Characterization of chemical composition and bacterial community of corrosion scales in different drinking water distribution systems†

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Characterization of the chemical composition and bacterial community of corrosion scales was studied in real drinking water distribution systems from eight cities. The results of X-ray powder diffraction (XRD) indicated that α-FeOOH and Fe3O4 were present in all corrosion scales, and green rust was only found in corrosion scales of pipes transporting surface water. The corrosion scales with a higher Fe3O4/α-FeOOH ratio showed less iron release than those with a lower ratio. Moreover, the results of 454 pyrosequencing revealed that Proteobacteria (46.5–84.3%) was the main bacterial phylum in all corrosion scales; however, the bacterial genera were very different in the pipes from eight cities. Nitrate-reducing bacteria (10.9–36.0%) were the main potential corrosive bacteria, and denitrifying genes including nirS, nirK and nosZ were all found in the corrosion scales. The results of most probable number (MPN) tests indicated that nitrate-reducing bacteria, Fe(III)-reducing bacteria and nitrate-dependent Fe(II)-oxidizing bacteria in corrosion scales were indeed active and had the function of Fe(III) oxidation and Fe(II) reduction. All the results suggested that there was an apparent relationship between the relevant biochemical functions (e.g., iron redox cycling) of the bacterial community and the formation of α-FeOOH and Fe3O4 in corrosion scales. Furthermore, the relative abundance of Desulfovibrio also correlated very well with the content of green rust in corrosion scales. These results will be very helpful for future control of iron release in water distribution systems.

1. Introduction

Unlined cast iron pipes have been used in drinking water distribution systems (DWDSs) for several centuries, and the corrosion and bacterial regrowth in pipes can cause deterioration of water quality.1–3 The formation of corrosion scales is a result of continued corrosion followed by a combination of precipitation and oxidation of corrosion products.4 Many corrosion scales are composed of goethite (α-FeOOH), lepidocrocite (γ-FeOOH), magnetite (Fe3O4), siderite (FeCO3), ferric hydroxide (Fe(OH)3), ferrous hydroxide (Fe(OH)2), and calcium carbonate (CaCO3).5–7

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The chemical composition of corrosion scales can affect the quality of distributed water in DWDSs. Yang et al. have indicated that thick and densely distributed corrosion scales with higher Fe3O4 content are more stable, and thin corrosion scales with higher α-FeOOH and FeCO3 content more easily release iron into the distributed water.8 The iron released from the corrosion scales will give drinking water a red, brown or yellow colour.4 Iron can also give tap water an unpleasant metallic taste9 and stain porcelain plumbing.10 Moreover, iron release plays great roles in biological stability in tap water; for example, iron is a key requirement for Legionella pneumophila growth and iron release can increase Legionella in tap water.11,12 The chemical composition of corrosion scales varies significantly with water quality and the hydraulic condition.13 Moreover, some studies have identified the bacterial community in the corrosion scales of cast iron pipes and indicated the effects of the bacterial community on the chemical composition of corrosion scales.14–16
Many bacteria play great roles in iron redox cycling, and this process also influences corrosion scale composition. Green rust can be formed during Fe(III) oxidation by the nitrate reducing Acidovorax sp. strain BoFeN1. Geobacter metallireducens could induce sequential nitrate reduction, Fe(III) reduction and nitrate dependent Fe(II) oxidation, and this process causes the transformation of the iron oxides. Our study also indicated that the nitrate-reducing bacteria (NRB) Acidovorax and Hydrogenophaga promoted the formation of Fe(III) oxides at the initial stage of corrosion. When the corrosion reaches a relatively stable stage, NRB Dechloromonas, iron-reducing bacteria (IRB) Rhodobacter and iron-oxidizing bacteria (IOB) Rhodomicrobium could induce iron redox cycling in the corrosion scales, and this accelerated the Fe₃O₄ formation and decreased the iron release. Because the NRB Dechloromonas was the main bacteria in the corrosion scales in our study, one bacterium, Dechloromonas hortensis, was used to test its function in different NO₃⁻-N concentrations. The results indicated that at lower NO₃⁻-N concentrations (2.2 mg L⁻¹), this bacterium induced Fe(III)/Fe(II) redox cycling in simulated DWDSs and resulted in higher Fe₂O₃ formation in corrosion scales and lower iron release. Moreover, Li et al. elucidated the same results by simulated DWDSs using groundwater and surface water with different NO₃⁻-N concentrations. Zhu et al. also found that NRB 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 DWDSs with UV/Cl₂ disinfection. Moreover, the water quality including the presence of SO₄²⁻, NO₃⁻ and natural organic matter (NOM) could influence the microbial oxidation and reduction of iron oxides. For example, in the presence of the IRB Shewanella putrefaciens CN32, NOM was found to enhance the microbial reduction of Fe(III) under anaerobic, circumneutral pH conditions.

