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Isolation and identification of bacteria implicated in microbiologically influenced corrosion of mild steel in tap water

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Abstract

When metal constructions are exposed to water, a prevalent issue is Microbiologically Influenced Corrosion (MIC). This study aims to isolate and identify the bacteria that cause microbiologically influenced corrosion (MIC) of mild steel in tap water over a period of 90 and 180 days, with a particular focus on the bacteria that are engaged in the corrosion process. At Federal University Oye-Ekiti (FUOYE), Nigeria, mild steel specimens were submerged in tap water from the New Faculty of Science Auditorium (NFSA) to simulate MIC conditions. Water samples were serially diluted and cultivated on a range of agar media, such as Nutrient and Eosine Methylene Blue, to assess the diversity and load of bacteria, which also accelerated the pace of corrosion. Important bacterial isolates identified by morphological and biochemical examination include species from genera known to accelerate corrosion, such as *Escherichia coli, Mycobacterium smegmatis,* and *S. aureus*. Additionally, the study assessed physicochemical properties like temperature, dissolved oxygen (DO), and pH. The results showed a consistent rise in corrosion rates when tap water changed, especially in terms of pH and DO levels, which were positively correlated with higher microbial loads and corrosion activity. The complexity of microbial interactions in MIC is highlighted by the discovery of Mycobacterium smegmatis as a member of the microbial consortium.

Keywords: Microbiologically Influenced Corrosion; Mild Steel; Biofilm Formation; *Mycobacterium smegmatis*; Corrosion rate

1. Introduction

The unavoidable process of a substance changing into a more chemically stable state is called corrosion (Harsimran *et al*., 2021). The type of corrosion known as "microbial influenced corrosion," which is a significant industry concern, is caused by microorganisms that change the chemistry of the media through their metabolic processes (Omar *et al*., 2021). The core issues with MIC remain unresolved despite a great deal of research and several publications. Microbial corrosion is often overlooked in industrial systems, leading to substantial waste and higher operating expenses (Javed, 2023).

Numerous microbial populations found in tap water, which is utilized extensively in many businesses, can hasten the corrosion of mild steel components. The goal of this investigation is to pinpoint the precise bacteria causing mild steel MIC in tap water systems. Designing specialized mitigation techniques that extend the lifespan of infrastructure and reduce maintenance costs requires an understanding of the microbiological composition and causes of corrosion (Wang *et al*., 2022). Higher corrosion rates are the result of MIC, which happens when bacteria come into contact with metal

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surfaces (Song and Gu, 2019). Mild steel components can be weakened by bacteria in tap water that adhere to metal surfaces and create corrosive metabolites (Kong *et al*., 2020).

Tap water is a common resource used in the residential, commercial, and industrial sectors. However, the presence of dissolved oxygen, minerals, and organic matter in tap water can produce an environment that promotes corrosion (Jia *et al*., 2025). A common component of water infrastructure and plumbing systems, mild steel is particularly vulnerable to MIC due to its composition and exposure to various bacterial populations in tap water. MIC in tap water systems poses significant environmental and financial difficulties. Costly repairs, downtime, and potential environmental pollution from leaks or ruptures are the outcomes of corrosion-related failures (Jones *et al.*, 2025).

The main interfacial process of corrosion is strongly influenced by the dissolved oxygen (DO) concentration, the presence and chemical makeup of salts, conductivity, redox potential, and pH of the associated electrolyte. Each of them may be affected in a highly localized way by microbial biofilms that colonize the contact. In the United States, the term "biocorrosion" is currently being used to describe the corrosion of implants within a living body due to both biotic and abiotic processes. This leads to misunderstandings with the term "biocorrosion," which is becoming more and more popular as a synonym for MIC in Europe and Latin America (Little *et al*., 2020).

In biofilms, microbial species work together in a synergistic way to support and sustain the community. Specific tasks carried out by each species in a biofilm aid in its upkeep. In biofilms, bacteria collaborate with one another (Knisz *et al*., 2023). On the other hand, biofilm resistance and degradation are caused by the same synergistic attribute (Jones *et al*., 2025). When mild steel surfaces are exposed to water, an unwanted accumulation of bacteria, plants, algae, or microscopic creatures is known as biofouling (Arshad *et al*., 2025). This is a well-researched phenomenon that can happen in freshwater systems as well as marine habitats. Mild steel is more susceptible to biofouling than other materials, including stainless steel, due to its iron content.

