

# Effect of Microbial Community on Iron Release under Low Velocity in Drinking Water Supply Pipes

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**Abstract:** Iron release from water supply network affects water quality safety. To investigate the change of microbial community structure and its effect on iron release in water supply pipes under leakage condition, the research was carried out based on the pilot platform used in actual water supply system. Microbial community structure was analyzed using high-throughput sequencing and the total iron index was measured by atomic flame absorption spectroscopy. The results showed that *Proteobacteria* had a large competitive advantage in the laminar flow rate, the average relative content increased more than 60%. At the level of class, the relative content of *Alphaproteobacteria*, *Gammaproteobacteria*, *Betaproteobacteria* increased larger than others. When the velocity was close to the critical flow rate, the microbial community was gradually stabilized. The total iron concentration decreased with the increase of flow rates, when the flow rate was 40% of the critical flow rate, the total iron concentration tended to be stable, and the promotion of total iron release was mainly *iron-oxidizing bacteria* and *sulfate reducing bacteria*, and the inhibition effect was mainly *nitrate reducing bacteria*. It has important reference significance for further controlling the microbial community and iron release in the water at the end of the pipe network.

## 1 INTRODUCTION

The implementation of urban-rural integrated water supply has effectively guaranteed the daily life of residents. However, color water events often occur at the end of some municipal pipe networks (Zhang, 2014; Li, 2016). There are many factors accounting for the phenomenon of color water. For example, the attenuation of dissolved oxygen and residual chlorine in the pipe network water under stagnation conditions leads to the rupture of the corrosion layer on the inner wall of the pipe and the release of a large amount of ferric hydroxide compounds (Zhi, 2014; Prest, 2016; Chen, 2013). Likewise, changes in hydraulic conditions such as abrupt changes in flow rate and direction would also lead to iron release (Tan, 2014). Recently, many studies have shown that the life activities of some microbial communities in the pipe network water are the main reasons for the deterioration of water quality (Li, 2016; Beale, 2012; Xu, 2014). Certain microbial communities affect water supply pipeline corrosion and consequently iron release (Tuovinen, 1980; Moradi, 2011), these microbial communities mainly include *iron-oxidizing bacteria*, *sulfate-reducing bacteria*, *sulfur-oxidizing bacteria*, *denitrifying bacteria*, *iron-reducing bacteria*, etc. For example, *Iron-oxidizing bacteria* can metabolize reduced iron and exist in water or in secretions in the form of Fe(OH)<sub>3</sub> suspended particles, which would cause the

phenomenon of color water in the pipeline network (Moradi, 2011; Gerke, 2008; Teng, 2008).

*Sulfate-reducing bacteria* can reduce sulfate into sulfur ions to obtain energy, and depolarize the metal surface cathodic by consuming hydrogen, which accelerates the electrochemical corrosion of pipelines. At the same time, the hydrogen sulfide gas produced is oxidized to sulfuric acid, which would cause serious pitting corrosion in the pipeline (Hu, 2014; R. A. King, 1971). In addition, some studies have shown that when *sulfate-reducing bacteria* and *iron-oxidizing bacteria* coexist, the corrosion rate will be more than 300 times that of electrochemistry (Venzlaff, 2013). Therefore, it is of great importance to study the effect of microbial community on iron release.

However, the water supply pipeline also has leakage problems. The net water leakage of the water supply network in more than 600 cities in China was estimated to be 16.23% in 2020, far exceeding the national standard that the leakage rate of cities should be controlled below 12% (Research Standard of Leakage Control Technology for Urban Water Supply Pipeline). While the leakage of pipes leads to waste of water resource, it will also cause certain economic losses to the water supply enterprises. Under severe circumstances, water supply accidents such as pipe bursts will occur, which will seriously affect the daily life of residents. However, when water in the pipe network is leaking, the indicators of corrosion

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microorganisms and the release of total iron in the water change, which was rarely concerned by scholars. Therefore, a series of seepage flow velocity gradients were set to study the change rule of drinking water microbial

community in water supply pipelines under different seepage flow rates, and the correlation between the corrosion microbial community and iron release in this study.

