Microbial community structure in biofilms and water of a drinking water distribution system determined by lipid biomarkers

M.M. Keinänen***, P.J. Martikainen**, L.K. Korhonen* and M.H. Suutari***,  

* Department of Environmental Health, National Public Health Institute, P. O. Box 95, FIN-70701 Kuopio, Finland  
** Department of Environmental Sciences, Bioteknia 2, University of Kuopio, P. O. Box 1627, FIN-70211 Kuopio, Finland  

Abstract The development of microbial communities in biofilms of a drinking water distribution system was monitored, and compared to the microbial communities in water. The microbial communities were studied by phospholipid fatty acid (PLFA) profiles. In drinking water samples the most common PLFAs, with the proportion of 60.9%, were monoenoic fatty acids, such as 16:1ω7c and 18:1ω7c, indicating high abundance of gram-negative bacteria. Instead, in biofilm samples saturated fatty acids, such as 16:0 and 18:0, indicating general biomass, accounted for 54.9–78.4% of the total PLFAs. The proportions of monoenoic fatty acids in biofilm increased from 11.5% to 31.2% with water aging from 22 h to 62 h in the distribution system. In conclusion, water aging affected the structure of microbial communities in biofilms, and the microbes in water differed from those in biofilms. These differences might also reflect the differences in the physiological state of the microbes, which is influenced by water chemistry and by the growth environment, i.e. water or biofilm.  

Keywords Biofilm; biomass; microbial community structure; phospholipid fatty acid  

Introduction  
Microbes in aquatic environments rapidly attach to solid surfaces and form biofilm (Block et al., 1993; Norton and LeChevallier, 2000). The formation of biofilms is of special interest in drinking water distribution systems, as biofilms can be associated with many technical and hygienic problems. They can induce scaling and corrosion of pipelines, cause taste and odor problems to drinking water, and provide protective places and reservoirs for pathogenic microbes.  

Classical methods of isolation and identification of microbes, as well as many modern molecular methods, are highly selective and fail to detect most organisms in different environments (Findlay, 1996). As much as 90 to 99% of drinking water bacteria are difficult or impossible to culture. Thus, culture methods usually dramatically underestimate the number and diversity of microorganisms present in drinking water (Szewzyk et al., 2000). Instead, the analyses of lipid biomarkers, such as phospholipid fatty acids (PLFAs) have enabled the characterization of microbial communities in different environments. Phospholipids compose the lipid bilayers of cell membranes of Bacteria and Eucarya, but not the cell membranes of Archaea. However, Archaea may not present significant contributions to the microbial communities of drinking water systems which generally are well oxygenated (Manz et al., 1993). The PLFA analysis produces a description of whole microbial communities, but does not have the specificity to identify the microbes at species or more detailed levels. On the basis of the fatty acid profile microbes can be grouped for example to gram-negative and gram-positive bacteria, actinomycetes and eucaryotic cells. The quantitative amounts of PLFAs can be used to estimate the viable microbial biomass, as PLFAs have a rapid turnover and their amounts in a cell are relatively constant (King et al.,
The PLFA profiles also describe the physiological state of microbial communities.

The PLFAs have been widely analyzed from different environmental samples such as soils and sediments since the 1970s. However, the PLFAs from drinking water biofilms have earlier been reported only a few times. Impacts of operation conditions on drinking water biofilters (Moll et al., 1998, 1999; Fonseca et al., 2001), and effects of chlorine, time of year (Smith et al., 2000) and phosphorus addition (Keinänen et al., 2002) on biofilm formation have been analyzed. In addition, microbial communities on corroded concrete surfaces in drinking water reservoirs have been characterized (Herb et al., 1995). We have now used PLFA analyses to monitor the effects of distance from waterworks on the biofilm microbial community structures compared to water in a full-scale drinking water distribution system.

