Cu-bearing stainless steel against microorganisms in tap water

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Abstract：Tap water is one of the most commonly used water resources in our daily life, where the pathogenic bacteria, such as *Staphylococcus aureus* and *Escherichia coli* may pose a potential health risk to humans. Furthermore, the mutualism of different pathogenic bacteria in actual tap water may diminish the antibacterial effect of antibacterial agents. This paper is to report performance of an innovative antibacterial Cu-bearing stainless steel (304Cu-bearing stainless steel (304CuSS)) against microbes in tap water, which possessed a broad-spectrum of antibacterial feature. The investigation involved the uses of heterotrophic plate-counting (HPC), substrate surface free energy (SFE), observing of the cell and subtract surface morphology by using scanning electron microscopy (SEM), copper ions release (2.8±1.2 **μ**g/cm^3 from the 304CuSS was measured by metals analysed by Atomic Absorption Spectrometry (AAS), and examining live/dead bacteria on normal 304SS and 304CuSS through confocal laser scanning microscopy (CLSM). The results showed that the 304CuSS not only killed most of the planktonic bacteria (max 95.8% killing rate), but also inhibited the bacterial bio-films formation on its surface, which contributing to the

reduction of pathogenic risk to the water surrounding environments. The observation also shown that the substrate surface free energy of 304SS was 0.5-4.5 $mJ \cdot m^{-2}$ higher than that of 304CuSS throughout the experimental work. And the released Cu ions tap water from the 304CuSS inhibited the growth of the biofilms and destroyed the bacterial cell walls resulting in the inhibition of the biofilm formation.

Keywords：Cu-bearing stainless steel; tap water; antibacterial ability; biofilm

1. Introduction

Tap water quality generally plays an important role in human health and routinely monitored in the distribution network but not inside households at the point of consumption. Though treated and deemed safe for human consumption, tap water still contains a certain level of bacteria, such as *Salmonella Enterica*, *Shigella Castellani*, *Vibrio Cholerae*, and *E. coli*, etc. [1-3]. It was found that materials used for making pipe and tap played one of the most important roles in promoting bacterial growth in buildings [4]. Up-to-date report on antibacterial effect of the agents and materials against the bacteria in tap water is very scarce. And it was found that materials used for making pipe and tap materials played one of the most important roles in promoting bacterial growth in buildings. In past decades, the number of outbreaks of waterborne diseases increased dramatically worldwide [5-8]. It was reported that around 4,000 to 6,000 people died of diarrhea per day globally, which caused by water pollution, especially in the case of children [9]. In 2010, a

population of approximately 4.3 million was infected with acute diarrhea in Brazil, and 4,000 of them died of the water related infections [7].

The tap water contamination problems caused by pathogenic bacteria have brought the worldwide attention with urgent demands for acquiring effective antibacterial materials [10-15] in order to inhibit the spreading of the pathogenic bacteria in the tap water. Over 95% of all types of living organisms are heterotrophic [19], able to use all the energy for growth and reproduction once released from water pipe through their taps. In fact, majority of the antibacterial tests have been aimed only at a few of the single bacterium. For instance, Azócar et al. (2012) found that a zirconia-polyether glycol film modified by silver nanoparticles inhibited the growth of *E. coli*, *S. aureus*, *Salmonella typhi* and *Listeria monocytogenes*, respectively [16]. Zhang et al. proved that the Cu modified stainless steel showed higher antibacterial efficiency (> 99.9%) against *E. coli* and *S. aureus* [17]. Tong et al. concluded that Cu(II)-exchanged montmorillonite interfered the growth of *E. coli K88* and *Salmonella choleraesuis* [18]. However, the mutualism of different pathogenic bacteria in the actual tap water may diminish or hinder the antibacterial effects of those reported materials [19]. Thus, it is meaningful to further study the antibacterial performance of the reported materials against the bacteria used in the actual tap water.

The growth and propagation of different bacteria in tap water are strongly in connection with the biofilms formation on their contacted materials [1]. The bacteria in biofilms are less sensitive to the hostile environment [20, 21], and thus are more possible to survive in the low-nutrient tap water [22-26] compared to those of planktonic cells. For example, once the *Salmonella Enterica* aggregates on a solid surface and its cluster turns into biofilms, they will become a potential risk to human health [27-29]. Biofilms can easily form on the solid surface without effective sanitary

67 measures, thus preventing the biofilm formation and killing the adherent bacteria are key steps in
68 antibacterial processes.

69 To solve above problems, an innovative Cu-bearing 304 type stainless steel (304CuSS) [30] was
70 investigated in this study focusing on its antibacterial ability and its inhibition of the biofilm
71 formation in tap water system. It is well known that the commercially available 304SS possessing
72 good mechanical performance and corrosion resistance, has been widely used in many fields such
73 as food processing and beverage storage equipment, medical devices and daily appliances, etc. [17,
74 31]. The innovative 304CuSS has been developed based on 304SS with copper addition into the
75 stainless steel formulation as listed in Table 1. The successful copper addition into stainless steel
76 still maintained its good mechanical performance and satisfied corrosion resistance [30], while
77 greatly broadening the spectrum of its applications with much enhanced antibacterial performance
78 against a variety of bacteria. The mechanism of antibacterial effect through Cu ions releasing from
79 the steel matrix has been reported by a number of papers and copper's antibacterial capability has
80 been reorganised since historical times [32, 33], and the 304CuSS with excellent antibacterial
81 performance will expand greatly the scope of general stainless steel applications in food, hygiene
82 and biological industries.

83 Therefore, the objectives of this work are investigating antibacterial performance and the relevant
84 mechanism of the 304CuSS against pathogenic bacteria in tap water. Methods of heterotrophic
85 plate-counting, contact angle measurements, SEM and examination on Cu^{2+} concentration in testing
86 fluids, surface free energy (SFE) and CLSM observations were used in this study to provide a
87 scientific basis for its practical application under the aqueous environments.