Therefore, many bacteria in corrosion scales could induce the iron redox cycling process, and this could promote Fe₃O₄ formation and decreased iron release. However, these studies were all done in the laboratory. The results in the laboratory cannot represent the process in real DWDSs completely because of the complexity of the actual environment. Until now, there has been little known about whether the bacteria in corrosion scales could induce iron redox cycling and whether this process has some relationship with the Fe₃O₄ formation in corrosion scales and iron release in real DWDSs. Therefore, the objective of this study was to analyze the characterization of the bacterial community and the chemical composition of corrosion scales in real drinking water pipes and to discuss the relationship between bacterial function and chemical composition in corrosion scales.

2. Materials and methods

2.1. Sample collection in actual DWDSs

Aged unlined cast iron pipe sections (approximately 20 years) were excavated from different DWDSs in eight cities of China during March–May 2014. Pipes from cities A–D were transporting groundwater, and pipes from E–H were transporting surface water. The diameter of all pipes was 100 mm. The hydraulic and water quality history of the sampling sites from June 2013 to May 2014 were derived from the database of the drinking water treatment plant in every city. In all sampling sites, the water flow velocity of all pipes was 0.1–0.5 m s⁻¹ according to the water consumption at different times. The value range of water quality including pH, Cl⁻, SO₄²⁻, alkalinity and chlorine residual in DWDSs is shown in Table S1.

The water was sampled from the taps of customers that were connected directly to the pipelines. The taps were cleaned using absolute ethyl alcohol and the tap water was run for an extensive time until the water temperature remained stable for 30 s to ensure that the water was coming through the main pipe and not stagnant water in order to obtain representative samples from the pipes. Ten-liter water samples were collected from each tap using sterile plastic carboys every 30 minutes. The water was sampled three times from each tap, and a total of 30 liters of water was collected. The mean average and standard deviation of water quality is shown in Table 1. The detected tap water quality was consistent with the values from the database of different drinking water treatment plants (Table S1†).

Because the pipes were still in service, the water inlet valve was closed after collecting the tap water, and then the water in the pipes was drained quickly. The exteriors of the pipes were cleaned using a chain cleaner prior to removal to minimize contamination within the pipes during the sampling. Pipe sections of 1 m in length were manually extracted using a hinged cutter, and the open ends were immediately sealed with sterile bags. The sampling was also done in triplicate, and then three pipe sections from each city were cooled with ice and quickly transported to the laboratory. The metal spatulas were all covered with brown paper and sterilized at 121 °C before DNA extraction. The water was sampled from the taps of customers that were transporting groundwater, and pipes from E–H were transporting surface water. The diameter of all pipes was 100 mm. The water flow velocity of all pipes was 0.1–0.5 m s⁻¹ according to the water consumption at different times. The value range of water quality including pH, Cl⁻, SO₄²⁻, alkalinity and chlorine residual in DWDSs is shown in Table S1.

2.2. Analysis of water quality and corrosion scales

The tap water quality parameters from different pipes including pH, Cl⁻, SO₄²⁻ and alkalinity were measured according to standard methods. Dissolved organic carbon (DOC) was analyzed using a total organic carbon analyzer...
Table 1  The main tap water quality parameters of different pipes

