Water Research 202 (2021) 117387

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Online microbial monitoring of drinking water: How do different techniques respond to contaminations in practice?

Jorien Favere [a,b,1], Fien Waegenaar [a,b,1], Nico Boon [a,b], Bart De Gusseme [a,b,*]

[a] Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, B-9000 Gent, Belgium
[b] Centre for Advanced Process Technology for Urban Resource Recovery (CAPTURE), P.O., Frieda Saeysstraat 1, B-9000 Gent, Belgium

ARTICLE INFO

Keywords:
Drinking water production
Online microbial monitoring
Flow cytometric fingerprinting
Biostability

ABSTRACT

Safeguarding the microbial water quality remains a challenge for drinking water utilities, and because of population growth and climate change, new issues arise regularly. To overcome these problems, biostable drinking water production and water reuse will become increasingly important. In this respect, high-resolution online microbial monitoring during treatment and distribution could prove essential. Here, we present the first scientific and practical comparison of multiple online microbial monitoring techniques in which six commercially available devices were set up in a full-scale drinking water production plant. Both the devices' response towards operational changes and contaminations, as well as their detection limit for different contaminations were evaluated and compared. During normal operation, all devices were able to detect abrupt operational changes such as backwashing of activated carbon filters and interruption of the production process in a fast and sensitive way. To benchmark their response to contaminations, the calculation of a dynamic baseline for sensitive separation between noise and events is proposed. In order of sensitivity, enzymatic analysis, ATP measurement, and flow cytometric fingerprinting were the most performant for detection of rain- and groundwater contaminations (0.01 – 0.1 v%). On the other hand, optical classification and flow cytometric cell counts showed to be more robust techniques, requiring less maintenance and providing direct information about the cell concentration, even though they were still more sensitive than plate counting. The choice for a certain technology will thus depend on the type of application and is a balance between sensitivity, price and maintenance. All things considered, a combination of several devices and use of advanced data analysis such as fingerprinting may be of added value. In general, the strategic implementation of online microbial monitoring as early-warning system will allow for intensive quality control by high-frequency sampling as well as a short event response timeframe.

1. Introduction

Microbial contamination is the most prevailing risk to public health associated with drinking water (WHO 2017). Safeguarding drinking water quality remains a challenge for drinking water utilities, with new issues arising regularly. Droughts and population growth are already stressing the drinking water provision, even in moderate climates (*e.g.* Limburg, Flanders, 2020). Due to global warming, outbreaks of waterborne diseases caused by opportunistic pathogens (*e.g. Pseudomonas aeruginosa*) are predicted to take place more frequently and have more severe consequences in the future (van der Kooij et al. 2013).

Currently, microbial regrowth in the distribution network is suppressed by adding a disinfection residual at the end of the treatment train, though this approach has several known drawbacks such as formation of harmful disinfection by-products, and induction of uncontrolled necrotrophic growth on the dead biomass (Chatzigiannidou et al. 2018, Li and Mitch 2018, Temmerman et al. 2006). Hence, drinking water utilities are looking into alternative ways to produce microbially safe drinking water without the use of disinfection residuals, as for example the production of biostable water (Nescerecka et al. 2014, Prest et al. 2016). The latter approach implies that the microbial community remains stable during distribution without the use of a disinfectant (Lautenschlager et al. 2013, Rittman and Snoeyinck 1984, van der Kooij 2000). This can be achieved by focusing on extensive

* Correspondence to: Bart De Gusseme, Ghent University; Faculty of Bioscience Engineering; Center for Microbial Ecology and Technology (CMET); Coupure Links 653; B-9000 Gent, Belgium.
E-mail address: bart.degusseme@ugent.be (B. De Gusseme).
[1] Jorien Favere and Fien Waegenaar have contributed equally to this manuscript and thus are sharing first authorship.

https://doi.org/10.1016/j.watres.2021.117387
Received 12 January 2021; Received in revised form 18 June 2021; Accepted 21 June 2021
Available online 26 June 2021

treatment such as nutrient limitation (*e.g.* assimilable organic carbon (AOC) < 10 μg/L), and intensive maintenance of the distribution system (Prest et al. 2013, van der Kooij et al. 1982, Van Nevel et al. 2013).

When producing and distributing biostable drinking water, monitoring of the microbial drinking water quality becomes even more important. Heterotrophic and selective plate counting are the legal standard, but are not sufficient for detection of short-term water quality changes (European Communities, 1998, Buysschaert et al., 2018; US Congress, 1996). Online monitoring systems are therefore gaining attention, as these allow a high-resolution quantification of the microbial dynamics, with a short time-to-result (*i.e.* 10 min to 2.5 hours).

A variety of online microbial monitoring techniques is commercially available. Online enzymatic techniques detect the total microbial load by commonly targeting the generic enzyme alkaline phosphatase (ALP) or the total coliforms and *Escherichia coli* by detection of the selective enzymes β-D-galactosidase and β-D-glucuronidase respectively (Hesari et al. 2016, Koschelnik et al. 2015). The microbial abundance is then quantified indirectly, by measuring the enzymatic hydrolysis of chromogenic and fluorogenic substrates (Rompré et al. 2002). Another indirect method for quantifying the microbial activity is online measurement of intracellular ATP, by detecting the fluorescent conversion of luciferin by the enzyme luciferase (de Vera and Wert 2019, Vang et al. 2014). Direct methods are commercially available as well. Online optical particle classifiers for example, use microscopic images for separation between bacteria and background particles (Højris et al. 2016, Højris et al. 2018). Online flow cytometry, which uses nucleic acid staining for quantifying the bacterial cell concentration, is another, well-established research technique, which has recently become commercially available as well (Besmer et al. 2014, Hammes et al. 2012). In combination with advanced data-analysis, it can even be used to assess the bacterial community characteristics (Props et al. 2016).

In this study, we present the first scientific and practical comparison of multiple online microbial monitoring techniques. Six devices were set up in a full-scale drinking water production plant. Both the devices' response towards operational changes and contaminations, as well as their detection limit for different contaminations were evaluated and compared. With this study, we aim to give an overview of the existing technologies on a scientific basis, in order to determine their compatibility with different applications in the drinking water sector or other industries.

2. Materials and Methods

2.1. Set-up

The study was performed in a full-scale drinking water production plant (De Watergroep, Kluizen, Belgium), where the drinking water is produced from surface water (60 000 m^3/day) using conventional techniques including coagulation, flocculation, sedimentation, flotation and filtration. The last steps of the treatment train include ozone disinfection, two-step activated carbon filtration and chlorination. The set-up was installed after the activated carbon filters, before chlorination, during seven weeks (Fig. SI.1). The sampling location was chosen to perform the experiments in a controlled full-scale environment without the presence of free chlorine. From October 21st until November 18th 2019, routine operational monitoring was performed. During this period, the devices were directly connected to the drinking water pipeline. From November 18th until December 11th 2019, the set-up was disconnected from the drinking water pipeline and experiments with different contaminations were performed (section 2.5). The set-up consisted out of a vessel (V = 100 L, well mixed), connected to a bleeding line coming from the main pipeline. From this vessel, the drinking water was pumped to the online monitoring devices (Fig. SI.2). During operational monitoring and flushing periods, the vessel served as an overflow to the sewer, and during the contamination experiments, the vessel was used for spiking and for water recirculation.

2.2. Online microbial monitoring devices

Six commercially available, online microbial monitoring devices were used. All techniques rely on different mechanisms for detection of the total microbial (mainly bacterial) concentration, and were operated with different measurement frequencies, ranging from 10 minutes to 2.5 hours. All costs are based on Belgian quotes, VAT excluded, provided by the suppliers and/or actual invoices for the devices and consumables that have been purchased. Since these costs will vary dependent on the (local) market conditions, the number of devices that are ordered (bulk vs. one), the volume of consumables that is purchased and/or customization, the costs are expressed as a range. The capital expenditures (CAPEX) are expressed as initial investment cost for the measuring device as we have used it in our research. The operational expenditures (OPEX) are expressed as a cost per sample, and as a range since these costs depend strongly on the consumable cost for e.g. chemicals.

