Microbiologically Influenced Corrosion Behavior of Carbon Steel in the Presence of Marine Bacteria *Pseudomonas* sp. and *Vibrio* sp.

Deli Cai, Jinyi Wu, and Ke Chai*

ABSTRACT: The microbiologically influenced corrosion (MIC) behavior of carbon steel is investigated in the presence of *Vibrio* and *Pseudomonas*. Sterilized natural seawater inoculated with *Pseudomonas*, *Vibrio*, and the mixture of *Pseudomonas* and *Vibrio*, separately, and they are utilized as the media for corrosion characterizations, which are closer to the natural environment in seawater. Weight loss measurements, electrochemical techniques (the open-circuit potential, electrochemical impedance spectroscopy (EIS), and potentiodynamic polarization curves), and surface analysis (scanning electron microscopy (SEM)) are performed to explore the synergistic effect of *Pseudomonas* and *Vibrio* on the corrosion behavior of carbon steel. As seen from the growth curves of bacteria, the growth and propagation of *Pseudomonas* and *Vibrio* are affected by their metabolic activities. Besides, the results obtained by SEM show that more severe pitting corrosion is observed on the coupons exposed to the sterilized natural seawater inoculated with the mixture of *Pseudomonas* and *Vibrio*. Further, the results from electrochemical measurements and weight loss measurements suggest that under the synergistic effect of *Pseudomonas* and *Vibrio*, the initial corrosion rate of carbon steel is inhibited, while the latter corrosion is enhanced.

1. INTRODUCTION

No matter whether in an aqueous environment or in high humidity conditions, any material is extremely susceptible to microorganisms, especially for metals. In the aqueous environment, microorganisms tend to attach to the surfaces of metal substrates and form a slimy biofilm, which mainly consisted of extracellular polymeric substances (EPSs), sessile cells, and corrosion products to protect them from external pressure and obtain nutrients for growth. It is widely accepted that the biofilm plays a crucial role in microbiologically influenced corrosion (MIC). MIC is a severe issue in quite a few industries, which accounts for 20% of all corrosion damage. Especially for carbon steel, which is widely used in the seawater environment due to its reasonable price and excellent properties, MIC is an inevitable issue that can cause severe corrosion.

MIC is not merely the result caused by a single species of bacteria, it is also usually induced by the synergistic effects of multiple species of microorganisms that coexist in the biofilm and the environment. A variety of microorganisms with different metabolic characteristics have been identified in MIC, among which *Pseudomonas* and *Vibrio* are indispensable. Our previous research studies suggested that *Pseudomonas* and *Vibrio* were found significantly abundant in the corrosion products of carbon steel immersed in natural seawater. Besides, a number of investigations have been performed to explore the corrosion behaviors of steel influenced by *Pseudomonas*, the results of which suggested that the presence of *Pseudomonas* not only can accelerate the corrosion process of 2205 duplex stainless steel and significantly promote the corrosion of 2707 hyper-duplex stainless steel but also results in extremely serious pitting corrosion of high-nitrogen nickel-free stainless steel. Several mechanisms have been proposed to account for the effects of *Pseudomonas* on the corrosion process of metals. Morales et al. and Franklin et al. proposed that differential aeration cells or metal ion concentration cells might be induced during the process of biofilm formation, which greatly changes the corrosion process of materials. Moreover, Pedersen et al. and Yuan et al. deemed that *Pseudomonas* sp. could produce organic acid, which promoted the passivity breakdown to accelerate the corrosion process of steel. However, different strains have different impacts on the corrosion of steel. In the presence of *Vibrio neocaledonicus* sp., the corrosion resistance of carbon steel increased by more than 60-fold, which showed a great corrosion inhibitory effect, while when *Vibrio natriegens* existed, a decrease in the charge transfer resistance and an increase in corrosion current densities indicated that the corrosion process of stainless steel was accelerated. Moreover, the research also revealed that in artificial seawater, the presence...
of Bacillus subtilis C2 significantly accelerated the initial corrosion and inhibited the latter corrosion.19

Nevertheless, most of the investigations only focus on the effects of a single species of bacteria on the corrosion behavior of metals, while microorganisms always coexist in the environment. The growth, activity, and distribution of Pseudomonas may be affected by other microbes in the heterogeneous natural biofilm and environment, which eventually leads to the change of corrosion behavior and mechanisms. For example, the corrosion process of cast iron diminished in the copresence of Pseudomonas aeruginosa and Desulfovibrio vulgaris, which is different from the case when D. vulgaris exists alone.20 However, the corrosion behavior of carbon steel under the influence of the metabolites of Pseudomonas and Vibrio is still not well understood. Therefore, it is highly desirable to study the corrosion conditions under mixed cultures of Pseudomonas and Vibrio.

Accordingly, the aim of this study is to explore the corrosion behavior of carbon steel in the presence of Pseudomonas and Vibrio. Instead of performing all of the experiments in the nutrient-rich media, sterilized natural seawater without any treatment, which is closer to the condition in the natural environment, was utilized for corrosion characterization. Therefore, a series of systematic comparisons about the corrosion behavior of carbon steel in single and mixed bacteria inoculated seawater were performed. Further, the effects of Pseudomonas and Vibrio on the corrosion behavior of carbon steel were explored using various techniques such as the electrochemical test, weight loss measurement, and scanning electron microscopy (SEM).

