Water Research 124 (2017) 435–445

Contents lists available at ScienceDirect

Water Research

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

Hotspots for selected metal elements and microbes accumulation and the corresponding water quality deterioration potential in an unchlorinated drinking water distribution system

Gang Liu [a, b, *], Yu Tao [c], Ya Zhang [d], Maarten Lut [a], Willem-Jan Knibbe [a], Paul van der Wielen [e, f], Wentso Liu [d], Gertjan Medema [b, e], Walter van der Meer [a, g]

[a] Oasen Water Company, P.O. Box 122, 2800AC, Gouda, The Netherlands
[b] Sanitary Engineering, Department of Water Management, Faculty of Civil Engineering and Geosciences, Delft University of Technology, P.O. Box 5048, 2600GA, Delft, The Netherlands
[c] Department of Chemical Engineering, Imperial College London, South Kensington, London, SW7 2AZ, United Kingdom
[d] Department of Civil and Environmental Engineering, University of Illinois at Urbana-Champaign, 205 North Mathews Avenue, Urbana, IL, 61801, United States
[e] KWR Watercycle Research Institute, P.O. Box 1072, 3430 BB, Nieuwegein, The Netherlands
[f] Laboratory of Microbiology, Wageningen University, P.O. Box 8033, 6700 EH, Wageningen, The Netherlands
[g] Science and Technology, University of Twente, P.O. Box 217, 7500AE, Enschede, The Netherlands

ARTICLE INFO

Article history:
Received 13 April 2017
Received in revised form
2 August 2017
Accepted 2 August 2017
Available online 4 August 2017

Keywords:
Drinking water distribution system
Material accumulation
Hotspot
Quality deterioration potential
Next generation sequencing

ABSTRACT

Biofilm formation, loose deposit accumulation and water quality deterioration in drinking water distribution systems have been widely reported. However, the accumulation and distribution of harbored elements and microbes in the different niches (loose deposits, PVC-U biofilm, and HDPE biofilm) and their corresponding potential contribution to water quality deterioration remain unknown. This precludes an in-depth understanding of water quality deterioration and the development of proactive management strategies. The present study quantitatively evaluated the distribution of elements, ATP, *Aeromonas* spp., and bacterial communities in distribution pipes (PVC-U, D = 110 mm, loose deposit and biofilm niches) and household connection pipes (HDPE, D = 32 mm, HDPE biofilm niches) at ten locations in an unchlorinated distribution system. The results show that loose deposits in PVC-U pipes, acting as sinks, constitute a hotspot (highest total amount per meter pipe) for elements, ATP, and target bacteria groups (e.g., *Aeromonas* spp., *Mycobacterium* spp., and *Legionella* spp.). When drinking water distribution system niches with harbored elements and microbes become sources in the event of disturbances, the highest quality deterioration potential (QDP) is that of HDPE biofilm; this can be attributed to its high surface-to-volume ratio. 16s rRNA analysis demonstrates that, at the genus level, the bacterial communities in the water, loose deposits, PVC-U biofilm, and HDPE biofilm were dominated, respectively, by *Polaromonas* spp. (2–23%), *Nitrosipra* spp. (1–47%), *Flavobacterium* spp. (1–36%), and *Flavobacterium* spp. (5–67%). The combined results of elemental composition and bacterial community analyses indicate that different dominant bio-chemical processes might occur within the different niches—for example, iron-arsenic oxidizing in loose deposits, bio-calumniation in PVC-U biofilm, and methane oxidizing in HDPE biofilm. The release of 20% loose deposits, 20% PVC-U biofilm and 10% HDPE biofilm will cause significant changes of water bacterial community.

1. Introduction

The deterioration of the quality of water during its transport through a drinking water distribution system (DWDS) has been widely observed in the form of increased particle load (Liu et al., 2016), heterotrophic plate counts (HPC), and *Aeromonas* plate counts; these are the traditional microbial indicators for regrowth (van der Wielen et al., 2016) at customers' taps. In extreme cases,

* Corresponding author. Room 4.41, Stevinweg 1, Building of CiTG, TU Delft, 2628CN, Delft, The Netherlands.
E-mail addresses: g.liu-1@tudelft.nl, ganghow@gmail.com, gang.liu@oasen.nl (G. Liu).

http://dx.doi.org/10.1016/j.watres.2017.08.002

aesthetic problems such as discolored water (dirty water, red water) may occur, or the water may contain heavy metals and health-problem associated (opportunistic) pathogens; these risks might increase in the event of disturbances (Sly et al., 1990; Vreeburg and Boxall, 2007; Li et al., 2010; Schwake et al., 2016; Liu et al., 2017).

A DWDS is a pressurized pipe network which delivers treated drinking water from a centralized treatment plant to the water meters at the consumers' buildings (Snoeyink et al., 2006). It is a complex system typically consisting of different kinds of pipe, including transportation pipes (which connect the treatment plant and reservoir with the supply areas, with a typical diameter of >200 mm); distribution pipes (the main pipes under the street, which distribute water within the supply area, with a typical diameter of 63–110 mm); and household connection pipes (which connect the distribution pipe networks to the water meters at the consumers' building, with a typical diameter of 25–32 mm). Water quality deterioration has been widely observed during transport in both distribution pipes and household pipes; the phenomenon has been attributed to the water's long retention time in these pipes and to the pipes' high surface-to-volume ratio (Tsvetanova and Hoekstra, 2010; Liu et al., 2013a,b,c). Moreover, there are different niches present within a pipe section, e.g., pipe surfaces and loose deposits (Liu et al., 2014; Proctor and Hammes, 2015; Prest et al., 2016; van der Wielen and Lut, 2016).

Although water quality has been significantly improved over the last decades as a result of new and/or improved treatment processes at the plants, the treated drinking water that enters a DWDS still contains particles, microorganisms, and nutrients (Liu et al., 2013a,b,c; Proctor and Hammes, 2015; Prest et al., 2016). During drinking water distribution, the niches present within a DWDS become sinks for particle accumulation and microbial growth, which gradually develop and stabilize over lengthy periods of time as the water flows through the system (Boe-Hansen et al., 2002; Liu et al., 2013a,b,c; Makris et al., 2014). The established sinks/niches have been studied and sub-divided into pipe scales (Sarin et al., 2001; Renner, 2008; Makris et al., 2014), biofilm matrices (Flemming and Wingender, 2010; Fish et al., 2016), and loose deposits (Smith et al., 1997; Gauthier et al., 1999), all of which can constitute reservoirs for organic compounds, heavy metals, and microbes (including pathogens) (Liu et al., 2017).

It is noteworthy that, in the event of a disturbance which destabilizes the established physiochemical and microbiological equilibriums, these different components can become resuspended in the drinking water and thereby result in a deterioration of water quality (Makris et al., 2014). Such destabilization can be caused by hydraulic turbulence of a peak velocity in the pipe (e.g., during a morning water-demand peak or a firefighting event) (Matsui et al., 2007; Vreeburg and Boxall, 2007); and/or by changes on the physiochemical and microbiological water characteristics (e.g., as a consequence of implementing disinfection strategies, switching source water, and changing treatment processes) (Li et al., 2010; Schwake et al., 2016; Liu et al., 2017).

To resolve these water quality and the related public health concerns associated with drinking water distribution, an understanding of the accumulation and distribution of material (e.g., cells, particles, and metals) across different niches in different DWDS pipes is critical. Although there is a consensus about the major part played by the DWDS in water-quality deterioration, the function of the DWDS as a sink (contaminants accumulation) and source (contaminants release), and the specific contribution of each niche remain unknown. This is especially true of full-scale distribution systems because of their low accessibility (Berry et al., 2006). The objectives of this study are (i) to identify hotspots for the accumulation of different microbial parameters and selected metals, and (ii) to determine the corresponding water quality deterioration potential (QDP) that predicts the possible contribution of each DWDS niche to drinking water deterioration in a full-scale DWDS.

2. Material and methods

2.1. Sampling

The sampling was conducted between February and March 2014 in the unchlorinated DWDS of the Oasen drinking water company in the central area of the Netherlands. At the drinking water treatment plant (DWTP) the abstracted groundwater is submitted to aeration, rapid sand filtration, softening, activated carbon filtration, and UV disinfection before the treated water is pumped into the distribution system. In the treated water, Fe, Mn, As, Al and *Aeromonas* spp. are below detection limit, with 23 ± 1.2 mg/l Ca and 8.0 ± 2.3 ng/l ATP.

As illustrated in Fig. 1 and Table S1, ten locations were selected for this study in the DWTP's supply areas. An integral sampling from PVC-U distribution pipes (D = 110 mm) was performed at each location, as previously described (Liu et al., 2014). In summary, water samples were collected from customers' taps connected directly to the main supply, and close to the hydrants for flushing loose deposits. Water samples were taken from each tap after the taps were left to run until the water temperature is constant. Loose deposits were collected at fire hydrants by flushing the distribution pipe with a velocity of 1.5 m/s (Vreeburg et al., 2008). Subsequently, two sections of the flushed pipe (length = 30 cm) were cut out to sample the biofilm in duplicate. The pipe sections were closed using pre-disinfected caps and filled with 1 l DNA-free water (Millipore, H20MB1006) to keep the inner surface wet during transportation. The HDPE household connection pipes (D = 32 mm) were taken in duplicate at each sampling location (l = 30 cm), closed using pre-disinfected caps, and filled with DNA-free water.

