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Research article

Effect of *Pseudomonas aeruginosa* on corrosion of X65 pipeline steel

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ABSTRACT

Microbiologically influenced corrosion (MIC) has caused great losses to many industries. This paper aimed to study the corrosion behavior of *P. aeruginosa* on X65 steel. The corrosion behavior of *P. aeruginosa* on X65 steel under aerobic and anaerobic conditions was studied by scanning electron microscopy, energy dispersive spectrometer and electrochemical analysis techniques. The results showed that the corrosion rate of X65 steel in bacterial environment is higher than that in sterile environment. In anaerobic environment, the corrosion of *P. aeruginosa* is mainly secreted acidic metabolites, and alkaline substances are corroded in aerobic environment. In general, the corrosion of X65 steel by *P. aeruginosa* in aerobic environment is more serious than that in anaerobic environment.

1. Introduction

Corrosion is a worldwide problem and brings huge economic consequences to all walks of life [1]. The oil and gas industry can bring considerable economic benefits, in recent years it has cost a fortune to replace or repair machines each year because of corrosion problems [2].

Microbiologically influenced corrosion (MIC) is the direct or indirect corrosion reaction of metals caused by the life activities of microorganisms attached to the surface of the material [3, 4]. Microbial corrosion widely exists in petroleum and natural gas industry and marine engineering. It is estimated that about 20% of corrosion losses are caused by microbial corrosion [5], and 34% of the oil companies' corrosion loss is related to microbial corrosion [6]. According to statistics, the annual corrosion cost in the world is about $4 trillion [7], and that in China is about $341 billion [8], resulting in huge economic losses.

Bacteria are single-cell organisms without real nuclei, and they are also the most important microorganisms involved in microbial corrosion. They are widely distributed in soil, water, air, food and skin [9]. Sulfate-reducing bacteria [10], sulfur-oxidizing bacteria [11], nitrate-reducing bacteria [12], acid-producing bacteria [13], iron-oxidizing bacteria [14] and iron-reducing bacteria [15] are all related to microbiologically influenced corrosion. The main cause of microbial corrosion of metal materials is the formation of biofilm on the surface of metal materials [10]. Biofilms consist of embedded sessile cells, extracellular polymeric substances (EPS), some organic and inorganic substances [16]. Bioelectrochemical studies have shown that bacteria attached to biofilms obtain electrons directly or indirectly from metal materials, leading to metal corrosion [17, 18]. Moreover, the metabolic activities of microorganisms can form various sites at the biofilm or metal interface, which are obviously different from other adjacent sites in chemical and physical properties, thus accelerating or slowing down the corrosion process of metals [19, 20].

Pseudomonas aeruginosa widely exists in natural environments such as soil, ocean and freshwater [21], and they are also abundant in oil pipelines [22]. *P. aeruginosa* is also the main microbial community causing microbial corrosion of steel materials in the marine environment [23]. Various studies have been reported that it can accelerate the corrosion of low carbon steel, stainless steel and aluminum alloy [24]. Therefore, the effect of *P. aeruginosa* on the corrosion of steel materials has attracted more and more attention. *P. aeruginosa* is a facultative anaerobic bacterium, which can provide energy to itself by nitrate as electron acceptor in anaerobic environment [12], When nitrate acts as an electron acceptor, nitrate can be reduced to NH_4^+ or N_2. The reactions involved are as follows:

$$NO_3^- + 8e^- + 10H^+ \rightarrow NH_4^+ + 3H_2O \quad (1)$$

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O \quad (2)$$

Although a large number of literatures have studied the corrosion effect of *P. aeruginosa* on steel materials, most of them are in the aerobic

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Table 1. The elementary composition of the X65 steel.

Elements	Mn	Mo	Si	Cr	Cu	Nb	C	V	Ti	Al	Ni	Fe
Wt%	1.56	0.39	0.23	0.18	0.16	0.05	0.1	0.03	0.03	0.03	0.01	Bal.

