Techneau, 06.
June 2006

Review and Selection of Monitoring Parameters and Methods

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TECHNEAU is an Integrated Project Funded by the European Commission under the Sixth Framework Programme, Sustainable Development, Global Change and Ecosystems Thematic Priority Area (contractnumber 018320).

Colophon

Title
TECHNEAU

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Quality Assurance
By partners within WP 5.5

Deliverable number
D 5.5.5

The overall aim of WP 5.5 is development of models about water quality changes in water distribution networks. Experimental verification of the models in lab-, pilot- and full-scale is indispensable stage in their development. In order to carry out experiments by different laboratories the analytical methods used for verification has to be selected and harmonised among partners. In this deliverable the routinely used methods and the ones in the stage which will be used by the authors in further experiments are comprehensively presented.

Importance

The goal of a drinking water distribution system is to deliver sufficient quantities of water where and when it is needed at acceptable quality. Although water quality may be acceptable when water leaves a treatment plant, transformations can occur though a distribution networks causing degradation of aesthetic (e.g. turbidity, smell, taste) and hygienic quality of water. The mechanisms effecting these changes are many including intrusion from broken pipes, corrosion of cast iron pipes, bacterial regrowth. An effective approach to control water quality is to apply water quality models. They enable a better understanding of the most important processes and hence open the way for efficient operation and maintenance measures. Most of the models require analyses of many parameters as input data for their verification in field- and lab-scale studies. As this study will be carried out in several laboratories with different analytical practices, there is a need to harmonize analytical methods between the partners. The aim of this deliverable was to review and select analytical methods that will be used in further laboratory-, pilot- and field-scale studies within WP 5.5 in TECHNEAU project.

Approach

Information from all partners were compiled and collapsed into the report using the common template. Some of the analytical details were adjusted, so the most of the partners are able to carry out analyses at their labs.

Result

34 methods were presented and described. Some of the methods are well established, thus only links to the particular standard are provided. Other methods are under stage of development therefore shall be improved during the project.

More information

The deliverable is subject to continuous upgrading during the TECHNEAU project.

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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	x	- Dune infiltration		**Control/instrumentation**		- Viruses		**Consumers / Risk**	
- Pretreatment		**Secondary treatment**		- Flow		- Parasites			
- Primary treatment		- Coagulation/flocculation		- Pressure		- Bacteria		**Trust**	
- Secondary treatment		- Sedimentation		- pH		- Fungi		- In water safety/quality	
- Sludge treatment		- Filtration		- Chlorine		**Aesthetic**		- In security of supply	
Treated water storage	x	- 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		- Taste		- For improved taste/odour	
Tap (Customer)		- 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**	x					- 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)*		T. Juhna, J. Rubulis , S.W.Osterhus, M. Esa, F. Hammes, B. Wricke , J. Menaia, P. Schaap, J. Vreeburg			
Database		Simple technology		*Organisation(s)*		RTU, SINTEF, TZW, LNEC, KIWA			
Spreadsheet		No/low skill requirement		*Contact name*		T. Juhna			
Model		No/low energy requirement		*Contact email*		talisj@bf.rtu.lv			
Research		No/low chemical requirement		*Quality controller name*		S.W.Osterhus, M. Esa, F. Hammes, B. Wricke , J. Menaia, P. Schaap, J. Vreeburg			
Literature review		No/low sludge production		*Quality controlerl organisation*		SINTEF, TZW, LNEC, KIWA			
Trend analysis		Rural location		Source					
Case study / demonstration		Developing world location		*Date prepared*		06/2006			
Financial / organisational				Date submitted (TKI)		06/2006			
Methodology	x			Date revised (TKI)		01/07			
Legislation / regulation									
Benchmarking									

Content

1 Introduction

The goal of a drinking water distribution system is to deliver sufficient quantities of water where and when it is needed at acceptable quality (Grayman et al, 2000). Although water quality may be acceptable when water leaves a treatment plant, transformations can occur though a distribution networks causing degradation of aesthetic (e.g. turbidity, smell, taste) and hygienic quality of water. The mechanisms effecting these changes are many including intrusion from broken pipes, corrosion of cast iron pipes, bacterial regrowth.

