Int. J. Electrochem. Sci., 14 **(2019)** 6468 – 6477, doi: 10.20964/2019.07.19

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Short Communication

Influence of *Pseudomonas aeruginosa* and Sulfate-reducing bacteria composite on the corrosion behavior of brass

Xiaodong Zhao[1,2], *Chaoming Yan*[2], *Jing Shao*[2], *Jie Yang*[1,*], *Jie Liu*[1], *Dongyu Sun*[1], *Shuai Wang*[1]

[1] School of Ocean, Yantai University, Yantai 264005, China;
[2] School of Naval Architecture and Mechanical-electrical Engineering, Zhejiang Ocean University, Zhoushan 316022, China
[*]E-mail: kittyangj@163.com

Received: 11 March 2019 / *Accepted:* 22 April 2019 / *Published:* 10 June 2019

In this paper, electrochemical methods such as open circuit potential(OCP) and electrochemical impedance spectroscopy(EIS) were used to assess the corrosion behavior of brass in sterile water and media containing *Pseudomonas Aeruginosa*, sulfate-reducing bacteria and their bacteria composite, respectively. The results showed that corrosion occurred to some extent in all the media. The corrosion rate of brass in the *Pseudomonas Aeruginosa* system is lower than that in the sulfate-reducing bacteria system. Especially in single *Pseudomonas Aeruginosa* system, the open circuit potential and total impedance value were comparatively higher than those in the other systems. In addition, the acceleration of corrosion in the composite system began from the 3rd day, later than that in the single sulfate-reducing bacteria system, indicating that the presence of *Pseudomonas Aeruginosa* might inhibit the activity of sulfate-reducing bacteria.

Keywords: Microbiologically influenced corrosion (MIC); Sulfate-reducing bacteria; *Pseudomonas aeruginosa*; Electrochemical impedance spectroscopy (EIS)

1. INTRODUCTION

In recent years, with the development and utilization of marine resources, the corrosion induced by microorganisms to marine engineering has increasingly attracted researchers' attention and become an urgent problem to be solved[1-2]. Microbiologically influenced corrosion(MIC) mainly refers to the corrosion behavior or failure of engineering material caused directly or indirectly by microorganisms in the marine environment such as seawater or sea mud. Up to now, most of the reports have focused on the corrosion induced by anaerobic bacteria, represented by sulfate-reducing bacteria (SRB)[3-6], while the research on aerobic bacteria has been less involved[7-8]. Moreover, most research work in MIC field are based on the culture of a single strain and carried out under bacterial and sterile conditions. The coexistence is especially prominent at oxic/anoxic interfaces because of the common competition among different bacterial species over organic carbon and nutrients. Obviously, these work cannot simulate the synergetic effect of multiple species on material corrosion in natural environment.

Cu and Cu alloys are widely used in marine engineering equipment due to their good thermal conductivity and corrosion resistance[9,10]. Cu alloys exist in the seawater with the ionization balance as:

$$Cu \leftrightarrow Cu^{2+} + 2e^{-} \qquad (1)$$

Cu^{2+} has a certain toxicity effect on the microbes, and it is difficult for microbes to survive in Cu^{2+} solution for a long time[11]. Therefore, the report related to Cu corrosion is also less. However, there are a wide variety of microbial species with resistance to Cu^{2+} in consortium in natural environment, including *Pseudomonas aeruginosa* (PAO-1, in short below) and SRB. SRB are anaerobes, while PAO-1 are facultative microorganisms capable of thriving in both anaerobic and aerobic conditions. Wagner[12] pointed out that H_2S produced by SRB might cause corrosion of Cu alloys. Chen[13] found that the metabolism of SRB reduced the anode area and promoted the localized corrosion of Cu alloys with CuS as the main corrosion product. Booth[14] showed that SRB caused corrosion to Cu and Cu alloys on the sulphate ore, resulting in a thick layer of copper sulfide with no adhesion or hexagonal copper sulfide. In addition, Yuan[15] found that the presence of PAO-1 induced the corrosion of 70/30 Cu/Ni alloys and San[16] reported that PAO-1 accelerated the failure of Ni-Cu coating.

