Biofilms in Drinking Water Distribution Systems

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Background

Microorganisms live within drinking water distribution systems (DWDS)[1] as planktonic cells or attached to the inner pipe surfaces as biofilms[2]. Biofilms account for ~95% of the microbial load[2] and have been found to have a different community composition to the planktonic cells in the bulk water[3].

A biofilm comprises cells embedded in a matrix (Fig.1) of extra cellular polymeric substances (EPS), which provides mechanical stability and 3D structure to the biofilm[4]. If the adhesive forces of the EPS matrix are overcome, for example following changes in shear stress at the pipe wall, biofilm will be mobilised into the water column[5]. The detached assemblages either reattach to a biofilm downstream or increase the microbial load within the water column causing degradation of water quality aesthetics (particularly discolouration), increasing microbiological quality failures and presenting a potential health risk[1,5,6].

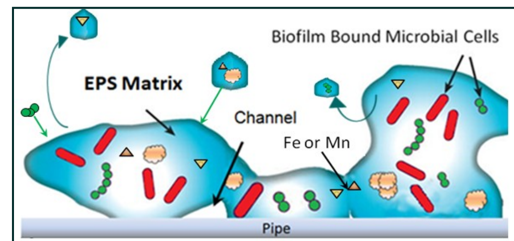

Fig. 1: Basic Biofilm Structure. Arrows show attachment, detachment or re-attachment

The microbial ecology of DWDS has previously been considered using laboratory bench-top experimental systems which do not accurately represent pipeline environmental pressures or the biofilm communities occurring within DWDS[7,8,9]. Additionally, despite EPS playing an integral role in biofilm stability [4] there is a paucity of data regarding environmental impacts upon its synthesis, composition and the stability that differing EPS matrices convey.

Drinking Water Distribution System Simulation Facility

(Bridges the gap between the dynamics of a live system and laboratory control of influential factors)

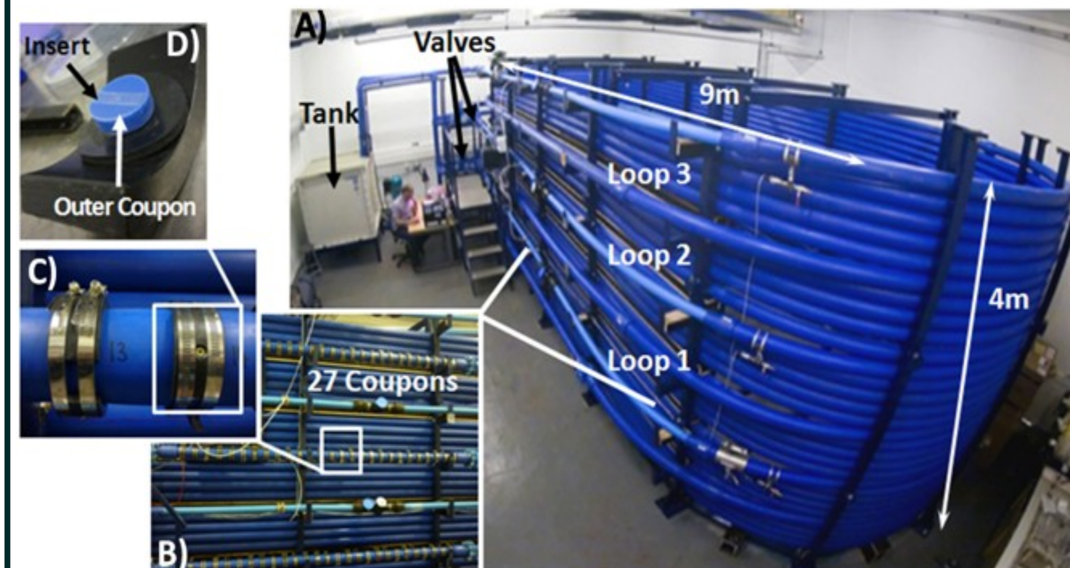

Fig. 2: DWDS Simulation Facility Overview A) Main pipe rig. Medium density polyethylene (MDPE) pipe separated into 3x200m loops by valves, diameter 79.3mm. B) Sections of MDPE 50mm diameter pipe with holes for coupon insertion, 27 per loop C) Coupon secured within pipe D) Removable coupon, fits flush to curvature of inner pipe wall.

