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A Study On The Impact Of Microbes On Oil Transporting Pipelines In Obiafun/Obrikom, Rivers State, Niger Delta Region, Nigeria

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ABSTRACT: *A study on the impact of microbes on oil transporting pipelines in Obiafun/Obrikom, Rivers State, Nigeria was conducted between 2011 and 2012. To harvest biofilms from the pipelines, ten coupons were placed into the inner surfaces of five pipelines (two per pipeline) and allowed for normal flow of petroleum for a period of 127 days. At the end of the 127 days, biofilms were scraped and used for the enumeration and identification of sulphate reducing bacteria (SRB), total heterotrophic bacterial and fungal counts. Corrosion rate was determined by weight loss method. The results revealed the following species of SRB, Desulfuromonas acetoxidans, Desulfosarcina variabilis and Desulfobulbus propionicus. The bacteria species identified were Bacillus Cereus, Klebsiella oxytoca, Pseudomonas aeroginosa, Serrratia marcescens and Halomonas subglaciescola. Among the bacterial species, Gram positive bacteria were more dominant with 62.5% occurrence and the Gram negative bacteria with 37.5%. the fungal isolates identified were mostly of the genera; Aspergillus, Verticillium, Saccharomyces and Microsporarium; Penicillum, Aureobasidium and Hormoconis. The mean values of corrosion rates in each pipelines were 1.6, 5.39, 1.0, 3.37 and 2.22 mpy respectively for 7 TUB, 6 LS, 6 25, 11ss and OBF31 pipelines. These results will provide baseline data for monitoring and controlling of biocorrosion in oil transporting pipelines.*

KEYWORDS: Impact, Microbes, Transporting pipelines, Obiofun/Obrikom

INTRODUCTION

Carbon steel pipelines are the most efficient and economic methods of transporting hydrocarbon products in the oil and gas industries. During oil and gas operations, pipelines networks are subjected to different corrosion deterioration mechanisms, including Microbiologically Influenced Corrosion (MIC) which results from accelerated deterioration caused by different microbial activities present in the hydrocarbon systems (Akpan *et al*, 2013a).

Microbiologically Influenced Corrosion (MIC) is of considerable concern to the oil and gas industries.

Microbioogically influenced corrosion has been reported in oil and gas treating facilities such as refineries and gas fractionating plants, pipeline systems and exporting terminals. MIC can be responsible for the increase in corrosion rates due to the presence of microbial activities that accelerate the rate of anodic and cathodic reactions (Mansfield 2007). But Bryant *et al.*, (1991) reported that corrosion rates depend largely on the total activity of hydrogenase within the biofilm rather than on microbial population size. The biofilm comprising SRB in a non-corroding pipeline had higher cell number but low hydrogenase activity, and showed a low

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corrosion (0.4mm iron oxidized per year). In contrast, biofilms with SRB in pipelines with intense corrosion (7.8mm iron oxidized per year) had lower cell densities but much higher total hydrogenase activity.

Microbiologically Influenced Corrosion (MIC) does not produce a defined type of damage, however it mostly results in a localized type of corrosion that manifests pitting, crevice corrosion, under-deposit corrosion, cracking enhanced erosion corrosion, and dealloying (Javaherdashri 2008). Microbial activities are thought to be responsible for greater than 75% of the corrosion in productive oil wells and for greater than 50% of failure of pipelines systems (Berhencourt *et al*, 2006, Flemming, 1996). MIC has been estimated to account for 20-30% of all internal pipeline corrosion costs. Different micro-organisms thrive in oil and gas transporting systems for the reason that all of the essential elements for life are present in this environment. Microbial life needs four basic things to thrive in an environment: a carbon source, water, and electron donor and an electron acceptor (Madigan, 2009).

