

KOA 99.079
june 1999

Biofilm Formation Potential of Pipe Materials in Plumbing Systems

Measurements results and evaluation

Kiwa

Biofilm Formation Potential of Pipe Materials in
Plumbing Systems

Measurement results and evaluation

Translated by BFGoodrich B.V.B.A. with
permission of VROM and Kiwa N.V.

BY ORDER OF
VROM

ORDER NUMBER
30.2461.016

AUTHORS
H.R. Veenendaal and D. van der Kooij

DEPARTMENT
Chemistry and Biology

Nieuwegein, June 1999

1999 Kiwa N.V.

KOA 99.079

**Research and
Consultancy**

Groningenhaven 7
Postbus 1072
3430 BB Nieuwegein
Telefoon (030) 606 95 11
Telefax (030) 606 11 65

BFGoodrich
Performance Materials

Chaussée de Wavre, 1945
B-1160 Brussels
Belgium
Tel.: + 32 2-678 19 11
Fax + 32 2-678 19 90
RCB/HRB 470685
TVA/BTW 408.454.528

IMPORTANT

Brussels, January 2001.

To Whom It May Concern:

This is a translation of the Kiwa publication "Biofilmvormingspotentie van leidingmaterialen voor binneninstallaties" - Meetresultaten en beoordeling, by order of VROM – order nr. 30.2461.016 – Authors: H.R. Veenendaal en D. van der Kooij , dated June 1999.

This publication has been translated into English by BFGoodrich with written permission of the Ministry of Public Housing, Urban Planning and Environment (VROM) and Kiwa N.V. BFGoodrich accepts no responsibility for the accuracy or validity of the information contained in this report nor for the English translation. In cases of doubt, the original Dutch version must be referred to.

BFGoodrich B.V.B.A.
TempRite® Division

S.P.R.L. BFGoodrich Chemical (België) B.V.B.A.

CONTENTS

SUMMARY	4
1 Acknowledgements	6
2 INTRODUCTION	8
3 ASSESMENT OF THE BIOFILM FORMATION POTENTIAL OF SELECTED MATERIALS	10
3.1 Set-up and performance of the study	10
3.1.1 Selection of the materials	10
3.1.2 Method	11
3.1.3 Selection of micro-organisms	12
3.2 Results	13
3.2.1 The biofilm formation potential (BFP) of the materials	13
3.2.2 Numbers of bacteria on the materials	15
3.2.3 Impact of the materials on the biomass concentration (BMC) in the test water	17
3.2.4 Number of bacteria in the water	19
3.3. The distribution of biomass over water and materials	20
3.4 Relationship of biomass formation and growth promotion of <i>Legionella</i> bacteria	22
3.5 Summary of results	24
4 BIOFILM FORMATION ON THE MATERIALS IN THE REFERENCE SET-UP	26
4.1 Set-up and performance of the study	26
4.2 The reference set-up	26
4.3 Test water	28
4.4 The biofilm monitor	28
4.5 Sampling method and parameters studied	29
4.6 Results	30
4.6.1 Measurements on the reference set-up	30
4.6.2 Measurements with the biofilm monitor	35
4.7 Summary of results	36
5 DISCUSSION	38
5.1 Impact of test conditions on biofilm formation	38
5.2 Choice of test method	40
5.3 Parameters and evaluation criteria	41
5.3.1 Parameters and basis of evaluation	41
5.3.2 Quality classes	43
5.3.3 Evaluation on the basis of the health risk associated with growth promotion of	44
5.3.4 Evaluation of pipe materials for domestic installations	46
6 CONCLUSIONS AND RECOMMENDATIONS	48
6.1 Conclusions	48
6.2 Recommendations	48
LITERATURE	50

ANNEX 1	Separate measurement values of the biofilm concentration on the materials
ANNEX 2	Separate measurement values of the <i>Legionella</i> concentration on the materials
ANNEX 3	Separate measurement values of the <i>Pseudomonas</i> concentration on the materials
ANNEX 4	Separate measurement values of the biomass concentration in the water
ANNEX 5	Separate measurement values of the <i>Legionella</i> concentration in the test water
ANNEX 6	Separate measurement values of the <i>Pseudomonas</i> concentration in the test water
ANNEX 7	Biofilm on the inner wall of the pipes (ATP data)
ANNEX 8	Measurements of water quality
ANNEX 9	Deposit rate of iron of the water
ANNEX 10	Deposit rate of manganese of the water
ANNEX 11	Prescription for determining the biofilm formation potential (LMB-006)
ANNEX 12	Water Quality of the Water treatment plant Tull en 't Waal (1992)

SUMMARY

To limit environmental pollution with copper, the Sustainable Construction Package recommends using plastic pipes instead of copper ones in plumbing installations. Plastics can release biodegradable compounds which promote the growth of micro-organisms. Thirteen different pipe materials used in drinking water installations were therefore tested for their promotion of the growth of micro-organisms in water. These materials include metals and plastics, whereby the choice of plastics was determined by the market share of the product concerned. The objective of the study was to :

- investigate the degree to which the various materials were growth-promoting in two different tests;
- make a start with the evaluation of materials on the basis of the degree of growth promotion.

All materials were tested in a static test, the BFP (biofilm formation potential) test. Then six materials were tested in a continuous flow test, the reference set-up, with which the hydraulic conditions which prevail in a drinking water installation for domestic use were simulated. In the BFP test, which is performed at 25 °C, the concentration of active biomass (adenosine triphosphate, ATP) on the material ('biofilm') and in the test water (filtrate of slow sand filters) is determined as a function of time, over a 16-week period. The BFP value of a material is defined as the average of the biofilm concentrations determined after 8, 12 and 16 weeks. On the basis of the BFP value and the biomass concentration in the water, the biomass production potential (BPP) per unit of surface area of the material can be determined. The reference set-up is fed with drinking water and after 8, 12 and 16 weeks the biofilm concentration was also measured in pipe segments. In both tests, the growth of *Legionella* bacteria on the material and in the water were determined at the same time. The biofilm formation rate of the feed water of the reference set-up is determined with the aid of a biofilm monitor, in which glass serves as support material for the biofilm.

The tested materials differed widely from one another in the BFP test. The lowest BFP value (41 pg ATP/cm²) was observed with stainless steel; values above 1000 pg ATP/cm² were found for one PE-based material and for silicone which was used as a positive control. In general, the multiplication of *Legionella* bacteria on the material or in the water was more vigorous the more growth-promoting the material (i.e. the higher the BFP value). The degree of *Legionella* bacteria growth promotion, calculated on the basis of the growth on the material and in the water, lay between values <1000 colony-forming units (cfu) per cm² (stainless steel, glass) and values higher than 10⁴ cfu/cm² for several PE-based materials. In the reference set-up, much less biofilm formation was observed than in the BFP test. The biofilm concentrations lay for the various materials between 80 pg ATP/cm² (PVC-C) and 240 ATP/cm² (copper), and were moreover significantly lower than in the biofilm monitor. The explanation for this is that water was continuously flowing through the biofilm monitor, whereby the biofilm formation was entirely determined by the supply of biodegradable substances in the water. As a result of the limited flow-through time (5 % of the total time), the supply of biodegradable substances with the water is much lower in the reference set-up than it was in the biofilm monitor. Moreover, the biofilm which is formed in the reference set-up in the standstill periods is probably rinsed away by the period flow of water. Under these circumstances, the impact of both the water and the materials on the biofilm formation is low. Also, as a result of the relatively low temperature (18 °C), *Legionella* bacteria were not observed in the reference set-up.

Factors such as temperature and residence time can differ greatly from installation to installation, and also over time. Relatively long residence times (no water consumption), increase of temperature and a greater surface area/volume ratio strengthen the growth of micro-organisms when materials release growth-promoting compounds. Given the absence of inspection of the water quality in such situations, preventive measures (design of the installation, choice of materials) are of great importance for limiting the increase of micro-organisms. It is therefore recommended to base the evaluation of materials for drinking water installations on their behaviour in the BFP test.

Materials can be compared with one another on the basis of their degree of growth promotion in the BFP test. However, an objective evaluation of the materials on the basis of the BFP or BPP value, possibly supplemented by data on the degree of growth promotion of *Legionella* bacteria, is not yet possible due to :

- lack of information about biofilm concentrations in drinking water installations;
- lack of information about the distribution of micro-organisms over biofilm and water in drinking water installations and in the BFP test under the impact of circulation or refreshment of the water;
- lack of a criterion for the maximum tolerable number of *Legionella* bacteria in the water.

In 2000, for legionella an MTC value of 50 cfu/l has been defined in legislation in The Netherlands.

Data on the biofilm concentrations in the distribution system are available, however, and offer points of support for evaluating the BFP values of materials in contact with drinking water. It is proposed to use as a point of departure the fact that the BFP or BP value of a material should not significantly contribute to the biofilm concentration on the pipe wall.

The requirements which are imposed on the growth-promoting characteristics of materials in contact with drinking water depend on the conditions under which these materials are applied (such as length of the pipe, surface area/volume ratio, temperature). It is therefore recommended to develop a system of quality classes (on the basis of BFP or BPP values) for materials in contact with drinking water.

1 ACKNOWLEDGEMENTS

The research described in this report was commissioned by the Ministry of Public Housing, Urban Planning, and Environment (Ministerie voor Volkshuisvesting, Ruimtelijke Ordening en Milieu, VROM), and was performed by Kiwa N.V. Research and consultancy, Project number 9712.0030. The research was supported by a project group with the following members:

Ing D. Vonk (Chairman)	VROM
Drs F.O. Dorgelo	VROM
Ing. L.J. van Driel	Kiwa N.V.
Prof dr ir D. van der Kooij	Kiwa N.V.
Dr ir E.J.T.M. Leenen	RIVM
Ing. K.H. Poortema	VEWIN
Drs P.G.G. Slaats	Kiwa N.V.
Drs R. Teunissen	RIZA
H.R. Veenendaal (secretary)	Kiwa N.V.

2 INTRODUCTION

Copper deriving from copper water pipelines accounts for a significant part of the copper pollution of the aquatic environment [Integrated Water Management Commission, 1997]. To limit this pollution, the Sustainable Construction Package recommends using water pipes made of plastic instead of copper inside residences.

Plastic pipe materials can release compounds which promote the growth of micro-organisms. Under certain circumstances, this can cause a multiplication of opportunistic-pathogenic bacteria such as *Legionella pneumophila*, *Mycobacterium* spp. and *Pseudomonas aeruginosa* [Colbourne and Pratt, 1984, Rogers et al., 1994, Nideveld et al., 1986, Groothuis et al., 1983]. The growth of by micro-organisms which are aesthetically undesirable can also be facilitated [Burman, 1979]. The release of biodegradable compounds by plastics generally involves compounds which are added to the plastic to improve the material's properties (stabilisers, anti-oxidants, dyes, etc.). In Germany and England, plastic materials in contact with drinking water are evaluated on their growth-promoting effect [British Standard, 1988, DVGW, 1990]. In the Netherlands, a working method has also been developed for this purpose, namely the determination of the biofilm formation potential (BFP) [Van der Kooij and Veenendaal, 1993]. Most pipe materials which are used in the Netherlands in distribution systems have been tested with this method. However, data concerning the growth-promoting characteristics of pipe materials for plumbing installation applications ('behind the water meter') are not yet available. Nor is information available on the impact of materials on the increase in practice of micro-organisms in drinking water installations. The impact of release of growth-promoting on the growth of micro-organisms can be relatively high in such systems, due to :

- the relatively high surface area/volume ratios;
- higher temperatures, and
- longer standstill periods (e.g. on weekends).

Contrary to these disadvantages, however, is the fact that the water in a drinking water installation with continuous flow generally only remains in a drinking water installation for a short period (several minutes) compared to the time it remains in the distribution systems (hours/days).

VROM commissioned Kiwa to study the biofilm-forming characteristics of plastic pipe materials for drinking water installations. This research included determination of the degree of biofilm formation on these materials in both a static (batch) test and a dynamic (continuous flow) test. The objectives of this study are :

- comparing selected materials with the aid of these methods;
- drafting an opinion for an evaluation criterion with regard to growth promotion.

As a first step, an inventory was drawn up of the plastic materials on the market and materials were selected for study. Then the biofilm formation potential (BFP) of these materials was determined in a static test. After that, several of these materials were studied in a test set-up under conditions which correspond with those in the domestic drinking water installation.

This report describes the results of these tests. Also an effort is made to evaluate plastic pipe materials for domestic installations on the basis of the biofilm-forming characteristics and growth promotion of selected micro-organisms.

3 ASSESMENT OF THE BIOFILM FORMATION POTENTIAL OF SELECTED MATERIALS

3.1 Set-up and performance of the study

3.1.1 Selection of the materials

Only certified materials are included in the study. An overview of the certified plastic pipe systems in the Netherlands is given in the list with approved systems [Kiwa, 1998].

The choice of the systems to be tested is based on the following criteria :

- material composition. At least two systems using a certain type of plastic were tested. In addition, one plastic expected on the market was included;
- production process. For the plastic pipe materials PE-X several production processes exist. Two systems are tested for PE-X : one produced according to the most widely applied production process (PE-Xc) and one system according to a less frequently applied process (PE-Xa);
- market share. Kiwa N.V. has a rough idea of the market shares of the various plastic pipe systems. For the copper pipes, use is also made of market share data in the possession of Kiwa N.V. For the delivery of the stainless steel, only one manufacturer is available.

The materials are delivered to Kiwa by the manufacturers or importers, whether or not provided with the related coupling pieces and necessary adhesive. The fittings and adhesive are not tested for biofilm formation potential.

Table 1. Pipe systems which are selected for the BFP test

Material	Description
PE-Xa	Polyethylene, cross-linked
PE-Xc (2x)	Polyethylene, cross-linked
Al/PE-MD	Polyethylene, cross-linked, internally reinforced with aluminium foil
PP-R (2x)	Polypropene (random copolymer)
PB (2x)	Polybutene
PVC-C (2x)	Post-chlorinated polyvinyl chloride
Cu half-hard radiated	Copper
Cu half-hard deoxidised	Copper
Stainless steel AISI 316	Stainless steel

The outer diameter of the materials amounts to around 15 mm. Pieces (rings) of the materials are cut off with a total surface of around 8 cm². Then these rings are rinsed for 1 hour in cold flowing drinking water. The control materials glass and stainless steel (and the stainless steel rings for the weighting of the materials), are cleaned by heating them for 4 hours at 550 °C.

3.1.2 Method

The method which is followed for determining the biofilm formation potential of materials in contact with drinking water is described in detail in annex 11. Below the method is given in abbreviated form. Representative samples (with a total surface of around 100 cm²) of the material to be studied were placed in 600 ml of biologically stable drinking water (filtrate of slow sand filters, representative for drinking water in the Netherlands) to which certain nutrient salts (potassium nitrate and potassium dihydrogen phosphate) and micro-organisms from river water (deriving from the Lek Canal) were added [Van der Kooij and Veenendaal, 1993]. This water with the materials was incubated in the dark at 25 ± 1 °C during a 16-week period. In this period the adenosine triphosphate (ATP) content is measured on the materials and in the water after various incubation periods. The ATP content is a measure for the concentration of active biomass. The biofilm formation potential (BFP) of a material is defined as the average biofilm concentration (expressed in pg ATP/cm²) after 56, 84 and 112 days of exposure. The biomass concentration (BMC) in the water is defined as the average concentration of biomass (expressed in pg ATP/ml) after 56, 84 and 112 days of exposure.

Water without addition of a material, water with glass and water with silicone hose were used as controls. To prevent floating, several materials (PE-Xa, PE-Xc, PP-R and PB) were weighted down with rings of stainless steel which were fastened to the materials with stainless steel wire. For this reason, a control of water with these stainless steel rings is also included in the test.

For technical reasons it was impossible to test all of the selected materials at the same time, and so three test series are used. With each series, the necessary controls (test water, glass, stainless steel and silicones) are taken. One of the materials (PE-Xa) was included in two tests. All samples were incubated during 112 days (16 weeks) at 25 °C, the maximum temperature allowed for drinking water (Drinking Water Decree). At this temperature, most water-borne bacteria multiply readily. During this period, the concentration of biomass on the material (biofilm) and in the water is periodically determined (after 7, 14, 28, 56, 84 and 112 days) with the aid of measurements of adenosine triphosphate (ATP). The biomass was detached from the material with the aid of ultrasonic vibrations (40 KHz).

Addition of selected micro-organisms

The BFP test was extended by inoculation with cultures of *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Mycobacterium* spp. and coliform bacteria (table 2), so that the number of colony-forming units per type of organism amounted to at most around 100 per ml. These were added in order to investigate whether the materials were capable of promoting the growth of these micro-organisms under the test conditions. The selection criteria of these micro-organisms are described in § 3.1.3.

Table 2. Added bacteria cultures

Micro-organism	Strain	Origin
<i>Legionella pneumophila</i>	M-980082	warm water
	M-980140	warm water
	Control strain <i>Legionella</i>	warm water
<i>Pseudomonas aeruginosa</i>	WP1	RIVM, water
	WP5	RIVM, water
	M-980447	Lek Canal
<i>Mycobacterium avium</i>	myc 97-01651	RIVM, water
<i>Mycobacterium kansasii</i>	myc 394	RIVM, water
<i>Mycobacterium fortuitum</i>	myc 97-1392	RIVM, water
<i>Escherichia coli</i>	WR1	RIVM, water
<i>Enterobacter cloacae</i>	WR3	RIVM, water
<i>Klebsiella pneumoniae</i>	873	drinking water

The concentrations of these added cultures were determined on the materials and in the water on days 56, 84 and 112 (after the number of added colony-forming units was determined on the day added). The number of *Legionella* bacteria was determined according to NEN 6265 (1991), the number of *Pseudomonas* according to NEN 6573 (1987), and the coliform bacteria according to NEN 6553 (1981).

The concentration of mycobacteria present on the materials and in the testing water was determined using the isolation method [Engel and Berwald, 1980].

Confirmations of suspected colonies (possible *Mycobacterium*) were performed with a specific dye for acid-proof rods (auramine dye).

The mineral oil on the copper pipes is determined using GC-FID (gas chromatography-flame ionisation detector).

3.1.3 Selection of micro-organisms

To assess the growth of (pathogenic) micro-organisms on materials for plumbing installations, the following micro-organisms were selected :

* ***micro-organisms from river water***

In order to incorporate the widest possible range of micro-organisms present in the aquatic environment into the evaluation of materials (in the static laboratory test). River water (taken from the Lek Canal) was added. Because this inoculation is passed through a 1.2 µm-membrane filter, all possible disturbing protozoa are removed.

* ***Coliform bacteria***

Coliform bacteria should not be present in drinking water. The selected strains all come from water.

* ***Pseudomonas aeruginosa***

The organism is an opportunistic pathogen. The selected strains all come from water.

* ***Legionella pneumophila***

This organism is also an opportunistic pathogen. Bacteria coming directly from the water (without being inoculated over onto culture media) with accompanying flora were used.

Mycobacteria

Certain mycobacteria are also opportunistic pathogens. Mycobacteria, including the selected strains, have been found in drinking water [Schulze-Röbbecke and Hagenau and Good, 1985].

3.2 Results

3.2.1 The biofilm formation potential (BFP) of the materials

The BFP values of the materials are included in table 3 and graphically presented in figure 1. The separate measured values of the biofilm concentration are graphically presented in annex 1. After 233 (test 1), 208 (test 2) and 190 (test 3) days, the biofilm concentration on the materials is determined once again. These results are also presented in table 3.

Table 3. The biofilm formation potential (BFP) (the average of the biofilm concentrations on days 56, 84 and 112) and the results of the measurements on days 233, 208 and 190.

Material	BFP ± sd (pg ATP/cm ²)	Biofilm concentration ± sd (pg ATP/cm ²) after 233 days
Test 1		
PE-Xa	1400 ± 200	350 ± 60
PE-Xc (1)	400 ± 60	390 ± 100
PP-R (1)	370 ± 110	200 ± 50
PP-R (2)	600 ± 100	230 ± 30
PB (1)	220 ± 70	180 ± 40
PVC-C (1)	140 ± 60	45 ± 4
PVC-C (2)	270 ± 80	82 ± 18
Test water (control)	Not applicable	Not applicable
Silicone hose (control)	1600 ± 500	210 ± 50
Glass (control)	12 ± 2	9.6 ± 3.7
Stainless steel (control)	70 ± 10	45 ± 8
Test 2		
Material	BFP ± sd (pg ATP/cm ²)	after 208 days
PE-Xc (2)	930 ± 340	640 ± 60
PE-Xa (repeated)	1300 ± 100	300 ± 80
PB (2)	650 ± 160	190 ± 10
Copper	590 ± 460	120 ± 10
Copper	350 ± 140	76 ± 19
SS	41 ± 14	23 ± 3
Test water (control)	n.a.	n.a.
Silicone hose (control)	990 ± 290	280 ± 20
Glass (control)	13 ± 2	23 ± 1
Stainless steel (control)	93 ± 15	51 ± 2
Test 3		
Material	BFP ± sd (pg ATP/cm ²)	after 190 days
A1/PE-MD	730 ± 140	920 ± 90
Test water (control)	n.a.	n.a.
Silicone hose (control)	1500 ± 600	1200 ± 500
Glass (control)	17 ± 6	10 ± 2

n.a. = not applicable

Table 3 shows clearly that only with stainless steel and glass was the BFP value lower than 100 pg ATP/cm². For a number of materials – namely PE-Xc (1), PP-R (1), PB (1) and the two PVC-C materials – the BFP values were lower than 500 pg ATP/cm². Only PE-Xa has a biofilm formation potential greater than 1000 pg ATP/cm².

For a number of materials, the biofilm concentration was initially relatively high. Stabilisation then occurred after 56 days of exposure to the test water (annex 1). However, for the two copper pipes and also for the PP-R the biofilm density continued to increase after 56 days. The relatively high BFP value of the two types of copper may be either connected with the presence of mineral oil residues on the pipe surface, or with the appearance of corrosion. Measurements showed that 0.7 µg of mineral oil/cm² was present on the inner wall of copper (1) and 1.9 µg of mineral oil/cm² on the outer wall. For copper (2) the amount of mineral oil on the inner wall was < 0.2 µg/cm² and on the outer wall 0.5 µg/cm². The BFP value of stainless steel (which served as weighting material) was higher than that of glass. For these materials some corrosion was observed, possibly as a result of heating at 550 °C. The BFP value of stainless steel has no impact on the BFP values of the materials which were weighted down with stainless steel.

The results of the measurements on the days 233, 208 and 190 show that the biofilm concentrations on copper (2) and stainless steel declined to values below 100 pg ATP/cm². This was also the case for the two PVC materials.

The reproducibility of the three separate tests appears to be good. The BFP values of the controls, but also those of the PE-Xa material included in two tests, correlate well with one another. Thus the BFP values of the materials which are evaluated in the three separate tests may be compared with one another.

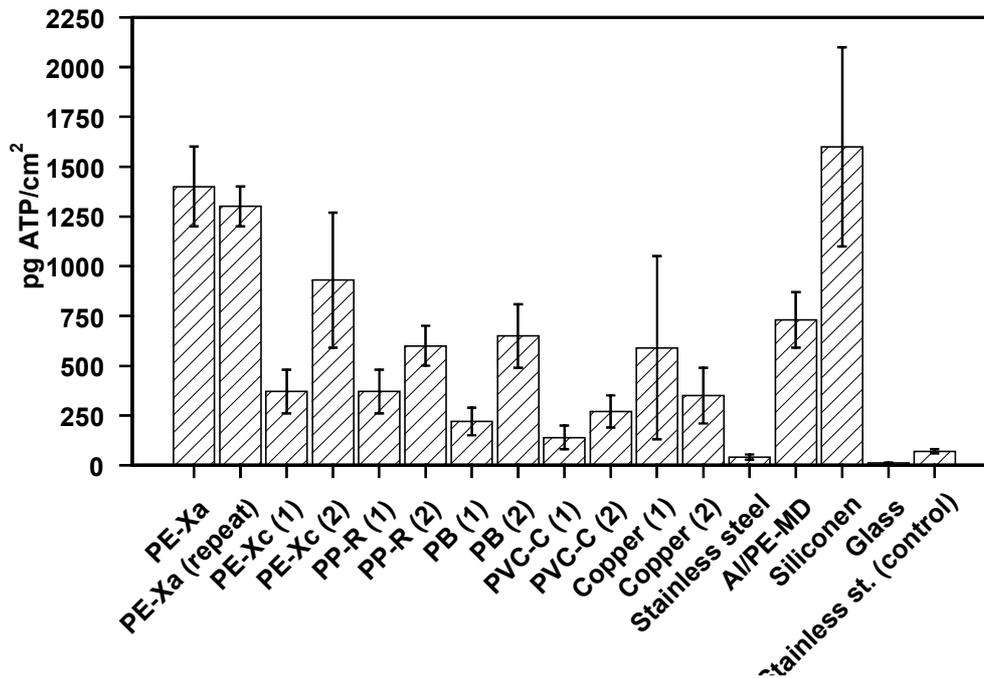


Figure 1. The biofilm formation potential (in pg ATP/cm² ± s.d.) of the materials

3.2.2 Numbers of bacteria on the materials

The colony counts of the various types of bacteria on the materials (the average of the numbers measured on day 56, 84 and 112) are presented in table 4.

Table 4. The colony numbers of the various types of micro-organisms on the materials, the average of the measured values on the days 56, 84 and 112

Material Test 1	<i>Legionella</i> (cfu/cm ²) ± sd		Trend	<i>Pseudomonas</i> (cfu/cm ²) ± sd		Trend	Coliform bacteria (cfu/cm ²) ± sd		Trend
PE-Xa	120 ±	90	↑	55 ±	41	↑	160 ±	270	↓
PE-Xc (1)	560 ±	450	↓	48 ±	38	↓	41 ±	71	↓
PP-R (1)	300 ±	190	-	160 ±	90	-	280 ±	480	↓
PP-R (2)	6200 ±	8700	↑	300 ±	320	↓	600 ±	1000	↓
PB (1)	4800 ±	1600	-	230 ±	150	-	550 ±	950	↓
PVC-C (1)	250 ±	30	-	150 ±	140	↓	300 ±	520	↓
PVC-C (2)	3400 ±	2200	-	160 ±	90	-	250 ±	30	↓
Test water (control)	n.a.			n.a.			n.a.		
Silicone hose (control)	40 ±	27	-	130 ±	110	-	120 ±	210	↓
Glass (control)	9,2 ±	11,5	-	7,4 ±	12,8	↓	3,7 ±	6,4	↓
Stainless steel (control)	190 ±	80	-	120 ±	110	-	n.a. ⁽¹⁾		
Material	<i>Legionella</i>			<i>Pseudomonas</i>			Coliform bacteria		

Test 2	(cfu/cm ²) ± sd		(cfu/cm ²) ± sd		(cfu/cm ²) ± sd	
PE-Xc (2)	1100 ± 800	↓	310 ± 110	-	n.a.	
PE-Xa (repeated)	16000 ± 16000	↓	180 ± 200	↓	n.a.	
PB (2)	2000 ± 1100	↓	440 ± 190	-	n.a.	
Copper (1)	770 ± 1210	-	n.a.		n.a.	
Copper (2)	61 ± 25	-	n.a.		n.a.	
Stainless steel	38 ± 24	-	200 ± 90	↓	n.a.	
Test water (control)	n.a.		n.a.		n.a.	
Silicone hose (control)	35 ± 18	↓	220 ± 90	-	n.a.	
Glass (control)	92 ± 95	-	13 ± 22	↓	n.a.	
Stainless steel (control)	350 ± 150	↑	12 ± 11	↓	n.a.	
Material	<i>Legionella</i>		<i>Pseudomonas</i>		Coliform bacteria	
Test 3	(cfu/cm ²) ± sd		(cfu/cm ²) ± sd		(cfu/cm ²) ± sd	
A1/PE-MD	12000 ± 14000	↓	n.a.		n.a.	
Test water (control)	n.a.		n.a.		n.a.	
Silicone hose (control)	130 ± 80	-	n.a.		n.a.	
Glass (control)	58 ± 20	-	n.a.		n.a.	

¹) not analysed/or not observed

The colony counts on various materials provide a less clear picture than the BFP values. The high spread between the measurement results on the days 56, 84 and 112 was caused primarily by increase or decrease of the colony counts in the period concerned (see annexes 2 and 3). The maximum values of the colony counts of *Legionella* bacteria were higher than 10⁴ cfu/cm² on the materials PP-R(2), PE-Xa (repetition) and on A1/Pe-MD. On the materials PE-Xc(1), PB(1), PB(2), PVC-C(2) and copper (1), the maximum colony numbers of *Legionella* were > 10³ cfu/cm². On copper(1) and stainless steel, the maximum colony number remained lower than 100cfu/cm². For several materials (PE-Xc(1), PE-Xc(2) and copper(1)) the colony number declined after 112 days to a value below 1000 cfu/cm². On PE-Xa (first test) and PP-R(2), the colony number of *Legionella* bacteria increased the longer the incubation in the test water. The colony numbers of *Pseudomonas* displayed an entirely different picture. No *Pseudomonas* was observed anywhere on the two copper materials.

Only in the first test are coliform bacteria observed (on day 56). It was determined that in the second and third test series, coliform bacteria were inoculated, but these organisms were not observed on the materials. The reason for this is not known. Growth of mycobacteria was not observed. A single *Mycobacterium* was found, once, on one material.

3.2.3 Impact of the materials on the biomass concentration (BMC) in the test water

The BMC values are included in table 5 and graphically presented in figure 3. The separate measured values which led to the BMC are presented graphically in annex 4. The results of the colony counts (the average number measured on days 56, 84 and 112) are presented in table 6.

