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Rapid Communication

Naturally effective inhibition of microbial corrosion on carbon steel by beneficial biofilm in the South China Sea

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ABSTRACT

Microbially influenced corrosion (MIC) of metals exerts a negative effect on the marine environment and causes a great loss of marine facilities. Corrosion prevention in an eco-friendly and sustainable way is a difficult problem to address, especially in the marine environment. In this work, *Nocardiopsis dassonville*, a corrosive bacteria isolated from the South China Sea was studied by using carbon steel. The results indicate that *N. dassonville* caused a corrosion loss of 7.68 mg cm^{-2} and a corrosion pit of 13.0 μm on the carbon steel surface, but the corrosion is inhibited in the presence of *Vibrio* sp. EF187016 in the medium. *Vibrio* sp. EF187016 preferentially occupied the carbon steel surface, forming a protective biofilm that hindered the attachment of *N. dassonville*. In addition, extracellular polymeric substances extracted from *Vibrio* sp. EF187016 was added to *N. dassonvillei* inoculated medium and showed a significant inhibition of MIC on carbon steel.

1. Introduction

Corrosion is a common threat to almost all industries, especially in marine infrastructures, and induces huge economic loss [1]. Microbially influenced corrosion (MIC) is considered an "invisible killer" of marine engineering materials because of its severe destructiveness and its complex underlying microbial and degradation mechanisms [2,3]. The South China Sea is one of the largest marginal seas in the tropical Pacific Ocean and covers an area of approximately 3,500,000 km^2 [4]. Severe MIC has been found for many years in this area [5]. Several mitigation techniques have been proposed to address MIC problems, including chemical, biological, and physical methods [6,7]. However, the possible environmental pollution caused by traditional corrosion mitigation techniques is concerning. In addition, bacteria could also gradually develop resistance or immunity [8]. Therefore, it is important to explore methods to combat MIC through non-synthetic and/or natural pathways.

Microbiome-inhibiting microbiologically induced corrosion (MIMIC) refers to using beneficial microorganisms to reduce the corrosion damage caused by corrosive microorganisms [9]. It can not only achieve the purpose of slowing MIC but also can eliminate or reduce the use of bactericide and corrosion inhibitors, reducing pollution and toxicity. The mechanisms of MIMIC mainly include biological competition, inhibition of microbial attachment, secretion of antibiotic substances, and phagocytic control [10]. Recent studies showed that extracellular polymeric substances (EPS), a kind of non-bactericidal activity material, excreted from bacteria can also form films on the metal surface, thus effectively preventing bacterial adhesion to the metal substrate [11,12]. EPS is produced by several different bacteria such as *Desulfovibrio vulgaris*, *Lactobacillus helveticus,* and *Vibrio* sp. QY101 has already shown its anti-biofilm ability on adhering surfaces [11–14]. However, their corrosion inhibition on MIC was still not fully understood. The biofilm disruption capacity of EPS was also proposed as one of the anti-corrosion mechanisms in addition to the adhesion-preventing effect [15]. Inspired by these results, EPS might have potential for application as an inhibitor in dealing with conventional corrosion and even microbial corrosion.

In this study, a novel electroactive Gram-positive bacterium, *Nocardiopsis dawsonville*, was isolated from the South China Sea and its corrosion properties on carbon steel were systemically studied by electrochemical analysis and surface characterization. The corrosion protection methods aimed at this bacteria were also considered by using living biofilm from *Vibrio* sp. EF187016 [16]. The potential of EPS secreted by *Vibrio* sp. EF187016 as a corrosion-inhibiting chemical that may also hinder the formation of biofilms was explored. Our objective is

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to assess the naturally effective inhibition of *Vibrio* sp. EF187016 biofilm on corrosive *N. dassonville* in the real marine environments, evaluating their corrosion protection applications within regional marine environments. By evaluating EPS resistance against *N. dassonville* corrosion, we aim to further explore compounds from biofilms as potential natural corrosion inhibitors.

2. Materials and methods

2.1. Metal substrate

The commercial X80 carbon steel with the chemical composition (wt %) of 0.07C, 1.82 Mn, 0.19 Si, 0.045 S, 0.045 P, 0.17 Ni, 0.01 Mo, 0.026 Cr and balance Fe was used in this study. The dimensions of all the tested samples were wire cut into 10 mm × 10 mm × 5 mm, and subsequently ground with SiC paper up to 1000 grit. Samples subjected to electrochemical measurements were elaborated into conducting working electrodes. Before tests, samples were ultrasonically cleaned in absolute ethanol for 20 min, dried in air, and sterilized with ultraviolet light for at least 30 min.

2.2. Bacterial cultivation and EPS extraction

Both *N. dassonville* and *Vibrio* sp. EF187016 were obtained from the South China Sea, Xiamen, China, (GPS: 21°12′36.0″N, 109°24′00″E). To simulate the practical marine environment, artificial seawater was used as the test solution. The composition of artificial seawater is (g/L): NaCl 24.53, KCl 0.69, $CaCl_2$ 1.16, Na_2SO_4 4.09, KBr 0.10, H_3BO_3 0.03, $NaHCO_3$ 0.20, $MgCl_2$ 5.2, $SrCl_2$ 0.025, and NaF 0.003. In addition, 1 g/L of yeast extract was added to ensure the growth and multiplication of bacteria. The liquids used in the experiments were autoclaved (121 °C, 20 min) and used after cool-down. All the experiments were conducted at 30 °C during the incubation period to ensure bacterial activity. The initial concentration of bacteria was 10^6 cells/mL for all the experiments.

The extraction of *Vibrio* sp. EF187016 EPS was carried out in line with our previously reported procedure [16]. The bacterial culture solution was subjected to centrifugation, filtration (0.22 μm sterile filter), and extraction processes to ultimately obtain the precipitated product of EPS. All the operations were performed in a biological safety cabinet to ensure complete sterility. Fourier transform-infrared (FTIR) spectrometer (Nico-let, Thermo, USA) was used to characterize the extracted EPS. Freeze dried EPS was mixed with KBr (0.5%) and the scanning range was 4000–400 cm^{-1} with the resolution of 4 cm^{-1}.

2.3. Immersion tests

Immersion tests were conducted in 100 mL conical flask, where a minimum of three carbon steel coupons were placed into 50 mL of each medium. The conical flasks were then aerobically incubated at 30 °C for 14 days. Four groups of different incubation medium were set up including sterile medium (control group), strain *N. dassonville*, the co-culture medium, and pre-grown medium. For the co-culture medium, *Vibrio* sp. EF187016 and *N. dassonville* were added simultaneously into artificial seawater medium and cultured for 14 days. *Vibrio* sp. EF187016 was inoculated in the 2216E medium for 3 days in order to form the mature biofilm on the surface [17] and then transferred to the artificial seawater medium. After that, *N. dassonville* was also inoculated in the same system and cultured with *Vibrio* sp. EF187016 for 14 days. This kind of sample was defined as pre-grown medium and was used to further investigate the inhibitory effect of *Vibrio* sp. EF187016 active biofilm.

