The International Journal Of Engineering And Science (IJES)
||Volume||2 ||Issue|| 5 ||Pages|| 39-46||2013||
ISSN(e): 2319 – 1813 ISSN(p): 2319 – 1805

Correlation between Microbial Populations Isolated From Biofilms of Oil Pipelines and Corrosion Rates

Godwin Umoren Akpan[1], Godwin Abah[2] and Boniface D. Akpan[2]
[1]*Department of Soil Science and Land Resources Management, University of Uyo, P.M.B. 1017, Uyo, Nigeria*
[2]*Department of Animal Science, University of Uyo, P.M.B. 1017, Uyo, Nigeria*

----------ABSTRACT----------
This study was designed to assess the correlation between microbial populations and corrosion rates in oil pipelines in Rivers and Delta States of Niger Delta, region, Nigeria. The coupons were inserted into the inner regions of pipelines at the pressure of 6000 pound per square inch (P.S.I) and allowed for normal petroleum flow for a period of 127 days. The corrosion analysis by weight loss method showed higher corrosion rates of pipelines in Delta State than Rivers State but were not significantly ($P < 0.05$) different from each other. The microbiological analysis also showed higher microbial population in biofilms from oil pipelines in Delta State than those detected from biofilms from oil pipeline in River State. The results further revealed that microbial species detected from biofilms of oil pipelines in Delta State constitute mostly Gram positive bacteria (Bacillus cereus, Bacillus subtilis, B. pumillus), acid producing bacteria (Klebsiella oxytoca, Pseudomonas aeruginosa) and acid producing fungi (Aureobasidium pullulan, Hormocous resimea and Aspergillus spp) than those from Rivers State. The correlation between microbial populations and corrosion rates showed positive corrosion rates. The results further showed that microorganisms were directly responsible for about 98% corrosion of oil pipelines in the area.

KEYWORDS: *Correlation, microbial populations, brofilms, corrosion rates, biocorrosion*

Date of Submission: 22 June 2013, Date of Publication: 10.July 2013

I. INTRODUCTION

Microbiologically influenced corrosion (MIC) is a well-recognized problem in the oil and gas industries. Microorganisms such as bacteria, algae and fungi can thrive under certain conditions, and accelerate the corrosion [1]. Biological organisms can enhance the corrosion process by their physical presence metabolic activities, and direct involvement in the corrosion reaction [1]. The occurrence of microbiologically influenced corrosion is often characterized by unexpected severe metal attack, the presence of excessive deposits and, in many cases, the rotten-egg odour of hydrogen sulphide (H_2S) [2]. It is established that the aggressive MIC takes place in the presence of microbial conditions in which many physiological types of bacteria including, sulphate oxidizing bacteria [3], Methanogens [4], nitrate reducing bacteria [5], Enterobacteria [6]. Thiosulphate and sulphate reducing bacteria (SRB) have long been reported as the main inducer of biocorrosion in metallic surfaces, especially under anoxic conditions [7], interact in complex ways within the structure of biofilms. Microbiologically induced corrosion does not produce a unique form of localized corrosion instead, it can result in pitting, crevice corrosion, under-deposit corrosion, and selective dealloying in addition to enhance galvanic and erosion corrosion [1]. Bacteria can initiate the required conditions for pitting or crevice corrosion and once localized corrosion has initiated, microbial reaction can maintain the conditions necessary (such as low oxygen) for continued pit/crevice growth.The rate at which pits propagate can be governed by organic acid produce by fungi in aerobic environments or by certain bacteria in anaerobic environments (As the pit grows iron-dissolves according to the anodic reaction,

$$Fe \longrightarrow Fe^{2+} + 2e \quad (1)$$

The cathodic reaction is reduction of dissolved oxygen outside the pit to form OH^- according to [8].

$$O_2 + 2H_2O + 4e^- \longrightarrow OH \quad (2)$$

The insoluble ferrous hydroxide corrosion product form by the reaction,

$$3Fe^{2+} + 6OH^- \longrightarrow 3Fe(OH)_2 \quad (3)$$

Bacterial degradation and corrosion of naphtha in transporting pipelines was studied by [9] using weight loss method. The result showed that degraded organic compounds in naphtha encourages the growth of

bacteria and enhances the formation of corrosion products like ferric oxides and manganese oxide. [10] opined that microorganisms (bacteria) are responsible for deterioration of construction materials and steel structures.

