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The effect of dissolved oxygen and *Shewanella algae* on the corrosion mechanism of titanium in a simulated marine environment

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ABSTRACT

The influence of dissolved oxygen (DO) and *Shewanella algae* on the corrosion behavior of pure titanium were systematically studied in this work. The formation of *S. algae* biofilms on titanium surface was facilitated by the anaerobic environment, which accelerated titanium corrosion. Upon the breakdown of passive film, *S. algae* acquired electrons from the titanium base substrate through extracellular electron transfer (EET) processes. Various EET-related genes were overexpressed by the low DO condition, leading to enhanced EET kinetics. High concentration DO increased TiO₂ content and the thickness of passive film, which enhanced the protective effect and mitigated microbiologically influenced corrosion.

1. Introduction

With the prosperous development of marine industry and the urge for applications operating under extreme conditions, the development and application of marine grade engineering materials also puts forward more stringent requirements [1]. Marine corrosion is considered to represent a severe and urgent hazard to high performance applications due to the aggressive nature of marine environments [2–4]. Corrosion not only induces huge economic losses but also poses a serious threat to structural integrity of marine structures as well as pollution of the marine ecosystem [5,6]. The severity of corrosion in marine environments originates from the highly conductive nature of seawater, its aggressive constituents such as chlorides, and the adhesion of marine organisms [7]. Microbiologically influenced corrosion (MIC) represents a significant portion of corrosion failure of metals in marine environments [8,9]. Aiming to pave the way for the detection of and effective protection against MIC, it is imperative to explore the underlying mechanisms and kinetics.

Commercially pure titanium (Ti) represents a new marine grade structural engineering metal due to its excellent mechanical properties

and corrosion resistance [10]. However, due to its good inherent biocompatibility, Ti has a high risk of suffering from MIC during long-term application [11,12]. For example, large perforation of a heavily scaled titanium condenser tube in a seawater environment has been reported due to scouring corrosion from turbulent flow in an area accumulating organic microbes [13]. Recent research of MIC of Ti has focused on the effect of microbial activity on the external environment and release of corrosive metabolites. For example, biofilms may lead to local acidification [12,14] and ions in metabolites may react with the metal surface, resulting in micro-pit formation [15,16]. Furthermore, it has been reported that MIC of Ti is related to extracellular electron transfer (EET) [17,18]. *Shewanella algae* is a common and electroactive facultative anaerobe in the marine environment [19,20]. Many studies have demonstrated that *S. algae* reduce high-valent metal ions to lower-valent states, thereby affecting the corrosion process at the metal-electrolyte interface [21,22].

In addition, also other environmental factors need to be taken into account in order to unravel the metabolic and corrosion mechanisms of MIC activity under practically relevant conditions. When serving in the marine environment, engineering metals may experience different and

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varying dissolved oxygen (DO) concentrations due to variations in location, including latitude, longitude, and depth of application. In itself, dissolved oxygen is a crucial factor that affects the degradation behavior of engineering metals in the marine environment [23]. DO is a strong depolarizing agent and most metallic materials suffer from cathodic depolarization corrosion when subjected to high DO conditions [24,25]. The DO influences the equilibrium potential of the cathodic reduction reaction from a thermodynamic point of view and its exchange current density from a kinetic perspective. Moreover, dissolved oxygen has an effect on the passivation behavior of metals [26–28]. DO can change the composition of the corrosion product or passive film on low carbon steel, acting as an oxidizing agent to convert iron from the mixed Fe(II) and Fe(III) form in Fe_3O_4 to the more stable Fe(III) form in $\gamma\text{-Fe}_2\text{O}_3$ [28]. An increased DO concentration is shown to lead to a decrease of defect density and thickening of the space charge layer in passive films on stainless steel, which reduced the corrosion rate [29]. However, in certain environments, high DO concentrations is reported to reduce the stability of the passive film [30,31]. Therefore, the passivation behavior of specific metals under different DO conditions needs to be discussed in relation to the specific environment. Li et al. [32] reported the joint effect of temperature and DO on Ti alloys and showed that high DO concentrations have a positive effect on the passivation behavior. However, along with the presence of microorganisms, the effect of DO on Ti corrosion is yet uninvestigated. It is an important open question that requires further investigation, as the DO may affect the growth and metabolic properties of microorganisms to influence the biofilm formation [27–29] and hence the overall MIC mechanism and kinetics. Therefore, it is imperative to not only study the individual effects of DO and microorganisms in the marine environment but also their interaction to better understand the corrosion mechanisms and kinetics of materials in practical marine environments.

In this study, the corrosion behavior of commercially pure Ti under the joint effect of DO and *S. algae* was investigated. Concerning the differences in DO concentration between marine surface and deep-water conditions, both oxygen-saturated and fully anaerobic conditions were set. Biofilms of *S. algae* were observed by scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). Electrochemical tests and pitting corrosion studies were performed to investigate the individual and combined effect of *S. algae* and DO on the corrosion behavior of pure Ti. The effect of set conditions on the passive film characteristics was analyzed by Mott-Schottky tests and X-ray photoelectron spectroscopy (XPS). Point defect modeling (PDM) was performed to unravel the passive film defect characteristics. RNA transcriptome experiments were performed to study the effect of DO on the expression of *S. algae* genes to fundamentally reveal the corrosion mechanism. This mechanistic study provides targeted guidance for the practical application and corrosion protection of titanium alloys in marine environment.

2. Materials and methods

2.1. Materials, bacterial strain and culture medium

The elemental composition of commercially pure Ti samples (wt%) was 0.01 Cu, 0.02 Fe, 0.01 C, 0.002 N, 0.10 O, 0.001 H (Ti balance). All materials were provided by the Institute of Metal Research, Chinese Academy of Sciences. For electrochemical testing, the samples were mounted into epoxy resin. For the growth of the biofilm and surface analysis, samples of 10 mm in diameter and 3 mm in thickness were employed. The exposed surfaces were polished by SiC paper up to 1000 grit, cleaned ultrasonically and sterilized before use.

S. algae MCCC 1A11468 was purchased from the Marine Culture Collection of China (MCCC) (Xiamen, China). The standard 2216E medium used in this study contains (g/L): 19.45 NaCl, 5.98 MgCl_2 , 3.24 Na_2SO_4 , 1.80 CaCl_2 , 0.55 KCl, 0.16 Na_2CO_3 , 0.08 KBr, 0.08 SrBr_2 , 0.034 SrCl_2 , 0.0024 NaF, 0.022 H_3BO_3 , 0.0016 NH_4NO_3 , 0.008 Na_2HPO_4 , 0.004

NaSiO_3 , 5.0 peptone, 1.0 yeast extract and 0.1 ferric citrate. The culture media was buffered at $\text{pH } 7.2 \pm 0.2$ and the initial cell concentration was approximately 10^6 cells/mL. The culture medium was autoclaved at 121°C for 20 min in order to obtain aseptic conditions. After cooling, the solution was transferred to sterilized glass bottles and aerated using N_2 and O_2 gases for 1 h at 25°C to reach saturation respectively. N_2 and O_2 were filtered through the 0.22-micron sterile centrifugal filter for sterility before flowing into the medium. Measured by an oxygen analyzer (YSI 550 A, China), the dissolved oxygen concentration (DOC) of the anaerobic culture solution was less than 0.1 ppm while the DOC of the O_2 -saturated culture solution was set and maintained at 20 ppm. Oxygen in an oxygen-saturated environment is supplemented by a needle every three days. The DOC in different mediums before and after immersion was listed in Figure S1. All experimental equipment was sealed in paraffin for the subsequent 14-day immersion time to sustain the DOC.

2.2. Biofilm characterization and surface observation

Morphological surface analysis of coupons exposed to the different culture media were performed after the 14-day exposure time. The morphology of biofilms on the coupon surfaces was studied by field emission-scanning electron microscopy (FE-SEM) (Ultra-plus, Zeiss, Germany). Prior to experiments, specimens were prepared by 4 % glutaraldehyde fixation for 4 hours and then dehydrated in ethanol with various concentrations (v/v) of 50 %, 60 %, 70 %, 80 %, 90 %, 95 %, and 100 %. Furthermore, the specimens were stained by the Live/Dead BacLight Bacterial Viability Kit L7012 (Life Technologies, USA) in the dark environment for 30 minutes in advance. Live and dead sessile cells were detected using a confocal laser scanning microscope (CLSM Model C2 Plus, Nikon, Japan). The biofilm thickness was measured by its digital 3D surface model.

2.3. Electrochemical tests

Electrochemical tests were performed with a Gamry 600 Electrochemical Workstation (Gamry Instruments, USA) using a conventional three-electrode system. A saturated calomel electrode (SCE) and a platinum sheet (10 mm $\times$ 10 mm $\times$ 0.1 mm) were used as a reference electrode and a counter electrode, respectively. All electrochemical measurements were performed after stabilization of the open circuit potential (OCP) for at least 20 mins. Linear polarization resistance (LPR) measurements were performed over a potential range of -5 mV to 5 mV versus OCP at a scanning rate of 0.333 mV/s. Electrochemical impedance spectroscopy (EIS) measurements were performed at a 5 mV amplitude sinusoidal voltage over a frequency range from 100 kHz to 0.01 Hz and the results were analyzed and fitted by ZsimpWin software. For potentiodynamic polarization tests, both anodic and cathodic plots were separately recorded at a sweep rate of 0.333 mV/s starting from OCP. Moreover, capacitance measurements (Mott-Schottky analysis) were performed through scanning from -0.6 V to $+1.6$ V vs. OCP at a sweep rate of 20 mV/s, with a sinusoidal wave modulated signal of 5 mV and a frequency of 1 kHz. All electrochemical tests under the same conditions were repeated at least three times for reproducibility analysis.

2.4. Pit morphology and stochastic pit growth analysis

The contour and depth of the corrosion pits were observed and measured by a CLSM machine (LSM710, Zeiss, Germany). Prior to this experiment, the adhering biofilm and corrosion products were removed from coupon surfaces following ASTM standard G1–03.

Each pit depth value was measured randomly from more than 10 identically exposed coupons, and the deepest pit depth for each investigated coupon was chosen to obtain ten maximum pit depths. The cumulative probability F can be calculated using $n/(N+1)$, where n is the ordinal number of the pit depths arranged from smallest to largest, and N

is the total number of selected pits. The formula for the Gumbel distribution of pit depths [31–33]:

$$Y = -\ln(-\ln F) \quad (1)$$

$$Y = \frac{d_i - \mu}{\alpha} \quad (2)$$

where Y is the reduced variant, d_i is the pit depth, μ is the center parameter, and α is the scale parameter. The probability of pit depth can be described by the following double exponential equation [34,35]:

$$P_{\max} = \mu + \alpha \ln S \quad (3)$$

$$P = 1 - \exp\left(-\exp\left(-\frac{d_i - (\mu + \alpha \ln S)}{\alpha}\right)\right) \quad (4)$$

where P denotes the probability of pit depth, S is the total area of the specimen, and $P_{\max}$ is the probability of maximum pit depth.

2.5. Passive film analysis

The composition of passive films formed on the different samples was studied via X-ray photoelectron spectroscopy (ESCALAB250, USA). The monochromatic X-ray source is Al $K\alpha$ radiation of 15 kV and 150 W at 50 eV passing energy with a step of 0.1 eV. The wide binding energy range extends from 0 to 1350 eV. The XPS data were analyzed by CasaXPS software (Version 2.3.15, USA), and the binding energy scale was calibrated by the standard C1s peaks.

The point defect density of the passive films was obtained by Mott-Schottky analysis. According to the conventional Mott-Schottky space-charge theory [36], the capacitance of a passive film (C_f) is considered to be constituted from the combination of three capacitances: the Helmholtz capacitance (C_H), the space-charge capacitance (C_{SC}) and the surface state capacitance (C_{SS}), which is given by:

$$\frac{1}{C_f} = \frac{1}{C_{SC}} + \frac{1}{C_H} + \frac{1}{C_{SS}} \quad (5)$$

Because both C_H and C_{SS} are larger than C_{SC} , the reciprocal of C_H and C_{SS} can be negligible [37]. As the space-charge of the passive film is under depletion conditions, a linear relationship is expected between $1/C_{SC}^2$ and the applied potential (E) for the film, which can be described by the following Eqs. (6) and (7) for different semiconductors:

$$\frac{1}{C_{sc}^2} = \frac{2}{\epsilon \epsilon_0 e N_d A^2} \left(E - E_{fb} - \frac{kT}{e} \right) \quad (\text{for n type}) \quad (6)$$

$$\frac{1}{C_{sc}^2} = \frac{2}{\epsilon \epsilon_0 e N_a A^2} \left(E - E_{fb} - \frac{kT}{e} \right) \quad (\text{for p type}) \quad (7)$$

where N_d and N_a represent the charge carrier densities of the donor and the acceptor, respectively, A is the surface area of working electrode, and ϵ the dielectric constant of the passive film (here ϵ is taken as 15.6 [38,39]), ϵ_0 the vacuum permittivity, e the elementary charge, k the Boltzman constant, T the absolute temperature and E_{fb} is the flat band potential.

2.6. Transcriptome analysis

Initially, *S. algae* was cultivated on pure Ti samples under oxygen-saturated and anaerobic conditions to promote the formation of biofilms. The biofilms were collected from the sample surface into a 4°C PBS solution using a sterilized brush-like applicator.

