

Proceeding Paper **Examining the Effect of Small-Amplitude Transients on the Shear Strength of Biofilms in Water Distribution Pipes †**

Mariele de Souza Parra Agostinho 1,2,* [,](https://orcid.org/0000-0002-5064-9815) Artur Sass Braga 3,[*](https://orcid.org/0000-0002-1425-7080) , Benjamin Anderson [3](https://orcid.org/0009-0001-9461-7620) , Yves Fillion [3](https://orcid.org/0000-0003-4309-0475) and Cristovão Vicente Scapulatempo Fernandes ²

- ¹ Parana Sanitation Company—Sanepar, Curitiba 80215900, Paraná, Brazil
- ² Department of Hydraulics and Sanitation, Federal University of Paraná—UFPR, Curitiba 80060000, Paraná, Brazil; cvs.fernandes@gmail.com
- ³ Department of Civil Engineering, Queen's University, Kingston, ON K7L 3N6, Canada; ben.anderson@queensu.ca (B.A.); yves.filion@queensu.ca (Y.F.)
- ***** Correspondence: marielespa@gmail.com (M.d.S.P.A.); asb9@queensu.ca (A.S.B.)
- † Presented at the 3rd International Joint Conference on Water Distribution Systems Analysis & Computing and Control for the Water Industry (WDSA/CCWI 2024), Ferrara, Italy, 1–4 July 2024.

Abstract: The dynamics of biofilm detachment from the pipe walls of drinking water distribution systems was investigated through experiments in a full-scale pipe loop laboratory system. Biofilms grown under steady-state and transitory flow rates were compared. Flow cytometry was used to quantify the microbial cells of the biofilms, and biofilm shear strength was evaluated based on their capacity to resist mobilization when subjected to elevated flushing flow rates. The results suggest that biofilms grown under transitory flow regimes may develop stronger adhesion strength to the pipe wall. This paper contributes to the enhanced understanding of biofilm behaviour in drinking water systems and its potential impacts on water quality.

Keywords: biofilms; small-amplitude transients; wall shear stress; water discolouration

Citation: Agostinho, M.d.S.P.; Braga, A.S.; Anderson, B.; Fillion, Y.; Fernandes, C.V.S. Examining the Effect of Small-Amplitude Transients on the Shear Strength of Biofilms in Water Distribution Pipes. *Eng. Proc.* **2024**, *69*, 204. [https://doi.org/](https://doi.org/10.3390/engproc2024069204) [10.3390/engproc2024069204](https://doi.org/10.3390/engproc2024069204)

Academic Editors: Stefano Alvisi, Marco Franchini, Valentina Marsili and Filippo Mazzoni

Published: 22 October 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license [\(https://](https://creativecommons.org/licenses/by/4.0/) [creativecommons.org/licenses/by/](https://creativecommons.org/licenses/by/4.0/) $4.0/$).

1. Introduction

The study of shear stress dynamics in pipes during hydraulic transients is pivotal for the design and operation of drinking water distribution systems (DWDSs). The seminal work by Brunone and Berni (2010) which employed ultrasonic Doppler velocimetry to measure instantaneous velocity gradients was instrumental in understanding wall shear stress (WSS) behaviour during turbulent transient pressurized flows. Their research on the variations in accelerating and decelerating flows underscored the need for precise modelling in engineering applications [\[1\]](#page-3-0). Another study has also proposed models to predict WSS in unsteady turbulent flows through a weighting function based on a two-region viscosity distribution, offering a versatile tool for unsteady flow modelling in pipes [\[2\]](#page-3-1).

Parallel to hydraulic transient research, previous research on the shear strength of biofilms in pipelines has been important to assess water distribution system performance and maintenance needs. Previous research has highlighted the complexity of biofilm detachment mechanisms and has revealed a significant correlation between local detachment events and the WSS induced by pipe flow [\[3\]](#page-3-2). This has underscored the influence of local biofilm morphology and hydrodynamic conditions on detachment rates [\[3\]](#page-3-2). Fish et al. (2017) investigated the impact of different hydraulic patterns on DWDS biofilms, linking hydraulic conditions to biofilm physical and community structures and their subsequent influence on water quality degradation [\[4\]](#page-3-3). Shen et al. (2015) explored the relationship between biofilm roughness, hydrodynamic conditions, and the adhesion and detachment of *Legionella pneumophila* (a known pathogenic bacteria) in DWDSs [\[5\]](#page-3-4). So et al. (2015) modelled the effects of biofilm morphology on detachment, emphasizing the role of local shear stress and biofilm cohesiveness in biofilm dynamics under pulse shear conditions [\[6\]](#page-3-5).

