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Formation of biofilms in drinking water distribution networks, a case study in two cities in Finland and Latvia

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Abstract The formation of biofilms in drinking water distribution networks is a significant technical, aesthetic and hygienic problem. In this study, the effects of assimilable organic carbon, microbially available phosphorus (MAP), residual chlorine, temperature and corrosion products on the formation of biofilms were studied in two full-scale water supply systems in Finland and Latvia. Biofilm collectors consisting of polyvinyl chloride pipes were installed in several waterworks and distribution networks, which were supplied with chemically precipitated surface waters and groundwater from different sources. During a 1-year study, the biofilm density was measured by heterotrophic plate counts on R2A-agar, acridine orange direct counting and ATP-analyses. A moderate level of residual chorine decreased biofilm density, whereas an increase of MAP in water and accumulated cast iron corrosion products significantly increased biofilm density. This work confirms, in a full-scale distribution system in Finland and Latvia, our earlier in vitro finding that biofilm formation is affected by the availability of phosphorus in drinking water.

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Introduction

In many industrial systems, including drinking water distribution networks, the bacterial biomass is associated with biofilm growing on the surfaces of the pipelines [13]. Biofilms reduce the aesthetic quality of distributed water, may increase the corrosion rate of pipes, and increase the survival of pathogenic microbes in drinking water distribution systems [22, 27]. Thus, if we are to find means to combat biofilms, more knowledge about the factors that influence biofilm formation is needed. These factors include microbial nutrients (e.g. the concentration of carbon, nitrogen and phosphorus) in water, temperature, pipe materials, disinfectants, bacteria in water and the hydraulic regime in drinking water supply systems [4, 5, 11, 17, 21, 31]. To restrict biofilm growth, final chlorination is most frequently applied to drinking water. However, due to the risk of elevating the levels of harmful disinfection by-products of chlorine in drinking water, other approaches to reduce biofilm formation have been considered. One such solution would be to remove the nutrients during water treatment, thus causing nutrient limitation for microbial growth [12].

In many countries, organic carbon limits microbial growth in drinking water and in drinking water biofilms [1, 10, 24]. There are several regions where the concentration of organic carbon in drinking water is high and the concentration of phosphorus low; in these waters bacterial growth is often limited by phosphorus. This kind of water occurs in Finland, Japan, Latvia and China [18, 25, 30]. Usually, efficient removal of phosphorus by chemical precipitation is the reason for phosphorus limitation [15]. It could be anticipated that in this kind of drinking water, the availability of phosphorus would also affect bacterial growth in biofilms.

Therefore, phosphorus removal from drinking water could be an effective way to control biofilm formation in the distribution net. However, bacterial growth is not directly coupled to phosphorus uptake because bacteria can store phosphorus and use it when phosphorus in the water becomes depleted [26, 29]. Therefore, the relationship between phosphorus and biofilm growth is not straightforward, and it is by no means certain that phosphorus removal from water would restrict biofilm development in a drinking water distribution system.

In carbon-limiting water it has been shown that phosphorus does not affect bacterial density in biofilms [1, 3, 24]. However, in in vitro experiments with water where phosphorus was limiting microbial growth of planktonic bacteria, the addition of phosphorus increased bacterial density in the biofilms [16]. Phosphorus is also known to affect biofilm growth in some natural aquatic ecosystems [20].

In this study, we installed identical biofilm collectors within the distribution networks and in the waterworks of two cities, Kuopio in Finland and the Latvian capital Riga. During the study, drinking water quality was monitored, especially for nutrients (carbon and phosphorus), and related to the development of bacterial numbers in biofilms. The aim was to identify factors affecting formation of biofilms in full-scale drinking water processing and distribution, with special attention being paid to phosphorus.

