

Environmental-Friendly Corrosion Inhibition of Carbon Steel Using *M. salsuginus* Bacteria

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ABSTRACT

The corrosion behaviour of carbon steel, X80 pipeline steel, was studied with the help of electrochemical and surface morphological techniques in a simulated marine environment, inoculated with marine bacterium *Marinobacter salsuginus*. The increase in linear polarization resistance, charge transfer resistance, and decrease in corrosion current density of the X80 pipeline steel immersed in biotic medium indicated its high corrosion resistance compared to the sample in abiotic medium. Scanning electron microscopy, confocal laser scanning microscopy and live/dead cells staining were employed to observe the biofilm morphology and bacterial viability after different immersion time. The results indicated that the corrosion inhibition efficiency was 74% higher in the biotic medium compared to that in abiotic medium; due to the formation of biofilm and the development of EPS layer on the metal surface. Thus, it was found that *M. salsuginus* can effectively mitigate the corrosion tendency of X80 pipeline steel.

Keywords: Corrosion inhibition, environmental-friendly, bacteria, carbon steel

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1. INTRODUCTION

Depending on the availability of the nutrients, bacteria secrete extracellular polymeric substance (EPS) which helps them adhere to the surface of the material [1–3]. Bacteria like *Pseudomonas fragi* and *Escherichia coli* secrete polysaccharide and producing biofilm on the material surface [4, 5]. The attachment of bacteria to the metal surface is a complicated process, affected by several factors such as environment, bacterial properties and the surface characteristics of the host material [6]. Bacterial colonization on the metal surface is a well-known phenomenon, which can enhance or inhibit the corrosion process [7]. The adhesion of organic and inorganic macromolecules, production of the extracellular polymeric substance (EPS), microbial growth, and hydrodynamic erosion can affect the biofilm formation [8]. Bacterial biofilm and their secreted EPS are considered having adverse effect on the corrosion property of the metals which has the tendency to increase the corrosion rate of various materials installed in different environments like soil, fresh water and sea water [9–11]. A number of bacteria such as *Hafnia alvei*, *Desulfovibrio desulfuricans*, *Bacillus* sp. and *Pseudomonas* sp. have been found accelerating the corrosion process of metals [9]. The corrosion caused by microorganisms is called microbiologically influenced corrosion (MIC) or bio-corrosion. Bio-corrosion of different metal alloys has been investigated and it was observed that most of the metal alloys are prone to MIC; even the high corrosion resistant alloys like duplex stainless steel and super austenitic stainless steel are not immune to the bacterial attack [12–16].

On the other hand, it has also been reported that biofilm can inhibit the corrosion of some metals. Jayaraman et al found a decrease in the corrosion of low carbon steel (SAE 1018) in the presence of *Pseudomonas fragi* biofilm, signifying the passivation of the surface by the biofilm [17]. Similarly, the corrosion rate of the aluminium alloy 2024 was increased when the bacterial cells died and were unable to further protect the surface from the corrosion [18]. Several studies have found that microorganisms are involved in the corrosion inhibition of the metals [18–26]. Pedersen and Hermansson observed a reduction in corrosion rate of mild steel exposed to *Pseudomonas* sp. S9 and *Serratia marcescens* EF190 biofilms [27, 28]. Research on the corrosion inhibition by microorganisms has made it clear that microbiologically influenced corrosion inhibition (MICI) is a more common phenomenon than it was considered in the past. It has been observed that bacteria do mitigate corrosion when the biofilm is alive [29]. Bacteria use the oxygen for respiration and stop it from reaching to the surface of the material which decrease the process of corrosion [17, 19, 30, 31]. The process of bacterial respiration played a vital role in controlling the cathodic corrosion at the surface of the mild steel [32, 33].

Carbon steel (X80 pipeline steel) is the most broadly used manufacturing material, which accounts for 85% of the annual steel production worldwide. Although it has weak resistance to corrosion, carbon steel has extensive use in marine environment, nuclear power plant, mining, transportation, petroleum production, chemical processing, refining pipelines, construction and metal processing equipment. The low corrosion resistance of carbon steel brings failures to this material, resulting in a huge economic losses in different sectors [34, 35]. To decrease the labour and cost needed for the renovation or modification of the materials used in different environments, the use of corrosion protection techniques are highly recommended. Various techniques like surface coating with organic paint, electroplating, and use of biocides have been used to control the corrosion of the materials. However, the high cost, toxicity and non-reliability of these techniques turned the attention of researchers towards the biofilm, which is a natural, nontoxic and cost effective method for the corrosion control [26].

