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JOURNAL OF IRON AND STEEL RESEARCH, INTERNATIONAL. 2015, 22(8): 715-720

Influence of Marine Aerobic Biofilms on Corrosion of 316L Stainless Steel

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Abstract: The influence of marine aerobic biofilms on the corrosion of 316L stainless steel (SS) in aerated and deaerated seawater was studied by electrochemical impedance spectroscopy (EIS), potentiodynamic polarisation curves, current-potential curves and scanning electron microscopy with energy-dispersive spectroscopy (SEM-EDS). EIS and SEM-EDS results showed that the aerobic biofilms inhibited 316L SS corrosion within the test duration. Comparison of results under aerated and deaerated conditions revealed that O_2 enhanced the inhibition efficiency of the aerobic biofilms. This result indicated that living cells were necessary for the aerobic biofilms to inhibit the corrosion of 316L SS. Polarization curves indicated that the biofilms mainly inhibited anode action. Current-potential curves under deaerated conditions showed that electron transfer processes occurred between microorganisms and electrodes. Moreover, 316L SS as an electron acceptor was protected from corrosion.

Key words: 316L stainless steel; microbiologically influenced corrosion; polarisation curve

Biofilms form on the surface of materials when they are immersed in natural seawater. The influence of marine microorganisms on metal corrosion has been reported in many studies. Microorganisms are found to accelerate metal corrosion, particularly local corrosion such as pitting corrosion, crevice corrosion and de-alloying corrosion[1]. Mansfeld[2] first introduced the concept and theory that regeneration biofilms were used to inhibit corrosion and reported that biofilms can inhibit uniform corrosion. Uniformly distributed biofilms also reportedly obstruct interface transmission or the oxygen consumption of organism on the surface of materials[3]. Mehanna et al.[4] found that Geobacter sulfurreducens can protect 304L stainless steel (SS) against pitting at low electron acceptor concentrations. In fact, different materials under the same conditions or the same materials under different conditions show varied corrosion behaviours and corrosion mechanisms[5]. Pseudomonas or Serratia reportedly accelerates the corrosion of iron, nickel and Cu-Ni alloys[5,6]. However, Videla and Guiamet[7] found that Pseudomonas and Serratia can inhibit the corrosion of aluminium and aluminium alloys under some other conditions. Thus, the phenomenon and mechanism of microbe-influenced corrosion are complicated and worthy of further discussion.

In this paper, the influence of marine aerobic biofilms on the corrosion of 316L SS in aerated and deaerated seawater was studied by electrochemical impedance spectroscopy (EIS), potentiodynamic polarisation curves, scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS). The results showed that electron transfer processes occurred between microorganisms and electrodes. Moreover, as an electron acceptor, 316L SS was protected from corrosion.

1 Experimental

1.1 Chemicals and materials

A 1 mm-thick 316L SS sample with composition of C 0.022, Mn 0.97, P 0.028, Si 0.69, S 0.003, Ni 10.03, Cr 16.28, Mo 2.16, N 0.015, and Fe balance in mass% was mechanically cut into 10 mm × 15 mm (by drilling a 2 mm hole in the edge) rectangle and 8 mm × 8 mm square specimens. The square specimens were exposed on one side, sealed with epoxy resin, and connected with copper lead. All specimens were polished with SiC papers up to 1 200 grit, washed with distilled water, ultrasonically degreased in acetone, and sterilised with 75% ethanol prior to immersion tests.

1.2 Biofilm formation in natural seawater

Before experiments, biofilms were naturally formed on the 316L SS specimens by immersing them in natural seawater for different durations in laboratory at room

Foundation Item: Item Sponsored by National Basic Research Program (973 Program) of China (2014CB643304, 2014CB643305); National Natural Science Foundation of China (51131008); The Open Fund of Key Laboratory of Marine Environmental Corrosion and Bio-fouling, Institute of Oceanology, Chinese Academy of Sciences (CKF201409)

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temperature. Seawater, pumped offshore of Huanghai Sea (Qingdao, China), was transported to the laboratory after sand filtration and stored in a 10 L glass tank. This seawater in the tank was periodically renewed once a day with natural seawater. The experimental equipment is shown in Fig. 1. During the immersing time, open-circuit potential (OCP) was collected by DDS16/32 dynamic signal test system V1.0 (Infotronix®, Beijing) with an Ag/AgCl electrode as reference electrode.

