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Stainless Steel Electrochemical Corrosion Behaviors Induced by Sulphate-Reducing Bacteria in Different Aerated Conditions

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The emphasis of this study lies in the differences of 304 stainless steel electrochemical corrosion behavior induced by the metabolic activity of Sulphate-reducing bacteria (SRB) in a growth cycle under aerobic (oxygen saturated and air saturated culture solutions) and anaerobic (nitrogen saturated culture solutions) conditions. The corrosion process, bio-film formation and the composition of the passive film in different conditions are investigated with electrochemical measurements, scanning electron microscopy and X-ray photoelectron spectroscopy. Both open circuit potential and electrochemical impedance spectra results indicate that the metabolic activity of SRB can accelerate the electrochemical corrosion process of stainless steel in both aerobic and anaerobic conditions, although the bio-film formed can inhibit the erosion of corrosive medium to some extent. The oxygen saturated environment can reduce the corrosion process by inhibiting the metabolic activity of SRB and promoting the passivation of stainless steel, but the air saturated environment presents little effect to the electrochemical property for the low concentration of oxygen. Furthermore, glomerate phenomenon can be observed from the biofilm formed in oxygen saturated culture solutions, indicating that SRB are prone to build localized anaerobic environment for its survival.

Keywords: Electrochemical corrosion behavior; Stainless steel; Sulphate-reducing bacteria; EIS.

1. INTRODUCTION

Microbiological induced corrosion (MIC) is a serious problem in the marine environment and many industries, such as power generation and petrochemical [1]. It's recorded that the material failure concerning MIC accounts for the 70%-80% of marine project materials and cost 3–5 billion dollars per year [2]. The service life and safety of the materials are significantly restricted by MIC, especially steel.

The research about MIC has attracted wide attention for decades [3-10]. Sulphate-reducing bacteria, as popular corrosion microorganism, are generally recognized as anaerobic microorganisms. Many mechanisms of MIC by SRB have been accepted widely, such as the cathodic depolarization mechanism [11], the local corrosion mechanism [12], and corrosion behavior of metabolic products [13, 14]. All these mechanisms are proposed based on the background of anaerobic environment. During the metabolic process of SRB, energy can be obtained mainly through the process of reduction of sulphate, and this process must be conducted under lower reduction potential. High oxygen concentration improves the reduction potential and inhibits the reduction of sulphate, and then inhibits the growth of SRB [15]. However, it was reported that SRB can bear oxygen concentration of up to 1.5mM using several defense strategies [16]. It implies SRB can survive in the aerobic condition in real ocean environment, such as the tidal zoon.

Oxygen is always viewed as corroding agent that promotes the process of MIC, because it mainly acts as depolarizing agent in the view of electrochemical reaction. Furthermore, the local increased partial pressure of oxygen can induce the ennoblement of potential resulted from the reversible potential of the oxygen electrode increasing [17]. It was proposed that the consumption of oxygen has the inhibition effect on the corrosion induced by aerobic biofilm [18, 19]. Von Rège and Sand [20] measured the corrosion rate of Q235 steel covered by biofilm in the culture medium with SRB on the continuous variety between anaerobic and aerobic conditions. The result showed that the maximum pitting depth of Q235 steel in aerobic condition is larger than in anaerobic condition. However, these researches focus on the action of oxygen as depolarizing agent, and ignore its effect on the metabolism of microorganism. The microbial activity has effect on the cathodic and / or anodic reactions on the surface of the metal [21]. It also changes the property and chemistry of the metal, affecting the electrochemical corrosion process.

We [22, 23] have studied the growth of SRB. It turned out that the SRB growth curves show a typical growth cycle that contains three stages (exponential growth, a stationary phase, and a death phase) in the aerobic and anaerobic conditions, and the oxygen dissolved in the culture solutions induces slow growth and fast decay of SRB. Furthermore, it also presents the variation of the concentration of sulfide anion, the vital metabolic product of SRB, under different conditions. The concentration of sulfide anion values in the anaerobic condition was higher than those in the aerobic conditions during the growth cycle. It is also proven that the corrosion rate of carbon steel in the anaerobic condition is lower than that in aerobic conditions through the analysis of the electrochemical result, which resulting from the protection of the complete and compact biofilm formed in anaerobic condition.

