



UNIVERSITY OF AMSTERDAM

# Implications of microbial adaptation for the persistency assessment of organic chemicals

**Baptiste A.J. Poursat**



# CEFIC-LRI ECO 29

Application of chemostat systems to include adaptation of microbial communities  
in persistency testing (CHEMADAPT)

2015-2019



UNIVERSITY OF AMSTERDAM



Dr. John Parsons  
Prof. Pim de Voogt  
Joost Dalmijn  
Rick Helmus

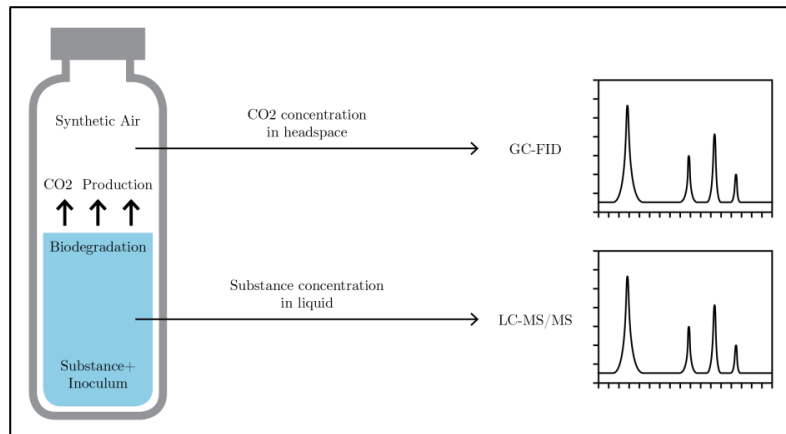
Dr. Wilfred Röling  
Dr. Rob van Spanning  
Martin Braster

**Research liaison team:**  
Graham Whale (Shell)  
Delina Lyon (Shell)  
David Saunders (Shell)  
Kees van Ginkel (Nouryon)  
Kathleen McDonough (P&G)  
Jason Snape (AstraZeneca)  
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Lucy Wilmot (ECETOC)

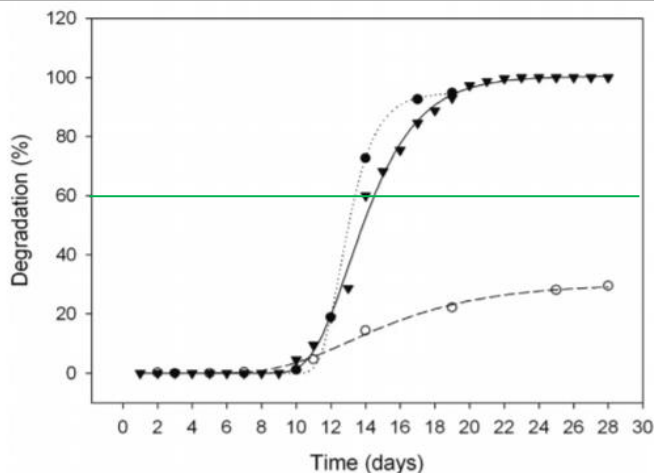
## Ready biodegradability tests (RBT)