The sampling procedure involved the following steps: obtaining the water samples, removing the household connection pipes, flushing the distribution pipe in the street for loose deposit sampling, and cutting out parts of the distribution pipe. During flushing, the turbidity was recorded online, the timing of loose deposit sampling at hydrant is calculated according to distance between flushed hydrant and cut pipe specimen as detail described in Fig. S1. The online recorded turbidity and measured ATP of flushed loose deposits were included in Fig. S2. All samples were kept at 0 °C as soon as they were taken and subsequently transported at 0 °C to the lab. To detach the bacteria from the surface of the loose deposits and pipe material, the samples were pre-treated three times using 2-min ultrasonication at 42 KHz (Magic-Knezev and van der Kooij, 2004). The obtained suspensions were used for further physiochemical and microbiological analysis. All samples were processed within 24 h of being taken.

2.2. Physiochemical analysis

Concentrations of Iron (Fe), Manganese (Mn), Calcium (Ca), Aluminum (Al), and Arsenic (As) were determined by inductively coupled plasma-mass spectroscopy (ICP-MS, PerkinElmer ELAN DRC-e), as previously described (Lytle et al., 2004; Peng et al., 2010; Liu et al., 2014). Quality control samples, including laboratory-fortified blanks and laboratory-fortified samples, were performed for every ten samples analyzed.

2.3. Microbiological analysis

2.3.1. Adenosine triphosphate (ATP)

The ATP concentrations, as a measure for active biomass, were

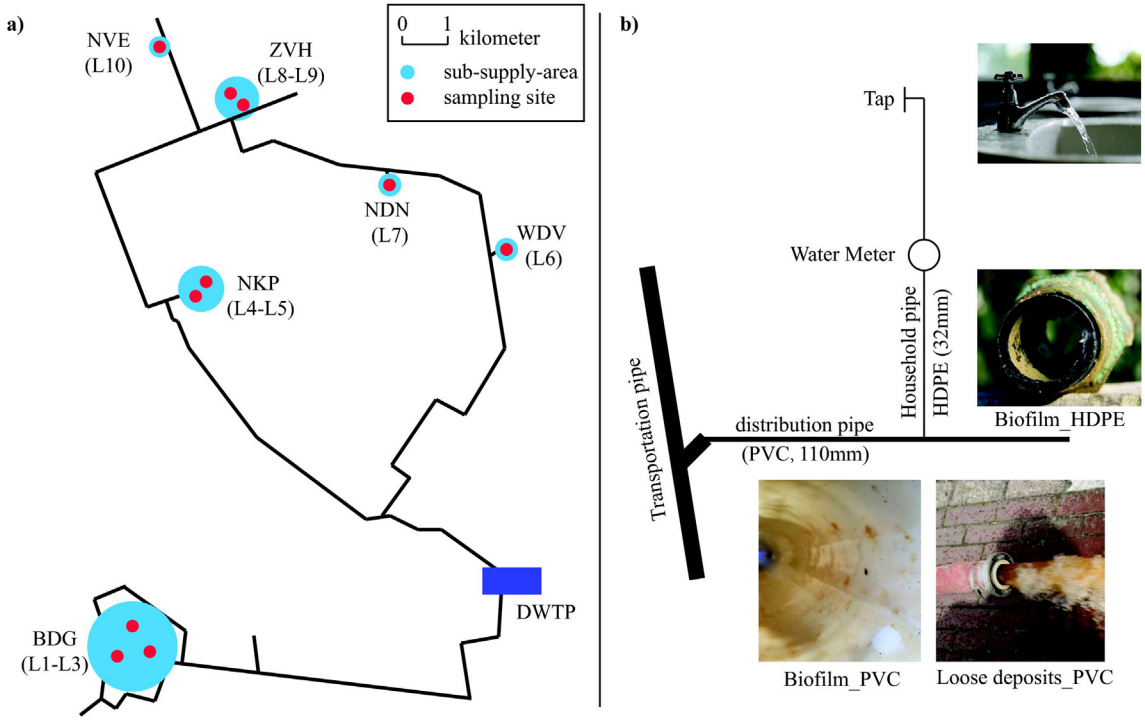

Fig. 1. a) Map of sampling locations illustrating the ten selected sites in the DWDS. The number of locations depended on the size of the sub-supply area: three locations were selected in BDG (L1, L2, L3), two in NKP (L4, L5) and ZVH (L6, L7), and one in WDV (L8), NDN (L9) and NVE (L10); b) At each location, water samples were taken from the customers' taps; loose deposits and biofilm were sampled from the distribution pipe (PVC-U, D = 110 mm), and from the connected household connection pipe (HDPE, D = 32 mm).

determined for all the samples. Total ATP concentration was determined, as described previously (Magic-Knezev and van der Kooij, 2004), by using the BacTiter Glo reagent and a luminometer. In summary, a water sample was warmed to 30 °C in a sterile Eppendorf tube, while the ATP reagent was simultaneously warmed. The sample and the reagent were combined after 2 min at 30 °C and then the luminescence was measured directly. The data were collected as relative light units and converted to ATP by means of a calibration curve made with a known ATP standard.

2.3.2. DNA extraction and Illumina Miseq sequencing

The DNA was extracted from water samples and other obtained suspensions after pre-treatment, using the FastDNA Spin Kit for Soil (Q-Biogene/MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions. The 16S rRNA gene amplification was carried out as previously described (Kozich et al., 2013). Briefly, the extract gDNA was amplified with a primer set (515F: 5′-GTGCCAGCMGCCGCGGTAA-3′ and 909R: 5′–CCCGTCAATTCMTTTRAGT-3′), targeting the V4–V5 hypervariable regions of both the Bacteria and Archaea domains. The primer was modified for the Illumina Miseq platform (Illumina, Inc., San Diego, U.S.) and the paired-end sequencing of the amplicons (2 × 300 bp) was done at the Roy J. Carver Biotechnology Center (University of Illinois at Urbana-Champaign). The sequencing data has been deposited in NCBI database, with reference code of SRR5807465-5807504, the sample origin of each sequencing library is provided in Table S2.

2.3.3. Sequence data processing

The sequences generated from the Illumina Miseq analysis of the 16S rRNA gene amplicons were processed (i.e., filtered, clustered, and taxonomically assigned and aligned) using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline with the default settings (Caporaso et al., 2010). The process consisted of quality checking, denoising, and a microbial diversity analysis. Both unweighted and weighted UniFrac distance matrices were constructed from the phylogenetic tree (built by a FastTree algorithm) and used to conduct a principal coordinate analysis (PCoA) (Liu et al., 2014). The core OTUs are defined as the OTUs with a defined cutoff of occupancy (100%, detected at every location) and relative abundance (>1%) within each phase/pipe (Ling et al., 2016). The OTUs were taxonomically classified as *Legionella* spp. and *Mycobacterium* spp at 97% confidence level.

2.3.4. Aeromonas spp.

A culture-based method was used to measure *Aeromonas* spp. The water samples and other obtained suspensions (100 mL) were filtrated over a 0.45 μm filter. Subsequently, the filter was incubated on *Aeromonas* dextrin agar (Merck), and agar plates were incubated for 24 h at 30 °C before the number of colony forming units (CFU) was determined (Havelaar et al., 1987).

2.4. Normalization of results and data analysis

2.4.1. Hotspots for selected microbial parameters and metal elements

Hotspots for the selected microbial parameters and metal elements are defined as niches that harbor disproportionately high amounts of biomass, *Aeromonas*, Fe, Mn, Ca, Al, or As, relative to the surrounding environments. To facilitate a cross-comparison among different locations and niches, the results obtained for each parameter were normalized and calculated back to their total amount per 1 m of pipe (OMP), according to their concentration and the corresponding surface areas using equation (1) for biofilm-related data; and according to their concentration and the corresponding volume using equation (2) for water or loose-deposit related data. The detailed information about the volume and surface area per OMP for each niche is given in Fig. S1-b.

$$T_{niche-OMP} = C_{niche-per\ surface\ area} \times S_{niche-OMP} \quad (1)$$

$$T_{niche-OMP} = C_{niche-per\ volume} \times V_{niche-OMP} \quad (2)$$

$T_{niche\text{-}OMP}$: Total amount of each parameter harbored by a niche.
C_{niche}: Concentration of each parameter harbored by a niche, concentration per volume of water/loose deposits, or concentration per surface area.
$S_{niche\text{-}OMP}$: Surface area available in the 1 m pipe section, e.g. pipe wall surface.
$V_{niche\text{-}OMP}$: Volume contained in the 1 m pipe section, e.g. volume of water contained within 1 m of pipe.