Table 5. The biomass concentration (BMC) in the test water (average of the concentrations measured on the days 56, 84 and 112)

Material Test 1	Biomass concentration \pm sd (pg ATP/ml)	Trend
PE-Xa	54 \pm 18	-
PE-Xc (1)	19 \pm 1	-
PP-R (1)	16 \pm 2	-
PP-R (2)	22 \pm 2	-
PB (1)	24 \pm 10	↓
PVC-C (1)	14 \pm 4	↓
PVC-C (2)	17 \pm 2	-
Test water (control)	5.9 \pm 2.0	-
Silicone hose (control)	49 \pm 26	↓
Glass (control)	8.0 \pm 0.4	-
Stainless steel (control)	9.0 \pm 2.2	-
Material Test 2	Biomass concentration \pm sd (pg ATP/ml)	
PE-Xc (2)	34 \pm 6	↓
PE-Xa (repeated)	30 \pm 5	↓
PB (2)	31 \pm 3	-
Copper (1)	11 \pm 5	↓
Copper (2)	8.0 \pm 3.1	↓
Stainless steel	13 \pm 6	↓
Test water (control)	6.6 \pm 2.6	↓
Silicone hose (control)	39 \pm 8	↓
Glass (control)	8.0 \pm 1.8	↓
Stainless steel (control)	12 \pm 1	-
Material Test 3	Biomass concentration \pm sd (pg ATP/ml)	
A1/PE-MD	51 \pm 9	-
Test water (control)	6.5 \pm 1.3	-
Silicone hose (control)	170 \pm 80	↓
Glass (control)	6.8 \pm 1.9	↓

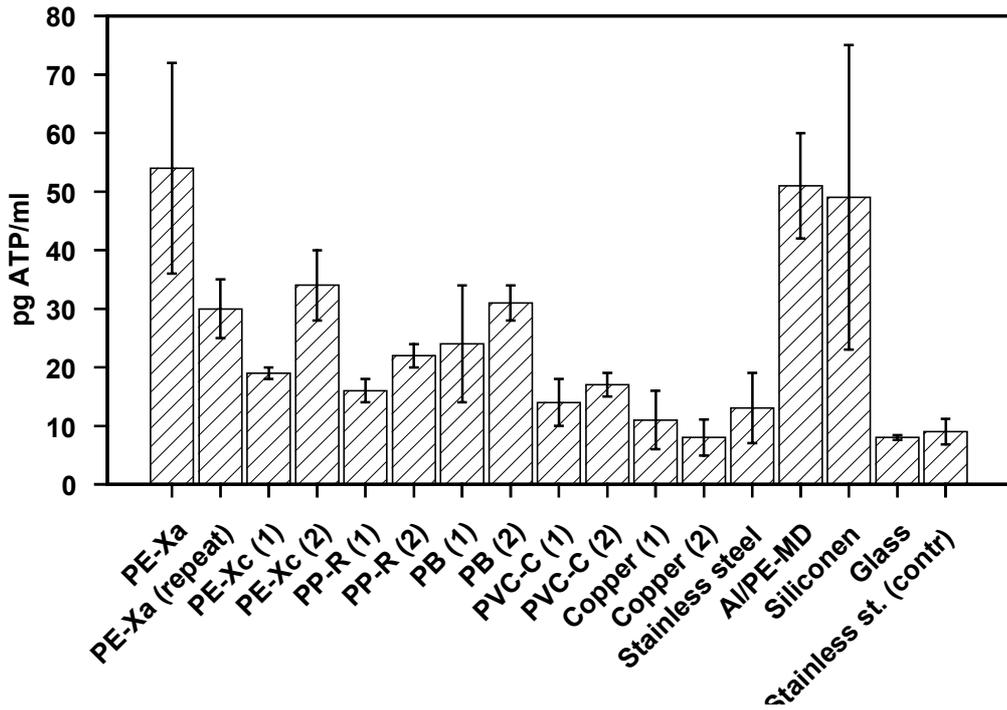


Figure 3. The biomass concentration (BMC, in pg ATP/ml) in water

The BMC values in the test water display the same picture as the BFP values of the materials. Here, too, reproducibility appears to be good. Higher BMC values are observed in the presence of the plastic materials than in the presence of glass or stainless steel.

3.2.4 Number of bacteria in the water

Table 6. Colony counts of *Legionella*, *Pseudomonas* and coliform bacteria in the test water (average of the measurements on days 56, 84 and 112)

Material Test 1	<i>Legionella</i> (cfu/ml) ± sd	Trend	<i>Pseudomonas</i> (cfu/ml) ± sd	Trend	Coliform bacteria (cfu/ml) ± sd	Trend
PE-Xa	89 ± 67	↓	22 ± 21	↓	80 ± 93	↓
PE-Xc (1)	168 ± 104	↓	63 ± 75	↓	170 ± 190	↓
PP-R (1)	330 ± 320	↓	3.0 ± 3.3	↓	5.0 ± 5.8	↓
PP-R (2)	17 ± 10	↓	46 ± 55	↓	70 ± 80	↓
PB (1)	170 ± 80	-	99 ± 10	-	67 ± 77	↓
PVC-C (1)	14 ± 16	↓	8.0 ± 8.4	↓	3.0 ± 3.8	↓
PVC-C (2)	8.0 ± 1.9	↓	29 ± 30	↓	55 ± 64	↓
Test water (control)	110 ± 40	↑	2.0 ± 1.9	↓	2.0 ± 1.9	↓
Silicone hose (control)	110 ± 60	↓	24 ± 34	↓	42 ± 48	↓
Glass (control)	20 ± 15	↓	n.a. ⁽¹⁾		2.0 ± 1.9	↓
Stainless steel (control)	110 ± 100	↓	n.a.		5.0 ± 5.8	↓
Material Test 2	<i>Legionella</i> (cfu/ml) ± sd		<i>Pseudomonas</i> (cfu/ml) ± sd		Coliform bacteria (cfu/ml) ± sd	
PE-Xc (2)	680 ± 810	↓	78 ± 62	↓	n.a.	
PE-Xa (repeated)	1200 ± 600	↓	72 ± 64	↓	n.a.	
PB (2)	200 ± 110	↓	32 ± 34	↓	n.a.	
Copper (1)	220 ± 40	-	n.a.		n.a.	
Copper (2)	160 ± 60	-	n.a.		n.a.	
Stainless steel	54 ± 41	↓	22 ± 20	↓	n.a.	
Test water (control)	71 ± 55	↓	12 ± 14	↓	n.a.	
Silicone hose (control)	300 ± 340	↓	10 ± 9	↓	n.a.	
Glass (control)	33 ± 44	↓	3.0 ± 3.8	↓	n.a.	
Stainless steel (control)	130 ± 140	↓	110 ± 110	↓	n.a.	
Material Test 3	<i>Legionella</i> (cfu/ml) ± sd		<i>Pseudomonas</i> (cfu/ml) ± sd		Coliform bacteria (cfu/ml) ± sd	
Al/PE-MD	1500 ± 900	↓	n.a.		n.a.	
Test water (control)	13 ± 5	↓	n.a.		n.a.	
Silicone hose (control)	1200 ± 1000	↓	n.a.		n.a.	
Glass (control)	7 ± 7	↓	n.a.		n.a.	

n.a. : not analysed or not observed

In the presence of most of the materials there initially appears a (strong) growth of *Legionella*, but the colony counts fell over the course of time (annexes 5 and 6). For a few materials, namely PE-Xa (repetition), PE-Xc(2) and Al/PE-MD, values above 1000 cfu/ml were observed. In the presence of the two PVC-C materials, the growth of *Legionella* bacteria in the water was low. After 112 days, the colony numbers of *Legionella* in the water in the presence of most materials had declined to values of around 100 cfu/ml or lower. In the presence of PE-Xa (repetition) and Al/PE-MD, the colony number after 112 days was still around 10³ cfu/ml. Any growth promotion caused by the stainless steel serving as weighting material can have had an impact on the colony numbers in the water with some materials which were weighted down with stainless steel rings.

In the presence of the two copper materials, no *Pseudomonas* bacteria were found in the water. The explanation for this must probably be sought in the sensitivity of

Pseudomonas bacteria for copper ions. *Legionella* is less sensitive in this respect [Habicht and Müller, 1988].

Only in the first series were the colony numbers of the coliform bacteria in the water with the test material generally higher than in the negative control (glass and test water without material). Growth of mycobacteria was not observed. A single *Mycobacterium* was found, once, in the test water.

3.3. The distribution of biomass over water and materials

Table 7. The biofilm formation potential (BFP), biomass concentration (BMC) and the biomass production potential (BPP) of the materials in the BFP test. BPP-bl water is the biomass production potential corrected for the biomass production in the water (blank); % mat, contribution (percentage) of the biomass on the material to the net BPP; % wat, contribution (percentage) of the biomass in the water to the net BPP.

Material	BFP pg ATP/cm ²	BMC ng ATP/l	BPP pg ATP/cm ²	BPP in water pg ATP/cm ²	% mat	% wat
Water, bl (1)	0	5.9	35.4	0		
Glas (1)	12	8	60	24.6	48.8	51.2
Stainless steel contr. (1)	70	9	124	88.6	79.0	21.0
Silicon (1)	1600	49	1894	1858.6	86.1	13.9
PE-Xa	1400	54	1724	1688.6	82.9	17.1
PE-Xc (1)	400	19	514	478.6	83.6	16.4
PP-R (1)	370	16	466	430.6	85.9	14.1
PP-R (2)	600	22	732	696.6	86.1	13.9
PB (1)	220	24	364	328.6	67.0	33.0
PVC-C (1)	140	14	224	188.6	74.2	25.8
PVC-C (2)	270	17	372	336.6	80.2	19.8
Water, bl (2)	0	6.6	39.6	0		
Glas (2)	13	8	61	21.4	60.7	39.3
Stainless steel contr. (2)	93	12	165	125.4	74.2	25.8
Silicon (2)	990	39	1224	1184.4	83.6	16.4
PE-Xc (2)	930	34	1134	1094.4	85.0	15.0
PE-Xa (rep.)	1300	30	1480	1440.4	90.3	9.7
PB (2)	650	31	836	796.4	81.6	18.4
Copper (1)	590	11	656	616.4	95.7	4.3
Copper (2)	350	8	398	358.4	97.7	2.3
Stainless steel	41	13	119	79.4	51.6	48.4
Water, bl (3)	0	6.5	39	0		
Glas (3)	17	7	59	20	85.0	15.0
Silicon (3)	1500	170	2520	2481	60.5	39.5
Al/PE-MD (1)	730	51	1036	997	73.2	26.8

The impact of a material on the growth of bacteria can be derived in the BFP test from the biomass concentration on the material (BFP) and the biomass concentration in the water (BMC). The biomass production potential (BPP) can be calculated from these parameters. Hence, $BPP \text{ (pg ATP/cm}^2\text{)} = BFP \text{ (pg ATP/cm}^2\text{)} + [BMC \text{ (pg ATP/ml)} \times \text{volume of water/surface area of the material}]$. The volume of the water amounted to 600 ml; the outside surface area of the materials was 100 cm². The BPP value is thus a measure for the entire quantity of active biomass (expressed as ATP) which is present per surface area in the defined period (56-112 days). The calculation of the BPP values is presented in table 7. This table also gives the percentage distribution of the biomass over the water (% wat) and the material (% mat). It becomes apparent that under the test conditions in general more than 70 to 80 % of the biomass was found on the material (BFP). For the materials glass and stainless steel, this was less than 30 to 40 %. A corresponding calculation is performed with the numbers of *Legionella* bacteria on the material and in the water. In this way, the total growth of *Legionella* bacteria (*Legionella* growth potential (LegGP)) is also calculated per surface area unit of the material concerned (table 8). Only with six materials was the major part of the *Legionella* bacteria present on the material (in the biofilm). For some materials, including the silicone material (positive control), the share of the *Legionella* growth on the materials was less than 6 % of the total growth of *Legionella*.

Table 8. The *Legionella* growth potential (LegGP) of the materials in the BFP test.

	LEG on material cfu/cm ²	Leg in water cfu/ml	LegGP cfu/cm ²	% mat	% wat
PE-Xa	120	89	654	18.3	81.7
PE-Xc (1)	560	168	1568	35.7	64.3
PP-R (1)	300	330	2280	13.2	86.8
PP-R (2)	6200	17	6302	98.4	1.6
PB (1)	4800	170	5820	82.5	17.5
PVC-C (1)	250	14	334	74.9	25.1
PVC-C (2)	3400	8	3448	98.6	1.4
Silicon (1)	40	110	700	5.7	94.3
Glas (1)	9	20	129	7.0	93.0
Stainless steel contr. (1)	190	110	850	22.4	77.6
PE-Xc (2)	1100	680	5180	21.2	78.8
PE-Xa (rep.)	16000	1200	23200	69.0	31.0
PB (2)	2000	200	3200	62.5	37.5
Copper (1)	770	220	2090	36.8	63.2
Copper (2)	61	160	1021	6.0	94.0
Stainless steel	38	54	362	10.5	89.5
Silicon (2)	35	300	1835	1.9	98.1
Glas (2)	92	33	290	31.7	68.3
Stainless steel contr. (2)	350	130	1130	31.0	69.0
Al/PE-MD (1)	12000	1500	21000	57.1	42.9
Silicon (3)	130	1200	7330	1.8	98.2
Glas (3)	58	7	100	58	42

3.4 Relationship of biomass formation and growth promotion of *Legionella* bacteria

One element which is important for evaluating the materials in the BFP test is the question of whether a relationship exists between the degree to which a material promotes biomass formation and the degree to which the growth of *Legionella* bacteria is promoted. Figure 4 presents, for the various materials, including the controls, the *Legionella* as a function of (respectively) the BFP values, the BMC values and the BPP values of these materials. The BPP values are corrected for the growth which is observed in the water without materials. The *Legionella* numbers and the BFP values are low for glass and stainless steel and high for several materials. If the data for silicone material are left out of consideration (growth took place primarily in the water), then it appears from application of Student's t-test that a highly significant ($P < 0.01$) positive relationship exists between the BFP value of a material and the number of *Legionella* bacteria on the material. Corresponding results were obtained by statistical evaluation of the relationships between numbers of *Legionella* bacteria and the biomass concentration in the water (BMC) respectively between the *Legionella* growth potential and the biomass production potential (BPP). The linear relationships which are derived are given below :

$$\text{Log} (\textit{Legionella}/\text{cm}^2) = 0.953 \times \text{log BFP} + 0.486 \quad (r^2 = 0.486)$$

$$\text{Log} (\textit{Legionella}/\text{ml}) = 0.674 \times \text{log BMC} + 1.372 \quad (r^2 = 0.357)$$

$$\text{Log} (\textit{Legionella}/\text{cm}^2) = 0.743 \times \text{log BPP} + 1.309 \quad (r^2 = 0.541)$$

Based on these results it can be concluded that, in the BFP, test more biomass formation goes hand in hand with more growth of *Legionella*. However, the relationships were not sufficiently clear to calculate accurate quantitative relationships between the parameters mentioned.

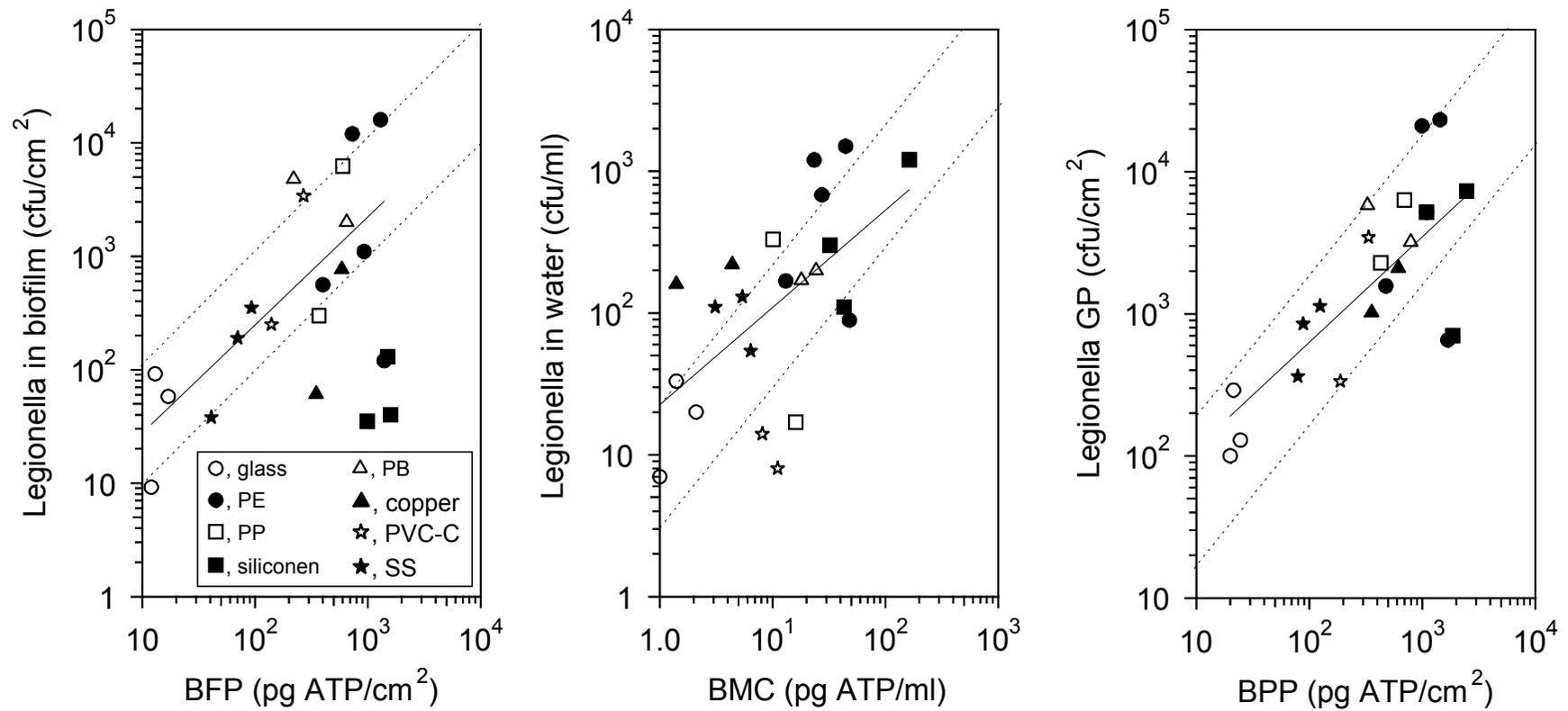


Figure 4. Relationship between the formation of biofilm and biomass and the number of Legionella bacteria. The full lines indicate calculated relationships; the dotted lines indicate the area within which a linear relationship could exist between the formation of biomass and the number of Legionella bacteria.

3.5 Summary of results

The results of the biofilm formation potential tests with the selected materials can be summarised as follows :

- With the aid of the BFP test, clear differences were demonstrated in the growth-promoting effect of the materials in contact with drinking water. The BFP test provided results with good reproducibility.
- All of the tested materials displayed stronger biofilm formation than glass.
- Of the tested materials, the stainless steel pipe material displayed the lowest biofilm formation potential. This material caused no growth promotion of *Legionella*, *Pseudomonas aeruginosa* and coliform bacteria.
- A relatively strong biofilm formation was observed on the two tested copper materials. This is probably the result of the presence of mineral oil on these materials. Growth promotion through corrosion cannot be excluded. After a long-term exposure (208 days) of the copper materials, the biomass concentration on the material fell to a low value (< 100 pg ATP/cm²).
- Of the plastics, the two PVC-C materials and BP(1) displayed the lowest BFP values. Only for the two PVC-C materials did the biofilm concentration drop after around 200 days to a level below 100 pg ATP/cm².
- The PE-based materials displayed the strongest biofilm formation and the strongest promotion of the growth of *Legionella* bacteria.
- Most of the biomass was located on the materials, *Legionella* bacteria were often located for the most part in the water.
- The growth of *Legionella* bacteria displayed significant positive correlations with the biomass concentration in the water, on the material and with the total biomass production (BPP).
- After an initial growth (dependent on the type of the material), the colony counts of *Pseudomonas* and *Legionella* in the water displayed a clear decline after a test period of 56 days; this decline was less pronounced on the materials.
- No *Pseudomonas aeruginosa* was observed on copper and in the water in the presence of copper.
- Growth of mycobacteria was not observed under the test conditions.
- Coliform bacteria were only observed in the first measurement of the first measurement series.

4 BIOFILM FORMATION ON THE MATERIALS IN THE REFERENCE SET-UP

4.1 Set-up and performance of the study

The degree of biofilm formation on the selected materials was tested under hydraulic conditions which correspond more with the situation in domestic installations. For this objective, a so-called reference set-up is used. Based on the results in the biofilm formation potential tests, six materials were selected for testing in the reference set-up. The materials are selected on the basis of composition, while per material type the materials were tested with the highest BFP value and the highest numbers of *Legionella* bacteria. The selected materials are given in table 9.

Table 9. The selected pipe materials for the reference set-up

Material	Description	Diameter (mm)	
		Outer	Inner
RVS AISI 316	Stainless steel	18	16
Copper (1)	Copper	15	13
PVC-C (2)	Post-chlorinated polyvinylchloride	16	12
PE-Xa	Polyethylene, cross-linked	16	11.5
PP-R (1)	Polypropylene (random copolymer)	16	10.5
Al/PE-MD	Polyethylene, cross-linked, internally reinforced with aluminium foil	16	12

A length of 5.7 meters of these materials is placed in the reference set-up.

4.2 The reference set-up

The reference set-up is designed in such a way that it simulates the last 5 meters of a domestic plumbing system. Figure 5 presents a schematic diagram. All coupling pieces used in the installation are made of stainless steel. The tested materials all had an outer diameter of 15, 16 or 18 mm. The drinking water is fed through unplasticized PVC water pipe because it is known that this material contributes virtually nothing to the biofilm formation. The reference set-up is constructed and operated according to the basic conditions indicated in draft DIN 50 932, part 1, "Korrosion der Metalle".

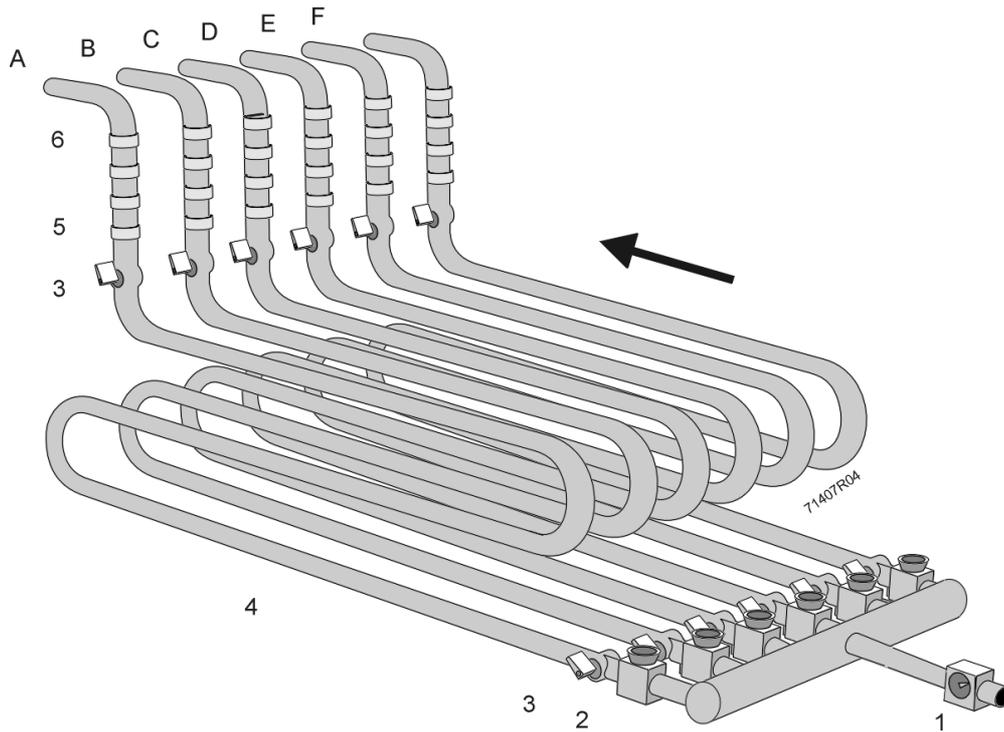


Figure 5 : Schematic overview of the reference set-up
 A through F, test pipes; 1, flowmeter; 2, dosage point; 3, shut-off valve; 4, test pipe; 5, removable part of test pipe; 6, discharge and/or sampling point.

Water is circulated through the pipes of the set-up according to a typical domestic pattern. Figure 6 gives an overview of the tap pattern during one day. Stagnation times are used varying from 0.5 to 8 hours, in accordance with draft DIN 50 931. Each day, 130 litres of water are fed through each pipe, the flow amounted to around 260 ± 50 l/hour. Each time it was tapped during around 30 seconds. The temperature of the water amounted to 18.6 ± 0.7 °C, the ambient temperature amounted to 21.2 ± 0.8 °C.

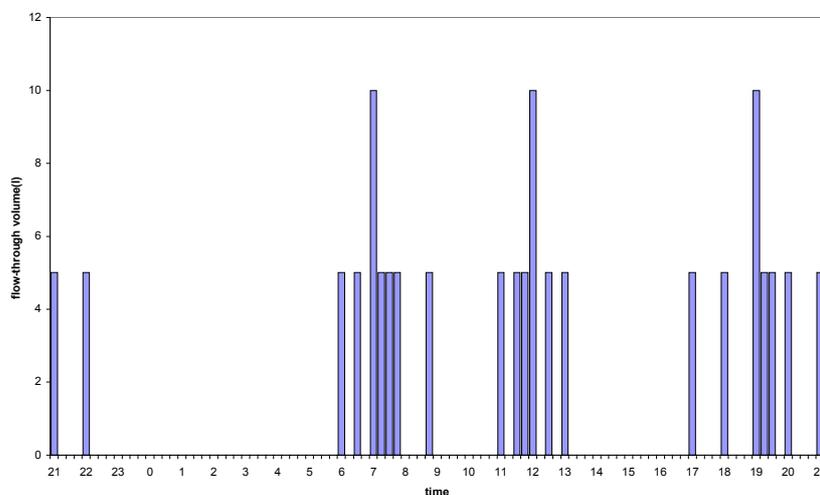


Figure 6. Tap pattern in the reference set-up (DIN 50 931)

In order to investigate whether opportunistic-pathogenic bacteria can increase/maintain themselves in the biofilm under the test conditions, a mixture of bacteria suspensions was dosed after 1 week and after 6 weeks of circulation in the set-up. Hereby the concentration was chosen so that after dosage per 100 ml pipe content 100 colony-forming units per variety of bacteria were present. This number is based on the number of colony-forming units in the dosed suspension. The composition of the dosed suspension is equal to that used in the BFP test and is presented in table 2.

4.3 Test water

The reference set-up was supplied with drinking water coming from water treatment plant Tull en 't Waal of NV Waterleidingbedrijf Midden-Nederland. This drinking water, which is prepared from anaerobic groundwater, may be regarded as representative for many types of drinking water in the Netherlands. The data on the composition of the drinking water are presented in annex 12 [VEWIN, 1994].

4.4 The biofilm monitor

Biofilm formation on surfaces in contact with water is also influenced by the concentration and the type of biodegradable substances in the water. The biofilm-forming characteristics of the water are determined with the aid of the biofilm monitor (Van der Kooij et al., 1997). A biofilm monitor consists of a vertically positioned glass column in which glass rings are placed on top of one another. The drinking water to be studied is circulated through this glass column at a constant rate of 0.2 m/s. This rate corresponds to the flow rate in the pipe network. Under these conditions, the formation of biomass on the rings is distributed equally over the column height. The increase of the biofilm concentration in the monitor is determined by periodically removing two glass rings and measuring the quantity of adenosine triphosphate (ATP) on them. The quantity of ATP is a measure for active biomass and can be measured quickly and sensitively. The Biofilm Formation Rate (BFR, $\mu\text{g ATP}/\text{cm}^2 \cdot \text{day}$) is defined as the linear increase of the ATP content of the biofilm as a function of time for a period between 0 and 100 days.

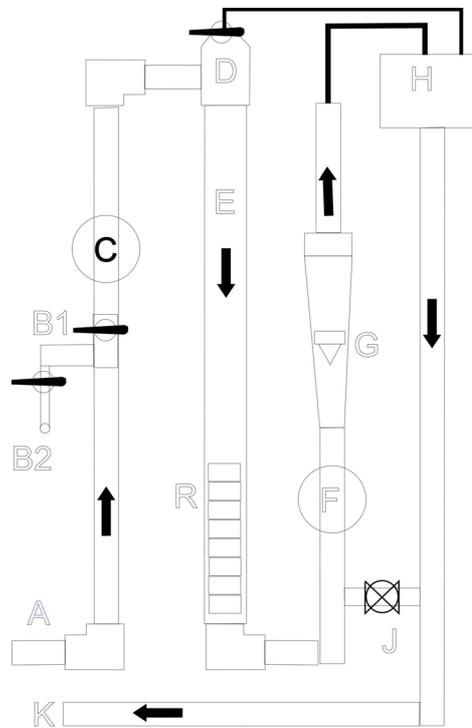
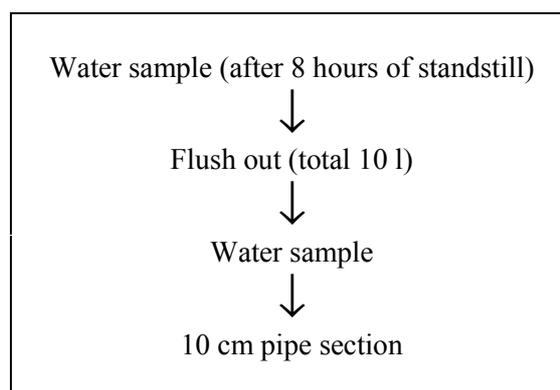


Figure 7. Schematic diagram of the biofilm monitor
A, water inlet point; *B1*, shut-off valve; *B2*, water sampling point; *C*, pressure regulator; *D*, air relief cock; *E*, glass column; *F*, water meter; *G*, flowmeter; *H*, overflow; *J*, shut-off valve; *K*, discharge; *R*, piled up glass rings.

4.5 Sampling method and parameters studied

The reference set-up

After 4, 8, 12, 16 and 20 weeks of continuous circulation, the reference set-up is sampled according to the following scheme :



All the water samples are then examined for the following parameters :

- Adenosine triphosphate (ATP), according to in-house regulation LMB-002
- colony number on glucose yeast extract agar at 22 °C, according to NEN 6560
- colony number on glucose yeast extract agar at 37 °C, according to NEN 6550
- *Pseudomonas*, according to NEN 6573
- coliform bacteria, according to NEN 6653
- *Legionella*, according to NEN 6562
- Mycobacteria, according to the method described by Engel and Berwald [1980]
- pH
- Dissolved organic carbon (DOC)
- copper (A.A.S.-flame)
- iron (A.A.S.-flame)

The surface of the pieces of pipe removed is examined for the following parameters :

- Adenosine triphosphate (ATP), according to in-house regulation LMB-002
- colony number on glucose yeast extract agar at 22 °C, according to NEN 6560
- colony number on glucose yeast extract agar at 37 °C, according to NEN 6550
- *Pseudomonas*, according to NEN 6573
- coliform bacteria, according to NEN 6653
- *Legionella*, according to NEN 6562
- Mycobacteria, according to the method described by Engel and Berwald [1980]
- copper (A.A.S.-flame)
- iron (A.A.S.-flame)

Samples from the reference set-up

The biomass is detached from the pieces of pipe with the aid of sterile cotton swabs. These cotton swabs are then placed directly into sterile tap water, and the biomass is then removed from the cotton swab with repeated ultrasonic vibrations (40KHz). The above-mentioned parameters are determined in the suspension thus obtained.

