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Biocorrosion Inhibition Efficiency of Locally Sourced Plant Extracts Obtained from Aloe Vera (*Barbadensis Miller*) and Scent Leaf (*Ocimum Gratissimum*)

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Abstract

Efficacy of Aloe Vera extract and scent leaf extract as biocorrosion inhibitors were evaluated using stainless steel coupons as the target surface/substratum. Stainless steel coupons were weighed and immersed in suspension containing (1) Iron Oxidizing Bacteria (IOB) and Scent Leaf Extract (SLE), (2) IOB and Aloe Vera extract (ALE), (3) Sulfate Reducing Bacteria (SRB) and SLE and (4) SRB and ALE respectively. Scent leaf and Aloe Vera extracts had 3.9 % and 1.4 % yield respectively. IOB had $\log_{10}$ IOB counts that ranged from 4.19 to 5.16 cfu/ml while SRB had $\log_{10}$ counts range of 4.11 to 5.16 cfu/ml. IOB isolates identified are *Leptothrix, Sphaerotilus* and *Gallionella* while that of SRB are *Desulfovibrio, Desulfotomaculum* and *Desulfovibrio*. In IOB culture, conductivity increased from 1.0130 to 3.90 s/m for ALE and 1.0140 to 2.80 s/m for SLE. The pH increased from 7.61 to 7.87 for ALE and 7.72 to 7.96 SLE medium. In SRB suspension, conductivity decreased for aloe Vera and increased in scent leaf. Iron II ions involving IOB in stainless steel coupons with Aloe Vera decreased by 56.7 % and decreased by 48.9 % for scent leaf medium while iron II ion involving SRB in suspension with ALE and SLE decreased by 43.01 % and 46.52 % respectively. Stainless steel immersed in SLE containing IOB had 272.7 mpy bio-corrosion inhibitions than those immersed in ALE and IOB (167.8 mpy). Biocorrosion was significantly ($p<0.05$) higher in IOB and SLE (99.1%) than in IOB and ALE suspension (74.8%). Steel immersed in SLE and SRB biocorroded at 206.6 mpy (64.2 %) and in ALE and SRB suspension, it biocorroded at 344.4 mpy (63.7 %). Bicorrosion monitoring and mitigation using locally sourced plant extracts as inhibitors could significantly reduce the spate of pipeline ruptures along pipeline Right of Ways in the Nigerian petroleum industries.

Keywords: Plant extracts, biocorrosion inhibitors, pipelines ruptures, stainless steel coupons.

Introduction

Biocorrosion occurs when complex microbial consortia interact with metallic surfaces through the establishment of multi species biofilms [1]. These products and/or their enzymes deteriorate the metallic material [2]. It is also known as microbial induced or influenced corrosion [3]. As reported by Koch *et al.* [4], the annual cost of all forms of corrosion in 2001 in oil and gas industries was 13.4 billion dollars. Out of this, microbial induced, initiated or influenced corrosion accounted for about 2 billion dollars. Biocorrosion occurs on metallic surfaces immersed in water and other environmental media [5-7]. Several inhibitors of acid corrosion of aluminum are known to be toxic and non-biodegradable [8-11]. As a result of increasing awareness on environmentally friendly practices for sustainable development, the demand for non-toxic biocorrosion inhibitors to replace relatively toxic ones has increased tremendously [12,13]. Thus, in recent years, several plant materials have been investigated for the inhibition of microbial corrosion of metals [14-19]. This is because green plants biomass (cell materials) contain naturally synthesized chemical compounds which are biocidal, biostatic, biodegradable, environmentally acceptable, inexpensive, renewable and readily available. The study was aimed at determining the biocorrosion inhibition efficiency of locally sourced plant extracts obtained from Aloe Vera (*Barbadensis miller*) and scent leaf (*Ocimum gratissimum*) on stainless steel coupons.

Materials and Methods

Sample Collection

Samples of Aloe Vera and scent leaf were obtained from a vegetable/fruit Market in Port Harcourt and transported in clean specimen bags to the University of Port Harcourt Reference Herbarium where they were identified as *Ocimum gratissimum* (Scent leaf) and *Barbadensis miller* (Aloe Vera) with voucher numbers assigned. Water sample was collected from a stagnant pond covered with ferns and raphia palms at a depth of 30 cm with sterile bottles in Africa Regional Aquaculture Centre (ARAC), Aluu. Clean sheets of stainless-steel coupons were obtained from Tricorr Technology Company in Port Harcourt, Rivers State.

Preparation of Corrosion Coupons

A rectangular specimen of stainless-steel (0.1-0.2 % carbon content and density of 7.02 g/cm^3) were cut into smaller sheets of area 2.0cm long, 1.0cm wide and 1.0cm thick (i.e. 2.0cm x 1.0 cm x 1.0 cm). The Aloe Vera extracts (ALE) as well as the Scent Leaf Extract (SLE) were then weighed separately and stored in a refrigerator at 4 ^oC for subsequent use.

Yield and Percentage Yield

The yield (g) and percentage yield were calculated using the expression:

$$\text{Yield} = \frac{X}{Y}(g)$$

$$\text{Percentage Yield } (\%) = \frac{X}{Y} \times 100$$

Where, g is gram, X is the weight of extract and Y is weight of plant sample.

Experimental Treatment Design

Duplicate samples of stainless-steel coupons were collected, weighed and suspended in broth medium labeled Set 1 through Set 5 (Table 1). Set 1 contained Stainless Steel Coupon (SSC) plus Winogradsky Broth (WGB) plus Iron Oxidizing Bacteria (IOB) pus Scent Leaf Extract (SLE). Set 2 had SSC plus WGB puls IOB plus Aloe Vera Extract (ALE). Set 3 contained SSC plus Postgate Broth (PGB) plus Sulfate reducing bacteria (SRB) plus SLE. More so, set 4 contained SSC plus PGB plus SRB plus ALE while Set 5 had SSC plus broth culture only to serve as control experiment (Table 1).

Setup	Experimental design
Set 1	Stainless steel + WGB + IOB + SLE
Set 2	Stainless steel + WGB + IOB + ALE
Set 3	Stainless steel + PGB + SRB + SLE
Set 4	Stainless steel + PGB + SRB + SLE
Set 5	Stainless steel + Broth (Control)

Table 1: Experimental Protocol.

***IOB:** Iron oxidizing bacteria, **SRB:** Sulfate reducing bacteria, **WGB:** Winogradsky broth, **PGB:** Postgate broth, **SLE:** Scent leaf extract, **ALE:** Aloe Vera extract.

Microbiological Analyses

Iron Oxidizing Bacteria (IOB) were enumerated from the pond water on Winogradsky agar using the pour plate method. Dry plates were incubated aerobically at 30 ^{o}C for 7 days [20]. Sulphate Reducing Bacteria (SRB) were enumerated from the pond water on Postgate agar medium.

Dry Petri plates were incubated in an Anaerobic jar containing gas pack to produce anaerobic condition at 30 ^{o}C for 14 days. Isolated bacterial colonies were counted, and the titers expressed in colony forming unit per milliliter (cfu/ml) of the original sample. The procedure previously described by Anichi and Abu [21] was adopted and discrete colonies were characterized and identified based on colonial morphology, Gram stain; motility test and spore stain reaction.

