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Direct evidence of microbiological water quality changes on bacterial quantity and community caused by plumbing system

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ABSTRACT

Drinking water quality deteriorates from treatment plant to customer taps, especially in the plumbing system. There is no direct evidence about what the differences are contributed by plumbing system. This study compared the water quality in the water main and at customer tap by preparing a sampling tap on the water main. The biomass was quantified by adenosine triphosphate (ATP) and the microbial community was profiled by 454 pyrosequencing. The results showed that in distribution pipes, biofilm contributed >94% of the total biomass, while loose deposits showed little contribution (< 2%) because of the low amount of loose deposits. The distribution of biological stable water had minor effects on the microbiocidal water quality regarding both quantity (ATP 1 ng/L vs. 1.7 ng/L) and community of the bacteria. Whereas the plumbing system has significant contribution to the increase of active biomass (1.7 ng/L vs. 2.9 ng/L) and the changes of bacterial community. The relative abundance of *Sphingomonas* spp. at tap (22%) was higher than that at water main (2%), while the relative abundance of *Pseudomonas* spp. in tap water (15%) was lower than that in the water from street water main (29%). Though only one location was prepared and studied, the present study showed that the protocol of making sampling tap on water main offered directly evidences about the impacts of plumbing system on tap water quality, which makes it possible to distinguish and study the processes in distribution system and plumbing system separately.

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Introduction

There is a broad conscious on water quality deterioration during drinking water distribution (Liu et al., 2013c; Van Der Kooij, 2000; Vreeburg and Boxall, 2007), especially the biological parameters that related to biostability and biosafety, such as the bacterial regrowth (Liu et al., 2013a; 2013b; 2013c), biofilm formation (Chaves Simões and Simões, 2013; Liu et al., 2020), loose deposits accumulation and the harbored bacteria enrichment (Gauthier et al., 1999; Lehtola et al., 2004; Liu et al., 2014), and the presence of (opportunistic) pathogens (Feazel et al., 2009; Wang et al., 2013; Wingender and Flemming, 2011). To guarantee the water quality at the tap and to fulfill the drinking water regulation, the water companies are required to take samples from the distribution system regularly (World Health Organization, 2003). Necessary actions should be taken based on the morning results obtained from the regular monitoring program.

To monitor biological water quality in distribution systems, heterotrophic plate counts (HPC) has been introduced since 1894 (Bartram et al., 2003) and it is still the primary parameter for assessing the general microbiological water quality (Chowdhury, 2012). The using of HPC is now increasingly challenged by the fact that it only counts media cultivable bacteria which account for a percentage between 0.001% and 6.5% of total bacteria in drinking water (Hammes et al., 2008). Cultivation independent methods were introduced in drinking water monitroing recently, e.g. adenosine triphosphate (ATP) (Liu et al., 2013b; Van der Wielen and Van der Kooij, 2010) and total cell count (TCC) by fluorescent microscopic count (Boe-Hansen et al., 2002) and flow cytometry cell count (Hammes et al., 2010; Liu et al., 2013b; Prest et al., 2014; Sklar, 2005). Most recently, with the development of molecular methods, a few studies have evaluated the use of next generation sequencing tools to assess the biological water quality changes and stability during distribution (Hwang et al., 2012; Lautenschlager et al., 2013; Pinto et al., 2014, 2012; Prest et al., 2014).

Regardless the monitoring methods used, till now, the distributed water samples were mainly collected randomly from distribution area at customers taps (World Health Organization, 2003). It has been reported that the plumbing system has significant influences on tap water quality, which is especially true regarding the microbiological parameters, such as quantity and community changes (Ji et al., 2015; Lautenschlager et al., 2010; Ling et al., 2018; Zlatanović et al., 2017) and growth of (opportunistic) pathogens induced by the long stagnation time (Falkinham et al., 2015; Rogers et al., 1994; Sarver and Edwards, 2011). This has attract special attention during the on-going COVID-19 pandemic, because the widely taken lockdown policy resulted in extra-long stagnation time of drinking water in the big public buildings (Proctor et al., 2020; Viglione 2020). For water quality studies in distribution system, especially microbiological studies, sterilization and pre-flushing before taking the samples at the tap were recommended and widely used to ensure the sampled water comes directly from the distribution pipes and to minimize the potential influences from the plumbing systems (Hammes et al., 2010; Liu et al., 2013b; Van der Wielen and Van der Kooij, 2010). However, there is no available information and/or direct evidence about the influences of plumbing system on the water quality at the taps, which mostly because of the difficulties of collecting samples right on the water main. As a result, it is still uncertain how representative the collected samples are for understanding the actual processes occurring during drinking water distribution, nor how much is contributed by distribution system and plumbing system individually.

