Environmental Science Advances

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Environ. Sci.: Adv.*, 2026, **5**, 1355

Examining the impact of small-amplitude hydraulic transients on biofilm development and adhesion in a full-scale, controlled water distribution environment

Mariele de Souza Parra Agostinho, *[a] Artur Sass Braga, [b] Benjamin Anderson, [b] Yves Filion[b] and Cristovão Vicente Scapulatempo Fernandes [c]

Biofilms play a crucial role in drinking water distribution systems (DWDS), as they influence microbial stability and water quality. While the effects of steady-state hydraulic conditions on biofilm formation and adhesion are well documented, the influence of small-amplitude hydraulic transients remains unexplored. This study examines the impact of small-amplitude transients on biofilm development and adhesion to the pipe wall, mobilization behavior during flushing, and microbial activity in the bulk water in a full-scale PVC distribution pipe laboratory. Over a 28 days biofilm conditioning period, biofilms were subjected to steady-state flow and periodic transient events, followed by controlled flushing to evaluate the detachment behavior. The key findings showed that a moderate transient pulse (20 kPa) enhanced biofilm adhesion to the wall and led to a more uniform biofilm distribution along the pipe wall. A stronger transient pulse (40 kPa) inhibited biofilm accumulation and promoted continuous detachment from the wall during the 28-days biofilm conditioning period. Pipe flushing confirmed that biofilms formed under moderate and strong transient conditions exhibited greater wall adhesion and resistance to mobiliation during flushing as compared to those grown under steady-state flow conditions. These findings suggest that controlled hydraulic transients may play a role in biofilm management strategies, offering the potential to optimize DWDS maintenance and reduce microbial mobilization risks. However, further research is needed to assess the long-term effects in real-world systems with diverse pipe materials, nutrient conditions, and disinfection regimes.

Received 14th November 2025
Accepted 20th March 2026

DOI: 10.1039/d5va00415b

rsc.li/esadvances

Environmental significance

Hydraulic transients influence biofilm behavior in water distribution systems. This study demonstrates that controlled, low-amplitude transients can either promote or inhibit biofilm growth. These findings contribute to our understanding of biofilms in drinking water infrastructure and highlight how network operational strategies can influence biofilm development and sediment accumulation on pipe walls, both key factors affecting drinking water quality.

Introduction

Biofilms are structured microbial communities embedded within an extracellular polymeric substance (EPS) matrix that enables microbial attachment to surfaces in aquatic environments, including drinking water distribution systems (DWDS).[1–3] While biofilms play beneficial ecological roles in natural systems, their development in DWDS presents significant operational and public health challenges. Biofilm accumulation is associated with water quality deterioration, taste and odor events, discoloration, biofouling, and increased maintenance costs.[4–6] Of particular concern is the enhanced resistance of biofilm-associated microorganisms to disinfectants such as chlorine, as cells embedded within EPS exhibit substantially greater tolerance than their planktonic counterparts.[7,8] Biofilms may also serve as reservoirs of antibiotic resistance genes,[7] further complicating risk management.[9,10] For these reasons, understanding the mechanisms governing biofilm growth, structural stability, and detachment under operational hydraulic conditions is essential for effective DWDS management.[2,3]

Hydraulic impact on biofilm growth dynamics

Hydrodynamic conditions play a central role in biofilm development within DWDS. The ability of microbial aggregates to

[a]*Parana Sanitation Company – Sanepar. Federal University of Paraná – UFPR, Curitiba, Paraná, Brazil. E-mail: marielespa@gmail.com*
[b]*Department of Civil Engineering, Queen's University, Kingston, Ontario, Canada. E-mail: asb9@queensu.ca; ben.anderson@queensu.ca; yves.filion@queensu.ca*
[c]*Federal University of Paraná – UFPR, Curitiba, Paraná, Brazil. E-mail: cvs.fernandes@gmail.com*

colonize pipe surfaces depends on their capacity to withstand wall shear stress (WSS) while maintaining adhesion to the pipe wall. Numerous investigations, particularly those employing pilot-scale pipe loops operated under controlled hydraulic regimes, have demonstrated that flow conditions strongly influence biofilm thickness, density, EPS production, microbial community composition, and structural compactness.[2,5,11–14] These experimental systems commonly simulate real distribution networks by imposing variable flow rate patterns, often through periodic adjustments that generate dynamic WSS ranges during biofilm growth.

Hydraulic variability has been shown to shape biofilm architecture and function. Increased flow variability is frequently associated with higher bacterial density but reduced EPS-to-cell ratios and lower microbial diversity.[4] Exposure to fluctuating velocities has also been linked to enhanced metabolic activity, indicating that hydraulic stress may act as a selective pressure promoting physiologically resilient communities. Surface properties further modulate these responses, as rougher pipe materials facilitate stronger initial adhesion, whereas sustained high shear conditions may inhibit biofilm accumulation.[15]

The importance of hydraulic history in determining biofilm structure has been highlighted in experimental DWDS studies. Douterelo *et al.*[1] demonstrated that both habitat type and hydraulic regime significantly influence bacterial community composition. Variable flow conditions promoted the formation of more compact biofilms that exhibited increased resistance to detachment. Such findings underscore the role of hydraulic conditioning in determining long-term biofilm stability. More recent work has reinforced the need to integrate hydraulic management with microbial monitoring, showing that flow regimes interact with environmental factors such as temperature[16] and that controlled flushing can effectively limit biofilm accumulation.[17] Collectively, these studies establish that biofilm growth dynamics in DWDS are intrinsically linked to the magnitude, variability, and history of applied shear forces.

Biofilm detachment under steady flow

While hydraulic conditions govern biofilm growth, they also control detachment processes. Biofilm detachment is commonly examined in the context of flushing operations, where rapid increases in flow velocity elevate WSS at the pipe–biofilm interface. Detachment occurs when hydrodynamic forces exceed either the cohesive strength of the biofilm matrix or the adhesive strength between the biofilm and the pipe surface, resulting in structural failure and biomass mobilization.[18]

Although flushing represents an intentional high-shear intervention, routine steady flow variations during normal operation can also generate shear stresses sufficient to destabilize biofilms. The structural properties developed during the growth phase determine the susceptibility of biofilms to such perturbations. Biofilms formed under steady hydraulic regimes often exhibit EPS-rich matrices that enhance cohesion and adhesion, conferring resistance to moderate shear stress, yet allowing substantial biomass accumulation that may mobilize during high-shear flushing.

Experimental evidence suggests that hydraulic conditioning influences detachment behavior. So *et al.*[19] reported that rougher biofilms were more prone to detachment under fluctuating shear forces, whereas Lemos *et al.*[20] found that biofilms developed under steady conditions exhibited greater shear resistance. These findings indicate that the detachment response is governed not only by instantaneous WSS magnitude but also by the prior hydraulic environment under which the biofilm was formed. Consequently, steady flow regimes can simultaneously promote structural resilience and increase the risk of episodic biomass release during operational disturbances.

Biofilm detachment under unsteady shear and transient flow

In addition to steady flow variations, transient hydraulic events introduce rapidly changing shear forces that can significantly influence biofilm stability. Most existing research has focused on high-amplitude hydraulic transients, such as water hammer events, which may cause structural damage to pipes and pumping systems.[21]

Early modeling work by Brunone and Berni demonstrated that transient flows produce fluctuating wall shear stresses distinct from those observed under steady conditions.[22] Subsequent experimental investigations by Wang *et al.*[23] provided empirical evidence linking local WSS variations during transient events to biofilm detachment and altered growth dynamics. Further experimental work by Weston *et al.*[24] indicated that the initial surge phase of a transient is particularly effective in mobilizing biofilm material. Complementary findings by Khu *et al.*[25] showed that fluctuating velocities and flow reversals can modify biofilm thickness, adhesion strength, and bacterial density, thereby influencing detachment behavior.