Specimens were aseptically removed from the system and gently rinsed in sterile seawater to remove any unattached cells before fixation with 4% glutaraldehyde solution (diluted with sterile seawater) for 30 min. The specimens were then analysed with an XL30 scanning electron microscope (PHILIPS, Holland) coupled with an INCA energy X-ray spectrometer.

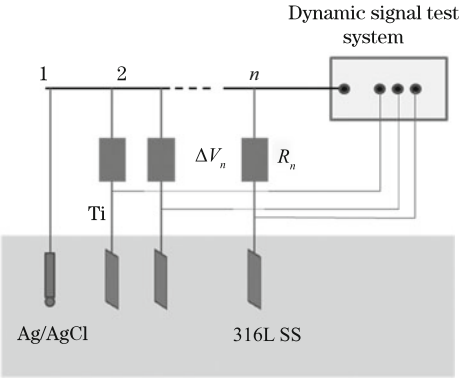


Fig. 1 Diagram of the experimental equipment

1.3 Electrochemical tests

During immersion, OCP or free corrosion potential (E_{corr}) was recorded by connecting a digital voltmeter with a computer and compared with Ag/AgCl electrode. Controlled experiments were performed in sterile seawater prepared from natural seawater by autoclaving at 121 °C and 0.138 MPa for 20 min.

Electrochemical experiments were performed in a conventional three-electrode cell at room temperature (15–20 °C) with PARSTAT 2273 potentiostat (Princeton Applied Research). A 316L SS, a 2.0 cm × 2.0 cm platinum foil and an Ag/AgCl electrode with a Luggin capillary were used as the working, counter, and reference electrodes, respectively. All potentials were expressed with respect to this reference electrode throughout the paper.

EIS measurement was carried out on steady-state OCP disturbed with amplitude of 10 mV ac sine wave. The frequency ranges from 100 kHz to 10 mHz. Potentiodynamic polarisation curves were then obtained at E_{corr} ranging from −250 mV to +500 mV at a scan rate of 0.5 mV/s. CorrWare with CorrView 2.0 and ZPlot with Zsimpwin software were used for the collection and analysis of potentiodynamic polarisation curve data and EIS data, respectively. Current-potential curves were obtained from OCP to –800 mV at a scan rate of 0.5 mV/s.

All experiments had three parallel samples to ensure the reproducibility of data.

2 Results and Discussion

2.1 Ennoblement of OCP of 316L SS electrode in seawater

The OCP or free corrosion potential of 316L SS electrodes was recorded during biofilm formation in natural and sterile seawater (Fig. 2). The OCP steadily maintained at approximately 0.25 V in sterile seawater. Ennoblement curves of 316L SS in natural seawater showed that the OCP was the same as that in sterile seawater in the first 2 days. The OCP then shifted to –0.1 mV in the following 3–5 days, rapidly increased exponentially, and reached a platform potential of approximately 0.33 V. This trend was similar to the ennoblement of passive metals in natural seawater commonly having an ennoblement width of approximately 0.3–0.5 V[8]. Based on the results in the first 2 days, unattached bacteria cannot result in the ennoblement of the OCP. Thus, biofilm formation on the 316L SS surface had a key function in the ennoblement process.

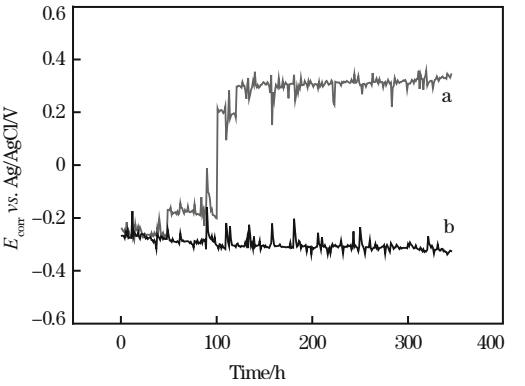


Fig. 2 E_{corr} of 316L SS immersed in natural (a) and sterile (b) seawater

2.2 Potentiodynamic polarisation curves

Fig. 3 shows the polarisation curves of 316L SS after immersion for 6 d in natural and sterile seawater. Anodic Tafel constant β_a and cathodic Tafel constant β_c, as well as corrosion current density I_{corr}, were calculated from the intersection of the anodic and cathodic Tafel lines in the polarisation curves at E_{corr}[9]. The results are listed in Table 1.