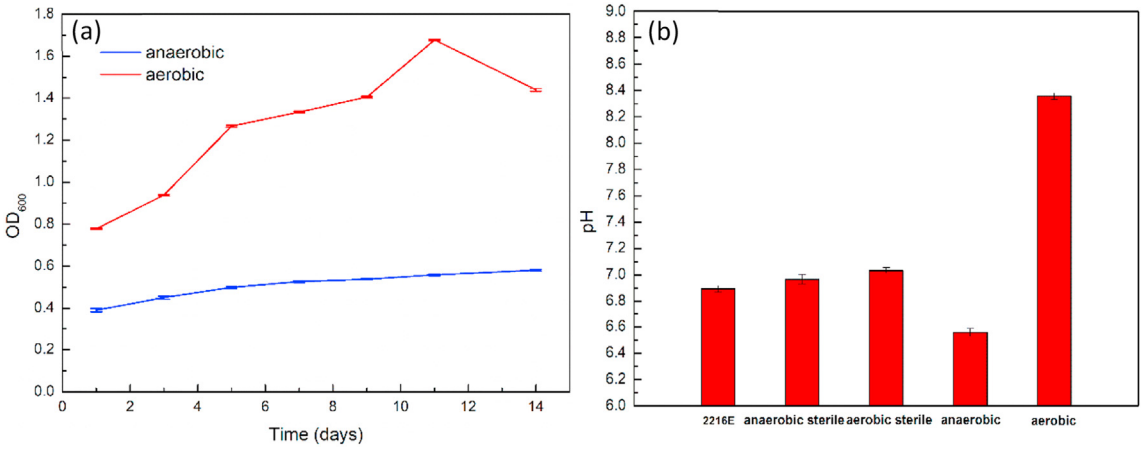

Figure 1. Growth curve of *P. aeruginosa* and *pH* value of medium with X65 steel immersed in different conditions for 14 days.

environment. There is still an anaerobic environment in the seawater environment, so the effect of *P. aeruginosa* on the corrosion of steel materials in an anaerobic environment needs to be studied. Pipeline steel is mainly used for automotive applications, and also plays an important role in the field of energy. The pipelines built are one of the most viable means of energy transportation in the world today and are a strategic tool for energy transportation [25, 26]. X65 pipeline steel has excellent properties, including high strength, high toughness, good weldability, excellent formability and corrosion cracking resistance and low production cost. It has been widely used in the oil and gas industry [27, 28]. X65 pipeline steel is the main material of submarine oil and gas pipeline. The pipeline constructed by this material is mainly used for transporting oil, natural gas and explosive medium [29]. But X65 pipeline steel is prone to serious corrosion in harsh seawater environment, especially microbial corrosion [30]. So, it is necessary to study the effect of microorganism on corrosion of X65 pipeline steel. In this paper, *P. aeruginosa* was cultured in aerobic and anaerobic environments respectively, and the corrosion effects of *P. aeruginosa* on X65 pipeline steel under aerobic and anaerobic conditions were mainly studied.

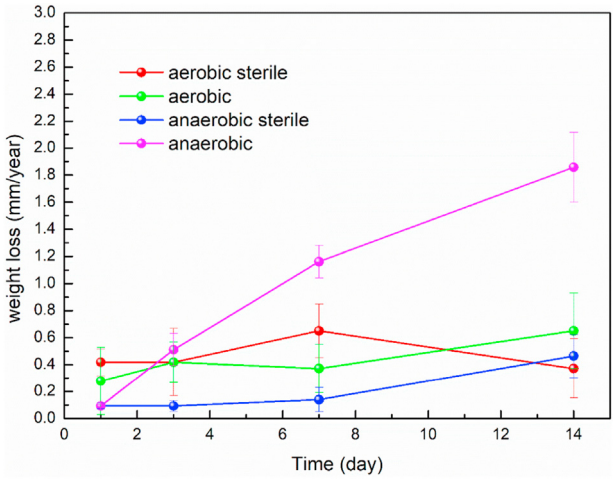

Figure 2. Weight loss results of X65 steel immersed in different media for 1, 3, 7 and 14 days.

2. Experimental

2.1. Experimental materials and solutions

All samples used in this study are X65 pipeline steel, the elements (wt %) of this steel are in Table 1. The sample size is 10 mm × 10 mm × 3 mm. Top surface of soaking sample (10 mm × 10 mm) is working face. Before soaking, sandpaper is used to treat the sample working face. The sample was cleaned by acetone ultrasonic. The electrochemical sample retains the top (10 mm × 10 mm) as the working face and welds the copper wire at the bottom sealed by epoxy resin, and the working face was treated with sandpapers. The sample was cleaned by acetone ultrasonic before the experiment, and then transferred to ultraviolet light for 1 h.

P. aeruginosa (MCCC 1A00099) used in the experiment was purchased from China Marine Microbes Conservation and Management Center. *P. aeruginosa* was packaged with glycerol and stored in liquid nitrogen at −80 °C. Before using, it was resuscitated in 2216E medium at 37 °C.

The solution used in the immersion experiment was 2216E medium. The chemical reagents used are analytical purity, and the solvent is deionized water. Before the experiment, the vessels, tweezers and liquid medium used in the experiment were sterilized by high temperature and high-pressure steam at 121 °C for 20 min, and then transferred to the ultraviolet lamp for 1 h. The anaerobically experiments were performed in a glove box (Smart 800/600, Eminex). The optical density at 600 nm (OD600) was adopted to characterize the growth of *P. aeruginosa* strain, and was measured by an ultraviolet spectrophotometer (Thermo Fisher, Bio Mate3S). To study the effect of metabolites, the pH value of the culture medium was continuously monitored by a pH meter (Mettler Toledo, S220-B). All measurements were performed in triplicate.

2.2. Weight loss tests

The required samples were weighed and recorded before the experiment, and the samples were taken out after soaking for 1, 3, 7 and 14 days, respectively. The rust remover was prepared by nitric acid, hexamethylenetetramine and deionized water to remove the biofilm and corrosion products on the surface of X65 steel. Then the samples were washed by deionized water and anhydrous ethanol, and finally the samples were dried. The weight of the sample after drying was weighed and recorded.

