Assessing the transition effects in a drinking water distribution system caused by changing supply water quality: an indirect approach by characterizing suspended solids

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Abstract

Worldwide, it is common that the drinking water distribution systems (DWDSs) may be subjected to changes of supply water quality due to the needs of upgrading the treatment processes or switching the source water. However, the potential impacts of quality changed supply water on the stabilized ecological niches within DWDSs and the associated water quality deterioration risks were poorly documented. In the present study, such transition effects caused by changing the supply water quality that resulted from destabilization of biofilm and loose deposits in DWDS were investigated by analyzing the physiochemical and microbiological characteristics of suspended particles before (T0), during (T3-weeks) and after upgrading the treatments (T6-months) in an unchlorinated DWDS in the Netherlands. Our results demonstrated that after 6 months’ time the upgraded treatments significantly improved the water quality. Remarkably, water quality deterioration was observed at the initial stage when the quality-improved treated water distributed into the network at T3-weeks, observed as a spike of total suspended solids (TSS, 50–260%), active biomass (ATP, 95–230%) and inorganic elements (e.g. Mn, 130–250%). Furthermore, pyrosequencing results revealed sharp differences in microbial community composition and structure for the bacteria associated with suspended particles between T0 and T3-weeks, which re-stabilized after 6 months at T6-months. The successful capture of transition effects was especially confirmed by the domination of Nitrospira spp. and Polaromonas spp. in the distribution system at T3-weeks, which were detected at rather low relative abundance at treatment plant. Though the transitional effects were captured, this study shows that the introduction of softening and additional filtration did not have an effect on the water quality for the consumer which improved considerably after 6-months’ period. The methodology of monitoring suspended particles with MuPFIs and additional analysis is capable of detecting transitional effects by monitoring the dynamics of suspended particles and its physiochemical and microbiological composition.

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1. Introduction

Drinking water treatments remove contaminants present in source water to make water potable. In both developing and
industrialized nations, a growing number of contaminants are entering water supplies from human activity: from pathogen/virus, heavy metals to micropollutants (Shannon et al., 2008; Ternes et al., 2015). Consequently, public health, environmental concerns and growing constraint to optimize the esthetical and comfort quality for the consumers (e.g. drinking water without chlorine taste and low in hardness) drive efforts to further treat waters previously considered clean, which has greatly promoted the development of water treatment science and technology over past decades (Shannon et al., 2008). In practice, the developments have been focusing on the upgrades of treatments and improvements of supply water quality regarding physiochemical and microbiological parameters, e.g. the concentrations of elements composition, nutrients concentration, cell number and microbial community (Liu et al., 2019; Xing et al., 2018b). However, the quality-changed drinking water still has to be delivered to customers’ taps through the old distribution systems in which biofilm and loose deposits have been established for decades (Liu et al., 2013b).

In drinking water distribution systems (DWDSs), over 98% of the total biomass was found to be contributed by the bacteria accumulated within loose deposits and biofilm (Liu et al., 2014). In particular, the biofilm in DWDSs has been widely documented because of its potential health risk (Batté et al., 2003; Chaves Simões and Simões, 2013; Flemming et al., 2002, Van Der Wende et al., 1988; Wingender and Flemming, 2011). As reported, biofilm can be as much as 10^6 CFU cm^-2 (Batté et al., 2003), 10^7 cells cm^-2 (Lehtola et al., 2006) or 10^9 pg ATP cm^-2 (Lehtola et al., 2006) depending on the measuring methods. The presence of biofilm promoted the deposition of elements such as manganese (Mn) and calcium (Ca) in a distribution system (Liu et al., 2017a; Sly et al., 1990). Similarly, loose deposits, reported to be reservoirs for inorganic elements, organic nutrients and bacteria (Gauthier et al., 1999; Lehtola et al., 2004; Liu et al., 2017a; Zacheus et al., 2001), can be as much as 24.5 g m^-1 in a full-scale distribution system (Carrière et al., 2005) and harbor comparable biomass (671–3738 ng m^-1 ATP) to biofilm (534 ± 23 ng m^-1 ATP) (Liu et al., 2014).

Under the regular water supply conditions, there is an equilibrium between the water and the solid phases in the network (e.g. loose deposits and biofilm). It is a common sense that water quality may deteriorate during distribution; the extreme cases have been observed and reported as dirty water (Sly et al., 1990) and discoloration (Vreeburg and Boxall, 2007; Xing et al., 2018a). For distribution of quality-changed water through old pipes, the equilibrium will be disturbed, and material harbored by distribution pipes (e.g. pipe scales, biofilm and loose deposits) will be destabilized and released into water column which can be potentially harmful (Feazel et al., 2009; Li et al., 2010; Liu et al., 2017b; Torvinen et al., 2004). As previously defined, such destabilization may be caused by physicochemical and microbiological water quality changes that break the established forces balance in pipe scales, biofilm and loose deposits, such as physical destabilization (e.g. reducing the weight of particles causing loose deposits resuspension), chemical destabilization (e.g. changes of pH, redox and ion composition can remobilize contaminants bound by pipe scales on metal pipes via desorption and/or dissolution), and microbiological destabilization (e.g. changes of nutrients concentration and composition can influence the microbial community and function in biofilm) (Liu et al., 2017b). It has been quantified that the release of 20% of either biofilm or loose deposits will cause significant changes in the bulk water bacterial community (Liu et al., 2017a). In practice, one example is the occurrence of red water in large areas of Beijing in 2008 when the city switched to better source water transported 1400 km from southern China, where increased sulfate in supply-water caused microbial community composition changes revealed by increase in sulfur oxidizing bacteria, sulfate reducing bacteria and iron oxidizing bacteria and red water events associated with high iron concentrations (Li et al., 2010). Recently, in the Flint drinking water crisis in Michigan, U.S., elevated blood lead levels were detected in children after water source changes (Hanna-Attisha et al., 2016), which has been attributed to the missing of orthophosphate corrosion inhibitor and lead leaching from the aging pipes into water column.

