

Article

Microbial Biosurfactant as Sustainable Inhibitor to Mitigate Biocorrosion in Metallic Structures Used in the Offshore Energy Sector

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Abstract

Microbiologically influenced corrosion (MIC) represents a critical challenge to the integrity of pipelines, piping, and metal structures in offshore environments, directly affecting the safety and operational costs of companies in the energy sector. However, conventional control methods, such as the use of chemical inhibitors, raise environmental and economic concerns. To face this problem, a biosurfactant produced by *Pseudomonas cepacia* CCT 6659 was tested as a biocorrosion inhibiting agent on carbon steel specimens immersed in seawater. For this purpose, static and dynamic conditions were simulated using different concentrations of the biosurfactant. Furthermore, analyses were performed using Scanning Electron Microscopy paired with Energy Dispersive Spectroscopy (SEM/EDS) to visualize the morphology of the biofilm and its chemical components. Laboratory tests indicated that the biosurfactant formulated in a 1:5 (*v/v*) ratio reduced the mass loss of test specimens (119.72 ± 2.64 g/m²) by no less than 57.3% compared to the control (280.28 ± 4.58 g/m²). Under dynamic conditions, the 1:2 (*v/v*) formulation showed greater protection, being able to reduce specimen corrosion (578.87 ± 7.01 g/m²) by 69.6% compared to the control (1901.41 ± 13.53 g/m²). SEM/EDS analyses revealed changes in surface composition and a reduction in corrosive elements associated with sulfur in the formed biofilms, which may be associated with a decrease in sulfate-reducing bacteria (SRB) activity, suggesting microbial inhibition by the biosurfactant. The results obtained in this study highlight the biosurfactant as a viable and ecological alternative to synthetic inhibitors, with potential application in the protection of metal structures exposed to corrosive environments in offshore energy systems, promoting greater durability, sustainability, and less environmental impact.



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Keywords: microbial corrosion; metal corrosion; biofilms; offshore energy systems; sustainability; seawater

1. Introduction

Metal corrosion is one of the main problems faced by several industries, causing economic losses, operational risks, and significant environmental impacts. In industrial environments, Microbiologically Influenced Corrosion (MIC) is of particular concern, being caused by the direct or indirect action of microorganisms such as Sulfate Reducing Bacteria (SRB). These microorganisms contribute to the structural weakening of metals and metal alloys through the production of corrosive metabolites, such as hydrogen sulfide, which accelerates the degradation of materials. Traditional methods of controlling MIC, such as the use of chemical inhibitors and protective coatings, have proven effective but raise questions related to their toxicity, high cost, and environmental impact, thereby demanding more sustainable and innovative alternatives [1,2].

The biocorrosion process begins with the adhesion of microorganisms onto the surface of the material and continues with the formation of a biofilm, which consists of a matrix of extracellular polymeric substances (EPS) secreted by the microorganisms themselves. This biofilm creates a protected microenvironment that favors the survival of microorganisms, whose metabolic activity modifies the chemical and electrochemical conditions close to the material surface, altering the pH, forming oxygen gradients (aerobic and anaerobic areas) and generating corrosive metabolic byproducts such as hydrogen sulfide [3,4].

Environmental factors, such as material composition, nutrient availability, oxygenation, and temperature, directly influence biocorrosion. Metallic materials such as carbon steel, stainless steel, and aluminum are particularly susceptible, especially in environments that favor microbial growth or present variations between aerobic and anaerobic conditions, promoting differential corrosion [5,6]. These effects are particularly relevant in seawater environments, which are naturally rich in sulfate ions, halotolerant microorganisms, and present a highly corrosive medium due to salinity and redox stratification. In this context, carbon steel is widely used as a metallic substrate due to its industrial use in pipelines and offshore structures, as well as its known susceptibility to MIC, making it a representative material for testing corrosion inhibition strategies under realistic marine conditions [7,8]. Considering all the problems caused by biocorrosion, the use of biosurfactants has emerged as a promising approach.

Biosurfactants, surface-active compounds of microbial origin, which can be produced from bacteria, yeasts, and filamentous fungi, represent sustainable and environmentally compatible alternatives to synthetic surfactants. High selectivity and stability under wide ranges of pH, temperature, and salinity characterize these compounds. Structurally amphipathic, biosurfactants comprise a hydrophilic moiety, which may include carbohydrates, amino acids, cyclic peptides, phosphates, alcohols, or carboxylic acids, and a hydrophobic moiety, generally composed of linear or branched, saturated or unsaturated fatty acids. Based on molecular weight, biosurfactants are classified into two main functional groups: low-molecular-weight compounds, such as lipopeptides, glycolipids, and phospholipids, primarily responsible for reducing surface and interfacial tension; and high-molecular-weight compounds, including lipoproteins and lipopolysaccharides, commonly referred to as bioemulsifiers due to their capacity to form and stabilize emulsions. This functional categorization underscores the versatile potential of biosurfactants for industrial and environmental applications, including biocorrosion control in harsh conditions, such as those found in the offshore energy sector [9–11].

Among the structurally diverse biosurfactants, glycolipids—particularly rhamnolipids—have shown considerable promise in corrosion inhibition due to their ability to interact effectively with metallic surfaces. Rhamnolipids consist of one or two fatty acid chains (typically C8–C16) linked to one or two rhamnose sugar units, conferring them the amphiphilic properties necessary for surface adsorption and protective film formation [12]. The biosurfactant produced by *Pseudomonas cepacia* CCT 6659, a rhamnolipid, has attracted growing attention owing to its notable performance under MIC scenarios. Studies indicate that this microbial surfactant not only hinders microbial adhesion and biofilm development on metallic substrates but also contributes to the formation of stable hydrophobic layers that impede corrosive agents [13,14]. In addition to their eco-friendly properties, biosurfactants may inhibit microbiologically influenced corrosion through multiple mechanisms, including the adsorption onto metallic surfaces to form hydrophobic protective films, interference with microbial adhesion, and possible direct antimicrobial action against biofilm-forming organisms, such as SRB [15].

Given the growing demand for environmentally sustainable technologies in the energy sector, especially in offshore operations, this study aimed to evaluate the use of microbial biosurfactants as MIC inhibitors in metallic systems exposed to seawater. For this purpose, metal specimens were submitted to microbiological and mass loss analyses as well as scanning electron microscopy (SEM) paired with energy dispersive spectroscopy (EDS) in order to characterize the action of the biosurfactant on their surfaces. By highlighting the viability of using the biosurfactant as a sustainable alternative, this research seeks to contribute to innovation in the management of the integrity of critical metallic assets used in pipelines and platforms in the energy industry.

2. Materials and Methods

2.1. Microorganism

The bacterial strain *Pseudomonas cepacia* CCT 6659, obtained from the culture bank of the André Tosello Foundation for Research and Technology, located in the city of Campinas, Brazil, was used as the biosurfactant producer. The cultures were subcultured every 30 days and maintained in inclined test tubes containing the solid medium (nutrient agar) under refrigeration at 5 °C.

2.2. Biosurfactant Production

Biosurfactant production was carried out as described by Soares da Silva et al. [16]. A mineral medium composed of 0.05% KH_2PO_4 , 0.1% K_2HPO_4 , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01% KCl, and 0.001% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was used after the addition of 2.0% residual canola oil from frying and 3.0% corn stearate as substrates. Cultivations were carried out at pH 7.0 and a temperature of 28 °C for 60 h, under agitation of 250 rpm and using an inoculum of 10^7 CFU/mL [16].

2.3. Biosurfactant Extraction

The biosurfactant was extracted from the cell-free fermented broth through liquid-liquid extraction with ethyl acetate (1:1 v/v), followed by solvent evaporation at 40 °C. The resulting residue was washed with hexane to eliminate hydrophobic impurities and treated with a base to promote crystallization and further purification, as described previously by Deng et al. [17] and Pal et al. [18]. The isolated biosurfactant was used at its critical micelle concentration (CMC) of 0.06% (600 mg/L). Its main composition, determined by Soares da Silva et al. [16], mainly consists of a mixture of mono- and di-rhamnolipids with fatty acid chains ranging from C8 to C16. This heterogeneity is typical of microbial biosurfactants

and is precisely this mixed composition that gives them their amphiphilic properties and their ability to form protective films on metal surfaces.

2.4. Evaluation of the Biosurfactant as an Inhibitor of Microorganisms Responsible for Metal Biocorrosion

To evaluate the corrosion potential over time, continuous immersion tests were performed under both static and dynamic conditions. In the dynamic assays, pumps operating at a frequency of 50/60 Hz and a power of 2.8 W, along with a Boyu/JAD S-510-01 air compressor capable of generating a pressure of 0.01 MPa and ensuring an air output of 4 L/min, were added to each system to promote continuous agitation and aeration of the liquid. Carbon steel 1045 was selected as the test material due to its widespread industrial use in marine and offshore environments, where it is particularly susceptible to microbiologically influenced corrosion, especially in the presence of sulfate-reducing bacteria. Its vulnerability and relevance in real-world applications make it a representative substrate for evaluating the anticorrosive potential of biosurfactants [8]. For the experiments, carbon steel test specimens (TSs) measuring 10 cm in length, 5.0 cm in width, and 0.10 cm in thickness were prepared for mass loss tests, microbiological analyses, and characterizations by Scanning Electron Microscopy paired with Energy Dispersive Spectroscopy (SEM/EDS), in order to examine biofilm formation on the surface of TSs.

Tests were conducted in 2 L glass vessels, where sterile systems were assembled under both static and dynamic conditions (Figure 1). Systems were evaluated in nine solutions prepared using seawater collected in the region of the industrial port of Suape (Ipojuca, Brazil), a coastal environment influenced by both industrial and estuarine activities. The seawater composition was based on the Environmental Quality Monitoring Report from DBF Consultoria (2019) [19], which includes data from 15 sampling stations. The main physicochemical seawater parameters are summarized in Table 1.

