EFFECT OF MICROBIOLOGICALLY INFLUENCE CORROSION OF DISSIMILAR WELDED JOINT BY PSEUDOMONAS AERUGINOSA

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Abstract. Microbiologically influenced corrosion (MIC) is a type of destructive corrosion that can be initiated, facilitated, or accelerated by the presence of bacteria. The study aims to assess the MIC behavior of dissimilar welded joint substrates exposed to the existence of the Pseudomonas aeruginosa inoculated nutrient-rich simulated sea water medium (NRSS). The formation of the biofilm layer, corrosion precipitates and pits on the dissimilar welded substrate is studied using field emission scanning electron microscopy (FESEM), X-ray diffraction, energy dispersive spectroscopy (EDS), weight loss, and corrosion rate methods. The corrosion rate of a welded coupon immersed in a bacteria-inoculated medium was higher than that of a sterile NRSS medium. The FESEM results revealed an aggressive role for pseudomonas aeruginosa biofilm and bacteria colonisation in inducing corrosion and producing significant pits on welded joints in the HAZ area. This study may contribute to a better understanding of the MIC behavior of dissimilar welded joints caused by bacterial colonisation and biofilm formation.

Keywords: dissimilar welded joint, pseudomonas aeruginosa, pitting, corrosion rate

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Introduction

Bio-Corrosion, also known as Microbiologically Influenced Corrosion (MIC), is an electrochemical process [1] that includes bacterial attachment and biofilm formation, which modify the electrochemical disorder at the metal substrate/solution interface, thus speeding up the MIC corrosion process [2-3]. Microorganisms such as bacteria, viruses, archaea, and even fungi can introduce and facilitate MIC. Microorganisms can attack most metal substrate materials, including low carbon steel, stainless steel, and copper [2-4], and significantly increase localised and pitting corrosion. MIC is a current and serious problem in a various environments, including the fresh water, seawater, soil, cooling water, and oil field produced water etc.

Bacteria are small microorganisms that are ubiquitous in the environment, particularly in the seawater. Stainless steel and low carbon steel, for example, are frequently welded together in marine environment, where they are subjected to extremely corrosive bacterium such as pseudomonas aeruginosa [5]. Pseudomonas aeruginosa is an aerobic bacterium, meaning it can live and proliferate in an oxygen-rich environment. To overcome the above problems, several researchers in recent times have focused on the application of fish collagen in skin regenerating template due to their lower production cost, ease of availability of raw materials and lower or no possibility of zoonosis as compared to mammalian source collagen [5]. However, the drawback of fish collagen is their weak mechanical properties and low biostability (fast degradation rate *in vitro* and *in vivo*). The strength and biostability of scaffolds directly influence on cell infiltration, adhesion, proliferation and new tissue development. Thus, the collagen needs to blend with other polymer to enhance their mechanical strength and biostability.

It is a motile, rod-shaped gram-negative bacterium easily transfers from one metal surface to another [5]. This bacterium can be found in large quantities in the marine environment [2, 6]. In the process of biofilm formation and growth, pseudomonas aeruginosa has been identified as an inventor colonist [6, 7]. In the marine environment, pseudomonas aeruginosa has been shown to increase the corrosion rate of carbon steel, stainless steel, and many former metal substrates [6-8]. Despite the fact that dissimilar welded metals such as low carbon steel and 316L stainless steel have been used in a variety of fields such as pipelines, offshore structures, and so on [9-10]. The applications of dissimilar welded joints in oil and gas are due to their low material cost, lightweight, and robust corrosion resistance [11-12]. While little is known about MIC mechanisms in the marine environment. There is still a scarcity of research on the MIC of dissimilar welded joints of low carbon steeland 316L stainless steel in the marine environment.

The corrosion behaviour of dissimilar welded joint low carbon steel and 316L stainless steel in the absence and presence of pseudomonas aeruginosa in the nutrient-rich simulated sea water medium (NRSS) to imitate the marine environment was explored in the current work. In the absence and presence of pseudomonas aeruginosa, an immersion study was carried out to investigate the corrosion behaviour of dissimilar welded joint low carbon steel and 316L stainless steel. The surface morphology, biofilm and pits formation, bacteria cell and EPS attachment of the samples in sterile and bacteria inoculated medium were studied using scanning electron microscopy (SEM), X-ray diffraction, and energy dispersive spectroscopy (EDS).

Materials and Methods

Dissimilar welded joint substrate material. The current investigation utilised low carbon steel and 316L stainless steel plate. Both materials were delivered in the form of a 12 mm thick plate. The chemical composition of the base and the filler used are shown in Table 1. The base material used in the present study for gas tungsten arc welding (GTAW) was both plates of size 100 mm × 50 mm × 12 mm, and the filler was ER316L electrode. The joint used for this project is a butt joint, and the geometry of the butt joint is a single V with a 60^{o} angle.