Stainless steels are alloys of great interest in marine applications for their high corrosion resistance; however, they cannot be free from microbial corrosion [24-27]. The microbial activity increases the susceptibility of the stainless steel corrosion and presents the phenomenon of ennoblement. The ennoblement increases the risk of initiation of pitting corrosion. It's recorded that the ennoblement is related to increased cathodic reduction rate [28, 29]. The existence of oxygen can modify the electrochemical performance of passive film, and it can also affect the metabolic activity of SRB. So the corrosion pattern and process of stainless steel induced by SRB in aerobic condition might be different with that in anaerobic condition.

In this paper, the electrochemical corrosion behavior of stainless steel in SRB culture solution under different aerated conditions was investigated with electrochemical measurements, SEM, XPS and so forth. The comparison of electrochemical performance among the 304 stainless steel in different aerated conditions was made based on the results of open circuit potential, Electrochemical impedance spectroscopy (EIS) and polarization curves. Furthermore, the effect of aerated environment on the MIC of stainless in a growth cycle of SRB is discussed from the points of bio-film morphology, passive film composition and so forth.

2. EXPERIMENTAL

2.1 The culture of SRB

The SRB seed used in this study was isolated from marine mud collected from the Bohai Sea, China. The purified seawater was collected from Huiquan Bay in Qingdao, China.

Analytical reagents of this study contained magnesium sulfate, ammonium chloride, sodium sulfate, calcium chloride, dipotassium phosphate, sodium lactate, and yeast extract. They were used to prepare the modified Postgate's culture medium with a pH value of 7.0. The culture medium was autoclaved at 121℃ for 20 minutes. After cooling at room temperature, they are sterilized by UV light for 30 minutes. Then the SRB culture was incubated in oxygen saturated solutions, air saturated solutions and nitrogen saturated solutions, respectively.

The culture solutions were aerated with nitrogen, air, or oxygen for 0.5 hour every day to ensure that the culture system was saturated, and they were cultivated at 30℃.

2.2 The pre-treatment of specimen

The 304 stainless steel electrode was polished to 2000# with silicon carbide, and it was ultrasonically cleaned in analytical reagent grade ethanol and Mili-Q water for 10 minutes, respectively. After that, the specimens are immersed into the solution.

2.3 Electrochemical experiments

The electrochemical experiments were conducted by using a CHI760C control system (CH Instruments, Inc.). They were implemented in traditional three electrodes system, in which, the working electrode is the 304 stainless steel with the area of 1 cm^2, the counter electrode was a platinum wire, and the reference electrode was a silver/silver chloride (3 molarity of Potassium Chloride) electrode. For the Electrochemical impedance spectroscopy measurement, the frequency range was 10 mHz-100 kHz and the amplitude of the sinusoidal voltage signal was 10 mV. The results of EIS were analyzed by fitting the data using Zsimpwin software. Polarization curves were recorded with a scan rate of 1 mV/s.

2.4 SEM experiments

After the immersion of 14 days, the specimens were taken from culture solutions and fixed with 1% glutaraldehyde in a phosphate buffer solution (PBS) for 1 hour, and then dehydrated with an ethanol gradient (at 50%, 75%, 95% and 99% for 10 minutes respectively，100% for 0.5h). The specimens were observed by using a Jeol JSM5900 LV scan electron microscope (Tokyo, Japan) at an acceleration of 20kV.

2.5 XPS experiments

The specimens removed from the culture solutions were washed by Mili-Q water and dried under nitrogen flow. The XPS experiment was implemented on a Thermo ESCALAB 250 photoelectron spectrometer equipped with an aluminum anode at a total power dissipation of 150W (15 kV, 10 mA).

