Multi-stage assessment of biofilm growth by drinking water bacteria on polymeric pipe materials

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Introduction

The presence of biofilms in drinking water distribution systems (DWDS) leads to a number of issues, i.e. secondary (biological) drinking water contamination, pipe damage and increased flow resistance. Among other operational factors, the selection of pipe material plays an important role in biofilm development. Up to now, the studies that have investigated this correlation provide contradictory results in terms of which material might be the most advantageous in the DWDS biofilm control strategy. Hence, to understand the influence of pipe material on biofilm formation, we focused on developing a standardized methodology that allows a multi-stage assessment of biofilm development on real pipe materials.

Results

Development of the methodology consisted of three steps: 1) material coupon sterilization, 2) biofilm cultivation and 3) biofilm analysis, using transparent polyvinyl chloride (PVC) as a study material. For the coupon sterilization, methods utilizing immersion in different disinfectant solutions with and without pre-cleaning by rubbing the coupons in a surfactant solution. The results showed that mechanical cleaning before washing is crucial and without it, reproducible sterilization was difficult to achieve. Biofilm formation on the PVC coupons was performed in a 6-well plate assay (24, 48 and 72 h; under agitation) using DWDS biofilm strains (Sphingomonas spp. and Pseudomonas extremorientalis) and Pseudomonas aeruginosa as a positive control. Bacterial fitness and ability to secrete EPS and form biofilms on the PVC surfaces were tested by monitoring optical density (OD600 nm), chemical oxygen demand (COD) and protein concentration. The formed biofilm and the morphology of attached bacteria were visualized using crystal violet staining (that allow qualitative (bright field microscopy) and quantitative (OD at 570 nm) evaluation), by scanning electron microscopy (SEM) and DNA staining (4,6-diamidino-2-phenylindole; DAPI) with fluorescence microscopy. Combination of those techniques gave a complete overview of patterns involved in biofilm development by selected drinking water bacterial strains in presence of a PVC surface. The developed methodology was also applied for the analysis of bacterial growth on real-grade pipe materials, such as PVC and polyethylene (PE), to understand their role in biofilm formation.

Conclusions

Implementation of various analytical and microscopic techniques is important in understanding mechanisms behind biofilm development in DWDS and the influence of pipe material in the process. The proposed approach allows the observation of biofilm formation in time, but also of the typical bacterial morphology of attached cells. In this study it was shown that to obtain reproducible results, it is crucial to select an appropriate sterilization technique and the influence of mechanical cleaning
cannot be ignored in preparation of polymeric surfaces.