Pyrosequencing analysis of bacterial community structure in an experimental drinking water distribution system

I. Douterelo , R. Sharpe, K. Fish and J. Boxall*

Department of Civil and Structural Engineering, Sir Frederick Mappin Building, University of Sheffield, Sheffield, S1 3JD, UK.

***E-mail:** I.Douterelo@sheffield.ac.uk

Introduction

Fig. 1: Biofilm development within drinking water pipes

Microbial biofilms formed on inner-pipe surfaces in drinking water distribution systems (DWDS) may affect the quality and safety of drinking water, particularly if mobilised from the pipe wall into the bulk water (Fig. 1).

Discolouration of drinking water is one of the main reasons for costumers complains to water companies in the UK. Mobilization of material accumulated on the inner-pipe surface occurs when shear stress within the pipes exceeds daily conditioning values. The mobilisation of material from the pipe wall results in discoloured water (Fig. 2).

Fig. 2 : Discolouration of drinking water

Objectives

- **To improve knowledge on bacterial ecology of DWDS using 454 pyrosequencing of the 16s rRNA gene**
- **To assess the potential mobilisation of biological material from pipe walls**

Materials and Methods

Research was carried out in a **full scale test-loop facility** to reproduce characteristics occurring in live distribution systems (Fig. 3). The facility was fed from the local network with a 24h water retention time to maintain a chlorine residual and nutrient supply. To facilitate DNA-based analysis of microbial biofilms HDPE **coupons** were inserted around the mid-length of each loop (Fig. 3).

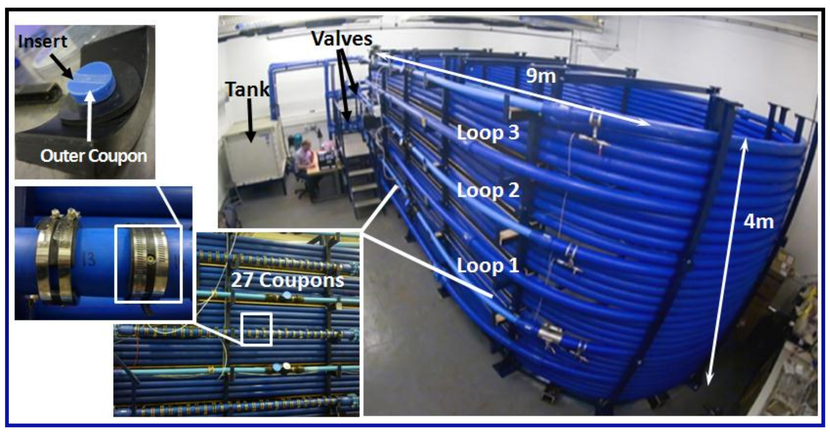

Fig .3 : Temperature controlled pipe-test facility at Sheffield University. Coupons are inserted along the length of each loop to allow for subsequent biofilm removal and examination.

After 28 days of biofilm growth at **steady state** conditions and at **16°C**, the test-loop facility was **flushed** by sequentially increasing the boundary hydraulic forces to investigate the mobilization of material from inner-pipe surfaces to the bulk water (Fig. 4). As shown in Figure 4, coupons and bulk water samples were collected pre- and post-flushing for physico-chemical and DNA-based analysis.

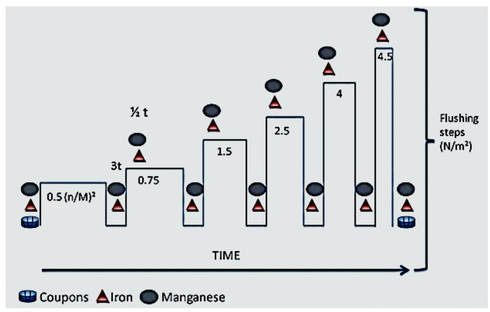

Fig. 4: Incremental shear stress applied to the experimental distribution system after 28 days of biofilm growth

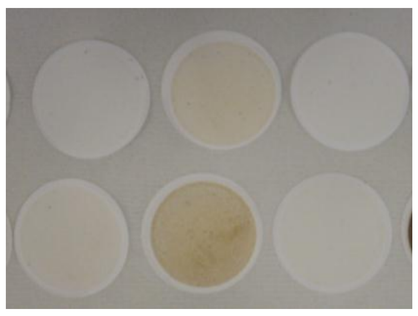

Fig. 5: Bacteria were concentrated from each water sample and biofilm suspension on 0.2-µm-pore-size nitrocellulose membrane filters for subsequent DNA extraction

DNA was extracted from biofilm and water samples (Fig. 5) and bacterial tag-encoded **FLX 454 pyrosequencing** (bTEFAP) was performed by the Research and Testing Laboratory (Lubbock, USA) using primers Gray28F and Gray519r which span the variable regions V1–V3 in the 16s rRNA gene. Sequences were analysed by Research and Testing Laboratory to obtain taxonomical assignments (Dowd *et al.*, 2008). The estimation of alpha and beta diversity was carried out using **QIIME** analysis pipeline (Caporaso, *et al.*, 2010).