II. MATERIALS AND METHOD

2.1.Determination of Corrosion Rates

Mild steel coupon with dimension 7cm x 2cm x 0.4cm for each. The exposed surface area of each coupon was 32.2cm^2, which was calculated as:

2(LW + LH + HW) (4)

Where

L = 7cm (length)

W = 2cm (width)

H = 0.4cm (height or thickness)

The density of each coupon: 7.57g/cm^3

Twenty (20) coupons of known weight were used on a whole in the experiment, 10 coupons were used per experimental site and 2 per pipeline. To obtain the biofilms samples, the mild steel coupons (Plate 1) having the same chemical composition as the involved pipelines were inserted at inner surfaces of the pipelines through the access values (Plate 2) and exposed to he flow of petroleum for a period of 127 days. The coupons were detached from the inner region of the oil pipelines at the end of the 127 days and the biofilms formed on the surfaces of each coupon were removed with sterile razor blade and collected into sterile bottles with 10 ml phosphate buffered saline pH 7.0 [11]. The biofilm samples were named after the identity of each coupon, corresponding to each pipeline (Tables 1 and 2). After the removal of the biofilms, the coupons were washed in acetone, dried and the final weight taken using ±0.0001 accuracy electric balance and the differences in weight (weight loss) used to determine corrosion rates using the formular:

Corrosion rate (mpy): $\frac{Area\ factor\ x\ (Wt\ loss)mg}{days\ exposed}$ (5)

Area factor:

Computed from the exposed surface area, and density [12].

III. MICROBIOLOGICAL ANALYSIS

3.1.Bacteria: The medium of choice for the cultivation of total heterotrophic bacterial counts (THBC) was nutrient agar with the following composition: Nutrient agar (Oxoid) containing 1.0g lemco powder, 2.0g yeast extract, 5.0g peptone, 5.0g NaCl, 15.0g agar, 1000 ml distill water. Postgate B medium: for the cultivation of sulphate reducing bacteria. The composition of the medium: KH_2PO_4 0.5g/ml, NH_4Cl 1.0g, Na_2SO_4 1.0g, $Cacl_2$.$6H_2O$ 0.1g, MgSO4.7 H_2O 2.0g, sodium lactate (60-70%) 5ml, yeast extract 1.0g Ascorbic acid 0.1g, thioglycolic acid 0.1g, $FeSO_4.7H_2O$ 0.5g, NaCl 26g, Distilled water 1000ml, pH 7.0.

Starkey broth: Composed of 3.0 g KH_2PO_4, 0.2g $MgSO_4$, 0.2g $CaCl_2$. $2H_2O$, 0.5g $(NH_4)_2SO_4$, trace of $FeSO_4$ in 1000 ml distilled water for sulphur oxidizing bacteria [13]

Fungi: *Sabrourand Dextrose* Agar (SDA) (Biolab) for the cultivation of total Heterotrophic bacterial counts (THFC) containing peptone 10g, glucose 20g, Agar 15g, Distilled water 1000ml. Ten fold serial dilution of the biofilm samples were made as described by [14, 15].

3.2.Inoculation and Incubation

One millitre of appropriate ten-fold serial dilution of biofilm samples were inoculated unto Nutrient agar, Postgate B, Starkey and SDA plates in triplicates using spread plate technique. Inoculated plates were incubated at 37^{o}C for 24 hours for the enumeration of total heterotrophic bacterial counts. The same procedure was used for the cultivation of sulphate reducing bacteria on postgate B medium stored in anaerobic jar and incubated at 37^{o}C for seven days and on SDA medium plates for the cultivation total heterotrophic fungal counts. The same procedure was used for the cultivation of sulphate oxidizing bacteria. At the end of each incubation period 18-24 hours for heterotrophic bacteria, 7 days for sulphate reducing bacteria and 7 days for fungal counts respectively, visible discrete colonies in inoculated plates were counted and the result recorded as colony forming unit per militre (cfu/ml) of biofilm sample.

3.3.Maintenance of Pure culture

Discrete colonies (of bacteria, sulphate reducing, sulphate oxidizing, bacteria, and fungi) were purified by repeated subculture unto NA, Postgate B, Starkey and SDA media. Pure cultures were preserved on NA slant, Postgate B slant, Starkey medium SDA Slants, stored at 4^{o}C for further test.

3.4.Characterization and Identification of Isolates

All bacterial isolates were characteristic and identified using the taxonomic schemes of [16, 17]. Briefly, the tests included Gram reaction, spore stain, oxidase test and motility test; indole test voges proskurar, methyl red test and sugar fermentation profile. Fungal isolates were characterized using the identification scheme of [18, 19]. Briefly, the wet mount method was carried out using lactrophenol in cotton blue stain.

