Effects of disinfection efficiency on microbial communities and corrosion processes in drinking water distribution systems simulated with actual running conditions

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ABSTRACT

The effects of disinfection efficiency on microbial communities and the corrosion of cast iron pipes in drinking water distribution systems (DWDSs) were studied. Two annular reactors (ARs) that simulated actual running conditions with UV/Cl2 disinfection and chlorination alone were used. High chlorine consumption and corrosion rate were found in the AR with UV/Cl2. According to functional genes and pyrosequencing tests, a high percentage of iron recycling bacteria was detected within the biofilm of the AR with Cl2 at the early running stage, whereas siderophore-producing bacteria were dominant in the biofilm of the AR with UV/Cl2. At the early running stage, the sequential use of UV light and an initial high chlorine dosage suppressed the biomass and iron-recycling bacteria in both bulk water and biofilms, thereby forming less protective scales against further corrosion, which enhanced chlorine consumption. Non-metric multidimensional scaling analysis showed that the bacterial communities in the ARs shaped from within rather than being imported by influents. These results indicate that the initial high disinfection efficiency within the distribution system had not contributed to the accumulation of iron-recycling bacteria at
Introduction

For more than a century, iron and steel pipes have been implemented in drinking water distribution systems (DWDSs) because of their high mechanical strength and cost-effectiveness (Mohebbi and Li, 2011). However, water quality can be deteriorated by pipe corrosion, which is derived from (electro)chemical processes and/or microbiological activities on the water-main surface, causing increase in turbidity, metal ion, disinfectant decay and even water episodes such as “red water” (Gerke et al., 2008; Kip and van Veen, 2015; Masters et al., 2015). During the corrosion process, metallic iron suffers oxidization and releases iron ions, which re-precipitate and develop corrosion scales. Notably, the compositions and structures of the scales tend to be heterogeneous and were generally considered to be influenced by water chemistry (e.g., pH, alkalinity, and DO) and/or hydraulic conditions (Peng et al., 2010). Accordingly, corrosion control strategies such as pH/alkalinity adjustments or corrosion inhibitors were developed, which, however, exhibited certain defects such as high cost and/or decrease in the biostability of the bulk water.

Recently, microbiologically influenced corrosion (MIC) has been gaining considerable attention because of its dual role in corrosion: i.e., it can either accelerate or inhibit corrosion (Kip and van Veen, 2015; Zhang et al., 2018). Generally, in metal transformation in aquatic environments, the primary bacterial species are sulfate-reducing bacteria (SRB), sulfur-oxidizing bacteria (SOB), iron-oxidizing bacteria (IOB), and iron-reducing bacteria (IRB). The first two species are usually associated with acceleration of corrosion because of acid (Jin et al., 2015) and sulfide (Sun et al., 2017) production, respectively. Similarly, IOB can accelerate corrosion by producing extracellular polymeric substances (EPS) and iron oxide precipitates (Liu et al., 2016), while IRB can generate Fe₃O₄, one of the main constituents of the hard, shell-like layers in tubercles (Yang et al., 2012; Yang et al., 2014a). Furthermore, the presence of iron-respiring bacteria in the biofilm can reduce the corrosion rate of steel by reducing ferric to ferrous ions and scavenging oxygen in the water column (Dubiel et al., 2002). The predominance of the bacteria involved in iron redox cycling promotes the formation of Fe₃O₄, which protects the iron from corroding (Wang et al., 2012). The corrosion process and corrosion products could be affected by the microbial composition of the biofilm. However, the shaping mechanism of the microflora in the biofilms toward corrosion control remains unclear.

Disinfection, in terms of residual disinfectants, is used to limit undesirable biofilm on water-main surface and bacteria in bulk water. Selective pressure on bacteria, however, may create distinct microbial communities with resilient bacteria (Roeder et al., 2010), which provide potentially beneficial opportunities for managing community structures in DWDSs. For instance, the sequential use of UV and chlorine exhibited a higher disinfection efficiency in both bulk water and biofilm (Liu et al., 2019). Chloramination has been reported to exert stronger selection pressure than chlorination on microflora (Hwang et al., 2012). Previously, studies have primarily investigated the corrosion-related bacterial communities in biofilms with different disinfection processes (Li et al., 2014; Zhang et al., 2019). For example, ClO₂ showed more pronounced effect on promoting corrosion than NaClO, whereas their disinfection effect on biofilm bacteria were dose dependent and species specific (Zhang et al., 2019). Nevertheless, the relationship between the corrosion-related microbes within DWDSs and those in inlets under actual running conditions, as well as the driving factors in corrosion-related bacteria succession, is still unclear. We hypothesized that the disinfection efficiency in a DWDS plays a key role in driving corrosion-related microflora.