A ColiMinder (VWMs GmbH, Austria), further referred to as "ENZ", was used as a first enzymatic technique to determine the total microbial concentration using the Alkaline Phosphatase Activity Assay Kit (VWMs GmbH, Austria). The enzyme alkaline phosphatase (ALP) converts the substrate 4-methylumbelliferyl phosphate (4-MUP) to 4-methylumbelliferone (4-MU) ($\lambda_{max,\ abs}$ = 365 nm) under alkaline conditions. The reagents were provided as standardized solutions in the QuickDetect Assay Reagents (VWMs GmbH, Austria) and were prepared and stored as recommended by the manufacturer. MilliQ (Merck, Belgium) with a trace amount of NaOCl was used for rinsing. The total microbial activity is measured as ALP activity, and is expressed as μU/100 mL, with one unit (U) ALP converting 1 mol 4-NPP per minute in glycine buffer (pH 10.4) at 37°C. A sample (6.5 mL) was taken every hour (operational monitoring and major contaminations) or every 30 minutes (minor contaminations), after which a standard cleaning procedure was performed.

A second online enzymatic device, BACTControl (microLAN, The Netherlands), further referred to as "ENZ-2", was also installed. This device targets different enzymes for different indicators of microbial contamination: β-D-galactosidase (coliforms), β-D-glucuronidase (*Escherichia coli*) and alkaline phosphatase (total microbial activity). The enzyme activity was detected by conversion of a standard substrate to 4-methylumbelliferone (4-MU) ($\lambda_{max,\ abs}$ = 365 nm). Standard reagent solutions were used and stored as provided and recommended by the manufacturer (microLAN, The Netherlands). Samples (100 mL) were filtered (0.45 μm) to concentrate the microorganisms, then incubated, after which the fluorescence was detected by a photodiode ($\lambda_{max,\ em}$ = 445 nm), and converted to enzymatic activity per time and volume (pmol MU x min/ 100 mL). Samples were taken every 2.5 hours (operational monitoring) or 2 hours (major contaminations). After each measurement, a cleaning procedure with buffer (<0.05 % HOCl) and heating was performed to eliminate residues in the system.

An online EZ-ATP (Hach, Belgium), further referred to as "ATP", was used for detection of intracellular ATP. The method is based on the ATP firefly assay (standard method ASTM D4012-15), using the Water-Glo™ reagents (Promega, Belgium). The amount of reaction product oxyluciferin ($\lambda_{max,\ abs}$ = 390 nm) is measured with a luminometer, and with calibration, converted into pg ATP/mL. The analysis consists of two consecutive measurements, of extracellular ATP (filtration) and total ATP (ultrasonic cell lysis). The intracellular ATP is then calculated as the difference between the total and extracellular ATP. A sample (1 mL) was taken every 30 minutes (operational monitoring) or every 20 minutes (major and minor contaminations). After every 10 measurements, an automatic cleaning cycle was performed with sodium hydroxide (1M) and hydrochloric acid (1M) to avoid biofilm formation and remove residual ATP.

Two online flow cytometric techniques were used in this study. The first online flow cytometric monitoring technique used an onCyt (onCyt Microbiology AG, Switzerland) add-on module, coupled to an Accuri C6 flow cytometer (BD Biosciences, Belgium). As this flow cytometer relies

on hydrodynamic focusing for the alignment of cells, it is further referred to as "FCM-H". The flow cytometer is equipped with a blue (20 mW, 488 nm) and a red laser (12.5 mW, 640 nm), two scatter detectors (side and forward scatter) configured on the blue laser, and four fluorescence detectors with bandpass filters. Three of the bandpass filters are for the blue laser emission (FL1: 533/30 nm, FL2: 585/40 nm, and FL3: 670 LP) and one is for the red laser emission (FL4: 675/25 nm). MilliQ (Merck, Belgium) was used as sheath fluid. Staining was performed using 1000x dilution in TRIS buffer (pH 8.2) of SYBR Green I concentrate (Invitrogen, Belgium), with 10 v% final concentration. Three samples (74.1 ± 2.8 µL) were taken every hour (operational monitoring and major contaminations) or 30 minutes (minor contaminations), and were incubated at 37°C for 20 ± 2 minutes prior to measuring. Between measurements, the incubation chambers were rinsed using MilliQ (Merck, Belgium). QC was performed using Spherotech 8-peak validation beads (BD Biosciences, Belgium).

The second online flow cytometer used in this study was a BactoSense (bNovate, Switzerland), which makes use of capillary alignment of particles. Hence, it is further referred to as "FCM-C". The flow cytometer is equipped with a blue 488 nm laser diode, two fluorescence detectors (FL1: 525/45 nm, FL2: 715 LP) and one side scatter detector (SSC: 488/10 nm). Staining was performed using SYBR Green I in DMSO (0.1 %), cleaning was performed using sterile water with 0.05 % sodium azide, and 0.1 % NaOCl. One sample (90 µL) was taken every hour (operational monitoring and major contaminations) or 30 minutes (minor contaminations).

An optical sensor, BACMON (GRUNDFOS, Denmark), further referred to as "OPT", was also installed. The device was connected to the pressurized water stream and measured at a fixed interval of 10 minutes during the study. The device consists of a flow cell (6 µL) and a camera that fixes and scans the sample respectively, after which a patented neural network classification algorithm classifies the particles on the images as "bacteria" and "non-bacteria", by characterizing every particle using 59 optical parameters and comparison with a preinstalled library (Højris et al. 2016, Højris et al. 2018). Between measurements, the flow cell was thoroughly rinsed with fresh drinking water.

2.3. Offline measurements

Validation of the microbial water quality was performed independently in the lab. Standard drinking water samples were taken near the sampling point by the drinking water utility every 4-5 days. Heterotrophic plate counts were analyzed on R2A (68 h at 36°C) and on yeast extract agar (68 ± 4 h at 22°C) consisting out of tryptone (6 g/L), yeast extract (3 g/L), and agar (12 g/L). A pour plate method was used in which 15 mL liquid agar was added to 1 mL sample. Colilert-18 and Quanti-Tray (IDEXX, Germany) were used for the detection of coliforms and *Escherichia coli,* and Enterolert (IDEXX, Germany) was used for the detection of enterococci. The concentration of carbon, nitrogen and phosphorous during the experiments was also measured in compliance with the drinking water regulations. The concentrations fluctuated slightly within the expected ranges for drinking water quality (3.0 ± 0.3 mg/L NPOC, 1.6 ± 0.5 mg/L total N, 0.01 ± 0.0 mg/L PO_4^{3-}).

During the contamination experiments (Nov 18th - Dec 11th), the total bacterial concentration of the raw contaminations was analyzed using an AccuriC6 Plus flow cytometer (BD Biosciences, Belgium). Its working mechanism is comparable to the Accuri C6 flow cytometer (see above). Staining was performed using SYBR Green I (SG, 100x concentrate in 0.22 µm-filtered DMSO, Invitrogen), with incubation for 20 minutes at 37°C (Props et al. 2016). Furthermore, standardized microbial analyses were performed in an external accredited lab. Samples were taken in sterile 0.5 L HDPE vials containing sodium thiosulfate to neutralize free chlorine, and were analyzed within 24 hours to determine the heterotrophic plate counts (68 ± 4 h at 22°C), and to detect coliforms (Colilert-18 and Quanti-Tray, IDEXX, Germany) and enterococci (Enterolert, IDEXX, Germany), according to WAC/V/A/001, WAC/V/A/002 and WAC/V/A/003 (VITO 2020).

