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Inhibition of Biogenic Sulphide Production and Biocorrosion of Carbon Steel by Sulphate-reducing Bacteria Using *Ocimum gratissimum* Essential Oil

O. M. Immanuel[1*], G. O. Abu[2] and H. O. Stanley[2]

[1]African Centre of Excellence for Oilfield Chemicals Research, Institute of Petroleum Studies, University of Port Harcourt, Rivers State, Nigeria.
[2]Department of Microbiology, University of Port Harcourt, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The inhibition effects of *Ocimum gratissimum* essential oil (OGEO) on biogenic sulphide production and biocorrosion of carbon steel by Sulphate-reducing bacteria (SRB) were investigated in the laboratory. SRB were isolated from injection and produced water from Bonga oilfield in Nigeria. Biogenic sulphide production experiment was setup to monitor sulphide production by SRB. The effects of temperature, pH and salinity on biogenic sulphide production were determined. High sulphide production was observed at temperatures ranging between 20℃ – 40℃ and pH 7 - 9. Sulphide production decreased as salinity increased. Significant production of sulphide (59-168 mg/l) was detected after 72 hours of incubation of $>10^5$ cells/ml SRB consortium under study conditions and the maximum of 293 mg/l was observed after 144 hours at 30℃ when pH was 7 and salinity 25 g/l. The minimum inhibitory concentration (MIC) and minimum bactericidal

**Corresponding author: E-mail: immanuelomega@gmail.com;*

concentration (MBC) of OGEO were determined to be the same at 0.52 mg/ml. Corrosion inhibition efficiency (IE) increased with increase in concentration of extract and decreased with increase in temperature. OGEO gave the highest IE of 69.61% at 20℃, 61.050% at 30℃ and 58.01% at 40℃ for the 14 days test. For the 28 days test, OGEO gave the highest IE of 71.03% at 20℃, 63.61% at 30℃ and 59.41% at 40℃. OGEO+50 ppm D-tyrosine ble nd gave IE ranging from 89.22 - 92.82% and 84.90 – 92.08% for 14 and 28 days test respectively. Adsorption mechanism of OGEO on carbon steel obeyed the Langmuir isotherm model. Thermodynamic parameters evaluated gave negative values for ΔH_{ads} and ΔS_{ads}, suggesting that the adsorption reaction is spontaneous at low temperature. OGEO showed activities against SRB isolated from oilfield environment and could be useful for the prevention of biocorrosion of carbon steel structures in this environment.

Keywords: Sulphate-reducing bacteria; biogenic sulphide; Ocimum gratissimum; biocorrosion.

1. INTRODUCTION

Oilfield microbial communities have gained attention as one of the factors that affect the economics of oil and gas operations. Only recently, microorganisms have been linked in several high profile failures of oil and gas transport infrastructure which include: failure of a light grade crude oil pipeline [1], failure in 8 months of a new pipeline carrying oil and produced water [2], failure of a seawater pipeline infrastructure [3] and leaks in the Trans-Alaskan Pipeline [4]. Failure of structural works used offshore due to sour corrosion, represents a more serious economic burden to the industry. Sulphate respiration by Sulphate-reducing prokaryotes (SRP) in environment rich in sulphate ions such as reservoirs, production wells and injection wells leads to considerable accumulation of sulphide. The undesirability of H_2S in reservoir and produced fluids is due to its deleterious effects which include decrease in the economic value of crude, Health Safety and Environment (HSE) risk due to its toxicity and corrosion of metal surfaces [5,6,7].

Microbial activities contribute significantly to the global economic burden of corrosion which is estimated to be greater than 3% of global Gross Domestic Product (GDP) at US $ 2.2 trillion [8], accounting for 40% of all localized corrosion [9,10]. The general mechanisms by which microbial communities promote corrosion is by action of corrosive sulphides, corrosive organic acids and by influence of extracellular polymeric substances (EPS) [11,12,13].

Sulphate-reducing bacteria (SRB) are the most serious culprits among bacteria often implicated in deterioration of oil and gas facilities [12,14]. This group of anaerobic bacteria has been isolated repeatedly at biocorrosion sites and in biofilms attached to steel structures [15,16]. The control of SRB using synthetic chemicals could impact negatively on the environment if non-biodegradable, hence the need for green biocides.

