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Short Communication

Corrosion Inhibition of Titanium by *Paecilomyces variotii* and *Aspergillus niger* in an Aqueous Environment

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Microbial activity can influence the corrosion behavior of metals through an inhibiting effect or an accelerating effect. Currently, research regarding microbiologically-influenced corrosion mainly focuses on bacteria such as sulfate-reducing bacteria and iron-oxidizing bacteria, but fungus can also influence corrosion processes of metals and materials. In this study, the corrosion behaviors of TA1 titanium were investigated through immersion in two fungi spore suspensions, containing *Paecilomyces variotii* and *Aspergillus niger*, which supplied an aqueous environment for up to 28 days. The reproduction of microorganisms and the formation of biofilms were observed using scanning electron microscopy. The effect of fungi upon the metal was measured with electrochemical methods. The results showed that the fungi had an inhibiting effect on the corrosion of titanium in an aqueous environment.

Keywords: Titanium, Fungi, Corrosion, EIS, MIC

1. INTRODUCTION

The microbiologically-influenced corrosion (MIC) that is usually associated with microbial activities can cause severe safety and reliability problems in the performance of metal materials [1, 2]. MIC exists in many industrial and natural environments, which can lead to significant economic losses [3]. Both bacteria and fungi microorganisms can cause MIC [4, 5]. The mechanisms of microorganisms that influence the corrosion processes are complex. Several mechanisms regarding their acceleration of corrosion have been proposed. For example, microbial activities can cause

corrosion products that contain chemical groups such as sulfides, acids, and ammonia, which can influence the corrosion process [6, 7]. Biofilms that are induced by microorganisms on metal materials can also increase the risk of localized corrosion, such as pitting and stress cracking [3]. Xinyan [8] investigated the corrosion behavior of 2024 aluminum alloys caused by *Aspergillus niger*, and results demonstrated that the corrosion rate was clearly accelerated in presence of *A. niger*. Studies have shown that microorganisms that are isolated from aircraft fuel tanks can accelerate the corrosion rate of aluminum alloys [9].

Simultaneously, in addition to having an accelerating effect on corrosion processes, an equally important consequence of MIC is its inhibiting effect on corrosion. For example, the microbial activities of microorganisms can neutralize corrosive species that come the metabolism [10]. The oxygen consumption that occurs in aerobic microorganisms may generate an oxygen-limiting environment, which can influence cathodic reactions [11]. Sometimes, the corrosion rate of metals decreases due to the barrier effect of the biofilms and corrosion products layers. Eimutis [5,12] investigated the corrosion behavior of metals in an aqueous environment with a spore suspension, and results showed that the fungi *Aspergillus niger* and *Penicillium frequentans* accelerate the corrosion of Al substrates, whereas the suspensions inhibit corrosion on Zn substrates.

Most current research has focused on the MIC of bacteria on metals, and not much attention has been paid to the MIC of fungi [13]. In this study, titanium (TA1) was chosen as a metal specimen and was immersed in spore suspensions of two common fungi, *Paecilomyces variotii* and *Aspergillus niger*, for 28 days. The surface morphologies of the metals and adhered biofilms were observed using scanning electron microscopy (SEM). The corrosion behaviors of the titanium specimens in the fungi spore suspensions were investigated using potentiodynamic polarization curves and electrochemical impedance spectroscopy (EIS).

2. EXPERIMENTAL SECTION

3. 2.1. Test specimens and nutrient medium solution

Titanium specimens were mechanically cut into dimensions of 10 mm × 10 mm × 4 mm and were then polished with 240, 400, 800, 1200, and 2000 grit. The specimens used for electrochemical tests were welded with copper wire and sealed in epoxy. All of the specimens were degreased with acetone and then cleaned in ethanol. The spores of each fungus were placed in sterile water and were diluted to a concentration of $(1 \pm 20\ \%) \times 10^7$ spores per liter. The number of spores was obtained using the plate counting method. The medium for the fungi was made by dissolving salts (Table 1) in 1 L of distilled water. Before the experiments, the solution was sterilized for at least 30 min under a UV lamp. All aseptic operations were performed on a Clean Bench.

Table 1. The chemical composition of the medium.

