Characteristics of biofilms and iron corrosion scales with ground and surface waters in drinking water distribution systems

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ABSTRACT

The composition of biofilm bacterial communities and iron corrosion scales were studied in two drinking water distribution systems transporting groundwater (DWDS-GW) and surface water (DWDS-SW). Loose corrosion products were formed containing α -FeOOH in DWDS-GW, while dense crystallized particles were formed in DWDS-SW, including α -FeOOH, Fe_3O_4 and CaCO_3 . The biofilms in the two systems had the same bacterial diversity, denitrifying functional genes and 16S rRNA gene copies, but the bacterial communities were very different. It was found that nitrate-reducing bacteria in biofilms that are associated with iron cycling played a large role in the formation of Fe_3O_4 and corrosion layers.

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1. Introduction

Cast iron pipes have been used in drinking water distribution systems (DWDSs) for over 500 years [1]. Corrosion of cast iron pipes is the most common distribution system problem [2–4], and it can cause three distinct but related problems: pipe mass loss, decreasing water capacity, and production of red water at the tap. Corrosion scales in iron pipes interact with finished water with resulting adverse effects on distribution water quality, possibly leading to release of undesirable substances.

In recent years, several serious red water cases have occurred due to switching of source water, such as in Southern California, Tucson, Arizona and Tampa, Florida in the United States [5–7]. In October, 2008, red water appeared in some areas in a city in Northern China soon after 80% of the local source water was replaced with source water from a neighboring province. It was noticed that the areas suffering from red water were historically supplied with local groundwater, but the areas without red water were historically supplied with local surface waters [8]. The Tucson, Arizona and Tampa, Florida red water cases were also caused by source water switches from groundwater to surface water. These phenomena indicated that the structure and morphology of corrosion scales played important roles in changes of distribution water quality from the interaction of corrosion scales with finished water. It was reported that thick corrosion scales or densely

distributed corrosion tubercles were mostly found in pipes transporting surface water, but thin corrosion scales and hollow tubercles were mostly discovered in pipes transporting groundwater [8]. Unstable and less protective corrosion scale is one of the main factors causing red water when the quality of water entering a distribution system changes significantly. Therefore, previous studies of iron pipe corrosion have focused on pipe degradation, corrosion scale formation, and iron release. A large number of factors were examined for their influence on iron pipe corrosion parameters, including water quality and composition, flow conditions, biological activity, and corrosion inhibitors [9–11]. In particular, some contradictory results have appeared on the effects of chloride and sulfate on iron corrosion [1,12,13], so the Larson-Skold Index does not provide an effective means of controlling iron corrosion, and biofilms may be used as an alternative method to combat corrosion [14].

Microbes perform oxidation and reduction reactions with iron to greatly affect the stability of minerals in the environment and are thought to play an important role in iron transformation [15,16]. Microbiologically influenced corrosion (MIC) affects diverse processes in DWDSs. Some theories about possible mechanisms of MIC exist, with the process depending on both the type of microorganisms involved and the environmental conditions experienced. The existing literatures on MIC are somewhat complex and confusing because bacterial activity at metal surfaces has been shown to both increase and in some cases reduce corrosion rates [17,18]. It is estimated that approximately 20% of annual damage due to corrosion in metals results from microbial activities, of which a

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significant part is due to aerobic corrosion influenced by iron-oxidizing bacteria (IOB) [19]. In addition, anaerobes such as sulfate-reducing (SRB) and iron-reducing bacteria (IRB) were reported to promote corrosion [20,21], while IRB exhibited a protective effect in other studies [22]. Recently, Xu et al. [23] found that a nitrate reducer was more corrosive than typical SRB by pitting data under strict anaerobic conditions. On the other hand, the nitrate-dependent Fe(II) oxidation from nitrate-reducing bacteria (NRB) metabolism has the potential to contribute to a dynamic iron redox cycle in freshwater sediments [16], which has been proven inhibiting iron corrosion [24]. Additionally, more and more studies have begun to show protective effects against corrosion by some bacterial biofilms on metal. Biofilms comprising iron-respiring bacteria, such as *Bacillus infernos*, *Desulfovibrio profundus*, and *Geothrix fermentans*, may reduce rather than accelerate the corrosion rate of steel [14]. Recent progress in corrosion inhibition using beneficial bacterial biofilms (BBB) has been reviewed [25]. Biofilm communities on corrosion scales are heterogeneous in DWDSs, so their effects on corrosion predominantly depend on the environmental characteristics of the metal/biofilm/medium interface.

In this present study, groundwater (GW) and surface water (SW) were selected to investigate the effects of water-bacterial community composition of biofilms and corrosion of cast iron pipes in DWDSs. Corrosion behavior and corrosion scales were characterized by X-ray powder diffraction (XRD), scanning electron microscopy (SEM) and Brunauer–Emmett–Teller (BET) analysis. Quantitative real-time PCR (qPCR) was used to monitor changes in the microbial abundances of biofilms inside corrosion scales according to specific groups: total bacteria based on the 16S rRNA gene and denitrifiers based on the functional genes *nosZ*, *nirK* and *nirS*. Most probable number enumerations of NRB, IRB and nitrate-dependent Fe(II)-oxidizing microorganisms were detected under different conditions for different corrosion scale biofilms. Pyrosequencing was used to monitor changes in diversity in the microbial community of biofilms, including corrosion-related bacteria. The relationship between corrosion and biofilms is discussed.

