

## Introduction

Water installations materials which support growth of biofilms pose a serious contamination risk for drinking water with hygienically relevant microorganisms such as *Pseudomonas aeruginosa* and *Legionella pneumophila*. Depending on the applied materials and the ambient conditions, like stagnation, different biofilm communities can be formed. Coliform bacteria may occur on installation materials which promote their growth and may be released into the flowing drinking water (Kilb et al., 2003).

In this project biofilm communities on different drinking water materials are compared with molecular methods (DGGE, cloning and sequencing). The intention was to find indicator organisms for drinking water materials.

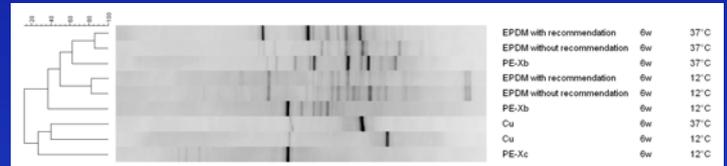


Figure 1 and 2: DGGE pattern similarity analysis of 6 week old biofilms from different materials and two different water temperatures

left: DOC of the groundwater 0,9 mg/ml (location 2)  
right: DOC of the groundwater ~ 3,3 mg/ml (location 4)

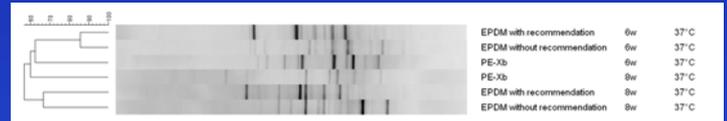
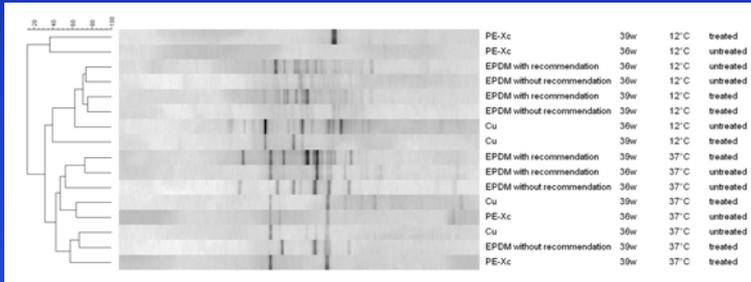


Figure 3 (left): DGGE pattern similarity analysis of biofilms from different materials at location 2 before (36w) and after (39w) treatment with 30 min impulse cleaning and chlorine dioxide (0,2 mg/ml) for 24 h

Figure 4 (right): DGGE pattern similarity analysis of biofilms from different materials at location 4 before (6w) and after (8w) contamination with *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Enterobacter amnigenus* and *Citrobacter freundii*

## Results and Conclusions

- The similarity between the biofilm population on different materials was low and regulated by the availability of the nutrients released by the materials.
- Growth supporting materials like EPDM showed a higher diversity of the biofilm population than inert materials. A higher DOC in the water resulted in a higher diversity in the biofilms.
- The first cloning results indicate that there may be no consistent biofilm community on the same material from different locations.
- It seems that the origin of the drinking water and the DOC play also an important role for the biofilm community structure.
- Mechanical treatment in connection with chemical disinfection using chlorine dioxide induced a new biofilm community.
- A contamination with water relevant pathogens resulted in a changed population structure on the same material.

## Materials and Methods

Different drinking water materials (copper, different PE and rubber qualities (EPDM)) are exposed in biofilm reactors in the drinking water installation at different sites (our institute (Location 1), research partner IWW, Mülheim an der Ruhr (Location 3) and in a pilot water house installation of our research partner (DVGW Forschungsstelle TUHH (Location 2 (DOC 0,9 mg/ml) and 4 (DOC ~3,3 mg/ml))).

