

A STUDY ON MICROBE-METAL INTERACTION: ALUMINIUM ALLOY (6061) AND *Bacillus cereus* IN SIMULATED MARINE ENVIRONMENT

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ABSTRACT

Metals in the marine environment is a lingering with the substantial calamity that poses serious environmental and safety hazards. Electrochemical and chemical interactions cause the gradual decaying of metals' exteriors and interiors. Marine microorganisms interact with metal and degrade it. Microbial corrosion is a special type of metal corrosion in which microbes act on metals. Microbes use different mechanisms to interact with metal and metalloids present in the environment. Few trace elements are required for their metabolism and absorb these metal ions present in a natural and synthetic environment. Some metal plays an important role in the physiological functions of an organism but if it is in excess may cause toxicity. In the present article, the interaction of Aluminum Alloy (AA) 6061 with *Bacillus sp* in a 3.5% NaCl (W/V) solution has been reported. The interaction study was performed under static and dynamic conditions for 4 weeks and 2 weeks respectively. Weight loss of the metal coupons was performed regularly. Microbial load in both conditions was checked by doing a viability cell count to ensure metal toxicity. Scanning Electron Microscope observation was done for biofilm formation on metal coupons.

Keywords: Microbial Corrosion, Biofilm, Electrochemical, Aluminum alloy, Exopolysaccharide.

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INTRODUCTION

Microbes act on metals differently depending on the metal and its physiological metabolism. The types of interaction include bio-precipitation, bioaccumulation, bio-sorption, bio-mobilization/ chelation, or biotransformation. In addition to revealing the fate of metals through their redox transformation and contamination, the emergence of such interactions also allows bacteria to become adapted to a metalloid environment. To use the interactions in the bioremediation of harmful substances, they are being explored.¹ Investigating these facets of microbe-metal interaction aids in the bioremediation of these metals from various contaminated settings and offers insightful information about the function of dwelling microorganisms on metal. The multidisciplinary research has great promise for future research into various molecular pathways for strategic application. For most metallic materials used in the marine environment, their mechanical, physical, and chemical properties can be damaged by the activity of the microorganisms that attach to the surfaces of the metallic structures. Under these circumstances, material deterioration increases the danger of premature and catastrophic breakdowns and raises the expense of upkeep and repairs to affected areas. Corrosion occurs in various ways. The most common type of corrosion is general corrosion, which is characterized by a chemical or electrochemical reaction that occurs across the exposed surface.^{2,3,4} The presence of microorganisms on metal surfaces causes the degradation of metal, which is known as microbiologically induced corrosion (MIC).⁵ Microbiologically induced corrosion is an inimitable form of biodeterioration of materials that occurs normally. It's important to know that MIC is not that divergent from other forms of aqueous electrochemical corrosion. Metals are not directly consumed by microorganisms. Rather, as a by-product of their energy-obtaining metabolism, certain forms of microorganisms create hostile conditions.⁶ The predominant source of microorganisms usually responsible

