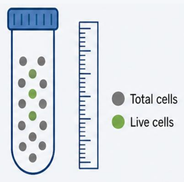
Flow-Cytometric Quantification of Live and Dead Bacterial Cells in Environmental Inocula for Biodegradation Screening Tests

Kelly Jobling[1], Mathew Brown[1] and Russell Davenport[1]

[1] School of Engineering, Newcastle University, Newcastle, NE1 7RU UK

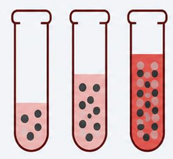
Email Contact: kelly.jobling@newcastle.ac.uk

Context



- Biodegradation screening tests (BSTs), such as ready biodegradability tests (RBTs) screen for chemical persistence but rely on poorly defined microbial inocula. [1]
- Standard methods quantify total biomass, not viable cells – the drivers of biodegradation
- Viable cell abundance is not quantified or controlled

The Problem

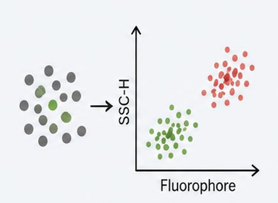


- Standard tests use microbial inocula below environmentally relevant cell concentrations (> 100x lower) [1]
- RBTs exhibit high variability and poor reproducibility.[2]
- False negatives are common – estimated to represent 20–80% test failures ("biodegradation lottery")[3]
- Functional diversity is reduced at low cell concentrations[4]

The Gap

- No universal methods to quantify viable cells
- Limited cross-matrix validation (Activated sludge (AS), fresh water, seawater)
- Unknown effects of handling and storage of samples on microbial viability

Approach



- Flow cytometry (FCM) and viability staining enable quantification of live/dead cells in samples for biodegradation testing.
- Applied across environmental inocula
- Evaluation of effect of preservation methods on viability
- Enables standardisation of inoculum based on viable cell abundance

Aim

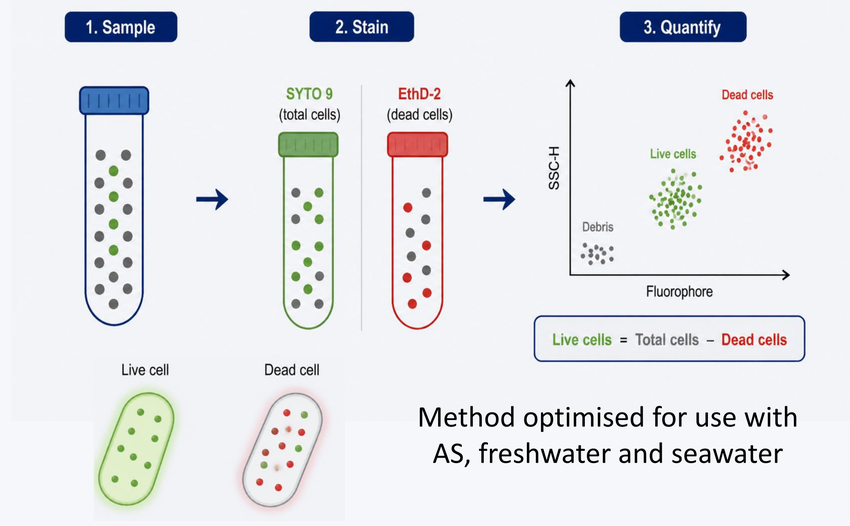
To develop a flow cytometric method for quantifying viable cells in environmental inocula, to improve standardisation and understanding of variability in ready biodegradation tests

Key Message

Biodegradation outcomes depend on viable cell abundance, now quantifiable using flow cytometry

