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Corrosion behavior of cold rolled steel in artificial seawater in the presence of *Bacillus subtilis* C2

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ABSTRACT

Effect of *Bacillus subtilis* C2 (BS) on the corrosion behavior of cold rolled steel (CRS) in artificial seawater has been studied. A visible decrease in pH value and a noticeable decrease in open circuit potential were observed in solutions containing BS compared to the sterile solutions. Biofilm was evidently observed on CRS surface after immersion in solution containing BS for some time, the biofilm increased and became more and more compact with increasing immersion time. A significant reduction in the latter corrosion rate was observed although the initial corrosion was clearly accelerated in the presence of BS.

1. Introduction

Microbiologically Influenced Corrosion (MIC) is a major problem in many industries such as oil and gas, as well as water utilities [1]. The term MIC is usually interpreted as to indicate an increase in corrosion rates due to the presence of bacteria that accelerate the rates of the anodic and/or cathodic corrosion reaction, while leaving the corrosion mechanism more or less unchanged [1]. However, an inhibiting effect of biofilm in aqueous environments (artificial seawater, for instance) has also been observed [2–4]. Thus, microorganisms may cause either microbially influenced corrosion acceleration (MICA) or inhibition (MICI).

One of the first studies of MIC involved sulfate-reducing bacteria (SRB) that thrive only under anaerobic conditions and are found widespread in many waters and soils [1]. SRBs easily reduce inorganic sulfates to sulfides in the presence of hydrogen or organic matter and are aided in the process by the presence of an iron surface. Kuehr [1] in 1923 proposed the so-called cathodic depolarization mechanism which assumes that the SRBs remove atomic hydrogen from the iron surface which causes accelerated corrosion of iron. Then, SBR became the most extensively studied microorganisms in relation to biocorrosion [5–9]. However, it is interesting that recent studies suggest that SRB need not be present in abundance in all microbial communities responsible for microbially influenced corrosion [10,11]. By contrast, *Bacillus* species were usually found in the corrosive surfaces of metals in many environments and some of them were identified as the dominant bacterial species [12–16]. Macdonald and Brözel [12] determined the community structure in the studied open recirculating cooling-water system and noted that they did not observe any sulfate-reducing bacteria or Aeromonas. Rajasekar et al. [13] described bacterial enumeration and identification in diesel and naphtha pipelines located in the northwest and southwest region in India, but sulfate-reducing bacteria were not detected in samples from both pipelines, the samples obtained from the diesel and naphtha-transporting pipelines showed the occurrence of 11 bacterial species in these fields, and 7 of these species are belong to *Bacillus* species. Giacobonea et al. [14] identified eighteen microorganisms and affirmed that *Bacillus cereus* was the predominant organism isolated from a spent nuclear fuel pool in Argentina. Bolton et al. [15] confirmed that *Bacillus pumilus* was one of the predominant bacteria in a corroding galvanized steel pipes conveying water for specialist applications. Marques et al. [16] pointed out that *Bacillus aquimaris* and *Bacillus licheniformis* were the predominant strains on the corrosion surface of carbon steel coupons using reactors containing produced water from a Brazilian oil platform.

In fact, as one of the most broad-spectrum species, *Bacillus* species can be readily isolated from soil and plant-associated environments, but are also found in other ecological niches such as deep-sea sediments, injection brine, fermented food, and the human gastrointestinal tract [17,18]. Of course, there will be existence of *Bacillus* species in the corrosion surfaces of metals in many environments. Therefore, the effects of *Bacillus* species on the corrosion of metals have attracted serious concern in the past decades [12–16,19–23]. However, only a few publications have dealt with MIC about *Bacillus*

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species, and the strains in these studies are rather extensive. 7 strains of *Bacillus* species including *Bacillus subtilis*, *Bacillus megaterium*, *B. pumilus*, *B. licheniformis*, *Bacillus brevis*, *B. cereus*, *Bacillus mycoides* have been reported in the literature available to date. Mansfeld et al. [21] showed that *B. subtilis* and *B. licheniformis* can inhibit the corrosion of cartridge brass, aluminum 2024 and carbon steel in artificial seawater; but Jack et al. [22] found that pure culture of *Bacillus* sp. induced greater corrosion of mild steel initially by 2- to 6-fold, and the rate of this corrosion decreased to that of a sterile control after 17 days; Juzeliūnas et al. [23] compared the corrosion activity of abiotic samples with that of the samples colonized with *B. mycoides* indicated microbially influenced corrosion acceleration for zinc, inhibition for aluminum, and indifference for mild steel. Bolton et al. [15] showed that *B. pumilus* accelerated the corrosion of galvanized coupons but did not increase the corrosion of steel. The results of Giacobonea et al. [14] showed that major pits covered with deposits were found on AA 6061 samples exposed to *B. cereus* but not on 99.999% Al. It easy to draw a conclusion from literatures that the results are in variety endless due to different strains of *Bacillus* species and different metals being used in different studies. And the results are likely to be different even when the same strain and metal are used in different studies. Just like MIC of other microorganisms, the mechanisms involved in MIC of *Bacillus* species are very complicated, as the process is affected by many factors, many mechanisms, such as formation of differential aeration cells caused by oxygen respiration, production of corrosive agents and organic and/or inorganic acids, metal-deposition, hydrogen embrittlement, and metal-binding effect of extracellular polymeric substance, inactivation of corrosion inhibitors and cathodic and anodic depolarization, were suggested, with no single mechanism identified as playing a major role in MIC of *Bacillus* species [1]. In recent years, popular viewpoint involved is that biofilm has a great impact on MIC, and some researchers also suggested that biofilm formed by some strains of *Bacillus* species e.g. *B. subtilis* may be beneficial to reduce MIC rates of metals [1,3,24–27]. For example, Du et al. [27] suggested that the compact biofilm formed by one strain of *Bacillus species* isolated from oil storage tank could effectively protect A3 steel from being corroded by solution, unfortunately, they did not showed the specific strain. Zuo et al. [3] demonstrated that *B. subtilis* retarded the corrosion of Al 2024 in AS by formation of a live biofilm, but they also deemed that the mechanism by which BS and other bacteria protect metallic surfaces from corrosion remained unclear. Furthermore, it is well known that many strains including *B. subtilis* can produce organic acid such as lactic acid in their physiological activities [28,29], and organic acid decreases pH values, but there are no studies involved in the effect of pH values in the presence of *B. subtilis*. Furthermore, microorganisms can colonize and form biofilm on metal surface during several days [5–7,19,20,27], the early corrosion information may provide helpful insights in establishing an accurate theoretical background on the microbiological influence on corrosion, but publications dealt with the corrosion in the early stage are rather scare.

