Synergistic Effect Between Sulfate-reducing Bacteria and *Pseudomonas Aeruginosa* on Corrosion Behavior of Q235 Steel

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Microbial community in nature is a whole in which the multiple species interact and restrict each other, leading to the metal corrosion as a synergistic result of microorganisms. In this paper, the corrosion behavior of Q235 steel in the culture media containing pure sulfate-reducing bacteria, *Pseudomonas aeruginosa* and their mixed cultures were analyzed by electrochemical methods and stereoscopic microscope. The results showed that the steel were subjected to corrosion to different degrees after immersion in the three cultures and with different characteristics. The corrosion of Q235 steel was the most severe in the pure SRB-containing culture, and then followed by that in pure PAO-containing culture. The corrosion process of Q235 steel in the mixed cultures was more complicated. The corrosion was alleviated compared to that in pure strain system, indicating that the coexistence of mixed strains might change the effect on biocorrosion process.

1. INTRODUCTION

Since microbiologically influenced corrosion was first proposed in 1891 [1], a great deal of research have been carried out focusing on the influence of various microorganisms on corrosion of marine engineering materials. The interaction between microorganisms and metal material in the literature was mainly focused on the corrosion acceleration induced by anaerobic microorganisms [2-4], especially pure strain, e.g., Aimeur [5] studied the influence of strain *Bacillus cereus* bacterium on corrosion behavior of carbon steel in natural seawater and found that the corrosion rate decreased

significantly in the presence of biofilm, and McBeth [6] investigated the effect of iron oxidizing bacteria on corrosion of low temperature steel in seawater. However, the microbial community in nature is a whole in which the multiple species interact and restrict each other, leading to the metal corrosion as a synergistic result of microorganisms. Recently, more and more researchers have begun to show interest in this field. Steele [7] found that the corrosion of 316L stainless steel was accelerated by the synergistic action of sulfate-reducing bacteria and aerobic bacteria by atomic force microscope. Li [8] pointed out that the synergistic action of *thiobacillus ferrooxidans* and *thiobacillus thiooxidan* aggravated the corrosion of Q235 steel, and the presence of *Thiobacillus thiobacillus* oxide weakened its localized corrosion. Therefore, the symbiosis of mixed bacteria may change the effect of pure strain of bacteria on microbial corrosion, and some of the mechanism is still unknown for the microbial community is very complicated.

Sulfate-reducing bacteria (SRB) are widely found in soil and seawater, and are identified as main microorganisms contained in highly corrosive environments to generate metabolites contributing to metallic MIC. The corrosion effects of sulfur and sulfur-iron compounds in the metabolites on metals have received increasing attention [9-11]. *Pseudomonas aeruginosa* (PAO) belongs to gram-negative bacilli and is commonly found in aquatic environments such as seawater [12], and it has been confirmed that *Pseudomonas sp.* are involved in the corrosion process of carbon steel, stainless steel and aluminum alloys [13-14]. The Q235 steels have been used in coastal engineering materials due to their good mechanical properties, good plasticity, weldability and low temperature resistance. Therefore, it is very meaningful to study the corrosion behavior of Q235 steel under conditions of pure SRB, PAO and their mixed strains. The objectives of this research are: 1) to introduce the concept of microbial diversity to MIC study more deeply; 2) to explore the corrosion behavior of Q235 steel involved with anaerobic and aerobic bacteria; 3) to investigate the synergistic effect of *Pseudomonas aeruginosa* and sulfate-reducing bacteria on the electrochemical performance of steel in simulated marine environment.

2. EXPERIMENTAL SECTION

2.1 Material

The chemical composition (wt.%) of the Q235 steel used in the experiment is as follows: C 0.18, Si 0.18, Mn 0.36, P 0.016, S 0.008, Al 0.011, Fe margin. The samples for electrochemical measurement were cut from a Q235 steel plate and sealed with epoxy resin leaving a square working area of 1cm^2 exposed to the electrolyte, and the non-working surface was weld with copper wire. The sample used for the observation of corrosion morphology was a cuboid with a dimension of 20mm×30mm×2mm. Before the experiment, the working surfaces were abraded with a series of silicon carbide papers (up to 1200#), and then washed with distilled water and degreased in acetone and dried. The electrode was kept in a deoxygenated chamber, sterilizing by ultraviolet lamp for 30 min prior to testing.

