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FOCUS ARTICLE

Understanding microbial ecology to improve management of drinking water distribution systems

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Microorganisms in drinking water distribution systems (DWDS), and in particular the microbial communities that form biofilms on infrastructure surfaces, drive critical processes impacting water quality. This paper reviews knowledge, research approaches, and monitoring methods to consolidate understanding of the microbial ecology of DWDS. The review highlights how microbial characteristics and subsequent behavior can be broadly classified as common or complex. Common behavior relates to the ubiquitous and continual development of biofilms, consistent core communities, and mediated material accumulation. In contrast, the complex aspect relates to the shape, structure, and composition of the microbiome, defined by site-specific properties such as supplied source water, pipe material, and hydraulic regimes. It is shown how the latest microbial tools and techniques can be applied to increase our understanding of DWDS ecology and how water utilities are starting to use this knowledge. This is not because of regulatory requirements, but in recognition that they provide valuable information facilitating proactive management and operation benefits to these critical yet aging systems, protecting water quality and public health in the process.

This article is categorized under:
Engineering Water > Sustainable Engineering of Water
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KEYWORDS
biofilms, drinking water distribution systems, management, public health

1 | MICROORGANISMS IN DRINKING WATER DISTRIBUTION SYSTEMS

Drinking water distribution systems (DWDS) are challenging environments for microbial growth with highly variable and uncertain physicochemical interactions across diverse pipe materials and dynamic hydraulic and disinfection regimes. Microorganisms can access the distribution system via treatment works, cross-connections, or contamination ingress (Besner, Prévost, & Regli, 2011) and exist as taxonomically diverse communities including bacteria, archaea, viruses, and protozoa (Douterelo et al., 2014). Regrowth and direct risks associated with the presence of pathogenic microorganisms in the bulk-water are often managed by the addition of a disinfectant residual. However, in addition to existing in a planktonic state (i.e., in the bulk-water), microorganisms can adhere to the pipe surfaces and form disinfectant-resistant biofilms (Szewzyk, Szewzyk, Manz, & Schleifer, 2000; Wang, Masters, Edwards, Falkinham, & Pruden, 2014). Indeed, it is known that the dominant organic component within DWDS is in the attached biofilm phase (Flemming, Percival, & Walker, 2002), where microorganisms coexist and interact (Stoodley, Sauer, Davies, & Costerton, 2002) impacting water quality.

Biofilms consist of cells attached to a surface and/or each other via an extracellular polymeric matrix comprising polysaccharides, proteins, lipids, extracellular DNA, and other associated particles (Hall-Stoodley, Costerton, & Stoodley, 2004; Lopes, Morin, Oliveira, & Melo, 2009; Neu & Lawrence, 2010). The extracellular polymeric substances (EPS) provides physical and chemical stability to biofilms, while offering protection against the direct action of external factors (Flemming & Wingender, 2010). In a DWDS context this includes resistance to the action of disinfectants and hydraulically induced shear forces (Cowle, Babatunde, Rauen, Bockelmann-Evans, & Barton, 2014). Additionally, within biofilms microorganisms act as a consortium, contributing via different metabolic capabilities to the acquisition and cycling of nutrients (Davey & O'Toole, 2000; Flemming et al., 2016). As a result of these survival advantages over the bulk-water environment, most viable microorganisms in DWDS are found within biofilms (Batté et al., 2003; Flemming et al., 2002). Biofilm formation on pipe surfaces can be rapid, even in networks which employ a disinfection residual. Morvay et al. (2011) reported that biofilm formation on different plumbing materials in chlorinated drinking water systems reached values of 10^7 cells/cm^2 after only 30 days. Studying the initial stages of biofilm formation in a high-density polyethylene-chlorinated DWDS, Douterelo, Boxall, et al. (2014) reported that the amount of biofilm cells can triple after 2 weeks of development under steady-state conditions. Importantly, the physical structure and chemical charges on cells and the EPS facilitates the adsorption and entrainment of material from the bulk water and can host opportunistic pathogens (Wingender & Flemming, 2011). This storing of material has the potential to compromise supplied water safety if subsequently mobilized and entrained into the bulk-water. This has been demonstrated internationally when the shear stress at the pipe wall (in response to increased flow) exceeds normal daily values, material (including biofilm) has been eroded and mobilized (Boxall & Saul, 2005; Ginige, Wylie, & Plumb, 2011; Husband & Boxall, 2010b). The result is not only aesthetically unacceptable discolored drinking water (Husband, Boxall, & Saul, 2008), but the release of previously adhered microorganisms (Douterelo, Sharpe, & Boxall, 2014; Lehtola et al., 2006). Hence physical, chemical, and microbiological water quality parameters are compromised.