Polarisation curves showed that 316L SS in natural seawater had a wider passive zone and a markedly lower corrosion current density (I_{corr}< 0.5 μA) than 316L SS in sterile seawater (Fig. 3 and Table 1). E_{corr} in natural seawater showed a positive shift. A compound can be recognised as an anodic- or cathodic-type inhibitor only when the change in E_{corr} is no less than 85 mV[10]. Table 1 showed that E_{corr} ennobled 256.8 mV. It indicated that the biofilms inhibited anode action[11]. Overall, the re-

sults indicated that the biofilms inhibited the corrosion of 316L SS.

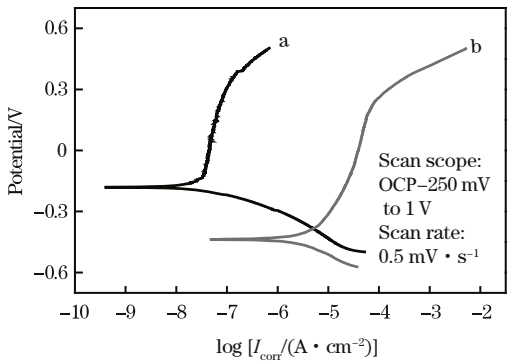


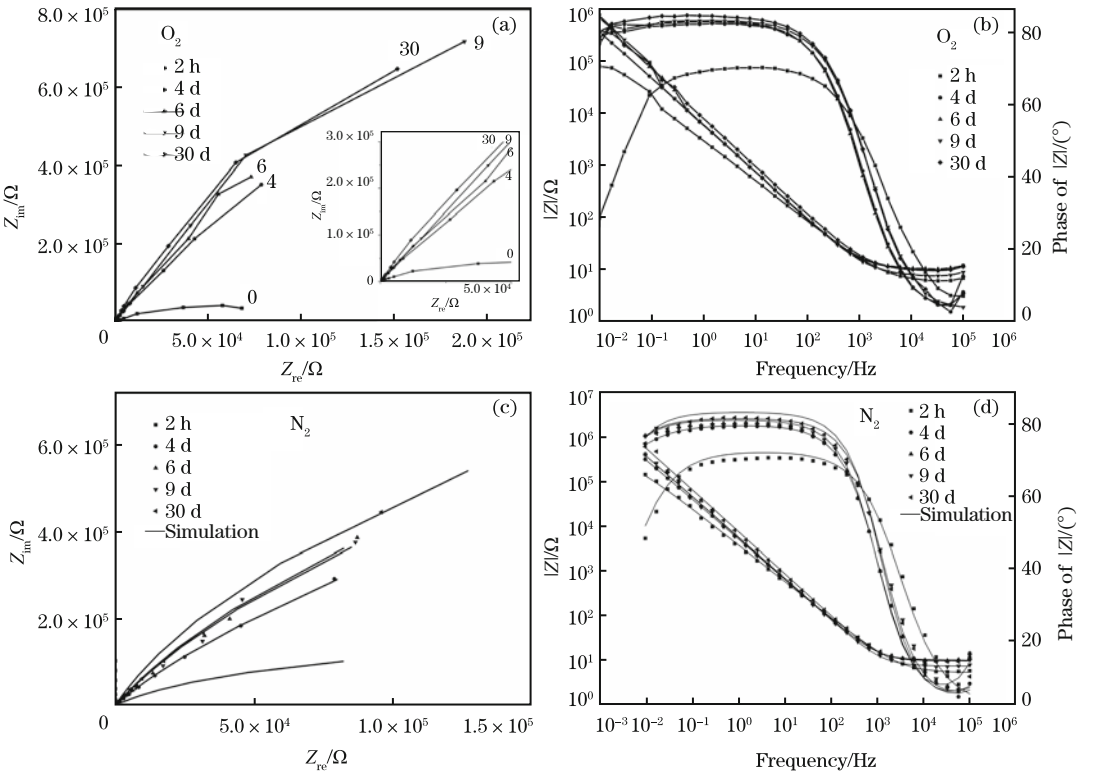
Fig. 3 Polarisation plot of 316L SS after immersion for 6 d in natural (a) and sterile (b) seawater