IV. RESULTS AND DISCUSSION

The results in Tables 1 and 2 shows the corrosion analysis at coupons retrieved from oil pipelines in Oshie flow station in Rivers state and Irri flow station in Delta State after 127 days of normal exposure to petroleum flow. The corrosion rates recorded per pipeline in Oshie flow station (Rivers State) are as follow: OSH 13= 2.57 mpy, OSH 04 = 2.86 mpy, OSH 13 = 2.36 mpy, OSH 17 = 1.47 mpy and EOC 04 = 1.07 mpy and the corrosion rate per pipeline in Irri flow station (Delta State) are as presented: Irri 02 = 6.81 mpy, Irri 06 = 4.50 mpy, Irri 07 = 2.72 mpy, Kwale 05 = 2.36 mpy and Kwale 06 = 2.49 mpy respectively. Comparatively, it is observed that the corrosion rates of pipelines in irri flow station (Delta State) are higher than pipelines in Oshie flow station (Rivers State), although not significantly ($P \geq 0.05$) different from each other, similarly, bacterial and fungal loads from Rivers and Delta States are presented in Figures 1 and 2. The result in Figure 1 shows bacterial and fungal populations in Rivers State pipelines. It is observed that there are more bacterial population than fungal populations in Rivers pipelines: OSH 01 pipeline, 26 x 10^4 cfu/L, bacteria and 19 x 10^3 cfu/L fungi, 30 x 10^4 cfu/L, bacteria has 29 x 10^4 cfu/L fungi, OSH 13 has 29 x 10^4 cfu/L bacteria and 13 x 10^3 cfu/L fungi, OSH 17 has 19 x 10^4 cfu/L bacteria and 16 x 10^3 cfu/L fungi and EOC has 23 x 10^4 cfu/L and 12 x 10^3 cfu/L bacteria and fungal population respectively. Also in Delta State the following microbial populations were recorded: Irri 02 has 45 x 10^4 cfu/L bacteria and 21 x 10^3 cfu/l fungi, Irri 06 has 38 x 10^4 cfu/L bacteria and 25 x 10^3 cfu/L fungi, Irri 07 has 26 x 10^4 cfu/L bacteria and 16 x 10^3 cfu/L fungi, Kwale 05 has 34 x 10^4 cfu/L bacteria and 19 x 10^3 cfu/L fungi and Kwale 06 has 30 x 10^4 cfu/L and 32 x 10^3 cfu/L fungi respectively.

High microbial population in Delta State pipelines may not necessarily be the reason for the high corrosion rates of pipelines in the sites. Corrosion rates may not be connected to quantity of microbes involves but corrosion appears to be worse when wide variety of microorganisms are present. This corroborates the observation by [20] that microbes isolation from fresh corrosion tubercles have yielded a wide variety of isolates that fall into a diverse number of physiological types among the heterotrophic bacteria isolated from pipelines. For example in this study different microbial species isolated in Rivers State include the following:- *Pseudomonas aeruginosa, Bacillus punilis, Alcanivorax borkumensis, Klebsiella oxytoca, Serretia marcescen, Acidithiobacillus forrooxidans* and two sulphate reducing bacteria: *Desulfobacter* postgatei and *Desulfonema limicola* (Fig. 3). In Delta State pipelines the heterotrophic bacteria detected are, *Bacillus subtilis, Bacillus cereus, Bacillus pumilus, Senratia marcescens, Pseudomonas aeruginosa, Klesiella oxytoca* and two sulphate reducing bacteria (*Desulfobacter postgatei* and *Desulfonema limicola) and one sulphate oxidizing bacterium (Acidithiobacillus ferrooxidans*) (Fig. 4).These results lend further evidence to the hypothesis that beside sulphate reducing bacteria, acid-forming bacteria play a key role in MIC. The involvement of *S. marcescens* is unexpected as this species has mot previously been reported to be associated with MIC in petroleum-transporting pipelines. *P. aeruginosa* and *Klesiella oxytoca* detected in pipelines in the two sites have minimal nutritional requirements and are often present in aquatic environments that are rich in organic pollutants such as gasoline solvents [21]. In addition, *P. aeruginosa* contributes to biofilm formation by producing exopolysaccharides and facilitating the attachment of other microorganisms [22] and hence accelerates the corrosion process [23].

