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Impacts of water treatments on bacterial communities of biofilm and loose deposits in drinking water distribution systems

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ABSTRACT

Treated drinking water is delivered to customers through drinking water distribution systems (DWDSs). Although studies have focused on exploring the microbial ecology of DWDSs, knowledge about the effects of different water treatments on the bacterial community of biofilm and loose deposits in DWDS is limited. This study assessed the effects of additional treatments on the bacterial communities developed in 10 months' old pilot DWDSs. The results showed a similar bacterial community in the pipe-wall biofilm, which was dominated by *Novosphingobium* spp. (20–82 %) and *Sphingomonas* spp. (11–53 %), regardless of the treatment applied. The bacterial communities that were retained in the distribution systems (including pipe-wall biofilm and loose deposits) were similar to the particle-associated bacteria (PAB) in the corresponding supply water. The additional treatments showed clear effects of the removal and/or introduction of particles. The genera *Aeromonas* spp., *Clostridium* spp., *Legionella* spp., and *Pseudomonas* spp., which contain opportunistic pathogenic species, were only detected among the PAB in ion exchange system. Our study demonstrated that the biofilm community is consistent across treatments, and the contribution from bacteria in loose deposits is important but can be controlled by removing particles. These findings offer more insight into the origin and development of microbial ecology in DWDSs and suggest paths for further research on the possibility of managing the microbial ecology in distribution systems.

1. Introduction

To develop effective control strategies that will ensure biological high-quality and bio-safe drinking water at customers' taps, it is necessary to understand the microbial ecology of drinking water distributions systems (DWDS) (n, Proctor and Hammes, 2015). Over last decades, it was believed that most of total microbial cells (>95 %) in DWDS are present in pipe-wall biofilm (Flemming, Percival et al., 2002) and studies have primarily focused on the pipe-wall biofilm (Batté et al., 2003; Chaves Simões and Simões, 2013; LeChevallier et al., 1987; Ren et al., 2024; Yao et al., 2023), such as the influence of disinfection strategies (Mathieu, Bouteleux et al., 2009, Hwang, Ling et al., 2012), nutrient levels (Van der Kooij, 1992, Juhna, Birzniece et al., 2007, Gouider, Bouzid et al., 2009), pipe materials (Hyun-Jung, Choi et al., 2011, Wang, Masters et al., 2014), and hydraulic conditions (Lehtola, Laxander et al., 2006, Douterelo, Sharpe et al., 2013, Mathieu, Bertrand et al., 2014).

Besides pipe-wall biofilm, there are multiple phases present in DWDS, all of which are important for its microbial ecology: bulk water flowing through the pipes, suspended solids (SS) that are particulate matter suspended and transported throughout the network, and loose deposits (LD) that are particulate matter accumulated/settled in the pipe

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(Hwang, Ling et al., 2012, Chaves Simões and Simões, 2013, Liu, Verberk et al., 2013, Liu, Bakker et al., 2014, Liu, Zhang et al., 2017). Among all phases, planktonic phase is arguably most relevant to the consumers (Proctor and Hammes, 2015). Biofilm and loose deposits are reservoirs for bacteria, which can be released into water, have higher resistance to disinfectant residuals (if applicable), and contain a number of micro-environments (e.g., anoxic and even anaerobic micro-environments) that promote the growth of diverse bacterial populations (Liu, Bakker et al., 2014, Liu, Tao et al., 2017). The integral study of DWDS microbiology of 110 mm PVC pipes in Dutch distribution system revealed that planktonic phase represents less than 2 %, pipe-wall biofilm accounted for 20–60 %, and the loose deposits harboured up to 80 % of bacteria in DWDS (Liu, Bakker et al., 2014, Proctor and Hammes, 2015, Liu, Tao et al., 2017), among which hygienically relevant microbes were detected, such as *Mycobacteria* (Torvinen, Suomalainen et al., 2004). It is clear that loose deposits in distribution system should be included in the assessments of DWDS microbial ecology (Chen et al., 2023).

Drinking water treatment is the main barrier to guarantee the bio-quality and bio-safety of drinking water at customers' taps. Yet, treated drinking water entering distribution system contains planktonic bacteria (PB), particle-associated bacteria (PAB) and nutrients (Liu, Verberk et al., 2013). Knowledge has been obtained on the effects of treatment processes on the bacterial community of bulk water bacteria in distribution systems (Pinto, Xi et al., 2012), the bacterial community of pipe-wall biofilm in pilot and full scale distribution systems (Shaw, Monis et al., 2014, Wu, Zhang et al., 2015). Studies have shown that the treatments which can remove particles can limit the amount of loose deposits in the followed distribution systems (Vreeburg, Schippers et al., 2008, Liu, Lut et al., 2013). However, it remains unknown whether or not the treatments play a role in shaping bacterial communities in biofilm and loose deposits.

The objective of this study is to assess the effects of additional water treatments on the bacterial communities of biofilm and loose deposits developed in pilot DWDSs. Planktonic bacteria (PB), particle-associated bacteria (PAB), and organic compounds were selectively removed by applying additional treatments, i.e., ion exchange (IEX) to reduce organic compound concentrations, ultrafiltration (UF) to reduce PB and PAB, and nanofiltration (NF) to reduce both organic compounds and PB/PAB.

2. Materials and methods

2.1. Drinking water production

This study was conducted at a drinking water production plant of the Oasen Water Company, the Netherlands. The treatment plant uses well-confined, anoxic groundwater as its source water. After abstraction, the water was treated by aeration, filtration, softening, carry-over sand filtration, activated carbon filtration, and UV disinfection. The finish water produced by this treatment process is hereinafter called "feed water".

2.2. Design of the study and selection of additional treatments

The feed water was further treated to achieve nutrients and/or bacterial changes in the water (Fig. 1). For short, ion exchange (IEX, Purolite® A860 resin) was used to reduce the concentration of organic compounds. Ultrafiltration (UF, S1.5 MB 2.0 membrane, pore size 0.02 μm; Dizzer) was used to remove particles and cells. Nanofiltration (NF membrane, NP90-2540; FILMTEC) was applied to reduce both nutrients and particles and cells. Our previous work reported the performance of the selected treatments: IEX efficiently removed DOC, UF produced particle-free water that has the same level of DOC as feed water, and NF produced water with low nutrient content and no particles over the study period of 10 months (Liu, Lut et al., 2013).

