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Comparative Study Of Microbial Diversity Inhabiting Biofilms Of Corroded Oil Pipelines In Delta And Rivers State Of Nigeria

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Abstract:
Corrosion is a leading cause of pipelines failure in the oil and gas industries because of the activities of microbial populations. The aim of the study was to assess microbial diversity associated with corrosive biofilms of oil pipelines in two sites (Warri in Delta and Oshie in Rivers States) within the Niger Delta region of Nigeria. Coupons were inserted into the inner surfaces of the pipelines (9'', 10'', 18'' and 24'' diameter) through the access valves for a period of 127 days. The coupons were detached from the inner surfaces of the pipelines at the end of 127 days and biofilms formed on the surfaces of each coupon were scraped with sterile razor blades and collected into sterile bottles containing 5 ml phosphate-buffered saline at pH 7 and taken to the laboratory for the cultivation fungi. The results from Rivers State (site 1) showed the following fungal species: Verticillium dahlae, Botrytis cineriae, Penicillium nalgiovese Humicola. grisae, Monilia balanitis,, Saccharomyces cerevisae, Aspergillus fumigates,. semitectum, Helminthosporium maydis and Eurotium repens. In Delta State (site 2) the fungal species detected were: Verticillium dahlae, Botrytis cinerae, Penicillium corylophilum Humicola grisae, Monilia balanitis,r, Saccharomyces ccerevisiae,Fusrium oxysporum, Aureobasidium pullulans, Aspergillus fumigatus and Hormoconis resinae. The results revealed a Sorenson's coefficient (cc) of 0.60, indicating that fungal communities in site 1 and 2 have a bit of overlap or similarity. The results further showed that diversity and evenness in site 2 are much higher than in site 1. Fungal populations in sites 2 are not only higher in the number of species present, but the individuals in the community are distributed equitably among these species. This shows that pipelines located in site 2 (Delta State) are likely to be severely corroded than site 1 (River State). Microbial communities in biofilms developed on surfaces of metals in natural environments are heterogeneous and there is significant uncertainty concerning how many of these organisms contribute to corrosion of metals. Therefore, the thorough knowledge of microbial species inhabiting biofilms of oil pipelines will give effective means of detecting, monitoring and control of corrosion of oil pipelines; this will significantly reduce pollution of agricultural lands and the environment by oil spillages in the Niger Delta.
Key words : *Microbial diversity,biofilms,corroded oil pipeline, corelation*

1.Introduction

Corrosion is a common form of structure degradation that reduces both the static and cyclic strength of a pipeline Nordin et al., (2011). There is always the chance that pipelines could leak or rupture, pipeline can cause serious human, environment and financial loses (Hopkin, 1995). Corrosion attacks are frequently responsible for most materials failure. Corrosion of underground metallic materials is a very serious and widespread problem. Structures such as natural gas and crude oil pipelines and water pipes are some of the structures reported to have been affected by soil corrosion around the world (Chukwu et al., 2008). In aqueous environments, iron materials are corroded not only by purely chemical or electrochemical reactions, but also by metabolic activities of microorganisms in a process termed microbially influenced (or induced) corrosion (MIC). Microbiologically influenced corrosion is defined as an electrochemical process where microorganisms initiate, facilitate or accelerate the corrosion reaction (Aruliah et al., 2010). Microbially influenced corrosion of materials is reported to account up to 50% of the damage cost (Hamilton, 1985; Fleming 1996). The industries that are suffering due to MIC most severely include the nuclear and fuel electric power generating sectors, pipelines, oil fields and offshore industry (Dowling and Guezennes, 1997). In some municipal systems such as drinking water distribution systems, high rates of MIC not only cause significant losses to the economy, but also directly affect public health (Volk et al., 2000). Sulphate reducing bacteria are proposed to be chiefly responsible for anaerobic corrosion, particularly in environments with high sulphate concentrations such as sea water (Cord- Ruwisch et al., 1987, Hamilton et al., 1988). The mechanism by which sulphate reducers accelerate metal corrosion has attracted many investigators, but details of the process are still inadequately understood (Hamilton, 1985; Cord-Ruwisch 2000). Fungi and algae are also involved in metal deterioration, in fuel and oil storage tanks, fungi species such as Aspergillus, Penicillium and Fusarium may grow on fuel components and produce carboxylic acids, which corrode iron (Little and Wagner, 1997, Little et al., 2001). In the presence of light, algae can produce organic acids and decrease the pH of the environment, thereby favouring corrosion (Mara and Williams 1992). The Fungus, Hormoconis resinae utilizes the hydrocarbons of diesel fuel to produce organic acids, the large quantities of organic by-products excreted by this fungus is capable of causing biocorrosion of storage tanks and transporting pipelines (Wingender et al., 1999). Other fungi detected that may contribute to biocorrosion of oil pipelines included Aspergillus Fumigatus which involves in iron reduction from Ferric State (Fe^{3+}) to the ferrous (Fe^{2+}) state (Haas, 2005). Fungi are capable of