In this study, we investigated the role of disinfection efficiency in microbial community-shaping, especially in controlling corrosion-related bacteria in both bulk water and biofilms, using two annular reactors (ARs) that were used to simulate actual running conditions for UV/Cl₂ disinfection and chlorination, respectively. The corrosion processes and scales were characterized via electrochemical and physicochemical measurements. Quantitative real-time PCR (qPCR) was applied to monitor the abundances of the bacteria in both the bulk water and biofilms. Pyrosequencing was used to characterize the diversity and taxonomy of the microbial communities. Furthermore, the association between the corrosion rate, biomass, and abundance of corrosion-related bacteria is discussed. Overall, an enhanced understanding of the relationship between bulk water and biofilm microbiomes, as well as the role of disinfection efficiency in driving corrosion-related bacteria, will contribute toward engineering desirable biofilm microbiomes for corrosion control.

1. Materials and methods

1.1. Set-up and operation of simulated DWDSs

Simulated distribution systems were set up using two ARs (1320LJ, BioSurface Technologies Co., USA). In the AR with UV/Cl₂ disinfection, raw water was treated with UV irradiation (40 mJ/cm²), which was obtained using an ultraviolet meter (UVT-201, Ushio Inc., Japan). Then, the irradiated water was chlorinated with NaClO. The AR with Cl₂ was disinfected using only NaClO (Appendix A Fig. S1). During the operational period, the effluents of both reactors were maintained at the same residual chlorine concentration of 0.08 mg/L; however, the influents were dosed with different amounts of NaClO. The set-ups of both ARs are provided in Appendix A.

1.2. Water quality analysis

Tested water (Appendix A Table S1) collected from the outlets of a sand filter in a drinking water treatment plant were
transported on ice to a laboratory, stored at 4 °C before analysis, and introduced into the simulated distribution system. Every week during operations, bulk water was collected for water quality analysis. Turbidity, total iron and residual chlorine were analyzed repeatedly for 3 times according to the standard method (EPA of China, 2002). DOC was measured with a TOC analyzer (Shimadzu TOC-V CPH, Japan). Moreover, we measured Cl\textsuperscript{−}, SO\textsubscript{4}\textsuperscript{2−}, NO\textsubscript{3}−, NO\textsubscript{2}−, and NH\textsubscript{4}+ using ion chromatography (Dionex, USA). One-way ANOVA analysis was used to compare the water quality parameters between the two ARs, with a significance threshold of a 0.05.

1.3. Microbial analysis

Primers targeting 16S rRNA were used for quantitative PCR (qPCR) analysis via a 7300-qPCR system. The qPCR was performed according to the reaction volume and the thermal cycling conditions as described previously (Suzuki et al., 2000). For the pyrosequencing tests, the V3–V6 regions within the 16S rRNA gene fragment were amplified using the primers of 341F (SI-CTACGGGAGGCAGCAG-3) and 1073R (SI-ACGAGCTGACGACARCC ATG-3) using a previously described procedure (Lai et al., 2014). 454 pyrosequencing was carried out on the GS-FLX Titanium platform at Majorbio BioPharm Technology Co., Ltd. (Shanghai, China). The mothur software was used to treat the obtained raw data, and representative sequences from each operational taxonomic unit (OTU) were selected for assignment to the SILVA database (http://www.arb-silva.de/). Non-metric multidimensional scaling (NMDS) were used to compare the compositions of the microbial communities in the bulk water and biofilms (Vignola et al., 2018). Principal component analysis (PCA) were performed to correlate the environmental variables and corrosion-related bacteria within the biofilms (Bouskill et al., 2012). The sampling technique, qPCR, and pyrosequencing data treatment are described in Appendix A.