2. RESULTS

2.1. Bacterium Number Analysis. Figure 1 shows the growth curves of Vibrio and Pseudomonas in different media. It could be seen that in the single bacterium environment, the count of Vibrio and Pseudomonas reached the maximum value on the 3rd and 5th days, respectively, while in the mixed culture medium, the count of Vibrio and Pseudomonas reaches the maximum value on the 5th day and 7th day, respectively. It is easy to find that in the mixed medium, the time when the cell count of Vibrio and Pseudomonas reaches the maximum value lags behind in the single bacterium culture medium, which mainly due to the competition between Vibrio and Pseudomonas over dissolved oxygen that is limited in the medium.21 With the increase of the immersion time, the concentration of the dissolved oxygen and nutrients in the mixed culture medium is consumed more quickly. Therefore, at the end of the experiment, the cell count of Vibrio and Pseudomonas in the mixed culture medium is about 1 order of magnitude lower than that in the single bacterium environment and the count of Pseudomonas is obviously less than that of Vibrio both in single and mixed bacteria media. Moreover, after 7 days of immersion, the count of Vibrio in the mixed medium becomes the highest in comparison to that in other media, which illustrates that the presence of Pseudomonas promotes the growth of Vibrio. Further, all of the results reveal that the growth and propagation of Vibrio and Pseudomonas are affected by each other’s metabolic activities. Figure 2 shows that Vibrio sp. produces acid. The pH value drops faster in the early stage of immersion and drops slowly in the later stage.

2.2. Average Corrosion Rate. To determine the influence of the synergistic effects of Vibrio and Pseudomonas on the corrosion extent of coupons, the average corrosion rate of coupons immersed in different media for different times is calculated by the data from the weight loss measurement. As shown in Figure 3, with the extension of the immersion time, the corrosion rates of coupons exposed to sterile seawater and the Vibrio-containing medium decrease gradually, whereas for coupons immersed in the mixed culture medium of Vibrio and Pseudomonas and the Pseudomonas-containing medium, the average corrosion rate increases and then decreases slightly. In addition, it is just a simple mixture of two bacteria to neutralize the corrosion rate. After 15 and 30 days of immersion in the mixed culture medium of Vibrio and Pseudomonas, the average corrosion rate of coupons becomes the largest compared with those in the other three environments. Consequently, the result illustrates that in the coexistence of Vibrio and Pseudomonas, their synergistic effect inhibits the initial corrosion of carbon steel and accelerates the latter corrosion.

2.3. Morphology Analysis by SEM. The surface morphology of coupons after 30 days of immersion in different media is characterized by SEM, and the results are shown in Figure 4. As it could be seen from Figure 4a,c, irregular,
heterogeneous, and dense corrosion products, which piled up and further formed cauliflower-like structures, are covered on the surface of coupons exposed to the mixed culture medium and the Pseudomonas-containing medium, while for the coupons immersed in the Vibrio-containing medium (Figure 4b), the products presented roseette-like structures formed by the overlap of platelet-like products.

Besides, in sterile seawater, corrosion products come into being massive and thick deposition on the coupons. Figure 4e,f shows that the incomplete and smooth biofilm composed of bacteria and abundant extracellular polymeric substances exists at some locations on the surface of the coupons, which are exposed to the Vibrio-containing medium and the mixed culture medium of Vibrio and Pseudomonas. The corrosion products and the metabolite form a compact corrosion product film, and due to the presence of this film, the transportation of corrosive species like oxygen as well as various ions was hampered and further affected the corrosion rate.22

Figure 5 shows the corrosion morphology of the coupons after removing the corrosion products and the biofilm. It is evident that after 30 days of immersion, the coupons exposed to the media containing bacteria suffer more severe corrosion, on which dense corrosion pits and spots, big or small, are presented. Moreover, the corrosion conditions of the coupons exposed to the mixed culture medium of Vibrio and Pseudomonas are more serious, the surfaces of which are much rougher and the corrosion holes and pits are bigger. Accordingly, the results reveal that the presence of bacteria aggravates the corrosion of carbon steel significantly after 30 days of immersion, and the synergistic effect of Vibrio and Pseudomonas on aggravating the corrosion of carbon steel is more evident.

2.4. Electrochemical Measurements. 2.4.1. Open-Circuit Potential. Figure 6 shows the variation of open-circuit potential values ($E_{ocp}$) with exposure time for coupons under different conditions. The difference in the bacterial metabolic activities, the attachment of the biofilm, and their corrosive characteristics for each test result in the fluctuation of the electrochemical data. It can be seen that the coupons immersed in the media with bacteria process far higher corrosion potential in comparison to those in sterile seawater. Similar results have also been reported by previous reports.23,24 In sterile seawater, the damage of the passivation film can be responsible for the variation of $E_{ocp}$ while in the biotic medium, $E_{ocp}$ is higher than that in sterile seawater, which is related to the biofilm and the corrosion product film formed by the bacteria.8 Simultaneously, in the mixed culture medium and the Vibrio medium, the values of the open-circuit potential shift evidently to the positive direction on the 5th day and then move back to the original level, while in the medium inoculated with Pseudomonas, the values of the open-circuit potential change little, keeping steady with time. Moreover, in the biotic media, the corrosion potential for the coupons exposed to the mixed culture medium is the lowest, and after 27 days of immersion, the values of the open-circuit potential shift to the negative direction evidently, which is consistent with the polarization curves.