Table 1. Chemical composition (wt. %) of the base metal and filler used

Alloy element	C	Si	Mn	P	S	Cr	Ni	Cu	Fe
Low Carbon Steel	0.174	0.176	0.624	0.028	0.021	0.104	0.078	0.223	Balance
316L Stainless Steel	0.028	0.248	1.46	0.041	-	17.80	9.80	0.551	Balance
Filler (ER316L)	0.056	0.658	1.35	0.035	-	16.00	9.53	0.119	Balance

Following the welding process, the welded pieces were subjected to X-ray radiography and a Dye Penetrant test. Faulty and poorly welded pieces were excluded. Welded pieces with no defects or faults were prepared for microbial-induced corrosion testing analysis. All test samples were cut into smaller pieces of around 50 mm × 10 mm × 12 mm using an CNC wire cut Machine Sodick AQ537L. All of the specimens were cleaned prior to the test. The cleaning procedure included grinding with SiC paper up to 1200 grit, carried out by grinding machine, and finally were cleaned using alcohol solution.

Preparation of NRSS medium. The NRSS medium was made according to the formulation of Yuan et al. [8-10], and the pH was adjusted to around 7.2 by adding 5 M NaOH. The medium consists of 23.476 g/L NaCl, 3.917 g/L Na_2SO_4, 0.192 g/L $NaHCO_3$, 0.664 g/L KCl, 0.096 g/L KBr, 10.61 g/L $MgCl_2.6H_2O$, 1.469g/ L $CaCl_2.2H_2O$, 0.026 g/L $H3BO_3$, 0.04 g/L $SrCl_2.6H_2O$, 3 g/ L bacteriological peptone, and 1.5 g/L yeast extract. Except for the yeast extract, which was purchased from Scharlay Company, all chemicals were purchased from QreC Company. Both media were autoclaved for 20 minutes at 121 ^{o}C and 15 psi pressure to ensure sterilisation.

Culturing of pseudomonas aeruginosa bacteria. Pseudomonas aeruginosa, bacteria code number DSM 50071, was used in this study and was obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DSMZ). The following steps were used to prepare and culture pseudomonas aeruginosa on agar plates:

P. aeruginosa bacteria 100 mL NRSS medium (batch 1)

↓

10 mL NRSS from batch 1 + 90 mL NRSS medium (batch 2)

↓

10 mL NRSS from batch 2 + 90 mL NRSS medium(batch 3)

↓

6 mL NRSS from batch 3 + 299.4 mL NRSS medium (prepared batch for immersion test)

To prepare each batch, the mixture was shaken in a shaker for 24 h at 30^{o}C at a speed of 150 rev min^{-1}. The optical density (OD) values were used to calculate the bacterial cell concentration. According to the standard calibration, an OD of 1.0 corresponds to ~10^{9} cells mL^{-1}. When the OD value was close to 1.0, the aseptically prepared specimens suspended on nylon strings were introduced into the inoculated medium [14-15].

Immersion test. The dissimilar welded joint samples were immersed in two types of NRSS medium for the immersion tests: (i) biotic medium (bacteria inoculated medium) and (ii) abiotic (control) medium (sterile medium). All of the samples were kept at room temperature in a stagnant environment. Every 7 days, 75% (225 mL) of the NRSS medium inoculated with pseudomonas aeruginosa, has drained away and replaced with an equivalent amount of fresh NRSS medium. This was done to keep the bacteria density close to the steady-state growth phase throughout the experiment.

The corrosion rate of the uncoated specimens was determined using the weight loss method. The values obtained were for qualitative observation, which provides some indicating of the coating materials corrosion performance. The dissimilar welded metal samples were cleaned in accordance with ASTM G1–72 after drying. It was then weighed to determine the weight loss as the difference between the initial and final weights. The corrosion rates values were calculated using the Faraday's law as indicated in equation (1):

$$\text{Corrosion rates (mm/year)} = \frac{K \times W}{A \times T \times D} \quad (1)$$

where: K = 8.76 × 10^{4}, T = time of exposure (hour), A = exposed surface area (cm^{2}), W = mass loss (g), and D = density (g/cm^{3})

FESEM/EDS analysis. A Field Emission Scanning Electron Microscope (FESEM, VP35 Zeiss Supra, Germany) equipped with an Energy Dispersive X-ray Spectrometer (EDS) was used to observe the biofilm layer formed on the dissimilar welded joint substrate.

Results and Discussion

The mechanism of MIC in sterile and inoculum solution was investigated using dissimilar metal welded joint specimens. Figure 1 shows a top view of dissimilar metal welded with different zones exposed to inoculated medium for MIC identification in three different zones: base metal, HAZ area, and weld metal with a corrosion problem. The following sections describe the visual inspection, immersion, and electrochemical and the corrosion behaviour of MIC at dissimilar metal welded joint surfaces in sterile NRSS and inoculate medium for 3, 7, 14, 28, and 42 days immersion times.

