Mitigation of microbial corrosion by Cu addition in X65 pipeline steel in presence of *Pseudomonas aeruginosa*

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Abstract

In this study, we focused on the corrosion behavior of X65 pipeline steel and X65Cu pipeline steel in sterile and *P. aeruginosa*-containing media. The resistance of Cu-bearing X65 pipeline steel against (microbiologically influenced corrosion) MIC caused by *P. aeruginosa* was investigated. Results demonstrate that the corrosion rate of X65Cu pipeline steel was lower than that of X65 pipeline steel whether it was in a sterile or bacteria-containing media. It is noticeable that, in the presence of *P. aeruginosa*, X65Cu pipeline steel can prevent the formation of biofilm to a certain degree and reduce the corrosion rate of X65Cu pipeline steel to less than half as that of X65 pipeline steel. In addition, the maximum pitting depth and average pitting depth of X65Cu pipeline steel were also much lower than that of X65 pipeline steel. The addition of Cu effectively reduced the corrosion of *P. aeruginosa* to X65 grade pipeline steel and improved the resistance to MIC pitting corrosion.

1 Introduction

Microbiologically influenced corrosion (MIC) is a type of destructive corrosion caused by the presence and activity of bacteria [1, 2]. It is recognized that MIC occurs widely in most industrial environments including marine, soil, etc, and 75% of the corrosion and semi-failure of buried oil well pipelines are related to it [3]. MIC can induce the corrosion perforation of buried pipelines and blockage of injection wells, resulting in potential safety risks during oil production and transportation [4].

Based on the up-to-date MIC research progress, it is well accepted that biofilm, a slimy layer formed on the metal substrate to prolong bacteria life and obtain nutrients for growth, is the main cause of MIC [5]. Namely, once the biofilm is inhibited, MIC could be mitigated. Therefore, different physical, chemical, and antibacterial materials have been developed to mitigate the MIC, such as pigging, biocides, biocide enhancers, antibacterial coatings, and antibacterial materials [6, 7]. New Cu-bearing pipeline steel is a new way of controlling the MIC for buried pipelines, especially for the external MIC of pipelines that other corrosion measures cannot be applied to [8, 9].

It was reported that X80 steel alloyed by 1 wt.% Cu with nano-sized Cu-rich precipitates distributed in the matrix expressed good MIC resistance against sulphate reducing bacteria (SRB) [10]. Both the results of the laboratory test and shale gas field test indicated that Cu-bearing carbon steel possessed obvious resistance to MIC [11]. The speculative antibacterial mechanism is due to the release of copper ions that are highly toxic to bacteria [12–15].

P. Aeruginosa, a gram-negative facultative aerobe, which is present in nearly all known environments, and it was frequently found in the oil pipeline involving MIC [16]. *P. aeruginosa* is also a typical bacterial species that can form biofilms. It adheres to the surface of the material, consumes oxygen for metabolic activities, and generates a battery with a difference in oxygen concentration at the bottom of the biofilm, which causes corrosion to the metal [17]. Research shows that it can accelerate the corrosion of carbon steel, stainless steel, and other metals [18–20]. The formation of biofilm and metabolites will cause the

oxidation and dissolution of alloying elements, destroy the passivation film, and accelerate the occurrence of pitting corrosion [21, 22]. A typical example is that *P. aeruginosa* can corrode carbon steel by means of extracellular electron transfer., which accelerated the metal to lose electrons and be oxidized [23]. However, the related research on the microbial corrosion resistance of Cu-bearing pipeline steel mainly focuses on the anaerobic SRB [10, 24], there are few reports on the corrosion resistance of the aerobic bacteria *Pseudomonas aeruginosa* (*P. aeruginosa*) [25–28].

In this study, MIC resistance against *P. aeruginosa* of Cu-bearing X65 pipeline steel was investigated. Scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), weight loss, and electrochemical measurements were used to characterize the surface variations and corrosion performance. This study aims to explore whether the Cu-bearing pipeline steel can resist the MIC against *P. aeruginosa*. The results will provide a new understanding of the MIC behavior of Cu-bearing pipeline steel and broaden its MIC resistance spectrum.

