INHIBITORY EFFECTS OF *Acanthus montanus* LEAVES EXTRACT ON MICROBIAL INFLUENCED CORROSION OF OIL PIPE LINE STEEL (CAUSED BY SULPHUR REDUCING BACTERIA) IN ANAEROBIC ENVIRONMENT

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ABSTRACT

The corrosion inhibitory effects of *Acanthus montanus* leaves extract on microbial influenced corrosion of oil pipe line steel (caused by sulphur reducing bacteria) in anaerobic environment was studied using weight loss method and media absorbance examination method. The test organism *Desulphurvibro specie* was isolated from rusted oil pipe line steel and Postgate medium was used for the isolation of the organism. The coupons used for study were prepared from the rusted oil pipe line steel supplied by *Altelerae Deconstriea Materae* Nigeria ltd. The results of the absorbance of the media showed that absorbance was lower in the media that contained *Acanthus montanus* leaves extract than that in the blank uninhibited medium. The reduction in absorbance is attributed to the inhibition on the growth of the test organism in the media containing the plant extract, which resulted to fewer particles to interact with light in the medium. The gravimetric results showed that weight loss reduced in the media containing the plant extract showing a reduction of corrosion rate in the inhibited media. The corrosion rate reduced as the concentration of the plant extract increased from 10mg/ml to 500mg/ml, but inhibition efficiency increased as the concentration of *Acanthus montanus* leaves extract increased. The inhibition is suggested to be through obstructing of the metabolic process of the agent of microbial influenced corrosion (Sulphur reducing bacteria) hindering the rate at which it reduces sulphate and oxides iron, coupled with the absorption of the extract molecules on the metal surface which serves as protective shield against the invading microbial. Adsorption of the extract molecules on the pipe line steel fitted into Langmuir adsorption isotherm, Temkins adsorption isotherm and Freudlich adsorption isotherm.

Key words: *Acanthus montanus, Desulphurvibro specie,* oil pipe line, anaerobic corrosion and adsorption

1.0 INTRODUCTION

Corrosion can be viewed as the process of returning metals to their natural state, i.e. the ores from which they were originally obtained. Approximately one-fifth of the iron and steel produced annually is used to replace rusted metal (Zumdahl, 1993). Mild steel and aluminum are used in fabricating various reaction vessels, reaction tanks, oil pipe lines etc, for industrial uses

due to their availability and low cost. However when used in aggressive media they tend to corrode which causes severe loss and malfunctioning of industrial equipment. Whenever corrosion is mentioned attention goes to acid or aerobic corrosion (In the presence of moisture and air) but observations has shown that corrosion also occur in anaerobic environments like buried pipe lines. This anaerobic corrosion is being caused and influenced by microorganisms present in biofilms because of their 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, this leads to deterioration and failure of pipes (Oyewole *et al*., 2011).

Microbiologically influenced corrosion (MIC) or bio-corrosion is a considerable problem for the oil and gas industry. MIC is considered one of the most damaging mechanisms to pipeline steel materials. The main types of bacteria associated with metals in pipeline systems are sulfate-reducing bacteria (SRB), iron and CO_2 reducing bacteria and iron and manganese oxidizing bacteria. Javaherdashti (2008), Little and Lee (2007). Among these, SRB have received much attention in the oil and gas industry and MIC investigations have revealed that these microorganisms have several detrimental metabolic activities including the ability to oxidize hydrogen as an electron donor, for metabolic life, use O_2 and Fe^{3+} as a terminal electron acceptor, utilize aliphatic and aromatic hydrocarbons as a carbon source, use very low levels of water for cellular maintenance and growth, couple sulfate reduction to the intracellular production of magnetite, compete with nitrate-reducing/sulfur-oxidizing bacteria (NRB–SOB) (since they may have a nitrite reducing activity) and cause elemental oxidation of iron Guan *et al*

(2016), Reutter *et al* (1994), Green et al (2003), Miranda *et al* (2006), Al Abbas (2013) and Green et al (2013).

