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# Table of content

# Summary

The biomass production potential (BPP) test, proposed to be incorporated in the European scheme for acceptance of construction products in contact with drinking water (CPDW), is a semi-static test for assessment of growth promoting properties under strictly defined conditions. The test is performed at the product's surface to volume contact ratio (S/V) of 0.16 $cm^{-1}$, that is quite different from the practice in buildings and domestic installations.

The goal of this study was to evaluate the importance of the S/V ratio on the performance of the BPP test and for correct assessment of growth promoting properties of CPDW and their impact on bacterial growth in water. The BPP of 10 pipe products, measured as pg ATP/$cm^2$, were compared under the S/V ratios of 0.16 $cm^{-1}$ and 1.6 $cm^{-1}$ in two consecutive trials. The BPP of most plastic pipe materials were higher under the S/V contact ratio of 1.6 $cm^{-1}$ in individual trials, but the 10-fold increase of the S/V ratio led up to 26 % increase of the BPP values in the first trial and up to 47 % in the second one. Similar trends for attached biomass production (AB) and planktonic biomass production (PB) were found. However, the differences of average BPP values between both ratios were statistically insignificant.

Our study confirmed that the Biomass Production Potential test at the originally proposed S/V contact ratio of 0.16 $cm^{-1}$ is a reliable approach for assessment of growth promoting properties of CPDW. The data showed that under the S/V ratio of 0.16 $cm^{-1}$ the BPP test achieves similar results as with a more realistic S/V ratio of 1.6 $cm^{-1}$.

However, the S/V contact ratio showed a significant effect on the planktonic biomass concentration (measured as ng ATP/l) and heterotrophic plate count in the test waters in contact with the tested pipe materials. Under the S/V ratio of 1.6 $cm^{-1}$ the tested plastic pipe materials caused from 3 to 14-fold higher PB concentrations in test water. That stronger effect on the water quality can be important from hygienic point of view. Therefore, the impact of the S/V contact ratio on drinking water quality should be taken into consideration for assessment of the products in contact with drinking water. For acceptance of the CPDW, besides a Pass/Fail Criterion for the BPP, a second criterion for evaluation of materials on their effect of drinking water quality needs to be developed and the planktonic biomass concentration could be useful for this purpose.

# List of abbreviations

| | |
|---|---|
| AB | Attached Biomass Production, pg $ATP/cm^2$ |
| AOC | Assimilable Organic Carbon, $\mu$g ac-C/L |
| ATP | Adenosine Triphosphate, ng/l |
| BP | Biomass Production, pg $ATP/cm^2$ |
| BPP | Biomass Production Potential, pg $ATP/cm^2$ |
| $BPP_{corr}$ | BPP of the product corrected by BPP of glass control, pg $ATP/cm^2$ |
| CEN | European Committee for Standardization |
| $CV_R$ | Coefficient of Variation of Reproducibility |
| CFU | Colony Forming Units |
| CPD | Construction Products Directive (89/106/EEC) |
| CPDW | Construction Products in Contact with Drinking Water |
| DWD | Drinking Water Directive (98/83/EC) |
| DWDS | Drinking Water Distribution Systems |
| EAS | European Acceptance Scheme |
| EPS | Extracellular Polymer Substances |
| EPDM | Ethylene Propylene Diene Monomer |
| HES | High Energy Sonication (ultrasonic homogenization) |
| HPC | Heterotrophic Plate Count |
| GS | Galvanized Steel |
| LES | Low Energy Sonication (ultrasonic bath) |
| MDOD | Mean Dissolved Oxygen Demand |
| MS | Member State |
| $NO_3$-N | Nitrates, mg $NO_3$-N/l |
| $PO_4$-P | Phosphates, mg $PO_4$-P/l |
| PB | Planktonic Biomass Concentration, ng ATP/l |
| PBP | Planktonic Biomass Production, ng $ATP/cm^2$ |
| PFC | Pass-Fail Criteria |
| PE | Polyethylene |
| PEx | Polyethylene cross-linked |
| PP | Polypropylene |
| PVCp | Polyvinylchloride plasticized |
| P | Probability |
| RSD | Relative Standard Deviation (Coefficient of Variation) |
| SS | Stainless Steel |
| S/V | Surface to Volume Contact Ratio, $cm^{-1}$ |
| t | t-distribution |
| $\|t\|$ | Calculated value of t in significance test |

# 1. Introduction

According the European Drinking Water Directive (DWD, 1998) the consumer's tap is the point of compliance of water quality. Article 10 of the DWD sets requirements for the quality of products used for building the new drinking water distribution systems. Migration of substances from the construction products to drinking water can compromise water quality by changing its organo-leptic characteristics (taste, odour, colour and turbidity) and chemical composition, enhancing bacterial growth in water and on pipes' surfaces or equipment.

Some Member States have their own methods for testing and regulations for acceptance of the construction products intended to contact with drinking water (CPDW). On the European level an approval scheme for these products is under development (EAS, 2005). As an important stage of approval process the European acceptance scheme (EAS) states the examinations of organo-leptic aspects, general hygiene, substances that pose a risk to health, as well as microbial growth promoting properties of the CPDW.

Different methods for assessment of bacterial re-growth based on migration of biodegradable organic compounds from the CPDW have been developed and standardized: the German method W270 measures the volume of biofilm slime formed (DVGW, 1998); the British method measures mean dissolved oxygen demand (MDOD) in water in contact (BS 6920, 2000); the Austrian method determines heterotrophic plate count (HPC) in water and adenosine triphosphate (ATP) of biofilm (ÖB 5018, 2002); the Dutch Biomass Production Potential (BPP) test measures ATP as parameter for active biomass on product's surface and in test water (Van der Kooij & Veenendaal, 2001).

The BPP test has been approved as the standard method for testing the CPDW in the Netherlands (NEN, 2004), but Pass-Fail criteria (PFC) are not available yet. Different approaches for definition of PFC have been discussed based on the BPP values from experimental testing of different products, the data for growth of pathogenic bacteria, i.e. *Legionella*, *Aeromonas* in biofilm or water at distribution systems, or the data for relation between the growth promoting properties of one and the same products at real distribution systems and in the BPP test (Van der Kooij & Veenendaal, 2001; Van der Kooij et al., 2002; 2006). On the basis of the assessment of the growth-promoting properties of 14 plastic materials by means of the German, British and Dutch test methods, assuming a proportional relationship, Van der Kooij & Veenendaal (2007) determined the BPP levels corresponding to the PFC values of the BS 6920 and the revised W270 test methods. Despite the insufficient data for calculation of quantitative relationship, it has been estimated that a BPP value of $2x10^4$ pg ATP/cm$^2$ corresponded with the PFC value of 2.4 mg $O_2$/l of the MDOD test and that a BPP value of $2x10^3$ pg ATP/cm$^2$ corresponded with the PFC value of 0.05 ml slime/800 cm$^2$ of the W270 test. These results have also demonstrated that the BPP test enables quantification of growth promoting properties of the materials at levels well below the limit of detection of the MDOD and W270 test methods.

In its 12$^{th}$ meeting in 2003 the Regulators Group proposed the BPP test to be used for assessment of microbial growth support properties of the construction products intended to contact with drinking water, as developed in the CPDW project (2003). An inter-laboratory testing of the BPP method has been carried out in 5 Member State laboratories (Van der Kooij *et al.*, 2006). The draft-standard of the BPP test has been proposed to CEN (CEN TC164/WG3, 2004).

The BPP test is a semi-static test that assesses the growth promoting properties of the CPDW in a test water under strictly defined conditions: temperature of 30°C, weekly replacement of test water, inoculation with diverse bacteria and product's surface to water volume contact ratio (S/V) of 0.16 cm$^{-1}$ (Van der Kooij *et al.*, 2003). The S/V ratio has been selected to ensure for the products having strong growth potential no growth limitation due to the depletion of oxygen/nutrients or to accumulation of growth inhibiting compounds and a sufficient surface area enables quantitative assessment of attached biomass on the products with low microbial growth potential. By reason of that it was considered that the test conditions resembling the real situation may compromise assessment of microbial growth potential of the CPDW. In addition, the British test is carried out under similar S/V ratio of 0.15 cm$^{-1}$ (BS 6920, 2000). However, the S/V ratio of 0.16 cm$^{-1}$ corresponding to pipe diameter of 25 cm, is unrealistic for the domestic installations, where the usual ratios are 2.1 $cm^{-1}$ for ½ inch pipes, 1.6 $cm^{-1}$ (1" pipes) or 0.7 $cm^{-1}$ (2" pipes). One might question if the BPP values and their relation with the planktonic biomass concentrations determined in the BPP test can be extrapolated directly to the domestic distribution systems and to water quality at the consumers' tap, and if the proposed semi-static BPP test is an overall approach for assessment of growth promoting properties of CPDW?

Very few data on the effect of S/V are available. Some assumptions in respect of the effect of S/V ratio have been done based on the bacterial growth rates in biofilm and in water. Boe-Hansen et al. (2002) determined in a model flowing system a higher activity of the bulk water bacteria than the biofilm bacteria in terms of culturability, cell-specific ATP content and cell-specific growth rate. Because of these different community properties the authors suggested that planktonic growth may be a significant factor for the net bacterial production in drinking water distribution systems at low S/V ratios (i.e. at large pipe diameters). Corfitzen et al. (2004) have studied the migration of biodegradable organic compounds from the polymer surfaces (called 'bioavailable migration') in abiotic (sterile) and biotic (in the presence of bacteria) conditions under different S/V contact ratios. In abiotic conditions the authors have found an inversely proportional relation between the 'bioavailable AOC migration per unit surface area of polyethylene material (measured as μg acetate-C/$cm^2$) and the S/V ratios, ranging from 0.07 to 1.38 $cm^{-1}$. It corresponds to low migration at small pipe diameter and indicates that in abiotic conditions 'bioavailable migration' depends on concentration of the substances released in water. However, in biotic test conditions (in the presence of bacteria together with the tested PE material) the S/V ratios in the range from 0.1 to 0.7 $cm^{-1}$ have not had any effect on the 'bioavailable migration' in water, since the migrating substances are continuously consumed by bacteria and in this way a maximum driving force for the migration is maintained (Corfritzen, 2004a).

The ***main goal*** of this study is to evaluate the importance of the S/V ratio for fulfilment of the BPP test of CPDW, respectively for enhancement of microbial growth by comparing: a) the BPP values of the pipe materials under the original S/V ratio of 0.16 $cm^{-1}$ and the 10-fold higher S/V ratio of 1.6 $cm^{-1}$; b) their impact on the bacterial growth in water under the both ratios.

The study on the influence of S/V contact ratio on the BPP and the PB values of pipe material could stretch the scale of the PB-BPP relationship and might facilitate a better selection of Pass/Fail criteria (PFC). It could contribute for assessment the effect of pipe materials for real bacterial exposure at the consumers' tap.

The ***main tasks*** of this experimental study are:

- to determine the BPP of pipe products for drinking water distribution under S/V contact ratios of 0.16 $cm^{-1}$ and 1.6 $cm^{-1}$;
- to assess how materials impact on bacteriological water quality under selected S/V ratios;
- to determine the reproducibility of the test by testing the same materials in two consecutive trials;
- to check the effectiveness of the procedure for biofilm removal.

The study is carried out for selected pipe materials, in combination with reference materials as negative and positive controls.

# 2. Materials and methods

## 2.1. Materials

Ten pipe products intended to contact with drinking water (Table 1) are under study:

- Polyethylene (PE) pipe produced by Aquatechnic s.r.l.;
- Polyethylene cross-linked (PEx) reinforced with aluminium foil (produced by Aquatechnic s.r.l.);
- 3 Polypropylene pipes (PP type 3, produced by Aquatechnic s.r.l.) having different wall thickness and diameter, as one of them containing aluminium foil for reinforcement;
- Stainless steel pipe (SS);
- Galvanized steel (GS);
- Plasticized PVCp (Italian trade mark IPL ARIANNA TA), flexible plastic tubing permitted for food contact (FCM, CEE/90/128);
- Ethylene Propylene Diene Monomer (EPDM) used as flexible tubing for hot water (produced by Trelleborg);
- Silicone tubing.

**Table 1. Description of pipe products under BPP test**

| Material | outer tube diameter, mm | wall thickness, mm |
|---|---|---|
| glass | 18 | 1.5 |
| SS | 15 | 1 |
| GS | 17 | 2.5 |
| PE | 20 | 3 |
| PEx | 14 | 2 |
| PP-1 | 20 | 3.5 |
| PP-2* | 25 | 3.5 |
| PP-3* | 20 | 2 |
| Silicone | 5 | 1 |
| EPDM | 13 | 1.5 |
| PVCp* | 16 | 3 |

*Additional materials tested only during the second trial

Glass and stainless steel materials are considered as a negative control, while the plasticized PVCp as a positive control.

## 2.2. Method for assessment of the BPP of products in contacts with drinking water

The products' samples are tested under two different surface to volume contact ratios – 0.16 $cm^{-1}$ and 1.6 $cm^{-1}$. The ratio of 0.16 $cm^{-1}$ originally proposed in the BPP test procedure (Van der Kooij *et al.*, 2003; 2006) corresponds to pipe diameter of 25 cm, while the S/V ratio of 1.6 $cm^{-1}$ - to pipe diameter of 2.4 cm (1").

Assessment of the BPP of the pipe products under the both S/V contact ratios is carried out following the procedure of the BPP test (Van der Kooij *et al.*, 2006). The samples of one and the same pipe material are incubated for a period up to 16 weeks at a constant S/V ratio of 0.16 $cm^{-1}$ or 1.6 $cm^{-1}$ at 30°C and a replacement of the test water. Weekly replacement of the test water prevents accumulation of metabolites and/or growth inhibiting compounds released from the products, oxygen depletion or growth limitation by inorganic substances.

Two consecutive experiments for assessment of the growth promoting properties of 5 selected pipe materials are carried out and the reproducibility of the BPP test is determined by calculation of the coefficient of variation of reproducibility ($CV_R$). The GS and silicone tested in the first trial are replaced in the second experiment with two PP pipe products.

Two preliminary experiments are carried out for comparison between the effectiveness of 3 procedures for biofilm detachment from the pipe surfaces - the original procedure as described in the BPP test (Van der Kooij *et al*., 2006) and two our laboratory procedures. All of them include the same steps of treatment - low energy sonication (LES), high energy sonication (HES) and swabbing the surface, accomplished in different order or way.

### 2.2.1. Test procedure for determination of the BPP

The preparation of material samples and examination of their BPP is done according the procedure of the harmonized method for determining the enhancement of microbial growth (Van der Kooij *et al.*, 2003, 2006).

The BPP test of each pipe material is conducted under both S/V contact ratios. In the first trial glass, PP-1 and PE materials, already evaluated under the S/V ratio of 0.16 $cm^{-1}$ in our previous study (Tsvetanova & Hoekstra, 2008), are tested without repetition. All rest materials are tested in two repetitions in glass jars, each containing 3 pieces of the material under the test (with total surface about 150 $cm^2$). Borosilicate glass rings with surface area of 16 $cm^2$ used as weight balance are attached to the test pieces that float by means of stainless steel wire.

The BPP test of the materials under the S/V contact ratio of 1.6 $cm^{-1}$ is carried out in 3 (4) repetitions in glass jars containing 3 (4) pieces of the product under test. Total number of pieces from each material tested under this S/V ratio is 9 (12).

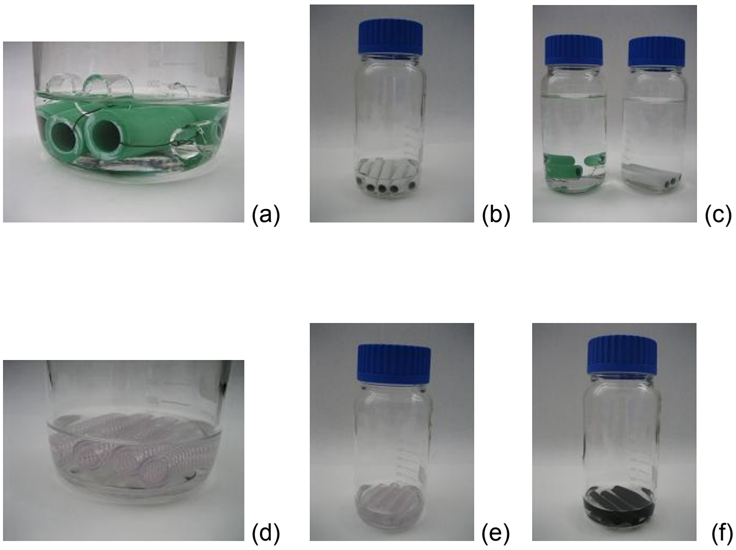


**Fig.1. Experimental procedure of the BPP test for plastic pipe materials under the both contact ratios**: (a), (b), (d), (e), (f) – test under S/V ratio of 1.6 $cm^{-1}$; (c) – test under S/V ratio of 1.6 $cm^{-1}$; the floating samples are submerged in water by means of glass rings as weight balance

***Sample cleaning***

Test pieces, each one with a surface area about 50 $cm^2$, are cut from the products under study.

Accurate measurement of the surface of the test pieces is done after the end of the test. The test pieces are cleaned by fully flushing with tap water for 30 min, soaking in Milli-Q water for 24 h and in the end rinsing repeatedly with Milli-Q water. Stainless steel wire and glass rings are heated at 450°C for 5 h.

### *Preparation of test water*

Milli-Q purified water (Elix Element system, Millipore) is used as test water in the BPP test. In the BPP test performed under the S/V ratio of 0.16 $cm^{-1}$ the test jars are filled with 900 ml of test water. 1 ml of sterile potassium nitrate solution (5 mg/l of $NO_3$-N), 1 ml of sterile potassium dihydrogen phosphate solution (2 mg/l of $PO_4$-P) and 9 ml lake water (as a bacterial inoculum) are added to each jar.