producing inorganic acid such as nitric acid (HNO_3), sulphurous acids (H_2SO_3), sulphuric acid (H_2SO_4), and carbonic acid (H_2CO_3). The fungus Aureobasiodium pullulans is capable of producing these acids, which are highly corrosive on oil production pipelines.

2.Materials And Methods

- Sampling Sties: The sampling sites included the Obiafun/Obrikom gas plant in Rivers State and the Kwale/Okpai gas plant in Delta State.
- Sampling of Biofilms: To obtain the biofilms, twenty mild steel coupons (Fig. 1) (surface area $35.2cm^2$ and density 7.57 g/cm^3 each) obtained from a commercial source (metal-samples company, Munford, Al).

Figure 1: Right and left coupons

The coupons used have the same chemical properties as the mild steel pipelines with the following chemical compositions: 0.06%C, 1.05% Mn, 0.27% Si, 0.06% P, 0.002% S, 0.02% Cr, 0.02%Ti, 0.05% Al, 0.02% Cu and 98.484$ Fe (Lynch, 1989) using metal sample corrosion monitoring systems (serial No. 16021 of metal sampling company, Munford, Al), coupons were inserted into the inner surfaces of the pipelines 24”, 18”, 9”, 14” and 10” diameter through the access valves (Fig . 2)for a period of 127 days.

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

At the end of the 127 days, the coupons were detached from the inner region of the pipelines and the biofilms formed on the surfaces of each coupon were scraped (Fig.3) with sterile razor blades and collected into sterile bottles containing 5ml phosphate-buffered saline at pH 7.0 according to Sambrook *et al.,* (1999), stored in a cooler of iced block and at the end of the day transferred to the laboratory for analysis.

Figure 3: Scraping of biofilms from coupons.

Each coupon was named after the pipeline involved, which were: OSH01, OSH 04, 0SH 13, OSH 17 and EOC 04 for Rivers State sampling site and in Delta State sampling site were: Irri 02, Irri 06, Irri 07, Kwale 05 and Kwale 06 respectively.

3.Determination of total Heterotrophic Fungal Counts (THFC)

Ten fold serial dilution of the biofilm samples were made as described by (Collin and Lyne 1978, Harrigan and McCance, 1976). Inoculation and incubation. One mililitre of appropriate tenfold serial dilution of biofilm samples were inoculated unto *Sabrourand Dextrose Agar* plates in triplicate using spread plates technique. Inoculated plates were incubated at 28°C for 5-7 days for the enumeration of total heterotrophic fungi. At the end of 7 days of incubation, visible discrete colonies in inoculated plates were counted and the results recorded as colony forming unit per mililitre (cfu/ml) of biofilm sample.

3.1.Maintenance of Pure Culture

Discrete colonies were purified by repeated subculture unto Sabrourand Dextrose Agar medium. Pure cultures were preserved on Sabrourand Dextrose Agar slant and stored at 4°C for further test.

4.Characterization And Identification Of Fungal Isolates

Fungal isolates were identified based on the taxonomic schemes of Lodder (1974) and Domsch et al., (1980). Briefly, the wet mount for examination and identification of fungal isolates was employed using lactophenol in cotton blue stain. A drop of lactophenol in cotton blue stain was placed on a clean slide, using sterile inoculating needle a loopful of 5-7 days cultures were transferred unto clean grease free slides. The isolates were flooded with lactophenol in cotton blue stain for 3-6 minutes. The slides were carefully covered with cover slips to avoid air bubbles and then mounted on the microscope. The slides were then placed under a x40 objective of the microscope to focus. The following features were looked for and recorded: colony colour, types of stoma, nature of hypha, special vegetative structures, asexual spores, special reproductive structures, conidial head and vesicle shapes.

5.Statistical Analysis

The statistical analysis used to compare fungal diversity in the two sampling sites (Rivers and delta States) was diversity induces.

