# Viability and metabolic activity of drinking water biofilm bacteria – assessment using EFM


## Summary

In this report an overview on different viability/activity detection methods is presented in respect of their suitability in combination with fluorescence in situ hybridization (FISH) and epifluorescence microscopy (EFM). Details concerning specific probes optimized within TECHNEAU project are considered as well. First, a literature review on the definition of viability is presented and the concept formulated. Viability can be operationally demonstrated by culture (turbidity, microcolonies, macrocolonies). Thus the methods based on the use of fluorescence can only evaluate some metabolic functions (activity) or the integrity of the cell structures, with a possible exception of Direct Viable Count (DVC) method. The exact mechanism of DVC, however, is not clear, although the cell elongation is assumed to be growth potential related. Next, the available methods are grouped and described, depending on their mode of action. Results, obtained in the Water Research Laboratory (WRL), Riga Technical University are described, where available. The most suitable methods were DVC, which gives an indication of viability since the method shows the potential to divide and CTC which indicates the respiratory activity. These methods were also used to study the fate and viability of *E. coli* in controlled conditions (see D.3.6.8.1 and the corresponding report) and in the treatment train of Riga Water Plant and distribution network (see D.3.6.8.2 and the corresponding report). Quantification aspects using FISH are discussed as well. Procedures for retaining maximum number of cells were investigated and it was found that if the whole DVC procedure was performed using the filters, about 80% of the cells were retained. Finally, the complete protocol for analysis of biofilm samples is given.


## Importance

Bacteria in the environment are exposed to different stresses through which they may become unculturable using the common media for these bacteria. Such bacteria, often called ABNC (active but not culturable), have acquired stress resistance by active mechanisms, which, in turn are genetically programmed but have developed recalcitrance to culture. However, such microorganisms can still possess metabolic activity and thus, the potential to infect the population, even if they are not detected using the conventional assay methods, employing culturing.

## Approach

Different activity measurement methods were compared and assessed in respect to their suitability in combination with EFM.

## Result

An assessment of available non-culture based methods which use EFM is given and the two most suitable methods, namely DVC and CTC analyses are selected for the field application. A complete protocol starting from the collection of samples till visualization is given as well.

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**TKI Categorisation**

| Classification | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Supply Chain** | | **Process Chain** | | **Process Chain (cont'd)** | | **Water Quality** | | **Water Quantity (cont'd)** | |
| | | | | | | | | | |
| **Source** | | **Raw water storage** | | **Sludge treatment** | | **Legislation/regulation** | | - Leakage | |
| - Catchment | | - Supply reservoir | | - Settlement | | - Raw water (source) | | - Recycle | |
| - Groundwater | | - Bankside storage | | - Thickening | | - Treated water | | | |
| - Surface water | | **Pretreatment** | | - Dewatering | | **Chemical** | | | |
| - Spring water | | - Screening | | - Disposal | | - Organic compounds | | | |
| - Storm water | | - Microstraining | | **Chemical dosing** | | - Inorganic compounds | | | |
| - Brackish/seawater | | **Primary treatment** | | - pH adjustment | | - Disinfection by-products | | | |
| - Wastewater | | - Sedimentation | | - Coagulant | | - Corrosion | | | |
| **Raw water storage** | | - Rapid filtration | | - Polyelectrolyte | | - Scaling | | | |
| - Supply reservoir | | - Slow sand filtration | | - Disinfectant | | - Chlorine decay | | | |
| - Bankside storage | | - Bank filtration | | - Lead/plumbosolvency | | **Microbiological** | | | |
| **Water treatment** | | - Dune infiltration | | **Control/instrumentation** | | - Viruses | | **Consumers / Risk** | |
| - Pretreatment | | **Secondary treatment** | | - Flow | | - Parasites | | | |
| - Primary treatment | | - Coagulation/flocculation | | - Pressure | | - Bacteria | X | **Trust** | |
| - Secondary treatment | | - Sedimentation | | - pH | | - Fungi | | - In water safety/quality | |
| - Sludge treatment | | - Filtration | | - Chlorine | | **Aesthetic** | | - In security of supply | |
| **Treated water storage** | | - Dissolved air flotation(DAF) | | - Dosing | | - Hardness / alkalinity | | - In suppliers | |
| - Service reservoir | | - Ion exchange | | - Telemetry | | - pH | | - In regulations and regulators | |
| **Distribution** | X | - Membrane treatment | | **Analysis** | | - Turbidity | | **Willingness-to-pay/acceptance** | |
| - Pumps | | - Adsorption | | - Chemical | | - Colour | | - For safety | |
| - Supply pipe / main | | - Disinfection | | - Microbiological | X | - Taste | | - For improved taste/odour | |

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|---|---|---|---|---|---|---|---|---|---|
| **Tap (Customer)** | X | - Dechlorination | | - Physical | | - Odour | | - For infrastructure | |
| - Supply (service) pipe | | **Treated water storage** | | | | | | - For security of supply | |
| - Internal plumbing | | - Service reservoir | | | | **Water Quantity** | | **Risk Communication** | |
| - Internal storage | | **Distribution** | | | | | | - Communication strategies | |
| | | - Disinfection | | | | **Source** | | - Potential pitfalls | |
| | | - Lead/plumbosolvency | | | | - Source management | | - Proven techniques | |
| | | - Manganese control | | | | - Alternative source(s) | | | |
| | | - Biofilm control | x | | | **Management** | | | |
| | | **Tap (Customer)** | | | | - Water balance | | | |
| | | - Point-of-entry (POE) | | | | - Demand/supply trend(s) | | | |
| | | - Point-of-use (POU) | | | | - Demand reduction | | | |

## TKI Categorisation (continued)

| **Contains** | | **Constraints** | | **Meta data** | | |
|---|---|---|---|---|---|---|
| Report | X | Low cost | | *Author(s)* | Talis Juhna | |
| Database | | Simple technology | | *Organisation(s)* | RTU | |
| Spreadsheet | | No/low skill requirement | | *Contact name* | Talis Juhna | |
| Model | | No/low energy requirement | | *Contact email* | talisj@bf.rtu.lv | |
| Research | X | No/low chemical requirement | | *Quality controller name* | Frederik Hammes and Claudia Beimfohr | |
| Literature review | | No/low sludge production | | *Quality controller organisation* | EAWAG and Vermicon | |
| Trend analysis | | Rural location | | Source | | |
| Case study / demonstration | | Developing world location | | *Date prepared* | 30/04/08 | |
| Financial / organisational | | | | Date submitted (TKI) | 30/04/08 | |

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|---|---|---|---|---|---|---|
| Methodology | X | | | Date revised (TKI) | 30/08/08 | |
| Legislation / regulation | | | | | | |
| Benchmarking | | | | | | |