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Effect of pipe material and low level disinfectants on biofilm development in a simulated drinking water distribution system*

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Abstract: The efficiency of chlorine and chloramines disinfection on biofilm development in a simulated drinking water distribution system was investigated by using heterotrophic bacterial spread plate technique. The experiments were carried out with four annular reactors (ARs) with stainless steel (SS) or copper (Cu) material slides. The results showed that there were fewer bacteria attached to Cu slides without a disinfectant compared with those attached to SS slides. When the water was disinfected with chloramines, the heterotrophic plate counts (HPCs) on the biofilm attached to the Cu slides were significantly lower (by 3.46 log CFU/cm^2) than those attached to the SS slides. Likewise, the biofilm HPC numbers on the Cu slides were slightly lower (by 1.19 log CFU/cm^2) than those on the SS slides disinfected with chlorine. In a quasi-steady state, the HPC levels on Cu slides can be reduced to 3.0 log CFU/cm^2 with chlorine and to about 0.9 log CFU/cm^2 with chloramines. The addition of chloramines resulted in a more efficient reduction of biofilm heterotrophic bacteria than did chlorine. We concluded that the chlorine and chloramines levels usually employed in water distribution system were not sufficient to prevent the growth and development of microbial biofilm. The combination of copper pipe slides and chloramines as the disinfectant was the most efficient combination to bring about diminished bacterial levels.

Key words: Copper (Cu), Stainless steel (SS), Biofilm, Heterotrophic plate counts (HPCs), Chlorine, Chloramines
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INTRODUCTION

Most bacterial growth cannot be inhibited on the surface of pipe materials, even though there is not enough microbial available forms of organic carbon to promote the reproduction of suspended cells (Haudidier *et al*., 1988; van der Wende *et al*., 1989; Camper *et al*., 1996; Srinivasan and Harringtona, 2007). The bacteria fixed to surfaces are more resistant to disinfectants than suspended bacteria in water (LeChevallier *et al*., 1988; Srinivasan *et al*., 1995; Cochran *et al*., 2000; Boe-Hansen *et al*., 2002; Morato′ *et al*., 2003), and may lead to a worsening of water quality. If the disinfectant residual is sufficient to inactivate bacteria that disassociate from the biofilm and get into the bulk water, there is no adverse impact on the user. On the contrary, if there is a large amount of disinfectant residual loss and a long residence time, bacteria that detach from the biofilm will not be inactivated. One study in a full-scale drinking water distribution system indicated that most bacteria derived from the biofilm of pipeline surface (LeChevallier *et al*., 1987). Flemming *et al*.(2002) evaluated that 95% of the all bacteria are adhered to the surface of pipeline, but only 5% is in the bulk water.

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Usually, chlorine and chloramines are common disinfectants in drinking water to kill bacteria, which are also the most common disinfection measures to prevent the formation of biofilm. Another method to control biofilm is a reduction in the levels of assimilable organic carbon (AOC) in the water, although the decrease in the AOC can inhibit deleterious microbial growth since it can limit some genera growth (LeChevallier *et al*., 1991; Volk and LeChevallier, 1999). The researchers noted that bacterial growth cannot be precluded by the limited nutrient level alone (Prévost *et al*., 1998; Flemming, 2002). The distribution system is often considered as a bioreactor, therefore the disinfectant residual at a certain concentration is regularly chosen as the most effective method for controlling the bacteria in bulk water and biofilm development of pipeline surface.

The pipe material used in water distribution systems is another important factor that influences the proliferation of the distribution system biofilm. It has been found that some pipes frequently experience problems with coliforms, taste and odor complaints. Also, there is a distinct development rate and microbe community structure of biofilm in different types of pipes (Lehtola *et al*., 2005).

Although the most usual measure taken against biofilm accumulation is to maintain a definite concentration of disinfectant residuals, significantly less quantitative information is available regarding a comparison between the effectiveness of chlorine or chloramines on the controlling biofilm with different pipe materials. Consequently, the aim of this work is to study the free chlorine or chloramines residuals necessary to control biofilm accumulation under controlled laboratory conditions, as measured by heterotrophic plate counts (HPCs). The results, although not directly comparable to field conditions, can provide a good indication as to how to control biofilm formation in actual distribution systems.

