Biofilms and bacteriological water quality in a domestic installation model simulating daily drinking water consumption
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ABSTRACT
The biofilm formation potential of a drinking water supply system is related to the chemical, microbiological and hydrodynamic characteristics of water, and to the pipe materials in contact with water flow. The goals of this study were: to determine the biofilm dynamics in a model of four drinking water installations, to simulate daily household water consumption; to compare the biofilms developed on different polymer pipe materials and their influence on bacteriological water quality. The results demonstrated that bacterial density of biofilms depended on pipe material type and was influenced by water temperature. The biofilms on polyvinylchloride chlorinated and polyethylene materials had higher bacterial density than biofilms on polypropylene (PP) brands. The effect of the materials, and respectively the biofilms, on drinking water quality was stronger in the overnight stagnation periods, especially during the initial weeks of model operation, than in periods of water consumption. Heterotrophic plate count (HPC) in stagnant or flowing waters and water temperature followed a similar curve pattern, demonstrating significant seasonal variations. In summer, the HPC values of stagnant waters were raised up to seven times higher than in winter and those of the outlet waters (during the consumption periods) were raised up to four times.

Key words | biofilm, daily drinking water consumption, domestic water installation, drinking water, pipe material, water quality

INTRODUCTION
Biofilms represent about 95% of the overall microbial biomass in drinking water distribution systems (DWDSs) (Flemming et al. 2002). The biofilm formation process is closely related to water characteristics (such as composition, temperature, flow velocity, shear stress, etc.) and to the substrate for microbial attachment, characterized by its roughness, hydrophobicity, surface energy and ‘biological affinity’ (Flemming 1991).

Van der Kooij et al. (2005) defined biofilm density in DWDSs as a result of the biofilm formation characteristics of water and biofilm formation potential of pipe products in contact. Since biodegradable organic substances available in water are essential for biofilm growth, their effect on the biofilm formation process have been thoroughly studied (Camper et al. 1996; Zacheus et al. 2000). Besides the evaluation of the biofilm formation rate of the drinking water itself (Van der Kooij et al. 1995) a method of assessment of the net biofilm formation potential (BFP) of a pipe product based only on the substances released from it during its contact with drinking water was developed (Van der Kooij & Veenendaal 2001). Quite a number of pipe materials were assessed and ranked depending on their biofilm and total microbial growth promoting potentials (Van der Kooij & Veenendaal 2001; Enkiri et al. 2006; Tsvetanova & Hoekstra 2009).

In contrast to the net biofilm formation potential of a pipe material, field and model biofilm studies have assessed overall biofilm growth based on the natural substances in water and the substances migrating from a pipe. Some
data on biofilm formation and the effect of pipe materials in real DWDSs could be traced: Hallam et al. (2001) observed differences between the biofilms on cast iron, polyethylene (PE) and polyvinylchloride’s (PVC) pipes removed from a city distribution system; Shin et al. (2007) detected the highest biofilm HPC level on old cast iron pipes and the lowest one on stainless steel pipes. Some model biofilm studies have also reported on differences between the biofilms on cast iron, stainless steel, PE or PVC pipe materials (Ollos et al. 1998; Niquette et al. 2000; Tsvetanova 2006), but this was contrary to the findings of other researchers for the same type of materials (Zacheus et al. 1999; Wingender & Flemming 2004; Manuel et al. 2007). While most studies in model DWDSs have simulated the biofilm formation process under continuous flow conditions, Vonk et al. (1999) focused on the effect of pipe materials in a reference set-up, simulating the intermittent water consumption of a domestic plumbing system. Under that flow condition, the lowest biomass was detected on polyvinylchloride chlorinated (PVC-C) pipes, while the highest HPC was in the biofilm on PE cross-linked. These very few data on microbial growth properties of pipe materials in real domestic installations or their models are the reason why the goals of this study were: to assess biofilm formation in a model of domestic drinking water installations simulating daily household consumption; and to compare biofilms developed on different pipe materials in these periodical flow conditions and their effect on bacteriological water quality.