The biofilm monitor

The biofilm monitor is sampled every two weeks. First a water sample was taken (supplied water), followed by sampling of two glass rings from the monitor. The glass rings are placed in a tube with sterile tap water immediately after their removal from the monitor.

4.6 Results

4.6.1 Measurements on the reference set-up

Biofilm on the inner wall of the pipes

The average values of the biofilm concentration measured on the inner wall of the pipes are included in table 10. The separate measured values are graphically depicted in annex 7. The measurement results of the colony counts are presented in table 11. The biofilm concentration on the materials is presented in figure 8.

Table 10. The biofilm concentration on the pipe wall (average of the measured values after 4, 8, 12, 16 and 20 weeks) and the average biofilm formation rate.

Material	Average biofilm concentration (pg ATP/cm ²) ± sd	Trend	Biofilm formation rate (pg ATP/cm ² .dag) ± sd
Stainless steel	100 ± 40	↑	1.47 ± 0.32
Copper (1)	240 ± 60	-	0.80 ± 1.05
PVC-C (2)	80 ± 90	↑	0.93 ± 0.35
PE-Xa	200 ± 30	-	1.61 ± 0.37
PP-R (1)	100 ± 80	↑	1.15 ± 0.31
Al/PE-MD	160 ± 30	-	0.86 ± 0.53

The highest biofilm concentration was observed on copper; the biofilm concentration was lowest on PVC-C. The quantity of biofilm which was formed on the PE-Xa and Al/PE-MD pipes was also substantially higher than the quantity of biofilm which formed on PVC-C. Stainless steel, PVC-C and PP-R demonstrated a rising trend during the test period of 140 days. The average BFR values over the test period were all lower than 2 pg ATP/cm².d. These relatively low values are probably the result of the alternation of periods of standstill with periods of flushing (see Discussion).

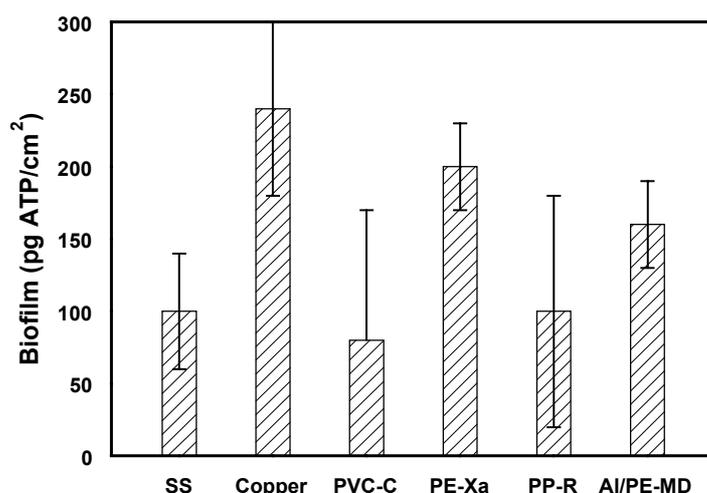


Figure 8. Average biofilm concentrations (in pg ATP/cm² ± s.d.) on the materials in the reference set-up.

Colony counts on the inner wall of the pipes

The average values of the measurements on the inner wall of the pipes of the number of colony-forming units on glucose yeast extract agar at 22 and 37 °C are presented in table 3. Significantly higher colony counts were encountered on Al/PE-MD than on the other tested materials. Striking hereby is that the colony number determined at 37 °C is equal to the colony number determined at 22 °C. All colony numbers displayed a falling trend over the 140-day test period. No coliform bacteria, *Legionella*, *Pseudomonas aeruginosa* and mycobacteria were found on the pipe wall.

Table 11. Colony numbers of the biofilm on the pipe wall, determined on glucose yeast extract agar at 22 and 37 °C (average of the measured values after 4, 8, 12, 16 and 20 weeks).

Material	Colony number 22°C (cfu/cm ²) ± sd	Trend	Colony number 37°C (cfu/cm ²) ± sd	Trend
Stainless steel	170 ± 150	↓	24 ± 33	↓
Copper (1)	110 ± 150	↓	10 ± 17	↓
PVC-C (2)	430 ± 650	↓	120 ± 180	↓
PE-Xa	320 ± 580	↓	47 ± 49	↓
PP-R (1)	150 ± 180	↓	9 ± 8	↓
Al/PE-MD	2500 ± 3400	↓	2500 ± 3300	↓

Iron and copper concentration on the inner wall of the pipes

Iron and manganese can accumulate in the biofilm. Detachment of the biofilm can lead to complaints about colour and turbidity. The observed concentrations of iron on the inner wall of the pipes are given in table 12. The iron concentration on the materials was low and no difference could be demonstrated between the tested materials for this parameter. Copper was only encountered in the biofilm in the copper pipes.

Table 12. Iron and copper concentrations on the inner wall of the pipes (average of the measured values after 4, 8, 12, 16 and 20 weeks).

Material	Iron (mg/cm ²) ± sd	Trend	Copper (mg/cm ²) ± sd	Trend
Stainless steel	0.40 ± 0.26	-	<0.1	-
Copper (1)	1.95 ± 1.10	-	54 ± 24	-
PVC-C (2)	0.50 ± 0.52	-	<0.1	-
PE-Xa	0.54 ± 0.44	-	<0.1	-
PP-R (1)	0.36 ± 0.36	-	<0.1	-
A1/PE-MD	0.57 ± 0.28	-	<0.1	-

Measurements of the water quality in the pipes

After 4, 8, 12, 16 and 20 weeks of operation of the reference set-up, measurements were performed of the water quality in the pipes at the same time as the pipe segments were removed. The samples are always taken immediately after a period of standstill (8 hours) and immediately after a flushing period. The results of the measurements (average of the results in weeks 4, 8, 12, 16 and 20, for the colony counts at 22 and 37 °C the median value and the maximum value) are presented in table 13. The ATP measurements are presented graphically in annex 8. No coliform bacteria, *Legionella*, *Pseudomonas aeruginosa* or mycobacteria were found in the water after standstill (8 hours) and after the pipes have been flushed out, despite dosing of these organisms after 4 and 12 weeks.

Table 13. Results of the measurements of the water quality in the reference set-up after standstill (8 hours) of the water in the pipes and after circulation. Presented are the average results after 4, 8, 12, 16 and 20 weeks of circulation. The median value and the maximum (max.) are presented for the colony numbers at 22 and 37 °C.

Stainless steel	Parameter	After standstill	After flushing
	ATP (ng/l)	4.0 ± 1.8	9.0 ± 5.9
Colony number 22°C on agar with glucose-yeast extract (cfu/ml)	10 (72)	48 (630)	
Colony number 37°C on agar with glucose-yeast extract (cfu/ml)	1 (16)	4 (420)	
Iron (mg/l)	<0.1	0.2 ± 0.2	
Copper (mg/l)	<0.1	<0.1	
NPOC (mg C/l)	1.6 ± 0.2	1.8 ± 0.2	
PH	7.9 ± 0.1	7.8 ± 0.0	

Copper (1)	Parameter	After standstill	After flushing
	ATP (ng/l)	12 ± 10	4.8 ± 1.7
	Colony number 22°C on agar with glucose-yeast extract (cfu/ml)	7 (123)	12 (290)
	Colony number 37°C on agar with glucose-yeast extract (cfu/ml)	<1 (16)	3 (12)
	Iron (mg/l)	<0.1	0.2 ± 0.2
	Copper (mg/l)	2.1 ± 0.8	0.2 ± 0.1
	NPOC (mg C/l)	1.6 ± 0.3	1.8 ± 0.2
PVC-C (2)	Parameter	After standstill	After flushing
	ATP (ng/l)	4.0 ± 1.0	4.4 ± 0.6
	Colony number 22°C on agar with glucose-yeast extract (cfu/ml)	30 (230)	32 (160)
	Colony number 37°C on agar with glucose-yeast extract (cfu/ml)	1 (18)	2 (25)
	Iron (mg/l)	0.1 ± 0.1	0.1 ± 0.0
	Copper (mg/l)	<0.1	<0.1
	NPOC (mg C/l)	1.7 ± 0.2	1.9 ± 0.2
PE-Xa	Parameter	After standstill	After flushing
	ATP (ng/l)	4.3 ± 1.1	4.9 ± 1.0
	Colony number 22°C on agar with glucose-yeast extract (cfu/ml)	24 (480)	37 (200)
	Colony number 37°C on agar with glucose-yeast extract (cfu/ml)	1 (69)	3 (60)
	Iron (mg/l)	0.1 ± 0.0	0.1 ± 0.1
	Copper (mg/l)	<0.1	<0.1
	NPOC (mg C/l)	1.8 ± 0.2	1.7 ± 0.2
PP-R (1)	Parameter	After standstill	After flushing
	ATP (ng/l)	4.3 ± 1.1	5.1 ± 1.2
	Colony number 22°C on agar with glucose-yeast extract (cfu/ml)	39 (170)	44 (170)
	Colony number 37°C on agar with glucose-yeast extract (cfu/ml)	2 (26)	2 (39)
	Iron (mg/l)	0.3 ± 0.5	0.1 ± 0.1
	Copper (mg/l)	<0.1	<0.1
	NPOC (mg C/l)	1.8 ± 0.2	1.9 ± 0.2
A1/PE-MD	Parameter	After standstill	After flushing
	ATP (ng/l)	6.7 ± 1.6	5.5 ± 1.0
	Colony number 22°C on agar with glucose-yeast extract (cfu/ml)	50 (5000)	55 (260)
	Colony number 37°C on agar with glucose-yeast extract (cfu/ml)	17 (5900)	11 (48)
	Iron (mg/l)	0.4 ± 0.6	1.3 ± 2.0
	Copper (mg/l)	<0.1	<0.1
	NPOC (mg C/l)	1.8 ± 0.3	1.8 ± 0.1
PH	7.9 ± 0.1	7.9 ± 0.1	

From the results of the measurements, presented in table 13, it appears that during the standstill period (8 hours) no significant increase (or decrease) takes place in the parameters studied. In none of the tested pipes, except for the copper pipe, could copper be demonstrated in the water (before and after standstill). The maximum colony numbers were virtually always found after 4 weeks of circulation of the reference set-up.

4.6.2 Measurements with the biofilm monitor

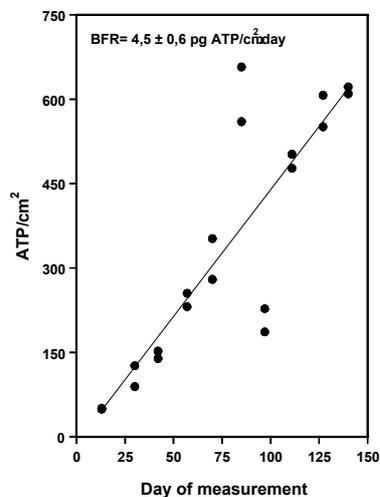
The biofilm-formation characteristics of the test water, as measured with the aid of the biofilm monitor are presented in table 14. The rate of biofilm formation, iron and manganese accumulation on the glass rings in the biofilm monitor is presented in figure 9, annex 9 and annex 10.

Table 14. BFR value, iron-accumulation rate (FeAR) and manganese accumulation rate (MnAR) observed in the biofilm monitor.

Parameter	Value
Biofilm (pg ATP/cm ² .day)	4.5 ± 0.6
FeDR (mg/cm ² .day)	0.07 ± 0.01
MnDR (µg/cm ² .day)	11 ± 3

The biofilm concentrations in the monitor after around 75 days were higher than the biofilm concentrations observed in the reference set-up, even with materials for which it is demonstrated with the BFP-test that they were promoted biofilm formation. The BFR value observed for water is relatively low in comparison with observations of a number of drinking water types. The ATP content of the water with which the monitor was fed was also low (3.9 ± 0.7 ng/l). This level is equal to the ATP concentrations observed in the water from the reference set-up (table 13).

Figure 9. Biofilm formation in the monitor



4.7 Summary of results

The results of the research with the reference set-up can be summarised as follows :

- In the reference set-up, the biofilm formation was lowest on PVC-C (80 pg ATP/cm²); the highest biofilm concentration was observed on copper (240 pg ATP/cm²).
- Biofilm formation on the materials in the reference set-up was lower than in the BFP test (with the exception of stainless steel).
- Biofilm formation on the materials in the reference set-up was lower than on glass in the biofilm monitor. The biofilm formation rate measured with the biofilm monitor amounted to 4.5 ± 0.6 pg ATP/cm².day. The average biofilm formation rate on the materials in the reference set-up amounted to 0.8 to 1.5 pg ATP/cm².day.
- In general, the colony numbers (22°C and 37°C) on the materials were low. The highest colony numbers were observed on Al/PE-MD.
- No *Legionella* bacteria, *Pseudomonas* bacteria, coliform bacteria or mycobacteria were observed with any of the materials.
- The highest colony numbers in the water were observed after 28 days, after standstill (8 hours). In general, standstill led to an increase of the colony number.
- The iron accumulation on the materials was low (< 1 mg/cm²) and virtually equal for the various materials.

5 DISCUSSION

5.1 Impact of test conditions on biofilm formation

The results of the study, described in chapters 3 and 4, make clear that the values of the biofilm formation potential of the materials measured in the (static) BFP test differ significantly from one another. The lowest value of the tested materials was 41 pg ATP/cm² (stainless steel); the highest value was 1400 pg ATP/cm² (PE-Xa). In the reference set-up (dynamic test) with which fewer materials were tested, the lowest value of the biofilm concentration was 80 pg ATP/cm² (PVC-C) and the highest value 240 pg ATP/cm² (copper). For stainless steel and PE-Xa, the average biofilm concentration in the reference set-up amounted to 100 and 200 pg ATP/cm², respectively. This biofilm concentration is significantly lower than the BFP values (with the exception of stainless steel). The differences in growth-promoting effect between the various materials thus appear in the reference set-up much less clearly than in the BFP test. When the comparison is made with the biomass production potential (BPP), which also includes the biomass which is formed under the influence of the material (in the BFP test), then the difference in distinguishing capacity between the tests is even greater. It is striking that the biofilm concentration on the materials in the reference set-up was even smaller than the biofilm concentration on glass in the biofilm monitor after 100 days. An explanation of these differences requires a closer analysis of the biofilm formation processes in the two tests and in the biofilm monitor.

The quantity of biomass which is formed on the surface of a material in contact with water depends on a number of processes/factors. These are listed in Table 15, which also indicates whether these processes play a role in the test concerned.

Table 15. Processes and factors which have an impact on the formation of biomass on the surface of materials under various test conditions.

Process	BFP test	Reference set-up	Biofilm monitor
Release of biodegradable compounds by the material	+	+	-
Supply of biodegradable compounds with the water	-	+	+
Dying off (including predation by protozoa)	+	+	+
Discharge of biomass with the flowing water	-	+	+

The rate of release of biodegradable compounds by the material probably depends mainly on the diffusion in the material itself and in principle is equal for the BFP test and the reference set-up. A condition for this is that the biodegradable compounds which become available on the surface of the material are directly used or discharged. However, it is also possible that growth occurs through deterioration of the material. Under the test conditions employed, the release of biodegradable compounds by the materials in the reference set-up was probably lower than in the BFP test, because the water temperature in the reference set-up was significantly lower (18.6 ± 0.7 °C) than in the BFP test, which was performed at 25 ± 1 °C.

The supply of easily biodegradable compounds with the water is very low in the BFP test, which is performed in the biologically stable filtrate of slow sand filters (low AOC content and a low biofilm formation rate). Possibly under the influence of corrosion of metals (whereby organic substances are bound by complex formation), certain organic compounds present in the water become available for growth of micro-organisms on the material. In the reference set-up, easily-biodegradable compounds are indeed supplied with the water. However, this supply is very low in comparison with the supply in the biofilm monitor. A consumption of 130 l/day and a flow of 260 l/hour results in a continuous flow of 0.5 hour per 24 hours, thus only 2 % of the time. There was a continuous flow through the biofilm monitor. A supply of biodegradable substances with the water which amounts to 2 % of the supply in the biofilm monitor delivers after 100 days a contribution to the biomass density of $100 \times 4.5 \text{ (BFS)} \times 0.02 = 9 \text{ pg ATP/cm}^2$. This contribution is less than 10 % and thus negligible vis-à-vis the biofilm concentrations observed on the materials in the reference set-up.

The degree of dying off of biomass (through endogenous respiration and predation by protozoa) depends on the concentration of the biomass and is also influenced by the temperature. There is less dying off at a low temperature than at a high temperature.

There is no discharge of biomass in the BFP test. Moreover, in this test, the impact of the material is determined by measuring the concentration of biomass in the water. In both the reference set-up and the biofilm monitor a strong discharge can occur. In the biofilm monitor there is a constant flow rate (0.2 m/s), whereby a biofilm of micro-organisms can develop which attaches relatively firmly. The discharge of biomass in the reference set-up is probably relatively high, because the biofilm is virtually entirely formed in the periods without flow. In the pipes of the reference set-up, a flow rate between 0.35 m/s (stainless steel) and (0.85 m/s (PP-R) predominates during tapping. At this rate, the flow pattern of the water in the pipes is turbulent. This means that during the short time that water is flowing through the pipes, a relatively strong erosion of the biofilm which formed during the standstill period can occur. Differences in flow rate in the various pipes may affect the degree of biofilm formation on the surface of the various materials in the reference set-up.

On the basis of the factors mentioned above, one can explain why in the reference set-up less biofilm formation was observed than in the BFP test, and also why the level of biofilm formation itself was lower than in the biofilm monitor. In the reference set-up, moreover, differences can be observed in biofilm concentration on the various materials. The highest biofilm concentrations were observed on the PE materials and on copper and the lowest values on PVC-C, stainless steel and PP-R. Already after 28 days of operation, a relatively high biofilm concentration was observed on several materials. This was the case for copper and the two PE-based materials. At the same time it is clear that, after an operation period of 140 days, no equilibrium situation had yet been reached for most materials. It is unclear whether further increase of the biofilm concentration can occur on these materials. More biofilm formation occurred on stainless steel than was expected on the basis of the biofilm formation rate observed in the biofilm monitor and the low percentage of the time that the reference set-up was under continuous flow. Factors which might play a role here are:

- the presence of an oil layer on the inside of the steel pipe (stainless steel used in the BFP test was heated to 550 °C before use);
- the degree of attachment of biomass to the pipe material (on steel possibly better attachment than on glass).

However, the study performed gives no answer about the possible role of these factors.

Another remarkable difference between the results in the BFP test and those in the reference set-up is that in the reference set-up no growth of *Legionella* bacteria was observed. The possibilities for growth of *Legionella* bacteria were limited in the reference set-up by the lower temperature of the water (< 20 °C) and the discharge of biomass as a result of the regular tapping of the water.

5.2 Choice of test method

Along with the determination of the degree of biofilm formation on the various materials under the different test conditions, one of the objectives of the study was to discuss the possibility of an evaluation of pipe materials on the basis of their growth-promoting characteristics. Such an evaluation requires a critical consideration of the measurement results obtained with the tests performed. A point of discussion hereby is the degree to which the test conditions are representative for situations in practice.

Conditions in practice (i.e. domestic drinking water installations) will in many cases correspond more closely to the conditions in the reference set-up than to those in the BFP test, i.e. the water is refreshed in the piping several times a day (in the reference set-up, 22 times a day). The observations indicate that the tested materials under conditions corresponding to the conditions in the reference set-up have virtually no impact on the microbiological quality of the water. Multiplication of undesired micro-organisms was not observed, and even the colony numbers determined at 22 and 37 °C remained low in the water before and directly after circulation. Only in the water from pipes of the material Al/PE-MD, the median value of the colony number determined at 37 °C was somewhat above the target level (10 cfu/ml) specified in the Drinking Water Decree (1984) for this parameter (table 13). On the basis of these results, one can conclude that application of the tested materials in practice under circumstances which correspond to the conditions in the reference set-up (frequent circulation, water temperature < 20 °C) will have little impact on the microbiological quality of the water.

In practice, however, the factors which can have an impact on the growth of micro-organisms in drinking water installations can vary widely. This is particularly true for the residence time (long-term stagnation), the water temperature (including seasonal influence, indoor temperature) and the contact time when flowing through (length of pipes). Even under unfavourable practical circumstances ('worst case' situations), the growth promotion by pipe materials should not cause microbial water quality problems. The microbiological quality of the water in drinking water installations is (virtually) not inspected and the protection of the microbiological quality therefore rests entirely on prevention. Against this background, it is recommended to use the behaviour of the materials in the BFP test (in which an extremely long residence time is combined with a relatively high water temperature) as the basis for evaluating the materials for application in drinking water installations. The question hereby is which evaluation criteria must be used.

5.3 Parameters and evaluation criteria

5.3.1 Parameters and basis of evaluation

Evaluation of the materials on the basis of growth promotion in the BFP test depends on the choice of the parameters (BFP, BMC, BPP, growth of *Legionella*, growth of other types of bacteria) and the criteria by which the measurement results can be tested. Tables 3, 4, 5 and 6 show that only the results which were obtained in the study concerning the parameters for biomass and the number of *Legionella* bacteria on the material and in the water come into consideration for an evaluation. Such an evaluation can then take place on the basis of :

1. A comparison with the practical data whereby the degree of biofilm formation in the BFP test is compared with observations on biofilm formation in drinking water installations and in the distribution system.
2. A scientifically well-founded approach including aspects such as health risk (infection risk) and aesthetic or technical objections linked with growth promotion are brought in;

These evaluation possibilities are examined in greater detail below.

Evaluation on the basis of biofilm formation

Aesthetic and technical objections

Aesthetic objections resulting from an excessive increase of micro-organisms include in particular the formation of flavouring substances by fungi and actinomycetes or the development of anoxia and the presence of animal organisms or particles of biomass in the water. An example is the growth of actinomycetes on rubber membranes in pressure vessels, which gives rise to taste problems. In England, the criterion for evaluating the growth properties of materials in contact with drinking water is based on the observation of visible growth on the materials. Such visible growth arises when the extra oxygen consumption of the material as determined in the Mean Dissolved Oxygen Difference (MDOD) test is higher than 2.3 mg/l (Colbourne, 1985). Due to the lack of data on the relationship between the MDOD value and the BFP value, this criterion cannot (yet) be translated into a BFP value. Another aesthetic aspect is the accumulation of iron and manganese in the biofilm, which could give rise to periodic complaints about the colour of the water. Here, too, quantitative data are lacking. A technical problem associated with biofilm formation is the increase of resistance in the pipe. Such a problem probably only arises in cases with an extreme level of biofilm formation. In sum, there is not enough information for defining a quantitative criterion for biofilm formation based on aesthetic and technical aspects with which the materials could be evaluated.

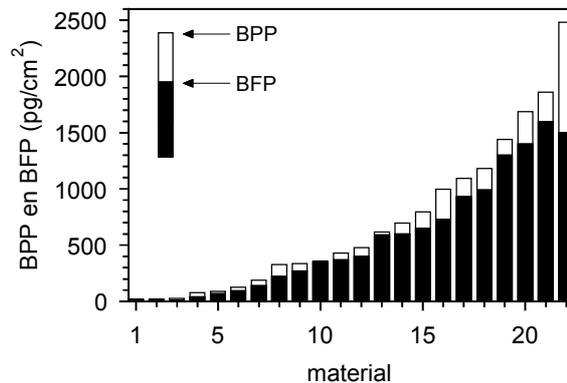


Figure 10 : The tested materials, including the control materials, ranked by the degree of biofilm formation in the BFP test. 1, 2 and 3, glass; 4, 5 and 6, stainless steel; 7, PVC-C(1); 8, PB(1); 9, PVC-C(2); 10, copper (2); 11, PP-R(1); 12, PE-Xc(2); 13, copper(1); 14, PP-R(2); 15, PB(2); 16, Al/PE-MD; 17, PE-Xc(2); 18, silicones; 19 and 20, PE-Xa; 21 and 22, silicones.

Comparison with practical situations

There is no data available on the biofilm density in drinking water installations in the Netherlands, and so a comparison of the biofilm formation observed in the BFP test with biofilm concentrations in drinking water installations is impossible. Within the framework of research on growth processes in distribution systems information has been collected on the biofilm-forming characteristics of drinking water and the biofilm concentration in distribution systems supplied with water without a disinfectant residual. From research performed with the biofilm monitor, it appeared that the biofilm formation rate (BFR) of the various drinking water types in the Netherlands lies between values $< 1 \text{ pg ATP/cm}^2 \cdot \text{day}$ (filtrate slow sand filters, drinking water prepared from oxygen-containing groundwater) to values greater than $50 \text{ pg ATP/cm}^2 \cdot \text{day}$ (drinking water prepared from anoxic groundwater). At the same time it appeared that the degree of regrowth of *Aeromonas* bacteria in the pipe network is related to the biofilm formation rate of the drinking water upon leaving the treatment plant (van der Kooij et al. 1999). A study on biofilm concentrations on the pipe wall (PVC), performed in 22 distribution systems, showed that the values of the biofilm concentrations varied from 40 pg ATP/cm^2 (min.) to 5800 pg ATP/cm^2 (max), with a median value of 670 pg ATP/cm^2 (van der Kooij et al. 1999). Levels above 1000 pg ATP/cm^2 were observed in pipeline networks with regrowth of *Aeromonas*. The mentioned biofilm concentrations are the result of picking up biodegradable compounds from the passing drinking water, because the biofilm formation potential of unplasticized PVC pipe materials is less than 100 pg ATP/cm^2 .

The data about the biofilm concentration on the pipe wall can be used for evaluating the BFP and BPP value of pipe materials. One could use as a starting point that a material may only contribute to a slight extent to the biofilm formation. In situations where the drinking water causes very little biofilm formation on the pipe wall, it is preferable to apply materials which cause as little extra biofilm formation as possible.

5.3.2 Quality classes

On the basis of the above-mentioned considerations, a classification into quality classes can be performed for materials in contact with drinking water. With the aid of the BFP test it can then be determined into what class certain materials fall. One possibility for such a classification on the basis of BFP values is offered in table 16. It is still unclear whether, with such a classification, one must proceed on the basis of the BFP value or the BPP value. For most materials, the difference between these parameters is small, however in the presence of some materials a relatively high level of biomass formation was observed in the water (figure 10).

Table 16. *Quality classes for materials in contact with drinking water, based on biofilm formation potential (BFP).*

Class	BFP (pg ATP/cm ²)	Material
A	≤ 100	Glass, stainless steel, PVC*, teflon*
B	100 < BFP ≤ 500	PVC-C (1), PVC-C (2), PB (1), Copper (2), PP-R (1), PE-Xc (1)
C	500 < BFP ≤ 1000	Copper (1), PP-R (2), PE-Xc (2), Al/PE-MD, silicones (2)
D	1000 < BFP ≤ 3000	PE-Xa, silicones (1), silicones (3)
E	BFP > 3000	Rubber types*, soft PVC*

*, result of research performed earlier.

Examples of materials belonging to the highest quality class (BFP or BPP value < 100 pg ATP/cm²) are: glass (however not usable for drinking water pipes; stainless steel, unplasticized PVC and PTFE. Examples of relatively strongly growth-promoting materials (BFP or BPP value > 3000 pg ATP/cm²) are : natural rubber and plasticized PVC. Such materials are not applied in contact with drinking water, except in situations where very small surface areas are exposed to the water (e.g. rubber rings in pipe couplings).

From the ranking of the materials on the basis of the BPP values (figure 10) it appears that a number of materials scored equal to or better than copper in the BFP test. It was already mentioned that the copper pipes showed a relatively strong biofilm formation in both the BFP test and the reference set-up. The biofilm concentration on the copper materials increased linearly as a function of time in the BFP test. From this, biofilm formation rates were calculated of around 4 (copper(2)) and 20 (copper(1)) pg ATP/cm².d. This biofilm formation is probably a result of the presence of mineral oil on the copper pipe, because the strongest biofilm formation is observed on the copper on which most mineral oil was present. However, if corrosion also plays a role, then it can be expected that the water composition too ((pH, inorganic and organic carbon) will have an impact on the growth promotion by copper. No further information is available on this.

These observations indicate that copper scores less well with regard to biological stability than was expected on the basis of the assumption that copper does not release any growth-promoting compounds and should even be growth-inhibiting. However, this growth inhibition effect does not apply for all micro-organisms. *Pseudomonas* varieties, and also *Aeromonas* varieties are relatively sensitive for copper, but low concentrations of copper have little effect on *Legionella* bacteria and *Mycobacterium*

varieties. The results of this study also demonstrated that copper had no or little effect on *Legionella*.

It may be concluded that, on the basis of biofilm formation/biomass production, a clear distinction can be made between materials which makes a division into quality classes possible. However, it is not yet possible to indicate for which application areas the materials of the various quality classes come into consideration. In the definition of the application areas, factors such as the length of the pipe, diameter (surface area/volume ratio) and water temperature play an important role.

5.3.3 Evaluation on the basis of the health risk associated with growth promotion of pathogenic bacteria

Potentially pathogenic micro-organisms, which can multiply in (drinking) water installations include *Legionella* spp., *Mycobacterium* spp. and *Pseudomonas* spp. One can assume that the more nutrients are present, the stronger will be the growth of such micro-organisms. Temperature, flow rate and residence time are also important. Under the test conditions, (virtually) no increase of *Pseudomonas* and *Mycobacterium* was observed. The growth of *Legionella* bacteria was observed in the BFP test and therefore the possibility of evaluating materials on the basis of (the risks attaching to) promotion of the growth of *Legionella* bacteria is discussed below.