Salts	Concentration ($g \cdot L^{-1}$)
KH_2PO_4	0.7
K_2HPO_4	0.7
NaCl	0.005
NH_4NO_3	1.0
$MgSO_4 \cdot 7H_2O$	0.7
$FeSO_4 \cdot 7H_2O$	0.002
$ZnSO_4 \cdot 7H_2O$	0.002
$MnSO_4 \cdot H_2O$	0.001
pH	6.0-6.5

2.2. Fungi corrosion test method

The beakers were autoclaved at 120℃ for 20 min. The specimens were immersed in ethanol (75%) and then sterilized for 30 min under a UV lamp. The spore suspensions were diluted 10 times in the medium solution (1:9, v/v), and the final concentration of spores in the solutions was $(1 \pm 20\ \%) \times 10^6$ spores per liter. Specimens were soaked in the solution in beakers. Subsequently, the specimens were transferred to an incubator, the environment of which was 30 ℃and 95% relative humidity (RH). The specimens were retrieved after 7, 14, and 28 days for analyses.

2.3. Surface morphologies and electrochemical test

The surface morphologies of specimens soaked in the aqueous environment were observed using an FEI Quanta250 scanning electronic microscope. To study the corrosion electrochemical behavior, a PAR VMP3 electrochemical workstation was used for potentiodynamic polarization scanning and EIS. A three-electrode system was used in this study: a saturated calomel electrode was the reference electrode, platinum was the counter electrode and titanium specimens were the working electrodes. Potentiodynamic polarization curves were measured from cathodic to anodic regions at a scan rate of 0.167 $mV \cdot s^{-1}$. The EIS measurements were conducted in the frequency range of 100 KHz to 10 mHz with amplitude perturbation of 10 mV. All measurements were performed at ambient temperature (25 ± 2)℃in suspension.

3. RESULTS AND DISCUSSION

3.1. Surface morphologies

Fig. 1 shows the surface morphologies of the titanium specimens that were soaked in the *P. variotii* spore suspension after different numbers of exposure days (from 0 to 28 days). It is apparent that after 7 days (Fig. 1b), the spores produced mycelia on the surfaces of the specimens, indicating the proliferation of *P. variotii*. After 14 days (Fig, 1b-c), some floccules were observed around the

mycelia, indicating the formation of biofilms and the production of a new generation. Due to the microbial activities of spores and mycelia on metal surfaces, the fungus mainly influenced the corrosion behavior of titanium after 7 days. After 28 days of exposure (Fig, 1d), more corrosion products were clearly observed surrounding the mycelia and spores, indicating that *P. variotii* showed good growth during the 28 days of exposure.

