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Effect of *Bacillus flexus* on the Degradation of Epoxy Resin Varnish Coating in Seawater

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Protective coatings have been being widely applied on the surface of metallic materials to protect against corrosion. However, there is still little known about the degradation of coatings caused by microbiological attack in seawater. As one of the most widespread species, *Bacillus* species are of great importance in the deterioration and degradation of materials. In this paper, the effect of *Bacillus flexus* on the degradation of an epoxy resin varnish coating in seawater was investigated. All the experiments were conducted in either sterile seawater or seawater containing *Bacillus flexus*. The electrochemical characteristics of the coatings in both kinds of seawater were monitored with electrochemical impedance spectroscopy (EIS). The reduction of the corrosion resistance of the coating exposed to seawater containing *Bacillus flexus* between 1 day of immersion and 19 days of immersion was evidently larger when compared with that in sterile seawater, which indicated that *Bacillus flexus* could remarkably decrease the corrosion resistance of the coating and potentially result in degradation of the coating. In addition, scanning electron microscopy (SEM) was employed for characterizing the surface morphology of the coatings before and after immersion. After 30 days of immersion, chalking traces and some tiny holes emerged on the surface of the coatings exposed to seawater containing *Bacillus flexus*, which was mainly ascribed to the biofilm formed by *Bacillus flexus* and their metabolic activities. The results demonstrated that the epoxy resin varnish coating was degraded by *Bacillus flexus*, which was further confirmed by the results obtained from Fourier transform infrared spectroscopy (FTIR).

Keywords: Epoxy resin varnish coating; *Bacillus flexus*; Degradation; Electrochemical impedance spectroscopy; Corrosion

1. INTRODUCTION

Metals are known to be susceptible to corrosion under aggressive environmental conditions. In many industries, such as those related to oil, gas and drinking water distribution, corrosion has become a predominant issue [1, 2], among which microbiologically influenced corrosion (MIC) cannot be ignored [3]. Especially in the marine environment, widely distributed microorganisms are prone to causing severe corrosion of materials, which significantly affects the performances of marine facilities such as underwater sonar systems and turbine blades. In aqueous environments, bacteria attaching on the surfaces of metals tend to form a layer of slimy biofilm, which plays an important part in the degradation and deterioration of the materials [4-6]. In a biofilm, the embedded microorganisms modify the electrochemical conditions at the metal-solution interface through their metabolic activities [7] and further affect the electrochemical reactions, which eventually results in the enhanced corrosion or retarded corrosion of metals [8-11].

Microorganisms can not only cause great corrosion damage to metallic materials but also degrade the nonmetallic materials. Polyester polyurethane, which is widely used in many industrial and medical applications, can be degraded by fungi such as *Nectria gliocladioides*, *Penicillium ochrochloron*, *Geomyces pannorum* [12] and *Aspergillus tubingensis* [13]. Polyurethane has served as a source of carbon for *Curvularia senegalensis*, *Fusariumsolani*, *Aureobasidium pullulans*, *Cladosporium* sp. and *Pestalotiopsis microspora* to meet their growth demands [14]. Kathiresan [15] identified eight fungal species and seven bacteria that could degrade the polythene from degrading polythene bags, among which five bacteria (*Streptococcus*, *Staphylococcus*, *Micrococcus*, *Moraxella*, and *Pseudomonas*) and two species of fungi (*Aspergillus glaucus* and *A. niger*) were predominant. Moreover, it has also been reported that by forming an intermediate product, 2-hydroxymuconic semialdehyde (2-HMSA), *Bacillus cereus* was able to degrade phenol through a meta-cleavage pathway, and the concentration of the degraded phenol could be up to as high as 2000 mg/L [16]. As one of the most widespread species, the *Bacillus* species have played a significant part in the degradation of organics and can degrade a variety of materials, such as p-cresol [17], phenanthrene [18] and polyester polyurethane [19].

Organic coatings have been extensively utilized to protect metallic materials from corrosion. In protective coatings, epoxy resin coatings are usually utilized for the protection of marine structures, which can be attributed to their outstanding performances, such as their excellent corrosion resistance, strong barrier effects and adhesion strength [20]. Our earlier studies [21, 22] have demonstrated that *Pseudomonas* sp. in seawater can degrade the epoxy resin varnish coating by oxidating the hydroxyl to carbonyl and the aluminum/epoxy coating by oxidating the epoxy to form hydroxyl through their metabolic activities. As far as we know, so far, only these works deal with the degradation of coatings by bacteria, which are not enough for revealing the coatings degradation by bacteria. In marine environment, *Bacillus* sp. is a typical bacteria, the metabolism of which differs from that of *Psudomonas* sp. and we have found that it is a predominant strain in the corrosion products of carbon steel which was exposed to natural seawater.

The aim of this paper is for investigating the effect of *Bacillus flexus* on the epoxy resin varnish coating degradation in seawater. Instead of implementing all the experiments in the nutrient-enriched media, sterilized natural seawater without any other treatment, which is closer to the conditions in the

natural marine environment, was utilized for corrosion characterization. Epoxy resin varnish coatings were exposed to seawater containing *B. flexus* and sterile seawater for 30 days, respectively. Various techniques, including electrochemical techniques, scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR), were used for characterizing the degradation of coatings.