Growth of Legionella in the test system

In the presence of growth-promoting materials, the growth of *Legionella* bacteria can be enhanced, thus giving rise to a health danger. *Legionella* bacteria appear widely dispersed in water and multiply in particular in biofilms at a sufficiently high temperature. From the results obtained with the BFP test it appears that a number of materials promote the growth of *Legionella* bacteria relatively strongly. Multiplication of *Legionella* bacteria was generally stronger the stronger the growth promotion of the material (figure 4). The clearest connection between the degree of growth promotion and the growth of *Legionella* bacteria appears from the relationship between the BPP values and the growth of *Legionella* bacteria on the materials and in the water (figure 4). However, the distribution is relatively large and the degree of growth promotion of *Legionella* bacteria cannot be derived accurately for a specific material simply from the BFP or the BPP value. A possible cause of this great distribution can be the water temperature (25 °C) used in the BFP test. This temperature is significantly lower than the optimum temperature (35 to 37 °C) for the growth of *Legionella* bacteria, making competition with other bacteria more difficult for *Legionella*. The potential for promoting the growth of these bacteria is probably more clearly expressed at temperatures closer to the optimal growth temperature of this organism, i.e. 30 – 37 °C.

Health risk

Can a health risk-based evaluation criterion be derived from the growth promotion of *Legionella* bacteria in the BFP test? In warm tap water systems in the Netherlands, *Legionella* bacteria are observed in numbers lying between 5×10^3 cfu/l to 2×10^5 cfu/l (Van der Kooij en Hoekstra, 1984). Meenhorst (1984) found numbers to several hundreds cfu/ml, and cases of legionnaires' disease were observed in concentrations > 40 cfu/ml. In Germany, a concentration of 10 cfu/ml has been proposed as the level above which corrective measures must be taken. In the presence of *L. pneumophila* serogroup 1, measures would also be necessary at lower levels (Exner et al. 1993). However, there is no standard value for the maximum number of *Legionella* bacteria, related to an infection risk (of e.g. 10^{-4} per person per year). Complicating factors for determining such a level (in water) are:

- infection occurs via aerosols which are formed with this water;
- the many varieties and serotypes of *Legionella* bacteria, which differ greatly from one another in their degree of virulence;
- the lack of data on the health risk upon exposure to *Legionella* bacteria ('infectious dose').

The concentrations of *Legionella* bacteria in the water of the BFP test were between 10 and 1500 cfu/ml, and were thus higher than the numbers in warm tap water systems, where legionnaires' disease is observed or where corrective measures are proposed. The conditions in the BFP test are relatively favourable for the growth of *Legionella* bacteria, despite the fact that the water temperature is not optimal for their growth. To 'translate' the results in the BFP test one needs data on the relationship between the number of *Legionella* bacteria in the biofilm and in the water at a certain rate of replacement of the water (in practice and in the test). Then a quantitative criterion is necessary for the number of *Legionella* bacteria in the water necessary to evaluate the result in the test. With the current data, it is not possible to derive a quantitative evaluation criterion for materials on the basis of growth promotion of *Legionella* bacteria in the BFP test.

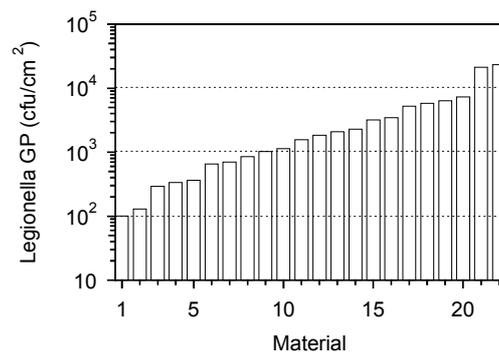


Fig 11. Growth potential of *Legionella* on different materials.
 1,2,3= glass; 4=PVC (1); 5=Stainless steel; 6= PE-Xa; 7= silicones (1); 8=Stainless steel control (1); 9= copper (2); 10= Stainless steel control (2); 11= Pe-Xc (1), 12= silicones (2); 13= copper (1), 14= PP-R (1); 15= PB (2); 16= PVC-C (2); 17= PE-Xc (2), 18= PB (1); 19= PP-R (2); 20= silicones (3); 21= Al/PE-MD; 22= PE-Xa (repeat)

5.3.4 Evaluation of pipe materials for domestic installations

The preceding considerations make clear that it is not yet possible to derive an objective evaluation criterion for materials in interior installations on the basis of growth promotion. The possibilities for arriving at an evaluation system have been discussed. The definition of quality classes, based on growth promotion and indicating the application area for each class, offers possibilities for arriving at a clear authorisation policy. One must investigate whether an evaluation on the basis of biofilm formation or biomass production is sufficient or whether the degree of multiplication of pathogenic micro-organisms, particularly *Legionella*, must be included in the categorisation into quality classes (and in the performance of the test). The VROM-Kiwa Commission on Health Aspects of Chemicals and Materials in contact with drinking water plays a central role in the evaluation of the impact of materials on the quality of drinking water. Given the potential health risks associated with the bacterial growth-promoting effect of materials in contact with drinking water, it would seem logical to involve this commission in the further development of the above approach.

6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Thirteen pipe materials, including copper and stainless steel, which are used in drinking water installations were tested for their promotion of bacterial growth. This study used two different test systems, i.e. the static biofilm formation potential (BFP) test and the reference set-up (continuous flow system).
 2. The BFP test showed that the materials clearly differ from one another with regard to promoting the growth of micro-organisms. The lowest growth promotion was observed with stainless steel; a growth promotion around 30 times stronger was observed for several PE-based materials.
 3. The biofilm formation on pipe materials was lower in the reference set-up than in the BFP test (with the exception of stainless steel). The explanation for this is that the biomass which was formed in the reference set-up was discharged with the water, while in the BFP test this was not the case. On none of the materials in the reference set-up, growth of *Legionella* bacteria, *Pseudomonas* bacteria, mycobacteria or coliform bacteria was observed. On the basis of these observations one can conclude that under normal circumstances the tested materials have little impact on the microbiological quality of the water.
 4. On copper a biofilm formation took place which was relatively strong (in comparison to the tested plastics and stainless steel), both in the BFP test and in the reference set-up. Probably this biofilm formation is linked to the presence of mineral oil on the copper. Possibly corrosion processes also play a role, whereby organic compounds from the water become available for micro-organisms.
 5. A significant relationship was observed between the degree of growth promotion measured as biofilm formation or biomass production and the growth promotion of *Legionella* bacteria. However, the degree of growth promotion of *Legionella* bacteria cannot simply be derived from the degree of biofilm formation or biomass production.
 6. Evaluation of the materials on the basis of biofilm formation or biomass production related to hygienic, aesthetic or technical aspects is still impossible. Also the lack of information on biofilm concentrations in drinking water installations makes it difficult to evaluate the results of the BFP test.
 7. Evaluation of the materials on the basis of growth promotion (in the BFP test) of *Legionella* bacteria is impossible due to the lack of information on the relationship between the numbers of *Legionella* bacteria in the water and in the biofilm under different hydraulic conditions, and the lack of a criterion for the number of *Legionella* bacteria in water.
- ☆ In 2000, an MTC value of 50 cfu/l has been defined in legislation in The Netherlands.

6.2 Recommendations

1. It is recommended to base the evaluation of materials with regard to growth promotion on the behaviour in the BFP test. It is also still important to determine in this test the growth of *Legionella* bacteria along with the biofilm formation/biomass production. Performance of the BFP test at 30 °C probably would probably give a better quantitative picture of the degree to which the growth of *Legionella* can be promoted than performance of the test at 25 °C.
2. It is recommended, for the evaluation of materials with regard to growth promotion, to set up a system of quality classes, as defined in the BFP test. The

required quality level depends on the conditions under which the material is applied (length of pipe, surface area/volume ratio, temperature, etc.).

3. It is recommended to perform further research in order to determine the required quality levels for the various applications. Such research would include :
 - collecting and assessing data about biofilm concentration, biofilm composition (including presence of *Legionella*) and concentrations of micro-organisms in the water in drinking water installations in relation to the conditions which prevail in these systems;
 - determining the effect of refreshing the water in the BFP test on the biofilm formation or biomass production and the distribution of biomass and micro-organisms (*Legionella*) over biofilm and water.

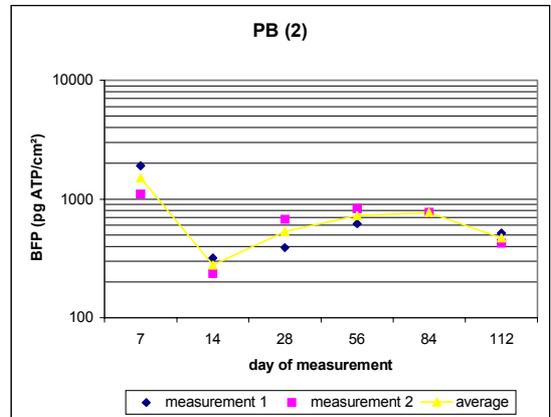
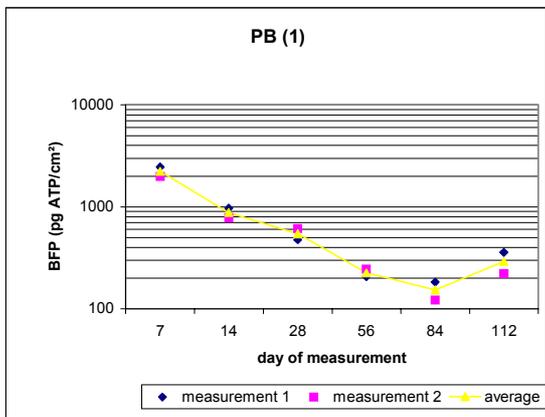
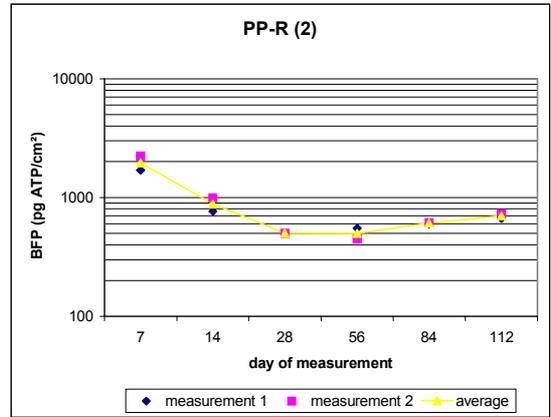
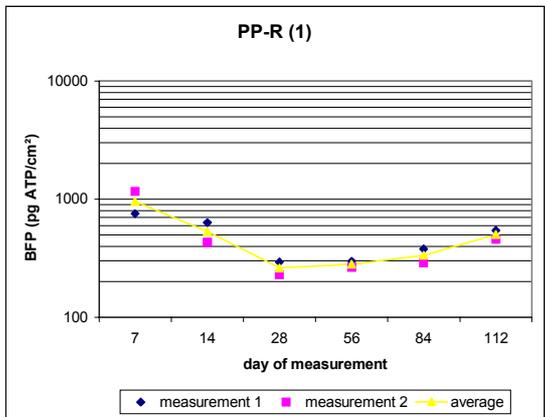
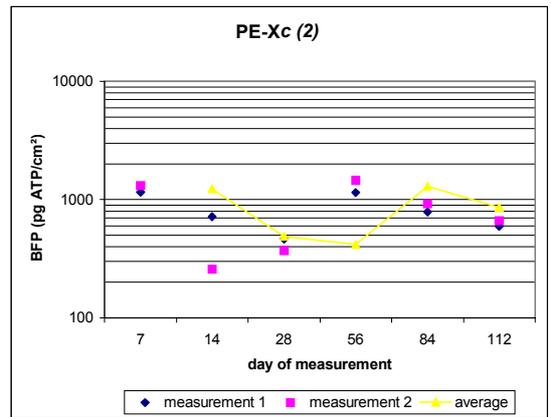
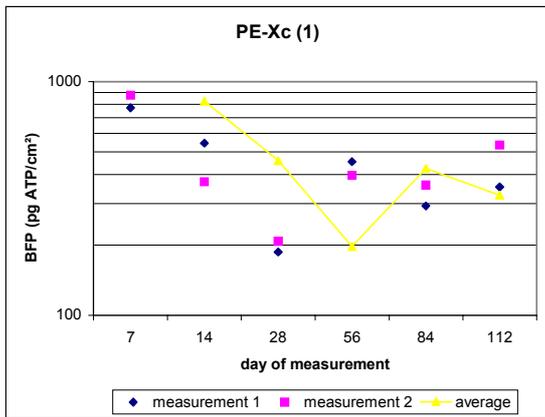
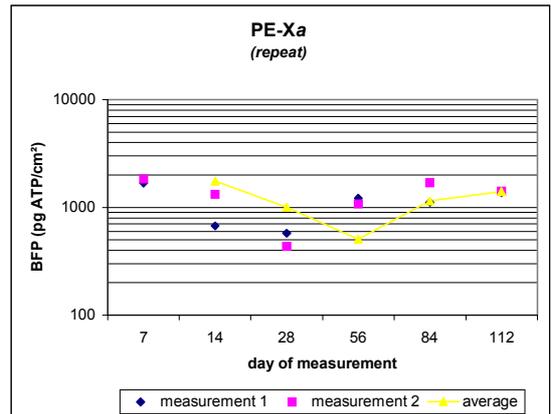
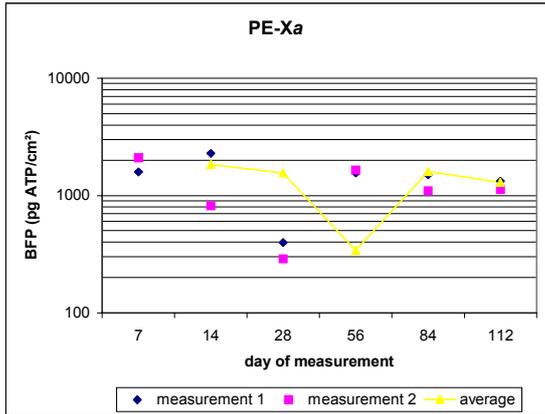
LITERATURE

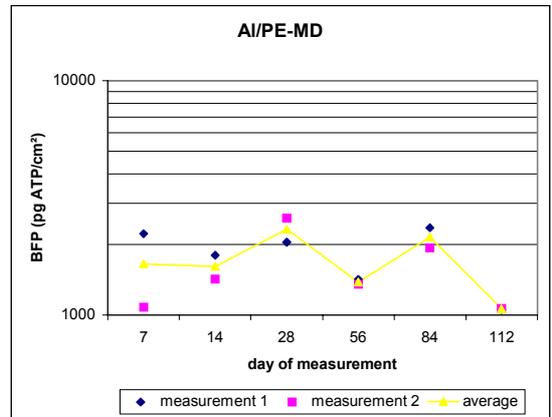
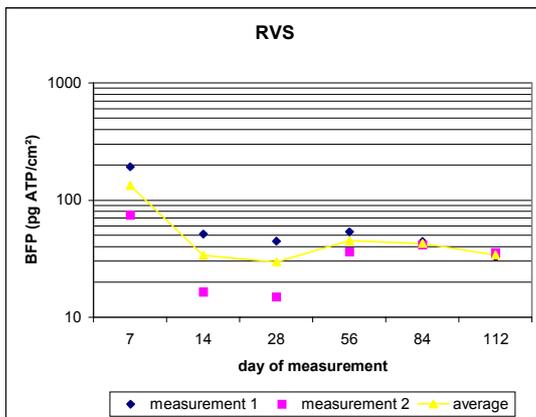
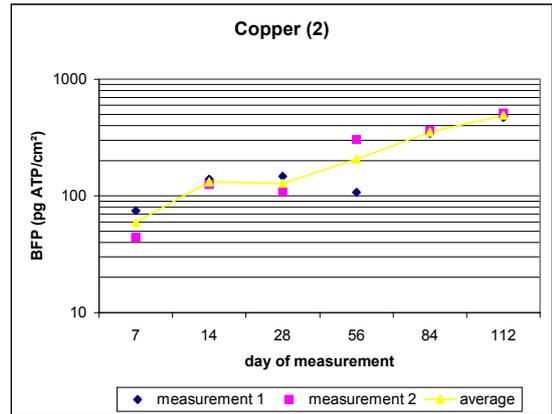
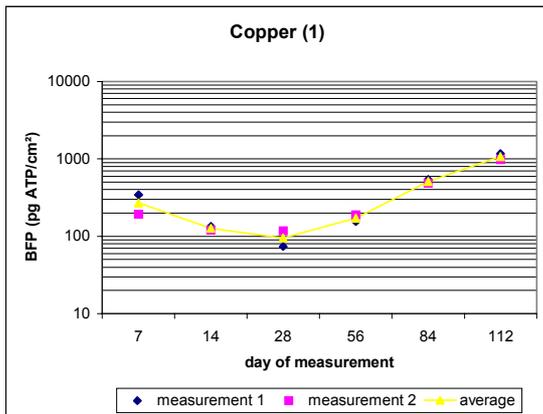
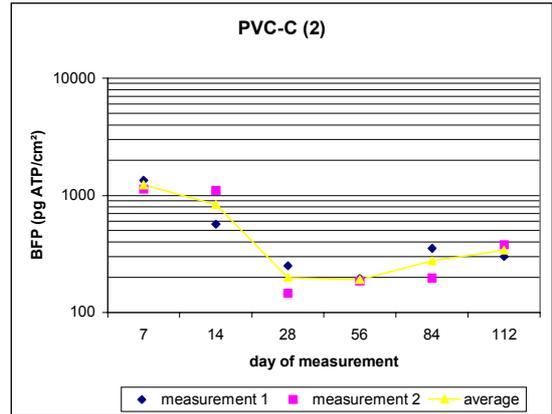
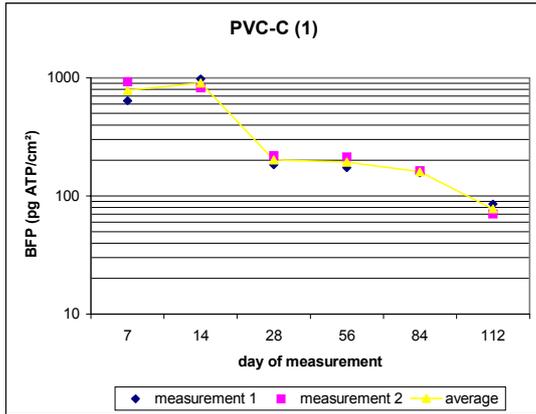
- BRITISH STANDARD (1988)
Suitability of non-metallic products for use in contact with water intended for human consumption with regard to their effect on the quality of water. Section 2.4 Growth of aquatic microorganisms.
British Standards Institution BS 6920 2.4
- BURMAN, N.P. AND COLBOURNE, J.S. (1979)
Effect of non-metallic materials on water quality.
J. Inst. Wat. Eng. Sci. 33:11-18
- COLBOURNE, J.S. AND PRATT, D.J. (1984)
Water fittings as sources of *Legionella pneumophila* in a hospital plumbing system. The Lancet; 28:210-213
- COLBOURNE, J.S. (1985)
Materials usage and their effects on the microbiological quality of water supplies. J. Appl. Bacteriol. Symp. Suppl. 47S-59S
- COMMISSIE INTEGRAL WATER BEHEER. Werkgroep IV (1997)
Handreiking regionale aanpak diffuse bronnen. CUWVO.
- CUWVO (1993)
Landelijk watersysteemrapportage 1992. Rapport RIZA-Lelystad
- DIN 50 931 TEIL 1 (1996)
Korrosion der metalle. Korrosionsversuche mit Trinkwässern. Teil 1: Veränderung der Trinkwasserbeschaffenheit. Deutsche Norm
- DVGW (1990)
Vermehrung von Microorganismen aut Materialien für den trinkwasserbereich. Prüfung und Bewertung. Technische Regeln. Arbeitsblatt W270. DVGW
- ENGEL, H.W.B. EN BERWALD, L.G. (1980)
Verder onderzoek naar het voorkomen van *Mycobacterium kansasii* in leidingwater. Rijksinstituut voor de Volksgezondheid, rapport nr 76/80 Bact, Bilthoven
- EXNER, M., G.J. TUSCHEWITZKI, B. LANGER F. WERNICKE UND ST JÉRÔME. PLEISCHL(1993)
Vorkommen und Bewertung von Legionellen in Krankenhäusern und anderen Grossgebäuden. Schriftenreihe des vereins für Wasser-, Boden und Lufthygiene 91:105-130
- GOOD, R.C. (1985)
Opportunistic Pathogens in the Genus *Mycobacterium*. Ann. Rev. Microbiol. 39, p. 347-369
- GROOTHUIS, D.G., BERWALD, L.G., AND BAAS, J.G. (1983)
Groeï van *Mycobacterium kansasii* in verschillende typen leidingwater; invloed van bebroedingstemperatuur en toevoeging van lood, koper polyvinylchloride (PVC) en tyleen. RIV-Rapport. 1983; rapportnummer 128009001.
- GROOTHUIS, D.G., BERWALD, L.G., HAVELAAR, A.H., AND BAAS, J.G. (1983)
Voorkomen van mycobacteriën in een zwembad met beweegbare bodem. RIV rapportnummer 128106001
- HABICHT, WILYA, AND MULLER, HANS. E. (1988)
Occurrence and Parameters of Frequency of *Legionella* in Warm Water Systems of Hospitals and Hotels in Lower Saxony. Zentralblatt Bakt. Hyg. B. ; 186:79-88
- HUISVOORSCHRIFT LMB-002 (1997)
Bepaling van het adenosinetrifosfaat-gehalte in water en suspensies. Versie 5
- JANSEN, P.F.C.
Duurzaam bouwen: Nationaal pakket woningbouw.
Rotterdam Stichting Bouwresearch SBR-publicatie359-H-96
ISBN 90-5367-175-7. Losbladig
- KIWA (1998)
Lijst met goedgekeurde systemen. Leidingssystemen van kunststof bestemd voor: drinkwaterinstallaties binnenshuis; verwarmingsinstallaties: radiatoraansluitingen; vloerverwarmingsinstallaties. Actueel tot 01-10-1998
- VAN DER KOOIJ, D., AND HOEKSTRA, A.C. (1984)
Legionella pneumophila in warmtapwaterinstallaties. Ziekehuishygiëne en infectiepreventie 84-5 p. 103-106

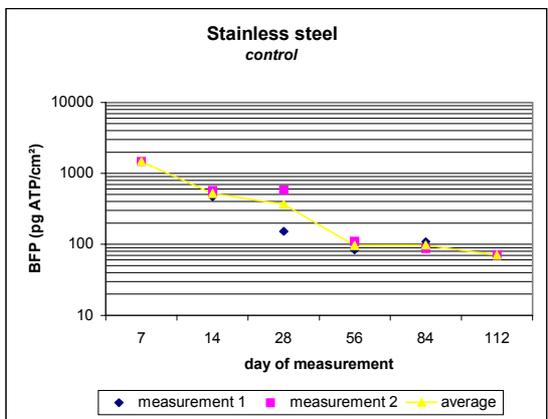
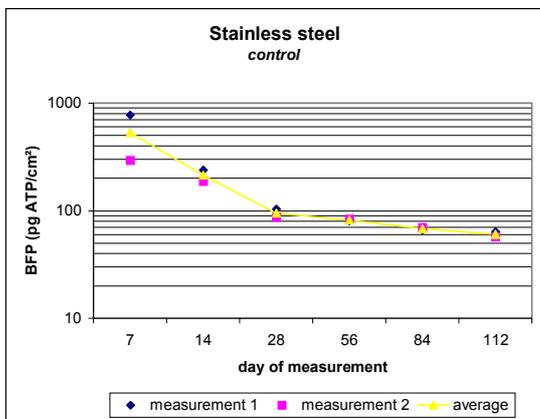
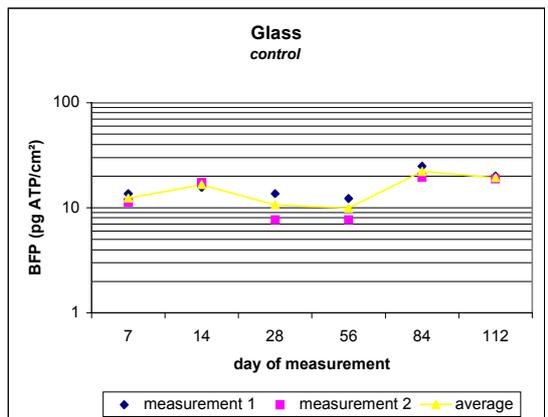
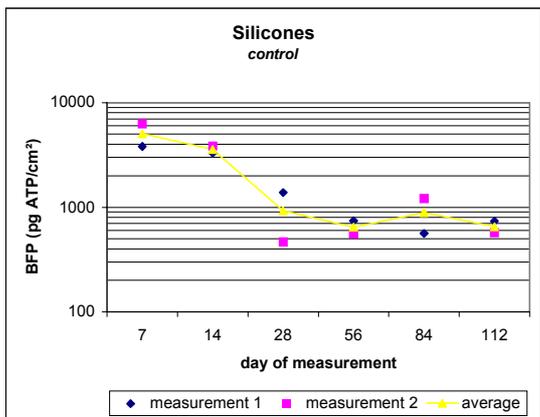
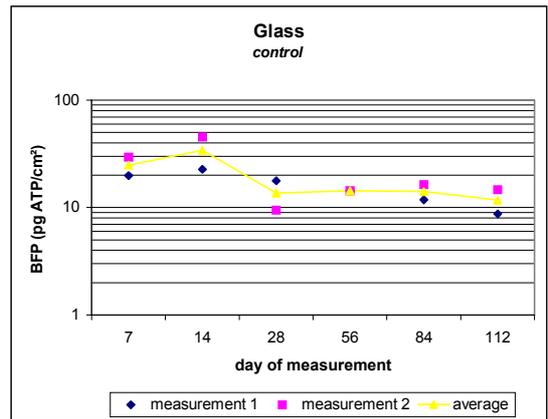
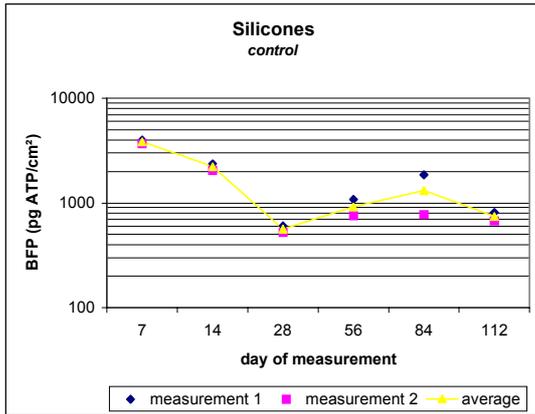
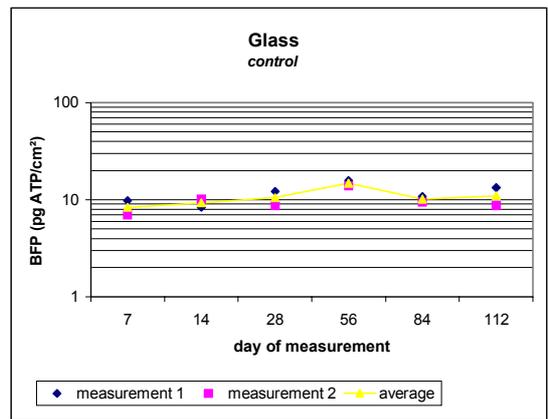
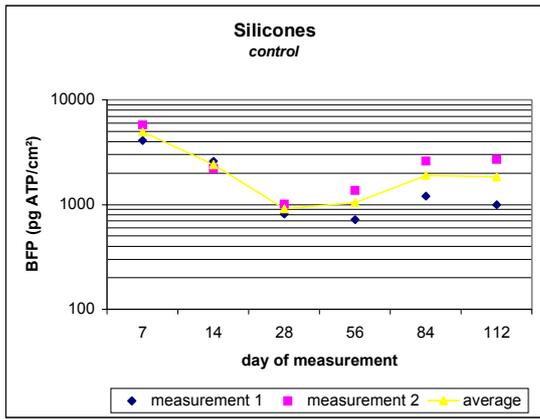
- VAN DER KOOIJ, D. AND VEENENDAAL, H.R. (1993)
 Biofilm development on surfaces in drinking water distribution systems. Proceedings 19th International Water Supply Congress. Oct 2-1993 Oct 8; SS 1:1.1-1.7
- VAN DER KOOIJ, D. AND VEENENDAAL, H.R. (1993)
 Assessment of biofilm formation potential of synthetic materials in contact with drinking water during distribution.
 Proceedings Water Quality Technology Conference, p. 1395-1407
- VAN DER KOOIJ, D, VROUWENVELDER, J.S., AND VEENENDAAL, H.R. (1997)
 Bepaling en betekenis van de biofilmvormende eigenschappen van drinkwater H₂O, 25, p. 767-771
- MEENHORST, P.L. (1984)
 1984. legionaire's disease. Some clinical, diagnostic and epidemiological aspects.
 Dissertatie. Leiden.
- NEN 6265 (1991)
 Bacteriologisch onderzoek van water. Onderzoek naar de aanwezigheid en het aantal kolonievormende eenheden (KVE) van *Legionella*-bacteriën.
 Nederlands Normalisatie Instituut, Delft (1^e druk)
- NEN 6550 (1979)
 Bacteriologisch onderzoek van water. Bepaling van het koloniegetal op glucose-gistextract bij 37 °C.
 Nederlands Normalisatie Instituut, Delft (1^e druk)
- NEN 6560 (1982)
 Bacteriologisch onderzoek van water. Bepaling van het koloniegetal op glucose-gistextract bij 22 °C.
 Nederlands Normalisatie Instituut, Delft (1^e druk)
- NEN 6573 (1987)
 Bacteriologisch onderzoek van water. Onderzoek met behulp van membraanfiltratie naar de aanwezigheid van het aantal kolonievormende eenheden (KVE) van *Pseudomonas aeruginosa*.
 Nederlands Normalisatie Instituut, Delft (1^e druk)
- NEN 6553 (1981)
 Bacteriologisch onderzoek van drinkwater. Onderzoek met behulp van membraanfiltratie naar de aanwezigheid van bacteriën van de coligroep.
 Nederlands Normalisatie Instituut, Delft (1^e druk)
- NIEDEVELD, COR.J., PET, FRED M., AND MEENHORST, PIETER L. (1986)
 Effect of rubbers and their constituents on proliferation of *Legionella pneumophila* in naturally contaminated hot water. The Lancet. July 26; 180-184
- ROGERS, JULIE, DOWSETT, A.B. DENNIS, P.J. LEE, J.V. AND KEEVIL, C.W. (1994)
 Influence of Plumbing Materials on Biofilm Formation and Growth of *Legionella pneumophila* in Potable Water Systems. Applied and Environmental Microbiology.; 60 (6): 1842-1851
- SHULZE-RÖBBECKE, R. UND C. HAGENAU
 Verhalten von Mikroorganismen und Viren beid der trinkwasseraufbereitung.
 Mykobakterien.
 DVGW-Schriftenreihe Wasser nr 110, p. 191-202
- SLAATS, P.G.G. (1996)
 Vergelijking materialen binnenleidingen
 Kiwa-rapport KOA 96.097
- VEWIN (1992)
 Statistisch onderzoek 1992
- VROUWENVELDER, J.S., en VAN DER KOOIJ, D.,
 Samenstelling en concentratie van biofilm op leidingwanden van distributiesystemen in Nederland. Kiwa-rapport 1999 (In voorbereiding).
- WATERLEIDINGBESLUIT (1984)