In this study, the influence of bacteria composite of PAO-1 and SRB on the corrosion behavior of brass was studied in order to introduce the concept of microbial diversity to MIC study more deeply and gain a better understanding of MIC.

2. EXPERIMENTAL SECTION

2.1 Material

The working electrodes used in this work were cut from a brass plate, containing (wt.%) Cu 61.43, Zn 37.63 and impurity balance. To prepare the samples for electrochemical testing, the samples were sealed with epoxy resin and poly-tetra-fluoroethylene, leaving an exposure surface of 10 mm^2. The surfaces of working electrode were abraded with a series of silicon carbide papers (up to 1500#), then rinsed with distilled water, sterilized with anhydrous ethanol, degreased with acetone, and dried for use.

2.2 Bacteria and culture

The SRB and the PAO-1 in this work were provided by Institute of Oceanology, Chinese Academy of Sciences. A modified Postgate's C medium which contained 0.5g KH_2PO_4, 1.0g NH_4Cl, 0.5g Na_2SO_4, 0.1g $CaCl_2$, 2.0g $MgSO_4$, 6ml $C_3H_5NaO_3$, 1.0g yeast extract, 0.3g $Na_3C_6H_5O_7 \cdot 2H_2O$ in 1 L sea water, was used for enrichment culture of SRB. As for PAO-1 enrichment culture, the medium contained 10g peptone, 5g yeast extract, 10g NaCl in 1L distilled water was used. The pH value was adjusted to 7.0~7.2. The prepared medium was sterilized in an autoclave at 121°C for about 20 min. The bacteria-containing medium was infused into the sterile medium with a volume ratio of 1:10, serving as single bacteria medium. Equal volume of SRB and PAO-1 single bacteria media were mixed, serving as a composite system.

2.3 Electrochemical measurement

Open circuit potential (OCP) and electrochemical impedance spectroscopy (EIS) measurement were carried out using an EG&G Parstat 2273 electrochemical system. All tests were performed in a classical three-electrode cell, where the brass electrode was used as working electrode (WE), a saturated calomel electrode (SCE) as reference electrode (RE) and a Pt wire (0.5mm in diameter) as counter electrode (CE). After each OCP test, the EIS was measured at a steady state of corrosion potential with a potential disturbance of 10 mV and frequency ranges from 0.01 Hz to 100 kHz.

3. RESULTS AND DISCUSSION

3.1 OCP measurement

Fig. 1 shows a diagram of the OCP of brass in the four systems as a function of time. The four systems are sterilized system, single PAO-1 system, single SRB system, and the composite system of PAO-1 and SRB, respectively.

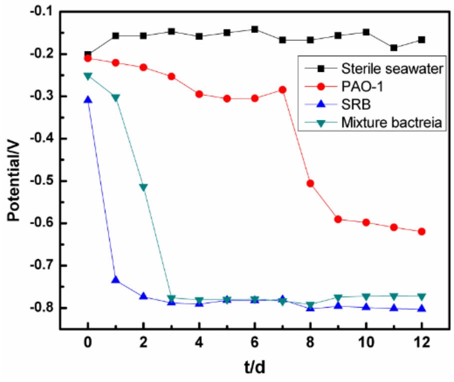


Figure 1. OCP of brass in the four systems over time

It is shown in Fig. 1 that the OCP of brass in the sterilized system changed little and shifted about 0.04 V positively after 12d than that of the beginning. It suggested that brass was stable in sterilized system and there was no obvious corrosion. With the participation of bacteria, the OCP of brass showed a significant negative shift. In the single PAO-1 system, the potential was relatively stable before 7d due to approximate balance of the corrosion tendency by the metabolism of bacteria with the corrosion resistance of biofilm formed on the surface of the sample. Then the potential quickly shifted negatively to about -0.62 V after 7~12 d. The reason lay in that with the consumption of dissolved oxygen, the corrosion process was accelerated for oxygen concentration difference. At the same time, the accumulation of alkali and amino-containing ferric carriers produced by PAO-1 also caused localized corrosion[17].