(Tenzin, 2025)

Figure 1 Microbiological Corrosion Patterns in Steel: Evidence of Sulfate-Reducing Bacteria Activity

Instead of referring to a single corrosion mechanism, the term "MIC" describes a collection of techniques whereby microorganisms' presence or activity affects the kinetics of corrosion reactions (Wei *et al*., 2025). In particular, the "three M's" (microorganisms, medium, and metals) must cooperate for MIC to form. Numerous mechanisms that might directly or indirectly affect the rate of metal corrosion are specified by the combination of these interactions. Although there have been a number of evaluations that specifically address MIC mechanisms, it can be challenging to distinguish between them due to the wide range of terminology used.

MIC mechanisms are often categorized based on the availability and/or presence of oxygen in a certain environment (Zhang *et al*., 2025). But in reality, microorganisms that may react directly with metal surfaces may occasionally have access to and/or absorb oxygen. As a result, there is often an oxygen gradient that might alter over time rather than a purely aerobic or anaerobic environment. Many laboratory-based MIC assays, on the other hand, are made to function

only in anaerobic or aerobic environments. In fact, more research on the impact of alternating oxygen in MIC tests may be possible (Wei *et al*., 2025).

Microbes create EPS, a slimy and complex mixture of proteins, polysaccharides, nucleic acids, and other chemical substances. The growth of bacteria and antimicrobial agents that depend on these nutrients for their proper operation may be inhibited by EPS's ability to trap vital nutrients within the biofilm (Sayahi *et al*., 2025). Although the antibiotic may still be effective at higher concentrations, this could lead to a bigger MIC. The effectiveness of antimicrobials is further reduced by the employment of efflux pumps, which are protein channels that actively pump antimicrobials out of the cell by certain microbes that are stuck in biofilms. The impact of EPS on efflux pump activity, however, is not fully understood and may vary depending on the particular organism and antibiotic (Fu *et al*., 2025).

2. Materials and methods

2.1. Study Area

The study was carried out in the Microbiology Laboratory at Federal University Oye Ekiti, Ekiti State, Nigeria

2.2. Sample Collections

The tap water samples used in this study was obtained from the New Faculty of Science Auditorium at Federal University Oye-Ekiti in Oye-Ekiti, Ekiti State. The water samples were collected into sterile plastic keg and then transported immediately to the microbiology lab for analysis. The Mild steel samples were obtained at the Department of Metallurgical and Materials Engineering, University of Lagos, Nigeria. The steel bars were wrapped with paper foil and packaged in an air-tight zip-lock bag, and then transported to the laboratory for analysis.

2.3. Enumeration of Total Bacterial Count

5.6 g of Nutrient agar was measured into a conical flask. Then, 2 g of agar powder was added to the Nutrient agar. 200 ml of distilled water was added using a graduated measuring cylinder. Visible lumps were removed from the media by stirring with glass rod. The conical flasks were covered with cotton plug and then placed in the autoclave and sterilized at 121°C for 15 minutes at 15 PSI. After autoclaving, the agar was allowed to cool for a few minutes. 1ml of diluent 5 and 7 of the water sample was dispensed aseptically into labelled Petri-dishes. Using the pour-plate technique, 20 ml of the Nutrient agar was aseptically dispensed into each Petri-dishes. The plates were incubated in both aerobic and anaerobic conditions for 24 hours. After incubation, distinct bacterial colonies were observed on the plate, and then counted with colony counter. The number of colonies was estimate to be the bacteria count.

$Colony\ forming\ unit\ ml = Average\ number\ of\ colonies\ x\ Dilution\ factor\ /Aliquot\ volume$ (Anthonia *et al*., 2025)

Nutrient Agar medium was prepared, sterilized in an autoclave, and then allowed to cool. The agar was then poured into the plates and allowed to gel. Using a gas-flamed inoculating loop and under aseptic conditions, each of the characterized isolates was carefully picked and streaked on the solidified agar medium. The petri plates were incubated for twenty-four (24) hours at 37°C. The above steps were repeated twice to ensure the discrete separation of the colonies.

