



# Article Effect of Pipe Materials on Bacterial Community, Redox Reaction, and Functional Genes

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Abstract: In the present study, the effect of pipe materials on water quality as well as the microbial community was researched with static devices as well as dynamic ones. Five kinds of pipe materials (SP: steel plastic composite pipe, SS: stainless steel pipe, DI: ductile iron pipe, CI: cast iron pipe, GS: galvanized steel pipe) were chosen, and the soaking experiment was carried out with bench-scale devices. To further investigate the performance of pipe materials over a long term, a pilot-scale simulated drinking water distribution system was constructed, and the water quality parameters were monitored for six months. The pipe materials were ranked as SP, DI, and CI by the order of increasing turbidity, COD<sub>Mn</sub>, and NH<sub>3</sub>-N. Furthermore, the biofilm samples were analyzed via pyrosequencing and COG functional categories. The DI biofilm possessed the highest bacterial diversity with a Shannon index of 3.56, followed by SP (3.14) and CI (0.77). The presence of nitrate-reducing bacteria (NRB), iron-oxidizing bacteria (IOB), iron-reducing bacteria (IRB), and sulfate-reducing bacteria (SRB)was identified, and NRB composed the largest share in all pipe materials (13.0%-17.2%), with other redox bacteria making up a minor proportion (0.02%-1.52%). NRB and IRB inhibited the corrosion process while IOB and SRB enhanced it. Most dominant genera present in samples were derived firstly from soil or active sludge, indicating a turbidity problem due to soil contamination in the distribution network.

**Keywords:** pipe materials; simulated drinking water distribution system; pyrosequencing; biofilm; corrosion

# 1. Introduction

Disinfected water experiences water deterioration in the drinking water distribution systems. The pipe material is considered to be one of the dominant factors in the process, owing to the release of nutrients and complicated physiochemical reactions with microbes [1]. In previous research, the effect of pipe materials on a series of substances has been reported. Particles are quantified and investigated, including corrosion scales [2,3], metal ions [4], organic matters [5], disinfection residuals [6], DBPs [7], nitrogen [8,9], and biofilm.

Biofilm plays a central role in reactions with pipe materials, and thus an increasing enthusiasm for biofilm investigation is found. The biomass quantification [10–12], bacterial numeration [6], microbe community structure [13,14], and identification of pathogens [15–17] are measured and analyzed. From different pipe materials, significant diversity of bacterial abundance and community distribution is found. Additionally, bacteria crucial in nitrification, corrosion, and chlorine resistance are found. The influence of phenotypic, chemotaxonomic, and phylogenetic characteristics of dominant bacteria on water parameters is not discussed.



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). A variety of pipe materials has been tested in terms of biofilm formation. In some essays, plastic-based materials (PVC, PE, HDPE) are observed to have better performance [6,18], while in other research, steel/iron-based materials [5] or cement-based materials are recommended. The steel-plastic composite pipe is a kind of lined material, with the combined property of steel, and HDPE. Ductile iron is commonly used in southern China. The effects of these materials on bacteria were rarely discussed before and need further research.

In the last decade, the use of next-generation sequencing has incredibly enhanced the understanding of the microbial ecology of different ecosystems [19]. With the KEGG Ortholog (KO) database of reference genomes, analysis tools such as the PICRUSt are widely applied to predict the functional composition of a metagenome [20]. Meanwhile, more functional genes have been shown to be linked to microbial metabolism [21,22]. To provide information on the microbial controlling strategy, the role of interspecies relationships in different pipe materials still needs further discussion, from the perspective of microbial ecology.

The simulation of distribution systems has been carried out with a bench-top device and a pilot-scale simulated system. The device avoids the uncertain influence of actual pipeline sampling (variation of pipe age, hydraulic condition, and temperature) and makes it convenient to sample and control. The pilot-scale simulated system, compared to a bench-top device, exhibits a better simulation of the real hydraulic condition [23].

In the present study, a bench-scale and a pilot-scale experiment were carried out to give an insight into the biological and chemical process both in a static and a dynamic model. A model pilot-scale distribution system was built for dynamic simulation to achieve better experimental results [24]. With the result of water quality parameters and 16S rRNA-based pyrosequencing, the optimal pipe materials were recommended and confirmed by the improvement of water quality in an engineering project.

