Biological degradation of high concentrations of 1,4-dioxane

from laboratory to field and back

Martin Slooijer
CEO GreenSoil Group

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GreenSoil Group

Belgium
Thor Park 8300
3600 Genk

Belgium
Kerkstraat 106
9050 Gent

The Netherlands
Buitendijks 19
3356 LX Papendrecht

Brazil
Av. Brigadeiro Faria Lima 1572
01451001 São Paulo

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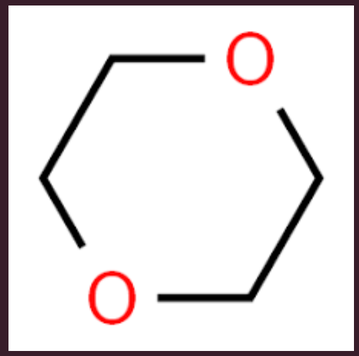
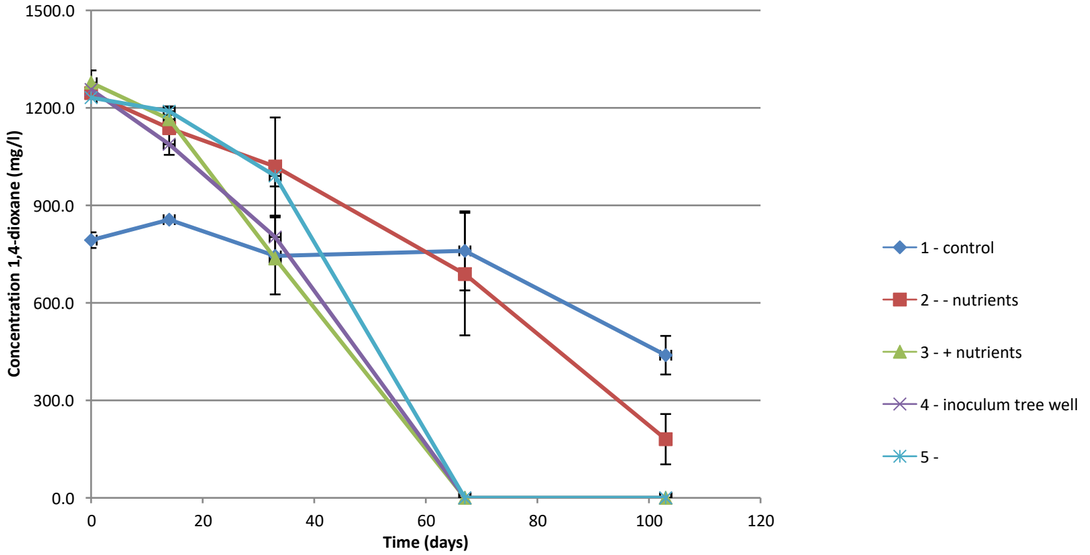
The project



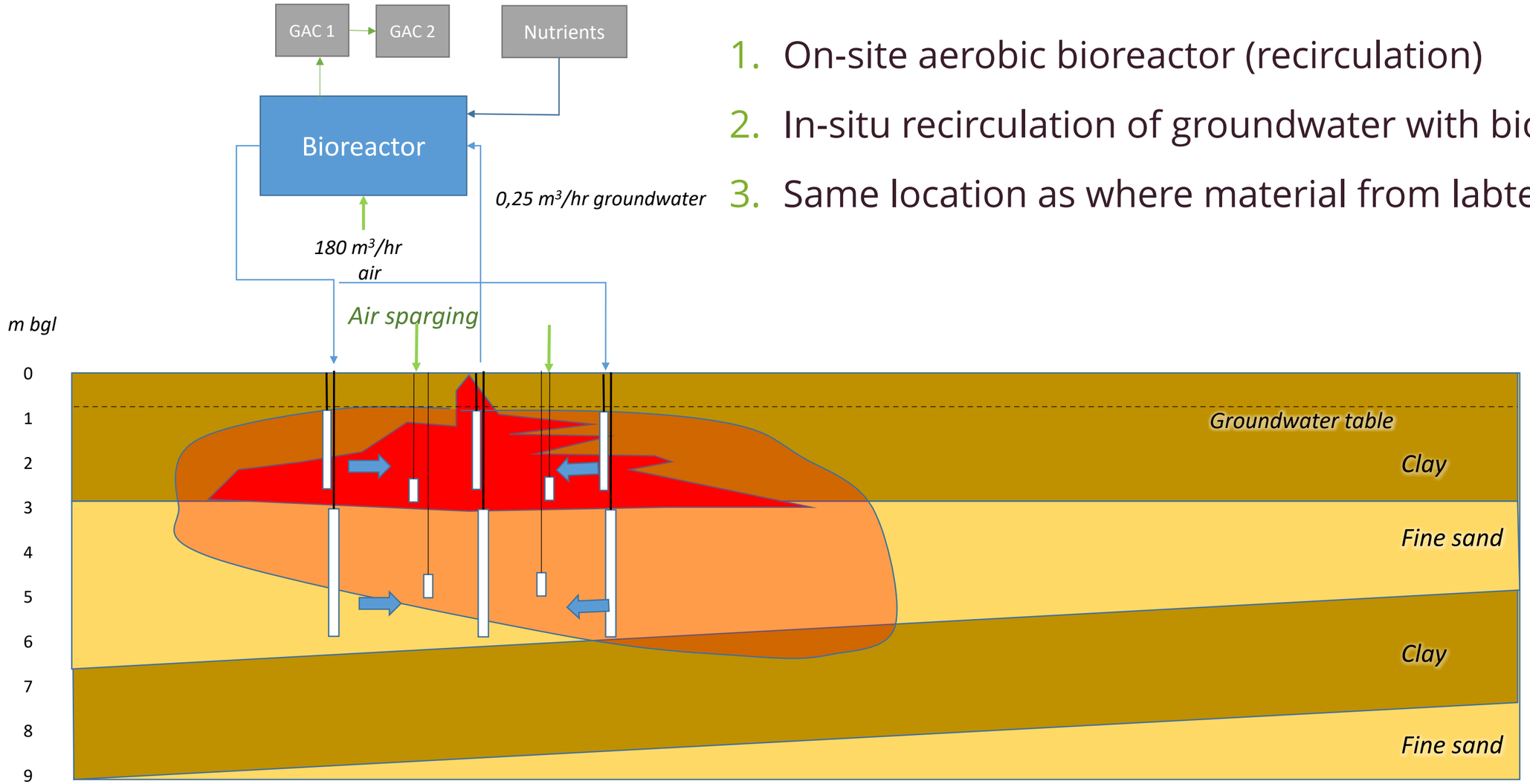
- Industrial site with high 1,4-dioxane contamination (> 500 mg/L)
- Successful feasibility test performed in 2017
- Field tests started in 2021: no clear signs of biodegradation for > 6 months
- Additional laboratory tests to gain more insight
- Parallel adaptation/optimalization of bioreactor