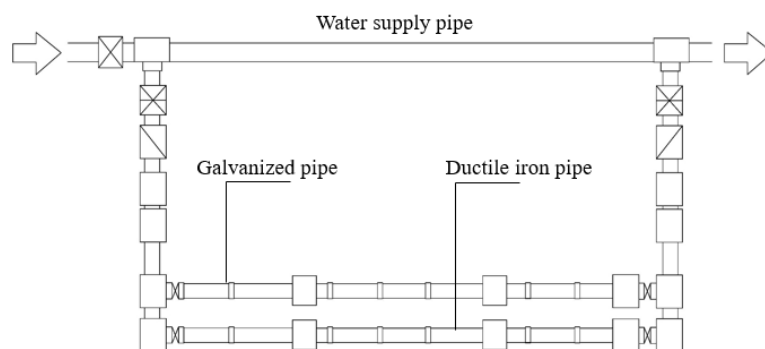


Fig.1 Schematic diagram of pilot system researched water supply pipelines

## 2 EXPERIMENTAL MATERIALS AND METHODS

### 2.1 Experimental Device

Figure 1 showed the pilot test platform at the end of the water supply network in a city along the coast of China. The pilot project was put into use in June 2015. It consisted of two groups of pipes. The pipes were galvanized pipes and ductile iron pipes. The pipe diameter is DN200, and each pipe section was about one meter long.

The pilot test platform was connected to the actual municipal pipeline network and was in acyclic operation. Both ends of the test platform were provided with manual valves that could be opened and closed for flow.

In this experiment, the water of two groups of water supply pipes, DN200 ductile iron pipe and galvanized pipe, were used as the seepage test objects. The two kinds of pipes were provided with water sample collection ports in parallel. When starting the experiment, the valves at both ends of the pipes should be slowly closed to avoid water or the sudden change of water flow conditions leading to the shedding of the biofilm in the pipeline and affecting the experimental results.

### 2.2 Water Quality Testing Indicators and Instruments

The water quality testing method was based on the national drinking water testing standard (GB/T5750.6-2006), and the instruments and methods were shown in Tab.1.

Tab. 1 Measurement instrument

Test items	Testing instruments and methods
Iron	ICP-MS
Turbidity	Portable Turbidity Meter
Free chlorine	DPD Spectrophotometry
Ammonia nitrogen	Nessler's reagent spectrophotometry
Alkalinity	Acid titration

### 2.3 Collection of Water Samples

In China, the leakage rate of urban water supply network is usually 5%~20% (Wei, 2016; Kuo, 2015). The stagnation time is generally 18 hours. According to the range of pipeline leakage rate of 5%-20%, the converted test seepage flow rates were: 0 ml/min, 250 ml/min, 350 ml/min, 450 ml/min, 550 ml/min, 900 ml/min, of which 900 ml/min was the critical layer Seepage flow at flow velocity. Converting the seepage flow into the tap water flow rate was: 0m/s,  $8.28 \times 10^{-4}$  m/s,  $1.16 \times 10^{-3}$  m/s,  $1.49 \times 10^{-3}$  m/s,  $1.82 \times 10^{-3}$  m/s,  $2.88 \times 10^{-3}$  m/s (critical flow velocity: when the flow velocity continues to increase, it will transform from laminar flow to turbulent flow, which is converted according to the critical Reynolds number of 2300). There were 6 groups of leakage flow velocity gradients, and these 6 groups of flow velocity gradients were all laminar flow velocity gradients scope. The corresponding letters were represented by letters A, B, C, D, E and F respectively, and the experimental time for each flow rate design was 18 hours. Water samples were collected every one hour, some of which were used for the detection of routine indicators on site, and some of which were stored at 4 °C at low temperature for later detection of relevant water quality parameters.

### 2.4 DNA Extraction

The collected water samples were extracted with the Power Soil DNA Kit for genomic DNA extraction. The extracted genomic DNA was detected by 1.0% agarose gel electrophoresis, and then placed in a -20 °C refrigerator for subsequent detection.

### 2.5 Illumina High-Throughput Sequencing

The extracted sample DNA was subjected to high-throughput sequencing, after obtaining the raw data, first perform quality control on the raw data to obtain the final sequence for analysis. Then the QIME software was applied to classify the sequences into multiple operational

units according to the sequence similarity (Caporaso, 2010).

The software mothur was used to operate according to the species richness of each sample in the OUT list (J L D Y, 2010).

## 2.6 Statistical Methods

Origin and IBM SPSS were used for statistical analysis of the experimental data, and one-way analysis of variance was used to test the statistical variance of the samples. The significant difference was assumed to be  $p=0.05$ . Environmental impact factor analysis was performed using Canoco for windows.

