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Abstract Drinking water distribution system biofilms were investigated for the presence of hygienically relevant microorganisms. Early biofilm formation was evaluated in biofilm reactors on stainless steel, copper, polyvinyl chloride (PVC) and polyethylene coupons exposed to unchlorinated drinking water. After 12 to 18 months, a plateau phase of biofilm development was reached. Surface colonization on the materials ranged between 4×10^6 and 3×10^7 cells/cm^2, with heterotrophic plate count (HPC) bacteria between 9×10^3 and 7×10^5 colony-forming units (cfu)/cm^2. Established biofilms were investigated in 18 pipe sections (2 to 99 years old) cut out from distribution pipelines. Materials included cast iron, galvanized steel, cement and PVC. Colonization ranged from 4×10^5 to 2×10^8 cells/cm^2, HPC levels varied between 10^1 and 2×10^5 cfu/cm^2. No correlation was found between extent of colonization and age of the pipes. Using cultural detection methods, coliform bacteria were rarely found, while *Escherichia coli*, *Pseudomonas aeruginosa* and *Legionella* spp. were not detected in the biofilms. In regular operation, distribution system biofilms do not seem to be common habitats for pathogens. However, nutrient-leaching materials like rubber-coated valves were observed with massive biofilms which harboured coliform bacteria contaminating drinking water.

Keywords Biofilms; coliforms; drinking water; HPC; *Pseudomonas*

Introduction

Internal surfaces of drinking water distribution systems are always colonized by microorganisms, mostly in the form of single cells or microcolonies (Ridgway and Olson, 1981; Pedersen, 1990; Servais *et al.*, 1995), but sometimes also as dense multiple cell-layer biofilms (Grubert *et al.*, 1992). It is estimated that 95% of the overall biomass in a distribution system is located on drinking water pipe walls, while only 5% occurs in the water phase (Flemming *et al.*, 2002). Thus, biofilms on pipe surfaces may provide a microbiological contamination potential, resulting in the deterioration of hygienic drinking water quality. There are indications that microorganisms with pathogenic properties can persist, and in some cases, multiply in biofilms; these organisms include bacteria, enteric viruses and protozoan parasites (for reviews, see Percival *et al.*, 2000; Flemming *et al.*, 2002). However, it is unknown if biofilms in drinking water distribution systems are a general and ubiquitous reservoir for hygienically relevant microorganisms. An extended study was undertaken to obtain an overview about the microbiological status of established biofilms in pipes of intact German drinking water systems. In parallel, early biofilm formation on different pipe materials was studied in drinking water biofilm reactors.

Methods

Installation and operation of drinking water biofilm reactors

Two biofilm reactors were operated in the present study. The first reactor was installed at the outlet of the waterworks Dorsten-Holsterhausen (Germany); this full-scale treatment plant supplied non-disinfected drinking water produced from groundwater. The mean water temperature during the 24-month study period was 11.7°C. The second reactor was

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installed within the distribution system at the city of Gladbeck approximately 15 km from the treatment plant. A biofilm reactor consisted of 5 circular columns (height: 760 mm, inner diameter: 100 mm) that were connected in series. Each column was composed of 5 vertically stacked polyethylene modules, which contained V4A steel, copper, polyethylene (PE) and polyvinyl chloride (PVC) coupons (22 mm × 75 mm). The reactors were sterilized with hydrogen peroxide (ca. 500 mg/l) for 24 h. After flushing of the systems, continuous flow with drinking water was started; the flow rate was adjusted to real flow conditions at the respective location in the water system. Sampling of the coupons was performed after 6, 12, 18 and 24 months. At each sampling date, one column was disconnected and immediately transported to the laboratory. After removal from the modules, 3 coupons per material were scraped with a sterile steel-blade and the biofilms were suspended in 30 ml deionized water for further microbiological investigation as described below.

