



TECHNICAL ARTICLE

Study of Microbiologically Influenced Corrosion of the Welded Stainless Steel 316L

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Submitted: 4 May 2022 / Revised: 31 October 2022 / Accepted: 25 November 2022 / Published online: 20 March 2023

Microorganisms are acknowledged to be responsible for corrosion failures worldwide; however, some recent studies have indicated that in some environments, bacteria activities can decelerate the corrosion rate of metals. This research investigated the microbial corrosion behavior of welded stainless steel 316L by sulfate-reducing, sulfur-oxidizing, and iron-oxidizing bacteria. The corrosion evaluations were performed using potentiodynamic and cyclic polarization tests. Furthermore, the scanning electron microscope and energy-dispersive spectroscopy analysis were executed after 90 days of bacteria exposure to determine the biofilm morphology changes over time. The results suggested that while the base metal (unwelded area) was unaffected by SRB, a vast increase in corrosion rate was observed in HAZ and welded area. On the other hand, analyzing the SEM morphology, EDS results, electrochemical tests and the corrosion rate obtained from Tafel extrapolation showed that SOB and IOB reduced the corrosion rate of the alloy and acted as inhibitors

Keywords iron-oxidizing bacteria, microbiologically influenced corrosion, sulfate-reducing bacteria, sulfur-oxidizing bacteria, weld zone

1. Introduction

The term microbiologically influenced corrosion (MIC) is used to indicate corrosion as a result of the presence and activities of microorganisms, and they are a type of organism that the naked eye cannot see. There are many microorganisms that affect corrosion, such as microalgae, bacteria, and fungi. The microorganisms do not produce and cause a unique type of corrosion; instead, they increase the intensity of other types of corrosion, including localized corrosion, stress corrosion cracking, and galvanic corrosion (Ref 1, 2).

The microbiologically influenced corrosion has been reported to decay almost all engineering metals and alloys, and it has been seen in many different environments, including seawater, fresh water, distilled/demineralized water, crude, and distillate hydrocarbon fuels, process chemicals, foodstuffs, soils, human plasma, saliva, and sewage. One of the reasons that make MIC dangerous is that it can occur in environments where corrosion would not be expected (e.g., low chloride waters), and the rates can be relatively high (Ref 1-3). Bacteria have a large number of varieties, including sulfate-reducing bacteria (SRB), sulfur-oxidizing bacteria (SOB), iron-oxidizing bacteria (IOB), and iron-reducing bacteria (IRB) (Ref 4).

Various researches have been carried out about MIC over the years. In 2019, Chen investigated the corrosion of Steel 907 influenced by SRB, and it was proven that SRB could enhance the samples' corrosion rate. Furthermore, it was demonstrated that after the introduction of SRB, the corrosion type of Steel 907 changed from uniform corrosion to localized corrosion (Ref 1).

Further, in 2021, the corrosion behavior of X80 pipeline steel in contact with SRB was investigated, and the MIC process and mechanisms were discussed. The results revealed intense pitting corrosion caused by SRB. In addition, the extracellular electron transfer MIC (EET-MIC) and metabolite MIC (M-MIC) mechanisms were studied (Ref 2).

In contrast, some studies claim that microbial activities might be able to increase the corrosion resistance of metals in a given situation. Lou proposed that microbiologically influenced corrosion inhibition (MICI) can be a fresh approach to preventing corrosion. The protective feature of microorganisms was first discovered in 1987 when Iverson suggested that bacteria activities can inhibit the corrosion of copper in seawater and freshwater. Biofilms contain different components of extracellular polymeric substances (EPS), and their role can differ depending on the complexity, concentration, charge, and adsorption of each EPS section (Ref 5).

For instance, some studies suggest that a small amount of EPS produced by thermophilic sulfate-reducing bacteria (t-SRB) in 3% NaCl solution at 30 C can inhibit corrosion. Meanwhile, a higher concentration of EPS enhances corrosion (Ref 6).

The different roles of microorganisms on the metals have made MIC a notable field of study. In recent years, many studies about microorganisms and their impact on various environmental conditions on microbial corrosion have been published, revealing that microorganisms can be both corrosive and preservative (Ref 7-12). Due to the diversity of microorganisms and the fact that they have evolved themselves to thrive under various environments, their behavior is hard to

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predict, and understanding their exact role in the corrosion process has become a difficult issue (Ref 13). Since even a specific type of bacteria in dissimilar situations can affect corrosion differently (Ref 6), finding an acceptable hypothesis about the corrosion influence of microorganisms requires many studies and experiments in various environments. Therefore, the objective of the present study is to investigate the influence of the three different bacteria, including sulfate-reducing bacteria (SRB), sulfur-oxidizing bacteria (SOB), and iron-oxidizing bacteria (IOB), on the corrosion resistance of welded stainless steel 316L. One of the notable cases in this research is the investigation of the different effects of bacteria on reducing or increasing corrosion resistance, which is considered in this research.

2. Material and Methods

2.1 Materials

The 316L specimens with a composition in Wt. % were as follows: (Fe: base, 0.0312 C, 0.499 Si, 1.393 Mn, 0.029 P, 0.012 S, 16.810 Cr, 2.138 Mo, 9.788 Ni, 0.036 Al, 0.027 Cu, 0.240 Co, 0.009 Nb, 0.004 Ti, 0.027 V, 0.017 W), which have been used in this study. All specimens used for the study were prepared from one 316L pipe, cut using the electrical discharge machining method, and joined using the GTAW method (according to Table 1) and standard method (according to ANSI B31.3 and ASME IX). Subsequently, specimens were cut off from the welding center area. Therefore, two targeted areas of welding zone and heat-affected zone (HAZ) were accessible in the prepared Samples. Due to the need to conduct different experiments, samples were prepared in different sizes. Therefore, thirty samples (30 × 30 × 8 mm) were prepared for electrochemical experiments, which included six samples for each test group (anaerobic control, aerobic control, SRB, SOB, and IOB Containing Medium). Furthermore, ten samples (30 × 10 × 8 mm) were prepared for microscopic experiments, including two samples for each test group. Prepared samples were polished using silicon papers (up to 1200); then, the samples were cleaned with deionized water (according to ASTM G1-03).

2.2 Microorganism Cultivation

The samples were abraded through 600, 800, and 1200-grit silicon carbide metallurgical papers, degreased in acetone, washed with anhydrous ethanol, dried with nitrogen gas, and stored in a desiccator until use. The test specimens were sanitized under a UV lamp for 30 min before incubation. Three types of bacteria, including *Desulfovibrio* sp. *Acidithiobacillus* sp. and *thiobacillus ferrooxidans*, were acquired from the

Persian Type Culture Collection (PTCC) and were grown for 10 days before inoculation. The culture medium was autoclaved at 121 °C for 20 min and then cooled to room temperature (25 ± 2 °C). It is notable that ferrous sulfate was added to the solution after autoclaving the culture medium. The SRB culture medium composition was (g/L): K₂HPO₄ 0.01, MgSO₄·7H₂O 0.2, (NH₄)₂Fe(SO₄)₂ 0.2, NaCl 10, yeast extract 1.0, vitamin C 0.1, in addition to 4.0 mL/L sodium lactate (pH 7.2). The IOB and SOB culture medium contained (g/L): K₂HPO₄ 0.5, NaNO₃ 0.5, CaCl₂ 0.2, MgSO₄·7H₂O 0.5, (NH₄)₂SO₄ 0.5 and ammonium iron citrate 10.0 (pH 6.5) (Ref 10).