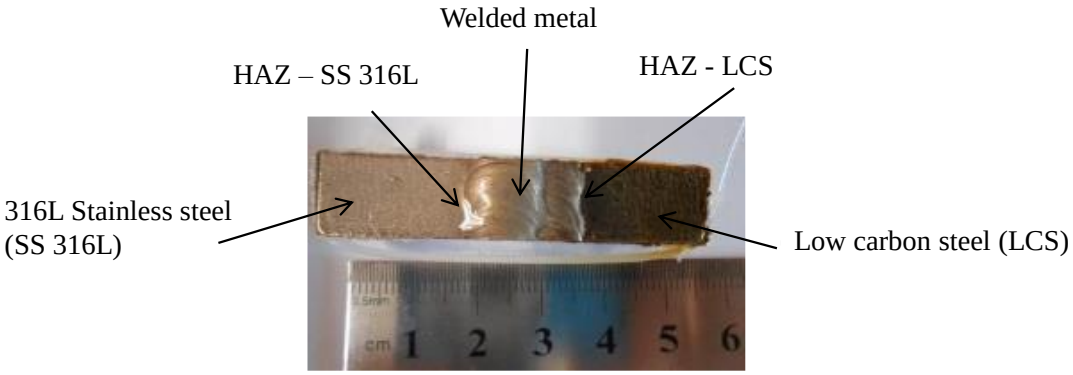

Figure 1. Top view of dissimilar welded metal with different zones exposed to inoculated medium.

Visual inspection. The visual appearance of the uncoated dissimilar welded joint substrate exposed to bacteria- inoculated medium with different immersion times (3, 7, 14, 21 and 42 days) is shown in Figures 2(a) to (e). The dissimilar welded joint structure comprises three different zone, i.e base metal HAZ areas and a single weld metal zone. The attachment of bacteria planktonic pseudomonas aeruginosa cells from the inoculated medium were attached uniformly on the dissimilar welded metal substrate. They formed a biofilm layer at the surface of the three different zones. A biofilm layer appears as a slimy white-coloured precipitate that forms on a dissimilar welded metal surface [2, 6-8, 10-17, 18] for stainless steel 316 material. While for the low carbon steel material covered by the brownish color of corrosion product. The initial activity of bacterial cells resulted in the formation of a biofilm layer on the dissimilar welded metal surface, which included all three zones, causing sever uniform and localised corrosion from 3 to 42 days immersion times.

The biofilm layer was formed on the uncoated substrate with corrosion products, particularly on the low carbon steel HAZ area and base metal, whereas the formation of an oxide layer at the surface provided excellent corrosion protection for the stainless steel 316L weld metal, HAZ area, and base metal. The thickness of the biofilm grew as the immersion duration increased from 3 to 42 days. A biofilm layer is also produced on the slimy white-coloured layer formed on the dissimilar metal welding surface.

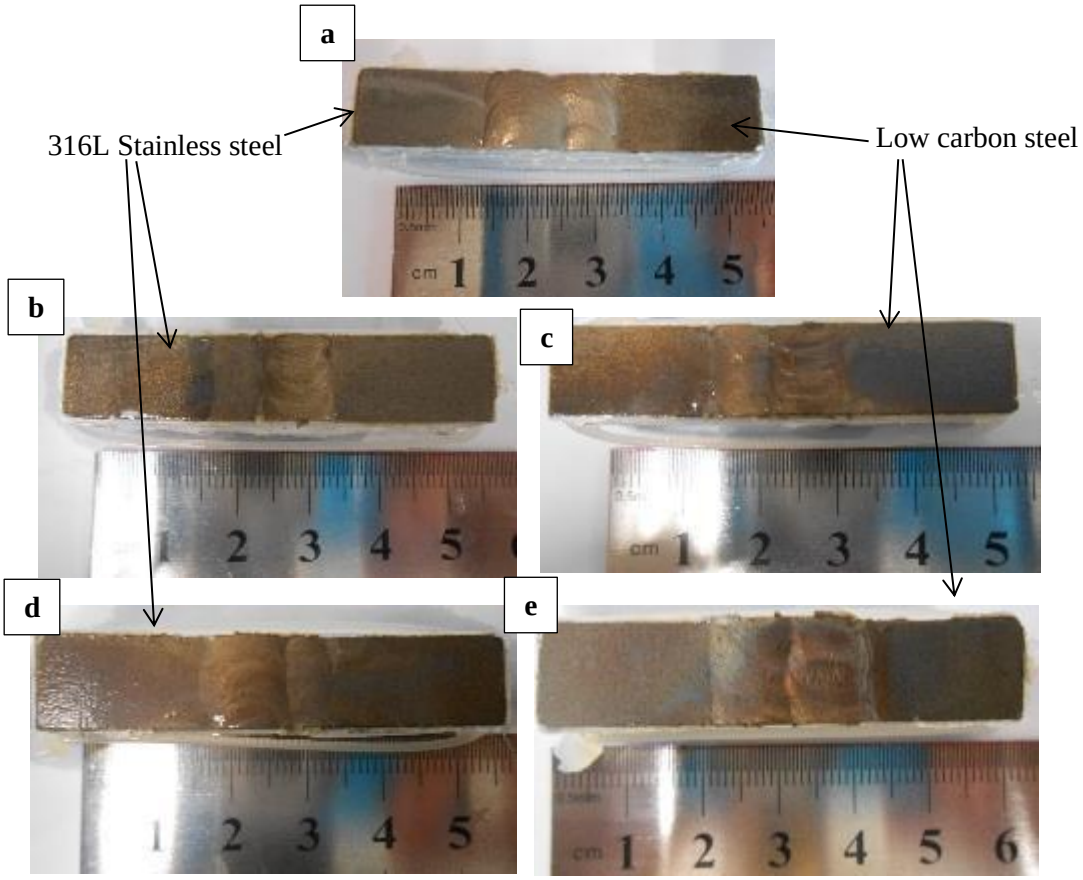

Figure 2. Visual inspection of uncoated dissimilar metal welded joint substrates exposed to inoculated medium after different immersion times (a) 3 days (b) 7 days, (c) 14 days, (d) 28 days, and (e) 42 days.