In the BPP test implemented under the S/V ratio of 1.6 $cm^{-1}$ the test jars are filled with 90 (120) ml of test water. The corresponding volumes of salt solutions and lake water are added to the flasks keeping the same composition of the test water like under the S/V ratio of 0.16 $cm^{-1}$.

### *Biomass removal from test materials*

Procedure for biomass detachment from test pieces includes 3 steps: treatment with low energy ultrasound; high energy sonication and wiping up the surfaces with sterile cotton swab. Duplicate aliquots of 0.1 ml sample are taken for ATP analyses after each treatment step (LES, HES and swabbing).

In the first trial biofilm removal is performed following our laboratory procedure N°1, while in the second trial following the originally proposed procedure of Van der Kooij *et al*. (2006). According the original procedure, all treatments of a test piece by LES, HES and swab are carried out in separate volumes of Milli-Q water collected after each replacement together in a sterile borosilicate glass bottle. The LES treatment is carried out consecutively 6 times for 2 min or 3 times for glass control in ultrasonic bath Transsonic T 780 (*Elma*) with frequency of 35 kHz, power 1.33 A, volume of 2.5 l. The HES treatment is performed by *Branson W-250 D* sonifier (20 kHz; 45% amplitude) for 2 min. In the end, each test piece is wiped out by sterile cotton swab and treated for 1 min by HES. The bottles with biomass suspension in kept in ice bath.

According our laboratory procedure all treatments (LES, HES and swabbing) of a test piece are carried out in the same volume of Milli-Q water in a glass beaker kept in an ice bath between the treatments.

### *ATP analyses*

For determination of the planktonic biomass (PB) concentration in the test waters in contact with the tested pipe products, the total ATP content is analysed on the days 56$^{th}$, 84$^{th}$ and 112$^{th}$. The biofilm suspensions (after LES, HES and swab treatment) are analysed for determination of total ATP content of the attached biomass (AB).

Individual ATP calibration curve is prepared for each separated day of analyses.

### *Calculation of BPP*

Biomass production (BP) per a unit of product's surface is calculated from the concentration of attached and suspended biomass, as follow:

$$\text{BP (pg ATP/cm}^2\text{)} = \text{AB (pg ATP/cm}^2\text{)} + \text{PB (pg/cm}^3\text{)} \times \text{V/S (cm)}$$

Biomass Production Potential (BPP) is the average concentration of active micro-organisms on the pipe surface and in the test water, measured as pg ATP per $cm^2$, after 56, 84 and 112 days of incubation under defined conditions.

$$BPP_{tested\ sample} = \text{average of BP values on } 56^{th}, 84^{th} \text{ and } 112^{th} \text{ d}$$

Net Biomass Production Potential of the tested pipe material ($BPP_{corr}$) is calculated by correction of its BPP with the BPP of the glass control (Van der Kooij and Veenendaal, 2007).

$$BPP_{corr} = BPP_{tested\ sample} - BPP_{glass}$$

### 2.2.2. Heterotrophic Plate Count

On the days $105^{th}$, $112^{th}$ the test waters in contact with the pipe materials under BPP test are analysed for determination of culturable bacteria number following ISO 6222-2000. HPC is determined on yeast extract agar after 3 days of incubation under 22°C.

### 2.2.3. Comparison between different procedures for biofilm removal

Two experiments are carried out to compare between the effectiveness of 3 different procedures for biofilm removal based on ultrasonic treatments and wiping with cotton swab. Two materials with different characteristics are used – solid PEx pipe and flexible PVCp connection. The biofilm was developed on the surfaces under the same conditions as in the BPP test. Three glass jars each one containing 900 ml test water and 6 pipe pieces are incubated for 4 weeks.

In the first trial the PVCp material (trade mark Rapigel) was incubated under the S/V ratio of 0.33 $cm^{-1}$, while PEx - under S/V ratio of 0.28 $cm^{-1}$. In the second trial the tested PVCp (trade mark ARIANNA) was incubated under the same S/V ratio of 0.33 cm-$^{1}$, but the biofilm on the PEx was developed under the S/V ratio of 0.22 $cm^{-1}$.

During the first trial all test pieces from one jar are analysed by one of the procedures. During the second trial, because of the significant differences found between the biofilms developed in the separate jars, 2 test pieces from each jar (total number of 6) are analysed by each treatment procedure.

Next three procedures of biofilm detachment are compared:

1) The procedure as proposed in the BPP test (Van der Kooij *et al.*, 2006). It includes consecutive LES and HES treatments and wiping a test piece with cotton swab in separate water volumes. Water volumes are mixed together and duplicate aliquots of 0.1 ml sample are taken for ATP analyses after each treatment.

2) In laboratory procedure (N°1) all treatments (LES, HES and swabbing) of a test piece are performed in the same water volume in the same order as proposed in the BPP test. Duplicate aliquots of 0.1 ml sample are taken for ATP analyses after each treatment procedure.

3) Laboratory procedure (Nº2) starts with wiping up the surface of each test piece by swab, combined with HES for 1 min, following by 6 times LES and in the end treatment by HES. The treatments are performed in the same water volume. Duplicate aliquots of 0.1 ml sample are taken for ATP analyses after each treatment procedure.

The highest among the ATP values from the treatment stages is used for calculation of the AB value. All results are analyzed statistically.

### 2.2.4. Statistical comparison between results

A significance test is used to show whether the difference between two results is significant. We are testing the truth of null hypothesis used to imply that there is no difference between two observed values other than attributed to random variation. Usually null hypothesis is rejected if the probability (P) of observed difference occurring by chance less than 0.05 and in such a case the difference is said to be significant at the 0.05 (or 5%) level (Miller & Miller; 1988). If the experimental value |t| is greater than a certain critical value of *t* (from the table for *t*-distribution at P = 0.05 and a degree of freedom n) then the difference between the two results is significant.

|t| is calculated using:

$$t = (x_1 - x_2)/\sqrt{(s_1^2/n_1 + s_2^2/n_2)}$$

Independently whether the standard deviations of the samples are significantly different or not the degree of freedom for the critical value of *t* was calculated using:

$$n = \{(s_1^2/n_1 + s_2^2/n_2)^2 / [(s_1^2/n_1)^2/(n_1+1) + (s_2^2/n_2)^2/(n_2+1)]\} - 2$$

where:

- $t$ - experimental value
- $n$ - degree of freedom
- $x_1$, $x_2$ - sample mean value
- $s_1$, $s_2$ - standard deviation
- $n_1$, $n_2$ - number of measurements

# 3. Results

## 3.1. Comparison between the procedures for biofilm removal

The results from the first trial for comparison between the effectiveness of the test procedures are present at Tables 2 and 3, and the data from the second trial are present at Tables 4 and 5. Detailed primary data are attached in Tables I and II of the Appendix.

**Table 2. Effectiveness of biofilm removal from PVCp material (trade mark Rapigel) depending on the treatment procedures (in 1st trial)**

| Treatment Procedure | Stages of procedure | Attached biomass pgATP/cm$^2$ | stdev | Efficiency of removal, % | RSD, % |
|---|---|---|---|---|---|
| 1) BPP - Van der Kooij | 1) 6 LES | 6289.1 | 588.7 | 93.1 | 9 |
| *et al.* (2006) | 2) HES | **6751.9*** | **713.8** | 6.9 | 11 |
| | 3) Swab+HES | 6722.4 | 885.4 | 0 | 13 |
| 2) Lab | 1) 6 LES | 2921.1 | 551.9 | 56.3 | 19 |
| Procedure N°1 | 2) HES | 5111.5 | 906.3 | 42.2 | 18 |
| | 3) Swab+HES | **5188.7** | **589.0** | 1.5 | 11 |
| 3) Lab | 1) Swab+HES | 4932.8 | 687.0 | 69.6 | 14 |
| Procedure N°2 | 2) 6 LES | **7092.1** | **679.4** | 30.4 | 10 |
| | 3) HES | 5754.0 | 565.8 | 0 | 10 |

* bold-faced data – the highest ATP value of the biofilm sample, determined in the stages of the tested treatment procedure; RSD – relative standard deviation.

**Table 3. Effectiveness of biofilm removal from PEx material depending on the treatment procedure (in 1st trial)**

| Treatment Procedure | Stages of procedure | Attached biomass pgATP/cm$^2$ | stdev | Efficiency of removal, % | RSD, % |
|---|---|---|---|---|---|
| 1) BPP - Van der Kooij | 1) 6 LES | 54.9 | 14.1 | 99.6 | 26 |
| *et al.* (2006) | 2) HES | 52.7 | 14.4 | 0 | 27 |
| | 3) Swab+HES | **55.1** | **18.7** | 0.4 | 34 |
| 2) Lab | 1) 6 LES | 40.8 | 10.4 | 76.9 | 26 |
| Procedure N°1 | 2) HES | 46.3 | 8.7 | 10.3 | 19 |
| | 3) Swab+HES | **53.1** | **8.1** | 12.7 | 15 |
| 3) Lab | 1) Swab+HES | 115.0 | 45.3 | 79.1 | 39 |
| Procedure N°2 | 2) 6 LES | 143.0 | 58.9 | 19.2 | 41 |
| | 3) HES | **145.4** | **68.6** | 1.7 | 47 |

* bold-faced data – the highest ATP value of the biofilm sample, determined in the stages of the tested treatment procedure; RDS RSD – relative standard deviation.

Statistical comparison between the procedures for biofilm removal from the surface of PVCp material Rapigel (Table 2) showed that the original BPP procedure and the Lab procedure N°2 had similar effectiveness. The significance test had t-values of 1.87 (after 1st stage), 0.48 (after 2nd stage) and 1.46 (after 3rd stage) lower than the critical t value of 2.07 (at degree of freedom n = 22 and P = 0.05).

The difference between the effectiveness of the Lab procedure N°1 and the other procedures was statistically significant at every stage of treatment and the ensured biofilm detachment was lower. The significance test between Lab procedure N°1 and the BPP procedure showed t-values of 15.10 (after 1st stage), 5.14 (after 2nd one) and 5.22 (after 3rd one), while between Lab procedures N°1 and N°2 the experimental t-values were 8.26 (after 1st stage), 6.33 (after 2nd one) and 2.5 (after 3rd one) when the critical t value was 2.07. For all experiments the repeatability of analyses was similar with a RSD ranging from 9 to 19%.

The LES treatment as a first stage of the original BPP procedure ensured detachment of most biofilm biomass (about 93%), while the both Lab procedures reached the same effectiveness after the second stage of treatment. For all 3 procedures the third stage had no effect.

The LES treatment of the BPP procedure fully removed the biofilm from the PEx material (Table 3) having low biofilm formation potential, while the HES and swabbing had contribution for biofilm removal during the both Lab procedures. In this trial no significant difference was found between biofilm amounts removed by BPP procedure and Lab procedure N°1, when the t-value was 1.36 (after 2nd stage) or 0.09 (after 3rd one), although after the 1st stage it was 4.52. Determinations of biofilm on PEx material had higher RSD in comparison with the evaluation of PVCp material having higher biofilm biomass.

The treatment by laboratory procedure N°2 gave higher result for the total attached biomass removed, but probable reason could be the higher microbial growth on the surface of test pieces in this single jar. Because of that, the comparison between these 3 test procedures during the second trial was carried out analysing 2 test pieces from each jar (total – 6 samples) to avoid probability for difference between biofilms' growth in separate jars. The results from the second trial (Table 4) showed big difference between biofilms on the test pieces of PVCp (mark ARIANNA) in each separate jar, demonstrated by the high RSD (69-80%) of the attached biomass values. However, this significant biofim heterogeneity might be a feature of that particular PVCp material (trade mark ARIANNA).

**Table 4. Effectiveness of biofilm removal from PVCp material (trade mark ARIANNA) depending on the treatment procedure (in 2nd trial)**

| Treatment Procedure | Stages of procedure | Attached biomass pgATP/cm$^2$ | stdev | Efficiency of removal, % | RSD, % |
|---|---|---|---|---|---|
| 1) BPP - Van der Kooij | 1) 6 LES | 2183.1 | 1598.8 | 88.5 | 73 |
| *et al.* (2006) | 2) HES | 2415.5 | 1663.3 | 9.4 | 69 |
| | 3) Swab+HES | **2467.2** | 1782.8 | 2.1 | 72 |
| 2) Lab | 1) 6 LES | 2366.1 | 1891.7 | 99.7 | 80 |
| Procedure N°1 | 2) HES | 2310.4 | 1693.9 | 0 | 73 |
| | 3) Swab+HES | **2372.3** | 1642.6 | 0.3 | 69 |

* bold-faced data – the highest ATP values of the biofilm sample, determined on the stages of the tested treatment procedure; RSD – relative standard deviation.

Comparison between the effectiveness of BPP procedure and Lab procedure N°1 (Table 4) showed insignificant difference between biofilm amounts removed after all stages of treatment of the PVCp material with the t-test values from 0.14 to 0.27 (degree of freedom, n = 22 at P = 0.05). The similar results were found comparing between the effectiveness of all procedures for biofilm removal from surface of the PEx material (Table 5). The t-values of the significance test between the BPP procedure and Lab procedure N°1 were 0.1, 2.07, 0.1 (degree of freedom n = 18 at P = 0.05), t-values between BPP procedure and Lab procedure N°2 were 0.11, 2.04, 0.05 (degree of freedom n = 18; P = 0.05) and t-values between Lab procedure N°1 and N°2 were 0.02, 0.23 or 0.67. The RSD was in the range of 12-33 %.

**Table 5. Effectiveness of biofilm removal from PEx material depending on the treatment procedure (in 2nd trial)**

| Treatment Procedure | Stages of procedure | Attached biomass pgATP/cm$^2$ | stdev | Efficiency of removal, % | RSD, % |
|---|---|---|---|---|---|
| 1) BPP - Van der Kooij | 1) 6 LES | 53.9 | 10.0 | 91.6 | 19 |
| *et al.* (2006) | 2) HES | 43.6 | 7.9 | 0 | 18 |
| | 3) Swab+HES | **58.8** | 7.3 | 8.4 | 12 |
| 2) Lab | 1) 6 LES | 53.4 | 13.9 | 84.8 | 26 |
| Procedure N$^o$1 | 2) HES | 53.5 | 14.6 | 0.2 | 27 |
| | 3) Swab+HES | **63.0** | 17.3 | 15.0 | 27 |
| 3) Lab | 1) Swab+HES | 53.3 | 17.5 | 90.9 | 33 |
| Procedure N$^o$2 | 2) 6 LES | 56.3 | 17.6 | 5.1 | 31 |
| | 3) HES | **58.6** | 16.4 | 3.9 | 28 |

* bold-faced data – the highest ATP values of the biofilm sample, determined in the stages of the tested treatment procedure; RSD – relative standard deviation

The results from the second trial shows that all compared procedures had similar effectiveness of biofilm removal from the test pieces. The LES treatment ensured detachment of about 90 % of biofilm biomass. The HES and swab stages guaranteed for removal of the rest of attached biomass, less of 10 %. No significant difference was found between effectiveness of the biofilm removal from solid (such as PEx) or soft (as PVCp) plastic pipe materials.

It could be summarized that no significant difference was found between the compared treatment procedures for biofilm removal in the second trial concerning their effectiveness. Under the Lab procedures N$^o$1 and N$^o$2 the separate treatments carried out in the permanent Milli-Q water volume created work facilities and were less time consuming. No matter what is the first stage of the treatment procedure it enables for removal of about or more than 90% of biofilm mass.

## 3.2. The BPP of pipe materials determined under two S/V contact ratios

### 3.2.1. Planktonic biomass concentration in the tests water in contact with pipe materials

*During the first experiment for determining the BPP of pipe materials*

The bacterial growth in the test water in contact with the tested pipe materials was measured as planktonic biomass (PB) concentration after 4, 5, 8, 12 and 16 weeks of incubation and are presented in Fig.2.

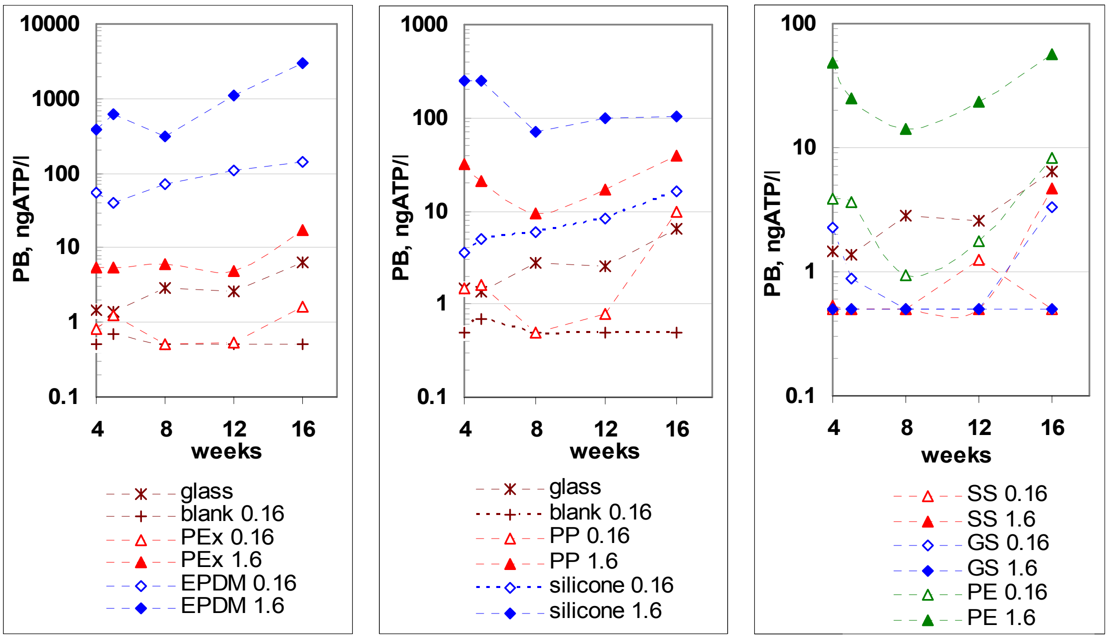


**Fig.2. Dynamics of planktonic biomass (PB) concentration in the 1st trial of the BPP test**

Weekly measurements of the planktonic biomass concentrations showed that the tested pipe materials differed in their capacity to release substances promoting microbial growth in the test water. The data showed that the PB concentration in the waters contacting with all plastic pipe materials under the S/V contact ratio of 0.16 $cm^{-1}$ is lower than under the S/V ratio of 1.6 $cm^{-1}$. Only metal materials (SS and GS) were an exception. Their PB concentration at the S/V ratio of 1.6 $cm^{-1}$ in most cases was under 0.5 ng ATP/l, the detection limit of the ATP method.

