Introduction

Less than 1 % of bacteria in drinking water are typically culturable on conventional growth media with the heterotrophic plate count (HPC) method. In addition, it is believed that some culturable bacteria can enter a state of unculturability when exposed to nutrient-poor environments. Therefore, a need exists for rapid and cultivation-free methods with which the viability/activity of drinking water microbial communities can be assessed.

Importance

Accurate assessment of viability/activity of the entire microbial community in drinking water can be used to judge the efficacy of disinfection treatment processes (e.g. ozonation, chlorination and membrane filtration), or to follow microbial events such as regrowth during activated carbon filtration or sand filtration. In addition, this will provide tools with which to study microbial proliferation during distribution network, thereby monitoring biological stability of drinking water.

Approach

This study investigated cultivation-free viability analysis using fluorescent staining methods coupled with flow cytometric detection/enumeration. The study focussed solely on quantification of cells in the planktonic phase. The results were normalised to flow cytometric total cell counts (TCC), and compared to conventional HPC and total adenosine tri-phosphate (ATP) analysis. Three fluorescent dyes, targeting different aspects of viability, were chosen based on available data in literature and preliminary experiments. These were propidium iodide (targeting membrane integrity), $DiBAC_3(4)$ (targeting membrane potential) and CFDA (measuring esterase activity).

Results

The results have shown propidiuim iodide in combination with SYBR Green I to be an easy method which can be used to assess severe cell damage as caused by ozonation, chlorination, heat and UV-A treatments. While $DiBAC_3(4)$ generally produced similar results to propidium iodide, the esterase activity assay (CFDA) tended to detect significantly less "viability" than the other dyes. ATP analysis was found to be a very sensitive indicator of viability as well, although care should be taken with free-ATP in water samples, especially during drinking water treatment with ozonation.

More information

Full details on this deliverable can be found under D3.3.8. Further information can be requested from:

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TECHNEAU Knowledge Integrator (TKI) categorisation

Categorisation of Knowledge Packages

Categorisation (i.e. classification, contains and constraints) of knowledge packages (KPs) can be carried out by 'checking' the appropriate boxes in the attached tables. For example, for a KP investigating point-of-use treatment suitable for a developing world country, the following might be checked:

Classification: Process chain – Tap (Customer) – Point-of-use (POU).
Contains: Report; Literature review.
Constraints: Low cost; Simple technology; No/low skill requirement; No/low energy requirement; No/low chemical requirement; No/low sludge production; Developing world location.

Note that only the lowest level classification needs to be checked, e.g. Point-of-use (POU) in the above example.

Meta data can be included under the 'More Information' section of the Executive Summary Report, i.e. Author(s), Organisation(s), Contact details (name and email), Quality controller (name and organisation) and Date prepared. (The TKI administrator can enter Source (= TECHNEAU), Date submitted (TKI) and Date revised (TKI)).