2 Experimental Materials And Method

2.1 Materials

Cu-bearing X65 pipeline steel and Cu-free steel were studied and named as X65Cu and X65 for short. The chemical compositions of the steels are listed in Table 1. Firstly, the experimental steels were melted in a 200 kg vacuum induction melting furnace, then the steel ingots were forged into blocks of 90 mm × 90 mm × 400 mm and austenitized at 1150 °C for 2 h. Finally, these blocks were hot rolled into 12 mm thick from the temperature of 1120 to 830 °C, then a fast cooling was applied to simulate the actual production process. To obtain the best antibacterial activity, the as-rolled plate was aged at 600 °C for 2 h [29]. The microstructures of steels were observed on a Zeiss LSM700 metallographic light microscope (LM). The ultrastructure was observed on an FEI Tecnai20 transmission electron microscope (TEM) at 200 kV.

Table 1
Chemical compositions of the experimental steels (wt%).

Steel	C	Si	Mn	P	S	Ni	Cu	Al	Nb	Ti
X65	0.028	0.18	0.59	0.008	0.0020	1.05	<0.01	0.026	0.018	0.017
X65Cu	0.029	0.19	0.59	0.009	0.0021	1.05	1.35	0.024	0.019	0.017

Coupons with a size of 10 mm×10 mm×5 mm were used for electrochemical tests. They were connected with Cu wire and embedded in an epoxy resin with exposing a surface area of 1 cm². The size of coupons used for immersion tests is 20 mm×20 mm×5 mm. All coupons were abraded with abrasive papers 180, 400, 800, and 1200 grit in sequence, and then cleaned with absolute ethanol and dried for use.

2.2 Bacteria and medium

P. aeruginosa MCCC 1A00099 was obtained from the Marine Culture Collection of China (MCCC). The bacteria were grown aerobically in 2216E medium based on artificial seawater, the composition of the medium was as follows: 19.45 g/L NaCl, 5.98 g/L MgCl₂, 3.24 g/L Na₂SO₄, 1.8 g/L CaCl₂, 0.55 g/L KCl, 0.16 g/L Na₂CO₃, 0.08 g/L KBr, 0.034 g/L SrCl₂, 0.08 g/L SrBr₂, 0.022 g/L H₃BO₃, 0.004 g/L NaSiO₃, 0.0024 g/L NaF, 0.0016 g/L NH₄NO₃, 0.008 g/L NaH₂PO₄, 5.0 g/L peptone, 1.0 g/L yeast extract, and 0.1 g/L ferric citrate. The medium was sterilized in an autoclave at 121°C for 30 min. The initial cell concentration was approximately 10⁶ cells/mL as determined by a hemocytometer under an optical microscope at 400 × magnification.

2.3 Electrochemical measurements

Electrochemical tests were carried out at an electrochemical workstation (Reference 600, Gamry, USA) using a three-electrode system, the reference electrode was a saturated calomel electrode (SCE), the counter electrode was a platinum sheet, and the working electrode were the steel coupons. The X65 and X65Cu steels were immersed in a sterile and *P. aeruginosa*-containing 2216E media sealed jar for 14 days. Open circuit potential (E_{OCP}) and linear polarization resistance (LPR) were measured on days 2, 4, 7, 9, 11, and 14, and the potentiodynamic polarization curves were measured on the 14th day. The LPR measurements were recorded at a scan rate of 0.125 mV/s in the range from -5 to 5 mV vs E_{OCP} , with a sampling period of 1 s. The potentiodynamic polarization scan rate was 0.5 mV/s and the potential range was -1200 to 1000 mV (vs. OCP).

2.4 Weight loss test and surface characterization

Before the immersion tests, the coupons were numbered and then weighed on an analytical balancer. Four parallel coupons were prepared for each group of experiments, in order to ensure the accuracy of the experimental results and exclude repeatability. The gap between the coupons in the jar was greater than 1 cm. The coupons were taken out after 14 days for dehydration, the specific steps are:

(1) Immersed the samples in 2.5% glutaraldehyde solution for 3 h to fix the formed biofilm on the coupon surfaces, and then dehydrated step-wise with 50, 70, 90, 100 vol% ethanol solutions. In order to enhance the conductivity of coupon surfaces, a gold film was sprayed on the surface of the coupons and then the biofilms were observed by SEM.

(2) To remove the rust layers, the coupons were immersed in a descaling solution in an ultrasonic environment for 5 minutes, then, washed with deionized water and absolute ethanol and their weights were recorded.

(3) At the same time, the corrosion morphology of the surfaces after removing the biofilms were observed by SEM.