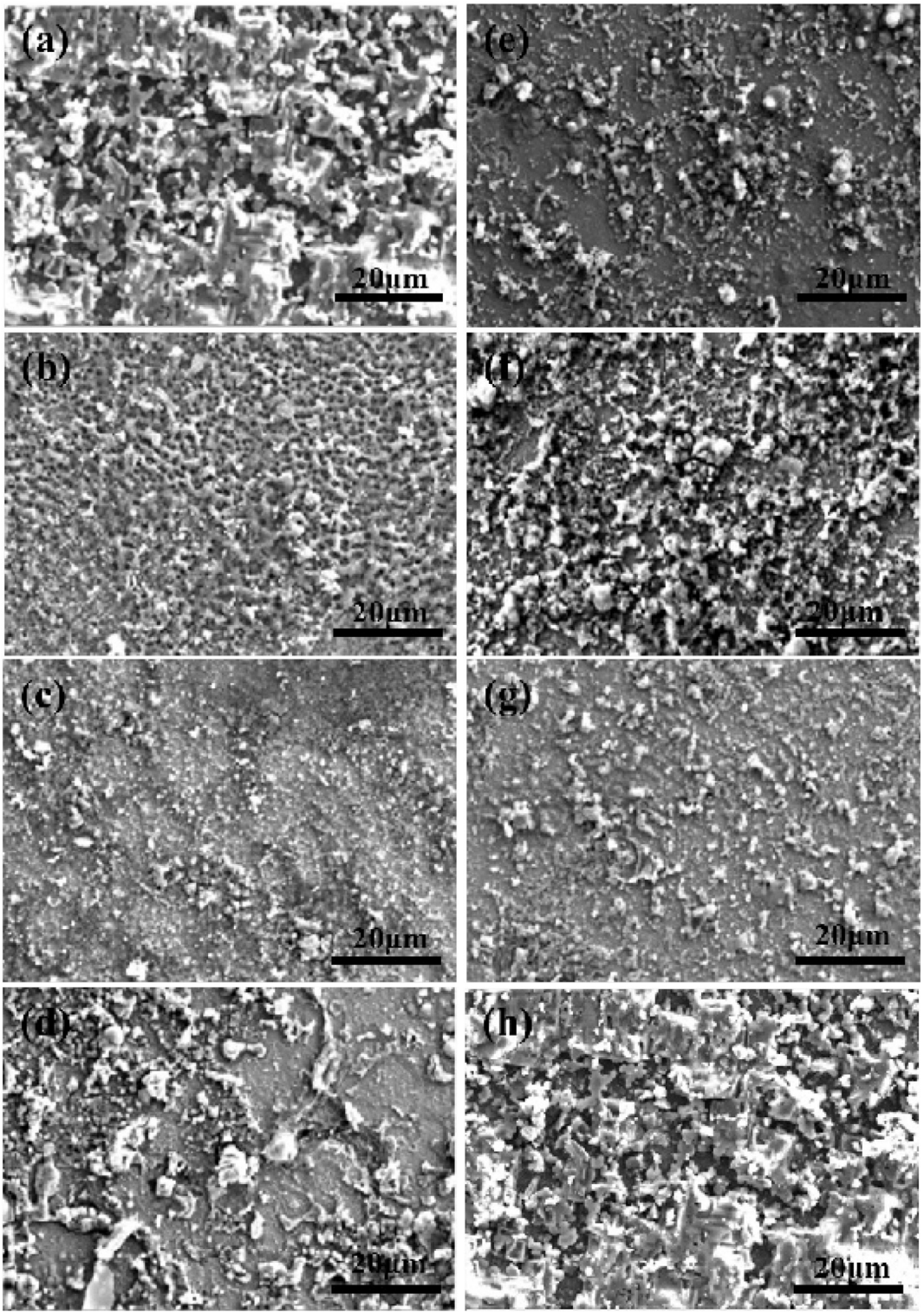

Figure 3. SEM images of X65 steel immersed in sterile anaerobic (a, b, c, d) and aerobic (e, f, g, h) conditions for 1, 3, 7, 14 days.

2.3. Surface analysis

The cell morphology and corrosion product film were observed by scanning electron microscope (SEM, Quanta FEG 250). The samples soaked in different cycles were taken out and rinsed with PBS to remove the floating *P. aeruginosa* cells and impurities on the sample surface. Firstly, the biofilm on the surface of the sample was fixed, and the sample was immersed in 2.5% glutaraldehyde solution for 24 h and stored in refrigerator at low temperature. After the samples were taken out, the bacteria were dehydrated step by step with different concentrations of ethanol solution. The concentration gradient was 50%, 60%, 70%, 80%, 90% and 100%, respectively. The dehydration time of each concentration gradient was 10 min [27]. Take out the sample and naturally dry, then vacuum and save. Before SEM observation, the sample surface was sprayed with gold to increase the conductivity.

