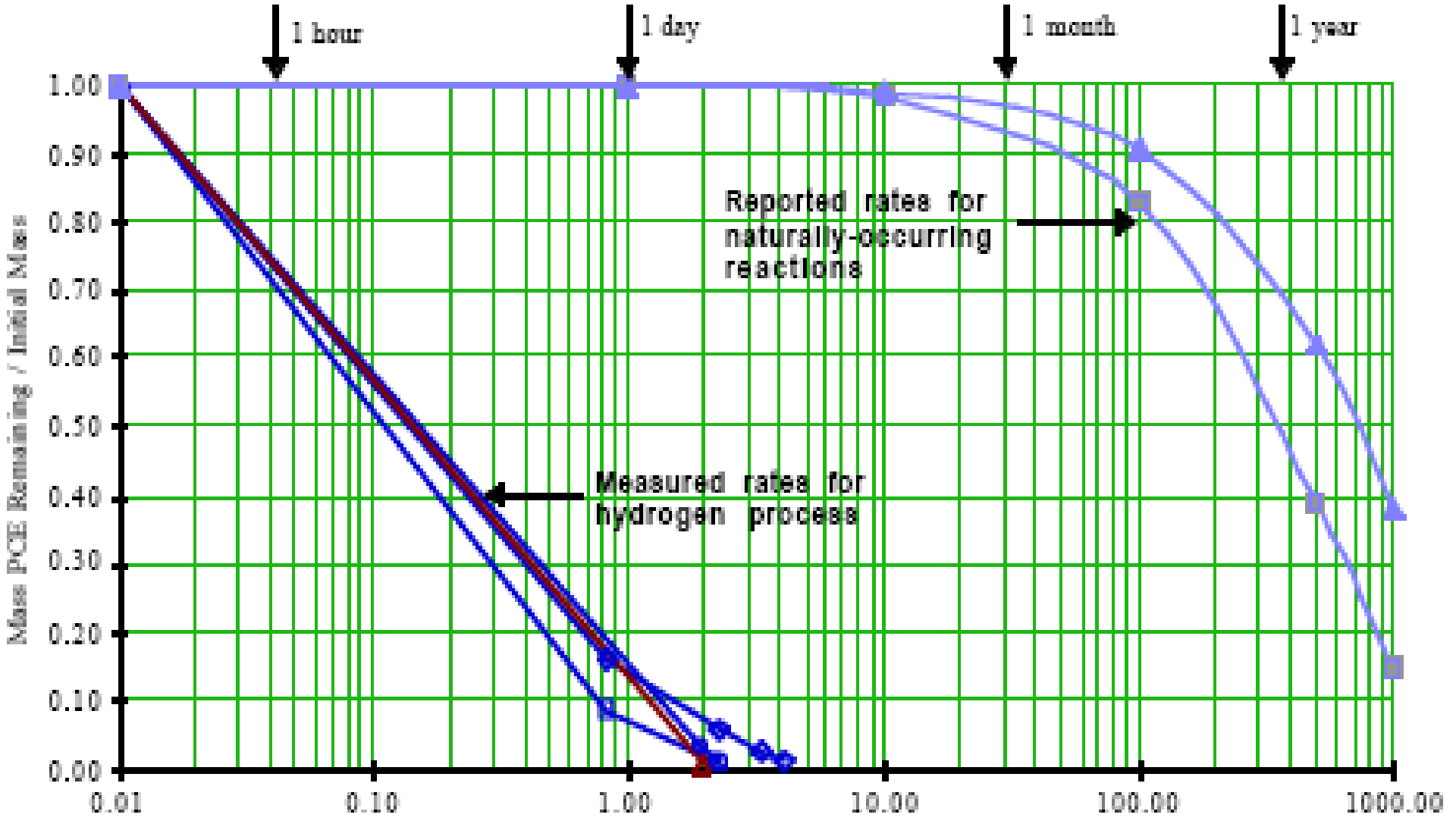
M14	131	1194	23	755
M17	95	1030	85	6

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.