Hydrocarbons in petroleum may serve as electron donors for sulfate reducing bacteria (SRB), which use sulphate as the terminal electron acceptor for respiration, resulting in sulfide production. The biogenic sulfide production results in metal bio-corrosion and reservoir souring, and SRB are typically the main bacterial group involved in these harmful processes in petroleum industries. The biogenic hydrogen sulfide production causes the acidulation and plugging of petroleum reservoirs and bio-corrosion of metal surfaces of pipelines and tanks (Nemati *et al.* 2001a). 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 (Zuo 2007). Finally, the accumulation of SRB biomass causes reduced oil recovery (Muyzer and Stams 2008; Nemati *et al.* 2001b; Postgate 1965). Therefore, in petroleum industries, it is mandatory to control and inhibit SRB growth, which is usually done by biocide dosage (Korenblum *et al*. 2013; Videla 2002).

Regardless of the effectiveness of these biocides, antimicrobial resistance often occurs, particularly in biocide treated biofilms (Fraise, 2002; Stewart and Costerton, 2001). 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 (Nemati *et al.* 2001b; Stewart 2002). 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 (Hammer *et al*. 1999; Solórzano-Santos and Miranda-Novales 2012). 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 (Reichling *et al*. 2009). The effect of different plant extracts on biofilms has already been demonstrated in the food industry and medical devices. 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 (Adukwu *et al.* 2012; Kavanaugh and Ribbeck 2012; Nahle *et al.* 2010; Nuryastuti et al. 2009)

Acanthus montanus plant is an ornamental plant of a great medicinal value in African community, and it can be used to cure many diseases like gonorrhea, syphilis, wounds and boils. Other uses of *Acanthus montanus* in herbal medicine include the treatment of hypertension, cardiac dysfunctions, hepatitis and heart diseases. Phytochemical screening conducted on leaves of *Acanthus montanus* by Odoh *et al,* (2010) revealed that it contains alkaloids, tannins, glycosides, flavonoids, steroids carbohydrates and terpenoids which are among the afore mentioned essential oils that were suggested to be the remedy for sustainable source for control of microbial influenced corrosion. Furthermore characterization and anti-microbial study carried out by Igwe and Nnaji (2014) revealed that *Acanthus montanus* leaf is constituted of 2,6-bis(1,1-dimethylethyl)-4-methylphenol(13.68%), allyl (2-tetrahydrofurylmethoxy) dimethylsilane (3.86%), sulfurous acid cyclohexylmethyl hexyl ester (5.67%), alpha-methyl 4-methylmannoside (8.41 %), hexadecanoic acid methyl ester (16.12 %), 11-octadecenoic acid methyl ester (19.03 %), docosane (5.85 %), N,N-dimethylvaleramide (18.62 %) and 2,6,10,15-

tetramethyl heptadecane (8.76 %). They (Igwe and Nnaji, 2014) reported as well that *Acanthus montanus* exhibited marked antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi* and *Proteus mirabilis*. On these grounds it will be reasonable to suggest that *Acanthus montanus* leaves extract will be a very good microbial corrosion inhibitor. A study on reported literatures revealed that the plant extract has been used against acid corrosion which yielded good result. Ibisi and Ufordiama (2016). The current study investigates the inhibitory effect of *Acanthus montanus* leaves extract on microbial influence corrosion of pipe line steel by sulphur reducing bacteria.

2.0 MATERIALS AND METHODS

2.1 COLLECTION AND IDENTIFICATION OF SAMPLE

The sample was collected from plant of interest around Michael Okpara University of Agriculture, Umudike, Abia State, and was identified by Prof. G.G.E. Osuagwu from Plant Science and Biotechnology Department, College of Natural and Applied sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.20 SAMPLE PREPARATION

The sample *Acanthus montanus* leaves were spread out on a laboratory work bench/table and inspected for the presence of variegated and extraneous materials such as dirt and insect larva. The inspected sample was cut into pieces to increase the surface area for easy drying and then oven dried at 50^0C. The dried sample was then pulverized to power form.