The biofilm suspension was resuspended and vortexed in 10 mL RNA extraction buffer (RNeasy kit, Ambion, USA). Samples were incubated at 65°C for 10 min and shaken every few minutes. The 2 μ L Superase-in RNase inhibitor (from RNeasy kit) and 50 μ L 0.5 M CaCl₂ were added into the 2 mL tubes, and the incubated sample was dispensed into tubes.

The mixture was centrifuged for 10 min. In the meantime, 50 μ L Plant RNA isolation aid and 4 μ L linear acrylamide (from Ambion, USA) were added to a new tube. The aqueous phase was then transferred in it. The 1 mL phenol-chloroform-isoamyl alcohol (stored at 4°C) preheated to 65°C was added and the mixture was rotated and centrifuged on high speed for 10 min. Then the aqueous phase was transferred to the tube with 100 μ L 3 M sodium acetate (RNase free) and 20 μ L Glycogen. Iso-propanol (1 mL) was added and the sample was precipitate at –30°C for 1 hour. The liquid was poured and then added 750 μ L cold 70 % ethanol into sample. After centrifuged, the ethanol was pumped and the pellet was air dried at room temperature for about 5–10 min. The sample was resuspended in 50 μ L TE buffer (from RNeasy kit), and RNA sample was collected after 10 minutes at room temperature. The concentration and purity of RNA were assessed using UV-Vis spectrophotometer (Nanodrop 2000, Thermo Scientific™, USA) while its integrity was examined through agarose gel electrophoresis. For a single library preparation, the RNA requirements were set as follows: total RNA quantity ≥ 1 μ g, concentration ≥ 35 ng/ μ L, OD_{260/280} ≥ 1.8 , and OD_{260/230} ≥ 1.0 . The samples were immediately frozen in liquid nitrogen and stored at –80°C before detection. The construction and RNA-Seq of the transcriptomic libraries were performed by the Personalbio gene technology Co., Ltd. (Nanjing, China) using the Illumina HiSeq™ platform. We used DESeq for differential analysis of gene expression, and the conditions for screening differentially expressed genes were: expression difference ploidy $|\log_2 \text{FoldChange}| > 1$, significance $p\text{-value} < 0.05$. Volcano plots of differentially expressed genes were plotted using R language (Ggplots2 software package) [40–42]. The volcano plot showed the distribution of genes, the difference in expression of genes and the significance of differences observed.

3. Result and discussion

3.1. Effect of the DO and *S. algae* on the corrosion conditions

3.1.1. *S. algae* biofilm formation on Ti

Fig. 1 shows the morphology of *S. algae* biofilms developed on the surface of Ti coupons after 7 and 14 days of immersion in anaerobic, and O₂-saturated media. In the anaerobic culture, *S. algae* adapted to the oxygen-limited conditions and formed a biofilm on Ti surface as from the 7th day (Fig. 1a). With immersion time, the biofilm became dense, multilayered and complex (Fig. 1b). This indicated that under anaerobic conditions, *S. algae* maintained a biofilm-forming capability. In contrast, when the oxygen was saturated, the cells sparsely distributed on Ti coupons and hardly exhibited a complete layer structure throughout the full immersion period (Fig. 1c and d). The sparsely formed biofilm under O₂-saturated condition indicated that high DOC hindered *S. algae* to form a dense biofilm on Ti surface.

3.1.2. Cell activity and biofilm thickness

The activity of sessile cells was studied and the thickness of the biofilm was measured by CLSM (Fig. 2). Under anaerobic condition, sessile cells were homogeneously and densely dispersed. The bright green dots indicated that these cells remain highly biologically active. From day 7–14, the maximum thickness of the biofilm increased from 28.8 μ m to 35.1 μ m (Fig. 2a and b), and the average thickness increased from 27.6 $\pm$ 1.3 μ m to 33.3 $\pm$ 1.9 μ m (Fig. 2e). Under the O₂-saturated condition, cells are dispersed unevenly on the surface of the Ti sample. Biofilms in some regions were shedding over time. For day 7 and day 14, the maximum thickness of biofilm was 17.2 μ m and 20.4 μ m (Fig. 2c and d) while the average thickness was 17.0 $\pm$ 2.2 and 19.8 $\pm$ 0.6 μ m (Fig. 2e), respectively. Consistent with the SEM results, CLSM images indicated that high DOC affected the dispersion and thickness of *S. algae* biofilm formation on Ti surfaces.

3.1.3. Medium analysis

The evolution of pH with immersion time in different culture media

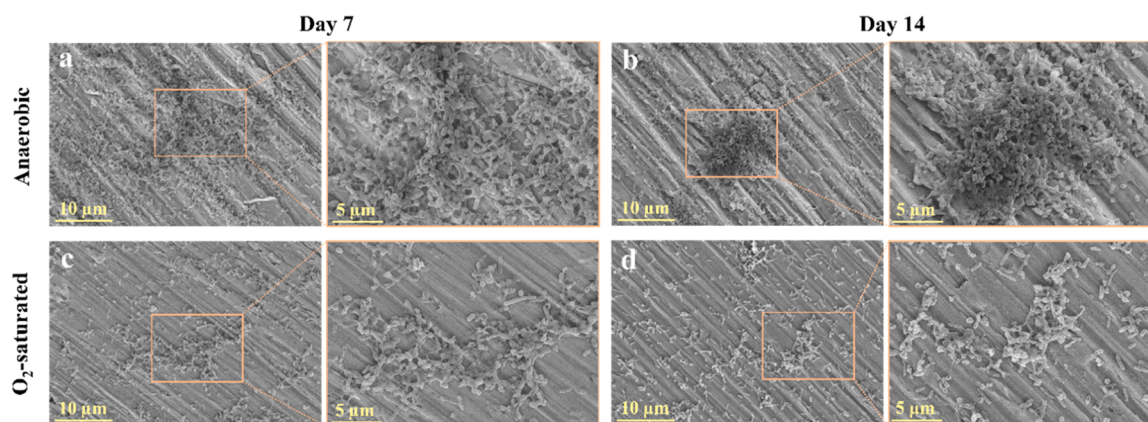


Fig. 1. SEM observation of *S. algae* biofilms attached on the Ti coupon surface in the anaerobic culture solutions at **a** Day 7 and **b** Day 14, in the O₂-saturated culture solutions at **c** Day 7 and **d** Day 14.

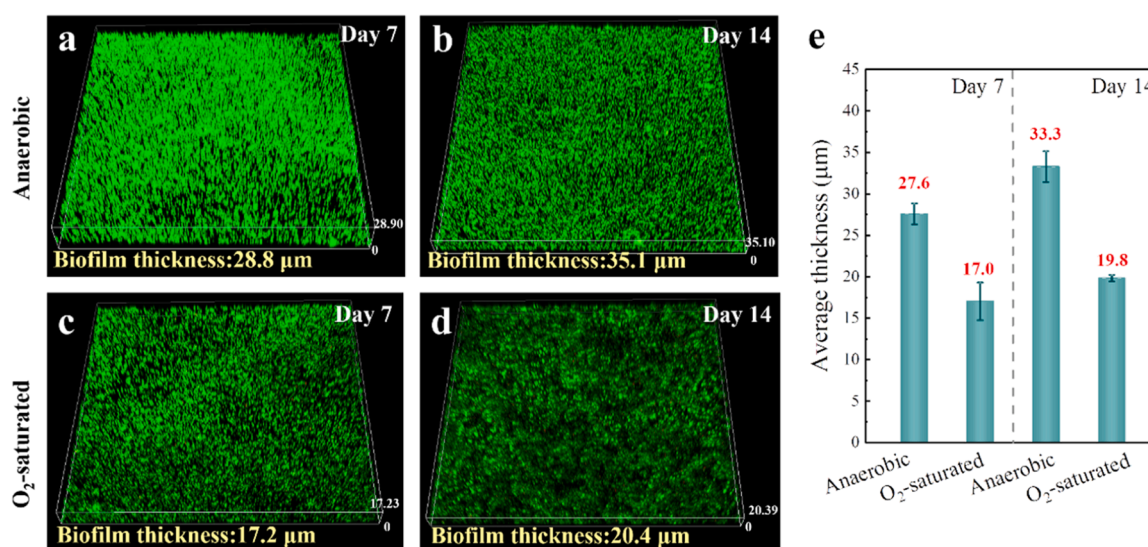


Fig. 2. CLSM analysis of *S. algae* biofilms attached on the Ti coupon surface. Green fluorescence represents active cells. Images of the thickest biofilm in the anaerobic culture solutions at **a** Day 7 and **b** Day 14, in the O₂-saturated culture solutions at **c** Day 7 and **d** Day 14. **e** Average thickness. Results are the mean and standard deviation ($n > 3$). Error bars represent standard deviations from at least three independent coupons.

was measured (Fig. 3a). The pH remained constant at 7.2 for the sterile medium in both anaerobic and O₂-saturated culture medium. With the inoculation of *S. algae*, the pH increased from 7.2 to 7.8, and then remained relatively constant towards the end of the incubation. The pH in aerobic environments was always slightly higher than that in

anaerobic environments. The nearly neutral pH indicated that Ti corrosion induced by *S. algae* was not alike acid corrosion which was reported to play a critical role in the corrosion of titanium dental implants in oral environment [43].

The growth curves in the different media demonstrated that the

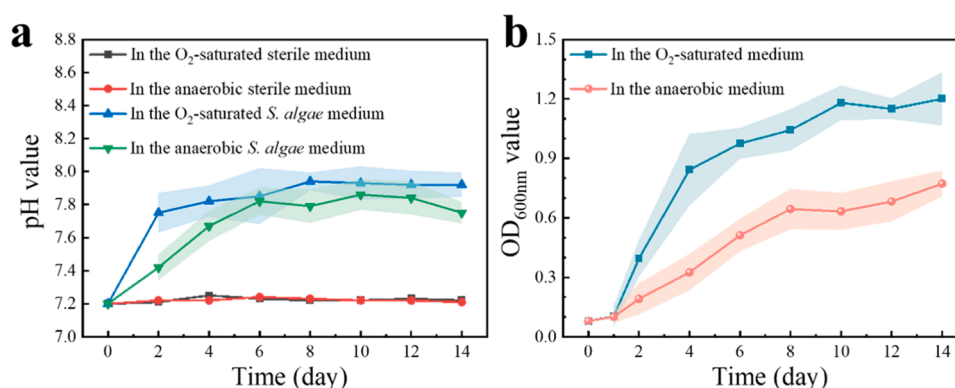


Fig. 3. **a** Variation of pH in the different mediums. **b** Growth curve of *S. algae* in the medium.

optical density (OD) values of cells in oxygen-saturated solutions were significantly higher than those in anaerobic solutions (Fig. 3b). This data indicated that *S. algae* cells preferred to grow in the medium rather than in biofilms on the metal surface at higher oxygen levels. Generally, *Shewanella* has respiratory diversity and is adept at forming complex biofilm, but there are many factors influencing cell differentiation and seeding dispersal within the biofilm, such as metabolite accumulation, donor insufficiency, or receptor species limitation [44–46]. When O_2 served as the sole electron acceptor, *Shewanella* species cells have been reported to show pronounced dispersive behavior and form hollow colonies [44,46]. This is consistent with the biofilm formation behavior of *S. algae* on pure Ti observed by SEM and CLSM. Overall, under anaerobic conditions, *S. algae* was more capable of forming biofilms on Ti surface.

3.2. Effect of DO and *S. algae* on the electrochemical behavior of Ti

3.2.1. OCP, LPR and potentiodynamic polarization analysis

Electrochemical techniques were applied to study the corrosion behavior of Ti as a function of absence or presence of *S. algae* and DO. The open circuit potential (E_{OCP}) and polarization resistance (R_p) values are presented in Fig. 4. As shown in Fig. 4a, the E_{OCP} value in the O_2 -saturated medium was more positive than that in the anaerobic media over the full immersion time. The addition of *S. algae* caused a significant decrease in E_{OCP} value. A more negative E_{OCP} value implies a higher intrinsic corrosion propensity [47]. Thus, the OCP data indicated that high DOC reduced the tendency of Ti to corrode, while *S. algae* increased the tendency. For LPR tests (Fig. 4b), the R_p values under different conditions showed no significant differences in the initial two days. The R_p value of Ti coupons under O_2 -saturated sterile medium increased from a minimum value of $6.6 \text{ M}\Omega\text{-cm}^{-2}$ to a maximum value of $16.9 \text{ M}\Omega\text{-cm}^{-2}$ during the whole immersion period, and the average value of R_p after the second day was $11.7 \pm 2.7 \text{ M}\Omega\text{-cm}^{-2}$. The upward trend of R_p values with time indicated that the Ti coupons underwent

passivation [48]. In contrast, the R_p values of coupons under anaerobic sterile condition were lower. The maximum R_p value was $7.9 \text{ M}\Omega\text{-cm}^{-2}$, with a mean value of $6.4 \pm 0.7 \text{ M}\Omega\text{-cm}^{-2}$ after the second day. Since the corrosion rate can be considered to be proportional to the inverse of the R_p value [49], the LPR result indicated that the DO had an inhibitory effect on the corrosion of Ti. In the presence of *S. algae*, the average R_p value for Ti under O_2 -saturated medium decreased to $2.8 \pm 0.5 \text{ M}\Omega\text{-cm}^{-2}$, and the average value under anaerobic medium decreased to $1.1 \pm 0.3 \text{ M}\Omega\text{-cm}^{-2}$. The R_p values of coupons under bacterial conditions decreased nearly tenfold compared to those under sterile conditions, indicating that the presence of *S. algae* biofilm significantly accelerated the corrosion rate. While DO slowed down the corrosion rate under *S. algae* conditions, the degree to which *S. algae* accelerated corrosion was much larger than the rate at which DO slowed down corrosion.