The objective of this study is to investigate the microbiological water quality changes during distribution. To obtain direct evidence and evaluate plumbing system's contribution, a sampling tap was pre-installed on the water main in the street. Integral samples were taken from different sampling points along the distribution and plumbing system (e.g., pumping station, tap made on the water main, house tap and hydrant) and from different phases (bulk water, suspended solids, pipe biofilm and loose deposits). The planktonic bacteria in bulk water and surface associated bacteria were quantified by measuring adenosine triphosphate (ATP) and the bacterial community was profiled by 454 pyrosequencing. By comparing the bacterial quantity and community at treatment plant, in distribution system and at customer's tap, the potential influences of plumbing system on microbiological water quality were highlighted.

1. Material and methods

1.1. *Drinking water production and distribution*

The study was conducted at one of drinking water production and distribution system of Dunea Water Company, the Netherlands. The treatment plant takes source water from Meuse River. The source water, after pre-treatment, is transported over 30 km to a dune area of natural lakes, where it recharges the groundwater. After an average residence time of 2 months, the water is abstracted from the dunes. Abstracted artificial recharge and recovery (ARR) water is post-treated by softening, powered activated carbon filtration, aeration, rapid sand filtration, and slow sand filtration before being pumped into the distribution system. The use of chlorination is avoided in the Netherlands.

1.2. *Design of the study and tap on the main*

To investigate the microbiological water quality changes and deterioration during distribution, a dead-end supply area was selected where the water age is expected to be long and loose deposits might form. As shown in **Fig. 1**, a sampling tap was pre-installed on the water main to compare the quality of samples directly from the water main and from the house tap. Water samples were collected at the pumping station of the treatment plant, and at the dead-end point in the distribution system. At the dead-end location, samples were collected from house tap, hydrant, and the tap pre-installed on the water main, respectively.

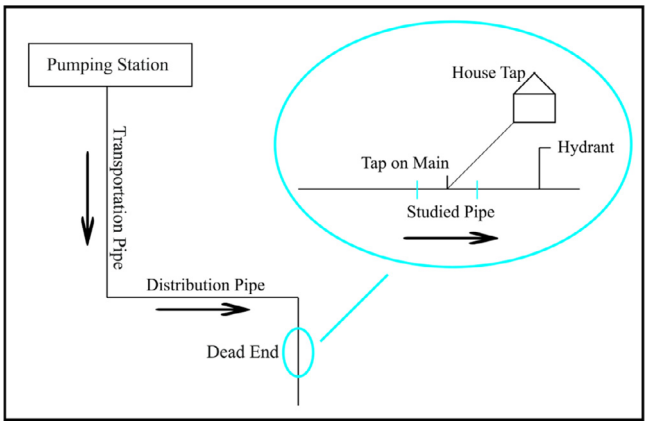

Fig. 1 – Schematic drawing of the study set-up. The studied site was selected at one dead-end point in the supply area. At the location, the distribution pipe on the street was pre-dug and sampling tap was pre-installed on the main. Water samples were taken at pumping station of treatment plant before the water entering distribution system (Water-PS), the pre-installed tap on the main (Water-main), house tap (Water-house) and the hydrant (Water-hydrant). Sand filter material were collected from operational slow sand filter bed (SSF). At the studied location, biofilm (BF), suspended solids (SS) and loose deposits (LD) were also sampled.