2.4.2. Water quality deterioration potential

The quantitative comparison of the total amount of each microbial parameter and selected metal elements per meter of pipe can identify hotspots for the accumulation of these parameters, but it cannot answer the question whether and to what extent these compounds influence water quality. The water-quality deterioration potential (QDP) is therefore proposed as a parameter which describes the maximum risk that the accumulated components represent for the deterioration of the water quality. Accordingly, the QDP is calculated assuming that the harbored microbial parameters and metal elements in each niche are released all at once into the contacting water column, as described in equation (3) (Liu et al., 2017).

$$QDP_{niche} = T_{niche-OMP}/V_{water-OMP} \quad (3)$$

QDP_{niche}: Quality deterioration potential caused by each niche.
$T_{niche\text{-}OMP}$: Total amount of material per OMP calculated by equation (1).
$V_{water\text{-}OMP}$: Volume of water contained in the OMP.

2.5. Data analysis

The potential influence of 5%, 10%, 20%, 30%, 50%, and 70% release of loose deposits and biofilm has been tested by subsampling loose deposits and biofilm community and adding to water community, respectively. Beta diversity and the significance of community variations were tested in QIIME on the generated data set by the principal coordinate analysis and beta significance test. To study the correlation between chemistry and dominate OTUs, the correlative analysis was conducted using Canonical Correspondence Analysis (CCA) (cca in R package "vegan").

The obtained data were statistically analyzed to determine whether the obtained data were significantly different among niches: 1) elements concentration; 2) microbe concentration; 3) QDP index; 4) the bacterial community among niches. The physiochemical parameters (elements, ATP, and Aeromonas) were tested by T test (two sample paired) using Past program (V3.15) (Hammer et al., 2008). The significance of community data was tested by QIIME using beta_significance.py. In both analysis, differences were considered significant when the p-value was lower than 0.05 ($p < 0.05$)."

3. Results

3.1. Accumulation and distribution of selected metal elements in a DWDS

3.1.1. Niches

Table 1 presents the concentrations of selected metal elements in the different niches. In the bulk water, the concentrations of Fe, Mn, Al, and As were all below the detection limit. A stable level of Ca concentration (23 ± 0.4 mg/l) was observed across the ten locations. Metal element accumulation associated with loose deposits and biofilm matrix was observed. For the flushed loose deposits, in contrast to the bulk water, Fe, Mn, As, and Al were enriched at average concentrations of 3.3 mg/l, 0.18 mg/l, 0.7 mg/l, and 0.06 mg/l, respectively; the exception was Ca, which could not be detected in the loose deposit fraction. Comparisons of the biofilm matrix formed on PVC-U and HDPE pipes showed that the PVC-U matrix harbored higher concentrations of Ca and Al, but lower concentrations of Fe, Mn, and As compared to the HDPE pipe matrix.

3.1.2. Hotspots

Following the normalization of the obtained data per meter of pipe, the comparison among niches showed clearly that the niche of loose deposits in 110 mm PVC-U pipes were hotspots for accumulation of Fe (27 ± 17 mg/m), Mn (1.6 ± 1.4 mg/m), and As (5.7 ± 3.1 mg/m) (Fig. 2a and c). Biofilm formed in PVC-U pipes was a hotspot for Al accumulation (1.5 ± 0.5 mg/m). The accumulation of Ca was only observed in biofilm formed on PVC-U pipes, in amounts comparable to those detected in the water column (225 ± 36 mg/m).

3.1.3. Water quality deterioration potential

Assuming that the accumulated material is released all at once into the water contained in a 1-m pipe section, the quality deterioration potential (QDP) calculated for the selected metal elements

Table 1
The accumulation of selected elements, ATP, Aeromonas spp. and the detected OTUs and alpha diversity (n = 10, average ± std.).

		Water	Loose deposits	Biofilm (PVC-U)	Biofilm (HDPE)
Selected elements	Fe	U.D.[a]	3.3 ± 2.4 mg/l	1.0 ± 0.5 μg/cm^2	4.5 ± 1.3 μg/cm^2
	Mn	U.D.	0.18 ± 0.21 mg/l	0.05 ± 0.03 μg/cm^2	0.16 ± 0.11 μg/cm^2
	Ca	23.0 ± 0.4 mg/l	U.D.	92 ± 40 μg/cm^2	17 ± 11 μg/cm^2
	As	U.D.	0.7 ± 0.3 mg/l	0.8 ± 0.3 μg/cm^2	2.4 ± 1.6 μg/cm^2
	Al	U.D.	64 ± 70 μg/l	430 ± 170 μg/cm^2	270 ± 140 μg/cm^2
Microbes	ATP	8.6 ± 1.3 ng/l	240 ± 170 ng/l	0.24 ± 0.24 ng/cm^2	0.76 ± 0.31 ng/cm^2
	Aeromonas	90 ± 70 (CFU/100 ml)	5000 ± 3000 (CFU/100 ml)	U.D.[a]	U.D.
16s rRNA sequencing	Sequences	9960 ± 7120	9690 ± 7160	9260 ± 5260	14750 ± 5100
	OTUs	864 ± 360	1510 ± 500	446 ± 153	1416 ± 503
	Filtered OTUs (n > 100)	84 ± 20	136 ± 30	48 ± 12	117 ± 20
	Core OTUs (occupancy = 100%, abundance> 1%)	13	14	4	15

[a] U.D. = Under the detection limit.

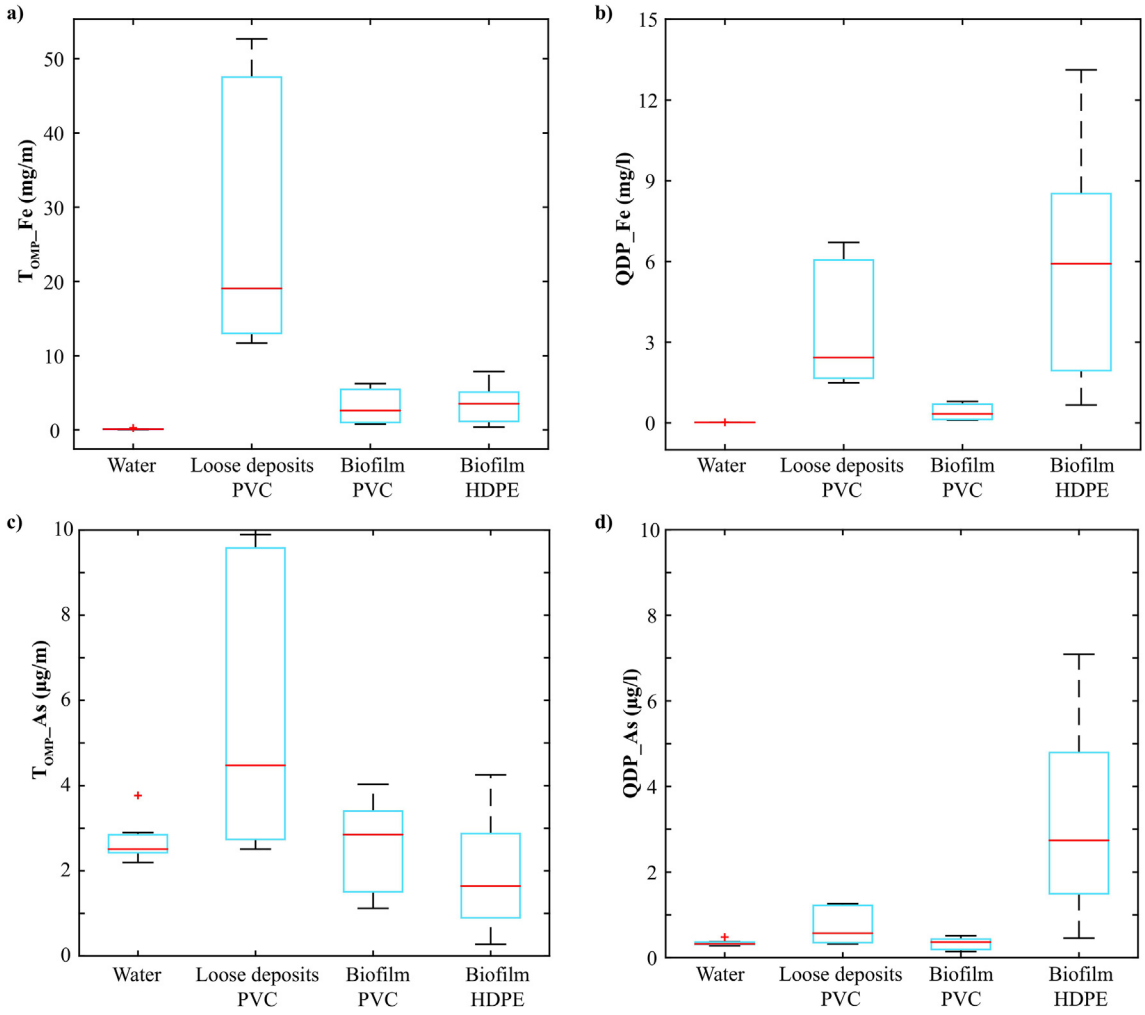

Fig. 2. Boxplot showing the distribution of Fe and As (mg/m) across niches normalized back to 1 m of pipe (n = 10); the values obtained from water samples are included to provide an indication of the relative values in loose deposits, PVC-U biofilm, and HDPE biofilm.

in HDPE biofilm was higher than that of loose deposits and biofilm on PVC-U pipes; this can lead to a maximum concentration increase in the water column of up to 13 mg/l Fe, 7 µg/l As, 0.6 mg/l Al, and 50 mg/l Ca (Fig. 2b and d and Fig. S3). The corresponding QDP_{Fe}, QDP_{Mn} and QDP_{Al} of loose deposits in the PVC-U pipes was higher than that of the biofilm on the same pipes ($p < 0.05$).