In the single SRB system, the corrosion potential shifted negatively in 2 days, and then tended to be stable. The following reaction was expected to occur in the early stage, which accelerated the corrosion process.

$$2Cu + S^{2-} + 2H_2O = Cu_2S + 2OH^{-} + H_2O \quad (2)$$

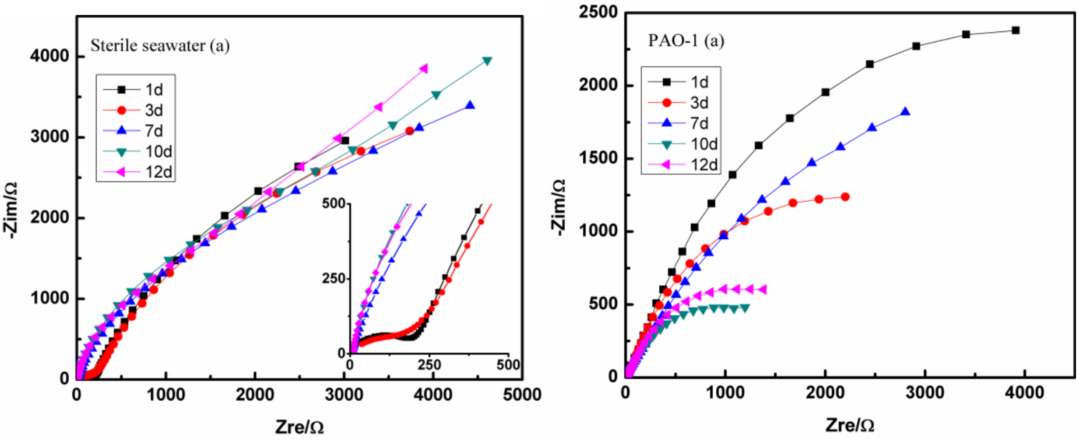
With the adhesion of corrosion products and extracellular polymer generated by bacterial metabolism to the sample surface, a protective film was formed, reducing the corrosion effect and the potential gradually stabilized.

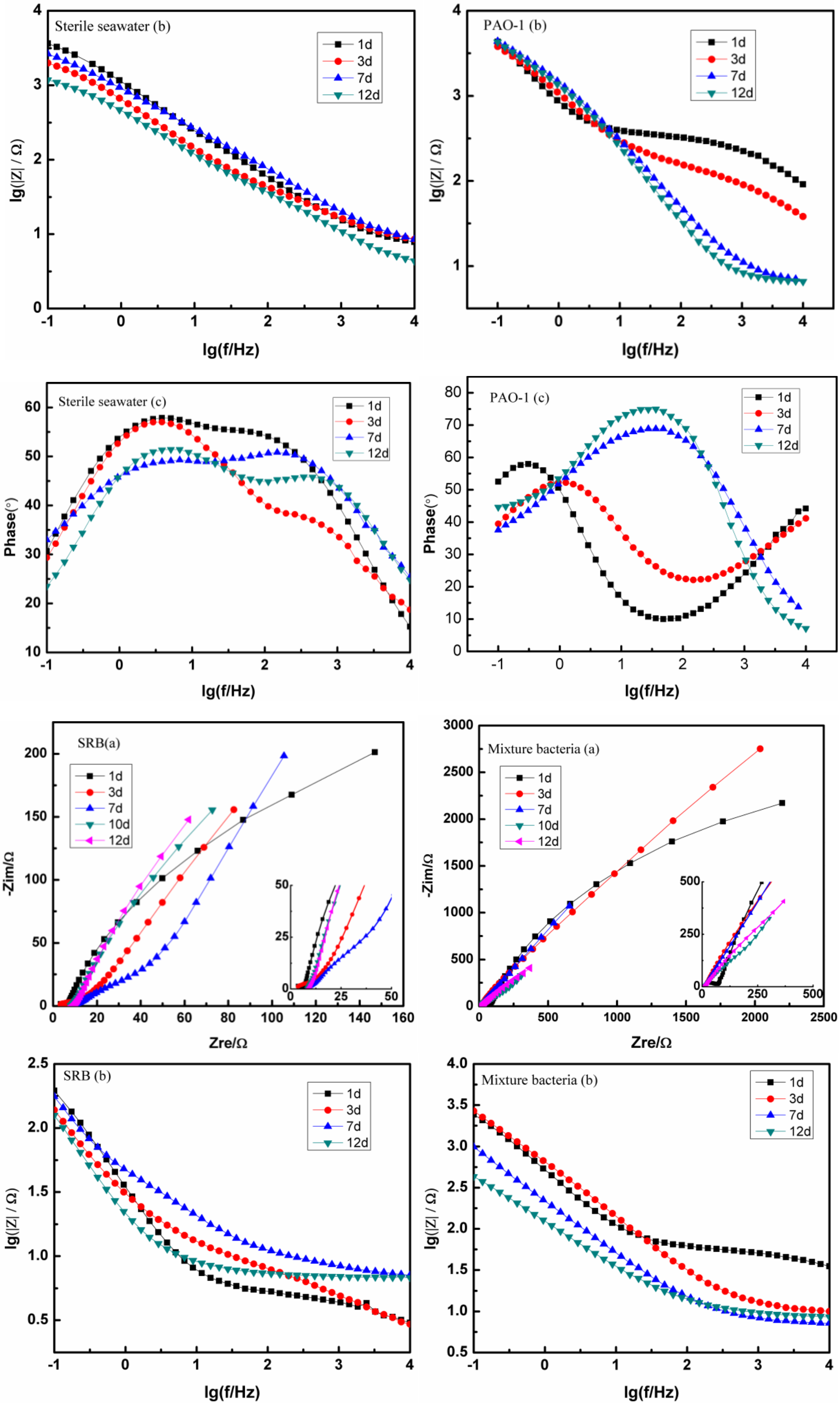
In the composite system, the potential negatively shifted significantly on the 1st day and the 3rd day, resulting from an anaerobic condition caused by PAO-1 and production of S^{2-} by SRB leading to corrosion acceleration separately. Three days later, the potential gradually stabilized at about -0.75V because the biofilm and corrosion products formed on the surface changed little.

