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Characterization of biofilm and corrosion of cast iron pipes in drinking water distribution system with UV/Cl₂ disinfection

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ABSTRACT

The effect of UV/Cl₂ disinfection on the biofilm and corrosion of cast iron pipes in drinking water distribution system were studied using annular reactors (ARs). Passivation occurred more rapidly in the AR with UV/Cl₂ than in the one with Cl₂ alone, decreasing iron release for higher corrosivity of water. Based on functional gene, pyrosequencing assays and principal component analysis, UV disinfection not only reduced the required initial chlorine dose, but also enhanced denitrifying functional bacteria advantage in the biofilm of corrosion scales. The nitrate-reducing bacteria (NRB) *Dechloromonas* exhibited the greatest corrosion inhibition by inducing the redox cycling of iron to enhance the precipitation of iron oxides and formation of Fe₃O₄ in the AR with UV/Cl₂, while the rhizobia *Bradyrhizobium* and *Rhizobium*, and the NRB *Sphingomonas*, *Brucella* producing siderophores had weaker corrosion-inhibition effect by capturing iron in the AR with Cl₂. These results indicated that the microbial redox cycling of iron was possibly responsible for higher corrosion inhibition and lower effect of water Larson–Skold Index (LI) changes on corrosion. This finding could be applied toward the control of water quality in drinking water distribution systems.

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1. Introduction

Iron and steel pipes have been used in water distribution systems for several centuries throughout the world, and are subjected to corrosion, causing deterioration of potable water quality due to unwanted chemical and biochemical reactions (McNeill and Edwards, 2001; Husband and Boxall, 2011). In the corrosion processes of iron pipes, iron ions were released into distributed water, and can re-precipitate forming corrosion scales, also referred to as tubercles (Gerke et al., 2008). The

processes can greatly affect water quality in distribution system, not only by releasing iron oxyhydroxides (red water), but also by reactions with e.g. chlorinated disinfection by-products (Chun et al., 2005), nitrates (Hansen et al., 1996) or natural organic matter (NOM) (Nawrocki et al., 2010). Typical iron corrosion products may be composed of the iron oxyhydroxides goethite (α -FeOOH) and lepidocrocite (γ -FeOOH), magnetite (Fe₃O₄), hematite (Fe₂O₃), ferrous hydroxide (Fe(OH)₂), ferric hydroxide (Fe(OH)₃), and siderite (FeCO₃) (Wang et al., 2012). The exact composition and structure of iron corrosion scales, however, varies significantly with

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water quality as well as the hydraulic conditions (Chun et al., 2005).

Recently, according to the report by Shi et al., in the drinking water distribution system of Beijing, dense corrosion scales were formed in pipes transporting surface water, while thin corrosion scales appeared in pipes transporting groundwater (Yang et al., 2012). Moreover, soon after 80% of the local source water was replaced by long-distance transported source water from a neighboring province in October, 2008, heavy red water occurred only in the pipes transporting groundwater, and the pipes transporting surface water did not encounter red water. The structure and character of corrosion scales on iron pipes could have great impact on the water quality in drinking water distribution systems (DWDS). Several environmental factors affect the corrosion rates and the composition of the corrosion products, such as metal type, chemical composition of the bulk water, electrochemistry, and microorganisms (Borenstein, 1994). Microbiologically influenced corrosion (MIC) has been of interest due to its complicated role in corrosion processes: it can either accelerate corrosion or inhibit corrosion. Recently more and more studies have begun to show protective effects by some bacterial biofilms on metal against corrosion (Zhang et al., 2010; Dubiel et al., 2002). For example, corrosion inhibition was caused by the reduction of ferric ions to ferrous ions and enhanced consumption of oxygen, both of which derive from iron-respiring bacteria respiration (Dubiel et al., 2002). The corrosion process and corrosion products can be affected by the bacterial characteristics of waters and biofilms in DWDS. However, the relationship between microorganisms and the character of corrosion scales in DWDS has still not been systematically investigated.

Disinfection is a key treatment step in biofilm control except pathogens, specifically premise plumbing pathogens control. Sequential UV chlor(am)ine disinfection is one potential way to control heterotrophic bacteria in distribution systems (Murphy et al., 2008). Rand et al. (2007) and Murphy et al. (2008) have also reported that sequential use of UV and chlorine could achieve greater log reductions in both effluent and biofilm than systems treated with chlorine-based disinfectants alone, maintaining water biological stability very well in drinking water distribution systems. However, to our knowledge, there were no studies to be conducted so far for characterization of bacterial community and corrosion with the combined disinfection process.

The objective of this study was to investigate the effects of the sequential use of UV light and chlorination on the corrosion of cast-iron coupons as well as biofilms and water quality in DWDS using annular reactors (ARs), with chlorination alone acting as a reference. In two ARs, the corrosion process and corrosion products were characterized by electrochemical and physicochemical measurements. Quantitative real-time PCR (qPCR) was used to monitor changes in the microbial community of biofilm inside corrosion scales according to specific groups: total bacteria by 16S rRNA gene and denitrifiers by the functional genes *nosZ*, *nirK* and *nirS*. Pyrosequencing was used to monitor changes in diversity in the microbial community of biofilms, including corrosion-related bacteria. The relationship of corrosion and biofilms was discussed.

