



## Inhibition of Bio corrosion of steel coupon by sulphate reducing bacteria and Iron oxidizing bacteria using Aloe Vera (*Aloe barbadensis*) extracts.

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**ABSTRACT;** The inhibition of biocorrosion of steel coupons by sulphate reducing bacteria (SRB) and Iron oxidizing bacteria (IOB) using Aloe vera (*Aloe barbadensis*) extract was tested. The water sample revealed a heterotrophic bacterial count of  $1.7 \times 10^3$  cfu/ml for the sulphate reducing bacteria and  $4.1 \times 10^3$  cfu/ml for the Iron oxidizing bacteria. Biocorrosion inhibition test using Aloe vera extract was prepared with 50 % w/v stock solution at 10 %, 20 %, 40 % and 80 % (v/v) concentrations. An increase in acidity was observed in the fresh post gate medium after 7 days to 6.6 with a slight drop to 6.5 after 28 days in the setup with SRB. In contrast, the pH of the setups with IOB showed an increase in alkalinity from 6.8 in fresh Winogradsky medium to 7.4 after 7 days. The conductivity of SRB setup increased from 3150  $\mu$ S/cm to 27100  $\mu$ S/cm after 7 days and decreased to 200  $\mu$ S/cm after 28 days. On the other hand, the IOB setup showed an increase in conductivity from 100  $\mu$ S/cm to 2820  $\mu$ S/cm after 28 days. The surfaces of the metal coupon used in the biocorrosion testing were slimy to touch, indicating possible biofilm production. The average corrosion rate from day 7 to day 28 for IOB and SRB are 6.606 mpy and 12.48 mpy respectively. The 80 % v/v concentration of Aloe vera extract gave the highest inhibition efficiency, ranging from 89 %-100 % for IOB and 66 %-100 % for SRB. Aloe vera extract inhibited corrosion of steel coupons mediated by aerobic iron oxidizing bacteria and anaerobic sulphate reducing bacteria. © JASEM

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**Keywords:** Aloe Vera extract, biocorrosion, metal coupons, oxidizing bacteria, reducing bacteria

Corrosion is metal deterioration due to diverse chemical attack by various interactions within the environment. It is a persistent challenge that is difficult to eliminate completely, but its prevention is easy and can be achievable. Corrosion occurs after disruption of the metal's protective barrier, followed by a variety of reactions. These reactions can be in form of change in metal properties, composition of the surface and the immediate environment. Certain chemicals act as either synthetic or natural inhibitors decreasing the metal's susceptibility to environmental degradation. There is an imminent need for the building of efficient corrosion inhibitors that are ecologically friendly (Abiola *et al.*, 2007; Sharma *et al.*, 2015). A number of natural products with known applicability as good corrosion inhibitors for metals have been investigated (Sangeetha *et al.*, 2012; Ulaeto *et al.*, 2012; Okafor *et al.*, 2013; Singh *et al.*, 2013; Gupta, 2014; Yamuna and Anthony 2014; Desai, 2015; Souza *et al.*, 2015). The close relationship between microfouling and corrosion process has recently gained increasing recognition by engineers and microbiologist. Microbiologically influenced corrosion (MIC) is usually understood as the deterioration of metal which is promoted and enhanced by the effect of microorganism. The main factor in microbiologically influenced corrosion, apart from other requirements such as energy and carbon source, electron donor, electron acceptor and water are microorganisms (Javaherdashti, 2008). When a metal is exposed to water, the water-borne

microorganisms colonize its surface forming biofilm through a series of steps (Marangoni *et al.*, 2013). Problems which occur when the biofilm builds up are termed biofouling (Zou, *et al.*, 2004). Microbial fouling not only cause microbiologically influenced corrosion but may also lead to other severe consequences such as product contamination, reduction in transfer or mechanical blockage (kumar *et al.*, 2011). Although metal corrosion can occur without microorganisms, microbial activities accelerate the reaction rate (Horn and Jones, 2002; Videla *et al.*, 1998; Schaechter 2004; Romeo 2008; Simões *et al.* 2010). Many bacteria have been shown to be associated with the process of biocorrosion since 1960s. According to Beech (2004), sulphate reducing bacteria (SRB), Iron/manganese oxidizing bacteria, Iron reducing bacteria, sulphur oxidizing bacteria and bacteria which produce organic acids and slimes are the dominant types of microorganism involved in microbiologically influenced corrosion in terrestrial and aquatic habitats. These microbes are found in natural biofilm as they all contribute to the complex consortia of biofilm (Beech and Coutinho 2003). Among others, SRB is not only the first group of interest in biocorrosion study (Videla *et al.*, 1991b) but has also been recognised as the most significant contributors to microbiologically influenced corrosion (Coetser and Cloete, 2005; Marangoni *et al.*, 2013). This study was carried out to assess theinhibition of biocorrosion of steel coupon