2. EXPERIMENTAL

2.1. Samples preparation

Cylindrical specimens prepared for electrochemical measurements have a size of Φ4 cm × 5 mm, which were processed from AISI 1045 carbon steel. Carbon steel coupons with a size of 18 × 18 × 2 mm were utilized as the substrates for surface analysis. Prior to being coated with the epoxy resin varnish coating, carbon steel coupons were sanded by a series of SiC abrasive papers (180, 400, 600 and 800). For all coupons, only one end was exposed, which would be utilized as the working surface, while the rest were cladded with epoxy resin in PVC pipes. Simultaneously, for electrochemical measurements, each coupon was soldered with a copper wire. Afterward, in order to obtain clean surfaces for promoting the adhesion of the coatings, all coupons were degreased in acetone, followed by rinsing with deionized water, dehydrating with anhydrous ethanol and finally drying at room temperature. Subsequently, clean working surfaces were evenly sprayed with the prepared epoxy resin varnish coating. The coating was prepared according to the weight proportion formulation: epoxy resin 60, mixed solvent 37, leveling agent 3 and curing agent 37. By dispersing at high speed, all components were mixed thoroughly to form the coating. After curing at room temperature for a week, coatings with dry film thickness of 70 ± 2 μm were obtained. Finally, all the coupons coated with the epoxy resin varnish coating were preserved for immersion experiments.

2.2. Preparation and identification of the bacterial strain

In this study, the bacterium was isolated from the corrosion products of AISI 1045 carbon steel, which had been exposed to natural seawater (South China Sea) for six months. In order to identify the bacterium isolated, 16S rDNA sequence of the bacterium was cloned by polymerase chain reaction (PCR), sequenced and eventually compared with reference strains held in the GenBank database. Further, the MEGA version 5.0 program was utilized for constructing the phylogenetic tree using the neighbor-joining method [23, 24].

2.3. Microorganism cultivation and inoculation

The *B. flexus* seed culture was cultivated in 2216E medium. The composition of 2216E medium was as follows (per liter natural seawater): peptone 5.0 g, yeast extract 1.0 g. The pH was adjusted with 1 mol/L NaOH solution to 7.8. Prior to inoculation, the medium was autoclaved at 121 ℃ for 20 min. After 2 days of incubation in 2216E medium at 26 °C, *B. flexus* seed culture was obtained. Then, the obtained *B. flexus* seed culture medium was inoculated into sterile seawater with a volume ratio of 1:100

and shaken for 24 h at 26 °C to prepare the seawater containing *B. flexus*. Finally, all the prepared coupons were separately immersed in seawater containing *B. flexus* and sterile seawater under aseptic conditions. The immersion experiments were performed on three replications.

2.4. Electrochemical analysis

An electrochemical workstation (Princeton Applied Research, PARSTAT 2273, US) was employed for performing the electrochemical measurements. Additionally, a three-electrode system was adopted, among which the reference electrode was a saturated calomel electrode (SCE) and the counter electrode was a Pt electrode. The working electrode was made of carbon steel coupons and was encapsulated with epoxy resin in a PVC pipe, leaving one side coated with the prepared coating acting as the working surface. The EIS was recorded at a 20 mV sinusoidal voltage and a frequency range of 10^{-2}-10^{5} Hz. Finally, the obtained EIS data was further analyzed with ZsimpWin software.

2.5. Surface characterization

Before and after immersion, scanning electron microscopy (FEI XL30 type) with a voltage of 10 kV was used for characterizing the surface morphologies of the epoxy resin varnish coatings. In addition, before examination by SEM, all coupons were coated with gold-palladium by sputtering.

2.6. FTIR analysis

FTIR spectra of coatings without any treatment and separately exposed to seawater containing *B. flexus* and sterile seawater for 30 days were obtained by using a Bruker IFS55 spectrometer with a frequency range of 450–4000 cm^{-1}. Before examination, the attachment on the surface of the coatings, including the deposition and biofilms, had already been drastically eliminated.

3. RESULTS

3.1. Identification of the bacterial strain

A strain of bacteria named HK58 was eventually isolated, which was found in abundance in the corrosion products of carbon steel exposed to natural seawater for six months. A phylogenetic tree was constructed (Fig. 1) basing on the analysis for the obtained 16S rDNA sequence. The sequence and phylogenetic analysis confirmed that the strain HK58 belonged to *B. flexus.* The 16S rDNA sequence of the isolated strain HK58 had been submitted to GenBank and the accession number was KX721506.

