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Short Communication

Effect of *Alcaligenes sp.* and sulfate-reducing bacteria on corrosion of Q235 steel in simulated marine environment

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Sulfate-reducing bacteria, as anaerobic microorganisms, are the most important and representative bacteria responsible for metal corrosion. *Alcaligenes sp.*, as a hydrocarbon-degrading bacterium, often coexists with sulfate-reducing bacteria in coastal environments, especially in storage tanks and pipelines. In this paper, the corrosion behavior of Q235 steel in the solution of pure sulfate-reducing bacteria, *Alcaligenes sp.* and their mixed bacteria were analyzed by open circuit potential, electrochemical impedance spectroscopy, polarization curves and stereoscopic microscope. All the electrochemical results and morphology analysis showed that the corrosion of Q235 steel was the most severe in the pure sulfate-reducing bacteria medium. The corrosion behavior in the mixed bacteria system was similar to that in a pure *Alcaligenes sp.* system, and the involvement of *Alcaligenes sp.* greatly weakened the local corrosion of Q235 steel by sulfate-reducing bacteria. It provides a new possible approach to increase the service life of the material when microbiologically influenced corrosion is dominated by sulfate-reducing bacteria.

Keywords: MIC; Sulfate-reducing bacteria; *Alcaligenes sp*; Mixed bacteria

1. INTRODUCTION

Since Garrett proposed in 1891 that microorganisms were closely related to the corrosion process of metals[1], a lot of research have been carried out on microbiologically influenced corrosion(MIC). In 1934, Kuhr et al[2] proposed the classical cathodic depolarization theory, which was of great significance to the research of MIC process. Up to now, deep research on MIC is still going on all over the world[3-6]. For example, the effect of Ag and Cu ions on the corrosion of 316L stainless steel in the

presence of *Desulfovibrio sp.* was studied by T. Unsal-Istek[7]. Erşan[8] found that Nitrate reducing $CaCO_3$ precipitating bacteria could survive and inhibit steel corrosion in the mortar. Chen[9] studied the long-term survival rate of *Desulfovibrio vulgaris* on carbon steel and the associated pitting corrosion. Liu[10] found that sulfate-reducing bacteria (SRB) and iron oxidizing bacteria have a synergistic effect on pitting corrosion of carbon steel, and iron oxidizing bacteria can inhibit the growth of planktonic SRB.

However, in the natural environment, the result of MIC is usually caused by a variety of bacteria. After the introduction of the concept of microbial diversity to MIC, this field has received more attention. Du[11] proved that the corrosion rate of steel was between two kinds of strains under the co-influence of *Thiobacillus thiooxidans* and *Bacillus*. Unlike a pure bacterial solution, round pitting was found in mixed bacteria culture. Duan[12] concluded through experiments that the corrosion weight loss of Q235 steel in the presence of *Pseudomonas* and iron bacteria was much lower than that of the pure bacteria. Iwona[13] used electrochemical methods to study the corrosion rate of carbon steel under the influence of *Sulfur oxidizing bacteria* and SRB in the South England Sea. It was found that the corrosion rate was much higher than those both in sterilized seawater and pure strain containing medium. Gevertz[14] claimed that after adding *Bacillus breris* to SRB, it produced antibiotics like substances to kill SRB directly thus preventing the metal corrosion. Batmanghelich[15] studied the influence of multispecies biofilms of *Pseudomonas aeruginosa* and *Desulfovibrio vulgaris* on the corrosion of cast iron. X Jiang[16] found that the presence of SRB and a small amount of saprophytic bacteria and iron bacteria facilitated formation fouling on the pipeline. However, the corrosion failure of the wastewater pipeline is due to MIC and under-deposition corrosion. It shows that the synergism of mixed bacteria has different effects on material corrosion. SRB is a kind of anaerobic microorganism and as one of the most important and representative bacteria causing metal corrosion[17]. As the most common research object of MIC, they are often found in coastal clay soil, polluted sea area and offshore oil extraction equipment[18,19]. *Alcaligenes sp.*(ASP) is one kind of hydrocarbon-degrading bacteria and belongs to heterotrophic nitrifying bacteria. Atlas[20] reported that under normal conditions, hydrocarbon-degrading bacteria accounted for only 1% of the microbial community. However, when oil pollution occurred, the proportion of degrading bacteria could be increased by 10 times. Chen isolated five strains including ASP from the Zhejiang coast in China and studied their biodegradation of crude oil[21]. Other scholars confirmed that the metabolites of hydrocarbon degrading bacteria included organic acids, surfactants, biopolymers, carbon dioxide, hydrogen, methane and hydrogen sulfide[22], which were prone to affect the material corrosion.

