Biocorrosion of 5083 aluminium alloy by *Citrobacter freundii* SKC-4 in seawater

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Abstract. The 5083-aluminium alloy, a widely utilized aluminium-magnesium alloy known for its high strength and excellent corrosion resistance, is commonly employed in various applications, including shipbuilding. Despite its inherent resistance to seawater, the presence of microorganisms such as sulphate reducing bacteria (SRB) significantly exacerbates its corrosion. In this study, immersion and electrochemical tests were performed on 5083 aluminium alloy in both sterilized seawater and seawater enriched with SRB *Citrobacter freundii* SKC-4. Prior to the corrosion tests, *Citrobacter freundii* SKC-4 was cultivated in modified Luria-Bertani medium under specific conditions. The results demonstrated increased corrosion rates in the presence of the bacteria, with extended immersion leading to greater weight loss of the alloy. Analyses using Scanning Electron Microscope-Energy Dispersive Spectroscopy Mapping and Fourier Transform Infrared confirmed the formation of biofilms by *Citrobacter freundii* SKC-4 on the alloy's surface, clearly indicating the occurrence of biocorrosion.

1 Introduction

Aluminium alloys are widely utilized in various applications owing to their superior characteristics, including being lightweight, robust, and having a high strength-to-weight ratio. The 5083-aluminium alloy, known for its excellent corrosion resistance and stability at low temperatures, is frequently used in industries such as shipbuilding, railcar manufacturing, and vehicle body construction. Its primary alloying component is magnesium, complemented by minor additions of manganese and chromium.

Corrosion is a primary factor leading to the degradation of alloys. Aluminium and its alloys exhibit considerable resistance to corrosion in aqueous environments due to the formation of a protective passive oxide layer on their surfaces. This layer effectively impedes further oxidation reactions, thereby also preventing reduction reactions on the alloy's surface. Nonetheless, the aggressive chloride ions found in seawater can compromise this passive layer, leading to localized corrosion. Additionally, the presence of microorganisms, such as bacteria in seawater, can alter the interaction between the alloy and the seawater, resulting in an alternative corrosion mechanism known as biocorrosion.

Biocorrosion refers to the accelerated degradation of metals caused by biofilms on their surfaces [1]. These biofilms, comprising bacterial colonies and extracellular polymeric substances produced through their metabolic processes, adhere to metal surfaces. A common bacterial type involved in biocorrosion in shipbuilding is sulphate-reducing bacteria (SRB). SRBs are anaerobic microorganisms that convert sulphates into sulphide ions. Previous studies have documented that SRBs exacerbate the corrosion of various metals including carbon steel, stainless steel, copper, brass, titanium, magnesium, and aluminium [2-11]. This research aims to assess the impact of SRB *Citrobacter freundii* SKC-4 on the corrosion behaviour of 5083 aluminium alloy in seawater. *Citrobacter freundii* SKC-4, isolated from Domas Crater, Tangkuban Perahu Mountain, West Java, Indonesia, is capable of thriving in a pH range of 1.5 to 8 and at temperatures between 20-70°C.

2 Materials and Methods

For the immersion test, 5083-aluminium alloy specimens were prepared with dimensions of 3 cm x 5 cm. For electrochemical testing, specimens with an exposed area of 1 cm² were connected to copper wires and then encased in resin. Prior to the

experiments, all specimens were cleaned and ground. Seawater from the Java Sea was filtered and sterilized in an autoclave at 1.2 atm pressure and a temperature of 121°C for 20 minutes to eliminate contamination from other microorganisms. *Citrobacter freundii* SKC-4 was cultured in a modified Luria-Bertani (LB) medium supplemented with 2 g/L $FeSO_4.7H_2O$, 5 g/L $Na_2S_2O_3.5H_2O$, 10 g/L tryptone, and 5 g/L yeast extract.

The immersion experiment was conducted in a beaker containing sterile seawater and a 10% volume/volume addition of bacteria-enriched sterile seawater, reaching a total volume of 250 mL. Following a specified immersion duration, each specimen was air-dried, then submerged in a 70% nitric acid (HNO_3) solution for five minutes to eliminate corrosion products. The weight difference between the initial and final states of each specimen was meticulously recorded. Additionally, electrochemical analysis was performed using a Gamry Reference 600 potentiostat. This setup included a silver/silver chloride (Ag/AgCl) reference electrode and a graphite counter electrode. The methodology involved potentiodynamic polarization measurements within a potential window of ±200 mV relative to the open circuit potential (OCP), at a scan rate of 0.167 mV/s.