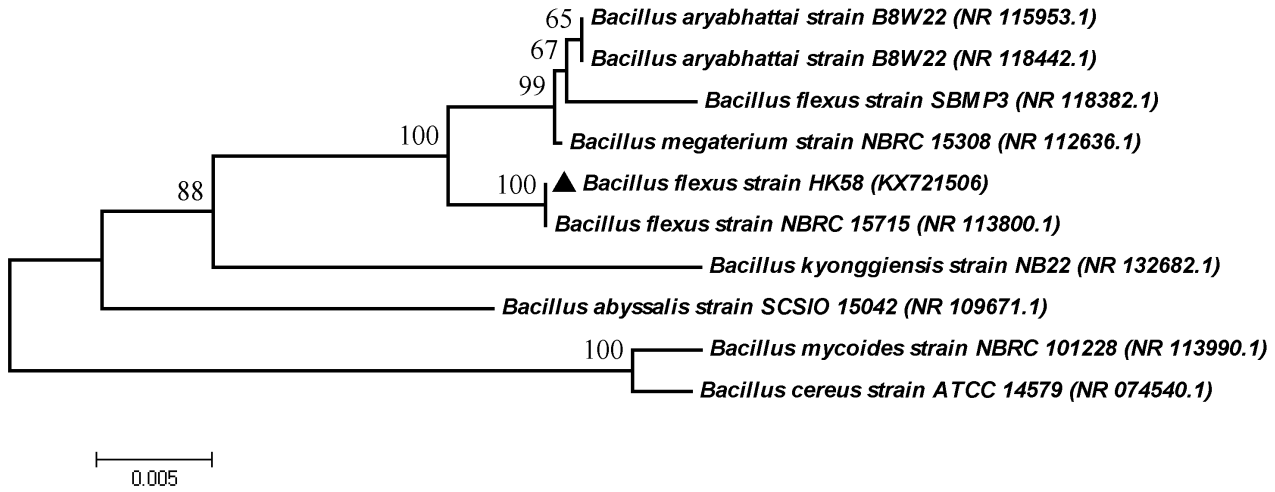

Figure 1. Phylogenetic tree on basis of the 16S rDNA gene sequences (approximately 1000 pb) of strain HK58, showing phylogenetic relationships between sequence of the strain and related sequences. Numbers at the nodes indicate the bootstrap values.

3.2. EIS characteristics

EIS is a powerful technique that has been widely employed for investigating the microbial corrosion behavior of coatings [25, 26]. In this paper, the electrochemical characteristics of the coatings immersed in both kinds of seawater were monitored by EIS. Fig. 2 shows the EIS results for coatings exposed to sterile seawater and seawater containing *B. flexus* for different time. The obtained results from the repeated experiments exhibited a similar trend.

As shown in the Nyquist plots (Fig. 2a and 2b), in both kinds of seawater, at 1 day of immersion, the capacitive arc diameters were approximate and larger than those recorded at other immersion times. Additionally, in sterile seawater (Fig. 2a), the capacitive arc diameter for the coatings decreased during 1-6 days, then increased from 6 days to 25 days and decreased slightly afterwards. While in seawater containing *B. flexus* (Fig. 2b), the capacitive arc diameter for the coatings decreased continuously during 1-19 days and increased from 19 days to 29 days. The minimum value could be observed at 19 days of immersion. Obviously, in seawater containing *B. flexus*, the decrease in the capacitive arc diameter between 1 day of immersion and 19 days of immersion was evidently larger compared with that in sterile seawater.

The results shown in the Bode plots (Fig. 2c and d) were in accordance with those presented in the Nyquist plots. In both kinds of seawater, the initial corrosion resistance for the coatings was approximately 10^8 $\Omega \cdot cm^2$, which were the largest compared with those recorded at other immersion times. Previous research had shown that for good coatings, the corrosion resistance was between 10^6 and 10^9 $\Omega \cdot cm^2$ [27, 28], which suggested that the coatings used in this study possessed good resistance against corrosion. Additionally, as shown in Fig. 2c, in sterile seawater, the corrosion resistance decreased to the minimum value at 6 days of immersion. In seawater containing *B. flexus* (Fig. 2d), the corrosion resistance values recorded at 1 day and 19 days of immersion corresponded to the maximum and minimum, respectively. Moreover, the corrosion resistance of coatings recorded in seawater

containing *B. flexus* decreased more than that in sterile seawater between 1 day immersion and 19 days immersion.

