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Microbial Influenced Corrosion of Copper and Inhibiting Effects of *Capsicum annuum* Extracts

Nwokorie, R.C.[1]; Ogbulie, J.N.[2]; Nweke C.O.[3]; Akujobi, C.O.[4]; Imo, E.O.[5*]

[1,2,3,4,5]Department of Microbiology, Federal University of Technology, Owerri, Nigeria

Corresponding Author: Imo, E.O.[5*]

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Abstract: This study investigated the potential of *Capsicum annuum* extract as a green biocide and corrosion inhibitor for copper in the presence of *Desulfotomaculum* sp, a sulfate-reducing bacterium implicated in biocorrosion. The phytochemical analysis of *C. annuum* revealed that the extract is rich in bioactive compounds, including capsaicinoids, fatty acids, and terpenes. The antimicrobial activity of the extract against *Desulfotomaculum* sp was evaluated using well-in-agar diffusion assay, which showed significant dose-dependent inhibition zones. The corrosion inhibition properties of the extract were assessed by monitoring the weight loss and corrosion rates of copper coupons coated with the extract in both control media and media containing *Desulfotomaculum* sp. The results showed that *C. annuum* extract, effectively reduced the corrosion rates of copper coupons, with the greatest inhibition observed in the control medium. The study proposes a dual mechanism of action, where the antimicrobial compounds in the extract inhibit the growth and metabolic activities of *Desulfotomaculum* sp, reducing the production of corrosive metabolites, while simultaneously forming a protective film or exhibiting antioxidant properties on the copper surface, directly inhibiting the corrosion process. These findings highlight the promising potential of *C. annuum* extract as a green, sustainable, and cost-effective solution for biocorrosion control in various industrial sectors.

Keywords: *Biocides, Corrosion, Inhibition, Plant Extracts, Metals.*

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I. INTRODUCTION

Corrosion is a major concern in various industries, especially in the oil and gas sector. [1, 2]. It is a natural process that leads to the deterioration of materials such as metals and alloys, resulting in significant economic losses and safety hazards. It involves the gradual deterioration of materials due to their reaction with the surrounding environment, resulting in the loss of structural integrity and functionality [3]. The detrimental impacts of corrosion range from compromised safety and environmental contamination to substantial economic losses [4]. As such, understanding the intricacies of corrosion, its various forms, influencing factors, and mitigation strategies is of paramount importance in maintaining the integrity and longevity of materials and structures.

Microbial-induced corrosion (MIC), also known as biocorrosion, is a form of corrosion where microorganisms play a crucial role in accelerating the degradation of metals [5]. *Desulfotomaculum* species are sulfate-reducing bacteria (SRB) that are commonly associated with MIC [6]. *Desulfotomaculum* species are sulphate-reducing bacteria (SRB) that are commonly associated with MIC. They are anaerobic, gram-positive bacteria commonly found in environments with high levels of organic matter and sulfate, such as oil and gas reservoirs. They are the second most abundant SRB group after *Desulfovibrio* species [7]. These bacteria can reduce sulphate to sulphide, which leads to the formation of corrosive H_2S gas. H_2S is highly reactive and can form corrosive compounds such as iron sulphide (FeS) on metal surfaces, leading to corrosion [8]. Studies have shown that *Desulfotomaculum* species are responsible for up to 50% of the total corrosion in oil and gas pipelines [7].

Desulfotomaculum species are adept at forming biofilms on copper surfaces. The extracellular polymeric substances (EPS) produced within these biofilms promote bacterial adhesion and protect them from environmental stressors. These biofilms create localized microenvironments that accelerate corrosion processes [9].

Therefore, developing eco-friendly and cost-effective strategies to control *Desulfotomaculum* growth is essential for mitigating biocorrosion. One potential solution is the use of plant-based extracts, such as *Capsicum annuum*, as green biocides. These green inhibitors are derived from plant extracts and exhibit biocompatibility with the environment.

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The understanding of these natural alternatives has the potential to transform corrosion protection strategies.