Surface characterization was performed using Scanning Electron Microscopy coupled with Energy Dispersive Spectroscopy Mapping (SEM-EDS Mapping). Prior to this analysis, specific preparation steps for the specimens were implemented. Following immersion in seawater containing bacteria, a 2.5% glutaraldehyde solution was applied to the specimen's surface, which was subsequently stored in a refrigerator for 24 hours to preserve the biofilms. Subsequent to this incubation, the specimen underwent a rinsing process in phosphate buffer solution, followed by sequential immersion in acetone solutions of increasing concentrations (25%, 50%, 75%, and 100%), with each step lasting for 15 minutes. The specimen was then left to air dry for a period of 24 hours. Additionally, Fourier transform infrared spectroscopy (FTIR) analysis was carried out on the corrosion products that were scraped off from the specimen's surface post-immersion.

3 Results and Discussion

3.1 Bacterial Growth Curve

The growth curve of *Citrobacter freundii* SKC-4 was established to ascertain its optimal growth timeframe. Fig. 1 illustrates the seven-day incubation growth profile of *Citrobacter freundii* SKC-4. Colony counts were conducted every 24 hours using the Total Plate Count (TPC) method. The growth duration of bacteria is influenced by various environmental factors, including hydrostatic pressure, solar radiation, temperature, salinity, pH, oxidation-reduction potential, and nutrient availability, as noted in reference [12]. Elevated salinity levels in seawater can lead to cellular membrane disruption. In response, bacteria may adapt by minimizing energy expenditure on metabolic activities, thereby impeding growth. The data reveal that the maximum colony count was observed on the second day. Consequently, for subsequent experiments, the bacteria harvested after two days of incubation were utilized.

3.2 Analysis of Sessile and Planktonic Phase

Fig. 2 illustrates the proportion of sessile to planktonic bacteria following immersion periods of 7, 14, and 30 days. Sessile bacteria refer to colonies adhering to the aluminium surface, whereas planktonic bacteria are those dispersed in seawater. The results indicate that, on day 7, a mere 9.5% of the bacterial colonies were sessile on the aluminium surface. This percentage of sessile bacteria increased over time. By day 14, the proportion of sessile bacteria rose sharply to 98.5%, while planktonic bacteria decreased to just 1.5%. On day 30, the sessile bacteria constituted 99.8% of the total colony, suggesting that *Citrobacter freundii* SKC-4 forms a biofilm on the 5083 aluminium alloy surfaces. It has been reported that the sessile growth of SRB during biocorrosion of aluminium alloys is favoured due to the protective and nutrient-rich environment of the biofilm, their heightened resistance to antibacterial agents, and a distinct transcriptional profile that enhances their survival and proliferation [13-18].

3.3 Immersion Test

Immersion experiments were carried out over periods of 7, 14, and 30 days using both sterile seawater and seawater inoculated with bacteria. Data on weight loss from these tests were employed to determine the corrosion rates of the materials. As depicted in Fig. 3, the corrosion rate of the 5083-aluminium alloy in seawater with *Citrobacter freundii* SKC-4 was markedly elevated for all tested durations of immersion. Notably, specimens immersed for 30 days exhibited significantly higher corrosion rates compared to those immersed for 7 and 14 days. In sterile seawater, aggressive ions are

known to compromise the protective oxide layer on the aluminium surface. Prolonged immersion leads to intensified degradation of this protective layer [19]. Conversely, in seawater enriched with bacteria, extended immersion periods allow for increased bacterial metabolic activity and colony formation, resulting in the development of a denser biofilm that envelops the aluminium surface.

3.4 Potentiodynamic Polarization Test

The potentiodynamic polarization curves depicted in Fig. 4 reveal that the corrosion current density of the 5083-aluminium alloy in seawater containing bacteria is approximately an order of magnitude higher than in sterile seawater. In both environments, the aluminium alloys exhibit active-passive behaviour. The passive region, characterized by a marginal increase in current, signifies the protective role of the oxide layer in inhibiting aluminium dissolution. This protective effect is attributed to the formation of a passive oxide film on the aluminium surface, serving as a barrier to further oxidation and corrosion [20]. Notably, despite a higher passive current density, the passive region in bacteria-enriched seawater is marginally broader compared to that in sterile seawater.

