

Synergistic Effect of *Iron Bacteria* and *Vibrio* on Carbon Steel Corrosion in Seawater

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The microbiologically influenced corrosion and electrochemical behavior of 45 steel were investigated in the presence of *vibrio* and *iron bacteria* using the mass loss determination, electrochemical analysis method and surface analysis method. The results showed that the corrosion of 45 steel was accelerated for the presence of the two bacterial species. 45 steel immersed in mixed medium containing both *vibrio* and *iron bacteria* showed a lower free corrosion current potential and a decrease in impedance with immersion time. Scanning electron microscopy (SEM) revealed that the mixed bacteria system caused more severe pitting corrosion of carbon steel.

Keywords: Microbiologically influenced corrosion; *Vibrio*; *Iron bacteria*; Synergistic effect

1. INTRODUCTION

Microbiologically induced corrosion (MIC) is a serious problem frequently encountered in natural marine environments. Because of the adhesion of microorganisms to metal surfaces in nature seawater, biofilms form gradually. Many studies show that the presence of biofilm leads to significant local differences in the species and concentration of inorganic and organic, dissolved oxygen levels and pH at metal/solution interface with bulk medium. These differences influence the rate of corrosion [1-6]. Hainan Province of China is located at the northern margin of tropics with typical tropical marine climate. For the higher temperature, microorganisms there grow vigorously all year around and

increase the corrosion rate. Wu et al. demonstrated that the average corrosion rate of 25 steel in natural seawater is 2.6 times higher than that exposed to sterile seawater in tropical marine environment after 365 days and various sizes of macro etch pits can be also observed on the coupons immersed in natural seawater for 365 days [7].

The preliminary work of our research group showed that *iron bacteria* and *vibrio* are discovered in large amount in corrosion products of mild carbon steel immersed in natural seawater. Large numbers of researches about single bacteria's influence on corrosion and electrochemical behavior of carbon steel by these two kinds of bacteria have been revealed [8-15]. According to the literature report, *iron bacteria* can oxidate dissolved ferrous ion to ferric ion and its metabolism on metal's surface promote the anode reaction of carbon steel accelerating the process of corrosion[16]. In addition, Nivens et al. investigated that both the growth of *vibrio* and the extracellular polymeric substance produced by it attached to the surface of steel are related to corrosive current density [17]. Although researches of the influence of single bacteria on metallic corrosion can interpret the process and mechanism of microbiologically induced corrosion to a certain extent, MIC in marine ecosystem is due to the synergistic effect of multiple bacteria rather than single one.

In present research, a comparative study of the corrosion behavior and mechanism of carbon steel in sterile, single bacteria and mixed bacteria inoculated seawater was carried out. Corrosion rate was assayed by mass loss method. To understand the corrosion process and corrosion mechanisms of mild carbon steel, electrochemical analysis (e.g., corrosion potential analysis, electrochemical impedance spectroscopy and polarization curve) was performed. SEM was used to investigate the surface morphology and the degree of corrosion damage after the removal of corrosion products. Composition of the corrosion products were determined by Energy dispersive spectroscopy (EDS).

2. EXPERIMENTAL

2.1 Preparation of coupons

The carbon steel (1045 grade) with a nominal composition of carbon 0.499 wt-%, manganese 0.596 wt-%, silicon 0.230 wt-% and balance iron was purchased from QiQiHar HongShun Heavy Industry Group Co. Ltd (China). A copper wire was soldered on one surface of the carbon steel coupon. Then, the total surface of the electrode was coated with epoxy resin leaving only the electrode surface exposed. The working area of coated electrode was 1cm^2 . Sheet coupons with a size of $50\times 25\times 3$ mm and $15\times 10\times 3$ mm were used in mass loss determination and SEM analysis respectively. The coupons were sanded sequentially by abrasive paper (120, 400, 800, 1200 and 1500) to a smooth surface then eliminated oil stain with acetone, rinsed with distilled water and ethanol, dried aseptically in air final placed into desiccators for reserve.

2.2 The cultivation of bacteria

The bacteria used in this study were separated from the corrosion products on the carbon steel coupons immersed in natural seawater for six months. *Iron bacteria* were cultured at 28 °C under

aerobic condition in ammonium ferric citrate medium (per liter of sterile sea water): ferric ammonium citrate 10 g, calcium chloride hexahydrate 0.2 g, magnesium sulfate 0.5 g, sodium nitrate 0.5 g, ammonium sulphate 0.5 g, dihydrogen phosphate 0.5 g, agar 20 g. The pH of medium was set to 7.0 with 1mol/L sodium hydroxide solution. *Vibrio* was cultivated at 37 °C under aerobic in 2216E medium (per liter of sterile sea water): peptone 5.0 g, yeast extract 1 g, agar 20 g. The pH was adjusted to 7.8 using 1 mol/L sodium hydroxide solution. The reagents used in this work were of analytical grade. Both kinds of culture media were autoclaved at 121 °C for 20 min. *Vibrio* media and *iron bacteria* media were prepared by inoculating culture media in sterile seawater with a volume ratio of 1:100 respectively. *Vibrio-iron bacteria* mixed media was prepared by the two kinds of single bacteria media with a volume ratio of 1:1. Bacteria were cultured thermostatically at 26 °C, which is the annual average temperature of Hainan Province. The media were replaced every 15 days to provide a nutrient-rich survival environment for bacteria. All the above work was completed in clean bench.

