A Study on Microbe-Metal interaction: Aluminium Alloy (6061) and Bacillus sp in simulated Marine environment

Joydeep Ghosal
Manipal Academy of Higher Education
Lavanya M
Manipal Academy of Higher Education
Divyashree M S (divyashree.ms@manipal.edu)
Manipal Academy of Higher Education

Research Article

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Abstract

The metals affect the growth of microorganisms and their activity. 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 natural and synthetic environment. Some of the metal play important role in physiological functions of organism but if it is in excess may cause toxic. But few metal doesn't have any significant role in organism's metabolism, but still microbes interact with them. Electrochemical and chemical interactions cause the gradual decaying of metals' exteriors and interiors. Metal degrades with the action of microorganisms. Microbial corrosion is a special type of metal corrosion in which microbes act on such metals where it does not have any intrinsic function. In present article interaction of Aluminioum Alloy (AA) 6061 with *Bacillus sp* in 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 were checked by doing viability cell count to ensure metal toxicity. Scanning Electron Microscope observation was done for biofilm formation on metal coupons.

1. Introduction

Microbes act on metals in different way depending on the metal and its physiological metabolism. The types of interaction includes bio-sorption, bioaccumulation, bio-precipitation, biotransformation or bio-mobilization/ chelation. The advent of such interactions not only divulge fate of metals by their redox transformation and toxicity but also unleash the adaptation of microorganisms in a metalloid environment. These interaction are being studied so that they can be applied for bioremediation of toxic element [1]. Exploring these aspects of microbe metal interaction provide valuable insight into the role of dwelling microbes on metal also helps for bioremediation of theses metals from various contaminated environments. The interdisciplinary study clasps much potential for future developments in understanding several molecular mechanisms 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 on the surfaces of the metallic structures. Material degradation under these conditions results in high costs of maintenance and repair of damaged areas, as well as high risks of premature and catastrophic failures. Metals corrode in a variety of ways. The most common type of corrosion is general corrosion, which is characterized by a chemical or electrochemical reaction that occurs uniformly across the exposed surface or over a wide region [2,3,4].

The presence and behavior of microorganisms cause degradation of metallic materials, which is known as microbiologically induced corrosion [5]. Microbiologically induced corrosion (MIC), is a unique form of bio deterioration of materials that occurs frequently. It's important to know that MIC is not that dissimilar from other forms of aqueous electrochemical corrosion. Metals are not directly consumed by

microorganisms in the same way as other organic compounds are. 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 bacteria. They are generally found in air, water, soil and food. Among the bacterial group, the typical bacteria generally causing microbiologically induced corrosion is sulfate-reducing bacteria (SRB). SRB are commonly discovered in sulfate-rich marine and freshwater sediments. Microbial communities rapidly colonize and strongly adhere to metal surfaces that are in contact with natural or industrial aquatic environments, known as ‘biofilms’. Biofilm formation protects microbial cells from the external environment, but this formation is also harmful to the lower substratum, in case of the physical degradation or bio-deterioration of the metal surface. SRB 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].

Aluminium 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 causing 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 microorganism. 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 due to their superior mechanical, physical, and tribological qualities when compared to base alloys. High specific strength, high wear and seizure resistance, high stiffness, improved 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 provide a number of advantages, including medium strength, formability, weld-ability, 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 alloy's micro-structural characterization and processing procedure are critical for that strategy [9–11].

Nevertheless, Aluminum alloys have a number of drawbacks, including a high coefficient of thermal expansion and poor tribological properties. Adding material for strengthening and improving and thus modelling such aluminum composites results in increased stiffness and durability, fatigue resistance, and improved tri-biological characteristics [12].

The current study investigated the influence of bacterial strain Bacillus which is known as metal resistant bacteria Bacillus being ubiquitous in nature, also dominates the areas containing contamination of 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 were done.

2. Materials And Methods

2.1 Metal Sample

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

2.2 Microorganism

Bacillus sp was used for the interaction was isolated and characterized earlier in lab. The culture was maintained and sub-cultured on nutrient agar medium (Hi-media, Mumbai, India) once in a month 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 32^{o}C and then inoculated into interaction medium at 10% (V/V) having 1.6 X 10^{7} CFU.

Media Preparation

The 3.5% NaCl solution was used as a test medium. The medium was prepared by mixing 35.24 g of sodium chloride in 1000 ml distilled water to get 3.5% NaCl 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 study [14].