1.3. *Sampling, samples preparation and pretreatment*

Bulk water samples were collected from the sampling ports after sterilized the sampling tap and flushing for about 10mins till constant temperature. The suspended solids, loose deposits and pipe wall biofilm were sampled and pretreated as described previously (Liu et al., 2014). In short, the suspended solids and associated bacteria were sampled by filtrating around 150–200 liters of water through 1.2 μm glass fiber filters. The loose deposits and associated bacteria were sampled by flushing the water main at a velocity of 1.5 m/sec over a length of 300 m' pipe with a diameter of 110 mm. Pipe wall biofilm was sampled by cutting out water main pipe (diameter 110 mm, PVC) after removing loose deposits by flushing. The cut pipes, a length of 30 cm was closed at two sides and filled with sterilized water to keep the pipe samples wet. All samples were transported to lab on ice and all analysis were performed within 24 hr. The collected suspended solids, loose deposits and pipe biofilm were pretreated by three-time ultrasonication. The obtained suspension was used for further ATP analysis and DNA extraction. All samples were taken duplicated, the duplicated samples were pooled for DNA extraction and Pyrosequencing.

1.4. *ATP analysis*

All collected samples were analyzed for ATP. Total ATP concentration was determined as described previously using the BacTier-Glo reagent and a luminometer (Magic-Knezev and van der Kooij, 2004). In short, 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.

1.5. *DNA extraction and 454 pyrosequencing*

The DNA was extracted from the bulk water samples and the pretreated suspension of suspended solids, pipe wall biofilm and loose deposits using FastDNA Spin Kit for Soil (Q-Biogene/MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions(Hwang et al., 2009; Tamaki et al., 2011) and was amplified with forward primer U515F (5′-Fusion A-Barcode-CA linker-GTGYCAGCMGCCGCGGTA-3′, covers 92.66% bacteria, 93.54% archaea) and reverse primer U1052R (5′-Fusion B-TC linker-TGCATGGYYGYCGYCAGYTC-3′, covers 95.10% bacteria, 90.95% archaea)(Wang and Qian, 2009). Pyrosequencing with titanium bulk sequencing methods (Roche, Branford, CT) was performed based upon the manufacturer's protocols developed at the Research and Testing Laboratory (Lubbock, TX, USA). Following the sequencing and image processing, the sequences were binned into individual multi-fasta files based on tag sequences and used for data analysis.

1.6. *Pyrosequencing data analysis*

The sequences generated from the pyrosequencing analysis of the 16S rRNA gene amplicons were processed (filtered, clustered, taxonomically assigned and aligned) using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline with default settings (Caporaso et al., 2010). The process consisted of quality checking and denoising, and microbial diversity analysis. In short, the flow diagrams were denoised and the UCLUST algorithm was used for operational taxonomic unit (OTU) assignment. Representative OTUs were selected based on the most abundant sequences, and the taxonomic assignment was conducted using the Ribosomal Database Project (RDP) classifier with datasets from Greengenes OTUs at a 0.8 minimum confidence level. Afterwards, the sequences were aligned using the Phyton Nearest Alignment Space Termination Tool (PyNAST) alignment algorithm. Weighted and unweighted UniFrac distance matrices were constructed from the phylogenetic tree (built by FastTree algorithm) and used to conduct principal coordinate analyses (PCoA).

2. Results

2.1. *Quantification measured by adenosine triphosphate*

The bacteria in water, suspended solids, loose deposits and pipe wall biofilm were measured by ATP and the results were shown in Fig. 2. The ATP of bulk water samples in distribution system was higher than at treatment plant. It was interesting to find that the water samples collected from the house tap contained higher ATP than from the tap on the main. The ATP of samples collected from hydrant under normal flow condition contained the highest ATP content of all the bulk water