Lastly, research from Weston et al. (2021) has demonstrated that material mobilization from pipe walls in an experimental setup predominantly occurred during the initial dynamic surge of the transient event [\[7\]](#page-3-6).

These studies collectively suggest that both the physical properties of biofilms, such as thickness and roughness, and the hydrodynamic conditions they are subjected to significantly influence biofilm formation, adhesion, and detachment processes. An improved understanding of these dynamics can assist water utilities in managing biofilm development in DWDSs and mitigate its impacts on drinking water quality. In this context, this paper aims to investigate the effect of small-amplitude transients on the shear strength of biofilms adhered to drinking water pipes. The working hypothesis of this paper is that regular, short-lived pressure waves produce regular short duration increases in WSS that force adaptations in the biofilm and increases its shear strength.

2. Material and Methods

Experiments were performed in the Drinking Water Distribution System Laboratory (DWDL)—a unique research facility in North America—using two identical pipe loops (A and B) of 200 m long full-scale PVC pipes with a 108 mm inner diameter. Both pipe loops operated with steady flow rates of 0.6 L/s (0.03 Pa) and a pressure of 280 kPa for 28 days. The experimental setup included a closed-loop system in which the water was not replaced or disinfected. Pipe loop B experienced consistent hydraulic transients—specifically, a 20 kPa surge every hour induced by the near-instantaneous closing of a solenoid valve, followed by a 30 s pause before reopening. In contrast, pipe loop A served as the control, and was maintained in a continuous steady state throughout the experiment. During periods of reduced flow rate, the steady-state WSS in the system decreased from 0.03 Pa at a flow rate of 0.6 L/s to 0.027 Pa, highlighting the impact of flow variations on shear stress levels within the pipes. In pipe loop B, the cumulative duration of transient events accounted for roughly 1.6% of the overall experimental period.

The experiment was started with the addition of an equal microbial population in both pipe loops. This initial microbial population was harvested from the local water supply using a granular activated carbon (GAC) filter. Once introduced into both pipe loops, the water was continuously amended with a small quantity of Nutrient Broth No.3 to allow for biofilm growth. After the conditioning period, each pipe loop was flushed with 3 incremental flushing steps using flow rates of 6.5 L/s, 11 L/s, and 14 L/s (wall shear stresses of 1.2 Pa, 3.1 Pa, and 5.0 Pa) to promote detachment of the accumulated biofilms and examine their adhesive shear strength. The bacterial cell concentration (BCC) within the bulk water and the bacterial cell density (BCD) on the pipe walls were evaluated through flow cytometry. Bulk water samples were taken in triplicate weekly during the biofilm growth phase and during each flushing step. Pipe wall samples were collected at the end of the growth phase (28 days) and after each one of the flushing steps (FS_1, FS_2, and FS_3). An average BCD was calculated using triplicate samples from five distinct locations distributed along the pipe loop in longitudinal and circumferential directions.

3. Results

Figure [1](#page-2-0) shows the BCD (a) and BCC (b) results across various experimental stages: the end of the growth phase (day 28) and the subsequent flushing steps (FS_1, FS_2, FS_3). It is noted that during the flushing operations, fresh drinking water was introduced into the loops to replace the flush water. This explains the larger values of BCC for the first flushing step (FS_1), since the BCC baseline for this step was the 28-day water after the conditioning phase, while the BCC values from FS_2 and FS_3 arose from biofilm detachment during those flush steps. The error bars of Figure [1](#page-2-0) represent the standard deviation of the samples, highlighting the spatial variability inherent in the biofilms.

