Assessing biological stability of drinking water without disinfectant residuals in a full-scale water supply system

Frederik Hammes, Cordula Berger, Oliver Köster and Thomas Egli

ABSTRACT

Biological stability refers to the inability of drinking water to support microbial growth. This phenomenon was studied in a full-scale drinking water treatment and distribution system of the city of Zürich (Switzerland). The system treats lake water with successive ozonation and biological filtration steps and distributes the water without any disinfectant residuals. Chemical and microbiological parameters, notably dissolved organic carbon (DOC), assimilable organic carbon (AOC), heterotrophic plate counts (HPC) and flow-cytometric total cell concentration (TCC), were measured over an 18-month period. We observed a direct correlation between changes in the TCC, DOC and AOC concentrations during treatment; an increase in cell concentration was always associated with a decrease in organic carbon. This pattern was, however, not discerned with the conventional HPC method. The treated water contained on average a TCC of 8.97×10^4 cells ml^{-1}, a DOC concentration of 0.78 mg l^{-1} and an AOC concentration of 32 $\mu g\, l^{-1}$, and these parameters hardly changed in the distribution network, suggesting that the treated water had a high level of biological stability. This study highlights the descriptive value of alternative parameters such as flow-cytometric TCC for drinking water analysis, and pinpoints some of the key aspects regarding biological stability in drinking water without disinfectant residuals.

Key words | assimilable organic carbon (AOC), biological stability, drinking water, flow cytometry, total cell concentration (TCC)

Frederik Hammes (corresponding author)
Cordula Berger
Thomas Egli
Swiss Federal Institute of Aquatic Science and Technology (Eawag),
Überlandstr. 133,
CH-8600, Dübendorf,
Switzerland
Tel.: +41 44 823 5350;
Fax: +41 44 823 5547
E-mail: *frederik.hammes@eawag.ch*;
www.eawag.ch

Cordula Berger
Oliver Köster
Zürich Water Supply (WVZ),
Hardhof 9, P.O. Box 1179,
CH-8021, Zürich,
Switzerland

Thomas Egli
Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich,
CH-8092, Zürich,
Switzerland

INTRODUCTION

Uncontrolled and excessive growth of bacteria in drinking water can lead to a deterioration of the aesthetic water quality, such as the development of undesirable tastes and odours or visual turbidity (van der Kooij 2000; Hammes *et al.* 2008). It can also lead to process malfunctioning: for example, the clogging of point-of-use filters, bio-fouling of distribution pipes and bio-corrosion (Lee *et al.* 1980). In a worst-case scenario, regrowth can allow the proliferation of pathogenic bacteria (Vital *et al.* 2007, 2008), resulting in a hygienic risk to the consumer. One common approach to limit potential regrowth in drinking water is the addition of disinfectants such as chlorine, chlorine dioxide or monochloramine after the treatment train (LeChevallier 1999; van der Kooij 2000). While this has proven effectiveness, it is also known that some bacteria are resistant to chlorine (Barbeau *et al.* 2005), that there is a health risk associated with disinfection by-products, and that a negative consumer perception associated with the chlorinous taste exists (Hambsch 1999; Uhl & Schaule 2004). Some European countries–notably the Netherlands, Germany, Austria and Switzerland–have taken the approach of distributing high quality drinking water without the use of additional residual disinfectants (Hambsch 1999; van der Kooij *et al.* 1999). Drinking water treatment in such countries aims to limit microbial regrowth through limitation of the nutrients essential for growth, which is

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usually (but not necessarily) organic carbon (Miettinen *et al.* 1997; van der Kooij 2000). Under these conditions, the need to understand and accurately monitor the general quality and microbial stability of drinking water has a high priority.

The inability of drinking water to support microbial proliferation is expressed as the biological stability (intermittently termed 'microbial stability', 'microbiological stability', 'biostability' or 'regrowth potential') of the water (Rittmann & Snoeyink 1984; Miettinen *et al.* 1997; van der Kooij 2000; Laurent *et al.* 2005). Simply viewed, biological stability is a function of biologically available organic carbon substrate, and 'instability' is measured as an increase of biomass and a concomitant decrease of substrate. However, different interpretations, coupled to preferences for different parameters and methods, are often applied to the concept of biological stability. For example, Rittmann & Snoeyink (1984) defined 'biostability' as the lack of microbial growth specifically in the absence of disinfectant residuals, while Srinivasan & Harrington (2007) modelled biological stability taking into account the presence of disinfectant residuals. Rittmann & Snoeyink (1984) and van der Kooij (2000) regarded both the quality of water, as well as the growth-supporting nature of the materials used for distribution, as important factors when considering biological stability. In this regard, van der Kooij (2000) highlighted assimilable organic carbon (AOC) and biofilm formation rate (BFR) as the key parameters of biological stability. Other groups have disregarded BFR and favoured biodegradable organic carbon (BDOC) as the most important water quality parameter for biological stability (Escobar & Randall 2001; Laurent *et al.* 2005). Moreover, depending on the composition of the water, inorganic nutrients (e.g. phosphate), rather than organic carbon, can also be growth limiting (Kerneïs *et al.* 1995; Miettinen *et al.* 1997).