2.21 Extraction Preparation

The extract was done as previously discussed in Oyewole *et al*., (2012). Aqueous solution was used for the extraction of *Acanthus montanus* leaf. One hundred grams of pulverized sample was suspended in 1000ml of aqueous solution and extracted using soxhlet extractor for 120hrs. The resultant mixture was filtered and the extract was concentrate using rotary evaporator.

2.3 MEDIA PREPARATION

Posgate medium was used for the isolation of the test organism. It was compounded from its ingredients (O.5 K_2HP0_4, 2gNaCl, 1g Na_2 SO_4, 0.1g $CaCl_2$, 3.5g $CH_3CHOHCOONa$ (Sodium lactate), 0.0002 $FeSO_4$. $7H_2O$, 1g yeast extract, 1 litre of distilled water and sterilized at 121^0C for 15-20 minutes. Oyewole et al., (2012), Elisa et al (2013).

2.4 ISOLATION OF Desulphovibrio Specie

Desulphovibrio Specie was isolated from corroded pipe line steel supplied by *Altelarae. Deconstriea Materea* Nigeria Ltd Port Harcourt Rivers state Nigeria. The sample was aseptically collected into a sterile sample bottle and brought to microbiology laboratory, Michael Okpara University of Agriculture Umudike (MOUAU). The corroded pipe was immersed in sterile water for 24hrs and a sample of the water was collected from it for analysis. Fivefold serial dilution was carried out and was placed on freshly prepared Postgate agar and incubated anaerobically at 37^0C for 3 days. One milliliter of pure isolate was sub-cultured into 9ml of Posgate broth and incubated anaerobically at 37^0C for 3-5 days (cheesbrough 2003), Cowan and Steel (1993), Onyewela *et al*., (2012).

2.5 PREPARATION OF THE STEEL INTO COUPONS

The corroded steel provided by *Altelerae Deconstriea Materiae* Nigeria Ltd was sandpapered in Metallurgical laboratory Federal University of Technology Owerri (FUTO) Imo state Nigeria, and 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 desicator prior to use.

2.6 DETERMINATION OF EFFECT OF PLANT EXTRACT ON THE TEST ORGANISM

Nine milliliter of Posgate was dispersed into 7 test tubes and various concentrations of the plant extract (10mg/ml, 50mg/ml, 200mg/ml, 300mg/ml, 400mg/ml, and 500mg/ml) was introduced into each tube separately. Control was also set up, without the plant extract, one piece of the pre-weighed coupons was introduced into each test tube containing the plant extract and same was done in the control (blank). The medium was sterilized using autoclave at 121^0C for 15-20min. One milliliter of the pure isolate was inoculated into each tube and the experiment was set up for 30 days. The rate of corrosion was determined using weight loss method.

2.7 WEIGHT LOSS ANALYSIS

The pre-weighed coupons immersed in the test medium were retrieved after 30 days of immersion and the weight loss was calculated using equation below;

$$\Delta W = W_1 - W_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Where ΔW is weight loss, W_1 is the weight of coupon before immersion and W_2 is weight of coupon after immersion. From weight loss data, corrosion rates of the coupons were calculated using the formula below.

$$CR = \frac{\Delta W}{AT} \quad \text{.....2}$$

Where CR is corrosion rate, A is area of the coupon and T is the time of immersion while ΔW retains its initial definition.

The inhibition efficiency and degree of the surface coverage of plant extract on the steel was calculated from the corrosion rate data using the formula below

$$\theta = (1 - \frac{CR_{in}}{CR_{bl}}) \quad \text{.....3}$$

$$\text{IE}(\%) = \left(1 - \frac{CR_{in}}{CR_{bl}}\right) X\ 100 \quad \text{.....4}$$

Where, θ is the degree of surface coverage, IE is inhibition efficiency, while CR_{in} and CR_{bl} represent corrosion rates in inhibited system and non-inhibited system respectively.