The OCP value of the brass in all three bacteria-containing media were smaller than that in sterile system, indicating that the corrosion resistance of the brass in the bacteria media was lower than that in the sterile solution. Meanwhile the potential in the composite system was lower than that in the single PAO-1 system and slightly higher than that in the single SRB system. In addition, the time when significant negative shift occurred was also between that in two single bacteria systems, indicating that the synergistic effect of the two bacteria accelerated the corrosion process of brass, and SRB dominated the corrosion behavior.

3.2 EIS measurement

The EIS measurement had little disturbance on the testing system which was expected to accurately quantify the metal corrosion process[18]. Fig. 2 shows the Nyquist and Bode diagrams measured on the surface of brass immersed in four media. It can be seen from Nyquist diagram that the capacitive arc radius in a sterile seawater system changed little at the same level of magnitude, which indicated that the corrosion rate of samples in the medium was relatively stable. In the composite medium, the capacitive arc radius was obviously smaller than that in the single strain system. After 1d, the emergence of the Warburg impedance meant that when the AC signal passed through the electrodes, not only the electrochemical polarization but also the concentration difference polarization occurred. This was due to the gradual loss of the PAO-1 biofilm on the surface of the samples and the subsequent adhesion of SRB in the later stage. The difference between the solution and the reactant concentration was also part of the reasons. As shown in Fig. 2, in a single SRB system, the capacitive arc radius varied greatly in 1~7 d, and the Warburg impedance also emerged in the later stage.




Sterile seawater (b)
1d
3d
7d
12d
lg(|Z| / Ω)
lg(f/Hz)
PAO-1 (b)
1d
3d
7d
12d
lg(|Z| / Ω)
lg(f/Hz)
Sterile seawater (c)
1d
3d
7d
12d
Phase(°)
lg(f/Hz)
PAO-1 (c)
1d
3d
7d
12d
Phase(°)
lg(f/Hz)
SRB(a)
1d
3d
7d
10d
12d
-Zim/Ω
Zre/Ω
Mixture bacteria (a)
1d
3d
7d
10d
12d
-Zim/Ω
Zre/Ω
SRB (b)
1d
3d
7d
12d
lg(|Z| / Ω)
lg(f/Hz)
Mixture bacteria (b)
1d
3d
7d
12d
lg(|Z| / Ω)
lg(f/Hz)