2. Materials and methods

2.1. Source water

This study focused on two principal water sources, groundwater (GW) and surface water (SW), in Northern China. The GW source is untreated groundwater prior to contact tank chlorination. The tested SW 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.

2.2. Pilot pipe distribution systems

Two pilot distribution systems were assembled with new cast iron pipe materials to transport GW and SW. The length of each line was approximately 50 cm. Pipe diameters were 10 cm. The elemental compositions (wt.%) of the cast iron pipe were C 3.21%, O 1.63%, Al 1.1%, Si 1.85%, and Fe 92.21%. The pilot systems were operated with a two-day hydraulic retention time (HRT) to reflect dead zones or worst-case conditions in an actual distribution system [7], in order to study the formation of corrosion scales and changes in biofilms. Chlorine was dosed from a stock solution of sodium hypochlorite (NaClO). The initial chlorine concentration in influents was approximately 10 mg L⁻¹ in the two DWDSs, and the average chlorine concentration in effluents of GW and SW DWDSs was 0.27 mg L⁻¹ and 0.13 mg L⁻¹, respectively.

2.3. Weight loss

To determine the corrosion rate in DWDSs, cast iron coupons, 80 × 15 × 5 mm, were immersed in covered 250 mL glass bottles filled with each of GW and SW containing chlorine disinfection, respectively. The bottles were kept in dark and triplicates were measured monthly. The elemental compositions (wt.%) of the iron coupons were 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%. The water was displaced with fresh water at 2 d intervals same as the tested pilot systems with a two-day hydraulic retention time. The weight loss method was utilized to determine the corrosion rate [24].

2.4. Physicochemical analysis

Water quality parameters, such as pH, alkalinity and chlorine concentration, were analyzed according to standard methods [26]. Chloride (Cl⁻), sulfate (SO₄²⁻) and nitrate (NO₃⁻) were analyzed by ion chromatography (DIONEX ICS-2000). Dissolved oxygen (DO) was monitored with an Orion 4 Star meter (Thermo, USA). The Larson–Skold Index (LI) was calculated using the concentration of chlorides, sulfates and bicarbonates [27]:

$$LI = \frac{[Cl^-] + 2[SO_4^{2-}]}{[HCO_3^-]}.$$

The corrosion scales were measured by an X-ray powder diffractometer (X'Pert PRO MPD, PANalytical, Almelo, The Netherlands) and photographed using scanning electron microscopy (SEM) (S-3000N, Hitachi, Japan). Nitrogen adsorption/desorption experiments were carried out on various samples using a Micromeritics ASAP 2020 analyser (Micromeritics ASAP 2020, USA).

2.5. Sampling and extraction of total DNA from water and biofilms

For bulk water samples, bacteria from 3 L of each water sample were harvested by membrane filtration with 0.22 μm Millipore GTTP filters and then transferred to sterile tubes for isolation [28]. For corrosion scales with biofilms, samples were collected with a sterile metal spatula scraping approximately 3 cm² surface area from 6 different locations on each pipe [29]. Then, the samples from the same pipe were mixed and lyophilized for further characterization.

DNA was extracted in triplicate samples with the Fast DNA[®] SPIN Kit for soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions [30]. DNA quality was checked on an agarose gel, and concentrations were measured with a NanoDrop (ND-1000, NanoDrop, USA). All DNA samples were stored at –80 °C for future use.

2.6. Quantitative PCR assay

To quantify all bacteria and denitrifiers in water and biofilm samples, quantitative PCR (qPCR) targeting 16S rRNA, *nirK*, *nirS* and *nosZ* genes was performed. For all bacteria, primer pairs 1369F and 1492R were combined with probe TM1389F [31]; for denitrifiers, *nirK* 876 and *nirK* 1040, R3cd and cd3aF, and *nosZ*-F and 1622R were used (Table 1) [32–34]. qPCR experiments were carried out with the Applied Biosystems 7300 qPCR system using premix Ex Taq[™] or SYBR[®] premix Ex Taq[™] (TaKaRa) in a 25 μL reaction volume. The annealing temperatures used were 60 °C for universal primer pairs, 55 °C for *nirK* 876/*nirK* 1040, 53 °C for R3cd/cd3aF and 58 °C for *nosZ*-F/1622R, which were determined by gradient PCR to be the most suitable for the present samples.

Table 1
Primer pairs used for qPCR analysis.