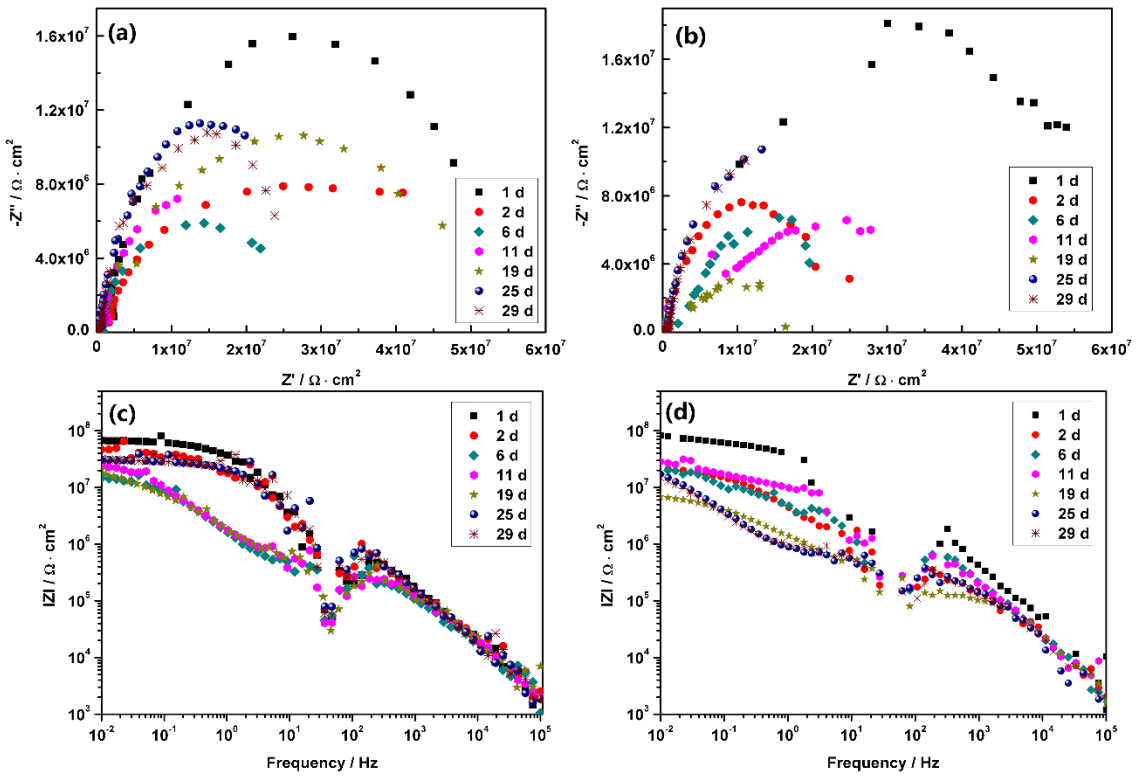

Figure 2. Nyquist (a, b) and Bode (c, d) plots of the coatings recorded at different time in sterile seawater (a, c) and seawater containing *B. flexus* (b, d).

The electrochemical results were further analyzed through simulating the EIS data with the electrical equivalent circuits (EECs) presented in Fig. 3a-e. The EEC shown in Fig. 3a was used for the coatings exposed to sterile seawater during 1-2 days of immersion, which shows only one time constant. In addition, the EEC exhibited in Fig. 3b was utilized to the coatings exposed to sterile seawater for the 6-29 days immersion period, which shows two time constants. In the EECs, R_s was the resistance of seawater, Q_{coat} was the constant phase element (CPE) relating to the coating capacitance and R_c was the coating resistance. C_{dl} and R_{ct} represented the double layer capacitance and charge transfer resistance, respectively. In seawater containing *B. flexus*, the EEC in Fig. 3c, used for 1-2 days of immersion was same as that in sterile seawater (Fig. 3a). The EECs shown in Fig. 3d and e were separately used for the coatings exposed to seawater containing *B. flexus* during 6-19 days and 25-29 days of immersion. In the EECs, $Q_{biofilm}$, $R_{biofilm}$ and W were the CPE relating to the capacitance of the biofilm, the biofilm resistance and the diffused resistor, respectively. Otherwise, the others circuit elements were consistent with those used in the EECs (Fig. 3a and b) corresponding to the coatings exposed to sterile seawater. Moreover, in the EEC shown in Fig. 3e, three time constants could be observed. According to the proposed equivalent circuit models, the fitted EIS parameters are presented in Table 1.

