Characterising the Physical Structure and Microbial Community Structure of Drinking Water Biofilms

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INTRODUCTION

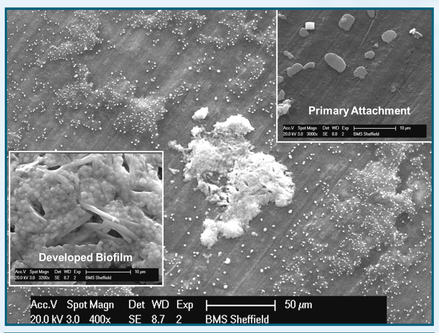


Fig. 1: Scanning electron microscope images of drinking water biofilm upon pipe surface.

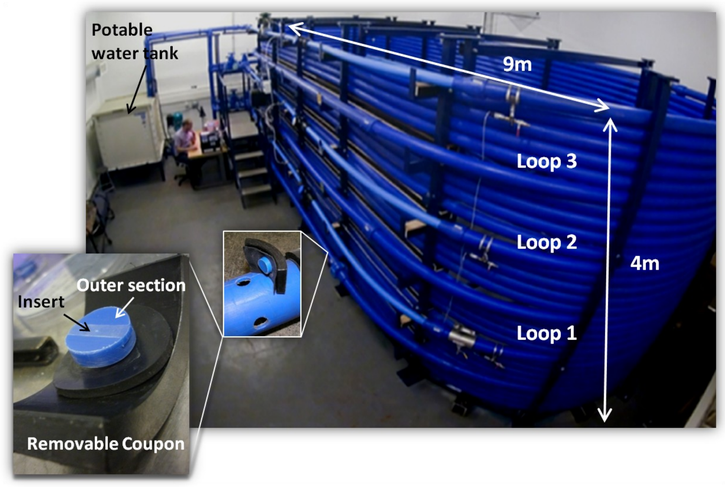
Mixed microbial species biofilms form upon the walls (Fig. 1) of drinking water distribution system (DWDS) pipelines. Biofilms adhere via extracellular polymeric substances (EPS), primarily carbohydrates and proteins, produced by microorganisms. The EPS provides physical stability to the biofilm. If the adhesive forces are overcome by shear stresses at the pipe wall, biofilm becomes mobilised into the bulk water, degrading water quality.

Gaining an insight into the physical structure of DWDS biofilms, particularly EPS characteristics and the influence of microbial community structure upon these, will increase understanding of DWDS biofilms which may aid development of strategies to manage biofilm mobilisation.

RESEARCH AIM

To combine fluorescence microscopic characterisation of drinking water biofilm (cells and EPS) physical structure and molecular analysis of microbial communities to enable characterisation of biofilm formation.

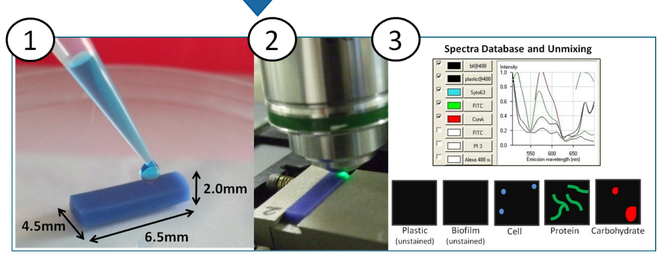
Full Scale Drinking Water Distribution System Test Facility



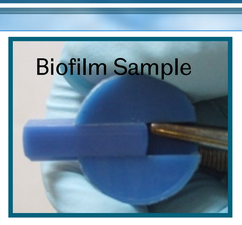
Biofilms were developed for 28 days (16°C, 0.4 ls^{-1} steady state), upon coupons within a temperature controlled facility fed with drinking water from the local distribution system.

Physical Structure

1. Stains targeting: cells (Syto 63), carbohydrates (Con-A tetrarhodamine) and proteins (FITC) were applied to Day 0 and Day 28 biofilm samples (n=5).
2. Confocal laser scanning microscopy (CLSM) produced Z-stack images at 5 fields of view per insert.
3. Each stain was unmixed using emission spectra fingerprints previously stored in a spectra database. Digital image analysis (DIA) was applied to these unmixed images.



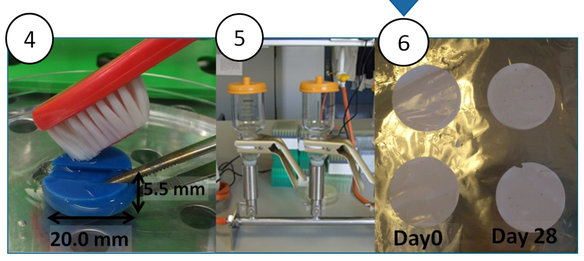
Fluorescent staining and imaging



Community Structure

4. Biofilm was removed from the outer coupon to produce a "biofilm suspension" in phosphate buffer.
5&6. The biofilm suspension was filtered (0.22µm pore nitrocellulose membrane) and DNA was extracted from the filter.

PCRs amplifying bacterial and archaeal 16S rRNA genes and fungal ITS regions were performed. Bacterial 16S rRNA community fingerprinting was carried out via Terminal-Restriction Fragment Length Polymorphism analysis.



Molecular analysis

Visualising and Quantifying the EPS and Cells

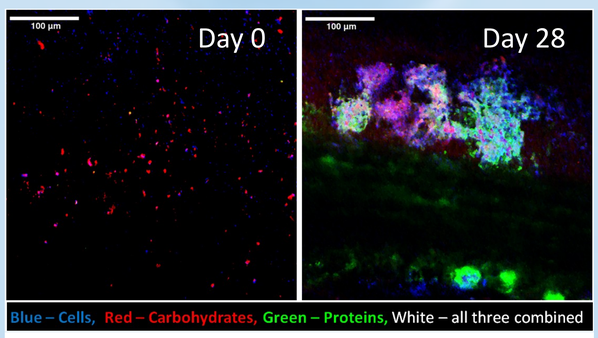


Fig. 2: CLSM image of the mid slice of a Day 0 and Day 28 biofilm.