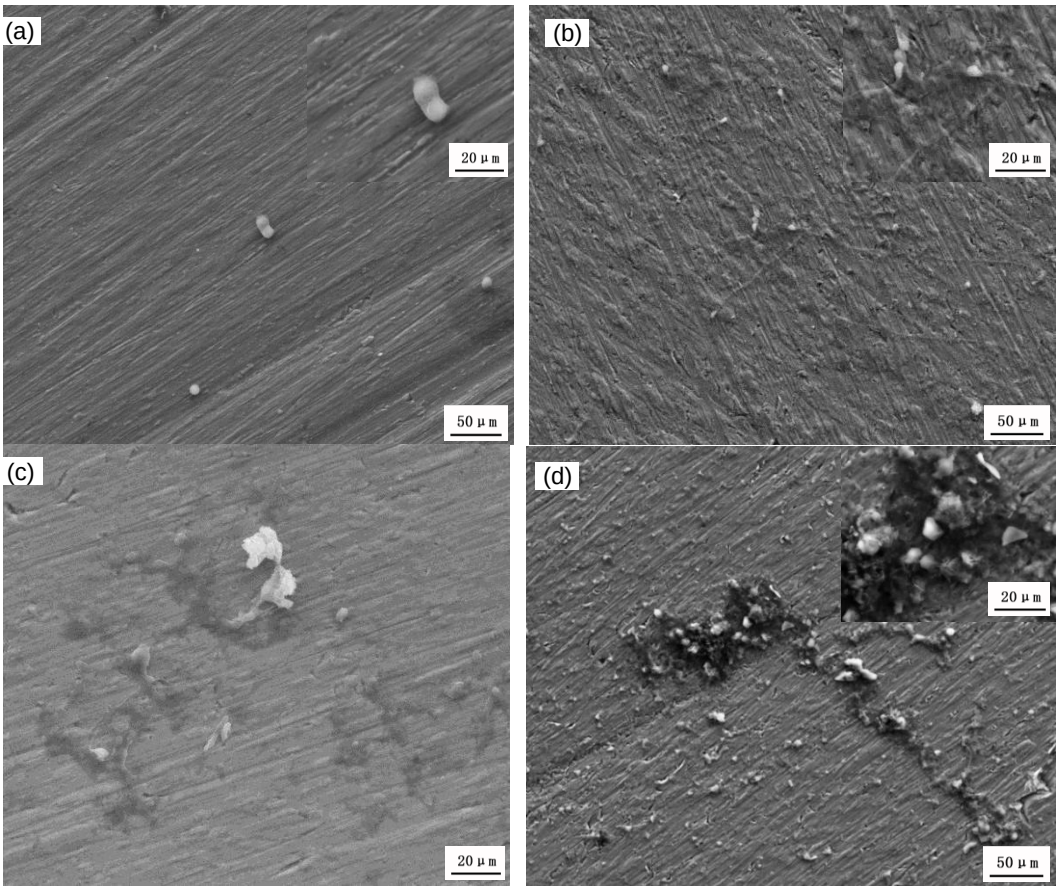

Figure 1. SEM images of biofilms of *Paecilomyces variotii* on titanium specimens in the spore suspension for (a) 0 d, (b) 7 d, (c) 14 d, and (d) 28 d.

Fig. 2 shows the surface morphologies of specimens that were soaked in the *A. niger* spore suspension for 7d, 14d and 28d. Compared to the SEM images of *P. variotii*, *A. niger* demonstrated a much lower proliferation rate on the titanium surface. After 7 days of exposure (Fig. 2a), only a few spores were observed on the metal surface. Between 14 and 28 days (Fig. 2b-c), spores of *A. niger* began to produce mycelia and then continued to grow, influencing the corrosion process. Although biofilms were observed after 14-28 day, the absence of clustered mycelia (such as shown by *P. variotii*) suggests a much slower growth of *A. niger*. Therefore, a weaker interaction occurs between *A. niger* and the titanium surface, leading to lower biofilm coverage.