2.4. Contamination experiments

Two contamination experiments were performed. In the first experiment (major contaminations), relatively high concentrations of different contaminations were added and were recycled through the pump-vessel system (section 2.1). Three different matrices were used as contamination: rainwater (runoff green roof, 3.32 ± 0.16 × 10^6 cells/mL) at Nov 19th – Nov 21st, in respectively 10, 5 and 0.5 v%, shallow groundwater (FARYS drinking water production plant, Oudenaarde, Belgium, 1.08 ± 0.00 × 10^6 cells/mL) at Nov 25th – Nov 27th, in respectively 10, 5 and 1 v%, and effluent of a wastewater treatment plant (WWTP Aquafin, Ghent, 9.93 ± 0.2 × 10^6 cells/mL) at Dec 2nd – Dec 4th, in respectively 5, 1 and 0.1 v%. After 24 hours, drinking water was used for diluting. Samples were taken after each spike for offline microbial analysis as validation (Table SI.3). Between contaminations with different matrices, recycling was stopped and fresh drinking water was used to flush the system for at least 72 hours.

In the second contamination experiment, smaller spikes were added with increasing concentrations, every 2 hours. For these experiments, two contamination matrices were used: rainwater (runoff green roof, 2.48 ± 0.09 × 10^6 cells/mL) at Dec 10th, in consequently 0.01, 0.1, 1 and 20 v%, shallow groundwater (FARYS drinking water production plant, Oudenaarde, Belgium, 2.88 ± 0.10 × 10^5 cells/mL) at Dec 11th, in consequently 0.01, 0.1, 1 and 10 v%. Between contaminations with the different matrices, recycling was stopped, and fresh drinking water was used to flush the system overnight. Samples were taken after each spike for offline microbial analysis as validation (Table SI.5).

2.5. Data analysis

Data of the devices, except for the flow cytometers, was provided in Microsoft Excel format, either by remote control or offline data collection, and was further analyzed in R (v3.6.3). For ATP, outliers (n = 4) related to operational interventions at the device were removed prior to processing, and for the other devices, the data was processed as such.

The flow cytometric data was obtained as Flow Cytometry Standard (.fcs) files (v3.1) and was processed in R (v3.6.3). *FlowCore* (v1.52.1) was used to import the .fcs files (Hahne et al. 2009). Based on the bivariate plot of green versus red fluorescence, bacterial cells were separated from background noise, as this is the configuration for the most optimal signal and noise separation in drinking water samples (Hammes and Egli 2005, Hammes and Egli 2010). A gate was drawn manually for each flow cytometer, and was kept constant throughout the experiments. For FCM-H, further data processing was done using *Phenoflow* (v1.1.2) to extract the cell concentration, as described by Props et al. (2016), and *FlowAI* (v1.16.0) was used to check the data quality and to remove anomalous values in terms of flow rate stability, signal acquisition and the dynamic range (Monaco et al. 2016). For FCM-C, data processing was performed similarly, but was based on the FL1 (green) and FL2 (red) channel output. Furthermore, advanced cytometric fingerprinting was performed on the data acquired with FCM-C during the minor contamination experiment, using probability binning approach of *flowFP* (v1.44.0), with a model grid with 5 recursions (25 bins) (Rogers and Holyst 2009). From these fingerprints, principal coordinates analysis (PcoA) and Bray-Curtis dissimilarity calculations were performed using *vegan* (v2.5.6) (Favere et al. 2020, Oksanen et al. 2019). Resampling to the lowest sample size (n = 16038 cells) was performed prior to PcoA and Bray-Curtis analysis to account for size-dependent differences. The fresh drinking water samples taken before the contaminations with different matrices (n = 26 for both rainwater and groundwater) were respectively used for baseline calculations for dissimilarity comparison (Fig. 4). The Bray-Curtis dissimilarity that was assigned to a sample was calculated as the average of the Bray-Curtis dissimilarities between that sample and all of the baseline

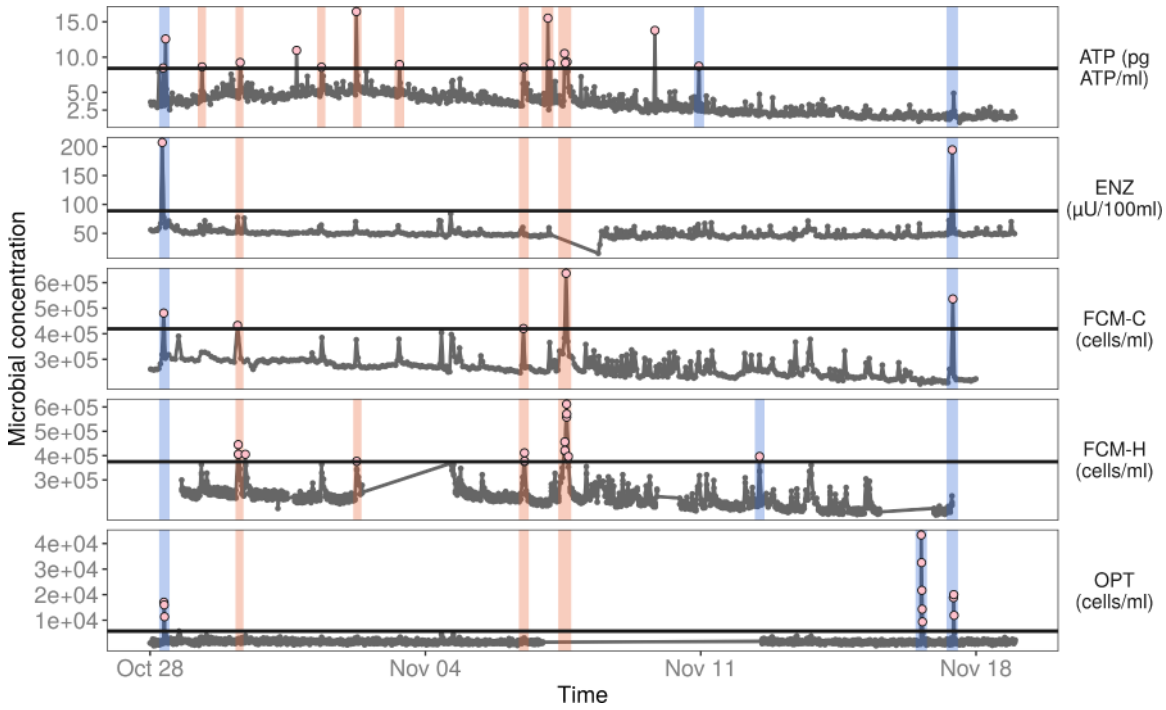

Fig. 1. Microbial concentration measured by different online microbial monitoring devices (rows) after activated carbon filtration in a drinking water production plant. The horizontal line is the baseline threshold for event detection, calculated as the average plus three times the standard deviation for each device during this monitoring period. Measurements above the baseline threshold are indicated in pink data points. Operational processes related to these events are highlighted: periods of backwashing (blue) and interrupted production (orange).

samples of the respective contamination.

3. Results

3.1. Baseline determination and response to operational changes

In the first stage of this study (October 21st – November 18th), the devices were used to follow up the operational dynamics of the drinking water production plant (Fig. 1). For this monitoring period, a baseline threshold was calculated for each device as the average plus three times the standard deviation to separate between noise (*e.g.* operational, instrumental) below the baseline, and events, defined as measurements above the baseline (Fig. 1 pink, Table SI.1). This baseline threshold was chosen as it is a conservative and rigid threshold for event detection. It is based on the normal distribution, where events above the baseline will only make up 0.13 % of the data (Favere et al. 2020, Howell et al. 1998). Even though some deviations from normality were observed using Q-Q plots (Fig. SI.3), this approach was chosen as the goal is to define a first robust and straightforward screening method for event detection. For further applications, the use of moving window analysis (see below) and/or transformations could be implemented to increase the sensitivity of the method.