Materials and methods

Sampling points

In Kuopio, the biofilm collectors were installed in two waterworks and at three points in the drinking water distribution system. The distribution system collectors were installed at the pumping stations, where collectors were connected to the drinking water pipeline in a toilet. One waterworks used surface water as its raw water (Surface plant). The water was first bank-filtered then chemically coagulated. The other waterworks (Recharged plant) recharged groundwater (bank filtration, dry and wet contact filters, and slow sand filtration). The collectors were installed in the waterworks before the chlorination unit because we especially wanted to study the effects of nutrients on biofilm growth, and after chlorination the formation of biofilms is controlled mainly by chlorine. In the distribution networks, one collector was in an area distributing recharged groundwater (approximately 4 km from the waterworks—Recharged net), and two collectors were installed at distribution networks distributing water from surface waterworks (5 and 10 km from the waterworks—Surface net 5 and 10 km, respectively). At Surface net 5 km there was some mixing of surface and recharged waters in distributing networks. The sampling points were chosen to represent, as well as possible, the network and two different waters produced in two different waterworks.

In Riga, the biofilm collectors were installed in three waterworks and at two points in the drinking water distribution networks. One waterworks produced drinking water from surface water (Surface plant) by coagulation-precipitation and biological treatment methods (ozonation followed by biologically active carbon filtration). The collector was installed at the plant before chlorination at the outlet from one of the filters used for the biological treatment. The second waterworks extracted groundwater from which about one-half was artificially recharged. The collector was installed in the waterworks (Groundwater plant) after chlorination (0.5–1.0 mg l^{-1}). The third waterworks extracted water from a deep confined aquifer (Artesian plant). The collector was installed at the plant before chlorination. In the distribution networks, one collector was installed in the area supplied with a mixture of water from Groundwater plant and Artesian plant (Groundwater net). Another collector was installed in an area of the distribution networks (Surface net) supplied from the Surface plant. The biofilm collectors were connected directly to the water mains in the networks.

Biofilms

The pipe systems (biofilm collectors) used for biofilm collection consisted of 21 pieces of polyvinyl chloride (PVC)-pipe (diameter 10 mm, length 10 cm), which were connected in line with stainless steel fittings. Water flowed through the collector at a velocity of 1 l min^{-1} (Re = 1,566, laminar flow), which was adjusted with a valve at the outlet. Before biofilm sampling, each piece of PVC-pipe was disconnected by closing both ends with ball valves. The pipes were removed with the water inside the tubes. At every sampling time, three 10-cm pieces were disconnected for analyses. Biofilms were removed by shaking with sterile 2 mm glass beads and rinsing twice with 5 ml sterile water. Before glass bead shaking, 1–2 ml water was removed from the pipes. The extraction from the 10-cm pieces, as well as the removed water, were analysed for acridine orange direct counts (AODC) [7], heterotrophic plate counts (HPC) on R2A-agar [23] and, in Kuopio, for adenosine triphosphate (ATP). When calculating the results, the amount of bacteria in drinking water was distinguished from the number of bacteria in the biofilm suspension.

For AODC, 2 ml biofilm extract was filtered on a black 0.22 μm Nuclepore membrane filter (Whatman, Clifton, N.J.) and stained with a 0.01% dilution of acridine orange. Bacteria were counted with an epifluorescence microscope (Olympus BH-2, Tokyo, Japan or Leica DMLB, Wetzlar, Germany). In heterotrophic plate count (HPC) analysis, R2A-agar plates were incubated for 7 days at 22°C before counting colony forming units (cfu). The content of ATP was determined by means of a Bio Orbit 1251 luminometer using protocols supplied by the manufacturer. The measured light output was converted to ATP concentration by using a

previously determined conversion factor. ATP was analysed only from biofilms growing in Kuopio.

To determine metal concentrations in the biofilms, samples were dried and metals extracted by heating the sample in solution of nitric acid and hydrogen peroxide. Concentrations of metals were detected by flame atomic adsorption spectrometer (Perkin-Elmer 403). The values obtained were converted to micrograms per square centimetre.