| Parameters                  | A         | B         | C         | D         | E         | F         | G         | H         |
|-----------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| pH                          | 8.52 ± 0.12 | 7.73 ± 0.11 | 7.87 ± 0.13 | 7.53 ± 0.10 | 8.36 ± 0.15 | 8.05 ± 0.12 | 7.91 ± 0.12 | 7.79 ± 0.08 |
| DOC (mg L⁻¹)                | 0.29 ± 0.03 | 0.35 ± 0.03 | 0.51 ± 0.04 | 1.94 ± 0.12 | 2.20 ± 0.15 | 1.97 ± 0.13 | 1.77 ± 0.11 | 1.43 ± 0.09 |
| NO₃⁻, N (mg L⁻¹)           | 0.44 ± 0.05 | 7.58 ± 0.52 | 9.69 ± 0.68 | 4.95 ± 0.24 | 2.95 ± 0.19 | 2.87 ± 0.14 | 2.38 ± 0.18 | 5.46 ± 0.61 |
| Cl⁻ (mg L⁻¹)                | 167.9 ± 15.8 | 26.1 ± 2.42 | 25.3 ± 2.15 | 27.1 ± 2.24 | 25.1 ± 2.16 | 39.4 ± 3.19 | 34.3 ± 3.24 | 48.8 ± 5.06 |
| SO₄²⁻ (mg L⁻¹)             | 171.7 ± 15.6 | 48.1 ± 5.84 | 36.8 ± 3.28 | 136.9 ± 12.8 | 99.7 ± 10.4 | 145.9 ± 13.2 | 106.5 ± 9.94 | 147.8 ± 13.6 |
| Alkalinity (mg CaCO₃ L⁻¹)  | 152.7 ± 14.8 | 182.0 ± 17.1 | 197.8 ± 18.5 | 228.9 ± 20.9 | 151.4 ± 14.8 | 150.2 ± 15.1 | 149.0 ± 13.7 | 189.3 ± 17.6 |
| Chlorine residual (mg L⁻¹) | 0.27 ± 0.03 | 0.25 ± 0.02 | 0.26 ± 0.02 | 0.26 ± 0.03 | 0.28 ± 0.02 | 0.24 ± 0.02 | 0.30 ± 0.03 | 0.26 ± 0.03 |

The results are shown as mean average and standard deviation.

(Phoenix 8000; Tekmer-Dohrmann, USA). Chlorine residual was measured using a HANNA HI93711 spectrophotometer (Italy). The difference of water quality was measured using analysis of variance (ANOVA) with a significance threshold of α = 0.05. P values of less than 0.05 were considered as statistically significant.

The corrosion scales from three pipe sections in each city were crushed using a sterile pestle and mortar in an anaerobic glove box which was equipped with an ultraviolet lamp. Crushed and homogenized scales from each city were analyzed using a powder X-ray diffractometer (XRD, X’Pert PRO MPD; PANalytical, The Netherlands) to determine the crystalline phase composition. The XRD patterns were analyzed using X’Pert HighScore Plus software (version 2.2.4) and the PDF 2004 database was used for crystalline phase identification. X-ray diffraction was used to semi-quantitatively determine the weight fraction of constituents.²³

2.3. The iron release in different pipes

In order to know the iron release of different pipes, two experimental facilities were set up in the laboratory, one using the pipe sections (1 m × 3) from city A, and the other one using pipe sections from city E (Fig. S1†). The water used was collected from a drinking water treatment plant in the north of China, which was treated by coagulation, flocculation, sedimentation, sand filtration, and biologically activated carbon filtration (prior to entering the chlorine contact tanks). The water quality is shown in Table S2.† Sodium hypochlorite was used as the disinfectant. The water flow rate was controlled at 36 L h⁻¹, and the water was cycled in the pipes for 24 hours to simulate the low-flow regions or intermittent water flow environment in actual pipes according to reported methods.⁶⁻²⁹ Then the effluents were collected and the total iron concentration was measured according to standard methods.¹⁰ The total iron concentration was measured from 6 days to 36 days until the concentration was stable in the effluents. The difference in total iron concentration in the effluents of the two pipes was also measured using ANOVA with a significance threshold of α = 0.05.

2.4. DNA extraction

During the collection of pipes, three pipe sections (1 m × 3) were sampled from each city. The corrosion scales collected from each pipe section were freeze dried using a FrezZone 2.5 L Benchtop system (Labconco, Kansas, MO). Then 0.5 g of crushed and homogenized corrosion scales from each pipe section was used for DNA extraction. The DNA of bacteria in corrosion scales was extracted using a FastDNA spin kit for soil (MP Biomedicals, Solon, OH, USA) following the manufacturer’s instructions. DNA quality was checked on an agarose gel, and concentrations were measured with a Nanodrop spectrophotometer (ND-1000, NanoDrop, USA). All DNA samples were stored at −80 °C until further processing.

To determine the method recovery efficiency, E. coli was used as a representative microorganism according to the method of Wang et al.²¹ A serial dilution of E. coli (ATCC 25922) was spiked into 50 mL of autoclaved water and subjected to freeze drying and/or DNA extraction. The recovery efficiency of the freeze drying method varied from 19.1% to 40.5% depending on the concentration of the sample, compared to direct DNA extraction (Fig. S2†).