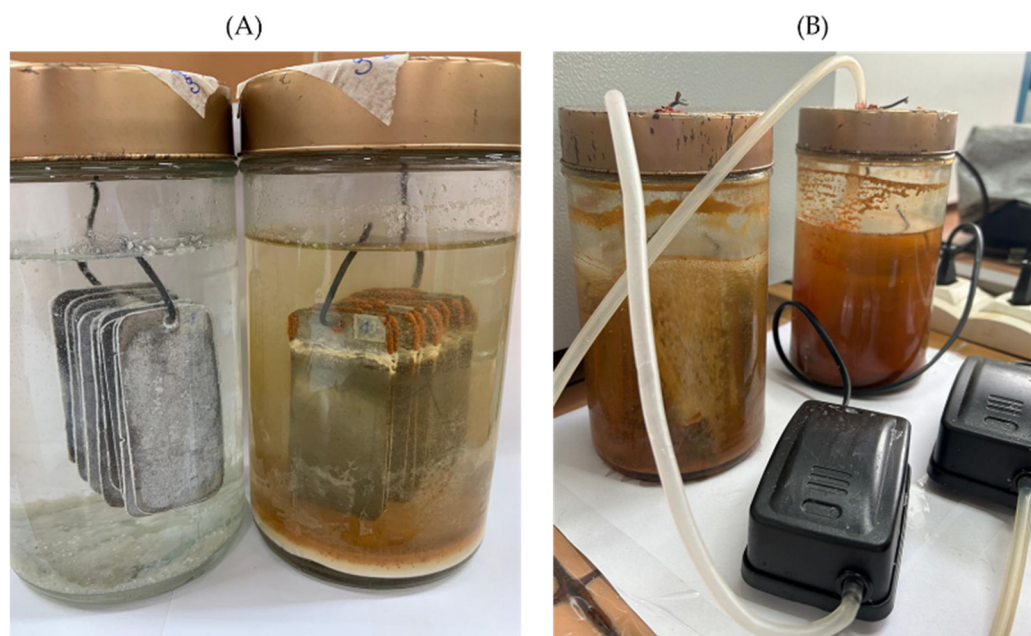


Figure 1. Illustrative diagram of the assembly of (A) static and (B) dynamic systems.

Table 1. Main physical-chemical parameters of seawater collected at the Suape industrial port (Ipojuca, Brazil), used to prepare solutions in biocorrosion tests.

Parameter	Value Range	Unit	Notes
Salinity	0.06–38.05	‰	Estuarine-to-marine variability
Temperature	27.16–32.09	°C	Tropical marine zone
pH	up to 8.54	–	–
Dissolved Oxygen	≥5.0	mg/L	Supports aerobic biofilm development
Total Phosphorus	up to 0.13	mg/L	Nutrient availability
Iron (Fe, dissolved)	up to 0.739	mg/L	Detected in natural concentration
Zinc (Zn)	0.12	mg/L	Trace metal
Oils and Greases	Not detected	–	Visual inspection

All values are expressed in wt% equivalents, where applicable, in accordance with standard elemental quantification protocols. The seawater composition accurately reflects real offshore conditions, ensuring environmental relevance for MIC assessment.

As detailed in Table 2, the isolated biosurfactant was applied at concentrations expressed as multiples of the Critical Micelle Concentration (CMC = 600 mg/L), namely ½ CMC, CMC, and 2 CMC. Fawzy et al. [20] evaluated biosurfactant concentrations ranging from 100 to 500 mg/L for corrosion inhibition, demonstrating that values in this range are commonly employed to ensure the formation of effective protective films on metal surfaces, in line with practices adopted for synthetic inhibitors. On the other hand, the formulated biosurfactant, consisting of the cell-free metabolic fluid, was used using 0.2% sodium benzoate as a preservative.

Table 2. Solutions formulated for biocorrosion evaluation experiments. They were prepared by adding the formulated biosurfactant in seawater in 1:2 and 1:5 (*v/v*) proportions or the isolated biosurfactant at concentrations of 1/2 CMC, CMC, and 2 CMC.

Test	Solution
Control	Seawater in natura
Condition 1	Seawater in natura + formulated biosurfactant at 1:2 (<i>v/v</i>)
Condition 2	Seawater in natura + formulated biosurfactant at 1:5 (<i>v/v</i>)
Condition 3	Isolated biosurfactant at 1/2 CMC in seawater in natura
Condition 4	Isolated biosurfactant at CMC in seawater in natura
Condition 5	Isolated biosurfactant (2 CMC) in seawater in natura
Control	Sterile seawater
Condition 1	Sterile seawater + formulated biosurfactant at 1:2 (<i>v/v</i>)
Condition 2	Isolated biosurfactant at 1% in sterile seawater

2.4.1. Mass Loss Tests

In the mass loss tests, 20 TSs were attached to the systems over a period of 120 days. Every 30 days, the extent of corrosion and other failures was carefully analyzed. The cleaning and preparation procedures followed the guidelines of the ASTM G1-03 standard [21], ensuring accurate removal of corrosion products without damaging the base metal. The

TSs were weighed on an analytical balance to determine the final mass (W , g) after the test and then to calculate the mass loss (L) per area, expressed in g/m^2 , using the formula:

$$L = \frac{W_0 - W}{A}$$

where W_0 is the mass (g) of each metal plate before being subjected to corrosion and A is the area (m^2) [22].

The inhibition efficiency (IE) was calculated using the following equation:

$$\text{IE}(\%) = \left(\frac{M_0 - M}{M_0} \right) \times 100$$

where M_0 represents the mass loss in the absence of the inhibitor (control), and M is the mass loss in the presence of the biosurfactant [23].

2.4.2. Microbiological Analyses

Microbiological monitoring of seawater planktonic microorganisms present in the formed biofilms was performed. The TSs were placed in vessels containing 30 mL of sterile reducing solution or sterile saline solution (30 g/L NaCl) for the qualification of anaerobic or aerobic microorganisms, respectively, and their entire surface was aseptically scraped with a sterile spatula to completely remove the biofilms formed on the surface. All media intended for the qualification of aerobic microorganisms were distributed in sterile test tubes and capped with cotton. For the cultivation of anaerobic microorganisms, all solutions and media were aseptically distributed into sterile, hermetically sealed glass vials, which were immediately capped and sealed to maintain anaerobic conditions.

Total Aerobic Heterotrophic Bacteria

Total Aerobic Heterotrophic Bacteria (TAHB) were qualified using Nutrient Broth medium containing 5.0 g/L meat peptone, 3.0 g/L meat extract, and 20.0 g/L sucrose, whose pH was adjusted to 7.0. The medium was then sterilized at 110°C for 15 min. After inoculation, the scrapings were incubated at $30 \pm 1^\circ\text{C}$ for 48 h. The growth of microorganisms was evidenced by the turbidity of the culture medium caused by the presence of microbial cells and their metabolites [24].

Total Anaerobic Heterotrophic Bacteria

To evaluate Total Anaerobic Heterotrophic Bacteria (TAnHB), we used thioglycolate fluid medium (Merck, Darmstadt, Germany), pH 7.0, sterilized at 121°C for 20 min. Inoculation was performed by adding 1 mL of the cell suspension diluted in the reducing solution to a penicillin bottle containing 9 mL of the medium, with the aid of 1 mL disposable syringes. The reducing solution, consisting of sodium thioglycolate 0.124 g/L, ascorbic acid 0.10 g/L, and rezarzurin 4.0 mL/L, pH 7.6, was sterilized at 121°C for 20 min.

After inoculation, the flasks containing the culture medium were sealed with rubber caps and metal seals in order to maintain anaerobiosis, and then incubated at $30 \pm 1^\circ\text{C}$ for 21 days. After this time, the growth of heterotrophic aerobic bacteria was evidenced by the turbidity of the culture medium, considered positive when visible cloudiness or sediment formation was observed in comparison to the sterile control [24].

Sulfate Reducing Bacteria

To check for the presence of sulfate-reducing bacteria (SRB), modified Postgate-B medium [25] was used, whose composition was 1.0 g/L NH_4Cl , 0.5 g/L KH_2PO_4 , 2.0 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 g/L NaSO_4 , 0.5 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g/L $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g/L ascorbic

acid, 1.0 g/L yeast extract, 4.0 mL of 0.025% (*w/v*) resazurin solution and 7.0 mL of 50% (*w/v*) sodium lactate solution. The final pH was adjusted to 7.6. The medium was modified by replacing thioglycolic acid of the original recipe with the addition of 0.1 mL/L of a 12.4 g/L sodium thioglycolate solution (reducing agent) in each vial of the dilution series. This reducing agent, together with ascorbic acid, allowed for a redox potential below -100 mV, a necessary condition for the growth of sulfate-reducing microorganisms [26]. According to Postgate [27], this medium can be used for diagnostic and culture maintenance purposes, and the growth of BRS is indicated by the black coloration resulting from the formation of iron sulfide (FeS) deposits, resulting from the reduction of sulfate to sulfide. The incubation of these microorganisms was carried out at 30 ± 1 °C for 28 days. After the incubation period, the darkening of the medium confirmed the growth of BRS.

2.4.3. Scanning Electron Microscopy Paired with Energy Dispersive Spectroscopy

Scanning Electron Microscopy (SEM) model MIRA 3, manufactured by TESCAN ORSAY HOLDING, a.s., Brno, Czech Republic, was used for the qualitative evaluation of biofilms present on metal surfaces [28]. In addition, Energy Dispersive Spectroscopy (EDS), coupled to SEM, was used to identify the chemical composition of biofilms and detect the presence of elements associated with corrosion, such as iron (Fe), sulfur (S), and oxygen (O), thereby allowing a more detailed analysis of the corrosive processes and the interactions between microorganisms and metal substrate. The selected TS size was 1.5 cm in length, 1.0 cm in width, and 0.10 cm in thickness.

2.5. Statistical Analysis

For mass loss experiments, quintuplicate tests were carried out, i.e., the results were obtained through the average of 5 TSs, aiming to obtain an average corrosion rate for each condition investigated. The data collected were expressed as the mean $\pm$ standard deviation of the tests performed in quintuplicate. The statistical analysis of variance of ANOVA was applied to determine the significance, where *p*-values < 0.05 were considered significant.

3. Results and Discussion

3.1. Evaluation of Biosurfactant as a Biocorrosion Inhibiting Agent

The graphs below show the results of static and dynamic tests performed with seawater, using different formulations and concentrations of biosurfactants. These results taken as a whole highlight the influence of the biosurfactant as a corrosion inhibitor in natural and sterile environments, highlighting the dynamics of action and performance variations over 120 days.

