New Approaches to Persistence Testing With Increased Cell Number Using Tangential Flow Filtration

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Background

Microbial biodegradation is the most important elimination pathway for chemicals in the environment. Current testing frameworks, while well standardised and consistent, are recognised as having some limitations with **difficult to assess chemicals** such as large, polar, or poorly soluble materials. Testing is often not reflective of real, variable environmental conditions, with little consideration for the microbes included in the test. **Tangential flow filtration** (**TFF**) techniques can be adopted to negate inconsistencies in traditional biodegradation tests, particularly aqueous samples with low microbial biomass, e.g. freshwater.

Increasing microbial biomass can increase the probability of a chemical encountering a **potential microbial degrader**, with a more representative proportion of the microbial community captured, tested in shorter timeframes, with tests generating data that can be used to support new in-silico approaches to predicting persistence of chemicals; ultimately **improving assessments of environmental risk**.

Methods

To investigate how new methods of **increasing microbial biomass** can be integrated with **existing screening methods**, and how **cell number** can impact **freshwater** biodegradation testing, river water was collected from the river Nene, Wellingborough, UK.

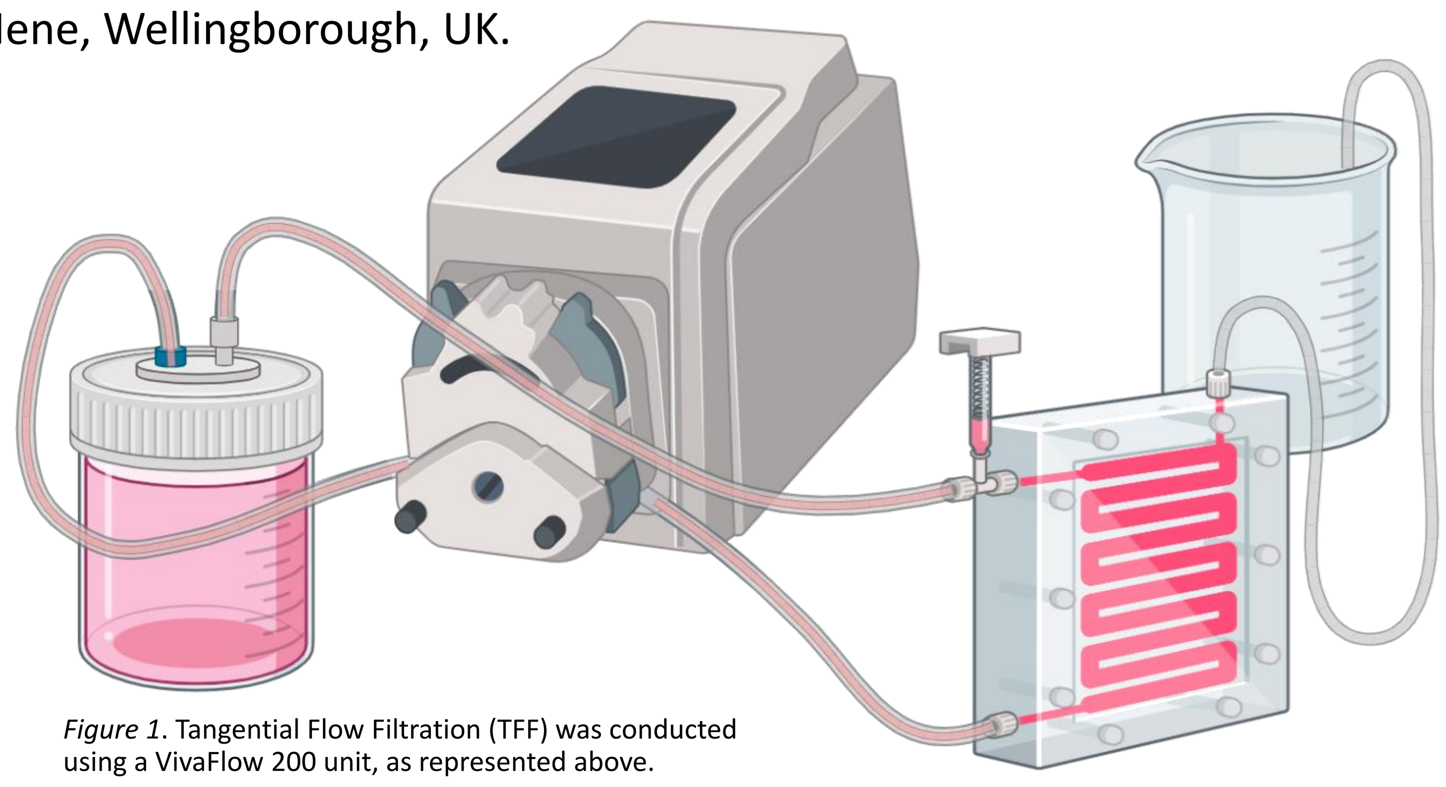
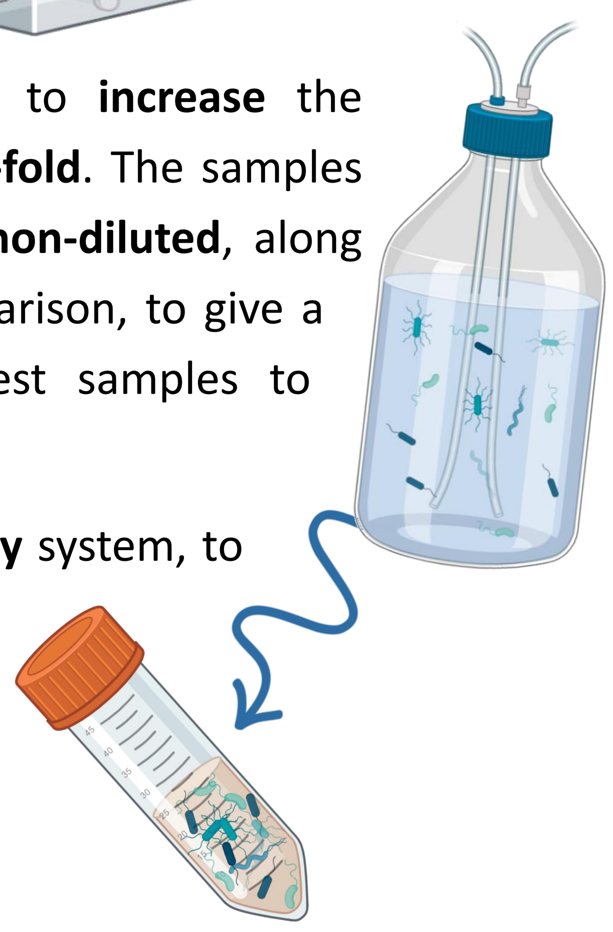