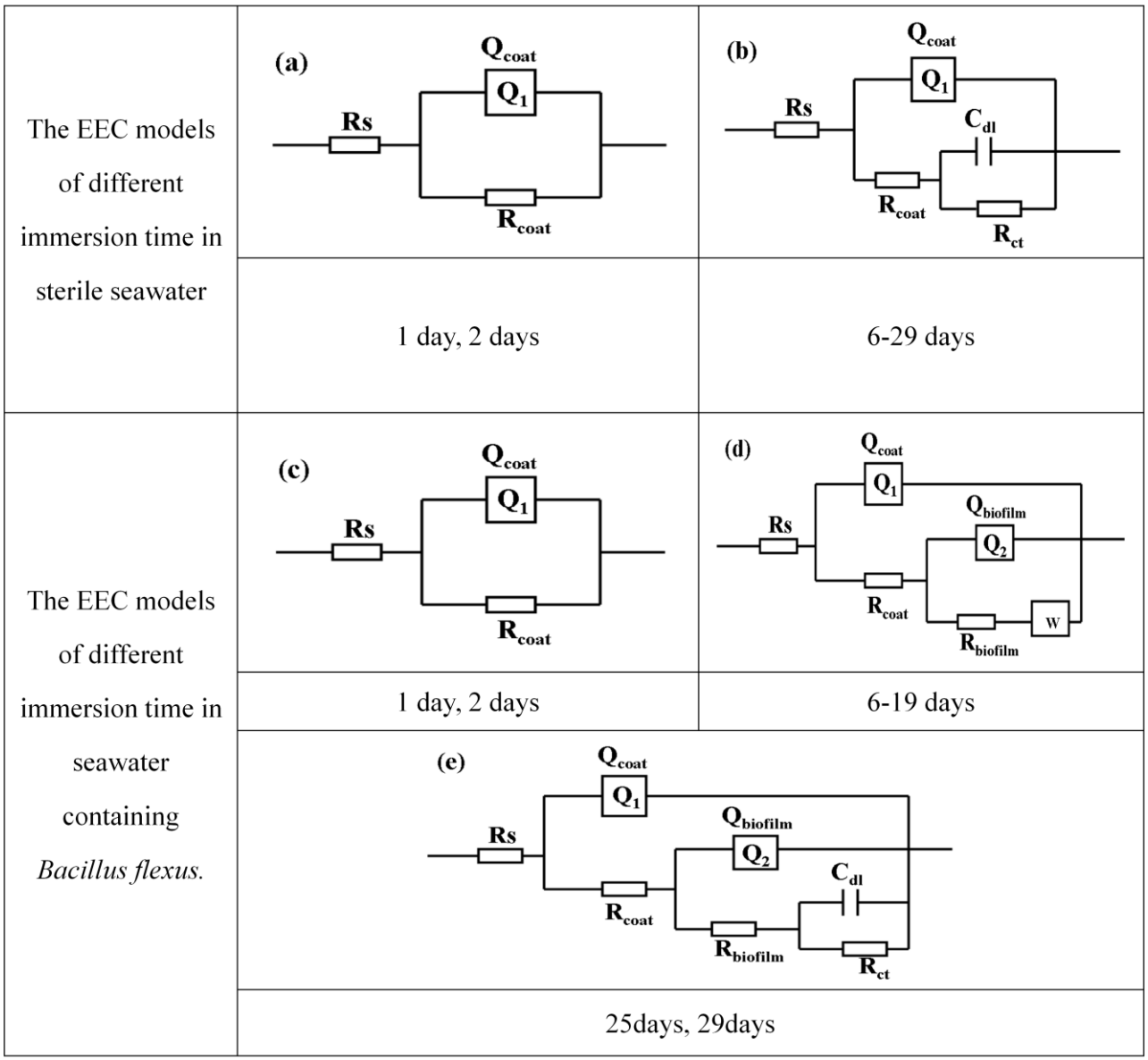

Figure 3. Electrical equivalent circuits employed to simulate the EIS data recorded in sterile seawater (a, b) and seawater containing *B. flexus* (c, d and e) at different immersion time.

As shown in Table 1, in both kinds of seawater, the seawater resistance R_s remained stable and quite low, which was ascribed to the good conductivity in the seawater. Additionally, based on the fitted results, Fig. 4 shows the variation of coating resistance with immersion time in sterile seawater and seawater containing *B. flexus*. In sterile seawater, the R_{coat} value decreased with a high speed from days 1-11. Afterwards, the R_{coat} value tended to stable, changing in a narrow range. In seawater containing *B. flexus*, the R_{coat} value decreased obviously from 1 day to 19 days of immersion, and then the R_{coat} value increased slightly. It was clear that the decrease in the R_{coat} values in seawater containing *B. flexus* was obviously higher than that in sterile seawater between 1 day and 19 days immersion. In addition, as seen in Table 1, in sterile seawater, the R_{ct} values increased continuously during days 6-25 and then decreased

slightly, during which the increase mainly resulted from the accumulation of corrosion products. Moreover, in seawater containing *B. flexus*, after 6 days of immersion, the $R_{biofilm}$ values increased, which was due to that the mature biofilm gradually formed on the coating surface. At the same time, an increase in the R_{ct} values from 25 days to 29 days of immersion could also be observed. The fitting results coincided with the results of Nyquist plots and Bode plots.

Table 1. EIS parameters for coatings exposed to sterile seawater and seawater containing *B. flexus* for different times.

Time (days)	R_S (Ω cm^2)	Q_{coat} (F cm^{-2})	R_{coat} (Ω cm^2)	R_{ct} (Ω cm^2)	C_{dl} (F cm^{-2})	$R_{biofilm}$ (Ω cm^2)	$Q_{biofilm}$ (F cm^{-2})	W (Ω cm^2)
In sterile seawater								
1	7.39	5.32×10^{-9}	8.03×10^{7}	-	-	-	-	-
2	8.24	8.21×10^{-8}	5.71×10^{7}	-	-	-	-	-
6	9.75	9.82×10^{-8}	1.11×10^{7}	1.75×10^{7}	3.58×10^{-7}	-	-	-
11	10.32	4.21×10^{-8}	5.83×10^{6}	2.05×10^{7}	3.15×10^{-7}	-	-	-
19	11.79	3.09×10^{-8}	6.47×10^{6}	3.36×10^{7}	2.27×10^{-7}	-	-	-
25	13.79	4.83×10^{-8}	6.42×10^{6}	3.63×10^{7}	1.22×10^{-7}	-	-	-
29	12.57	4.02×10^{-8}	5.53×10^{6}	2.32×10^{7}	3.4×10^{-7}	-	-	-
In seawater containing *B. flexus*								
1	10.89	4.39×10^{-9}	7.79×10^{7}	-	-	-	-	-
2	10.34	6.83×10^{-8}	4.13×10^{7}	-	-	-	-	-
6	11.97	8.44×10^{-8}	1.10×10^{7}	-	-	15.16	7.84×10^{-5}	2.41×10^{-4}
11	18.64	4.16×10^{-8}	4.22×10^{6}	-	-	24.91	6.22×10^{-5}	2.54×10^{-4}
19	15.14	2.55×10^{-8}	1.46×10^{6}	-	-	85.62	7.33×10^{-5}	4.87×10^{-3}
25	16.88	4.19×10^{-8}	2.53×10^{6}	2.68×10^{7}	3.59×10^{-7}	134.37	7.67×10^{-4}	-
29	18.44	5.24×10^{-8}	2.19×10^{6}	2.89×10^{7}	4.45×10^{-7}	227.53	1.16×10^{-4}	-

