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Regrowth of Microorganisms from Treatment to Tap in Operational Drinking Water Supply Networks †

Isabel Carneiro [1,*], Katherine Fish [1], Peter Jarvis [2], John Haley [3], Fiona Webber [4], Paul Gaskin [5] and Joby Boxall [1]

[1] Department of Civil and Structural Engineering, The University of Sheffield, Sheffield S1 3JD, UK; k.fish@sheffield.ac.uk (K.F.); j.b.boxall@sheffield.ac.uk (J.B.)
[2] Water Science Institute, Cranfield University, Bedford MK43 0AL, UK; p.jarvis@cranfield.ac.uk
[3] UK Water Industry Research, London SE1 2RE, UK; john.a.haley@outlook.com
[4] Scottish Water, Stepps G33 6FB, UK; fiona.webber@scottishwater.co.uk
[5] Dŵr Cymru Welsh Water, Cardiff CF3 0LT, UK; paul.gaskin@dwrcymru.com
* Correspondence: icarneirocardosodasilva1@sheffield.ac.uk
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Abstract: This research provides new understanding of the (re)growth of microorganisms within drinking water distribution networks. Flow cytometry data quantifying total and intact cell counts from consumers' taps are currently rare, and its value for aiding understanding of the growth of microorganisms is unknown. In this study, changes in microbial concentrations from the treatment works to customers' taps were measured (using flow cytometry) in two UK drinking water distribution networks. Throughout each network, five locations were sampled for five consecutive days within a week, and to assess seasonal impacts, this was repeated twice in each network. Significant growth of microorganisms was observed in both networks during autumn, particularly at higher-water-age taps. These results give novel emphasis to the active impact of the distribution system on microbiological growth by using flow cytometry data collected in a systematic way from treatment through to customers' taps. Such understanding is essential to achieving the delivery of safe and aesthetically pleasing drinking water to customers.

Keywords: biological stability; water quality; drinking water distribution systems; bacteria

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1. Introduction

Achieving biological stability is essential to the delivery of safe and aesthetically pleasing drinking water. Uncontrolled (re)growth of microorganisms in drinking water supply systems (DWSSs) can deteriorate water quality and impact taste, color, and odor [1–5]. Water utilities are increasingly going beyond regulatory requirements of heterotrophic plate counts and using flow cytometry techniques to collect total and intact cell count data from treatment works and service reservoir outlets. However, this information is still limited at customer taps, and its significance for determining a system's biological stability is unknown. This work aims to understand the value of measuring cell counts from tap water samples for assessing biological stability. It investigates the impact of water age and seasonality on (re)growth of microorganisms by sampling customer taps with a range of travel times during two periods of the year.

2. Materials and Methods

2.1. Sampling Point Selection

Two operational DWSSs (A and B) were selected in two different regions of the UK. In DWSS A, water is abstracted from a river and the water treatment process consists of coagulation, clarification, a rapid gravity filter, granular activated carbon adsorbers, and

chlorine dosing. It has the capacity to treat 49 MLD. The service reservoir is located 4.6 km downstream with a total storage capacity of 2073 m^3 of water and operational retention time of 19 h during summer and 20 h in winter. In DWSS B, water is abstracted from upland reservoirs, and treatment consists of coagulation, clarification filtration, and chlorination with the capacity to treat 230 MLD of water. For each network, maps and hydraulic models were studied to carefully select sampling points within district metered areas downstream of a single service reservoir and treatment works (Figure 1). Sample points consisted of the water treatment work's final water, a service reservoir outlet in system A and 3 (DWSS A) or 4 (DWSS B) network points to cover young, medium, and older residence times for each network. In DWSS B, two older water taps (Networks 3 and 4, Figure 1B), were added to replace a younger one (Network 0) during the second round of sampling. Figure 1 displays an overview of both systems.