After leaving treatment plants water quality will deteriorate and a greater contact time with the DWDS infrastructure is likely to accelerate this degradation. Distributed water reaches consumers after hours or days with the water age dependent on the system hydraulic retention time, a function of supply demands and network distances (Machell & Boxall, 2012). Factors influencing degradation include pipe material, local hydraulics, decay of disinfectant residual, changes in water temperature, and microbial regrowth (Machell & Boxall, 2012; Machell, Boxall, Saul, & Bramley, 2009). Pipe characteristics (e.g., material, roughness, diameter, etc.) also influence biofilm composition, morphology and pipe wall attachment processes (Douterelo, Husband, Loza, & Boxall, 2016; Keevil, 2003; Niquette, Servais, & Savoir, 2000). Hydraulic regimes influence the amount of biofilm and strength of attachment to pipes (Sharpe, Biggs, & Boxall, 2017) and the microbial diversity of these ecosystems (Douterelo, Sharpe, & Boxall, 2013; Fish, Osborn, & Boxall, 2017b) while temperature can affect biofilm growth rates (Donlan, 2002; van der Wielen & van der Kooij, 2010). It is also acknowledged that disinfectant residuals do not prevent microbial colonization and that organisms in DWDS can develop resistance (Berry, Xi, & Raskin, 2006; Codony, Morato, & Mas, 2005), potentially posing a greater risk to water quality.

Many of the adaptive traits that allow microorganisms to thrive and successfully respond to environmental changes in DWDS are encoded at a genetic level. Indeed, recently acquired molecular knowledge has yielded not only a better understanding of the diversity of microbial communities in relation to environmental factors in DWDS, but also highlights functions and associations with key processes (e.g., corrosion, disinfection by-products, discolouration, resistance to disinfection, and antibiotics; Fish, Osborn, & Boxall, 2017a; Montoya-Pachongo et al., 2017; Zhou, Zhang, Zhang, Li, & Mao, 2017; Zhang et al., 2018). The majority of this knowledge, however, has yet to be integrated or developed for the benefit of end-users (regulators, engineers, water companies, and consumers). Therefore, this paper reviews the latest research approaches and monitoring methods to consolidate understanding of microbial ecology in DWDS and how this can inform management practices, with reference to UK case studies.

2 | INDUSTRY TECHNIQUES AND APPROACHES TO MONITOR MICROORGANISMS AND DETECT FAILURES

Water utilities use standard methods, embedded in legislation, to monitor the microbial quality and hence safety of water within DWDS. The methods commonly used are based on quantification of planktonic microorganisms via heterotrophic plate counts and fecal indicators (e.g., *Escherichia coli* and coliforms). These methods have not fundamentally changed since cholera outbreaks were first associated with drinking water and DWDS infrastructure was first being built, often associated with John Snow's investigations in 1854 as stated in Szewzyk et al. (2000). Despite the assumption at the time that cholera was an airborne transmitted disease, John Snow identified a particular water pump in London as the source of an outbreak of cholera (Snow, 1855). Culture-dependent methods are convenient diagnostic tools used by water companies given that they are simple to perform and relatively low cost. However, culturing methods are time intensive with companies waiting days for results