88

2. Materials and methodologies

2.1. Materials and sampling

Standard sheet samples of 304SS were purchased from Taiyuan Steel Co. in China and those of 304CuSS were melted in a 25 kg vacuum induction-melting furnace and forged to plates by a 50 kg air hammer. The chemical compositions of the experimental stainless steels are shown in Table 1. The 304CuSS was solution treated at 1040℃ for 0.5h, and then aged at 700℃ for 6 h to precipitate the saturated Cu-rich phase from the steel matrix. The earlier study had shown the TEM image of the microstructure of Cu-rich precipitates within the steel matrix, and the size of the Cu-rich precipitates was about 50 nm [30]. These Cu-rich phase precipitates could enable proper amount of Cu ions (Cu^{2+}) to be released from the surface of the steel into the water or any fluids or solution and thus offer the Cu-steel antibacterial ability [31]. After the heat treatment, the sample sheets were cut into sample pieces with dimensions of 10 × 10 × 1 mm^3 in general, and 40 × 40 × 2 mm^3 for Cu ions releasing test as well, and mechanically polished using $1000^{\#}$ SiC papers, and then cleaned ultrasonically in an acetone bath, followed by an ethanol bath (KQ-500DB, Kun Shan Ultrasonic Instruments Co., Ltd, China) for 15min respectively. After blow-drying, the samples were sterilized under UV for 30min [31].

Table 1 Chemical composition of the experimental steels (wt. %).

Materials	Cr	Ni	Cu	C	Si	Fe
304CuSS	18.66	9.78	3.88	0.026	0.048	Balance
304SS	18.39	10.12	—	0.028	0.052	Balance

In order to verify the antibacterial ability of the 304CuSS in the actual tap water, tap water samples were randomly collected from water taps in separate household in Shenyang (China) The

water were qualified according to GB-T 5749-2006 (China) and HPC [34]. All glassware used for sampling in this study was sterilised.

2.2. Antibacterial test

The plate-count bacteria standard used was based on GB-T 5750-2006 (China) with the testing methodology as close as possible to the WHO Heterotrophic plant count (HPC) standard for examining diversified planktonic bacteria, which was used broadly to define the wide range of microorganisms that include bacteria, yeasts and moulds [4, 34]. The diversity of bacteria in drinking water system was as similar as in other freshwater systems [4], where reports showed the bacterial communities were dominated by Proteobacteria (Alpha-, Beta-, Gammaproteobacteria), Cyanobacteria and Bacteroidetes [35]. Species of Pseudomonas, Aeromonas, Acinetobacter, Corynebacterium, Flavobacterium, sulphatobacteria and ferrobacteria were also frequently found in drinking water systems cross world [35-37]. Nevertheless the purpose of this work was not to identify specific species in tap water, but to determine whether or not the 304CuSS possesses antibacterial efficacy against bacteria in the tap water system. Hence, the procedure of antibacterial test was as follows:

Tap water bacteria preparation: The Luria–Bertani (LB) medium was used with compositions of beef extract 5.0g/L, NaCl 5.0g/L, peptone 10.0g/L, agar 20.0g/L, and distilled water 1000ml, with pH value of 7.2 ± 0.1 [32].

A volume of 800μl [14] tap water was dropped into 24-well plates with different samples (one sample in each well) and then incubated in an incubator (DNP-9272, Jinghong Laboratory Instrument Co., Ltd, Shanghai, China) at 25℃ for 24 h, 48 h and 72 h, respectively. After contact

with the sample steels, 1 ml of tap water was serially diluted and added onto the nutrition agar plates, respectively. The plates were counted after the nutrition agar plates were incubated at 37℃ for 24 h. Each experiment was performed in triplicate.

2.3. Surface free energy measurement

A volume of 800μl fresh tap water was added into each well of the 24-well plates (There was one sample in each well), and then they were incubated in an incubator at 25℃ for 24 h, 48 h and 72 h, respectively. The tap water in different plates was removed and the samples were rinsed with distilled water for three times. The bacterial biofilms on the steel substrate surfaces were air-dried to a certain state [38, 39]. Contact angle measurements were performed by a goniometer (JC2000C, Shanghai Zhongchen, China) for 5 times on each of the steel surfaces based on the reported procedure [38]. The test liquids used were deionized water and 1-Bromonaphthalene. The surface free energies were then calculated by using Owens–Wendt–Rabel–Kaelble (OWRK) theory as well as Owens two liquid methods [24, 40-43], and the contact angles (θ) and surface free energy (SFE) are expressed as:

$$\frac{1+\cos\theta}{2}\cdot\frac{\gamma_L}{\sqrt{\gamma_L^n}}=\sqrt{\gamma_S^p}\sqrt{\frac{\gamma_L^p}{\gamma_L^n}}+\sqrt{\gamma_S^n} \quad (1)$$

$$\gamma_S=\gamma_S^p+\gamma_S^n \quad (2)$$

Where θ is the contact angle between the liquid and the solid, γ_S represents the surface free energy of solid, γ_L describes the surface free energy of liquid (*p*=polar, *n*=nonpolar). The test liquids were

150 deionized water and 1-Bromonaphthalene, in which the nonpolar components were 21.8 $mJ \cdot m^{-2}$ and
151 44.6 $mJ \cdot m^{-2}$, respectively, and the polar components were 51 $mJ \cdot m^{-2}$ and 0 $mJ \cdot m^{-2}$, respectively [44,
152 45]. Also each measurement was performed in triplicate.

153

154 ***2.4. DAPI staining***

155 DAPI (4', 6-diamidino-2-phenylindole) is a fluorescent chemical capable of forming the
156 fluorescent complexes with double stranded DNA and yielding strong fluorescent signal [46-49].
157 The maximum of fluorescence was observed at a wavelength of 461nm. DAPI staining was used for
158 observing the sessile bacteria on the surface of steel samples.

159 After the steel samples were immersed in 800μl tap water for 24 h, 48 h and 72 h, respectively, a
160 volume of 0.8μl DAPI stock solution was added to stain the steel samples, and the final working
161 concentration of DAPI was 1 μg/ml. After 15min in the dark chamber at room temperature, the steel
162 samples were taken out from the tap water, and washed with phosphate buffer solution (PBS)
163 (pH=7.4±0.1) for 3 times, and then dried at room temperature. The samples were analysed under a
164 CLSM (C2 Plus, Nikon, Japan) [46, 49].