Together, these studies demonstrate that biofilm mobilization is governed not only by extreme hydraulic interventions but also by the dynamic shear variability inherent to distribution systems. Nevertheless, most transient-focused investigations have examined large-amplitude pressure waves capable of exceeding biofilm shear strength and posing structural risk to infrastructure.

Research gap and objectives

Modern DWDS operate under continuously dynamic hydraulic conditions. Electromechanical components, including fixed-speed and variable-speed pumps and control valves, frequently adjust their operation to match fluctuating water demand. These adjustments generate recurrent, short-lived pressure and velocity fluctuations multiple times per hour.[26] Although such small-amplitude hydraulic transients are intrinsic to routine system operation, their influence on biofilm growth, structural development, and mechanical stability has not been systematically investigated.

Existing transient-related studies have largely treated hydraulic transients as acute disturbance events characterized by high-amplitude pressure waves associated with pipe burst risk or catastrophic failure. In contrast, the potential role of

small-amplitude transients as repetitive mechanical conditioning stimuli has received little attention. In this study, small-amplitude hydraulic transients are defined as pressure fluctuations in the range of 20–40 kPa that induce localized WSS variations without approaching structural failure thresholds. These conditions reflect typical operational fluctuations rather than extreme events.

It is hypothesized that repeated small-amplitude WSS oscillations may influence biofilm structural development, adhesion strength, and adaptive responses to shear, thereby affecting resilience to subsequent high-shear events such as flushing. To date, no study has systematically evaluated the impact of such operationally representative transients on biofilm formation and stability in a full-scale, controlled laboratory pipe system.

The overarching aim of this study is to examine the effects of small-amplitude hydraulic transients on biofilm development in a full-scale laboratory PVC pipe rig under controlled conditions. Specifically, this work seeks to determine how these transients affect biofilm growth and spatial distribution along the pipe wall, biofilm mechanical resilience under elevated WSS during flushing, and microbial activity within the bulk water phase.

Material and methods

The experiments were conducted over a 28 days period in the Drinking Water Distribution Laboratory (DWDL) at Queen's University. The DWDL is a unique research facility in North America that can partially replicate the full hydraulic, physic-chemical, and microbiological conditions of real distribution systems in a controlled environment. The DWDL is comprised of two pipe loops, each with a length of 193 m of IPEX Blue Brute PVC pipe Class 235 (DR18) and an internal diameter of 108 mm. Water is introduced into each loop from a tank with a volume of 3.6 m^3 and two variable-speed centrifugal pumps. Each experiment was divided into two phases: a biofilm conditioning phase, during which biofilms were allowed to develop under controlled hydraulic conditions, and a flushing phase, where biofilm detachment was assessed under increasing shear stress.

A total of three experiments were performed. In the first experiment (Exp A), a steady-state flow rate of 0.6 L s^{-1} was maintained at 280 kPa without induced transients during the 28-days period. In the second experiment (Exp B), a steady-state flow rate of 0.6 L s^{-1} was maintained at the same pressure but a small-amplitude 20 kPa positive transient pressure wave was generated with a solenoid valve installed at the outlet of a pipe loop (Fig. 1). During the 28 days biofilm conditioning phase, the solenoid valve was partially closed once every hour to temporarily reduce the flow rate to 0.54 L s^{-1} for a duration of 30 seconds. Following this period, the valve was reopened to restore the steady-state flow condition of 0.6 L s^{-1} within the pipe loop. During the 28-days biofilm conditioning phase, a total of 672 initial positive transient pressure pulses (20 kPa) were generated with the solenoid valve. A third experiment (Exp C) was performed where the solenoid valve was partially closed and then re-opened to generate a 40 kPa initial positive transient pressure pulse at every hour of the 28-days biofilm conditioning period. The solenoid valve operations in Exp B and C were calibrated to ensure controlled, reproducible wall shear stress (WSS) fluctuations in the pipe loop. The initial positive transient pressure pulses of 20 kPa and 40 kPa generated in Exp B and C produced flow variations of 10% and 20% in the pipe loop. These flow variations are representative of transient events frequently encountered in operational DWDSs due to routine activities such as pump startups, valve closures, and demand fluctuations.[27,28] Unlike major transient events caused by pump failures or sudden valve closures that produce large pressure waves and flow accelerations and decelerations, the controlled solenoid valve operations were designed to produce small, repeatable pressure waves and flow accelerations/decelerations in the pipe loop.

The biofilm conditioning phase was set to 28 days based on previous full-scale laboratory studies in which biofilms reached structural and metabolic stability within this timeframe.[1,4] Biofilm development was categorized into three stages: an initial

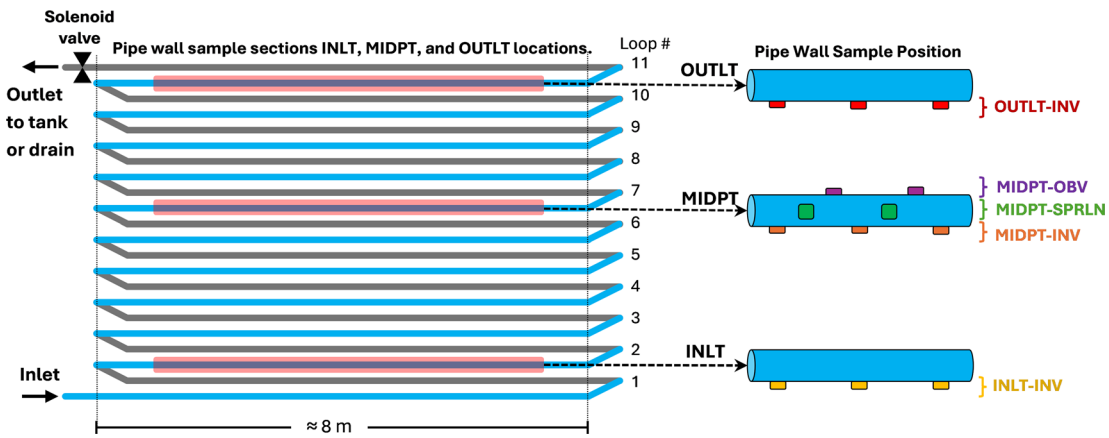

Fig. 1 Location of pipe wall sample sections (INLT, MIDPT and OUTLT) in the pipe loop, and the pipe wall sample positions: (1) at the pipe inlet/invert (INLT-INV); (2) at the pipe midpoint/invert (MIDPT-INV); (3) at the pipe midpoint/springline (MIDPT-SPRLN); (4) at the pipe midpoint/obvert (MIDPT-OBV); and (5) at the pipe outlet/invert (OUTLT-INV).

Table 1 Water quality parameters for the Kingston system and water in the pipe loops during Exp A, B, and C during the biofilm conditioning phase

Water quality parameter[a]	Kingston system[c]	Experiment A	Experiment B	Experiment C
pH	7.20–8.15[d]	7.75	7.92	8.10
Water temperature (°C)	12.3[e]	16.7	16.9	16.1
Dissolved oxygen (mg L^{-1})[b]	N/A	13.1	13.1	13.0
Specific conductivity (uS per cm)	303–324[d]	313	275	286
Turbidity (NTU)	0.056–0.197[e]	0.287	0.342	0.226

[a] Water quality parameters are averages of measurements taken during the 28-days biofilm conditioning period in Pipe Loops A and B, unless otherwise specified. [b] Dissolved oxygen values reported correspond to average, minimum, and maximum values measured during 28-d biofilm conditioning period. [c] Source water quality parameters taken from 2023 King Street Water Treatment Plant Annual Report, Utilities Kingston. [d] Measured in Kingston distribution system. [e] Measured at discharge works of Kingston King St. WTP.

phase (days 0–7), characterized by bacterial attachment and EPS production; an intermediate phase (days 7–21), where biofilms developed three-dimensional structures and increased microbial diversity; and a stabilization phase (days 21–28), during which biofilm adhesion strengthened and resistance to shear stress increased.