After preparing the culture medium, the acquired bacteria and samples were added to the solutions. Each test group used a different Erlenmeyer flask for the electrochemical tests and the microstructure analysis. The sulfate-reducing bacteria are anaerobic; therefore, a layer of paraffin was added to the top of the solution to the medium oxygen could be limited. The six Erlenmeyer flasks with the aerobic condition and four Erlenmeyer flasks with the anaerobic condition were prepared.

2.3 Electrochemical Measurements

The potentiodynamic and cyclic polarization tests were carried out in a standard three-electrode cell in 500 mL solution. The Ag/AgCl electrode was used as a reference electrode and platinum as a counter electrode. For each sample, a specific culture medium with their bacteria was used as an electrolyte solution for the experiments. The prepared samples for electrochemical measurements were immersed in the medium for 14 days. The electrochemical tests were performed using a potentiostat (Model Autolab type III) with Nova software version 2.1 according to ASTM G61. The corrosion measurements consist of stabilizing the working electrode in the corrosion test electrolyte at the open circuit potential (OCP) for 1 h with a scan rate of 0.2 mV·S⁻¹ within the range of - 300 to 500 mV at room temperature. And the polarization curves were analyzed using Nova 2.1 software.

The potentiodynamic and cyclic polarization tests for each sample were performed in the three different Zone (welding zone, heat-affected zone (HAZ), and base metal). These experiments were done for all cultured groups. All experiments were carried out at an ambient temperature and repeated at least three times. The tests were started after the open circuit potential of the samples reached a steady-state condition.

2.4 Scanning Electron Microscope (SEM)

After 90 days, ten prepared samples were extracted from their medium, and a visible biofilm was produced at their surface. A gold coating was applied on the surface of the samples via the sputtering method (Quorum Technologies model Q150R-ES). Each sample's corrosion product analysis

Table 1 Applied welding specification on the stainless steel 316L

Weld position	Process	Filler	Amp	Volts	Travel speed, cm/min	Gas flow rate
5G	GTAW	ER316L	70–110	9–15	4–8	7–11

was conducted using scanning electron microscopy (TESCAN-Vega 3). The images were produced in 3000 \times magnification (10 μm). The microstructure analysis of the samples was performed in each target zone and test group mentioned by scanning electron microscopy. (SEM, Leica Cambridge, Stereoscan S360, UK). A few samples were selected for examining the surface via energy-dispersive spectroscopy (EDS) after performing the SEM analysis.

3. Results and Discussion

Figure 1, 2, 3 shows the results of potentiodynamic polarization test. The corrosion parameter of the samples during the polarization test for each bacteria is listed in Table 2. These results indicate that corrosion resistance was reduced due to the presence of SRB, so the SRB increased the current density of welded stainless steel 316L compared to the anaerobic control sample. However, it should be noted that

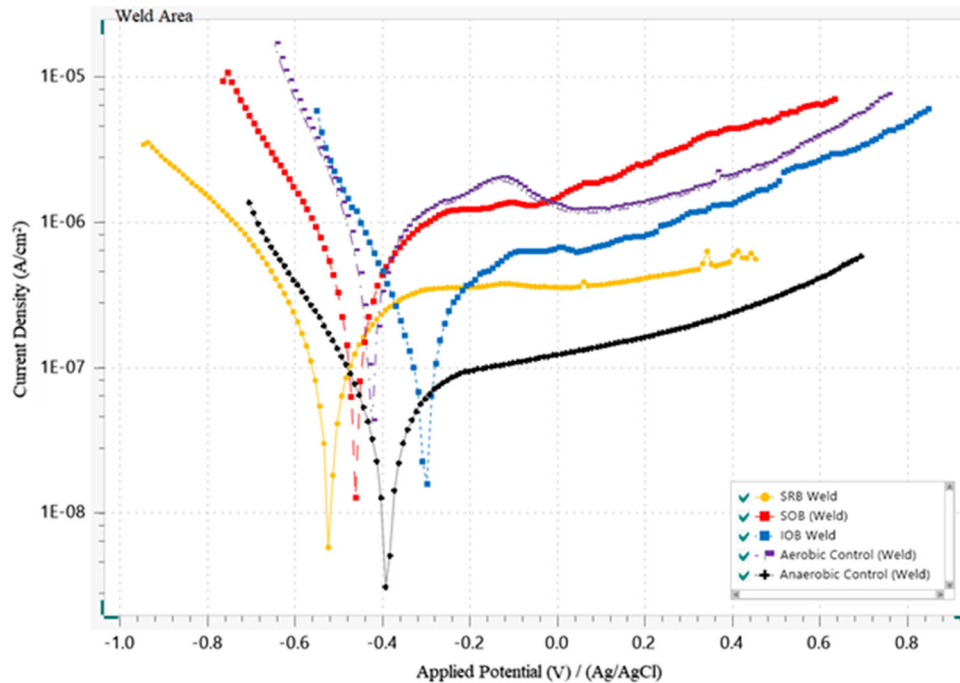


Fig. 1 The potentiodynamic curves for Weld zone of SS 316L immersed in control and with bacteria testing mediums