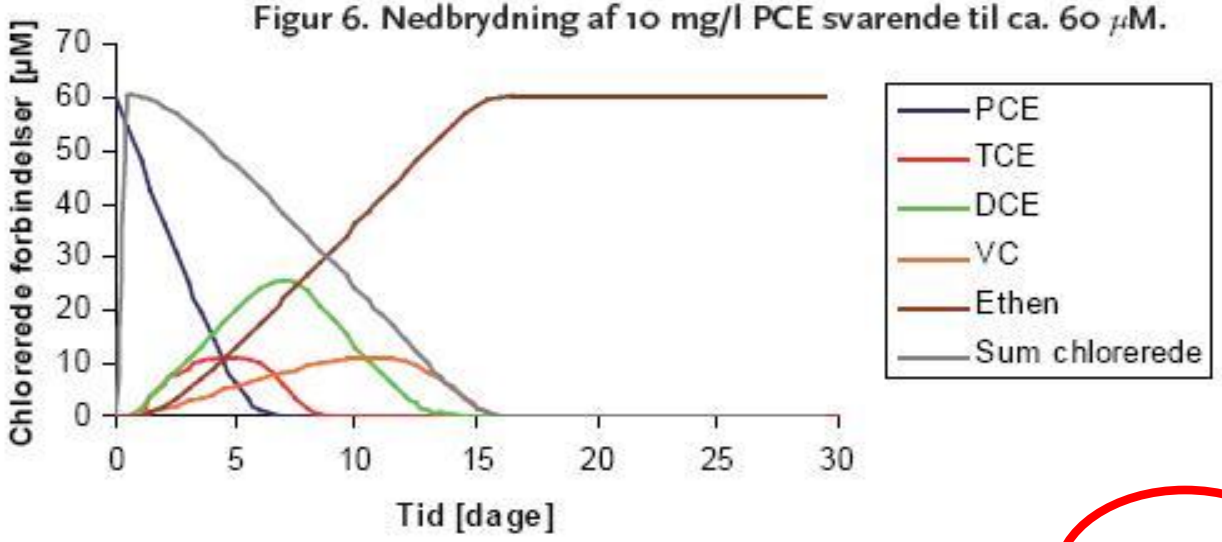
De diffusie van 3 meter in deze bodem is voor acetaat te ver.

Snelle en langzame omzetting

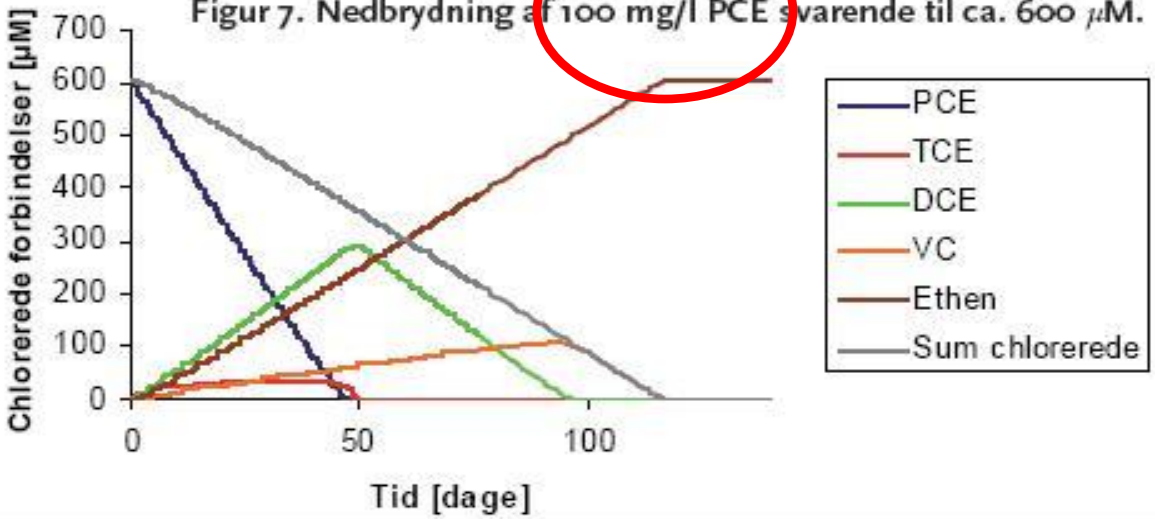


Snelle omzetting in lab

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

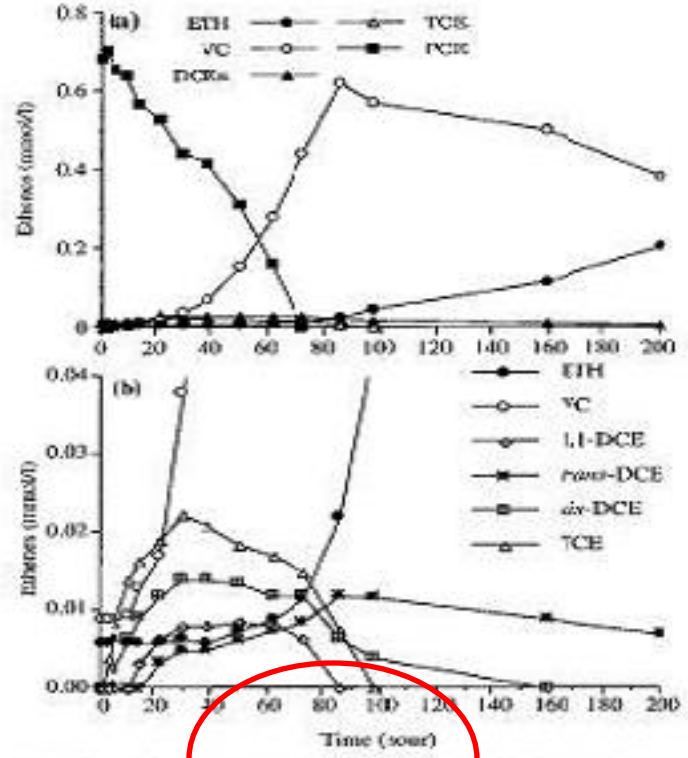


FIG. 2. (a) Product formation by PCE-grown strain 195 inoculated into medium to which one dose of PCE (0.7 mmol/liter) was added. (b) Plot with an expanded scale, showing intermediates in PCE metabolism, including individual DCE isomers.