Figure 1. (a) The average bacteria cell density (BCD) on the pipe walls and (**b**) the average bacteria cell concentration (BCC) in the bulk water at the end of the growth phase (28 days) and for each flushing tep (FS_1, FS_2, and FS_3) for pipe loop A (control) and pipe loop B (small-amplitude transients) step (FS_1, FS_2, and FS_3) for pipe loop A (control) and pipe loop B (small-amplitude transients).

detachment of the biofilm, as evidenced by the sharp increase in BCC (Figure [1b](#page-2-0)) during flushing and the corresponding decrease in BCD (Figure [1a](#page-2-0)) in comparison to the values measured at 28 days. Subsequent flushing steps within pipe loop A (FS_2 and FS_3) did not yield significant changes in BCD on the pipe wall when compared to FS_1. Further, the decrease in BCC during these flushing stages also suggested a decrease in biofilm detachment. The small BCD values in pipe loop A (Figure [1a](#page-2-0)) after the last flushing stage suggest that only small quantities of biofilms with high adhesion strength remained on the pipe wall after this last flush step. In pipe loop A (used as the control), the initial flushing step (FS_1) led to a notable

Conversely, pipe loop B exhibited no substantial variations in BCD on the pipe wall (Figure [1a](#page-2-0)). This finding is corroborated by the bulk water analyses from the first and second flushing steps (Figure [1b](#page-2-0)), which did not show an increase in cell count (BCC) compared to the preceding step. However, an unexpected increase in BCC in pipe loop B $\frac{1}{2}$ compared during the final fluching step. $\frac{1}{2}$ (Eigure, 1b) with counts reaching double was observed during the final flushing step FS_3 (Figure 1b), with counts reaching double was observed during the final flushing step FS_3 (Figure [1b](#page-2-0)), with counts reaching double the level found in FS_2. the level found in FS_2.

4. Discussion

4. Discussion The experiments provided preliminary evidence that pipe loops subjected to identical water quality and identical maximal flushing WSS yet exposed to slightly different flow conditions—one with constant WSS (pipe loop A) and the other with periodical unsteady WSS (pipe loop B)—developed biofilms with distinct shear strength profiles. The results suggest that the presence of regular, small-amplitude transients assisted in the development of biofilms with higher levels of shear strength (adhesion to the pipe walls). This distinction aligns with previous research [\[8\]](#page-3-7) that demonstrates the substantial influence of both the steadiness and duration of shear stress on biofilms' structure, mechanical properties, and microbial composition. According to Yang et al. [\[9\]](#page-4-0), such exposure to unsteady WSS can lead to biofilms with enhanced shear strength and potentially altered composition, suggesting an adaptive response to dynamic stress conditions This adaptive behaviour is theorized to be a survival mechanism, allowing biofilms to optimize their structure for behaviour is theorized to be a survival mechanism, allowing biofilms to optimize their nutrient acquisition and resistance against shear forces.

The results observed from the variable WSS conditions in pipe loop B have profound The results observed from the variable WSS conditions in pipe loop B have profound implications for biofilm formation and removal, since these demonstrate that conditions implications for biofilm formation and removal, since these demonstrate that conditions common to DWDSs can lead to "permanent biofilm accumulation" (un-flushable). This phenomenon is not merely a function of the biofilm's physical robustness but also of its phenomenon is not merely a function of the biofilm's physical robustness but also of its adaptive capacity and ecological resilience. As noted by Lemos et al. [\[10\]](#page-4-1), different shear adaptive capacity and ecological resilience. The noted by Echos et al. [10], different shear stress patterns can lead to varied biofilm phenotypic characteristics, including thickness, stress patterns can lead to varied biofilm phenotypic characteristics, including thickness, biomass production, and the content of extracellular polymeric substances. This adaptive biomass production, and the content of extracellular polymeric substances. This adaptive phenomenon underscores the necessity of adopting an integrated biofilm management phenomenon underscores the necessity of adopting an integrated biofilm management strategy in DWDSs. Traditional methods focused solely on physical removal or chemical

disinfection may prove insufficient against biofilms that have adapted to withstand variable shear stress conditions. A multifaceted approach that combines mechanical disruption, targeted chemical treatments, and perhaps even biological control measures may be required. Tailoring these interventions to the specific hydraulic and microbial dynamics observed in DWDSs could enhance the efficacy of biofilm management practices.

5. Conclusions

The findings of this study have pivotal implications for the design and management of water distribution systems, particularly concerning the operational strategies aimed at controlling biofilm development and ensuring the potability of distributed water. The augmented shear strength of biofilms under transient conditions warrants further exploration into their mechanical properties and shear stress resistance profiles. Understanding these aspects is critical for refining maintenance practices, such as flushing and disinfection protocols, potentially leading to more effective management of biofilm presence in water distribution networks.