for inducing microbial corrosion in metals are bacterial species. They are ubiquitous in nature and generally found in air, water, soil, and food. Among the bacterial genera, the typical bacterium that generally causes microbiologically induced corrosion is sulfate-reducing bacteria (SRB). SRB is commonly discovered in sulfate-rich marine and freshwater sediments. 'Biofilms' are microbial communities that quickly colonize and firmly attach to metal surfaces in contact with industrial or natural aquatic environments. While biofilm growth safeguards microbial cells from the outside environment, it also harms the lower layer when the metal surface deteriorates physically or biologically. SRBs are also commonly found in biofilm developed on the surfaces of industrial water systems such as heat exchangers, oil fields pipelines, sewage systems, and cooling towers resulting in biofouling and bio-corrosion^{7,8} Aluminum and its alloys are extensively used in industry because they combine good physical properties, adequate mechanical performance and quite good resistance to uniform corrosion. Unfortunately, they are susceptible to the MIC which causes enormous economic losses. During the processing and service of aluminum alloys, emulsion, oil stain, human excreta, and water could remain on the surface, which provides conditions for the growth of microorganisms. In general, microorganisms may cause either microbially influenced corrosion acceleration (MICA) or inhibition (MICI). Aluminum and its alloys have long been utilized successfully in structural applications. It is gaining significant industrial importance with superior mechanical, physical, and tribological qualities when compared to base alloys. High specific strength, high wear and seizure resistance, high stiffness, high-temperature strength, regulated thermal expansion coefficient, and improved damping capacity are only a few of these qualities. Due to its good mix of strength, ductility, and corrosion resistance, Al-Mg-Si(-Cu) alloys, commonly known as 6xxx series alloys, have been considered the most promising choices for automotive body panel applications. This aluminum alloy series has excellent corrosion resistance in various conditions. Since the 1930s, aluminum alloys have been the preferred material for aircraft manufacturing. The aircraft sector uses a lot of 2xxx and 7xxx alloys, although 6xxx aluminum alloys are becoming increasingly popular. 6xxx alloys have several advantages, including medium strength, formability, weldability, corrosion resistance, and low cost. After heat treatments, he claims that 6xxx can be utilized instead of more expensive 2xxx and 7xxx alloys in a range of applications, including aircraft fuselage skins and automobile body panels and bumpers. As a result, the microstructural characterization and processing procedure are critical for that strategy.⁹⁻¹¹ Nevertheless, Aluminum alloys have several drawbacks, including a high coefficient of thermal expansion and poor tribological properties. Adding material for strengthening and improving and thus modeling such aluminum composites results in increased stiffness and durability, fatigue resistance, and improved tri-biological characteristics.¹² The current study investigated the influence of the bacterial strain *Bacillus* which is a known metal-resistant bacterium that is ubiquitous and can be enumerated from the sample contaminated with heavy metals. They can effortlessly convert heavy metals into their non-lethal forms. Interaction of *Bacillus sp* with aluminum alloy 6061 (AA6061) in 3.5% NaCl medium under static and dynamic conditions was done.

EXPERIMENTAL

Metal Sample

Aluminum alloy 6061 coupons were formed from Al alloy 6061 rods using a wire EDM (Electrical Discharge Machine). The dimensions of the coupons were 12mm × 2mm where 12mm is the diameter and 2mm was the thickness.¹³ The metal coupons were polished using sand papers to remove the surface irregularity, and roughness and to get a smooth surface. Different grades of sandpaper were used (up to 1500 grit) to polish the metal coupons. These metal coupons were used in both static and dynamic studies.

Microorganism

Bacillus cereus was used for the interaction and was isolated from the soil and characterized in the laboratory. The culture was maintained on a nutrient agar medium (Hi-media, Mumbai, India) and sub-cultured, for every 2 weeks, and stored at 4°C for further use.

Inoculum

A loop full of *Bacillus* stock culture was taken and introduced to a 5 ml sterile nutrient broth (Hi-Media, Mumbai India) and incubated for 18 h at 30°C and then the inoculum having 1.6×10^7 CFU. strength transferred into an interaction medium (10% V/V).

Media Preparation

The medium was prepared by mixing 35.24 g of sodium chloride in 1000 ml distilled water to get 3.5% NaCl a solution. The 3.5% sodium chloride concentration used here is because the average salinity in seawater is 3.5%. This test medium was used for both static and dynamic studies.¹⁴

Interaction study

Bacillus inoculum that is in the exponential phase was introduced to the 50 ml of interaction medium/test medium in a 250 ml flask. Metal coupons were immersed in the conical flasks. All the tests were done in duplicate. One set was kept in stationery condition at Room temperature (28 ± 2 °C) for 2 weeks and another set was kept on a shaker with 180 rpm for 4 weeks for dynamic study. A control set without bacteria was maintained in both cases. The bacterial growth curve was performed by turbidometry. Total cell count was done every 24 h for both static and dynamic flasks. Viable cell count was determined in terms of colony-forming units by the total plate count method. Biofilm formation on the metal was evaluated by Scanning Electron Microscope (SEM).

