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Severe microbiologically influenced corrosion of S32654 super austenitic stainless steel by acid producing bacterium *Acidithiobacillus caldus* SM-1

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ABSTRACT

Microbiologically influenced corrosion (MIC) of S32654 (654SMO) super austenitic stainless steel (SASS) by acid producing bacterium (APB), *Acidithiobacillus caldus* SM-1, a strain of sulfur-oxidizing bacteria (SOB) used in biohydrometallurgy field, was investigated using electrochemical measurements and surface characterizations during a 14-day immersion test. The results indicated that S32654 SASS was susceptible to MIC by APB, and *A. caldus* SM-1 was capable of producing an aggressive acidic environment underneath the biofilm, resulting in the dissolution of the passive film and severe pitting attacks against S32654 SASS, which is commonly regarded as a corrosion resistant material.

1. Introduction

Microbiologically influenced corrosion (MIC), which is very harmful to many engineering materials [1], is ubiquitous in all kinds of environments including oceans, oilfields, and cooling water systems [2,3]. A large number of examples are reported in the literature [4,5] showing serious economic losses globally caused by MIC. Anaerobic MIC is mainly characterized into two types based on anaerobic respiration and fermentation of microorganisms [6–10]. Electrogenic microbes are capable of obtaining electrons released from oxidation of energetic metals through extracellular electron transfer (EET) for the reduction of a non oxygen oxidant such as sulfate and nitrate in the cytoplasm [11]. This is known as EET-MIC. Venzlaff et al. [12] found that sulfate-reducing bacteria (SRB) can uptake electrons from iron directly by cytochrome C on the cell membrane, a pathway called direct electron transfer (DET). Zhang et al. [13] reported that electron mediator riboflavin and FAD (flavin adenine dinucleotide) accelerated the MIC caused by SRB through mediated electron transfer (MET). Metabolites-MIC (M-MIC) is caused by the secreted metabolites of microbes such as organic acids that are produced in anaerobic fermentation in the absence of an externally supplied electron acceptor. Acid producing bacteria (APB) are well-known for lowering pH underneath their biofilm [14]. Like M-MIC, aerobic iron oxidizing bacteria can also decrease the pH to a very low value, resulting in an acid attack [15]. However, not enough is known for the MIC caused by acidophilic bacteria, which can produce a bulk-fluid pH below 2.

Bioleaching, an environmental-friendly and low-cost technique, is commercially used in mining of some metals such as copper through biosolubilization of metal oxides and metal sulfides. Sulfur-oxidizing bacteria (SOB) play an important role in bioleaching. *Acidithiobacillus caldus* is mainly distributed in acid mine drainage [16] and has been detected frequently as one of the dominant SOB in biomining. It gains energy from sulfur oxidation and plays a very important role in the global sulfur cycle [17]. *A. caldus* SM-1, an obligatory acidophilic bacterium (optimum pH for growth less than 4.0), is Gram-negative with a rod shape (0.4×2.0 μm). This aerobe is motile with one or more flagella. It uses CO_2 as carbon source in its autotrophic growth [18]. The major roles of *A. caldus* SM-1 in biomining processes are as follows: (1) to oxidize elemental sulfur and reduce inorganic sulfur compounds (RISCs), thus producing the acidity which is essential for biomining; and (2) to remove the accumulated elemental sulfur that would otherwise inhibit the oxidation of ores [19]. As an important bacterium for bioleaching, little is known for its corrosivity.

In this study, *A. caldus* SM-1 strain was isolated from bioreactor treating gold-bearing concentrates [20]. Most bioreactors are made of 316 L stainless steel (316 L SS). However, corrosion resistant material such as 316 L SS [21–23] and duplex stainless steel [24–26] are not immune to MIC attack. Considering the extreme acidity of *A. caldus* SM-1, a strong corrosion resistant material, S32654 (654 SMO) super austenitic

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stainless steel (SASS) with a high pitting resistance equivalent number (PREN) value of 56 calculated by wt% Cr + 3.3 wt% Mo + 16 wt% N was selected to explore the corrosion behavior of *A. caldus* SM-1 in this work [24,27,28]. An effort was made in this study to investigate the MIC behavior of *A. caldus* SM-1 and elucidate its MIC mechanism.

2. Experimental method

2.1. Isolation, identification and cultivation of the bacterium

The *A. caldus* SM-1 strain was isolated from a bioleaching bioreactor. It was identified by the Institute of Microbiology, Chinese Academy of Sciences (Beijing, China) [20]. The initial *A. caldus* SM-1 cell concentration was approximately 10^5 CFU ml^{-1} right after inoculation. The cell counts were determined using a hemocytometer under an optical microscope at 400× magnification. *A. caldus* SM-1 was cultivated in the liquid medium described by Duquesne et al. [29] and Johnson et al. [30]. The medium contained 0.5 g $MgSO_4 \cdot 7H_2O$, 3 g $(NH_4)_2SO_4$, 0.5 g $K_2HPO_4 \cdot 3H_2O$ and 0.1 g KCl, 0.01 g $Ca(NO_3)_2$, per litre of distilled water. The solution pH was adjusted to 4 with a 10% sulfuric acid, and then the medium was autoclaved for 30 min at 115 °C and subsequently cooled at room temperature. The trace element solution (10 ml) was filter sterilized and then added into 100 ml medium with 1 g sulfur powder. The trace elements solution contained 11 mg $FeCl_3 \cdot 6H_2O$, 0.5 mg $CuSO_4 \cdot 5H_2O$, 2.0 mg H_3BO_4, 2.0 mg $MnSO_4 \cdot H_2O$, 0.8 mg $Na_2MoO_4 \cdot 2H_2O$, 0.6 mg $CoCl_2 \cdot 6H_2O$, 0.9 mg $ZnSO_4 \cdot 7H_2O$ and 10 ml distilled water. All the tests were conducted in three medium systems, the medium containing bacterial culture is represented as *A. caldus* SM-1 medium and the one without bacterial culture is referred as abiotic medium containing sulfur. To evaluate the influence of sulfur powder, another control group, in which sulfur powder was not added in the medium, is considered as abiotic medium. Finally, the pH of these medium systems was set at 3.30 with 10% sulfuric acid solution. The incubation lasted for 14 days at 37 °C and the pH was measured every three days.