2.2 Source and culture of bacteria

The strains of sulfate-reducing bacteria and *Pseudomonas aeruginosa* were provided by Chinese Academy of Sciences, Institute of Oceanology. A modified Postgate's C medium, which contained 0.5g

KH_2PO_4, 1.0g NH_4Cl, 0.06g $CaCl_2 \cdot 6H_2O$, 0.06g $MgSO_4 \cdot 7H_2O$, 0.004g $FeSO_4 \cdot 7H_2O$, 6ml 70% sodium lactate, 1g yeast extract and 0.3g sodium citrate in 1 L seawater, was used for the enrichment culture of SRB. The culture medium for PAO includes 10g peptone, 5g yeast, 10g NaCl in 1L distilled water. The pH value was adjusted to 7.5~7.8 with 1 mol·L^{-1} NaOH. The medium was then autoclaved at 121℃ for 20 min. The culture medium containing bacteria was introduced into the correspondingly sterile medium at 1:10 volume ratio to prepare a pure strain containing solution. The mixed bacteria culture was prepared by mixing the same volume of SRB and PAO containing culture together at 1:1 volume ratio. Then, the culture media were kept in a incubator with a temperature of 38℃.

2.3. Electrochemical measurement and corrosion morphology analysis

The electrochemical experiments were performed in a classical three-electrode cell, with a platinum wire (0.5 mm in diameter) used as the counter electrode, and a saturated calomel electrode (SCE) as the reference electrode. The three-electrode system was immersed in the bacterial solution at room temperature (20~25℃) and the OCP and EIS measurements were performed daily. All tests were operated using an EG&G Parstat2273 electrochemical system. On the basis of the open corrosion potential measurement, the electrochemical impedance spectroscopy (EIS) of the samples were measured in three media, respectively. The test of EIS was performed in the frequency range of $10^{-2} \sim 10^5$ Hz and the amplitude of the sinusoidal voltage signal was 10 mV.

The samples for observation of the corrosion morphology were immersed in the above bacterial culture media for 12 days. Then after washed with distilled water and degreased in acetone and dried, the samples were observed under a stereomicroscope.

3. RESULTS AND DISCUSSION

3.1 OCP measurement

The open circuit potential (E_{ocp}) of Q235 steel was measured in three media. The potential curves over time were obtained, as shown in Fig. 1.
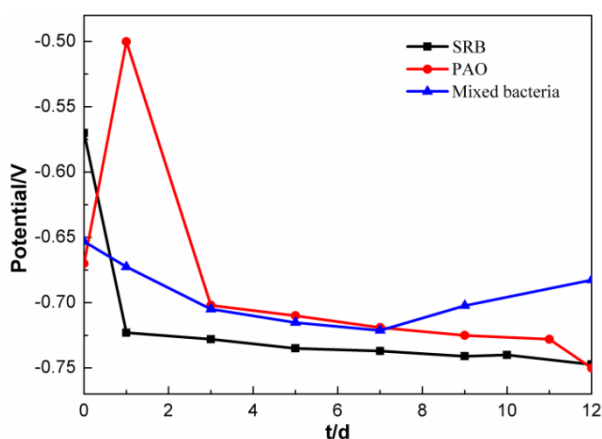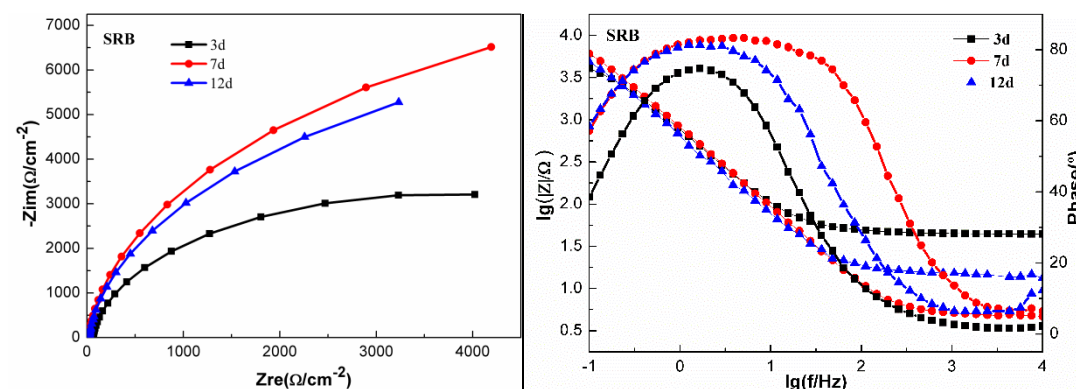