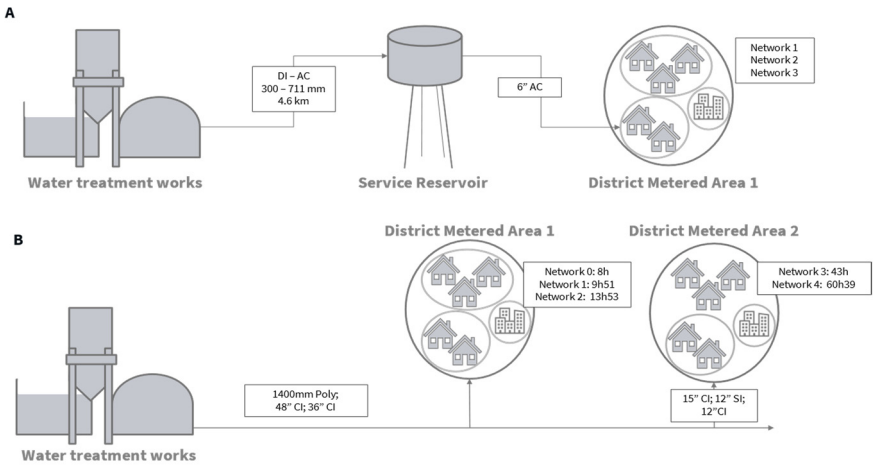

Figure 1. Schematics of DWSS (**A**,**B**) displaying connectivity between sampling points. Estimated travel time and pipe length and diameter are indicated where known. DI: ductile iron, AC: asbestos cement, Poly: polyethylene, CI: cast iron, SI: spun iron.

2.2. Water Samples

For each DWSS, water samples were collected for five days (Monday–Friday) from all five of the selected points (Figure 1). This was repeated twice for each system at an approximately 3-month interval to capture seasonality (autumn/winter). Samples were collected in 500 mL sterile plastic bottles preprepared with sodium thiosulfate and transported to the University of Sheffield for microbiological analysis within 24–32 h. Free chlorine concentration and water temperature were measured on-site at the time of sampling.

2.3. Water Quality: Microbial Concentration and Viability

Total and intact cell counts (TCCs and ICCs) were measured in triplicate (n = 3) using the flow cytometry protocol described elsewhere [1]. Briefly, 500 µL samples were stained with SYBR Green I for total cells or SYBR Green I and propidium iodide for intact cells and incubated at 37 °C for 10 min. Measurements were performed using an Attune NxT flow cytometer (Thermo Fisher Scientific, Waltham, MA, USA).

3. Results

Figure 2 displays changes in ICC and free chlorine residual during the first round of sampling in autumn. Flow cytometry results show repeatability between triplicate measurements and were consistent throughout the week. In both DWSSs, flow cytometry analysis of tap water samples was able to capture microbial growth as water traveled through the network. The highest intact cell counts are accompanied by lowest free chlorine concentrations in both networks at the oldest water sampling point. In DWSS A,

the average water temperature was 14.1 °C leaving the treatment works. This increases through the network, reaching 16.1 °C at the oldest sampling point.

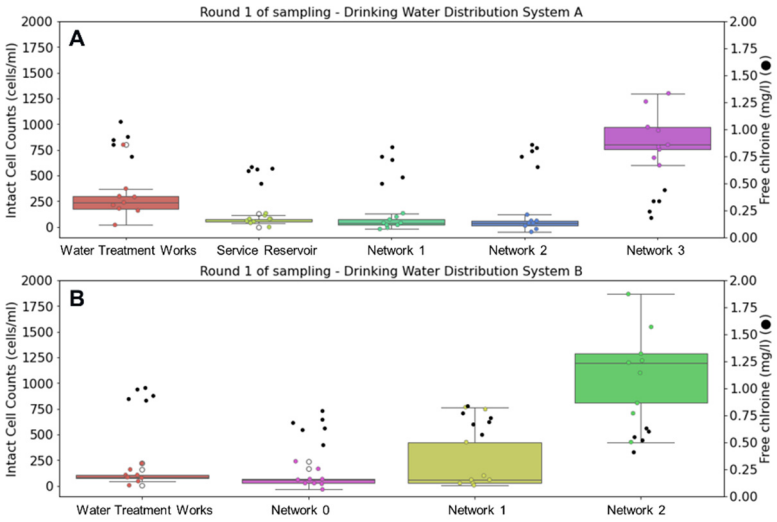

Figure 2. Comparison of intact cell counts (ICCs) (left) and chlorine concentrations (•) (right) at drinking water supply systems (**A**,**B**) during autumn sampling. Whisker plots show data distribution of triplicate samples collected over three days (n = 9) for ICCs, where horizontal lines indicate from top to bottom maximum, upper quartile, median, lower quartile, and minimum values of the dataset.