2.3.1. Characterization and identification of bacterial isolates

The morphological and biochemical characteristics of the isolates in pure cultures were determined using general microbiological procedures as described by Cheesbrough (2010). Gram staining reaction, catalase test, oxidase test, methyl-red test, indole test, citrate utilization test, Acid fast staining, Spore Staining, Urease Test, Sugar Fermentation, Mr-Vp Test, Starch Hydrolysis Test, Haemolysis Test, Indole Test, Triple Sugar Iron Test, and sugar fermentation test were carried out on the isolates.

2.3.2. Physicochemical analysis of the tap water, Mild steel

The Tap water samples were subjected to chemical and physical analysis in accordance with APHA guidelines (2012) in order to determine its color and odor. Physico-chemical parameters analyzed include pH, total suspended solids, total dissolved solid, dissolved oxygen

2.4. Measurement of the thickness of mild steel samples

The Vernier calliper used was first calibrated and then used to measure the thickness of the galvanized steel samples

2.5. pH of water samples

The pH of water is a measurement of its acidity or basicity; acidic water has more hydrogen ions (H+), while basic water has more hydroxyl ions (OH-). The probe was rinsed with distilled water before use. The sample water samples were then dispensed into a beaker until it was enough to cover the tip of the electrode. The pH measurement of the sample was read after there was no fluctuations in the values displayed on the meter.

2.6. Temperature

The temperature of the sea water medium was determined by dipping the thermometer in the sea water until the reading is stabilized; i.e. until the mercury ring stopped moving and the final record was taken.

2.7. Total Dissolved Oxygen

The Dissolved oxygen was measured using a dissolved oxygen meter.The solubility of oxygen in water is dependent on the water temperature, salinity and atmospheric pressure

2.8. Weight

The weight of the steel was aseptically measured using an analytical weighing balance and was recorded for days 0, 90 and 180.

2.8.1. Determination of total dissolved solid (tds) in water samples

The materials needed for this experiment includes: crucible, oven, filter paper, conical flask, funnel, desiccator, measuring cylinder, analytical balance.

2.8.2. Procedure

The crucible was sterilized by washing and cleaning it with 70% ethanol then placed in the hot-air oven and allowed to dry at 105 0C for 30 minutes. The crucible was allowed to cool in a desiccator for few minutes, and the dry weight was measured using the analytical balance. 150 ml of the water sample was measured using the measuring cylinder, and filtered with whatmann filter paper. The filtered tap water was then dispensed into the crucible and dried in the oven at 105 0C. The crucible was then allowed to cool at room temperature inside the desiccator. Then the crucible was weighed again using the analytical balance. The total dissolved solid of the sea water was determined by using the equation below:

Weight of the residue =

Where:

- W2 = Weight of the crucible with residue
- W1 = Initial dry weight of the crucible
- Total dissolved solids = weight of the residue(mg) / the volume of the tap water in litres
- In other words, Total Dissolved Solid = Vol. of sample (ml)

2.8.3. Determination of total suspended solid (TSS) in water samples

Materials needed for Total Suspended Solids are: filter paper, Oven, conical flask, analytical balance,

2.8.4. Procedure

A dust-free Whatsmann filter paper was placed in the oven and heated at 105 0C to dryness in order to remove moisture acquired from the environment by the filter paper and to get the actual net-weight of the filter paper. The filter paper was then weighed on the analytical balance. The sea water was properly mixed together and 25 ml of the Tap water was filtered using the folded filter paper. The wet filter paper was placed on a foil paper, and placed inside the oven at 105°C then allowed to heat to dryness for 1 hour, the filter paper was places in the desiccator in order for it to cool, Using the analytical balance, the weight of the filter paper was measured.