2.5. Pyrosequencing

Primers 341F (5’-CCTACGGGAGGCAGCAG-3’) and 1073R (5’-ACGGAGGTGACACRCATG-3’) were used to amplify partial regions (V3-V6) of 16S rRNA genes.³³ 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). A Roche massively parallel 454 GS-FLX Titanium sequencer (Roche 454 Life Sciences, Branford, CT, USA) was used for the pyrosequencing. 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. Total bacterial communities were analyzed for the number of operational taxonomic units (OTUs) 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/). The difference in relative abundance of the bacterial genera in the corrosion scales was also
measured using ANOVA with a significance threshold of $\alpha = 0.05$.

2.6. Quantitative real time PCR
Quantitative real time polymerase chain reaction (qPCR) analysis of 16S rRNA gene for total bacteria was performed using the primer pairs 1369F (5'-CGTGTAACCTCTTCTGGG-3') and 1492R (5'-GGWACTCGTGCTAGCAGTCTGA-3') with the probe 1389F (5'-CTTGTACACCCCGCCGTTCA-3'). In addition, genes encoding nitrite reductase ($nirS$) and nitrous oxide reductase ($nosZ$) are the most common molecular markers for denitrifiers. Three pairs of primers, nirK876 (5'-ATYGGCGGAYGCGAGA-3'/nirK1040 (5'-GCCCTGATCGGRTTGTGGTT-3'), nirScd3aF (5'-GTSAACTGTSAGARACGG-3')/nirSR3cd (5'-GASTTCCGRTSGTCTCCG-3') and nosZ-F (5'-CGYGTTCGCTCAGCGCAGCAG-3')/nosZ1622R (5'-CGSACCTTSTSCGCTGCGG-3'), were used to amplify $nirK$, $nirS$ and $nosZ$, respectively. PCR amplifications were carried out in 96-well optical plates on an Applied Biosystems 7300 qPCR system with 7300 SDS software (Applied Biosystems). All samples, including standards and negative controls, were analyzed in triplicate. The slopes of standard curves and amplification efficiency values for quantification were as follows: $-3.356$ and $98.6\%$ for $nirK$, $-3.388$ and $97.3\%$ for $nirS$, $-3.352$ and $98.8\%$ for $nosZ$, and $-3.409$ and $96.5\%$ for 16S rRNA gene.

2.7. Most probable number enumeration
The abundance of culturable nitrate-reducing bacteria, Fe(III)-reducing bacteria and nitrate-dependent Fe(II)-oxidizing bacteria in the corrosion scales of different pipelines was quantified using a three-tube most probable number (MPN) technique. Triplicate pressure tubes containing sterile, anaerobic (N$_2$: CO$_2$: 90:10, V/V) AGW medium (10 mM PIPES, 2 mM NaHCO$_3$, 5 mM NH$_4$Cl, 0.5 mM KH$_2$PO$_4$, pH 6.8) were inoculated with homogenized corrosion scales. For testing of the three bacteria in the corrosion scales, the AGW medium was amended according to previous studies. For testing of the acetate-oxidizing nitrate-reducing bacteria, tubes were amended with 5 mM NaHCO$_3$ and 10 mM Na-acetate from sterile, anaerobic stock solutions. For acetate-oxidizing Fe(III)-reducing bacteria, the medium was amended with 10 mM of synthetic hydrous ferric oxide prior to autoclaving, and then amended with 10 mM Na-acetate and 2 mM FeCl$_2$ (as a reducing agent) from sterile, anaerobic stock solutions. The medium for Fe(II)-oxidizing nitrate-reducing bacteria was amended with $0.5$ mM Na-acetate, 5 mM NaNO$_3$ and 10 mM FeCl$_2$ from sterile, anaerobic stock solutions. Positive results for acetate-oxidizing nitrate reducers were determined by checking for depletion of NO$_3^-$ by ion chromatography. Visual assessment of blackening of the medium, and formation of reddish-brown precipitates, was used to identify positive results for Fe(II) reducers and nitrate-dependent Fe(II) oxidizers, respectively.

After the MPN enumerations, the Fe(II) oxidation and Fe(III) reduction products caused by the bacterial function in the corrosion scales of pipe E under different conditions were analyzed using a powder X-ray diffractometer (XRD, X’Pert PRO MPD; PANalytical, The Netherlands) to determine the crystalline phase composition.