However, until now, our understanding of the water quality deterioration risk associated with biofilm and loose deposits destabilization in distribution systems during switching supply water quality is limited. This has been mainly attributed to the lack of accessibility of real distribution systems for study (Berry et al., 2006) and the dilution effects of large volumes of water that keep flowing through the system increasing the difficulty of detection (Liu et al., 2017b). The suspended particles, especially the associated bacteria, have been used to study the effects of mixing water on bacterial community (Liu et al., 2016) and used as SourceTracker to study the contribution of biofilm detachment and loose deposits resuspension to the tap water bacteria (Liu et al., 2018). To overcome the above-mentioned difficulties, monitoring the variations of suspended solids characteristics can be used as an indirect approach without deconstructing distribution pipes or interrupting water supply services, while still being able to detect the changes with serious implications for health risks and esthetical water quality. This study followed the upgrade of treatments in an unchlorinated drinking water supply system in the Netherlands, monitored the suspended solids in treated and distributed water before (T0), during (T3-weeks) and after the treatment upgrade (T6-months). The objective was to capture and study the potential release of elements and biomass caused by biofilm and loose deposits destabilization subjected to the changes in the supply water quality caused by the introduction of new softening and rapid sand filtration steps (for example: decrease of hardness and suspended particle load) through monitoring and characterizing suspended particles in the drinking water leaves treatment plant and distributed water at the customers’ taps.

2. Material and methods

2.1. Treatment plant and sampling locations

The drinking water treatment plant produces drinking water from anoxic groundwater (3.8 Mm^3/year). Before introducing new treatment steps (softening, second rapid sand filtration and adding carbon dioxide), the water was treated by aeration and rapid sand filtration before being pumped into the distribution system. The sampling locations were selected at the treatment plant before the water entered the distribution system (TP, 0 km): locations at customers’ taps at DS1 (5 km to TP), DS2 (11 km to TP), and DS3 (17 km to TP). The distribution networks in the study area is 110 mm PVC-U pipes (water main pipe). The treatment processes and sampling locations are illustrated in Fig. S1. The produced water quality before and after treatment changes is given in Table 1.

2.2. Sampling of suspended solids

The suspended solids (SS) were sampled by multiple particle filtration systems (MuPfIsSs) as previously described (Liu et al., 2013a). In short, the system has four filtration lines in parallel with water meters in each line to measure the volume of water flow filtered. The SS were sampled by filtering approximately 200 L of water through glass fiber filters (Whatman, 1822–047, 1.2 μm) over a period of 3 h under tap pressure (~2.0 bar). The filter pore size was selected according to our previous study (Liu et al., 2013a). Before
each sampling, the water tap was flushed until a constant temperature at the tap to make sure the water from distribution system was taken (-5 min s for a typical Dutch household).

The sampling of suspended solids was conducted over three time periods: before (1 month, T0, in March), during (at the 1st, 2nd and 3rd week immediately after the introduction of new treatment steps, T3 weeks, in April), and stabilized after treatment upgrades (6 months, T6 months, in October). Comply with the stable climate temperature in the three different sampling periods at the study area in the Netherlands, the temperature in the distribution system were also comparable (~11–15 °C). Therefore, the samples from both the source ground water and the distribution sites were not subjected to the potential influences of temperature fluctuations from seasons.

For each period, triplicate samples were obtained by running MuPFiSS on the same day of the week for three consecutive weeks at all sampling locations. For each run of MuPFiSS, four filters were collected in parallel, three of which were sent for TSS, elements and ATP/DNA analysis, respectively. The 4th filter was set as back up in case of any filter broken during the sampling. For T6 months, the third time-consuming was contaminated, therefore results from duplicate samples were presented. In total, 32 samples were collected for the whole period of this study at each location (12 from T0, 12 from T3 weeks, 8 from T6 months), which resulted in 128 filters from all locations (32 × 4 = 128). For each parameter of TSS, elements and ATP/DNA sequencing, 32 filters have been analyzed. Every time, water samples were collected together with the MuPFiS run from the nearest tap (32 water samples along with the filter samples).

### 2.3. Sample preparation

Four samples can be obtained by each MuPFiS run for different analyses. The filters for particle-associated bacteria analysis were inverted and submerged into 5 mL of autoclaved tap water with glass beads immediately after filtration. As described previously (Liu et al. 2013a, 2016), all of the samples were maintained in a cooling box and transported to the laboratory within 2 h after sampling; the bacteria were detached from the particles by a low energy ultrasonic treatment performed 3 times, for 2 min each (Branson ultrasonic water bath, 43 kHz, 180 W power output, 10 L sonication chamber). The obtained suspensions were used for particle-associated bacteria (PAB) quantification and DNA extraction. The other filters were kept for total suspended solids (TSS) and elemental composition analyses.

### 2.4. Sample analysis

#### 2.4.1. Total and volatile suspended solids analysis (TSS and VSS)

Suspended material is collected on the filter for mass measurement. Prior to filtration, filters were pre-dried in the oven for 2 h at 105 °C. Gravimetric analyses were conducted by weighing the filters before and after filtration (drying at 105 °C), providing the TSS, and after a second filtration (combusting in a muffle furnace at 550 °C) for 2 h, providing the VSS (American Water Works Association 1998).

#### 2.4.2. Inductively coupled plasma-mass spectroscopy (ICP-MS)

Concentrations of several elements in the samples, generated using sequential extractions and filtration experiments utilizing filters with varying sizes, were determined by inductively coupled plasma-mass spectroscopy (ICP-MS) (PerkinElmer ELAN DRC-e ICP-MS). The elements quantified in these measurements included iron (Fe), calcium (Ca), and manganese (Mn). Quality control samples, including laboratory-fortified blanks and laboratory-fortified samples, were performed for every 10 samples analyzed. Average elemental recoveries ranged from 85.2 to 92.8% for the laboratory-fortified samples.

#### 2.4.3. Adenosine triphosphate (ATP)

To study the biological properties of collected suspended solids (SS), the suspension obtained after the above-described pre-treatment was analyzed according to adenosine triphosphate (ATP) content. The ATP of SS was defined as attached ATP (A-ATP) and measured as previously described (Liu et al., 2013a). In short, the released ATP from cells by nucleotide-releasing buffer (NRB, Celsis) was measured by the intensity of the emitted light in a luminometer (Celsis Advance<sup>TM</sup>) calibrated with solutions of free ATP (Celsis) in autoclaved tap water following the procedure as given by the manufacturer.

#### 2.4.4. DNA extraction and 454 pyrosequencing

DNA was extracted from the suspension using the FastDNA Spin Kit for Soil (Qiagen/MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions (Hwang et al., 2011; Tamaki et al., 2011) and was amplified with the bacterium-specific forward primer 27 F and the reverse primer 534 R (Hong et al., 2010). DNA extraction were formed on unused filters to be used as blank, none of which contained sufficient DNA performing downstream sequencing analysis. The 454 pyrosequencing was performed with a 454 Life Sciences GS FLX series genome sequencer (Roche, Switzerland). The obtained DNA sequences were deposited in the DDBJ sequence read archive (Accession Number: PRJNA498802).