The visual appearance of the dissimilar welded metal substrates exposed to sterile NRSS medium at different immersion times is shown in Figure 3. In Figures 3(a) to (e), it shows the different immersion test sample from 3, 7, 14, 21 and 42 days respectively in sterile medium which means absent of pseudomonas aeruginosa bacterium. Due to the salt concentration in the medium, no biofilm formation occurred on the uncoated substrate, and corrosion products only appeared on the surface, particularly on the low carbon steel HAZ area and base metal. The formation of corrosion products increased as the immersion period increased from 3 to 42 days. Due to the lack of bacteria in the medium, no slimy white-coloured layer formed on the dissimilar welded metal surface.

Weight loss and corrosion rate measurement. The weight loss method was used in the initial study of corrosion rate measurement, in which each dissimilar welded metal substrate was cleaned and dried before being weighed before and after the immersion test. Figure 4 displays the weight loss results after immersion test. Based on the equation (1), the corrosion rate results were computed. They are only used to prove and support the visual inspection results with the existence of rust at the welded surface that contributes to corrosion behaviour with the presence and absence of pseudomonas aeruginosa bacteria in NRSS medium. The weight loss results show the increasing of weight loss since the exposure times also increased.

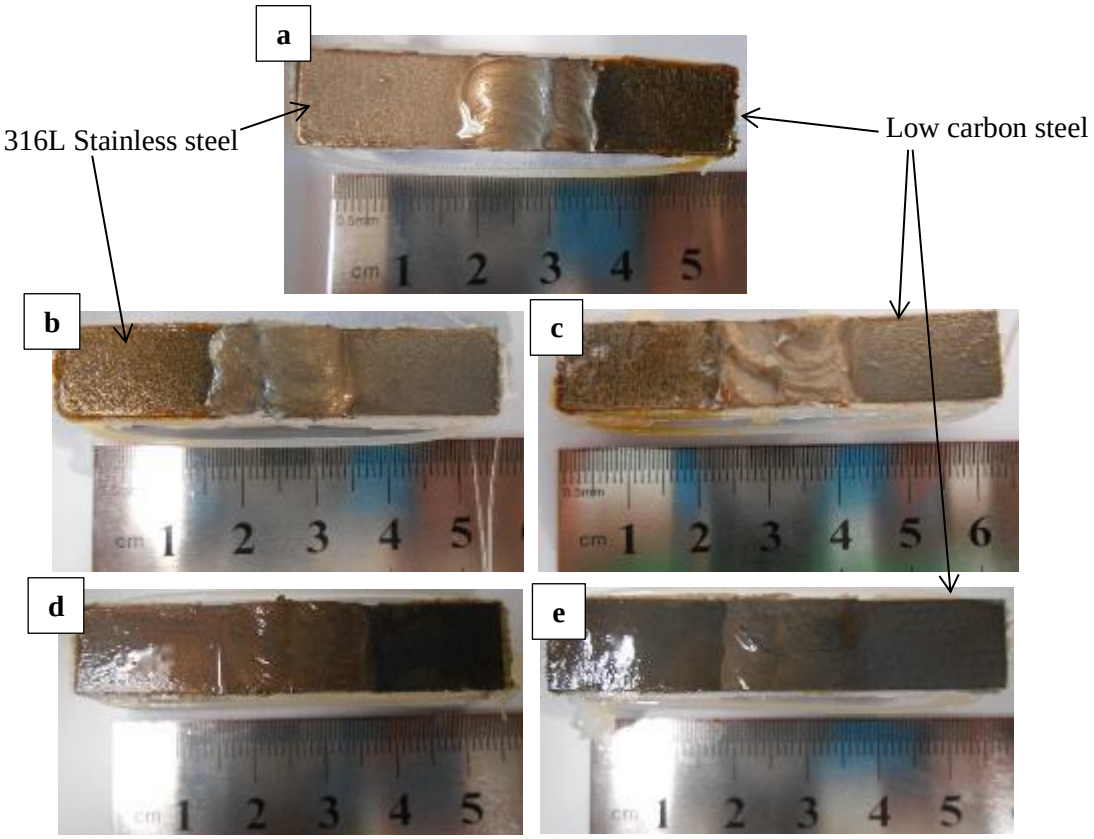

Figure 3. Visual inspection of dissimilar metal welded joint substrates exposed to sterile NRSS medium after different immersion times (a) 3 days (b) 7 days, (c) 14 days, (d) 28 days and (e)42 days.

