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Nitrate-Mediated Corrosion Control in Microbial Biofilms: A Sustainable Approach

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Abstract

Corrosion is the deterioration of a material by a chemical attack or reaction under the influence of the surrounding environment. Metal corrosion can be due to the attack of Sulphate Reducing Bacteria which causes heavy economic losses to the industries. Sulphate Reducing Bacteria can be inhibited by the growth of Nitrate Reducing Bacteria and that could be a better eco-friendly solution to fight corrosion. In the present study, the effect of increasing concentration of 1%, 3% and 5% sodium nitrate in a biofilm community was studied using metals such as Mild steel, Stainless steel and Copper. The test metal coupons after exposure to increasing concentration of sodium nitrate were examined for weight loss and corrosion rate was determined. The corrosion rate had decreased to a greater extent in 1% of sodium nitrate. The biofilm bacterial community obtained from the test metal coupons had increased number of nitrate reducing bacteria which could be a promising reason for corrosion inhibition. It is concluded that increasing nitrate concentration can attract Nitrate Reducing Bacteria (NRB) which can outcompete Sulphate Reducing Bacteria (SRB) in a biofilm community and thus inhibits corrosion.

Key words: Corrosion, Biofilm, Sulphate Reducing Bacteria, Nitrate Reducing Bacteria.

INTRODUCTION:

Corrosion is a natural process. The word 'corrode' is derived from the Latin word "*corrodere*", which means, "to gnaw to pieces". Corrosion, is the deterioration of a metal due to the interaction of the metal and its environment [(1)]. Various environmental factors influence

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both the rate of corrosion and the nature of the corrosion products, including the type of metal, the chemical composition of the surrounding water, electrochemical conditions, and the presence of microorganisms [2]. Metals corrode when they come into contact with water and oxygen. In aerobic environments, corrosion progresses as long as there is a continuous electron flow—meaning oxygen is consistently supplied at the cathode, while insoluble corrosion products, such as iron oxide, are cleared away at the anode [1]. Corrosion involves both biological and non-biological factors. Microbially influenced corrosion on metal surfaces is influenced by the metabolic activity of microbial communities, which in turn is shaped by the chemical and physical conditions of the environment. In cooling water systems, metal corrosion is impacted by oxygen levels and can occur purely through abiotic mechanisms. [3].

A biofilm is an organized assembly of bacterial cells embedded in a self-generated matrix and attached to either a living or non-living surface. This form of growth offers protection and enhances the bacteria's ability to survive in harsh environmental conditions [4]. While biofilms can consist of a single microbial species, they more often contain a diverse community made up of multiple microbial species [5]. Biofilms can develop on a broad range of surfaces, such as living tissues, implanted medical devices, pipelines in industrial or drinking water systems, and natural aquatic environments [4].

Once bacteria attach to an inert surface or living tissue, the attachment stabilizes, leading to the formation of micro colonies. The bacteria then begin to reproduce and release chemical signals that enable communication between cells. When the concentration of these signals reaches a critical threshold, it triggers genetic pathways responsible for exopolysaccharide production [6]. Through this process, bacteria continue to reproduce within the surrounding exopolysaccharide matrix, leading to the development of a micro colony [7].

In natural environments, many microorganisms exist within synergistic, mixed-species biofilms. These biofilms play a pivotal role in microbially influenced corrosion (MIC), as electro active sessile cells embedded in the biofilm matrix—rather than planktonic cells—are capable of extracting electrons directly from high-energy metallic surfaces. Moreover, biofilms can concentrate corrosive metabolites such as organic acids at the metal–biofilm interface. The volumetric density of sessile cells within biofilms can exceed that of planktonic cells by two to three orders of magnitude. Despite comprising a smaller proportion of the total microbial population, these sessile cells are tightly confined within thin layers typically ranging from 50 to 200 µm in thickness. This structural organization results in a unique and localized biochemical environment that can significantly influence corrosion dynamics [8].