The potentiodynamic polarization curves provide information on the corrosion current density under each condition. In Fig. 4c, the polarization curve shifted positively in O_2 -saturated sterile seawater media compared to the anaerobic media in both bacterial and sterile environments. This phenomenon was due to the increase of oxygen partial pressure at the electrode surface with high DOC, thus resulting in the negative shift of corrosion potential [50]. In the presence of *S. algae*, the polarization curves of the electrodes also showed a negative shift in corrosion potential (Fig. 4d). Furthermore, the corrosion current density (i_{corr}) from fitting with Tafel extrapolation were shown in Fig. 4d to quantify the corrosion rate. The i_{corr} in the O_2 -saturated sterile seawater medium was $4.9 \pm 0.3 \text{ nA cm}^{-2}$, lower than i_{corr} in the anaerobic sterile medium ($8.3 \pm 1.4 \text{ nA cm}^{-2}$). In the presence of *S. algae*, Ti under anaerobic medium exhibited a significantly increased i_{corr} at $22.6 \pm 0.8 \text{ nA cm}^{-2}$, which was about three times higher than that in sterile medium. In the O_2 -saturated condition, the i_{corr} of Ti decreased to approximately $16.2 \pm 1.2 \text{ nA cm}^{-2}$. This result agreed with LPR data and confirmed that *S. algae* significantly accelerated corrosion and a high DOC has a protective effect on Ti.

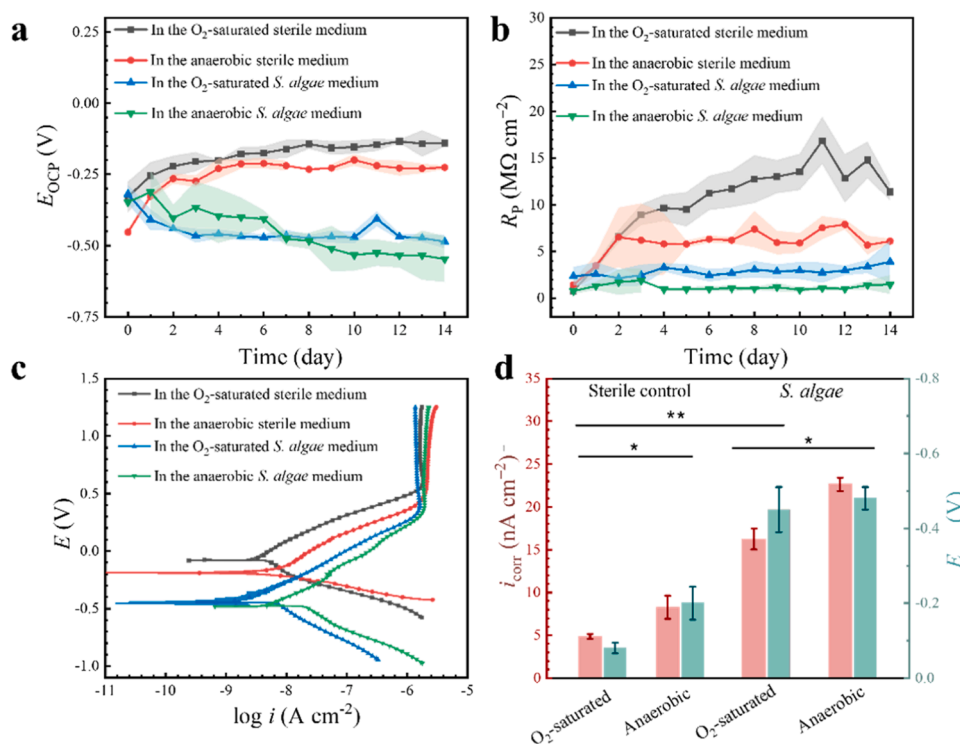


Fig. 4. Corrosion behavior of Ti in the different media: Variations of **a** E_{OCP} and **b** R_p data over time, **c** potentiodynamic polarization curves of the samples after immersion for 14 days, and **d** fitted i_{corr} and E_{corr} values. Error bars represent standard deviations from at least three independent coupons. An asterisk represents a statistically significant difference (* $p < 0.05$, ** $p < 0.01$).

3.2.2. Electrochemical impedance spectroscopy analysis

The EIS results for the Ti coupons in different media for 3, 7 and 14 days are shown in Fig. 5. In the Nyquist plots, flat and incomplete capacitive arcs were observed in all environments, which also describe the typical passivation characteristics of passive films grown on Ti [32, 51]. For the coupons immersed in the solution for 14 days, the arc radius in the low-frequency region of the Nyquist plots (Fig. 5a-c) decreased in presence *S. algae* representing a lower corrosion resistance [52] versus that under sterile conditions. In the corresponding Bode plots (Fig. 5d-f), all curves also exhibited two partially overlapping time constants, and the phase angles approaching -90° from the low to medium frequencies. Generally, the higher impedance values at low frequency in Bode plots ($|Z|_{0.01 \text{ Hz}}$) represent a higher corrosion resistance [53]. The result in Fig. 5g showed that *S. algae* decreased the corrosion resistance while the oxygen increased it. For two time constants, the one in the mid-high frequency range is indicative of the capacitive and resistive behavior in the surface film, and the one in the low-frequency region reflects the charge transfer process in the metal-to-electrolyte/surface film interfacial region, all in all representing the impedance response of the passive layer [54–56]. Therefore, the equivalent electrical circuit shown in Fig. 5h, which is widely used for passivating systems, was chosen to fit the impedance spectrum results [32,57,58]. This equivalent circuit model is composed of the electrolyte resistance (R_s), the oxide layer resistance or the mixture resistance of oxide layer and biofilm (R_f), the charge-transfer resistance (R_{ct}) and the constant phase element

(CPE, Q). It is necessary to use the constant phase element instead of an ideal capacitance on account of the dispersion effect of the heterogeneity caused by electrode corrosion in the medium. Q_f represents the capacitive properties of the oxide layer, and Q_{dl} represents the capacitive properties of electric double-layer[59–62]. The impedance of CPE can be determined by:

$$Z(\text{CPE}) = [Y_0(j\omega)^n]^{-1} \quad (8)$$

where n and Y_0 are the CPE parameters, ω is the angular frequency (in rad s^{-1}), and $j^2 = -1$. The Y_0 is the conductance; n is the coefficient of dispersion reflecting the surface roughness. The Q is ideal capacitor (C) when n equals to 1.

The fitted EIS data are listed in Tables S1–S3. The R_{ct} value is inversely proportional to the corrosion rate, and the variations in R_{ct} resistance are displayed in Fig. 5i. The R_{ct} ranks as following: O_2 -saturated sterile medium > Anaerobic sterile medium > O_2 -saturated *S. algae* medium > Anaerobic *S. algae* medium. The corrosion rate reflected by R_{ct} results was the same as that of R_p as studied with LPR. All electrochemical test results lead to the same conclusion, the corrosion rates for different conditions were ordered as: Anaerobic *S. algae* medium > O_2 -saturated *S. algae* medium > Anaerobic sterile medium > O_2 -saturated sterile medium.

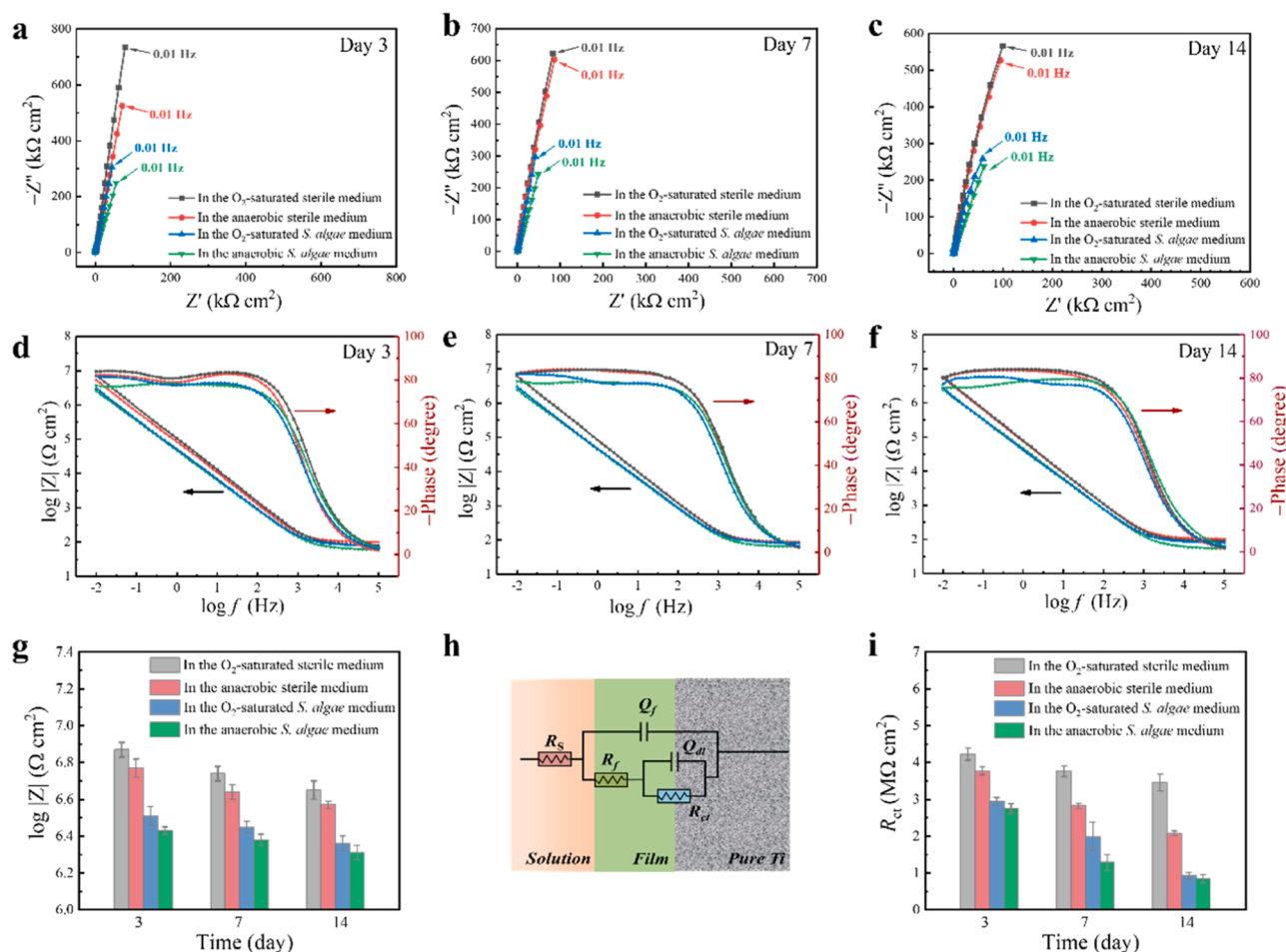


Fig. 5. The Nyquist and Bode raw plots of Ti coupons exposed to the different mediums after (a, d) day 3, (b, e) day 7 and (c, f) day 14. g the $\log |Z|$ values at 0.01 Hz of coupons exposed to the different mediums. h Equivalent fitting circuit. The equivalent circuit model is composed of the electrolyte resistance (R_s), the oxide layer resistance or the mixture resistance of oxide layer and biofilm (R_f), the charge-transfer resistance (R_{ct}), the capacitive properties of the oxide layer (Q_f) and the capacitive properties of electric double-layer capacitance at the film/solution interface (Q_{dl}). i The R_{ct} value from EIS data fitting. Results are the mean and standard deviation ($n > 3$). Error bars represent standard deviations from at least three independent coupons.

3.3. Effect of DO and *S. algae* on the pitting corrosion of Ti

3.3.1. Pitting observation and statistics

In order to determine whether biofilm growth on the Ti surface resulted in pitting, the corrosion morphologies of Ti coupons after removing the biofilms and corrosion products were analyzed. The morphologies of the deepest pits are shown in Fig. 6a–d. Under sterile condition, very shallow pits were formed on the Ti surface after 14 days of immersion (Fig. 6a and b). It indicated that pure Ti possessed good resistance to pitting corrosion in the sterile seawater medium. With the addition of *S. algae*, the localized corrosion was significantly aggravated and the depth of pits increased. Under O₂-saturated *S. algae* conditions, the maximum pit depth reached 4.6 μm (Fig. 6c), much higher than the maximum pitting depth of 1.6 μm in sterility (Fig. 6a). Under anaerobic *S. algae* conditions, the maximum pit depth reached 5.1 μm (Fig. 6d), more than twice the maximum pit depth of 2.0 μm under sterile condition (Fig. 6b). These observations indicated that *S. algae* destabilized the passive film of Ti enabling visible pitting at its surface. On the other side, the presence of oxygen lowered the extent of localized corrosion of Ti coupons.

