Feasibility of FISH-FCM for drinking water microbial population analysis

Introduction

Fluorescent in situ hybridization (FISH) uses rRNA-targeting oligo-nuleotide probes to visualize and identify specific bacteria or bacterial groups in natural environments. The FISH method is normally done in combination with epi-fluorescence microscopy, but flow cytometry (FCM) has been proposed previously as a method to achieve rapid and quantitative results. However, some practical problems, typically associated with drinking water bacteria, such as weak fluorescence signals (from low RNA content in the cells) and cell loss during the FISH procedure (specifically washing steps) have hampered the practical implementation of this approach.

Importance

Rapid determination of the population composition of a drinking water sample has great value for assessment of changes in the water quality during treatment and distribution.

Approach

We have used a published FISH-FCM protocol as basis of the work and adapted this to target the specific problems of the method. We employed double staining with DAPI, together with the FISH probes, and multi-laser FCM analysis in order to avoid the washing step after hybridisation of the probed with the targeted cells. We furthermore tested several filtration-resuspension protocols as means to overcome the first washing step after permeabilisation.

Result

The double staining, double laser approach was effectively employed to avoid the second washing step (after hybridisation) in the FISH protocol. However, none of the filtration-resuspension steps proved to be successful from a quantitative perspective in order to avoid the first washing step (after permeabilisation and fixation). Cell loss occurred during all protocols tested. As a result we concluded that the FISH-FCM method does not hold a practical advantage over the conventional microscopy-FISH method at this stage.

More information

Full details on this deliverable can be found under D3.3.2 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)).

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

- Internal plumbing		- Service reservoir				**Water Quantity**		**Risk Communication**	
- Internal storage		**Distribution**						- Communication strategies	
		- Disinfection	x			**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)	x			- Demand/supply trend(s)			
		- Point-of-use (POU)	x			- Demand reduction			

TKI Categorisation (continued)

Contains		**Constraints**		**Meta data**					
Report	x	Low cost		*Author(s)*		Frederik Hammes			
Database		Simple technology		*Organisation(s)*		EAWAG			
Spreadsheet		No/low skill requirement		*Contact name*		Frederik Hammes			
Model		No/low energy requirement		*Contact email*		Frederik.hammes@eawag.ch			
Research	x	No/low chemical requirement		*Quality controller name*					
Literature review		No/low sludge production		*Quality controlerl organisation*					
Trend analysis		Rural location		Source					
Case study / demonstration		Developing world location		*Date prepared*		26-06-2007			
Financial / organisational				Date submitted (TKI)		28-06-2007			
Methodology	x			Date revised (TKI)					
Legislation / regulation									
Benchmarking									