Investigating Bacterial Community Response During Discolouration Events in an Experimental Water Distribution System

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INTRODUCTION

Discolouration of potable water, due to fine insoluble particles, is a major cause for customer contacts to water companies. Within water distribution systems (WDS) particulate material accumulates on pipe walls. The stability and amount of material within these layers is known to be influenced by the maximum shear stress exerted by daily flow profile but the processes and mechanisms explicitly involved are poorly understood. Mobilisation of material into the bulk water occurs when the shear stress exceeds the conditioning values (Fig. 1). Whilst discolouration is predominantly an aesthetic issue association with microbiological content has been made. Biofilm microbiological loads are known to be significant within WDS. It seems likely that microbial biofilms may be important in understanding causes and consequences of discolouration. The internationally unique temperature controlled pipe-rig test facility at the University of Sheffield (Fig. 2) can be used to bridge the gap between field and bench scale studies by simulating field conditions in a controlled laboratory setting to determine the underpinning factors that contribute to discolouration and the role biofilm microorganisms play.

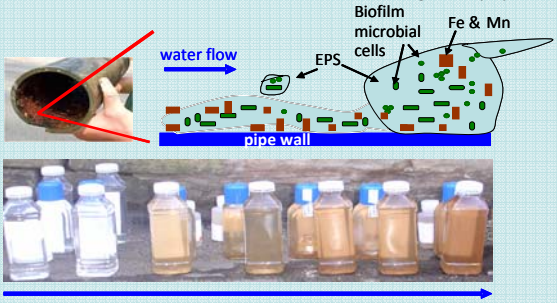

Fig. 1. Increased shear stress (right to left) above the daily conditioning shear resulting in discolouration of drinking water as material associated with the pipe wall is mobilised.

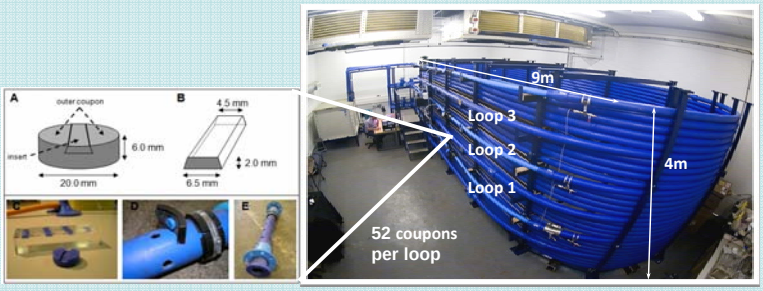

Fig. 2. The temperature controlled pipe loop test facility at the University of Sheffield. Excerpt the Pennine Water Group coupon (Deines *et al.,* 2010), 52 coupons are inserted along the length of each loop to facilitate examination of the pipewall biofilm.

AIM

To examine the response of WDS bacterial communities to an experimental discolouration event using the internationally unique pipe-loop test facility at the University of Sheffield (Fig. 2).

OBJECTIVES

1: To test the hypothesis that biofilm stability is influenced by the maximum conditioning shear stress of the water flowing through it.

2: Determine the affect of incremental shear stress flushing steps on biofilm bacterial community structure conditioned at three different shear stresses.

METHODS

Pipe wall material was developed in each loop for 28 days at 8°C using the following constant shear stress; **Loop 1 low - 0.11 N/m², Loop 2 medium - 0.22 N/m²** & **Loop 3 high – 0.44 N/m².** After 28 days, each loop was individually flushed according to figure 3. Each flushing step was conducted for three turnovers of water. Turbidity changes in the bulk water were continuously measured and water samples were collected for DAPI and Mn analysis after one turnover. 5 coupons were taken from each loop before and after the flushing event. Biofilm was removed from the coupons using a nylon brush as described by Deines *et al.,* 2010, filtered and DNA was extracted. T-RFLP was conducted using F63-FAM & R518 primer set, followed by individual digest with *CfoI* and *AluI*. T-RFLP profiles were aligned using T-Align (Smith *et al.,* 2005) and subsequently analysed using a Bray-Curtis similarity matrix in PRIMER-6. Q-PCR was conducted using the primer set F1369 & R1492 with *Taq*Man probe 1389 according to Smith *et al.,* 2006.