The use of green biocides is the world best practices in microbial control. Ecofriendly biocides effective against a broad spectrum of bacteria are been sought by the petroleum industry as alternatives to those currently in use. Phytochemicals from plants offer promising potentials for use as biocides. The potential for use of phytochemicals as biocides is further boosted by their proven anticorrosion properties [17,18]. The plant *O. gratissimum* has been reported to have antimicrobial effect against pathogenic bacteria, such as *Escherichia coli, Listeria monocytogenes, Klebsiella* sp., *Salmonella typhimurium, Shighella flexneri* and *Staphylococcus aureus* [19,20,21,22]. *O. gratissimum* prevents dental biofilms and gingivitis [23] and inhibits biofilm formation in urinary catheter [24]. This study seeks to investigate the antimicrobial and anticorrosion potential of *O. gratissimum* essential oil against SRB.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction of Essential Oil

The plant *O. gratissimum* used for this study was obtained from a vegetable garden in Yenagoa, Bayelsa State and identified at the Department of Plant Science Herbarium, University of Port Harcourt, where voucher specimen was deposited.

The essential oil of *O. gratissimum* was extracted by steam distillation using Clavenger apparatus as described by Asawalam et al. [25]. One kilogram (1 kg) weight of fresh leaves was used.

The condensing essential oil was collected in n-hexane, which was later removed by distillation. Extracted oil was dried over anhydrous sodium sulphate and stored in sterile amber-coloured vials at 4℃.

2.2 Isolation of SRB

Sulphate-reducing bacteria were isolated from injection and produced water obtained from Bonga oilfield situated 120 km off the West Coast of Nigeria in Oil Mining License (OML) 118. Isolation of SRB was done using modified Postgate B medium containing in 1 litre: 1.0 g NH_4Cl, 0.5 g KH_2PO_4, 2.0 g $MgSO_4.7H_2O$, 1.0 g $CaSO_4$, 1.0 g Na_2SO_4, 5.0 mL sodium lactate 60%, 1.0 g yeast extract, 0.1 g ascorbic acid, 0.1 g sodium thioglycolate, 0.5 g $FeSO_4.7H_2O$, 25 g sodium chloride and 15 g agar. The pH was adjusted by the addition of 5M NaOH to make the final pH of the medium 7.0. The ferrous salt was sterilized separately and added to medium after cooling immediately before use. Nine millilitre (9 ml) of culture broth was dispensed into sterile 10 ml screw capped tubes. One milliliter (1) ml of sample was directly used as inoculum. All tubes were incubated at 30℃ for 7 days and observed for blackening by SRB due to H_2S formation. Repeated sub-culturing in agar plates was carried out to obtain a pure culture of SRB. Incubation was done in a Coy anaerobic chamber. A mixed SRB consortium (SRB-C) was obtained for biogenic sulphide production and corrosion experiments.

2.3 Antimicrobial Assay of Extract

One millilitre (1 ml) of extract was added to sterile vacuum tubes (Skytec) containing 8 ml of sterilized Postgate B broth. Each tube was inoculated with 1 ml of mixed culture of SRB-C with concentration of about 10^5cells/ml. The control tube was left untreated. Appropriate concentrations for susceptibility test were obtained by serial dilution in vacuum tubes (Skytec), containing sterile Postgate B medium to determine the minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC). The concentration range for this study was 0.1 mg ml^{-1}- 2.0 mg ml^{-1}. The least concentration of extract added that did not result in the formation of a black precipitate in the tube served as the minimum inhibitory concentration (MIC). The minimum bactericidal concentration (MBC) was determined as the lowest concentration of extract that resulted in no growth of SRB. This was determined by inoculating 1 ml of the treated and untreated cell suspensions from the MBC tubes in fresh 9 ml Postgate B medium and then incubated for 7 days at 30℃ and observed for growth. All tests were performed in triplicates.

2.4 Estimation of Biogenic Sulphide Productivity of SRB

To determine biogenic sulphide productivity by SRB, 5 ml seed culture of SRB-C with concentration of about 10^5cells/ml was placed in 90 ml of modified Postgate B medium in a 100 ml serum bottle and incubated for 192 hrs at 30℃. The total dissolved sulphide in the medium was determined spectrophotometrically with methylene blue according to APHA, 1989 [26]. Sample was nitrogen purged for 30 minutes. A needle was inserted within the sample, and a plastic catheter was introduced to collect released H_2S by reaction with cadmium sulphate, which forms a yellow precipitate. The H_2S was released from the precipitate in an acid medium through reaction with N, N-dimethyl-p-diphenylamine and iron (III) ion, which results in the formation of methylene blue. Absorbance was measured at 620 nm.

2.5 Effect of Temperature on Biogenic Sulphide Production

The effect of temperature on biogenic sulphide production was studied at 20℃, 30℃, 40℃, 50℃ and 60℃. Growth conditions and sulphide measurement were performed as described above.