3. RESULTS AND DISCUSSION

3.1 Electrochemical feature of the 304 stainless steel

3.1.1 Open circuit potential

Fig.1 shows the open circuit potential (E_{OC}) values obtained from 304 stainless steel in oxygen saturated, air saturated and nitrogen saturated culture solutions without and with SRB within 14 days. In sterile environment, the E_{OC} values are relatively stable in the three kinds of sterile solution (oxygen saturated, air saturated and nitrogen saturated culture solution). It is implied that the existence of passive film can inhibit the erosion of corrosive ions in seawater (such as chloride ion). It should be noticed that the E_{OC} of specimen in oxygen saturated culture solution is 0.1 V more positive than that in nitrogen saturated culture solution after the immersion of 14 days. This is attributed to the existence of oxygen, which can promote the passivation of stainless steel immersed in solution. Different with the sterile solution, the E_{OC} values of specimens shift towards negative direction suddenly after the three kinds of solutions are inoculated with SRB. Although the E_{OC} values do not provide any direct information on the corrosion kinetics, the decrease of E_{OC} suggests that the metabolic activity of SRB can increase susceptibility of the stainless steel corrosion in all the three kinds of aerated environment. During the growth cycle of SRB (14 days), the E_{OC} in oxygen saturated solution shows slightly increase trend, and the E_{OC} in nitrogen saturated solution presents decrease trend. After the immersion of 14 days, the value of E_{OC} in oxygen saturated solution is 0.2 V more positive than that in nitrogen saturated solution. So it seems that the effect of aerated environment on the electrochemical performance of specimens in inoculated solution is more obvious than that in sterile solution. This can be due to the fact that the presence of oxygen can affect the electrochemical performance of specimens in two ways, one is its effect on the passivation of stainless steel, and the other is its inhibition effect to the metabolic activity of SRB [15, 22]. Furthermore, the E_{OC} of specimen in air saturated inoculated

solution presents similar variation trend with that in nitrogen saturated solution, and the difference of E_{OC} between the two cases is very slight, implying that the content of oxygen in air saturated solution presents little influence to the electrochemical performance of stainless steel.

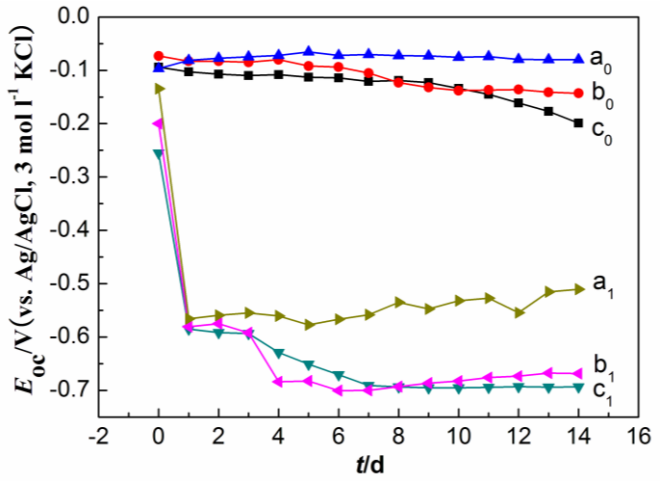
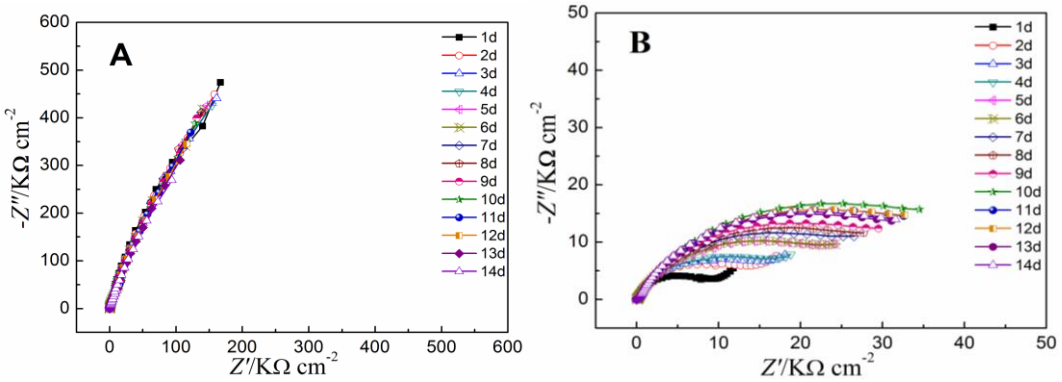


Figure 1. The E_{OC} values obtained on 304 stainless steel immersed in O_2-saturated (a_0, a_1), air-saturated (b_0, b_1), and N_2-saturated (c_0, c_1) culture solutions without (a_0, b_0, c_0) and with (a_1, b_1, c_1) SRB for 14 days.