One of the more contentious aspects of biological stability is which microbiological parameter should be used to describe and monitor this phenomenon. The most used parameter is conventional heterotrophic plate counts (HPC) (Hambsch 1999; Carter *et al.* 2000; Müller *et al.* 2003; Srinivasan & Harrington 2007), even though it is well known that the HPC method does not accurately reflect total microbial abundance in drinking water (Yokomaku *et al.* 2000; Hoefel *et al.* 2003; Hammes *et al.* 2008).

Although the concept of biological stability is well known among drinking water microbiologists, little experimental and/or field data exist that underpin the main factors involved, and that adequately demonstrate the presence/absence of biological stability. One reason for this is the complexity of the problem: different countries often have completely different water qualities, different treatment technologies and employ different analytical tools for monitoring (Miettinen *et al.* 1997; van der Kooij 2000; Laurent *et al.* 2005). Another reason is a shortage of methods specifically describing the two main parameters: namely biologically available substrate (organic carbon) and microbial biomass. With regard to the latter, we have previously shown that flow-cytometric total cell concentration (TCC) is an important parameter for drinking water treatment and distribution systems, and that it holds more descriptive value for the treatment process than conventional HPC measurements (Hammes *et al.* 2008; Siebel *et al.* 2008).

Here we present a case study of a full-scale drinking water treatment system that treats surface water through successive ozonation and filtration steps and where the treated water is distributed without the addition of disinfectant residuals. Conventional drinking water parameters (HPC and DOC) were complemented with AOC and flow-cytometric TCC measurements during an 18-month sampling campaign. This study highlights the descriptive and complementary value of these two additional parameters for monitoring the microbial quality of drinking water, and it contributes to a better understanding of the fundamental principles of biological stability of drinking water.

MATERIALS AND METHODS

Preparation of AOC-free glassware

Borosilicate glass sampling bottles (250 ml) with glass caps were used for sampling, while 20 ml borosilicate glass vials were used for the AOC assays. Sterile, carbon-free glassware was prepared by heat-treatment (500°C, 6 h) as described previously (Greenberg *et al.* 1993; Hammes & Egli 2005). Teflon-coated caps for the AOC vials were cleaned of

residual AOC by soaking in warm persulfate (60°C, 1 h) as described previously (Greenberg *et al.* 1993).

Layout of the full-scale drinking water treatment plant and sampling sites

The full-scale plant that was monitored in this study produces roughly 50% of the drinking water for the city of Zürich (Switzerland) by treating surface water (Lake Zürich) through sequential ozonation and filtration steps. The treatment train consists of the following specific steps (Figure 1): (1) pre-ozonation (ozone dose 1.1 mg l^{-1} ± 25%, hydraulic contact time 50 min, ozone residual after contact time *c.* 0.15 mg l^{-1}); (2) rapid sand filtration (RSF) (double layer filter with 50 cm of pumice stone and 80 cm of quartz sand; filter flow rate 1.4–4 m h^{-1}); (3) intermediate ozonation (ozone dose 0.5 mg l^{-1}, hydraulic contact time 26 min, ozone residual after contact time 0.28 mg l^{-1}); (4) granular active carbon (GAC) filtration (double layer filter with 130 cm of GAC (Norit ROW 0.8 supra) and 40 cm of quartz sand, filter flow rate 4.6–13.2 m h^{-1}); (5) slow sand filtration (SSF) (quartz sand ~65 cm, filter flow rate 0.18–0.52 m h^{-1}); and (6) reservoir in the plant. Samples (250 ml) were taken roughly every two weeks over an 18-month period. Samples were taken before and after each step and also of the raw water and of the water coming from the reservoir (Reservoir 1) in the plant after treatment. Two points in the distribution network (DN) were sampled. Both points received their water from the same reservoir (Reservoir 2) in the distribution network which is located 2.4 km from the treatment plant (Figure 1). Point DN-1 was located a further 1 km from this reservoir, and point DN-2 was located 6.1 km from the reservoir. Assuming a retention time of one day in the reservoir, the water collected at points DN-1 and DN-2 have hydraulic retention times in the network of 29 h and 43.5 h, respectively. All samples were collected in AOC-free glassware and transported in cold-storage containers to the laboratory, where they were processed within four hours of sampling.

