Application of chemostat systems for adaptation of microbial communities in persistency testing

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LRI The Long-range Research initiative

Background

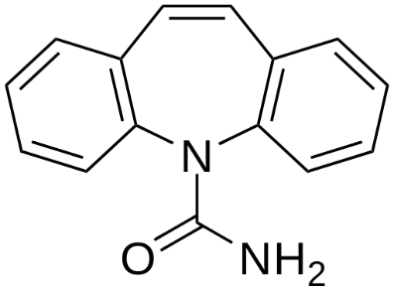
- Changes in catabolic capabilities and microbial communities upon exposure show that biodegradation half-lives change with time.
- OECD ready biodegradability tests do not take account of adaptation of microorganisms as it is known to occur in the natural environment.
- The evolution and spread of antibiotic resistance should be considered as a model to demonstrate the ecological importance and significance of adaptation research.

General approach

- Cultivation of microbial communities in chemostat systems
  - Continuous growth under defined conditions
- Expose to persistent chemicals and follow
  - Changes in community
  - Changes in biodegradation rates
  - Effects of environmental conditions (e.g. nutrients)
- Determine effects of adaptation on results of biodegradability testing

Chemostat cultures exposed to carbamazepine

Does long term exposure in chemostat leads to the biodegradation of Carbamazepine?