2. Materials and methods

2.1. Materials and preparation

The cast iron coupons, with the composition of C 19.08%, O 6.09%, Si 2.06%, Ca 0.58%, P 0.65%, S 1.60%, Fe 65%, Cu 1.98%, Mn 0.92%, Zn 2.04%, were used in this study. Prior to experiment, the coupons were sequentially ground using a series of grit (180, 500, 800, and 1200) silicon carbide energy paper to a smooth surface. The polished coupons were rinsed with sterile deionized water thrice, degreased in acetone, followed by sterilizing in 70% ethanol for 8 h, and then dried aseptically in a laminar flow cabinet. The coupons were exposed to UV light for 30 min, then they were weighed prior to use.

2.2. Model distribution system

Two annular reactors (ARs) (Model 1320LJ, BioSurface Technologies Co., USA) were installed to simulate the drinking water distribution system. The ARs consisted of two concentric glass cylinders and a rotating inner drum that housed cast iron coupons (20 coupons in each reactor). Each coupon had an exposed surface area of 17.5 cm² for biofilm growth. Two reactors were operated in parallel conditions, i.e., at a rotational speed of 50 rpm, which translated to a shear stress of 0.25 N/m², corresponding to a flow rate of approximately 0.3 m/sec in a 100-mm-diameter smooth pipe (Wang et al., 2012). The hydraulic retention time for the reactors was 6 h, as determined by the injection rate (2.5 mL/min). All glass surfaces and any exposed surfaces were covered with aluminum foil to reduce the potential for phototrophic growth within the system.

In one AR system, water as influents was first treated with UV irradiation at a fluence of 40 mJ/cm². Prior to startup of the trial, the UV lamp was cleaned and soaked in 70% ethanol for 24 h. The fluence was determined by ultraviolet irradiation meter. Then, the AR was conducted with free chlorine as the secondary disinfectant. Chlorine was dosed from a stock solution of sodium hypochlorite (NaClO). The other one AR was conducted with free chlorine as the secondary disinfectant only (Fig. S1). The two ARs were operated in two stages. At stage I, the initial chlorine concentration was about 0.85 mg/L in the influents with addition of NaClO in the two ARs. ARs were given 50 days to form a relatively stable corrosion scales. Then, at the stage II, in order to maintain the same effluent residual chlorine concentration of 0.08 mg/L in both ARs, different amount of NaClO was dosed into the influent. In addition, when the corrosion of iron coupons became steady on 300 d for the two ARs, the stability of the formed corrosion scales was examined by more corrosive water with the influent Larson-Skold index (LI) increasing. The influent LI 2, 4, 8 were obtained by adding Na₂SO₄ with the SO₄²⁻ concentration 182, 412, 872 mg/L in the AR with Cl₂, and 187, 417, 878 mg/L in the AR with UV/Cl₂, respectively. LI was calculated using the Eq. (1) (Ishii and Boyer, 2011):

$$LI = \frac{[Cl^-] + 2[SO_4^{2-}]}{[HCO_3^-]} \quad (1)$$

2.3. Water quality analysis

The tested water (Table S1) was treated by chemical precipitation and flocculation, settling, sand filtration, and biologically-activated carbon filtration (prior to entering the chlorine contact tanks) in a drinking water treatment plant in north of China. All samples were stored at 4 °C before analysis and transporting into distribution systems.

Effluent samples were taken weekly from each AR. Water quality parameters, such as turbidity, anions, total iron and residual chlorine, were measured repeatedly for three times according to the standard methods (EPA of China 2002). Data for total iron and turbidity were the arithmetic mean of three measured values with error bars showing one standard deviation. Dissolved organic carbon was analyzed via a total organic carbon analyzer (Phoenix 8000; Tekmar-Dohrmann, USA). Differences in water quality parameters between the two ARs were analyzed using analysis of variance (ANOVA) with a significance threshold of $\alpha = 0.05$. All information about sampling was shown in Supporting Information.

2.4. Corrosion rate measurement

Weight loss method was utilized to determine the corrosion rate. Briefly, the scraped coupons were rinsed with 70% ethanol, gently wiped with cotton swabs, freeze-dried for a day and weighted to determine the weight loss. The corrosion rate was calculated by using the formula below (ASTM G 31, 1994):

$$\text{corrosion rate (mm/year)} = \frac{8.76 \times 10^4 \times W_{\text{loss}}}{A \times D \times T} \quad (2)$$

where W_{loss} is weight loss in gram; A is the surface area of the specimen in cm^2 ; D is the density of the test coupons in g/cm^3 ; and T is the corrosion time in h . In addition, all electrochemical measurements were described in Supporting Information.

2.5. Characterizing corrosion scales

Crystalline phase was analyzed using an X-ray powder diffractometer (XRD, X'Pert PRO MPD; PANalytical, The Netherlands). The morphology of the corrosion products was examined by Focused Emission Scanning Electron Microscopy (FESEM, Hitachi, SU8020), operating at 1.0 kV.