and the approach commonly accounts for less than 1% of the total diversity in environmental samples (Riesenfeld, Schloss, & Handelsman, 2004). In addition, traditional bacterial indicators are failing to capture potential issues and consequently outbreaks of, for instance *Cryptosporidium* and *Giardia* (protozoa), which have been frequently reported in European countries (Putignani & Menichella, 2010). Similarly, emergent waterborne pathogens, which are not detected by traditional methods, are increasingly being observed in DWDS due to deterioration in quality of source waters and in changing environmental conditions (Levy, Woster, Goldstein, & Carlton, 2016). This is aggravated by the short duration and sporadic nature of many microbial water quality events within DWDS. To address these issues a revolution in speed, applicability, and information resulting from monitoring techniques is required. Such techniques should, ideally, not be restricted by the need to collect discrete samples.

One of the available techniques that can provide rapid and direct detection of microorganisms that is attracting interest among water utilities is flow cytometry (FC). FC is a fast and reliable method that by the addition of dyes that bind to cellular DNA and then passing the sample through narrow channels detects fluorescence to quantify individual bacterial cells and viability, providing a more comprehensive quantification of the total number of cells in water samples than traditional plate counts (De Roy, Clement, Thas, Wang, & Boon, 2012; Wang, Hammes, De Roy, Verstraete, & Boon, 2010). FC has been established as a reference method in Switzerland by the Swiss Federal Institute of Aquatic Science and Technology (Van Nevel et al., 2017) and has the potential of detecting specific microorganisms such as *Cryptosporidium* and *Giardia* (Efstratiou, Ongerth, & Karanis, 2017). FC is also emerging as a potentially useful tool for biofilm cell quantification, although its application to adhered cell clusters may be susceptible to errors, a challenge which needs to be overcome with care to develop a universal protocol and facilitate comparison between studies (Kerstens et al., 2015; Waller, Packman, & Hausner, 2018).

Two other methods in drinking water research to estimate biomass and bacterial growth are the quantification of adenosine triphosphate (ATP) and of assimilable organic carbon (AOC). ATP is a molecule that serves as the currency of energy in cells and the presence of intracellular ATP is used as an indication of the quantity of viable microorganisms in a sample (Nescerecka, Juhna, & Hammes, 2016). ATP analysis however has potential drawbacks due to interference caused from extracellular ATP, most likely released from damaged cells (Hammes, Goldschmidt, Vital, Wang, & Egli, 2010). ATP has proved useful to help determining microbial biomass in drinking water filters (Lizanne, Van Dyke, Anderson, & Huck, 2014), detecting microbial ingress from sewage systems and source water in DWDS (Vang, Corfitzen, Smith, & Albrechtsen, 2014) and monitoring biofouling in plumbing materials (Ginige, Garbin, Wylie, & Krishna, 2017). AOC refers to the fraction of biodegradable organic matter that can be converted to biomass or mineralized by bacteria in a given period of time (Polanska, Huysman, & van Keer, 2005). AOC has been used as a complementary method to study bacteria regrowth in drinking water systems (Liu et al., 2002), determining the effectiveness of disinfection regimes on pipeline materials (Lehtola et al., 2005) and monitoring bacterial dynamics in DWDS (Prest, Weissbrodt, Hammes, van Loosdrecht, & Vrouwenvelder, 2016). ATP and AOC quantification methods can be used in combination with other techniques, such as FC, to enable better insights into the response of microorganisms to specific treatments or conditions (Vital et al., 2012; Lautenschlager et al., 2013) or to determine the biological stability of DWDS (Lautenschlager et al., 2013). Latest generation prototype FC and ATP instrumentation are suitable for online operation and they can provide a time series of data at localized sites (Hammes et al., 2012). Application of these (and future) automated approaches could transform our knowledge and understanding of the spatial and temporal variability of microbial communities throughout DWDS, while simultaneously increasing the speed at which failures and pathogens outbreaks are detected.

