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GROWTH EVALUATION OF SULPHATE-REDUCING BACTERIA (SRB) ASSOCIATED WITH BURIED MILD STEEL RODS

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ABSTRACT

Introduction: Sulphate-reducing bacteria are the major cause of corrosion of underground structure corrosion of underground structures made of mild steel.
Objective: This study set out to evaluate the growth pattern of sulphate-reducing bacteria associated with buried mild steel rods in Akure metropolis, Nigeria
Methodology: Mild steel rods (12 mm diameter, area 39.97 cm^2), were collected from Ondo State Water Corporation (ODSWC), Akure, Nigeria and were buried and monitored for a period of three months. Percentage composition of the mild steel rods was determined. Isolation of sulphate-reducing bacteria was carried out using compounded Postgate C medium. Turbidimetric growth pattern and biochemical characteristics of the isolates were determined using standard protocols.
Results: *Citrobacter freundii, Citrobacter gilleni, Enterobacter aerogenes, Pseudomonas aeruginosa, Desulfovibrio vulgaris* and *Bacillus megaterium* were the bacterial consortia isolated from the buried mild steel rods. Turbidity was observed to be at low level on the 5th day of sulphate-reducing bacteria (SRB) growth, but increased to optimum on the 10th day of incubation and reduced drastically on the 15th day. The growth outline of sulphate-reducing bacteria (SRB) was confirmed by the swelling turbid periodic growth for all four mediums. Iron (Fe) had the highest percentage mineral composition of 98.7 % whereas lanthanum had the least composition at 0.00031 %.
Conclusion: Findings of this study revealed the presence of sulphate-reducing bacteria (SRB) on buried mild steel rods of a water distribution system suggesting induction of corrosion of the underground mild steel pipes.

Keywords: Turbidity; sulphate-reducing bacteria; mild steel rods; mineral composition; bacteria; corrosion

INTRODUCTION

Metal corrosion is caused by (bio) chemical processes in which the metal releases electrons or ions. Anodic reactions are triggered by corrosive substances such as acids. Microbes increase cathodic processes by utilizing hydrogen, and they may also stimulate corrosion by secreting enzymes and acidic metabolites **(Procópio, 2020)**. The main types of bacteria associated with the corrosion of iron and steel are sulfate-reducing bacteria (SRB), sulfur-oxidizing bacteria, iron oxidizers, iron reducers, manganese oxidizers and microbes that secrete organic acids and produce extracellular polymeric substances (EPS) **(Hamilton, 2003)**.
Microbial corrosion, primarily caused by sulphate-reducing bacteria, is a major cause of underground structure corrosion, particularly pipeline corrosion. Although various microorganisms, primarily bacteria, may contribute to corrosion, the sulphate-reducing bacteria are mainly responsible for corrosion of iron and steel **(Okabe *et al*., 1995**; **Sass and Cypionka, 2004; Zhang *et al*., 2011)**. The bacterial species enhances the corrosion of pipeline material via either reduction or oxidation of the metal; SRB species generate H_2S which accelerates the corrosion of mild steel **(Wang *et al*., 2015)**. Sulfur-oxidizing bacteria convert sulphide to sulphate using a sulphide-oxidizing enzyme, and iron- and sulfur-oxidizing bacteria of the genus *Thiobacillus* are only active in acidic environments **(El-Shamy *et al*., 2015)**. Sulphate-reducing bacteria are anaerobic and non-pathogenic bacteria, but they are capable of causing economic and environmental problems (**Lopes *et al*., 2018**).
Due to the emission of corrosive H_2S, sulphate-reducing bacteria (SRB) have been shown to be the most aggressive bacterium in bio-corrosion induction **(Mansour and Elshafei, 2016)**. In many industries, SRB have a negative impact by forming toxic biofilms on metal surfaces that may affect anodic and cathodic processes, thereby creating ideal circumstances for microbial corrosion induction **(Mansour and Elshafei, 2016)**. Many additional bacteria, including sulphate-reducing bacteria like *Desulphovibrio* and *Desulfotomaculum*, may corrode iron anaerobically. The generation of sulphuric acid, sulfate, and H_2S by these bacteria to oxidize iron to iron oxide suggests their very corrosive action **(Coetser and Cloete, 2005)**.
Corrosion of ferrous metals in soil have significant negative impact on oil and water pipelines, storage tanks, and cooling water pipeline distribution systems **(Cole and Marney, 2012)**. These materials are subjected to various types of corrosion, including localized, pitting, crevice, and uniform corrosion **(Wu *et al*., 2013)**. Corrosion is the primary source of material degradation and destruction in both natural and man-made materials. It is estimated that approximately 25 % of annual steel production is destroyed by corrosion. Soil as a corrosive environment is probably of greater complexity than any other environment. The corrosion process of buried metal structures is extremely variable and may range from rapid to negligible **(Arriba-Rodriguez *et al*., 2018)**. The aim of this study was to evaluate the growth pattern of sulphate-reducing bacteria (SRB) on buried iron rods collected from a tropical vegetation cover in the vicinity of Ondo State water corporation, Akure, Nigeria. . This is to gain a better understanding of how sulphate-reducing bacteria (SRB) induce corrosion on underground mild steel pipes of a water distribution system.

