Corrosion Science 87 (2014) 407–415

Contents lists available at ScienceDirect

Corrosion Science

journal homepage: www.elsevier.com/locate/corsci

Corrosion behavior of copper under biofilm of sulfate-reducing bacteria

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ARTICLE INFO

Article history:
Received 29 April 2014
Accepted 2 July 2014
Available online 9 July 2014

Keywords:
A. Copper
B. XPS
C. Microbiological corrosion

ABSTRACT

The effect of sulfate-reducing bacteria (SRB) on corrosion behavior of copper was investigated using surface analysis and electrochemical measurements in seawater. Results demonstrated that SRB adhere onto copper surface to form biofilm and that the resulting corrosion product is mainly composed of cuprous sulfide. Cuprous sulfide and EPS are helpful for SRB adhesion on copper by providing a barrier against copper toxicity. In SRB growth cycle, corrosion rate is related to metabolic activity. Especially during exponential growth and stationary phases, SRB metabolism decreases the anodic zone area and promotes localized corrosion of copper.

1. Introduction

Microorganisms tend to attach to surfaces and then grow, replicate, and produce extracellular polymeric substances (EPS), thereby forming a cohesive structure known as biofilm. This process corrodes metal substrate through a route known as microbiologically induced corrosion (MIC). According to estimates, MIC accounts for about 20% of all corrosion damage of metals and building materials, and the direct cost of MIC is estimated to total $30–50 billion per year [1]. SRB are anaerobic microorganisms that are ubiquitous in environment [2]. SRB are considered the main microorganisms that cause MIC, and SRB-induced corrosion constitutes half of all MIC cases [3]. Many studies have been conducted to investigate steel corrosion induced by SRB, and several mechanisms have been reported [4–7]. For instance, metal corrosion can be accelerated through consumption of cathodic hydrogen via hydrogenase catalysis during SRB metabolism (cathodic depolarization theory) [4]. The metabolic product (H_2S) of SRB also accelerates metal corrosion [5]. EPS, which is the main component of SRB biofilm, affects corrosion processes by strongly complexing action with metal ions [6]. Some SRB promote corrosion by direct electron exchange between metal surface and microbial cells [7].

Copper and its alloys are commonly used in structures and components exposed to seawater and other marine environments due to their corrosion resistance, machinability, thermal and electrical conductivities. In general, copper and its alloys are impervious to the effects of MIC because elemental copper and its compounds have a broad spectrum of antimicrobial activity against Gram-negative and positive bacteria, fungi, and viruses by disrupting plasma membrane integrity or damaging DNA and proteins [8]. Microbes are rapidly killed on metallic copper surfaces at a rate of at least 1×10^7–1×10^8 colony forming units per hour, and live microorganisms rarely recover from copper surfaces after prolonged incubation [9]. Thus, limited attention has been given to MIC of copper. In recent years, several investigations have suggested that a number of microbes, such as *Pseudomonas fluorescens*, have numerous survival mechanisms for tolerating copper toxicity; these mechanisms include export of copper ions outside the cell [10], energy-dependent efflux of copper ions [10,11], and enzymatic detoxification/reduction [12]. Microbes are believed to be able to adhere to copper surface and influence corrosion process [13]. To the best of our knowledge, limited information on SRB-induced MIC in copper is available. Thus, studies on SRB-induced corrosion of copper are highly valuable.

For engineering metal, localized corrosion is a significant factor that affects its service life. It is a typical form of MIC for the characteristic of heterogeneous electrochemistry on biofilm/metal interfaces [14–16]. In investigations of localized metal corrosion mechanisms, it is essential to determine electrochemical parameters at local areas of metal surface. However, conventional electrochemical methods utilized in MIC studies are hard to verify the mechanisms of microorganism-induced localized corrosion, because they can only obtain average data/information about an electrochemically active surface. In recent years, localized electrochemical methods, such as scanning reference electrode technique and scanning vibrating electrode technique, were utilized to investigate localized corrosion processes on metals [17,18]. These techniques can obtain localized electrochemical parameters by detecting ionic current flows in electrolyte phase. However, in

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http://dx.doi.org/10.1016/j.corsci.2014.07.001
0010-938X/

MIC investigation, the distribution of ionic currents in electrolyte is complex and difficult to detect accurately for existence of heterogeneous biofilm covering the metal. As a technique for obtaining localized electrochemical information, wire beam electrode (WBE) method has been successfully used to investigate characteristic of heterogeneous electrochemistry in localized corrosion [19,20]. Actually, WBE setup consists of numerous metallic wires, which are individual electrochemical sensors. These wires enable WBE system to measure electrochemical parameters, such as electronic current and corrosion potential distribution, at localized areas of electrode surface [21]. Thus, WBE is speculated to be effective for investigations of localized corrosion mechanisms under biofilms.

In present study, surface analysis techniques were used to investigate SRB adhesion on copper surface as well as the resulting corrosion products. Electrochemical measurement techniques and WBE method were utilized to monitor the overall and local electrochemical processes of copper during growth cycle of SRB. Based on these results, corrosion mechanism of copper induced by SRB was clarified.

2. Experimental

2.1. Materials

Disk coupons with a diameter of 10 mm and thickness of 4 mm were cut from copper (>99.9%, mass%) plates and used for electrochemical measurements, and surface and component analyses. For electrochemical measurements, coupons were embedded in a mold of non-conducting epoxy resin with their circular cross-sections left exposed. Electrical connection was realized via a copper wire soldered to sample. WBE used in this study was manufactured from 121 identical pure copper wires (99.9%, mass%; 1.34 mm diameter). Total working area of electrode array (Fig. 1A) was approximately 1.71 cm^2. All wires were regularly arranged in an 11×11 matrix and embedded in epoxy resin at intervals of 1 mm from each other.