Designation	Sequence (5'–3')	Target
1369F	CGGTGAATACGTTTCYCGG	All bacteria 16S rRNA
1492R	TACGGYTACCTTGTACGACTT	
Probe TM1389F	TACGGYTACCTTGTACGACTT	
<i>nirK</i> 876	ATYGGCGVCAYGGCGA	Denitrifying bacteria <i>nirK</i>
<i>nirK</i> 1040	GCCTCGATCAGRTTGTGTT	
cd3aF	AACGSAAGGARACSGG	Denitrifying bacteria <i>nirS</i>
R3cd	GASTTCGGRTGSGTCTTSAYGAA	
<i>nosZ</i> -F	CGYTGTTCMTGACAGCCAG	Denitrifying bacteria <i>nosZ</i>
1622R	CGSACCTTSTGCCSTYCGG	

2.7. Pyrosequencing analysis

DNA was amplified with bacterium-specific forward primer 341F (5'-Fusion A-Barcode-CA linker-CCTACGGGAGGCAGCAG-3') and reverse primer 1073R (5'-Fusion B-TC linker-ACGAGCTGAC-GACARCCATG-3') [30]. The PCR products of three independent reactions were pooled together, purified with the AxyPrep DNA Gel Extraction Kit (Axygen, USA), and quantified using a TBS-380 Fluorometer (Turner Biosystems, USA). Before pyrosequencing, PCR products from different samples were normalized to equimolar amounts in the final mixture, which was used to construct PCR amplicon libraries. The pyrosequencing was carried out on a Roche massively parallel 454 GS-FLX Titanium sequencer (Roche 454 Life Sciences, Branford, CT, USA) according to standard protocols. Pyrosequencing flowgrams were converted to sequence reads using MOTHUR software (<http://www.mothur.org/>) and then analyzed using the UCHIME (<http://www.drive5.com/uchime>) standard pipeline. Sequence reads were initially filtered and denoised to remove low quality or ambiguous reads. High quality sequences (>200 bp in length, quality score > 25, exact match to barcode and primer, and containing no ambiguous characters) remained with an average length of 436 bp. The total bacterial communities were analyzed for the number of operational taxonomic units (OTUs) and species richness using the Mothur program. The numbers of OTUs were estimated at 97% 16S rRNA gene sequence similarity. Taxonomic positions of representative sequences were assigned using the SILVA database (<http://www.arb-silva.de/>).

2.8. Most probable number (MPN) enumerations

In the all MPN experiments, for corrosion scales with biofilms, samples were scraped with a sterile metal spatula on the surface of each pipe. Upon return to laboratory, corrosion scales were homogenized and placed in an N₂ atmosphere at room temperature. The abundance of culturable acetate-oxidizing NRB, acetate-oxidizing IRB and nitrate-dependent Fe(II)-oxidizing microorganisms of biofilms inside corrosion scales were estimated using a three-tube MPN technique [16]. Triplicate pressure tubes containing sterile, anaerobic (N₂) AGW medium (10 mM PIPES, 2 mM NaHCO₃, 5 mM NH₄Cl, 0.5 mM KH₂PO₄, pH 6.8) were inoculated with serially diluted homogenized corrosion scales. For enumeration of acetate-oxidizing NRB, tubes were amended with 5 mM NaNO₃ and 10 mM Na-acetate. Acetate-oxidizing, IRB were enumerated in medium amended with 10 mM synthetic hydrous ferric oxide, 10 mM Na-acetate and 2 mM FeCl₂ (as a reducing agent). The medium for enumeration of nitrate-dependent Fe(II)-oxidizing bacteria was amended with 0.5 mM Na-acetate, 5 mM NO₃⁻ and 10 mM FeCl₂. Pressure tubes were incubated statically in the dark at 30 °C for 10 weeks. Positive results for acetate-oxidizing nitrate reducers were determined checking for depletion of NO₃⁻ (to <1 mM). Visual assessment of medium blackening and formation of reddish-brown precipitates was used to identify positive results

for Fe(III) reducers and nitrate-dependent Fe(II) oxidizers, respectively. Hydrous ferric oxide was synthesized according to the described method [35].

2.9. Statistical analysis

Student's paired *t*-tests were performed on experimental data using SPSS software (version 16.0), and *p* < 0.05 was considered statistically significant.

3. Results

3.1. Water quality of water sources and effluents

Significant differences between the water quality of groundwater (GW) and surface water (SW) are listed in Table 2. In GW, the concentration of nitrate-N was 5.04–7.89 mg L⁻¹, LI was 0.22–0.31; and these values were 0.87–2.17 mg L⁻¹, 0.60–1.05 in SW, respectively. In the effluents from DWDSs, DO decreased (*p* < 0.05) to approximately 1 mg L⁻¹. Alkalinity in GW and SW DWDSs decreased to 118 mg L⁻¹ (*p* < 0.05) and 89 mg L⁻¹ (*p* < 0.05), respectively; the pH and SO₄²⁻ did not change significantly, and together with the change in chloride concentration, resulted in an increase in the LI index. The total iron concentration of effluents in DWDS-GW was 1.78 mg L⁻¹ at 7 d, and increased to 2.32 mg L⁻¹ at 11 d. Then it showed a little fluctuation at around 2 mg L⁻¹ from 11 d to 50 d. After that, it gradually decreased to 1.62 mg L⁻¹ at 73 d, but quickly decreased to 0.33 mg L⁻¹ at 91 d, then fluctuated from 0.57 to 0.96 mg L⁻¹ during 101 to 168 d, and increased to approximately 1.09 mg L⁻¹ after 168 d (Fig. 1). In DWDSs transporting SW, the concentration of total iron in effluents fluctuated from 1.05 to 1.92 mg L⁻¹ before 70 d, then decreased to 0.57 at 76 d, but increased to approximately 1.83 mg L⁻¹ after

Table 2
Water quality of water source and effluents.

