Laboratory investigation of the microbiologically influenced corrosion (MIC) resistance of a novel Cu-bearing 2205 duplex stainless steel in the presence of an aerobic marine Pseudomonas aeruginosa biofilm

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The microbiologically influenced corrosion (MIC) resistance of a novel Cu-bearing 2205 duplex stainless steel (2205 Cu-DSS) against an aerobic marine Pseudomonas aeruginosa biofilm was investigated. The electrochemical test results showed that $R_p$ increased and $i_{corr}$ decreased sharply after long-term immersion in the inoculation medium, suggesting that 2205 Cu-DSS possessed excellent MIC resistance to the P. aeruginosa biofilm. Fluorescence microscope images showed that 2205 Cu-DSS possessed a strong antibacterial ability, and its antibacterial efficiency after one and seven-days was 7.75\% and 96.92\%, respectively. The pit morphology comparison after 14 days between 2205 DSS and 2205 Cu-DSS demonstrated that the latter showed a considerably reduced maximum MIC pit depth compared with the former (1.44 \textmu m vs 9.50 \textmu m). The experimental results suggest that inhibition of the biofilm was caused by the copper ions released from the 2205 Cu-DSS, leading to its effective mitigation of MIC by P. aeruginosa.

Keywords: 2205 Cu-DSS; Pseudomonas aeruginosa; MIC; biofilm; antimicrobial

Introduction

Duplex stainless steel (DSS) is constituted of approximately equal volume fractions of ferrite ($\alpha$) and austenite ($\gamma$), possessing the advantages of both austenitic and ferritic stainless steels (SSs). Therefore, it exhibits a desirable combination of high strength and excellent corrosion resistance, and it is widely used in the marine environment (Jeon et al. 2013; Lee et al. 2014; Li, Ren et al. 2014). DSS contains high levels of chromium, molybdenum and nickel, resulting in excellent corrosion resistance and a high value of pitting resistance equivalent number (PREN). The PREN value is calculated from $[\text{wt}\% \text{ Cr} + 3.3 (\text{wt}\% \text{ Mo} + 0.5 \text{ wt}\% \text{ W}) + 16 \text{ wt}\% \text{ N}]$ (Lee et al. 2014). Normally, the PREN of DSS exceeds 35. For a super duplex SS (SDSS), the value of PREN is up to 40–45 (Elhoud et al. 2010). When exposed to the marine environment, in spite of its high PREN value, DSS still suffers from severe localized corrosion, such as the pitting corrosion caused by microorganisms, known as microbiologically influenced corrosion (MIC) (Moradi et al. 2014). Liu (2014) found that the 2205 DSS was susceptible to MIC, and the 2205 DSS pipe on board a new yacht failed due to an extremely high corrosion rate (40 mm year$^{-1}$). Moradi et al. (2014) found that a high concentration of chloride ion was present in the biofilm structure, leading to a loss of the amount of Cr in 2205 DSS underneath the biofilm and causing pitting corrosion.

Pseudomonas aeruginosa is a Gram-negative motile rod-shaped bacterium, widely distributed in nature (Hamzah et al. 2013). It is reported that P. aeruginosa is frequently found in gasoline tanks, and it is considered a dominant bacterium in the marine environment (Manga et al. 2012). P. aeruginosa is said to be the pioneer colonizer in the process of biofilm formation in the aquatic environment (San et al. 2014) and it has been confirmed that it is an important corrosive bacterium in the marine environment, resulting in economic and environmental damage (Abdolahi et al. 2014). Researchers have investigated the MIC of some carbon steels and SSs and found these materials were severely corroded by P. aeruginosa (Yuan & Pehkonen 2007; Abdolahi et al. 2014; Hamzah et al. 2014).