3.2. Accumulation and distribution of ATP and Aeromonas in a DWDS

3.2.1. Niches

Table 1 shows measured ATP and *Aeromonas* in each niche. Active biomass concentrations as measured by ATP ranged from 4.0 to 10.0 ng/l in bulk water, but was 20 times higher in loose deposits. ATP concentrations in the biofilm on HDPE pipes were about three times higher than in the biofilm on PVC-U pipes.

In water, *Aeromonas* spp. was detected at nine of the ten locations, with numbers ranging from 10 to 200 CFU per 100 ml. Among the other niches, *Aeromonas* spp. was detected in higher numbers in loose deposits (on average 5000 CFU per 100 ml) compared to water, but could not be detected in any of the biofilms.

3.2.2. Hotspots

Comparisons between niches showed that the niche of loose deposits harbored the highest amount of ATP per meter of pipe ($1.9 \pm 1.4 \times 10^3$ ng/m) and was a hotspot for ATP accumulation (Fig. 3a, $p < 0.05$). The ATP concentrations in the biofilms on PVC-U and HDPE pipes were comparable. Regarding the cultivable *Aeromonas*, the results demonstrated that the loose deposits were also a hotspot for *Aeromonas* accumulation, harboring more than 10^6 CFU of *Aeromonas* per meter of PVC-U pipe (Fig. 3c).

3.2.3. Water quality deterioration potential

Similar to the QDP for selected metal elements, the QDP for ATP was highest for the biofilm niche on HDPE compared to that of loose deposits and biofilm on PVC-U pipes (Fig. 3b). It can lead to a maximum increase of ATP up to 1.2×10^3 ng/l, which is more than 100 times higher than the ATP concentration in normal tap water. In contrast, the QDP for *Aeromonas* was the highest for loose deposits, indicating that the release of loose deposits *Aeromonas* ($QDP_{Aeromonas\text{-}loose\ deposits}$) can create a peak concentration as high as 7.8×10^5 CFU/100 ml in the water column (Fig. 3d).

3.3. Bacterial community structure and diversity

3.3.1. Niches

In total 436,754 sequences were obtained from 40 samples, which were assigned to 14,613 OTUs. The rarefaction curves were

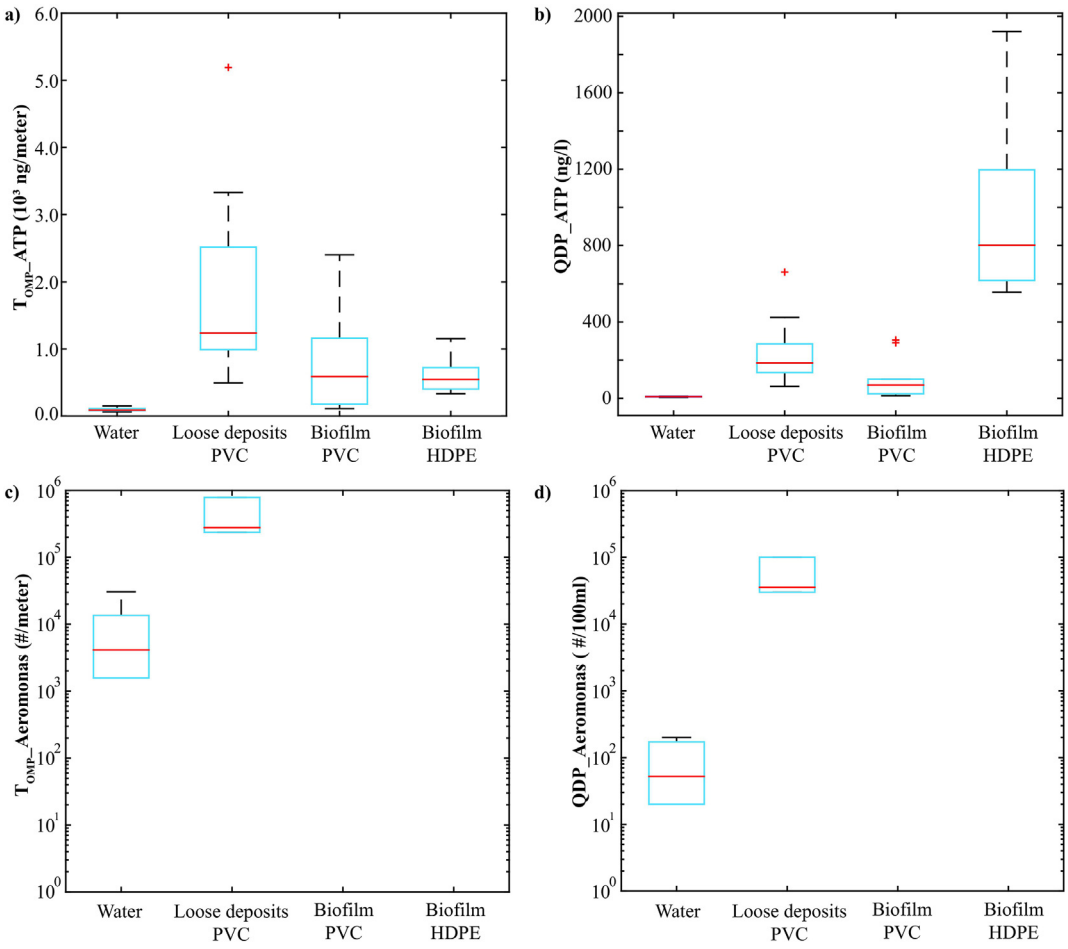

Fig. 3. Boxplot showing the distribution of active biomass (ATP and Aeromonas) across niches normalized back to 1 m of pipe (n = 10); the values obtained from water samples are included to provide an indication of the relative values in loose deposits, PVC-U biofilm, and HDPE biofilm.

established toward a plateau after 5000 reads were sequenced, indicating that enough sample coverage was obtained in this study (Fig. S4). An overview of obtained sequences, detected OTU numbers, OTU number after filtering out rare ones (<100 hits), and number of core OTUs (occupancy = 100%, abundance>1%) within each niche are summarized in Table 1.

Results showed that the sequences and total number of detected OTUs were comparable among the four niches. After filtering out the rare ones with low counts (<0.01%), OTU numbers decreased dramatically. The ranking of the niches by the number of harbored OTUs, after filtering out rare OTUs, was, in descending order: loose deposits, biofilm on HDPE pipes, water, and biofilm on PVC-U pipes. Regarding the bacterial community structure and composition at the phylum level, all samples were dominated by Proteobacteria (50–80%) and Bacteroidetes (5–32%) (Fig. S5). At the genus level, the bacterial communities were dominated by *Polaromonas* spp. (2–23%), *Nitrospira* spp. (1–47%), *Flavobacterium* spp. (1–36%), and *Flavobacterium* spp. (5–67%), respectively, for water, loose deposits, biofilm on PVC-U pipes, and biofilm on HDPE pipes (Fig. 6).

When focusing on the core OTUs, 32 OTUs were found, none of which was shared by all four niches (Fig. 4). One OTU (denovo565558) was shared by three niches, namely, loose deposits, biofilms on PVC-U, and biofilm on HDPE pipes; whereas three OTUs (denovo565558, denovo50188, denovo131517) were shared by biofilms on PVC-U and HDPE pipes. Loose deposits, biofilm on HDPE pipes, and water contained 7, 6, and 6 unique core OTUs, respectively, whereas the biofilm on PVC pipes did not contain any unique core OTU (Venn diagram, Fig. S6). The detailed taxonomy information of the core OTUs is summarized in Fig. S7 in a phylogenetic tree.

Correlative analysis between elements and microbiological parameters was performed by conducting canonical correspondence analysis (CCA) (Fig. 5). Results showed that there is a clear positive correlation among the analyzed elements of Fe, Mn, and As. Moreover, there is strong association between microbial genera and elements: 1) denovo668026, denovo349612, denovo254704, denovo138720, denovo603885 and Fe–Mn–As; 2) denovo1313959, denovo645721, denovo1886283, denovo1499877, denovo50188, denovo1851036, denovo274653 and Al; and 3) denovo131517, denovo541245 and Ca.