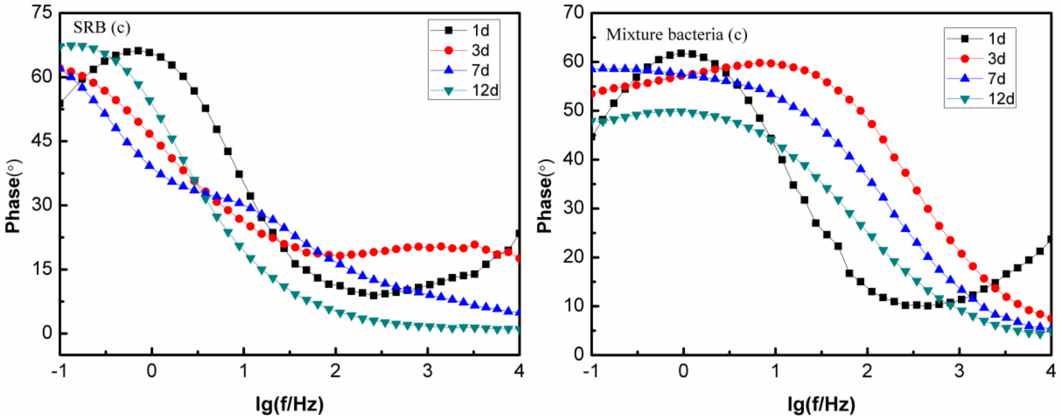


Figure 2. Nyquist and Bode diagrams of brass immersed in four media(a represents the Nyquist diagram, b represents the magnitude diagram, and c represents the phase-angle diagram)

Fig. 2 shows the Bode diagrams measured on the surface of brass immersed in four media as a function of time. From the amplitude diagram, it is seen the slope of the 1st day in the sterile seawater system and that of the 7th day, 12th day in the single SRB system at low and medium frequencies were close to -1, indicating that the dispersion effect was little at this time while relatively large in other cases. It might be related to the roughness of the electrode surface and the structural looseness, or the composition of electrolyte[19].

From the phase angle diagram, it is found that there were two obvious peaks in the sterile seawater system, showing two layers of dense protective films were formed on the surface of the electrode. On the 3rd and 7th day in the single SRB system, in addition to the obvious peaks in low-frequency regions, smaller peaks also appear at the mid-high frequencies, indicating that the protective film formed on the electrode surface in early stage was loose. Both the composite system and the single PAO-1 system showed peaks at the intermediate frequency and the similar impedance characteristics. The difference was that in the composite system there were also peaks at low frequency due to the formation of two different biofilms on the sample surface. For environmental advantage and growth characteristics, an aerobic biofilm was formed by PAO-1 at first, then with the oxygen consumption in the solution, the proliferation of anaerobic SRB resulted in the formation of SRB biofilms on the surface of the sample.

The equivalent circuit models in Fig. 3 are used to fit the EIS experimental data for brass immersed in four media in order to show the change of impedance more clearly, and the fitting parameters derived are shown in table 1. The R(Q(R(QR)) model shown in Fig. 3(a) is adoptable for sterile seawater system and single PAO-1 system, while the R(QR)(Q(RW)) model shown in Fig. 3(b) is adoptable for single SRB system and composite system. The two models consist of solution resistance(R_s), the film resistance(R_b), charge transfer resistance(R_{ct}), the Warburg impedance(W), film capacitance(Q_b), and electrical double layer capacitance(Q_{dl}).

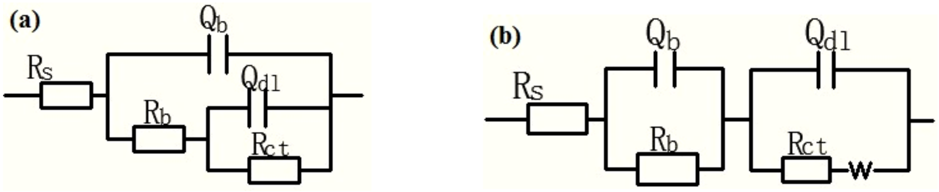


Figure 3. The equivalent circuit models used to fit the EIS experimental data for brass immersed in (a) sterile seawater system and single PAO-1 system, (b) single SRB system and composite system