2.3 EIS measurement

EIS is used to characterize membrane features. Thus, this method can accurately measure membrane capacitance and resistance on bilayer membranes. In this study, EIS measurements were performed and are plotted in Fig. 4. The results of EIS were analyzed with Zsimpwin software. The quality of the fit to the equivalent circuit was based on the χ^2 value between the experimental data and the fitted results, and the average variance χ^2 reaches 10^{-3}[12]. A single-layer equivalent circuit (Fig. 5(a)) was used to simulate the EIS data of 316L SS after immersion for 2 h and a double layer (Fig. 5(b)) after immersion for 4, 6, 9 and 30 d. R_s denotes the solution resistance between the working electrode and reference electrode, whereas R_p is the polarised resistance that can be the characterization of the corrosion rate of the metal, and the corrosion rate increases when R_p decreases. R_b is resistance of the surface biofilm. Q_p and Q_b are the constant phase angle elements (CPEs, derived capacitance) of the double and film layers, respectively. The constant phase angle element impedance is described as: $Z_{CPE}=Y_0^{-1}(j\omega)^{-n}$ ($0<n<1$), where Y_0 is a constant phase element and n is an empirical exponent; the value of n can reflect the degree of heterogeneities on the sample surface. Z_w is Warburg impedance. The fitted parameters are listed in Table 2.

Table 1 Polarization parameters for 316L SS after immersion for 6 d in natural and sterile seawater

	E_{corr} *vs.* Ag/AgCl/mV	I_{corr}/(μA·cm^{-2})	β_a/(mV·dec^{-1})	β_c/(mV·dec^{-1})
Natural seawater	180.5	0.033 93	1 479	1 374
Sterile seawater	437.3	14.14	64.12	378.2



Zero day refers to the beginning of the immersion experiment.

Fig. 4 EIS of 316L SS immersed in seawater for 2 h and 4, 6, 9 and 30 d and bubbled with O_2 or N_2

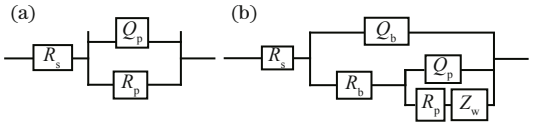


Fig. 5 Equivalent circuit models after immersion for 2 h (a) and 4, 6, 9 and 30 d (b)

As shown in Fig. 4, the centre of the capacitance semicircle of 316L SS electrode laid below the real axis at the beginning of immersion experiment. This result showed that the capacitance was diffused because of the inhomogeneous surface. The surface tended to be homogeneous with prolonging time, as proven by the recovery of the capacitance semicircle (after immersion for 4, 6, 9 and 30 d). Table 2 shows that R_p increased and Q_p decreased with prolonging immersion time under aerated conditions (bubbling with O_2). This result indicated that the aerobic biofilms inhibited 316L SS corrosion[13]. In this paper, n_2 can be used as a parameter of surface inhomogeneity; its decrease indicated increased surface metal roughening[14]. The n_2 values increased noticeably from 0.814 to 0.966 after immersion for 6 d and then became almost equal to 1.000 from 6 d to 30 d. These findings indicated that the surface roughening of 316L SS decreased with the increase of aerobic biofilm attachment. The n_1 values were between 0.910 and 1.000, which indicated that the biofilms on the 316L SS surface tended to integrate throughout the entire immersion process[15]. From 4 d to 6 d, the increase in Q_b values indicated that the adsorption/desorption equilibrium shifted towards adsorption[16]. From 6 d to 30 d, the Q_b values did not obviously change, indicating the adsorption/desorption equilibrium. Electrochemical experiments showed that the inhibition characteristics obtained from EIS reasonably well agreed with potentiodynamic polarisation.

Table 2 Fitted parameters for impedance of 316L SS in seawater with exposure time

