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Accelerated corrosion of 2304 duplex stainless steel by marine *Pseudomonas aeruginosa* biofilm

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ABSTRACT

Microbiologically influenced corrosion (MIC) in the marine environment is a serious threat to the service life of marine materials. MIC pitting corrosion rate is usually much faster than the general corrosion process. The 2304 duplex stainless steel (DSS) is an excellent alternative to 316L SS in marine applications, while its MIC behavior is barely known. In this work, surface analysis and electrochemical techniques were used to study the corrosion behavior of 2304 DSS caused by the ubiquitous marine aerobe *Pseudomonas aeruginosa*. Compared with the abiotic control, the largest pit depth showed that the *P. aeruginosa* biofilm greatly accelerated the pitting corrosion (11.0 μm vs. 4.8 μm for the abiotic control). The presence of *P. aeruginosa* biofilm oxidized the passive film of 2304 DSS from Cr_2O_3 to CrO_3, which was a water-soluble compound, resulting in the decrease of the relative Cr content and destruction of the passive film. The linear polarization resistance (LPR), electrochemical frequency modulation (EFM), electrochemical impedance spectroscopy (EIS) and polarization curve analyses all demonstrated that 2304 DSS was susceptible to MIC.

1. Introduction

Duplex stainless steels (DSSs) combine great mechanical performance and high corrosion resistance properties of austenitic and ferritic phases. Thus they are widely used in industrial applications (Jiang et al., 2013; Yang et al., 2016; Zhou et al., 2016). Under rapid development in recent years, different contents of DSS are designed by balancing their attractive performance price ratio. The commercial DSSs with comparatively low concentrations of nickel, molybdenum and other precious metal elements (SAF 2304 and 2101 DSS), duplex stainless steel, super duplex stainless steel and hyper duplex stainless steel (HDSS) can satisfy various industrial demands due to different thermal stability of microstructure, strength and corrosion resistance (Zanotto et al., 2014). The economical DSS with low nickel is developed in order to satisfy the market requirement for low cost, and to serve as an excellent alternative to replace the conventional austenitic stainless steels. In our previous study, the microbiologically influenced corrosion (MIC) resistance behavior of 2707 HDSS was found to be far superior to other stainless steels such as 2205 DSS (Li et al., 2016b). It has been reported that the traditional DSS such as 2205 DSS was not immune to MIC attack in the presence of marine bacteria (Liu, 2014; Xia et al., 2015). However, little is known about the MIC behavior of low-cost 2304 DSS in the marine environment.

It is well recognized that MIC can severe corrosion, usually pitting corrosion on steels (Beech et al., 2005; Faimali et al., 2010; Hilbert et al., 2003; Lanneluc et al., 2015; H. Li et al., 2017; P. Li et al., 2017; Usher et al., 2014a; Vastra et al., 2016; Videla and Characklis, 1992). Some researchers discussed the role of the electron transfer between the metal surface and the bacterial cells, which is the key bottleneck for MIC by electrogenic biofilms such as sulfate reducing bacteria (SRB) and nitrate reducing bacteria (NRB) (Enning et al., 2012; Gu et al., 2009; Venzlaff et al., 2013a; Xu et al., 2013a; Xu and Gu, 2014a; Zhang et al., 2015).

MIC causes tremendous economic damages to marine assets (Li et al., 2015). In fact, MIC has been confirmed as the main cause for many accidents and failures (Ching et al., 2016). In 2006, the leak of Alaska pipeline triggered a huge spike in the world's crude oil prices (Jacobson, 2007). MIC was the only remaining suspect in this leak. In

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the US alone, the corrosion losses attributed to MIC reach billions of dollars every year (Walsh et al., 1993). Apart from these economic losses, the serious environmental pollution problems caused by MIC attracted more and more public attentions, especially since the 2006 Alaska pipeline leak (Gu and Xu, 2013; Jacobson, 2007).

The corrosion mechanisms of MIC have been investigated by researchers over the years (Cheng et al., 2009; Diósi et al., 2003; Eckert and Skovhus., 2016; Nan et al., 2015; San et al., 2013; Yin et al., 2009). Most MIC studies were focused on anaerobic MIC due to sulfate-reducing bacteria (SRB) (Gu et al., 2011; Beech, 2003; AlAbbas et al., 2013). Zhang et al. found that the pitting corrosion of 304 stainless steel induced by the *Desulfovibrio vulgaris* biofilm was accelerated by electron mediators riboflavin and flavin adenine dinucleotide, which proved that cross-cell wall electron transfer was a bottleneck in their SRB MIC (Zhang et al., 2015). The mechanistic model based on charge transfer and mass transfer in the presence of SRB was proposed by Xu and his co-workers to explain the MIC caused by SRB bioenergetics (Xu et al., 2016). For 2205 DSS, the presence of SRB biofilm changed the cathodic behavior and reduced the corrosion resistance because of the formation of sulphides and hydroxides (Dec et al., 2016). So far, the investigations of MIC due to aerobic bacteria were far less than the anaerobic ones. This is because the oil and gas industry faces mostly MIC by anaerobic biofilms. *Pseudomonas aeruginosa* is frequently found in marine environments. It has been confirmed that it corrodes metallic materials including stainless steels (Cournet et al., 2010; Yuan et al., 2007a,b; Morales et al., 1993). The formation of the *P. aeruginosa* biofilm on these steels contributed to MIC pitting corrosion.

Previous studies have reported the corrosion behavior of 2304 DSS in the environments containing aggressive ions (Cl^-) or acidic solutions (Chen et al., 2012; Tan et al., 2011). In this study, the susceptibility of 2304 DSS to marine MIC in the presence of the aerobic *P. aeruginosa* was investigated through the surface analysis and electrochemical techniques.