by sulphate reducing bacteria and Iron oxidizing bacteria using Aloe vera (*Aloe barbadensis*) extract.

## MATERIALS AND METHOD

*Preparation of steel Coupon Specimen;* The steel bar used in this experiment was cut mechanically into strips of size 1.8 cm × 1 cm × 0.2 cm, polished with fine grade emery papers, washed, sterilized in an autoclave and weighed using electronic balance. The sterilized metal coupons were wrapped in a sterile foil until they were used. The scrapings were collected and wrapped in a sterile foil for Atomic Absorption Spectrophotometer (AAS) analysis.

*Preparation of Aloe Vera extract;* The sample was pulverized with a victory grinder and soaked in 99 % Ethanol for 24 h. The extract was filtered into a clean dry pre-weighed beaker through cheese cloth. The residue in the cheese cloth was severally soaked and filtered until the colour of the extract showed no trace of active ingredient under examination. The extract was placed in Plus (II) Gallenkamp Oven maintained at 78°C to vaporize the solvent (Ethanol). The residue was reweighed in the balance and the residue weight was calculated.

Weight of Residue = (Weight of residue + Beaker) – (Weight of Beaker)

*Preparation of Stock solution* The stock solution was prepared by weighing 8g of the Aloe vera extract in 16 ml of deionised water. This gave a concentration of 50 % w/v. From the stock solution, 10 %, 20 %, 40 % and 80 % v/v concentrations were made as follows: 0.5 mls of the stock solution was added to 5 mls, 2.5 ml, 1.25 ml, and 0.625 ml of deionised water.

*Isolation and identification of sulphate reducing and iron oxidizing bacteria:* A sterile bottle was used to collect water samples were collected from Ikpokulu River in Borikiri, Port Harcourt metropolis, Rivers State, Nigeria at a depth which the pipelines were placed (30 cm). The sample was collected close to corroded badges which have been abandoned for a long time. A ten-fold serial dilution was carried out on the water samples up to 10<sup>-3</sup>. Enumeration of the sulphate reducing bacteria was carried out by culturing 0.1 ml of the dilutions on postgate agar medium with the spread plate method and incubated in an anaerobic jar at 30°C for 14 days. Iron oxidizing bacteria were isolated by plating 0.1ml of the diluted samples on Winogradsky medium, with the spread plate technique and incubated anaerobically at 30°C for 7 days. Identification of isolates were carried out using pure cultures of distinct organisms on nutrient agar slant were subjected to confirmatory

biochemical tests including Gram stain, spore stain, motility and IMVIC. The isolates were identified by comparing the biochemical comparing the biochemical characteristics of already described taxa (David and John, 1994).

*Biocorrosion and inhibition testing against the bacteria using Aloe Vera extract;* A loopful of discrete colonies of iron oxidizing bacteria was suspended in Winogradsky broth and Other Winogradsky broth solutions containing 10 %, 20 %, 40 % and 80 % of Aloe vera extracts. Forty (40) pre-weighed metal coupons (steel coupon) labelled I<sub>1</sub> - I<sub>40</sub> were placed into Bijou bottles containing the set ups in pairs. The set up were incubated aerobically at 30°C. The coupons were withdrawn after 7days, washed, dried and re-weighed. The procedure was repeated for a 7days interval three times (14, 21 and 28 days). A loopful of sulphate reducing bacteria was suspended in fresh Postgate broth and another set up containing Postgate broth and 10 %, 20 %, 40 % and 80 % Aloe vera extract respectively. Forty (40) pre-weighed metal coupons (Steel coupons) labelled S<sub>1</sub> - S<sub>40</sub> were placed in pairs inside Bijou bottles containing both set up and incubated anaerobically at 30°C for 7 days. The same procedure was repeated for a 7 day interval thrice (14, 21 and 28 days). The controls for both Iron oxidizing bacteria and Sulphate reducing bacteria set up comprised uninoculated sterile media. The loss in weight of the metal coupons was calculated and the corrosion rates were calculated as according to Davis, (2000) as follows:

$$R = KW/ATD$$

Where; K=Constant equal to 3.45×10<sup>6</sup>; W=Weight loss in gram; A=Area to the nearest 0.01cm<sup>2</sup>; T=Time of exposure in hours; D=Density of metal (Steel) coupon; Inhibition efficiency (%) was also determined by the following formula:

$$\text{Inhibition efficiency (\%)} = \frac{CR_B - CR_I}{CR_B} \times 100$$

Where; CR<sub>B</sub> and CR<sub>I</sub> are corrosion rates in the absence and presence of inhibitor respectively.

*Physicochemical analysis;* Measurements of the pH of the samples and experimental set ups were carried out using a pH meter (model 5021). The instrument was standardized and the electrode dipped into the solutions whose pH was to be determined. The steady displayed reading was the pH of the sample solution. The conductivity of the experimental samples was measured by first flushing the electrode with distilled water to get a zero reading from the meter before

dipping the electrode into the samples and the conductivity values recorded in Millisiemens per meter (Ms/m). The conductivity meter used was JENWAY model 4010. Atomic absorption spectrophotometry was used to analyze the  $\text{Fe}^{2+}$  of the set ups.

## RESULTS AND DISCUSSIONS

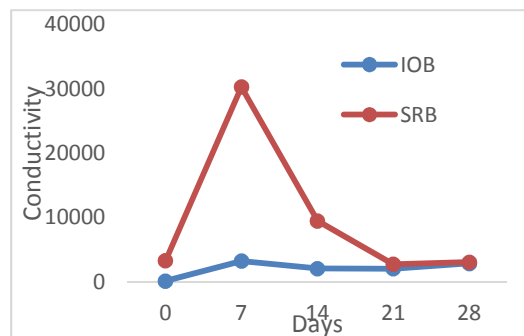
The biocorrosion of Steel coupons by SRB and Iron oxidizing bacteria using Aloe vera extract as a green inhibitor of biocorrosion was evaluated. There are about seventy-five potentially active constituents including minerals, sugars and vitamins enzymes among others (Hart and James, 2014). They also contain monosaccharide and Anthraquinones which are phenolic compounds, some of which acts as antibacterial and antiviral compounds. These Anthraquinone compounds have been used to inhibit corrosion (Amitha and Bharathi, 2012). The Steel coupons contained Chromium, Copper, Zinc, and Iron with concentration (mg/kg) of 895.63, 3989.00, 8225.00, and 344963.00 respectively with a density of  $7.89\text{g/cm}^3$ . The predominant composition of the Steel coupon is Iron, on which corrosion basically occurs most. An average Iron oxidizing bacterial count of  $4.1 \times 10^3$  cfu/ml was obtained with an average sulphate reducing bacteria of  $1.7 \times 10^3$  cfu/ml in the water sample. After the test period, a change in the physical appearance of the steel coupons was observed; the coupons placed in the Iron oxidizing bacterial culture were rusty and had a slimy surface, indicating biofilm formation, while the coupons subjected to the SRB culture appeared black indicating Iron sulphide deposition (Anichi and Abu, 2012). The average corrosion rate of the Steel coupons incubated in suspensions of Iron oxidizing bacteria in the absence of inhibitor (Aloe vera extract) was determined. The average corrosion rate after 7 days was 1.103mpy and increased to 8.961mpy and 9.191mpy after 14 days and 21 days respectively. The corrosion rate however decreased to 7.169mpy after 28 days, 11.51mpy after 21 days and 28 days respectively. The average corrosion rate of Steel