Parameters	GW	GW-effluent	SW	SW-effluent
pH	7.60–8.06 ^a	8.20 ± 0.38 ^b	7.62–8.10	8.04 ± 0.29
DO (mg L ⁻¹)	7.35–7.46	0.95 ± 0.16	7.93–8.27	1.17 ± 0.08
Cl ⁻ (mg L ⁻¹)	14.13–16.02	31.45 ± 2.80	19.85–22.37	43.06 ± 3.30
SO ₄ ²⁻ (mg L ⁻¹)	21.41–24.85	19.09 ± 1.91	50.22–76.46	53.92 ± 2.30
NO ₃ ⁻ -N (mg L ⁻¹)	5.04–7.89	–	0.87–2.17	–
Alkalinity (mg ^c L ⁻¹)	155–198	118 ± 7	106–138	88 ± 5
LI	0.22–0.31	0.61 ± 0.15	0.60–1.05	1.41 ± 0.17

^a Value range.

^b Mean ± standard deviation.

^c mg CaCO₃.

Fig. 1. Total iron concentration in effluents of GW and SW DWDSs with increasing time.

Table 3
Corrosion rate (mm/year) in two waters for different time.

Time	GW	SW
31 d	0.150 ± 0.005	0.156 ± 0.000
65 d	0.0646 ± 0.001	0.142 ± 0.005

Fig. 2. Changes of nitrate and ammonium in influents and effluents in GW (A) and SW (B) DWDSs. Error bars represent one standard deviation.

91 d, and stabilized at approximately 1.03 mg L⁻¹ except the point at 210 d (Fig. 1). Furthermore, the total iron concentration of effluents in the two DWDSs showed no significant difference ($p = 0.68$). However, for 31 d and 65 d, the average weight loss values of the corrosion coupons were about 0.15, and 0.0646 mm/year in GW DWDSs, while that ones were about 0.156 and 0.142 mm/year (Table 3) in SW DWDSs, indicating that iron release in two systems was not agreed with corrosion rate of iron.

In addition, the two DWDSs exhibit greatly changes of NO₃⁻-N with increasing time (Fig. 2). In GW DWDSs, NO₃⁻-N removal greatly increased about 3.53 mg L⁻¹ before 64 d, then gradually increased to 5.55 mg L⁻¹ at 135 d, and tended to a stable value of 4.28 mg L⁻¹, while the concentration of NH₄⁺-N in effluent increased to a maximum about 0.78 mg L⁻¹ throughout experimental time, indicating that most of nitrate were not transformed to ammonium. Also the nitrite concentration was below the detection limit during this period. The results suggested that most of nitrate was biologically denitrified to N₂, not converted to ammonium by the reduction of zero-valent iron [36,37]. The same phenomena occurred in SW DWDSs, where 1 mg L⁻¹ of NO₃⁻-N was removed after 130 d.

3.2. Characterization of corrosion scales on cast iron pipes

Fig. 3 shows the XRD patterns of corrosion scales at different time points from DWDSs transporting GW and SW. In the GW DWDSs, the predominant crystalline corrosion product was goethite (α -FeOOH) with peak intensities increasing with increasing

Fig. 3. XRD patterns of corrosion scales on cast iron pipes in DWDS-GW (A) and DWDS-SW (B) at different times (Ca-CaCO₃, G- α -FeOOH, L- γ -FeOOH, M-Fe₃O₄).

exposure. In addition, CaCO₃ was observed in the 79 d sample, and then disappeared in other samples. In the SW DWDSs, the main crystalline compounds were α -FeOOH and γ -FeOOH in the sample after a 79 d exposure. The peak intensity of α -FeOOH increased up to 220 d and then decreased from 220 to 550 d, whereas the proportion of Fe₃O₄ increased with increasing time. The intensity of γ -FeOOH peaks decreased from 79 to 220 d, and then increased with time, indicating that γ -FeOOH was unstable [9]. The results suggested that Fe₃O₄ was formed from the transformation of α -FeOOH and γ -FeOOH. CaCO₃ appeared in samples from the 550 d exposure. Figs. 4 and 5 show representative SEM micrographs of the corrosion scales on the GW and SW DWDSs at different time points. The GW DWDSs exhibited loosely amorphous corrosion scales before 137 d, and filaments of α -FeOOH had formed by 220 d. In the SW DWDSs, well-crystallized particles were observed at 79 d, indicating the formation of a dense oxide layer, and more densely crystallized particles appeared at 137 and 220 d, indicating stable protective layer formation. The BET surface areas of the corrosion scales were approximately 85.5 and 18.8 m² g⁻¹ from GW and SW DWDSs, respectively. This result confirmed that loose corrosion layers formed on the pipe transporting GW, while a compact corrosion layer formed on the pipe transporting SW, which agreed with the description from Yang et al. [8]. Furthermore, as a reference, in sanitized SW with chlorination, the corrosion scales were characterized by XRD and SEM in order to illustrate the impact of water chemical parameters and microorganisms on the structure of iron corrosion products. The main compounds were α -FeOOH and CaCO₃ (Fig. 6). These peak intensities increased with increasing time. There was no Fe₃O₄ formation in corrosion scales through the entire experimental period in sanitized water, and the corrosion products exhibited flower-like crystals and porous structures (Fig. 7), and its BET surface area was approximately 50.8 m² g⁻¹.