The water without additional treatment ("feed water"), the IEX-treated water ("IEX water"), the UF permeate ("UF water"), and the NF permeate ("NF water") were the waters prepared and supplied to the downstream pilot distribution systems (PDSs) to study the bacterial communities of biofilm and loose deposits developed in the corresponding PDSs.

2.3. Pilot distribution systems (PDSs)

Four PDSs were built using 400 m (8 × 50 m) of PE tubing (inner diameter 6 mm, product code PLN; Festo). The nutrient (DOC and assimilable organic carbon) release potential of the tubing was tested

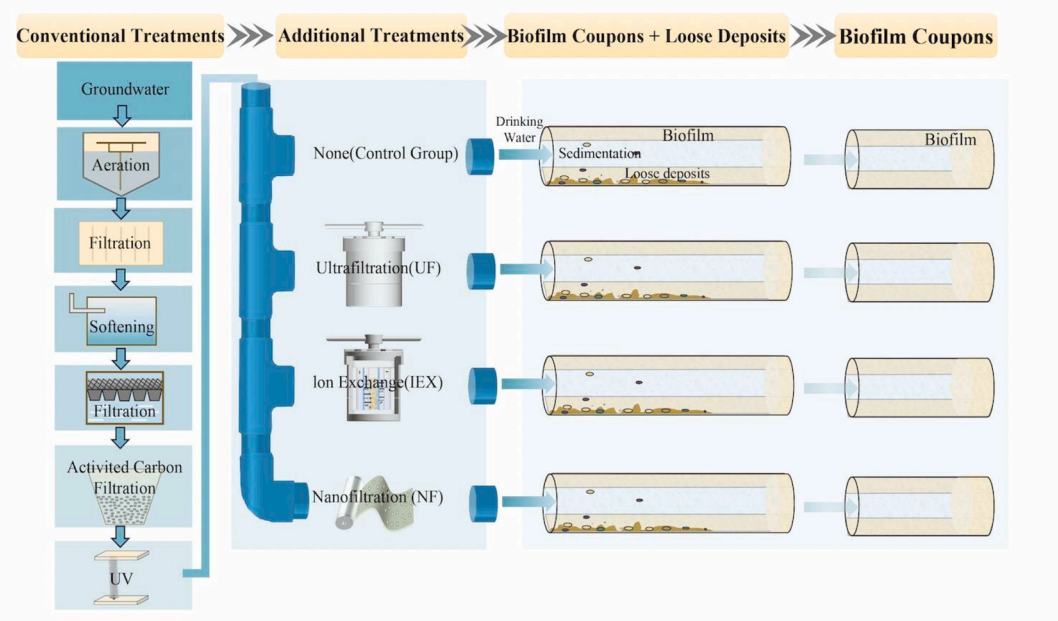

Fig. 1. The layout of the study set-up: the correlation and influences of additional treatments on the potential risks associated with the mobilized bacteria reservoir of loose deposits and biofilm in DWDS are shown in the figure.

before it was selected for use. The results showed that the amount of nutrient released was very low (the methods and results are provided in Fig. S1). The residence time in the PDS was 24 h. The feed flow was 0.5 l/h, and the water flow was laminar. A stable pressure was used to feed the PDS. At the end of the PDS, removable coupons made from the same PE tubing were installed for the biofilm sampling (the details are described in Fig. S2). Each coupon contained two pieces of 25 cm tubing (for duplicate measurements). The systems were flushed with sterilized demineralized water before the study was started. No disinfectant was applied prior to or during the study, as per Dutch drinking water practice. The pilot system was located in the basement of the groundwater pumping station. The water temperature was stable (~12 °C).

2.4. Sampling, samples preparation, and pretreatment

Water and suspended solids. Planktonic bacteria were sampled by taking 500 mL water samples. Bacteria on suspended particles were sampled and pretreated as previously described (Liu, Ling et al., 2013), i.e., by filtering 150–200 L of water through 1.2-μm glass fiber filters. The samples were detached and eluted from the filters by ultrasonication at 43 kHz, three times for 2 min. The suspensions obtained were used for further DNA extraction and pyrosequencing.

Before the water entered the PDS, bulk water planktonic bacteria (PB) and suspended particle-associated bacteria (PAB) were collected on three occasions: at the start, half-way, and at the end of the 288-day research period. During the 24th week (6th month), duplicate biofilm samples were taken for DNA extraction. The UPGMA tree revealed the similarity and stability of PAB and PB feeding the PDSs over the study period (Fig. S3), and confirmed the reliability of the pilot distribution system and reproducibility of the results (Fig. S4).

Pipe-wall biofilm. At the conclusion of this study (day 288), biofilm samples were taken from the coupons installed at the end of the PDS. After the water traveled through 400 m of tubing at a very low velocity, particles settled at the proximal part of the PDS. Visual inspection confirmed that the biofilm collectors at the distal part did not capture any loose deposits, the detail on visual inspection is given in Fig. S2.

The coupons with contained biofilm were ultrasonicated at 43 kHz, three times for 2 min. The obtained suspensions were collected for DNA extraction and pyrosequencing. The biofilms formed in PDSs supplied with feed water, IEX water, UF water and NF water are hereinafter called Feed-BF, IEX-BF, UF-BF and NF-BF.

Biofilm plus loose deposits. At day 288, an autopsy study was conducted on all of the 400 m of the PE tubing in each PDS. Samples of the mixture of biofilm plus loose deposits were obtained. The 400 m of tubing consisted of 8 pieces, each measuring 50 m in length. Every 50 m, the tubing was closed by Festo valves for pretreatment. For the 50-meter tubing, a pump was used each time to empty the water from the tubing and to refill it with autoclave-sterilized ATP-free water. The same procedure of biofilm coupons pretreatment was followed, the 8 times suspensions obtained from each PDSs were mixed for DNA extraction and pyrosequencing. The mixture of biofilms and loose deposits formed in PDSs supplied with feed water, IEX water, UF water and NF water are hereinafter called Feed-D, IEX-D, UF-D and NF-D.

