Correlation between materials, water quality, and biofilm diversity in drinking water biofilms

Roeder, R., Tarne, P., Heeg, K., and Szewzyk, U.

Berlin University of Technology, Department of Environmental Microbiology, Franklinstrasse 29, D-10587 Berlin, Germany

Email: r.roeder@mailbox.tu-berlin.de , ulrich.szewzyk@tu.berlin.de

Introduction

Water installation materials which support growth of biofilms pose a serious contamination risk for drinking water with hygienically relevant microorganisms. Depending on the applied materials and the ambient conditions, different biofilm communities will develop.
In this project biofilm communities on different drinking water materials are compared using molecular methods (DGGE, cloning and sequencing). The objective was to correlate factors from the water, the different materials and the composition of the resulting biofilms.

Conclusions

- The microbial diversity on growth-supporting materials like EPDM is comparatively high.
- The similarity between the biofilm populations on different materials exposed in the same drinking water was low and influenced by the nutrients released by the materials.
- The composition of the biofilm population is influenced both by the material and the origin of the drinking water.
- Both contamination of an existing biofilm with target organisms and disinfection had a great impact on the biofilm population.

Materials and Methods

Exposed drinking water materials:

- Copper
- PE-Xb (silane-cross-linked polyethylene)
- PE-Xc (electron-ray cross-linked polyethylene)
- EPDM (ethylene-propylene-diene-monomer) with and without recommendation (KTW-C and DVGW W270)

Locations of biofilm development:

L1: Drinking water from recharged, reduced groundwater of eastern Germany
L2: Drinking water from reduced groundwater of northern Germany[1] (DOC 0,8 mg/ml)
L3: Drinking water from river bank filtration of western Germany[2]
L4: Drinking water from reduced groundwater of northern Germany[1] (DOC 3,1 mg/ml)

Biofilm reactors

Biofilm-Reactors used at the different locations (left to right: L1, L2 and L4, L3)

Analyzed biofilm samples:

a. 6 weeks old, cold-water biofilms[1,2] from four different locations L1 to L4

b. Contaminated* biofilms[1] on drinking water materials (location L2) before and after cleaning and disinfection (3 weeks regrowth):
Mechanical (30 min air/water impulse cleaning) and chemical treatment with chlorine dioxide (0,2 mg/l, 24 h)

* Biofilms were contaminated with *Pseudomonas aeruginosa AdS*, *Legionella pneumophila AdS* and *Enterobacter amnigenus* at week 8

c. Disinfected biofilms[3] in silicone hoses after 2 weeks of regrowth
with chlorine dioxide (continuous), hydrogen peroxide with fruit acid, hydrogen peroxide combined with silver or with silver and peracetic acid (circular flow)

[1] Biofilm samples of Location L2 and L4 were applied by DVGW-Forschungsstelle TUHH
[2] Biofilm samples of Location L3 were applied by IWW, Mülheim an der Ruhr
[3] Biofilm samples of silicone tubes were applied by Institute for Hygiene and Public Health, University Bonn

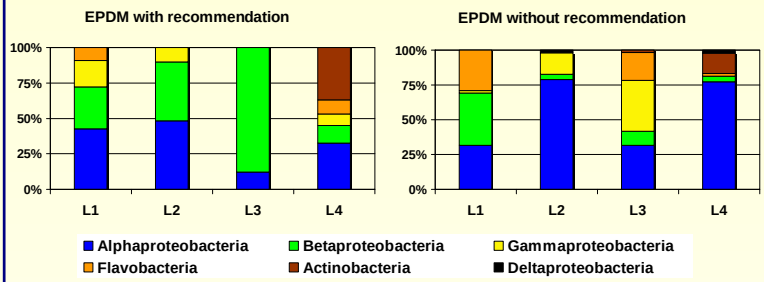
Molecular methods:

- Fingerprinting with DGGE (denaturing gradient gel electrophoresis): V3 region of 16S rDNA (Muyzer et al., 1993)
- Cloning with PCR products of 16S rDNA (Marchesi et al., 1998) and TOPO TA Cloning KIT for Sequencing. Dependent on the DGGE pattern sequencing of about 500 bp of 50-60 (EPDM) and 10-20 (PE-Xc) clones, respectively.

Results

Biofilm community analysis by cloning and sequencing

- Biofilms grown on the same materials in different waters developed dissimilar microbial communities.
- Growth-supporting materials like EPDM showed a higher diversity than inert materials, however the populations on EPDM were also influenced by the water type.