3. Results and discussion

3.1. Water quality analysis
The main tap water quality parameters of different pipes are summarized in Table 1. The water source of pipes A-D was groundwater, and the water source of pipelines E-H was surface water. The water from pipes A and B was treated with chlorine dioxide only, and from pipes C and D was treated with chlorine only. The water from pipes E and F was treated with coagulation, sedimentation, filtration and chlorine disinfection, and from G and H was treated with coagulation, sedimentation, filtration, ozonation, biologically activated carbon and chlorine disinfection. The pH was 7.5-8.5, and chlorine residual was 0.2-0.3 mg L$^{-1}$ for the eight tap waters. There was no significant difference between the eight tap waters ($p > 0.05$). However, DOC, NO$_3^-$-N, Cl$^-$, SO$_4^{2-}$ and alkalinity were very different in different tap waters ($p < 0.05$). Therefore, the water source and treatment process may have a great influence on the water quality in different cities. pH, chlorine residual and dissolved organic carbon (DOC) and nitrate were considered to be the most important factors affecting bacterial communities. Chloride and sulphate had been shown to increase water corrosivity, while higher alkalinity had been demonstrated to reduce the corrosion rate. Sun et al. and Yang et al. have also indicated that chloride, sulphate and alkalinity affected the potential corrosive bacteria and chemical composition of corrosion scales in the DWDSs. Therefore, the quality of the water in DWDSs could affect the chemical composition and bacterial communities in corrosion scales.

3.2. Chemical composition of the corrosion scales
The morphology of the corrosion scales of different pipes is shown by photographs in Fig. S3. The corrosion scales densely attached to the surface of the pipes, and tubercles formed on the surface of most pipes. Fig. 1 shows the crystalline composition of the corrosion scales in different pipes. XRD patterns for each sample are also shown in Fig. S4. The content of goethite (α-FeOOH) changed from 13% to 52%, while the content of magnetite (Fe$_3$O$_4$) changed from 19% to 48%. Previous research had indicated that thick and stable scales had a much higher Fe$_3$O$_4$/α-FeOOH ratio (>1.0), while thin and unstable corrosion scales had a lower Fe$_3$O$_4$/α-FeOOH ratio and higher content of γ-FeOOH. In our study, the average ratio of Fe$_3$O$_4$/α-FeOOH was about 1.14. Therefore, most of the corrosion scales were thick and stable. However, in corrosion scales of pipe A, the ratio was 0.41, and the content of lepidocrocite (γ-FeOOH) was 34%. This indicated that the corrosion scales in pipe A may be less stable than...
the corrosion scales from pipes B–H. In tap water of pipe A, DOC concentration was lower (0.29 ± 0.03 mg L\(^{-1}\)), and Cl\(^-\) (167.9 ± 15.8 mg L\(^{-1}\)) and SO\(_4^{2-}\) (171.7 ± 15.6 mg L\(^{-1}\)) were higher than those of tap waters from pipes B–H (Table 1). Therefore, lower DOC and higher Cl\(^-\) and SO\(_4^{2-}\) concentration may not be helpful for the stability of corrosion scales in pipes. Moreover, the average content of Fe\(_3\)O\(_4\) in groundwater and surface water pipes was 33.8% and 32.3%, respectively. The average content of \(\alpha\)-FeOOH in groundwater and surface water pipes was 47.8% and 26.3%, respectively. Therefore, the bacterial genera in corrosion scales from pipes E–H, and its relative abundance was from 28.8% to 56.8% (Fig. 3A). Moreover, Deltaproteobacteria was also high in corrosion scales of pipes E–H, and its relative abundance was from 7.25% to 23.9% (Fig. 3A).

In addition, the analysis based on the genus level allowed us to further verify the bacterial community composition in different samples (Fig. 3B). In the corrosion scales of pipe A, the main bacterial genus was Methylocystis, and its relative abundance was 22.1%. In corrosion scales of pipe B, the main bacterial genus was Acidovorax (18.6%), and the main bacterial genera were Comamonas (6.87%), Flavobacterium (9.66%) and Propionivibrio (8.90%) in corrosion scales of pipe C. However, in corrosion scales of pipe D, the main bacterial genera were Acinetobacter (11.9%) and Comamonas (17.2%). Therefore, the bacterial genera in corrosion scales from pipes A–D which were transporting groundwater changed greatly. However, in corrosion scales of pipes E–H which were transporting surface water, the main bacterial genera were Comamonas (10.6–12.7%) and Desulfovibrio (5.06–23.7%). Although the main bacterial genera had some similarity in some corrosion scales from pipes E–H, the bacterial genera in corrosion scales of these pipes were also changed. For
example, the relative abundance of *Flavobacterium* was high in corrosion scales of pipes E and G, while *Variovorax* was also high in corrosion scales of pipes F and H. In general, the relative abundance of bacterial genera in corrosion scales was very different in eight cities ($p < 0.05$), which may be due to the different water quality in the eight drinking water distribution systems.