3.1.2 Electrochemical impedance spectroscopy

Fig.2 shows the Electrochemical impedance spectroscopy obtained on 304 stainless steel immersed in （A, B） nitrogen- , (C, D) air- and （E, F） oxygen-saturated conditions without (A, C, E) and with (B, D, F) SRB during 14 days, respectively. In sterile culture solutions, the impedance results of stainless steel represent few changes with immersion time under the three kinds of aerated conditions (Fig.2 A, C and E), implying that the stainless steel is relatively stable in sterile culture solution for the existence of passive film. This result coincides with that of open circuit potential. However, in comparison with sterile culture solution, the impedance magnitude of stainless steel is reduced in the culture solution inoculated with SRB under all three kinds of condition, indicating that the metabolic activity of SRB can destroy the passive film and accelerate the corrosion process.



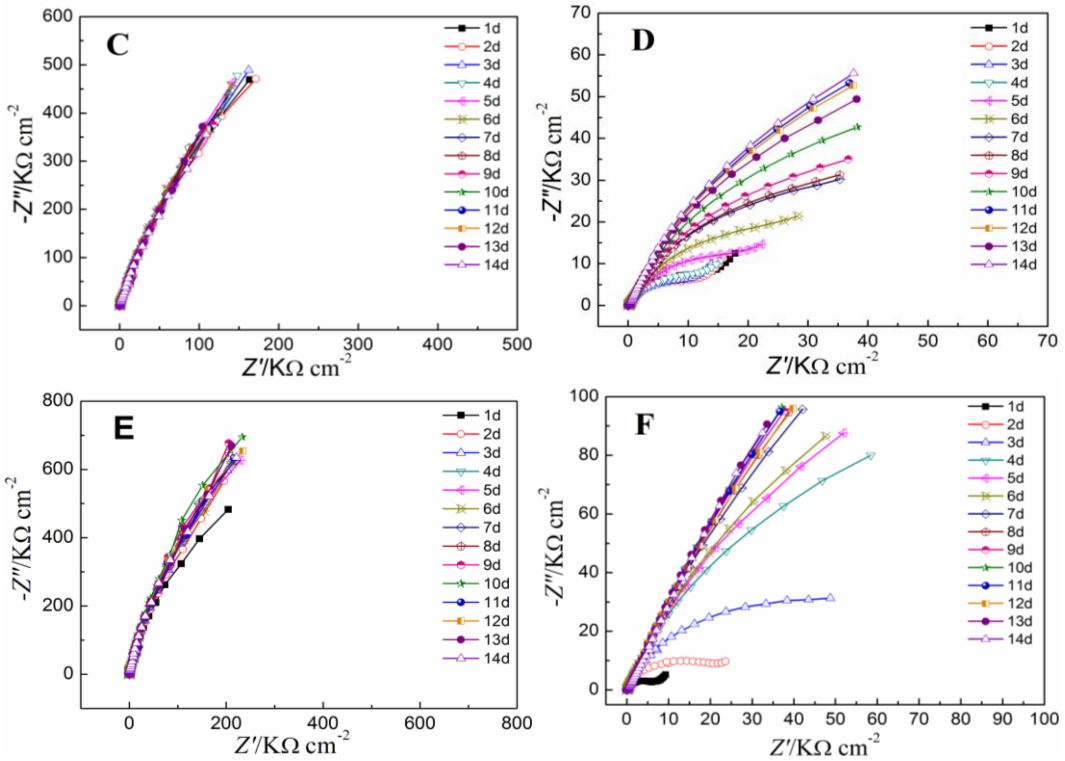


Figure 2. EIS obtained on 304 stainless steel immersed in （A, B） N_2- , (C, D) air- and （E, F） O_2-saturated conditions without (A, C, E) and with (B, D, F) SRB during 14 days.

Fig.3 shows the equivalent circuit models which are used to fit experimental impedance diagrams of specimen in nitrogen-, air-, and oxygen-saturated culture solutions (A) without and (B) with SRB. As we know, stainless steel possesses thin and stable passive film which forms spontaneously on surface, so the EIS data fitting adopts a model with two time constants in sterile system, including passive film and double layer (Fig.3 A).