The effect of EPS on the corrosion of carbon steel was also investigated in artificial seawater medium inoculated with *N. dassonville*. Two types of samples were prepared: one without EPS layer and one with EPS layer. For the sample with EPS layer, the powdered EPS was weighed and dissolved in artificial seawater at solid/liquid ratio of 1 g/L. Samples were immersed in the sterile artificial seawater containing EPS for 5 h and then transferred to the medium inoculated with *N. dassonvillei* for 14 days.

2.4. Electrochemical tests

The experiments were performed using a conventional three-electrode system: the reference electrode was a saturated calomel electrode, the auxiliary electrode was a platinum electrode, and the working electrode was the test sample. All test cells were incubated in a water bath at temperature controlled at 30 °C during the whole experiment (14 days). Open circuit potential (OCP), linear polarization resistance (LPR), electrochemical frequency modulation (EFM), electrochemical impedance spectroscopy (EIS), and potentiodynamic polarization tests were carried out using a Gamry electrochemical workstation (Reference 600, Gamry Instruments, USA). The potential scan rate for the LPR tests was set to 0.125 mV/s and the scan range was from −5 mV to +5 mV (vs. E_{OCP}). The EFM measurements were conducted by applying a potential perturbation signal with an amplitude of 5 mV, base frequency of 0.01 Hz, multiplier A and B of 2 and 5, respectively. The data processing was performed using Echem Analyst 6.33. The EIS was measured in the frequency range of 100– 0.01 Hz, and an AC potential was applied with an amplitude of ±5 mV. The data obtained from the tests were fitted by Zsimpwin software. The potentiodynamic polarization was performed on the last day of the incubation with a scan range of −0.5 to +0.3 V (vs.

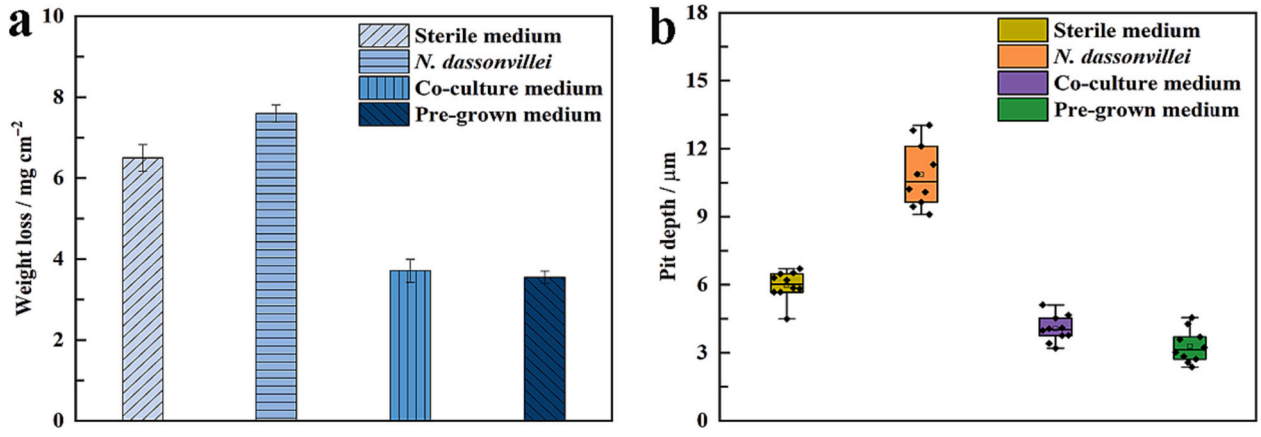

Fig. 1. Corrosion of coupons in different media after incubation for 14 days: (a) weight loss, and (b) statistics of maximum pit depth.