3.3.2. Hotspots for Mycobacterium spp. and Legionella spp.

Besides the results on cultivable *Aeromonas* spp., the 16s rRNA gene sequence results revealed the accumulation and distribution of two other genera (*Mycobacterium* spp. and *Legionella* spp.) among the four niches in the DWDS (Fig. S8). Compared to the core OTUs, *Mycobacterium* spp. and *Legionella* spp. had a lower relative abundance and occupancy. Neither of these genera were detected in the biofilm formed on PVC-U pipes. *Legionella* spp. was observed in niches of water, loose deposits, and biofilm on HDPE pipes, at the relative abundances of 0.01%, 0.01%, and 0.003%, and occupancies of 40%, 70%, and 50%, respectively. In contrast, *Mycobacterium* spp. was only detected in loose deposits and biofilms on HDPE pipes, which accounted for 0.003% and 0.007% of the total OTUs, and has

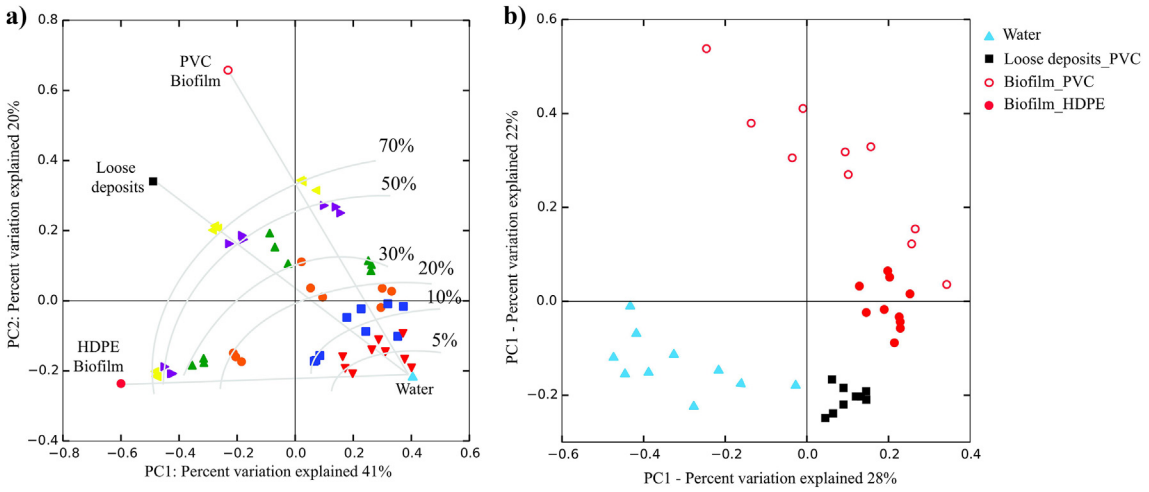

Fig. 4. Relative abundance of core OTUs (>1% relative abundance, 100% occupancy) and their taxonomy classification at the identified level (mostly genus level).

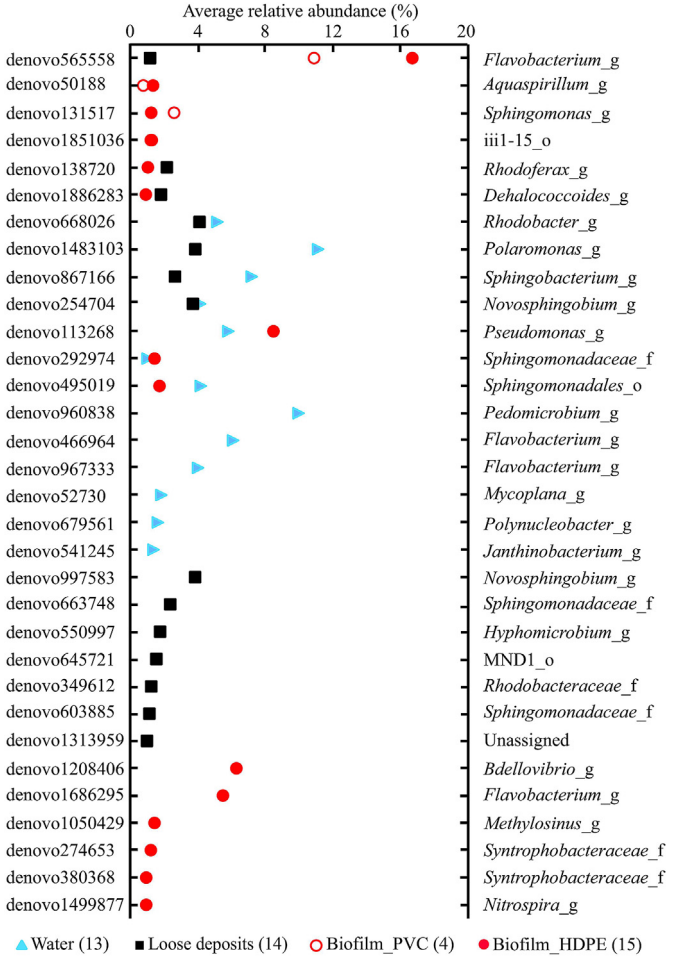

Fig. 5. CCA ordination plot of the relationship between elements and detected core OTUs.

an occupancy of 60% in both of these niches.

3.3.3. *Water quality deterioration potential*

For the bacterial community similarity comparison, the beta diversity analysis revealed four different clusters in the four niches (Fig. 6a PCoA based on unweighted UniFrac metrics). The observed differences between results based on unweighted UniFrac metrics and weighted UniFrac metrics suggesting fluctuations in the relative abundance of microbes among different locations (Fig. S9, PCoA based on weighted UniFrac metrics). Moreover, across the sampling locations, high similarity was found for bacterial communities in loose deposits and biofilm on HDPE pipes, whereas more variations were observed in water and biofilm on PVC-U pipes from different locations (beta significance test results given in Table S4).

In a step beyond the quantitative assessment of microbiological QDP, the bacterial community study based on 16s rRNA gene sequencing revealed that the release of microbes from other niches into the water would change the water bacterial community. The beta diversity and the significance of community variation analysis

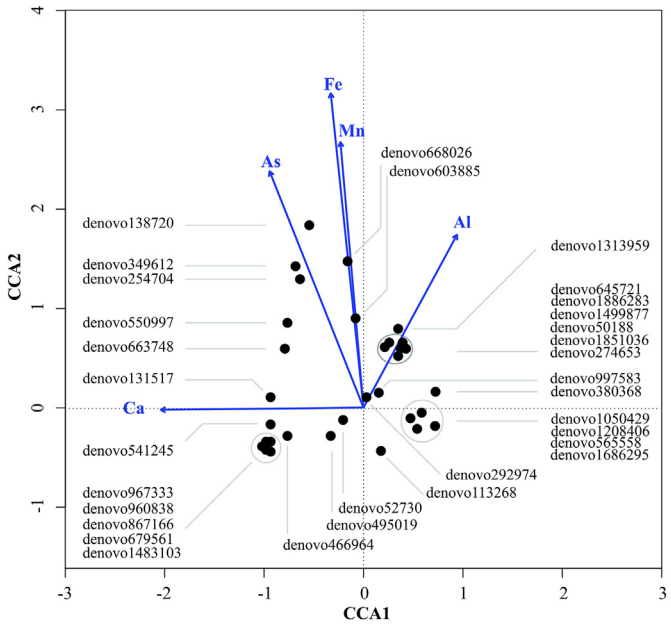

Fig. 6. a) PCoA plot of the potential influence of loose deposits and biofilm release on the water bacterial community, the release ratio of 5%, 10%, 20%, 30%, 50%, and 70% were tested. Statistical test results are shown in Table S3 reveal that the release of 20% loose deposits, 20% U-PVC biofilm and 10% HDPE biofilm will lead to significant changes on the water bacterial community; b) PCoA plot generated using WUnF metrics for all sampling locations and phases.

revealed that the release of 20% loose deposits, 20% PVC-biofilm, and 10% HDPE-biofilm leads to significant changes in the water bacterial community ($P \leq 0.01$) (Fig. 6b, Table S3). Moreover, other new OTUs might be introduced into the water column through the resuspension of the loose deposits and detachment of pipe surface biofilm. Taking *Mycobacterium* spp. and *Legionella* spp. as examples, the resuspension of loose deposits or detachment of biofilms in HDPE pipes can lead to an increase in *Legionella* and *Mycobacterium* levels.

4. Discussion

4.1. Hotspots for material accumulation: niches in the DWDS as sinks

In the present study, accumulation of selected metal elements and bacteria in loose deposits, and biofilms on PVC-U and HDPE pipes was observed, even for parameters that were under the detection limit in water. The detection of considerable amounts of elements and microbes in biofilms on both PVC-U and HDPE pipes corresponds with previous research on the biofilm matrix in DWDSs, which has been attributed to the extracellular polymeric substance (EPS) matrix produced by the microflora in the biofilm, where organics, inorganics, and cell aggregates can accumulate in (LeChevallier et al., 1987; Van Der Wende, Characklis et al., 1989; Costerton et al., 1995; Flemming and Wingender, 2010; Wang et al., 2012). The observation of high amounts of selected metal elements and microbes in loose deposits also corresponds with previous reported studies, which concluded that loose deposits can act as a reservoir for inorganics and microbes (Gauthier et al., 1999; Zacheus et al., 2001; Peng et al., 2010; van der Wielen and Lut, 2016).