### REACH- Annex VII 1-10 t/year

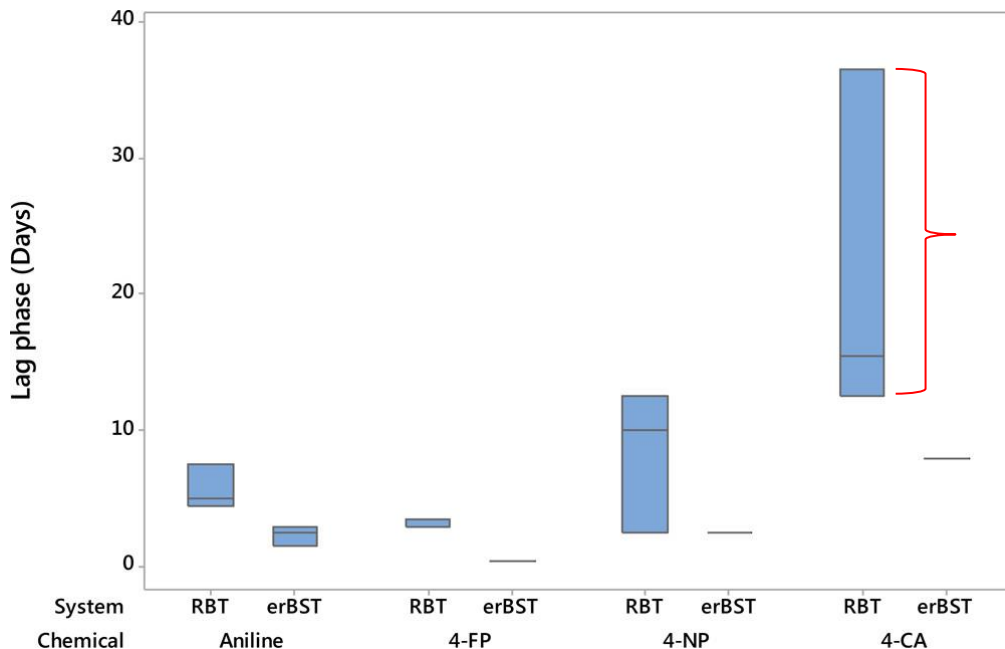
- Short term toxicity on freshwater aquatic invertebrate (Daphnids) (OECD 202)
- Alga or Lemna sp growth inhibition test (OECD 201, 221)
- **Ready Biodegradability (OECD 301, 310)**
- Water solubility (OECD 105) & partition coefficient (n- octanol/water) (OECD 107, 117)



Ready biodegradable chemicals are expected to be quickly degraded in **most aerobic aquatic environment**



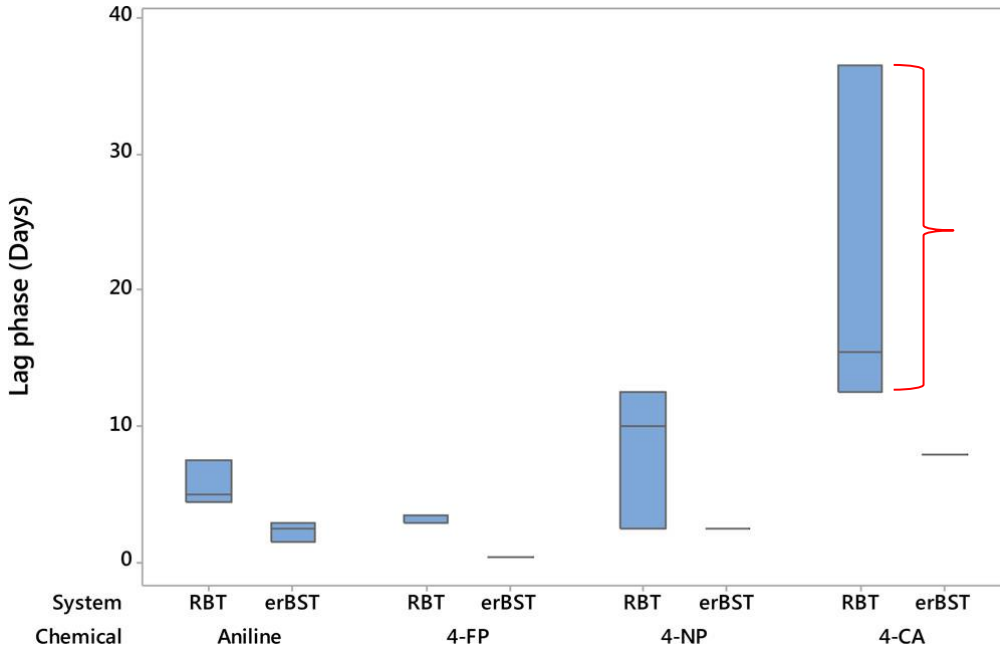
## OECD RBTs results can be highly variable



Boxplots indicating the range of lag phase durations for triplicate biodegradation tests, in erBSTs (erBST) and OECD RBTs (RBT) for a group of chemicals with varying environmental persistence.