In order to validate whether the variation of bacterial genera in corrosion scales may be due to the different water quality in eight cities and not to the random variation of the sampling and the 454 pyrosequencing method, three samples from pipe B which was transporting groundwater and three samples from pipe F which was transporting surface water were also analyzed separately using 454 pyrosequencing (Fig. 4). In the three samples of corrosion scales from pipe B, the main bacterial genera were *Acidovorax*, *Arthrobacter*, *Mycobacterium*, *Methylocystis*, *Hyphomicrobiurn*, *Flavobacterium*, *Desulfovibrio*, *Comamonas*, *Aerrobacter*, *Actinobacter* and *Acidovorax*.

### 3.4. The potential corrosive bacteria and their function

The bacterial communities in corrosion scales were further analyzed in terms of their potential function with respect to iron corrosion. Many studies have indicated that the iron-oxidizing bacteria (IOB), iron-reducing bacteria (IRB), sulfur-oxidizing bacteria (SOB), sulfate-reducing bacteria (SRB), nitrobacteria (NOB), and nitrate-reducing bacteria (NRB) could influence the corrosion process and the composition of the corrosion scales.14,15,43,44 The potential corrosive bacteria were identified at the genus level, and the classification was made according to the above reports (Fig. 5 and Table S3†).

The abundance of nitrogen-respiring bacteria related to iron corrosion was found to be 10.9–36.0% (NRB) and 0.03–0.61% (NOB), including *Nitrospira* and *Nitrospira*.14,15 NRB were predominant in all the corrosion scales from different pipes. The most abundant NRB was *Comamonas*, which was related to Fe(II) oxidation and nitrate reduction.45 The second most abundant bacteria were iron-respiring bacteria, including IRB (1.87–10.6%) and IOB (0.47–6.05%). *Gallionella* and *Geothrix* were the most common IOB and IRB present in corrosion scales, respectively,23,44 the relative abundance of which was 0–4.66% and 0–6.40%, respectively. The third most abundant bacteria were sulfur cycling bacteria, including SOB (0.02–10.2%) and SRB (0.43–23.7%). *Sulfuricella* was the most abundant SOB in corrosion scales of pipe B, and the relative abundance was 7.32%. *Desulfovibrio* was the most abundant SRB in corrosion scales of pipes E and G, and the relative abundance was 23.7% and 17.9%, respectively.

![Fig. 3](image)

**Fig. 3** Relative abundance of bacterial classes (A) and genera (B) in corrosion scales from different pipes. The genera which were lower than 5% in all samples are not shown in this figure.

![Fig. 4](image)

**Fig. 4** The random variation of relative abundance of bacterial genera in three corrosion scale samples from pipe B which was transporting groundwater and pipe F which was transporting surface water.

![Fig. 5](image)

**Fig. 5** Relative abundance of potential corrosive bacteria at genus level in corrosion scales of different pipes.
From the analysis above, the relative abundance of NRB was the highest among the potential corrosive bacteria in all of the corrosion scales. Therefore, the denitrifying functional genes including nirS, nosZ and nirK were analyzed quantitatively by qPCR (Fig. S6†). In corrosion scales, the gene copy number of nirS, nosZ and nirK changed greatly in different pipes. The total denitrifying gene copy numbers, as the percentage of total bacterial 16S rRNA gene copy numbers, changed from 1.8% to 13.7%. Furthermore, MPN showed that the abundance of culturable Fe(III)-reducing bacteria was 9.5 × 10^7–7.5 × 10^8 cells per g, nitrate-dependent Fe(II)-oxidizing bacteria was 1.5 × 10^7–3.8 × 10^8 cells per g, and nitrate-reducing bacteria was 4.5 × 10^6–1.1 × 10^11 cells per g in the corrosion scales of different pipes (Table 2).