4.1.1. Loose deposits as hotspots for accumulation of elements and microbes

The quantitative comparison of the accumulations of selected metal elements, ATP, and *Aeromonas* between the four DWDS niches in our study revealed that loose deposits were hotspots for Fe, Mn, As, and biomass accumulation, but not for Ca and Al. This may be because in the bio-chemical reactor of loose deposits, the co-presence of Fe, Mn, As and related bacteria favored their complexation and co-accumulation. For example, As (Ⅲ) is oxidized more rapidly when Fe, Mn, and related bacteria are present in the environments (Jones et al., 2012; Liu et al., 2014; Bai et al., 2016); this corresponds to the observation that the iron oxidizing bacteria *Rhodobacter* spp. (Emerson et al., 2010). was detected as one of the core OTUs in loose deposits in our study. The accumulated Fe in loose deposits, in turn, supported *Rhodoferax* spp. (iron reduction bacteria, *Rhodoferax ferrireducens*) become one of the core OTUs (Finneran et al., 2003). The current 16s rRNA analysis can offer bacterial information to the genus level; metagenomics will be necessary to obtain more solid functional insights into the microbes and the above-mentioned bio-chemical reactions. Moreover, the loose deposit particles may contain specific nutrients that favor the attachment and subsequent growth of microbes, while the greater mobility of loose deposits compared to biofilms can also increase the particles' contact with water and enhance their capturing ability (Liu et al., 2014).

Both cultivation and molecular results showed that some bacteria groups (e.g., *Aeromonas* spp. and *Mycobacterium* spp.) were only detected in the loose deposit niches. This may be explained by the origin of these bacteria, which is associated with suspended particles that originate in the treatment plant and settle and grow within the loose deposits during distribution (Brazos and O'Connor, 1996; Vreeburg and Boxall, 2007; Liu et al., 2013a,b,c; Proctor and Hammes, 2015; Liu et al., 2016), or/and by the fact that the growth of these bacteria is favored by the presence of multiple micro-environments (anoxic and sub-anoxic conditions), which form and develop within the loose deposits (Liu et al., 2014). However, the detection of *Mycobacterium* spp. at genus level does not indicate the existence of bio-safety problems, because the detected member may not be the pathogenic species. As reported by van der Wielen and van der Kooij (2013) pathogenic mycobacteria have not been found in drinking water in the Netherlands (van der Wielen and van der Kooij, 2013).

4.1.2. Biofilm as a hotspot for Ca accumulation

In contrast to other measured elements, Ca accumulation was only observed in biofilms on PVC-U pipes, not in those on HDPE pipes nor in loose deposits. This indicates that the dominant processes that occur in pipe surface biofilms might differ from those occurring in loose deposits and, similarly, that the processes might differ between PVC-U and HDPE biofilms. The Ca concentrations detected in water and biofilm were similar, suggesting that the Ca concentration in water is mainly determined by the Ca concentration in the treated water leaving the treatment plant. The observation of Ca in PVC-U biofilms in the present study corresponded with the finding that Ca is a critical factor for biofilm formation under low substrate concentrations (Hijnen et al., 2016). To capture Ca, biofilm and its extracellular matrices absorb $Ca2+$, and promote calcium carbonate formation by providing additional nucleation sites. Bifilm formed on U-PVC and HDPE pipes are different, as indicated by PCoA analysis in Fig. 6a. This maybe the reason that Ca did not accumulate in HDPE pipes, since the extraceller matrix components and secreted organic matter of biofilm determine the growth of calcium carbonate deposits and crystal growth. The possible assembly of mineral scaffolds in PVC-U biofilm can, in turn, play a cardinal and conserving role, by providing high resistance to environmental stresses (e.g., antibacterial agents, hydraulic turbulence, and water quality changes), thus promoting the biofilm's resilience and limiting the penetration of antibacterial agents (Oppenheimer-Shaanan et al., 2016).

In contrast to Ca, the concentrations of Fe, Mn, and As in HDPE biofilms were much higher than those in PVC-U biofilms. As observed in loose deposits, iron-related *Rhodoferax* spp. bacteria were detected as core OTUs in the biofilms on HDPE pipes (but not in the PVC-U biofilm), suggesting that similar bio-chemical processes occur in the HDPE biofilm niches (but not in the niche of PVC-U biofilm niches). Moreover, among the core OTUs in HDPE biofilm are methanotrophs (denovo1050429 assigned to *Methylosinus* spp.) and nitrifiers (denovo1499877 assigned to *Nitrosipra* spp.), indicating that the bio-chemical processes of methane oxidation and nitrification may take place in HDPE biofilms. Previous studies have extensively demonstrated their presence in drinking water at taps when groundwater is used as the source water (Ling et al., 2016).

The above-mentioned differences between biofilms on PVC-U and HDPE pipes may reflect the distinct properties of the two types of pipe material. The material plays an important role in the quantity (Van der Kooij and Veenendaal, 2001) and bacterial community of the formed biofilm (Hyun-Jung et al., 2011; Ji et al., 2015; Zhang et al., 2017), since they leach different amounts and types of compounds. Additional differences between these two niches—such as running patterns (continuous vs. intermittent), hydraulics, and residence times—might also explain the observed biofilm differences (Douterelo et al., 2013; Wang et al., 2014; Stanish et al., 2016).

4.2. Water quality deterioration potential: niches in the DWDS as sources

When the equilibrium between the water column and the

niches in the DWDS is disturbed, either by hydraulic changes or water quality changes, the DWDS niches can be transformed from sinks to sources and thus cause a deterioration in water quality. There is a broad consensus that the distribution process is a major contributor to water quality deterioration (Van Der Wende et al., 1989; Van der Kooij, 1992; Matsui et al., 2007; Vreeburg and Boxall, 2007; Liu et al., 2014). To explore beyond this consensus, the present study has quantified the quality deterioration potential (QDP_{niche} and $QDP_{parameter}$) for the different niches in an operational distribution system on physiochemical and microbiological parameters.

4.2.1. Water quality deterioration potential associated with each niche

Comparisons of the water quality deterioration potential associated with each niche (QDP_{niche}) showed that biofilm formed on HDPE pipes had the highest QDP for Fe, Mn, As, Al, and ATP, followed by $QDP_{loose\ deposits}$, and $QDP_{PVC\text{-}U}$. The differences with regard to the pipes' QDPs can be explained, according to equation (2), by the different volumes of water contained in one pipe meter, namely, PVC-U: 7.85 L and HDPE: 0.6 L. Moreover, the household HDPE pipes are normally dead ends, with an intermittent flow (longer residence time) and small diameters (high surface/volume ratio), all of which increases the potential risks of water quality deterioration within them (Lautenschlager et al., 2010; Tsvetanova and Hoekstra, 2010). Since these household pipes are also in close proximity to the consumer's tap, they will have a more direct influence on the drinking water quality at the tap than will the more distant distribution pipes (e.g., transportation pipes). The higher QDP_{HDPE} compared to $QDP_{loose\ deposits}$ and $QDP_{PVC\text{-}U}$ for Fe, Mn, As, Al and ATP points to the importance of understanding which microbiological and physiochemical parameters accumulate, and how to clean these household pipes efficiently. It should be noted that household HDPE pipes, as the last meters before the water meter, constitute a small portion of the total pipe length in a distribution system, when compared to the (PVC-U) distribution pipes. Consequently, in practice, the general water quality deterioration caused by PVC-U pipes could actually be much higher when the total length of these pipes is taken into consideration. In conclusion, we recommend that the contribution of pipe length to the QDP should be the object of further investigations.

Since Ca accumulated mainly in the niche of biofilms on PVC-U pipes, the increase in Ca concentration in the water will have been the result of biofilm detachment in these pipes ($QDP_{PVC\text{-}U}$). *Aeromonas* spp. and *Mycobacterium* spp. accumulated only in loose deposits ($QDP_{loose\ deposits}$), meaning that an increase in their numbers in water will mainly occur when the loose deposits are resuspended (van der Wielen and Lut, 2016). Resuspension of loose deposits or biofilm detachment may introduce more microbes into water because of the diverse bacterial community of these niches; this might even increase the presence of opportunistic pathogens in the water (Torvinen et al., 2004; van der Wielen and van der Kooij, 2013; van der Wielen and Lut, 2016).

4.2.2. Overall water quality deterioration potential

The material harbored by different niches may be released into the water under different circumstances and to different degrees. For example, the loose deposits can be resuspended by hydraulic disturbances, with the level of water quality deterioration depending on the turbulence created by peak flows. This is demonstrated by the reported increase of particle loads observed at customers' taps as a consequence of hydraulic changes during morning peaks (Matsui et al., 2007; Liu et al., 2016). At the velocity of 0.14 m/s, ferric chloride particles were also resuspended in the distribution pipes with a diameter of 100 mm (Vreeburg and Boxall, 2007). The destabilization of biofilm niches may take longer because of their higher resistance to environmental dynamics due to EPS, mineral scaffolds, entanglement of biopolymers, and viscous bonds between the attached surface and biofilm matrix (Flemming and Wingender, 2010; Oppenheimer-Shaanan et al., 2016). However, shock releases can still occur when a DWDS is subjected to sharp hydraulic disturbances or water quality disturbances. One such case occurred as water discoloration was observed in 80% of the supply area of a Beijing treatment plant after it switched its source water; the system was operating under regular hydraulic conditions and high loads of biomass and inorganics were detected in the red water (Li et al., 2010).