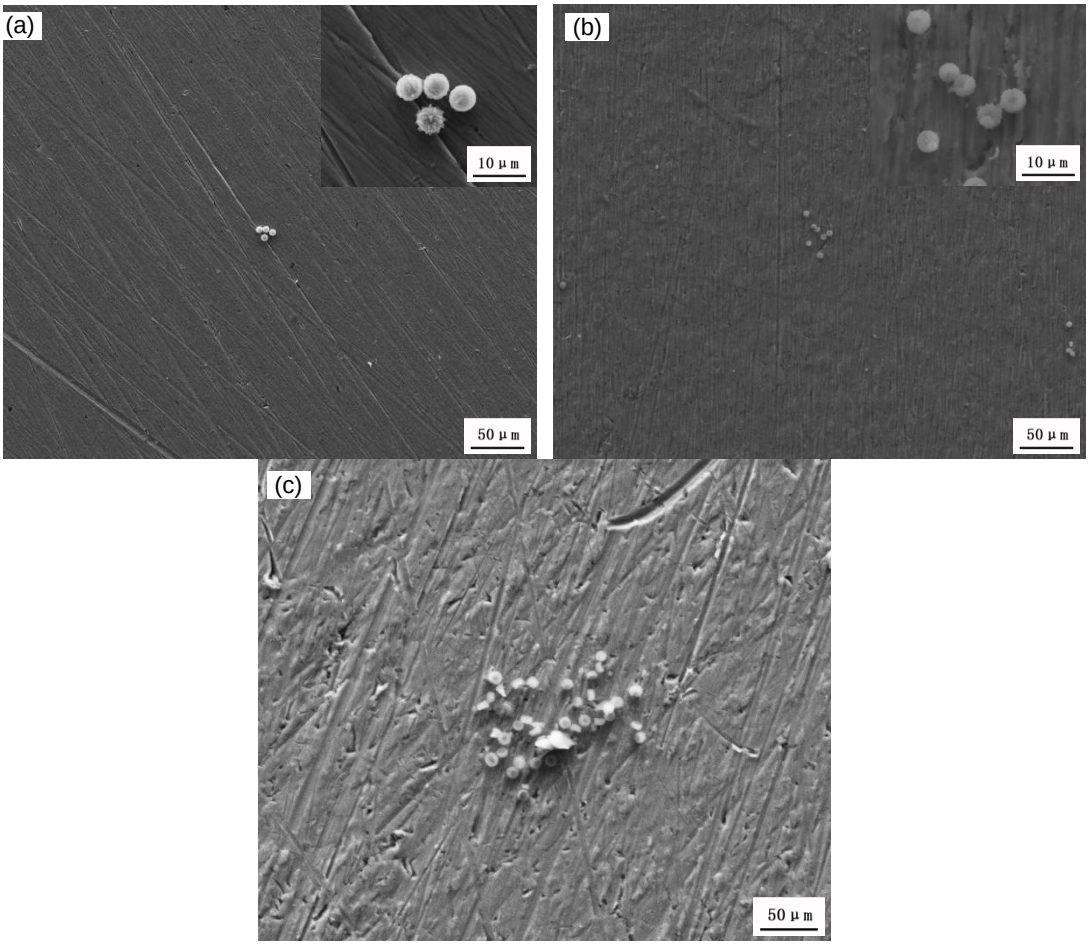

Figure 2. SEM images of biofilms of *Aspergillus niger* on titanium surfaces in the spore suspension for (a) 7 d, (b) 14 d, and (c) 28 d.

3.2. Potentiodynamic polarization curves

Fig. 3 presents the polarization curves of the titanium specimens that were soaked in a spore suspension of *P. variotii* for up to 28 days. The curve for day 0 has a passivity region between 0.25 V to 1.5 V, which is attributed to the intrinsic passivity of the titanium specimens. After 7 days of exposure, a second passivity region begins to form between -0.30 V and 0.25 V, indicating that the microbial activities influence the corrosion behavior of titanium. After 7 days of exposure, the biofilms may hinder the corrosive particles that are in contact with the titanium, resulting a slower corrosion reaction. However, the organic acids that are secreted by fungi that are adhered to the surface can destroy the integrity of the metal surfaces; as a result, the i_{corr} increases after 14 days of exposure. However, with the proliferation and production of *P. variotii*, the hindering effect of the biofilms gradually takes advantage again, thereby decreasing the i_{corr} between 14 and 28 days. The biofilms change significantly, which is consistent with the morphologies that were observed in the SEM images. Although the second passivity is associated with the protective effect of the adhered fungi biofilms, the presence of the passive region even after 28 days of exposure to the spore suspension confirmed the stability of biofilms [14]. Thus, the biofilms that formed on the titanium in the *P. variotii* suspension have a strong inhibiting effect on the metal surfaces. In a study by Zuo [15], the

biofilms of *Bacillus subtilis* that had adhered to Al 2024 inhibited corrosion, and the passivity was found and the time constant was changed in the presence of biofilms.

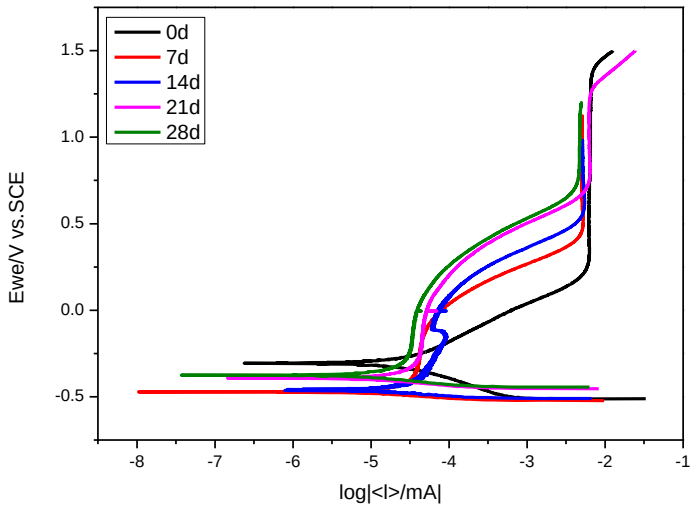

Figure 3. Potentiodynamic polarization curves of titanium soaking in a spore suspension of *Paecilomyces variotii* for different exposure days