The two flow cytometers, FCM-C and FCM-H, detected respectively $3.1 \pm 0.5 \times 10^5$ cells/mL and $2.3 \pm 0.5 \times 10^5$ cells/mL, whereas the ATP concentration was 3.6 ± 1.6 pg ATP/mL. The total activity detected by ENZ was on average 53.2 ± 11.9 µU/100 mL, which is in the range of expected values for activity after activated carbon filtration (40 - 60 µU/100 mL), as defined by the manufacturer. OPT measured on average $1.6 \pm 1.4 \times 10^3$ cells/mL, with a high relative standard deviation due to the big difference between the baseline values and the peak events. The absolute values of the cell concentration detected by OPT are around 2 log units lower than the total cell concentration measured by the flow cytometers. This difference may be allocated to the working mechanism, as this device reports correct particle sizes in the range of 0.77 - 3 µm, but smaller particles or bacteria may not be detected or classified correctly.

As the measurement frequency was device-specific, the percentage of events above the baseline was calculated relative to the number of measurements within this period. The flow cytometers (1.44 % and 1.58 % for FCM-C and FCM-H respectively), and ATP (1.33 %) registered the largest percentage of events above the baseline threshold, and ATP detected the most independent events in absolute terms. On the other hand, OPT and ENZ classified respectively only 0.36 % and 0.64 % of the measurements as events, but showed a clear distinction between noise and events. After determination of the events above the baseline threshold for each device, common peaks (n = 5) were determined as events detected by two or more devices. FCM-C detected all common peaks, ATP detected four out of five common peaks, FCM-H and OPT three and ENZ two. During the monitoring period, ENZ-2 was not functioning properly due to crystallization of the reagents at temperatures below 15°C and the relatively high amount of particles in the water. The data was considered to be unreliable, hence, data from this device was not taken into account for analysis and discussion.

In a next step, the events were linked to operational changes. Backwashing of the activated carbon filters was shown to be related to several peaks (Fig. 1 blue, Fig. SI.4 B). Moreover, all backwashing events that have occurred in this period were detected by one or more devices. Also, interruption of the production process was linked to an increase in the microbial concentration, as detected by several devices. Interrupted production, meaning that there was no net flow through the installation, resulted in an increase of the water temperature as well (Fig. SI.4 A).

3.2. Response to contaminations and dynamic baseline calculation

In the second stage of this study (Nov 18th – Dec 11th), the devices were disconnected from the drinking water network, and contaminations were added to the vessel in the set-up. In a first experiment, relatively high concentrations of rainwater, groundwater and effluent of a WWTP were added with decreasing concentrations. Similar to the previous experiment, a baseline was calculated to separate noise and

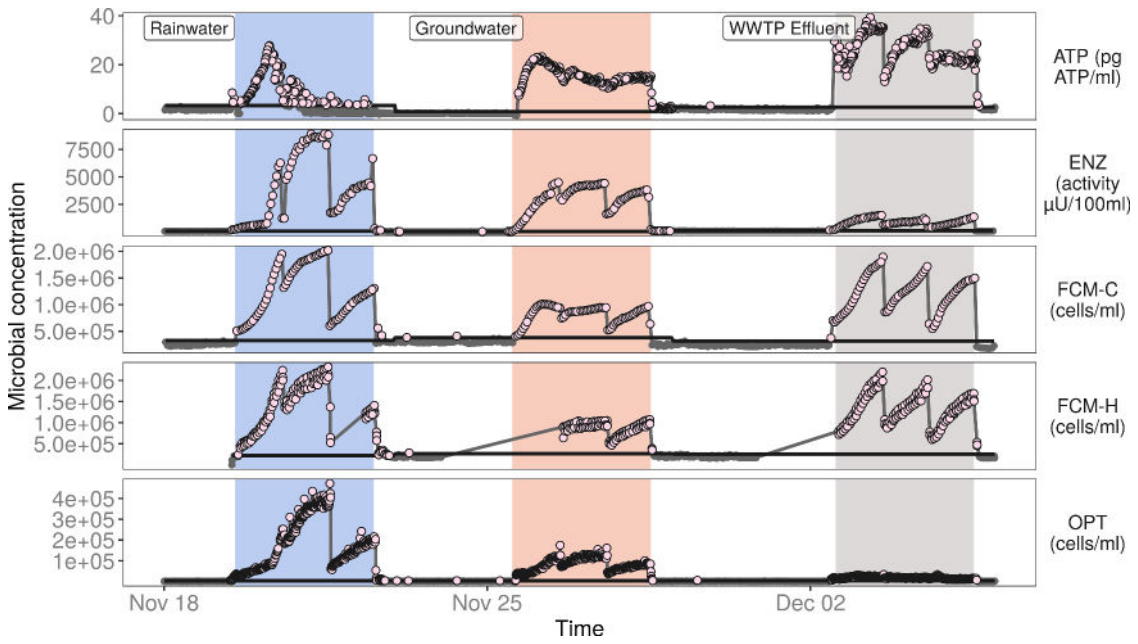

Fig. 2. Microbial concentration measured by different online microbial monitoring devices (rows) after activated carbon filtration in a drinking water production plant. The horizontal line is the baseline threshold for event detection, calculated as the average plus three times the standard deviation for each device over this monitoring period. Events above the baseline value are indicated in pink data points. Contamination was performed with three subsequent spikes (24 h/spike) with decreasing concentrations of three matrices: rainwater (blue, Nov 19th – 21st), groundwater (orange, Nov 25th – 27th) and effluent of a WWTP (grey, Dec 2nd – 4th). The system was refreshed withdrinking water between different contaminations.

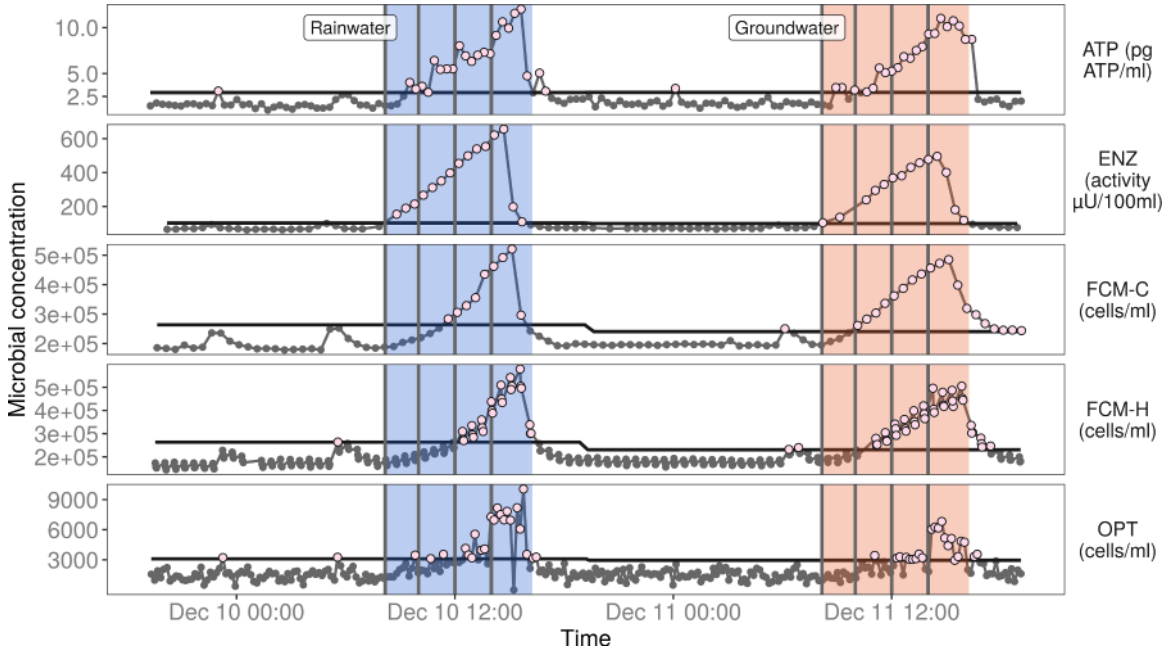

Fig. 3. Microbial concentration measured by different online microbial monitoring devices (rows) after activated carbon filtration in a drinking water production plant. The horizontal line is the baseline threshold for event detection, calculated as the average plus three times the standard deviation for each device based on the drinking water samples before each contamination. Events above the baseline value are indicated as pink data points. Contamination was performed with four subsequent spikes (2 h/spike) with increasing concentrations of two matrices: rainwater (blue, Dec 10th) and groundwater (orange, Dec 11th). Between the two contaminations, the system was refreshed with drinking water

