Impact of different materials on Biofilm communities in drinking water installations

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Introduction

Pathogenic microorganisms in drinking water like for instance *P. aeruginosa* and *Legionella* pose a threat for human health. They settle in biofilms, which protect them against decontamination and offer an optimal habitat. Depending on the applied materials and the ambient conditions, like stagnation, different biofilm communities can be formed. Materials which support growth of biofilms present a serious risk for the contamination of drinking water with hygienically relevant microorganisms. Coliform bacteria may occur on installation materials which promote their growth and they may be released in the flowing drinking water (Kilb et al., 2003). Inert materials like glass and PE-HD were dominated by beta-Proteobacteria and a new genus *Aquabacterium* was described as dominant for drinking water systems (Kalmbach et al ., 1997). 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.

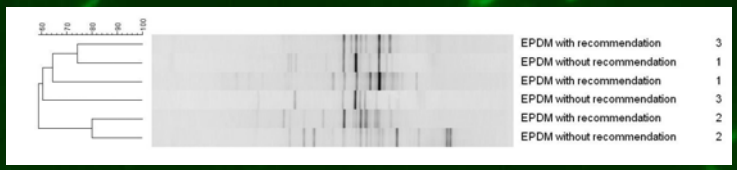


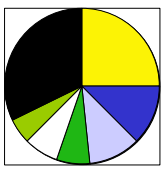
Figure 1 and 2: DGGE pattern similarity analysis
left: Biofilms on different materials grown at our institute at different locations (1a - 1c)
right: Biofilms on EPDM with and without recommendation at three different locations:
Location 1: drinking water from recharged groundwater of east Germany
Location 2: drinking water from recharged groundwater of north Germany
Location 3: drinking water from river bank infiltration of west Germany

Results and Conclusions

- **Growth supporting materials like EPDM showed a higher diversity of the biofilm population as inert materials.**
- **The similarity between the biofilm population on the different materials is low and regulated by the availability of the nutrients released by the materials.**
- **The genus *Aquabacterium* is ubiquitous found in drinking water biofilms.**
- **There is no consistent biofilm community on the same material from different locations. The analysis of the DGGE pattern of both EPDM qualities showed a low similarity.**
- **It seems that the origin of the drinking water plays also an important role for the biofilm community structure.**

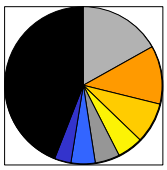
EPDM with recommendation

Location 1



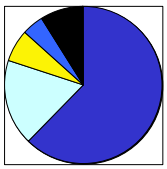
- *Sphingopyxis sp.*
- *Aquabacterium citratiphilu*
- *Pseudoxanthomonas sp.*
- *Flavobacterium sp.*
- *Leptothrix sp.*
- *Acidovorax defluvii*
- other

Location 2



- *Acidovorax sp.*
- *Sphingopyxis sp.*
- *Sphingomonas sp*
- *Sphingobium sp.*
- *Legionella anisa*
- *Aquabacterium parvum*
- *Aquabacterium citratiphilum*
- other

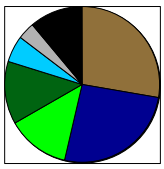
Location 3



- *Aquabacterium citratiphilum*
- *Methylibium aquaticum*
- *Sphingopyxis sp.*
- *Aquabacterium parvum*
- other

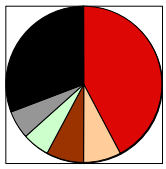
EPDM without recommendation

Location 1



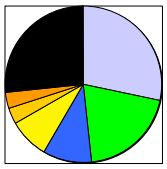
- *Rhizomonas sp.*
- *Aquabacterium sp. Aqua1a*
- *Flavobacterium terrigena*
- *Flavobacterium kamogawaensis*
- *Aquabacterium commune*
- *Acidovorax defluvii*
- other

Location 2

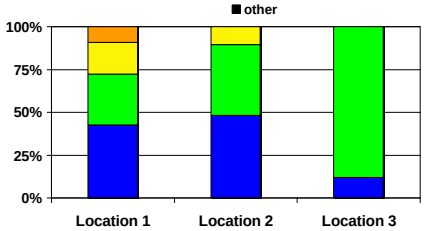


- *Azospirillum sp.*
- *Rhodovarius lipocyclicus*
- *Rhizosphere soil bacterium*
- *Pseudomonas fluorescens*
- *Legionella anisa*
- other

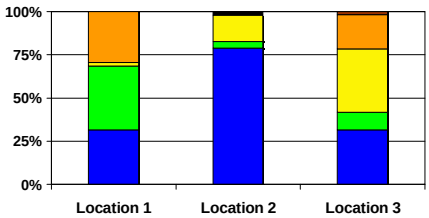
Location 3



- *Pseudoxanthomonas sp.*
- *Flavobacterium terrigena*
- *Aquabacterium parvum*
- *Sphingopyxis sp.*
- *Sphingomonas sp.*
- *Sphingobium sp.*
- other



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



Location 1:
drinking water from recharged groundwater of east Germany
Location 2:
drinking water from recharged groundwater of north Germany
Location 3:
drinking water from river bank infiltration of west Germany

Figure 3 and 4: Biofilm communities on EPDM with recommendation examined by 16S rDNA cloning
top: the main closest relatives
down: main Groups of bacteria

Figure 5 and 6: Biofilm communities on EPDM without recommendation examined by 16S rDNA cloning
top: the main closest relatives
down: main Groups of bacteria

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)). The biofilm communities on the 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 were 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

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We would like to thank our research partner (www.biofilm-hausinstallation.de) for providing biofilm samples.

References

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IMPACT OF DIFFERENT MATERIALS ON BIOFILM COMMUNITIES IN DRINKING WATER INSTALLATIONS

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ABSTRACT

Pathogenic microorganisms in drinking water like *P. aeruginosa* and *Legionella* pose a threat for human health. They settle in biofilms, which protect them against decontamination and offer an optimal habitat. Materials which support growth of biofilms present a serious risk for the contamination of drinking water with hygienically relevant microorganisms. The biofilm communities 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: IWW, Mülheim an der Ruhr). Also we examined biofilm communities at two different water temperatures (12 °C, 37 °C) from a pilot water house installation of our research partner in Hamburg (DVGW Forschungsstelle TUHH). 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. 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.

Acknowledgements

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