Physicochemical Analyses

These included measurement of pH (Hanna pH meter model Hl 8314, USA) which was on the water sample from where the test organisms were isolated and that of conductivity (HAC conductivity meter, model 2845500, USA) of the source water and the experimental setups [22]. The iron II ion content of the experiment was also analyzed using an atomic absorption spectrometer (GBC Avanta PM AAS model A6600, Australia).

Biocorrosion Inhibition Assay

A suspension of Iron Oxidizing Bacteria (IOB) was prepared by dispensing a loopful of discrete colonies of iron bacterial isolates in freshly prepared Winogradsky broth. The broth contained all the components of Winogradsky medium except agar agar. Eight pre-weighed coupons of the stainless steel labeled A1-A8 were placed in pairs, inside test bottles containing sterile Winogradsky broth. The set ups were incubated aerobically at 30 ^{o}C.

Thereafter, 2 coupons were withdrawn with sterile forceps after 7 days; washed, dried and reweighed. This procedure was repeated after 14, 21and 28 days respectively. Similar procedure was repeated with Postgate broth inoculated with a loopful of discrete black colonies of Sulphate Reducing Bacteria (SRB). Coupons for SRB were labeled S1-S8 and in duplicate. Test bottles containing SRB suspension and stainless-steel coupons were incubated in an anaerobic jar containing gas pack at 30 ^{o}C.

The controls were comprised of un-inoculated sterile Winogradsky and Postgate broth media for IOB and SRB respectively. In the treatment setups, SLE and ALE were added separately to each suspension. Changes in weight of the stainless-steel coupons were recorded at an interval of 7 days for a period of 28 days.

Rate of Biocorrosion Inhibition

The Corrosion Rate (C.R) in Mils Per Year (mpy) was calculated using the formula of Davis [23] as expressed thus:

$$\text{Corrosion Rate (C. R)} = \frac{\text{K} \times \text{W}}{\text{A} \times \text{T} \times \text{D}}$$

Where, K = constant equal to 3.45 x 10^6, W = weight loss in g or mg, A = area of specimen in cm^2, T = time of exposure in hour (h) and D = density of stainless-steel coupons measured in g/cm^3.

Minimum Inhibitory Concentration

For Minimum Inhibitory Concentration (MIC) test, different concentrations of extracts were prepared using the Kirby-Bauer Disc method described by Prescott *et al.* [24]. In this method, 1.0 g of each extract (ethanolic) was separately dissolved in 5 ml each of Dimethyl Sulfoxide (DMSO) and sterile water to get a concentration of 200 mg/ml and this was labeled as solution 1. Standard dilution susceptibility test technique was employed [25,26]. A previously prepared broth containing various concentrations of the extracts were inoculated with the standard inoculums of each test isolate followed by incubation at 37 ^{0}C for 24-48 h.

Thereafter, the tubes were observed for the presence or absence of growth which was determined by the appearance of turbidity in the test tubes. The degree of sensitivity of the bacterial isolates to the different concentrations of plant extracts in suspension containing the stainless-steel coupons were reported as + and ++, indicating the less (+) to high (++) sensitive microorganisms. Two milliliters (2 ml) of solution 1 was diluted 2-fold. A total of 8 times corresponding to 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.75 mg/ml, 3.13 mg/ml, 1.56 mg/ml and 0.78 mg/ml each for both the aqueous and ethanolic extracts was performed.

Statistical Analysis

The data generated were subjected to statistical analysis using the two-way analysis of variance (ANOVA) to determine levels of significance (p=0.05) of data at 95 % confidence interval.

Results

The efficacy of locally sourced plant-based biocorrosion inhibitors obtained from Aloe Vera and scent leaf extracts were evaluated using stainless steel as the target surface/substratum. The results obtained during the 28-day study period using duplicate samples are shown in Figs. 1-10 and Tables 2-4 respectively. The stainless-steel coupons used in this study had a mass of 1.050 g and density of 7.02 g/cm^3. The Aloe Vera plant extracts yielded 0.014 g, corresponding to 1.4 % yield while the scent leaf extracts yielded 0.039 g which represented 3.9 % yield.

The average IOB and SRB counts from pond water in African Regional Aquaculture Centre (ARAC), Aluu, were too few to count in all the samples examined at the beginning on day zero. This result conforms to Bloomfield *et al.* [20]. The water samples gave average IOB counts of 1.54x10^4 and 1.46x10^5 cfu/ml respectively for samples A1 and A2 on day 7 of the study. Samples A3 and A4 had average IOB counts of 3.19x10^4 and 1.33x10^5 cfu/ml respectively after day 14.

On day 21, IOB counts were 1.63x10^4 and 1.19x10^5 cfu/ml respectively for A5 and A6. The IOB counts on day 28 decreased to 2.99x10^4 cfu/ml for A7 and 1.42x10^5 cfu/g for A8 (Figure 1). Fig. 2 indicated the results obtained for SRB with counts of 1.47x10^4 and 1.43x10^5 cfu/ml respectively for S1 and S2 during the day 7 study. The counts deceased drastically to 1.33x10^4 and 1.16x10^{5}cfu/ml on day 14 for SRB in S3 and S4 samples respectively. Thereafter, on day 21, the bacterial population had significantly (p>0.05) reduced to 1.28x10^4 and 1.16x10^5 cfu/ml for S5 and S6 respectively. Counts of 1.29x10^4 and 1.38x10^5 cfu/ml were obtained for S7 and S8 respectively.

Tables 2 and 3 show the identification and characterization of Iron Oxidizing Bacteria (IOB) and Sulfate Reducing Bacteria (SRB) isolates from the pond water sample. The colonial morphology of IOB isolates revealed various convex, concave, smooth and serrated edge colonies with a rusty brown coloration. The non-motile rods with a sheath were identified as *Leptothrix* species while the motile straight rods were identified as *Sphaerotilus* species. Furthermore, the motile bean-shaped rods bacteria isolated were identified as *Gallionella* species (Table 2).

The SRB isolates obtained were mostly circular, convex and black in color. The bacterial isolates showed motility in broth media like those previously obtained by Solomon *et al.* (2018c, d). They were spiral to viroid shaped, Gram negative, non-spore forming and motile cells. These were identified as *Desulfovibrio* species [2,27,28].

There were also singly curved rods, Gram positive, spore forming and motile cells which were identified as *Desulfotomaculum* species. Others were singly and spiral-shaped, Gram negative, none spore forming and motile cells and were identified as *Desulfovibrio* species (Table 3).