The objective of this investigation is to determine the effect of *B. subtilis* C2 (BS) on the corrosion of cold rolled steel (CRS) in artificial seawater (AS) at the early stages. Meanwhile, a possible mechanism is presented to explain the experimental observation.

2. Experimental methods

2.1. Material

The experiments were performed with cold rolled steel (CRS) specimens with the following chemical composition (wt.%): C 0.050, Si 0.02, Mn 0.28, Cu 0.25, Ni 0.25, S 0.023, Cr 0.15, P 0.019, Fe remainder.

2.2. Bacterium

B. subtilis (BS) was obtained from Laboratory for Conservation and Utilization of Bio-Resources & Key Laboratory for Microbial Resource of the Ministry of Education, Yunnan University (Kunming, China). The bacterial strain was cultured in Luria Bertani (LB) agar at 37 ± 2 °C.

2.3. Medium

All tests were conducted using a nutrient-rich simulated seawater-based medium (AS). CRS was exposed to AS prepared as Vätäänen nine salts solution [30] (VNSS: NaCl: 17.6 g/l, $NaHCO_3$: 0.08 g/l, KBr: 0.04 g/l, $CaCl_2{\cdot}2H_2O$: 0.41 g/l, $SrCl_2{\cdot}6H_2O$: 0.008 g/l, Na_2SO_4: 1.47 g/l, KCl: 0.25 g/l, $MgCl_2{\cdot}6H_2O$: 1.87 g/l, H_3BO_3: 0.008 g/l, $FeSO_4{\cdot}7H_2O$: 0.01 g/l, Na_2HPO_4: 0.01 g/l, peptone: 1.0 g/l, starch: 0.5 g/l, glucose: 0.5 g/l, yeast extract: 0.5 g/l). The pH of the medium was adjusted to 7.5 ± 0.1 using a 1 M NaOH solution and sterilized by autoclaving for 20 min at 121 °C and at 100 kPa.

2.4. Growth phase experiments

Growth phase experiments were performed to determine the growth kinetics of BS in AS medium. A loop of BS cells from a slant culture of fresh nutrient agar was used to inoculate a 250 ml Erlenmeyer flask containing 100 ml Luria Bertani (LB) broth (pH 7.0). The flask was incubated on a rotary shaker at 150 rpm at 36 °C for 20 h until the BS was grown to an OD_{550} of 1.1, then 2 ml bacterial culture was taken out by sterile pipette and inoculated in AS to obtain BS inoculated medium with a volume of 150 ml in 250 ml flask. The flask was incubated with a stirring speed of 150 rpm using a polytetrafluoroethylene magnetic stirring at 25 °C by a thermostatically controlled water tank. In this process, three parallel flasks were performed. During incubation, 2 ml culture from each of three parallel flasks was collected and pooled every 4 h, the combined samples were then subjected to cell density measurement. The optical density of the culture at 550 nm (OD_{550}) was measured over time using a photometer. The experiment was terminated until bacterial cells reached decline phase. All the experiments were performed in triplicate.

2.5. Fluorescent microscopy (FM)

The CRS tablets (10 × 10 × 1 mm) were abraded with emery paper from 100 to 2000, then rinsed with distilled water, degreased with acetone (CH_3COCH_3), and dried with a warm air stream, sterilized with 2% glutaraldehyde solution for 1 h and rinsed with sterile distilled water. The treated CRS tablets were exposed to the BS inoculated medium without renewing the fresh medium under the conditions as described in growth phase experiments for 10, 24, 32, 72, 96 and 240 h, respectively. At the predetermined period of bacterial incubation, the specimens were retrieved and washed twice with a sterile phosphate buffered saline (PBS) solution to remove the dead and loosely attached bacteria. The specimens were washed thrice with the sterile PBS solution and deionization water, followed by staining with 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) solution for 15 min. The specimens with immobilized bacterial cells were imaged under 100 × magnifications using a Nikon E800 fluorescence microscope, equipped for epifluorescence with a mercury lamp.

2.6. pH tests

pH tests were also performed in AS solutions containing working electrode with and without BS before electrochemical