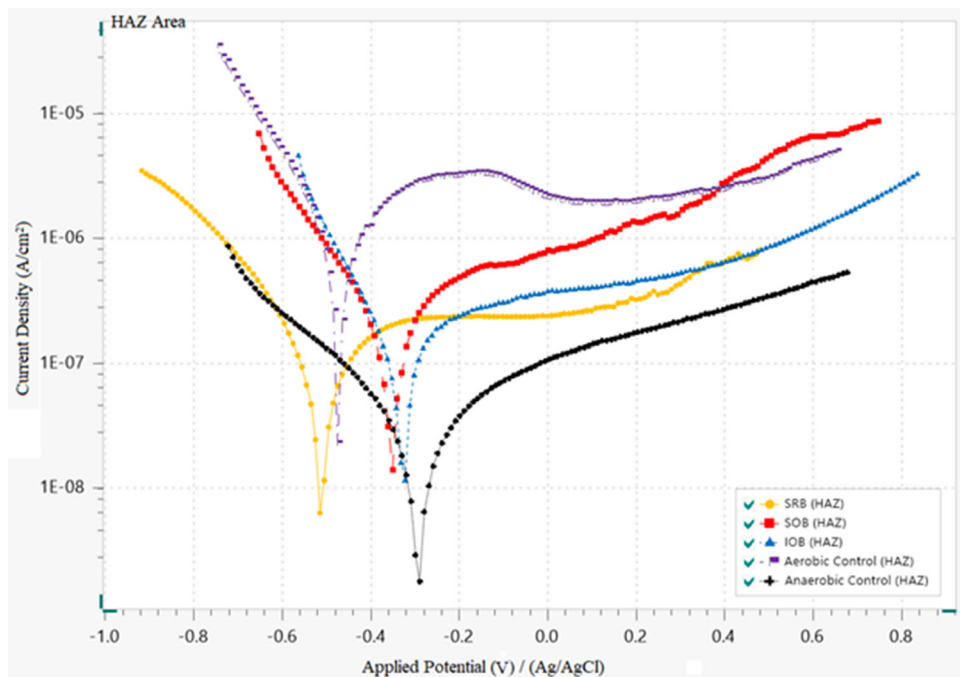


Fig. 2 The potentiodynamic curves for HAZ of SS 316L immersed in control and with bacteria testing mediums

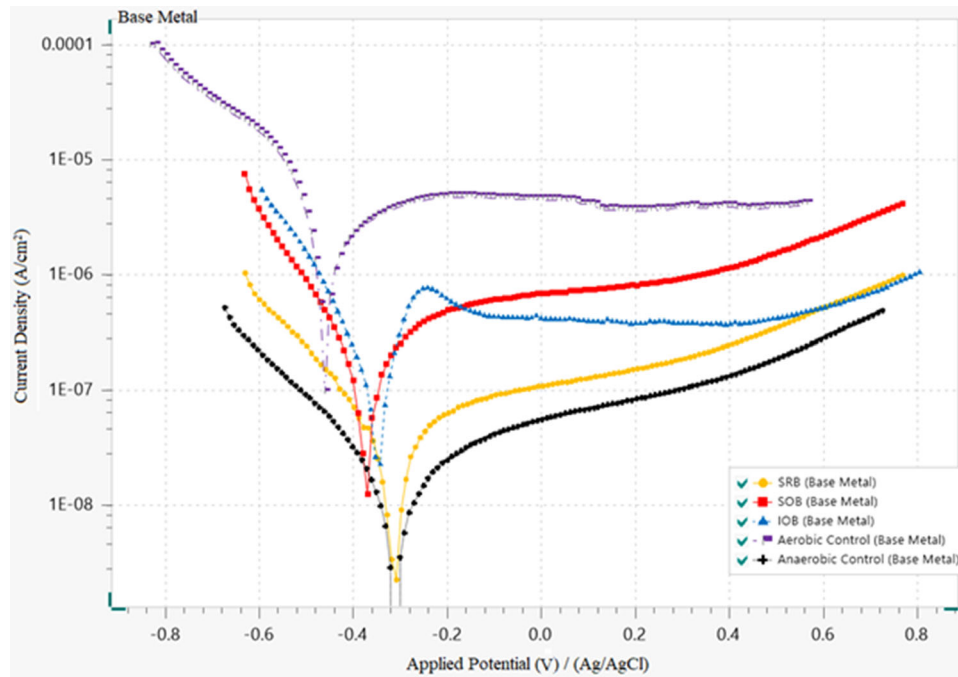


Fig. 3 The potentiodynamic curves of base metal of SS 316L immersed in testing mediums

Table 2 The results of polarization test for SRB and anaerobic control samples

	SRB weld area	Control weld area	SRB HAZ	Control HAZ	SRB base metal	Control base metal
E_{corr} (V)	- 0.520	- 0.388	- 0.510	- 0.292	- 0.305	- 0.310
R_p (Ω)	5.93×10^5	16.36×10^5	7.29×10^5	30.27×10^5	18.52×10^5	47.48×10^5
C.R. (mm/year)	6.20×10^{-4}	1.87×10^{-4}	5.69×10^{-4}	2.30×10^{-4}	1.79×10^{-4}	1.52×10^{-4}

Table 3 The results of polarization test for SOB and anaerobic control samples

	SOB weld area	Control weld area	SOB HAZ	Control HAZ	SOB base metal	Control base metal
E_{corr} (V)	- 0.462	- 0.425	- 0.352	- 0.472	- 0.372	- 0.461
R_p (Ω)	2.01×10^5	1.00×10^5	3.61×10^5	0.62×10^5	3.58×10^5	0.23×10^5
C.R. (mm/year)	22.5×10^{-4}	46.5×10^{-4}	18.5×10^{-4}	72.7×10^{-4}	7.43×10^{-4}	167×10^{-4}

Table 4 The results of polarization test for IOB and anaerobic control samples

	IOB weld area	Control weld area	IOB HAZ	Control HAZ	IOB base metal	Control base metal
E_{corr} (V)	- 0.301	- 0.425	- 0.325	- 0.472	- 0.346	- 0.461
R_p (Ω)	3.49×10^5	1.00×10^5	4.57×10^5	0.62×10^5	2.82×10^5	0.23×10^5
C.R. (mm/year)	17.8×10^{-4}	46.5×10^{-4}	14.8×10^{-4}	72.7×10^{-4}	15.0×10^{-4}	167×10^{-4}

the current density of the welded zone is higher than the current density of HAZ and base metal. Also, it can be seen that the IOB and SOB significantly affect the current density of SS

316L. The IOB and SOB decrease the current density of SS 316L compared to the aerobic control sample.

The electrochemical measurements of potentiodynamic polarization test were obtained via Tafel extrapolation (Table 2,

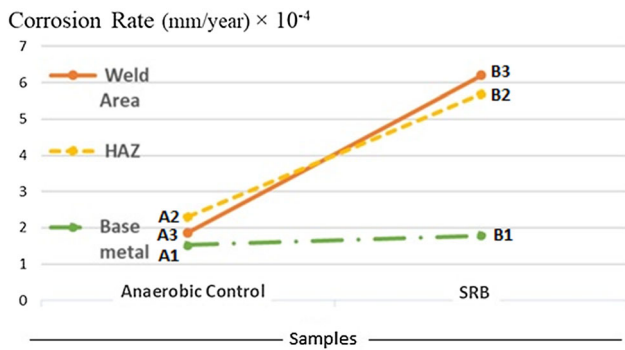


Fig. 4 The corrosion rate variation of specimens exposed to SRB compared to control samples