Figure 1. Variation of E_{ocp} of Q235 steel in three media over time

It is seen from Fig. 1 that the corrosion tendency in pure SRB system is large. After a sharp decline in 1 d, there was a slight negative shift in E_{ocp} during the whole process. The production of extracellular polymer substances (EPS) contributed to the adhesion of SRB and the formation of biofilm [15-16] and then FeS was produced by a combination reaction as a layer of corrosion product film formed on the surface of the steel, resulting in the inhibition of corrosion of Q235 steel in the later stage. In PAO system, there was a large positive shift in E_{ocp} within 1 d at the beginning of the experiment, showing that a layer of barrier film for some reason was formed the surface of the steel thus changed the state of the electrode. Subsequently the corrosion rate was accelerated due to the organic acids secreted by PAO promoting the passive decomposition [17]. As free Fe^{2+} ions in solution served as signals for biofilm development of PAO [18], 3d later, a biofilm was formed on the electrode surface, which acted as a barrier and alleviated the corrosion rate. The obvious negative shift in the last stage should be deduced that the decrease of dissolved oxygen restricted the growth of PAO. When it entered a declining period the protective film began to fall off and the corrosion rate was accelerated.

In the mixed bacteria system of SRB and PAO, the E_{ocp} changed little due to the synergistic effect of the two bacteria. The negative shift of potential in 3 d might be caused by Cl^- in the solution and the corrosion initiated by PAO adhering to the surface of the steel, since PAO could enhance the passive destruction of the material in Cl^- containing solution [19]. Due to the rapid growth of PAO after 3 d, the biofilm formed on the electrode surface began to reduce the corrosion rate. After 7 d, the potential moved positively and did not show a stable state. In the presence of PAO a relatively anoxic environment was created and SRB began to grow rapidly on the surface of the electrode. The thickness of the protective film was increased although S^{2-} produced by SRB metabolism still caused corrosion at the time, thus the corrosion resistance of protective film was more obvious. Furthermore, the activity of SRB might be suppressed by pyocyanins produced by PAO metabolism, resulting in the positive shift of potential.

3.2 EIS measurement

EIS was used to study the electrochemical reaction of metal/biofilm interface and the formation of corrosion products and biofilm in MIC [13-20]. Fig. 2 shows the Nyquist and bode diagrams of Q235 steel immersed in different media for 3d, 7d and 12d.
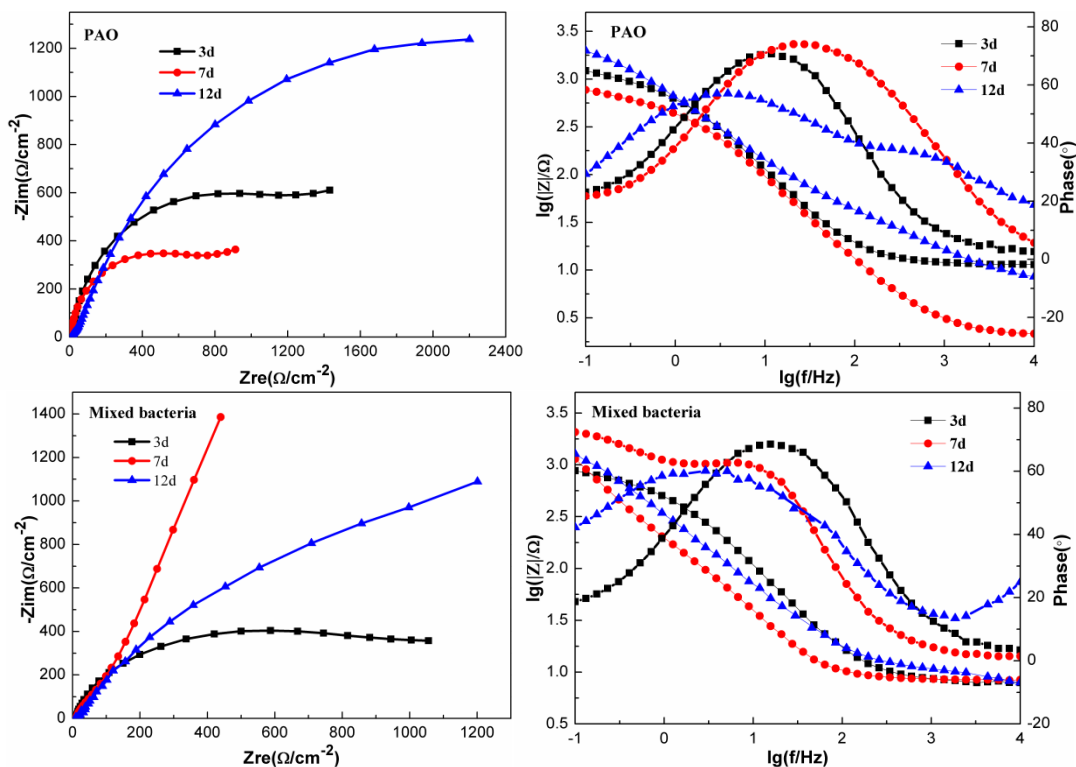
Figure 2. Nyquist and bode diagrams of Q235 steel immersed in different media for 3 d, 7 d and 12 d