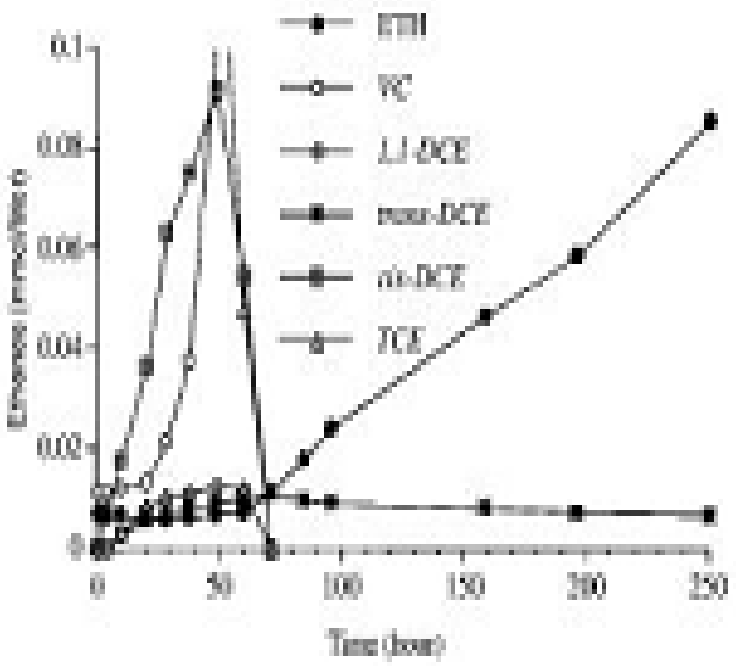
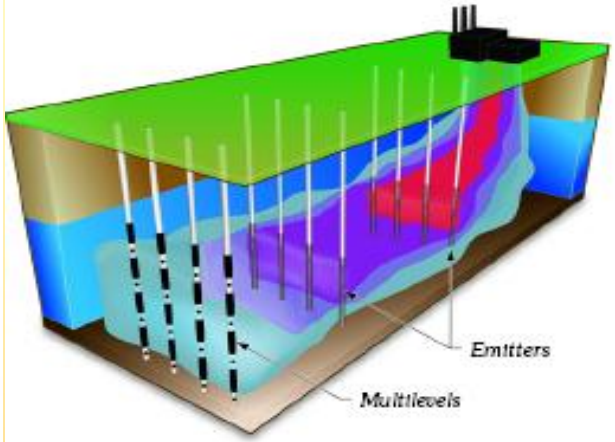


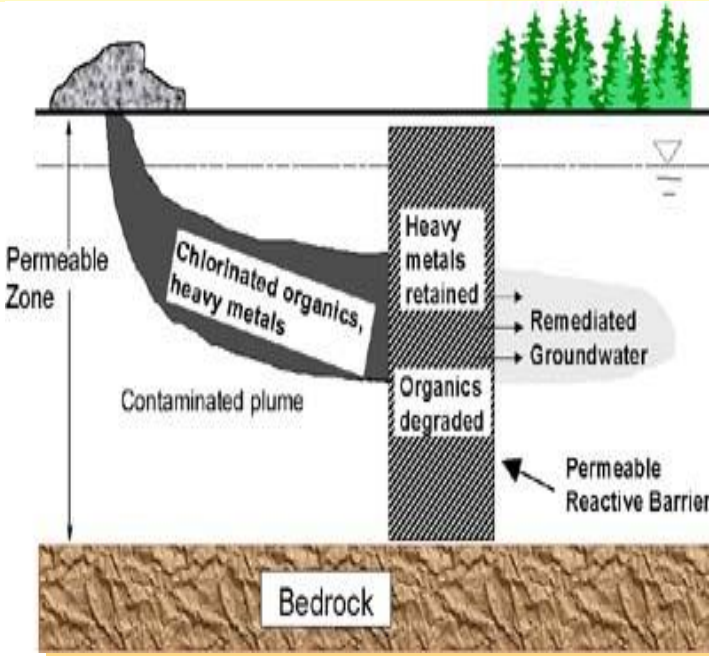
FIG. 3. Intermediate formation by TCE-grown strain 195 inoculated into medium to which a single dose consisting of 0.35 mmol of TCE per liter was added.