#### 2. Materials and Methods

# 2.1. Static Soaking Experiment

Five kinds of pipe material were soaked in two kinds of water, prepared in the laboratory, and collected from a distribution site, respectively. All pipes were purchased from Chengdu ChuanLi Intelligence Fluid Equipment Co. Ltd. (Chengdu China). The water preparation was based on the methodology of standard for assessment of drinking water distribution equipment and protective materials. The characteristics of the water mixed in the laboratory were determined as turbidity of 0.12 NTU, chlorine residuals of 2 mg/L, and a total number of bacteria of 0 CFU/mL. Correspondingly, the characteristics of the water collected in a real distribution site were determined as turbidity of 0.53 NTU, chlorine residuals of 0.08 mg/L, and a total number of bacteria of 3100 CFU/mL. Heshanqiao note was chosen as it has the most severe problem of water deterioration.

Sixty centimeters in length and 20 mm in diameter, the pipe materials were thoroughly washed out and disinfected with sodium hypochlorite. The pipes were soaked at 25 °C in the constant-temperature incubator. To simulate a real circumstance, the pipes were shaded. Turbidity, BDOC, DOC, and TPC were measured every day for a week, reflecting water quality in different water ages.

#### 2.2. Dynamic Distribution System Setup and Operation

A model pilot-scale distribution system was built in Suzhou, Taihu lake region (Figure 1). This system included three loops, using SS pipe, CI pipe, and DI pipe. The pipes were purchased from Chengdu ChuanLi Intelligence Fluid Equipment Co. Ltd. (Chengdu, China). Every loop was equipped with an inlet valve, flowmeter, and pumps. To simulate the real circumstances in pipelines as precisely as possible, the distribution system was fed with water from the clean water basin in the Xiangcheng water treatment plant consistently. The flow rate was maintained at  $5.6 \text{ m}^3/\text{h}$ .



Figure 1. The simulated drinking water distribution system.

After a stabilizing period of three months, the turbidity,  $COD_{Mn}$ , and  $NH_3$ -N were measured every two weeks for eight months. Biofilm samples were collected at the end of the term.

#### 2.3. Pyrosequencing of Bacterial 16S rRNA Genes

Biofilm samples were selected for bacterial community profiling by pyrosequencing, targeting 16S rRNA genes, which was performed at the I-Sanger Bioinformatics Analysis Cloud Platform, the Majorbio (http://www.i-sanger.com/, accessed on 4 March 2022). In total, 40,242, 40,237, and 36,911 pyrosequences were obtained from three biofilm samples, and they were swabbed from DI, CI, and SS. The length of sequences varied between 271 and 499. In total, 334 OTUs were defined based on clustering. Pyrosequences were assigned to 20 phyla, of which proteobacteria were the most abundant.

# 2.4. Data Analysis and Statistics

Kruskal–Wallis ANOVA was used to evaluate the effects of pipe materials on turbidity, BDOC, DOC, and TPC, respectively. Statistical significance was set at p < 0.05. Multiple comparison tests were performed to find out which pair of the five pipe materials were significantly different. Kruskal–Wallis ANOVA and multiple comparison tests were performed with OriginPro 2021.

A redundancy analysis was carried out with Canoco 4.5. Water quality statistics from the pilot-scale experiment and the transformation engineering project were analyzed with the above multivariate statistical analysis tools.

# 3. Results

## 3.1. The Difference in Water Quality Due to Leaching Matters from Pipe Materials

In the bench-scale experiment, pipe materials were soaked with water samples from the laboratory and in situ distribution system. During seven days, leaching components as well as bacterial growth were analyzed with indicators involving turbidity, BDOC, DOC, and TPC (Figure 2).



Figure 2. Comparison of effluent from different pipe materials during a seven-day soaking experiment.