Pipe cut-outs

A total of 18 pipe sections were removed from different German drinking water distribution systems (Table 1). Prior to removal of the pipe sections, water samples were collected at hydrants downstream of the pipeline. Free chlorine concentration measured by the N,N-diethyl-*p*-phenylenediamine colorimetric method was determined on site using a portable spectrophotometer. The external pipe surfaces were disinfected with 10% hydrogen peroxide for 5 min and rinsed with sterile-filtered (0.2 µm) deionized water. Pipe lengths of 1 m were removed using mechanical cutters. The pipes were immediately sealed with sterile plastic foil and transported to the laboratory. Deposits from cast-iron pipes were scraped from the internal surfaces using a sterile stainless steel pipe; in the case of PVC pipes and cement-lined pipes, 100 g of sterile glass beads (5 mm diameter) in 150 ml of deionized water were filled into the pipes, the open ends of the pipes were closed with plastic foil and the pipes were manually shaken for 5 min. The resulting suspension was collected without the glass beads, the pipes were re-filled with 150 ml of water and the procedure was repeated. After up to three repetitions, all suspensions were mixed and analyzed microbiologically as described below.

Microbiological methods

Total cell counts in water samples and biofilm suspensions were determined by staining of the bacteria for 20 min with 4',6-diamidino-2-phenylindole (5 µg/ml) and subsequent quantification by epifluorescence microscopy at 1,000-fold magnification.

The heterotrophic plate count (HPC) was performed by the spread plate method on R2A agar (Reasoner and Geldreich, 1985); colony-forming units (cfu) were determined after incubation of the plates at 20°C for 7 d.

Coliform bacteria in drinking water were analyzed qualitatively in 100 ml-samples by enrichment culture in lactose peptone broth (Oxoid) according to the German Drinking Water Regulations (1990). In biofilm suspensions, *E. coli* and coliform bacteria were quantified by the most probable number (MPN) method in lactose peptone broth after incubation at 36°C for 2 days. In the case of acid and gas formation in the broth cultures, subcultivation on DEV Endo agar (Merck) at 36°C for 24 h was carried out. In addition, spread plates with biofilm suspensions were prepared on m-Endo agar LES (Difco), m-T7 agar (Difco), Chromocult Coliform agar (Merck), and Lactose TTC agar with Tergitol 7 (Merck), and incubated at 36°C for 48 h. Differentiation of typical colonies was performed by biochemical tests according to the German standard DIN 38411 (1991). Coliform bacteria were identified using the API 20 E system (bioMérieux).

P. aeruginosa was analyzed by the MPN method in arginine brilliant green glucose peptone broth (Merck) after incubation at 36°C for 48 h (Schubert, 1989). Subcultures were

grown on cetrimide agar at 36°C for 24 h. The detection of other pseudomonads was performed by the spread plate method on *Pseudomonas* Agar with CFC selective supplement (Oxoid). The plates were incubated at 30°C for 48 h. Representative colonies of each colony type were subcultivated on *Pseudomonas* Agar F (Merck) at 30°C for 24 h to obtain pure cultures. The isolates were identified by their cytochrome oxidase reaction (Bactident-Oxidase, Merck) in combination with the API 20 NE identification system (bioMérieux). Some isolates were additionally analyzed with the Biolog system, using GN2 MicroPlates (Oxoid).

The cultural determination of *Legionella* spp. was performed in untreated, heat-treated and acid-treated biofilm suspensions according to ISO 11731 (1998), using the spread plate method on GVPC agar (Oxoid); the plates were incubated for 10 d at 36°C.