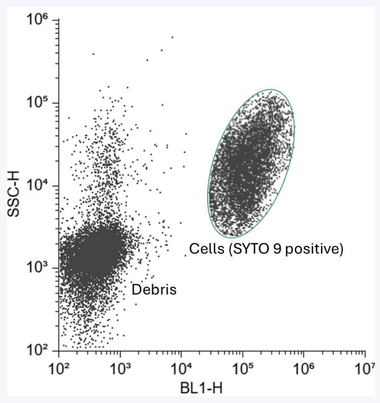
Method Development and Application

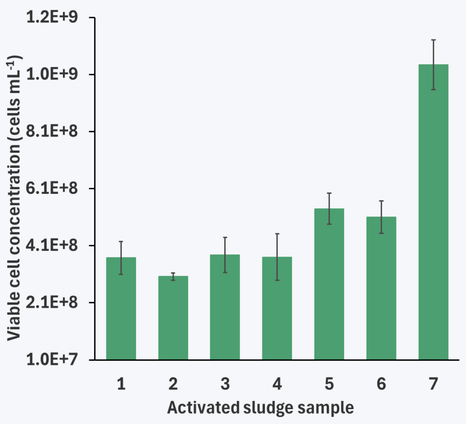
Workflow for quantification of viable cells using FCM



Method optimised for use with AS, freshwater and seawater

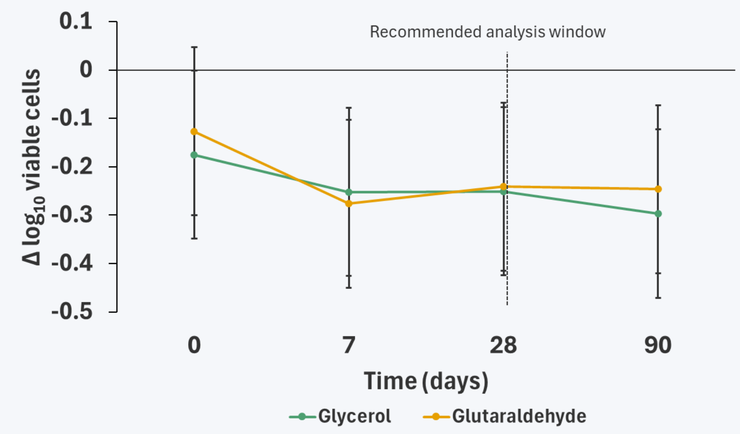
FCM reveals variability in viable cell abundance across inocula

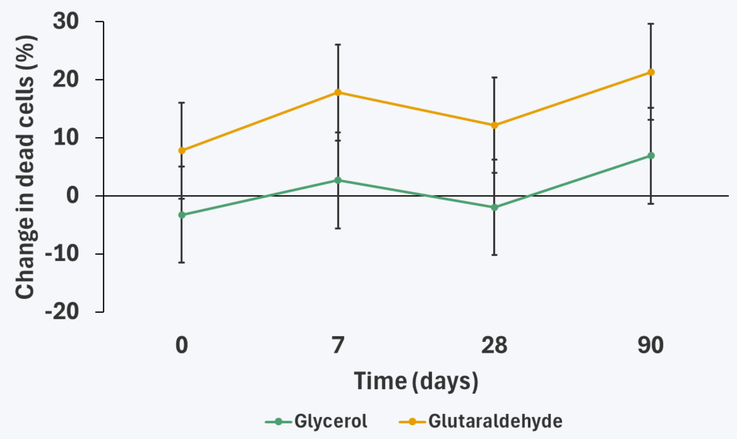




- FCM enabled discrimination of viable microbial cells - representative plot (left)
- AS samples showed substantial variation in viable cell abundance (right) – cell numbers ranging from 3 x 10^8 – 1 x 10^9 cells/ml
- Differences in viable cell abundance may contribute to BST variability