Hydrocarbon acts as an excellent food source for a wide variety of micro-organism, water also exists in mixed solution with hydrocarbon. Other elements including Sulphur, Nitrogen, Carbon, and Phosphorus are needed. The main type of micro-organisms associated with metals in pipeline systems are sulphate- reducing and Sulphate – oxidizing bacteria,, iron and CO_2 reducing bacteria, iron and manganese oxidising bacteria, and acid producing fungi (Beech and Sunner 2004, Akpan *et al*, 2013). The Microbiologically Influenced Corrosion process starts by the attachment of plantonic micro-organisms to a metal surface that then leads to the formation of a complex biofilms. During the growth of the biofilm, and through their metabolic activities, bacteria catalyze numerous invisible slow electrochemical reactions at the cell metallic surface intervere. There metabolic reactions may be corrosive in nature or may dissolve a protective surface-oxide film (Keevil 2001). The MIC process starts with a biofilm formation on a metal substrate. Immobile cells attach to the steel substrate, grow, reproduce and produce an extra-cellular polymeric substance (EPS) that results in a complex biofilm formation (Little and Lee 2007). The biofilm formation encompasses three different stages, the first of which start with the absorption of macromolecules, such as protein, lipids, polysaccharides and lumic acids that work as a conditioner of the steel surface. These macromolecules change the physical chemistry of the interface including the hydrophobicity and electrical change. During this stage, micro-organisms and surface and aqueous-medium Characteristics play a significant role in the extent of bacterial transfer rate, adhesion and resultant biofilm size. Microbial characteristics include surface change, cell size, and hydrophobicity. Surface properties included chemical compositions, roughness, inclusions, crevice, oxides, or coating, whereas the aqueous-medium properties include flow regime of the system and ionic strength (Little and Lee, 2007).

When sessile cells reside on a steel surface, their metabolic products introduce multiple cathodic reactions and thus promoting corrosion. Several work have been done, on the impact of oil spill on microbes and the Niger Delta environment, but there is dearth of information on the impact of microbes on oil transporting pipelines in the Niger Delta Region of Nigeria. The objective of this research was therefore designed to study the impact of microbes on oil transporting pipelines in Niger Delta.

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MATERIALS AND METHODS

Sampling of Biofilms

To obtain the biofilm samples, ten mild steel coupons (surface are $4cm^2$ and density $7.57g/cm^3$) were placed at the inner surface of the pipelines through the access value (Fig. 1) at five different pipelines made up of the same materials and the coupons and exposed for 127 days to the flow of petroleum. The involved pipelines were located at the Obiafun/Obrikum gas plant, Rivers State, Niger Delta region of Nigeria, where crude oil and gas are extracted. The coupons were detached from the inner region of oil pipelines and the biofilms formed on the surfaces of each coupon were removed with sterile razor blade and collected into sterile bottle with 20ml phosphate-buffered saline, pH7 (Sambroak and Rusell *(* 2001). The biofilm samples were named as 7TGB, 6LS, 6SS, 11SS and 0BF31, corresponding to each oil pipeline.

After the removal of the biofilms, the coupons were washed in acetone, dried and the final weight taken using $\pm$ 0.0001 accuracy electric balance and the differences in weight (weight loss) used to determine corrosion rate using the formular:

$$\text{Corrosion rate (MPY)} = \frac{\text{Area factor x Weight loss}}{\text{Day exposed}}$$

Fig 1: Access Valve

ISSN 2056-7537(print), ISSN 2056-7545(online)

Microbiological Analysis

i. **Bacteria:** The medium of choice for the cultivation of total heterotrophic bacterial counts (THBC) was nutrient agar with the following composition.

ii. **Nutrient Agar (Oxoid)**: Containing 1.g Lamco powder, 2.0g Yeast extract 5.0g Peptone, 5.0g NaCl, 15,0g Agar, 1000ml Distilled water.

iii. **Postgate B Medium**: For the cultivation of sulphate reducing bacteria. The composition of the medium are: KH_2PO_4 O.5g/ml, NH_4Cl 1.0g, Na_2SO_4 1.0g, $CaCl_2$ $6H_2O$ 0.1g, $MgSO_4$ $7H_2O$ 2.0g, Sodium Lactate (60-70%) 5ml, Yeast extract 1.0g, Ascorbic acid 0.1g, thioglycelic acid 0.1g, $FeSO_4$ $7H_2O$ 0.5g, NaCl 26.0g, Distilled water 1000ml, pH7.0 (Postgate 1984).

iv. **Fungi**: Sabround Dextrose Agar (SDA) (Biolab) for the cultivation of total Heterotrophic fungal counts (THFC) containing: Peptone 10g, glucose 20g, Agar 15g, Distilled water 1000ml, Barnet and Hunter 1987).

v. **Inoculation and Incubation:** One militre of appropriate ten-fold serial dilution of biofilm samples were inoculated unto nutrient agar, Postgate B and SDA plates in triplicates using spread plate technique. Inoculated plates were incubated at 37^0C 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 37^0C for 7 days and on SDA medium plates for the cultivation of total heterotrophic fungal count and incubated for 5 days.

vi. **Maintenance of Pure Culture:** Discrete colonies of heterotrophic bacteria, sulphate reducing bacteria and fungi were purified by repeated sub-culture unto NA, Postgate B and SDA media. Pure cultures were preserved on NA, Postage B and SDA Slants respectively, stored at 4^0C for further use.