2.3 Interaction study

Bacillus inoculum that are in exponential phase was introduced to the 50 ml of interaction medium/test medium in 250 ml flask. metal coupons were immersed in the conical flasks. All the test were done in duplicates. One set was kept in stationery condition at Room temperature (28 ± 2 ^{o}C) for 2 weeks and another set was kept on shaker with 180 rpm for 4 weeks for dynamic study. Control set without bacteria was maintained in both the cases. Bacterial growth curve was performed by terbidometry. Total cell count was done for every

24 h for both static and dynamic flasks. Viable cell count was determined in terms of colony forming units by total plate count method. Biofilm formation on the metal was evaluated by SEM.

2.4 Analysis

2.4.1 Weight Loss Technique

The weight of the metal coupons were measured for both static and dynamic study using a weigh balance. The weight was measured to check whether any weight was lost in the coupons due to corrosion. Initial weight of the coupons were noted before immersing into the conical flasks. After every three days, the metal coupons were taken out of their conical flasks using a forceps and was 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 lose 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]:

$$\text{Corrosion rate (mils/year)} = \frac{K \times W}{A \times t \times D} \quad (2)$$

Where, $K =$ constant, 3.45 x10^6

$W =$ Mass loss, (g).

$A =$ Area of the metal coupon,(cm^2).

$t =$ Time, days.

$D =$ Density of the metal, (g/cm^3).

2.4.2. Visualization of metal surface and biofilm

In 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 it 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 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 was 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].

3. Results And Discussions

Bacterial Cell Count

The cell count for the static and dynamic study was performed on a daily basis. Figure 1A represents the graph of total cell count of static study. We can observe that the trend suddenly decreases and stay lows but remains almost constant. It was also observed during the inoculation that the strain 62 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 its later exponential stage or was on the verge of entering into its stationary stage, which explains the sudden decrease in the trend and later becoming constant. The cell count was performed for both main and duplicate flask. Both the flasks gave similar results [18,19].

Figure 1B displays the graph of cell count for dynamic study. It can observe 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 its later exponential stage or was on the verge of entering into its stationary stage, which explains the sudden decrease in the trend, becoming constant and later entering the death phase. The cell count was performed for both main and duplicate flask. Both the flasks gave similar results.

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. From the trend, we can infer that there is almost negligible weight loss taking place in the metal coupon. The reasons for 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 both main and duplicate flask. Both the flasks gave similar results [20,21].

Figure 2B displays the graph of weight loss of dynamic study. The control flasks of both static and dynamic conditions was studied. It was observed that more weight loss was present in the dynamic study than in the static study. The weight loss was performed for both main and duplicate flask. Both the flasks gave similar results. Comparing the trends of weight loss of both graphs, we can infer that there is more weight loss in the dynamic study in 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 surface of the coupon in both static and dynamic conditions. Also pH plays an important role in 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 and since it is an unidentified strain, it is not known that this bacterial strain can induce corrosion. In the control flasks, the corrosive wear plus the chloride ions subjecting to more weight loss in the dynamic coupon than the static coupon.

Corrosion Rate Analysis

The corrosion rate was calculated for both static and dynamic study. With respect to 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. 3A represents the graphs of corrosion rate of the static study. The corrosion rate was measured for both main and duplicate flask. Both the flasks gave similar results. From the corrosion rate trend of static study, we can observe that initially it increases and then remains constant. From this, we can infer that initially there was weight loss (at initial stage) and then the weight loss factor becomes constant so the corrosion rate also becomes constant.

Figure 3B displays the graph of corrosion rate of the dynamic study. The corrosion rate was measured for both main and duplicate flask. Both the flasks gave similar results. For the dynamic study, the corrosion rate trend is similar to that of the static study, increases initially and then becomes constant. From the below trend it is similar inferences to that of the corrosion rate of the static study. The trend is a little different from the static study is because of more weight loss in the dynamic than in the static study.

Surface Analysis

The polished aluminum alloy coupons were observed under an optical microscope under three state of the coupons: before polishing, after polishing and immersed in 3.5% NaCl with bacterial strain in static study (Fig. 4) The coupon, which was used for static study, was found to be very less corroded. In some regions, very few pits were observed. The corrosion resistant behavior of the metal coupon might be because the test medium and the bacterial strain was less reactive towards the metal. The process may be slow and it may take longer period for the degradation [25].