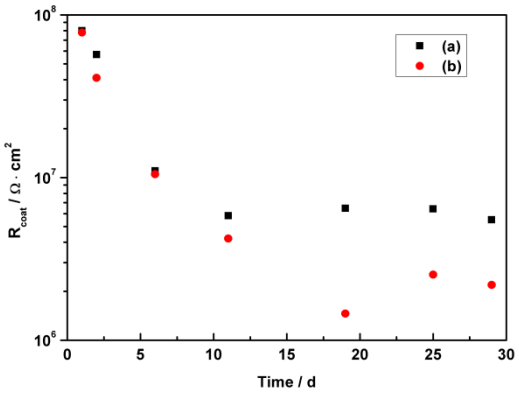

Figure 4. Variation in the R_{coat} values with immersion time in sterile seawater (a) and seawater containing *B. flexus* (b).

3.3. SEM analysis

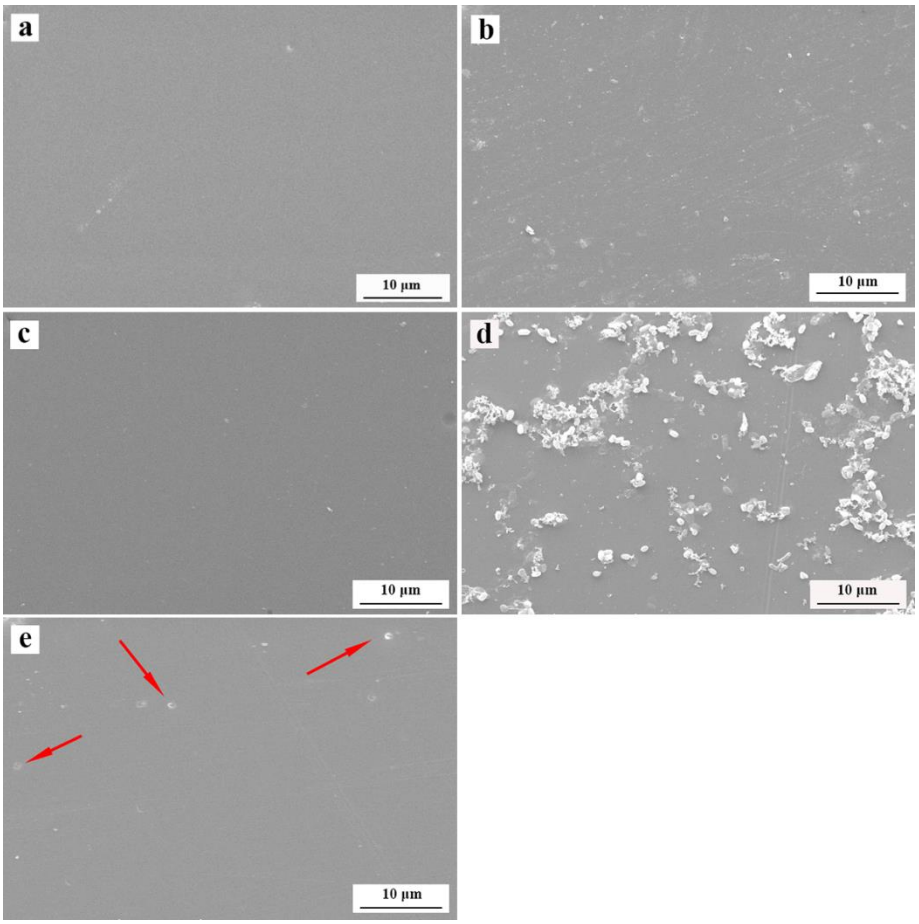

Figure 5. Surface morphologies of coatings without immersion (a), after being immersed in sterile seawater for 30 days (b) and after removing the deposition (c), and after being immersed in seawater containing *B. flexus* for 30 days (d) and after removing attachment including biofilm and deposition (e).