Water analyses

Total organic carbon (TOC) was analysed by a high temperature combustion method with a Shimadzu 5000 TOC analyser (Kyoto, Japan). Water was acidified and purged before analysis. Assimilable organic carbon (AOC) was analysed by a modification [19] of the original method [10]. The modification included addition of inorganic nutrients to ensure that only the AOC content restricted microbial growth, i.e. AOC was measured as $AOC_{potential}$ [19]. The growth of *Pseudomonas fluorescens* P17 (ATCC 49642) and *Spirillum* sp. strain NOX (ATCC 49643) in water samples was calculated to correspond to acetate equivalents. In water samples containing chlorine, residual chlorine was removed by the addition of 50 µl 0.02 M (for 100 ml) sodium thiosulphate.

Microbially available phosphorus (MAP) concentrations were analysed by a bioassay where the maximum growth of *P. fluorescens* P17 (ATCC 49642) in sterilised water samples is related to the phosphorus concentration [14]. Inorganic salts (except phosphorus) and sodium acetate were added to the water to ensure that growth of the test bacteria was limited solely by phosphorus. The maximum microbial cell production (cfu ml^{-1}) was converted to a phosphorus concentration using a previously determined empirical yield factor.

HPC and total concentrations of bacteria in drinking water were analysed as described above for biofilms. Free residual chlorine was analysed in Kuopio using a Palinest Micro 1000 chlorometer (Palintest, Gateshead, UK) and total residual chlorine in Riga by the *N,N*-diethyl-1,4-phenylenediamine method.

Statistical analyses

Spearman rank correlations were calculated with the program SPSS 10.1 (Statsoft, Tulsa Okla.). Regression analysis was performed with the Microsoft Excel program (Microsoft, Seattle, Wash.).

Results and discussion

Water quality

The concentrations of microbial nutrients in drinking water from Kuopio and Riga differed (Table 1). In Riga, the concentration of AOC in drinking water was markedly higher than the corresponding value in Kuopio. The TOC content was also higher in all sampling points in Riga except at the outlet from the artesian plant. The high content of AOC in treated surface water is, perhaps, a result of ozonation of a source water with a high content of natural organic matter, mainly humic substances. Ozone degrades organic matter into a form that is more available to microbes [10, 9]. The reason for the high AOC content in the artesian water remains unknown. Even though the AOC content was quite high in the artesian water and in the treated surface water, only a small fraction ($< 10\%$) of it could be utilised by bacteria because of the shortage of MAP in these waters. This was proved by the increase in the AOC yield after the addition of nutrients in AOC analyses. In AOC analyses with nutrient addition, all other inorganic nutrients but no organic carbon are added to the water [19]. We compared the results analysed with and without

Table 1 Chemical and microbiological characteristics of water at the biofilm sampling points. Values are average ±SD; number of analyses is in parenthesis. *T* Temperature (°C), *TOC* total organic carbon (mg l^{-1}), *AOC* potential assimilable organic carbon (µg AOC-C l^{-1}), *MAP* microbially available phosphorus (µg MAP l^{-1}), *HPC* heterotrophic plate counts (cfu ml^{-1})