METHODOLOGY

Sample collection

Mild steel rods (12 mm diameter, area 39.97 cm^2), used for this study were obtained from Ondo State Water Corporation (ODSWC), Alagbaka, Akure, Ondo State. They were buried for a period of three months and closely monitored.

Determination of percentage composition of mild steel

Mild steel (12 mm) commercially obtained were analyzed at the SPECTRO, AMETEK Materials analysis division, New Jersey, United States. Cylindrical mild steel samples were cut with an average length of 10 mm with their exposed surface ends metallo-graphically prepared in accordance with American society for Testing and Materials **(ASTM G1-03, 2011)**.

Media preparation

Postgate C medium for Sulphate-reducing bacteria **(Yahaya *et al*., 2011)** was prepared according to the chemical composition as shown in Table 1. The prepared medium was adjusted to pH 7.5, thereafter, dispensed into test tubes, then surged with nitrogen free oxygen gas for 2 minutes before being clamped and sterilized in autoclave at 121℃ for 15 minutes.

Isolation of sulphate-reducing bacteria

SRB were isolated from the buried mild steel rod samples periodically on a monthly basis. Samples were inoculated in the freshly prepared Postgate C medium in test tubes and were incubated under anaerobic condition at 37°C for 7- 22days **(Yahaya *et al*., 2011).**

Table 1 Chemical composition of Postgate C medium

S/N	Ingredients	Concentration (g/L)
1	Sodium Lactate	6.0
2	Na_2SO_4	4.5
3	NH_4Cl	1.0
4	Yeast Extract	1.0
5	KH_2PO_4	0.5
6	$C_6H_5Na_3O_7.2H_2O$	0.3
7	$CaCl_2.6H_2O$	0.06
8	$MgSO_4{\cdot}7H_2O$	0.06
9	$FeSO_4.7H_2O$	0.004
10	Distilled water	1000

Experimental procedure

The experiment was carried out under anaerobic condition. Four (4) anaerobic vials were prepared containing steel coupon in each of the vial and surged with nitrogen free oxygen gas for 2-minutes, before clamped with rubber and aluminum cap. The vials were then autoclaved in order to ensure sterile condition and to eliminate the possibility of contamination. The remaining vials were injected with the isolated Sulphate-reducing bacteria (SRB). Then, the vials were kept inside the incubator at 37 °C and the turbidity measurement was taken at 5 days interval for the duration of 15 days **(Yahaya *et al*., 2011)**.

Turbidimetric determination of sulphate reducing bacteria inoculated in Postgate C medium

Spectrophotometer PEC Medical 721 (New Jersey, United States) was used to determine the optical density (O.D.) of the assigned broth bacterial culture at 600 nm wavelength.

Biochemical characrteristics of bacterial isolates from buried mild steel rods

All the bacterial isolates were identified using standard biochemical techniques **(Váradi et al., 2017).** First, all isolates were subcultured on Postgate C medium agar petriplates at 37 °C at 7-22 days **(Yahaya *et al*., 2011)**. Biochemical tests such as Gram stain, catalase, motility, hydrogen sulphide production, urease, indole, oxidase, nitrate production, starch hydrolysis, methyl red, Voges Proskauer, and sugar fermentation tests were conducted.

RESULTS

Biochemical characteristics of bacterial isolates from buried mild steel rods

Gram negative rods comprising; *Citrobacter freundii, Citrobacter gilleni, Enterobacter aerogenes, Pseudomons aeruginosa* and *Desulfovibrio vulgaris* coupled with Gram positive *Bacillus megaterium* were isolated from buried mild steel (Table 3).