ANNEX 1

Individual measurement values of the biofilm concentration on the materials

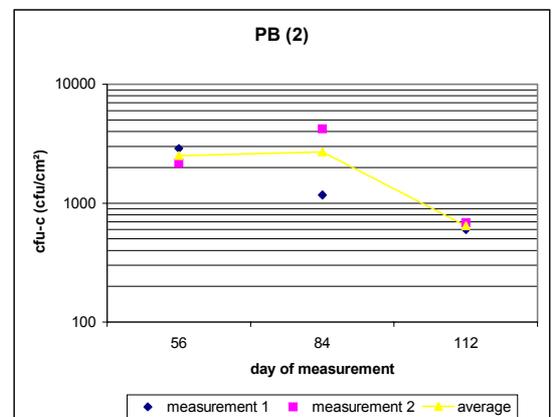
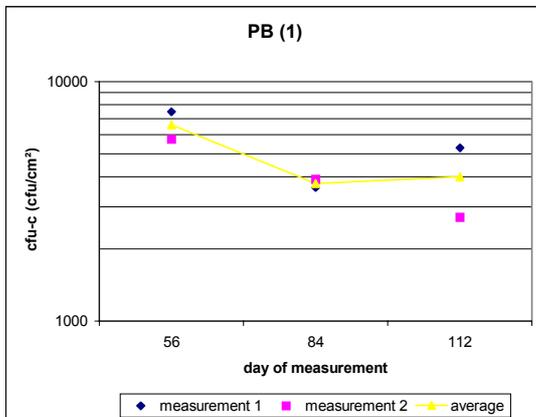
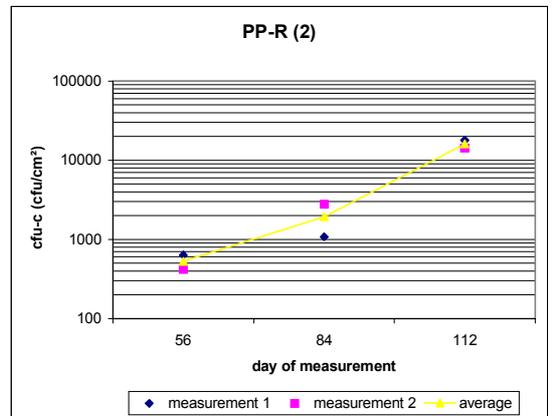
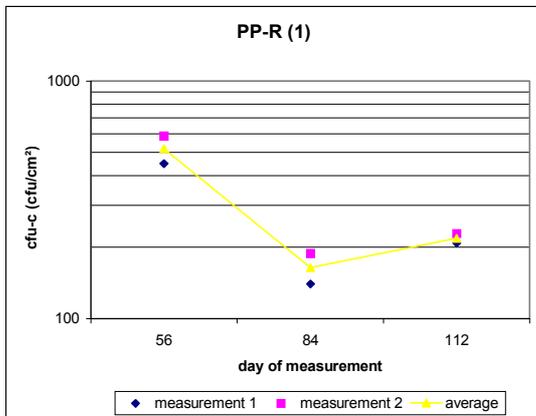
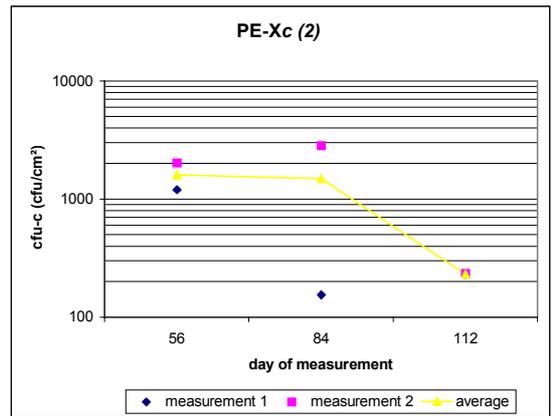
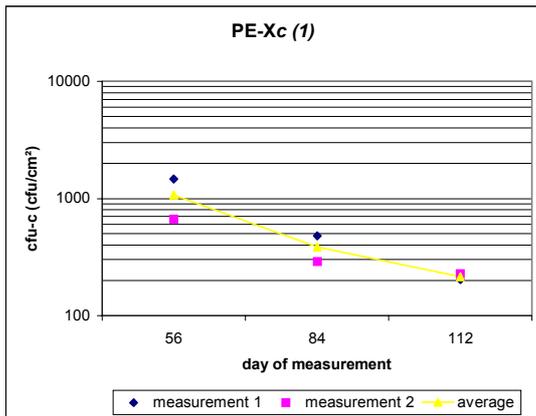
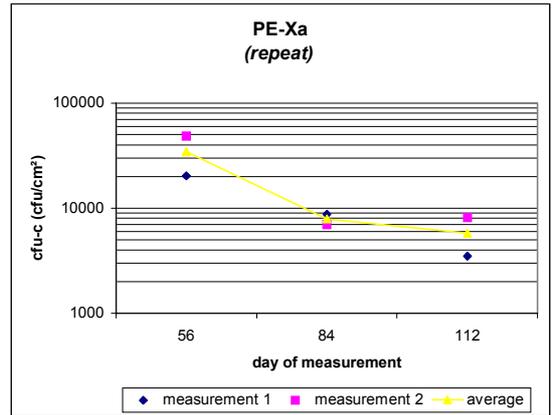
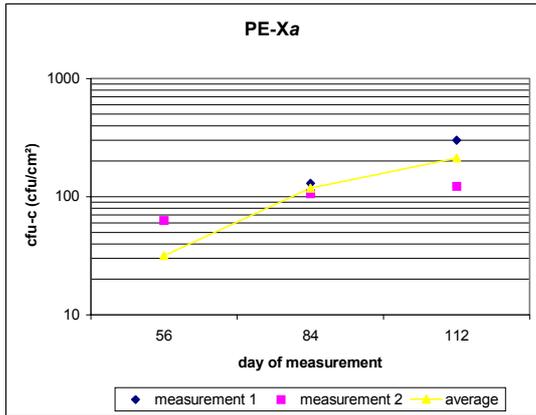


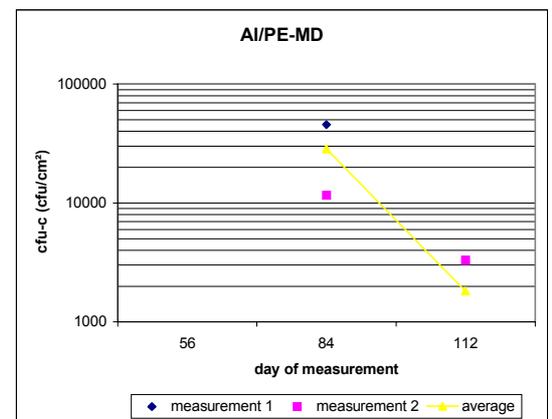
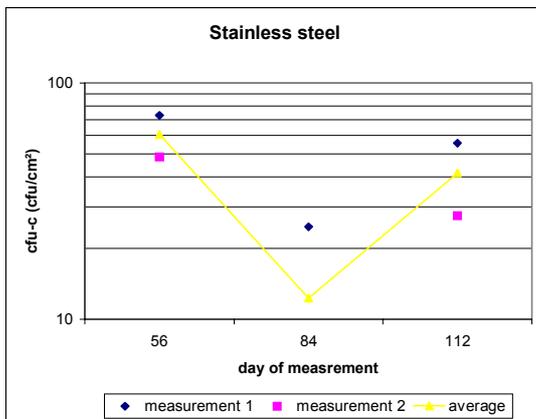
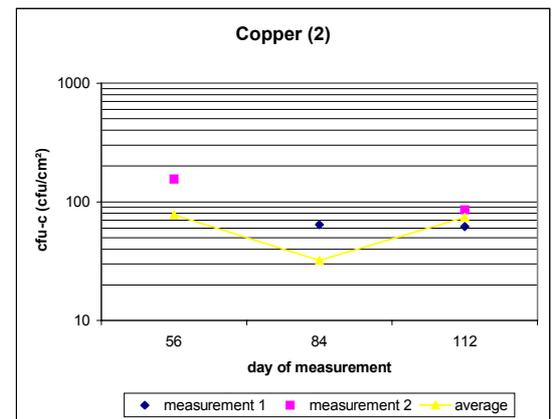
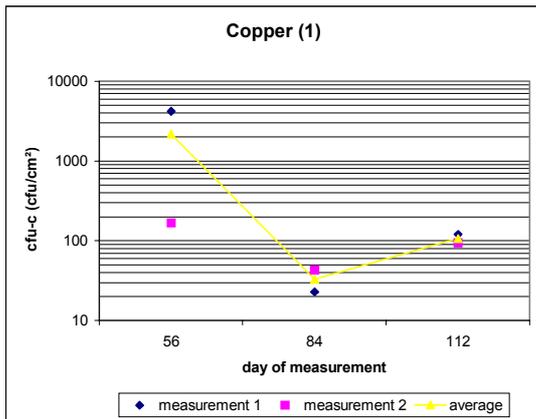
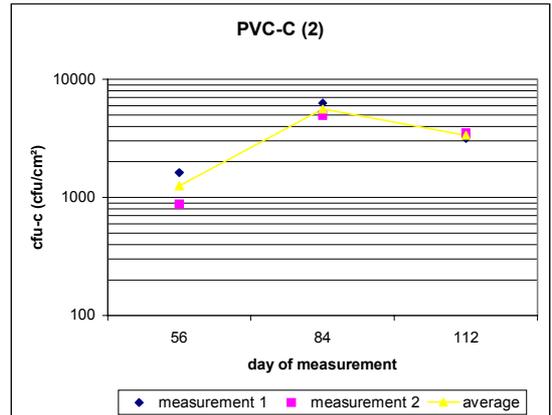
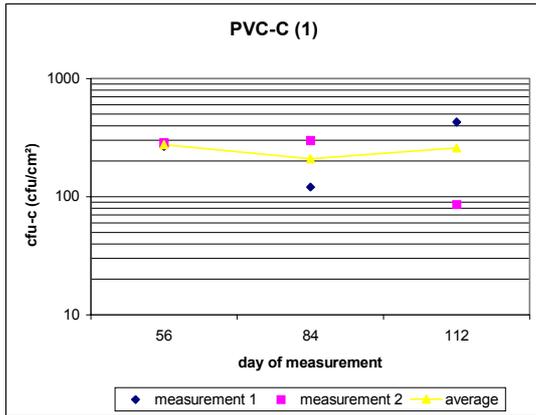


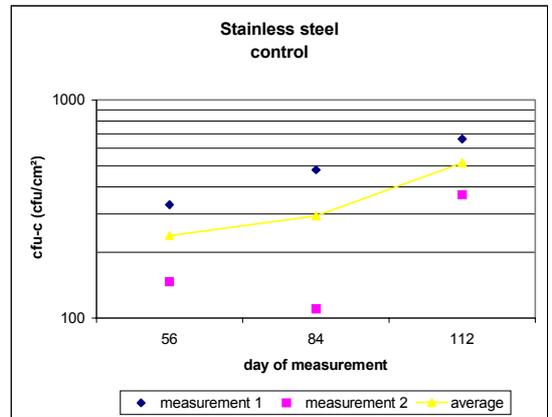
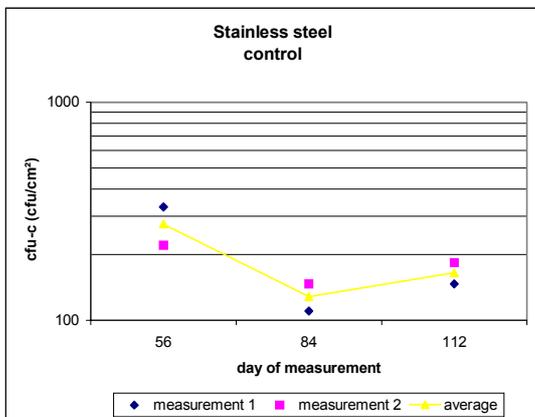
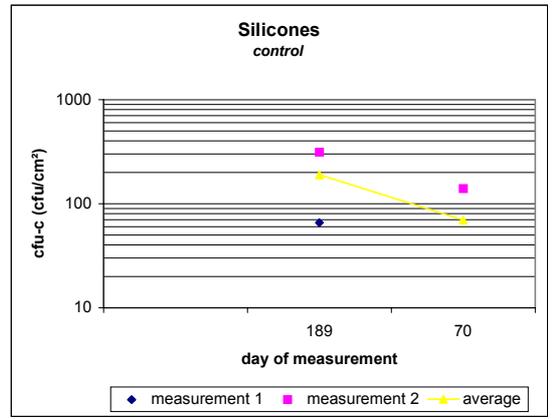
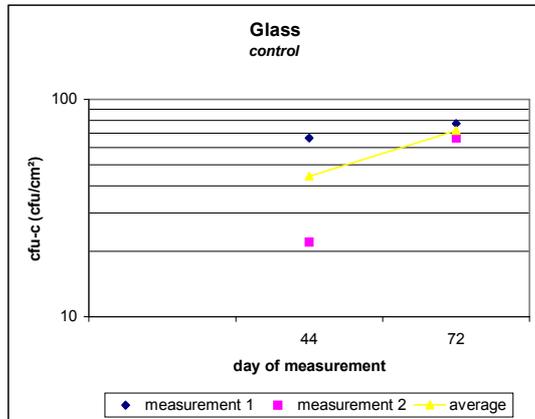
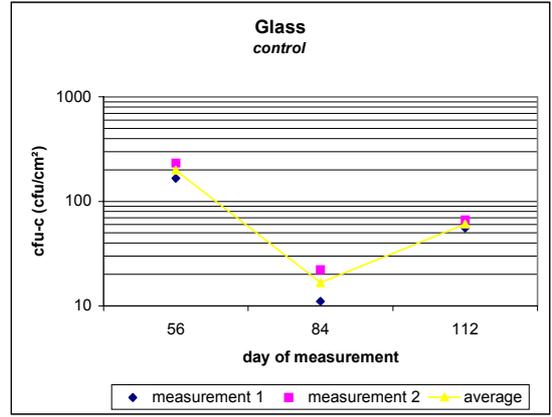
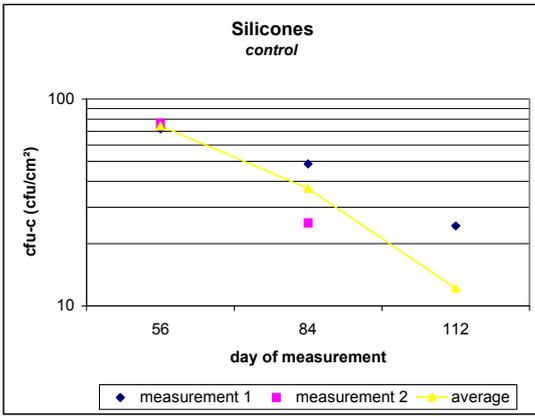
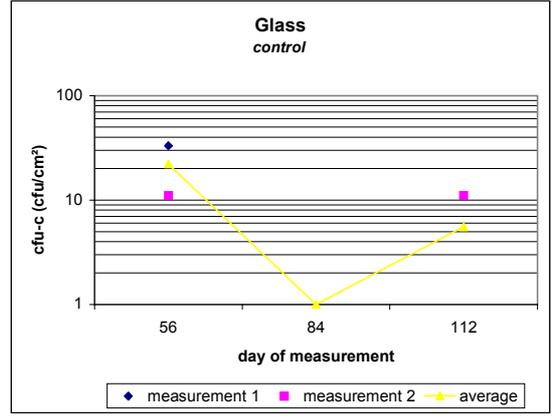
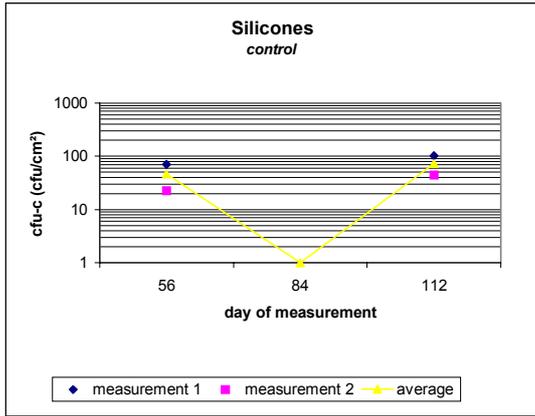


ANNEX 2

Individual measurement values of the *Legionella* concentration on the materials

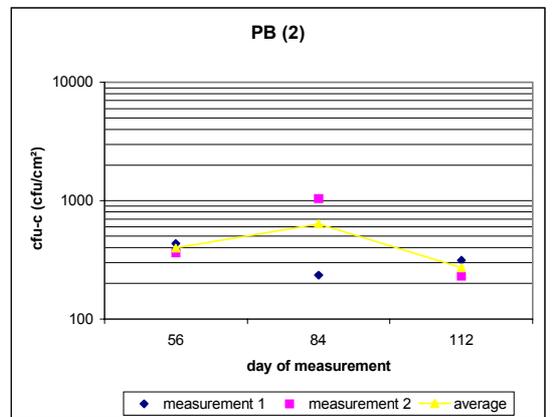
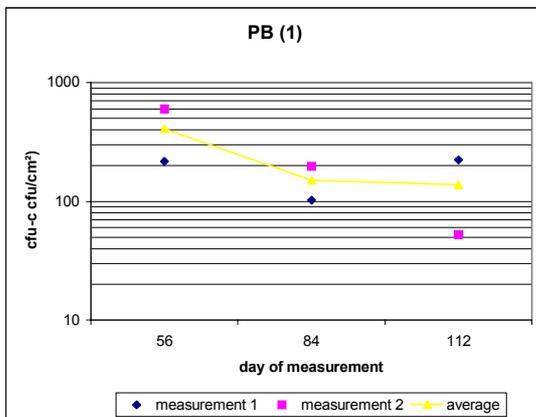
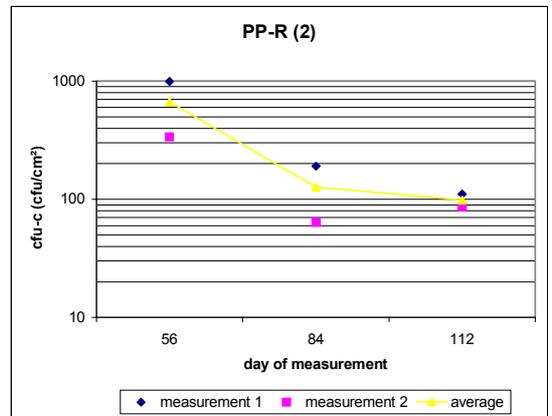
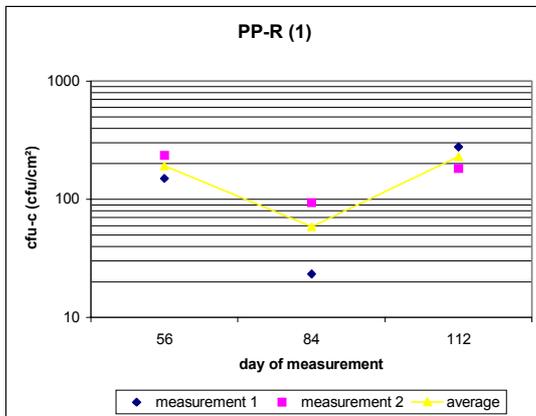
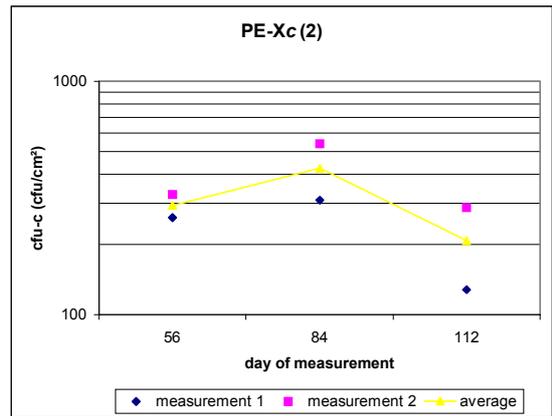
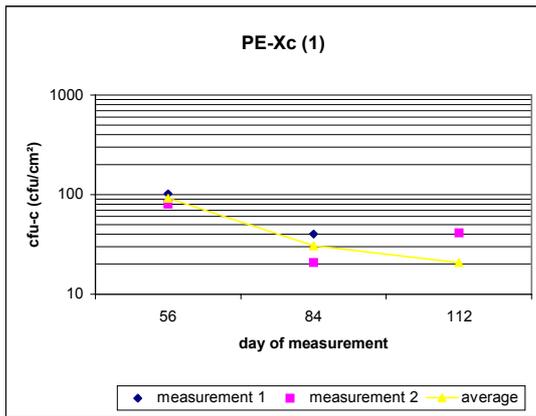
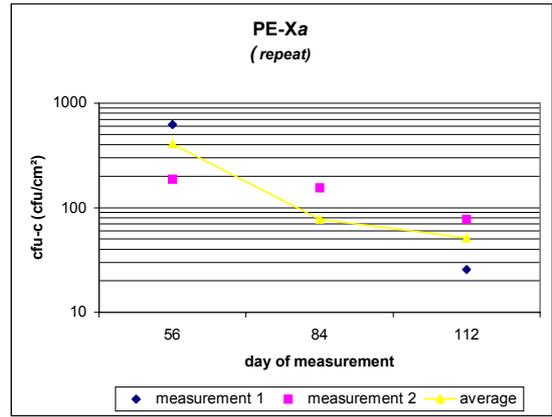
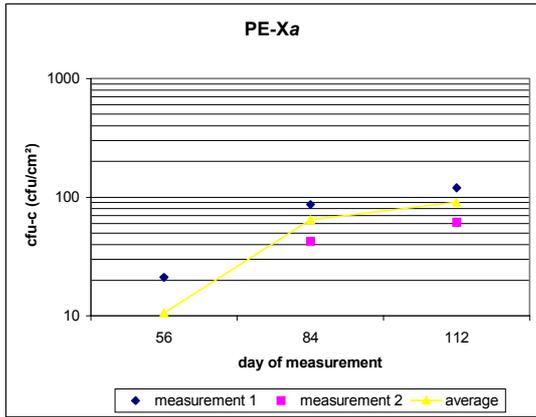


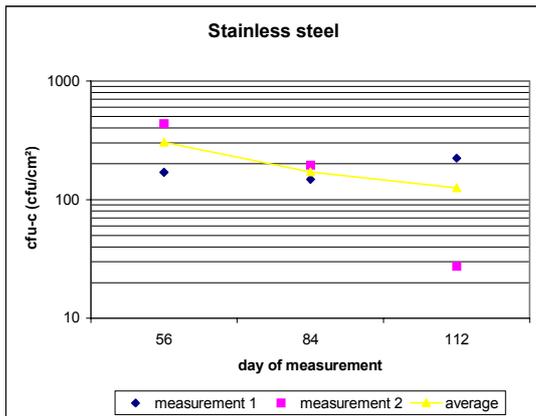
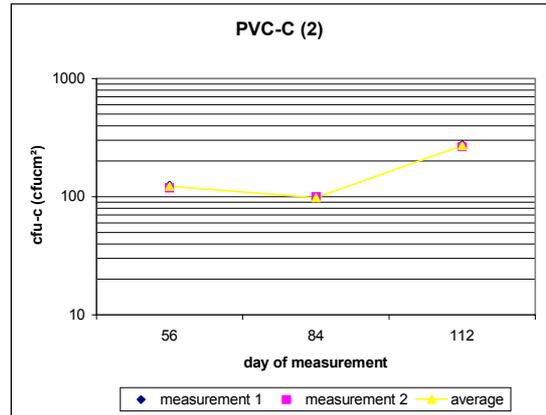
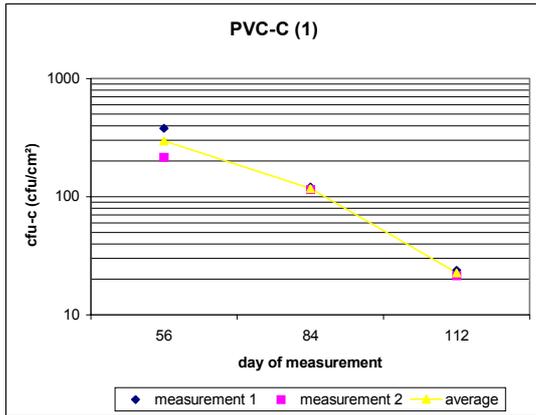


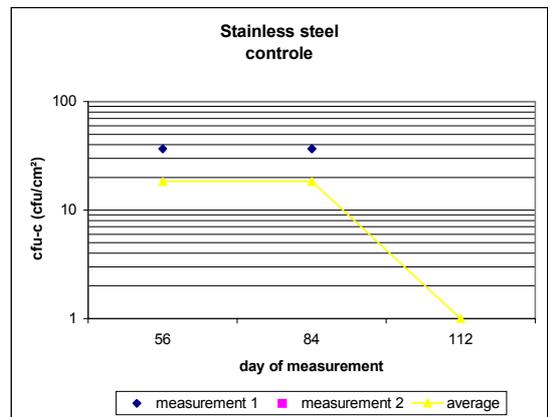
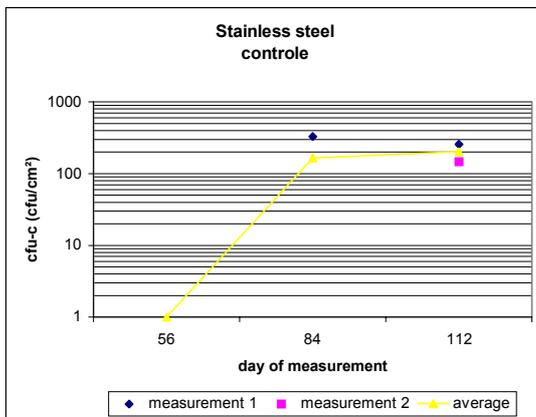
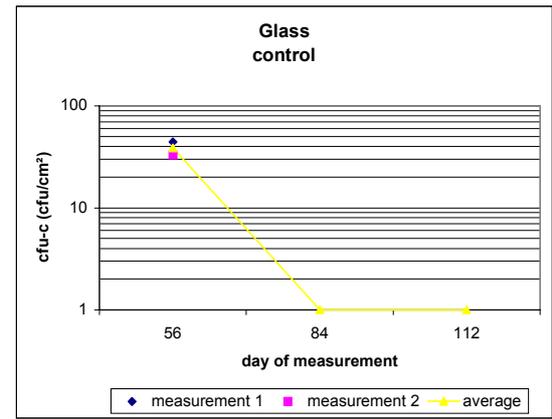
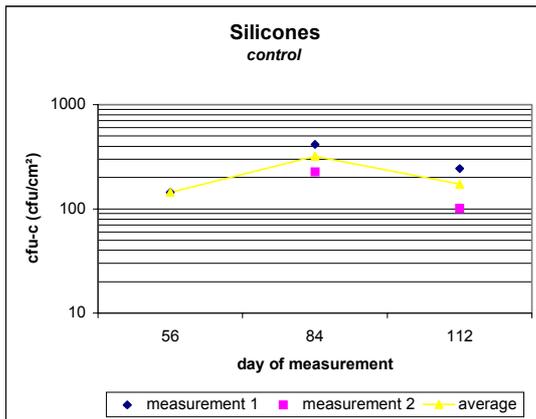
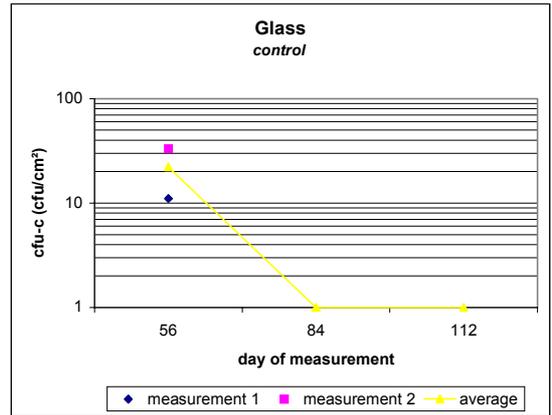
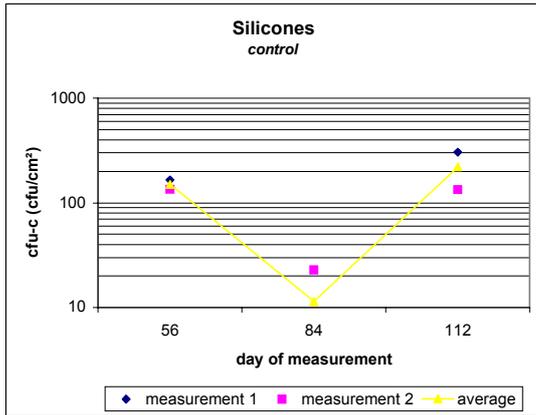


ANNEX 3

Individual measurement values of the *Pseudomonas* concentration on the materials