Q235 steel is widely used in marine engineering such as oil tanks and pipelines in coastal areas. SRB and ASP often co-exist in the environment of oil tanks. In this paper, corrosion behaviour of Q235 steel under conditions of pure SRB, ASP, and their mixed bacteria were discussed by electrochemical methods and stereoscopic microscope. 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 hydrocarbon degrading bacteria; 3) to investigate the synergistic effect of ASP and SRB 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 used in the electrochemical tests were cut from Q235 steel plates and sealed with epoxy resin. A 1 cm^2 square working area was exposed to the electrolyte. The sample used for the observation of corrosion morphology was a cuboid with a dimension of 20 × 30 × 2 mm. As the surface roughness of the material can affect the adhesion of bacteria[23], 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 with acetone. The electrodes were placed in a deoxygenation chamber and tested after UV disinfection for 30 minutes.

2.2 Bacteria and culture

The SRB and ASP were provided by Chinese Academy of Sciences, Institute of Oceanology. A modified Postgate's C medium, which contained 0.5 g KH_2PO_4, 1.0 g NH_4Cl, 0.06 g $CaCl_2·6H_2O$, 0.06 g $MgSO_4·7H_2O$, 0.004 g $FeSO_4·7H_2O$, 6 mL 70% sodium lactate, 1 g yeast extract and 0.3 g sodium citrate in 1 L seawater, was used for the enrichment culture of SRB. The improved crude oil medium was used for the enrichment culture of ASP, including 4g crude oil, 0.2 g K_2HPO_4, 0.2 g NH_4NO_3 in 1 L seawater[21]. 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-containing solution was prepared by mixing the same volume of SRB and ASP containing culture together. Finally, the medium was kept in an incubator at a temperature of 38 ℃.

2.3 Electrochemical tests and corrosion morphology analysis

The electrochemical experiments were performed in a classical three-electrode cell, with a platinum wire (0.5mm in diameter) used as the counter electrode, and a saturated calomel electrode (SCE) as the reference electrode. The electrochemical properties of open circuit potential (OCP), electrochemical impedance spectrum (EIS) and polarization curve were tested by electrochemical workstation (PARSTAT2273, USA). EIS was performed in the frequency range of 10^{-2} ~ 10^5 Hz and the amplitude of the sinusoidal voltage signal was 10 mV. The polarization curve was tested in a scanning range of -350 mV to +350 mV at a scanning rate of 0.333 mV/s.

The samples for observation of the corrosion morphology were immersed in the above bacterial culture media for 13 days. The samples were washed with distilled water, degreased and dried with acetone, and observed under a stereoscopic microscope.

3. RESULTS AND DISCUSSION

3.1 OCP measurement

The E_{ocp} of Q235 steel was measured in three bacterial media. The relation curve of potential and time is obtained, as shown in Fig. 1.

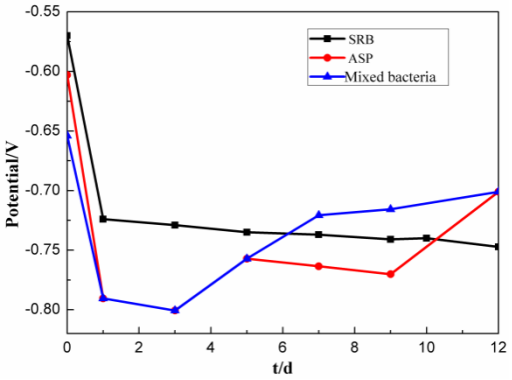


Figure 1. Variation of E_{ocp} of Q235 steel in SRB, ASP and mixed bacteria media over time