First laboratory test (2017)



- Performed with Deltares
- Slurry test (contaminated soil and groundwater)
- Complete degradation of 1,270 mg/l 1,4-dioxane in biostimulated conditions (oxygen + nutrients) in 40-50 days

Pilot : bioreactor + field (start 2021)



1. On-site aerobic bioreactor (recirculation)
2. In-situ recirculation of groundwater with biosparging
3. Same location as where material from labtest was obtained

The challenges

1. pH increase in bioreactor to ± pH 9
2. No clear signs of biodegradation during first 6 months

(Possible) explanations

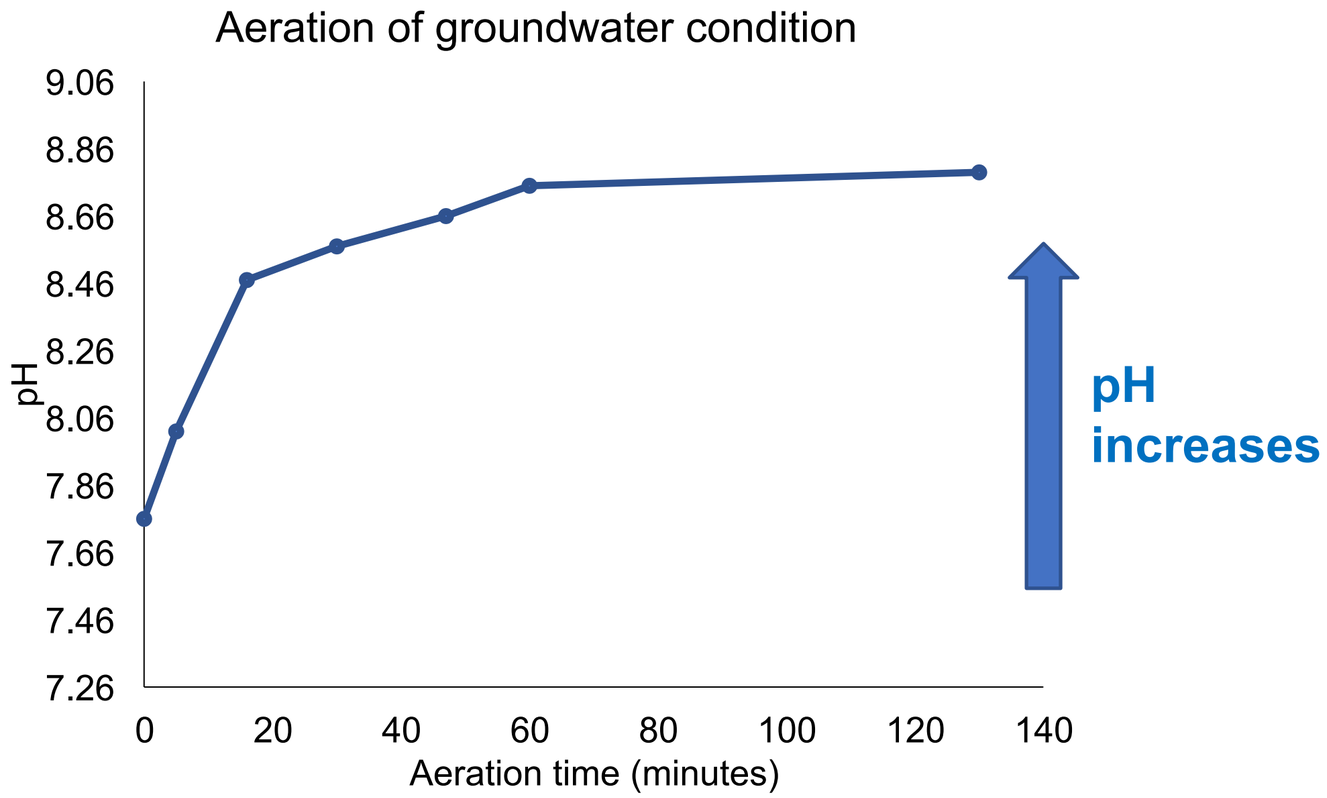
1. pH increase in bioreactor to ± pH 9

pH increase due to aeration (CO_2 stripping)

2. No clear signs of biodegradation during first 6 months

Due to pH? Presence of soil required?