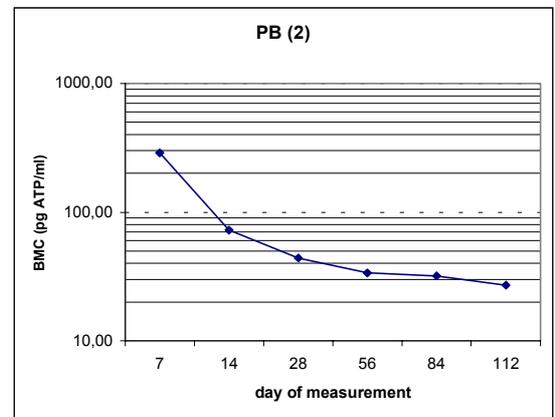
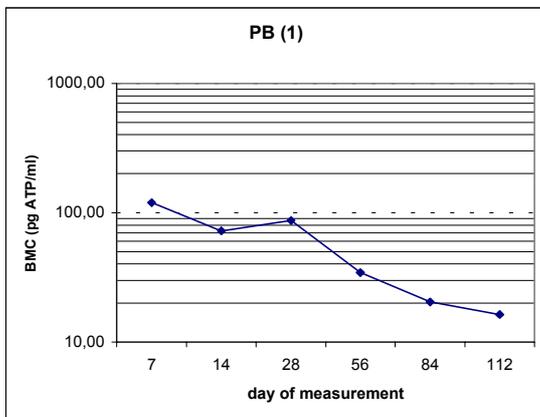
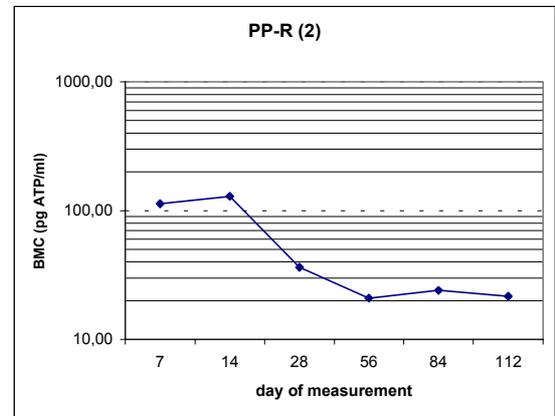
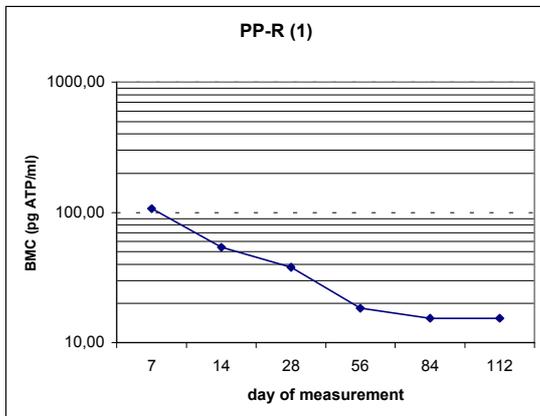
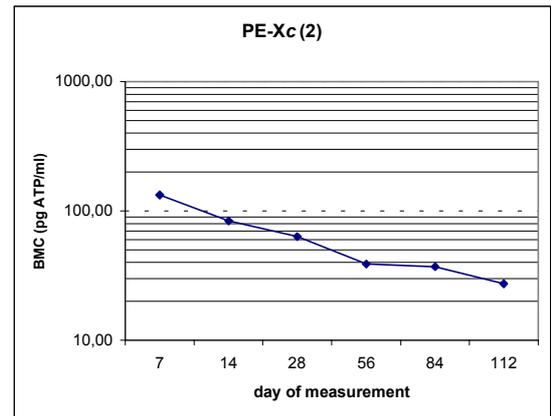
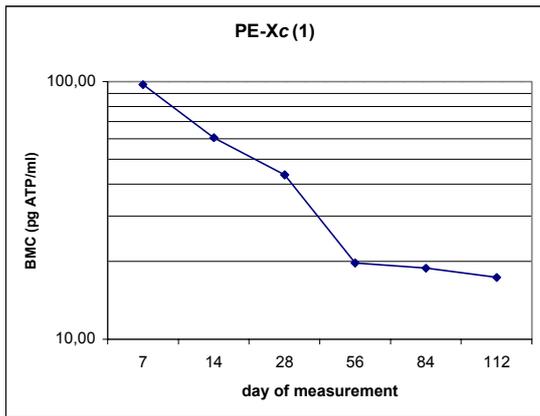
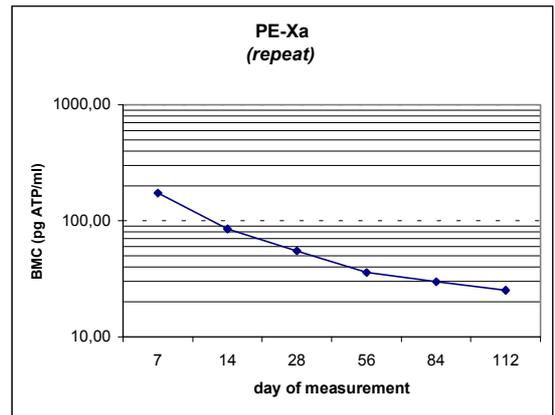
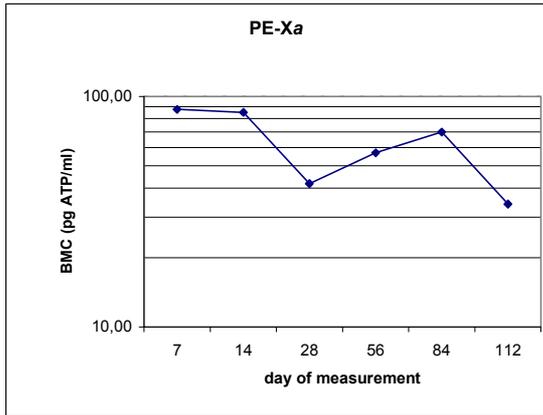


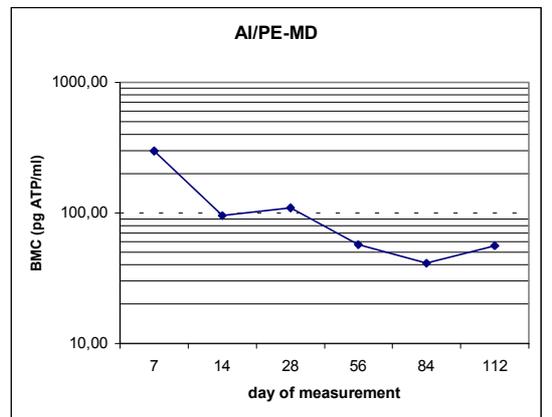
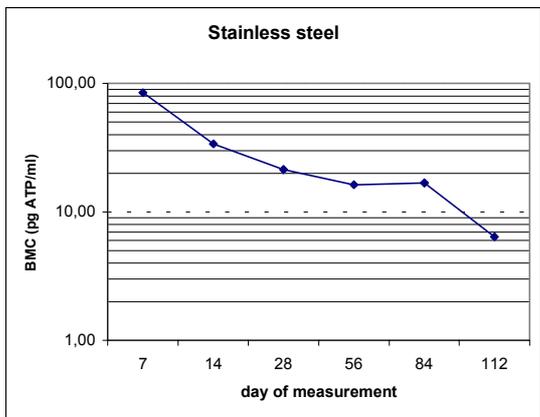
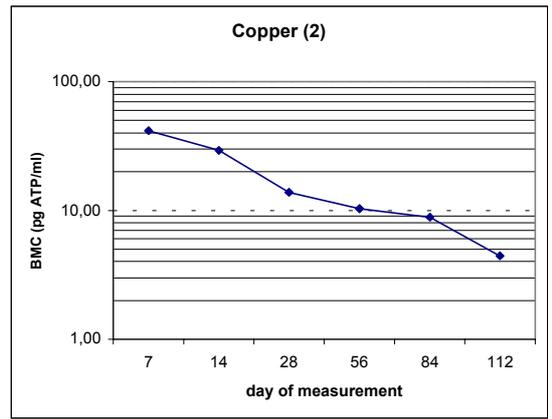
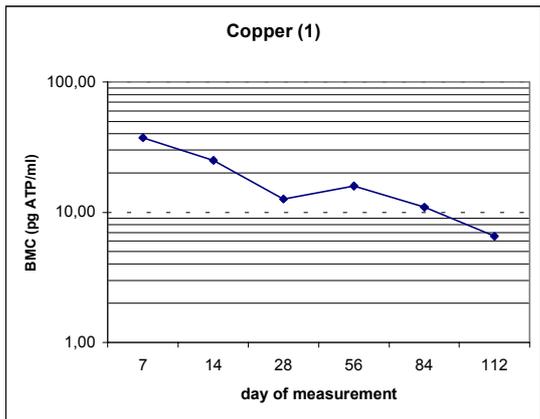
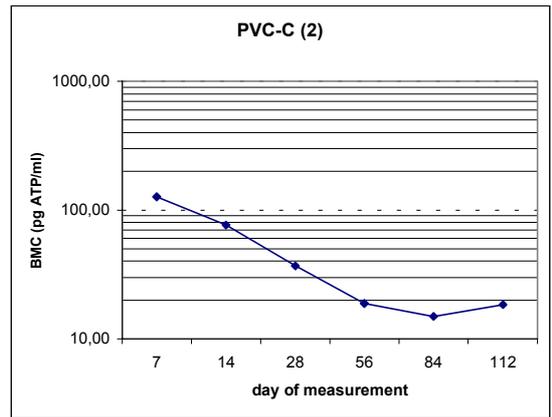
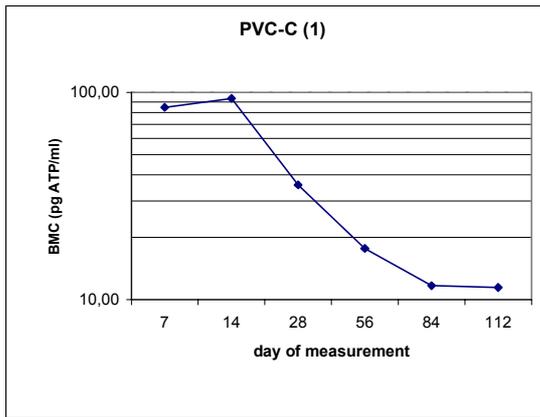


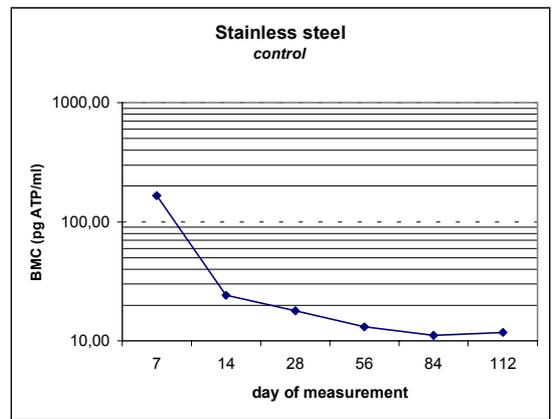
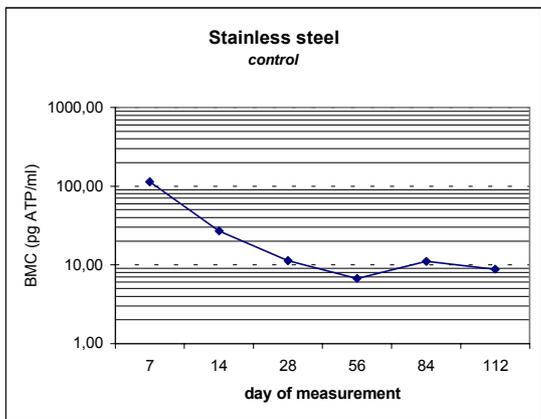
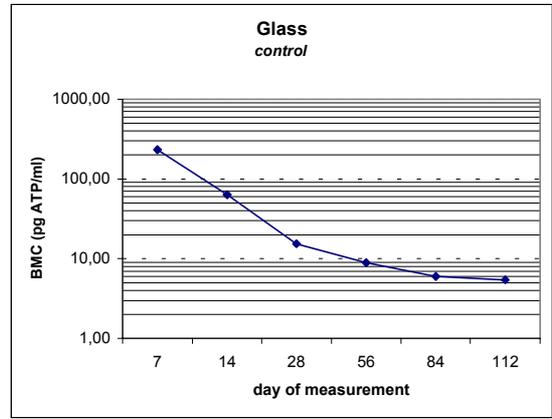
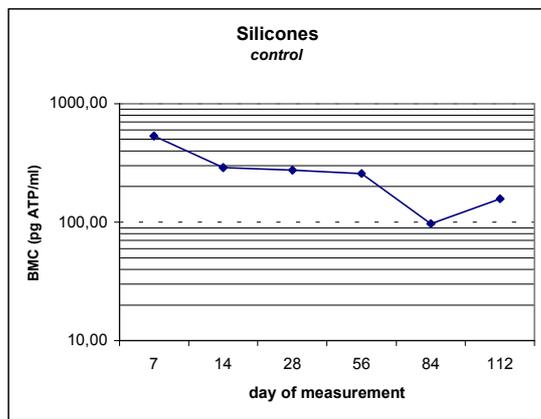
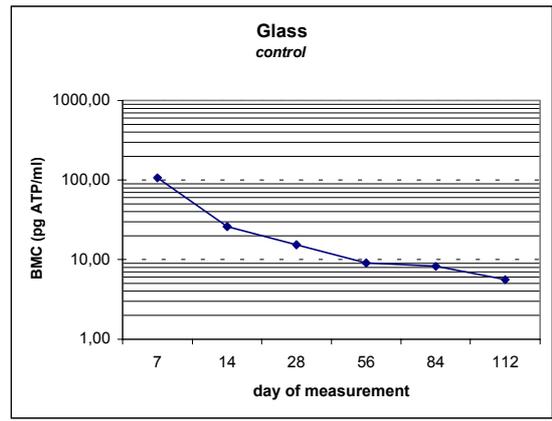
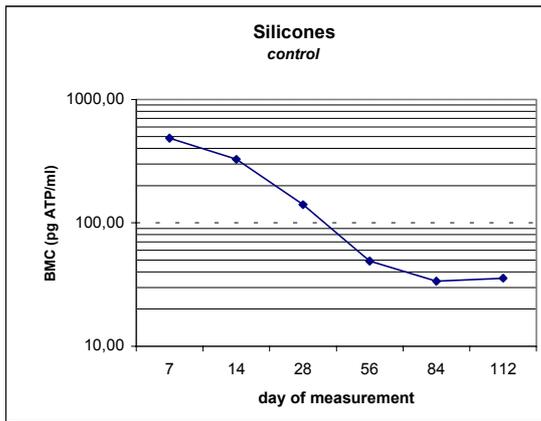
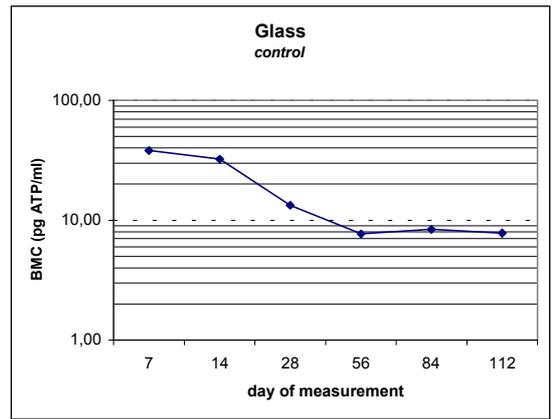
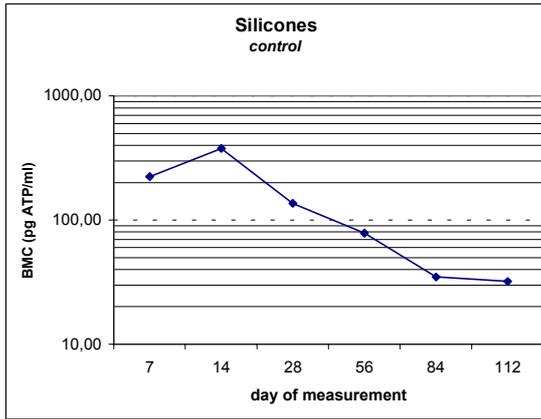


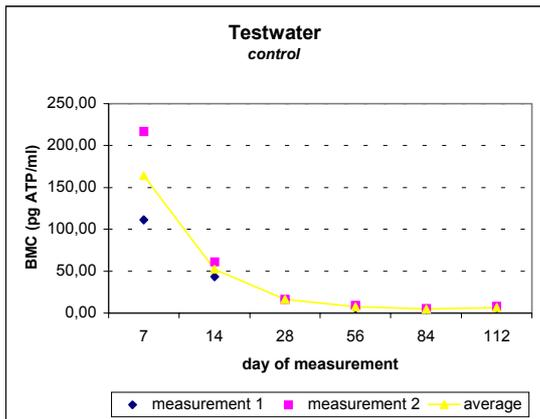
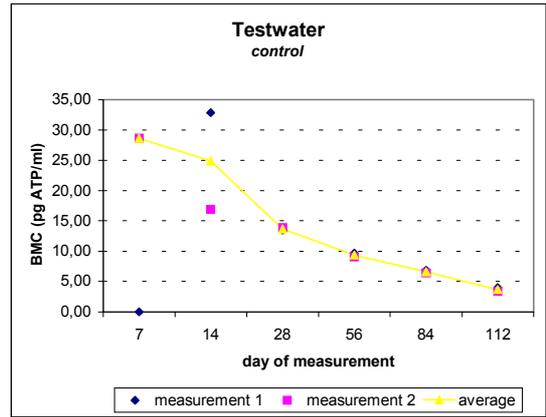
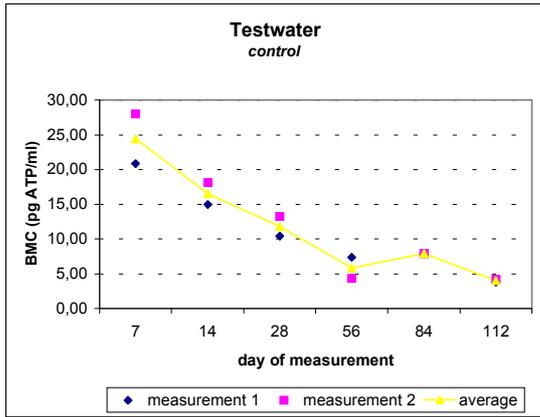
ANNEX 4

Individual measurement values of the biomass concentrations in the water



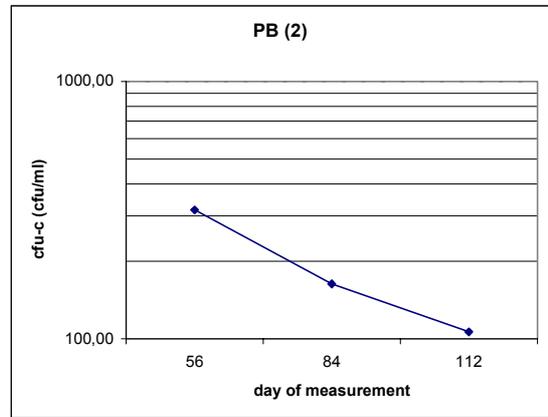
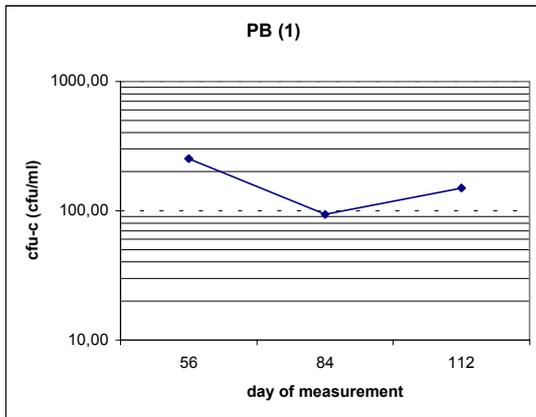
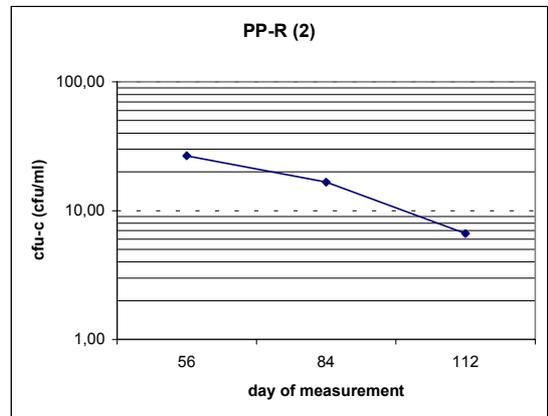
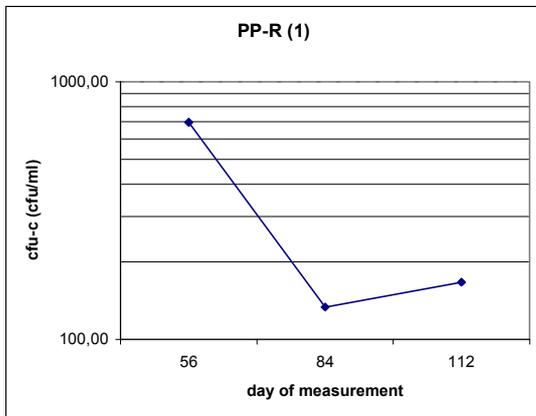
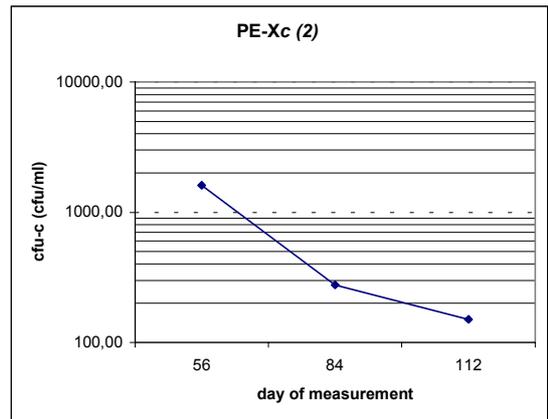
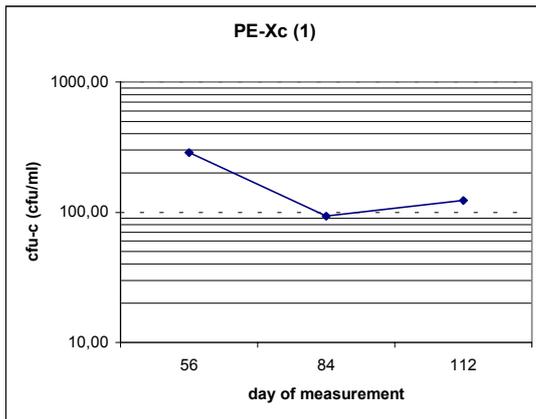
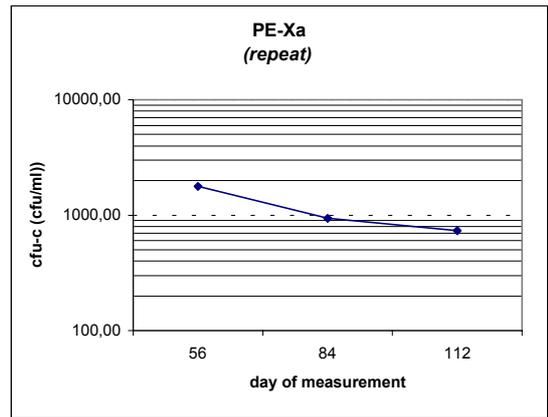
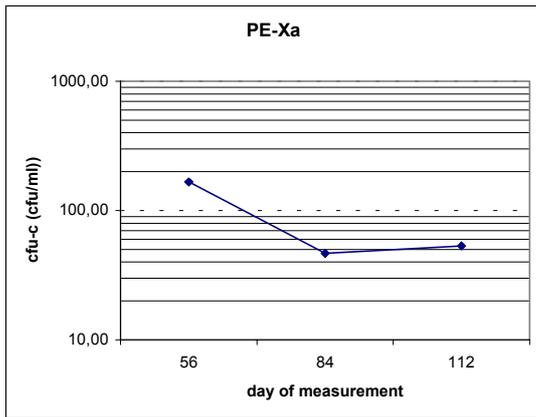


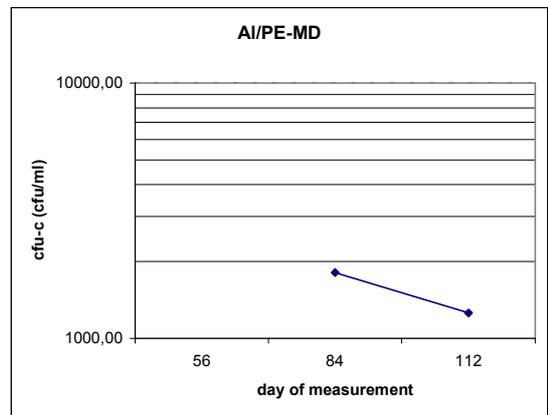
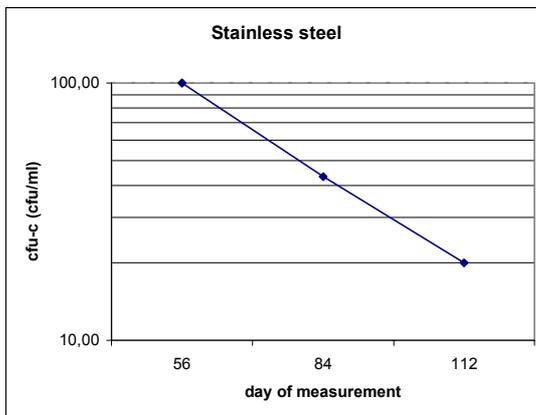
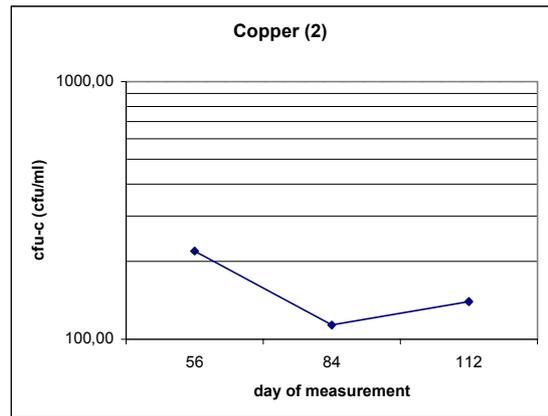
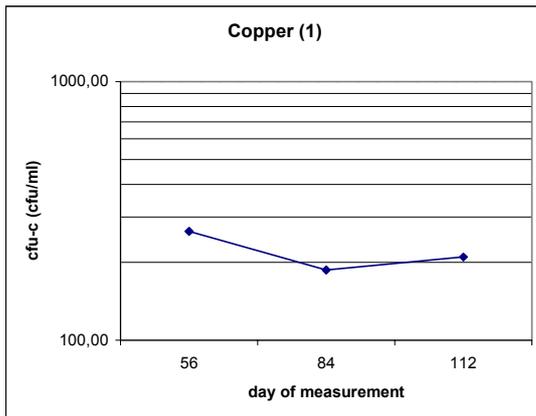
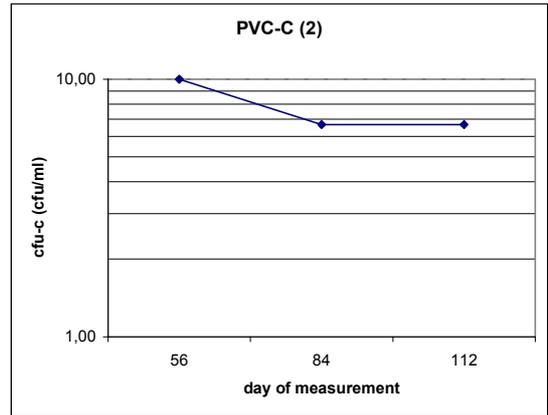
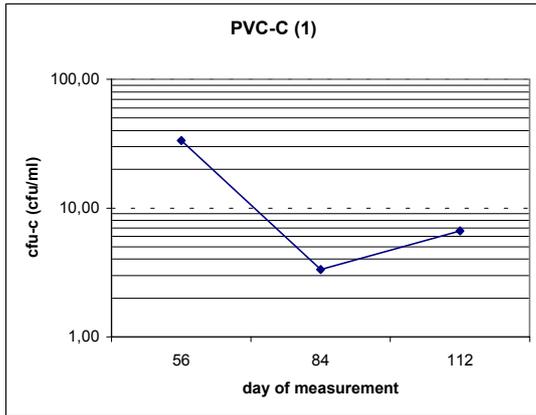