Characterization and Identification

Pure cultures of microbial isolates were identified based on cultural parameters, microscopic techniques and biochemical tests including; Gram's reaction, oxidaze test, motility test, indole test, VP and MR tests carbohydrate utilization (Cruickshank et al., 1976). Identification of the bacterial isolates was accomplished by comparing the characteristic of the cultures with that of known taxa as in (Holt et al. 1994).

Fungal isolates were characterized using the identification scheme of (Barnet and Hunter, 1987). Briefly, the wet mount method was carried out using lactophenol in cotton blue stain.

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RESULTS

Microbiological Analysis

i. Microbial Counts

The total sulphate reducing bacteria per pipeline are shown in Table 1. The total SRB per pipeline are: TGB 1.6 x 10^4cfu/ml, 6LS 1.0 x 10^4cfu/ml, 6SS 3.6 x 10^4, 11SS 1.0 x 10^4cfu/ml, OBF31 1.10 x 10^4cfu/ml.

Total heterotrophic bacteria (THBC) are presented in Table II are: TGB 5.0 x 10^4cfu/ml, 6LS 6.0 x 10^4cfu/ml, 6SS 2.5 x 10^4cfu/ml, 11SS 43 x 10^4cfu/ml and OBF31 5.6 x 10^4cfu/ml.

Total fungal counts are as presented in Table III: 7TGB = 1.6 x 10^3cfu/ml, 6LS = 1.10 x 10^3cfu/ml, 6SS = 1.0 x10^3cfu/ml, 11SS = 1.5 x 10^3cfu/ml and OBF31 = 1.2 x 10^3cfu/ml. Among the identified bacteria flora, gram positive were more dominant with 62.5% occurrence while gram negative was 37.5%

ii. Microbial Isolates from the Pipelines

The Sulphate reducing bacteria isolated from pipeline are: *Desulfuromonas acetoxidans, Desulfosarcina variabilis, Desulfobulbus propionicus.* Other bacteria isolates isolated were; *Bacillus subtilis, Bacillus cereus, Klebsiella oxytoca, Pseudomonas aeruginosa, Halomonas subglaciescola, Serratia marcescen, Staphylococcus epidermidis* and *Staphylococcus aureus.*

The fungal isolates were mostly of the genera *Aspergillus, Verticillium, Saccharomyces* and *Microsporum*

Corrosion Rates per Pipeline

The results for corrosion rates per pipeline are as shown on Table 4, and are as follows: TGB had 1.73 and 1.47 with the mean of value of 1.6mpy, 6LS had 5.42 and 5.36mpy with the mean value of 5.39mpy, 6SS had corrosion rates of 1.13 and 1.06mpy with the mean value of 1.10mpy, 11SS had the corrosion rates of 4.61 and 2.13mpy with the mean value of 3.37mpy and OBE31 had the corrosion rates of 2.15 and 2.29mpy with the mean value of 2.22mpy.

Table 1: Sulphate Reducing Bacteria Isolated from Oil Pipelines

N/N	Facility Location	Coupon ID	TSRB cfu/ml	Identified organism
1.	Oshie	7TGB	1.6 x 10^4	Desulfuromonas acetoxidans
2	Oshie	6LS	1.0 x 10^4	Desulfobulbus propionicus
3.	Oshie	6SS	3.6 x 10^4	Desulfosarcina Variabilis
4.	Oshie	11SS	1.0 x 10^4	Desulfosarcina variabilis
5	EOC	OBF31	1.10 x 10^4	Desulfobulbus propionicus

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Table 2: Total Heterotrophic Bacteria