Analysis

Weight Loss Technique

The weight of the metal coupons was measured for both static and dynamic studies using a weight balance. The weight was measured to check whether any weight was lost in the coupons due to corrosion. The initial weight of the coupons was noted before immersing them into the conical flasks. After every three days, the metal coupons were taken out of their conical flasks using forceps and were kept in a petri dish. The coupons were washed with distilled water and then dried using tissue papers and a blow dryer. To remove any loose corrosion products, the coupon was dipped in 70% nitric acid. The coupon was dried. Then the coupon was placed on the weighing balance with an accuracy of 0.1 mg and the weight reading was noted.¹⁵ Based on the weight loss measurements, the corrosion rate was calculated using the formula:¹⁶

$$\text{Corrosion rate (mils/year)} = \frac{K \times W}{A \times t \times D}$$

Where, K = constant, 3.45×10^6

W = Mass loss, (g).

A = Area of the metal coupon, (cm²).

t = Time, days.

D = Density of the metal, (g/cm³).

Visualization of Metal Surface and Biofilm

In the present study, the metal coupon surfaces were observed for physical change as well as microbial observation. For biofilm studies, conical flasks with metal coupons in them were left undisturbed for one week. Then the coupons were dipped in 2.5% glutaraldehyde solution for 8 hours. Here, the glutaraldehyde solution acts as a fixation solution for the biofilm to adhere to the surface of the coupon. After eight hours, the coupons were taken out of the fixation solution and were subjected to ethanol solutions of gradient concentrations (20%, 40%, 60%, 80%, and 100%) for five minutes each. After the ethanol solution, the metal coupons were kept in a desiccator for 24 hours. After this, the coupons were observed under a scanning electron microscope (SEM).¹⁷

RESULTS AND DISCUSSION

Bacterial Cell Count

The cell count for the static and dynamic study was performed daily. Figure-1A represents the graph of the total cell count of the static study. We can observe that the trend suddenly decreases and stay low but remains almost constant. It was also observed during the inoculation that the strain was growing at a very higher rate. Moreover, from this trend, we can infer that during the initial stages, the cells might have been at their later exponential stage or were on the verge of entering into their stationary stage, which explains the sudden decrease in the trend and later becoming constant. The cell count was performed for both the main and duplicate flask. gave similar results.^{18,19} Figure-1B displays the graph of cell count for the dynamic study. It can be observed that trend suddenly decreases, becomes almost constant, and then starts decreasing. From this trend, we can infer that similar to that of the static study the cells might have been at

their later exponential stage or were on the verge of entering into their stationary stage, which explains the sudden decrease in the trend, becoming constant and later entering the death phase. The cell count was performed for the both main and duplicate flasks. Both the flasks gave similar results.

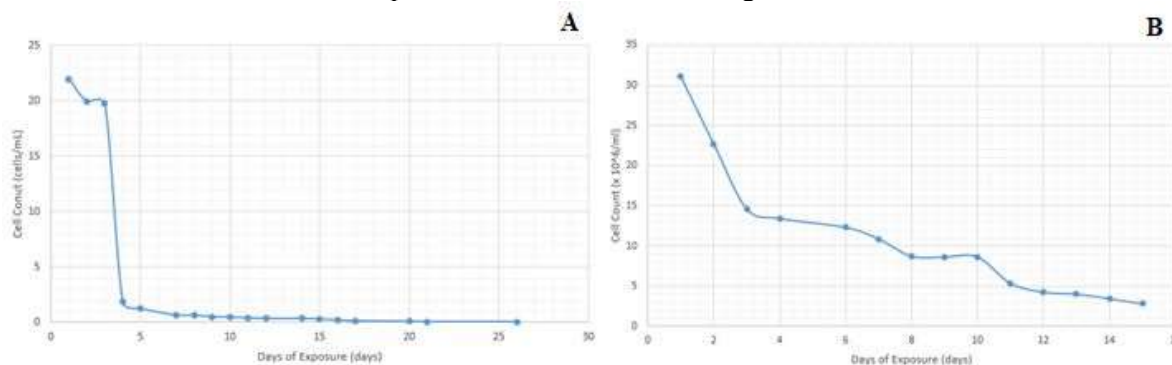


Fig.-1: The Graph of Cell Count in the Microbe Metal Interaction. Fig.-A: Total Cell Count for the Flask Maintained in Static Condition, Fig.-B: Total Cell Count for the Flask Maintained in Dynamic Condition