Aim

To determine the effects of different environmental conditions upon biofilms developed within a full scale DWDS simulation pipe-rig facility (Fig. 2) and to assess their influence on drinking water quality. In particular focus on:

- Characterising biofilm community composition
- Characterising biofilm architecture (EPS quantity/ composition, thickness, cell abundance)
- Determining the stability of the established biofilms when exposed to increasing shear stresses
- Determining differences in structure and function between planktonic cells and biofilm

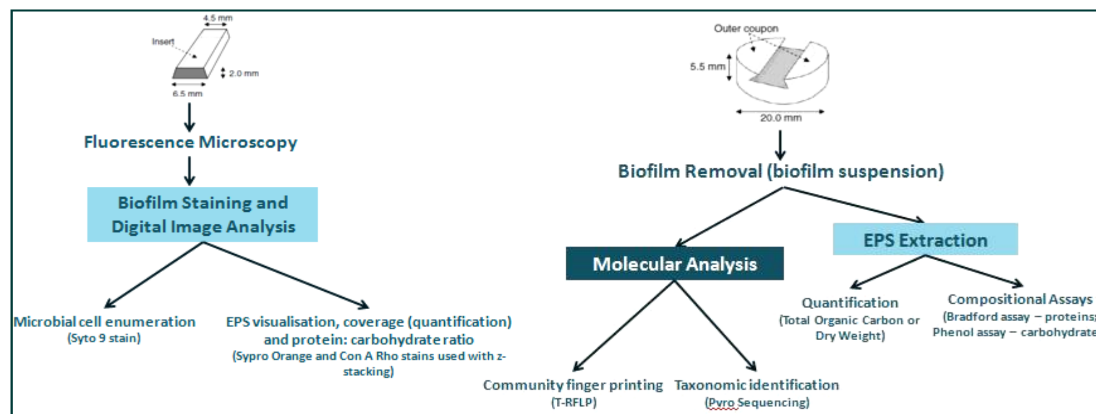

Fig. 3: Biofilm Sample Analysis. Insert designed for microscope analysis, coupon for molecular analysis

Progress To Date

Biofilm samples developed on coupons (Fig. 2) under steady state flows (0.2 l s^{-1}, 0.4 l s^{-1}, 0.8 l s^{-1}) were used to develop and optimise EPS analysis techniques— extraction and fluorescence microscopy (Fig. 3). Simultaneously, molecular fingerprinting (T-RFLP) and pyrosequencing were used to characterize the microbial communities (Fig. 3).

- **EPS extraction**, quantification and compositional assay protocols developed and optimised but technique not sensitive enough to detect the volumes of biofilm present (data not shown).
- **Staining and Digital Image Analysis (DIA)**
  - Suitable fluorophores for cells, protein and carbohydrate fractions of EPS identified. Confocal laser scanning microscope programs currently being optimally configured and triple staining technique being developed.
  - Preliminary samples imaged (see Fig. 4) and DIA used to determine the coverage of EPS, protein: carbohydrate, cell numbers and determine the biofilm thickness. (DIA optimisation is currently ongoing).
- **Molecular Analysis**
  - T-RFLPs allowed for the characterization of the dominant bacterial groups (most abundant) within biofilms and planktonic samples under different conditions .
  - Pyrosequencing analysis generated a more accurate description of the bacterial communities and is a powerful technique to detect changes at species level in response to environmental change (Fig. 5).

