

**KWR 07.068**  
September 2007

# Assessment of the microbial growth potential of materials in contact with treated water intended for human consumption

A comparison of test methods



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# Colophon

**Title**

Assessment of the microbial growth potential of materials in contact with treated water

**Project number**

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**Project manager**

drs L. W. C. A. van Breemen

**Client**

Department of Housing, Spatial Planning and Environment (VROM)

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*This report is not published and delivered to the client*

# Preface

The investigation described in this report has been conducted for the Department of Housing, Spatial Planning and Environment (VROM), zaaknummer 505005.1072; contact Ir. W. Cramer. Project progress was discussed with ir. W. Cramer, drs. W. van de Meent and drs. L.W.C.A. van Breemen (project manager).



# List of abbreviations

AB	Attached biomass ('biofilm')
AOC	Assimilable Organic Carbon ( $\mu\text{g C/l}$ )
ATP	Adenosine triphosphate
BP	Biomass Production ( $\text{pg ATP/cm}^2$ )
BFP	Biofilm Formation Potential ( $\text{pg ATP/cm}^2$ )
BPP	Biomass Production Potential ( $\text{ng ATP/l}$ ; $\text{pg ATP/cm}^2$ )
CEN	European Normalization Centre
CFU	Colony Forming Units (bacteria)
CPDW	Construction Products in Contact with Drinking Water
COV	Coefficient of Variation (relative standard deviation, %)
DOC	Dissolved Organic Carbon ( $\text{mg/l}$ )
EAS	European Acceptance Scheme
EC	European Commission
EPDM	Ethylene Propylene Diene Monomer
EPS	Extra cellular polymeric substances
PE	Polyethylene
HES	High Energy Sonication (ultrasonic homogenization)
HPC	Heterotrophic Plate Count ( $\text{CFU/ml}$ ; $\text{CFU/cm}^2$ )
Hz	Hertz, unit of frequency (cycles per second)
KWR	Kiwa Water Research
LES	Low Energy Sonication (in water bath)
MDOD	Mean Dissolved Oxygen Difference ( $\text{mg/l}$ )
NPOC	Non-purgable organic carbon ( $\text{mg/l}$ )
PB	Planktonic Biomass ( $\text{ng ATP/l}$ )
PBP	Planktonic Biomass Production ( $\text{pg ATP/cm}^2$ )
PFC	Pass-Fail Criteria
PVC	Polyvinylchloride
PVC-C	Chlorinated PVC
PVC-P	Plasticised PVC
PVC-U	Unplasticised PVC
RLU	Relative Light Units
SB	Suspended biomass (= planktonic biomass)
SILR	Silicone Rubber
SS	Stainless Steel
SP	Slime production
S/V	Surface area to Volume contact ratio ( $\text{cm}^{-1}$ )
TDC	Total direct cell count (with microscopy)
TWUL	Thames Water Utilities Ltd.
TZW	Technologie Zentrum für Wasser
W	Watt, unit of power (Joules/second)



## Summary

In the Netherlands, the Biomass Production Potential (BPP) test, as recently harmonised and further standardised in international investigations funded by the European Commission, will be used for determining the microbial growth potential of materials in contact with drinking water. To obtain information needed for defining pass-fail criteria (PFC), the BPP test has been applied to a series of selected materials in contact with drinking water which were also tested with the BS6920 test, which measures the Mean Dissolved Oxygen Difference (MDOD) and the W270 test, which measure the slime production (SP). The objective of this investigation was to determine which BPP levels correspond to the PFC values of the BS6920 and W270 tests. A total of 14 different materials, including glass and soft PVC (PVC-P), were used in the investigation. The selected materials in contact with drinking water in the BPP test showed large differences in the concentrations of attached biomass (biofilm) and planktonic biomass (PB). The BPP values ranged from about 45 pg ATP/cm<sup>2</sup> for glass to more than 46000 pg ATP/cm<sup>2</sup> for SBR. The concentration of attached biomass measured with ATP analysis is related to the total concentration of bacterial cells. A biofilm concentration of 1000 pg ATP/cm<sup>2</sup> corresponds with about 1.9x10<sup>7</sup> cells/cm<sup>2</sup>. The BPP value at which the ATP concentration in the test water is not elevated after one week of stagnation ('no-effect level') was <100 pg ATP/cm<sup>2</sup> (not corrected for the blank with glass). The value is <50 pg ATP/cm<sup>2</sup> after correction.

Three of the 14 tested materials (EPDMA, SBR and PVC-P) gave a significantly elevated MDOD value. These data were insufficient for calculation of an eventual quantitative relationship between the BPP values and the MDOD values of the tested materials. From the comparison of the results of MDOD with BPP values, assuming a proportional relationship, it was derived that the PFC value for the MDOD test (2.4 mg O<sub>2</sub>/l) corresponded with a BPP value of about 2x10<sup>4</sup> pg ATP/cm<sup>2</sup>.

Four of the 14 selected materials (EPDM a and b, SBR and PVC-P) gave an elevated biomass volume (slime production, SP) in the W270 test, which was applied as original procedure (sedimentation) and as revised procedure (centrifugation). Also here, insufficient data were obtained for calculation of an eventual quantitative relationship between the BPP values and the SP values of the tested materials. From the comparison of the results of the W270 test with the BPP values, assuming a proportional relationship, it was estimated that the PFC value for the revised W270 test (0.05 ml slime/800 cm<sup>2</sup>) corresponded with a BPP value of about 2x10<sup>3</sup> pg ATP/cm<sup>2</sup>. No relationship could be derived from values of MDOD and SP because most of the values observed for the selected materials were below the detection level of both methods.

The results demonstrate that the BPP test enables quantification of the growth-promoting properties of materials at levels which are well below the

level of detection of the MDOD and W270 methods. Furthermore, the PFC values for materials tested with the MDOD and W270 procedures relate to BPP values at which clearly elevated concentrations of planktonic biomass were observed in the BPP test, which simulates a stagnation period of one week. It is proposed to determine the relationship between BPP values and the growth of undesirable bacteria, viz. *Legionella*, in the biofilm to obtain additional information about the significance of BPP values of materials in contact with treated water.

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# 1 Introduction

## 1.1 Background

Treated water intended for human consumption is in intensive contact with the surface of distribution system structures and installations inside houses and buildings before it is used. Physico-chemical and microbiological processes at the surfaces of materials in contact with water have an impact on water quality. The main processes are: (i) release of inorganic compounds (e.g. corrosion), (ii) release of organic compounds and (iii) attachment, growth and release of micro-organisms. This report will focus on the microbiological processes. Attachment and growth of micro-organisms on a surface in contact with water is a well known phenomenon, occurring in both natural environments and in engineered systems. The utilization of biodegradable compounds present in the water induces multiplication of the attached micro-organisms leading to the formation of a biofilm. The microbial community of the biofilm consists of bacteria and eukaryotic organisms, viz. fungi, protozoa and metazoa. The extent of biofilm formation is affected by environmental conditions (nature of the surface, hydraulic regime, temperature) and the concentration and nature of the compounds available as source of energy and carbon for micro-organisms. These compounds may originate from the water and/or may be released by certain materials in contact with the water.

In drinking water distribution systems, biofilm formation leads to an accumulation of micro-organisms, and may impair water quality. Water quality deterioration includes: (i) increase heterotrophic plate counts, (ii) increased counts of coliform bacteria, (iii) multiplication of opportunistic pathogenic bacteria (*Aeromonas* spp., *Pseudomonas* spp., *Mycobacterium* spp and *Legionella* spp.), (iv) aesthetic problems (taste, odour, turbidity) and also the presence of invertebrates, visible to the naked eye, e.g. *Asellus aquaticus*. Biofilm formation processes can be limited by maintaining a disinfectant residual in the water, and/or the removal of biodegradable compounds from the water and the use of materials that do not promote biofilm formation. This report deals with the assessment of the enhancement of microbial growth by materials in contact with treated water intended for human consumption.

Amplification of microbial growth by materials in contact with treated water has been observed for about a century (Schoenen and Schöler, 1984). A variety of materials is involved with this problem, e.g. coatings for reservoirs and pipes causing increased colony counts (Bernhardt und Liesen, 1988; Ellgas and Lee, 1980), redwood tanks inducing multiplication of coliform bacteria (Seidler et al. 1977) and rubber materials enhancing the multiplication of *Legionella* (Colbourne et al. 1984; Nideveld et al. 1986; Schoenen et. al. 1988 and Rogers et al 1994). The use of polymeric materials (plastics) in contact with drinking water is increasing as is the number of types of materials. Synthetic materials are composed of a polymer and

additives for protection or functionality, e.g. antioxidants, stabilizers, fillers, antistatics, etc. The potential negative impact of the use of these complex materials on water quality has raised concern and attracted attention in many countries. This issue is also addressed in legislation of the European Community. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption requires in Article 10 that 'member states shall take all measures to ensure that no substances or materials for new installations used in the preparation or distribution of water intended for human consumption or impurities associated with such substances or materials for new installations remain in water intended for human consumption in concentrations higher than is necessary for the purpose of their use and do not, either directly or indirectly, reduce the protection of human health provided for in this Directive' [European Commission, 1998]. Consequently, standardised test methods and quality criteria are needed to assess the suitability of such substances and materials. Several member states have already implemented a series of measures related to this issue. In the Netherlands, specific legislation dealing with materials and chemicals used in contact with drinking water has been developed and enforced (VROM, 2002a).

## 1.2 Test methods and pass-fail criteria

Test methods for determining the growth-promoting properties of materials in contact with treated water have been developed and implemented in a few member states. These methods are:

- the mean dissolved oxygen difference (MDOD) test, which is the standard method (BS 6920) in the United Kingdom (BSI, 2000);
- the Code of Practice W270, which is the standard method in Germany (DVGW, 1998);
- the Austrian method assessing the increase of the heterotrophic plate count of the water under test conditions (Önorm, 2002);
- the biomass production potential (BPP) test, which is the standard method (NVN 1225) in the Netherlands (NEN, 2004).

Harmonization of test methods is part of the European policy aiming at reducing trade barriers. Investigations, financially supported by the European Commission and a few national governments, have been conducted to achieve harmonization of methods for testing construction products in contact with drinking water (CPDW). These investigations have led to adaptations of the BPP test and to further standardization of the test conditions (Van der Kooij et al., 2003; Van der Kooij et al., 2006). Pass-fail criteria (PFC) had not yet been defined for the BPP test in the Netherlands. The availability of an improved and harmonized procedure enables to make progress in collecting information for defining PFC. Therefore, further investigations were conducted in The Netherlands to collect such information.

### 1.3 Research objectives

The first step in the process of collecting information for defining PFC for the BPP test is a comparison of the results of the BPP test with those of the MDOD test and the W270 test on a number of selected materials. The following questions were addressed:

- do results obtained with the BPP test show relationships with results of the MDOD and W270 tests ?
- how do the PFC values applied for the MDOD test and the W270 test relate to BPP values ?

The comparison of the BPP test with the MDOD test and the W270 test is described in this report.



## 2 Methods and materials

### 2.1 Research outline

A number of selected materials were tested in the three procedures. Each method was applied by a laboratory specialised in conducting the involved test, viz. Thames Water Utilities Ltd. (TWUL) for MDOD, Technologie Zentrum für Wasser (TZW) for W270 and Kiwa Water Research (KWR) for the BPP test. The selected materials included a variety of products and also positive and negative controls, with the intention to cover a wide range a growth promoting properties. Materials had been prepared and distributed by KWR to ensure that all tests were conducted with sample pieces of identical materials. The materials used in the study were not specifically approved for use in contact with water intended for human consumption, and may not be representative for such materials.

### 2.2 Test methods

#### 2.2.1 *Growth of aquatic micro organisms test (BS 6920)*

In the test described in British Standard 6920, the oxygen consumption in the presence of the material to be tested is used as the parameter for microbiological growth support (Colbourne and Brown, 1979). The principle of the test is based on incubation of sample pieces at a defined surface (S) to volume (V) ratio in tap water in the dark at 30 °C. This tap water is inoculated with a fresh sample of river water. Twice a week the water in the containers is replaced and the oxygen concentration is measured in the fifth, sixth and seventh week of the test, immediately before the water is changed. Typical values for the mean dissolved oxygen difference (MDOD) range from about 0.5 mg/l (blank with glass) to values of about 8 mg/l for paraffin wax (positive control). If a value for the MDOD after seven weeks is  $>1.69$  mg/l, but  $<2.0$  mg/l, then the test will be continued for a further two weeks. A large number of materials has been tested with this method, which has been described in BS 6920 and applied for several decades. Materials with an MDOD  $> 2.4$  mg/l generally are considered not to be suited for use in contact with water intended for human consumption (Colbourne, 1985). In more detail, if a sample is tested as a single sample and the MDOD is  $\geq 1.7$  mg/l, it is not suitable. However, if the MDOD values is less than 2.9 mg/l for the sample, then two further samples can be tested and if the average MDOD result for the three samples is then less than 2.4 mg/l the material is suitable.

#### 2.2.2 *Code of Practice W270*

The method described in W270, developed in Germany (Schoenen and Schöler, 1983), was published as standard method in 1998 (DVGW, 1998). The method is applied in a flow-through (dynamic) system, with sheets (800 cm<sup>2</sup>) of materials in contact with continuously flowing tap water. The volume of slime scraped from the surface of the tested material is used as the parameter for microbial growth. In this report these volumes will be described as slime production (SP). Typical SP values range from less than 0.1 ml (Stainless Steel

blank) to more than 15 ml on solvent-containing bitumen or plasticized PVC (PVC-P). Materials with an SP value > 0.1 ml are considered unsuitable for use in contact with drinking water. Higher SP levels can be allowed for certain applications with a low surface to value ratio (DVGW, 1998). The MDOD and SP tests have been compared on a number of PVC materials (Schoenen and Colbourne, 1987). Apart from one material, the test results in combination with the defined criterion gave the same conclusion regarding pass/failure of the tested materials. Recently, the W270 method has been revised by shortening the exposure period to three months in combination with the application of centrifugation to the substances scraped from the exposed material. With this approach lower volumes of biomass can be detected (DVGW, 2007). The pass-fail criterion for the revised method is  $0.05 \pm 0.02$  ml/800 cm<sup>2</sup>.

### 2.2.3 *The Biomass Production Potential (BPP) test*

The BPP test is derived from the Biofilm Formation Potential (BFP) test (Van der Kooij and Veenendaal, 1993). In these tests adenosine triphosphate (ATP) is used as parameter for active biomass. ATP is an energy-rich compound, which is present in all living (= active) organisms. ATP analysis enables the detection of very low concentrations of active biomass. The detection limit of the ATP analysis when applied directly to (tap) water is 1 ng/l, but equipment and chemicals are available for detection to a level of about 0.1 ng/l. The result of the analysis is available within a few minutes. ATP analysis can be used for determining the concentration of active biomass in drinking water samples, for determining the biofilm concentration on walls of distribution system pipes and in the biofilm monitor for determining the Biofilm Formation Rate (BFR) values of drinking water (Van der Kooij et al., 1995). A database of biomass concentrations in water, in biofilms and on materials facilitates the interpretation of individual measurements (Unifying Biofilm Analysis) (Van der Kooij et al., 2003).

In the BPP test the production of active biomass (ATP) as a function of time in the presence of the material to be tested is determined. The BPP test initially was carried out as a static test without replacement of the water (van der Kooij et al., 2001). Based on the results of a European research project, water replacement at a weekly interval was introduced (Van der Kooij et al. 2003). This approach simulates a situation in a system with stagnancy. The concentration of active biomass, measured as ATP, is in balance with the supply of biodegradable compounds originating from the material. The Biomass Production (BP, pg ATP/cm<sup>2</sup>) is calculated from the concentration of attached biomass (AB, biofilm) and the concentration of planktonic (suspended) biomass (PB) using the SV<sup>-1</sup> ratio. The BPP value is the average of the BP values obtained on days 56, 84 and 112. The Biofilm Formation Potential (BFP) is the average value of the AB values on these days. The BPP and BFP parameters are all expressed as the quantity of active biomass per surface unit of the tested material (pg ATP cm<sup>-2</sup>). Details of the applied procedure are given in Appendix 1, which is the proposed CEN method.