**Fig 1:** pH of suspension containing Iron oxidizing and sulphate reducing bacteria

coupons in the presence of inhibitor (Aloe vera extract) at any concentration was shown to be lower than the average corrosion rate in the absence of inhibitor (Aloe vera extract) in solutions containing Sulphate reducing bacteria and Iron oxidizing bacteria. Previous study by Obuekwe *et al.* (1981) showed that mild steel was extensively pitted with a concurrent formation of sulphide and ferrous ions. Corrosion rate was shown to increase at the initial formation of sulphide, but was slowed by the subsequent formation of the ferrous protective film. The formations of soluble iron have prevented protective layer formation. The corrosion of the Steel coupon is mostly on the Iron component, which was observed in the high levels of soluble  $\text{Fe}^{2+}$  in the culture solutions of Iron oxidizing bacteria and Sulphate reducing bacteria respectively. Moulin *et al.* (1998) reported high corrosion rates attributed to high levels of soluble iron on carbon steel.  $\text{Fe}^{2+}$  availability have been reported to be regulated by hydrogenase formation of *Desulfovibrio vulgaris* giving an alternative mechanism affecting corrosion (Cheung and Beech, 1996). The composition of the Steel coupon showed the predominant metal to be Iron with a concentration of 344963.00mg/kg. The water sample had an initial pH of 6.1, the Iron oxidizing bacteria increased from 6.8 in the freshly prepared solution to 7.8 after 28 days of incubation. A decrease resulted in Sulphate reducing bacteria from 8.4 in the freshly prepared solution to 6.5 after 28 days (Fig. 1). The production of hydrogen sulphide may be responsible for the acidity of the experimental set (Elke *et al.*, 2000). The fresh Postgate medium pH with the SRB showed an increase in acidity from 8.4 to 6.6 after 7 days and dropped to 6.5 after 28 days. While, IOB showed an increase in alkalinity from 6.8 to 7.4 after 7 days, this drift can be attributed to formation of oxides and hydroxides of iron. On the other hand, there was a decrease in pH in the set up containing Sulphur reducing bacteria from 8.4 in the freshly prepared solution to 6.5 after 28 days.

The conductivity increased from  $100 \mu\text{S/cm}$  to  $3200 \mu\text{S/cm}$  after 7 days and decreased to  $2820 \mu\text{S/cm}$  after 28 days with the Iron oxidizing bacteria. But for Sulphate reducing bacteria a drastic increase from  $3150 \mu\text{S/cm}$  to  $27100 \mu\text{S/cm}$  was observed but after 7 days, dropped to  $7400 \mu\text{S/cm}$ ,  $700 \mu\text{S/cm}$  and  $200 \mu\text{S/cm}$  after 14 days, 21 days and 28 days respectively (Fig. 2).



**Fig 2:** Conductivity ( $\mu\text{s}/\text{cm}$ ) of suspension containing IOB & SRB

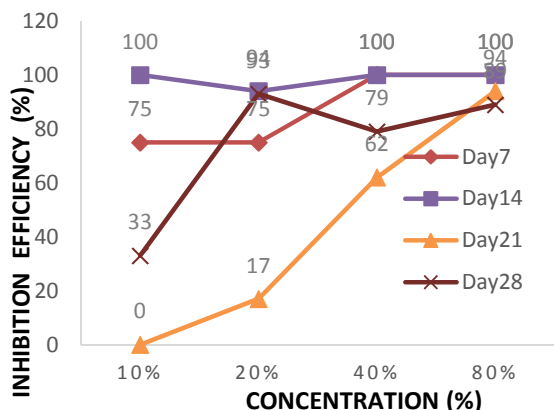
This decrease may be attributed to the presence of bacteria genera such as *Crenothrix Leptothrix*, *Sphaerotilus*, *Siderocapsa*, *Gallionella*, and *Clonothrix* which are characteristically non-ionic resulting in the biotransformation of oxides of metals such as iron and manganese (Gounot, 1994). The conductivity of the set up containing sulphate reducing bacteria increased drastically from  $3150\mu\text{S}/\text{cm}$  to  $27100\mu\text{S}/\text{cm}$  after 7 days as a result of the hydrogen sulphide formation ( $\text{H}_2\text{S}$ ) which is a weak acid. The increase in Iron (II) ions presence from Sulphate reducing bacteria and Iron oxidizing bacteria over a period of 28 days is an indication of biocorrosion (Figs. 3). A study by Malik *et al.*, (1992), showed a decreasing corrosion with a corresponding increase in pH. Corrosion rate was also shown to increase with a linear increase of chloride. The corrosion rate of the Steel coupon in the absence of inhibitor was shown to increase within 21 days of incubation with increasing pH and decreased by 22% after 28 days with a drop in pH in the set up containing Iron oxidizing bacteria. On the other hand, the set up containing Sulphate reducing bacteria showed an increase in corrosion with decreasing pH. The average corrosion rate of the Steel coupon in the absence of inhibitor (Aloe Vera extract) was shown to increase within 14 days with