In this study, the inhibiting effect of extracts of *Capsicum annuum* on the *Desulfotomaculum* sp influenced corrosion of copper including evaluation of the phytochemical constituents of the extract was investigated. Anticorrosion effects of the extract on the corrosion associated *Desulfotomaculum* sp was assessed using gravimetric. Antimicrobial screening to determine the growth inhibition of the extract and the minimum inhibitory concentration were assessed using agar-in-well diffusion method.

II. MATERIAL AND METHODS

- Metal Coupons: The copper metal used in this study was obtained from the material and metallurgical department of the University.

The copper metal was first cut into coupons of specific dimension 2cm x 2cm x 0.14 cm. Thereafter the coupons were polished with silicon carbide abrasive paper (from grade no. 400 to 1000), then cleaned with distilled water, dried in acetone and weighed with electronic weighing balance (Nicolet Model 37500). Weighed coupons were stored in moisture-free desiccators prior to use.

Isolation and identification: The bacterial isolate used in this experiment was isolated from biofilm covering abounded corroded hydrocarbon tank. The composition of the medium (Postgate B broth medium)– Oxoid used for the cultivation and identification *Desulfotomaculum* sp was K_2HPO_4 1.0g/L; NH_4Cl 2.0g/L; $CaSO_4.2H_2O$ 1.3g/L; $MgSO_4.7H_2O$ 4.0g/L; Lactic acid (88%)2.7g/L [10, 11]. All the ingredients were mixed in 100mL of deionized water and the pH of the medium was adjusted to 7.5.

A 10-fold serial dilution of the corroded metal sample suspension was prepared by weighing out 1 g of the scrapped layers of the corroded metal tank, into 9 mL of sterile distilled water in sterile 20 mL test tube. This constituted 10^{-1}dilution. The corroded metal suspension was vigorously shaken for 3 minutes by hand. After shaking, the 10^{-1}dilution was allowed to stand for 30 seconds. Then using a sterile pipette, 1mL was removed from the middle of the suspension and transferred into 9 mL sterile distilled water to achieve 10^{-2}dilution. The content of the 10^{-2}dilution test tube was shaken and dilution continued until the 10^{-7}dilution was obtained. After the serial dilutions, aliquots (0.1 mL) of dilutions 10^{-5} to 10^{-7} were inoculated in Postgate B broth medium (Oxoid) and incubated anaerobically at room temperature for 14 days. Microbial colonies isolated were characterized using sterile nutrient agar media. *Desulfotomaculum* sp was identified by gram staining and biochemical tests. The isolate is spore-forming, spherical, motile and gram-negative bacteria. It grows optimally at 30– 32 ºC and pH 6.8–7.2. The cells are curved rods shaped. The stock culture was maintained in a medium under nitrogen atmosphere at 4ºC.

A. Plant Extract:

The dried *Capsicum annuum* were washed with distilled water and pulverized with a blender. With the used of Soxhlet extractor, the stock solution was prepared using standard procedure outlined by [12] Dan (2009). From the individual stock inhibitor test solutions were prepared in the desired concentration range by diluting with distilled water. Quantitative and qualitative phytochemical screening of the extract was done using standard laboratory procedures [13].

B. Antimicrobial Screening

The *C. annum* extract was assayed for antimicrobial activity by the agar diffusion method. About 100g of the dried and powered seed was extracted with 200mL each of dichloromethane using Soxhlet extractor. Thereafter, Petri dishes were filled with Postgate B agar medium. Using a sterile cork borer of 6 mm in size, each plate was punched and 0.1mL of the extract poured in the hole. Bored hole filled with sterile distilled water was used as control. Each sample was prepared in triplicate. The medium was then inoculated with the with 0.1 mL Mac Farland Standard inoculum of *Desulfotomaculum* sp. The radius of the zone of inhibition was measured from the edge of the disc to the edge of the zone.