#### 2.4.5. Sequencing data processing

The sequences generated from pyrosequencing were processed by removing low quality sequence ends (threshold: Q = 20), primers, and singleton.UCHIME software was used to identify and remove chimeras (Edgar et al., 2011). Afterwards, the sequences

### Table 1

Water quality before and after changing the treatments.

| Parameters          | Before treatment changes (Finished water, n = 6) | After treatment changes |
|---------------------|--------------------------------------------------|-------------------------|
|                     | 3 weeks (n = 6)                                  | 6 months (n = 6)        |
| Turbidity (NTU)     | 0.20 ± 0.09                                      | 0.15 ± 0.06             |
| PH                  | 7.41 ± 0.03                                      | 7.53 ± 0.04             |
| ATP (ng l<sup>-1</sup>) | 4.0 ± 0.9                                      | 3.6 ± 0.4               |
| TCC (cells ml<sup>-1</sup>) | 1.6 × 10<sup>4</sup> ± 1.5 × 10<sup>4</sup> | 1.5 × 10<sup>3</sup> ± 3.5 × 10<sup>3</sup> |
| TOC (mg l<sup>-1</sup>) | 1.7 ± 0.3                                      | 1.7 ± 0.2               |
| Ca (µg l<sup>-1</sup>)  | 84.1 ± 2.8                                      | 78.4 ± 0.8             |
| Mg (µg l<sup>-1</sup>)  | 10.4 ± 1.5                                      | 10.8 ± 0.7             |
| NH<sub>4</sub> (µg l<sup>-1</sup>) | 0.04 ± 0.02                                    | <0.01                   |
| Fe (µg l<sup>-1</sup>)  | 0.012 ± 0.004                                   | <0.002                  |
| Mn (µg l<sup>-1</sup>)  | 0.014 ± 0.007                                   | <0.005                  |
were trimmed, resulting in an average sequence length of 230 bp. The merged alignments of the sequences were obtained via the infernal aligner from the Ribosomal Database Project (RDP) pyrosequencing pipeline (http://pyro.cme.msu.edu/) and the NAST alignment tool from Greengenes, based on the software developed by the Biotechnology Center at the University of Illinois (UI) (http://acai.igb.uiuc.edu/bio/merge-nast-infernal.html). The RDP Classifier was used for the taxonomical assignments of the aligned 454 pyrosequences at the 97% sequence similarity cut-off. The total PAB communities from the different sampling points were analyzed for the number of operational taxonomic units (OTUs), species richness, and biodiversity using the Quantitative Insights INTO Microbial Ecology (QIIME) program (Caporaso et al., 2010).

Core OTUs were defined as the OTUs with a cutoff of relative abundance (>1%) in each sampling period. The core genus is defined corresponded to taxonomy information of the core OTUs. Alpha-diversity indices were calculated based on the rarefied OTU table at a depth of 5000 sequences per sample (rarefaction analysis). Beta diversity comparison was calculated at sequence depth of 1046, which could cover all the sequenced samples. The unweighted and weighted UniFrac distance matrices were constructed from the phylogenetic tree and used to conduct the principal coordinate analyses (PCoA) using R vegan package (Noyce et al., 2016). Venn diagrams were drawn using R VennDiagram package to analyze overlapped and unique OTUs among different sampling locations at each sampling period (Chen and Boutros, 2011). Heatmap was implemented by R heatmap packages (Kolde, 2013).

2.4.6. Statistically analysis

Different statistical tools were applied using Past and R (vegan package), including: (1) one-way analysis of variance (ANOVA) tests to determine the significance of differences on physicochemical and microbiological parameters; (2) one-way permutational analysis of variance (PERMANOVA) based on Bray-Curtis similarity matrices to test the significance of differences regarding the beta diversity of bacterial communities (Anderson and Walsh, 2013). The differences were considered significant when the p-value was lower than 0.05 (P < 0.05).

3. Results

3.1. Water quality improvements

Generally, the water quality clearly improved after upgrading the treatments (Table 1): turbidity removal improved by more than 50% (P < 0.05), meanwhile 15%, 35% (P < 0.05), and 7% more TOC, Ca and Mg were further removed, respectively. The NH₄, Fe and Mn that were detected before were under the detection limit after upgraded treatments (P < 0.05). About 20% extra active biomass reduction (P < 0.05), as quantified by both ATP and TCC, was achieved by the introduction of additional treatments. A stable pH was maintained by CO₂ dosing. Thus, the most noticeable water quality improvement is the Ca concentration reduction.

3.2. Suspended particles

In this drinking water supply system, up to 40 µg l⁻¹ TSS was detected (Fig. 1). The value of TSS at the treatment plant decreased slightly after introducing the additional treatments (T₃-weeks), by 11%, P > 0.05). Another significant decrease was further achieved when the additional treatments were applied for 6 months (T₆-months), by 91%, P < 0.05). During the distribution of water under the regular conditions at T₀, the TSS decreased along the distribution network from treatment plant (−40 µg l⁻¹) to DS3 (−10 µg l⁻¹). After introducing additional treatments, although TSS reduction was achieved after 6 months at the three locations in the distribution system (at T₆-months, by 3–13% comparing to T₀, P < 0.05). At T₃-weeks, the TSS levels were comparable between treatment plants and distribution sites, while at T₆-months the TSS levels increased slightly from treatment plant to distribution sites (not significant, P > 0.05). A remarkable initial increase was observed during the switching of the supply water quality (at T₃-weeks, by 50–260% comparing to T₀, P < 0.05). Based on the change of TSS at the same locations in time one could see an increase at T₃-weeks Compared to T₀ which might indicate remobilization of TSS from the network due to destabilization processes. Looking into the fractions of TSS (FSS and VSS, Fig. 1), it is observed that the VSS at T₀ and T₃-weeks was higher than the concentration of VSS at T₆-months at the treatment plant. Meanwhile, in distribution system the VSS fraction at T₃-weeks is higher than at T₀ and T₆-months indicating the biological nature of destabilization and remobilization of suspended solids during the transitional period.