2.6 Effect of pH on Biogenic Sulphide Production

The effect of pH on sulphide production by SRB was investigated at pH values within the range of 6 to 11 by adjusting pH value of Postgate B medium with addition of NaOH and HCl. Incubation was at 30℃.

2.7 Effect of Salinity on Biogenic Sulphide Production

The effect of salinity on sulphide production by SRB was investigated using salinity values of 25 g/L, 30 g/L, 35 g/L, 40 g/L and 45 g/L. These salinities were chosen to accommodate moderate to high salinities.

2.8 Gravimetric Experiment

Carbon steel coupons (0.1% C, 0.4% Mn, 0.03 % S, 0.06 % P and balance Fe) of dimension 4.5 cm× 1.0 cm× 0.23 cm, were smoothly polished using emery paper, cleansed briefly in 18% HCl, neutralized in sodium bicarbonate, washed with distilled water, rinsed in acetone, air dried and weighed using a digital weighing balance Citizen CY-204 with readabilty of 0.0001 g. Metal coupons were conditioned using varying concentrations of extracts, 50 ppm D-tyrosine and MIC+50 ppm D-tyrosine for 24 hrs before placement into 100 ml serum bottles containing 90 ml Postgate B. Five millilitres (5 ml) of seed culture of SRB-C was used to inoculate the tubes. Blank control was untreated coupon in medium not inoculated with SRB cells. Cell control was untreated coupon in medium inoculated with SRB cells. At the end of the 14 and 28 days test period, metal coupons were cleansed briefly in acid, neutralized with sodium bicarbonate, rinsed in acetone, air dried and reweighed. Changes in weight were recorded. Experiments were performed in triplicate and the mean values of the weight loss were reported. Corrosion rate (CR) was calculated using the formula of RCS [27], equation 1. The inhibition efficiency (IE) and the surface coverage (Θ) were calculated using equation 2 and 3 respectively.

$$CR = KW/DAT \quad (1)$$

Where K = rate constant 22,300; W = weight loss in grammes; D = density in gcm^{-3}, A = exposed area in $in.^2$ and T = time of exposure in days.

$$(IE) = 100\,[1 - (W_2/W_1)] \quad (2)$$

Where W_1 is the corrosion rate in the absence of the inhibitor, W_2 is the corrosion rate in the presence of the inhibitor.

$$\Theta = IE/100 \quad (3)$$

Data obtained from weight loss measurements were used for adso-kinetic studies of the extracts.

The adsorption equilibrium constant K_{ads} was deduced from the plot of C/Θ against C, using equation (4).

$$C_{inh}/\Theta = 1/K_{ads} + C_{inh} \quad (4)$$

Where, C is the concentration of inhibitor.

The Gibbs free energy (ΔG_{ads}) of adsorption was obtained from the relation:

$$\Delta G_{ads} = -RTIn(55.5K_{ads}) \quad (5)$$

Where, R is the universal gas constant (8.314 $Jmol^{-1}K^{-1}$), T is the operating temperature and 55.5 is the molecules of water displaced by adsorbed molecules of inhibitor.

Enthalpy change (ΔH_{ads}) and entropy change (ΔS_{ads}) of adsorption were deduced from the linear plot of ΔG_{ads} against T, using equation 6:

$$\Delta G_{ads} = \Delta H_{ads} - T\Delta S_{ads} \quad (6)$$

3. RESULTS AND DISCUSSION

3.1 Inhibition of Sulphide Production

The minimum inhibitory concentration (MIC) of OGEO against SRB-C was determined to be 0.52 (mg/ml). The minimum bactericidal concentration (MBC) was same as MIC. The *O. gratissimum* plant used in this study is a eugenol chemotype made up of 58.2% of eugenol. Eugenol has proven bioactive properties. In the present study we have demonstrated that OGEO showed activities against SRB isolated from oilfield (Fig. 1). Hydrogen sulphide production cause blackening of medium due to reaction with iron to form insoluble sulphide. SRB and archaea are known to be involved in dissimilatory reduction of sulphate to sulphide. Results in Fig. 1 showed that blackening of growth medium only occurred when extract concentration was below the MIC. Eugenol, a principal constituent of OGEO is cytotoxic causing cellular lipid membrane alteration and inhibition of nucleic acid synthesis [28], inhibitory against food spoilage bacteria [29] and microorganism causing biocorrosion of titanium [30]. For plant extract to be able to cause irreversible damage to the bacteria, it should be able to alter or cross the membrane barrier of SRB. The nature of outer membrane of Gram-negative bacteria such as SRB makes them insusceptible to toxic compounds [31]. Cell membrane is selective by nature. Essential oils are lipophilic and can bind to the hydrophobic lipid layer of cell membrane to cause damage. The inhibition of sulphide production by SRB in this study might be as a result of the possible interaction of OGEO with cell membrane.