3.5 SEM and FTIR Analyses

The Scanning Electron Microscope (SEM) imagery in Fig. 5 and the Fourier Transform Infrared (FTIR) spectroscopy data in Fig. 6 demonstrate the formation of biofilms on the surface of 5083 aluminium alloy in seawater inoculated with *Citrobacter freundii* SKC-4, indicative of biocorrosion. The FTIR spectrum identifies various chemical bonds present on the biofilm's surface, including the Al-OH bond at a wavenumber of 915 cm^{-1}, the H-S bond within the range of 2530-2580 cm^{-1}, the O-H bond at 3446.79 cm^{-1}, C-H and -CH_3 bonds at 1385 cm^{-1}, -NH_2 and C-N bonds at 1540 cm^{-1}, and C-N and C=O bonds at 1660 cm^{-1}, as referenced in literature [21-24].

4 Conclusions

From the comprehensive experimental data, it is evident that the sulphate-reducing bacterium *Citrobacter freundii* SKC-4 forms a biofilm which significantly accelerates corrosion on the surface of 5083-aluminium alloy. The maximum observed corrosion rate was 0.8078 mm/year in aluminium samples following a 30-day immersion in seawater containing this bacterium. Surface characterization techniques further substantiated the existence of biofilm on the aluminium surface.

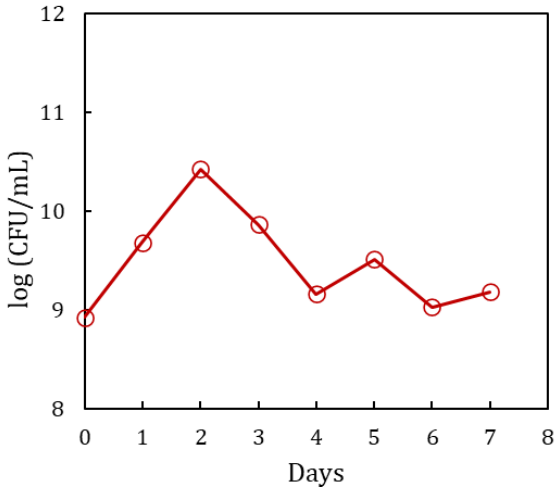

Fig. 1. Bacterial growth curve

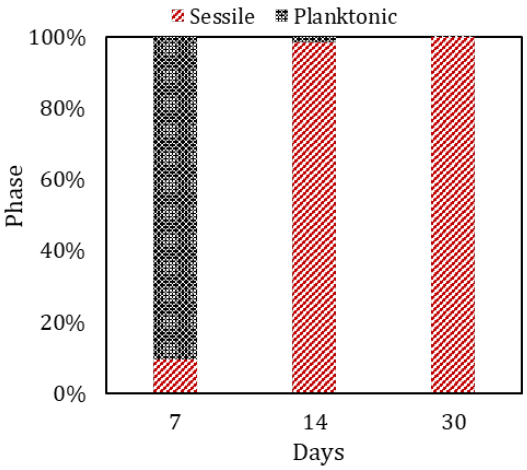

Fig. 2. Proportion of sessile to planktonic bacteria

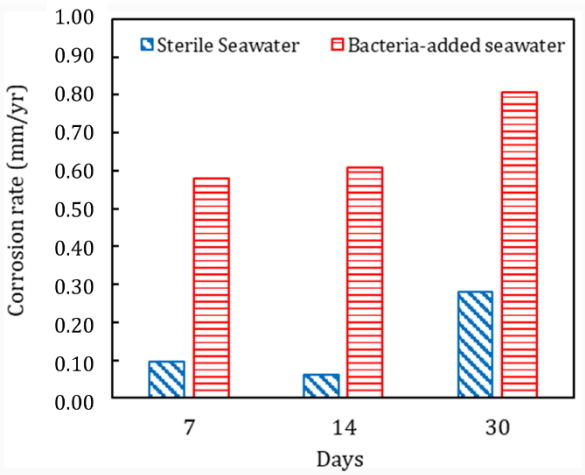

Fig. 3. Comparison of corrosion rates in 5083 aluminium alloy in seawater, with and without bacterial presence