2.4.2. Electrochemical Impedance Spectroscopy (EIS) Analysis. Time-dependent Nyquist plots of the coupons immersed in different media are shown in Figure 7. Generally, a larger semicircle diameter in Nyquist plots usually indicates higher electrical resistance at the metal—solution interface, which implies lower corrosion rates.25−27 In the Nyquist plots (Figure 7a−d), the diameter of the Nyquist plots for the coupons exposed to different media changes much with exposure time. In the presence of Vibrio alone (Figure 7b), the diameter of the Nyquist plot increases during 0−3 days, which suggests that a protective film composed of EPS and corrosion products forms on the coupons. Then, the diameter of the Nyquist plot decreases gradually and finally increases to the maximum value after 30 days of immersion. The increase of the Nyquist plot diameter can be attributed to the protection offered by the biofilm—corrosion product film, while the decrease of the Nyquist plot diameter indicates the breakdown of the protective corrosion product film.28,29 Besides, the variation in the Nyquist plot diameter for the coupons immersed in the medium inoculated with Pseudomonas (Figure 7c) is similar to that in the medium containing only Vibrio. However, different from the case of medium containing Vibrio or Pseudomonas alone, the diameter in the Nyquist plots for the coupons exposed to the mixed culture medium of Vibrio or Pseudomonas reaches the maximum value at the 7th day and then decreases consistently during 7−30 days. The result suggests that the initial corrosion is weakened and the latter corrosion is accelerated. While in sterile seawater, the maximum value of the diameter of the Nyquist plots emerges after 30 days of exposure.

The Bode phase angle versus log frequency plots of the coupons exposed to different media for different times are shown in Figure 8. As seen from the Bode phase angle plots (Figure 8a) for the coupons immersed in the mixed culture medium inoculated with Vibrio and Pseudomonas, two peaks indicating two time constants emerge at the initial time, one of which is at the low-frequency side, while the other one locates at a high frequency. However, with the prolonging of the immersion time, the only peak moves to the low-frequency side, suggesting that a more integrated film is formed on the coupons. In addition, for the coupons exposed to the media inoculated with Vibrio and Pseudomonas alone, the variation in the Bode phase angle plots is extremely similar to that in the mixed culture medium. Nevertheless, different from the situations of the medium inoculated with Vibrio and the mixed culture medium of Vibrio and Pseudomonas, after 30 days of immersion, two peaks emerge in the Bode phase angle plots of the coupons immersed in the medium inoculated with Pseudomonas, one of which locates at the middle frequency, while the other one emerges at the low frequency. Usually, two time constants are due to the development of a two-layer structure during the corrosion process, and in the Bode plots, the
peak at the higher frequency is most likely due to the biofilm, whereas the peak at the lower frequency can be attributed to the electrical double layer, which is confirmed by the fitting results of impedance measurements. Moreover, as can be seen from Figure 8a, after 30 days of immersion, the impedance values at the low frequency for the coupons exposed to the mixed culture medium decrease obviously, which is a sign of the presence of a porous or patchy film and indicates the breakdown of the corrosion product film and the biofilm.

To get a quantitative measure of the electrochemical parameters at the metal/electrolyte interface, ZSimpWin software was utilized to fit the EIS data. Figure 9 shows the equivalent circuit model where the fitted results of the electrical components are listed in Table 1. The quality of fitting was judged by $\chi^2$ and all of the $\chi^2$ values were around $10^{-3}$, which indicated a good fit of the EIS data. In the equivalent circuit model (Figure 9), which has been used in previous reports, $R_s$, $R_{ct}$, $Q_{dl}$, $R_f$, and $Q_f$ correspond to the solution resistance, charge transfer resistance, capacitance of the double layer, resistance of the film, and capacitance of the film. In Table 1, $R_s$ is quite low and changes little with exposure time, owing to the good conductivity in the medium with and without bacteria. Generally, the charge transfer resistance $R_{ct}$ is the parameter that is usually used to characterize the corrosion rate. In sterile seawater and the medium inoculated with *Vibrio*, the charge transfer resistance, $R_{ct}$, has a clear trend of increasing with the immersion time, suggesting the decrease of the corrosion rate of the coupons with time, which also confirms the results from the weight loss measurement, whereas in the mixed medium and the medium inoculated with *Pseudomonas*, $R_{ct}$ fluctuates from 0 to 7 days and then tends to be stable. As shown in Table 1, it is evident that $R_{ct}$ in the mixed culture medium is higher than those in sterile seawater and the single bacterial system at the initial time and subsequently becomes relatively smaller than those in
other media, indicating that the synergistic effect of Vibrio and Pseudomonas can inhibit the initial corrosion and accelerate the latter corrosion of the coupons.

2.4.3. Potentiodynamic Polarization Curve Analysis. Figure 10 exhibits the potentiodynamic polarization curves of the coupons exposed to different media at different times. As shown in Figure 10a, after 7 days of immersion compared with the curves recorded in biotic media, which are quite close, the curves recorded in sterile seawater possess higher anodic current densities and more negative corrosion potential, suggesting that the coupons exposed to sterile seawater are more susceptible to be corroded and oxidized, while from Figure 10b,c, with the increase of the immersion time, the curves recorded in the mixed culture medium display lower corrosion potential and relatively higher current densities. In the presence of Pseudomonas and Vibrio, the corrosion current densities obviously increased with the extension of the immersion time from 7 to 15 days and then a slight decrease can be observed after 30 days of immersion. Besides, a significant negative shift in corrosion potential from −0.783 to −1.016 V is observed during 7–30 days. The potentiodynamic polarization curve results that display the current densities in Table 2 are consistent with the weight loss measurement and EIS data, which confirms that the presence of Vibrio and Pseudomonas inhibits the initial corrosion of carbon steel, while enhances the latter corrosion.