After experimental period of 1, 3, 5, 7, 15, 30 days, corrosion products which attached on sample surfaces were scraped by sterile knife and diluted with sterile water respectively. Then plate counting method was used to calculate the quantities of bacteria in corrosion product in different systems.

2.3 Mass loss determination

The corrosion products on the interface of carbon steel coupons were removed with chemical cleaning method (36% hydrochloric acid, 1 L; antimonous oxide, 20 g; stannous chloride, 50 g) then washed with distilled water and analytically pure ethanol. Finally the samples were weighed after being kept in dryer for 24 h. Three replicate coupons were used and the corrosion rate was calculated based on the data obtained from the weight loss determination. The average corrosion rate (v) was calculated from equations (1):

$$v(\text{mm/a})=(K \times W)/(A \times T \times D) \quad (1)$$

Where K is 3.65×10^3 ; W is the lost weight of samples, g; T is corrosion time, day; A is total area of samples, cm^2 ; D is the density of samples, g/cm^3 respectively.

2.4 Surface analysis

Scanning electron microscopy (SEM) and Energy dispersive spectroscopy (EDS) techniques were individually performed at the surface of carbon steel coupons immersed in different media after 7, 15, 30 days. A scanning electron microscope S-3000N with the beam voltage at 10kV was used to visualize the morphology of corrosion product film.

2.5 Electrochemical analysis

Electrochemical measurements were carried out in a three-electrode cell exposed in the four different systems and the temperature was kept at 26 °C. Potentials were measured vs. the saturated calomel electrode (SCE). All the electrochemical experiments were taken by the Princeton Applied

Research PARSTAT 2273. The scan of cyclic voltammetry were started at -1.4V/SCE up to 0.4V/SCE and then back to -1.4V/SCE, with a scan rate dE/dt of 2.0 mV/s. The Electrochemical impedance spectroscopy (EIS) was measured the free-corrosion potential with an excitation signal of 10 mV, and the test frequency in the range of 0.005 to 100000 Hz.

3. RESULTS

3.1 Microbiological analysis

Fig.1 shows the variations of bacteria counts with immersion time in different media. The quantities of bacteria in corrosion product all reached 10^5 - 10^6 CFU/g after 1 day's exposure. In *vibrio* inoculated media, after the adjustment period, from 1st to 7th day, the count of bacteria increased gradually and achieved to maximum, 2.5×10^7 CFU/g. In *iron bacteria* media, the quantity of bacteria reached maximum on the third day then decreased and tended to stability. While in *vibrio-iron bacteria* mixed system, both the adhesion to metal surfaces and the growth of *vibrio* were inhibited at initial stage. Later the bacteria count increased sharply to 7.0×10^7 CFU/g on the 7th day and then declined rapidly. The quantity of *vibrio* was about one order of magnitude lower than that in single bacteria system after 15 days and the difference value changed little with immersion time. On the contrary, the quantity of *iron bacteria* tended to stability after 7 days and was higher than that in single bacteria environment.

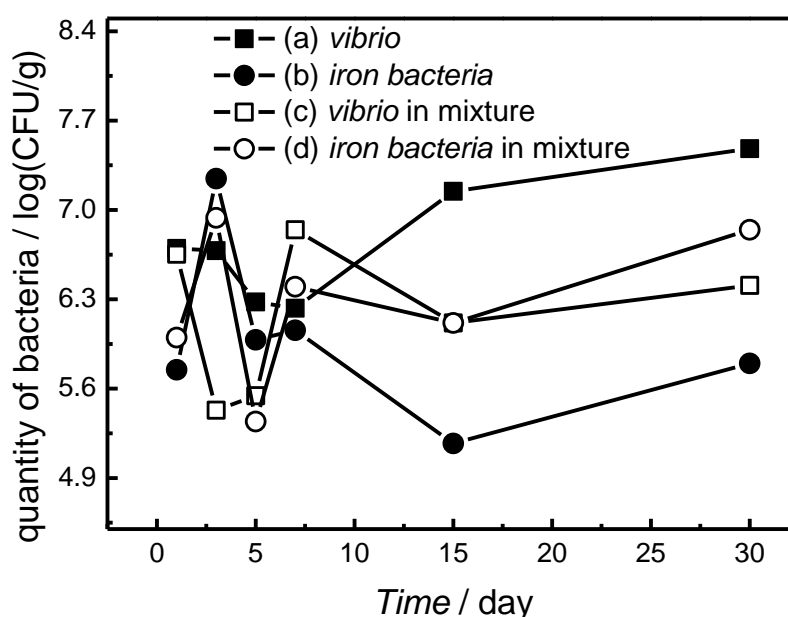


Figure 1. (a) *vibrio* and (b) *iron bacteria* contents in corrosion products in single bacteria medium and (c) *vibrio* and (d) *iron bacteria* contents in corrosion products in mixed bacteria medium with immersion time

