MICROBIOLOGICAL EVALUATION OF VARIOUS OILFIELD WASTEWATERS FROM OBEN LAND RIG LOCATION IN EDO STATE, NIGERIA

Abstract

Oilfield wastewater contains toxic and hazardous substances that are detrimental to water quality and microorganisms. It is estimated that large quantities of wastewater from operations of oil industries in Nigeria is discharged into water environment without adequate treatment. The microbiological evaluation of various of oilfield wastewater on the microbial population and diversity of aquatic environment was studied bi-weekly for a period of two months. Water samples collected 10cm from pit 1, pit 2, pit 3, camp pit 1, and camp pit 2 were analyzed for total heterotrophic bacterial count, hydrocarbon utilizing bacterial count, total fungal count, and hydrocarbon utilizing fungal count, and for microflora using standard microbiological methods. Total heterotrophic bacteria (THB) counts ranged from 4.0×10^4 Log10cfu/ml to 4.4×10^4 Log10cfu/ml, total fungal (TF) count ranged from 2.0×10^4 Log10cfu/ml to 2.2×10^4 Log10cfu/ml. The total hydrocarbon utilizing bacterial (THUB) count ranged from 3.0×10^4 Log10cfu/ml to 3.1×10^4 Log10cfu/ml, while the total hydrocarbon utilizing fungal (THUF) count ranged from 1.0×10^4 Log10cfu/ml to 1.1×10^4 Log10cfu/ml. the percentage (%) of occurrence for the total hydrocarbon utilizing bacterial (THUB) ranged from 30.7% to 210.5%, while the total hydrocarbon utilizing fungal (THUF) ranged from 86.9% to 1285.7%. Statistical analysis showed that there was no significant difference in the THB between the pits and the sampling stations. The types of bacteria isolated in the study included *Kurthia* spp, *Bacillus* spp, *Pediococcus* spp, *Enterococcus* spp, *Aeromonas* spp, *Micrococcus* spp, *Pseudomonas* spp, *Escherichia coli*, *Alcaligenes* spp and *Lactobacillus* spp. The fungi isolated included *Aspergillus fumigatus*, *Penicillium brevicompactum*, *Rhizopus oryzae* and *Fusarium* spp. The bacterial and fungal counts in the study revealed the impact of oilfield wastewater on aquatic microbes. The high prevalence of hydrocarbon utilizing microorganisms revealed that the water studied contained active indigenous hydrocarbon utilizers which can be harnessed for bioremediation process.

Keywords: Oilfield wastewater, Hydrocarbon Utilizing Bacteria/ Hydrocarbon Utilizing Fungi

INTRODUCTION

Oilfield wastewater or produced water is a formation and injection water that contains production chemicals that is generated during the production of crude oil and gas from onshore and offshore wells (Neff *et al.*,2011a). The production water is a complex mixture of dissolved and particulate organic and inorganic chemicals in water that ranges from essentially freshwater to concentrated saline brine. Produced waters most abundant organic chemicals are water soluble low molecular weight organic acids and monocyclic aromatic hydrocarbons.

Produced water is a complex mixture with many variables influencing its characteristics, including the age and location of the oil field, the geological characteristics of the formation from which the water is originating, the type of hydrocarbon product being produced, the production history of the reservoir, and the operational conditions under which it originates (Fakhru'l-Razi et al., 2009). While the composition of produced water is considered highly variable (Durrell et al., 2006) and constituent concentrations can vary between different sources by orders of magnitude (Neff et al., 2011a; Fakhru'l-Razi et al., 2009), it is similar across oil production facilities in terms of its major constituents (Fakhru'l-Razi et al., 2009). Fakhru'l-Razi et al. (2009) summarize the components of produced water to include crude oil, which is a mixture of aliphatic and aromatic hydrocarbons; dissolved formation minerals, including heavy metals and radioactive materials; production chemicals, which are typically synthetic additives; solids such as formation solids, corrosion and scale materials, bacteria, waxes and asphaltenes; and dissolved gases. Oil is a generic term representing a wide array of compounds, mainly hydrocarbons, which may be present in produced water as dispersed droplets and/or dissolved in the water phase, depending on their solubility and structural properties (OGP, 2005). Aliphatic hydrocarbons are typically found in the dispersed phase, while carboxylic acids are most often found in the dissolved phase. Aromatics can be in either, or sometimes in both, depending on their molecular weight and structural complexity, with lower molecular weight compounds tending to be relatively more water soluble and thus more often present in the water (dissolved) phase (OGP, 2005). Produced water is generated in large volumes in the production phase of conventional oil wells. Approximately 1.1 m3 is generated for each 1.0 m3 of oil produced worldwide (Neff et al., 2011a), making it definitively the largest waste stream associated with the production process (Arctic Monitoring and Assessment Programme (AMAP), 2010). Produced water is typically treated to remove the dispersed crude oil content (that is, droplets of

