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# Spatial and temporal analogies in microbial communities in natural drinking water biofilms

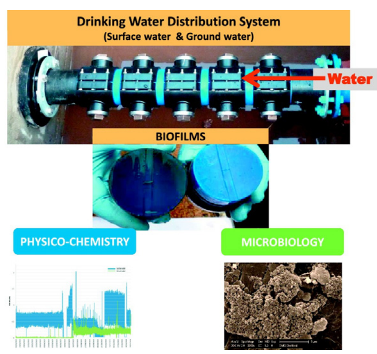
I. Douterelo [a,*], M. Jackson [b], C. Solomon [b], J. Boxall [a]

[a] Pennine Water Group, Department of Civil and Structural Engineering, Mappin Street, University of Sheffield, Sheffield S1 3JD, UK
[b] Wessex Water, Claverton Down Rd, Bath, Somerset BA2 7WW, UK

## HIGHLIGHTS

- Main abiotic factors affecting microbial distribution were flow, temperature and pH.
- Internal factors were central in shaping biofilm formation and composition.
- Ubiquitous core bacterial community observed in all biofilm samples.
- The less abundant bacteria were responsible for most of the variability.
- This information is essential for the management of drinking water systems.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Biofilms are ubiquitous throughout drinking water distribution systems (DWDS), playing central roles in system performance and delivery of safe clean drinking water. However, little is known about how the interaction of abiotic and biotic factors influence the microbial communities of these biofilms in real systems. Results are presented here from a one-year study using *in situ* sampling devices installed in two operational systems supplied with different source waters. Independently of the characteristics of the incoming water and marked differences in hydraulic conditions between sites and over time, a core bacterial community was observed in all samples suggesting that internal factors (autogenic) are central in shaping biofilm formation and composition. From this it is apparent that future research and management strategies need to consider the specific microorganisms found to be able to colonise pipe surfaces and form biofilms, such that it might be possible to exclude these and hence protect the supply of safe clean drinking water.



## 1. Introduction

Drinking Water Distribution Systems (DWDS) are complex ecosystems where biotic and abiotic factors interact along an amalgamate of pipes, storage tanks and other infrastructure extended through vast areas in a buried environment. Many of the interactions are microbially mediated and microorganisms play a central role in determining the quality of the drinking water arriving at customers' taps. Most of the microorganisms living in DWDS are attached to pipe surfaces forming mixed-species biofilms. Biofilms can be considered as microbial factories in constant operation where specific processes can take place such

* Corresponding author.
*E-mail address:* i.douterelo@sheffield.ac.uk (I. Douterelo).

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as pipe corrosion, residual disinfectant decay or trapping/accumulation of inorganics. Determining the potential for biofilm growth and their composition and structure in DWDS is essential, since biofilm affect the performance of these systems and ultimately the delivery of safe clean drinking water. Understanding the effect of environmental change on biofilm composition and structure in DWDS is challenging mainly due to the difficulty of accessing these buried ecosystems. Commonly observed effects of external factors on biofilms in DWDS are changes in microbial composition and structure (Douterelo et al., 2014), in the components of the extracellular polymeric matrix (EPS) (Fish et al., 2015), changes in density and in chemical and electrical properties (Mukherjee et al., 2012; Janjaroen et al., 2013) and in cell-cell interaction (quorum sensing) (Lee et al., 2014). However, to what extent the combination of biotic and abiotic factors, under realistic conditions, affects the development and composition of natural biofilms in DWDS remains unknown.

We have only a limited vision of the microbial ecology of DWDS since most studies have generally focused on free-living organisms from tap samples (*e.g.* Holinger et al., 2014; Donohue et al., 2015) or water treatment plants (Kasuga et al., 2010; Liu et al., 2013). Studies that have explored biofilms have tended to be in artificial systems in isolation without establishing associations with realistic environmental parameters or between free-living organisms and the attached communities co-habiting the same system (Giao et al., 2008; Moritz et al., 2010). Studies in different environments have shown that natural biofilms respond to their environment and adapt to changes by means of a diverse range of mechanisms (Stewart and Franklin, 2008). Limited information exists regarding whether there are common microbial patterns in biofilms dynamics over time and across different locations. To have a better insight into microbial assembly of natural biofilms and ecological factors influencing their development in DWDS we have used a short-term approach (re-growth every three months) and a long term approach (succession over a one-year period). This will provide a comprehensive vision of how biofilm develop in real systems, allowing for exploration of patterns of behaviour such as seasonal shifts in the structure and composition of biofilms in DWDS.