Weight Loss Analysis

The weight loss for the static study and dynamic study was performed after every three days. Figure-2A represents the weight loss graph of the static study. From the graph given below, we can observe that the trend remains constant and there is almost negligible weight loss taking place in the coupon, the reason for the lack of weight loss can be the coupon is corrosion-resistant, which is because of oxygen dissolved in water, contributes to the formation of the natural oxide layer when corrosion occurs. And the corrosion rate decreases as the immersion period increases. The weight loss was performed for the both main and duplicate flasks. Both the flasks gave similar results.^{20,21} Figure-2B displays the graph of weight loss in a dynamic study. The control flasks of both static and dynamic conditions were studied. It was observed that more weight loss was present in the dynamic study than in the static study. The weight loss was performed in duplicates. Both the flasks gave similar results. Comparing the trends of weight loss in both graphs, we can infer that there is more weight loss in the dynamic study in the presence and absence of the bacterial strain compared to the static study. The reason for weight loss taking place in dynamic conditions might be that the coupon is constantly in motion so it undergoes corrosive wear.²² Another reason might be the chloride iron causing pits on the surface of the coupon in both static and dynamic conditions. Also, pH plays an important role in the corrosion of aluminum alloy though in this study it did not cause much damage to the coupons.²³ In the static study, a biofilm was formed. In the control flasks, the corrosive wear plus the chloride ions subjected to more weight loss in the coupon under dynamic than the static coupon.

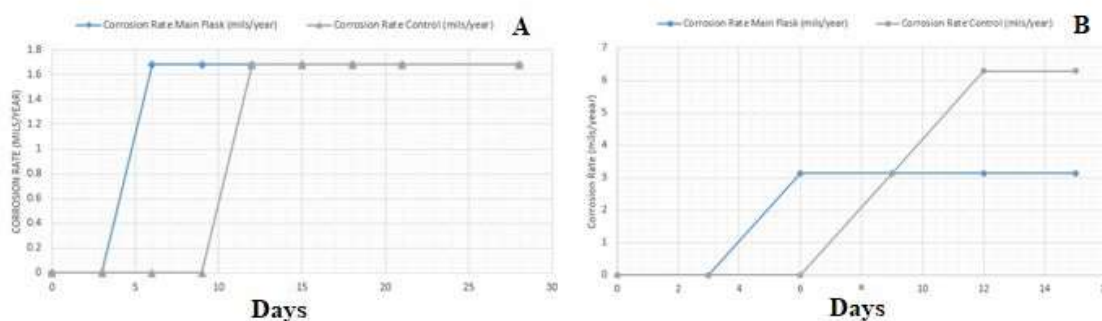


Fig.-2: The Graph of Corrosion Rate of (A) Static Condition and (B) Dynamic Study

Analysis of Corrosion Rate

The rate of corrosion was calculated for both static and dynamic studies. Concerning the determination of corrosion rate, the most accurate and precise method is probably mass loss measurement. If weight loss was present, the corrosion rate will increase initially, and then it will decrease. The decrease might be due to the exhaustion of corrosion products.²⁴ In the present study, Fig.-2A represents the graphs of the corrosion rate

of the static study. The corrosion rate was measured for the both main and duplicate flasks. Both the flasks gave similar results. From the dynamic study, we can observe that the corrosion rate increases initially and then remains constant. It correlates with that of the weight loss analysis, where there was a weight loss at the initial stage and then the weight loss factor becomes constant so the corrosion rate also becomes constant. Figure-2B displays the graph of the corrosion rate of the dynamic study. The corrosion rate for the dynamic and for the static studies trend was the same, increasing initially and then becoming constant.