A contamination of biofilms with *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Enterobacter amnigenus* und *Citrobacter freundii* and a mechanical and chemical treatment with chlorine dioxide were realized by our research partner at the pilot water house installation.

Biofilm communities on different materials are compared with a fingerprinting method and cloning. 16S rDNA polymerase chain reaction (PCR) of the V3 region and denaturing gradient gel electrophoresis (DGGE) analyses detect changing DNA band patterns depending on the material and the ambient conditions (Muyzer et al., 1993).

Cloning of 16S rDNA was performed with the products of the primers 63f and 1387r (Marchesi et al., 1998) and the TOPO TA Cloning KIT for Sequencing. Around 400 – 500 bp of 50-60 clones each were sequenced for the first screening.

### Acknowledgements

This investigation was financially supported by the German Federal Ministry of Education and Research (BMBF). We would like to thank our research partner ([www.biofilm-hausinstallation.de](http://www.biofilm-hausinstallation.de)) for providing biofilm samples.

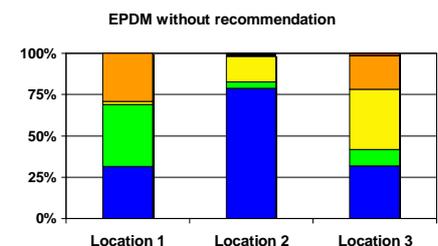
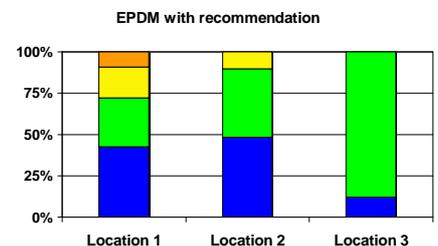


Figure 5: the main closest relatives in 6 weeks old biofilms on EPDM with and without recommendation examined by 16S rDNA cloning

- Alphaproteobacteria
- Betaproteobacteria
- Gammaproteobacteria
- Flavobacteria
- Actinobacteria
- Deltaproteobacteria

Location 1: drinking water from recharged groundwater of Eastern Germany  
Location 2: drinking water from reduced groundwater of Northern Germany  
Location 3: drinking water from river bank infiltration of Western Germany

### References

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# Influence of drinking water installation materials on biofilm composition

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Water installations materials which support growth of biofilms pose a serious risk for the contamination of drinking water with hygienically relevant microorganisms. In water installations many different materials are used over a long period of time. Depending on the applied materials and the ambient conditions, like stagnation and water temperature, different biofilm communities are formed. In former examinations biofilm populations on inert materials like glass and PE-HD were dominated by beta-Proteobacteria and two-thirds of the biofilm population was in a viable but nonculturable state (VBNC). Up to now mainly indirect methods exist for the assessment of biofilm formation on materials, which measure the thickness of biofilms, the consumption of oxygen or the activity of microorganisms. There is only little information available about the influence of house installation materials on the composition of a biofilm population.

In this project, biofilms on different materials, which are normally used in water installations (Copper, different PE and rubber qualities), are investigated. The materials are exposed in biofilm reactors in a drinking water house installation at different sites (our institute, research partner all-over Germany). Also we examined native, contaminated (*P. aeruginosa*, *L. pneumophila*, *E. amnigenus*) and disinfected biofilms on different materials at two different water temperatures (12 °C, 37 °C) from a pilot water house installation of our research partner in Hamburg. The biofilm communities on the different materials are compared with a fingerprinting method (Denaturing gradient gel electrophoresis (DGGE)). Changing DNA band patterns depending on the material and the ambient conditions (e.g. water temperature) are identified. Selected DNA bands are analyzed by cloning and sequencing to identify indicator organisms for the materials. *P. aeruginosa* and *L. pneumophila* will be quantified by real-time PCR at different points in time. With the results of this research probes for Fluorescence In Situ Hybridization (CARD-FISH) and primers for quantitative PCR (qPCR) will be developed for further investigations of the biofilm composition.

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