C. Gravimetric Experiments

The precleaned and weighed coupons were suspended in flask containing the test solutions using suspended with a cotton thread which passes through the hole in each coupon. To determine weight loss with respect to time, the coupons were retrieved at 7 days intervals progressively for 42 days. To test for inhibitory effects of *C. Annuum,* the prepared metal coupons were dipped in a flask containing 20mL of the extract and allowed to dry at room temperature (28±2ºC). Thereafter, the coupons were inoculated with a standardized suspension of *Desulfotomaculum* sp in a conical flask and incubated anaerobically in an anaerobic jar with gas park. In the control, metal coupons treated with extracts were placed in Postgate broth medium but not inoculated with the test organism. Medium with metals was incubated at room temperature (28±2ºC). The entire experiments were uniformly prepared in triplicates. The experiment was retrieved at 7 days intervals for a period of 42 days and analysed for the gravimetric corrosion measurement. The weight loss was taken to be the difference between the weight of the coupons at a given time and its initial weight. All tests were run in triplicate, and the data showed good reproducibility. Average values for each experiment were obtained and used in subsequent calculations.

$$Wl = Wi - Wf \quad (1)$$

Where:

Weight loss (WL) = Initial weight (WI) - Final weight (WF)

$$CR = 534 \frac{W}{DAT} \quad (2)$$

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Where:
CR= corrosion rate in mpy;
W= weight loss in grams;
D = density of copper in g/cm;
T = exposure time in hours
A = total surface area in cm;
534 = corrosion constant

III. RESULTS

A. Phytochemical Analysis

Table 1 shows the results of the phytochemical screening, and the percentage amounts of key phytochemical constituents present in *C. annuum* seed extract. They include 2 – (1 – mercapto-1-methylethyl) – 5 – methyl trans Cyclohexanone, Pentadecane, 6, 6 – Diethyloctadecane, 2, 6, 10, 14 – tetramethyl pentadecane, Tetradecanoic acid, Pentadecanoic acid, Heptadecane, Methylester Hexadecanoic acid, n -Hexadecanoic acid, Methylester 9, 12 – Octadecadienoic acid, (Z, Z) – 9, 12 – Octadecadienoic acid, 2, 5 – Dihydroxybenzoic acid 3TMS derivative, Octadecamethyl cyclononasiloxane, Nonivamide, Capsaicin, Dihydrocapsaicin, 2, 3 – dihydroxypropyl ester – 9, 12 – octadecadienoic acid (Z, Z), Eicosamethyl Cyclodecasiloxane, Vitamin E, and Gamma Sitosterol.

Table 1 Quantitative Phytochemical Constituents of *C. annuum*

Parameters	**Relative amount (%) of key phytochemical constituent**
2 – (1 – mercapto-1-methylethyl) – 5 – methyl trans Cyclohexanone	0.854
Pentadecane	1.576
6, 6 – Diethyloctadecane	1.730
2, 6, 10, 14 – tetramethyl pentadecane	2.359
Tetradecanoic acid	4.620
Pentadecanoic acid	1.956
Heptadecane	1.454
Methylester Hexadecanoic acid	3.039
n -Hexadecanoic acid	24.62
Methylester 9, 12 – Octadecadienoic acid	3.596
(Z, Z) – 9, 12 – Octadecadienoic acid	32.47
2, 5 – Dihydroxybenzoic acid 3TMS derivative	1.486
Octadecamethyl cyclononasiloxane	1.196
Nonivamide	2.227
Capsaicin	6.803
Dihydrocapsaicin	5.723
2, 3 – dihydroxypropyl ester – 9, 12 – octadecadienoic acid (Z, Z)	1.138
Eicosamethyl Cyclodecasiloxane	0.928
Vitamin E	1.223
Gamma Sitosterol	0.996

B. Antimicrobial Activity of the Extract

Table 2 shows the summary of the minimum inhibitory concentration (MIC) of the extracts. The MIC closely followed the same trend as the growth inhibition effect of the extracts. The highest growth inhibitory activity was obtained from the stock solution.

The results of the bactericidal activity of the extract of *C. annuum* against *Desulfotomaculum* sp are shown in Table 2. The highest growth inhibitory activity was obtained from the stock solution of the extract.