In Figure 2, referring to seawater in natura, a continuous increase in mass loss over time can be observed in the absence of biosurfactant, showing natural changes in the system. The biosurfactant formulations presented satisfactory performance over the 120 days, with emphasis on the one prepared in the proportion of 1:5 (*v/v*), which recorded a mass loss value (119.72 ± 2.64 g/m²) 57.3% lower than that observed in the control condition (280.28 ± 4.58 g/m²). In the condition “isolated biosurfactant (CMC) in seawater in natura”, mass loss showed temporal variation, indicating an unstable corrosion behavior. The recorded values were 124.41 ± 2.71 g/m² at 30 days, 78.40 ± 1.95 g/m² at 60 days, and 168.08 ± 3.32 g/m² at 120 days. Although the initial mass loss was close to that of the control, a reduction was observed at 60 days, suggesting a transient inhibitory effect of the biosurfactant on the corrosion process. The subsequent increase may be attributed to the degradation or dilution of the biosurfactant, or to changes in the microbial community and physicochemical conditions over time. This transient inhibitory effect may be related to the limited stability or gradual desorption of the biosurfactant over time. Similar behavior has

been reported for biosurfactant-based inhibitors, whose protection tends to decrease due to environmental factors, medium dynamics, or compound degradation [20,29].

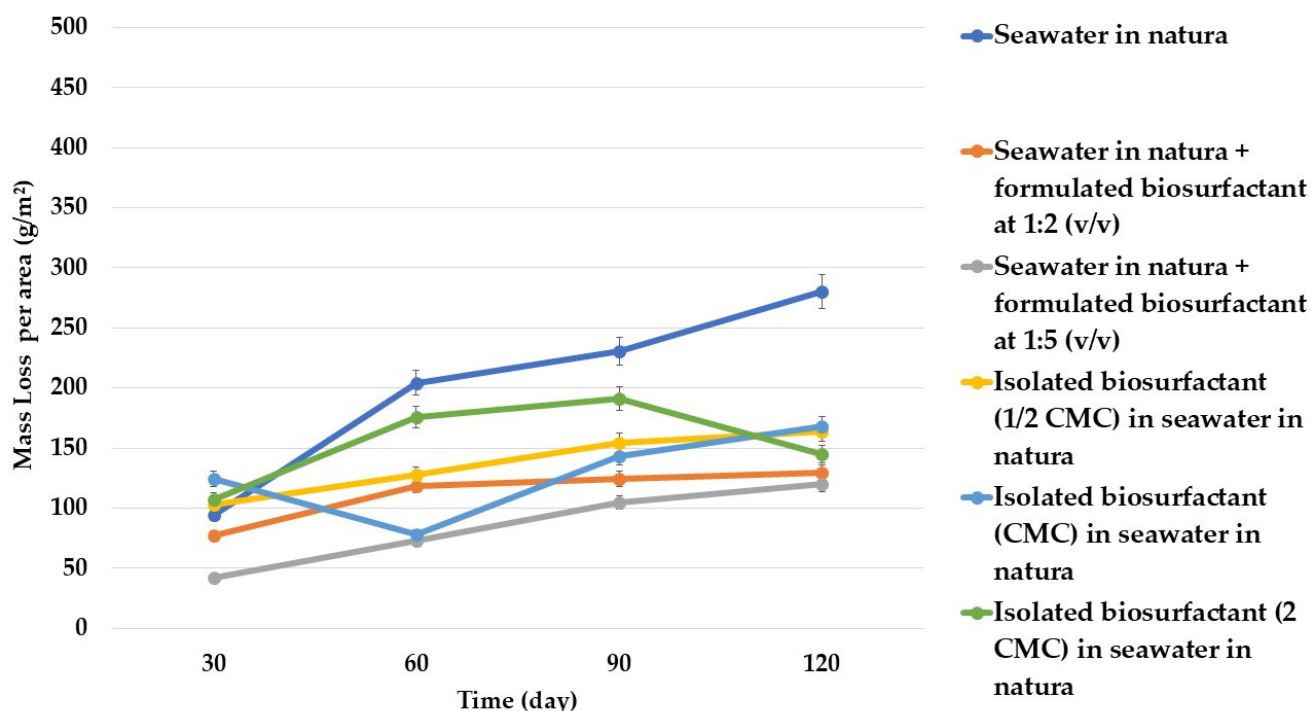


Figure 2. Results of the action of *Pseudomonas cepacia* CCT 6659 biosurfactant as a biocorrosion inhibitor on metal parts expressed as mass loss per area over time. The tests were performed in static systems in seawater in natura.

The results described by Olivia et al. [30], who used coupons immersed in biosurfactant produced by *Penicillium citrinum* at concentrations of 5, 7.5 and 10% (v/v), demonstrated that the biosurfactant at a concentration of 10% (v/v) had the highest corrosion inhibition efficiency (58.02%) and was more effective than the synthetic non-ionic surfactant Tween 80. Li et al. [31] reported that a rhamnolipid produced by *Pseudomonas aeruginosa* inhibited corrosion caused by *Bacillus licheniformis* by 68.4%, reinforcing the viability of biosurfactants as an alternative to traditional chemical inhibitors.

Figure 3, which refers to sterile seawater, shows the impact of the microbiota on the biosurfactant performance. In the absence of living organisms, the results were more expressive for sterile water without additives. However, the addition of the biosurfactant led to better performance, i.e., lower mass losses in metal structures. In particular, the biosurfactant formulation prepared in a 1:2 (v/v) ratio stood out, as it guaranteed, after 120 days, a mass loss value ($123.94 \pm 2.69 \text{ g/m}^2$) 46.7% lower than that obtained in the control ($232.39 \pm 4.0 \text{ g/m}^2$). Similar results were reported by Parthipan et al. [32], who also observed the effectiveness of biosurfactants in reducing corrosion in abiotic systems. They attributed the significant corrosion in the abiotic control system to the high chloride content, which was further intensified by corrosive bacterial strains, leading to mass losses of up to $44.5 \pm 1 \text{ mg}$. However, with the use of a glycolipid biosurfactant, the mass loss was drastically reduced (4–6 mg) in 20 days, both in abiotic systems and in the presence of microbial consortia. These findings support our results, indicating that even in sterile and highly saline environments, the use of biosurfactants can significantly mitigate corrosion processes.

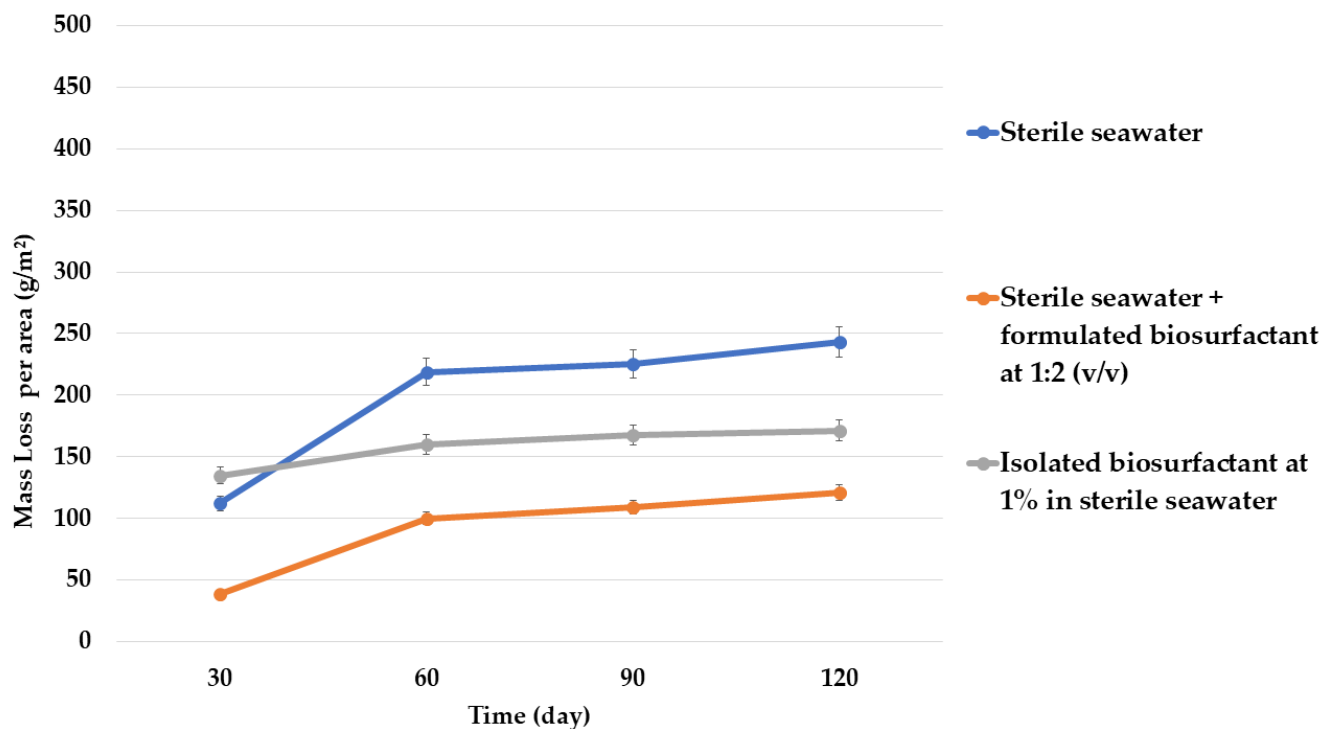


Figure 3. Results of the action of *Pseudomonas cepacia* CCT 6659 biosurfactant as a biocorrosion inhibitor on metal parts expressed as mass loss per area over time. The tests were performed in static systems in sterile seawater.

Previous studies revealed that biosurfactants can interfere with biofilm formation by modifying the hydrophobicity of the metal surface, making it less favorable for microbial adhesion [31,33]. The hydrophilic part of the rhamnolipid molecule, made up of rhamnose sugar units, mainly handles adsorption onto the metal surface through hydrogen bonding and electrostatic interactions with oxide or hydroxide groups naturally found on carbon steel. Conversely, the hydrophobic fatty acid chains (lipid part) stretch outward, creating a tight barrier that blocks corrosive ions and reduces microbial adhesion. This amphiphilic structure explains how rhamnolipids can both change surface energy and prevent biofilm formation. This structural detail emphasizes the importance of knowing whether the biosurfactant is mono- or di-rhamnolipid, as well as the fatty acid chain length, to improve understanding and optimize its adsorption and protective abilities [34]. This corroborates the findings of this study, since the presence of the biosurfactant reduced corrosion even in dynamic systems, over the 120 days of testing (Figures 4 and 5). In fact, in these tests, the use of aquarium pumps represents an important dynamic element, since the continuous agitation promoted by this equipment is able to directly influence the interaction among the biosurfactant, the compounds present in the water, and the microorganisms (when present). Therefore, the dynamics generated may have accelerated processes that, under static conditions, would occur more slowly [35,36].

