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Microbiologically influenced corrosion of 316L stainless steel in the presence of *Chlorella vulgaris*

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ABSTRACT

Chlorella vulgaris is a commonly found alga in the marine environment and has been widely associated with steel corrosion and biofouling. In this study, the corrosion of 316L stainless steel (SS) in the presence of *C. vulgaris* was monitored using electrochemical measurements like open circuit potential (OCP), electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization curves. Surface and corrosion product analysis was conducted using scanning electron microscopy (SEM), energy dispersive spectrum (EDS) and X-ray photoelectron spectroscopy (XPS). SEM images showed the tendency of *C. vulgaris* cells to cluster together by their exuded extracellular polymeric substances (EPS) and easily adhere to the SS surface, subsequently causing microbiologically influenced corrosion (MIC). The pitting corrosion was observed in the presence of *C. vulgaris* cells with pit depth of 20 μm formed on the SS surface after 21 days of incubation, indicating that *C. vulgaris* accelerated the localized corrosion of 316L SS. XPS and EDS analysis indicated that there were more organic C, O and P in the biofilm in the presence of *C. vulgaris*, suggesting the better growth of *C. vulgaris* on the 316L SS surface. In the daytime, *C. vulgaris* was more active and corrosive as it could produce oxygen through photosynthesis, resulting in increase of the dissolved oxygen (DO) concentration in the bulk solution and biofilm. These results indicated that the change of DO was closely related to the corrosion process.

1. Introduction

Microbiologically influenced corrosion (MIC) is a common phenomenon associated with marine, oilfield and soil environment, and has been widely studied area of research (Brauer et al., 2017; Liu and Cheng, 2017; Liu et al., 2017c; Zarasvand and Rai, 2014). MIC, an electrochemical corrosion, usually can considerably accelerate metal corrosion rates and promote the deterioration of the metal materials (Batmanghelich et al., 2017; Liu et al., 2017a). MIC is closely related to biofilm activity, and the formation of biofilm on metal surface can influence the anodic and cathodic corrosion processes (Beech and Sunner, 2004; Liu et al., 2017b). Microorganisms on metal surface can also modify the physical and chemical properties of corrosion interface and influence the formation of corrosion products, thus indirectly affecting the metallic corrosion process (Enning and Garrelfs, 2014; Videla and Herrera, 2009).

Stainless steels (SS) have been widely used in various environments due to their good corrosion resistance. However, serious pitting corrosion of SS is commonly observed, which leads to the destruction of construction materials (Bachmann and Edyvean, 2006). MIC is caused by a variety of microorganisms, such as bacteria, archaea, fungi, algae, and others, acting together to create conditions resulting in metal loss (Sharma et al., 2017). Shi et al. (2003) found that manganese oxidizing bacteria (MOB) were directly involved in pit initiation of 316L SS, which accelerated pitting corrosion. Dec et al. (2016) observed that sulfate reducing bacteria (SRB) could destroy the passive film of 2205 duplex SS through the formation of different sulphides, which also subsequently caused pitting corrosion. However, there are few reports related to effects of *Chlorella vulgaris* on the SS corrosion which is a commonly found microalga in the marine environment.

In recent years, eutrophication has been considered as a worldwide problem in water ecosystem, and its biggest threat is causing algal blooms (Liu et al., 2015b; Raja et al., 2008). Many lakes in the most parts of the world, including Asia, America, Europe, and Africa have been confronted with the problem of algal blooms (Han et al., 2013; Rovira and Pardo, 2006; Stepanauskas et al., 2002). The presence of large amounts of algal bloom not only deteriorates the water environments, but also contributes to the metallic corrosion. The adhesion of

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large amounts of algae on metal surface can cause biofouling (Poulsen et al., 2014). Biofouling is a worldwide problem in marine systems (Videla, 2001), which cost an estimated $1 billion per year for the US Navy alone (Callow and Callow, 2002). Algae is one of the main microorganisms causing biofouling (De Muynck et al., 2009; Youngblood et al., 2003). The adhesion of microorganisms can easily modify the properties of the metal surface and affect the metal corrosion. Therefore, it is clear that one of the important consequence of biofouling is the metallic corrosion and further deterioration of the metal structure following corrosion. Hence, it is important to study corrosion behavior and mechanism of metal induced by algae, which is considered as the first step for the control of algae corrosion and biofouling.

Our previous study indicated that *C. vulgaris* could easily attach to the surface of carbon steel, and considerably accelerate the pitting corrosion of carbon steel (Liu et al., 2015b). However, effects of *C. vulgaris* on corrosion of stainless steel is still not known. It is very important to investigate the effects of attachment of *C. vulgaris* on SS corrosion, especially on localized corrosion which can damage SS infrastructure in the marine environment.

In this work, corrosion of 316L SS in the absence and presence of *C. vulgaris* was studied using electrochemical measurements (i.e. open circuit potential, OCP, electrochemical impedance spectroscopy, EIS, potentiodynamic polarization curves) and surface analysis (i.e. scanning electron microscopy, SEM, three-dimensional stereoscopic microscope and X-ray photoelectron spectroscopy, XPS). The aim of this work is to investigate the effect of attachment of *C. vulgaris* on stainless steel surface and subsequent localized corrosion caused, especially pitting corrosion.