Results and discussion

Formation of biofilms in drinking water biofilm reactors

In order to follow the kinetics of biofilm formation depending on pipe material and flow distance in the distribution system, stainless steel, copper, PVC and PE coupons were exposed to drinking water in biofilm reactors at the outlet of a groundwater treatment plant and within the distribution system. General surface colonization was studied by the determination of total cell counts and viable cell counts (HPC). After 12 to 18 months, the plateau phase of biofilm development was reached in both reactors independent of the coupon material. Then, cell densities were similar in the biofilm reactors at the waterworks and in the distribution system, indicating that during transport of drinking water in the unchlorinated system, no change of water quality had taken place that might have influenced biofilm growth. After an exposure time of 18 months, no significant difference in the extent of biofilm formation between steel, PVC and PE was observed, whereas copper revealed slightly elevated cell densities (2.4-fold to 8.6-fold higher total cell counts) compared with the other materials. After 18 months, the total cell counts on all materials ranged from 3.6×10^6 to 3.1×10^7 cells/cm^2, and the HPC levels ranged from 8.6×10^3 to 7.1×10^5 cfu/cm^2. The total cell counts were generally 100-fold to 1,000-fold higher than the corresponding HPC values. These results are in agreement with observations of Pedersen (1990) and Zacheus *et al.* (2000), who also found no significant difference in biofilm formation of steel and plastic-based surfaces after exposure to drinking water for several months. Bacterial colonization of copper in drinking water systems has also been reported by Tuschewitzki (1990) and Schwartz *et al.* (1998), indicating that heterotrophic water bacteria are able to grow on copper surfaces in drinking water environments.

Established biofilms in drinking water pipes

The deposits from 18 pipes of different German drinking water distribution systems were studied microbiologically using the same methods that were applied for the analysis of biofilm reactor coupons. Pipe sections that had been in use between 2 and 99 years were cut out from intact distribution networks, whose routinely analyzed bacteriological and chemical water quality complied with the requirements of the German Drinking Water Regulations (1990). The drinking water was either non-chlorinated or contained relatively low levels of free chlorine residuals at the sampling sites (Table 1). The total cell counts in drinking water taken from the pipelines to be sampled ranged from 1.9×10^4 to 5.6×10^5 cells/ml. Visual inspection of cut-out pipe sections revealed that cast-iron pipes contained relatively thick and heterogeneous mineral incrustations of brown colour, whereas the cement-lined iron pipes and PVC pipes contained thin and evenly distributed deposits.

The total cell counts on the internal surfaces of the 18 pipes ranged from 4.1×10^5 to 2.0×10^8 cells/cm^2, while the HPC levels ranged from 1.2×10^1 to 2.0×10^5 cfu/cm^2 (Table 1).

Table 1 Microbial analysis of 18 drinking water pipes cut out from different German drinking water distribution systems

City	Free chlorine (mg/ml)	Pipe material	Age (yr)	Area sampled (cm^2)	Total cell count (cells/cm^2)	HPC (cfu/cm^2)	Coliforms (cfu/cm^2)
Berlin	n. c.	Cast iron	2	3,009	4.9×10^7	9.9×10^3	n. d.
Duisburg	0.05	Cement-lined	8	3,272	8.0×10^5	1.2×10^1	0.04
Berlin	0.13	Galvanized steel	10	976	2.0×10^8	1.2×10^5	n. d.
Rhade	n. c.	PVC	12	3,142	1.3×10^6	2.8×10^3	n. d.
Duisburg	0.13	Cement-lined	20	3,343	3.0×10^5	$1,5 \times 10^2$	n. d.
Berlin	n. c.	PVC	24	5,511	1.6×10^7	7.8×10^3	0.26
Berlin	n. c.	PVC	24	2,779	7.4×10^6	3.1×10^3	n. d.
Duisburg	< 0.01	Cast iron	26	3,189	6.7×10^6	1.5×10^4	n. d.
Duisburg	0.01	Cast iron	27	3,810	2.4×10^7	5.0×10^4	n. d.
Duisburg	< 0.01	PVC	28	3,110	4.8×10^5	6.3×10^2	n. d.
Duisburg	< 0.01	Cast iron	30	3,340	1.1×10^7	3.8×10^5	n. d.
Duisburg	< 0.01	PVC	34	3,456	1.9×10^6	3.0×10^1	n. d.
Mülheim	< 0.01	Cast iron and cement-lined	37	3,244	6.1×10^6	2.0×10^5	n. d.
Mülheim	0.07	Cast iron	49	2,010	5.8×10^7	2.5×10^2	n. d.
Duisburg	0.02	Cast iron	62	2,540	2.1×10^7	1.0×10^5	n. d.
Berlin	< 0.02	Cast iron	73	2,942	n. a.	1.4×10^5	n. d.
Mülheim	< 0.01	Cast iron	81	3,236	n. a.	1.7×10^5	n. d.
Oberhausen	0.01	Cast iron	99	3,202	2.6×10^7	1.3×10^4	n. d.

n. c., not chlorinated; n. d., not detected; n. a., not analyzed

The areal cell counts suggested a relatively thin and patchy colonization, or maximally monolayer biofilms (Griebe and Flemming, 2000); only at a density of above 10^8 cells/cm^2, as was observed for the galvanized steel pipe (Table 1), could a biofilm with several cell layers be assumed.