Copper exhibits its antibacterial property in several different forms, including free Cu$^{2+}$, complexes of Cu$^{2+}$ and CuO nano particles (Sharifahmdian et al. 2015; Sun et al. 2015). Several antibacterial mechanisms for copper have been proposed (Sun et al. 2015): (1) elevated Cu$^{2+}$ levels inside a cell cause oxidative stress and the formation of biocidal hydrogen peroxide; (2) excess copper

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weakens cell membrane integrity, causing leakage of specific essential nutrients; and (3) copper binds with some proteins, leading to their dysfunction and bioactivity losses. Many antibacterial materials, such as copper-coated metals and nano-copper materials, have been created (Sharifahmdian et al. 2015). Some studies (Ren et al. 2011; Xiang et al. 2012; Nan et al. 2015) found that Cu-bearing SSs could precipitate the copper-rich phase after the solution and aging treatment. Ren et al. (2011) investigated the antibacterial efficacy of a Cu-bearing SS against Escherichia coli and they attributed its antibacterial property to the precipitation of the ε-Cu phase. Hong and Koo (2005) studied the corrosion behavior of a copper containing 304 SS, and suggested that the release of Cu$^{2+}$ from the copper-rich phase could kill sessile bacteria.

It is well known that MIC pitting corrosion is caused by microbial biofilms (Xu et al. 2013). Xu and Gu (2014) found that in order to obtain maintenance energy for survival, sessile cells at the bottom of an electrogenic biofilm on a carbon steel surface, when starved of organic carbon (their normal electron donor), switched to oxidizing elemental iron to obtain electrons via direct or indirect electron transfer. Zhang et al. (2015) proved that the Desulfovibrioa vulgaris biofilm on a SS accelerated pitting corrosion and weight loss when an electron mediator was added to promote electron transfer between the metal and the sessile cells. Electrochemical evidence for electron transfer between a corrosive biofilm and a metal surface in MIC was provided by Enning et al. (2012) and Venzlaff et al. (2013). MIC mitigation relies on the biofilm treatment. A recent study showed that 304L-Cu SS was far more resistant to E. coli MIC compared with 304L SS (Nan et al. 2015). Copper has also been added to ferritic and austenitic SSs to improve their resistance to uniform corrosion in acid and chloride solutions (Hermas et al. 1995; Soursisseau et al. 2005; Pardo et al. 2006). Inspired by these studies, this work investigated a novel 2205 Cu-DSS for its efficacy against MIC by marine P. aeruginosa biofilms. Electrochemical analysis, surface analytical methods and corrosion product analysis were performed.

Materials and methods

Materials

The 2205 DSS was purchased from the Taiyuan Iron & Steel (Group) Co. Ltd, Taiyuan, Shanxi, China. The 2205 Cu-DSS was made in a 25 kg vacuum induction furnace and hot forged to Φ 30 mm bars at the Institute of Metal Research (IMR), Chinese Academy of Sciences, Shenyang, China. The chemical compositions (wt%) of the 2205 Cu-DSS and 2205 DSS specimens shown in Table 1 were determined by the Department of Materials Analysis and Testing of IMR. The specimens were first annealed at 1,050°C for 1 h and then quenched in water. The aim of the heat treatment was to allow copper to dissolve to supersaturation in the matrix of each specimen and to form a balanced microstructure of the α and γ phases. To precipitate the Cu-rich phase in the matrix of each specimen, the specimens were aged at 540°C for 4 h after the solution treatment (Ren et al. 2012). The temperature and time of heat treatment were previously reported (Xiang et al. 2010, 2012). The specimens were cut into square coupons with dimensions of 10 mm × 10 mm × 5 mm. Prior to the experiments, coupons were polished and cleaned, based on the procedure described by Xu et al. (2012).

Culture medium and inoculum

P. aeruginosa MCCC 1A00099 was obtained from the Marine Culture Collection of China (MCCC), Xiamen, China. All the experiments were conducted in marine 2216E liquid medium (Qingdao Hope Bio-technology Co. Ltd, Qingdao, China) with the following composition: 19.45 g l$^{-1}$ NaCl, 5.98 g l$^{-1}$ MgCl$_2$, 3.24 g l$^{-1}$ Na$_2$SO$_4$, 1.8 g l$^{-1}$ CaCl$_2$, 0.55 g l$^{-1}$ KCl, 0.16 g l$^{-1}$ Na$_2$CO$_3$, 0.08 g l$^{-1}$ KBr, 0.034 g l$^{-1}$ SrCl$_2$, 0.08 g l$^{-1}$ SrBr$_2$, 0.022 g l$^{-1}$ H$_2$BO$_3$, 0.004 g l$^{-1}$ NaSiO$_3$, 0.0024 g l$^{-1}$ NaF, 0.0016 g l$^{-1}$ NH$_4$NO$_3$, 0.008 g l$^{-1}$ Na$_2$PO$_4$, 5.0 g l$^{-1}$ peptone, 1.0 g l$^{-1}$ yeast extract and 0.1 g l$^{-1}$ ferric citrate. The pH of the medium was 7.6 ± 0.2. It was sterilized by autoclaving for 15 min at 121°C. The initial cell concentration immediately after inoculation was ~10$^6$ cells ml$^{-1}$ measured by hemocytometer under an optical microscope at 400× magnification.

