Electronic Supplementary Material (ESI) for *Water*.

Effect of Pipe Materials and Interspecific Interactions on Biofilm Formation and Chlorine Resistance: Turn Enemies into Friends

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Table S1. The properties of the tested pipe material segments

	PE	Cast iron
Absolute roughness（mm）	0.009~0.010	0.15~0.5
Chemical property	Chemical stability	Relatively stable
service life（year）	About 10	About 10
Large segment size(cm)	7.2×2.4×0.28	7.2×3.2×1.2
Small segment size(cm)	1×1×0.28	1×1×1

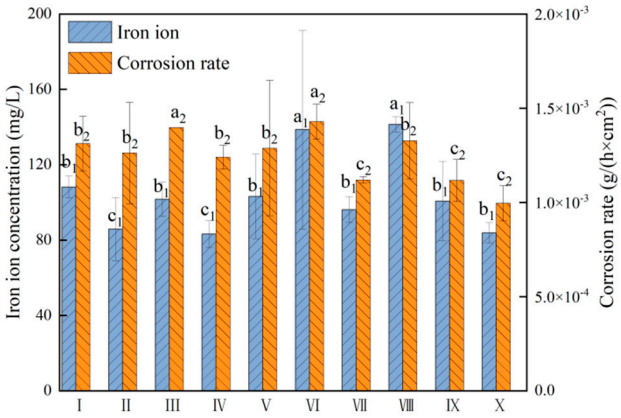


Fig. S1. Iron ion concentration and the corrosion rate of cast iron pipe. The a, b, c in figure represented the results of cluster analysis. The a_1, b_1, c_1 was the results of iron ion concentration while the a_2, b_2, c_2 was the results of corrosion rate. I: *Sphingomonas* sp. + *Acidovorax defluvii*, II: *Sphingomonas* sp. + *Bacillus cereus*, III: *Sphingomonas* sp. + *Acinetobacter* sp., IV: *Sphingomonas* sp. + *Microbacterium laevaniformans,* V: *Acidovorax defluvii* + *Bacillus cereus*, VI: *Acidovorax defluvii* + *Acinetobacter* sp., VII: *Acidovorax defluvii* + *Microbacterium laevaniformans*, VIII: *Bacillus cereus* + *Acinetobacter* sp., IX: *Bacillus cereus* + *Microbacterium laevaniformans*, X: *Acinetobacter* sp. + *Microbacterium laevaniformans*. The groups corresponding to I~X in the following figure are the same as those in Fig. S1.

Corrosion of cast iron pipe occurred in all the groups and the iron ions concentration was high in the environment, while no iron ions were detected in PE pipe. With the corrosion of cast iron pipe, iron ions were released into the environment, and significant correlation was noted between iron ions concentration and corrosion rate (r=0.646, p<0.001).

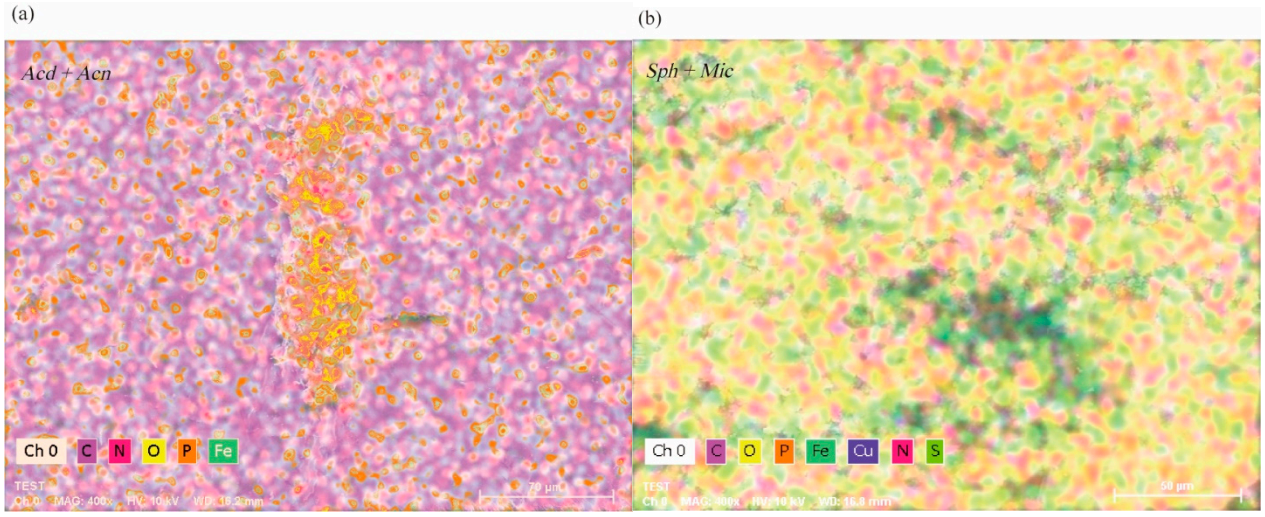


Fig. S2. SEM-EDS of the represented bacteria in two pipe materials. (a) SEM-EDS of the *Acidovorax defluvii* + *Acinetobacter* sp. in PE pipe, (b) SEM-EDS of the *Sphingomonas* sp. + *Microbacterium laevaniformans* in cast iron pipe.