Carbamazepine
Formula : $C_{15}H_{12}N_2O$

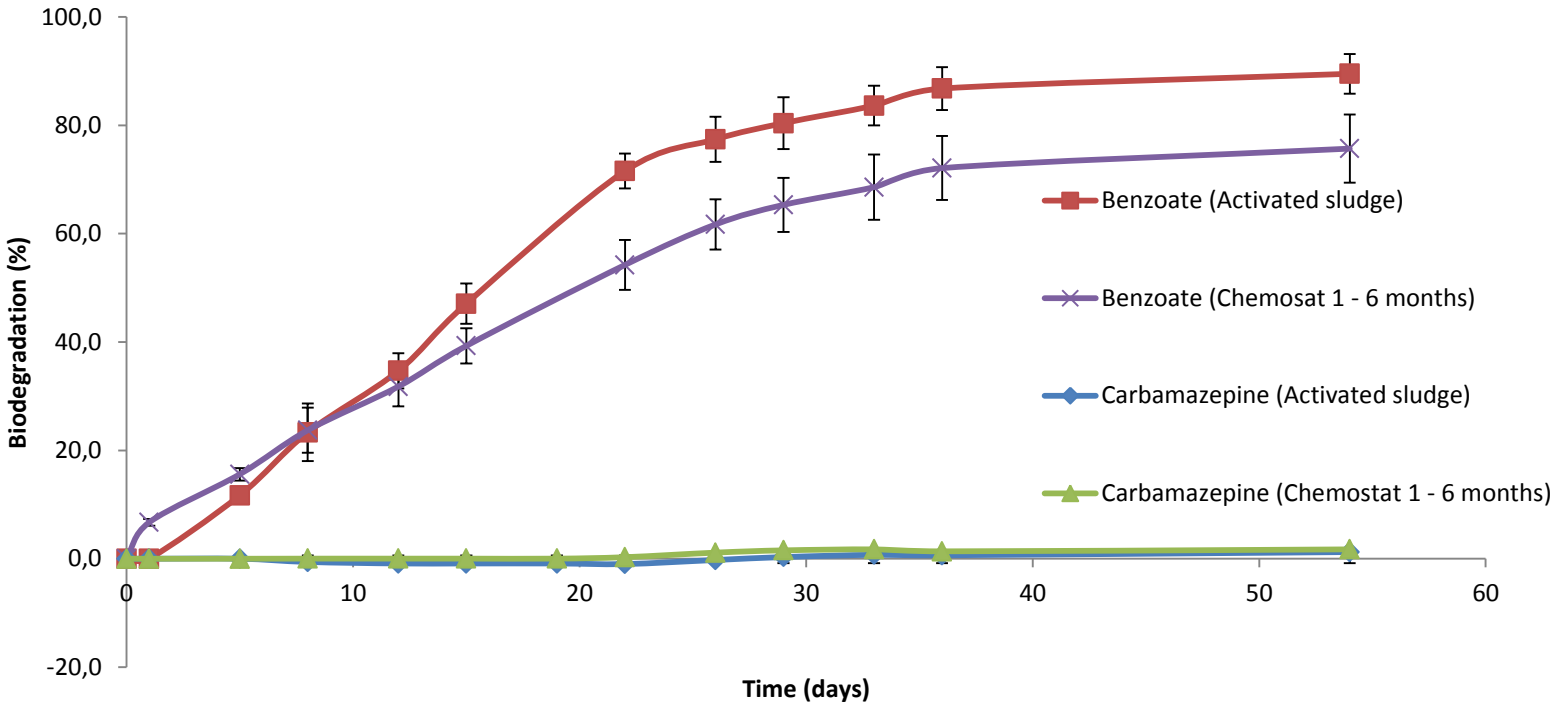
Molecular mass : 236.3 g/mol

Log Kow: 2,45

- Carbamazepine (CBZ) is a pharmaceutical product used in the treatment of epilepsy and neuropathic pain.
- Ubiquitously present in municipal wastewater and it is resistant to biodegradation.
- Persistent to biological treatment (10% removal in WWTP)
- Persistent in the environment

Biodegradation of carbamazepine in closed bottle

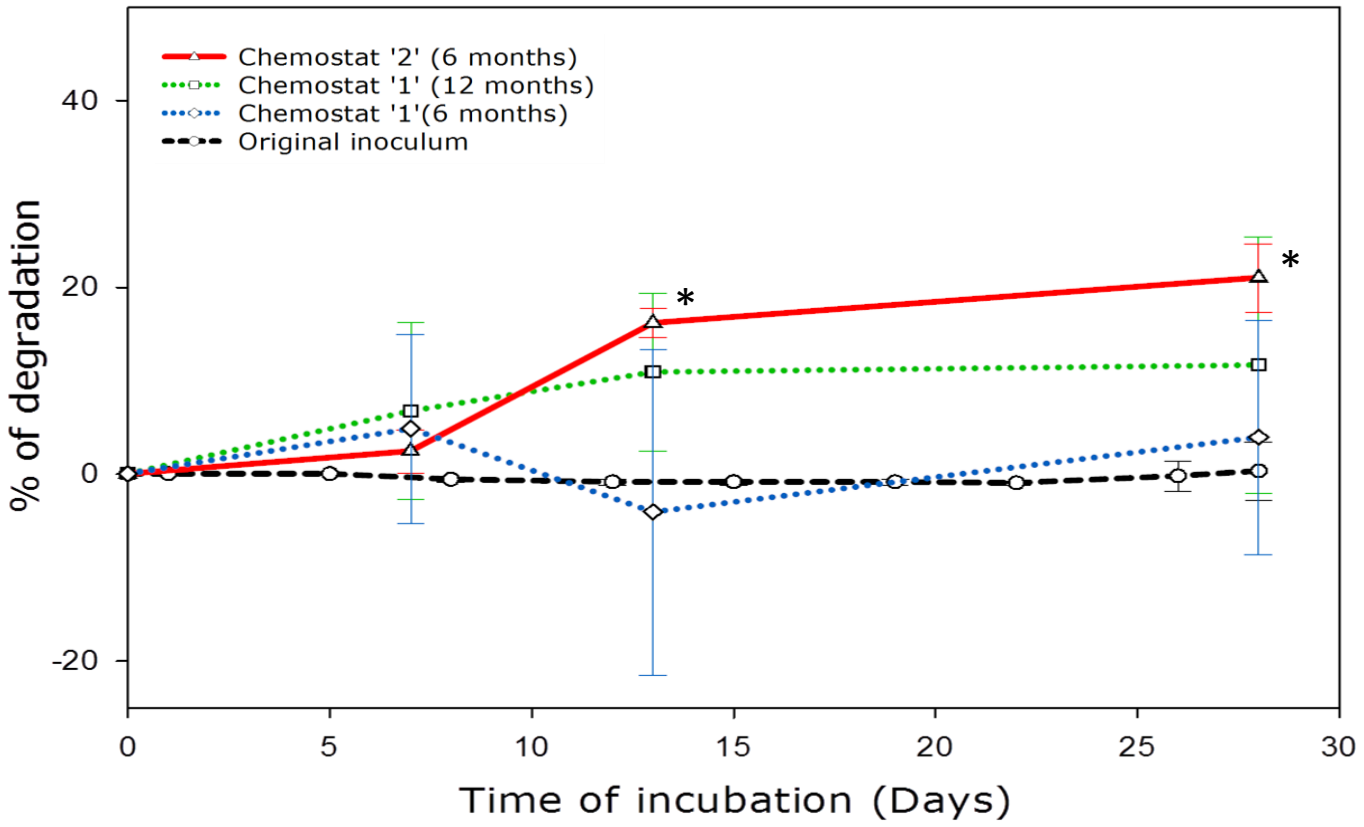
- Closed bottle (**OECD 310**)
- CO_2 production measured by GC-FID
- 15 mg/l of tested chemicals