Fig. 4. SEM micrographs of corrosion scales in a GW pipe for different amounts of time (A: 79 d; B: 137 d; C: 220 d).

Fig. 5. SEM micrographs of corrosion scales in a SW pipe for different amounts of time (A: 79 d; B: 137 d; C: 220 d).

3.3. Denitrifying functional genes and most probable number enumerations

The Fe(II) oxidation and Fe(III) reduction were induced by respiration of some denitrifying bacteria [16], which may have affected the formation of corrosion products. Therefore, denitrifying functional genes were analyzed quantitatively by qPCR. Fig. 8 shows *nosZ*, *nirS*, *nirK*, and 16S rRNA gene copy numbers at different stages for the two DWDSs. In the source water and effluents from DWDSs, the 16S rRNA gene copy number was 2.02×10^7 copies mL⁻¹ in source GW, and it was not significantly different after 137 d ($p > 0.05$) and 220 d ($p > 0.05$) in GW effluent (Fig. 8A), but it was approximately 1.23×10^{11} copies g⁻¹ in the biofilm (Fig. 8C). For

source SW, the 16S rRNA gene copy number was 2.24×10^7 copies mL⁻¹, and it was approximately 6.30×10^6 copies mL⁻¹ in SW effluent (Fig. 8B), but it was 1.05×10^{11} copies g⁻¹ in the corrosion scale biofilm (Fig. 8C). The sum of *nosZ*, *nirS* and *nirK* increased from 1.48×10^5 copies mL⁻¹ in source GW to 3.65×10^6 copies mL⁻¹ in GW effluent, and it was 3.79×10^9 copies g⁻¹ in the GW corrosion scale biofilm. The sum of *nosZ*, *nirS* and *nirK* increased from 1.42×10^5 copies mL⁻¹ in source SW to 3.38×10^5 copies mL⁻¹ in SW effluent, and it was 3.47×10^9 copies g⁻¹ in the SW corrosion scale biofilm. Furthermore, in the biofilms within corrosion scales, MPN for NRB, IRB and nitrate-dependent Fe(II)-oxidizing bacteria were determined under different culture conditions. For GW and SW DWDSs, in the presence of NO₃⁻ and acetate, culturable NRB were

Fig. 6. XRD patterns of corrosion scales on cast iron coupons in sanitized SW water (Ca–CaCO₃, G–α-FeOOH).

Fig. 7. SEM micrographs of corrosion scales formed on cast iron coupons in sanitized SW water.

detected approximately 2.7×10^7 and 7.5×10^6 cells g^{-1} , and in the presence of Fe(III) and acetate, the abundances of culturable IRB were approximately 10^3 and 10^7 cells g^{-1} , respectively, whereas approximately 10^8 cells g^{-1} and 10^6 cells g^{-1} of culturable (MPN assay) nitrate-dependent Fe(II)-oxidizing microorganisms were detected with Fe(II) and NO₃[−] (Table 4). According to the previous work [16,38], biological nitrate-dependent Fe(II) oxidation came from IRB and NRB, and NRB also could reduce Fe(III) at the lower concentration of NO₃[−] (<0.5 mM). These results indicated that NRB in DWDSs are able to induce oxidation of Fe(II) or reduction of Fe(III) in the presence or absence of nitrate. Moreover in GW DWDSs, nitrate-dependent Fe(II) oxidation was predominant within corrosion scales, but the reduction of Fe(III) by NRB and IRB were predominant inside corrosion scales in SW DWDSs.

3.4. Bacterial diversity and composition in biofilms and water

Pyrosequencing yielded a total of 51,421 high-quality 16S rRNA gene sequences for all samples. Table 5 shows the diversity indices of bacteria for different samples. The Shannon index was 4.79 and 5.83 for raw GW and SW, respectively, and became 2.62 and 4.19 for the effluents. The results indicated that raw SW had higher bacterial diversity than raw GW, and chlorine disinfection inhibited bacterial activity in the DWDSs. However, the biofilms in both systems had nearly the same Shannon index, indicating the same bacterial diversity within the biofilms.

Table 6 shows a taxonomic breakdown at the class level for GW and SW samples. In raw water, the major classes were *Alphaproteobacteria* (25.4%), *Gammaproteobacteria* (23.2%), and *Sphingobacteria* (20%); *Betaproteobacteria* (6.6%), and *Actinobacteria* (3.5%) constituted the other minor classes. In effluent, *Alphaproteobacteria* became the most abundant class (86.7%), *Betaproteobacteria* increased to 10.6%, *Sphingobacteria* decreased to 2.4%, and *Gammaproteobacteria* and other bacteria disappeared. For biofilm samples from the GW DWDSs at 137 d, the dominant class was *Betaproteobacteria* (79.4%), and the other minor classes were *Alphaproteobacteria* (13.1%), *Sphingobacteria* (5.6%), and *Gammaproteobacteria* (1.7%); however at 220 d, *Betaproteobacteria* decreased to 40.2%,

Fig. 8. The abundance of bacterial 16S rRNA, *nosZ*, *nirK* and *nirS* genes in GW water (A) and SW water (B) and corrosion scales (C) at different time points in GW and SW DWDSs. Error bars represent one standard deviation.