### 2.2.4 Test characteristics

The main characteristics of the applied test are presented in Table 2.1. Main differences between the methods include the biomass parameter, viz. oxygen (MDOD), slime volume (W270) and biomass activity (BPP). Furthermore, the MDOD test and the BPP test are conducted under static conditions (batch test) at a defined temperature, whereas the W270 test is conducted as a dynamic test at ambient temperature. Both the MDOD test and the BPP test can be conducted with different sample shapes, provided that the outer surface area is defined. The W270 test requires the use of flat plates, which in most cases must be prepared for the test on request.

Table 2.1 Characteristics of the applied test methods

Condition/parameter	MDOD (UK)	W270 (Germany)	BPP* (NL)
Temperature (°C)	30 ± 1	Ambient (> 6°C)	30 ± 1
Surface (cm <sup>2</sup> ) of sample pieces	150 (or 75) cm <sup>2</sup>	800 cm <sup>2</sup>	50 (or 25) cm <sup>2</sup>
Volume (V) of water (cm <sup>3</sup> )	1000	100,000	900
S/V (cm <sup>-1</sup> )	0.15	n.a.**	0.166
Replacement	Twice a week	Continuously	Weekly
Water type	Tap water#	Tap water#	Tap water#
Duration (weeks)	7.5	12	16
Microbiological activity	Oxygen	Slime volume	ATP

\*, adapted method; \*\*, n.a., not applicable; #, without disinfectant residual

## 2.3 Other analytical methods

### 2.3.1 Total direct cell count (TDC)

The total direct cell count (TDC) was applied to determine the total number of bacterial cells visible with the microscope. Acridine-orange staining was applied following a standardised procedure (Hobbie et al. 1977). A microscope (Leica DMR RXA) was used at 100x10 magnification to count the fluorescent bacteria.

### 2.3.2 Heterotrophic plate count (HPC)

The heterotrophic plate count (HPC) of water was determined using Plate Count agar plates (NEN-EN-ISO 6222). One ml of a (decimally diluted) water sample) was added to molten agar. Plates were incubated at 22 °C for 72 hours. Subsequently, colonies visible to the naked eye are counted. This method is used for routine monitoring of the quality of drinking water in the distribution system. The maximum value (geometric mean over a one year period) for this parameter in drinking water is 100 CFU/ml (VROM, 2001).

### 2.3.3 Non-purgable organic carbon (NPOC), copper and pH

These parameters were determined following standardized procedures.

## 2.4 Materials

A variety of materials was selected for the study. A requirement was the use of a number of different materials, with a range of values for the promotion of growth of micro-organisms during contact with water. The use of flat plates

in the W270 test demanded the acquisition of flat plate materials for the preparation of samples for all tests.

Table 2.2 Material types used in the study

Material type	Origin	Thickness (mm)	Comment
Glass	Stock	3.0	Plates
Stainless steel, 316 .	Stock	1.5	Plates
Copper	Copper Benelux	1.5	Plates
Unplasticised PVC (PVC-U)	Vink	3.0	Plate
Chlorinated PVC (PVC-C)	Stock	3.3	Plate
Silicon rubber	Holimex	3.0	Sheet
Polyethylene 40 (PE40)	Wavin	3.0.	Prepared on request**
Polyethylene 80 (PE80)	Wavin	3.0	Prepared on request**
Polyethylene 100 (PE100)	Vink	3.0	Plate
EPDM Rubber A	Holimex	3.0	Sheet
SBR rubber	Holimex	3.0	Sheet
Plasticised PVC (PVC-P)	Vink	3.0	Sheet
Polypropylene (PP)	Vink	3.0	Plate
EPDM Rubber B	Obtained from TZW	3.0	Used in previous research at TZW

\*\* PE40 and P80 plates of 25 x 25 mm, thickness 3 mm, have been prepared by Wavin using PE40 type DOW 150E (natural) and yellow coloured PE80 type Total Fina 3802YCF. Melted at 180 °C. No solvent was used during preparation.

#### 2.4.1 Sample preparation and sample treatment



Fig. 2.1 Material samples prepared for the BPP test

Samples were cut to the required size using a metal saw, which had been cleaned with solvents prior to its use. Materials samples were sent to TWU (2 samples of 6x12 cm for each material) and TZW (12 samples of 20x20 cm for each material). TWU conducted the MDOD test following BS 6920. TZW

conducted the W270 test following the original procedure (DVGW, 1998) and the revised procedure (DVGW, 2007).

The sample materials used in the BPP test were provided with holes (1 mm) to enable attachment of stainless steel wire (length 6-7 cm; d = 0.8 mm) to prevent intensive contact with each other or the bottom of the container. A glass ring (i.d. 14 mm, o.d. 18 mm, L 6.3 cm) was connected to the PE and PP samples to prevent floating of these materials (Fig. 2.1). KWR used sample pieces of 3x4 cm in the adapted BPP test, as described in the draft BPP protocol (Appendix 1). Samples had been cleaned by flushing with tap water (without disinfectant residual) during 1h, 24 h storage in tap water, followed by 1 h flushing. The glass rings and the stainless steel wire had been heated in an oven at 550 °C for 4 h. Each flask contained 6 sample pieces in 900 ml of water at the beginning of the test. Material samples, collected on days 56, 84 and 112, were treated with high energy sonication (HES) to remove attached biomass. For this purpose, the samples was placed in 70 ml of autoclaved tap water, contained in a 100 ml glass beaker. HES treatment was done during 1 min at 45% capacity using a Branson W-250 systems (20 kHz) with sonotrode tip, model 1020). Hard materials received two HES treatments; soft materials received three HES treatments, followed by swabbing with sterile cotton swabs. One to three sticks were used for each side of the material sample to achieve complete removal of the biomass remaining after HES treatment. The swabs were placed in sterile water and HES treated to suspend the biomass.

#### 2.4.2 Water type

The tap water available at the laboratory was used in the study. This water is prepared from groundwater by aeration and rapid sand filtration without chemical disinfection. The NPOC concentration is about 1.8 ppm; the AOC concentration is < 5 µg C/l; the Biomass Production Potential (BPP) is < 5 ng ATP/l. Further details are given in the Table 2.2.

Table 2.2. Quality characteristics of the locally available tap water

<i>Parameter</i>	<i>Avg. value</i>	<i>Parameter</i>	<i>Avg. value</i>
pH	7.9	Calcium (mg/l)	71.2
Conductivity (mS/m)	38.4	Magnesium (mg/l)	5.8
CO <sub>2</sub> (mg/l)	6.5	Total hardness (mmol/l)	2.0
HCO <sub>3</sub> (mg/l)	255	Nitrate (mg /l)	0.72
CO <sub>3</sub> <sup>2-</sup> (mg/l)	<2	Nitrite (mg/l)	<0.007
Chloride (mg/l)	9.9	Ammonia (mg /l)	<0.04
Sulphate (mg/l)	<1.0	Dissolved organic carbon (TOC) mg/l	2.0
Sodium (mg/l)	12.3	Iron (mg/l)	0.08
Potassium (mg/l)	0.94	Manganese (mg/l)	0.03

The water was supplemented with phosphate and nitrate as described in the draft standard (Appendix 1).



# 3 Results

## 3.1 Biomass Production Potential (BPP)

The results of the BPP test are presented in Table 3.1. The observations on planktonic biomass (PB), attached biomass (AB) and BP values are presented in more detail in the following paragraphs.

### 3.1.1 Planktonic biomass (PB)

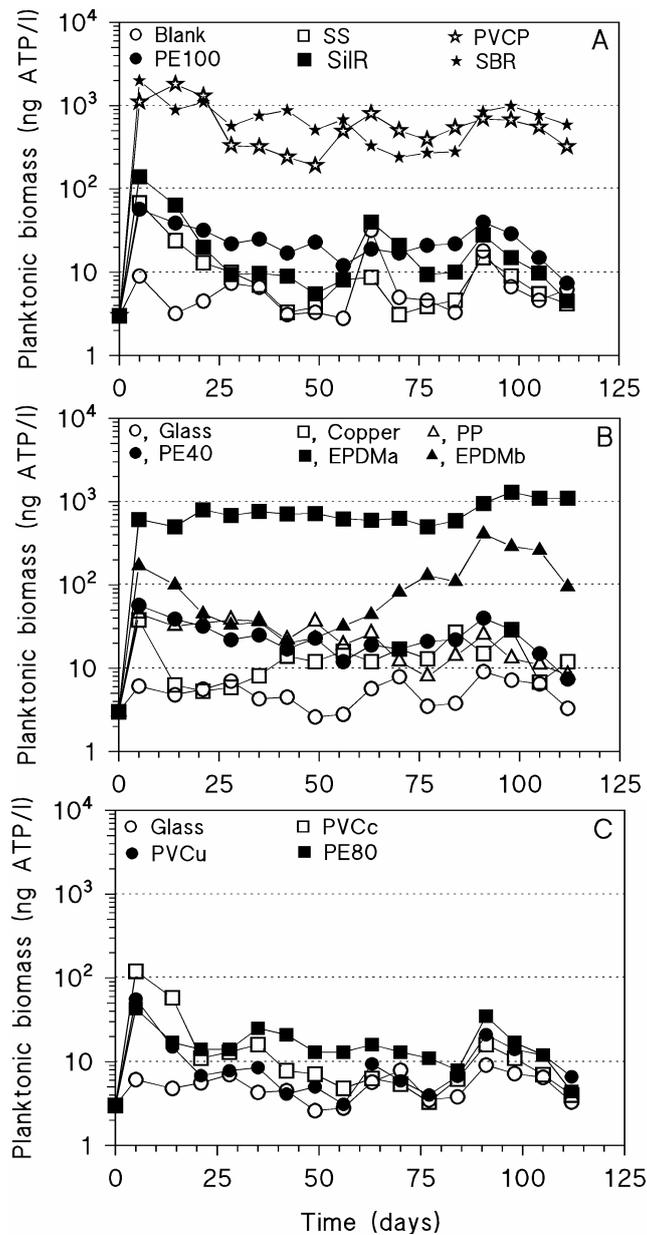


Fig. 3.1. Concentration of planktonic (suspended) biomass in the presence of the tested materials. For each material, the results of one flask are shown.

The concentration of planktonic biomass (PB) was measured in the water directly before the weekly replacement. The PB concentration in the blank and with glass remained below 10 ng ATP/l in the test period. A few materials induced a PB increase which declined within a month. Average PB values of glass, SS, PVC-U, PVC-C, PE80 and SilR were below 10 ng/l. PB values for copper, PE40, PE100, PP and EPDMb were between 10 and 100 ng/l and PB values exceeding 100 ng ATP/l were observed with PVC-P, SBR, and the EPDM materials. Incidentally, the PB value was above 1000 ng ATP/l. Table 3.1 shows that the average of the PB values on days 56, 84 and 112 ranged from 3.1 (glass) to 638 (EPDMa).

### 3.1.2 Attached biomass (AB)

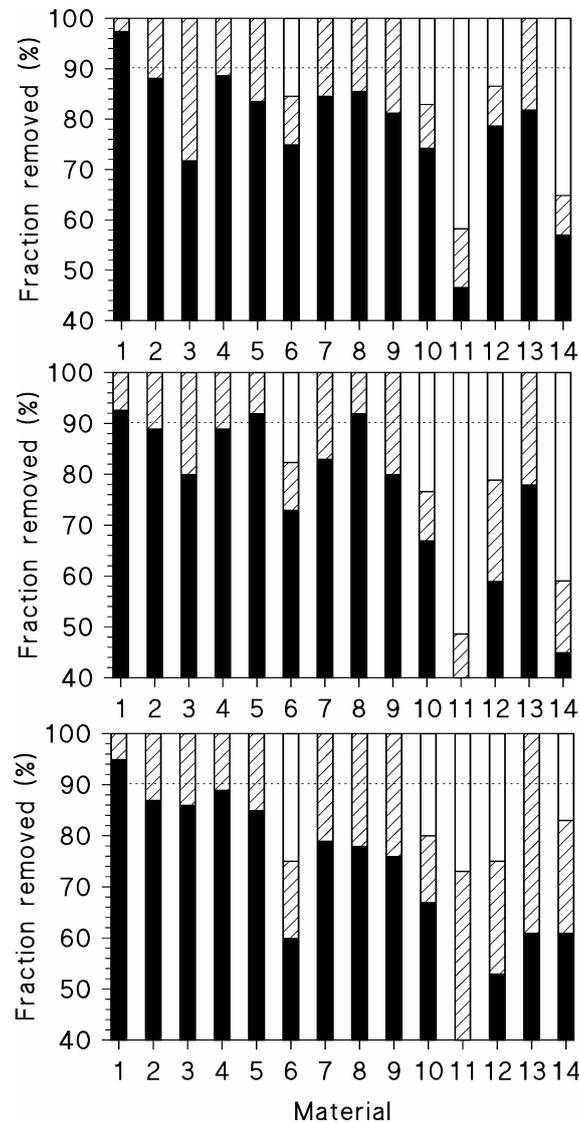


Fig.3.2 Effect of High Energy Sonication (HES) and swabbing on biomass removal from the materials. Black bar: relative yield (%) of first HES treatment; hatched bar, relative effect (%) of second HES treatment; white bar, relative effect of swabbing. 1, glass, 2 Stainless Steel, 3 Copper, 4 PVC-U, 5 PVC-C, 6 SilR, 7 PE40, 8 PE80, 9 PE100, 10 EPDMa, 11 SBR, 12 PVC-P, 13 PP, 14 EPDMb.

Biomass was removed from the hard materials by applying two series of HES treatment. The sample pieces of the soft materials (SilR, EPDM, SBR and PVC-P) were subjected to three series of HES treatment, followed by swabbing. Swabbing yielded a considerable proportion of AB, ranging from 14 (SilR, day 56) to 51% (for SBR, day 84). Generally, the lowest yield with HES (< 40%) was observed with SBR (Fig. 3.2). With a few materials (e.g. SilR, EPDM, PVC-P), the relative yield of HES decreased from day 56 to day 112, indicating that the intensity of attachment of the microorganisms to the surface of the materials increased.

The significance of the ATP measurements of attached biomass was verified with total direct cell counts (TDC). A clear relationship was observed between TDC values and ATP concentrations in the biofilm (Fig. 3.3). From the TDC values and the ATP concentrations, it can be calculated that the median value of the ATP content of a single cell is 0.052 fg ATP/cell (90 percentile: 0.13 fg ATP/cell). A level of 1000 pg ATP/cm<sup>2</sup> corresponds with about 10<sup>7</sup> bacteria/cm<sup>2</sup>. The slope of the regression line indicates that the ATP concentration of the cells decreases with decreasing concentration of the biofilm. The smallest (less active cells) were observed in the biofilm on copper and glass; the most active cells on EPDM and PE100.