As *Acidovorax defluvii* + *Acinetobacter* sp. in PE pipe and *Sphingomonas* sp. + *Microbacterium laevaniformans* in cast iron pipe exhibited higher biofilm biomass and thus could present more elements for observation, they were also analyzed by

SEM-EDS, and the results showed the presence of many oxygen elements on the surface of the cast iron pipe material, confirming corrosion of cast iron.

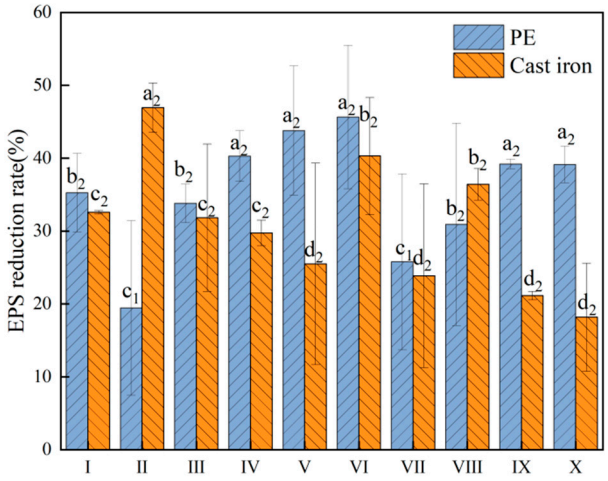


Fig. S3. Dual-species biofilm EPS reduction in two pipe materials after chlorination. The a, b, c, d in figure represented the results of cluster analysis. The a_1, b_1, c_1 were the result of PE pipe while a_2, b_2, c_2, d_2 were the result of cast iron pipe.

The EPS reduction for different bacteria in different pipes significantly varied, and the same dual-species biofilm in different pipe materials exhibited diverse EPS reduction, resulting in varied protective effect on the dual-species biofilms. Besides, the dual-species groups presenting higher EPS reduction also showed higher biofilm biomass reduction, implying decreased protective effect of EPS.

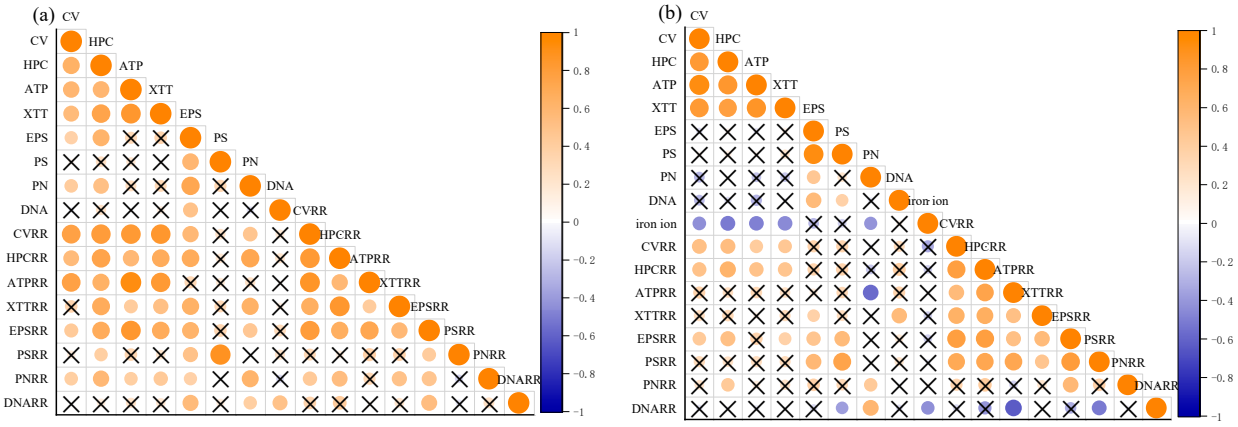


Fig. S4. Correlation heat map of all data of two pipe materials. (a) PE pipe, (b) Cast iron pipe. Orange represents positive correlation while blue represents negative positive correlation. The size and color of circle means the correlation coefficient value. The cross represents no significant correlation. PS represents polysaccharide, PN represents protein, RR represents reduction.

Figure S4 shows the correlation heat maps of the dual-species biofilms in the two pipe materials. Before chlorination, CV,

HPC, ATP, and XTT values were significantly correlated in both the pipes, indicating that biofilm biomass was related to bacterial activity. Iron ions were negative correlated with the bacterial activity and biofilm biomass, confirming that high iron ions concentration can inhibit bacterial growth. After chlorination, the biofilm biomass reduction was correlated with the EPS reduction, suggesting that lower EPS reduction resulted in better chlorine resistance of the dual-species biofilms.