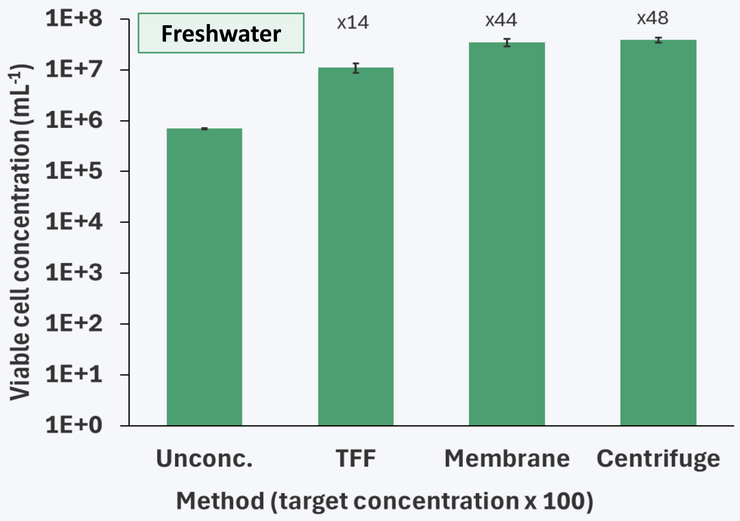
Delayed FCM analysis is feasible - analyse within *28 days* and preserve in *glycerol*

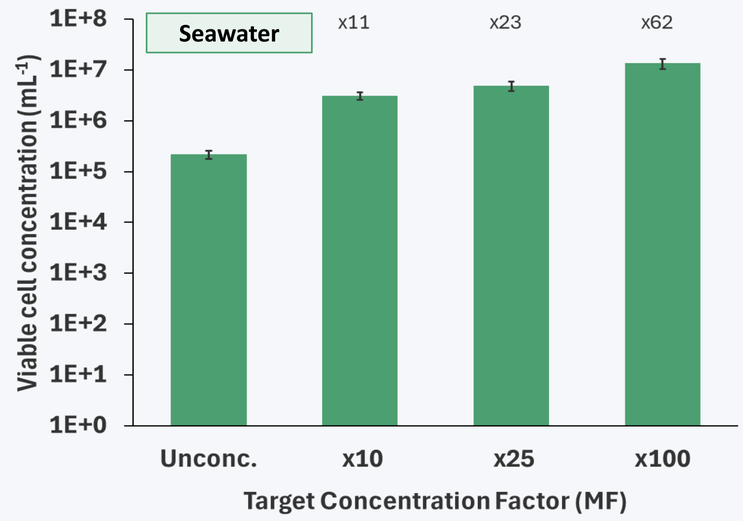




- Immediate FCM analysis is not always feasible during high-throughput biodegradation experiments
- No significant difference in viable cells between preservation methods (left, p = 0.420)
- Significant viability decline observed at 90 days (p <0.001), therefore analysis recommended within 28 days
- Glycerol preservation maintained lower proportions of dead cells than glutaraldehyde (right, p <0.001)

FCM quantifies the impact of sample preparation on viable cells





- Standard BSTs often use microbial inocula concentrations below environmentally relevant cell concentrations
- FCM quantified the impact of sample preparation workflows on viable cell recovery in freshwater and seawater samples
- Achieved viable cell recovery differed substantially between concentration methods and target concentration factors
- FCM enables quantitative evaluation of workflows designed to achieve environmentally relevant viable cell abundances

Conclusions

- Viable cell abundance can be **rapidly and reproducibly quantified** using FCM across environmental samples
- **Uncontrolled variation in viable cells** likely contributes to poor reproducibility in BSTs
- **Delayed FCM analysis is feasible**, but samples should ideally be analysed within 28 days and preserved in glycerol to minimise viability loss
- FCM provides a quantitative tool to **optimise and standardise inoculum preparation**, improving the reliability of biodegradation screening

Acknowledgements and References

Funding provided by the International Collaboration on Cosmetics Safety (ICCS)

[1] Kowalczyk, A. et al 2015. Refinement of biodegradation test methodologies and the proposed utility of new microbial ecology techniques. Ecotoxicology and Environmental Safety
[2] Martin, T. J et al 2017. Environmentally relevant inoculum concentrations improve the reliability of persistent assessments in biodegradation screening tests. Environmental Science & Technology
[3] Ott, A., et al. Increased cell numbers improve marine biodegradation tests for persistence assessment. Sci. Total Environ
[4] Goodhead, A.K., et al. 2014. Standard inocula preparations reduce the bacterial diversity and reliability of regulatory biodegradation tests. Environ Sci Pollut