The survival and increase in acid bacteria, iron bacteria and manganese oxidizing bacteria are due to the existence of favourable conditions and the ability of these organisms to utilize hydrocarbon as nutrients source. Strains such as *Bacillus sp* and *P. aeruginosa* use ferrous ions as electron donors and gain energy from the oxidation of Fe^{2+} to Fe^{3+} [24]. The free energy change in Fe^{2+} oxidation at low pH is large enough to be coupled to ATP synthesis, it is rather low at neutral pH, and iron bacteria cannot grow at pH above 4. This reaction is given as $\mathbf{Fe^{2+} + ¼O_2 + H^+ \longrightarrow Fe^{3+} + ½\ H_2O}$ (6)

In this study, the oxidation of ferrous to ferric ion by *P. aeruginosa, Bacillus subtilis, B. cereus, B. pumilus* indicates that the bacteria promoted ferric formation at low pH. *Desufobacter* postgatei and *Desulfonema limicola* are two very important sulphate reducing bacteria detected in both Rivers and Delta State pipelines that play an important role in metal corrosion by reducing sulphate and/or sulphur to hydrogen sulphide (H_2S). Hydrogen sulphide reacts with iron and form black ferrous sulphide (FeS), it also reacts with water to produce an acid condition, accelerating the corrosion process of pipelines in the two sites. The detection of sulphate oxidizing bacterium, *Acidithiobacillus ferrooxidans* that was detected in three pipelines in Delta and

Rivers states respectively, actively participates in corrosion of steel metals. This *acidophylic* oxidizing bacterium oxidizes reduced form of suphur to sulphuric acid by this reaction

$$2S + 3O_2 + H_2O \longrightarrow 2H_2SO_4 \quad (7)$$

Most of the fungi detected are capable of growth in fuel, notably among them are Pencillium spp, Paccilomyces spp, Aspergillus spp and Fusarium spp., Hormoconis resinae and Aureobasidium pullulan isolated only in pipelines in Delta State are mainly acids producers. Hormoconis resinea has been identified by [25] as the organism that produces dodecanoic acid as a major organic acid involved in the corrosion of aluminium fuel tanks. Aureobasidium pullulans also isolated only in pipeline in Delta State is capable of producing sulphorus and sulphuric acids as metabolic by-products. The acid metabolic by-products by this microbe is highly corrosive on oil production pipelines [26]. Other microbial consortia isolated from biofilms in pipelines in Rivers State were: Batrytis cinerea, Verticillium dahlae, Fusarium oxysporum, Aspergillus funigatus, Saccharomyces cerevisiae, Aspergillus frequentans, Penicillium glabrum, Aspergillus cylopitn (Fig. 5) and those detected from pipelines in Delta State are as follows: Aureobasidium pullulans, verticillium dahlae, Monilia balanitis, Hormoconis resinae (formerly classified as Cladosporum resinae), Paecilomyces variotii, Penicillium conglophilium, Humicola grisae, Aspergillus fumigatus and Saccharomyces cerevisiae (Fig. 6). These filamentous fungi are capable of causing severe damage to oil pipelines, jet fuel storage tanks, military and civilian aircraft operations. This study establishes correlations between the presence of various types of microorganisms in complex biofilms and metal correlation rates. (Figure 3 and 4). By the results in Figures 3 and 4 with positive correlation coefficient, it is clear that microorganisms are directly responsible for the corrosion of metals.

Table 1: Corrosion Analysis of Coupons Retrieved from oil Pipeline in Oshie flow station (Rivers State)

S/NO	Facility location	Coupon	Duration (days)	Initial weight (g)	Final weight(g)	Weight loss (g)	Corrosion rate (mpy)
1	Oshie F/S	OSH 01 (7 TBG)	127	38.68 33	37.3466	1.3367	2.565
2	Oshie F/S	OSH 04 (6LS)	127	38.5632	37.4918	1.3647	2.86
3	Oshie F/S	OSH 13 (6 SS)	127	37.4259	36.1985	1.2274	2.36
4	Oshie F/L	OSH 17 (11 SS)	127	37.7074	36.0656	1.6418	1.47
5	EOC	EOC 04 OBF 31 LS	127	38.9448	37.4325	1.5223	1.07

Table 2: Corrosion Analysis of Coupons Retrieved from oil Pipeline in Irri flow station (Delta State)