2.6. 454 Pyrosequencing

DNA was extracted in triplicate using the FastDNA spin kit for soil (Qbiogene, Solon, OH) and mixed uniformly. All DNA samples were stored at -80°C for future use. Primers 341F (5'-CCTACGGGAGGAGCAGCAG-3') and 1073R (5'-ACGAGCTGACGACARCCATG-3') were used to amplify partial regions (V3–V6) of 16S rRNA genes (Lai et al., 2014). Each PCR product was tagged by inserting the 12-bp error-correcting Golay barcode to the forward primer and was gel-purified for pyrosequencing (Li et al., 2013). The pyrosequencing was performed on the GS FLX Titanium platform by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). All of the raw sequence data were processed using Mothur Software

(Zhao et al., 2011). Some Sequences were removed, including shorter than 200 bps, primer mismatches, and a quality score lower than 25. Mothur was used to cluster the operational taxonomic units (OTUs) based on $\geq 97\%$ identity. Taxonomic positions of representative sequences were taxonomically assigned on the SILVA database (<http://www.arb-silva.de/>) (Zhao et al., 2013). Principal component analysis (PCA) has been used to correlate both physicochemical and clone library matrices (Bouskill et al., 2012). It was used to correlate the bacteria in genus level and corrosion current densities (i_{corr}) in this paper by CANOCO program (CANOCO 4.5 for Windows).

2.7. Quantitative PCR assay

Primers targeting *nirS*, *nirK*, *nosZ* and 16S rRNA genes were utilized in quantitative PCR (qPCR) analysis via a 7300 qPCR system and the 7300 system SDS software (Applied Biosystems). The analysis procedures were described in Supporting Information.

3. Results

3.1. Chlorine consumption and iron release

In the two ARs before 50 d (stage I), the initial chlorine dose in the influents of ARs was around 0.85 mg/L while the residual chlorine in the effluent could hardly be detected to ensure that a relatively stable corrosion scale was established. No significant difference was observed in initial chlorine ($p = 0.18$) and residual chlorine ($p = 0.53$) between the two ARs at this period by ANOVA analysis (Fig. S2). In the AR with UV/Cl₂ at this stage, the total iron concentration of the effluents reached 0.52 mg/L within the first 20 days, then it gradually stabilized at approximately 0.11 mg/L around 50 d (Fig. 1A). For the AR with Cl₂, total iron concentration was 0.25 mg/L over the first 20 days followed by stabilization at 0.1 mg/L (Fig. 1A). A similar result can be seen in the turbidity value with stabilization around 50 d (Fig. S3). The two ARs exhibited similar iron concentration ($p = 0.37$) and turbidity ($p = 0.26$) of effluents by ANOVA analysis. The changes of iron release and turbidity indicated that relatively stable corrosion scale was established at around 50 d.

In stage II, for the same residual chlorine (0.08 mg/L) in the two ARs, a lower initial chlorine dose was required for the AR with UV/Cl₂ (average at 2.02 mg/L) than that for the AR with Cl₂ (average at 2.35 mg/L). A significant difference was observed in initial chlorine dose ($p = 0.0001$) between the two ARs by ANOVA analysis. In addition, the required chlorine gradually decreased from 2.17 mg/L to 1.83 mg/L with increasing time in the AR with UV/Cl₂, indicating that the iron corrosion rate greatly decreased with time (Fig. S2). As to the iron release at stage II, the total iron concentration of the effluents in the AR with UV/Cl₂ was stable at a concentration around 0.11 mg/L (Fig. 1B), while in the AR with Cl₂, there was an apparent increase (average at 0.20 mg/L) in total iron release before 140 d, followed by a stable total iron level at 0.14 mg/L (Fig. 1B). Overall, higher turbidity (average at 0.37 NTU) was measured in the effluents of the AR with Cl₂ than that of the AR (average at 0.27 NTU) with UV/Cl₂ during stage II (Fig. S3). ANOVA

Fig. 1 – Diagram of total iron release before (A) and after (B) 50 d in effluents of two ARs. Error bars show one standard deviation.

analysis also showed that total iron ($p = 0.0001$) and turbidity ($p = 0.003$) were very different between the two ARs at stage II.

Some researches have indicated a significant linear relationship between iron release and the Larson-Skold index (LI), and high corrosion rates tended to result from waters with $LI > 1.2$ (Melidis et al., 2007; Ishii and Boyer, 2011). Therefore, the stability of the corrosion scales formed in the two ARs was examined by increasing water corrosivity with increasing LI after 300 d. With the addition of Na_2SO_4 , the LI of the influent increased from 1.2 to 2, 4 and 8. Correspondingly, the total iron (Fig. 1B) and turbidity (Fig. S3) in the effluent did not significantly change in the AR with UV/ Cl_2 , while higher total iron and turbidity appeared in the AR with Cl_2 alone. A significant difference was observed in total iron ($p = 0.001$) and turbidity ($p = 0.0001$) between the two ARs by ANOVA analysis.

3.2. Corrosion process with different disinfection

Polarization curve and EIS measurements are electrochemical technique for the characterization of electrochemical reactions at the metal/biofilm interface and the formation of corrosion and biofilm (Mansfeld and Little, 1991; Yuan et al., 2007). In the experiment, polarization curves were measured

for the corroded coupons in the two ARs (Fig. S4). The corrosion potentials, E_{corr} and corrosion current densities were obtained by quantitative analysis (Table S2). In the AR with UV/ Cl_2 , corrosion current densities, i_{corr} , exhibited the highest value $54.85 \mu\text{A cm}^{-2}$ at 50 d, then decreased to $27.5 \mu\text{A cm}^{-2}$ at 210 d, and corrosion potentials, E_{corr} , underwent a slightly positive shift from ~ -0.774 to ~ -0.664 V after 210 d. The results indicated that the corrosion rate decreased after 50 d, due to the inhibition effect of the corrosion layer on the electrode reactions. In contrast, in the AR with Cl_2 alone, i_{corr} increased and then decreased, indicating the corrosion rate increased with time before 140 d, then decreased due to the formation of a corrosion oxide layer. Similarly, E_{corr} exhibited a negative shift from 50 d to 140 d, implying corrosion enhancement, then a slightly positive shift, indicating corrosion inhibition. The results indicated that after 140 d, the corrosion reached a stable period, and a protective layer formed on the coupon in the DWDS with Cl_2 .

