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The Inhibitive Behaviors of *Costus afar* Leaves Extract on Sulphur Reducing Bacteria

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Authors' contributions

This work was carried out in collaboration between both authors. Author NEI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author COO managed the analyses of the study. Authors NEI and COO managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The inhibitive/adsorptive behavior of *Costus afar* leaves extract on the growth of sulphate reducing bacteria and anaerobic corrosion of pipeline steel caused by sulphate reducing bacteria were studied using gravimetric and media absorbance examination techniques. The test organism *Desulphurvibro specie* was isolated from corroded pipe line steel supplied by *Altelerae Deconstriea Materae* Nigeria ltd. Postgate medium was used for the isolation of the organism, and the coupons used for the study were prepared from the rusted pipeline steel. The absorbance of the media values decreased in the media containing *Costus afar* leaves extract from the absorbance values of the blank uninhibited medium. The decrease in the absorbance level has been suggested to be attributed to inhibition of Sulphate reducing bacteria in the inhibited media which resultantly led to light passing through with less interactions than the absorbance observed in the blank medium. The gravimetric results showed decrease in weight loss and corrosion rate in the medium containing *Costus afar* leaves extract from the blank, and the decrease in corrosion rate was more pronounced as the concentration of the *Costus afar* leaves extract increased in the media. Inhibition efficiency of the plant extract increased with increase in extract concentration. Besides

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obstructing the metabolic process of the microbial influenced corrosion agents (sulphate reducing bacteria), the plant extract molecules got absorbed to the pipeline steel surface serving as a protective shield against the invading microbial influenced corrosion agents (sulphate reducing bacteria). Adsorption of the extract molecules fitted into Langmuir adsorption isotherm.

Keywords: Anaerobic corrosion; Desulphurvibro specie; corrosion inhibition; postgate medium; Costus afar.

1. INTRODUCTION

Corrosion is the deterioration or degradation of metals or alloys, when they are destructively attacked by their environment. It is an electrochemical process which generally occurs in the presence of oxygen, aqueous electrolyte solution and moisture. [1]. It could be over the entire surface of a metal or alloy, and it occurs electrochemically between two different metallic materials or between two points on the surface of alloys of different chemical activity [2]. Besides aerobic environs corrosion also occurs in anaerobic environments like buried pipelines. This anaerobic corrosion is caused and influenced by microorganisms present in biofilms. The microorganism`s ability to utilize these metals as a source of carbon and energy required for their growth and metabolism using products of their metabolic activities such as enzymes, exopolymers, organic and inorganic acids, as well as ammonia and hydrogen sulphide, leads to deterioration and failure of pipes [3].

Microbiologically influenced corrosion (MIC) or bio-corrosion is a considerable problem for the oil and gas industry [4]. It is considered one of the most damaging mechanisms to pipeline steel materials [5]. Microorganisms are thought to be responsible for greater than 20% of pipeline systems failures [6]. The main types of bacteria associated with metals in pipeline systems are sulfate-reducing bacteria (SRB*),* iron reducing bacteria, CO_2 reducing bacteria, iron oxidizing bacteria and manganese oxidizing bacteria [6,7]. Among these, sulfate reducing bacteria have received much attention in the oil and gas industry and microbial influenced corrosion. However, investigations have shown that these micro-organisms have several detrimental metabolic activities including ability to;

- Oxidize hydrogen as an electron donor for metabolic life [6,7]
- Use O_2 and Fe^{3+} as a terminal electron acceptor [8]
- Utilize aliphatic and aromatic hydrocarbons as a carbon source [9].
- Use low levels of water for cellular maintenance and growth [10].
- Couple sulfate reduction to the intracellular production of magnetite [11].
- Compete with nitrate-reducing bacteria/ sulfur-oxidizing bacteria (NRB–SOB) (since they may have a nitrite reducing activity) [12,13]
- Cause elemental oxidation of iron [14]

Basically, prevention and treatment of microbial influenced corrosion is aimed mainly at destroying the microbial cell and/or preventing the development of biofilms [15].