The Total Suspended Solids was determined by calculating the weight

Weight of the residue =

Where

- W2 = Weight of the filter paper with dried residue
- W1 = Initial dry weight of the filter paper
- Total Suspended Solids = weight of the residue(mg) / the volume of the tap water in litres
- In other words, Total Suspended Solid = Vol. of sample (ml)

3. Result

3.1. Bacteriological enumeration

The samples underwent series of serial dilutions with dilution 10-3 and 10-5 being plated in different medium such as Mac Conkey agar, Nutrient agar, and Eosine Methylene Blue for day 0, 90 and 180. After 24-hour of incubation: Figure 4.1. shows the final result of the microbial load which was counted using colony counter and the accurate amount of CFU in the tap water was estimated using the formular (formular) of the Total bacteria count.

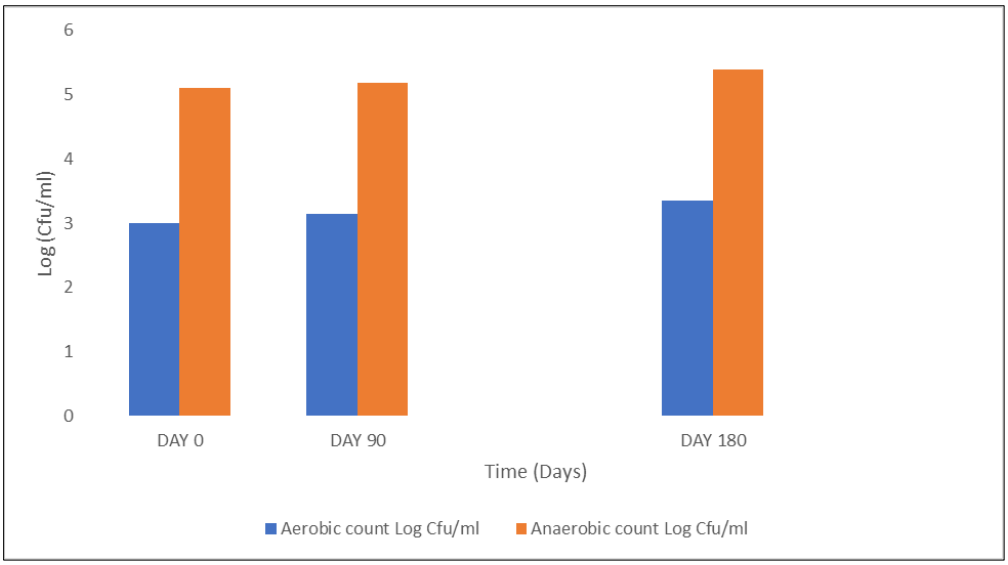

Figure 1 Bacteria count for aerobic and anaerobic count

3.2. Cultural and morphological characteristics of bacteria isolate

Table 1 Demonstrates the cultural and morphological characteristics of bacteria isolated from the tap water that contains the mild steel, it was observed that majority of the isolates are irregular in shape, entire margin, convex elevation and possess the cream colour

Isolate	Shape	Colour	Margin	Elevation	Surface	Size
MSTNA1	Circular	Cream	Entire	Convex	Shiny	Large
MST NA1(a)	Irregular	Cream	Entire	Convex	Shiny	Large
MST EMB1	Irregular	Cream	Undulate	Convex	Dull	Medium
MSTNA3	Circular	Cream	Entire	Convex	Dull	Small
MST EMB2	Circular	Cream	Entire	Flat	Dull	Medium
MTNA5	Circular	Cream	Entire	Undulate	Shiny	Medium

M-mild steel; T-Tap water; NA-Nutrient Agar; EMB-Eosine Methylene Blue; AE- aerobic; ANA -anaerobic; 5- diluent 5; 3-Diluent

