Short-term microbial dynamics in a drinking water plant treating groundwater with occasional high microbial loads

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ABSTRACT

Short-term fluctuations in bacterial concentrations in drinking water systems, occurring on time scales of hours-to-weeks, are essentially unexplored due to a lack of microbial monitoring tools that allow high frequency measurements. Here, we applied fully automated online flow cytometry to measure the total cell concentrations (TCC) in both raw water (karstic groundwater) and treated water (flocculation — ultrafiltration (UF) — ozonation — granular active carbon (GAC) filtration) during a period of 70 days at high temporal resolution (n > 4000 for both water types). We detected and characterized in considerable detail aperiodic fluctuations in the raw water following regional precipitation, with TCC increasing up to 50-fold from a dry weather baseline of approximately 120 cells μl^{-1} to an event peak of > 5000 cells μl^{-1}. Moreover, we observed the buffering of the treatment plant against these fluctuations, but in addition we recorded a completely unexpected periodic fluctuation of TCC in the treated water after GAC filtration. We concluded that the latter was the result of fluctuating water abstraction from the treatment plant reservoir by two connected water utilities, which resulted in variations in water throughput in the plant. This in turn influenced bacterial detachment and dilution in the GAC filter. This study provides strong evidence of multiple different microbial dynamics occurring in a drinking water treatment system. Given numerous possible sources of natural and operational fluctuations in raw water and drinking water treatment plants, such microbial fluctuations should be expected in many systems. The high-frequency monitoring approach presented herein can improve the understanding and eventual mitigation of such fluctuations.

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1. Introduction

Water treatment systems are designed and operated to ensure safe and clean drinking water of consistent quality (LeChevallier and Au, 2004; World Health Organization, 2011). However, a variety of short-term fluctuations are often inherent to drinking water treatment systems. This can include fluctuations in raw water quality, intermittent water extraction, periodic particle filter backwashing and varying water throughput (Stevenson, 1997; Kistemann et al., 2002; LeChevallier and Au, 2004). Unknown (or poorly characterized) fluctuations are generally undesired in production processes because they make it harder to control and ensure the quality of the output. Arguably, an ideal treatment system is one that buffers completely against uncontrollable fluctuations in source water, while not introducing new and unknown fluctuations in the treated water.

Proper process monitoring is a crucial component for all production processes. An example from drinking water treatment is the real-time monitoring and steering of ozonation reactors with online ozone-sensors (Kaiser et al., 2013). Suitable monitoring tools for microbiology that allow for high temporal resolution and short analysis times were not available until recently. Conventional cultivation-based methods are by default too slow, while most alternative methods (e.g., microscopy, PCR) have not yet been automated successfully or are not yet sufficiently quantitative for process monitoring and control. Nevertheless, attempts to detect short-term microbiological fluctuations in water treatment and distribution systems were made during the last few years through labor-intensive manual measurements of high frequency grab samples (Nescerecka et al., 2014; Prest et al., 2014). Furthermore, microbial detection methods are increasingly being automated. For example, Besmer et al. (2014) showed automated FCM

measurements to be feasible and similar attempts were made for adenosine tri-phosphate (ATP) (Vang et al., 2014) and for optical-based bacterial detection systems (Hojris et al., 2016).

Short-term microbial dynamics in drinking water treatment plants are largely unknown due to the absence of relevant data sets, given the above-described shortage of suitable detection methods. In particular, the link between operational procedures and microbial dynamics is largely unexplored. This lack of knowledge hinders identification of potentially problematic dynamics and the operational causes thereof, as well as consequent process optimization. Moreover, unawareness of microbial dynamics questions the reliability and validity of current (microbial) quality controls based on infrequent grab samples. For example, Kistemann et al. (2002) highlighted and quantified the large contribution of microbial peak loads (of short duration, after precipitation events) to total annual loads in natural reservoirs. If such peaks fall between grab samples and are missed, their (potentially negative) influence cannot be assessed and is thus underestimated. Previous studies on microbial dynamics in different natural and engineered aquatic ecosystems (Besmer et al., 2014; Nescerecka et al., 2014; Hojris et al., 2016) and specifically on the vulnerability of (karstic) groundwater (Farnleitner et al., 2005; Butscher et al., 2011; Knierim et al., 2015) suggested that such peak loads in raw water and other short-term fluctuations in both raw and treated water are indeed to be expected.