After the MPN enumerations, the Fe(II) oxidation and Fe(III) reduction products caused by the bacteria in the corrosion scales from pipe E under different conditions were measured using XRD. In AGW medium with 10 mM FeCl_2, reddish-brown precipitates were formed. They were made up of goethite (α-FeOOH) (Fig. 6), which came from the oxidation of Fe(II) by the bacteria in the corrosion scales. The concentrations of sodium acetate, NaNO_3 and FeCl_2 in solution decreased to 0.43, 3.33 and 6.49 mM, respectively, indicating the process of nitrate-dependent Fe(II)-oxidation. In the AGW medium with 10 mM synthetic hydrous ferric oxide, the colour became black. The synthetic hydrous ferric oxide was amorphous; after the MPN experiment, magnetite (Fe_3O_4) and goethite (α-FeOOH) were precipitated (Fig. 6). Moreover, the concentration of sodium acetate decreased from 10 mM to 7.39 mM, and that of FeCl_2 decreased from 2 mM to 0.18 mM. This indicated that the bacteria in corrosion scales could use acetate and Fe(II) as electron acceptors to reduce Fe(III). Because the MPN experiment showed the number of culturable nitrate-reducing, Fe(III)-reducing and nitrate-dependent Fe(II)-oxidizing bacteria in corrosion scales, the results indicated that the bacterial communities could drive active iron redox cycling in the corrosion scales from different pipes.

### 3.5. Relationship between bacterial community and chemical composition of corrosion scales

The Fe\(^{2+}\) produced by chemical corrosion can be oxidized to Fe\(^{3+}\) by IOB, and rapid precipitation of bacteriogenic iron oxides could occur.\(^{44}\) The formed amorphous biomineralization products can further transform to α-FeOOH.\(^{46}\) In addition, NRB was also shown to produce Fe(III) oxides including α-FeOOH during the nitrate-dependent Fe(II) oxidation.\(^{34,45,47}\) The biogenic nitrite, which was very reactive in regard to nitrate, abiotically oxidized Fe(II) to green rust (GR).\(^{46}\) Moreover, DO in the corrosion scales was 0.20–0.45 mg L\(^{-1}\) according to a previous study.\(^{15}\) Under this anoxic condition, IRB could respire with Fe(III) oxides and induce Fe_3O_4 formation.\(^{15,36}\) Moreover, under anoxic or anaerobic conditions, NRB could induce the iron redox cycling process in corrosion scales, promoting Fe_3O_4 formation.\(^{20,21,44}\) Our previous work also indicated the effects of IOB, IRB and NRB on the corrosion of cast iron pipes, which had only chemical corrosion, using sterile water acting as a reference.\(^{21}\) In this study, the bacterial communities in corrosion scales of real drinking water distribution pipes were characterized. The results showed that there were many potential corrosive bacteria including IOB, IRB and NRB in corrosion scales of different pipes (Fig. 5). MPN results also indicated that these bacteria in corrosion scales were indeed active and had the function of Fe(II) oxidation and Fe(III) reduction, and this iron redox cycling process also could induce formation of different corrosion products including α-FeOOH and Fe_3O_4 (Table 2 and Fig. 6). Coincidentally, the relative abundance of corrosion potential bacteria