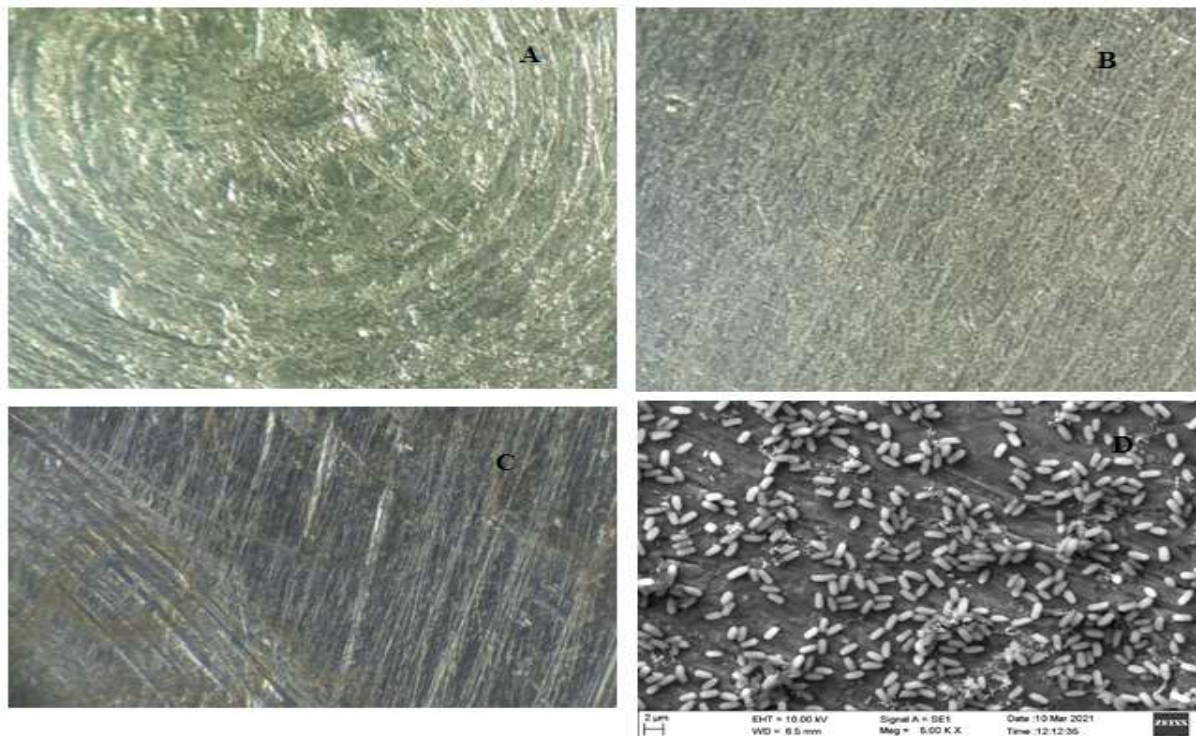


Fig.-3: Optical Microscopic Photographs Results. (A) The Surface of the Aluminum Alloy 6061 Before Polishing, (B) After Polishing the Metal Coupon, (C) After Immersing in 3.5% NaCl (D) Bacterial Cells Grown on the Metal Surface

Surface Analysis

The polished aluminum alloy coupons were observed under an optical microscope under three states of the coupons: before polishing, after polishing, and immersed in 3.5% NaCl with bacterial strain in the static study (Fig.-3). The coupon, which was used for the static study, was found to be less corroded, with only a few pits on the surface. The corrosion-resistant behavior of the metal coupon might be because the test medium and the bacterial strain were less reactive toward the metal. The process may be slow and it may take a longer period for the degradation.²⁵

Evaluation of Biofilm by Scanning Electron Microscopy

Figure-4 represents the SEM image of a 6061-aluminum alloy metal coupon after exposure to the test medium with the presence of the bacterial strain. From Fig.-4A, it is observed that there is biofilm formation on the surface of the metal coupon indicating the growth of the bacteria in the medium and the pits formed on the surface (Fig.-4B). The chances of tribological deformation on the surface are supplemented by the presence of pits.¹³ The biofilm also showed the presence of some extracellular polymeric material (EPS) agglutinating bacterial cells (Fig.-4C and 4D). EPS is a crucial component of the biofilm matrix, enabling cell adherence to the surface and fostering a solid bond between different microorganisms.²⁶

CONCLUSION

The current investigation analyzed the bio-corrosion of AA6061 subjected to 3.5 % NaCl solution with the presence of bacterial strain in both static and dynamic conditions. The alloy used in this study was found to be corrosion-resistant. The bacterial strain displays a minimal role in corroding the alloy may be because aluminum metal is not involved in any of the bacterial metabolic processes, The generation of oxidizing

agents by microbes through their metabolism is required for them to participate in the reaction process²⁷, so the interaction between the metal and bacteria may be slow in turn the damage occurs to the metal may be negligible which can be considered as a corrosion resistant nature.