Table 3 Biochemical characteristics of bacterial isolates obtained from location 1 iron sample after 30 days

Isolate No.	Catalase	Starch	Motility	H_2S	Urease	Indole	Casein	Glucose	Nitrate	Methyl Red	VP	Citrate	Mannitol	Oxidase	Probable organism
1	+	+	+	-	-	-	+	A	+	-	-	+	-	-	*Bacillus megaterium*
2	+	+	+	+	+	+	+	AG	+	+	-	+	-	-	*Citrobacter freundii*
3	+	-	-	+	+	-	+	AG	+	+	-	+	-	-	*Citrobacter gilleni*
4	+	+	+	+	+	-	+	AG	-	-	-	+	-	-	*Desulfovibrio vulgaris*
5	+	+	+	+	+	-	+	AG	+	-	-	+	-	-	*Enterobacter aerogenes*
6	+	+	+	+	+	-	+	AG	+	-	-	+	+	+	*Pseudomonas aeruginosa*

Legend: + - positive, - - negative, A - acid production, AG - acid and gas production, VP - Voges Proskauer

Turbidimetric profile and growth rate of sulphate-reducing bacteria from buried mild steel rods

Turbidity was observed to be at low level on the 5th day of sulphate-reducing bacteria (SRB) growth. But, it increased to optimum on the 10th day of incubation and reduced drastically on the 15th day of incubation in the Postgate C medium. Figure 1 shows the growth rate of sulphate-reducing bacteria (SRB) at diverse time interval with the stipulated temperature for growth set at 37 °C during the course of the experimental protocol. In order to get consistent growth model of SRB, turbidity measurement was recorded at 5 days interval for 15 days. The black colour of the medium and rancid egg odor is the pointer of the SRB growth within 5 days after the inoculation of SRB seeded into the medium. The growth outline of sulphate-reducing bacteria (SRB) was confirmed by the swelling turbid periodic growth for all four mediums. The premise deduced in the growth rate graph showed that optimum turbidity (Abs) was observed at day 10, before it gradually decreased through day 5 and day 10 for the four (4) respective mild steel samples.

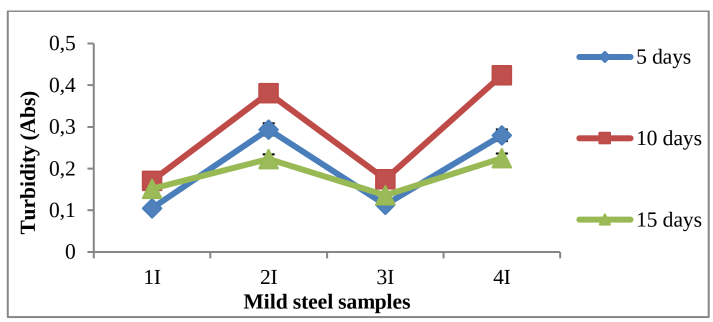

Figure 1 Turbidimetric profile and growth rate of sulphate-reducing bacteria from buried mild steel rods

Legend: 1I – Inoculated medium containing first mild steel sample; 2I – Inoculated medium containing second mild steel sample; 3I – Inoculated medium containing third mild steel sample; 4I – Inoculated medium containing fourth mild steel sample

Percentage mineral composition profile of mild steel/iron rods

Buried mild steel rods (12 mm diameter, area 39.97 cm^2) metallurgically-analyzed revealed the percentage composition of Carbon (C) 0.122, Silicon (Si) 0.110, Manganese (Mn) 0.361, Phosphorus (P) 0.0339, Sulphur (S) 0.0433, Chromium (Cr) 0.178, Molybdenum (Mo) 0.0146, Nickel (Ni) 0.0881, Aluminium (Al) 0.0062, Cobalt (Co) 0.0074, Copper (Cu) 0.229, Tin (Sn) 0.0395, Arsenic (As)

0.0061, Nitrogen (N) 0.0068, Lanthanum (La) 0.00031, Zinc (Zn) 0.0054, Boron (B) 0.00065, Tellurium (Te) 0.0071, Selenium (Se) 0.0131, Antimony (Sb) 0.0138, Calcium (Ca) 0.00097 and Iron (Fe) 98.7 (Table 2). Iron (Fe) had the highest percentage mineral composition of 98.7 % whereas lanthanum had the least composition at 0.00031 %.