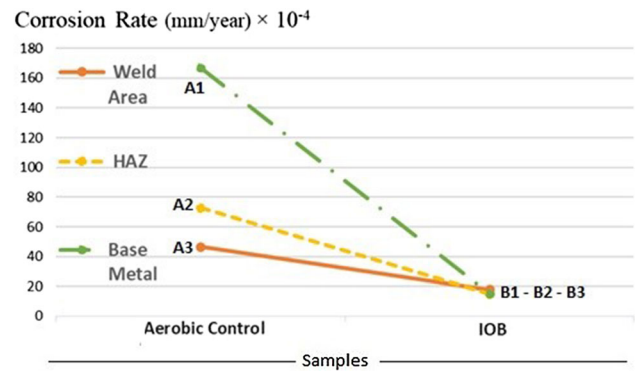


Fig. 6 The corrosion rate variation of specimens exposed to IOB compared to control samples

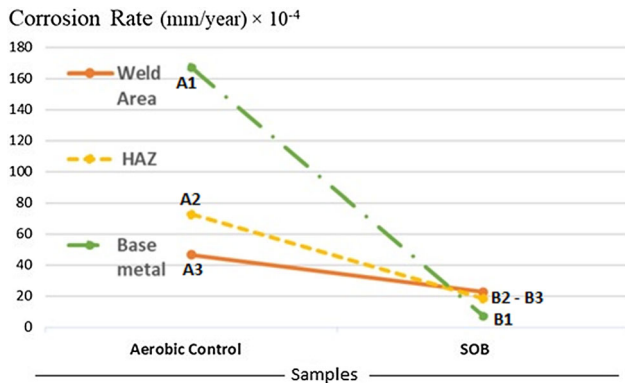


Fig. 5 The corrosion rate variation of specimens exposed to SOB compared to control samples

3, 4). Table 2 shows the corrosion rate ($C.R$), corrosion potential (E_{corr}), and polarization resistance (R_p) of welded specimens after exposure to the SRB environment. According to these results, the SRB increased corrosion rate and reduced E_{corr} and R_p of the alloy. The weld zone and HAZ had shown a significant increase in the corrosion rate. It may be concluded that welded SS 316L is susceptible to MIC caused by SRB in the weld zone and HAZ. Also, according to the obtained results, it is clear that SRB does not affect the corrosion resistance of the base metal.

Figure 4 to 6 summarizes the corrosion rate variation of specimens exposed to each bacteria compared to control samples. (A1 – A2 – A3) points are the calculated corrosion rate of SS 316L in the control medium, and (B1 – B2 – B3) show the corrosion rate of the samples exposed to each bacteria. These figures show the exact amount of increase and decrease in corrosion rate.

According to Table 3 and Fig. 5, the corrosion rate of SS 316L has been reduced after exposure to SOB, and an increase in corrosion potential and polarization resistance was observed. However, weld zone and HAZ have also experienced declining corrosion rates, and welding appears to have a negative effect on the protective properties of SOB. The highest decrease in corrosion rate was noticed in the base metal. The results of Fig. 6 are shown in Table 4. It can be concluded that the IOB was able to increase the corrosion potential and polarization

resistance, thus reducing the corrosion rate of SS 316L. Moreover, the corrosion rate in the base metal has decreased significantly.

The results of cyclic polarization tests are shown in Fig. 7, 8, 9. According to Fig. 7, SRB and anaerobic control samples have similar protection potential (E_{pp}) and pitting potential (E_p). Therefore, the SRB does not have a remarkable effect on the pitting corrosion of the welded stainless steel. It is evident that the sample has a negative loop which implies that a stable passive protective layer was formed on the surface of the sample (Ref 12). Likewise, after being exposed to SRB, it can be seen that the curve has a backward return, and a passive layer was formed on the surface. However, after increasing the applied potential, the protective layer was breached, and the current density rate was increased. The protection and pitting potential of the other two bacteria (IOB and SOB) were similar in the welding zone. In the anaerobic control sample, the polarization curve has a positive hysteresis loop (forward return), which indicates the protective layer is not created on the control sample, and the control sample had a high corrosion rate (Ref 12). It can be concluded that SS 316L in contact with IOB and SOB can produce a stable protective layer, and these bacteria have a protective effect on the alloy.

According to cyclic polarization curves in Fig. 8, the protection potential and pitting potential of the samples in the HAZ have insignificant differences, which means different bacteria did not affect the beginning of pitting and formation of the passive layer. Also, the anaerobic control sample has a negative loop which includes that a stable passive protective layer has been created on the surface of the sample. It may be noted that in the culture medium, the SRB can destroy the passive layer on the surface of stainless steel 316L on the HAZ. For the other two bacteria (IOB, and SOB) in the HAZ, it can be seen that the cyclic polarization curves have a negative loop. As a result, 316L SS in contact with IOB and SOB bacteria in the culture medium can produce a passive layer, and these bacteria have a protective effect on the alloy, and 316L SS in contact with the culture medium acted as an electron acceptor and was effectively protected against corrosion.

It can be seen in Fig. 9 that the 316L SS samples (base metal) have negative loops. And protection potential (E_{pp}) and pitting potential (E_p) had a slight difference. Also, the different bacteria did not have many effects at the beginning of pitting and formation of the passive layer. It is perceptible that a good

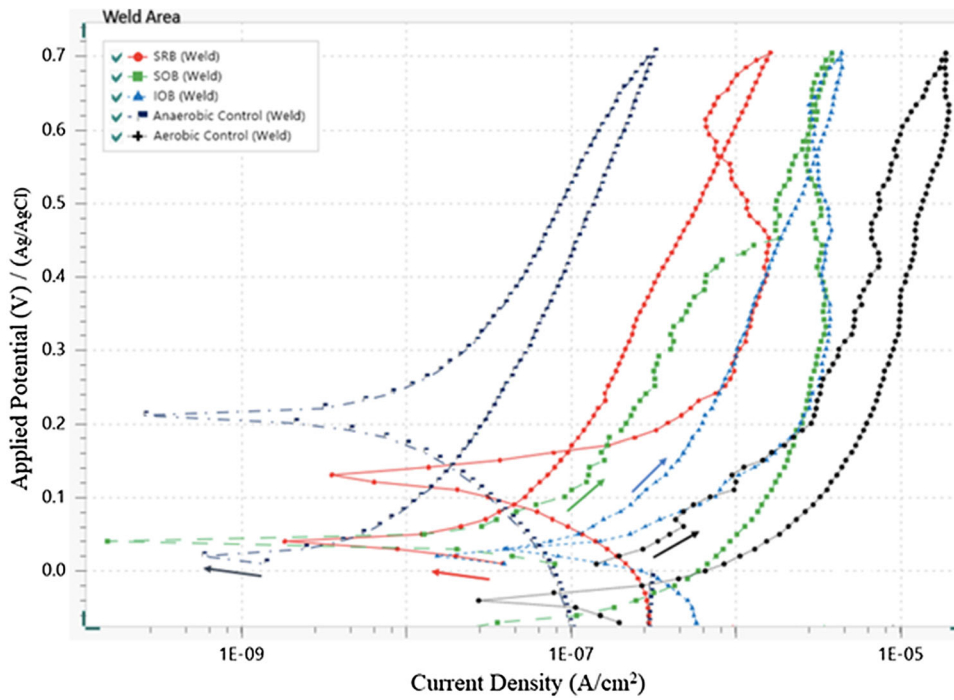


Fig. 7 The cyclic polarization curve for weld zone of SS 316L immersed in testing mediums