The soaking experiment gave a profile of the soaking water quality indicators related to the leaching process by varying the soaking water. The characteristics of the soaking water, collected from distribution systems or prepared in the laboratory, are listed in Table 1. The initial bacterial concentration was much higher from the distribution system node (3100 CFU/mL), with a relatively lower chlorine residual (0.08 mg/L) and higher turbidity. For metal pipe materials tested (SS, DI, CI, and GS), the leaching levels of metal ions, mainly iron and zinc, are higher when a higher level of chlorine is presented [25–27]. Besides the chlorine residuals, the initial bacteria concentration also proceeds the bacterial growth in the pipe, which accelerates the corrosion and subsequent iron release [28].

Table 1. (	Characteristics	of the	soaking	water.
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Water Sources	Turbidity (NTU)	Chlorine Residual (mg/L)	Total Plate Count Bacteria (CFU/mL)
Laboratory	0.12	2	0
Distribution system node	0.53	0.08	3100

The influence of pipe materials on water quality was evaluated by using Kruskal–Wallis ANOVA analysis. As illustrated in Table 2, pipe materials had a significant influence on water quality measured by turbidity, DOC, and BDOC (Figure 3). Considering the relatively short period, changes in the turbidity, DOC, and BDOC were mainly caused by the diffusion process, and the pipe material did play an important role. However, no insignificant influence by TPC was observed. Although there were no obvious differences observed in TPC between different materials, it was not to say the effect of the pipe material

on microbes was negligible. The total plate counts were only a reflection of quantities of microbes that could be fostered without the special requirement of nutrition and oxide. It did not reflect the structure of the microbe community, and further investigation should be carried out.

**Table 2.** Effects of pipe materials on water quality in water from different sources using Kruskal–Wallis ANOVA. Factors with a *p*-value smaller than 0.05 are highlighted in bold.

Sources of Immersing Water	Laboratory	Distribution System Node
Turbidity	0.001	0.021
TPC	0.578	0.116
DOC	0.182	0.006
BDOC	0.004	0.024



**Figure 3.** Multiple comparison test of the BDOC between different kinds of pipe material using water collected from the distribution system node (the type of critical value to use for the multiple comparisons is Kruskal–Wallis test, and the significance level is 0.05).

The SP is a kind of steel pipe lined with HDPE. For organic pipes, residual chlorine, temperature, and time have a positive effect on the released organics [29]. Organic compounds leaching from PE pipes are mostly antioxidants and plasticizers, which serve as nutrients for bacteria. Besides, PE releases biodegradable organic compounds and phosphorus which promote biofilm development on plastic surfaces [30]. Lehtola et al. observed a significant rate of phosphorus that was released from PE pipes into the water for 2–3 weeks, and the biofilm development was influenced by the phosphorus availability in the water systems [31].

# 3.2. The Difference in Water Quality Due to the Activity of Microorganisms

Redundancy analysis was carried out with Canoco 4.5 to investigate the influence of pipe materials, as well as temperature and chlorine residuals. The water quality was expressed as a linear combination of turbidity,  $COD_{Mn}$ , and  $NH_3$ -N. The analysis demonstrated that the water quality was highly correlated with the environmental factors (*p*-value = 0.0040, Monte Carlo test). Briefly, 18.7% of the information corresponded to the first sequencing axis, and 9.1% corresponded to the second axis.

As illustrated in Figure 4,  $COD_{Mn}$  corresponded positively with NH<sub>3</sub>-N, whereas no obvious correlation between  $COD_{Mn}$  and turbidity was observed, with the angle between the two vectors equal to nearly 90°. Among all the environmental factors, pipe materials (CI)

were located the most far away from the coordinate origin, indicating a violent influence on the water quality. In the direction of  $COD_{Mn}$  and  $NH_3$ -N, the projection of CI was the longest, followed by DI and SP. The negative projection of SP and DI revealed a relatively good property in maintaining water quality, whereas CI showed an opposite tendency. However, in the direction of turbidity, no obvious difference was confirmed between these three pipe materials.



**Figure 4.** Redundancy analysis (RDA) ordination triplot of environment factors and water quality indicators over a long period. Red triangles represent three kinds of pipe material; red arrows represent variables: temperature and chlorine residuals; blue arrows represent parameters: turbidity, COD<sub>Mn</sub>, and NH<sub>3</sub>-N.