2.5. DNA extraction and sequencing

DNA was extracted from the bulk water samples, pretreated suspensions of suspended particle-associated bacteria, the pipe-wall biofilm, and the biofilm plus loose deposits (obtained as described above), using FastDNA Spin Kits for Soil (Q-Biogene/MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions (Hwang, Ling et al., 2011, Tamaki, Wright et al., 2011). The DNA was amplified with forward primer U515F (5′-Fusion A-Barcode-CA linker-GTGYCAGCMGCCGCGGTA-3′, which covers 92.66 % of bacteria and 93.54 % archaea), and the reverse primer U1052R (5′-Fusion B-TC linker-TGCATGGYYGYCGYCAGYTC-3′, which covers 95.10 % bacteria, 90.95 % archaea) (Wang and Qian, 2009). Pyrosequencing with titanium bulk sequencing methods (Roche, Branford, CT) was performed using 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. The obtained DNA sequences were deposited in the DDBJ sequence read archive (Accession Number: DRA002415).

2.6. Pyrosequencing data analysis

The sequences generated from the pyrosequencing 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, Kuczynski et al., 2010). The process consisted of quality checking, denoising, and a microbial diversity analysis. In short, the flow diagrams were denoised, and the UCLUST algorithm was used to assign operational taxonomic units (OTUs). 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. The sequences were then aligned using the Python Nearest Alignment Space Termination Tool (PyNAST) alignment algorithm. Network analysis was performed and visualized by Cytoscape (V3.2.1). Unweighted UniFrac distance matrices were constructed from the phylogenetic tree (built by a FastTree algorithm) and used to conduct a principal coordinate analysis (PCoA) (Liu, Bakker et al., 2014).

3. Results

3.1. General information regarding bacterial communities

Diverse bacteria were detected in the unchlorinated water and the downstream pilot distribution systems. In total, 92,683 16S rRNA pyrosequences were obtained from the 12 samples and further separated into 890 OTUs, based on a similarity cutoff of 97 %. The sequences obtained were assigned to 11 phyla. In general, *Proteobacteria* was the most abundant phylum and accounted for 85–99 % of the total OTUs across all samples. Within *Proteobacteria*, *Alphaproteobacteria* was the most dominant (75–98 %), followed by *Betaproteobacteria* (0–3 %), *Deltaproteobacteria* (0–3 %), and *Gammaproteobacteria* (0.1–10 %). At the genus level, the detected OTUs were mainly composed of *Sphingomonas* spp. (11–84 %), *Sphingopyxis* spp. (0.2–63 %), *Novosphingobium* spp. (0.5–82 %), and *Afipia* spp. (0–14 %). The detail of phyla and genera detected from all the samples are given in Fig. SX and Table SX.

3.2. Comparison of bacterial diversity

The diversity and complexity of the bacterial communities are assessed by the number of OTUs (Fig. 2A), Chao1 (Fig. 2B) and phylogenetic diversity values (PD, Fig. 2C). Results show that the planktonic bacteria had the lowest OTU number and diversity. The OTU number and diversity of bacteria associated with suspended solids were much higher than planktonic bacteria, which was comparable with biofilm. For PDSs supplied with membrane filtrated water, the OTU number and diversity of biofilm without and with loose deposits are comparable.

The highest OTU number and diversity were observed when the bacteria harbored by loose deposits were taken into account for the systems supplied with feed water and IEX water, indicating diverse and complex bacterial community of loose deposits.

3.3. Comparison of bacterial community composition

The bacterial community composition was compared by calculating UniFrac distance metric coupled with principal coordinates analysis

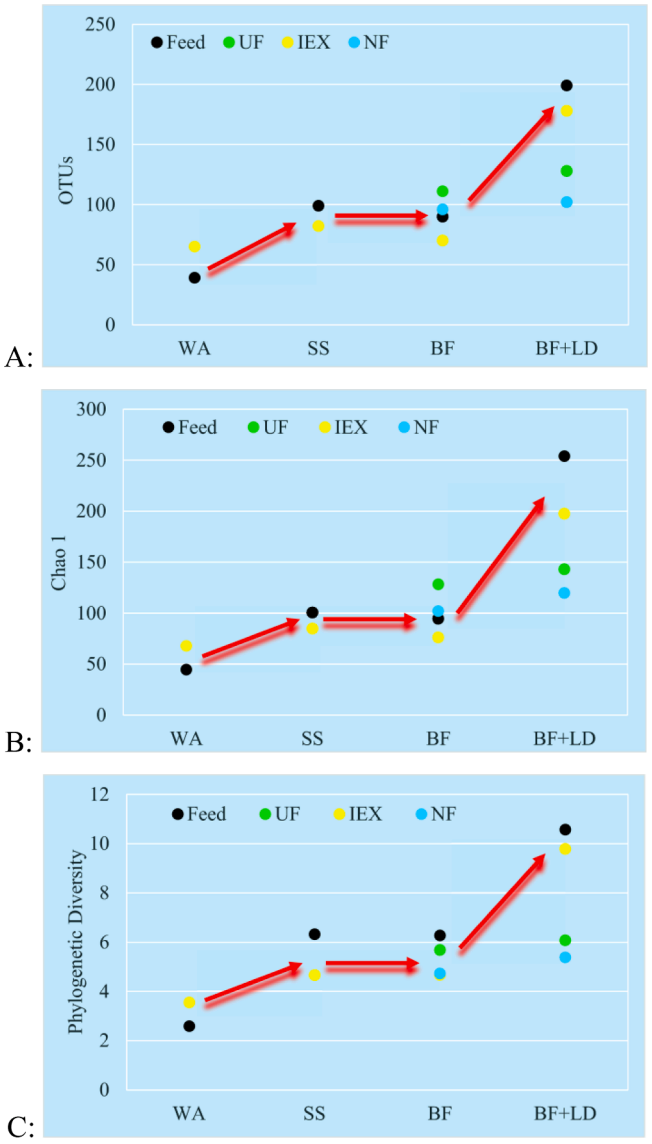

Fig. 2. A) Number of OTUs detected in different phases; B) Chao 1 index; C) Phylogenetic diversity (PD) values. All the parameters were generated and calculated by Qiime alpha diversity script. (Different from counting the OUT tables).