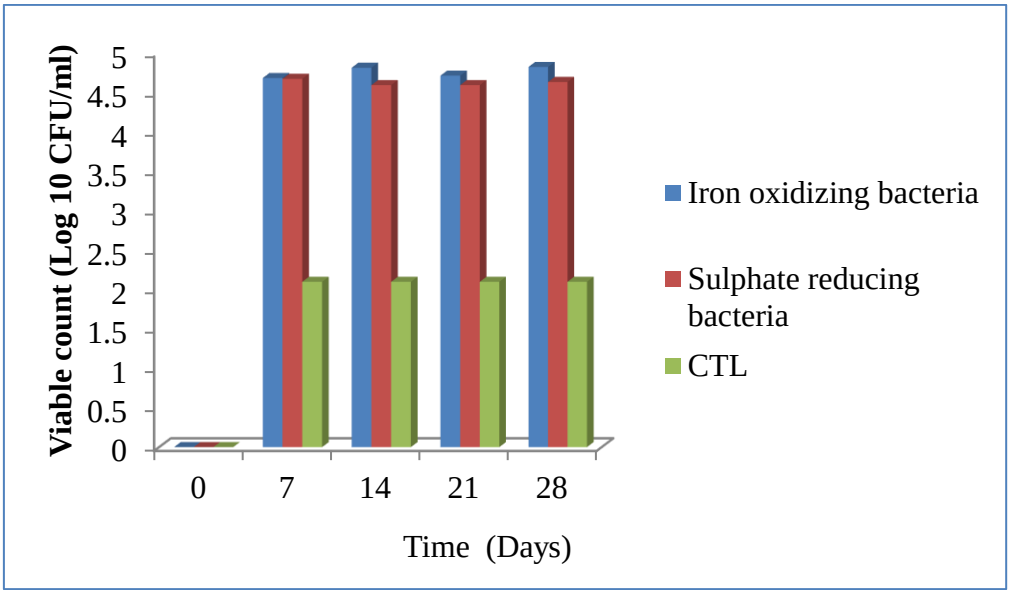

Figure 1: Changes in Iron oxidizing and sulfate reducing bacterial counts in suspension containing scent leaf extract.

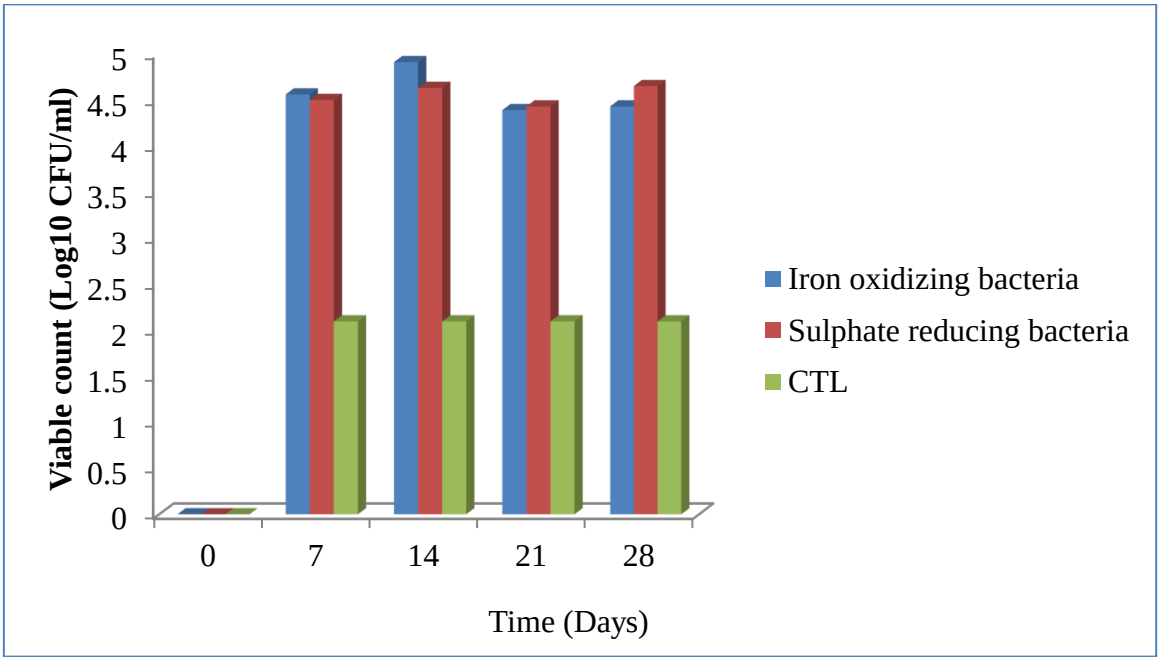

Figure 2: Changes in Iron oxidizing and sulfate reducing bacterial counts in suspension containing Aloe Vera extract.

Colonial Morphology	Gram Reaction	Spore Reaction	Motility Test	Probable Identity
Convex, concave, smooth and serrated edged colonies with a rusty brown color	G-ve, short rods with a sheath	_	_	*Leptothrix*

Convex, concave, smooth and serrated edged colonies with a rusty brown color	G-ve, bean-shaped rods	–	+	*Gallionella*
Convex, concave, smooth and serrated edged colonies with a rusty brown color	G-ve, straight rods	–	+	*Sphaerotilus*

Key: +/-: Presence/absence of spores and or motility, **-ve:** Gram negative

Table 2: Identification and Characterization of Iron Oxidizing Bacterial Isolates.

Code	Colonial morphology	Gram Stain	Spore	Motility	Probable identity
SI	Spiral to viroid shaped	G-ve, short rods with a sheath	-	+	*Desulfovibrio*
S2	Singly curved rods	G+ve, rods	+	+	*Desulfotomaculum*
S3	Spiral to viroid shaped	G-ve, short rods with a sheath	-	+	*Desulfovibrio sp*.
S4	Singly spiral shaped	G-ve, rods	-	-	*Desulfovibrio*
S5	Singly curved rods	G+ve, rods	+	+	*Desulfotomaculum*
S6	Singly spiral shaped	G-ve, rods	-	-	*Desulfovibrio*
S7	Spiral to viroid shaped	G-ve, short rods with a sheath	-	+	*Desulfovibrio*
S8	Singly curved rods	G+ve	+	+	*Desulfotomaculum*

Key: +/-: Presence/absence of spores and or motility, **-ve:** Gram negative

Table 3: Identification and Characterization of Sulfate reducing Bacterial Isolates.

Identifications of SRB were based on the schemes of David and John [29]. The coupons in the iron bacterial culture appeared rusty in color and rough on touch. The surface was slimy on touch before vigorous washing, suggesting biofilm development. This corroborated with David [23]. Coupons in SRB became black which could be due to the deposition of iron II sulphide compounds [30]. The black coloration disappeared after exposure to air and could be due to the oxidation of sulphide to sulphate.

Results obtained for Minimum Inhibitory Concentration (MIC) of extracts obtained from Aloe Vera and Scent leaf are presented in Table 4. Out of the three iron oxidizing bacteria (*Leptothrix*, *Gallionella* and *Spaerotilus*) isolates, two (*Leptothrix* and *Gallionella*) were less sensitive to scent leaf extract while the remaining one (*Spaerotilus*) was highly sensitive to the scent leaf extract. Two of the SRB (*Desulfovibrio* and *Desulfotomaculum*) isolates were less sensitive while *Desulfotomaculum* was highly sensitive to the scent leaf extract and *Desulfovibrio* gave less sensitivity to the extract. Aloe Vera leaf extract indicated that four genera (*Gallionella*, *Spaerotilus*, *Desulfovibrio* and *Desulfotomaculum*) were sensitive to the extract while the remaining two genera (*Leptothrix* and *Spaerotilus*) were less sensitive to the plant extracts investigated.