## 3.RESULTS AND DISCUSSION

### 3.1 Composition of Microbial Community in Water Under Low Flow Rate Conditions

It could be seen Figure 2 that at the phylum level whether it was a galvanized pipe or a ductile iron pipe, the relative content of *Proteobacteria* in the initial water phase accounted for between 45.80% and 61.23%. The relative contents of other phyla-level microbial communities were lower than *Acidobacteria* (3.21%~9.24%), *Bacteroidetes* (3.70%~7.21%), *Actinobacteria* (4.15%~8.45%) and *Chloroflexi* (5.69%~8.12%).

Taking *Proteobacteria* analysis as an example, under the lower laminar flow velocity, for example, the average relative content of galvanized pipe and ductile iron pipe network water in the first to third groups were 45.80%, 48.70%, 49.20% and 46.40%, 50.20% and 51.94% at the initial time, which increased to 70.24%, 78.40%, 66.61% and 78.90%, 72.81% and 60.58% respectively after 18 hours, and the increase amounts were 24.44%, 29.70%, 17.40% and 32.51%, 22.62% and 8.64% respectively.

*Proteobacteria* had a greater survival advantage compared to other bacteria when the layerotic flow rate is low, and the average relative content increased to more than 60%. However, due to the low content, the change was not significant.

Taking the fifth and sixth groups as examples, When the seepage flow velocity gradually approached the critical laminar flow velocity the initial moments of the water of the galvanized pipe and ductile iron pipe network were 58.90%, 59.52% and 61.23%, 59.34%, respectively After 18 hours, the *Proteobacteria* in the water reached 62.33%, 63.80% and 65.45%, 64.30%, respectively. The increase ratios were 3.43%, 4.28% and 4.22%, 4.96% respectively, the increase had been significantly reduced relative to the low flow rate, these showed that the flow velocity has a significant impact on the microbial community. When the flow velocity was close to the critical laminar flow velocity, the relative content of the microbial community changed slightly, but it had basically stabilized.

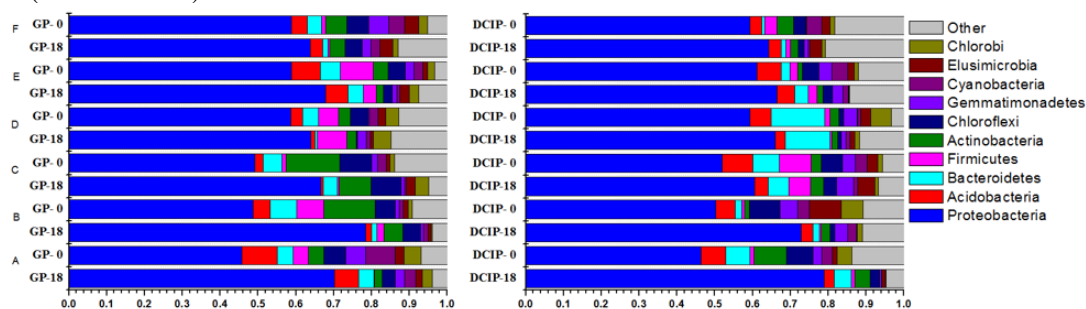


Fig.2 microbial community composition at the phylum level under different seepage velocities

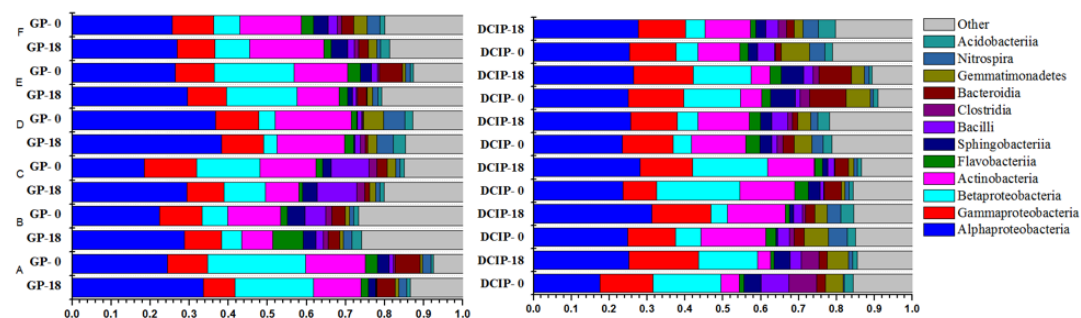


Fig.3 microbial community composition at the class level under different seepage velocities

Analyzing the data at the class level, as shown in Figure 3, it can be found that different seepage flow rates have obvious effects on the relative content of microbial communities. The main class-level bacterial communities contain *Alphaproteobacteria*, *Gammaproteobacteria*, *Betaproteobacteria*, *Actinobacteria*, *Flavobacteriia*, *Sphingobacteriia*, *Bacteroidia*, *Bacilli*, etc.

