Molecular analysis of bacterial biofilm communities in response to environmental perturbations within drinking water distribution systems

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Aim of the Study

Discolouration of drinking water is one of the main reasons for costumers complains to water companies. Discolouration occurs when accumulated particles attached to the inner-pipe walls are mobilised into the network due to changes in shear stress. Although, it has been suggested that microbial biofilms may play an important role in discolouration, there is little experimental evidence supporting this hypothesis. The aim of this study is to investigate the response of the bacterial communities to an ***experimental discolouration event*** using a pilot-scale test facility representative of a full-scale drinking water distribution systems (DWDS).

Background

➢Drinking water distribution systems (DWDS) contain microorganisms which survive disinfection and under certain environmental conditions attach to the internal pipe surface and form biofilms (Fig. 1).

➢Microbial biofilms can generate various problems in the DWDS such as changes in water quality (e.g. **discolouration**, taste, odour), promotion of opportunistic pathogen survival and corrosion of pipes (Szewzyk *et al*., 2000).

➢Mobilisation of material accumulated on the inner-pipe surface occurs when shear stress within the pipes exceed daily conditioning values. The mobilisation of material from the pipe wall results in discoloured drinking water.

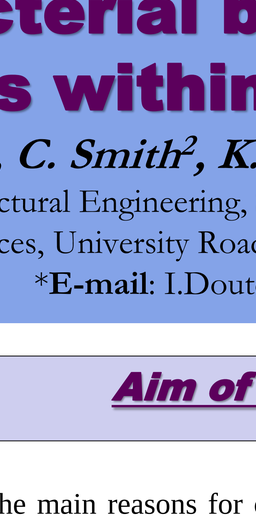

Fig. 1: Biofilm development within drinking water pipes.

Material and Methods

Biofilm samples were obtained from coupons inserted into the internationally unique temperature controlled test pipe facility at the University of Sheffield (Fig. 2). To reproduce the dynamics occurring in real systems, the facility operated at three different hydraulic regimes (0.11 N/m^2, 0.22 N/m^2 and 0.44 N/m^2) and at a temperature of 8°C.

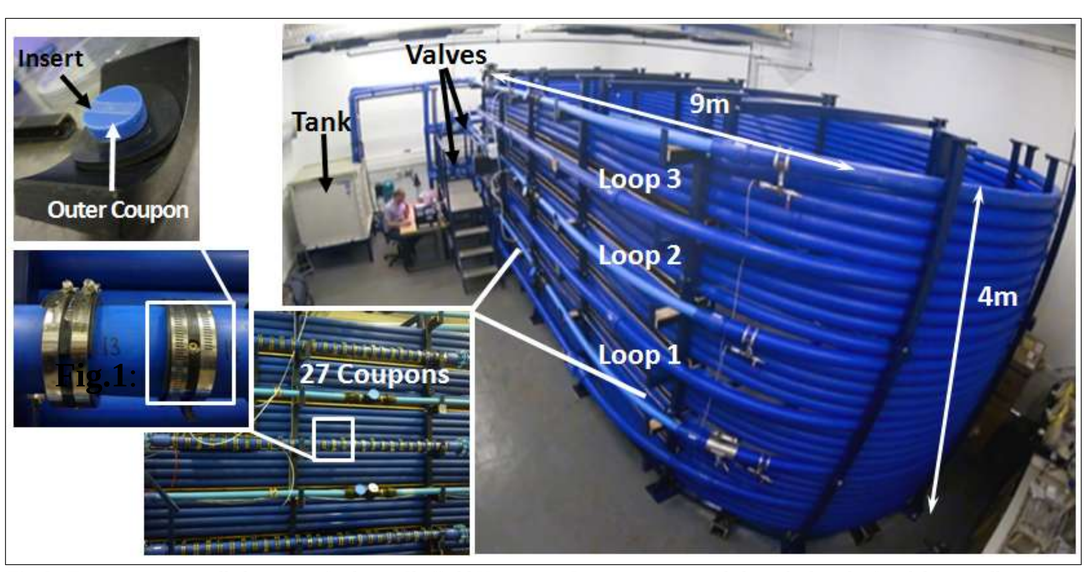

Fig. 2: Temperature controlled pipe-test facility at Sheffield University. Coupons are inserted along the length of each loop to allow for subsequent biofilm removal and examination.

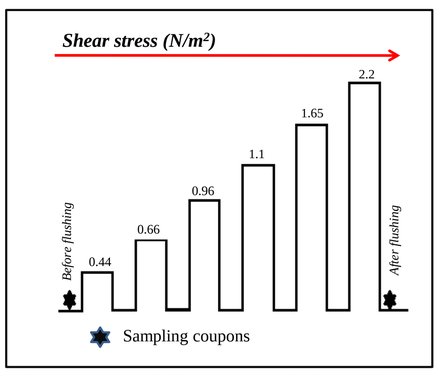

Fig. 3: Incremental shear stress applied to each loop after 28 days biofilm development.