The total cell counts in the cast-iron pipes of the present study ($0.7 \times 10^7 - 5 \times 10^7$ cells/cm^2) corresponded to cell counts determined on cast-iron coupons ($0.3 \times 10^7 - 2.6 \times 10^7$ cells/cm^2) that had been exposed in different French drinking water distribution systems (Servais *et al.*, 1995). The viable counts of the biofilms of the present study were within the range of HPC levels reported for 20 pipe coupon samples ($1.0 \times 10^1 - 2.2 \times 10^6$ cfu/cm^2) ranging in age from 8 to 90 years from distribution systems in the USA (LeChevallier *et al.*, 1987); these samples were mostly cast iron, cement-lined and asbestos cement. HPC levels within similar orders of magnitude between 1.0×10^3 and 5.2×10^6 cfu/cm^2 were also determined for biofilms of 8 drinking water pipes 0.5 to 10 years old (cast iron, cement-lined, PVC) from the distribution system of Leicester, UK (Hallam *et al.*, 2001).

The percentages of viable (HPC) to total cell counts varied between 0.0004% and 3.5%; in most cases, the HPC values were several orders of magnitude lower than total cell counts (Table 1). In the biofilm reactors described above, the proportion of colony counts in relation to total cell counts ranged from 0.01% to 2.63% on the material coupons which had been exposed for 6 to 24 months to drinking water. Percival *et al.* (1998) reported a percentage of HPC to total cell counts of 1.9% to 11.2% on steel surfaces exposed to drinking water for over 12 months; Boe-Hansen *et al.* (2002) found a culturable fraction of steel surface-attached bacteria in the range beween 0.004% and 24% during a study period of 522 days, where the highest percentages were achieved in the young biofilm. Thus, low culturability typical of bacteria in oligotrophic drinking water environments also seems to be characteristic of bacteria in several months to years old drinking water biofilms. Possible explanations may be unfavorable culture conditions or the adoption of a viable but non-culturable state by the biofilm bacteria.

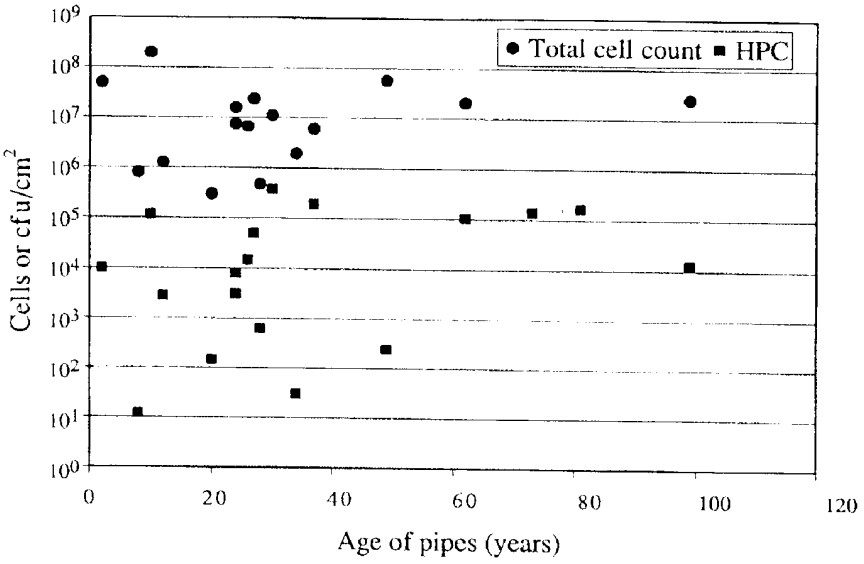

Figure 1 Bacterial densities (total cell counts, HPC) in biofilms from drinking water pipes of different ages

