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Incorporation and Mobilisation of Health-Related Organisms from within Drinking Water Biofilm †

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Abstract: The present study explored the incorporation of health-related organisms within drinking water biofilms and bulk water quality using benchtop-scale distribution systems. The annular reactors simulated the dead-end pipes of distribution systems with low shear stress but maintained a chlorine residual and formed young biofilms on plastic surfaces. Spiked coliforms and *Escherichia coli* were introduced to the annular reactors after 1 month of growth. Although initially detected in the spike, the coliforms were inactivated in the bulk water phase, likely due to environmental stresses, such as nutrient starvation and residual chlorine. Also, coliform incorporation within biofilm was only detected in a single coupon in the reactor 24 h post-spike (with 100% lake water), suggesting they were not incorporated or under the detection limit.

Keywords: annular reactor; biofilm; coliforms; distribution systems

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1. Introduction

Drinking water biofilms are formed by the attachment of planktonic cells on pipe walls and can serve as a reservoir or a shield for bacteria and pathogens to survive against environmental stresses, such as residual disinfectants and low nutrient contents [1]. Health-relevant organisms such as coliforms, *Escherichia coli*, *Legionella*, and other pathogens are of particular concern to water utilities and can be embedded and incorporated within drinking water biofilm [2]. Once incorporated, these organisms can persist within biofilms for months. These processes are not well understood. We also do not understand how and if pathogens incorporated within these biofilms can be mobilised into the water column and cause secondary contamination of drinking water.

The present study investigated coliform incorporation following different exposures of coliforms within drinking water biofilm formed from the indigenous community under hydraulic conditions replicating the drinking water distribution system (DWDS). The growth phase allowed biofilm formation and was followed by a 'spike' in natural contaminants (including coliforms, amongst other potential opportunistic microorganisms) with behaviour including any incorporation of health-related organisms allowed to occur. The hydraulic conditions were then increased to replicate flushing conditions to gain an understanding of the mobilisation of biofilm, including any health-relevant organisms, back into the bulk water.

2. Materials and Methods

2.1. Experimental Set-Up

The incorporation of health-related indicator organisms within drinking water biofilms was explored using benchtop-scale systems. During the growth phase, the hydraulic regimes simulated low shear stress, similar to low-demand areas of networks or a dead-end pipe, but with a chlorine residual maintained. Systems were then spiked with the environmental contaminant (48 h) prior to a flushing phase to assess mobilisation. Bulk water and biofilm analysis included cell quantification and viability (via flow cytometry) and coliform quantification (via Colilert MPN).

Three laboratory-scale annular reactors (ARs; 1420LJ, BioSurface Tech, Bozeman, MT, USA) were used to grow drinking water biofilms for 41 days, fed with chlorinated tap water (from the local supply network and surface water source) and operated at a 22 RPM rotational speed (Reynolds number, 1660; shear stress, 0.005 N m^{-2}) (Figure 1a). Tap water was introduced to the AR from reservoir containers (15 L) with a 30 mL min^{-1} flow rate via a peristaltic pump, which was refreshed three times per week. High-density polyethlene (HDPE) coupon slides were cleaned, autoclaved, and inserted into coupon slots (total number = 20, per AR). Biofilms were sampled at the start and end of this biofilm development phase (Figure 1b).

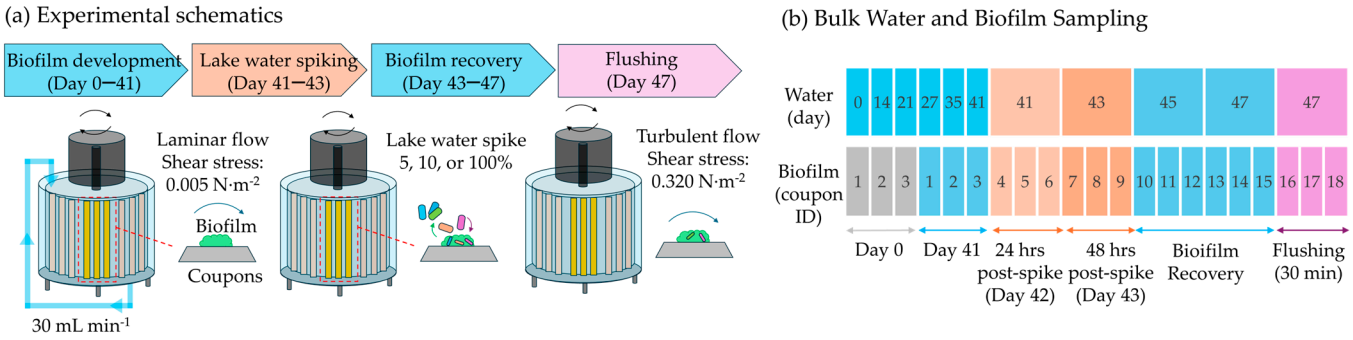


Figure 1. Experimental schematics of the annular reactor and biofilm coupon sampling.