165

166 ***2.5. Live/dead staining***

167 The LIVE/DEAD BacLight Bacterial Viability Kit (Invitrogen, Molecular probes, Darmstadt,
168 Germany) was used to stain the sessile bacteria attached on the steel surface, and detect the
169 biologically active. Other functions included inactivating the bacteria and evaluating the
170 antibacterial performance. The kit utilized mixture of two nucleic acid stains, green-fluorescent

SYTO 9 stain and red-fluorescent propidium iodide stain. When staining with proper amount of this mixture, the live bacteria with intact cell walls showed fluorescent green, whereas bacteria with damaged cell walls exhibited fluorescent red [50].

After steel samples were immersed in 800μl tap water for 24 h, 48 h and 72 h, respectively, they were taken out and washed with PBS for 3 times, and then dried at room temperature. The samples were analysed under a CLSM [49].

2.6. SEM observation

After immersed in tap water for different times, the steel samples were fixed in the 4% glutaraldehyde solution for 4h at room temperature and rinsed for 3 times with PBS. The dehydration process was performed by the following steps: 1ml of 25%, 50%, 75% and 100% ethanol was separately dropped onto the samples for 15minutes, and then the samples were dried at room temperature followed by gold sputter-coating. The morphologies of the bacteria adhered on the substrate surfaces were observed on a SEM (SUPRA 55, CARL ZEISS, Germany) [11, 43, 51].

2.7. Copper ions release measurement

The samples of 304CuSS with size of 40 mm × 40 mm × 2 mm were immersed in a sterile container with 12ml tap water, the same ratio of sample surface area and tap water volume as other samples. After incubated in an incubator at 25℃ for 24 h, 48 h and 72 h, respectively, the tap water was collected and then the quantity of Cu ions was measured by an AAS (Z-2000, Hitachi, Japan).

2.8. Statistical analysis

All data in this study were presented in the mean ± SD (Standard Deviation). Independent t-test computing with SPSS 13.0 was used to compare the data of planktonic cell counts, contact angles and surface free energies between 304CuSS and 304SS.

3. Results and discussion

3.1. Antibacterial performance

Table 2 The HPC / planktonic bacteria counting (CFU/ml) was carried out in tap water after contact with samples. for different times.

Time [h]	**304CuSS** [CFU/ml]	**304SS** [CFU/ml]
24	60±20	100±50
48	130±60	3200±350
72	2620±120	10640±420

As shown in Table 2, the colony forming units (CFU) of planktonic bacteria in tap water after contact with two steel samples for 24 h showed no significant difference ($p > 0.05$). Whereas, after the steel samples were immersed in tap water for 48 h, the CFU of planktonic bacteria in the tap water contacted with 304CuSS (130±60 CFU/ml) was much lower than that of 304SS (3200±350 CFU/ml), with antibacterial rate of 95.9%. After 72 h, the CFU of planktonic bacteria in the tap water contacted with 304SS rapidly increased to 10640±420 CFU/ml, while on the contrary, the CFU of planktonic bacteria in the tap water contacted with 304CuSS increased only to 2620±120 CFU/ml. The HPC results indicate that 304CuSS had a strong antibacterial effect against the planktonic bacteria in the tap water. Killing mechanisms demonstrated by published papers showed

that after samples contacted with tap water, trace amount of Cu ions diffused into the tap water from the surface of 304CuSS, which destroyed the bacterial cell walls and inhibited the growth of the bacteria [52].

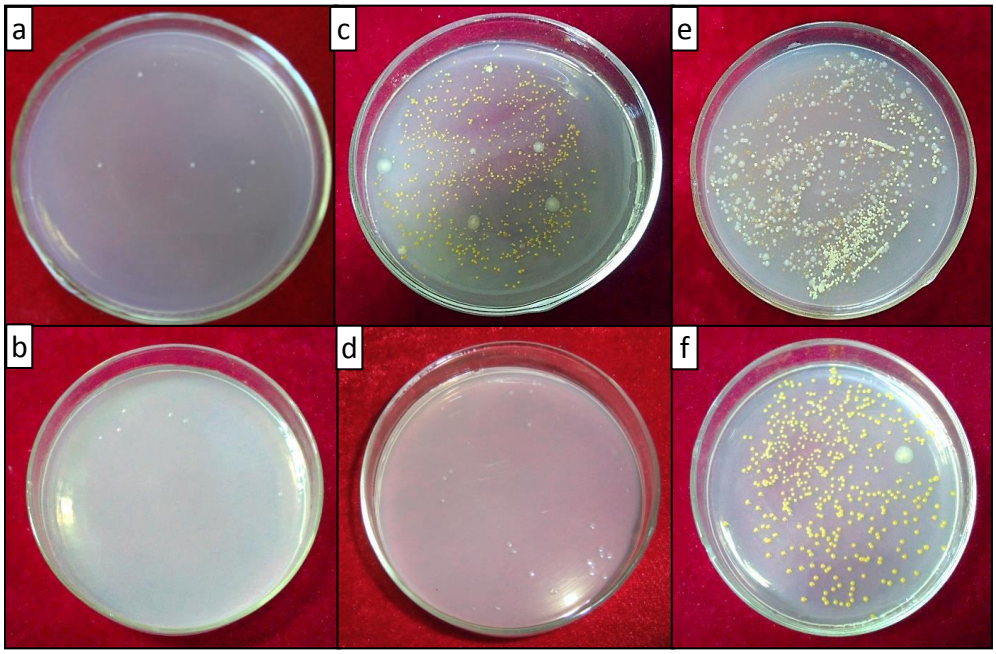


Fig. 1 Photos of bacterial cell count of the tap water immersed with different stainless steels, (a) 304SS and (b) 304CuSS for 24 h; (c) 304SS and (d) 304CuSS for 48 h; (e) 304SS and (f) 304CuSS for 72 h.

Fig. 1 illustrated the images of the planktonic bacterial colonies (with colours) in petri dishes after contact with steel samples. After contact with 304SS for 48 h, the colours and morphologies of planktonic bacterial colonies of the tap water changed, and there were more than two kinds of planktonic bacterial colony judging by colours in the petri dish (Fig. 1c), while contact with 304CuSS, there was only one kind of bacterial colonies (Fig. 1d). After 72 h, the bacteria in tap water contacted with 304SS were in colours of white, yellow and shiny yellow (Fig. 1e), while only colours in white and yellow appeared after contact with 304CuSS (Fig. 1f). The number of the total bacterial colonies shown in Fig. 1f (304CuSS for 72 h) is much less than that in Fig. 1e (304SS for 72 h). Thus it can be reasonably deduced that the 304CuSS greatly inhibited the planktonic bacteria from growth and propagation, demonstrating a good antibacterial ability against bacteria in tap

224 water.