MATERIALS AND METHODS

The study was carried out in a model of four domestic drinking water installations (Figure 1) comprising four water pipelines made from PVC-C, PE and polypropylene brands PP-1 and PP-2. Each pipeline (with a total length of 2.7 m) simulated the water installation of a household with a daily water consumption of about 126 L/d.

Each pipeline included an end part, referred to as a biofilm ‘tube-in-tube’ reactor, designed as an outer tube where about 20 removable test pipe pieces were placed, forming an inner tube with the same diameter as the main pipeline. The model was fed by tap water with a joint pipe part. Water meters and stop valves were installed at the end of each pipeline to be used for the adjustment of flow velocity and daily water consumption. The flow velocity was 0.3 m/s (equal to 2 L/min). The Reynolds number was 3400.

Operational conditions

Each pipeline simulated the daily water consumption of a household in the morning, at noon, and in the evening. Three working regimes (A with duration of 12 min, B of 6 min and C of 5 min) were used for simulation of water consumption: in the morning – BAC; at noon – CAC; and in the evening – BAB. Between two consecutive regimes of water consumption the magnetic valve was shut-off and the water inside the model remained still from 1 to 8 hours. The total daily flow-through time was 63 min (4.4% of the total daily operation time).

Biofilm analysis

One test piece was taken from each pipeline of the model at certain intervals of time. After the outer surface of the test piece was disinfected by alcohol, the inner surface was wiped with a sterile cotton swab dipped in a physiological solution. Next, the test piece underwent three consecutive treatments for 4 min in separate solution volumes in an ultrasonic bath (Apronex; 40 kHz). The individual portions were pooled. The biofilm suspension was analyzed for
viable heterotrophic bacteria by the pour plate count technique (R2A agar; 7 d; 22°C). Periodically, coliforms and enterococci were analyzed.

**Water analysis**

Comparison of inlet and outlet drinking waters was made to assess the impact of pipe material, with respect to the biofilm formed, on water. Two types of outlet water samples were collected from each pipeline – after 8-hours, overnight stagnation periods and during water consumption period (water flow regime A). The inlet water was thoroughly flushed before taking the samples of inlet and flow waters. Water samples were analyzed for HPC according EN ISO 6222 (yeast extract agar; 3 d; 22°C). *Escherichia coli* and coliforms were detected according EN ISO 9308-1 standard and enterococci according EN ISO 7899-2. The chemical composition of feeding water was analyzed periodically following the operative EU standards.

**Statistical analyses**

A significance t-test for comparison between a pair of biofilm (or water) samples was used, as the truth of a null hypothesis was tested. The difference between logarithms of two compared values was considered significant when the experimental value of t was greater than the t-value from t-distribution at P of 0.05 and degree of freedom n (Miller & Miller 1988).

**RESULTS AND DISCUSSION**

During the model operation the feeding water met the requirements for drinking water quality – nitrate content of 2.3 ± 1.8 mg/l, ammonium below 0.01 mg/l, phosphate of 21.5 ± 10.6 μg/l, oxidisability of 1.8 ± 0.8 mg O2/l. Only the residual chlorine content was permanently low (0.02 ± 0.01 mg/l) because of the dead-end building location and low water consumption in this section. Bacteriological water quality was good – no *E. coli*, coliforms and enterococci were detected. As in domestic installations, the water temperature in the model to a large extent followed the room temperature varying in the range 10–21°C (data presented in Figures 2–4).

**Biofilm dynamics in the domestic drinking water installation model**

The dynamics of biofilm formation on the pipes (as HPC) under the periodic flow conditions are presented in Figure 2.

![Figure 2](https://iwaponline.com/ws/article-pdf/12/6/720/417007/720.pdf)
No coliforms and enterococci were detected in biofilms during the model operation.

All pipe tubes showed different initial bacterial colonization on the 3rd week of model operation. The biofilms on PP-1 and PE achieved a pseudo-steady state after 5 weeks, while one on PVC-C achieved it after 17 weeks. The biofilm on PP-2 pipe attained a steady-state at least after 20 weeks, as a result of intense initial colonization, probably due to a stronger initial migration of substances from the pipe, followed by a significant decrease of biofilm density.