Electrochemical studies

A conventional three-electrode glass cell with a liquid volume of 500 ml was used for the electrochemical studies (Beese et al. 2013). A platinum plate electrode (10 mm × 10 mm × 1 mm) was used as the counter electrode, and the saturated calomel electrode (SCE) was used as the reference electrode. To create a working electrode sample, each vertically placed sample was connected with a copper wire at the back and then mounted in epoxy resin that covered the entire back, leaving an exposed area of 1 cm$^2$ at the front. The linear polarization resistance (LPR) and cyclic potential dynamic polarization were performed using an Autolab potentiostat/galvanostat (Reference 600™, Gamry Instruments Inc., Warminster, PA, USA). The LPR measurements were recorded at a scan rate of 0.125 mV s$^{-1}$ in the range of −5 to 5 mV vs the open circuit potential ($E_{OCP}$), and at a sampling frequency of 1 Hz. The cyclic potential dynamic polarization curves were recorded at a forward scan rate of 0.1667 mV s$^{-1}$ from −200 mV vs the $E_{OCP}$ to the potential corresponding to the anodic
current value of 100 μA cm\(^{-2}\) followed by reverse scan to \(E_{ocp}\), and a sampling frequency of 1 Hz (Antony et al. 2008). The electrochemical data were analyzed using Echem AnalystTM (http://www.gamry.com/products/potentiostats/reference-600/) software packages. To check the reproducibility, each experiment was conducted three times.

**Surface analysis**

The largest pit depth was measured under a Zeiss confocal laser scanning microscope (CLSM) (LSM 710, Zeiss, Jena, Germany) after the biofilms were removed, according to the Chinese National Standards (CNS) GB/T4334.4-2000 (Chinese National Standards 2000). For the CLSM surface analysis, the entire coupon surface was first scanned at a low resolution to confirm the location with the deepest pits. This region was then examined at a higher resolution to produce a detailed pit depth profile for reporting. The corrosion products were tested using X-ray photoelectron spectroscopy (XPS, ESCALAB250 surface analysis system, Thermo VG, Waltham, MA, USA). XPS measurements were conducted by utilizing a monochromatic X-ray source (a Al kα electrode at 15 kV and 150 W). The pass energy of the spectra within the range of 0–1,350 eV and the high resolution spectra were recorded using 50 eV passing energy and 0.1 eV step.

**Biofilm staining**

Biofilms on 2205 Cu-DSS and 2205 DSS coupons were stained with a live/dead BacLight bacterial viability kit (Invitrogen, Eugene, OR, USA) according to the manufacturer’s recommendations. The kits contained a mixture of the green-fluorescent SYTO 9 stain and the red-fluorescent propidium iodide stain. When observed under CLSM, live cells showed a green fluorescence at an excitation wavelength of 488 nm, while dead cells appeared red, and partially damaged/dead cells yellow at an excitation wavelength of 559 nm. After the 2205 Cu-DSS and 2205 DSS coupons had been incubated in 2216E medium inoculated with \(P.\ aeruginosa\) for one, seven, and 14 days, the coupons were taken out, gently washed with a phosphate buffer saline (PBS) solution to remove planktonic cells, and then dried at room temperature. A Nikon CLSM (C2 Plus, Nikon, Tokyo, Japan) was used to observe the stained cells in the biofilm. The biofilm thickness was measured in the 3-D scanning mode. For each exposure time, triplicate coupons were measured, and 10 sets of CLSM images were taken for each coupon. The numbers of both live and dead sessile \(P.\ aeruginosa\) cells were quantified using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