Moreover, the pitting density on the coupons under different conditions was determined and presented in Fig. 6e. The pitting density of Ti coupon in the presence of *S. algae* was significantly higher than in the sterile condition, but the difference between the aerobic and anaerobic conditions was not significant. Fig. 6f shows the statistics of the ten maximum pit depths of Ti coupons after 14 days of immersion. The pit depth of Ti coupons in O₂-saturated sterile medium ranged from 0.4 to 1.6 μm, and the pit depth in the anaerobic sterile medium ranged from 0.9 to 2.0 μm. In comparison, the pit depth of Ti coupons in O₂-saturated *S. algae* medium ranged from 1.3 to 4.6 μm, and the pit depth in the anaerobic *S. algae* medium ranged from 1.6 to 5.1 μm. In brief, the pit depth showed the same trend in the electrochemical corrosion rate (For instance, the i_{corr} value of Fig. 4d): Anaerobic *S. algae* medium > O₂-

saturated *S. algae* medium > Anaerobic sterile medium > O₂-saturated sterile medium. The results indicated that *S. algae* enhanced pitting while oxygen weakened it. When *S. algae* and oxygen were simultaneously present, the effect of *S. algae* was the predominant factor.

3.3.2. Stochastic modeling of pit initiation and growth

In order to further reveal the pit formation mechanism under different conditions, Fig. 6g shows the Gumbel distribution of the pit depths. This distribution was obtained from the cumulative probability of pit depth presented in Figure S2. Under sterile conditions, there was only one linear region of pure Ti in both aerobic and anaerobic media. This demonstrated that under sterile conditions, only one corrosion mechanism controlled the pitting behavior, represented by the corrosive chloride ions in the seawater medium. Pitting in this case was meta-stable. However, pure Ti in the *S. algae*-containing medium had two linear regions, indicating that the pitting behavior was controlled by two corrosion mechanisms [63]. The pits developed from the meta-stable to stable state at a transition depth of about 2.5 μm. In addition, the scale parameter α and the location parameter μ (Table S4) from the Gumbel distribution were analyzed to predict the probability of pits (Fig. 6h). The slope of pitting probability in sterile medium was greater than that of *S. algae*-containing medium, suggesting that the probability of forming larger pits was lower under sterile conditions, whereas *S. algae* increased the probability of larger pits. Further, the slopes were all smaller in the O₂-saturated culture solution, which indicated that oxygen had an inhibitory effect on the pitting occurrence.

3.4. Effect of DO and *S. algae* biofilm on the passive film properties of Ti

3.4.1. Passive film thickness

The passive film is the first barrier to protect titanium substrate from corrosion [44]. The corrosion resistance is related to the properties of passive film, including thickness, composition and point defects [64,65].

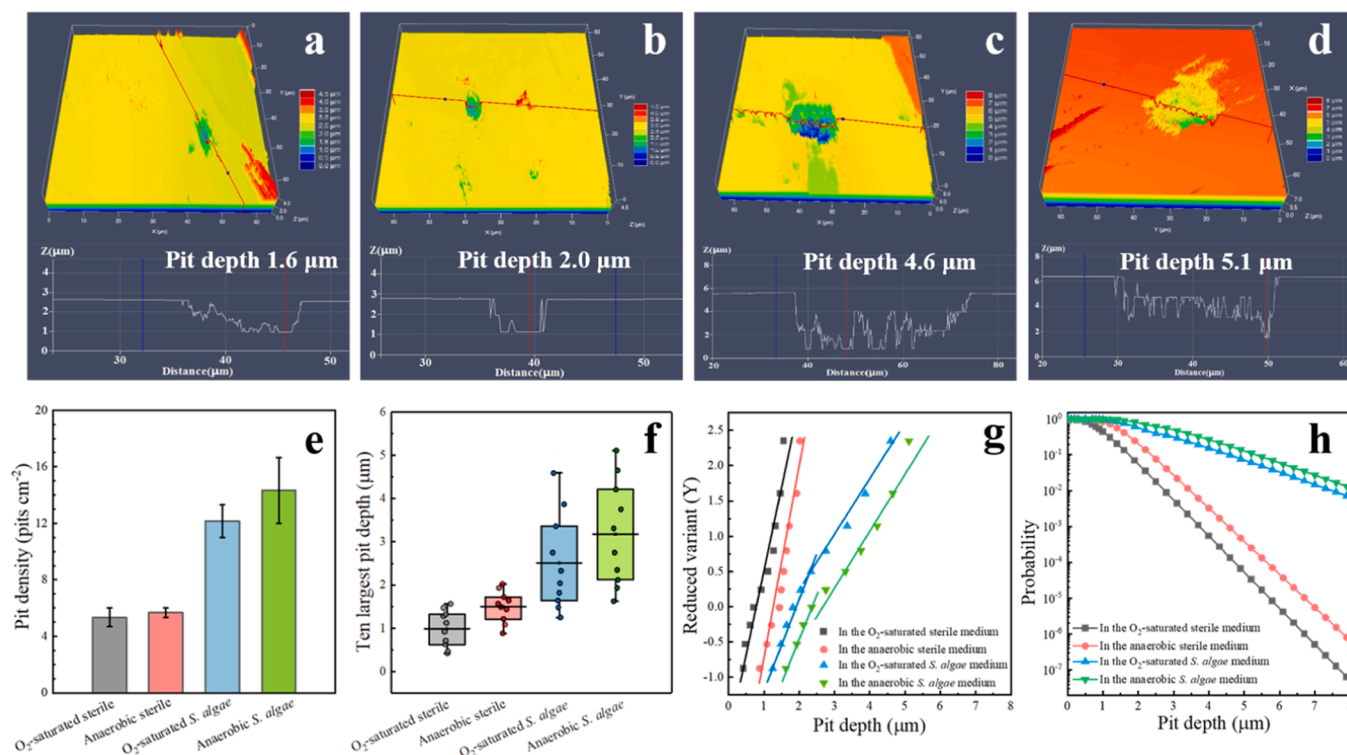


Fig. 6. CLSM 3-D images for the deepest pits on Ti after immersion of 14 days: **a** in the sterile medium of O₂-saturated culture solutions and **b** anaerobic culture solutions, **c** in the presence of *S. algae* of O₂-saturated culture solutions and **d** anaerobic culture solutions. **e** Statistics of pitting density. **f** Ten largest pit depths in the different culture media after 14 days of incubation. Statistics of pit depth: **g** Gumbel probability plots for the pit depth and **h** probability of various pits of Ti in the different mediums.

Therefore, the properties of passive films were investigated and analyzed from these three perspectives. The approximate thickness of passive film could be obtained by XPS depth profiles. The oxide scale equivalent thickness was inferred from the half height of initial maximum oxygen-atoms concentration [66–68]. As shown in Fig. 7a and b, the thickness of Ti passive film in the O₂-saturated sterile environment (approximately 13.6 nm) was slightly higher than that for the anaerobic sterile environment (approximately 11.6 nm). This result indicated a positive effect of oxygen on passive film formation. However, the thickness was significantly reduced when the *S. algae* was inoculated. The thickness of passive film in the O₂-saturated *S. algae* environment was approximately 9.4 nm (Fig. 7c) while in the anaerobic *S. algae* environment it was approximately 7.3 nm (Fig. 7d). The trend of passive film thickness was: O₂-saturated sterile medium > Anaerobic sterile medium > O₂-saturated *S. algae* medium > Anaerobic *S. algae* medium. The decrease in thickness represented the negative effect of *S. algae* on passive film formation, which underpins the observations of accelerated corrosion under these conditions. While *S. algae* showed a negative effect on the passive film, oxygen enabled promotion of the passivation process to enhance the thickness of the passive film, thereby reducing some of the overall corrosion rate.

3.4.2. Passive film composition

In addition to depth profiles, XPS test provided the composition information of passive films (Fig. 8). In the XPS survey spectra (Figure S3), Ti and O elements were detected for all conditions, indicating that the passive film mainly consisted of titanium oxide. Further, detailed spectral analysis of the Ti element was performed to determine the specific

types of titanium oxides and the relative percentages of each titanium oxide were calculated from the integral area of corresponding elements [69,70]. Generally, the relative content of TiO₂ in passive film plays a crucial role in the corrosion resistance of Ti coupons[56]. A higher ratio of TiO₂ indicates enhanced stability and better corrosion resistance of the passive film[32]. In the O₂-saturated sterile medium (Fig. 8a, b), 73.6 % of titanium at 3 nm and 58.0 % of titanium at 6 nm of passive film existed as constituent of TiO₂. While in the anaerobic sterile medium (Fig. 8c, d), the corresponding relative proportion of titanium oxide was different and the TiO₂ content decreased to 68.1 % and 48.3 % respectively. This result indicated that oxygen elevated the TiO₂ content in the passive film. In the presence of *S. algae*, the proportion of TiO₂ was further reduced while the proportion of other oxides was increased. The percentages of TiO₂ under aerobic condition at 3 nm and 6 nm were 41.1 % and 8.6 % (Fig. 8e, f). The percentage of TiO₂ under anaerobic condition at 3 nm was 27.5 % while the presence of TiO₂ was not detected at a depth of 6 nm (Fig. 8g, h). The order of TiO₂ content in passive film of under each condition was the same as that for the passive film thickness (Fig. 7). Therefore, it can be concluded that *S. algae* accelerated corrosion was due to the destruction of the passive film, which was manifested in the reduction of TiO₂ content. High DOC tempered the MIC effect by increasing the TiO₂ content.

3.4.3. Point defect density of passive film

When the protective properties of passive film are decreased, the metal atoms will dissolve rapidly into the solution through short-circuit diffusion channels to form metal ions, leaving a large number of point defects on the surface. The corrosion resistance of Ti is related to the

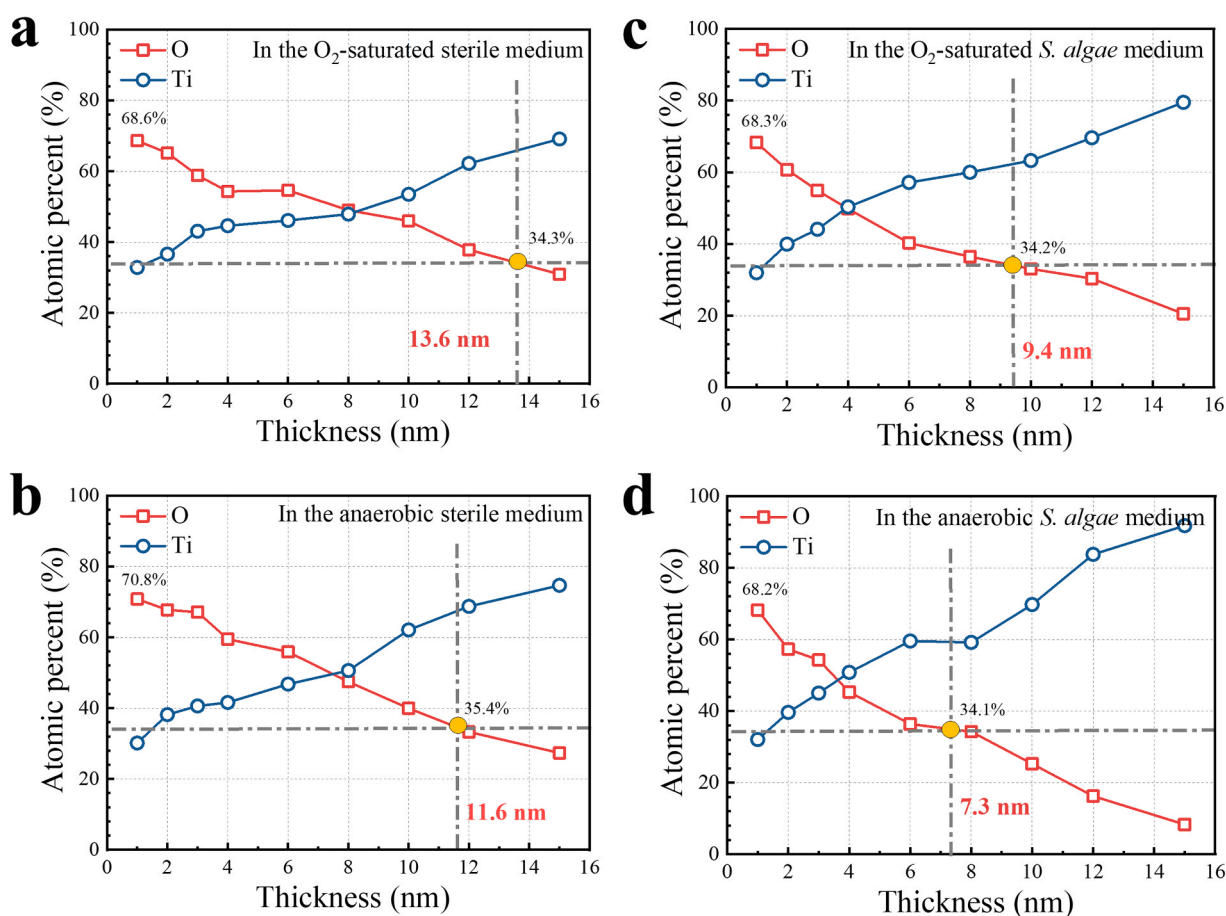


Fig. 7. Depth profiles of the passive films formed on the Ti immersed in the different media after 14 days: a in the sterile medium of O₂-saturated culture solutions and b anaerobic culture solutions, c in the presence of *S. algae* of O₂-saturated culture solutions and d anaerobic culture solutions. Results are the mean and standard deviation ($n > 3$). Error bars represent standard deviations from at least three independent coupons.

