J Therm Spray Tech
DOI 10.1007/s11666-017-0535-8

PEER REVIEWED

Colonization of Bacteria on the Surfaces of Cold-Sprayed Copper Coatings Alters Their Electrochemical Behaviors

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Submitted: 1 April 2016 / in revised form: 22 January 2017

Abstract Copper coatings were fabricated on stainless steel plates by cold spraying. Attachment and colonization of *Bacillus* sp. on their surfaces in artificial seawater were characterized, and their effects on anticorrosion performances of the coatings were examined. Attached bacteria were observed using field emission scanning electron microscopy. Electrochemical behaviors including potentiodynamic polarization and electrochemical impedance spectroscopy with/without bacterial attachment were evaluated using commercial electrochemical analysis station Modulab. Results show that *Bacillus* sp. opt to settle on low-lying spots of the coating surfaces in early stage, followed by recruitment and attachment of extracellular polymeric substances (EPS) secreted through metabolism of *Bacillus* sp. The bacteria survive with the protection of EPS. An attachment model is proposed to illustrate the bacterial behaviors on the surfaces of the coatings. Electrochemical data show that current density under *Bacillus* sp. environment decreases compared to that without the bacteria. Charge-transfer resistance increases markedly in bacteria-containing seawater, suggesting that corrosion resistance increases and corrosion rate decreases. The influencing mechanism of bacteria settlement on corrosion resistance of the cold-sprayed copper coatings was discussed and elucidated.

Keywords bacterial attachment · cold spray · copper coating · corrosion

Introduction

Corrosion and fouling are primary problems to metal devices and vessels immersed in seawater, which induce enormous economic damage to maritime activities (Ref 1-3). Painting is the most convenient method to prevent corrosion/fouling of marine structures, storage tanks, ship bottoms, etc. (Ref 4). However, paint coatings are subjected to failure especially on the surfaces of worn parts (Ref 5). Cold gas dynamic spraying (also called cold spraying) is a relatively new coating process, which was developed in the mid-1980s in Novosibirsk (Ref 6, 7). Cold spraying employs Laval nozzle to produce high velocity gas flow, and particles are deposited through kinetic energy of impact (Ref 8-11). Compared to traditional thermal sprayed coatings, cold-sprayed coatings present denser microstructure, higher deposition efficiency, higher bonding strength and higher purity (Ref 12). Therefore, cold-sprayed coatings are expected to be used as marine anticorrosion or antifouling coatings. Many efforts have been made pertaining to marine applications of cold-sprayed

This article is an invited paper selected from presentations at the 7th Asian Thermal Spray Conference (ATSC 2015) and has been expanded from the original presentation. ATSC 2015 was held in Xi'an, China, September 23-25, 2015, and was organized by the Asian Thermal Spray Society in association with Xi'an Jiaotong University, State Key Laboratory for Mechanical Behavior of Materials.

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coatings, for example, Marrocco et al. (Ref 13) deposited titanium coatings by cold spraying and revealed the evolution of their corrosion resistance as a function of coating porosity. Vucko et al. (Ref 14) fabricated Cu/polymer composite structures using cold spraying and evaluated their antifouling property. Moreover, corrosion resistance of cold-sprayed tantalum-based (Ref 15, 16) and aluminum-based amorphous alloy coatings (Ref 17), nickel coatings (Ref 18) and zinc coatings (Ref 19) have also been studied. Vucko et al. (Ref 14) focused on antifouling property of cold-sprayed Cu/polymer composite structures. Ding et al. (Ref 5) prepared Cu-Cu_2O composite coatings by cold spraying and also investigated the evolution of their antifouling property as a function of Cu_2O content in the coatings. Sanpo et al. evaluated antibacterial behavior of cold-sprayed ZnO-Al coatings (Ref 20), HA-Ag/PEEK coatings (Ref 21) and Chitosan-Cu/Al coatings (Ref 22).