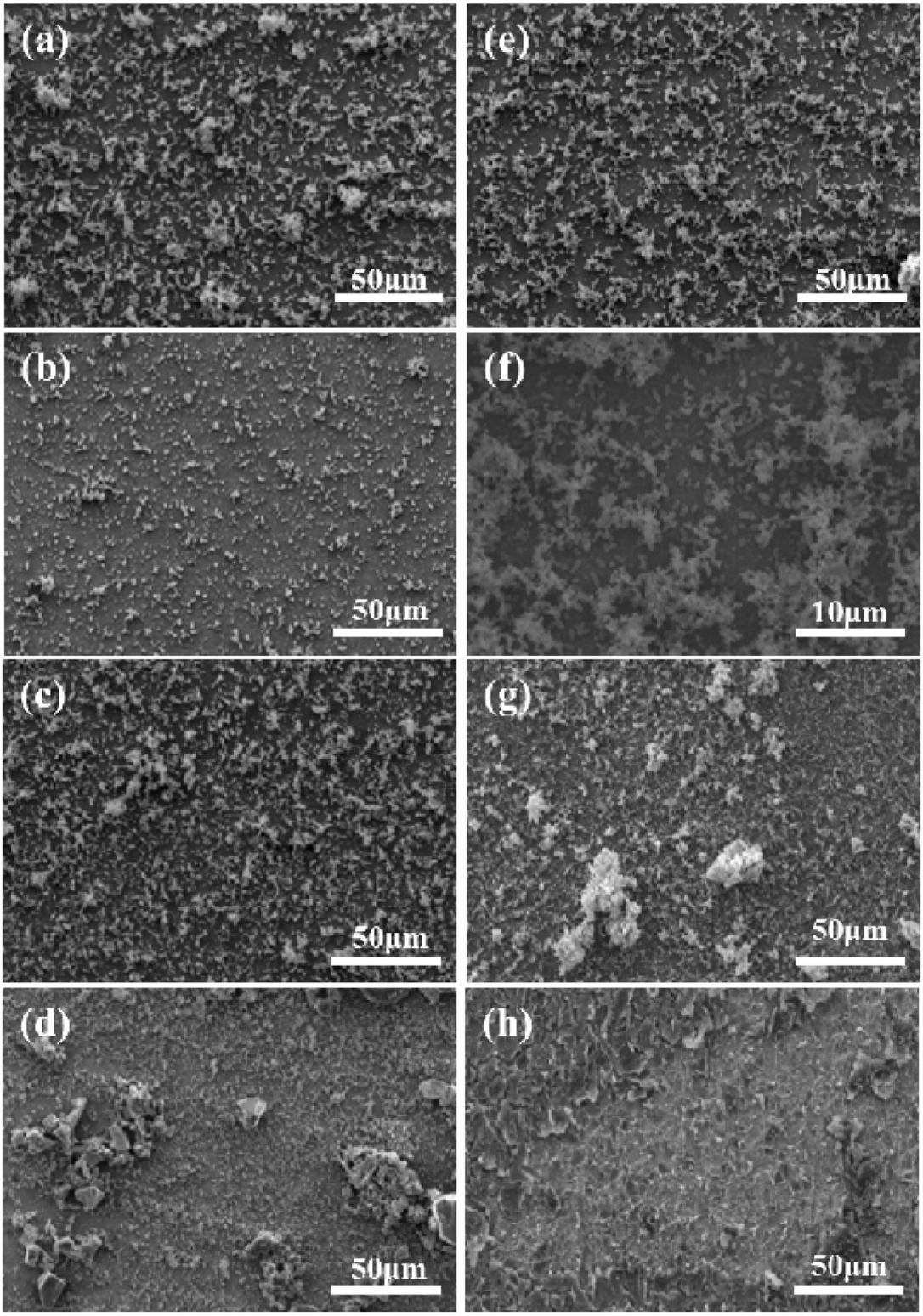

Figure 4. SEM images of X65 steel immersed in anaerobic (a, b, c, d) and aerobic (e, f, g, h) medium inoculated with *P. aeruginosa* for 1, 3, 7, 14 days.

2.4. Electrochemical measurements

Electrochemical experiments were performed by electrochemical workstation (Shanghai Chenhua). Three-electrode system was used in the test. The working electrode was X65 steel sealed with epoxy resin, the counter electrode was graphite electrode, and the reference electrode was saturated calomel electrode. Firstly, open circuit potential (OCP) was measured, then electrochemical impedance spectroscopy (EIS) test was operated, the test frequency range is 100,000 Hz–0.01 Hz, sinusoidal disturbance amplitude is 0.05 V. The EIS data were processed by ZSimpWin software to establish the equivalent circuit model. Each group was tested three times to ensure repeatability.