1.4. Characterizations of corrosion scales

The crystalline phases of the corrosion products were analyzed by an X-ray powder diffractometer (XRD, Rigaku D/Max-rA, Japan) and identified by Jade XRD software. The morphologies of the corrosion scales were examined using a field-emission scanning electron microscope (FESEM, Hitachi SU8020, Japan) operated at 1.0 kV.

1.5. Corrosion rate measurements

The corrosion rates (mm/year) of the iron coupons were measured by the weight-loss method and evaluated by the following formula (ASTM G 31, 1994):

$$\text{corrosion rate} = \frac{8.76 \times 10^4 \times W_{\text{loss}}}{A \times D \times T}$$

where, $W_{\text{loss}}$ (g), $A$ (cm\textsuperscript{2}), and $D$ (g/cm\textsuperscript{3}) represent the weight loss, surface area, and density, respectively, of the test coupons. $T$ (hr) represents the corrosion time, and the sampling and electrochemical measurements are provided in Appendix A.

2. Results

2.1. Chlorine consumption and iron release

Appendix A Fig. S2 shows the variations of the residual chlorine in the influents and effluents of both ARs. During the entire running period, the residual chlorine in both effluents was maintained at 0.08 mg/L with no observable significant difference ($p = 0.743$). In the early running days, the required chlorine in the influents underwent decreases, followed by a decrease to a stable stage in both ARs. For example, in the AR with UV/Cl\textsubscript{2}, the chlorine concentration in the influent reached 6.02 mg/L within the first 20 days, then decreased to 2.66 mg/L around day 70, followed by gradual stabilization at 1.28 mg/L with increase in running time (Appendix A Fig. S2A). For the AR with Cl\textsubscript{2}, the required chlorine reached 3.48 mg/L around 20 days, and then gradually decreased to 2.11 mg/L around day 70, followed by stabilization around 1.13 mg/L along with the running time (Appendix A Fig. S2B). These results indicate that chlorine consumption in the AR with UV/Cl\textsubscript{2} was higher than in the AR with Cl\textsubscript{2} during the running days.

Fig. 1 shows the total iron concentrations (TICs) in the two ARs. High TICs followed by stable iron release were found in both ARs. Typically, the TIC reached 0.32 mg/L within first 20 days in the effluents of the AR with UV/Cl\textsubscript{2}, followed by a decrease to 0.16 mg/L around day 70, and then gradual stabilization at 0.11 mg/L around day 240. In the AR with Cl\textsubscript{2}, the TIC in the effluent increased to 0.21 mg/L within 20 days, followed by a gradual decrease to 0.11 mg/L around day 70, and stabilization at 0.08 mg/L with increasing time. We obtained similar results in the effluent turbidity of the two ARs (Appendix A Fig. S3). The operational periods of both ARs show two phases: rapid corrosion stage (Stage I) and stable stage (Stage II), which represent the running times before and after day 70, respectively. In Stage II, the average TIC and turbidity were 0.10 mg/L and 0.16 NTU, respectively, in the AR with UV/Cl\textsubscript{2}. These values were higher than those for the AR with Cl\textsubscript{2} (0.08 mg/L and 0.11 NTU, respectively).

![Fig. 1 – Diagram of total iron release in effluents of two ARs. Error bars show one standard deviation.](image-url)
2.2. Corrosion processes and rates

Polarization curves were obtained to identify the corrosion processes at the metal/biofilm interfaces (Appendix A Fig. S4). Appendix A Table S2 lists the corrosion potentials ($E_{corr}$) and corrosion current densities ($i_{corr}$). In the AR with UV/Cl₂, $i_{corr}$ was 36.18 $\mu$A/cm² at day 20, which then slightly decreased to 35.58 $\mu$A/cm² at day 70, followed by a further reduction to 31.25 and 16.91 $\mu$A/cm² at day 210 and day 350, respectively. Moreover, $E_{corr}$ underwent a positive shift from $-0.759$ V (day 20) to $-0.559$ V (day 350). The results indicated that corrosion rate was largely decreased in Stage II because of the corrosion layer that had formed on the coupons. However, in the AR with Cl₂, $i_{corr}$ was 32.90 and 30.38 $\mu$A/cm² at day 20 and day 70, respectively, and then decreased considerably to 20.30 at day 210, followed by 12.50 $\mu$A/cm² at day 350. The results showed that corrosion rate in the AR with Cl₂ was lower than that in the AR with UV/Cl₂. The corrosion layer formed in the AR with Cl₂ showed more protection on the coupons in Stage I. Moreover, the corrosion rates determined by the weight-loss method were consistent with the results of electrochemical measurements (Appendix A Table S3).