3.2 Average corrosion rate

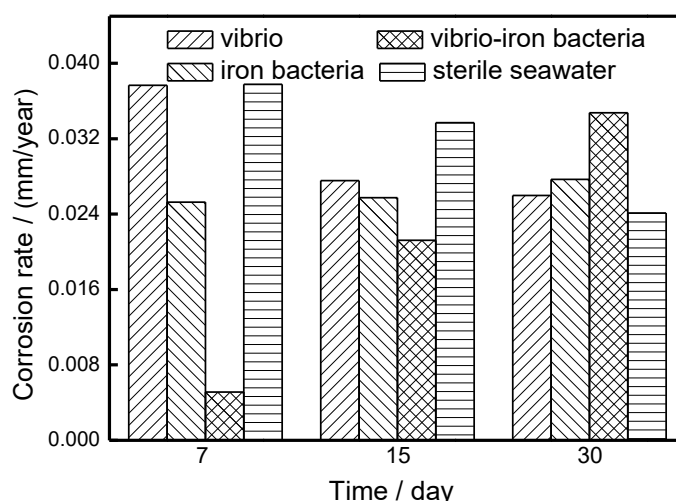


Figure 2. Histogram of corrosion mass loss rate of mild carbon steel exposed to *vibrio* media, *iron bacteria* media, *vibrio-iron bacteria* media and sterile seawater with immersion time

Fig.2 illustrates the corrosion rate vs. time experimental histogram of mild carbon steel coupons which were exposed to the four conditions respectively. The corrosion rates of coupons immersed in different systems showed different changing trend. The corrosion rate of samples exposed in sterile seawater and *vibrio* media decelerated gradually. While in *iron bacteria* and *vibrio* combination bacteria system the corrosion rates displayed oppositely changing trend. The results indicated that at the initial stage, *vibrio-iron bacteria* combination system inhibited the corrosion of carbon steel but with the prolonging of exposure time the mixed bacteria system accelerated the corrosion rate significantly.

3.3 Surface analysis

As Fig. 3a shows, the specimen exposed to *vibrio* media for 30 days was covered with a homogeneous and plain microbial film. By zooming in, large numbers of spherical and rod-shaped corrosion products were encapsulated in biofilm (Fig. 3b). The dense biofilm might inhibit general corrosion to some extent, but the differences of chemical composition and concentration between the areas attached by biofilm and surrounding environment would create favorable condition for pitting corrosion. In *iron bacteria* media, many irregular tuberculate deposits can be visible on the corrosion product layer. The tuberculate deposits mainly consisted of spherical and sheet corrosion products, which embed in biofilm and resulted in the destruction of both the stability and the corrosion resistance performance of the biofilm-corrosion products complex film. In *vibrio* and *iron bacteria* combination media, the layers covered on specimens are uniform and dense. Relatively fewer tuberculate deposits attached to the corrosion products film (Fig. 3e). By zooming in, some cracks were existed on the film (Fig. 3f). The cracks might provide routes for dissolved oxygen to metal surfaces and created local oxygen concentration cell, which aggravated local corrosion.