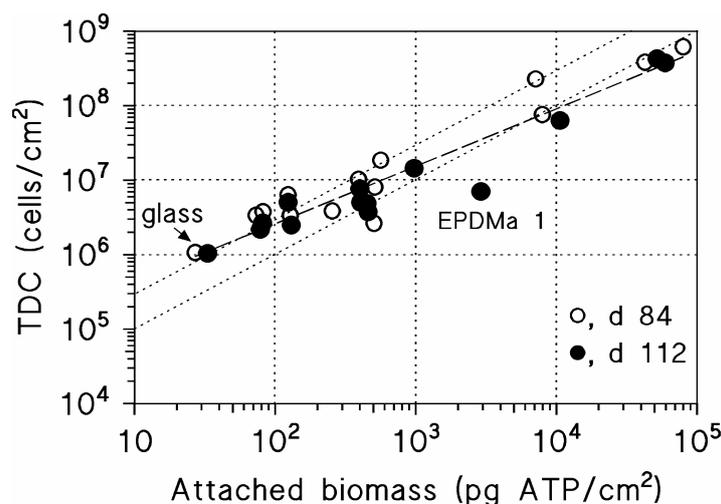


Fig. 3.3 Relationship between Total Direct bacterial Count (TDC) and the concentration of active biomass (ATP) on the materials on days 84 and 112. Dotted lines represent proportional relationships. Broken line is regression line ( $\log \text{TDC} = 0.77 \log \text{ATP} + 4.8$ ;  $r^2 = 0.91$ ;  $p < 0.001$ ).

### 3.1.3 Biomass Production (BP)

The biomass production (BP) is calculated from the concentration of attached biomass (AB, biofilm) and the concentration of planktonic biomass (PB), measured on days 56, 84 and 112. The BP values show some changes in the course of the experiment, e.g. a decline with copper, and an increase with EPDMa, EPDMb and PVC-P. For the other materials no systematic change was observed within the test period. The BPP value is the average of the BP values. As a result of changing BP values, the standard deviation of the BPP

values attained a level of more than 20% with a few materials, viz. EPDM and SBR (Table 3.1).

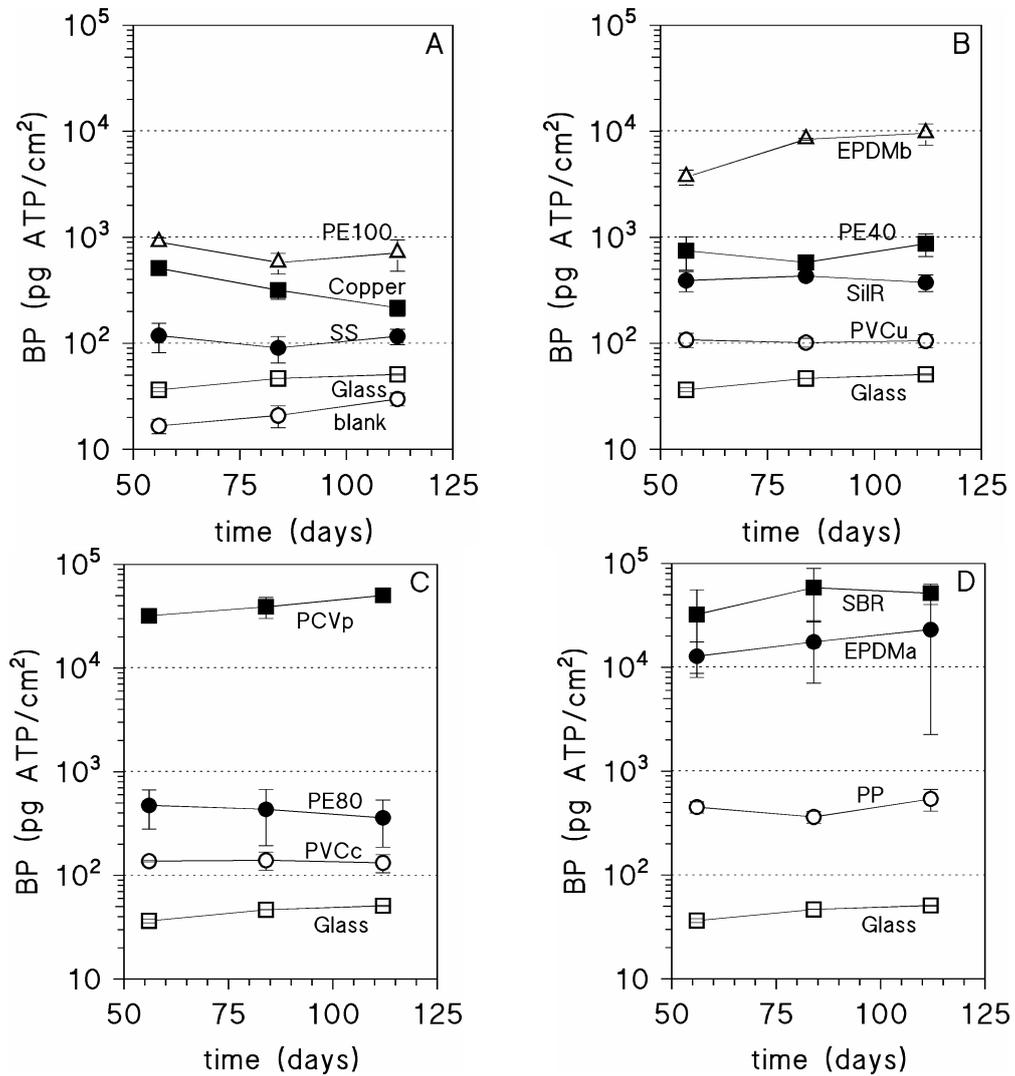


Fig. 3.4. Biomass production (BP) of the materials at days 56, 84 and 112.

Table 3.1. Results of the Biomass Production Potential (BPP) test\*. BPP values not corrected for values of control with glass.

Material	PB (ng ATP/l)	day 56		day 84		day 112		BPP ± std (pg ATP/cm <sup>2</sup> ) (COV)#
		PB** (pg ATP/cm <sup>2</sup> )	AB** (pg ATP/cm <sup>2</sup> )	PB (pg ATP/cm <sup>2</sup> )	AB (pg ATP/cm <sup>2</sup> )	PB (pg ATP/cm <sup>2</sup> )	AB (pg ATP/cm <sup>2</sup> )	
Blank	4.1 ± 1.3	16.6 ± 2.6	0	20.8 ± 4.9	0	29.8 ± 4.1	0	22.4 ± 6.7 (29.9%)
Glass	3.1 ± 0.4	15.5 ± 1.2	19.8 ± 2.9	16.6 ± 4.8	31.5 ± 3.8	17.2 ± 0.5	34.9 ± 1.5	45.1 ± 8.8 (19.5%)
SS	6.1 ± 2.0	31.4 ± 21.5	79 ± 14	26.2 ± 0.4	70.8 ± 16	32.3 ± 11.4	86.5 ± 8.1	109 ± 11 (10%)
Copper	21.5 ± 10.6	72.8 ± 26.0	373 ± 87	185.6 ± 45	182 ± 62	91.4 ± 33	160 ± 40	355 ± 97 (27%)
PVC-U	4.9 ± 1.4	21.7 ± 7.5	84 ± 14.9	31.7 ± 5.3	76 ± 10	29.4 ± 7.9	79.2 ± 10	107 ± 1.7 (1.5%)
PVC-C	4.5 ± 1.0	21.9 ± 3.6	113 ± 3.9	25.2 ± 9.0	110 ± 13	19.1 ± 1.8	103.4 ± 18	131 ± 7.4 (5.6%)
PE40	13.3 ± 8.6	116 ± 70	587 ± 127	107 ± 19	562 ± 213	45.9 ± 7.0	854 ± 166	757 ± 124 (16.3%)
PE80	8.4 ± 3.3	48.1 ± 29.5	410 ± 160	40.5 ± 2.0	397 ± 169	23.5 ± 0.2	356 ± 167	425 ± 41 (9.6%)
PE100	21.9 ± 8.6	113 ± 45.3	681 ± 157	139 ± 37	449 ± 58	67.6 ± 41	691 ± 229	713 ± 110 (15.4%)
PP	15.1 ± 3.6	93.5 ± 11.3	319 ± 59	80.2 ± 7.7	319 ± 47	59.8 ± 21.6	456 ± 86	442 ± 64 (14.4%)
SilR	7.0 ± 2.8	48.2 ± 10.5	302 ± 65	43.5 ± 9.9	385 ± 48	21.4 ± 1.6	374 ± 46	391 ± 39 (9.9%)
EPDMa	638 ± 259	2925 ± 235.9	12139 ± 3845	3003 ± 74	15246 ± 8071	3507 ± 2762	21264 ± 21719	19361 ± 4948 (25.5%)
EPDMb	68 ± 34	155 ± 18.7	3636 ± 418	499 ± 111	7912 ± 2683	403 ± 134	8661 ± 1763	7089 ± 2874 (40.5%)
SBR	307 ± 238	2587 ± 1067	32453 ± 20503	1083 ± 414	50238 ± 22969	1622 ± 1782	51253 ± 19012	46406 ± 9873 (21.2%)
PVC-P	337 ± 207	1524 ± 1364	32259 ± 5588	1623 ± 1580	37322 ± 4782	1013 ± 857	45085 ± 5414	39609 ± 6184 (15.6%)

\*Average values of duplicate test, with standard deviation; \*\* PB, planktonic biomass; AB, attached biomass, #, COV coefficient of variation (= relative standard deviation).

### 3.1.4 Relationship between attached and planktonic biomass

Attached biomass (BFP, which is the average of the observed AB values) represented the major proportion of the BPP value (Table 3.2). The BFP and BPP values observed with glass are entirely attributed to the presence of biodegradable compounds in the water used for the test. Correction of the BFP and BPP values observed with the materials with the value for glass gives the effect of the material. From these corrected values, the contribution of planktonic biomass (PBP) to the net BPP value is calculated. With 7 materials, the net PB production was less than 10% of the BPP value corrected for the blank (water). Values over 10% were observed with a few materials.

Table 3.2 BFP and BPP values corrected for the glass control

Material	BFP (pg ATP/cm <sup>2</sup> )	BFP <sub>cor</sub> * (pg ATP/cm <sup>2</sup> )	BPP (pg ATP/cm <sup>2</sup> )	BPP <sub>cor</sub> * (pg ATP/cm <sup>2</sup> )	PBP** (%)
Water	0	0	22.4 ± 6.7	0	100
Glass	28.7 ± 7.3	0	45.1 ± 8.8	0	n.a.
SS	78.8 ± 13.7	50.0	109 ± 11	64	27
Copper	238 ± 116	210	355 ± 97	310	34
PVC-U	80 ± 11	51	107 ± 1.7	62	16
PVC-C	109 ± 13	80	131 ± 7.4	86	8
PE40	668 ± 208	639	757 ± 124	346	8,2
PE80	387 ± 152	359	425 ± 41	712	7.4
PE100	607 ± 189	578	713 ± 110	379	14.9
PP	364 ± 90	336	442 ± 64	397	16.1
SILR	354 ± 61	325	391 ± 40	346	5.6
EPDMa	16216 ± 12888	16188	19361 ± 4948	19316	16.5
EPDMb	6736 ± 2864	6708	7089 ± 2874	7044	4.8
SBR	44642 ± 20936	44613	46406 ± 9873	46361	3.3
PVC-P	38222 ± 7287	38194	39609 ± 6184	39564	4.3

\*, corrected for value of glass; \*\*, PBP, planktonic biomass production, based on corrected values [ $=100 \cdot (BPP_{cor} - BFP_{cor}) / BPP_{cor}$ ]; n.a., not applicable.

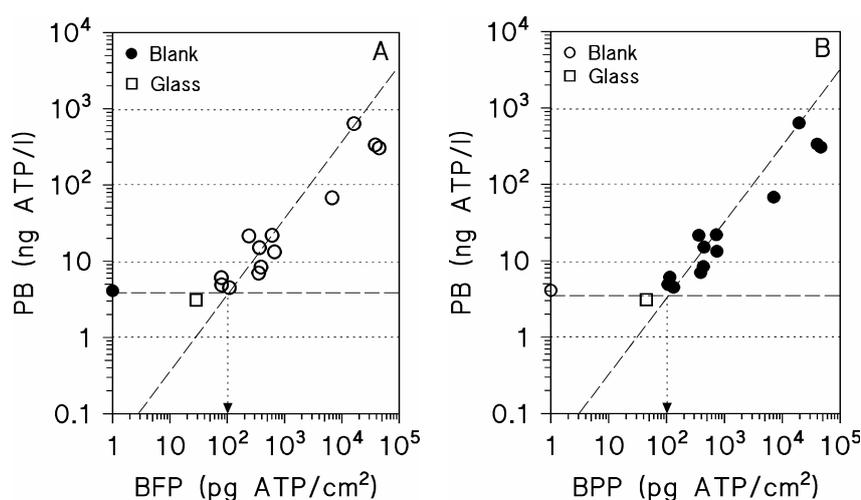


Fig. 3.5. Relationship between average PB values and values of BFP (A) and BPP (B) of the tested materials ('Biostability diagram'). The PB value of the blank is presented on the Y-axis. The horizontal broken line represents the 'no-effect level'.

The average values of the concentrations of planktonic biomass (PB), measured at days 56, 84 and 112 are related to the average values of the concentrations of attached biomass (Biofilm Formation Potential, BFP) and the BPP values (Fig. 3.5). These BPP values were not corrected for the blank or the value for glass. From the proportional relationship between PB and BFP, resp. BPP, it can be derived that values for BFP and BPP (not corrected for blank) exceeding 100 pg ATP/cm<sup>2</sup> result in elevated PB values. Hence, the no-effect level in the BPP test, corrected for the blank (glass), is < about 60 ATP/cm<sup>2</sup>.

### 3.1.5 Additional quality effects

On day 112, a number of additional water quality parameters were determined, viz. the heterotrophic plate count (HPC), according to the standard procedure for routine monitoring of drinking water in the distribution system (pour plate of Yeast Extract Glucose Agar, 72 hrs at 22 °C), the NPOC concentration, the concentration of copper (in few flasks) and the pH of the water (Table 3.3). The HPC values exceeded the value of 100 CFU/ml in the blank, with glass, copper, and the soft materials (SiR, EPDM, SBR and PCVp). Elevated NPOC values (>1.8 ppm) were observed with all soft materials, indicating that these materials released organic compounds after 112 days of incubation in water at 30 °C. From these NPOC values, an estimation can be made of the NPOC release in a period of one week. These values ranged from not detectable (< 5 mg C/m<sup>2</sup>.week) to 85 mg C/m<sup>2</sup>.week for EPDMa. These values are also shown in Table 3.3. The concentration of copper in the tap water was low (about 1 µg/l). Values of about 1 mg/l were observed in the presence of copper material. The pH values showed little variation; the lowest values were observed with the materials with the highest BPP values.

Table 3.3 Specific water quality parameters measured on day 112. Values for each flask are given.

Material	HPC (CFU/ml)	NPOC (mg/l)	NPOC release [mg/(m <sup>2</sup> .week)]	Copper (µg/l)	pH
Blank	243; 334	1.7; 1.8	na	1.4; 0.8	8.4; 8.6
Glass	124; 66	1.8; 1.8	<5	nd	8.5; 8.4
SS	46; 57	1.7; 1.8	<5	0.7; 1.1	8.6; 8.6
Copper	280; 104	1.8; 1.8	<5	970; 1230	8.6; 8.7
PVC-U	34; 24	1.7; 1.8	<5	nd	8.6; 8.6
PVC-C	32; 19	1.8; 1.7	<5	nd	8.7; 8.6
PE40	87; 14	1.8; 1.8	<5	nd	8.5; 8.5
PE80	76; 73	1.8; 1.8	<5	nd	8.7; 8.5
PE100	79; 87	1.7; 1.9	<5	nd	8.6; 8.6
PP	59; 89	1.8; 1.8	<5	nd	8.3; 8.1
SiR	161; 93	1.9; 2.3	25	nd	8.6; 8.6
EPDMa	119000; 23100	2.9; 4.1	85	nd	8.3; 8.1
EPDMb	265; 915	2.3; 2.5	30	nd	8.6; 8.5
SBR	17000; 14200	3.0; 3.7	60	nd	8.2; 7.9
PVC-P	19300; 35000	2.6; 2.7	40	nd	8.2; 8.1

\*, n.a. not applicable; nd, not determined

### 3.2 Mean Dissolved Oxygen Difference (MDOD)

The results of the MDOD test, reported by TWUL, are presented in Table 3.4. The results of the BPP test are presented for comparison. Negative MDOD values have been reported with a number of materials, including the negative reference, glass, SS, PVC-U, and PE40. Any value that is different from the negative control is considered to differ; however in order to validate the test the MDOD of the negative reference must be  $0.0 \pm 0.6$  mg/l. MDOD values  $\geq 0.6$  mg/l were observed with three materials, viz. EPDMa, SBR and PVC-P. Fig. 3.6 depicts a comparison between the results of these two tests.