In fact, corrosion and fouling coexist and interrelate in the marine environment (Ref 23-26). For bulk materials, microorganisms within biofilms formed on metal surfaces affect cathodic and/or anodic reaction kinetics and change electrochemical conditions at metal/solution interfaces, leading to acceleration or inhibition of corrosion. Enzymatic activity in biofilms also influences the corrosion of metal surfaces in the marine environment. Extensive research on microbially influenced corrosion (MIC) has been reported (Ref 27-31). Benboudiz-rolletn et al. (Ref 32) reported that *sulfate-reducing bacteria* favored the growth condition where *V. natriegens* and *Desulfovibrio vulgaris* have developed an extensive biofilm, which could further induce or enhance corrosion on metal surfaces. Other research indicated that biofilm can reduce the corrosion rate of metals such as copper and brass by reducing oxygen concentration on metal surfaces (Ref 33).

The distinguishable differences between bulks and thermal sprayed coatings are predominately porosity and surface characteristics, which play important roles in marine corrosion and fouling. Among them, surface characteristics were well investigated (Ref 34-39). Bagherifard et al. (Ref 34) pointed out that the nanostructured features on severely shot peened 316L stainless steel surfaces reduced gram-positive bacterial adhesion. Epstein et al. (Ref 35) also investigated control of the surface nanostructure to bacterial biofilm growth. However, influence of bacterial attachment on corrosion behavior of thermal sprayed coatings was barely reported, although the function of bacterial attachment on corrosion was well investigated for bulk materials. Recently, the influence of bacterial colonization on electrochemical behavior of arc-sprayed Zn coatings and Al coatings in seawater was reported (Ref 40). Compared to thermal sprayed coatings, cold-sprayed coatings have dense microstructure and low oxidation, shedding light on potential antifouling applications of cold-sprayed copper-based coatings. Nevertheless, it is not well understood yet how the bacterial attachment affects the corrosion behaviors of cold-sprayed marine coatings. In this work, copper coatings were fabricated by cold spraying and the influence of colonization of typical marine bacteria, *Bacillus* sp., on their surfaces on their corrosion behaviors was examined and elucidated.

Experimental details

Coating preparation

Copper powder with the mean diameter of 28.8 μm (99.9%, BGRIMM Advanced Materials Science and Technology Co., Ltd, China) was used to prepare copper coatings, and the surface morphology of the powder and the coatings is shown in Fig. 1. Grit-blasted stainless steel

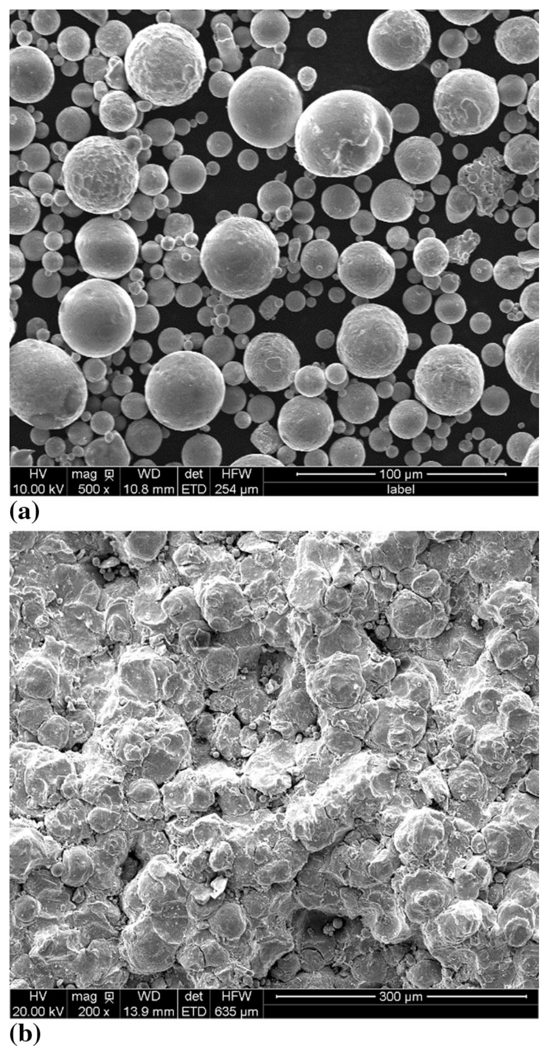

Fig. 1 Surface morphologies of the copper powder (a) and the cold-sprayed copper coatings (b)