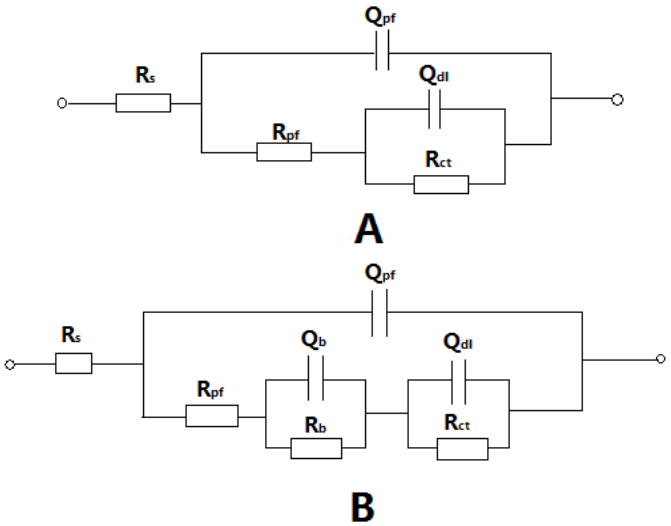


Figure 3. Equivalent circuit models proposed to fit experimental impedance diagrams for the specimens immersed in N_2-, air-, and O_2-saturated culture solutions without (A) and with (B) SRB.

Different with that, bio-film can form on the surface of stainless steel soon after being exposed to the culture solution with SRB. Thus, a three time constants model [30, 31], which contains passive film, bio-film and double layer, is used to fit the EIS data in inoculated culture solution (Fig.3 B). In the two equivalent circuit models, R_s is the solution resistance, Q_{pf} is the capacitance of the passive film, R_{pf} is the resistance of the passive film, Q_{dl} is the capacitance of the double layer, R_{ct} is the charge transfer resistance, Q_b is the capacitance of bio-film, R_b is the resistance of bio-film. Among them, double layer forms on the interface between electrode and surrounding electrolyte. Capacitors in EIS experiments often act like a constant phase element in token of CPE. The presence of CPE can be explained by dispersion effects that are caused by microscopic roughness of a surface or biofilm malformation on the sample surface [30, 32].

The charge transfer resistance, R_{ct}, is usually viewed as a parameter indicating the corrosion rate. As shown in Fig.4 (A), R_{ct} of specimen in sterile solutions is relatively stable during the immersion period with a value of about 2×10^6 Ωcm^{-2}. However, R_{ct} of specimen in inoculated solutions is more than 1 order of magnitude lower than that in sterile solutions (Fig.4 B), proving that the metabolic activity of SRB can accelerate the electrochemical corrosion process of stainless steel in three kinds of aerated condition. As we have published in reference [22], the growth curves of SRB in all three kinds of condition display a typical three-stage growth cycle, with an exponential growth phase, a stationary phase, and a death phase. Fig.5 presents the variation of SRB number and the concentration of sulfide anion in the three kinds of condition, and it has been published in reference [22]. The metabolite, especially sulfide anion, is regarded as the main reason for the MIC induced by SRB, so it can be predicted that its concentration can affect the corrosion rate of stainless steel. Unexpectedly, it seems that there is little apparent relationship between the concentration of sulfide anion and R_{ct} by comparing their results in Fig.4 (B) and Fig.5. Taking the nitrogen saturated condition as an example, the concentration of sulfide anion increases during the exponential growth phase of SRB. If the other factors are ignored, it can be predicted that the corrosion rate of stainless steel will increase with time in this phase, because the passive film can be destroyed gradually by corrosive ions under anaerobic condition. However, the value of R_{ct} increases with the growth of SRB in this phase (Fig.4 B). Furthermore, it can be noticed that the value of R_{ct} begins to decrease at the primary stage of death phase. Since the pH value of culture solution, which is another possible factor affecting corrosion process of stainless steel, is stable at the range from 6 to 7 during the growth cycle of SRB, it is reasonable to refer that the bio-film formed on surface is an important factor that affects MIC. With the growth of SRB, bio-film forms on stainless steel and it can act as barrier to the erosion of corrosive ions. In the death phase, the bio-film begins to degenerate and lose protection performance gradually. Similar with the case in nitrogen saturated solution, the R_{ct} increases during the exponential growth phase and stationary phase in oxygen saturated solution. However, in the death phase, the R_{ct} does not decline, but remains stable at the value of about 70 $k\Omega cm^{-2}$. In the exponential growth phase and stationary phase, both oxygen and bio-film contribute to the gradual increase of R_{ct} by strengthening the passive film and forming protective film. In the death phase, the sulfide anion concentration gets lower, and the passive film is relatively stable in this condition. In this case, the effect of bio-film to the electrochemical performance of stainless steel is too slight to affect the performance of stainless steel. It should be noticed that the variation trend of R_{ct} in air is similar with that in nitrogen, even