The PB concentration in the test waters varied significantly in the course of the BPP test. Its dynamics in water contacting with PP and PE tends to a decrease until the 8th week of the test period and followed by a gradual increase with the highest value on the 16th week of incubation. Only silicone showed a decrease of the PB concentration under the S/V ratio of 1.6 $cm^{-1}$, while the PB in test water contacting with EPDM material increased. These results differ from our previous findings (Tsvetanova & Hoekstra, 2008) observing the highest planktonic growth during the first 4-5 weeks of contact for all materials and followed by a very slow decrease to a relatively stable planktonic growth's level.

*During the second experiment for determining the BPP of pipe materials*

During the second trial the PB concentrations in the test waters also were higher under the S/V ratio of 1.6 $cm^{-1}$ (Fig.3). The PB values of the flexible plastic connections as EPDM and PVCp varied during the test period more than the PB values of other plastic materials. The PB concentration could be an important parameter from consumers' exposure point of view because it is an indicator of immediate impact of the pipe products on water quality.

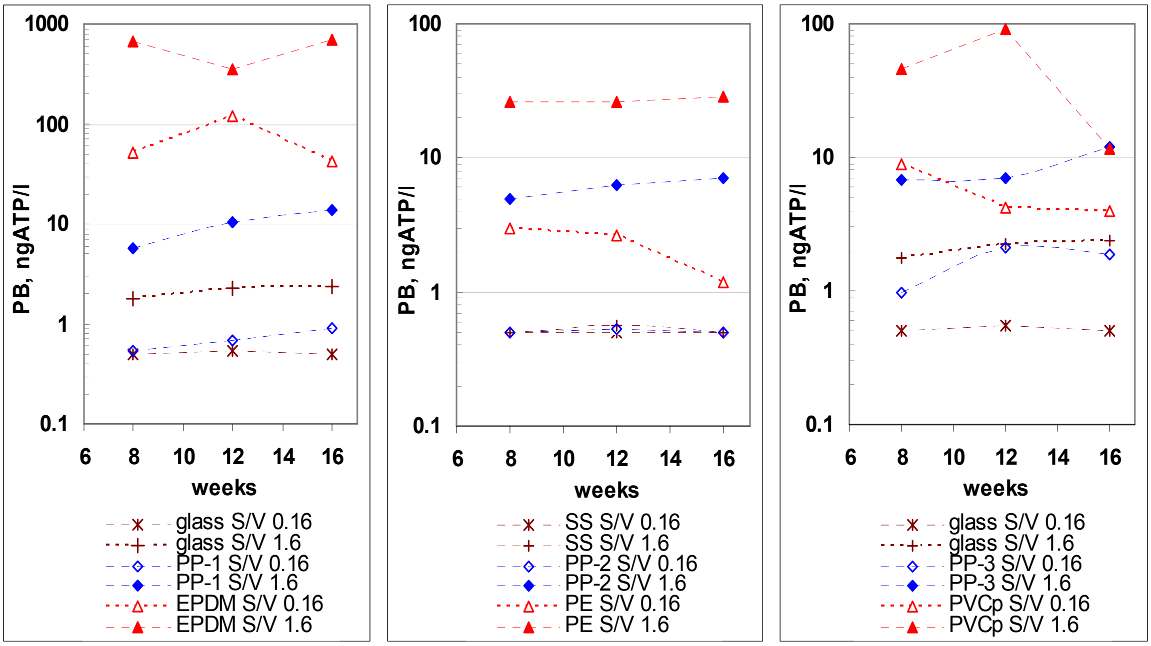


**Fig.3. Dynamics of the planktonic biomass (PB) concentration in the 2nd trial of the BPP test**

During both trials the PB concentration under the S/V ratio of 0.16 $cm^{-1}$ is about one order lower than under the S/V ratio of 1.6 $cm^{-1}$. The data presented reveal stronger impact of the pipe materials on the water quality under higher S/V ratio. Actually, under the S/V ratio of 1.6 $cm^{-1}$ a unit of pipe product's surface contacts with 10-fold smaller water volume and higher product impact is a result of larger amount of substances supporting microbial growth. Where as diffusion depends on concentration gradients, the saturation could not be reached in the 7 days incubation period during which bacteria consume the substances in water and their depletion maintains the driving force for diffusion process.

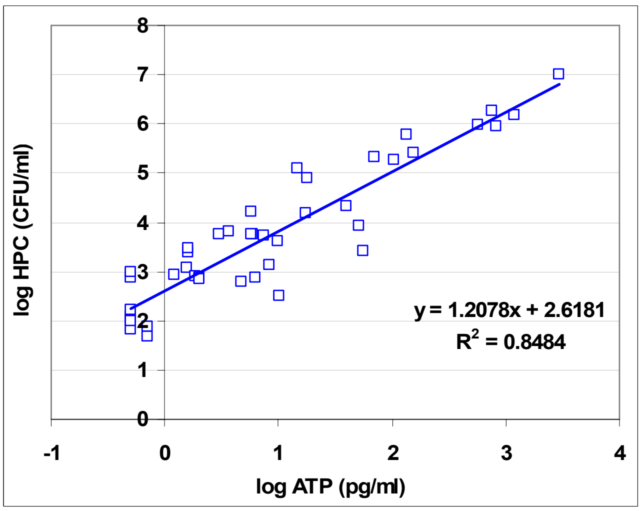


**Fig.4. Relation between HPC bacteria and planktonic biomass (PB) concentration in the test waters after 16 weeks of contact with tested materials at both S/V ratios**

Simultaneous measurements of the PB concentration and the number of culturable HPC bacteria in the test waters on the 16th week of both BPP trials showed (Fig.4) that ATP values correlated well with the HPC values and the HPC/ATP ratio is about $6x10^3$ CFU/pg ATP. The results correspond with the findings of Van der Kooij & Veenendaal (2001) for HPC/ATP ratio about $10^3$ CFU/pg ATP and conclusion that only a fraction of bacterial cells are culturable.

### 3.2.2. Dynamics of Biomass Production

The dynamics of the biomass production (BP) of the tested pipe materials during the first trial for determination of their BPP is presented at the Fig.5 and in details in Tables 6 and 7. Detailed primary data are enclosed in Table III of *Appendix*. Independently of the S/V contact ratio, the BP values showed some changes in the course of the test as it was already shown for the planktonic biomass concentrations. At the S/V ratio of 0.16 $cm^{-1}$, the BP of most materials (SS, GS, PP, PEx and silicone) increased during the test period having the highest values on the 16th week. The same materials had more stable behaviour at ratio of 1.6 $cm^{-1}$, only the biomass production of SS and EPDM materials increased during the test period.

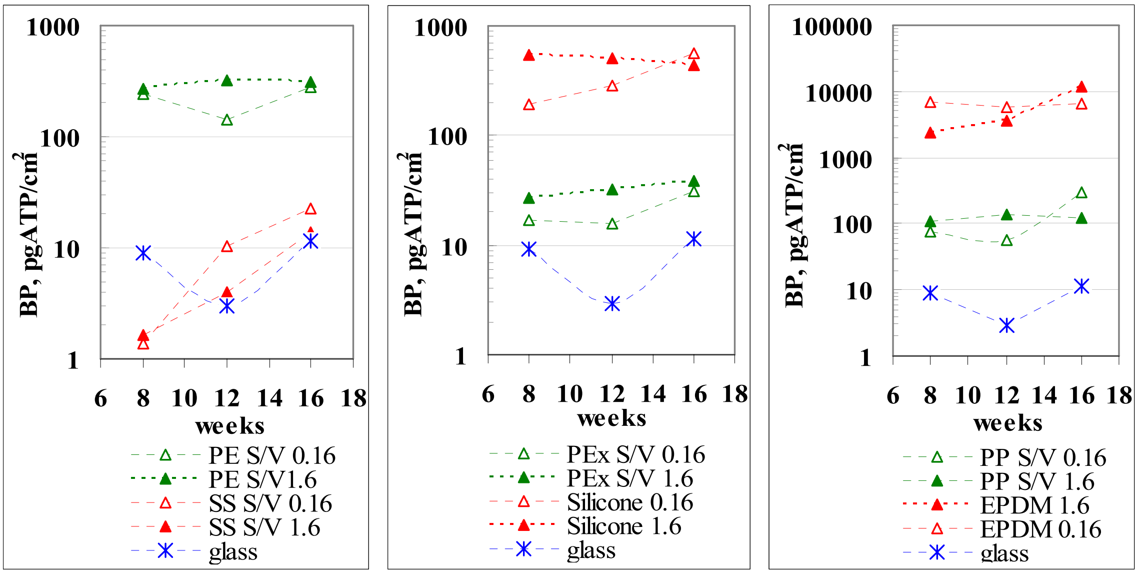


**Fig.5. Dynamics of Biomass Production, BP, during the 1st trial for assessment of the BPP of pipe materials in contact with drinking water under the S/V ratios of 0.16 $cm^{-1}$ or 1.6 $cm^{-1}$**

As a result of the variations of the BP during the test, the coefficients of variation (i.e. relative standard deviation, RSD) of the BPP values of the plastic materials ranged from 18 to 54 % at the S/V ratio of 0.16 $cm^{-1}$, with exception of PP-1 (Table 6). At the S/V ratio of 1.6 $cm^{-1}$ (Table 7) the RSD of the BPP of the tested plastic materials was 20-44 %, with exception of EPDM. The metal materials (negative control) showed the highest RDS which is related to their very low BPP and the analysis being close to detection limit of ATP.

The RSD for some plastic materials (PE, PEx) is close to 20%, the target value for the BPP test, as it was discussed by Van der Kooij *et al.* (2006) after the inter-laboratory validation of the BPP method at 5 Member State's laboratories. In that exercise the participating laboratories had performed the BPP test of 5 pipe materials with average RSD of 24-40%, but individual values varied in much more wider interval.

**Table 6. Biomass production, biomass production potential and corrected biomass production potential ($BPP_{corr}$) of the pipe materials under S/V ratio of 0.16 $cm^{-1}$ ($1^{st}$ trial)**

| Sample N° | Material | Biomass production, BP, $pgATP/cm^2$ | | | BPP, $pgATP/cm^2$ | Stdev | RSD, % | $BPP_{corr}$ $pgATP/cm^2$ |
|---|---|---|---|---|---|---|---|---|
| | | 56 d | 84 d | 112 d | | | | |
| 1 | Blank | 3 | 3 | 9.5 | **5.2** | 3.7 | 72.5 | |
| 7 | GS | 5.6 | 5.8 | 24.2 | **11.8** | 8.2 | 69.2 | 4.0 |
| 8 | | 8.2 | 7.0 | 20.3 | | | | |
| 12 | SS | 1.4 | 10.2 | 22.6 | **11.4** | 10.7 | 93.6 | 3.5 |
| 14 | PE | 241.8 | 142.3 | 275.5 | **219.9** | 69.2 | 31.5 | 212.0 |
| 19 | PP-1 | 75.9 | 57.7 | 293.7 | **142.5** | 131.3 | 92.2 | 134.6 |
| 24 | PEx | 13.4 | 17.1 | 28.1 | **21.3** | 8.3 | 38.9 | 13.4 |
| 25 | | 20.0 | 14.7 | 34.4 | | | | |
| 29 | Silicone | 202.9 | 342.9 | 467.2 | **350.6** | 189.1 | 53.9 | 342.7 |
| 30 | | 187.7 | 231.8 | 670.9 | | | | |
| 35 | EPDM | 5932.0 | 5877.1 | 7823.6 | **6448.1** | 1175.7 | 18.2 | 6440.2 |
| 36 | | 7978.4 | 6002.9 | 5074.3 | | | | |

$BPP_{corr}$ – BPP value corrected by value of glass negative control; RSD – relative standard deviation; each BP value is average of two repetitive ATP measurements of the PB and the AB.

The data for dynamics of the biomass production in the $2^{nd}$ trial of the BPP test are presented in Fig.6 and in detail in the Tables 8 and 9. Detailed primary data are enclosed in Table IV of *Appendix*. Under both compared S/V ratios, the BP values varied during the test period, depending on the plastic materials. The BP went down for PEx, PVCp, and PP-2 or slightly increased for PP-1 (at the S/V of 0.16 $cm^{-1}$) and PP-3 (at the S/V of 1.6 $cm^{-1}$). The relative standard deviation RSD of the BPP values ranged from 12 to 46 % at the S/V of 0.16 $cm^{-1}$ and from 18 to 48 % at the S/V of 1.6 $cm^{-1}$. The materials having low BPP, such as SS and glass, had the highest values of RSD.

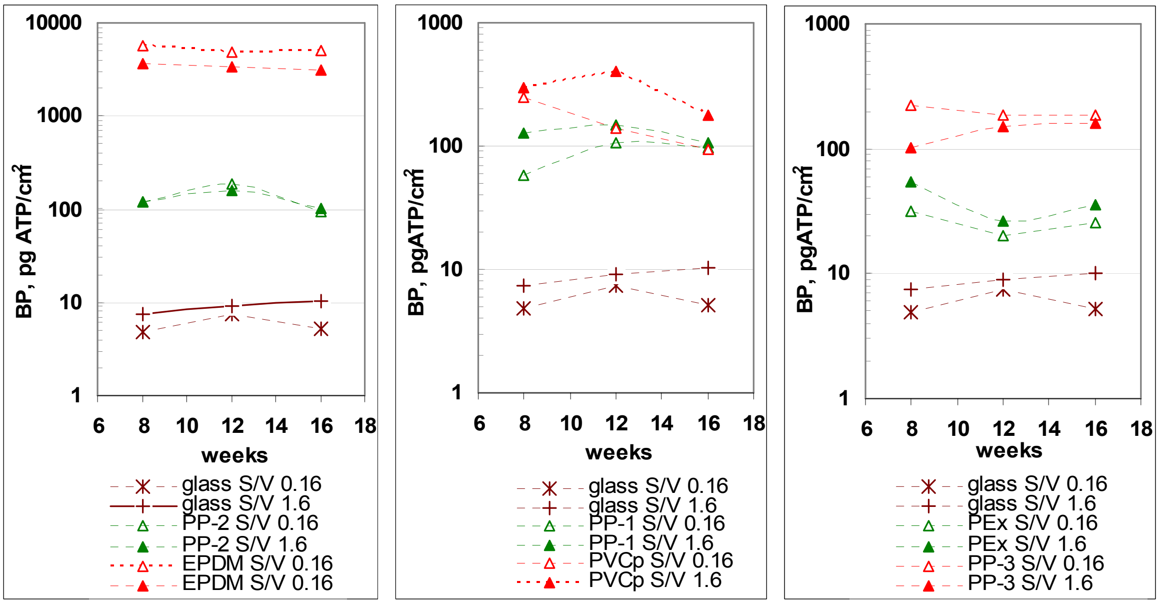


**Fig.6. Dynamics of Biomass Production (BP) during the $2^{nd}$ trial for assessment of the BPP of pipe materials in contact with drinking water under the S/V ratios of 0.16 $cm^{-1}$ or 1.6 $cm^{-1}$**

**Table 7. Biomass production, biomass production potential and corrected biomass production potential ($BPP_{corr}$) of the pipe materials under the S/V ratio of 1.6 $cm^{-1}$ during the BBP test (1st trial)**

| Sample | Material | Biomass production BP, pgATP/$cm^2$ | | | BPP, pgATP/$cm^2$ | Stdev | RSD, % | $BPP_{corr}$, pgATP/$cm^2$ |
|---|---|---|---|---|---|---|---|---|
| | | 56th day | 84th day | 112th day | | | | |
| 2 | Blank | 3 | 3 | 3.4 | **3.2** | 0.3 | | |
| 3 | | 3 | 3 | 3.6 | | | | |
| 4 | Glass | 12.8 | 1.7 | 10.6 | **7.9** | 4.3 | 54.1 | |
| 5 | | 6.6 | 3.7 | 13.1 | | | | |
| 6 | | 7.8 | 3.5 | 10.9 | | | | |
| 9 | GS | 1.5 | 1.2 | 1.1 | **1.2** | 0.2 | 16.1 | **0** |
| 10 | | 1.5 | 1.0 | 1.1 | | | | |
| 11 | | 1.5 | 1.0 | 1.3 | | | | |
| 13 | SS | 1.6 | 4.0 | 13.9 | **6.5** | 6.5 | 99.8 | **0** |
| 15 | PE | 258.9 | 352.0 | 451.9 | **300.2** | 60.8 | 20.2 | **292.4** |
| 16 | | 247.2 | 357.5 | 246.5 | | | | |
| 17 | | 264.7 | 271.5 | 276.9 | | | | |
| 18 | | 310.8 | 303.4 | 261.6 | | | | |
| 20 | PP-1 | 113.9 | 221.3 | 125.1 | **124.0** | 33.8 | 27.3 | **116.1** |
| 21 | | 111.4 | 104.5 | 154.2 | | | | |
| 22 | | 99.6 | 105.0 | 108.4 | | | | |
| 23 | | 122.3 | 108.9 | 113.4 | | | | |
| 26 | PEx | 24.7 | 27.2 | 38.8 | **32.3** | 6.5 | 20.1 | **24.5** |
| 27 | | 22.9 | 30.2 | 36.0 | | | | |
| 28 | | 32.2 | 40.1 | 39.0 | | | | |
| 31 | Silicone | 845.7 | 267.9 | 377.3 | **496.4** | 220.6 | 44.4 | **488.5** |
| 32 | | 852.1 | 702.3 | 497.4 | | | | |
| 33 | | 267.3 | 453.6 | 491.2 | | | | |
| 34 | | 180.5 | 618.8 | 402.5 | | | | |
| 37 | EPDM | 2213.6 | 3174.6 | 15761.8 | **5882.1** | 4842.7 | 82.3 | **5874.2** |
| 38 | | 3326.9 | 2767.6 | 10402.9 | | | | |
| 39 | | 1466.4 | 4575.4 | 9249.6 | | | | |