To control water quality changes water suppliers are monitoring water quality at different location in water supply system. Another effective approach to control water quality is to apply water quality models. They enable a better understanding of the most important processes and hence open the way for efficient operation and maintenance measures. Most of the models require analyses of many parameters as input data for their verification in field- and lab-scale studies. As this study will be carried out in several laboratories with different analytical practices, there is a need to harmonize analytical methods between the partners.

The aim of this deliverable is to review and select analytical methods that will be used in further laboratory-, pilot- and field-scale studies within WP 5.5 in TECHNEAU project.

The following report contains two sections. In the first section the list of water quality monitoring parameters and partners who are going to use them in the study are presented. Some of the parameters are **routine parameters** for which standards are available and they are often used by water industry. Others are specific parameters (**research parameters**) which are mainly used for research purposes, and many cases are in the stage of development.

In the second section the analytical methods for measuring the parameters are described in details.

References

Grayman W.M., Rossman L.A. and E.E.Geldreich. (2000) Water quality; Chapter 9. In: Water Distribution Systems Handbook. Larry W.Mays ed. American Water Works Association. McGraw-Hill. Inc.

2 List of analytical methods

Analytical method	Partner						Research parameter	Routine parameter
	TZW	KIWA	SINTEF	RTU	LNEC	EAWAG		
1	2	3	4	5	6	7	8	9
Total organic carbon (TOC)/dissolved organic carbon (DOC)			x	x				x
Biodegradable NOM fractions (with LC-UV-OCD)	x						x	
Biodegradable dissolved organic carbon (BDOC)			x	x			x	
Assimilable organic carbon (AOC)				x	x	x	x	
Microbially available phosphorus (MAP)				x			x	
Measurement of adenosine triphosphate (ATP)		x		x		x		x
Total cell number (TBN) in water	x			x				x
Rapid enumeration of total cell number with flow cytometry						x	x	
Enumeration of culturable microorganisms (as HPC) (water/biofilm)			x	x	x			x
Cultivation-independent viability assessment with flow cytometry						x	x	
Enumeration of bacteria in pipe sediments					x		x	
Resuspension Potential Method	x	x					x	
Time Integrated Large Volume Sampling (TILVS)		x					x	
Biofilm removal procedure	x			x			x	
Corrosion rate by weight loss measurements (*)			x				x	
Weight of corrosion product accumulation (*)			x				x	
Analysis of corrosion products by Scanning Electron Microscopy (*)			x				x	
Analysis of corrosion products by X-ray (*)			x				x	
Acid capacity	x							x
Alkalinity			x	x				x
Aluminium			x	x				x
Ammonium			x					
Calcium (and sum of calcium and magnesium)			x	x				x
Chloride (and other anions)	x		x					x
Total chlorine and free chlorine			x	x				x
Color			x	x				x
Conductivity			x	x				x
Iron	x		x	x				x
Dissolution of iron oxides							x	
Manganese	x		x	x				x
Nitrate			x	x				x
Nitrite				x				x
Total nitrogen			x	x	x			x
Oxygen	x							x
pH value	x		x	x				x
Total phosphorus			x	x				x
Silicates			x					x
Turbidity	x		x					x

(*) – reference to the report by TZW, where methods for corrosion experiment with test rigs will be described.

3 Description of analytical methods

3.1 Total organic carbon /dissolved organic carbon

European Standard CEN 1484. Water analysis - Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC).

3.2 Biodegradable NOM fractions

Method by TZW

Scope

The LC-OCD (Liquid Chromatography - Organic Carbon Detection) technique is able to give both quantitative and qualitative information on NOM. Quantification is based on carbon mass determination (like in TOC analysis) and qualitative analysis is based on the gel chromatographic separation of NOM prior to analysis. To be able to do that carbon mass determination is made with an organic carbon detector (OCD) which produces a TOC-value about every second.

The OCD is based on a *Gräntzel* gravity-flow thin-film reactor. The organic and inorganic carbon species (OC, IC) are separated in the upper part of the reactor by means of continuous acidification and nitrogen stripping. The quantitative oxidation of the organic bound carbon takes place in an oxygen-free atmosphere via radiolytic dissociation (wavelength 185 nm) of small amounts of water into highly reactive oxygen radicals. This technique is a VUV (vacuum-UV) technique. The reaction product carbon dioxide is measured by non-dispersive infrared absorption. The detection limit for TOC is in the low-ppb concentration range (1 -10 ppb).