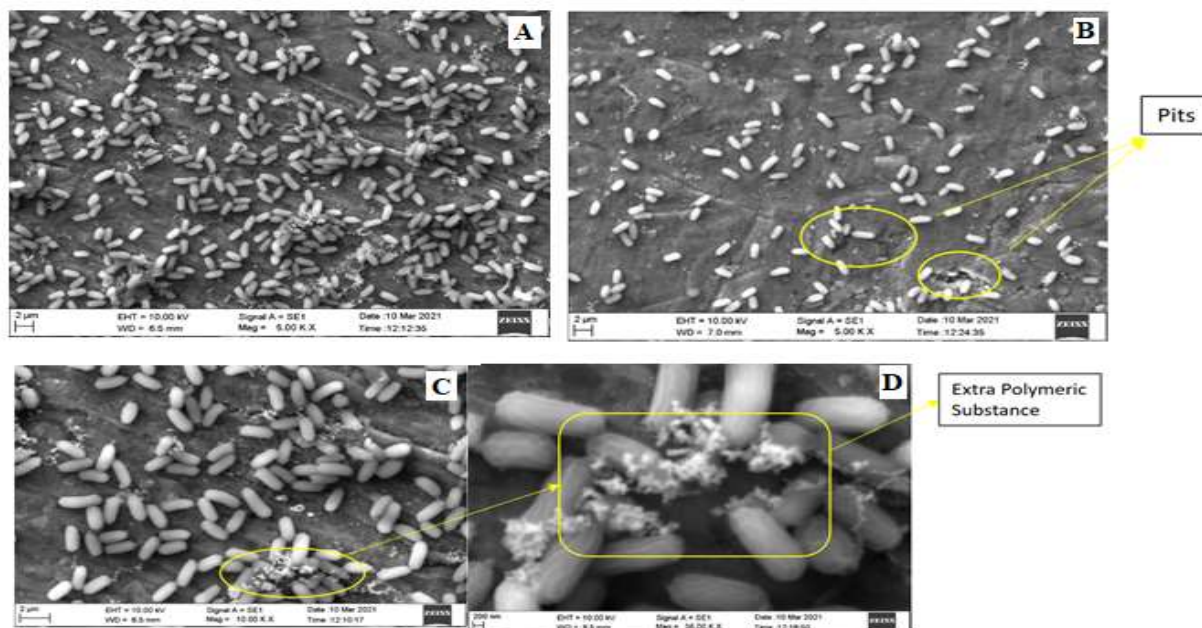


Fig.-4: Scanning Electron Microphotographs. (A) Biofilm Formation on Surface of Al Alloy 6061 Immersed in 3.5 % NaCl Solution with Bacterial Strain (5K X magnification) (B) Pit Formation on the Surface of the Alloy (5K X Magnification). (C) Presence of Extra Polymeric Substance (The white color material in the figures) on The Bacterial Cell Surface SEM -10K X Magnification, (D) Polysaccharide with Higher Magnification- 35K X Magnification

The minimum weight loss in turn was maybe due to the chloride ions. The dynamic study presented a higher corrosion rate in comparison to the static study, which may be due to the wear-corrosive factor. The corrosion rate for both static and dynamic conditions displayed a similar trend because of the corrosion-resistant nature of the metal. The construction of a protective barrier on the surface of the aluminum alloy was caused by the growth of a biofilm being agglutinated by an extracellular polymer matrix, according to the SEM study.

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CONFLICT OF INTERESTS

No potential competing interest was reported by the authors.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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