(4) Subsequently, The diameter, depth and number of corrosion pits on the coupon surfaces were measured by CLSM.

3 Results

3.1 Microstructure

Figure 1 shows the microstructures of two experimental steels. Bolt X65 and X65Cu steels show the same polygonal ferrite microstructure with the grain size varying in the range of 10–20 μm . TEM images and the corresponding EDS mapping results (Figure 1(c, d)) show the presence of massive nano-sized Cu-rich precipitates in X65Cu steel.

3.2 Open circuit potential

Figure 2 shows the E_{OCP} values of X65 and X65Cu steels in sterile and *P. aeruginosa*-containing media as a function of immersion time. In sterile medium, the E_{OCP} of X65 and X65Cu steels stabilized around –600 mV to –700 mV during immersion. Whereas in the bacteria-containing medium, the E_{OCP} values of both steels decreased sharply in the first 4 days. The E_{OCP} of X65 steel decreased from –402 mV to –680 mV, and the E_{OCP} of X65Cu steel decreased from –432 mV to –654 mV. The negative shift of E_{OCP} may be due to the formation of biofilms [30]. After being immersed for 4 days, the corrosion system and the biofilm on the coupon surfaces were in a relatively stable state, the E_{OCP} of the two steels became stable and a gap of about 40 mV appeared.

It is generally known that the formation of biofilm shifts E_{corr} to a negative direction due to the electronegativity of bacterial cells and the accumulation of FeS products.

3.3 Linear polarization resistance

Figure 3 shows the variations of linear polarization resistance (R_p) with the immersion time for two steels in the sterile and *P. aeruginosa*-containing media. According to Stern–Geary formula [31–33] (Eq. 1):

$$i_{\text{corr}} = \frac{\beta_a \times \beta_c}{2.303 R_p \times (\beta_c - \beta_a)} \quad (1)$$

where i_{corr} is the corrosion current density, R_p is the polarization resistance, β_a and β_c are the Tafel slopes of anode and cathode. It can be seen that i_{corr} is inversely proportional to R_p . For the sterile media, as shown in Fig. 3(a), the R_p variations show a similar trend for two steels, whose curves increase first and then decrease, that is to say, the corrosion rate decreases first and then increases, and the corrosion rate is lowest on the 7th day. For the bacteria-containing medium, the R_p of X65 and X65-Cu steels decreased rapidly first and finally stabilized gradually. Moreover, the R_p of X65-Cu steel was always higher than that of X65 steel in aseptic or bacterial culture. This result indicates a better corrosion resistance for X65-Cu steel.

3.4 Potential dynamic polarization

Figure 4 shows the potentiodynamic polarization curves of the two steels after 14 days of immersion in the sterile and *P. aeruginosa*-containing medium. For a sterile environment, it is seen that the shapes of

the two curves are identical, indicating that the mechanisms of the anode process and cathode process of two steels do not change essentially. In the presence of bacteria, the anode reaction process of the two steels is significantly different. The Tafel extrapolation method was used to estimate the corrosion current densities of X65 steel and X65Cu steel in a sterile environment (66.7 μA and 21.5 μA), and the corrosion current density in a bacteria-containing environment (10.2 μA and 7.57 μA , respectively).

Compared to X65 steels, X65Cu steels have a lower i_{corr} whether they are in the medium with and without *P. aeruginosa*. These are consistent with the previous research results, the addition of Cu element effectively improves the corrosion resistance of X65 grade pipeline steel.

3.5 Biofilm observation

Figure 5 shows SEM image of the rust layer and biofilms of X65 and X65Cu steels cultured in sterile (Figure 5(a, b)) and *P. aeruginosa* (Figure 5(c, d)) medium for 14 days. In the sterile medium, it can be seen that the substrate of X65 steel was exposed and a few corrosion products were attached. It has been washed away by ethanol solution during dehydration because of the loose rust layer. In addition to corrosion products on the surface of X65-Cu steel, a residual outer rust layer and a stable inner rust layer can also be seen in the microscopic picture.

For the bacteria-containing medium, A large numbers of clusters consisting of corrosion products and rod-shaped bacterial cells can be seen attached to the surfaces of the X65 steel. The biofilm on the X65Cu steels was discrete with a loose and uneven corrosion product layer. The biofilm layer on the surfaces of the X65 steel was more homogeneous and intact than that on the X65Cu steels surfaces, and more sessile bacteria adhered.