The less sensitive bacteria in the presence of scent leaf were 20% while the percentage of bacteria that indicated sensitivity was 80%. This indicates that it has high inhibitory effect on the bacteria genera. In the presence of Aloe Vera leaf extract, 60% of the bacteria genera were resistant while 40% of were sensitive to the extract, indicating low inhibition of bio-corrosion of the stainless-steel coupons used in conducting this research (Table 4).

Bacterial Isolates	Scent leaf extract	Aloe Vera extract
Leptothrix	++	++
Gallionella	++	+
Spaerotilus	++	++
Desulfovibrio	+	+
Desulfotomaculum	++	+
Highly sensitive of all isolates	80%	40%
Less sensitive of all isolates	20%	60%

Key: ++: highly sensitive, **+:** less sensitive

Table 4: Activity of the plant Extracts Against the Bacterial Isolates.

Figure 3 and 4 show the changes in weight of stainless-steel coupons suspended in Aloe Vera and scent leaf extracts in the presence of SRB and IOB respectively after the 28-day study period using duplicate samples. The mean rate of bio-corrosion of stainless-steel coupons in suspension of IOB and scent leaf extract during the study period are as presented in Figs. 3 and 4. After day 7 and 14 of the study, stainless steel coupons suspended in IOB and ALE had mean corrosion rate of 332.03 mpy and 216.11 mpy respectively. Bio-corrosion inhibition rate further dropped to 39.49 mpy and 83.59 mpy on day 21 and 28 respectively. This is shows that the stainless-steel coupons immersed in SRB broth was bio-corroded by74.8% after 28-day study. More so, stainless steel coupons suspended in IOB and SLE had mean biocorrosion inhibition rate of 849.07 mpy and 67.47 mpy on days 7 and 14 respectively.

Thereafter, on day 21, a decrease in mean bio-corrosion inhibition rate up to 165.04 mpy was recorded and was observed to further dropped to 7.32 mpy after day 28. Comparatively, stainless steel coupons in suspension of iron oxidizing bacteria and scent leaf extract media bio-corroded significantly ($p<0.05$) at 99.1 % as against iron oxidizing bacteria and Aloe Vera leaf extracts which had 74.8 % mean biocorrosion inhibition effectiveness.

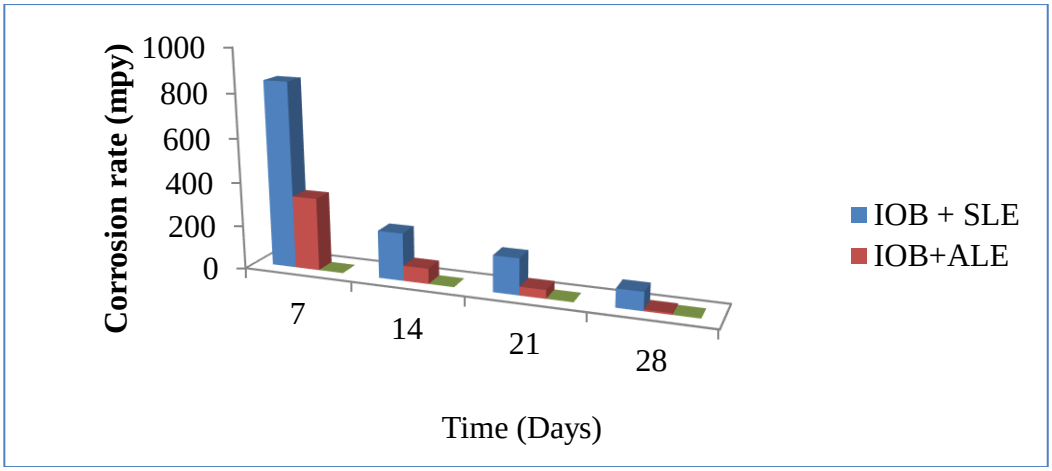

Figure 3: Mean corrosion rate of stainless-steel coupons in suspension of IOB + SLE and IOB + ALE during the study period.

Stainless-steel suspended in SRB and SLE showed no significant ($p>0.05$) bio-corrosion inhibition at the onset of the study, during day 0 but after day 7, the SRB and ALE suspension had mean bio-corrosion inhibition rate of 615.05 mpy and by the 14th day, mean bio-corrosion rate had decreased significantly ($p<0.05$) to 281.19 mpy (Fig. 4). There was a further decrease in the rate of bio-corrosion inhibition of the stainless-steel coupons to 257.67 mpy after day 21 before it finally reduced to 223.60 mpy by the 28th day. The stainless-steel coupons suspended in SRB and SLE had mean bio-corrosion rate of 389.79 mpy on day 7 and after day 14, it however, reduced significantly ($p<0.05$) to 66.92 mpy. By day 21, mean bio-corrosion rate had decreased to 230.13 mpy before it dropped to 139.68 mpy after day 28. Results obtained indicated that the mean rate of bio-corrosion inhibition of stainless-steel coupons slightly decreased after day 28 in suspension containing IOB and SLE. This indicated that IOB was more effective in inducing the bio-corrosion of stainless-steel coupons than were SRB (Figure 4). The conductivity of the source water sample was 1.0130 s/m.

The levels of measured parameters in the sourced water are consistent with the conditions in an environment that favors microbial stabilization and activity [24,31]. The conductivity of the various setups with the iron bacteria increased significantly ($p<0.05$) from day 7 of the study to 1.0140 s/m in fresh medium and thereafter, decreased to 0.70 s/m after day 14 of incubation. By day 21, there was a drastic increase in conductivity of the medium to 3.30 s/m and by the 28th day, the conductivity had risen to 3.90 s/m. The conductivity of the set up with the SRB dropped from 19300 s/m in the fresh medium to 18508 s/m on day 7 and then rose steadily to 19590 s/m after day 28 of the study as presented in Figure 5 and 6.