Fig. 1. Levels of inhibition with various concentrations of OGEO

Where A= 0 mg/ml, B=0.26 mg/ml, C=0.52 mg/ml, D=0.78 mg/ml, E=1.04 mg/ml, F=1.30 mg/ml and G=Blank control

3.2 Effect of Temperature on Sulphide Production

Fig. 2 shows result for biogenic sulphide production measured 24 hourly at varying temperatures. The amounts of sulphide produced ranged from 8 mg/l to 293 mg/l. Sulphide production was not detected before 24 hrs of incubation at all temperatures. At 30℃ measured sulphide showed higher concentrations than at other study temperatures, ranging from 19 mg/l - 293 mg/l. No sulphide was produced at 50℃ and 60℃.

3.3 Effect of pH on SRB Growth and Sulphide Production

Fig. 3 shows result for the effect of pH on biogenic sulphide production at 30℃ incubation temperature. Result showed that amounts of sulphide produced ranged from 6 mg/l to 291 mg/l. Maximum sulphide production was observed at pH 7, according to Fig. 3. No activity was observed at pH 11.

3.4 Effect of Salinity on Biogenic Sulphide Production

Fig. 4 shows the effect of salinity on biogenic sulphide production at 30℃ incubation temperature. Maximum sulphide production of 293 mg/l was observed at salinity of 25 g/l. Sulphide production decreased with increasing salinity.

Given the necessary conditions to thrive like moderate, pH, temperature and salinity, presence of electron donors in form of organic acids and electron acceptor in form of sulphate, in a reduced environment devoid of oxygen, SRB can produce substantial amount of sulphide. The potential of SRB to produce sulphide under varying conditions prevalent in the environment from which they were isolated was investigated.

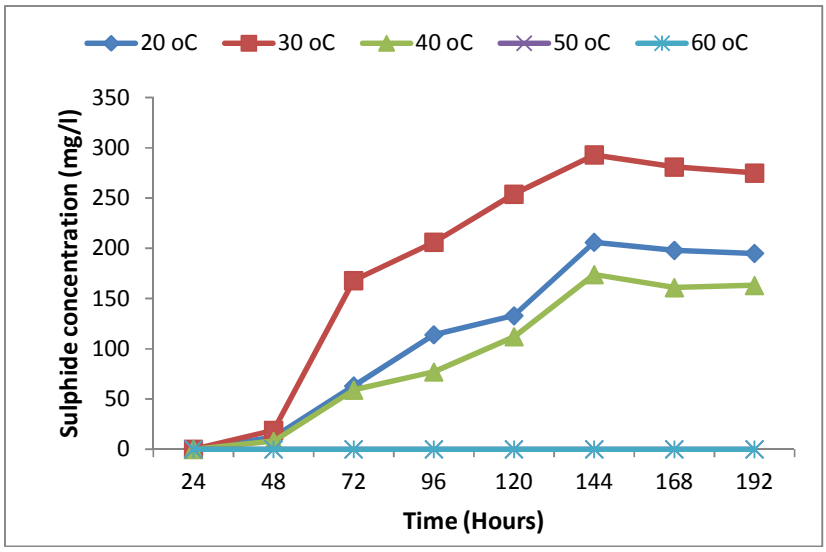

Fig. 2. Plot of biogenic sulphide concentration against time at various temperatures

