Cite this: *Environ. Sci.: Processes Impacts*, 2014, 16, 576

Formation and release behavior of iron corrosion products under the influence of bacterial communities in a simulated water distribution system†

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To understand the formation and release behavior of iron corrosion products in a drinking water distribution system, annular reactors (ARs) were used to investigate the development processes of corrosion products and biofilm community as well as the concomitant iron release behavior. Results showed that the formation and transformation of corrosion products and bacterial community are closely related to each other. The presence of sulfate-reducing bacteria (SRB, e.g. *Desulfovibrio* and *Desulfotomaculum*), sulfur-oxidizing bacteria (SOB, e.g. *Sulfuricella*), and iron-oxidizing bacteria (IOB, e.g. *Acidovorax*, *Gallionella*, *Leptothrix*, and *Sphaerotilus*) in biofilms could speed up iron corrosion; however, iron-reducing bacteria (IRB, e.g. *Bacillus*, *Clostridium*, and *Pseudomonas*) could inhibit iron corrosion and iron release. Corrosion scales on iron coupons could develop into a two-layered structure (top layer and inner layer) with time. The relatively stable constituents such as goethite (α -FeOOH) and magnetite (Fe_3O_4) mainly existed in the top layers, while green rust ($\text{Fe}_6(\text{OH})_{12}\text{CO}_3$) mainly existed in the inner layers. The IOB (especially *Acidovorax*) contributed to the formation of α -FeOOH, while IRB and the anaerobic conditions could facilitate the formation of Fe_3O_4 . Compared with the AR test without biofilms, the iron corrosion rate with biofilms was relatively higher ($p < 0.05$) during the whole experimental period, but the iron release with biofilms was obviously lower both at the initial stage and after 3 months. Biofilm and corrosion scale samples formed under different water supply conditions in an actual drinking water distribution system verified the relationships between the bacterial community and corrosion products.

Received 17th October 2013

Accepted 7th January 2014

DOI: 10.1039/c3em00544e

rsc.li/process-impacts

Environmental impact

Iron pipe corrosion and the release of corrosion products could greatly affect the integrity of water distribution infrastructure and the safety of drinking water at consumer ends. Corrosion scales formed by the accumulation of corrosion products under different water supply conditions had different morphological and compositional characteristics, which are closely associated with the processes of iron corrosion and release. Biofilms developed on the internal surface of distribution pipes could play crucial roles in corrosion reactions and the composition of corrosion scales. Therefore, understanding the effects of bacterial community structure on the iron corrosion, iron release and associated corrosion by-products could provide scientific support for drinking water quality and distribution infrastructure maintenance.

Introduction

Internal corrosion of cast iron pipes is ubiquitous in drinking water distribution systems (DWDSs). The corrosion of cast iron pipes is not only responsible for the destruction of the pipe

material, but also for the deterioration of potable water quality. Corrosion scales formed by the accumulation of corrosion products can reduce the water transportation capacity and offer habitation for pathogenic and opportunistic bacteria.^{1,2} “Red water” or “discolored water” episodes caused by severe iron corrosion product release are among the main reasons for consumer complaints. Yang *et al.* reported that corrosion scales formed under different water source histories exhibited significantly different morphological and structural characteristics, and the properties of corrosion scales could be crucial aspects associated with the processes of iron corrosion and release.³ Magnetite (Fe_3O_4) was reported to be an important mineral in protecting iron pipes from extensive iron release.^{3,4}

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3em00544e

A DWDS can be regarded as a large scale biological reactor that hosts a wide variety of microorganisms both in bulk water and on the pipe surfaces (as biofilms), which could lead to high disinfectant demand and dissolved oxygen (DO) consumption.^{5–7} Some microorganisms on metal surfaces could affect the kinetics of cathodic and/or anodic corrosion reactions, and thus lead to acceleration or inhibition of corrosion processes. Bacteria associated with iron corrosion include sulfate-reducing bacteria (SRB), sulfur-oxidizing bacteria (SOB), iron-reducing bacteria (IRB), and iron-oxidizing bacteria (IOB).^{8–10} It has been reported that some bacteria within a biofilm could precipitate certain authigenic iron minerals, and thus influence the composition of corrosion scales.¹¹ Research by Lytle *et al.* also found that the sulfide produced by SRB could affect the iron geochemistry of the corrosion scales in DWDSs.⁴ However, many previous studies focused on the microbially influenced corrosion (MIC) processes of metallic materials,^{12–14} little attention was paid to the effect of biofilm communities on the composition of corrosion products and the key factors influencing corrosion product release behavior.

Sarin *et al.* had reported that iron corrosion and iron release were two different processes in themselves, and that the iron release was often unrelated to the corrosion rate.¹⁵ However, the essential difference between both processes has not been fully recognized in drinking water treatment and supply industries. Iron corrosion mainly focuses on the mass loss of metallic iron, while iron release mainly focuses on the amount of iron corrosion products entering into bulk water from the corroded surface. Iron release behavior could be influenced by many aspects, including the corrosion rate of metals, water chemistry, hydraulic conditions, and dissolution of corrosion scale constituents.^{9,16} The concentration of total iron in bulk water (iron released) and the corrosion rate of metal iron had been used without thorough differentiation.