Table 2 Percentage mineral composition of mild steel rods

Mineral composition	Percentage (%)
Aluminium (Al)	0.0062
Antimony (Sb)	0.0138
Arsenic (As)	0.00061
Boron (B)	0.00065
Calcium (Ca)	0.00097
Carbon (C)	0.122
Chromium (Cr)	0.178
Cobalt (Co)	0.0074
Copper (Cu)	0.229
Iron (Fe)	98.7
Lanthanum (La)	0.00031
Manganese (Mn)	0.361
Molybdenum (Mo)	0.0146
Nickel (Ni)	0.0881
Nitrogen (N)	0.0068
Phosphorus (P)	0.0339
Selenium (Se)	0.0131
Silicon (Si)	0.110
Sulphur (S)	0.0433
Tellurium (Te)	0.0071
Tin (Sn)	0.0395
Zinc (Zn)	0.0054

DISCUSSION

The sulphate-reducing bacteria isolated from buried mild steel samples in this study have been reported to possess immense potential applications in desulphurization process as reported by **Babu *et al*. (2014)**. They also isolated the sulfur-specific bacteria from Indian refinery plant sites and used in desulfurization process as well as *Enterobacter* sp., and *Pseudomonas* sp. Which bears semblance to the outcome of the biochemistry of bacteria associated with buried mild steel rods in our study. Similarly, *Bacillus* species which was also implicated as a sulphate-reducing bacteria in our study was enumerated from oil-contaminated soil and used in Bio-desulfurization **(Shamrao Patil et al., 2018)**. **Philip *et al*. (1998)** found that *Bacillus* species were also involved in heavy metal reductions which is parallel to the sulphate-reducing property of *Bacillus* species isolated from buried mild steel rods in this study.

Although carbon source was used in the Postgate C medium adopted in this study, yet diverse strains of SRB has specific medium for growth. This indicated that each mineral composition in the mild steel showed different effect on the growth of the sulphate-reducing bacteria. Additionally, the presence of iron (Fe) in the mild steel rod metallurgically-analyzed is a fundamental constituent which is accountable for supporting the microbial activity of SRB. As a rider to this, biologically-free iron cannot be useful for SRB because iron (Fe) is the most significant constituent required for the growth of SRB. Furthermore, local sulphate-reducing bacteria (SRB) strain can be isolated from crude oil sample as well to contrast the active growth of dissimilar SRB strain from onshore and offshore exploitation sites **(Pott *et al*., 2012; Potty and Sobaimi, 2013)**.

The turbidity of the broth culture of Postgate C medium used in this study indicated the presence and growth of Sulphate-reducing bacteria (SRB) in the liquid culture **(Csuros, 1999)**, which confirms the expected growth of SRB in our study. The more turbid the medium, the higher the number of SRB present in the medium. **Postgate (1984)** stated that the characteristic obnoxious odour from hydrogen sulphide and black coloured solution are mainly due to the evidence of SRB growth and its metabolism in the Postgate C medium utilized for SRB cultivation in our study. Consequently, the turbidity measurement gives enough confirmatory information without any iota of doubt which is needed in envisaging the growth of SRB from mild steel rods.

The result of the percentage mineral composition of metallurgically-analyzed mild steel rods such as 98.7 % Iron (Fe), 0.122 % Caron (C), 0.229 % Copper (Cu), 0.0138 Antimony (Sb) is in agreement with the observation of **Loto and Tobiloba (2018)**, where the authors characterized the percentage mineral composition of mild steel with emphasis on corrosion inhibition properties of the synergistic effect of 4-hydroxy-3-methoxybenzaldehyde and hexadecyltrimethylammonium bromide on mild steel in dilute acid solutions. The high percentage composition of iron may be attributed to iron being an essential component of mild steels activity of Sulphate-reducing bacteria (SRB) (**Loto and Tobiloba, 2018)**.

CONCLUSION

Findings of this study revealed the presence of sulphate-reducing bacteria (SRB) such as *Citrobacter freundii, Citrobacter gilleni, Enterobacter aerogenes, Pseudomonas aeruginosa*, *Desulfovibrio vulgaris* and *Bacillus megaterium* on buried mild steel rods of a water distribution system suggesting induction of corrosion of the underground mild steel pipes.

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Authors' inputs: DJA designed the study. IOO developed the methodology and acquired the data. IOO wrote the manuscript, AOO and DJA corrected the manuscript, provided administrative support and aptly supervised the study. All authors read and approved the final manuscript.

Conflict of Interest: The authors have no conflict of interest to declare.

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