(PCoA, Fig. 3). Results showed that the Feed-WA and IEX-WA clustered together, illustrating that additional treatment by IEX had minor effects on the community of planktonic bacteria (not significant, $P = 0.13$). On the contrary, the differences between Feed-SS and IEX-SS demonstrating IEX had significant contribution ($P < 0.05$) on shaping the community of bacterial associated with suspended solids which is because of the release of IEX resins with associated bacteria.

Interestingly, the biofilm bacterial community compositions are not significantly different from one another, except for NF-BF and Feed-BF ($P < 0.05$), indicating the simultaneously and efficiently removing of organic compounds and cells by NF significantly changed the biofilm community. The differences between NF-BF and NF-D ($P = 0.11$), UF-BF and UF-D ($P = 0.05$) are not significant.

Further, the integral bacterial community that taking the contribution from loose deposits into account were different from biofilm. IEX-D are similar to IEX-SS ($P = 0.4$), and Feed-D is similar to Feed-SS ($P = 0.2$), indicating the bacteria associated with suspended particles in the supply water contribute significantly to the integral bacterial communities formed in the downstream distribution system, and the IEX-D were similar to the bacterial community of the bacteria associated with suspended particles in the corresponding supply water. The results showed that the bacterial communities of the biofilm from different PDSs were similar (Fig. 4), indicating that the treatments that removed particles/PAB, nutrients (measured as DOC), and planktonic bacteria (PB) from the feed water played a minor role in shaping the biofilm bacterial community. This finding suggested that the biofilm community was governed by processes in the network rather than the treatments applied. Previous studies have found that bacterial communities of biofilms were influenced by available nutrients, such as organic carbon.

3.4. Key OTUs/Taxa highlighted by network analysis

The specific bacteria OTUs within each sample were presented in the network figure by their sharing degree (the number of samples that detected the OTU), and the number of sequences detected in the samples that belongs a given OTU (Fig. 4, degree > 2 OTUs were shown). The OTUs with high abundances and present in multiple phases were highlighted in the center of Fig. 4. A full list of OTU number and its assigned genus shown in Fig. 4 is given in the Table 1.

Results showed that the OTUs presented in all 12 samples were OTU-#97 (degree = 12; 1871 sequences; assigned to *Novosphingobium* spp.), OTU-#101 (degree = 12; 2495 sequences; assigned to *Sphingopyxis* spp.), OTU-#624 (degree = 12; 6079 sequences; assigned to *Sphingobium* spp.) and OTU-#1042 (degree = 12; 2385 sequences; assigned to family of *Sphingomonadaceae*, unknown genera). These all samples/phases shared OTUs should not be all originated from supply water, because the treatments of membrane has filtrated out the OTUs in the supply water.

The most abundant OTUs were OTU-#296 accounted for the highest number of detected sequences (30,000 sequences, degree = 9, assigned to *Sphingomonas* spp.) and OTU-#224 (10163 sequences, degree = 11, assigned to *Novosphingobium* spp.).

Remarkably, the network analysis showed that the OTUs that assigned to genera containing opportunistic pathogenic pieces (e.g. OUT-#775 *Clostridium* spp. and OUT-#335 *Mycobacterium* spp.) were only detected in IEX-D was originated from IEX-SS (can be traced to the IEX resins used).

4. Discussion

In natural water systems, the availability of nutrients has been reported to be a major driver of bacterial richness (OTUs). In fresh water systems, it is confirmed by a study that covered 14 oligotrophic lakes (TOC 2.0–10.0 mg/L, 664–1677 OTUs were detected) (Hewson, Vargo et al., 2003, Logue, Langenheder et al., 2011). Our observation agrees with the general trend: drinking water contains a very low nutrient level (extreme oligotrophic compared to lake water, 0.2–1.5 mg DOC L^{-1}), and fewer OTUs were detected (Fig. 2, 40-200 OTUs).

Despite the vast diversity of bacterial groups in DWDSs, consistent with the present study, the dominance of *Proteobacteria* at the phylum level has been widely reported previously (Kalmbach, Manz et al., 1997, Schwartz, Hoffmann et al., 1998, Schmeisser, Stöckigt et al., 2003, Williams, Domingo et al., 2004, Williams, Santo Domingo et al., 2005, Eichler, Christen et al., 2006, Douterelo, Husband et al., 2014, Shaw, Monis et al., 2014). More specifically, the dominance of *Alphaproteobacteria* and *Sphingomonas* spp. at the class and genus level was agreed with our previous studies of multiple phases in Dutch unchlorinated drinking water treatment plants and the followed distribution systems (Liu, Ling et al., 2013, Liu, Bakker et al., 2014).

4.1. Influence of additional treatments on biofilm bacterial community

4.1.1. Biofilm diversity

However, within these extremely oligotrophic drinking water environments, the trend was not observed for biofilm bacteria. Though this appears to be contradictory, the loose deposits bacteria were excluded.

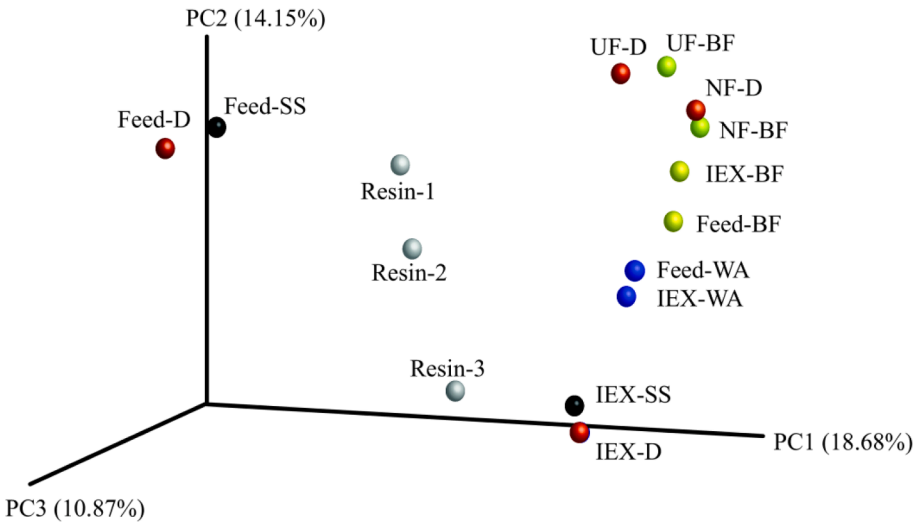

Fig. 3. The bacterial community composition similarity among water, biofilm and loose deposits. PCoA plot of all samples based on weighted UniFrac metrics (all samples).