The impedance spectra of the corroded coupons exhibited different changes in the two ARs with exposure time (Fig. S5). Clearly, in the UV/ Cl_2 DWDS, the diameter of the impedance loops markedly increased with time, indicating a decrease of the corrosion rate after 50 d. However, in the Cl_2 alone DWDS, the diameters of impedance loops decreased from 50 d to 140 d, and later increased with time, indicating that the corrosion rate was accelerated before 140 d, then the corrosion rate decreased. Furthermore, according to a described method (Gerengi et al., 2009), all impedance spectra were analyzed with an equivalent circuit, where R_s refers to resistance of electrolytes in bulk, R_{ct} refers to charge transfer resistance at metal surface, W refers to Warburg impedance, Q refers to constant phase element of the electrical double layer (Fig. S6). The analyzed values showed that Q parameter meaning double layer capacitance gradually decreased, while the charge transfer resistance, R_{ct} increased with time, confirming improvement of corrosion inhibition with time in the AR with UV/ Cl_2 (Table S2). Differently, in the AR with Cl_2 alone, before 140 d, the Q increased, while R_{ct} decreased with time, indicating an increase in the corrosion rate of the iron coupons. After 140 d, the Q decreased, and R_{ct} increased with time, indicating enhancement of corrosion inhibition with time (Table S2). Similarly, for stage I and stage II, the average weight loss values of the corrosion coupons were about 0.287 and 0.153 mm/year in the AR with UV/ Cl_2 , while that ones were about 0.195 and 0.168 mm/year, which confirmed the results of electrochemical measurements (Table 1).

3.3. Characterization of corrosion scales

Fig. 2 shows the XRD patterns of different corrosion scales on iron coupons in the two ARs. Green rusts were observed in

Table 1 – Corrosion rate (mm/year) in two ARs at different running stages^a.

	AR with UV/ Cl_2	AR with Cl_2
Stage I	0.287 ± 0.015	0.195 ± 0.03
Stage II	0.153 ± 0.011	0.168 ± 0.006

^a Values are mean $\pm$ standard deviation of two replicates.

corrosion scales of both ARs at stage I, which were layered double hydroxides containing Fe(II) and Fe(III), then disappeared in scales at stage II. Goethite (α -FeOOH), Calcite (CaCO_3), and Magnetite (Fe_3O_4) also were detected in all samples, however, their intensities were higher in the AR with UV/ Cl_2 than those in the AR with Cl_2 . In addition, the SEM micrographs of corrosion scales on the cast iron coupons under different conditions were obtained (Fig. S7). The corrosion products gradually formed round particles with increasing time in the AR with UV/ Cl_2 , while the products formed in the AR with Cl_2 alone exhibited a laminar structure at stage I, and partly converted into round particles with increasing time, implying the round particles were more stable. The results also indicated that the stable protective layer formed faster in the AR with UV/ Cl_2 than in the AR with Cl_2 alone.

3.4. Denitrifying functional gene abundance of biofilm

It has been reported that the oxidation and reduction of iron were induced by respiration of some denitrifying bacteria (Weber et al., 2006a). Therefore, denitrifying functional genes were analyzed quantitatively by qPCR. Fig. 3 shows the *nosZ*,

nirS, *nirK*, and 16S rRNA gene copy numbers of biofilm at different stages for the two ARs. The 16S rRNA gene increased through all stages, indicating an increase of microbial biomass. At stage II, the two ARs had nearly same 16S rRNA gene copy numbers, indicating same microbial biomass. However, the denitrifying functional gene copy numbers exhibited different changes with time in the two ARs. The three functional gene numbers greatly increased with increasing 16S rRNA gene numbers in the AR with UV/ Cl_2 , the *nirS* increased from 5.73×10^7 to 1.64×10^{10} copies/g (Fig. 3), and the total denitrifying gene numbers were 33.1% of 16S rRNA at stage II (Fig. S8). In the AR with Cl_2 , the three functional gene numbers slightly increased, and the total denitrifying gene numbers were 15.1% of 16S rRNA at stage II (Fig. S8).

Correspondingly, in bulk water, total of denitrifying functional genes exhibited a great increase at stage II through the AR with UV/ Cl_2 , while that one did not greatly change at stage I and stage II in the AR with Cl_2 alone. The 16S rRNA gene copy numbers decreased through all stages for the two ARs (Fig. S9). Significantly, the 16S rRNA gene copy numbers in bulk water with UV/ Cl_2 were smaller than that ones with Cl_2 alone, indicating the more inactivation of bacteria by UV (Fig. S9).