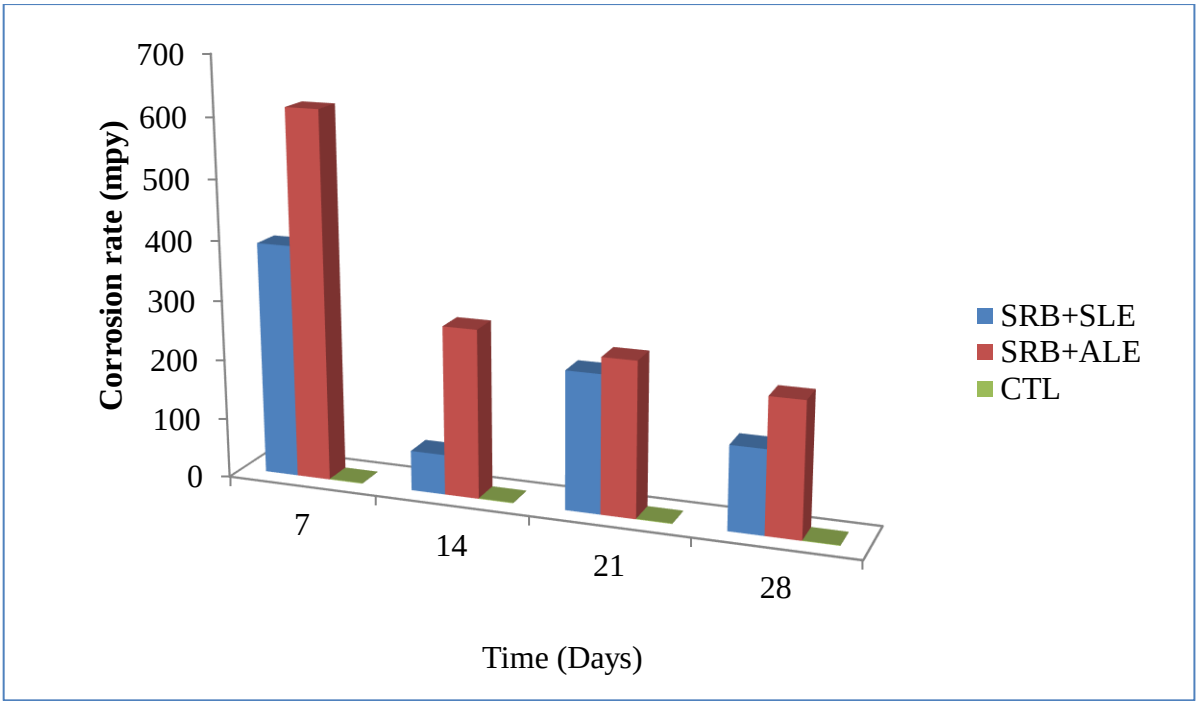

Figure 4: Mean corrosion rate of stainless-steel coupons in suspension of SRB+ SLE and SRB+ALE during the study period.

Figure 3 and 4 show that stainless steel coupons immersed in SLE suspension corroded microbiologically at the rate of 272.7 mpy in the presence of IOB while the same substratum immersed in ALE suspension induced bio-corrosion significantly (p<0.05) at the of 167.8 mpy in the presence of IOB. Biocorrosion induction was 99.1 % in IOB and SLE suspension while 74.8 % biocorrosion inhibition was recorded for IOB suspended in ALE medium. Comparatively, stainless steel coupons immersed in SLE suspension corroded microbiologically at the of 206.6 mpy after 28 day in the presence of SRB and 344.4 mpy in the presence of ALE and SRB during the 28-day study period. Furthermore, 64.2 % biocorrosion inhibition was recorded in SRB and SLE suspension while 63.7 % biocorrosion induction was attained in suspension containing SRB and ALE respectively.

The changes in iron II ion concentration involving IOB in coupons suspended with ALE and SLE are as shown in Fig.7. Results obtained on day 7 indicated that ALE had iron II ion content of 488.25 mg/l while SLE recorded 510.41 mg/kg. These decreased after day 14 to 409.40 mg/l for ALE and 438.36 mg/l for SLE. By day 21 of the study, values of 310.44 and 373.23 mg/l were obtained for ALE and SLE respectively. Iron II ion content in the ALE and SLE setups after day 28 of the study decreased to 211.57 mg/l for Aloe Vera and 261.08 mg/l for sent leaf in the iron oxidizing bacteria system. The changes in iron II ion concentration involving SRB in coupons suspended with ALE and SLE are as shown in Fig. 8. Iron II ion concentrations in ALE and SLE suspension on day 7 were 511.10 and 565.36 mg/l respectively. These further decreased to 456.89 and 498.18 mg/l for ALE and SLE respectively after day 14. Furthermore, after day 21, Iron II ion content in ALE and SLE medium decreased significantly (p<0.05) to 361.83 and 402.69 mg/l and values of 291.28 mg/l and 302.36 mg/l was obtained in medium containing ALE and SLE respectively. Figure 4.6 shows the changes in pH in the presence of stainless-steel material coupons in suspension containing IOB.

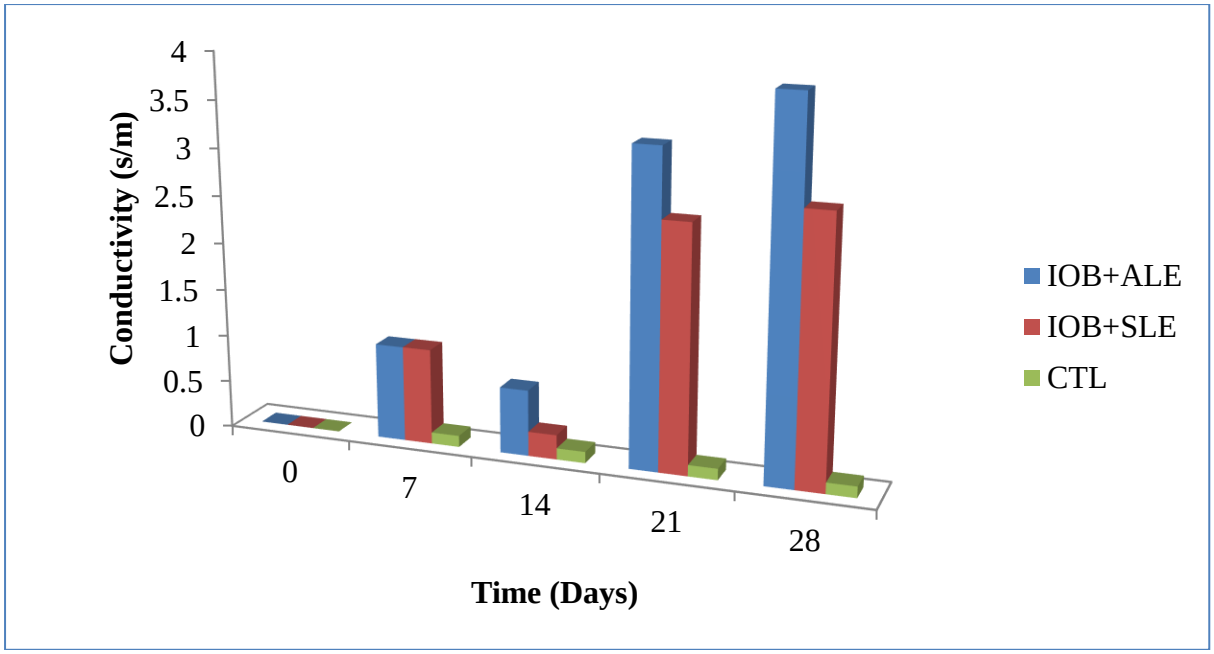

Figure 5: Changes In conductivity in the presence of stainless-steel suspension contain IOB+ALE and IOB+SLE during the study.