Previous research suggests that the microbial ecology of DWDS will be affected by source water characteristics (Gomez-Alvarez et al., 2015) the type of treatment (Hwang et al., 2012; Pinto et al., 2012) and hydraulic conditions in the system (Douterelo et al., 2013). However, what remains unknown is to which extend external variation will affect attachment to the pipes of certain microorganisms and biofilm formation, composition and dynamics over time. The objectives of the research reported here are to establish the effect of external factors, including different source waters, on the microbial ecology within operational DWDS and to examine patterns of biofilm formation and growth that can inform efficient management of these systems.

## 2. Materials and methods

### 2.1. Biofilm sampling devices and sampling sites

*In situ* biofilm sampling devices (Fig. 1) made of High Density Polyethylene (HDPE) were used to study two DWDS with different source waters (physico-chemical characteristics shown in Table 1) and hydraulic regimes (Fig. 2). Each sampling device was fitted into a real system in an available space of 150 mm at both sites and contained 10 modified (increased diameter to improve representative sampling) Pennine Water Group (PWG) coupons (Deines et al., 2010) that enable the study of naturally occurring biofilms *in situ* without the need for cutting, scraping or flushing the pipes. In addition, the use of PWG coupons allows for studying biofilms on pipe surfaces without distorting boundary layer hydraulic conditions including shear stress and turbulence driven processes such as nutrient exchange. Using these coupons two different processes were studied: 1) quarterly biofilm re-growth and 2) biofilm succession over a one-year period. The sampling devices were first installed in February 2013, with first assessment of 3 month-old biofilm development used to test a range of different techniques to evaluate best biofilm monitoring practices (Douterelo et al., 2016). From May 2013 and every three months thereafter the same three coupons were replaced with sterile coupons in order to study biofilm re-growth dynamics at different seasons starting from a completely

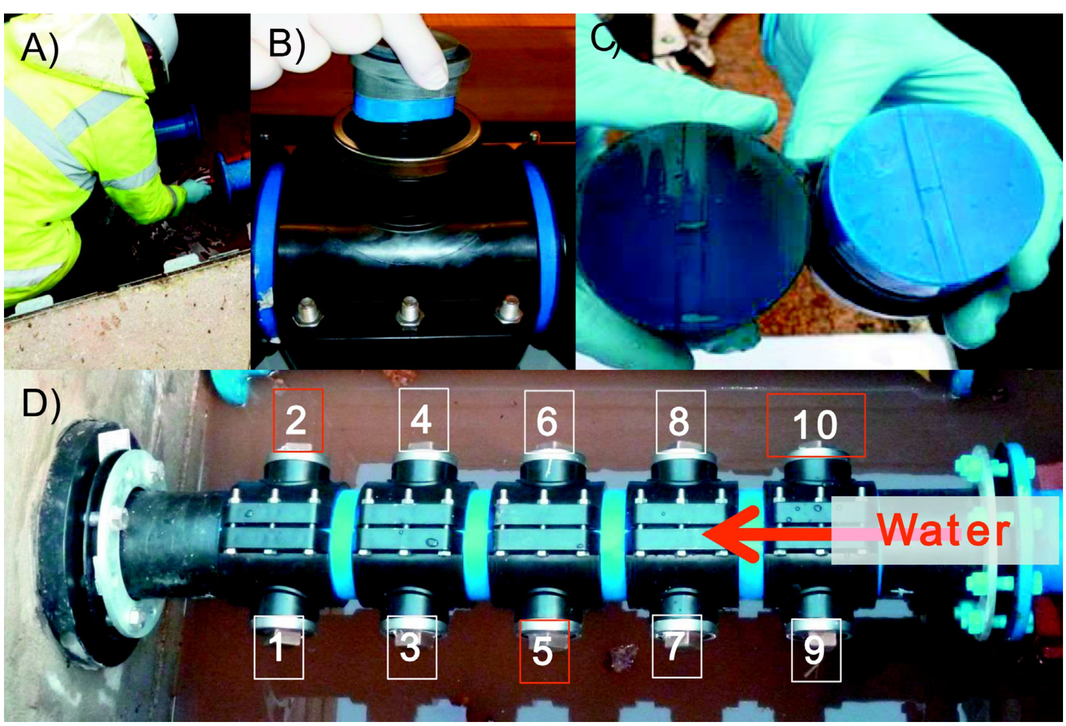


**Fig. 1.** A) Replacement of a section of pipe for installation of coupon devices. B) Insertion of a coupon in one of the holders of the biofilm sampling device. C) Coupons after sampling showing areas used for biofilm removal. D) Picture showing the distribution of the coupons in the device, coupons in red (2, 5 and 10) were used for re-growth studies, the other coupons were used for long term studies to study succession. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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