crude oil, typically ranging from 1 to 10 um in size) (Neff et al., 2011a) before it is either discharged as a waste material into the sea, or is re-injected into a sub-sea formation for disposal (Ekins et al., 2007; Yeung et al., 2015).

Offshore drilling for oil and gas produces large amount of oil field waste water which is usually discharged into the aquatic environment though it undergoes some form of treatment before being discharged. Oil field waste water is discharged into sea after it has been separated from oil drawn from the reservoir (Jerry *et al.*,2006). Water co-produced with oil and gas and separated for discharge (oilfield wastewater) retains up to 50mgL-1 of separate phase oil as small droplets and also may contain up to 35mgL-1 of dissolved hydrocarbons (Koons *et al.*,1979). The numerous inorganic constituents dissolved in formation water can be potentially or actually far more hazardous than the crude oil itself (Wardley-Smith, 1979). The ecological health of many river systems is threatened by the discharge of toxic compounds and the accumulation of these contaminants in these aquatic environments (Pruell *et al.*,1990).

The most abundant organic chemicals in most treated produced waters are water-soluble low molecular weight organic acids (primarily mono- and di-carboxylic acids) and monocyclic aromatic hydrocarbons (MAHs) including benzene, ethyl benzene, toluene, and xylenes (Neff et al., 2011a). Produced water components thought to contribute most to the ecological risk in marine environments based on their chemical characteristics are the MAHs, polycyclic aromatic hydrocarbons (PAHs), related heterocyclic aromatic compounds, and sometimes one or more metals such as iron, lead, mercury, and zinc (OGP, 2005). Therefore, this study investigated the microbiological evaluation of various oilfield wastewaters from Oben Land Rig location in Edo State**.**

MATERIALS AND METHODS

Description of study Area

Oben is a community in Edo State, it is known to be part of the Niger Delta oil producing community. The oilfield site is known as OML4, where all the oil production processes are carried out and waste water discharged.

Collection of Samples

Oilfield wastewater was collected from Oben flow Station; an onshore oil production platform located in Oben in Edo State, Nigeria. At each Land rig site, the water samples were collected using a specially prepared pail. The pail was rinsed with the samples before collection from each Pit point. The samples were put in a properly labelled and sterilized McCartney bottles for microbiological analysis and stored in an ice packed cooler. The collected and appropriately

labeled oilfield wastewater were immediately transported to the laboratory for analysis within 24 hours for processing and analyses.

Media Preparation

Nutrient Agar was used for Total Heterotrophic bacterial count; Potato dextrose agar was used for total fungal count while Mineral salt agar medium prepared according to the modified minimal salts medium (MSM) composition of Mills *et al.* (1978) was used for the isolation of total hydrocarbon utilizing bacteria and fungi. Minimal salts medium (MSM) composition is [MgSO4.7H2O (0.42g), KCl (0.29g), KH2PO4 (0.83g), Na2HP04 (1.25g) NaNO3 (0.42), agar (20g)] in 1Litre of distilled water. The mixture was thoroughly mixed and autoclaved at 15psi at 121°C for 15mins and was allowed to cool to 45°C. The medium was prepared by the addition of 1% (v/v) crude oil sterilized with 0.22µm pore size Millipore filter paper (Obire, 1988) to sterile MSM, which has been cooled to 45^{O}C under aseptic condition. The MSM and crude oil were then mixed thoroughly and aseptically dispensed into sterile Petri dishes to set.

