

EXPLORING THE GREEN POTENTIAL OF RHIZOPUS-DERIVED BIOSURFACTANT FOR CORROSION INHIBITION IN MILD STEEL

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Abstract

Corrosion poses significant challenges in construction and manufacturing, impacting aesthetics and structural integrity. This study evaluates the efficacy of Glycerolipids biosurfactant, derived from the soil fungus *Rhizopus*, in inhibiting corrosion on mild steel bars. The biosurfactant was cultivated in Mineral Salt Medium (MSM) broth supplemented with waste frying oil as the sole carbon source via aerobic batch fermentation for 40 days, with harvesting at a concentration of 1gml⁻¹, meeting emulsification index, drop collapse, and oil spreading test criteria. Biosurfactant's inhibition efficiency was evaluated through Scanning Electron Microscope analysis, weight loss experiments, and electrical resistivity tests. The specimens were immersed for 100 days in saline solutions with biosurfactant concentrations ranging from 5% to 20% (v/v). Comparative analyses were conducted with control specimens treated with the synthetic surfactant, Tween 80. The findings establish a direct correlation between biosurfactant concentration and corrosion inhibition efficacy, resulting in a reduced corrosion rate of mild steel bars. The results indicated that adding biosurfactant up to 17.5% of the volume of saline solution inhibited corrosion at 71.94% efficiency, with a corrosion rate of only 0.0201 mm/year. These findings highlight the potential of Glycerolipids biosurfactant as an eco-friendly corrosion inhibitor for mild steel.

Keywords: Corrosion inhibitor, Glycerolipids biosurfactant, Mild steel, *Rhizopus oryzae*, Waste frying oil.

1. Introduction

Corrosion is a natural phenomenon wherein metal undergoes degradation through chemical reactions occurring between the metal and its surrounding environment [1]. This ongoing reaction entails the oxidation of the metal, accompanied by the loss of electrons, leading to the formation of rust and other forms of degradation, such as cracking, spalling, delamination, hence affecting the performance [2]. In the construction industry, the corrosion of steel rebar remains a significant concern, jeopardizing the durability of reinforced concrete structures. Environmental factors such as carbonation and chloride diffusion exacerbate degradation processes, emphasizing the urgent need for effective corrosion inhibitors [3].

The application of corrosion inhibitors represents a widely recognized approach in the prevention and delay of the corrosion and degradation of mild steel reinforcement [4]. Classification of corrosion inhibitors can be established according to their application techniques, modes of protection, and composition. The composition can either be organic or inorganic. They operate by impeding the corrosive mechanism through the creation of a protective layer termed anodic inhibitors, or by augmenting polarity and diminishing corrosion potential, which are referred to as cathodic inhibitors. Additionally, there are dual-action inhibitors. These inhibitors can be directly applied onto the reinforcement, added into fresh concrete as admixture or additives, or applied onto the surface of a reinforced concrete structure [5]. While the use of inhibitors is a practical method, the reliance on conventional chemical inhibitors raises long-term environmental apprehensions. Organic inhibitors, particularly biosurfactants, offer a sustainable alternative with low-cost synthesis and high effectiveness in inhibiting corrosion [6].

The non-organic inhibitors commonly employed for corrosion mitigation are expensive and possess inherent toxicity. Despite their efficacy, concerns about the environmental impact and sustainability of the non-organic inhibitors have fuelled a growing interest in alternative solutions [7]. Therefore, there is a significant requirement to substitute the detrimental inhibitors with alternative solutions that are cost-effective, environmentally friendly, sustainable, and non-hazardous [8].

Studies conducted on biosurfactants as corrosion inhibitors reveals both successes and limitations in their application. Research by Parthipan et al. has delved into glycolipid biosurfactants produced by bacteria *Pseudomonas* as corrosion protection for carbon steel with inhibition efficiency of 87% [9]. Gana et al. reported that biosurfactant derived from *Bacillus* species inhibit the corrosion rate of metal in the oil industry [10]. Plaza and Achal in their review suggested that some microbials such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, and *Acinetobacter calcoaceticus* are good biosurfactant producer with ability to mitigate biocorrosion [11]. The biosurfactants produced in these previous studies have shown promising results in forming protective layers on metal surfaces, hindering corrosion processes. However, not all microbes are corrosion inhibitors producers as some are biocorrosion inducers.

Lactobacillus, *Acetobacter*, *Azospirillum*, and *Azotobacter* are types of microbes that metabolize to secrete organic and inorganic acids to induce corrosion [12]. These previous studies provide valuable insights into the biosurfactants and their microbial producers to facilitate the development of more effective and sustainable corrosion-minimizing methods and materials. The success of these

studies contributes to the growing recognition of biosurfactants as sustainable alternatives in corrosion protection.