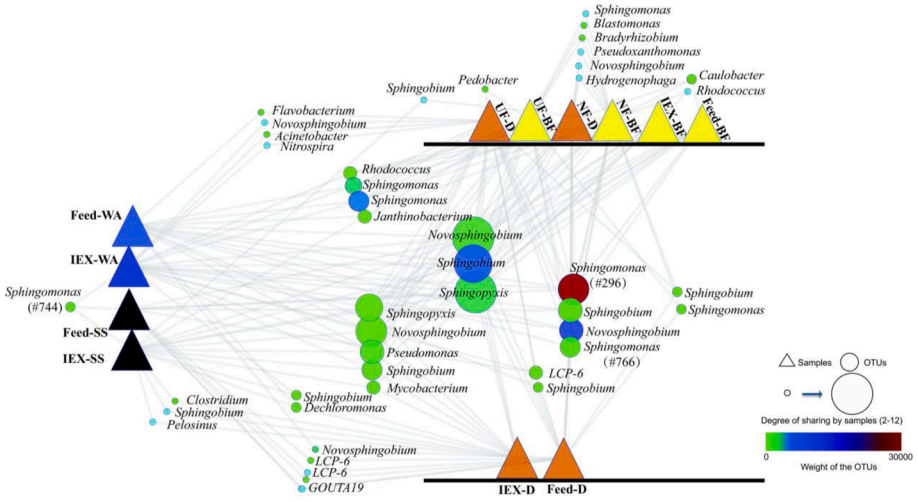

Fig. 4. Network figure of the specific bacteria OTUs within each sample and the number of sequences detected in the samples that belongs a given OTU. Nodes indicating OTUs (degree > 2) are colored by their weights, and the size is proportional to degree.

Table 1
The OTU number and its assigned genus and the degree and E-weight for each out.

OTUs	Assigned Taxa (genera)	Degrees	E-weight/Sequences
#97	*Novosphingobium* spp.	12	1871
#101	*Sphingopyxis* spp.	12	2495
#624	*Sphingobium* spp.	12	6079
#1042	*Sphingomonadaceae,* unknown genus	12	2835
#224	*Novosphingobium* spp.	11	**10,163**
#296	*Sphingomonas* spp.	9	**30,010**
#766	*Sphingomonas* spp.	7	5507
#335	*Mycobacterium* spp.	7	807
#460	*Pseudomonas* spp.	7	1428
#593	*Comamonadaceae,* unknown genus	7	2397
#846	*Syntrophobacteraceae,* unknown genus	7	5199
#977	*Sphingomonas* spp.	6	1048
#970	*Sphingomonas* spp.	5	3124
#369	*Novosphingobium* spp.	5	2460
#1088	*Sphingomonadaceae,* unknown genus	4	2729
#524	*Sphingomonadaceae,* unknown genus	4	1014
#775	*Clostridium* spp.	2	207

Regarding the biofilm formation during water distribution, the removal of DOC effectively controlled the amount of biofilm formed in the PDS; specifically, the highest concentrations were found after UF treatment, followed by IEX, and then by NF (Table 1) (Liu, Lut et al., 2013). Observations from a microbial ecology perspective were different. Our results showed that the bacterial communities of the biofilm from different PDSs were similar to one another (Fig. 4), which is consistent with previous study of biofilms formed in distribution system supplied by water produced by different treatments (Shaw, Monis et al., 2014). In contrast, Wu et al., found that different drinking water purification strategies could result in different biofilm communities (Wu, Zhang et al., 2015). It is noticed that similar biofilm communities were found in the systems without disinfectant residual and oxidation processes. The treatments investigated by Wu et al., involved chlorination in both cases, and a two-stage ozonation in one of the treatment lines. It is likely that the differences observed by Wu et al., are caused by the different disinfection (ozonation) process, because it is well known that disinfection has clearly influence on biofilm bacterial communities.

The similar biofilms formed in PDSs regardless the treatments applied indicating that water treatments played a minor role in shaping the biofilm bacterial community. This finding suggested that the biofilm

community was governed by processes in the network rather than the treatments applied. Previous studies have found that bacterial communities of biofilms were influenced by available nutrients, such as organic carbon (Karthikeyan, Korber et al., 2001, Pang and Liu, 2006) and phosphates (Keinänen, Korhonen et al., 2002). This led us to hypothesize that the similar biofilm in different PDSs could have been the result of nutrients released from the tubing material (Yu, Kim et al., 2010, Hyun-Jung, Choi et al., 2011, Wang, Masters et al., 2014). However, the concentration of released nutrients is very low (Fig. S1), and the amount of biofilm formed on the same material is different which correlated very well with the organic carbon concentrations in the treated water (Table 1). It is hypothesized that the nutrients released from the tubing material can be the determine factor on the initial community of biofilm, while the nutrients level in the supply water is the limit factor on the quantity of biofilm.

Another possible explanation for the similarity in the communities observed after the different types of treatment relates to the development stage of the biofilms concerned. The duration of the present study was too short (initial phase) to allow for the development of biofilm (Shaw, Monis et al., 2014), compared to the case of field distribution systems, which typically involve durations of decades (Martiny, Jørgensen et al., 2003, Liu, Bakker et al., 2014). Bacterial community composition and structure are subject to change over time (Martiny, Jørgensen et al., 2003, Henne, Kahlisch et al., 2012). It has been reported that in the initial phase (i.e., the period relevant to this study), bacteria (pioneer colonizers) are selected by their ability to adhere to the surface rather than by the water's nutrient content (Bos, Mei et al., 1999, Pang and Liu, 2006). As pertains to the present study, *Sphingomonas* spp. as the biofilm dominated genus can irreversibly attach to surfaces by producing exopolysaccharides around the cells to form biofilm (White, Suttont et al., 1996, Busse, Kämpfer et al., 1999, Azeredo and Oliveira, 2000, Vuoriranta, Männistö et al., 2003, Bereschenko, Stams et al., 2010).

