

Towards the development and design of in-situ faecal matter sensing platforms for aquatic environments.

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Abstract

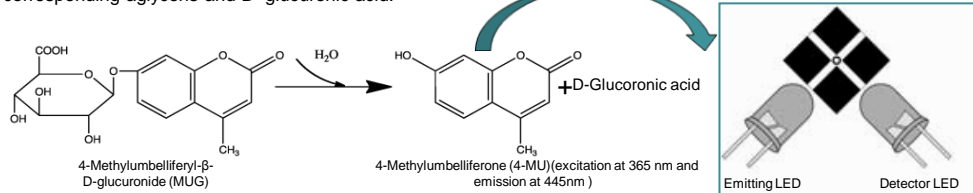
Standard methods for the assessment of faecal contamination of water rely on laboratory-based techniques, which are time-consuming, labour-intensive and unable to be employed for continuous monitoring. Currently, 18 hours are required after sampling for the analysis to be performed. Our focus is to develop a remote sensing platform able to continuously monitor and provide near real-time measurements of *Escherichia Coli* (*E. Coli*) in environmental waters.

The detection and quantification of *E. Coli* is studied using the activity of β -D-glucuronidase (GUD) marker enzyme.

Current work

•Working Principle

Measurement of *E. coli* in a water sample is achieved using Beta-D-glucuronidase fluorogenic (Scheme 1) or chromogenic substrates (Scheme 2); GUD catalyses the hydrolysis of β -D-glucuronides based substrates to corresponding aglycons and D-glucuronic acid.



Scheme 1. The hydrolysis of 4-methylumbelliferyl- β -D-glucuronide (MUG) releases fluorescent 4MU and the intensity of the measured fluorescent signal is proportional to the amount of enzyme present, therefore, indicating the number of *E. coli* present in the sample.

Figure 2. A schematic of an LED-LED system (L. Tymecki, R. Koncki, *Analyst*, 2011, 136,73)

Introduction

The microbiological quality of water is measured using indicator bacteria. These are bacteria, which themselves are not necessarily pathogenic, but indicate the presence of pathogenic bacteria due to the faecal contamination of water.

With the implementation of Directive 2006/7/EC by 2015, the EU is changing the way the microbiological contamination of water is measured, interpreted and classified. The directive includes the change of faecal indicator bacteria to Intestinal Enterococci and *E. coli*

Escherichia Coli (*E. coli*)

- Best indicator for faecal contamination according to EPA and WHO.
- Almost exclusively associated with faecal sources.



Figure 1. Low-temperature electron micrograph of a cluster of *E. coli* bacteria.

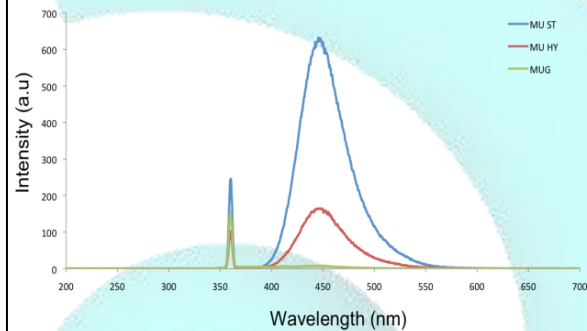
The assay of the marker enzymes can be suitable for continuous monitoring; 97% of *E. coli* strains are GUD positive, therefore in this study GUD is used as a marker enzyme for *E. coli*.

When glucuronides are present they induce *E. coli*'s synthesis of GUD, which is used to hydrolyze the sugars for use as energy or carbon sources.

Ideal Sensor Characteristics

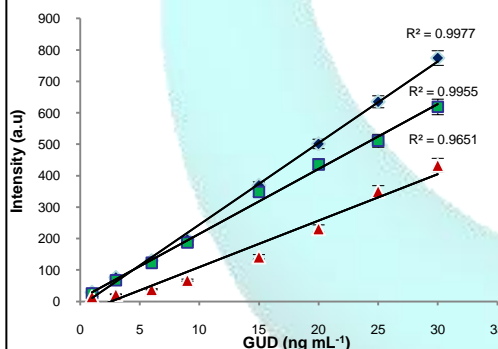
- Cost effective
- Resolution
- Repeatability
- Accuracy
- Robustness
- Sustainability
- Remote-sensing
- Sensor network
- Continuous Monitoring

Results



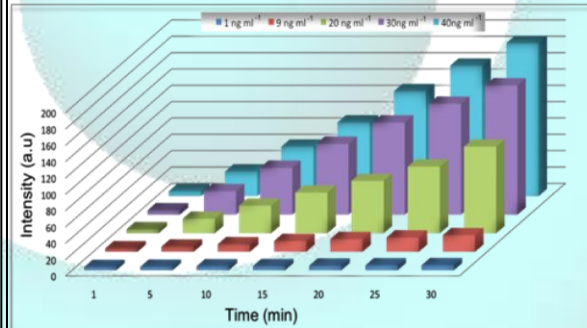
Graph 1. Spectra recorded at 360 nm excitation wavelength, showing the maximum emission at 445 nm for the commercial fluorophore (MU ST) and for the hydrolysed fluorophore (MU HY)

•Temperature Study



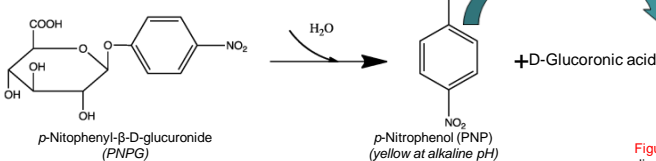
Graph 2. GUD hydrolysis rate at 44°C \blacktriangle , 37°C \blacksquare and 20°C \blacklozenge , and the correlation between GUD concentration and the intensity recorded at 445 nm.

•Time Study



Graph 3. GUD serial dilutions hydrolysis rate, in the presence of 4-MUG, over 30 min, at 37°C.

Future Work



Scheme 2. The hydrolysis of *p*-Nitrophenyl- β -D-glucuronide (PNPG)

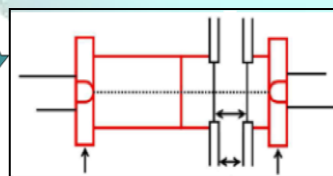


Figure 3. A schematic of the integrated paired emitter-detector diode flow analysis device used for colorimetric detection (M. O Toole, D. Diamond, *Sensors* 2008, 8, 2453-2479).

Acknowledgements

EC FP7 People Programme and the Marie Curie Foundation for funding this project.