Table 4

MPN enumerations of nitrate-reducing, Fe(III)-reducing and nitrate-dependent Fe(II)-oxidizing microorganisms in corrosion scales of DWDSs.

Culture conditions	MPN (cells g ⁻¹)		95% confidence interval	
	GW DWDS	SW DWDS	GW DWDS	SW DWDS
Acetate + NO ₃ ⁻	2.7 × 10 ⁷	7.5 × 10 ⁶	5.7 × 10 ⁶ –1.2 × 10 ⁸	1.9 × 10 ⁶ –2.1 × 10 ⁷
Acetate + Fe(III)	5.8 × 10 ³	4.6 × 10 ⁷	1.1 × 10 ³ –2.3 × 10 ⁴	1.7 × 10 ⁷ –1.9 × 10 ⁸
Fe(II) + NO ₃ ⁻	1.6 × 10 ⁸	1.3 × 10 ⁶	2.8 × 10 ⁷ –6.6 × 10 ⁸	2.2 × 10 ⁵ –5.2 × 10 ⁶

Table 5

Community diversity estimates of the bacteria within bulk water and corrosion scales at different time points in GW and SW DWDSs.

Samples	OTUs	Diversity indices	
		Shannon	Simpson
GW	784	4.79	0.0302
GW-220 d	443	2.62	0.03357
BGW-137 d	762	4.15	0.0534
BGW-220 d	620	4.14	0.0719
SW	1286	5.83	0.01
SW-220 d	567	4.19	0.0414
BSW-137 d	317	3.29	0.0944
BSW-220 d	581	4.13	0.0614

GW (groundwater source); GW-220 d (groundwater effluent at 220 d); BGW (corrosion scales of GW DWDSs); SW (surface water source); SW-220 d (surface water effluent at 220 d); BSW (corrosion scales of SW DWDSs).

Alphaproteobacteria was 12.9%, *Sphingobacteria* increased to 30.2%, *Holophagae* (13.4%) and *Gemmatimonadetes* (2.9%) appeared, and *Gammaproteobacteria* disappeared. In raw SW, classes *Alphaproteobacteria* (35.8%), *Betaproteobacteria* (27%), and *Gammaproteobacteria* (12.5%) were included. *Sphingobacteria* was 11.3%, and *Actinobacteria* was 3.5%. In effluent, the relative abundance of *Alphaproteobacteria* and *Betaproteobacteria* increased to 50.4% and 40.7%, respectively, *Sphingobacteria* decreased to 8.1%, and other bacteria were below 1%. For biofilm samples from the SW DWDS at 137 d, the groups were *Alphaproteobacteria* (23.1%), *Betaproteobacteria* (68.8%) and *Sphingobacteriia* (7.8%); however at 220 d, *Alphaproteobacteria* decreased slightly to 14.5%, *Betaproteobacteria* increased to 78.3%, and *Sphingobacteria* was 7%. None of these classes changed greatly, indicating the stability of the biofilm in the DWDSs transporting SW.

Furthermore, the community structures of these dominant bacteria were compared at the genus level for different samples (Table 7). The compositions of corrosion-related bacteria underwent large changes in bulk water and biofilms on pipes transporting GW and SW. In raw GW, the dominant bacteria were IOB *Sediminibacterium* [39] (19.2%), with other iron-respiring bacteria and NRB below 0.8%. In effluent, the *Sediminibacterium* decreased to 2.4%. NRB associated with redox cycling of iron (NRB-Fe) appeared at 5.8%, including *Zoogloea* [36,40] (2.75%), *Aquabacterium* [41]

(1.54%), *Azospira* [42] (0.63%), *Dechloromonas* [16] (0.42%), and *Hyphomicrobium* [36,43] (0.29%). In addition, the NRB producing siderophore (NRB-S) *Sphingomonas* [44] (2.6%) appeared. In the biofilm of the GW DWDS at 137 d, the IOB *Sediminibacterium* was 5.6%, and NRB-Fe were 26.8%, including *Azospira* (15.5%), *Dechloromonas* (5.9%), *Acidovorax* [45] (3.2%), and *Rhodanobacter* [46] (1.6%). *Bradyrhizobium* (8.4%) and *Sphingomonas* (0.1%) were NRB-S [47]. At 220 d, IOB *Sediminibacterium* increased to 30.1%. The *Geothrix* [48] (12.4%) IRB appeared, while NRB-Fe decreased to 20%, including *Azospira* (9.6%), *Dechloromonas* (6%), and *Zoogloea* (3.6%). *Bradyrhizobium* decreased to 4.99%, and *Sphingomonas* was 0.1%. In raw SW, the IOB *Sediminibacterium* was 9.5% and IRB *Rhodobacter* [49] was 1.2%. NRB-S *Sphingomonas* was 8.4%. In effluent, the IOB *Sediminibacterium* decreased to 7.5%, and *Rhodobacter* decreased to 0.28%. NRB-Fe increased to 28.4%, including *Zoogloea* (13.1%), *Azospira* (7.1%), *Dechloromonas* (5.2%), and *Hyphomicrobium* (2.8%), but *Sphingomonas* increased to 16.9%. In the biofilm of SW DWDSs at 137 d, *Sediminibacterium* was 7.8%, and no significant IRB were detected. However, the NRB-Fe comprised 58.7%, mainly including *Acidovorax* (56.8%) and *Rhodopseudomonas* [36] (1.5%). *Sphingomonas* was 6%. At 220 d, NRB-Fe increased to 64.6%, including *Azospira* (29.9%), *Dechloromonas* (17.7%), *Zoogloea* (8.8%), *Rhodopseudomonas* (8.3%) and *Acidovorax* (5%). *Sediminibacterium* was 7%. NRB-S *Sphingomonas* decreased to below 1%.