3.5. Bacterial community structure of biofilms

Pyrosequencing yielded a total of 27,032 high-quality sequences of the 16S rRNA gene for all samples. Table S3 shows the observed OTUs and diversity indexes of the bacteria phylotypes. Meanwhile, the estimated Shannon index for all samples expressed lower diversity of microbial community at stage II for both reactors. The OTU richness expressed by Chao 1 index indicates the highest OTU richness in UV/ Cl_2 reactor at stage I. The taxonomic breakdown at the class level was provided for all samples (Fig. S10). The bacterial community composition exhibited notable differences in the two ARs at different stages. In the AR with UV/ Cl_2 , at stage I, Alphaproteobacteria (48%) was the dominant class, other minor classes were Actinobacteria (15%), Acidobacteria (10%), Acidimicrobiidae

Fig. 2 – X-ray diffraction (XRD) patterns of corrosion scales on iron coupons at different stages in the AR with UV/ Cl_2 (A) and Cl_2 alone (B).

Fig. 3 – Quantitative PCR results on *nirK*, *nirS*, *nosZ* and 16S rRNA gene copy numbers in biofilm of different corrosion scales (expressed as log₁₀ gene copies per gram of corrosion scales). Error bars represented the standard deviation from the average.

(6%), Gammaproteobacteria (4%) and Betaproteobacteria (3%), while at stage II, Betaproteobacteria became the most abundant class (55%), Alphaproteobacteria, Acidobacteria and Actinobacteria decreased to 28%, 2% and 2% respectively. In the AR with Cl_2 alone, at stage I, the major classes were Alphaproteobacteria (39%) and Actinobacteria (32%), the minor groups were Betaproteobacteria (7%) and Gammaproteobacteria (5%) and Thermoleophilia (6%), while at stage II, Alphaproteobacteria and Betaproteobacteria dramatically increased to 62% and 27% respectively. Other classes greatly decreased to less than 3%.

Furthermore, the community structures of these dominant bacteria are compared at the genus level for different biofilms (Fig. 4). The compositions of bacteria related with iron cycling underwent great changes in different ARs and different stages. In the AR with UV/ Cl_2 , at stage I, the relative abundance of nitrate-reducing bacteria (NRB) was 9.9%, and among them, 3.9% NRB were related to redox cycling of iron, such as *Hyphomicrobium* (3.7%) (Stein et al., 2001), and 6% NRB produced siderophores, including *Sphingomonas* (Duckworth et al., 2009), *Brucella* (Roop, 2012), *Acinetobacter* (Proschak et al., 2013) and *Azospirillum* (Loaces et al., 2011). The iron-respiring bacteria were 11.7%, predominantly including iron-reducing bacteria (IRB) *Acidobacterium* (10.2%) (Nancuqueo and Johnson, 2010) and iron-oxidizing bacteria (IOB) *Pedomicrobium* (1.3%) (Braun et al., 2009). The rhizobia including *Bradyrhizobium*, *Rhizobium* and *Mesorhizobium* were 2.3%, which produced siderophores (Arif et al., 2012). At stage II, the dominant genera NRB were 64.1%, and among them, *Dechloromonas* (Weber et al., 2006a) and *Hyphomicrobium* were 45.23% and 1.3% inducing redox of iron, and 17.5% NRB produced siderophores. While IRB, including *Rhodobacter* (Dobbin et al., 1996), *Ferribacterium* (Cummins et al., 1999), *Acidobacterium* and *Albidiferax* (Lu et al., 2013), decreased to 3.6%, and IOB such as *Sediminibacterium* (Wang et al., 2012) and *Pedomicrobium* were 0.5%, the rhizobia were 2.6%. In the AR with Cl_2 alone, at stage I, the relative abundance of the same rhizobia was 7.7%, including *Bradyrhizobium*, *Rhizobium* and *Mesorhizobium*, while the relative abundance of NRB was 12.8%, including 6.5% NRB inducing redox of iron, such as *Hyphomicrobium* (3.5%) and *Herbaspirillum* (3%) (Geissler et al., 2011), and 6.3% NRB producing siderophores, such as *Sphingomonas*, *Acinetobacter* and

Azospirillum. The IRB *Acidobacterium* was at 1.5%. At stage II, the relative abundance of the rhizobia increased to 25.3%, and the NRB increased to 26.3%; however, among them, the NRB associated with iron redox cycling decreased to 4%, while the NRB producing siderophores were at 22.3%, including *Sphingomonas*, *Brucella*, *Acinetobacter* and *Azospirillum*. The IRB *Albidiferax* became 3.3% of the total bacteria. Therefore, the changes of NRB relative abundance were agreed with those ones of denitrifying functional gene numbers in the two ARs. The results indicated that UV disinfection enhanced denitrifying functional bacteria advantage in the biofilm of corrosion scales.

4. Discussion

The combination of UV irradiation as a primary disinfectant and free chlorine as a secondary disinfectant has been shown not only to prevent subsequent microbial regrowth but also to produce a synergetic disinfection (Shang et al., 2007). Therefore, the UV inactivation of bacteria in the influent possibly led to the advantage of denitrifying functional bacteria in biofilm and bulk water through the AR with UV/ Cl_2 . On the other hand, the above corrosion data suggested that different bacterial community composition of biofilm may affect the corrosion process of iron. Although at stage I, there were same water chemical parameters due to same initial chlorine dose, different corrosion rate occurred in the two ARs, indicating microorganism played a great role in the corrosion of iron. Moreover, at stage II, in order to maintain same residual chlorine (0.08 mg/L), a less initial chlorine dose was required in the AR with UV/ Cl_2 than that one with Cl_2 alone. The results suggested that less chlorine was consumed by the corrosion in the AR with UV/ Cl_2 , indicating the tested chlorine dose had less influence than microorganism on the corrosion under the experimental conditions.