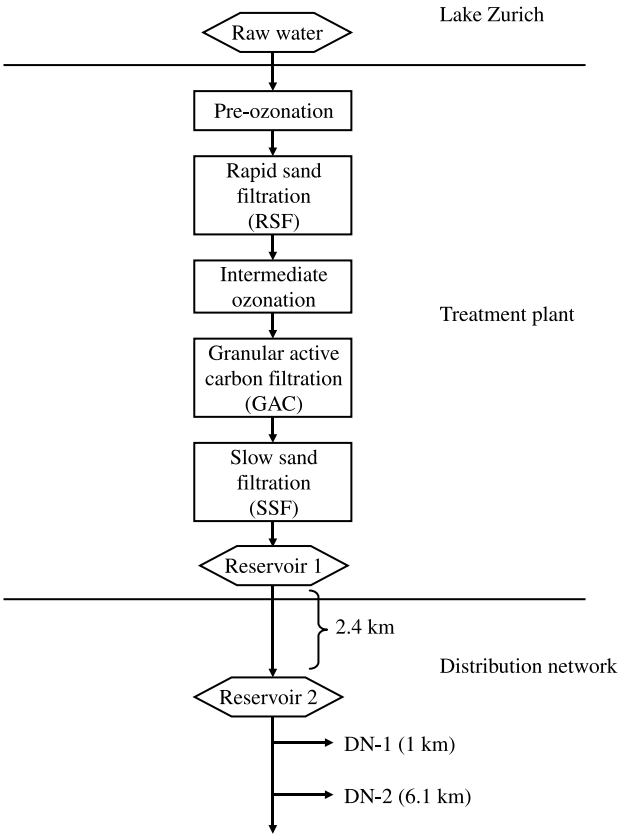

Figure 1 | Schematic presentation of the full-scale drinking water treatment plant (Lengg, Zürich, CH) monitored in this study. Water samples were taken during 18 months before and after every treatment step and, additionally, at two points in the distribution network (DN-1 and DN-2).

Total cell counts with fluorescence staining and flow cytometry (FCM)

Bacterial cells were stained with 10 µl ml^{-1} SYBR® Green I (1:100 dilution in dimethylsulfoxide (DMSO); Invitrogen) and incubated in the dark for at least 15 min before measurement. Where necessary, samples were diluted in filtered (0.22 µm; Millex®-GP, Millipore) bottled mineral water (Evian, France) just before analysis, so that the concentration measured with the flow cytometer was always less than 2×10^5 cells ml^{-1}. Flow cytometry was performed using a PASIII flow cytometer (Partec, Hamburg, Germany) equipped with a 25 mW solid state laser (488 nm) and volumetric counting hardware. Green fluorescence was collected in the FL1 channel (520 ± 20 nm), red fluorescence (also resulting from SYBR® Green I) was collected in the FL3 channel (> 615 nm) and all data were processed with the Flowmax software (Partec). Electronic gating with the software was used to separate positive signals from noise. All samples were collected as logarithmic (3 decades) signals and were triggered on the green

fluorescence channel (FL1). The standard instrument error on the FCM measurements was experimentally determined to be below 5%.

Conventional water parameters

The heterotrophic plate count (HPC) method was performed according to the Swiss guidelines for drinking water (SLMB 2000). In short: 1 ml of the water sample was transferred to a sterile Petri dish and mixed with about 15 ml plate count agar (PCA, Oxoid). The agar was kept at 46°C before plating. The samples were incubated at 30°C for 3 days. Phytoplankton in the lake water was measured as described in Müller *et al.* (2003). Dissolved organic carbon (DOC) (after 0.45 μm filtration) and particulate organic carbon (POC) (concentrated on a fibreglass filter (Whatman GF/F)) were measured on a Dimatoc 2000 TOC analyser equipped with a Dima 1000 Universal detector. Water temperature was measured on site during sampling.