Thus, it is necessary to evaluate the roles of bacteria in the formation and release of corrosion products by using well designed corrosion experiments and powerful biofilm characterization techniques. In the present study, annular reactors (ARs), which were widely used to simulate DWDSs,^{9,17,18} were applied to conduct iron corrosion and release experiments. The main objective of this work is to understand the formation and transformation processes of corrosion products under the influence of bacterial communities in DWDSs, and the relationship between iron corrosion and corrosion product release was further elucidated by comparing with ARs running in parallel without biofilms. The possible roles played by certain functional bacterial communities in the formation of iron corrosion products with different characteristics were discussed. Furthermore, iron corrosion scale and biofilm samples formed under different water supply conditions in an actual DWDS were also characterized to verify the relationships between the bacterial community and corrosion products.

Materials and methods

Dynamic AR test

The ARs (Model 1320LJ, BioSurface Technologies Corp., USA) consist of two concentric glass cylinders and a rotating inner

Table 1 Average feed water quality parameters of raw tap water and disinfected tap water used for AR tests

Water quality parameter	Raw tap water	Disinfected tap water
pH	7.82	7.85
Turbidity (NTU)	<1.0	<1.0
Conductivity ($\mu\text{S cm}^{-1}$)	500	515
Alkalinity (mg L^{-1} as CaCO_3)	137	137
Cl^- (mg L^{-1})	32	35
SO_4^{2-} (mg L^{-1})	72	72
NO_3^- (mg L^{-1} N)	3.4	3.4
NO_2^- (mg L^{-1} N)	<0.001	<0.001
Ca^{2+} (mg L^{-1})	23	23
Mg^{2+} (mg L^{-1})	9.2	9.6
DO^a (mg L^{-1})	7.2	7.2
TOC^b (mg L^{-1})	1.9	8.1
Total nitrogen (mg L^{-1} N)	1.6	3.0
LR ^c	1.75	1.81

^a DO: dissolved oxygen. ^b TOC: total organic carbon.

^c Larson ratio (LR) = $\frac{[\text{Cl}^-] + 2[\text{SO}_4^{2-}]}{[\text{alkalinity}]}$, units of $[\text{Cl}^-]$, $[\text{SO}_4^{2-}]$ and [alkalinity] in meq. L^{-1} .²⁶

drum that houses 20 flush-mounted removable cast iron coupons. Each coupon has an exposed surface area of 17.4 cm^2 for biofilm growth. In order to investigate the iron corrosion and iron release behavior as well as the roles of bacteria in the formation and transformation of corrosion products under the influence of bacterial communities, raw tap water collected from our lab without further disinfection (with low residual chlorine of $0.05\text{--}0.07 \text{ mg L}^{-1}$) was fed into an AR with a peristaltic pump, and the coupons were withdrawn at pre-determined time intervals for corrosion product and bacterial community analysis. Another AR was run in parallel for effluent water quality analysis. AR tests using feed water treated with polyquaternary ammonium (50 mg L^{-1} as active ingredient, a kind of non-oxidant disinfectant) were also conducted to provide somewhat comparative results in the absence of biofilms. The polyquaternary ammonium was a commercial product purchased from a chemical company in Beijing, China.

The typical water quality parameters of the raw tap water and disinfected tap water are presented in Table 1. Iron coupons were prepared following the standards for AR tests. The ARs were operated at a rotational speed of 50 rpm, and the hydraulic retention time (HRT) for the reactors was 2 h.^{17,18} Prior to experimental use, the ARs were disinfected as described in the literature.¹⁸ All glass surfaces and any exposed surface were covered with aluminum foil to reduce the potential for phototrophic growth within the system.

Pipe section collection in an actual DWDS

Four old (approximately 20 years old), unlined cast iron pipe sections were excavated from four different sites of the DWDS of a Northern city in China. These sites were supplied with finished waters from different water treatment plants and different water sources. Of the four pipe sections, Pipe A was transporting surface water (SW-1); Pipe B and Pipe C

were transporting blended surface water and groundwater (B: SW-2 and GW-1; C: SW-3 and GW-1), since they were excavated from the boundary of SW and GW service areas; Pipe D was transporting groundwater (GW-2). Immediately after excavation, pipe sections were rapidly transported to the lab cooler and biofilm samples were collected within 24 h. Water source types and main finished water quality parameters related to the obtained pipe sections are summarized in the ESI (Table S1†).

Analysis of water quality, corrosion rate and corrosion scales

Water quality parameters, such as pH, turbidity, alkalinity, conductivity, chloride (Cl^-), sulfate (SO_4^{2-}), nitrate (NO_3^-), and nitrite (NO_2^-) ions, were measured. The pH and conductivity were measured using a Portable Multiparameter meter (Sension156 HACH, USA), and the turbidity was analyzed using a 2100P Turbidimeter (HACH, USA). The SO_4^{2-} , Cl^- , NO_3^- , and NO_2^- were measured using an Ion Chromatography System-1000 (Dionex, USA). The total organic carbon (TOC) was measured with a TOC analyzer (TOC-VCPH, SHIMADZU, Japan), and the total nitrogen was determined by the $\text{K}_2\text{S}_2\text{O}_8$ -spectrophotometric method. Total iron, calcium ions (Ca^{2+}), and magnesium ions (Mg^{2+}) were analyzed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Perkin-Elmer Optima 2000, USA). Unless otherwise specified, all chemicals used in this study were of Analytical Reagent (AR) grade. Ultrapure nitric acid was used to digest samples for total iron analysis. Sampling for water quality analysis was performed at a minimum of two times per week, while the turbidity and total iron were monitored at set hours every 2 days before 90 days, and every 4 days after 90 days.