	Predominant closest relatives in PE-Xc biofilms	
L1	*Dechloromonas sp.*	
L2	*Acidovorax sp.*	*Acidovorax temperans*
L3	Candidatus *Reyranella massiliensis*	
L4	*Undibacterium sp.*	

Disinfection of biofilms on drinking water materials with chlorine dioxide

- Mechanical treatment in connection with chemical disinfection using chlorine dioxide induced new biofilm communities on drinking water materials.

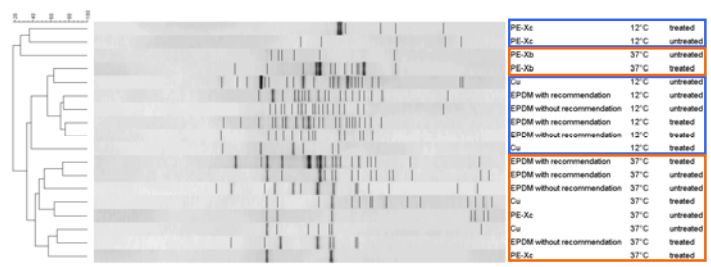


Figure 1: DGGE pattern similarity analysis of cold water (blue) and warm water (orange) biofilms at location L2 before (36 w old) and after (3 weeks regrowth) mechanical and chemical treatment

Disinfection of biofilms in silicone hoses

- Chemical treatment induced a selection pressure and resulted in a new biofilm population after 2 weeks of regrowth.

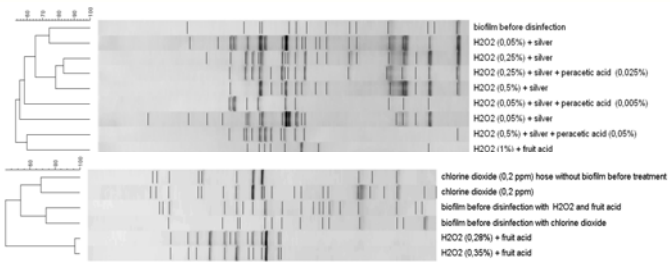


Figure 2a and 2b: DGGE pattern similarity analysis of biofilms (a:14 month/ b:10 month) before and after treatment with different disinfection methods

Acknowledgements
This investigation was financially supported by the German Federal Ministry of Education and Research (BMBF). We would like to thank our research partner for providing biofilm samples:
Benölken, J. and Bendinger, B. from the DVGW-Forschungsstelle TUHH
Lenz, J., Gebel, J. and Exner, M. from the Institute for Hygiene and Public Health, University Bonn
Schaule, G. and Flemming, H.-C. from IWW, Mülheim an der Ruhr

References

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C.; Hiom, S. J., Dymock, D. and Wade, W. G. (1998), Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA., Appl Environ Microbiol 64(2), 795--799.
Muyzer, G., de Waal, E. C. and Uitterlinden, A. G. (1993), Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA., Appl Environ Microbiol 59(3), 695--700.

Correlation between materials, water qualities, and biofilm diversity in drinking water biofilms

R. Roeder, P. Tarne, K. Heeg, and U. Szewzyk
Department of Environmental Microbiology, University of Technology Berlin, Franklin-strasse 29, D-10587 Berlin Germany, Tel: +49-30-314 73460, www.umb.tu-berlin.de
email: rosemarie.roeder@tu-berlin.de, ulrich.szewzyk@tu.berlin.de

Biofilm communities grown on different drinking water pipe materials (Copper, different PE and rubber qualities) at different sites of Germany (reduced groundwater, recharged groundwater, river bank filtration) are analysed. The objective was to correlate factors from the water, the different materials and the composition of the resulting biofilms.

The biofilms of the pilot plant (DVGW Research Center TUHH) were also contaminated with *Pseudomonas aeruginosa* and *Legionella pneumophila* and were treated mechanically in combination with chemical disinfection (chlorine dioxide). Additional evaluations on the effect of other disinfectants on biofilm communities, were carried out with biofilms grown on silicone.

The biofilm communities on different materials were compared with a fingerprinting method (DGGE) and cloning (16S rDNA). The different biofilm communities had high diversity on rubber and comparative low diversity on PE-X. DGGE patterns and cloning results of the same materials exposed at different sites showed a relative low similarity. Consequently, the composition of the biofilm population is influenced both by the material and the origin of the drinking water. A contamination with water relevant pathogens resulted in a changed population structure. Chemical treatment induced a selection pressure and resulted in a new biofilm population.

Acknowledgements
Part of this investigation was financially supported by the German Federal Ministry of Education and Research (BMBF).
We would like to thank our research partners (www.biofilm-hausinstallation.de) for providing biofilm samples.