The corrosion rate for both media was calculated after each immersion test with exposure times ranging from 3 to 42 days, and the results are shown in Figure 5. The corrosion rates for the steel substrate in bacteria-inoculated medium and the dissimilar welded metal substrate in sterile NRSS medium were found to gradually decrease over time.

The increase in corrosion products on the steel substrate surface may result in oxide formation as a protective layer, slowing the corrosion rate. The dissimilar welded joint substrate corrosion rate in the bacteria inoculated medium is higher than that in the sterile NRSS medium (Figure 5). Figure 6 shows the schematic diagram the formation of pseudomonas aeruginosa biofilm induces the corrosion damage on the welded substrate via the activation of differential aeration cells by generating the anode and cathode sides of area. The centre area on the welded surface basically under a low partial pressure of oxygen that normally occurs under the biofilm which becomes anodic relative to the welded surface nearby that has not been fully covered by the biofilm formation become the cathode. The cells differential aeration can cause the corrosion damage on welded surface. The presence of Cl^- ions in NRSS medium could synergistically enhance corrosion process. Clearly it shows the interaction of both pseudomonas aeruginosa biofilms and chloride (Cl^-) ions have an aggressive role in the occurrence of pitting corrosion on welded surface [11-16].

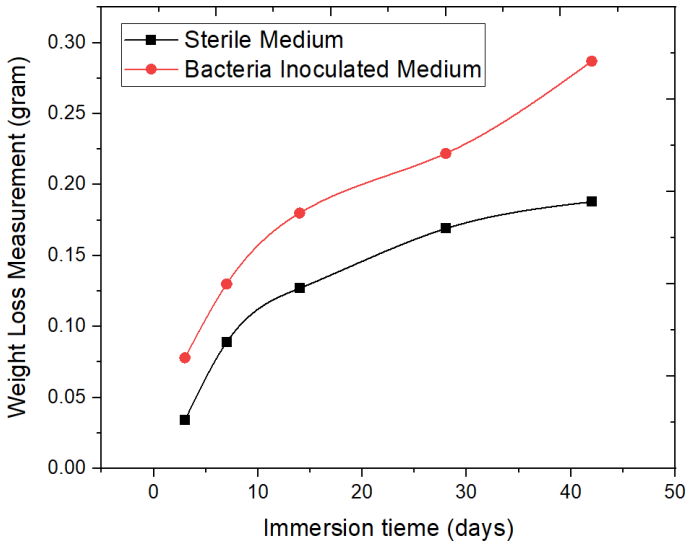

Figure 4. Weight loss values of dissimilar welded joint substrate immersed in sterile NRSS and bacteria inoculated medium.

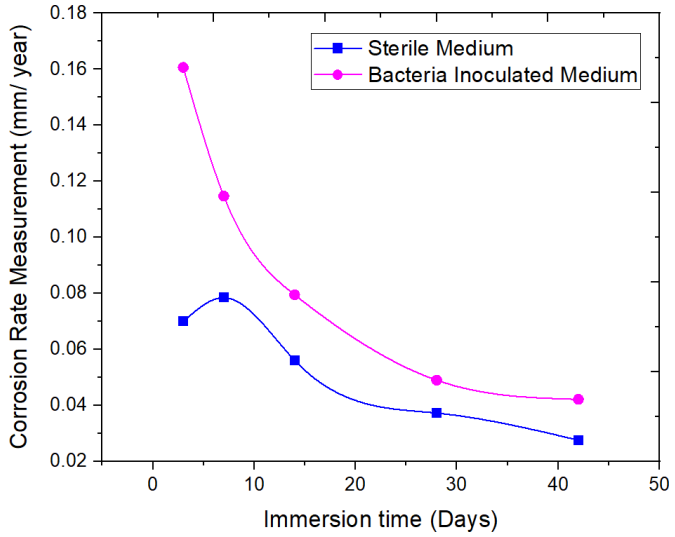

Figure 5. The corrosion rate for dissimilar welded joint substrates exposed to sterile NRSS andbacteria inoculated medium at varying immersion times.