Microbiological Analysis of the Oilfield Wastewater

Determination of Total Heterotrophic Bacterial (THB) Count of Oilfield Wastewater

The total heterotrophic bacterial (THB) count was determined using the nutrient agar and spread plate technique as described by Prescott *et al.* (2005). An aliquot (0.1ml) of each serially diluted sample using dilution factors of 10^{-5} for Raw wastewater, 10^{-4} for oilfield wastewater and inoculated onto different sterile nutrient agar plates in triplicates. The plates were incubated at 37°C in an inverted position for 24 hours. After incubation, colonies that developed on the plates were counted and only counts of between 30 and 300 were recorded. The average values of replicate plates were calculated and expressed as colony forming unit cfu/ml for oilfield wastewater.

Comment [D1]: Need to add the image of THB colonies.

Determination of Total Fungi Count of Samples of Oilfield Wastewater

The total count of fungi in the samples was also determined by the spread plate technique. An aliquot (0.1ml) of serial dilution (10^{-2}) of each of the various samples was plated onto separate Potato dextrose agar plates to which 0.1 ml of streptomycin solution was incorporated to suppress bacterial growth. The plates were incubated at 28°C for 5-7 days and the discrete

colonies that developed were enumerated as the viable counts (CFU) of fungi in the oilfield wastewater (Obire and Wemedo, 1996).

Comment [D2]: Need to add image of Fungi developed in plate

Total Fungi

Hydrocarbon Utilizing Bacterial Count (HUB) of Samples

Total hydrocarbon utilizing bacteria count of oilfield wastewater samples was determined by inoculating 0.1ml of the serially diluted samples (10^{-4}) on mineral salt agar. The Vapor Phase Transfer method was adopted by the use of sterile filter paper discs that were soaked in filter sterilized crude oil which served as the only carbon source in the mineral salt agar (Obire and Wemedo,1996). The sterile crude oil-soaked filter papers were aseptically transferred to the inside cover of the inoculated Petri dishes and incubated for 5 days at room temperature. Colonies that develop were counted; average of duplicate colonies calculated colony forming units per ml (cfu/ml) of oilfield wastewater calculated.

Comment [D3]: Need to add image of Colonies developed in plate

Hydrocarbon Utilizing fungal Count (HUF) of Samples

Total hydrocarbon utilizing fungal count of oilfield wastewater was determined by inoculating 0.1ml of the serially diluted samples ($10^{-2)}$ on mineral salt agar. The mineral salt medium will be supplemented with streptomycin (0.1ml) to suppress bacterial growth (Obire and Wemedo, 1996). The Vapor Phase Transfer method was adopted by the use of sterile filter paper discs that were soaked in filter sterilized crude oil which served as the only carbon source in the mineral salt agar. The sterile crude oil-soaked filter papers were aseptically transferred to the inside cover of the inoculated Petri dishes and incubated for 5 days at room temperature. Colonies that develop were counted; average of duplicate colonies calculated colony forming units per ml (cfu/ml) of oilfield wastewater was calculated.

Characterization and Identification of Bacterial and Fungal Isolates from Samples

The cultural, morphological, microscopic characteristics of the isolates from the study were observed and recorded. The morphological and biochemical tests conducted using the isolates included Gram staining, motility, catalase, oxidase, citrate utilization, sugar fermentation, hydrogen supplied production, indole production, methyl red and Voges Proskauer test. Results of the morphological and biochemical characteristics of the isolates were compared with those of known Taxa using Bergey's manual of determinative bacteriology (1994). For the presumptive

identification of fungal isolates, pure fungal cultures were observed while still on plates and after wet mount in lactophenol on slides under the compound microscope. Observed characteristics such as vegetative hyphae and reproductive structures were recorded and compared with the established identification key of Barnett and Hunter (1972) and Malloch (1997).

Statistical Analysis

Statistical analysis was also conducted using Duncan Multiple Range test and Analysis of variance to determine whether there is significant difference between various concentration of oil field wastewater and period of incubation.