225 ***3.2. Surface free energy***

226 Both surface free energy and the polar component of 304SS are higher than 304CuSS. It has been
227 known [53] that the wettability of a material depends upon the surface free energy, and the increase
228 of the polar component contributes to the increase of the wettability. The lower the polar component,
229 the less likely the surface to be wet-out. The number of organic groups and the surface properties or
230 composition of the metals are the factors that may affect the polar component [53, 54]. For the
231 oxidation of a given metal, carbon, oxygen and nitrogen are adopted onto the surface of the metal to
232 form different organic compounds that become the source of the growth and propagation of bacteria
233 [43, 55]. Thus, the polar component has a strong relationship with the wettability. Table 3 and Fig. 2
234 show the variation of contact angle and surface free energy of steel samples within 72 h.

235 **Table 3** Contact angles of steel samples

Samples	Time [h]	Contact angle [°]	
		Deionized water	1-Bromonaphthalene
304SS	0	37.70±1.35	11.5±0.14
304CuSS		46.70±0.14	16.20±0.99
304SS	24	30.70±0.06	12.50±0.35
304CuSS		32.91±0.12	9.60±0.85
304SS	48	27.67±1.18	11.21±0.77
304CuSS		29.67±1.41	14.77±0.80
304SS	72	22.58±0.12	14.83±0.24

304CuSS		53.00±0.67	13.83±1.04

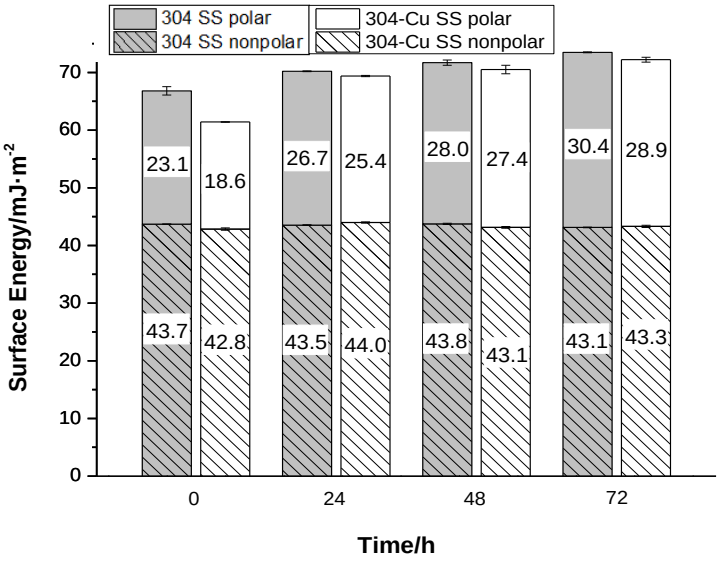


Fig. 2 Variations of surface free energy of steel samples after removing planktonic bacterial cells with time.

The contact angles and the surface free energies of the samples changed obviously after the adhesion of microorganisms on their surfaces as reported by other researchers [55]. Prior to experiment, the nonpolar components of both steels were approximately identical, while the polar component of the 304SS was 4.5 mJ·m^{-2} higher than that of 304CuSS, and the surface free energy was 5.4 mJ·m^{-2} higher than that of 304CuSS. With the extension of immersion time, the nonpolar component of both stainless steels kept almost the same range from 42 to 43mJ·m^{-2}, while the polar component and surface free energy significantly changed for both. After exposure to tap water for 24 h, the polar component and surface free energy of 304CuSS were 1.27 mJ·m^{-2} and 1.4 mJ·m^{-2} lower than those of 304SS, respectively. When it came to 48 h, the polar component and surface free energy of 304CuSS (27.4 mJ·m^{-2} and 70.5 mJ·m^{-2}) were lower than those of 304SS (28.0 mJ·m^{-2} and 71.8 mJ·m^{-2}), respectively. After 72 h, the polar component and surface free energy of 304CuSS were still 1.5 mJ·m^{-2} and 1.3 mJ·m^{-2} lower than those of 304SS, respectively. Thus we

reached the conclusion that the polar component was the main factor that changed within the immersion time, and the polar component of the surface of 304CuSS was lower than that of the surface of 304SS [53, 54].

3.3. SEM images of bacteria

It was found that the bacteria in the tap water gradually adhered to the substrate surfaces. In Fig. 3a-b, both rod-like and ball-like bacteria were found on the surfaces of both steels and showed highly discrete distributions. The number of bacteria on the surface of 304SS was slightly more than that on the surface of 304CuSS. After 48 h, the number of bacteria on the surface of 304SS rapidly increased and even formed clusters, as shown in Fig. 3c, while relatively much less bacterial observed on 304CuSS (Fig. 3d).