Table 1. Parameter fitting values of each element in the equivalent circuit

Medium	t d	R_s $\Omega\cdot cm^2$	$Q_b\times10^{-4}$ （uF/cm²） Hz^{1-n_1}	n_1	R_b $k\Omega\cdot cm^2$	$Q_{dl}\times10^{-4}$ （uF/cm²） Hz^{1-n_2}	n_2	R_{ct} $k\Omega\cdot cm^2$	$W\times10^{-4}$ （uF/cm²） Hz^{1-n_2}
Sterile	1	6.50	2.02	0.70	1002	24.27	0.91	4978	-
	3	6.57	2.71	0.65	82.64	1.20	0.82	3109	-
	7	5.80	7.49	0.90	1.66	3.52	0.55	7108	-
	12	2.96	2.57	0.69	77.57	2.85	0.70	1532	-
PAO-1	1	0	0.10	0.62	363.5	2.98	0.84	1282	-
	3	0	0.67	0.53	195	2.02	0.74	9133	-
	7	6.48	1.12	0.81	2799	3.01	0.74	9935	-
	12	3.60	0.91	0.90	1782	2.99	0.61	2624	-
SRB	1	0	10.02	0.47	5.55	60.11	0.87	381.2	131.2
	3	0.03	113.0	0.77	1242	570.2	0.10	2.42	11.3
	7	6.41	806.3	0.55	6.33	165.0	0.93	190.8	70.1
	12	6.76	491.2	0.50	8.44	106.8	0.96	170.7	41.2
Mixed bacteria	1	0	0.36	0.55	59.03	4.35	0.81	4474	25.81
	3	5.05	872.6	0.08	0	2.90	0.77	903.3	2.70
	7	3.279	0.01	0.78	3.71	13.15	0.67	1755	0.93
	12	0	0.10	0.38	8.77	25.30	0.62	1217	13.58

Two time constants showed up in the sterile seawater medium due to the oxide film on the surface itself and a protective film formed with the attachment of the material in the solution. The R_{ct} value decreased from 4978 to 3109 $\Omega\cdot cm^2$, and then greatly increased to 7108 $\Omega\cdot cm^2$ after 7d. It revealed that the oxide film on the electrode surface recovered after damaged to a certain extent, and the film resistance increased. At the same time, the Q_{dl} value increased from 2.427E-5 $\Omega^{-1}\cdot cm^{-2}$ to 3.52E-4 $\Omega^{-1}\cdot cm^{-2}$, followed by a slight decrease. The n_2 value underwent a process of decrease first and then increase, indicating that densification of protective film on the electrode surface had deteriorated in its early stage and improved after 7d.

In the presence of bacteria, in a single PAO-1 system, the oxide film on the surface of the electrode suppressed the corrosion, and the adhesion of PAO-1 also had a certain effect of corrosion resistance. Then the ammonia produced by the metabolism of PAO-1 accelerated the corrosion of brass. Correspondingly, the R_{ct} values increased greatly within 3d, and R_b, R_{ct} value decreased from 7 to 12d, proving the corrosion was accelerated after 7 d. In a single SRB system, the n_2 value decreased to 0.0986 from 1d to 3d, at the same time the Q_{dl} value rose to about 0.95, indicating that the surface roughness and corrosion current density of the electrode were relatively uniform. The R_{ct} value increased to 190 $\Omega\cdot cm^2$ after 3 d, indicating that the resistance in the circuit increased and the corrosion slowed down and proving the voltage variation in the self-corrosion potential. Different from the single SRB system, the composite system changed more obviously in 1~3d. The R_b value decreased by a 10^7 times magnitude. Although the R_{ct} value increased, the overall corrosion rate of the sample increased significantly. At this time, PAO-1 entered its recession period, and the protective film on the electrode surface began to fall off. The S^{2-} reduced by SRB promoted the corrosion. After 3d, the R_{ct} value increased from 903.3 to 1217 $\Omega\cdot cm^2$, revealing that the corrosion resistance of samples in the later stage was enhanced. Q_b represented the tendency of adsorption equilibrium. The increase of the value indicated the formation of the biofilm and the decrease showed the weakening biofilm[20]. It is seen that Q_b varied within different orders of magnitude, indicating the microbiological activities on the surface of the electrode were more complicated in the composite system.