Polymer filled columns are used for the chromatographic separation process. The dominating separation criterion is the molecular size (size exclusion chromatography, SEC). Secondary chromatographic effects, including hydrophobic interaction and ionogenic interaction, are also active and help to characterize the chromatographic fractions.

In order to identify biodegradable organic fractions LC-OCD measurements are performed from incubated BDOC samples (as per description *Biodegradable organic carbon detection BDOC*) simultaneous to conventional DOC measurement.

References

Huber, S.A.; Frimmel, F.H.: A Liquid Chromatographic System with Multi-Detection for the Direct Analysis of Hydrophilic Organic Compounds in Natural Waters. Fresen. J. Anal. Chem. 342, 198-200 (1991).

Huber, S.A.; Frimmel, F.H.: A New Method for the Characterization of Organic Carbon in Aquatic Systems. Intern. J. Environ. Chem., 49, 49-57 (1992).

3.3 Biodegradable dissolved organic carbon (BDOC)

Method by TZW

Scope

The BDOC is the fraction of the dissolved organic carbon (DOC) in water which can be metabolized by bacteria within a period of a few days to a few months.

Procedure

The BDOC is determined by using a fixed biofilm. The biofilm is cultured on sintered glass carrier beads (i.e. SIRAN, Schott Ref. SIKUG 012/05/300/A) by incubating the carrier in a filter column (5 cm x 40 cm) at the sample tap of finished water (without chlorine) in a water works with a flow rate of approximately 200 mL/min. To adapt the microorganisms, the carriers are stored for 28 days in a glass flask with drinking water (weekly water exchange). 100 g of the wet carrier are filled in a 500 mL glass bottle. The carrier are washed three times with the sample water (shake carefully on a horizontal shaker for 20 min for every washing step). After that the bottle is filled with the sample water and incubated in the dark on a horizontal shaker (shake carefully) at 20°C. The samples for the TOC analysis are taken immediately at the beginning of the incubation period and afterwards at the 2nd, 5th, 7th, 14th, 21st, and 28th day. The biodegradable dissolved organic carbon (BDOC) is obtained from the difference between the initial DOC and the minimal value of DOC measured during incubation.

Method by SINTEF

Procedure

We have used the method by Servais et al. (1989). The samples are filtered through 0.2 µm filter. A suspended inoculum, filtered through 2 µm filter, is added to the samples (volume 1% of the sample volume). The samples are incubated for 4 weeks at 20°C and after which DOC is analysed. The control samples have been acidified to around 2 and stored at +4°C.

References

Servais, P., Anzil, A. and Ventresque, C. (1989) Simple method for determination of biodegradable dissolved organic carbon in water. Appl. Environ. Microbiol. 55(10), 2732-2734.

3.4 Assimilable organic carbon (AOC)

Method by RTU

a. The Dutch standard method NEN 6271.
b. AOC method modification

Scope

The AOC bioassay using *Pseudomonas fluorescens* P-17 and *Aquaspirillum* sp. NOX involves growth to a maximum density of a small inoculum in a batch culture of pasteurized test water. Pasteurization inactivates native microflora.

The test organisms are enumerated by spread plate method for heterotrophic plate counts and the density of viable cells is converted to AOC concentrations by an empirically derived yield factor for the growth of P. fluorescens P-17 on acetate-carbon and Aquaspirillum sp. NOX on oxalate-carbon as standards. The number of organisms at stationary phase is assumed to be the maximum number of organisms that can be supported by the nutrients in the sample and the yield on acetate carbon is assumed to equal the yield on naturally occurring AOC (van der Kooij 1982, Kaplan and Bott 1989). The underlying assumption of the AOC bioassay is that the bioassay organism(s) represent the physiological capabilities of the distribution system microflora. In some waters (e.g humic waters) inorganic nutrients regulate bacterial growth (Miettinen et al., 1999). Thus, to ensure that carbon is limiting bacterial growth, enough of inorganic nutrients are added in sample of test water. In theory, concentrations of less than 1 µg C/L can be detected. In practice, organic carbon contamination during glassware preparation and sample handling imposes a limit of detection of approximately 5 to 10 µg AOC/L. High concentration of metals (esp. Al, Cu) is toxic for strain P. fluorescens, which makes this procedure unsuitable for waters containing these metals.