Hydrocarbons in petroleum may serve as electron donors for sulfate reducing bacteria (SRB), which use sulfate as the terminal electron acceptor for respiration, resulting in sulfide production. The biogenic sulfide production results in metal bio-corrosion and SRB are typically the main bacterial group involved in these harmful processes in petroleum industries. The biogenic hydrogen sulfide production causes the bio-corrosion of metal surfaces of pipelines and tanks [16]. Moreover, the sulfide is explosive in high concentrations. SRB may grow in pipes and tanks forming biofilms, leading to the biodegradation of the metal surface [17]. Finally, the accumulation of SRB biomass causes reduced oil recovery [18,19,20]. Therefore, in petroleum industries, it is mandatory to control and inhibit SRB growth, which is usually done by biocide dosage [5,21]. Regardless of the effectiveness of these biocides, antimicrobial resistance often occurs, particularly in biocide treated biofilms [22,23]. In addition, the residual concentration, toxicity and persistence of biocides in industrial effluents are of high environmental concern. Hence, alternatives for SRB control are of great interest to the petroleum industry [19,24].

Less expensive and environmental friendly treatments are sought by the petroleum industry as alternatives to the use of synthetic biocides.

Essential oils from plant extracts are mixtures of lipophilic and volatile substances, which are known to have components with antibacterial and/or antifungal activity, and are potential sources of novel inhibitory substances [25,26]. The composition of essential oils/phytochemicals is different among species and plant parts. The oil's main components are terpenes and terpenoids, which are aromatic and aliphatic acid, esters and phenolic compounds [27]. The effect of different plant extracts on biofilms has already been demonstrated in the food industry and medical devices [11]. In addition, unlike other natural antimicrobial compounds, essential oils show inhibition on planktonic and sessile microbial growth at the same concentration. Thus, the ability to form biofilms does not provide extra protection for the organism when using essential oils as an antimicrobial agent [28-31] These reasons spur our interest and suggestion that *Costus afar* plant will possess good microbial influenced corrosion inhibition properties since it possesses antibacterial and/or antifungal activity and it is a potential source of novel inhibitory substances [25,26].

1.1 *Costus afar* as Microbial Influenced Corrosion Inhibitor

The plant *Costus afar* is among the 150 species of stout, perennial and rhizomatous herbs of the genus *Costus*. It can be found in the forest belt of Senegal, South Africa, Guinea, Niger, Sierra Leone and Nigeria [32,33]. *Costus afar* is commonly called bush cane or by other names in the Nigerian languages viz: Ireke omode (Yoruba), Opete (Igbo), Mbritem (Efik/Ibibio). *Costus afar* contains several bioactive metabolites [34], hence its extensive use in folkloric medicine as remedy for cough, rheumatic pains, sleepiness and cardio tonic. Tea from the dried aerial parts is used for hypertension while the leaves are used as poultry feed additives to increase both the size and number of eggs of treated birds [33]. Studies on different parts of *Costus afar* reveal invitro and invivo pharmacological activities [35], essential oil, topical anti-inflammatory activity, phytochemical composition and antioxidant activities [36], and antimicrobial activity [37]. A few chemical studies have shown that *Costus afar* contains oxalic acid, lanosterol, trigogenin, new diosgenin, stigmasterol, Sitosterol, costugenin, a new steroidal saponin aferoside. [34]. The corrosion inhibition potentials of *Costus afar* on mild steel in acidic medium have also been reported [38]. The present study investigates the inhibitive/adsorptive behaviors of *Costus afar* leaves extract on the growth of sulphate reducing bacteria and anaerobic corrosion of pipeline steel caused by sulphate reducing bacteria.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Sample

The sample was collected from the plant of interest around Michael Okpara University of Agriculture Umudike, Umuahia, Abia state, and was identified by Professor G.G.E Osuagu from the department of Plant Science and Biotechnology, college of Natural and Applied Science, Michael Okpara University of Agriculture, Umudike, Abia state, Nigeria.