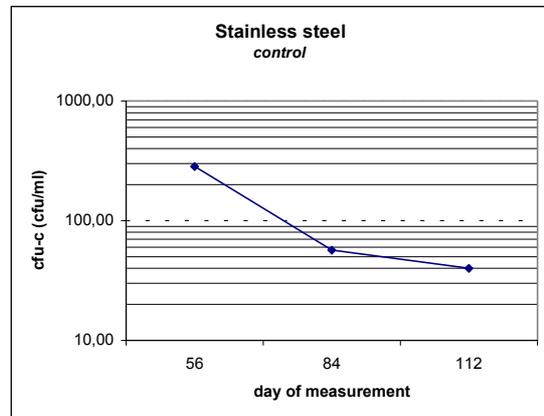
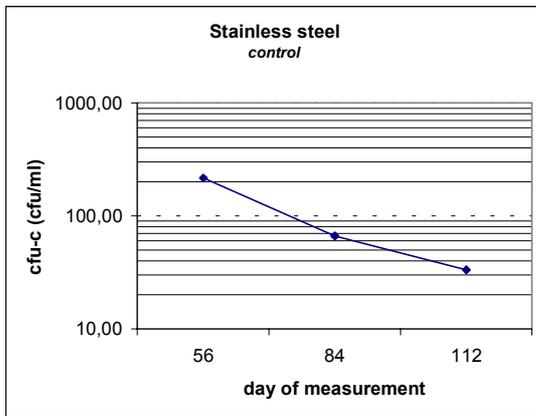
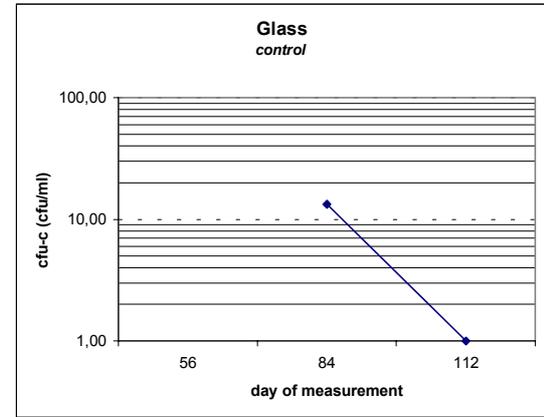
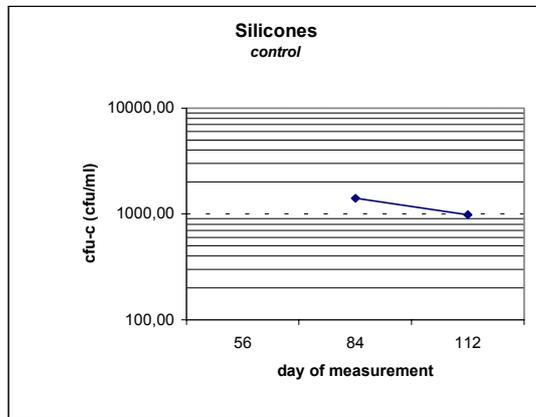
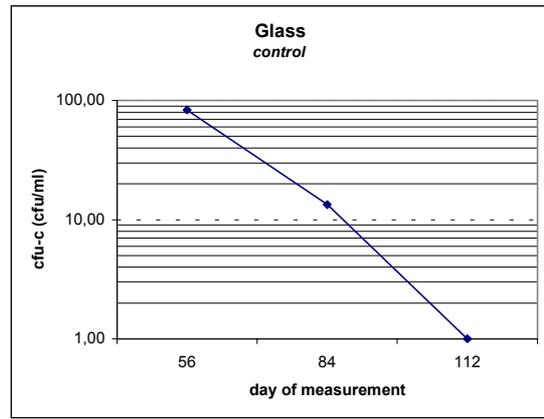
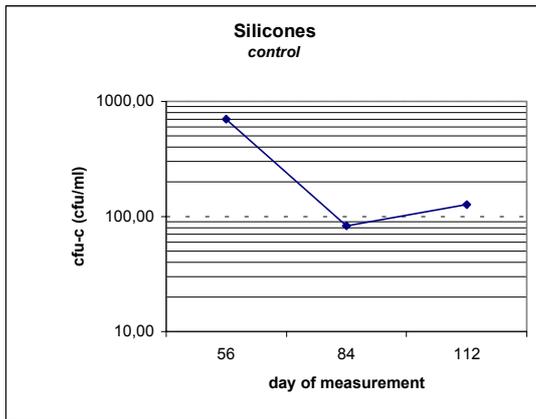
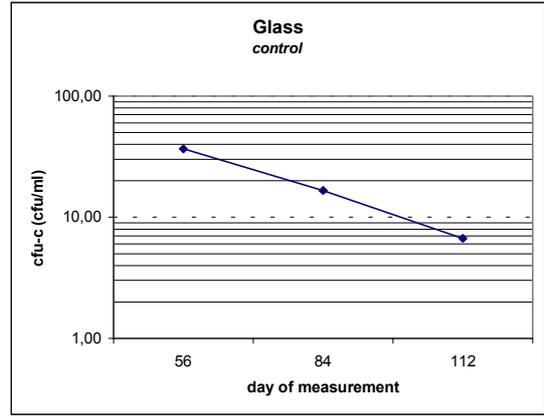
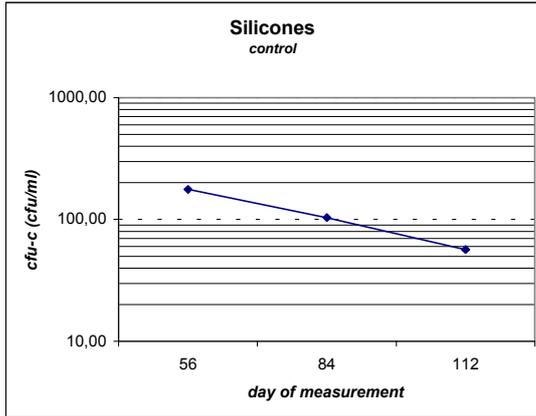


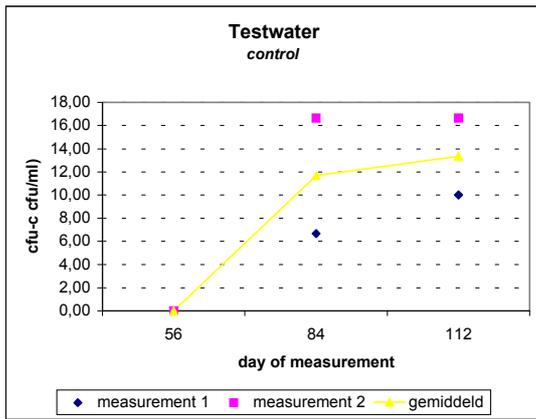
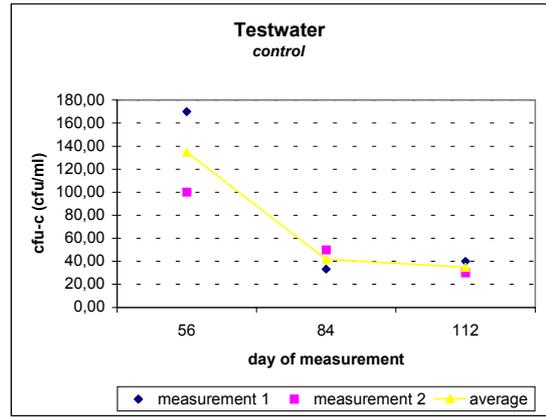
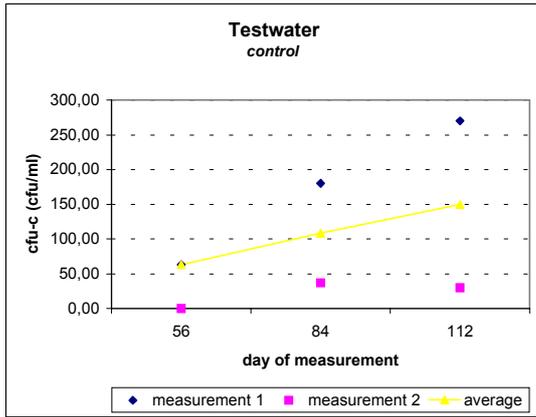
ANNEX 5

Individual measurement values of the *Legionella* in the test water



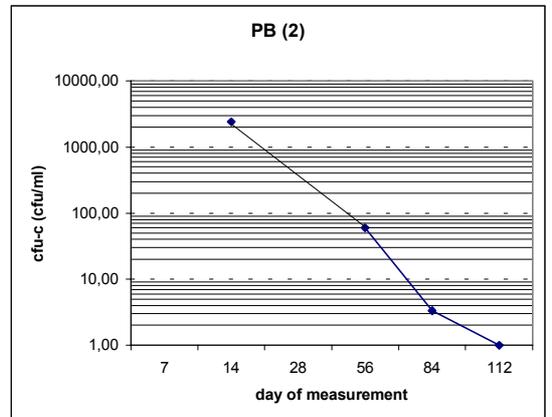
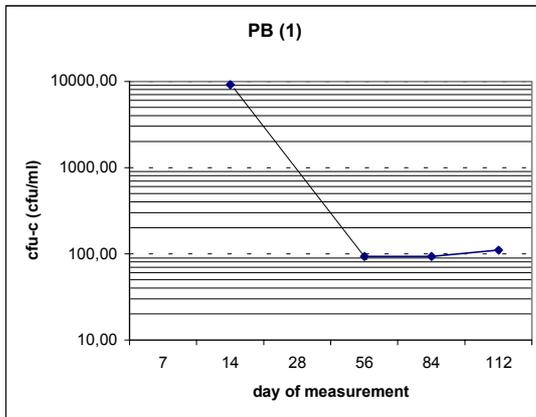
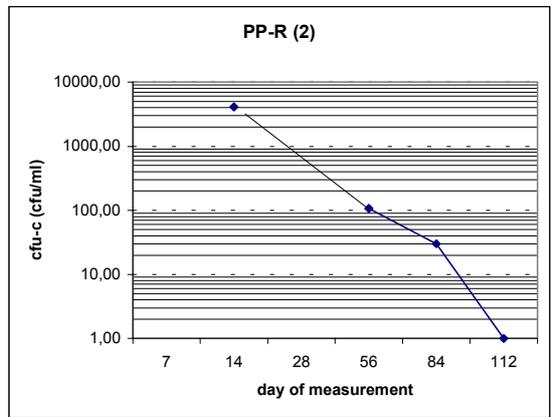
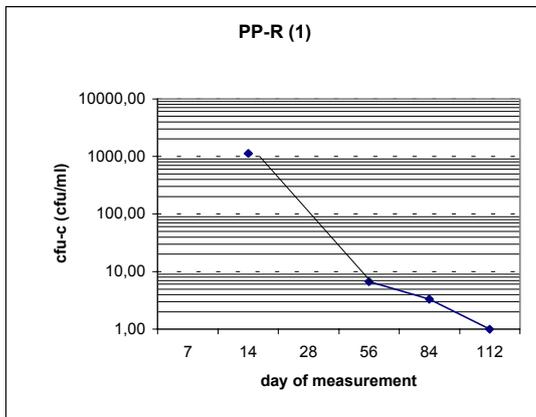
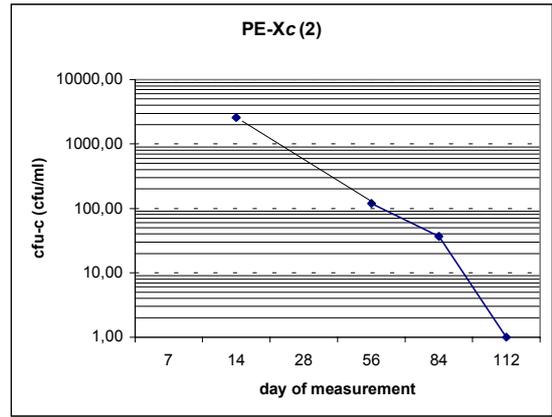
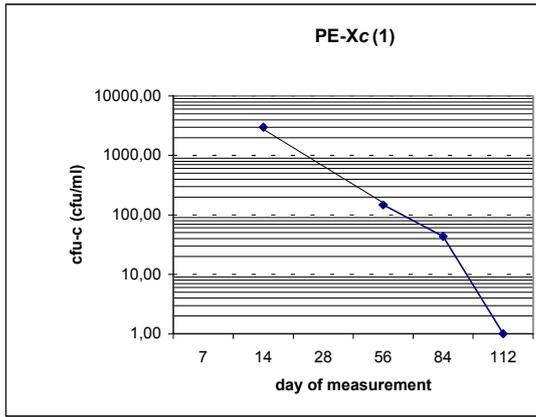
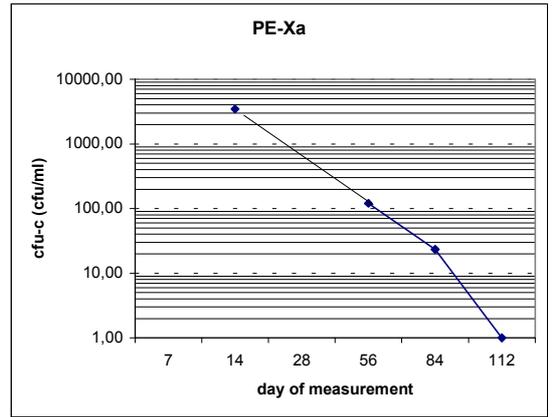
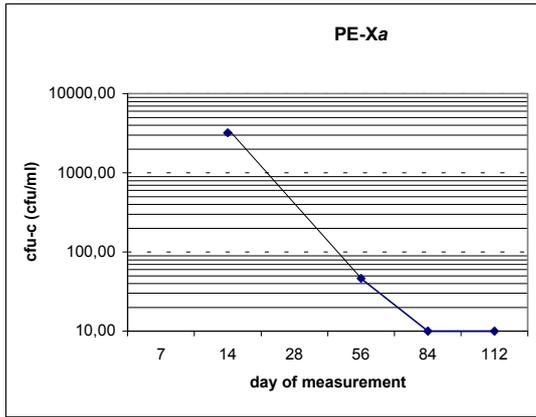


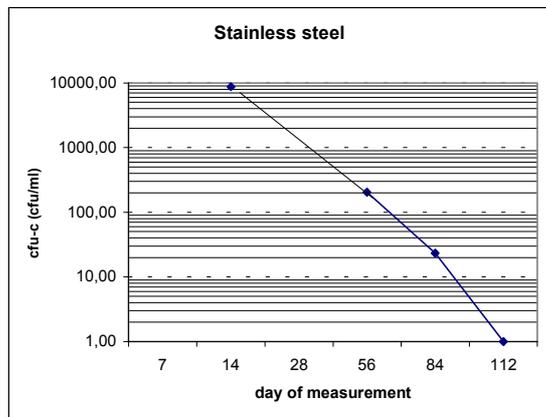
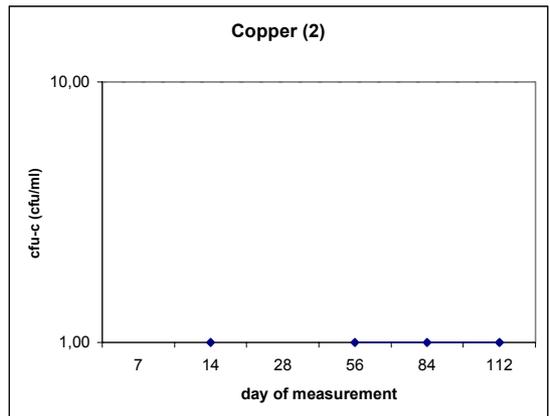
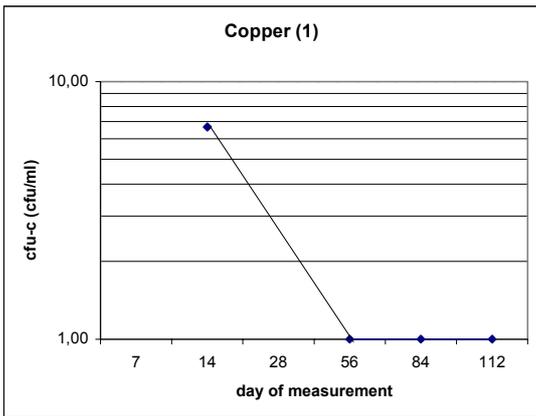
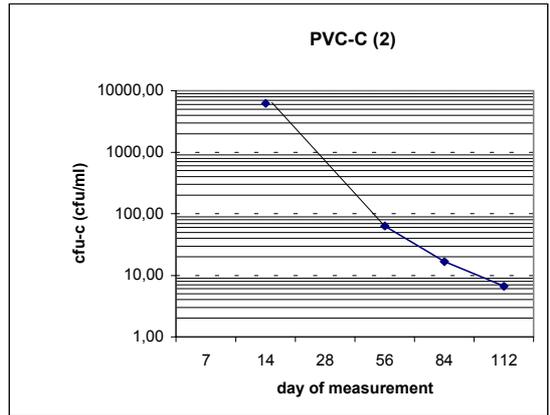
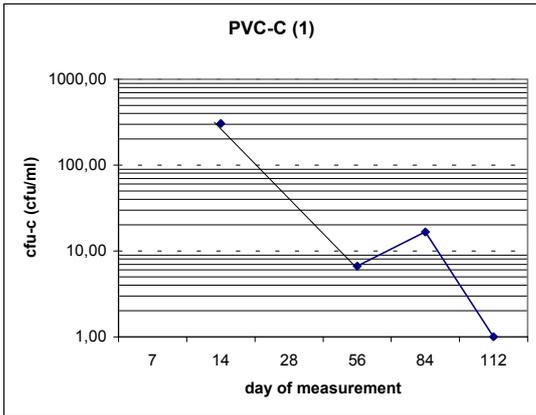


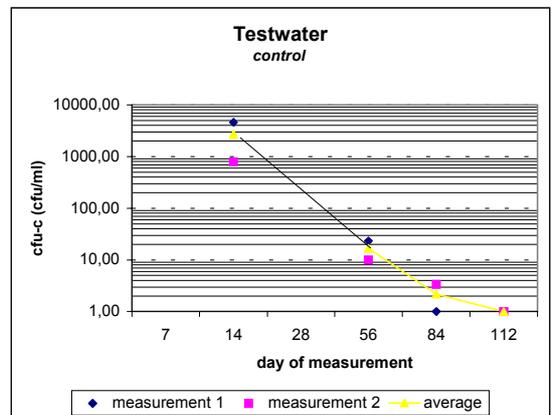
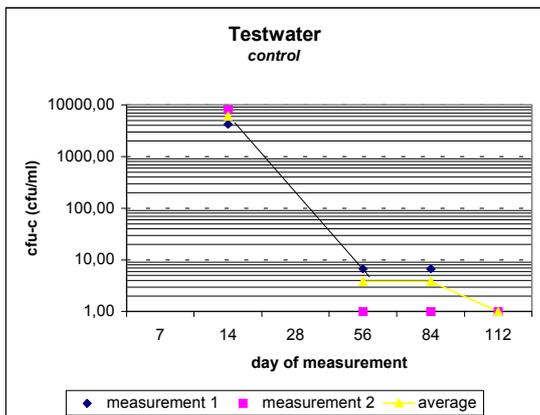
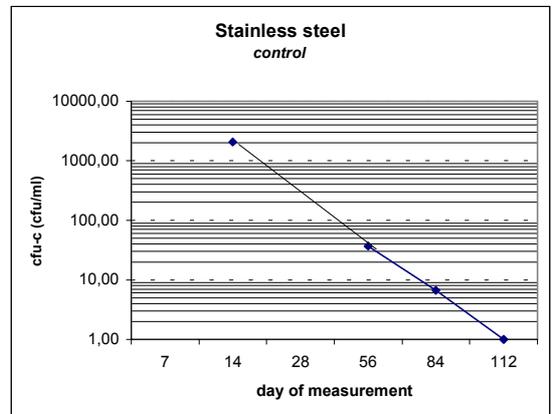
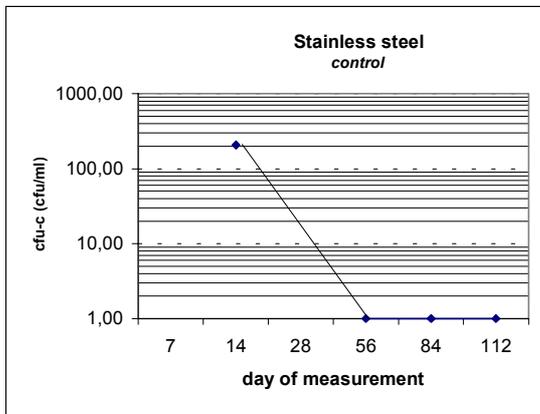
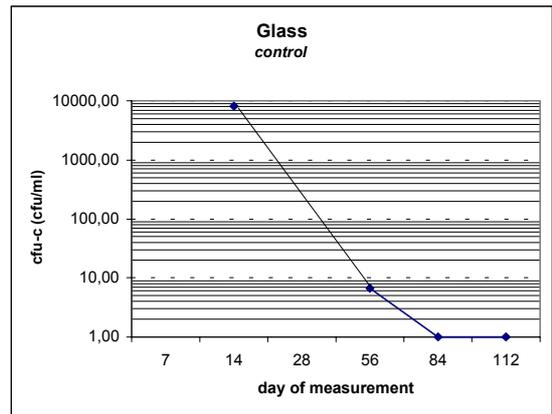
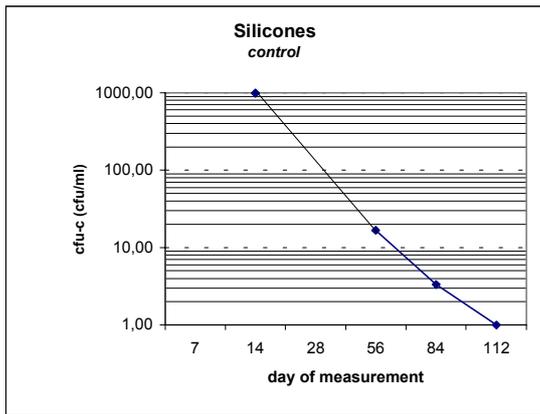
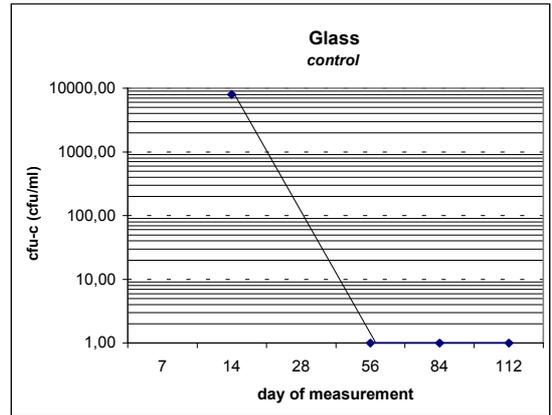
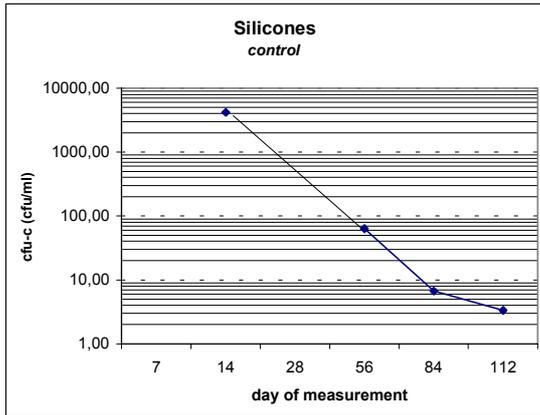


ANNEX 6

Individual measurement values of the *Pseudomonas* concentration in the test water

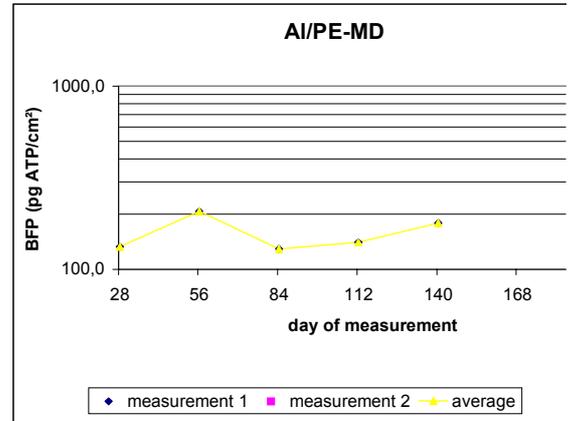
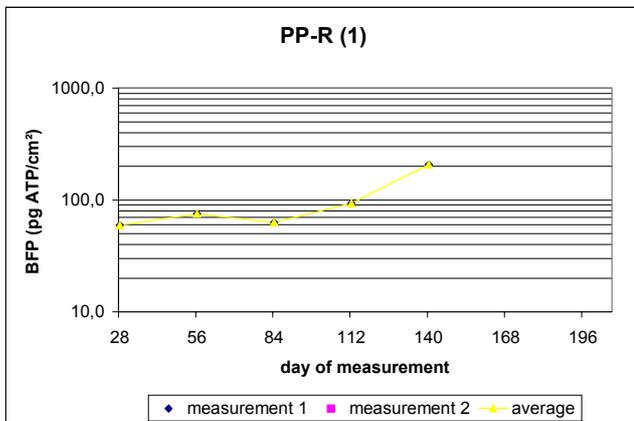
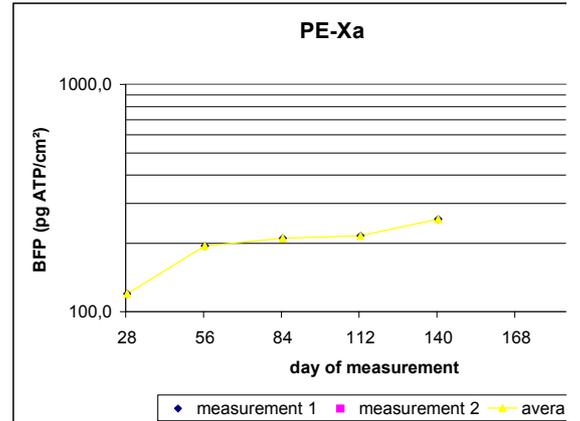
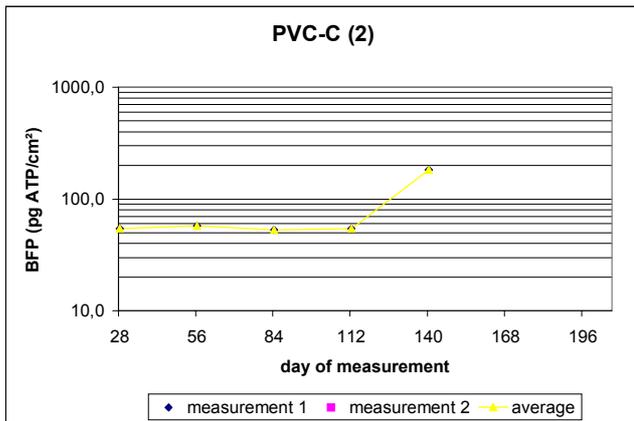
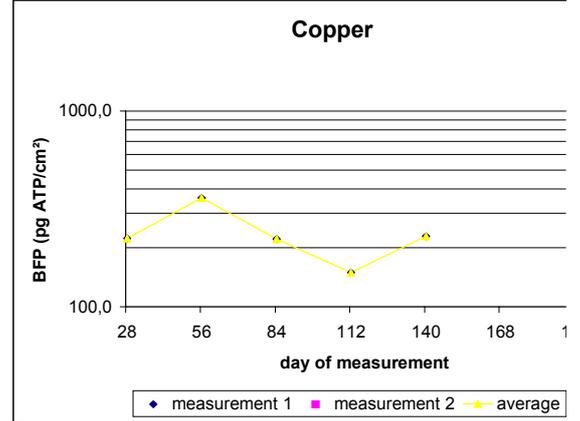
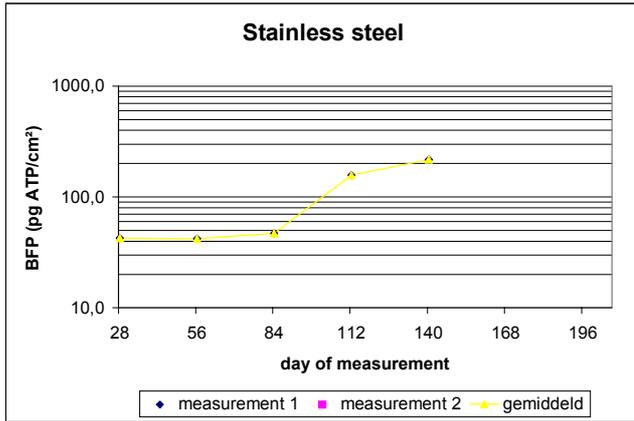




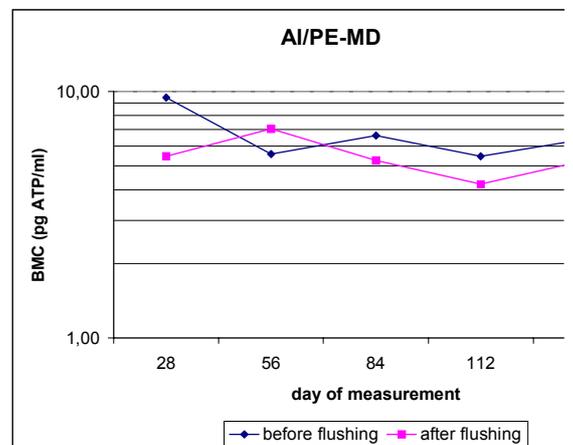
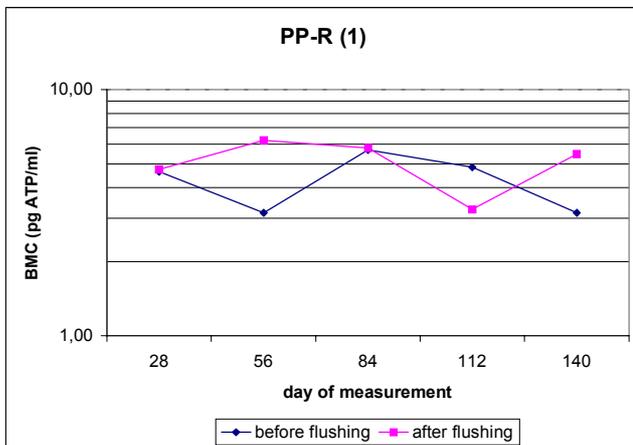
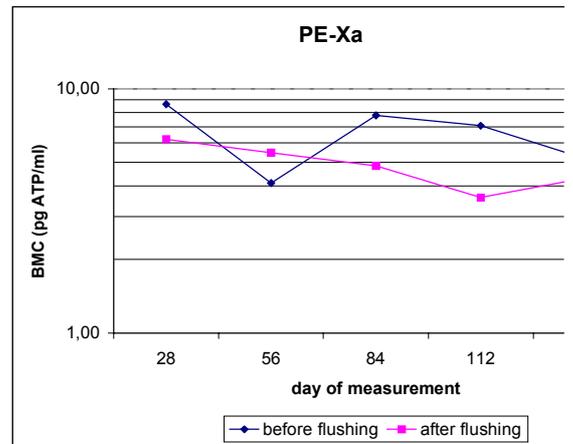
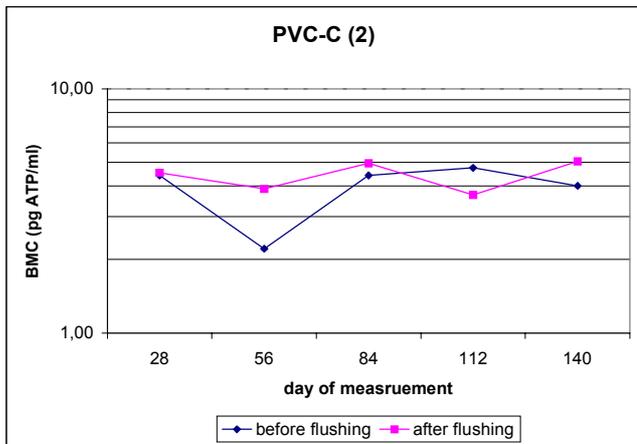
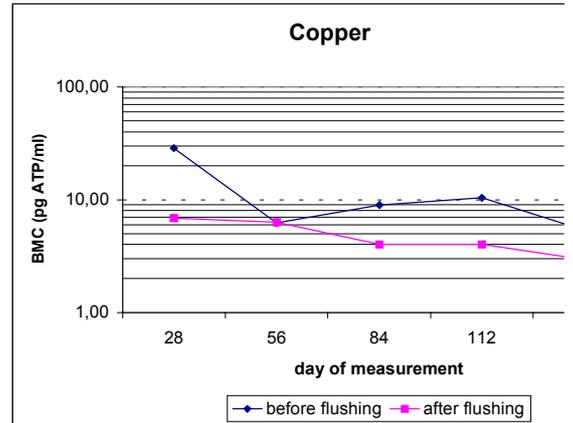
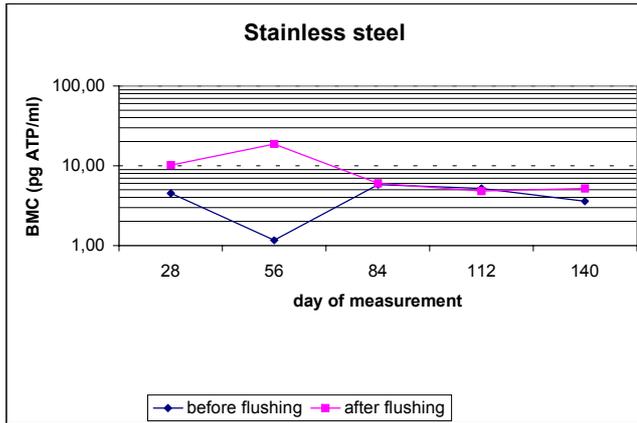