The corrosion products on the surface of the samples were analyzed using EDS, the results are shown in Fig. 6, and the chemical composition of the biofilm are listed in Table 2. O and Fe elements account for the majority, indicating that iron oxides are the main components of the corrosion products in the biofilm. The C content of X65Cu steel is lower than that of X65 steel in different media. The content of C, O and P on the surface of X65 steels in the bacterial environment was significantly more than that of X65Cu steel which is relevant to the reduction of adhesive biofilm. Meanwhile, a certain amount of Cu was detectable on the X65Cu steels surface.

Table 2
EDS results corresponding to Fig. 6 (wt. %).

Steel	C	O	P	Mg	S	Ca	Fe	Ni	Cu
X65 in sterile medium	11.96	11.27	–	–	–	–	73.34	2.40	–
X65Cu in sterile medium	8.63	18.34	18.34	–	0.58	1.28	59.30	59.30	8.14
X65 in <i>P. aeruginosa</i> medium	15.24	31.58	2.71	1.36	–	2.87	44.42	1.83	–
X65Cu in <i>P. aeruginosa</i> medium	7.28	11.36	2.48	–	0.33	0.95	69.23	1.90	6.47

3.6 Corrosion morphology observation

Figure 7 shows the corrosion morphology of the coupons after washing off the biofilm. The surface morphologies of two steels immersed in the sterile medium (Figure 7(a, b)) are uniformly corroded, and the grain boundaries of the steel can be clearly seen. X65-Cu pipeline steel is less corroded, and the appearance of matrix grain boundaries is not as clear as that of X65 pipeline steel, and the scratches left by surface pretreatment are more obvious (Figure 7(b)). The coupons immersed in the bacterial-containing medium express uniform corrosion and pitting at the same time (Figure 7(c, d)). Among them, there were more pits in X65 steels than in X65Cu steels, and these pits are deeper and larger. It is apparent that the corrosion of X65 steel by *Pseudomonas aeruginosa* is more serious than that of X65Cu steel. The corrosion morphology of the two steels matches well with the electrochemical results, it shows that X65Cu possessed a stronger pitting corrosion resistance of steels.

3.7 Weight loss analysis

The corrosion rate (V_c) was calculated according to the following formula (Eq. 2):

$$V_c = \frac{87600(W_0 - W_1)}{t \rho A} \quad (2)$$

where V_c (mm/year) is the corrosion rate, t (h) is the soaking time, ρ (g/cm³) is the density of X65 pipeline steel, A (cm²) is the exposed area of the coupons and W_0 and W_1 are the weight of the coupons before and after immersion, respectively. Fig. 8 shows the V_c calculated by weight loss. As can be seen that the V_c of X65 steels is greater than that of X65Cu steels significantly, whether they were in a sterile or bacteria-containing environment, and the V_c of X65 steels in the presence of *P. aeruginosa* is at least twice higher than that of X65Cu steels. The results indicates that the addition of Cu element effectively improves the corrosion resistance of X65 grade pipeline steel.

3.8 Pit morphology and depth

Figure 9 shows the CLSM images of the largest pit depths on the surfaces of X65 and X65Cu steels immersed in *P. aeruginosa*-containing media, and Table 3 summarizes the detailed data of pitting pits, the largest pit depth on the surfaces of the X65 steel is 46.05 μm and the average pit depth is $34.27 \pm 6.33 \mu\text{m}$. In comparison, the largest and average pit depths of X65Cu steels were considerably reduced to 25.05 μm and $20.19 \pm 2.94 \mu\text{m}$, which implies that localized corrosion caused by *P. aeruginosa* is mitigated, a successful enhancement of the pitting corrosion resistance is acquired due to the release of Cu ions.

Table 3
Pit depths of the surfaces of X65 and X65Cu steels inoculated with *P. aeruginosa* for 14 days.

Steel	Largest pit depth (μm)	Average pit depth (μm)
X65	46.05	34.27 ± 6.33
X65Cu	25.05	20.19 ± 2.94

4 Discussion

Figure 10 shows a simple corrosion mechanism diagram of X65 and X65Cu steel immersed in sterile and *P. aeruginosa*-containing medium.