It is seen from the Nyquist diagram that in a pure SRB system, the radius of capacitive impedance loop increased first and then decreased, showing that the corrosion tendency decreased first and then increased. The Nyquist diagram in mixed bacteria system showed the same tendency overall. However, in a pure PAO system, it was a opposite situation that the radius of capacitive impedance loop decreased first and then increased.

In addition, it is seen from the corresponding bode diagram that the total impedance at low frequencies in a pure SRB system changed little, but still showed a slight increase over time on the whole. There was a time constant in the phase diagram, and the peak value in the lower frequency range could be attributed to the electric double layer (EDL) [13]. In a pure PAO system, the total impedance at low frequency decreased first and then increased in 3~12 d, and peaks and valleys occurred in the lowest frequency range, which was due to the bacterial activity [21]. Before 7 d, the PAO entered a stable growth period, and the ability to adhere to the surface of the electrode was limited. In addition, the width angle of EDL was large, so the peak value of biofilm might be covered, and the maximum phase could provide information about relaxation time constant [22]. In the phase diagram, the peak at higher frequency was most likely to be caused by biofilm, and the one at lower frequency was due to EDL. There were two obvious peaks at higher frequency in the phase diagram on the 12 d. In the mixed bacteria system, the total impedance increased slightly at low frequency, and the overall change was less obvious than that in the pure system, which might indicate that the symbiotic environment of bacteria was more stable. There were two obvious peaks on 7 d, revealing that SRB began to grow at this time

and the adhesion led to the increase of the double layer capacitance in the extreme low frequency region. This appeared slowly in the low frequency region.

According to the above characteristics, the equivalent circuits in Fig. 3 were obtained by fitting with Zsimp-win software. In all circuits, R_s represents the solution resistance, R_b represents the protective layer resistance, R_{ct} represents charge transfer resistance, Q_b represents the protective layer capacitance and Q_{dl} represents the electric double layer capacitance. Q is the constant phase element CPE. When n = 1, CPE = C, and the capacitance is an ideal capacitance. The results of fitting the components are shown in Table 1.
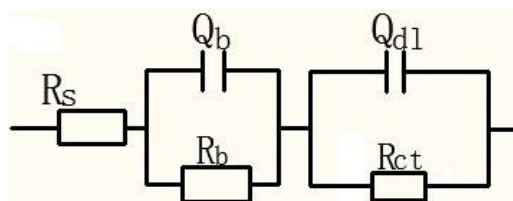

Figure 3. The equivalent circuit model used to fit the EIS experimental data for Q235 steel in different media

Table 1. Fitting parameters derived from equivalent circuit model in Fig. 3

Medium	t (d)	R_s ($\Omega \cdot cm^2$)	$Q_b \times 10^{-4}$ ($\Omega^{-1} \cdot cm^{-2}$)	n_1	R_b ($\Omega \cdot cm^2$)	R_{ct} ($\Omega \cdot cm^2$)	$Q_{dl} \times 10^{-4}$ ($\Omega^{-1} \cdot cm^{-2}$)	n_2
SRB	3	4.95	7.55	0.81	5.04	6164	2.09	0.94
	7	1E-7	9.18	0.82	5.49	1.41E4	1.89	0.95
	12	1E-8	2.45	0.94	1.23E4	17.33	0.50	1
PAO	3	12.99	13.50	0.80	1276	750.8	2.11	0.94
	7	2.29	18.30	0.71	925.9	436.4	2.45	0.95
	12	1E-7	3.59	0.69	3739	23.68	8.04	0.70
Mixed	3	7.94	2.65	0.92	490.1	674.9	18.53	0.77
	7	8.40	14.60	0.94	53.65	3.69E4	13.01	0.95
	12	2.53	7.62	0.73	2748	7.02	5.50	0.89