2.2 Sample Preparation and Extraction

The sample *Costus afar* leaves were spread out on a laboratory work bench and inspected for the presence of extraneous materials such as dirt and insects larva. The inspected sample was cut into pieces to increase the surface area for easy drying. It was then further oven dried at a temperature of 50°C and pulverized to powdery form. The Extraction was carried out as discussed by Oyewole et al. [39]. About a hundred grams of the powdered sample in one liter of distilled water was extracted using a soxhlet extractor for twenty four hours. The extract was then concentrated using a rotary evaporator.

2.3 Media Preparation

Postgate medium was used for the isolation of the test organism and this was compounded from 0.5 g Potassium phosphate (K_2HPO_4), 2 g Sodium Chloride (NaCl), 1 g Sodium Sulphate (Na_2SO_4), 0.1 g Calcium Chloride ($CaCl_2$), 3.5 g Sodium Lactate ($CH_3CHOHCOONa$), 0.0002 g Iron (ii) Sulphateheptahydrate ($FeSO_4.7H_2O$), 1 g Yeast extract, 1litre of distilled water, sterilized at 121°C for fifteen to twenty minutes [39].

2.4 Isolation of *Desulphovibrio* specie

Desulphovibrio specie was isolated from corroded pipeline steel supplied by the *Atelerie Deconstreae Materea* Limited, an oil servicing company located at Trans Amadi Industrial Layout, Portharcourt, Rivers State, Nigeria. The

corroded sample was collected into a sterile sample bottle and brought into microbiology laboratory of Michael Okpara University of Agriculture Umudike, Umuahia, Abia state, Nigeria. The corroded pipe was immersed in sterile water for twenty four hours and a sample of the water was collected from it for analysis. Five folds serial dilution was then carried out using the water sample and these were placed on a freshly prepared Postgate agar and incubated anaerobically at 37°C for three days. One milliliter of pure isolate was sub-cultured into nine milliliter of Postgate broth and incubated anaerobically at 37°C for three to five days, [39,40,41].

2.5 Preparation of the Steel into Coupons

The corroded steel provided was sandpapered at the department of Metallurgy, Federal University of Technology Owerri, Imo state, Nigeria, and was further cut into $5mm\,X\,5mm\,X\,1mm$. The coupons were washed with distilled water and dried in acetone. The dried coupons were weighed and stored in air tight desiccators prior to use.

2.6 Determination of Effect of *Costus afar* Extract on Test Organism

Nine milliliters of Postgate broth was dispensed into seven test tubes and the plant extract was introduced into each of the test tubes in different concentrations (10 mg/ml, 50 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml and 500 mg/ml). A Control was also set up, without the plant extract. A piece of the pre-weighed coupons was then introduced into each of the test tubes containing various concentrations of the plant extract and the blank respectively. The medium was sterilized with an autoclave at 121°C for fifteen to twenty minutes after which one milliliter of the pure isolate was inoculated into the test tubes and the experiment was setup for thirty days in anaerobic jar. The temperature of the medium was maintained at 37°C throughout the duration of the experiment. The rate of corrosion was then determined using weight loss method.

2.7 Weight Loss Method

The pre-weighed coupons immersed in the various test tubes were retrieved after thirty days and the weight loss was measured and calculated using equation 2.1.

$$\Delta W = W_1 - W_2 \quad (2.1)$$

Where, ΔW is the weight loss, W_1 is the weight of coupon before the immersion, W_2 is the weight of the coupon after immersion. From the weight loss data, the corrosion rates of the coupons were calculated with the formula in equation 2.2.

$$CR = \frac{\Delta W}{At} \quad (2.2)$$

Where; CR is the rate of corrosion, A is the area of the coupon, ΔW is the weight loss, t is the time of immersion.