From the previous research, a significant gap emerges in the current understanding, particularly concerning the use of fungal biosurfactants to alleviate corrosion in mild steel reinforcement. Additionally, the exploration of waste frying oil as a sustainable substrate for biosurfactant production, particularly derived from the filamentous soil fungus *Rhizopus Oryzae*, constitutes an underexplored avenue in the field of corrosion inhibition.

This research addressing this critical gap by evaluating the corrosion inhibitory potential of *Rhizopus*-derived biosurfactant, synthesized using waste frying oil as a substrate. The aim of this study is to evaluate the efficacy of the biosurfactants derived from *Rhizopus Oryzae*, an indigenous soil fungus, as a green alternative for corrosion inhibition in mild steel bars. The research objectives include the production of biosurfactant, followed by the assessment of inhibition performance through Scanning Electron Microscope (SEM) imaging, weight loss experiments employing gravimetric analysis, and electrical resistivity testing.

Rhizopus Oryzae was chosen due to its rapid growth and easy reproducibility. Importantly, this fungus excels in producing significant quantities of biosurfactant when utilizing waste frying oil as a substrate. It has demonstrated effectiveness under conditions of high salinity, elevated temperature, and extreme pH. The biosurfactant derived from *Rhizopus Oryzae* has proven capabilities in reducing water surface tension and exhibits high emulsification properties, indicating its potential effectiveness as a corrosion inhibitor.

Waste frying oil, a byproduct abundantly generated from various food-related industries, is typically disposed of, making it generally available at a lower cost. This characteristic ensures that waste frying oil serves as a readily available and scalable resource for biosurfactant production, and in some cases, it can be obtained for free from partnering industries. The dual advantage of cost-effectiveness and accessibility enhances the economic viability and sustainability of biosurfactant production using waste frying oil as a carbon source [13].

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The outcomes of the research pertaining to *Rhizopus*-derived biosurfactant present potential applications in the construction industry. Incorporating the biosurfactant in the formulation of coatings or paints for steel structures would provide a protective layer against corrosion. Additionally, developing admixtures for concrete that include the biosurfactant could enhance the durability of reinforced concrete structures.

2. Experimental Procedures

The experimental procedures were segmented into distinct phases, encompassing fungal isolation, biosurfactant production, screening methods, strain identification and fungal characterization, and corrosion inhibition testing.

2.1. Material preparation

The materials used in this research are Glycerolipids biosurfactant and mild steel bars (MS144:2014, G250) with a chemical composition shown in Table 1. The steel bars are plain in surface and are round sections. A total of eighteen (18) specimens were cut, each measuring 15mm in length with a diameter of 10mm. To prepare the test specimens, a fine brush and a range of emery papers were used for abrasion, progressing from coarser (220) to finer (1200) grades. The specimens underwent a thorough cleaning and degreasing process involving distilled water, acetone, and deionized water [14].

Following the cleaning procedure, the specimens were air-dried for thirty minutes at room temperature. The cleaning process is crucial for ensuring the removal of contaminants and preparing the specimens for subsequent inhibitor solution immersion. The cleanliness of the mild steel bars is essential as it directly influences the accuracy of measurements, weight assessments, and, ultimately, the corrosion inhibition testing. Prior to submerging the specimens in the inhibitor solutions, individual measurements and weight assessments were conducted on each specimen to determine their respective densities. Figure 1 provide a visual representation of the pre-treated specimens, emphasizing their condition before further experimentation.

Table 1. Chemical composition of mild steel bar (MS144:2014) grade 250.

Chemical Composition (%)	C	Si	Mn	P	S	V	Cu	Ni	Cr	Mo	Nb	C _{eq}
	X100			X1000			X100			X10000	X100	
	19	17	66	21	18	3	19	6	12	1	50	34



Fig. 1. Mild steel bar specimens.

2.2. Fungal isolation

The *Rhizopus oryzae* used in this research was sourced from the Faculty of Applied Science, Universiti Teknologi MARA, Kota Samarahan. Cryotube cryovials were employed for the storage of both master and working stocks. These were meticulously organized in properly labelled cryoboxes and stored at a temperature of 4°C. The isolation of the fungus involved the use of petri dishes containing Malt Dextrose Agar (MDA) for up to 10 days, with an incubation temperature of 37°C. All isolation procedures were conducted in triplicate to ensure reliability and consistency.