Consistent with the observations on TSS changes, the elemental analysis showed the same decrease along distribution system under regular distribution conditions at T₀ (sum of Fe, Mn and Ca, showed as concentrations for each element in Fig. 2). The elemental composition results revealed that the decreased TSS may relate to the decrease of Fe from treatment plant to DS3, where Ca and Mn remained the similar concentrations. During the introduction of additional treatments (T₃-weeks), there was no significant changes regarding the concentrations of Fe, Ca, and Mn at treatment plant, where all concentrations decreased significantly at T₆-months (P < 0.05). In the distribution system, clear improvements were observed as decrease of Fe, Mn and Ca concentrations at all distribution sites at T₆-months after 6 months operation of introduced treatments. Similar as observed at T₀, at T₆-months concentrations of Fe decreased while the concentrations of Mn and Ca remained similar from treatment plant to locations in distribution system. In the distribution system, at T₃-weeks Mn increased by 130–250% when Fe and Ca remained stable. Especially at DS1, the Mn concentration was more than 3 times higher than at treatment plant but decreased to the similar concentrations as treatment plant at DS2 and DS3, which were still much higher than Mn concentration at the same location at T₀ and T₆-months.

![Fig. 1. Particle load before (T₀, black), during (T₃-weeks, red) and after (T₆-months, blue) upgrading the treatments measured by total suspended solids (TSS), volatile suspended solids (VSS) from treatment plant (TP) to distribution system (DS1, DS2 and DS3).](http://example.com/f1.png)
3.3. Quantification of suspended particle-associated bacteria (A-ATP)

The active biomass associated with suspended solids were measured by ATP and represented as attached ATP (A-ATP) per mass of suspended solids (ng mg\(^{-1}\)) (Fig. 3). The A-ATP concentration increased during distribution at the three time slots. However, at T3-weeks (shortly after treatment adaption) the initial A-ATP increased at the treatment and subsequently the increase of A-ATP was significantly higher compared to T0 and T6-months. Generally, A-ATP initially increased at T3-weeks (by 95–230% compared to T0) and then decreased at T6-months below its original values (by 25–46% compared to T0). Regardless of the sampling period, it was observed that the further going into the distribution system, the higher the A-ATP of the suspended solids. At the treatment plant, the changes of A-ATP in time (T0, T3-weeks and T6-months) were different from observations on TSS that the A-ATP already showed an increase at T3-weeks when TSS slightly decreased (not significant). While, the changes of A-ATP at the distribution sites were consistent with that of TSS. In space, the constant and significant increases of A-ATP from treatment plant to distribution sites were also different from the changes of TSS, which was especially true for the observations at T0.

3.4. Communities of bacteria associated with suspended particles

In total, 148,922 16S rRNA pyrosequences were obtained and further assigned as 4918 OTUs based on a similarity cutoff of 97%. The rarefaction curve reached a plateau after 5000 sequence reads were obtained, indicating that enough sample coverage was obtained for most of the samples (Fig. S2). The obtained sequences were assigned to 20 phyla (Fig. S3). Proteobacteria was the most abundant phylum, which accounted for 42–93% of the total OTUs across all samples. Within Proteobacteria, Alphaproteobacteria (24–78%), Gammaproteobacteria (4–53%) and Betaproteobacteria (1–41%) were the most abundant classes. At the genus level, the detected OTUs were mainly composed of Sphingomonas spp. (0–43%), Pseudomonas spp. (0–35%), Legionella spp. (0–29%), Nitrospira spp. (0–27%), Sphingobium spp. (0–22%) and Pseudomonas spp. (0–21%) (Fig. 4).

At T0 before upgrading the treatments, Pseudomonas spp. (35%) and Pseudomonas spp. (21%) were the most abundant genera at the treatment plant. The microbial community remained relatively stable during distribution, within which Methyllosinus spp. (6–10%) was the main member. When it comes to the core OTUs (defined as OTUs with relative abundance greater than 1%), 23 OTUs were found across all samples. Among the core OTUs at DS1, DS2 and DS3, 9/17, 10/17 and 9/18 OTUs were present, respectively, in the treated water (11 core OTUs) (Fig. S4a). In the distribution system, 14/17 core OTUs were shared by all locations (DS1, DS2 and DS3).
In contrast, at T3-weeks during the treatment upgrading, Legionella spp. (28%) was the most abundant genus at the treatment plant. Comparing this to T0, the microbial community of suspended particle-associated bacteria in the distribution system showed a wider variation (Fig. 4). Nitrospira spp. (27%), Legionella spp. (29%) and Polaromonas spp. (31%) were the dominating genera at DS1, DS2 and DS3, respectively (Fig. 4). In total, 33 core OTUs were found in all samples, among which 6/18, 12/16 and 5/17 core OTUs at DS1, DS2 and DS3 were present at the treatment plant (15 core OTUs) (Fig. S4b). However, only 7/17 core OTUs were shared by the three locations.

At T6-months, Sphingomonas spp. (43%) and Sphingobium spp. (22%) were dominant at the treatment plant. Compared to T3-weeks, the bacterial communities became relatively stable after 6 months’ operation of the upgraded treatments (Figs. 4 and 5). Among the 3 locations, Sphingomonas spp. (17–23%) was the main member, except Acinetobacter spp. (38%) accounted for the highest abundance at DS3. Regarding the core OTUs, 23 core OTUs were found in all samples, 11/19, 9/13 and 6/8 core OTUs at DS1, DS2 and DS3 were present at treatment plant (11 core OTUs), respectively (Fig. S4c). Moreover, 6 core OTUs were shared by the three locations in the distribution system (average 13 core OTUs).

The principal coordinates analysis (PCoA), using unweighted and weighted UniFrac distance, showed clear differences among the three periods of T0, T3-weeks and T6-months (PERMANOVA, F = 9.643, P = 0.001), which fell into three clusters (Fig. 5 and Fig. S6). The cluster of T6-months showed an undeniable distance from the other two clusters (D_{T0-T3-weeks} = 0.34 ± 0.06, D_{T0-T6-months} = 0.47 ± 0.05, D_{T3-weeks-T6-months} = 0.47 ± 0.05). Noticeably, the communities of bacteria associated with suspended particles at the treatment plant at T0 were similar to that of T3-weeks (PERMANOVA, F = 22.71, P > 0.100), which were significantly different from those of T6-months (PERMANOVA, F = 18.06, P = 0.003). Moreover, across the three locations in the distribution system, high similarity was found for bacterial communities before treatment upgrades at T0 (PERMANOVA, F = 2.002, P > 0.05) and 6 months after treatment upgrades at T6-months (PERMANOVA, F = 1.671, P > 0.05), while sharp variations were observed right after treatment upgrading at T3-weeks (PERMANOVA, F = 8.381, P = 0.003, Figs. 4 and 5).
4. Discussion

From a long perspective, in this case after 6 months, the upgrading of treatments clearly improved the water quality. However, it is important to notice the so-called transition effects during the initial stage of switching (i.e., during the first 3 weeks), which is defined as water quality deterioration caused by the physicochemical and microbiological characteristic changes of the supply water quality (Liu et al., 2017b; Wu et al., 2015). For the very first time, this study captured the effects of changing supply water quality on the water quality deterioration indirectly through studying the suspended particles over three periods: T0 (before upgrade treatments), T3-weeks (during upgrade treatments) and T6-months (after upgrade treatments).