To obtain an integrated view of the variable circumstances and the water quality deterioration potential associated with different niches for a certain parameter, the actual $QDP_{parameter}$ can be calculated according to the following equation:

$$QDP_{parameter} = \sum_{1}^{n} K_n \cdot QDP_n \quad (4)$$

$QDP_{parameter}$: overall quality deterioration potential can be caused by harbored material in different niches; the number of niches is n. For the present study, three niches have been studied so that $n = 3$.

K_n: coefficient for the QDP from the niche, the value ranges between 0 and 1. $K_n = 1$ when all harbored material associated with the niche is released into water; $K_n = 0$ when no harbored material is disturbed and none is released into water. The value of K should be further studied and defined. This paper takes $K = 1$ so as to calculate the maximum QDP.

QDP_n: the quality deterioration potential caused by niche 'n'. This is calculated according to equation (3) and the results shown in Figs. 2 and 3.

According to currently available knowledge, when there is only a hydraulic disturbance caused by a regular morning demand peak, K_n for the biofilms niches can be estimated as 0 (minor release, almost 0), whereas the K_n for the niche of loose deposits ($K_{loose\ deposits}$) is between 0 and 1 depending on the created disturbance. As discussed above, the detected *Aeromonas* spp. is a result of the resuspension of loose deposits and the associated release of the bacteria into the water column. In such cases, $K_{loose\ deposits}$ during regular operation can be calculated by the concentration measured in the water column (deteriorated quality) and the maximum deterioration potential (QDP), according to equation (4) (K = deteriorated quality/QDP). The calculated $K_{loose\ deposits}$ ranges from 0 to 0.06, suggesting a very small portion of the loose deposits (<6%) is disturbed by the daily operational hydraulics.

A $K_{loose\ deposits}$ of 1, which represents the maximum deterioration potential, can occur when there is a sufficiently high increase of velocity in the distribution pipes (e.g., 1.5 m/s for flushing). The $QDP_{parameter\_maximum}$ calculation, according to equation (4), will therefore be dominated by the contribution from loose deposits. In the event that the supply water quality changes, for instance, due to a switching of source water (Li et al., 2010; Schwake et al., 2016; Liu et al., 2017) or a change in treatment, the value of K_n for all niches will shift up toward 1. Further investigation will be needed to obtain an actual value for K_n for loose deposit resuspension and biofilm detachment under different disturbances.

4.3. Practical importance of hotspots and QDP evaluation

The present study extends the current understanding of biofilm

formation and loose deposit accumulation in water distribution systems, by showing the distribution of elements and microbes among the different niches, through the quantitative assessment of "hotspots" and connecting these directly to water quality by determining their "QDPs". The hotspots and the QDP evaluation, provide the basis for taking management decisions and practical, decisive preventive actions to avoid unwanted esthetic and/or public health problems associated with water quality in the case of destabilization events in a DWDS (e.g., Flint water crisis and Beijing discoloration) (Li et al., 2010; Gostin, 2016; Schwake et al., 2016). For example, our results demonstrate that loose deposits can be a hotspot for selected metal elements, ATP, *Aeromonas* spp., and *Mycobacterium* spp. accumulation in a DWDS. Moreover, loose deposits can be resuspended into the water column by daily hydraulic variations (Vreeburg and Boxall, 2007). Therefore, it is important to control and clean the loose deposits in a DWDS when there are morning-peak related water-quality problems. This can be done by: 1) limiting the particles in the supply water through improved treatment (Liu et al., 2013a,b,c); 2) maintaining a self-cleaning velocity for the distribution system design (Vreeburg et al., 2009); and 3) conducting regular flushing activities during normal operations and before introducing supply water of new quality (Lehtola et al., 2004; Vreeburg and Boxall, 2007; Liu et al., 2017).

The household HDPE pipes contribute the highest QDP. Although hydraulic disturbances probably do not detach as many elements and microbes from the HDPE biofilms compared to their resuspension of loose deposits, the potential should not be ignored (e.g., released Pb and *Legionella* spp. in Flint water crisis). It is important that the material accumulation and the releasing mechanisms be evaluated, since they are still insufficiently understood. Moreover, the household HDPE pipes are dead-ends and have a small diameter, which makes them difficult to clean efficiently. The influence of household pipe biofilms on drinking water-quality deterioration should therefore be studied in more detail, a task that was beyond the range of our study.

Despite the efforts made to investigate the accumulation of the elements and microbes and their release into the distribution system, the mechanism of substrate transfer between bulk water and the niches in the DWDS is still not clear. Moreover, as mentioned above, the actual QDP and the specific contributions of loose deposits and biofilm will depend greatly on the local circumstances, such as the water usage pattern, real-time flow rate, pipe material, and the heterogeneity of the plumbing system. Comprehensive studies and complete databases of online water monitoring, together with biofilm and loose deposits information that covers treatment plant, distribution system and customers' taps, will be needed for an in-depth understanding. Depending the demand and interests, there is also a great opportunity to extend the QDP concept to more parameters (e.g. QDP_{carbon}).

5. Conclusion

- Loose deposits are hotspots for the accumulation of Fe, Mn, As, Al and ATP, *Aeromonas* spp. only accumulated in the niche of loose deposits.
- Household HDPE pipes have the highest water-quality deterioration potential, except for *Aeromonas* spp..
- The release of 20% loose deposits, 20% U-PVC biofilm and 10% HDPE biofilm can lead to significant changes of the water bacterial community.
- The hotspot and QDP evaluation can be used to guide water utilities in taking efficient actions to avoid unwanted water-quality problems.

Acknowledgements

The authors would like to acknowledge Harmen van der Laan and Kevin Holthuijsen from Oasen Drinkwater for their assistance on planning the project and conducting the complex sampling in the field distribution system.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.08.002.