4. Discussion

In DWDSs, iron pipe corrosion, chlorination and transformation of nitrate resulted in the large changes in the bacterial communities from water sources to effluents for GW and SW. However, both the effluents and the biofilms exhibited nearly the same bacterial community types in the two systems at 220 d. The composition of the bacterial communities from both water sources had a large influence on the communities of the biofilms, leading to different bacterial community compositions in the two systems. Furthermore, all the biomass in the effluent of the GW DWDSs was greater than in the SW effluent, which was due to bacteria detaching from the loose corrosion layer in the GW DWDSs. However at 220 d, there was almost same biomass inside the corrosion scales for the two systems. Significantly, denitrifying functional gene copy numbers increased with increasing corrosion time in the effluent

Table 6

Relative abundance of the major phylotypes at class level within bulk water and corrosion scales at different time points in GW and SW DWDSs.

	GW	GW-220 d	BGW-137 d	BGW-220 d	SW	SW-220 d	BSW-137 d	BSW-220 d
<i>Actinobacteria</i>	3.54	0.00	0.03	0.00	1.04	0.04	0.00	0.00
<i>Alphaproteobacteria</i>	25.43	86.62	13.05	12.94	35.77	50.35	23.10	14.46
<i>Betaproteobacteria</i>	6.60	10.62	79.38	40.20	27.03	40.7	68.82	78.32
<i>Deltaproteobacteria</i>	0.38	0.05	0.00	0.16	2.77	0.04	0.00	0.00
<i>Flavobacteria</i>	0.00	0.00	0.00	0.00	2.40	0.00	0.02	0.00
<i>Gammaproteobacteria</i>	23.16	0.11	1.74	0.16	12.46	0.18	0.25	0.02
<i>Gemmatimonadetes</i>	0.08	0.05	0.01	2.91	0.43	0.00	0.00	0.00
<i>Holophagae</i>	0.00	0.11	0.16	13.38	0.00	0.02	0.00	0.11
<i>Sphingobacteria</i>	20.00	2.39	5.62	30.19	11.34	8.06	7.82	7.03
Others (unknown etc.)	20.81	6.76	0.05	0.61	0.01	0.06	0.00	0.06

Only bacterial species representing 1% or more of total bacterial sequencing reads are shown.

Table 7

Relative abundance of corrosion-related bacterial genera in bulk water and corrosion scales at different time points in GW and SW DWDSs.

	GW	GW-220 d	BGW-137 d	BGW-220 d	SW	SW-220 d	BSW-137 d	BSW-220 d
IOB								
<i>Sediminibacterium</i>	19.19	2.36	5.62	30.11	9.54	7.46	7.82	7.03
IRB								
<i>Geothrix</i>	0.00	0.00	0.00	12.39	0.00	0.00	0.00	0.00
<i>Rhodobacter</i>	0.69	0.02	0.05	0.29	1.22	0.28	0.00	0.00
NRB-Fe								
<i>Acidovorax</i>	0.02	0.21	3.15	0.44	0.02	0.23	56.83	5.03
<i>Azospira</i>	0.00	0.63	15.52	9.60	0.00	7.13	0.00	29.94
<i>Aquabacterium</i>	0.00	1.54	0.01	0.02	0.70	0.00	0.00	0.00
<i>Dechloromonas</i>	0.00	0.42	5.94	6.02	0.00	5.17	0.00	17.65
<i>Zoogloea</i>	0.00	2.75	0.58	3.56	0.00	13.1	0.00	8.82
<i>Rhodanobacter</i>	0.00	0.00	1.60	0.07	0.00	0.00	0.00	0.00
<i>Rhodopseudomonas</i>	0.12	0.00	0.02	0.24	0.06	0.02	1.53	8.28
<i>Hyphomicrobium</i>	0.04	0.29	0.00	0.05	0.23	2.78	0.04	0.11
NRB-S								
<i>Sphingomonas</i>	0.26	2.6	0.14	0.10	8.38	16.87	5.95	0.06
<i>Bradyrhizobium</i>	0.16	0.00	8.38	4.99	0.06	0.00	0.08	1.16

Only bacterial species representing 1% or more of total bacterial sequencing reads are shown.