4.1. T0: suspended particles from treatment plant settled during distribution

Comparing the suspended particle-associated bacteria (PAB) at the treatment plant and distribution sites, the sharing of core membership (up to 75%) and high similarity of the bacterial community (PCoA, Fig. 5) revealed that under regular operation at T0 the PAB present in the distribution system mainly originated from the PAB in the treated water. This finding is consistent with our previous studies in the Dutch unchlorinated drinking water supply system that assessed the formation of different niches in the distribution system (Liu et al., 2014) and the origin of bacteria in drinking water (Liu et al., 2018), illustrating that the suspended particles in the distribution system are part of the suspended particles entering and flowing through the distribution networks. Meanwhile, the total suspended solids (TSS) decreased from the treatment plant along the distance in the distribution system. This indicated that the suspended solids (SS) in the treated water entering the distribution system partly settled in the network because of the precipitation of metal oxides or calcium carbonates, post-flocculation or biological growth that led to particle agglomeration (Gauthier et al., 1999). The elemental composition results revealed the possible precipitation of Fe and Mn by a decrease in Fe and Mn concentrations, while the A-ATP results revealed the possible biological growth by an increase of ATP when going further into the distribution system from treatment plant to DS3.

4.2. T3-weeks: changing supply water quality and transition effects

During changes to the supply water quality (T3-weeks), previously reported discolored water events (Li et al., 2010) and public health problems (Hanna-Attisha et al., 2016) were not found in the present study. The transition effects caused by the changing of supply water quality and the destabilization of established physiochemical and microbiological equilibrium in DWDS were captured by monitoring the pre-concentrated suspended solids. Regarding the timeline of destabilization, it happened right after the introduction of the new treatments (within 1st week), which lasted three weeks or longer.

4.2.1. Physicochemical deterioration

At T3-weeks, after introducing upgraded treatments, one of the clear improvements was the decreased TSS at the treatment plant compared to the TSS at T0. At T3-weeks, when there is slightly less TSS entering the distribution system, it is remarkable to observe that more TSS were collected in the distribution system compared to TSS collected at T0, suggesting the potential contribution of suspended particles release from the distribution system. Such release of suspended particles may come from destabilization of biofilm, loose deposits or pipe scales caused by changes in the water characteristics (Liu et al., 2017b; Makris et al., 2014). The loss of clear trend of TSS in space from treatment plant to DS3 and the large variations of TSS values measured at each distribution site at T3-weeks might be caused by the destabilization of uneven distributed loose deposits and biofilm in the network and the variable local hydraulics (Douterelo et al., 2013; Liu et al., 2014).

Regarding the chemical parameters, the same trend as seen for the TSS was observed for Mn: less particulate Mn entered the distribution system, but a dramatic increase in particulate Mn was observed in the distribution system at T3-weeks compared to at T0 (especially at DS1). Together, the increase of TSS and particulate Mn in the distribution system indicates that the release of suspended particles from the distribution system likely comes from the resuspension of loose deposits and/or the detachment of biofilm, as previous studies have found that loose deposits and biofilms were hotspots for Mn accumulation (Cerrato et al., 2006; Liu et al., 2017a).

4.2.2. Microbiological deterioration

At each location in the distribution system, the A-ATP was much higher at T3-weeks than at T0, which is consistent with the observation on VSS (representing biological particulates, Fig. 1) and Mn, as mentioned above. However, because the A-ATP at the treatment plant was also increased due to the destabilization of treatments (e.g., last step sand filtration), it is difficult to distinguish the observed increases of A-ATP at distribution sites at T3-weeks were caused by either higher A-ATP in the treated water or the release of A-ATP from the distribution system. The latter should be the case because the community of bacteria associated with suspended particles at the treatment plant remained very similar to T0 both of which may originate from the release of particles from the last step sand filters, but the increased A-ATP in the distribution system has a totally different community compared to that of T0 (PCoA clusters, Fig. 5, P < 0.05), which contributed by the release of biomass from loose deposits or biofilm. This can also be supported by the fact that Legionella spp., which was commonly detected in drinking water biofilms (Richards et al., 2015; Rodriguez-Martinez et al., 2015), was most abundant in the treated water at T3-weeks. Regarding the increase of A-ATP at the treatment plant, most likely it was caused by biomass detachment from the sand filters during the application of new treatments (Pinto et al., 2012). While, the high similarity among bacterial communities does not mean no changes on the bacterial community composition, because the changes of certain member (OTUs) in the community might not be revealed by the similarity analysis of PCoA (Legendre and Anderson, 1999).

The community of suspended particle-associated bacteria across different locations clustered together demonstrated stable microbial community composition and structure at T0. However, at T3-weeks, the observation of different dominant genera and the dissimilarity across different locations in the distribution system, especially the dissimilarity observed for each location between T0 and T3-weeks, indicated the occurrence of pronounced disturbances because of the distribution of quality-improved water. This is because the microbial communities in drinking water are sensitive to water quality changes (i.e., disinfectants, nutrients concentration and composition), which inducing different selection pressures on microbial population and community diversification (Gomez-Alvarez et al., 2016). For example, in cases of water quality improvements (e.g., AOC reduction), the biological activity in the water and the biofilm will decrease (Van der Kooij, 1992; Van der Wielen and Van der Kooij, 2010; Liu et al., 2013b). As a result, the biomass and EPS production will be reduced which will lead to a reduction of the bio-adhesion to the attached surface and cause the release of biofilm into bulk water (Liu et al., 2017a). Such release of biofilm into drinking water can be problematic, since biofilm is reservoir for pathogens in drinking water (Wingender and Flemming, 2011).
Although the loose deposits and biofilm sampling was not included in this study, the changes in core community members at T3-weeks provides a possible indication for the destabilization of DWDS microbial ecology (e.g. Legionella spp. Polaromonas spp. and Nitrospira spp.). Legionella spp. was commonly detected in drinking water biofilms (Richards et al., 2015; Rodríguez-Martínez et al., 2015), the increase in its relative abundance and A-ATP at T3-weeks indicates the possible release of biofilm from the distribution system into bulk water subjected to the changes in supply water quality. Legionella spp., a member of this genus widely known to be an opportunistic pathogen (i.e. Legionella pneumophila) (Falkinham et al., 2015; Richards et al., 2015), however, the detection of Legionella spp. at the genus level does not indicate bio-safety problems, especially in the case in the Netherlands, because the detected member may not be the pathogenic species as scanned earlier in Dutch drinking water systems (van der Wielen and van der Kooij, 2013).