Evaluation of the biofilms by Scanning Electron Microscopy

Figure 4 represents the SEM image of 6061 aluminum alloy metal coupon after exposure to the test medium with the presence of the bacterial strain. From Fig. 5A, 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. 5B). The chances of tribological deformation on the surface is supplemented by the presence of pits [13]. The biofilm also showed the presence of some extracellular polymeric material (EPS) agglutinating bacterial cells. EPS is an integral part of the biofilm matrix, mediating the adhesion of cells to the surface, and promoting a strong link between one microorganism and to the other [26].

The present study evaluated the bio-corrosion of 6061 aluminum alloy exposed 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 minimal role in corroding the alloy may be because aluminum metal is not involved in any of the bacterial metabolic process, The generation of oxidising agents by microbes through their metabolism is required for them to participate in the reaction. Microorganisms play a role in starting or worsening the electrochemical processes on metals by directly

participating in one or both of the reactions [27], so the interaction between the metal and bacteria may be slow inturn the damage occur to the metal may be negligible which can be considered as a corrosion resistant nature. The minimum weight loss that was exhibited 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 similar trend because of the corrosion resistant nature of the metal coupon. SEM analysis revealed the development of a biofilm were agglutinated by an extracellular polymer matrix, resulting in the formation of a protective barrier on the aluminum alloy surface. The biofilm developed had a discontinuous look, with areas of the metallic substrate devoid of microbial adhesion.

Declarations

The authors declare no conflict of interest involved related to this research. The research work does not involve experimentation on animals or humans.

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Author Contributions

The above work was carried out by Joy deep under the guidance of Dr Divyashree. And metal coupons were provided by Dr Lavanya.

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Data Availability N/A.

Code Availability N/A.