Comparing the three pipe materials, the distance between the SP and CI was the longest, indicating a great difference between these two materials. Based on the projection in the direction of  $COD_{Mn}$  and  $NH_3$ -N, the property of the pipe materials could be listed in the following order: SP > DI > CI, which was consistent with the outcome of a bench-scale experiment. Higher levels of  $COD_{Mn}$  were observed in DI and CI, revealing an inclination in biofilm formation and detachment in iron pipes versus plastic-lining materials. A higher level of  $COD_{Mn}$  correlated with biofilm detachment. EPS was produced by the attached community to provide structure and stability for the biofilm. If the EPS adhesive strength deteriorated or was overcome by external shear forces, biofilm was mobilized into the water, potentially leading to the degradation of the water quality. With carbohydrates as the dominant component in the biofilm, the level of organic matter measured by  $COD_{Mn}$  is raised as a result of the detachment [32]. Microbes' preference for iron materials is also confirmed by results in the previous report, with higher TPC and HPC in iron pipes than that in plastic ones [33].

In addition to the influence of pipe materials, the temperature trended to vary in a way with the change of all the water parameters, supporting the assumption that water deteriorates fiercely with the rise of temperature. On the one hand, the temperature is the main reason that causes the change in the diversity of bacteria [34]. On the other hand, biofilm formation and the release of EPS are much slower and much more separated at a lower temperature, which accounts for water deterioration [35].

#### 3.3. Phylogenetic Assessment of Microbial Community

A total of 117,390 pyrosequences were obtained from three biofilm samples, with an average sequence yield of  $39,130 \pm 1569$  for each sample (range 36,911-40,242). Based on the clustering of these sequences at 97% gene similarity, 110, 202, and 205 OTUs were found in the biofilms from the CI pipe, DI pipe, and SP pipe, respectively. The CI biofilm possessed the lowest bacterial diversity with the Shannon index (0.77) and Simpson index

(0.66), while no notable differences were confirmed between DI and SP with the Shannon and Simpson indexes (Table 3). In terms of community richness, SP demonstrated the highest community richness expressed by the Chao and Ace indices, followed by DI and CI.

Table 3. Alpha-diversity indexes of the bacterial.

Sample	Shannon	Simpson	Ace	Chao
SP	3.14	0.10	226.17	225.30
CI	0.77	0.66	119.17	118.75
DI	3.56	0.05	207.34	205.24

The community richness of SP (HDPE-lined steel pipe) was the highest, indicating that plastic could stimulate biofilm growth. The mechanism of the stimulation is attributed to the release of biodegradable organic compounds [36] and phosphorus from this material.

The taxonomic distributions of phylogenetic groups for biofilms are listed in Figure 5. The pyrosequences were assigned to 14 phyla, of which Proteobacteria was the most abundant, followed by Bacteroidetes and Firmicutes. On the genus level, the composition of biofilm samples is shown in Figure 5. The SP sample was dominated by several genera including *Bradyrhizobium*, *Sphingobium*, and *Novosphingobium*. The CI sample accommodated two major genera, *Brevundimonas*, and *Brevibacillus*, with a minor portion of other genera. On the contrary, the genera in the DI sample exhibited were well distributed among the genera spectra. The diversity of the CI samples was relatively low, which was also confirmed by the Shannon Index.



Community barplot analysis

Figure 5. Taxonomic distribution of phylogenetic groups for biofilms from SP, CI, and DI at the genus level.

The phenotypic, chemotaxonomic, and phylogenetic characteristics of dominant bacteria in SP, DI, and CI are listed in Table 4. In SP, *Novosphingobium*, *Sphingobium*, and *Bradyrhizobium* were found with a higher abundance than the other materials. All three genera are  $\alpha$ -proteobacteria, and they share common features: Gram-negative; mesophilic and neutrophilic; chemo-organotrophic; aerobes or facultative anaerobes. *Sphingobium* can be found in soil, pentachlorophenol-contaminated soil, and clinical specimens. They can degrade a variety of chemicals in the environment, such as aromatic and chloroaromatic compounds, phenols like nonylphenol and pentachlorophenol, herbicides, propionic acid, hexachlorocyclohexane, and polycyclic aromatic hydrocarbons. These compounds exist in SP as a result of organics leaching and provide nutrients for *Sphingobium*. Closely related to *Sphingobium, Novosphingobium* is also strictly aerobic and chemo-organotrophic. Especially, it can reduce nitrate to nitrite. *Novosphingobium* is a genus of Gram-negative bacteria that includes N. taihuense, which can degrade aromatic compounds such as phenol, aniline, nitrobenzene, and phenanthrene [37]. *Bradyrhizobium*, a genus of slow-growing, root nodule bacteria from leguminous plants, fixes atmospheric nitrogen (dinitrogen) when in the symbiotic state within root nodules [38].

