

Executive Summary

Introduction

Monitoring of microbiological contaminants in water supplies requires fast and sensitive methods for the specific detection of indicator organisms or pathogens. The Fluorescence *in situ* Hybridisation (FISH) technology has been proven to represent a sensitive and rapid molecular method for the specific detection of microorganisms. Within the TECHNEAU project a protocol for the simultaneous detection of *E. coli* and coliform bacteria based on the FISH technology was developed (Deliverables 3.4.1 and 3.4.15). Moreover, FISH-protocols for the detection and quantification of *Pseudomonas aeruginosa* (Deliverable 3.4.14) and *Legionella* spp. (Deliverable 3.4.16) have also been elaborated. The developed FISH protocols consist of two different approaches. One approach allows the direct detection of single bacterial cells on filter membranes. The second approach combines an incubation of the filter membranes on a nutrient agar plate and the subsequent detection of the grown micro-colonies.

Importance

Methods applicable for the analysis of drinking water samples have not only to be specific, but also sensitive enough to detect 1 cell/100 mL. For the single cell approach the microscopic evaluation becomes extremely labour-intensive, as the complete filter must be manually scanned using a high microscopic magnification to detect every single fluorescent cells present on the membrane. Hence, there is a need for automation of cell detection and counting in order to make the single cell approach practicable for routine analyses of bacteria in drinking water samples.

Approach

In this study, methods for the automated quantification of the fluorescent cells were developed and evaluated. In advance, a theoretical market study was performed in order to investigate different technologies, which can be applied for the quantification of the FISH-stained single cells and micro-colonies. Two systems for each approach were chosen and further tested: a laser-based system (AES Chemunex) as well as a microscope-based system (Gevitec) for the single cell approach, and a stereomicroscope-based (Leica) and a LED-reader-based (Synentec) system for the micro-colony approach.

Result

The outcome of this study was that no successful automatic quantification of single cells could be established. Regarding the laser-based system ChemScan RDI from AES Chemunex sensitivity issues arose, which could not be solved. The main drawback of the microscope-based system from Gevitec was the lack of automatic focus, which made the time requirements for a complete filter scan tremendously high. Thus under present circumstances, the automated quantification of fluorescent single cells is not an option for routine evaluation of drinking water samples.

In contrast to this the micro-colony approach has the potential of practical use. If a stereo-microscope is applied, a basic quantification macro is available, which was established and successfully validated in this study. Even manual quantification of the micro-colonies is not very time consuming and can therefore be used in routine, too. The LED-based technology however would still need some developmental effort to produce a prototype. All in all it turned out, that FISH-labelled micro-colonies detected and counted with a stereo-microscope is a promising and robust technology for routine analysis of drinking water. The time requirement for the whole procedure (filtration, incubation, FISH labelling and automated evaluation) is normally less than 1 working day. Hence this procedure is considerably faster than traditional culture-based methods.

More information

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