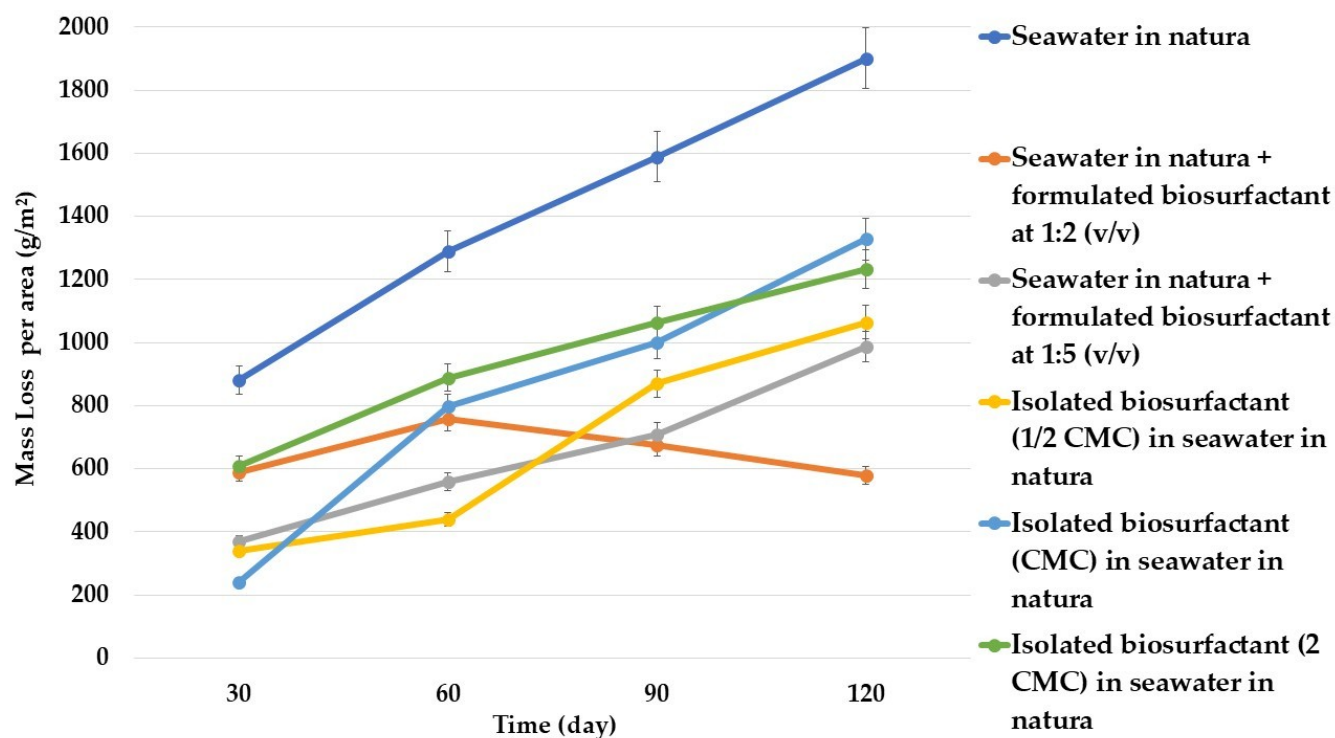


Figure 4. Results of the action of *Pseudomonas cepacia* CCT 6659 biosurfactant as a biocorrosion inhibitor on metal parts expressed as mass loss per area over time. The tests were performed in dynamic systems in seawater in natura.

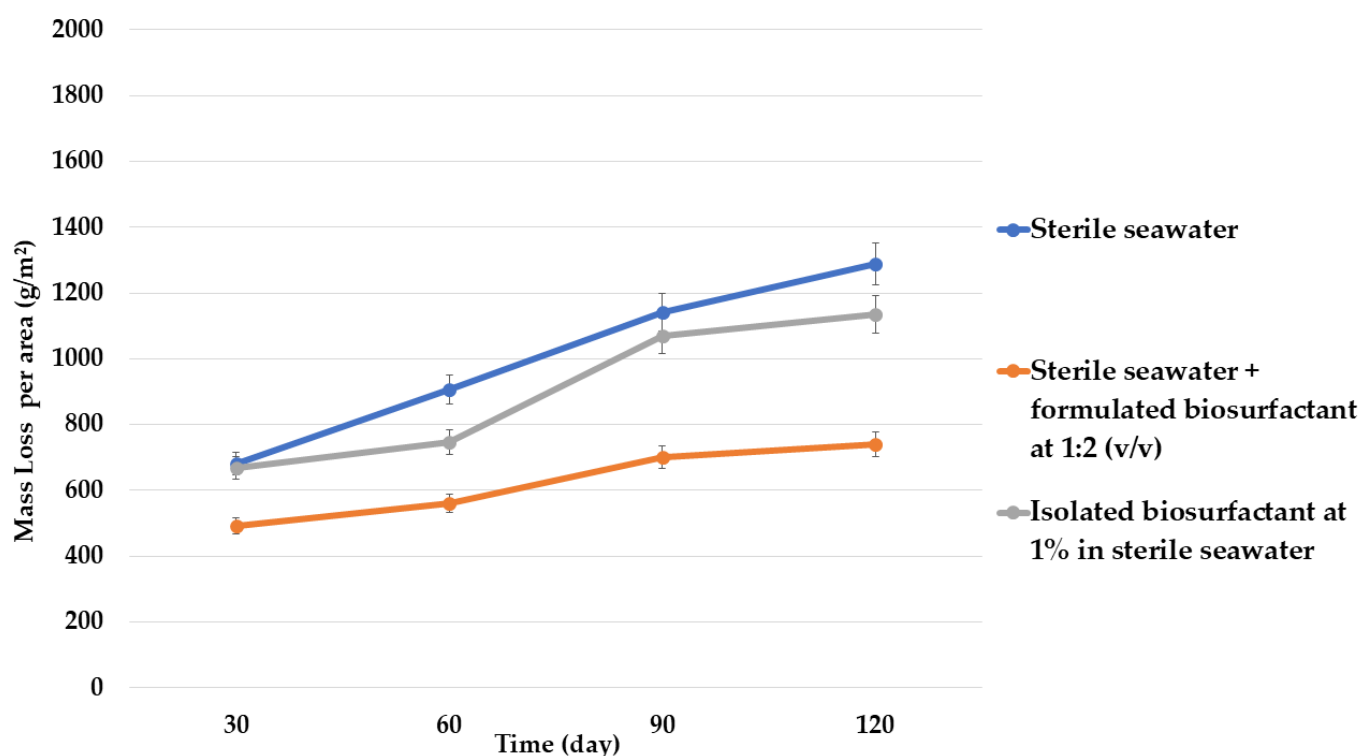


Figure 5. Results of the action of *Pseudomonas cepacia* CCT 6659 biosurfactant as a biocorrosion inhibitor on metal parts expressed as mass loss per area over time. The tests were performed in dynamic systems in sterile seawater.

It is possible that agitation in the dynamic system intensified the interaction between the compounds present in the system, increasing their bioavailability for degradation.

Although dynamic conditions are often associated with reduced inhibition due to possible removal of protective films, in this study, the biosurfactant maintained high efficiency under agitation. This can be attributed to its physicochemical stability and strong surface affinity, which favor the formation of a stable adsorbed layer even under flow. Similar effects were observed by Purwasena et al. [37], who reported efficient corrosion protection by biosurfactants under continuous flow, and by Wang and Yan [38], who emphasized that certain biosurfactants remain active under dynamic conditions due to their interfacial properties. This effect was particularly evident in seawater in natura, where the autochthonous microbiota acted synergistically with the biosurfactant, leading to a gradual increase in corrosion over 120 days (Figure 4). However, the biosurfactant formulated in a 1:2 (*v/v*) ratio stood out in inhibiting corrosion, leading to a mass loss after 120 days ($578.87 \pm 7.01 \text{ g/m}^2$), 69.6% lower than that obtained in the control condition ($1901.41 \pm 13.53 \text{ g/m}^2$). Mao et al. [39] investigated the synergistic effect of the ecological surfactant gemini ester in combination with saline additives in preventing corrosion and evaluated its efficiency by measuring mass loss, potentiodynamic polarization, and electrochemical impedance spectroscopy. Even though the surfactant alone exhibited high corrosion prevention efficiency (94.5%) at the concentration of $6.9 \times 10^{-5} \text{ M}$, its maximum efficiency (98.4%) was achieved in combination with the saline additives.

In the case of sterile seawater (Figure 5), the absence of microbiota may have limited the biodegradative processes, restricting the action of the biosurfactant to physicochemical reactions. Agitation may have favored only the dispersion of the compounds and the direct interaction of the biosurfactant with the medium. This explains the superior results obtained under the conditions tested, especially using the biosurfactant formulated in a 1:2 (*v/v*) ratio, which exhibited the lowest corrosion rate after 120 days ($739.44 \pm 8.06 \text{ g/m}^2$) compared to that observed in the control ($1288.55 \pm 10.96 \text{ g/m}^2$), yielding a reduction in mass loss of 42.7%. Similar results were obtained by Li et al. [31], who demonstrated that the rhamnolipid produced by *P. aeruginosa* not only inhibited the corrosion of X70 carbon steel in simulated seawater but also acted effectively against microbiologically influenced corrosion induced by *B. licheniformis*. The 68.4% reduction in corrosion current density and the inhibition of bacterial growth at concentrations above 125 mg/L highlight the dual functionality of biosurfactants: antimicrobial activity and anticorrosive protection. Although antimicrobial activity was not an active factor in our experiments, the similarity of the results supports the effectiveness of biosurfactants even in sterile environments, reinforcing that their physicochemical properties alone can significantly contribute to corrosion mitigation.