From **Martin et al (2017); Environ. Sci. Technol. 2017, 51, 5, 3065-3073**

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Some of the proposed solutions are to:

Increase the inoculum biomass

Increase test volume

**Pre-expose the inoculum to the test compound**

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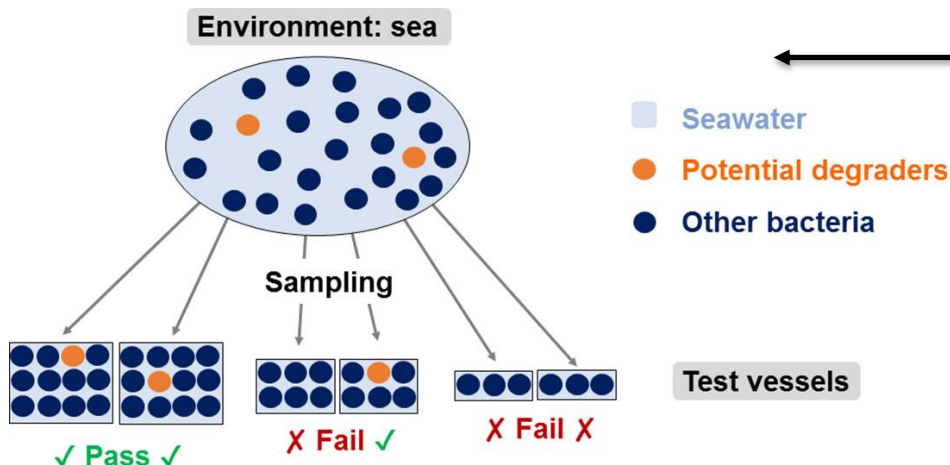
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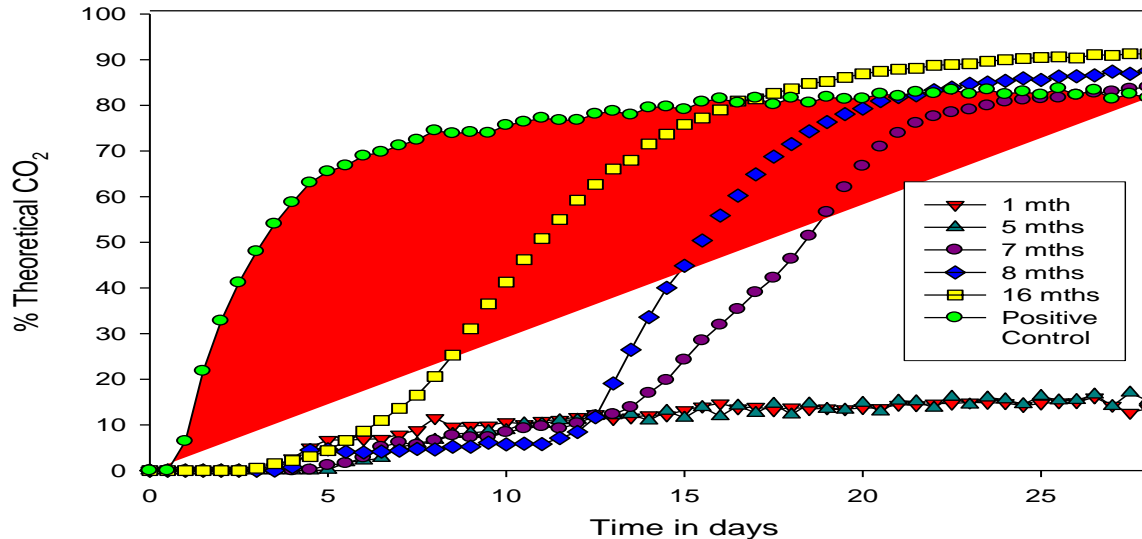


Graphical abstract from Ott et al (2019): Improving the biodegradability in seawater test (OECD 306); Science of the total env.