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Figures

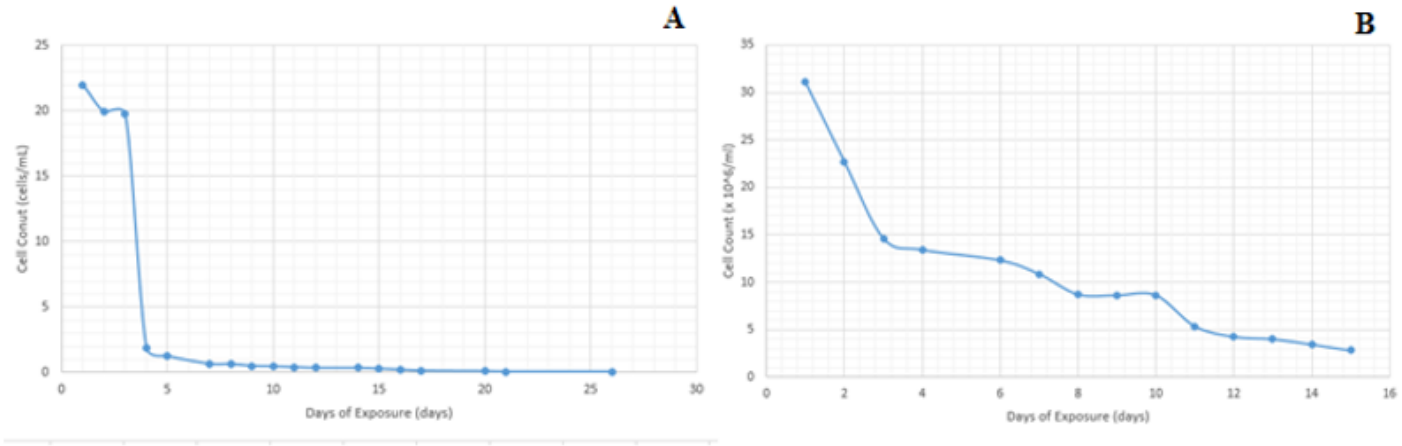

Figure 1

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

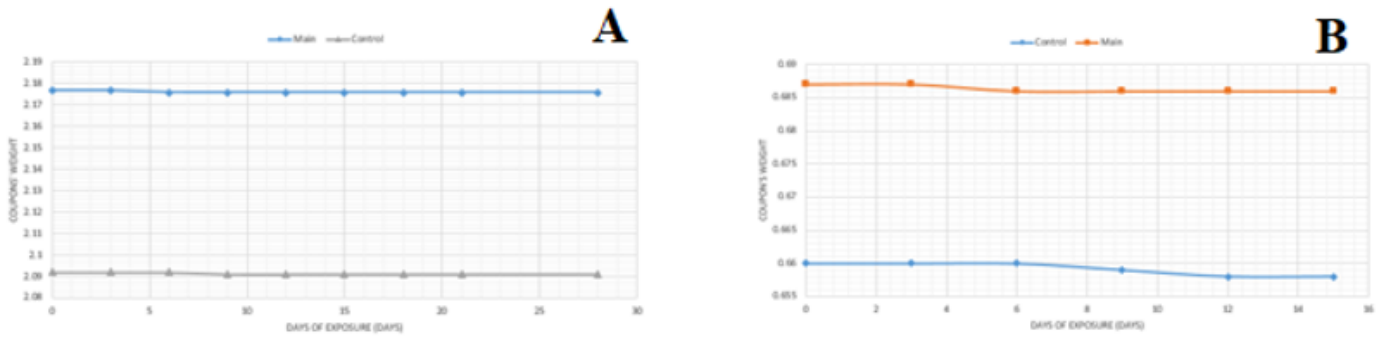

Figure 2

The graph of weight loss for static condition (A) and for Dynamic study (B).

Figure 3

The graph of corrosion rate of static condition (A) and Dynamic study.