The factor n can be used as an index of surface inhomogeneity, and its reduction is related to the increase of surface roughness [23]. In a pure SRB system, the R_{ct} increased first and then decreased. It could be deduced that the corrosion rate decreased first and then increased, and the inhomogeneity of the mental surface decreased first and then increased.

In the pure PAO system, the increase of Q_{dl} revealed in 3~7 d that the electrode was in a adsorption state and a protective film was gradually formed. The increase of n_2 indicated that the inhomogeneity of electrode surface was decreasing. These two sets of parameters support each other. In the mixed bacteria system, the R_{ct} increased about 50 times in 3~7 d showing the corrosion tendency was small. However, R_{ct} and Q_{dl} decreased while R_b increased significantly on 12 d. The results showed that

the growth of SRB was promoted in the anoxic environment provided by PAO and the corrosion resistance of electrode was gradually enhanced due to the symbiotic effect of mixed bacteria.

3.3 Surface morphology

Fig. 4 shows the surface morphology of Q235 steel immersed in SRB, PAO and their mixed bacteria containing media for 12 days, respectively.
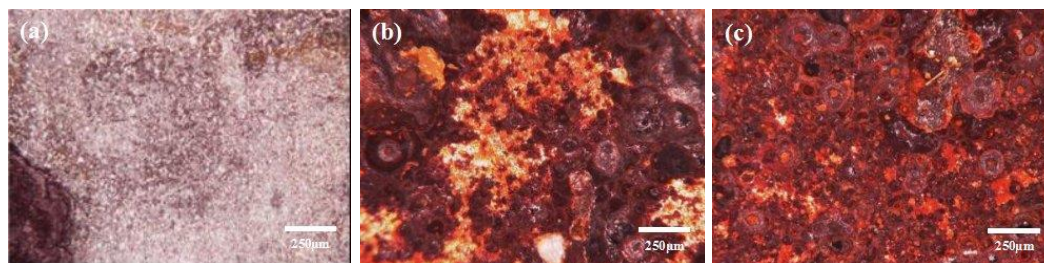

Figure 4. Surface morphology of Q235 steel immersed in (a)SRB, (b)PAO and their (c)mixed bacteria containing media for 12 days

After immersion for 12 d, the surface of all samples have been subjected to corrosion to different degrees and with different characteristics. It was found that large area of localized corrosion occurred in pure SRB system for the oxygen concentration difference induced by the EPS and H_2S produced during the process of film formation [24-25]. In Fig. 4(b), the corrosion of Q235 steel in PAO system was mainly characterized by corrosion pits and cracks. In addition, there were a lot of corrosion products on the surface of the steel caused by the accumulation of alkaloids and amino-containing iron carriers in the metabolites of PAO, leading to localized corrosion [26]. However, in mixed bacteria system shown in Fig. 4(c), there were corrosion pits smaller than those in Fig. 4(b) and a small amount of corrosion products on the steel surface. The corrosion in the mixed bacteria system was alleviated compared to that in pure culture, which indicated that the corrosion behavior of Q235 steel was affected in the symbiotic state of PAO and SRB. The morphology of corrosion products supported the previous electrochemical analysis.

3.4 Discussion

MIC is caused by the presence and activity of microorganisms, and the corrosion rate is changed by a series of biological activities and interactions of bacteria. The growth and reproduction of bacteria are also affected by temperature, pH, oxygen, osmotic pressure and so on. The pH value in this study is set to 7.5-7.8, which is the most suitable weak alkaline environment for SRB growth. The cell walls of common bacteria exhibit electronegativity, which helps them to adhere to metal substrates [27]. When electrochemical reaction occurs, the cathodic reaction and anode reactions are as follows:

$$2H_2O + 4e^- + O_2 = 4OH^- \tag{eq.1}$$
$$Fe = Fe^{2+} + 2e^- \tag{eq.2}$$

Subsequently, the corrosion product, mainly FeS was formed:
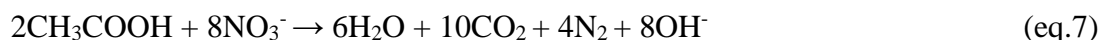
$$Fe^{2+} + S^{2-} = FeS \tag{eq.3}$$