References

Kaplan L.A., Bott T.L. 1989. Measurement of assimilable organic carbon in water distribution systems by a simplified bioassay technique. In Advances in Water Analysis and Treatment, Proc. 16th Annu. AWWA Water Quality Technology Conf.,Nov. 13-17, 1988, St. Louis, Mo., p. 475. American Water Works Assoc., Denver,Colo.

Miettinen I.T., Vartiainen T. and Martikainen P.J. 1999. Determination of assimilable organic carbon in humus-rich drinking waters. Water Res. 33 (10): 2277-2282.

Reasoner D.J., Geldreich E.E. 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol. 49:1-7.

Swanson K.M.J., Busta F.F., Peterson E.H., Johnson M.G. 1992. Count methods. In Compendium of methods for the microbiological examination of foods. Vanderzant C., Splittstoesser D.F., eds. APHA, Washington, 75-95.

Van der Kooij D., Visser A., Oranje J.P. 1982. Multiplication of fluorescent pseudomonads at low substrate concentrations in tap water. Antonie van Leeuwenhoek 48:229-243.

Method by EAWAG

AOC is measured in a bioassay using a site-specific natural microbial community and fluorescence staining coupled with flow cytometry for enumeration (Hammes and Egli, 2005; Hammes et al., 2006). As in the Dutch standard method, the maximum growth (stationary phase) of a relatively small inoculum (c.a. 1 x 10^4 cells/mL) is measured. Incubation is done at 30 °C and stationary phase is reached between 24 – 48 h. Fluorescence staining with SYBR®green, SYTO9, DAPI or any other dye which targets all bacterial cells can be used for enumeration. The best experience currently is with SYBR®green. The cell concentration is measured with flow cytometry.

Epifluorescence microscopy can also be used but will be more arduous. We use a PARTEC flow cytometer equipped with volumetric cell counting, but other instruments would also suffice. A broad mixture of synthetic substrates (organic acids, amino acids and sugars) is used as a positive control, and the detection limit of the method is around 5 µg AOC/L. The method generally produces higher AOC values than the Dutch standard method, probably as a result of a broader substrate range of the natural community. The method furthermore offers the advantage of following microbial growth kinetics or differential staining, due to the rapid nature of flow cytometric analysis. Standardisation of the method is part of the Techneau project (WP3.3).

References

Hammes F.A. and Egli, T. (2005) New method for assimilable organic carbon determination using flow-cytometric enumeration and a natural microbial consortium as inoculum. Environmental Science and Technology **39**, 3289-3294.

Hammes F., Salhi E., Köster O., Kaiser H.-P., Egli T. and von Gunten U. (2006) Mechanistic and kinetic evaluation of organic disinfection by-product and assimilable organic carbon (AOC) formation during the ozonation of drinking water. *Water Research, **In Press,** Available online 13 June 2006*

3.5 Microbially available phosphorus (MAP)

Method by RTU

Scope

The MAP bioassay using *Pseudomonas fluorescens* P-17 involves growth to a maximum density of a small inoculum in a batch culture of pasteurized test water. Pasteurization inactivates native microflora. The test organism is enumerated by spread plate method for heterotrophic plate counts and the density of viable cells is converted to MAP concentrations by an empirically derived yield factor for the growth of *Ps. fluorescens* P-17 on phosphate phosphorus as standard. The number of organisms at stationary phase is assumed to be the maximum number of organisms that can be supported by the nutrients in the sample. The yield on phosphate-phosphorus (PO_4-P) is assumed to be equal the yield on naturally occurring MAP. *Ps. fluorescens* P-17 has phosphatase activity. The underlying assumptions of the MAP bioassay are that the carbon and inorganic nutrients, with the exception of phosphorus, are present in excess, i.e., that phosphorus is limiting (Lehtola et al., 1999). Concentrations 0.08-10 µg MAP/L can be detected (Lehtola et al.,1999). High concentration of metals (esp. Al, Cu) are toxic for strain *Ps. fluorescence*, which makes this procedure unsuitable for waters containing these metals.