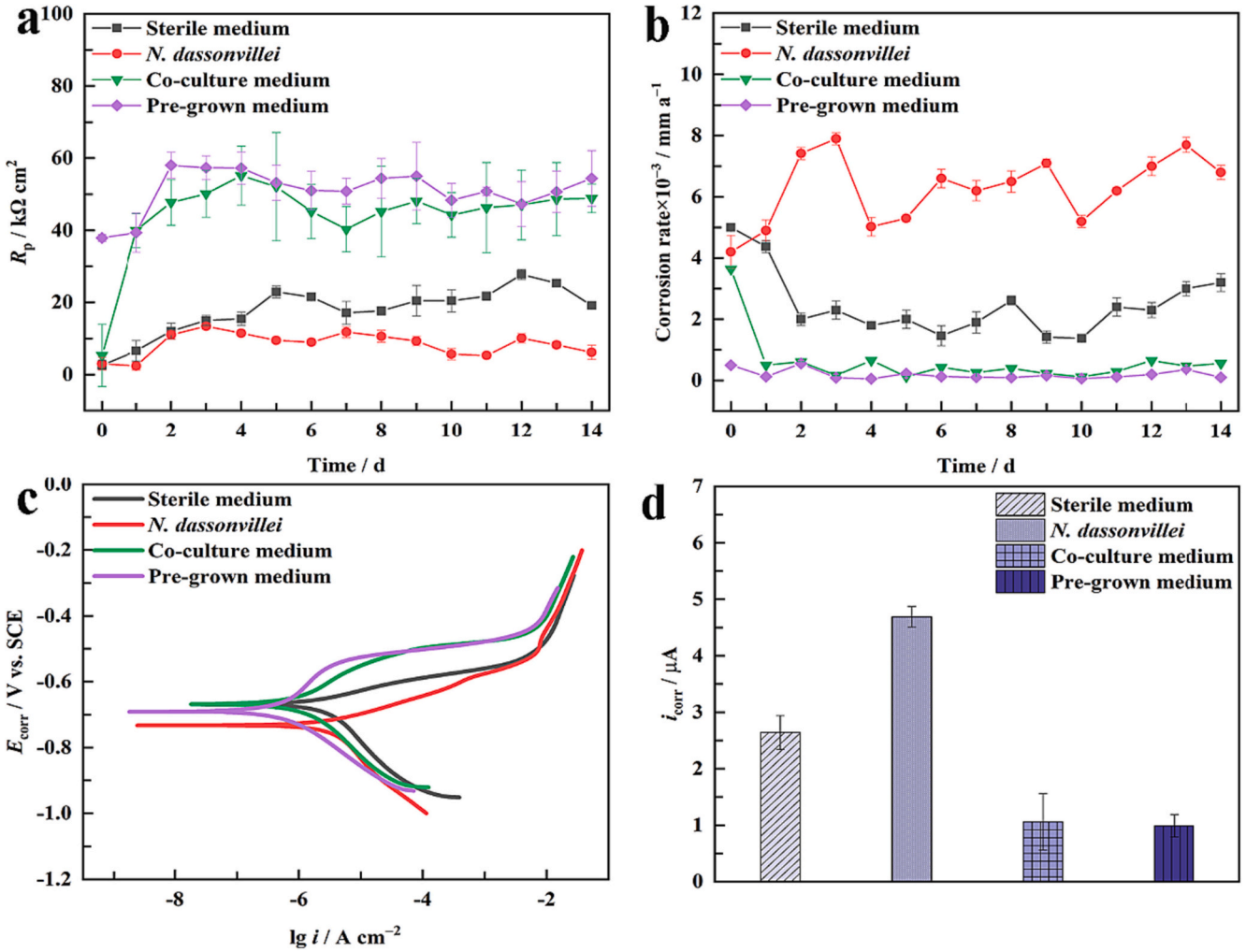

Fig. 2. Electrochemical measurements of coupons in the different media: (a) R_p, (b) corrosion rate, (c) polarization curves, and (d) i_{corr} from Tafel analysis of (c).

E_{OCP}) and a scan rate of 0.333 mV/s. All tests were conducted three times to assess the reproducibility.

2.5. Biofilm characteristics

To observe the morphology of bacterial biofilms on sample surfaces, field emission scanning electron microscopy (FESEM, UltraPlus, Zeiss, Germany) was applied. After incubation in the different media, samples were rinsed with phosphate buffered saline (PBS) solution to remove loosely attached biofilms and planktonic cells, and then placed in 3% (*v/v*) glutaraldehyde solution for 3 h to immobilize the sessile cells. The biofilms were dehydrated successively in 50%, 60%, 70%, 80%, 90%, 95%, and 100% (v/v) ethanol solutions for 8 min in each step. All the samples were mounted on pin stubs using double-sided carbon tape and imaged at 20 kV.

2.6. Corrosion morphology and weight loss

CLSM (LSM710, Zeiss, Germany) was used to evaluate the corrosion morphology and pitting depth after 14 days of incubation. Before measuring, the specimens were treated according to ASTM G1–03 [18]. The sequence of treatment steps was ultrasonic cleaning, acid cleaning and scrubbing samples. The samples were also tested for corrosion weight loss. The weight of the samples was determined using an analytical balance (Shimadzu-AUW220D, Japan) with a readability of 0.01 mg. To assess the level of reproducibility, 3 to 5 parallels were set up for each group of samples. The corrosion inhibition efficiency (*IE*%) was calculated using the following Eq. (1):

$$IE\% = \frac{W_0 - W_1}{W_0} \times 100\% \quad (1)$$

W_0 is the weight loss in the control group, W_1 is the weight loss in the inoculated medium with different strains.

3. Results

3.1. Weight loss and pitting corrosion

The results of corrosion weight loss are shown in Fig. 1a. After 14 days of incubation, the weight loss of samples in the presence of *N. dassonville* is 7.60 mg cm^{-2}, higher compared to that in the sterile medium (6.50 mg cm^{-2}), indicating that *N. dassonville* has a promoting effect on corrosion of carbon steel. However, the weight loss of carbon steel in pre-grown medium and co-culture medium i.e. 3.73 and 3.55 mg cm^{-2}, respectively, is lower than the value obtained in the sterile medium. The results indicate that *Vibrio* sp. EF187016 might had a mitigating effect on the corrosion of carbon steel caused by *N. dassonville*. The inhibition efficiency after 14 days of incubation was approximately 53% and 51% for the co-culture and pre-grown medium, respectively.

Fig. S1 shows the maximum pit depth and morphology of the sample surfaces after 14 days of incubation in the different media. The related