3. DISCUSSION
The effects of microorganisms on metal corrosion are related to various factors like their species, concentration, and metabolic activity. Pseudomonas sp., an aerobic microorganism, has been found as one of the most abundant aerobic strains in seawater, which can influence the corrosion behavior of materials by forming a heterogeneous biofilm and further results in differential aeration cells. Therefore, countless electrochemical cells begin to form on the surface of materials and further change the corrosion process of materials, while as a facultative anaerobe, Vibrio consumes oxygen rapidly when oxygen is adequate, and contrarily, under hypoxic conditions, fermentation metabolism is predominant. In this study, Figure 1 shows the growth curves of Vibrio and Pseudomonas in different media, which suggests that the growth and propagation of Vibrio and Pseudomonas are greatly affected by each other’s metabolic activities. This is due to the competition between Vibrio and Pseudomonas over dissolved oxygen and limited nutrients. As
seen from the weight loss measurements, after 7 days of immersion in different media, the average corrosion rates of the coupons immersed in Vibrio, Pseudomonas, and Vibrio and Pseudomonas containing media are 88.4, 66.5, and 81.6% of that in sterile seawater, which indicates that the corrosion of carbon steel in the biotic environment is inhibited at the initial time. This is due to the fact that Vibrio and Pseudomonas consume oxygen rapidly during the normal metabolism activity. By examination, after 7 days of immersion, the dissolved oxygen concentration in sterile seawater is about 6.3 mg/L, while in the mixed culture medium, it is just about 2.8 mg/L. As is known, the limited dissolved oxygen in media not only affects the growth of bacteria but also has a great impact on the rate of the cathodic reaction process.31 The reduction of oxygen can affect the cathodic reaction and further change the corrosion process of carbon steel. Besides, the complete biofilm–corrosion product film gradually forms on the surface of coupons, which would hinder the transportation of substances, like oxygen and some ions, which also retards the corrosion of carbon steel.32,33 As shown in Figure 7, the impedance value becomes maximum after 7 days of immersion. Vibrio is a kind of acid formation bacteria, which can produce acid in its metabolic activities and decrease the pH values, accelerating the corrosion rate of metals. Therefore, the corrosion inhibition in the Vibrio-containing medium is not as strong as in the Pseudomonas-containing medium. Moreover, the polarization curves shown in Figure 9 suggest that lower corrosion potential and higher corrosion current density for the coupons are recorded in sterile seawater, which confirms that the corrosion of the coupons in the biotic environment is inhibited at the initial time.

With the increased immersion time, a denser and thicker corrosion product film forms on the surface of the coupons, which results in the decrease of the average corrosion rate of the coupons in different media from 15 to 30 days. After 30 days of immersion, the average corrosion rate in the mixed culture medium is 121.5% of that in sterile seawater, indicating that the corrosion in the mixed culture medium is enhanced significantly. Nevertheless, in the single bacterial system, there are no obvious effects in accelerating the corrosion of the coupons. The EIS results show that in the mixed culture medium, the corrosion resistance of the coupons reaches the maxima after 7 days of immersion, indicating that a compact film, which consist of corrosion products and the biofilm, is formed on the surface of the coupons. Due to the presence of this film, the corrosion of carbon steel is retarded at the initial immersion time. As the immersion time increases, the corrosion resistance decreases gradually, suggesting that the film is destroyed. Additionally, in comparison to that in sterile seawater, the corrosion resistance is evidently higher in the mixed culture medium at the initial immersion stage and subsequently become slightly smaller than that in sterile seawater, which indicates that in the mixed culture medium, the corrosion of the coupons is retarded at the initial immersion time, while enhanced at the latter immersion stage. Different from the case of the mixed culture medium, in the Pseudomonas sp. inoculated medium and the Vibrio sp. inoculated medium, the corrosion resistances of the coupons achieves the maxima after 30 days. To better understand the EIS results, the results are further analyzed by fitting the equivalent circuit, which confirmed the results by EIS measurements.

Figure 7. Nyquist plots (a–d) recorded on coupons exposed to Vibrio sp. and Pseudomonas sp. (a), Vibrio sp. (b), and Pseudomonas sp. (c) containing media and sterile seawater (d) for different times.
In the mixed culture medium, the anaerobic area under the deposition of corrosion products and the area where oxygen concentration is relatively enriched create the oxygen concentration cells gradually, which accelerates the corrosion process of the coupons, inducing more severe corrosion (Figure 5a). Simultaneously, a large amount of corrosion products pile up beneath the biofilm and further destroy the biofilm. As can be seen from Figure 4e,f, an incomplete and smooth biofilm can be observed on the local position of the coupons after 30 days of exposure, which confirms the destruction of the biofilm. Accompanying the destruction of the biofilm, the oxygen concentration cells form easily where the biofilm is destroyed, accelerating the corrosion. As shown in the Nyquist plots (Figure 7a), in the mixed culture medium, the maximum value of the diameter in the Nyquist plot is observed at the 7th day, which can be attributed to the formation of the complete and compact biofilm—corrosion product film, whereas it decreases during 7–30 days, which is mainly due to the destruction of the film. Moreover, heterogeneous distribution of Vibrio and Pseudomonas makes the difference in dissolved oxygen more complex, which promotes the corrosion of the coupons. In the mixed culture medium, Vibrio, as a kind of acid formation bacteria, can reduce the localized pH on the surface of the coupons where they are attached to, which caused severe pitting corrosion. This observation is supported by the SEM images shown in Figure S, where evident pitting traces are observed. Consequently, after 30 days of exposure, the average corrosion rate becomes the highest compared with those in other environments, which suggested that the synergistic effects of these two kinds of bacteria promote the corrosion of metals.

4. CONCLUSIONS

The corrosion behavior of AISI carbon steel in natural seawater in the presence of Vibrio and Pseudomonas has been investigated using weight loss measurement, surface analysis, and electrochemical techniques. The changes in growth curves for bacteria indicate that the growth of Vibrio and Pseudomonas is affected by each other’s metabolic activities. In the presence of Vibrio and Pseudomonas, the average corrosion rate of carbon steel is inhibited at the initial stage, while with the increase of the immersion time, the corrosion is enhanced. SEM micrographs show that in the mixed medium, more severe pitting corrosion can be observed on the coupons after removing the corrosion products, which is mainly ascribed to the synergistic effect induced by the mixture of Vibrio and Pseudomonas. The

Figure 8. Bode plots (a–d) recorded on coupons exposed to Vibrio sp. and Pseudomonas sp. (a), Vibrio sp. (b), and Pseudomonas sp. (c) containing media and sterile seawater for different times.