2.3. Morphology and physicochemical characteristics of corrosion scales

Appendix A Fig. S5 shows the visual appearance of the harvested corrosion scales in both ARs. The corrosion layers in both ARs were initially loose and transformed into compact layers in Stage II with more black layers observed in the AR with Cl₂. To reveal the microstructure of the corrosion scales in both ARs, SEM images are shown in Appendix A Fig. S6. These microscopic images show the variety of crystallographic structures in the scales of both ARs. In the AR with UV/Cl₂, the tubercles showed a porous structure at day 20 and roughly shifted to round particles at day 70, followed by an enlargement of the particles with increasing time. In the AR with Cl₂, the corrosion products shifted from the amorphous structure at day 20, to porous crystals at day 70, lamellar crystals at day 210 to dense crystals at day 350. These results indicate that the porous tubercles had formed at Stage I in the AR with UV/Cl₂ whereas the dense tubercles had formed in the AR with Cl₂. Furthermore, we obtained the XRD results of the corrosion scales on the iron coupons in both ARs (Fig. 2).

![XRD patterns of corrosion scales on iron coupons at different running days in AR with UV/Cl₂ (a) and Cl₂ alone (b).](image)

Fig. 2 – XRD patterns of corrosion scales on iron coupons at different running days in AR with UV/Cl₂ (a) and Cl₂ alone (b).

layer with a magnetite content that was higher than that in the AR with UV/Cl₂, which experienced a porous corrosion layer with higher calcite content.

2.4. Bulk water chemistry

Cl⁻, NO₃⁻, and SO₄²⁻ were reported to be involved in the chemical corrosion process (Ishii and Boyer, 2011; Masters et al., 2015). Therefore, water chemistry parameters, i.e., Cl⁻, SO₄²⁻, NO₃⁻, NO₂⁻, NH₄⁺, and NO₂⁺ were analyzed in both influents and effluents of the two ARs along with the running time. As shown in Appendix A Figs. S8 and S9, similar concentrations (or values) of Cl⁻ ($p = 0.9230$), NO₃⁻ ($p = 0.8097$), and SO₄²⁻ ($p = 0.9953$) were found in the influents. The Cl⁻ values (average = 22.51 mg/L) in the effluent of the AR with UV/Cl₂ were higher than those (average = 20.39 mg/L) in the AR with Cl₂ ($p = 0.0133$) at Stage I. However, there is no significant difference at Stage II ($p = 0.1471$), indicating that more ClO₂⁻ had been reduced by Fe⁰ to Cl⁻ at Stage I in the AR with UV/Cl₂. This reduction had probably been caused by the formation of fewer protective corrosion layers (Wang et al., 2012). In addition, Fe⁰ can promote the transition from NO₃⁻ to NO₂⁻ and NH₃ (Masters et al., 2015). NO₃⁻ was found to decrease in the effluents of both
ARs at Stage I (Appendix A Fig. S9). However, the concentrations of NH$_4^+$ and NO$_2^-$ at Stage I in the AR with UV/Cl$_2$ were higher than those in the AR with Cl$_2$, suggesting a more significant loss of Fe$^0$ in the former AR because of fewer passivation layers against NO$_3^-$. The results indicate that the scales, which had formed during the early running stage in the AR with UV/Cl$_2$, promoted the chemical consumption of ClO$^-$ and NO$_3^-$, and caused higher influent demand for NaClO to maintain the required residual chlorine.