events. However, because the drinking water quality shows long-term variations, *e.g.* a seasonal temperature decrease of 4°C during the baseline measurements, the baseline microbial concentration and activity will also differ over time (Nescerecka et al. 2018, Pinto et al. 2014). This is why during the contamination experiments, the baseline was calculated using a moving window analysis (MWA) based on the drinking water samples measured before each respective contamination (Fig. 2, Fig. 3, Table SI.2) (Marzorati et al. 2008, Wittebolle et al. 2005).

A similar reaction behavior was observed for all devices and spikes (Fig. 2). After each spike, the microbial concentration increased sharply, after which an immediate decrease was observed due to the dilution with fresh drinking water. Then, the bacterial concentration increased

Table 1
Overview of contaminations detected by the devices. The ratio indicates the samples above the baseline compared to the total amount of samples measured during this spike. The color code indicates the success rate of response to the respective contaminations of drinking water (0-25%: red, 25-50%: orange, 50-75%: yellow, 75-100%: green).

	Rainwater (v%)				Groundwater (v%)			
	0.01 %	0.1 %	1 %	20 %	0.01 %	0.1 %	1 %	10 %
OPT (cells/mL)	1/11	2/11	7/12	10/11	0/11	1/12	8/11	10/11
FCM-C (cells/mL)	0/3	1/4	4/4	4/4	0/4	4/4	4/4	4/4
FCM-C fingerprint (Bray Curtis)	1/4	4/4	4/4	4/4	2/4	4/4	4/4	4/4
ENZ (µU ALP/100mL)	3/3	4/4	4/4	4/4	2/2	3/3	4/4	5/5
ATP (pg ATP/mL)	2/6	6/6	6/6	6/6	3/6	4/5	6/6	6/6
FCM-H (cells/mL)	0/12	0/12	9/9	12/12	0/9	6/9	12/12	12/12
Coliform plate counts (CFU/100 mL)	0	0	1	1	0	0	1	0
Enterococci plate counts (CFU/100 mL)	0	0	0	0	0	1	0	2

again strongly, and this fast regrowth was promoted by a temperature increase from the heat of the recirculation pump. This was confirmed with independent flow cytometry, on samples taken after 30 minutes of recycling (Table SI.3). Overall, the contamination using rainwater (collected as runoff from a green roof) resulted in the highest responses of the devices, and the groundwater contamination resulted in the lowest responses. As shown in Fig. 2, the contamination with effluent of a WWTP resulted in cell concentrations similar to the rainwater contamination (FCM-C, FCM-H), but resulted in a lower enzymatic activity response than the groundwater contamination (ENZ). OPT only reacted slightly to the WWTP effluent contamination compared to the other contaminations, though still with cell concentrations above the baseline (Højris et al. 2016). Due to technical issues, ATP was not functioning properly during the rainwater contamination, and FCM-H had several downtime periods.

As validation, the online measurements were compared to plate counts from grab samples (Table SI.3). The coliforms and enterococci plate counts indicated the presence of a contamination, and showed a decreasing trend with decreasing concentrations of every spike. Contrary to the results obtained from the online devices targeting the total microbial concentration, coliforms and enterococci plate counts indicated that WWTP effluent was the most contaminated, whereas rainwater was the least contaminated regarding the hygienic water quality. The total colony counts were above the upper limit for quantification (300 CFU/ 100 mL), as the sampling point was before the chlorination step (data not shown).

3.3. Detection limit for different contaminations of drinking water

The results from the previous experiment showed that devices were able to detect contaminations in a fast and consistent way. However, the volume ratio of these contaminations compared to the drinking water were relatively high (0.5 – 10 v%), and as refreshment of the drinking water was only performed after 24 hours of recirculation, extensive regrowth could occur (Fig. 2). Hence, a second contamination experiment was performed by spiking the contaminations in lower volume ratios, starting from 0.01 v% and gradually increasing every two hours up to 10 - 20 v%. As rain- and groundwater intrusion in the drinking water network are two major hazards for drinking water utilities in practice, the experiment was focused on these two contaminations (Fig. 3). The dynamic baseline was calculated for each contamination using a moving window to account for systematic changes in water characteristics, as explained above (section 3.2). In this context, the baseline forms the boundary between events and noise, and thus, represents the detection limit of the devices for the different contaminations in question. Subsequently, the sensitivity of each device towards the different contaminations was objectively quantified as the success rate of detection of a certain contamination, calculated as the amount of samples with values above the baseline, divided by the total amount of samples taken during the respective spike (Table 1). ENZ was able to

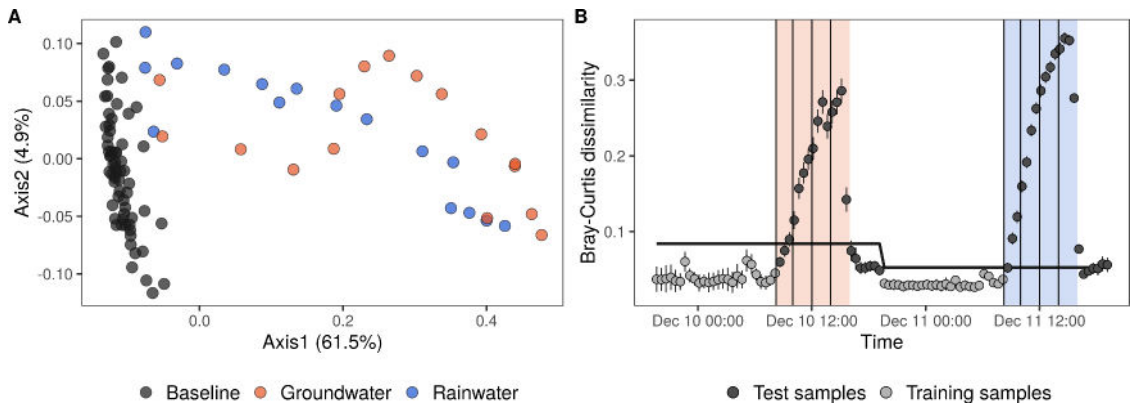

Fig. 4. (A) PcoA analysis of the cytometric fingerprints of FCM-C during spikes with increasing concentrations (Dec 10th – 11th). Data points are colored according to the respective contamination. Samples taken during flushing with drinking water between spikes are labelled as 'baseline'. (B) Bray-Curtis dissimilarity of the cytometric fingerprints. Samples in grey (n = 26) before each contamination are used for baseline calculation and comparison. Samples in black are used as test dataset for contamination detection, in which the Bray-Curtis dissimilarity assigned to a sample was calculated as the average Bray-Curtis dissimilarity between that sample and all baseline samples. Contamination was performed with four subsequent spikes (2 h/spike) with increasing concentrations of two matrices: rainwater (blue, Dec 10th) and groundwater (orange, Dec 11th).

detect contaminations of 0.01 v%, followed by ATP, able to detect 0.1 v % of each contamination. The total cell concentration using flow cytometry (FCM-C and FCM-H) could detect 1 v% of each contamination (Fig. 3, Table 1), and using optical classification, between 1-10 or 20 v% of the contaminations could be detected. These results indicate that the sensitivity of enzymatic techniques (ATP, ENZ) towards rain- and groundwater contamination is the highest, followed by flow cytometry (FCM-C, FCM-H) and then the optical technique (OPT) (Fig. 3), or, in other words, that a rainwater or groundwater contamination resulted in the first place in an increase in enzymatic activity and ATP concentration. As ENZ was able to pick up all concentrations, its detection limit may even be lower than the lowest concentrations tested in this study.