To understand the coliform incorporation within biofilms, after 41 days, the reactors were spiked with different proportions of lake water and exposed to the spike for two days in the closed system (5, 10, and 100% v/v lake water to tap water in a 1 L reactor volume) (Figure 1). The lake water was collected from Crookes Valley, Sheffield. The presence/absence and concentrations of total coliform and *Escherichia coli* were determined by Colilert tray assays (IDEXX, UK). The lake water was positive for total coliform and *Escherichia coli* (271 and 53 MPN per 100 mL, respectively), but *Legionella* species were not detected (Legiolert, IDEXX). Biofilms were collected 24 and 48 hrs post-spike, as well as during a recovery/regrowth phase after the spiking, in which the ARs were once again supplied with tap water (Figure 1b). For flushing, the reactors were run with turbulent flow for 30 min (250 RPMs; Reynolds number, 18,890; shear stress, 0.32 N m^{-2}).

2.2. Bulk Water and Biofilm Sample Analysis

Free and total chlorine, temperature, turbidity, pH, and ORP (oxidation–reduction potential) were recorded as bulk water quality, Table 1. Water quality was measured weekly for inlet (fresh) tap water and for two-day-old water in each reactor. Biofilm was sampled at days 0, 40, post-spike (24 and 48 h), at the end of the recovery/regrowth phase and post-flushing by removing coupons (n = 3, surface area of each 18.75 cm^2) and brushing into 30 mL phosphate-buffered solution [3]. Total and intact cell concentrations (TCC and ICC) were measured (via flow cytometry) for bulk water and biofilm samples using Attune NxT cytometer (Thermo Fisher Scientific, Waltham, MA, USA). Planktonic and biofilm coliform quantification was undertaken via Colilert assays (presence/absence, most probable number).

Table 1. Bulk water quality for annular reactors (ARs) during biofilm development (n = 18, weekly triplicates).

Bulk Water	Inlet	AR1	AR2	AR3
[(a)] TCC ($\times 10^3$ cells mL^{-1})	3.3 ± 6.4	0.9 ± 0.3	1.5 ± 2.8	1.4 ± 1.1
[(b)] ICC ($\times 10^3$ cells mL^{-1})	1.7 ± 4.1	0.3 ± 0.3	0.7 ± 1.2	0.5 ± 0.5
Free chlorine (mg L^{-1})	0.63 ± 0.05	0.34 ± 0.24	0.34 ± 0.26	0.33 ± 0.23
Total chlorine (mg L^{-1})	0.68 ± 0.06	0.37 ± 0.23	0.39 ± 0.26	0.38 ± 0.24
Temperature (°C)	9.8 ± 1.0	12.3 ± 0.9	12.5 ± 0.8	12.5 ± 0.8
Turbidity (NTU)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
[(c)] ORP (mV)	672 ± 16	635 ± 74	625 ± 87	643 ± 77
pH	7.43 ± 0.15	7.28 ± 0.06	7.27 ± 0.05	7.28 ± 0.05

[(a)] total cell count, [(b)] intact cell count, and [(c)] oxdiation–reduction potential, ± standard deviation.

3. Results and Discussions

3.1. Bulk Water Quality and Biofilm Formation

During biofilm development, coliforms were not found in inlet drinking water or 2-day-old drinking water in the reactors. Inlet TCC and ICC, Figure 2, fluctuated over the course of sampling, but bacterial cells were dramatically inactivated, and ICC ranged below 0.7 × 10^3 cells mL^{-1} inside the reactors. Free chlorine was consumed by 48% in the reactors, but a residual of 0.37–0.39 mg L^{-1} remained (on average) higher than real dead-end pipes (<0.20 mg L^{-1}). During biofilm formation (days 0–41), coliforms were not found in drinking water biofilms due to the absence of seeding coliforms in inlet water. Even though the biofilm developed under the same conditions, the biofilm cell counts fluctuated and ranged from 768 ± 305 to 3253 ± 1779 cells cm^{-2} between reactors, which was dramatically low compared to previous studies under turbulent regimes [4,5]. The biofilm cell content could vary due to the laminar flow regime, as had been reported in other studies [5,6]. The cell mobilisation from young biofilm was insignificant during the flushing.