Assimilable organic carbon (AOC)

AOC was determined with a batch growth assay as described previously (Hammes & Egli 2005; Vital *et al.* 2007). In short: the pasteurised and filtered water samples (15 ml) were inoculated with 10 μl (1×10^4 cells ml^{-1} initial concentration in the assay) of a bacterial AOC test-inoculum. These suspensions were then incubated at 30°C for three days (until stationary phase was reached) and the resulting growth was measured with flow cytometry (see above). The AOC test-inoculum comprised autochthonous bacteria from the treatment plant that was studied, and was prepared as described previously (Vital *et al.* 2007). The same bacterial community was used for all AOC determinations throughout the present study. As standard quality control prior to use, the performance of this inoculum was compared with bacterial AOC test-inocula used in previous studies in our group (Hammes *et al.* 2006; Vital *et al.* 2007), using different types of natural surface water as media. A difference of less than 10% in the average AOC values was deemed acceptable for use. AOC (μg l^{-1}) is estimated from cell concentrations (cells ml^{-1}) using a theoretical conversion factor (Hammes *et al.* 2006) (Equation 1). All assays were performed in triplicate. The detection limit of the method was 10 μg l^{-1} and the average standard deviation was ± 10%.

$$\mathrm{AOC}(\mu\mathrm{g\,l^{-1}}) = \frac{\text{net grown cells (cells l}^{-1})}{\text{conversion factor } (1 \times 10^7 \text{ cells } \mu\mathrm{g^{-1}})} \quad (1)$$

Data presentation and calculations

Given the large set of data collected over an 18-month period, we have opted to present the data as box plots to illustrate the spread of the data, and we have used the geometrical mean values of all data for the calculations.

RESULTS AND DISCUSSION

Water supply without the addition of disinfection residuals

The configuration of the Zürich drinking water treatment plant targets specifically the production of biologically stable, high quality drinking water that can be distributed without the need for additional disinfectants (Figure 1). The raw water (Lake Zürich) has a low organic carbon content (POC = 0.22 (±0.1) mg l^{-1}; DOC = 1.3 (±0.1) mg l^{-1}; AOC = 0.023 (±0.017) mg l^{-1}), which means that relatively low ozone dosages are required during treatment. Two ozonation steps are meant to serve as double disinfective barriers against any malignant microorganisms that may enter the system through the raw water, while also oxidising possible micropollutants in the water (Müller *et al.* 2003; Von Gunten 2003). The ozonation also transforms stable, dissolved natural organic matter (NOM) molecules and organic particles (e.g. phytoplankton) into typical AOC molecules (Von Gunten 2003; Hammes *et al.* 2006, 2007). Three separate biological filtration processes (rapid sand filtration, granular activated carbon filtration and slow sand filtration) are the basis for the removal of organic carbon from the water. We have demonstrated previously that these filters are biologically active with high AOC removal capacity (Hammes *et al.* 2006; Velten *et al.* 2007). As a result, regrowth of microorganisms occurs in the biological filters together with the removal of biologically available

organic carbon. The combination of low organic carbon concentrations and an autochthonous community of natural planktonic bacteria stabilises the treated water, which is then distributed without additional disinfection.

General microbiological quality of the water in the treatment train

Flow-cytometric total cell concentration (TCC) was a sensible and reliable parameter to describe bacterial removal and regrowth during drinking water treatment and in the distribution system during the 18-month sampling campaign

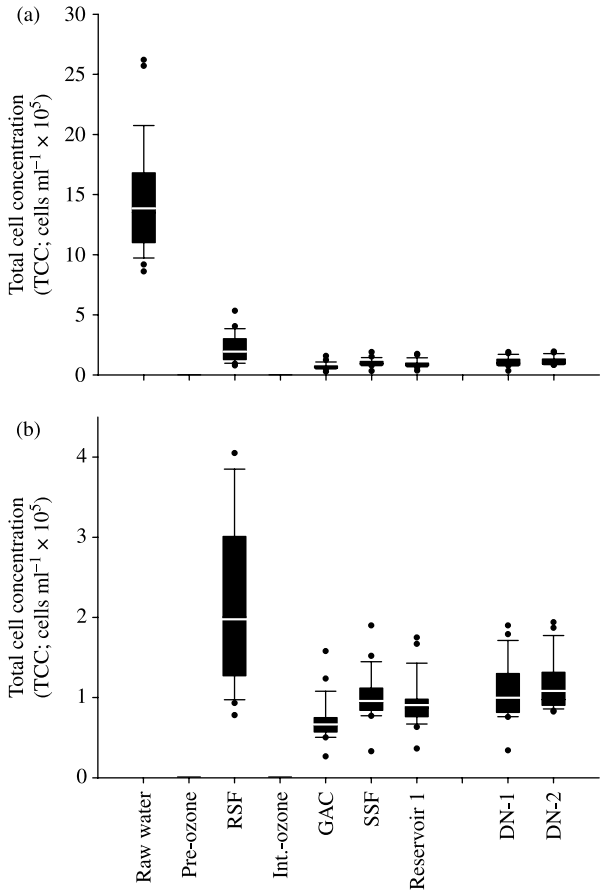

Figure 2 | (a) Changes in the total cell concentration during drinking water treatment over an 18-month period, as measured with flow cytometry; (b) detailed overview of the treatment process (excluding raw water data). RSF = rapid sand filtration; Int-ozone = intermediate ozonation; GAC = granular active carbon filtration; SSF = slow sand filtration; DN = distribution network; BDL = below detection limit (< 1,000 cells ml^{-1}). Black bars represent the 75 percentiles, whisker lines represent the 90 percentiles and black dots represent the outlier values. White bisects are the geometrical mean values for all data points, used for all calculations ($n = 27$).