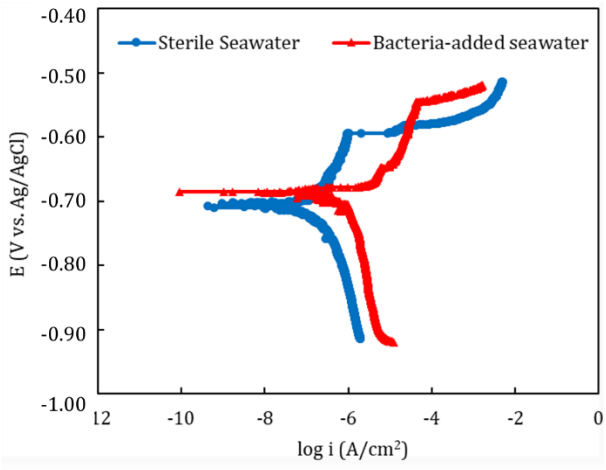

Fig. 4. Potentiodynamic polarization curves of 5083 aluminium alloy in seawater, comparing conditions with and without bacterial presence

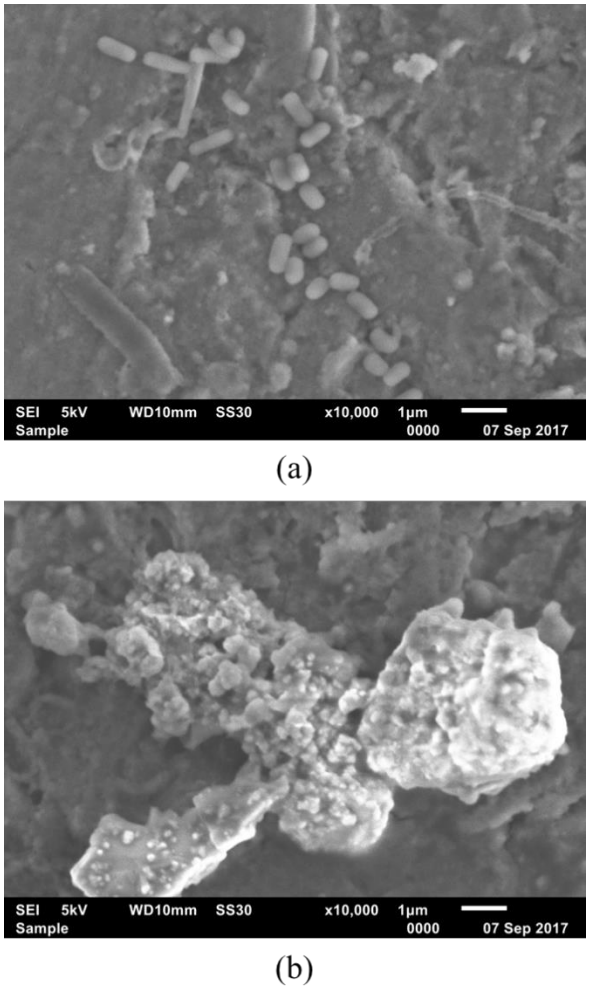

Fig.5. SEM images depicting (a) bacteria attachment and (b) biofilm formation on 5083 aluminium alloy following immersion in seawater inoculated with *Citrobacter freundii* SKC-4

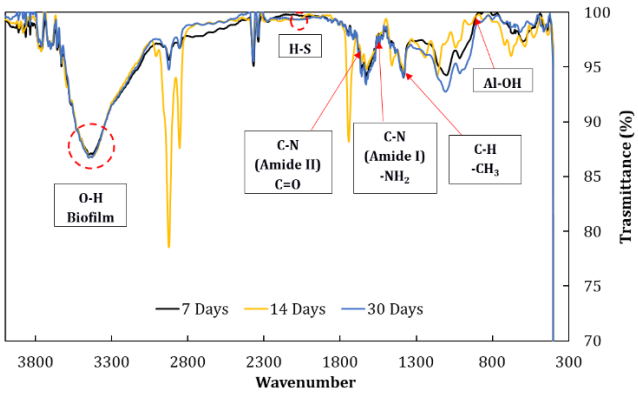

Fig. 6. FTIR spectra of 5083 aluminium alloy post-immersion in seawater inoculated with *Citrobacter freundii* SKC-4

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