The corrosion behavior of Cu-bearing pipeline steel X65Cu in sterile medium is similar to that of Cu-free X65 steel, corrosion of steels were caused by the co-occurrence of electrochemical reactions of anodic iron oxidation and cathodic dissolved oxygen reduction (Eq. 3, 4). $\text{Fe}(\text{OH})_2$ formed by the combination of Fe^{2+} and OH^- will oxidize to form unstable $\text{Fe}(\text{OH})_3$ and finally dehydrate into FeOOH (Eq 5, 6, 7), as shown in Fig. 10(a).

Keiser et al. [34] believed that the protective inner rust layer is mainly composed of $\alpha\text{-FeOOH}$. Yamashita et al. [35] suggested that in the long-term corrosion process, the amorphous FeOOH material will eventually transform into $\alpha\text{-FeOOH}$ which is a stable rust layer composition. On the one hand, the addition of Cu can help the transformation of $\alpha\text{-FeOOH}$, which is conducive to the formation of the rust layer. On the other hand, the dissolution of the matrix produces Cu segregation, which makes the rust layer denser and has better protection (Fig. 10(b)). Thus, the corrosion rate of X65Cu steels is always smaller than that

of X65 steels in a sterile medium, as shown by the values of linear polarization resistance and weight loss experiments.

It is well known that C content affects the intergranular corrosion and pitting behavior of X65 steels. The reduction of C content will reduce the susceptibility to intergranular corrosion and improve the corrosion resistance [36–38]. In this work, the C content of X65Cu steels was much lower than X65 steels. It is reasonable to speculate that X65Cu steels with comparatively lower C content may exhibit better corrosion resistance in the absence of microorganisms.

In *P. aeruginosa*-containing media, due to metabolism, aerobic bacteria form biofilms in the initial stage, which makes the environment have reducing properties, increase the electron transfer rate and promote the progress of the reaction (Eq. 3, 4) so as to accelerate the corrosion (Fig. 10(c)). However, bacteria decay with time, leading to the corrosion system and the biofilm on the coupon surfaces in a relatively stable state as shown in Fig. 2(b). The R_p of X65-Cu steel was always higher than that of X65 steel which indicates a better corrosion resistance of X65-Cu steel. Potentiodynamic polarization curves also suggest that the presence of Cu increases E_{corr} and decreases I_{corr} . It prevents the occurrence of corrosion by inhibiting the anodic dissolution reaction of the metal.

In comparison with X65 steels, the corrosion rate of X65Cu steel was much lower because of fewer bacteria attached to the surface and a lower biofilm coverage, and MIC was effectively inhibited. The presence of Cu may be a major factor in the disruption of *P. aeruginosa* biofilms. As shown in Fig. 10(d), when the corrosive *P. aeruginosa* biofilm forms on the X65Cu steels surfaces, The release of Cu ions effectively kills sessile bacteria and destroys the structure of the biofilm, which is consistent with its documented antibacterial properties. Xia [23] et al. reported that Cu ions released from Cu-rich phases play a dominant role in antimicrobial activity. Nan et al. [39] studied the antibacterial properties of Cu-containing stainless steel, and found it has superior antibacterial performance against most gram-negative bacteria and gram-positive bacteria, showing a strong bactericidal effect with a bactericidal rate of over 99%.

The main components of the biofilm, the content of C and O on the surface of X65 steels in the bacterial environment are higher than that of X65Cu steel significantly. The increase in P content is also attributed to the formation of biofilms on the coupon surface because the basic skeleton of the cell membrane is the phospholipid bilayer [40]. The record of Cu indicates that X65Cu steel releases Cu ions in the culture medium and acts on the biofilm. The above phenomena show that X65-Cu pipeline steel has superior bacteriostasis, the release of Cu ions causes a large number of deaths of *P. aeruginosa*, and it was unable to adhere, aggregate, and grow on the coupon surfaces to form a biofilm. Hence, the excellent resistance to microbial corrosion is achieved.

It is also noteworthy that the corrosion rate of X65Cu steels in bacteria-containing conditions is lower than that of sterile conditions (Fig. 8). This is because the Cu can increase the volume fraction of the austenite region, and the austenite phase is a weak phase where pitting corrosion occurs preferentially,

which leads to a decrease in the corrosion resistance of steels in a sterile solution. Nan et al. [39] believed that the decrease in pitting resistance potential of antibacterial stainless steel after the action of *E. coli* is due to the accelerated dissolution of Cu ions in the Cu-rich phase on its surface. Therefore, adding Cu element to improve the corrosion resistance of X65-Cu pipeline steel in *P. aeruginosa* solution is at the expense of corrosion resistance in sterile solution. But under normal circumstances, the main cause of pipeline steel failure is the occurrence of pitting corrosion, so pitting corrosion resistance is more important.

