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Influence of sulphate-reducing bacteria on environmental parameters and marine corrosion behavior of Q235 steel in aerobic conditions

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ABSTRACT

The growth cycle of sulphate-reducing bacteria (SRB), *Desulfovibrio caledoniensis*, and the effect of SRB on the environmental parameters and corrosion behavior of Q235 steel during a growth cycle in aerobic (air- and O_2-saturated culture solutions) and anaerobic (N_2- saturated culture solutions) conditions were investigated. Oxygen dissolved in the culture solutions induced slow growth and fast decay of SRB. The growth process of SRB under anaerobic and aerobic conditions influenced sulphide anion concentration ($C_{s^{2-}}$), pH, and conductivity (κ). The values of $C_{s^{2-}}$ and κ under aerobic conditions were lower than those under anaerobic conditions, and the pH values increased from O_2- to air- to N_2-saturated culture solutions. Aerobic conditions induced the open circuit potential (E_{OC}) to shift in the positive direction after the stationary phase of SRB growth. The charge transfer resistance (R_{ct}) increased quickly during the exponential growth phase, almost maintained stability during the stationary phase, and decreased after the stationary phase in all three conditions, and the impedance magnitude decreased from O_2- to air- to N_2-saturated culture solutions. The biofilms induced by SRB were observed by scanning electron microscopy (SEM) under aerobic and anaerobic conditions, and energy dispersive spectroscopy (EDS) was performed in abiotic and SRB-containing systems to distinguish the corrosion products. The reasons for the effects of SRB on the environmental parameters and corrosion behavior of carbon steel are discussed.

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1. Introduction

Sulphate-reducing bacteria (SRB) are ubiquitous in the marine environment, where they use sulphate as a terminal electron acceptor for degradation of organisms, resulting in the production of sulphide [1]. The relevant activities of SRB induce the rock-ribbed problem because one of metabolic products, sulphide, is highly corrosive, toxic, and reactive. In accordance with this, SRB cause corrosion of steel, which has led to serious environmental problems and enormous economic losses [2].

SRB generally are recognized as anaerobic microorganisms, but it was recently reported that they can bear oxygen concentrations of up to 1.5 mM using several defense strategies [3]. SRB possess various self-protection enzymes that facilitate survival during periods of oxygen exposure. Some SRB can generate the relevant superoxide dismutase and catalase enzymes necessary for scavenging the toxic and deleterious reduced dioxgen species hydrogen peroxide and superoxide [4–8]. Another important point for the survival of SRB in the oxic systems is that cytochrome *bd* oxidase and cytochrome *c* oxidase are the terminal membrane oxygen reductases capable of consuming oxygen [9–11]. In addition, some species of SRB in the marine environment coexist with the aerobic native microbial community [12,13].

Since the 1960s, considerable efforts have been made to investigate the mechanisms of microbially influenced corrosion (MIC) by SRB. Several mechanisms of MIC of metal materials under anaerobic condition are widely accepted, such as the cathodic depolarization theory [14], the local corrosion mechanism [15], and corrosion behavior of metabolic products [16,17]. Moreover, in our previous study we reported the effect of the growth process of SRB on the corrosion behavior of carbon steel in anaerobic conditions [18]. Currently, the influence of biofilms on the MIC of carbon steel in artificial seawater conditions has gained increasing attention among researchers [13,19–21]. The electrochemical reactions that occur during metal corrosion in the presence of a SRB biofilm are as follows [14]:

The anodic reaction:

$$Fe \rightarrow Fe^{2+} + 2e^{-} \quad (1)$$

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The cathodic reactions:

$$2H_2O + 2e^- \rightarrow 2H_{ad} + 2OH^- \quad (2)$$

$$SO_4^{2-} + 8H_{ad} \rightarrow S^{2-} + 4H_2O \quad (3)$$

Reactions for the corrosion product:

$$Fe^{2+} + S^{2-} \rightarrow FeS \quad (4)$$

$$3Fe^{2+} + 6OH^- \rightarrow 3Fe(OH)_2 \quad (5)$$

SRB consume the atomic hydrogen accumulated at the cathode and use sulphate as the electron acceptor and reduce it to the sulphide. Finally, the production of sulphide from SRB metabolism and ferrous ion from anodic dissolution lead to the formation of the corrosion products FeS and $Fe(OH)_2$.

To explore the growth of SRB and the effect of SRB on the environmental parameters and corrosion behaviors of carbon steel in a growth cycle under aerobic conditions, we measured the sulphide anion concentration ($C_{s^{2-}}$), pH, and conductivity (κ) of the research system. We also used scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), the open circuit potential (E_{oc}), and electrochemical impedance spectroscopy (EIS) to investigate the effects of SRB on carbon steel.

2. Experiments

2.1. Materials and reagents

Q235 steel (ASTM A284 Grade D) with a mass percent of elements of 0.18 C, 0.22 Si, 0.60 Mn, 0.02 S, and 0.016 P was cut into cylinders (5 mm diameter and 5 mm height) for use as working electrodes. The cylinders were embedded in a mold of non-conducting epoxy resin. Electrical connection was via a copper wire soldered onto the tip of the sample.