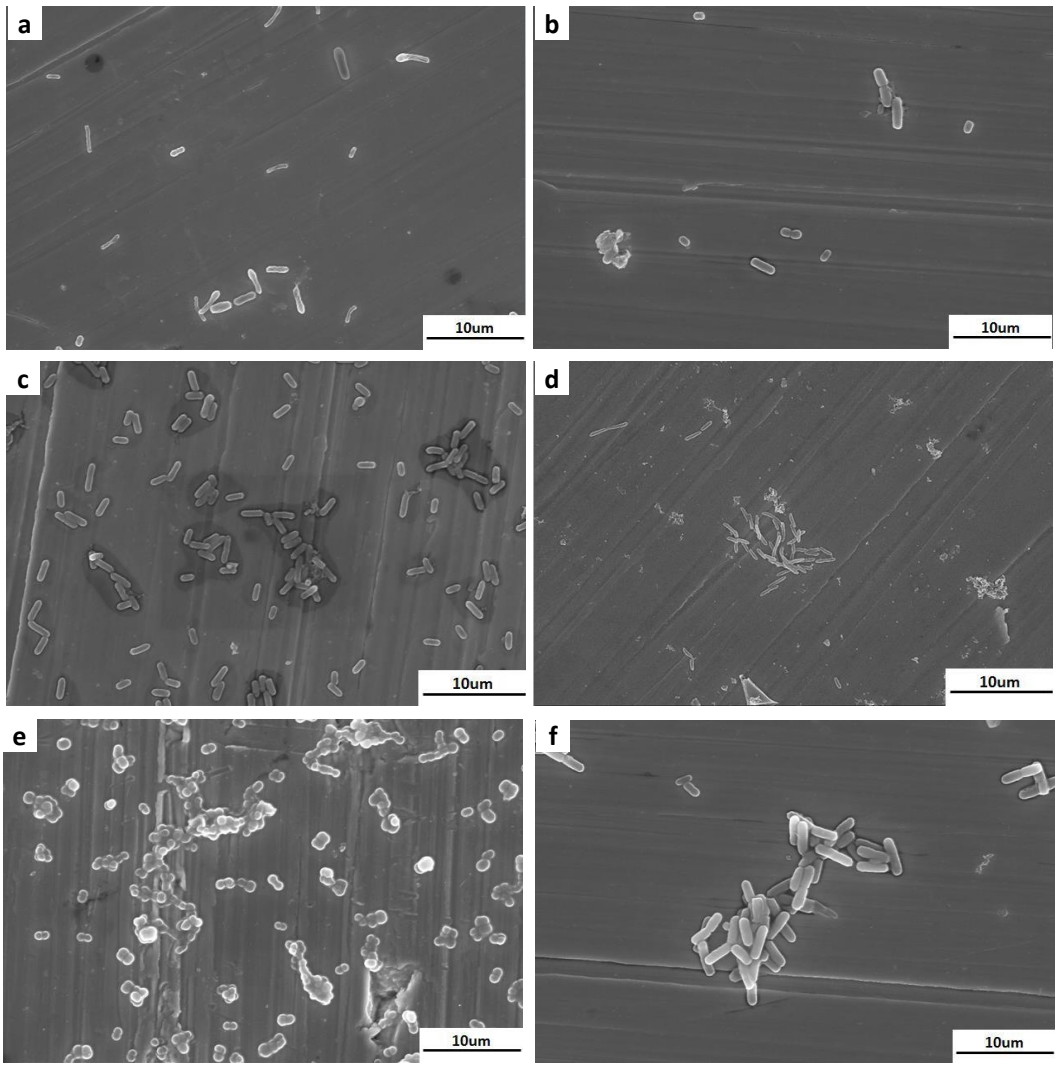


Fig. 3 SEM images of bacteria after contact with steel samples with different times (h): (a) 304SS and (b) 304CuSS for 24 h; (c) 304SS and (d) 304CuSS for 48 h; (e) 304SS and (f) 304CuSS for 72 h.

After 72 h, the bacteria on the 304SS became more intensive, as shown in Fig. 3e. However the bacteria on the 304SS were still much less (Fig. 3f). It can be seen from Fig. 3 that the number of bacteria on the surface of 304CuSS was always less than that of the 304SS, and much more bacterial clusters were formed on the surface of the 304SS. The reason might be that the Cu ions released from the surface of 304CuSS could inhibit the growth and propagation of the bacteria [56], thus hinder the conversion from the planktonic cells to the adherent biofilm. Whereas, the bacteria contacted with the 304SS intended to adherent to the surface and thus could grow and propagate by the protection of biofilm.

3.4. DAPI-staining

The number of adherent bacteria on the steel surfaces with DAPI staining is observed in Fig. 4. Five in some random positions were chosen for counting and imaging.

An observation by CLSM as shown in Fig. 4a - f illustrated that the number of bacteria on the surface of 304CuSS was obviously less than those on the surface of 304SS. For example, 4.1×10^3 cm^{-2} and 2.9×10^3 cm^{-2} were counted spots in Fig. 4a and b. And the numbers of adherent bacteria shown in Fig. 4d and Fig. 4f were much less than those in Fig. 4c and e, respectively. After 72 h, the adherent bacteria on surface of 304SS increased dramatically to more than 2×10^5 cm^{-2}, while those on surface of 304CuSS were relatively less (2.85×10^4 cm^{-2}). The bacteria on the surface of 304CuSS grew more slowly than those on the surface of 304SS, and they could hardly convert into biofilms.