and biofilm inside corrosion scales, and most of NO_3^- -N was removed by denitrification in the two DWDSs. The concentration of NO_3^- -N ($5\text{--}7.9\text{ mg L}^{-1}$) in GW were much higher than SW ($0.9\text{--}2.2\text{ mg L}^{-1}$), leading higher denitrification reaction, inducing higher nitrate-dependent Fe(II) oxidation than SW DW. The MPN enumerations also suggested that nitrate-dependent Fe(II) oxidation occurred predominantly by NRB-Fe and IOB in GW DWDS, whereas the reduction of Fe(III) and the oxidation of Fe(II) occurred via the anaerobic respiration of NRB-Fe in SW DWDSs. These different iron redox processes may affect the re-precipitation of released iron ions, the formation of corrosion products and the characteristics of microorganisms in the two DWDSs. The corrosion scales were thin and loosely bound reddish filaments of α -FeOOH in the DWDS transporting GW while the dominant corrosion-related bacteria were IOB *Sediminibacterium* (30.1%), IRB *Geothrix* (12.4%), and NRB-Fe *Azospira* (9.6%), *Dechloromonas* (6%), and *Zoogloea* (3.6%) within the biofilm. Conversely, the corrosion scales were thick, hard-shell tubercles consisting of Fe_3O_4 and α -FeOOH, and the main corrosion-related bacteria were *Azospira* (29.9%), *Dechloromonas* (17.7%), *Zoogloea* (8.8%) and *Rhodopseudomonas* (8.3%) in the DWDSs transporting SW. In the sanitized SW (without biofilm), loose corrosion scales without Fe_3O_4 were formed, although the water had the same chemical parameters as the SW DWDSs. The results indicated that the biofilm may play a greater role than water chemical parameters in formation of Fe_3O_4 and the dense oxide layer. Previous works have verified that the reddish filament morphology of α -FeOOH was mainly formed from the microbial oxidation of Fe(II) in a geological environment [50]. Moreover, some studies indicated that anaerobic iron redox cycling could be catalyzed by a single group of *Azospira*, *Dechloromonas* and IRB, and favor the formation of Fe_3O_4 [15,16], causing formation of more compact corrosion scales [8,51]. In addition, nitrate-dependent Fe(II) oxidation has been suggested to have a direct role in the formation of Fe_3O_4 ; however, its role was weaker than that of anaerobic iron redox cycling [15]. In the corrosion processes of iron pipes, iron ions were released into distributed water, and can re-precipitate forming corrosion scales [4]. The total iron concentration in bulk water depended on iron corrosion rate, re-precipitation rate and dissolution rate of corrosion scales [52]. In SW DWDSs, the re-precipitation rate of released iron ions was higher than that one in GW DWDSs due to anaerobic iron redox cycling, leading to similar total iron concentration in both DWDSs. Formation of Fe_3O_4 and a dense corrosion layer greatly depended on the composition of the bacterial community in the biofilm inside the

corrosion layer and the concentration of nitrate microbial reduction in DWDSs.

5. Conclusions

The results showed that a loose corrosion layer was formed containing reddish filaments of α -FeOOH with a BET surface area of $85.5\text{ m}^2\text{ g}^{-1}$ in DWDS-GW, but in DWDS-SW, a dense corrosion layer was formed, including crystallized particles of α -FeOOH, γ -FeOOH, Fe_3O_4 and CaCO_3 with a BET surface area of $18.8\text{ m}^2\text{ g}^{-1}$. In sanitized SW, α -FeOOH and CaCO_3 were formed with a loose and porous morphology with a BET surface area of $50.8\text{ m}^2\text{ g}^{-1}$. These results indicated that microorganisms played a larger role in the formation of Fe_3O_4 and the properties of the corrosion layer than water chemical parameters. Both bulk bacteria in the effluent and biofilm inside the iron corrosion products had almost the same bacterial community composition for the two DWDSs with GW and SW. The total biomass, including total microorganisms and denitrifying functional bacteria, in the effluent of the GW DWDSs was much larger than in the SW DWDSs due to the loose corrosion layer in the GW DWDSs. However, bacterial diversity, denitrifying functional genes and 16S rRNA gene copy numbers of biofilms were nearly the same in the two systems after 220 d, but the composition of the bacterial communities was very different due to the effect of the water source. With formation of the corrosion layer, the dominant corrosion-related bacteria were IOB *Sediminibacterium* (30.1%), IRB *Geothrix* (12.4%), and NRB associated with iron redox cycling (NRB-Fe) (20%), including *Azospira*, *Dechloromonas* and *Zoogloea* within the biofilm in DWDS-GW at 220 d, and NRB-Fe (64.7%) were dominant, including the above NRB-Fe and *Rhodopseudomonas*, in DWDS-SW. Based on the MPN enumerations and qPCR of denitrifying functional bacteria, the formation of corrosion products was mainly affected by nitrate-dependent Fe(II) oxidation of NRB-Fe and Fe(II) oxidation of IOB *Sediminibacterium* in the DWDSs transporting GW, but reduction of Fe(III) and oxidation of Fe(II) occurred from the anaerobic respiration of NRB-Fe in the DWDSs transporting SW.

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