Continuous fluorometric method based on β-D-Glucuronidase for rapid detection of Escherichia Coli in water.

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Introduction

At present standard methods employed for the microbiological monitoring of bathing waters require at least 18 hours to perform and are based on culturing techniques. This is a huge drawback when immediate action is required. Real-time and on-line monitoring are key factors for consideration in current method development for continuous indicator organism detection in order to meet early warning requirements and water safety plans. The European Union has introduced a new Bathing Water Directive that is going to be implemented in all member states from 2008-2013. According to this directive classification of bathing water is in “excellent”, “good” and “sufficient” quality is based on the microbial indicators E.Coli and intestinal enterococci.

Methods

Utilising β-D-Glucuronidase (GUD) activity as an indicator of Escherichia Coli presence use labelled glucuronides to produce optical signals. Fluorometric assays for the measurement of Escherichia Coli/GUD activity are traditionally performed using the fluorogenic substrate 4-methylumbellifere-β-D-glucuronide (4-MUG) which upon hydrolysis releases the fluorophore 4-methylumbellifere (4-MU). The major drawback of 4-MU is its high $pK_a$ (7.8), which causes only partial dissociation at pHs around the optimum pH for GUD activity (6.5-7.0). To overcome this issue researchers have employed discontinuous enzyme assays which require the addition of alkali.

In this context we explore the spectrophotometric properties of three fluorogenic substrates and their respective aglycons (Fig. 1) for the continuous measurement of GUD activity and we apply the developed method for the rapid detection of Escherichia Coli in environmental water samples.

Results

UV VIS spectroscopy was used to determine the absorption $A_{max}$ for the fluorophores and substrates at different pH values and the protonation/deprotonation behaviour of the fluorophores (Fig. 2.)

Fluorescence spectroscopy was used to determine the $pH$ dependent fluorescence of the umbelliferone based fluorophores. When the excitation wavelength is selected to maximise the emission, the fluorescence intensity of 6-CMU in the 6.8-7.5 pH range is 6 times higher than that of 4-MU and 2.5 times higher than the fluorescence of 3-CU (Fig. 3).

The influence of substrate on the excitation and emission spectra of the fluorophores.

At $pH$ 6.8, the excitation spectra of 3-CU and 4-MU are strongly affected by the presence of substrate due to inner filter effects (Fig. 4 a,b) while 6-CMU is not (Fig. 5 c).

Conclusions

A continuous fluorometric method for the measurement of E.Coli GUD activity has been developed using 6-CMUG and offers a more straightforward approach for the evaluation of kinetic data. Benefits of this method as compared to a continuous one, include less sample manipulation, less reagent consumption, less experimental errors and better LOD. The method was applied for the detection of E. Coli from environmental water samples and was successful in predicting E. Coli concentrations below the EU threshold for “excellent quality”, in 1h.

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