Figure 9. Equivalent circuit used to fit the EIS data.
Table 1. Electrochemical Parameters Obtained from the EIS Results in Different Media

| system                  | time (days) | $R_\text{i}$ (Ω cm$^2$) | $C_i$ (μF/cm$^2$) | $R_\text{ct}$ (Ω cm$^2$) | $C_\text{dl}$ (μF/cm$^2$) | $R_\text{ct}$ (Ω cm$^2$) |
|-------------------------|-------------|--------------------------|------------------|--------------------------|---------------------------|--------------------------|
| *Pseudomonas sp.*—*Vibrio sp.* | 0           | 9.749                    | 200              | 375.4                    | 1400                       | 2711                     |
|                         | 1           | 10.39                    | 1000             | 335                       | 800                        | 750.9                    |
|                         | 3           | 10.21                    | 2500             | 249.8                    | 1700                       | 1677                     |
|                         | 5           | 11.29                    | 6600             | 78.89                    | 3200                       | 673.1                    |
|                         | 7           | 8.753                    | 6400             | 170.5                    | 3000                       | 2190                     |
|                         | 15          | 9.953                    | 16400            | 21.68                    | 5600                       | 2030                     |
|                         | 30          | 12.79                    | 17200            | 46.16                    | 12300                      | 1971                     |
| *Vibrio sp.*            | 0           | 9.283                    | 1300             | 13.18                    | 1600                       | 1131                     |
|                         | 1           | 10.49                    | 1800             | 198.4                    | 1600                       | 1899                     |
|                         | 3           | 10.08                    | 2900             | 203.5                    | 1600                       | 1923                     |
|                         | 5           | 8.835                    | 300              | 1.639                    | 1100                       | 1222                     |
|                         | 7           | 11.51                    | 5600             | 89.49                    | 2000                       | 1402                     |
|                         | 15          | 19.08                    | 15200            | 35.01                    | 5600                       | 1332                     |
|                         | 30          | 16.07                    | 8700             | 243.6                    | 7400                       | 2080                     |
| *Pseudomonas sp.*       | 0           | 10.24                    | 200              | 453.9                    | 1700                       | 1587                     |
|                         | 1           | 11.13                    | 600              | 450.3                    | 1100                       | 809.4                    |
|                         | 3           | 10.7                     | 2000             | 178.1                    | 1000                       | 1279                     |
|                         | 5           | 9.461                    | 8800             | 45.98                    | 2700                       | 1626                     |
|                         | 7           | 10.88                    | 4400             | 112.6                    | 1600                       | 1610                     |
|                         | 15          | 11.78                    | 9600             | 29.93                    | 3800                       | 1402                     |
|                         | 30          | 19.84                    | 5600             | 64.02                    | 8500                       | 2281                     |
| sterile seawater        | 0           | 14.91                    | 600              | 2.633                    | 4900                       | 1234                     |
|                         | 1           | 9.456                    | 2600             | 67.1                     | 1400                       | 1433                     |
|                         | 3           | 10.43                    | 5000             | 65.64                    | 2900                       | 1589                     |
|                         | 5           | 11.86                    | 15000            | 7.494                    | 3800                       | 1630                     |
|                         | 7           | 16.13                    | 14900            | 19.08                    | 4100                       | 1533                     |
|                         | 15          | 12.73                    | 16700            | 29.37                    | 5900                       | 1860                     |
|                         | 30          | 17.63                    | 16100            | 32.09                    | 7100                       | 1970                     |

Electrochemical results show that the open-circuit potential (OCP) of carbon steel shifts toward the positive direction at the initial stage and then shifts toward the negative direction after 24 days of immersion in the mixed medium, suggesting carbon steel is more susceptible to be corroded at the latter stage. The variation of the charge transfer resistance ($R_\text{ct}$) obtained from EIS and the potentiodynamic polarization curves confirm the conclusion that the corrosion rate of carbon steel is inhibited at the initial stage and the latter corrosion is enhanced.

5. MATERIALS AND METHODS

5.1. Coupon Preparation. AISI 1045 carbon steel with the elemental composition of 0.42% C, 0.596% Mn, 0.230% Si, 0.028% S, 0.020% Cr, 0.014% Cu, and remainder Fe was provided by QiQiHar HongShun Heavey Industry Group Co., Ltd (China). Cylindrical coupons with a diameter of 10 mm and a height of 5 mm were used for electrochemical measurements. After copper wires were soldered, the coupons were embedded in epoxy resin leaving only one end surface (1 cm$^2$) exposed. For weight loss measurements and corrosion morphology analysis, sheet coupons with dimensions of 50 × 25 × 3 and 15 × 10 × 3 mm$^3$ were utilized, respectively. Besides, holes with a size of 3 mm$^2$ were drilled on one side of the coupons, where a copper wire would be connected. Finally, all coupons were sequentially polished with a series of grit SiC papers (180, 400, 800, and 1200), degreased with acetone, rinsed with deionized water repeatedly, immersed in absolute ethanol, dried at room temperature, and finally, placed into desiccators for preservation. Prior to be immersed in the different media, all coupons were sterilized by immersing in 70% ethanol for 30 min and dried aseptically in a biosafety cabinet under UV radiation.