The comparison between the static and dynamic tests revealed that agitation accelerated the processes dependent on the mixing of the components present in the system, reducing the time required to observe significant changes. However, it is important to emphasize that, in real environments, such as metal parts exposed to salinity, natural dynamic conditions may be less intense than in the controlled experimental system, which may result in differences in the observed performance. Studies in the literature reinforced the critical role of agitation in the efficiency of biosurfactants. According to Rahman et al. [40], agitation increases the bioavailability of hydrophobic organic compounds, enhancing their biodegradation. On the other hand, Das and Mukherjee [41] pointed out that very high concentrations of biosurfactants can generate interference due to excessive agitation, impairing micelle stability. In addition, it is known that agitation improves the oxygenation of the system, which favors the activity of aerobic microorganisms in seawater in natura.

Figure 6 illustrates the status of the test specimens (TSs) after the corrosion process. In the first image, corresponding to the control condition, marked corrosion is observed, evidencing intense metallic degradation in seawater. In the other two images, which refer

to the use of the isolated and formulated biosurfactant as additives for corrosion inhibition, a significant reduction in corrosive effects can be noted. These results demonstrate the high potential of using the biosurfactant, especially in its formulated version, as an effective strategy for controlling MIC, contributing to the reduction of corrosive damage in marine environments. Soares da Silva et al. [42] used a coating matrix composed of vegetable resin as a plasticizer, oleic acid, ethanol, and CaCO_3 to incorporate the *P. cepacia* biosurfactant. The addition of the biosurfactant at twice its Critical Micelle Concentration (CMC) led to a reduction in the mass loss of the carbon steel specimen from 123.6 to 82.2 g/m², while in the galvanized iron sheets, the mass loss decreased from 285.9 to 226.7 g/m² at the same biosurfactant concentration. In another test, synthetic enamels supplemented with the same microbial biosurfactant were investigated. The alkyd resin-based paint ensured lower mass loss in the samples (46.0 g/m²) compared to the control without biosurfactant (58.0 g/m²). Using the paints formulated with oil-based resin and petroleum derivatives, the mass loss decreased from 53.0 to 24.1 g/m² and from 82.2 to 27.6 g/m², respectively.

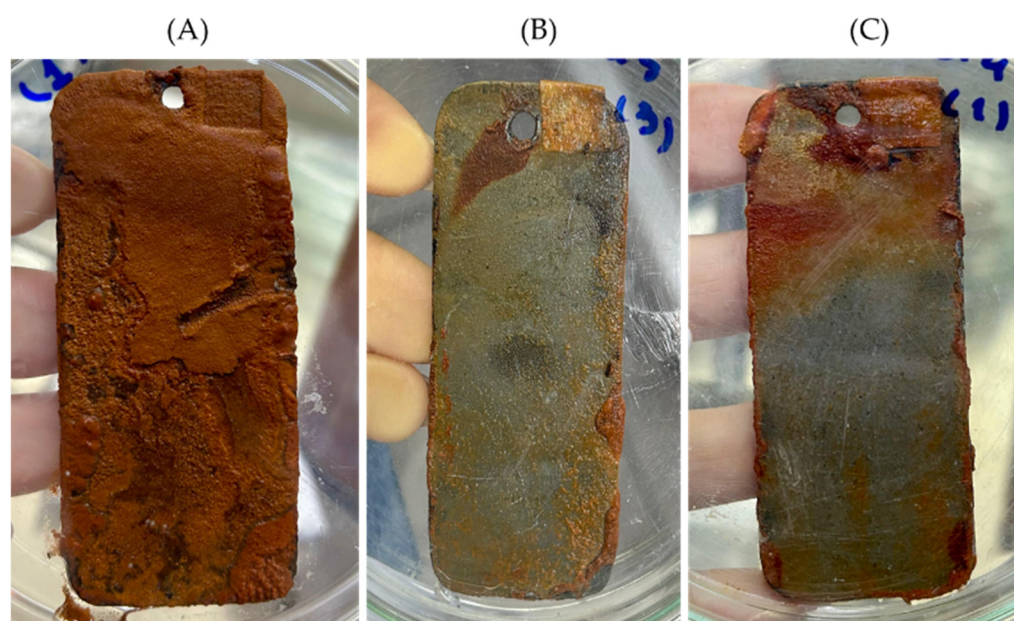


Figure 6. Biofilm formed on the surface of carbon steel sheets (A) under control conditions, (B) in the presence of the formulated biosurfactant, and (C) in the presence of the isolated biosurfactant.

3.2. Autochthonous Microorganisms in Static and Dynamic Systems

The qualification analysis of autochthonous microorganisms, namely Total Anaerobic Heterotrophic Bacteria (TAnHB), Total Aerobic Heterotrophic Bacteria (TAHB), and SRB, in static systems over 120 days revealed the influence of fluid conditions on microbial growth (Table 3). Seawater in natura supported the continuous presence of these microorganisms, probably due to the availability of nutrients and sulfate. The addition of formulated and isolated biosurfactant limited or inhibited the growth, especially of SRB, which are well known for their resistance to antimicrobial compounds. Sterile seawater prevented microbial development, but in some supplemented conditions, growth resumed, indicating possible bacterial presence or adaptation. These results reflect microbial dynamics influenced by factors such as substrate availability, chemical inhibition, and the ability of bacteria to adapt themselves to the environment. Salgar-Chaparro et al. [43] studied the impact of nutrient levels on biofilm characteristics, biocide efficacy, and associated MIC risk using multi-species biofilms from two consortia from different oil fields. The study demonstrated that continuous nutrient flow for microbial growth resulted in greater activity, thickness, and robustness of biofilms formed on carbon steel, which induced greater localized corrosion

compared to biofilms formed under batch nutrient conditions. There was a high incidence of anaerobic bacteria under all conditions, probably because, as the system was static and there was no oxygen renewal, the aerobic bacteria quickly consumed the oxygen dissolved in the medium, thus favoring the subsequent development of anaerobic bacteria [44].

Table 3. Qualitative analyses of autochthonous microorganisms in static systems. TAHB = Total Aerobic Heterotrophic Bacteria; TAnHB = Total Anaerobic Heterotrophic Bacteria; SRB = Sulfate Reducing Bacteria.

Fluid Conditions	30 Days			60 Days			90 Days			120 Days		
	TAHB	TAnHB	SRB	TAHB	TAnHB	SRB	TAHB	TAnHB	SRB	TAHB	TAnHB	SRB
Seawater in natura	+	+	+	—	+	+	+	+	+	+	+	+
Seawater in natura + formulated biosurfactant at 1:2 (v/v)	+	+	—	+	+	+	+	+	+	+	+	—
Seawater in natura + formulated biosurfactant at 1:5 (v/v)	+	+	—	+	+	—	+	+	—	+	+	—
Isolated biosurfactant (1/2 CMC) in seawater in natura	+	—	+	—	—	+	—	—	—	—	—	—
Isolated biosurfactant (CMC) in seawater in natura	+	+	+	+	—	+	—	—	—	—	—	—
Isolated biosurfactant (2 CMC) in seawater in natura	+	—	+	+	—	+	—	—	—	—	—	+
Sterile seawater	+	—	+	—	—	+	—	—	+	+	—	+
Sterile seawater + formulated biosurfactant at 1:2 (v/v)	+	+	—	+	+	—	+	+	—	+	+	—
Isolated biosurfactant at 1% in sterile seawater	+	—	—	+	+	—	+	+	—	+	—	—

The results in the dynamic systems (Table 4) indicate that seawater in natura favored the MIC due to the continuous presence of SRB up to 60 days, possibly due to microbial competition and nutrient reduction. In the presence of the formulated biosurfactant, there was a progressive disappearance of bacteria after 60 days, indicating that biofilms conferred high microbial resistance, while in the presence of the isolated one, there was initial growth followed by reduction of all microorganisms after 90–120 days. The microbial growth was less in sterile seawater, reinforcing the biological nature of MIC. These results indicate that corrosion can be mitigated by biosurfactants and strategies based on microbiota regulation, contributing to the control of MIC in marine environments.

Table 4. Qualitative analyses of autochthonous microorganisms in dynamic systems. TAHB = Total Aerobic Heterotrophic Bacteria; TAnHB = Total Anaerobic Heterotrophic Bacteria; SRB = Sulfate Reducing Bacteria.

Fluid Conditions	30 Days			60 Days			90 Days			120 Days		
	TAHB	TAnHB	SRB	TAHB	TAnHB	SRB	TAHB	TAnHB	SRB	TAHB	TAnHB	SRB
Seawater in natura	+	+	+	+	+	+	+	+	+	—	—	—
Seawater in natura + formulated biosurfactant at 1:2 (v/v)	+	+	+	—	—	—	—	—	—	+	—	—
Seawater in natura + formulated biosurfactant at 1:5 (v/v)	+	+	+	+	—	+	+	+	—	+	+	—
Isolated biosurfactant (1/2 CMC) in seawater in natura	+	—	+	+	—	+	+	+	—	+	+	—
Isolated biosurfactant (CMC) in seawater in natura	+	+	+	—	+	—	—	+	—	—	+	—
Isolated biosurfactant (2 CMC) in seawater in natura	+	—	+	+	+	+	+	+	—	—	+	—
Sterile seawater	—	+	—	—	+	+	+	+	—	—	—	—
Sterile seawater + formulated biosurfactant at 1:2 (v/v)	+	+	—	+	—	+	—	—	—	—	+	—
Isolated biosurfactant at 1% in sterile seawater	+	—	+	—	+	—	—	+	—	—	+	—

Stancu [45] detected aerobic and anaerobic bacteria at low levels in water samples and isolated three bacterial strains using a sample enrichment procedure, which, based on phenotypic and genotypic characteristics, were identified as *Stenotrophomonas maltophilia* IBB Cn1 (MT893712), *Stenotrophomonas maltophilia* IBB Cn2 (MT893713), and *Bacillus thuringiensis* IBB Cn3 (MT893714). The bacteria in the water sample were able to initiate corrosion of A570 and 1045 carbon steel. SRB were detected in higher numbers than heterotrophic bacteria and iron-oxidizing bacteria at the end of the biocorrosion tests. Carbon steel coupons showed macroscopic and microscopic changes in surface characteristics, which may have resulted from the formation of biofilm on their surfaces and the accumulation of corrosion products. The corrosion rate varied from one type of carbon steel to another, depending on the incubation conditions and the chemical composition of specimens. Faccioli et al. [13] observed a decrease in the sessile microbial population over time, probably due to the non-renewal of nutrients throughout the test and/or the dynamics of biofilm formation itself.

