Towards rationally designed hazard, risk and persistency assessment: Putting the "bio" back into biodegradability tests

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1. Introduction

Regulatory emphasis has recently shifted to identifying and prioritising chemicals that are persistent, liable to bioaccumulate and are toxic (e.g. REACH). Chemicals with these properties have previously been shown to be those most harmful to human health and the environment. Biodegradation is one of the most important fate processes that determines persistence, i.e. the rate a chemical degrades in the environment. The ability to estimate or predict biodegradation is critical in determining eventual environmental concentrations, likely exposure and ultimately the risk of long-term adverse effects of chemical substances on biota, including humans. Information on the degradability of chemicals is used in hazard assessment (e.g. for classification, packaging and labelling), environmental risk assessment (for Chemical Safety Assessment) and persistency assessments (for PBT/ vPvB assessment).

Ready biodegradability tests (RBTs) have been the central foundation for understanding the biodegradation of chemicals in regulatory frameworks for hazard and environmental risk assessments for 2-3 decades. They are highly prescribed, standardised and conservative regulatory tests that measure the relative biodegradability of chemicals (e.g. OECD 301 tests). Fulfilling the pass criteria for the RBT is a reliable indicator that the chemical can biodegrade quickly in most environments through routine use, but failing the pass criteria is not necessarily a reliable indication of persistence. Consequently, RBTs offer little potential to prioritise chemicals based on their relative persistence. They also exhibit high levels of variation.

This has recently been recognised in REACH guidance which advocates the introduction of a new tier of enhanced tests to enable efficient and effective identification of persistent chemicals (ECHA, 2008). Reliable extrapolation from any small-scale systems to predict local and regional environmental impacts depends on the test systems truly being representative of the real environment, including the nature of the microbial populations present. Enhancements may therefore include increasing inocula concentrations to environmentally-equivalent concentrations, thereby incorporating increased and realistic microbial diversity. It is therefore necessary to understand the relationship between bacterial diversity and biodegradation outcome in such enhanced tests. In order to gain this understanding, we have been applying the latest molecular detection methods and theoretical ecology models to quantify the distribution, abundance and diversity of bacteria in different environmental compartments and their influence on biodegradation outcome, with the eventual aim of developing scientifically sound screening tests for persistence.

2. Materials and methods

- Multiple locations from different environmental compartments (soils, activated sludge, lakes, rivers, and estuarine, coastal and sea waters) were sampled and prepared for use as inocula in minitiuarised high-throughput biodegradation tests (BTs). Locations were chosen to represent a broad range conditions likely to have an impact on microbial diversity (e.g. pristine versus eutrophic sites).

- Inocula were concentrated by filtration and a range of cell densities were prepared by serial dilution to give final concentrations of 10^4 - 10^9 cells ml^{-1}. Replicates of each dilution were inoculated into 96-well plates (one plate per dilution) containing the test compound (diluted to 10mg/l carbon in sterile mineral medium) and incubated at 30°C for either 28 days (as per OECD RBT protocol) or 20°C 60 days.

- Test compounds included 4-hydroxybenzoic acid (4-HBA), 4-nitrophenol (4-NP), 4-chlorophenol (4-CP) and 4-fluorphenol (4-FP) to represent a range of intrinsically different biodegradabilities. Parent compound disappearance was evaluated at the end of the test using a novel colorimetric assay based on azo-dye coupling (Goodhead et al., 2008). Those showing greater than 70% parent compound disappearance were scored as positive providing a probability of biodegradation for each inoculum dilution, which was also used to determine specific degrader abundances by the most probable number method; MPN.

- Samples were also taken for bacterial community analysis by using denaturing gradient gel electrophoresis (DGGE), which briefly incorporates; DNA extaction, PCR amplification of the 'universal' evolutionary biomarker - 16S rRNA gene fragments, and separation of the fragments based on sequence on a denaturing fingerprint gel. Each band in the resulting pattern represents a taxon and the number of bands (band richness) is indicative of the diversity of the predominant members of the community. This data was used to compare patterns of taxa frequency-abundance with those predicted by a neutral community model (NCM; Sloan et al., 2006), which is explicitly based on ecological theories of community assembly.

3. Results and discussion

Bacterial community composition in many environmental compartments, except activated sludge, conformed to the NCM suggesting that predominant bacterial taxa assemble from a common source community for each compartment.

The equivalent of 59,700 individual BTs have been completed so far from 51 locations in 7 compartments.

Enhanced inocula concentrations result in greater probablities of biodegradation for all test compounds. Less than 20% of inocula sources demonstrated any ready biodegradability of 4-NP at OECD-recommended inocula concentrations in the 28-day tests.

The order of relative biodegradabilities of test compounds was generally 4-NP>4-CP, and 4-HBA>4-NP>4-FP for 28 and 60-day tests respectively.

Activated sludge and lakes showed the greatest variability in biodegradation outcomes between inocula sourced from different locations, and coastal waters showed the least in 28-day tests.

Pooled data from all environmental compartments demonstrated a weak but significant correlation (P < 0.01) between bacterial diversity and biodegradation potential for 4-NP, but not for 4-CP, a relationship that held for most individual compartments at approximately the 10% error-rate.
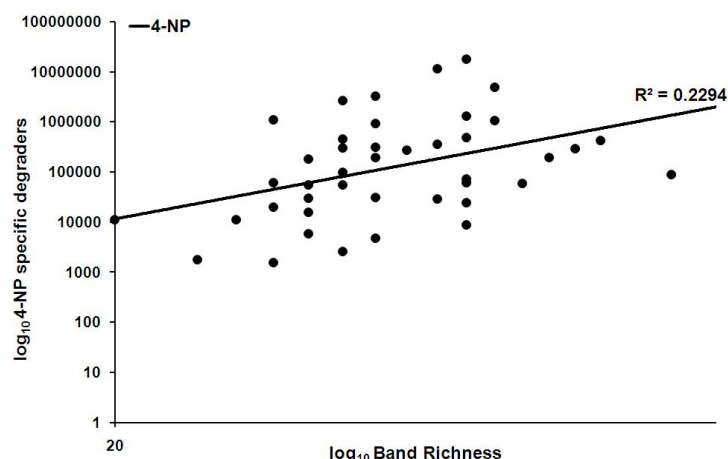

Figure 1. Influence of bacterial diversity on biodegradation potential measured as the abundance of 4-NP-degaders

4. Conclusions

Enhanced screening tests were successfully used to prioritise the relative biodegradability/persistence of chemicals. Inocula source, concentration and bacterial community composition and diversity play an important role in biodegradation outcome and its variation. An understanding of bacterial diversity could be used to select sources and in conjunction with novel models lead to better prediction of biodegradtion

5. References

[1] ECHA (2008) Guidance on information requirements and chemical safety assessment Chapter R.7b: Endpoint specific guidance
[2] Goodhead, AK, Snape, JR, Head, IM, Davenport, RJ (2008) Rapid biodegradability estimation of phenolic compounds. 18th SETAC Europe Annual Meeting, Poland.
[3] Sloan, W.T., Lunn, M., Woodcock, S., Nee, S., Head, I.M., and Curtis, T.P. (2006). Quantifying the roles of immigration and chance in shaping prokaryote community structure. Env.Microbiol., 8: 732-740.