$BPP_{corr}$ – BPP value corrected by value of glass negative control; RSD - coefficient of variation (relative standard deviation); each BP value is average of two repetitive ATP measurements of the PB and the AB concentrations

**Table 8**. **Biomass production, biomass production potential and corrected biomass production potential ($BPP_{corr}$) of the pipe materials under the S/V ratio of 0.16 $cm^{-1}$ during the BPP test (2nd trial)**

| Material | Biomass production BP, pgATP/$cm^2$ | | | BPP, pgATP/$cm^2$ | Stdev | RSD, % | BPPcorr, pgATP/$cm^2$ |
|---|---|---|---|---|---|---|---|
| | 56th day | 84th day | 112th day | | | | |
| blank | 3.6 | 3 | 3.0 | **3.2** | 0.3 | 10.6 | |
| glass | 4.7 | 8.9 | 5.6 | **5.8** | 3.2 | 55.6 | |
| | 5.0 | 6.1 | 4.7 | | | | |
| SS | 7.4 | 4.9 | 18.4 | **8.1** | 5.1 | 63.4 | **2.3** |
| | 6.7 | 4.9 | 6.4 | | | | |
| PE | 167.4 | 164.4 | 116.9 | **124.9** | 40.7 | 32.6 | **119.1** |
| | 120.8 | 124.6 | 55.4 | | | | |
| PEx | 32.2 | 22.6 | 23.2 | **25.7** | 5.7 | 22.1 | **19.9** |
| | 30.8 | 17.3 | 28.1 | | | | |
| PP-1 | 48.7 | 98.4 | 92.6 | **87.1** | 24.3 | 27.9 | **81.2** |
| | 67.5 | 114.8 | 100.3 | | | | |
| PP-2 | 118.1 | 134.9 | 100.8 | **133.8** | 17.1 | 12.7 | **128.0** |
| | 127.9 | 233.7 | 87.6 | | | | |
| PP-3 | 172.3 | 172.0 | 131.5 | **197.3** | 23.5 | 11.9 | **191.5** |
| | 267.9 | 204.7 | 235.4 | | | | |
| EPDM | 4310.2 | 3845.9 | 2955.4 | **3385.3** | 1092.1 | 32.3 | **3379.5** |
| | 3106.0 | 2915.8 | 3178.6 | | | | |
| PVCp | 240.7 | 144.2 | 65.0 | **168.7** | 73.6 | 45.8 | **156.9** |
| | 262.1 | 174.4 | 126.1 | | | | |

$BPP_{corr}$ – BPP value corrected by value of glass negative control; RSD - coefficient of variation (relative standard deviation); each BP value is average of two repetitive ATP measurements of the PB and AB concentrations.

**Table 9. Biomass production, biomass production potential and corrected biomass production potential ($BPP_{corr}$ ) of the pipe materials under S/V ratio of 1.6 $cm^{-1}$ during the BPP test (2nd trial)**

| Material | Biomass production BP, pgATP/$cm^2$ | | | BPP, pgATP/$cm^2$ | Stdev | RSD, % | BPPcorr, pgATP/$cm^2$ |
|---|---|---|---|---|---|---|---|
| | 56th day | 84th day | 112th day | | | | |
| blank | 3.6 | 3 | 3 | **3.2** | 0.3 | 10.6 | |
| glass | 10.7 | 12.2 | 13.7 | **9.2** | 3.7 | 39.8 | |
| | 4.2 | 6.0 | 8.7 | | | | |
| SS | 3.9 | 2.2 | 13.8 | **4.8** | 4.4 | 91.6 | **0** |
| | 3.7 | 2.1 | 3.3 | | | | |
| PE | 152.3 | 143.2 | 130.0 | **236.3** | 114.1 | 48.3 | **227.0** |
| | 436.9 | 142.7 | 407.8 | | | | |
| | 160.6 | 368.1 | 176.5 | | | | |
| | 156.8 | 259.4 | 300.7 | | | | |
| PEx | 29.3 | 23.3 | 31.6 | **39.0** | 17.9 | 45.9 | **29.8** |
| | 46.1 | 15.6 | 33.1 | | | | |
| | 70.2 | 30.7 | 47.7 | | | | |
| | 74.8 | 36.1 | 29.7 | | | | |
| PP-1 | 134.7 | 118.3 | 103.1 | **137.9** | 24.2 | 17.5 | **128.6** |
| | 131.4 | 167.8 | 169.2 | | | | |
| | 134.0 | 161.6 | 109.5 | | | | |
| | 110.6 | 152.6 | 161.8 | | | | |
| PP-2 | 129.3 | 173.9 | 102.2 | **129.9** | 45.2 | 34.8 | **120.6** |
| | 93.1 | 122.3 | 120.3 | | | | |
| | 108.4 | 244.1 | 85.8 | | | | |
| | 142.9 | 106.3 | | | | | |
| PP-3 | 83.8 | 133.0 | 179.5 | **137.4** | 32.6 | 23.7 | **128.1** |
| | 101.2 | 130.0 | 169.7 | | | | |
| | 120.0 | 167.3 | 120.3 | | | | |
| | 105.0 | 171.2 | 167.5 | | | | |
| EPDM | 5518.0 | 4391.6 | 5380.1 | **5160.0** | 1177.3 | 22.8 | **5150.8** |
| | 6010.5 | 3089.7 | 3787.8 | | | | |
| | 5681.9 | 6838.3 | 5742.2 | | | | |
| PVCp | 261.7 | 343.8 | 155.7 | **295.1** | 143.3 | 48.6 | **285.9** |
| | 380.4 | 620.7 | 150.0 | | | | |
| | 261.6 | 246.6 | 235.8 | | | | |

$BPP_{corr}$ – BPP value corrected by value of glass negative control; RSD - coefficient of variation (relative standard deviation); each BP value is average of two repetitive ATP measurements of the PB and the AB concentrations.

In summary, the BP of the tested pipe materials at both S/V ratios varied more or less during the test period depending on the material type. The BP of the tested pipe materials showed lower coefficients of variation in the second trial for assessment of their BPP, particularly at the S/V ratio of 0.16 $cm^{-1}$.

### 3.2.3. Biomass production potential of the tested pipe materials

The BPP of the pipe materials tested under both S/V ratios in the first trial (and already presented in Tables 6,7) are shown graphically at Fig.7, while the BPP values determined in the second trial (Tables 8,9) are shown at Fig.8.

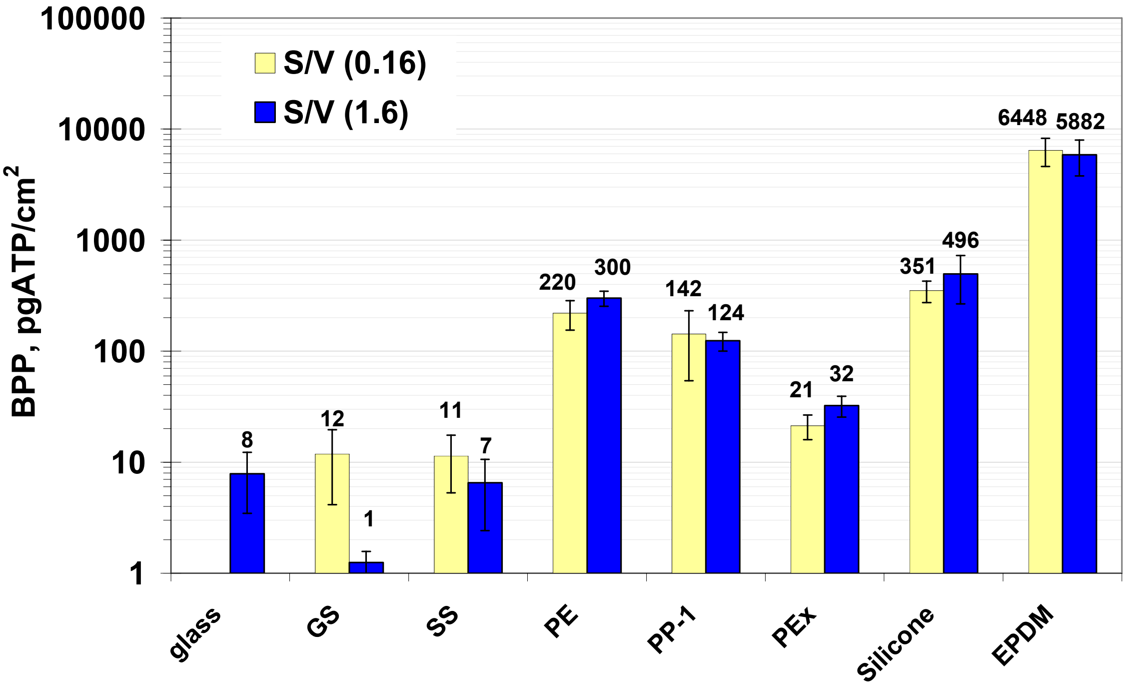


**Fig.7. Biomass production potential of the pipe materials in contact with test water under the S/V ratios of 0.16 $cm^{-1}$ or 1.6 $cm^{-1}$ (1st trial);** number above bar – the BPP value of the material under the test

Under both S/V ratios the behaviour of the tested pipe materials depended on the material type. In the first trial the BPP values of PE and PEx pipes and silicone tubing were significantly higher at the S/V ratio of 1.6 $cm^{-1}$, while for SS, PP-1 and EPDM materials the difference between BPP values was insignificant. Only the GS showed higher BPP values under the lower S/V ratio. All statistical data from significance t-test between the BPP (and their component data, as PBP and AB) of the tested pipe materials, based on all individual ATP measurements, are presented in Table V in *Appendix*.

During the second trial the BPP values of most tested materials (PE, PEx, PP-1, EPDM and PVCp) trend to be significantly higher at the higher S/V ratio. Only the BPP values of SS and PP-2 were similar under the both ratios, while for PP-3 the higher BPP was found at the lower S/V ratio.

If all data of the two trials are grouped no significant difference between the both S/V ratios is found for SS, PP-1, PP-2 and EPDM (see Table VI in *Appendix*).

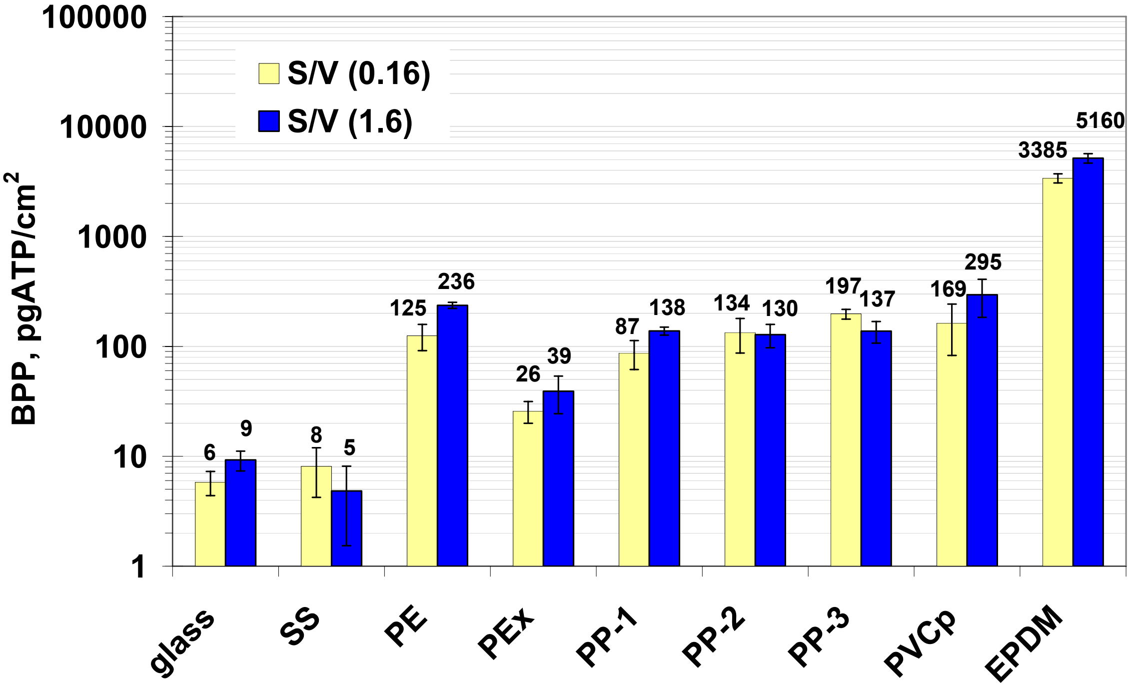


**Fig.8. Biomass production potential of the pipe materials in contact with test water under the S/V ratios of 0.16 cm$^{-1}$ or 1.6 cm$^{-1}$ (2$^{nd}$ trial);** number above bar – the BPP value of the tested material

Reproducibility of the test, defined as the coefficient of variation of reproducibility ($CV_R$) was calculated on the base of the BPP values for one and the same material during the both consecutive trials. Data are presented in Table 10 and in details in Table VII in *Appendix*. The $CV_R$ varied from 13% to 44% for the materials tested at the S/V ratio of 0.16 cm$^{-1}$. The test carried out at higher S/V ratio showed a better reproducibility in the range from 7% to 21%, due to the larger number of test waters and the double number of test pieces analysed.

**Table 10. Coefficient of variation of reproducibility ($CV_R$) of the BPP of the same pipe materials determined in two successive trials**

| Material | $CV_R$ of BPP, % | |
|---|---|---|
| | S/V of 0.16 cm$^{-1}$ | S/V of 1.6 cm$^{-1}$ |
| glass | | 11 |
| SS | 24 | 21 |
| PE | 39 | 17 |
| PP-1 | 34 | 7 |
| PEx | 13 | 13 |
| EPDM | 44 | 9 |

Reproducibility of determinations of planktonic biomass concentrations in test waters in contact at the S/V ratio of 0.16 cm$^{-1}$ was between 13-33%, with exception of PP-1 (Table 11). Detailed data can be found in Table VIII in *Appendix*. The reproducibility of the PB determinations of the pipe materials at the S/V ratio of 1.6 cm$^{-1}$ was less than the reproducibility of the BPP determinations at the same S/V ratio and $CV_R$ varied from 23% to 45% (with exception of SS).

**Table 11. Coefficients of variation of reproducibility ($CV_R$) of the PB concentrations in the test water in contact with the same materials in two successive trials under both S/V contact ratios**

| Material | $CV_R$ of PB concentration, % | |
|---|---|---|
| | S/V of 0.16 cm-1 | S/V of 1.6 $cm^{-1}$ |
| glass | | 29 |
| SS | 13 | 82 |
| PE | 33 | 11 |
| PP-1 | 96 | 45 |
| PEx | 18 | 23 |
| EPDM | 30 | 40 |

The data from the BPP test are presented in detail in Table 12 and 13. The value of $BPP_{corr}$ is calculated by subtraction of the BPP of glass to compensate the effect of test water and to obtain the net BPP value of the material. The tested materials showed large differences in the $BPP_{corr}$, hence, the test method is robust to differentiate between growth support properties of pipe materials. The BPP values were similar or lower than ones found in model or real drinking water distribution systems (DWDS) due to the effects of the chemical and hydraulic water conditions.

**Table 12. The parameters measured in the BPP test of pipe materials under the S/V ratio of 0.16 $cm^{-1}$**

| Material | Planktonic Biomass concentration, PB, ngATP/l | Planktonic Biomass production, PBP, pgATP/$cm^2$ | Attached Biomass production, AB, pgATP/$cm^2$ | BPP, pgATP/$cm^2$ | $BPP_{corr}$ | PBP/BPP % |
|---|---|---|---|---|---|---|
| **First Test** | | | | | | |
| GS | 1.5 (1.4) | 8.6 (8.4) | 3.2 (1.1) | 11.8 (7.9) | 6 | 73 |
| SS | 0.6 (0.1) | 3.0 (2.1) | 8.4 (8.4) | 11.4 (9.5) | 5 | 26 |
| PE | 3.7 (3.7) | 22.2 (21.8) | 197.7 (52.0) | 219.9 (62.4) | 214 | 10 |
| PP-1 | 3.7 (4.7) | 21.8 (28.0) | 120.6 (90.0) | 142.5 (117.7) | 136 | 15 |
| PEx | 0.9 (0.6) | 5.3 (3.6) | 16 (4.7) | 21.3 (7.9) | 15 | 25 |
| Silicone | 10.1 (4.9) | 60.1 (29.4) | 290.4 (153.0) | 350.6 (180.3) | 345 | 17 |
| EPDM | 108.1 (34.4) | 639.0 (197.0) | 5809.0 (1172.5) | 6448.1(1834.2) | 6442 | 10 |
| **Second Test** | | | | | | |
| glass | 0.5 (0.1) | 3.2 (0.3) | 2.8 (1.6) | 5.8 (1.7) | | 53 |
| SS | 0.5 (0) | 3.0 (0) | 5.0 (4.9) | 8.1 (4.9) | 2 | 48 |
| PE-3 | 2.3 (1.1) | 13.3 (6.3) | 111.6 (33.6) | 124.9 (38.8) | 119 | 11 |
| PEx-2 | 0.7 (0.3) | 4.0 (1.6) | 25.0 (6.1) | 25.8 (5.4) | 20 | 3 |
| PP-1 | 0.7 (0.3) | 4.1 (1.6) | 83 (23.2) | 87.1 (23.6) | 81 | 5 |
| PP-2 | 0.5 (0) | 5.9 (4.8) | 131.4 (51.8) | 134.4 (51.9) | 128 | 2 |
| PP-3 | 1.6 (0.6) | 6.6 (4.2) | 196.0 (51.4) | 197.3 (47.1) | 191 | 1 |
| PVCp | 5.7 (2.5) | 33.3 (15.5) | 138.9 (58.2) | 168.7 (70.3) | 163 | 8 |
| EPDM | 70.7 (47.9) | 386.5 (229.6) | 2998.7 (501.6) | 3385.3 (539.6) | 3379 | 11 |

* Average value and standard deviation (in brackets) are calculated on the base of all ATP measurements (including repetitions).