3.3 Discussion

Schiffrin[21] found that MIC of Cu alloys could be attributed to the long-term modification of the oxide layer to a sulfide-rich coating, i.e. sulfides such as CuS, ZnS generated on the surface of the alloys. SRB reduce SO_4^{2-} in the medium to S^{2-}, which induces the corrosion of brass. As shown in Fig. 1, after the brass immersed in the SRB solution for 1d, the open circuit potential quickly shifted negatively, indicating that the S^{2-} began to participate in the reaction and the corrosion tendency increased. The fact that values of R_s, R_b, R_{ct}, and W in the single SRB system shown in table 1 were significantly smaller after 1d and it was easier for the corrosion reaction also proved the above viewpoint. In addition, Shalaby[22] added 1 ppm inorganic sulphides to the SRB culture. By potential dynamics observation and morphological studies, the Cu alloys were subjected to more severe corrosion.

Seen from Fig. 2, diffusion resistance occurred in the presence of sulfate-reducing bacteria, allowing the electrode reaction to be controlled by the charge transfer and the diffusion process together, and electrochemical polarization and concentration polarization co-existed. This was shown in the bode plots as the continuous existence of two time constants. The impedance in the high frequency region increased over the immerse time, resulting from the corrosion product layer. Little[23] also obtained similar impedance response to Cu and Cu alloys in his work and attributed it to surface layer formation and the layer-mediated charge transfer and mass transfer controlled reactions.

Since the formation of biofilm is due to the colonization of bacteria by strong adhesion to the surface and proliferation, attachment of bacteria to metal substrate is an important variable to consider. The DLVO (named after Boris Derjaguin and Lev Landau, Evert Verwey and Theodoor Overbeek) stability theory of colloids proposed by Lyklema[24] was often used to explain the bacterial adhesion. However, in the interactive environment involving more than one bacteria, this theory did not give a specific explanation. Since the initial adhesion of microorganisms is decisive for the adhesion strength of the entire biofilm, it is also related to the physical and chemical properties of the initial surface of the bacteria and that of the substrate.

From the experimental results we found that by adding PAO-1, the corrosion of brass influenced by SRB was inhibited to some extent. This might due to the adhesion and proliferation of PAO-1, accompanied by the production of certain extracellular polymeric substance(EPS) and exotoxin, changed the chemical properties of the substrate surface, and negatively affected the activity of SRB. In addition, in the later stage of the experiment, PAO-1 might use peptone (organic carbon and nitrogen source) as an electronic donor to use oxygen in NO_3^- for anaerobic respiration. The total reaction process can be expressed as:

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O \tag{3}$$

During the process, the bacteria reduced NO_3^- to NO_2 first. Kaster[25] pointed out that nitrite had high reactivity with isomerous sulfate reductase and reduced to nitrogen gradually, thereby accelerating the death of SRB.

Some researchers suggested that enzymes in bacterial extracellular polymers might be responsible for the increase of cathodic current[26]. Catalase is proposed to be the most commonly bacterial metabolites with catalytic activity, which can be produced by both SRB and PAO-1. Busalmen[27] showed the first increase of the cathode current during using the cell-free culture of PAO-1, and described the coupling mechanism of electrochemical oxygen reduction and enzyme activity. The role of catalase depends on the generation of H_2O_2 in the electrochemical process and the accumulation at the interface. Pyocyanin produced by PAO-1 can penetrate the cells and induce the production of hydrogen peroxide[28].

In the early stage of the experiment, PAO-1 produced catalase by its metabolism as follows:

$$O_{2(sol)} \xrightarrow{K_d} O_{2(ads)} \square \quad H_2O_{2(ads)} \rightarrow OH^- \tag{4}$$

Busalmen[29] found that the presence of catalase increased the current density by 35% and suggested that catalase decomposed the surface peroxides and resulted in the increase of oxygen. This change was little while had practical significance on the study of enzymes.

4. CONCLUSION

Microbiologically influenced corrosion is generally associated with a variety of mechanisms. Biofilm plays a crucial role in controlling the corrosion of material. On one hand, biofilms block the corrosive substances in the medium. On the other hand, some extracellular polymers are also corrosive to the substrate. In addition, biofilms may also interact with the original protective film of the material and influence on the corrosion.