**Fig 3:** Iron (II) ion ( $\text{mg}/\text{l}$ ) of suspension containing Iron Oxidizing and Sulphur Reducing Bacteria

decreasing pH. However, there was a decrease of 43% after 21 days and 51% after 28 days. There is a significant relationship conductivity and corrosion rate of the solutions containing the SRB and Iron oxidizing bacteria. As the conductivity of the solution containing the Iron oxidizing bacteria decrease from day 7 to day 21, the corrosion rate increased and at day 28 when the conductivity of the solution increased, the corrosion rate decreased. Steel coupon corrosion in the solution containing the Sulphate reducing bacteria was highest with a decrease in conductivity from day 7 to day 14, and then decreases from day 14 to day 28. Also, as the concentration of Aloe Vera extract increases, the corrosion rate was shown to decrease in both solutions of the Sulphate reducing bacteria and Iron oxidizing bacteria (Fig. 4 and 5). A study by Zhang *et al.*, (2003) showed that steel corrosion depends on the formation of dissolved oxygen, chloride ions, carbonate and calcium ions.

Some research groups have studied the use of naturally occurring substances to inhibit metal corrosion, with extracts of *Delonix regia* exhibiting aluminum corrosion in acidic solutions. (Abiola *et al.*, 2007)



**Fig 4.**inhibition efficiency of aloe vera extract in suspension of iron oxidizing bacteria

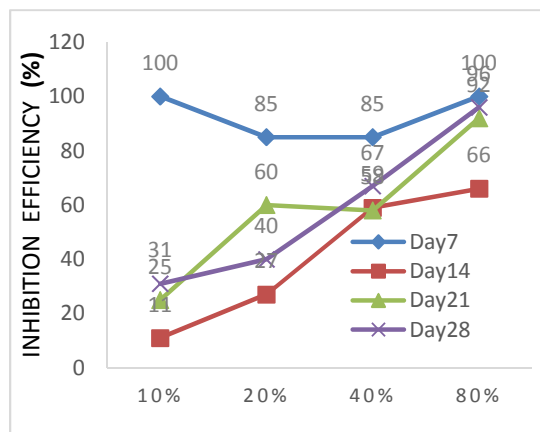
A study by Eddy *et al.*, (2009) showed variations in the temperature, concentration, immersion period, electrode potentials and pH in the adsorption of *Musa sp* on mild steel, which was spontaneous in line with the physical adsorption, and the Langmuir and Frumkin adsorption isotherms. The inhibition efficiency is shown to be affected by the concentration of the Aloe vera extract and the time of exposure in days. An increase in extract concentration resulted in an increase in inhibition efficiency. This is may be because the quantitative phytochemical analysis of Aloe vera extract during which the particular secondary metabolite responsible for biocorrosion inhibition of Steel coupons which could have been isolated and used precisely was not carried out. Hence, the optimum concentration of the active component of Aloe vera extract needed for biocorrosion inhibition was not determined.

Biofilm development by microbes that adhere to industrial surfaces has been shown to cause corrosion of metals, indicating that inhibition of biofilm production of surface bacteria is a simple way of decreasing and inhibiting corrosion.

**Conclusion;** The inhibition of corrosion by Aloe vera was observed in extract concentrations ranging from 10 – 80% (v/v). Aloe vera extracts, a green inhibitor, has the potential to inhibit biocorrosion and can be considered as an ecologically friendly approach for corrosion mitigation.

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**Fig. 5** Inhibition efficiency of Aloe vera extract in suspension of Sulphate reducing bacteria

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