Polaromonas spp. have been widely observed in ultra-oligotrophic freshwater environments (Magic-Knezev et al., 2009). At T0, Polaromonas spp. was detected in high abundance (35%) at the treatment plant, while they decreased to below 5% in the distribution system. Our previous study of the Dutch unchlorinated drinking water system found that Polaromonas spp. in bulk water, in which study it is also found that Polaromonas spp. was detected in loose deposits (sampled by flushing distribution pipes through hydrant), but not in pipe wall biofilms in terms of core genus (Liu et al., 2014). When it comes to T3-weeks, the relative abundance of Polaromonas spp. lessened (2%) at the treatment plant, but was much greater (2-31%) in the distribution system compared to T0 (especially at DS3), confirming the potential contribution/release of loose deposits to the increase in TSS at the taps. Similarly, Nitrospira spp., which accounted for an abundance in the distribution system at T3-weeks (especially at DS1), was only detected in loose deposits and suspended solids as core genus (Liu et al., 2014), indicating the possible release of loose deposits contributing to the increase in TSS after introducing quality-improved supply water.

4.3. T6-months: re-stabilizing of DWDS microbial ecology

At T6-months, after 6 months’ operation of the upgraded treatments, the spike in TSS at T3-weeks faded away together with the related particulate Mn, turbidity, A-ATP and the sudden changes in the community composition and structure. Comparing the results from T6-months to T0, clear improvements were observed with the decrease in TSS, particulate Mn and A-ATP. The less particle load entering distribution system will limit the accumulation of loose deposits in distribution system, which will reduce the flushing frequency (Jan Vreeburg et al., 2008). The achieved stable improvements, together with the stable bacterial community associated with particles from the treatment plant to the locations across the distribution systems, indicated that the dependence among treatment plant and distribution sites and the stabilization of the drinking water distribution system has been re-established. It is known that the destabilization and re-stabilization of microbial ecology may take time (Allison and Martiny, 2008; Liu et al., 2017b), but no information is available from real cases on how long it will take. Based on the present study, it is clear that the destabilization occurred right after introducing new treatments, lasting for more than three weeks. While the re-stabilization was achieved after 6 months, further investigation is needed to determine whether a shorter period for re-stabilization can be achieved.

In contrast to the trend of TSS decrease along the distances at T0, the TSS became slightly higher while going further into the distribution system. The different trends observed may be because of the better removal of particles after introducing new treatments. When the particles in the treated water reduced in size and number, the dominating process during distribution was no longer particle sedimentation but the growing of attached bacteria on the suspended particles (Liu et al., 2014). This is consistent with the corresponding slight increase in A-ATP and a similar community of suspended particle-associated bacteria from the treatment plant to different sampling sites in the distribution system.

At T6-months in the re-stabilized water supply system, the top five dominant genera became Sphingomonas spp., Pseudomonas spp., Sphingobium spp., Sphingopyxis spp. and Novosphingobium spp. (in descending order), all of which are commonly found in drinking water systems (Douterelo et al., 2017; Ling et al., 2016; Liu et al. 2014, 2016). The different core genera can be explained by the new treatments and different operations of the treatment steps (filters) (Pinto et al., 2012), which further indicate the possibility of managing drinking water microbes through engineering approaches (Liu et al., 2018; Pinto et al., 2012; Wang et al., 2013).

4.4. Capture and investigate transition effects through studying suspended solids

Based on this study, the transitional effects can be generally summarized as: 1) de-stabilization: observed as a spike in the TSS after switching to upgraded treatments, which might associate with the release from biofilm and/or loose deposits in a distribution system; 2) re-stabilization: observed as improvements after operating the upgraded treatments for a period of six months. Special attention should be given to the de-stabilization and release of loose deposits and biofilm into bulk water because both niches are hotspots for heavy metals and (opportunistic) pathogens (Torvinen et al., 2004; Wang et al., 2012). The analysis on (opportunistic) pathogens was not included in this study, it is highly recommended to be investigated in future studies.

Worldwide, changing supply water quality may cause transitional effects which could lead to serious water quality problems: from an esthetic quality perspective (e.g. discoloration) to biological and chemical safety issues (e.g. Pb and Legionnaires’ disease) (Liu et al., 2017b; Zahran et al., 2018). Such transitional effects deserve more attention. Yet, there is no methodology illustrating how the transition effects can be captured and investigated. The present study demonstrated an indirect approach by studying the physiochemical and microbiological characteristics of suspended solids over the different periods (T0, T3-weeks and T6-months) from treatment plant to distribution sites in a full scale drinking water supply system, which successfully overcame the challenges of field distribution network accessibility (non-destructive) and dilution effects (concentrated) (Liu et al., 2017b).

From a broader perspective, this methodology can be adapted and applied for transitional effects evaluation in other drinking water supply systems subjected to changes of either source water or treatment processes. By characterizing suspended particles in time (T0, T3-weeks, T6-months) and space (from treatment plant to distribution sites), the following critical questions that correlated with important drinking water quality issues for customers can be answered:

(1) Whether transitional effects occur? Have the distribution system loose deposits and biofilm destabilized and released into bulk water, and how much?

These questions can be answered by comparing the load of suspended particles (TSS).

(2) What has been released into bulk water? Will the release lead to serious water quality problems/risks (e.g. Pb,
opportunist pathogens? Are there any suggestions should be given to water utility managers and/or customers?

These questions can be answered by analyzing the changes on physiochemical and microbiological composition regarding the suspended particles.

(3) Where the released TSS may originate from? How should the problem be managed?

Finding the origin of released TSS will be very important for the utility to find proper managing strategy to prevent unwanted water quality problems at customers’ taps. The source of released TSS can be tracked using the bacterial community fingerprint by SourceTracker method. We have demonstrated the application of SourceTracker method to assess the origin of bacteria in distribution system and tap water in our early work (Liu et al., 2018).