2.2. Metal material

S32654 SASS (solution-treated at 1200 °C for 1 h) was provided by the School of Metallurgy, Northeastern University (Shenyang, China). The S32654 SASS plate was cut into square coupons with the dimensions of 10 mm × 10 mm × 3 mm. The chemical element composition of S32654 specimens obtained from the Department of Materials Analysis and Testing, Institute of Metal Research (Shenyang, China) is listed in Table 1 and the mechanical properties were reported by Li et al. [31]. The specimens before MIC immersion test were mechanically abraded by silicon carbide papers from 400, 600, 800, 1000 until 1200 grits. The coupons were then rinsed with distilled water, degreased in absolute ethanol, dried in warm air and weighed using an analytic balance (ATY124, Shimadzu, Japan, ±0.1 mg). For the coupons used in the electrochemical tests, the side connecting with copper wire was embedded in epoxy resin entirely and the other side was polished in the same way as with the samples of MIC immersion test. All the specimens were sanitized under an ultraviolet lamp for 20 min before use.

2.3. Electrochemical tests

The open circuit potential (OCP), linear polarization resistance (LPR), electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization curve were performed using a potentiostat (Reference 600, Gamry Instruments, Inc., USA). The classical three-electrode glass cell system was used in this study, and the glass cell was sterilized before testing. Saturated calomel electrode (SCE) and platinum plate served as reference and counter electrodes, respectively. S32654 SASS specimens embedded in epoxy resin with an exposed surface area of 1 cm^2 were used as working electrodes one at a time. The potential of linear polarization resistance (LPR) was swept in range from −5 to 5 mV (vs. E_{OCP}) at the scan rate of 0.125 mV/s. EIS was obtained at open circuit potential by supplying an alternating current voltage of 5 mV over frequencies ranging from 10^{-2} to 10^5 Hz. The EIS data were fitted and analyzed using the ZSimpWin software Version 3.30 (Princeton Applied Research, USA). The potentiodynamic polarization curve was measured at a scan rate of 0.5 mV/s.

2.4. Weight loss measurement

All the coupons were weighed using an analytical balance. After immersion testing of 14 days, the coupons were taken out from the culture medium, ultrasonically washed with distilled water and absolute ethanol for 15 min (DSA100-SK2, Desen, China), and then immersed in the solution containing 10 ml HNO_3 (ρ = 1.42 g ml^{-1}), 2 ml HF (ρ = 1.155 g ml^{-1}, 47%~53% HF) and 100 ml distilled water for 5 min according to the Chinese National Standards (CNS) GB/T4334.4-2000. Next, the coupons were cleaned with distilled water again. After that, the coupons were cleaned with absolute ethanol for three times, each time for 20 min in the ultrasonic bath, and then dried in warm air. The corrosion rate (mmy^{-1}) was calculated using the following equation [32]:

$$V_{corr} = \frac{8.76 \times 10^4 \Delta m}{\rho A t} \quad (1)$$

where V_{corr}, Δm, ρ, A and t were corrosion rate (mmy^{-1}), weight loss (g), coupon density (g cm^{-3}) and exposed area of specimens (cm^2), and immersion time (h), respectively.

2.5. Surface morphology analysis

The biofilm morphology on the coupon surface was examined using a field emission scanning electron microscopy (FESEM, Ultra-Plus, Zeiss, Germany), and its accompanied energy dispersive spectroscopy (EDS) unit was used for element analysis of the bare matrix surface and biofilm at the same time. The coupons were immersed in a 2.5% (v/v) glutaraldehyde solution for 8 h to fix the biofilm firstly, and then the coupons were dehydrated successively with ethanol solutions (50%, 60%, 70%, 80%, 90%, 95% and 100% by volume) for 10 min each. It is widely accepted that MIC was caused by the biofilm [6]. Thus, the thickness of the biofilm is considered to be one of the significant factors to MIC. The live/dead BacLight Bacterial Viability Kit (Invitrogen, Thermo Fisher Scientific, USA) was used to stain the biofilms for 20 min in the dark at room temperature with 2 ml mixture of SYTO-9 and PI dyes (1.5 μl each component in 1 ml saline solution). A confocal laser scanning microscopy (CLSM) (Model solution-treated C2 Plus, Nikon, Tokyo, Japan) was used to measure the thickness of the *A. caldus* SM-1 biofilm after live/dead staining. SYTO-9 dye exhibited green fluorescence at the excitation wavelength of 488 nm to observe live cells, and PI showed red fluorescence at the excitation wavelength of 559 nm to detect dead cells [33]. The biofilm thickness was measured from 3-D mode CLSM images. According to the Chinese National Standards

Table 1
Chemical composition of S32654 SASS.

Elements	C	Mn	P	S	Si	Cr	Ni	N	Mo	Cu	Fe
Wt%	0.012	2.92	0.005	0.002	0.39	24.45	22.58	0.54	7.38	0.46	Bal.