2.5. Quantitative PCR of total bacteria

qPCR for the bacterial 16S rRNA gene was utilized to evaluate microbial abundances in the bulk water and biofilms on different running days (Fig. 3). The 16S rRNA gene copies increased in the effluent of both ARs during all stages, indicating bacterial regrowth. Moreover, the 16S rRNA gene copies in both the influent and effluent increased with running time which was in accordance with the decrease in residual chlorine. Notably, the abundance of the bacteria was largely inhibited at the early running stage but greatly increased with time in the AR with UV/Cl$_2$, whereas the increase in the biomass in the AR with Cl$_2$ was much more moderate. For example, the 16S rRNA gene of the influent and effluent in the AR with Cl$_2$ increased from $6.33 \times 10^7$ and $3.05 \times 10^{10}$ copies/L (day 20) to $1.90 \times 10^{10}$ and $4.62 \times 10^{11}$ copies/L (day 350), respectively. However, in the AR with UV/Cl$_2$, the 16S rRNA gene of the influent and effluent increased from $4.60 \times 10^7$ and $2.96 \times 10^8$ copies/L (day 20) to $1.85 \times 10^9$ and $1.02 \times 10^{11}$ copies/L (day 350), respectively. The results indicate that the biomass had become more effectively inactivated, especially at the early running stage, in both the bulk water and biofilms of the AR with UV/Cl$_2$.

2.6. Bacterial community

Appendix A Table S4 shows the obtained OTUs, Chao 1 index, and Shannon diversity indices for all samples. The lower Shannon index was found in the effluents and biofilms of the AR with UV/Cl$_2$. Moreover, similar results were found for the OTU richness in the bulk water and biofilms of both ARs, indicating that the UV/Cl$_2$ treatment had reduced the microbial diversity more effectively in the effluents and biofilms.

The bacterial communities at the class level in the bulk water and biofilms of both the simulated distribution systems exhibited notable differences at both stages (Appendix A Fig. S10). Proteobacteria, Planctomycetia, and Sphingobacteria were found to be dominant in the raw water at both stages in the Cl$_2$ AR, whereas the UV treatment tended to inactivate the Planctomycetia and Sphingobacteria but enrich Proteobacteria in raw water. For example, Proteobacteria were the dominant class in the raw water of the AR with Cl$_2$ with a relative abundance of 50.2% at Stage I and 54.3% at Stage II. The values in the AR with UV/Cl$_2$ were 92.1% in Stage I and 77.5% in Stage II, suggesting the enrichment of Proteobacteria in the raw water. However, after chlorination, the most abundant bacterial class in the influents during both stages in both ARs was Actinobacteria, suggesting the potential chlorine resistance of this bacterial group. Although Proteobacteria were found to be dominant in the effluents and biofilms of both ARs, a higher percentage of the bacteria was found in the effluents (96.5% and 81.0% at Stages I and II, respectively) and biofilms (91.8% and 74.2% at Stages I and II, respectively) in the AR with UV/Cl$_2$ than that in the AR with Cl$_2$ (effluents: 96.2% and 52.5%; biofilms: 82.2% and 77.3% at Stages I and II, respectively). These results indicated that the composition of the microbial community in the biofilm was similar to that in the effluent.