S/N	F/L	C/ID	THBC (Cfu/ml)	Identified organisms
1	Oshie	7TGB	5.0×10^4	*Bacillus subtilis*$^+$ *Bacillus cereus*$^+$, *Klebsiella oxytoca*$^-$ *Pseudomonas aeruginosa*$^+$
2	Oshie	6LS	6×10^4	*Halomonas subglaciescola,*$^+$ *Serratia marcescens* $^-$ *Bacillus cereus*$^+$
3.	Oshie	6SS	2.5×10^4	*Staphylococcus*$^+$ *epidermidis,* *Halomonas subglaciescola* *Bacillus subtilis*
4.	Oshie	11SS	43×10^4	*Serratia marcescens* *Klebsiella oxytoca* $^-$ *Pseudomonas aeruginosa*$^+$ *Bacillus cereus*$^+$
5	EOC	OBF31	5.6×10^4	*Staphylococcus aureus*$^+$ *Bacillus cereus*$^+$ *Seratia marcescens*

F/L = Facility Location, C/ID = Coupon Identity

Table 3: Total Fungal Count

S/N	F/L	C/ID	TFC(Cfu/ml)	Identified organisms
1	Oshie	7TGB	5.8×10^3	*Aspergillus fumigatus* *Verticillium dahlae* *Aspergillus ochraceus* *Saccharomyces cerevisiae*
2	Oshie	6LS	2.40×10^3	*Aspergillus fumigatus* *Aspergillus parasiticus* *Saccharomyces cerevisiae*
3.	Oshie	6SS	2.8×10^3	*Saccharomyces cereisiae* *Microsporum gypseum*
4.	Oshie	11SS	2.0×10^3	*Fusarium frequentans* *Fusarium oxysporun* *Saccharomyces cerevisiae*
5	EOC	OBF31	2.3×10^3	*Microsporum gypseum* *Saccharomyces cerevisiae* *Aspergillus fumigatus*

F/L = Facility Location, C/ID = Coupon Identity

ISSN 2056-7537(print), ISSN 2056-7545(online)

Table 4: Corrosion Rates of each pipeline

S/N	F/L	C/ ID	Duration (Day)	Initial weight (g)	Final weight (g)	Weight loss (g)	Corrosion rates (mpy)
1	Oshie	7TGB	127	38.5726	38.2119	0.3607	1.73
				38.7944	38.4894	0.305	1.47
2	Oshie	6LS	127	38.9498	37.8222	1.1276	5.39
				38.1765	37.0614	1.1151	5.36
3	Oshie	6SS	127	37.4732	37.2377	0.2355	1.10
				37.3786	37.1592	0.2194	1.06
4	Oshie	11SS	127	37.6524	36.8025	0.4435	3.37
				37.6524	37.2089	0.12114	2.13
5	EOC	OBF31	107	38.5523	38.0038	0.5286	2.29

DISCUSSION

This work on the impact of microbes on oil transporting pipelines in Obiafun/Obrikom, Niger Delta Region, Nigeria was designed to provide baseline study for the understanding of the diversity of microbial species involved in corrosion, and will be useful for development of a new approach for the detection, monitoring, and control of microbiological influenced corrosion. Microbiological contamination of fuels has been a cause of intermittent operational problems throughout the world, and over the years the frequency and severity appears to have been increasing dramatically (Hamilton 1985, Muthukumar et al 2003b). Microbial activity can result in fuel degradation that may lead to such problem as unacceptable level of turbidity, corrosion of storage tanks, pipelines and souring of stored products.

Sulphate Reducing Baceria (SRB) enhance metal corrosion by reducing sulphate and/or sulphur to hydrogen sulphide (H_2S) Hydrogen sulphade (H_2S) reacts with water to produce an acid condition, accelerating corrosion process.

In this study, the presence of *Desulfuromonaes acetoxidans, Desulfobulbus propionicus* and *Desulfosarcina variabilis* may be due to the availability of intermediate hydrocarbon degradation products which served as energy source for the physiological activities of the sulphate reducing bacteria.

The supply of utilizable hydrocarbon degradation products may well explain why corrosion occurs in crude oil pipelines in the Niger Delta region of Nigeria very often. Several investigators have also isolated sulphate reducing bacteria from microbial communities involved in microbiologically influenced corrosion in oil and gas-transporting pipeline (Miranda – Tello et al, 2004). Among the heterotrophic bacteria isolated, Gram positive bacteria were more dominant than Gram negative bacteria, with 62.5 and 37.5% respectively. Gram positive bacteria are more actively involved in corrosion of oil pipelines because of their endowed capacity to inhabit such stressed environment as oil pipeline. These results corroborate previous observations by Jan – Roblero et al., (2004) who had similar view.