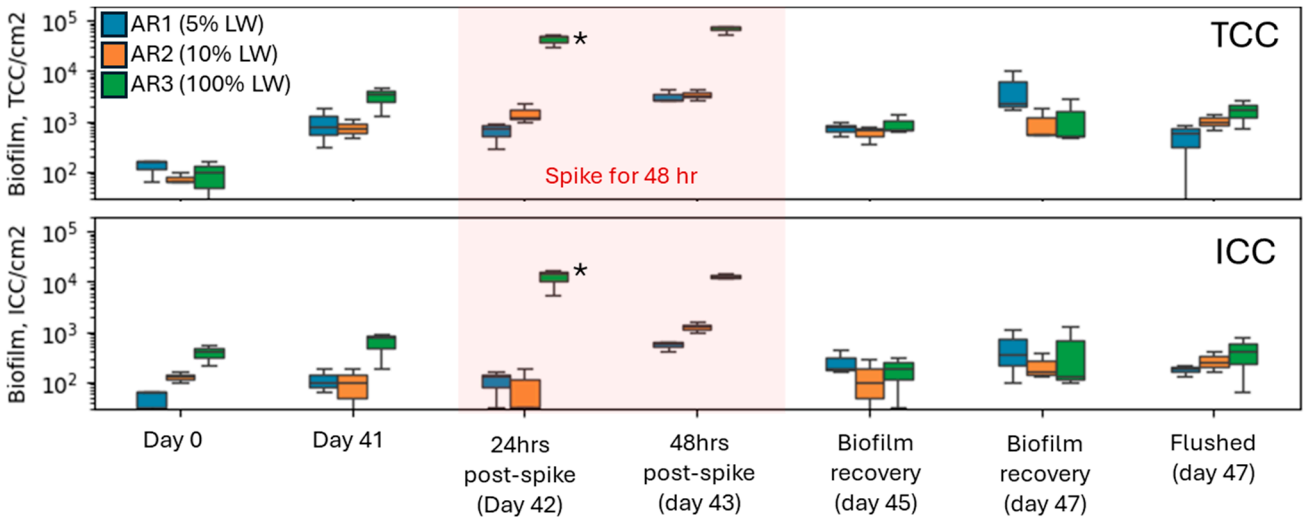


Figure 2. Biofilm total and intact cell accumulation and mobilisation throughout the experiment. * indicates the biofilm where coliforms were detected. During the spike, AR1 = 5% lake water, AR2 = 10% lake water, and AR3 = 100% lake water (LW).

3.2. Coliform Spike and Incorporation

The lake water spikes of 5, 10, and 100% contained 0.08, 0.17, and 1.40 × 10^6 TCC mL^{-1}, respectively. The lake water contained 0.53 × 10^6 ICC mL^{-1}, whereas 5 and 10% diluted lake water contained less than 50 ICC mL^{-1}. Therefore, the free chlorine residual in the tap water (which was higher than in a typical dead-end pipe) may have inactivated the

lake water microbiome in 5 and 10% spike water. Chlorine residual plays the primary role in mitigating the planktonic regrowth, or biofilm seeding, caused by potential pathogens (or undesired micro-organisms) [3]. This could suggest that the chlorine residual within the reactors may have been sufficient to inactivate coliforms and *Escherichia coli* in the first 24 h as they were not observed in spiked water for ARs 1 and 2 (lake water 5 and 10%, respectively). Conversely, coliforms were detected in 2-day-old spiked water in AR3 (100% spike), which did not have a chlorine residual, but *Escherichia coli* was not detected, suggesting this was inactivated by stressful conditions such as nutrient starvation.

During 24 hrs post-spike, the biofilm cell counts dramatically increased up to 3.3-, 4.4-, and 44.8-fold for ARs 1, 2, and 3, respectively. Interestingly, coliforms and *Escherichia coli* were typically not detected in the post-spike biofilm samples except for one coupon (24 h post-spike for AR3). This could suggest that coliforms preferred to be in the planktonic form for the conditions investigated. Coliforms were not well incorporated within drinking water biofilm during acute exposure, were metabolically inactive, or were under the detection limit. Previous studies have highlighted the incorporation of organisms, such as *Legionella pneumophila* or *Pseudomonas aeruginosa*, adapted to oligotrophic conditions [1]. However, this incorporation can be boosted by mature biofilms and turbulent hydraulic regimes. Further experiments with biofilm age and a turbulent hydraulic regime are required to understand the behaviours of health-related organisms under various hydraulic conditions and the chances of biofilm attachment/incorporation [6].

4. Conclusions

This study highlights that coliform incorporation within biofilms is only observed in the early phase of the spike. Biofilm age and operating conditions (constant laminar flow and chlorine residual) in this study were unfavourable conditions for coliform proliferation/incorporation. Young biofilm did not significantly mobilise during flushing.

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