

Incorporation of hygienically relevant bacteria into drinking water biofilms in response to changes in material quality due to disinfection and sanitation measures.

Moritz, M. M.¹, Schaule, G.², Flemming, H.-C.^{1,2} and Wingender, J.¹

¹Biofilm Centre, Aquatische Mikrobiologie, Fachbereich Chemie, Universität Duisburg-Essen, D-47057 Duisburg, Germany

²IWW Water Centre, Moritzstraße 36, D-45476 Mülheim an der Ruhr, Germany

Microbial biofilms that inevitably form on the inner surfaces of drinking water distribution systems have the potential to act as reservoirs for hygienically relevant bacteria such as *Pseudomonas aeruginosa*, *Legionella pneumophila* and coliforms. The bacteria can be released into the water phase, which poses a threat to human health. The present study focuses on the situation in household installations where factors such as elevated temperature, extended periods of water stagnation and the quality of piping materials may have an effect on biofilm development. There has been evidence that due to the leaching of biodegradable substances from elastomeric and plastic materials the growth of biofilms and the incorporation and persistence of hygienically relevant bacteria in these biofilms are supported. Disinfection and cleaning measures can lead to enhanced release or even to the production of biodegradable substances. In the present work the influence of disinfection and sanitation measures on drinking water biofilms and on the incorporation of *P. aeruginosa* and *L. pneumophila* into these biofilms is investigated.

Four different materials were tested, both untreated and after artificial aging, in order to investigate the influence of possible material alterations due to disinfection with respect to biofilm growth and incorporation of hygienically relevant bacteria: 1. ethylene-propylene-diene-monomer (EPDM), 2. silane cross-linked polyethylene (PE-X b), 3. electron-ray cross-linked PE (PE-X c) and 4. copper. Artificial aging was achieved by a treatment with 5 ppm hypochlorite at 3 bar and 40 °C for 4 weeks. Drinking water biofilms were grown on coupons of these materials in a stainless steel tank under flow-through conditions (20 L/h) at room temperature. Coupons with 14 day old biofilms were transferred to 80 mL stainless steel flow-through reactors and inoculated with a suspension of *P. aeruginosa*, *L. pneumophila* and *Enterobacter nimipressuralis* (10^6 cells/mL each) in drinking water. After a static incubation for 24 h, reactors were connected to a drinking water tap and perfused with drinking water continuously for four weeks.

After 14 days, biofilms with different cell densities (total cell counts) had developed on the four materials in the following order: EPDM (10^7 cells/cm²) > PE-X b and copper (10^6 cells/cm²) > PE-X c (10^5 cells/cm²). The levels of culturable heterotrophic plate count bacteria were about 1 log unit lower than the total cell count in biofilms grown on elastomeric and plastic materials. In the case of copper the difference amounted to 2 log units. The duration of persistence as well as the cell density of *P. aeruginosa* and *L. pneumophila* varied depending on bacterial species and material. *P. aeruginosa* persisted for 28 days in biofilms grown on EPDM, PE-X b and PE-X c, but was unable to colonize copper biofilms. Genotyping of *P. aeruginosa* biofilm isolates by pulsed-field electrophoresis analysis revealed the same macrorestriction patterns compared with the original strain used for inoculation, confirming that the original strain had persisted in the biofilm. *L. pneumophila* incorporated into biofilms grown on any of the materials, whereas the concentration of *L. pneumophila* was highest in biofilms grown on PE-X c and lowest on PE-X b after 4 weeks. Experiments using the artificially aged materials did not reveal any significant differences regarding biofilm formation and the incorporation of *P. aeruginosa* and *L. pneumophila* into biofilms. Thus, an influence of the aging procedure could not be detected. Application of the fluorescence in situ hybridisation (FISH) method using specific oligonucleotide probes for the detection of *P. aeruginosa* and *L. pneumophila* showed that the bacteria often persisted over longer periods of time and in higher concentrations than reflected by culture-based methods. These results indicate that part of the bacterial population incorporated into the biofilm entered a viable but non culturable (VBNC) state, in which they are not detectable with the standard methods but are still present and can be hygienically relevant.

ACKNOWLEDGEMENT

This work was financially supported by the German Federal Ministry of Education and Research (grant number 02WT0836). The technical assistance of S. Eppmann and A. Dannehl is gratefully acknowledged.