References

Lehtola M.J., Miettinen I.T., Vartiainen T., Martikainen P.J. 1999. A new sensitive bioassay for determination of microbially available phosphorus in water. Appl.Environ. Microbiol. 65:2032.

Reasoner D.J., Geldreich E.E. 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol. 49:1.
Swanson K.M.J., Busta F.F., Peterson E.H., Johnson M.G. 1992. Count methods. In Compendium of methods for the microbiological examination of foods. Vanderzant C., Splittstoesser D.F., eds. APHA, Washington, 75-95.

3.6 Measurement of adenosine triphosphate (ATP)

Method by EAWAG

The BacTiter-Glo™ Microbial Cell Viability Assay (Promega), based on the luciferine-luciferase assay, and a luminometer (Turner BioSystems, Sunnyvale; USA) is used. Prior to analysis, 100 µL of an ATP reagent is pre-warmed in a water bath together with 150 µL water sample in separate, sterile Eppendorf tubes. After 2 min incubation at 30 °C, 100 µL of the water sample is transferred into the 100 µL reagent (ratio 1:1) and mixed with the transfer pipette. The Eppendorf tube is briefly dried with paper and then promptly placed into the luminometer for measurement of light intensity expressed in Relative Light Units (RLU). RLU are converted into ATP concentrations by means of an ATP standard curve which was generated for each new reagent using a pure ATP standard. For drinking water and surface water, a working range of 0.0 to 0.1 nM for the standard range is usually sufficient. Nanopure water is used as a blank. An ATP signal of 2000 - 3000 RLU (c.a. 0.01 nM) was considered the lower detection limit above which statistically accurate measurements were possible (standard deviation < 5 %).

Method by KIWA

The measurement of ATP is based on the production of light in the luciferine-luciferase assay. ATP will be released from suspended cells with nucleotide-releasing buffer (NRB, Celsis). The intensity of the emitted light will be measured in a luminometer (Celsis Advance™) calibrated with solutions of free ATP (Celsis) in autoclaved tap water following the procedure as given by the manufacturer. The detection limit is 1 ng ATPL~^{1} of sample. Standard additions of free ATP dissolved in autoclaved tap water are used for recovery experiments.

3.7 Total cell number (water/biofilm)

Method by RTU

Scope
This method describes a procedure for counting all bacteria in water and homogenised and/or diluted biofilm samples using the dye 4´, 6-diamidino-2-phenylindole (DAPI). There are other fluorochromes which can be used for the same purpose e.g. Acidine Orange and Syto 9. Acridine Orange is the dye which is often used by the semiconductor industry to estimate the total cell number in ultra pure water. The procedure follows in this case the ASTM Standard Test Method Designation F 1095-8. The advantage of Acridine

Orange is that it will show bright cells with the disadvantage that the stain is much more unspecific to noncellulare material. The total cell number of biofilms can be evaluated directly without removing the biofilm by using the Confocal Laser Scanning Microscope. Staining will be performed the same way like water samples (filter method). Counting is performed with epiflourescence microscope.

References

Hobbie, J.E., R.J. Daley, Jaspers, S. (1977): Use of Nucleopore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33: 12225-1228.

ASTM Standard Test Method: Designation: F 1095 – 8 (Reapproved 1994). Rapid enumeration of bacteria in Electronic-Grade Purified Water Systems by Direct Count Epifluorescence Microscopy.

Materials

4´, 6-Diamidino-2-phenylindole (DAPI), black Nucleopore filter (pore size 0.2 µm), non-fluorescent immersion oil.

All reagents should be filtered through a cellulose nitrate filter with the pore size of 0.2 µm.

Procedure

Place one black polycarbonate membrane filter (0.2 µm pore size, laser beamed) on top of the sampling port, assuring that the shiny side of the filter is facing upwards. Filter an aliquot of the sample and stop the filtration process immediately when the sample is filtered through. Supplement with 1 mL DAPI (10 µg/mL) and add 1 mL Triton X-100 (0.1 %). The final concentration should be 5 µg/mL. The incubation time should be 15 to 20 minutes. Then the supernatant is filtered through and the filter with the stained bacteria placed in a petri dish or any other box to let it air dry. If the filter will be stored more than 2 days, add formaldehyde (1 % v/v) to the DAPI solution. The air dried filter is prepared for the microscope by embedding it in immersion oil on the surface of a clean microscope slide; like a sandwich the filter is between the immersion oil and a clean glass coverslip. The enumeration of bacteria and other microorganismns are performed with a magnification of at least 1000 fold in a epifluorescence microscope. All blue stained cells are counted in randomly chosen microscopic viewing fields delineated by the eyepiece micrometer. There should be 10-50 cells per viewing field. In minimum 300 bacteria should be counted or so many viewing fields that the coefficient of variation of < 30% is obtained.