3.0 RESULTS AND DISCUSSIONS

Table 1 contains the results of absorbance shown by the blank uninhibited medium which contain only desulphurvibrio specie (Sulphate reducing bacteria) in the anaerobic medium, and the ones that contain various concentrations of the *Acanthus montanus* leaves extract. Observation from the table shows that there is a reduction in the absorbance shown by the media that contains *Acanthus montanus* leaves extract from the value shown by the medium that does

not contain the plant extract. This obviously, means that there is more growth of desulphurvibrio Specie in the blank than it did in the media containing the plant extract. This observation is an indication of inhibition of the growth of the bacteria by *Acanthus montanus* leaves extract in the anaerobic medium.

Table 1: Absorbance of the Media

Conc. (mg/ml)	**Absorbance (nm) at 510cm**	**Molar absorptivity**
Blank	1.22	1.22
10	0.97	0.10
50	0.89	0.02
200	0.81	$4.00. \times 10^{-3}$
300	0.47	1.57×10^{-3}
400	0.38	9.50×10^{-4}
500	0.29	5.80×10^{-4}

The inhibition became pronounced as the concentration of the plant extract increased from 10mg/ml to 500mg/ml. This can be deduced from the decrease in the values of absorbance as the concentration of *Acanthus montanus* leaves extract increases. Another indication which confirms the inhibition was that, the media that contain the plant extract did not turn black as did the one without the extract at the end of the experiment as a result of presence of sulphide. This observation suggest the inhibition effect of *Acanthus montanus* extract on the growth of sulphate reducing bacteria and corrosion caused by the bacteria.

The relationship between absorbance and concentration of the medium is expressed by the Beer-Lambert law following equation below

$A \propto CL$ ……5

On removing the proportionality sign we have

$A = \mathcal{E}LC$ ……6

Where A is the absorbance of the medium, C is the concentration of the extract, L is the length of the light part which is equal to the width of the cuvette and $\mathcal{E}$ is molar absorptivity. The molar absorptivity is calculated using equation 6 above.

The results of molar absorptivity of the media studied are presented in table 1. Form the results the values of molar absorptivity reduced in the inhibited system and the reduction became more magnified as the concentration of the extract increases from 10mg\ml to 500mg\ml. The reduction no doubt can be as a result of more *Acanthus montanus* extract molecules in the system which inhibits the bacteria growth the more. The molar absorptivity is a measure of probability of the electronic transition. The proportionality of light absorbed will depend on how many particles or molecule it interacts with. The blank showed very high absorbance and high molar absorptivity because the sulphur reducing bacteria to interact with the light, but the media containing *Acanthus montanus* leaves extract showed low absorbance and low molar absorptivity, because there were lesser sulphur reducing bacteria in the media to interact with the light.

There are different mechanisms by which anaerobic sulphur reducing can facilitate corrosion which have been suggested by Beese *et al* (2013). Example of mechanisms include cathodic depolarization, iron sulfide galvanic coupling and direct electron uptake. Beese *et al* (2013)., Lewandoalski and Beyenal (2009). The main electrochemical reaction involves the production of sulphide via sulphate reducing bacteria metabolic activities. Sulphate reducing bacteria utilize cathodic hydrogen via hydrogenase enzyme to obtain the required electrons to reduce sulphate to sulphide by the following reaction. Lewandouski and Beyanal (2009).

$SO_4 + 8H^+ + 8e^- \rightarrow HS + OH^- + 3H_2O$ ……..7

In a de-aerated environment, hydrogen is produced by the water dissociation cathodic reaction as shown by equation.