**Measurement of copper ion release**

The coupons were immersed in 5 ml centrifuge tubes containing 2 ml of sterilized 2216E medium. The \(\text{Cu}^{2+}\) concentration was measured after incubation for one, three, five, seven, nine, and 14 days by using atomic absorption spectroscopy (AAS) (Z-2000, Hitachi, Tokyo, Japan). A graphite furnace was adopted for atomization. \(\text{Air-C}_2\text{H}_2\) was used as the gas at a flow rate of 1.81 min\(^{-1}\). The flow rate of the oxidant gas was 15.0 l min\(^{-1}\) with a pressure of 160 kPa. The lamp current was 280 mA, the wavelength was 324.8 nm and the slit width was 1.3 nm.

**Results**

**Linear polarization resistance**

In this work, the corrosion rates of 2205 DSS and 2205 Cu-DSS were measured through \(i_{corr}\), which was calculated from the Stern–Geary equation (Stern & Geary 1957). The Tafel slope \(B\) was assumed a typical value of 26 mV, because \(i_{corr}\) was relatively insensitive to \(B\) (Zou et al. 2011; Beese et al. 2013). Figure 1a shows the variation in the polarization resistance \((R_p)\) vs exposure time for 2205 DSS and 2205 Cu-DSS in medium inoculated with and without \(P.\ aeruginosa\) at 30°C. The initial \(R_p\) values of 2205 DSS in the uninoculated medium, 2205 DSS in the inoculated medium and 2205 Cu-DSS in the uninoculated medium were all equal to 200 kΩ cm\(^2\). The \(R_p\) of 2205 DSS in the uninoculated medium increased sharply on the third day, and reached its maximum value of 1,210 kΩ cm\(^2\) on the sixth day. The \(R_p\) of 2205 Cu-DSS increased sharply on the ninth day, and reached 1,430 kΩ cm\(^2\) on the 14th day. This suggests that its passivity peaked three additional days later, but it reached a higher level compared with 2205 DSS in the uninoculated medium. In comparison with 2205 Cu DSS, the \(R_p\) of 2205 DSS in the inoculated medium fluctuated and increased slowly, but it never shot up, suggesting that the latter did not possess MIC resistance.

<table>
<thead>
<tr>
<th>Element</th>
<th>Cu-DSS (wt%)</th>
<th>Si</th>
<th>Mn</th>
<th>P</th>
<th>S</th>
<th>Ni</th>
<th>Cr</th>
<th>Mo</th>
<th>Cu</th>
<th>N</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSS (wt%)</td>
<td></td>
<td>0.04</td>
<td>0.01</td>
<td>0.006</td>
<td>0.0034</td>
<td>6.03</td>
<td>23.63</td>
<td>3.90</td>
<td>3.02</td>
<td>0.23</td>
<td>Balance</td>
</tr>
</tbody>
</table>

Table 1. Chemical composition of 2205 Cu-DSS and 2205 DSS (wt%).
The $i_{corr}$ value vs incubation time for 2205 DSS and 2205 Cu-DSS with and without *P. aeruginosa* are shown in Figure 1b. The inhibition efficiency, $\eta_p$, was calculated according to Equation 1 below (San et al. 2014):

$$\eta_p = \frac{i_{corr\,(uninh)} - i_{corr\,(inh)}}{i_{corr\,(uninh)}} \times 100\% \quad (1)$$

where $i_{corr\,(uninh)}$ and $i_{corr\,(inh)}$ are the uninhibited and inhibited corrosion current densities in the presence of *P. aeruginosa*, respectively. The $\eta_p$ values calculated from Equation 1 are shown in Table 2. In all cases the $\eta_p$ showed a general trend that shifted upward initially and then started to decrease to a stable level on the 10th day. The $\eta_p$ value was 65.7% on the 14th day for 2205 Cu-DSS in the presence of *P. aeruginosa* compared with that of 2205 DSS.

**Tafel polarization measurement**

Figure 2 shows the Tafel plots of 2205 DSS and 2205 Cu-DSS with and without *P. aeruginosa* on the 14th day of incubation. The electrochemical parameters (corrosion potential, $E_{corr}$; corrosion current density, $i_{corr}$; cathodic Tafel slope, $\beta_c$; and anodic Tafel slope, $\beta_a$) are listed in Table 3.