Time		R_s/($\Omega\cdot cm^2$)	Q_b		R_b/($\Omega\cdot cm^2$)	Q_p		R_p/($\Omega\cdot cm^2$)	Z_w/($\Omega\cdot s^{-1/2}\cdot cm^2$)	χ^2
			Y_0/($\Omega^{-1}\cdot cm^{-2}\cdot s^{n_1}$)	n_1		Y_0/($\Omega^{-1}\cdot cm^{-2}\cdot s^{n_2}$)	n_2			
2 h	O_2	6.150				5.862×10^{-5}	0.814	1.847×10^{5}		1.02×10^{-2}
	N_2	5.470				5.847×10^{-5}	0.809	3.442×10^{5}		6.17×10^{-3}
4 d	O_2	9.9×10^{-3}	1.492×10^{-8}	1.000	10.29	3.240×10^{-5}	0.899	3.592×10^{6}	$2.158\ 2\times10^{-6}$	2.23×10^{-3}
	N_2	1.001×10^{-4}	1.257×10^{-8}	1.000	10.27	3.698×10^{-5}	0.893	1.867×10^{6}	$3.612\ 0\times10^{-6}$	2.05×10^{-3}
6 d	O_2	10.780	1.775×10^{-5}	0.970	224.1	6.057×10^{-6}	0.966	8.353×10^{6}	$3.147\ 1\times10^{-6}$	1.88×10^{-2}
	N_2	10.360	3.010×10^{-5}	0.910	290.1	6.281×10^{-6}	0.977	3.886×10^{6}	$7.588\ 1\times10^{2}$	1.00×10^{-2}
9 d	O_2	8.097	1.551×10^{-5}	0.983	245.4	6.813×10^{-6}	1.000	2.584×10^{6}	$8.914\ 2\times10^{6}$	3.00×10^{-2}
	N_2	7.548	2.502×10^{-5}	0.932	138.4	7.312×10^{-6}	0.925	1.000×10^{6}	$1.170\ 0\times10^{-6}$	9.91×10^{-3}
30 d	O_2	9.998	1.543×10^{-5}	0.954	232.4	4.293×10^{-6}	0.949	4.018×10^{6}	2.534	5.93×10^{-3}
	N_2	9.858	1.002×10^{-5}	0.952	321.5	5.252×10^{-6}	0.953	3.295×10^{6}	$4.960\ 1\times10^{3}$	1.58×10^{-2}

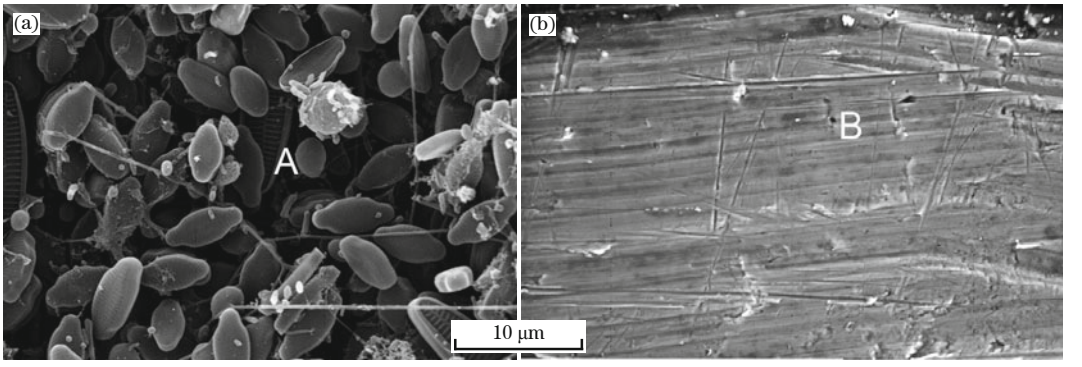
To investigate the influence of bacterium activity on the corrosion of 316L SS, EIS under deaerated (bubbled N_2) conditions was performed. The results are shown in Fig. 4 and Table 2. R_p of 316L SS electrode without biofilms under aerated conditions decreased compared with R_p under deaerated conditions. However, R_p of 316L SS electrode with biofilms under aerated conditions increased compared with R_p under deaerated conditions. These results showed that O_2 accelerated the corrosion of 316L SS without biofilms and decreased the corrosion of 316L SS with biofilms. As shown in Table 2, marine aerobic biofilms inhibited the corrosion of 316L SS both under aerated and deaerated conditions. The inhibition ability of marine aerobic biofilms was enhanced by O_2, which indicated that living cells were necessary for corrosion inhibition.

Studies have reported that some biofilms can inhibit the corrosion of carbon steel, copper, and aluminium. This inhibition was attributed to the consumption of O_2 by activity bacterium on the metal surface[3,17,18]. The present results showed that O_2 was necessary for biofilms to inhibit 316L SS corrosion. Thus, the inhibition activity of marine aerobic biofilms can be attributed not only to the consumption of O_2 but to the activity of compounds metabolised by aerobic microorganisms.