Results

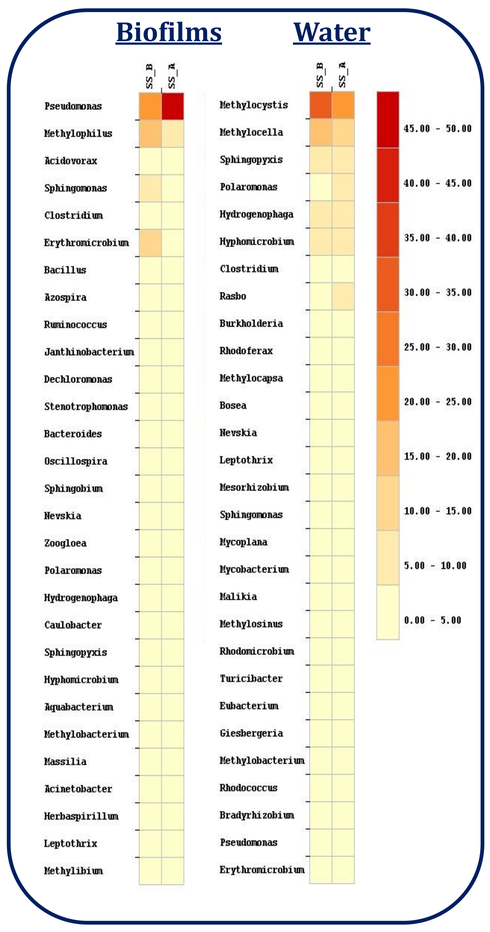

Fig. 6: Heatmaps showing the percentages of the most abundant species at genus level within bulk water and biofilms

Pyrosequencing analysis showed significant differences ($p<0.05$) in the relative abundance of bacteria at different taxonomic levels between **biofilm and water samples**. *Gammaproteobacteria* (27-50%) at class level and *Pseudomonas* (up to 48%) at genus level were predominated in biofilms, while *Alphaproteobacteria* (68-77%) and species belonging to the genera *Methylocystis* (23-31%) and *Methylocella (17-21%)* were abundant in water samples (Figs. 6 and 7).

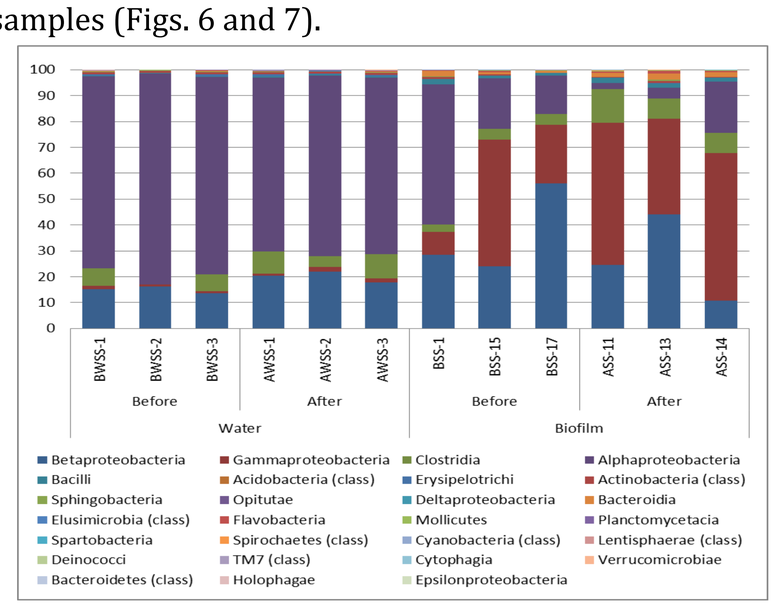

Fig. 7: Bacterial community composition of biofilm and bulk water samples at class level

After flushing the pipe-test facility, considerable shifts in *Gammaproteobacteria* (increased 23 %) and *Alphaproteobacteria (decreased 21 %)* relative abundance were observed in biofilm samples. At genus level, the main changes in relative species abundance within biofilms were in the genera *Pseudomonas, Methylophilus, Sphingomonas* and *Erythromicrobium* (Figs. 6 and 7).