Under the conditions of lower flow rate (taking the first to third groups of flow rates as an example), the average relative content of *Alphaproteobacteria* in the water of the galvanized pipeline at the initial time was about 24.40%, 22.34% and 18.54%, respectively, after 18 hours, the average relative content of this bacteria reached 33.54%, 28.70% and 29.32%, and the increases were 9.14%, 6.36%

and 10.78% respectively. Taking the fifth and the sixth group as an example, When the seepage flow rate continued to increase the average relative content in the water at the initial moment was 26.40% and 25.60%, respectively, and after 18 hours, the average relative content of the bacteria reached 27.50% and 26.88%, respectively, and the increase was only 1.10% and 1.28%, respectively.

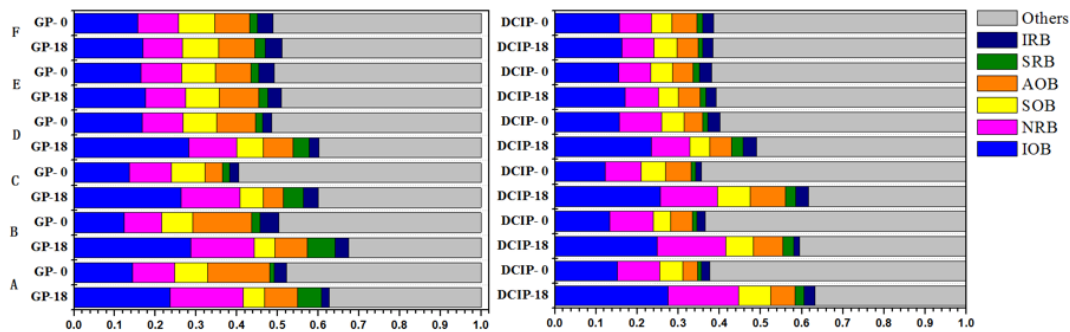
Similarly, taking the first to third groups of flow rates as an example, the water in ductile iron pipeline also showed a similar law. At the initial time, under the condition of low flow rate, the average relative content of *Alphaproteobacteria* increased by 7.70%, 6.47% and 4.55% respectively after 18 hours, from the perspective of increase in the amount of galvanized pipe was more obvious, which also indirectly proved that pipe differences would affect the bacterial community content.

Taking the fifth and sixth groups as examples, when the seepage velocity increased, the average relative content of *Alphaproteobacteria* increased by 1.46% and

2.35% respectively. Under the condition of low initial seepage flow rate, the relative average content of other microorganisms

*Gammaproteobacteria*, *Betaproteobacteria* and *Actinobacteria* showed an attenuation trend relative to the initial time. When the seepage flow rate gradually approached the critical laminar flow rate, the content of these microbial communities was still almost the same as the initial time after 18 hours.

The data of two kinds of pipes, galvanized pipe and ductile iron pipe, showed that at the level of low laminar flow velocity, there were differences among different microbial communities. The average relative content of *Alphaproteobacteria* increased with time, while *Gammaproteobacteria*, *Betaproteobacteria* and *Actinobacteria* decrease, *Alphaproteobacteria* had a great survival competitive advantage. With the increase of laminar flow velocity, the average relative content of microbial community in water would no longer change with time, but gradually became stable.



**Fig.4** microbial community composition at the genus level under different seepage velocities

According to the classification of bacteria by function, it was found that there were corrosive bacteria in the water, including iron oxidizing bacteria, iron reducing bacteria, sulfate reducing bacteria, sulfate oxidizing bacteria, ammonia oxidizing bacteria, nitrate reducing bacteria, etc. When the seepage flow rate was low, it could be found that the average relative content of iron-oxidizing bacteria and sulfate-reduced gold in the water of galvanized pipes and ductile iron pipes had increased to varying degrees after 18 hours compared with the initial time.