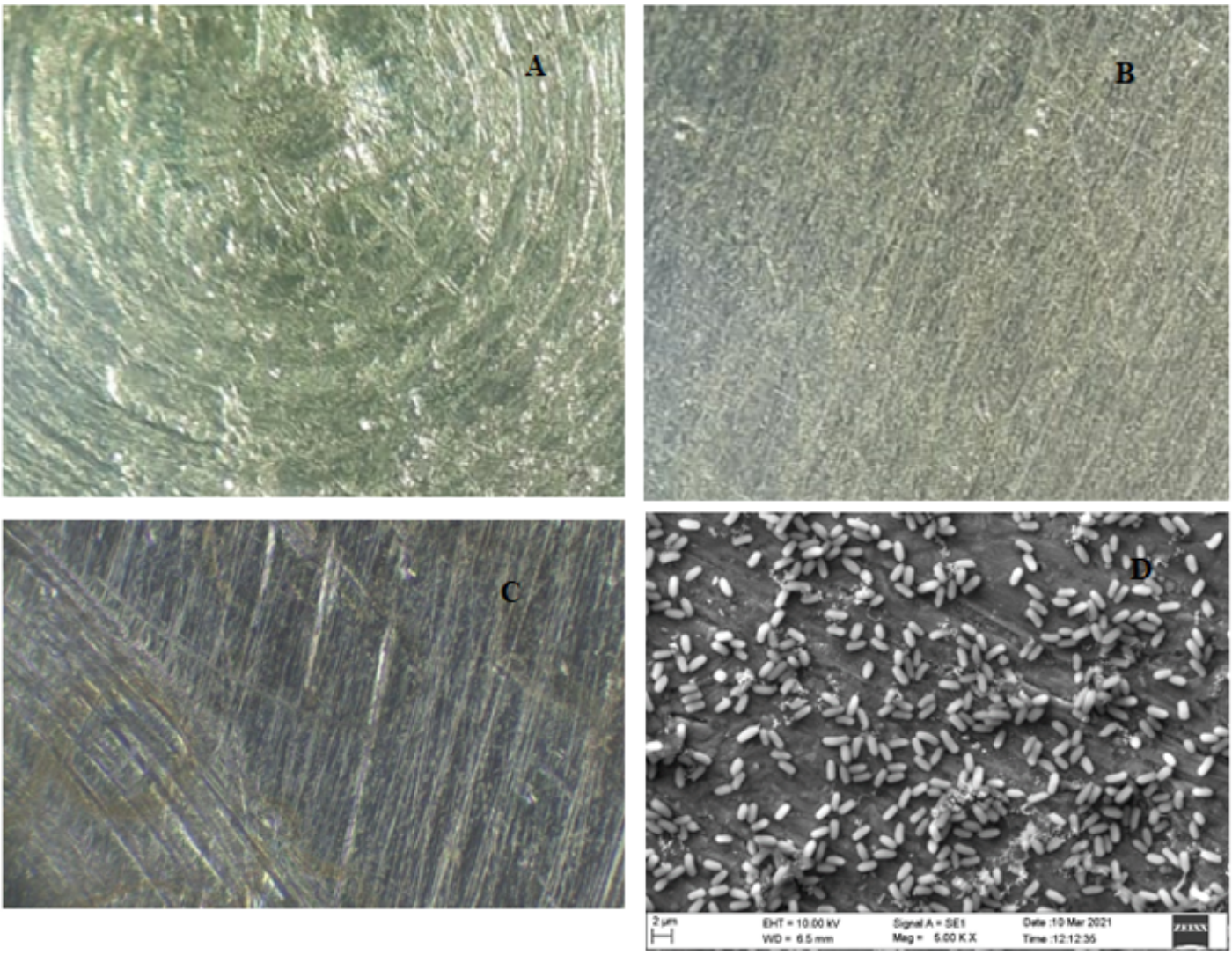

Figure 4

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

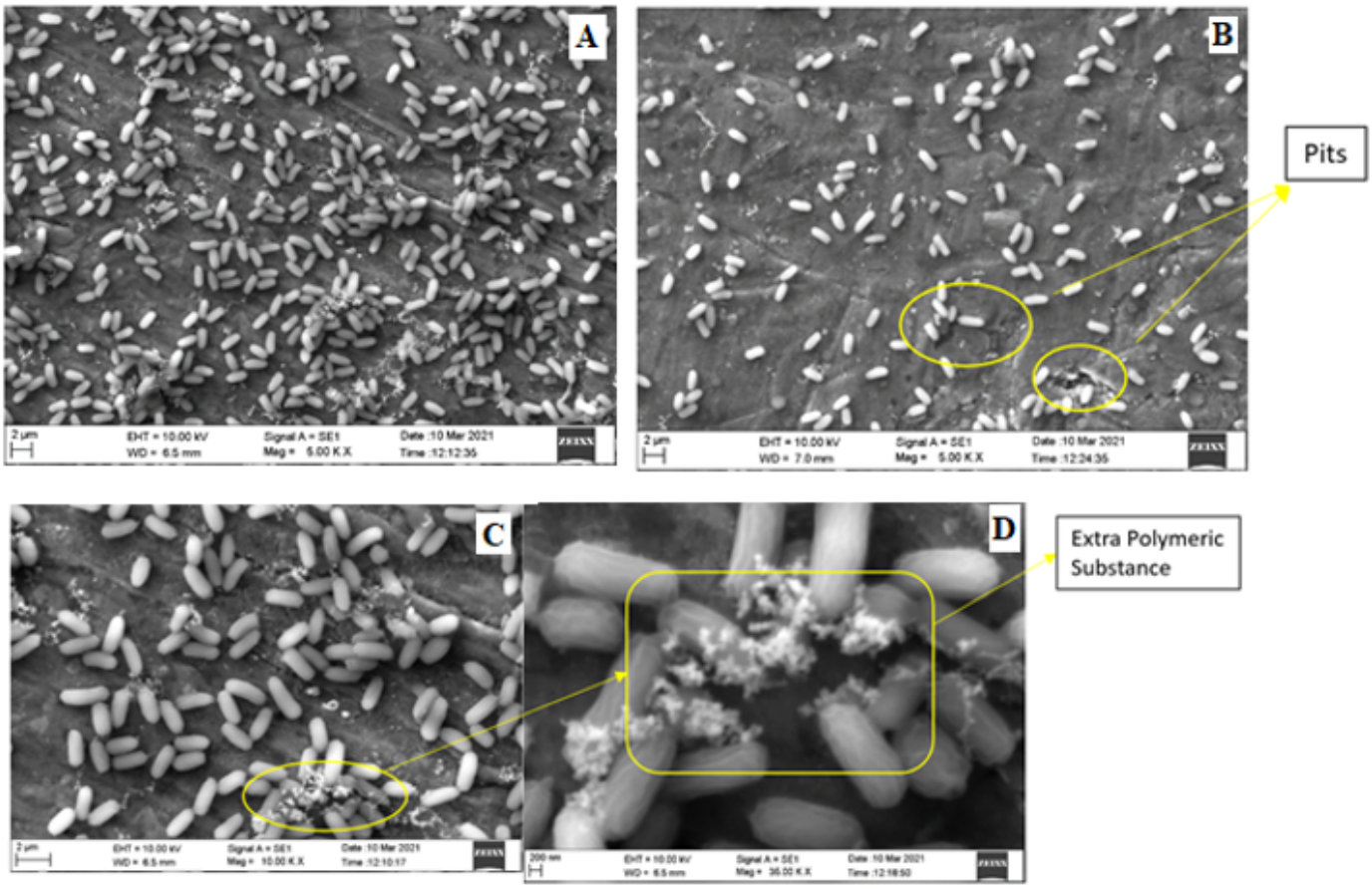

Figure 5

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