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Assessing microbiological water quality in drinking water distribution systems with disinfectant residual using flow cytometry

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ABSTRACT

Flow cytometry (FCM) as a diagnostic tool for enumeration and characterization of microorganisms is rapidly gaining popularity and is increasingly applied in the water industry. In this study we applied the method to obtain a better understanding of total and intact cell concentrations in three different drinking water distribution systems (one using chlorine and two using chloramines as secondary disinfectants). Chloramine tended to result in lower proportions of intact cells than chlorine over a wider residual range, in agreement with existing knowledge that chloramine suppresses regrowth more efficiently. For chlorinated systems, free chlorine concentrations above 0.5 mg L^{-1} were found to be associated with relatively low proportions of intact cells, whereas lower disinfectant levels could result in substantially higher percentages of intact cells. The threshold for chlorinated systems is in good agreement with guidelines from the World Health Organization. The fact that the vast majority of samples failing the regulatory coliform standard also showed elevated proportions of intact cells suggests that this parameter might be useful for evaluating risk of failure. Another interesting parameter for judging the microbiological status of water, the biological regrowth potential, greatly varied among different finished waters providing potential help for investment decisions. For its measurement, a simple method was introduced that can easily be performed by water utilities with FCM capability.

1. Introduction

Monitoring the microbiological cleanliness and hygiene of water distribution systems (WDS) is of critical importance to protect public health especially in times challenged with ageing drinking water infrastructure. In the absence of faecal indicators in treated water, heterotrophic plate counts (HPCs) constitute a standard tool to assess general microbiological quality (Bartram et al., 2004; Robertson and Brooks, 2003). Although not an indicator of adverse human health effects, increases in counts can indicate problems with raw water

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quality, water treatment, or in the distribution system (Health Canada, 2012). HPC concentrations in distribution systems are influenced by various water parameters including disinfectant residual, availability of biodegradable nutrients, pipe materials and roughness, surface area-to-volume ratios, stagnation, temperature and hydraulic variations (Allen et al., 2004; Carter et al., 2000; Chowdhury, 2012; LeChevallier, 2003). Although HPC numbers can occasionally be elevated, operational decision makers are faced with the problem of long lists of zero count samples. As distribution systems are obviously not sterile, this reflects that the conventional approach is of limited value as a diagnostic tool. Different factors can contribute to the absence of counts including sublethal injury (due to residual disinfectant), non-culturability of organisms with commonly used growth media, inadequate growth temperature or time or excess presence of non-heterotrophic organisms (Health Canada, 2012). This together with the fact that traditional HPC procedures enumerate only approx. 1% of the total bacterial population in drinking water as observed by direct microscopy (van der Kooj, 2003; Wagner et al., 1993), makes more comprehensive cultivation-independent methods appear attractive.

One of the most promising developments in microbiological monitoring of water quality is flow cytometry (FCM). Robust protocols are available that have undergone thorough testing over the recent years (Berney et al., 2008; Hammes et al., 2008). Applications range from assessing absolute numbers of cells in different water types, monitoring treatment efficacy, changes in microbial numbers with increasing water age, or assessment of the physiological status of subpopulations (Hammes and Egli, 2010a; Hewitt and Nebe-Von-Caron, 2004; Wang et al., 2010). The success in academic research and the availability of affordable instrumentation has resulted in increased awareness of FCM in the water industry, which was greatly enhanced by the fact that Switzerland as the first country incorporated FCM-based standardized and validated method no. 333.1 for determining total cell counts and distinct bacterial populations in freshwater into the Swiss Food Compendium as a recommended test method (SLMB, 2012). The fast analysis time with results available within 15–20 min compared to at least 2 days for HPC assays increases the appeal. Bacterial numbers detected in drinking water by FCM are typically several orders of magnitude greater than the numbers of HPC (Hammes et al., 2008; Hoefel et al., 2003).

Despite the promise of the method, applications to full scale chlorinated/chloraminated distribution systems are currently rare. This study aimed at gaining insight into differences in microbiological water quality for three selected distribution networks receiving water that has undergone different treatment trains, supplemented with either with free chlorine or chloramines as secondary disinfectants. Apart from mere measurement of cell concentrations, we aimed to define useful practical indicators for operational decisions. Parameters measured included disinfectant concentrations and concentrations of total and intact cells. As microbial numbers can widely vary between different waters, the absolute numbers of microorganisms were used to determine the relative proportions of intact cells. The correlation between disinfectant concentrations and percentage of intact cells was considered interesting as chlorine concentrations had previously been shown to be inversely proportional to cell survival (Helbling and VanBriesen, 2008). Moreover a simple method to determine regrowth potential was applied to gain further insight into how different raw and finished waters compare.

2. Materials and methods

2.1. Samples and treatment trains

Water samples were taken from a variety of different Water Treatment Works (WTW; samples referred to as Treatment Works Final Water) and corresponding distribution systems/service reservoir outlets in the Scottish Water distribution network (treated distributed water). Free chlorine was used as secondary disinfectant in distribution system I, chloramine in systems II and III. System III received water from two treatment works: WTW III-A and –III-B. All of these WTWs were fed by surface water sources with treatment trains shown in Table 1. In addition to Treatment Works Final Water, samples from 11 service reservoirs for each of the three distribution systems were considered in this study.

2.2. Sampling procedure

Sampling was performed according to Scottish Water sampling procedures. Taps were flushed for 3 min and then flame sterilized using a blow torch for 30 s. After flushing the tap for an additional 30 s, water was sampled into sterile 500 mL sample bottles (Aurora Scientific, Bristol, U.K.) containing pre-aliquoted thiosulfate to eliminate residual disinfectant. Samples were stored and transported in a refrigerated van at 5 °C (±3 °C) and arrived in the laboratory within 24 h of sampling. No data was available to allow accurate assessment of the distances from the treatment works to the sample points in the network, due to the complexity of the distribution systems, which meant that water could take different routes to the same point depending on which valves were open or closed. Residence times are highly dependent on the water demand, which in turn varies significantly according to factors such as weather and fluctuations in the transient population.

2.3. Chlorine measurements

The measurement of free and total chlorine residuals was performed at the time of sampling, using a pocket colorimeter (Hach-Lange, Salford, U.K.) set to the Low (LO) range mode. Sample cells were filled with 10 mL of water and any liquid spills were wiped off the outside of the cells using paper tissue. After reading the blank, the free and total chlorine DPD powders were added to the samples and cells were gently swirled for 30 s, followed by a 1 min incubation prior to measurement.

2.4. Flow cytometric measurements

For measurements of total cell concentrations (TCC), SYBR Green I (10, 000 × stock, cat. no. S-7567; Life Technologies Ltd.,