3.3. Scanning Electron Microscopy

Biocorrosion is a recurring problem in marine environments, where microbial biofilms promote intense corrosive processes on metal surfaces. The presence of bacteria adhered to metal material is generally associated with an increased corrosion rate, especially when corrosive microorganisms such as SRB and other biofilm-forming species are involved [46,47]. However, the results obtained in this study demonstrate that, despite the presence of bacteria in the samples treated with formulated biosurfactant, the mass loss associated with corrosion was significantly reduced in this condition, when compared to the control exposed only to seawater in natura.

The selection of conditions for Scanning Electron Microscopy (SEM) analysis was based on the results of the mass loss tests, in which the formulated biosurfactant showed better performance compared to the other conditions. It was decided to use systems with seawater in natura instead of sterile seawater in order to also investigate the marine microbiota present and its influence on the biocorrosion process.

The SEM analysis revealed the occurrence of structures typical of biofilm formation in samples subjected for 30 and 120 days to both static and dynamic experimental conditions in seawater in natura, either in the absence or the presence of biosurfactant (Figures 7–10). The lower corrosion rate in the presence of biosurfactant suggests that its action is not based solely on the total elimination of microbiota, but also on altering the biofilm structure and modulating the interaction between microorganisms and the metal surface. This hypothesis is corroborated by studies in the literature that demonstrate that biosurfactants can influence bacterial adhesion, biofilm formation, and associated corrosive processes [48].

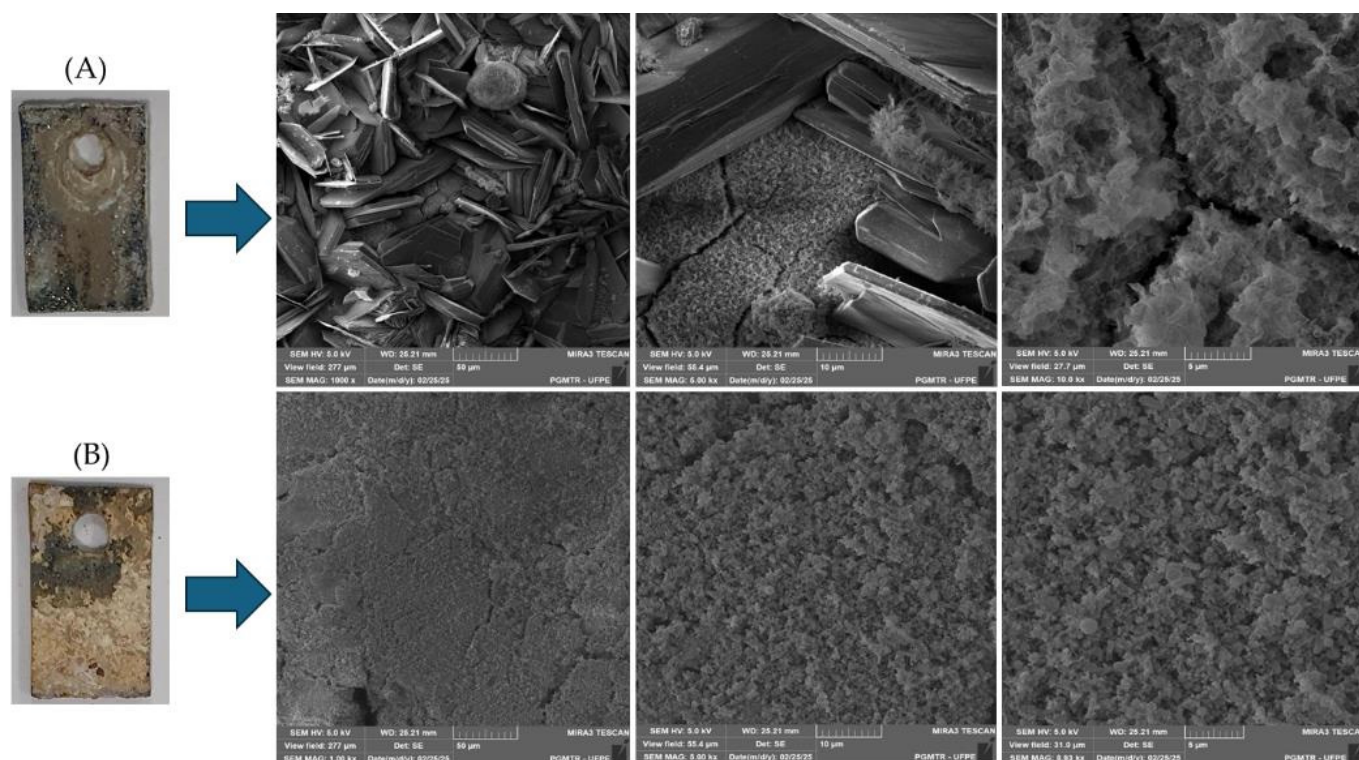


Figure 7. Scanning Electron Microscopy micrographs of static system specimens after 30 days in seawater in natura. Conditions: (A) control; (B) formulated biosurfactant at 1:5 (v/v).

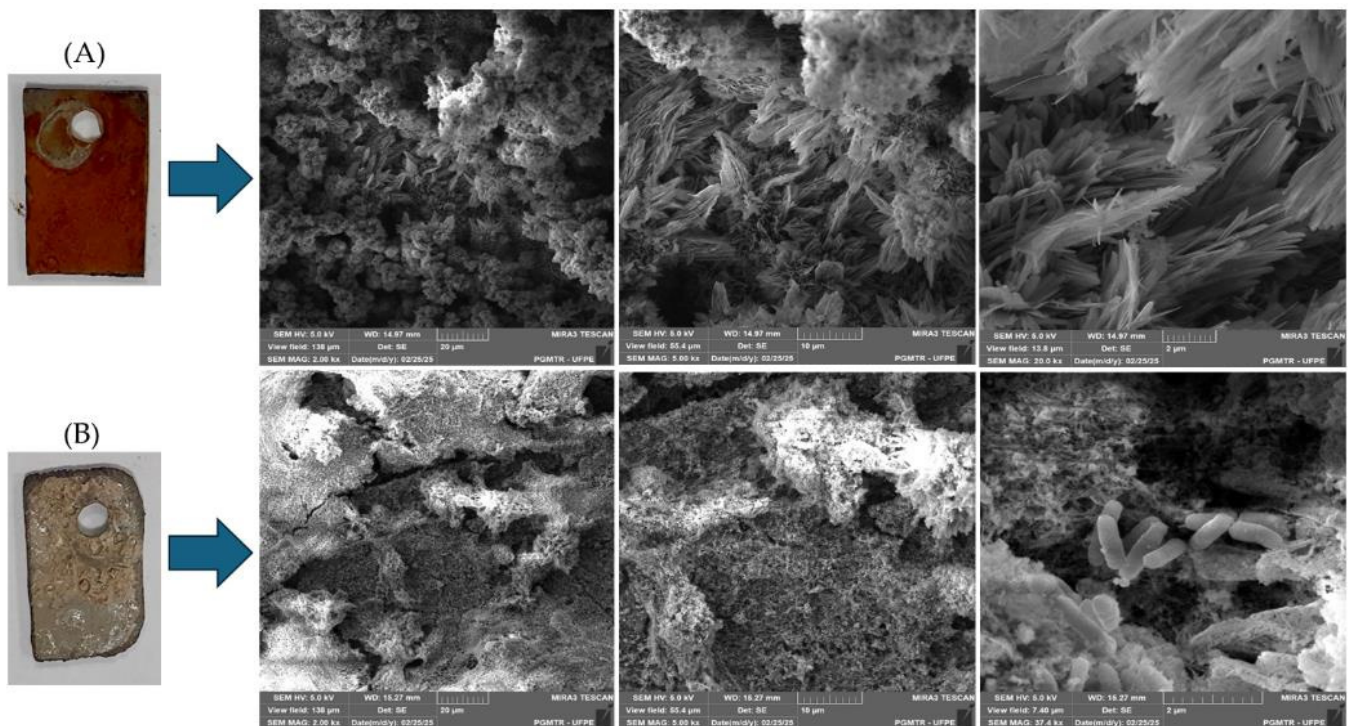


Figure 8. Scanning Electron Microscopy micrographs of dynamic system specimens after 30 days in seawater in natura. Conditions: (A) control; (B) formulated biosurfactant at 1:5 (v/v).