The inhibitory efficiency and the degree of surface coverage of *Costus afar* plant extract on the corroded steel were calculated from the corrosion rate data, using equations 2.3 and 2.4;

$$\theta = 1 - \left(\frac{CR_{in}}{CR_{bl}}\right) \quad (2.3)$$

$$IE(\%) = \left(1 - \left(\frac{CR_{in}}{CR_{bl}}\right)\right) X100 \quad (2.4)$$

θ is the degree of surface coverage, IE is the inhibitory efficiency, CR_{in} is the corrosion rate in inhibited system, CR_{bl} is the corrosion rate in non-inhibited system.

3. RESULTS AND DISCUSSION

3.1 Microbial Inhibition

The effect of *Costus afar* extract on the growth of *Desulphovibrio species* is evident in the absorbance shown by the medium containing the plant extract in different concentrations compared with that of the blank. The result is graphically represented in Fig. 1, which is a plot of absorbance against concentration of the media studied. Observations from the graph debits a decrease in the absorbance of the media containing *Costus afar* leaves extract, from that shown by the blank. The decrease in absorbance became more pronounced as the concentration of the plant extract increased from 10 mg/ml to 500 mg/ml. The reason for this decrease in absorbance no doubt is likely to be that there was more growth of *Desulphovibrio sp*. in the blank, while the less growth of the bacteria in the media containing the plant extract resulted in more light passing through the medium. It can also be observed that the absorbance decreased more as the concentration of the extract increased from 10 mg/ml to 500 mg/ml which

simply means that the inhibition increases as the concentration of *Costus afar* leaves extract increase.

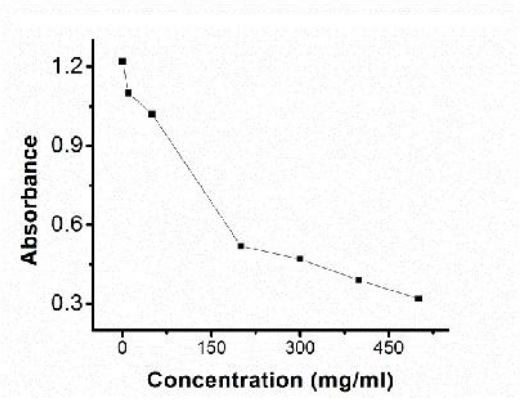

Fig. 1. Plot of Absorbance against concentration of the media

Desulphovibrio sp influence corrosion by reducing sulphates, to sulphide compounds [10]. The sulphide thus formed, reacts with iron in pipes to form iron (II) sulphide – a corroded product [42-46]. Besides the inhibition of the growth of sulphate reducing bacteria, the extract can as well get absorbed to the metal surface and form a barrier layer on the pipeline surface which serves as protective shield against the invading microbial thereby preventing corrosion. [47].

3.2 Gravimetric Results

The weight loss results of the blank (control) and the inhibited medium is graphically represented in Fig. 2. Observations from the graph show decrease in weight loss as the concentration of *Costus afar* leaves extract increase from 10mg/ml to 50 mgml. The graphical representation of corrosion rate of the iron pipe in anaerobic microbial influence corrosion is depicted in Fig. 3. A study of the graph shows that corrosion rate decreased in presence of the plant extract and this effect became more pronounced with increase in the concentration of the *Costus afar* leaves extract. This decrease in corrosion rate shown means that, the plant extract inhibited microbial influenced corrosion caused by sulphate reducing bacteria in anaerobic medium. This observation is related to what was observed in Oyewole et al. [39], from their study on inhibitory effect of nicotine on corrosion caused by *Desulphovibro specie* isolated from water pipes in Minna Nigeria.