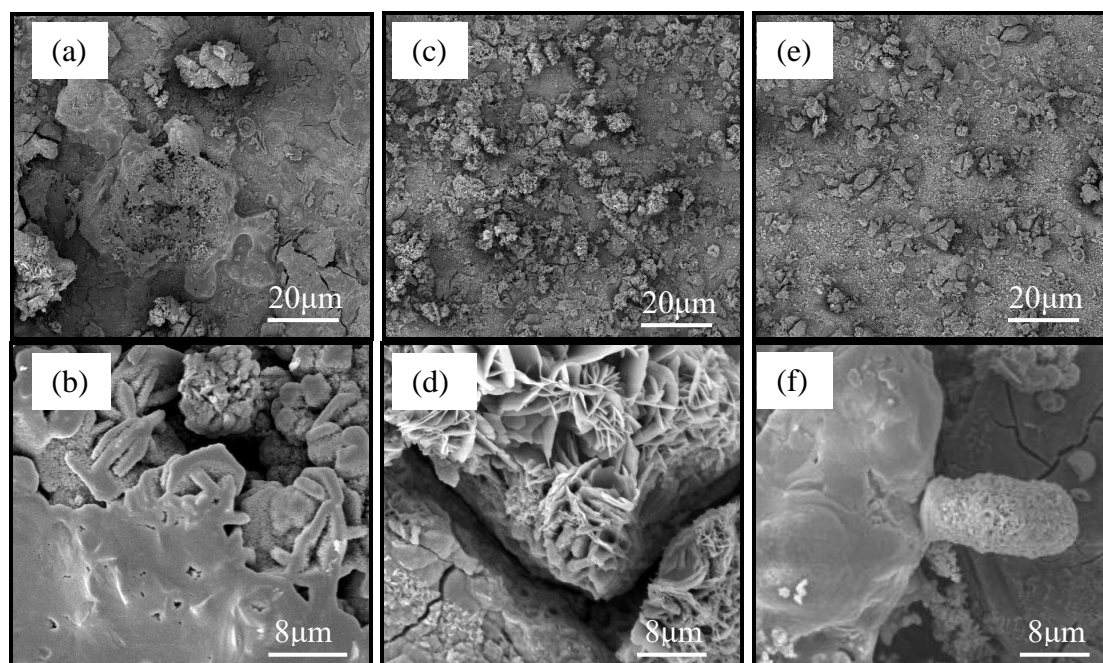


Figure 3. Corrosion morphologies of carbon steel immersed in different systems for 30 days *vibrio* (a, b), *iron bacteria* (c, d) and *vibrio-iron bacteria* (e, f)

EDS semiquantitative analysis of the deposit indicated that iron and oxygen were the main components as shown in table 1. In *vibrio* system and mixed bacteria system, the relatively higher carbon content might be caused by the organic materials of bacteria and metabolism product such as extract extracellular polymers (EPS) contained various polysaccharide and protein [18].

Table 1. EDS analysis of the 45 steel immersed in different systems for 30 days (mass%)

	Fe	O	C	Si	Ca	Na	Mg	S
<i>Vibrio</i>	52.39	36.33	6.45	1.69	1.47	1.02	0.65	-
<i>Iron bacteria</i>	55.52	37.98	1.74	0.59	-	-	1.90	-
<i>Vibrio-Iron bacteria</i>	47.24	43.57	4.40	-	0.72	0.83	1.85	1.39

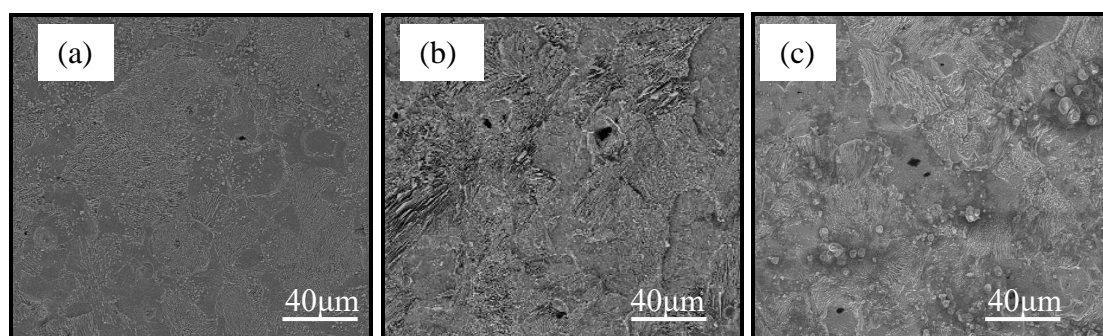


Figure 4. Surface morphology of carbon steel immersed in different systems for 30 days after removing the corrosion products (a) *vibrio*, (b) *iron bacteria* and (c) *vibrio-iron bacteria*

After removing the biofilm and corrosion products, the typical morphology of pits on the surfaces of specimen immersed in biotic systems are visible in Fig. 4. Some larger and wider pits were observed in mixture system as shown in Fig. 4c. The phenomenon demonstrated, under the same chemical and physical condition, the localized corrosion damage on the carbon steel surface immersed in the mixed bacteria systems was more severe than that in single bacteria seawater.

3.4 Electrochemical analysis

3.4.1 Open circuit potential

The open-circuit potential vs. time for carbon steel coupons exposed to four different systems are shown in Fig.6. In sterile seawater, the open-circuit potential fluctuated at the beginning stage and then gradually tended to be stable after 17 day. In biotic system the microbio attached on the coupon surfaces and formed relatively complete corrosion product films consisted of bacteria, metabolite and corrosion products. The film played a role of mechanical barrier and led to the ennoblement of the potential at initial stage. With prolonging the duration, in *vibrio* and *iron bacteria* single bacteria system the potential entered into a stable fluctuation state. While in *vibrio-iron bacteria* combination media the potential remained steady in the middle period and trended to negative direction after 21 days. This indicated that the relatively dense and homogeneous layers were destroyed and lost the protective property, which coincided with the weight lose results.