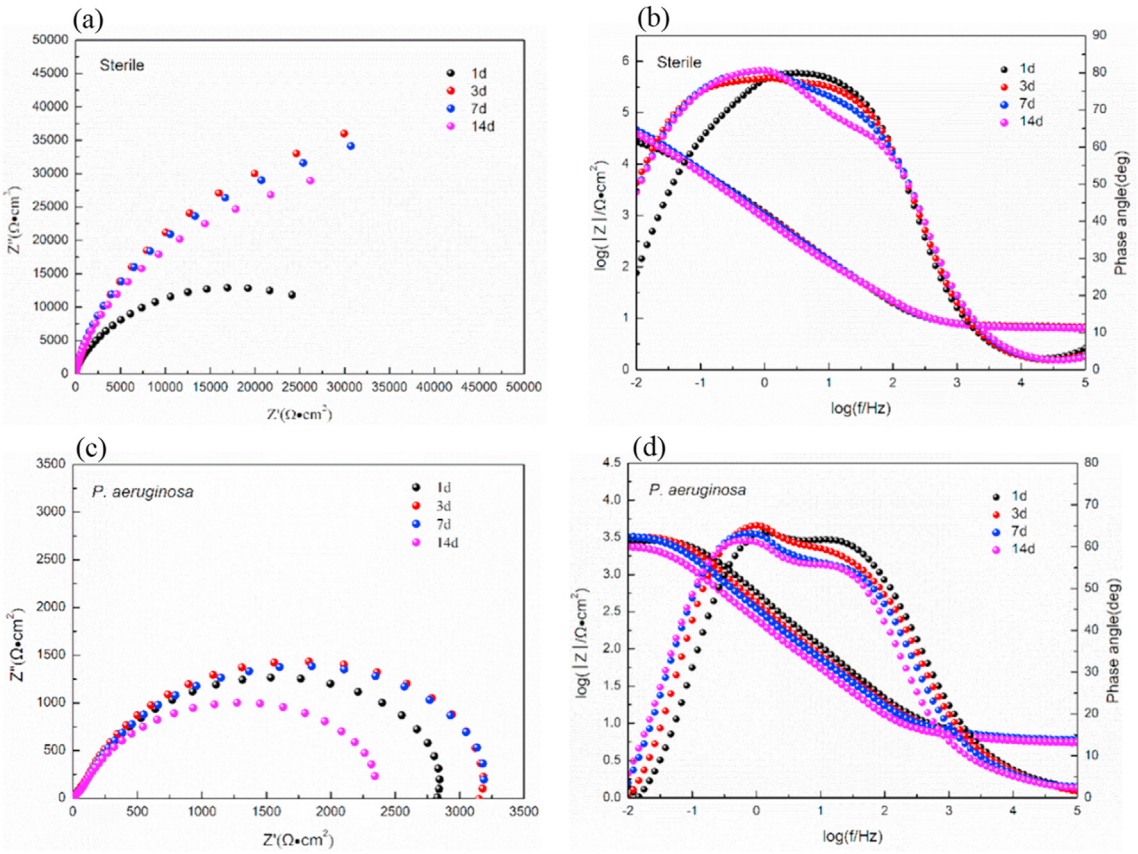

Figure 5. EIS plots of X65 steel immersed in aerobic sterile (a, b) and *P. aeruginosa* (c, d) medium for different time periods.

2.5. pH analysis

After the samples soaked in different cycles were taken out, 20 mL was poured out from the remaining solution into the sub-bottle. First, calibrate the pH meter (PHS-3CB) before each use, then measure the pH of the solution and record it.

3. Results and discussion

3.1. Growth of P. aeruginosa

Figure 1a shows the growth curve of *P. aeruginosa* in its aerobic and anaerobic culture medium. An exponential growth stage appeared within days, the concentration of the *P. aeruginosa* cells in the culture medium reached its maximum value on the 11th day in aerobic culture while on the 14th day in anaerobic culture.

Figure 1b shows the *pH* values of 2216E medium and X65 steel immersed in sterile anaerobic, sterile aerobic, *P. aeruginosa* anaerobic and *P. aeruginosa* aerobic conditions for 14 days, respectively. The pH value of the sterile group was stable at about 7, which was not significantly different from that of 2216E, indicating that there was no component in the solution that could affect the *pH* of the medium without the participation of *P. aeruginosa* bacteria. The culture medium of X65 steel immersed in anaerobic condition of *P. aeruginosa* was acidic, indicating that anaerobic respiration of *P. aeruginosa* produced acid metabolites and caused corrosion of X65 steel. In aerobic condition, the *pH* of the culture medium was significantly alkaline, indicating that *P. aeruginosa*, as a nitrate-reducing bacteria, produced ammonia under aerobic conditions, which was an alkaline substance, resulting in corrosion of X65 steel.

3.2. Weight loss tests

Figure 2 shows the weight loss of X65 steel after 1, 3, 7 and 14 days of immersion in sterile anaerobic, sterile aerobic, *P. aeruginosa* anaerobic and *P. aeruginosa* aerobic conditions. With the increase of immersion time, the weight loss values of the sterile group were maintained at a small value within the error range of the balance. The weight loss value of the samples inoculated with *P. aeruginosa* has been increasing under anaerobic conditions. Under aerobic conditions, although the weight loss value of the first three days was basically unchanged, the weight loss value increased after 7 days. The results showed that *P. aeruginosa* cause corrosion of X65 steel, the corrosion of X65 steel by *P. aeruginosa* was more severe after 7 days of inoculation under anaerobic conditions.