Molecular methods are based on the information contained in nucleic acids (DNA/RNA) and are useful tools to detect and monitor microorganisms in aquatic environments (Clark et al., 2018), including DWDS. Molecular methods require the extraction of nucleic acids, followed by the amplification of a target gene or genes via polymerase chain reaction (PCR) and post-PCR analysis. Among all the molecular methods, quantitative PCR (qPCR) is highly valuable at quantifying and detecting pathogens (Aw & Rose, 2012), and as such has been used to detect *Cryptosporidium* (Liang & Keeley, 2012) and adenoviruses in drinking water (Albinana-Gimenez et al., 2009). Another molecular tool with great potential to serve as a monitoring and diagnostic tools is next generation sequencing (NGS). Briefly, sequencing involves determining the order of nucleotides within a DNA molecule, from marker genes to establish taxonomic assignments through to entire genomes of an environmental sample. NGS is becoming more accessible (in part due to reduced costs) and is capable of enhancing the understanding of microbial ecology and functionality within many habitats, including DWDS. NGS platforms have improved the depth of sequencing as they can produce thousands of short reads in a single run, this allows the detection of whole communities including less-abundant members (Metzker, 2010). NGS methods were limited in their ability to sequence complete marker genes. Nowadays, it is possible to, for example, sequence the entire 16S rRNA gene, offering the possibility of a more precise taxonomic identification. To quantify specific pathogenic species and their associated risk, it is recommended to use more sensitive techniques such as qPCR. However, such techniques when using DNA do not differentiate between viability and presence, so care is needed in interpreting results and assessing risks and should include consideration of the years over which attached materials accumulate and develop within DWDS. To determine the viability of cells, there are different methods

available that can be explored in the context of DWDS monitoring (Emerson et al., 2017). Diversity estimates and improved understanding on how the environment within DWDS influences microbial behavior and on how microorganisms might affect water quality can also be usefully investigated (Douterelo, Husband, & Boxall, 2014; Tan et al., 2015). By using more accurate and reliable indicators than traditional methods, NGS will allow the development of new monitoring tools and diagnostics of failures in DWDS.

While culture-dependent methods remain the regulatory required technique for use by water utilities, molecular methods are starting to be used by some water companies to generate a deeper understanding of performance within their networks and hence better manage them to protect public health. The initial emphasis is on using FC to enumerate planktonic cells and applying PCR-based approaches to detect pathogens. The constant improvement in sequencing methods, increasing quantity of sequences obtained and read length, while reducing costs and complexity, is also likely to see their growing uptake. These techniques that can facilitate direct monitoring of pathogens and indicators in DWDS (i.e., system failures), still, however, require refinement in order to achieve standardization, thus allowing comparison between systems, supporting wider application.

3 | LIMITATIONS AND ACTUAL CHALLENGES OF STUDYING AND MONITORING DWDS

Water utilities invest time and money trying to control microbial deterioration during distribution. The lack of realistic and applicable knowledge regarding the microbial ecology and in particular the role of biofilms in water quality deterioration, makes existing management strategies unsustainable. This lack of detailed understanding is associated with several limitations and challenges regarding the study and monitoring of DWDS.

3.1 | Lack of reliable sampling and monitoring systems during distribution

Tap water is generally of the highest quality as confirmed by discrete regulatory-driven water sampling in most developed countries. However short duration, spatially disparate events are known to occur in DWDS as evidenced by recorded water quality failures and customer complaints. In order to more fully understand these events, sampling is required that offers both greater spatial and temporal coverage, and/or captures longer-term accumulation processes at the pipe wall. Samples obtained from DWDS, for either regulatory or research purposes, are generally from the bulk water (Wang et al., 2014; Li et al., 2018; Mao et al., 2018; Potgieter et al., 2018). Given that biofilms represent more than 95% of the biomass in DWDS (Flemming et al., 2002), monitoring based on bulk water alone is therefore a clear limitation. Changes occurring within DWDS are influenced and often dominated by the interface between the pipe surfaces and the bulk water (Sekar et al., 2012), consequently by not analyzing biofilms, potential microbial and other risks are not being monitored.