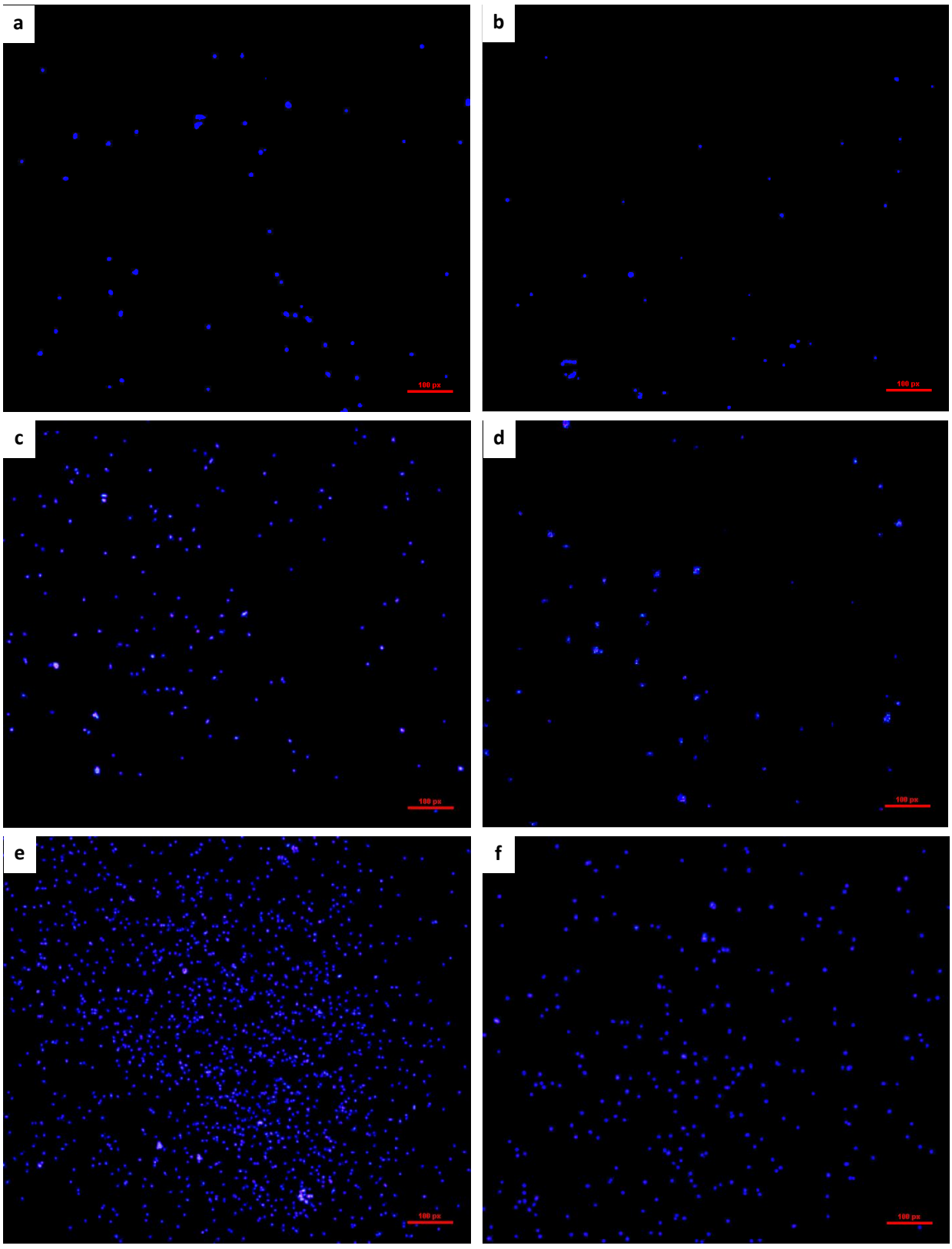


Fig. 4 DAPI staining: visualization of bacteria adhered to sample surfaces in consecutive time, (a) 304SS and (b) 304CuSS for 24 h; (c) 304SS and (d) 304CuSS for 48 h; (e) 304SS and (f) 304CuSS for 72 h.

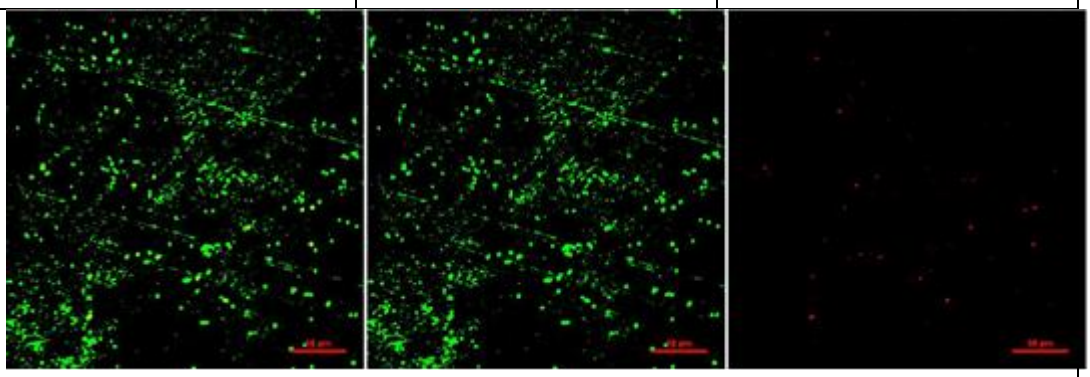
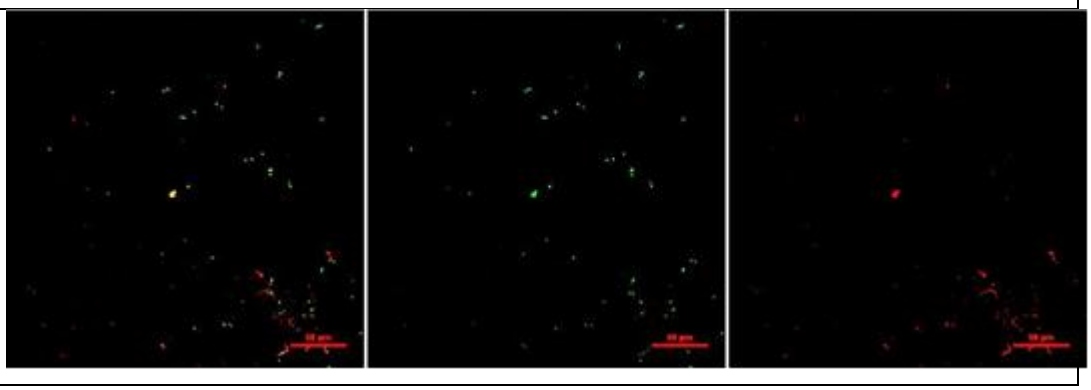
Contact time	Samples	Bacteria	Biologically active bacteria	Biologically inactive bacteria
24 h	304SS			
	304CuSS			

Fig. 5 Live/dead staining of adherent bacteria on the surfaces of 304SS and 304CuSS after 24 h.

3.5. Live/dead-staining

The numbers of biologically active bacteria on the steel surfaces are the real reflection of bacterial killing capability of the antibacterial stainless steel. 5 randomly positioned samples were chosen for observation. As shown in Fig. 5-7, the number of biologically active and inactive bacteria in the adherent state varied with the contact time of the sample steels.

The total bacterial number and the number of biologically active bacteria on the surface of 304SS were always higher than those on the surface of the 304CuSS after contacting with tap water for 24 h, 48 h and 72 h, respectively. After 24 h, the number of adherent bacteria on 304SS increased and began to form into biofilms, while there was only few bacteria adherent to the surface of the

304CuSS and almost half of them were biologically inactive, as shown in Fig. 5. When it came to 48 h, adherent bacteria on the surface of 304SS became dense, whereas the number of adherent bacteria on the surface of 304CuSS increased slightly (Fig. 6).