To determine the effect of incremental shear stress (Fig. 3) on biofilm bacterial community structure, three coupons were removed from each loop at the start and end of the mobilisation event.

DNA was isolated from biofilms formed on the pipe-wall of **Loop 2 (0.22 N/m^2)** after 28 days of growth and specific regions of the 16S rRNA gene were amplified by PCR. Bacterial communities inhabiting the biofilms were characterised using a molecular fingerprinting technique, terminal restriction fragment length polymorphisms (T-RFLPs), in combination with pyrosequencing.

T-RFLP analysis was conducted using F63-FAM & R518 primer set, followed by individual digest with CfoI and AluI (Fig. 4). T-RFLP profiles were aligned using T-Align (Smith *et al*., 2005). Relatedness of samples were calculated using the Bray-Curtis similarity calculation and the similarity was illustrated using a dendrogram.

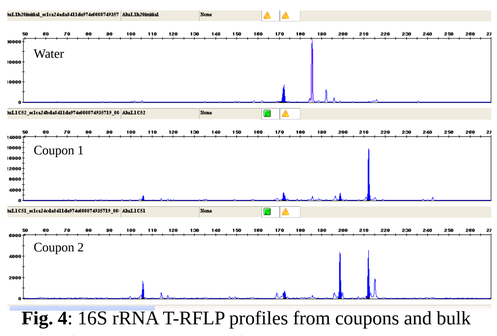

Fig. 4: 16S rRNA T-RFLP profiles from coupons and bulk water from the pipe-rig test facility.

Pyrosequencing

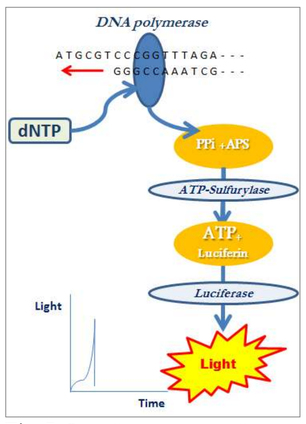

Fig. 5: Steps in a pyrosequencing reaction.

Bacterial tag-encoded FLX amplicon pyrosequencing (Fig. 5) was performed using primers Gray28F and Gray519R (Dowd *et al*., 2008).

Two types of analysis were carried out:

A) To calculate the percentage of bacteria from each division, sequences were filtered and chimeras removed using B2C2 (Gontcharova *et al*., 2010). Sequences were assembled into clusters and queried using a distributed BLASTm.NET algorithm against a bacterial database derived from NCBI (Dowd *et al* ,2008)

B) To obtain rarefaction curves, species richness and diversity sequences were filtered with Mothur (http://www.mothur.org) and then subjected to the RDP pyrosequencing pipeline (http://pyro.cme.msu.edu/)

Results

T-RFLP analysis

Loop 2 – medium shear stress 0.22 (N/m)²

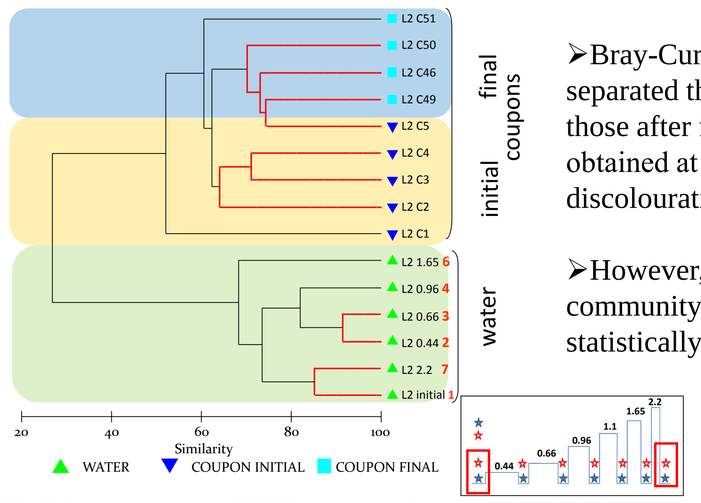

Fig. 6: Dendrogram showing T-RFLP analysis of bulk water samples obtained at different shear stress and coupon samples before and after flushing.

➢Bray-Curtis similarity analysis (Fig. 6), clearly separated the coupon samples before flushing, from those after flushing and from bulk water samples obtained at different shear stress's during the discolouration event.

➢However, the variation detected in the bacterial community structure before and after flushing was not statistically significant.

