



Investigation into the OECD 309 Surface Water Mineralisation Test

Exploring Impacts of Sample Collection and Storage, Experimental Factors, and Reference Compounds

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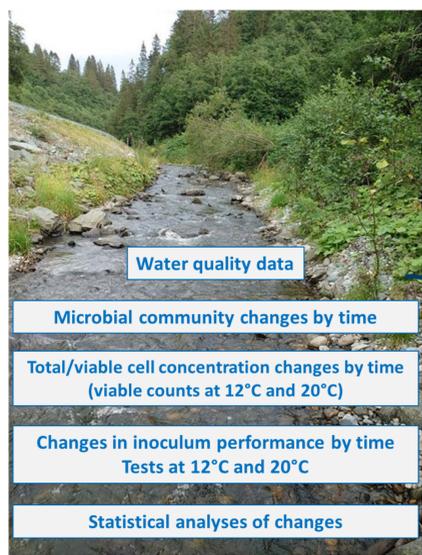
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Background

- Biodegradation is an important process for the removal of several environmental organic contaminants and is a major determinant of both chemical persistence and chemical exposure.
- Guidelines for chemical hazard assessment in certain regional and global regulations, such as the European chemicals regulation REACH, set thresholds for persistence in environmental compartments (e.g., degradation half-life in water) that need to be evaluated quantitatively.
- OECD309 Surface water mineralisation test is a simulation test for determination of persistence by aerobic mineralization of test substances in natural non-amended and non-acclimated water.
- In this ongoing work, the OECD 309 Surface Water Mineralisation Test is investigated in detail by providing:
 - an in-depth assessment of inoculum quality and viability;
 - identifying and validating more relevant reference substances that better reflect the vital status and composition of the inoculum and
 - providing a data-based evaluation of the test robustness and applicability at the new mandated test temperature (12°C in freshwater/9°C in seawater).

Sample Collection and Storage

- Waters from different geolocalities (Norway, UK and Germany) and with different temperatures at sampling.
- Assess inoculum 'quality' on three interacting levels



1. Changes in biodiversity and biomass
2. Changes in inoculum viability and activity
3. Performance changes in biodegradation of selected reference substances

Effect of Storage on Inoculum 'quality'

- Reference substances: Sodium benzoate, aniline, and 4- isopropyl phenol
- Small differences in biodegradation potential observed related to test temperatures (12/20°C).
- Biodegradation potential of the inoculum is affected by storage at 4°C .
- Changes in microbial community structures observed from 1 week of storage at 4 °C, independent of temperature at sampling (fig 2 and 3)

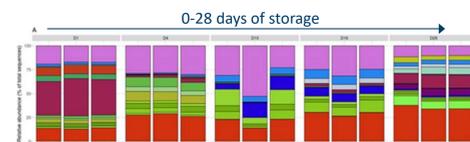


Figure 2: Microbial community composition

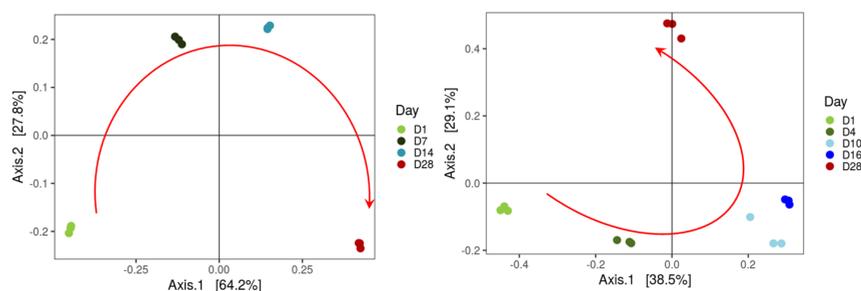


Figure 3: Beta diversity. Microbial community composition during storage, based on Bray-Curtis dissimilarity

Reference compounds and experimental factors

- The OECD309 test is to be performed with substances not readily biodegradable in screening tests, however the existing reference substances aniline and sodium benzoate are readily biodegradable.
- Nine substances have been investigated in a screening program for the suitability as alternative reference substances. 3 substances with different degradation rates were selected for further testing (figure 1)
 - fast – Aniline,
 - with lag - phase and average degradation rate - Caffeine
 - very slow / incomplete – 2,4-D
- Possible variations in experimental setups and their influence on test outcomes have also been investigated (e.g., test concentration and temperature, closed vs open systems, sacrificial vs sequential sampling, dosing method).

- Water source: River 'LEINE' (52° 11' 39.419" N; 9° 47' 7.49" O)
- Sampling dates: May and July 2022
- Test conditions: 12°C in the dark, with continuous stirring. Mineralisation was determined via oxygen depletion

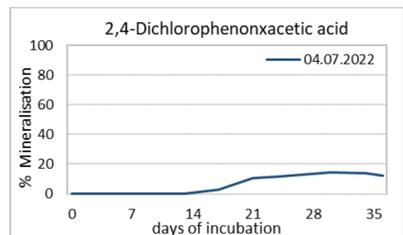
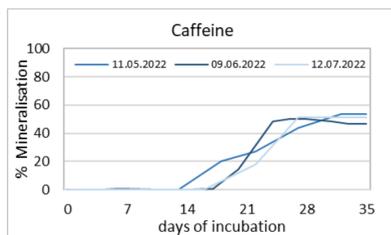
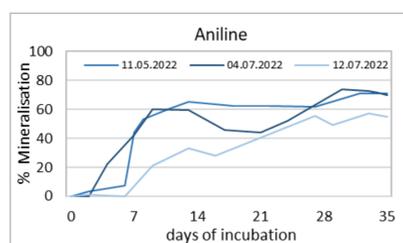


Figure 1: Mineralization of candidate reference substances

Perspective

- We are planning to propose a revision of OECD TG 309, for the development of an improved test design and procedure
- A pre-test (to end in May) is carried out to check varying environmental and laboratory conditions, with two different locations of the water, a variety in temperature and two concentrations of three candidate reference substances.
- An international ring-test based on these results will take place between May and November this year (2023) to investigate the performance of candidate reference compounds, influence of experimental setups, and the robustness/refinement of validity criteria (including at 12°C).
- **Ring-test participants welcome!** Please contact Chris Hughes or Kate Schofield from Ricardo (Booth 59) or Noack Lab (Booth 5) for more information..



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Acknowledgments

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