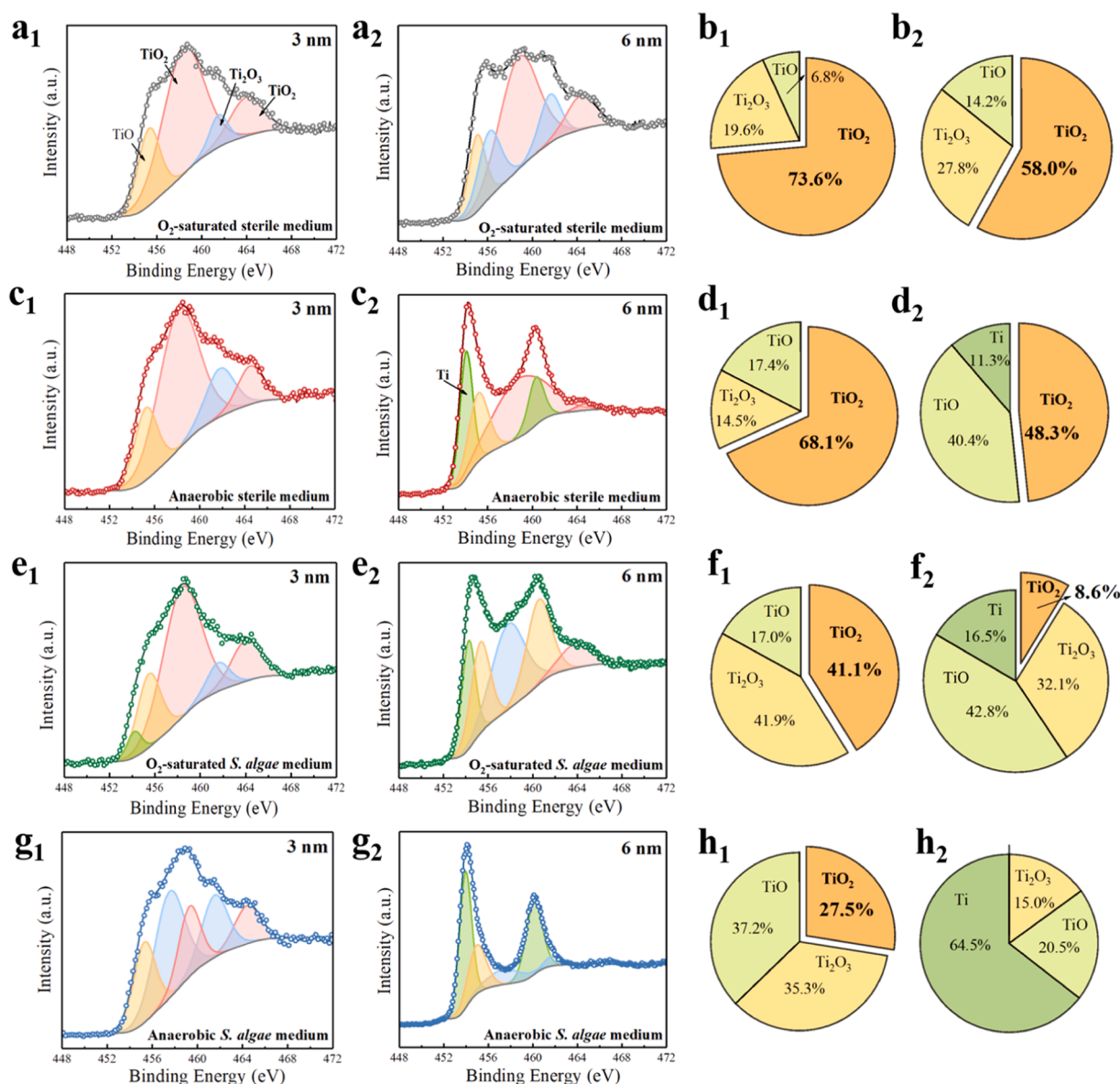


Fig. 8. Detailed XPS spectra at 3 nm/6 nm and cation percentages of Ti 2p peaks after incubation for 14 days in the different medium: (a₁, a₂, b₁, b₂) O₂-saturated sterile medium, (c₁, c₂, d₁, d₂) anaerobic sterile medium, (e₁, e₂, f₁, f₂) O₂-saturated *S. algae* medium and (g₁, g₂, h₁, h₂) anaerobic *S. algae* medium.

concentration and diffusivity of point defects in passive film [71,72]. Mott-Schottky results of pure Ti exposed to the different medium for 1 day, 4 days, 7 days, 10 days, and 14 days are shown in the left column of Fig. 9. For all plots, positive slopes were obtained for each condition, implying that the passive film of Ti coupons behaved as n-type semiconductors in all environments. The presence of oxygen or bacteria did not change the semiconductor type of the passive film. The acceptor density (N_d) of the passive films were calculated according to the slopes of Mott-Schottky curves and listed in the right column of Fig. 9. It could be seen that the N_d increased gradually with time under all mediums, indicating that the densification of the passive film decreased with increasing immersion time. Besides, the N_d in the sterile environment was much smaller than that in the *S. algae* inoculated condition and the samples in the presence of oxygen maintained a lower defect density than samples without oxygen. The donor density ranking was in the order of Anaerobic *S. algae* medium (Fig. 9h) > O₂-saturated *S. algae* medium (Fig. 9f) > Anaerobic sterile medium (Fig. 9d) > O₂-saturated sterile medium (Fig. 9b). Therefore, *S. algae* increased the N_d value of passive film while the DO decreased the N_d value. In passive films, the

defect density in TiO₂ is higher than that in Ti₂O₃; and the content of Ti₂O₃ is usually proportional to the defect density. Therefore, there was consistency between the pitting results and the XPS results. Combined with the thickness and composition data of passive film (Fig. 7 and Fig. 8), it could be concluded that *S. algae* contributed to the destruction of the passive film, while DO has a protective effect to it.

3.4.4. Analysis of the semiconductor property of passive film

Point defect modeling (PDM) serves to interpret kinetic information about the growth and decomposition of passive films [73]. In this study, the PDM model was employed to discuss the effect of DO and *S. algae* on passive film. The slope of curves and the fitted data in Fig. 9 showed that the passive film of Ti is an n-type semiconductor. For the n-type semiconductor, oxygen vacancies ($V_O^{\bullet\bullet}$) and cation vacancy (V_{Ti}^{4-}) are the major point defect types [39,74]. Reactions for the production of oxygen vacancies ($V_O^{\bullet\bullet}$) and cation vacancy (V_{Ti}^{4-}) in pure Ti are [39]:



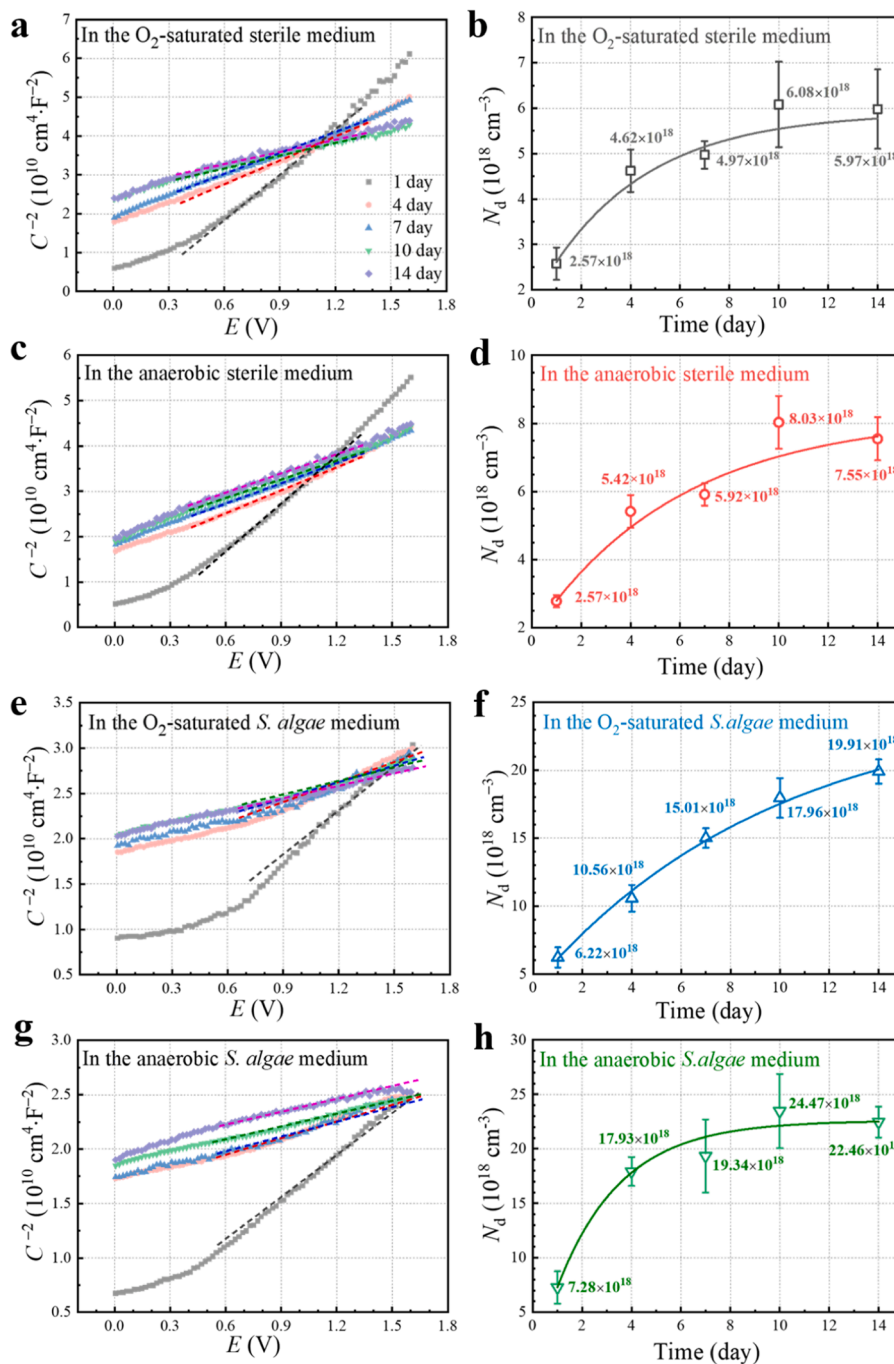


Fig. 9. Mott-Schottky plots and the variations of fitted donor densities after different days: (a, b) in the sterile medium of O₂-saturated culture solutions and (c, d) anaerobic culture solutions, (e, f) in the presence of *S. algae* of O₂-saturated culture solutions and (g, h) anaerobic culture solutions. The dots are experimentally measured data and the lines are the fitting curves. Results are the mean and standard deviation ($n > 3$). Error bars represent standard deviations from at least three independent coupons.



Where, Ti_{Ti} corresponds to Ti ions in the cation site of the passive film, $\text{V}_{\text{O}}^{\bullet\bullet}$ corresponds to oxygen vacancies in the passive film which carries two positive charges and $\text{V}_{\text{Ti}}^{4-}$ corresponds to Ti ion vacancies in the passive film which carries four negative charges. Since the passive film is semiconducting, the above two reaction for the appearance of point defects is spontaneous. However, external environmental factors (dissolved oxygen and microorganisms) can impact the rate of the reaction or stimulate the generation of new reaction, thus influencing the point defect density.

Oxidation of metals occurs at the passive film/ substrate interface and oxygen vacancies are generated [32,59]. The $\text{V}_{\text{O}}^{\bullet\bullet}$ will move to the passive film/solution interface due to diffusion effects, thus the concentration of oxygen vacancies in the passive film shows an index decay trend. Oxygen in solution can adsorb on the surface of passive film and occupy the anionic vacancies to participate in the forming passive film, and the occupancy rate is proportional to the amount of oxygen adsorbed on the surface of the material. The reaction is [59,75]:



Where, O_{O} corresponds to oxygen ion in the anion site of passive film.

When the concentration of oxygen in the solution is higher, it is favorable to the occupancy of oxygen vacancies and the formation of oxides in the passive film. Due to partial oxygen vacancies ($V_{\text{O}}^{\bullet\bullet}$) being occupied, the point defect in the passive film decreased with high DOC (as shown in Fig. 9).