Simulation pH increase in laboratory



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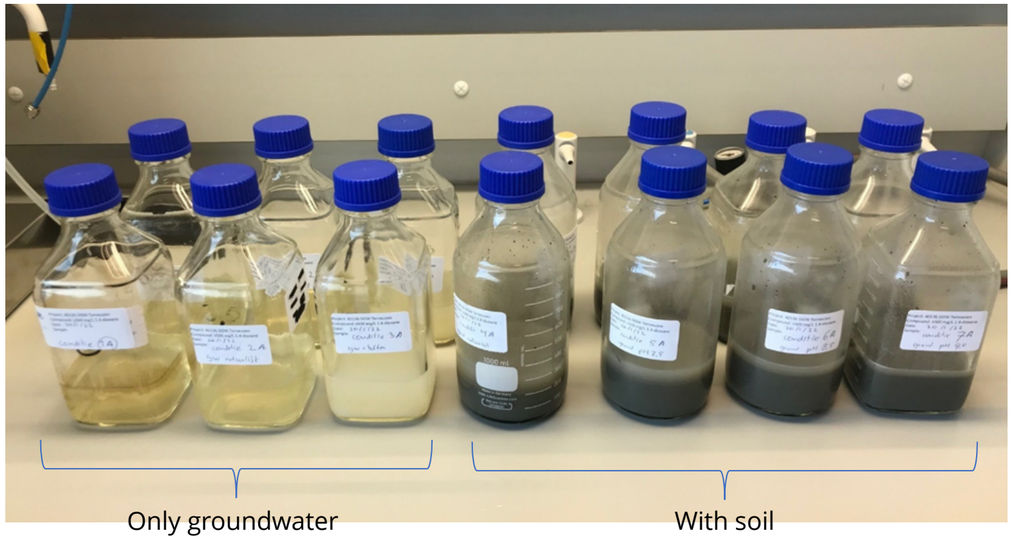
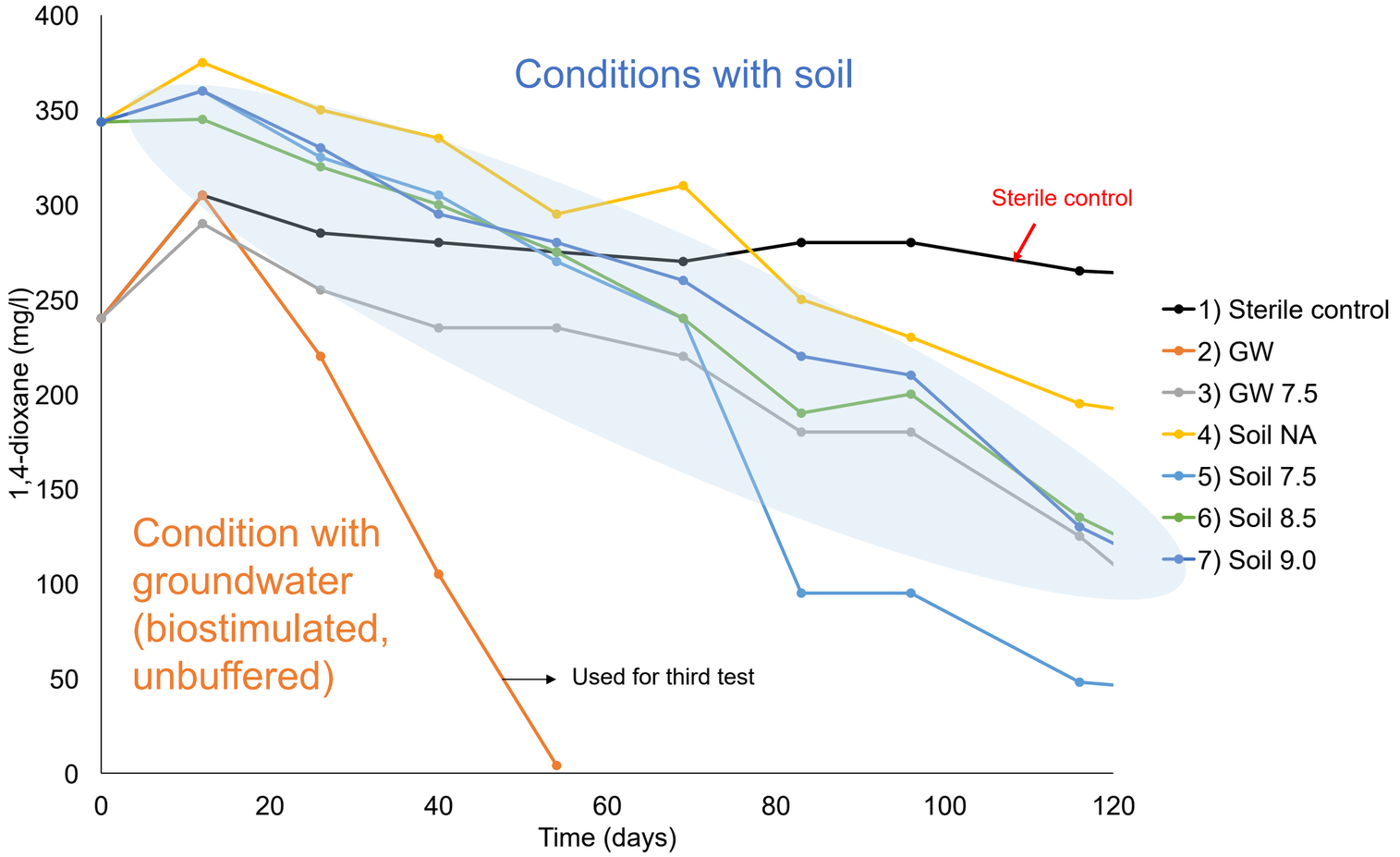
Second laboratory degradation test (2021 – 2022)

- Soil required for 1,4-dioxane degradation?
- High pH inhibiting for biodegradation?
- More insight into degradation process and enzymes/microorganisms involved