4.2. Influence of additional treatments on the integral bacterial community

Compared to biofilm, our knowledge about the bacteria associated with loose deposits is limited. To the best of our knowledge, the present study is the first to examine the effect of treatment processes on the integral DWDS microbial ecology that includes the contributions of loose deposits. In the present study, although the dominance of *Proteobacteria* (more specifically, *Alphaproteobacteria*) is consistent with our previous study in field distribution systems, the percentage is much higher (39 % versus 75 %) (Liu, Bakker et al., 2014). At the genus level, the dominance of *Sphingomonas* spp. and *Sphingopyxis* spp. is consistent with the previous study, but substantially fewer genera > 1 % were detected (19 in the previous study versus 6 in the present study). This difference may be due to the fact that the loose deposits in the field distribution system can be generated from other sources than treatment processes – e.g., pipe corrosion or pipe bursts – or be the result of pipes that are tens of years old.

Our results showed that the additional treatments had clear effects on the bacterial community of the biofilm plus loose deposits. Because the biofilms formed were similar among all PDSs (as discussed in the previous section), any evident differences suggested an important contribution of bacteria from loose deposits. It is not surprising that no loose deposits and the associated bacteria were found in the PDS supplied with particle-free water produced by membrane filtration (Vreeburg, Schippers et al., 2008, Liu, Lut et al., 2013). Compared with the feed water, the IEX treatment showed a clear influence on the DWDS bacterial community when loose deposits were considered. This observation indicated the influence of IEX on governing the bacteria in loose deposits. Because its minor influence on the PB community (Fig. 4, IEX-WA versus Feed-WA), the observed contribution was attributed to either the impact on the suspended PAB or the selective removal of organic compounds. The former was confirmed on the basis of the observed similarity between the community of PAB in the IEX water and the community of collected PDS bacteria (biofilm plus loose deposits). This conclusion is reasonable because the IEX resin beds provide favorable growth media for bacteria (Flemming, 1987), which may lead to the microbial fouling of the beds (LAL, 2001). The released resin and the associated bacteria introduced into IEX water (Fig. 4, IEX-SS) became the seeds/origin for the bacteria in loose deposits (Fig. 4, IEX-D). This process can be proved by our previous field distribution study, in which it is concluded that the loose deposits originated from the sedimentation of suspended particles (Liu, Bakker et al., 2014). During this developmental phase (i.e., a period of less than a year), the sedimentation process is dominant. After an aging process (over decades), during which an EPS layer is built up, different groups may play the role of the loose deposit bacterial community because of the availability of multiple micro-environments inside loose deposits.

In addition, more bacterial genera were detected when loose deposits were included. Remarkably, the genera *Aeromonas* spp., *Clostridium* spp., *Legionella* spp., and *Pseudomonas* spp., which contain opportunistic pathogens, were only detected in loose deposits. These findings further confirmed that although the PAB accounted for less than 3 % of the drinking water bacteria in drinking water treatment plants (Liu, Ling et al., 2013) and distribution systems (Liu, Bakker et al., 2014), it is still considered to be a potential microbial safety issue (Liu, Ling et al., 2013, Liu, Lut et al., 2013). Bacteria such as *Aeromonas* spp., *Caulobacter* spp., *Legionella* spp., and *Pseudomonas* spp. were only detected in the PAB in the IEX system indicating the IEX resins introduced bacteria while removing nutrients from water, and provides evidence that drinking water production material should be pre-disinfected.

4.3. Insights into the origin and development of microbial ecology in DWDSs

Limited knowledge is available on how the type and concentration of biomass in the supply water influencing the microbial ecology of DWDSs. Insights into the development of DWDS microbial ecology can be obtained by comparing the PB and PAB in the supply water and developed microbial ecology in PDSs. The similarity of PB and the biofilm bacterial communities in the feed water and IEX systems (Fig. 4, PCoA) showed that the biofilm bacterial community probably originated from PB that had attached and proliferated in the supply water. However, similar biofilms also formed in UF-water and NF-water supplied PDSs (without PB sources), which suggested that the pipe biofilm was governed by processes in the distribution system. This finding agrees with the previous findings that removing only the cells, but not the nutrients (e.g., by ultrafiltration), cannot regulate biofilm formation (Okabe, Kokazi et al., 2002, Liu, Lut et al., 2013). Previous studies that supplied same water to pipes of different material has observed different communities in the formed biofilm (Yu, Kim et al., 2010, Hyun-Jung, Choi et al., 2011). Combing the observations with our results that supplied different waters to same pipe material, it lead us to the hypothesis that the pipe material rather than the supply water is the determine factor of the bacterial community of biofilm.

The bacteria collected from the PDSs (pipe-wall biofilm plus loose deposits) were similar to the PAB in the corresponding supply water but different from the PB. This finding indicated that the bacteria from the loose deposits had a greater influence on the integral bacteria in the PDS than pipe-wall biofilm. It has been hypothesized in previous studies that the bacteria associated with loose deposits originated from the PAB in the supply water (Gauthier, Portal et al., 2001, Vreeburg and Boxall, 2007, Liu, Ling et al., 2013, Liu, Verberk et al., 2013, Liu, Bakker et al., 2014). The present study is the first to provide such evidence.

From the management perspective, water utilities could select proper pipe material to possibly manage the biofilm community, reduce the nutrients level to regulate the amount of biofilm, and remove particles/PAB entering distribution system to limit loose deposits and the

harbored bacteria accumulation.

The results from this comparison study of the three additional treatment processes offer valuable information to facilitate the design of specific treatment processes, and/or the selection of treatment processes to upgrade current treatment plants. It is evident that after passing through ultrafiltration and nanofiltration, PAB was removed from the feed water, whereas after passing through the IEX system, PAB was introduced instead. On the other hand, IEX system had good efficiency in limiting both growths in loose deposits and in biofilm. Consequently, the effects of PAB on biofilms and microbial communities in the bulk water need to be considered when designing treatment processes for DWTPs.