- Diversity index is a mathematical measure of species diversity in a given community.
- Based on the species richness (the number of species present) and species abundance (the number of individuals per species).
- The more species you have, the more diverse the area.
- However, there are two types of induces, dominance indices and information statistics indices.
- The equation for the two indices used for thus study are:
- Shannon Index (H) $= \sum_{s} Pi\ In\ pi$
- Simpson Index (D) $= \frac{1}{\sum_{i=1}^{s} Pi^2}$

The Shannon index is an information statistic indeces, which mean it assumes all species are presented in a sample and that they are randomly sampled. In the Shannon index, P is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individual found (N), in is the natural log, Σ is the sum of the calculations and s is the number of species.The Simpson index is a dominance index because it gives more weight to common or dominant species. In the Simpson index, P is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), Σ is the sum of the calculation s, and s is the number of species.

6.Community Similarity

- Calculating community similarity (what the communities have in common in terms of species).
- We used Sorenson's co-efficient
- Sorenson's coefficient gives a value between O and 1, the closer the value is to 1, the more the communities have in common a complete community overlap is equal to 1, complete community dissimilarity equal to 0.
- The equation is:

Sorenson's coefficient (CC) = $\frac{2C}{S1+S2}$

where: C is the number of species the two communities have in common, S1 is the total number of species found in community 1, and S2 is the total number of species found in community 2 (Torsvik et al. 1996).

7.Results and Discussion

The results in tables 1 and 2 showed the fungal species isolated from biofilms of corroded oil pipelines in Rivers (site 1) and Delta (site 2) States, respectively. The fungal species isolated from biofilms of corroded oil pipeline in Rivers State (site 1) are as follow: Verticillium dahlae, Botrytis cinerae, Penicillium nalgiovense, Humicola grisae, Monilia balanitis, Aspergillus niger, Saccharomyces ccerevisiae, Aspergillus flavus, Helminthosporium maydis, Aspergillus fumigatus and Aspergillus terreus (Table 1).

	Species	fi	filogfi	logfi	log^2fi	filog^2fi	pi	pilnpi	lnpi	lnpi^2	pilnpi^2	(ni/N)^2	ni(ni-1)	ni(ni-1)/N(N-1)
1	*V. dahlae*	5	3.49485002	0.69897	0.4885591	2.4427953	0.0892857	-0.2157066	-2.4159138	5.8366394	0.5211285	0.0079719	20	0.0064935
2	*B.cinerae*	2	0.60205999	0.30103	0.0906191	0.1812381	0.0357143	-0.1190073	-3.3322045	11.103587	0.3965567	0.0012755	2	0.0006494
3	*P. nalgiovense*	2	0.60205999	0.30103	0.0906191	0.1812381	0.0357143	-0.1190073	-3.3322045	11.103587	0.3965567	0.0012755	2	0.0006494
4	*H.grisae*	3	1.43136376	0.4771213	0.2276447	0.6829341	0.0535714	-0.1567896	-2.9267394	8.5658035	0.4588823	0.0028699	6	0.0019481
5	*M.balanitis*	1	0	0	0	0	0.0178571	-0.0718813	-4.0253517	16.203456	0.2893474	0.0003189	0	0
6	*A. niger*	3	1.43136376	0.4771213	0.2276447	0.6829341	0.0535714	-0.1567896	-2.9267394	8.5658035	0.4588823	0.0028699	6	0.0019481
7	*S.cerevisiae*	5	3.49485002	0.69897	0.4885591	2.4427953	0.0892857	-0.2157066	-2.4159138	5.8366394	0.5211285	0.0079719	20	0.0064935
8	*A. flavus*	1	0	0	0	0	0.0178571	-0.0718813	-4.0253517	16.203456	0.2893474	0.0003189	0	0
9	*H.maydis*	6	4.6689075	0.7781513	0.6055194	3.6331162	0.1071429	-0.2393135	-2.2335922	4.9889342	0.5345287	0.0114796	30	0.0097403
10	*A.fumigatus*	3	1.43136376	0.4771213	0.2276447	0.6829341	0.0535714	-0.1567896	-2.9267394	8.5658035	0.4588823	0.0028699	6	0.0019481
11	*A. terreus*	1	0	0	0	0	0.0178571	-0.0718813	-4.0253517	16.203456	0.2893474	0.0003189	0	0
	Sum	**32**	**17.1568188**	**4.209515**	**2.4468097**	**10.929985**	**0.5714286**	**-1.5947539**	**-34.586102**	**113.17717**	**4.6145883**	**0.0395408**	**92**	**0.0298701**

Table 1: Computation for Diversity and Dominance Indices for Site 1

ℓn = natural log; log = logarithm, Pi = proportional abundance of the ith species, N = total number of individuals of all species,