Materials and methods

The drinking water and biofilm samples were collected from a drinking water distribution system in Finland. The distribution system received drinking water, which was produced from surface water using chemical coagulation, rapid sand filtration and disinfection by ozone and chloramine. The biofilms were allowed to develop for 80 days on experimental polyvinylchloride (PVC) pipes (area 62.8 cm²) connected to the full-scale drinking water distribution system in summer 1997. The drinking water flow rate in the PVC biofilm collector was 1.1 min⁻¹. Water samples of 20 litres were taken one week after the biofilm sampling from the same sites where biofilms were grown. Water ages (retention times) at the sampling sites were 22 and 62 hours. The physical and chemical characteristics of the drinking water leaving from the waterworks during the sampling period were as follows: temperature, 9.4°C; pH, 8.7; electric conductivity, 15.5 mS m⁻¹; chemical oxygen demand, 1.5 mg Mn l⁻¹; turbidity, 0.04 FTU; color <0 mg Pt l⁻¹; hardness, 0.6 mmol l⁻¹; alkalinity, 0.8 mmol l⁻¹; total organic carbon (TOC), 2.8 mg l⁻¹; total chlorine (Cl₂), 0.6 mg l⁻¹. Phosphorus limited the microbial growth in the distribution system, as the Finnish drinking water sources have a high content of organic matter but a low content of inorganic nutrients (Miettinen et al., 1996).

The water samples and detached biofilms in water were filtered, frozen, and freeze-dried. The phospholipid fatty acids were analyzed as presented earlier (Keinänen et al., 2002). Shortly, the lipids were first extracted with 28.2 ml of chloroform:methanol:phosphate buffer pH 7.4 (1:2:0.8 vol/vol/vol) (Bligh and Dyer, 1959; White et al., 1979; Frostegård et al., 1991). Lipids were separated from the solvent phase after the addition of chloroform and buffer (final ratios of solvents 1:1:0.9 vol/vol/vol). Lipids were then fractionated in a silica column to neutral, glyco- and phospholipids with 10, 20 and 10 ml of chloroform, acetone and methanol, respectively (King et al., 1977; Frostegård et al., 1991). PLFAs were further saponified, methylated, extracted (Suutari et al., 1990) and analyzed as methyl ester with gas chromatography equipped with mass selective detector (GC-MS) using selected ion monitoring (Tunlid et al., 1989; Keinänen et al., 2002). The detection limit for PLFAs was approximately 5 µg for 20 fatty acids. The quantitative amounts of PLFAs were converted to cell estimate using the following conversion factors. On average bacteria contain 100 µmol PLFAs g⁻¹ dry weight, and 1 g of bacteria (dry wt) is equivalent to 2.0 x 10¹² cells (Balkwill et al., 1988). The analysis of a sample takes 2–3 working days in laboratory, and the material costs are approximately 5 euros per sample.

Results and discussion

The phospholipid fatty acids analysis identified 26 fatty acids in drinking waters and 22 in biofilms. The fatty acid profiles were grouped to straight-chain saturated, straight-chain
mono-unsaturated and cyclopropane, terminally branched saturated, middle-branched saturated, branched mono-unsaturated and polyunsaturated PLFAs (Figure 1).

In drinking water samples the fatty acid profiles were quite similar with water age of 22 h or 62 h, and the results are presented as average values (Figure 1). In drinking waters the most abundant PLFAs (60.9%) were the cyclopropane and straight-chain mono-unsaturated fatty acids, especially 16:1\text{ω7c} and 18:1\text{ω7c}, characteristic of gram-negative bacteria (White et al., 1996). Earlier studies have also shown that the gram-negative bacteria are the most abundant microbes in drinking waters. In contrast, the terminally branched saturated PLFAs, characterizing gram-positive bacteria (White et al., 1996), consisted of 6.0% of total PLFA profile in drinking waters. The proportion of straight-chain saturated fatty acids, generally found in all microbes, was 22.5%. The polyunsaturated fatty acids, characterizing fungi, algae and protozoa (White et al., 1996), were detected in amounts of 8.0%. The quantitative amount of PLFAs in drinking waters was 52 \pm 16 \text{nmol l}^{-1}, corresponding (1.0 \pm 0.3) \times 10^9 \text{cells l}^{-1}. As a result, water age had minor effects on microbial communities in the drinking waters.