Sampling point	T	TOC	AOC	MAP	HPC[a]	Chlorine
Riga						
Groundwater plant	9.1 ± 0.8 (7)	7.3 (1)	538 ± 229 (9)	3.39 ± 2.42 (9)	70 (8)	0.34 ± 0.09 (8)
Groundwater net	7.4 ± 0.7 (3)	5.9 (1)	664 ± 292 (9)	2.88 ± 2.02 (9)	6,900 (9)	< 0.1 (6)
Artesian plant	7.5 ± 0.1 (6)	1.0 (1)	761 ± 525 (10)	0.34 ± 0.30 (10)	70 (10)	Before chlorination
Surface plant	9.9 ± 6.6 (11)	8.2 (1)	765 ± 227 (10)	0.17 ± 0.17 (10)	1,600 (9)	Before chlorination
Surface net	15.4 ± 2.2 (4)	NA[b]	695 ± 426 (9)	0.62 ± 0.91 (8)	3,400 (9)	ND[c]
Kuopio						
Recharged plant	6.6 ± 4.1 (6)	1.9 ± 0.1 (2)	31 ± 20 (7)	0.56 ± 0.66 (7)	210 (6)	Before chlorination
Recharged net	8.8 ± 5.2 (6)	2.4 ± 0.8 (4)	40 ± 13 (9)	0.43 ± 0.14 (9)	380 (11)	0.16 ± 0.11 (4)
Surface plant	8.7 ± 1.7 (6)	2.9 ± 0.3 (3)	24 ± 8 (8)	0.18 ± 0.10 (7)	300 (8)	Before chlorination
Surface net 5 km	10.7 ± 2.8 (5)	2.5 ± 0.8 (7)	39 ± 12 (9)	0.63 ± 0.63 (9)	3,300 (12)	0.08 ± 0.07 (5)
Surface net 10 km	14.4 ± 2.4 (6)	2.4 ± 0.2 (4)	34 ± 9 (9)	0.58 ± 0.71 (9)	24,000 (12)	0.04 ± 0.03 (5)

[a]Geometric mean value
[b]Not analysed
[c]Not detected

nutrient addition and found that with nutrient addition, the test produced concentrations of AOC about ten times higher than without nutrients (results not shown). Low levels of MAP were observed in artesian water, recharged groundwater and treated surface water both in Riga and Kuopio. In Kuopio, phosphorus has been previously recognised as being the limiting nutrient for bacterial growth in drinking water [15, 16]. In Riga, the content of MAP in the outlet from the groundwater plant and in the groundwater net was high (Table 1). The high MAP content in groundwater agrees with earlier findings in untreated Finnish groundwaters [15].

Drinking water temperature varied between 6.6 and 15.4°C and was highest in networks distributing surface water (Table 1), especially during summertime.

At all sampling sites, except in the groundwater plant in Riga, the total residual chlorine level was lower than 0.3 mg l^{-1}. The highest number of heterotrophic bacteria (by HPC) was found in the Kuopio surface network, even though the AOC and MAP concentrations were relatively low (Table 1). The reason was probably the high temperature and long residence time of the water (3–4 days).

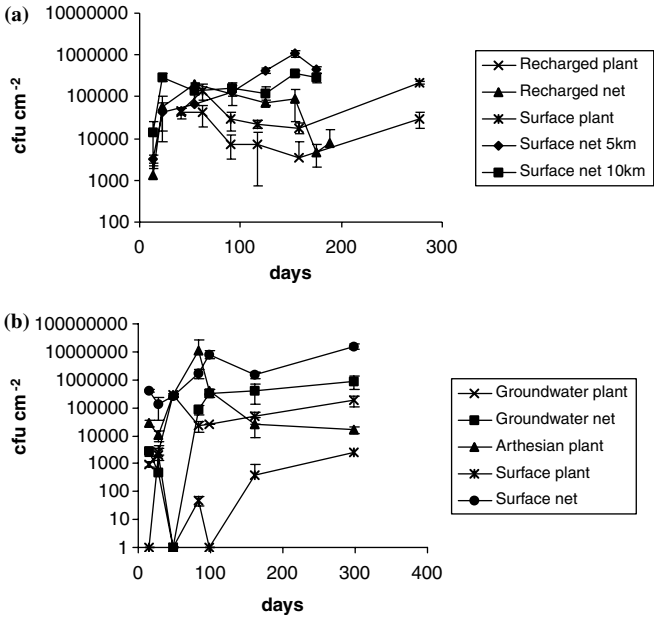

Fig. 1 Biofilm formation on polyvinyl chloride (PVC) pipes in **a** Kuopio and **b** Riga, determined as numbers of heterotrophic microbes (cfu) per square centimetre. *Error bars* SD ($n = 3$)

Biofilm formation

The formation of biofilms was analysed for HPC, total bacteria number and ATP content (the latter only in Kuopio). Biofilms reached their steady state after 2–3 months, after which the microbial concentration did not increase significantly (Figs. 1, 2). At room temperature in the laboratory, biofilms may reach steady state within 3 weeks [16].