[doi.org/10.1016/j.scitotenv.2019.02.167](https://doi.org/10.1016/j.scitotenv.2019.02.167)

# Microbial adaptation can influence the test results

## Biodegradation of L-GLDA in RBT shifts with exposure time



Biodegradation by activated sludge as a function of time following initial shipment of product with L-GLDA. Itrich et al. (2015) *Environ. Sci. Technol.* 49, 13314-13321.

Natural microbial exposure to organic pollutants may increase the test results variability

# How can we include microbial adaptation in RBTs to reduce variability?

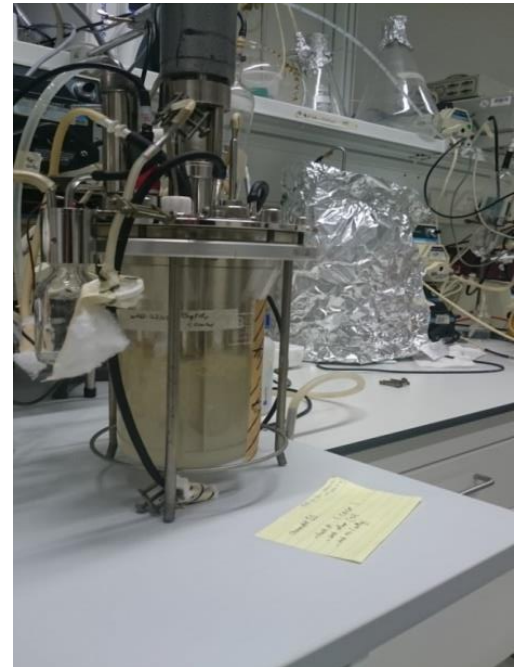
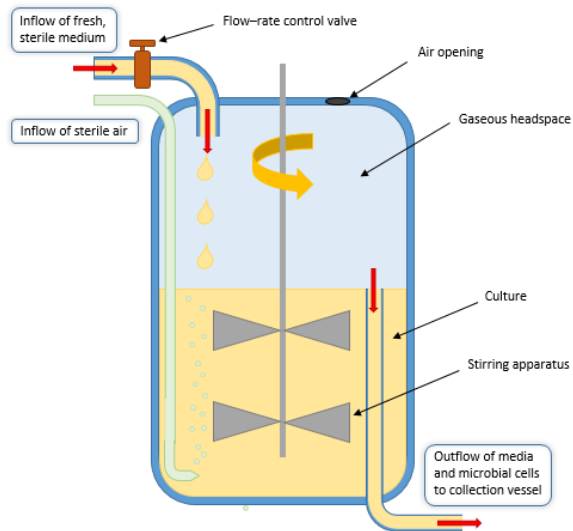
## **Objectives:**

1. Review the literature on microbial adaptation and its implications for RBTs
2. Investigate the effect of long-term exposure to an organic pollutant on:
  - The inoculum
  - Ready biodegradability tests results
3. Propose a modified ready biodegradability test to include adaptation



# Exposure to the test chemical in **batch** (days) and **chemostat systems** (months)

Does long term exposure in chemostat lead to adaptation and reduce RBTs variability?