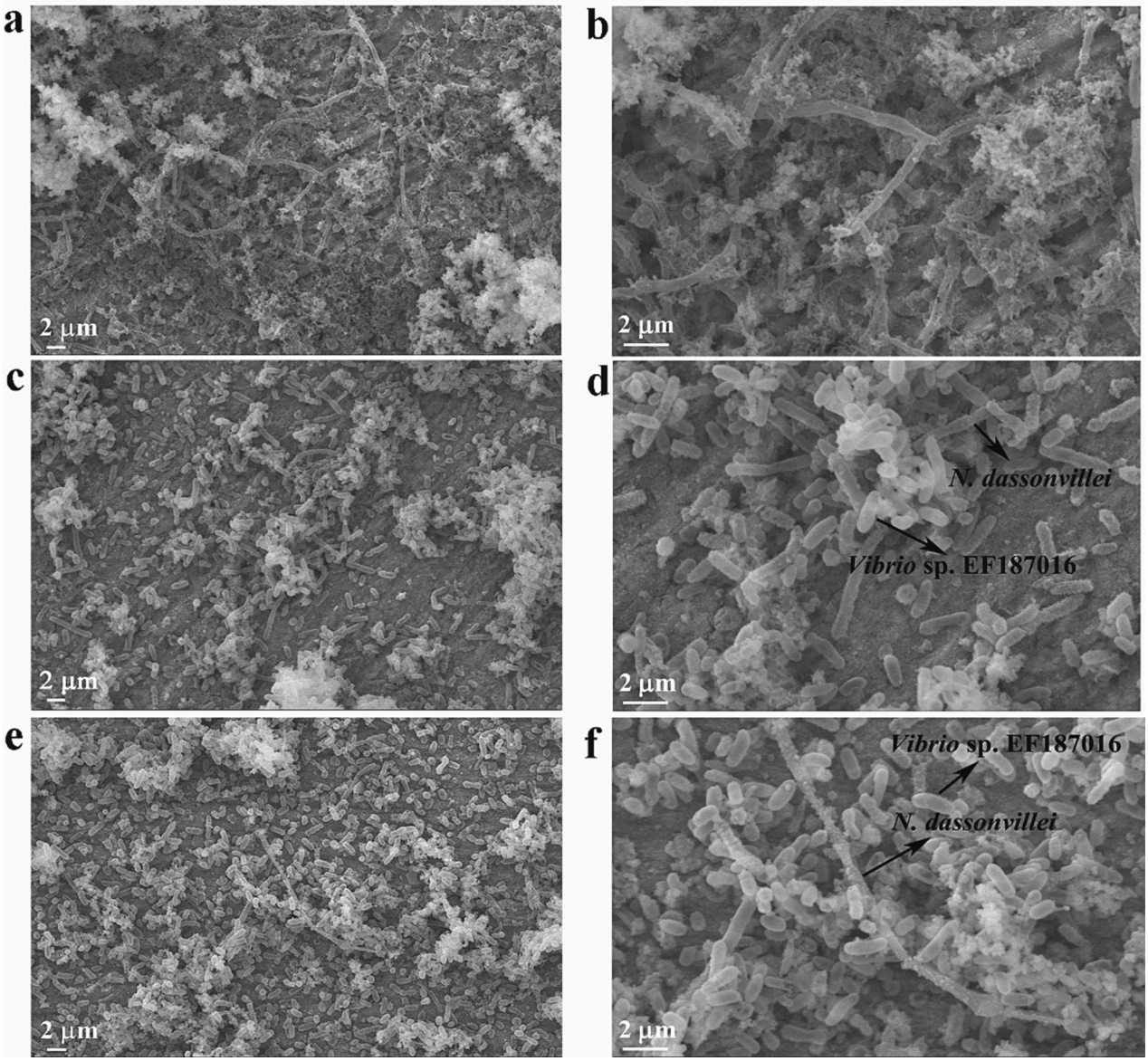

Fig. 3. FESEM images of biofilm on coupons after 14 days of incubation: (a, b) *N. dassonvillei*, (c, d) co-culture medium, (e, f) pre-grown medium.

pit statistics are listed in Fig. 1b. For the sterile medium, the maximum pit depth is 6.7 μm and the value is about twice that (13.0 μm) in the medium inoculated with *N. dassonville*. It can be inferred that *N. dassonville* can promote the formation of pits and accelerate the local corrosion. Chloride ions in the medium have been reported to migrate into the substrate through the heterogeneous biofilm thus leading to corrosion pits [19]. In both co-culture and pre-grown medium, the value of the pits on the surfaces suggests mitigated pitting corrosion with the largest pitting depth of 5.1 and 4.5 μm, respectively. The results confirm that the *Vibrio* sp. EF187016 biofilm protects carbon steel against enhanced pitting corrosion. As a result, *Vibrio* sp. EF187016 is shown to inhibit extensive pitting corrosion triggered by corrosive bacteria in the co-culture and pre-grown medium. The data in Fig. S2 shows the pH variation in each medium during the 14 days of incubation. The pH in all medium was between 7– 8, indicating that the corrosion of the sample seems to have no relationship with the acidic solution.

3.2. Electrochemical analysis

In sterile medium, the R_p is about 4.2 kΩ cm^2 at 0 day (Fig. 2a). After that, R_p remains around 20.5 kΩ cm^2 during the 14 days of incubation. On the other hand, the value of R_p in the medium containing *N. dassonville* fluctuates around 10.5 kΩ cm^2, which is less than that in the sterile medium. Interestingly, when *Vibrio* sp. EF187016 is present in the medium, R_p increases significantly after 1 day and sustains this upward trend during the leftover experimental period. The highest R_p is found in the pre-grown medium which remained at 55.1 kΩ cm^2 at the end of incubation, whereas, an R_p of 45.0 kΩ cm^2 in the co-culture medium is observed. It demonstrates that the medium with the addition of *Vibrio* sp. EF187016 can effectively show protection against MIC of carbon steel by *N. dassonville*. The corrosion rate curves obtained by EFM are shown in Fig. 2b. Compared to the sterile samples, the presence of *N. dassonville* led to an increased rate of corrosion, indicating that *N. dassonville* colonized on the surfaces could induce MIC. However, in the co-culture and pre-grown medium, the largest corrosion rate was effectively inhibited by the presence of *Vibrio* sp. EF187016. The corrosion rate was even lower than that in the control medium. EIS was performed to investigate the corrosion kinetics of the samples (Fig. S3). In order to get a quantitative measure of the electrochemical parameters at the metal/electrolyte interface, EIS data were modeled using equivalent electrical circuits shown in Fig. S4. In addition, the corresponding electrochemical data were also given in Table S1. The results of EIS are

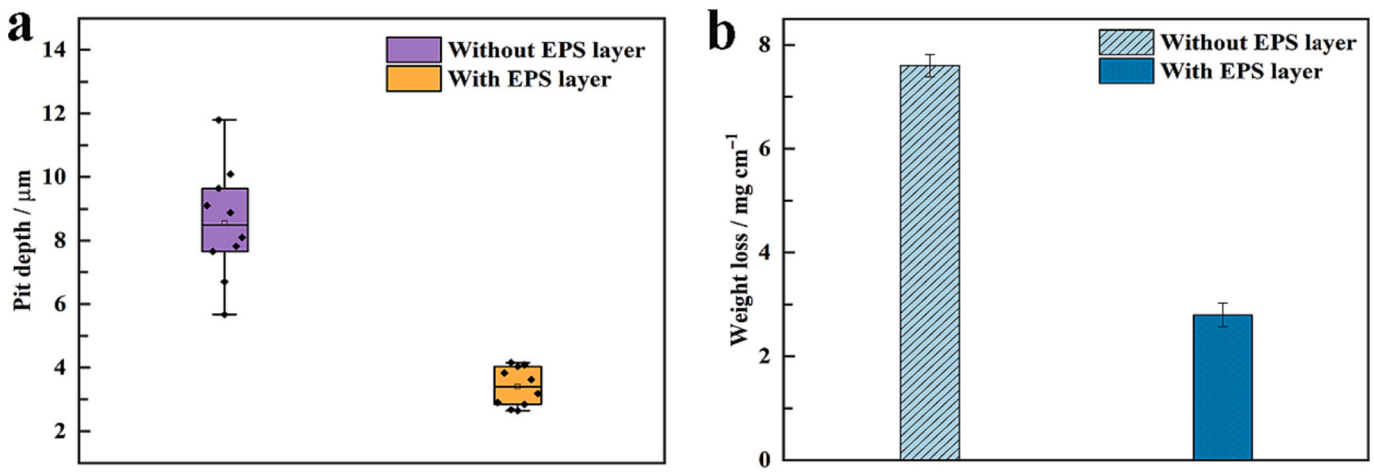

Fig. 4. Corrosion of different coupons in *N. dassonvillei* inoculated medium after 14 days: (a) statistics of maximum pit depth and (b) weight loss.

consistent with the data obtained from LPR, and it is confirmed again that *Vibrio* sp. EF187016 inhibits the MIC of carbon steel caused by *N. dassonville*.

