Dissertation Miriam Moritz

"Integration of hygienically relevant bacteria in drinking water biofilms grown on domestic plumbing materials"

Abstract

Biofilms in domestic plumbing systems can represent a reservoir for potentially pathogenic bacteria including *Pseudomonas aeruginosa*, *Legionella pneumophila* and coliform bacteria. The selection of materials utilised in domestic plumbing as well as their exposure to disinfectant stress ("ageing") may affect the extent of biofilm formation, the composition of biofilm populations and the incorporation and persistence of hygienically relevant bacteria in drinking water biofilms. The presence of protozoa may additionally influence the persistence and multiplication of potentially pathogenic bacteria.

Drinking water biofilms were grown on coupons of plumbing materials including ethylene-propylene-diene-monomer (EPDM) rubber, silane cross-linked polyethylene (PE-Xb), electron-ray cross-linked PE (PE-Xc) and copper under constant flow-through of cold tap water at ambient temperature (19.0 °C ± 3.1 °C). The materials were tested both untreated and after treatment with sodium hypochlorite (5 ppm, 3 bar, 40 °C, 4 weeks) or chlorine dioxide (5 ppm, 3 bar, 40 °C, 4 weeks) in case of EPDM, PE-X b and c or after exposure to unchlorinated drinking water for ≥ 6 months in case of copper. After 14 days, the biofilms were spiked with *Pseudomonas aeruginosa*, *Legionella pneumophila* and *Enterobacter nimipressuralis* (10^6 cells/mL each). The test bacteria were environmental isolates from contamination cases in drinking water systems. After static incubation for 24 h, water flow was resumed and continued for four weeks. Total cell count and heterotrophic plate count (HPC) of biofilms were monitored, and the population diversity of was determined using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). *P. aeruginosa*, *L. pneumophila* and *E. nimipressuralis* were quantified, using standard culture-based methods or culture-independent fluorescence in situ hybridisation (FISH). 14 day-old biofilms grown on untreated EPDM, PE-Xb, PE-X c and copper were analysed for the presence of total protozoa and the amoebal genera *Acanthamoeba* and *Hartmannella* using FISH.

After 14 days total cell counts and HPC values were highest on EPDM followed by the plastic materials and copper. The diversity of biofilm populations was higher in biofilms grown on synthetic materials (EPDM, PE-Xb, PE-Xc) compared to biofilms

grown on copper. Amoebae were present in drinking water biofilms grown on all domestic plumbing materials tested, with *Acanthamoebae* and *Hartmannella* being the prevalent genera. Material ageing did not significantly influence biofilm formation and biofilm population diversity. After inoculation, *P. aeruginosa* persisted for 28 days in biofilms on EPDM, PE-Xb and PE-Xc, but was unable to colonise copper biofilms. *L. pneumophila* persisted in biofilms on any of the materials for 28 days. *E. nimipressuralis* was not detected in any of the biofilms. The aged materials did not show significant differences compared to untreated materials regarding the incorporation of *P. aeruginosa* and *L. pneumophila* into biofilms. Application of FISH showed that *P. aeruginosa* and *L. pneumophila* often persisted in higher concentrations than detected by culture-based methods indicating that part of the *P. aeruginosa* and *L. pneumophila* populations entered a viable but non-culturable (VBNC) state, in which they were not detectable with standard culture methods.

Additional investigations on *P. aeruginosa* pure cultures showed that copper can be one of the stress factors inducing the VBNC state in drinking water and drinking water biofilms of domestic plumbing systems. Planktonic and biofilm-associated *P. aeruginosa* in the VBNC state became culturable again upon the release of copper stress by incubation in the presence of the chelator diethyldithiocarbamate (DDTC).

The results show that biofilm formation measured as total cell count and HPC as well as biofilm population diversity are material dependent, but not influenced by material ageing. *P. aeruginosa* and *L. pneumophila* are able to incorporate into and persist in drinking water biofilms grown on materials relevant in domestic plumbing. Material ageing did not have an influence on pathogen persistence. The detection of amoebae in all drinking water biofilms suggests that these organisms can interact and probably serve as a host for hygienically relevant bacteria, at least for *L. pneumophila*. The concentrations of *P. aeruginosa* and *L. pneumophila* in drinking water biofilms may be underestimated by conventional culture-based methods. Hygienically relevant bacteria that are not culturable may still be viable and of hygienic relevance as they can retain their virulence or regain it upon resuscitation.