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

Afgraven en alleen in de pluim

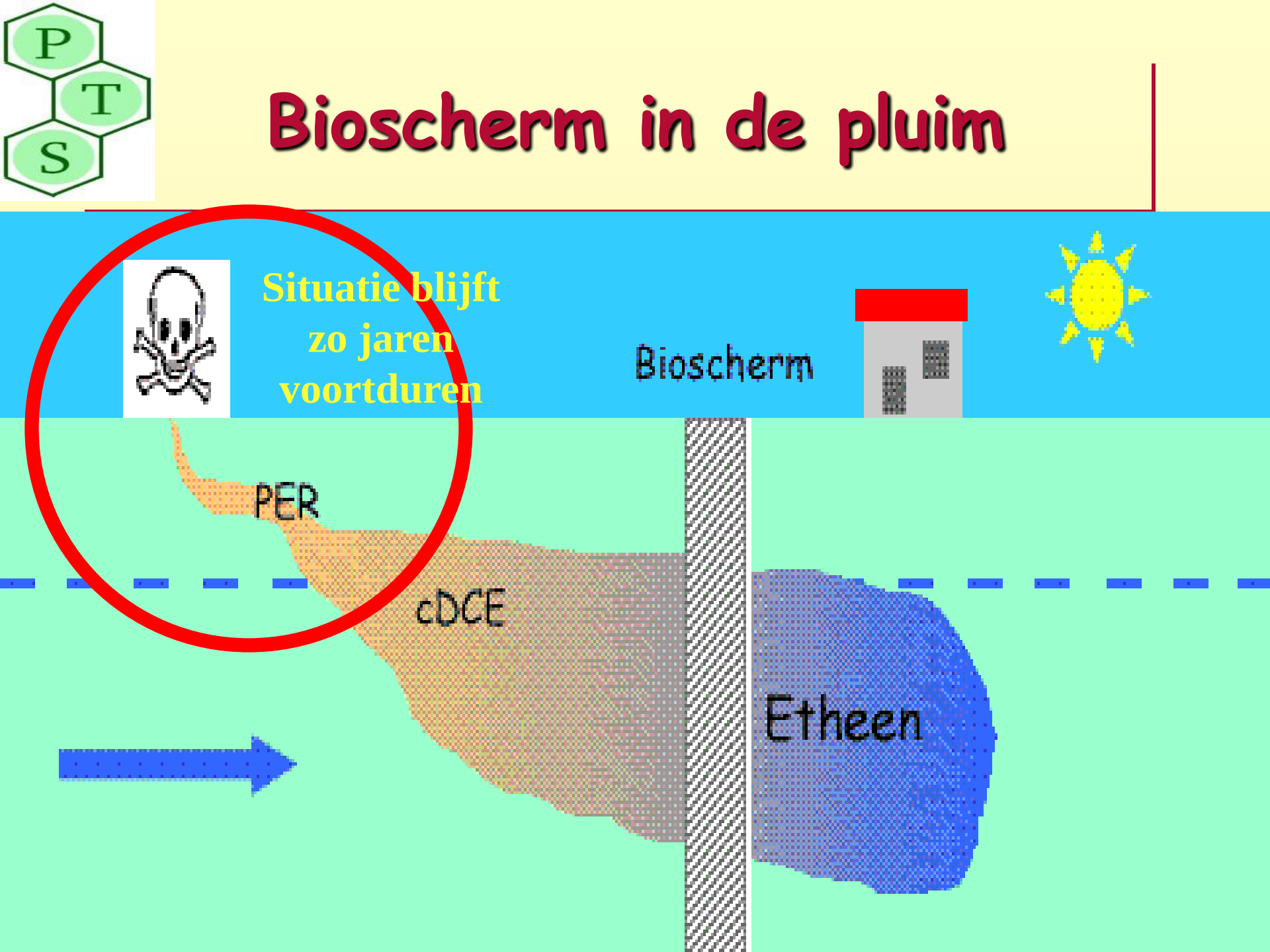


Schematic showing staged plume remediation using Waterloo Emitters, monitored by a transect of CMT or Waterloo Multilevel Systems