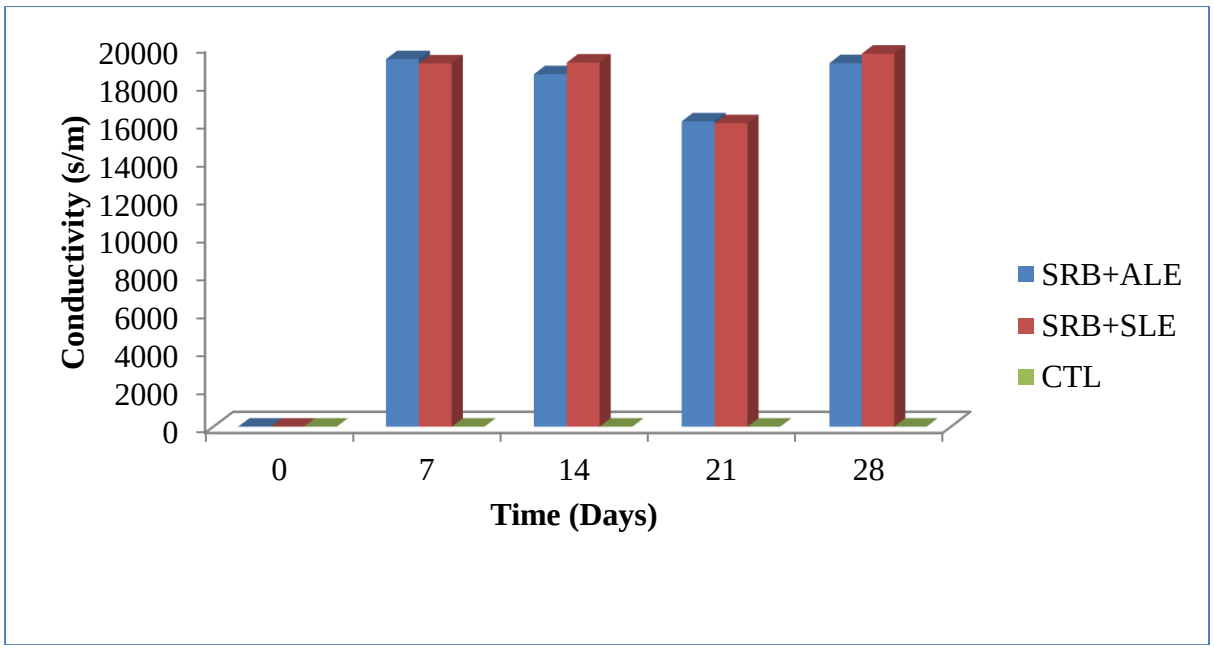

Figure 6: Changes in conductivity of stainless-steel coupons suspended in SRB + ALE and SRB+SLE during the study period.

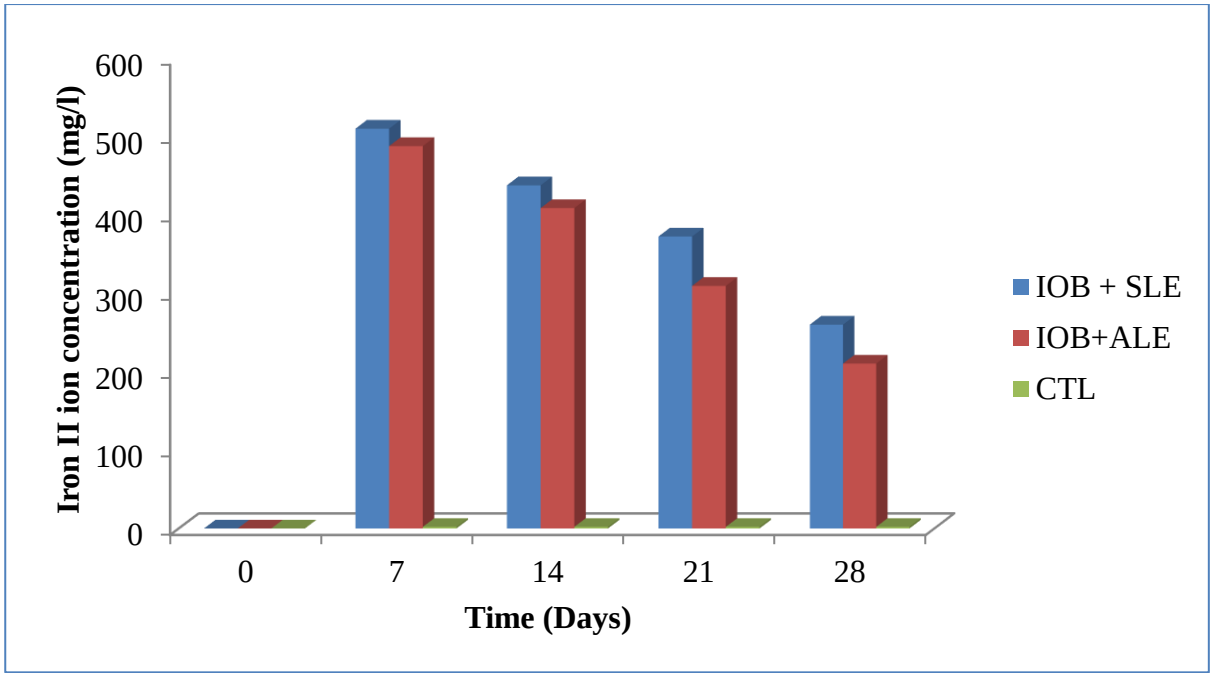

Figure 7: Changes I Iron II ion concentration stainless steel coupons suspended in IOB+ALE and IOB+SLE during the study period.