3.8 Rapid enumeration of total cell number with flow cytometry

Method by EAWAG

Total cells in a water sample are stained with SYBRgreen (15 minutes in the dark). Alternative dyes can be used, based on the characteristics of the flow cytometer (e.g. DAPI with a UV laser, SYTO9 and SYBRgold with a blue laser). The best experience in our group with drinking water is using

SYBRgreen. We use a PARTEC (PASIII and/or Cyflow SPACE) equipped with volumetric counting for the analysis. A sample is processed in about 3 minutes with an operational error of about 4 %. The detection limit of this method is usually between 1 – 5 x 10^3 cells/mL for drinking water bacteria.

3.9 Enumeration of culturable microorganisms (water/biofilm)

A) European Standard: EN ISO 6222. Water quality – Enumeration of culturable micro-organisms – Colony count by inoculation in a nutrient agar culture medium.
B) Number of colony forming units on R2A nutrient agar

Scope
The bacterial colony forming units (CFU) are enumerated by spread plate method for heterotrophic plate counts.

Reference
Reasoner DJ and Geldreich EE (1985). A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microb. 49: 1-7.

Procedure
Heterotrophic plate counts (HPC) are estimated by spread plating method using 0.1 mL or 1 mL sample. The medium is R2A-agar (Difco, USA) (Reasoner and Geldreich 1985). R2A-agar plates are incubated for 7 days at 22 ± 2°C before the colony forming units (CFU) are counted by eye.
Membrane filter method will be used if the cell density of the sample is too low for direct spread plate. The membrane filters have a diameter of 47 mm, a pore size of 0.2 µm. The sample up from 10 mL will be filtered and the membrane filter placed on one R2A agar plate. Be careful: air bubbles under the filter should be avoided.
Results are expressed as the mean number of bacterial CFU per mL of water sample

Methods by TZW

A) Enumeration of culturable microorganisms is performed with German standard method DIN 38411 K5 as prescribed in German Drinking Water Regulations (1990). This standard is comparable to EN ISO 6222 with the following exceptions:
- Plate count agar medium without yeast extract.
- Incubation for 48 hours at 20 °C and 36 °C

Due to these differences, colony counts are generally slightly lower than by EN ISO 6222.

Since 2001, EN ISO 6222 is a part of the German Drinking Water Regulations, but water supply companies are also allowed to continue using DIN 38411 K5. (Therefore, it is much more appreciated, and we still use it, too.)

B) Heterotrophic plate counts (R2A) are estimated as described above except pour plating using 0.1 mL or 1 mL sample instead of spread plating. Agar medium must not be hotter than 45 °C when plating. Colonies are counted by eye by means of magnifying-glass (4 to 6 times magnified).
In our experience, differences between spread plated and pour plated colony counts were negligible.

Reference

German Drinking Water Regulations:
BGBl (Bundesgesetzblatt); 1990; Teil I, p. 2613ff: Verordnung über Trinkwasser und über Wasser für Lebensmittelbetriebe (Trinkwasserverordnung – TrinkwV) vom 12. Dezember 1990.

3.10 Cultivation-independent viability analysis with flow cytometry

Method by EAWAG

A current Eawag project concerns optimization of cultivation-free viability analysis for drinking water bacteria. Since this may be suited for the Techneau project we can include it provisionally. The basic of the procedure is the same as described for the total cell counting (above). Essential to this approach is that no pre-treatment (sonication, lysis buffer or fixation) should be applied to the samples, and that samples must be processed rapidly after sampling. A total cell dye (e.g. DAPI or SYBRgreen) is used to mark all bacterial cells, and dyes targeting specific areas of viability is used separate or in combination with the total cell dye. Examples of the latter are: propidium iodide (cell wall integrity), ethidium bromide (membrane pumping activity), DiBAC (membrane potential), as well as dyes targeting esterase activity, redox potential and RNA content in the cells.