To facilitate rapid microbial colonization of the pipe wall surfaces, an Initial Bacterial Broth (IBB) was prepared using effluent from a Granular Activated Carbon (GAC) filter combined with a nutrient supplement. Specifically, 18 L of GAC effluent water was mixed with 1.5 L of NutriSelect® Plus No. 3 nutrient broth (13 g L^{-1}) and incubated at 16 °C under continuous agitation for seven days to promote microbial growth. Before application, the bacterial concentration of the IBB was assessed in triplicate using flow cytometry, yielding an average of 2.3×10^5 cells mL^{-1}. To initiate the experiment, 2 L of the IBB was added to the inlet of each tank that supplied the pipe loops. This inoculation step enabled the establishment of biofilms on the internal surfaces of the pipe loops. To support biofilm growth, Nutrient Broth No. 3 was continuously added to the tanks that supply the pipe loops at a rate of 0.60 mg L^{-1} per day.

Table 1 summarizes the water quality characteristics observed both in the Kingston municipal distribution system, which supplies the DWDL, and within the pipe-loop setups used during the 28 days biofilm conditioning phase in Experiments A, B, and C. Water temperature was kept stable at 16 °C, a value representative of summer conditions commonly observed in Canadian drinking water networks.[11] The specific conductivity data, as shown in Table 1, reflect the ionic concentration of the water supplemented with nutrient broth. To facilitate biofilm growth, oxygen was introduced into the storage tanks *via* aeration, as detailed in Table 1.

Kingston's potable water originates from Lake Ontario, near the confluence with the St. Lawrence River (Utilities Kingston 2023).

The King Street Water Treatment Plant—located within a 5 km radius of the DWDL—employs multiple treatment steps including pre-chlorination, screening, coagulation/flocculation, filtration, and final chlorination. After treatment, sodium hypochlorite is dosed into a clear well to establish an average residual chlorine concentration of 0.17–2.97 mg L^{-1}, which is maintained throughout the distribution network.[29] The treated water reaches the DWDL through a 150 mm cast iron main installed in the 1970s.

Following the biofilm conditioning phase, each pipe loop was flushed at a flow rate of 6.5 L s^{-1}, corresponding to a wall shear stress of 1.2 Pa 1.2 Pa (calculated *via* the Darcy–Weisbach equation). This step assessed the biofilm's adhesion strength and resistance to detachment developed under the different hydraulic regimes.

During flushing, fresh drinking water was continuously supplied to the tanks at the inlet of the pipe loops to ensure that mobilized biofilm materials were flushed out rather than reintroduced into the loops.

Microbial analysis was conducted by monitoring bacterial cell concentration (BCC), adenosine triphosphate (ATP) concentrations, and volatile suspended solids (VSS) in the bulk water to assess microbial metabolic activity. The BCC of the bulk water samples was assessed using a flow cytometer model SH800 from SONY. Samples were fixed with a 5% glutaraldehyde solution, stained with SYTO BCTM fluorescent dye that targets DNA of bacterial cells, and counted with the equipment against a standard suspension of reference beads with a known concentration. The rate of bioactivity of the bulk water was assessed using rapid ATP tests from Lumina Ultra. VSS was determined by filtering, drying, and weighing the solid fraction of the sample. The solid fraction was autoclaved at a temperature of 550 °C to determine the mass of the volatile fraction.

A removable pipe coupon[30] system was used to obtain representative biofilm samples from five locations along both longitudinal and circumferential positions of the pipe loops (Fig. 1). To characterize bacterial cell density (BCD) and ATP density at the pipe wall, biofilms were retrieved from the pipe coupons with a sterile cotton swab. The swabs were immersed in an aqueous solution, and the biofilm material was resuspended through centrifugation for 1 minute. Following this, bacterial cell count and ATP analyses were performed on the biofilm material suspended in solution in a similar manner to those performed on the bulk water samples.

Results

Impact of transients on cell density at the pipe wall during biofilm conditioning and flushing

Fig. 2 shows the BCD distribution along the pipe walls at 14 and 28 days and during the flush (FS). By day 14, Exp B exhibited the highest BCD (4.73×10^4 cells cm^{-2}), followed by Exp C ($3.38 \times$

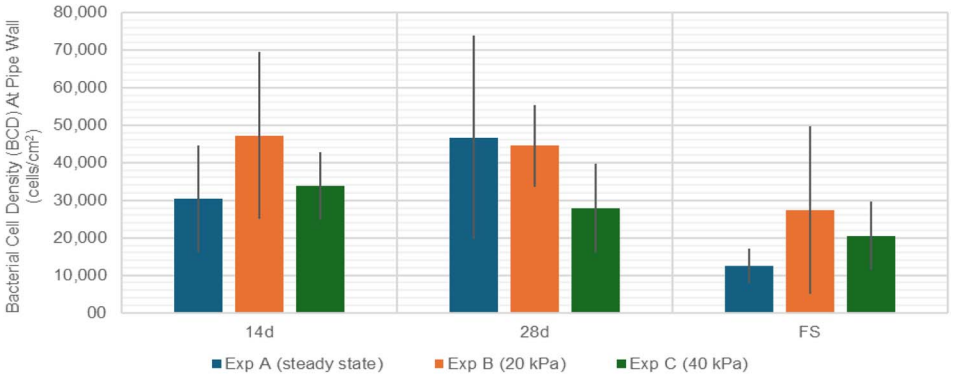

Fig. 2 Bacterial cell density at the pipe wall (BCD) at 14 days and 28 days of the biofilm conditioning phase and after the flushing step of Exp A, B, and C. C. The BCD values are averages calculated with measurements taken in triplicate across the three circumferential positions. The error bars represent the variance from the average by ± 1 standard deviations.

10^4 cells cm^{-2}) and Exp A (3.03×10^4 cells cm^{-2}). This indicates that moderate transients (20 kPa) promoted stronger biofilm adhesion compared to steady-state flow or higher transient conditions.

At 28 days, BCD remained highest in Exp A (4.67×10^4 cells cm^{-2}), suggesting that steady-state conditions favor biofilm accumulation over time. In contrast, Exp B exhibited a slight reduction in BCD (4.45×10^4 cells cm^{-2}), possibly due to periodic detachment caused by the transients. Pipe-loop C had the lowest BCD at 28 days (2.79×10^4 cells cm^{-2}), indicating extensive removal of loosely attached cells. This is further illustrated in Fig. 2, where the decrease in cell density between day 28 and the flush step FS is smallest for Exp C. This confirms that while fewer cells persisted on the pipe wall during the biofilm conditioning in Exp C, those that remained exhibited stronger adhesion.

After flushing step FS, Exp B retained the highest BCD (2.73×10^4 cells cm^{-2}), which highlights the resilience of biofilms formed under the 20 kPa transients. By contrast, Exp A exhibited the lowest BCD (1.25×10^4 cells cm^{-2}) after flushing and experienced the greatest level of detachment from flushing. Despite experiencing greater material detachment during the biofilm conditioning phase, the biofilms in Exp C (40 kPa transients) exhibited higher BCD than those in the steady-state Exp A after the flushing step FS. This suggests that while stronger transients initially caused more disruption in the biofilm conditioning phase, the biofilms that remained on the pipe wall developed a higher shear strength, and were more resistant to subsequent detachment.