3.3. Surface analysis

Figure 3 shows the SEM images of X65 steel immersed in sterile anaerobic and aerobic conditions for 1, 3, 7, 14 days. It can be seen that the corrosion products are relatively uniform after soaking for 1, 3, 7 and 14 days in sterile environment, whether in aerobic or anaerobic conditions, indicating that the corrosion rate of steel is not significantly increased or decreased under this condition.

Figure 4 shows the SEM images of X65 steel immersed in the medium inoculated with *P. aeruginosa* for 1, 3, 7 and 14 days under anaerobic and aerobic conditions. The biofilm on the surface of X65 steel is mainly composed of surface bacteria and extracellular polymers [28] and superimposed with corrosion products to form a composite product film, which is distributed in clusters on the surface of X65 steel. In the presence of bacteria and anaerobic conditions, the corrosion product clusters on the steel surface decreased from day 1 to day 3 and increased from day 3

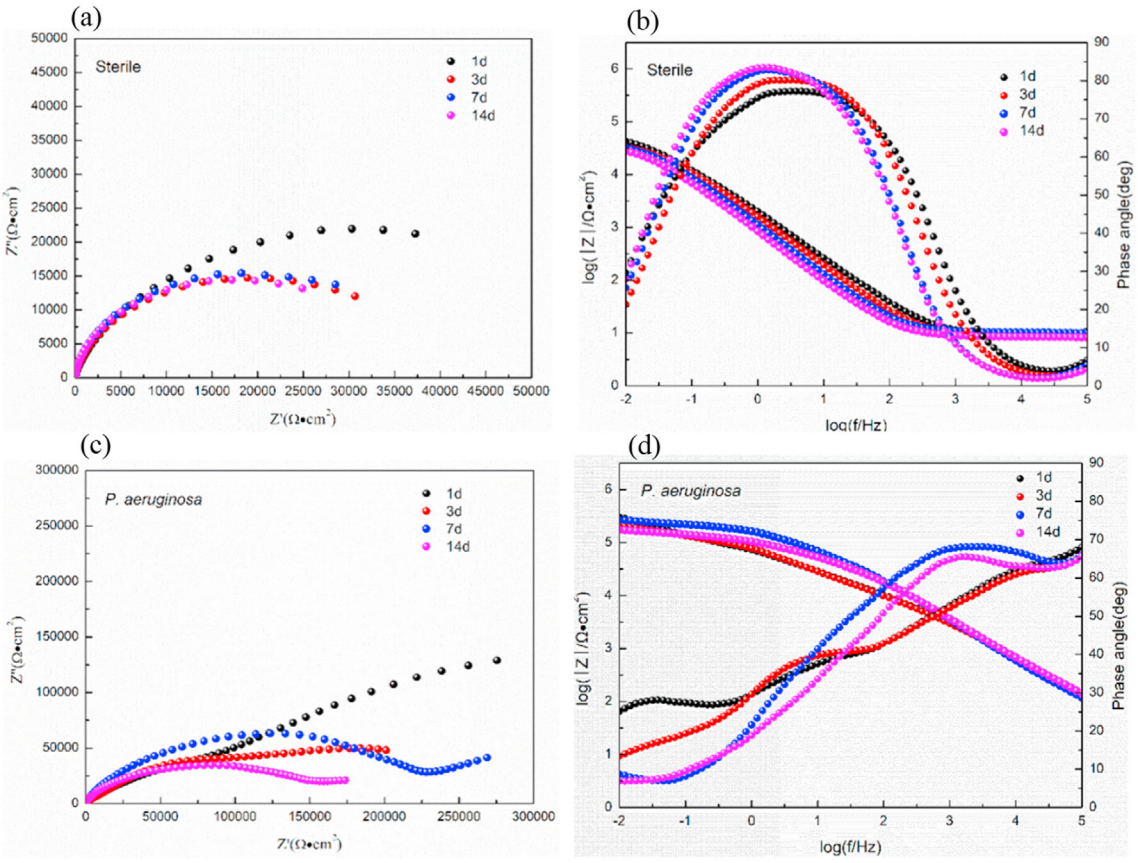

Figure 6. EIS plots of X65 steel immersed in aerobic sterile (a, b) and *P. aeruginosa* (c, d) medium for different time periods.

to day 14, indicating that the corrosion products were the least on day 3, the corrosion rate decreased from day 1 to day 3 and increased from day 3 to day 14. Under aerobic conditions, the corrosion product clusters on the steel surface increased from day 1 to day 3, indicating that the corrosion rate on day 3 was higher than that on day 1. On the seventh day, the corrosion products on the steel surface are denser, which may protect the steel and slow down the corrosion rate. On the 14th day, *P. aeruginosa* had been unable to survive under aerobic conditions, so only a layer of embroidery could be seen on the steel surface.