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Commonly used corrosion prevention methods include protective coatings, corrosion inhibitors, polymers, anodic and cathodic protection, and the use of corrosion-resistant metals and alloys [9]. However, these approaches can be costly, toxic, and sometimes ineffective. An alternative strategy involves the addition of nitrate, which enhances the activity of native nitrate-reducing, sulfide-oxidizing bacteria, thereby decreasing the likelihood of corrosion [10].

This study focused on inhibiting metal corrosion by exposing three different metals mild steel, stainless steel and copper coupons to three different concentrations of sodium nitrate salt thereby increasing the available nitrate source in a biofilm community which develops over the metal coupons under study and identification of the bacteria from the exposed metal coupons after one month of immersion in sodium nitrate salt solution. This study will definitely be an eco-friendly and a biological solution to combat metal corrosion and will aid in preventing the economic losses caused by corrosion.

MATERIALS AND METHODS:

Substratum used for biofilm growth:

Rectangle shaped mild steel, stainless steel, copper coupons with a surface area of 10.08 cm^2, 10.25 cm^2, and 10.08 cm^2 respectively were used as substratum for biofilm growth. Before exposure the metal coupons were polished with emery paper and washed with distilled water, ethanol and acetone for its surface sterilization. Then the metal coupons were weighed.

Preparation of Sodium nitrate salt solution:

Sodium nitrate salt solution was prepared as three different concentrations of 1%, 3% and 5% by adding 1g, 3g and 5g of sodium nitrate to 100 ml of sterile distilled water respectively.

Exposure of metal coupons to sodium nitrate salt solution:

After sterilization the metal coupons such as mild steel, stainless steel, copper were immersed in sodium nitrate salt solution in three different concentration. Mild steel, stainless steel and copper were exposed to 1%, 3% and 5% sodium nitrate solution respectively. And this setup was kept under static condition at 37°C for 30 days. After exposure for 30 days the weight loss of the metal coupons were recorded. Control metal coupons were kept immersed in distilled water without sodium nitrate and kept at 37°C for 30 days. The physical appearance was recorded by comparison with the control.

Evaluation of nitrate mediated corrosion inhibition of the metals (mild steel, stainless steel, copper):

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Physical Appearance:

After exposure of metal coupons in Sodium nitrate salt solution for a period of 30 days, the metal coupons were retrieved and the appearance of the metal coupons was observed. The test metal coupons were compared with that of the control metal coupons.

Weight loss:

Before the exposure of metal coupons in sodium nitrate salt solution, the metal coupons (mild steel, stainless steel, and copper) were weighed and the initial weight was recorded. After exposure the metal coupons were retrieved, corrosion products and biofilm were removed and dried in an oven and weighed again to determine the weight loss. The weight loss of test metal coupons was compared with the weight loss of the control metal coupons.

Corrosion rate:

Using the initial weight and final weight of the metal coupons the corrosion rate ($mg.Dm^{-2}.d^{-1}$) were then calculated using the formula:

$$C = W_1\text{-}W_2 / At$$

Where,

C is the corrosion rate ($mg.\ Dm^{-2}.d^{-1}$).

W_1 and W_2 are the weight in gram of metal coupons before and after immersion.

A is the area of the panels (cm^2).

t is the duration of immersion in days.

Isolation and identification of bacteria from the biofilms obtained from the test and control metal coupons:

Coleville synthetic brine Agar medium was used for the isolation of bacteria from the biofilms of the exposed metal coupons. The biofilm formed over the test metal coupons (Mild steel, Stainless steel, Copper) was removed by scrapping and the suspension obtained from test metal coupons was spread plated on sterile Coleville synthetic brine medium plates and incubated for 48 hours at 37° C. After incubation the colonies in the plates were carefully observed and based on the colony morphology nine distinct colonies were identified and the isolates were named as IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8 and IS9. The isolates were identified by various biochemical tests such as Nitrate reduction test, Indole test, Methyl red test, Voges- Proskauer test, Citrate test, Catalase test, Starch hydrolysis and Urease test.

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RESULTS:

The test metal coupons such as mild steel, stainless steel and copper were exposed to three different concentrations of sodium nitrate while control metal coupons were exposed to distilled water without sodium nitrate. After exposure of the metal coupons with 1%, 3% and 5% of sodium nitrate the coupons were retrieved from the test and control beakers of 1%, 3% and 5% sodium nitrate and the physical appearance was observed.