For example, lower or equal AB values can be found comparing between the data from the BPP test and a biofilm study in a model drinking water system (4 h stagnation; AOC of 17.8 ± 5.7 ac-C/l, biofilm age of 1.5 years) made from one and the same PE material. In this study the biofilm densities were 681(319) pg ATP/cm$^2$ under flow velocity of 0.5 m/s, 822 (341) pg ATP/cm$^2$ under 0.7 m/s or 1592 (853) pg ATP/cm$^2$ under 1 m/s (unpublished data). Hallam et al. (2001) detected biomass density of 670 pg ATP/cm$^2$ or 865 pg ATP/cm$^2$ on the PE pipe section of a real distribution system. This shows the BPP test allows comparison between the net BPP of materials and their effect for bacterial re-growth, while the model studies present the behaviour of pipe materials depending on characteristics of water and operational conditions in distribution systems. The comparison shows that BPP test could be useful means to foresee the contribution of materials to microbial growth potential of the DWDS.

Like in the drinking water distribution systems where biofilm is the main part of the bacterial biomass, the attached biomass (AB) is the main part of total bacterial biomass determined in the BPP test. What will be the bacterial number in drinking water in contact with pipe materials, respectively PB concentration, is important from consumers' exposure point of view. On the base of the recalculated data (Tables 12, 13) for the ratio between the planktonic biomass production (PBP) and total biomass production of the materials (PBP/BPP) it can be found that the PB production of the plastic pipe materials contributed from 8 % to 25 % of the total BPP. The materials characterized by a high BPP and a relative higher PBP/BPP ratio have a higher potential to impair bacteriological water quality. For metals the PBP/BPP ratio is higher (up to 73%), but because of the very low BPP their effect on the water quality is negligible.

**Table 13. The parameters measured in the BPP test of pipe materials under S/V ratio of 1.6 cm$^{-1}$**

| Material | Planktonic Biomass concentration, PB, ngATP/l | Planktonic Biomass production, PBP, pgATP/cm$^2$ | Attached Biomass production, AB, pgATP/cm$^2$ | BPP, pgATP/cm$^2$ | $BPP_{corr}$ | PBP/BPP % |
|---|---|---|---|---|---|---|
| **First test** | | | | | | |
| glass | 3.2 (2.1) | 2.0 (1.3) | 5.4 (3.3) | 7.9 (4.3) | | 31 |
| GS | 0.5 (0) | 0.3 (0) | 0.9 (0.2) | 1.2 (0.2) | 0 | 24 |
| SS | 1.9 (2.2) | 1.4 (1.6) | 5.2 (4.3) | 6.5 (5.8) | 0 | 21 |
| PE | 24.3 (15.7) | 16.1 (10.1) | 281.4 (59.0) | 300.2 (59.7) | 292 | 6 |
| PP-1 | 16.9 (11.1) | 11.9 (7.3) | 111.2 (33.3) | 124.0 (33.2) | 24 | 17 |
| PEx | 7.4 (5.0) | 5.6 (3.6) | 26.7 (4.9) | 32.3 (6.4) | 116 | 10 |
| Silicone | 83.9 (24.3) | 50.1 (14.5) | 441.7 (211.1) | 496.4 (215.9) | 489 | 11 |
| EPDM | 1038.6 (1000.9) | 906 (724.8) | 5009.3 (4089.9) | 5882.1 (4701.0) | 5874 | 15 |
| **Second Test** | | | | | | |
| glass | 2.1 (0.5) | 1.9 (0.9) | 7.4 (3.7) | 9.2 (3.6) | | 20 |
| SS | 0.5 (0.1) | 0.3 (0) | 4.5 (4.2) | 4.8 (4.2) | 0 | 7 |
| PE | 28.2 (17.3) | 16.8 (10.4) | 220.2 (105.9) | 236.3 (112.3) | 227 | 7 |
| PEx | 10.3 (14.0) | 6.6 (8.4) | 37.6 (11.0) | 39.0 (17.6) | 30 | 4 |
| PP-1 | 8.7 (3.4) | 5.0 (2.0) | 135.4 (28.7) | 137.9 (24.2) | 129 | 2 |
| PP-2 | 5.9 (1.4) | 3.7 (1.1) | 126.3 (44.3) | 129.9 (44.1) | 121 | 3 |
| PP-3 | 7.5 (2.8) | 4.3 (1.5) | 132.2 (31.0) | 137.4 (32.1) | 128 | 4 |
| PVCp | 51.9 (33.9) | 30.8 (20.1) | 266.2 (121.4) | 295.1 (139.8) | 286 | 10 |
| EPDM | 577.9 (246.6) | 341.7 (147.5) | 4833.9 (1097.3) | 5160.0 (1145) | 5151 | 6 |

* Average value and standard deviation (in brackets) are calculated on the base of all ATP measurements (including repetitions).

The growth-promoting potential of the products intended to contact with drinking water could be assessed on the base of their BPP, but also on the base of bacterial number in the test waters, measured as PB concentration.

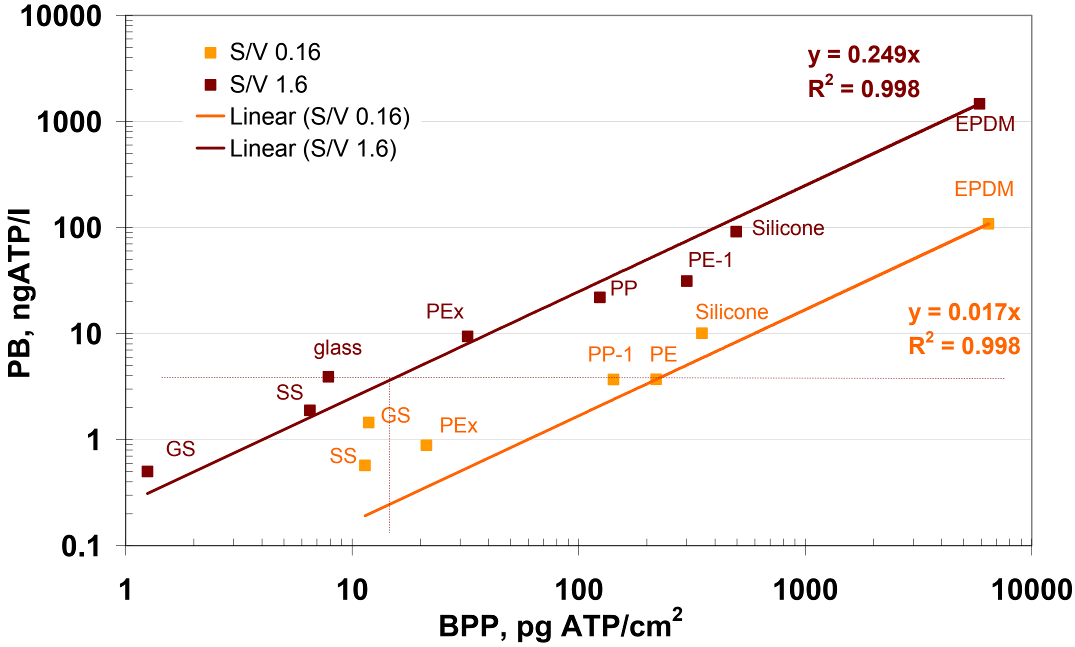


**Fig.9. Relation between the BP concentrations and the BPP of the tested pipe materials under the both S/V ratios in the 1st trial**

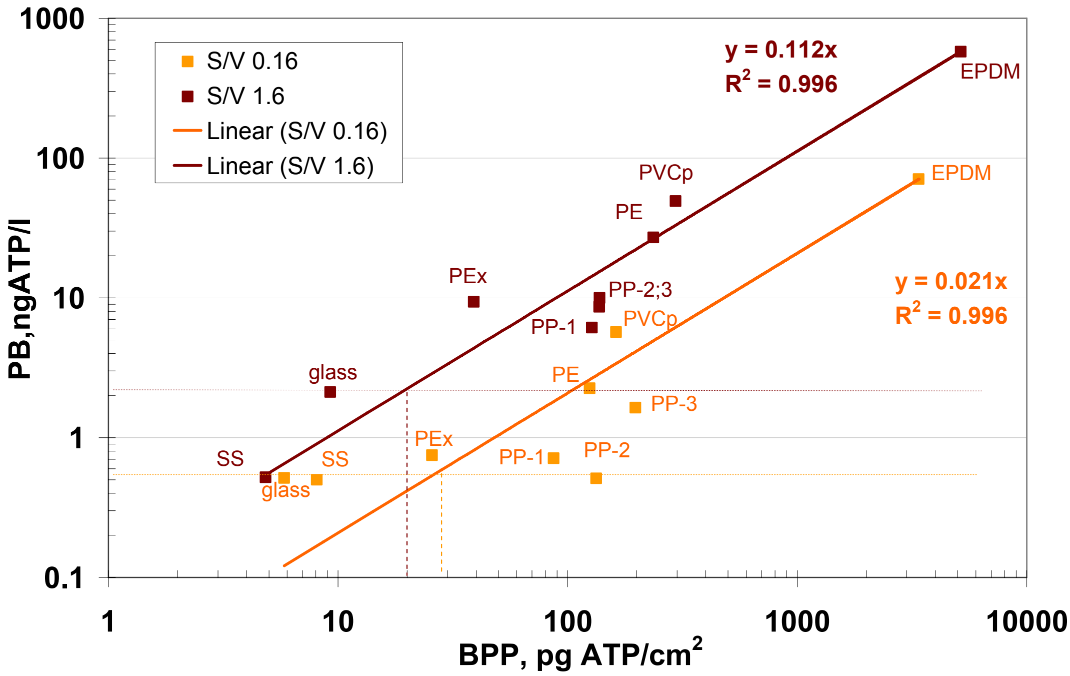


**Fig.10. Relation between the BP concentrations and the BPP of the tested pipe materials under the both S/V ratios in the 2nd trial**

Fig.9 and Fig.10 present the relations detected between the planktonic growth in the test waters measured as PB concentration and the BPP of the tested pipe materials during both experiments for assessment of their growth promoting properties. The comparison between the linear relations found for the growth promoting properties of the CPDW under both S/V ratios shows stronger influence of materials on the water quality under the S/V ratio of 1.6 $cm^{-1}$. It could be indicated by the coefficients of the linear equations (presented on Figures 9 and 10).

At the lower S/V ratio all tested materials promote microbial growth in the test water in comparison with glass negative control, but only PB concentrations of silicone (in first trial) and EPDM (in both trials) exceeded 10 ng/l, the value considered as a limit for biological stability of drinking water. At the higher S/V ratio the PB concentrations of more materials exceeded 10 ng/l, such as PP-1 and silicone (in first trial), PEx and PVCp (in second one), and PE and EPDM (in both trials).

## 4. Discussion

The results from the BPP test presented on Fig.11 confirm that the tested plastic pipe materials had large differences in their capacity to support microbial growth, as has been already observed at S/V contact ratio of 0.16 $cm^{-1}$ (Van der Kooij *et al.*, 2003; 2006; Tsvetanova & Hoekstra, 2008). The $BPP_{corr}$ of the tested pipe materials at both S/V ratios can be ranked in the order

GS < SS < PEx < PP < PE < PVCp < Silicone < EPDM.

The only exception is the similar growth potential of GS and SS at the lower S/V ratio.

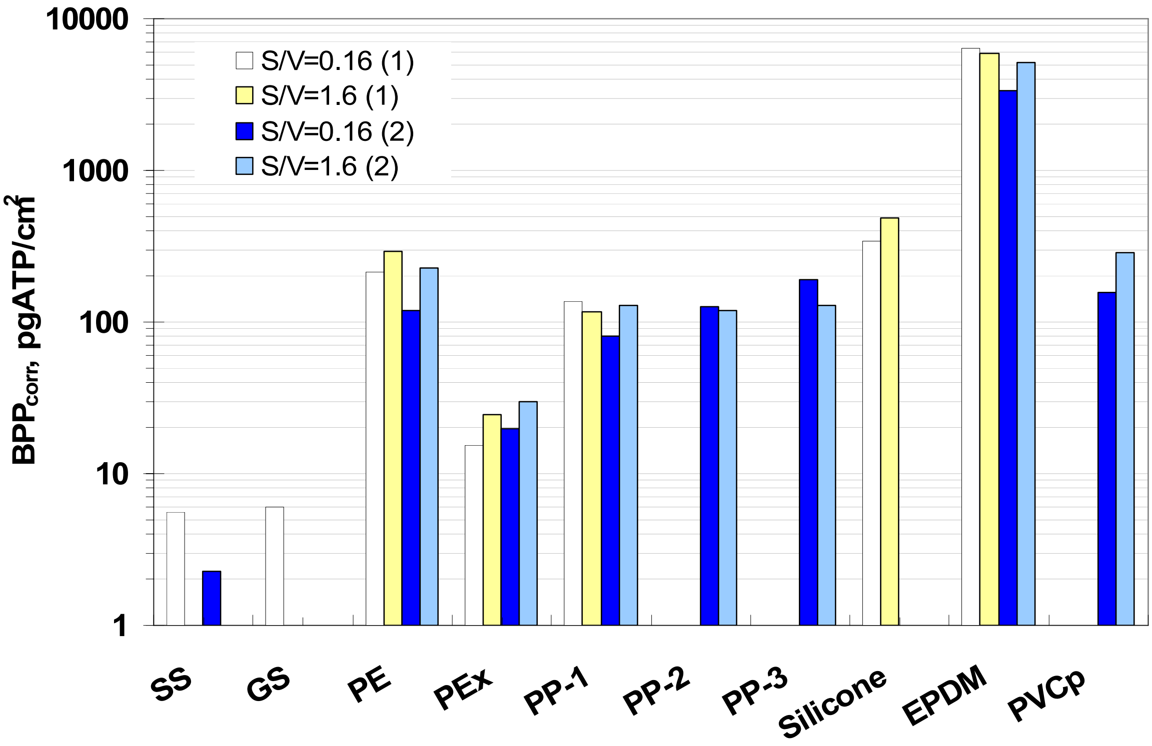


**Fig.11. The $BPP_{corr}$ of the tested pipe materials under the S/V contact ratios of 0.16 $cm^{-1}$ or 1.6 $cm^{-1}$;** in brackets: (1) – data from the first trial; (2) – data from the second trial.

The BPP values of the materials tested were in quite similar ranges under the both S/V ratios: from 21 pg ATP/$cm^2$ for PEx to 6400 pg ATP/$cm^2$ for EPDM at the S/V ratio of 0.16 $cm^{-1}$ and from 39 pg ATP/$cm^2$ to 5900 pg ATP/$cm^2$ for the same materials at the S/V ratio of 1.6 $cm^{-1}$. The BPP of glass controls at the both ratios in the second trial were 5.8 pg ATP/$cm^2$ and 9.2 pg ATP/$cm^2$, as well the BPP values of SS were similar. Only for GS pipe a trend to lower BPP value under the S/V ratio of 1.6 $cm^{-1}$ was found. The release of zinc into test water was most intensive during the first 3 weeks of contact and stabilized at a level of 2-4 mg/l for the S/V ratio of 0.16 $cm^{-1}$, and at 30-80 mg/l for the higher S/V ratio (Fig.12). A toxic effect of Zn ions could be a probable reason for the lowest BPP at S/V of 1.6 $cm^{-1}$, as it was observed for bacterial activity in marine biofilms (Fang *et al.*, 2002). The behaviour of the new GS pipe material in the BPP test was different than in drinking water systems. Silhan *et al.* (2006) found the highest biomass density (measured as ATP or HPC) on again used (old) GS pipe in comparison with plastic pipes. This high microbial growth was explained by adsorption of organic substances on the iron oxides suggesting that the zinc layer was damaged. In contrast to the high biomass density on the surface, the used GS pipe sections showed the lowest HPC in the water compared to the plastic pipes. This is in agreement with our results (Table 15) from the BPP test.