$2H_2O + 2e \rightarrow H_2 + 2OH^-$ ……8

Electron transport reactions lead to proton motive force formation that supplies energy to the cells. Little and Lee (2007). Some biological produced sulphide ions will convert to hydrogen sulphide especially at acidic pH according to equation below:

$AS^- + H^+ = H_2S$ ……9

The production of hydrogen sulphide and the oxidation of iron in anaerobic medium promotes the formation of iron sulphide according the equation below Jauaherdashti (2008), Guan *et al* (2016), Little and Lee (2007).

$Fe \rightarrow Fe^{2+} + 2e$ ……10

$Fe^{2+} + H_2S \rightarrow FeS + 2H^+$ ……11

$3Fe^{2+} + 6OH^- \rightarrow 3Fe(OH)_2$ ……12

In uninhibited or *Acanthus montanus* extract free medium, the sulphate reducing bacteria metabolite activities have enhanced the process through the depolarization of hydrogen production of suphide and the formation of semi conductive iron sulphide film on the surface Guan *et al* (2016), Lewandewaski and Bayenal (2009).

Moreover, a protective shielding layer might have developed around the metal surface under media conditions owing to the organic nature of the *Acanthus montanus* leaves extract. This protection shield might provide further protection of the steel surface against the corrosion and the growth in the medium. So the microbial influenced corrosion is inhibited by *Acanthus montanus* extract by both inhibition of the growth of the sulphate reducing bacteria cells and adsorption of the plant extract molecules on the metal surface forming a barrier between the metal and the bacteria.

4.1 GRAVIMETRIC ANALYSIS

The variation of the weight loss is graphically represented in figure 1. Observation from the graph shows reduction in weight loss of the metal immersed in the media containing *Acanthus montanus* extract from the one immersed in the extract free medium. Figure 2 is graphical representation of corrosion rate against concentration of the plant extract studied. From the graph, it can be deduced that corrosion rate reduce in the medium containing the *Acanthus montanus* extract and the reduction became more pronounced as the concentration of the

Acanthus montanus extract increases. This reduction in corrosion rate indicates inhibition of the microbial influenced corrosion by the plant extract.