In this study, four annular reactors (ARs) were applied to simulate a drinking water distribution system with two-fold goals: (1) to examine the impact of the pipeline material on the formation of biofilm and suspended bacteria; (2) to determine the influence of chlorine or chloramines disinfection on the adhered and suspended bacteria proliferation.

MATERIALS AND METHODS

Characteristics of the tap water

The experiment was carried out with tap water supplied by Harbin No. 3 Water Plant. The water contained high concentrations of nutrients and a chloramines residual of 0.60~0.75 mg/L; measured water quality parameters are listed in Table 1.

Table 1 Quality characteristics of tap water supplied by Harbin No. 3 Water Plant

Parameter	Average value
TOC (mg/L)[a]	4.42
AOC (μg ac-C/L)[b]	350
COD_{Mn} (mg/L)[c]	3.36
HPC (CFU/ml)	1.07×10^3
Chloramines (mg/L)	0.60~0.75
Turbidity (NTU)	1.5
Ammonia-N (mg/L)	0.43
Nitrate-N (mg/L)	1.38
Nitrite-N (mg/L)	0.003
pH	7.3

[a] TOC: toal organic carbon; [b] AOC: assimilable organic carbon; μg ac-C/L: μg acetate carbon/L; [c] COD: chemical organic carbon

Reactor design and operation

The ARs used were fabricated in the laboratory. On the whole, the ARs are composed of two concentric glass annuluses and a rotatable inner rotor that has 20 removable slides. The feed water runs into the area between the inner rotor and the internal glass annulus. Each slide is installed on the inner rotor surface with a surface area of 18.2 cm^2 for biofilm adhered. Recirculated water between the two concentric glass annuluses keeps the temperature constant.

ARs were applied to simulating water temperature, nutrient substance concentration and shear stresses of drinking water distribution system. The slide materials used in these experiments were stainless steel (SS) and copper (Cu). All influents were supplied with tap water to the reactors via peristaltic pumps with a residence time of 53 min (Hebei Lange Company of China). Before the experiment, the reactors were taken apart, cleaned thoroughly, reassembled, and disinfected, including the SS and Cu slides. Fig.1 is a diagram of the experimental setup.

Heterotrophic plate counts

Culturable heterotrophic bacterial numbers were got by the spread plate technique. Samples were diluted appropriately, and placed on R2A (Sigma) agar plates for HPCs. The incubations were carried out at 25 °C for 7 d (Williams and Pirbazari, 2007). Replicate plates for both biofilm and bulk samples were analyzed for each dilution within the appropriate counts between 30 and 300 CFU selected for enumeration. The density of cells in the biofilm was reported as CFU/cm^2.

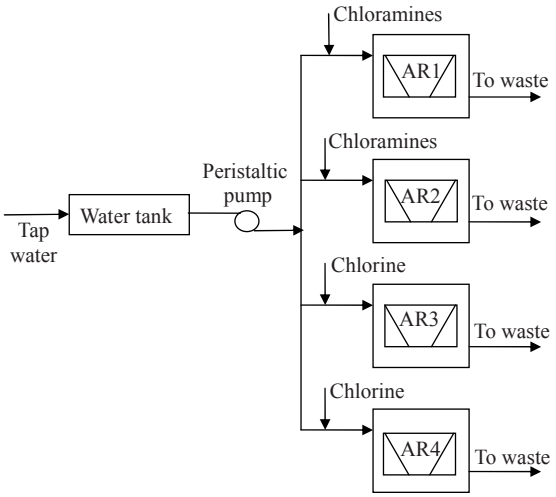

Fig.1 Diagram of the experimental setup

Assimilable organic carbon

The bacterial aftergrowth potential was analysed by a modification. The samples (40 ml) were pasteurized (70 °C, 30 min), inoculated by two bacteria, *Pseudomonas fluorescenc*e strain P17 and *Aquaspirillum* sp. strain NOX. The two yield coefficients for the growth of the bacteria on acetate (Y(P17)= 1.7×10^7 CFU/μg C, Y(NOX)=0.8×10^7 CFU/μg C) were used for calculating the AOC value, respectively (van der Wende *et al*., 1989).