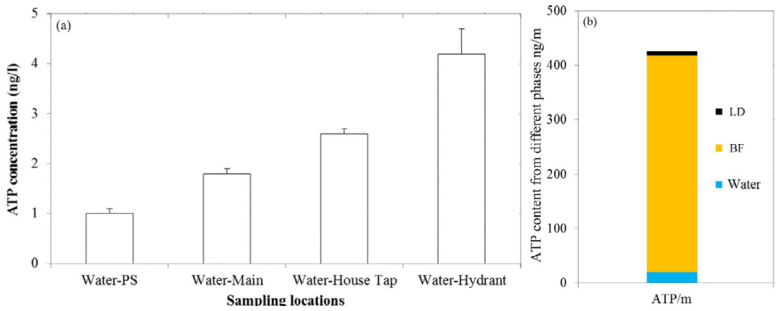

Fig. 2 – (a) ATP concentrations from water samples collected from different sites, at pumping station, from the tap on the main, the tap in the house, and directly from hydrant; (b) comparison of bacterial abundance (comparison of biomass as inferred from ATP results) of different phases within a length of one-meter water main pipe (PVC, 110 mm). Normalization was based on the surface area for biofilm, mass of loose deposits and the volume of water over one meter.

samples. In distribution system, the ATP of the four phases within one-meter pipe was compared to show the relative abundances of different phases. Results showed that most of the contributions (> 94%) was from pipe surface biofilm, little was from loose deposits (< 2%), the rest was in bulk water (< 4%).

2.2. *Diversity and composition of microbial communities revealed by pyrosequencing*

2.2.1. *Bacterial diversity*

In total, 40,942 16S rRNA pyrosequences were obtained from the 9 samples and further separated into 862 OTUs based on a similarity cutoff of 95%. For different phases, highest species richness (Chao1) and diversity index (Shannon) at genetic distances of 5% were observed at samples taken from slow sand filter, followed by particle associated bacteria in loose deposits and suspended solids (Fig. 3). Lower species richness and diversity were observed for bulk water and biofilm samples, among which the biofilm samples were only higher than bulk water samples collected at treatment plant and from hydrant. The rarefaction curves for each sample (observed OTUs, Fig. 3) showed that the bulk water samples had less observed OTUs than particle-associated bacteria (filter material, suspended solids and loose deposits). The OTUs observed in pipe biofilm was somewhere in the middle of bulk water samples.

Regarding the bulk water samples, the Chao1 richness estimator, the Shannon diversity index and observed OTUs estimated at 5% cut-offs showed that the bacterial richness and diversity in descending order is water (Tap) > water (Main) > water (PS) > water (Hydrant). Similarly, the bacterial richness and diversity of bacteria associated with suspended solids collected from distribution system was higher than that from treatment plant.

2.2.2. *Planktonic bacteria from different sampling points*

The obtained sequences of planktonic bacteria were assigned to 18 phyla (Fig. 4). Among all planktonic bacteria from *Proteobacteria* was the most abundant phylum that accounted for 66%–85% of the total OTUs across all collected water samples. Among the sub-phylum, Alphaproteobacteria (19%–45%), Betaproteobacteria (12%–46%) and Gammaproteobacteria (8%–32%) were abundant. Deltaproteobacteria was detected, but with a low percentage (1%–2%). Another three phylum were detected that accounted for more than 1%: Actinobacteria (6%–17%), *Firmicutes* (1%–12%) and Bacterioidetes (1%–8%). At genera level, the detected OTUs were mainly comprised of *Herbaspirillum* spp. (11%–42%), *Pseudomonas* spp. (4%–29%), *Ochrobactrum* spp. (3%–14%) and *Pimelobacter* spp. (2%–7%).