Four cast iron coupons were taken out for the measurement of the corrosion rate at each time. The corrosion rate was the mean value of the four cast iron coupons, which was measured by the weight loss method as described previously,¹⁹ using the following equation:

$$\text{Corrosion rate (mm per year)} = 87\,600 W / A t \rho$$

where W is the weight loss (g), A is the exposure area (cm^2), t is the operating time (h) and ρ is the density of cast iron (7.87 g cm^{-3}).

The corrosion products were scraped from the inner wall of the coupons with a sterile spatula in an anaerobic glove box at 30, 60, 76, and 163 days and pretreated for X-ray diffraction (XRD) analysis. At 76 days and 163 days, the corrosion products of the cast iron coupons exhibited a two-layered structure, namely the top layer (T) and the inner layer (I) (Fig. S1†). The two different layers were analyzed separately. Detailed procedures are provided in the ESI, S1.†

The pretreatment procedures and the XRD (D/max-rA, Rigaku, USA) operation parameters were the same as previously described.³ Pattern analysis was performed using the ICDD (International Centre for Diffraction Data), and the K value method was used in the semi-quantitative analysis of the crystalline phase composition of corrosion products.

DNA extraction

Bacterial community in AR tests. The corrosion scales with bacteria were scraped using sterile spatulas from cast iron coupons at the operating times of 7, 30, 60, 76, and 163 days, then pulverized in an anaerobic glove box and freeze-dried. The anaerobic glove box, equipped with an ultraviolet lamp, was sterilized for 30 min before use. The scrapers, medicinal ladles, agate mortars, and the other tools used for pretreatment of corrosion scales were all sterilized before use. For the 76 days and 163 days samples, the top layers and the inner layers were also classified and analyzed separately.

DNA was extracted from $0.5 \pm 0.0001 \text{ g}$ freeze-dried samples using a FastDNA spin kit for soil (MP Biomedicals, Solon, USA) facilitated with a FastPrep-24 bead beater system following the manufacturer's instructions, and then quantified with a Nano-drop-1000 spectrophotometer (Thermo Scientific, Wilmington, USA). All DNA samples were stored at -80°C until further processing.

Bacterial community of biofilms in an actual DWDS. Approximately 20.0 cm^2 of biofilm samples was scraped using sterile spatulas from the top, middle, and bottom of each pipe section. Three parts of biofilm samples were mixed homogeneously for DNA extraction. Before DNA extraction, the biofilm samples were washed three times using aseptic, phosphate-buffered saline (pH 7.0) and centrifuged at 10 000 rpm ($10\,400 \times g$) at 4°C for 15 min. After centrifugation, DNA was extracted using a FastDNA spin kit for soil as used in the above method. The biofilms were named A, B, C, and D according to the excavated pipes.

Pyrosequencing and statistical analysis

The V1–V3 regions within the 16S rRNA gene fragment were amplified from biofilm DNA by PCR using the barcoded universal primers 8F ($5'\text{-AGAGTTTGATCCTGGCTCAG-3'}$) and 533R ($5'\text{-TTACCGCGCTGCTGGCAC-3'}$) containing the A and B sequencing adaptors, respectively (provided origin of 454 Life Science, Branford, USA).²⁰ PCR amplification was performed as described previously.²¹ Positive PCR products were purified with an AxyPrep DNA Gel Extraction Kit (Axygen, USA). The DNA concentration of purified amplicons was measured using a TBS-380 Fluorometer (Turner Biosystems, USA). Prior to sequencing, the amplifications from each reaction mixture were mixed in equal amounts based on the concentration and subjected to emulsion PCR, and amplicon libraries were generated as recommended by 454 Life Sciences. Sequencing was performed from the primer B end using the 454/Roche B (Roche Diagnostics, Indianapolis, USA) sequencing primer kit with the Genome Sequencer GS-FLX according to the protocols.

Pyrosequencing flowgrams were converted to sequence reads using the MOTHUR software (<http://www.mothur.org/>) and then analyzed using the UCHIME (<http://drive5.com/uchime>) standard pipeline. Sequence reads were initially filtered and denoised for removing low quality or ambiguous reads. The treated sequences were then subjected to systematic checks to remove replicates, duplicates, barcodes, primer sequences, and low quality reads. High quality sequences (>200 bases in length,

quality score > 25, exact matches to barcode and primer, and those containing no ambiguous characters) briefly remained with an average length of 475 bases.

Sequences were clustered into the operational taxonomic units (OTUs) by setting a 0.03 distance limit (equivalent to 97% similarity) using the MOTHUR program. Species richness estimators (abundance-based coverage estimator [ACE] and Chao),^{22,23} the Shannon diversity index as well as Simpson's diversity index were generated in MOTHUR for each sample. Shannon–Wiener curves were performed on R (<http://www.r-project.org/>). Sequences were phylogenetically assigned to taxonomic classifications using MOTHUR *via* the SILVA database (SILVA SSU111, <http://www.arb-silva.de>) with a set confidence threshold of 80%. After phylogenetic allocation of the sequences down to the phylum, class, and genus level, relative abundance of a given phylogenetic group was set as the number of sequences affiliated with that group divided by the total number of sequences per sample.²⁴ Sperman's rank and Student's *t*-test were determined by using the SPSS 18.0 (SPSS Inc., Chicago, USA). Distribution heatmap of genus-level bacterial communities was implemented by vegan package of R in Linux. The bacterial phylogenetic tree on the left was created by the approximately-maximum-likelihood method using FastTree,²⁵ and the relationship among samples was determined by Bray–Curtis and the complete clustering method.