(Figure 2(a), (b)). The raw water, originating from 30 m depth of lake Zürich, contained 1.38 × 10^6 cells ml^{-1} (geometrical mean; $n = 27$), which is typical for natural surface water in the region (Wang *et al.* 2007; Hammes *et al.* 2008). Twice during the treatment, all planktonic bacteria in the water are completely destroyed by ozonation to levels below the flow-cytometric quantification limit (Figures 1 and 2; see also Hammes *et al.* 2008), safeguarding the system against any potential incursion of pathogenic bacteria in the raw water. Subsequently, benign heterotrophic microbial communities regrow in the various biological filters, feeding on the organic carbon originating from the ozonated raw water, and thus filling the niche that was created with the ozonation process. As a result, the treated water (Figure 2, 'reservoir') contained a stable planktonic community of 8.97 × 10^4 cells ml^{-1}, which is a typical value for public drinking water and bottled water where residual disinfectants are not used (Hammes *et al.* 2008; Berney *et al.* 2008). Notably, fewer cells are produced during biofiltration after each ozonation step (1.98 × 10^5 cells ml^{-1} after RSF; 0.7 × 10^5 cells ml^{-1} after GAC). This can be attributed to changes in the organic carbon quantity and quality in the influent of each filtration step (discussed below).

HPC is the conventional parameter for characterising microbial numbers in drinking water (Carter *et al.* 2000; Allen *et al.* 2004; Uhl & Schaule 2004), and as such, variations of the HPC method are usually included in guidelines or legislation for drinking water treatment (Hambsch 1999; SLMB 2000; Uhl & Schaule 2004). HPC (30°C; 3 days) values for the Zürich treatment and distribution system were typically low (< 10 cfu ml^{-1}) and meet the local water quality criteria (Figure 3). Note that there was no evident correlation between the TCC and the HPC in the treatment train (Figures 2 and 3(a)), which concurs with numerous previous reports that only a small percentage of planktonic bacteria in drinking water is in fact culturable (Yokomaku *et al.* 2000; Berney *et al.* 2008; Hammes *et al.* 2008). Also, the HPC values did not accurately reflect the main microbiological events (i.e. die-off through ozonation and regrowth during biological filtration) that occurred in the treatment train (Figure 3(b)). As a result, all calculations below were done with the TCC data. It is noted that different HPC methods often give different results, specifically, R2A agar and lower incubation temperatures giving higher HPC values (Leclerc & Moreau

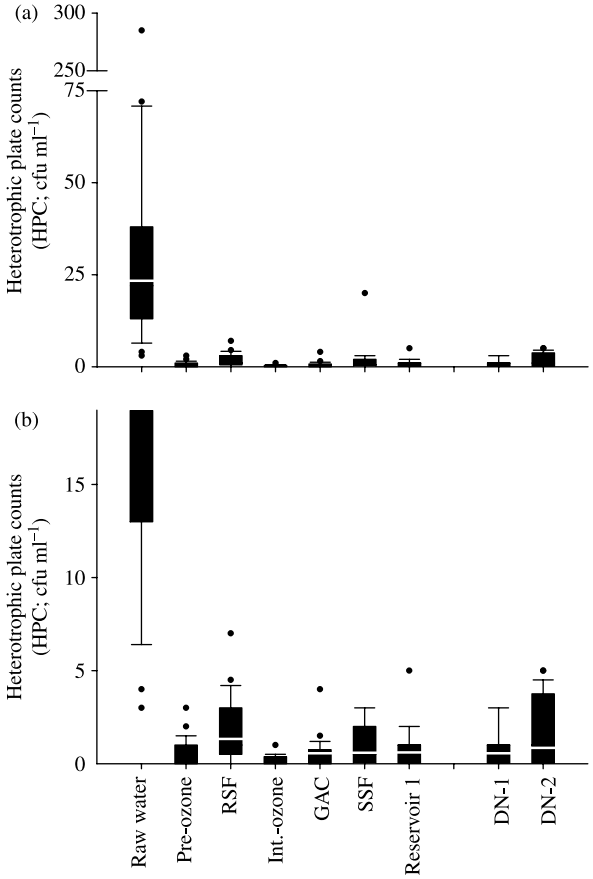

Figure 3 | (a) Changes in the heterotrophic plate counts (HPC) during drinking water treatment over an 18-month period; (b) detailed overview of the treatment process (excluding raw water data). See Figure 2 for key to abbreviations and scale ($n = 27$).