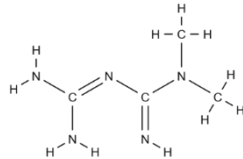
# Experimental protocol

Inoculum: Activated sludge

RBT: OECD 310

Primary biodegradation + transformation products: LC-MS/MS (Qtrap)

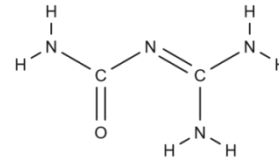
Community profiling by 16S rRNA gene amplicon sequencing



**Metformin**

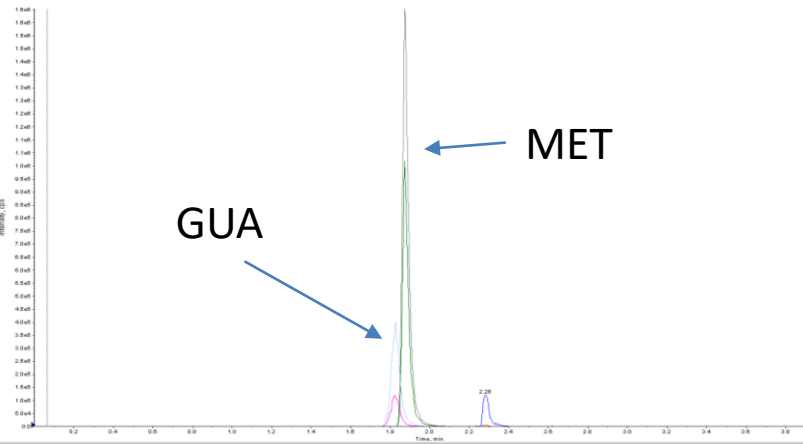
**Inherently biodegradable**

**Biotransformed**



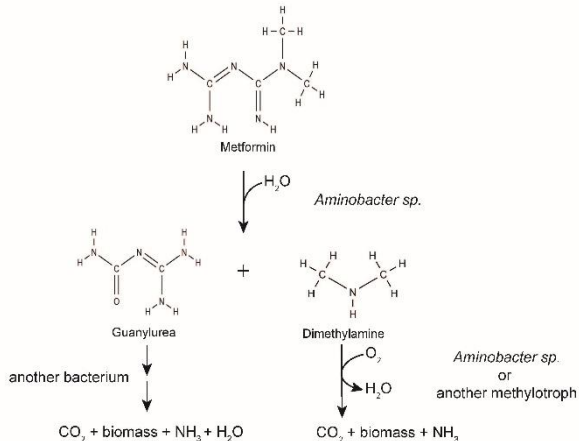
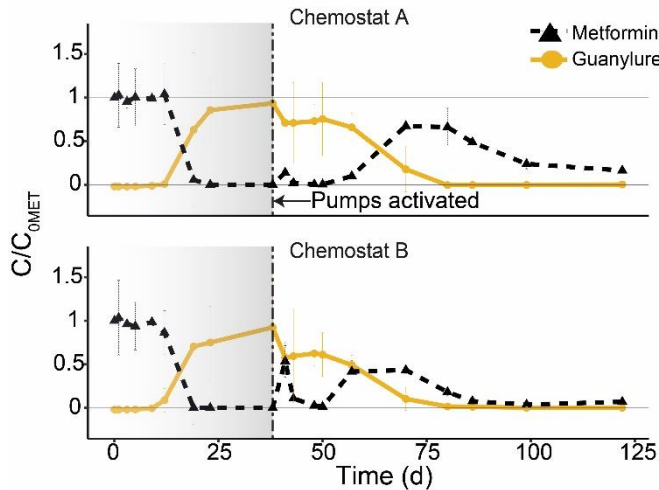
**Guanylurea**