In the present study the biofilm of a marine bacterium *M. salsuginosus* was examined for its corrosion inhibiting behaviour. *M. salsuginosus* is a Gram-negative bacterium having motile nature. It is an aerobic halophilic-natured bacterium which grows at temperature of 35–37 °C and pH of 7.5–8.0. Corrosion behaviour of carbon steel (X80 pipeline steel) was studied in simulated marine environment inoculated with *M. salsuginosus*. The study was supported by electrochemical technique using open circuit potential (OCP). Moreover, the scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) were performed for surface analysis. The bacterial viability was then investigated with the help of live/dead cells staining experiment.

2. MATERIALS AND METHODS

2.1. Materials

The chemical composition of the material is listed in Table 1. For all experiments, samples with dimension of 10 mm × 10 mm × 5 mm were cut from the carbon steel bar, which were then sequentially abraded using silicon carbide paper from 240 to 1000 grit. The samples were then washed with deionized water and degreased by sonication in ethanol for 15 min. For electrochemical study, the samples were connected with copper wire and embedded in epoxy resin leaving only 1 cm² exposed surface. Prior to the experiment, the samples were sterilized in ethanol followed by sanitization under UV light for 25 min.

2.2. Bacterial Growth

2216E culture medium which is a simulated marine environment was used for this study. The culture medium was with the following compositions (g/L): Na₂SO₄ (3.24), MgCl₂ (5.98), peptone (5.0), NaCl (19.45), KBr (0.08), KCl (0.55), H₃BO₃ (0.022), NaF (0.0024), Na₂CO₃ (0.16), NaSiO₃ (0.004), NH₄NO₃ (0.0016), SrCl₂ (0.034), NaH₂PO₄ (0.008), SrBr₂ (0.08), yeast extract (1.0) and ferric citrate (0.1).

The medium was prepared by dissolving 37.4 g of the 2216E culture medium in 1000 mL water, which was then sonicated and autoclave-sterilized at 121 °C for 20 min. Bacteria in culture medium were incubated for 24 h and the initial bacterial concentration used for the experiment was 10^6 cells/mL.

2.3. Electrochemical experiment

The electrochemical techniques such as OCP, LPR, EIS and potentiodynamic curves were measured by using potentiostat (Reference 600™, Gamry instruments, inc., USA). Saturated calomel electrode and platinum electrode were used as a reference and counter electrodes respectively. Carbon steel coupon connected with copper wire was used as the working electrode. The experiments were made in an electrochemical glass cell having 250 mL of culture medium. The LPR measurements were performed at the scan rate of 0.125 mV/s, within the potential range of -5 to 5 mV vs. OCP.

2.4. Live/dead Cells Staining

Live/dead cells staining experiment was performed to investigate the bacterial viability. LIVE/DEAD BacLight Viability Kit (Invitrogen, Eugene, OR, USA) containing two fluorescence dyes including SYTO-9 and propidium iodide was used for staining the cells. SYTO-9 is a green colour pigment, representing live bacterial cells while propidium iodide is a red colour pigment, representing dead bacterial cells. The samples were first immersed in bacteria inoculated solution for 7 and 14 days. Then phosphate buffer saline solution (PBS) was used to wash the samples, which were then incubated in a complete dark environment using 1 mL PBS solution containing SYTO-9 and propidium iodide dyes. The bacterial sustainability was observed with the help of Nikon CLSM (C2 Plus, Nikon, Japan), by applying 488 nm and 559 nm wavelengths to detect the live and dead bacterial cells respectively.

2.5. Characterization of Surface Morphology

The samples were incubated in sterile and bacteria inoculated culture media for different times. To remove the planktonic cells and the organic and inorganic debris present in the culture medium, the coupons were washed with PBS solution. The washed coupons were subsequently soaked in 4% glutaraldehyde solution to kill and immobilize the bacterial cells on the material surface [36]. The coupons were then dehydrated using ethanol of different concentration (50%, 60%, 70%, 80%, 90% and 100%, v/v). To increase the surface conductivity, the samples were sputter-coated with a thin film of gold. The prepared samples were then examined for the biofilm morphology with the help of a scanning electron microscopy, SEM (Ultra-Plus, Zeiss, Germany). The samples after immersion in biotic and abiotic media for 7 and 14 days were examined with the help of a Zeiss confocal laser scanning microscope (LSM 710, Zeiss, Germany). Before observation, samples were soaked in ethanol for 10 min followed by washing in 18% HCl solution containing 20 g/L hexamethylenetetramine to remove the biofilm and corrosion product from the surface.