It is seen from Fig. 1 that in a pure SRB system there was a negative and rapid shift in E_{ocp}, and then it varied within a small range. The electrode surface initially had no protective film to hinder the corrosive medium. Then the bacteria began to multiply rapidly and biofilm appeared on the surface of the electrode. As the life activities of bacteria, the following reactions occur at the interface:

$$Fe^{2+} + S^{2-} = FeS \quad (1)$$

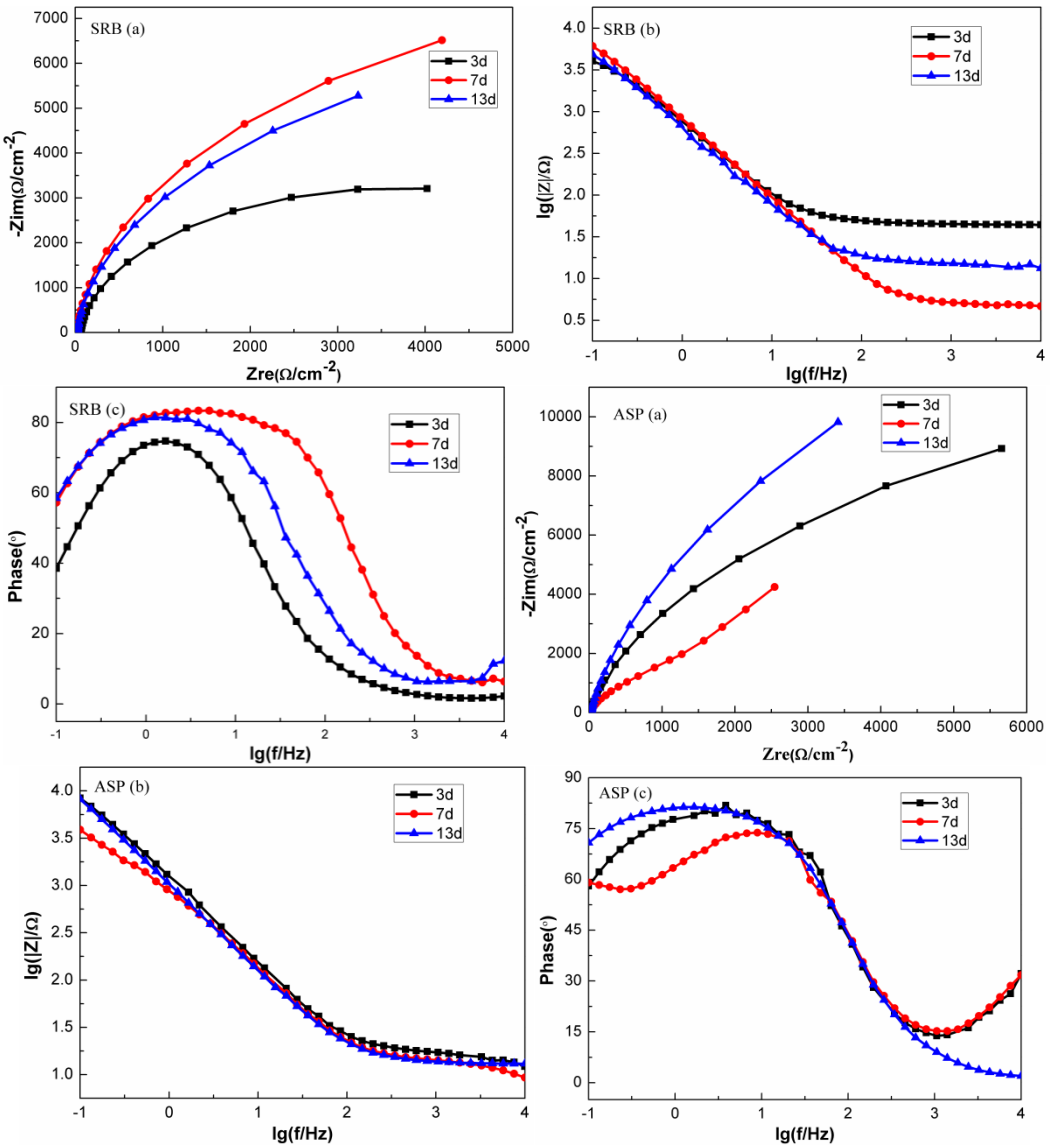
And the corrosion product film was formed on the electrode surface, which played a protective role and slowed down the corrosion. The variation in a pure ASP system was comparatively complex. The E_{ocp} negatively shifted before biofilm formed on the surface of the electrode at first. During the period of 1~5d, biofilm formed by ASP on the electrode surface had a temporary protective effect. The change during 5~9d was caused by the inhomogeneous biofilm and the difference of oxygen concentration on the electrode surface. In addition, acids in metabolites such as H_2S and other organic acids also caused damage to the protective film. During the period from 9d to 13d, a corrosion product film appeared on the surface of the electrode, and the potential moved forward again. Some scholars have pointed out that the enzymes produced by microorganisms are also the cause of positive potential transfer[24,25].