The results of corrosion morphology is consistent with the results of CLSM images, indicating that X65Cu has better MIC pitting resistance. A successful pitting corrosion mitigation was achieved on X65Cu steels surfaces, by inducing the death of bacteria that cause MIC pitting corrosion. The death of the bacteria decreases the probability of pitting occurrence, which had led to the maximum pitting depth and average pitting depth of X65Cu steels being much lower than that of X65 steels.

5 Conclusions

In this study, a Cu-bearing pipeline steel (X65Cu) was developed and compared to regular X65 grade pipeline steel in the aspects of MIC behavior in the media with and without *P. aeruginosa*. The following conclusions can be drawn:

- (1) The addition of Cu is conducive to the formation of the rust layer which has better protection. Thus, the corrosion rate of X65Cu steels was always smaller than that of X65 steels in a sterile medium.
- (2) In *P. aeruginosa*-containing media, the presence of Cu increased E_{corr} and decreased i_{corr} of X65 grade pipeline steel. It prevented the occurrence of corrosion by inhibiting the anodic dissolution reaction of the metal.
- (3) When the corrosive *P. aeruginosa* biofilm formed on the X65Cu steels surfaces, the release of Cu ions effectively destroyed the structure of the biofilm and killed the sessile bacteria, which is consistent with its documented antibacterial properties. A successful pitting corrosion mitigation was achieved on X65Cu steels surfaces, by inducing the death of bacteria that cause MIC pitting corrosion.
- (4) Cu element can increase the volume fraction of the austenite region, and the austenite phase is a weak phase where pitting corrosion occurs preferentially, which leads to a decrease in the corrosion resistance of steels in a sterile solution.

Declarations

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References

1. Little BJ, Lee JS (2014) *Int Mater Rev* 59:384–393
2. Qian H, Zhang D, Lou Y, Li Z, Xu D, Du C, Li X (2018) *Corros Sci* 145:151–161
3. Jiang B, Du C, Li X, Li XG (2008) *Petrochem Corros Prot* 25:1–4
4. Videla HA (2002) *Int Biodeterior Biodegradation* 49:259–270
5. Simões M, Simões LC, Vieira MJ (2010) *LWT* 43:573–583
6. Xu D, Jia R, Li Y (2017) *World J Microb Biot* 33:97
7. Popoola LT, Grema AS, Latinwo GK, Gutti B (2013) *Int J Ind Chem* 4:1–15
8. Yang K, Shan YY, Shi XB (2017) China. Patent, 201510419067.3,
9. Shan YY, Yang K, Shi XB (2017) China. Patent, 201510418577.9,
10. Shi XB, Yan W, Xu D, Yan M, Yang C (2018) *J Mater Sci Technol* 34:2480–2491
11. Yu H, Li Z, Xia Y, Qi Y, Li Y, Liu Q (2021) *Anti-Corros Method M* 68:302–309
12. Hong IT, Koo CH (2005) *Mater Sci Eng A* 393:213–222
13. Ren L, Nan L, Yang K (2013) *Mater Des* 32:2374–2379
14. Nan L, Yang WC, Liu YQ, Xu H, Li Y (2008) *J Mater Sci Technol* 24:197–201
15. Nan L, Liu Y, Yang MLÜK (2008) *J Mater Sci Mater Med* 19:3057–3062
16. Hamzah E, Hussain MZ, Ibrahim Z, Abdolahi A (2013) *Corros Eng Sci Technol* 48:116–120
17. Jia R, Yang D, Xu D, Gu T (2017) *Front Microbiol* 8:2335
18. Xia J, Yang C, Xu D, Sun D, Nan L, Sun Z (2015) *Biofouling*, 31481–492
19. Li H, Zhou E, Ren Y, Zhang D, Xu D, Yang C (2016) *Corros Sci* 111:811–821
20. Li H, Yang C, Zhou E, Yang C, Feng H, Jiang Z (2017) *J Mater Sci Technol* 33:1596–1603
21. Zhou E, Li H, Yang C, Wang J, Xu D, Zhang D, Gu T (2018) *Int Biodeterior Biodegradation* 127:1–9
22. Zhao Y, Zhou E, Xu D, Yang Y, Zhao Y, Zhang T (2018) *Corros Sci* 143:281–291
23. Jia R, Unsal T, Xu D, Lekbach Y, Gu T (2019) *Int Biodeterior Biodegradation* 137:42–58
24. Shi XB, Yan W, Yan MC, Wang W, Yang ZG, Shan YY, Yang K (2017) *Acta. Metall. Sin. (Engl Lett)* 30:601–613
25. Lou Y, Lin L, Xu D, Zhao S, Yang C, Liu J (2016) *Int Biodeterior Biodegradation* 110:199–205
26. Li P, Zhao Y, Liu Y, Zhao Y, Xu D, Yang C (2017) *J Mater Sci Technol* 33:723–727
27. Moradi M, Song Z, Yang L, Jiang J, He J (2014) *Corros Sci* 84:103–112
28. Xu D, Xia J, Zhou E, Zhang D, Li H, Yang C (2017) *Bioelectrochemistry* 113:1–8
29. Shi X, Yan W, Wang W, Yang Z, Shan Y, Yang K (2016) *ISIJ Int* 56:2284–2289
30. Yang J, Shao J, Wu L, Li Y (2019) *Int J Electrochem Sci* 14:9193–9205
31. Vasyliiev GS (2015) *Corros Sci* 98:33–39
32. Aljohani TA, Hayden BE (2013) *Electrochim Acta* 111:930–936