Figure 1. Tangential Flow Filtration (TFF) was conducted using a VivaFlow 200 unit, as represented above.

Tangential flow filtration techniques were used to **increase** the **freshwater microbial biomass** by **10-fold** and **100-fold**. The samples were tested both **diluted** in minimal media and **non-diluted**, along with filtered but un-altered river water as a comparison, to give a **range of microbial biomass densities** within test samples to investigate an 'ideal' range.

The samples were tested in an **OxiTop respirometry** system, to detect biodegradation **of Polyethylene glycol** (PEG) (35,000 MW), in an adapted assay comparable to an OECD Guideline **301F** Test.



Results

Increasing the **number of microbes** in **river water** can enable representative and robust screening, to determine whether a chemical could be biodegradable, by **increasing the rate of biodegradation**, within short testing timeframes.

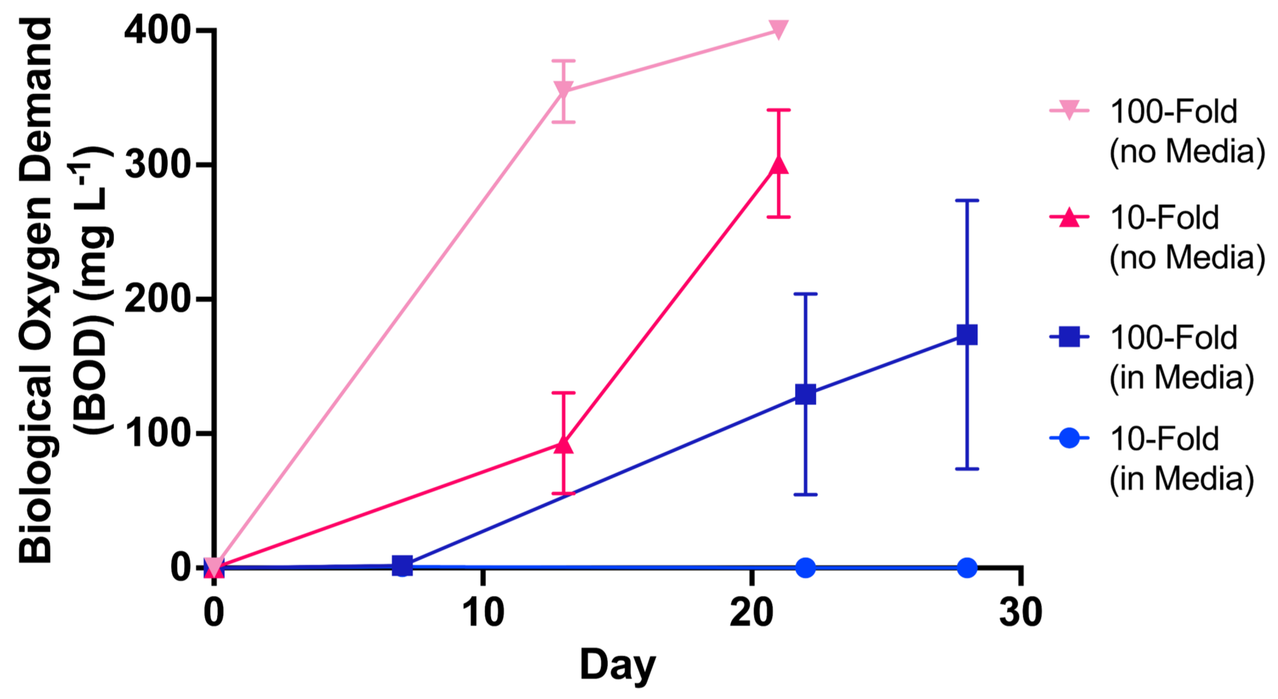


Figure 2. Biological Oxygen Demand, over time, of increased microbial biomass inocula incubated with PEG in an OxiTop system. Comparison un-altered river water did not exhibit detectable degradation of PEG and is not included in this plot. Error bars represent +/- standard error of the mean. Measuring range for an OxiTop with 164 mL sample volume is 0 - 400 mg L^{-1}.

Preliminary tests with increased microbial biomass inocula by TFF in an **OxiTop** assay demonstrated that increasing microbial biomass **decreases** the **lag** in biodegradation rates of PEG, and the increased cell number increased **detectable biodegradation** of PEG.

Samples of un-altered river water **did not** demonstrate **detectable biodegradation** across the duration of the tests.