737, 630, 649 and 755 OTUs were detected in the water samples from treatment plant, water main, house tap and hydrant, respectively. Differences of planktonic bacterial communities were observed from different water samples (Figs. 4 and 5). The water samples collected from treatment plant, water main, and house tap were similar, the hydrant planktonic bacteria were found to be a totally different cluster. Among the similar communities of water samples from treatment plant, water main and house tap, there were 37, 95, 88 OTUs only detected from water taken from treatment plant, water main and house tap. More specific, the planktonic bacteria in treated water at pumping station were dominated (> 10%) by *Brevundimonas* spp. (17%), *Herbaspirillum* spp. (12%), *Caulobacter* spp. (12%) and *Sporosarcina* spp. (10%); in water at house tap were dominated by *Sphingomonas* spp. (22%), *Pseudomonas* spp. (15%) and *Herbaspirillum* spp. (15%); in water sampled directly from water main were dominated by *Pseudomonas* spp. (29%), *Brevundimonas* spp. (15%) and *Herbaspirillum* spp. (12%); in the hydrant water were dominated by *Herbaspirillum* spp. (42%), *Pseudomonas* spp. (14%) and *Ochrobactrum* spp. (12%).

2.2.3. *Community of pipe surface biofilm*

635 OTUs were detected in the collected pipe biofilm that assigned to 9 phyla, among which *Proteobacteria* accounted for 97% of the total OTUs. Among the sub-phylum, *Alphaproteobacteria* (60%), *Betaproteobacteria* (10%) and *Gammaproteobacteria* (26%) were abundant. At genera level, the detected OTUs were mainly comprised of *Sphingomonas* spp. (50%), *Stenotrophomonas* spp. (15%), *Pseudomonas* spp. (6%), *Sphingopyxis* spp. (3%), *Herbaspirillum* spp. (3%), *Brevundimonas* spp. (1%), *Massilia* spp. (1%) and *Hyphomicrobium* spp. (1%).

2.2.4. *Community of particle-associated bacteria*

641 OTUs were detected in the bacteria in slow sand filter (SSF) that assigned to 17 phyla. These phyla, in the descending order, were Proteobacteria (34%), Actinobacteria (22%), Plancto-

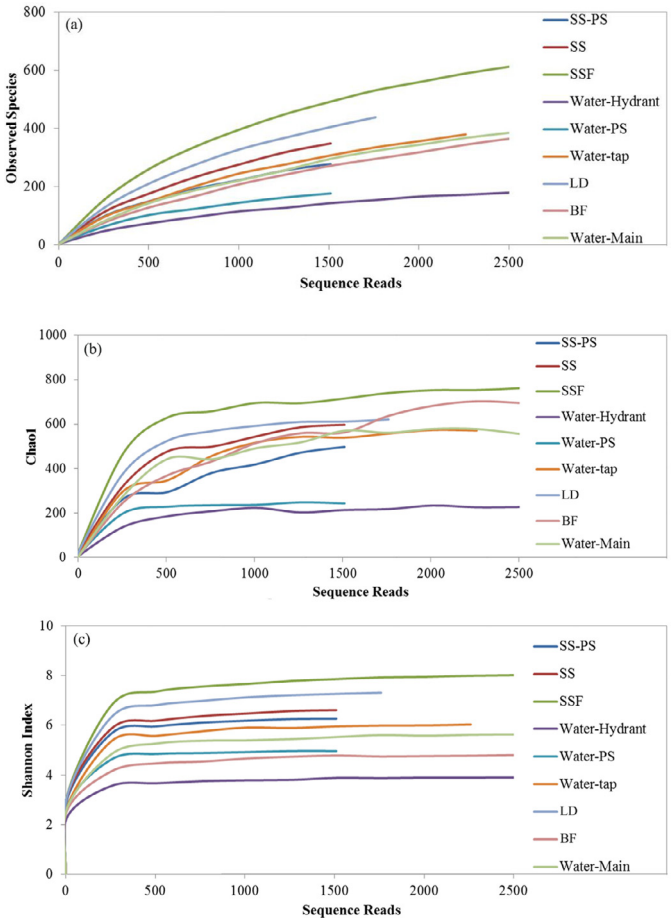

Fig. 3 – Rarefaction curves at 95% of sequence similarity. Rarefaction curves were obtained for observed OTUs (a), Chao 1 index richness estimator (b) and Shannon diversity estimator (c).