2002; Uhl & Schaule 2004; Berney *et al.* 2008). For example, in a previous study of the same treatment plant, Müller *et al.* (2003) reported significantly higher HPC values with a lower incubation temperature (20°C/3 d) for both the raw water and the effluent of the rapid sand filter, compared with the results from the present study. Whether these differences are due to environmental changes in the raw water, or a result of different HPC methods that were used, is not evident. In the present study we have followed specifically the HPC method as set out in the Swiss guidelines for drinking water analysis (SLMB 2000). However, irrespective of the HPC method applied, the data suggest that rapid FCM analysis of the TCC in the water is more useful than HPC analysis for assessment of the general microbial quality of drinking water during treatment.

Organic carbon parameters

The increase of bacteria (regrowth) during different filtration steps, as measured with the total cell concentration (Figure 2), was equally reflected in simultaneous decreases observed in two main organic carbon parameters (DOC and AOC) (Figures 4 and 5). Changes in the DOC concentration were reflective of the different treatment processes (Figure 4). The raw water had a low DOC concentration (1.3 mg l^{-1}) and ozonation did not alter this concentration. With the applied ozone dosage one would not expect complete mineralisation to carbon dioxide, but merely a conversion of the carbon towards AOC-like molecules such as organic acids and aldehydes (Von Gunten 2003; Hammes *et al.* 2006). Therefore, the DOC concentration before and after ozonation was identical (Figure 4), while the AOC concentration changed significantly during this step (Figure 5). The filtration steps all resulted in the partial removal of DOC. Rapid sand filtration (empty filter bed contact time: 0.92–0.32 h) removed on average 248 µg l^{-1} (19%), granular activated carbon filtration (empty filter bed contact time: 0.37–0.13 h) removed 102 µg l^{-1} (10%) and slow sand filtration (empty filter bed contact time: 3.61–1.26 h) removed a further 164 µg l^{-1} (13%). On average, much more DOC than AOC was removed in the entire treatment (515 µg l^{-1} DOC vs. 93 µg l^{-1} AOC). Although this depended on the time of year

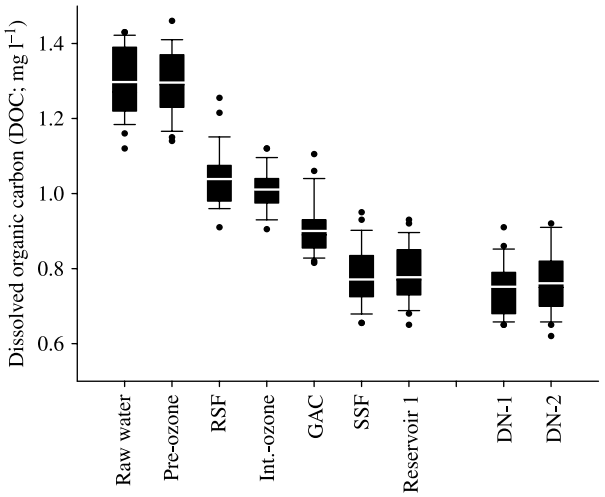

Figure 4 | Box plot of changes in the DOC concentration during drinking water treatment. Data were collected over an 18-month period. See Figure 2 for key to abbreviations and scale ($n = 27$).

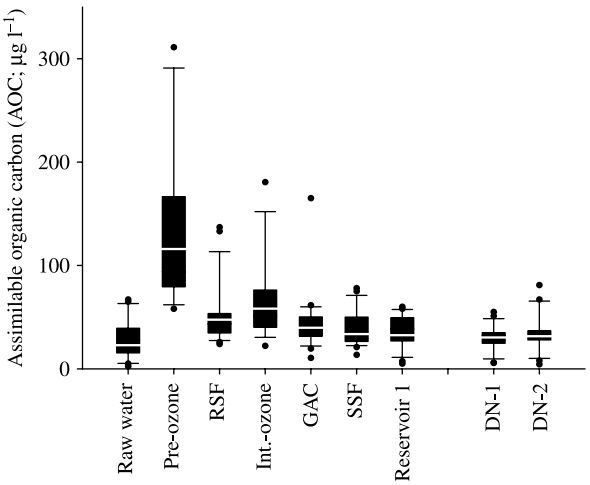

Figure 5 | Box plot of changes in the AOC concentration during drinking water treatment. Data were collected over an 18-month period. See Figure 2 for key to abbreviations and scale ($n = 27$).

and other factors, typically only 18% of the removed DOC was measured as AOC. We have often observed that when planktonic bacteria grow in natural water, nearly all consumed DOC can be measured as AOC, but when the same water is treated with sand or GAC filters, the DOC reduction is significantly higher than the AOC change (unpublished data). One explanation for this phenomenon might be sorption to the particle surfaces and slow turnover of high molecular weight DOC compounds (e.g. polymers) in the sand filters and GAC reactors.