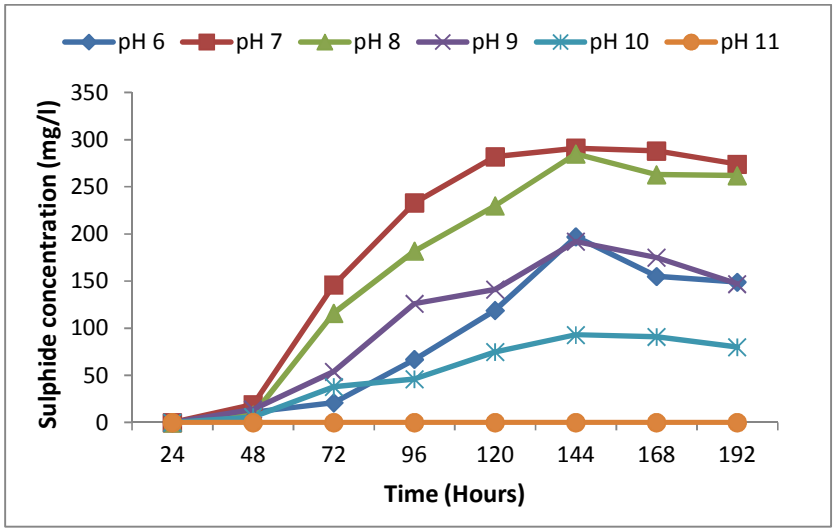

Fig. 3. Plot of biogenic sulphide concentration against time at various pH

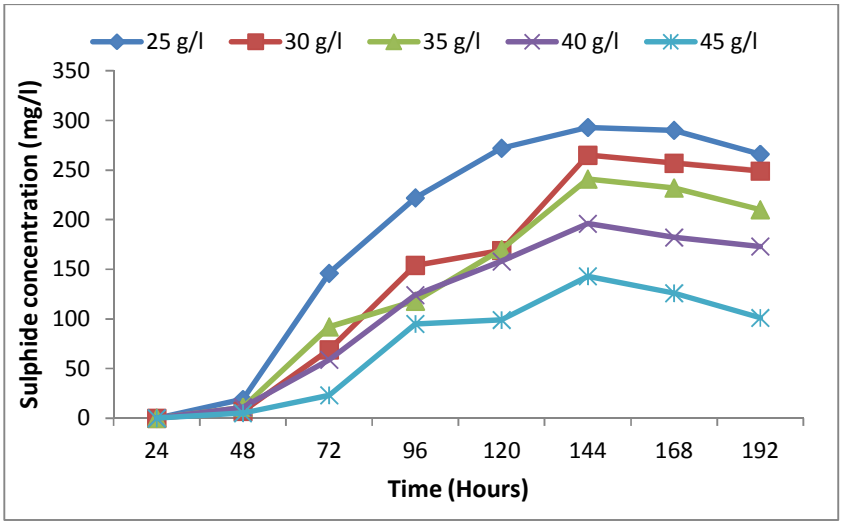

Fig. 4. Plot of biogenic sulphide concentration against time at various salinities

Postgate B medium is suitable for culturing and detecting the common culturable SRB [32]. Our findings revealed that SRB grown in Postgate medium B in anaerobic vial produced the most amount of sulphide (293 mg/l) after 144 hrs at 30℃, pH 7 and salinity of 25 g/l. Although the seed inoculum contained > 10^5 SRB cells/ml, no immediate sulphide production was observed within the first 24 hrs. This period (lag phase) is consistent with bacteria cells as they adjust to a new medium. The amount of sulphide produced after 144 hrs did not differ significantly ($P<0.05$) from values at 168 hrs and 192 hrs. This could be attributed to the inhibitory effect of high concentration of sulphide on SRB growth.

Hydrogen sulphide (H_2S) is toxic to SRB, inhibiting their growth and ability to utilize substrate [33]. Sulphide concentration ranging from 100 to 547 mg/l can inhibit the growth of SRB [34,35]. H_2S can also have indirect toxicity on SRB by precipitating insoluble iron sulphide [36], thereby making the important mineral unavailable to the growing cell. As more nutrients become depleted in the growth matrix cells enter the stationary stage and proceed to the decline phase. In the present study it can be hypothesized that H_2S inhibition of growth and sulphate utilization became prominent after the 168 hrs of incubation as measured dissolved sulphide showed a slight decrease from 293 mg/l to 281 mg/l.

4. CORROSION RATE AND CORROSION INHIBITION

Corrosion rate measured in the presence and absence of OGEO at 20℃, 30℃ and 40℃ for the 14 days test are shown in Fig. 5. Fig. 6 shows corrosion rates at varying temperatures for the 28 days test. Results indicated that corrosion rate decreased with increase in concentration of extract and increased with increase in temperature. Blank control depicts chemical corrosion induced by the chemical nature of the growth medium. There was no significant difference ($p<0.05$) between the corrosion rate in blank control and corrosion rates when concentrations above the MIC of extracts were applied. From the results of inhibition efficiency with OGEO shown in Table 1 and 2, it can be seen that inhibition efficiency increased with increase in concentration of extract and decreased with increase in temperature. Potentiating the extract with D-tyrosine gave better inhibition efficiency.