Results

Feed water quality characteristics for AR tests

The main water quality parameters of raw tap water and disinfected tap water were relatively stable during the experimental period. Compared with raw tap water, the disinfected tap water had higher concentrations of TOC and total nitrogen, while the

other water quality parameters including the Larson ratio (LR)²⁶ were similar (Table 1). The discrepancy of pH in the raw and disinfected tap water was negligible. It is indicated that the addition of polyquaternary ammonium as an organic-nitrogen non-oxidative bactericide increased the organic and nitrogen content but had little effect on the water quality parameters that related to corrosivity, such as pH, alkalinity, LR, *etc.*

To verify the disinfection efficiency of polyquaternary ammonium, the heterotrophic bacteria count (HPC) in the disinfected water and on the cast iron coupons in contact with disinfected tap water were monitored. The HPC was below the detection level throughout the experimental period, which indicated that a relatively good sterile condition was achieved by polyquaternary ammonium addition.

Changes of corrosion rate, iron release and corrosion products with experimental time

Corrosion rate and iron release. Fig. 1a shows the changes of iron corrosion rate with time in the AR tests under raw and disinfected tap water conditions. The curve of the iron corrosion rate with raw tap water exhibited three different stages. At the period of 4–30 days (Stage I): the corrosion rate is rather high at the initial time (4–7 days), and decreased sharply at 14 days, then increased again at 30 days. After 30 days, the corrosion rate increased rapidly from 0.21 to 0.34 mm per year at 76 days (Stage II), later decreased to 0.17 mm per year at 135 days and remained at this level thereafter (Stage III). The variation trends of corrosion rate at Stages II and III were consistent with the research work by Wang *et al.*,⁹ which indicated that the iron corrosion rate was increased at 30–70 days and decreased after 70 days. The changes of the corrosion rate with disinfected tap water were relatively simple. It is 0.174 mm per year at 30 days,

Fig. 1 Changes of corrosion rate and total iron concentration with time under raw and disinfected tap water conditions in AR tests (a: corrosion rate of cast iron coupons; b: total iron concentration of effluents).

then decreased gradually to 0.12 mm per year at 110 days and maintained at this level.

As shown in Fig. 1b, the total iron concentration under raw tap water conditions is rather high (1.66 mg L^{-1}) at the time of 4 days, and decreased rapidly to 0.22 mg L^{-1} at the time of 36 days. After 36 days, it was increased gradually from 0.22 to 0.512 mg L^{-1} at 76 days, and decreased once again and remained at about 0.02 mg L^{-1} . The concentration of total iron with disinfected tap water was 1.92 mg L^{-1} at 4 days, and then it decreased gradually and maintained at about 0.17 mg L^{-1} after 100 days. The turbidity of effluents exhibited similar variation trends with the concentration of total iron (Fig. S2†). The correlation coefficients of the total iron concentration and turbidity with raw and disinfected tap water were 0.81 and 0.89, respectively, which indicated that the iron in water is mainly in particulate form.

Composition of corrosion products. Calcium carbonate (CaCO_3), green rust ($\text{Fe}_6(\text{OH})_{12}\text{CO}_3$), goethite ($\alpha\text{-FeOOH}$), and Fe_3O_4 were the main crystalline minerals of the corrosion scales under raw tap water conditions. During the period of 7–60 days, the relative abundances of CaCO_3 and $\alpha\text{-FeOOH}$ were gradually increased with time, whereas the content of $\text{Fe}_6(\text{OH})_{12}\text{CO}_3$ was decreased. For the samples at 76 days and 163 days, the XRD results showed that $\alpha\text{-FeOOH}$ and Fe_3O_4 mainly existed in the top layer, and green rust mainly existed in the inner layer (Table 2a).

Table 2 Relative abundance (%) of corrosion products on cast iron coupons with raw and disinfected tap water in AR tests (a: raw tap water; b: disinfected tap water)

a				
Sample	CaCO_3	$\text{Fe}_6(\text{OH})_{12}\text{CO}_3$	$\alpha\text{-FeOOH}$	Fe_3O_4
14 days	8	64	28	— ^a
30 days	16	32	52	—
45 days	23	23	54	—
60 days	34	11	54	1
76 days (I) ^b	51	26	19	4
76 days (T) ^c	50	4	39	7
110 days (I)	58	22	10	10
110 days (T)	52	—	35	13
163 days (I)	54	18	19	9
163 days (T)	49	2	31	18

^a “—” – not detected. ^b I – inner layer. ^c T – top layer.

b					
Sample	CaCO_3	$\text{Fe}_6(\text{OH})_{12}\text{CO}_3$	$\alpha\text{-FeOOH}$	Fe_3O_4	FeCO_3
14 days	25	63	12	—	—
30 days	61	20	19	—	—
45 days	66	15	19	—	—
60 days	77	11	12	—	—
76 days (I)	72	12	13	—	3
76 days (T)	71	5	24	—	—
110 days (I)	65	15	16	—	4
110 days (T)	77	—	19	4	—
163 days (I)	68	9	14	3	6
163 days (T)	72	—	21	7	—

Under disinfected conditions, the main crystalline minerals of the corrosion products were similar to those under raw tap water conditions. However, the relative abundances of iron oxides such as $\text{Fe}_6(\text{OH})_{12}\text{CO}_3$, $\alpha\text{-FeOOH}$, and Fe_3O_4 were much lower compared with those under raw tap water conditions, while the content of CaCO_3 was higher.