though its value is higher than that in nitrogen saturated condition during the growth cycle of SRB. It implies that oxygen in air saturated solution is not enough to construct stable passive film in inoculated culture solution.

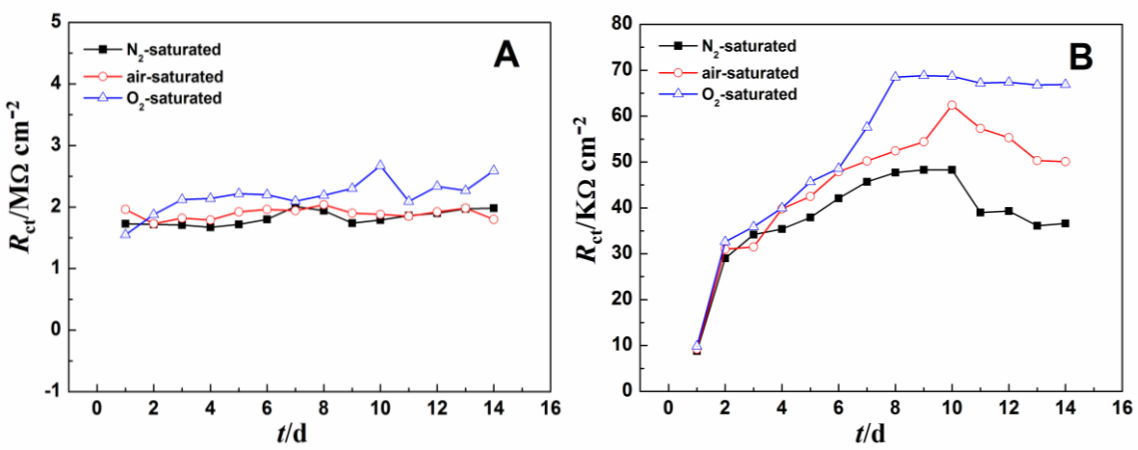


Figure 4. The R_{ct} of the specimens immersed in N_2-, air-, and O_2-saturated culture solutions without (A) and with (B) SRB for 14 days.

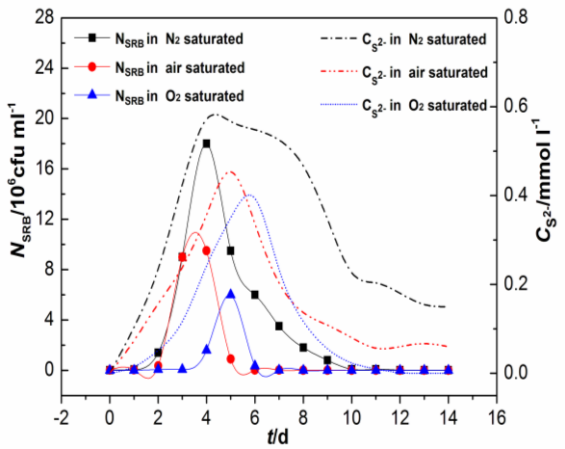


Figure 5. The growth curves of SRB and the concentration of S^{2-} in different aerated environments. [22]

3.1.3 The polarization curves

Fig.6 shows the polarization curves of the 304 stainless steel immersed in the nitrogen- , air- and oxygen-saturated culture solutions with SRB for 14 days. The breakdown potential of specimen in oxygen-saturated condition is about 0.3V, which is 0.2V higher than the other two conditions. Furthermore, the polarization current density of the stainless steel in oxygen saturated condition is lower than the other two cases. It is proven that the oxygen is beneficial to the self-recovery of passive film [33, 34], which results in the lower corrosion rate in oxygen saturated condition than that in other

condition. Moreover, the lower concentration of metabolic production (such as sulfide anion) in oxygen saturated condition might be another reason for the lower corrosion rate. It should be noticed that the polarization curves of samples in air and nitrogen saturated culture solution with SRB are almost overlapped. Especially, the breakdown potential of sample in air saturated condition is almost close to that in nitrogen saturated condition. It is indicated that the air saturated environment presents little influence on the recovery property of passive film.