IRB, IOB, and some nitrate-reducing bacteria (NRB) could be involved in the redox cycling of iron, which promotes the
formation of dense scales against further corrosion (Dubiel et al., 2002; Herrera and Videla, 2009; Wang et al., 2012; Weber et al., 2006), whereas siderophore-producing bacteria (SPB) showed weaker corrosion inhibition (Grateron et al., 2007; Raymond and Dertz, 2004). Therefore, the community structures in the bulk water and biofilms were compared at the genus level to determine the composition of the bacteria involved in iron cycling and siderophore production at the two running stages (Fig. 4). We have provided information about the microbial compositions in Appendix A Table S5. During the fast corrosion stage, the iron recycling bacteria (IOB, IRB, and NRB) were more abundant in the effluent and biofilm of the AR with Cl2 (Stage I). However, the abundance of iron recycling bacteria increased in the bulk water and biofilm of the AR with UV/Cl2 at Stage II. For example, at Stage I in the AR with Cl2, the relative abundance of iron recycling bacteria increased in the bulk water and biofilm of the AR with Cl2, while the relative abundance of SPB was 6.9%, including 0.4% IRB, 19.9% IOB, and 0.3% NRB (Appendix A Table S5). In the influent of the AR with Cl2, the relative abundance of iron recycling bacteria decreased to 6.6% but that of SPB increased to 87.7%, among which Mycobacterium were 86.7% (Ferreras et al., 2005). In the effluent, however, the relative abundance of iron recycling bacteria were 36.7% and that of SPB was 46.2%, including 24.5% IOB, 0.9% IRB, and 10.7% NRB. In the biofilm, we observed 38.4% of iron recycling bacteria and 21.1% of SPB. Similarly, at Stage II, the iron recycling bacteria were 12.7% and SPB were 10.5% in raw water. Chlorination triggered an increase of SPB to 39.8% in the influent. Notably, the abundances of iron recycling bacteria (19.9%) and SPB (9.7%) in the effluent at Stage II were lower than those at Stage I, including iron-respiring bacteria (4.4% IOB and 6.8% IRB). At Stage I in the AR with UV/Cl2, the relative abundance of the iron recycling bacteria and SPB in the raw water was 51.6% and 4.4%, respectively, including 46.4% IOB and 1.7% IRB. Moreover, the chlorination-enriched SPB to 35% in the influent; however, SPB were the predominant bacteria in the effluents and biofilms, reaching levels of 58.4% and 45.1%, respectively. At Stage II, 14.4% iron recycling bacteria and 12.5% SPB were detected in the raw water, whereas iron recycling bacteria decreased to 3.4% after chlorination. The iron recycling bacteria in the effluent (13.3%) and biofilm (23.5%) were more abundant at Stage II than at Stage I (3.2% in effluent and 8.9% in biofilm). However, the SPB in the effluent (13.7%) and biofilm (17.1%) were less abundant at Stage II than at Stage I (58.4% in effluent and 45% in biofilm). The percentages of IRB in the effluents and biofilms of the AR with UV/Cl2 increased at Stage II (Appendix A Table S5). However, the IRB in the AR with Cl2 were more abundant than those in the AR with UV/Cl2 at both stages. These results indicate that the bacteria involved in iron recycling were more dominant in the effluents and biofilms of the AR with Cl2 at Stage I, whereas the SPB were more dominant in the AR with UV/Cl2. It has been reported that IRB could induce green rust formation (Jin et al., 2015), which is consistent with the higher proportion of green rust in the AR with Cl2. At Stage II, the abundance of the iron recycling bacteria increased in the biofilm of the AR with UV/Cl2. However, the total abundances of the iron recycling bacteria and SPB in the biofilm of the AR with UV/Cl2 were lower than those in the AR with the Cl2 showing less protection against corrosion.

3. Discussion

UV irradiation, followed by chlorination, has been shown to exhibit a synergistic effect in the disinfection of both the bulk water and biofilms (Shang et al., 2007; Liu et al., 2019). Therefore, the sequential use of UV and Cl2 disinfection may inactivate microorganisms more effectively in both the bulk water and biofilm of the AR with UV/Cl2. In this study, during the entire running period, although the same effluent chlorine concentration (0.08 mg/L) was maintained for both ARs, a lower chlorine dosage was required in the influents of the AR with Cl2, especially at the early running stage (Stage I). Meanwhile, dense corrosion scales with Fe3O4 were formed in the AR with Cl2 at this stage. It was demonstrated that iron recycling bacteria were dominant in the biofilm of the AR with Cl2 at Stage I. However, the water chemistry, such as Cl−, NO3−, and SO42− in the influents of the two ARs were similar, whereas lower corrosion rates were found in the AR with Cl2. Therefore, the lower chlorine consumption had probably
been caused by the iron surface rather than influenced by the water chemistry in the influent because passivation layers with stable structures had formed in the AR with Cl₂ at this stage. However, fewer protective layers caused more severe corrosion and the loss of iron in the AR with UV/Cl₂, as demonstrated by the increase in effluent Cl⁻, NO₂⁻, and NH₄⁺.

Furthermore, these results indicate that the microbial quantity and/or community, rather than the water parameters, may have been responsible for the differences in corrosion scales and process.

At Stage I in the AR with Cl₂, higher 16S rRNA gene copies were found in the effluents and biofilms. However, more iron recycling bacteria (38.4%) were detected in the biofilms, which developed corrosion scales with a higher proportion of magnetite that protected the test coupons from further corrosion and reduced the chlorine consumption at Stage II. At Stage I in the AR with UV/Cl₂, lower bacterial gene copies were detected in the effluent and biofilm. A lower abundance (8.9%) of bacteria involved in iron recycling and formation of fewer protective corrosion layers was detected in the biofilm and the scales, respectively. Hence, a higher chlorine dosage was required to maintain the effluent residual chlorine at Stage I; however, the higher influent chlorine dosage inhibited the biomass. Therefore, the serious corrosion at Stage I can be explained by the high corrosion rate caused by chlorine via (electro)chemical reaction and the reduced protection offered by the corrosion scales and biofilm at the early running stage. However, the microbes involved in iron recycling at this stage were more abundant in the biofilm of the AR with Cl₂, thus offering the iron more protection from corrosion and a lower iron release in the bulk water. With the formation of the corrosion scales at Stage II, the corrosion rate was reduced and a lower chlorine dosage was required to maintain the residual chlorine in the effluents thus a stability in iron release was obtained. At this stage in the AR with Cl₂, a more stable component (Fe₃O₄) was present in the corrosion scales and caused less iron release. These results also suggest that the initial disinfection efficiency (represented by the biomass quantity) in the distribution system may play an important role in selecting the iron recycling bacteria within the bulk water and biofilm for corrosion control.