In the mixed system of SRB and ASP, the E_{ocp} was similar to that in a pure ASP system before 5 days, since ASP played a major role in the aerobic environment at the beginning of the experiment, and the effect of anaerobic bacteria (SRB) was little. The change in 5~13d was due to the fact that ASP belonged to gram-negative bacteria, and the presence of lipopolysaccharide in the outer layer of gram-negative cells was reported to have a special effect with Fe^{2+}[26]. At the same time, with the decrease of dissolved oxygen, ASP entered its recession period and extracellular polymers (such as lipopolysaccharide which could release toxins) acted as surface activity inhibitors. The corrosion

tendency was decreased for the hindering of the adhesion of other microorganisms to the electrode surface[27].

3.2 EIS measurement

The EIS can provide more information about the kinetics and the structure of the electrode interface than the conventional method. Fig. 2 shows the EIS of Q235 steel immersed in different media for 3d, 7d and 13d.



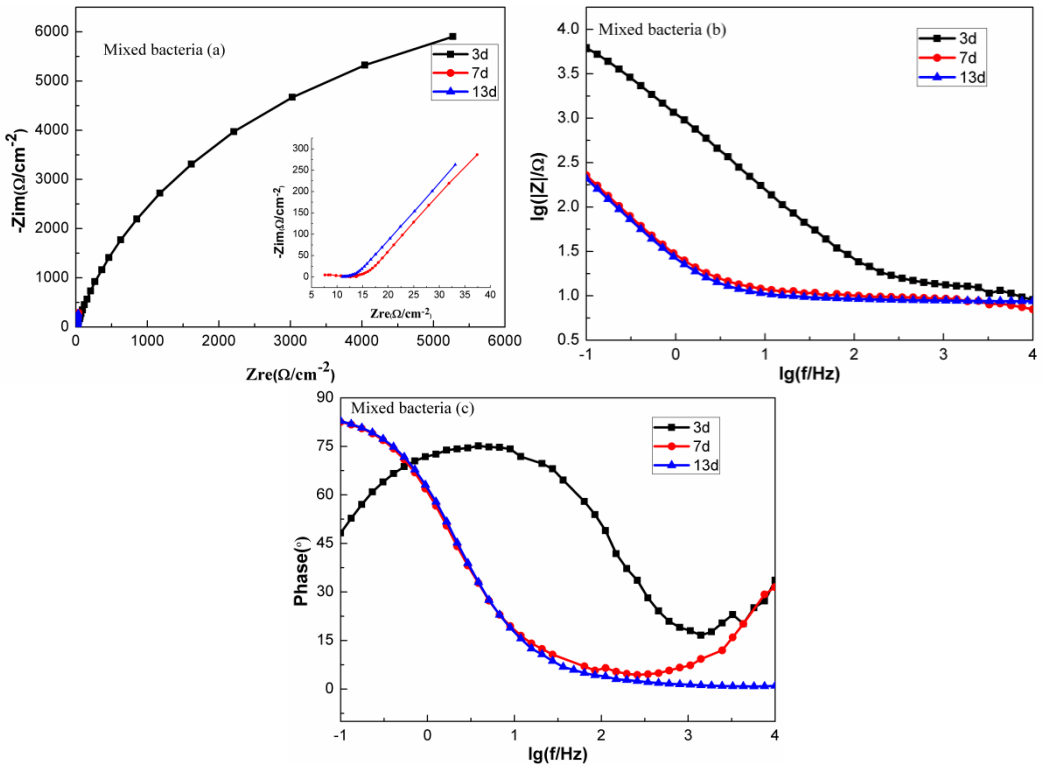


Figure 2. Nyquist and Bode diagrams of Q235 steel immersed in SRB, ASP and mixed bacteria media (a represents the Nyquist diagram, b represents the magnitude diagram, and c represents the phase angle diagram) for 3d, 7d and 13d

It is seen from the Nyquist diagram that in a pure SRB system, the radius of capacitive impedance loop increased first and then decreased, which was due to the rapid growth of SRB and biofilm formed on the surface of the material during the period of 3~7d. At the same time, the corrosion product such as FeS adhered to the surface of the material and slowed down the corrosion. During the period of 7~13d, with the transformation of corrosion product, the protective film began to fall off and the corrosion was accelerated.