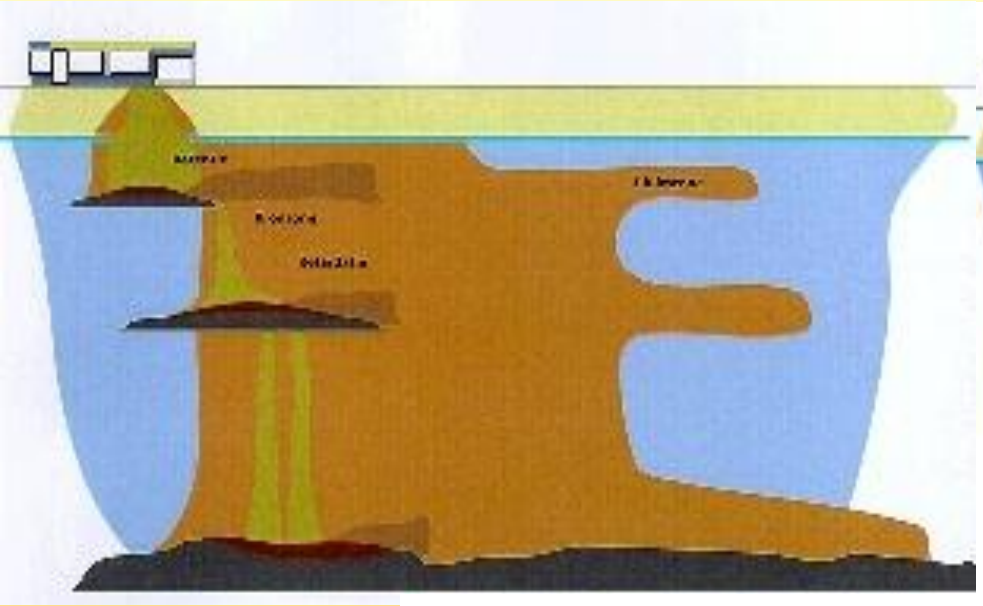


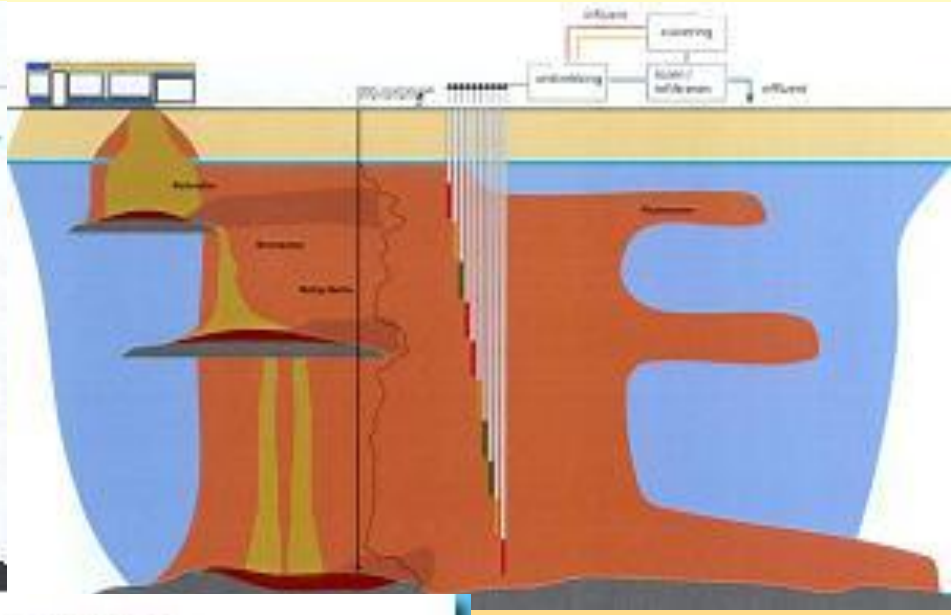
Geen opties

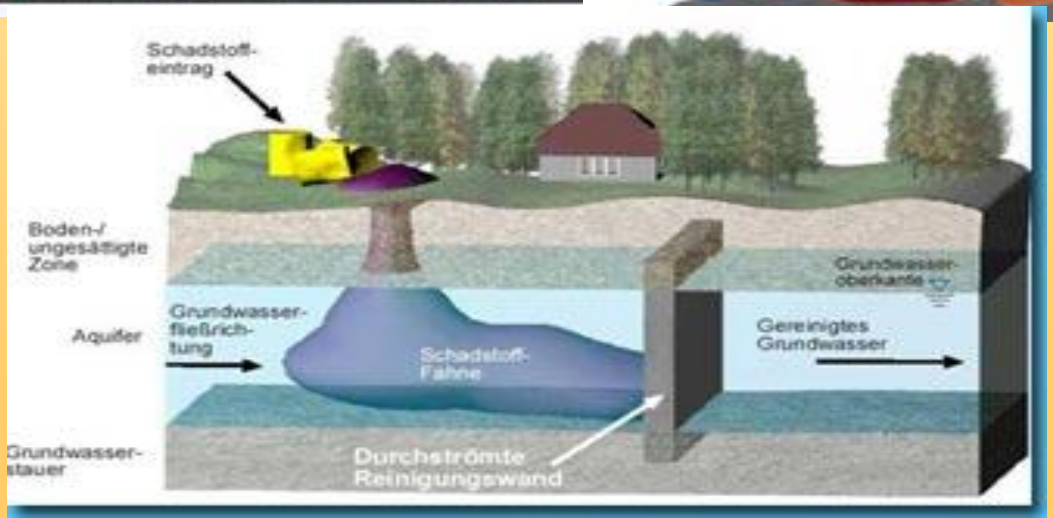
Bioscherm in de pluim



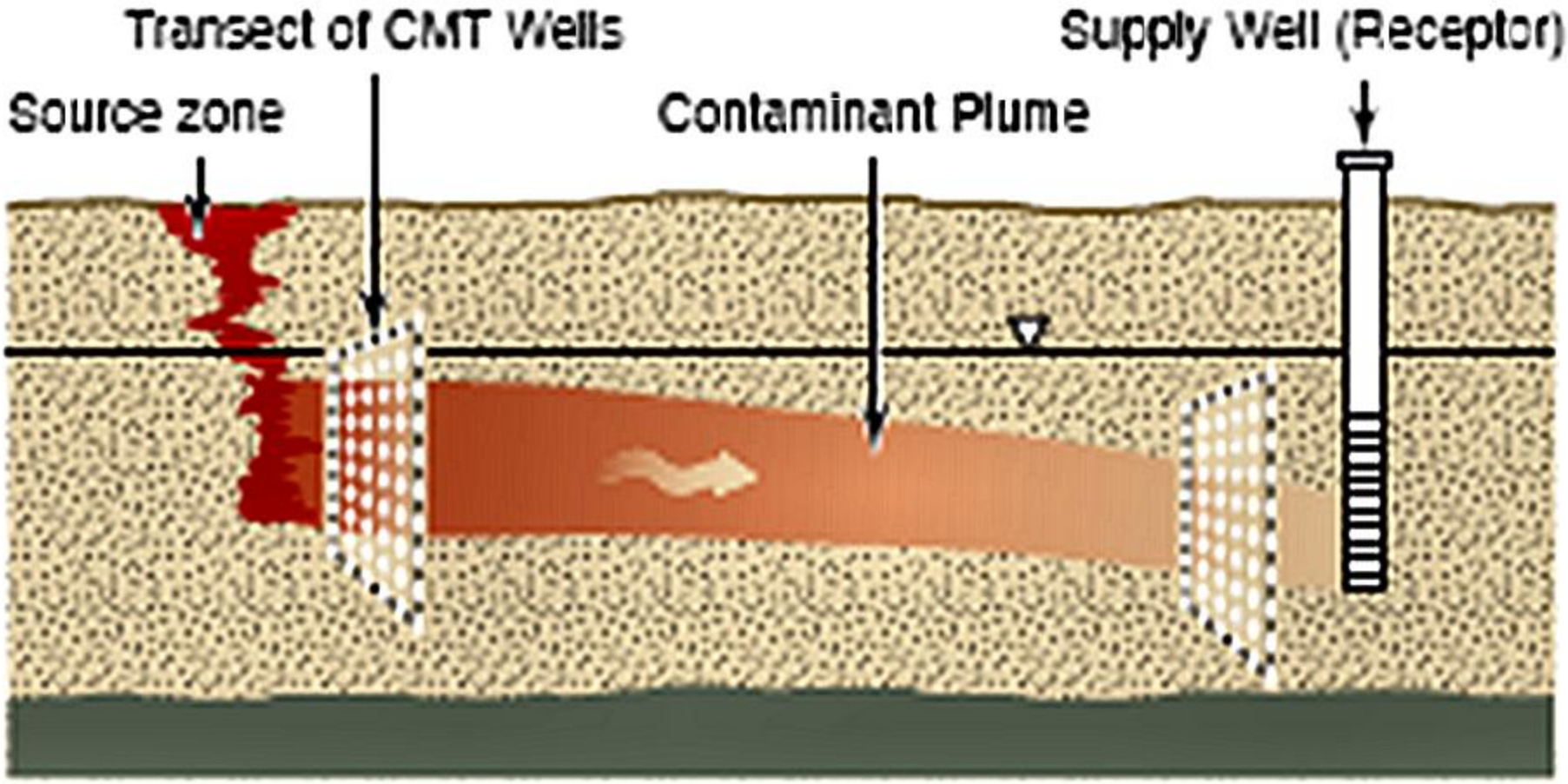