2.3. Biosurfactant production

For biosurfactant production, the fungal mycelium was extracted from the Petri dish after 10 days of isolation and inoculated into Mineral Salt Medium (MSM) broth containing 2.5 g/L (w/v) NaNO₃; 2.0 g/L (w/v) K₂HPO₄; 0.2 g/L (w/v) MgSO₄; 0.2 g/L (w/v) NaCl and 0.1 g/L (w/v) CaCl₂·6H₂O. Waste frying oil served as the sole carbon source at a concentration of 5% (v/v) of the total volume of the fermentation broth. The cultivation process was carried out at ambient temperature for a duration of 40 days. The waste frying oil underwent a thorough purification procedure involving twin filtrations with muslin cloth to eliminate all solid particulates. Only WFO with a pH above 7 was used as a substrate, as fungi have a higher production yield under this pH condition. The production of biosurfactant was conducted through cultivation in MSM broth under aerobic fermentation, utilizing batch processing and submerged cultivation [15]. The fermentation process was carried out in a customized plastic fermenter equipped with an oxygen aerator. Biosurfactant harvested after 40 days is shown in Fig. 2.

The biosurfactant was harvested when it reached maximum concentration of 1gml⁻¹ and passed all the screening tests namely emulsification index, drop collapse and oil spreading tests. Crude biosurfactants produced were extracted from fermentation broth using the liquid-to-liquid method by centrifugation and filtration. Liquid to liquid extraction was performed by combining equal volumes of the supernatant and ethyl ether in a separating funnel.



Fig. 2. Crude biosurfactant (after 40 days of fermentation).

2.4. Screening and selection of potential biosurfactant producers

Qualitative Drop Collapse Test and Oil Displacement Assay were used as screening methods to evaluate biosurfactant production. The Emulsification Index Test (E₂₄) was used as a quantitative measure [16]. The screenings were performed on the 7th, 10th, 14th, and 40 days within the fermentation duration. All screening procedures were conducted in triplicate to ensure consistency and reliability in the obtained results. From the three screening tests conducted, the biosurfactant secreted by *Rhizopus* exhibited the significant emulsification activities and low surface tension value.

2.4.1. Drop collapse test

This test relies on the droplet collapse of biosurfactant upon encountering a hydrophobic surface, providing a quantifiable measure indicative of the prevalence of biosurfactants [17]. The assay was conducted in a 96-microwell plate. A 2μL of

sterile motor oil was added into the wells and left equilibrated at 37°C for 1 hour. Subsequently, 5µL of supernatant was placed at the central region of the wells above the oil layer, and the geometry of the resulting oil droplets was assessed after one minute. Collapsed droplets indicated the presence of biosurfactant, while beaded droplets indicated the absence of biosurfactant [18].

2.4.2. Oil displacement test / oil spreading assay

The progress of biosurfactant production was monitored by assessing biosurfactant activity through the Oil Displacement Test. The underlying principle of this test, pioneered by Morikawa et al. in 2000, involves reducing the surface tension between water and oil phases. This test is instrumental in gauging the maturity of the biosurfactant and evaluating its surfactant activity. This technique is regarded as highly sensitive, reliable, rapid, and straightforward to execute [19].

In this experimental setup, a thin layer of oil formed atop 20 ml of distilled water in a petri dish by adding 2 ml of motor oil. Subsequently, a 1 ml cell-free culture broth was deposited in the middle of the oil layer. The presence and effectiveness of the biosurfactant were assessed based on the formation of a distinct clear zone within the oil-water mixture. The diameter of this clear zone served as a measure of the biosurfactant's efficiency.

2.4.3. Emulsification index (E24) test

A widely utilized and straightforward method in emulsification analysis is the Emulsification Index (E24), first introduced by Cooper and Goldenberg in 1987. This method proves to be highly efficient and is frequently employed in various scientific studies. It involves quantifying the percentage of the emulsified layer to the overall volume of the compound [20].

Figure 3(a) illustrates the results of the Emulsification Index Test conducted on the 10th day of biosurfactant cultivation, depicting the emulsified layer of a mixture of cooking oil and biosurfactant. While in Fig. 3(b), are the emulsification of Tween 80 and biosurfactant in motor oil after 24 hours of vortex. To quantify the emulsification characteristics, a cell-free broth was supplemented with motor oil at an equal volume ratio of 1:1 before undergoing vigorous shaking with a vortex mixer at high speed for two minutes. The vortexed mixture was left at room temperature for 24 hours. After 24 hours, the height of the emulsion layer was measured. The emulsion index, denoted as E24, was then calculated as the ratio of the emulsion layer's height to the total height of the liquid. The experiment was conducted in triplicates. The formula for the calculation of Emulsification Index (E24) is:

$$E_{24} = \frac{\text{Height of emulsion layer}}{(\text{Total height of the liquid}) \times 100} \quad (1)$$

This formula expresses the emulsification index as a percentage, providing a measure of the emulsifying efficiency in the liquid [20].