Biofilm sampling

The slides were taken out from the reactors for biofilm analysis and substituted with a sterile slide. Two slides were used to confirm the results reproducibility when sampling; however, this procedure was reduced to one slide when it was found that there were no obvious differences in bacterial counts. The deposits on the slides were scraped with 2~3 pieces of sterilized cotton swabs in 10 ml of sterile water, and then the deposits on the cotton swabs were released by ultrasonic treatment in a water bath (25 min, 250 W). After ultrasonic treatment, the samples were shaken for 30 s using a vortex agitator. The detached biomass (biofilm suspensions) was diluted with sterile saline solution for bacterial enumeration. The results were reported as the number of CFU per square centimeter of slide surface.

Data analysis

Plate count data were based on the average of the three plates from the appropriate dilution on a per-milliliter (suspended bacteria) or per-square-centimeter (biofilm). Duplicate slides were removed and analyzed for the number of culturable heterotrophic bacteria. There was good agreement among replicates at various locations within the reactors with the standard error of bacterial counts between slides being less than 10%. The statistical analysis for the data was calculated with a Microsoft Excel 2007 program.

RESULTS

Effect of slide material and chloramines on biofilm formation

To assess the effect of both types of slide material and chloramines levels, two ARs were operated in parallel by using two different slide materials (SS and Cu). The experiments were divided into those with and without chloramines present. In the first stage of the experiment (1~33 d), chloramines were neutralized by the continuous addition of a $Na_2S_2O_3$ solution. In the second stage of treatment (34~66 d), the chloramines levels were 0.60~0.75 mg/L, typical values was usually found in the distribution systems of water supplies.

The stabilization time required for a biofilm to enter into a stable state depends on the environmental conditions. LeChevallier *et al*.(1990) found that a steady biofilm could form on a pipeline surface during 14 d of accumulation. Other studies came to the conclusion that 21 d was the typical time essential to the biofilm equilibrium stage (Hallam *et al*., 2001). Under low nutrient condition water of 5 μg ac-C/L AOC, the biofilm steady state was reached after about

385 d (Boe-Hansen *et al*., 2002). Usually, the steady state was assumed to have been reached when only a relatively small variation in the HPCs was examined in serial samplings. The results indicate that, as shown in Fig.2, significant biofilm formation (on SS) could be detected at around 72 h in the absence of chloramines. After 22 d, the density of culturable microorganisms remained stable at about 5 log CFU/cm^2. After 33 d without neutralization of the chloramines, the density of culturable microorganism decreased. In the final stage of the experiment, the number of the attached bacteria on the SS slide was 4.3 log CFU/cm^2. In water distribution systems, a steady state for biofilm was usually reached where the numbers of attached bacteria vary between 5 log CFU/cm^2 and 7 log CFU/cm^2 (LeChevallier *et al*., 1987). The count of attached biomass measured in this work is similar to the values previously found. Compared with SS, there were fewer bacteria attached to the Cu slides, where the number of bacteria was about 0.9 log CFU/cm^2 when chloramines were applied.

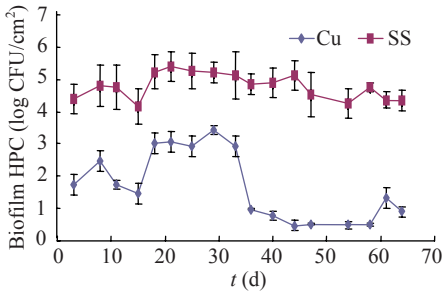

Fig.2 Biofilm formation on SS and Cu coupon without (1~33d) and with chloramines (34~66 d)

Effect of material and chlorine on biofilm formation

Fig.3 indicates the biofilm levels in the presence of chlorine on the SS and Cu slides in the AR3 and AR4 units. The biofilm heterotrophic bacteria grown in the presence of chlorine addition (0.6 mg/L) reached a maximum on Day 10 after the system startup. The results indicated that, in the presence of chlorine, significant biofilm formation (on SS slides) could be detected after 72 h. In these cases, the density of culturable microorganisms remained stable at about 4.5 log CFU/cm^2 after 6 d. The biofilm HPC levels in the presence of chlorine on Cu slides reached about 3.0 log CFU/cm^2 and remained stable between Day 16 and Day 19. After 19 d, the density of culturable microorganisms decreased to about 2.58 log CFU/cm^2.