**Table 4.** Phenotypic, chemotaxonomic, and phylogenetic characteristics of dominant bacteria in SP, DI, and CI. NA: not acquired.

-	Genus	Phylum and Class	DNA (mol% GC)	Habitats/First Derived From	Characteristics	References
SP	Novosphingobium	Proteobacteria, α-proteobacteria	62–67	soil, stocked distilled water, coastal plain sediments, uidizedbed reactor	non-sporulating rods, Gram-negative, strictly aerobic, chemo-organotrophic	[37]
-	Sphingobium	Proteobacteria, α-proteobacteria	62–67	soil, pentachlorophenol- contaminated soil, and clinical specimens	non-sporulating rods, Gram-negative, strictly aerobic, chemo-organotrophic	[37]
-	Bradyrhizobium	Proteobacteria, α-proteobacteria	62–66	slow-growing, Root Nodule Bacteria	short rods, Gram-negative, aerobic, chemo-organotrophic, optimal temperature: 25–30 °C	[38]
DI	Dechloromonas	Proteobacteria, β-proteobacteria	NA	a pulp and paper plant waste pulp sludge	rod-shaped, Gram-negative, facultative anaerobe; the predominant chlorate-reducing bacteria	[39]
-	Pseudorhodoferax	Proteobacteria, β-proteobacteria	69–70	activated sludge; soil	short rods, Gram-negative, aerobic, chemo-organotrophic, optimal temperature: 30 °C	[40]
-	Edaphobacter	Acidobacteria, Acidobacteria	55.8–56.9	soils, low carbon concentrations, and neutral to slightly acidic conditions	non-spore-forming, short, ovoid rods, Gram-negative, aerobic, chemo-organotrophic, optimal temperature: 30 °C	[41]
-	Phreatobacter	Proteobacteria, α-proteobacteria	NA	ultrapure water	motile rods, Gram-negative, strictly aerobic	[42]
-	Simplicispira	Proteobacteria, β-proteobacteria	63–65	freshwater, wastewater, Antarctic mosses	Spirilla, Gram-negative, aerobes and facultative anaerobes, mesophilic	[43]
CI	Brevundimonas	Proteobacteria, α-proteobacteria	65–68	NA	short rods, Gram-negative, aerobic, chemo-organotrophic, optimal temperature: 30–37 °C	[44]
-	Brevibacillus	Firmicutes, Bacilli	42.8–57.4	NA	rods, Gram-positive or variable, aerobic, optimal temperature: 30 °C	[45]

In DI, a moderate-high level of *Dechloromonas*, *Pseudorhodoferax*, *Edaphobacter*, *Phreatobacter*, and *Simplicispira* was identified. *Dechloromonas* is the predominant chlorate-reducing bacteria in the environment. As a facultative anaerobe, it can oxidize acetate with  $O_2$ ,  $ClO_3^-$ ,  $ClO_4^-$ , or  $NO_3^-$  as alternative electron acceptors [46]. *Pseudorhodoferax* is aer-

obic and chemo-organotrophic. The species is isolated from activated sludge and soil. Both *Pseudorhodoferax* and *Dechloromonas* are located in the β-subclass of proteobacteria. *Edaphobacter* belongs to Acidobacteria. It is adapted to environments with low carbon concentrations and neutral to slightly acidic conditions, which is consistent with drinking water distribution systems [47]. *Phreatobacter* is phylum Proteobacteria, Gramnegative, and inhibited by the presence of high concentrations of NaCl. The type species, *Phreatobacter oligotrophus*, is isolated from the ultrapure water of the water purification system of a power plant [42]. The optimum environmental condition for its growth is 25–37 °C, pH 6–8. Additionally, it does not produce acid production from all carbon sources of the API 50CH test. *Simplicispira* comprises anaerobes and facultative anaerobes, accustomed to the neutral and moderated environment. The common habitat for *Simplicispira* is freshwater, wastewater, and Antarctic mosses. It is capable of denitrification [43]. It provides a possible explanation for the negative correlation between DI materials and the concentration of NH<sub>3</sub>-N in Figure 4.