Results

Comment [D4]: Need to represent the result data to be presented in tabular form

The microbiological counts obtained in the various sampling points as showed in Fig 1. The Total Heterotrophic Bacteria Count (THB) ranged from 4.4 Log10cfu/ml to 4.5 Log10cfu/ml. The highest count was recorded in the Camp pit1& 2 (4.5 Log10cfu/ml), while the lowest was observed in the Pit 1,2& 3(4.4 Log10cfu/ml). The total fungal counts (TFC) ranged from 2.0Log10cfu/ml to 2.2 Log10cfu/ml. The highest count was recorded in the Camp pit 1&2 (2.2 Log10cfu/ml), while the Lowest was observed in the Pit 1&2 (2.0 Log10cfu/ml). The Total Hydrocarbon Utilizing Bacteria (HUB) count ranged from 3.0 Log10cfu/ml to 3.1 Log10cfu/ml. The highest count was observed in the Pit 1 2&3 (3.1 Log10cfu/ml), while the lowest was recorded in Camp pit 1& 2 (3.0 Log10cfu/ml). The total Hydrocarbon Utilizing Fungi (HUF) counts ranged from 1.0 Log10cfu/ml to 1.1 Log10cfu/ml. The highest was recorded in the Pit 1(1.1 Log10cfu/ml), while the lowest was recorded in the Camp pit 1&2 (1.0 Log10cfu/ml). The microbiological counts obtained in the various sampling points in Log10cfu/ml are as shown in Fig 1.

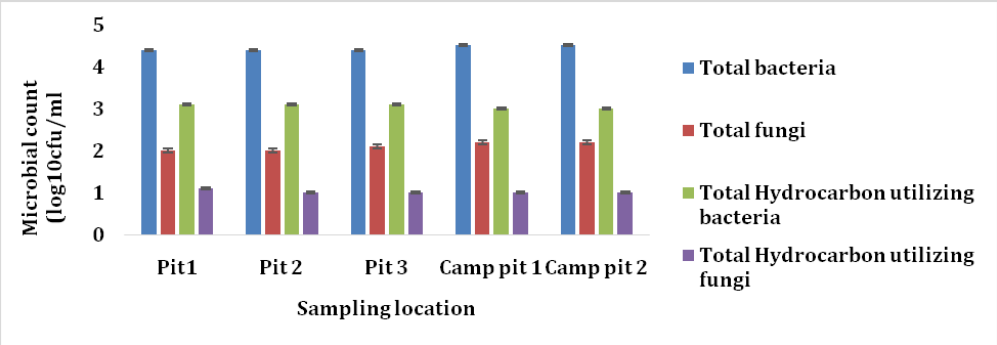

Fig 1: Microbial Counts of Oben

The predominant bacteria are of the genera; *Kurthia* spp, *Bacillus* spp, *Pediococcus* spp, *Enterococcus* spp, *Aeromonas* spp, *Micrococcus* spp, *Pseudomonas* spp, *Escherichia coli*, *Alcaligenes* spp and *Lactobacillus* spp. The hydrocarbon utilizing bacteria isolated from pit 1; *Bacillus* spp, pit 2; *Kurthia* spp. While the fungi are *Aspergillus fumigatus*, *Penicillium brevicompactum*, *Rhizopus oryzae* and *Fusarium* spp that were isolated from the oilfield wastewater from Oben are as shown in Table 1 and Table 2 respectively.

Table 1: **Bacteria isolated from the Oilfield Wastewater from Oben**

Isolate codes	**Organism**
O1	*Enterococcus* spp
O2	*Aeromonas* spp
O3	*Kurthia* spp
O4	*Bacillus* spp
O6	*Alcaligenes* spp
O7	*Pseudomonas* spp
O8	*Bacillus spp*
O9	*Bacillus* spp
O10	*Bacillus* spp

O11	*Micrococcus* spp
O12	*Escherichia* spp
O13	*Bacillus* spp
O14	*Bacillus* spp
O15	*Pseudomonas* spp
O16	*Bacillus* spp
O17	*Enterococcus* spp
O18	*Micrococcus* spp
O19	*Kurthia* spp
O20	*Bacillus* spp
O21	*Bacillus* spp
O22	*Pseudomonas* spp
O23	*Alcaligenes* spp
O24	*Alcaligenes* spp
O25	*Bacillus* spp
O26	*Lactobacillus* spp
O27	*Micrococcus* spp
O28	*Pediococcus* spp
O29	*Alcaligenes* spp
O30	*Bacillus* spp