5. Conclusion

This study highlights the importance of including loose deposits in exploring the microbial ecology in DWDS. Although the study period involved in this study – just under one year – may only be relevant for the starting phase, the results provided insight for a better understanding of DWDS microbial ecology. The main findings are:

- In the extra low nutrients drinking water, the lower the nutrients concentration, the more detected OTU number;
- The pathogenic bacterial taxa were only detected among the particle associated bacteria;
- The pipe-wall biofilm is hardly influenced by additional treatments and governed by processes in the distribution system;
- The loose deposits have clear contribution to the distribution system microbial ecology, and it is important to be included in the DWDS microbial ecology study;
- The integral bacteria retained in the PDS (pipe-wall biofilm plus loose deposits) were similar to and governed by the PAB in the corresponding supply water.

CRediT authorship contribution statement

Yue Zhang: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Xiaoming Li:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Anran Ren:** Software, Investigation. **Mingchen Yao:** Methodology, Investigation. **Chen Chen:** Resources. **Haichen Zhang:** Project administration, Resources. **Walter van der Meer:** . **Gang Liu:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Azeredo, J., Oliveira, R., 2000. The role of exopolymers in the attachment of Sphingomonas paucimobilis. Biofouling 16 (1), 59–67.
Batté, M., Appenzeller, B.M.R., Grandjean, D., Fass, S., Gauthier, V., Jorand, F., Mathieu, L., Boualam, M., Saby, S., Block, J.C., 2003. Biofilms in Drinking Water Distribution Systems. Rev. Environ. Sci. Biotechnol. 2 (2), 147–168.
Bereschenko, L.A., Stams, A.J.M., Euverink, G.J.W., Van Loosdrecht, M.C.M., 2010. Biofilm formation on reverse osmosis membranes is initiated and dominated by Sphingomonas spp. Appl. Environ. Microbiol. 76 (8), 2623–2632.
Bos, R., Mei, H.C., Busscher, H.J., 1999. Physico-chemistry of initial microbial adhesive interactions–its mechanisms and methods for study. FEMS Microbiol. Rev. 23 (2), 179–230.
Busse, H.J., Kämpfer, P., Denner, E.B.M., 1999. Chemotaxonomic characterisation of Sphingomonas. J. Ind. Microbiol. Biotechnol. 23 (4–5), 242–251.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pẽa, 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. Methods 7 (5), 335–336.
Chaves Simões, L., Simões, M., 2013. Biofilms in drinking water: Problems and solutions. RSC Adv. 3 (8), 2520–2533.
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.
Chen, Lihua, Li, Xuan, Medema, Gertjan, Walter van der Meer, Liu*, Gang, 2023. Transition effects in an unchlorinated drinking water system following the introduction of partial reverse osmosis. Nature Water 1, 961–970.
Douterelo, I., Husband, S., Boxall, J., 2014. "The Bacteriological Composition of Biomass Recovered by Flushing an Operational Drinking Water Distribution System.". Water Research 54, 100–114.
Eichler, S., Christen, R., Höltje, C., Westphal, P., Bötel, J., Brettar, I., Mehling, A., Höfle, M.G., 2006. Composition and dynamics of bacterial communities of a drinking water supply system as assessed by RNA- and DNA-based 16S rRNA gene fingerprinting. Appl. Environ. Microbiol. 72 (3), 1858–1872.
Flemming, H.-C., 1987. Microbial growth on ion exchangers. Water Res. 21 (7), 745–756.
Flemming, H. C., S. L. Percival and J. T. Walker (2002). "Contamination potential of biofilms in water distribution systems." water Science and Technology 2: 271-280.
Gauthier, V., Portal, J.M., Yvon, J., Rosin, C., Block, J.C., Lahoussine, V., Benabdallah, S., Cavard, J., Gatel, D., Fass, S., 2001. Characterization of Suspended Particles and Deposits in Drinking Water Reservoirs. 1, 89–94.
Gouider, M., Bouzid, J., Sayadi, S., Montiel, A., 2009. Impact of orthophosphate addition on biofilm development in drinking water distribution systems. J. Hazard. Mater. 167 (1–3), 1198–1202.
Henne, K., Kahlisch, L., Brettar, I., Höfle, M.G., 2012. Analysis of structure and composition of bacterial core communities in mature drinking water biofilms and bulk water of a citywide network in Germany. Appl. Environ. Microbiol. 78 (10), 3530–3538.
Hewson, I., Vargo, G., Fuhrman, J., 2003. Bacterial diversity in shallow oligotrophic marine benthos and overlying waters: effects of virus infection, containment, and nutrient enrichment. Microb. Ecol. 46 (3), 322–336.
Hwang, C., Ling, F., Andersen, G.L., Lechevallier, M.W., Liu, W.T., 2011. Evaluation of methods for the extraction of DNA from drinking water distribution system biofilms. Microbes Environ. 27 (1), 9–18.
Hwang, C., Ling, F., Andersen, G.L., LeChevallier, M.W., Liu, W.T., 2012. Microbial community dynamics of an urban drinking water distribution system subjected to phases of chloramination and chlorination treatments. Appl. Environ. Microbiol. 78 (22), 7856–7865.
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.
Juhna, T., Birzniece, D., Rubulis, J., 2007. Effect of phosphorus on survival of Escherichia coli in drinking water biofilms. Appl. Environ. Microbiol. 73 (11), 3755–3758.
Kalmbach, S., Manz, W., Szewyk, U., 1997. Dynamics of biofilm formation in drinking water: Phylogenetic affiliation and metabolic potential of single cells assessed by formazan reduction and in situ hybridization. FEMS Microbiol. Ecol. 22 (4), 265–279.
Karthikeyan, S., Korber, D.R., Wolfaardt, G.M., Caldwell, D.E., 2001. Adaptation of bacterial communities to environmental transitions from labile to refractory substrates. Int. Microbiol. 4 (2), 73–80.
Keinänen, M.M., Korhonen, L.K., Lehtola, M.J., Miettinen, I.T., Martikainen, P.J., Vartiainen, T., Suutari, M.H., 2002. The microbial community structure of drinking water biofilms can be affected by phosphorus availability. Appl. Environ. Microbiol. 68 (1), 434–439.
Lal, M., 2001. Microbial fouling of an anion exchange resin. Curr. Sci. 80 (9), 1104.
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., Laxander, M., Miettinen, I.T., Hirvonen, A., Vartiainen, T., Martikainen, P. J., 2006. The effects of changing water flow velocity on the formation of biofilms and water quality in pilot distribution system consisting of copper or polyethylene pipes. Water Res. 40 (11), 2151–2160.
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 unchlorined drinking water from three treatment plants by cultivation-independent methods. Water Res. 47 (10), 3523–3533.