Although less AOC than DOC was removed, the changes in AOC concentrations showed an expected descriptive pattern of the treatment train (Figure 5). The AOC concentration in the raw water was low ($23\,\mu g\,l^{-1}$), as is expected for a stable natural water system. Pre-ozonation resulted in an increase of the AOC concentration (mean = $113\,\mu g\,l^{-1}$), but the amount of AOC that was formed during ozonation varied seasonally (range: $50–311\,\mu g\,l^{-1}$), with typically more AOC formed during colder raw water temperature periods (data not shown). This corroborates a previous observation of higher AOC concentrations after ozonation in winter months (Müller *et al.* 2003). However, it was not evident from the data whether the increased AOC concentrations could be ascribed directly to an influence of temperature, or rather to seasonal changes in the raw water composition (e.g. algal blooms). We have previously shown that ozonation of filtered (0.22 µm) water from lake Zürich forms predominantly organic acids (acetate, oxalate and pyruvate) which are typical AOC molecules (Hammes *et al.* 2006), while the ozonation of phytoplankton in the raw water could have contributed to the AOC formation as well (Müller *et al.* 2003; Hammes *et al.* 2007). However, in the current study, no specific correlation was found between the phytoplankton concentration in the raw water and the concentration of AOC that was formed (data not shown). Rapid sand filtration resulted in the average removal of 59% of the total AOC (down to a mean value of $46\,\mu g\,l^{-1}$), which underlined the biological nature of this treatment step, as was also demonstrated previously (Müller *et al.* 2003; Hammes *et al.* 2006). Intermediate ozonation resulted in only little additional AOC formation (from $46\,\mu g\,l^{-1}$ to $58\,\mu g\,l^{-1}$), probably because the first ozonation step already oxidised most of the easily oxidisable carbon. Subsequently, the granular active carbon filter and the slow sand filter removed a further 31% and 6%, respectively, of the remaining AOC from the water, resulting in a final AOC concentration of $32\,\mu g\,l^{-1}$. The AOC concentrations reported in the present study are slightly higher than those reported in a previous study of the same treatment plant, but follow the same overall trend (Müller *et al.* 2003). The higher values can be attributed to methodological differences in the measurement of AOC from the two studies.

A correlation between cell growth and carbon removal

It is important to note that in all cases where cell growth was observed (thus a biomass increase), it coincided with a decrease in both AOC and DOC concentrations (thus a substrate decrease) (Figures 2, 4 and 5). In the filtrate of the rapid sand filter, an average cell concentration of 1.98×10^5 cells ml^{-1} was recorded (Figure 2), which coincided with the removal of $248\,\mu g\,l^{-1}$ DOC (Figure 4) and $67\,\mu g\,l^{-1}$ AOC (Figure 5). Based on the DOC values, this constitutes a numerical cell yield of 7.98×10^5 cells $\mu g\text{-}C^{-1}$ (assuming steady state conditions of the biofilm biomass on the filter media and not considering any potential losses through the filter backwashing process). The yield from the granular activated carbon filter (also backwashed) was lower (6.57×10^5 cells $\mu g\text{-}C^{-1}$) and from the slow sand filter it was considerably lower (1.77×10^5 cells $\mu g\text{-}C^{-1}$).

The lower yields in the two later reactors can be ascribed to a probable reduction in the quality of the DOC, as easy utilisable AOC molecules would be removed first in the rapid sand filter. This is corroborated by the percentage of AOC that was removed as DOC in the various reactors (RSF = 27%; GAC filter = 18%; SSF = 3%). However, the data used for these calculations are only the bulk measurements before and after each reactor, and do not take into account potential turnover in the reactors, that is, that some cells might also be retained or even grazed by higher organisms in the slow sand filter. This would naturally alter the precise yield values, particularly for the slow sand filter. However, for practical reasons this aspect was not considered in this study.