There appeared to be no correlation between bacterial densities (total and viable cell count) and the age of the pipes (Figure 1). Since the minimum age of the pipes was 2 years, it can be concluded that the plateau phase (pseudo-steady state) of pipe wall colonization can be reached after maximally 2 years in operation under the drinking water conditions examined in the present study. In agreement with this assumption was the observation of biofilm development in the biofilm reactors mentioned above, where the biofilm plateau phase was reached within this time period, and then the extent of surface colonization on all materials examined in the biofilm reactors was in the same orders of magnitude as in the drinking water pipes (Table 1). Thus, it can be expected that the pseudo-steady state of drinking water biofilms on inert materials can be reached after a few months of exposure. The plateau phase of drinking water biofilm formation has been described to occur within one week at water temperatures above 15°C (Donlan *et al.*, 1994), 3 weeks (Block *et al.*, 1993; Hallam *et al.*, 2001), 4 months (Percival *et al.*, 1998) or 200 days (Boe-Hansen *et al.*, 2002), while in other studies a delay in biofilm development to the plateau phase over several months was observed, probably due to water temperatures below 15°C (Donlan *et al.*, 1994), the presence of disinfectant residuals or the grazing activity of protozoa (Pedersen, 1990).

Interestingly, there seemed to be no influence of free chlorine residuals in the drinking water systems on bacterial colonization of the pipes (Table 1). In the presence of the relatively low free chlorine levels measured in the present study, bacterial colonization seemed not to be severely inhibited. Pedersen (1990) also observed a significant surface colonization of 4.9×10^6 cells/cm^2 on steel and PVC exposed for 167 days to drinking water containing 0.1 mg/l free chlorine. However, it was reported that chlorine levels higher than those in the drinking water networks of the present study (> 0.1 mg/l) can result in the control of the extent of biofilm formation in drinking water systems (Servais *et al.*, 1995; Ollos *et al.*, 2003).

The tendency of surface colonization of the 18 pipe sections was in the order of cement-lined pipes ≤ PVC pipes ≤ cast-iron pipes (Table 1). The lower colonization of cement-lined and PVC pipes may have been due to the higher pH values in deposits from these pipes; at pH values above 7.5, a decrease of total and viable cell counts was observed. The most sparsely colonized surfaces occurred in the case of the two cement-lined pipes with pH values of the deposits of 9.1 and 9.2, respectively. Thus, alkaline pH conditions at the interface of pipe wall and water phase may delay and control the long-term formation of drinking water biofilms.

Coliform bacteria in drinking water biofilms

Young biofilms. In the biofilms on all material coupons in the drinking water biofilm reactors, *E. coli* and coliform bacteria were not detected; the only exception was a coliform-positive biofilm sample on a PVC coupon in the waterworks reactor after 6 months of operation. The coliforms were detected at a concentration of 15 cfu/cm^2 and were identified as a *Citrobacter* species. The coliforms were not detected during longer exposure times.

Old biofilms. In samples from the internal pipe surfaces, coliform bacteria were found in 2 out of the 18 biofilms at relatively low levels (Table 1), while coliforms were not detected in 100-ml sample volumes of drinking water from the same pipes. The isolate from the PVC pipe biofilm was identified as the environmental coliform species *Rahnella aquatilis*; the isolate from the cement-lined pipe was grouped as a coliform bacterium according to the German Drinking Water Regulations (1990), but it was not identified any further. *E. coli* was not detected in the pipe biofilms with any of the methods used.