Alleen behandeling van de pluim







Weer niet de bron maar de pluim



CMT Transects for Mass Flux Assessment

Voorstel tot saneren

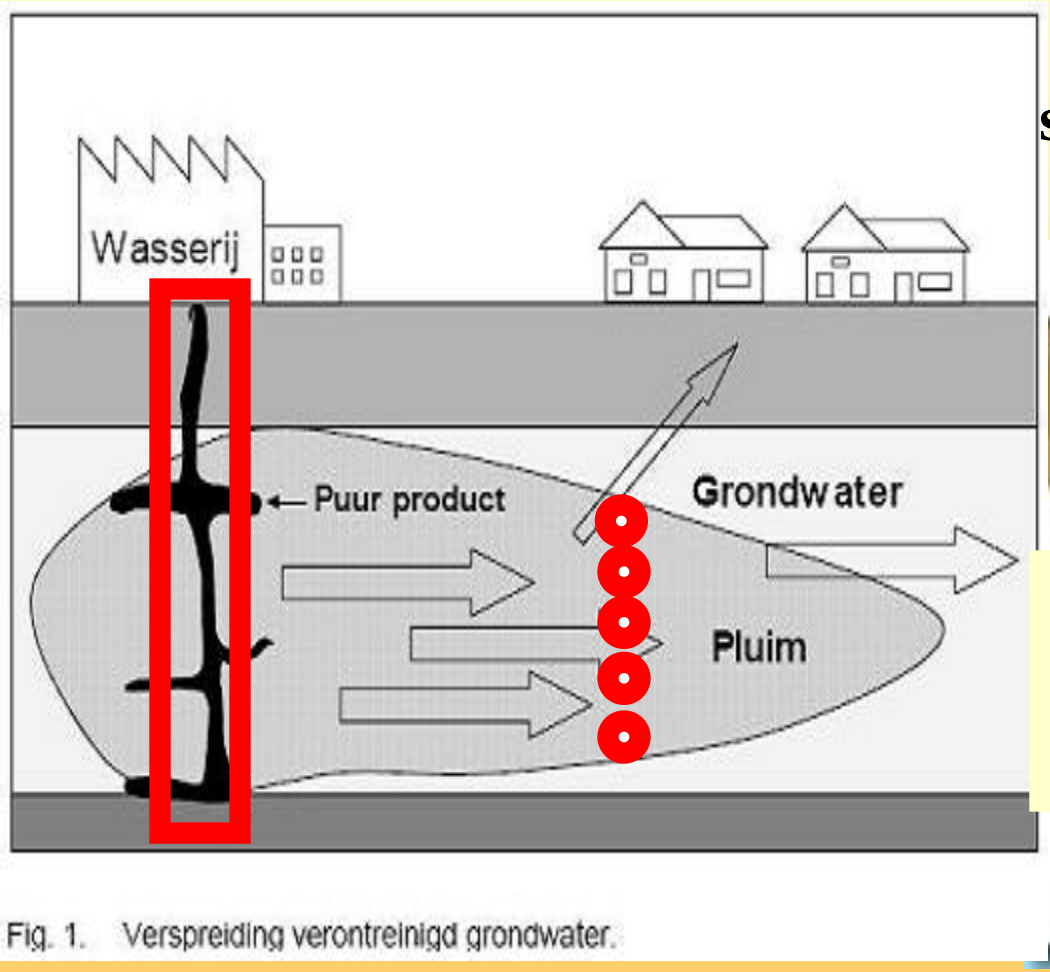