plates (316L) with a dimension of 2 cm × 2 cm × 0.2 cm were used as substrates. A high pressure cold spray system (CS-2003, Xi'an Jiaotong University, China) was used to deposit the coatings. Nitrogen (550 °C, 2.5 MPa) was used as the main gas, and the spray distance was set as 20 mm.

Assessment of Biological Behaviors

Bacillus sp. (1A00791, MCCC, China), a typical rod-shaped gram-positive bacterium, forming biofilm with its abundant secreted EPS to cause biofouling, was employed to study the influence of bacterial attachment on corrosion behavior of the cold-sprayed copper coatings. *Bacillus* sp. bacteria were cultured in 2216E (CM 0471) media prepared by dissolving 1 g yeast extract, 3 g peptone, 1 g beef extract and 0.01 g $FePO_4$ in 1000 ml artificial seawater (ASW) prepared according to ASTM standard D1141-98 (2003). The testing conditions involving typical sea bacteria cultured in ASW were proven effective in biofilm-related research (Ref 28, 41). Peptone was added into the solution as carbon and energy sources for the bacteria (Ref 42). The media containing the bacterial strain were shaken for 24 h at 37 °C. The inoculation medium was prepared by adding *Bacillus* sp. for an initial concentration of 10^5 CFU/ml at 25 °C under aerobic condition. Bacterial number was determined based on the standard calibration with an assumption that an OD value of 1.0 was equivalent of 10^9 cells/ml. The copper coatings were immersed in the ASW and the bacteria-containing ASW for 3 days and 7 days, respectively. All samples were immersed in 2.5 vol.% glutaraldehyde solution for 8 h, and then dehydrated by immersing successively in 25% ethanol for 5 min, 50% ethanol for 5 min, 75% ethanol for 5 min, 90% ethanol for 5 min, and 100% ethanol for 40 min. Attachment of the bacteria on the copper coatings was examined by field emission scanning electron microscopy (FESEM, FEI Quanta FEG 250, USA). Bacterial and EPS coverage on the coatings was evaluated using EDS mapping analysis, and ten pictures for each coating were acquired and analyzed using the software Image J.

Electrochemical Characterization

The coatings with/without bacterial attachment were taken out from the inoculation media and immersed in ASW to evaluate the impact of the colonized bacteria on the electrochemical behavior of the coatings. Potentiodynamic polarization and electrochemical impedance spectroscopy (EIS) of the copper coatings were evaluated using electrochemical analysis station Modulab (Modulab, Solartron Analytical Co., Ltd, UK). Three classic electrodes were employed to perform electrochemical testing, a 1-cm^2 platinum plate was used as the counter electrode, a saturated calomel electrode (SEC) was employed as the reference electrode, and the testing specimen with an exposure area of 1 cm^2 was used as the working electrode. Polarization curves were acquired by linear sweep voltammetry at a sweeping rate of 0.5 mV/s from -500 mV to 500 mV. Six specimens for each testing sample were examined to get an average data.

EIS was measured at an applied AC signal of 10 mV and the frequency ranging from 100 kHz to 0.01 Hz. The acquired data were fitted and analyzed using ZSimpWin software based on an equivalent circuit model. The equivalent circuit model was chosen based on time constants and fitting quality.