In Riga, the lowest microbial concentration in biofilms was observed in the outlet from surface water plant, although the biofilm collector was installed before chlorination (Figs. 1, 2). One possible reason for this finding was the very low concentration of MAP in the treated surface water (Table 1). Also in Kuopio, the number of HPC and concentration of ATP in biofilms were lowest in the outlet from the water plant (Figs. 2 and 3), probably because of the minute number of bacteria in the water. It was interesting that in Kuopio the total number of bacteria was highest in biofilms from the surface water plant. This means that in the surface plant there was a high number of total bacteria in biofilms, but chemical water treatment subsequently clearly decreased their viability.

Although the MAP level was relatively low (Table 1), the bacterial number in the biofilm of the distributed surface water in Riga was very high; in fact it was higher than that in biofilms of distributed water in Kuopio (Figs. 1, 2). This could be explained by the high water temperature over the study period and especially by the poor condition of the pipe where the biofilm collector was installed. The pipe was severely corroded and thus released corrosion products into the PVC collector. The high iron content was confirmed by metal analyses of the

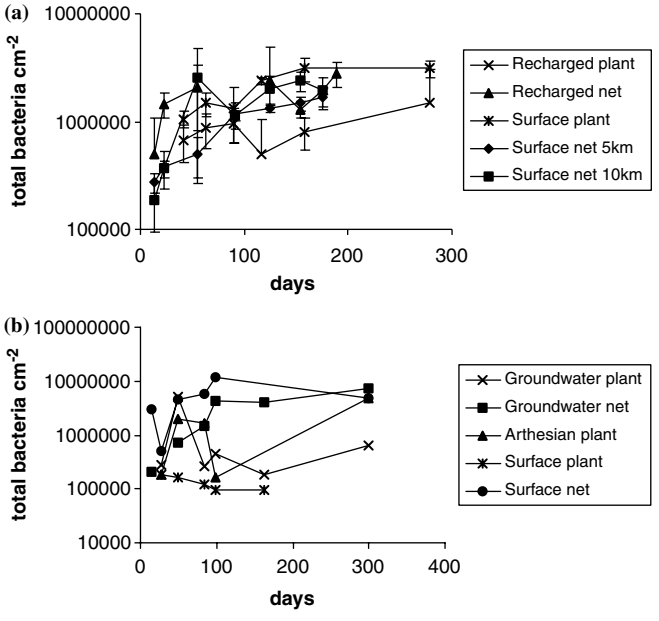

Fig. 2 Biofilm formation in **a** Kuopio and **b** Riga on PVC pipes, determined as numbers of total bacteria per square centimetre. *Error bars* SD ($n = 3$)

deposits (Table 2). Corrosion products increase the number of attachment sites for bacteria and decrease the disinfection efficiency, which results in more biofilm. Corroded pipes are known to adsorb organic matter from water and to make it more available for bacteria [6]. This causes an increase in the biofilm density in carbon-limiting waters. In the data from both cities, the concentration of iron correlated with HPC in biofilms with age over 100 days ($r = 0.73$, $P = 0.016$). Previous

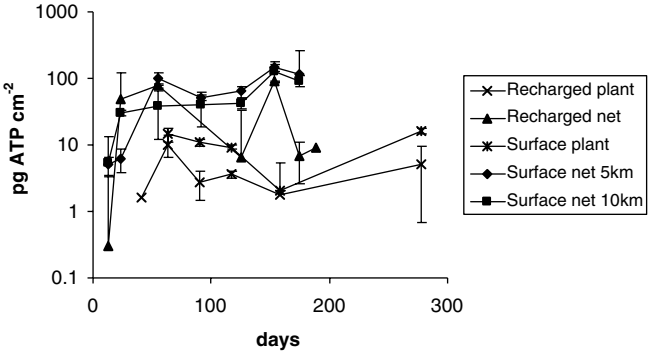

Fig. 3 Biofilm formation in Kuopio on PVC pipes determined as ATP content in biofilms per square centimetre. *Error bars* SD ($n=3$)