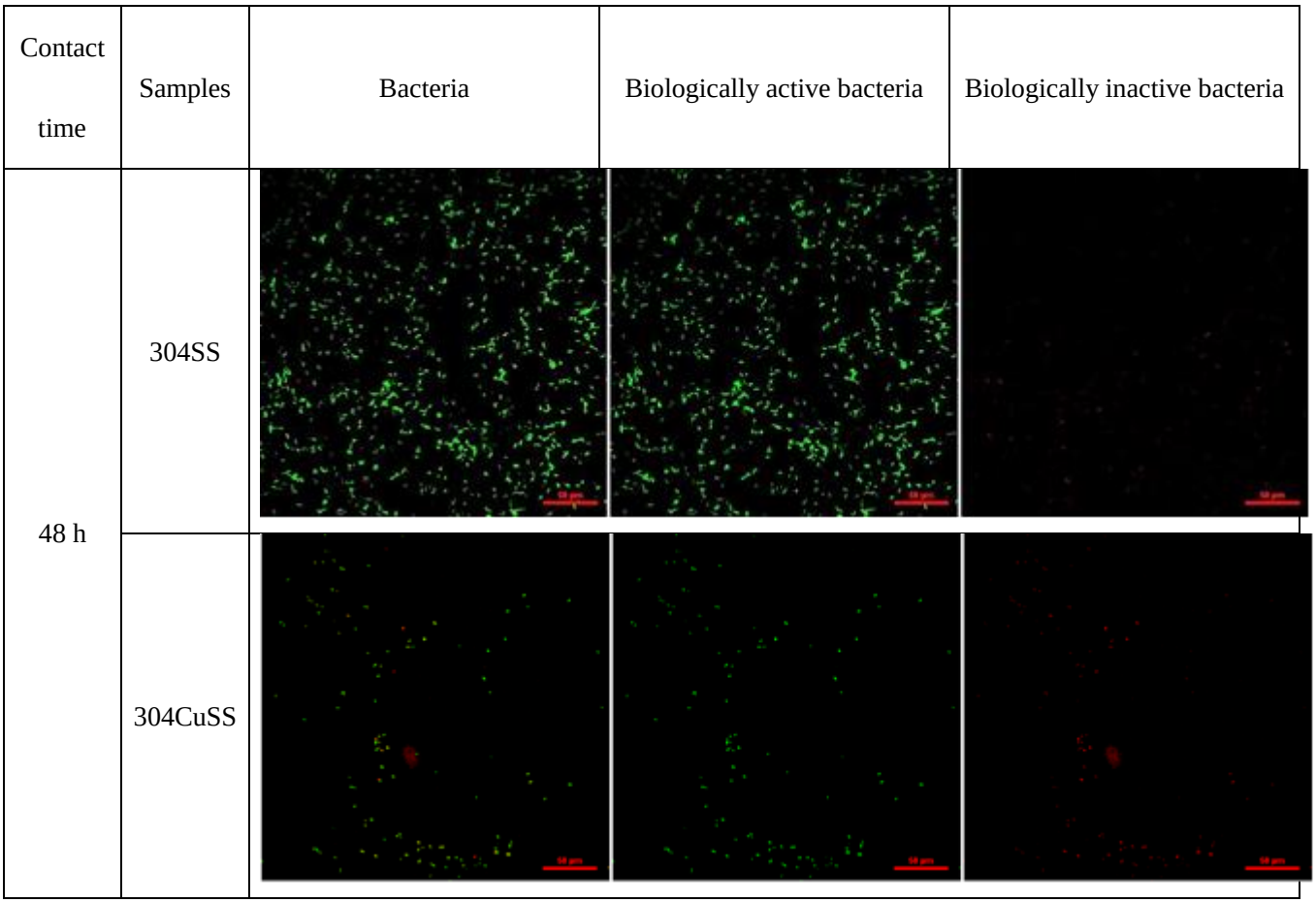


Fig. 6 Live/dead staining of adherent bacteria on the surfaces of 304SS and 304CuSS after 48 h.

After 72 h, the quantity of biologically active bacteria on the surface of 304SS was much bigger and the biofilm was dense, while most of the adherent bacteria on the surface of 304CuSS were biologically inactive (Fig. 7). As shown in Fig. 5-7, adherent bacteria on the surface of 304SS grew faster and converted into biofilms, while the number of adherent bacteria on the surface of 304CuSS increased slightly and the number of biologically inactive bacteria increased. We can conclude that Cu ions released from the surface of 304CuSS killed most of the bacteria adherent to the surface, thus the adherent bacteria could not transform themself into biofilm [52].