Table 2 Biochemical characterization of isolates

ISOLATE	GRAMS RXN	OXIDASE	CATALASE	UREASE	STARCH	CITRATE	INDOLE TEST	Gas	H_2S	LACTOSE	GLUCOSE	MANNITOL	MOTILITY	BIOFILM	HAEMOLYSIS	MR	VP	ACID-FAST	SPORE	PROBABLE MICROORGANISM
MSTNA 1	+/rod	-	+	+	-	-	-	+	+	+	+	+	+	+	ɣ	+	-	+	-	*Mycobacterium smegmatis*
MST NA2	+/cocci	-	+	+	-	-	+	+	-	+	+	+	+	+	β	+	+	+	-	*Staphylococcus aureus*
MST EMB1	-/rod	-	+	+	-	-	+	+	-	+	+	+	+	+	ɣ	+	-	+	-	*Escherichia coli*
MSTNA 3	+/rod	+	+	+	-	-	+	+	+	+	+	+	+	+	A	+	-	+	-	*Mycobacterium smegmatis*
MST EMB2	-/rod	-	+	+	-	-	+	+	-	+	+	+	+		A	+	+	+	-	*Escherichia coli*

3.3. Physicochemical Characteristics

Table 2 displays the physiological characteristics of the sample and it shows that the colour of the tap water changes as the as the day goes on it was also observed that the water changes from pure to yellow at day 90 and becomes more deeper at day 180, in which the control (Mild steel in Distilled water) changes from colourless to black.

Table 2 Physiological characteristics of Tap water sample

Code	Time (Days)	Temp. ^{o}C	Ph	DO (mg/l)	TSS (mg)	TDS (mg)	Color
MT	0	27	7.1	3.5	127.9	1.5	Pure
MD		27	7.0	3.5	26.2	7.2	Pure
MT	90	27.4	7.5	3.8	80.8	161.7	Yellow
MD		30	7.4	3.9	72.0	6.8	Black
MT	180	27.9	7.6	4.4	21.2	198.6	Amber
MD		28	8.0	4.8	183.3	1.2	Black

MT-Mild steel in tap water; MD- Mild steel in Distilled water; TDS: Total dissolve solid, TSS: Total soluble solid, DO: Dissolved oxygen

From the table, it was observed that the weight and diameter increased as the rate of corrosion increases this implies that the biocorrosion add to the weight of the steel thereby enhancing and enlarging the thickness of the steel

Table 3 Presents the Weight loss, Diameter and rate of corrosion

Period (Days)	Sample	Weight (mg)	Diameter (mm)	Rate of corrosion (Mg/mm^2/yr)
0	MT	0	2.9	0
	MD	0	3.1	0
90	MT	1545.7	2.8	132
	MD	198.5	2.8	16.96
180	MT	1743.8	3.2	69.64
	MD	363.7	3.1	14.52

4. Discussion

Microbiologically Influenced Corrosion (MIC) in tap water is especially harmful to mild steel. MIC occurs when the corrosion process is accelerated by microbial activity. Understanding the bacterial species involved and the physicochemical properties influencing corrosion rates is essential for creating effective mitigation strategies (Li *et al*., 2020). In MIC research, a variety of bacterial species, such as acid-producing bacteria (APB), iron-oxidizing bacteria (IOB), and sulfate-reducing bacteria (SRB), have been connected to corrosion processes. By forming biofilms on metal surfaces, these microorganisms accelerate corrosion and encourage metal breakdown. For instance, SRBs generate hydrogen sulfide as a metabolic byproduct, which subsequently transforms into iron sulfide, which corrodes mild steel (Beech and Sunner, 2022).

According to the results in this study, it was also observed that the dissolved oxygen increased as the day increases. The electrochemical behavior of mild steel in tap water at different dissolved oxygen (DO) concentrations and immersion times has been studied under dynamic conditions using electrochemical techniques. The results show that both DO and immersion period influence the morphology of the corrosion products. In comparative tests, the corrosion rate was systematically found to be lower in solutions with lower DO, lower HCO3− concentrations and longer immersion time (Li *et al*., 2020).

According to Zuo et al. (2022), samples with higher levels of microbial colonization and adverse physicochemical conditions such as high DO exhibited a higher rate of corrosion. Temperature affects electrochemical reactions and

microbial metabolism; at higher temperatures, corrosion processes tend to accelerate (Zuo *et al*., 2022). The temperature in this study rose marginally from day 0 to day 180, from 27°C to 27.9°C, which may eventually lead to a higher rate of corrosion. DO levels are crucial for determining the kind of corrosion. Aerobic bacteria, such as those found in this study, require oxygen for metabolic functions, which might accelerate corrosion by producing oxides and other chemicals. DO levels increased from 3.8 mg/L on day 90 to 4.4 mg/L on day 180, indicating an increase in corrosion rate.