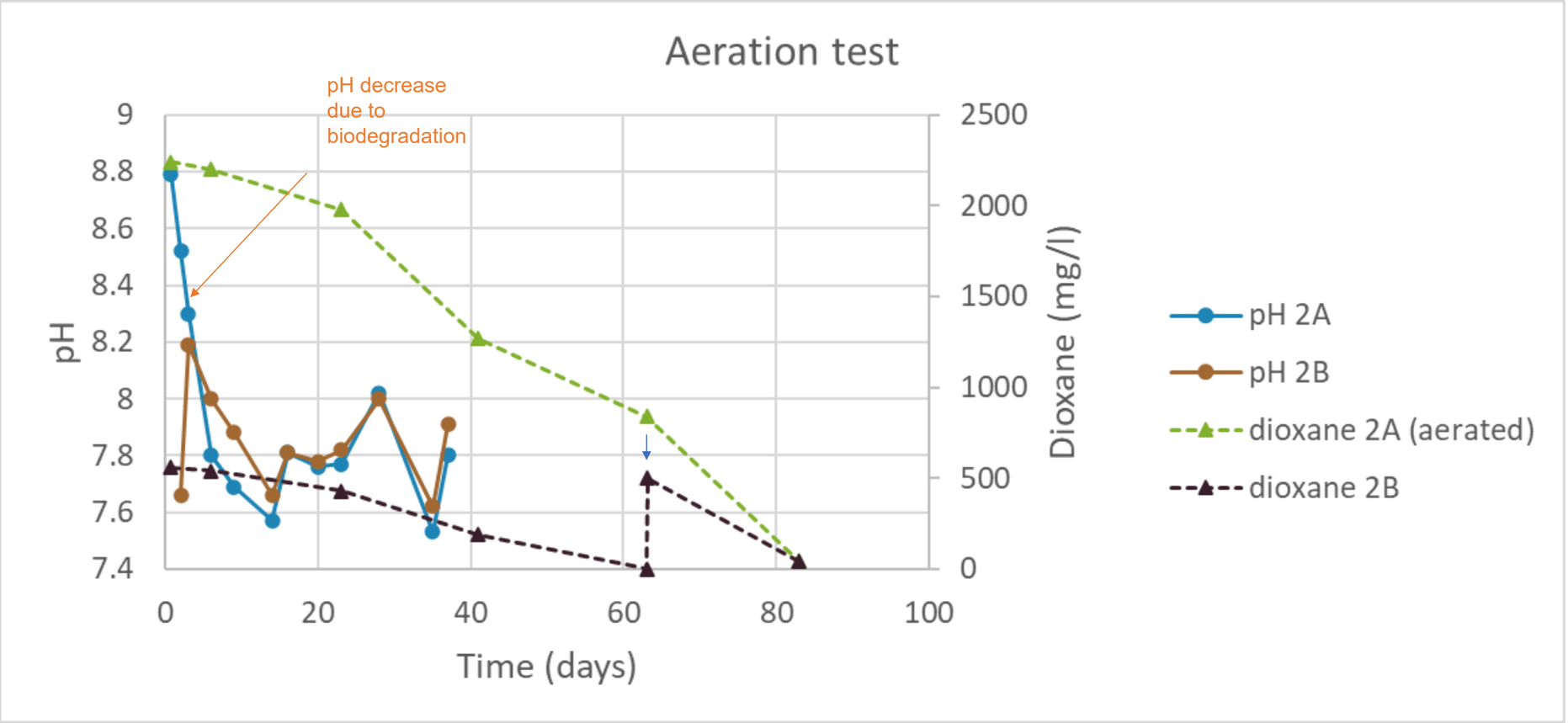


Second lab test : influence soil



Second lab test : Degradation + aeration

Active aeration (20 min) at t=0



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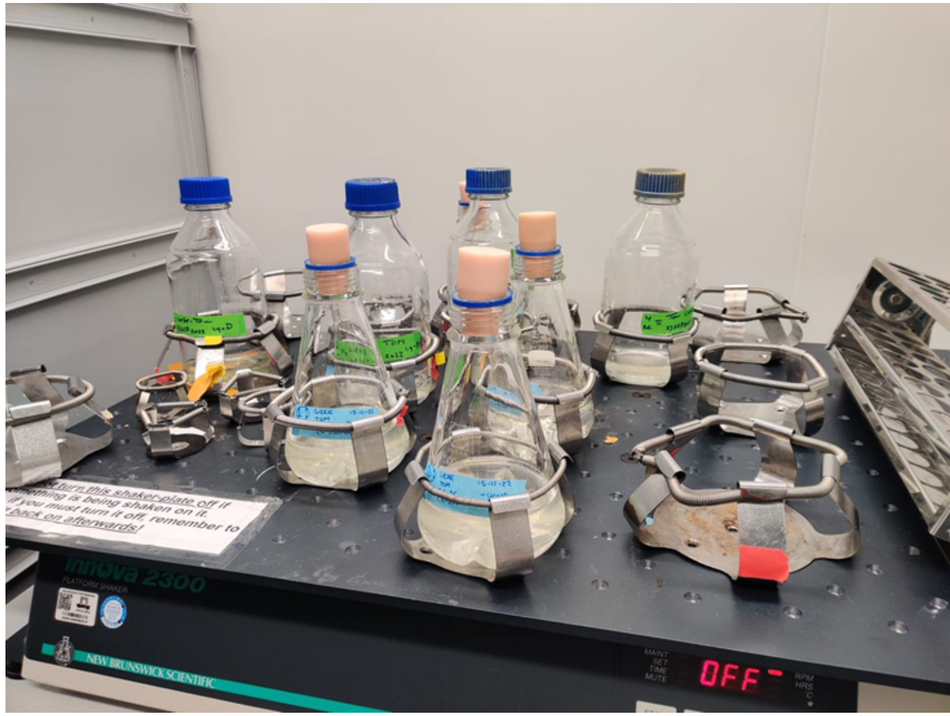
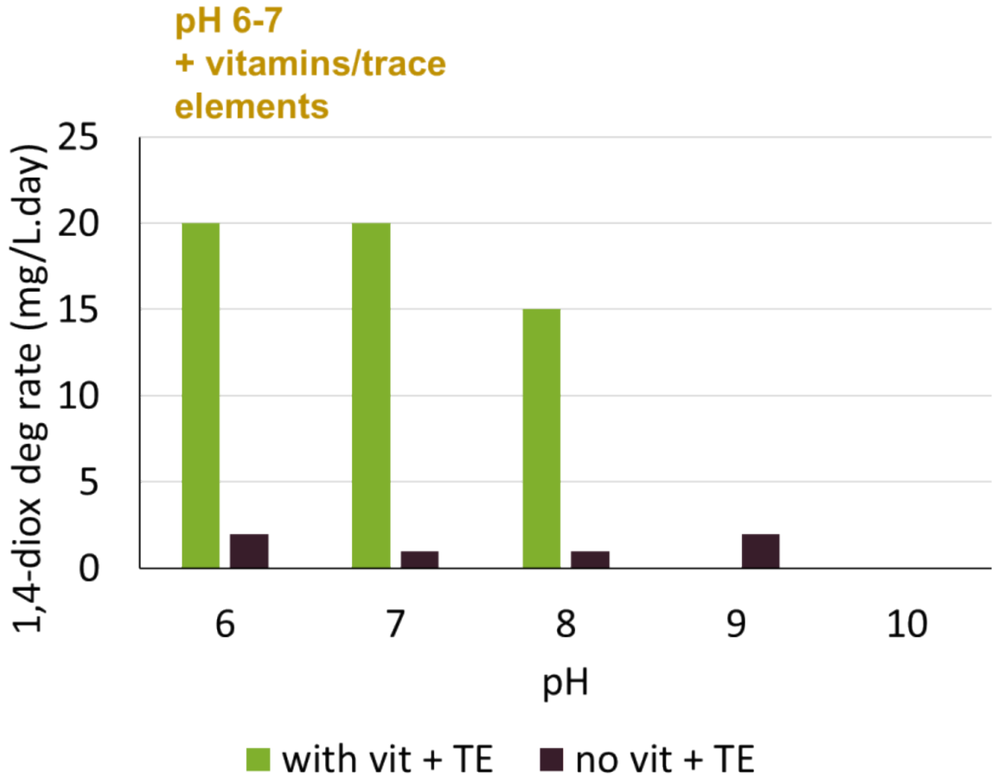
Additional labtest done Wageningen University (WUR)