Fig. 3 indicates a time series of turbidity measured at the outlet of the pipe loop during the flushing stage of Experiments A, B, and C. Exp A exhibited the highest turbidity peak (0.9 NTU) and the greatest level of detachment. This further suggests that the steady-state conditioning imposed in Exp A produced weakly adhered biofilms. Exp B reached a lower turbidity peak (0.6 NTU) which suggests a controlled detachment during flushing. The moderate transients (20 kPa) imposed in the biofilm conditioning phase of Exp B may have enhanced biofilm adhesion and controlled detachment in flushing. By contrast, Exp C exhibited the lowest turbidity (0.3 NTU) which suggests that the strong transient conditions (40 kPa) imposed during the biofilm conditioning phase may have produced the most adhesive biofilms with the least detachment during the flushing phase.

Impact of transients on longitudinal location of cells on pipe wall during biofilm conditioning and flushing

Fig. 4 indicates the BCD measured at the longitudinal positions (INLT, MIDPT, OUTLT) in Exp A, B, and C during the biofilm

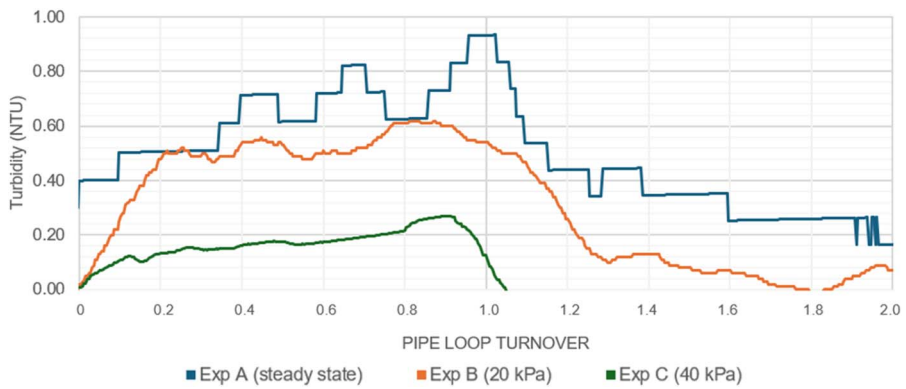

Fig. 3 Times series plot of turbidity measured at the outlet of the pipe loop during the flushing stage of Experiments A, B, and C.

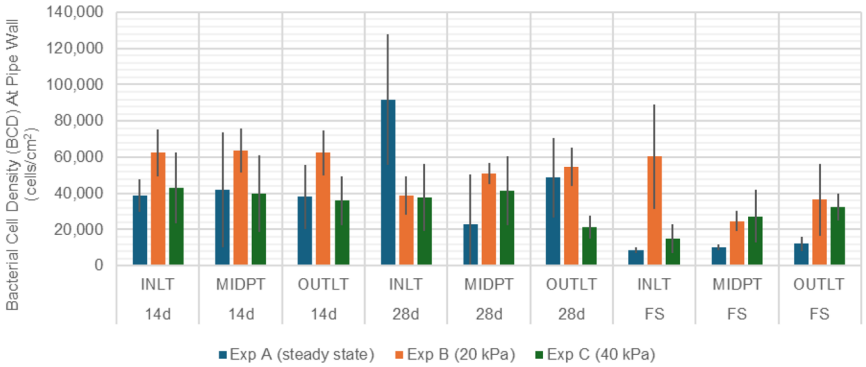

Fig. 4 Bacteria cell density (BCD) at pipe walls at 14 days and 28 days of the conditioning phase and the flushing step (FS) of Experiments A, B and C. The BCD values are averages calculated with measurements taken in triplicate at the invert (INV) pipe position. The error bars represent the variance from the average by ± 1 standard deviation.

conditioning and flushing phases. During the biofilm conditioning phase at 14 days in Fig. 4, Exp B (20 kPa transients) exhibited the highest average BCD across all longitudinal positions. For instance, at the inlet (INLT), the BCD was 6.24 × 104 cells cm^{-2}, compared to 3.87 × 104 cells cm^{-2} in Exp A and 4.29 × 104 cells cm^{-2} in Exp C. Again, in Exp B, the bacterial cell density measured at the midpoint (MIDPT) and outlet (OUTLT) was 6.35 × 104 cells cm^{-2} and 6.25 × 104 cells cm^{-2}, respectively—both higher than the values in Exp A and Exp C. These results again suggest that the moderate transients of Exp B (20 kPa) enhanced biofilm adhesion along the pipe. In Exp C (40 kPa), the observed reduction in BCD at the outlet (OUTLT, 3.60 × 10^4 cells cm^{-2}) near the solenoid valve may indicate that the shear stress from stronger transients exceeded the biofilm strength threshold, promoting regular detachment and inhibiting biofilm adhesion.

Fig. 4. Bacteria cell density (BCD) at pipe walls at 14 days and 28 days of the conditioning phase and the flushing step (FS) of Experiments A, B and C. The BCD values are averages calculated with measurements taken in triplicate at the invert (INV) pipe position. The error bars represent the variance from the average by ± 1 standard deviation.

Fig. 4 also indicates that in Exp A (steady state), the BCD measured at the inlet (INLT) increased markedly to reach 9.17 × 10^4 cells cm^{-2} at 28 days. This suggests that biofilm was able to grow and accumulate in a localized manner near the pipe–loop entry point. In Exp B, the biofilm showed a more uniform distribution, with BCD values ranging from 3.88 × 10^4 cells cm^{-2} at the inlet (INLT) to 5.49 × 10^4 cells cm^{-2} at the outlet (OUTLT). This suggests that moderate transients (20 kPa) contributed to a more uniform biofilm growth and presence along the pipe. In Exp C, low levels of BCD were measured at the outlet (OUTLT; 2.13 × 10^4 cells cm^{-2}), which reinforces the hypothesis that a higher transient pressure pulse (40 kPa) led to more frequent detachment during the conditioning phase and hindered the development and stability of the biofilm.

To assess the biofilm resistance to detachment, a quantitative analysis of the relative change in BCD between Day 28 and FS was performed. At the inlet (INLT), Exp A saw a reduction in BCD of 90.7% after flushing, Exp B saw an increase of 55.6% in BCD, and Exp C saw a reduction of 60.2% in BCD. At the midpoint (MIDPT), Exp A saw a reduction in BCD of 55.9%, Exp B saw a reduction in BCD of 51.7%, and Exp C saw a reduction in BCD of 34.2%. At the outlet (OUTLT), Exp A saw a reduction in BCD of 74.7%, Exp B saw a reduction in BCD of 33.3%, and Exp C saw an increase in BCD of 52.3%. These results confirm that biofilms conditioned under moderate (20 kPa) and strong (40 kPa) transient events developed stronger adhesion

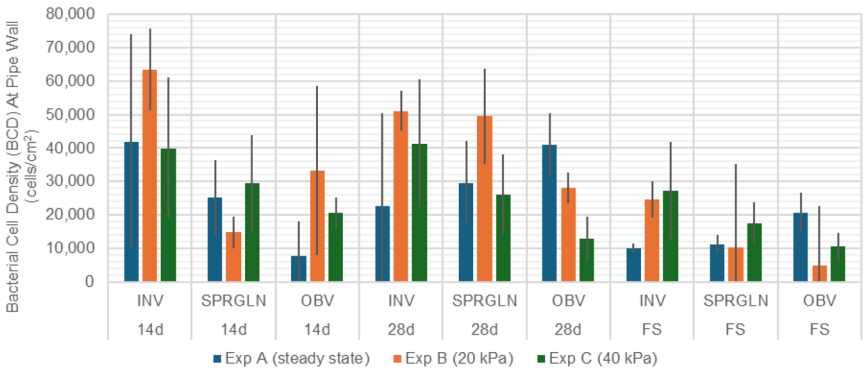

Fig. 5 Bacterial cell density (BCD) at pipe wall at the mid-point longitudinal location (MIDPT) of the pipe loop and in the three circumferential positions (INV, SPRGLN, OBV) of the pipe, at 14 days and 28 days of the biofilm conditioning phase and the flush.

properties than those formed under steady-state. The increases in BCD between Day 28 and after the flush may reflect a localized biomass accumulation during flushing. This phenomenon may be attributed to the mobilization and subsequent redeposition of detached biofilm clusters downstream, resulting in a localized increase in BCD at downstream sampling locations.