Table 2 Antimicrobial Activity of *C. annuum* against *Desulfotomaculum* sp

Mean zone of inhibition (mm)			
100%	50%	25%	12.5%
15	12.3	0	0

C. Corrosion Inhibition Results

- *Gravimetric Data*

The inhibitive effects of *C. annum* extract on the corrosion behavior of Cu in the presence of *Desulfotoculum* sp was studied using gravimetric technique. Figure 1 and Figure 2 shows the weight loss and corrosion rates of Cu in the presence and absence of the inhibitor respectively. The plots show that *C.annuum* extract effectively retarded Cu corrosion at the concentration studied. Furthermore, the corrosion rate was found to decrease with exposure time.

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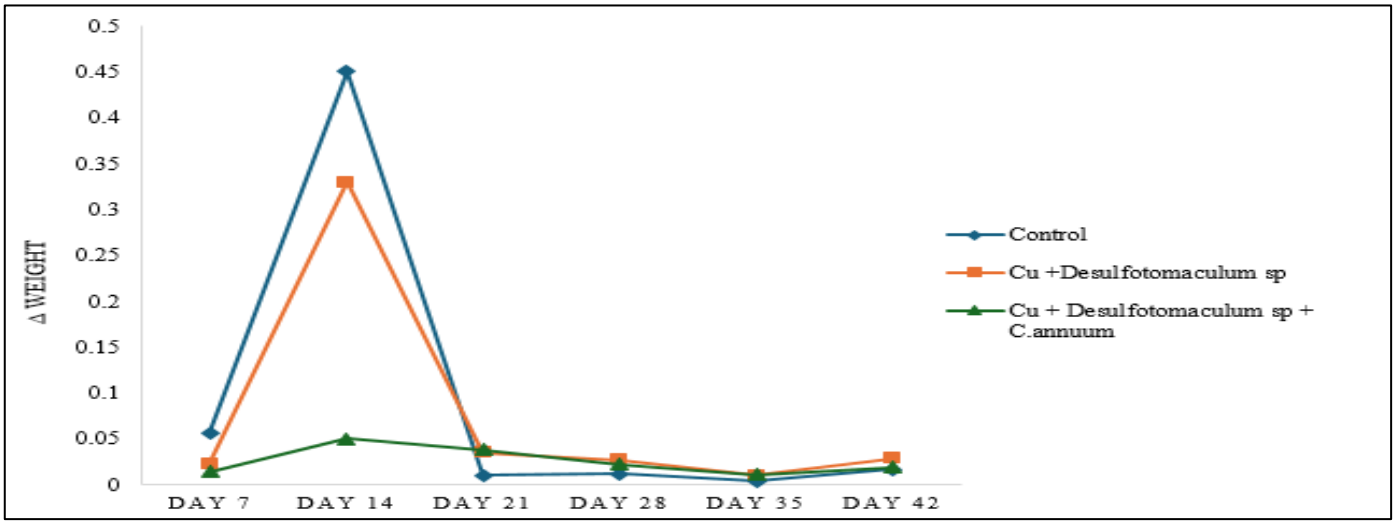

Fig 1 Weight Loss of *Dsulfotomaculum* sp Influenced Corrosion of Cu in the Presence and Absence of *C. annuum* Extract