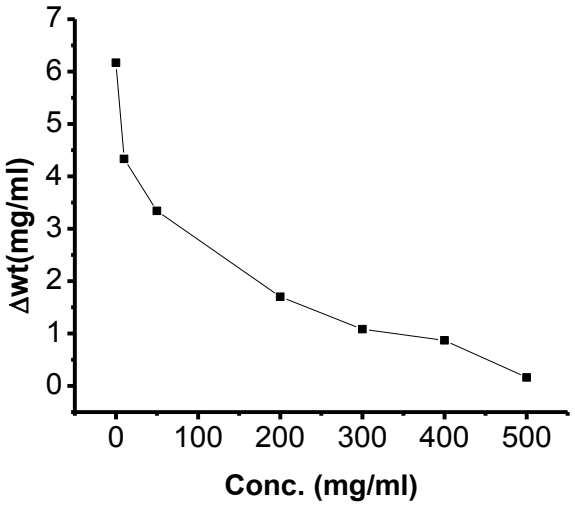

Figure 1: Weight Loss against Concentration

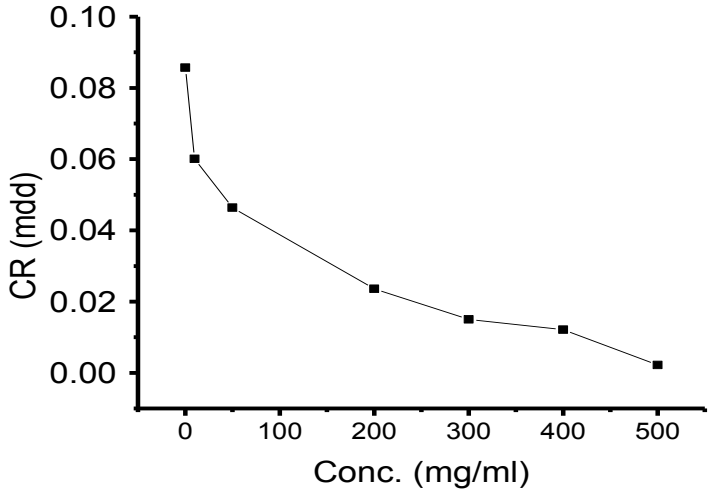

Figure 2: Corrosion Rate against Concentration

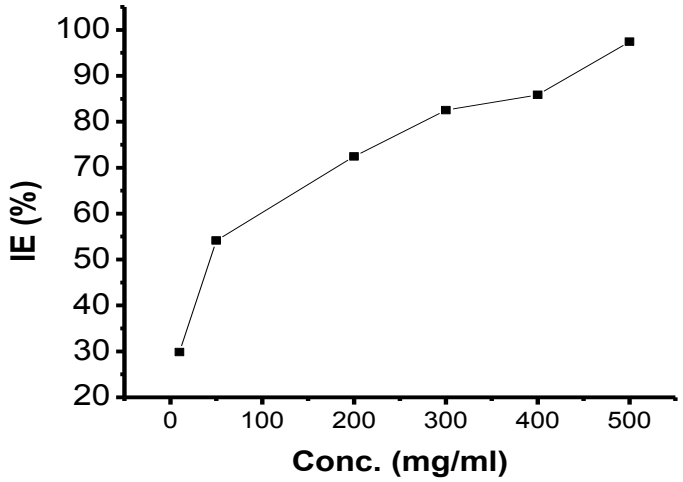

Figure 3: Inhibition Efficiency against Concentration

Graphical representation of inhibition efficiency against concentration of one plant extract is shown in figure 3. From the graph, it can be observed that the inhibition efficiency increased as the concentration of the plant extract increased from 10mg/ml to 500mg/ml. This shows that the more the extract molecules the more inhibition. Besides inhibiting the growth of sulphate reducing bacteria cells another way the plant extract molecules inhibit corrosion caused by sulphate reducing bacteria is by being adsorbed to the metal surface, thereby creating a barrier between the metal surface and the corrosive specie which hinders the formation of iron II sulphide.

4.2 ADSORPTION CONSIDERATION

Corrosion inhibition is accompanied by one or more of several mechanisms. Some inhibitors retard corrosion by adsorption to form a thin invisible film only a few molecules thick while others form visibly bulky precipitates, which coat the metal and protect it from attack.