3. RESULTS AND DISCUSSION

3.1. Electrochemical experiment

3.1.1. Open circuit potential (OCP) and linear polarization resistance (LPR) analysis

The open circuit potential (OCP) of the material in biotic and abiotic media is given in Fig. 1a and b. The OCP of the material in biotic medium was comparatively more negative than the material in abiotic medium. The lower value of OCP could be due to the bacterial attachment on the material surface. Bacteria are known to secrete extracellular polymeric substance (EPS), which possess high efficiency to make complexes with the ions formed during surface oxidation. OCP is a thermodynamic process. More negative OCP indicates higher thermodynamic tendency to oxidation [37]. The OCP of the coupon in biotic medium had a value of -678 mV, which was relatively lower than the coupon in sterile medium (-642 mV), representing the surface oxidation due to the bacterial attachment. After 6 days of immersion, the OCP shifted positively reaching a peak value of -651 mV. Thereafter, the OCP maintained a slight stability for the remaining days of immersion.

The increase in OCP was attributed to the formation of biofilm and the development of a protective layer of EPS on the surface, which stopped the surface oxidation of the coupon in biotic medium.

3.2. Surface morphology study

3.2.1. Scanning electron microscopy (SEM)

The surface of the X80 coupons immersed in biotic and abiotic medium was investigated for the study of biofilm morphology. As shown in Fig. 2a and b, the coupons in abiotic medium were observed with corrosion product on their surfaces. X80 pipeline steel owns very low corrosion resistance when it comes in contact with corrosive environment. This material is unable to form a stable oxide layer which could protect the material surface from corrosion. The non-appearance of protective layer made the material weak in corrosion resistance. The material was investigated after 7 and 14 days (Fig. 2a, b) and it was observed that the surface is highly oxidized, which could be the main reason of increased corrosion rate in abiotic medium. The iron was working as an anode, by oxidation it was providing iron ions and free electrons released, while oxygen acted as a cathode, as it was receiving electrons from the solution and getting reduced. The SEM experiment was consistent with the electrochemical analysis.

3.2.2. Investigation of the bacterial viability and pitting corrosion

CLSM was used for measuring the pitting corrosion of the X80 steel after immersion for 7 and 14 days in biotic and abiotic medium. As shown in Fig. 3a and b, the sample had a higher thickness of biofilm (50 µm) depth after it was initially dipped for 7 days in abiotic medium, while in biotic medium had depleted biofilm of 46 µm without any pit appearing on it. This signifies its high corrosion resistance and that some bacteria biofilm responsible for corrosion inhibition had been eaten up at 14 days. Hence, it showed that the surface was protected by the biofilm and the EPS from the contact of the aggressive elements.

4. CONCLUSIONS

The corrosion of X80 pipeline steel was successfully controlled using marine bacterium *M. salsuginosus*. The inhibition efficiency in the presence of the bacterial medium was very high compared to the sterile medium. Both the biofilm and EPS were involved in the corrosion inhibition of the sample. The oxidation was stopped by the EPS film which was formed through complexation with the iron ions, while the biofilm controlled the cathodic reaction by using the oxygen for its metabolic activities. On the basis of the present study we can prefer these bacteria as an effective, renewable and natural source of corrosion control of X80 steel.