The surface morphologies of coatings before and after 30 days of exposure to seawater containing *B. flexus* and sterile seawater are presented in Fig. 5. The initial surface morphologies for the coating

before immersion were intact and smooth, without any damaged traces (Fig. 5a). In addition, after being immersed in sterile seawater for 30 days, there were no evident changes on the coating surface except for some deposition and soaking traces (Fig. 5b) and after removing the deposition only some slight soaking traces could be observed (Fig. 5c). Whereas after 30 days of exposure to seawater containing *B. flexus,* the biofilm composed of bacteria and their metabolic products covered the surface of the coating (Fig. 5d), which usually was responsible for the damage to the protective coating. Further, after removing the biofilm and deposition, some tiny holes and chalking traces emerged on the coating surface (Fig. 5e).

3.4. FTIR analysis

Fig. 6 shows the FTIR spectra of coatings before and after being exposed to different environments for 30 days. In all cases, the strong absorption bands that corresponded to the vibration of -OH and -CH_X were at 3410-3430 cm^{-1} and 2800-3000 cm^{-1}, respectively [29]. Additionally, the peaks due to the aromatic ring vibration could be observed at 1510 cm^{-1} and 1610 cm^{-1}. The peaks generated by C-O stretching in the epoxy group were at at 1246 cm^{-1} and 831 cm^{-1}. The 1640 cm^{-1} peak corresponded to the vibration of the carbonyl group [30]. Moreover, the peaks at 1450 cm^{-1} and 1100 cm^{-1} resulted from the vibrations of the C–H bending of the aliphatic groups [31] and the ether linkage vibration (C–O–C) [32], respectively.

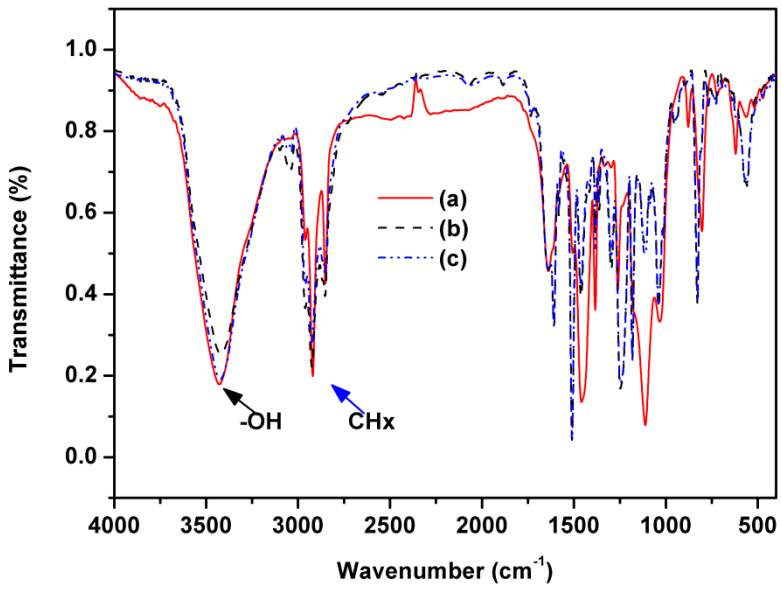

Figure 6. FTIR spectra of the coatings after being immersed in seawater containing *B. flexus* for 30 days (a), the coatings without immersion (b) and the coatings after 30 days of exposure to sterile seawater (c). Before examination, the deposition and biofilm on the surface of coatings were removed thoroughly.

As shown in Fig. 6, there was no evident difference in the absorption band at 3410 cm^{-1}-3430 cm^{-1} for coatings which were exposed to sterile seawater and seawater containing *B. flexus.* However, the absorbance of the -OH peak for the coatings without any treatment was evidently lower than those exposed to seawater. Moreover, the FTIR spectra for the coatings without any treatment and the coatings

exposed to sterile seawater were almost the same except for the -OH peak, which suggested that after being exposed to sterile seawater for 30 days the coating changed little. In seawater containing *B. flexus*, significant changes could be observed in the fingerprint region at 550-1700 cm^{-1} in FTIR spectra for the coatings. In comparison with the coatings before immersion and after exposure to sterile seawater, the absorbance of aliphatic –CH groups (1450 cm^{-1}) and the ether linkage groups (1100 cm^{-1}) for the coating exposed to seawater containing *B. flexus* obviously increased, and the decrease in the peaks (1510 cm^{-1}, 1610 cm^{-1}, 1246 cm^{-1} and 830 cm^{-1}) could be observed. The results proved that *B. flexus* degraded the coating.