In CI, *Brevundimonas* and *Brevibacillus* were dominant. *Brevundimonas*, a large portion of all samples, exhibited a dominant position in the distribution system. It belongs to  $\alpha$ -proteobacteria. The strains exhibit a limited nutritional spectrum; only DL-P-hydroxybutyrate, pyruvate, L-glutamate, and L-proline are used by 90% or more of the strains as sole carbon and energy sources. Additionally, pantothenate, biotin, and cyanocobalamin are required as growth factors [44]. From the above description, it can be inferred that the nutrition of *Brevundimonas* is restricted. Thus, the carbon source may come from other organisms' metabolic products, and a mutually promoted relationship with other microbes exists. *Brevibacillus* belongs to Firmicutes, strictly aerobic (except for *Brevibacillus Zaterospoius*). Optimum growth occurs at pH 7.0. The optimum growth temperature of nine species (all species except Brevibacillus themoruber) is 30 °C. Some species are capable of reducing nitrate to nitrite [45].

#### 3.4. Presence of Redox Bacteria and Chlorine-Resistant Bacteria

To further analyze the role of redox bacteria and the metabolic cycle of iron, nitrogen, and sulfate in different pipe materials, the OUT proportions are compared between different pipe materials in Figure 6. In all kinds of pipes, the presence of NRB, IOB, IRB, and SRB was identified, whereas nearly none of the AOB, NOB, and SOB were found. NRB composed the largest share in all pipe materials (13.0–17.2%), with other redox bacteria making up a minor proportion (0.02–1.52%). The highest abundance of NRB was presented in SP, followed by CI and DI. In terms of IOB, IRB, and SRB, the highest abundance was presented in DI, and extremely few IOBs were identified in CI.



**Figure 6.** OUT proportions of redox bacteria in different pipe materials. Abbreviations are used for NRB, IOB, IRB, and SRB [48–50].

Owing to the chlorine residuals in the influent, chlorine-resistant bacteria were identified in all the pipe materials. The abundance of chlorine-resistant bacteria was 0.57%, 0.55%, and 0.49% for SP, CI, and DI, respectively. SP had the largest proportion, but the distinction was not conspicuous. Research indicated that multispecies biofilms are generally more resistant to disinfection than single-species biofilms [51]. Therefore, more chlorine-resistant bacteria can be attributed to a higher community richness of SP.

As seen from Table 5, chlorine-resistant bacteria existed in all pipe materials, and the proportion in SP was the highest. Therefore, SP favored microorganisms with tolerance to chlorine. Correspondingly, the COG categories including cell wall/membrane/envelope biogenesis and defense mechanisms had the biggest share in SP. The reaction site of disinfection was reported to be the cell wall [52]. Additionally, the number of induced membrane-associated genes increases with increasing chlorine doses [53].

Chlorine-Resistant Bacteria Genus	SP	DI	CI
Acinetobacter [54]	3	8	28
Bacillus [55]	14	22	66
Francisella [54]	0	0	0
Legionella [56]	129	11	0
Methylobacterium [57]	0	0	0
Moraxella [17]	0	0	0
<i>Mycobacterium</i> [58,59]	5	54	0
Pseudomonas [54,60]	40	93	122
Sphingomonas [61]	0	0	0
Staphylococcus [54]	2	4	15
The proportion of the chlorine-resistant bacteria	0.57	0.49	0.55

Table 5. Proportion of Chlorine-resistant bacteria genus.

# 3.5. Effect of Pipe Materials on COG Functional Categories

An analysis of genes in COG functional categories was carried out to compare the proteomic variation in different pipe materials (Table 6). Generally, the occupation of COG groups does not diversify drastically from each other, representing a high degree of similarity in protein function. The proportion of unknown functional genes in the three samples was the highest, which were 10.66% (DI), 11.72% (CI), and 9.48% (SP), respectively, indicating that some functions of these samples had not been successfully annotated by the COG database, so more annotation information was necessary. Except for unknown function genes and general function prediction-only genes, amino acid transport and metabolism genes accounted for the highest proportion, which were 8.31% (DI), 7.23% (CI), and 7.84% (SP), respectively.