5.2. Microbe Cultivation and Inoculation. *Pseudomonas* sp. is one of the most abundant aerobic strains in seawater that are of great importance in the corrosion process of metals in marine environments.\(^3\) The bacteria, both *Pseudomonas* and *Vibrio*, used in this study were originally isolated from the corrosion products on the surface of carbon steel coupons, which were immersed in natural seawater for six months. After being isolated and identified according to Bergey’s Manual of Determinative Bacteriology,\(^3\)\(^7\) the strains were preserved for experiments. The determined 16S rDNA sequences of these two strains were included in the GenBank databases under accession numbers KX268356 (*Pseudomonas* sp.) and (Vibrio sp.). *Pseudomonas* and *Vibrio* were cultured in a 2216E culture medium, separately, the composition of which was (per liter of sterilized natural seawater): 5.0 g of peptone, 1.0 g of yeast extract, 20.0 g of agar, and the pH was adjusted to 7.8 using a 1 mol/L sodium hydroxide solution. All of the culture media were autoclaved at 121 °C for 20 min and then *Pseudomonas* and *Vibrio* were cultivated under aerobic conditions in the 2216E medium at 26 °C for 24 h, individually. Further, the *Vibrio* and *Pseudomonas*-containing media were prepared separately by inoculating prepared culture media in sterile seawater with a volume ratio of 1:100. In addition, the *Vibrio—Pseudomonas* mixed culture medium was prepared by mixing these two kinds of single bacterium media with a volume ratio of 1:1. Sterile seawater was used as a control medium. Significantly, to create a natural seawater approximate environment, all of the media were refreshed every 15 days. All of the immersion experiments were performed at a temperature of 26 °C, which is the annual average temperature of Hainan Province. After 0, 1, 3, 5, 7, and 15 days of
immersion in different media, the counts of bacteria in media were periodically evaluated using a plate counting method.

**5.3. Weight Loss Measurement.** To monitor the corrosion extent of the coupons, weight loss measurements were performed. After 7, 15, and 30 days of immersion in different media, coupons for weight loss measurements were taken out and subsequently removed the biofilm and corrosion products with Clarke’s solution (36% HCl, 1 L; Sb₂O₃, 20 g; SnCl₂, 50 g). Then, the coupons were rinsed with distilled water, cleaned with analytically pure ethanol, and finally, weighed after being dried in a desiccator for 24 h. Further, the data obtained from three replicate coupons were used to calculate the average corrosion rate according to eq 1

\[
V(\text{mm/a}) = \frac{K \times W}{A \times T \times D}
\]

where \(K\) is \(3.65 \times 10^3\), \(W\) is the lost weight of the coupons (g), \(T\) is the corrosion time (day), \(A\) is the total area of the coupons (cm²), and \(D\) is the density of the coupons (g/cm³).

**5.4. Characterization of the Corrosion Surface Morphology and Corrosion Products.** To observe the surface morphology of corrosion products, after 30 days of immersion in different media, the coupons were taken out, fixed and dehydrated following the procedures reported in the literature. Eventually, the morphology of corrosion products was observed by scanning electron microscopy (SEM). Besides, prior to observe the corrosion morphology of the coupons beneath the corrosion products and the biofilm after 30 days of immersion in four different environments, the coupons were taken out, rinsed with sterile deionized water, and the corrosion products and the biofilm was removed with Clarke’s solution sequentially. Then, scanning electron microscopy (SEM) was performed to observe the corrosion morphology of the surfaces of the coupons.

**5.5. Electrochemical Measurements.** As a nondestructive technique, electrochemical impedance spectroscopy (EIS) is being widely used to explore the electrochemical reactions at

---

Table 2. Current Densities Obtained from the Potentiodynamic Polarization Curve Results in Different Media

| system          | \(T\) (days) | \(i_{cor} \) (μA/cm²) | \(E_{corr} \) (mV vs SCE) | \(\beta_a \) (mV/dec) | \(\beta_c \) (mV/dec) |
|-----------------|--------------|------------------------|----------------------------|-----------------------|-----------------------|
| Vibrio sp.      | 7            | 5.37                   | -739.05                    | 66.3                  | -71.03                |
|                 | 15           | 4.17                   | -819.42                    | 100.71                | -54.67                |
|                 | 30           | 5.29                   | -837.32                    | 120.49                | -23.16                |
| Pseudomonas sp. | 7            | 2.29                   | -781.19                    | 65.78                 | -181.24               |
|                 | 15           | 5.75                   | -799.09                    | 123.95                | -71.63                |
|                 | 30           | 5                      | -841.19                    | 110.85                | -25.72                |
| Pseudomonas sp. | 7            | 3.16                   | -768.99                    | 71.78                 | -191.95               |
| vs Vibrio sp.   | 15           | 5.03                   | -989.19                    | 40.55                 | -117.41               |
| sterile seawater| 7            | 5.32                   | -1016.9                    | 63.19                 | -123.15               |
|                 | 15           | 5.31                   | -828.96                    | 70.61                 | -58.33                |
|                 | 30           | 4.75                   | -864.72                    | 120.58                | -29.76                |

---

Figure 10. Potentiodynamic polarization curves recorded on coupons immersed in different media for 7 days (a), 15 days (b), and 30 days (c).
metal/biofilm interfaces. The open-circuit potential (OCP), EIS, and potentio-dynamic polarization curves were performed on an electrochemical workstation (Princeton Applied Research, PARSTAT 2273, software PowerSuite) with a three-electrode system, in which a saturated calomel electrode and a platinum electrode were used as the reference and counter electrodes, respectively. Besides, carbon steel coupons for electrochemical measurements were used as the working electrode. Electrochemical impedance spectroscopy (EIS) was performed at OCP with a sinusoidal voltage signal of 10 mV in a frequency range of 0.005–1 000 000 Hz. Moreover, a suitable equivalent circuit model for the EIS data was obtained using ZSimpWin software. Potentiodynamic polarization curves were measured by the potential-sweep method, keeping the OCP at a sweep rate of 4 mV/s. All of the experiments were repeated at least three times.