Impact of transients on circumferential location of cells on pipe wall during biofilm conditioning and flushing

The effect of small-amplitude transients on the spatial distribution of biofilms around the circumferential position of the pipe was also examined. Fig. 5 indicates the BCD measured at the midpoint location (MIDPT) in the three circumferential positions (INV, SPRGLN, OBV) of the pipe in Exp A, B, and C during the biofilm conditioning and flushing phases. Fig. 5 shows that at 14 days, biofilm growth was highest at the invert position (INV) in all three Experiments A, B, and C. At 14 days, biofilm growth was at its lowest level at the obvert position (OBV) in Experiments A, B, and C. This pattern suggests that biofilm formation is initially more favorable at the invert position and less so at the springline and obvert positions under both steady-state and transient conditions.

Fig. 5 also shows that by 28 days, biofilm distribution was more uniform across all circumferential positions in Exp A, B and C. Specifically, in Exp B the biofilm had a relatively uniform BCD distribution, while in Exp A the biofilm showed localized growth at the obvert (OBV, 4.10×10^4 cells cm^{-2}). The results suggest that the steady-state flow imposed in Exp A promoted biofilm accumulation at the obvert position, whereas in Exp B and C the transients promoted growth at the invert and springline positions.

Fig. 5 shows that, following the flushing step FS, biofilm detachment occurred at all circumferential positions in Exp A, B and C, but to varying degrees. Quantitative analysis of the relative change in BCD between Day 28 and FS indicates that at the invert (INV) the reductions were 55.9% in Exp A, 51.7% in Exp B and 34.2% in Exp C. At the springline (SPRGLN), the reductions were 62.0%, 79.4% and 32.5% for Exp A through C. While at the obvert (OBV), the reductions amounted to 49.9%, 83.0% and 19.1% for Exp A through C. After the flush FS, Exp B showed high BCD levels of 2.47×10^4 cells cm^{-2} at the invert (INV) and BCD levels of 2.47×10^4 cells cm^{-2} at the springline (SPRGLN). Similarly, Exp C showed high levels of BCD of 2.73×10^4 cells cm^{-2} at the invert (INV) and BCD levels of 1.04×10^4 cells cm^{-2} at the obvert (OBV).

These results show that biofilms formed under steady-state conditioning (0 kPa, Exp A) exhibited the weakest adhesion, while those formed under moderate and strong transients (20 kPa-Exp B and 40 kPa-Exp C) exhibited the highest strength and ability to resist high shear during the flush. This indicates that biofilms formed under transients, particularly moderate ones, develop stronger adhesion and resist detachment more effectively.

The results demonstrate clear differences in biofilm adhesion and distribution in both the longitudinal locations (INLT, MIDPT, OUTLT) and circumferential positions (INV, SPRGLN, OBV) across all three experiments and their associated conditioning hydraulics. Steady-state conditions (Exp A) promoted localized biofilm accumulation, with notable increases at the inlet (INLT) longitudinal location and obvert (OBV) circumferential position. However, these biofilms demonstrated weaker adhesion overall and were more readily detached during flushing events. Biofilms formed under moderate transients (20 kPa, Exp B) exhibited strong adhesion and uniform distribution along the pipe. These biofilms were characterized by higher BCD values, particularly at the downstream location (OUTLT) and at the invert (INV) and springline (SPRGLN) circumferential positions. They also showed a greater resistance to detachment during flushing than the biofilms in Exp A (steady state). The biofilms grown under strong transients (40 kPa, Exp C) generally showed a uniform distribution, with higher BCD values at the invert (INV) and springline (SPRGLN) positions. These biofilms generally exhibited the greatest level of adhesion and the greatest resistant to detachment during the flushing phase.

Impact of transients on microbial activity in the bulk water

Fig. 6a–c indicates the bacterial cell concentration (BCC), adenosine triphosphate (ATP) and volatile suspended sediments (VSS) concentration in the bulk water during the biofilm conditioning and flushing phases of Experiments A, B, and C. The results in Fig. 6a–c show that in all experiments there was a gradual increase in microbial indicators that reflects bacterial growth and biological activity over the 28 days biofilm conditioning period. It was not possible to determine how much of the increase in microbial activity was owing to planktonic biological growth in the bulk water and how much of the increase was owing to biofilm detachment from the wall being introduced as biological material into the bulk water.

Fig. 6a–c indicate a modest increase in BCC and ATP and a more important increase in VSS in the bulk water after the flush for Exp A and C. This result accords with the previous observations that biofilm detachment occurred in the flush for these experiments. As such, this detachment may have introduced new material in the bulk water to increase the value of the biological parameters. Fig. 6a–c also shows that in Exp B, BCC, ATP, and VSS values decreased after flushing. This result is contradictory to what was found in Fig. 2–5, where biofilm detachment was found to occur in the flushing phase. However, this decrease in bulk water parameters may be the result of a combination of factors, including greater structural stability of biofilms formed under moderate transients (reducing the release of viable cells), variations in planktonic populations, or experimental variability.

Discussion

The findings demonstrate hat small-amplitude hydraulic transients influence not only biofilm growth and spatial distribution, but also the mechanical resilience of biofilms under cyclic hydrodynamic forcing, as reflected in their detachment behaviour and associated changes in bulk water microbial activity.