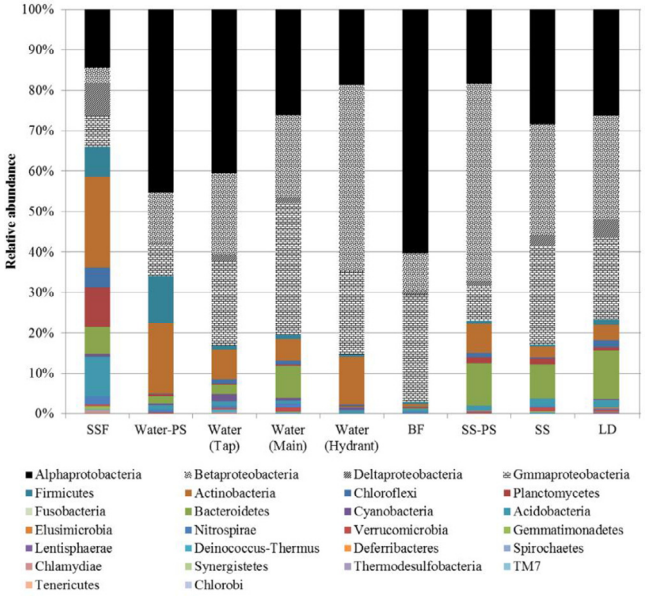

Fig. 4 – Relative abundance of bacterial phyla of bacteria from water, suspended solids, loose deposits and biofilm. The dominant phylum, Proteobacteria, is shown as four classes in the upper panel in the figure.

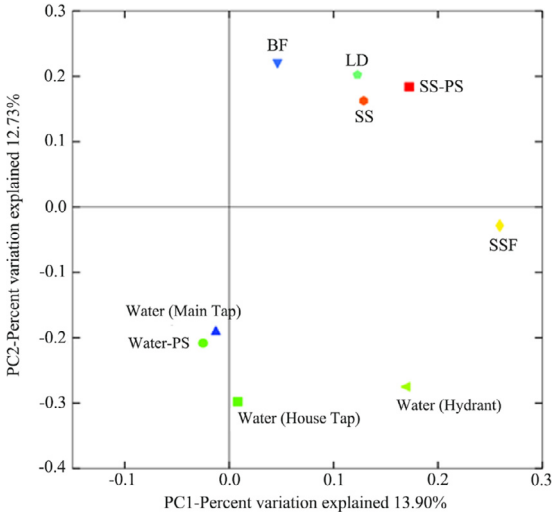

Fig. 5 – PCoA plot generated using WUnF metrics for drinking water bacteria from different sampling sites and different phases.

mycetes (10%), Acidobacteria (10%), Firmicutes (7%), Bacterioidetes (7%), Chloroflexi (5%), and Nitrospirae (2%). At genera level, there were 22 genera accounted for percentage higher than 1%. These OTUs were mainly comprised of *Arthrobacteria* spp. (11%), *Acidobacterium* spp. (6%), *Rhodopirellula* spp. (4%), *Hyphomicrobium* spp. (4%), *Holophaga* spp. (4%), *Sporosarcina* spp. (4%), *Flavobacterium* spp. (3%) and *Frankia* spp. (3%).

In the suspended solids, 654 and 689 OTUs were detected at treatment plants and water main, that assigned to 11 and 14 phyla, respectively. The community compositions and structures of suspended particle-associated bacteria at pumping station and water main were similar, both of which were dominated by Proteobacteria (77% at pumping station, 82% at water main) and Bacteroidetes (11% at pumping station, 8% at water main). Among the sub-phylum, at treatment plant Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria were abundant and accounted for 18%, 49%, and 9%; the three sub-phyla were also abundant at water main that accounted for 28%, 27% and 24%. At genera level, there were 16 genera accounted for percentage higher than 1%. These OTUs were mainly comprised of *Methylotenera* spp. (13%-PS, 3%-Main), *Methylophilus* spp. (10%-PS, 1%-Main), *Methyloverstailis* spp. (9%-PS, 5%-Main), *Pseudomonas* spp. (2%-PS, 13%-Main), *Polaromonas* spp. (2%-PS, 6%-Main), *Brevundimonas* spp. (5%-PS, 5%-Main).