Table 3.4 Results of the MDOD test (TWUL)

Material	Surface area (mm <sup>2</sup> )	MDOD (mg/l)	BFP (pg ATP/cm <sup>2</sup> )	BPP (pg ATP/cm <sup>2</sup> )
Negative reference (glass plate)	Standard	-0.2	nt*	nt
Positive reference (paraffin wax block)	standard	6.5	nt	nt
Water	n.a.	n.a.	0	22.4 ± 6.7
Glass	15514	-0.2	28.7 ± 7.3	45.1 ± 8.8
SS	14917	-0.2	78.8 ± 13.7	109 ± 11
Copper	15204	0.0	238 ± 116	355 ± 97
PVC-U	15549	-0.3	80 ± 11	107 ± 1.7
PVC-C	15756	0.1	109 ± 13	131 ± 7.4
PE40	15255	-0.1	668 ± 208	757 ± 124
PE80	15487	0.3	387 ± 152	425 ± 41
PE100	15382	0.2	607 ± 189	713 ± 110
PP	15635	0.0	364 ± 90	442 ± 64
SILR	15807	-0.1	354 ± 61	391 ± 40
EPDMa	16054	4.7	16216 ± 12888	19361 ± 4948
EPDMb	15648	0.4	6736 ± 2864	7089 ± 2874
SBR	15841	5.0	44642 ± 20936	46406 ± 9873
PVC-P	15836	4.0	38222 ± 7287	39609 ± 6184

\* n.a., not applicable; nt, not tested

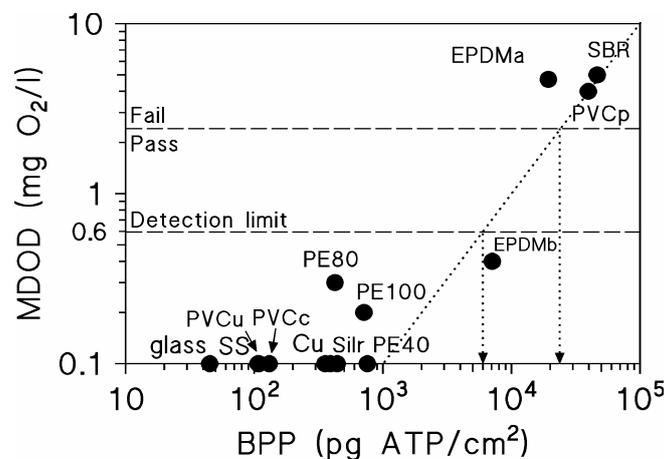


Fig. 3.6. Results of MDOD test in relation to results of BPP test. Negative MDOD values are presented as 0.1 mg/l. Dotted lines based on assumption of proportional relationship between MDOD and BPP.

Based on the assumption of a proportional relationship between MDOD and BPP values a line is drawn in Fig 3.6, using the MDOD values above the detection level and the position of the highest BPP value at the MDOD values below the detection limit. This line serves only as an indication, because insufficient suited pairs of data are available to calculate a relationship. The observations suggest that (i) BPP values below about 5000 pg ATP/cm<sup>2</sup> do not give an MDOD value above the detection limit and (ii) the PFC value for the MDOD test (2.4 mg/l) corresponds with a BPP value of about 2x10<sup>4</sup> pg ATP/cm<sup>2</sup>.

### 3.3 Code of practice W270 (TZW)

The W270 test was conducted at TZW following the original protocol (OP), with measurements after 3 months and 6 months of exposure. Slime volumes with OP were based on sedimentation at 6 °C for 24 h. In the revised protocol (RP), measurements were conducted after 1, 2 and 3 months, and the slime volume was determined after centrifugation at 3000 x g during 10 minutes. Table 3.5 shows the results of the OP after 3 and 6 months of exposure and the results of the RP method after 1, 2 and 3 months of exposure. Most observations with OP were below the detection limit (0.1 ml). Higher SP values were observed with copper, EPDM a and b, SBR and PVC-P. The substances obtained from copper most likely included inorganic compounds, but analytical results were not given. The SP values obtained with the RP were about 50% lower than the values observed with OP. This difference is attributed to the formation of a more compact pellet with centrifugation as compared to sedimentation.

Table 3.5 Results of the W270 method.

Material	Slime volume (ml/800 cm <sup>2</sup> )		BFP (pg ATP/cm <sup>2</sup> )	BPP (pg ATP/cm <sup>2</sup> )
	OP*	RP**		
Glass	0; <0.1	0.01; 0.01; 0.02	28.7 ± 7.3	45.1 ± 8.8
SS	0; <0.1	0.01; <0.01; <0.01	78.8 ± 13.7	109 ± 11
Copper	0.7; 0.2	0.03; 0.06; 0.09	238 ± 116	355 ± 97
PVC-U	0; 0	<0.01; <0.01; <0.01	80 ± 11	107 ± 1.7
PVC-C	<0.1; 0	<0.01; <0.01; 0.01	109 ± 13	131 ± 7.4
PE40	<0.1; 0	<0.01; 0.01; 0.02	668 ± 208	757 ± 124
PE80	<0.1; <0.1	0.02; 0.02; 0.02	387 ± 152	425 ± 41
PE100	0; 0	0.01; <0.01; <0.01	607 ± 189	713 ± 110
PP	<0.1; 0	0.02; 0.02; 0.02	364 ± 90	442 ± 64
SILR	<0.1; <0.1	0.08; 0.02; 0.01	354 ± 61	391 ± 40
EPDMa	13; 14	4.5; 11.5; 9.75	16216 ± 12888	19361 ± 4948
EPDMb	0.5; 0.3	0.22; 0.17; 0.12	6736 ± 2864	7089 ± 2874
SBR	6.0; 7.0	1.6; 5.0; 3.5	44642 ± 20936	46406 ± 9873
PVC-P	3.8; 3.3	0.88; 0.73; 1.25	38222 ± 7287	39609 ± 6184

\*, OP original protocol, results after 3 and 6 months; \*\*RP, revised protocol, harvest after 4, 8 and 12 weeks.

A comparison between the results of the W270 method and the BPP method is presented in Fig. 3.7. This figure shows that materials with SP values  $\leq 0.1$  ml (OP) correspond with BPP values  $< 1000$  pg ATP/cm<sup>2</sup>. SP values  $\leq 0.05$  ml (RP) correspond with BPP values  $< 2000$  pg ATP/cm<sup>2</sup>. Copper is an exception, due to inorganic deposits. Lines based on a proportional relationship are presented, but a proportional relationship between biomass volume and biomass activity (ATP) may not exist. Insufficient data pairs in the range between 0.1 and 10 ml of biomass volume are available for calculation of a possible relationship.

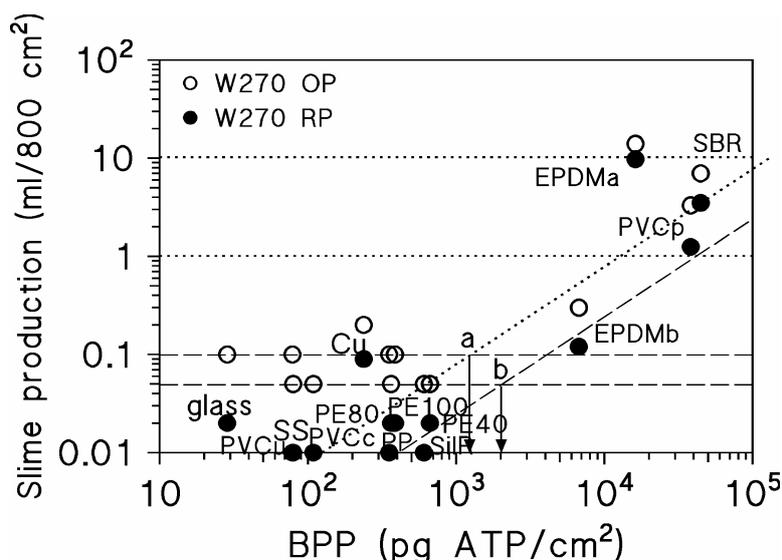


Fig. 3.7. Comparison of the results of the W270 test with the results of the BPP test. Open symbols: W270 OP; closed symbols: W270 RP. Broken lines: Pass-fail levels of W270 OP (0.1 ml/800 cm<sup>2</sup>) and W270 RP (0.05 ml/800 cm<sup>2</sup>). Arrows a and b indicate BPP values corresponding to PFC values of W270 test.

### 3.4 Comparison of W270 with MDOD

The available data enable a comparison between the results of the MDOD and W270 methods (Table 3.6 and Fig. 3.8). This comparison is hampered by the low level of growth enhancement of most materials. The growth potential of the materials glass, SS, PVC-U, PVC-C, PE100, SilR were below the detection limits of both methods. SP values for copper and EPDMb were above 0.1 ml, but MDOD values were below the detection level of the test. The materials PVC-P, SBR and EPDMa failed in both tests. Lines of proportionality between the results of both tests show that the SP value on EPDMa is relatively high. From these lines it can be derived that the PFC value for the MDOD test is clearly ( $> 10$  x) above the PFC value (0.1 ml) of the W270 test. Again, insufficient data pairs are available to show if a quantitative relationship between MDOD and SP values exists.

Table 3.6 Comparison of the results of the W270 method and the MDOD test.

Material	W270, OP* Biomass volume (ml/800 cm <sup>2</sup> )	W270, RP** Biomass volume (ml/800 cm <sup>2</sup> )	MDOD (mg/l)
Glass	0; <0.1	0.01; 0.01; 0.02	-0.2
SS	0 ; <0.1	0.01; <0.01; <0.01	-0.2
Copper	0.7; 0.2	0.03; 0.06; 0.09	0.0
PVC-U	0; 0	<0.01; <0.01; <0.01	-0.3
PVC-C	<0.1; 0	<0.01; <0.01; 0.01	0.1
PE40	<0.1; 0	<0.01; 0.01; 0.02	-0.1
PE80	<0.1; <0.1	0.02; 0.02; 0.02	0.3
PE100	0; 0	0.01; <0.01; <0.01	0.2
PP	<0.1; 0	0.02; 0.02; 0.02	0.0
SILR	<0.1; <0.1	0.08; 0.02; 0.01	-0.1
EPDMa	13; 14	4.5; 11.5; 9.75	4.7
EPDMb	0.5; 0.3	0.22; 0.17; 0.12	0.4
SBR	6.0; 7.0	1.6; 5.0; 3.5	5.0
PVC-P	3.8; 3.3	0.88; 0.73; 1.25	4.0

\*, W270 OP original protocol, result after 3 months and 6 months \*\*RP, revised protocol; harvest after 4, 8 and 12 weeks.

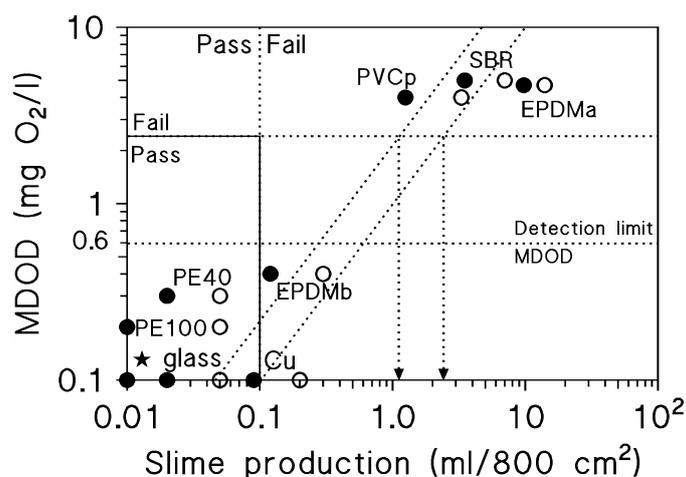


Fig. 3.8. Comparison of results of the MDOD test and W270 test. Open symbols, W270 OP; closed symbols: W270 RP. Materials with negative or zero MDOD values and biomass volumes  $\leq 0.01$  ml are not included. Dotted lines indicate assumed proportional relationships.



## 4 Discussion

### 4.1 Characteristics of test methods

The methods applied in the investigations described in this report are based on different approaches regarding the technical aspects of the test and the definition of PFC values.

The MDOD test determines the difference between the oxygen concentration in tap water without sample pieces (blank) and in tap water with samples of the material to be tested. Tap water with borosilicate glass serves as negative reference. The oxygen concentration is measured directly before replacement of the water. An MDOD value of  $0.0 \pm 0.6$  mg/l is accepted for the glass reference. The saturation concentration of oxygen in fresh water at 30 °C is 7.6 mg/l. Consequently, the growth-promoting properties which can be observed in the MDOD test cover a range of about 0.6 to 7.6 mg/l; the detection limit being about 10 times lower than maximum MDOD value. The MDOD value for the positive control ( $7.5 \pm 2.5$  mg/l) may not represent the real growth potential of the material (paraffin wax) due to oxygen limitation. Also MDOD values close to the positive control may not represent the real growth potential of the material because of a reduced oxygen uptake at low concentrations. The MDOD value is calculated as the difference of the arithmetic mean of the three measured concentrations of dissolved oxygen in the test water control and in the sample container. Information about these oxygen concentrations and their variations was not provided for the materials tested in this investigation.

In the W270 test samples of the materials are incubated in flowing tap water at ambient temperature. In this test, the volume of the slime scraped from the surface of the sample (800 cm<sup>2</sup>) is the parameter for growth promotion. The relatively large surface area requires specific production of test pieces for the test. This requirement may be considered as a disadvantage of the W270 procedure, because acquiring such samples pieces can take several months and conditions applied for preparation may differ from those applied for the regular production of the involved material. In the original protocol (OP), the volume of the produced slime (SP) is read after storage of the collected substances for 24 h in a refrigerator at 5 °C. In the revised protocol (RP) the collected material is subjected to centrifugation, which results in a more rapid sedimentation and a smaller final volume (DVGW, 2007). With the W270 OP, the SP value for the glass reference is < 0.1 ml and about 6 ml/800 cm<sup>2</sup> for the positive control (paraffin). Higher values (> 10 ml) have been reported for a number of materials e.g. rubbers and plasticized PVC (Schoenen und Schöler, 1983; Schoenen und Colbourne, 1987). Hence, the SP values for the blank (glass) and the positive control in the W270 method cover a range of 60 times the value of the blank. This range may exceed 100 times with materials producing more slime than paraffin.

The BPP value for glass was about 45 pg ATP/cm<sup>2</sup> and the highest value, observed with SBR, was more than 46.000 pg ATP/cm<sup>2</sup> (Table 3.1). The BPP test thus enables to quantify the growth-promoting properties of materials differing more than 1000 times in microbial growth enhancement. This range is even larger, because a detection level of 0.5 pg ATP/ml and a surface area of about 28 cm<sup>2</sup> of the sample gives a detection level for attached biomass of a few pg ATP/cm<sup>2</sup>. This level is clearly below the biofilm concentration observed on glass, which is attributed to the effect of the test water. The BPP test thus enables quantification of very low levels of growth enhancement.