S/NO	Facility location	Coupon	Duration (days)	Initiate weight (g)	Final weight(g)	Weight loss (g)	Corrosion rate (mpy)
1	Irri F/S	Irri 02 (Irri 4) LS	127	37.9351	34.6912	3.2439	6.81
2	Irri F/S	Irri 06 (Irri 2T)	127	38.5668	36.4127	2.1441	4.50
3	Irri F/S	Irri 07 ISK 4 LS	127	38.5835	37.2864	1.2971	2.72
4	Kwale GP	Kwale 05	127	38.5128	37.2831	1.2297	2.36
5	Kwale GP	Kwale 06 (GLS)	127	38.5376	37.2522	1.2854	2.49

Corrosion rate (mpy) = Area factor x $\frac{\text{(Get loss) mg}}{\text{days exposed}}$

= Area factor = surface area x density = 266.464

Table 3: Occurrence of Bacterial Isolate per Pipeline in Oshie Flow State

Bail isolates	Rivers State				
	OSH 01	OSH 04	OSH 13	OSH 17	EOC 04
P. aeruginosa	+	+	+		
B. pumilius		+	+	+	
A. borkumensis	+			+	
K. oxytocs	+	+	+		
S. marcescens		+	+	+	*
A. ferrooxidans	+	+	+		*
Desulfobacter postgatei	+	+			
Desulfonema limicola	+			+	*

+ = indicates the pipelines which the isolates were found

Table 4: Occurrence of Bacterial Isolates per Pipeline in Irri Delta State

Bail isolates	Delta State				
	Irri 02	Irri 06	Irri 07	Kwale 05	Kwale 06
A. ferrooxidans	+				
D. limicola	+	+	+	+	
D. postgatei	+		+		+
P. aeruginosa	+	+		+	
S. marcescens	+	+	+		+
R. oxytoca		+	+	+	
M. Janneschii					
B. subtilis	+				+
B. cereus		+	+	+	+
B. pumilis	+	+		+	+

+ = indicates the pipelines which the isolates were found

Table 5: Occurrence of Fungal Isolates per Pipeline in Rivers State

Fungal isolates	Rivers State				
	OSH 01	OSH 04	OSH 13	OSH 17	EOC 04
Verticullium dahlae	+				+
Batrytis cinerae	+				
Fusarium oxysporum		+			
Aspergillus fumigatus		+			
Microsporum gyseum			+		
Saccharomyces cerevisiae			+		
Aspergillus frequentans				+	
Penicilium glabrum				+	
Aspergillus cytopitan					+

+ = indicates the pipelines which the isolates were found

Table 6: Occurrence of Fungal Isolates per Pipeline in Delta State

Fungal Isolates	Irri 02	Irri 06	Irri 07	Kwale 05	Kwale 06
Auerobacidium pullulans	+				
Verticillium dahlae	+				
Monilia balanitis		+			
Hormoconis resinae		+		+	
Penicillium corylophilum			+		
Aspergillus fumigatus				+	
Helminthasporium maydis					+
Paecilomyces variotii		+			