For future applications, it is recommended that when the distribution system is accessible, studying the suspended solids generated by de-stabilization together with the sampling of loose deposits and biofilm from the target distribution network. By such complete study, the spiked TSS can be source tracked to its origin, based on which the corresponding strategy can be selected, such as flushing the distribution system if the TSS originated from loose deposits, or ice pigging if the TSS originated from pipe wall biofilm. Another recommendation is that the suspended solids should be monitored and sampled online (online filtration every 1 h, or 2–3 h), because both the quantity and characteristics of suspended solids in the distribution system are highly dependent on the hydraulic conditions (Fish et al., 2017; Matsui et al., 2007; Sekar et al., 2012). It has been reported that the diurnal hydraulic changes had significant effects on the bulk water bacterial community (Bautista-de Los Santos et al., 2016). Besides, the obtained online results will be able to offer high resolution background to distinguish the irregular de-stabilization from regular hydraulic disturbances. Considering the non-periodic release of biofilm and loose deposits, the online system will increase the success rate and avoid the possibility of missing the release events comparing to take suspen-sed solids samples offline.

5. Conclusions

Through characterizing the suspended particles before, during and after introducing additional treatment steps, we have indirectly investigated the transitional effects at three locations in field distribution network. The following conclusions were drawn from this study. Despite the difficulties for conducting field studies, it is encouraged to have more sampling locations for a global understanding throughout the network.

- The water quality significantly improved after 6 months’ time operation of the additional treatments;
- Remarkably, temporarily water quality deterioration with no consumers effect was observed at the initial stage when the quality-improved treated water distributed into the network at T3-weeks, observed as a spike of total suspended solids (TSS, 50–260%), active biomass (ATP, 95–230%) and inorganic elements (e.g. Mn, 130–250%);
- Pyrosequencing results revealed sharp differences in microbial community composition and structure for the bacteria associated with suspended particles between T0 and T3-weeks, which re-stabilized after 6 months at T6-months;
- Though the transitional effects were captured, the study shows that the introduction of softening and additional filtration did not have an effect on water quality for the consumer which improved considerably after 6-months’ period. The methodology of monitoring suspended particles with MuPPFs and additional analysis is capable of detecting transitional effects by monitoring the dynamics of suspended particles and its physiochemical and microbiological composition.

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.

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Appendix A. Supplementary data

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References

Allison, S.D., Martiny, J.B., 2008. Resistance, resilience, and redundancy in microbial communities. Proc. Natl. Acad. Sci. 105 (Suppl. 1), 11512–11519.
Anderson, M.J., Walsh, D.C.L. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? Ecol. Monogr. 83 (4), 557–574.
Batté, M., Appenzzeller, B.M.R., Grandjean, D., Fass, S., Gauthier, V., Jorand, F., Mathieu, L., Boualam, M., Saby, S., Block, J.C., 2003. Biofilms in drinking water distribution systems. Rev. Environ. Sci. Biotechnol. 2 (2), 147–168.
Bautista-de Los Santos, Q.M., Schroeder, J.L., Blakemore, O., Moses, J., Haffey, M., Sloan, W., Pinto, A.J., 2016. The impact of sampling, PCR, and sequencing replication on discerning changes in drinking water bacterial community over diurnal time-scales. Water Res. 90, 216–224.
Berry, D., Xi, C., Raskin, L., 2006. Microbial ecology of drinking water distribution systems. Curr. Opin. Biotechnol. 17 (3), 297–302.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knight, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Prieur, R., Reid, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335.
Carriere, A., Gauthier, V., Desjardins, R., Barbeau, B., 2005. Evaluation of loose depositions in distribution systems through unidirectional flushing. J. Am. Water Work. Assoc. 97 (9), 82–92.
Cerato, J.M., Reyes, L.P., Alvarado, C.N., Dietrich, A.M., 2006. Effect of PVC and iron materials on Mn(II) deposition in drinking water distribution systems. Water Res. 40 (14), 2720–2726.
Chaves Simoes, L., Simoes, M., 2013. Biofilms in drinking water: problems and solutions. RSC Adv. 3 (8), 2520–2533.
Chen, H., Boutros, P.C., 2011. VennDiagram: a package for the generation of highlycustomizable Venn and Euler diagrams in R. BMC Bioinf. 12, 35.
Douterelo, L., Jackson, M., Solomon, C., Boxall, J., 2017. Spatial and temporal analogies in microbial communities in natural drinking water biofilms. Sci. Total Environ. 581–582, 277–288.
Douterelo, L., Sharpe, R.L., Boxall, J.R., 2013. Influence of hydraulic regimes on bacterial community structure and composition in an experimental drinking water distribution system. Water Res. 47 (2), 503–516.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27 (16), 2194–2200.
Falkingham 3rd, J.O., Hibbitts, E.D., Arndout, M.J., Pruden, A., Edwards, M.A., 2015. Epidemiology and ecology of opportunistic premise plumbing pathogens: Legionella pneumophila, Mycobacterium avium, and Pseudomonas aeruginosa. Environ. Health Perspect. 123 (8), 749–758.
Finkel, L.M., Baungartner, L.K., Peterson, K.L., Frank, D.N., Harris, J.K., Pace, N.R., 2009. Opportunistic pathogens enriched in showerhead biofilms. Proc. Natl. Acad. Sci. U.S.A. 106 (38), 16393–16398.
Fish, K., Osborn, A.M., Boxall, J.R., 2017. Biofilm structures (EPS and bacterial communities) in drinking water distribution systems are conditioned by hydraulics and influence discolouration. Sci. Total Environ. 593–594, 571–580.
Flemming, H.C., Percival, S.L., Walker, J.T., 2002. Contamination potential of biofilms in water distribution systems. Water Sci. Technol. 2, 271–280.
Gauthier, V., Gérard, B., Portal, J.M., Block, J.C., Gatel, D., 1999. Organic matter as
Loose deposits in a drinking water distribution system. Water Res. 33 (4), 1014–1026.

Hanna-Attisha, M., LaChance, J., Sadler, R.C., Champney Schnee, A., 2016. Elevated blood lead levels in children associated with the Flint drinking water crisis: a spatial analysis of risk and public health response. Am. J. Public Health (0), 1–8.

Hong, P.Y., Hwang, C., Ling, F., Andersen, G.L., LeChevallier, M.W., Liu, W.T., 2010. Pyrosequencing analysis of bacterial biofilm communities in water meters of a drinking water distribution system. Appl. Environ. Microbiol. 76 (16), 5631–5635.

Hwang, C., Ling, F., Andersen, G.L., LeChevallier, M.W., Liu, W.T., 2011. Evaluation of methods for the extraction of DNA from drinking water distribution system biofilms. Microb. Environ. 27 (1), 9–18.

Kolde, R., 2013. A Package for Drawing Pretty Heatmaps in R. Pheatmap: Pretty Heatmaps.

Legendre, P., Anderson, M.J., 1999. Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. Ecol. Monogr. 69 (1), 1–24.