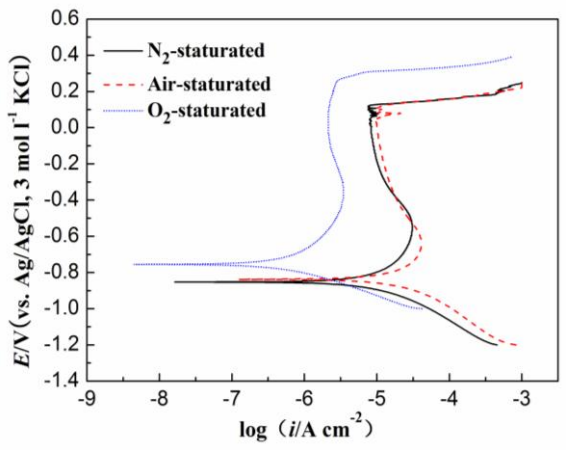
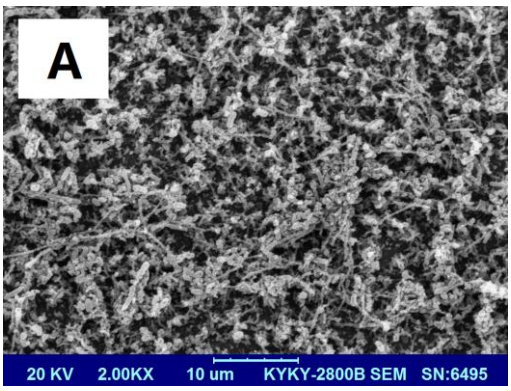


Figure 6. Polarization curves of 304 stainless steel immersed in the N_2-, air- and O_2-saturated culture solutions with SRB.

3.2 The morphology of biofilm

Fig.7 shows the SEM images of the SRB biofilm obtained in (A) nitrogen-saturated, (B) air-saturated and (C) oxygen-saturated culture solutions after a growth cycle (14 days). In both nitrogen and air saturated culture solution, relatively homogeneous and dense bio-film which is composed of SRB and metabolite can be found on stainless steel surface. It seems that there is no essential difference on bio-film morphology between the two cases. However, glomerate phenomenon appears on the biofilm formed in oxygen saturated culture solution. It can be inferred that SRB are prone to get together to build localised anaerobic environment for survival in oxygen saturated culture solution [35].



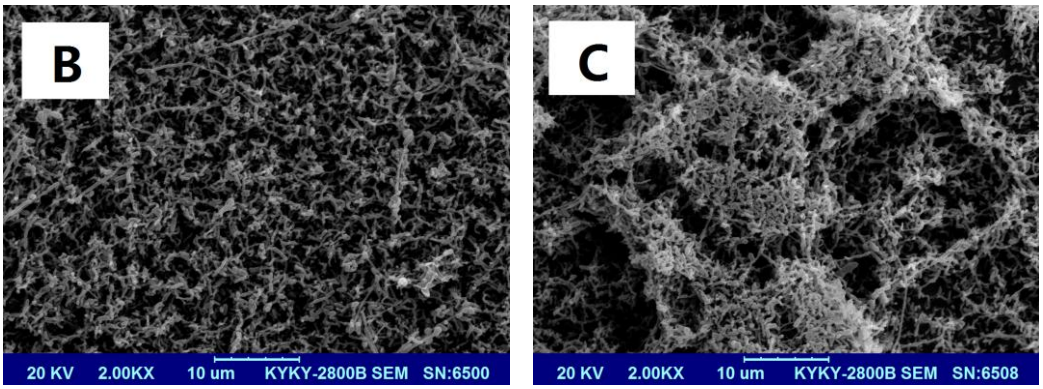


Figure 7. The biofilm topography on the 304 stainless steel immersed in the (A) N_2-, (B) air- and (C) O_2- saturated culture solutions with SRB for 14 days.