Oxygen consumption generally is considered to be a parameter for biological activity and may represent biological activity when chemical oxidation can be excluded. However, when oxygen uptake is limited in the test, also the activity is limited. ATP represents active biomass; the compound is not present in dead biomass (Holm-Hansen and Booth, 1966). The relationship observed between the ATP concentration and the total concentration of bacterial cells (Fig. 3.3) confirms that ATP is well suited for determining the concentration of active bacteria in the BPP test. From the median value of the ATP content per bacterial cell (0.052 fg/cell), it can be calculated that a BPP value of 1000 pg ATP/cm<sup>2</sup> corresponds with  $1.9 \times 10^7$  cells/cm<sup>2</sup>. The significance of the volume of produced slime (SP) as growth parameter is less clear. The material scraped from the surface of the exposed material may include compounds not related to biological activity, e.g. corrosion products (as reported for copper but not quantified) or other constituents originating from the tested materials. Furthermore, the SP value may include an unknown amount of dead biomass, not directly related to the actual utilisation of biodegradable compounds from the material. Finally, the nature of microbial growth, e.g. presence of certain extracellular polymeric substances (EPS), may have an impact on the properties of the biomass, affecting its sedimentation. Hence, the observed slime volume may not be proportional to the amount of viable biomass and the composition may differ between materials, and depend on the temperature. The relatively high SP of EMPMA as compared to the values for BPP and MDOD might be due to the formation of voluminous slime. As a result, SP values and MDOD, and also SP and ATP may not show clear relationships, although generally a higher SP value may relate to elevated values for MDOD and BPP.

A further aspect related to the parameter used for microbial growth is its direct significance in relation to the quality of drinking water. Oxygen is present in drinking water, usually at concentrations close to saturation. Strong oxygen consumption in drinking water during distribution is undesirable, because it may result into local oxygen depletion, followed by microbial activity at anaerobic conditions, causing problems with taste, odour and colour. An MDOD value of 7.6 mg/l (positive control) implies that oxygen depletion occurs within 4 days at a surface-to-volume (S/V) ratio of 0.15 cm<sup>-1</sup>. In practice, at a lower temperature, oxygen uptake may be slower than under the conditions of the test (30 °C). From the MDOD value, also the oxygen uptake rate (mg/m<sup>2</sup>.d) of the biomass on the materials can be estimated, e.g. an MDOD of 1 mg/l corresponds with an uptake rate of about

16 mg/(m<sup>2</sup>.d). With such an uptake rate, an estimation can be derived for the oxygen concentration in water in contact with the involved material, depending on contact time and SV ratio. However, the impact on the oxygen concentration in drinking water is not considered for the evaluation of the MDOD of the tested material.

For the SP values, the comparison between the test parameter and water quality is even more difficult, because information about the relationship between SP values on the material surface and in the test water is not collected in the W270 test. Estimations for slime volumes in treated water may be derived from total cell counts and cell volumes. However, these volumes do not take into account the amount of EPS and water as present in slime scraped from materials. As a result, the interpretation of the observed SP values can only be derived from the ranking of the materials and possibly from information about the volume of slime observed on surfaces in situations in practice, in relation to water quality problems.

The BPP test gives information about the concentration of active biomass on the surface of the material and in the water. These data enable ranking of the materials, but the concentrations in the water and on the material can be used directly for comparison with situations in practice. Information about ATP concentrations in drinking water is available. Values over 10 ng ATP/l are considered relatively high in drinking water distributed without a disinfectant residual in the Netherlands and values below 1 ng/l are low (Van der Kooij, 1992; Van der Kooij, 2003). ATP also is a useful parameter for quantification of microbiological activity in water treatment and distribution. Information is available about ATP concentrations of biofilms in distribution systems, in granular activated carbon filters, in membrane systems and in experimental water installations (Van der Kooij et al., 2003; Magic-Knezev and Van der Kooij, 2004; Van der Kooij et al., 2005). A further argument in favour of the ATP analysis is that the result is obtained within a few minutes, once a sample with suspended micro-organisms is available. For biofilm analysis, detachment of micro-organisms is required, which can be considered as a disadvantage compared to the MDOD test.

The repeatability of the test results also is an important performance characteristic of the method. The standard deviation of the BPP values ranged from values over 20% to a few values below 10% (Table 3.1). No information about the standard deviations of the results of the MDOD and W270 tests was obtained. Standard deviations < 10% have been reported for MDOD values of 3 to 5 mg/l and 100% for an MDOD value of about 0,1 mg/l (Colbourne and Brown, 1979). Reproducibility values <10% have been reported for paraffin wax (Ashworth and Colbourne, 1987). For the repeatability (*r*) of the arrhythmic mean of the MDOD result a value of 15% is mentioned in BS 6290 and a reproducibility (R) of 20%. The negative MDOD values observed for a number of materials remain unexplained. From results reported for the W270 test (original procedure) it can be derived that standard deviations, based on a test in duplicate with a number of different PVC-P sheets, ranged from less than 10% to a value over 40% (Schoenen and Colbourne, 1987). Relative

standard deviations of about 30% have also been reported recently with paraffin wax in the W270 following the original procedure and the revised procedure (DVGW, 2007). The PFC value proposed for the revised procedure ( $0.05 \pm 0.02$  ml) suggests a relative standard deviation of about 40% at low SP levels.

The PFC value for the MDOD method in the UK is based on the formation of visible slime when the oxygen consumption exceeds a certain value (2.4 mg/l) (Colbourne, 1985). The detection limit (0.1 ml/800 cm<sup>2</sup>) is used as PFC value in the original protocol of the German W270 method (DVGW, 1998). For the BPP test, a PFC value has not yet been defined. The detection limit of the BPP method (a few pg ATP/cm<sup>2</sup>) is even below biofilm concentrations observed in highly oligotrophic environments and therefore cannot be used as the PFC value for materials in contact with treated water intended for human consumption. Comparison of the BPP method with the MDOD method and the W270 is a first step in the process of collecting information needed to define a PFC value for the BPP test.

## 4.2 Comparison of test results

### 4.2.1 Ranges of values

The majority of the tested materials gave insufficient promotion of growth for a readable result in both the MDOD and W270 tests. A few materials gave clear results in the MDOD and W270 tests but these values were all of the same order of magnitude and only one material had a BPP value between 10<sup>3</sup> and 10<sup>4</sup> pg ATP/cm<sup>2</sup>. Consequently, insufficient data points were obtained for calculation of the relationship between BPP values and values for MDOD and SP, respectively. The requirement of using identical materials in the different tests, in combination with the requirement of using flat sample pieces for the W270, resulted in the use of materials with unknown growth-promoting properties. In a number of cases growth promotion was less than observed earlier for pipe materials of similar materials (e.g. PE and silicon rubber; Van der Kooij and Veenendaal, 2001; Van der Kooij et al., 2002). The results demonstrate that the detection level of the BPP test is clearly below the level of detection of the MDOD and W270 methods.

### 4.2.2 Pass-fail criteria

The second question to be answered with the investigation concerned the comparison of the PFC values for the MDOD and the W270 tests with BPP values. Materials with a BPP value below 1000 pg ATP/cm<sup>2</sup> gave MDOD and SP values at or below the detection limit of these methods. The position of a number of data points at or below the detection limit of the W270 – method (0.1 ml) is uncertain, because information about the standard deviation was not provided. Assumption of proportional relationships between BPP and MDOD or SP give indications for BPP values related to the PFC values of these tests. These values are estimated at 2.10<sup>4</sup> pg ATP.cm<sup>2</sup> for MDOD (2.4 mg/l) and 2000 pg ATP.cm<sup>2</sup> for SP 0.05 ml/800 cm<sup>2</sup> (Fig. 3.6, Fig. 3.7). At these BPP values, the ATP concentration in the water in the BPP test, which simulates a situation with one-week stagnancy at a defined S/V ratio

( $0.166 \text{ cm}^{-1}$ ), is  $> 10 \text{ ng ATP/l}$  (Fig. 3.5). Also a level of  $1000 \text{ pg ATP/cm}^2$  increases the ATP concentration of the water to a value above  $10 \text{ ng ATP/l}$ . Fig. 3.5 shows that the 'no-effect level' of the BPP and BFP value was below  $100 \text{ pg ATP/cm}^2$ . The S/V ratio applied in the BPP test corresponds with an internal pipe diameter of 24 cm. Distribution system pipes transporting the water to houses and buildings, and pipes inside buildings have a 2 to 10 times smaller diameter, i.e. a 2 to 10 times higher S/V ratio. A BPP test at a higher S/V ratio may lead to a lower no-effect level than the value obtained in this investigation.

HPC values which were more than 100 times the maximum acceptable concentration for drinking water ( $100 \text{ CFU/ml}$ ) were observed in the BPP test with EPDMa, SBR and PVC-P (Table 3.3). These materials had BPP values  $> 10^4 \text{ pg ATP/cm}^2$  and also the values for MDOD and SP were above the PFC levels defined for these tests. These observations confirm that the MDOD and W270 procedures are suited to prevent the use of materials which have a strong negative impact on the bacteriological quality of water. The material EPDMb, which also gave an elevated HPC value in the BPP test, passed the MDOD test, but the SP level was above the PFC value in the W270 procedure. Most materials with BPP values ranging from 100 to  $1000 \text{ pg ATP/cm}^2$  had HPC values  $< 100 \text{ CFU/ml}$ , but HPC values exceeded  $100 \text{ CFU/ml}$  in the blank, with glass, copper and SilR. These observations suggest that the biofilm on SS, PVC-U, PVC-C, PE and PP may have a favourable effect on the HPC value in the water. This effect is attributed to the presence of unculturable bacteria in the biofilm, competing with culturable bacteria, because the PB concentrations in water with these materials ( $10 \text{ ng ATP/l}$ ) indicate that the total number of bacteria is higher than  $10^5 \text{ cells/ml}$ . Consequently, HPC values are not suited to evaluate BPP values.

Elevated NPOC values were observed only with the soft materials (Table 3.3), which also had the highest BPP values and the highest HPC values. Compounds released from the material probably promoted the growth of planktonic bacteria. However, the elevated NPOC concentrations observed after one week of contact between the sample pieces (with the biofilm) and the water (just before replacement) may represent compounds which either are not or only slowly biodegraded. Elevated concentrations of planktonic biomass were also observed in the BPP test, without increase of the NPOC concentration. Hence, NPOC analysis may give an indication about the ability of a material to promote microbial growth, but a sensitive bioassay is needed to obtain a quantitative determination of the growth potential.

The BPP test gives quantitative information about the microbial growth potential of materials in contact with drinking water at levels which remain undetected by the MDOD and W270 methods. The low detection level of the BPP test makes it possible to distinguish between the effect of the water (using a blank and a glass control) and the effect of the material and to determine the level at which a material has no effect on the concentration of active biomass in the water. These levels are far below the PFC values in the MDOD and W270 procedures.

A significant water quality effect related to biofilm formation is the growth of *Legionella* in water installations. Certain protozoa grazing on the bacteria in the biofilm can serve as host for *L. pneumophila* (Rowbotham, 1980, Kuiper et al. 2004). *L. pneumophila* has been observed in biofilms at concentrations below 500 pg ATP/cm<sup>2</sup> in static tests and in a model warm water system (Van der Kooij et al., 2002; Van der Kooij et al., 2005). These observations indicate that prevention of the enhancement of growth of *Legionella* by materials in contact with drinking water requires lower BPP values than those corresponding with the PFC values for MDOD and SP (W270), and even lower than the detection limits of these methods. More information about the relationship between BPP values and growth of *Legionella* is needed for the definition of PFC values for materials in contact with drinking water.

# 5 Conclusions and recommendations

## 5.1 Summary and conclusions

- Complete removal of attached biomass (biofilm) from soft materials requires the application of swabbing after two HES treatments;
- The selected materials in contact with drinking water in the BPP test showed large differences in the concentrations of attached biomass and planktonic biomass (PB). The BPP values ranged from about 45 pg ATP/cm<sup>2</sup> for glass to more than 46000 pg ATP/cm<sup>2</sup> for SBR;
- The concentration of attached biomass measured with ATP analysis is directly related to the total concentration of bacterial cells. A biofilm concentration of 1000 pg ATP/cm<sup>2</sup> corresponds with about 1.9×10<sup>7</sup> cells/cm<sup>2</sup>;
- The concentrations of attached biomass, measured on days 56, 84 and 112, varied with some materials and showed a decline (copper) or an increase (EPDM, PVC-P);
- The concentration of planktonic biomass in the test water is not elevated after one week of stagnation ('no-effect level') at BPP values <100 pg ATP/cm<sup>2</sup> (not corrected for the blank with glass). The BPP value after correction is < c. 50 pg ATP/cm<sup>2</sup>;
- Three of the 14 tested materials gave a significantly elevated MDOD value. These materials were EPDM, SBR and PVC-P;
- Insufficient suited data pairs were obtained for calculation of an eventual quantitative relationship between the BPP values and the MDOD values of the tested materials;
- From the comparison of the results of MDOD with BPP values, assuming a proportional relationship, it can be derived that the PFC value for the MDOD test (2.4 mg/l) corresponds with a BPP value of about 2×10<sup>4</sup> pg ATP/cm<sup>2</sup>;
- Four of the 14 selected materials gave an elevated biomass volume (SP) in the W270 test. These materials were EPDM a and b, SBR and PVC-P;
- Insufficient suited data pairs were obtained for calculation of an eventual quantitative relationship between the BPP values and the SP values of the tested materials;
- From the comparison of the results of W270 test with the BPP values, assuming a proportional relationship, it can be derived that the PFC value for the W270 test (0.05 ml) corresponds with a BPP value of about 2×10<sup>3</sup> pg ATP/cm<sup>2</sup>;
- No relationship could be observed between values of MDOD and SP because most values were below the detection level of both methods;
- The BPP test quantifies the growth-promoting properties of materials at levels which are below the level of detection of the MDOD and W270 methods.

## 5.2 Recommendations

- Further investigations to determine the relationship between BPP values and the concentration of *Legionella* in the biofilm and in the water.

## 6 References

- Ashworth, J. and J.S. Colbourne. 1987. The testing of non-metallic materials for use in contact with potable water and the interrelationships with in service use. *Industrial Microbiological testing*: 151-169.
- Bernhardt, H. und H.U. Liesen, 1988. Trinkwasserverkeimungen in Verteilungsnetzen durch Korrosionsschutz auf Bitumenbasis. *GWF Wasser-Abwasser* 129(1):28-32.
- BSI 2000. Suitability of non-metallic products for use in contact with water intended for human consumption with regard to their effect on the quality of the water. Part 2: Methods of test- Section 2.4: Growth of aquatic microorganisms. BS 6920-2.4.
- Colbourne, J.S. and D.A. Brown, 1979. Dissolved oxygen utilization as an indicator of total microbial activity on non-metallic materials in contact with potable water. *J. Appl. Bacteriol.* 47:223-231.
- Colbourne, J.S., D.J. Pratt, M.G. Smith, S.P. Fisher-Hoch and D. Harper, 1984. Water fittings as sources of *Legionella pneumophila* in a hospital plumbing system. *Lancet* January 28:210-213.
- Colbourne, J., 1985. Materials usage and their effects on the microbiological quality of water. *J. Appl. Bacteriol. Symp. Suppl.* 47S-59S.
- DVGW, 1998. Vermehrung von Mikroorganismen auf Materialien für den Trinkwasserbereich-Prüfung und Bewertung-. *Technische Regeln Arbeitsblatt W270*. Eschborn.
- DVGW, 2007. Research into the refinement of the DVGW Code of practice W 270. 'Surface biomass of micro-organisms on materials used in drinking water supply'. DVGW Report W6/03/03, CEN/TC164/WG3 N885
- Ellgas, W.M. and R. Lee, 1980. Reservoir coatings can support bacterial growth. *J. Am. Water Works Assoc.* December 693-695.
- European Commission, 1998. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. *Official Journal of the European Communities* L 330: 32-53.
- Frensch, K., J.U. Hahn, K. Levsen, J. Niessen, H.F. Schöler und D. Schoenen, 1987. Koloniezahlerhöhungen in einem Trinkwasserbehälter verursacht durch Lösemittel des Anstrichmaterials. *Zbl. Bakt. B.* 184:556-559.
- Hobbie J.E., Daley R.J. and Jasper S. (1977). Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33 (5), 1225-1228.
- Holm-Hansen, O. and C.R. Booth 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. *Limnol. Oceanogr.* 11: 510-519.
- Kuiper, M.W., B.A. Wullings, A.D.L. Akkermans, R.R. Beumer and D. van der Kooij. 2004. Intracellular proliferation of *Legionella pneumophila* in *Hartmannella vermiformis* in aquatic biofilms grown on plasticized polyvinyl chloride. *Appl. Environ. Microbiol.* 70(11):6826-6833.
- Magic-Knezev, A. and D. van der Kooij. 2004. Optimisation and significance of ATP analysis for measuring active biomass in granular activated carbon filters used in water treatment. *Wat. Res.* 38:3971-3979.
- Niedeveld, C.J., F.M. Pet and P.L. Meenhorst, 1986. Effect of rubbers and their constituents on proliferation of *Legionella pneumophila* in naturally contaminated hot water. *Lancet* July 26: 180-184.