Prior to experiments, the exposed surfaces of samples, including disk coupons and WBE, were sequentially ground with a series of mesh silicon carbide emery papers (400, 800, 1200, and 2000) to smoothen them. The samples were then rinsed with deionized water, degreased with absolute ethyl alcohol, dried with pure nitrogen, and subsequently sterilized by exposure to ultraviolet radiation for 30 min before use.

2.2. Microorganism cultivation

Bacterial sample was isolated from marine sludge collected from the Bohai Sea of China. The modified Postgate's culture solution used in this work contained 0.5 g of KH_2PO_4 (Sinopharm Chemical Reagent Co., Ltd.), 1 g of NH_4Cl (Sinopharm Chemical Reagent Co., Ltd.), 0.1 g of $CaCl_2$ (Sinopharm Chemical Reagent Co., Ltd.), 2 g of $MgSO_4$ (Sinopharm Chemical Reagent Co., Ltd.), 0.5 g of Na_2SO_4 (Sinopharm Chemical Reagent Co., Ltd.), 4 mL of sodium lactate (Sinopharm Chemical Reagent Co., Ltd.), and 1 g of yeast extract (Thermo Fisher Biochemical) per liter of natural seawater, which was collected from Huiquan Bay in Qingdao, China. The pH of this solution was adjusted to 7.2 ± 0.1 using 1 M NaOH solution. In the culture medium, sodium lactate served as electron donor and sulfate served as electron acceptor for SRB growth.

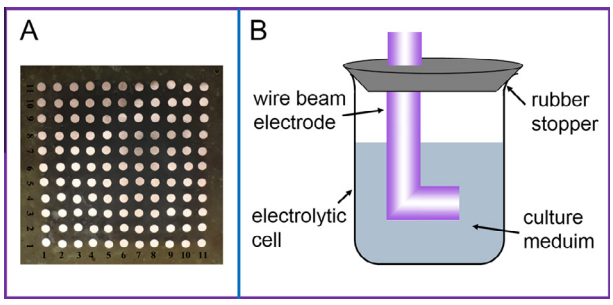

Fig. 1. (A) The digital photo of WBE and (B) the schematics of WBE set-up.

Culture medium was poured into a 1 L beaker (as an electrolytic cell, Fig. 1B), deoxygenated by N_2 sparging for 1 h, and then autoclaved at 121 °C for 30 min. After cooling, sterile WBE, rubber stopper, and beaker with culture medium were rapidly assembled, as shown in Fig. 1B. This setup was inoculated with the 4-day-old bacteria sample at room temperature (25 ± 2 °C) and subsequently sealed and stored in a temperature incubator at 30 °C.

2.3. Surface and component analysis

Scanning electron microscopy (SEM) was utilized to observe morphologies of biofilm over coupon surface and substrate. Biofilm was visualized after preparation using following procedure: Samples were exposed to 2.5% glutaraldehyde for 1–2 h and serially dehydrated with an ethanol gradient (at 30%, 50%, 70%, 90%, and 100% for 15 min). Coupons were then dried at critical point and sputter-coated with gold prior to observation. Biofilm and corrosion products were removed from coupon surfaces by following procedure: Samples were treated by ultrasonic cleaning in absolute ethanol for 15 min to remove biofilm and then subsequently treated with 10% dilute sulfuric acid for 1 min to remove corrosion products. A scanning electron microscope (KYKY-2008B) was used to visualize biofilm and substrate morphologies.

Chemical composition information of copper surface immersed in sterile and SRB media for 14 days was obtained by X-ray photoelectron spectroscopy (XPS) (Thermo ESCALAB 250, Al Kα radiation).

2.4. Electrochemical experiments

Open-circuit potential (E_{oc}) and electrochemical impedance spectroscopy (EIS) experiments were conducted in a cell with three electrodes using a CHI760C (CH Instruments, Inc.) control system in sterile and SRB media. In three-electrode system, copper electrode, Pt wire, and silver/silver chloride (Ag/AgCl, 3 M KCl) (CH Instruments, Inc.) were used as working, counter, and reference electrodes, respectively. Each impedance spectrum was obtained at E_{oc} under excitation of a sinusoidal wave with an amplitude of 5 mV and within frequency range of 10^5 Hz to 10^{-2} Hz. EIS results were analyzed by fitting data using ZSimpWin software. All electrochemical experiments were performed at 25 ± 2 °C.

The current distribution of WBE was measured using a test device (NI PXI-1042Q) consisting of NI PXI-8108 embedded controller and modular instruments: NI PXI-2535, PXI-4022, and PXI-4071, similar to those described in literature [22]. PXI-8108 is a 5-slot PXI chassis with an integrated MXI-Express controller. PXI-4071 is a 7.5-digit digital multimeter. PXI-4022 is a high-speed, high-precision guard and current amplifier that can detect picoampere current levels with femtoampere noise with PXI-4071. PXI-2535 is a high-density field-effect transistor switch matrix module featuring 544 crosspoints, a 4×136 one-wire matrix configuration (136 channels), switching speeds reaching 50,000 crosspoints/s, and unlimited simultaneous connections. This PXI system was directly controlled by a computer.

After WBE was immersed in culture medium, all wire sensors were individually connected in sequence to permit electrons to move freely between wires, similar to a one-piece electrode. Galvanic current distribution was monitored by PXI-4071 and