■ AUTHOR INFORMATION

Corresponding Author
Ke Cai – College of Chemical Engineering and Technology, Hainan University, Haikou 570228, China; Phone: +86-0898-68638682; Email: 793643781@qq.com

Authors
Deli Cai – College of Chemical Engineering and Technology, Hainan University, Haikou 570228, China; orcid.org/0000-0002-6388-1736
Jinyi Wu – College of Chemical Engineering and Technology, Hainan University, Haikou 570228, China

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c05402

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was financially supported by the National Natural Science Foundation of China (Nos. 51261006 and 51161007).

■ REFERENCES

(1) Artham, T.; Sudhakar, M.; Venkatesan, R.; Madhavan Nair, C.; Murty, K. V. G. K.; Doble, M. Biofouling and stability of synthetic polymers in sea water. Int. Biodeterior. Biodegrad. 2009, 63, 884–890.
(2) Belkaid, S.; Ladjouzi, M. A.; Hamdani, S. Effect of biofilm on naval steel corrosion in natural seawater. J. Solid State Electrochem. 2011, 15, 525–537.
(3) Dong, Z. H.; Liu, T.; Liu, H. F. Influence of EPS isolated from thermophilic sulphate-reducing bacteria on carbon steel corrosion. Biofouling 2011, 27, 487–495.
(4) Simões, M.; Simões, L. C.; Vieira, M. J. A review of current and emergent biofilm control strategies. LWT–Food Sci. Technol. 2010, 43, 573–583.
(5) Jia, R.; Yang, D.; Xu, D.; Gu, T. Electron transfer mediators accelerated the microbiologically influence corrosion against carbon steel by nitrate reducing Pseudomonas aeruginosa biofilm. Bioelectrochemistry 2017, 118, 38–46.
(6) AlAbbasi, F. M.; Williamson, C.; Bhola, S. M.; Spear, J. R.; Olson, D. L.; Mishra, B.; Kakovbia, A. E. Influence of sulfate reducing bacterial biofilm on corrosion behavior of low-alloy, high-strength steel (API-5L X80). Int. Biodeterior. Biodegrad. 2013, 78, 34–42.
(7) Xia, J.; Yang, C.; Xu, D.; Sun, D.; Nan, L.; Sun, Z.; Li, Q.; Gu, T.; Yang, K. Laboratory investigation of the microbiologically influenced corrosion (MIC) resistance of a novel Cu-bearing 2205 duplex stainless steel in the presence of an aerobic marine Pseudomonas aeruginosa biofilm. Biofouling 2015, 31, 481–492.
(8) Li, H.; Zhou, E.; Ren, Y.; Zhang, D.; Xu, D.; Yang, C.; Feng, H.; Jiang, Z.; Li, X.; Gu, T.; Yang, K. Investigation of microbiologically influenced corrosion of high nitrogen nickel-free stainless steel by Pseudomonas aeruginosa. Corros. Sci. 2016, 111, 811–821.
(9) McNeill, L. S.; Edwards, M. Iron pipe corrosion in distribution systems. J. - . Am. Water Works Assoc. 2001, 93, 88–100.
(10) Wu, J. Y.; Chai, K.; Xiao, W. L.; Yang, Y. H.; Han, E. H. The single effect of microbe on the corrosion behaviors of 25 steel in seawater. Acta Metall. Sin. 2010, 46, 755–760.
(11) Xu, D.; Xia, J.; Zhou, E.; Zhang, D.; Li, H.; Yang, C.; Li, Q.; Lin, H.; Li, X.; Yang, K. Accelerated corrosion of 2205 duplex stainless steel caused by marine aerobic Pseudomonas aeruginosa biofilm. Bioelectrochemistry 2017, 113, 1–8.
(12) Li, H.; Zhou, E.; Zhang, D.; Xu, D.; Xia, J.; Yang, C.; Feng, H.; Jiang, Z.; Li, X.; Gu, T.; Yang, K. Microbiologically Influenced Corrosion of 2707 Hyper-Duplex Stainless Steel by Marine Pseudomonas aeruginosa Biofilm. Sci. Rep. 2016, 6, No. 20190.
(13) Morales, J.; Esparza, P.; González, S.; Salvareza, R.; Arévalo, M. P. The role of Pseudomonas aeruginosa on the localized corrosion of 304 stainless steel. Corros. Sci. 1993, 34, 1531–1540.
(14) Franklin, M. J.; White, D. C.; Isaacs, H. S. Pitting corrosion by bacteria on carbon steel, determined by the scanning vibrating electrode technique. Corros. Sci. 1991, 32, 945–952.
(15) Pedersen, A.; Kjelleberg, S.; Hermansson, M. A screening method for bacterial corrosion of metals. J. Microbiol. Methods 1988, 8, 191–198.
(16) Yuan, S. J.; Choong, A. M. F.; Pehkonen, S. O. The influence of the marine aerobic Pseudomonas strain on the corrosion of 70/30 Cu–Ni alloy. Corros. Sci. 2007, 49, 4352–4385.
(17) Moradi, M.; Song, Z.; Tao, X. Introducing a new bacterium, Vibrio necaleonidus sp., with the highest corrosion inhibition efficiency. Electrochim. Commun. 2015, 51, 64–68.
(18) Cheng, S.; Tian, J.; Chen, S.; Lei, Y.; Chang, X.; Liu, T.; Yin, Y. Microbiologically influenced corrosion of stainless steel by marine bacterium Vibrio nitritogens: (1) Corrosion behavior. Mater. Sci. Eng., C 2009, 29, 751–755.
(19) Qu, Q.; He, Y.; Wang, L.; Xu, H.; Li, L.; Chen, Y.; Ding, Z. Corrosion behavior of cold rolled steel in artificial seawater in the presence of Bacillus subtilis C2. Corros. Sci. 2015, 91, 321–329.
(20) Wu, J.; Zhang, D.; Wang, P.; Cheng, Y.; Sun, S.; Sun, Y.; Chen, S. The influence of Desulfovibrio sp. and Pseudoalteromonas sp. on the corrosion of Q235 carbon steel in natural seawater. Corros. Sci. 2016, 112, S52–S62.
(21) Batmanghelich, F.; Li, L.; Seo, Y. Influence of multispecies biofilms of Pseudomonas aeruginosa and Desulfovibrio vulgaris on the corrosion of cast iron. Corros. Sci. 2017, 121, 94–104.
(22) Javed, M. A.; Stoddart, P. R.; Wade, S. A. Corrosion of carbon steel by sulphate reducing bacteria: Initial attachment and the role of ferrous ions. Corros. Sci. 2015, 93, 48–57.
(23) Liu, H.; Xu, D.; Doo, A. Q.; Zhang, G.; Ly, Y.; Liu, H. Study of corrosion behavior and mechanism of carbon steel in the presence of Chlorella vulgaris. Corros. Sci. 2015, 101, 84–93.
(24) Dexter, S. C.; Zhang, H. J.; Chandrasekaran, P. Biofouling Effects on Corrosion of Stainless Alloys in Seawater. In Mycotoxins, Wood Decay, Plant Stress, Biocorrosion, and General Biodeterioration; Llewellyn, G. C.; Dashke, W. V.; O’Rear, C. E., Eds.; Springer US: Boston, MA, 1994; pp 553–571.
(25) Liu, H.; Gu, T.; Asif, M.; Zhang, G.; Liu, H. The corrosion behavior and mechanism of carbon steel induced by extracellular polymeric substances of iron-oxidizing bacteria. Corros. Sci. 2017, 114, 102–111.
(26) Qu, Q.; Li, S.; Li, L.; Zu, L.; Ran, X.; Qu, Y.; Zhu, B. Adsorption and corrosion behaviour of Trichoderma harzianum for AZ31B magnesium alloy in artificial seawater. Corros. Sci. 2017, 118, 12–23.
(27) Yu, L.; Duan, J.; Xu, Y.; Huang, Y.; Hou, B. Accelerated aerobic corrosion of electroactive sulfate-reducing bacteria by electrochemical impedance spectroscopy and chronoamperometry. Electrochem. Commun. 2013, 26, 101–104.