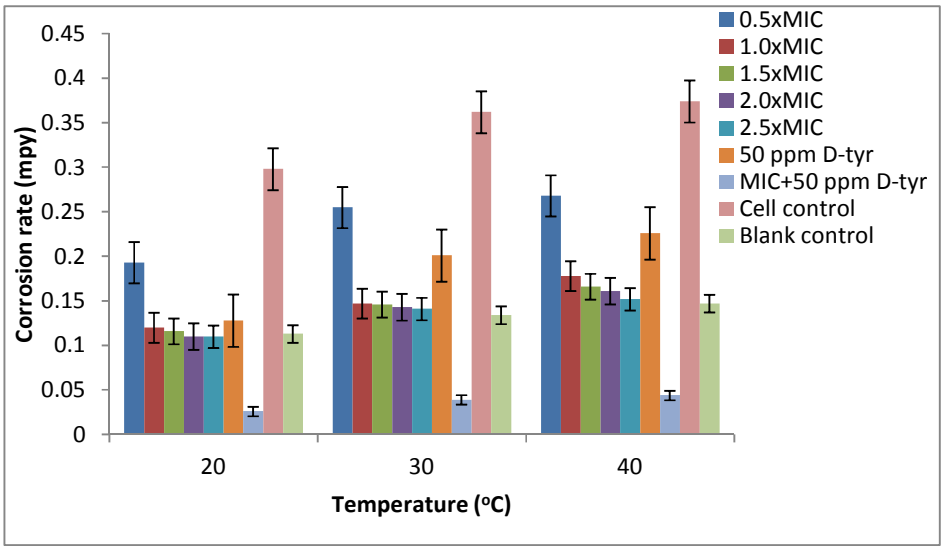

Fig. 5. Corrosion rate with varying temperature in the presence and absence of OGEO (14-days test)

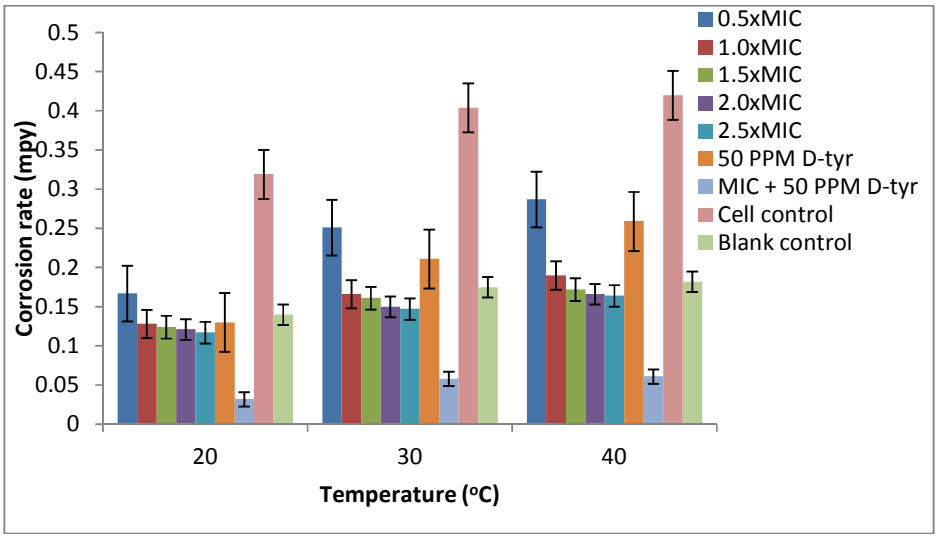

Fig. 6. Corrosion rate with varying temperature in the presence and absence of OGEO (28-days test)

Table 1. Variation of inhibition efficiency with temperature in the presence and absence of OGEO (14-days test)

Treatment	Inhibition efficiency (%I)		
	20°C	30°C	40°C
0.5 x MIC	35.23	29.56	28.34
1.0 x MIC	59.73	59.39	52.41
1.5 x MIC	61.07	59.67	55.61
2.0 x MIC	63.09	60.50	56.95
2.5 x MIC	63.09	61.05	59.36
50 ppm D-tyrosine	57.05	44.48	39.57
MIC + 50 ppm D-tyrosine	91.27	89.23	88.23

Table 2. Variation of inhibition efficiency with temperature in the presence and absence of OGEO (28-days test)

Treatment	Inhibition efficiency (%I)		
	20°C	30°C	40°C
0.5 x MIC	47.64	28.960	31.67
1.0 x MIC	59.87	58.910	54.76
1.5 x MIC	61.13	60.150	59.05
2.0 x MIC	62.07	62.870	60.48
2.5 x MIC	63.32	63.610	60.95
50 ppm D-tyrosine	59.25	47.77	38.33
MIC + 50 ppm D-tyrosine	89.97	85.640	85.48

Control of biocorrosion by microorganisms can be achieved if the organisms causing the menace can be killed or inhibited from forming metabolites or biofilms on metal surface. The corrosion data revealed activities at concentrations below and above MIC. The corrosion rate data showed that weight loss did not change much at day 28 compared to day 14 test. Metals left in the presence of a corrodant like H_2S would invariably be corroded and the longer the metal is exposed the more the expected corrosion. The prolonged exposure of the metal to the sour environment could account for the higher corrosion rate in the 28 days test even though sulphide production ceases with cell death. This is assumed to be so because H_2S has inhibitory effect on SRB such that as the toxic compound accumulates in the growth matrix, microbial activities decline and cells die.