Fig. 2c shows the polarization curves and the relevant corrosion parameters calculated based on the Tafel fit. The principle about how to fit the polarization curve were supplemented in Fig. S5. The anodic and cathodic branches in the polarization curves of samples in the bacteria medium are different from those in the sterile medium, which indicates that the existence of biofilms affected the anodic and cathodic reactions [20]. The negative shift of E_{corr} and the larger value of i_{corr} (Fig. 2d)

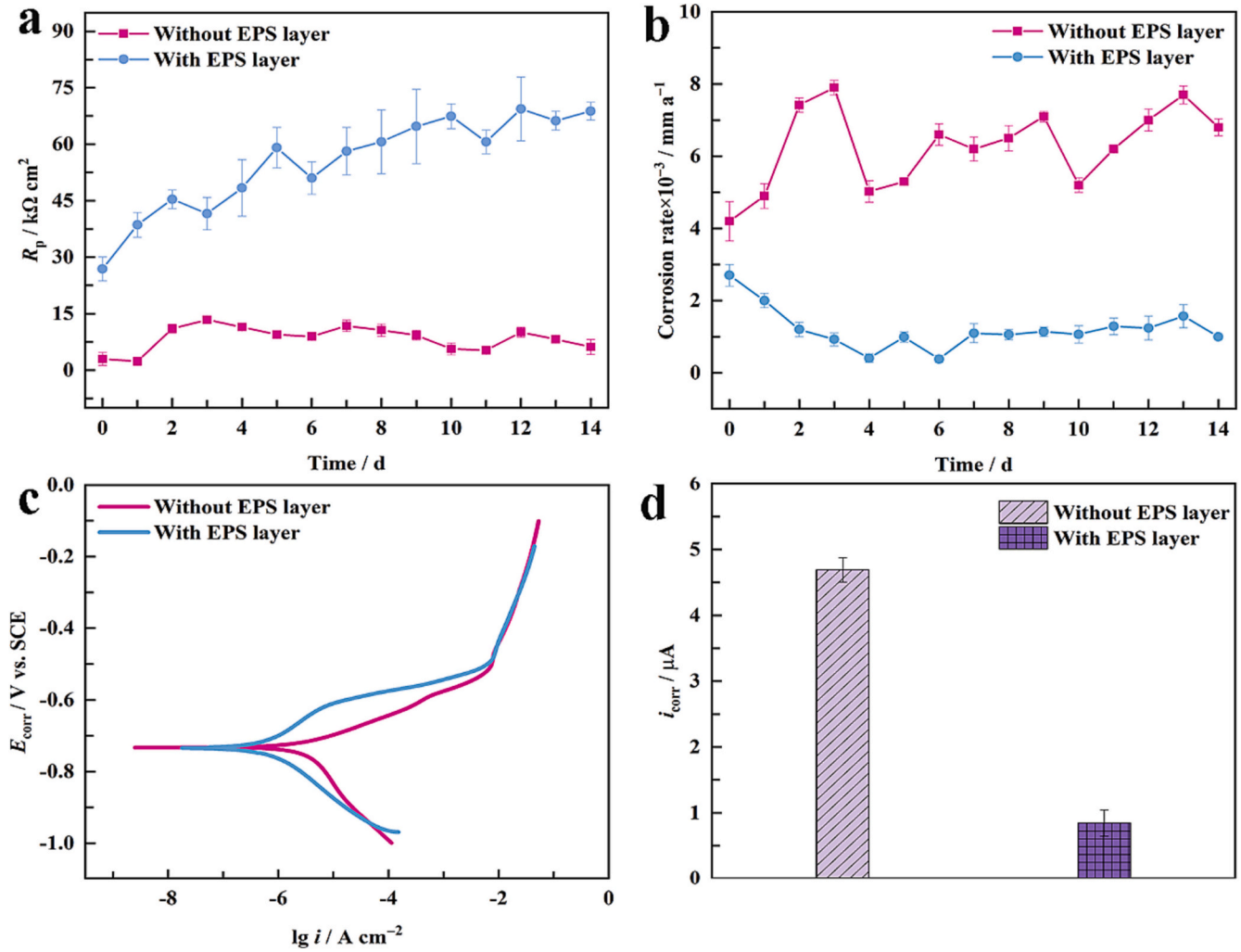

Fig. 5. Electrochemical measurements of different coupons in *N. dassonvillei* inoculated medium: (a) R_p, (b) corrosion rate, (c) polarization curves, and (d) i_{corr} from Tafel analysis of (c).

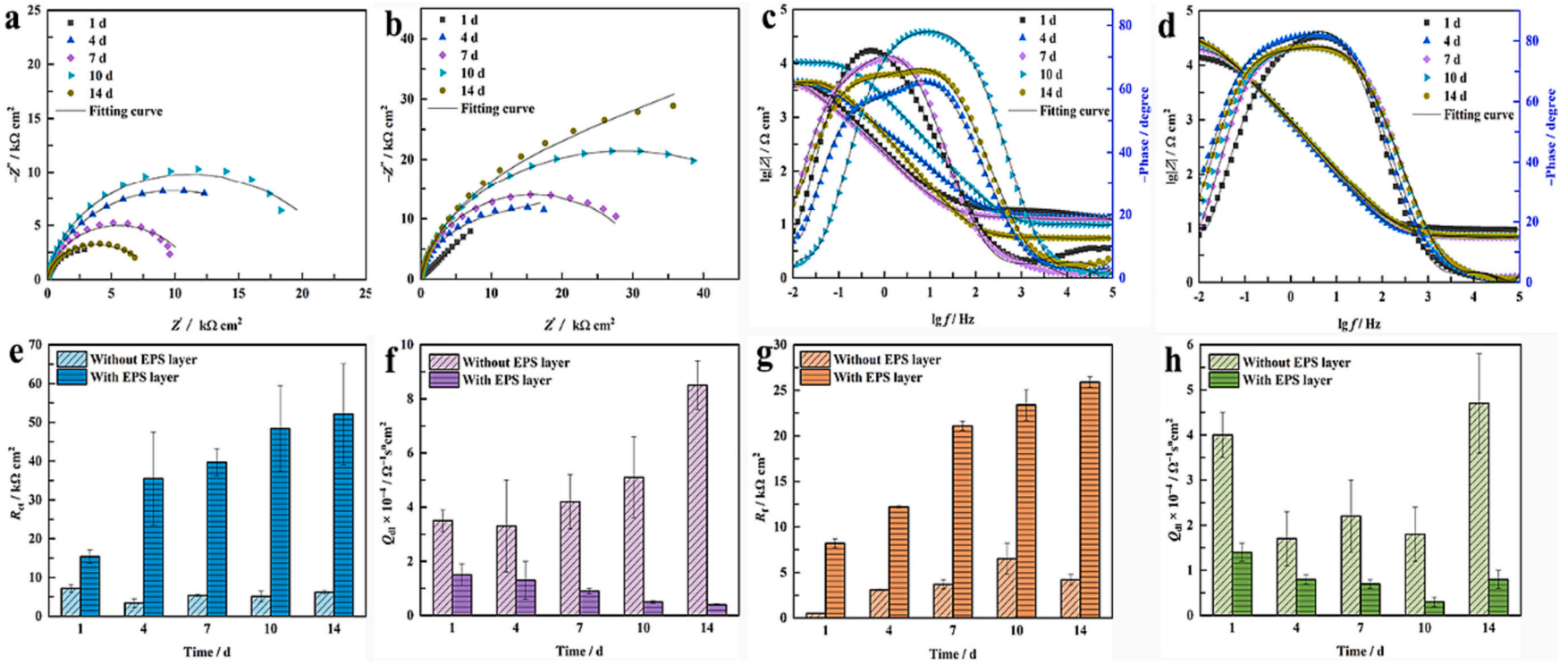

Fig. 6. Impedance spectrum and fitting parameters of coupons (a, c) without and (b, d) with EPS layer exposed to *N. dassonvillei* inoculated medium: (e) R_{ct}, (f) Q_{dl}, (g) R_f, (h) Q_f.