https://doi.org/10.1021/acsomega.0c05402
(28) Chongdar, S.; Gunasekaran, G.; Kumar, P. Corrosion inhibition of mild steel by aerobic biofilm. *Electrochim. Acta* **2005**, *50*, 4655—4665.

(29) Cogan, S. F. Neural Stimulation and Recording Electrodes. *Annu. Rev. Biomed. Eng.* **2008**, *10*, 275—309.

(30) Yin, Y.; Cheng, S.; Chen, S.; Tian, J.; Liu, T.; Chang, X. Microbiologically influenced corrosion of 303 stainless steel by marine bacterium *Vibrio natriegens*: (II) Corrosion mechanism. *Mater. Sci. Eng., C* **2009**, *29*, 756—760.

(31) Rezakhani, D. The effects of temperature, dissolved oxygen and the velocity of seawater, on the corrosion behavior of condenser alloys. *Anti-Corros. Methods Mater.* **2011**, *58*, 90—94.

(32) Jayaraman, A.; Earthman, J. C.; Wood, T. K. Corrosion inhibition by aerobic biofilms on SAE 1018 steel. *Appl. Microbiol. Biotechnol.* **1997**, *47*, 62—68.

(33) Saravanan, P.; Prabagaran, S. R.; Nancharaiah, Y. V.; Krishnaveni, M.; Venugopalan, V. P.; Jayachandran, S. Isolation and characterization of *Pseudalteromonas ruthenica* (SBT033), an EPS-producing biofilm bacterium from the seawater intake point of a tropical power station. *World J. Microbiol. Biotechnol.* **2008**, *24*, 509—515.

(34) Beech, I. B.; Zinkevich, V.; Hanjangsit, L.; Gubner, R.; Avci, R. The effect of *Pseudomonas NCIMB 2021* biofilm on AISI 316 stainless steel. *Biofouling* **2000**, *15*, 3—12.

(35) Busalmen, J. P.; Vázquez, M.; de Sánchez, S. R. New evidences on the catalase mechanism of microbial corrosion. *Electrochim. Acta* **2002**, *47*, 1857—1865.

(36) Valcarce, M. B.; de Sánchez, S. R.; Vázquez, M. Localized attack of copper and brass in tap water: the effect of *Pseudomonas*. *Corros. Sci.* **2005**, *47*, 795—809.

(37) Buchanan, R. E.; Gibbons, N. E. *Bergey’s Manual of Determinative Bacteriology*, 8th ed.; Williams & Wilkins Co., 1994; Vol. 65, p 315.

(38) Walker, J. T.; Keevil, C. W. Study of microbial biofilms using light microscope techniques. *Int. Biodeterior. Biodegrad.* **1994**, *34*, 223—236.

(39) Manohar, A. K.; Bretschger, O.; Nealson, K. H.; Mansfeld, F. The use of electrochemical impedance spectroscopy (EIS) in the evaluation of the electrochemical properties of a microbial fuel cell. *Bioelectrochemistry* **2008**, *72*, 149—154.