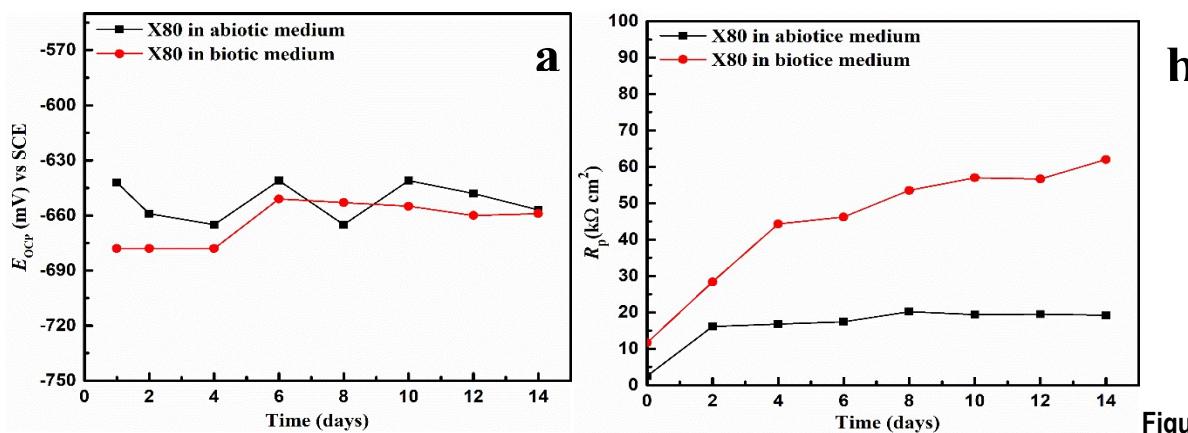


Figure 1.

Variations in open circuit potential (a) and linear polarization resistance (b) of X80 steel immersed in biotic and abiotic media for 14 days.

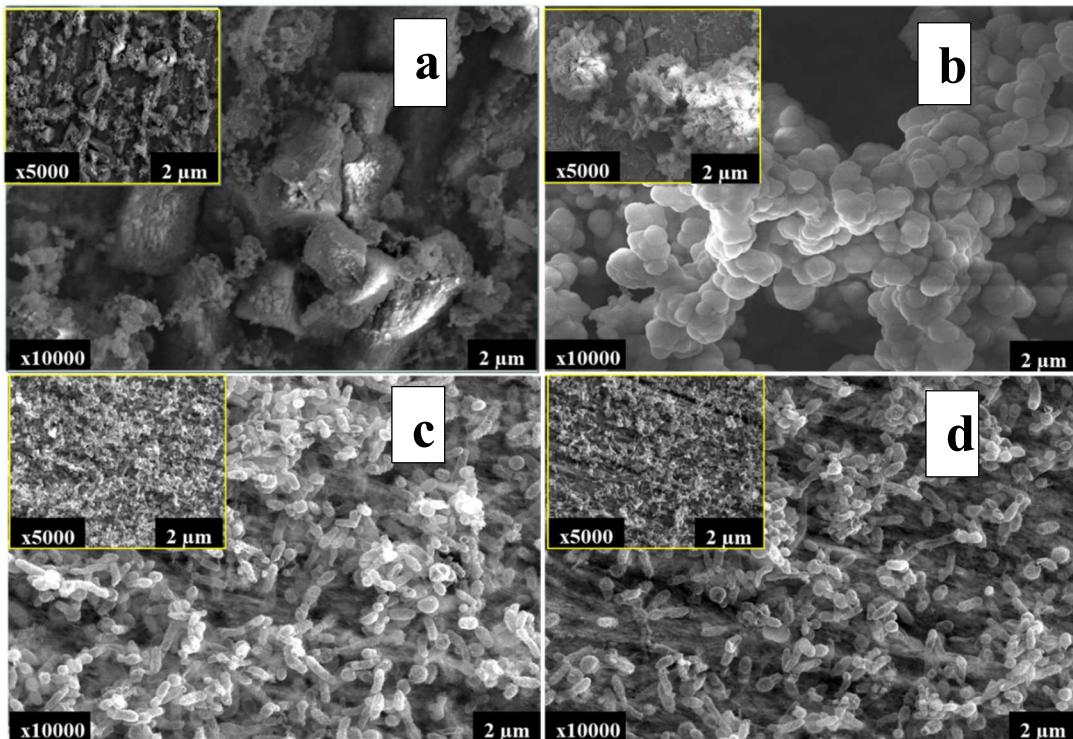


Figure 2. SEM images, (a, b) showing the oxide layer on the material surface in sterile medium and examined after 7 and 14 days while (c, d) showing biofilm on the coupon surface in biotic medium examined after 7 and 14 days respectively.