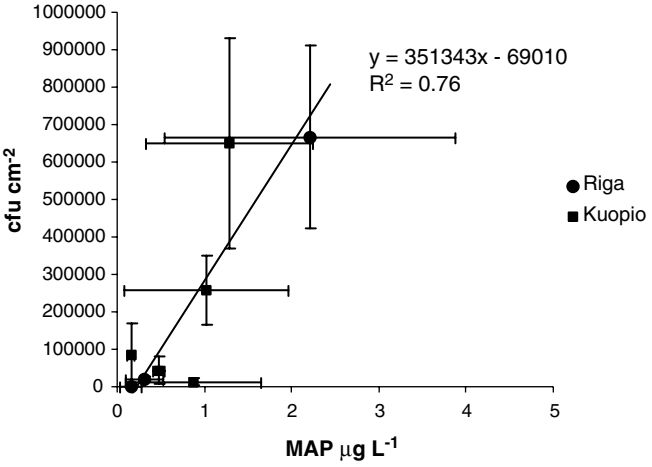

Fig. 4 Relationship between heterotrophic bacterial number in biofilm (cfu cm^{-2}) and microbially available phosphorus (MAP) in the water supply systems of Kuopio (*filled squares*) and Riga (*filled circles*) 100 days after the start of the experiment. Data points are mean values; *bars* ±SE

studies have shown that phosphate anions can be adsorbed on iron oxyhydroxide [2]. Thus we propose that corrosion products increase biofilm growth also in phosphorus-limiting waters because they may accumulate phosphorus from water needed for biofilm bacteria growth. Hence, corrosion should be eliminated in order to decrease biofilm growth both in carbon- and phosphorus-limiting drinking waters.

Other metals may also affect the growth of biofilms—copper especially is known to be toxic for several bacterial species (e.g. *Legionella*) [8]. Manganese and copper may also inhibit microbial growth in biofilms [32]. However, bacteria (*P. aeruginosa*) in biofilms are still more resistant to copper than planktonic bacteria in water [28]. In this study, the concentrations of metals in biofilms were usually higher in Riga than in Kuopio (Table 2). This is probably attributable to the more corroded condition of the pipes in the distribution networks and the higher content of iron in the groundwater. In Kuopio, only in the case of copper were concentrations were slightly higher than in Riga. The copper probably originates from copper pipes that were fitted before the biofilm collectors. There were no significant correlations between the concentration of any metal other than iron and biofilm growth.

We calculated the Spearman rank correlations for biofilms over 100 days old for both cities. HPC in water correlated with HPC in biofilms ($r=0.60$, $P=0.002$). The content of ATP in biofilms correlated with HPC in biofilms ($r=0.52$, $P=0.022$) and temperature correlated with HPC ($r=0.60$, $P=0.005$) and ATP in biofilms ($r=0.62$, $P=0.017$). Temperature also correlated with HPC in drinking water ($r=0.70$, $P=0.001$). However, there was no correlation with HPC in water and microbial concentration in biofilms aged under 50 days, i.e. the concentration of bacteria affected the steady state level of biofilms but not the biofilm formation rate.