+ = indicates the pipelines which the isolates were found

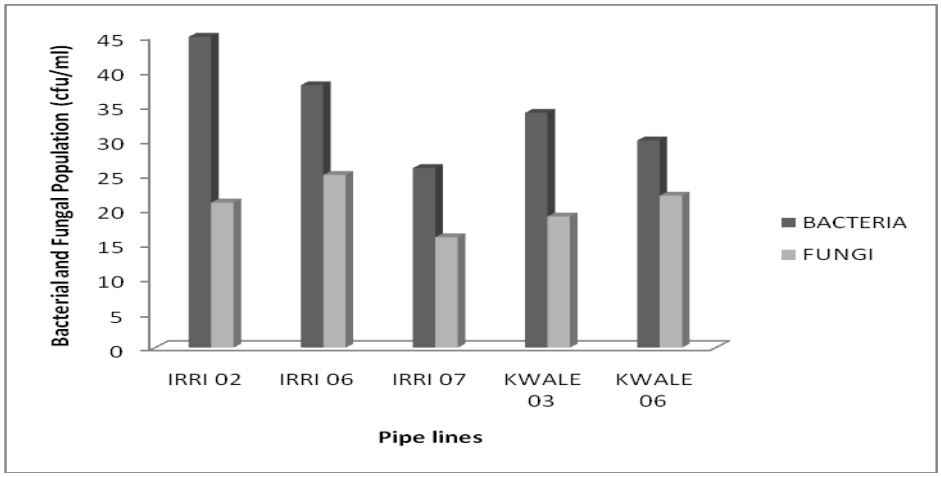

Fig. 1: Bacterial and Fungal Population in Delta State

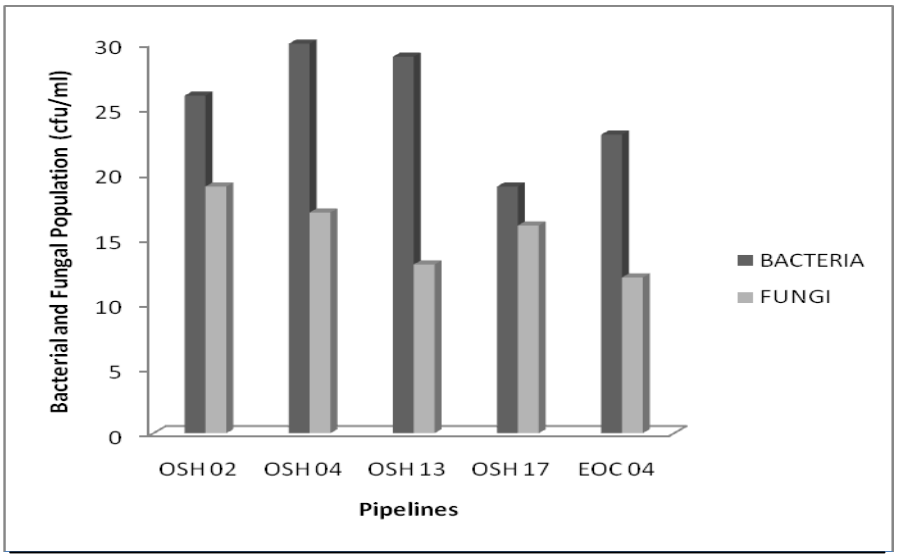

Fig. 2: Bacterial and Fungal Population in Rivers State

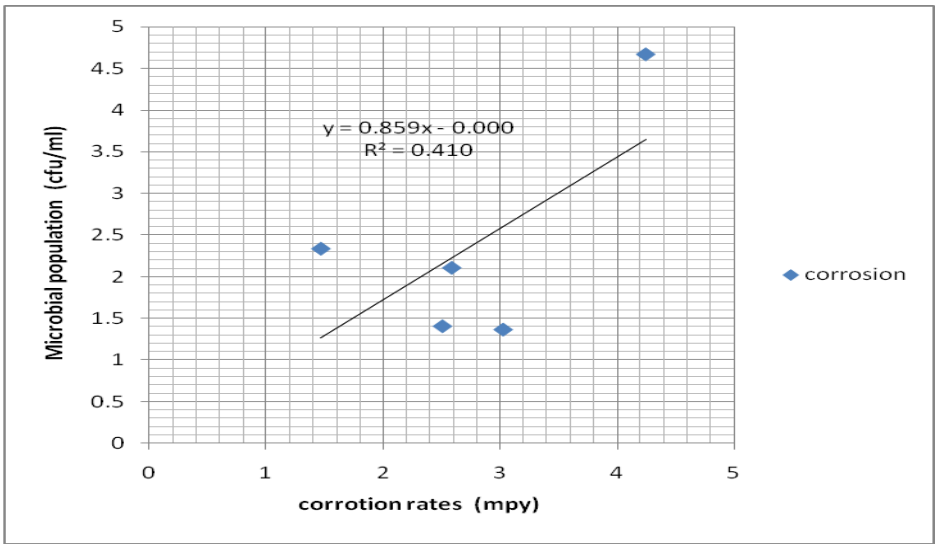

Fig. 3: Correlation between Microbial population and corrosion rates in Rivers State.