- Carbamazepine is a persistent molecule
- No degradation after 6 months of incubation in chemostat 1

Biodegradation of carbamazepine

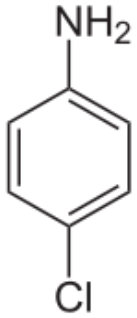
- Batch culture with mineral medium (OECD 310)
- Concentration measured by LC-MS/MS
- 15 mg/l of tested chemical



- After 6 months, microbial community from chemostat 2 shows significant capacity to degrade carbamazepine by up to 20 %.

Chemostat cultures exposed to 3 chemicals

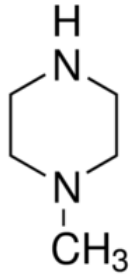
Chemostat 1 (4-CA)



4-Chloroaniline

- Erratic behaviour in RBTs
- Reduction of the lag phase from 88 to 9 days after pre-exposure in SCAS for 5 weeks.

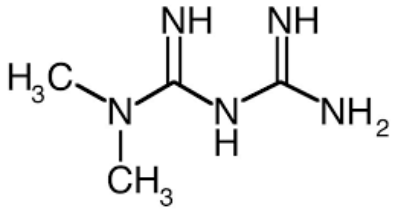
Chemostat 2 (NMP)



N-Methylpiperazine

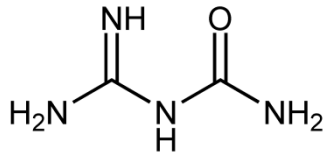
- Degradation after pre-exposition in Semi Continuously-fed Activated Sludge (SCAS) unit.

Chemostat 3 (Met)