Evaluation of nitrate mediated corrosion inhibition of the metals (mild steel, stainless steel, copper):

Physical appearance:

The control mild steel, stainless steel and copper metal coupons were completely attacked and covered with corrosion products, while the test mild steel, stainless steel and copper metal coupons remained partially attacked and corrosion products were lowered as compared with that of the control shown in Figure No. 1, 2 & 3.

Weight loss:

The weight loss of the control and test MS metal coupons after exposure was determined as shown in Table No. 1. The MS test coupons exposed to 1%, 3% and 5% of sodium nitrate showed a weight loss of 0.09g, 0.12 g and 0.17g respectively, while MS control coupons showed a weight loss of 0.21g. The weight loss of the control and test SS metal coupons after exposure was determined shown in Table No. 2. The SS test coupons exposed to 1%, 3% and 5% showed a weight loss of 0.02g, 0.04g and 0.08g respectively, while SS control coupons showed a weight loss of 0.14g. The weight loss of the control and test Copper metal coupons after exposure was determined shown in Table No. 3. The copper test coupons exposed to 1%, 3% and 5% showed a weight loss of 0.0001, 0.0002 and 0.0004 respectively, while copper control coupons showed a weight loss of 0.0007 g.

Corrosion rate:

Corrosion rate of mild steel test metal coupons exposed to 1%, 3% and 5% was determined as 0.0002 $mg.dm^{-2}.d^{-1}$, 0.0003 $mg.dm^{-2}.d^{-1}$ and 0.0005 $mg.dm^{-2}.d^{-1}$ respectively, while the corrosion rate of control coupons was determined as 0.0006 $mg.dm^{-2}.d^{-1}$. Corrosion rate of stainless steel test metal coupons exposed to 1%, 3% and 5% was determined as 0.00006 $mg.dm^{-2}.d^{-}1$, 0.0001 $mg.dm^{-2}.d^{-1}$ and 0.0003 $mg.dm^{-2}.d^{-}1$, while corrosion rate of control coupons was determined as 0.0004 $mg.dm^{-2}.d^{-1}$. Corrosion rate of copper test metal coupons exposed to 1%, 3% and 5% was determined as 0.001 $mg.dm^{-2}.d^{-1}$, 0.0002 $mg.dm^{-2}.d^{-1}$, 0.0004 $mg.dm^{-2}.d^{-}1$, while corrosion rate of control coupons was determined as 0.0007 $mg.dm^{-2}.d^{-1}$.

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EVALUATION OF PHYSICAL APPEARANCE, WEIGHT LOSS AND CORROSION RATE OF MILD STEEL AFTER EXPOSURE TO THREE CONCENTRATIONS OF SODIUM NITRATE

Figure: 1 - Physical appearance

1% MILD STEEL

1st day **30th day**

3 % MILD STEEL

1st day **30th day**

5% MILD STEEL

1st day **30th day**

CONTROL MILD STEEL

1st day **30th day**

TABLE 1: Determination of weight loss of Mild Steel

S. No.	Metal Coupon	Initial Weight (W_1) g	Final Weight (W_2) g	Weight Loss (g) (Initial Weight – Final Weight)
1.	1% MS	27.23	27.14	0.09
2.	3 % MS	26.22	26.10	0.12
3.	5 % MS	26.27	26.10	0.17
4.	Control	26.71	26.50	0.21

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TABLE 2: Determination of corrosion rate for Mild steel

S. No	Metal Coupon	Concentration of Sodium Nitrate	Corrosion rate ($mg.dm^{-2}.d^{-1}$)
1.	Mild steel	1 %	0.0002
2.		3 %	0.0003
3.		5 %	0.0005
4.		Control	0.0006

EVALUATION OF PHYSICAL APPEARANCE, WEIGHT LOSS AND CORROSION RATE OF STAINLESS STEEL AFTER EXPOSURE TO THREE CONCENTRATIONS OF SODIUM NITRATE