Biofilms on rubber-coated valves. In the course of the present investigation, case histories of coliform occurrences in several German drinking water systems provided the opportunity to study their relationship to drinking water biofilms. On the basis of a systematic sampling strategy, biofilms on rubber-coated valves were supposed to be related to the coliform episodes. A total of 21 rubber-coated valves from 6 different water systems were analyzed microbiologically; details of the study are described in Kilb *et al.* (2003). The rubber-coated valves had been in use for 3 weeks to 4 years. Visual inspection of the valve surfaces after recovery from the pipe systems showed that in most cases, thick slimes or crusty deposits were clearly visible with the naked eye, while the inner surfaces of adjacent drinking water pipes were always visually free of deposits. The total cell numbers on the rubber surfaces varied between 2.7×10^6 and 1.8×10^9 cells/cm^2, the concentration of HPC bacteria ranged from $3.0 \times 10^5 - 5.4 \times 10^9$ cfu/cm^2. For biofilms from 18 rubber-coated valves, the proportion of HPC in relation to total cell count was found to vary from 2% to 73%. This relatively high percentage of culturable biofilm bacteria indicated favorable nutrient conditions on the valve surfaces compared to the drinking water biofilms mentioned above where HPC usually represented lower percentages of the total cell count. Coliform bacteria were found in 15 out of 21 biofilms from the rubber-coated valves with numbers ranging from 1.0 MPN/cm^2 to 4.7×10^3 MPN/cm^2. In 11 cases, coliforms were detected both in drinking water and in biofilm samples. In 14 of the 15 coliform-positive biofilm samples, *Citrobacter* species were identified, while in one biofilm *Enterobacter cloacae* was detected. When isolated both from biofilms and from water samples, the coliform species from biofilms had the same biochemical profiles in the API 20 E system as had the coliform isolates from the respective drinking water samples.

Observations from field studies and experimental systems indicate that coliform bacteria are able to survive and multiply in biofilms even under low-nutrient and low-temperature conditions of distribution systems (LeChevallier *et al.*, 1987; Camper *et al.*, 1991). As a consequence, it is generally assumed that coliforms are able to persist and grow in drinking-water biofilms (Camper, 1994). Release of coliform bacteria from biofilms into the bulk water may be one of the causes of sporadic or intermittent events of coliform occurrence during routine drinking water analysis. In the present study, biofilms on the inert materials investigated in the biofilm reactors and drinking water pipes only rarely contained coliform bacteria in relatively low numbers; these results correspond to reports in the literature of occasional occurrences of coliform bacteria on the internal surfaces and in deposits of drinking water distribution systems without any apparent failures in the water treatment (LeChevallier *et al.*, 1987; Sartory and Holmes, 1997; Zacheus *et al.*, 2001).

However, biofilms on a number of rubber-coated valves were identified as a reservoir of coliform bacteria. These case histories demonstrate that specific, discrete locations within a distribution system can act as point sources of drinking water contamination; from a public health perspective, this phenomenon is of importance since contamination of drinking water with coliforms from distribution system biofilms can interfere with the indicator function of coliforms for faecal or other undesirable exogenous contaminations and mask true failures in water treatment and maintenance of the network. A possible explanation for extensive biofilm formation on rubber-coated valves may be the availability of utilizable carbon compounds, for example low-molecular-weight additives such as paraffins used as softeners, that leached from the coating material, providing nutrients at the valve surfaces.

Pseudomonads in drinking water biofilms

In the present study, the important opportunistic pathogen *P. aeruginosa* was detected by cultural methods neither in young biofilms in the biofilm reactors and in old biofilms from the 18 drinking water pipes nor in water samples taken from the respective water systems. *B. cepacia* (formerly *Pseudomonas cepacia*) as another hygienically relevant pathogen was also not found in any of the biofilm samples. The concentrations of other *Pseudomonas* species within the biofilms in the biofilm reactors and from the drinking water pipes were relatively low; *Pseudomonas* spp. were detected in 15 of the 18 pipe biofilms at concentrations varying between 0.1 cfu/cm^2 and 4.4 × 10^2 cfu/cm^2, and on the steel, PVC and PE coupons at concentrations ranging from 6.1 cfu/cm^2 to 2.4 × 10^2 cfu/cm^2. Among the species identified, *P. fluorescens* was the most frequent isolate (in all of 20 *Pseudomonas*-positive biofilms on material coupons in the biofilm reactors; in 10 of 12 *Pseudomonas*-positive drinking water pipe biofilms); other species were *P. stutzeri*, *P. putida* and *P. mendocina*. On the copper coupons in the biofilm reactors, *Pseudomonas* species were not detected with one exception (coupon after 24 month exposure in the distribution system). These observations indicate that in contrast to other heterotrophic water bacteria (see above), pseudomonads seem to be relatively sensitive to copper ions and thus do not colonize copper surfaces, or pseudomonads may enter a non-culturable state, so that they cannot be detected by the culture methods used.