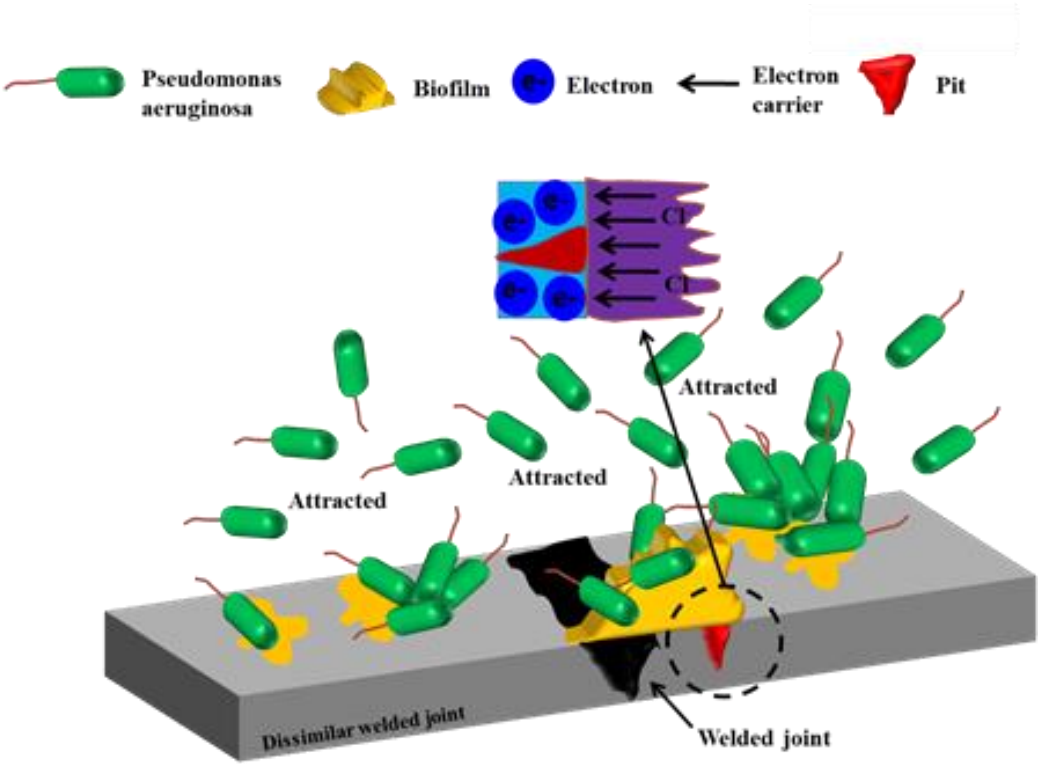

Figure 6. Schematic illustration of microbial-influence corrosion (MIC) mechanism at dissimilar metal welded joint surface.

The present of the bacterium pseudomonas aeruginosa in the NRSS medium causes an aggressive attack or 'eating' of the metal surface, increasing the corrosion rate. Furthermore, the biofilm formation on the dissimilar metal welded joint substrate surface causes chemical changes in the composition of the dissimilar metal welded joint surface. Moreover, the increased corrosion rate is caused by severe damage to the low carbon steel surfaces, resulting in in the formation of differential aeration cells [14 - 16]. According to the findings, the corrosion rate for dissimilar metal welded joints in bacteria inoculated medium is higher than that for dissimilar metal welded joint in sterile NRSS medium.

Different base metal (low carbon steel and 316L stainless steel) and filler metal (stainless steel) compositions may cause galvanic corrosion, resulting in an electrochemical potential difference and making some weld regions more active. For marine environments, it is necessary to carefully select the appropriate filler metal [1, 17]. Practitioners typically choose the weld filler so that the joint is considered stainless rather than carbon steel. The weld bead should ideally be covered so that only the 'parent' stainless steel is visible. This is done to prevent galvanic corrosion cells from forming across the joint with a composition 'gradient' [18, 20]. In general, a protective coating is applied to both the dissimilar welded joint and the parent metal to reduce the chances of galvanic corrosion in any environment caused by variations in the weld metal composition [18-20]. As a result, because all of the dissimilar welded specimens were coated with self-healing coating for MIC studies, galvanic corrosion issues can be avoided for this study.

Surface characterization by FESEM/EDS. Figure 7 shows a FESEM image of dissimilar welded metal substrates with three main zones on the cross area, which are weld metal, HAZ area with two different metals, and two different base metal materials after 28

days exposure in pseudomonas aeruginosa inoculated medium. The bacteria covered the entire area of the welded structure at the difference area namely base material, HAZ and weld metal uniformly. HAZ and low carbon steel base material were discovered as the most severe area, because the bacterial cells covered the entire surface, promoting localised corrosion. Due to the corrosion protection provided by the oxide film, there were fewer bacteria cells in the weld metal, HAZ area, and stainless steel 316L base metal as shows in Figure 7(a), (b) and (c). The worst corroded areas were found at low carbon steel base material and HAZ are closed to LCS base material as demonstrate in figure 7(d) and (e) because the pseudomonas aeruginosa cell and EPS attached at this area.