In this study, compared with the sterile environment, both PAO-1 and SRB promoted the corrosion process of brass. Brass had the highest total impedance in a single PAO-1 system and relatively good corrosion resistance. The corrosion of brass in the composite system was similar to that in the single SRB system after immersed for 3 days. The potential shifted negatively to -0.75 V in the composite system after about 3 days, later than that in the single SRB system. And the potentials of the composite system were comparatively higher than those of the single SRB, indicating that PAO-1 inhibited the growth of SRB in a certain period of time.

ACKNOWLEDGMENTS
This research was supported by Foundation of Key Laboratory of Marine Materials and Related Technologies, Ningbo Institute of Materials Technology and Engineering, Chinese Academy of Sciences (Grant No.2017Z01). The support from Scientific Research Foundation of Yantai University (Grant No. HX17B38) and Equipment Development Department of Central Military Commission and China Shipbuilding Industry Corporation unite foundation (No.6141B04080206) is also gratefully acknowledged.

References

1. T.L. Skovhus, R.B. Eckert and E. Rodrigues, *J. Biotechnol.*, 256(2017)31.
2. M. Setareh and R. Javaherdashti, *Mater. Corros.*, 54(2015)259.
3. X. Zhao, J. Duan and B. Hou, *Can. Metall. Quart.*, 53(2014)450.
4. D. Sun, W. Ming and X. Fei, *Mat. Sci. Eng. A*, (2018)721.
5. H. Li and D. Xu, *Plos One*, 10(2015)e0136183
6. Y. Li, D. Xu and C. Chen, *J. Mater. Sci. Technol.*, 34(2018)3.
7. A. Abdolahi, E. Hamzah and Z. Ibrahim, *Brit. Corros. J.*, 50(2015)538.
8. R. Jia, D. Yang and D. Xu, *Sci. Rep.-UK*, 7(2017)6946.
9. G. Liu, J. Qian and M. A. Yan, *Electrochemistry*, 8(2002)191.
10. M. B. McNeil, J. M. Jones and B. J. Little, *Corrosion*, 47(1991)674.
11. W. P. Iverson, *Adv. Appl. microbiol.*, 32(1987)1.
12. P. Wagner and B. Little, *Mater. Performance*, 32(1993)65.
13. S. Chen, P. Wang and D. Zhang, *Corros. Sci.*, 87(2014)407.
14. G. H. Booth and A. K. Tiller, *Corros. Sci.*, 8(1968)583.
15. S. J. Yuan, A. M. F. Choong and S. O. Pehkonen, *Corros. Sci.*, 49(2007)4352.
16. N. O. San, H. Nazır and G. Dönmez, *Corros. Sci.*, 79(2014)177.
17. K. Chai, Q. Luo and J. Wu, *J. Chin. Soc. Corros. prot.*, 33(2013)481.
18. F. Mansfeld, *J. Appl. Electrochem.*, 25(1995)187.
19. B. Y. Cui, Z. B. Zhang and S. Z. Huo, *Corros. Sci. Prot. Technol.*, 6(1994)123.
20. S. Chongdar, G. Gunasekaran and P. Kumar, *Electrochim. Acta*, 50(2005)4655.
21. D. J. Schiffrin and S. R. D. Sanchez, *Corrosion*, 41(1985)31.
22. H. M. Shalaby, A. A. Hasan and F. Al-Sabti, *Brit. Corros. J.*, 34(1999)292.
23. B. J. Little and F. B. Mansfeld, *Mater. Corros.*, 42(1991)331.
24. W. Norde and J. Lyklema, *Colloid. Surface*, 38(1989)1.
25. K. M. Kaster, A. Grigoriyan and G. Jenneman, *Appl. Microbiol. Biot.*, 75(2007)195.
26. V. Scotto and M. E. Lai, *Corros. Sci.*, 40(1998)1007.
27. J. P. Busalmen, M. A. Frontini and S. R. D. Sanchez. *Developments in marine corrosion*, (1998)119.
28. G. Davis and P. J. Thornalley, *BBA-Bioenergetics*, 724(1983)456.
29. J. P. Busalmen, M. Vazquez and S. R. D. Sanchez, *Electrochim. Acta*, 47(2002)1857.