compared to those in the sterile medium indicate an acceleration of the corrosion rate of carbon steel in the presence of *N. dassonville*. The sharp change in cathodic branching current with voltage (Fig. 2c) in *N. dassonvill* inoculated medium also indicates the facilitation of reduction rates by this kind of bacteria. Nevertheless, inhibition of the cathodic branch by *Vibrio* sp. EF187016 biofilm suggests that the biofilm can also inhibit corrosion by hindering the transfer of the dissolved oxygen and thus the following cathodic reaction. In addition, when *Vibrio* sp. EF187016 is present in the medium, a passivation-like phenomenon appears in the anode branch, which does not appear in the sterile and *N. dassonville* inoculated medium. This suggests that a protective biofilm formed on the surfaces in the presence of *Vibrio* sp. EF187016 which can decrease anodic dissolution. The corrosion current density (i_{corr}) is in the order of: *N. dassonville* (4.7 μA cm^{-2}) > sterile medium (2.6 μA cm^{-2}) > co-culture (1.9 μA cm^{-2}) > pre-grown (1.0 μA cm^{-2}). Electrochemical results show that the protective film formed by *Vibrio* sp. EF187016 on the surfaces of carbon steel can sufficiently mitigate the MIC of *N. dassonville*, and improve the corrosion resistance. The superior corrosion inhibition demonstrated by *Vibrio* sp. EF187016 in the pre-grown medium suggests that preferential biofilm formation is the key reason why corrosion protection is observed.

3.3. Biofilm morphology characteristics

Fig. 3 represents the morphology of the biofilms on the coupon surfaces in the different culture medium after 14 days of incubation. As is known, *Vibrio* sp. EF187016 is characterized by its limited length, smooth surface and arc shape curvature [21]. *N. dassonville* is long and slender with a rough surface [22]. In Fig. 3a and b, *N.. dassonville* adhered well to the surfaces and formed reticulate biofilm, leading MIC of carbon steel surfaces. It is noteworthy that, for the medium involving two bacteria, the *Vibrio* sp. EF187016 is evenly attached to the surfaces in clusters, while the *N. dassonville* is wrapped in the colonies of *Vibrio* sp. EF187016 in an elongated state (Fig. 3c and d). Based on the surface morphology observation and the results from the corrosion tests, it can be inferred that for the co-culture and pre-grown medium, *Vibrio* sp. EF187016 is the dominant strain, preferentially attaching and occupying the entire surfaces. What is then formed on the surfaces is a protective film, blocking the corrosive agents present in the external solution. Hence, harmful bacteria (*N. dassonville*) in the medium cannot attach and form a biofilm to induce corrosion.

3.4. Corrosion characterization of carbon steel with EPS modification

In summary, the inhibitory effect of *Vibrio* sp. EF187016 on MIC caused by *N. dassonvillei* mainly depended on its protective biofilm on the carbon steel surface, which served as a protective layer blocking harmful bacteria from attaching to the substrate. As is well known, EPS, an important component of mature biofilm, is involved in maintaining biofilm stability and mediating biofilm expression with various functions [23]. According to our previous work, EPS from *Vibrio* sp. EF187016 was the key to its corrosion inhibition ability. It can form a protective film layer on the carbon steel surfaces to protect the substrates against corrosion in 3.5 wt% NaCl. Corrosion inhibition can be achieved without relying on the living biofilm [16]. Inspired by the results, we tested the possibility of using *Vibrio* sp. EF187016 EPS to counteract the MIC caused by *N. dassonvillei*.

The pitting corrosion morphology on the surfaces of different samples was observed by CLSM and depth statistics were performed. As mentioned above, the maximum pit depth observed in *N. dassonvillei* inoculated medium is 13.0 μm (Fig. S1b) and this value is also shown in Fig. S6a to facilitate the comparison between different media. However, on the surfaces with EPS layer, the pitting corrosion is largely inhibited and the maximum pit depth is reduced to 4.1 μm. The depths of the 10 largest pits in different areas on the surfaces were counted (Fig. 4a). It can be seen that EPS presented as a protective layer and effectively suppressed the pitting corrosion caused by *N. dassonvillei*. In addition, Fig. 4b represents the corrosion weight loss results after 14 days of incubation. The samples with EPS layer show less weight loss after incubation. These indicate that, in *N. dassonvillei* inoculated medium, EPS layer present on the surfaces can protect against corrosion. In addition, compared to the inhibition efficiency of the living biofilms (51% for the co-culture and 53% for the pre-grown medium as mentioned above), EPS itself can inhibit pit formation by 66%.

Time resolved electrochemical monitoring is shown in Fig. 5. Both R_p and corrosion rate further confirmed the corrosion protection ability of EPS indicated by the smaller corrosion rate obtained in the presence of EPS. During the 14 days of the test period, the surfaces of samples with EPS layer always show enhanced resistance against corrosion by *N. dassonvillei*. Potentiodynamic polarization also shows the suppression of both anodic and cathodic reactions with EPS layer adhered. As can be seen from the evolution in the curve, passivation occurs at the anodic branch, while the rate of cathodic reaction slows down. Similar to living biofilms, EPS also decreased anodic dissolution and slowed down the oxygen reduction reaction, thereby protecting the substrates from the