3.3 The passive film composition

In order to clarify the effect of aerated environment on the passive film, the composition of passive film in two different kinds of culture solution (oxygen and nitrogen saturated) with SRB is investigated. It should be mentioned that the surface of specimen is sputtered with argon for 90 s before XPS test to avoid the influence of bio-film and corrosion product (such as sulfide) to the result. Fig.8 shows the chromium 2p spectra of the 304 stainless steel immersed in the (A) nitrogen- and (B) oxygen-saturated culture solutions with SRB for 14 days. As shown in Fig.8, the chromium element in passive film is in the form of chrome, chromic hydroxide, and chromium oxide in both nitrogen and oxygen saturated conditions. It is reported that chromium oxide in passive film is more corrosion-resistant than chromic hydroxide [36-38].

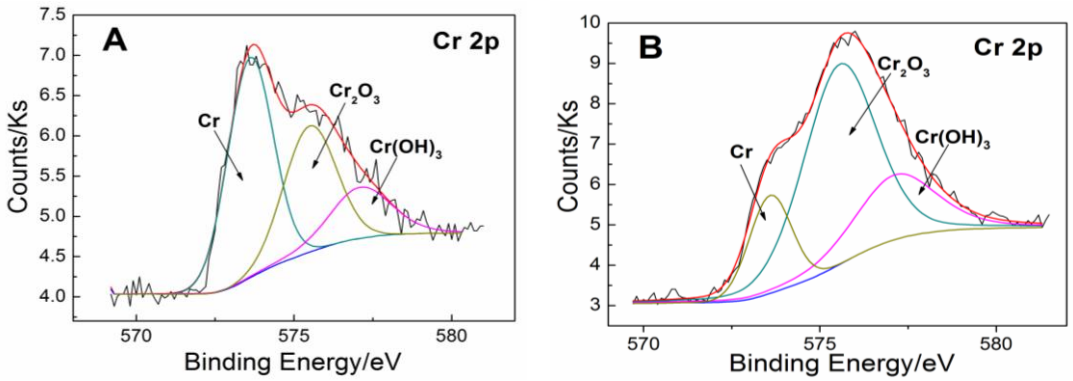


Figure 8. Cr 2p spectra of the 304 stainless steel immersed in the (A) N_2- and (B) O_2- saturated culture solutions with SRB for 14 days.

According to the ratio analysis result, it is found that the ratio of chromium oxide and chromic hydroxide in nitrogen saturated condition with a value of 1.9 is lower than that in oxygen saturated condition (with a value of 2.5). This can be attributed to the effect of oxygen to the self-repairing

property of passive film, and it can explain the fact that the stainless steel in oxygen saturated solution with SRB is more corrosion-resistant than that in nitrogen saturated solution. Furthermore, it should be noticed that the chromium element in passive film in nitrogen saturated solution is mainly in the form of chrome. The result is consistent with the inference that the protective oxide layer can be destroyed and the metal chromium is exposed in anaerobic condition.

4. SUMMARY

In this paper, the influence of aerated environment to the MIC of stainless steel by SRB in an open system is investigated. This research is helpful to clarify the corrosion mechanism of MIC in real ocean environment. The conclusions can be drawn as follows:

(1) In both aerobic and anaerobic condition, the metabolic activity of SRB can accelerate the electrochemical corrosion process of stainless steel. The bio-film can act as barrier to inhibit the erosion of metabolite to some extent in the growth cycle, especially in the exponential growth phase and stationary phase.

(2) The electrochemical results indicate that oxygen saturated environment can reduce the MIC process and raise breakdown potential of stainless steel by promoting its passivation and inhibiting the metabolic activity of SRB in comparison with other two cases. However, the air saturated environment presents little essential effect to the MIC for the low concentration of oxygen.

(3) Different with the homogeneous bio-film formed in nitrogen and air saturated conditions, the bio-film presents glomerate and heterogeneous morphology in oxygen saturated condition, because SRB are prone to get together to build localized anaerobic environment for survival.

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