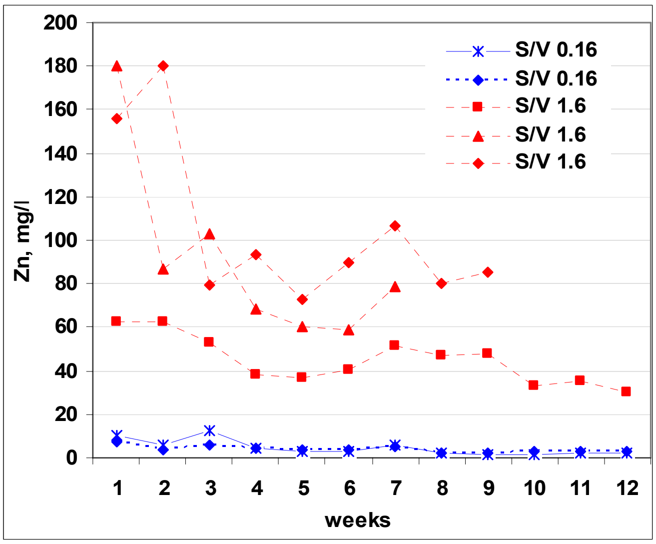


**Fig.12. Concentration of Zn (II) in the test waters in contact with GS material in two repetitive jars of the BPP test at S/V ratio of 0.16 $cm^{-1}$ or in 3 repetitive jars at S/V ratio of 1.6 $cm^{-1}$**

Comparing the individual data at both S/V ratios shows that the BPP values of PE and PEx were significantly higher at the S/V ratio of 1.6 $cm^{-1}$ in both trials whereas silicone and PVCp were higher in their single trial (see *Appendix*; Tables V, VI). The BPP of PP-1 and EPDM at the S/V ratio of 1.6 $cm^{-1}$ was higher in the second trial, but not in the first one. The BPP values of PP-2 were similar at the both S/V ratios and that of PP-3 was even significant lower at higher S/V ratio.

The 10-fold increase of the S/V contact ratio for plastic materials resulted in average increase of the BPP only with 26 % in first trial and 47 % in second trial. However, the statistical comparison between the average BPP values for SS, PE, PEx, PP-1 and EPDM materials pooling the results of both consecutive trials shows that the differences between growth support potentials of each material at both S/V ratios were statistically insignificant (Table 14).

**Table 14. Statistical data from t-test between the BPP of the tested material under the both S/V ratios**

| Materials | Degree of freedom, n | \|t\|* | t (P=95%) |
|---|---|---|---|
| SS | 2 | 2.19 | 4.3 |
| PE | 3 | 1.67 | 3.18 |
| PP-1 | 1 | 0.57 | 12.7 |
| PEx | 3 | 3.04 | 3.18 |
| EPDM | 1 | 0.38 | 12.7 |

* based on the comparison between the average BPP values from both trials for one and the same material under both S/v ratios; |t| – calculated value depending of a degree of freedom and probability; t - critical value (at degree of freedom n and probability 0.05)

On the other hand, the increase of the BPP values at the higher S/V ratio was found for pipe materials either having high or low growth promoting properties. Thus, it can be supposed that no growth limitation due to oxygen depletion or accumulation of growth inhibiting compounds in the test water was due to the higher S/V contact ratio.

Since the attached biomass production is the predominant part of their BPP, the same trends were found for that parameter (*Appendix*; Tables VI). Comparing between the PB concentrations (as ng ATP/l) showed that plastic materials have stronger impact on the test waters in contact under the S/V ratio of 1.6 $cm^{-1}$. However, converting the PB concentration into the PB production (PBP, pg ATP/$cm^2$) a statistically insignificant difference also was observed between the calculated PBP values for both S/V ratios, i.e. the contribution of PBP to the BPP is similar.

Our results for all 8 tested plastic pipe materials are in agreement with the findings of Corfitzen *et al.* (2004) for PE material, that the varying S/V ratios do not affect the migration of biodegradable substances in static biotic conditions, and respectively the after-growth potential of the materials. Theoretically, migration of substances from surfaces into water is controlled by diffusion process in the solid-liquid boundary layer, with a driving force the difference between the equilibrium and actual concentrations in water phase. Thus, the lower the actual concentration in water, the stronger driving force for the migration. During the migration process the concentration in water could lower the driving force. In flowing drinking water systems the driving force is kept on a maximum due the short contact time and is independent of the S/V ratio. In the BPP test a similar situation occurs. The expected higher concentration of the migrating substances in the test water at the S/V ratio of 1.6 $cm^{-1}$ is reduced due to continuous utilization by micro-organisms and a similar driving force is maintained as at the 10-fold lower S/V ratio. As a result the effect of the S/V ratio on the migration process and on the microbial growth potential of the pipe materials is practically negligible. The thick biofilm, as one on the EPDM, probably could limit the transport of substances into the water. In case of massive biofilms, bacteria detachment and/or slough of biofilm could have significant contribution for a high planktonic biomass concentration in water.

The diffusion depends on the initial concentration of migrant(s) in product, density, surface area of contact, time of contact, etc. The significant differences found between the BPP values of the tested three PP products at the S/V ratio of 0.16 $cm^{-1}$ could due to the wall thickness, aluminium foil for enforcement in the PP-3 material, non-homogeneity of product or lot. Supposing that these 3 pipe products are made from the same PP material we can calculate coefficient of reproducibility $CV_R$ of the BPP test and that was 32%. Under the S/V ratio of 1.6 $cm^{-1}$ insignificant difference was found between all tested PP products and the $CV_R$ was 5%.

The BP values on the 56$^{th}$, 84$^{th}$ and 112$^{th}$ day of BPP test indicated that the migration of assimilable organic substances did not level down significantly during the all period of the test. What the long-term effect of the products on the water in contact will be the BPP test is not to suppose, because of the complexity of the diffusion process and biological utilization of the migrants. May be migration modelling and solution of the differential diffusion equation, based on the data about chemical composition and probable migrants (monomers, additives, etc.) from the polymeric pipe products, in connection with the BPP and PB data probably could predict the long-term effect of pipe products on bacterial growth in distribution systems and on bacteriological water quality.

From the assumption for a possible inhibition effect of the substances released from the product and accumulated into the test water can be expected that it may concern the planktonic growth, especially at high S/V ratio. Comparison between the planktonic biomass concentrations in test waters (measured as ng ATP/l) shows that the samples of tested plastic materials have stronger effect on the bacteriological water quality at the S/V ratio of 1.6 $cm^{-1}$ (Fig.13). It is logical that the net microbial growth potential ($BPP_{corr}$) of the tested materials under the compared S/V ratios and their net planktonic biomass concentration ($PB_{corr}$) will indicate the same trend of impact (Fig.14). Under higher S/V ratio the materials have from 3 to 10-fold higher PB concentrations in first trial and from 4 to 14-fold higher PB values in the second one. Such an effect on drinking water quality could be important from consumer's exposure point of view.

The Figures 13 and 14 also show the proportional relations observed between the PB concentration in test waters and the BPP of the tested pipe materials under both S/V ratios. Since the PB concentration of the tested materials is a result of bacterial re-growth in water and detachment of biofilm bacteria, the latter depending on the surface properties of materials, physiological status of bacteria, concentration of migrating substances and competition during their utilization, the proportional relations could represent and emphasize only on the general trend of effect of the S/V ratio on the PB.

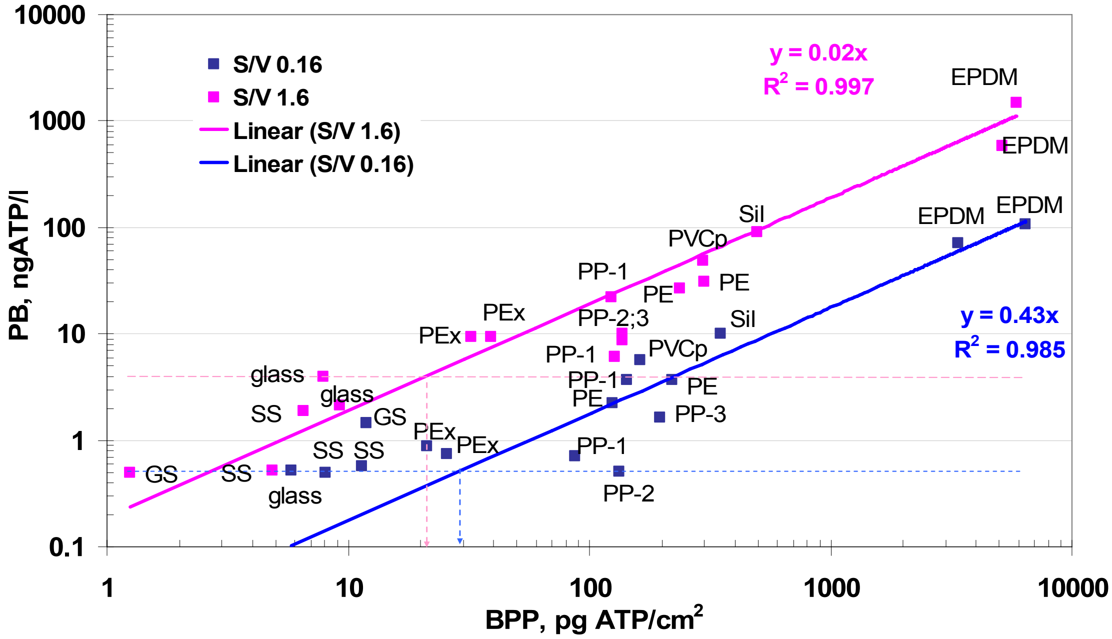


**Fig.13. Relation between the planktonic biomass (PB) concentration and the biomass production potential (BPP) of the tested materials;** the horizontal broken line represents the no-effect level of the PB of glass control; the vertical arrow represents no-effect BPP value; the line represents a proportional relation between the PB and the BPP values.

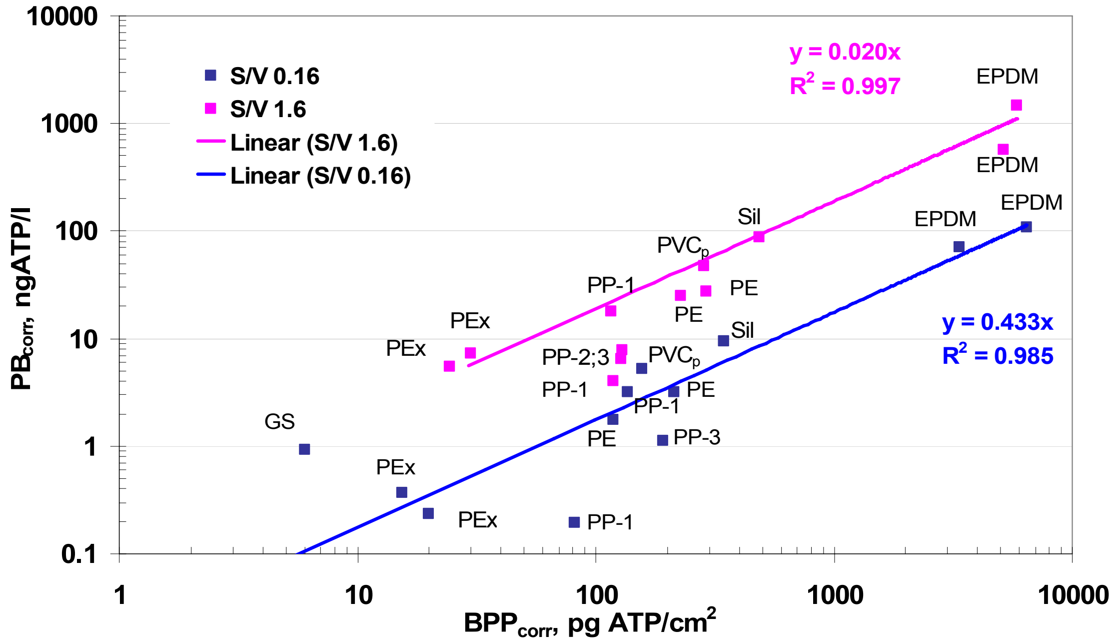


**Fig.14. Relation between corrected planktonic biomass ($PB_{corr}$) concentration and corrected biomass production potential ($BPP_{corr}$) of the tested pipe materials**

Although the PB concentrations determined under the specific conditions of the BPP test present a worse case of water stagnation in distribution systems (7 days), they can manifest the impact of the products on bacteriological water quality. At the S/V ratio of 0.16 $cm^{-1}$ the PB values of plastic pipe materials did not exceed 10 ng ATP/l, the value considered as a limit for biological stability of drinking water, with exception of EPDM. The same plastic materials at the S/V ratio of 1.6 $cm^{-1}$ promoted the planktonic growth resulting in biologically unstable water. This trend was confirmed by no-effect BPP values, determined from the linear PB-BPP relation under both S/V ratios – higher no-effect BPP was found at lower S/V ratio. Taking into account all these data we consider the acceptance of products intended to contact with drinking water should not be based only on a Pass/Fail Criterion for the BPP. The impact of the S/V contact ratio on drinking water quality could be important for assessment of materials from the consumers' exposure point of view. Materials have different effect on the PB concentration and this aspect could be taken as a second criterion for evaluation.

The number of culturable heterotrophic bacteria in drinking water is an indicator for the effectiveness of the treatment process and/or biofilm status of the DWDSs (Van der Kooij, 2000). The data for HPC bacteria in the test water after 7 day contact with the tested materials (Table 15) confirmed the stronger effect on bacteriological water quality under the S/V ratio of 1.6 $cm^{-1}$ already found out for the PB concentrations (as ng ATP/ml).

**Table 15. Culturable bacteria numbers in the test waters in contact with the tested pipe products before weekly replacement on 105$^{th}$ and 112$^{th}$ day of the BPP test**

| First trial | HPC (CFU/ml) | | Second trial | HPC (CFU/ml) | |
|---|---|---|---|---|---|
| | S/V 0.16 $cm^{-1}$ | S/V 1.6 $cm^{-1}$ | | S/V 0.16 $cm^{-1}$ | S/V 1.6 $cm^{-1}$ |
| blank | 659 (162) | 742 (115) | blank | 36 (18) | 25 (13) |
| glass | | 513 (405) | glass | 131 (69) | 1075 (120) |
| SS | 977 (98) | 717 (212) | SS | 95 (55) | 64 (24) |
| GS | 318 (190) | 777 (84) | PEx | 52 (28) | 400 (370) |
| PE | 1263 (120) | 904 (137) | PE | 688 (291) | 15661(2499) |
| PEx | 684 (81) | 2005 (813) | PP-1 | 304 (14) | 5180 (254) |
| PP-1 | 7050 (8365) | 8079 (7897) | PP-2 | 353 (324) | 10030 (5607) |
| Silicone | 1222 (311) | 12725 (2964) | PP-3 | 1990 (2183) | 7416 (3787) |
| EPDM | 470750 (104926) | 5161000 (8587103) | PVCp | 8104 (7124) | 37020 (44307) |
| | | | EPDM | 107500 (50750) | 1096200 (314984) |

In brackets – confident interval; number of analyses for single material – from 4 to 8.

Detailed data for HPC in the test waters in contact with tested CPDW at both S/V ratios are present at *Appendix* in Table IX (from the first trial) and Table X (from second one). The number of HPC bacteria in the test water in contact with all materials was bigger under the higher S/V ratio and the difference is statistically significant, with the exception for PE and PP in the first trial. It is logical that HPC of blank and SS under both ratios were similar.

# 5. Conclusions

The BPP test is a useful means to evaluate the contribution of CPDW to microbial growth potential of the distribution systems. The BPP test allows comparison between the net BPP of CPDW and their effect on bacterial re-growth.

Taking into consideration the results from the study we can summarize that the BPP test at the originally proposed S/V contact ratio of 0.16 $cm^{-1}$ is a good approach for assessment of growth promoting properties of CPDW in semi-static conditions. The data show that under the S/V contact ratio of 0.16 $cm^{-1}$ the BPP test can achieve similar results as with the S/V contact ratio of 1.6 $cm^{-1}$, the more realistic ratio for the domestic water installations.

Despite the insignificant effect of the S/V contact ratio on the BPP of CPDW, their effect on the water quality, measured as planktonic biomass concentration or HPC, can be underestimated at the S/V ratio of 0.16 $cm^{-1}$. Therefore, taking into account the effect of pipe materials on bacteria number in test water (measured as PB concentration or HPC), the impact of the S/V contact ratio on drinking water quality could be important for assessment of materials from the consumers' exposure point of view.

The products intended to contact with drinking water should be evaluated not only on the basis of the BPP values, but also on their effect on bacteriological water quality. For acceptance of the CPDW, besides a Pass/Fail Criterion for the BPP, a second criterion for evaluation of materials on their effect of drinking water quality needs to be developed to avoid an underestimation of the human health effect.