ANNEX 7

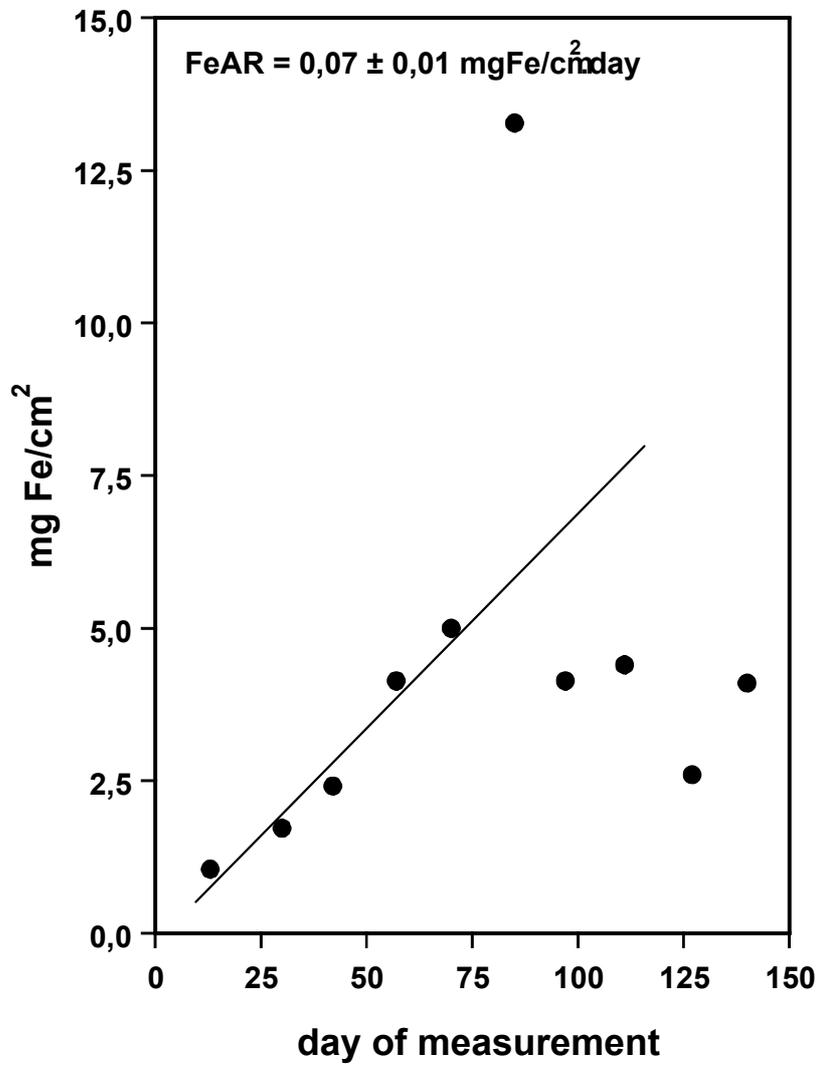
Biofilm on the inner wall of pipes (ATP-data)



ANNEX 8
Measurement values for water quality (ATP-measurements)

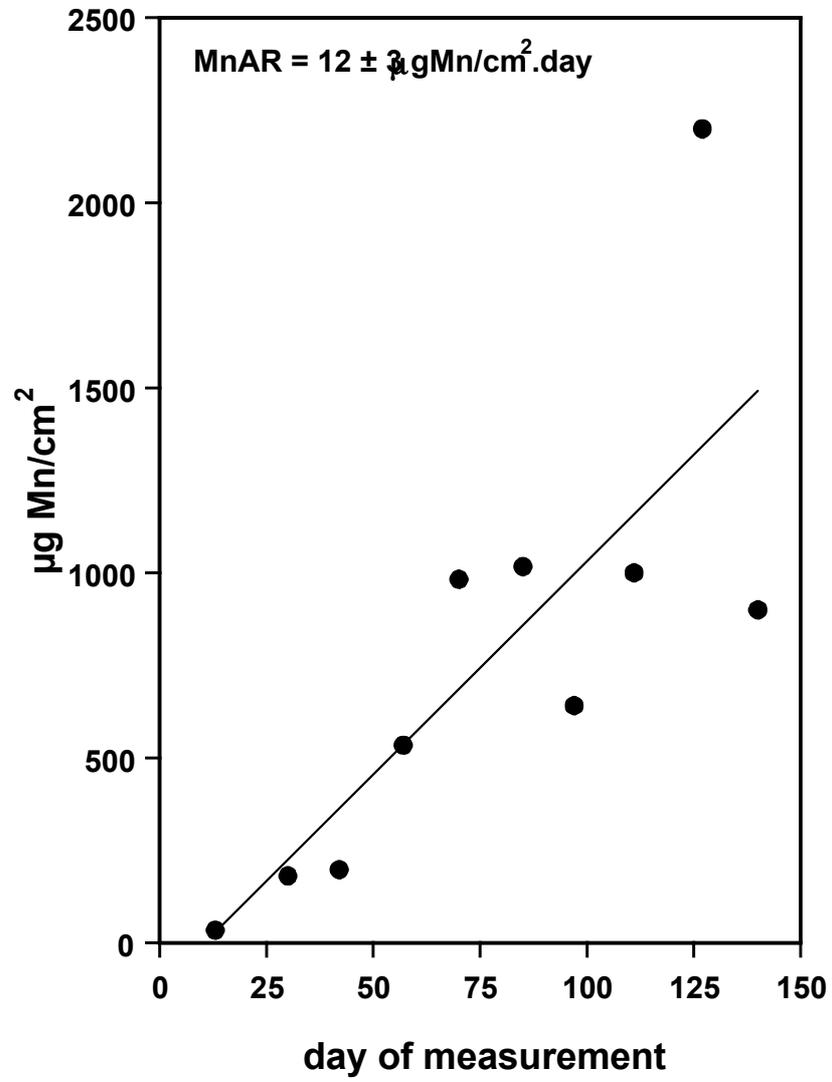


ANNEX 9
Rate of iron deposit formation in water



ANNEX 10

Manganese accumulation in the biofilm monitor



ANNEX 11

Prescription for determining the biofilm formation potential (LMB-006)

Kiwa In-house regulation

Title

Determination of the biofilm formation potential of materials which come into contact with drinking water

Number	Version	date	page	authorisation initials
LMB-006	2	7 January 1999	1 of 11	

1 Subject

This regulation describes the determination of the degree to which biofilm is formed on materials in test water in the presence of the micro-organisms naturally to be found in drinking water in combination with a river water inoculation as a result of the release of growth-promoting compounds by the materials in question.

2. Applicability

This regulation applies to all types of materials with a solid surface which come into contact with drinking water and which do not contain or release any toxic compounds. Included here are primarily the synthetic materials and materials which contain organic compounds which can be used by micro-organisms. The method can also be applied to materials which come into contact with water in other applications, e.g. with swimming pool water, medical equipment or food packaging.

The objective of this provision is the determination of the capacity of materials to promote the growth of micro-organisms.

3 Definitions

- 3.1 Biofilm Density (BD) : the ATP content per cm² of material.
- 3.2 Biofilm formation potential (BFP) : the average density of active biomass on the surface of the material, measured as adenosine triphosphate (ATP) per cm² of material, after 8, 12 and 16 weeks, incubated in test water under defined conditions.
- 3.3 Biomass concentration (BMC) : the average quantity of active micro-organisms, measured as adenosine triphosphate (ATP), produced per cm² of material in test water, after 8, 12 and 16 weeks of incubation.

4 Principle

Representative samples of the material to be studied are placed in drinking water to which certain minerals and micro-organisms from river water are added. These samples are incubated during a 16-week period, whereby the formation of a biofilm on the material is monitored, using adenosine-5'-

Kiwa In-house regulation

Title

Determination of the biofilm formation potential of materials which come into contact with drinking water

Number	Version	date	page	authorisation initials
LMB-006	2	7 January 1999	2 of 11	

triphosphate (ATP) measurements which are performed with pieces of the material which are periodically taken out of the flasks. At the same time, the ATP content in the test water is measured, with the same frequency.

5 Safety and environment

The biological waste is separately collected and disposed of in accordance with KCB-005.

6 Controls

Include the following controls together with each determination. Treat the controls identically to the samples.

6.1 Procedure blank

The procedure blank consists of inoculated test water to which no materials have been added.

The biomass concentration must be smaller than 15 ng ATP/l.

At the same, if this is used in the test, a procedure blank is performed of stainless steel.

The biofilm formation potential of stainless steel must be less than 150 pg ATP/cm². The biomass concentration must be less than 20 ng ATP/l.

If the procedure blank does not satisfy the mentioned criteria, then the test must be repeated. Investigate the reason why the values were exceeded.

6.2 Positive control

The positive control consists of silicone hose.

The biofilm formation potential of silicone hose must be higher than 10³ pg ATP/cm². The biomass concentration must be more than 50 ng ATP/l.

If the positive blank does not satisfy these criteria, then the test must be repeated. Investigate the reason why the values were exceeded.

6.3 Negative control

The negative control consists of glass.

The biofilm formation potential of glass must be less than 50 pg ATP/cm². The biomass concentration of glass must be less than 10 pg ATP/ml.

Kiwa In-house regulation

Title

Determination of the biofilm formation potential of materials which come into contact with drinking water

Number	Version	date	page	authorisation initials
LMB-006	2	7 January 1999	3 of 11	

If the negative control does not comply with these criteria, the test should be repeated. Check the cause of the excess.

7 Reagents and materials

Only use analytically pure reagents and demineralised water

7.1 Potassium dichromate solution

7.1.1 Composition

K ₂ Cr ₂ O ₇	100 g
H ₂ SO ₄ (95-98%)	900 ml
Demi water	1000 ml

7.1.2 Preparation

Dissolve the potassium dichromate in demineralised water
Add sulphuric acid to this potassiumbichromate solution, cool with ice.
After cooling, add demineralised water until the crystals are dissolved.

7.2 Potassium dihydroxyphosphate solution

7.2.1 Composition

KH ₂ PO ₄	0.2 g
Water	100 ml

7.2.2 Preparation

Dissolve the potassium dihydroxyphosphate in water in an AOC-free volumetric flask (6.3.1) and autoclave (15 min, 121 °C ± 1°C) this solution

7.3 Potassium nitrate solution

7.3.1 Composition

KNO ₃	1 g
Water	100 ml

7.3.2 Preparation

Dissolve the potassium nitrate in water in an AOC-free volumetric flask (8.3.1) and autoclave (15 min, 121 °C ± 1°C) this solution

7.4 Dilutions

Dilutions are made with autoclaved (15 min, 121 °C ± 1°C) tap water of drinking quality with a copper content below 50µg/l and a pH-value between 6.5 and 8.5

Kiwa In-house regulation

Title

Determination of the biofilm formation potential of materials which come into contact with drinking water

Number	Version	date	page	authorisation initials
LMB-006	2	7 January 1999	4 of 11	

- 7.5 Test water
Drinking water free of growth-inhibiting compounds (such as free chlorine) with a
- copper content < 50µg/l
 - pH between 6.5 and 8.5
 - high bacteriological stability, i.e. with an AOC-content < 10 and a DOC-content < 2mg/l

Remark: a slow filtrate on sand usually complies with these conditions

7.5.1 Composition

Test water	600 ml
Potassium nitrate solution	1.45 ml
Potassium dihydrogenphosphate solution	0.55 ml

7.5.2 Preparation

Weigh the AOC-flasks. Then pour approximately 600 ml test water in these flasks, according to LMB-018. Close the flasks with glass stoppers and bring them to the laboratory, where they are handled immediately. Weigh the flasks again and bring the volume to 600 ml ± 20 ml, then pour new water. Add the potassium nitrate and the potassium dihydrogenphosphate using AOC-free pipettes (8.3.2) aseptically to the flasks. Measure the pH of the test water from one of the flasks. If the pH does not comply with the standard (pH 6.5-8.5), taken new water.

7.6 Inoculation material

7.6.1 Composition

Fresh river water	250 ml
-------------------	--------

7.6.2 Preparation

Place a filter holder with a filter membrane (8.3.5) over the vacuum container and connect it to the vacuum pump.

Place a 250 ml container in the vacuum container under the filter holder and filter the river water to remove all protozoa.

Collect the filtrate in a sterile flask. The inoculation material is ready to use.

7.7 Cold flowing water, drinking water quality

Kiwa In-house regulation

Title

Determination of the biofilm formation potential of materials which come into contact with drinking water

Number	Version	date	page	authorisation initials
LMB-006	2	7 January 1999	5 of 11	

8 Apparatus and devices

8.1 Apparatus

The temperatures for sterilisation, cooling and incubation were fixed according to the procedures KLMB-004

- 8.1.1 Oven for dry sterilisation of glass at 150-175 °C.
- 8.1.2 Oven for heat treatment of pipettes at 250 °C
- 8.1.3 Oven at 550°C for heat treatment of conical flasks and stainless steel (SS) used to add weight to the material.
- 8.1.5 Incubator set to 25°C ± 1°C.
- 8.1.6 Analytical scale with a precision of 0.1 mg
- 8.1.7 Scale with a precision of 10 mg
- 8.1.8 Vortex mixer
- 8.1.9 pH meter
- 8.1.10 Ultrasonic water bath
- 8.1.11 Apparatus for measurement of ATP
- Vacuum pump
- 8.1.12 Thermometer 0-30°C

8.2 Sterile glassware

The glassware must be able to withstand repeated heating at 250°C or 550°C. Borosilicate and Pyrex are suitable. AOC-free volumetric flasks, pipettes and tubes for dilution should be cleaned according to 8.3

- 8.2.1 1000 ml Erlenmeyers with an inner diameter at the neck of 25 mm, with a polished glass stopper.
- 8.2.2 100 ml beaker glasses
- 8.2.3 AOC-free glass pipettes with 0.01 ml divisions
- 8.2.4 16-18 culture tubes, 160 mm, sterile, with autoclavable stopper
- 8.2.5 21-24 culture tubes, 200 mm, sterile, with autoclavable stopper
- 8.2.6 Measuring cylinder (4=45 cm, d=15 cm) with glass siphon
- 8.2.7 Autoclavable dispenser for volumes up to 10.0 ml
- 8.2.8 250 ml graduated cylinder
- 8.2.9 250 ml glass containers
- 8.2.10 500 ml beaker glasses

Kiwa In-house regulation

Title

Determination of the biofilm formation potential of materials which come into contact with drinking water

Number	Version	date	page	authorisation initials
LMB-006	2	7 January 1999	6 of 11	

8.3 Cleaning the glassware

8.3.1 AOC-free flasks

Wash the Erlenmeyer's in the washing machine. Then put a chrome nickel hook between the neck and the bottle. Sterilise the bottles for 4 hours at $550\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$.

8.3.2 Pipettes

Set glass pipettes vertically in a glass measured cylinder with a sulphuric acid dichromate solution. The next day, place these pipettes in the glass cylinder with the siphon system and wash them with drinking water for 4 hours. Allow the water to run out of the pipettes, put them in steel cookers and sterilise for 4 hours at $250^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$. These pipettes may definitely **NOT** contain cotton plugs!

8.3.3 Dilution tubes

Wash culture tubes in the racks in the washing machine. Place the caps on the tubes. Sterilise the tubes for 4 hours at $150\text{ }^{\circ}\text{C} - 175\text{ }^{\circ}\text{C}$.

8.4 Devices

8.4.1 Steel lockable cookers, for dry sterilisation of pipettes.

8.4.2 Tube racks, for large and small tubes.

8.4.3 Chrome nickel steel hooks (ϕ 1.0 mm, length 90 mm).

8.4.4 Tweezers, with rounded-off corners for picking up membrane filters, materials and chrome nickel steel hooks.

8.4.5 Membrane filters, pore size 1.2 μm , diameter 47 mm.

8.4.6 Sliding callipers, accuracy 0.01 cm.

8.4.7 Shears, (Stanley) knife and/or saw and tongs for processing the material, all grease-free.

8.4.8 Melting ice.

8.4.9 Sterile pipettes of 10 ml.

Test material for controls :

- Rings of silicone hose with a surface area of 8 cm^2 .
- Glass rings (ϕ 15 mm) with a surface area of 8 cm^2 .
- Stainless steel rings and wire (ϕ 0.8 mm) to weigh down the materials. Both of heavy-duty quality, DIN 125A.

Lay the rings and pieces of wire in a glass tray and then sterilise them for 4 hours at $550\text{ }^{\circ}\text{C}$.

Use cleaned tongs for further handling of the rings (the weighting of the materials).

Before use, clean the tongs 3 times with petroleum ether, then 3 times with acetone and then rinse well under flowing water. Then dry with a tissue.

Kiwa In-house regulation

Title

Determination of the biofilm formation potential of materials which come into contact with drinking water

Number	Version	date	page	authorisation initials
LMB-006	2	7 January 1999	7 of 11	

9 Sample to be studied

Before transport from manufacturer to laboratory, the samples must be packaged in such a way that no contamination can occur with dust, grease or oil. At the same time, they must be protected against the influence of heat, sunlight and volatile chemicals. In the laboratory, they can be stored in the dark at room temperature, unless specified otherwise by the manufacturer.

Note : Do not test any samples written on with ink or pencil lead or samples with adhesive residues from labels. Make sure that hands are clean when handling the samples so as to prevent contamination with skin grease. If materials have to be cut, this may not be done with oil or other biodegradable compounds.

24 pieces of around 8 cm² are needed per material. Check in advance whether these pieces float. If they do, then these pieces must be individually weighted down using stainless steel rings and stainless steel wire.

10 Method

Perform the tests in a room where the atmosphere is free of volatile organic compounds, given that these can dissolve in such concentrations in the test water that they cause a (strong) bacterial growth which will mask the growth resulting from the sample to be studied.

10.1 Inoculation and incubation

Wash the samples on the day that the test is started for 1 hour in a 500 ml-beaker with cold flowing drinking water.

For each sample, two flasks with test water must be used (flask A and flask B) Add the pieces of material to each flask. Then inoculate with 1 ml of the inoculation material (7.6).

Set glass stoppers over the closed flasks in order to prevent contamination by dust. Incubate, without shaking, the flasks for 16 weeks in an incubator at 25°C ± 1 °C.

10.2 Determination of the growth

The biofilm density on the material (bottle A) and the biomass concentration (bottle B) are determined by means of ATP measurements.

Kiwa In-house regulation

Title

Determination of the biofilm formation potential of materials which come into contact with drinking water

Number	Version	date	page	authorisation initials
LMB-006	2	7 January 1999	8 of 11	

10.2.1 Material phase

Determine the biofilm density (BD, pg ATP/cm²) on the surface of the materials on days 56, 84 and 112. If the project leader so desires, one can also measure on days 7, 14 and 28.

For this, take two pieces of material with a calcined and cooled pincer from the water of beaker A. Place each piece in a large test tube with 10 ml of sterile tap water. Vibrate this piece during 2 minutes in an ultrasound vibrating water bath. Fill this water bath with demineralised water so that the water level in the tube is equal to the water level in the water bath. Pipette the 10 ml from the tube, allow 9 ml to flow out and catch 1 ml in a sterile test tube. Place this tube in melting ice. Add once again 10 ml of sterile tap water to the material and repeat the procedure. Check the temperature of the vibrating water bath regularly. If it rises above 25 °C, cool the water by adding ice. Vibrate each material, with the exception of glass and stainless steel, 6 times. Vibrate glass and stainless steel 3 times. The ATP content is determined from the collected volume (6 or 3 ml). From this, the biofilm density on the material can be calculated (11.1).

Measure the materials and calculate their surface area.

10.3 Liquid phase

Determine the growth curves using periodical ATP measurements (T= 56, 84 and 112) of the test water of flask B. Possibly, at the request of the project leader, one can also take measurements on days 7, 14 and 28.

Swirl the flasks manually just before taking the samples. In so doing, make sure that the glass plugs of the flasks do not get moistened. If necessary, dry the stopper and neck of the flask in a cleanly burning gas flame.

For the sample-taking, use only AOC-free pipettes (8.3.2).

10.4 Additional analyses (optional)

If the project leader wishes, additional analyses can be performed during the test and at the end of the test period. Table 1 presents a list of these additional analyses.

Kiwa In-house regulation

Title

Determination of the biofilm formation potential of materials which come into contact with drinking water

Number	Version	date	page	authorisation initials
LMB-006	2	7 January 1999	9 of 11	

Parameter	Procedure	Volume
Coliform bacteria	LMB-028	100 ml
Colony number at 22 °C	LMB-032	≤ 1 ml
Colony number at 37°C	LMB-032	≤ 1 ml
Colony number at 25 °C on R ₂ A medium	LMB-014	≤ 1 ml
<i>Aeromonas</i> spp.	LMB-022	≤ 1 ml
<i>Legionella</i> spp.	LMB-027	≤ 1 ml
<i>Pseudomonas aeruginosa</i>	NEN-6573	≤ 1 ml
Total organic carbon		
pH	NEN 6411	

11 Identification and Quantification

11.1 Calculation of the biofilm density (BM) per cm²

Calculate the biofilm density on the material using the following formula:

$$BD = \frac{ATP_{\text{collected volume}} \times \text{added volume}}{\text{Surface material}}$$

11.2 Calculation of the biofilm formation potential (BFP)

Calculate the biofilm formation potential on the material using the following formula:

$$BFP = \frac{BD_{\text{day 56}} + BD_{\text{day 84}} + BD_{\text{day 112}}}{3}$$

11.3 Calculation of the biomass concentration (BMC)

Calculate the biomass concentration on the material using the following formula:

Kiwa In-house regulation

Title

Determination of the biofilm formation potential of materials which come into contact with drinking water

Number	Version	date	page	authorisation initials
LMB-006	2	7 January 1999	10 of 11	

$$\text{BMC} = \frac{\text{ATP}_{\text{ml day 56}} + \text{ATP}_{\text{ml day 84}} + \text{ATP}_{\text{ml day 112}}}{3}$$

Calculate the standard deviation as well.

12 Characteristic value

Not applicable

13 Report

Include the following details in the report:

- details necessary for sample identification
- the method used: according to LMB-006
- the quantity of ATP detected on the material in pg per cm² and in the water in pg per ml, in graphs.
- The biofilm formation potential (BFP) and the biofilm formation concentration (BMC) of the material with their standard deviations.
- The results of possible optional analyses.
- Possible special observations during the treatment
- All procedures not described in the prescriptions that might have influenced the results.

14 Literature

- 14.1 NEN 6271: Bacteriologisch onderzoek van water. Bepaling van het gehalte gemakkelijk assimileerbaar koolstof (AOC).
- 14.2 Van der Kooij, D.A. Visser, and W.A.M. Hijnen 1982. Determining the concentration of easily assimilable organic carbon in drinking water. J. Am. Water Works Assoc. 74: 540-545
- 14.3 Van der Kooij, D. 1992. Assimilable organic carbon as an indicator of bacterial regrowth. J. Am. Water Works Assoc. 84: 57-65
- 14.4 Van der Kooij, D., H.R. Vennendaal. 1992. Assesment of the biofilm formation characteristics of drinking water. Proceedings AWWA Water Quality Technology Conference, Toronto, Canada. November 15-19; pp 1099-1110.

Kiwa In-house regulation

Title

**Determination of the biofilm formation potential of materials
which come into contact with drinking water**

Number	Version	date	page	authorisation initials
LMB-006	2	7 January 1999	11 of 11	

14.5 Van der Kooij, D., J.S. Vrouwenvelder, H.R. Veenendaal and M.J.C. van Raalte-Drewes, 1994. Multiplication of aeromonads in ground-water supplies in relation with the biofilm formation characteristics of drinking water. Proceedings AWWA Water Quality Technology Conference, November 6-11, San Francisco, California

14.6 Van der Kooij, D., H.R. Vennendaal. 1993. Assesment of the biofilm formation characteristics of synthetic materials in contact with drinking water during distribution. Proceedings AWWA Water Quality Technology Conference, Miami, Florida, November 7-11, pp 1395-1407

15 Remarks

None

16 Annexes

16.1 Annex 1: Information sheet

16.2 Annex 2: ATP measurement results sheet

16.3 Annex 3: Measurements results sheet surface calculation

16.4 Annex 4: ATP calculations sheet for samples

ANNEX 12

Water Quality Water Treatment Plant Tull and 't Waal (1992)

Water distribution company : WMN				Year			Measurement location: 029-05 A01		
Pump Station : 029805 Tull en 't Waal				1992			Pure		
Laboratory : WMN									
Contact person : drs. F.A. Jutte									
KIWA	Wb	Name of parameter	Measurement unit	av.	min	max	av.	min	max
120	8	Temperature	°C				13	12	13
122	12	Oxygen, dissolved	mg/l O2				5.13	4.5	7.1
126	2	Degree of turbidity	FTE				0.16	0.10	0.55
128	3	Suspended matter	mg/l						
140	65	Total available chlorine	mg/l Cl2						
141	65	Free available chlorine	mg/l Cl2						
160	64	Total beta radioactivity	Bq/l						
162	64	Remaining beta radioactivity (tot. -K40)	Bq/l						
164	63	Tritium	Bq/l						
170	6	Odour dissolving factor							
172	7	Taste dissolving factor					1.0	1.0	1.0
174	7	Odour, qualitative							
176	7	Taste, qualitative							
180	9	Acidity	pH				7.7	7.6	7.9
182		Equilibrium pH	pHs						
200	5	EC (elect. Conduct. , 20°C)	mS/m				38.8	38.3	39.6
202	4	Dry remains, 105°C	mg/l						
220	21	Carbon dioxide	mg/l CO2				8.3	6.0	9.2
222	21	Hydrogen carbonate	mg/l HCO3				265	260	270
230	27	Chloride	mg/l Cl				9.4	9.1	9.6
232	22	Sulphate	mg/l SO4				<2.0	<2.0	<2.0
240	28	Sodium	mg/l Na				12.5	11.7	12.8
242	29	Potassium	mg/l K				1.05	0.71	1.18
244	30	Calcium	mg/l Ca				71.9	71.1	72.7
246	31	Magnesium	mg/l Mg				5.89	5.81	5.98
250		Total hardness	mmol/l				2.04	2.01	2.06
270	18	Ammonium	mg/l N				<0.02	<0.02	0.02
274		Nitrogen, Kjeldahl	mg/l N						
276	17	Org. combined nitrogen	mg/l N						
280	19	Nitrite	mg/l N				<0.005	<0.005	0.005
282	20	Nitrate	mg/l N				0.14	0.12	0.15
284	23a	Orthophosphate	mg/l P				0.027	<0.005	0.038
286	23	Total phosphate	mg/l P						
288	24	Silicate	mg/l Si				8.6	8.5	8.6
300	37	Iron	mg/l Fe				0.04	0.02	0.10
304	36	Manganese	mg/l Mn				<0.01	<0.01	0.02
310	32	Aluminium	µg/l Al						

314	42	Arsenic	µg/l As						
316	46	Barium	µg/l Ba						
318	47	Beryllium	µg/l Be						
322	33	Boron	mg/l B						
324	45	Cadmium	µg/l Cd						
326	34	Chromium	µg/l Cr						
330	40	Copper	µg/l Cu						
328	39	Cobalt	µg/l Co						
330	40	Copper	µg/l Cu						
332	49	Mercury	µg/l Hg						
334	50	Lead	µg/l Pb						
340	38	Nicker	µg/l Ni						
342	44	Selenium	µg/l Se						
350	35	Vanadium	µg/l V						
352	48	Silver	µg/l Ag						
354	41	Zinc	µg/l Zn						
380		Bromide	mg/l Br						
382	26	Fluoride	mg/l F			0.03	0.03	0.03	
386	25	"total" cyanide	µg/l CN						
400	16	Organic carbon	mg/l C						
404	14	Chem. Oxygen compounds	mg/l O2						
406	15	Biochem. Oxygen compounds	mg/l O2						
408	13	Oxidisability with KmnO4	mg/l O2			1.38	1.34	1.45	
412	1	Colour intensity, Pt/Co-scale	mg/l Pt			6.5	5.9	7.3	
420	52	An. Act. Deterg.	mg/l Na-Lauryl-SO4						
426	54	Phenols, water vapour vol.	µg/l C6H5OH						
428	51	Mineral oil, IR-method	mg/l						
432	56	EOX (extr. org. bound halog.)	µg/l Cl						
434	57	VOX (vol. org. bound halog.)	µg/l Cl						
451	55	Trihalomethanes, sum	µg/l						
459	59	PAK, 6 of Borneff	µg/l						
466	62	Chol.est inhibitors	µg/l paraoxon						
600	70	Colony number 3d., 22°C, casting plate	n/ml			>48	1	>300	
602	70	Colony number 2d., 37°C, casting plate	n/ml			4	1	74	
610	66	MPN bact. Coliform 37 °C	MPN/100ml			<1	<0.3	<1.0	
620	67	MPN bact. Coliform 44 °C	MPN/100ml			<1	<0.3	<1.0	
630	68	Faecal streptococci	n/100ml						
644	69	Clostridia, spores SO3-red.	n/100ml						
650	71	Salmonellae	n/100ml						
1028		Bromodichloromethane	µg/l						
1033		Dibromochloromethane	µg/l						
1058		Tribromomethane	µg/l						
1064		Trichloromethane	µg/l						
8361		Hexachlorobenzene (HCB)	µg/l						