Table 6. Comparison of COG functional categories percentage for SP, CI, and DI.

Description	COG Functional Categories Percentage (%)		
	DI	CI	SP
RNA processing and modification	0.03	0.00	0.04
Chromatin structure and dynamics	0.03	0.00	0.04
Energy production and conversion	6.87	6.17	6.99
Cell cycle control, cell division, chromosome partitioning	0.98	1.01	1.04
Amino acid transport and metabolism	8.31	7.23	7.84
Nucleotide transport and metabolism	2.27	2.49	2.17
Carbohydrate transport and metabolism	5.50	4.17	5.29
Coenzyme transport and metabolism	3.71	3.41	3.85
Lipid transport and metabolism	4.87	5.35	5.02
Translation, ribosomal structure, and biogenesis	5.22	5.98	5.36
Transcription	6.63	6.21	6.15

Table 6. Cont.

Description	COG Functional Categories Percentage (%)			
	DI	CI	SP	
Replication, recombination, and repair	5.23	5.07	5.60	
Cell wall/membrane/envelope biogenesis	6.98	6.83	7.25	
Cell motility	1.83	2.09	1.72	
Posttranslational modification, protein turnover, chaperones	4.32	5.10	4.60	
Inorganic ion transport and metabolism	6.00	6.30	6.41	
Secondary metabolites biosynthesis, transport, and catabolism	2.54	2.25	2.78	
General function prediction only	8.17	8.29	7.97	
Function unknown	10.66	11.72	9.48	
Signal transduction mechanisms	6.02	6.31	6.07	
Intracellular trafficking, secretion, and vesicular transport	2.34	2.49	2.75	
Defense mechanisms	1.48	1.52	1.58	
Extracellular structures	0.00	0.00	0.00	
Nuclear structure	0.00	0.00	0.00	
Cytoskeleton	0.00	0.00	0.01	

Nevertheless, more distinctions were found between CI and the others. In CI, amino acid transport and metabolism genes, energy production and conversion genes, and carbohydrate transport and metabolism genes accounted for the lowest proportion. Meanwhile, CI, with the highest proportion of cell motility genes and relatively poor water quality, suggested that the correlation between water quality deterioration and cell motility genes is due to cell detachment.

# 4. Discussion

Different concentrations of organic matter are presented in the effluent of different pipe materials. Variation in ammonium concentration in different pipe materials is attributed to the microorganism's metabolic process for nitrogen. The ammonium ion is partially oxidized via nitrite to nitrate in the drinking water distribution systems. Additionally, the nitrate generated by nitrification is partly utilized by nitrate-reducing (and denitrifying) bacteria [62]. More ammonium is affirmed in CI, suggesting the absence of nitrifying bacteria. For SP, ammonia nitrogen was rarely monitored in bulk water. It demonstrated a good performance of SP in water quality maintenance.

Nitrates may also undergo denitrification in steady water, i.e., they would support bacterial existence in the distribution system. Such an environment forms favorable conditions for sulfate-reducing bacteria and, in all of the steady water samples, we have easily (but qualitatively) detected sulfides [63].

# 4.1. Bacteria's Common Requirement of Habitat from Different Pipe Materials

In this work, Gram-negative, chemo-organotrophic, mesophilic and neutrophilic, and aerobic bacteria were found to be the major groups in the distribution system. Most of them belonged to  $\alpha$ - and  $\beta$ -Proteobacteria, which was similar to previous work [64]. Some dominant genera were first derived from soil or active sludge, which agreed with the turbidity problems observed due to soil contamination in the distribution network. While other dominant genera were first derived from pure water, the oligotrophic nutrient-deprived habitat suggested an adaption to environments with low carbon concentrations.