Metformin

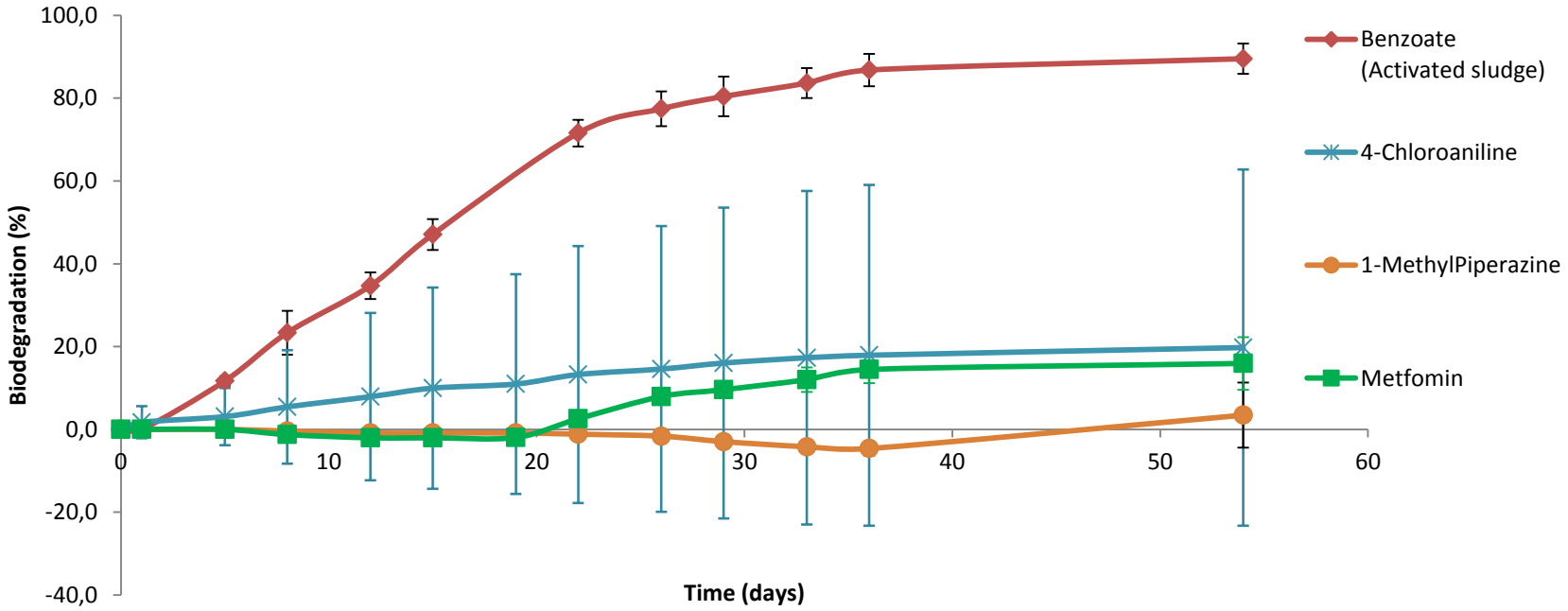
- Inherently biodegradable
- Persistent transformation product: Guanylurea



Chemostat 4 (Blank): Acetate

Biodegradation tests

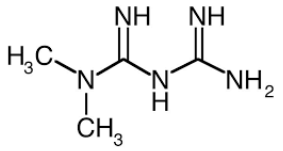
- Closed bottle (**OECD 310**)
- CO_2 production measured by GC-FID
- 15 mg/l of tested chemicals
- Inocula: Activated sludge



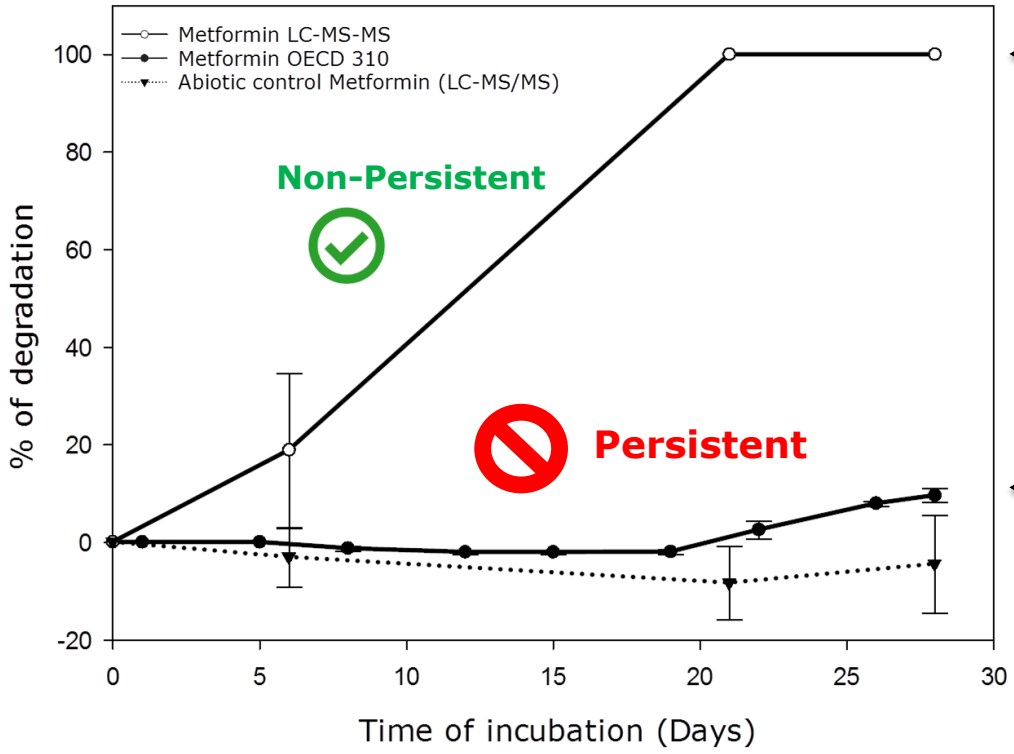
- No significative biodegradation
- None of these molecules can be considered as readily biodegradable