The data suggest that drinking water biofilms of public distribution systems in regular operation are not a common habitat for *P. aeruginosa* and thus are no source of contamination with this species. However, under certain conditions such as during repair works, installation of new pipelines, storage of water, and in certain areas such as tubes in dental unit water systems, plumbing systems of hospitals or household installations, *P. aeruginosa* has been shown to persist and multiply in biofilms and to be the cause of serious infections (Botzenhart and Döring, 1993; Barbeau *et al.*, 1996; de Victorica and Galván, 2001; Anaissie *et al.*, 2002). The other pseudomonads identified in the drinking water biofilms of the present study with no or only low pathogenic potential seem to be typical inhabitants of aquatic biofilms in natural environments and technical water systems (Read and Costerton, 1987; Barbeau *et al.*, 1996). On the basis of this study, it can be assumed that, except for *P. aeruginosa*, the *Pseudomonas* species identified occur ubiquitously on drinking water materials with the exception of copper, and belong to the autochthonous microbial community in drinking water biofilms, where they represent a small proportion of the total heterotrophic bacterial community.

Legionellae in drinking water biofilms

Using the culture method according to ISO 11731 (1998), *Legionella pneumophila* and other *Legionella* species were detected neither in biofilms on steel, copper, PVC and PE coupons exposed in the drinking water biofilm reactors nor in any of the 18 biofilm samples

from the drinking water pipes. Using methods that were not based on culturing such as polymerase chain reaction, southern blot hybridization and *in situ* hybridization, non-pneumophila *Legionella*, but not *L. pneumophila*, were detected in biofilms on PE, PVE and steel surfaces that had been exposed in a drinking water system for three weeks (Schwartz *et al.*, 1998). These observations suggest that the biofilms examined in the present study ranging in age between 6 months and 99 years in the biofilm reactors and in the drinking water pipes either did not harbour *Legionella* bacteria or contained them below the detection limit of the culture methods used, or these bacteria were present in a viable non-culturable state.

Conclusions

The work presented here indicates that biofilms are ubiquitous in drinking water distribution systems but they do not represent a common habitat for hygienically relevant organisms. Biofilm formation is usually not confluent but patchy. Only a minor fraction of the population can be detected by the HPC method, indicating a low nutrient status and low physiological activity. After a period of about 12 to 18 months, a final level of colonization seems to be reached which is not exceeded even after many decades of operation of the distribution system. No significant difference in the colonization of the investigated materials could be detected. The range of bacterial densities (total cell counts, HPC) determined in the pipe biofilms seem to represent baseline levels of colonization in intact drinking water distribution systems which meet the bacteriological requirements of legal drinking water regulations. Although the bacterial densities can be relatively high at surfaces, no relevant contamination of the drinking water could be detected with the methods used in this study. In parallel to the work presented above, the biofilm samples of the present study were investigated for the occurrence of additional obligate and facultative pathogens by other laboratories in a joint research project (compiled in Flemming, 2003). Neither pathogenic *Yersinia* spp., *Campylobacter* spp., *Helicobacter pylori*, colon cell-adhesive aeromonads nor protozoan parasites such as *Giardia* and *Cryptosporidium* could be detected. Atypical mycobacteria, aeromonads, and free living amoebae, all with low infectious potential, were found occasionally. Fungi with apathogenic or low pathogenic potential were frequently found at low numbers and seem to belong to "normal" biofilm populations in drinking water systems.

However, when plumbing materials are used which release biodegradable substances, massive biofilm growth can be supported. Such biofilms have been found to be habitats for hygienically relevant organisms (coliform bacteria) and seem to allow for their growth with subsequent contamination of the drinking water. These materials can be avoided in drinking water systems, whereas they are common in household plumbing systems. Here, they clearly represent a hygienic risk, in particular for convalescent and otherwise immunocompromised persons.

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