The PLFA profiles of drinking water and biofilm samples were different (Figure 1). In drinking water samples the proportion of straight-chain mono-unsaturated and cyclopropane fatty acids was 2.0 and 5.3 times higher than in biofilms with water age of 22 h and 62 h, respectively. In contrast, the proportion of straight-chain saturated fatty acids was 3.5 times higher in biofilm with water age of 22 h hours than in drinking waters, and 2.4 times higher in biofilm with water age of 62 h than in drinking waters. The amount of polyunsaturated fatty acids was slightly higher in drinking water than in biofilms. Thus, fungi, algae and protozoa might have been more common in waters than in biofilms. Branched monounsaturated fatty acids were detected only in drinking waters. Norton and LeChevallier (2000) have also reported that the bacterial populations between aquatic phase and biofilm differ remarkably. In conclusion, the biofilm formation for 80 days affected the microbial community structure compared to the aquatic phase. The differences in PLFAs might also reflect the differences in physiological stage of microbes, which is affected by water chemistry and by the growth environment, i.e. water or surface. A biofilm is generally found to be a favorable habitat for microbes, in comparison to an aquatic phase.

![Figure 1](https://iwaponline.com/wst/article-pdf/47/5/143/422474/143.pdf)

**Figure 1** The proportions of phospholipid fatty acid (PLFA) types in drinking waters (H\textsubscript{2}O) and in biofilms from a drinking water distribution system with water age of 22 h or 62 h. The results are expressed as mean \pm sd (n = 2). The PLFA type nomenclature: saturates = straight-chain saturated, mono-enoics = straight-chain mono-unsaturated and cyclopropane, ter br saturates = terminally branched saturated, mid br saturates = middle-branched saturated, br mono-enoics = branched mono-unsaturated, and polyenoics = polyunsaturated PLFAs.
The distance from waterworks affected the PLFA composition of biofilms, because the proportion of straight-chain saturated fatty acids was higher and that of straight-chain monounsaturated and cyclopropane fatty acids smaller in biofilm with water age of 22 h than in biofilm with water age of 62 h (Figure 1). These differences might be due to changes inside the gram-negative bacteria community, and/or the same gram-negative bacteria had different physiological stages with water age of 22 h than 62 h. Earlier studies have also shown that gram-negative bacteria are the most abundant microbial group in drinking water biofilters and biofilms (Moll et al., 1998, 1999; Smith et al., 2000; Fonseca et al., 2001; Keinänen et al., 2002). In addition, the proportion of gram-positive bacteria might have increased in the network further away from waterworks, because the terminally branched saturated fatty acids were more abundant in biofilms with water age of 62 h than 22 h. The differences in PLFAs might also reflect favorable changes for microbes in chemical and physical conditions of the system, such as decline of residual chlorine with water aging (Miettinen et al., 1997; Norton and LeChevallier, 2000). In addition, the quantitative amount of PLFAs was higher with water age of 62 hours (340 ± 64 pmol cm⁻², corresponding (6.8 ± 1.3) × 10⁶ cells cm⁻²) than with water age of 22 hours (216 ± 5.2 pmol cm⁻², corresponding (4.3 ± 0.1) × 10⁶ cells cm⁻²).

It is important to understand and monitor the microbial community dynamics in biofilms to minimize the potential risk of technical and microbiological problems in the drinking water systems. The PLFAs offer a useful tool to analyze the community structure, viable biomass and the physiological state of microbes. The method can be combined with molecular techniques, such as DNA gene probes, to get more detailed information concerning the sub-populations of microbes (Findlay, 1996; White et al., 1996). Attention should be paid to the sensitivity of the method, achieved by purity of solvents, reagents and glassware and the use of selected ion monitoring in MS detection. In this study we have shown that the differences in microbial communities of drinking water biofilms can be characterized by monitoring PLFAs.

Conclusions
This present work indicated that: (a) the microbial communities consisted predominantly of gram-negative bacteria in drinking waters, and in biofilms collected from drinking water with water age of 22 h or 62 h; (b) the phospholipid fatty acid compositions indicated that microbial communities differed between biofilms and drinking waters, and between biofilms collected from drinking water with the age of 22 and 62 h; (c) the PLFA analysis may be an appropriate tool at least for scientific studies to monitor the microbial community structure and biomass in biofilms and water of drinking water distribution systems independently of culturability.

Acknowledgements
The study was supported by the Finnish Research Programme on Environmental Health (project 42676), Academy of Finland (projects 34538 and 51999), Ministry of Agriculture and Forestry of Finland, and the Finnish Graduate School in Environmental Science and Technology (EnSTe). Special thanks to Dr. Outi Zacheus and Mrs. Marjo Tiittanen for collaboration in providing samples.

References