As shown in Figure 2, the Tafel plot curves of 2205 DSS shifted markedly towards the positive direction in the presence of *P. aeruginosa* compared with the abiotic curves. The curves of 2205 Cu-DSS in the presence of *P. aeruginosa* shifted only slightly upward compared with the abiotic curves. However, there was also a considerable shift towards the direction of a lower $i_{corr}$ as indicated by Figure 2 and Table 3. When incubated with *P. aeruginosa*, the $i_{corr}$ of 2205 DSS was 0.20 μA cm$^{-2}$, five times that for 2205 Cu-DSS (0.04 μA cm$^{-2}$), suggesting that 2205 Cu-DSS exhibited better corrosion resistance to MIC by *P. aeruginosa* after long-term incubation. Its $\eta_p$ value calculated from Equation 1 was 80%, consistent with the data in Figure 1b and Table 2.

In the presence of *P. aeruginosa*, the $\beta_c$ values of 2205 DSS and 2205 Cu-DSS were both higher than their corresponding values in the uninoculated medium, suggesting that cathodic oxygen reduction under the biofilm was inhibited after a prolonged incubation period that probably resulted in a much lower oxygen concentration under the aerobic biofilm. The inserted diagram in Figure 2 reveals that the pitting potentials of 2205 DSS and 2205 Cu-DSS in the presence of *P. aeruginosa* were 1.050 V (vs SCE) and 0.977 V (vs SCE), respectively.

**Surface and corrosion products analysis**

When exposed to *P. aeruginosa*, the coupon surfaces were all covered by a thick dark green layer containing...
the biofilm and corrosion products. The composition of the corrosion products layer was analyzed by XPS. Figure 3a shows the wide spectra of the layer. Its relative concentrations of elements (RACE) values are shown in Table 4. For both 2205 DSS and 2205 Cu-DSS in the presence of \textit{P. aeruginosa}, the RACE values of carbon and oxygen in the outer layer were higher than those on the coupons in the uninoculated medium, which could be attributed to the formation of the \textit{P. aeruginosa} biofilm. Based on the RACE results, the Cl values of 2205 Cu-DSS were all much higher than those of 2205 DSS with or without exposure to \textit{P. aeruginosa}. The Cl 2p spectra obtained from the 2205 DSS and 2205 Cu-DSS coupons after exposure for 14 days to \textit{P. aeruginosa} are shown in Figure 3b and c. Cu$_2$(OH)$_3$Cl exhibited a stronger peak at a binding energy of ~199.3 eV in Figure 3b, indicating that copper ions were released from the 2205 Cu-DSS matrix. Cu$_2$(OH)$_3$Cl was not detected in the case of 2205 DSS, as shown in Figure 3c.

After coupon surface cleaning using nitric-hydrofluoric acid, the pit morphology was detected on the surfaces of 2205 DSS and 2205 Cu-DSS that had been exposed to \textit{P. aeruginosa} for 14 days. The CLSM 3-D images of the pit morphology on the bare coupon surfaces are presented in Figure 4. In Figure 4a and b, the 2205 DSS coupon exposed to \textit{P. aeruginosa} showed the largest pit depth of 9.5 μm with a surface diameter of 50 μm. In comparison, the largest pit depth on the 2205 Cu-DSS exposed to \textit{P. aeruginosa} for 14 days was only 1.4 μm, with a surface diameter of 10 μm. The pit morphology results are consistent with the electrochemical results in Figures 1 and 2.

![Figure 2](image-url)  
Figure 2. Tafel plots of (a) 2205 DSS in the uninoculated medium; (b) 2205 DSS in the medium inoculated with \textit{P. aeruginosa}; (c) 2205 Cu-DSS in the uninoculated medium; and (d) 2205 Cu-DSS in the medium inoculated with \textit{P. aeruginosa} after incubation for 14 days. (The whole cyclic polarization curves of (b) and (d) are embedded in the top right corner.)

Table 3. The polarisation parameters of 2205 DSS and 2205 Cu-DSS in the sterile medium and in the presence of \textit{P. aeruginosa}.