Lehtola, M.J., Saxen, M., Miettinen, I.T., Hirvonen, A., Vartiaen, T., Martikainen, P.J., 2006. The effects of changing water flow velocity on the formation of biofilms and water quality in pilot distribution system consisting of copper or polyethylene pipes. Water Res. 40 (11), 2151–2160.

Lehtola, M.J., Nissinen, T.K., Miettinen, I.T., Martikainen, P.J., Vartiaen, T., 2004. Removal of soft deposits from the distribution system improves the drinking water quality. Water Res. 38 (3), 601–610.

Li, D., Li, Z., Yu, J., Cao, N., Liu, R., Yang, M., 2010. Characterization of bacterial community structure in a drinking water distribution system during an occurrence of red water. Appl. Environ. Microbiol. 76 (21), 7171–7180.

Ling, F., Hwang, C., LeChevallier, M.W., Andersen, G.L., Liu, W.T., 2016. Core-satellite populations and seasonality of water meter biofilms in a metropolitan drinking water distribution system. ISME J. 10 (3), 582–595.

Liu, G., Bakker, G., Li, S., Vreeburg, J.H.G., Boxall, D.J.B., 2007. Discolouration in potable water distribution systems: undermining the regrowth of opportunistic waterborne pathogens in drinking water distribution systems. Sci. Total Environ. 378 (16), 1467–1475.

Liu, G., Verberk, J.Q.J.C., Dijk, J.C., 2013b. Bacteriology of drinking water distribution systems: an integral study of bulk water, suspended solids, loose deposits, and pipe wall biofilm. Environ. Sci. Technol. 47 (18), 10117–10128.

Matsui, Y., Yamagishi, T., Terada, Y., Matsuhashi, T., Inoue, T., 2007. Suspected particles and their characteristics in water mains: developments of sampling methods. J. Water Supply Res. Technol. – Aqua 56 (1), 13–24.

Noyce, G.L., Fulthorpe, R., Gogoilevski, A., Hazlett, P., Tran, H., Basiliko, N., 2016. Soil microbial responses to wood ash addition and forest fire in managed Ontario forests. Appl. Soil Ecol. 107, 368–380.

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.

Richards, C.L., Broadaway, S.C., Eggers, M.J., Doyle, J., Pyle, B.H., Camper, A.K., Ford, T.E., 2015. Detection of pathogenic and non-pathogenic bacteria in drinking water and associated biofilms on the crown reservation, Montana, USA. Microb. Ecol. 76 (1), 52–63.

Rodriguez-Martinez, S., Shirabay, Y., Pecellin, M., Brettar, I., Höfle, M., Halpern, M., 2015. Spatial distribution of Legionella pneumophilia MLVA-genotypes in a drinking water system. Water Res. 77, 119–122.

Sekar, R., Deines, P., Machell, J., Osborn, A.M., Biggs, C.A., Boxall, J.B., 2012. Bacterial water quality and network hydraulic characteristics: a field study of a small, looped water distribution system using culture-independent molecular methods. J. Appl. Microbiol. 112 (6), 1220–1234.

Shannon, M.A., Bohn, P.W., Elimelech, M., Georgiadis, J.G., Marinis, B.J., Mayes, A.M., 2008. Science and technology for water purification in the coming decades. Nature 452 (7185), 301–310.

Sly, L.J., Hodgkinson, M.C., Arunpairojana, V., 1990. Deposition of manganese in a drinking water distribution system. Appl. Environ. Microbiol. 56 (3), 628–639.

Ternes, T., Joss, A., Oehlmann, J., 2015. Occurrence, fate, removal and assessment of emerging contaminants in water in the water cycle (from wastewater to drinking water). Water Res. 72, 1–2.

Torvinen, E., Suomalainen, S., Lehtola, M.J., Miettinen, I.T., Zacheus, O., Paulin, L., Karila, M.L., Martikainen, P.J., 2004. Mycobacteria in water and loose deposits of drinking water distribution systems in Finland. Appl. Environ. Microbiol. 70 (4), 1973–1981.

Van Der Weede, E., Characklis, W.G., Smith, D.B., 1989. Biofilms and bacterial drinking water quality. Water Res. 23 (10), 1313–1322.

van der Wielen, P.W., van der Kooij, D., 2013. Nontuberculous mycobacteria, fungi, and opportunistic pathogens in unchlorinated drinking water in The Netherlands. Appl. Environ. Microbiol. 79 (3), 825–834.

Vreeburg, J.H.G., Boxall, D.J.B., 2007. Discoulourisation in potable water distribution systems: a review. Water Res. 41 (3), 519–529.

Wang, H., Edwards, M., Falkinham III, J.O., Pruden, A., 2012. Molecular survey of the occurrence of Legionella spp., Mycobacterium spp., Pseudomonas aeruginosa, and amoeba hosts in two chloraminated drinking water distribution systems. Appl. Environ. Microbiol. 78 (17), 6285–6294.

Wang, H., Edwards, M.A., Falkinham III, J.O., Pruden, A., 2013. Probiotic approach to pathogen control in premise plumbing systems? A review. Environ. Sci. Technol. 47 (18), 10177–10128.

Wingender, J., Fleming, H.C., 2011. Biofilms in drinking water and their role as reservoir for pathogens. Int. J. Hyg. Environ. Health 214 (6), 417–423.

Wu, H., Zhang, J., Mi, Z., Xie, S., Chen, C., Zhang, X., 2015. Biofilm bacterial communities in urban drinking water distribution systems transporting waters with different purification strategies. Appl. Microbiol. Biotechnol. 99 (4), 1947–1955.

Xing, X., Wang, H., Hu, C., Liu, L., 2018a. Characterization of bacterial community and iron corrosion in drinking water distribution systems with O3-biological activated carbon treatment. J. Environ. Sci. (China) 69, 192–204.

Xing, X., Wang, H., Hu, C., Liu, L., 2018b. Effects of phosphate-enhanced ozone biotreatment on formation of disinfection byproducts and occurrence of opportunistic pathogens in drinking water distribution systems. Water Res. 139, 168–176.

Zacheus, O.M., Lehtola, M.J., Korhonen, L.K., Martikainen, P.J., 2001. Soft deposits, the key site for microbial growth in drinking water distribution networks. Water Res. 35 (7), 1757–1765.

Zahran, S., McElmurry, S.P., Kellogg, P.E., Mushinski, D., Press, J., Love, N.G., Sadler, R.C., Swanson, M.S., 2018. Assessment of the Legionnaires’ disease outbreak in Flint, Michigan. Proc. Natl. Acad. Sci. 115 (8), E1730–E1739.