When those sampling points with water containing chlorine >0.3 mg l^{-1} (Riga Groundwater plant) and with a high amount of corrosion products (Riga Surface net) were excluded from the calculations, HPC in biofilms older than 100 days correlated with MAP in water (Fig. 4). Even though the variation in the results was high, we noted that water with the highest MAP concentrations also exhibited the highest number of HPC in biofilms. In these data, MAP concentrations above 1 µg l^{-1} strongly increased the number of HPC in the biofilms. This agrees well with earlier in vitro results [16]

Table 2 Average concentration (µg cm^{-2}) of Ca, Mg, Mn, and some heavy metals in biofilms of Kuopio and Riga at the end of the experiment

	Ca	Mg	Fe	Mn	Zn	Pb	Cu
Riga							
Groundwater plant	39.89	12.50	277.43	5.72	19.05	7.38	4.29
Groundwater net	10.72	7.26	122.79	28.28	3.13	2.01	2.38
Artesian plant	13.84	10.94	4.84	0.04	6.18	0.36	20.84
Surface plant	16.79	7.14	3.93	0.38	1.31	0.12	0.81
Surface net	18.46	8.33	402.46	0.24	5.12	0.94	3.93
Kuopio							
Recharged plant	5.06	4.04	7.22	0.02	1.26	0.23	8.78
Recharged net	6.21	4.73	19.17	0.57	2.18	0.83	2.83
Surface plant	1.82	1.24	8.51	0.52	0.75	0.24	3.34
Surface net 5 km	5.82	4.04	10.19	0.13	0.29	0.16	1.30
Surface net 10 km	7.57	1.83	5.88	1.80	1.08	0.24	1.47

that showed that addition of a small amount of phosphorus strongly increased the microbial concentration in the biofilms. Our results confirm that the effect of phosphorus on biofilm formation in a full-scale water distribution system is similar to that depicted in laboratory scale.

In conclusion, this study confirmed our earlier finding that biofilm formation in drinking water distribution networks is controlled by many factors, including temperature, corrosion products and the limiting nutrient in the water. In this study we show for the first time in two independent large-scale systems that a high content of phosphorus in the water may enhance the formation of biofilms within distribution networks. This knowledge can be used by the water industry to find solutions on how best to control biofilm formation within their drinking water distribution networks.