3.2 | Limited knowledge based on real DWDS and applicability of research studies to real systems

The pipe assets of operational DWDS are costly and difficult to access as they are often buried. The further difficulty of accessing the internal surfaces within operational networks makes the study of biofilms challenging and so the majority of microbial diversity studies in DWDS are restricted to bulk water samples (Pinto et al., 2012). While these provide an excellent resource for the background transmission of microorganisms, particularly bacteria as the most commonly studied organisms, these studies do not convey information regarding biofilms. Research studies on the microbial ecology of DWDS have also often been obtained from experiments performed using artificial, nonrealistic systems, using small-scale laboratory reactors and/or assessing a few selected microorganisms under controlled conditions which do not represent the dynamics of real networks (Douterelo, Boxall, et al., 2014). As a consequence, there is a limited vision of the real biofilm microbial ecology. Evidence from studies under representative conditions are required to determine how the environment and microorganisms interact and the impact on water quality, and conversely how the environmental conditions impact the microbial community.

Another gap in understanding DWDS microbiology is the lack of information required to link microbial diversity (who is there?) and function (what are they doing?) (Clark et al., 2018). In addition, most routine microbial techniques used to study microorganisms are not optimized for application to DWDS. Techniques therefore need to be optimized and adapted to yield valid and informative data. The techniques used in scientific research are often time and resource intensive, and despite their value, such as to inform modeling and microbial risk assessments, real-world application and use by water utilities is currently restricted due to technical complexities.

4 | USEFUL APPROACHES TO EXPLORE AND UNDERSTAND THE MICROBIAL ECOLOGY AND RELATED INTERACTIONS WITH INFRASTRUCTURE AND WATER QUALITY IN DWDS

To overcome monitoring limitations and challenges, innovative methods and approaches have been used by research groups, such as at the University of Sheffield, to study operational and realistic laboratory simulated DWDS (see Figure 1 for an example). These include:

1. Temperature-controlled full-scale laboratory test facility incorporating realistic flow and pressure profiles;
2. Biofilm sampling devices extensively developed and trialed under realistic conditions and installed in situ in UK operational drinking water networks; and
3. Application of extensively monitored managed flushing trials to study the mobilization characteristics and composition of material attached to pipe surfaces.

Full-scale DWDS experimental pipe systems (Figure 1) have transformed drinking water research by recreating realistic hydraulic, microbial, and physicochemical conditions while retaining laboratory-level control of environmental parameters and sampling regimes. Many reactors and devices to study biofilms cause significant distortion of the boundary layer and hence are not representative of pipe wall boundary layer forces and the turbulent regime that drives nutrient and material exchange to and from the boundary zone (Gomes, Simoes, & Simoes, 2014). The boundary layer is a small zone at the interface between a fixed surface (boundary) and the moving fluid. In this zone, the hydraulic conditions are different to those of the main bulk water flow—conditions here are laminar even when the main flow is fully rough turbulent. In this context, changes to the fixed surface (physical shape, roughness), relative size (surface to volume), and the forces driving the flow (i.e., pressure vs. rotational force) will directly impact on the conditions of shear stress and nutrient exchange that the biofilms experience. Specifically designed to address this issue, Pennine Water Group coupons (PWG, Figure 1b and c) were developed to prevent disruption of the critical boundary layer hydraulics (Deines et al., 2010), while also providing a removable surface to analyze biofilms. The triple loop design of the facility allows multiple investigations of operational variables, as has been reported in several original research articles including insights into the initial stages of biofilm formation and material

FIGURE 1 (a) Real-scale DWDS laboratory facility at Sheffield University, (b) PWG coupons are inserted along the pipe, (c) PWG coupons showing insert and outer part of the coupon. (d) Biofilm sampling devices used to sampling biofilm in situ during field work. (e) Flushing equipment used in real DWDS