- NEN, 2004. Bepaling van de biomassa-productiepotentie (BPP) van kunststof materialen metalen en coatings in contact met (leiding)water. Ontwerp NVN 1225, Normcommissie 349 164 'Drinkwatervoorziening'.
- NEN-EN-ISO 6222. Tellen van kweekbare micro-organismen. Bepaling van het koloniegetal.
- Önorm B 5018-1, 2002. Prüfung der Verkeimungsneigung von Trinkwasserrohren. Teil 1: Prüfverfahren. Österreichisches Normungsinstitut, 1020 Wien.
- Rogers, J., A.B. Dowsett, P.J. Dennis, J.V. Lee and C.W. Keevil, 1994. Influence of plumbing materials on biofilm formation and growth of *Legionella pneumophila* in potable water systems. *Appl. Environ. Microbiol.* 60:1842-1851.
- Rowbotham, T.J. 1980. Preliminary report on the pathogenicity of *Legionella pneumophila* for freshwater and soil amoebae. *J. Clin. Pathol.* 33:1179-1183
- Schoenen, D. and H.F. Schöler, 1983. *Trinkwasser und Werkstoffe: Praxisbeobachtungen und Untersuchungsverfahren; Praxisbeobachtungen und Untersuchungsverfahren*. DVGW-Schriftenreihe, Wasser Nr. 37. Gustav Fischer Verlag, Stuttgart.
- Schoenen, D. und J.S. Colbourne, 1987. Microbiological evaluation of drinking water construction materials. *Zbl. Bakt. Hyg. B.* 183. 505-510.
- Schoenen, D. R. Schulze-Röbbecke und N. Schirdewahn, 1988. Mikrobielle Kontamination des Wassers durch Rohr- und Schlauchmaterialien. 2. Mitteilung: Wachstum von *Legionella pneumophila*. *Zb. Bakt. Hyg. B* 186:326-332.
- Seidler, R.J., J.E. Morrow, S.T. Bagley, 1977. *Klebsiella* in drinking water emanating from redwood tanks. *Appl. Environ. Microbiol.* 33:893-900.
- Van der Kooij, D. and H.R. Veenendaal 1993. Assessment of the biofilm formation potential of synthetic materials in contact with drinking water during distribution. pp. 1395-1407 *In Proc. Am. Water Works Assoc. Water Qual. Technol. Conf. Miami (Fla), 7-11 November 1993.*
- Van der Kooij, D., H.R. Veenendaal, C. Baars-Lorist, D.W. van der Klift and Y.C. Drost. 1995. Biofilm formation on surfaces of glass and teflon exposed to treated water. *Wat. Res.* 29(7):1655-1662.
- van der Kooij, D. and H.R. Veenendaal, 2001. Biomass production potential of materials in contact with drinking water: method and practical importance. *Water Sci. Technol. Water Supply* 1(3), 39-45.
- van der Kooij, D., H.R. Veenendaal, N.P.G. Slaats and D. Vonk, 2002. Biofilm formation and the multiplication of *Legionella* on synthetic pipe materials in contact with treated water under static and dynamic conditions. In *Legionella* (eds R. Marre et al), ASM press, Washington D.C. pp 176-180.
- Van der Kooij, D. 2003. Managing regrowth in drinking water distribution systems. Pp. 199-232. *In Heterotrophic plate counts and drinking water safety*. Eds. J. Bartram, J. Cotruvo, M. Exnes, C. FRicker, A. Glasmacher. IWA Publishing London, UK.
- Van der Kooij, D., J.S. Vrouwenvelder and H.R. Veenendaal. 2003. Elucidation and control of biofilm formation processes in water treatment and distribution using the unified biofilm approach. *Water Sci. Technol.* 47 (5): 83-90.
- van der Kooij, D., H.J. Albrechtsen, C.B. Corfitzen, J. Ashworth, I. Parry, F. Enkiri, B. Hamsch, C. Hametner, R. Kloiber, H.R. Veenendaal, D. Verhamme, E.J. Hoekstra, 2003. CPDW project: Assessment of the microbial-growth support potential of products in contact with drinking water. *Report EUR 20832 EN*.
- Van der Kooij, D., H.R. Veenendaal, W.J.H. Scheffer. 2005. Biofilm formation and multiplication of *Legionella* in a model warm water system with pipes of copper, stainless steel and cross-linked polyethylene. *Wat. Res.* 39:2789-2798.
- Van der Kooij, D., P.K. Baggelaar, H.R. Veenendaal, L. Moulin, C.B. Corfitzen, H.J. Albrechtsen, D. Holt and B. Hamsch. 2006. Standardising the Biomass Production

Potential method for determining the enhancement of microbial growth by construction products in contact with drinking water. Report EC Grant Agreement nbr SI2. 403889.

VROM, 2001. Besluit van 9 januari 2001 tot wijziging van het Waterleidingbesluit in verband met de richtlijn betreffende de kwaliteit van voor menselijke consumptie bestemd water. *Staatsblad* 31:1-25.

VROM, 2002a. Regeling materialen en chemicaliën leidingwatervoorziening. *Staatscourant* 241 pp. 25-36.



# Appendix 1: Draft BPP method, CEN TC164/WG3/AHG3 (August 2006)



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UDC

Key words: Factory made or factory applied materials; water quality; migration test method

English version

**Influence of materials on water intended for human consumption  
- Promotion of Microbiological Growth**

Influence sur l'eau des matériaux  
destinés à entrer en contact avec  
l'eau destinée à la consommation humaine -  
Développement de micro-organismes  
aquatiques

Einfluß von Bedarfsgegenständen auf  
Trinkwasser –

This draft European Standard is submitted to CEN members for Member body enquiry.  
It has been drawn up by Technical Committee CEN/TC 164 "Water supply".

If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

This draft European Standard shall be established by CEN in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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**CEN**  
European Committee for Standardization  
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Europäisches Komitee für Normung

**Central Secretariat: rue de Stassart 36, B - 1050 Brussels**

## Introduction

Water intended for human consumption comes into contact with construction products during storage, transportation and distribution, including water systems inside buildings. The materials used in these products are selected on the basis of technical requirements and criteria regarding their influence on the water quality, e.g. release of toxic substances and effects on odour, flavour or colour of the water. However, water quality problems may also arise when such materials enhance the multiplication of micro-organisms. Examples of problems that can occur include: increased heterotrophic plate counts and growth of undesired types of bacteria (e.g. *Legionella*, coliforms, *Pseudomonas*). Therefore, materials should also be tested for their potential to promote growth of microorganisms. This standard describes a method, the Biomass Production Potential (BPP) test, which determines the production of biomass of microorganisms in the presence of a material incubated in biologically stable water.

The Biomass Production Potential (BPP) test is based on a combination of two principles as follows:

- 1) determination of the biomass concentration with adenosine tri-phosphate (ATP) analysis, **and**
- 2) semi-dynamic test conditions with replacement of the test water once a week

ATP is a carrier of chemical energy and is present in all living (active) organisms. It provides the energy for metabolic processes in the cell. The ATP level is a measure for the concentration of active biomass and reflects the availability of biodegradable compounds serving as a source of energy. The ATP concentration in the cell decreases rapidly when the food supply decreases. This property means that the concentration of ATP provides a quantitative indication of the potential of a material to promote microbial growth.

## 1. Scope

This European Standard specifies a procedure to determine the ability of non-cementitious and non-metallic materials to promote the growth of microorganisms in water.

This standard is applicable to materials intended to be used under various conditions for the transport and storage of water intended for human consumption, including raw water used for the production of water intended for human consumption.

## 2. Normative references

This European Standard incorporates, by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter.

For dated references, subsequent amendments to, or revision of, any of these publications apply to this European Standard only when incorporated in it by amendment or revision.

For undated references the latest edition of the publications referred to applies.

EN ISO 3696:1995, Water for analytical laboratory use – Specification and test methods

## 3. Definitions

**3.1 suspended-biomass concentration:** the concentration of active micro-organisms in the test water and expressed as amount of adenosine tri-phosphate (ATP) per ml of the test water

**3.2 biofilm (attached biomass) concentration:** the concentration of active microorganisms on the surface of the material and expressed as amount of adenosine tri-phosphate (ATP) per cm<sup>2</sup> of the material

**3.3 biomass production (BP):** the sum of the amount of metabolically active biomass present on the surface of materials and the concentration of suspended active biomass present in the water in contact with the material, measured on the same day; concentrations are calculate as pg ATP/cm<sup>2</sup>

**3.4 biomass production potential (BPP):** a measure of the amount of metabolically active biomass growing on the surface of the material and in the water in contact with it (after taking into account the growth associated with the negative reference material (5.10.2)), measured as adenosine triphosphate (ATP) per cm<sup>2</sup> of the test material, after incubation for 56, 84 and 112 days under defined conditions.

**3.5 ATP:** adenosine tri-phosphate – the compound produced by the metabolic activities of all living organisms; used, in the context of this method, as a measure of the amount of metabolically active biomass in water and on the material surface

**3.6 AOC:** assimilable organic carbon

**3.7 product:** a manufactured item, in its finished form, that comes into contact with water intended for human consumption, or a component part of a manufactured item, and made from one or more materials.

NOTE: this test method is suitable for use with individual materials, or products made solely from one material. It is unsuitable for use with assembled products made from more than one material.

**3.8 homogeneous material:** a material where the water contact surface is made from the same material as the remainder of the material.

**3.9 non-homogeneous material:** a material where the water contact surface is made from a material that differs from those comprising the remainder of the material. Special test pieces, manufactured with the water contact material on all surfaced shall be used for test purposes.

**3.10 test sample:** a sample of a material submitted for testing.

**3.11 test piece:** the test sample, or a part of it, that is tested.

**3.12 tap water:** water intended for human consumption (see also 5.2).

**3.13 prewashing water:** water used for prewashing test samples (see also 5.3)

**3.14 factory made materials:** materials made in a factory under controlled conditions as part of the manufacturing process

**3.15 site applied materials:** materials manufactured on site, e.g. sealing membranes, or materials mixed, applied and cured on site, e.g. coatings, sealants and adhesives

## 4. Principle

Representative samples of the material to be tested are incubated in tap water containing specified inorganic supplements and inoculated with a mixture of naturally occurring micro-organisms derived from river water. These material samples are incubated for a period of 16 weeks at a constant surface to volume ratio of 0.16 cm<sup>-1</sup>. The surface to volume ratio is kept constant by removing a proportional volume of water from the test containers after sampling (removal) of the test pieces for biofilm determination (3.2).

The test water is replaced once a week.

Formation of biomass on the material surface (biofilm) and in the water is determined with adenosine triphosphate (ATP) measurements after 8, 12 and 16 weeks of incubation. The ATP concentration is used as a measure for the presence of active microbial biomass, and the biomass associated with a unit surface area of the test material is calculated from the concentrations of attached and suspended biomass.

Validation of the results is achieved by testing glass controls and reference materials in parallel with the materials under test.

The test is shown schematically in Annex D.

## 5. Reagents

Use analytical grade reagents unless otherwise specified.

**5.1 test water**, free from toxic effects on bacteria, with a high degree of biological stability, sufficient inorganic nutrients, and conforming with the specification given in table 1.

Table 1 Quality criteria for test water

Quality parameter	Concentration (range)	Comment
Hydrogen ion (pH) concentration	6.5 – 8.5	
Free-chlorine	<0.2	This can be removed using sodium thiosulphate, activated carbon or by standing the water for 48 hours at 4°C
Oxygen (mg/l)	> 6.5	
PO <sub>4</sub> <sup>3-</sup> -P (mg/l)	2.0 – 6.7	Add potassium dihydrogen orthophosphate (5.5) if needed
Nitrate-N	5 – 11.3	Add potassium nitrate (5.6) if needed
Ammonia-N (mg/l)	< 0.05	
Copper (mg/l)	< 0.05	
Silver (mg/l)	< 0.01	
Total dissolved organic carbon (mg/l)	< 2	
Biostability (BP, ng ATP/l)	< 10	This degree of biostability corresponds with an AOC value < 10 µg C/l

**5.1.1 biostability of the test water** – assessment of the biological stability of the test water shall be determined as BPP (3.4), ng ATP/l, determined in accordance with Annex A.

**5.1.2 free-chlorine** – this shall be ≤ the limit of detection of the method used to assess the free-chlorine concentration

**5.2 tap water**, water intended for human consumption conforming with the EC Directive 98/83/EC (on the quality of water intended for human consumption) and with a free chlorine content less than 0.2 mg/l as Cl<sub>2</sub>

**5.3 reagent water**: water conforming to Grade 3 of EN ISO 3696

**5.4 dilution water**: autoclaved reagent water (5.3)

### 5.5 potassium dihydrogen orthophosphate solution

#### Composition

KH <sub>2</sub> PO <sub>4</sub>	0.79 g
Reagent water (5.3)	100 ml

#### Preparation

Dissolve the potassium dihydrogen phosphate in reagent water and autoclave this solution at (121 ± 10)°C for 15 min. Store in tightly closed containers at 4°C for a maximum period of 1 month.

## 5.6 potassium-nitrate solution

### Composition

KNO <sub>3</sub>	3.24 g
Reagent water (5.3)	100 ml

### Preparation

Dissolve the potassium nitrate in demineralised water and autoclave this solution at  $(121 \pm 10)^{\circ}\text{C}$  for 15 min. Store in tightly closed containers at  $4^{\circ}\text{C}$  for a maximum period of 1 month.

**5.7 inoculum**, consisting of a fresh sample taken from a lowland surface water suitable for abstraction for water intended for human consumption and conforming with -

- pH – 5.5 to 9.0
- Copper –  $<0.05$  mg/l

### Preparation for use -

Assemble a membrane filtration unit with the membrane filter (6.7). Flush the membrane filter with about 250 ml of reagent water (5.3) to remove soluble organic carbon compounds from the membrane – discard this water. Filter the river water through the membrane and collect the filtrate in a sterile flask. Store for a maximum of 1 week at  $4^{\circ}\text{C}$ .

Note: very turbid water (after filtration) or water visibly green with algal growth, should not be used as an inoculum

## 5.8 Cleaning liquids for glassware

**5.8.1 hydrochloric acid**, concentrated (30% mass per volume) analytical reagent grade.

**5.8.2 hydrochloric acid solution**, prepared by slowly adding  $(0.5 \pm 0.01)$  litre of concentrated hydrochloric acid (5.8.1) to  $(0.5 \pm 0.01)$  litre of reagent water (5.3).

NOTE: Care is needed because the solution may generate heat.