2.4 EDS and current-potential curve analysis

SEM-EDS analysis was performed to assess the state of 316L SS, and the results are shown in Fig. 6 and Table 3. Fig. 6 demonstrates that the 316L SS surface was covered by thick biofilms containing alga, bacteria and compounds metabolised by microorganisms. Plot A was used to analyze the composition of 316L SS surface with biofilms (Fig. 6). The elements included C, O, Na, S and Ca, which indicated that the biofilms were mainly formed by organic compounds and partially formed by

Ca and Na salt deposition. Plot B was used to analyze the composition of 316L SS surface after removing the biofilms. Compared with the original 316L SS sample, the elements C and O obviously increased because of the oxidation film layer and extracellular polymer substance (EPS)-metal complex film layer. EPS has an important function in the integrity of biofilms. EPS contains a polysaccharose with a carboxyl group that is complex with metallic atoms to change the corrosion behaviour of the metal[19]. The polysaccharose of EPS can be adsorbed onto the metal surface through chemisorption as a result of sharing or transfer of electrons from organic molecules to the metal surface, thereby forming a coordinate type of bond. Consequently, 316L SS was effectively protected. It can be seen from Table 3 that Mn signals are depleted at plot B, possibly because the manganese signals were much weaker than those of iron and chromium as demonstrated in Ref. [20] and maybe because of the superstratum of oxidation film layer and EPS-metal complex film layer.



(a) Attached biofilm (plot A); (b) Removing the biofilms (plot B).

Fig. 6 Secondary electron images of 316L SS immersed in seawater for 6 months

Table 3 EDS results corresponding to Fig. 6 (normalized) mass%

	C	O	Na	Mn	S	Ca	Si	Cr	Ni	Mo	P	N	Fe
Plot A	15.71	63.63	0.5		0.21	19.95							
Plot B	4.78	1.56					0.57	16.18	9.86	2.24			64.8
316L SS	0.022			0.92	0.003		0.69	16.28	10.03	2.16	0.028	0.015	69.83

To verify the electron transfer from organic molecules to metal, the current-potential curve was tested after immersion for 6 d in natural deoxygen and sterile deoxygen seawater. The results are plotted in Fig. 7, which shows that the reduction current strongly increased when the 316L SS electrode was coated with biofilms in anaerobic seawater. The deoxidised potential ennobled 0.3 V, which indicated that electrons were transferred more easily. The present results showed that marine biofilms can transfer electrons to electrodes and catalyse the reduction reaction simultaneously.

Over the last few years, bacteria have been demonstrated to develop unexpected strategies for directly exchanging electrons with solid surfaces[21,22]. In previous study[23], the authors found that aerobic biofilms attached onto conductor materials can also be closely related to electron transfer. This may be an extensive phenomenon. Bacteria and alga living in biofilms and colonising on the surface of conductor materials may have an important function in directly or indirectly transferring electrons to 316L SS. In this paper, 316L SS as an electron acceptor was effectively protected against corrosion.

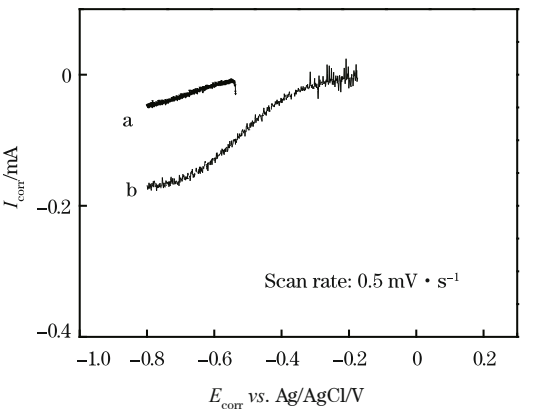


Fig. 7 Current-potential curve of 316L SS immersed for 6 d in sterile (a) and natural (b) deoxygen seawater

3 Conclusion

The influence of marine aerobic biofilms on the corrosion of 316L SS in aerated and deaerated seawater was studied by EIS, potentiodynamic polarisation curves and SEM-EDS. The results showed that the marine aerobic biofilms can effectively inhibit 316L SS corrosion.

The homogeneity of biofilms on the surface of 316L SS is important to the corrosion inhibitive property. Comparison of EIS results under aerated and deaerated conditions revealed that living cells were necessary for the aerobic biofilms to inhibit the corrosion of 316L SS. Combining with the current-potential results, it is presumed that 316L SS as an electron acceptor was protected against corrosion. Electron transfer from bacteria to conductor materials (SS and others) may be a universal phenomenon, and electron transfer mechanisms require further studies.

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