Characteristics of the bacterial communities at different operation times in AR tests

From the iron coupons in contact with raw tap water, seven biofilm samples were collected at different experimental days and pyrosequencing was run to characterize the community structure and transformation process of the bacterial community. A total of 90 587 valid sequences and 5004 OTUs at 97% similarity level were obtained (Table S2†). The Shannon diversity indices of all samples already reached stable values at the sequencing depth used in this study, which means that most diversity had already been captured although new phylotypes might be expected with additional sequencing (Fig. S3†). All

Fig. 2 Bacterial distribution of 30 most abundant genera among seven samples with raw tap water in AR tests. Double hierarchical dendrogram shows the bacterial distribution. The color intensity of the scale indicates relative abundance of each genus. Relative abundance was defined as the number of sequences affiliated with that genera divided by the total number of sequences per sample (I: inner layer; T: top layer).

Table 3 Relative abundance (%) of corrosive bacteria (at the genus level) on ARs test coupons and actual DWDS biofilms

	Genera	7 days	30 days	60 days	76 days (I)*	76 days (T)**	163 days (I)	163 days (T)	A	B	C	D
SRB ^a	<i>Desulfovibrio</i>	—	—	0.03	0.27	0.01	0.36	0.24	0.02	0.19	0.06	0.05
	<i>Desulfotomaculum</i>	—	—	—	0.06	—	—	—	0.23	0.06	—	—
SOB ^b	<i>Sulfuricella</i>	—	—	13.94	24.84	1.47	16.65	2.77	0.02	0.03	0.3	0.02
	<i>Acidithiobacillus</i>	—	—	—	—	—	—	—	0.07	—	—	—
	<i>Alicyclobacillus</i>	—	—	—	—	—	—	—	0.08	0.2	—	—
IRB ^c	<i>Bacillus</i>	0.01	—	—	0.04	0.07	0.11	1.47	19.45	9.03	2.32	0.1
	<i>Clostridium</i>	0.1	—	—	0.04	—	0.06	0.02	0.67	0.77	—	—
	<i>Pseudomonas</i>	0.02	—	—	—	—	0.04	0.16	1.09	0.82	1.72	1.03
	<i>Escherichia-Shigella</i>	—	—	—	—	—	—	—	0.24	0.08	0.53	0.05
IOB ^d	<i>Acidovorax</i>	0.21	17.98	3.29	0.05	0.49	0.08	0.46	0.47	2.14	0.78	0.09
	<i>Gallionella</i>	0.01	—	0.25	1.4	0.03	1.53	0.1	0.07	—	0.03	—
	<i>Leptothrix</i>	0.01	—	0.21	0.23	0.32	0.51	0.6	—	0.02	—	—
	<i>Sphaerotilus</i>	—	—	—	—	0.03	0.11	0.26	—	—	—	—
	<i>Pedomicrobium</i>	—	—	—	—	—	—	—	0.08	—	—	0.04

^a SRB: sulfate-reducing bacteria. ^b SOB: sulfur-oxidizing bacteria. ^c IRB: iron-reducing bacteria. ^d IOB: iron-oxidizing bacteria. *I: inner layer; **T: top layer.

obtained sequences were classified from phylum to genus according to the program MOTHUR. *Proteobacteria* (mainly consisted of *Alphaproteobacteria* and *Betaproteobacteria*) was the most predominant group in all bacterial communities (Fig. S4†). Fig. 2 shows that the composition of the bacterial communities at the genus level changed with time, and the bacterial communities in the top and inner layers were separated, suggesting the existence of distinct community structures between top and inner corrosion scales.

Based on the results obtained by pyrosequencing and previously published reports,^{8,27–34} potential corrosive bacterial genera were identified in the seven bacterial communities of 7 days, 30 days, 60 days, 76 days (I), 76 days (T), 163 days (I) and 163 days (T). The relative abundance of bacteria associated with iron corrosion at the genus level is listed in Table 3. Some notable characteristics could be observed. First, the relatively higher abundance of sequences related to *Sulfuricella* was detected in samples of 60 days, 76 days (I) and 163 days (I), and the highest abundance was present in the sample of 76 days (I). Most of the sequences related to *Sulfuricella* showed high similarity to *Sulfuricella denitrificans* in the present study, which is known as SOB.²⁷

Second, *Desulfovibrio* (SRB) was only detected until 60 days, and the relative abundance of *Desulfovibrio* was increased after 60 days. For the samples at 76 days and 163 days, it mainly existed in the inner layers. Another SRB genus *Desulfotomaculum* was only detected in the sample of 76 days (I).