Microorganisms are hardly directly involved in the reaction of point defects. Since the synergistic effect between *S. algae* and chloride ions in the corrosion process has been reported, the effect of *S. algae* on point defects can be established by corrosive ions in seawater [17]. Besides, biofilms can cause local submembrane enrichment of chloride ions and enhance their effects. According to PDM, the aggressive chloride ions could adsorb at the surface and occupy the oxygen vacancy at the film/solution interface, resulting in the enhanced generation of cation vacancies ($V_{\text{Ti}}^{4\bullet}$) via Schottky-pair (Null) reaction [50,51]:



The presence of electron vacancies results in more chloride ions being attracted to the surface of the passive film causing a greater degree of ion enrichment. The generated $V_{\text{O}}^{\bullet\bullet}$ reacts further with Cl^- to recycle more and more cation/oxygen vacancy pairs. Even though some of the oxygen vacancies are consumed, the reaction produces more pairs of vacancies. Wherein, the cationic vacancies ($V_{\text{Ti}}^{4\bullet}$) react with Ti ions at the metal/passive film interface[75]:



This reaction is protective for the substrate and is part of the repassivation process of the passive film. If the cationic vacancies at the interface between the metal and the passive film are completely consumed by this annihilation reaction, the substrate exhibits repassivation, at which time the pitting is meta-stable. However, when such cationic vacancies are not completely annihilated, the excess vacancies aggregate to form irreversible voids, leading to the localized dissolution of the passive film and the formation of stable pitting. In conjunction with pitting data presented in Fig. 6, pits on pure Ti surfaces were in a steady state mode in the presence of *S. algae*. Hence, the large amount of pitting defects caused by *S. algae* were not completely eliminated, ultimately resulting in irreversible passive film damage and ongoing pitting corrosion of the Ti surface.

The point defect density of passive film (Fig. 9), the i_{corr} value (Fig. 4d) and the pitting degree (Fig. 6) of pure Ti in four conditions corroborated in the same order; and they were in the opposite order to the R_p value (Fig. 4b), R_{ct} value (Fig. 5i), passive film thickness (Fig. 7) and TiO_2 ratio (Fig. 8). All the data together indicated that *S. algae* accelerated the corrosion of pure Ti by destroying the passive film, while the repairing effect of oxygen on passive film reduced the corrosion rate.

3.5. Effect of DO on the EET-MIC of *S. algae*

In addition to the effect of DO and microorganisms on metal, the effect of DO on the living activities of *S. algae* also influenced the EET-MIC process[20]. *Shewanella* spp. have a diverse range of electron transfer mechanisms: they can directly acquire electrons from metal via terminal reductases and outer membrane c-type cytochrome proteins with redox activity; they utilize electrically conductive pili for long-distance electron transfer processes [76]; and they can secrete electron shuttles such as riboflavin, flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN) to accomplish the mediated electron transfer [76–79]. In order to clarify the influence of DO on various gene expression levels within the cell, high-throughput RNA sequencing was performed. According to the volcano plot for the transcriptome data in Fig. 10, eight genes were significantly overexpressed under anaerobic conditions among the nine differentially expressed genes related to EET. As listed in Table 1, the overexpressed genes are involved in various

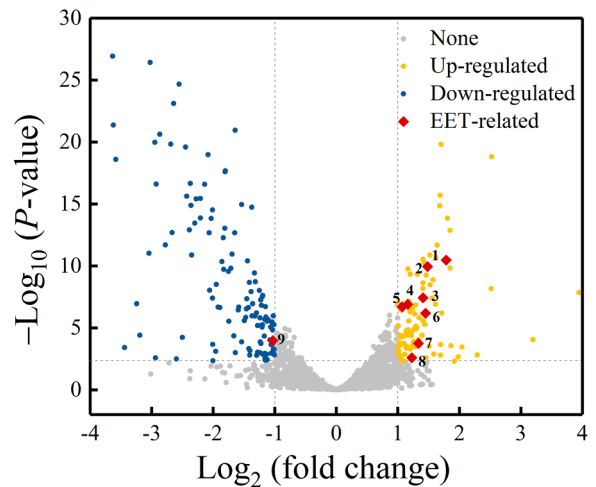


Fig. 10. Volcano plot of transcription group. The experimental group is the biofilm on coupons under anaerobic conditions, while the control group is the biofilm on coupons under O_2 -saturated conditions. The horizontal coordinate is $\log_2(\text{Fold Change})$ and the vertical coordinate is $-\log_{10}(p\text{-value})$. The two vertical dashed lines are the 2-fold expression difference thresholds; the horizontal dashed line is the p -value of 0.05 threshold. Yellow dots indicate up-regulated genes in this group, blue dots indicate down-regulated genes in this group, and gray dots indicate non-significant differentially expressed genes. The red squares mark the genes associated with EET and metal reduction.

Table 1

The function analysis of EET-related gene (marked in Fig. 10).

Plot	$\log_2$ fold change	gene function
1	1.78	Metal ion binding
2	1.48	Nanowires
3	1.41	Cytochrome-c
4	1.16	Metal ion binding (oxidation/reduction)
5	1.07	Metal ion binding
6	1.45	Cytochrome-c
7	1.33	Flavin binding
8	1.23	Metal ion binding
9	-1.03	Flavin binding

functions, including metal ion binding, nanowires, cytochrome-c, and flavin binding, which are all associated with the ability of *S. algae* to accommodate an EET process. The overexpressed EET-related genes may lead to more severe damage to the passive film due to the existence of *S. algae* [80,81]. When the passive film damage by *S. algae* occurred, more intact surface area could serve as cathodic sites and some weakened spots on Ti served as anodic sites where *S. algae* take in electrons for nitrate respiration. Overexpressed EET genes facilitated *S. algae* to enhance electron uptake from metals. Extensive damage to the passive film led to exposure of the underlying Ti substrate, enhancing acquisition of electrons by *S. algae* directly from the substrate by EET, which leads to severe corrosion of the Ti substrate.

3.6. Mechanistic diagrams

To compile the findings towards better understanding of the effect of DO and *S. algae* on pure Ti, a mechanistic diagram is postulated in Fig. 11. The mechanisms presented include the following:

- The oxygen vacancies ($V_{\text{O}}^{\bullet\bullet}$) and cation vacancy ($V_{\text{Ti}}^{4\bullet}$) are the major point defect types in passive film of Ti. The chloride ions in seawater provide more vacancy pairs in the passive film. Vacancy condensation is formed by the accumulation of point defects between the passive film and the substrate. However, pure Ti

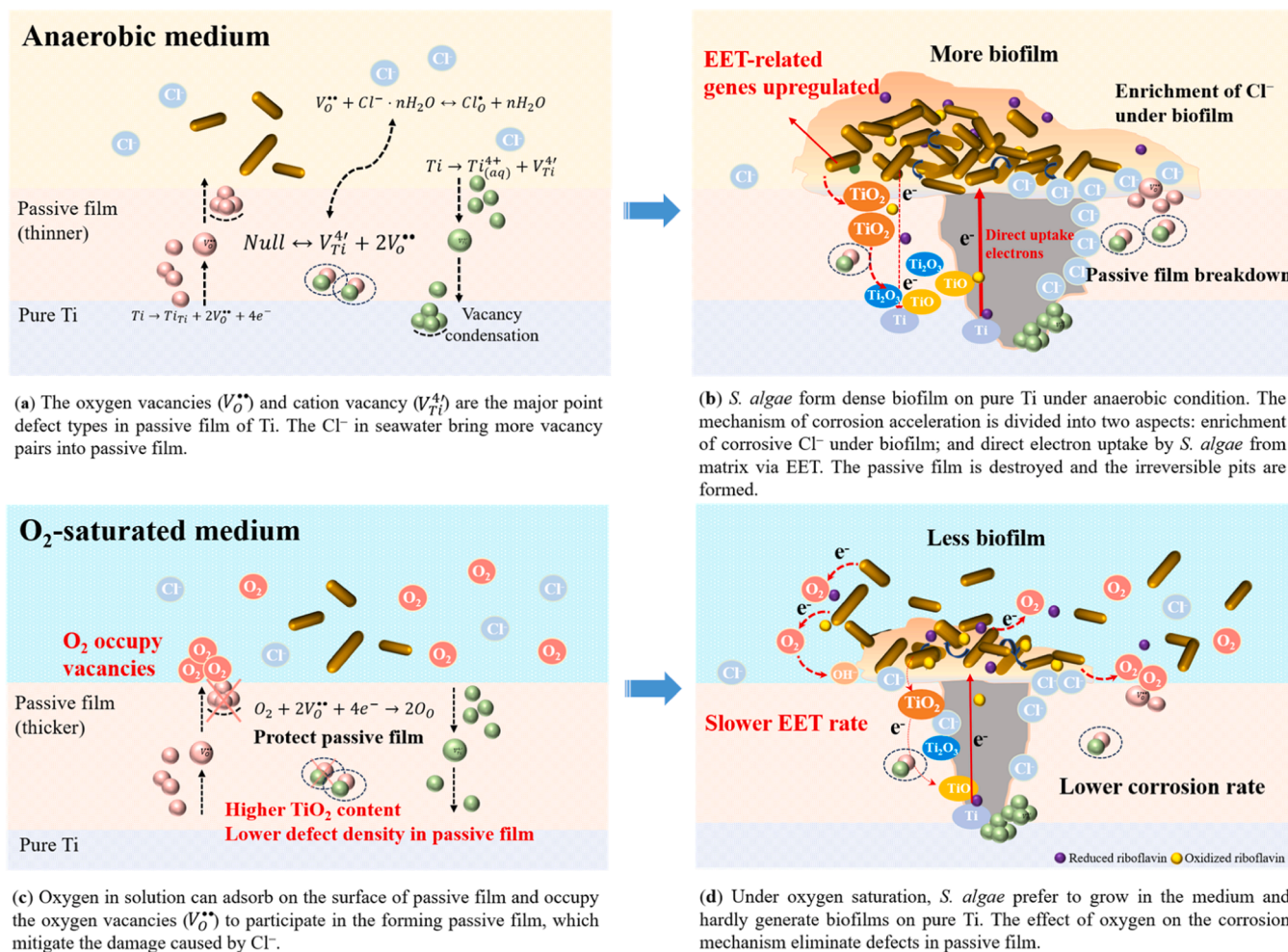


Fig. 11. Mechanistic diagrams showing the effect of DO and *S. algae* on corrosion of commercially pure Ti.

exhibited good overall corrosion resistance due to the protective power of the passive film.

- (b) *S. algae* formed a dense biofilm on pure Ti under anaerobic condition. The mechanism of corrosion acceleration is divided into two aspects. On one hand, *S. algae* altered the corrosive environment: *S. algae* biofilm caused enrichment of corrosive chloride ions, and the synergistic effect of *S. algae* on chloride ions accelerated corrosion. On the other hand, various EET-related genes were over-expressed under anaerobic conditions. Thus, the electron transfer efficiency between *S. algae* and pure Ti was increased. The TiO_2 content in the passive film was lowest under anaerobic condition (Fig. 8g, h), and the damage of passive film by *S. algae* induced a higher defect density (Fig. 9g, h) and more severe corrosion (Figs. 4–6).
- (c) Oxygen in solution can adsorb on the surface of passive film and occupy oxygen vacancies (V_o^{**}) to participate in the passive film formation, which mitigate the damage caused by chloride ions. Therefore, the passive film showed higher TiO_2 content (Fig. 8) and lower defect density in the presence of oxygen (Fig. 9).
- (d) Under oxygen saturation, *S. algae* prefer to grow in the medium (Fig. 3b) and hardly generate biofilms on pure Ti (Fig. 1a, b). Oxygen acted as preferential electron acceptor in the aerobic environment and was reduced to OH^- , so the pH value of aerobic environment was higher (Fig. 3a). The damage of the passive film was reduced, resulting in the lower corrosion rate.

4. Conclusions

In conclusion, as the depth increases in a marine environment, the DOC reduces and the environment changes from an O_2 -saturated state to an anaerobic state and the following effects can be deduced from the current study:

- S. algae* accelerated the corrosion rate and increased the pitting susceptibility of pure Ti in both oxygen saturated and anaerobic environments. The Gumbel distribution of pit depths indicated that the *S. algae* biofilm induced a new corrosion mechanism. Accelerated corrosion was caused by the degradation of the passive film, which was indicated by the reduced thickness and TiO_2 content, as well as the increased defect density.
- High DOC favored the passivation behavior of Ti, resulting in a lower corrosion rate and pitting susceptibility. Oxygen promoted the re-passivation process to enhance the thickness of passive film with a higher proportion of TiO_2 and lower defect density. However, the destructive effect of *S. algae* was stronger than the protective effect of high DOC.
- The Point Defect Model was used to analyze the performance of the passive film. The passive film of Ti is n-type semiconductor. A high DOC was favorable to the occupancy of oxygen vacancies, while *S. algae* increased the production of Schottky defective pairs by synergizing with chloride ions.

- (4) The EET-related genes of *S. algae* were overexpressed under anaerobic conditions. The absence of oxygen increased the EET-MIC rate of *S. algae* on pure Ti.

CRediT authorship contribution statement

Arjan Mol: Writing – review & editing. **Fuhui Wang:** Writing – review & editing. **Zhong Li:** Writing – review & editing. **Mingxing Zhang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Yuting Jin:** Visualization, Investigation, Formal analysis, Data curation. **Jiahang Li:** Visualization, Formal analysis, Data curation. **Dake Xu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Jiaqi Li:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.corsci.2024.112400](https://doi.org/10.1016/j.corsci.2024.112400).