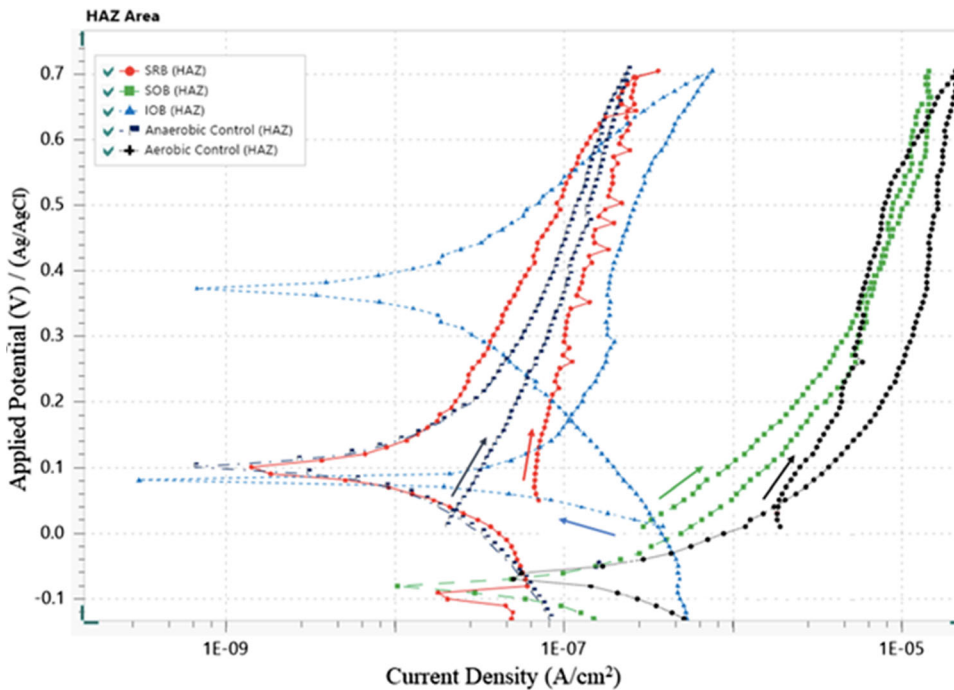


Fig. 8 The cyclic polarization curves for HAZ of SS 316L immersed in testing mediums

protective passive layer was formed on the 316L SS samples in this environment. Therefore, the corrosion resistance in the culture medium increased.

The results of SEM are shown in Fig. 10, 11, 12, 13. The images were produced in secondary electron mode with 3000X magnification (10 μm). Figure 10a-c contains the SEM images

of the samples before the corrosion tests in base metal, HAZ, and weld areas. Figure 11(a-c) shows SEM images of the stainless steel 316L alloy surfaces after 90 days' exposure to the culture medium with IOB, and Fig. 11(d) shows the surface of the aerobic control sample. Due to the activity of microorganisms, extracellular polymeric substance (EPS) has been

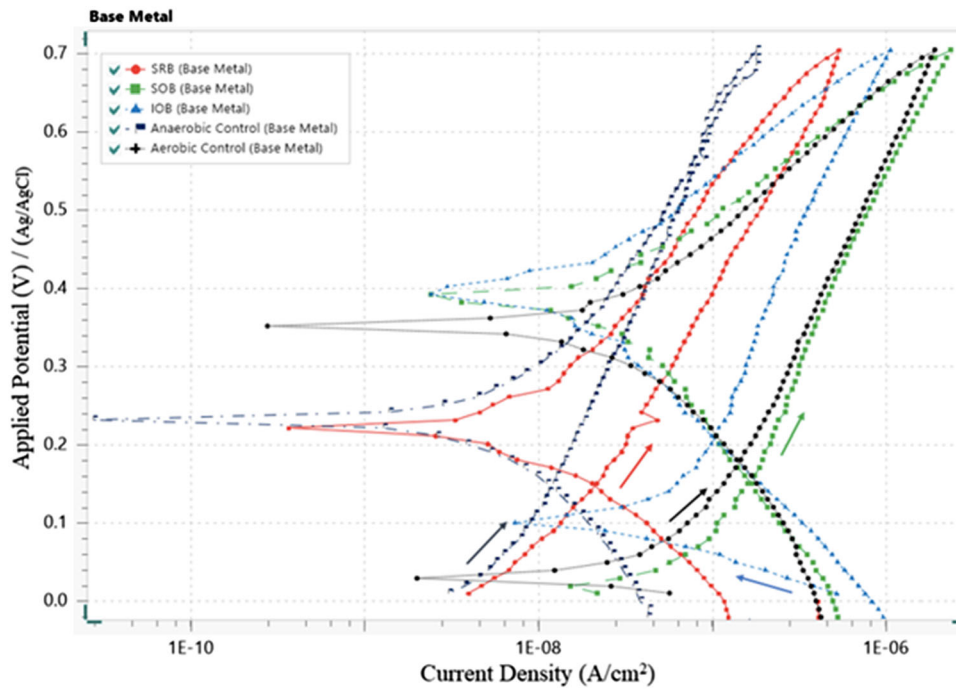


Fig. 9 The cyclic polarization curves for base metal of SS 316L immersed in testing mediums

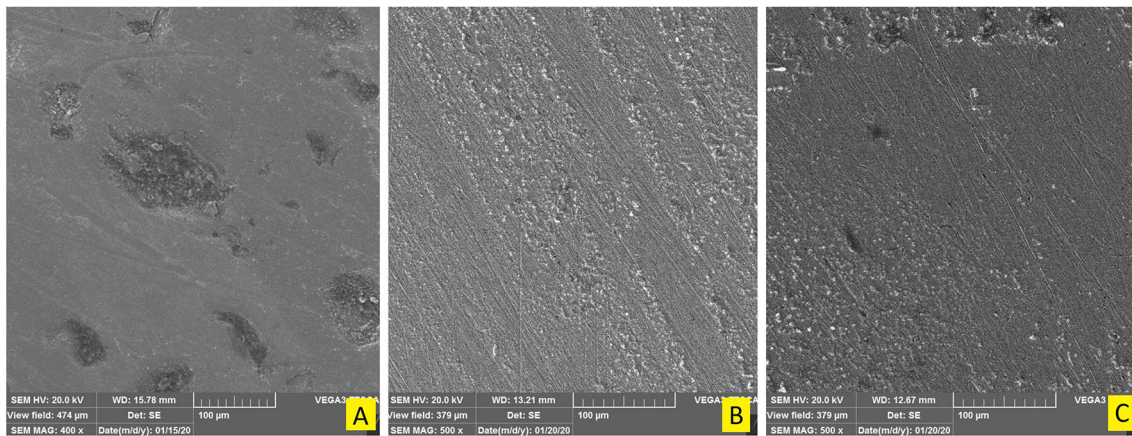


Fig. 10 The secondary electron images of 316L SS before the corrosion tests. (a) base metal—(b) HAZ area—(c) weld Area

produced on the surface of the alloy, while no biofilm and EPS are visible on the control sample.