Additionally, the flow cytometric data obtained by FCM-C was analyzed through cytometric fingerprinting from which the phenotypic beta diversity (*i.e.* inter-sample diversity) was calculated using PcoA analysis (Fig. 4 A). This analysis showed that the contaminations contained phenotypically different communities from the baseline measurements. Furthermore, time-wise analysis revealed a "boomerang effect", in which increasing concentrations resulted in a gradually more phenotypically different community from the baseline (further away on PcoA plot), after which flushing with drinking water resulted in a gradual change to a phenotypically more similar community compared to the baseline (back to baseline on PcoA plot). Both contaminations resulted in a similar extent of phenotypic changes in the microbial community, compared to the baseline.

This was also observed in the Bray-Curtis dissimilarity between cytometric fingerprints. The Bray-Curtis dissimilarity is a straightforward parameter expressing the difference between fingerprints, with identical fingerprints having a Bray-Curtis dissimilarity of zero. Samples taken during flushing with drinking water served for baseline calculation (0.085 and 0.053 for resp. rainwater and groundwater) and as training set (Fig. 4 B). The Bray-Curtis dissimilarity of a sample taken during the contaminations was then calculated as the average dissimilarity between this sample and all of the baseline samples. This parameter was more sensitive than the flow cytometric cell counts as such (Table 1). Also, in contrast to the cell concentrations, the Bray-Curtis dissimilarity indicated that the groundwater contamination resulted in a bigger change in the phenotypic microbial community structure than the rainwater contaminations (Fig. 3, Fig. 4 B).

The online results were validated with grab sample plate counting (Table 1, Table SI.5). The heterotrophic plate counts (coliforms and enterococci) indicated a presence of contamination, but lacked a clear trend. The plate counts were around the detection limit of 1 CFU per 100 mL, with no enterococci detected in the rainwater contamination.

4. Discussion

4.1. Common operational changes affect the short-term microbial dynamics of drinking water

In the first part of this study, we studied the response of different online microbial monitoring devices towards common operational changes. Similar average values for all microbial parameters have been reported in literature for unchlorinated drinking water (Højris et al. 2016, Liu et al. 2013a, Vang et al. 2014). Backwashing of the activated carbon filters was linked to an increase in the microbial concentration and/or activity, as detected by several devices (Fig. 1, Fig. SI.4 B). During this process, the water is sent through the filter in an up-flow, and the bed is expanded (± 30 %) for removal of biomass and particles. The microbial community in activated carbon filters is known to shape the microbial community of the drinking water, and the influence of backwashing on the biomass in the filter has been reported by several authors (Gibert et al. 2013, Lautenschlager et al. 2014, Lohwacharin et al. 2015, Pinto et al. 2012). After backwashing, filter ripening, *i.e.* a stabilization period of around 10 minutes, is necessary before reconnection to the drinking water supply, to prevent the loss of particles in the final drinking due to the increase in flow (Suthaker et al. 1998). Still, in this study, all events of backwashing were detected by one or more devices. Backwashing a filter with a higher flow (*e.g.* change from -800 m^3/h up to 500 m^3/h at Nov 17th), resulted in detection by more devices, indicating that abrupt changes in the flow can cause short-term increases in microbial abundance and/or activity. Likewise, an increase in turbidity of the filtrate after backwashing has been reported in literature (Ahmad et al. 1998).

Interruption of the production process also increased the microbial activity and/or concentration (Fig. 1). During interruption, the water remained stagnant in the installation, and a slight, but clear increase (order of 0.1 - 1°C) in water temperature was observed (Fig. SI.4 A). The increase in microbial abundance and/or activity can hence be explained by a combination of the stagnant water with increasing temperature facilitating growth, and, as previously mentioned, detachment of microorganisms by a sudden increase in flow during start-up (Besmer and Hammes 2016, Liu et al. 2013b, Nescerecka et al. 2018). Overall, using these online devices, the short-term dynamics of the microbial community were quantified, independent of their technology. This temporal resolution and short time-to-result is a straightforward added value compared to the grab sample plate counts. This way, online microbial monitoring techniques can assist in optimizing the operational performance of a production site, and can be implemented as first barrier for safeguarding the drinking water quality, to optimize operational performance, or can serve as guidance for more targeted plate count sampling.

4.2. Data analysis and management of early-warning systems for detecting contaminations

One of the major challenges during online monitoring is the handling of the immense amount of data that is being generated. In this study, we have first proposed the use of a straightforward and conservative baseline for operational event detection using online microbial monitoring techniques. This approach has shown to work for detection of events linked to operational changes, when combining the data generated by all monitoring devices and operational data (Fig. 1). As mentioned above, the online microbial monitoring techniques can in this respect serve as a control parameter for drinking water quality.

When going a step further, namely implementing these techniques as an early-warning system, the calculation of a dynamic baseline is necessary for anticipating to gradually changing water quality (Fujioka et al. 2019). This approach was applied using a moving window during the contamination experiments (Fig. 2, Fig. 3). The results from the first contamination experiment showed that these devices react clearly and in a similar way to different contaminations and could hence be implemented as early-warning systems. Nevertheless, these simulated contaminations were unrealistically high in concentration. Therefore, a second contamination experiment was performed with a lower range of relevant contaminations. In practice, intrusion of rainwater and groundwater in the network can occur through bad connections by consumers or pipe breaks and leaks (Vanysacker et al. 2019). The results from this experiment indicated that enzymatic techniques (ENZ and ATP), together with flow cytometric fingerprinting (Bray-Curtis dissimilarity) were able to pick up contaminations of rain- and groundwater of 0.01 - 0.1 v%, or 100 mL to 1 L in 1 m^3, a concentration that can realistically be achieved when wrongly connecting a rainwater pump without safety valves (Table 1) (Vanysacker et al. 2019). Hereby, it should be noted that the actual detection limit of ENZ may even be lower than the concentrations tested in this study. The low detection limit of monitoring devices based on enzymatic activity was also reported in a study of Tatari et al. (2016). Also, Vang et al. (2014) reported that detection of intracellular ATP was more sensitive towards contamination in drinking water than the total direct cell counts, as the latter includes both viable and non-viable cells. Flow cytometric fingerprinting may overcome this limitation, as demonstrated by the use

Table 2
Summary of different characteristics of online microbial monitoring devices, based on own experience obtained during this study (maintenance time and level of expertise, data analysis) and on information of the manufacturers. The reported maintenance time of each device is expressed in hours per three months (quarter). The CAPEX and OPEX are given as investment cost and the cost per sample, respectively. These costs are expressed VAT excluded, and as a range, since they will vary dependent on (local) market conditions, order number or volume, and the measuring frequency.

Device	Maintenance time (h/quarter)	Maintenance	Highest frequency	Remote control	Data analysis	CAPEX (x 1000 €)	OPEX (€)
ATP	6	Difficult	10 min	No	Basic	25 – 35	2.0 - 2.1
OPT	0.5	Basic	10 min	Yes	Basic	10 – 20	0.01 - 0.05
ENZ	4	Medium	15-20 min	Yes	Medium	35 – 45	3.9 - 5.0
FCM-C	0.5	Basic	30 min	Yes	Medium	35 – 45	1.5 - 2.0
FCM-H	20	Difficult	10 min	Partial	Difficult	95– 105	0.1 - 0.2

of the Bray-Curtis dissimilarity as sensitive and straightforward parameter derived from the cytometric fingerprint. Its use in event detection has been demonstrated previously (Favere et al. (2020)), though in the current study we demonstrate its sensitivity and added value compared to total cell counts.