O 1-30; Oben

Table 2: **Fungi Isolated from Oilfield Wastewater from Oben**

Isolates code	Organism

OB6	*Fusarium* spp
OB7	*Rhizopus oryzae*
OB1	*Penicillium brevicompactum*
OB14	*Aspergillus fumigatus*

Keys; OB- Oben

DISCUSSION

The present study revealed the microbial population and diversity of bacteria and fungi in oilfield wastewater from Oben. Microbial populations play a role in degradation of hydrocarbon contaminations, Leahy and Colwell (1990) concluded that hydrocarbon biodegradation depends on the composition of the microbial community and its adaptive response to the presence of hydrocarbons. Hydrocarbon utilizing bacteria and fungi counts were higher in the oilfield wastewater in the various months of study.

The result showed that more heterotrophic bacteria count was obtained from the Camp Pit 1&2(4.5 Log_{10}cfu/ml) and the least count was found in Pit 1,2&3 (4.4 Log_{10}cfu/ml) these can be attributed to the less oilfield wastewater been deposited there than in the Pit 1,2&3. The high fungi count was obtained in the Camp Pit 1&2 (2.2 Log_{10}cfu/ml), followed by Pit 3 (2.1 Log_{10}cfu/ml) and the least found in the Pit 1&2 (2.0 Log10cfu/ml), this can attribute to lesser activities being carried out there in the Pit 1&2. The high hydrocarbon utilizing bacteria count was obtained in the Pit 1,2&3(.3.1 Log_{10}cfu/ml), followed by the camp Pit 1(3.0 Log_{10}cfu/ml), the least count was obtained in the Camp Pit 2(3.0 Log_{10}cfu/ml), while the high hydrocarbon utilizing fungi count was obtained in the Pit 1(1.1 Log_{10}cfu/ml) and the least in pit 1&2 and Camp Pit 1&2 (1.0 Log_{10}cfu/ml), this high hydrocarbon utilizing bacteria and fungi count found in the Pit 1 can be attributed to more hydrocarbon content being deposited there since it is close to the oilfield station. The high prevalence of hydrocarbon utilizing bacteria and fungi found in this study shows that the inorganic and organic constituent found in the oilfield wastewater has served as nutrient for bacteria and fungi thus enhancing their growth. The continuous discharge of treated oilfield wastewater will have a deleterious effect on the proper functioning of the soil and aquatic environments thereby affecting aquatic and agricultural resources that are of economic importance (Obire, and Amusan, 2003).

The isolated Bacteria (*Kurthia* spp, *Bacillus* spp, *Pediococcus* spp, *Enterococcus* spp, *Aeromonas* spp, *Micrococcus* spp, *Pseudomonas* spp, *Escherichia coli, Alcaligenes* spp and *Lactobacillus* spp.) and Fungi (*Aspergillus fumigatus, Penicillium brevicompactum, Rhizopus oryzae* and *Fusarium* spp) were hydrocarbon utilizing bacteria and fungi which indicated that the oilfield waste water contained high hydrocarbon contents. Similar organisms were also isolated by Aleruchi and Obire (2019) indicating high hydrocarbon content contained in the oilfield waste water that is been discharged into Oben oilfield.

CONCLUSION AND RECOMMENDATION

The occurrence of these microorganisms in the wastewater may be to the constant exposure of these microorganisms to hydrocarbon (oily) components of the wastewater which could have made the organisms to have the ability to utilize and grow in the presence of the hydrocarbon. The high population of hydrocarbon utilizers in the sampling stations suggests that the hydrocarbon utilizers were adapted to the quantity of hydrocarbons in the environment and hereby increased the number of hydrocarbon utilizers in the polluted area. The study also revealed that most of the organisms isolated as total heterotrophic bacteria and total fungi were part of the utilizers. The response of these microorganisms in the oil polluted environment suggests that the isolated bacteria and fungi could utilize the oil as energy and carbon source which serves as nutrient for their growth and thus could be effective in the cleanup of the polluted sites as a bioremediation agent.

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