Third, the genera *Bacillus*, *Clostridium*, and *Pseudomonas* were only detected in the samples at the time of 7 days, 76 days and 163 days. Previous research reported that a great many species (e.g. *Bacillus* sp., *B. subtilis*, *B. infernus*, *B. sp. nov.*, *B. circulans*, *Clostridium* sp., *Pseudomonas* sp. et al.) belonging to these genera had the ability to reduce ferric iron to ferrous iron, known as IRB.^{8,28–32} In the present study, *Bacillus* and *Pseudomonas* mainly existed in the top layers of the samples at 76 and 163 days, and the highest abundances were present in the sample of 163 days (T).

Fourth, *Acidovorax*, *Sphaerotilus*, *Gallionella* and *Leptothrix* (IOB)^{33,34} were also detected in the samples. Particularly, *Acidovorax*, as the most dominant IOB, was present all through the experimental period. In addition, for the layered corrosion scales, *Acidovorax* and *Sphaerotilus* mainly existed in the top layers (samples at 76 and 163 days), but *Gallionella* mainly existed in the inner layer. *Leptothrix* existed in both inner and top layers.

Characteristics of corrosion scales and biofilms in an actual DWDS

Morphological and structural characteristics of corrosion scales. Corrosion scales of Pipes A, B and C were the typical four-layered thick corrosion scales or tubercles (Fig. 3). The

Fig. 3 Appearance of corrosion scales on cast iron pipes in an actual DWDS (a) Pipe-A; (b) Pipe-B; (c) Pipe-C; (d) Pipe-D.

micro-structural features of such kind of tubercle-formed and thick corrosion scale had been described in detail,^{2,3} which included a loosely attached top surface layer, a hard shell-like layer, a porous core, and a corroded floor. Compared with Pipes A, B, and C, corrosion scales on Pipe D were much thinner and relatively smooth (nearly no corrosion tubercles on the inner pipe surfaces).

Sarin *et al.* and Yang *et al.* found that Fe_3O_4 and $\alpha\text{-FeOOH}$ were the main constituents of the hard, shell-like layers.^{2,3} Yang *et al.* further observed that the thin scales formed under GW supply conditions had no Fe_3O_4 detected or with a much lower

$\text{Fe}_3\text{O}_4/\alpha\text{-FeOOH}$ ratio.³ Other ferrous iron oxides such as FeCO_3 , green rust, and $\text{Fe}(\text{OH})_2$ (mainly existed in the porous core) also existed at a relatively higher content in corrosion scales of SW pipes. In the scales of GW pipes, less stable iron oxides such as akaganeite ($\beta\text{-FeOOH}$), lepidocrocite ($\gamma\text{-FeOOH}$) as well as amorphous iron oxides were relatively higher than those of the SW pipes.

Characteristics of the biofilm communities. Through 454 pyrosequencing analysis, 45 538 valid reads and 3822 OTUs at 97% similarity level were obtained from all four biofilm samples. The Shannon diversity indices of the four samples already reached stable values, which indicated that almost all diversity information had been captured (Fig. S5†). *Proteobacteria* (mainly consisted of *Betaproteobacteria*) was the most predominant phylum in all samples. The phyla *Firmicutes* and *Actinobacteria* mainly existed in biofilms with SW, and appeared at much lower proportions in biofilms with GW (Fig. 4). Evaluation at the genus level showed that these two phyla were primarily comprised of *Bacillus* and *Mycobacterium*, respectively. The majority of these organisms found in DWDSs were aerobic, Gram-negative bacteria, and capable of degrading a wide range of organic compounds.

As shown in Table 3, the most abundant sequences related to *Bacillus* (IRB) were detected in Sample A (19.45%). Compared with Sample A, Sample D contained a much lower proportion of *Bacillus* (0.10%). The relative content of *Bacillus* in Samples B and C was between that of Samples A and D, which was 9.03% and 2.32% respectively. The percentages of the genus *Acidovorax* (IOB) in Samples A, B, and C (0.47%, 2.14% and 0.78%, respectively) were also much higher than that in Sample D (0.09%). SRB (*Desulfovibrio*, *Desulfotomaculum*) and SOB (*Acidithiobacillus*, *Alicyclobacillus* and *Sulfuricella*) were also detected, but they are not rich in all biofilm samples compared with IRB and IOB. The relative abundance of these potentially corrosive microorganisms in samples under GW conditions was markedly lower than that in other samples.

Discussion

Iron corrosion and iron release

Fig. 1 demonstrates the corrosion rates of cast iron coupons (a) and the corresponding iron release (b) under raw and disinfected conditions all changed with time. The correlation coefficients of the total iron concentration and iron corrosion rate with raw and disinfected tap water were 0.82 and 0.93, respectively, which indicated that iron release had a good positive relationship with the iron corrosion rate under both experimental conditions. However, it should be noticed that the corrosion rate of cast iron coupons with disinfected tap water was obviously lower than that with raw tap water all the time (Student's *t*-test, $p < 0.05$), but the iron release corresponding to disinfected tap water was relatively higher after 80 days ($p < 0.01$). This phenomenon demonstrated that a higher corrosion rate might not always mean higher iron release although a good correlation between them existed within one group.

