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>
<thead>
<tr>
<th>Bacteria</th>
<th>Excellent</th>
<th>Good (Obligatory)</th>
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<tbody>
<tr>
<td><em>E. coli</em></td>
<td>≤ 250 CFU /100 mL</td>
<td>≤ 500 CFU /100 mL</td>
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<tr>
<td>Enterococci</td>
<td>≤ 100 CFU /100 mL</td>
<td>≤ 200 CFU /100 mL</td>
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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.

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-Methylumbellifereone (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.

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)
- Tripletic sampling
- Portability

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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