All concentrations of OGEO showed some anticorrosion activities. Concentrations below the MIC of biocides are often bacteristatic. This investigation is the first study highlighting the anticorrosion potential of OGEO. The inhibition efficiency increased significantly with increase in concentration. OGEO gave the highest inhibition efficiency of 69.61% at 20℃, 61.050% at 30℃ and 58.01% at 40℃ for the 14 days test. For the 28 days test, OGEO gave the highest inhibition efficiency of 71.03% at 20℃, 63.61% at 30℃ and 59.41% at 40℃. OGEO+50 ppm D-tyrosine blend showed significantly higher inhibition efficiency than other treatments. OGEO+50 ppm D-tyrosine blend gave inhibition efficiency ranging from 89.22 - 92.82%, and 84.90 – 92.08% for 14 and 28 days test respectively. D-tyrosine has good inhibition efficiency against corrosion of carbon steel [37]. Our study showed that D-tyrosine enhanced the inhibition efficiency of OGEO. Several studies have reported that corrosion rate of carbon steel in acid medium reduces with increase in concentration of extracts [38,39]. Although increased anticorrosion activity was observed when concentrations higher than the MIC were used, it does not necessarily suggest that increasing concentration would continual lead to better efficiency. The effective concentration for microbial control remains the MIC but because our extracts adsorbed to the metal surfaces, concentration higher than the MIC could give a better corrosion inhibition. Hence, we attributed the corrosion inhibitory behavior of our extracts to their antimicrobial properties as well as to the adsorption of their molecules on the metal surface.

When corrosion inhibition is believed to be by adsorption, the mechanism is best described using adsorption isotherms. In this study Langmuir adsorption model was used to predict the mechanism (Fig. 7). The plots all showed a good fit with R^2>0.9, which implies the corrosion inhibition mechanism followed the Langmuir adsorption model. The Linearity of Langmuir isotherm adsorptions indicated the formation of a monolayer of extract on the carbon steel surface [40]. Adsorption of absorbate molecules to metal surfaces can be as a result of physical or chemical interaction between them. Physisorption can arise due to the weak electrostatic interaction between charged metal surface and the adsorbate molecules for Gibb's free energy values around -20 kJ/mol or lower [41]. The negative value of the ΔG_{ads}, obtained in this study (Table 3) implies that the adsorption process was spontaneous and adsorption mechanism can be assumed to be by physisorption. When inhibition efficiency decreases with increase in temperature,

physisorption can also be assumed [42]. Physisorption is believed to inhibit corrosion by blocking the surface of the metal from chemical attack [43]. From the point of view of biocorrosion, physisorption could prevent microbial attachment and attack by their metabolites.

High and positive K_{ads} values indicate conditions for good adsorption and strength of adsorption [44,45]. The K_{ads} values in our study decreased with increase temperature, consistent with the observations of other authors [46,40]. The obtained value for heat of adsorption (-29.13 kJ/mol) in the presence of OGEO, established that the process was exothermic at study temperatures (Table 3). ΔH_{ads} and ΔS_{ads} were both negative as deduced from the free energy plot (Fig. 8), suggesting that the reaction is spontaneous at low temperature. Negative value of entropies in the presence of OGEO at T implies an increased probability of extracts molecules adsorbing on carbon steel [46].

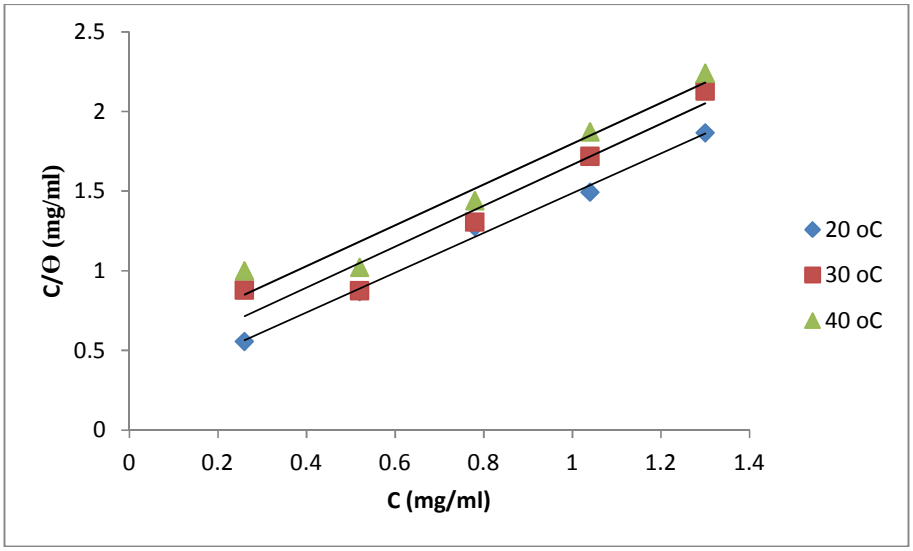

Fig. 7. Langmuir adsorption isotherm for inhibition of corrosion of low carbon steel in presence of OGEO