2. Materials and methods

2.1. Coupon preparation

316L SS coupons were used for this study and the alloy composition of this material consisted of (wt%) Cr 17.85; Ni 13.90; Mo 2.70; Mn 0.68; C 0.02%; Si 0.62; P 0.007; S 0.001and Fe Balance. All SS coupons were abraded through 600, 800 and 1200-grit silicon carbide metallurgical paper before use, then degreased in acetone, washed with anhydrous ethanol, dried with pure N_2 (99.99% purity, v/v). These coupons were subsequently radiosterilized using a UV lamp for 30 min before tests. SS coupons with the diameter of 15 mm and thickness of 2 mm were used for surface analysis, including biofilm observation and corrosion product morphology using SEM. SS coupons with exposed surface area of 0.785 cm^2 were used for electrochemical measurements.

2.2. Microbiological cultivation and inoculation

The details related to the growth and procurement of *C. vulgaris* strain used in this study has been reported in our previous work (Liu et al., 2015b). The medium used for the growth of *C. vulgaris* consisted of (g L^{-1}): carbamide (carbonyl diamide) 0.5; $CaCl_2{\cdot}2H_2O$ 0.0116; $MgSO_4$ 0.2; KH_2PO_4 0.01533; K_2HPO_4 0.24, under an aerobic chamber and it was sterilized by autoclaving at 121 °C for 20 min. This culture medium was also used for preforming the control electrochemical experiments where no inoculum was added to the control test cells, while 10% inoculum of a metabolically active *C. vulgaris* culture was used for inoculation of experimental cells. These electrochemical test cells were incubated at room temperature (~20 °C) under a light: dark (11 h: 13 h) cycle, to simulate the natural conditions.

2.3. Surface analysis

The biofilm and corrosion product morphology as well as their composition in the presence and absence of *C. vulgaris* cells were studied using SEM and EDS. Prior to SEM observation, all biofilm-covered coupons were removed from test solution, then were fixed using glutaraldehyde and dried using ethanol in serial dehydration at various concentration as previously described in detail (Liu et al., 2015a). Afterwards, all coupons were dried under pure N_2 (99.99% purity, v/v). Prior to SEM observation, the coupon surface was coated with a thin Au film to improve the electroconductivity.

Three-dimensional stereoscopic microscope (Model VHX-10000) was used to observe the adhesion of *C. vulgaris* cells on coupon's surface, and the corrosion morphologies of coupons after removing corrosion products. For further studying the corrosion products in detail, XPS was conducted using AXIS-ULTRA DLD-600 W spectrometer equipped with dual Al-Mg anode having Mg Kα radiation (hν 1253.6 eV) as the source.

2.4. Electrochemical measurements

The open circuit potential (OCP), electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization curves were conducted using an electrochemical workstation (Model CS350, Corrtest, China) for the study of electrochemical corrosion process in real time. A three-electrode setup was used for conducting these corrosion tests where working electrode used was 316L SS coupon as described above, counter electrode used was a platinum plate and a saturated calomel electrode (SCE) was used as reference. Electrochemical impedance spectroscopy (EIS) was conducted by applying a sinusoidal voltage signal of 10 mV in a frequency range of 10^{-2} to 10^5 Hz. EIS data were fitted using the Zview2 software (Scribner, Inc.) with an equivalent circuit model. Potentiodynamic polarization curves were measured by scanning the potential from −200 mV to +250 mV vs. OCP at a sweep rate of 0.5 mV s^{-1}. All the tests were conducted in triplicate at room temperature (~20 °C).

3. Results

3.1. Adhesion of C. vulgaris on 316L SS surface

C. vulgaris has a spherical shaped cell with average diameter between 2 and 8 μm. They have very similar structural properties to plants as they can photosynthesize and are also capable of surviving during unfavorable conditions by modifying their protein, starch, and lipid contents and by producing exopolysaccharides (Safi et al., 2014). Fig. 1 shows the morphology of *C. vulgaris* cells acquired through SEM and three-dimensional stereoscopic microscope measurements. The spherical *C. vulgaris* cells with similar size of about 2–3 μm were seen clearly both in SEM and microscope images. Some *C. vulgaris* cells tend to cluster (Fig. 1b) in the initial incubation time. Some viscous substances enfolded *C. vulgaris* cells and connected cells to each other. The viscous substances are mainly extracellularly secreted by these marine organisms and more commonly known as extracellular polymeric substance (EPS) which helps in formation of a protective and hydrated microenvironment for the organism to survive in a stressed environment including adhesion to substrate like steel (Lenhart et al., 2014; Liu et al., 2017a; Rossi and De Philippis, 2015).

The transformation of the surface morphology and adhesion pattern of *C. vulgaris* on the 316L SS surface can be visualized in Fig. 2. The green color represents *C. vulgaris*. At the start of the incubation period, very few cells were seen attached to the surface of the SS coupon by the 2nd day (Fig. 2a), but as the incubation period progressed to 21 days, a thick algal mat of *C. vulgaris* cells could be seen covering almost the whole surface of the SS coupon (Fig. 2b). Similar morphological pattern was also observed in the SEM images (Fig. 1) confirming the growth pattern behavior of *C. vulgaris* cells and their tendency to spread and adhere to the entire surface of SS coupon and contributing to corrosion in the process.

3.2. Biofilm characterization

Fig. 3 shows the SEM characterization of biofilms on the SS coupon