#### 4.2. Nutrients in the Effluent as a Result of Biofilm Detachment

In the pilot scale experiment, the maximum nitrogen and organic matter were observed in CI. It suggested the most serious deterioration problem in CI and may prosper the bacteria regrowth downstream. The organic matter in effluent mainly comes from the detachment of biofilm and EPS by hydraulic shear force. In CI, the COG functional group controlling cell motility exhibited the highest abundance in accordance with the cod concentration results.

Except for biofilm detachment, the metabolites in the respiration process also enter the effluent. Thus, the nutrients' concentration is also related to metabolic intensity. In CI, the abundance of two functional groups was the lowest: amino acid and carbohydrate, whose transport and metabolism resulted in the release of organic matter into the bulk water instead of being utilized by microorganisms.

## 4.3. Corrosion Bacteria in Different Pipe Materials

For iron pipes, corrosion plays an important role in water deterioration. The process is related to several bacteria capable of utilizing iron, nitrogen, and sulfate.

In SP, the dominant genus *Dechloromonas* and *Bradyrhizobium* inhibited the corrosion process, in accordance with a better effluent water quality. In previous research, Dechloromonas was reported to cast the greatest corrosion inhibition by inducing the redox cycling of iron to enhance the precipitation of iron oxides and the formation of Fe<sub>3</sub>O<sub>4</sub> [15,48]. *Rhizobia Bradyrhizobium*-producing siderophores have a weaker corrosion-inhibition effect by capturing iron to inhibit the dissolution of iron and iron corrosion [1,48].

In DI, the highest abundance of IRB, IOB, and SRB was identified, indicating the existence of corrosion. IRB can reduce ferric iron to ferrous iron, which was reported to have the ability to inhibit iron corrosion [65]. Additionally, the EPS at the metal surface can impede the dissolution of ferrous iron corrosion products [66]. IRB can also favor the formation of Fe<sub>3</sub>O<sub>4</sub> [67]. Conversely, the presence of SRB and IOB in biofilms can speed up iron corrosion. The IOB contributes to the formation of a-FeOOH. Beneath the iron-rich tubercles produced by IOB, SRB may increase rapidly. SRB is usually associated with anaerobic iron corrosion by producing hydrogen sulfide (H<sub>2</sub>S) as a corrosive agent. The interaction between SRB/IOB can accelerate the iron corrosion process [68]. These bacteria play a collaborative as well as an opposite role in the corrosion process (Figure 7).



Figure 7. The relationship between bacteria (IOB, SRB, and IRB) and DI pipe corrosion.

# 5. Conclusions

The pipe materials had a salient effect on water quality and microbial community. In the static soaking experiment, the pipe material apparently affected the water quality determined by turbidity, DOC, and BDOC, while having no significant effect on TPC. In the dynamic simulation of the distribution system, the performance of the pipe materials was ordered as SP, DI, and CI with respect to water quality maintenance. The presence of NRB, IOB, IRB, and SRB was identified, and NRB composed the largest share in all pipe materials (13.0%–17.2%), with other redox bacteria composing a minor proportion (0.02%–1.52%). In DI, the highest abundance of IRB, IOB, and SRB was identified, indicating the existence of

corrosion. SP favored chlorine-resistant bacteria, and the COG categories including cell wall/membrane/envelope biogenesis and defense mechanisms had the biggest share. CI, with the highest proportion of cell motility genes, suggested that the correlation between water quality deterioration and cell motility genes is due to cell detachment. The turbidity problems caused by soil contamination in the distribution network led to the most dominant genus being derived firstly from soil or active sludge.

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# Nomenclature and Acronyms

DBP: disinfection byproduct; SP: steel plastic composite pipe; SS: stainless steel pipe; DI: ductile iron pipe; CI: cast iron pipe; GS: galvanized steel pipe; COG: clusters of orthologous genes; NRB: nitrate-reducing bacteria; IOB: iron-oxidizing bacteria; IRB: iron-reducing bacteria; SRB: sulfate-reducing bacteria; PVC: polyvinyl chloride; PE: polyethylene; HDPE: high-density polyethylene; BDOC: biodegradable dissolved organic carbon; DOC: dissolved organic carbon; TPC: total plate counts; OTU: operational taxonomic unit; EPS: extracellular polymeric substances; HPC: heterotrophic plate count; AOB: ammonia-oxidizing bacteria

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