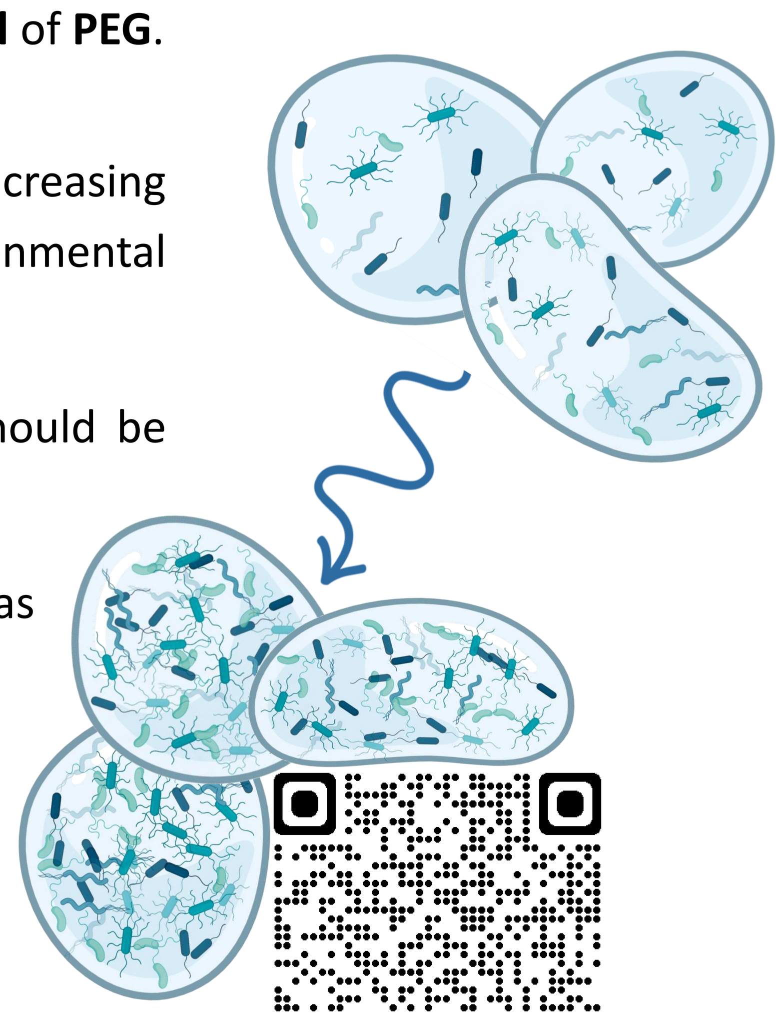
A **10-fold increase** in freshwater microbial biomass enabled robust detection of biodegradation, within the measuring range of the system, with little background noise.

Conclusions

- **Persistence**, as a chemical's **recalcitrance to transformation**, including biodegradation, isn't a static property of a chemical. It varies based on how a chemical's intrinsic properties interact with **microbial communities** and the **variation** in the environment they are found in.
- The microbial biomass in these tests was **increased**, ensuring the microbes included are **representative of the real environment**, and contain a larger snapshot of the **catabolic diversity** within the habitat.
- Tangential flow filtration is one adaptation to traditional tests that has been proposed, to aid understanding and evidence a chemical's **environmental fate**, and support more consistent testing for **hard to assess chemicals**, and has been shown here to be an ideal method for increasing cell number to easily assess the **biodegradation potential** of **PEG**.

Future Perspectives

- To gain accurate insights into a chemical's potential persistence in the environment, under **relevant environmental conditions**, increasing numbers of methods to **investigate degradation processes** are being discovered and tested, expanding understanding of environmental processes globally.
- While this study focused on freshwater, **all environmental compartments**, with their **associated microbial communities,** should be considered when assessing a chemicals potential persistence in the environment, including soil, marine systems, and sediment.
- Efforts could be taken to **quantify** and assess **viability** of the bacteria that are being used within testing, using methods such as flow cytometry, direct microscopic counts, tetrazolium dyes, or new methodology approaches.
- A single test will never fully reflect the **environmental conditions** that a chemical is released into, but screening methods are a **useful tool**, and further testing in a variety of conditions and locations, over periods of time, can help ascertain the true potential for environmental persistence of a chemical.



References: Davenport, R., Curtis-Jackson, P., Dalkmann, P., Davies, J., Fenner, K., Hand, L., McDonough, K., Ott, A., Ortega-Calvo, J. J. & Parsons, J. R. 2021. Scientific concepts and methods for moving persistence assessments into the 21st Century. *Integrated Environmental Assessment and Management*. | Martin, T. J., Goodhead, A. K., Snape, J. R. & Davenport, R. J. 2018. Improving the ecological relevance of aquatic bacterial communities in biodegradability screening assessments. *Science of the Total Environment*, 627, 1552-1559. | Ott, A., Martin, T. J., Snape, J. R. & Davenport, R. J. 2020b. Increased cell numbers improve marine biodegradation tests for persistence assessment. *Science of the Total Environment*, 706, 135621. | ***Acknowledgements:*** Preliminary testing of PEG in an OxiTop respirometry system was undertaken at Unilever's Safety and Environmental Assurance Centre, by Katie Endersby and Dave Gore.

Diagrams created using BioRender.

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