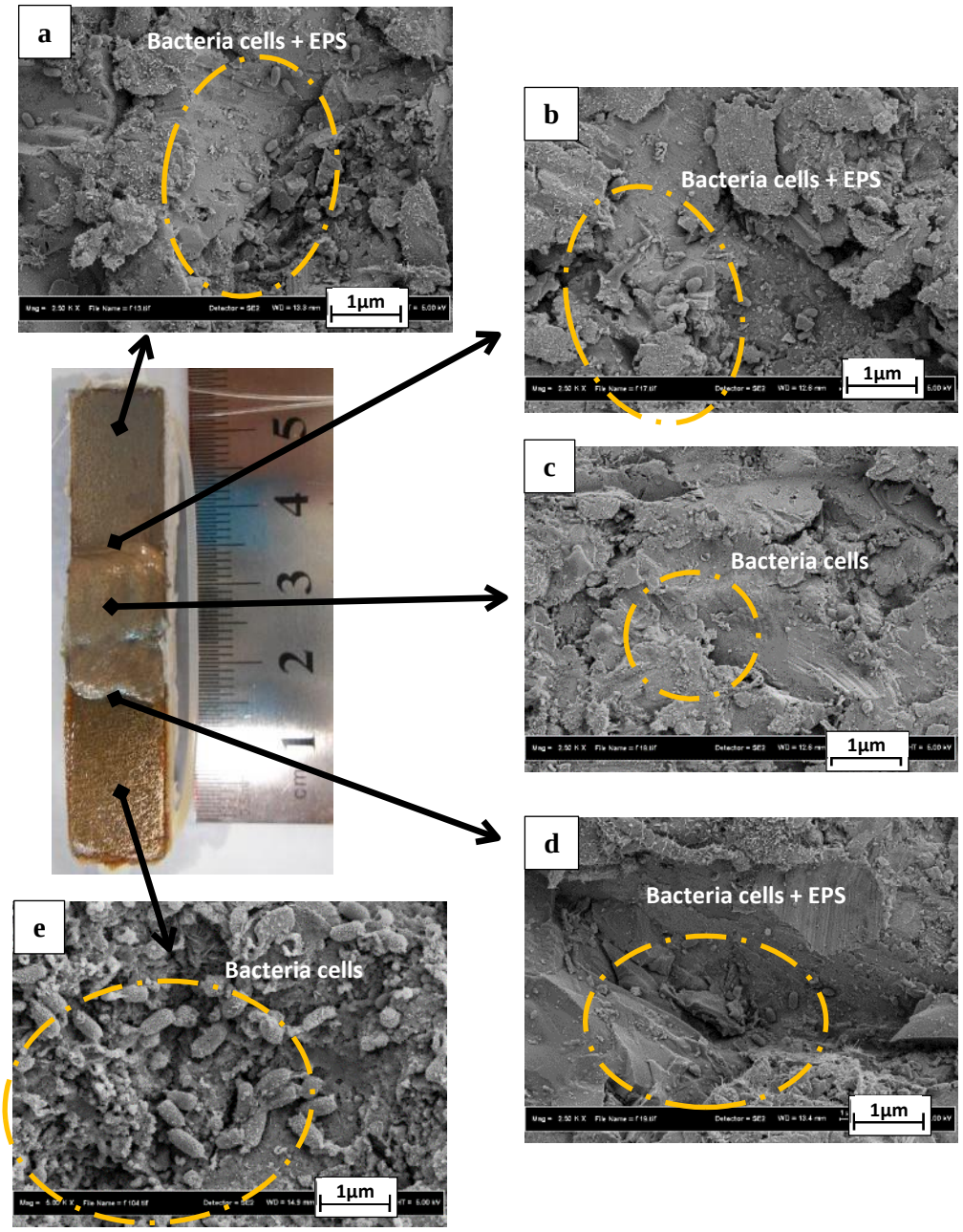

Figure 7. FESEM micrographs of dissimilar metal welded joint substrate after 28 days of exposure pseudomonas aeruginosa inoculated NRSS medium (a) 316L stainless steel (SS 316L), (b) HAZ- SS316L, (c) Welded metal, (d) HAZ- LCS and (e) Low carbon steel (LCS).

Figure 8 depicts FESEM micrographs of dissimilar welded metal substrates before and after 42 days of exposure to sterile NRSS medium; other specimens immersed for different lengths of time, 3, 7, 14, and 28, displayed the same morphology and characteristics. Figures 8(a) and (b) show no bacteria cells or colonies, biofilm, or corrosion products on the welded structure before and after immersion. According to observations, the mineral attachment and corrosion product increased the thickness due to the extended exposure time on the dissimilar welded metal substrate after being in the sterile NRSS medium.

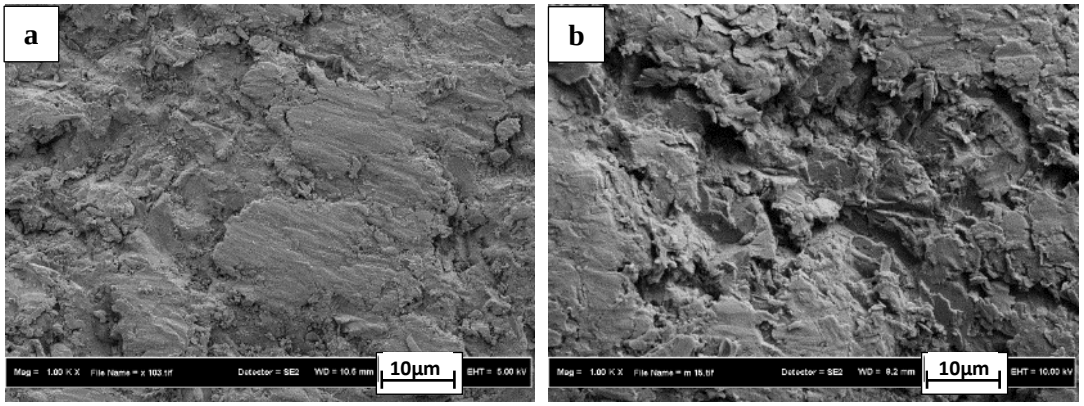

Figure 8. FESEM micrographs of dissimilar welded joint substrate average (a) before immersion test and (b) 42 days after exposure to sterile NRSS medium.