In table 2 the total suspended solids was observed to increase with the rate of corrosion and this can act as biofilm nucleation sites, promoting microbial colonization of metal surfaces (Li *et al.,* 2020). Biofilms can trap dispersed particles, resulting in microenvironments favorable to MIC. High levels of TSS can erode protective coatings or oxide layers on metals, exposing new metal surfaces to corrosive attack (Yang *et al*., 2020). This physical damage can accelerate corrosion, particularly in flowing water systems where suspended particles are more dynamic. Suspended residue can influence oxygen transport in water. In high-TSS situations, oxygen penetration to the metal surface may be limited, resulting in anaerobic conditions that promote the growth of anaerobic bacteria such as SRBs, which create corrosive hydrogen sulfide

Table 2 also indicated that high total dissolved solids (TDS) levels enhance the rate of corrosion, hence promoting electrochemical reactions that propel corrosion processes. (Song and Gu, 2019). Accelerated corrosion is caused by an increase in ionic strength, which improves ion transport between anodic and cathodic sites on the metal surface. Corrosive substances like chloride ions, which are known to pierce metal's protective oxide layers and encourage pitting corrosion, can be found in dissolved solids (Zuo et al., 2022). Passive films can be broken by chlorides, which increases metals' vulnerability to assault. High TDS conditions can support the growth of microbial populations, especially those engaged in MIC, which increases corrosion and biofilm formation (Jia *et al*., 2025).

Prior research has indicated the important function of SRBs in anaerobic settings, including water storage tanks and pipelines, where they are supported by the sulfate ions in the water. IOB, on the other hand, are common in aerobic environments where they facilitate the production of rust by oxidizing ferrous ions to ferric ions (Javed *et al.,* 2023). By generating organic acids that decrease pH and promote metal solubility, APB contributes to MIC It is necessary to culture samples on selective media such, Nutrient agar, and Eosin Methylene Blue agar in order to isolate bacteria from corroded mild steel in tap water. The implicated bacterial species can be identified with the aid of morphological and biochemical test. This test discovered several bacteria that are known to contribute to MIC through different ways, such as Mycobacterium smegmatis, Staphylococcus aureus, and Escherichia coli (Jia et al., 2021).

Biofilms have the ability to form cells with varied oxygen concentrations. Galvanic corrosion could result from oxygen depletion in the regions beneath the biofilm relative to the surrounding environment (Li *et al*., 2020). By producing a variety of corrosive metabolites as a result of its metabolic activity, M. smegmatis can contribute to corrosion. The production of organic acids like acetic and lactic acid by M. smegmatis' metabolic processes might reduce the pH at the metal surface and hasten corrosion (Song and Gu, 2019). M. smegmatis can cohabit alongside sulfate-reducing bacteria (SRB) in biofilms, offering an environment that promotes SRB activity even though it is not an SRB. In order to create iron sulfide, which reacts with iron to cause pitting and crevice corrosion, SRBs can synthesize hydrogen sulfide, a corrosive agent (Zuo *et al*., 2022). M. smegmatis and other bacteria in biofilms can work in concert to increase the overall corrosive effect on metals. Because of their combined metabolic activity and greater biofilm complexity, M. smegmatis can interact with other bacteria, including *Staphylococcus aureus* and *Escherichia coli*, to cause more aggressive corrosion processes

5. Conclusion

The research sheds light on the main bacterial species implicated in the corrosion process and the microbiologically influenced corrosion (MIC) of mild steel in tap water. Bacterial strains such Mycobacterium smegmatis and Escherichia coli were isolated and identified, indicating their possible contributions to accelerated corrosion rates through a variety of metabolic pathways. Results from the experiment indicate that changes in water physicochemical parameters like pH, total suspended solids (TSS), and total dissolved solids (TDS) are strongly correlated with the presence of specific bacteria. According to the results, pH levels gradually rose when bacteria were present. This can speed up corrosion by compromising the integrity of protective oxide coatings on mild steel surfaces.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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