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# APPENDIX

## Table I. Comparison between different procedures for biofilm removal (1st trial)

| Treatment procedure | Attached biomass, AB, ngATP/cm² | | | | | |
|---|---|---|---|---|---|---|
| | PVCp Rapigel | | | PEx | | |
| **Procedure of Van der Kooij *et al.*** | **6 LES** | **HES** | **swab+HES** | **6 LES** | **HES** | **swab+HES** |
| 1 | 7027.4 | 7486.4 | 7936.3 | 39.0 | 35.2 | 27.7 |
| 1 | 6715.1 | 7502.3 | 8114.5 | 37.5 | 32.4 | 36.0 |
| 2 | 6993.9 | 6796.3 | 6800.1 | 50.1 | 47.7 | 47.1 |
| 2 | 6725.8 | 7067.5 | 6707.0 | 49.3 | 41.6 | 51.9 |
| 3 | 6723.2 | 7344.8 | 7361.9 | 51.4 | 66.7 | 58.6 |
| 3 | 6701.8 | 7940.2 | 7699.4 | 56.5 | 51.8 | 84.5 |
| 4 | 6078.9 | 6485.5 | 5974.5 | 53.7 | 54.2 | 45.7 |
| 4 | 6064.1 | 6412.2 | 5903.5 | 54.4 | 51.7 | 67.2 |
| 5 | 5635.2 | 5667.7 | 5440.7 | 73.7 | 73.1 | 77.2 |
| 5 | 5350.6 | 6238.9 | 5812.0 | 83.3 | 72.2 | |
| 6 | 5688.5 | 6025.6 | 6434.8 | | | |
| 6 | 5764.1 | 6055.6 | 6483.6 | | | |
| **Average** | **6289.1** | **6751.9** | **6722.4** | **54.9** | **52.7** | **55.1** |
| **stdev** | **588.7** | **713.8** | **885.4** | **14.1** | **14.4** | **18.7** |
| **Lab procedure N°1** | **6 LES** | **HES** | **swab+HES** | **6 LES** | **HES** | **swab+HES** |
| 7 | 3393.0 | 5992.5 | 6056.0 | 30.0 | 38.7 | 44.4 |
| 7 | 3503.3 | 5849.5 | 6201.7 | 28.9 | 39.3 | |
| 8 | 2655.7 | 5051.1 | 4784.1 | 41.7 | 52.6 | 57.7 |
| 8 | 2944.6 | 4793.9 | 4705.9 | 41.8 | 52.6 | |
| 9 | 2196.4 | 3668.0 | 4330.5 | 42.2 | 61.8 | 64.6 |
| 9 | 2347.3 | 3661.4 | 4674.2 | 42.7 | 56.6 | |
| 10 | 3065.5 | 5400.9 | 5404.7 | 25.0 | 40.7 | 51.4 |
| 10 | 3739.0 | 5848.5 | 5233.7 | 26.9 | 40.3 | |
| 11 | 2170.0 | 4483.0 | 4866.8 | 23.7 | 39.6 | 47.3 |
| 11 | 2400.6 | 4412.4 | 4865.3 | 23.8 | 40.9 | |
| 12 | 3425.3 | 6039.2 | 5783.3 | | | |
| 12 | 3212.1 | 6137.6 | 5358.8 | | | |
| **Average** | **2921.1** | **5111.5** | **5188.7** | **32.7** | **46.3** | **53.1** |
| **stdev** | **551.9** | **906.3** | **589.0** | **8.3** | **8.7** | **8.1** |
| **Lab procedure N°2** | **Swab+ HES** | **LES** | **HES** | **Swab+ HES** | **LES** | **HES** |
| 13 | 4765.5 | 6060.2 | 4915.2 | 62.8 | 99.1 | 87.1 |
| 13 | 4882.9 | 5661.2 | 4776.8 | 62.2 | 95.0 | |
| 14 | 4567.3 | 7391.9 | 5578.5 | 75.2 | 112.2 | 117.7 |
| 14 | 4300.4 | 7471.6 | 5931.5 | 74.1 | 113.8 | |
| 15 | 5086.4 | 7331.4 | 6067.0 | 166.6 | 250.4 | 264.1 |
| 15 | 4175.2 | 7809.8 | 6229.1 | 151.2 | 254.6 | |
| 16 | 6176.3 | 7576.3 | 6733.5 | 85.6 | 126.0 | 133.9 |
| 16 | 4411.6 | 7437.3 | 6290.7 | 80.8 | 126.0 | |
| 17 | 4683.9 | 6751.6 | 5584.7 | 83.3 | 130.4 | 124.2 |
| 17 | 4974.9 | 6591.0 | 5467.9 | 78.6 | 122.4 | |
| 18 | 4779.2 | 7303.7 | 5493.6 | | | |
| 18 | 6390.4 | 7719.5 | 5979.4 | | | |
| **Average** | **4932.8** | **7092.1** | **5754.0** | **92.0** | **143.0** | **145.4** |
| **stdev** | **687.0** | **679.4** | **565.8** | **36.3** | **58.9** | **68.6** |

## Table II. Comparison between different procedures for biofilm removal (2nd trial)

| Treatment procedure | Attached biomass, AB, ngATP/cm$^2$ | | | | | |
|---|---|---|---|---|---|---|
| | PVCp ARIANNA | | | PEx | | |
| **Procedure of Van der Kooij** | **6 LES** | **HES** | **swab+HES** | **6 LES** | **HES** | **swab+HES** |
| 1 | 4190.9 | 4113.6 | 4529.7 | 74.0 | 57.4 | 75.3 |
| 1 | 3818.8 | 3920.1 | 4408.0 | 67.6 | 55.1 | 62.2 |
| 2 | 3762.6 | 3921.8 | 4426.1 | 56.5 | 46.7 | 54.5 |
| 2 | 3882.6 | 4089.6 | 4340.8 | 59.4 | 47.3 | 52.5 |
| 3 | 186.7 | 201.5 | 214.7 | 52.4 | 40.4 | 55.1 |
| 3 | 158.2 | 182.7 | 232.3 | 48.5 | 46.1 | 63.2 |
| 4 | 248.6 | 295.2 | 327.9 | 45.5 | 34.2 | 51.1 |
| 4 | 243.7 | 352.5 | 336.9 | 46.0 | 33.6 | 47.6 |
| 5 | 2714.4 | 3060.9 | 2824.3 | 56.7 | 45.0 | 61.5 |
| 5 | 2570.6 | 3395.8 | 2999.5 | 56.7 | 46.2 | 63.5 |
| 6 | 2169.3 | 2758.9 | 2494.4 | 42.9 | 36.1 | 60.1 |
| 6 | 2250.3 | 2693.5 | 2471.4 | 40.5 | 35.5 | 59.4 |
| **Average** | **2183.1** | **2415.5** | **2467.2** | **53.9** | **43.6** | **58.8** |
| **stdev** | **1598.8** | **1663.3** | **1782.8** | **10.0** | **7.9** | **7.3** |

| **Lab procedure** | **6 LES** | **HES** | **swab+HES** | **6 LES** | **HES** | **swab+HES** |
|---|---|---|---|---|---|---|
| 7 | 2934.3 | 3732.5 | 4125.2 | 77.1 | 75.1 | 91.4 |
| 7 | 2914.3 | 3906.3 | 4114.1 | 72.6 | 76.8 | 91.5 |
| 8 | 5611.4 | 4898.1 | 4582.7 | 38.6 | 40.1 | 51.7 |
| 8 | 5663.3 | 4631.0 | 4399.4 | 36.3 | 41.3 | 51.7 |
| 9 | 283.3 | 352.3 | 441.0 | 41.2 | 35.3 | 42.3 |
| 9 | 284.0 | 369.8 | 455.9 | 40.6 | 35.5 | 42.5 |
| 10 | 297.8 | 389.9 | 474.7 | 44.5 | 47.0 | 50.8 |
| 10 | 299.8 | 416.7 | 495.4 | 46.2 | 47.9 | 51.5 |
| 11 | 2497.6 | 2292.3 | 2355.1 | 58.9 | 65.9 | 74.4 |
| 11 | 2626.0 | 2369.0 | 2517.4 | 60.3 | 62.4 | 73.4 |
| 12 | 2521.6 | 2204.4 | 2271.6 | 60.8 | 59.1 | 66.0 |
| 12 | 2459.5 | 2162.6 | 2234.7 | 63.7 | 56.1 | 68.3 |
| **Average** | **2366.1** | **2310.4** | **2372.3** | **53.4** | **53.5** | **63.0** |
| **stdev** | **1891.7** | **1693.9** | **1642.6** | **13.9** | **14.6** | **17.3** |

| **Lab procedure** | **swab+HES** | **LES** | **HES** | **Swab+ HES** | **LES** | **HES** |
|---|---|---|---|---|---|---|
| 13 | 3508.0 | | | 66.6 | 70.6 | 71.7 |
| 13 | 3435.7 | | | 62.4 | 67.5 | 71.1 |
| 14 | 476.5 | | | 48.5 | 52.9 | 51.8 |
| 14 | 466.0 | | | 50.7 | 50.9 | 54.0 |
| 15 | 3343.9 | | | 38.4 | 40.3 | 46.3 |
| 15 | 3228.1 | | | 40.4 | 41.6 | 47.5 |
| 16 | 452.0 | | | 37.5 | 41.7 | 37.9 |
| 16 | 428.3 | | | 35.5 | 39.6 | 39.1 |
| 17 | 1597.9 | | | 85.5 | 88.4 | 85.6 |
| 17 | 1575.3 | | | 83.9 | 86.0 | 85.3 |
| 18 | 1651.6 | | | 44.5 | 48.4 | 58.8 |
| 18 | 1579.2 | | | 45.3 | 47.3 | 53.7 |
| **Average** | **1811.9** | | | **53.3** | **56.3** | **58.6** |
| **stdev** | **1257.9** | | | **17.5** | **17.6** | **16.4** |

## Table III. All measurements of the BP and the BPP in the first BPP test of the pipe materials

| Sample | Material – S/V ratio | Biomass production BP, pgATP/cm$^2$ | | | BPP, pgATP/cm$^2$ | Stdev | RSD, % |
|---|---|---|---|---|---|---|---|
| | | 56$^{th}$ day | 84$^{th}$ day | 112$^{th}$ day | | | |
| 1 | blank-0.16 | 3 | 3 | 8.7 | 5.2 | 3.4 | 66 |
| | | 3 | 3 | 10.3 | | | |
| 2 | blank-1.6 | 3 | 3 | 2.9 | 3.2 | 0.3 | |
| | | 3 | 3 | 3.8 | | | |
| 3 | | 3 | 3 | 3.9 | | | |
| | | 3 | 3 | 3.3 | | | |
| 4 | glass-1.6 | 12.7 | 1.7 | 10.4 | 7.9 | 4.2 | 53 |
| | | 12.9 | 1.8 | 10.8 | | | |
| 5 | glass-1.6 | 8.2 | 3.5 | 13.2 | | | |
| | | 5.1 | 3.9 | 13.0 | | | |
| 6 | glass-1.6 | 8.0 | 3.5 | 10.6 | | | |
| | | 7.5 | 3.5 | 11.2 | | | |
| 7 | GS - 0.16 | 6.0 | 5.5 | 24.0 | 11.8 | 7.9 | 67 |
| | | 5.2 | 6.2 | 24.3 | | | |
| 8 | GS - 0.16 | 8.2 | 6.8 | 17.6 | | | |
| | | 8.1 | 7.2 | 23.0 | | | |
| 9 | GS-1.6 | 1.5 | 1.0 | 1.1 | 1.2 | 0.2 | 16 |
| | | 1.5 | 1.3 | 1.1 | | | |
| 10 | GS-1.6 | 1.5 | 1.0 | 1.1 | | | |
| | | 1.5 | 1.0 | 1.1 | | | |
| 11 | GS-1.6 | 1.5 | 1.0 | 1.3 | | | |
| | | 1.5 | 1.0 | 1.3 | | | |
| 12 | SS-0.16 | 1.4 | 9.8 | 22.5 | 11.4 | 9.5 | 84 |
| | | 1.3 | 10.5 | 22.7 | | | |
| 13 | SS-1.6 | 1.6 | 3.9 | 13.7 | 6.5 | 5.8 | 89 |
| | | 1.7 | 4.2 | 14.1 | | | |
| 14 | PE-0.16 | 229.6 | 142.7 | 273.7 | 219.9 | 62.4 | 28 |
| | | 254.1 | 142.0 | 277.3 | | | |
| 15 | PE - 1.6 | 259.7 | 353.5 | 445.8 | 300.2 | 59.7 | 20 |
| | | 258.0 | 350.4 | 458.0 | | | |
| 16 | PE - 1.6 | 239.6 | 357.4 | 256.0 | | | |
| | | 254.9 | 357.6 | 237.0 | | | |
| 17 | PE - 1.6 | 258.5 | 267.7 | 287.2 | | | |
| | | 270.9 | 275.4 | 266.6 | | | |
| 18 | PE - 1.6 | 303.7 | 304.1 | 265.4 | | | |
| | | 318.0 | 302.7 | 257.7 | | | |
| 19 | PP - 0.16 | 75.1 | 57.4 | 281.6 | 142.5 | 117.7 | 83 |
| | | 76.7 | 58.1 | 305.8 | | | |
| 20 | PP - 1.6 | 112.9 | 217.6 | 123.8 | 124.0 | 33.2 | 27 |
| | | 115.0 | 225.0 | 126.5 | | | |
| 21 | PP - 1.6 | 108.6 | 105.4 | 157.2 | | | |
| | | 114.3 | 103.6 | 151.3 | | | |
| 22 | PP - 1.6 | 99.6 | 108.5 | 111.8 | | | |
| | | 99.6 | 101.5 | 104.9 | | | |
| 23 | PP - 1.6 | 122.2 | 107.8 | 109.7 | | | |
| | | 122.3 | 110.1 | 117.0 | | | |
| 24 | PEx - 0.16 | 13.9 | 16.7 | 26.5 | 21.3 | 7.9 | 37 |
| | | 12.9 | 17.5 | 29.7 | | | |
| 25 | PEx - 0.16 | 19.5 | 14.0 | 35.0 | | | |
| | | 20.4 | 15.5 | 33.9 | | | |

**Table III. (Continuation)**

| Sample | Material – S/V ratio | Biomass production BP, pgATP/cm$^2$ | | | BPP, pgATP/cm$^2$ | Stdev | RSD, % |
|---|---|---|---|---|---|---|---|
| | | 56$^{th}$ day | 84$^{th}$ day | 112$^{th}$ day | | | |
| 26 | PEx -1.6 | 25.3 | 26.3 | 38.2 | 32.3 | 6.4 | 20 |
| | | 24.1 | 28.1 | 39.3 | | | |
| 27 | PEx -1.6 | 23.5 | 29.1 | 35.4 | | | |
| | | 22.2 | 31.3 | 36.6 | | | |
| 28 | PEx -1.6 | 32.9 | 38.8 | 38.7 | | | |
| | | 31.5 | 41.4 | 39.2 | | | |
| 29 | Silicone - 0.16 | 198.9 | 344.0 | 474.0 | 350.6 | 180.3 | 51 |
| | | 207.0 | 341.8 | 460.4 | | | |
| 30 | Silicone - 0.16 | 191.6 | 234.2 | 680.4 | | | |
| | | 183.8 | 229.3 | 661.4 | | | |
| 31 | Silicone - 1.6 | 841.9 | 268.4 | 396.6 | 496.4 | 215.9 | 44 |
| | | 849.5 | 267.4 | 358.0 | | | |
| 32 | Silicone - 1.6 | 852.1 | 720.9 | 493.6 | | | |
| | | 852.1 | 683.6 | 501.2 | | | |
| 33 | Silicone - 1.6 | 261.2 | 464.8 | 487.8 | | | |
| | | 273.3 | 442.4 | 494.6 | | | |
| 34 | Silicone -1.6 | 186.5 | 617.8 | 405.4 | | | |
| | | 174.5 | 619.7 | 399.5 | | | |
| 35 | EPDM 0.16 | 6059.3 | 5791.7 | 8032.2 | 6448.1 | 1129.1 | 18 |
| | | 5804.7 | 5962.6 | 7615.1 | | | |
| 36 | EPDM-0.16 | 7815.4 | 5995.4 | 5151.0 | | | |
| | | 8141.4 | 6010.4 | 4997.6 | | | |
| 37 | EPDM - 1.6 | 2246.1 | 2977.8 | 15415.7 | 5882.1 | 4701.0 | 80 |
| | | 2181.1 | 3371.4 | 16107.9 | | | |
| 38 | EPDM - 1.6 | 3287.4 | 2694.5 | 10230.8 | | | |
| | | 3366.4 | 2840.6 | 10575.1 | | | |
| 39 | EPDM - 1.6 | 1424.5 | 4587.2 | 9058.4 | | | |
| | | 1508.3 | 4563.6 | 9440.9 | | | |

**Table IV. All measurements of the BP and the BPP from the second BPP test of the pipe materials**

| Sample | Material – S/V ratio | Biomass production BP, pgATP/cm$^2$ | | | BPP, pgATP/cm$^2$ | Stdev | RSD, % |
|---|---|---|---|---|---|---|---|
| | | 56$^{th}$ day | 84$^{th}$ day | 112$^{th}$ day | | | |
| 1 | Blank - 0.16 | 4.2 | 3 | 3 | 3.2 | 0.5 | 15 |
| | | 3 | 3 | 3 | | | |
| 2 | Blank - 1.6 | 3 | 3 | 3 | 3.2 | 0.5 | 15 |
| | | 4.2 | 3 | 3 | | | |
| 3 | Glass - 0.16 | 4.7 | 7.6 | 5.0 | 5.8 | 1.7 | 29 |
| | | 4.7 | 10.1 | 6.3 | | | |
| 4 | Glass - 0.16 | 4.7 | 7.2 | 5.0 | 9.2 | 3.6 | 39 |
| | | 5.4 | 5.0 | 4.4 | | | |
| 5 | Glass - 1.6 | 8.9 | 12.4 | 14.0 | | | |
| | | 12.5 | 12.0 | 13.5 | | | |
| 6 | Glass - 1.6 | 4.4 | 6.2 | 8.8 | | | |
| | | 4.0 | 5.7 | 8.6 | | | |
| 7 | SS - 0.16 | 8.0 | 4.9 | 18.8 | 8.1 | 4.9 | 61 |
| | | 6.7 | 4.9 | 18.0 | | | |
| 8 | SS - 0.16 | 6.8 | 4.9 | 5.9 | | | |
| | | 6.6 | 4.9 | 6.8 | | | |
| 9 | SS - 1.6 | 3.9 | 2.3 | 14.1 | 4.8 | 4.2 | 87 |
| | | 3.9 | 2.1 | 13.4 | | | |
| 10 | SS - 1.6 | 3.8 | 2.1 | 3.3 | | | |
| | | 3.7 | 2.1 | 3.3 | | | |
| 11 | PE - 0.16 | 166.2 | 162.4 | 117.5 | 124.9 | 38.9 | 31 |
| | | 168.5 | 166.5 | 116.2 | | | |
| 12 | PE - 0.16 | 120.9 | 130.9 | 55.1 | | | |
| | | 120.8 | 118.3 | 55.6 | | | |
| 13 | PE - 1.6 | 153.8 | 145.6 | 131.7 | 236.3 | 112.3 | 48 |
| | | 150.7 | 140.7 | 128.4 | | | |
| 14 | PE - 1.6 | 435.3 | 123.3 | 404.0 | | | |
| | | 438.5 | 162.0 | 411.7 | | | |
| 15 | PE - 1.6 | 159.2 | 367.6 | 141.5 | | | |
| | | 162.1 | 368.5 | 211.6 | | | |
| 16 | PE - 1.6 | 161.7 | 252.6 | 309.3 | | | |
| | | 151.9 | 266.2 | 292.1 | | | |
| 17 | PEX - 0.16 | 33.7 | 22.3 | 21.4 | 25.7 | 5.6 | 22 |
| | | 30.6 | 22.8 | 25.0 | | | |
| 18 | PEX - 0.16 | 31.9 | 16.8 | 27.0 | | | |
| | | 29.7 | 17.8 | 29.2 | | | |
| 19 | PEX - 1.6 | 28.3 | 24.6 | 31.2 | 39.0 | 17.6 | 45 |
| | | 30.4 | 22.1 | 32.1 | | | |
| 20 | PEX - 1.6 | 43.5 | 14.9 | 34.9 | | | |
| | | 48.6 | 16.2 | 31.3 | | | |
| 21 | PEX - 1.6 | 72.7 | 29.3 | 48.7 | | | |
| | | 67.8 | 32.1 | 46.8 | | | |
| 22 | PEX - 1.6 | 77.6 | 37.2 | 28.6 | | | |
| | | 72.0 | 35.1 | 30.8 | | | |
| 23 | PP-1 - 0.16 | 48.5 | 97.4 | 93.8 | 87.1 | 23.6 | 27 |
| | | 49.0 | 99.4 | 91.3 | | | |
| 24 | PP-1 - 0.16 | 67.2 | 115.3 | 89.6 | | | |
| | | 67.9 | 114.3 | 111.0 | | | |