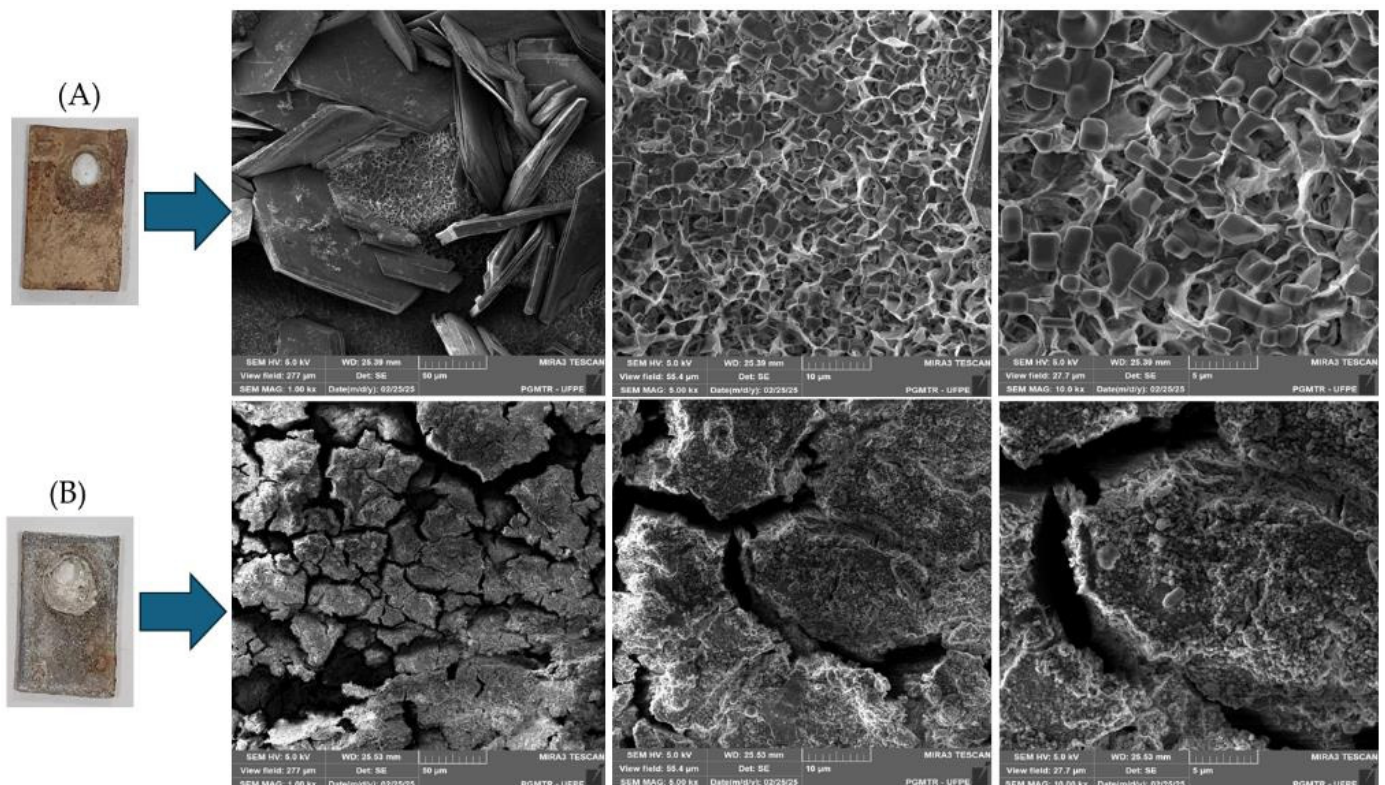


Figure 9. Scanning Electron Microscopy micrographs of the static system specimen after 120 days in seawater in natura. Conditions: (A) control; (B) formulated biosurfactant at 1:5 (v/v).

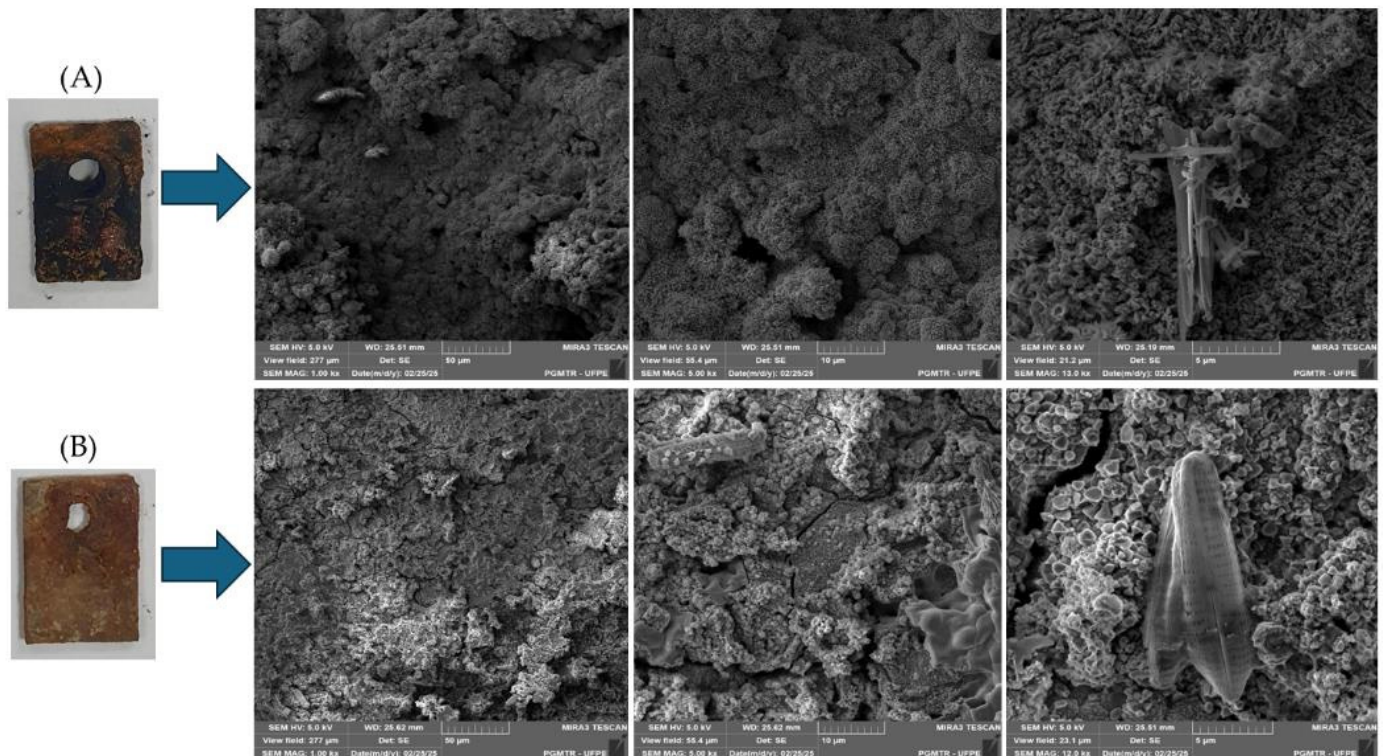


Figure 10. Scanning Electron Microscopy micrographs of dynamic system specimens after 120 days in seawater in natura. Conditions: (A) control; (B) formulated biosurfactant at 1:5 (v/v).

Based on the images of specimens treated at the two selected time points—at the beginning and end of the tests—it is evident that granular structures are present, alongside bacterial and phytoplankton cells typical of marine microbiota. This suggests that the sample was subjected to conditions conducive to biofilm formation. These visual characteristics align with those described in the literature as typical of marine biofilms [49]. Furthermore, the identification of both heterotrophic aerobic and anaerobic bacteria, as well as sulfate-reducing bacteria, within the system further supports this conclusion. While specific staining of the extracellular polymeric matrix was not conducted, the combination of morphological evidence and the microbiological context allows for a qualitative inference regarding the presence of biofilm.

These findings indicate that there was no significant biocidal action by the *Pseudomonas cepacia* CCT 6659 biosurfactant investigated in this study, which had been previously characterized as a rhamnolipid [16]. Previous studies indicated that biosurfactants, such as rhamnolipids and surfactin, can significantly reduce the initial adhesion of microorganisms to metal surfaces, interfering with the formation of structured and highly corrosive biofilms such as those of SRB, while allowing colonization by other less aggressive microorganisms [46,47]. Thus, the lower corrosion rate observed in this study may have been due to a change in the microbial community of the biofilm, making it less prone to inducing intense corrosive reactions. Didouh et al. [50], through SEM analyses, confirmed the presence of thick biofilm clusters and corrosion spots in carbon steel samples exposed to injection water. In contrast, in crude oil samples, less biofilm formation and reduced corrosion levels were observed.

Jin et al. [51], who used SEM to examine the morphologies of biofilms formed by *Geobacter sulfurreducens* ACL_{HF} and *Vibrio* sp. EF187016 strains, as well as by a mixed consortium including precultured *Vibrio* biofilm, observed that a significant number of bacterial cells adhered uniformly to the surface of carbon steel, forming intact biofilms. *Vibrio* sp. EF187016 exhibited the highest biofilm formation capacity. Purwasena et al. [37],

through SEM analyses, observed that coupons without the addition of biofilm consortium did not exhibit corrosion products on their surface. In contrast, all coupons inoculated with biofilm exhibited signs of corrosion. Both coupons treated without antimicrobials and those treated with glutaraldehyde revealed corrosion products with morphology similar to stacked sheets. On the other hand, specimens treated with biosurfactant showed corrosion products organized in a centralized, flower-like structure. These results demonstrated that biofilm formation on the steel surface was positively correlated with corrosion.

3.4. Characterization by Energy Dispersive Spectroscopy

The analysis of the EDS spectra was performed for the static and dynamic systems after 30 days, both in the control condition and in the presence of formulated biosurfactant at 1:5 (*v/v*), with the aim of evaluating the biofilm and its components. The choice of the 30-day exposure time was due to the fact that, in closed systems, a too-long exposure would have resulted in excessive degradation of the biofilm, making detailed analysis difficult and compromising the accurate visualization of its composition. This occurs because, in these systems, the absence of renewal leads to the gradual depletion of nutrients, promoting the progressive biofilm degradation over time [43]. In general, the addition of the biosurfactant to seawater *in natura* resulted in significant changes in the elemental composition of the TS surface.

In particular, the corrosion inhibition observed in the static system in the sample treated with the formulated biosurfactant can be explained by several factors involving changes in the chemical composition of the surface, as shown in the Energy Dispersive Spectroscopy (EDS) spectra (Figure 11).

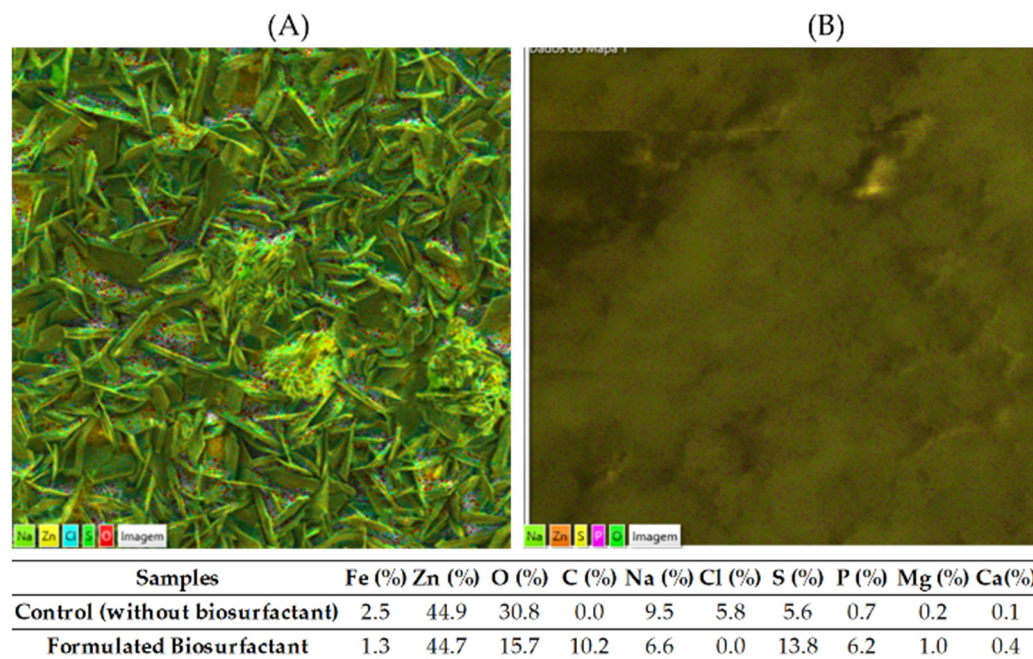


Figure 11. Scanning Electron Microscopy visualization and semiquantitative characterization of the elements present on the surfaces of the test specimens, after 30 days in a static system, obtained by Energy Dispersion Spectroscopy. Conditions: (A) control; (B) formulated biosurfactant at 1:5 (*v/v*).