<table>
<thead>
<tr>
<th></th>
<th>Sterile medium</th>
<th>After inoculation</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2205 DSS</td>
<td>2205 Cu-DSS</td>
<td>2205 DSS</td>
<td>2205 Cu-DSS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{corr}$/mV (vs SCE)</td>
<td>-308.2</td>
<td>-478.5</td>
<td>-135.2</td>
<td>-437.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$i_{corr}$/μA cm$^{-2}$</td>
<td>0.01</td>
<td>0.13</td>
<td>0.20</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta_a$ (V/dec)</td>
<td>0.18</td>
<td>0.99</td>
<td>0.56</td>
<td>0.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta_c$ (V/dec)</td>
<td>-0.09</td>
<td>-0.08</td>
<td>-0.58</td>
<td>-0.20</td>
<td></td>
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</tbody>
</table>
Live/dead cell staining of the biofilm

The biofilms on 2205 DSS and 2205 Cu-DSS coupons after incubation with *P. aeruginosa* for one, seven, and 14 days were characterized by CLSM. Figure 5a shows that after incubation for one day, the *P. aeruginosa* biofilm established on the 2205 DSS surface was uniform; and the dead (red) sessile cells were hardly detectable. However, the biofilm formed on the 2205 Cu-DSS surface was much more dispersed, and the dead sessile cells were more numerous than those on the 2205 DSS surface after incubation for one day as shown in Figure 5b. After seven days, the 2205 Cu-DSS surface showed a

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**Figure 3.** (a) The wide XPS spectra of the surfaces of the 2205 DSS and 2205 Cu-DSS in medium with and without *P. aeruginosa* after incubation for 14 days; (b) the high resolution XPS spectra of Cl 2p for 2205 Cu-DSS after exposure to *P. aeruginosa* for 14 days; and (c) the high resolution XPS spectra of Cl 2p for 2205 DSS after exposure to *P. aeruginosa* for 14 days.
Table 4. Relative atomic concentrations of the main constituents on the surface of 2205 DSS and 2205 Cu-DSS in the sterile medium (A, C) and *P. aeruginosa* inoculated (B, D) medium after 14 days.

<table>
<thead>
<tr>
<th>Sample</th>
<th>C</th>
<th>N</th>
<th>O</th>
<th>Cl</th>
<th>Mg</th>
<th>Fe</th>
<th>Cu</th>
<th>Na</th>
<th>Cr</th>
<th>Mo</th>
<th>bdl</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>54.69</td>
<td>8.82</td>
<td>23.87</td>
<td>5.15</td>
<td>2.06</td>
<td>0.24</td>
<td>–</td>
<td>3.24</td>
<td>bdl</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>63.66</td>
<td>4.17</td>
<td>23.92</td>
<td>4.01</td>
<td>2.40</td>
<td>bdl</td>
<td>–</td>
<td>0.71</td>
<td>bdl</td>
<td>bdl</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>47.44</td>
<td>6.29</td>
<td>19.85</td>
<td>10.97</td>
<td>0.02</td>
<td>1.02</td>
<td>bdl</td>
<td>6.08</td>
<td>0.64</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>53.96</td>
<td>4.03</td>
<td>21.3</td>
<td>10.24</td>
<td>5.25</td>
<td>0.05</td>
<td>bdl</td>
<td>5.25</td>
<td>bdl</td>
<td>bdl</td>
<td></td>
</tr>
</tbody>
</table>

*bdl: below detection limit.*

Figure 4. CLSM 3-D images of (a) 2205 DSS and (b) 2205 Cu-DSS incubated in medium inoculated with *P. aeruginosa* for 14 days.
Figure 5. Epifluorescence microscope images to show the growth of the *P. aeruginosa* biofilm on the surface of (a) 2205 DSS after one day; (b) 2205 Cu-DSS after one day; (c) 2205 DSS after seven days; and (d) 2205 Cu-DSS after seven days. The calculated numbers of live/dead cells are shown in (e) and (f). The biofilm thickness values for 2205 DSS and 2205 Cu-DSS coupons are presented in (g).
strong antibacterial ability compared with 2205 DSS as shown in Figure 5c and d. The biofilm on the 2205 DSS was still intact, while the biofilm structure could no longer be seen on the 2205 Cu-DSS surface. A relatively large quantity of dispersed dead sessile cells and a small amount of live sessile cells were present on the 2205 Cu-DSS surface. The antibacterial efficiency $K$ of 2205 Cu-DSS was calculated from Equation 2 (Li, Nan et al. 2015):