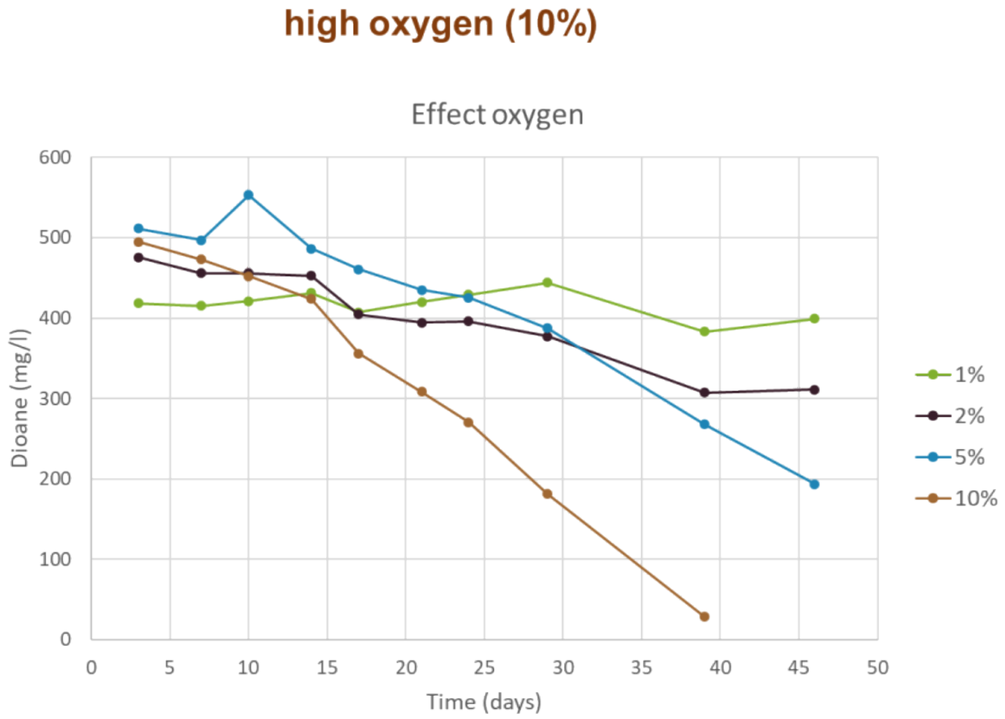
- Further investigate effect pH on biodegradation and microbial community
- Get further insight into degradation process -> develop qPCR targets to follow up biodegradation in the field



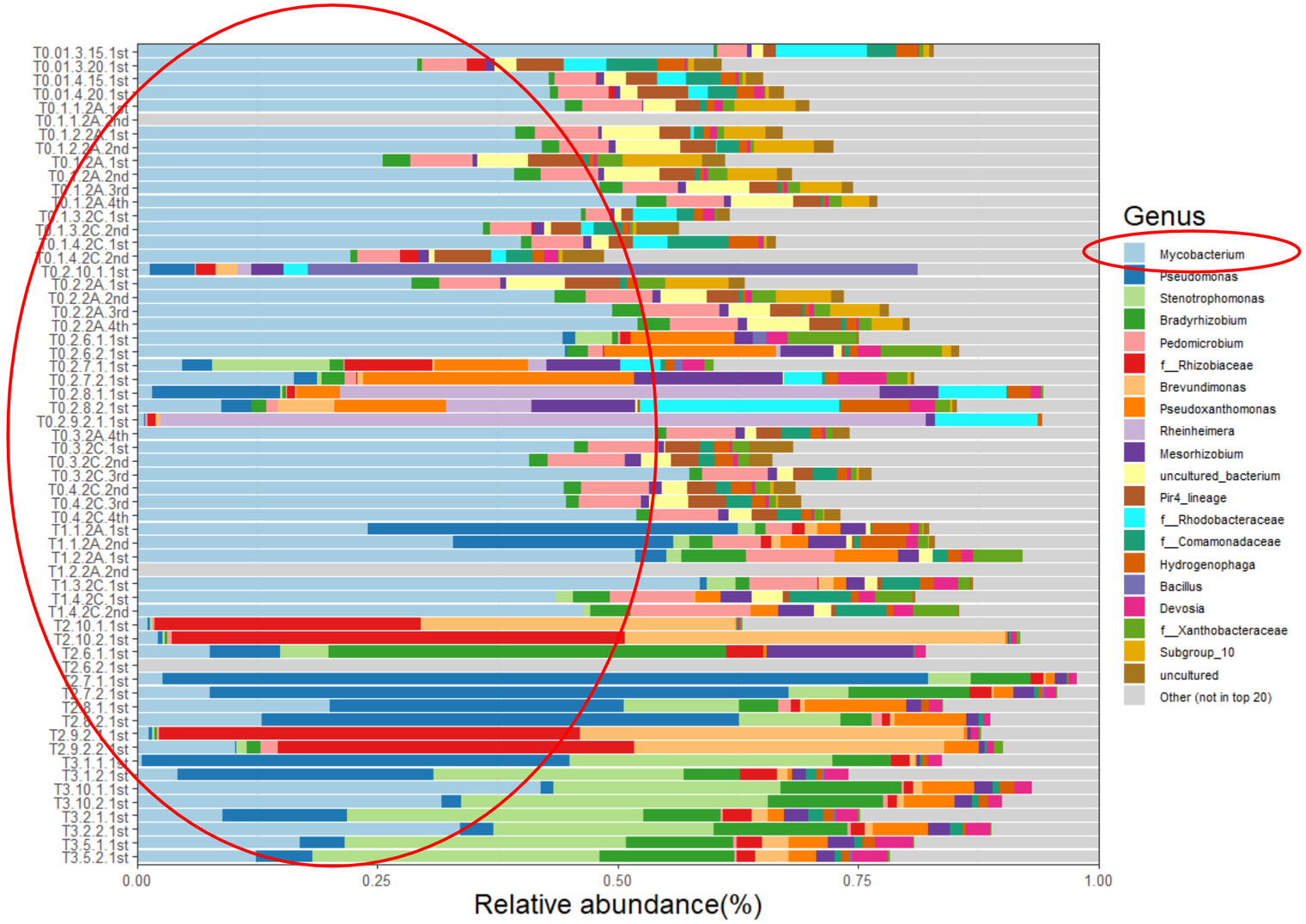
WUR micorcosms

Determine ideal conditions and limitations: pH, vitamins and trace elements, oxygen