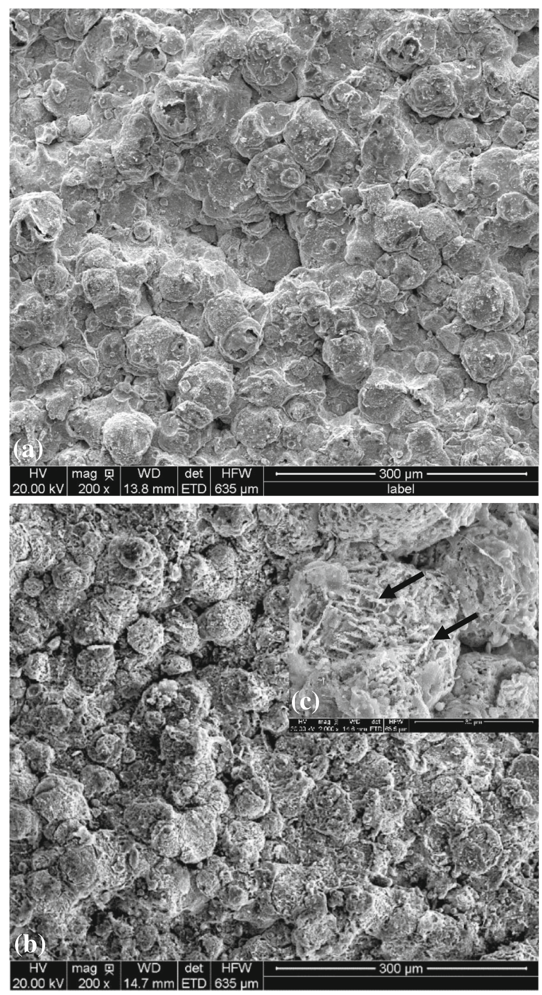

Fig. 2 Surface morphologies of the copper coatings immersed in ASW for 3 days (a) and 7 days (b, c)

Results and Discussion

Surface morphology of the cold-sprayed copper coatings after immersing in the ASW and the bacteria-containing ASW for 3 days and 7 days was characterized, which is shown in Fig. 2 and 3, respectively. Some loose floccules are found on the surface of the coating immersed in ASW (Fig. 2a), which is markedly different from the surface of the as sprayed coating (Fig. 1b). The loose floccule structural feature was enhanced as the immersion time was prolonged to 7 days (Fig. 2b), and enlarged floccules are seen (Fig. 2c). The floccule structures (marked by black arrows in Fig. 2c) on deformed particles were analyzed using EDS, which were identified as copper oxides (CuO and Cu_2O). The copper coatings were also incubated in the bacteria-containing ASW to reveal the influence of the bacterial settlement on anticorrosion performances of the coatings. Interestingly, it is noted that no loose corrosion products are found on the coatings immersed in the bacteria-containing ASW. Instead, some rod-like structures are clearly seen (marked by white arrows in Fig. 3a), which are identified as settled bacteria. It is well established that copper ions can restrain adhesion of bacteria. In this case, the antifouling efficacy of copper ions depends on the rate of their release from the coatings. The bacterial adhesion would be restrained once the release rate reaches a critical value. To investigate the influence of the bacterial adhesion on corrosion of the coatings, the typical marine bacterium *Bacillus* sp. was employed. The settlement of *Bacillus* sp. on the coating surface can be contributed to the protective effect of EPS. SEM observation suggests that the bacterial attachment may restrain the corrosion process in the marine environment. It can also be found that some bacteria settle on the low-lying spots (as marked by white arrows in Fig. 3a) on the coating surface in early stage of the incubation. EPS is clearly seen on the surfaces of the copper coatings immersed in the bacteria-containing ASW for 7 days (Fig. 3b). EPS was secreted by settled bacteria, which could protect the bacteria from attacking of copper ions and provided a suitable environment for the settlement of following bacteria. Quantitative analyses of the coverage of the bacteria and EPS on the coating surfaces show that the coverage increases from $2.86 \pm 0.87\%$ to $25.1 \pm 6.78\%$ as the immersion time of the coatings in the bacteria-containing ASW is prolonged from 3 to 7 days.