It is interesting to note that, for the same treatment plant, Müller *et al.* (2003) detected only significant regrowth in the rapid sand filters, and only in the winter periods. These authors furthermore suggested that an AOC concentration of 100 $\mu g l^{-1}$ is required to stimulate significant regrowth (Müller *et al.* 2003). Similarly, Carter *et al.* (2000) did not detect a clear correlation between organic carbon (e.g. AOC) and bacterial concentrations (as HPC) in distributed drinking water. Although this seems contradictory to the present study, it is important to note that the conclusions from both aforementioned studies (Carter *et al.* 2000; Müller *et al.* 2003) were based on HPC values and not on total cell concentrations. The clear correlation between changes in total cell concentrations and AOC and DOC concentrations in the present study is further evidence that the total cell concentration is one of the most accurate microbiological parameters with which changes during drinking water treatment can be described (Hammes *et al.* 2008).

Biological stability of treated water

Total DOC removal during treatment was 40% and resulted in a final concentration of 780 $\mu g l^{-1}$ in the treated water. Throughout the 18 months of this study, hardly any decrease in the DOC concentration of the treated water was observed in the distribution system (20–30 $\mu g l^{-1}$; Figure 4), and likewise for the AOC concentrations (2–4 $\mu g l^{-1}$; Figure 5). A paired student t-test suggested that these minute decreases were significant at point DN-1 but not at point DN-2 (Table 1). Notably, van der Kooij (2000) proposed an AOC concentration of less than 10 $\mu g l^{-1}$ for biological stability of non-chlorinated water. We have shown previously that the AOC method used in this study typically presented higher values than the method used by van der Kooij (2000) (Hammes & Egli 2005), which may explain the difference between the proposed value (10 $\mu g l^{-1}$) and the observed value (32 $\mu g l^{-1}$). The TCC showed a small but significant increase (11–18%) during distribution (Figure 2(b); Table 1). Interestingly, if one applies the yield factors derived from the treatment train data ($2–8 \times 10^5$ cells $\mu g\text{-}C^{-1}$; above), the observed DOC decrease (20–30 $\mu g l^{-1}$) accounts for about $0.5–2 \times 10^4$ cells ml^{-1}, which would be enough to explain the 11–18% TCC increase (Table 1). This suggests that TCC is a parameter that can be used as a sensitive indicator for biological stability and growth in treatment and distribution systems.

Higher water temperatures could have promoted bacterial regrowth during distribution (Kerneïs *et al.* 1995; Uhl & Schaule 2004). The temperature in the treatment plant varied only slightly during the sampling period (range: 3.9–7.1°C), but the distribution network was subject to significantly higher temperatures and larger fluctuations in

Table 1 | Geometrical mean values ($n = 27$) for the treated water (reservoir) and distribution network (DN) sampling points during the entire sampling period (18 months). Significant variations between the distribution network values and the treated water are indicated

	Reservoir	DN-1	DN-2
pH	8.04	8.04	8.04
Temperature (°C)	6.15	8.37*	8.80*
DOC ($mg l^{-1}$)	0.78	0.75*	0.76
AOC ($\mu g l^{-1}$)	32	28*	30
HPC ($cfu\ ml^{-1}$)	1	1	1
TCC ($cells\ ml^{-1}$)	8.97×10^4	$1.00 \times 10^{5*}$	$1.09 \times 10^{5*}$

*$P < 0.05$ (paired student t-test).

temperature (range: 3.6–17.7°C), particularly during the summer periods. However, no correlation was found between increased temperatures and increased cell concentrations (data not shown). There are no existing guidelines for biological stability with respect to TCC. If biological stability of drinking water is interpreted in the strictest sense as the absence of any change in the concentrations of either growth-supporting nutrients or biomass, a certain level of instability was measured (Table 1). However, the minor change in TCC (11–18%), and the fact that the organic carbon concentrations hardly changed during distribution, suggests in our opinion that the distributed water displayed a high level of biological stability.

CONCLUSIONS

- Two alternative methods for the monitoring and characterisation of drinking water have been tested: namely, flow-cytometric total cell concentration (TCC) and assimilable organic carbon (AOC). These two methods have been shown to be useful for describing microbiological changes during treatment in a full-scale water supply system.
- From this study we conclude that the cultivation-independent parameter, TCC, allows a more quantitative description of biological stability than conventional heterotrophic plate counts.
- During the 18-month sampling campaign, the treated drinking water had an average TCC of 8.97×10^4 cells ml^{-1}, DOC of 0.78 $mg\,l^{-1}$ and AOC of 32 $\mu g\,l^{-1}$. These parameters changed only minutely during distribution, suggesting that the treated water had a high level of biological stability.
- Monitoring of straightforward parameters for substrate (DOC) decrease and biomass (TCC) increase allows a basic understanding of growth and biological stability in drinking water treatment and distribution systems.

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