Mineralisation or primary biodegradation?

- CO_2 production measured by GC-FID
- Primary biodegradation by LC-MS/MS
- 15 mg/l of tested chemicals
- Inocula: Activated sludge



Metformin



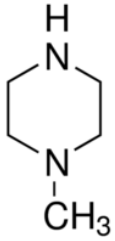
LC-MS/MS

CO_2 production

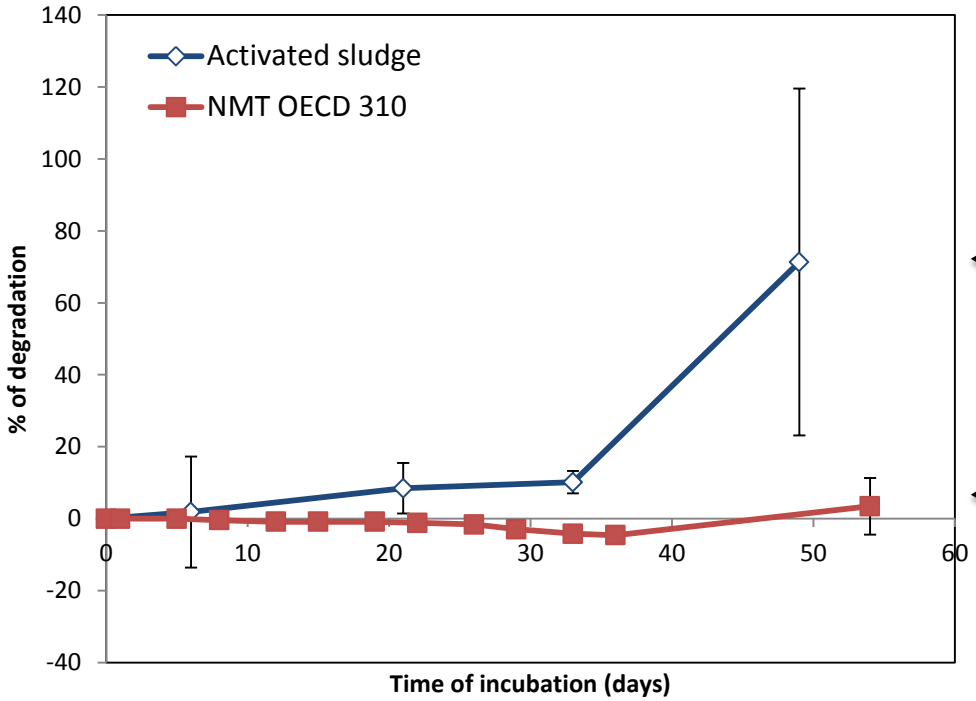
Transformation product “guanylurea” has been detected but not quantified yet.