2. Materials and methods

2.1. Materials

The 2304 DSS used in this study was manufactured by Taiyuan Iron & Steel (Group) Co., Ltd, and the main chemical composition (wt %) of 2304 DSS is: 0.47% Si, 1.52% Mn, 23.21% Cr, 4.54% Ni, 0.38% Mo, 0.12% N, 0.28% Cu and Fe for balance. The mechanical properties of 2304 DSS were measured at room temperature (25 °C). The elongation, sectional shrinkage, R_m and $R_{p0.2}$ of 2304 DSS were 37.0%, 80%, 765 MPa and 550 MPa, respectively. The 2304 DSS was cut into coin-shaped coupons with diameter 10 mm and height 5 mm. Prior to the MIC tests, all the coupons were abraded with silicon carbide papers to 1200 grit, and then they were ultrasonically rinsed with deionized water followed by alcohol for 15 min, successively. All coupons used in the test were immediately immersed in the culture medium after alcohol sterilization and drying under ultraviolet radiation for 30 min.

In this study, the MCCC (Marine Culture Collection of China) 1A00099 marine strain *P. aeruginosa* was used. The 2216E culture medium for bacterial growth was purchased from Qingdao Hope Biotechnology Co., Qingdao, China. The components of the marine 2216E culture medium were composed by (g/L): 19.45 NaCl, 5.98 $MgCl_2$, 3.24 Na_2SO_4, 1.8 $CaCl_2$, 0.55 KCl, 0.16 Na_2CO_3, 0.08 KBr, 0.034 $SrCl_2$, 0.08 $SrBr_2$, 0.022 H_3BO_3, 0.004 Na_2SiO_3, 0.0024 NaF, 0.0016 NH_4NO_3, 0.008 NaH_2PO_4, 5.0 peptone, 1.0 yeast extract and 0.1 ferric citrate. The initial pH of the culture medium was 7.2 ± 0.2. After autoclaving at 121 °C for 20 min and air cooling inside a biosafety hood, the 2216E medium was inoculated with *P. aeruginosa* at 37 °C.

2.2. Surface analysis

The surface analysis techniques including a field emission scanning electron microscope (FESEM) with energy-dispersive X-ray spectroscopy (EDS) (Ultra-Plus, Zeiss, Germany), a confocal laser scanning microscope (CLSM, C2 Plus, Nikon, Japan) were used to characterize biofilms. The corrosion products on the coupon surface after 14 days in the abiotic and *P. aeruginosa* broths were detected using X-ray photoelectron spectroscopy (XPS, ESCALAB250 surface analysis system, Thermo VG, USA). To obtain the pit depth profile, coupon surfaces were cleaned according to the Chinese National Standards (CNS) GB/T4334.4–2000, and then examined using CLSM (LSM 710, Zeiss, Germany). Detailed conditions of these analyses were described previously (Li et al., 2016a).

2.3. Electrochemical measurements

A conventional three-electrode glass cell equipped with the electrochemical workstation (Reference 600, Gamry Instruments, Inc., USA) and was used to evaluate the corrosion resistance of 2304 DSS in the presence of *P. aeruginosa* at 37 °C (Zhao et al., 2017). The reference electrode was the saturated kangon electrode and the counter electrode was a platinum sheet (10 mm × 10 mm). Linear polarization resistance (LPR), electrochemical frequency modulation (EFM) and electrochemical impedance spectroscopy (EIS) techniques were used to compare the change of the corrosion rate in the presence of *P. aeruginosa*. The polarization curves were also measured in the 2216E culture medium with and without *P. aeruginosa* using the Gamry potentiostat. The LPR scan rate was 0.125 mV/s, and the potential ranged from -15 to 15 mV. The EFM measurements were performed under 0.01 Hz potential perturbation frequency. The EIS data were tested with sinusoidal voltage at 5 mV of open circuit potential (E_{oc}), and the frequency ranged from 0.01 Hz to 100 kHz. The polarization curves were recorded after 14 days in the culture medium with and without *P. aeruginosa*, with the scan rate of 0.5 mV/s. These tests were measured in a three-electrode system (Fig. S1), with the Pt plate (10 mm × 10 mm) as the counter electrode and the saturated calomel electrode (SCE) as the reference electrode. The 2304 coupon acted as the working electrode with an exposed circular area of 0.79 cm^2. All electrochemical tests were repeated three times.

2.4. Chromium ions concentration measurement

Ten coin-shaped coupons with diameter 10 mm and height 1 mm were put into a bottle with a volume of 500 mL, in which 350 mL of culture medium was added with and without *P. aeruginosa*. Triplicate samples were measured to determine the concentration of hexavalent chromium ions using diphenylcarbazide spectrophotometric method according to Chinese standards GB7467-1987. The inductively coupled plasma mass spectrometry was also used to detect the concentration of chromium ions.

3. Results

3.1. Surface topography

P. aeruginosa cells adhered on the coupon surface gradually to form a biofilm. As shown in Fig. 1, *P. aeruginosa* cells were rod-shaped with a length of 1–3 μm. Pitting corrosion was observed underneath the biofilm after 14 days (Fig. 1b). The corresponding EDS analysis results (Fig. 1c and d) clearly demonstrate that the elemental contents of the abiotic coupon did not change significantly after immersion of 14 days, indicating that the passivation film remained relatively stable. In contrast, underneath the *P. aeruginosa* biofilm, the contents of Fe, Cr and Ni decreased (Table S1). The amount of Fe, Cr and Ni was 69.23%, 23.08%, and 4.85% for the coupon immersed in the abiotic medium, while they decreased to 56.07%, 19.43% and 3.14% in the *P. aeruginosa* broth, respectively. In addition, the carbon and nitrogen contents (16.24% and 1.47%) in the *P. aeruginosa* broth were much higher than