**5.8.3 nitric acid**, concentrated (65% mass per volume) analytical reagent grade.

**5.8.4 nitric acid solution**, prepared by slowly adding  $(0.5 \pm 0.01)$  litre of concentrated nitric acid (5.8.3) to  $(0.5 \pm 0.01)$  litre of reagent water (5.3).

NOTE: Care is needed because the solution may generate heat.

**5.8.5 sulphuric acid**, concentrated (density 1.84 g/ml) analytical reagent grade.

## 5.9 Test reference materials

**5.9.1 positive reference material** - plasticised PVC (PVC-P) tubing manufactured using phthalate plasticizers and shown to support microbiological growth.

Under the conditions of testing this material shall give a biomass production potential (BPP) value (10.2) of greater than  $10\,000$  pgATP/cm<sup>2</sup>.

**5.9.2 negative reference material** - borosilicate glass rings (outer diameter 18 mm, wall thickness 2 mm; length 15 mm) with a total external surface of about 17 cm<sup>2</sup>

## 5.9.3 sodium acetate solution

### Composition

Sodium acetate (water free)	25.63 g
Reagent water (5.3)	100 ml

### Preparation

Dissolve the sodium acetate in the reagent water. Add  $(1 \pm 0.1)$  ml of this solution to  $(9 \pm 0.1)$  ml of autoclaved tap water contained in an AOC-free culture tube with stainless steel cap and place this tube in water at  $(60 \pm 2)^{\circ}\text{C}$  for 30 minutes.

NOTE: this solution must be used within a few hours and a fresh solution prepared for each test.

#### Use

Add  $(0.4 \pm 0.05)$  ml of this solution with a 1 ml pipette to a flask containing 600 ml of test water.

## 6. Apparatus

**6.1 Vessels, containers, stoppers and connectors and general laboratory apparatus** shall consist of a material, such as glass, PTFE or stainless steel, that is inert under the specified test conditions

NOTE: The material PTFE should only be used when there is a small contact area with the test water. Thus PTFE is unsuitable for containers.

**6.2 Plates of stainless steel, mild steel, (sand-blasted) glass or concrete/cement-mortar plates**, for testing linings or the material itself. The plates must be covered completely with the test material.

**6.3 Test containers** – suitable test containers include screw topped glass jars with a volume of 1000 ml, an internal neck diameter of 57 mm, provided with a lid with PTFE inlay

**6.4 Test tubes** – sterile glass tubes of suitable diameter for use for sterile dilution water or in sonication of test samples, with suitable caps

**6.5 Incubator (or hot room)**, capable of maintaining the test temperature of  $(30 \pm 2)^\circ\text{C}$  (in the absence of light), and free from volatile organic compounds

NOTE: volatile organic compounds in the air may cause microbial growth in the test containers, masking the growth attributable to the test samples.

**6.6 Sterilisation ovens** – capable of maintaining temperatures of  $(160 \pm 10)^\circ\text{C}$ ,  $(250 \pm 10)^\circ\text{C}$  and  $(550 \pm 20)^\circ\text{C}$ .

**6.7 Membrane filters** – pore size  $1.2 \mu\text{m}$  – used for filtering the inoculum (5.8)

### 6.8 Cleaning

**6.8.1. general laboratory glassware and stainless steel plates** - clean by washing with a biodegradable laboratory detergent, followed by rinsing with either hydrochloric acid solution (5.8.2) (except for stainless steel), or nitric acid solution (5.8.4) and finally by thoroughly rinsing with reagent water (5.3). Drain and dry them in a hot air cabinet.

**6.8.2 test containers (6.3)** - wash the test jars, lids and inlays/seals in a washing machine using the cleaning sequence in accordance with 6.8.1. After washing, flush with reagent water (5.3). Drain and dry them in a hot air cabinet.

**6.8.3 test tubes for dilution water (6.4)** - wash in a washing machine using the cleaning sequence in accordance with 6.8.1. After washing drain the tubes, cover with caps and heat at 150 to 175°C for 4 hours.

## 7. Test samples and test pieces

### 7.1 Sampling, transport and storage of test samples

Sample materials in accordance with the relevant product/ system standard or with the relevant national regulations when applicable.

The test material samples should originate from a normal production batch, which has not received any special treatment, and represent the material used in contact with drinking water

Products such as washers and O-rings can be tested as such provided that these materials are of suitable size. Large products (e.g. pipes) are cut to sample pieces with the desired surface area and size.

Ensure that the surface of test pieces intended to come into contact with test water shall be free from adhesive tape, labels, ink or pencil marks. Take care to ensure that the transport and storage conditions shall not influence the test results.

NOTE 1: If the test samples have to be stored, then this should be done in the absence of light at  $(23 \pm 5) ^\circ\text{C}$ , in stainless steel containers, tissue-paper, glassware or other materials, which do not influence the results of the test, except where the supplier of the test samples provides alternative written storage instructions which are those that the materials/products are subject to in practice.

NOTE 2: Storage envelopes or pockets should not be sealed, dusting powder should not be used and cleaning should not be carried out unless any of these procedures form part of the usual production procedures. Where appropriate, storage containers should be cleaned using the same procedures as are used for the test containers.

## **7.2 Test piece preparation**

### **7.2.1 General**

Prepare test pieces (3.11) in such a way that only the surface intended to come into contact with drinking water is exposed to the test water (5.1). The total surface area of the sample exposed to water should be  $50 \text{ cm}^2$ ; to achieve this one sample may be made up of more than one test piece.

For homogeneous materials it is acceptable to expose the whole test piece to the test water, including surfaces not intended to come into contact with drinking water. Calculate the total surface area of the test piece and the surface-area-to-volume (S/V) ratio actually to be used.

NOTE: If a homogeneous material has to be cut to obtain the required test piece size, this should be done in a manner that ensures the area of the cut edges is as small as possible.

### **7.2.3 Factory applied linings**

Test pieces with factory applied linings shall be prepared by the manufacturer or a contractor in accordance with the manufacturer's written instructions under supervision of the responsible body. Test panels/plates shall be fully coated in accordance with the standard factory procedure. If this is impractical, then a method giving an equivalent material surface to that produced by the standard factory procedure shall be used.

### **7.2.4 Site Applied Materials**

The manufacturer shall provide a copy of the detailed instructions for application which accompany the materials(s).

NOTE: The application instructions should cover aspects such as:

- surface preparation;
- mix ratios and method of mixing;
- method of application;
- minimum cure temperature and time;
- material film thickness;
- associated materials, e.g. primers and undercoats.

The manufacturer shall provide all necessary information on material and chemical safety.

Test samples shall be prepared on site by the manufacturer/contractor under the supervision of the test laboratory in accordance with the manufacturer's written instructions. If no specialised equipment for application is required then the test samples may be prepared by the test laboratory under conditions that simulate site application. Where it is found necessary to deviate from these instructions, this shall only be done with the prior agreement of the test laboratory and manufacturer or contractor.

If transportation of test samples to the test laboratory is necessary, then this period of time shall be part of the cure conditions (e.g. time and temperature).

The samples shall be delivered within the curing period.

The test laboratory shall prepare a detailed record of test sample preparation and curing conditions.

Care shall be taken to ensure that materials and test samples are not contaminated during transport.

Start testing as soon as the curing period has been completed.

## 8. Pretreatment of test pieces

WARNING: Do not use any other cleaning procedures than those specified in this section, unless they are specified as part of the normal installation and/or commissioning procedures for the test material/product.

### 8.1 General

8.1.1 Test pieces are pretreated prior to testing by procedures involving flushing, stagnation and prewashing.

8.1.2 If it is not possible, because of laboratory time constraints, to carry out the pretreatment and test procedure without a break, only a break during the pretreatment procedure is acceptable.

### 8.2 Pre-treatment

#### 8.2.1 Flushing

Flush test pieces with tap water (5.2) in an appropriate vessel, e.g. a beaker, having a flow of water from the bottom upwards such that the calculated speed with regard to the upper open surface of the vessel is 1 to 3 m/min for (60 ± 5) min.

#### 8.2.2 Stagnation with test water

8.2.2.1 Test pieces shall be immersed in test water (5.1) for a period of (24 ± 1) hours at (23 ± 2) °C.

8.2.2.2 Remove the water.

8.2.2.3 Prewash the test pieces according to 8.3.

### 8.3 Prewashing

8.3.1 Flush test pieces according to 8.2.1

8.3.2 Rinse the test pieces with test water (5.1) for (2±0.5) min.

## 9. Test procedure

NOTE: the timing and sequence of the overall process, including the critical steps, is illustrated below. For further details see Annex A.

Week	Test water (±20) ml	additions		ATP determinations
		inoculum	supplements	
0	900	Yes	As needed	-
1 to 7 inclusive	900	No	As needed	-
8 (56 days)	600	No	As needed	Yes
9 to 11 inclusive	600	No	As needed	-
12 (84 days)	300	No	As needed	Yes
13 to 15 inclusive	300	No	As needed	-
16 (112 days)	<i>End of test</i>			Yes

### 9.1 Test Precautions

#### 9.1.1 Safety

As well as general consideration of safety, particular care shall be taken in this test since some of the organisms present may be pathogenic. Since the conditions of the test may

permit pathogens present in the inoculum to grow until present in large numbers, take special precautions in the handling and disposal of all test samples and water after incubation.

NOTE: Any written safety guidance should be supplemented by thorough training and supervision.

### **9.1.2 ATP Contamination**

Skin is a source of ATP; thus contact between skin and apparatus, test samples or test liquids, may give rise to ATP contamination. Therefore prevent direct contact between skin, test apparatus, reagent and test samples by using polyethylene gloves and forceps. Use separate gloves for each type of material. Limit contact between gloves and test materials as much as possible. Ensure that all apparatus is thoroughly cleaned before use in this test.

## **9.2 Preparation of the test container/system**

Using a clean 1 litre graduated measuring cylinder, add 900 ml of test water (5.1) to each test container.

Using ATP-free pipettes add solutions of potassium di-hydrogen orthophosphate (5.5) and potassium nitrate (5.6) to ensure that the final concentrations of phosphate and nitrate in the test system meet the requirements given in Table 1.

Check that the pH conforms to the range given in Table 1 – if it is outside this range then adjust the pH of the water using either dilute hydrochloric acid or sodium hydroxide solutions as needed.

Add 9 ml of the inoculum (5.7)

## **9.3 Test samples**

Add three test samples, each with a total surface area of 50 cm<sup>2</sup>, to each of two test containers plus test water/inoculum (9.2).

## **9.4 Incubation**

Incubate the test containers with test samples at (30±2)°C in the absence of light (6.5).

## **9.5 Reference/validation samples**

Set up four containers in accordance with 9.2; to each of two containers add three borosilicate glass samples, each with a total surface area of 50cm<sup>2</sup> (5.9.2); to each of the other two containers add three lengths of plasticised PVC tubing each with a total surface area of 50cm<sup>2</sup> (5.9.1).

Incubate the test containers with reference samples at (30±2)°C in the absence of light (6.5).

## **9.6 Changing of test water**

After incubation for seven days, remove both the test sample and reference sample containers from the incubator (6.5).

Gently swirl each contained by hand before opening.

Note : the object is to gently mix the water in the container without disturbing any biofilm present on the surface of the test pieces.

Open each container and decant the water from each container to waste. Add (900±20) ml of fresh test water plus any supplements required (5.5 and 5.6), close each container and return to the incubator.

Repeat this change of water every 7 days for 16 weeks with the following exceptions:

**9.6.1 During weeks 8, 12 and 16** (56, 84 and 112 days) carry out ATP measurements on both the test water and test samples before replacing the test water (in accordance with 9.6).

**9.6.2 During weeks 9 to 12** only add (600±20) ml of test water to keep a constant surface area to volume test ratio, plus appropriate (reduced) volumes of supplements

**9.6.3 During weeks 13 to 16** add only (300±20) ml of test water to keep a constant surface area to volume test ratio, plus appropriate (reduced) volumes of supplements.

### **9.7 Measurement of ATP concentrations**

Before changing the test water during weeks 8, 12 and 16, determine the ATP concentrations in the test water and on the surface of one of the three test samples in each test jar, in accordance with **Annex C**.

#### **9.7.1 Suspended biomass**

At each of the three test periods (56, 84 and 112 days) gently swirl each jar by hand (see **9.6**) just before removing a sample of the test water for ATP analysis. Remove a sufficient quantity of water from each flask for the ATP determination in accordance with **Annex C**.

#### **9.7.2 Attached biomass**

At each of the three test periods (56, 84 and 112 days) remove a test sample (**3.11**) from each test container and determine the biomass concentration (pg ATP/cm<sup>2</sup>) on the surface of the material samples in accordance with **Annex C**.

##### **9.7.2.1 Attached Biomass removal**

Remove this attached biomass in accordance with **Annex B**.

Determine the ATP concentration of the total water volume obtained; this ATP concentration is used in the calculation of biomass products (**10.1**).

Discard the test sample.

### **9.8 Analysis**

Determine the concentration of ATP in both the test water and in the solution prepared from the attached biomass (**9.7.2**), in accordance with **Annex C**.

### **9.9 Sample surface areas**

Accurately determine the external surface area of each test sample after the ATP analysis on it has been completed; use this surface area to calculate the biomass production (pg ATP/cm<sup>2</sup>) in accordance with **10.1** and **10.2**.

## **10. Calculation of test results**

**10.1 Biomass Production (BP)** Calculate the BP value for each material from the ATP concentrations as observed on the material sample and in the test water – see details below. The average BP value is the biomass concentration on the material (pg ATP/cm<sup>2</sup>) and the biomass concentration in the test water x V/S after 56, 84 and 112 days of incubation. Also determine the standard deviation (SD) of the mean of the BP values.

AB= attached biomass = biofilm (pg ATP/cm<sup>2</sup>)(**3.2**)

SB= suspended biomass (pg ATP/ml) in test water (**3.1**)

BP= biomass production (pg ATP/cm<sup>2</sup>)(**3.3**)

BPP=biomass production potential (pg ATP/cm<sup>2</sup>)(**3.4**)

V= Volume of test water

S = surface area of exposed test sample

AB (pg ATP/cm<sup>2</sup>) = ATP (pg/ml) x volume of sonicate (ml) / (surface area of test sample (cm<sup>2</sup>)).

SB (in test water) (pg ATP/ml)

BP (pg ATP/cm<sup>2</sup>) = AB + (SB x Volume/Surface area)

BPP = (BP day 56 + BP day 84 + BP day 112)/3

Standard deviation = stdev (BP day 56 + BP day 84 + BP day 112)

**10.2 Biomass Production Potential (BPP)** The Biomass Production Potential (BPP) is calculated as the average value of the BP values observed on days 56, 84 and 112 respectively, minus the average BP value for the glass control, and is expressed as pg ATP/cm<sup>2</sup>.

$$BPP_{\text{material}} = BPP_{\text{tested sample}} - BPP_{\text{glass}}$$

## 11. Reproducibility & Repeatability

Awaiting information on this aspect.
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## 12. Test Report

The test report shall include the following information:

### 12.1 General information

- name and address of test laboratory and location where the test was carried out when different from the address of the testing laboratory;
- unique identification of report ( such as serial number ) and of each page, and total number of pages of the report;
- name and address of client;
- description and identification of the test item;
- the proposed use of the material;
- a signature and title or an equivalent marking of person (s ) accepting technical responsibility for the test report and date of issue;
- a statement to the effect that the test results relate only to the items tested;
- a statement that the report shall not be reproduced except in full without the written approval of the testing laboratory;

The information on the material shall at least include;

- trade name or designation of manufactured material ;
- complete identification and date of receipt of test item and date of performance of test;
- for factory and site applied materials - the names of the primers and undercoats used, the wet (or dry) film thickness of each coat/lining applied, and the curing conditions used;
- details of the test piece preparation;
- the name of the manufacturer of the material;
- the production place and date;
- the organisation submitting the sample;

- the organisation responsible for preparing the test pieces;
- description of sampling procedure.