4. DISCUSSION

In this study, the electrochemical characteristics of the coatings were characterized with EIS. Among the obtained EIS results, the capacitive arc diameter and corrosion resistance presented in the Nyquist plots and Bode plots, respectively, as well as the R_{coat} value, were of great importance for characterizing the corrosion resistance of the coatings. Generally, in Nyquist plots, a capacitive arc with a larger diameter is usually a sign of the higher electrochemical resistance at the interface [33]. In both kinds of seawater, the diameters of the capacitive arcs for the coatings recorded at 1 day of immersion were approximately the same and larger than those recorded at other immersion times. The result indicated that at the initial immersion time, the coatings possessed excellent corrosion resistance, and the presence of *B. flexus* in seawater had little impact on corrosion resistance of the coatings. However, with prolonged immersion time, the capacitive arc diameter first decreased and subsequently increased. In sterile seawater, the decrease mainly resulted from the permeation of seawater and others corrosive species in seawater, while the increase was mainly ascribed to the accumulation of corrosion products [34, 35]. Additionally, it was noted that the decrease in capacitive arc diameter between 1 day of immersion and 19 days of immersion in seawater containing *B. flexus* was obviously larger in comparison with that in sterile seawater. Moreover, the results presented in the Bode plots and the variations of the R_{coat} values were in accordance with that exhibited in Nyquist plots, which further validated the electrochemical results. Importantly, except for the bacterium, *B. flexus*, there was no distinction between the two kinds of seawater, and we therefore concluded that the presence of *B. flexus* in seawater decreased the corrosion resistance of the coating significantly and potentially resulted in the degradation of the coating.

By fitting the experimental data, the EIS results were further analyzed. The EECs models showed that whether in sterile seawater or seawater containing *B. flexus*, with prolonging the immersion time, the number of time constants increased. In sterile seawater, the EEC in Fig. 3a for 1-2 days of immersion showed only one time constant, While after 6 days of immersion, the corresponded EEC in Fig. 3b presented two time constants, which indicated that corrosion reaction occurred under the coating. Additionally, in seawater containing *B. flexus*, the time constants observed in Fig. 3c-d increased from 1 to 3 with the extension of immersion time, suggesting that the biofilm formed on the coating surface and subsequently corrosion reaction happened on the substrate beneath the coating. Moreover, according to the fitted EIS parameters, the presence of *B. flexus* in seawater aggravated the decrease of the R_{coat} values.

It might be because in seawater, the presence of *B. flexus* caused damage to the coating, destroying the integrity of the coating and further enhancing the permeation of seawater and other corrosive species. In addition, in both kinds of seawater, the R_{ct} values increased with the immersion time. In sterile seawater, the accumulation of corrosion products was mainly responsible for the increase of the R_{ct} values [36]. While in seawater containing *B. flexus*, apart from the accumulation of corrosion products, the formation of biofilm was also of great importance for the increase in the R_{ct} values [37, 38]. The biofilm on the coating surface could be observed by SEM. The fitting results further corroborated the conclusion that *B. flexus* not only decreased the corrosion resistance of coating significantly but also possibly resulted in the degradation of the coating.

The SEM images showed that after exposure to seawater containing *B. flexus* for 30 days, a large amount of bacteria adhered to the coating surface, forming a biofilm. Smironov [39] previously demonstrated that organic acids extensively existed in the products of microbial corrosion. The formation of organic acids and other metabolites in the metabolism process could result in the deterioration of the coatings. Simultaneously, after removing the deposition and biofilm, chalking traces and some tiny holes emerged on the surface of the coatings after exposure to seawater containing *B. flexus*, while there was only slight soaking traces on the surface of the coatings exposed to sterile seawater. The results coincided with the results of the EIS and together demonstrated the destruction and degradation of the coating by *B. flexus*.

Based on the electrochemical measurements and SEM results, the FTIR results further revealed the reason for the degradation of the coating. According to the FTIR results, the higher absorbance of the hydroxyl group (3410-3430 cm^{-1}) for the coatings after 30 days of exposure to both kinds of seawater was observed, which could be attributed to the penetration of seawater. In addition, significant changes observed at the fingerprint region further demonstrated that *B. flexus* degraded the epoxy resin varnish coating mainly by destroying the aromatic rings and epoxy groups in the coating. The destructions might be associated to the bacterial metabolism. Researches showed that bacteria could resulted in the fission of the aromatic ring in the various organics, such as benzene, toluene, ethylbenzene and xylene (BTEX), trough their metabolic activity [40-43]. The epoxy group in an epoxycyclohexane and styrene oxide and its derivatives could be degraded by bacterial epoxide hydrolase [44, 45]. The increase of the absorbance at the peak (1100 cm^{-1}) was due to the formation of the ether linkage. In the process of polyacrylamide degradation, dehydration reaction could occur under the catalysis of bacteria, forming an ether linkage in the carbon chain [46]. The FTIR results further confirmed that *B. flexus* degraded the coating.

5. CONCLUSION

In this paper, to determine the effect of *B. flexus* on the degradation of epoxy resin varnish coating in seawater, various techniques, including electrochemical technique, SEM and FTIR, were employed. The EIS results showed that *B. flexus* remarkably decreased the capacitive arc diameter, corrosion resistance and R_{coat} value, which indicated that the presence of *B. flexus* in seawater decreased the corrosion resistance of the coating significantly and possibly resulted in the degradation of the coating. The SEM images corroborated the EIS results, and together demonstrated that *B. flexus* degraded the

coating after 30 days of immersion. Based on the results of EIS and SEM, the FTIR results further confirmed that *B. flexus* degraded the coating mainly by destroying the aromatic rings and epoxy groups in the coating.

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