Here we used two different automated flow cytometry-based monitoring systems with high (20 min) and ultra-high (1 min) temporal resolution to investigate short-term microbial dynamics in raw and treated karstic groundwater during several months. The specific goals of this study were: (1) To assess the scale of fluctuations in bacterial concentrations in the raw groundwater; (2) To assess whether the treatment successfully buffers against these fluctuations; (3) To investigate and quantify how operational processes affect microbial concentrations in treated water. The novelty of this study is the high frequency data, which characterize in detail precipitation-induced raw water fluctuations and revealed operationally induced fluctuations in treated water.

2. Materials and methods

2.1. Study site

This study was conducted at a drinking water treatment plant in Northeastern Switzerland, treating groundwater from two nearby karstic springs. The water throughput varied between 518 m^3 d^{-1} and 1372 m^3 d^{-1} (average 777 m^3 d^{-1}), with two water utilities regularly abstracting water from the plant. The groundwater is extensively treated in a multi-barrier treatment train (Fig. 1). First, flocculation and sedimentation decrease turbidity to protect the subsequent ultrafiltration (UF) membranes. Thereafter, ozonation is followed by granular activated carbon (GAC) biofiltration and final disinfection with ultraviolet (UV) light. Water throughput is automatically increased stepwise in the treatment plant when the water level in the treated water reservoir drops below certain thresholds due to water abstraction from the connected water utilities.

2.2. Online flow cytometry

Continuously flowing sampling bypass lines for the spring water and the treated water respectively were installed in the treatment plant (Fig. 1). An automated sampling, staining and incubation module was connected to the sampling lines and combined with an Accuri C6 flow cytometer (BD Accuri, San Jose CA, USA) as described previously (Besmer et al., 2014). In short, water samples were

drawn every 20 min and mixed with a fluorescent stain (SYBR Green I [Life Technologies, Eugene OR, USA]; final concentration 1:10,000). The mixture was then incubated for 10 min at 37 °C and subsequently transferred to the flow cytometer where it was measured at a flow rate of 66 µl min^{-1} for 90 s with a lower threshold on the green fluorescence (FL1-H) channel set at 1000. After each sampling and measurement, the staining module was rinsed with nanopure water (deionized, 0.22 µm filtered). Extended cleaning with hypochlorite and detergent was performed after every 100 samples. For data analysis, all flow cytometry data was exported as individual fcs (Flow Cytometry Data File Standard) files (one per measurement) and batch processed with custom software. To this end, fixed gates (Prest et al., 2013) were used to separate bacteria from background signals and to determine the concentration of bacteria for each measurement.

2.3. Real time flow cytometry

A different automated staining and incubation module was combined with an Accuri C6 flow cytometer (BD Accuri, San Jose CA, USA) for ultra-high frequency real-time analysis. In short, water samples were drawn continuously from a water bypass line at a rate of 0.3 ml min^{-1} by a high-precision pump. The sample was mixed continuously with a fluorescent stain (SYBR Green I [Life Technologies, Eugene OR, USA]; final concentration 1:10,000; rate: 0.3 ml min^{-1}) in a mixing chamber. The stained samples were incubated for 10 min at 37 °C in an incubation loop and then directed to the flow cytometer at a flow rate of 0.6 ml min^{-1}, where they were measured continuously (flow rate: 14 µl min^{-1}; mode: "unlimited run"; lower threshold on the green fluorescence (FL1-H): 1000). For data analysis, the measurements were binned to 1 min intervals. The same fixed gates as above (Section 2.2 (Prest et al., 2013)) were used to separate bacteria from background signals.

2.4. Hydrological measurements and operational information

Precipitation data were collected as 30 min cumulative values at a meteorological station located approximately 3.5 km east of the treatment plant. Operational data (i.e. spring discharge, turbidity, water throughput, water abstraction) were obtained from the treatment plant operator and were collected as 1 h cumulative values.

2.5. Data analysis

The online flow cytometry system sequentially processed raw water and treated water. It is known that cross contamination can occur between sequential flow cytometry samples (Van Nevel et al., 2013). The observed cross-contamination of the treated water measurement by precipitation-induced peak loads in the parallel raw water measurements was removed from the dataset based on a simple simulation. The cross-contamination was estimated to result in a mixture of 3.3% raw water in 96.7% treated water. This was then calculated for each time point by multiplying the respective raw water concentration with 3.3% and adding it to the product of a constant (average) concentration of 110 cells µl^{-1} and 96.7% (see Fig. S2 for more details). The simulated cross-contamination effect was then deducted from the measured data with only the observed short-term fluctuations remaining.

2.6. Modeling of bacterial concentrations in treated water

In order to better understand fluctuations in the total cell concentration (TCC) of the treated water that were observed in the