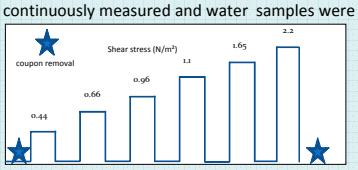

Fig. 3: Schematic of incremental shear stress applied to each loop. Star indicates coupon removal.

RESULTS

Results 1: Mobilistation of pipe wall material into bulk water during flushing event

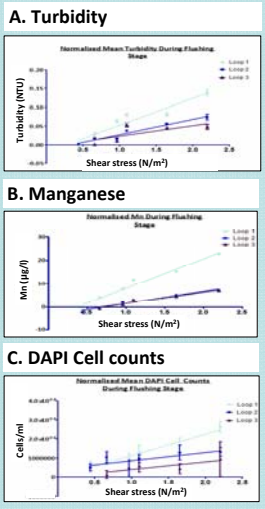

Fig. 4: Normalised increase in A) tubidity, B) manganese and C) DAPI cell counts in the bulk water after each flushing step for loop 1, 2 and 3.

Results 2: Pipe-wall bacterial community structure pre- and post- flushing.

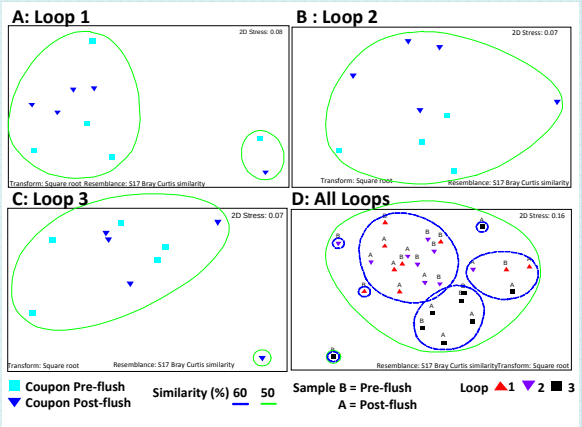

Fig. 5: (A-C) MDS analysis of T-RFLP data from loop 1, 2 & 3 respectively and (D) combined T-RFLP data from all 3 loops before & after flushing. ANOSIM analysis did not show any statistical difference in community structure before and after the flushing event for any loop.

Results 3: Quantitative changes in 16S rRNA gene copy numbers from pipe wall pre- and post-flushing.

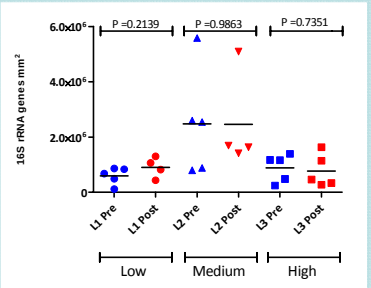

Fig. 6: 16S rRNA gene copy numbers per mm² of pipe wall, quantified pre- and post-flushing for each loop. No statistical difference in gene copy numbers pre- and post- flushing was observed for any of the three loops ($P < 0.05$).

SUMMARY

1: Loop 1, conditioned during the 28 day growth phase at the lowest shear stress (0.11 N/m2) resulted in the greatest amount of material associated with the pipe-wall is released into the bulk water as evidenced by the highest turbidity, Mn and DAPI cell counts of the three loops (Fig. 1). The increase in DAPI count cell numbers indicates that biological material is released into the bulk water during a discolouration event.

2: Neither the diverse bacterial community structure nor 16S rRNA gene copy numbers on the pipe-wall of the three loops varied significantly between loops or after the flushing event.

In conclusion, pipe-wall material was mobilisied into the bulk water during the experimental discolouration event, the amount mobilised was influenced by the daily conditioning shear. Neither the conditioning shear stress or the incremental shear stress applied during the flushing altered the pipe-wall bacterial community structure.

References: Deines *et al.*, 2010 Appl. Microbiol.Biotechnol. 87: 749 -746; Smith *et al.,* 2005 FEMS Microbiol. Ecol. 54: 375-380; Smith *et al.,* 2006 Environ. Microbiol. 8: 804 -815.