The presence of a biosurfactant in the solution can lead to the adsorption of organic molecules on the metal surface, forming a physical barrier that prevents direct contact of the metal with corrosive agents such as oxygen (O), chlorine (Cl), and ions dissolved in seawater. This may have led to the significant increase observed by other authors in the incidence of carbon and sulfur on the surface of samples, which suggests the formation

of a protective film composed of organic compounds of the biosurfactant [7,38]. In the present study, the EDS spectrum showed a reduction in the oxygen content from 30.8 to 15.7% in the presence of the biosurfactant. Corrosion in marine environments occurs mainly by oxidation reactions, where dissolved oxygen reacts with the metal to form corrosive oxides and hydroxides. The reduction of oxygen on the metal surface suggests that the biosurfactant inhibited the availability of this essential reagent for corrosion [52,53].

In the EDS spectrum of the sample treated with biosurfactant (Figure 12), a reduction in sodium (Na) content from 9.5% to 6.6% and in that of Cl from 5.8% to a value below the detection limit was observed, which suggests that the biosurfactant may have altered the mobility or solubility of corrosive ions, reducing their degrading effect on the metallic material [38]. Variations in the presence of sulfur (S) in corrosion products are relevant, since this element is associated with the activity of sulfate-reducing bacteria and the formation of iron sulfides (FeS). In the control condition, 5.6% sulfur was observed, while in the sample with the formulated biosurfactant, this value increased to 13.8%, possibly due to the retention of sulfur metabolites promoted by the biosurfactant. The absence of agitation in the static system may have favored the deposition of these compounds, intensifying their detection by EDS. Nevertheless, the mass loss data indicate that, despite the higher content of retained sulfur, the biosurfactant acted as an inhibitor, reducing the corrosion rate. The incidence of iron (Fe), which is one of the main indicators of corrosion for being one of the first elements to be oxidized and dissolved in marine environment, was reduced from 2.5 to 1.3%, suggesting a lower corrosion rate probably resulting from the formation of a protective barrier interfering with its dissolution as well as with the deposition of corrosive oxides.

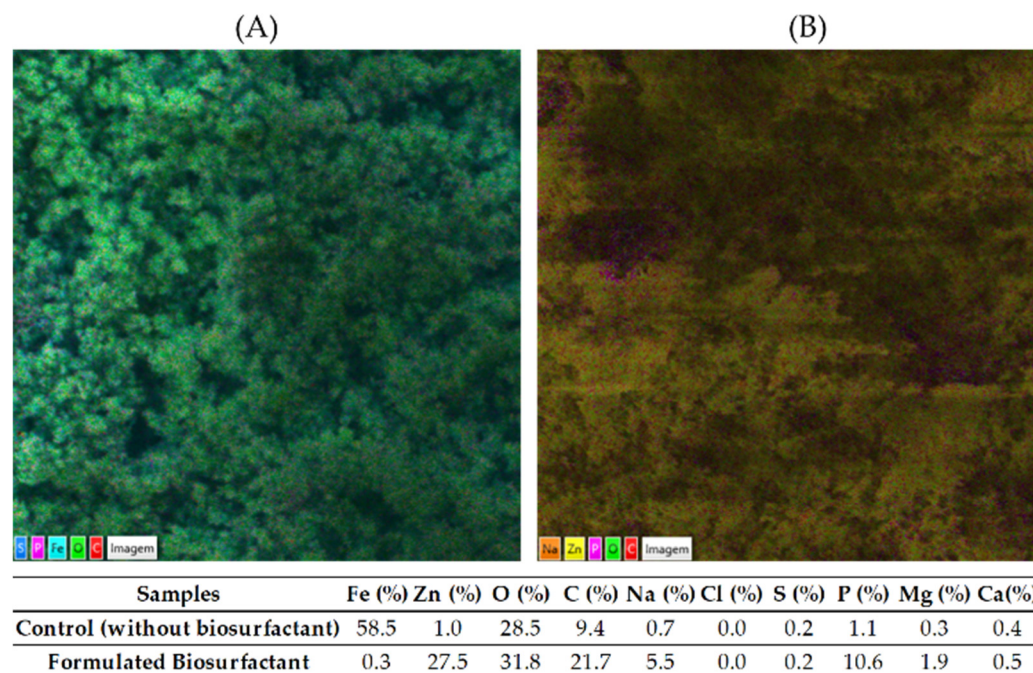


Figure 12. Scanning Electron Microscopy visualization and semiquantitative characterization of the elements present on the surfaces of the test specimens, after 30 days in a dynamic system, obtained by Energy Dispersion Spectroscopy. Conditions: (A) control; (B) formulated biosurfactant at 1:5 (*v/v*).

The elemental chemical characterization of the surfaces of samples subjected to treatment in a dynamic system revealed equally important findings (Figure 12).

A clear contrast was observed between the static and dynamic systems regarding the surface elemental composition revealed by EDS. In the static condition, zinc appeared in a higher proportion than iron (44.9% Zn vs. 2.5% Fe in the control), suggesting the presence

of a superficial zinc layer that remained mostly intact due to the lower mechanical stress. In contrast, in the dynamic control system, the zinc content dropped to 1.0%, while the iron content increased to 58.5%, indicating the possible removal of the zinc coating and exposure of the steel substrate. Notably, under dynamic conditions with the biosurfactant, zinc levels rose again (27.5%) and iron dropped to 0.3%, suggesting a protective effect of the biosurfactant in maintaining the surface barrier. Similar effects have been reported in the literature, where the use of surfactants has been shown to enhance the compactness and protective performance of surface films, especially those containing zinc and phosphate compounds [54], and to influence the elemental distribution on coated surfaces, depending on agitation and the presence of additives [55].

It is important to emphasize the drastic reduction in Fe content on the analyzed surface (from 58.5 to 0.3%). While in the control system, the high concentration of Fe and O indicated intense formation of iron oxides, in the presence of the biosurfactant, there was a significant increase in the levels of Zn (from 1.0% to 27.5%) and phosphorus (P) (from 1.1 to 10.6%). This suggests that protective compounds, such as phosphates and carbonates, may have formed on the surface, acting as a physical barrier against corrosion [55,56]. For sulfur (S), both conditions presented low levels (0.2%), which suggests that the flow regime hindered the deposition of sulfur compounds, contributing to the mitigation of biocorrosion. These results reinforce the influence of the hydrodynamic regime on both the performance of the biosurfactant and the intensity of corrosion induced by microorganisms. The increase in the C content from 9.4 to 21.7% and P may have been due to the formation of biofilms, whose presence is reinforced by the role of the biosurfactant in promoting microbial growth [57].

Purwasena et al. [37] used EDS to characterize corrosion products on metal coupons exposed to biofilms, identifying high concentrations of O, P, and Fe, suggesting the formation of oxides such as Fe_3O_4 , Fe_2O_3 , and possibly P_2O_5 , the latter attributed to phosphate addition in the saline medium. The chemical composition correlated with biofilm presence, highlighting the microbial influence on the corrosion process. Changes in the morphology of the deposits depending on the antimicrobial treatment applied, along with the presence of phosphorus, also suggest the involvement of microbial metabolites in the complexation of metal ions, emphasizing the role of surface conditions in the formation of corrosion products. According to Boxin et al. [58], energy-dispersive X-ray spectroscopy (EDS) was employed to characterize the corrosion products formed on X52 steel after 14 days of immersion in produced seawater, under both biotic and abiotic conditions. In the biotic system, Fe, O, S, P, Ca, and Na were detected, with notably high sulfur content (5.62 wt%) and the presence of phosphorus, attributed to the activity of sulfate-reducing bacteria (SRB), which promote the formation of iron sulfides and sulfur-rich compounds known to be highly aggressive. In contrast, corrosion products formed in the abiotic system showed a distinct morphology and were composed mainly of Fe, O, Na, Cl, and Ca, with no detection of S or P, indicating a purely chemical corrosion mechanism. The comparison between systems clearly demonstrates that microbial activity significantly alters the composition and structure of corrosion deposits, intensifying corrosion processes in terms of both aggressiveness and complexity.

These observations indicate that the tested biosurfactant acted as an agent able to mitigate biocorrosion, reducing microbial adhesion to the metal without eliminating it completely. Its action is associated with interference in the initial adhesion of bacteria, modulation of biofilm composition, and dispersion of its consolidated structures, making the environment less prone to corrosion. These findings highlight the potential of biosurfactants as sustainable alternatives to synthetic corrosion inhibitors, with promising

applications in the protection of metal structures exposed to severe marine conditions, contributing to the preservation and longevity of materials in aggressive environments.

4. Conclusions

The results of this study demonstrated the great potential for the application of *Pseudomonas cepacia* CCT 6659 biosurfactant as a biotechnological inhibitor of microbiologically influenced corrosion, with an effective reduction in metal mass loss under conditions simulating real marine environments. The efficacy of the biosurfactant was evident in both static and dynamic conditions, being more pronounced in seawater in natura, where it promoted the structural integrity of carbon steel. The biosurfactant also showed relevant antimicrobial effects on microorganisms associated with biocorrosion, such as sulfate-reducing bacteria, which suggests its action not only as a physical barrier, but also as an antimicrobial agent, hindering the formation of corrosive biofilms. Scanning Electron Microscopy analyses showed reduced biofilm formation and deposition of corrosion products on surfaces treated with the biosurfactant, especially under dynamic conditions. Energy Dispersive Spectroscopy analyses confirmed a lower presence of elements associated with corrosion, such as sulfur, reinforcing the protective action of the biosurfactant on the integrity of the material. These findings indicate that the biosurfactant can be used in anticorrosive protection strategies in offshore systems, as part of ecological primers or smart coatings. Thus, this study contributes to the development of cleaner and more sustainable technologies for the energy sector, aligned with international guidelines and environmental commitments of companies in the oil, gas, and renewable energy sectors.

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