However, with the consumption of oxygen by PAO activities and cathodic reaction, ferrous sulfide film (corrosion product film) formed in the absence of dissolved oxygen are usually unstable and subjected to penetration because of the crystal lattice of the layer. It will further lead to the formation of an active corrosion battery between ferrous sulfide (cathode) and exposed steel surface (anode) [28]. In addition, the corrosion of the steel can be further accelerated in the presence of Cl^- in the medium by the reactions as follows:

$$Fe^{2+} + 2H_2O + 2Cl^- \rightarrow Fe(OH)_2 + 2HCl \tag{eq.4}$$
$$Fe(OH)_2 + 3Cl^- \rightarrow FeCl_3 + 2OH^- \tag{eq.5}$$
$$FeCl_3 + 3H_2O \rightarrow Fe(OH)_3 + 3HCl \tag{eq.6}$$

The corrosion product, $Fe(OH)_3$, was unstable and could not prevent the steel from corrosion [29].

PAO is capable of reducing iron to ferrous ion thereby promoting oxidization [30]. When the steel surface is covered with biofilm, it is easy to accelerate the corrosion due to the presence of differential aeration cells. PAO produces iron carrier, pyochelin and pyoverdin [31], during metabolism process, which are low molecular weight compound with high affinity for iron and capable of dissolving iron ions. In addition, the EPS include proteins, polysaccharides, uronic acids, nucleic acids and fatty acids, which have anion functional groups, e. g., -COOH, -$CH_3COCOOH$, PO_4^{3-}, SO_4^{2-}. These functional groups can bind to metals and be used as corrosion inducer. Coumet [32] found that pyocyanine secreted by PAO accelerated the electron transfer between metal substrate and PAO, inducing serious pitting corrosion. The most common low carbon chain fatty acid is acetic acid, which is highly corrosive to carbon steel when concentrated in microbial sediments. Pedersen [33] reported that *Pseudomonas* promoted the passive decomposition of metals by releasing organic acids, resulting in an increase in the corrosion rate of metals.

$$2CH_3COOH + 8NO_3^- \rightarrow 6H_2O + 10CO_2 + 4N_2 + 8OH^- \tag{eq.7}$$

It is seen from the EIS test that the electrochemical corrosion process of Q235 steel in mixed bacteria system is more complicated. The interaction between bacteria and metal as well as the interaction between bacteria can affect the results. The aerobic PAO first adhered to the electrode surface. With the decline of oxygen content in the solution, the sulfate-reducing bacteria began to enter the proliferation stage. However, PAO used peptone as nitrogen source for denitrification, and the by-product of the reaction might have toxic effect on the growth of sulfate-reducing bacteria [34].

In addition, pyocyanin, the product of PAO, may also take part in the reaction. Jacob [35] indicated that pyocyanins had an inhibitory effect on the sensitive strains. In the presence of the protease-resistant R-type pyocyanins, the synthesis of DNA, RNA and protein of some sensitive strains could be prevented, resulting in the ribosome damage and deceleration of the bacterial respiration. Thus, the presence of PAO inhibited the reduction of SRB from producing S^{2-} and further alleviated the corrosion of steel.

4. CONCLUSION

The purpose of this study is to understand the effect of multiple bacteria on corrosion of Q235 steel by using a series of electrochemical analysis methods and microscopic techniques, and the following conclusions are obtained:

(1)   The samples of Q235 steel were subjected to corrosion to different degrees after immersion in three media and with different characteristics. The corrosion rate was accelerated in pure PAO system for that the organic acids secreted by PAO promoted the passive decomposition, and the pyocyanins secreted by PAO accelerated the electron transfer between the metal substrate and PAO.

(2)   The corrosion of Q235 steel was the most severe in the pure SRB-containing medium. Large area of localized corrosion occurred in pure SRB system for the oxygen concentration difference induced by the EPS and H_2S produced during the film formation process.

(3)   The symbiosis of mixed bacteria may change the effect of pure strain of bacteria on mental corrosion. The electrochemical corrosion process of Q235 steel in mixed bacteria system is more complicated. Although the growth of SRB was promoted in the anoxic environment provided by PAO, it might also be suppressed by the by-product of denitrification reaction and the pyocyanins secreted by PAO.