In 2010, the biofilm samples showed significant differences in their bacterial densities, with two exceptions: the biofilms on PP-2 and PVC-C in the 5th week (|t| of 1.6 < t of 2.8 at n = 4) and those on PE and PVC-C pipes in the...
17th week ($|t| < 0.8$ of $2.8$). The observed differences might be a result of the complex effect of several factors: the microbial growth promoting properties of pipe materials themselves, pipe surface properties and biofilm structure, which has an effect on mass exchange processes and cell activity. In the course of time, the microbial growth effect based on migrated substances and pipe surface properties could be concealed or overwhelmed by the flow regime, because water flow flushes the pipe wall throughout the water consumption periods and could partly detach the biofilm, especially weakly adhered cells. At the same time, the initial adhesion of substances and cells from water could change the surface properties of the pipe walls and, in this way, could diminish the differences between pipe materials. Probably for such reasons the initial difference between biofilms in the PP-1 and PP-2 pipe brands disappeared after 6 months and a similar bacterial density ($|t| < 0.9$ of $2.2$ at $n$ of $12$) was kept throughout 2011. However, a different trend was clearly observed for the PVC-C and PE pipes: in 2011 the biofilms on them showed an exponential growth and then a steady-state. The biofilms on the PE pipe increased significantly, enlarging in this way the difference with those on PVC-C and in the whole of 2011 the biofilms on PE, PVC-C and PP pipelines were significantly different ($1$–$3\log\text{CFU/cm}^2$). These results confirmed convincingly the findings of Vonk et al. (1999), attained in a similar flow model, of a difference between biofilms dependent on pipe material type, although the ranking of the tested materials was different, probably related to the different brands under study.

The differences between the biofilms on PP, PE and PVC-C pipes detected in our study were in correspondence also with the findings of Simoes et al. (2006) and Cloete et al. (2003) for a significant influence of the support materials on biofilm HPC level, but opposed to Manuel et al. (2007). In our study the tested PE pipe promoted the highest HPC biofilm density, despite the more retrospective data of Kerr et al. (1999) for a slow accumulation and limited biofilm formation. PVC-C, considered as suitable for plumbing applications due to its low biofilm formation and very smooth surface structure (Van der Kooij & Veenendaal 2001; Yu et al. 2010), demonstrated a comparatively high biofilm density.

Comparing the pattern of the curves of HPC and water temperature, a temperature effect related to material type could be observed. Significant correlations between the biofilm HPC density and water temperature were detected in 2011 ($r = 0.86$ for PVC-C; $r = 0.99$ for PE), but a small temperature effect was shown for PP brands. These results were in correspondence with the finding of Hallam et al. (2003) for approximately 50% higher biofilm activity at a water temperature of $17\,\text{C}$ than at $7\,\text{C}$ and with the data of Ndiougue et al. (2005) for an increase of the biofilm HPC level with temperature (6–18 $\text{C}$).

**Effect of biofilms on stagnant drinking water**

Comparison between inlet water and the waters running out after the overnight stagnation period in the pipelines demonstrated (Figure 3) a significant deterioration of the bacteriological water quality, as a result of bacterial re-growth in the water and biofilm detachment. The results showed a significant increase of culturable bacteria number in the stagnant waters, especially during the initial 10 weeks of the model’s operation. Then, the pipe materials promoted the strongest bacterial re-growth, probably due to the large quantity of easily migrated substances and active cell detachment from fast-growing biofilms.

The PE and PVC-C materials, promoting a higher biofilm growth (Figure 2), had a higher effect on water than the PP brands. In autumn 2010, the PVC-C pipe kept on causing the highest HPC values (being four times lower than in summer), but after the winter (41st week) HPC fell down more than 1 order. In the same period, HPC of the stagnant waters in contact with the rest of the materials decreased by approximately 2 orders.

In summer 2011, bacterial contamination of the waters in the PVC-C and PE pipes was raised up to seven times higher than in the winter; this could be related to more active biofilm growth and to temperature conditions, while in the PP pipes it remained unchanged. Bacterial contamination of all stagnant waters in 2011 was lower than the previous year and was clearly dependent on material type. For all sampling dates, statistically significant differences between the HPC values of all stagnant waters in contact with the tested materials were found, with only a few exceptions for the PP brands.