Molecular works



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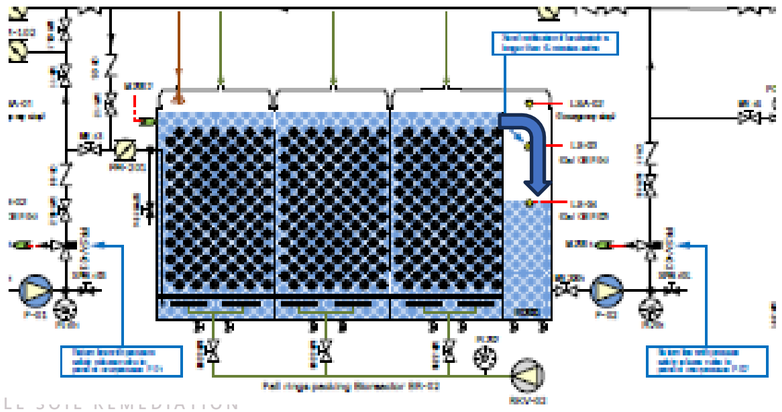
Conclusions second labtest

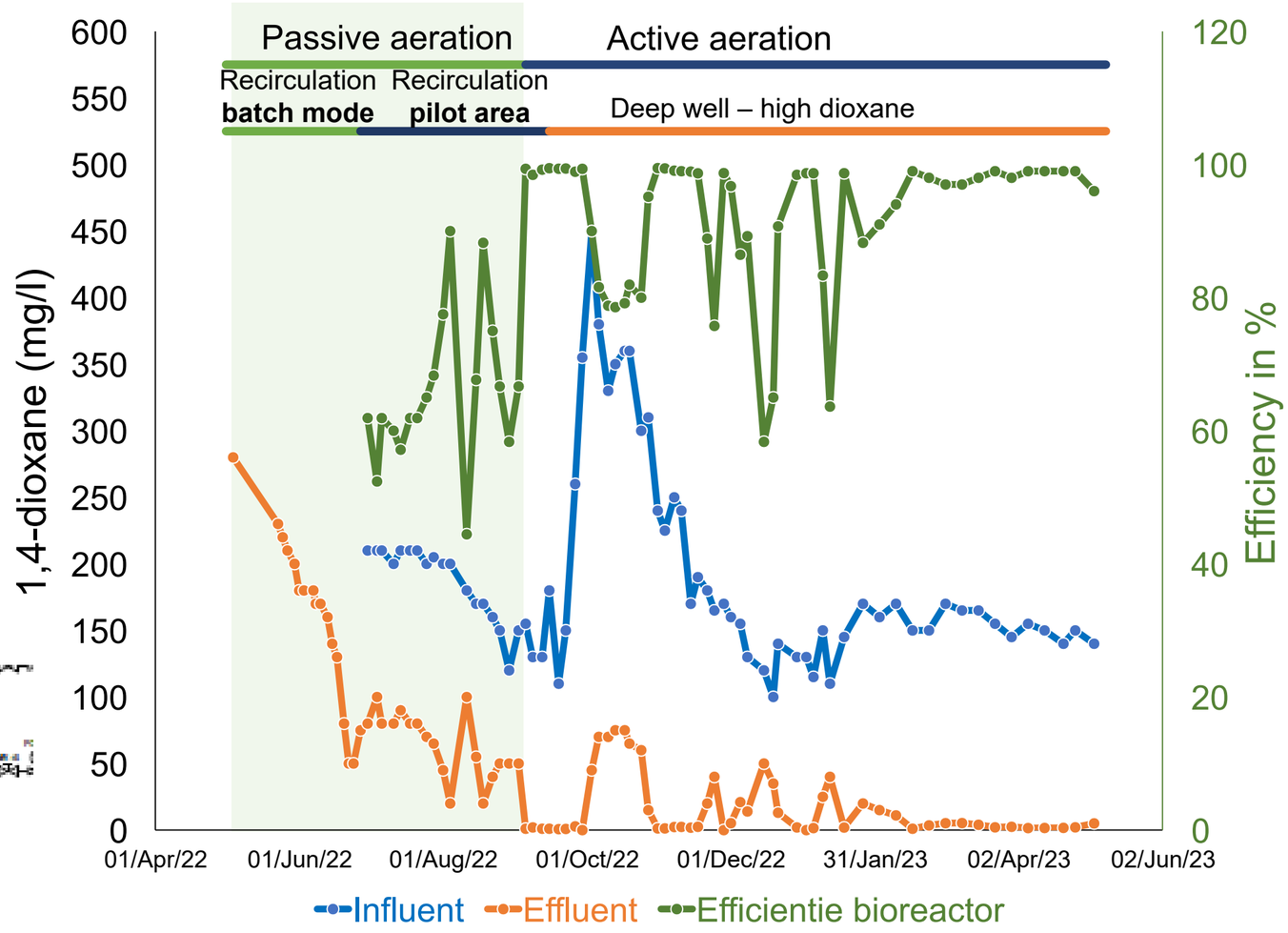
- pH increase due to stripping (CO2)
- No soil required for biodegradation
- No clear negative effect of high (up to 9) pH in presence of soil
- Lower degradation rate at higher pHs in water only cultures
- Short-term high pH not affecting biodegradation
- Once active: pH ↓ (likely due to CO2-production)
- Mycobacterium most likely dioxane degrading candidate

Pilot installation : Bioreactor

1. Recirculation batch mode
2. Passive aeration

➢ **Establish 1,4-dioxane biodegradation without increasing pH**





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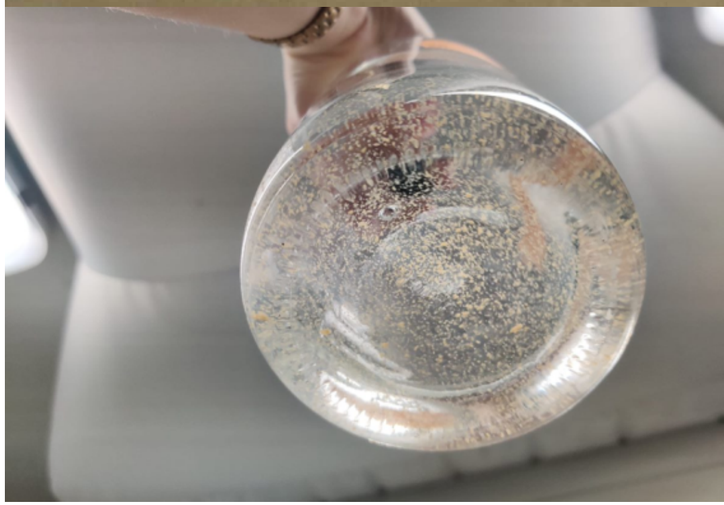
Biomass

Biomass observations:

- Clear water + flocs
- Effluent bioreactor also very clear
- No clear biomass attached on carrier material bioreactor (present as non-attached flocs?)
- Low yield of biomass



Results In-situ Pilot

- Soil Samples show:
  - Layer 1.5-2.0 average reduction **99%**
  - Layer 2.5-3.0 average reduction **73%**
- Groundwater Samples show:
  - Average reduction of **96%**
- Pilot System / Bioreactor has effectively worked well since September 2022.