2.5. Fungal strain identification and biosurfactant characterization

The stocks of *Rhizopus oryzae* utilized in this research were provided by the Faculty of Applied Science, Universiti Teknologi MARA (UiTM), Kota Samarahan. The isolated fungus subsequently underwent morphological characterization to confirm its species and strain.

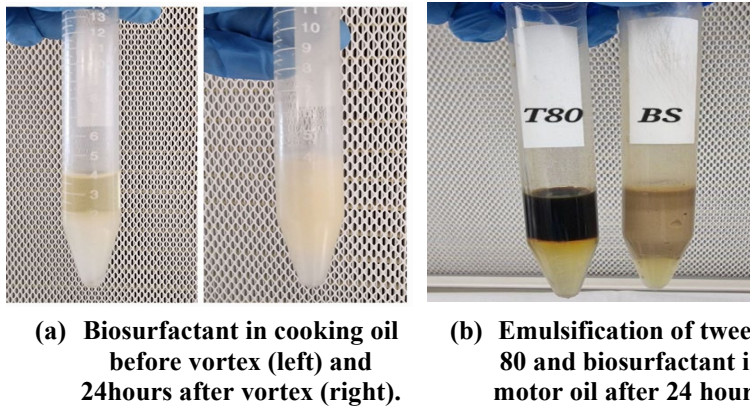


Fig. 3. Result of the emulsification index test after 24 hours.

2.5.1. Fungal strain identification

Microscopic observations of the fungus mycelium were conducted to assess various characteristic features, including the height, size, margin surface, and colour of the mycelium during its growth on agar plates. These observations were made after 7 to 14 days of isolation in the incubator at a temperature of 37°C.

Figure 4 provides a visual representation of the fungal growth on MDA after 7 to 10 days of isolation. Initially, the mycelium appears white and evolves into shades of brown, brownish-grey, grey, and eventually black when reaching maturity, effectively covering the entire petri dish. The fungus displays rapid growth with stolon's extending at various points to the substrate, facilitated by rhizoids. These morphological characteristics align with the known features of *Rhizopus oryzae*, confirming the strain's identity. This microscopic examination supporting its classification within the *Rhizopus oryzae* species [21].

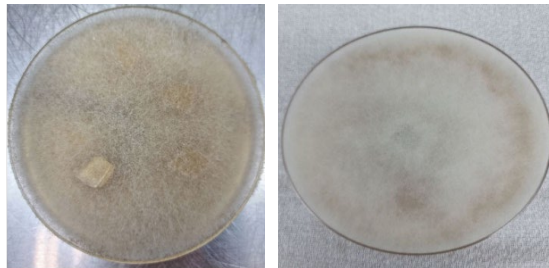


Fig. 4. Fungi growth on the malt dextrose agar after 7 days (left) and 10 (right) days of isolation.

2.5.2. Biosurfactant characterization

The identification of glycerolipids compounds was carried out by liquid chromatography quadrupole time-of-flight mass spectrometry (LCQTOF-MS). This analysis was done in both positive and negative ion modes. The analysis was performed using a mass spectrometer (Vion IMS QTOF, Waters, USA). The

compounds were identified by their chemical formula, mass spectra, mass errors, observe retention time (Rt) and adducts [22].

2.6. Evaluation of the corrosion inhibition efficiency

From the qualitative and quantitative screening tests conducted for potential biosurfactant producers, it was inferred that the biosurfactant produced by soil fungi, *Rhizopus oryzae* has the highest emulsification ability. From the LCQTOF-MS analysis, the biosurfactant produced was characterized as glycerolipids. It was then used in the corrosion inhibition studies to evaluate its ability as green corrosion inhibitor. The studies consist of two quantitative tests, namely Gravimetric Test and Electrical Resistance Test. Surface morphology of the treated specimens were confirmed using Scanning Electron Microscope analysis (SEM) and Energy Dispersive X-ray Spectroscopy (EDX).