NMDS analysis based on Bray–Curtis dissimilarity distances were applied to reveal the differences in the microbial community structures in the two ARs at different stages (Fig. 5). The communities developed in the biofilms tended to be more similar to those in the effluent rather than the influent and raw water. Furthermore, the similarity between the biofilm communities in the two ARs was closer with a lower influent chlorine dosage at Stage II. The amount of residual chlorine at Stage II was evidently lower than that at Stage I for the two ARs, indicating that the disinfection dosage (or disinfection efficiency) was a vital selection pressure on the microflora within the simulated DWDSs. In summary, these results indicate that the microbial communities in the biofilm were determined within the distribution systems rather than introduced by the influents. The disinfection efficiency may play a key role in shaping community structures in the biofilms, especially at the early running stage.

PCA was used to explore the connection among corrosion-related bacteria (at the genus level), 16S rRNA, the Chao 1 index, the Shannon diversity indices, and i_corr (Fig. 6). The PCA ordination result showed that i_corr was inversely correlated with the total bacterial gene copies, the Chao 1 index, and the Shannon diversity indices. In the biofilms of the two ARs, the corrosion-related bacteria exhibited different compositions at

![Fig. 5 - NMDS generated by Bray–Curtis index for samples from two ARs. UV-I, UV-II, C-I, and C-II represent samples of AR with UV/Cl₂ and AR with Cl₂ alone at Stages I and II, respectively.](image-url)
Stage I. In the AR with UV/Cl2, the corrosion-related bacteria were mostly SPB, such as Sphingomonas (22.8%), followed by Bradyrhizobium (8.9%), 4.4% Brevundimonas (Singh et al., 2016), and Novosphingobium (2.5%). Furthermore, IOB (1.5% Acidovorax (Zhang et al., 2019) and Aquabacterium (1.0%)), IRB (0.04% Ralstonia (Lin et al., 2007) and 1.5% Microbacterium (Zhou et al., 2016)), and NRB (Hyphomicrobium (Stein et al., 2001)) were found to be positively correlated with $i_{corr}$. However, at Stage I in the AR with Cl2, the corrosion-related bacteria included IOB such as Sideroxydans (19.2%), Sediminibacterium (6.4%), 1.9% Rhodobacterium (Kappler and Straub, 2005), and 0.2% Leptothrix (Yang et al., 2014b), as well as IRB such as Rhodobacter (5.5%). Notably, the total relative abundances of IRB and IOB in the biofilms of the AR with Cl2 were higher than those in the AR with UV/Cl2, the metabolism of which led to the cycling of Fe(II)/Fe(III) and a higher proportion of Fe3O4 in the corrosion scales of the AR with Cl2 (Dubiel et al., 2002; Wang et al., 2012; Zhu et al., 2014). However, in the AR with UV/Cl2, SPB were abundant, producing and importing siderophores to become biochelators that captured iron to inhibit the dissolution of iron and iron corrosion (Raymond and Dertz, 2004; Grateron et al., 2007). At this stage, the sequential use of UV and Cl2 exhibited higher efficiency in the inhibition of biomass and iron recycling bacteria in both the bulk water and biofilms to form fewer passivation layers on the coupons. Notably, Sphingomonas was the most abundant (22.8%) SPB in the biofilms of the AR with UV/Cl2. Sphingomonas is widely distributed and resistant to many disinfectants (such as chlorine and/or UV) and toxic chemicals (Koskinen et al., 2000; Marizcurrena et al., 2017); therefore, the abundance of Sphingomonas was probably because of its resistance to the high disinfection efficiency of the AR with UV/Cl2. However, fewer passivation layers caused higher chlorine consumption, which promoted a higher chlorine dosage in the influent, as well as less biomass and fewer iron recycling bacteria.