Figure: 2 – Physical appearance

1 % STAINLESS STEEL

1st day **30th day**

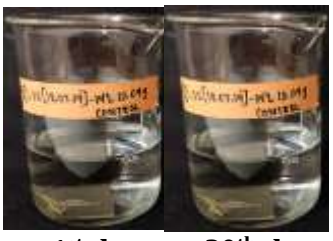
3 % STAINLESS STEEL

1st day **30th day**

5 % STAINLESS STEEL

1st day **30th day**

CONTROL STAINLESS STEEL



1st day **30th day**

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TABLE 3: Determination of weight loss of stainless steel

S. No	Metal Coupon	Initial Weight (W1) g	Final Weight (W2) g	Weight Loss (g) (Initial Weight – Final Weight)
1.	1% SS	12.00	11.98	0.02
2.	3% SS	11.18	11.14	0.04
3.	5% SS	12.13	12.05	0.08
4.	Control	12.09	11.95	0.14

TABLE 4: Determination of corrosion rate for Stainless steel

S. No	Metal Coupon	Concentration of Sodium Nitrate	Corrosion rate
1.	Stainless steel	1 %	0.00006
2.		3 %	0.0001
3.		5 %	0.0003
4.		Control	0.0004

EVALUATION OF PHYSICAL APPEARANCE, WEIGHT LOSS AND CORROSION RATE OF COPPER AFTER EXPOSURE TO THREE CONCENTRATIONS OF SODIUM NITRATE

Figure 3 – Physical appearance

1 % COPPER **3 % COPPER**

1st day **30th day** **1st day** **30th day**

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5 % COPPER **CONTROL COPPER**

1st day **30th day** **1st day** **30th day**

TABLE 5: Determination of weight loss of copper

S. No	Metal Coupon	Initial Weight (W_1) g	Final Weight (W_2) g	Weight Loss (g) (Initial Weight – Final Weight)
1.	1%	75.18	75.14	0.04
2.	3%	60.09	60.00	0.09
3.	5%	61.93	61.80	0.13
4.	Control	64.34	64.10	0.24

TABLE 6: Determination of corrosion rate for Copper

S. No.	Metal Coupon	Concentration of Sodium Nitrate	Corrosion rate
1.	Copper	1 %	0.0001
2.		3 %	0.0002
3.		5 %	0.0004
4.		Control	0.0007

Isolation and identification of bacteria from the biofilms obtained from the test and control metal coupons:

The metal coupons which are exposed to sodium nitrate were used for the isolation of the bacteria. The exposed metal coupons were scraped and a suspension was obtained. This suspension was spread plate on Coleville synthetic brine agar medium. These plates were incubated at 37^0C for 24 hours. After incubation the colonies grown was observed. Nine bacterial isolates with different colony morphology were identified and named as IS 1, IS

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2, IS 3, IS 4, IS 5, IS 6, IS 7, IS 8, and IS 9. Various tests were carried out to characterize the isolates. They are IMViC test, Catalase test, Nitrate reduction test, starch hydrolysis, Urease test and shown in Table. No. 7.

TABLE 7: BIOCHEMICAL CHARACTERISTICS OF THE NINE ISOLATES

TEST	IS 1	IS 2	IS 3	IS 4	IS 5	IS 6	IS 7	IS 8	IS 9
Nitrate Test	+	+	+	+	+	-	+	+	-
Indole Test	+	+	+	+	+	-	+	+	+
Methyl Red Test	-	-	-	-	-	-	-	+	+
Voges Proskauer Test	-	-	-	-	-	-	-	-	-
Citrate Test	-	-	-	-	+	-	-	-	+
Urease Test	-	+	-	-	+	+	-	-	-
Catalase Test	-	-	-	-	-	-	+	-	-
Starch Hydrolysis Test	-	-	-	+	-	-	+	-	-

CONCLUSION:

Corrosion is the deterioration of a material by a chemical attack or reaction under the influence of the surrounding environment. Corrosion is a continuous process which could be difficult to control and terminate. Traditionally, reduction of corrosion has been managed by various methods including cathodic protection, process control, reduction of the metal impurity content, and application of surface treatment techniques as well as incorporation of suitable alloys. However, the use of corrosion inhibitors has proven to be the easiest and cheapest method for corrosion protection and prevention in acidic media. These inhibitors slow down the corrosion rate and thus prevent monetary losses due to metallic corrosion on industrial vessels, equipment, or surfaces. Corrosion control can be achieved by physical, chemical and biological methods. Among these biological methods is cheapest, eco-friendly, cost effective. Metal corrosion can be due to the attack of some bacteria which causes heavy economic losses to the industries. These corrosion causing bacteria can be inhibited by the growth of some other competing bacteria and that could be a better eco-friendly solution to fight corrosion.

In the present study, the effect of increasing concentration of sodium nitrate in a biofilm community was studied using metals such as Mild steel, Stainless steel and Copper. The test

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metal coupons after exposure to increasing concentration of sodium nitrate was examined for weight loss and corrosion rate. The biofilm formed over the test metal coupons was used to isolation bacteria in Coleville synthetic brine agar media. Nine bacterial colonies were isolated, identified and characterized.

Due to an increase in nitrate source, the biofilm bacterial community obtained from the test metal coupons (MS, SS, Copper) had more number of nitrate reducing bacteria. And this increase in the number of nitrate reducing bacteria could be a promising reason for corrosion inhibition taken place in this study. This study clearly depicts that sodium nitrate has a corrosion inhibitory effect on the metals such as Mild Steel, Stainless Steel and Copper by altering the diversity of microbial flora in the biofilm community. This concept can be extensively used as a corrosion control strategy to be applied in industries which employs Mild steel, Stainless steel and Copper.

REFERENCES:

1. Hamilton, WA 1985, 'Sulphate – reducing bacteria and anaerobic corrosion', Journal of Annual Review Microbiology, vol. 39, pp. 195-217.
2. Borenstein, SW 1994, 'Microbiologically Influenced Corrosion handbook', Wood head, Cambridge, England.
3. Potekhina, JS, Sherisheva, NG, Povetkina, LP, A. P. Pospelov, AP, Rakitina, TA, Warnecke, F & Gottschalk , G 1999, 'Role of microorganisms in corrosion inhibition of metals in aquatic habitats', Journal of Applied Microbiology and Biotechnology, vol. 52, pp. 639-646.
4. Prakash, B, Veeregowda, BM & Krishnappa, G 2003, 'Biofilms: A survival strategy of bacteria', Journal of Current Science, vol. 85, pp. 1299-1307.
5. Anwar Huq, Chris White House, A, Christopher Grim, J, Muniral Alam & Rita Colwell, R 2008, 'Biofilms in water, its role and impact in human disease', Current opinion in Biotechnology, vol. 19, pp. 244-247.
6. Costerton, JW, Stewart, PS and Greenberg, EP 1999, 'Bacterial biofilms: a common cause of persistent infections' Journal of Science, vol. 284, pp. 1318-1322.

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11 Iss 12, 2022

7. Mc Kenney, D, Hubner, J, Mulier, E, Wang, Y, Goldman, DA & Pier, GA 1998,'Journal of Immunology and Infectious diseases, vol. 66, pp. 4711-4720.
8. Dou, W, Xu, D & Gu, T 2021, 'Biocorrosion caused by microbial biofilms is ubiquitous around us', Microbial Biotechnology, vol. 14, no. 3, pp. 803-305.
9. Koch, GH, Brongers, MPH, Thompson, NG, Virmani, YP & Payer, JH 2001, Corrosion costs and preventive strategies in the United States, Report, CCTL, NACE & FHWA, USA.
10. Diane Gevertz, Anita J, Telang, Gerrit Voordouw, Gary E, Jenneman 2000, 'Isolation and Characterization of Strains CVO and FWKO B, Two Novel Nitrate-Reducing, Sulfide-Oxidizing Bacteria Isolated from Oil Field Brine', Applied and Environmental Microbiology, vol. 66, no. 6. pp. 2491-2501.