**Persistent**





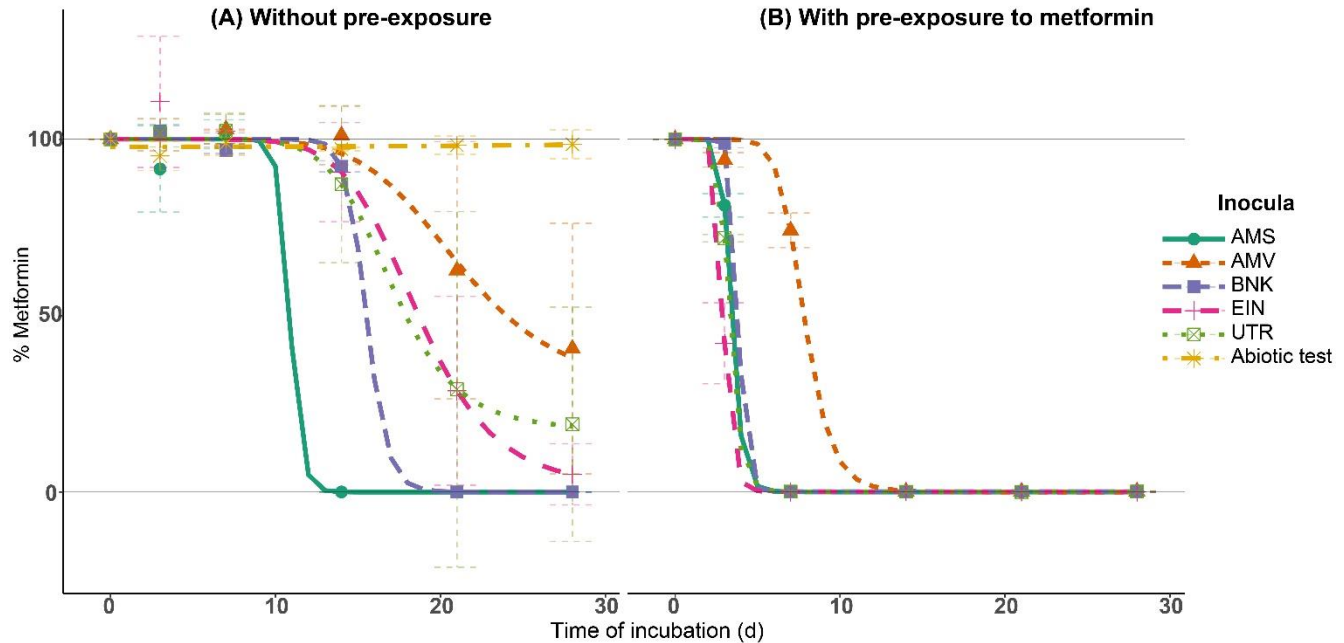
# Metformin and guanyurea can be degraded in chemostat



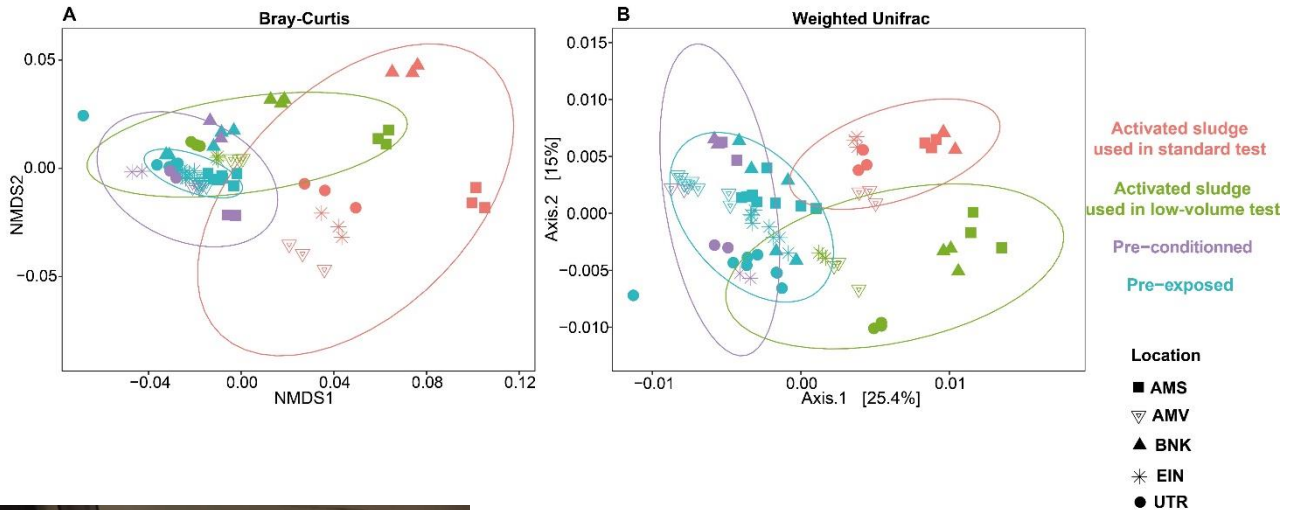
Guanyurea can be degraded while it was expected to be persistent