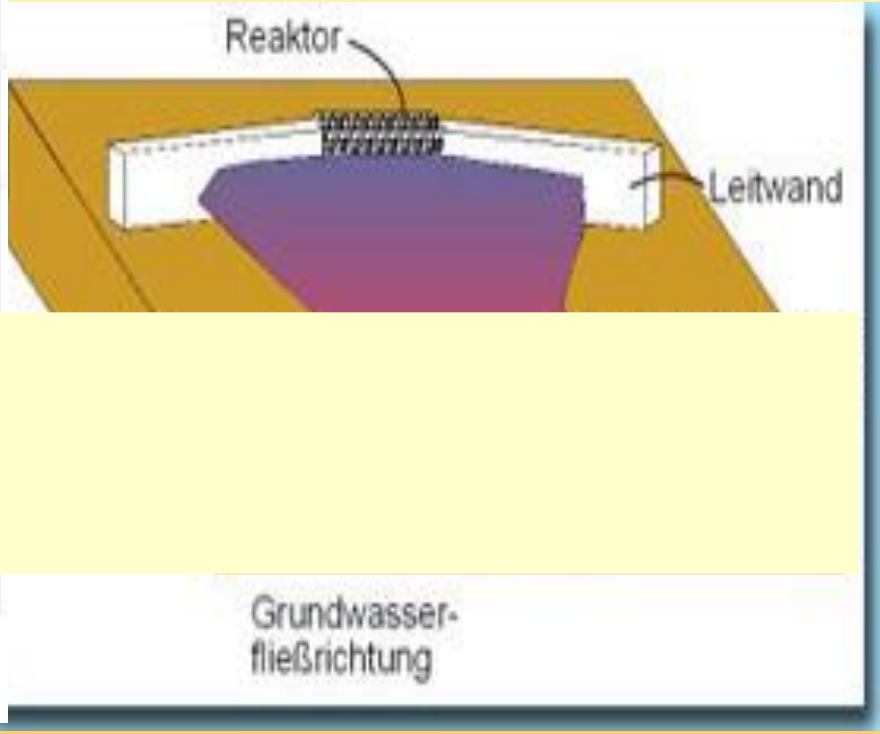


Fig. 1. Verspreiding verontreinigd grondwater.

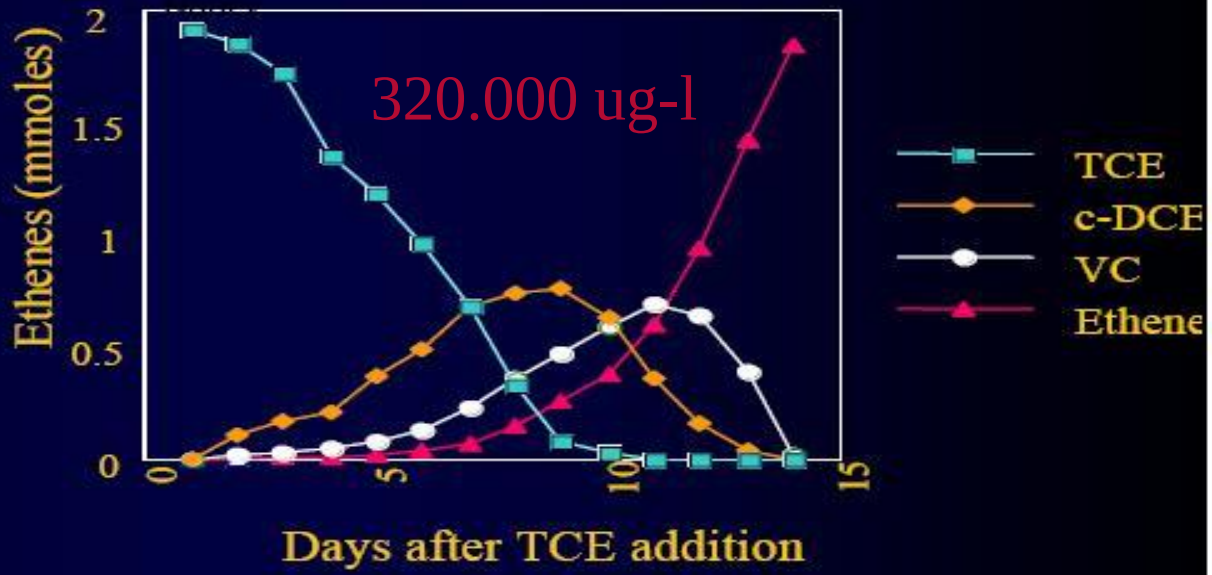
Geen duur scherm die de situatie jarenlang in standhoud omdat de bron weg is