3.11 Enumeration of bacteria in pipe sediments

Method by RTU

To estimate bacteria number in pipe sediments of municipal distribution network samples were taken before pipeline flushing. The pipeline cleaning was done by compressed air-water flushing, i.e. compressed air and water pulses were passed through the pipeline. Compressed air and water were drawn into the pipeline through the fire hydrant. The water flow during cleaning was turbulent, and the flow rate of water pulses inside the pipelines was 3-12 m/s. It took about 1 h to clean the pipe.
Soft deposit samples were collected during the compressed air-water flushing. Samples were collected at the beginning of the cleaning when the thickest deposits were coming from the pipe. Collected samples of deposits were dissolved (see 3.25 Dissolution of iron oxides) and analyzed for total bacterial number.

References

Lehtola M.J., Miettinen I.T., Vartiainen T., Martikainen P.J. 2004. Removal of soft deposits from the distribution system improves the drinking water quality. Water Res 38(3), 601-610.

3.12 Resuspension Potential Method

Method by KIWA

Scope and Procedure

Irrespective of the origin, the presence and mobility of deposits determines the discolouration risk. The Resuspention Potential Method (RPM) as developed in the Netherlands (Vreeburg 2004) is based on measuring the mobility of the material in a network.

The RPM consists of a controlled and reproducible increase of the velocity in a pipe. The hydraulic shear stress as a result of the increased velocity causes particles to mobilize, affecting the turbidity of the water. The method is mainly applied in 100-150 mm pipes hence the absolute difference in shear stress caused by the uniform velocity increase is not very large. The turbidity effect is monitored and translated to a ranking of the discolouration risk. The method is applied as follows:

- Crack the fire hydrant which will be used for the disturbance
- Isolate the pipe for which the discolouration risk is to be assessed, as for uni-directional flushing (Antoun, 1999). The isolated length should be at least 315 meter.
- Open the fire hydrant such that the velocity in the pipe is increased by an additional 0.35 m/s above the normal velocity and maintain this flow for fifteen minutes. Close hydrant after 15 minutes, the flow reduces to normal (Total length affected is 315 m). The velocity of 0.35 m/s was empirically determined (Vreeburg 2004).
- Monitor turbidity in the pipe throughout the fifteen minutes and until turbidity returns to the initial level.
- Open valves to restore normal flow pattern.

Reference

Vreeburg J.H.G., Schaap, P.G, Dijk, J.C. van (2004) "Measuring Discolouration Risk: Resuspention Potential Method: Leading Edge Technology, Prague, IWA

3.13 Time Integrated Large Volume Sampling (TILVS)

Method by KIWA

An experimental online sampling set-up for filtering water. The sampling set-up consists of a True-dos pump, a water weir and a stainless steel filter unit. Filters are used for different particle analysis techniques. The pump delivers a constant flow of water to the filter, allowing the total volume filtered over the duration of the sampling period to be calculated. Flow rates ranging from 0.5-4.0 l/h over a period of 19-72 hours. Normally samples are collected on 0.45

µm cellulose acetate filters, though 0.2 µm polycarbonate and 1.2 µm cellulose acetate are also useable.
Filters are prepared by rinsing with deionised water and drying in a 105 °C oven. All glass and plastic ware used for the preparation and handling of the filters will be acid bathed in a 1:10 dilution of AR grade nitric acid (Merck) before use.

3.14 Biofilm removal

Methods by RTU

Biofilm removal with vortexing
For indirect enumeration the biofilm bacteria are scraped mechanically with a razor blade and disaggregated on a vortex for 3 minutes. With this bacterial suspension the total cell number and the heterotrophic plate count are performed.

Biofilm removal with glass beads
Biofilms from pipeline sample were removed by shaking with sterile 2 mm glass beads and rinsing twice with 5 ml sterile water. The obtained suspension is collected and used for further analyses.

Biofilm removal with sonification
Coupons that are colonized with biofilms are taken from the sampling devices without discontinuing the water flow within the distribution system. They are then placed in sterile flasks containing 25 ml of bacterial cell-free distilled water. Less than 30 minutes later, the biofilm is dispersed by a gentle sonication (ColeParmer, USA, 2 min., 20µA, 22 KHz, diameter 3mm). The probe is placed 1 cm above coupon, inside bacterial cell-free distilled water. The bacterial content of the resulting suspensions must be analysed within one hour.