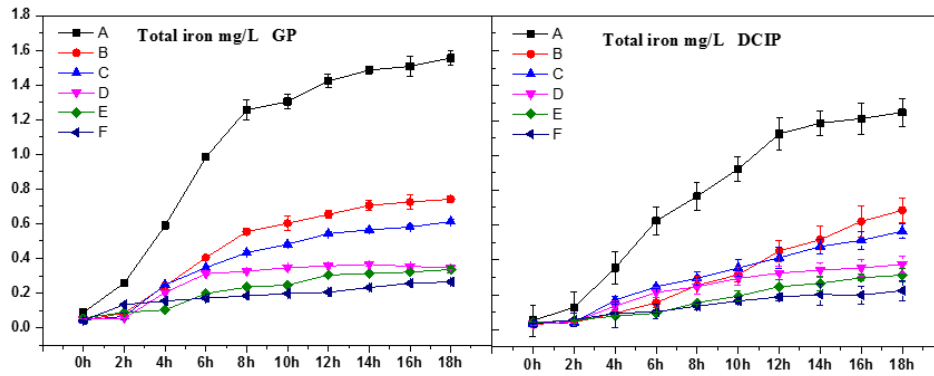
The average relative content in the water phase of zinc pipe and ductile iron pipe increased by 9.14%, 16.36%, 12.78% and 12.30%, 11.54% and 13.45%, respectively. The average relative content of sulfate reducing bacteria in the water phase of galvanized pipe and ductile iron pipe. The increase was 4.79%, 4.70%, 3.15% and 1.30%, 1.50%, 1.49% respectively. From the data, it could be seen that the change of flow rate has a significant effect on the content of *Iron-oxidizing bacteria* and *Sulfate-reducing bacteria*. In the laminar flow velocity range, the flow velocity increases and the growth of corrosive bacteria was inhibited. The *iron-oxidizing bacteria* in galvanized pipes and ductile iron pipes were mainly *Acidovorax* (3.40%-5.6%), *Gallionella* (5.78%-6.41%), *Leptothrix* (3.59%-4.95%), while *Sulfate-reducing bacteria* were mainly *Desulfovibrio* (1.23%-2.34%) and *Desulfotomaculum* (0.15%-2.94%) were the main ones.

The presence of these species could accelerate corrosion of pipes.

### 3.2 Correlation Analysis Between Microbial Communities and Iron Release

The variation trend of total iron concentration in the water of galvanized pipe and ductile iron pipe under different flow rates was described in Figure 2-4. It showed that the maximum total iron concentration in pipe network water under stagnant flow conditions was 1.556 mg/L and 1.246 mg/L respectively. Under the same duration, the total iron concentration in the pipeline water gradually decreased with the increase of flow velocity.

For the same flow rate, with the extension of time, the total iron concentration index of the pipe network water showed an upward trend. It was showed in Figure 5 that in the shortest 2 hours, the total iron index of the pipe network water had exceeded the upper limit of the total iron concentration of 0.3mg/L in the drinking water quality standard. At the same time, the total iron concentration of the pipe network water exceeded the standard after 8 hours under most leakage flow rates. When the seepage velocity was greater than  $1.49 \times 10^{-3}$  m/s (about 40% of the critical velocity), the total iron concentration was basically stable at the concentration level of 0.3 mg/L.



**Fig.5** Variation of total iron concentration of galvanized pipe and ductile iron pipe at different flow rates

From the biological point of view, the correlation and significance level between the total iron in the water and the microbial community in the galvanized pipe and the ductile iron pipe are shown in Table 2 and Table 3, respectively.

The total iron concentration of galvanized and ductile iron pipes at the gate level was significantly higher than that of *Proteobacteria* ( $r=0.615$ ,  $p=0.03$  and  $r=0.592$ ,  $p=0.045$ ), *Bacteroidetes* ( $r=0.669$ ,  $p=0.017$  and  $r=0.659$ ,  $p=0.02$ ) was significantly positively correlated, and significantly negatively correlated with *Acidobacteria* ( $r=-0.579$ ,  $p=0.048$  and  $r=-0.593$ ,  $p=0.042$ ). From the analysis of the data at the class level, it could be concluded that *Alphaproteobacteria* had a significant positive correlation with the total iron concentration ( $r=0.713$ ,  $p=0.034$ ) and ( $r=0.653$ ,  $p=0.041$ ), and *Gammaproteobacteria* had a significant positive correlation with the total iron concentration of galvanized pipes and ductile iron pipes. There was also a significant positive correlation between iron. It could be also seen from the data that *Betaproteobacteria* and *Flavobacteriia* were mainly negatively correlated with

total iron concentration, but the correlation was not very strong.