Mineralisation or primary biodegradation?

- CO_2 production measured by GC-FID
- Primary biodegradation by LC-MS/MS
- 15 mg/l of tested chemicals
- Inocula: Activated sludge



N-Methylpiperazine



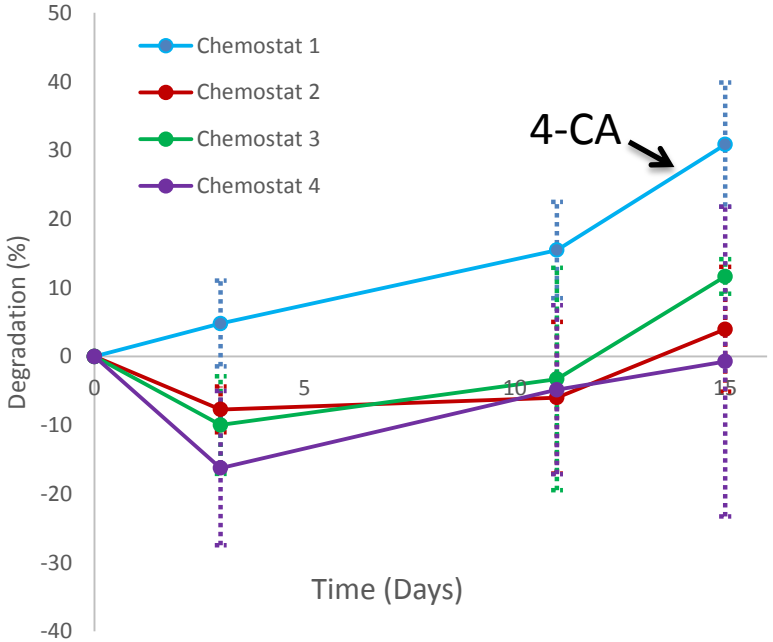
LC-MS/MS

CO_2 production

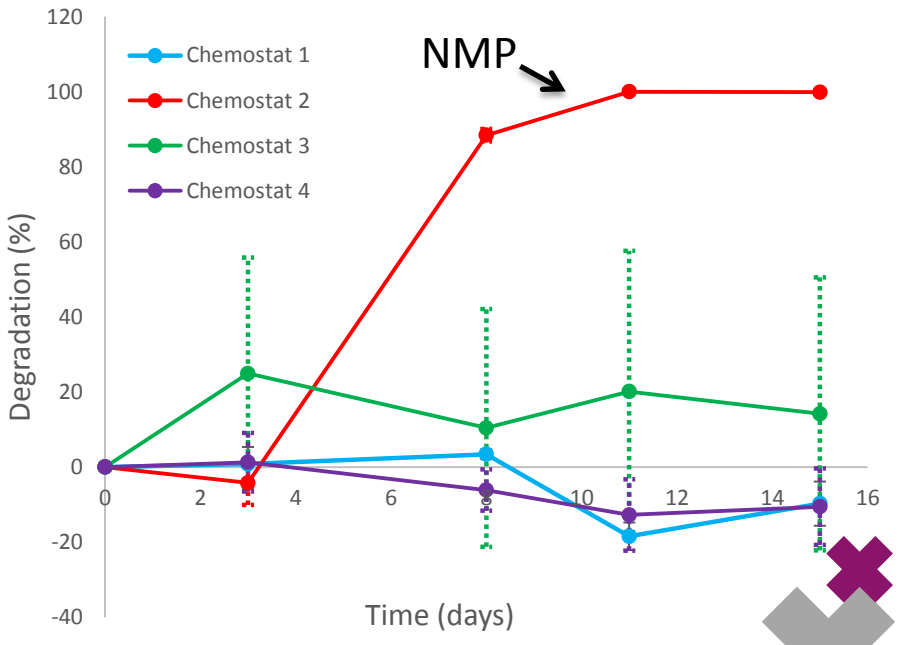
Biodegradation tests after 3 months exposure in chemostat