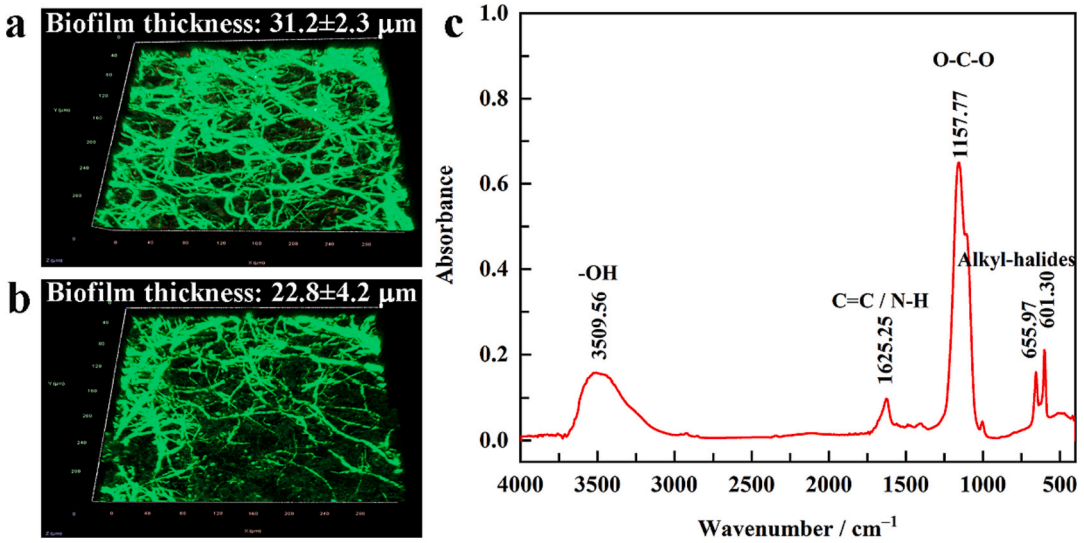

Fig. 7. The attachment of *N. dassonville* on the sample surfaces: (a) without EPS layer, (b) with EPS layer, and (c) FTIR characterization of EPS.

microbial corrosion induced by *N. dassonvillei*. After 14 days of incubation, the value of i_{corr} of the samples covered with EPS layer is reduced to 0.8 μA cm^{-2} compared to the value obtained without the presence of EPS as shown in Fig. 5d.

The impedance spectra of samples with and without EPS layer in the *N. dassonvillei* inoculated medium are shown in Figs. 6a-d. According to the Nyquist plots, it can be seen that different samples immersed in *N. dassonvillei* inoculated medium show different capacitive loops. For samples with EPS layer, during the whole incubation process, the diameter of capacitive loops increased in the experiment duration, which was larger than that of the samples without EPS layer. Larger semicircle diameters in Nyquist plots are usually a sign of higher electrical resistances at the interface of the coupons with an electrolyte; indicative of lower corrosion rates and vulnerability [24], confirming the inhibition effects of EPS layer. As for the bode plots, two-time constants were considered for different sample surfaces, the one at middle frequency was related to metal/electrolyte interface, and the second one at low frequency was ascribed to the membrane interface [25]. As shown in Fig. 6b, for the sample without EPS layer, the phase angle of the peak approaches −70° on the first day and is adjusted downward to −60° on the 14th day. This indicates that the surface corrosion resistance of samples deteriorated during the incubation process in the presence of corrosive *N. dassonvillei*. By comparison, for the sample with EPS layer, the two-time constants overlap at middle frequency, and the corresponding phase angle peaks approach −80°.

From the calculated EIS parameters, the samples with EPS layer show an extremely high charge transfer resistance (R_{ct}) value compared to the samples without EPS layer in the *N. dassonvillei* inoculated medium after 1 day. During the incubation period, the large R_{ct} value obtained with EPS layer might be attributed to the formation of a protective layer at the metal/solution interface, which indicates that the EPS adsorbs on the matrix at the metal-solution interface, resulting in a resistance effect and hindering the charge transfer [25]. Fig. 6f shows that the thickness of the electrical double layer on the samples with EPS layer increases with decreasing Q_{dl} value. On the other hand, R_f and Q_f reflect the capacitance and resistance of the surface film. Much higher R_f was found on the surfaces with EPS layer, revealing a high resistance mainly due to the adsorbed EPS. After 14 days of incubation, the R_f for the surfaces with EPS layer is almost five times higher than that on the surfaces without EPS layer. Furthermore, the lower Q_f on the surfaces with EPS layer indicated the weak electrical capacity. This means that the surface of the samples with EPS addition had a denser and thicker film layer, which provides effective protection for the substrate [26]. EPS formed a physical barrier by adsorption on the surfaces of the coupon, blocking the penetration of corrosive substances in electrolytes (bacteria, O_2, and Cl^-) [27].

Fig. 7 shows the three-dimensional CLSM images obtained from different sample surfaces. After 7 days of incubation, less *N. dassonvillei* adhered to the EPS-covered regions. The biofilm thickness on the surfaces without EPS layer is 31.2 ± 2.4 μm, the value reduces to 22.8 ± 3.0 μm for the surfaces with EPS layer. The results obtained here confirm that the presence of EPS on the sample surfaces affected the attachment of *N. dassonvillei* and the biofilm thickness became thinner. The presence of EPS played a role in inhibiting biofilm formation, which may be due to the presence of anti-biofilm polysaccharides in EPS [28].

Fig. 7c shows the FTIR spectrum of EPS characteristic functional groups. The FTIR analysis reveals a broad stretching vibration peak at ~3509 cm^{-1} corresponding to the hydroxyl group carbohydrates of protein. The peak appears at 1625 cm^{-1} can be ascribed to C═O stretching vibration and variable angle vibration of N—H bending of the amide. The absorption peak at 1157 cm^{-1} is attributed to C–O–C stretching vibrations from the polysaccharide. The absorption peak at 655 and 601 cm^{-1} is related to stretching vibration of alkyl-halides [29]. The ability of EPS to inhibit corrosion is due to its molecular structures which are relatively similar to conventional organic corrosion inhibitors. These compounds possess the ability to adsorb on metal surfaces and to form a protective layer that protects them from corrosion [30]. The layer formed by EPS protected the substrate and at the same time influenced the physical and chemical properties (hydrophobicity, roughness, and its charge) of the surface.

4. Discussion

4.1. Corrosion acceleration by N. dassonville

The results obtained here clearly show that the corrosion of X80 carbon steel was promoted by the inoculation of *N. dassonvillei*. The data from Fig. 3 show that when carbon steel coupons were immersed in the simulated seawater medium, *N. dassonville* formed a biofilm on the surface, which increased the rate of anodic metal dissolution and cathodic oxygen reduction (Fig. 2). The conductive biofilms allow for the exchange of electrons between bacteria and the carbon steel surfaces, which catalyzes corrosion reactions [32]. Here, on the carbon steel matrix, Fe^0 tends to lose electrons, and the electrons can be transferred to the oxygen through the conductive biofilm, ultimately leading to the dissolution of the substrates, similar to the mechanisms proposed in many previous reports by using other corrosive bacteria [33–35]. Our results indicated that the electrons required for this reduction were provided by the oxidation of the underlying metal.