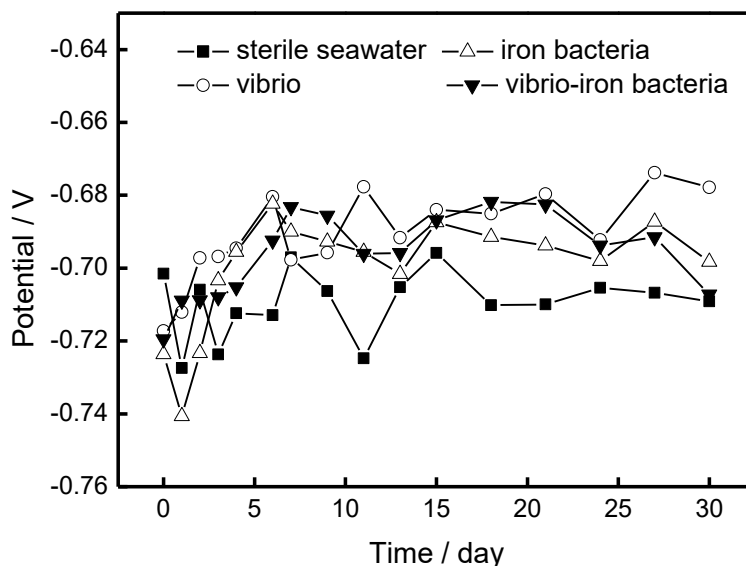


Figure 5. Evolution of the open circuit potential of carbon steel exposed to sterile seawater, *vibrio* media, *iron bacteria* media and *vibrio-iron bacteria* mixed media with immersion time

3.4.2 EIS results

Fig.6 shows the EIS for coupons immersed in different media after 0 day, 1 day, 3 days, 7 days, 15 days and 30 days.

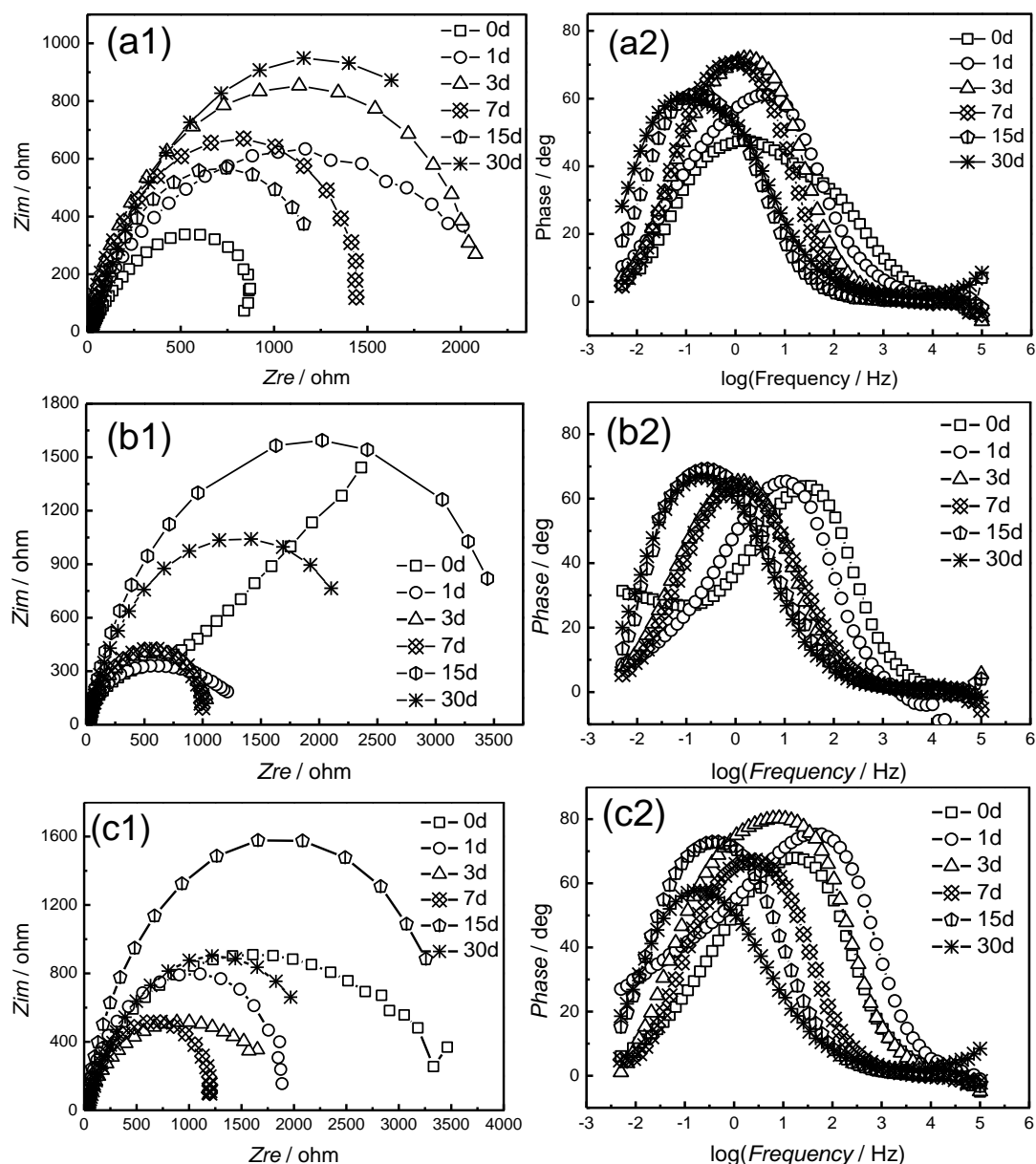


Figure 6. EIS diagrams of 45 Steel after different days of immersion in *vibrio* system (a1, a2), *iron bacteria* system (b1, b2), and *vibrio-iron bacteria* system (c1, c2)