With the formation of corrosion layers at Stage II, the biofilms exhibited positive correlations with 16S rRNA, the Chao 1 index, and the Shannon diversity indices. However, a negative correlation is observed with $i_{corr}$, suggesting microbial corrosion passivation. At Stage II in the AR with UV/Cl2, the corrosion-related bacteria were SPB (1.3% Rhizobium (Arif et al., 2012), Mycobacterium (9.9%), 1.2% Mesorhizobium (Arif et al., 2012), 0.2% Nocardia (Schneider et al., 2007), and 0.1% Rhodococcus (Carrano et al., 2001)), IOB (7.9% Gallionella (Yang et al., 2014b) and 2.5% Acidiferrobacter (Ahoranta et al., 2017)), IRB (0.2% Acidobacterium (Nancucacho and Johnson, 2010) and 0.2% Anaeromyxobacter (Pan et al., 2017)), and NRB (4.7% Hydrogenophaga). In the AR with Cl2, however, the corrosion-related bacteria were IRB (1.7% Pseudomonas (Pan et al., 2017), 1.0% Desulfovibrio (Herrera and Videla, 2009), Acidobacterium (0.3%), 0.2% Bacillus (Herrera and Videla, 2009), and Anaeromyxobacter (0.2%)), IOB (Gallionella (3.5%) and Acidiferrobacter (5.1%)), and SPB (Pseudomonas (13.7%), Mesorhizobium (0.8%), Nocardia (0.2%), and Rhodococcus (0.1%)).

Previously, studies have reported that oxygen could be effectively blocked and anaerobic conditions could be created when the corrosion layer was thicker than ~8 mm (Jin et al., 2015), which could facilitate the proliferation of
IRB. Therefore, the increase in the iron recycling bacteria, including the IRB in the biofilm of the AR with UV/Cl$_2$, had probably been caused by the anaerobic environment during the formation of the corrosion scales at Stage II. Moreover, the total relative abundance of IRB was higher in the AR with Cl$_2$ than in the AR with UV/Cl$_2$ at Stage II. This enhanced the precipitation of iron oxide, the formation of Fe$_3$O$_4$, and the consumption of oxygen, thus causing the further passivation of iron coupons. The increase in the iron recycling bacteria in the biofilms of the AR with UV/Cl$_2$ had been accompanied by a decrease in the SPB, indicating competition for an ecological niche. In summary, these results suggest that high disinfection efficiency should be avoided for the iron recycling bacteria to accumulate at the early running stage which could be beneficial to the formation of Fe$_3$O$_4$, which can develop protective layers against further corrosion.

4. Conclusion

The results verified that a higher corrosion rate and iron release had occurred in the distribution systems with UV/Cl$_2$ that was simulated with actual running conditions. With an initial high chlorine dosage, the UV/Cl$_2$ treatment showed higher disinfection efficiency in biomass and iron recycling bacteria control in both bulk water and biofilms within the AR, especially at the early running stage. NMDS analysis showed that the microbial communities in the biofilms had developed in the ARs rather than having been imported by the influents. The PCA analysis showed that the total bacteria gene copies and Shannon diversity indices were inversely correlated with $i_{corr}$. At Stage I in the biofilms of the AR with Cl$_2$, IOB Sideroxydans and IRB Rhodobacter were the major corrosion-related bacteria, the metabolism of which induced the recycling of Fe(II)/Fe(III), thereby enhancing the precipitation of iron and Fe$_3$O$_4$ to form stable corrosion scales that prevented further corrosion. At Stage II in the AR with UV/Cl$_2$, the corrosion-related bacteria were SPB, which produced siderophores to capture iron and led to the formation of porous scales that exhibited less protection against corrosion. Moreover, the abundances of iron recycling bacteria and SPB were lower in the AR with UV/Cl$_2$ than in the AR with Cl$_2$ during the running time. These bacteria exhibited slower corrosion inhibition, which contributed to higher corrosion consumption and iron release in the effluents. The initial disinfection efficiency proved to be an important pressure in the selection of the iron recycling bacteria.

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Appendix A. Supplementary data

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REFERENCES