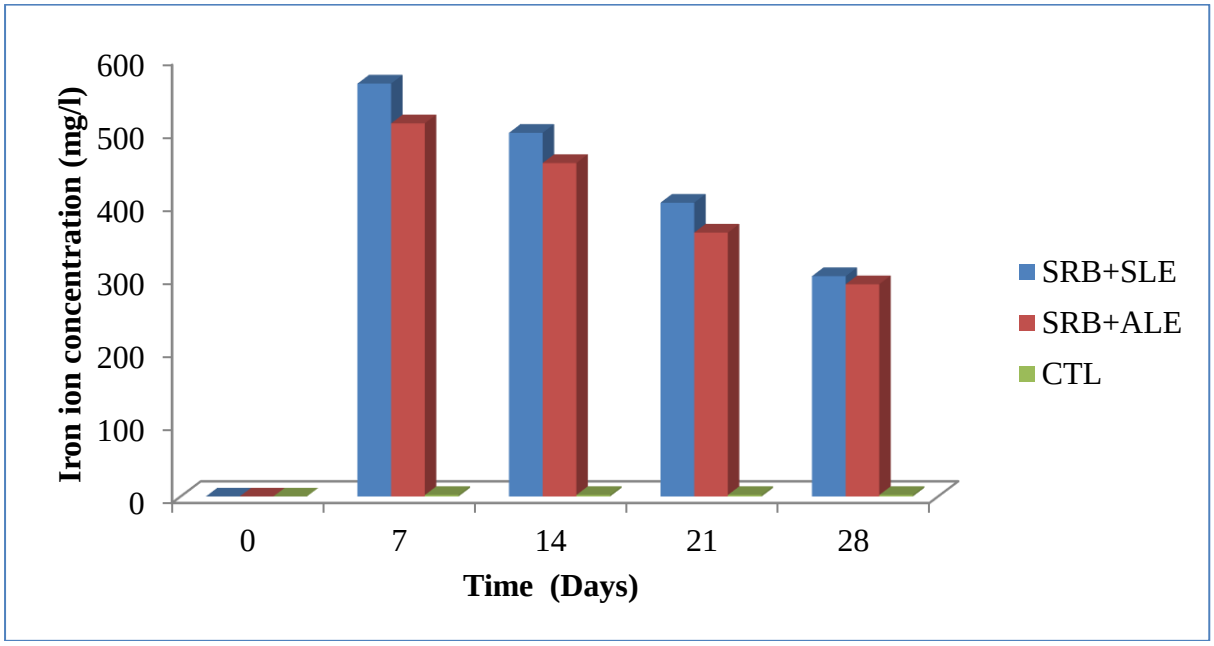

Figure 8: Changes in Iron II ion concentration of stainless-steel coupons suspended in SRB+ALE and SRB+ SLE during the study period.

Fig. 9 and 10 shows the changes in pH in the presence of stainless-steel material coupons in suspension of IOB and SRB respectively. The pH of the original water sample was 7.61 while that of the setup with the IOB changed from 7.61 in the fresh medium containing ALE to 7.87 after 28 days and 7.72 in SLE medium on day 7 to 7.96 after day 28 (Figure 9). The pH of the experimental setup with SRB changed significantly (p<0.05) from 7.87 in the fresh water medium to 8.18 after day 28 of the study for ALE suspension and 7.75 was obtained on day 7 increased to 8.13 after day 28 of the study for suspension containing SLE (Figure 10).

The set up with the Iron oxidizing bacteria showed a significant ($p<0.05$) change in pH from 7.61 to 7.87 after 28 days of incubation with Aloe Vera extract and 7.72 to 7.96 with sent leaf extract, respectively. The set up with the sulphate reducing bacteria had pH changed from 7.87 to 8.13 after 28 days of incubation with Aloe Vera extract and 7.75 to 8.13 with sent leaf extract suspension. Acidic environment with pH < 6 or alkaline environment with pH > 8 is more corrosive than an environment with pH in the range of 6-8 [20]. The role of microorganisms in the deterioration of concrete and other surfaces has been recognized [30, 32,33]. Among the genera that cause microbial corrosion, iron oxidizing bacteria as well as the sulphate reducing bacteria are of great importance due to their wide distribution in the environment, specific metabolic activities and the prevailing anoxic conditions where pipes are laid [28,34].

Apart from producing Hydrogen Sulphide (H_2S) that stimulates the absorption of hydrogen into the structural metals leading to embrittlement, SRB can induce corrosion by formation of biofilms on surfaces of metals and concretes. They form the biofilms deriving energy through their fermentative activities. The bacteria seem to be able to make the stainless steel alkaline. This is usually the condition of freshly cured cement [21]. The acidic nature of the set up with the SRB may be due to the production of acidic hydrogen sulphide [25]. The increase in conductivity of the set ups with the SRB could be attributed to hydrogen sulphide (H_2S, a weak acid) formation. Microbially induced metal corrosion can also be brought about by many environmental microorganisms including *Serratia marcescens* and *Pseudomonas* species [35] which have been implicated in bioremediation of crude oil in aged oil-impacted sites [36,37].

However, the major, or the most notorious and most insidious cause of microbiologically induced metal corrosion is the obligate anaerobic bacteria, especially the SRB. Results of Minimum Inhibitory Concentration (MIC) indicated that both extracts were inhibitory to the isolates although ALE had more inhibitory effect on biocorrosion inducers of metallic surfaces than its SLE counterpart. The juice extracts may have been adsorbed onto the steel surface through their polar atoms, hence forming protective films.

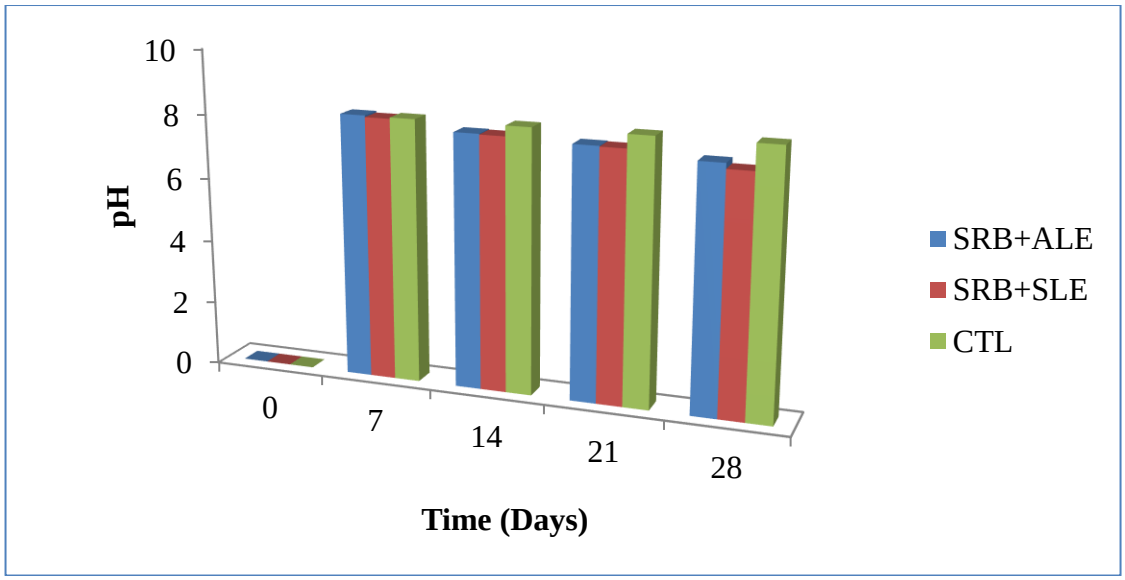

Figure 9: Changes in pH in the presence of stainless coupons suspended in SRB+ALE and SRB+SLE during the study period.