As shown in Nyquist plot, in *vibrio* media, the impedance magnitude increased on 0-3 days, which suggested biofilm formed on the sample surface gradually and inhibited corrosion. However, the not intact biofilm led to the reaction between corrosive solution and local freshly exposed metal surface. As electrochemical impedance spectrum showed that the real part at low frequency of carbon steel contracted at the beginning of immersion time [19]. Subsequently the diameter of EIS decreased and then increased to maximum after 30 days' immersion. In *iron bacteria* media, Warburg impedance emerged at low frequency region on 0 day. The Low frequency area angle was approximately 30 degree. The Warburg impedance disappeared with the immersion time. The diameters of impedance loops in the Nyquist plots were quite the similar on 1-7 days and reached the maximum value on fifteenth day. Then the diameters of impedance loops decreased with the immersion time for the flaking of biofilm-corrosion products complex film. In *vibrio-iron bacteria* media, the capacitive

impedance loop at low frequency area was a squished capacitive impedance loop, which might be because the dispersion effect caused by the absorbents such as microbes, hydrogen ions and chloride ions absorbing on metal surface [20]. With the immersion time, the diameters of impedance loops increased first and then decreased. It reached maximum at 15th day.

As shown in Fig. 6(a2, b2, c2), two time constants were visible in Bode plots in different environments with immersion time. This phenomenon reflected that biofilm or biofilm-corrosion products complex film generated on the samples' surfaces. With the prolonging of corrosion time, the peaks moved to the lower frequency. This indicated that the integrated membranes were formed on metal surface [21].

In order to interpret the EIS spectra, ZSIMP-WIN software was utilized to fit EIS spectra data in every system. For the diffusion process caused by roughness of microscopic surface, constant phase angle element (CPE) Q was used to fit. The equivalent circuit models are shown in Fig. 7. Table 2 shows the values obtained through the simulation of the resistance of the solution (R_s), CPE of the electrical double layer (Q_{dl}), charge transfer resistance of the electrical double layer (R_{ct}); CPE of the biofilm-corrosion products complex film (Q_p), resistance of the biofilm-corrosion products complex film (R_p) and Warburg impedance (W). R_s was quite low and stable which depended on the ionic concentration, the type of ions and temperature of sea water [22]. The expressions of Faraday impedance of equivalent circuits, Z_a and Z_b , are as follows:

$$Z_a = R_s + \frac{1}{Y_p + \frac{1}{R_p} + \frac{1}{Y_{dl} + \frac{1}{R_{ct}}}} \quad (2)$$

$$Z_b = R_s + \frac{1}{Y_p + \frac{1}{R_p + Z_w} + \frac{1}{Y_{dl} + \frac{1}{R_{ct}}}} \quad (3)$$

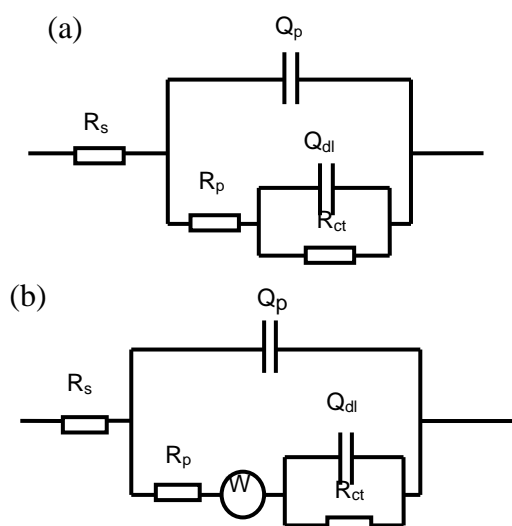


Figure 7. Equivalent circuit models for electrodes in different bacterial cultures

In *vibrio* system, Q_p first increased and then tended to stable from 0 to 15 days. The changing tendency illustrated that the adsorption equilibria on electrodes shifted to adsorption and proved biofilm formed on the electrodes' surfaces. In *iron bacteria* media, the changing trend of Q_p was quite similar as in *vibrio* system at initial stage. In *vibrio-iron bacteria* media, Q_p increased from 1 to 15 days, which indicated biofilm formed on the metal surfaces and inhibited the metal corrosion. Then for the rupture of composite membrane, Q_p decreased later.

Compared the total impedance provided by mixed bacteria system with single bacteria systems. From 0 to 7 days, total impedances of mixed bacteria system were larger than that of *iron bacteria* system after same days' immersion. Compared with *vibrio* system, the total impedances of mixed bacteria system were significantly larger at initial stage. So the synergistic effect of the two kinds of bacteria inhibited corrosion of metal. However with the prolonging of exposure time, the total impedances of mixed bacteria system were smaller than that of the two single bacteria systems gradually. Corrosion rate of samples in mixed bacteria system increased and was larger than that in single bacteria media with immersion time. This is in line with the previous results.