2.6.1. Gravimetric test

The attendant impact of the inhibitors against corrosion of steel bar was obtained by weight loss experiment (gravimetric analysis) to assess the corrosion rate and the inhibition efficiency (%). Before the experiment, the specimens were accurately weighed individually. Subsequently, the specimens were immersed in 0.9% NaCl with and without the addition of inhibitors (negative control). The biosurfactant's concentration ranges between 5%, 7.5%, and 10%. To assess the efficacy of biosurfactants compared to inorganic surfactants, we utilized a commercially available surfactant known as Tween 80 (P8074) [23]. Tween 80 is a low-molecular-weight surfactant extensively used in various oil-in-water emulsion products due to its superior emulsifying properties [24].

The dry and clean specimens were submerged in the solution and allowed to remain at room temperature. Each piece of mild steel bar was affixed with a thread to facilitate its suspension in the medium. After 14th, 28th and 100th days immersion periods, the specimens were extracted from the solution to quantify their weight loss. To ensure accurate measurements, prior to weighing, each specimen underwent meticulous cleaning with a soft cloth and fine brush to eliminate any traces of rust. A 30-minute air-drying period at room temperature ensued before the weighing process. All the experiments were conducted in triplicate to ensure reliability and credibility of the specimen's average weight loss. The formula of corrosion rate is provided below [11]:

$$\text{Corrosion Rate } (Cr) \text{ mm/year} = \frac{[87600 \times C]}{A \times T \times D} \quad (2)$$

where: C= weight loss, in gram, A= surface area of specimen, in cm², T = time of exposure, in hour, D = density of specimen, in gcm⁻³.

The inhibition efficiency (IE) of corrosion inhibitors on steel rebars were calculated using this formula [25]:

$$IE (\%) = \left(1 - \frac{C_{corr}}{C_{uncorr}}\right) \times 100 \quad (3)$$

where: IE is the inhibition efficiency in percentage, C_{corr} is the corrosion rate in the presence of an inhibitor, C_{uncorr} is the corrosion rate without any inhibitor (the control corrosion rate).

2.6.2. Electrical resistivity test

The electrical resistivity test is utilized to gain insights into the internal corrosion rate within the steel bars. The specimens, measuring 15mm in length with a 10mm diameter, were immersed in a 0.9% NaCl solution with and without the addition of inhibitors (used as a negative control). The concentration of the biosurfactant ranged from 5% to 15%. To assess the efficacy of biosurfactants compared to inorganic surfactants, a commercially available surfactant, Tween 80 (P8074), was used.

Following a cleaning process involving a fine brush and a range of emery papers (grades 220 to 1200), the specimens underwent a degreasing process using distilled water, acetone, and deionized water. Subsequently, the specimens were submerged in the corrosion inhibitors solution at room temperature for a duration of 100 days. Each mild steel bar was affixed with a thread to facilitate its suspension in the medium. After the 100th day of immersion, the specimens were carefully removed from the solution and underwent meticulous cleaning with a soft cloth and fine brush to eliminate any traces of surface rust and biofilm prior to the test.

An electrical resistivity test was conducted to measure the electrical resistance of the specimens by passing a current through them and measuring the voltage across them. The resistance was then calculated using Ohm's law. A digital multimeter was used to determine the electrical resistance of the corroded specimens, set to measure resistance (Ohms). The multimeter probes were connected to both ends of the specimen, and the resistance reading was recorded.

This test provides information about the corrosion rate of each tested specimen. Corrosion increases the electrical resistance of a metal; therefore, the higher the corrosion rate, the higher the material's electrical resistivity.

2.6.3. Scanning electron microscope analysis (SEM) and energy dispersive X-ray spectroscopy (EDX)

The morphology and surface characteristics of mild steel surfaces subjected to varying concentrations of Tween 80 and biosurfactant treatment after 100 days were examined using a Scanning Electron Microscope (SEM). The biosurfactant adsorption on the steel bar is essential to ensure the formation of the uniform film of adsorbed surfactant molecules on the steel surface. The ability of biosurfactants to adsorb is related to their ability to aggregate to form micelles and to form a protective layer at the metal surface. This layer reduces or prevents corrosion of the materials.

Prior to analysis, the specimens underwent cleaning with a fine brush and clean, soft cloths to eliminate traces of rust and biofilm. The mild steel specimens were affixed to carbon studs using double-sided tape. Imaging analysis was performed using a QUANTA FEI 650 SEM at magnifications of 500x and 1000x. Specific regions of interest (ROIs) were focusing on areas exhibiting visible signs of corrosion and surface alterations. Regions with features like rust formation, pitting, or any observable changes induced by the treatments were targeted for detailed analysis. This approach allowed for a targeted investigation of the area's most susceptible to corrosion.