The extracellular polymeric substance is an essential part of the biofilms and has a 3D complex and uniform structure. Mediating the adhesion of bacteria and cells to the surface, assisting and developing a strong link between a microorganism and the others, and protecting the microorganisms are among EPS' tasks. EPS can act as a trap around the biofilms and obtain organic materials and ions for the microorganisms (Ref 14, 15). According to previous studies, EPS usually contains polysaccharides, proteins, humic acids, uronic acids, and deoxyribonucleic acids. The presence of EPS on the metal surface can change the morphology and chemistry of corrosion products and affect corrosion resistance differently (Ref 16, 17). Biofilms and their effects on corrosion have always been a

debate among researchers. Some studies found that biofilms can make the corrosion process more aggressive; meanwhile, some researchers indicated that the corrosion rate had decreased in the presence of biofilms (Ref 18).

Over the last few years, scientists have found that microorganisms can develop an unexpected role in directly exchanging electrons with solid surfaces. In previous research, it has been found that an electron transfer can occur when aerobic biofilms are attached to the conductor surface, and bacteria may have a direct or indirect effect on protecting metals (Ref 19, 20).

In Fig. 11, it can be seen that a large amount of EPS was produced on the surface. According to the results of the electrochemical examination, the IOB was able to decrease the corrosion rate of SS 316L, so it can be concluded that EPS had a role in increasing the corrosion resistance, so in the culture

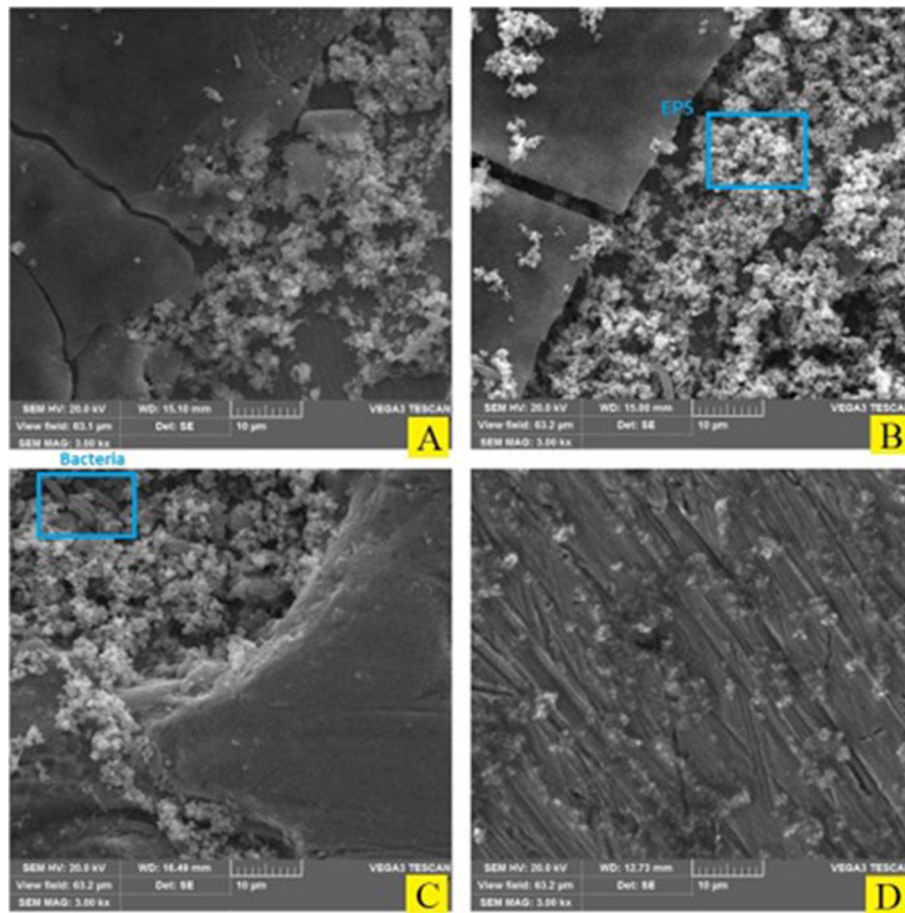


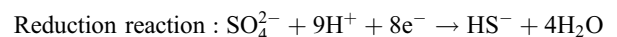
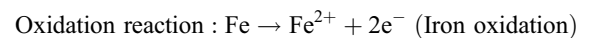
Fig. 11 The secondary electron images of 316L SS were immersed in the culture medium with and without IOB for 90 days. (a) weld zone with IOB—(b) HAZ with IOB—(c) base metal with IOB—(d) aerobic control sample

medium, IOB has a protective effect on welded SS 316L. Based on past research, EPS can transfer or share electrons from organic molecules of biofilms to the metal surface through chemisorption and electron tunnelling, thereby creating a coordinate type of bond. This process can effectively protect the metal's surface and decrease the corrosion rate (Ref 20). According to Fig. 12, it can be seen that SOB has also produced EPS and caused a similar effect on the welded SS316L, and the alloy was effectively protected.

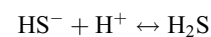
In a recent study, different mechanisms for the protection feature of microorganisms were discussed. Some aerobic microorganisms produce EPS and consume the oxygen near the metal surface, leading to the formation of an oxygen-free or low oxygen area, which can lead to corrosion inhibition. Moreover, another mechanism suggests that EPS components can form a corrosion inhibition barrier on the metal surface. The barrier effect of EPS relies on microbial species and environmental factors, including flow rate, temperature, ion species, and EPS concentration (Ref 5).

The SEM images shown in Fig. 13 indicate that SRB has also created EPS; however, after comparing these images with electrochemical test results, we can see that, unlike two other tested bacteria, SRB has increased the corrosion rate of welded stainless steel 316L. So SRB's EPS does not have a protective effect on welded SS 316L.

Studies about the corrosion mechanism of SRB suggest the biocatalytic cathodic sulfate reduction (BCSR) theory, which introduces two types of MIC: extracellular electron transfer MIC (EET-MIC) and metabolite MIC (M-MIC) (Ref 21, 22). EET-MIC or EET is a process in which SRB collects extracellular electrons and transfers them into the cytoplasm for energy production. Generally, SRB uses organic carbon sources as the electron donor, and sulfate acts as the electron acceptor. However, biofilms produced by SRB on the metal surface are dense, and it might block the SRB from acquiring organic carbon from the solution. Thus, SRB on the metal surface can obtain its needed survival energy as the electron donor. During the EET, the following two reactions occur:



Furthermore, the HS^{-} produced by SRB can decrease the pH near the metal surface and initiate the acidic metabolites (M-MIC) process through the following reaction, which increases the corrosion (Ref 2).



