

Does long term exposure leads to biodegradation of Carbamazepine?

CEFIC-LRI Eco29 "Application of chemostat systems to include adaptation of microbial communities in persistency testing" (CHEMADAPT) (2015-2019)

Baptiste A.J. Poursat^{1,2}, Martin Braster², Rick Helmus¹, Wilfred F.M. Röling^{†2}, Rob J.M. van Spanning², Pim de Voogt¹, Hans V. Westerhoff², John R. Parsons¹

¹: Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands.
²: Department of Molecular Cell Biology, Faculty of Earth and Life Sciences, Vrije Universiteit, De Boelelaan 1108, 1081 HZ Amsterdam, The Netherlands.



Introduction

Persistence is a key property in assessing the environmental risks of organic chemicals. However, simple screening biodegradability tests are not suited to determining degradation rates under environmentally realistic conditions. The short exposure times do not allow for adaptation of microbial communities to a new chemical, while in the natural environment microorganisms tend to adapt to pollutants upon long term exposure, allowing for the development of efficient and fast degradation over time.

The aim of this project is to study the microbial adaptation to persistent chemicals. This will be achieved by using continuous culture systems, which will allow the development of guidelines for persistency tests in which the role of adaptation is accounted for.

Biodegradation of Carbamazepine

Our first tested chemical is a pharmaceutical product Carbamazepine (CBZ).

- The inoculum was a secondary sludge microbial community from a wastewater treatment plant (WWTP; Amsterdam West, The Netherlands), cultivated in chemostat and batch culture systems in presence of carbamazepine.
- CBZ concentration is measured with a LC-MS/MS (figure 2).
- Persistency of CBZ has been assessed in batch culture before exposure of the inoculum in chemostat using the biodegradation test OECD 301A (figure 3).

Carbamazepine
Formula : C₁₅H₁₂N₂O
Molecular mass : 236.3 g/mol

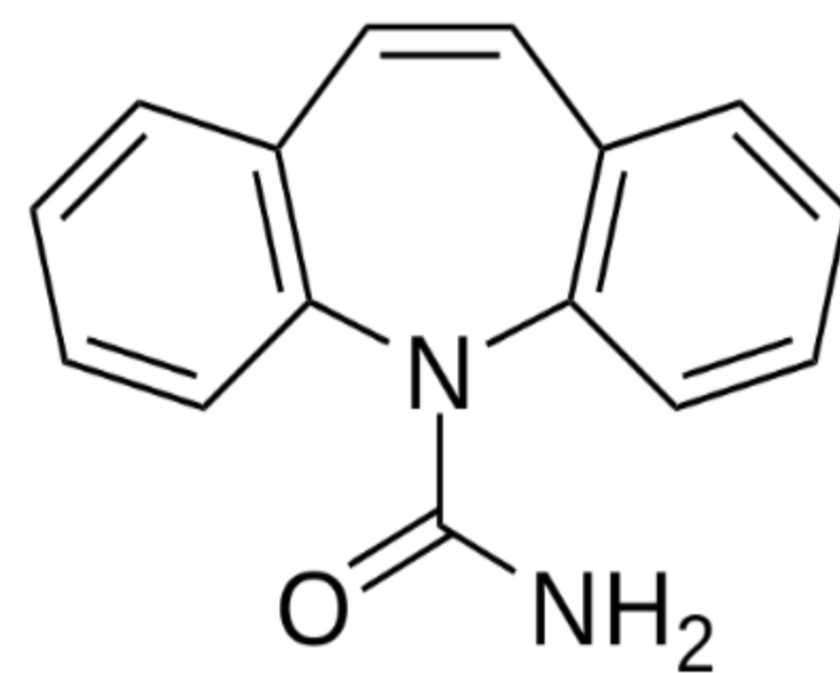


Fig.1 : Chemical structure of Carbamazepine

Long term exposure to Carbamazepine

- In chemostat, fresh medium enters the bioreactor while spent medium and biomass continuously leave the bioreactor, allowing continuous growth under defined conditions, for in theory, infinite periods of time.
- Chemostat (D:0,04) are used to grow WWTP microbial communities and to expose them for a long term to Carbamazepine (6 µg/l).