After SEM imaging, elemental analysis was directly carried out on the chosen ROIs using Energy Dispersive X-ray Spectroscopy (EDX). The EDX analysis was conducted using an Oxford Instruments X-Max model. The electron beam voltage was maintained at 10 kV during imaging to discern the elemental composition of the treated mild steel specimens that had been subjected to varying concentrations of Tween 80 and biosurfactant. This comprehensive analysis aimed to correlate the observed morphological changes with the elemental composition, providing insights into the corrosion inhibition effects of the treatments.

3. Results and Discussion

3.1. Gravimetric test

The mass reduction of specimens after 100 days of accelerated corrosion in a sodium chloride (NaCl) solution is detailed in Table 2. On average, specimens without any corrosion inhibitor lost 0.1020g of weight in NaCl. When incorporated at varying concentrations (5%, 7.5%, and 10% v/v), Tween 80 exhibited corrosion inhibition properties, with corresponding weight reductions measured as 0.0828g, 0.0692g, and 0.0632g, respectively. In contrast, specimens treated with biosurfactant at concentrations (5%, 7.5%, and 10% v/v) displayed lower weight losses of 0.0702g, 0.0582g, and 0.0514g. The results showed that the biosurfactant exhibits significant efficacy in mitigating corrosion, particularly at concentrations above 7.5%.

Table 2. Characteristics and weight reduction of specimens in the control solution and inhibitors at different concentrations recorded after 100 days of immersion.

Media	Initial Weight (gram)	Weight (gram) at 336 Hours (D14)	Weight (gram) at 672 Hours (D28)	Weight (gram) at 2400 Hours (D100)	Weight Loss (gram) on D100	Corrosion Rate (Cr) mm/year	Inhibition Efficiency (IE %)
Control	9.3075	9.3030	9.2928	9.2055	0.1020	0.0752	0.0000
T80 (5%)	8.5884	8.5855	8.5812	8.5056	0.0828	0.0648	13.7955
T80 (7.5%)	8.5638	8.5621	8.5584	8.4946	0.0692	0.0545	27.5403
T80 (10%)	8.7208	8.7193	8.7154	8.6576	0.0632	0.0492	34.6268
BS (5%)	9.1674	9.1637	9.1550	9.0972	0.0702	0.0525	30.1537
BS (7.5%)	9.0848	9.0816	9.0739	9.0267	0.0582	0.0437	41.9129
BS (10%)	9.2283	9.2260	9.2191	9.1769	0.0514	0.0381	49.3226

In the initial exposure periods spanning from 14 to 28 days, specimens immersed in Tween 80 demonstrated a noteworthy reduction in corrosion rates. As depicted in Fig. 5, the concentration-dependent trend revealed lower corrosion rates with increasing amounts of Tween 80. However, as the accelerated corrosion testing extended to 100 days, a distinctive shift was observed. The specimens treated with biosurfactant consistently exhibited lower corrosion rates compared to those treated with Tween 80. The beneficial effects of biosurfactant became increasingly evident with concentrations up to 10% (v/v). At this concentration, the

corrosion rate decreased to a remarkable 0.0381 mm/year. In comparison, specimens without any inhibitor exhibited a significantly higher corrosion rate of 0.0752 mm/year, while specimens treated with Tween 80 at a 10% concentration displayed a corrosion rate of 0.0492 mm/year. This corrosion rate is 29% higher than that of specimens treated with biosurfactant at equal concentration.

Figure 6, the corrosion rate of both Tween 80 and biosurfactant is illustrated across a range of concentrations, extending up to 20% (v.v). Notably, the biosurfactant demonstrates an optimal inhibition efficiency at 17.5% (v.v), beyond which its effectiveness reaches a plateau.

The relationship between concentration of inhibitors and inhibition efficiency is presented in Fig. 7. The coefficient of determination (R^2) value, calculated at 0.934, signifies a strong and statistically significant relationship between the concentration of biosurfactant and its inhibition efficiency. This comprehensive analysis establishes a coherent narrative across Figs. 5-7 elucidating the nuanced variations in corrosion rates with different inhibitors and concentrations over varying exposure periods.