Fig. 4 shows the polarization curves for the titanium specimens that were soaked in a spore suspension of *A. niger* for different numbers of days. As discussed above for *P. variotii*, the activities of microorganisms mainly affect the process of the anodic reaction. *A. niger* produced biofilms on the titanium specimens; thus, a second passive region between - 0.25 V and 0.25 V was observed after 7 days, indicating the protective effect of the biofilms. After 7 days, the biofilms formed on the surface, resulting in a lower i_{corr}. After 14 days, the corrosive effect of the organic acids that were secreted by *A. niger* influenced the integrity of surface; thus, an increase of i_{corr} was observed after 14 days of exposure. Then, the inhibition effect of the biofilms increased and i_{corr} decreased. However, with prolonged immersion in an aqueous environment, the biofilms locally began to degrade. Therefore, the i_{corr} of curves increased again after 21 days of exposure.

Zhang [14] demonstrated that the surface coverage of biofilms on 304 stainless steel specimens increased as immersion time increased, and reached a maximum at 21 days. Then, there was a decrease after 21 days. As such, exposure to the *A. niger* spore suspension for more than 21 days may result in local degradation of the biofilms. Thus, prolonged soaking in a spore suspension may cause degradation of the biofilms, which leads to a reduced inhibition effect on the titanium specimens after 21 days.

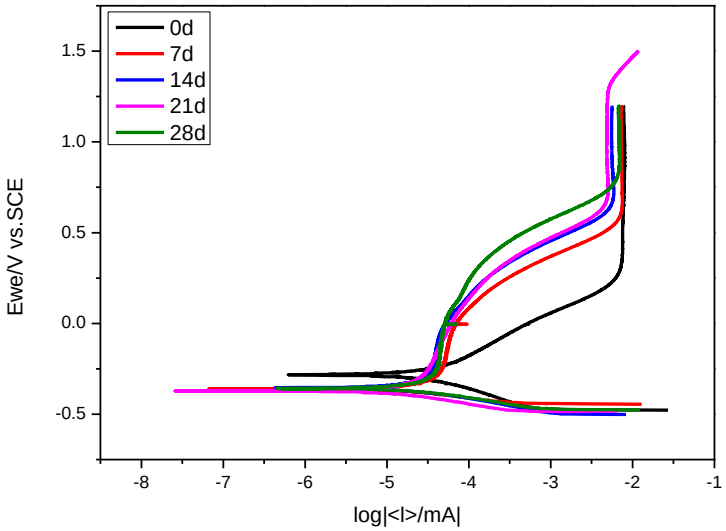

Figure 4. Potentiodynamic polarization curves of titanium soaking in a spore suspension of *Aspergillus niger* for different numbers of exposure days.

Typically, a lower i_{corr} indicates a lower corrosion rate; thus, with immersion for 28 days, the corrosion process was inhibited because of the formation of *P. variotii* and *A. niger* biofilms. From 0 to 7 days, the formation of biofilms reduced the corrosion rate. From 7 to 14 days, the acids that were secreted by microorganisms increased the value of i_{corr}. However, after 14 days of exposure, the effect of biofilms was enhanced due to the proliferation and reproduction of mycelia. As a whole, the fungi biofilms had an inhibition effect on the corrosion of titanium.

Table 2. Electrochemical parameters of potentiodynamic polarization curves for the *Paecilomyces variotii* spore suspension.

	E_{corr} *(mV vs. SCE)*	i_{corr} *(mA·cm^{-2})*	$-b_c$
0 d	-309.4	3.311×10^{-5}	0.338
7 d	-471.9	4.713×10^{-6}	0.036
14 d	-459.4	2.362×10^{-5}	0.058
21 d	-381.3	1.711×10^{-5}	0.049
28 d	-371.9	5.754×10^{-6}	0.070

Table 3. Electrochemical parameters of potentiodynamic polarization curves for the *Aspergillus niger* spore suspension.