accumulation (Douterelo, Boxall, et al., 2014) and the impact of temperature and hydraulics on biofilms and discoloration (Fish et al., 2017a; Sharpe et al., 2017). One of the key findings regarding the influence of hydraulic regimes on biofilm formation is that where there is the greatest variation in flow demands, the least amount of mobilizable material was observed (Douterelo et al., 2013). Operationally this means that a low and relatively stable flow is generally optimal for pump and flow management, and potentially leakage control; under these conditions the amount of material (cells and EPS) and hence water quality risk through discoloration or elevated chemical/biological determinants is increased (Fish et al., 2015; Sharpe et al., 2017). These results demonstrate that appropriately scaled facilities such as these are integral to conduct biofilm research which can then be delivered to full field conditions. For instance, extensive international trials have led to the validation of the prediction of discoloration in distribution systems (PODDS) model that was initially investigated in laboratory trials (Husband et al., 2008). This concept and accompanying model are now used as a standard management tool to control discoloration and has led to multi-million pound benefits by reducing cleaning and maintenance costs in UK networks (Husband & Boxall, 2010a, 2011; Husband, Fish, Douterelo, & Boxall, 2016; Machell & Boxall, 2012).

By not disrupting the boundary hydraulic conditions, as shown in Figure 1d, PWG coupons allow in situ monitoring of biofilm development. This facilitates detailed analyses and understanding of the development of mixed species biofilms in DWDS supplied by different water sources (Douterelo, Jackson, Solomon, & Boxall, 2016, 2017). These studies have shown higher diversity and richness in surface water supplied biofilms. The exceptionally low diversity with few dominant genera in the groundwater biofilm samples was also linked to the low-flow hydraulic conditions in the studied section. This indicates that local environmental conditions influence biofilm formation, composition, and biomass. The in situ biofilm sampling also allowed the temporal study of biofilm succession and regrowth. Independent of the characteristics of the incoming water and marked differences in hydraulic conditions between sites and over time, a core microbial community was observed in all samples (Douterelo et al., 2017). This suggests that internal factors (microbial interactions) are central in shaping biofilm formation. With all studies indicating the endemic and persistent presence of biofilms, future research and ultimately management strategies could consider how to manage the microbial interactions within the biofilm community. This could favor positive characteristics with the potential to exclude undesirable or pathogenic members, such as using a pro-biotic approach and avoiding chemical treatments.

The application of planned flushing regimes in real operational networks to study the mobilization and composition of the material attached to pipe surfaces has yielded new knowledge on the causes of drinking water discoloration and on associated microbial risks. PODDS has been employed to predict discoloration and manage DWDS interventions eliminating the need for expensive and disruptive invasive cleaning strategies (Husband & Boxall, 2010a). The concept of cohesive material layers of different shear strengths attached to pipe surfaces is directly analogous to the observed properties of biofilms, that is, layers and clusters of varying composition and attachment strengths on the pipe wall (Figure 2). Thus it was proposed that the turbidity response simulated by PODDS modeling is based on describing the shear strength behavior of biofilms. With this understanding it becomes possible to use managed increases in flow and flushing (Figure 1e), to sample and hence study the role of microbial biofilms in the processes of discoloration, including the accumulation of particulates, such as iron, manganese, and lead. Using the concept of cohesive layers to inform flushing programs, adjacent polyethylene and cast iron pipe sections from within the same supply zone were subjected to flow increases above normal (Douterelo et al., 2017; Douterelo, Husband, & Boxall, 2014). Concurrent turbidity and sampling found that while a lower turbidity response was observed from the plastic pipes, there was greater bacterial diversity and richness. In the cast iron pipes a high relative abundance of bacteria that are able to utilize different iron and manganese compounds such as *Lysinibacillus* spp., *Geobacillus* spp. and *Magnetobacterium* spp were found. Although the mobilization behavior displayed a common, predictable response, this work demonstrates distinctly different and complex communities develop even when fed by the same water.

5 | OVERVIEW OF KEY MICROBIAL FINDINGS IN DWDS

The use of realistic yet controllable laboratory facilities, combined with planned and extensive trials in operational networks and the use of non hydraulically compromising coupons in both situations has facilitated detailed investigations into microbial community, composition, and structure in DWDS. The main findings can be summarized as:

1. Hydraulic regimes affect biofilm amount, composition, and physical structure.
2. The microbial composition of biofilms significantly differs from that present in bulk water.
3. Biofilms demonstrate adaptation to specific network features and respond to system changes. This can be adapted for network maintenance, for example, flushing to physically remove pipe wall biofilms.
4. Water quality failings can be associated with network changes (physical, chemical, and biological) that cause destabilization and subsequent mobilization of biofilms.