In a pure ASP system, the radius of capacitive impedance loop decreased first and then increased. The reason is that during the 3~7d period, ASP consumed a large amount of dissolved oxygen and produced acidic substances through metabolism, resulting in the increase of corrosion tendency. Then the protective film generated and the corrosion tendency was reduced. In addition, the Warburg impedance appeared in about 7 d. However, the Warburg impedance occurred in the mixed strain system in about 7 d and 13 d, and the formation of protective film was more complicated.

In order to further characterize the impedance characteristics, ZSimp-win software is used to fit the EIS obtained from the above different media, the corresponding equivalent circuits are shown in Fig. 3. In all circuits, R_s represents the solution resistance, R_b represents the protective layer resistance, R_{ct} represents charge transfer resistance, W represents the Warburg impedance, Q_b represents the protective layer capacitance and Q_{dl} represents the electric double layer capacitance. The results of fitting the components are shown in Tab. 1.

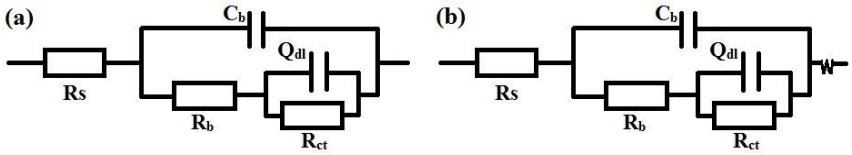


Figure 3. The equivalent circuit model used to fit the EIS experimental data for Q235 steel in SRB, ASP and mixed bacteria media(The equivalent circuit b is used for 7d and 13d of Q235 steel in the mixed bacteria system, and the equivalent circuit a is used for the rest.)

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 $k\Omega \cdot cm^2$	$Q_{dl} \times 10^{-4}$ $(\Omega^{-1} \cdot cm^{-2})$	n_2	R_{ct} $k\Omega \cdot cm^2$	$W \times 10^{-4}$
SRB	3	4.94	1.58	0.91	40.02	0.53	1	6198	-
	7	2.78	0.31	0.73	28.74	1.64	0.97	1.56E4	-
	13	1E-5	0.07	0.71	17.84	2.38	0.95	1.24E4	-
ASP	3	1E-7	0.44	0.8	22.2	1	0.96	2.85E4	-
	7	14.43	1.82	0.87	4122	2.63	1	1.41E4	-
	13	14.51	0.92	0.94	13.17	0.68	0.89	4.41E4	-
Mixed bacteria	3	1E-7	0.41	0.65	16.56	1.25	0.90	1.49E4	-
	7	2E-7	0.07	0.85	9.43	71.45	0.88	3.25E4	766.5
	13	1E-7	1.84	0.59	8.91	79.93	0.87	4.22E4	769.3

In a pure SRB system, the biofilm developed continuously from 3d to 7d, and the corrosion product film developed from 7d to 13d. It revealed that the corrosion rate in the system was decreasing, which was consistent with the previous electrochemical analysis. The existence of SRB can produce a large amount of H_2S, which increases active charge transfer by reacting with Fe^{2+} to form ferrous sulfide deposits[28].

In the mixed bacteria system, the synergistic effect of the two strains complicated the process. In the first three days, a dense protective film developed on the electrode surface and slowed down the corrosion. Both 7d and 13d showed Warburg impedances, suggesting that the constant presence of diffusion impedance between 7~13d, and the overall corrosion tendency was small. The value of R_{ct} in 13d increased compared with that in 7d and the corrosion rate was still decreasing. It is basically consistent with the electrochemical analysis.