4.3. Practical comparison for implementing online microbial monitoring techniques

This study is, to our knowledge, the first to present a scientific and practical comparison of a wide range of online microbial monitoring techniques on a full-scale drinking water production plant. A summary of the characteristics of the devices, based on our experience obtained during this study, is given in Table 2. As FCM-H is a prototype used in research, the maintenance time is the highest of all devices, with the maintenance requiring an in-depth knowledge of the device. Also, the data analysis requires advanced programming skills, whereas the data obtained from ATP and OPT is the easiest to extract. OPT and FCM-C are the most robust systems, requiring little maintenance and allowing full remote control. FCM-C and ENZ have a highest sampling frequency of respectively 30 and 15-20 minutes, though most devices allow for measuring every 10 minutes. The CAPEX of all devices is comparable, except for FCM-H as this machine was originally developed for research applications. The OPEX depends mainly on the requirements of chemicals. This is the reason why OPT is the least expensive technology, as it does not use any chemicals. For the other techniques, the price of the consumables is comparable, and can usually be suppressed with bulk orders. In comparison with conventional plate counting, the cost per sample of the online monitoring devices is low, due to the requirement of less labor, consumables and infrastructure (Van Nevel et al. 2017). Furthermore, flow cytometric fingerprinting is an added value to the use of flow cytometry, allowing sensitive and straightforward detection of contaminations, even on robust sensors such as FCM-C. Also, fingerprinting shows if an increase in cell concentration is related to a shift in the microbial community or not, thus indicating if this event is resulting in a change in microbial water quality or not. Different strategies have been developed for research purposes already, however, straightforward implementation of this data analysis would be of great added value for the industry, in combination with easy-to-interpret visualization of the data for operational control (Props et al. 2016, Rogers and Holyst 2009, Safford and Bischel 2019).

Overall, the choice for implementing a certain technique depends mainly on the type of application. For example, unmanned water towers require a more robust technique with less maintenance, that are compatible with pressurized streams, such as OPT or FCM-C. On the other hand, high-risk production steps in *e.g.* drinking water production or wastewater reuse facilities need very sensitive techniques for safeguarding the microbial water quality, so in this case, implementation of ATP or ENZ may be more suited. Also, it should be noted that the detection limit of a certain technique may vary according to the drinking water characteristics and the used device (Hammes et al. 2008, Vang et al. 2014). For example, Hammes et al. (2008) demonstrated that ATP measurements after ozonation resulted in severe interferences by extracellular ATP. Furthermore, a combination of different techniques can provide a more holistic overview of the microbial dynamics. For example, the combination of ATP and flow cytometry has already been reported by several authors, as this combines quantification of the cell concentration with the activity of the biomass, which can result in complementary information of the microbial community characteristics throughout *e.g.* drinking water production steps or the distribution network (Hammes and Egli 2010, Lautenschlager et al. 2013, Prest et al. 2016, Safford and Bischel 2019). Finally, as all monitoring systems require a certain level of knowledge, operational skills, data handling, and come with a certain investment cost, implementing biological online monitoring systems becomes most interesting when these systems are strategically implemented at certain critical control points (CCP) in the production process. Because of the speed of analysis, the results are available faster and more data points are available. As such, the online monitoring systems allow to follow the production process continuously and to analyze data trends in relation with the operational conditions. This way, they can serve as early-warning systems, in assistance and complementary to the more targeted routine sampling.

5. Conclusion

As biostable drinking water production and water reuse will become more and more important, online microbial monitoring of drinking water during treatment and distribution is a fast and sensitive tool to ensure safe drinking water. In this study, it was concluded that the tested online microbial monitoring devices could detect abrupt changes such as backwashing of activated carbon filters and interruption of a full-scale production process. A dynamic baseline calculation allowed sensitive separation between noise (*e.g.* operational, instrumental) and contaminations of rainwater, groundwater and effluent of a WWTP. Enzymatic analysis, ATP measurement and flow cytometric fingerprinting showed to be the most sensitive techniques for detection of rain- and groundwater contaminations, even more sensitive than plate counting. However, optical classification and flow cytometric cell counts are more robust techniques, requiring less maintenance and providing a direct cell concentration. The main message of this study is hence that the choice for a certain device or technique will depend on the type of application and is a balance between sensitivity, cost and maintenance. Furthermore, a combination of several techniques (*e.g.* ATP and FCM) may be of added value as this may provide a more holistic overview of the microbial dynamics. In general, the strategic implementation of online microbial monitoring as early-warning system will allow for intensive quality control by high-frequency sampling as well as a short event-response timeframe.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded by the FWO Flanders [grant number 3S85419]

and by the FWO-SBO Biostable project [grant number S006221N]. The work was financially and logistically supported by De Watergroep, FARYS, Pidpa and Water-link. The authors would like to thank Benjamin Buysschaert, Han Vervaeren, Simon Vanzavelberg, Jerom Vranken, Sander Latte, Katrien De Maeyer and Koen Joris for their practical and intellectual contribution to this project. Also, we would like to thank Stijn Mortier, Erwin Van Poucke, Alexander and Annick from the drinking water production plant for the technical assistance and practical information, and Nathalie Van Eeghem and Kristin Van Hecke for analyzing the lab samples.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117387.