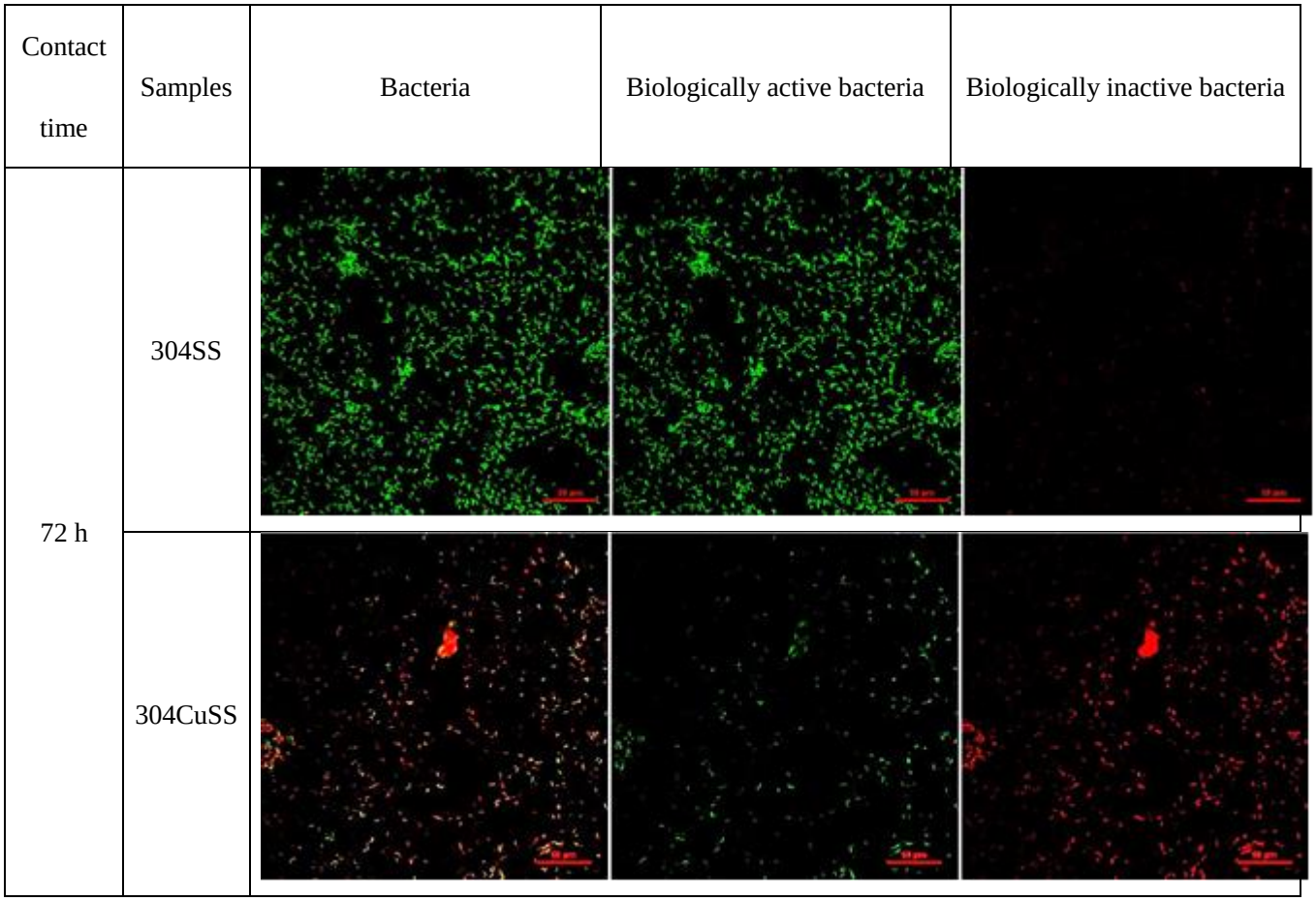


Fig. 7 Live/dead staining of adherent bacteria on the surfaces of 304SS and 304CuSS after 72 h.

3.6. Copper ions release

Table 4 Release profiles of 304CuSS in tap water

Sample	Release of Cu ions (μg/cm^2)		
	24 h	48 h	72 h
304CuSS	0.5±0.5	2.4±1.6	2.8±1.2

The release of Cu ions from the 304CuSS was measured to evaluate to what level of the Cu ions release could produce the antibacterial effect. As shown in Table. 4, the Cu ions release was slow in the first stage, after that a rapid release happened. Release of Cu ions after 48 h was about 5 times of the first 24 h, and the release after 72 h increased slightly. It can be found in Table. 2 that

304CuSS showed good antibacterial ability after immersions in tap water for 48 h and 72 h. Thus the amount of Cu ions released from 304CuSS corresponded to antibacterial effect. Meanwhile, it was examined that 304SS in water did not shown any release of Cu^{2+} with extended time (Ref.)

3.7. Mechanism of bacteria and biofilm inhibition

Bacteria exist in tap water with two different "life styles": one is the planktonic cell and the other is the biofilm. As shown in Fig. 8a, the planktonic bacterial cells could be predicated as colonized on the 304SS at first, release the extracellular polymeric substances (EPS) and then form bacterial biofilm on the steel surface [27]. It can be seen from Fig. 1 and Table 2 that the 304CuSS possessed strong antibacterial ability against the planktonic cells in tap water. Based on the description shown in Fig. 8b, the released Cu ions from the 304CuSS surface produced the antibacterial function and inhibited the growth of the biofilms [52]. Cu ions was able to be dissolved into tap water, and destroyed the bacterial cell walls and then killed the bacteria [12, 30, 51, 52, 56, 57] resulting in the inhibition of the biofilm formation. Polar bonds served as the primary adsorption sites for the polar molecules and the surfaces, which could influence the adhesion force [53].

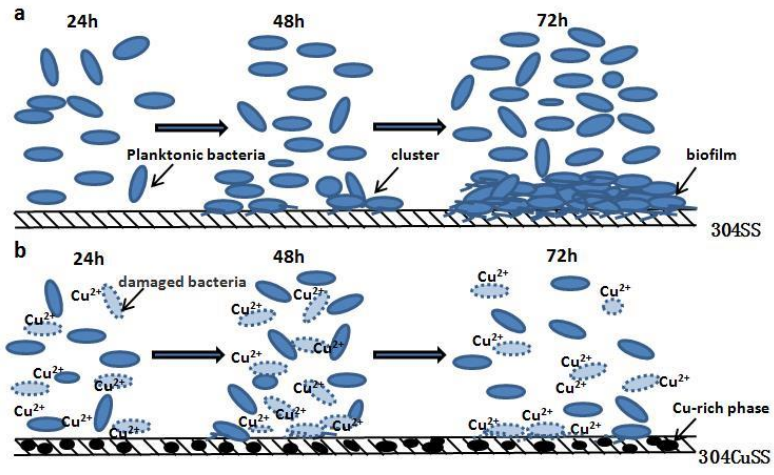


Fig. 8 a. Schematic process of transition from the planktonic cells to biofilm of bacteria on 304SS; b. Inhibition of the cluster-related cell process of bacteria on 304CuSS.

4. Conclusions

Bacterial adhesion to the stainless steel is a complex process effected also by in relation with the bacterial cell density, nutrient availability [27], hydrophobicity and pH [43, 51, 58]. However, one of the major findings in this paper was effects of the surface free energy and their polar component of 304BCuSS to the heterotrophic bacterial adhesion in line with some papers reported that the lower surface free energy of materials reduces the bacterial adhesion and biofilm formation [43, 59, 60]. The higher polar component of the 304SS compared to that of the 304CuSS after immersed in tap water, resulted in a higher sessile bacteria formation in line with the reported sessile bacteria increase proportional to the rising of polar component [40, 59, 61, 62]. The longer immersion time allowed much more amount of organic compounds being increased, thus the oxygen polar group increased [53, 63]. And therefore, the amount of adherent bacteria was more [40, 41, 53, 64-67]. This paper proved that 304CuSS has significant ability in against the bacteria, and in inhibiting the biofilm formation on its surface compared with that of 304SS, which significantly decreased the pathogenic risk to water and its surrounding environment.

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