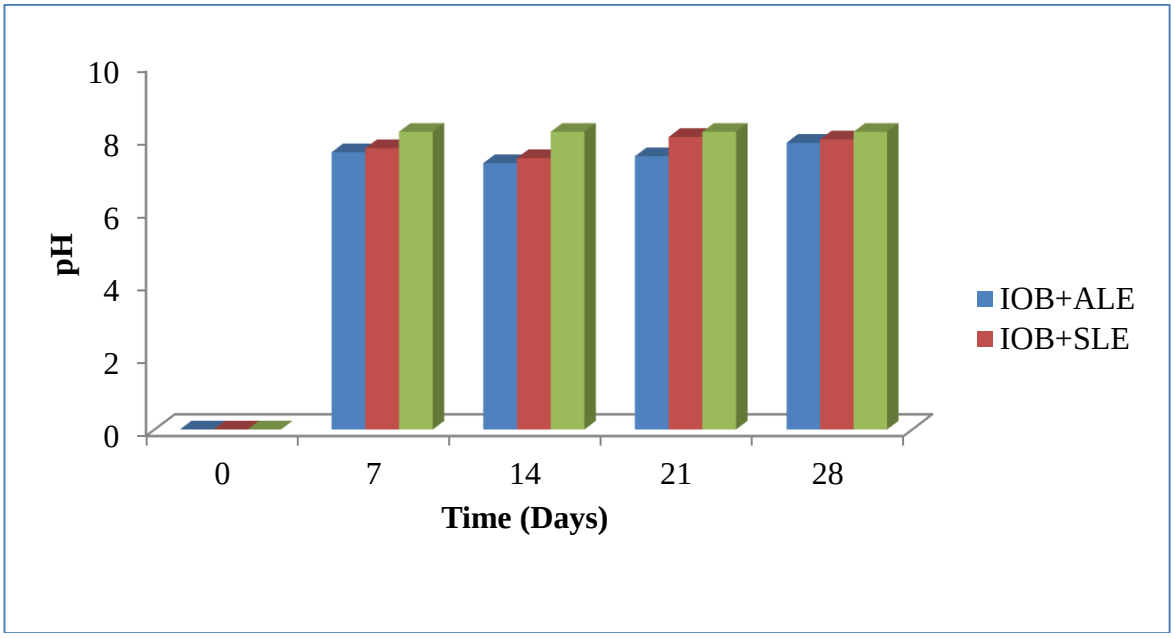

Figure 10: Changes in pH in the presence of stainless-steel coupons suspended in IOB+ALE and IOB+SLE during the study period.

Discussion

Oil pipelines are protected by several means of coating, but the efficiency of the coating will depend on the resistance of the coating material to microbial attack. Stainless steel coating material offers good protection to pipes and adds weight to reduce buoyancy of the pipes in aquatic environments, but its lifespan depends on its resistance to microbial biocorrosion. The conductivity of the set ups with Iron oxidizing bacteria decreased. This may be due to the presence of nonionic species such as oxides. The decrease in the amount of ferrous ion in both set ups suggests transformation of ferrous ion to other forms of iron that were not identifiable by the spectrometric analysis or deposition of the iron II ion content as insoluble oxides.

The sulphate reducing bacteria with its lower pH and lower conductivity shows more tendencies to corrode the concrete material. A direct proportionality has been shown between concrete conductivity and corrosion rate [31,38]. There was a general decrease in the amount of ferrous ion (Fe^{2+}) in the set ups with both the iron oxidizing bacteria and sulphate reducing bacteria during the study period. Identifications of sulphate reducing bacteria were based on the schemes of David and John [29]. Changes in acidity could also be from metabolism of organic molecules in the media used (Winogradsky and Postgate). Acidic pH has also been reported from Humic Acid (HA) formed from plant leaves [39]. In addition, the acidity of the water bodies may have resulted from substances such as oxides of sulfur, nitrogen and carbon that have entered the atmosphere through gas flaring (gas flaring is a common feature in the study area) which are converted to Sulphur acid, nitric acid and carbonic acid, after rainfall as earlier reported by Rim-Rukeh *et al.* [40].

The pH values of the water samples are within the range 4-9 identified by Costerton *et al.* [1] to be suitable for bacteria growth. The water samples gave an average count of 10^4 cfu/ml and $1.42x10^5$ cfu/ml after day 28 day for both IOB and sulphate reducing bacteria. It has been

previously reported [41] that total bacterial population varied from 10^5cfu/ml to 10^6cfu/ml in all water samples analyzed, indicating adequate bacterial population for microbiologically influenced corrosion activity [16]. It has been suggested that sulphate reducing bacterial count of 10^4 cells/ml is a clear indication of possible corrosion problem, while a relative population of 10^6 cells/ml of microorganisms is a concern of potential corrosion problem in an environment [42] and is in tandem with this work The result of the microbiological analysis showed that sulphate reducing bacteria can be isolated from commonly occurring stagnant aquatic environments such as ponds. The low counts of the sulphate reducing bacteria may be due to their strict anaerobic demands. Iron oxidizing bacterial counts was relatively high. This may be attributable to the fact that the environmental conditions for their growth were easier to simulate in the laboratory.

There were changes in the physical appearance of the materials such as color and texture. The result of the biocorrosion testing revealed that there was colonization of the coupons by the organisms. The defacement and slimy nature of the surfaces of the coupons could be attributable to biofilm formation and deposition of bacterial cells or their metabolites. This finding is in tandem with that of Wen and Gu [43].

Several workers have investigated the biodeterioration/biocorrosion of concrete pipeline coating material by SRB and IOB in the laboratory [30,41,44]. Aloe Vera and Scent leaf extracts were very effective in inhibiting the acid corrosion of steel in acid solutions, with high inhibition efficiency at optimum concentration and the inhibitory action of the extract is ascribed to the presence of phytochemicals in the plant [45,46].

Both locally sourced biocorrosion inhibitors used in this study had indicated that scent leaf extract significantly ($p<0.05$) induced biocorrosion of stainless steel coupons in the presence of Iron oxidizing bacteria (99.1%) than its Aloe Vera extract counterpart which had 74.8% biocorrosion inhibition efficiency. On the same vein, in the presence of sulfate reducing bacteria, scent leaf extract was found to actively inhibit biocorrion (64.2%) than Aloe Vera leaf extract (63.7%). In the presence of Aloe Vera leaf extract, 20% of the isolates were resistant while 80 % were sensitive, respectively. This, again, demonstrated that Scent Leaf Extract (SLE) is an effective and a potent biocorrosion inhibitor of stainless-steel coupons than its Aloe Vera Leaf Extract (ALE) counterpart.

Conclusion

Both plant extracts investigated in this study were effective in inhibiting microbial corrosion of stainless-steel coupons. Although, scent leaf extract showed more inhibitory potential on biocorrosion inducers (bacteria) of stainless-steel materials used than Aloe Vera leaf extract. The high bacteria counts obtained for iron oxidizing bacteria as against its sulphate reducing bacteria counterpart is an indication that iron oxidizing bacteria are more involved in biocorrosion of metallic material than their sulfate reducing bacteria counterpart.

Recommendations

To prevent or reduce the rate of biocorrosion and improve the lifespan of industrial materials, expertise of microbiologists should be part of the process of the formulation and testing of the stainless-steel materials. Use of biocides, possibly green biocides, which should not impact negatively on the environment, should be incorporated in the formulation of the stainless

steel. There should be routine checks on the laid pipelines to dislodge the IOB and SRB-forming biofilms on the existing pipes.

Plants are readily available, and their extracts could help in the reduction of microbial induced corrosion of metals and concrete materials. Biocorrosion monitoring and mitigation using locally sourced plant-based materials may assist in reducing the spate of pipeline ruptures along the thousands of kilometers of pipelines Right of Ways (RoWs) in the Nigerian Petroleum Industry. This will further lead to environmental sustainability and ecosystem balance. Further research is recommended in the area of biocide treatment of oil production systems.

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