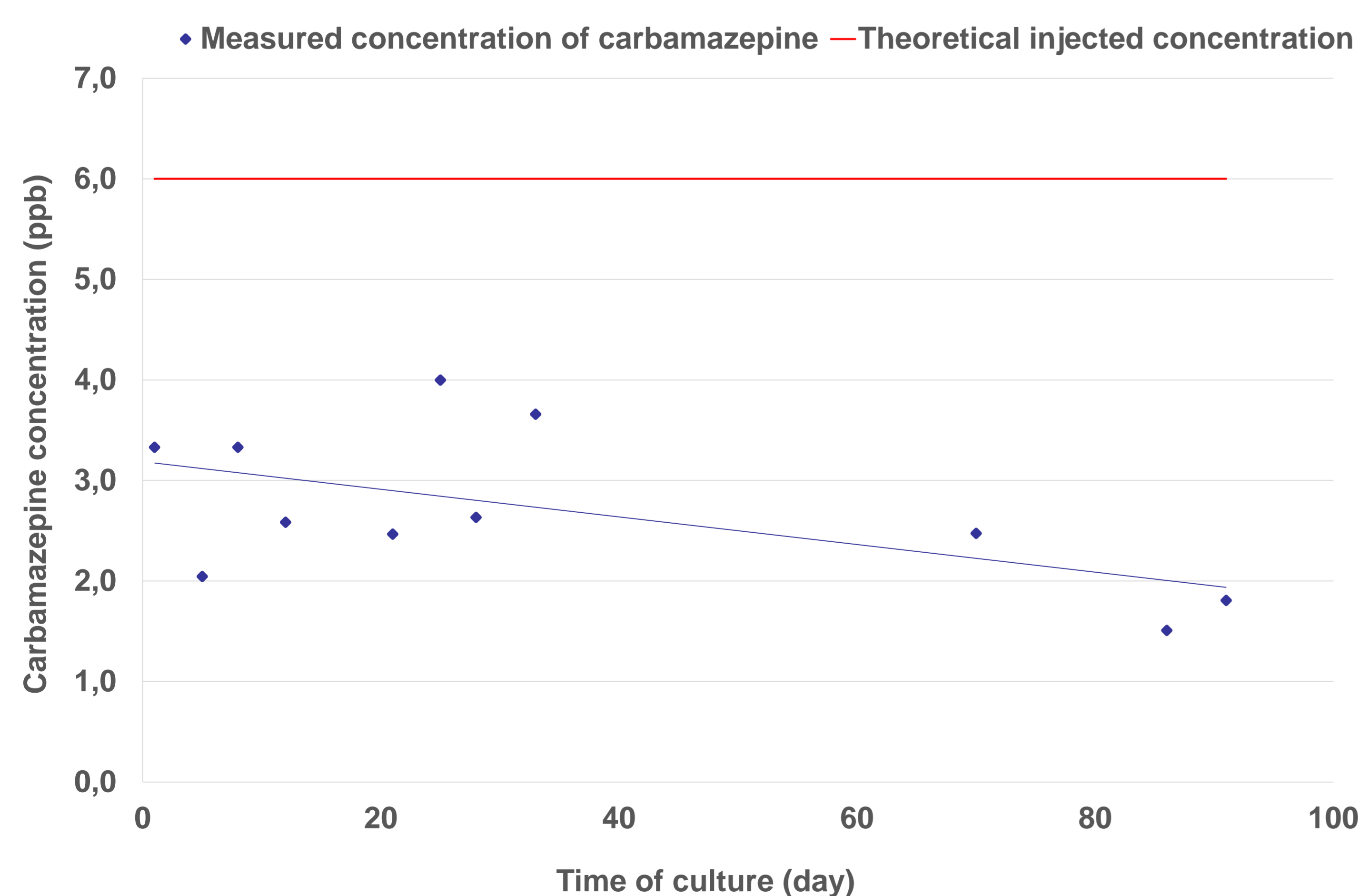


Fig. 2 : Measured (blue) and theoretical (red) concentration of Carbamazepine in chemostat.

Fate of Carbamazepine in biodegradation testing

Biodegradability of CBZ has been assessed, before exposure in chemostat, using the ready biodegradation test OECD 301A.

- Degradation has been followed by DOC (dissolved organic carbon) analyses of aerobic batch culture over a 28-day period. Sodium benzoate has been used as positive control of biodegradation activity.