Author Contributions: Conceptualization, M.d.S.P.A. and A.S.B.; methodology, A.S.B., M.d.S.P.A., B.A., C.V.S.F. and Y.F.; investigation, A.S.B., M.d.S.P.A. and B.A.; resources, A.S.B. and Y.F.; writing, M.d.S.P.A., A.S.B., B.A., Y.F. and C.V.S.F.; visualization, M.d.S.P.A.; supervision, A.S.B., Y.F. and C.V.S.F.; project administration, Y.F.; funding acquisition, Y.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets presented in this article are not readily available because the data are part of an ongoing study. Requests to access the datasets should be directed to the corresponding author.

Acknowledgments: CAPES—Print: Brazilian Institutional Internationalization Program.

Conflicts of Interest: Author Mariele de Souza Parra Agostinho was employed by the Parana Sanitation Company—Sanepar. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- 1. Brunone, B.; Berni, A. Wall Shear Stress in Transient Turbulent Pipe Flow by Local Velocity Measurement. *J. Hydraul. Eng.* **2010**, *136*, 10. [\[CrossRef\]](https://doi.org/10.1061/(ASCE)HY.1943-7900.0000234)
- 2. Vardy, A.E.; Brown, J.M.B.; He, S.; Ariyaratne, C.; Gorji, S. Applicability of Frozen-Viscosity Models of Unsteady Wall Shear Stress. *J. Hydraul. Eng.* **2015**, *141*, 04014064. [\[CrossRef\]](https://doi.org/10.1061/(ASCE)HY.1943-7900.0000930)
- 3. Wang, S.; Zhu, H.; Zheng, G.; Dong, F.; Liu, C. Dynamic Changes in Biofilm Structures under Dynamic Flow Conditions. *Appl. Environ. Microbiol.* **2022**, *88*, 22. [\[CrossRef\]](https://doi.org/10.1128/aem.01072-22) [\[PubMed\]](https://www.ncbi.nlm.nih.gov/pubmed/36300948)
- 4. Fish, K.; Osborn, A.M.; Boxall, J. 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. [\[CrossRef\]](https://doi.org/10.1016/j.scitotenv.2017.03.176) [\[PubMed\]](https://www.ncbi.nlm.nih.gov/pubmed/28360007)
- 5. Shen, Y.; Monroy, G.L.; Derlon, N.; Janjaroen, D.; Huang, C.; Morgenroth, E.; Liu, W.-T. Role of biofilm roughness and hydrodynamic conditions in Legionella pneumophila adhesion to and detachment from simulated drinking water biofilms. *Environ. Sci. Technol.* **2015**, *49*, 4274–4282. [\[CrossRef\]](https://doi.org/10.1021/es505842v) [\[PubMed\]](https://www.ncbi.nlm.nih.gov/pubmed/25699403)
- 6. So, M.; Terashima, M.; Goel, R.; Yasui, H. Modelling the Effect of Biofilm Morphology on Detachment. *J. Water Environ. Technol.* **2015**, *13*, 49–62. [\[CrossRef\]](https://doi.org/10.2965/jwet.2015.49)
- 7. Weston, S.L.; Collins, R.P.; Boxall, J.B. An experimental study of how hydraulic transients cause mobilisation of material within drinking water distribution systems. *Water Res.* **2021**, *194*, 116890. [\[CrossRef\]](https://doi.org/10.1016/j.watres.2021.116890) [\[PubMed\]](https://www.ncbi.nlm.nih.gov/pubmed/33592354)
- 8. Rochex, A.; Godon, J.J.; Bernet, N.; Escudié, R. Role of shear stress on composition, diversity and dynamics of biofilm bacterial communities. *Water Res.* **2008**, *42*, 4915–4922. [\[CrossRef\]](https://doi.org/10.1016/j.watres.2008.09.015) [\[PubMed\]](https://www.ncbi.nlm.nih.gov/pubmed/18945468)
- 9. Yang, J.; Cheng, S.; Li, C.; Sun, Y.; Huang, H. Shear Stress Affects Biofilm Structure and Consequently Current Generation of Bioanode in Microbial Electrochemical Systems (MESs). *Front. Microbiol.* **2019**, *10*, 398. [\[CrossRef\]](https://doi.org/10.3389/fmicb.2019.00398) [\[PubMed\]](https://www.ncbi.nlm.nih.gov/pubmed/30894842)
- 10. Lemos, M.; Mergulhão, F.; Melo, L.; Simões, M. The effect of shear stress on the formation and removal of Bacillus cereus biofilms. *Food Bioprod. Process.* **2015**, *93*, 242–248. [\[CrossRef\]](https://doi.org/10.1016/j.fbp.2014.09.005)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.