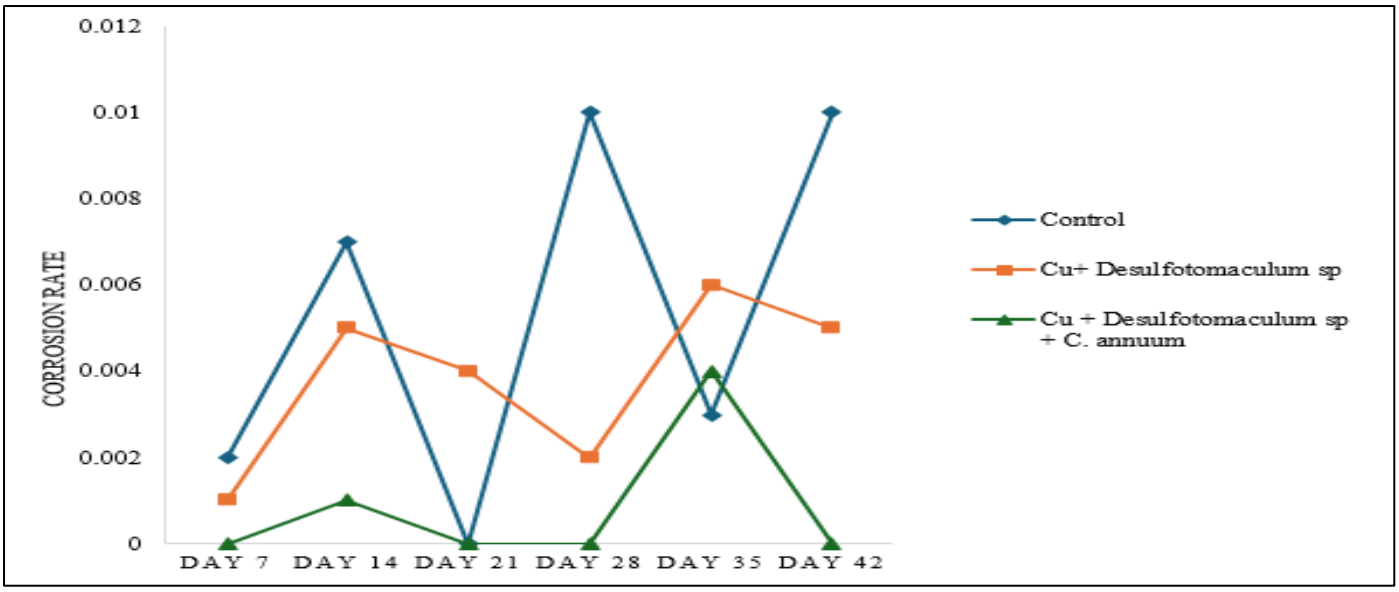

Fig 2 Corrosion Rate of *Dsulfotomaculum* sp Influenced Corrosion of Cu in the Presence and Absence of *C. annuum* Extract.

IV. DISCUSSION

The phytochemical analysis of *C. annuum* extract revealed a rich composition of bioactive compounds, primarily capsaicinoids, fatty acids, and terpenes (Table 1). The presence of these compounds is consistent with previous studies on *C. annuum* and their extracts [14, 15] (Tsao and Zhou, 2000; Reyes-Escogido *et al.,* 2011). The high concentrations of capsaicinoids, particularly capsaicin (6.803%) and dihydrocapsaicin (5.723%), are notable findings. These alkaloid compounds are responsible for the characteristic pungency of chili peppers and have been extensively studied for their biological activities. Reyes-Escogido et al. [15] reviewed the pharmacological properties of capsaicinoids and highlighted their antimicrobial, anti-inflammatory, and antioxidant activities. The antimicrobial activity of capsaicinoids is attributed to their ability to disrupt bacterial cell membranes, leading to the leakage of cellular contents and eventual cell death [16]. This mechanism of action could explain the potent antibacterial activity observed against *Desulfotomaculum* sp.

Furthermore, the high content of fatty acids, particularly (Z, Z)-9,12-Octadecadienoic acid (32.472%) and n-Hexadecanoic acid (24.624%), is noteworthy. These compounds are known to contribute to the antioxidant properties of chili peppers and their extracts. Gao et al. [17] reported that fatty acids, particularly unsaturated ones like linoleic acid ((Z, Z)-9,12-Octadecadienoic acid), exhibit strong antioxidant activities by scavenging free radicals and inhibiting lipid peroxidation. This antioxidant activity could play a role in the corrosion inhibition observed in the present study, as oxidation is a key process in metal corrosion.

The presence of terpenes, such as Caryophyllene (10.908%), is also significant. Terpenes are known for their diverse biological activities, including antimicrobial and anti-inflammatory properties [14, 18]. Their hydrophobic nature allows them to interact with bacterial cell membranes, causing disruption and cell death [19]. This mechanism could contribute to the antibacterial activity of the chili pepper extract against *Desulfotomaculum* sp.

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Overall, the phytochemical composition of the *C. annuum* extract, with its rich blend of capsaicinoids, fatty acids, and terpenes, provides a plausible explanation for the observed antibacterial and corrosion inhibition properties. The synergistic effects of these bioactive compounds could contribute to the extract's effectiveness as a green biocide and corrosion inhibitor.