3.4. Electrochemical analysis

EIS is performed under stable OCPs. Figures 5 and 6 are the changes of Nyquist diagram and Bode diagram of X65 steel immersed in different culture conditions for 1,3,7 and 14 days. In EIS spectra, the change of Nyquist diameter and Bode phase angle can directly reflect the corrosion rate [29]. It can be seen from the Nyquist plot that the diameter of the Nyquist ring of the medium inoculated with *P. aeruginosa* was smaller than that of the sterile medium. The results showed that *P. aeruginosa* promoted the corrosion process of X65 steel, which was consistent with the weight loss test results.

Under sterile and anaerobic conditions, the diameter of Nyquist ring first increased and then decreased, with the largest diameter at day 3, indicating that X65 steel is the most resistant to corrosion on the third day. Under sterile aerobic conditions, the diameter of Nyquist ring decreases continuously, indicating that the corrosion of X65 steel is increasing. Under the condition of bacteria and anaerobic, the diameter of Nyquist ring first increased and then decreased, and the diameter was

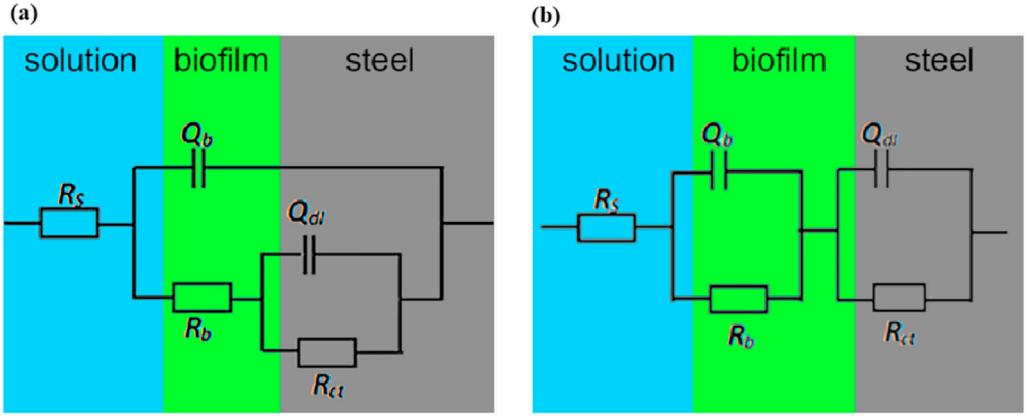

Figure 7. Electrochemical equivalent circuit used to fit EIS data.

Table 2. EIS fitting parameters of X65 steel immersed in aerobic sterile and *P. aeruginosa* medium for different time periods.

Duration/day	R_s (Ω cm^2)	Q_f (Ω^{-1} cm^{-2} s^n)	R_f (Ω cm^2)	Q_{dl} (Ω^{-1} cm^{-2} s^n)	R_{ct} (Ω cm^2)
Sterile					
1	6.3 ± 0.4	5.9E-05 ± 4.7E-06	1.8E + 04 ± 1.6E + 03	1.6E-04 ± 6.2E-05	3.9E + 04 ± 2.8E + 03
3	7.2 ± 0.6	7.7E-05 ± 5.9E-06	8.7E + 03 ± 1.5E + 02	3.4E-04 ± 4.1E-05	3.6E + 04 ± 1.6E + 03
7	8.0 ± 0.5	1.2E-04 ± 3.1E-05	2.3E + 00 ± 8.2E-01	1.6E-04 ± 3.8E-07	3.2E + 04 ± 4.0E + 02
14	7.8 ± 0.9	6.8E-04 ± 1.4E-05	6.1E + 00 ± 5.3E-01	1.1E-04 ± 1.1E-06	2.3E + 04 ± 3.9E + 03
P. aeruginosa					
1	7.0 ± 0.6	1.4E-06 ± 9.7E-07	4.3E + 04 ± 3.2E + 03	9.7E-06 ± 1.6E-06	4.0E + 05 ± 2.1E + 03
3	6.9 ± 1.1	9.7E-06 ± 1.2E-06	8.2E + 04 ± 7.2E + 03	1.2E-05 ± 2.2E-06	6.3E + 04 ± 8.3E + 02
7	5.6 ± 1.5	1.2E-05 ± 7.1E-06	9.5E + 04 ± 5.5E + 03	1.4E-05 ± 4.3E-06	1.8E + 04 ± 2.4E + 02
14	7.2 ± 0.8	1.6E-05 ± 1.0E-05	1.5E + 05 ± 7.9E + 03	1.7E-05 ± 1.4E-06	1.9E + 04 ± 8.9E + 02

Table 3. EIS fitting parameters of X65 steel immersed in anaerobic sterile and *P. aeruginosa* medium for different time periods.