4.2. Alleviation effect of Vibrio sp. EF187016

The corrosion inhibition ability of *Vibrio* sp. EF187016 has already been investigated [16]. Our results further indicate that this kind of bacteria also can inhibit MIC caused by *N. dassonville*. In the co-culture and pre-grown medium, the acceleration of corrosion by *N. dassonville* was significantly mitigated in the presence of *Vibrio* sp. EF187016. The SEM images (Fig. 3) indicated that *Vibrio* sp. EF187016 might be the dominant species on the surface of carbon steel as more amounts of this kind of species were observed compared to *N. dassonville*. The different sizes of these two kinds of bacteria might be the reason why this phenomenon was observed. *Vibrio* sp. EF187016 colonizes the surfaces, making the attachment of *N. dassonville* less possible. The compact biofilms of *Vibrio* sp. EF187016 can inhibit the transportation of some corrosive species, e.g. oxygen, thus the occurrence of the corrosion. Compared to the co-culture medium, the lower corrosion current density of coupons in the pre-grown medium (1.9 vs 1.0 μA cm^{-2}) (Fig. 2d) further confirmed the crucial importance of the formation of the protective biofilm from *Vibrio* sp. EF187016. Inspired by the results obtained in this work, natural beneficial biofilms found in some marine environments can be utilized to protect against metal corrosion and might be a potential environmental-friendly, cost-effective living material in MIMIC.

4.3. Potential of Vibrio sp. EF187016 EPS as a natural corrosion inhibitor

EPS modified carbon steel exhibited excellent corrosion resistance properties against *N. dassonville* (Figs. 4–6). The results indicate that naturally bacterial secretions such as EPS might be candidates for carbon steel corrosion protection. Initially, EPS was physically adsorbed on the surface of the coupon due to the van der Waals and electrostatic forces [36]. In addition, the functional groups in the EPS such as –OH and C–O–C have negative charges in the process of deprotonation which occurs in the attachment process [27]. The negative charge on the functional groups facilitates the chelation of iron, forming the metal-EPS complex, involved in the development of a protective film and the following protection of carbon steel [36,37]. Furthermore, polysaccharides outside the organic layer of EPS were mainly involved in the anti-adhesive properties of EPS, enhancing the inhibition efficiency [38]. It was reported that EPS, particularly polysaccharides, can form a matrix composed of largely anionic molecules [39]. The charge properties of the EPS matrix repelled the adhesion of the negatively charged

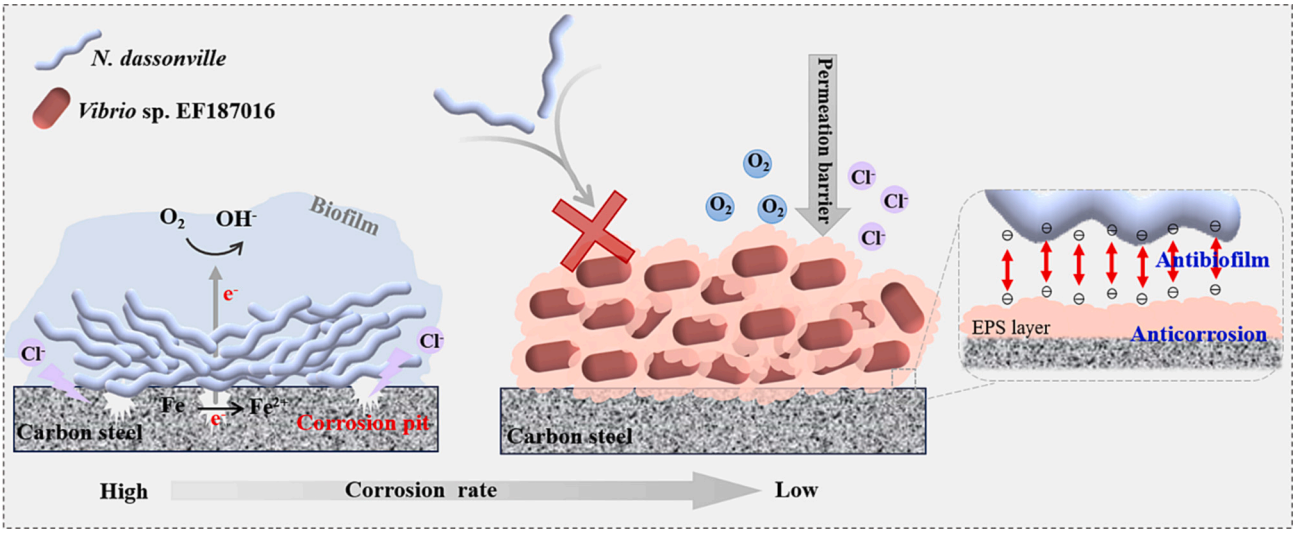

Fig. 8. Schematic illustration of the proposed mechanism.

N. dassonvillei cells on the carbon steel surfaces, alleviating the corrosion by *N. dassonvillei*. Overall, the schematic of the proposed mechanism is presented in Fig. 8.

5. Conclusion

The corrosion properties of a novel isolated bacterium, *N. dassonvillei*, from the South China Sea, were investigated in this work. *N. dassonvillei* induced severe MIC on carbon steel through electroactive biofilm, leading to a twofold increase in corrosion current and pit depth compared to the abiotic coupons. The corrosion caused by *N. dassonvillei* was mitigated in the presence of *Vibrio* sp. EF187016. *Vibrio* sp. EF187016 had the species advantage over *N. dassonvillei*, which can preferentially occupy carbon steel surfaces. The results of a slower corrosion rate in the pre-grown medium compared to those in the co-culture medium emphasized the vital role of preferential attachment of *Vibrio* sp. EF187016 biofilm in *N. dassonvillei* induced corrosion protection. Furthermore, EPS, as a natural compound derived from *Vibrio* sp. EF187016 biofilm exhibited effective resistance against corrosion caused by *N. dassonvillei*. The data obtained in this work suggest that the local resources including the living bacteria (*Vibrio* sp. EF187016 here) as well as the derived EPS are directly utilized to protect against microbial corrosion of carbon steel.

Author statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Yu Gao: Writing – original draft, Methodology, Formal analysis, Data curation. **Jingru Zhang:** Methodology, Investigation. **Donglei Wang:** Methodology, Formal analysis. **Jiaxin Fan:** Investigation, Formal analysis, Data curation. **Arjan Mol:** Writing – review & editing. **Fuhui Wang:** Writing – review & editing, Funding acquisition. **Danni Zhang:** Writing – review & editing, Visualization, Supervision. **Dake Xu:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data presented in this study are available upon request.

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Appendix A. Supplementary data

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