Contamination of tap water by hygienically relevant bacteria from biofilms in drinking water installations and effects of disinfection

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Biofilms in drinking water installations are a potential source for hygienically relevant bacteria. In contrast to drinking water distribution systems household installations have elevated water temperatures, mainly stagnating water and a high diversity of materials. These factors obviously influence the colonization of biofilms by pathogens and their dissemination into the water phase. In the present study the long time potential risk of tap water contamination by hygienically relevant bacteria after a single contamination incident is investigated. Experiments are performed in a close-to-practice test unit with a water consumption profile representative for a household with four persons according to DIN 50931-1. Contamination potentials were studied for different water qualities, temperatures (12 and 37°C) and installation materials.
Four different pipe or tubing materials used in drinking water installations were tested in a total length of 10 m each: (i) ethylene-propylene-diene-monomer (EPDM) with recommendation (WR) achieving the specification of the technical guidelines of the DVGW-German Gas and Water Association standard W 270 and the category “C” specification of the German Commission for Drinking Water (KTW), (ii) EPDM without recommendation (WOR) not achieving these two specifications, (iii) electron-ray cross-linked polyethylene (PE-Xc) achieving specifications of DVGW standard W 270 as well as KTW category “A” and (iiii) copper (Cu).
Biofilm formation on the different materials in two drinking water qualities with dissolved organic carbon (DOC) concentrations of 0.8 mg/L and 3.1 mg/L was tested. On EPDM–WOR more biofilm was formed (according to total cell counts) than on EPDM-WR. On PE-Xc and Cu clearly lower amounts of biofilm had developed. In water with high DOC-concentration more biofilm on the tested materials was formed than in water with low DOC-concentration. After 8 weeks of running time biofilms had reached near steady state and subsequently were contaminated with a mixture of hygienically relevant bacteria (*Legionella pneumophila* and *Pseudomonas aeruginosa*) simulating a heavy single pollution incident of a drinking water installation.
L. pneumophila was able to colonize the biofilms, to multiply in the biofilms and to contaminate the 8 h stagnating water. This was observed at 37°C for all tested installation materials. The concentrations of Legionella in the biofilms and in the 8 h stagnating water increased with increasing amount of biofilm which had developed on the different materials.
In water with a low DOC-concentration the culturability of *P. aeruginosa* in biofilms decreased after 3 to 8 weeks of exposure to below detection level. Depending on the material and temperature *P. aeruginosa* was detected for a longer running time in stagnating water than in the corresponding biofilm. In water with a high DOC-concentration *P. aeruginosa* was never detected by cultivation methods but was detected with the noncultural FISH-method. Obviously, in biofilms *P. aeruginosa* fell into a viable but non culturable (vbnc) state.
Mechanical (30 min air/water impulse cleaning) and subsequent chemical treatment with up to 28 mg/L ClO_2 (start-concentration) for 24 h were not sufficient to eliminate Legionella on both EPDM-qualities. Even an additional disinfection-dose with 18 mg/L ClO_2 applied 24 h after the disinfection with 28 mg/L ClO_2 did not eliminate Legionella in the biofilms on EPDM-WR and EPDM-WOR. On copper and PE-Xc a single disinfection dose of 28 mg/L ClO_2 eliminated Legionella from the biofilms.
Hence, under the applied close to practice conditions hygienically relevant bacteria were able to colonize drinking water biofilms on the tested installation materials, survive or multiply in biofilms and disseminate into stagnant drinking water resulting in contaminated tap water.

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