Electronic Supplementary Material (ESI) for ***Environmental Science-Water Research & Technology***

Microbiological safety of drinking water assessment in outdoor pipe materials: biofilm formation and chlorine resistance of typical bacteria

Zebing Zhu, *[ab] Siyang Xu, [a] Yunyan Pei, [a] Lili Shan,*[a] Wanjun Zheng,[a] Xiajun Bao,[a] and Yixing Yuan[b]

a. School of Civil Engineering and Architecture, East China Jiao Tong University, Nanchang, 330013, China. E-mail: hitzzb@126.com; hitshanlili@163.com ddress here.

b. State Key Laboratory of Urban Water Resource and Environment, School of Envi-ronment, Harbin Institute of Technology, Harbin, 150090, China

Table S1 The ODc on the two pipes during the culture time

Time(d)	1	2	3	4	5
Polyethylene	0.246	0.271	0.279	0.324	0.377
Cast iron	0.124	0.163	0.189	0.253	0.316

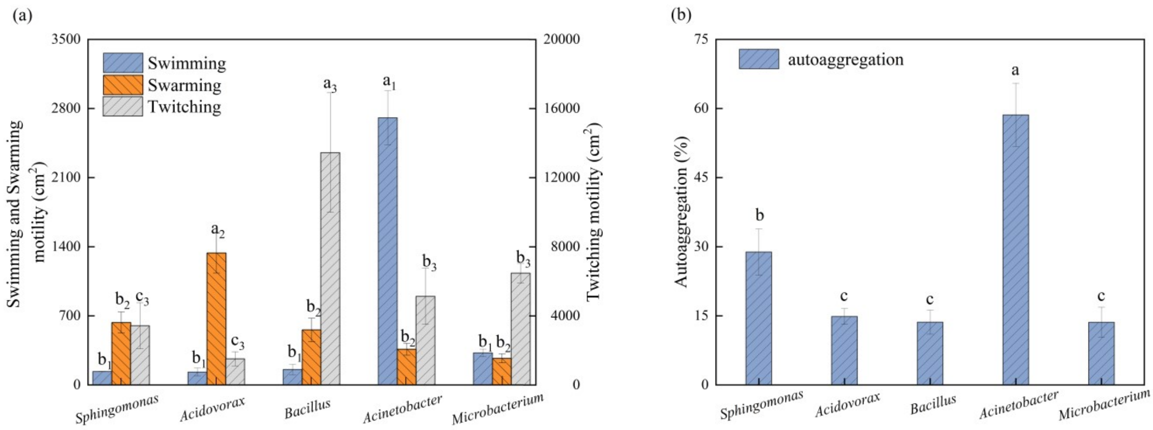

Fig. S1 The characterization of suspended bacteria associated with suspended bacteria initial adhesion. (a) motility, (b) autoaggregation. The a, b, c in figure represented the results of cluster analysis. The a_1, b_1, c_1 were the result of swimming motility while a_2, b_2, c_2 and a_3, b_3, c_3 were the result of swarming motility and twitching motility, respectively.

Both cell motility[1] and autoaggregation[2] are characteristics associated with initial adhesion of suspended bacteria, and bacteria with better cell motility and autoaggregation may have an advantage during the adhesion process in different pipe materials.

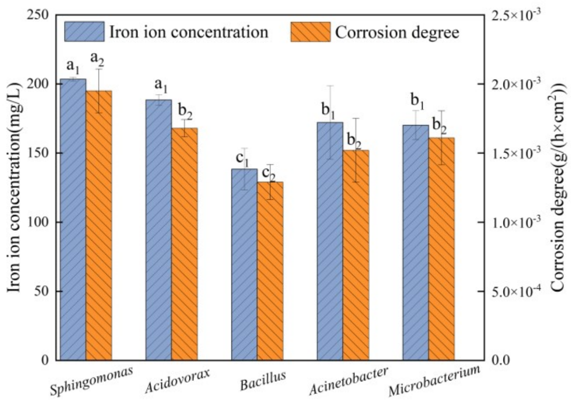

Fig. S2 Iron ion concentration and the corrosion rate of cast iron pipe. The a, b, c in figure represented the results of cluster analysis. The a_1, b_1 was the results of iron ion concentration while the a_2, b_2, c_2 was the results of the corrosion degree.