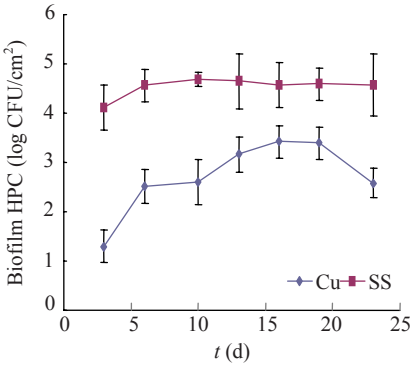

Fig.3 Biofilm formation on SS and Cu coupon in the AR3 and AR4

Influent and effluent HPC levels

As shown in Table 2, the effluent HPC levels are the sum of the influent HPC plus any additional cells arising from detached biofilm. The increased count of bacteria in the distribution system is believed to derive from bacterial reproduction, supposing that there are no other sources (e.g., external contamination events). The following equation shows a simple balancing of the bulk bacteria in the simulated distribution system when decay processes are neglected:

$$V\frac{\mathrm{d}X_{\mathrm{bulk}}}{\mathrm{d}t}=Q\left(X_{\mathrm{bulk}}-X_{\mathrm{bulk,inlet}}\right)+\mu_{\mathrm{bulk}}X_{\mathrm{bulk}}V+r_{\mathrm{det}}A, \tag{1}$$

where V is the volume (ml), X_{bulk} is the bulk phase bacterial concentration (cells/ml), $X_{\mathrm{bulk,inlet}}$ is the inlet bulk phase bacterial concentration (cells/ml), t is the

Table 2 Comparisons of influent and effluent HPC between different ARs (log CFU/ml)

	Material	HPC		
		Max	Min	Average
Influent		3.98	2.69	3.45
Effluent				
No disinfectant	Cu	6.13	5.06	5.58
	SS	6.24	5.12	5.67
Chloramines	Cu	5.11	4.52	4.83
	SS	5.21	4.69	5.01
Chlorine	Cu	4.45	3.73	4.10
	SS	4.65	4.12	4.45

time (h), Q is the flow rate (ml/h), μ_{bulk} is the bulk phase bacterial net growth rate (d^{-1}), A is the surface area (cm^2), and r_{det} is the surface detachment rate (cells/(cm^2·h)) (Boe-Hansen *et al*., 2002).

When disinfectants are added, there is a balance between the production of bacteria in the biofilm and the effect of disinfection in both the biofilm and effluent culturable cell numbers. There is also a difference in the way that the data are shown in Table 2. The effluent HPC data are shown with the range and average for each type of material. These are given to show the inherent variability in each of the experiments, as well as to illustrate the general trends in the datasets. Before the results for the effluent counts are provided, it is important to state that there was no obvious distinction between the mean influent HPC levels during the experiments. These results demonstrate the stability in the number of cells entering the reactors across the entire experimental period.

The bulk phase bacteria in effluent markedly increased over that in the influent, even in the case of AR4 with Cu slides. Lehtola *et al*.(2004) found that Cu ions led to reduced microbial numbers in bulk water. Some studies show that Cu can be toxic to bacteria at certain concentrations (Blanc *et al*., 1989). However, the reverse was found from the microbial numbers in the AR4 with Cu slides. There are only small differences among the effluent HPC levels for SS and Cu slides with chlorine or chloramines as the disinfectant. In this study, the tap water contains high concentrations of AOC (350 μg ac-C/L) that promotes bacterial growth. There is a connection between the high concentration of suspended bacteria and the proliferation of bacteria and abscission in the biofilm (Block *et al*., 1993; Camper *et al*., 1996; Cochran *et al*., 2000; Chandy and Angles, 2001).

Effect of disinfectant type on the maximum biofilm HPC level

Fig.4 illustrates the level of biofilm HPCs with the addition of chlorine or chloramines and without a disinfectant for the different slide materials investigated. Each bar stands for the mean HPCs of biofilm when the biofilm formation appeared to be steady.