Ni = total number of individual , ni = the number of individual in the ith species, fi = abundance

a) Margdef's species richness (d)

$$d = \frac{15-1}{\ell_n 36} = \frac{14}{3.5835} = 3.9068$$

b) Shannon- Wiener Index (H)

$$H = \frac{36 \log 36 - 20.3944}{36} = \frac{56.0269 - 20.3944}{36}$$

$$H = \frac{35.6325}{36} = 0.9898$$

Shannon Index (H^1) = (–1.7492) = 1.7492

c) Evenness Index (E^1) = $\frac{H^1}{\ell_n S} = \frac{1.59707}{\ell_n 15}$

$E^1 = 0.5897$

d) Simpson's Dominance Index (C)

$$\Sigma\left(\frac{n_i}{N}\right)^2 = 0.0485$$

Simpson Index (D) = 0.0377

Simpson Index (D^1) = $\frac{1}{0.0377} = 26.5252$

The fungal species isolated from biofilms of corroded oil pipelines from Delta State (site 2) are as follow: Verticillium dahlae, Botrytis cinerae, Pencillium corylophplum, Humicola grisae, Auerobasidium pullulans, Monilia grisae, Hormoconis resinae, Saccharomyces cerevisiae, Aspergillus fumigatus, Fusarium semitectum, Bacteridium sp and Eurotium repens (Table 2). The species common to the two sites are: Verticillium sp, Batrytis cinerae, Penicullium nalgiovense, Humicola grisae, Monilia balanitis, Saccharomyces cerevisiae, and Aspergillus fumigates. The species isolated from site 1 (Table1) and not found in site 2 (Table 2) are: Aspergillus niger, Aspergillus flavus, Helminthrosporum maydis and Aspergillus terreus. The species found only in site 2 are as follows: Penicillium corylophilum, Fuarium oxysporum, Eurotium repens, Hormoronis resiane and Aureobasidium pullulans. The results showed a Sorvenson's coefficient (cc) of 0.60 indicating that the fungal communities in site 1 and 2 have quite a bit of overlap or similarity.

		Station 2 Delta (sample 1)												
	Species	**fi**	**filogfi**	**logfi**	**log^2fi**	**filog^2fi**	**pi**	**pilnpi**	**lnpi**	**lnpi^2**	**pilnpi^2**	**(ni/N)^2**	**ni(ni-1)**	**ni(ni-1)/N(N-1)**
1	*V.dahlae*	6	4.6689075	0.778151	0.6055194	3.6331162	0.1071429	-0.2393135	-2.2335922	4.9889342	0.5345287	0.0114796	30	0.0097403
2	*B.cinerera*	5	3.49485002	0.69897	0.4885591	2.4427953	0.0892857	-0.2157066	-2.4159138	5.8366394	0.5211285	0.0079719	20	0.0064935
3	*P.corylophilum*	5	3.49485002	0.69897	0.4885591	2.4427953	0.0892857	-0.2157066	-2.4159138	5.8366394	0.5211285	0.0079719	20	0.0064935
4	*H.grisae*	6	4.6689075	0.778151	0.6055194	3.6331162	0.1071429	-0.2393135	-2.2335922	4.9889342	0.5345287	0.0114796	30	0.0097403
5	*A. pullans*	3	1.43136376	0.477121	0.2276447	0.6829341	0.0535714	-0.1567896	-2.9267394	8.5658035	0.4588823	0.0028699	6	0.0019481
6	*M.balanitis*	2	0.60205999	0.30103	0.0906191	0.1812381	0.0357143	-0.1190073	-3.3322045	11.103587	0.3965567	0.0012755	2	0.0006494
7	*H. resinae*	2	0.60205999	0.30103	0.0906191	0.1812381	0.0357143	-0.1190073	-3.3322045	11.103587	0.3965567	0.0012755	2	0.0006494
8	*S.cerevisiae*	3	1.43136376	0.477121	0.2276447	0.6829341	0.0535714	-0.1567896	-2.9267394	8.5658035	0.4588823	0.0028699	6	0.0019481
9	*A. fumigatus*	1	0	0	0	0	0.0178571	-0.0718813	-4.0253517	16.203456	0.2893474	0.0003189	0	0
10	*F. semitectum*	1	0	0	0	0	0.0178571	-0.0718813	-4.0253517	16.203456	0.2893474	0.0003189	0	0
11	*Bacterium sp*	1	0	0	0	0	0.0178571	-0.0718813	-4.0253517	16.203456	0.2893474	0.0003189	0	0
12	*E.repens*	1	0	0	0	0	0.0178571	-0.0718813	-4.0253517	16.203456	0.2893474	0.0003189	0	0
	Sum	**36**	**20.3943626**	**4.510545**	**2.8246844**	**13.880167**	**0.6428571**	**-1.749159**	**-37.918307**	**125.80375**	**4.9795821**	**0.0484694**	**116**	**0.0376623**