For the bacteria associated with loose deposits formed in water main, 620 OTUs were detected that assigned to 16 phyla. Similar to the bacteria associated with suspended particles at water main, the loose deposits bacteria were dominated by Proteobacteria (77%) and Bacteroidetes (12%). Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria accounted for 26%, 26% and 20% of the total OTUs. At genera level, there were 21 genera accounted for percentage higher than 1%. These OTUs were mainly comprised of *Sphingomonas* spp. (8%), *Pseudomonas* spp. (13%) and *Flavobacterium* spp. (6%).

3. Discussion

3.1. *Bacterial quantity changes*

The obtained ATP values from the present study were conformed with the previous yearly biological stability study in the same treatment plant and the downstream distribution area (Liu et al., 2013b). Though the produced water is characterized as biological stable according to AOC measurements (data not shown, < 10 μg C/L), microbial processes occurring everywhere in distribution system from the treatment to customer's tap. For bulk water, regardless of where did the water samples were taken in the distribution system, the ATP values were higher than that measured at the treatment plant, indicating the occurrence of microbial activity during distribution, which has been widely observed and reported worldwide (Hammes et al., 2010; Liu et al., 2013b; Nescerecka et al., 2014; Prest et al., 2014; Van Der Kooij 2000; Van der Wielen and Van der Kooij, 2010). Compared to our previous research in unchlorinated distribution system in the Netherlands (Liu et al., 2014) and other distribution studies worldwide (Liu et al., 2013c), the formed biofilm and the amount of loose deposits obtained in the present study were in the lower range. This may be due to the lower AOC concentrations and the low particle load in the treated water at pumping station (Liu et al., 2013b). In general, the sampling protocol used in the present study allowed a comparison of relative contributions from different phases in one-meter-long water main pipe. The finding that most of contributions from biofilm and loose deposits agreed with previous studies (Batté et al., 2003; Flemming et al., 2002; Liu et al., 2014, 2013a). It is noticed that little contribution ($< 2\%$) was found associated with loose deposits compared to our previous study in another Dutch distribution system (Liu et al., 2014). The observation is different from previous reported high contribution of loose deposits ($> 85\%$), which could be explained by the low amount loose deposits formed at the studied location (flushed samples turbidity of 3–8 NTU in the present study vs. 50–150 NTU for the previous study). The high contribution of biofilm may pose potential risk during turbulences, which could detach biofilm and cause particulate and biological matter peaks at customer tap, such as sudden changes in water quality and/or local hydraulics (Chen et al., 2020; Liu et al., 2017).

3.2. *Bacterial community changes*

Though ATP increased during drinking water distribution, the corresponding bacterial community remained similar with only minor differences (Fig. 5, PCoA), confirming that the distribution of biological stable water has minor effects bacterial community's structure and composition (Lautenschlager et al., 2013; Liu et al., 2014; Prest et al., 2014). This is especially true comparing the water samples at treatment plant and water samples taken from the sampling tap on water main. Whereas, larger dissimilarity was observed between water from the tap on the main and water from the tap in customer's house kitchen, indicating significant contribution of plumbing system to tap water microbes, which might be induced by the high surface-volume ration, high temperature and long stagnation time (Ling et al., 2018; Zlatanović et al., 2017). For the bacterial community of water taken from hydrant, it was a totally different cluster because of the turbulence created by opening the hydrant and the extra-long retention time of water in the dead-end hydrant. Beside, high similarities were also found between the bacteria associated with suspended solids at treatment plant and distribution water main, suggesting same stability of bacteria as observed in the planktonic bacteria in bulk water.

The bacterial community developed in distribution system within pipe biofilm and loose deposits were similar with each other, both of which were similar to the community of bacteria associated with suspended particles, but different from that of planktonic bacteria in bulk waters. This is conformed with our previous finding on the correlation and exchange of bacteria among different phases within distribution system (Liu et al., 2014). By including bulk water and suspended solids samples at treatment plant, the present study revealed that the suspended particle-associated bacteria in treated water leaves treatment plant might seed the growth of bacteria on pipe surface and in loose deposits. It should be mentioned that only one location was investigated in the present study and the supply water was biological stable with low particle and nutrients load, further studies covering multiple lo-

cations in different distribution systems (with and without disinfectant residuals) are recommended to draw solid conclusions.