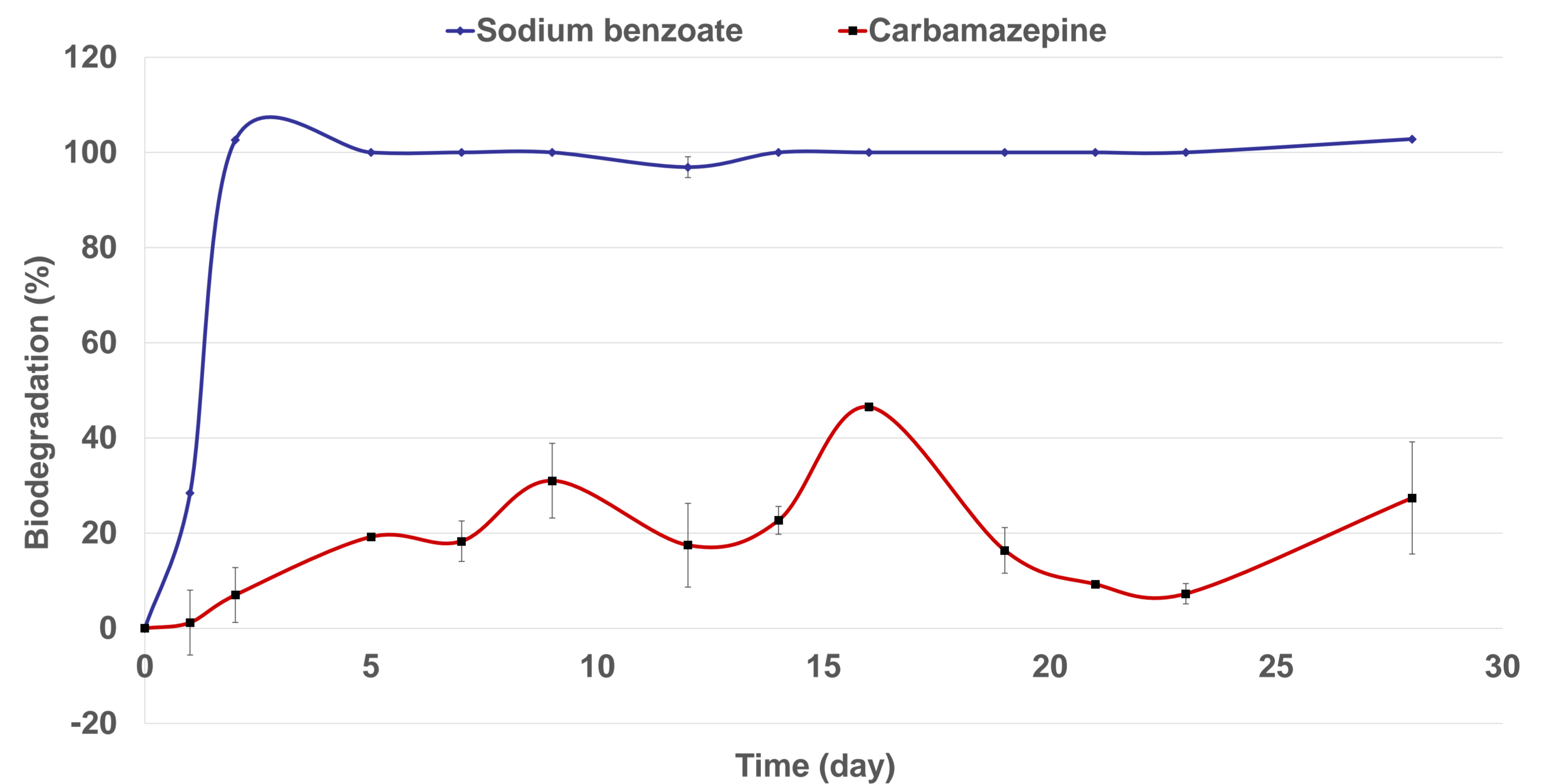


Fig.3 : Biodegradation (% of removal compared to initial concentration) of CBZ (red) and sodium benzoate (blue) in biodegradation testing (OECD 301A).

- Results of the test OECD 301A show that Carbamazepine is not readily biodegradable.
- We can observe a fluctuation of the degradation rate of CBZ over the time due to a variation in the DOC concentration.

Future prospects

The next steps for this first experiment are:

- Assess the impact of the long term exposure of Carbamazepine on the WWTP microbial community by metagenomic, metatranscriptomic and physiological analysis.
- Measuring the potential degradation product of Carbamazepine by LC-QTOF-MS.
- Perform another ready biodegradation test of Carbamazepine (OECD 301) on the exposed microbial community.

Conclusion

Results of this project are expected to improve understanding of the role of adaptation in biodegradation of persistent chemicals. This will contribute to the development of protocols allowing microbial adaptation to be include in enhanced screening or simulation tests in order to improve the environmental risk assessment .

This research work is funded by the CEFIC-LRI. ECO29-UvA: Application of chemostat systems to include adaptation of microbial communities in persistency testing (CHEMADAPT).

Selected references

OECD 301, Test No. 301: Ready biodegradability. Paris: Organisation for Economic Co-operation and Development, 1992.
van der Meer, J.R., 2006. Environmental pollution promotes selection of microbial degradation pathways. *Front. Ecol. Environ.* 4, 35–42.



Contact details:
B.A.J.Poursat@uva.nl
J.R.Parsons@uva.nl

Institute for Biodiversity and Ecosystem Dynamics
Universiteit van Amsterdam