	Before Pilot				In Pilot Area									
Point	7662	7663	7664	Ave.	14810	14811	14812	14813	14814	14816	14817	14819	Ave.	Decr.
Year	2021	2021	2021		2024	2024	2024	2024	2024	2024	2024	2024		
Layer														
1,0-1,5	104	43	10	52										
1,5-2,0	336	409	313	353	12	1,2	3,6	1,5	3,3	2,4	6,7	2,3	4	**99%**
2,0-2,5	381	405	506	431										
2,5-3,0	1154	399	485	679	207	146	255	167	305	324	15	33	182	**73%**

Groundwater Shallow Layer				
Point	Date	Value	% Decrease periode	% Decrease total
Average	June-21	930		
Average	June-22	560	39,78%	**39,78%**
Average	June-23	147	73,81%	**84,23%**
Average	April-24	37	74,55%	**95,99%**

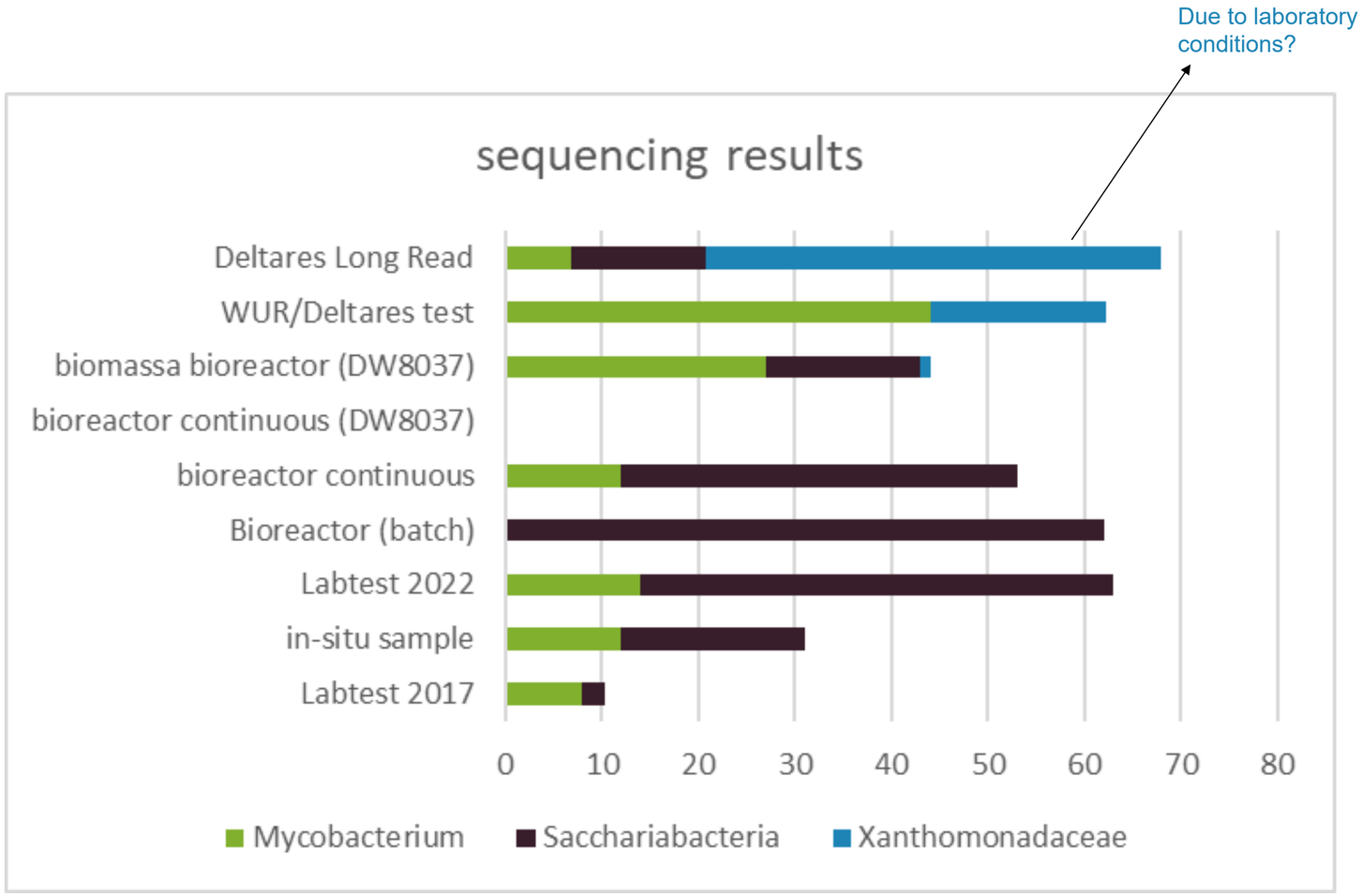
Combination of all tests

- Degradation rates

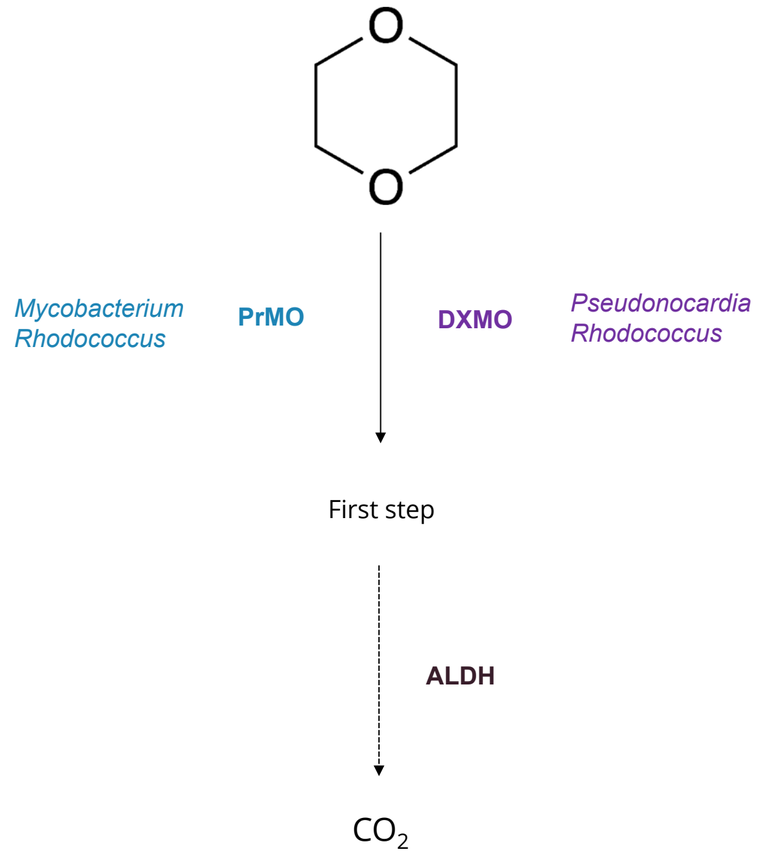
Test/system	Estimated degradation rate (mg/l.day)
First labtest 2017	29-49
Second labtest 2021	10-23
Labtest WUR	8-47 (avg 20)
Bioreactor	Max 69.7

Combination of all tests

- Sequencing results



Combination of all tests



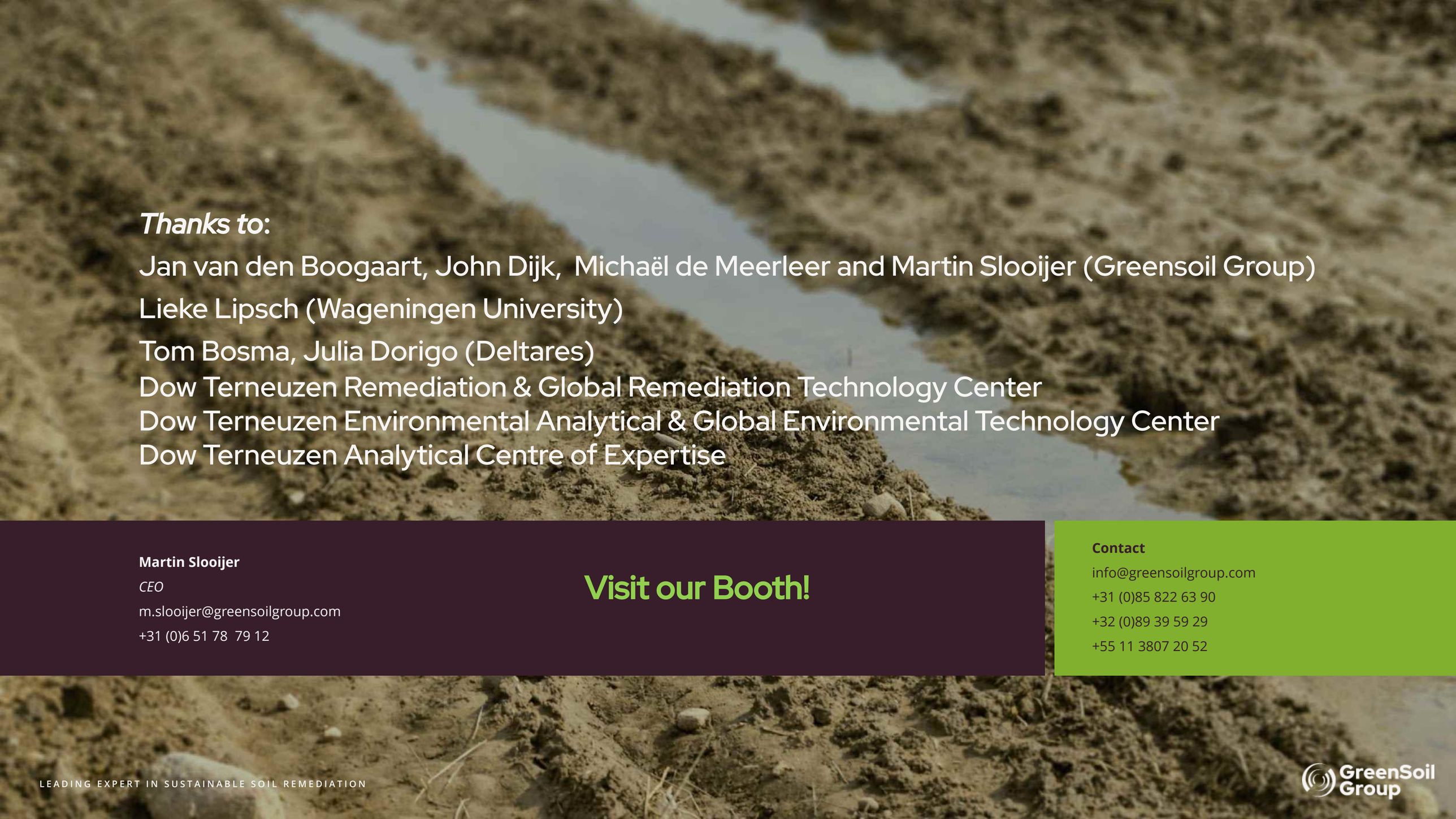
- Genes encoding for key enzymes

Enzyme	Labtest 2017	Field/labtest 2022	Labtest 2023
		Gene copies/ml	
DXMO	10^4	-	10^3
ALDH	10^4	10^1	10^4
PMO	?	?	10^4
SDIMO	?	-	

- Likely low biomass yield + floc forming (heterogeneity) caused unrepresentative DNA samples/low reads
- Different microbial community in laboratory?

Conclusions

- Degradation 1,4-dioxane more complex than initially thought
- Stable process once active, but difficult to start-up (long lag-phase)
- With knowledge from labtests, a stable bioreactor with >99% efficiency was established
- Degradation pathway/mo’s involved still being investigated



***Thanks to*:**

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Dow Terneuzen Remediation & Global Remediation Technology Center

Dow Terneuzen Environmental Analytical & Global Environmental Technology Center

Dow Terneuzen Analytical Centre of Expertise

Martin Slooijer

CEO

m.slooijer@greensoilgroup.com

+31 (0)6 51 78 79 12

Visit our Booth!

Contact

info@greensoilgroup.com

+31 (0)85 822 63 90

+32 (0)89 39 59 29

+55 11 3807 20 52

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