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Reference

1. Appenzeller BMR, Batte M, Mathieu L, Block J-C, Lahoussine V, Cavard J, Gatel D (2001) Effect of adding phosphate to drinking water on bacterial growth in slightly and highly corroded pipes. Water Res 35:1100–1105
2. Appenzeller BMR, Duval YA, Thomas F, Block J-C (2002) Influence of phosphate on bacterial adhesion onto iron oxyhydroxide in drinking water. Environ Sci Technol 36:646–652
3. Batté M, Koudjonou B, Laurent P, Mathieu L, Coallier J, Prévost M (2003) Biofilm responses to ageing and to a high phosphate load in a bench-scale drinking water system. Water Res 37:1351–1361
4. Block JC (1992) Biofilms in drinking water distribution systems. In: Melo LF et al (eds) Biofilms science and technology. Kluwer, Dordrecht, pp 469–485
5. Camper AK, Jones WL, Hayes JT (1996) Effect of growth conditions and substratum composition on the persistence of coliforms in mixed-population biofilms. Appl Environ Microbiol 62:4014–4018
6. Camper AK, Brastrup K, Sandvig A, Clement J, Spencer C, Capuzzi AJ (2003) Effect of distribution system materials on bacterial regrowth. J Am Water Works Assoc 95:107–121
7. Hobbie JE, Daley RJ, Jasper S (1977) Use of nucleopore filters for counting bacteria by fluorescence microscopy. Appl Environ Microbiol 33:1225–1228
8. Kim BR, Andersson JE, Mueller SA, Gaines WA, Kendall AM (2002) Literature review—efficacy of various disinfectants against *Legionella* in water systems. Water Res 36:4433–4444
9. Kooij D van der (1990) Assimilable organic carbon (AOC) in drinking water. In: McFeters GA (ed) Drinking water microbiology, progress and recent developments. Springer, Berlin Heidelberg New York, pp 57–87
10. Kooij D van der, Visser A, Hijnen WAM (1982) Determination of the concentration of easily assimilable organic carbon in drinking water. J Am Water Works Assoc 74:540–545
11. Kooij D van der, Veenendaal HR, Baars-Lorist C, van der Klift DW, Drost YC (1995) Biofilm formation on surfaces of glass and teflon exposed to treated water. Water Res 29:1655–1662
12. Kooij D van der, van Lieverloo JHM, Schellart JA, Hiemstra P (1999) Distributing drinking water without disinfectant: high achievement or height of folly? J Water SRT Aquat 48:31–37
13. Laurent P, Servais P, Randon G (1993) Bacterial development in distribution networks; Study and modeling. Water Supply 11:387–398
14. Lehtola MJ, Miettinen IT, Vartiainen T, Martikainen PJ (1999) A new sensitive bioassay for determination of microbially available phosphorus in water. Appl Environ Microbiol 65:2032–2034
15. Lehtola MJ, Miettinen IT, Vartiainen T, Martikainen PJ (2002) Changes in content of microbially available phosphorus, assimilable organic carbon and microbial growth potential during drinking water treatment processes. Water Res 36:3681–3690
16. Lehtola MJ, Miettinen IT, Martikainen PJ (2002) Biofilm formation in drinking water affected by low concentrations of phosphorus. Can J Microbiol 48:494–499
17. Mathieu L, Block JC, Dutang M, Maillard J, Reasoner D (1994) Control of biofilm accumulation in drinking water distribution systems. Water Supply 111:365–376
18. Miettinen IT, Vartiainen T, Martikainen PJ (1997) Phosphorus and bacterial growth in drinking water. Appl Environ Microbiol 63:3242–3245
19. Miettinen IT, Vartiainen T, Martikainen PJ (1999) Determination of assimilable organic carbon in humus-rich drinking waters. Water Res 33:2277–2282
20. Mohamed MN, Lawrence JR, Robarts RD (1998) Phosphorus limitation of heterotrophic biofilms from the Fraser river, British Columbia, and the effect of pulp mill effluent. Microb Ecol 36:121–130
21. Niquette P, Servais P, Savoir R (2000) Impacts of pipe materials on densities of fixed bacterial biomass in a drinking water distribution system. Water Res 34:1952–1956
22. Percival SL, Walker JT (1999) Potable water and biofilms: a review of the public health implications. Biofouling 42:99–115
23. Reasoner DJ, Geldreich EE (1985) A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol 49:1–7
24. Rompré A, Prevost M, Coallier J, Brisebois P, Lavole J (2000) Impacts of implementing a corrosion control strategy on biofilm growth. Water Sci Technol 41:287–294
25. Sathasivan A, Ohgaki S, Yamamoto K, Kamiko N (1997) Role of inorganic phosphorus in controlling regrowth in water distribution system. Water Sci Technol 35:37–44
26. Schlegel HG (1997) General microbiology, 7th edn. Cambridge University Press, Cambridge
27. Storey MV, Ashbolt NJ (2001) Persistence of two model enteric viruses (B40-8 and MS-2 bacteriophages) in water distribution pipe biofilms. Water Sci Technol 43:133–138
28. Teitzel GM, Parsek MR (2003) Heavy metal resistance of biofilm and planktonic *Pseudomonas aeruginosa*. Appl Environ Microbiol 69:2313–2320
29. Vadstein O (2000) Heterotrophic, planktonic bacteria and cycling of phosphorus. Phosphorus requirements, competitive ability, and food web interactions. Adv Microb Ecol 16:115–167
30. Yu X, Zhang XJ, Wang ZS (2003) Improving removal efficiency of organic matters by adding phosphorus in drinking water biofiltration treatment. Biomed Environ 16:29–39
31. Zacheus OM, Iivanainen EK, Nissinen TK, Lehtola MJ, Martikainen PJ (2000) Bacterial biofilm formation on polyvinyl chloride, polyethylene and stainless steel exposed to ozonated water. Water Res 34:63–70
32. Zacheus OM, Lehtola MJ, Korhonen LK, Martikainen PJ (2001) Soft deposits, the key site for microbial growth in drinking water distribution networks. Water Res 35:1757–1765