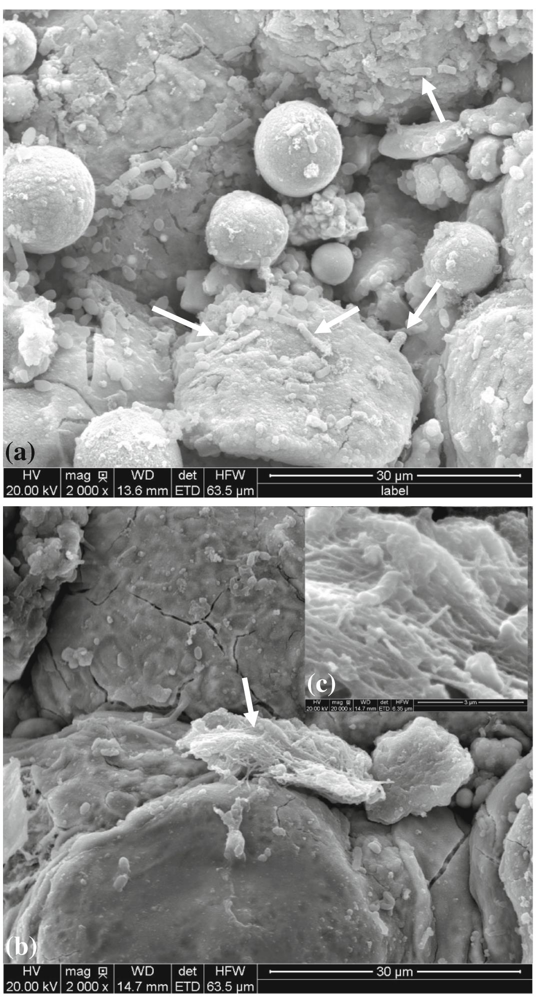

Fig. 3 Surface morphologies of the copper coatings immersed in the bacteria-containing ASW for 3 days (a) and 7 days (b, c). (the white arrow points to typical EPS)

Electrochemical testing of the coatings with/without settlement of the bacteria on their surfaces suggests pronounced influence of the colonized bacteria (Fig. 4). The electrochemical corrosion parameters such as corrosion potential (*E*) and corrosion current density (*I*) were obtained from the potentiodynamic polarization curves. Three samples were employed for the testing to obtain reliable data, and statistical significance of the values,

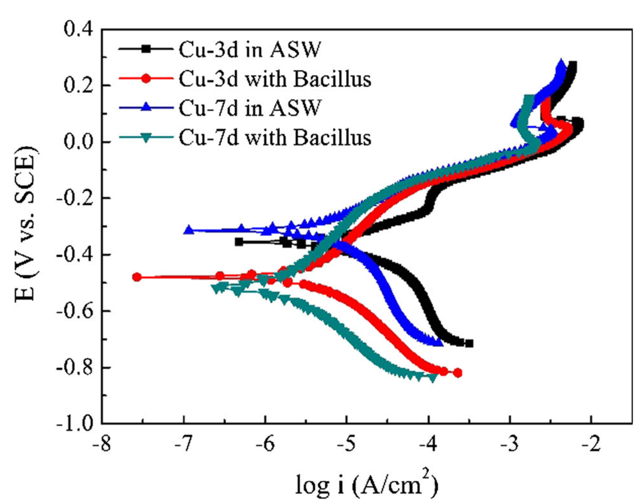

Fig. 4 Potentiodynamic polarization curves of the copper coatings

including the average values (E_{mean}, I_{mean}) and the relative standard deviations (E_{RSD}, I_{RSD}), is also provided in Table 1. It can be found that the corrosion current density of the coatings with colonized bacteria on their surfaces is 2.34 μA/cm^2, slightly lower than that of the coatings immersed in the bacteria-free solution for 3 days (2.46 μA/cm^2). A similar trend can also be found in the case of the copper coatings immersed for 7 days. The average corrosion current density of the coatings decreases from 2.38 to 2.21 μA/cm^2 as the bacteria-containing ASW was used. It could be considered that bacterial settlement slightly improves the corrosion resistance of the coatings, which could be partly due to the incontinuous nature of the biofilm formed by the colonized bacteria. It is anticipated that full coverage of colonized bacteria would give rise to significantly altered electrochemical performances of the coatings. In fact, our group has already realized that the presence of homogeneous bacterial biofilm on aluminum coating triggered occurrence of pitting corrosion (Ref 43). Anode polarization slope is also usually used to evaluate corrosion resistance of coatings. It is noted that anode polarization slope of the coatings with colonized is bigger than that of the coatings without bacteria settlement, suggesting that bacterial attachment alleviates the corrosion and enhances the corrosion resistance of the cold-sprayed copper coatings.