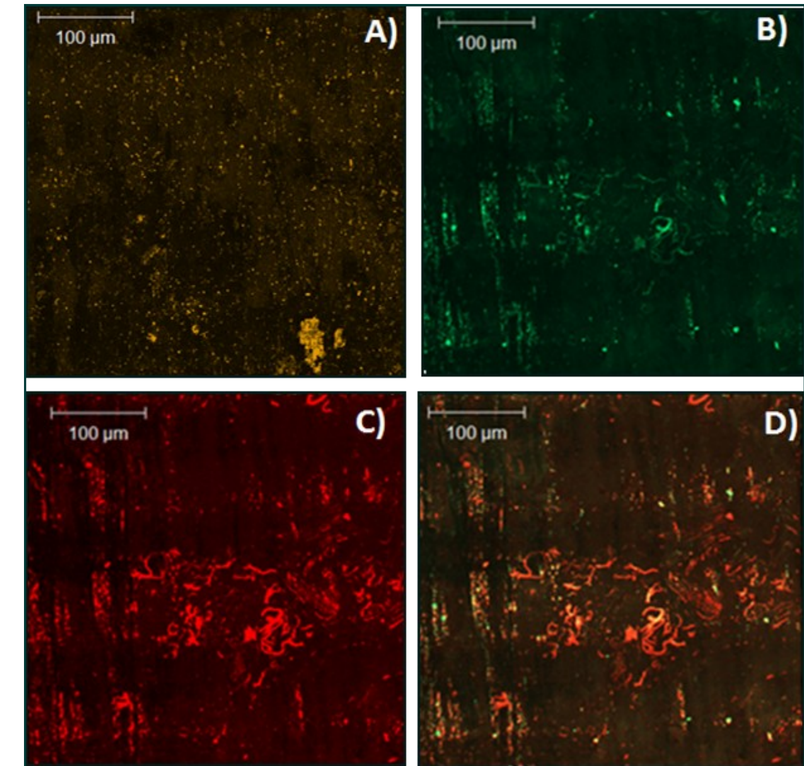

Fig. 4: Biofilm Fluorescent Staining. A) Sypro Orange staining of proteins B) Syto 9 staining of DNA C) Con A Rho staining of carbohydrate D) Cell and carbohydrate images overlaid. Scale bars as indicated, biofilms developed under steady state flow rates at 16°C for 14 days.

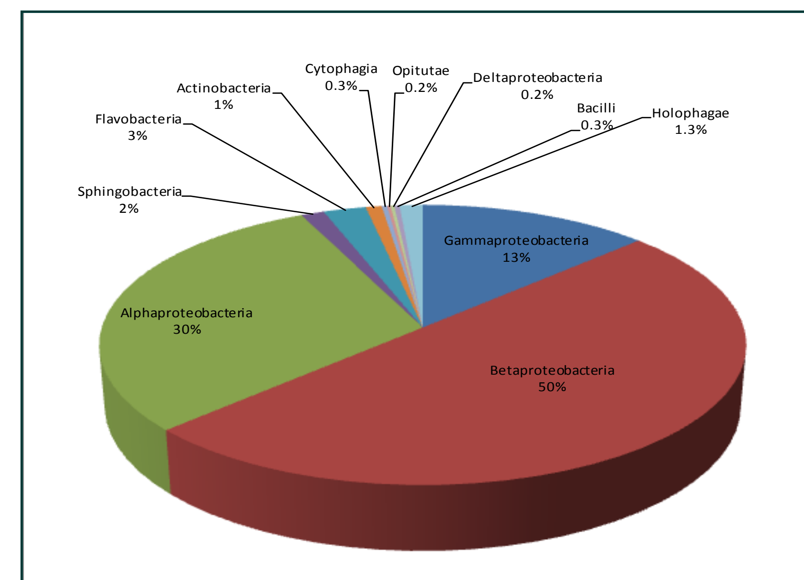

Fig. 5: Biofilm Community Analysis. Percentage of bacterial groups within biofilms developed on the inner pipe surface after 28 days of growing under steady state flow rates at 8°C.

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