Sarin *et al.* reported that corrosion scale constituents could influence iron release.¹⁵ In this work, the composition of

Fig. 4 Taxonomic classification of pyrosequences from the four biofilm samples in an actual DWDS (a: at the phylum level; b: at the class level). Sequences that could not be classified into any known group and some smaller phyla (classes) (relative abundance $< 0.1\%$) in amount were assigned as "Unclassified".

corrosion scales was different under two conditions. The relative abundances of Fe_3O_4 ($p < 0.05$) and $\alpha\text{-FeOOH}$ ($p < 0.01$) with raw tap water were significantly higher than those with disinfected tap water (Table 2). In an actual DWDS, $\alpha\text{-FeOOH}$ and Fe_3O_4 were reported to be important minerals in corrosion scales with a layered structure, and such constituents could play crucial roles in protecting iron pipes from extensive iron release.^{3,4} In addition, as mentioned above, both IRB and IOB can participate in the precipitation of iron corrosion products.^{9,32–34} Therefore, the corrosion scales as well as the bacterial community properties could greatly influence the iron release process, and moreover, the concentration of total iron in bulk water could not reliably reflect the iron corrosion rate.

Corrosion rate and bacterial communities

During the initial stage (Stage I), the iron corrosion rate with raw tap water was similar to that with disinfected tap water before 7 days in ARs, which indicated that corrosion of the cast iron was mainly caused by electrochemical corrosion at the initial stage (Stage I). With time increased (7–14 days), the corrosion scales of cast iron coupons became thicker, which could serve as the barrier between the water and iron surface, and thus prevent further corrosion.³⁵

At Stage II (30–76 days), the corrosion rate with raw tap water increased, which was much higher than that with disinfected tap water ($p < 0.05$). Meanwhile, the relative abundances of some potential corrosive bacteria such as SRB (*Desulfovibrio*), SOB (*Sulfuricella*), and IOB (*Sphaerotilus*, *Gallionella* and *Leptothrix*) were all increased during this period. Research work by Xu *et al.* reported that SRB is usually associated with anaerobic iron corrosion by producing hydrogen sulfide (H_2S) as a corrosive agent.³⁶ H_2S could be converted to sulfuric acid (H_2SO_4) by SOB, resulting in serious iron corrosion.³⁷ In the present study, SRB and SOB were mainly found in the inner layer (under anaerobic conditions), and the corrosion rate of cast iron coupons at Stage II was positively correlated with SRB ($R = 0.74$) and SOB ($R = 0.97$). This result might indicate that growth and interaction of SRB and SOB were mainly associated with anaerobic iron corrosion. In addition, SOB could convert ferrous sulfide to H_2SO_4 , releasing ferrous iron in the process, which in turn could be used by IOB. Beneath the iron rich tubercles produced by IOB, SRB might increase rapidly. The interaction between SOB/IOB and SRB/IOB could accelerate the iron corrosion process.³⁶ In the present study, IOB existed in both inner and top layers, which was also positively correlated with the iron corrosion rate ($R = 0.69$). These results indicated that the growth and interaction of SRB, SOB, and IOB could speed up the iron corrosion at Stage II. The iron release with raw tap water at Stage II (30–76 days) was in accordance with the iron corrosion rate, which implied that SRB, SOB, and IOB also increased iron release during this period.

At Stage III (after 76 days), the corrosion rate of cast iron coupons with raw tap water was decreased. The pyrosequencing analysis showed that even though the relative abundances of some corrosive bacteria *e.g.* *Desulfovibrio*, *Sphaerotilus*, *Gallionella*, and *Leptothrix* were increased, the percentage of

Sulfuricella and *Acidovorax* were all decreased greatly. The decrease of these genera might reduce the corrosion rate to some extent. In addition, it should be noticed that the relative abundances of IRB related bacterial genera, such as *Bacillus* and *Clostridium*, were all increased at Stage III, and *Pseudomonas* only detected at 163 days. IRB could reduce ferric iron to ferrous iron, which was reported to have the ability to inhibit iron corrosion.^{16,38} Some species belonging to these genera such as *Bacillus* sp., *Pseudomonas* sp. and *Clostridium* sp. are also slime-forming microorganisms which could produce copious quantity of extracellular polymeric substances (EPS) during biofilm formation on the metal, and the EPS at the metal surface could impede the dissolution of ferrous iron corrosion products.⁹ Moreover, IRB could also favor the formation of Fe_3O_4 (details below), which was reported to protect iron pipes from extensive iron release in DWDSs.^{3,4} Therefore, the growth of IRB might be one of the factors decreasing iron corrosion and iron release.

Corrosion products and bacterial communities

Iron-oxidizing bacteria and $\alpha\text{-FeOOH}$ formation. Corrosion of iron involves the formation of ferrous iron (Fe^{2+}) from elemental iron (eqn (1)), and Fe^{2+} may then diffuse into water; $\alpha\text{-FeOOH}$ can be formed from the reactions (eqn (2) and (3)), according to the Siderite model proposed by Sontheimer *et al.*³⁹

IOB can impact the formation of iron minerals by their metabolic activity and by forming the nucleation surface. For instance, IOB promote the oxidization of Fe^{2+} to Fe^{3+} by using dissolved oxygen (DO), and rapid precipitation of bacteriogenic iron oxides could occur. Freshly formed amorphous biomineralization products can further transfer to more crystalline $\alpha\text{-FeOOH}$.⁴⁰ Moreover, IOB can induce ferric hydroxide precipitation as a secondary by-product, and the ferric hydroxide may then serve as a precursor for more stable iron oxides, such as $\alpha\text{-FeOOH}$.⁴¹ Some species belonging to the genus *Acidovorax* (such as *Acidovorax* sp. BoFeN1 and *Acidovorax* sp. strain 2AN) were also shown to produce $\alpha\text{-FeOOH}$ during the Fe(II) oxidation.^{41,42}