A model for this process is shown in Fig. 14.

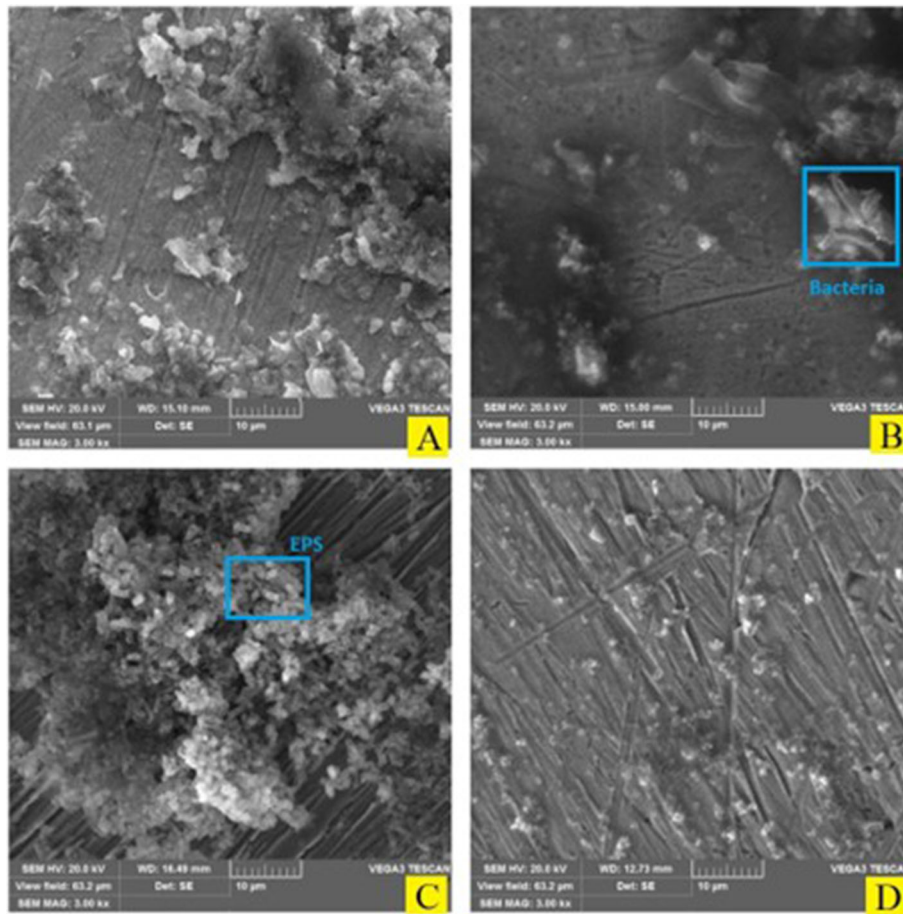


Fig. 12 The secondary electron images of 316L SS were immersed in the culture medium with and without SOB for 90 days. (a) weld zone with SOB—(b) HAZ with SOB—(c) base metal with SOB—(d) aerobic control sample

Figure 15 presents SEM morphology and EDS results for control, IOB, SOB and SRB samples. According to the results, the IOB sample has experienced an increase in biofilm and EPS-forming components such as phosphorus and oxygen. Moreover, a high concentration of EPS can be observed on the surface, resulting in a corrosion inhibition barrier of biofilms (Ref 5). The SOB results also show an increase in EPS-forming components; however, the surface's oxygen concentration was reduced compared to the control sample, and the SEM morphology suggests that SOB produces a lower concentration of EPS. These results recommend that SOB has consumed and lowered the oxygen concentration on the metal surface and reduced the corrosion rate (Ref 5). The EDS result for SRB influenced sample implies that while EPS-forming components were observed on the surface, the sulfur concentration on the surface was increased. As mentioned previously, sulfur is one of the components in corrosion products (Ref 2). Furthermore, SEM images suggest that SRB produced a localized concentration of EPS and led to localized corrosion occurring in SS316L.

4. Conclusion

After performing electrochemical measurements, SEM, and EDS analysis, it is concluded that SRB has a corrosive effect on the welded stainless steel 316L. The welded zone and HAZ of the stainless steel had the most corrosion rate enhancement, and the base metal had almost complete resistance against SRB.

According to the obtained results from electrochemical experiments, SEM, and EDS analysis, SOB can decrease the corrosion rate of stainless steel 316L. The SEM images showed that microorganisms produced EPS, and it is assumed that EPS was able to protect the surface metal by transferring electrons from organic molecules to the metal surface.

Electrochemical evaluation, SEM, and EDS analysis showed that IOB had reduced the corrosion rate of stainless steel 316L. The SEM analysis revealed that IOB was able to produce EPS and protect the metal's surface via electron transfer from organic molecules to the metal surface.

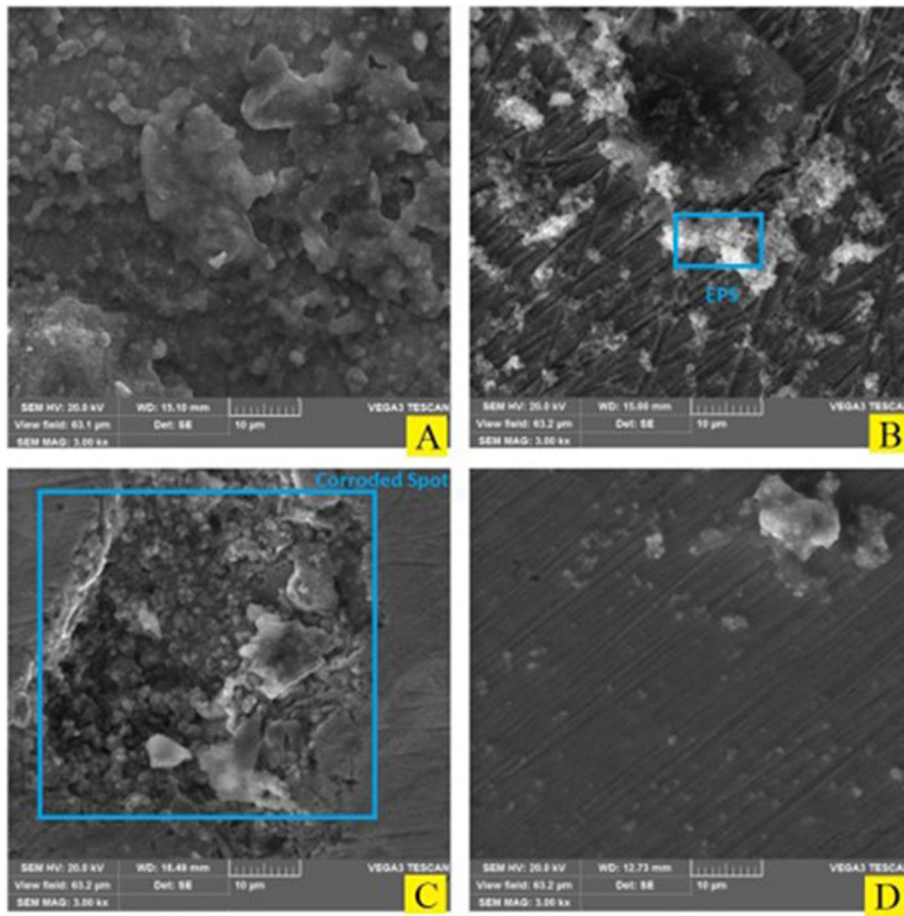


Fig. 13 The secondary electron images of 316L SS were immersed in the culture medium with and without SRB for 90 days. (a) weld zone with SRB—(b) HAZ with SRB—(c) base metal with SRB—(d) anaerobic control sample