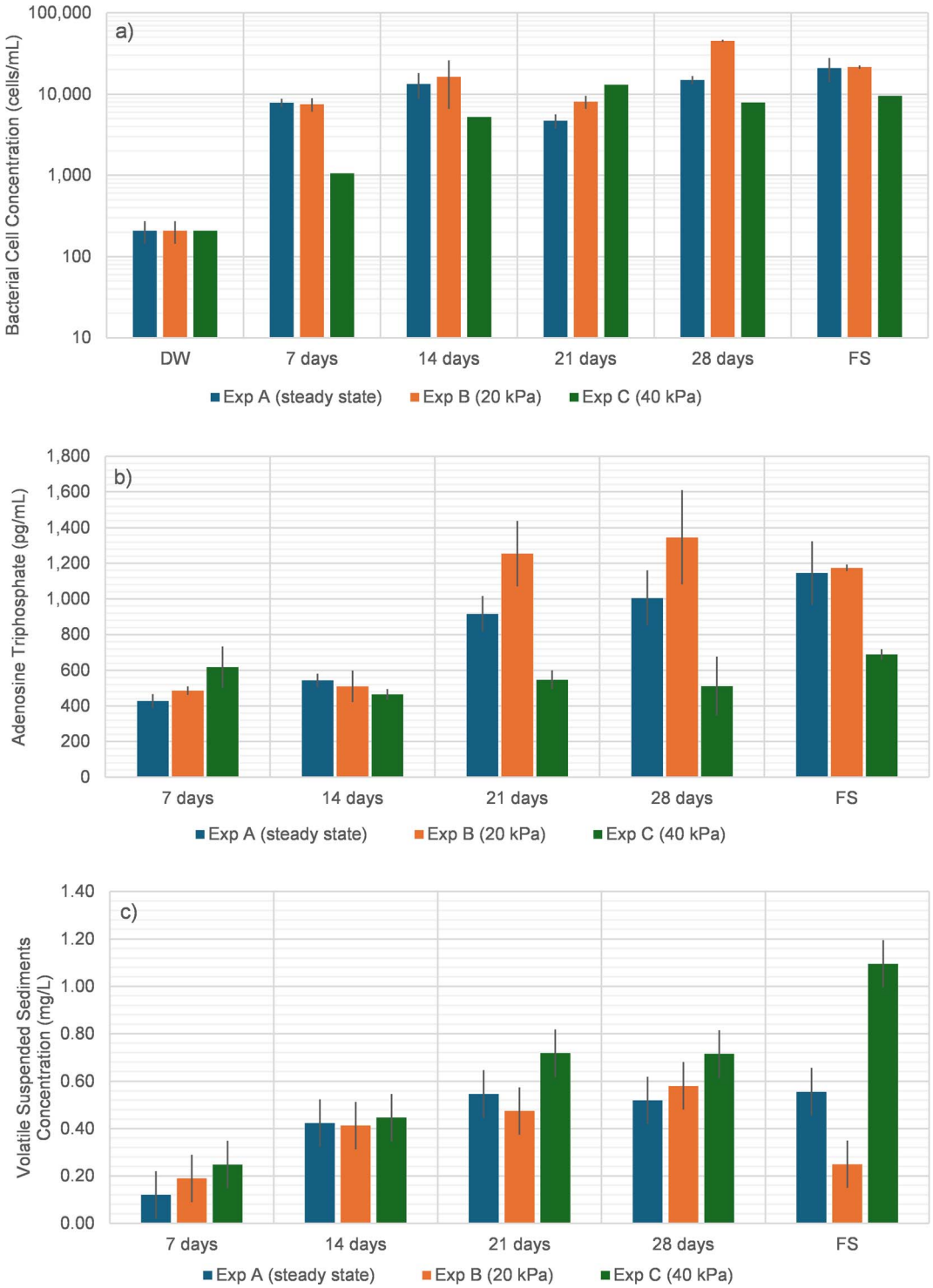

Fig. 6 (a) Bulk cell concentration (BCC), (b) adenosine triphosphate (ATP), and (c) volatile suspended solids concentration measured in the bulk water in Experiments A, B, and C. The plotted values are averages calculated with measurements from bulk water samples taken in triplicate. The error bars represent the variance from the average by ± 1 standard deviations.

Biofilm conditioning

The results indicate that moderate transients (20 kPa) in Exp B facilitated biofilm formation and distribution in the loops, particularly at the inlet (INLT) and midpoint (MIDPT) longitudinal locations, and the circumferential positions of the invert (INV) and springline (SPRGLN). Stronger transients (40 kPa) in Exp C induced more frequent detachment throughout the biofilm conditioning phase, yielding consistently lower BCD values at both the outlet and obvert positions and highlighting the adverse effect of high transient shear stress on biofilm

adhesion. These observations align with Rochex *et al.*,[31] who demonstrated that excessive shear stress disrupts microbial attachment and biofilm adhesion, thereby limiting surface accumulation. The results support the threshold-effect hypothesis of Thomen *et al.*,[32] which posits that an optimal balance between nutrient transport and mechanical stress is necessary to sustain biofilm development. Moreover, periodic exposure to elevated levels of shear stress at the wall may have triggered adaptive responses such as increased EPS production and community restructuring thereby enhancing biofilm mechanical resilience under repeated shear fluctuations.[24,33]

In this context, the study by Chen *et al.* (2022) provides a robust conceptual framework for interpreting these observations. Although their work focused on biofilm-bound sandy systems, the authors demonstrated that biofilms exhibit a significant adaptive response to cyclic changes in shear stress, leading to enhanced resilience over time.[34] By applying this lens to the present study, the results suggest that the cyclic nature of the small-amplitude transients (20–40 kPa) functioned as a mechanical conditioning agent. This process likely selected for more resilient microbial clusters and stimulated the development of a more cohesive matrix, allowing the biofilm to maintain structural integrity even when subjected to subsequent high-shear flushing events. Interpreting the findings through the lens of biofilm resilience to cyclic hydrodynamic forcing—rather than adhesion strength alone—provides a mechanistic explanation for the reduced detachment observed following conditioning under moderate transients.

Flushing phase

The flushing phase of the experiments further highlighted differences in biofilm resilience. Biofilms in Exp B and C exhibited a greater resistance to detachments evidenced by a lower percent reduction in BCD at the pipe wall after the flushing step FS relative to the steady state experiment (Exp A). This finding is consistent with previous research that suggests that periodic shear stress can induce adaptive restructuring of the biofilms, increase EPS production, and enhance the mechanical strength of biofilms.[33] Steady-state conditions in Exp A promoted biofilm accumulation at specific positions, such as the obvert, but these biofilms exhibited lower structural resilience to high-shear disturbance, lending to substantial detachment during flushing, as evidenced by the high percent reduction in BCD at the pipe wall observed after flushing. This finding is consistent with studies that suggest steady-state hydraulic conditions facilitate biofilm accumulation but not structural resilience.[35]

Microbial activity in bulk water

In the steady state and transient experiments, microbial activity in the bulk water increased throughout the 28 days biofilm conditioning period, but it was unclear whether this was due to planktonic biological growth or from continuous biofilm detachment. The indicators of microbial activity (BCC, ATP, and VSS) in the bulk water in Exp A and C did increase after flushing which suggests that biofilm detachment did occur during flushing. These findings align with previous studies that highlight the role of hydraulic disturbances in enhancing nutrient transport and microbial retention on pipe walls, which promote biofilm growth.[4] Additionally, the periodic detachment induced by transient shear stress may create ecological niches that allow microbial communities to adapt and develop structurally robust biofilms.[24]

These findings have practical implications for the management of biofilms in water distribution systems. There are two opportunities here. First, the generation of controlled hydraulic transients could be strategically applied to periodically mobilize low-strength biofilms and other material in pipes to clean the pipes on a near-continuous basis. Second, the generation of controlled hydraulic transients could be used to promote biofilms with enhanced strength and resistance to detachment. This could potentially reduce the risk of extensive biofilm detachment and release of metals and other toxins anchored in the biofilm during unexpected high-shear events (*e.g.*, pump trip, pipe burst, rapid opening of fire hydrant) and cause serious discolouration episodes. However, the exact magnitude of controlled transients needed to achieve these two goals is likely system-specific and will depend on a number of factors such as pipe material, system configuration, composition and age of biofilms among others. The results also underscore the need for integrated biofilm management strategies that account for both hydraulic and microbial dynamics. Advanced hydraulic and discoloration modelling and monitoring tools could help identify optimal transient regimes that balance biofilm control and system efficiency while minimizing the risk of pipe damage.[36]

Limitations

While this study provides novel insights into the influence of small-amplitude hydraulic transients on biofilm development, several limitations must be acknowledged. One key limitation is the use of smooth PVC pipes in the experimental setup, which differs significantly from older, rougher materials such as cast iron (CI) commonly found in real water distribution systems. Research has shown that rougher pipe surfaces provide more microscopic niches and surface irregularities that enhance biofilm adhesion and growth.[1,15] As a result, biofilms in older distribution systems may exhibit different structural and adhesive properties compared to those observed in this study.