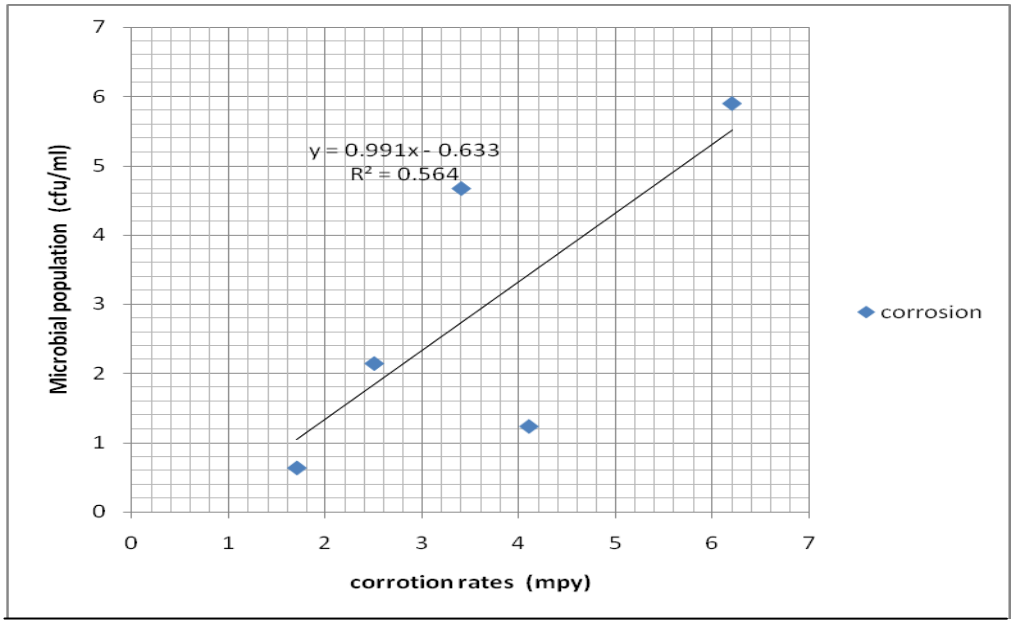

Fig. 4: Correlation between Microbial population and corrosion rates in Delta State.

Plate 1: Right and left coupons

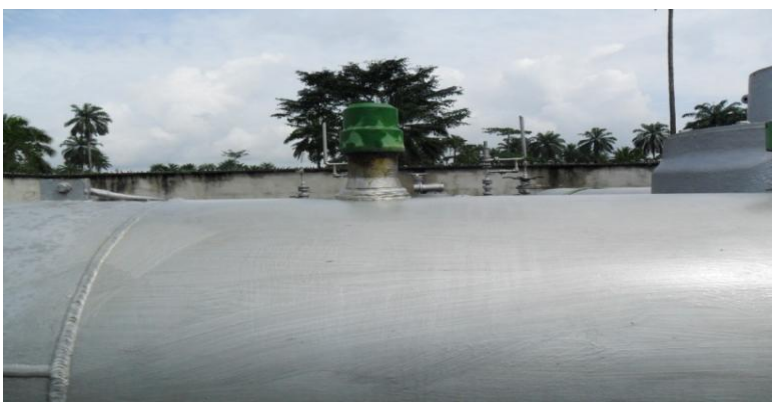

Plate 2: Access valve for insertion of coupon in a 24" pipeline.

V. CONCLUSION

This is the study exploring the correlation between microbial diversity in a corrosive biofilm associated with steel pipelines and corrosion rates. The microbial diversity in a corrosive biofilm associated with steel pipelines subjected to normal operation conditions. The results showed that the diversity of microbial community was diverse in the analysed biofilms and many physiological groups such as sulphate reducing bacteria (SRB) (*D. posgatei* and *D. limicola*), sulphur oxidizing bacterium (*A. ferrooxidans*), acid-forming bacteria (*K. oxytoca, B. cereus* and *B. subtilis*). Iron-oxidising bacteria (*P. acruginosa* and *B. subtilis*) were identified. Also detected were acid forming fungi (*Pullulans* and *H. resinae.*The correlation analysis was positive, indicating that microbial population inhabiting biofilms of oil pipelines are directly responsible for the corrosion of oil pipeline. It is likely that with an improved understanding of the compositions and variability of microbial communities present in ail and gas pipelines, we will be able to develop better means of monitoring and preventing microbiologically influenced corrosion

REFERENCES

[1] L. Stanley and B. L. Jones. Microbe-assisted external corrosion in oil and gas pipelines. *Pipeline worlds*. Vol. 1; 2004. 5-10.

[2] H. A. Videla. *Manual of Biocorrosion*. Florida. CRC. Lewis Publishers, 1996. 273p.

[3] G. Hernandez-Eugenio, M. L. Fardean, J. L., Cayol, B. K. Patel, P., Thomas, H., Macaric, J. L., Garcia, B. Olivier,. Clostridium thiosulpfatir-educens sp. nov. a proteolytic, thiosulfate and sulfur-reducing bacterium isolated from an up low anaerobic sludge blanket (UASB) reactor, *International Journal of Systematic Evolution Microbiology,* 53, 2002. 1461-1468.

[4] T. Zhang, H. H. P. Fang, B. C. B. Ko. Methanogen population in a marine biofilm corrosive to mild steel. *Applied Microbiology Biotechnology* 63, 2004. 101-106.

[5] R. E. Eckford and P. M. Fedorak. Planktonic nitrate reducing bacteria and sulfate reducing bacteria in some western Canadian oil field waters. *Journal of Industrial Microbiology and Biotechnology,* 29, 2002. 83-92.