Principal component analysis (PCA) was used to correlate the corrosion-related bacteria in genus level and the corrosion current density (i_{corr}) in this research (Fig. 5). The biofilm in the two ARs at stage I, including the genera *Pedomicrobium* (IOB), *Acidobacterium* (IRB), *Hyphomicrobium* (NRB) and *Herbaspirillum* (NRB), was positively correlated with i_{corr} , enhancing corrosion of iron coupon. Glycolic acid was produced by the growth of *Acidobacterium*, leading extremely acidic environments to enhancing corrosion of iron coupon, while *Hyphomicrobium* and *Herbaspirillum* were related to nitrate-dependent Fe(II) oxidation, they and IOB *Pedomicrobium* played corrosive role for the iron coupons at stage I. In the AR with UV/ Cl_2 , *Acidobacterium*, *Hyphomicrobium* and *Pedomicrobium* were dominant for 10.2%, 3.7% and 1.29% respectively. Moreover, their total relative abundances were higher than those of *Hyphomicrobium* (3.45%), *Acidobacterium* (1.48%) and *Herbaspirillum* (3.02%) in AR with Cl_2 , leading to higher corrosion rate and faster precipitation of iron oxides in the AR with UV/ Cl_2 at stage I.

At the stage II, with the formation of corrosion layer, the biofilms in the two ARs exhibited negative correlation with i_{corr} , indicating microbial corrosion inhibition. The main bacteria were *Dechloromonas*, *Ferribacterium*, *Sphingomonas*, *Brucella*, *Sediminibacterium*, *Bradyrhizobium*, *Rhodobacter*, *Albidiferax*

Fig. 4 – Corrosion-related bacterial genera by relative abundance in biofilms from different corrosion scales.

Fig. 5 – Principal component analysis (PCA) based on a correlation matrix combining corrosion current density (i_{corr}) and the abundance of corrosion-related genera in the biofilm of cast iron coupons. (UI, UII, CI, CII represented the sample from AR with UV/Cl₂ and AR with Cl₂ alone at stage I and stage II, respectively.

and *Rhizobium* in the following order of decreasing effect of the genera on i_{corr} . It indicated that *Dechloromonas* (NRB) and *Ferribacterium* (IRB) played a great role in the corrosion inhibition of cast iron coupons. The two genera only presented in the AR with UV/Cl₂, at stage II, and the relative abundance of *Dechloromonas* (NRB) was 45.23%, correspondingly, the denitrifying functional gene copy numbers were 33.1%. The results indicated most of *Dechloromonas* had denitrifying function. It has been reported that in NO₃ concentration less than 31 mg/L, the oxidation and reduction of iron occurred simultaneously by the respiration of *Dechloromonas*, resulting in Fe₃O₄ formation (Weber et al., 2006a, 2006b). Under the above conditions, the cycling of Fe(II)/Fe(III) were driven by *Dechloromonas* because the concentration of NO₃ was less than 10 mg/L in the influent, and the interaction of iron and NRB could enhance the precipitation of iron oxide, the formation of Fe₃O₄ and consumption of oxygen, causing higher corrosion inhibition. The next corrosion-inhibition bacteria *Sphingomonas* (9.03%), *Brucella* (7.84%) produced and imported siderophores to be biochelators capturing iron to inhibit the dissolution of iron and iron corrosion (Grateron et al., 2007; Little et al., 2007; Raymond and Dertz, 2004). These microbial roles could avoid the iron release when the corrosivity of influent water increased in the AR with UV/Cl₂. In contrast, the main corrosion-inhibition bacteria were these bacteria produced siderophores, including *Sphingomonas* (9.64%), *Bradyrhizobium* (10.4%), *Brucella* (12.31%) and *Rhizobium* (14.7%) in the AR with Cl₂, while the NRB related with iron cycling was very low. The main corrosion inhibition was carried out by siderophores capturing iron from these bacteria growth. The lower corrosion-inhibition effect led to the higher iron release for higher corrosivity of influent water in the AR with Cl₂.

5. Conclusions

The results verified that the corrosion occurred faster and was more rapidly inhibited to form more stable protective layer in the AR with UV/Cl₂ than in that one with Cl₂ alone. UV disinfection not only reduced the required initial chlorine dose, but also enhanced denitrifying functional bacteria advantage in the biofilm of corrosion scales. PCA analysis showed that the NRB *Dechloromonas* and IRB *Ferribacterium* exhibited greatest inhibition effect on the iron corrosion, while the rhizobia *Bradyrhizobium* and *Rhizobium*, and the NRB *Sphingomonas* and *Brucella* presented weaker effect on the corrosion inhibition. The NRB *Dechloromonas* was main corrosion-inhibition bacteria in the AR with UV/Cl₂. Its metabolism induced the cycling of Fe(II)/Fe(III) to increase the precipitation of iron oxides and Fe₃O₄ formation, enhancing the stability of corrosion scales, inhibiting the iron release for influent water with higher corrosivity. In the AR with Cl₂, the dominant rhizobia and NRB *Sphingomonas* *Brucella* inhibited the corrosion by producing siderophores to capture iron, leading slower corrosion inhibition, which was contributed to the iron release for the higher influent water corrosivity.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2014.04.035>.