References

Ahmad, R., Amirtharajah, A., Al-Shawwa, A., Huck, P.M., 1998. Effects of backwashing on biological filters. J. - Am. Water Works Assoc. 90 (12), 62–73.
Besmer, M.D., Hammes, F., 2016. Short-term microbial dynamics in a drinking water plant treating groundwater with occasional high microbial loads. Water Res. 107, 11–18.
Besmer, M.D., Weissbrodt, D.G., Kratochvil, B.E., Sigrist, J.A., Weyland, M.S., Hammes, F., 2014. The feasibility of automated online flow cytometry for in-situ monitoring of microbial dynamics in aquatic ecosystems. Front. Microbiol. 5, 265.
Buysschaert, B., Vermijs, L., Naka, A., Boon, N., De Gusseme, B., 2018. Online flow cytometric monitoring of microbial water quality in a full-scale water treatment plant. npj Clean Water 1 (16).
Chatzigiannidou, I., Props, R., Boon, N., 2018. Drinking water bacterial communities exhibit specific and selective necrotrophic growth. npj Clean Water 1 (1).
de Vera, G.A., Wert, E.C., 2019. Using discrete and online ATP measurements to evaluate regrowth potential following ozonation and (non)biological drinking water treatment. Water Res. 154, 377–386.
European Communities, 1998. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Official J. 41, 32–54.
Favere, J., Buysschaert, B., Boon, N., De Gusseme, B., 2020. Online microbial fingerprinting for quality management of drinking water: Full-scale event detection. Water Res. 170, 115353.
Fujioka, T., Ueyama, T., Mingliang, F., Leddy, M., 2019. Online assessment of sand filter performance for bacterial removal in a full-scale drinking water treatment plant. Chemosphere 229, 509–514.
Gibert, O., Lefevre, B., Fernandez, M., Bernat, X., Paraira, M., Calderer, M., Martinez-Llado, X., 2013. Characterising biofilm development on granular activated carbon used for drinking water production. Water Res. 47 (3), 1101–1110.
Hahne, F., LeMeur, N., Brinkman, R.R., Ellis, B., Haaland, P., Sarkar, D., Spidlen, J., Strain, E., Gentleman, R., 2009. flowCore: a Bioconductor package for high throughput flow cytometry. BMC Bioinformatics 10, 106.
Hammes, F., Berney, M., Wang, Y., Vital, M., Koster, O., Egli, T., 2008. Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. Water Res. 42 (1-2), 269–277.
Hammes, F., Broger, T., Weilenmann, H.U., Vital, M., Helbing, J., Bosshart, U., Huber, P., Odermatt, R.P., Sonnleitner, B., 2012. Development and laboratory-scale testing of a fully automated online flow cytometer for drinking water analysis. Cytometry A 81 (6), 508–516.
Hammes, F., Egli, T., 2005. New method for assimilable organic carbon determination using flow-cytometric enumeration and a natural microbial consortium as inoculum. Environ. Sci. Technol. 39 (9), 3289–3294.
Hammes, F., Egli, T., 2010. Cytometric methods for measuring bacteria in water: advantages, pitfalls and applications. Anal. Bioanal. Chem. 397 (3), 1083–1095.
Hesari, N., Alum, A., Elzein, M., Abbaszadegan, M., 2016. A biosensor platform for rapid detection of E. coli in drinking water. Enzyme Microb. Technol. 83, 22–28.
Højris, B., Christensen, S.C., Albrechtsen, H.J., Smith, C., Dahlqvist, M., 2016. A novel, optical, on-line bacteria sensor for monitoring drinking water quality. Sci. Rep. 6, 23935.
Højris, B., Kornholt, S.N., Christensen, S.C.B., Albrechtsen, H.J., Olesen, L.S., 2018. Detection of drinking water contamination by an optical real-time bacteria sensor. H2Open J. 1 (2), 160–168.
Howell, D., Rogier, M., Yzerbyt, V., Bestgen, Y., 1998. Statistical methods in human sciences. Wadsworth, New York.
Koschelnik, J., Vogl, W., Epp, M. and Lackner, M. (2015) Rapid analysis of β-D-glucuronidase activity in water using fully automated technology, pp. 471-481, Vienna.
Lautenschlager, K., Hwang, C., Ling, F., Liu, W.T., Boon, N., Koster, O., Egli, T., Hammes, F., 2014. Abundance and composition of indigenous bacterial communities in a multi-step biofiltration-based drinking water treatment plant. Water Res. 62, 40–52.
Lautenschlager, K., Hwang, C., Liu, W.T., Boon, N., Koster, O., Vrouwenvelder, H., Egli, T., Hammes, F., 2013. A microbiology-based multi-parametric approach towards assessing biological stability in drinking water distribution networks. Water Res. 47 (9), 3015–3025.
Li, X.F., Mitch, W.A., 2018. Drinking Water Disinfection Byproducts (DBPs) and human health effects: multidisciplinary challenges and opportunities. Environ. Sci. Technol. 52 (4), 1681–1689.
Liu, G., Van der Mark, E.J., Verberk, J.Q., Van Dijk, J.C., 2013a. Flow cytometry total cell counts: a field study assessing microbiological water quality and growth in unchlorinated drinking water distribution systems. Biomed. Res. Int. 2013, 595872.
Liu, G., Verberk, J.Q., Van Dijk, J.C., 2013b. Bacteriology of drinking water distribution systems: an integral and multidimensional review. Appl. Microbiol. Biotechnol. 97 (21), 9265–9276.
Lohwacharin, J., Phetrak, A., Takizawa, S., Kanisawa, Y., Okabe, S., 2015. Bacterial growth during the start-up period of pilot-scale biological activated carbon filters: Effects of residual ozone and chlorine and backwash intervals. Process Biochem. 50 (10), 1640–1647.
Monaco, G., Chen, H., Poidinger, M., Chen, J., de Magalhaes, J.P., Larbi, A., 2016. flowAI: automatic and interactive anomaly discerning tools for flow cytometry data. Bioinformatics 32 (16), 2473–2480.
Nescerecka, A., Juhna, T., Hammes, F., 2018. Identifying the underlying causes of biological instability in a full-scale drinking water supply system. Water Res. 135, 11–21.
Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'hara, R., Simpson, G.L., Solymos, P., Stevens, M.H.H. and Wagner, H. (2019) Vegan: community ecology package. R package version 2.5-4.
Pinto, A.J., Schroeder, J., Lunn, M., Sloan, W., Raskin, L., 2014. Spatial-temporal survey and occupancy-abundance modeling to predict bacterial community dynamics in the drinking water microbiome. MBio 5 (3) e01135-01114.
Pinto, A.J., Xi, C., Raskin, L., 2012. Bacterial community structure in the drinking water microbiome is governed by filtration processes. Environ. Sci. Technol. 46 (16), 8851–8859.
Prest, E.I., Hammes, F., Kotzsch, S., van Loosdrecht, M.C., Vrouwenvelder, J.S., 2013. Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method. Water Res. 47 (19), 7131–7142.
Prest, E.I., Hammes, F., van Loosdrecht, M.C., Vrouwenvelder, J.S., 2016. Biological stability of drinking water: controlling factors, methods, and challenges. Front. Microbiol. 7, 45.
Props, R., Monsieurs, P., Mysara, M., Clement, L., Boon, N., Hodgson, D, 2016. Measuring the biodiversity of microbial communities by flow cytometry. Methods Ecol. Evol. 7 (11), 1376–1385.
Rittman, B., Snoeyinck, V.L., 1984. Achieving biologically stable drinking water. J. Am. Water Works Ass. 76 (10), 9.
Rogers, W.T., Holyst, H.A., 2009. FlowFP: a bioconductor package for fingerprinting flow cytometric data. Adv. Bioinformatics, 193947.
Rompré, A., Servais, P., Baudaert, J., de-Roubin, M.-R., Laurent, P., 2002. Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. J. Microbiol. Methods 49, 31–54.
Safford, H.R., Bischel, H.N., 2019. Flow cytometry applications in water treatment, distribution, and reuse: a review. Water Res. 151, 110–133.
Suthaker, S., Smith, D.W., Stanley, S.J., 1998. Optimisation of filter ripening sequence. J. Water Supply: Res. Technol. —AQUA 47 (3), 107–118.
Tatari, K., Corfitzen, C.B., Albrechtsen, H.J., christensen, S.C.B., 2016. Sensors for microbial drinking water quality. Technical University of Denmark.
Temmerman, R., Vervaeren, H., Noseda, B., Boon, N., Verstraete, W., 2006. Necrotrophic growth of Legionella pneumophila. Appl. Environ. Microbiol. 72 (6), 5.
US Congress, 1996. Safe Drinking Water Act (SDWA). Public Health Service, United States, pp. 964–1043.
van der Kooij, D., 2000. Biological stability: a multidimensional quality aspect of treated water. Water, Air Soil Pollution (123), 25–34.
van der Kooij, D., van der Wielen, P.W., Rosso, D., Shaw, A., Borchardt, D., Ibisch, R., Apgar, D., Witherspoon, J., di Toro, D.M., Paquin, P.R., 2013. Microbial growth in drinking water supplies. Iwa Publishing, London, United Kingdom.
van der Kooij, D., Visser, A., Hijnen, W.A.M., 1982. Determining the concentration of easily assimilable organic carbon in drinking water. J.-Am. Water Works Assoc. 74 (10), 540–545.
Van Nevel, S., De Roy, K., Boon, N., 2013. Bacterial invasion potential in water is determined by nutrient availability and the indigenous community. FEMS Microbiol. Ecol. 85 (3), 593–603.
Vang, O.K., Corfitzen, C.B., Smith, C., Albrechtsen, H.J., 2014. Evaluation of ATP measurements to detect microbial ingress by wastewater and surface water in drinking water. Water Res. 64, 309–320.
Vanysacker, L., De Gusseme, B., Buysschaert, B., Van den Belt, K. and De Winter, B. (2019) The risk of rainwater reuse in household installations, Berlin.
VITO, 2020. EMIS Compendium voor de monsterneming, meting en analyse van water (WAC). Flemish Government.
WHO, 2017. Guidelines for drinking-water quality. World Health Organisation.