Biofilm removal by scraping
Method by TZW

For the microbiological characterization in the real pipes the biofilm is removed by scraping and suspended in a defined volume of sodium chloride solution (0.9 %).
Biofilm in the biofilm test rig tubes are detached by shaking with sodium chloride solution (0.9 %) and small glass beads (diameter 1 – 2 mm).

3.15 Acid capacity (KS 4,3)

Method by TZW
The German standard method DIN 38409-H7

3.16 Alkalinity

ISO 9963-2:1995. Water quality - Determination of alkalinity - Part 2: Determination of carbonate alkalinity

3.17 Aluminium

ISO 10566:1994. Water quality - Determination of aluminium - Spectrometric method using pyrocatechol violet

3.18 Ammonium

ISO 5664:2000. Water quality - Determination of ammonium - Distillation and titration method

3.19 Calcium (and sum of calcium and magnesium)

ISO 6058:1984. Water quality - Determination of calcium - EDTA titrimetric method
ISO 6059:1984. Water quality - Determination of the sum of calcium and magnesium - EDTA titrimetric method

3.20 Chloride (and fluoride, nitrite, orthophosphate, bromide, nitrate and sulfate ions)

EN ISO 10304-1:1995. Water quality. Determination of dissolved anions by liquid chromatography of ions. Determination of fluoride, chloride, nitrite, orthophosphate, bromide, nitrate and sulfate ions. Method for water with low contamination

3.21 Total chlorine and free chlorine

ISO 7393 - 1: 2001. Water quality - Determination of free chlorine and total chlorine - Part 1: Titrimetric method using N, N-diethyl-1, 4-phenylenediamine

3.22 Color

ISO 7887:1994. Water quality - Examination and determination of color

3.23 Conductivity

ISO 27888:1993. Water quality - Determination of electrical conductivity

3.24 Iron (and aluminium, calcium, manganese and silicium ions)

Method by TZW

DIN EN ISO 11885: 1996. Water quality - Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy

Method by RTU

ISO 6332: 1988. Water quality - Determination of iron - Spectrometric method using 1,10-phenanthroline

3.25 Dissolution of iron oxides

1 ml of the sample was fixed with glutaraldehyde (2.5% final concentration). A particle-free oxalate solution (8.9 ml of 28 g of ammonium oxalate and 15 g of oxalic acid per liter) was added to dissolve the iron forms. The iron was extracted for at least 15 min with occasional mixing on a Vortex mixer. A subsample (1 ml) was passed through a Nuclepore filter (0.2- µm pore diameter) and fixed with formaldehyde for further bacterial enumeration

References

Lovley D and Phillips J.P.E. (1988) Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron and manganese. J. Appl. Env. Microb. 54(6): 1472-1480.

3.26 Manganese

Method by RTU

ISO 6333:1986. Water quality - Determination of manganese - Formaldoxime spectrometric method

Method by TZW

DIN EN ISO 11885: 1996. Water quality - Determination of 33 elements by inductively coupled plasma atomic emission spectroscopy

3.27 Nitrate

ISO 7890-3:1988. Water quality - Determination of nitrate - Part 3: Spectrometric method using sulfosalicylic acid

3.28 Nitrite

ISO 6777: 2001. Water quality - Determination of nitrite - Molecular absorption spectrometric method

3.29 Total nitrogen

ISO EN 12260:2003. Water quality – Determination of bound nitrogen (TNb) – following oxidization of nitrogen oxides

3.30 Oxygen

ISO 5814:1990. Water quality - Determination of dissolved oxygen - Electrochemical probe method

3.31 pH value

Method by TZW

The German standard method DIN 38404-C5

Method by RTU

ISO 10523: 2002. Water quality - Determination of pH

3.32 Total phosphorus

ISO 6878:2004. Water quality – Determination of phosphorus – Ammonium molybdate spectrometric method

3.33 Silicates

ISO 16264:2002. Water quality -- Determination of soluble silicates by flow analysis (FIA and CFA) and photometric detection

3.34 Turbidity

ISO 7027:1990. Water quality; Determination of turbidity