- Batch culture with mineral medium (**OECD 310**)
- Concentration measured by LC-MS/MS
- 15 mg/l of tested chemical
- Chemostat 1 : 1,5 mg/l of 4-Chloroaniline
- Chemostat 2 : 1,5 mg/l of N-Methylpiperazine
- Chemostat 3 : 1,5 mg/l of Metformin
- Chemostat 4 : Blank

Biodegradation of **4-Chloroaniline**

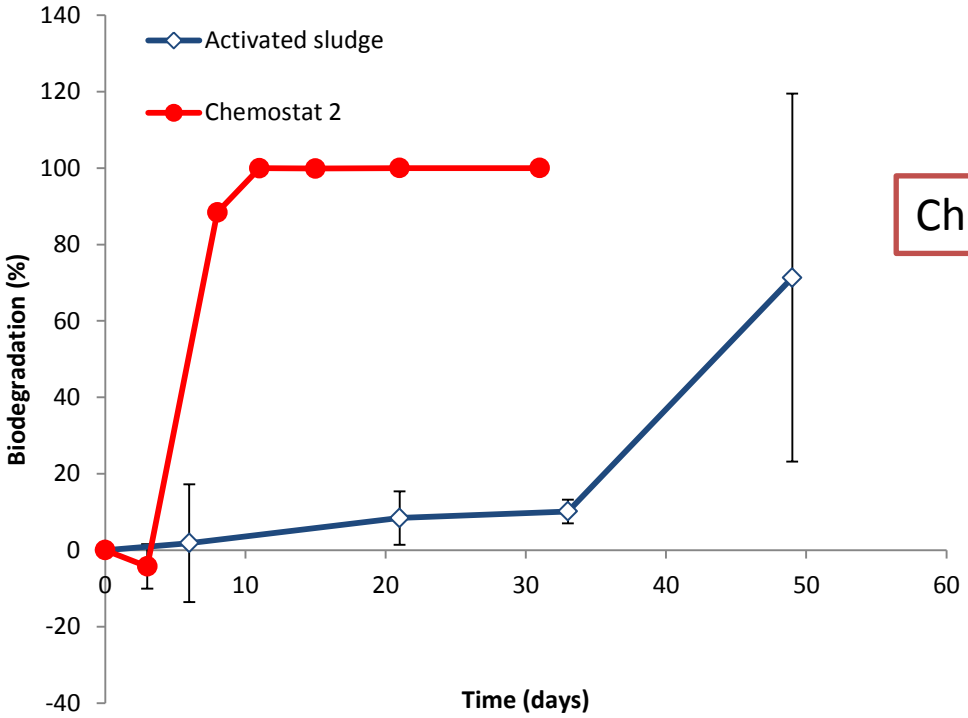


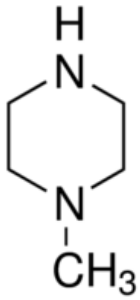
Biodegradation of **N-Methylpiperazine**



Effect of adaptation on biodegradation test of NMP

- Batch culture with mineral medium (OECD 310)
- Concentration measured by LC-MS/MS
- 15 mg/l of tested chemical
- **Chemostat 2 : 1,5 mg/l of N-Methylpiperazine**





N-Methylpiperazine

Chemostat 2 after 3 months

Activated sludge

After 3 months of exposure in chemostat, the microbial community can degrade N-methylpiperazine within 10 days

Preliminary conclusions

- **Carbamazepine** is a persistent molecule even after long term exposure.
  - But the last results show some adaptation, resulting in ca. 20% removal.

- Long term exposure of **N-methylpiperazine** in chemostat leads to its complete removal
  - Identification of the adaptation mechanisms
  - Implication for a standard ready biodegradation test (**OECD 310**)
  - Identification of potential transformation products.

- First results for **4-Chloroaniline** show enhanced removal following long term exposure in chemostat.

- Analyses of **Metformin** samples in progress

Acknowledgements

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