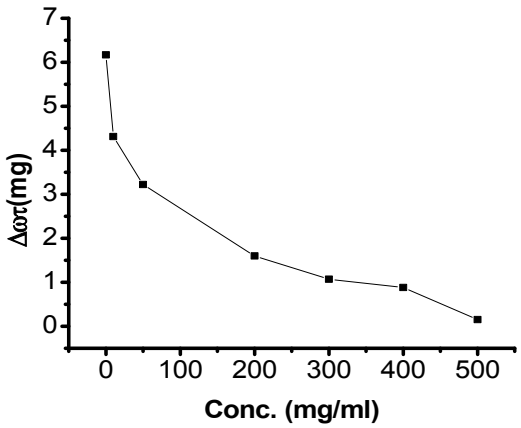

Fig. 2. Plot of weight loss against concentration of *Costus afar*

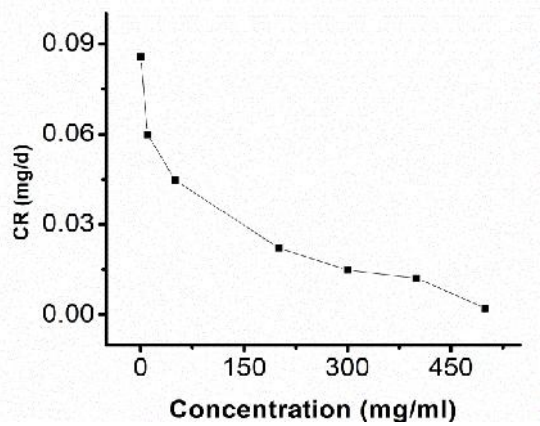

Fig. 3. Corrosion rate against concentration of *Costus afar*

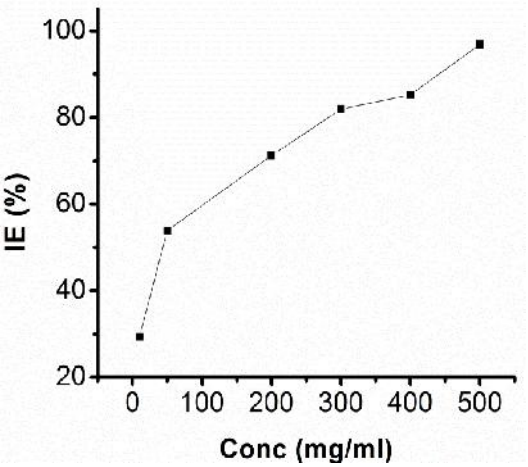

Fig. 4. Inhibition efficiency against concentration of *Costus afar*

Fig. 4 shows graphical representation of inhibition efficiency of *Costus afar* leaves on corrosion caused by *Desulphovibrio sp*. in anaerobic medium. Observation from the graph shows increase in inhibition efficiency as the extract concentration increases from 10 mg/ml to 500 mg/ml. This inhibition could be as a result of the extract molecules absorption to the surface of the pipe line steel and, or the inhibition on the growth of sulphate reducing bacteria by the plant extract. [3]. Studies carried out previously on *Costus afar leaves,* by Omokhua, and Ikpeazu et al. [48,49], reveal that *Costus afar* leaves extract is constituted of organic compounds like kaempferol, 3-flouro-β,5-dihydroxyl-N-methyl benzeethanamine, Dextroamphetamine, Hydroxyurea, Epinephrine etc, with structures in the figures below.