	E_{corr} *(mV vs. SCE)*	i_{corr} *(mA·cm^{-2})*	$-b_c$
0 d	-273.8	4.677×10^{-5}	0.330
7 d	-368.9	1.380×10^{-5}	0.119
14 d	-350.0	2.454×10^{-5}	0.197
21 d	-386.8	1.148×10^{-5}	0.169
28 d	-361.9	2.754×10^{-5}	0.179

3.3. EIS study

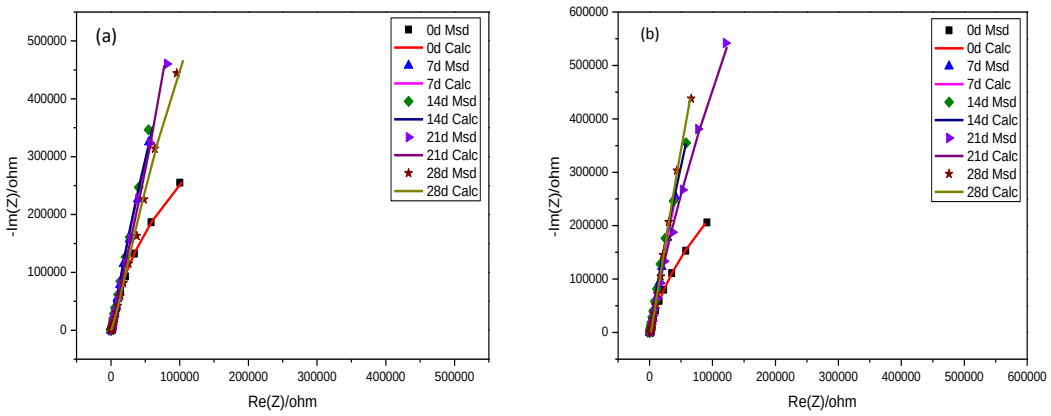

Figure 5. Nyquist diagrams for titanium specimens in spore suspensions for different numbers of exposure days: (a) *Paecilomyces variotii* and (b) *Aspergillus niger*.

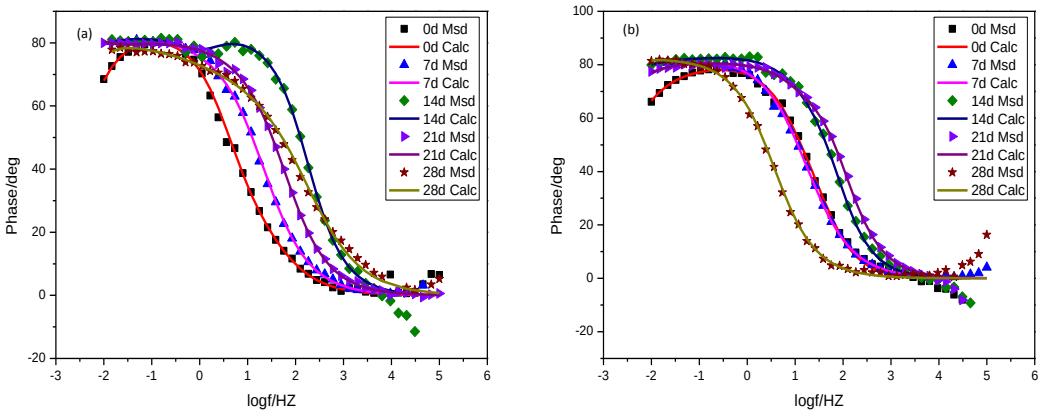

Figure 6. Bode diagrams for EIS of titanium specimens that were soaked in spore suspensions for different numbers of exposure days: (a) *Paecilomyces variotii* and (b) *Aspergillus niger*.

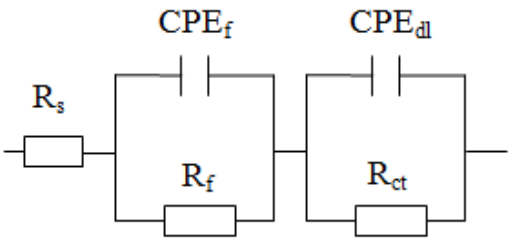

Figure 7. The Equivalent circuit for EIS of titanium that was soaked in fungi spore suspensions for up to 28 days (for both *P. variotii* and *A. niger*).

Fig. 5 shows the results in the form of Nyquist plots for the titanium specimens for up to 28 days. Fig. 6 shows the Bode diagrams that resulted from the EIS study. Fig. 6 shows the corresponding

equivalent circuits of EIS. There are two time constants in the equivalent circuits of specimens that were soaked in *P. variotii*. CPE_{dl} and R_{ct} represent the electrical double layer, CPE_f and R_f reflect the biofilms on the specimen [16]. After 7 days of exposure in the *P. variotii* suspension, the value of R_f increases significantly, indicating the formation of biofilms. Consistent with the polarization curves, with the microbial activities of biofilms from 7 days to 14 days, the organic acids secreted by the biofilms threatened the integrity of the metal surface, resulting in a temporary decrease of Y_0 of the biofilms. However, the proliferation of microorganisms enhances the hindering effect of the biofilms; thus, the value of Y_0 decreased again after 21 days.