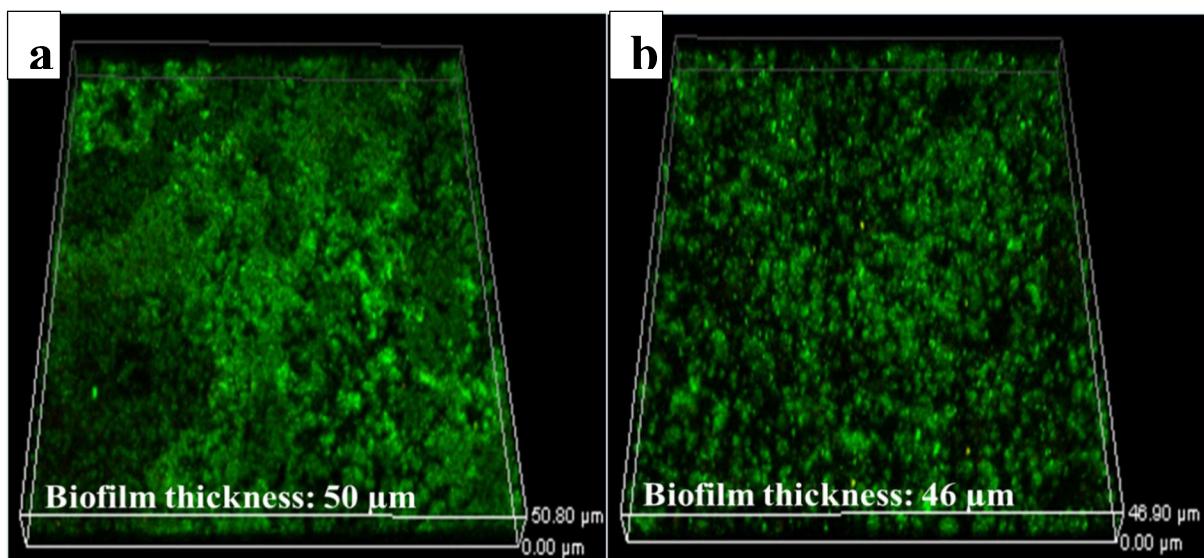


Figure 3. CLSM live/dead cells experiment after 7 (a) and 14 days (b)

Table 1. Elemental composition of X80 steel

X80 steel	Material											Element
	Cu	Ni	Cr	Nb	C	Mo	Si	Mn	S	P	V	
	0.20	0.29	0.08	0.008	0.028	0.22	0.28	1.90	0.002	0.012	0.03	Bal

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COMPARATIVE STUDY OF MICROSTRUCTURAL CHARACTERISATION AND TENSILE PROPERTIES OF WROUGHT AND ADDITIVELY MANUFACTURED Ti – 6Al – 4V SAMPLES

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Abstract

Titanium alloys are extensively applied in several engineering design due to their superior properties. The manufacturing of titanium products seems to be difficult when a traditional method is used because it is always time-consuming, waste high amount of material, coupled with the manufacturing costs. Selective laser melting (SLM), an additive manufacturing technique, has recently gained attention owing to its capability to produce near net shape components with little production time. SLM has been made known to be an attractive manufacturing method for the production of α/β titanium alloys particularly Ti – 6Al – 4V. A full understanding of the relationship between the process, microstructure and mechanical properties of the components manufactured by this technology is however critical for the establishment of SLM as an alternative manufacturing route. The purpose of this study is therefore to determine the microstructure, chemical composition, tensile properties and hardness for the wrought and additive manufactured SLM samples. Microstructure, mechanical properties and hardness were analysed in both longitudinal and transverse directions of wrought and SLM bar. It was established that the additively manufactured bar has higher yield strength, ultimate tensile strength and hardness than the wrought bar. The difference in the properties of SLM and wrought can be ascribed to the difference in microstructure due to processing conditions.

Keywords: Selective laser melting, titanium alloys, microstructure, mechanical properties, tensile properties

INTRODUCTION

Titanium is a low-density element, and records show that approximately 60% of the density of steels, titanium and its alloys and other superalloys can be strengthened significantly by alloying and deformation processes. Titanium alloys are extensively used in aerospace, automotive, biomedical as well as chemical industry due to their excellent properties such as corrosion resistance and high-strength ratio (Lutjering and Williams, 2007).

Ti – 6Al – 4V alloy is distinctive since it combines attractive properties with characteristic of workability that enables it to be produced in all types of mill products (in both large and small sizes), fabricability which allows the mill products to be made into complex shapes. Ti – 6Al – 4V became the standard alloy against which other alloy must be compared when selecting a titanium alloy for specific application (ASTM, 2014). Boyer (1996) explained that this titanium alloy is the most applied in aerospace engineering due to its high mechanical properties, low density and excellent corrosion properties. Leyens and Peters (2003) also agreed that titanium alloys are used in aerospace application where the combination of weight, strength, corrosion resistance and or high temperature stability of aluminium alloys, high strength steels or nickel – based (Nb) superalloys are not enough. Owing to Ti – 6Al – 4V biocompatibility couple with