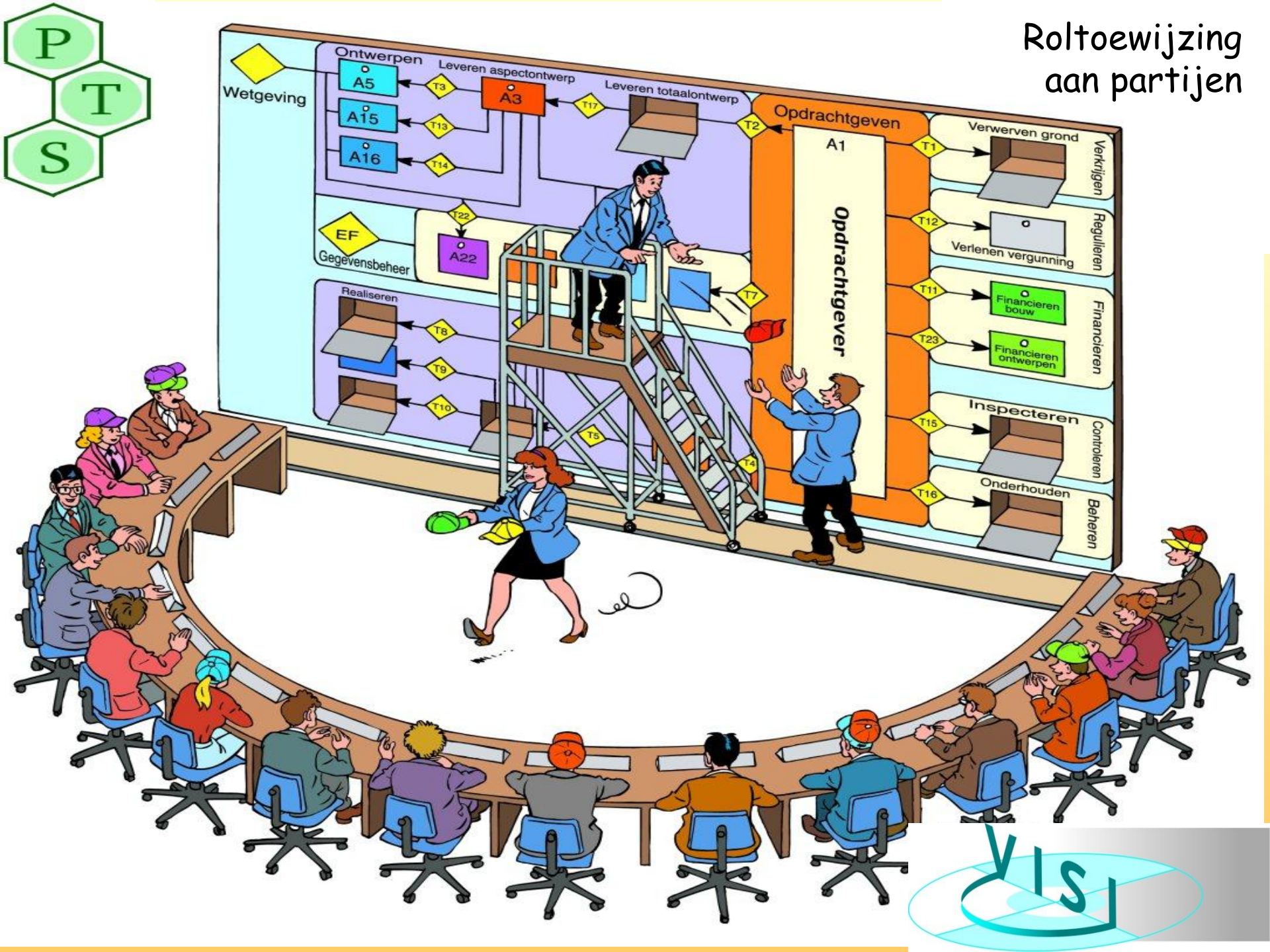
Geen lactaat maar acetaat en het gaat nog sneller

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

Dr. G.J.J. Beukeveld

Renoirhof 161, 1628 XC Hoorn

GJJ.Beukeveld@inter.nl.net

Secretariaat: Hotel zur Ems

Emsstraße 12

48499, Salzbergen (D)

webmaster@hotelzurems.de

Telefoon: 0049-59761011