EIS was measured to disclose the influence of the bacterial adhesion on corrosion behavior of the copper coatings, and the results are shown in Fig. 5 and Table 2. It can be found that the impedance acquired from Nyquist plots of the coatings immersed in the bacteria-containing ASW is higher than that obtained from the coatings immersed in the bacteria-free ASW (Fig. 5a). This suggests that the adhesion of the bacteria gives rise to enhanced corrosion resistance of the copper coatings. Bode plots (Fig. 5b and c) show that the impedance of the copper coatings decreases as the frequency increases, and the copper coatings immersed in the bacteria-containing ASW show much higher total impedance magnitude than those immersed in the bacteria-free ASW at a low frequency region. Apart from the phenomenon that the colonization of *Bacillus* sp. restrains the corrosion of the copper coatings, the bacterial adhesion also presumably brings about other impacts, for example, increase in drag force, rise in fuel consumption and the augmentation of navigation noise.

An equivalent circuit model is proposed to illustrate the corrosion regime of the bacteria-settled copper coatings in the marine environment, in which the contributions of electrical double layer and biofilm formation are both taken into account (Ref 25). In this case, two-time constant equivalent circuit (Fig. 6) was used to fit the electrochemical data, and the second time constant is related to the bacteria/electrode interface. Equivalent circuit model consists of three parts: (1) solution resistance R_s; (2) parallel combination of bacteria/electrode interface capacitance Q_{coat} (constant phase element, CPE) and resistance R_{coat}; (3) parallel combination of double layer capacitance Q_{dl} and charge-transfer resistance R_{ct}. Here, Q_{coat} and R_{coat} refer to the capacitance and resistance of the cold-sprayed coatings in bacteria-free ASW and bacteria-containing ASW. Q_{dl} and R_{ct} represent the interfacial capacitance and resistance between the coatings and the substrates immersed in bacteria-free ASW or bacteria-containing ASW. Taking into account the electrochemical impedance parameters of the copper coatings listed in Table 2, R_{coat} increases as the exposure time of the copper coatings in the solution was prolonged to 7 days. This might be explained by formation of oxides layer on their surfaces. In the bacteria-containing ASW, R_{coat} drops as the coatings were immersed for 3 days and then increases as the exposure time was prolonged to 7 days. The changes may be due to inhibited formation of the oxides layer in an early stage of incubation in the bacteria-containing solution, and as a consequence, ion diffusion was blocked after EPS formation. Dispersive exponent n is a parameter with the value ranging from 0 to 1, and directly related to the dispersive behavior. When n approaches 1, the value of CPE impedance tends to the conventional capacitor (Ref 44). Exponent n_2 decreases as the immersion time of the coatings was prolonged. It is reported that bacteria settlement would result in increase in surface roughness (Ref 3), which can be used to explain the decrease in n_2. Charge-transfer resistance R_{ct} also increases for the copper coatings immersed in the bacteria-containing ASW for 3 and 7 days, which can also prove the restrain effect of bacteria settlement on the corrosion behavior of the cold-sprayed

Table 1 Polarization parameters of the copper coatings

Sample	E_{mean} (V versus SCE)	E_{RSD}	I_{mean}, μA/cm^2	I_{RSD}
After 3 days				
Cu coatings in sterile ASW	−0.36	7.76×10^{-3}	2.46	0.21
Cu coatings in bacteria-containing ASW	−0.45	2.05×10^{-3}	2.34	0.24
After 7 days				
Cu coatings in sterile ASW	−0.34	1.84×10^{-2}	2.38	0.19
Cu coatings in bacteria-containing ASW	−0.45	7.91×10^{-2}	2.21	0.13