Another limitation concerns the methods used to grow bacteria to inoculate the pipe loops. The experimental setup included two granular activated carbon (GAC) filters—one to remove chlorine and another to provide a surface for bacterial growth before these were introduced into the pipe loops. This approach likely introduced a selection bias by favoring bacteria that thrive in a chlorine-free environment while excluding chlorine-resistant or low-nutrient-adapted species. The absence of these species in the experimental conditions may have influenced the composition and structural properties of the biofilms. In addition to this, the study was performed in a high-nutrient, chlorine-free environment, whereas real distribution systems typically operate under low-nutrient conditions with residual disinfectants that play a crucial role in shaping microbial communities. The absence of these real-world constraints may

have affected biofilm growth rates, shear response, and mechanical properties, potentially leading to an overestimation of biofilm resilience under cyclic hydrodynamic forcing relative to operational networks.The representation of hydraulic transients in the experimental setup is another factor to consider. The transient pulses introduced in this study were designed to generate short-lived acceleration and deceleration phases in flow, rather than to precisely replicate transient events that occur in real distribution systems (*e.g.*, pump trips, hydrant operations, and valve closures). While this approach allowed for the isolation of the impact of transient-induced shear stress on biofilm behaviour, the magnitude, frequency, and duration of these laboratory-generated transients may not fully capture the complexity of hydraulic transient fluctuations in real systems. The duration of the experiment, limited to 28 days, is also a constraint. While this timeframe was sufficient to observe an evolution in biofilm formation and adhesion, it may not fully capture the long-term biofilm dynamics and adaptation processes that occur in real distribution systems. Biofilms in operational networks are subjected to seasonal variations, prolonged exposure to fluctuating hydraulic conditions, and interactions with accumulated pipe deposits, all of which could influence their long-term behaviour in ways not accounted for in this study.

Despite these limitations, this study represents a critical first step forward in understanding the impact of hydraulic transients on biofilm development in drinking water distribution systems. Rather than treating hydraulic transients solely as acute disturbance events, this work demonstrates that operational-scale cyclic perturbations can function as mechanical conditioning stimuli that modulate biofilm resilience. By isolating transient effects, this study provides unique evidence of how biofilms respond to dynamic hydraulic conditions, a factor often overlooked in conventional biofilm management strategies. Furthermore, the findings highlight the potential role of controlled hydraulic transients in influencing biofilm stability, which may lead to new strategies to mitigate biofilm-related water quality issues.

The insights gained here establish a foundation for future research, emphasizing the need for field-scale validation and the inclusion of additional variables such as pipe material diversity, and realistic nutrient and disinfection regimes. By framing the results within the concept of biofilm resilience to cyclic hydrodynamic forcing, this work contributes to a more mechanistic understanding of how dynamic hydraulic conditions shape microbial ecosystem development indrinking water distribution networks.

Conclusions

This study provided novel insights into the impact of small-amplitude hydraulic transients on biofilm formation, adhesion, and detachment in drinking water distribution systems. The key findings highlighted that moderate transients (20 kPa) produced biofilms that generally had a uniform distribution in the pipe loop, a high bacterial cell density on the wall with a strong wall adhesion and a good ability to resist wall shear stresses during flushing. Stronger transients (40 kPa) promoted biofilm that also had a uniform distribution in the pipe. However, the stronger transients caused a continuous biofilm detachment from the pipe wall during the biofilm conditioning phase, and a correspondingly lower bacterial cell density on the wall. The biofilms that managed to remain adhered to the wall during the biofilm conditioning phase however exhibited a greater resistance to the high shear stress imposed by flushing. These findings support the hypothesis that periodic hydraulic disturbances can enhance the structural integrity of biofilms. By contrast, steady-state conditions (no transients) produced biofilms that were less uniformly distributed in the pipe, had weaker adhesion, and that were more susceptible to detachment under increased shear stress during flushing. These results underscore the complex interplay between hydraulic conditions and biofilm dynamics, and demonstrate that flow variability is a crucial factor in microbial ecosystem development within DWDS.

The results have important practical implications for biofilm management in DWDSs. The strategic generation of controlled hydraulic transients may offer a potential approach for regulating biofilm formation while minimizing excessive accumulation or sudden detachment events that could lead to water quality issues such as discoloration and microbial contamination.

Overall, this study advances the understanding of how hydraulic transients influence biofilm development, reinforcing the need for integrated biofilm management strategies that consider both hydraulic and microbial dynamics. By bridging the gap between hydraulic engineering and microbial ecology, these findings contribute to the development of more effective water quality control practices, enhancing the sustainability and safety of drinking water distribution networks.

Author contributions

Conceptualization: M. S. P. A., A. S. B.; methodology: A. S. B., Y. F., M. S. P. A., B. A., C. V. S. F.; investigation: A. S. B., M. S. P. A., B. A.; resources: A. S. B., Y. F.; writing – original draft: M. S. P. A., A. S. B., B. A.; writing – review & editing: Y. F., C. V .S. F.; visualization: M. S. P. A.; supervision: A. S. B., Y. F., C. V. S. F.; project administration: Y. F.; funding acquisition: Y. F.

Conflicts of interest

There are no conflicts to declare.

Data availability

All data supporting the findings of this study are included in the manuscript and in its supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5va00415b.

Acknowledgements

CAPES – Print: Brazilian Institutional Internationalization Program. CNPq – Brazilian Council of Research.

References

1 I. Douterelo, R. Sharpe and J. Boxall, Influence of hydraulic regimes on bacterial community structure and composition in an experimental drinking water distribution system, *Water Res.*, 2013, **47**(2), 503–516, DOI: 10.1016/j.watres.2012.09.053.

2 S. Liu, C. Gunawan, N. Barraud, S. A. Rice, E. J. Harry and R. Amal, Understanding, Monitoring, and Controlling Biofilm Growth in Drinking Water Distribution Systems, *Environ. Sci. Technol.*, 2016, **50**(17), 8954–8976, DOI: 10.1021/acs.est.6b00835.

3 I. Douterelo, K. Fish and J. Boxall, Succession of bacterial and fungal communities within biofilms of a chlorinated drinking water distribution system, *Water Res.*, 2018, **141**, 74–85, DOI: 10.1016/j.watres.2018.04.058.

4 K. Fish, A. Osborn and J. Boxall, Biofilm structures (EPS and bacterial communities) in drinking water distribution systems are conditioned by hydraulics and influence discolouration, *Sci. Total Environ.*, 2017, **593–594**, 571–580, DOI: 10.1016/j.scitotenv.2017.03.176.

5 H. C. Flemming, J. Wingender, U. Szewzyk, *et al.*, Biofilms: An emergent form of bacterial life, *Nat. Rev. Microbiol.*, 2016, **14**(9), 563–575, DOI: 10.1038/nrmicro.2016.94.

6 U. Simunič, P. Pipp, M. Dular and D. Stopar, The limitations of hydrodynamic removal of biofilms from the dead-ends in a model drinking water distribution system, *Water Res.*, 2020, **178**, 115838, DOI: 10.1016/j.watres.2020.115838.

7 W. Li, Q. Tan, W. Zhou, J. Chen, Y. Li, F. Wang and J. Zhang, Impact of substrate material and chlorine/chloramine on the composition and function of a young biofilm microbial community as revealed by high-throughput 16S rRNA sequencing, *Chemosphere*, 2019, **242**, 125310, DOI: 10.1016/j.chemosphere.2019.125310.

8 X. Miao, X. Han, C. Liu and X. Bai, Intrinsic chlorine resistance of bacteria modulated by glutaminyl-tRNA biosynthesis in drinking water supply systems, *Chemosphere*, 2022, **308**, 136322, DOI: 10.1016/j.chemosphere.2022.136322.

9 C. Calero Preciado, S. Husband, J. Boxall, G. del Olmo, V. Soria-Carrasco, S. K. Maeng, *et al.*, Intermittent water supply impacts on distribution system biofilms and water quality, *Water Res.*, 2021, **201**, 117372, DOI: 10.1016/j.watres.2021.117372.