$$K = \frac{N_{ctrl} - N_{anti}}{N_{ctrl}} \times 100\%$$ (2)

where $N_{ctrl}$ represents the total number of sessile cells on the 2205 DSS surface (control) and $N_{anti}$ the total number of sessile cells on the 2205 Cu-DSS surface. The total number of sessile bacteria was the sum of the quantity of the live and dead bacteria. Figure 5e and f shows the numbers of live and dead bacteria on the 2205 DSS and 2205 Cu-DSS surfaces after incubation for one and seven days, respectively. They indicate that the antibacterial efficiency of 2205 Cu-DSS after seven days (96.92%) was much higher than that after one day (7.75%).

The biofilm thickness was measured in a 3-D mode under CLSM. Figure 5g shows that the biofilm thickness values of 2205 DSS and 2205 Cu-DSS in the presence of $P. \ aeruginosa$ were 68.30 ± 6.16 μm and 60.70 ± 7.80 μm, respectively. After exposure for seven days, the biofilm thickness values of 2205 DSS and 2205 Cu-DSS were 53.70 ± 2.91 μm and 47.81 ± 3.64 μm, respectively. In both cases, the biofilm on the 2205 Cu-DSS was thinner than that on 2205 DSS. The Cu addition in the matrix of 2205 Cu-DSS endowed it with antibacterial ability, resulting in a thinner biofilm.

**Discussion**

The elements Fe, Cr, Mo and Cu were undetectable or low in quantity in the biofilm when 2205 Cu-DSS was incubated with the medium inoculated with $P. \ aeruginosa$ after 14 days, probably because XPS did not penetrate to the bottom of the relatively thick biofilm. The same observation (except Cu) was made for 2205 DSS. Yuan and Pehkonen (2007) observed the same results when the biocorrosion behavior of the 304 SS in the presence of $P. \ aeruginosa$ was investigated.

Many researchers regard the cuprous dichloride anion ($\text{CuCl}_2^{2-}$) as the main form of soluble cuprous species in seawater or a NaCl solution (Kear et al. 2004; Badawy et al. 2005). Ma et al. (2015) proposed that $\text{Cu}_2(\text{OH})_2\text{Cl}$ was the oxidation product of Cu(I) oxide which increased the corrosion resistance of SS. This may explain why 2205 Cu-DSS showed a better MIC resistance performance than 2205 DSS.

In the presence of $P. \ aeruginosa$, the pH of the bulk culture medium was slightly changed from 7.8 initially to 8.2 finally on the 14th day. Although the pH under a biofilm can differ from that in the bulk fluid by up to two units (Hidalgo et al. 2009), the relatively high pH suggested that organic acid attack was not a factor in this study. Figure 1b shows that the corrosion current density of 2205 DSS in the presence of $P. \ aeruginosa$ was higher than that of 2205 DSS in the uninoculated medium. This may be attributed to the catalysis of $P. \ aeruginosa$ on the reduction of oxygen (Cournet et al. 2010), which accelerated the of corrosion process. Busalmen et al. (2002) also demonstrated that the cathodic current density could be accelerated by the presence of a $P. \ aeruginosa$ biofilm.

Figure 6 shows the release of Cu$^{2+}$ from 2205 Cu-DSS vs incubation time in the uninoculated medium. On the third day, the Cu$^{2+}$ concentration reached a peak value of 31.7 ppb. However, after three days, the Cu$^{2+}$ concentration started to decrease rapidly, reaching 1.8 ppb on day seven. It then increased to ~5 ppb and remained constant. This confirmed that the 2205 Cu-DSS matrix was able to release Cu$^{2+}$ into the medium and maintained a Cu$^{2+}$ concentration in the medium. Cu$^{2+}$ formed the protective Cu$_2$(OH)$_3$Cl layer on the 2205 Cu-DSS surface due to corrosion, consuming part of the Cu$^{2+}$ ions and preventing further corrosion of the surface.