Duration/day	R_s (Ω cm^2)	Q_f (Ω^{-1} cm^{-2} s^n)	R_f (Ω cm^2)	Q_{dl} (Ω^{-1} cm^{-2} s^n)	R_{ct} (Ω cm^2)
Sterile					
1	6.7 ± 0.2	1.8E-05 ± 3.9E-06	1.8 ± 0.6	5.5E-04 ± 1.3E-05	7.5E + 03 ± 3.6E + 03
3	6.2 ± 0.3	4.2E-05 ± 3.7E-06	2.5 ± 0.4	4.5E-04 ± 2.6E-05	8.3E + 04 ± 1.0E + 02
7	6.4 ± 0.8	1.8E-04 ± 5.1E-05	28.3 ± 8.2	3.1E-05 ± 2.8E-06	7.1E + 04 ± 1.1E + 02
14	6.4 ± 0.6	1.9E-04 ± 5.2E-05	36.2 ± 5.3	2.9E-05 ± 8.9E-06	6.2E + 04 ± 8.3E + 02
P. aeruginosa					
1	5.9 ± 0.2	4.4E-05 ± 1.2E-05	1.8E + 03 ± 2.4E + 02	6.9E-04 ± 1.6E-05	4.1E + 02 ± 2.1E + 01
3	5.6 ± 0.4	7.4E-05 ± 2.1E-06	2.1E + 03 ± 1.3E + 02	8.7E-04 ± 2.2E-05	3.5E + 02 ± 8.3E + 00
7	6.0 ± 0.2	7.4E-05 ± 8.1E-06	2.2E + 03 ± 3.3E + 02	1.3E-03 ± 9.3E-06	1.9E + 02 ± 2.4E + 00
14	5.6 ± 0.2	1.2E-04 ± 5.6E-05	2.9E + 03 ± 2.0E + 02	1.6E-03 ± 1.4E-05	1.7E + 02 ± 8.9E + 00

the largest on the third day, indicating that the corrosion of X65 steel experienced a process of first decreasing and then increasing. It can be seen from the phase angle diagram of Bode diagram (Figure 5c) that the phase angle decreases continuously from day 3 to day 14, which is generally due to the destruction of biofilm structure, thereby increasing the corrosion rate of X65 steel. In addition, the corrosive ions in the solution may enter the substrate surface, resulting in pitting [30].

Under aerobic conditions, the diameter of Nyquist ring decreased from day 1 to day 3, increased from day 3 to day 7, and decreased from day 7 to day 14. Due to the formation of an embroidery layer on the metal surface under aerobic conditions, the corrosion of *P. aeruginosa* on X65 steel was slowed down, so X65 steel was the most resistant to corrosion on the first day. Corrosion rate of *P. aeruginosa* on X65 steel increased by dissolution of embroidery layer on the third day. It can be seen from Figure 3(b) that the corrosion product was more compact on the day 7, which played a protective role and slowed down the corrosion of X65 steel. On the day 14, there was only one layer of embroidery on the steel surface, indicating that the corrosion of *P. aeruginosa* on X65 steel was very serious.

Figure 7 shows the equivalent circuit fitted by the measured impedance data, where R_s represents the electrolyte resistance, R_b and Q_b represent the resistance and capacitance of the biofilm, R_{ct} and Q_{dl} represent the charge transfer resistance and double-layer capacitance, respectively. The fitting results are shown in Tables 2 and 3. The charge transfer resistance R_{ct} is closely related to the corrosion rate, and higher values represent lower corrosion rates [31].

It can be seen from Tables 1 and 2 that the R_{ct} values of the sterile group were basically larger than those of the bacterial group, so *P. aeruginosa* promoted the corrosion of X65 steel. Under *P. aeruginosa* anaerobic condition, the biofilm resistance R_b increased continuously and reached the maximum on the day 7, indicating that the biofilm and corrosion products accumulated on the electrode surface. Then R_b decreased, indicating that the film adhered to the steel surface became porous or pore size increased. The R_{ct} value was the largest on the third day, indicating that the corrosion rate of steel was the smallest on the third day. Under the aerobic condition of *P. aeruginosa*, a layer of embroidery was formed on the surface of steel on the first day, so the biofilm resistance R_b was the largest on the first day. At the same time from the value of R_{ct} can also be seen in the first day of corrosion rate is relatively small.

4. Conclusions

Compared with the sterile system, the medium inoculated with *P. aeruginosa* increased the corrosion rate of X65 steel, indicating that *P. aeruginosa* could aggravate the corrosion of X65 steel. The corrosion degree of *P. aeruginosa* in aerobic environment is more serious than that in anaerobic environment, indicating that oxygen can promote the corrosion of *P. aeruginosa* on X65 steel. Corrosion of X65 steel caused by anaerobic respiration and secretion of acidic substances by *P. aeruginosa* in anaerobic environment. Under aerobic conditions, nitrate ions react with oxygen to form ammonia, resulting in alkaline substances that cause corrosion to X65 steel.

Declarations

Author contribution statement

Jianhua Tang: Conceived and designed the experiments.
Ruiqi Guo: Performed the experiments; Wrote the paper.
Xin Zhang: Contributed reagents, materials, analysis tools or data.
Xu Zhao: Analyzed and interpreted the data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

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