[6] D. Bermont-Bouis, M. Janvier, P. A. Grimont, I. Dupont and T. Valleys. Both Sulfate-reducing bacteria and Enterobacteracea take parts in Marine biocoorsion of carbon steel. *Journal of Applied Microbiology*. 102, 2007. 161-168.

[7] I. B. Beech, J. A. Sunner. *Sulphate reducing bacteria and their role in of corrosion of ferrous materials.* In: Barton, L.L., Hamilton, W. A. (eds) Sulphate reducing bacteria: environmental and engineered systems. Cambridge University Press, Cambridge, 2007. 459-483.

[8] A. Rajasekar, S. Ponmariappan, S. Maruthanathu and N. Palaniswamy Bacterial degradation and corrosion of Naphtha in Transporting pipeline, *Current Microbiology*. 55, 2007. 374-381.

[9] S. Maruthamuthu, N. Muthukumar, M. Natesan and N. Palaniswamy. Role of air microbes on atmospheric corrosion. *Current Science*, Vol. 94, No. 3, 2008.

[10] J. Sambrook, E. F. Fritsch and T., Maniatis. *Molecular Cloning*. A laboratory manual (3rd ed.) cold spring harbor laboratory Press. New York, 2001. 235-137.

[11] E. A. Ghazy, M. S. Mahmoud, M. N. Asker, M. M. Mohamoud, A. Elsoud, and A. Sami. Cultivation and detection of sulfate reducing bacteria (SRB) in sea water. *Journal of American Science*. 7(2), 2011. 604-608.

[12] H. Collins, and F. M. Lyne. *Microbiological methods*. Great Britain. Butterworth and Company Limited. 1976.

[13] Starkey Broth LRL. Starkey and V. G. Collin. Autotrophys. In: Methods in microbiology. J. R. Nomis, D. W. Ribbons (eds), New York, Academic Press, 38. 56-78.

[14] E. F. Harrigan and M. E. McCance. *Laboratory methods in Food and Diary Microbiology.* London Academic Press. 1976.

[15] S. T. Cowan and K. J. Steel. *Manual for the identification of medical bacteria,* (2nd ed.) Cambridge University Press, 1985. 42-110.

[16] J. G. Holt, R. K. Noel, H. A. S. Peter, T. S. James and T. W. Stanley. *Berggeys Manuel of Determinative Bacteriology,* (9th ed.). Williams and Wilkin Publishing Co. U.S. A. 1994. 787.

[17] Lodder, J. *The Yeast,* (2nd Ed.) Northern Holland Publishing Co. Amsterdam and American Publishing Co. Incorporated, New York. 1974.

[18] K. H. Domsch, H. Gams and T. H. Anderson. Compendium of soil fungi. London: Academy Press, 1980.

[19] M. J. Franklin, D. E. Nivens, M. W. Mihelman, A. A. Vass, R. F. Jack, N. J. E. Dowling, R. P. Mackowski, S. L. Duncan, O. B. Ringlebry and D. C. White. *An analogue MIC system with specific bacterial consortia to test effectiveness at material selection and counter measures corrosion NACE proceedings of conference*, New Oceans. 1989. 89- 573.

[20] X. Y. Zhu, J. Lubeck, J. Kilbane. Characterization of microbial communities in gas industry pipelines: *Applied Environmental Microbiology*, 69, 2003. 5354-5363.

[21] M. W. Lechevallier, C. D. Cawthon, R. G. Lee. Factors promoting survival of bacteria in Chlorinated water supplies. *Applied Environmental Microbiology*, 54, 1988. 649-654.

[22] J. F. Batista, R. F. Perein, J. M. Lopes, M. F. Carvallao, M. J. Fein, M. A. Reis. In situ corrosion control in Industrial water systems. *Biodegradation*, 11, 2000. 441-448.

[23] D. W. S. Westlake, K. M. Semple, C. O. Obuekwe. Corrosion by ferric-iron reducing bacteria isolated from oil production systems. In: Dexter Sc. (ed.) Biologically induced corrosion. *NACE, Houston,* 1986.193-200.

[24] E. R. Schiapparelli and B. R. Meybaum. The role of dodecanoic acid in the microbiological corrosion of jet aircraft integral fuel tanks. *International Biodeterioration Bulletin* 16(3), 1980. 61-66.

[25] B., P. Little, Wagner and J. Jacobus. The Impact of sulfate-reducing bacteria on melded copper nickel seawater piping systems. *Materials performance.* 27(8), 1988. 57-61.