A metformin degrader (*aminobacter* sp.) was isolated from the chemostat community

# Short pre-exposure in batch can reduce test variability

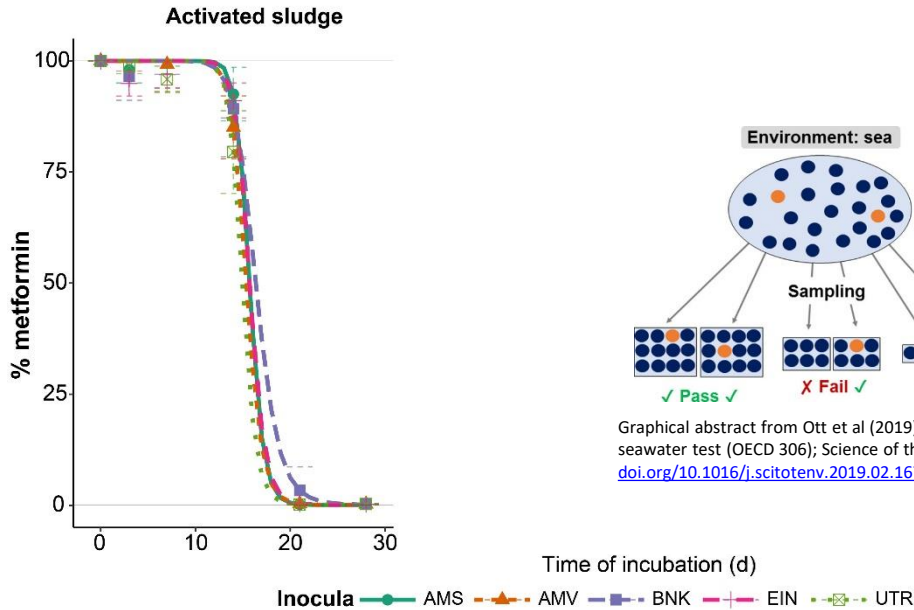


# Inocula were different based on their location of sampling

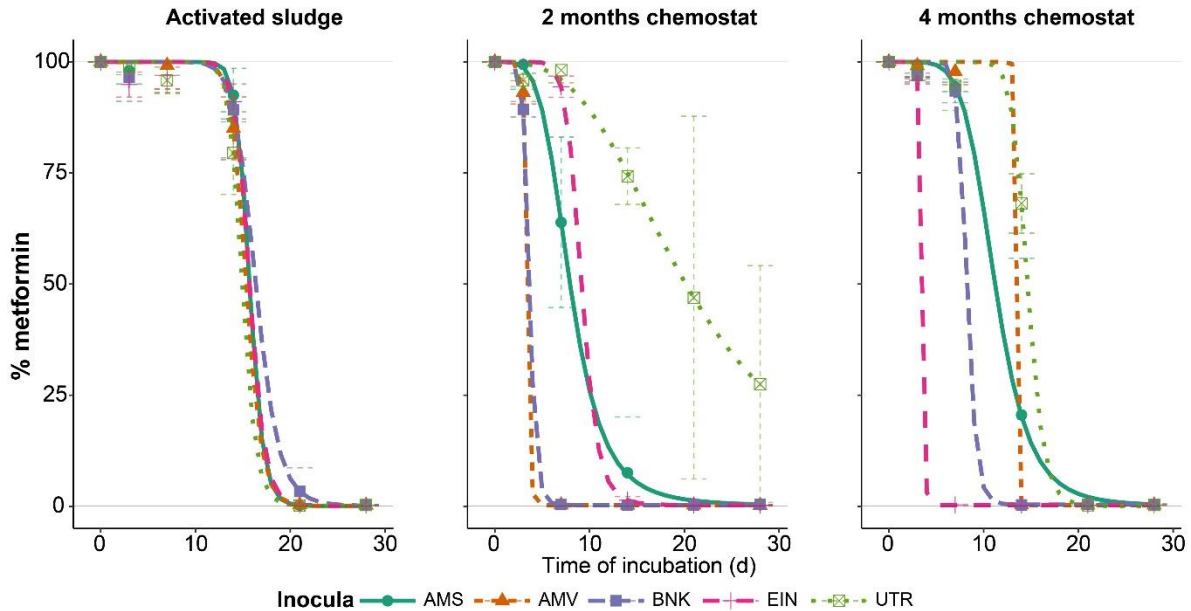


Increased variability in lower volume test

# Increasing the test volume increased the absolute number of degrading microorganisms

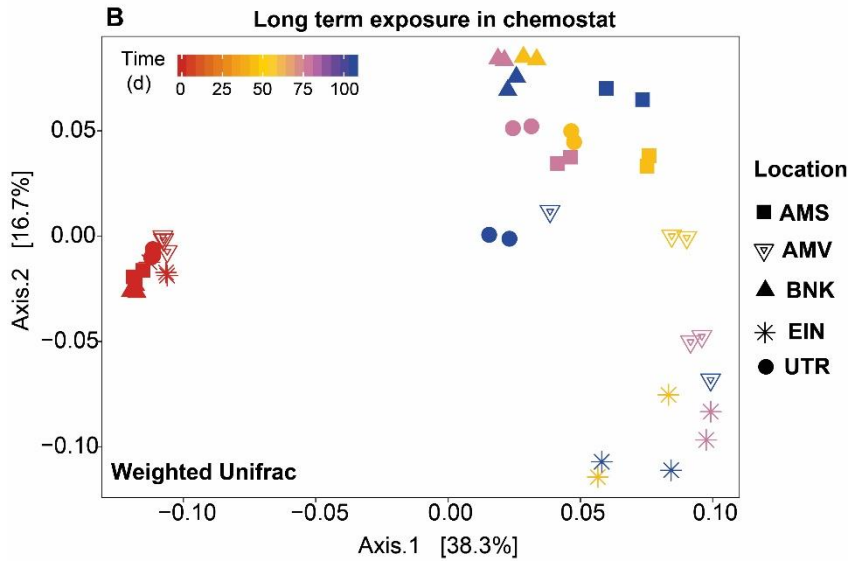


# Long term exposure in chemostat can induce variability in the RBT results





# Long term exposure in chemostat can induce variability in the RBT results



**Chemostat culture changed the communities composition and activity**

No correlation between the abundance of *aminobacter* sp. and the enriched bacteria (differential abundance analysis) with the biodegradation results

# Conclusion

Results of biodegradability tests may depend on:

- Source and exposure history of the inoculum
- Volume of the test

Long-term exposure in chemostat cultures may

- Enhance removal of some chemicals
- Lead to more variation in tests results

16s rRNA gene amplicon sequencing does not allow to identify adaptation mechanisms

- Further research must be conducted before to include adaptation in RBTs
  - We do not recommend to use chemostat systems to expose the inoculum before RBTs

# Thank you

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Baptiste.Poursat@wur.nl  
Poursat.baptiste@gmail.com