3.3. *Influences of plumbing system on tap water quality*

Though only one location was prepared, to the best of our knowledge, this is the first study that prepared a sampling tap on the water main and had direct comparison of water quality in the distribution pipe and at the water tap in customer's kitchen. Comparing the results obtained from those two sampling points, it is clear that plumbing system gave major contribution to the microbiological water quality changes from both quantitative (ATP, Fig. 2a) and community perspectives (PCoA, Fig. 5). For example, such as the higher relative abundance of *Sphingomonas* spp. at tap (22%) than at water main (2%), while lower relative abundance of *Pseudomonas* spp. in tap water (15%) than water from street main (29%). For the present study, the samples were taken after sterilized and flushed the kitchen tap as widely used, which means more strict protocols might be needed to completely avoid the potential influences of plumbing system for drinking water distribution system research. It is essential to have right samples for comparison so as to properly study and understand the microbiological quality changes during drinking water distribution.

The situation could be worse for customer consumption than it is for research purpose, since there is normally neither sterilization nor flushing procedure before the water is taken. Regarding the consumers, there are 283 million people in U.S. (Salehi et al., 2020) and 518 million people in China (Statistics, 2019) receive drinking water from a public water supply system, and although not surveyed, it could be assumed that the water passes through building plumbing. The water quality deterioration caused by plumbing system for the huge population is well recognized, in extreme cases there could be aesthetics and public health risks (Julien et al., 2020; Rhoads et al., 2016). Recently, the widespread adoption of innovative water conservation strategies, green buildings, and sustainable water infrastructures were found to favor the increase of water age and levels of pathogen genetic markers, and the decrease of water chemistry and microbiology (e.g. absent of disinfectant residuals) (Salehi et al., 2020). For example, in a net-zero energy house, where a solar "pre-hear" water tank was installed, the water retention time increased from 1 day to 2.7 days (Rhoads et al., 2016). This means the balance between water quality and green infrastructure should be considered before the innovative construction approaches to be implemented. Regarding customer water consumption, special attention and proper guidelines should be given to customers water consumption, especially for the big buildings and after holidays and/or lockdowns because of pandemic or other reasons (Proctor et al., 2020; Viglione, 2020). To manage the water quality deterioration associated with plumbing system, some actions have been proposed, including possible probiotic approach (Wang et al., 2013), produce and supply biological stable water, use biologicals table plumbing material and minimizing the water age by building design.

4. Conclusion

Though only one location was prepared and studied, the present study showed that the sampling protocol of making sampling tap on water main offered directly evidences about the impacts of plumbing system on tap water quality, which makes it possible to distinguish and study the processes in distribution system and plumbing system separately. The water quality deterioration should be considered from both research and customer's consumption perspectives. The following conclusions could be drawn:

- The distribution of biological stable water has minor effects on the microbiocidal water quality regarding both ATP (1 ng/L vs. 1.7 ng/L) and the bacterial community (Fig. 5, PCoA).
- The plumbing system has significant contribution to the increase of active biomass (1.7 ng/L vs. 2.9 ng/L) and changes of bacterial community composition and structure (Fig. 5, PCoA). The relative abundance of *Sphingomonas* spp. at tap (22%) was higher than that at water main (2%), while the relative abundance of *Pseudomonas* spp. in tap water (15%) was lower than that in the water from street main (29%).
- In distribution pipes, biofilm contributed > 94% of the total biomass, while loose deposits showed little contribution (< 2%) because of the low amount of loose deposits.
- It is the suspended particle associated bacteria leaves treatment plant rather than planktonic bacteria seeded the bacterial growth in biofilm and loose deposits.
- The plumbing system should be better studied and managed to ensure the tap water quality for consumers, especially after extended stagnation due to holidays or lockdowns.

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