When $P. \ aeruginosa$ cells adhered to the 2205 Cu-DSS surface and began to attack the matrix, corrosion of Cu-Cu would take place, triggering the release of Cu$^{2+}$ ions. It is well known that Cu$^{2+}$ ions can effectively kill sessile bacteria and destroy the biofilm structure (Gorman & Humphreys 2012). This probably explains why the $i_{corr}$ of 2205 Cu-DSS was lower than that of 2205 DSS in the presence of $P. \ aeruginosa$ after nine days because the $P. \ aeruginosa$ biofilm was destroyed.
Biofilm treatment is vital in MIC mitigation. In this study, the experimental data illustrate that with the addition of Cu to 2205 DSS, the biofilm coverage, the sessile *P. aeruginosa* cell number and the largest MIC pit depth on 2205 Cu-DSS coupons were all significantly reduced due to the antibacterial ability of Cu$^{2+}$ released from the metal (Teitzel & Parsek 2003; Nan et al. 2008). The 14-day data of live/dead biofilm staining were not obtained because after 14 days, most of the sessile cells on 2205 DSS and 2205 Cu-DSS surfaces were dead due to the depletion of the nutrients in the 2216E medium.

Figure 7 illustrates how Cu$^{2+}$ was released from the Cu-rich phase from the 2205 Cu-DSS matrix and how *P. aeruginosa* MIC was mitigated. In the early stage, Cu$^{2+}$ was easy to release because of the corrosion of the matrix by the *P. aeruginosa* biofilm. With the release of Cu$^{2+}$ ions, the biofilm was gradually inhibited and eventually most of the sessile cells, especially those that were directly on the metal surface, were killed by the Cu$^{2+}$ ions as shown in Figure 7b after long-term immersion.

It is well known in the literature that SS welds are more prone to MIC attack. Post-weld heat treatment is a prerequisite for 2205-Cu DSS to exhibit its antibacterial ability in an entire structure. This treatment may not be practical *in situ*. Thus, special attention should be paid to MIC at the welds. This concern should be addressed before 2205-Cu DSS becomes a viable commercial success. Additional research regarding the weldability of 2205-Cu is needed. For example, its mechanical properties such as toughness may be influenced by the presence of the alpha prime. Because MIC pipeline or storage tank leaks are often pinhole leaks, the largest pit depth is a critical parameter in MIC. The results of this study prove that the novel 2205 Cu-DSS exhibited better MIC resistance than the commercial 2205 DSS.

**Conclusions**

The corrosion behavior of a novel 2205 Cu-DSS in the presence of an aerobic marine *P. aeruginosa* biofilm was investigated by analyzing electrochemical test data, sessile cell kills, corrosion pit sizes and corrosion products. The electrochemical tests indicated that 2205 Cu-DSS had better MIC resistance than the common 2205 DSS in the presence of *P. aeruginosa* after incubation for nine days. The XPS results revealed that a protective Cu$_2$(OH)$_3$Cl layer was formed on the 2205 Cu-DSS surface. The pit morphology suggested that the pitting corrosion resistance of 2205 Cu-DSS was significantly better than that of 2205 DSS in the presence of *P. aeruginosa*. The antibacterial efficiency of 2205 Cu-DSS after incubation for one and seven days with *P. aeruginosa* was found to be 7.75% and 96.92%, respectively, demonstrating excellent antibacterial efficacy after incubation for seven days. The biocorrosion caused by *P. aeruginosa* biofilm was successfully mitigated by the 2205 Cu-DSS after incubation for nine days. This could be explained by the fact that the release of Cu$^{2+}$ from the metal triggered by the initial *P. aeruginosa* biofilm attack effectively eradicated the biofilm subsequently.

The pitting corrosion caused by the corrosive *P. aeruginosa* biofilm in marine environments has been a serious problem. This study showed that addition of the Cu element in the currently used DSS created a new metal with a superior antimicrobial ability against the marine *P. aeruginosa* biofilm. This will likely result in
field applications, such as seawater cooling systems, as an environmentally friendly and cost-effective alternative to biocide treatment of biofilms.

**Conflict of interest disclosure statement**
No potential conflict of interest was reported by the authors.

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