References

- [1] K. Rohith, S. Shreyas, K.B. Vishnu Appaiah, R.V. Sheshank, B.B. Ganesha, B. Vinod, Recent material advancement for marine application, *Mater. Today Proc.* 18 (2019) 4854–4859, <https://doi.org/10.1016/j.matpr.2019.07.476>.
- [2] S. Chen, D. Zhang, Corrosion behavior of Q235 carbon steel in air-saturated seawater containing *Thalassospira* sp., *Corros. Sci.* 148 (2019) 71–82, <https://doi.org/10.1016/j.corsci.2018.11.031>.
- [3] E.R. Melchers, Effect of nutrient-based water pollution on the corrosion of mild steel in marine immersion conditions, *Corrosion* 61 (2005) 237–245, <https://doi.org/10.5006/1.3280633>.
- [4] D.J. Blackwood, C.S. Lim, S.L.M. Teo, X. Hu, J. Pang, Macrofouling induced localized corrosion of stainless steel in singapore seawater, *Corros. Sci.* 129 (2017) 152–160, <https://doi.org/10.1016/j.corsci.2017.10.008>.
- [5] D. Xu, Y. Li, F. Song, T. Gu, Laboratory investigation of microbiologically influenced corrosion of C1018 carbon steel by nitrate reducing bacterium *Bacillus licheniformis*, *Corros. Sci.* 77 (2013) 385–390, <https://doi.org/10.1016/j.corsci.2013.07.044>.
- [6] S.J. Hashemi, N. Bak, F. Khan, K. Hawboldt, L. Lefsrud, J. Wolodko, Bibliometric analysis of microbiologically influenced corrosion (MIC) of oil and gas engineering systems, *Corrosion* 74 (2017) 468–486, <https://doi.org/10.5006/2620>.
- [7] M. Stipanicev, F. Turcu, L. Esnault, E.W. Schweitzer, R. Kilian, R. Basseguy, Corrosion behavior of carbon steel in presence of sulfate-reducing bacteria in seawater environment, *Electro Acta* 113 (2013) 390–406, <https://doi.org/10.1016/j.electacta.2013.09.059>.
- [8] R. Javaherdashti, Microbiologically Influenced Corrosion – An Engineering Insight, *Brit. Corros. J.* 29 (2008) 181–182, <https://doi.org/10.1179/000705994798267674>.
- [9] R. Jia, T. Unsal, D. Xu, Y. Leckbach, T. Gu, Microbiologically influenced corrosion and current mitigation strategies: a state of the art review, *INT BIODETER BIODEGR* 137 (2018) 42–58, <https://doi.org/10.1016/j.ibiod.2018.11.007>.
- [10] J.R. Myers, H.B. Bomberger, F.H. Froes, Corrosion behavior and use of titanium and its alloys, *JOM* 36 (1984) 50–60, <https://doi.org/10.1007/BF03338589>.
- [11] S. Yan, G.-L. Song, Z. Li, H. Wang, D. Zheng, F. Cao, M. Horynova, M.S. Dargusch, L. Zhou, A state-of-the-art review on passivation and biofouling of Ti and its alloys in marine environments, *J. Mater. Sci. Technol.* 34 (2018) 421–435, <https://doi.org/10.1016/j.jmst.2017.11.021>.
- [12] R.C. Costa, V.L. Abdo, P.H.C. Mendes, I. Mota-Veloso, M. Bertolini, M.T. Mathew, V.A.R. Barão, J.G.S. Souza, Microbial corrosion in titanium-based dental implants: how tiny bacteria can create a big problem? *J. Bio Tribo Corros.* 7 (2021) 136, <https://doi.org/10.1007/s40735-021-00575-8>.
- [13] H.M. Shalaby, H. Al-Mazeedi, H. Gopal, N. Tanoli, Failure of titanium condenser tube, *Eng. Fail. Anal.* 18 (2011) 1990–1997, <https://doi.org/10.1016/j.engfailanal.2011.05.008>.
- [14] J.C.M. Souza, P. Ponthiaux, M. Henriques, R. Oliveira, W. Teughels, J.-P. Celis, L. A. Rocha, Corrosion behaviour of titanium in the presence of *Streptococcus mutans*, *J. Dent.* 41 (2013) 528–534, <https://doi.org/10.1016/j.jdent.2013.03.008>.
- [15] T.S. Rao, A.J. Kora, B. Anupkumar, S.V. Narasimhan, R. Feser, Pitting corrosion of titanium by a freshwater strain of sulphate reducing bacteria (*Desulfovibrio vulgaris*), *Corros. Sci.* 47 (2005) 1071–1084, <https://doi.org/10.1016/j.corsci.2004.07.025>.
- [16] R. Harada, E. Kokubu, H. Kinoshita, M. Yoshinari, K. Ishihara, E. Kawada, S. Takemoto, Corrosion behavior of titanium in response to sulfides produced by *Porphyromonas gingivalis*, S0109564117301574, *Dent. Mater.* (2017), <https://doi.org/10.1016/j.dental.2017.10.004>.
- [17] Z. Li, J. Wang, Y. Dong, D. Xu, X. Zhang, J. Wu, T. Gu, F. Wang, Synergistic effect of chloride ion and *Shewanella algae* accelerates the corrosion of Ti-6Al-4V alloy, *J. Mater. Sci. Technol.* 71 (2021) 177–185, <https://doi.org/10.1016/j.jmst.2020.07.022>.
- [18] T. Unsal, L. Xu, R. Jia, P. Kijla, S. Kumseranee, S. Punpruk, M.E. Mohamed, M. A. Saleh, T. Gu, Microbiologically influenced corrosion of titanium by *Desulfovibrio vulgaris* biofilm under organic carbon starvation, *Bioelectrochemistry* 149 (2023) 108307, <https://doi.org/10.1016/j.bioelechem.2022.108307>.
- [19] H.M. Holt, B. Gahrn-Hansen, B. Bruun, *Shewanella algae* and *Shewanella putrefaciens*: clinical and microbiological characteristics, *Clin. Microbiol. Infect.* 11 (2010) 347–352, <https://doi.org/10.1111/j.1469-0691.2005.01108.x>.
- [20] C.E. Turick, F. Caccavo, L.S. Tisa, Electron transfer from *Shewanella algae* BrY to hydrous ferric oxide is mediated by cell-associated melanin, *Fems. Microbiol. Lett.* (2010) 99–104, [https://doi.org/10.1016/S0378-1097\(03\)00096-X](https://doi.org/10.1016/S0378-1097(03)00096-X).
- [21] H. Guha, K. Jayachandran, F. Maurrasse, Kinetics of chromium (VI) reduction by a type strain *Shewanella alga* under different growth conditions, *Environ. Pollut.* 115 (2001) 209–218, [https://doi.org/10.1016/S0269-7491\(01\)00108-7](https://doi.org/10.1016/S0269-7491(01)00108-7).
- [22] Y. Fu, Y. Zhang, B. Li, D. Liang, S. Lu, Y. Xiang, B. Xie, H. Liu, K.H. Nealson, Extracellular electron transfer of *Shewanella oneidensis* MR-1 for cathodic hydrogen evolution reaction, *Electro Acta* 305 (2019) 528–533, <https://doi.org/10.1016/j.electacta.2019.03.085>.
- [23] F. Xue, X. Wei, J. Dong, I.-I.N. Etim, C. Wang, W. Ke, Effect of residual dissolved oxygen on the corrosion behavior of low carbon steel in 0.1M NaHCO₃ solution, *J. Mater. Sci. Technol.* 34 (2018) 1349–1358, <https://doi.org/10.1016/j.jmst.2017.11.004>.
- [24] L. Caceres, T. Vargas, M. Parra, Study of the variational patterns for corrosion kinetics of carbon steel as a function of dissolved oxygen and NaCl concentration, *Electro Acta* 54 (2009) 7435–7443, <https://doi.org/10.1016/j.electacta.2009.07.078>.
- [25] H. Zeng, Y. Yang, M. Zeng, M. Li, Effect of dissolved oxygen on electrochemical corrosion behavior of 2205 duplex stainless steel in hot concentrated seawater, *J. Mater. Sci. Technol.* (2020), <https://doi.org/10.1016/j.jmst.2020.06.030>.
- [26] Z. Cui, S. Chen, Y. Dou, S. Han, L. Wang, C. Man, X. Wang, S. Chen, Y.F. Cheng, X. Li, Passivation behavior and surface chemistry of 2507 super duplex stainless steel in artificial seawater: Influence of dissolved oxygen and pH, *Corros. Sci.* 150 (2019) 218–234, <https://doi.org/10.1016/j.corsci.2019.02.002>.
- [27] K.S. Raja, D.A. Jones, Effects of dissolved oxygen on passive behavior of stainless alloys, *Corros. Sci.* 48 (2006) 1623–1638, <https://doi.org/10.1016/j.corsci.2005.05.048>.
- [28] W.C. Baek, T. Kang, H.J. Sohn, Y.T. Kho, In situ surface enhanced Raman spectroscopic study on the effect of dissolved oxygen on the corrosion film on low carbon steel in 0.01 M NaCl solution, *Electro Acta* 46 (2001) 2321–2325, [https://doi.org/10.1016/S0013-4686\(01\)00442-X](https://doi.org/10.1016/S0013-4686(01)00442-X).
- [29] H. Zeng, Y. Yang, M. Zeng, M. Li, Effect of dissolved oxygen on electrochemical corrosion behavior of 2205 duplex stainless steel in hot concentrated seawater, *J. Mater. Sci. Technol.* 66 (2021) 177–185, <https://doi.org/10.1016/j.jmst.2020.06.030>.
- [30] J.H. Zheng, W.F. Bogaerts, K. Phlippe, Effects of dissolved oxygen and hydrogen peroxide on the corrosion potential of 316L stainless steel in hot lithium hydroxide solution, *Fusion. Eng. Des.* 24 (1994) 299–307, [https://doi.org/10.1016/0920-3796\(94\)90028-0](https://doi.org/10.1016/0920-3796(94)90028-0).
- [31] X. Yue, Effect of Traces of Dissolved Oxygen on the Passivation Stability of Super 13Cr Stainless Steel Under High CO₂/H₂S Conditions, *Int. J. Electrochem. Sci.* (2017) 7853–7868, <https://doi.org/10.20964/2017.08.33>.
- [32] X. Li, L. Wang, L. Fan, Z. Cui, M. Sun, Effect of temperature and dissolved oxygen on the passivation behavior of Ti-6Al-3Nb-2Zr-1Mo alloy in artificial seawater, *J. Mater. Res. Technol.* 17 (2022) 374–391, <https://doi.org/10.1016/j.jmrt.2022.01.018>.
- [33] M. Gholami, M. Hoseinpoor, M.H. Moayed, A statistical study on the effect of annealing temperature on pitting corrosion resistance of 2205 duplex stainless steel, *Corros. Sci.* 94 (2015), <https://doi.org/10.1016/j.corsci.2015.01.054>.
- [34] S.Y. Paredes-Dugarte, B. Hidalgo-Prada, Statistical analysis of the optical interferometry of pitting process in aluminum 3003 sheets exposed to saline environment, *Proce. Mater. Sci.* 8 (2015) 82–90, <https://doi.org/10.1016/j.mspro.2015.04.051>.
- [35] Y. Yang, T. Zhang, Y. Shao, G. Meng, F. Wang, New understanding of the effect of hydrostatic pressure on the corrosion of Ni-Cr-Mo-V high strength steel, *Corros. Sci.* 73 (2013) 250–261, <https://doi.org/10.1016/j.corsci.2013.04.013>.