References

1. J. H. Garrett, *Brit. Med. J.*, 1584(1891)1026.
2. J. Yang, J. Shao and L. Wu, *Int. J. Electrochem. Sci.*, 14 (2019) 9193.
3. X. Zhao, K. Chen and J. Yang, *Int. J. Electrochem. Sci.*, 14 (2019) 875.
4. X. Zhao, K. Chen and J. Yang, *Int. J. Electrochem. Sci.*, 14 (2019) 886.
5. N. Aimeur, K. Houali and L. Hamadou, *Corros. Eng. Sci. Techn.*, 50(2015)579.
6. J. M. McBeth, B. J. Little and R. I. Ray, *Appl. Environ. Microb.*, 77(2011)1405.
7. A. Steele, D. T. Goddard and I. B. Beech, *Int. Biodeterior. Biodegrad.*, 34(1994)35.
8. S. Li, Y. Wang and J. Liu, *J. Chin. Soc. Corros. Prot.*, 29(2009)182.
9. H. Venzlaff, D. Enning and J. Srinivasan, *Corros. Sci.*, 66(2013)88.
10. X. Zhao, K. Chen and J. Yang, *RSC Adv.*, 8(2018)38118
11. J. Chen, J. Wu and P. Wang, *J. Mater. Eng. Perform.*, 28(2019)1469.
12. L. C. Xu, H. H. Fang and K. Y. Chan, *J. Electrochem. Soc.*, 146(1999)4455.
13. S. J. Yuan, A. M. F. Choong and S. O. Pehkonen, *Corros. Sci.*, 49(2007)4352.
14. J. Morales, P. Esparza and S. González, *Corros. Sci.*, 34(1993)1531.
15. J. Michalska, B. Chmiela and W. Simka, *Mater. Corros.*, 69(2018)8.
16. F. L. Heggendorn, F. Agm and D. C. Ferreira, *Int. J. Dent.*, 79(2018)115.
17. A. Pedersen, S. Kjelleberg and M. Hermansson, *J. Microbiol. Method.*, 8(1988)191.
18. E. Banin, M. L. Vasil and E. P. Greenberg, *P. Natl. Acad. Sci. USA.*, 102(2005)11076.
19. J. Morales, P. Esparza and S. Gonzalez, *Corros. Sci.*, 34(1993)1531.
20. A. K. Manohar, O. Bretschger and K. H. Nealson, *Bioelectrochemistry*, 72(2008)149.

21. F. Mansfeld, Y. Wang and S. H. Lin, *Electrochemical Impedance: Analysis and Interpretation*, ASTM. STP 1188, Philadelphia, 1993, 297.
22. W. S. Tail, *An Introduction to Electrochemical Corrosion Testing for Practicing Engineers and Scientists*, University of Wisconsin-Madison, 1994.
23. A. A. Oskuie, T. Shahrabi and A. Shahriari, *Corros. Sci.*, 61(2012)111.
24. J. R. Postgate, *The Sulphate-Reducing Bacteria*, Cambridge University Press, New York, 1984.
25. S. A. Wade, P. L. Mart and A. R. Trueman, *Corros. Mater.*, 36(2011)68.
26. K. Chai and Q. Luo, *J. Chin. Soc. Corros. Prot.*, 33(2013)481.
27. R. B. Frankel and D. A. Bazylinski, *Rev. Mineral. Geochem.*, 54(2003)95.
28. W. A. Hamilton, *Biofouling*, 19(2003)65.
29. A. Heyer, F. D'Souza and C. F. L. Morales, *Ocean. Eng.*, 70(2013)188.
30. C. Pillay and J. Lin, *Int. Biodeter. Biodegr.*, 83(2013)158.
31. J. Brandel, N. Humbert and M. Elhabiri, *Dalton T.*, 41(2012)2820.
32. A. Cournet, M. Bergé and C. Roques, *Electrochim. Acta*, 55(2010)4902.
33. A. Pedersen, S. Kjelleberg and M. Hermansson, *J. microbiol. meth.*, 8(1988)191.
34. K. M. Kaster, A. Grigoriyan and G. Jennneman, *Appl. Microbiol. Biot.*, 75(2007)195.
35. F. Jacob, *Ann. Inst. Pasteur.*, 86(1954)149.