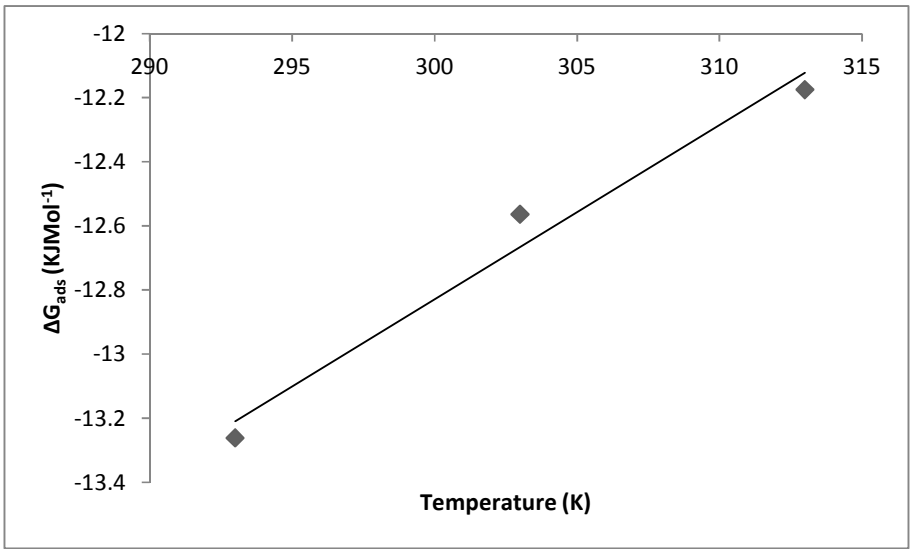

Fig. 8. A plot of free energy of adsorption ΔG_{ads} against temperature

Table 3. Thermodynamic parameters for adsorption of OGEO on carbon steel at different temperatures from Langmuir adsorption isotherm

Temperature (K)	R^2	K_{ads}	ΔG_{ads} ($kJmol^{-1}$)	ΔH_{ads} ($kJmol^{-1}$)	ΔS_{ads} ($Jmol^{-1}K^{-1}$)
293	0.994	4.167	-13.261	-29.13	-54
303	0.941	2.639	-12.563	-29.13	-54
313	0.95	1.038	-12.174	-29.13	-54

The chemical structures of eugenol and D-tyrosine showed the presence of anchoring functional groups which contained one or more hetero atoms such as N and O, as in hydroxy, epoxy, amino and carbonyl (Figs. 9 and 10). The anchoring groups allows for binding onto the metal surface.

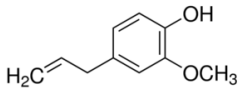

Fig. 9. Molecular structure of eugenol

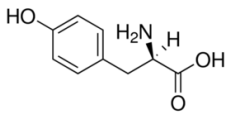

Fig. 10. Molecular structure of D-tyrosine

Microbial attack of steel leads to loss in weight due to the oxidation of Fe^0 and subsequent precipitation as FeS. The process is electrochemical in nature as defined by the anodic and cathodic reaction and differs from chemical corrosion in that it is biocatalysed. An increase in biomass of microbial cells at corrosion site is often taken as an indication of their involvement in the process, particularly when the only source of Fe an essential nutrient in cellular respiration is the metal itself. Observation that weight loss measurement is more correlated to microbial activities than electrochemical measurements which relate more to conductivity has been reported [47]. It was shown in this study that corrosion rate was higher when dissolved sulphide was at the most and least in the presence of OGEO which inhibited its production.

5. CONCLUSION

This work has demonstrated the antimicrobial and anticorrosion activities of *O. gratissimum.* This commonly used culinary and medicinal plant has been shown to be useful in the inhibition of corrosion and biogenic sulphide production by SRB in the laboratory. The results of this study give hope of finding suitable green phytochemical for oilfield operations to prevent souring and corrosion. Sourcing for antimicrobial agents from plants has the advantage that they are readily available, cheap and biodegradable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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