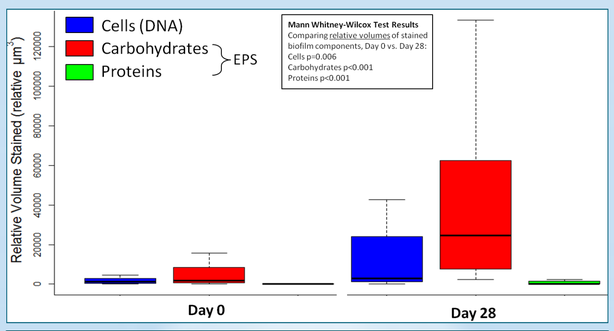


Fig.4: Volumes of each biofilm component relative to the Z-stack dimensions

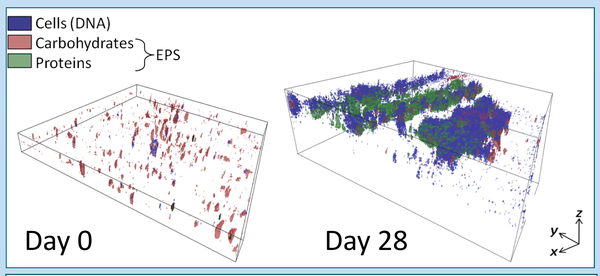


Fig. 3: 3D projection of the carbohydrates and proteins of the EPS and the biofilm cells throughout Z-stacks of Day 0 and 28 biofilms. (In both cases XY=420µm^2; Day 0 maximum Z=35.4µm, Day 28 maximum Z=127.4µm).

- Compared to Day 28 biofilms, Day 0 biofilms had less protein content and were thinner with smaller microcolonies as regards area covered (Fig.2) and 3D distribution (Fig. 3).
- The volume of all three biofilm components (Fig. 4) increased significantly during development. Carbohydrate volumes exceeded cell and protein volumes.
- EPS (mainly carbohydrate) accounted for the greatest proportion of the biofilm (Fig. 4) at both Day 0 (72%) and Day 28 (80%).

Microbial Community Analysis

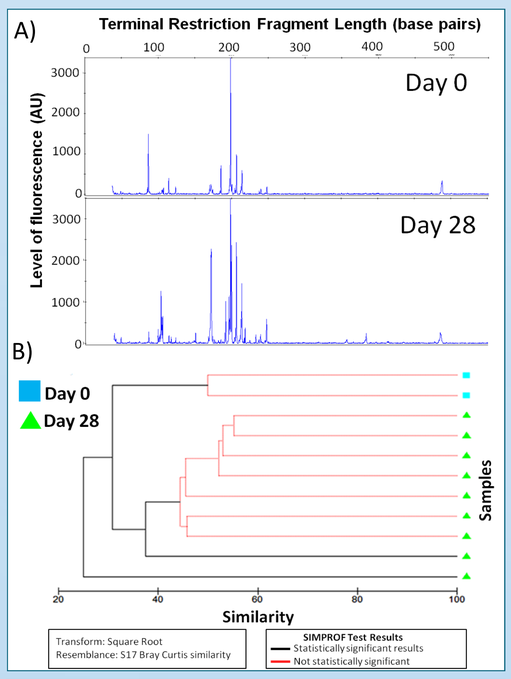


Fig. 5: T-RFLP analysis of amplified bacterial 16S rRNA genes.
A) Electropherogram examples; B) Dendogram, showing the similarity between samples based on T-RF presence/absence. SIMPROF results as indicated in key.

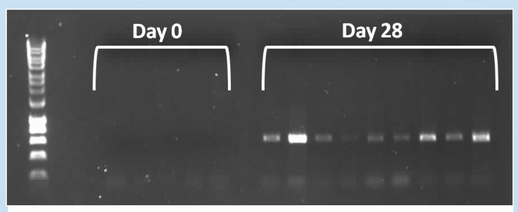


Fig. 6: Amplified fungi ITS region PCR products (using FAMITS1/ITS4)

- Bacterial 16S rRNA genes were amplified from two Day 0 samples and all Day 28 samples.
- T-RFLP community fingerprints (Fig. 5A) showed similarities between the two time points but a greater number of T-RFs were observed at Day 28. Resemblance analysis (Fig. 5B) with SIMPROF testing showed (statistically significant) distinct groupings of the Day 0 and Day 28 samples, with an outlying Day 28 sample. ANOSIM confirmed a difference between the time points (Global R=0.414) but this was not found to be significant (p=0.091).
- Archaeal 16S rRNA genes could not be amplified at any time point.
- Amplification of the Fungi ITS region was visualised in Day 28 samples only (Fig.6).

CONCLUSIONS

We present for the <u>first time</u> data generated using a novel tool combining characterisation of the physical and community structure of microbial biofilms from a DWDS test facility which is directly representative of real networks.

- Distinct physical structures are visualised and quantified at the start and end of the biofilm development. EPS was found to account for the majority of the biofilm and was dominated by carbohydrates in each case, which may be the critical component in early biofilm adhesion.
- Primary bacterial colonisers dominated the microbial community throughout biofilm development although diversity increased at Day 28 as indicated by a greater number of 16S rRNA T-RFS and the presence of fungi.

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