The **Chao1 richness indicator**, calculated at 95 % similarity cut off showed that bacterial species richness was higher in biofilm than in bulk water samples. Species richness also decreased in post-flushing biofilm samples. **Shannon Diversity index (H')** indicated that the bacterial community within biofilms was more diverse before flushing than after flushing the tested pipes (Fig. 8).

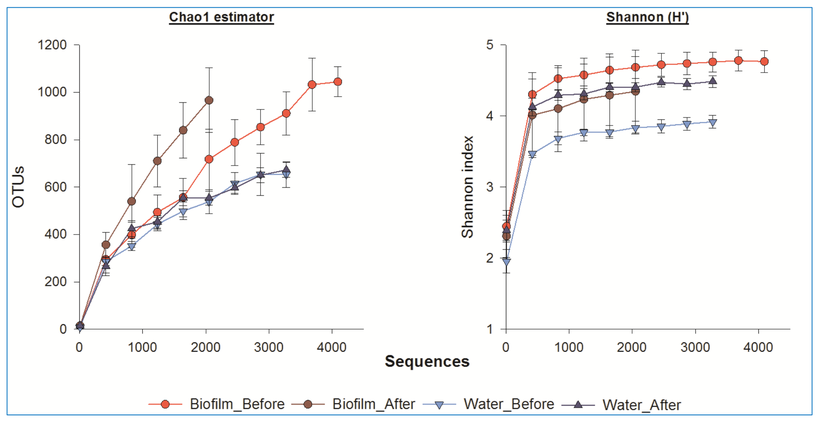

Fig. 8: Rarefaction curves at 95 % of sequence similarity for water and biofilm samples. Rarefaction curves were obtained for observed OTUS, Chao1 richness estimator and Shannon diversity index. Bars are indicating standard error.

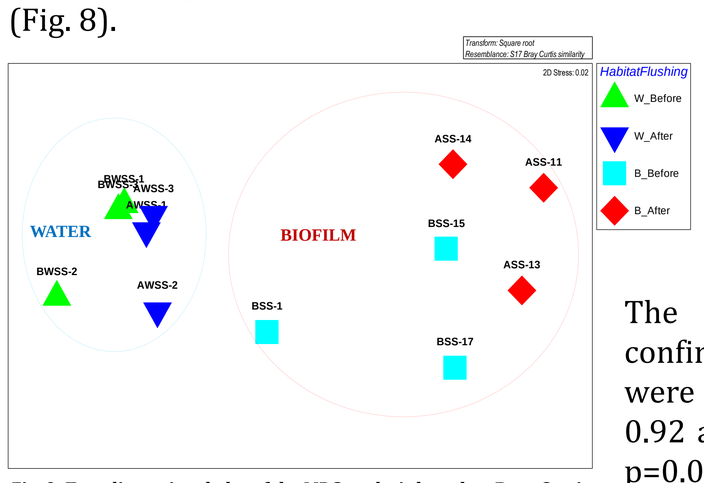

Fig. 9: Two-dimensional plot of the MDS analysis based on Bray-Curtis similarities of the percentage sequence abundance. W = water and B = biofilm.

The non metric Multi-Dimensional Scaling **(MDS)** analysis of relative sequence abundance at 95 % similarity cut off, showed a clear separation among **biofilm and bulk water** samples (Fig. 9).

The analysis of similarities (ANOSIM) confirmed that water and biofilm samples were significantly different (class level; R= 0.92 and p= 0.002 and species level; R=0.99, p=0.002). ANOSIM also showed significant differences between pre- and post-flushing biofilm samples (class level; R= 0.33, p= 0.01 and species level; R=0.37, p=0.01).

Conclusions

- **Pyrosequencing analysis highlighted that bulk water and biofilms had significant different bacterial structure and composition**
- **Flushing did not completely removed biofilms from the pipes but altered the pipe-wall bacterial community structure**
- **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

- Dowd *et al.,* 2008. Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: bacterial diversity in the ileum of newly weaned Salmonella infected pigs. Foodborne Pathog Dis 5(4):459-472.
- Caporaso et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature methods, 7 (5),335-336.