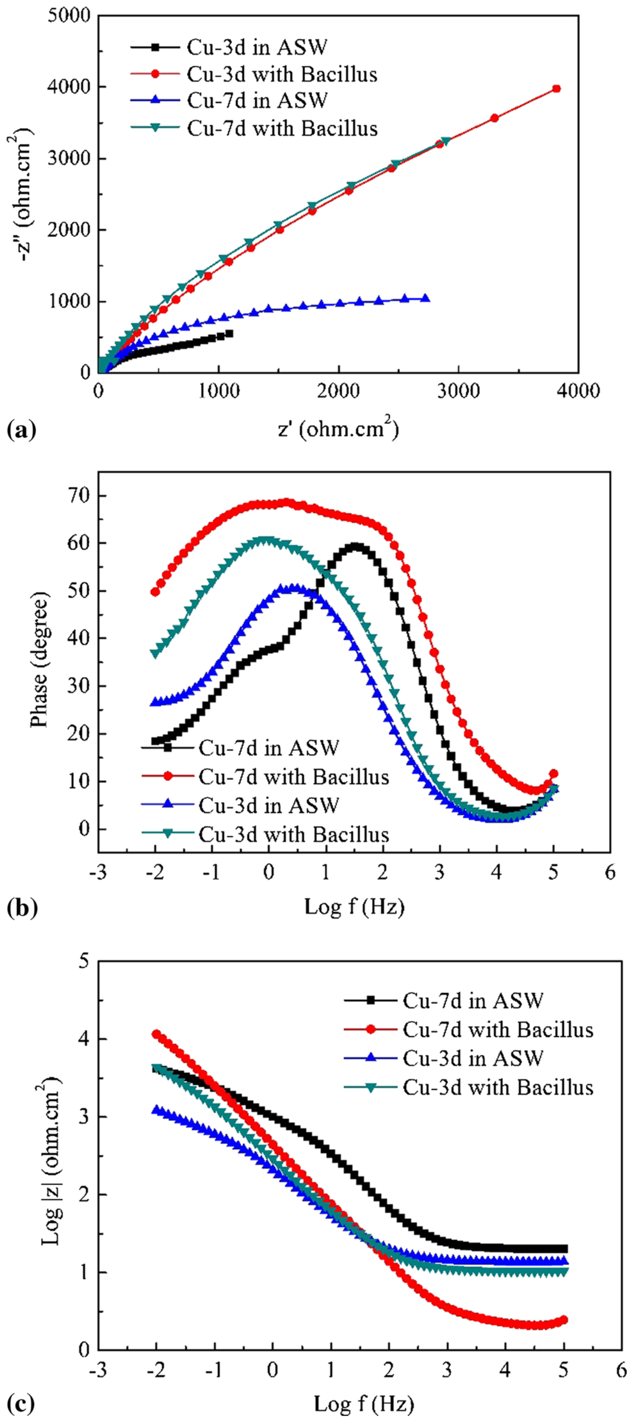

Fig. 5 Nyquist plots (a) and bode plots (b, c) of the copper coatings

coatings. For bulk metals, biofilm usually plays an important role on hindering the corrosion process. Jayaraman et al. (Ref 45-47) demonstrated that protective biofilms reduced the corrosion rate of mild steel, apparently by reducing the oxygen concentration at the metal surface. Due to the porous structure of the cold-sprayed coatings, the bacterial biofilm also plays other roles. Ding et al. (Ref 48) claimed that the corrosion of cold-sprayed copper coatings in the marine environment involved:

$$Cu \Leftrightarrow Cu^{+} + e^{-} \quad \text{(Eq 1)}$$

$$Cu^{+} + 2Cl^{-} \Leftrightarrow CuCl_2^{-} \quad \text{(Eq 2)}$$

It is known that chloridion transmission is important for the corrosion of the coatings in the marine environment. As observed in Fig. 3, EPS could be easily formed on the surfaces of the coatings. EPS could on one hand restrain the transmission of chloridion into the inner of the coatings, and on the other hand limit transmission of corrosion product ($CuCl_2$) into the marine environment. Moreover, the complexation effect between EPS and cupric ions can also contribute to the limitation effect of the biofilm to corrosion of the coatings (Ref 49, 50), which could decrease copper ion concentration in the vicinity of the coating surfaces. Accordingly, more and more bacteria survive and settle on the coatings. Based on the above discussion, an attachment model is proposed to illustrate the settlement process of *Bacillus* sp. on the surface of the cold-sprayed copper coating (Fig. 7). *Bacillus* sp. prefers to settle on the low-lying spots of the coating surfaces in early stage of incubation, followed by EPS secretion through metabolism of *Bacillus* sp.. Finally, subsequent *Bacillus* sp. settles readily on the coating surface with the protection of EPS.

Conclusions

Cold-sprayed copper coatings were employed to investigate the influence of settlement of *Bacillus* sp. bacteria on their corrosion behaviors in artificial seawater. Surface morphology and electrochemical behaviors of the coatings with/without attachment of the bacteria on their surfaces were examined. The bacteria can easily settle on the copper coatings with the protection of extracellular polymeric substances secreted by the bacteria attached on the low-lying spots in early stage of incubation. Electrochemical testing reveals that the colonization of the bacteria restrains the corrosion and improves the corrosion resistance of the cold-sprayed copper coatings. The results shed some light on future in-depth investigation of biocorrosion of cold-sprayed coatings in the marine environment.

Table 2 Electrochemical impedance parameters of the copper coatings

Sample	R_s, Ω cm^2	R_{ct}, Ω cm^2	R_{coat}, Ω cm^2	Q_{CT}, μF cm^{-2}	n_1	Q_{coat}, μF cm^{-2}	n_2	*Chi-square*
Sterile ASW-Cu coatings								
After 3 days	4.56	2.45×10^3	26.56	5.62	0.82	94.01	0.43	9.03×10^{-3}
After 7 days	12.06	5.38×10^3	64.09	271.00	0.81	491.20	0.39	1.21×10^{-3}
Bacteria-containing ASW-Cu coatings								
After 3 days	2.07	3.32×10^3	6.30	18.20	1.00	538.10	0.75	1.18×10^{-3}
After 7 days	10.46	6.69×10^3	535.20	52.45	1.00	2229.00	0.63	0.97×10^{-3}

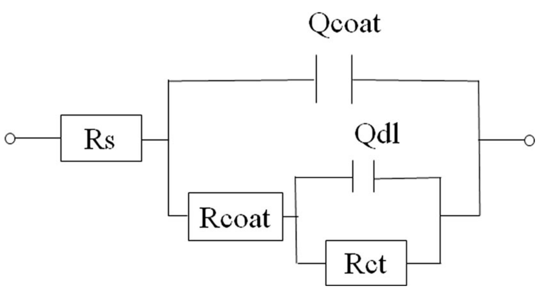

Fig. 6 Equivalent circuit model for the copper coatings

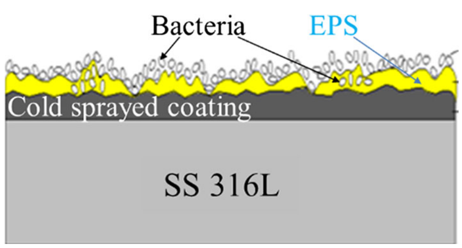

Fig. 7 Schematic depiction illustrating the bacterial adhesion on cold-sprayed copper coatings

Acknowledgments This research was supported by Project of Scientific Innovation Team of Ningbo (Grant # 2015B11050), National Natural Science Foundation of China (Grant # 31500772 and 41476064), State Key Laboratory for Mechanical Behavior of Materials (Grant # 20151710), and State Key Laboratory for Marine Corrosion and Protection of Luoyang Ship Material Research Institute (LSMRI) (Grant # KF160409).

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