R_{ct} is the index for evaluation of the corrosion rate. Cetin D pointed out that the increase of charge transfer resistance would increase the porosity of the protective film and the potential of the capacitive element in parallel with it[29]. It can be seen that during 3~13d R_{ct} increased first and then decreased in a pure SRB system, indicating that the corrosion rate decreased first and then increased, which is consistent with the above analysis. It also suggested that the porosity on the steel surface increased in the early stage and then decreased, which showed that the pitting corrosion of the steel occurred soon after the experiment.

Different from this, the slight increase of R_{ct} in the mixed bacteria system within 7~13d indicated that the porosity increased gradually. In addition, the diffusion impedance appeared in the mixed bacteria

system after 7 days, so the change of corrosion rate could not be evaluated simply by value of R_{ct}. In a pure ASP system, the change of R_{ct} was the most obvious. However, it can still be judged that the value of R_{ct} in the ASP system is greater than that in the SRB system, which indicates that the Q235 is subjected to less corrosion persecution in the ASP system.

3.3 Polarization curve

The polarization curve shows the relationship between corrosion potential and corrosion current density. Fig. 4 shows the polarization curve of Q235 steel after immersion in different media for 3d, 7d and 13d.

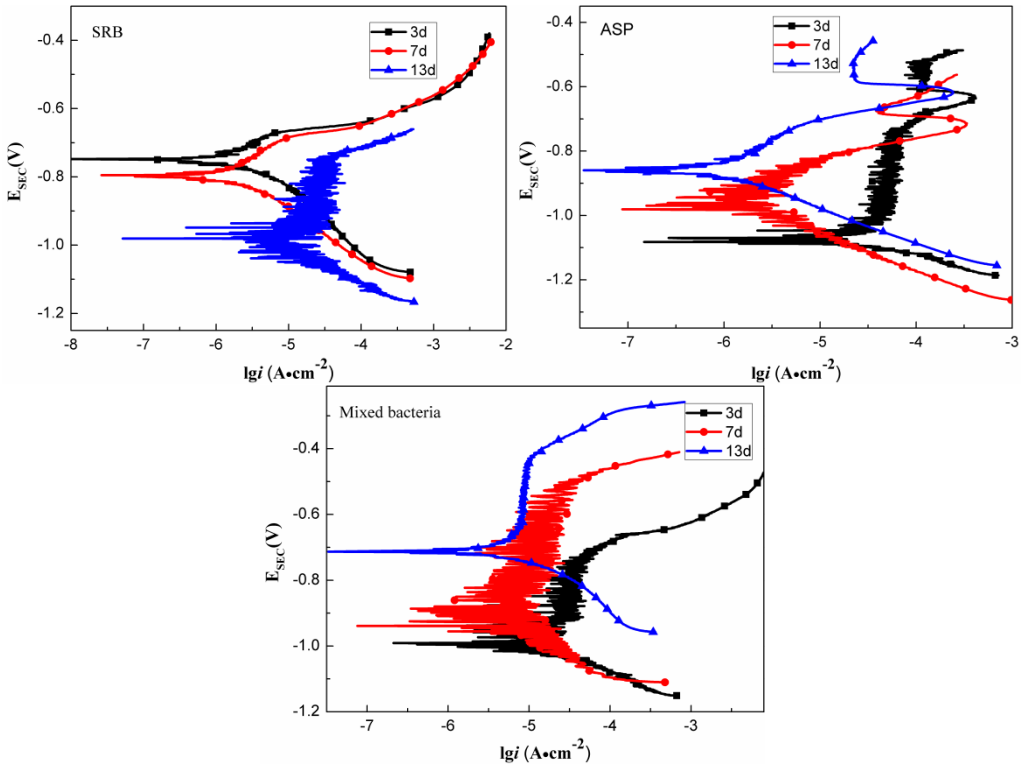


Figure 4. Polarization curves of Q235 steel immersed in SRB, ASP and mixed bacteria media for 3d, 7d and 13d

It can be seen from Fig. 4 that all the basic shapes of the polarization curve are similar, showing that the microbial activity does not change the properties of the electrode in reaction process but only the corrosion rate. The passivating areas appear in the polarization curves of the three systems. The passivation area of SRB system appeared at the later stage of the experiment, indicating that the corrosion product film was formed on the electrode surface, which slowed down the corrosion. The passivating areas in ASP system was narrowed, indicating that the acidic product produced by the metabolism of bacteria might damage the protective film on the electrode surface. The electrode anode in the mixed bacteria system was in the passivating area for a long time, which indicated that the passivating film of

Q235 steel was relatively complete in the mixed bacteria and the corrosion resistance was relatively strong.