| Pipelines | Acetate + NO_3^- | Acetate + Fe(III) | Fe(II) + NO_3^- |
|-----------|-----------------|-----------------|-----------------|
|           | MPN (cell per g) | 95% confidence interval | MPN (cell per g) | 95% confidence interval | MPN (cell per g) | 95% confidence interval |
| A         | 9.5 × 10^7      | 1.5 × 10^7–3.9 × 10^8 | 9.5 × 10^7      | 1.5 × 10^7–3.9 × 10^8 | 9.5 × 10^7      | 1.5 × 10^7–3.9 × 10^7 |
| B         | 1.1 × 10^10     | 1.5 × 10^10–4.8 × 10^10 | 7.5 × 10^9      | 1.4 × 10^9–2.3 × 10^9 | 7.5 × 10^9      | 1.4 × 10^9–2.3 × 10^9 |
| C         | 3.0 × 10^10     | 3.6 × 10^10–4.9 × 10^10 | 4.5 × 10^9      | 7.3 × 10^9–2.2 × 10^9 | 4.5 × 10^9      | 7.3 × 10^9–2.2 × 10^9 |
| D         | 1.1 × 10^11     | 1.3 × 10^11–4.8 × 10^11 | 2.5 × 10^9      | 4.3 × 10^9–1.3 × 10^9 | 2.5 × 10^9      | 4.3 × 10^9–1.3 × 10^9 |
| E         | 2.0 × 10^9      | 3.3 × 10^9–4.7 × 10^9  | 4.5 × 10^8      | 7.3 × 10^8–2.2 × 10^8 | 4.5 × 10^8      | 7.3 × 10^8–2.2 × 10^8 |
| F         | 3.0 × 10^9      | 3.6 × 10^9–4.9 × 10^9  | 2.5 × 10^8      | 4.3 × 10^8–1.3 × 10^8 | 2.5 × 10^8      | 4.3 × 10^8–1.3 × 10^8 |
| G         | 4.5 × 10^10     | 6.9 × 10^10–2.3 × 10^11 | 4.5 × 10^7      | 7.3 × 10^7–2.2 × 10^7 | 9.5 × 10^7      | 1.5 × 10^7–3.9 × 10^7 |
| H         | 4.5 × 10^8      | 7.3 × 10^8–2.2 × 10^9  | 2.5 × 10^6      | 4.3 × 10^7–1.3 × 10^7 | 2.5 × 10^6      | 4.3 × 10^7–1.3 × 10^7 |
was higher in corrosion scales of pipe E, and the Fe$_2$O$_4$/α-FeOOH ratio was also higher, but its iron release was lower compared with that of pipe A (Fig. 1, 2 and 5). This was consistent with other studies, which indicated the effects of IOB, IRB and NRB on corrosion and the effects of Fe$_2$O$_4$/α-FeOOH ratio on iron release. Moreover, SRB Desulfovibrio was also found in the corrosion scales of pipes E–H in which the content of GR was high. Fig. S7† also shows that the content of GR and the relative abundance of SRB Desulfovibrio correlated very well using analysis of standard linear regression and Pearson test ($r = 0.963$, $p = 0.037$). This result suggested that the formation of GR may have some relationship with the presence of SRB Desulfovibrio in the corrosion scales. Pineau et al. had verified that the presence of green rust in the inner parts of corrosion scales was helpful for the growth of SRB. The SRB Desulfovibrio could induce pitting corrosion; however, the α-FeOOH and Fe$_3$O$_4$ formed by the transformation of green rust could protect the iron from corrosion. The above results indicated that the potential corrosive bacteria, Fe$_2$O$_4$ and α-FeOOH were present in all the corrosion scales of different drinking water distribution pipes. The potential corrosive bacteria in corrosion scales were active and had the function of iron redox cycling. There was some relationship between the biochemical function of the bacterial community and the formation of Fe$_2$O$_4$ and α-FeOOH in corrosion scales. Moreover, the content of GR correlated very well with the relative abundance of Desulfovibrio. However, in the real drinking water distribution pipes, the water quality may also affect the bacterial community and chemical composition of corrosion scales. Continuous observation of the actual drinking water distribution systems is needed in order to better understand the interaction between the bacterial community and the chemical composition of corrosion scales.

4. Conclusions

The results verified that the main potential corrosive bacteria in corrosion scales were nitrate-reducing bacteria. These bacteria in corrosion scales were indeed active and had the function of Fe(n) oxidation and Fe(m) reduction. Moreover, α-FeOOH and Fe$_3$O$_4$ were the main chemical components of corrosion scales, and green rust was only found in corrosion scales of pipes transporting surface water. There was an apparent relationship between the relevant biochemical functions (e.g., iron redox cycling) of the bacterial community and the formation of α-FeOOH and Fe$_3$O$_4$ in corrosion scales. The corrosion scales with a higher Fe$_2$O$_4$/α-FeOOH ratio showed less iron release than those with a lower ratio. This information will be useful for future control of iron release in actual DWDDSs. Because the bacterial genera in corrosion scales are very different in the eight cities, continuous observation of the bacterial community and chemical composition of corrosion scales in actual DWDDSs is needed in order to take measures to promote the formation of Fe$_2$O$_4$, which will be helpful for future control of iron concentration in tap water. Furthermore, the content of green rust and the relative abundance of sulfate-reducing bacteria Desulfovibrio correlated very well in corrosion scales. Further work is required to study the interaction between green rust and Desulfovibrio in corrosion scales in order to control the corrosion and iron release of actual cast iron pipes caused by the sulfate-reducing bacteria.

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