There were obvious differences in the HPC values of Cu with the addition of chlorine or chloramines, with the chloramines HPC values being lower by 2 log CFU/cm^2. However, for SS slides, there was a little difference with the addition of chlorine or chloramines. The biofilm HPCs of the Cu slide were significantly different and lower (by 3.46 log CFU/cm^2) than the SS slides treated with chloramines. Also the biofilm HPCs on the Cu slides were lower (by 1.19 log CFU/cm^2) than the SS slides treated with the chlorine.

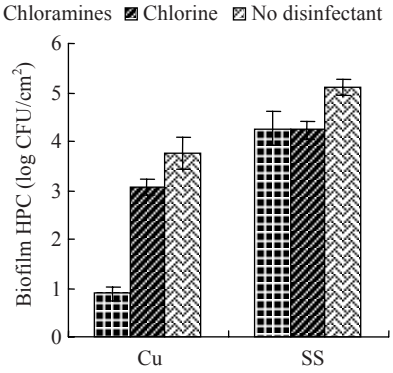

Fig.4 Effect of disinfectants and materials on biofilm formation

DISCUSSIONS

Some previous studies indicate that pipe materials can influence the biofilm development (Niquette *et al*., 2000; Schwartz *et al*., 2003). Our work also showed pipe materials may influence the biofilm accumulation and the quality of water in distribution system. Compared with Cu slides, the biofilm HPC on the SS slides had a statistically higher density of bacteria, which is consistent with the previous studies. Close to the pipeline surface, where the biofilm is adhered, the chlorine or chloramines concentration is so low that the bacteria number cannot be controlled because it may be depleted by corrosion or dissolved natural organic matter. Copper pipes are known as one of the most resistant to pollution materials with a property of the toxicity of copper ions to microorganisms, especially for bacteria in biofilm (Slowey *et al*., 1967; Santo *et al*., 2008). The combined effect of the Cu biocides with disinfectants, however, has not been tested, and there may be some synergistic effect.

Specifically, the presence of chloramines or chlorine does not completely prevent the biofilm formation. Contrary to the suspended heterotrophic bacteria, chloramines cause as much a decrease in biofilm HPC levels as does chlorine in AR with Cu

slides. However, with biofilm present, the capacity of the disinfectant to control microbial levels is reduced. For this reason, it is important to ensure sufficient disinfectant levels at all points in order to preclude biofilm formation and to guarantee the microbial quality of water.

The Ministry of Health of the People's Republic of China Standards for Drinking Water Quality defines the maintenance of a disinfectant residual as the best available technology to control the detrimental bacteria in distribution systems, and recommends a minimum residual goal of 0.3 mg/L (total residual disinfectant, mg/L). Both chlorine and chloramines at these usual levels result in low levels of suspended bacteria, yet this concentrated disinfectant is insufficient to preclude microbial biofilm formation on the pipeline inner surfaces. Even though disinfectants are widely used to preclude the development of biofilm, their efficiency on biofilm is always far less than their efficiency on bacteria in suspension.

CONCLUSION

Biofilm formation was affected by the type of disinfectant and pipe material. Compared with chlorine, chloramines treatment was more effective in controlling biofilm formation both for SS and Cu materials, and especially for Cu. In a quasi-steady state, the HPC levels on Cu slides can be reduced to 3.0 log CFU/cm^2 with chlorine and to about 0.9 log CFU/cm^2 with chloramines. Chlorination at the usual levels results in low levels of suspended bacteria, but it is not enough to preclude the growth and development of biofilm on the inner surfaces of pipelines.

The tested pipe materials (SS and Cu) did affect bacterial accumulation both with chlorine and chloramines. Compared with SS, there were fewer bacteria attached to the Cu slide with chloramines or chlorine as the disinfectant.

Based on the data presented in this study, new criteria should be developed to evaluate the best disinfection method to control the formation of biofilm. Although the solution will depend on the unique circumstances of each system, considerations, such as the penetration of biofilm, taste and odor production, trihalomethane formation, corrosion control and disinfectant stability.

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