It is worth emphasizing that the corrosion potential and corrosion current density in the polarization curve are the factors best to reflect the corrosion strength. As time progressed, the corrosion potential in the SRB solution was always negatively shifted and the corrosion current density shifted first and then shifted. These states indicate that the corrosion is generally intensified, but the corrosion tendency decreases at 7d. The positive shift of corrosion potential and the negative shift of corrosion current density in ASP system showed that the corrosion of Q235 steel gradually decreased. In the solution of mixed bacteria, the corrosion potential shifted positively, and the corrosion current density shifted negatively. Especially in the 7~13d period, the results show that the presence of mixed bacteria slows down the corrosion, and ASP plays a role in inhibiting SRB corrosion. The results are very consistent with the OCP and EIS.

Table 2. Fitting parameters of the polarization curve of the steel after immersed in SRB, ASP and mixed bacteria media for 13 days

	E_{corr} (V)	I_{corr} (μA/cm^2)	B_a(mV·dec^{-1})	B_c(mV·dec^{-1})
SRB	-0.80	15.16	131	120
ASP	-0.94	2.30	770	498
Mixed bacteria	-0.88	3.30	131	79

Tab. 2 shows the fitting parameters of the polarization curve of the steel after immersion in different media for 13 d. E_{corr} and I_{corr} are the main parameters for analysis, and decrease of corrosion voltage and increase of corrosion current density reveal the acceleration of corrosion. It can be seen from the table that the corrosion rate of Q235 steel is the greatest in pure SRB system. In a pure ASP system, the corrosion current density is small, and the reason for this state may be that a large amount of oxygen in the solution is consumed as the life activities of ASP. The corrosion positive of Q235 steel in mixed bacteria system was lower than both that in pure bacterial solution, and the corrosion current density was smaller. It was also confirmed that certain polymers produced by ASP might inhibit the activity of SRB and improve the corrosion of Q235 steel.

3.4 Surface morphology

Fig. 5 shows the surface morphology of Q235 steel immersed in SRB, ASP and their mixed bacteria media for 13 days.

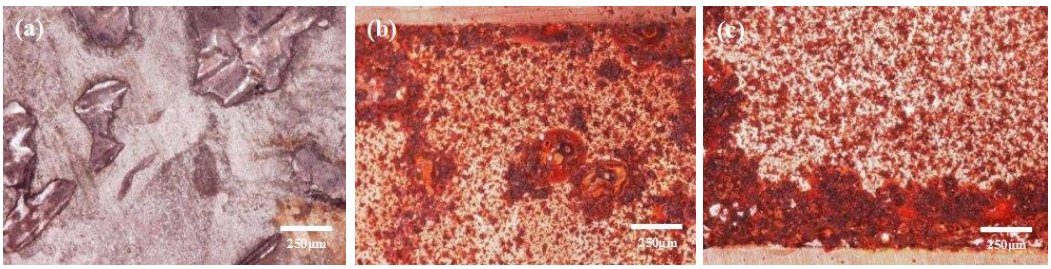


Figure 5. Surface morphology of Q235 steel immersed in (a)SRB, (b)ASP and (c)Mixed bacteria containing media for 13 days

After immersion for 13 days, the surface of all samples have been subjected to corrosion to different degrees and with different characteristics. Local corrosion, especially pitting, occurred in pure SRB system for partial exfoliation of corrosion product film from the substrate. The reason mainly lay in the oxygen concentration difference induced by the EPS and H_2S produced during the process of film formation[30, 31]. In a pure ASP system, corrosion pits were more obvious, which mostly due to the destruction of protective film by acid products such as organic acids. Although the corrosion behavior of Q235 steel also occurred in the mixed bacterial solution, the bonding density of the protective film was higher than that of the pure strain system, which indicated that it provided relatively good blocking effect in the mixed medium. As the corrosion behavior of Q235 steel in mixed bacteria system was analyzed, it was found that corrosion started from the boundary of the working surface and gradually developed inward. In this case, the corrosion of Q235 steel is relatively light, which is more conducive to the use of materials. The corrosion morphology was basically consistent with the previous electrochemical analysis, which also indicated that the addition of ASP slowed down the corrosion attack of SRB on Q235 steel.