Compared to the EIS results for *P. variotii*, the R_f of biofilms that were soaked in the *A. niger* spore suspension is much lower, indicating the lower amount of surface area coverage and thickness of *A. niger* on the metal surface. With the proliferation of fungi, the Y_0 of biofilms decreases consistently, which is attributed to the development of biofilms. After 14 days of exposure, the value of n equals 1, indicating that the *A. niger* biofilms on the titanium are more homogeneous than *P. variotii*, which may be due to the slower rate of proliferation and generation of *A. niger*. In addition, compared to the fluctuating trend of Y_0 for *P. variotii*, the continuous declining trend of Y_0 in *A. niger* also demonstrates the lower microbial activities of *A. niger*.

Table 4. The fitting results of EIS for titanium that was soaked in a spore suspension of *Paecilomyces variotii*.

	$R_f (\Omega \cdot cm^2)$	$Y_0 (\Omega^{-1} \cdot cm^{-2} \cdot s^n)$	n	$C_f (F \cdot cm^{-2})$
0 d	340.1	1.307×10^{-4}	0.7101	3.667×10^{-5}
7 d	6.723×10^{4}	8.862×10^{-5}	0.7135	1.815×10^{-4}
14 d	9.163×10^{4}	1.147×10^{-4}	1	1.147×10^{-4}
21 d	1.227×10^{5}	6.291×10^{-5}	0.7669	1.171×10^{-4}

Table 5. The fitting results of EIS for titanium that was soaked in a spore suspension of *Aspergillus niger*.

	$R_f (\Omega \cdot cm^2)$	$Y_0 (\Omega^{-1} \cdot cm^{-2} \cdot s^n)$	n	$C_f (F \cdot cm^{-2})$
7 d	175.7	5.203×10^{-4}	0.6049	1.029×10^{-4}
14 d	123.1	1.011×10^{-4}	1	1.011×10^{-4}
21 d	81.24	9.398×10^{-5}	1	9.398×10^{-5}
28 d	4670	5.932×10^{-5}	1	5.932×10^{-5}

3.4. Corrosion mechanism

The results from potentiodynamic polarization curves and EIS demonstrate that *A. niger* and *P. variotii* mainly have an inhibitive effect on the corrosion of titanium. Basically, the inhibitive mechanisms that are caused by microbiological influences can be divided into the following reasons: (1) biofilms produced in metabolic cycles have a barrier effect that can hinder the interaction between corrosive ions or species and the material surface [17,18]; (2) oxygen consumption, especially in the

microbial activities of aerobic microorganisms [19]; and (3) secretion of chemical complexes which suppresses corrosion reactions [20].

Before 14 days of exposure, the mechanism of inhibition mainly originated from the metabolic products of microorganisms [21]. Previous studies have indicated that complexes that contain chemical groups such as amide and carboxyl groups are conducive to suppressing anodic dissolution at localized sites. However, with the secretion of inhibitors, some organic acids that can destroy metal surfaces also exist between metal surfaces and mycelia. In previous studies, researchers have demonstrated that citric acid that is secreted by *A. niger* can corrode metal [22] and oxalic acid that is secreted by *A. niger* is one of the main factors in the corrosion of AA2024 aluminum [5]. Thus, the inhibition of corrosion was suppressed. After 14 days of exposure, the inhibition of corrosion was enhanced due to the formation of biofilms, which produce a barrier effect. In this study, because the cathodic polarization curves demonstrated a similar trend, oxygen limitation was not a main factor.

4. CONCLUSIONS

The inhibition effects by *P. variotii* and *A. niger* on the corrosion of titanium specimens in an aqueous environment were investigated in this study. SEM observations indicated that *P. variotii* rapidly induced the formation of biofilms on titanium, whereas *A. niger* showed much slower reproduction. In addition to the original passive region in the polarization curves, a second passive region revealed the formation of biofilms on titanium, which enhanced the corrosion resistance properties of titanium. EIS studies also confirmed the formation of biofilms on titanium, and the parameters of the outer layer revealed the microbial activities in biofilms.

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