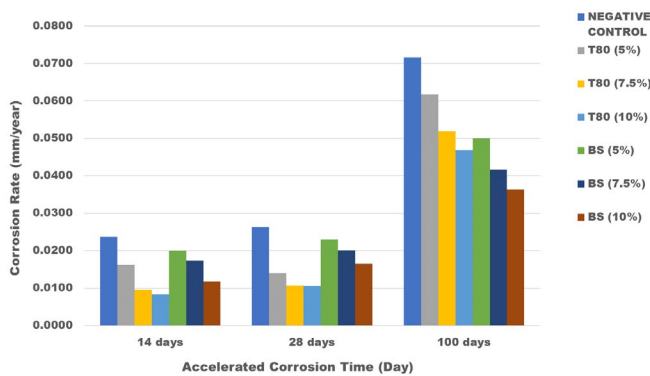


Fig. 5. Effect of inhibitor concentrations on corrosion rates throughout exposure duration.

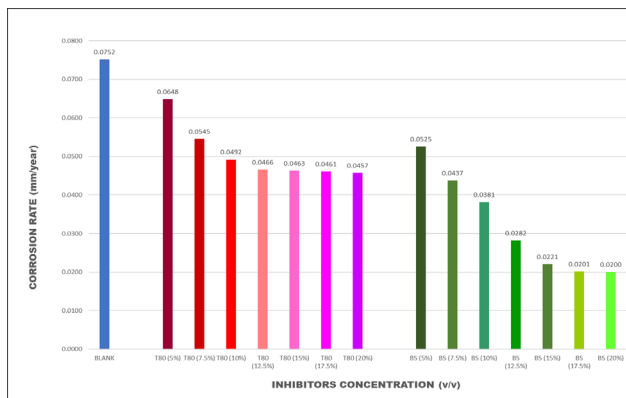


Fig. 6. Average corrosion rate (mm/year) for specimens immersed in the control solution, tween 80, and biosurfactant at various concentrations after 100 days of immersion.

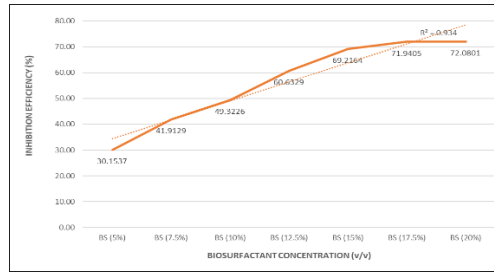


Fig. 7. Corrosion inhibition efficiency of inhibitors at various concentrations after 100 days of treatment.

3.2. Electrical resistivity test

The data presented in Fig. 8, derived from the electrical resistance test, provides insights into the corrosion behaviour of specimens immersed in NaCl solutions with and without the presence of corrosion inhibitors. The control specimen (blank) exhibited the highest electrical resistance, aligning with expectations as corrosion processes typically result in the formation of corrosion byproducts, impeding the flow of electric current and leading to increased electrical resistance. In contrast, specimens immersed in a saline solution containing 10% (v/v) biosurfactant displayed the lowest electrical resistance.

This observation suggests that the presence of biosurfactant influenced the corrosion process, leading to a reduction in electrical resistance. The lower electrical resistance in this scenario indicates a potential enhancement in corrosion inhibition, possibly attributed to the protective properties of the biosurfactant. These findings contribute valuable insights into the electrochemical aspects of corrosion inhibition, shedding light on how different inhibitors may impact the corrosive behaviour of mild steel.

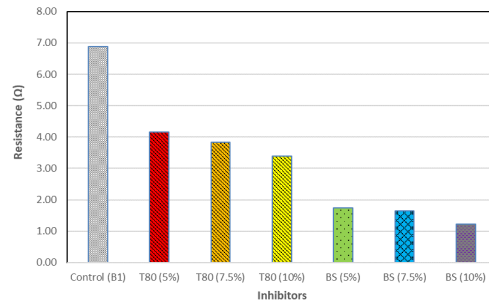


Fig. 8. Electrical resistance of specimens after 100 days of immersion in various medium.

3.3. Scanning electron microscope analysis (SEM) and energy dispersive x-ray spectroscopy (EDX)

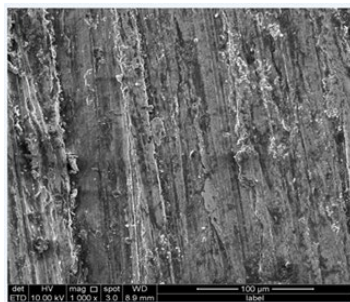
After 100 days of immersion, SEM-EDX analysis was conducted on specimens extracted from saline solutions containing inhibitors at various concentrations. The observed surface morphologies in the SEM-EDX analysis provide crucial insights into the effectiveness of the corrosion inhibitors and their impact on the overall corrosion behaviour of mild steel.