At the genus level, *iron-oxidizing bacteria*, *sulfate-reducing bacteria*, *sulfate-oxidizing bacteria* and *ammonia-oxidizing bacteria* played a role in promoting the release of total iron, and iron-reducing bacteria and *nitrate-reducing bacteria* played an inhibitory role. In terms of correlation and significance level, *Iron oxidizing bacteria* ( $r=0.677$ ,  $p=0.016$  and  $r=0.655$ ,  $p=0.021$ ) played a major role in promoting the release of total iron in the water of galvanized and ductile iron pipes, respectively ( $r=0.677$ ,  $p=0.016$  and  $r=0.655$ ,  $p=0.021$ ), and *sulfate-reducing bacteria* ( $r=0.477$ ,  $p=0.049$  and  $r=0.438$ ,  $p=0.054$ ), the main inhibitory effect was *Nitrate-reducing bacteria* ( $r=-0.632$ ,  $p=0.032$  and  $r=-0.689$ ,  $p=0.042$ ). Statistical analysis was carried out on the total iron release of galvanized pipes and ductile iron pipes from the levels of phylum, class and genus.

These microbial communities could promote or inhibit the release of total iron. The same was that the types of microorganisms that promote or inhibit the release of total iron in pipe network water of different pipes were the same.

**Tab.2** Correlation level and significance level of total iron release and microorganism

phylum	<i>Proteobacteria</i>	<i>Acidobacteria</i>	<i>Bacteroidetes</i>	<i>Firmicutes</i>	<i>Actinobacteria</i>
<i>r</i>	0.615*	-0.579*	0.669*	-0.152	-0.307
<i>p</i>	0.030	0.048	0.017	0.437	0.331
Class	<i>Alphaproteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Betaproteobacteria</i>	<i>Actinobacteria</i>	<i>Flavobacteriia</i>
<i>r</i>	0.713*	0.268	-0.384	0.052	-0.152
<i>p</i>	0.034	0.528	0.195	0.871	0.638
genus	IOB	SRB	SOB	NRB	AOB
<i>r</i>	0.677*	0.477*	0.377	-0.632	0.384
<i>p</i>	0.016	0.049	0.127	0.032	0.218

Note: \* means  $p<0.05$ , \*\* means  $p<0.01$ ;

**Tab.3** Correlation level and significance level of total iron release rate and microorganism

phylum	<i>Proteobacteria</i>	<i>Acidobacteria</i>	<i>Bacteroidetes</i>	<i>Firmicutes</i>	<i>Actinobacteria</i>
<i>r</i>	0.592*	-0.593*	0.659*	-0.142	-0.244
<i>p</i>	0.045	0.042	0.020	0.659	0.444
Class	<i>Alphaproteobacteria</i>	<i>Gammaproteobacteria</i>	<i>Betaproteobacteria</i>	<i>Actinobacteria</i>	<i>Flavobacteriia</i>
<i>r</i>	0.653	0.665*	-0.093	0.375	-0.218
<i>p</i>	0.041	0.018	0.775	0.229	0.496

genus	IOB	SRB	SOB	NRB	AOB
<i>r</i>	0.655*	0.438	0.341	-0.689	0.364
<i>p</i>	0.021	0.054	0.141	0.042	0.129

Note: \* means  $p < 0.05$ , \*\* means  $p < 0.01$ ;

## 4 CONCLUSION

According to the results of different gradient seepage experiments on the pilot platform in the actual water supply network, the following conclusions can be drawn:

1) *Proteobacteria* had a great competitive advantage in the case of laminar flow velocity, and the average relative content increases to more than 60%. At the class level, the average relative content of *Alphaproteobacteria*, *Gammaproteobacteria*, and *Betaproteobacteria* increased greatly. When the flow rate was close to the critical flow rate, the microbial community gradually stabilized.

2) The total iron concentration of the galvanized pipe and ductile iron pipe network water decreased with the increase of the flow rate. When the flow rate was 40% of the critical flow rate, the total iron concentration tended to be stable, which promoted the release of total iron mainly due to *Iron Oxidation Bacteria* and *Sulfate-Reducing Bacteria*, the main inhibitory effect was *Nitrate-Reducing Bacteria*.

3) By adjusting the flow rate gradient at the end of the water supply network, the distribution of microbial communities related to iron release in the water of the network could be controlled, so as to reduce iron release and reduce the water quality safety risk of the network.

## ACKNOWLEDGMENTS

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