10 S. L. Weston, R. P. Collins and J. B. Boxall, A novel demonstration of adhered material mobilisation by hydraulic transients, *Environ. Sci.:Water Res. Technol.*, 2019, **5**(12), 2132–2141, DOI: 10.1039/C9EW00686A.

11 A. S. Braga and Y. Filion, Initial stages of particulate iron oxide attachment on drinking water PVC pipes characterized by turbidity data and brightfield microscopy from a full-scale laboratory, *Environ. Sci.:Water Res. Technol.*, 2022, **8**, 1195–1210, DOI: 10.1039/D2EW00010E.

12 I. Douterelo, S. Husband, V. Loza and J. Boxall, Dynamics of biofilm regrowth in drinking water distribution systems, *Appl. Environ. Microbiol.*, 2016, **82**(14), 4155–4168, DOI: 10.1128/AEM.00109-16.

13 T. Wang, X. Dai, T. Zhang, C. Xin, Z. Guo and J. Wang, Formation and Microbial Composition of Biofilms in Drip Irrigation System under Three Reclaimed Water Conditions, *Water*, 2022, **14**(8), 1216, DOI: 10.3390/w14081216.

14 E. Tsagkari, S. Connelly, Z. Liu, A. McBride and W. T. Sloan, The role of shear dynamics in biofilm formation, *npj Biofilms Microbiomes*, 2022, **8**, 33, DOI: 10.1038/s41522-022-00300-4.

15 M. Cowle, G. Webster, A. Babatunde, B. Bockelmann-Evans and A. Weightman, Impact of flow hydrodynamics and pipe material properties on biofilm development within drinking water systems, *Environ. Technol.*, 2020, **41**(28), 3732–3744, DOI: 10.1080/09593330.2019.1619844.

16 K. Fish, R. Sharpe, C. Biggs and J. Boxall, Impacts of temperature and hydraulic regime on discolouration and biofilm fouling in drinking water distribution systems, *PLOS Water*, 2022, **1**, e0000033, DOI: 10.1371/journal.pwat.0000033.

17 X. Zhang, D. Wei, C. Li, Q. Zhao, Z. Liu and H. Zhang, The impact of flushing the drinking water distribution system pipeline on the quality of drinking water and microorganisms, *J. Water Process Eng.*, 2025, **69**, 106865, DOI: 10.1016/j.jwpe.2024.106865.

18 S. Husband, K. Fish, I. Douterelo and J. Boxall, Linking discolouration modelling and biofilm behaviour within drinking water distribution systems, *Water Sci. Technol. Water Supply*, 2016, **16**(4), 942–950, DOI: 10.2166/ws.2016.045.

19 M. So, M. Terashima, R. Goel and H. Yasui, Modelling the effect of biofilm morphology on detachment, *J. Water Environ. Technol.*, 2015, **13**(1), 49–62, DOI: 10.2965/jwet.2015.49.

20 M. Lemos, F. Mergulhão, L. Melo and M. Simões, The effect of shear stress on the formation and removal of Bacillus cereus biofilms, *Food Bioprod. Process.*, 2015, **93**, 242–248, DOI: 10.1016/j.fbp.2014.09.005.

21 P. Boulos, B. Karney and D. Wood, Hydraulic Transient Guidelines for Protecting Water Distribution Systems, *J. AWWA*, 2005, **97**(5), 111–124, DOI: 10.1002/j.1551-8833.2005.tb10892.x.

22 B. Brunone and E. Berni, Wall shear stress distribution in transient turbulent pipe flow by local velocity measurement, *J. Hydraul. Res.*, 2010, **136**(10), 716–726, DOI: 10.1061/(ASCE)HY.1943-7900.0000234.

23 S. Wang, H. Zhu, G. Zheng, F. Dong and C. Liu, Dynamic changes in biofilm structures under dynamic flow conditions, *Appl. Environ. Microbiol.*, 2022, **88**(22), e01072–22, DOI: 10.1128/aem.01072-22.

24 S. Weston, R. Collins and J. Boxall, An experimental study of how hydraulic transients cause mobilisation of material within drinking water distribution systems, *Water Res.*, 2022, **194**, 116890, DOI: 10.1016/j.watres.2021.116890.

25 S.-T. Khu, C. Xin, T. Wang, Y. Zhang and X. Zuo, Effects of hydraulic conditions on biofilm detached in drinking water distribution system, *J. Water Process Eng.*, 2023, **53**, 103882, DOI: 10.1016/j.jwpe.2023.103882.

26 M. Agostinho, A. Braga, B. Anderson, Y. Filion and C. Fernandes, Examining the effect of small-amplitude transients on the shear strength of biofilms in water distribution pipes, *Eng. Proc.*, 2024, **69**, 204, DOI: 10.3390/engproc2024069204.

27 B. W. Karney, Y. R. Filion. Energy dissipation mechanisms in water distribution systems. In: Proceedings of the 4th ASME JSME Joint Fluids Engineering Conference. 2003. Paper FEDSM2003-45244, DOI: 10.1115/FEDSM2003-45244.

28 O. B. G. Del, S. Husband, C. Briones, A. Soriano, C. Calero Preciado, J. Macian and I. Douterelo, The microbial ecology of a Mediterranean chlorinated drinking water distribution system in the city of Valencia (Spain), *Sci. Total Environ.*, 2021, **754**, 142016, DOI: 10.1016/j.scitotenv.2020.142016.

29 Utilities Kingston. King Street Water Treatment Plant 2023 Annual Report. Kingston, Ontario, Canada, 2023.

30 A. S. Braga and Y. A. Filion, A novel monitoring scheme to detect iron oxide particle deposits on the internal surface of PVC drinking water pipes, *Environ. Sci.:Water Res. Technol.*, 2021, **7**(11), 2116–2128, DOI: 10.1039/D1EW00614B.

31 A. Rochex, J. J. Godon, N. Bernet and R. Escudié, Role of shear stress on composition, diversity and dynamics of biofilm bacterial communities, *Water Res.*, 2008, **42**(20), 4915–4922, DOI: 10.1016/j.watres.2008.09.015.

32 P. Thomen, J. Robert, A. Monmeyran, A.-F. Bitbol, C. Douarche and N. Henry, Bacterial biofilm under flow: First a physical struggle to stay, then a matter of breathing, *PLoS One*, 2017, **12**(4), e0175197, DOI: 10.1371/journal.pone.0175197.

33 J. Yang, S. Cheng, C. Li, Y. Sun and H. Huang, Shear stress affects biofilm structure and consequently current generation of bioanode in microbial electrochemical systems (MESs), *Front. Microbiol.*, 2019, **10**, 398, DOI: 10.3389/fmicb.2019.00398.

34 X. Chen, C. Zhang, I. H. Townend, Z. Gong, Q. Feng and X. Yu, The resilience of biofilm-bound sandy systems to cyclic changes in shear stress, *Water Resour. Res.*, 2022, **58**(5), e2021WR031098, DOI: 10.1029/2021WR031098.

35 Y. Shen, G. L. Monroy, N. Derlon, D. Janjaroen, C. Huang, E. Morgenroth, *et al.*, Role of biofilm roughness and hydrodynamic conditions in *Legionella pneumophila* adhesion to and detachment from simulated drinking water biofilms, *Environ. Sci. Technol.*, 2015, **49**(7), 4274–4282, DOI: 10.1021/es505842v.

36 A. E. Vardy, J. M. B. Brown, S. He, C. Ariyaratne and S. Gorji, Applicability of frozen-viscosity models of unsteady wall shear stress, *J. Hydraul. Eng.*, 2015, **141**(7), 04014064, DOI: 10.1061/(ASCE)HY.1943-7900.0000930.