REFERENCES

- Arif, K., Archana, G., Anjana, D.J., 2012. Engineering heterologous iron siderophore complex utilization in rhizobia: effect on growth of peanut and pigeon pea plants. *Appl. Soil. Ecol.* 53, 65–73.
- ASTM Standard G 31, 1994. Standard practice for laboratory immersion corrosion testing of metals. In: *Annual Book of ASTM Standards*, vol. 03. ASTM International, West Conshohocken, PA, 02.
- Borenstein, S.W., 1994. *Microbiologically in Corrosion Handbook*. Woodhead Publishing.
- Bouskill, N.J., Eveillard, D., Chien, D., Jayakumar, A., Ward, B.B., 2012. Environmental factors determining ammonia-oxidizing organism distribution and diversity in marine environments. *Environ. Microbiol.* 14 (3), 714–729.
- Braun, B., Richert, I., Szwedzyk, U., 2009. Detection of iron-depositing *Pedomicrobium* species in native biofilms from the Odertal National Park by a new, specific FISH probe. *J. Microbiol. Methods* 79 (1), 37–43.

- Chun, C.L., Hozalski, R.M., Arnold, W.A., 2005. Degradation of drinking water disinfection byproducts by synthetic goethite and magnetite. *Environ. Sci. Technol.* 39 (21), 8525–8532.
- Cummings, D.E., Caccavo, F., Spring, S., Rosenzweig, R.F., 1999. *Ferribacterium limneticum*, gen. nov., sp. nov., an Fe(III)-reducing microorganism isolated from mining-impacted freshwater lake sediments. *Archives Microbiol.* 171 (3), 183–188.
- Dobbin, P.S., Warren, L.H., Cook, N.J., McEwan, A.G., Powell, A.K., Richardson, D.J., 1996. Dissimilatory iron(III) reduction by *Rhodobacter capsulatus*. *Microbiology* 142 (4), 765–774.
- Dubiel, M., Hsu, C.H., Chien, C.C., Mansfeld, F., Newman, D.K., 2002. Microbial iron respiration can protect steel from corrosion. *Appl. Environ. Microbiol.* 68 (3), 1440–1445.
- Duckworth, O.W., Holmstroem, S.J.M., Pena, J., Sposito, G., 2009. Biogeochemistry of iron oxidation in a circumneutral freshwater habitat. *Chem. Geol.* 260 (3–4), 149–158.
- EPA of China, 2002. Analysis Method for Water and Waster Water, fourth ed. Press of Chinese Environmental Science.
- Geissler, A., Law, G.T.W., Boothman, C., Morris, K., Burke, I.T., Livens, F.R., Lloyd, J.R., 2011. Microbial communities associated with the oxidation of iron and technetium in bioreduced sediments. *Geomicrobiol. J.* 28 (5–6), 507–518.
- Gerengi, H., Darowicki, K., Bereket, G., Slepski, P., 2009. Evaluation of corrosion inhibition of brass-118 in artificial seawater by benzotriazole using dynamic EIS. *Corros. Sci.* 51 (11), 2573–2579.
- Gerke, T.L., Maynard, J.B., Schock, M.R., Lytle, D.L., 2008. Physiochemical characterization of five iron tubercles from a single drinking water distribution system: possible new insights on their formation and growth. *Corros. Sci.* 50 (7), 2030–2039.
- Grateron, C., Barbosa, O., Rueda, N., Ortiz-Lopez, C., Torres, R., 2007. AS7, a bacterial siderophore valuable as a high performance corrosion inhibitor. *J. Biotechnol.* 131 (2), S87–S88.
- Hansen, H.C.B., Koch, C.B., NanckeKrogh, H., Borggaard, O.K., Sorensen, J., 1996. Abiotic nitrate reduction to ammonium: key role of green rust. *Environ. Sci. Technol.* 30 (6), 2053–2056.
- Husband, P.S., Boxall, J.B., 2011. Asset deterioration and discolouration in water distribution systems. *Water Res.* 45 (1), 113–124.
- Ishii, S.K.L., Boyer, T.H., 2011. Evaluating the secondary effects of magnetic ion exchange: focus on corrosion potential in the distribution system. *Desalination* 274 (1–3), 31–38.
- Lai, C.Y., Yang, X., Tang, Y., Rittmann, B.E., Zhao, H.P., 2014. Nitrate shaped the selenate-reducing microbial community in a hydrogen-based biofilm reactor. *Environ. Sci. Technol.* 48 (6), 3395–3402.
- Li, D., Alidina, M., Ouf, M., Sharp, J.O., Saikaly, P., Drewes, J.E., 2013. Microbial community evolution during simulated managed aquifer recharge in response to different biodegradable dissolved organic carbon (BDOC) concentrations. *Water Res.* 47 (7), 2421–2430.
- Little, B., Lee, J., Ray, R., 2007. A review of 'green' strategies to prevent or mitigate microbiologically influenced corrosion. *Biofouling* 23 (2), 87–97.
- Loaces, I., Ferrando, L., Scavino, A.F., 2011. Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. *Microb. Ecol.* 61 (3), 606–618.