References

Bai, Y., Yang, T., Liang, J., Qu, J., 2016. The role of biogenic Fe-Mn oxides formed in situ for arsenic oxidation and adsorption in aquatic ecosystems. Water Res. 98, 119–127.
Berry, D., Xi, C., Raskin, L., 2006. Microbial ecology of drinking water distribution systems. Curr. Opin. Biotechnol. 17 (3), 297–302.
Boe-Hansen, R., Albrechtsen, H.J., Arvin, E., Jørgensen, C., 2002. Bulk water phase and biofilm growth in drinking water at low nutrient conditions. Water Res. 36 (18), 4477–4486.
Brazos, B.J., O'Connor, J.T., 1996. Seasonal effects on generation of particle-associated bacteria during distribution. J. Environ. Eng. 122 (12), 1050–1057.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pēa, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, 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. Meth. 7 (5), 335–336.
Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., Lappin-Scott, H.M., 1995. Microbial biofilms. Annu. Rev. Microbiol. 49 (1), 711–745.
Douterelo, I., Sharpe, R., Boxall, J., 2013. Influence of hydraulic regimes on bacterial community structure and composition in an experimental drinking water distribution system. Water Res. 47 (2), 503–516.
Emerson, D., Fleming, E.J., McBeth, J.M., 2010. Iron-oxidizing bacteria: an environmental and genomic perspective. Annu. Rev. Microbiol. 64, 561–583.
Finneran, K.T., Johnsen, C.V., Lovley, D.R., 2003. Rhodoferax ferrireducens sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe (III). Int. J. Syst. Evol. Microbiol. 53 (3), 669–673.
Fish, K.E., Osborn, A.M., Boxall, J., 2016. Characterising and understanding the impact of microbial biofilms and the extracellular polymeric substance (EPS) matrix in drinking water distribution systems. Environ. Sci. Water Res. Technol. 2, 614–630.
Flemming, H.-C., Wingender, J., 2010. The biofilm matrix. Nat. Rev. Microbiol. 8 (9), 623–633.
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.
Gostin, L.O., 2016. Politics and public health: the flint drinking water crisis. Hastings Cent. Rep. 46 (4), 5–6.
Hammer, Ø., Harper, D., Ryan, P., 2008. PAST-Palaeontological Statistics, Ver. 1.89. Paleontological Museum, University of Oslo, Norway. http://folk.uio.no/ohammer/past/index.html.
Havelaar, A., During, M., Versteegh, J., 1987. Ampicillin-dextrin agar medium for the enumeration of Aeromonas species in water by membrane filtration. J. Appl. Bacteriol. 62 (3), 279–287.
Hijnen, W., Schultz, F., Harmsen, D., Brouwer-Hanzens, A., van der Wielen, P., Cornelissen, E., 2016. Calcium removal by softening of water affects biofilm formation on PVC, glass and membrane surfaces. Water Sci. Technol. Water Supply ws2016021.
Hyun-Jung, J., Choi, Y.J., Ka, J.O., 2011. Effects of diverse water pipe materials on bacterial communities and water-quality in the annular reactor. J. Microbiol. Biotechnol. 21 (2), 115–123.
Ji, P., Parks, J., Edwards, M.A., Pruden, A., 2015. Impact of water chemistry, pipe material and stagnation on the building plumbing microbiome. PloS One 10 (10), e0141087.
Jones, L.C., Lafferty, B.J., Sparks, D.L., 2012. Additive and competitive effects of bacteria and mn oxides on arsenite oxidation kinetics. Environ. Sci. Technol. 46 (12), 6548–6555.
Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79 (17), 5112–5120.
Lautenschlager, K., Boon, N., Wang, Y., Egli, T., Hammes, F., 2010. Overnight stagnation of drinking water in household taps induces microbial growth and changes in community composition. Water Res. 44 (17), 4868–4877.
LeChevallier, M.W., Babcock, T.M., Lee, R.G., 1987. Examination and characterization of distribution system biofilms. Appl. Environ. Microbiol. 53 (12), 2714–2724.
Lehtola, M.J., Nissinen, T.K., Miettinen, I.T., Martikainen, P.J., Vartiainen, 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.L., Li, S., Vreeburg, J.H.G., Verberk, J.Q.J.C., Medema, G.J., Liu, W.T., Van Dijk, J.C., 2014. Pyrosequencing reveals bacterial communities in unchlorinated drinking water distribution system: an integral study of bulk water, suspended solids, loose deposits, and pipe wall biofilm. Environ. Sci. Technol. 48 (10), 5467–5476.
Liu, G., Ling, F., Magic-Knezev, A., Liu, W., Verberk, J.Q.J.C., Van Dijk, J.C., 2013a. Quantification and identification of particle associated bacteria in unchlorinated drinking water from three treatment plants by cultivation-independent methods. Water Res. 47 (10), 3523–3533.
Liu, G., Ling, F., van der Mark, E., Zhang, X., Knezev, A., Verberk, J., van der Meer, W., Medema, G., Liu, W., van Dijk, J., 2016. Comparison of Particle-associated Bacteria from a Drinking Water Treatment Plant and Distribution Reservoirs with Different Water Sources. Scientific reports 6.
Liu, G., Lut, M.C., Verberk, J.Q.J.C., Van Dijk, J.C., 2013b. A comparison of additional treatment processes to limit particle accumulation and microbial growth during drinking water distribution. Water Res. 47 (8), 2719–2728.
Liu, G., Verberk, J.Q.J.C., Dijk, J.C., 2013c. Bacteriology of drinking water distribution systems: an integral and multidimensional review. Appl. Microbiol. Biotechnol. 97 (21), 9265–9276.
Liu, G., Zhang, Y., Knibbe, W.-J., Feng, C., Liu, W., Medema, G., van der Meer, W., 2017. Potential impacts of changing supply-water-quality on drinking water distribution: a review. Water Res. 116, 135–148.
Lytle, D.A., Sorg, T.J., Frietch, C., 2004. Accumulation of arsenic in drinking water distribution systems. Environ. Sci. Technol. 38 (20), 5365–5372.
Magic-Knezev, A., van der Kooij, D., 2004. Optimisation and significance of ATP analysis for measuring active biomass in granular activated carbon filters used in water treatment. Water Res. 38 (18), 3971–3979.
Makris, K.C., Andra, S.S., Botsaris, G., 2014. Pipe scales and biofilms in drinking-water distribution systems: undermining finished water-quality. Crit. Rev. Environ. Sci. Technol. 44 (13), 1477–1523.
Matsui, Y., Yamagishi, T., Terada, Y., Matsushita, T., Inoue, T., 2007. Suspended particles and their characteristics in water mains: developments of sampling methods. J. Water Supply Res. Technol. - AQUA 56 (1), 13–24.
Oppenheimer-Shaanan, Y., Sibony-Nevo, O., Bloom-Ackermann, Z., Suissa, R., Steinberg, N., Kartvelishvily, E., Brumfeld, V., Kolodkin-Gal, I., 2016. Spatio-temporal assembly of functional mineral scaffolds within microbial biofilms. npj Biofilms Microbiome. 2, 15031.
Peng, C.Y., Korshin, G.V., Valentine, R.L., Hill, A.S., Friedman, M.J., Reiber, S.H., 2010. Characterization of elemental and structural composition of corrosion scales and deposits formed in drinking water distribution systems. Water Res. 44 (15), 4570–4580.
Prest, E.I., Hammes, F., van Loosdrecht, M.C., Vrouwenvelder, J.S., 2016. Biological stability of drinking water: controlling factors, methods, and challenges. Front. Microbiol. 7.
Proctor, C.R., Hammes, F., 2015. Drinking water microbiology—from measurement to management. Curr. Opin. Biotechnol. 33, 87–94.
Renner, R., 2008. Pipe scales release hazardous metals into drinking water. Environ. Sci. Technol. 42 (12), 4241–4241.
Sarin, P., Snoeyink, V.L., Bebee, J., Kriven, W.M., Clement, J.A., 2001. Physico-chemical characteristics of corrosion scales in old iron pipes. Water Res. 35 (12), 2961–2969.
Schwake, D.O., Garner, E., Strom, O.R., Pruden, A., Edwards, M.A., 2016. Legionella DNA markers in tap water coincident with a spike in legionnaires' disease in flint, MI. Environ. Sci. Technol. Lett. 3 (9), 311–315.
Sly, L.I., Hodgkinson, M.C., Arunpairojana, V., 1990. Deposition of manganese in a drinking water distribution system. Appl. Environ. Microbiol. 56 (3), 628–639.
Smith, S.E., Bisset, A., Colbourne, J.S., Holt, D.M., Lloyd, B.J., 1997. The occurrence and significance of particles and deposits in a drinking water distribution system. J. N. Engl. Water Works Assoc. 111 (2), 135–144.
Snoeyink, V., Hass, C., Boulos, P., Burlingame, G., Camper, A., Clark, R., Edwards, M., LeChevallier, M., McMullen, L., Moe, C., 2006. Drinking Water Distribution Systems: Assessing and Reducing Risks.
Stanish, L.F., Hull, N.M., Robertson, C.E., Harris, J.K., Stevens, M.J., Spear, J.R., Pace, N.R., 2016. Factors influencing bacterial diversity and community composition in municipal drinking waters in the Ohio River Basin, USA. PLoS One 11 (6), e0157966.
Torvinen, E., Suomalainen, S., Lehtola, M.J., Miettinen, I.T., Zacheus, O., Paulin, L., Katila, 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.
Tsvetanova, Z.G., Hoekstra, E.J., 2010. The effect of the surface-to-volume contact ratio on the biomass production potential of the pipe products in contact with drinking water, 10, 105–112.
Van der Kooij, D., 1992. Assimilable organic carbon as an indicator of bacterial regrowth. J. Am. Water Works Assoc. 84 (2), 57–65.
Van der Kooij, D., Veenendaal, H.R., 2001. Biomass production potential of materials in contact with drinking water: method and practical importance. Water Sci. Technol. Water Supply 1 (3), 39–45.
Van Der Wende, 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., Bakker, G., Atsma, A., Lut, M., Roeselers, G., de Graaf, B., 2016. A survey of indicator parameters to monitor regrowth in unchlorinated drinking water. Environ. Sci. Water Res. Technol. 2, 683–692.
van der Wielen, P.W., Lut, M.C., 2016. Distribution of microbial activity and specific microorganisms across sediment size fractions and pipe wall biofilm in a drinking water distribution system. Water Sci. Technol. Water Supply ws2016023.
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., Blokker, E.J.M., Horst, P., Van Dijk, J.C., 2009. Velocity-based self-cleaning residential drinking water distribution systems. Water Sci. Technol. Water Supply 9, 635–641.
Vreeburg, J.H.G., Boxall, D.J.B., 2007. Discolouration in potable water distribution systems: a review. Water Res. 41 (3), 519–529.
Vreeburg, J.H.G., Schippers, D., Verberk, J.Q.J.C., van Dijk, J.C., 2008. Impact of particles on sediment accumulation in a drinking water distribution system. Water Res. 42 (16), 4233–4242.
Wang, H., Masters, S., Edwards, M.A., Falkinham, J.O., Pruden, A., 2014. Effect of disinfectant, water age, and pipe materials on bacterial and Eukaryotic community structure in drinking water biofilm. Environ. Sci. Technol. 48 (3), 1426–1435.
Wang, Z., Hessler, C.M., Xue, Z., Seo, Y., 2012. The role of extracellular polymeric substances on the sorption of natural organic matter. Water Res. 46 (4), 1052–1060.
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.
Zhang, C., Li, C., Zheng, X., Zhao, J., He, G., Zhang, T., 2017. Effect of pipe materials on chlorine decay, trihalomethanes formation, and bacterial communities in pilot-scale water distribution systems. Int. J. Environ. Sci. Technol. 14 (1), 85–94.