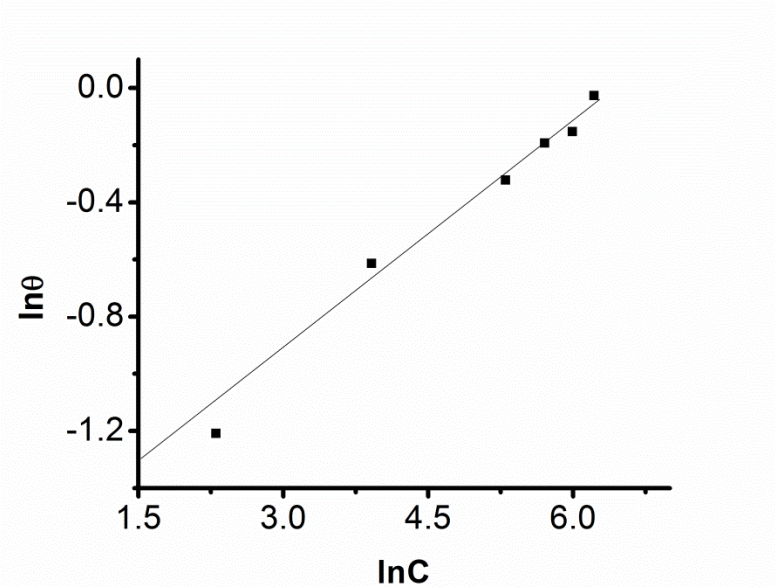

Figure 4: Freudlich adsorption isotherm

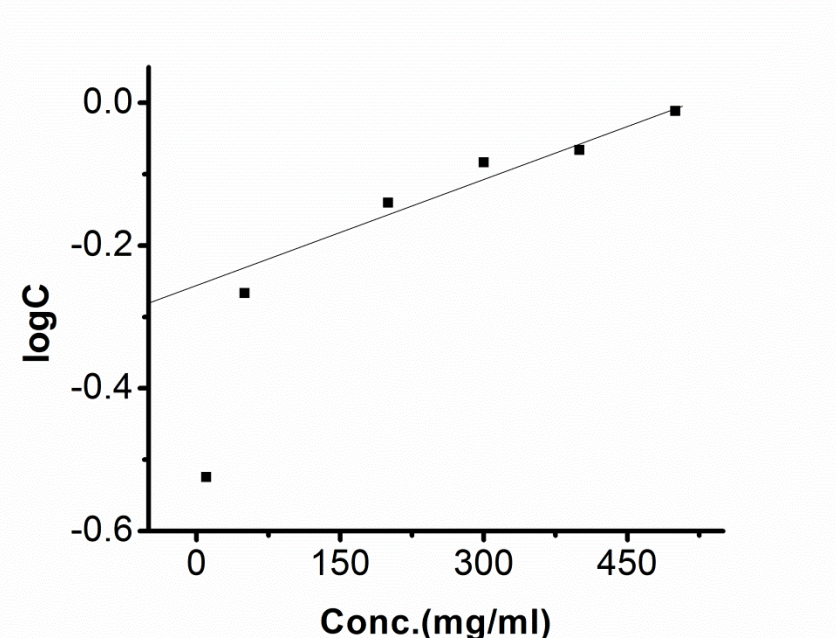

Figure 5: Temkins adsorption isotherm

The relationship between the amounts of substance (inhibitor) adsorbed on the unit area of the metal surface and the concentration of the inhibitor molecules in the solution at a given temperature is given by adsorption isotherm. Belhachemi and Addoun (2011)

Isotherms provide important clues about the nature of metal-inhibitor interaction. Frequently used adsorption isotherms are Langmuir, Temkim, Freundlich, Bockris- Swindles, Flory Huggins and Frumkin isotherms.