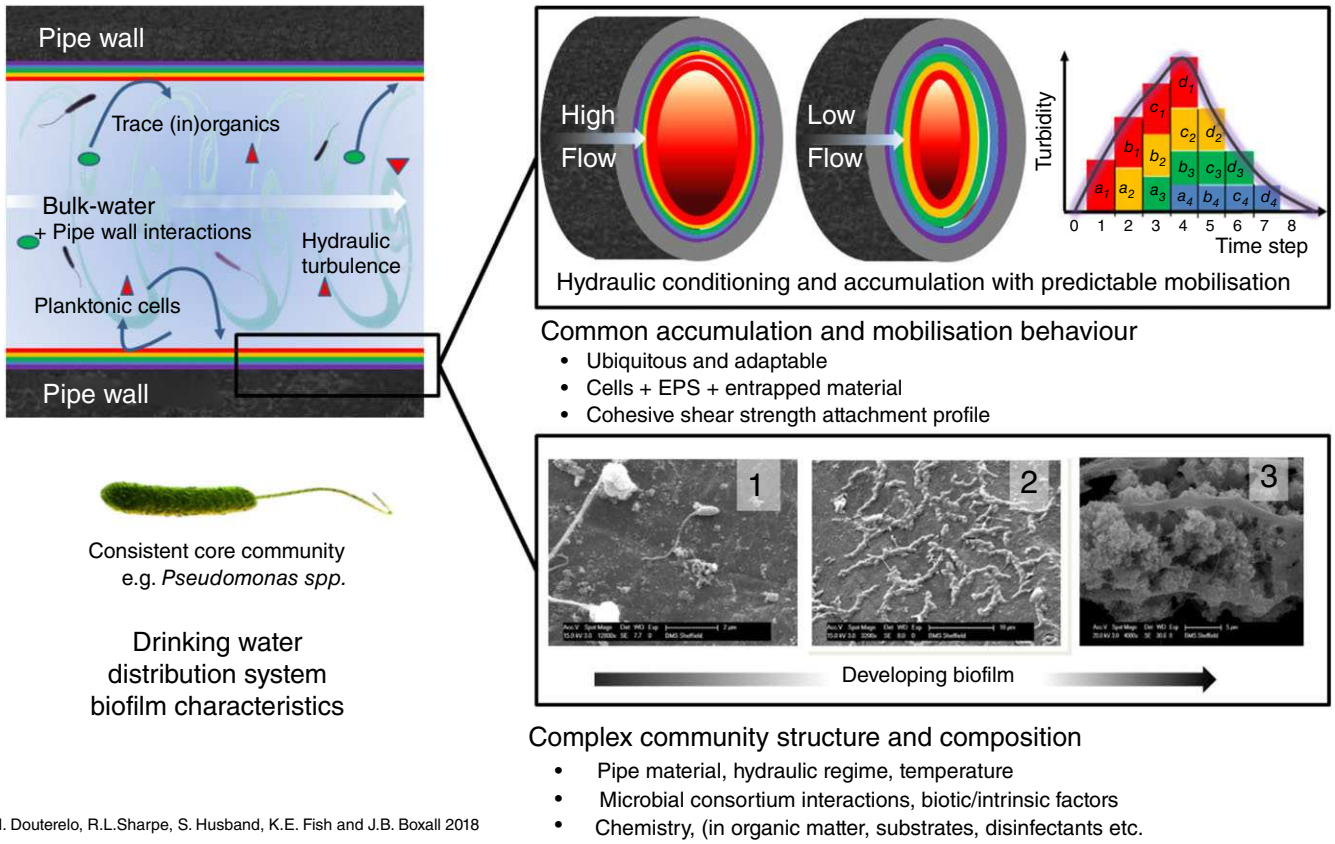

FIGURE 2 Schematic showing the concept of cohesive material layers of different shear strengths attached to pipe surfaces and its association to the observed properties of biofilms, that is, layers and clusters of varying composition and attachment strengths on the pipe wall