**Table IV. (Continuation)**

| Sample | Material – S/V ratio | Biomass production BP, pgATP/cm$^2$ | | | BPP, pgATP/cm$^2$ | Stdev | RSD, % |
|---|---|---|---|---|---|---|---|
| | | 56$^{th}$ day | 84$^{th}$ day | 112$^{th}$ day | | | |
| 25 | PP-1 - 1.6 | 137.2 | 117.4 | 105.6 | 137.9 | 24.2 | 18 |
| | | 132.2 | 119.2 | 100.6 | | | |
| 26 | PP-1 - 1.6 | 133.4 | 166.2 | 161.3 | | | |
| | | 129.4 | 169.4 | 177.2 | | | |
| 27 | PP-1 - 1.6 | 134.9 | 158.6 | 108.2 | | | |
| | | 133.1 | 164.7 | 110.9 | | | |
| 28 | PP-1 - 0.16 | 113.0 | 151.5 | 147.0 | | | |
| | | 108.1 | 153.8 | 176.6 | | | |
| 29 | PP-2 - 0.16 | 118.1 | 138.4 | 100.4 | 134.4 | 51.9 | 39 |
| | | 118.1 | 131.4 | 101.2 | | | |
| 30 | PP-2 - 0.16 | 127.9 | 232.9 | 86.9 | | | |
| | | | 234.6 | 88.4 | | | |
| 31 | PP-2 - 1.6 | 130.3 | 175.1 | 103.6 | 129.9 | 44.1 | 34 |
| | | 128.3 | 172.7 | 100.9 | | | |
| 32 | PP-2 - 1.6 | 93.1 | 124.6 | 122.5 | | | |
| | | 93.2 | 120.0 | 118.0 | | | |
| 33 | PP-2 - 1.6 | 107.1 | 245.3 | 84.6 | | | |
| | | 109.6 | 242.9 | 86.9 | | | |
| 34 | PP-2 - 1.6 | 142.9 | 104.7 | | | | |
| | | 142.9 | 107.8 | | | | |
| 35 | PP-3 - 0.16 | 166.8 | 172.0 | 134.7 | 197.3 | 47.1 | 24 |
| | | 177.9 | 172.0 | 128.3 | | | |
| 36 | PP-3 - 0.16 | 259.9 | 204.6 | 236.5 | | | |
| | | 275.8 | 204.8 | 234.3 | | | |
| 37 | PP-3 - 1.6 | 81.6 | 132.8 | 177.1 | 137.4 | 32.1 | 23 |
| | | 86.0 | 133.3 | 182.0 | | | |
| 38 | PP-3 - 1.6 | 97.6 | 131.1 | 179.4 | | | |
| | | 104.8 | 129.0 | 159.9 | | | |
| 39 | PP-3 - 1.6 | 117.9 | 169.7 | 119.5 | | | |
| | | 122.1 | 164.9 | 121.2 | | | |
| 40 | PP-3 - 1.6 | 111.5 | 172.5 | 161.3 | | | |
| | | 98.5 | 169.8 | 173.6 | | | |
| 41 | PVCp - 0.16 | 237.5 | 137.7 | 69.6 | 162.7 | 71.5 | 44 |
| | | 243.9 | 150.7 | 60.4 | | | |
| 42 | PVCp - 0.16 | 262.6 | 122.3 | 123.9 | | | |
| | | 261.5 | 154.4 | 128.2 | | | |
| 43 | PVCp - 1.6 | 253.1 | 339.1 | 158.0 | 295.1 | 139.8 | 47 |
| | | 270.2 | 348.5 | 153.3 | | | |
| 44 | PVCp - 1.6 | 378.1 | 617.9 | 150.5 | | | |
| | | 382.8 | 623.6 | 149.5 | | | |
| 45 | PVCp - 1.6 | 262.6 | 246.0 | 194.3 | | | |
| | | 260.6 | 247.2 | 277.3 | | | |
| 46 | EPDM - 0.16 | 4268.4 | 3864.4 | 2888.5 | 3385.3 | 539.6 | 16 |
| | | 4352.0 | 3827.5 | 3022.2 | | | |
| 47 | EPDM - 0.16 | 3105.9 | 2918.7 | 3142.8 | | | |
| | | 3106.1 | 2912.9 | 3214.4 | | | |
| 48 | EPDM - 1.6 | 5508.5 | 4407.7 | 5235.9 | 5160.0 | 1145.0 | 22 |
| | | 5527.6 | 4375.4 | 5524.3 | | | |
| 49 | EPDM - 1.6 | 6034.6 | 2955.6 | 3772.9 | | | |
| | | 5986.3 | 3223.8 | 3802.8 | | | |
| 50 | EPDM - 1.6 | 5763.5 | 6886.0 | 5661.8 | | | |
| | | 5600.4 | 6790.6 | 5822.6 | | | |

### Table V. Significance test between the BPP, PB and AB productions of the pipe materials under both S/V ratios based on all individual ATP measurements for each material in both consecutive trials

| Material | Significance test for BPP | | | Significance test for PB production | | | Significance test for AB production | | |
|---|---|---|---|---|---|---|---|---|---|
| | n | \|t\| | t (0.05) | n | \|t\| | t (0.05) | n | \|t\| | t (0.05) |
| **First Trial** | | | | | | | | | |
| GS | 11 | 4.64 | 2.23 | 11 | 3.43 | 2.23 | 12 | 7.22 | 2.18 |
| SS | 10 | *1.07* | 2.23 | 11 | *1.50* | 2.23 | 8 | *0.84* | 2.31 |
| PE | 8 | 2.85 | 2.31 | 6 | *0.66* | 2.45 | 10 | 3.43 | 2.23 |
| PP-1 | 5 | *0.38* | 2.57 | 5 | *0.87* | 2.57 | 5 | *0.25* | 2.57 |
| PEx | 22 | 4.03 | 2.09 | 25 | *0.20* | 2.09 | 27 | 6.04 | 2.04 |
| Silicone | 28 | 2.14 | 2.04 | 16 | *1.08* | 2.12 | 32 | 2.45 | 2.04 |
| EPDM | 20 | *0.49* | 2.09 | 18 | *1.41* | 2.10 | 21 | *0.78* | 2.09 |
| **Second Trial** | | | | | | | | | |
| glass | 17 | 2.78 | 2.10 | 11 | 4.35 | 2.18 | 15 | 3.90 | 2.14 |
| SS | 23 | *1.74* | 2.09 | 20 | 137.85 | 2.09 | 23 | *0.28* | 2.10 |
| PE | 33 | 4.36 | 2.04 | 19 | *1.01* | 2.04 | 31 | 4.58 | 2.04 |
| PEx | 31 | 3.38 | 2.04 | 17 | *1.23* | 2.10 | 36 | 4.40 | 2.04 |
| PP-1 | 24 | 6.03 | 2.09 | 23 | *1.27* | 2.04 | 29 | 5.88 | 2.04 |
| PP-2 | 19 | *0.25* | 2.09 | 10 | *1.42* | 2.23 | 19 | *0.28* | 2.09 |
| PP-3 | 17 | 3.97 | 2.12 | 15 | *1.80* | 2.14 | 16 | 3.95 | 2.12 |
| PVCp | 28 | 3.27 | 2.04 | 22 | *0.34* | 2.09 | 27 | 3.83 | 2.04 |
| EPDM | 27 | 5.70 | 2.04 | 20 | *0.57* | 2.09 | 27 | 6.19 | 2.04 |

### Table VI. Significance test between the BPP of the pipe materials under both S/V contact ratios based on all individual ATP measurements for each material in both trials

| Materials | Degree of freedom n | \|t\| | t (P=95%) |
|---|---|---|---|
| Glass* | 12 | 2.71 | 2.04 |
| SS | 32 | 1.96 | 2.04 |
| GS* | 11 | 4.64 | 2.23 |
| PE | 47 | 5.43 | 2.01 |
| PEx | 65 | 4.81 | 2.01 |
| PP-1 | 19 | 1.46 | 2.09 |
| PP-2* | 19 | 0.25 | 2.23 |
| PP-3* | 17 | 3.97 | 2.23 |
| EPDM | 57 | 0.90 | 2.01 |
| Silicone* | 28 | 2.14 | 2.04 |
| PVCp* | 28 | 3.27 | 2.23 |

* Materials tested in one trial

**Table VII. Coefficients of variation of reproducibility ($CV_R$) of the BPP of the same pipe materials determined in two successive trials**

| Materials | BPP, pg ATP/$cm^2$ | | $CV_R$, % |
|---|---|---|---|
| | First trial | Second trial | |
| SS (0.16 $cm^{-1}$) | 11.4 | 8.1 | 24 |
| SS (1.6 $cm^{-1}$) | 6.5 | 4.8 | 21 |
| PE (0.16 $cm^{-1}$) | 219.9 | 124.9 | 39 |
| PE (1.6 $cm^{-1}$) | 300.2 | 236.3 | 17 |
| PP-1 (0.16 $cm^{-1}$) | 142.5 | 87.1 | 34 |
| PP-1 (1.6 $cm^{-1}$) | 124.0 | 137.9 | 7 |
| PEx (0.16 $cm^{-1}$) | 21.3 | 25.7 | 13 |
| PEx (1.6 $cm^{-1}$) | 32.3 | 39.0 | 13 |
| EPDM (0.16 $cm^{-1}$) | 6448.1 | 3385.3 | 44 |
| EPDM (1.6 $cm^{-1}$) | 5882.1 | 5160.0 | 9 |

**Table VIII. Coefficients of variation of reproducibility ($CV_R$) of the PB concentrations in test water in contact with the same materials in two successive trials**

| Materials | PB, ng ATP/l | | $CV_R$, % |
|---|---|---|---|
| | First trial | Second trial | |
| glass (1.6 $cm^{-1}$) | 3.2 | 2.1 | 29 |
| SS (0.16 $cm^{-1}$) | 0.6 | 0.5 | 13 |
| SS (1.6 $cm^{-1}$) | 1.9 | 0.5 | 82 |
| PE (0.16 $cm^{-1}$) | 3.7 | 2.3 | 33 |
| PE (1.6 $cm^{-1}$) | 24.3 | 28.2 | 11 |
| PP (0.16 $cm^{-1}$) | 3.7 | 0.7 | 96 |
| PP (1.6 $cm^{-1}$) | 16.9 | 8.7 | 45 |
| PEx (0.16 $cm^{-1}$) | 0.9 | 0.7 | 18 |
| PEx (1.6 $cm^{-1}$) | 7.4 | 10.3 | 23 |
| EPDM (0.16 $cm^{-1}$) | 108.1 | 70.7 | 30 |
| EPDM (1.6 $cm^{-1}$) | 1038.6 | 577.9 | 40 |

### Table IX. HPC bacteria in the test waters in contact with the tested pipe materials during the BPP test (1st trial)

| Sample | HPC after 15 weeks, CFU/ml | | | | HPC after 16 weeks, CFU/ml | | | |
|---|---|---|---|---|---|---|---|---|
| | S/V ratio 0.16 $cm^{-1}$ | | S/V ratio 1.6 $cm^{-1}$ | | S/V ratio 0.16 $cm^{-1}$ | | S/V ratio 1.6 $cm^{-1}$ | |
| | N | stdev | N | sStdev | N | stdev | N | stdev |
| blank | 745 | 21 | 838 | 152 | 573 | 30 | 819 | 21 |
| blank | | | 548 | 14 | | | 762 | 54 |
| glass | | | 292 | 17 | | | 776 | 4 |
| SS | 985 | 95 | 833 | 1 | 968 | 45 | 602 | 14 |
| GS | 358 | 125 | 820 | 28 | 158 | 92 | 733 | 4 |
| GS | 608 | 53 | | | 299 | 261 | | |
| PE | 1121 | 159 | 857 | 109 | 1291 | 56 | 963 | 35 |
| PP | 10667 | 1629 | 3867 | 95 | 2567 | 153 | 12290 | 1711 |
| silicone | 1256 | 74 | 11433 | 627 | 1740 | 28 | 14587 | 580 |
| silicone | 763 | 144 | | | 1010 | 212 | | |
| EPDM | 492000 | 16971 | 522000 | 82024 | 256667 | 45709 | | |
| EPDM | 540000 | 28284 | | | 568000 | 96167 | 9800000 | 1131371 |
| PVCp | 1056000 | 90510 | 3900000 | 141421 | 1480000 | | 868000 | 62225 |
| PVCp | 1040000 | 1244508 | | | 1810000 | 65054 | | |
| PEx | 629 | 62 | 1717 | 240 | 817 | 38 | 2430 | 240 |
| PEx | 629 | 41 | | | 683 | 65 | | |

### Table X. HPC bacteria in the test waters in contact with the tested pipe materials during the BPP test (2nd trial)

| Sample | HPC after 15 weeks, CFU/ml | | | | HPC after 16 weeks, CFU/ml | | | |
|---|---|---|---|---|---|---|---|---|
| | S/V ratio 0.16 $cm^{-1}$ | | S/V ratio 1.6 $cm^{-1}$ | | S/V ratio 0.16 $cm^{-1}$ | | S/V ratio 1.6 $cm^{-1}$ | |
| | N | stdev | N | stdev | N | stdev | N | stdev |
| blank | 36 | 7 | 25 | 5 | | | | |
| glass | 185 | 40 | 1075 | 48 | | | | |
| glass | 77 | 23 | | | | | | |
| SS | 154 | 25 | 76 | 21 | 8 | 3 | 47 | 2 |
| SS | 153 | 21 | | | 6 | 1 | | |
| PEx | 101 | 21 | 613 | 81 | 15 | 1 | 80 | 9 |
| PEx | 51 | 5 | | | 16 | 8 | | |
| PP-1 | 307 | 12 | 5180 | 28 | | | | |
| PP-1 | 300 | 0 | | | | | | |
| PP-2 | 93 | 12 | 13273 | 1158 | 94 | 6 | 5165 | 64 |
| PP-2 | 1097 | 134 | | | 16 | 6 | | |
| PP-3 | 65 | 5 | 9940 | 295 | 18 | 3 | 3387 | 501 |
| PP-3 | 6760 | 339 | | | 2080 | 113 | | |
| PE | 825 | 247 | 15660 | 1005 | | | | |
| PE | 597 | 220 | | | | | | |
| PVCp | 14000 | 0 | 58067 | 29753 | 265 | 7 | 5450 | 778 |
| PVCp | 17900 | 707 | | | 250 | 42 | | |
| EPDM | 157000 | 7071 | 1218000 | 269985 | 48500 | 707 | 913500 | 19092 |
| EPDM | 117000 | 4243 | | | | | | |

**European Commission**

**EUR 23663 EN – Joint Research Centre – Institute for Health and Consumer Protection**
Title: **Impact of surface to water volume contact ratio on the biomass production potential of the products in contact with drinking water**

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**Abstract**
The biomass production potential (BPP) test is a semi-static test for assessment of growth promoting properties of construction products in contact with drinking water (CPDW) under defined conditions. The test is performed at the product's surface to water volume contact ratio (S/V) of 0.16 $cm^{-1}$, that is quite different from the practice in buildings and domestic installations.

The goal of this study was to evaluate the importance of the S/V ratio for performance of the BPP test and for correct determining the enhancement of microbial growth by CPDW. The BPP of 10 pipe products were compared under the S/V ratios of 0.16 $cm^{-1}$ and 1.6 $cm^{-1}$ in two consecutive trials. Our study found out that the BPP test at the originally proposed S/V contact ratio is a reliable approach for assessment of growth promoting properties of CPDW. The data showed that under the S/V ratio of 0.16 $cm^{-1}$ the test achieves similar results for the BPP of the tested pipe materials as with a more realistic S/V ratio of 1.6 $cm^{-1}$. However, the S/V ratio showed a significant effect on the planktonic biomass concentration and heterotrophic plate count in the test waters in contact with the tested pipe materials and that stronger effect on the water quality can be important from hygienic point of view. Therefore, the impact of the S/V contact ratio on drinking water quality should be taken into consideration for assessment of the products in contact with drinking water. For acceptance of the CPDW, besides a Pass/Fail Criterion for the BPP, a second criterion for evaluation of materials on their effect of drinking water quality needs to be developed and the planktonic biomass concentration could be useful one for this purpose.

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