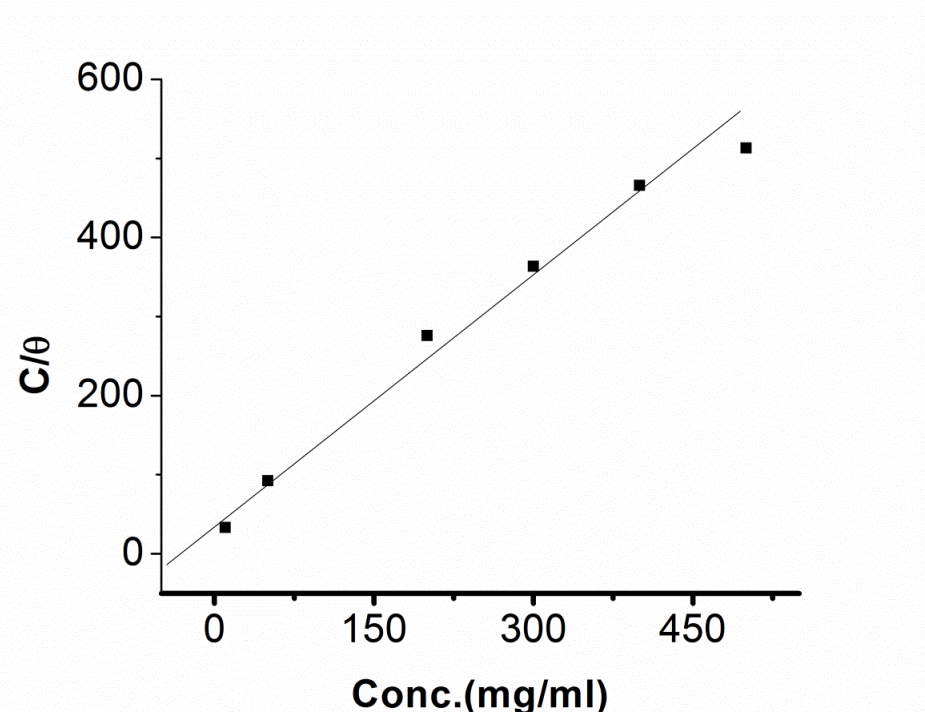

Figure 6: Langmuir adsorption isotherm

Figures 4,5 and 6 are plots of Freudlich adsorption isotherm, Temkins adsorption isotherm, and Langmuir adsorption isotherm respectively. Observations from the plots show that the graphs are all linear with intercepts, which shows that adsorption of the plant extract molecules studied fitted into Freudlich, Temkins, and Langmuir adsorption isotherms.

The relationship between the degree of surface coverage (θ) and *Acanthus montanus* leaves extract can be represented by the Langmuir adsorption isotherm

$$\frac{C}{\theta} = C + \frac{1}{k_{ad}} \text{ - - - - - - - - - - - - - - - - - 13}$$

Where K_{ad} is the constant for adsorption which is related to the free energy of adsorption ($\Delta G^0_{ad)}$) by equation 6

$$K_{ad} = \frac{1}{55.5} \exp\left(\frac{\Delta G^0_{ad}}{RT}\right) \text{----------14}$$

ΔG_{ad} is change in free energy of adsorption, R is universal gas constant, T is temperature of the system studied, C and θ are concentration of the extract and degree of surface coverage respectively. The calculated values of ΔG_{ad} are shown in table 2.

A negative sign of free energy of adsorption indicates spontaneous adsorption of the extract molecules to the surface of the pipe line steel in the anaerobic corrosive medium studied. Ngobiri *et al* (2015),, Ngobiri *et al* (2013), Ejikeme et al *2015*, Ibisi and Okoroafor *2016*). It can be observed from the results of free energy of adsorption, showed negative values at extract concentrations of 200mg/ml, 300mg/ml and 400mg/ml, the results in 10mg/ml, 50mg/ml and 500mg/ml were positive. This indicates that *Acanthus motanus* leaves extract molecules spontaneously adsorbed to the pipe line steel surface in 200mg/ml, 300mg/ml and 400mg/ml while in extract concentrations of 10mg/ml 50mg/ml, and 500mg/ml adsorptions were not spontaneous

Table 2: Results of Free Energy of Adsorption

Conc. (mg/ml)	**Free energy of adsorption ($KJmol^{-1}$)**
Blank	2.22
10	6.97
50	0.70
200	- 0.81
300	-0.35
400	-0.44
500	3.70

5.1 CONCLUSION

From the study, it can be concluded that *Acanthus montanus* leaves extract inhibits microbial influenced corrosion through obstructing of the metabolic process of the agent of microbial influenced corrosion (Sulphate reducing bacteria) hindering the rate at which it reduces sulphate and oxides iron, and the absorption of the extract molecules on the metal surface which serves as protective shield against the invading microbial. Adsorption of the extract molecules on the pipe line steel fitted into Langmuir adsorption isotherm, Temkins adsorption isotherm and Freudlich adsorption isotherm. From the trend in results of free energy of adsorption, the extract molecules in concentrations of 200mg/ml, 300mg/ml and 400mg/ml were spontaneously adsorbed to the pipe line steel surface, but adsorptions were not spontaneous in extract concentrations of 10mg/ml, 50mg/ml and 500mg/ml.The more the extract molecules, the more the degree of

surface coverage on the metal and the inhibition. Thus, the inhibition efficiency increased with increase in extract concentration.

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