5. *Pseudomonas sp.* was the main colonizer identified in DWDS with increasing presence for lower hydraulic conditions.
6. Networks operated at lower and more steady (over daily periods) velocities can develop larger communities and potentially less stable, biofilm structures.
7. Pipe material influenced microbial composition, for example, increased presence of specialized microorganisms able to metabolize iron compounds in cast iron pipes.
8. Source water has a controlling effect on biofilm formation. This is most likely a result of substrate loading and supply.
9. Biofilms, and most likely EPS development, promote the entrainment of particulate material such as precipitated iron and manganese, key components of customer observed discoloration. Such understanding can help with the management and estimation of discoloration accumulation rates, key to scheduling interventions and managing the associated risks.
10. The EPS of biofilms provides a range of adhesive strength characteristics that are directly analogous to the cohesive layer strength concepts captured in empirical discoloration models, suggesting that understanding biofilms is key to managing discoloration.
11. Key physico-chemical factors affecting the microbial ecology of DWDS, correlated with turbidity levels in field experiments, particularly temperature and substrate loading. Hence managing loading at treatment works and scheduling interventions with respect to seasonal temperatures can be effective in management strategies.
12. Independent of the system analyzed, most of the differences in microbial diversity were due to less abundant species; however, core-communities were found in all the systems studied.
13. Fungi together with bacteria form part of the core communities, but often showed a lag during biofilm development and were more stable over time in comparison to bacteria.

All the results indicate that while systems vary and responses need tailored approaches to suit these, biofilms and associated common behaviors are ubiquitous. If we are therefore to manage these critical infrastructure assets and maintain the highest quality water, we need to embrace these complex microbial ecosystems. The elimination of biofilms in easily accessed well-controlled, consistent environments is problematic, hence the eradication of biofilms from within the old, complex, nonsterile environment of DWDS is effectively impossible. The challenge is to develop a comprehensive understanding such that instead of relying on chemical control, ongoing network management can focus on encouraging conditions that promote healthy microbial communities that resist pathogens and safeguard water quality.

It is important that research and innovation continues, such as the application of online systems to capture the short duration and sporadic nature of many water quality events. In the future, some of the techniques and approaches described here may become routine. However, water network operators now have the ability to go beyond regulatory requirements and utilize new tools and techniques to gain a deeper understanding. This will enable the implementation of management strategies and proactive interventions to further protect public health within constrained investment budgets such that resources can be best targeted to support the aging infrastructure asset base.

6 | CONCLUSIONS

This research highlights (see Figure 2) how microbial properties and subsequent network behavior can be broadly classified as common or complex;

- Common: ubiquitous and continual biofilm development with material accumulation including rate consistent with supplied water quality, predictable shear stress-associated mobilization, likely key community members that are associated with EPS production (e.g., *Pseudomonas* spp.)
- Complex: site-specific properties that define community and structure. This includes continual biofilm adaptation to varying environmental factors for example, temperature, disinfectant residual, hydraulic regimes, water chemistry etc.

Findings from purpose built laboratory facilities that have also been corroborated through extensive field trials demonstrate distinct bulk water and biofilm microbiomes present in water distribution systems. Although there is inevitable cross-over with the planktonic component, it is the traditionally more difficult to study complex biofilms that are critical to asset and water quality performance. With advances in genetic techniques allowing greater detail to be derived from studies, common behavioral traits facilitate innovative and viable techniques to investigate biofilms without direct access, such as managed hydraulic mobilization. This is only truly achievable with in situ techniques to capture the ever-changing conditions, such as bespoke coupon systems that maintain realistic shear and material transport profiles. This work highlights that the ability to study biofilms is both feasible and critical for strategy development to control and engineer these systems to provide the greatest operational benefits.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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