Figures 9(a) and (b) depict the EDS spectrum of a bacteria cell and the formation of a biofilm on a welded substrate. C, O, Si, Cl, Ca, and Na are elements found in the biofilm layer (Figure 9(c)). The chemical composition of the biofilm layer is related to the presence of high levels of carbon and oxygen. Corrosion products comprise Fe and O caused by biofilm growth combined with hostile ions like Cl^- and O_2 which can cause corrosion as shown in figure 9(d). The presence of Ca, Cl, Si, and Na in the EDS results is related to the NRSS medium's composition. Figure 8(d) shows the EDS results for a dissimilar metal weld joint before immersion testing, revealing that only Fe and O are present on the welded substrate (LCS material side).

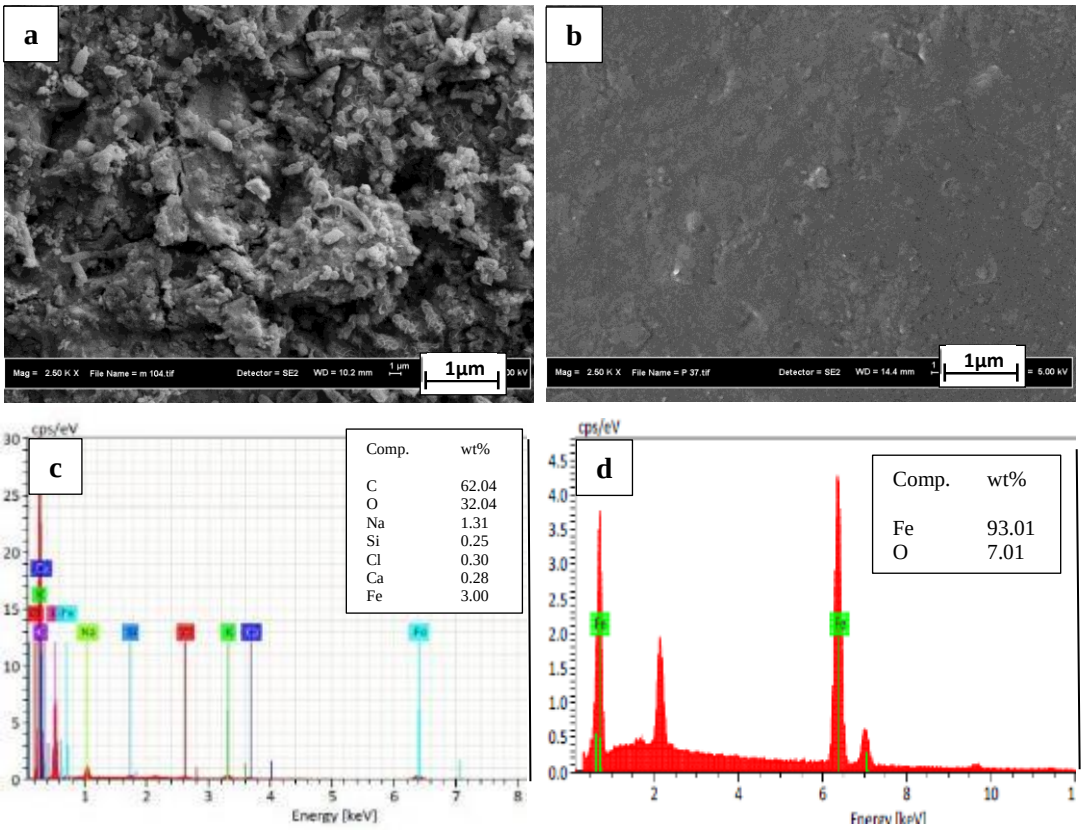

Figure 9. FESEM images and EDS spectrum of the (a) biofilm and pseudomonas aeruginosa bacteria on dissimilar welded joint after 42 days of immersion in bacteria inoculated medium and (b) DMW before immersion test.

Conclusion

The corrosion behaviour of dissimilar welded joints in the absence and presence of a marine aerobic strain pseudomonas aeruginosa in a nutrient-rich simulated seawater-based medium was investigated. The weight loss and corrosion rate results from the immersion test showed that, despite a slight increase in the bacteria-inoculated medium compared to the sterile nutrient-rich medium, it was relatively low due to the absence of pseudomonas aeruginosa. Compared to other areas, the HAZ close to low carbon steel is more severe on bacteria inoculated medium.

FESEM images of the surface and interface revealed that the heterogeneity and coverage of biofilms formed intact the entire dissimilar welded surface augmented exposure time, and that extensive uniform and localised corrosion occurred beneath the biofilms.

EDX spectra confirmed that localised corrosion was caused by the synergistic effect of violent chloride ions and the colonisation of bacterial cells and their EPS, which resulted in the breakdown of the passive oxide film and thus increased the likelihood of MIC.

In contrast, no apparent signs of uniform and localised corrosion were detected on the welded surface after exposure in the sterile nutrient-rich medium, except for the formationof thin conditioning layers and mineral deposition.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare

Compliance with Ethical Standards

The work is compliant with ethical standards

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