It is known that microbial activity in a DWDS is closely related to the seasonal fluctuations of water temperature and
that problems with bacteriological water quality are more common in summer when the reaction rate is higher (Holden et al. 1995; Ollos et al. 1998). Our results demonstrated a similar run of the HPC and water temperature curves, as the correlation coefficient \( r \) for all materials in 2010 were in the range of 0.95–0.98. In 2011, the stagnant water inside the PVC-C and PE pipes showed better correlation with temperature (\( r \)-values of 0.76 or 0.66, respectively) than the PP brands did.

Comparing the stagnant water contacted with each pipe material throughout both consecutive summer seasons of the model’s operation, an apparent decrease of HPC (about 2–3 orders) was detected in the second summer. These data upheld the view that water temperature is an important factor for microbial growth, but initial migration of biodegradable substances, pipe surface properties and biofilm peculiarities (such as architecture, thickness, firmness, etc.) could have a more substantial effect on the bacterial contamination of stagnant waters than water temperature.

**Effect of biofilms on water quality during the water consumption periods**

During the drinking water consumption periods the culturable bacteria number in the outlet waters increased significantly in comparison with the inlet water, as a result of biofilm detachment (Figure 4). The highest increase of water pressure immediately after opening the tap and the shear of water flow were the main reasons for cell detachment under the regimes of periodic water consumption.

During the first 10 weeks of the model’s operation the waters running out from all pipelines had the highest HPC values, but only the biofilm on the PVC-C pipe had statistically significant effect on bacteriological water quality (\( \Delta t \) of 2.5 > \( t \) of 2.1; \( n = 18 \)). During autumn 2010, HPC of all outlet waters fell about two to four times, but the effect of the PVC-C pipe remained the highest, as for the overnight stagnant water. In summer 2011, the HPC values of all outlet waters slightly increased. The HPC values showed significant variation, especially in 2011, and the individual effect of each pipe material could be differentiated with difficulty. Because of that, a comparison based on the average HPC values of the inlet and outlet waters was made. The HPC value of inlet water was 34 ± 16 CFU/ml, while for outlet waters they were: 57 ± 25 CFU/ml for PP-1; 60 ± 42 CFU/ml for PP-2; 77 ± 40 CFU/ml for PVC-C and 90 ± 49 CFU/ml for PE. Based on data comparison, the noticeable impact of the biofilms on water quality was statistically confirmed: for PP-1 \( |t| \) of 2.9 > 2.1 at \( n = 16 \); for PP-2 \( |t| \) of 2.3 > 2.1 at \( n = 20 \); for PVC-C tof 3.4 > 2.1 at \( n = 20 \); for PE tof 3.8 > 2.1 at \( n = 12 \). This trend differed from the data from Vonk et al. (1999) who found little impact of pipe materials on HPC, pathogens numbers or ATP content in the waters collected from a similar domestic installation model. This is probably due to the longer operation time of our model and to unfavorable operational conditions (drinking water practically free of residual chlorine and summer water temperature of about 20 °C).

**CONCLUSIONS**

The study, simulating domestic installations in a model with periodic water consumption, has extended our knowledge of biofilm formation in real domestic plumbing made from polymer pipe materials. The results showed that:

– Biofilm formation on the pipelines depended on the material type. The biofilms on PVC-C and PE pipes had higher bacterial density than the biofilms on PP brands.

– Biofilms developed on the pipelines had a substantial effect on the viable bacteria numbers in the overnight stagnant waters and in the outlet waters during the daily water consumption periods. HPC of both water types and water temperature followed a similar curve pattern, demonstrating significant seasonal variations. In summer HPC was raised up to seven times higher in the stagnant waters and up to four times higher in the outlet waters than the winter. Although water temperature is important for microbial growth control, in new domestic installations the other factors, such as initial migration of biodegradable substances, pipe surface properties and periodic water flow, could have a more significant influence.

**REFERENCES**

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