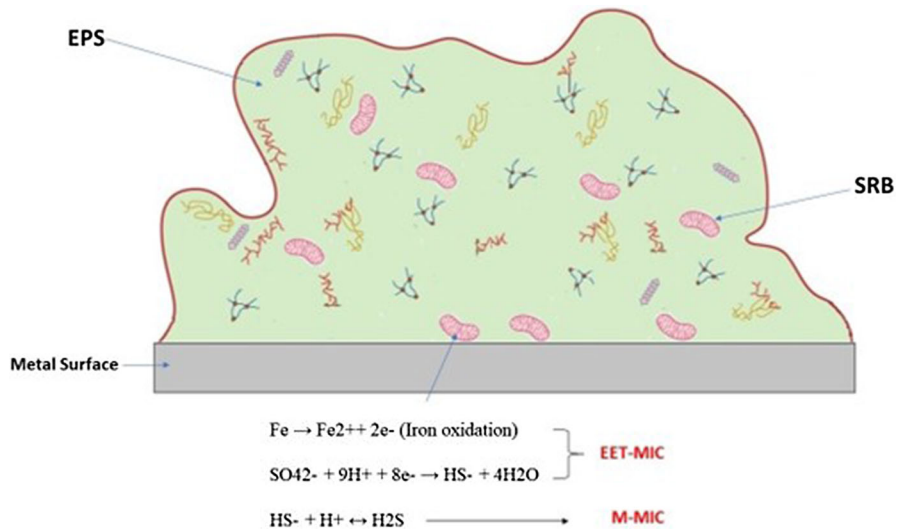


Fig. 14 A model for SRB corrosion mechanism

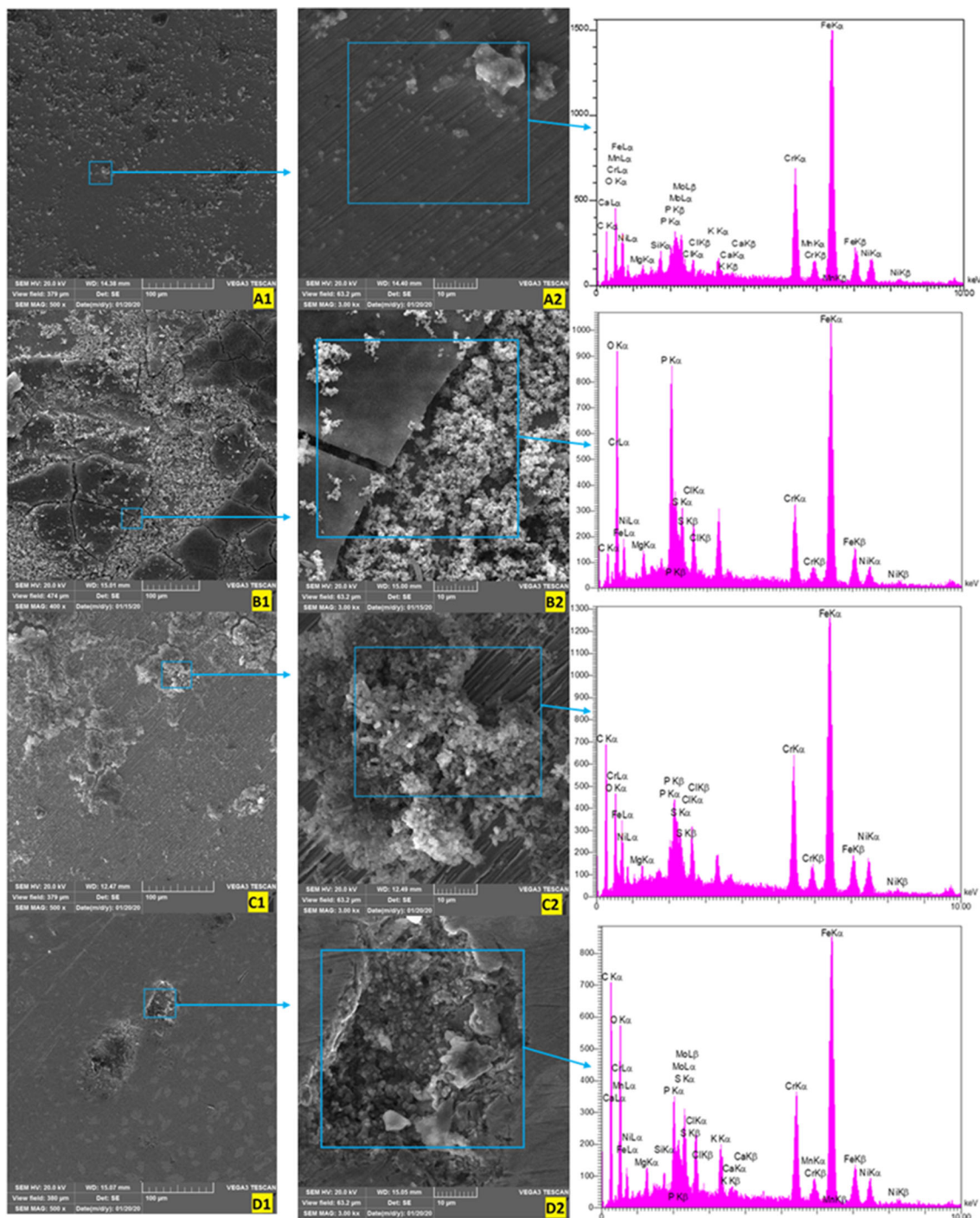


Fig. 15 The secondary electron images and energy-dispersive spectroscopy of 316L SS in control medium and the bacteria culture medium after 90 days of immersion. (a1-a2) control Sample—(b1-b2) IOB sample—(c1-c2) SOB sample—(d1-d2) SRB sample

Acknowledgments

This research has been performed using the facilities by Shiraz Branch, Islamic Azad University, Shiraz, Iran.

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