With the corrosion of the cast iron pipe, some iron ions were released into the environment, and the corrosion degree was significantly correlated with iron ion concentration (r=0.804, p<0.001). Although all the five bacterial strains grew in the presence of high iron ion concentration, the effect of corrosion of cast iron was severe on *Sphingomonas* and *Acidovorax*. This may be owing to the fact that EPS can promote corrosion of cast iron in the early stage[3], and that both *Sphingomonas* and *Acidovorax* in cast iron pipe exhibited high EPS content (Fig. 5), which may have led to higher degree of corrosion. In contrast, no iron ion was detected in the PE pipe.

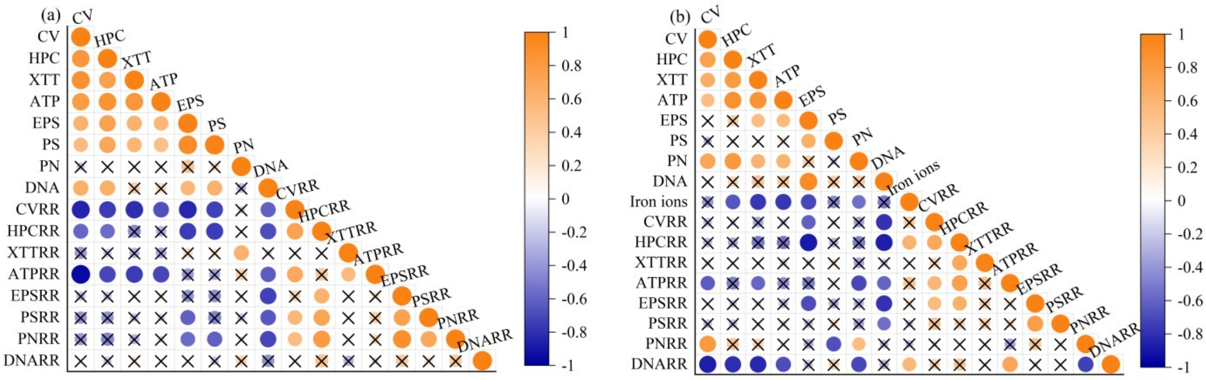

Fig. S3 Correlation heat map of all data of two outdoor pipe materials. (a) PE pipe, (b) Cast iron pipe. Orange represents positive correlation while blue represents negative positive correlation. The size and color of circle means the correlation coefficient value. The cross represents no significant correlation. RR represent reduction rate.

Figure S3 shows the correlation heat maps of the dual-species biofilm in the two outdoor pipe materials. In both PE and cast iron pipes, HPC, CV, ATP, and XTT were significantly correlated, indicating that bacterial activity was related to biofilm biomass. The iron ion content was negatively correlated with the bacterial activity in cast iron pipe, implying that iron ion can inhibit bacterial activity. In addition, the EPS content was also negatively correlated with the iron ion concentration. After chlorination, the biofilm biomass reduction rate was correlated with the EPS reduction rate, suggesting that bacteria with low EPS reduction rate in the dual-species biofilm have better chlorine resistance.

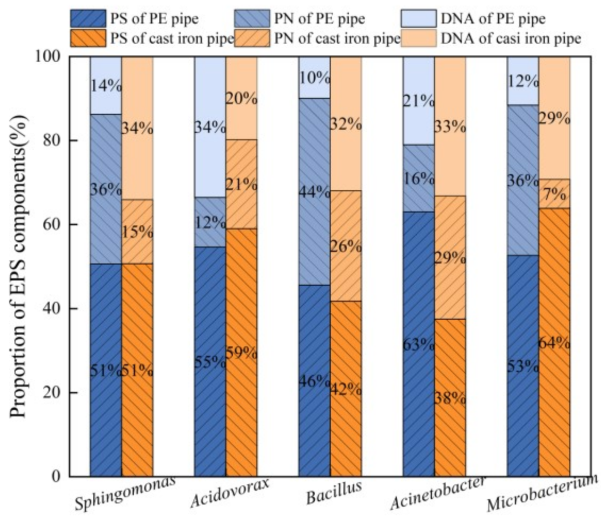

Fig. S4 The proportion of EPS components of the five bacterial biofilm in two pipe materials.