The presence of acid-forming bacteria *Klebsiella oxytoca*, *Bacillus cereus* and *Serratia marcescens* in biofilms collected from the pipelines suggests that acid-producing bacteria may

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also contribute significantly in corrosion. *Klebscella oxytoca* which was detected only in two pipelines, is often present in soil and water and fixes nitrogen under anaerobic or microaerobic condition. It produces nitrates and/or nitric acid that may contribute to metal corrosion.

Pseudomonas aeruginosa and *Klebsiella oxytoca* isolated have minimal nutritional requirement and often present in aquatic environments that are rich in organic pollutants such as gasoline and solvents. In addition, Pseudomonas aeruginosa contributes to biofilm formation by producing exopolysaccharides and facilitating the attachment of other microorganisms (Akpan et al, 2013, Lechevallier et al. 1988) and hence accelerates the corrosion processes (Batista et al. 2000).

The survival and increase in acid bacteria, iron bacteria and manganese oxidising bacteria may probably be due to existence of favourable condition and the ability of these organisms to utilize hydrocarbon as nutrients source. The strains such as *Bacillus cereus, Bacillus subtilis* and *Pseudomonas aeruginosa* use ferrous ions as electron donors and gain energy from the oxidation of Fe^{2+} to Fe^{3+} (Akpan et al 2013b).

In this present research, the oxidation of ferrous to ferric ion by *Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis* and *Klebsiella oxytoca* indicates that the bacteria promotes ferric formation at low pH as evidenced by the ferric oxides on the coupons used in this study (Fig 2). The presence of ferric oxide indicates the role of iron-oxidising bacteria in the formation of corrosion products/sludge in oil pipelines. (Fig. 2) revealed the presence of ferrous and ferric sulphate in the biofilm, which indicated the role of iron and manganese bacteria, while acid producers caused the formation of sulphate in water and biofilm. On the inner wall of the pipelines severe corrosion was evident on the pits on the coupons used in this research. (fig.3) in addition, *Halomonas subglaciescola* was detected. This chemoorganotrophic bacterium, frequently isolated from marine and saline environments, produce organic acids from sugars (Andrea et al. 2001, Mata et al., 2002) and have been associated with biofilms or used as models in adhesion studies to solid substrates.

Fig 2: Coupons before insertion into oil Pipelines

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Fig 3: Biofilms on oupons after 127 days in oil Pipelines

Stimulation of the anodic reaction by acidic metabolites, such as organic acids, enhances corrosion. *Halomonas subglaciescola* have relevant physicochemical properties, such as a stable viscosity over a wide pH and salinity. As a result of their adhesion capacities, the EPS – producers strain isolated or detected in this work could constitute the planktornic fraction that initiates the interaction of the cells with the metal surface, the first step in bioflim formation.

Among the fungal isolates detected in biofilm from oil pipelines are those that have reported ability to grow in oil. For example; *Fusarium oxysporum, Microsporum gypseum, Aspergillus fumigatus* can grow on oil component and produced carboxylic acids which corrode iron. The acids frequently produced by fungi are formic, citric and acetic acids. These acids are damaging to metals including pipelines. These acids may contributes significantly to corrosion of oil pipelines by reducing iron from ferric state (Fe^{3+}) to the ferrous (Fe^{2+}) State (Haa, 2005).

The corrosion rate of each pipeline was determined by weight loss method. The corrosion rates in the pipelines are between low to high. The occurrence of corrosion in the pipelines may be due to preference of acid producing bacteria and fungi and spore forming bacteria, which most are Gram positive bacteria. The corrosion rates may probably be due to the fact that the micro-organisms inhabiting the pipelines might have developed resistant against the biocides and corrosion inhibitors used by the oil company and use them as substrate for their growth. The pit corrosion observed on the coupons indicated that the pipelines after about 5 years may need attention to avoid sudden spills (Fig 4).

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Fig 4: Pit Corrosion on Coupons after 127 days in oil Pipeline

CONCLUSION

A study on the impact of microbes on oil transporting pipelines was conducted. The study revealed that Gram positive bacteria dominated the oil pipelines with 62.5% and Gram negative bacteria 37.5%. Acid producing fungi were also in abundance in the pipelines and these contributed to the pit corrosion observed on the coupons. These findings call for regular monitoring and application of biocides and corrosion inhibitors to protect the pipelines.

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