Table 2. Parameter values of elements in the equivalent circuit models

system	Time day	R_s $\Omega \cdot \text{cm}^2$	Q_p $\mu\text{F}^{-1} \cdot \text{cm}^{-2}$	R_p $\Omega \cdot \text{cm}^2$	Q_{dl} $\mu\text{F}^{-1} \cdot \text{cm}^{-2}$	R_{ct} $\Omega \cdot \text{cm}^2$	W $\mu\Omega \cdot \text{cm}^2$	Equivalent circuit
<i>Vibrio</i>	0d	9.422	621.5	58.2	980.6	945.1	-	(a)
	1d	10.53	826.5	848.7	1514	1297	-	(a)
	3d	10.1	980.9	930.8	937.7	1234	-	(a)
	7d	11.52	1509	744.4	970.3	761	-	(a)
	15d	19.1	4204	358.3	1768	1031	-	(a)
	30d	16.57	4064	662.1	1286	2038	-	(a)
<i>Iron bacteria</i>	0d	9.92	136.6	247	1541	570.1	505.7	(a)
	1d	9.02	744.6	0.13	265.2	1534	-	(a)
	3d	10.29	1925	719.4	3624	374	-	(a)
	7d	10.87	1603	187	149.9	882.9	-	(a)
	15d	16.99	293.5	11.39	1131	4002	-	(a)
	30d	14.95	567.6	5.763	2743	2773	-	(a)
<i>Vibrio-iron bacteria</i>	0d	10.15	151.9	702.3	384.8	3032	-	(b)
	1d	10.22	1003	1015	1361	937.6	-	(a)
	3d	20.06	587	182.9	1105	1810	-	(a)
	7d	9.584	898.5	518.5	702.2	730	-	(a)
	15d	12.85	1469	33.31	419.7	3710	-	(a)
	30d	25.07	1253	23.73	1418	2595	-	(a)

3.4.3 Polarization Curves

Polarization curves of carbon steel immersed in different media for 5 days and 30 days are shown in Fig. 8. In the three systems, corrosion potential moved negatively after 30 days' immersion, and the phenomenon in mixed bacteria system was especially obvious. The corrosion potential decreased from -0.895 V on 5th day to -0.976 V on 30th day. To further analyze the corrosion states of

metal in different environments. By fitting the data of weak polarization region, which located between the linearized current regions and the Tafel regions, electrochemical corrosion parameters such as corrosion current density (I_{corr}), cathodic (β_b) and anodic (β_a) Tafel slopes are given in table 3. The corresponding coefficients of determination (R^2) were good ($R^2 > 0.99$). After 5 days' exposure, I_{corr} of electrodes in mixed bacteria system was smaller than that in single bacteria systems and increased with immersion time. The cathodic Tafel slope increased in the presence of *iron bacteria* after 30 days in both single bacteria and mixed bacteria systems.

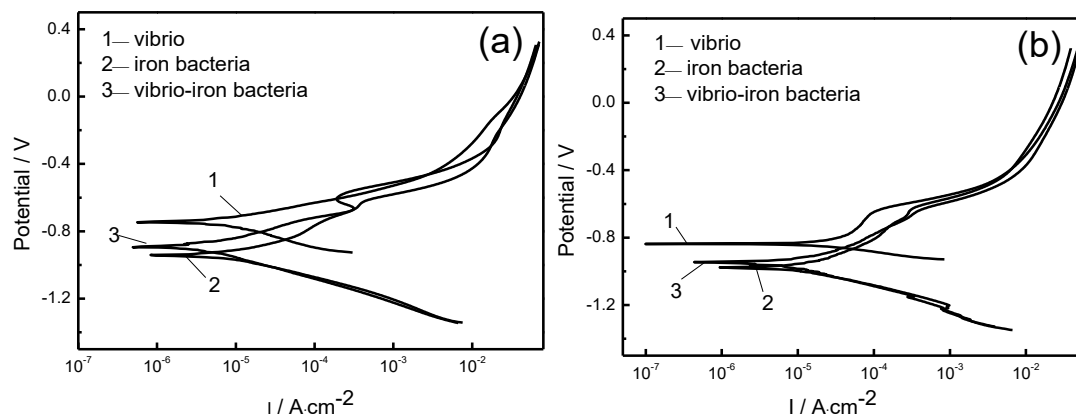


Figure 8. Polarization curves of the 45 steel in different systems after 5 days (a) and 30 days (b)

Table 3. Tafel analysis of polarization curves of the 45 steel in different systems after 5 days and 30 days

system.	5 d				30 d			
	$I_{\text{corr}}/(\mu\text{A}\cdot\text{cm}^{-2})$	β_a/mV	β_b/mV	R^2	$I_{\text{corr}}/(\mu\text{A}\cdot\text{cm}^{-2})$	β_a/mV	β_b/mV	R^2
<i>vibrio</i>	6.69	44.8	49.8	0.9974	30.31	109.22	33.4	0.9996
<i>Iron bacteria</i>	10.48	60.22	62.85	0.9997	18.07	74.29	177.85	0.9955
mixture	4.19	54.63	59.46	0.9960	11.34	89.16	85.68	0.9990