The proportion of EPS components of the five bacterial biofilms in the two outdoor pipe materials was examined. In both PE and cast iron pipes, polysaccharide was the major component of the bacterial EPS, followed by protein and extracellular DNA exhibiting similar proportion.

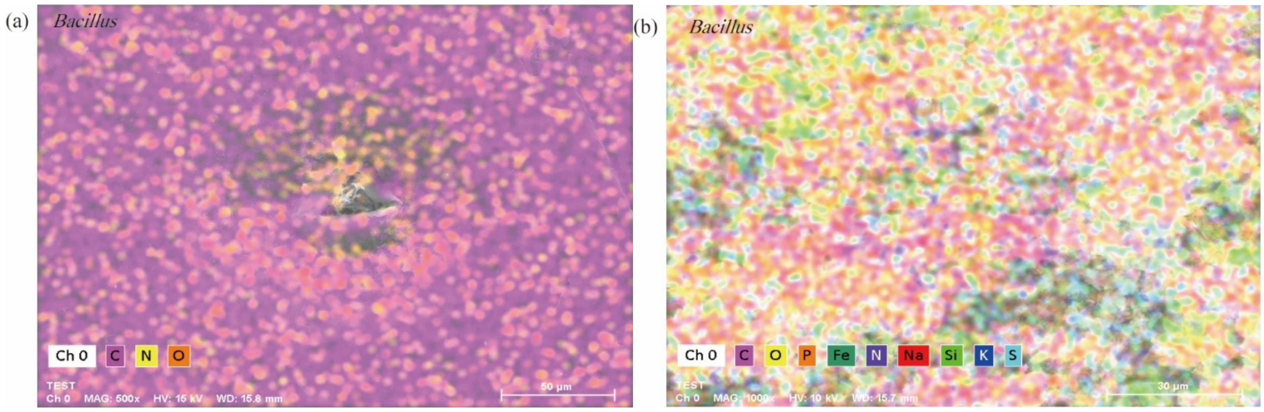

Fig. S5 SEM-EDS of the represented bacteria in two pipe materials. (a) SEM-EDS of the *Bacillus* in PE pipe, (b) SEM-EDS of the *Bacillus* in cast iron pipe.

To highlight the differences in the bacterial growth environment, *Bacillus*, which presented high biofilm biomass in both PE and cast iron pipes, was further analyzed by SEM-EDS.

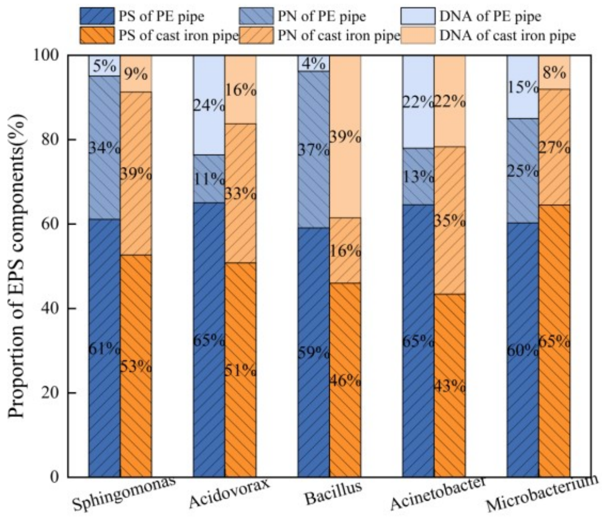

Fig. S6 The proportion of EPS components of the five bacterial biofilm in two outdoor pipe materials after chlorination.

Chlorination altered the proportion of EPS components. The polysaccharide content increased after chlorination to offer chlorine resistance to bacteria. However, this change in polysaccharide content varied with different bacteria and pipe materials, resulting in different chlorination effects.

Notes and references

1 W. Steinchen, S. Ahmad, M. Valentini, K. Eilers, M. Majkini, F. Altegoer, M. Lechner, A. Filloux, J. C. Whitney and G. Bange, Molecular Microbiology, 2021, 115, 1339-1356.

2 L. A. Simoes, A. C. de Souza, I. Ferreira, D. S. Melo, L. A. A. Lopes, M. Magnani, R. F. Schwan and D. R. Dias, Journal of Applied Microbiology, 2021, 131, 1983-1997.

3 J. Jin and Y. Guan, Bioresource Technology, 2014, 169, 387-394.