- [36] G. Vázquez, I. González, Diffusivity of anion vacancies in WO₃ passive films, *Electro Acta* 52 (2007) 6771–6777, <https://doi.org/10.1016/j.electacta.2007.04.102>.
- [37] J. Amri, T. Souier, B. Malki, B. Baroux, Effect of the final annealing of cold rolled stainless steels sheets on the electronic properties and pit nucleation resistance of passive films, *Corros. Sci.* 50 (2008) 431–435, <https://doi.org/10.1016/j.corsci.2007.08.013>.
- [38] H. Luo, C.F. Dong, K. Xiao, X.G. Li, Characterization of passive film on 2205 duplex stainless steel in sodium thiosulphate solution, *Appl. Surf. Sci.* 258 (2011) 631–639, <https://doi.org/10.1016/j.apsusc.2011.06.077>.
- [39] Z. Cui, S. Chen, Y. Dou, S. Han, L. Wang, C. Man, X. Wang, S. Chen, Y.F. Cheng, X. Li, Passivation behavior and surface chemistry of 2507 super duplex stainless steel in artificial seawater: Influence of dissolved oxygen and pH, *Corros. Sci.* 150 (2019) 218–234, <https://doi.org/10.1016/j.corsci.2019.02.002>.
- [40] H.Y. Tang, D.E. Holmes, T. Ueki, P.A. Palacios, D.R. Lovley, Iron corrosion via direct metal-microbe electron transfer, *mBio* 10 (2019), <https://doi.org/10.1128/mBio.00303-19>.
- [41] T.S. Gunasekera, R.C. Striebig, S.S. Mueller, E.M. Strobel, O.N. Ruiz, Transcriptional profiling suggests that multiple metabolic adaptations are required for effective proliferation of *Pseudomonas aeruginosa* in jet fuel, *Environ. Sci. Technol.* 47 (2013) 13449–13458, <https://doi.org/10.1021/es403163k>.
- [42] P.M. Shrestha, A.-E. Rotaru, Z.M. Summers, M. Shrestha, F. Liu, D.R. Lovley, Transcriptomic and genetic analysis of direct interspecies electron transfer, *Appl. Environ. Microb.* 79 (2013) 2397, <https://doi.org/10.1128/AEM.03837-12>.
- [43] S. Sridhar, T.G. Wilson, Jr., K.L. Palmer, P. Valderrama, M.T. Mathew, S. Prasad, M. Jacobs, I.M. Gindri, D.C. Rodrigues, In vitro investigation of the effect of oral bacteria in the surface oxidation of dental implants, *Clin. Implant Dent. R.* 17 (2015) e562–e575, <https://doi.org/10.1111/cid.12285>.
- [44] K.M. Thormann, R.M. Saville, S. Shukla, Induction of Rapid Detachment in *Shewanella oneidensis* MR-1 Biofilms, *J. Bacteriol.* (2005), <https://doi.org/10.1128/JB.187.3.1014-1021.2005>.
- [45] J.S. McLean, P.D. Majors, C.L. Reardon, C.L. Bilskis, S.B. Reed, M.F. Romine, J. K. Fredrickson, Investigations of structure and metabolism within *Shewanella oneidensis* MR-1 biofilms, *J. Microbiol. Meth.* 74 (2008) 47–56, <https://doi.org/10.1016/j.mimet.2008.02.015>.
- [46] C. Wu, Y.-Y. Cheng, H. Yin, X.-N. Song, W.-W. Li, X.-X. Zhou, L.-P. Zhao, L.-J. Tian, J.-C. Han, H.-Q. Yu, Oxygen promotes biofilm formation of *Shewanella putrefaciens* CN32 through a diguanylate cyclase and an adhesin, *Sci. Rep.* 3 (2013) 1945, <https://doi.org/10.1038/srep01945>.
- [47] D. Liu, H. Yang, J. Li, J. Li, Y. Dong, C. Yang, Y. Jin, L. Yassir, Z. Li, D. Hernandez, D. Xu, F. Wang, J.A. Smith, Electron transfer mediator PCN secreted by aerobic marine *Pseudomonas aeruginosa* accelerates microbiologically influenced corrosion of TC4 titanium alloy, *J. Mater. Sci. Technol.* 79 (2021) 101–108, <https://doi.org/10.1016/j.jmst.2020.11.042>.
- [48] M. Saleem Khan, Z. Li, K. Yang, D. Xu, C. Yang, D. Liu, Y. Lekbach, E. Zhou, P. Kalnaowakul, Microbiologically influenced corrosion of titanium caused by aerobic marine bacterium *Pseudomonas aeruginosa*, *J. Mater. Sci. Technol.* 35 (2019) 216–222, <https://doi.org/10.1016/j.jmst.2018.08.001>.
- [49] D. Xu, Y. Li, F. Song, T. Gu, Laboratory investigation of microbiologically influenced corrosion of C1018 carbon steel by nitrate reducing bacterium *Bacillus licheniformis*, *Corros. Ence* 77 (2013) 385–390, <https://doi.org/10.1016/j.corsci.2013.07.044>.
- [50] Z. Cui, S. Chen, Y. Dou, S. Han, L. Wang, C. Man, X. Wang, S. Chen, Y.F. Cheng, X. Li, Passivation behavior and surface chemistry of 2507 super duplex stainless steel in artificial seawater: influence of dissolved oxygen and pH, *Corros. Sci.* 150 (2019) 218–234, <https://doi.org/10.1016/j.corsci.2019.02.002>.
- [51] K. Azumi, M. Seo, Changes in electrochemical properties of the anodic oxide film formed on titanium during potential sweep, *Corros. Sci.* 43 (2001) 533–546, [https://doi.org/10.1016/S0010-938X\(00\)00105-0](https://doi.org/10.1016/S0010-938X(00)00105-0).
- [52] N. Agarwal, K.A. Gallagher, S. Keaveny, J.G. Carton, D. Brabazon, M.A. Obeidi, Influence of processing parameters on the corrosion resistance of additively manufactured nitinol parts for biomedical applications, *Result Mater.* 21 (2024) 100536, <https://doi.org/10.1016/j.rinma.2024.100536>.
- [53] C. Su, W. Wu, Z. Li, Y. Guo, Prediction of film performance by electrochemical impedance spectroscopy, *Corros. Sci.* (2015), <https://doi.org/10.1016/j.corsci.2015.05.029>.
- [54] Y. Bai, C. Zhao, J. Yang, J.Y.H. Fuh, W.F. Lu, C. Weng, H. Wang, Dry mechanical-electrochemical polishing of selective laser melted 316L stainless steel, *Mate. Des.* 193 (2020) 108840, <https://doi.org/10.1016/j.matdes.2020.108840>.
- [55] H. Qian, D. Xu, C. Du, D. Zhang, X. Li, L. Huang, L. Deng, Y. Tu, J.M.C. Mol, H. A. Terry, Dual-action smart coatings with a self-healing superhydrophobic surface and anti-corrosion properties, *J. Mater. Chem. A* 5 (2017) 2355–2364, <https://doi.org/10.1039/C6TA10903A>.
- [56] J. Pouilleau, D. Devilliers, F. Garrido, S. Durand-Vidal, E. Mahé, Structure and composition of passive titanium oxide films, *Mat. Sci. Eng. B* 47 (1997) 235–243, [https://doi.org/10.1016/S0921-5107\(97\)00043-3](https://doi.org/10.1016/S0921-5107(97)00043-3).
- [57] X. Yang, C. Du, H. Wan, Z. Liu, X. Li, Influence of sulfides on the passivation behavior of titanium alloy TA2 in simulated seawater environments, *Appl. Surf. Sci.* 458 (2018) 198–209, <https://doi.org/10.1016/j.apsusc.2018.07.068>.
- [58] B. Munirathnam, R. Narayanan, L. Neelakantan, Electrochemical and semiconducting properties of thin passive film formed on titanium in chloride medium at various pH conditions, *Thin Solid Films* 598 (2016) 260–270, <https://doi.org/10.1016/j.tsf.2015.12.025>.
- [59] C.H. Hsu, F. Mansfeld, Technical note: concerning the conversion of the constant phase element parameter Y_0 into a Capacitance, *Corros* 57 (2001) 747–748, <https://doi.org/10.5006/1.3280607>.
- [60] B. Hirschhorn, M.E. Orazem, B. Tribollet, V. Vivier, I. Frateur, M. Musiani, Determination of effective capacitance and film thickness from constant-phase-element parameters, *Electrochim. Acta* 55 (2010) 6218–6227, <https://doi.org/10.1016/j.electacta.2009.10.065>.
- [61] Y.W. Cui, L.Y. Chen, P. Qin, R. Li, Q. Zang, J. Peng, L. Zhang, S. Lu, L. Wang, L. C. Zhang, Metastable pitting corrosion behavior of laser powder bed fusion produced Ti-6Al-4V in Hank's solution, *Corros. Sci.* 203 (2022) 110333, <https://doi.org/10.1016/j.corsci.2022.110333>.
- [62] G.J. Brug, A.L.G. van den Eeden, M. Sluyters-Rehbach, J.H. Sluyters, The analysis of electrode impedances complicated by the presence of a constant phase element, *J. Electroanal. Chem.* 176 (1984) 275–285, [https://doi.org/10.1016/S0022-0728\(84\)80324-1](https://doi.org/10.1016/S0022-0728(84)80324-1).
- [63] J.J. Park, S.I. Pyun, Stochastic approach to the pit growth kinetics of Inconel alloy 600 in Cl⁻ ion-containing thiosulphate solution at temperatures 25–150 degrees C by analysis of the potentiostatic current transients, *Corros. Sci.* 46 (2004) 285–296, [https://doi.org/10.1016/S0010-938X\(03\)00158-6](https://doi.org/10.1016/S0010-938X(03)00158-6).
- [64] X. Yang, C. Du, H. Wan, Z. Liu, X. Li, Influence of sulfides on the passivation behavior of titanium alloy TA2 in simulated seawater environments, *Appl. Surf. Sci.* 458 (2018) 198–209, <https://doi.org/10.1016/j.apsusc.2018.07.068>.
- [65] Y. Bai, X. Gai, S. Li, L.-C. Zhang, Y. Liu, Y. Hao, X. Zhang, R. Yang, Y. Gao, Improved corrosion behaviour of electron beam melted Ti-6Al-4V alloy in phosphate buffered saline, *Corros. Sci.* 123 (2017) 289–296, <https://doi.org/10.1016/j.corsci.2017.05.003>.
- [66] Z.B. Wang, H.X. Hu, Y.G. Zheng, Determination and explanation of the pH-related critical fluoride concentration of pure titanium in acidic solutions using electrochemical methods, *Electro Acta* 170 (2015) 300–310, <https://doi.org/10.1016/j.electacta.2015.04.165>.
- [67] L. Zhang, Y. Duan, R. Gao, J. Yang, K. Wei, D. Tang, T. Fu, The effect of potential on surface characteristic and corrosion resistance of anodic oxide film formed on commercial pure titanium at the potentiodynamic-aging mode, *Materials* 12 (2019) 370, <https://doi.org/10.3390/ma12030370>.
- [68] L. Avril, S. Reymond-Laruinaz, J.M. Decams, S. Bruyère, V. Potin, M.C.M. de Lucas, L. Imhoff, TiO₂ anatase films obtained by direct liquid injection atomic layer deposition at low temperature, *Appl. Surf. Sci.* 288 (2014) 201–207, <https://doi.org/10.1016/j.apsusc.2013.10.007>.
- [69] L. Zha, H. Li, N. Wang, In situ electrochemical study of the growth kinetics of passive film on TC11 alloy in sulfate solution at 300 °C/10 MPa, *Materials* 13 (2020) 1135, <https://doi.org/10.3390/ma13051135>.
- [70] L. Wang, H. Yu, S. Wang, B. Chen, Y. Wang, W. Fan, D. Sun, Quantitative analysis of local fine structure on diffusion of point defects in passive film on Ti, *Electro Acta* 314 (2019) 161–172, <https://doi.org/10.1016/j.electacta.2019.05.048>.
- [71] K. Azumi, M. Nakajima, K. Okamoto, M. Seo, Dissolution of Ti wires in sulphuric acid and hydrochloric acid solutions, *Corros. Sci.* 49 (2007) 469–480, <https://doi.org/10.1016/j.corsci.2006.04.017>.
- [72] L. Wang, H. Yu, S. Wang, B. Chen, D. Sun, Quantitative analysis of local fine structure on diffusion of point defects in passive film on Ti, *Electro Acta* 314 (2019), <https://doi.org/10.1016/j.electacta.2019.05.048>.
- [73] X. Du, W. Shi, S. Xiang, Effect of low dissolved oxygen concentration on the defects and composition of regenerated passive film of Ti-6Al-4V alloy under continuous wear, *RSC Adv.* 13 (2023) 20135–20149, <https://doi.org/10.1039/d3ra03865c>.
- [74] D.D. Macdonald, The history of the point defect model for the passive state: a brief review of film growth aspects, *Electro Acta* 56 (2011) 1761–1772, <https://doi.org/10.1016/j.electacta.2010.11.005>.
- [75] J. Yao, C. Dong, Passive film on 2205 duplex stainless steel studied by photo-electrochemistry and ARXPS methods, *Corros. Sci.* (2019), <https://doi.org/10.1016/j.corsci.2018.10.020>.
- [76] D.I. Seo, J.B. Lee, Effects of competitive anion adsorption (Br or Cl) and semiconducting properties of the passive films on the corrosion behavior of the additively manufactured Ti–6Al–4V alloys, *Corros. Sci.* 173 (2020), <https://doi.org/10.1016/j.corsci.2020.108789>.
- [77] Y.A. Gorby, S. Yanina, J.S. McLean, K.M. Rosso, D. Moyle, A. Dohnalkova, T. J. Beveridge, I.S. Chang, B.H. Kim, K.S. Kim, D.E. Culley, S.B. Reed, M.F. Romine, D.A. Saffarini, E.A. Hill, L. Shi, D.A. Elias, D.W. Kennedy, G. Pinchuk, K. Watanabe, S. Ishii, B. Logan, K.H. Nealson, J.K. Fredrickson, Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms, *P. Natl. Atl. A. Sci.* 103 (2006) 11358–11363, <https://doi.org/10.1073/pnas.0604517103>.
- [78] E. Marsili, D.B. Baron, I.D. Shikhare, D. Coursolle, J.A. Gralnick, D.R. Bond, *Shewanella* secretes flavins that mediate extracellular electron transfer, *P. Natl. Acad. Sci. USA* 105 (2008), <https://doi.org/10.1073/pnas.0710525105>.
- [79] M. Breuer, K.M. Rosso, J. Blumberger, J.N. Butt, Multi-haem cytochromes in *Shewanella oneidensis* MR-1: structures, functions and opportunities, *J. R. Soc. Interface* 12 (2015) 20141117, <https://doi.org/10.1098/rsif.2014.1117>.
- [80] D. Coursolle, D.B. Baron, D.R. Bond, J.A. Gralnick, The Mtr Respiratory Pathway Is Essential for Reducing Flavins and Electrodes in *Shewanella oneidensis*, *J. Bacteriol.* 192 (2010) 467–474, <https://doi.org/10.1128/JB.00925-09>.
- [81] Z. Li, W. Chang, T. Cui, D. Xu, D. Zhang, Y. Lou, H. Qian, H. Song, A. Mol, F. Cao, T. Gu, X. Li, Adaptive bidirectional extracellular electron transfer during accelerated microbiologically influenced corrosion of stainless steel, *Commun. Mater.* 2 (2021) 1–9, <https://doi.org/10.1038/s43246-021-00173-8>.