MESTECH
Marine & Environmental Sensing Technology Hub

A rapid, field portable test for faecal indicator detection

Brendan Heery, Ciprian Briciu-Burghina, Dermot Brabazon and Fiona Regan
Marine and Environmental Sensing Technologies Hub (MESTECH), National Centre for Sensor Research (NCSR), School of Chemical Sciences, Dublin City University, Dublin.
brendanheery@gmail.com

Introduction

The EU Bathing Water Directive 2006/7/EC sets limits for the microbial contamination in waters used for recreation. *E. coli* and Enterococci are used as indicators of faecal pollution. Table 1 shows the specified limits in Colony Forming Units (CFU) for marine and transitional waters.

Table 1. Bathing Water Directive *E.Coli* and Enterococci limits

Bacteria	Excellent	Good (Obligatory)
E.Coli	≤ 250 CFU /100 mL	≤ 500 CFU/100 mL
Enterococci	≤ 100 CFU /100 mL	≤ 200 CFU /100 mL

Commercially available culture based detection methods are slow. Colilert 18 and Petri-Film take 18 hours and 22 hours incubation respectively. This period, plus the time to take the sample and transport to the lab, means that a result is obtained next day. There is a demand for "Rapid" or same day test methods preferably *in-situ* and autonomous.

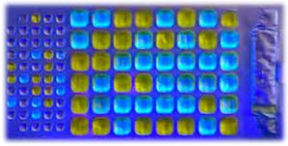

Fig 1. Colilert 18

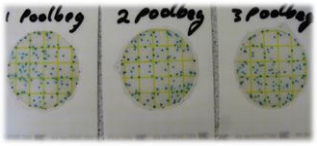

Fig 2. PetriFilm

Detection methodology

A fluorescence based enzymatic assay is used to detect *E.coli.* A chemical substrate e.g. 4-Methylumbelliferyl-ß-D-Glucoronide (4-MUG) is introduced to the water sample and taken up by the ß-Glucoronidase (GUD) positive *E.coli*. The substrate is hydrolysed to release a fluorescent molecule 4-Methylumbelliferone (4-MU) and a sugar. The resultant fluorescence can then be measured and used to quantify the bacteria. Figure 3 illustrates the process. This direct fluorescence approach can yield results in as little as 1 hour. A number of variations on this assay have been trialled to improve specificity and reliability.

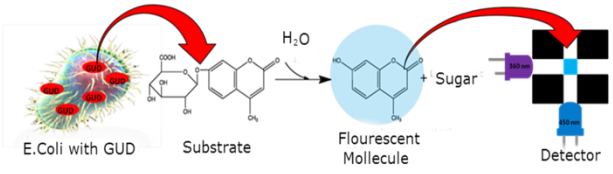

Fig 3. Enzyme substrate chemistry

Instrumentation development

A portable bench top instrument (ColiSense) was built to carry out sample analysis and trial different methods. Features include :

- Fluorescence detection (Ex: 365nm, Em: 445 nm)
- Incubation (Temperature controlled at 44°C)
- Triplicate sampling
- Portability

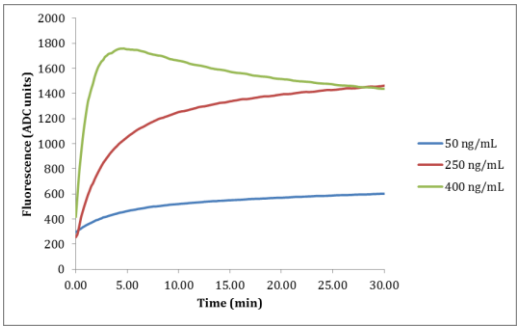

Fig 4. ColiSense response to GUD concentrations in 500 µM 6-Chloro-4-methylumbelliferyl ß-D-glucuronide (6-Cmug)

Conclusions

A prototype system (ColiSense) has been developed which is capable of performing a number of fluorescence based assays for faecal indicator detection. Detection times as low as 1 hour have been achieved. Reliability remains an issue.

Acknowledgements

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