Figure 3 displays changes in intact cell counts and free chlorine residual during the second round of sampling in winter. Water temperature leaving the treatment works of DWSS A averaged 4.8 °C and reached a maximum of 7.1 °C in the network. At considerably lower water temperatures, no growth or chlorine decay was observed in either of these systems, despite the addition of older water sampling points in DWSS B.

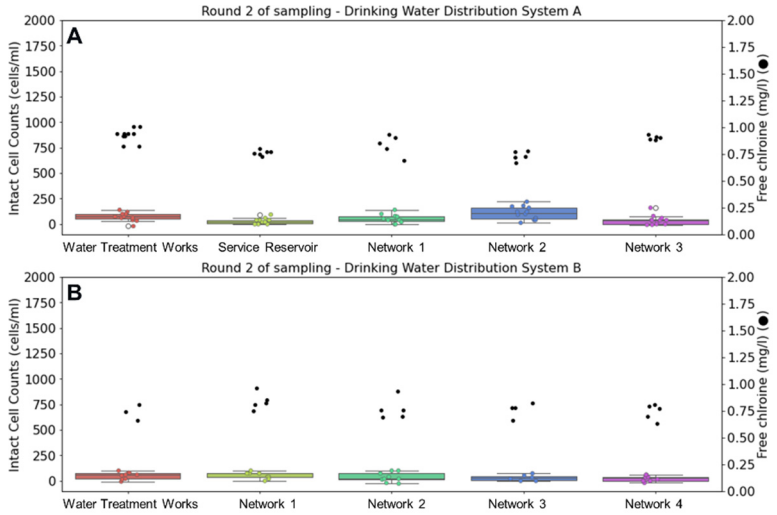

Figure 3. Comparison of intact cell counts (ICC) (left) and chlorine concentrations (right) at drinking water supply systems (**A**,**B**) during winter sampling. Whisker plots show ICC data distribution of triplicate samples collected over four days (n = 12) for DWSS A and three days (n = 9) for DWSS B. Horizontal lines indicate, from top to bottom, maximum, upper quartile, median, lower quartile, and minimum values of the dataset.

4. Discussion

This work studied changes in microbial concentrations in two operational DWSSs, from treatment to tap, going beyond typical flow cytometry sampling points at the water

treatment works and service reservoirs. In DWSS A, considerable growth was observed at the furthest point of the network, with a threefold increase in intact cell counts compared to the outlet of the treatment and thirteenfold compared to the outlet of the reservoir for the first round of sampling. Similar results were observed for DWSS B, with an elevenfold increase in intact cell counts at the furthest network tap sampled in the first round of sampling. This suggests flow cytometry analysis of samples from customer taps can provide further unique insights into a systems' biological (in)stability that cannot be conveyed by only sampling treatment works and service reservoirs. When sampling was repeated during winter, approximately three months later, no growth was observed at any of the sampling points. At lower temperatures, chlorine decay was less extensive, which was likely both a complex cause and effect of biological stability. Substantial seasonal differences have also been observed in previous studies [2–5], reinforcing the importance of considering seasonal variations when studying biological stability.

5. Conclusions

- Flow cytometry data from drinking water samples collected at network taps provided valuable insight into the systems' biological stability by capturing growth beyond typical flow cytometry sampling analysis points (WTW and SR).
- Seasonal variations in biological stability were observed within two different systems. Both DWSSs were less stable, displaying more growth during autumn, when water temperatures reached 16 °C, but were completely stable during winter, when water temperatures reached a maximum of only 7 °C.
- Understanding the factors affecting growth of microorganisms from treatment works to tap can inform improved design and management of water distribution networks to assure delivery of safe and aesthetically pleasing drinking water.

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Conflicts of Interest: Author Fiona Webber was employed by the company Scottish Water. Author Paul Gaskin was employed by the company Dŵr Cymru Welsh Water. 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.

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