3.5 Discussion

The corrosion form induced by SRB for carbon steel is pitting. Javed[32] found that bacteria cells preferentially adhered to the grain boundaries of steel samples. Although there was no evidence on the corrosion surface of intergranular corrosion materials, loose corrosion products deposited within the grain boundaries. Alcaligenes can produce an extracellular polymeric substance (EPS) consisting of four sugar repeating units of D-glucose, D-glucuronic acid, d-glucose and l-rhamnose[33]. The EPS have high molecular weight and unique viscoelastic and rheological properties[34]. Therefore, it may make the biofilm produced by ASP cover better on the surface of Q235 steel. As shown in Fig. 5, the Q235 samples after 13d immersion showed a more uniform and dense covering state in the system containing ASP.

The underlying mechanism of microbial corrosion can be attributed to the role of protein molecules in controlling the movement of reactants to metal surfaces. It is seen from the experiment that the corrosion rate in SRB system is relatively large, since the H_2S produced by the reduction reaction is corrosive to a certain extent. It has been reported that SRB sessile cells harvest extracellular electrons from elemental iron oxidation for energy production in their metabolism. This results in SRB biofilms

form much denser biofilms on carbon steel surfaces[35]. In addition, there are channels and voids capable of carrying aqueous electrolytes in biofilm, which lead to the persistent attack of Cl^- on the surface of materials[36]. However, the effect of microorganism on corrosion of Q235 steel is reduced by addition of *Alcaligenes sp.*. RE Eckfordproved that the presence of nitrate reducing bacteria in almost all oil fields[37], and SRB might be stimulated by these bacteria thus leading to the greatly decline of the H_2S production. As one of the nitrate reducing bacteria, *Alcaligenes sp.* also has the same ability.

Since *Alcaligenes sp.* is a kind of facultative anaerobic bacteria, oxygen becomes a limiting nutrient in aerobic environment and the transfer rate of oxygen controls the reaction rate of the whole process[38]. It is the reason that Warburg impedance occurs in the EIS. Under hypoxic conditions, ASP can accomplish respiration by denitrification. Denitrification refers to reduction of nitrate to nitrite and may be further reduced to N_2. Nitrite hinders the growth of SRB since it inhibits the production of sulfonic acid reductase, which is the key to the sulfate reduction by SRB. It has also been reported that the by-products of denitrification may be toxic to the activity of SRB[39]. The mechanism of corrosion induced by SRB is summarized as follows: Hydrogen sulfide is used to stimulate the part of the cathode that corrodes cells[40]; Cathodic reaction is stimulated by solid $FeSO_4$ through the reaction of Fe^{2+} with sulfide ions produced by bacteria[41]; Sulphur produced by bacteria stimulates the anode reaction, causing the metal to dissolve[42]; Steel is corroded directly by metabolic coupling[43]. It can be seen from Fig. 5 that the corrosion rate of Q235 steel is obviously slower in mixed bacteria system than that in SRB system. Ontiveros[44] pointed out that the reduction of SO_4^{2-} caused by electronic donor competition could be suppressed by nitrate. It is then deduced that adding a certain amount of nitrate reducing bacteria such as *Alcaligenes sp.* can weaken the corrosion of Q235 steel by sulfate-reducing bacteria.

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 participation of ASP greatly weakened the localized corrosion of Q235 caused by SRB.

(2) The corrosion of Q235 steel in the mixed system of SRB and ASP was similar to that in a pure ASP syetem. ASP played a decisive role in the corrosion of materials and suppressed SRB to a great extent, which might be related to the inhibition of microbial activity of SRB by certain polymers produced by ASP, such as lipopolysaccharide can release toxins.

(3) In the mixed bacteria system, the protective film formed on the surface of the steel is the most compact, which has a barrier effect on the corrosion of Q235 steel.

The results show that the corrosion degree of SRB on Q235 carbon steel is affected by adding ASP. This work might provide a new approach to increase the service life of the material using mixed species to change the covering condition of the protective film when microbial corrosion is dominated by SRB.

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