Table 2: Computation for diversity and dominance indices for site 2

ℓn = natural log; log = logarithm, Pi = proportional abundance of the ith species, N = total number of individuals of all species, Ni = total number of individual , ni = the number of individual in the ith species, fi = abundance

a) $d = \frac{S-1}{\ell_n N} = \frac{15-1}{\ell_n 32} = \frac{14}{3.4657}$ 4.0395,

(b) Shannon- Wiener Index (H)

$$H = \frac{32 \log 32 - 17.15682}{32} = \frac{31.0080}{32} = 0.9690$$

Shannon Index (H^1) = (−1.5948) = 1.5948

c) Evenness Index (E)

$$(E^1) = \frac{H^1}{\ell_n S} = \frac{1.5948}{\ell_n 15} = 0.5889$$

d) Simpson's Dominance Index (C)

C= 0.0395

Simpson Index (D) = 0.0299

$$\text{Simpson Index } (D^1) = \frac{1}{0.0299} = 33.4482$$

	Rivers (Site 1)	**Delta (Site 2)**
Species Richness Index	3.9068	4.0395
Shannon-Wiener Index (H)	0.9898	0.9690
Shannon's Index (H^1)	1.7492	1.5948
Evenness Index (E^1)	0.5897	0.5889
Dominance Index (C)	0.0485	0.0395
Simpon's Index (D)	0.0317	0.0299
Simpson's Index (D^1)	26.5252	33.4482

Table 3: Summary of Results

It is observed from the results that species richness in site 2 (Delta State) are much higher than in site 1 (Rivers State). Also specie abundance in site 2 (Delta State) is not only greater, but the individuals in the community are distributed more equitably among these species. Evenness index (E') in site 1 is significantly higher than site 2 (Table 3).

In the samples collected from River State (site 1), there are 11 species and 36.36% belong to one genus, that is the Aspergillus and about 63.64% are distributed among 7 species. It is also important to note that the more diverse a community is the most likely it is severely affected by corrosion. Pipeline in site 2 is more likely to be affected by corrosion than pipeline in site 1. This is so, because the site contains fungi such as acid producing fungi (Hormoconis resinae and Aureobusidium pullulans) and iron reducing fungi such as Aspergillus fumigatus and Aspergillus terreus. The fungi listed above may involved in metal deterioration. In fuel and oil storage tanks fungi species such as Aspersillus, Penicillium and Fusarium may grow on fuel components and produce carboxylic acids which corrode the iron (Little et al., 2001). The problems associated with microbial growth are as follows: in diesel fuel, microbial contamination may contribute to aging instability, microbially induced corrosion of the storage tanks and pipe work, formation of microbial mats, with the ability to block filters and pipelines, and to increase wear in pump (Solana and Gaylarde 1995). Bacteria showed decreasing capabilities to degrade alkanes with increasing claim length, but filamentous fungi do not exhibit preference for specific chain length. The acid frequently produced by fungi are fumic, citric and acetric acids. These acids are damaging to metals, glass, masonry and other materials, and may contribute significantly to corrosion of oil pipelines (Little and

Stachle, 2001). Fungi are capable of producing copious quantities of either organic or inorganic acids as metabolic by-products. Microbially produced inorganic acids are nitric acid (HNO_3). Sulphurous acid (H_2SO_3), sulphuric acid (H_2SO_4), nitrous acid (HNO_2) and carbonic acid (H_2CO_3). Sulphurous acid and sulphuric acid are mainly secreted by bacteria of the genera. Thiobacillus and the fungus *Aureobasidium* which was isolated from biofilms samples from Delta State (site 2) only. The third type of inorganic acid, the carbonic acid is produced by many life forms. Carbonic acid, especially if present in high concentrations, can react with calcium hydroxide and forms the insoluble calcium carbonate ($CaCO_3$) and the water-soluble calcium hydrogen carbonate ($Ca(HCO_3)_2$). This is aggressive carbonic acid (Videla, 1996).

8.Conclusion

This is the study exploring directly fungal diversity in a corrosive biofilms of oil pipelines subjected to normal flow conditions. The diversity indices provide more information than simply the number of species present, in the oil pipelines but serve as valuable tools that enable researchers to quantify diversity in a pipeline and describe its structures. These data allow a better, appreciation of the composition and variability of oil pipeline fungal communities, and may contributes to a new improved ways to detect, monitor, and control microbial corrosion in oil and gas industry pipelines.

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