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EBNet Environmental Biotechnology
Engineering and Physical Sciences Research Council
Biotechnology and Biological Sciences Research Council

FLUORESCENT MICROBIOFILTER ASSAY FOR RAPID REAL-TIME MONITORING OF ORGANIC MICROPOLLUTANTS BIODEGRADATION

AIM

Microbial communities on biofilters form stochastically, based on the chemistry and biology in the raw water. We contend that targeted design of microbial ecology for degradation of difficult-to-treat organic micropollutants (OMPs) could be achieved by optimising said chemistry and biology using genetic algorithms. While this would require *in situ* testing of the responses of microbial communities to OMPs, no fast, inexpensive, reliable analytical methods to measure OMPs in microbial cultures are currently available.

Current gold standard methods provide precise and accurate results, but at best hours and most likely days after sampling, by which time conditions in the biological systems may have changed drastically. We aim to design and validate a new microwell plate assay to measure the biological removal of OMPs in microscale biofilters.

The plate wells will be filled with a transparent porous medium that acts as the substratum for biofilms, whose degradation of fluorescently tagged OMPs will be monitored using fluorescent excitation-emission. By the end of the project, we hope to deliver a robust, precise and sensitive assay that can be further integrated to high-throughput robotic platforms for rapid and site-specific optimisation of microbial seeds of full-scale biofiltration systems.

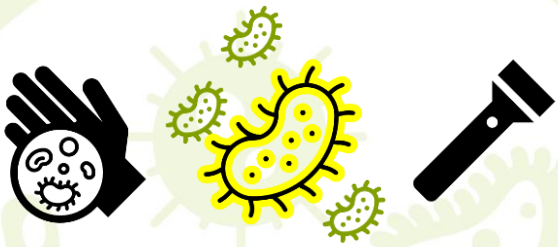
RESULTS

With the EBNet PoC funding, we were able to demonstrate the feasibility of a high throughput assay for rapid selection of microbial seeds for the degradation of 17ß estradiol (E2).

Click chemistry was employed to attach a linker on a labile hydrogen of E2, and a green fluorescent BODIPY to the linker. The fluorescent tag enabled specific detection and quantification of the tagged compound in the media using a spectrofluorometer, with limits of detection much lower than standard analytical methods.

We further demonstrated in batch planktonic experiments that the fluorescent E2 could be taken up and degraded by known heterotroph E2 degraders; we were also able to accelerate this with an additional carbon source. Cell culture inserts for wells with a porous membrane at the base were shown to be a suitable medium for growth of the biofilm.

We were able to establish the conditions, necessary controls, quality control and quality assurance, and final protocol of a 24-well plate assay to select the most efficient of three degrading communities using real time fluorescence monitoring in a plate reader.



WHAT NEXT?

Research is continuing within the EPSRC Decentralised Water Technologies (EP/V030515/1) programme grant, in which Caroline Gauchotte-Lindsay (CGL) is a CoI. Validation with relevant natural communities will be carried out within this grant. Once this has been validated, she will take the project to the Converge Challenge.

OUTPUTS:

Video: https://youtu.be/SiC_MceeNAs

BODIPY-labelled estrogens for fluorescence analysis of environmental microbial degradation
By: Felion, C., Lopez-Gonzalez, R., Sewell, A. L., Marquez, R. and Gauchotte-Lindsay, C. In: ACS Omega, 7(45), pp. 41284-41295.