## **12.2 Information on the test procedure**

The information on the test procedure shall include;

- reference to this standard and (if applicable) to the referring standard or national regulation ;
- number of test pieces used together in a migration;
- volume of the test water (V) in litres ;
- surface area of test piece exposed to the test water (S) in square decimetres calculated from the actual dimensions of the test pieces ;
- S/V ratio used;
- source of the test water and (if applicable) details of preparation;
- any deviation from the test procedure specified in this standard ;
- any factors which may have affected the results, such as any incidents or any operating details not specified in this standard ;
- special observations, during preparation of the samples and/or during testing;
- all treatments/procedures used which are not described in the standard, and which may have affected the results
- dates of start and completion of the test.

## **12.3 Test results**

The test results shall, at least, include

- the BP values on days 56, 84, and 112 as observed with the material samples, the average BP value and the standard deviation;
- the BP values of glass control on days 56, 84 and 112 and the average BP value;
- the BP values of the positive control (PVC-P) on days 56, 84 and 112 and the average BP value;
- the BPP value of the material(s) tested;

## **Annex A (Normative). Biostability of the Test Water**

### **A.1 Introduction**

The quality criteria for test water listed in **Table 1** of this standard, < 10 ng ATP/l, corresponds with an AOC value (**3.6**) of < 10 µg C/l. Use the following method to demonstrate conformity of the test water with this requirement.

### **A.2 Determination of the biological stability of test water and the need for trace element addition**

Add 600 ml of the water to be assessed for its suitability for use in the BPP test, and complying with the quality criteria mentioned in Table 1, to each of 6 borosilicate flasks (1 l), provided with either polyester or polypropylene screw caps with PTFE liners. Before use clean the flasks, caps and liners in accordance with **6.8.2**, and then heat the flasks at  $(550\pm 20)^{\circ}\text{C}$  for 4 hrs and allow to cool.

Add 1 ml of each of the stock solutions of trace elements (**5.5** and **5.6**) to four of these flasks. Add 1 ml of a freshly prepared sodium acetate solution (**5.9.3**) to two flasks without added trace elements and to two flasks with trace elements, to obtain a final concentration of sodium acetate of  $(5\pm 0.1)$  mg C/l. Add 1 ml of the inoculum (**5.7**) to all the flasks and incubate these in the absence of light at  $(30\pm 2)^{\circ}\text{C}$ .

Determine the concentration of ATP after 0, 1, 3, 4 and 7 days of incubation. When the concentration remains constant, or declines, within this period the test can be terminated.

Calculate the average value of the maximum concentration of ATP in each set of duplicate flasks.

**A.2.1 biostability** - the test water is suited for use in the BPP test if the BPP value < 10 ng ATP/l.

**A.2.2 trace elements** - addition of trace elements (TE) to the test water is required when testing shows that the BPP value of the test water, supplemented with TE and sodium acetate, is below the BPP value in the absence of added TE and TE plus sodium acetate.

## **Annex B (Normative). Attached Biomass Removal**

### **B.1 Introduction**

The method for removing biomass from materials should be capable of removing > 90% of the attached biomass, when measured as ATP. Different (combinations of) techniques can be used to achieve removal, but the efficiency of the selected procedure must be demonstrated. Suitable procedures are detailed below.

### **B.2 Low energy sonication (LES)**

Give three treatments of (120±10) seconds LES, with collection of the sonicated suspension and replacement of the water following each treatment, to the glass (negative control). Give 6 treatments of (120±10) seconds to all other samples. Combine the suspensions produced for each control or test sample and stored then in tubes immersed in melting ice.

After this treatment, swab the entire surface of the sample piece with one or more sterile cotton swabs, until the surface is entirely clean. Place the swab(s) in autoclaved tap water, and sonicate for (120±10) seconds.

Measure the ATP concentration of the combined suspensions and calculate the concentration of the attached biomass as pg ATP/cm<sup>2</sup>.

Notes:

- a. This LES procedure is suited for use on hard materials when the contribution of ATP from swabbing is less than 10% of the total ATP concentration obtained with LES and swabbing.
- b. A total of 6 LES treatments is needed for the removal of biomass from soft materials. Subsequently, swabbing must be applied. To assess the suitability of the treatments, additional swabbing should be conducted and the amount of removed biomass should be measured separately. LES followed by swabbing is sufficient, when the second swabbing yields less than 10% of the total amount removed. PVC-P is a suitable material to use in verifying the efficiency of the treatment.

### **B.3 High energy sonication (HES)**

Apply the HES for (120±10) seconds in accordance with the equipment manufacturer's instructions. During sonication keep the sample in contact with melting ice.

For hard materials one treatment is sufficient. To assess the suitability of the equipment (setting), a second sonication must be made after replacing the test water. Less than 10 % of the concentration of attached biomass should be obtained from the second treatment to demonstrate that one treatment is effective. Swabbing can be applied to verify the efficacy of one HES treatment.

For soft materials 2 HES treatments must be applied, with water replacement between the treatments.

The equipment and settings must be evaluated with a sample of PVC-P (positive control) by applying a third HES treatment. This third treatment should yield less than 10% of the total of all biomass removed. Swabbing can also be used to demonstrate the efficiency of the applied HES treatments.

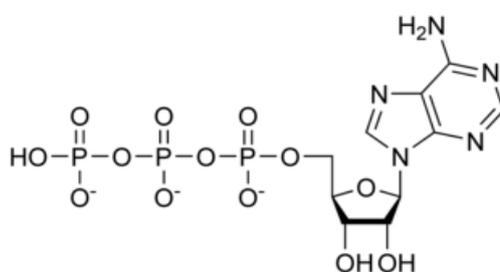
## Annex C (Normative). Protocol of ATP analysis

### C.1. Introduction and Scope

ATP is a measure of the quantity of active biomass. The procedures described in this Annex are applicable to the measurement of ATP concentrations obtained from materials in contact with water. The detection limit of the procedure is about 1 ng ATP/l. The detection limit for biofilms obtained from pipe and construction materials is calculated on the basis of the surface area of material processed. Excessive pH concentration, excess chloride, calcium, or copper concentrations and the colour of the sample may affect the accuracy of the ATP measurement. Other factors affecting measurement include low (< 18°C) or high (>25°C) temperatures of the sample and the reagents.

### C.2. Terms and definitions

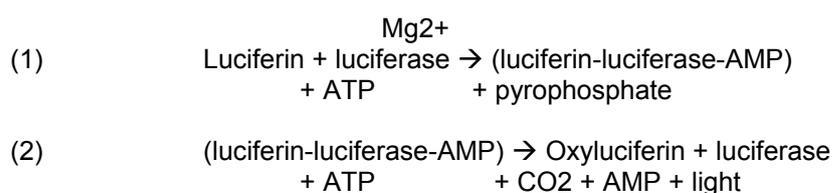
Adenosine triphosphate is an energy-rich compound that is present in all living cells and consequently provides a measure of active biomass. The chemical formula is  $C_{10}H_{16}N_5O_{13}P_3$ . The structure of the ATP molecule is given below:



Energy for a living cell is obtained by the hydrolysis of ATP to adenosine diphosphate (ADP) and/or to adenosine monophosphate (AMP).

### C.3. Principle

ATP measurement is based on the reaction of luciferin with luciferase (originating from fireflies) which occurs in the presence of free ATP. During this process, light is produced.



Under optimal conditions, 1 photon of light is produced per molecule of ATP. The light generated is measured using a sensitive photometer and expressed in Relative Light Units (RLU's). The ATP content of the sample is then calculated with the aid of a conversion factor.

### C.4. Apparatus and glassware

Standard microbiological laboratory equipment. In particular:

#### C.4.1 Apparatus for sterilisation by steam (autoclave)

Sterilise glassware and tap water for 20 minutes at (21±1)°C.

#### C.4.2 Luminometer - with a minimum sensitivity of 1 pg ATP/ml.

#### C.4.3 Balance - with an accuracy of 1 mg

C.4.4 Freezer - with a temperature <65°C.

**C.5. Reagents and auxiliary substances**

Prepare all solutions as prescribed by the manufacturer.  
Allow all reagents to reach (22.5)C before use (about 15 minutes).

*C.5.1 Sterile water*

Autoclaved tap water with a low copper (<0.05 mg/l) and ATP (≤5 ng ATP/l) concentrations.

**C.5.2 ATP solutions for standard dilutions and calibration curves**

*C.5.2.1 Composition*

In case of a calibration curve in buffer, the dilution medium is the reconstitution buffer supplied with the reagent kit; in case of a calibration curve in tap water, the dilution medium is sterilised test water (5.1).

*C.5.2.2 Preparation of ATP solutions*

Pipette the dilution media (5.2.1.) accurately into the ATP standard vial as described by the manufacturer.

- Dissolve the ATP standard by mixing carefully (concentration 10<sup>6</sup> ng ATP/l);
- ALLOW THIS SOLUTION TO STAND FOR 1 HOUR BEFORE USING FURTHER (to assure homogeneity).

In undissolved form, the ATP standard is unstable at room temperature; however, the standard remains stable for longer at -70 °C. The solution must not be refrozen after thawing.

Start with the ATP dilution of 10<sup>6</sup> ng ATP/l. Make further dilutions according to Table II.1.

**Table C.1. Standard dilutions for ATP calibration**

Standard dilution [pg ATP/ml]	Preparation						
	ml		Dilution			MI	
10.000	0.1	of	1.000.000	pg ATP/ml	to	9.9	'Blank'
1.000	1.0	of	10.000	pg ATP/ml	to	9.0	'Blank'
750	3.0	of	1.000	pg ATP/ml	to	1.0	'Blank'
500	2.0	of	1.000	pg ATP/ml	to	2.0	'Blank'
250	1.0	of	1.000	pg ATP/ml	to	3.0	'Blank'
100	0.1	of	10.000	pg ATP/ml	to	9.9	'Blank'
75	3.0	of	100	pg ATP/ml	to	1.0	'Blank'
50	2.0	of	100	pg ATP/ml	to	2.0	'Blank'
25	1.0	of	100	pg ATP/ml	to	3.0	'Blank'
10	0.1	of	1.000	pg ATP/ml	to	9.9	'Blank'
7.5	3.0	of	10	pg ATP/ml	to	1.0	'Blank'
5	2.0	of	10	pg ATP/ml	to	2.0	'Blank'
2.5	1.0	of	10	pg ATP/ml	to	3.0	'Blank'
0							'Blank'

To produce a calibration curve in buffer: the 'Blank' is reconstitution buffer. To produce a calibration curve in drinking water the 'Blank' is sterile filtered (0.20 µm) autoclaved drinking water.

Aliquots (e.g. 0.3 ml) of each dilution are transferred to suitable tubes (or containers) and stored at a temperature of ≤70°C; at this temperature the standard dilutions remain stable for a period of at least 6 months. Standard dilutions should not be refrozen after thawing.

**C.5.3 Luciferin/luciferase (enzyme) solution**

**C.5.3.1**                    *Composition*  
Luciferin/luciferase  
Reconstitution buffer

**C.5.3.2**                    *Preparation*

- Dissolve the Luciferin/luciferase in the reconstitution buffer as described by the manufacturer.
- Dissolve the Luciferin/luciferase by gentle swirling.
- ALLOW THIS SOLUTION TO STAND FOR 15 MINUTES AT (22±5)°C BEFORE USING FURTHER (to assure homogeneity).
- Use this solution within 4 hours.

**C.5.4** *ATP extraction reagent*

Handle the ATP extraction reagent as described by the manufacturer. Allow it to reach (22±5)°C before use.

## **C.6.    Sampling**

Where possible start the investigation immediately after sampling, and always within (24±1) hours.

All types of samples must be kept on melting ice until measurement is undertaken in order to prevent changes in the microbial activity of the sample. When a portion of the sample (e.g. 100 µL) is transferred to the measuring cuvette it will rapidly equilibrate to (22±5)°C. The sample should be mixed with reagents at (22±5)°C.

Prior to subsampling for ATP measurement homogenise the samples by Vortex mixing for 5 seconds.

## **C.7.    Procedure**

Use the Luminometer in accordance with the manufacturer's instructions.

**C.7.1** *Blank water*

Measure 2 samples of the dilution medium.

**C.7.2** *Control dilutions*

Thaw standard dilutions and measure either a series for a calibration curve or measure at least two concentrations in duplicate, e.g. 1000 ng ATP/l and 10 ng ATP/l.

## **C.7.3** *Samples*

Measure all samples in duplicate. After measuring the ATP concentration of the last sample, undertake further measurements, in duplicate, on the blank water (C.7.1) and control solutions (C.7.2).

## C.8. Expression of results

### C.8.1 Water samples

Calculate the ATP content using the following formula:

$$Total\ ATP = \frac{mean\ RLU - mean\ blank\ water}{mean\ slope}$$

where:

total ATP: The ATP content in ng/l of sample or in pg/ml of sample;

Mean RLU: The arithmetic mean of the four RLU values of the blank water (two before the samples and two after the samples);

Mean slope: A value laid down on the basis of historic data. This value is the mean gradient of calibration curves.

### C.8.2 Material samples

Calculate the ATP content using the following formula:

$$Total\ ATP = \left( \frac{mean\ RLU - mean\ blank\ water}{mean\ slope} \right) \cdot \frac{volume}{surface}$$

where:

Total ATP: The ATP content in ng/l of sample or in pg/ml of sample;

Mean RLU: The arithmetic mean of the two RLU values of the samples;

Mean blank water: The arithmetic mean of the four RLU values of the blank water (two before the samples and two after the samples);

Mean slope: A value laid down on the basis of historic data. This value is the mean gradient of calibration curves;

Volume: The total volume obtained of the pre-treatment of the material;

Surface: the total water exposed surface of the sample.

## C.9. Test record

The test record shall contain at least the following information:

- reference to this procedure
- all details necessary for complete identification of the sample
- the results expressed in accordance with **C.8**
- any particular occurrence(s) observed during the course of the analyses and any operation(s) not specified in the method which may have influenced the results.

## C.10. Quality assurance

### Quality control of calibration curves

The RLU response on a given measuring day depends on the activity of the enzyme and the room temperature. New standard dilution must therefore always be calibrated against old standard dilutions. All dilutions are measured in duplicate.  $R^2$ -values for the calibration curves must be higher or equal to 99% (0.990). Deviation between slopes of the old and the new calibration curve must be within 5%. At deviations above 5% the new standard dilutions must be rejected. The slopes of the calibration curves should be recorded on a control chart. Control charts must be made of data of at least 6 calibration curves.

To minimise the effect of uncertainty with a single calibration curve, the average slope of a series of calibration curves can be used to calculate the ATP value. The average slope must be based on historical values. Deviation between slopes of calibration curves should normally be within 5%.

*NOTE: When starting up a database of values to calculate the average slope, a minimum of 10 different dilution series must be used, made with a minimal of three different batches ATP standard solutions.*

When using an average slope value for calculation of ATP-values, it must be controlled on each measuring day that enzyme activity corresponds to the historical values. At least two standard dilutions (e.g. 10 pg ATP/ml and 1,000 pg ATP/ml) must be measured in duplicate on each measuring day, but frozen complete calibration curves can also be used. The RLU responses must be collected on a control chart. These control charts must be made of at least 10 measurements each. In case the RLU exceed the chart limits, a full set of standard dilutions must be measured on the given measuring day, and the slope of this calibration curve used for calculation of the ATP-value.

*NOTE: If the laboratory is not temperature regulated, it may be necessary to measure a full dilution series on all days with peak high or low temperatures (e.g. peak summer and winter periods). In these extreme situations different temperature ranges can selected for preparing calibration curves. The corresponding calibration curve should be chosen dependant on the laboratory temperature on when samples are measured.*

## Annex D (Informative). Flow Diagram of the Test Procedure