In Figs. 9(b) and (c), the specimen treated with biosurfactant at a 10% concentration exhibits a notably smoother surface with minimal biofilm attachment. This outcome suggests that the biosurfactant not only acts as a corrosion inhibitor but also plays a significant role in preventing the formation and attachment of biofilm on the mild steel surface. The smooth surface indicates a reduced susceptibility to pitting and general corrosion, reinforcing the corrosion inhibition properties of the biosurfactant.

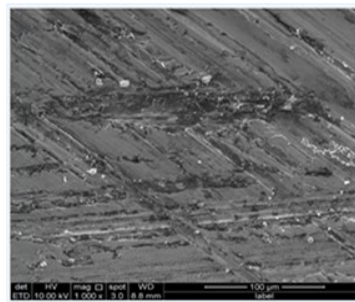
In contrast, the visual analysis of the control specimen reveals severe pitting and extensive general corrosion as shown in Fig. 9(a). This indicates the vulnerability of mild steel to corrosion in the absence of any inhibitor, emphasizing the protective role of inhibitors in corrosive environments.

Specimens treated with Tween 80 display an irregular surface, which can be attributed to the occurrence of pitting corrosion as shown in Fig. 9(d). This observation aligns with the higher corrosion rates noted in the discussion, emphasizing the limitations of Tween 80 in inhibiting corrosion compared to biosurfactant.

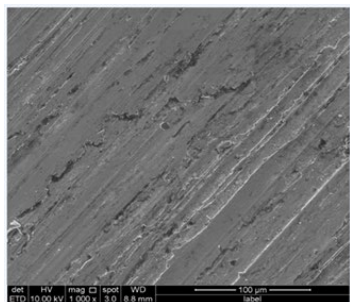
Therefore, the SEM-EDX analysis not only provides a visual representation of the surface morphologies but also reinforces the implications of these morphologies for corrosion inhibition. The smoother surface and absence of biofilm in the biosurfactant-treated specimen correlate with lower corrosion rates, highlighting the potential of biosurfactant as an effective corrosion inhibitor for mild steel in saline solutions.



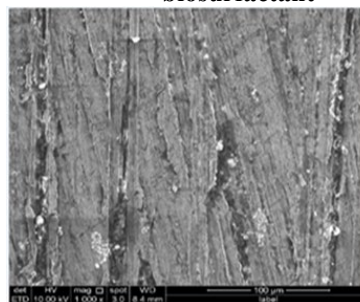
(a) Control specimen



(b) Specimen treated with 5% biosurfactant



(c) Specimen treated with 10% biosurfactant.



(d) Specimen treated with Tween 80

Fig. 9. EDX image of specimens after 60 days immersion in corrosion inhibitors.

4. Conclusion

In conclusion, this research has effectively assessed the corrosion inhibition potential of biosurfactant derived from *Rhizopus Oryzae* on mild steel. The findings reveal that an elevated biosurfactant concentration correlates with improved corrosion inhibition efficiency, resulting in a significant reduction in the corrosion rate. The optimal concentration is identified at 17.5% (v/v), and a minimum treatment time of 14 days is required to observe increased efficiency. These results emphasize the substantial potential of Glycerolipids as a green corrosion inhibitor for mild steel bars.

Being a product of microbial fermentation, the biosurfactant will break down naturally over time, minimizing its impact on ecosystems. This production process contributes to waste reduction by repurposing waste frying oil as the exclusive carbon source, to reduce production costs and mitigate the environmental footprint associated with waste disposal. The corrosion inhibition properties of the biosurfactant offer a sustainable alternative to conventional methods, effectively reducing corrosion rates without introducing harmful substances. This research contributes to the ongoing paradigm shift toward environmentally conscious corrosion prevention methods, promoting a sustainable and eco-friendly approach to infrastructure maintenance.

Future studies should investigate diverse exposure conditions and explore biosurfactant admixtures in concrete formulations, assessing compatibility and impact on strength and durability. Further exploration on various metals used in construction may unveil novel, eco-friendly corrosion prevention strategies for different materials.

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Nomenclatures

R^2 Coefficient of determinant

Greek Symbols

Ω Electrical resistance, Ohm

Abbreviations

BS	Biosurfactant
C	Carbon
C_{eq}	Carbon equivalent
Cr	Chromium
Cu	Copper
IE	Inhibition efficiency
MDA	Malt dextrose agar
Mn	Manganese
Mo	Molybdenum
MSM	Mineral salt medium

NaCl	Sodium chloride
Nb	Niobium
Ni	Nickel
P	Phosphorous
S	Sulphur
Si	Silicon
V	Vanadium

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