



# Experiences with Enhanced Biomass Biodegradation Studies to Prioritise on Persistency as Advocated under REACH

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## Introduction & Aims

Biodegradation is an important but poorly understood fate process that can result in the loss or transformation of a chemical substance 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. Degradation, including biodegradation, is therefore a key parameter in any approach used to estimate the risk of long-term adverse effects on biota. 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 persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) assessments. 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. RBTs are highly conservative tests that have received widespread use to understand and measure relative biodegradability and persistence of chemicals. However, the basis of RBT are undermined by their unreliability and poor standardisation. In this study, an 'Enhanced' approach to biodegradation screening was applied to investigate the effect of variations in inoculum source, density and diversity on biodegradability. Standard guidelines of inocula preparation were shown to reduce the detectable diversity and biodegradability compared to original source material.

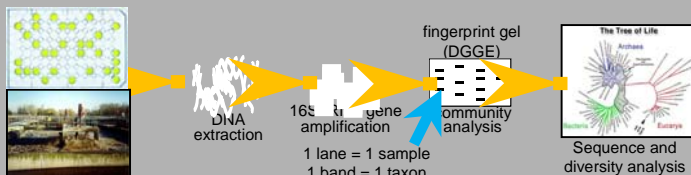
## Enhanced Biomass Studies

Within the REACH guidance it has been recognised that a new tier of enhanced biodegradation screening tests may enable the efficient and effective identification of chemicals that are likely to persist in the environment. As such REACH advocated four methods of enhancement. These were:

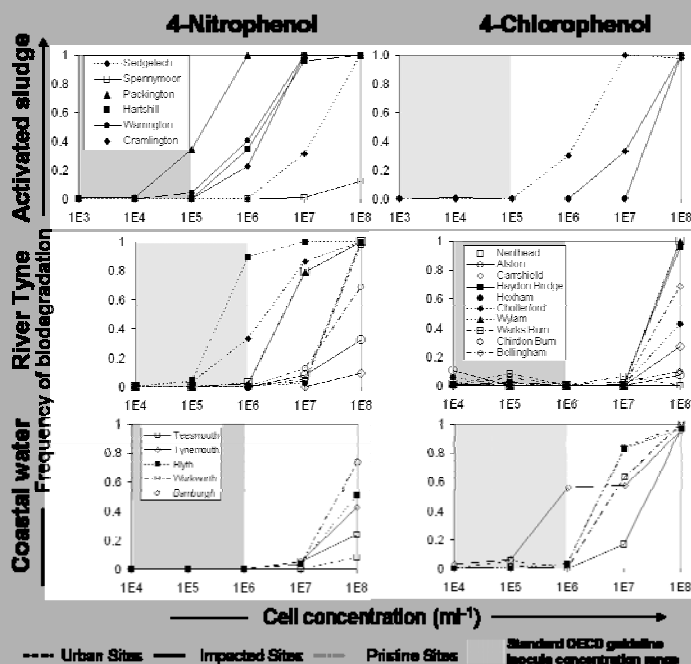
- Extension of the test duration beyond 28 days
- Increased vessel size or enhanced biomass density
- Conducting two ready tests in series (i.e. single subculture)
- Semi-continuous biodegradation assessments with environmental waters.

## Materials & Methods

- Inocula prepared from activated sludge, river water and marine water
- Total cell counts determined by epifluorescence microscopy using a universal DNA stain
- Serial 10-fold dilutions inoculated into 96-well RBT incubated at 30°C for 28 days with the test chemical as the sole carbon source (10 mg C l<sup>-1</sup>) with appropriate controls
- Colour change from yellow to colourless scored as positive degradation
- Diversity analysis using molecular techniques targeting the 16S rRNA gene

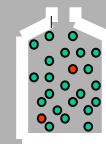


## Test Results

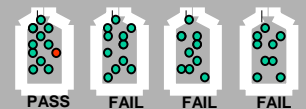
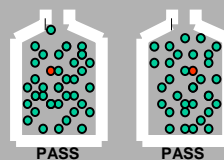


## RBT Lottery

Specific degrader



Inherently assumes that all bacteria/functions are ubiquitous but relies on chance inclusion of rare species



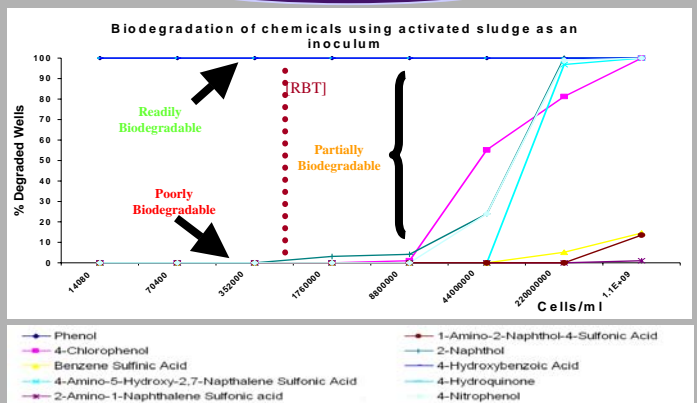
- OECD guideline inocula are poorly standardised

- 1 drop (~50µl) to 5 ml (3 orders of magnitude)
- Different environmental sources and pre-treatments

- Inherently variable and unreliable

- Low replication
- High failure rate

## Ranking on Persistence



## Species Richness

Activated sludge (AS)  
OECD guideline inoculum from AS (~50µl)  
OECD guideline inoculum from AS (5ml)

## Conclusions

- Increased bacterial densities result in greater frequency of biodegradation with inocula from all environmental sources
- Inocula from different environmental sources have different biodegradation potentials, but do not necessarily correspond to perceived levels of diversity (soil>river>AS>marine)
- AS shows the greatest variation in biodegradation potential
- Proof of concept established as a possible RBT replacement that can prioritise and rank chemicals based on relative persistency
- Full-scale validation required (under funding by CEFIC LRI – ECO11)