The isolation and characterization of a *Desulfotomaculum* sp from a corroded hydrogen carbon holding tank is significant, as these bacteria are known to play a crucial role in biocorrosion processes. *Desulfotomaculum* species belong to the group of sulfate-reducing bacteria (SRB), which are anaerobic, spore-forming organisms that utilize sulfate as a terminal electron acceptor during respiration [20]. The key characteristic observed in our isolate is the production of hydrogen sulfide (H_2S), a highly corrosive byproduct of SRB metabolism. This aligns with the findings of [21], who highlighted the role of H_2S in accelerating corrosion by promoting the formation of iron sulfides, which are less protective than iron oxides.

The presence of *Desulfotomaculum* sp. in a corroded hydrogen carbon holding tank is not surprising, as these environments often provide favorable conditions for SRB growth. Anaerobic conditions, the presence of sulfate ions, and the availability of organic matter as electron donors support the proliferation of SRBs like *Desulfotomaculum* [22]. Furthermore, the ability of *Desulfotomaculum* species to form endospores contributes to their survival and persistence in adverse conditions [23].

The isolation of this bacterium from a corrosive environment underscores its potential role in biocorrosion processes. Several studies have reported the involvement of *Desulfotomaculum* species in microbially influenced corrosion (MIC) of various metals, including carbon steel [24], stainless steel [25], and copper [26]. The production of H_2S and other metabolic byproducts by these bacteria can directly accelerate corrosion or create conditions that favour further corrosion reactions.

The results of this study support the premise that *Desulfotomaculum* sp. plays a significant role in biocorrosion processes, particularly in environments where anaerobic conditions and the presence of sulfate ions are prevalent. The ability of *C. annuum* extract to inhibit the growth of this bacterium, as demonstrated by the antibacterial assay (Table 2), suggests its potential application as a green biocide for mitigating biocorrosion.

The antibacterial activity of *C. annuum* extract against *Desulfotomaculum* sp, as demonstrated by the well diffusion assay (Table 2), is a significant finding in the context of biocorrosion control.

The dose-dependent inhibition of bacterial growth observed in this study is consistent with previous reports on the antimicrobial properties of chili pepper extracts. Koffi-Nevry *et al.* [27] reported similar dose-dependent inhibition zones for capsicum extracts against various bacterial strains, including *Escherichia coli* and *Staphylococcus aureus*. They attributed this activity primarily to the presence of capsaicinoids, the pungent compounds found in chili peppers.

The observed antibacterial activity against *Desulfotomaculum* sp. suggests that the bioactive compounds in *C. annuum* extract could inhibit the growth and metabolic activities of these SRB. Capsaicinoids are known to disrupt bacterial cell membranes, leading to cell death [28]. Additionally, the fatty acids and terpenes present in the extract may contribute to the overall antimicrobial activity through their antioxidant and membrane-disrupting properties [19, 17].

The inhibition of SRB growth and metabolism is particularly relevant for biocorrosion control, as these bacteria are known to play a significant role in MIC processes. The production of corrosive metabolites, such as H_2S, by SRBs can accelerate corrosion reactions and promote the formation of non-protective corrosion products [29]. By inhibiting the growth of *Desulfotomaculum* sp the *C. annuum* extract could potentially mitigate the biocorrosion processes associated with these bacteria [30].

While the well diffusion assay provides valuable insights into the antimicrobial potential of the *C. annuum*, it is important to note that the observed inhibition zones may not directly correlate with the inhibitory concentrations required in practical applications. Further studies are needed to determine the minimum inhibitory concentrations and minimum bactericidal concentrations (MBCs) of the extract against *Desulfotomaculum* sp. under various environmental conditions relevant to biocorrosion scenarios.

V. CONCLUSION

Overall, the results from this study indicate that *C. annuum* extract has promising potential as a green biocide for inhibiting the growth and metabolic activities of biocorrosive bacteria like *Desulfotomaculum* sp.

DECLARATION

The authors declare that there is no conflict of interest.

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