- Lu, S., Chourey, K., Reiche, M., Nietzsche, S., Shah, M.B., Neu, T.R., Hettich, R.L., Kusel, K., 2013. Insights into the structure and metabolic function of microbes that shape pelagic iron-rich aggregates ("iron snow"). *Appl. Environ. Microbiol.* 79 (14), 4272–4281.
- Mansfeld, F., Little, B., 1991. A technical review of electrochemical techniques applied to microbiologically influenced corrosion. *Corros. Sci.* 32 (3), 247–272.
- McNeill, L.S., Edwards, M., 2001. Iron pipe corrosion in distribution systems. *J. Am. Water Works Assoc.* 93 (7), 88–100.
- Melidis, P., Sanozidou, M., Mandusa, A., Ouzounis, K., 2007. Corrosion control by using indirect methods. *Desalination* 213 (1–3), 152–158.
- Murphy, H.M., Payne, S.J., Gagnon, G.A., 2008. Sequential UV- and chlorine-based disinfection to mitigate *Escherichia coli* in drinking water biofilms. *Water Res.* 42 (8–9), 2083–2092.
- Nancucheo, I., Johnson, D.B., 2010. Production of glycolic acid by chemolithotrophic iron- and sulfur-oxidizing bacteria and its role in delineating and sustaining acidophilic sulfide mineral-oxidizing consortia. *Appl. Environ. Microbiol.* 76 (2), 461–467.
- Nawrocki, J., Raczyk-Stanislawiak, U., Swietlik, J., Olejnik, A., Sroka, M.J., 2010. Corrosion in a distribution system: steady water and its composition. *Water Res.* 44 (6), 1863–1872.
- Proschak, A., Lubuta, P., Gruen, P., Loehr, F., Wilharm, G., De Berardinis, V., Bode, H.B., 2013. Structure and biosynthesis of fimsbactins A-F, siderophores from *Acinetobacter baumannii* and *Acinetobacter baylyi*. *ChemBioChem* 14 (5), 633–638.
- Rand, J.L., Hofmann, R., Alam, M.Z.B., Chauret, C., Cantwell, R., Andrews, R.C., Gagnon, G.A., 2007. A field study evaluation for mitigating biofouling with chlorine dioxide or chlorine integrated with UV disinfection. *Water Res.* 41 (9), 1939–1948.
- Raymond, K.N., Dertz, E.A., 2004. Biochemical and Physical Properties of Siderophores. ASM Press.
- Roop II, R.M., 2012. Metal acquisition and virulence in *Brucella*. *Animal Health Res. Rev.* 13 (1), 10–20.
- Shang, C., Cheung, L.M., Liu, W., 2007. MS2 coliphage inactivation with UV irradiation and free chlorine/monochloramine. *Environ. Eng. Sci.* 24 (9), 1321–1332.
- Stein, L.Y., La Duc, M.T., Grundl, T.J., Nealson, K.H., 2001. Bacterial and archaeal populations associated with freshwater ferromanganous micronodules and sediments. *Environ. Microbiol.* 3 (1), 10–18.
- Wang, H.B., Hu, C., Hu, X.X., Yang, M., Qu, J.H., 2012. Effects of disinfectant and biofilm on the corrosion of cast iron pipes in a reclaimed water distribution system. *Water Res.* 46 (4), 1070–1078.
- Weber, K.A., Urrutia, M.M., Churchill, P.F., Kukkadapu, R.K., Roden, E.E., 2006a. Anaerobic redox cycling of iron by freshwater sediment microorganisms. *Environ. Microbiol.* 8 (1), 100–113.
- Weber, K.A., Achenbach, L.A., Coates, J.D., 2006b. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nat. Rev. Microbiol.* 4 (10), 752–764.
- Yang, F., Shi, B.Y., Gu, J.N., Wang, D.S., Yang, M., 2012. Morphological and physicochemical characteristics of iron corrosion scales formed under different water source histories in a drinking water distribution system. *Water Res.* 46 (16), 5423–5433.
- Yuan, S.J., Choong, A.M.F., Pehkonen, S.O., 2007. The influence of the marine aerobic *Pseudomonas* strain on the corrosion of 70/30 Cu–Ni alloy. *Corros. Sci.* 49 (12), 4352–4385.
- Zhang, Y., Griffin, A., Edwards, M., 2010. Effect of nitrification on corrosion of galvanized iron, copper, and concrete. *J. Am. Water Works Assoc.* 102 (4), 83–93.
- Zhao, H.P., Van Ginkel, S., Tang, Y.N., Kang, D.W., Rittmann, B., Krajmalnik-Brown, R., 2011. Interactions between perchlorate and nitrate reductions in the biofilm of a hydrogen-based membrane biofilm reactor. *Environ. Sci. Technol.* 45 (23), 10155–10162.
- Zhao, H.P., Ontiveros-Valencia, A., Tang, Y.N., Kim, B.O., Ilhan, Z.E., Krajmalnik-Brown, R., Rittmann, B., 2013. Using a two-stage hydrogen-based membrane biofilm reactor (MBfR) to achieve complete perchlorate reduction in the presence of nitrate and sulfate. *Environ. Sci. Technol.* 47 (3), 1565–1572.