33. Zou Y, Wang J, Zheng YY (2011) Corros Sci 53:208–216
34. Keiser JT, Brown CW, Heidersbach RH (1983) Corros Sci 23:251–259
35. Yamashita M, Miyuki H, Matsuda Y, Nagano H, Misawa T (1994) Corros Sci 36:283–299
36. Gholami M, Hoseinpoor M, Moayed MH (2015) Corros Sci 94:156–164
37. Jiang J, Xu D, Xi T, Shahzad MB, Khan MS, Zhao J, Fan X, Yang C, Gu T, Yang K (2016) Corros Sci 113:46–56
38. Lv JL, Liang TX, Dong LM, Wang C (2016) Corros Sci 104:144–151
39. Nan L, Liu YQ, Yang WC (2007) Acta Metall Sin 43:1065–1070
40. Keller R, Berge D, Nouwen N (1996) Bioelectrochemistry 35:3063–3071

Figures

Figure 1

Optical microstructures images of X65 (a) and X65Cu steels (b); TEM images (c) and the corresponding EDS mapping results (d) of X65Cu steels.

Figure 2

Variations of E_{ocp} with immersion time for X65 and X65Cu steels in sterile (a) and *P. aeruginosa*-containing media (b).

Figure 3

Variations of R_p with immersion time for X65 and X65Cu steels in sterile (a) and *P. aeruginosa*-containing media (b).

Figure 4

Polarization curves for X65 and X65Cu steels in sterile and *P. aeruginosa*-containing media at the 14th day.

Figure 5

SEM images of X65 rust layer and biofilms after 14 days incubation in sterile (a) and *P. aeruginosa*-containing medium (c); X65Cu rust layer and biofilms incubation in sterile (b) and *P. aeruginosa*-containing medium (d).

Figure 6

EDS images of the corrosion product layers on the X65 rust layer and biofilms after 14 days incubation in sterile (a) and *P. aeruginosa*-containing medium (c); X65Cu rust layer and biofilms incubation in sterile (b) and *P. aeruginosa*-containing medium (d).

Figure 7

SEM images of X65 corrosion morphology after 14 days incubation in sterile (a) and *P. aeruginosa*-containing medium (c); X65Cu steels corrosion morphology after 14 days incubation in sterile (b) and *P. aeruginosa*-containing medium (d).

Figure 8

V_c of X65 and X65Cu steels in sterile and *P. aeruginosa*-containing media.

Figure 9

The largest pit depth measured by CLSM on the surfaces of X65 (a) and X65Cu steels (b) inoculated with *P. aeruginosa* for 14 days.

Figure 10

Corrosion mechanism diagrams of X65 in sterile (a) and *P. aeruginosa*-containing medium (c); X65Cu steels in sterile (b) and *P. aeruginosa*-containing medium (d).