Pyrosequencing analysis

➢The predominant bacteria phylum in the biofilm samples were *Betaproteobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria*. After flushing there was an increase in *Betaproteobacteria* (13%) and *Gammaproteobacteria* (3%) and a decrease in *Alphaproteobacteria* (13%), *Sphingobacteria* (1.75%), *Flavobacteria* (1.4 %) and other less represented phyla (Fig. 7).

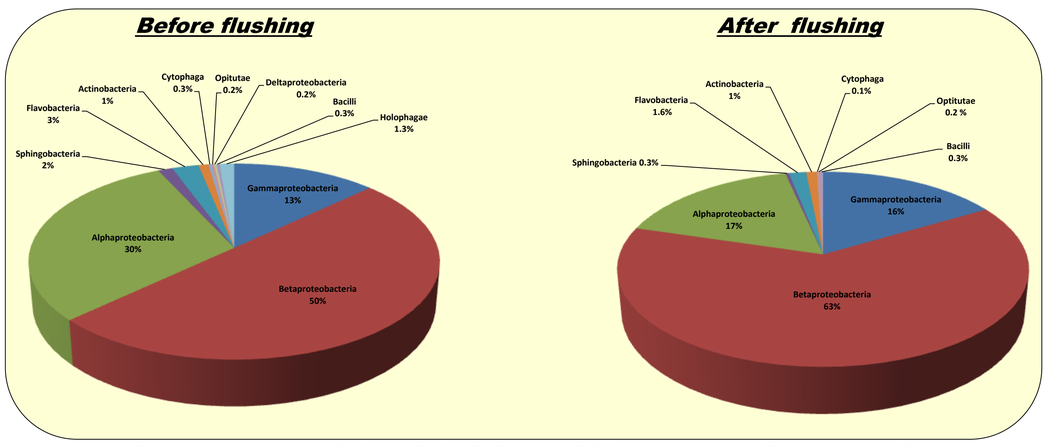

Fig. 7: Bacterial community composition before and after flushing revealed by pyrosequencing. The percentage of bacteria from each phylum was calculated and results of > 1% are shown.

➢The **Chao1 richness indicator**, calculated at two different similarity cut offs showed that richness in the biofilm samples decreased after flushing. Also, the **Shannon Diversity index (H')**, which indicate the relative OTU abundance, revealed that the bacterial community before flushing was more diverse (Fig . 8).

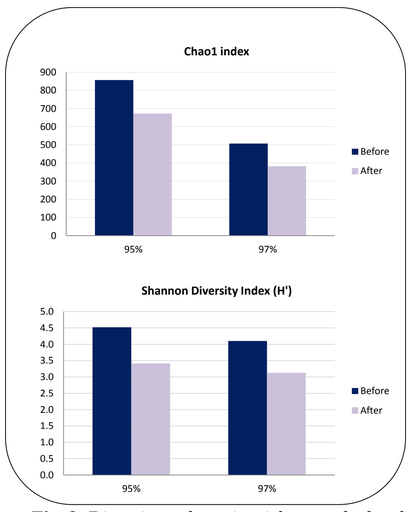

Fig. 8: Diversity and species richness calculated at two different cut offs 95 and 97% 16S rRNA gene similarity.

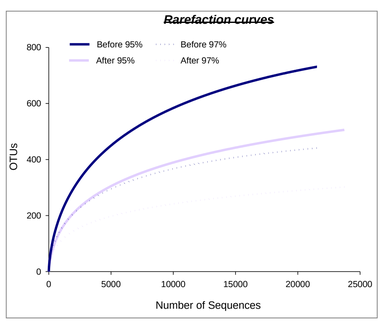

Fig. 9: Rarefaction curves before and after flushing defined at 95 and 97% 16S rRNA gene similarity.

➢**Rarefaction curves** approximated toward a plateau after 15,000 sequences, indicating that enough sample coverage was obtained (Fig. 9). Curvature towards the horizontal indicates that increased sequencing effort is required to observe new OTUs when only rare OTUs remain to be detected.

Conclusions

1. T-RFLP allowed for an assessment of the dominant members of bacterial communities within biofilms. However, pyrosequencing analysis yielded valuable taxonomical information, allowing for the estimation of the relative abundance of different members of bacterial communities.
2. T-RFLP analysis did not show a statistical significant difference in the microbial community structure before and after flushing.
3. Pyrosequencing analysis revealed a change in bacterial community structure before and after the discoloration event. Furthermore, higher bacterial diversity and species richness were detected before flushing, indicating that pipe-wall material might have been mobilised into the bulk water during the experimental discolouration event.

References

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❖Smith *et al*., 2005. T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. FEMS Microbiol Ecol 54:375-380.

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