In the present study, $\alpha\text{-FeOOH}$ was mainly found in the top layer of the corrosion scales, and the relative abundance of $\alpha\text{-FeOOH}$ in the ARs under non-disinfected conditions was significantly higher than that under disinfected conditions ($p < 0.01$). For the samples at 76 and 163 days under non-disinfected water conditions, the genus *Acidovorax* also mainly existed in the top layers. Correlation analysis showed that the relative abundance of $\alpha\text{-FeOOH}$ had a significant positive correlation with *Acidovorax* ($R = 0.80$, $p < 0.01$) in AR tests. Moreover, in the pipes from the actual DWDS, the relative abundances of IOB (especially *Acidovorax*) in the biofilms formed on the hard, shell-like layers of thick corrosion scales were much higher than those formed on thin scales, and $\alpha\text{-FeOOH}$ was one of the main constituents of the hard, shell-like layers. The results of this

study indicated that the growth or action of IOB (especially *Acidovorax*) contributed to the formation of α -FeOOH in the DWDS.

Iron-reducing bacteria and Fe₃O₄ formation. Under suboxic to anoxic conditions, ferric hydroxide could be converted to Fe₃O₄, FeCO₃ and iron sulfides through various reductive processes,⁴¹ and biological iron reduction was involved in the formation of Fe₃O₄.⁴³ Research by Lovley *et al.* found that the formation of Fe₃O₄ in anaerobic sediments resulted from the oxidation of organic matter coupled to the reduction of ferric oxide to Fe₃O₄, and the dissimilatory iron-reducing bacteria were the catalysts for this reaction (eqn (4)).⁴⁴ The IRB could respire with oxidized iron in the form of amorphous ferric oxyhydroxides or crystalline iron oxides, and induce the precipitation of Fe₃O₄ under anaerobic conditions.^{10,44,45} For example, α -FeOOH can be reduced to Fe₃O₄ by IRB with a soluble electron;⁴⁶ β -FeOOH can also be reduced to Fe₃O₄ by IRB but exogenous electron carrier substances are not necessary.⁴⁵ γ -FeOOH may also be transformed to Fe₃O₄ by adsorbing Fe²⁺ (eqn (5)).⁴⁷

In the present work, a majority of the microorganisms found in the DWDS were aerobic, and a previous study found that the concentration of DO was decreased at the biofilm–water interface due to direct DO consumption by these organisms.⁴⁸ Thus, anaerobic conditions favored the formation of Fe₃O₄ under biofilms. In the dynamic AR test, Fe₃O₄ and IRB were all abundant in the top layers. Correlation analysis showed that the relative abundance of Fe₃O₄ was positively correlated with IRB ($R = 0.92$, $p < 0.05$). As mentioned earlier, Fe₃O₄ was one of the main constituents of the hard shell-like layers of thick corrosion scales in the real DWDS, but the thin scales had no Fe₃O₄ detected or with a much lower content. The pyrosequencing analysis of biofilms in the real DWDS showed that the IRB was also abundant in the biofilm formed on the hard shell-like layers. These results testified that the IRB as well as the gradient anaerobic conditions play crucial roles in the formation of Fe₃O₄ in the DWDS.

Conclusions

In this study, annular reactor tests demonstrated that the corrosion rate of cast iron coupons in raw tap water with biofilms was higher than that under disinfected conditions (using polyquaternary ammonium as a disinfectant). However, the iron release was obviously lower under raw tap water conditions (except for the period of 36–80 days). The results confirmed that the concentration of total iron in bulk water could not reliably reflect the corrosion rate of metal.

Pyrosequencing analysis of bacterial communities in the ARs showed that the growth and interaction of SRB (*Desulfovibrio*, *Desulfotomaculum*), SOB (*Sulfuricella*), and IOB (*Acidovorax*, *Gallionella*, *Leptothrix* and *Sphaerotilus*) accelerated iron

corrosion, while IRB (*Bacillus*, *Clostridium* and *Pseudomonas*) inhibited iron corrosion and iron release.

α -FeOOH and Fe₃O₄ mainly existed in the top layers of corrosion scales formed under raw tap water conditions, which was in agreement with the scenario in an actual DWDS. Correlation analysis showed that the relative abundance of IOB (especially *Acidovorax*) and IRB positively correlated with α -FeOOH ($R = 0.80$, $p < 0.01$) and Fe₃O₄ ($R = 0.92$, $p < 0.05$), respectively. The pyrosequencing analysis of biofilms in both ARs and the actual DWDS revealed that IOB (especially *Acidovorax*) contributed to the formation of α -FeOOH, while IRB and the gradient anaerobic conditions contributed to the formation of Fe₃O₄ in the DWDS.

Acknowledgements

This work was financially supported by National Natural Science Foundation of China (no. 51178450 and no. 51378493) and National Water Pollution Control and Treatment Special Key Project of China (2012ZX07404-002, 2012ZX07408-002).

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