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., 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. Tech. 48 (10), 5467–5476.
Liu, G., Tao, Y., Zhang, Y., Lut, M., Knibbe, W.-J., van der Wielen, P., Liu, W., Medema, G., van der Meer, W., 2017a. Hotspots for selected metal elements and microbes accumulation and the corresponding water quality deterioration potential in an unchlorinated drinking water distribution system. Water Res. 124, 435–445.
Liu, G., Zhang, Y., Knibbe, W.-J., Feng, C., Liu, W., Medema, G., van der Meer, W., 2017b. Potential impacts of changing supply-water quality on drinking water distribution: A review. Water Res. 116, 135–148.
Logue, J.B., Langenheder, S., Andersson, A.F., Bertilsson, S., Drakare, S., Lanzén, A., Lindström, E.S., 2011. Freshwater bacterioplankton richness in oligotrophic lakes depends on nutrient availability rather than on species–area relationships. ISME J. 6 (6), 1127–1136.
Martiny, A.C., Jørgensen, T.M., Albrechtsen, H.J., Arvin, E., Molin, S., 2003. Long-Term Succession of Structure and Diversity of a Biofilm Formed in a Model Drinking Water Distribution System. Appl. Environ. Microbiol. 69 (11), 6899–6907.
Mathieu, L., Bouteleux, C., Fass, S., Angel, E., Block, J.C., 2009. Reversible shift in the α-, β- and γ-proteobacteria populations of drinking water biofilms during discontinuous chlorination. Water Res. 43 (14), 3375–3386.
Mathieu, L., Bertrand, I., Abe, Y., Angel, E., Block, J., Skali-Lami, S., Francius, G., 2014. Drinking water biofilm cohesiveness changes under chlorination or hydrodynamic stress. Water Res. 55, 175–184.
Okabe, S., Kokazi, T., Watanabe, Y., 2002. Biofilm Formation Potentials in Drinking Waters Treated by Different Advanced Treatment Processes. 2, 97–104.
Pang, C.M., Liu, W.-T., 2006. Biological filtration limits carbon availability and affects downstream biofilm formation and community structure. Appl. Environ. Microbiol. 72 (9), 5702–5712.
Pinto, A.J., Xi, C., Raskin, L., 2012. Bacterial community structure in the drinking water microbiome is governed by filtration processes. Environ. Sci. Tech. 46 (16), 8851–8859.
Proctor, C.R., Hammes, F., 2015. Drinking water microbiology—from measurement to management. Curr. Opin. Biotechnol. 33, 87–94.
Ren, Anran, Yao, Mingchen, Fang, Jiaxing, Dai, Zihan, Li*, Xiaoming, van der Meer, Walter, Medema, Gertjan, Rose, Joan B., Liu*, Gang, 2024. Bacterial Communities of Planktonic Bacteria and Mature Biofilm in Service Lines and Premise Plumbing of a Megacity: Composition, Diversity, and Influencing factors. Environmental International 108538.
Schmeisser, C., Stöckigt, C., Raasch, C., Wingender, J., Timmis, K.N., Wenderoth, D.F., Flemming, H.C., Liesegang, H., Schmitz, R.A., Jaeger, K.E., Streit, W.R., 2003. Metagenome Survey of Biofilms in Drinking-Water Networks. Appl. Environ. Microbiol. 69 (12), 7298–7309.
Schwartz, T., Hoffmann, S., Obst, U., 1998. Formation and bacterial composition of young, natural biofilms obtained from public bank-filtered drinking water systems. Water Res. 32 (9), 2787–2797.
Shaw, J.L., Monis, P., Fabris, R., Ho, L., Braun, K., Drikas, M., Cooper, A., 2014. Assessing the impact of water treatment on bacterial biofilms in drinking water distribution systems using high-throughput DNA sequencing. Chemosphere 117, 185–192.
Tamaki, H., Wright, C.L., Li, X., Lin, Q., Hwang, C., Wang, S., Thimmapuram, J., Kamagata, Y., Liu, W.T., 2011. Analysis of 16S rRNA amplicon sequencing options on the roche/454 next-generation titanium sequencing platform. PLoS One 6 (9).
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.
Van der Kooij, D., 1992. Assimilable organic carbon as an indicator of bacterial regrowth. Journal / American Water Works Association 84 (2), 57–65.
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.
Vuoriranta, P., Männistö, M., Soranummi, H., 2003. Occurrence of Sphingomonas sp. bacteria in cold climate drinking water supply system biofilms. Water Sci. Technol. Water Supply 3 (1–2), 6.
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. Tech. 48 (3), 1426–1435.
Wang, Y., Qian, P.Y., 2009. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS One 4 (10).
White, D.C., Suttont, S.D., Ringelberg, D.B., 1996. The genus Sphingomonas: Physiology and ecology. Curr. Opin. Biotechnol. 7 (3), 301–306.
Williams, M.M., Domingo, J.W.S., Meckes, M.C., Kelty, C.A., Rochon, H.S., 2004. Phylogenetic diversity of drinking water bacteria in a distribution system simulator. J. Appl. Microbiol. 96 (5), 954–964.
Williams, M., Santo Domingo, J., Meckes, M., 2005. Population diversity in model potable water biofilms receiving chlorine or chloramine residual. Biofouling 21 (5–6), 279–288.
Wu, H., Zhang, J., Mi, Z., Xie, S., Chen, C., Zhang, X., 2015. Biofilm bacterial communities in urban drinking water distribution systems transporting waters with different purification strategies. Appl. Microbiol. Biotechnol. 99 (4), 1947–1955.
Yao, Mingchen, Zhang, Yue, Dai, Zihan, Ren, Anran, Fang, Jiaxing, Li, Xiaoming, van der Meer, Walter, Medema, Gertjan, Rose, Joan B., Liu*, Gang, 2023. Mingchen Yao, Building Water Quality Deterioration during Water Supply Restoration after Interruption: Influences of Premise Plumbing Configuration. Water Research 241, 120149.
Yu, J., Kim, D., Lee, T., 2010. Microbial diversity in biofilms on water distribution pipes of different materials. 61: 163-171.