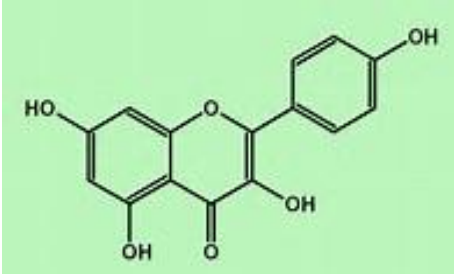

Fig. 5. Kaempferol

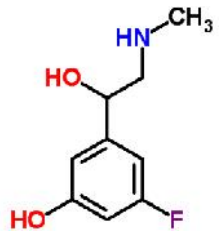

Fig. 6. 3-flouro-β,5-dihydroxyl-N-methyl benzeethanamine

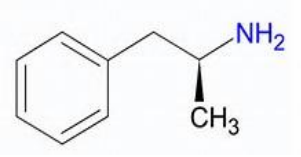

Fig. 7. Dextroamphetamine

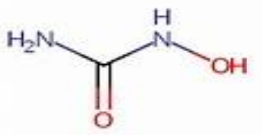

Fig. 8. Hydroxyurea

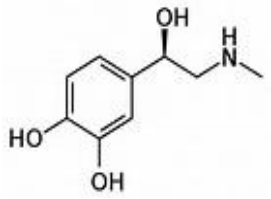

Fig. 9. Epinephrine

Since *Costus afar* leaves extract contains so many compounds with amine, hydroxyl, carbonyl and floro functionalities, which are ligands and can therefore form complexes with the metal surface thereby getting adsorbed to the metal. [50]. It is then difficult to single out the compound that is responsible for the adsorption of the plant extract on the metal surface. However more than one compound if not all, that contain functional groups with hetero atoms can take part in the adsorption/ inhibition of sulphate reducing bacteria.

3.3 Adsorption Consideration

The relationship between the degree of surface coverage and *Costus afar* leaves extract can be represente[132] y the Langmuir absorption isotherm as state[esente[ad] lated in] uation 3.1.

$$\frac{C(1}{\frac{C(1-(}{\theta}} = \frac{1}{Kad} \qquad (3.1)$$

Where C is the concentration of the plant extract, θ is degree of surface coverage and K_{ad} is the constant for adsorption. The equation is related to free energy of ads**adsor**(ΔG^0ad) by the expr[d b] in equatio[sorption 2] 3.2.

$$\frac{\theta)}{} = \frac{1}{55.5}\exp(\frac{\Delta}{\frac{ad}{}}) \qquad (3.2)$$

Where, T is temperature, R is gas constant and other parameters retain their previous definitions. Fig. 10 shows the plot of C/θ against concentration of the *Costus afar* leaves extract to be linear with intercept 1/K which suggest that the experiment fits into Langmuir adsorption isotherm. The calculated values of free energy of adsorption are shown in Table 1. The negative sign observed in the values obtained in 200 mg/ml, 300 mg/ml and 400 mg/ml indicates that *Costus afar* leaves extract molecules was spontaneously adsorbed to the pipeline steel surface in these concentrations of the plant extract in the anaerobic corrosive medium studied. [51] In contrary extract concentrations of 10 mg/ml, 50 mg/ml and 500 mg/ml showed

positive values which indicates that adsorption is not spontaneous and has to be induced in these concentrations [52].

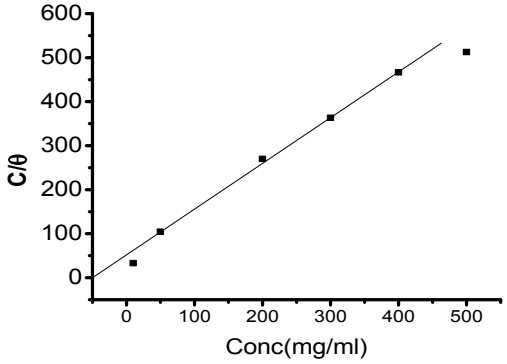

Fig. 10. Langmuir absorption isotherm

Table 1. Results of free energy of adsorption

Concentration (mg/ml)	Free energy(ΔG°ad) KJ/mol
10	2.2468
50	0.0462
200	−0.5948
300	−0.3329
400	−0.4629
500	3.8301

4. CONCLUSION

From the results of the analysis carried out, it can be concluded that;

i. *Costus afar* leaves extract inhibits the growth of sulphate reducing bacteria and corrosion caused by the bacteria in anaerobic medium.
ii. The inhibition efficiency increases as the concentration of the plant extract increases.
iii. The microbial influenced corrosion inhibition by the plant extract was as a result of the inhibition of the growth of the *Desulphovibrio sp*. and or the adsorption of the plant extract molecules on the surface of the metal.
iv. *Costus afar* leaves extract spontaneously adsorbed to the pipeline steel surface and the adsorption fitted into Langmuir adsorption isotherm

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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