4. DISCUSSION

The results of this study manifest that under the same physical and chemical condition, the presence of microbe inhibited corrosion at the initial stage. Corrosion rate of metal in mixed bacteria system was significantly lower than that in single bacteria systems after 7 day's immersion. It was 13.6% and 20.3% of the corrosion rate in *vibrio* system and *iron bacteria* system respectively. This is due to *iron bacteria* consuming oxygen during normal metabolism. Otherwise as facultative anaerobe, *vibrio* consume the oxygen rapidly for respiration when oxygen concentration is high [23]. While in the low oxygen concentration, fermentation metabolism is the main metabolism type. So at the initial stage of this experiment, the presence of *vibrio* can decrease the oxygen content at metal/medium

interface rapidly. By test, dissolved oxygen content in mixed bacteria media was 1.55 mg/L, which was only 25.0% of sterile seawater after 3 days' exposure. The reduction of oxygen in large amount reduced the rate of cathodic process and retarded the corrosion of metal [24]. There was no obvious effect on corrosion rate of samples in *vibrio* media at initial stage. This is because *vibrio* is a kind of acid formation bacteria. Its metabolites are corrosive, which can worsen media environment and accelerate corrosion rate of metal. Meanwhile *vibrio* can influence pitting corrosion by reducing the local pH where it attached to. In mixed bacteria system, *iron bacteria* get energy by oxidating dissolved ferrous ion to ferric ion in metabolic processes and form ferric hydroxide, which deposit on the metal surface [25]. Iron hydroxide reacts with acid and reduces the concentration of hydrogen ions, which decreases the effect of *vibrio* and control the process of corrosion.

With the prolonging of immersion time, a relatively complete biofilm-corrosion products complex film formed on metal surface and hindered the mass transfer processes. Corrosion rates of metal in biotic systems were all lower than that in sterile seawater after 15 day's immersion. In mixed bacteria system, the presence of the two kinds of bacteria consume a large amount of oxygen and the lower oxygen concentration provide nice living condition for *iron bacteria*. The *iron bacteria* contents in corrosion products increased gradually after 7 days and could reach one order larger than that in single bacteria system after same days' immersion. For the presence of large amount of *iron bacteria*, they produce iron hydroxide which attached on metal surfaces as rusty slime. The layer on the samples' surfaces form a diffusion barrier to resistance the effect of erosive ions. As shown in the analysis results of EIS, the value of total impedance reached to maximum for 15 days' immersion in mixed bacteria system.

At the last stage, in *vibrio-iron bacteria* media, the dense layer attached on metal surfaces may lead to significant decrease of oxygen concentration at carbon steel/solution interface which resulted in a rate reduction of cathodic process and a shift of E_{corr} to negative values during specimen exposure (Fig. 8b). Organic acids secreted by *vibrio* can reduce the localized pH on coupons surface. The deposit on coupons surface retard the diffusion of hydrogen ions, creating hydrogen ion concentration cells and promote the localized concentration cell corrosion. Meanwhile, iron hydroxide, obtained as a result of metabolism of *iron bacteria*, binded with EPS and formed irregular tuberculate deposits covering on the metal surface finally [26]. Tuberculate deposits acted as diffusion barriers. The anaerobic area (anode) under the localized deposit and the oxygen-enriched area (cathode) surround it create the oxygen concentration cells which induce the severe localized corrosion (Fig. 4c). At the same time, a large number of spherical corrosion products piled up in biofilm and extruded with each other for inner stress. This led to the rupture of biofilm and oxygen concentration cells can easy formed around the cracks increasing corrosion tendency. In electrochemical analysis, in *vibrio-iron bacteria* combination media the potential trended to negative direction after 21 days and the total impedance reduced relatively after 30 days' immersion. By the comprehensive analysis above, with the prolonging of exposure time, corrosion rate increased gradually. The synergistic effects of the two kinds of bacteria promote the corrosion of metal.

4. CONCLUSIONS

Experiment results show that at the initial stage, the aggregation of *vibrio* and *iron bacteria* inhibited the corrosion of mild carbon steel, while with the prolonging of time the corrosion rate increased gradually. The corrosion rates of coupons in mixed bacteria media were 13.6% and 133.8% of that in sterile seawater after 7 days' and 30 days' immersion respectively.

SEM images illustrate that iron hydroxide binded with EPS and formed irregular tuberculate deposits covering on the metal surface in mixed bacteria system. And some cracks were existed on the film which might lead to severely pitting corrosion.

Electrochemical results show the synergy of *vibrio* and *iron bacteria* ennobled the open circuit potential at initial stage and then the potential shift negative after 21 days. EIS results show that for the formation of biofilm on metal surfaces, Q_p , CPE of the biofilm-corrosion products complex film increased gradually from 1 to 15 day. Then the rupture of complex film led to the decrease of Q_p . At the same time, the total impedance of mixed media system at 30 day reduced compared with that at 15 day. These illustrate the presence of *vibrio* and *iron bacteria* accelerate corrosion rate of coupons with the prolonging of exposure time.

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