A series of chemicals, including magnesium sulphate (Shanghai Meixing Chemical Co., Ltd.), ammonium chloride (Shanghai Yunling Chemical Plants), sodium sulphate (Sinopharm Chemical Reagent Co., Ltd.), calcium chloride (Tianjin BASF Auxiliary Chemicals Co., Ltd.), dipotassium hydrogen phosphate (Tianjin Guangcheng Chemical Reagent Co., Ltd.), sodium hydroxide (Tianjin Rgent Chemicals Co., Ltd.), sodium lactate (Tianjin Fuchen Chemical Reagent Factory), and yeast extract (Beijing Aoboxing Biotech Co., Ltd.), were used to prepare the modified Postgate's culture medium. Ultra-high purity nitrogen and oxygen (>99.999%, Qingdao Heli Gas Co., China) were used for the preparation of the N_2- and O_2-saturated culture solutions, respectively.

2.2. The culture of SRB

The seed bacteria were isolated from marine sludge collected from the Bohai Sea of China. The purified seawater used in this work was collected from Huiquan Bay in Qingdao, China.

The modified Postgate's culture solution contained 2.0 g magnesium sulphate, 0.5 g dipotassium hydrogen phosphate, 1.0 g ammonium chloride, 0.5 g sodium sulphate, 0.1 g calcium chloride, 1.0 g yeast extract, and 2.0 ml sodium lactate in 1 l seawater. The pH value was adjusted to 7.0 using the appropriate amount of sodium hydroxide before the solution was autoclaved at 121 °C for 20 min. After cooling, the SRB culture was incubated in sterilized glass bottles aerated using N_2, air, and O_2 gases for 1 h at 30 °C in a temperature incubator.

2.3. SEM and EDS analysis

SEM and EDS analysis were conducted at the surface of the carbon steel after 11 days of exposure to artificial seawater supplemented with nutrients, under both biotic and abiotic conditions. For the carbon steel soaking in SRB-containing medium, SRB-coated carbon steel had to be fixed in 5% glutaraldehyde for 3 h and then washed with PBS and Mili-Q water. The sample was then immersed in a gradient series of ethanol (50%, 75%, and 99%) solutions for dehydration. These experiments were performed using a Jeol JSM 5900 LV scan electron microscope (Tokyo, Japan) at an acceleration of 25 kV.

2.4. Measurements of the number of SRB and of environmental parameters

The experiments were performed to illustrate the growth curve of SRB and to determine the changes in environmental parameters (e.g., $C_{s^{2-}}$, pH, and κ values) that occurred during the SRB growth process in N_2-, air-, and O_2-saturated culture solutions. A 5 ml sample of SRB seeds was first transferred into a 250 ml sterilized bottle containing 200 ml of culture solution. The culture solutions were subsequently aerated with N_2, air, or O_2 for 1 h each day to ensure that the culture system was kept saturated. Following the aeration, the bottles were sealed immediately with olefin and incubated in a constant temperature incubator at 30 °C. Meanwhile, the number of active SRB (N_{SRB}) in the N_2-, air-, and O_2-saturated culture solutions was estimated using the most probable number (MPN) method according to the American Society of Testing Materials (ASTM) standard D4412-84.

The $C_{s^{2-}}$ values were detected using a sulphide-selective electrode (Shanghai Kangyi Instrument Co., Ltd.) according to the ASTM standard D 4658-03. The values of pH and κ in the three systems were measured using an Orion 5 star meter purchased from Thermo Fisher Company.

2.5. Electrochemical experiments

The E_{oc} and EIS experiments were conducted in a cell with three electrodes using a CHI760C (CH Instruments, Inc.) control system in the N_2-, air-, and O_2-saturated culture solutions during the SRB growth process. The electrodes used were as follows: a Q235 steel (5 mm diameter) working electrode; a Pt wire counter electrode; and a silver/silver chloride (Ag/AgCl, 3 M KCl) reference electrode (CH Instruments, Inc.). The results of EIS were analyzed by fitting the data using Zsimpwin software.

Before the experiments, the Q235 steel electrode was polished with 3000# and 5000# silicon carbide paper and 1 μm and 0.05 μm alumina powder. The electrode then was ultrasonically cleaned in Mili-Q water for 10 min. All electrochemical experiments were performed at 25 ± 2 °C.

3. Results and discussion

3.1. The growth curves of SRB

Fig. 1 shows the growth curves of SRB in N_2-, air-, and O_2-saturated culture solutions. The growth curves of SRB in all three conditions displayed a typical three-stage growth cycle, with an exponential growth phase, a stationary phase, and a death phase. Compared to the anaerobic condition (N_2-saturated, curve a), SRB in the aerobic conditions (curves b and c) exhibited a slow growth rate and a fast decay rate during the growth cycle. The experimental results agree with the enumeration of SRB in an O_2-saturated condition reported by Krekeler et al. [22].

These results illustrate the toxicity of oxygen to SRB. The effect of oxygen usually is related to the sensitivity of several proteins in SRB, such as hydrogenases [23], aldehyde dehydrogenase [24], and NAD-dependent alcohol dehydrogenase [25]. SRB use several strategies

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